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by

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ABSTRACT

A detailed caging experiment was conducted in the southern Gulf of St. Lawerence and on the Scotian Shelf – areas of known high concentrations of commercially valuable snow crab (*Chionoecetes opilio*) and, where substantial reserves of oil and natural gas are believed to exist. The possible effects of seismic exploration on snow crab populations was examined in 2003 off western Cape Breton and results suggested some abnormalities in certain crab appendages and organs; however, it was found that caging itself may have had a negative impact on crab health, masking potential effects of exposure to seismic energy. The current study: 1) established expected ranges for applicable health parameters (condition of organs and appendages, morphometric relationships, hepatopancreas energy (lipid and glycogen) content, hemolymph biochemistry analysis) for free-ranging mature male and female snow crab in these environments; 2) assessed the effects associated with 2 weeks, 6 months, and 12 months of caging on these health parameters; and, 3) examined the effects of two methods of crab collection (trapping, trawling) on snow crab health by hemolymph biochemistry analysis.

It was concluded that factors such as sampling period (seasonal effects), sampling site (regional effects), and crab sex must be considered when using these health parameters. The condition of appendages and organs and morphometric relationships may be used to detect short-term abnormalities with cautious consideration for environmental and life cycle induced variations. Hemolymph biochemistry profiles were sensitive indicators of caging stress, detecting differences in concentrations of metabolites such hemolymph protein concentration between free and caged crabs as early as two weeks when the groups were directly compared. Reference intervals for biochemistry parameters were not as sensitive as direct comparisons but, could be improved if larger sample sizes (≥ 120) were used to generate them. Biochemistry parameters had moderate success at estimating hepatopancreas lipid content only in some, but not all, crab groups. It is anticipated that these estimates could be improved if total body (hepatopancreas, muscle, and gonad) energy (lipid, glycogen, and protein) stores were assessed and then compared to hemolymph parameters. Such global assessment should account for the effects of redistribution of energy reserves in females for oocyte production and other potential seasonal and sex-related variation in the energy reserves used by snow crab.

Other interesting findings, beyond the scope of this study to investigate further, included the detection of two previously unreported viruses (one localized to the hepatopancreas, one systemic but most prominent in the gills) and the first observation of a one year (instead of a two year) reproductive cycle at two of the three southern Gulf of Saint Lawrence sampling sites.

Results from the caging experiments suggest that caging itself significantly stresses the crabs and thus cage-held crabs may not be representative of the free-ranging population. Mortality increased with the length of caging for both male and female crabs. Furthermore, collecting crabs by trapping may create a sampling-induced bias towards nutritionally stressed (hungry)

crabs and against physically and physiologically damaged crabs. Significant differences between free and caged crabs were observed in plasma biochemistry profiles, the hepatopancreas (overall condition, histology and biochemical analysis), gill fouling species, stomach contents and the reproductive cycle in females.

Future studies examining the impact of environmental stresses such as seismic shooting/testing should consider collecting crab by trawling rather than trapping due to: 1) the possibility of performing immediate dissection/obersvation onboard in order to reduce the emersion period; 2) the vast choice of crab size range, sex, and maturity available; 3) the reduced sampling bias; and, 4) the more natural setting than trapping. If an alternative method to caging is not feasible, a meticulous research plan must be prepared to simultaneously measure and distinguish two independent stressors i.e. seismic noise and caging induced stresses.

SUMMARY

Due to the growing human pressures on the oceans, the need to better assess the health of aquatic populations becomes increasingly important. A better understanding of the normal physiological, morphological and behavioral variations from healthy snow crab populations is essential and should be an ongoing process.

Biological baseline information for snow crab was examined for future assessment of the possible impact of seismic noise on snow crab, *Chionoecetes opilio,* albeit no seismic activity was performed during this study. Since caged animals are often used concomitantly with seismic exploration (and since the possible induced stress due to caging on crab remains unknown), this study compared various appendages, organs and feeding behavior between caged and free crab. Free crab were collected by trapping at three sampling stations in the southern Gulf of St. Lawrence and one on the Scotian Shelf while caging occurred at 2 of the 3 sampling stations in the southern Gulf of St. Lawrence.

Stress can be measured at different levels varying in sensitivity and specificity (biochemical, physiological, individual and population). The most sensitive tools (i.e. DNA integrity, metabolites) detect the first signs of a stress reaction within a system or individual but the outcome is usually of low relevance and reversible. The least sensitive tools (sex ratio, reproductive integrity) often detect effects at the population level and are of high ecological and economic importance. Ideally, stressors imposed simultaneously would create reactions measurable at different levels or have known specific responses. Otherwise, distinguishing the effects between two stressors concomitantly will remain equivocal.

This study had three objectives: first, to determine the normal physiological variations of organs, and appendages and their morphometric relationships, of free-ranging snow crabs found in their

natural state within their primary habitat; second, to evaluate the possible pathologic effects of caging for three different immersion periods (2 weeks, 6 months and 12 months); and third, to compare the effects ofcrab collection methods (trapping versus trawling) on snow crab health by hemolymph biochemistry analysis.

Crabs were collected by using traps at four stations- three in the southern Gulf of St. Lawrence (Grande-Rivière, Margaree Harbor, and Cheticamp) and one on the Scotian Shelf (Louisbourg) in spring and fall 2012 and 2013. In the fall 2013, crab collection was only amenable in Margaree Harbor and Cheticamp. In addition, caged crabs were immersed at two stations, Margaree Harbor and Cheticamp for 2 weeks (16/17 days), 6 months (214/222 days) and 12 months (345/355 days, respectively).

The collection of biological baseline information included: size-weight (carapace width (CW)-body weight, CW-ovary weight, CW-hepatopancreas weight) and size-fecundity relationships; examination of the physical/morphological condition of antennules, statocysts (group hairs) and gills;, identification of gill fouling species and their abundances; determination of ovary, hepatopancreas and egg color by the visual observations and a colorimeter; determination of embryonic developmental stages; examination of feeding behaviors (by stomach content and stable isotopic analyses); measurements of larval morphometry; histological examination of gills, ovaries and hepatopancreas; and, biochemical analyses of hepatopancreas (lipid and glycogen contents) and hemolymph. Statistical analyses of selected parameters for their natural/seasonal variability and for treatment effect (caging vs free) were also conducted.

Some difficulties in at-sea trapping due to: 1) bad weather (consequently sampling period was extended); 2) commercial fishing (sampling stations were in the middle of commercial fishing ground and in some cases, study traps were empty when retrieved/lifted); and, 3) the biological cycle and natural abundance of different crab categories (difficultly trapping immature females and adolescent males and lack of mature females at pre-determined stations known for high concentrations of females based on stock assessments and local fishermen's experience and knowledge), were encountered. Consequently, crab categories were modified from "immature females, mature females, adolescent males and commercial size adult males" to "mature females, undersize adult/pygmy males and commercial size/large adult males"). The second, deeper station off the Scotian shelf near Louisbourg was also abandoned due to the lack of females.

Some difficulties in trap setting were also encountered due to unfavourable weather conditions and vessel schedule. As such, four planned sampling dates were rescheduled for a later time. These delays in sampling dates can significantly affect results, especially when examining reproductive parameters such as embryonic and ovary development and fecundity. Using different vessels with different sampling teams may resolve this problem although it is not cost effective.

There was significant difference in mean crab carapace width within each crab category (large male, pygmy male and mature female). This was especially notable for pygmy male crabs, as only a limited number of crabs were caught from this category. If a narrow size range of crab is required for a particular study, a longer sampling period and/or more intense sampling effort per station may be necessary. Analysis of variance-covariance by location, year and year-location interaction showed that crab carapace size vs body-weight relationships were significantly different in terms of location but not in terms of treatment (caging vs free).

GENERAL FINDINGS AND INDICATORS

Gills

Gill condition

The degree of condition of gills was determined in a 2003-04 study on the impact of seismic noise on snow crab for the sonified and unsonified individual. The condition of gills was classified into three categories i.e. clean, intermediate and dirty (filled with compacted sediment-like substances). In the current study, we classified the gill condition into four categories i.e. clean, relatively clean (trace of dirtiness), intermediate and dirty. The majority of gills were either clean (96%) or relatively clean (3%). Very few samples (1%) were classified as having an intermediate condition and none of the samples examined was classified as dirty in contrast to the 2003-2004 study where 94% of sonified crabs had 'dirty' gills. In healthy snow crab, gill lamellae are continuously cleaned/swept by the setose epipods of the first, second and third maxillipeds. There was no effect of caging on gill condition (% of clean gills) in any crab category (large males, pygmy males or mature females).

Gill histology

The histological evaluation of gill tissue did not reveal any particular characteristic unique to caged crabs. Inflammatory changes were minimal although a slight tendency for increased hemocyte nodule formation was noted in six month caging samples for pygmy male and female crabs compared to mature male crabs at both Cheticamp and Margaree Harbor stations. Coarse granulation was noted on light microscopy in the epithelium of eight crabs, all but one from Cheticamp or Margaree Harbor stations, which was shown to be due to a previously unreported intracytoplasmic virus with transmission electron microscopy. Gill would be an excellent tissue to use for screening purposes in the event that further work is to be done to better characterise this new virus regarding possible implications to the snow crab population. Three lesions not related to caging were also identified. Firstly, eosinophilic hyaline deposits within the vascular spaces of gill lamellae, especially the bases and tips tended to occur with greater intensity in the fall. It is speculated that this may be the consequence of dehydration of gill tips during transport with subsequent activation of the prophenoloxidase activity of hemocyanin. Secondly, abnormal

epithelial cells (smaller, dense nuclei with cytoplasmic vacuolation) were observed most often in the spring 2013 samples, in smaller pygmy male and female crabs. A toxic or infectious cause could be considered. Finally, microabscesses were noted infrequently but most often (4/6) in free male crabs from Grande-Rivière.

Gill fouling species and their abundance

Among the gill lamellae, the ten most frequently encountered groups/species identified were: Type-I: Harpacticoida copepod, Leophonte sp.;Type II: Harpacticoida copepoda, Tisbe celata; Type III: Bryozoa; Type IV: Turbellaria flatworms, Ectocotyla hirudo and E. multitesticulata; Type V: Nematoda, Unknown genus and species; Type VI: Tisbe celata nauplii; Type VII: Kinorhyncha, Echinoderes elongates; Type VIII: Polychaeta larvae, Ophryotrocha geryonicola; Type IX: Mites (Halacaridae); Type X: Turbellaria, egg capsules. Types VIII, IX and X were very rare. In terms of the total abundance of gill fouling species, type VII was the most abundant followed by types I, V, II and VI. The highest average number of gill fouling species per crab (carrying at least one individual of a given type of gill fouling species) was also the type VII (5,002/crab) followed by types I, VI, V, II. The frequency of prevalence was the highest for type V followed by types II, I, VI, VII, IV, III and X. The degree of impact may be assessed by the species composition and their abundance among snow crab gill lamellae.

There was a significant difference ($p = 0.001$) between free and caged crab for gill fouling species type VI (Harpacticoida, copepoda nauplii), VII (Kinorhyncha, Echinoderes elongates) and X (Turbellaria, egg capsules) for all crab categories (large males, pygmy males and mature females). The type VI and VII were more abundant in free crabs and type X was more abundant in caged crabs. However, it is difficult to understand the cause of difference without any information on the life cycle and habitat preference of these species.

Antennules

Antennule condition

Under normal conditions, the majority of antennules should not bear any substances/dirtiness and if so, substances should be of organic origin (e.g. fouling species) instead of sediment components. The condition of the outer flagellum and aesthetasc hairs of the antennules were classified into four categories: 1) clean, 2) relatively clean (some dirtiness on the aesthetasc hairs), 3) intermediate and 4) dirty. Out of 1,125 antennules observed, the majority (97%) were classified as clean and relatively clean. Only 3% of antennules had an intermediate or dirty condition. In this present study, the substances observed in “dirty antennules” seemed to be of organic origins. No damaged aesthetasc hairs were observed.There was no caging effect on the condition (% of clean antennules) of antennules for any crab category (large males, pygmy males

and mature females). These results are in contrast to the 2003-2004 study where sediment was noted on 52 % of antennules.

Statocysts

Statocyst condition

During the practice dissection phase, dissecting whole staocysts without damaging the external membranous structure was extremely difficult (in more than 85% of cases, the outer membrane was damaged with possible displacement of the internal statolith and damage to thread hairs). During the seismic study in 2003/2004, it was noted that when the statolith was displaced, the group hairs also showed pronounced dirtiness. Therefore, a dissection protocol for collection of group hairs, which caused minimum artefact, was established and served as a good proxy for the statocyst conditions.

The condition of statocysts (group hairs) was classified into four categories: 1) clean, 2) relatively clean, 3) intermediate and 4) dirty. All statocysts were classified either clean (97%) or relatively clean (3%), which is comparable to 100% clean condition based on Moriyasu *et al.* (2011). There was no trace of displaced statoliths onto group hairs comparable to that observed by Moriyasu *et al.* (2011) on the snow crab exposed to the seismic noise. Under normal conditions, group hairs in statocysts should not bear any substances/dirtiness. There was no caging effect on the condition (% of clean statocyst) of statocysts for any crab category (large males, pygmy males and mature females).

Ovary

Ovary color measurements

Both types of observations (visual observations and colorimeter measurements) suggested that ovary color in caged (6 and 12 months) females were not comparable to that observed in free females. This suggests that the developmental cycle was disturbed in crab caged for more than 6 months. In free females, the color transition phase over the 12 month observation period seemed to be different in Baie des Chaleur compared to free females in the three other stations (Margaree Harbor, Cheticamp and Louisbourg). Females in Baie des Chaleurs might have a 2-year reproductive cycle whereas free females in other (more southern) locations may have one year reproductive cycle.

Carapace size-ovary weight relationship

Although there was no significant difference in carapace width and wet ovary weight relationships in terms of location, year and location-year interaction, there was a tendency of an

annual variation pattern in regression residual being low in spring and high in fall, except for in Grande-Rivière station which suggests that the possible difference in reproductive schedule also suggested by other parameters (egg and ovary colors, fecundity and ovary histology). There was a significant difference for 6- and 12-months caged crabs. This observation together with other findings (egg color, ovary color, fecundity and ovary histology) suggests that caging mature females may result in desynchronization of reproductive cycle.

Ovary histology

Histological changes in the ovaries were recognizable in caged females regardless the caging duration (two weeks, 6 and 12 months). A slight increase in oocyte resorption was noted after two weeks, evidence of delayed and/or aborted spawning at six months, and delayed recovery and/or marked resorption of oocytes at 12 months. Changes observed after two weeks seem more likely to be related to handling and/or caging stress while, changes noted at six and 12 months are more likely related to decreased nutritional stores. The histological data from the free crabs suggests that snow crabs are following a one year spring spawning cycle in Cheticamp, Margaree Harbor, and Louisbourg while crabs collected from Grande-Rivière are following a two year spring spawning cycle.

Embryo

Embryo color measurements/Embryonic stages

Determination of embryonic stages and egg color measurements suggested that the reproductive cycle in free females from Margaree Harbor, Cheticamp and Louisbourg was a one year cycle instead of a 2-year cycle that was assumed prior to this project. Only females in Baie des Chaleurs might have had a two-year reproductive cycle during the observation period of the study (possibly influenced by bottom water temperature regime).

Carapace size-fecundity relationship

There was a significant difference in carapace width and fecundity (estimated number of eggs per clutch) relationships in terms of location, and year in both spring and fall seasons. However, no clear seasonal tendency in regression residual was observed in free crabs among four stations. For caged vs free crabs there was a significant difference for 12 month caged vs free. Possibly this difference is due to desynchronization of reproductive cycle caused by caging treatment resulting in egg loss in caged females. Caged females showed a delay in embryonic development compared to free females. Temperature probes attached to cages showed a non-negligible fluctuation in water temperature and if free females maintain their position within certain water temperature range by small-scale movement in order to control embryonic development schedule,

a delay in embryonic development in caged female crabs may be explained by their restricted movement.

Hepatopancreas

Hepatopancreas condition

The majority of the outer hepatopancreatic walls were considered as 'smooth' or relatively convoluted. There was no highly convoluted hepatopancreas wall observed such as those observed (in limited number) in the 2003 seismic ensonified samples. In addition, there was a higher percentage of crabs bearing moderately convoluted hepatopancreas walls in caged samples after 6 months of immersion compared to free crabs. However, as there is no relationship established between the morphology of the outer hepatopancreas wall and snow crab feeding behavior, these results have to be interpreted with caution.

There was a caging effect on the hepatopancreas condition (% of smooth outer walls) in mature female crabs ($p = 2.018e-06$). No significant difference was observed in large males or pygmy males ($p = 0.001$). The significance level was at $p= 0.02504$ and 0.01818 for large males and pygmy males, respectively, suggesting that the hepatopancreas outer wall condition shows some degree of modification by caging treatment. It is possible that decreased feeding activity and/or the quality of prey items may have impacted the morphological condition of the hepatopancreas.

Hepatopancreas color measurements

Although there was a high variability when measuring hepatopancreas color values in terms of location, year and location-year interaction, there was a seasonal tendency of color values showing that the redness value of hepatopancreas increased from spring to fall and decreased again towards the following spring. It was noted that the majority of cases (except for large males in Margaree Harbor) a significant difference in hepatopancreas color in 12-month caged vs free crabs (caged crab hepatopancreas was greener than that of free crab), indicating some changes in hepatopancreas color after 12 months of retention.

Carapace size-Hepatopancreas weight relationship

Analysis of variance-covariance by location, year and year-location interaction in the spring showed that crab carapace size vs hepatopancreas-weight relationships were significantly different in terms of location and year/location interaction for the three categories of crab. However, there was no clear seasonal or annual tendency in regression residual. The comparison between caged and free crabs suggested that caging may have impacted on carapace size-hepatopancreas weight relationship for all three categories of crab especially for large males

where this ratio was higher in caged crabs, due to decreased hepatopancreas weight, however this requires further investigation to draw any conclusion.

Hepatopancreas histology

Histological examination of hepatopancreas tissue identified seasonal and cage-related differences in R-cell vacuolation (interpreted as lipid),similar to those seen for directly measured lipid content, and RI cell abundance i.e., both having generally lower values in the spring than the fall in free crabs and lowere after 12 months of caging. This pattern was most consistent for pygmy male and mature female crabs, except in Grande Riviére. Histology is a useful tool for rapid estimation of lipid reserves but direct measurement is recommended when accurate values are required. In contrast to the 2003-2004 study, inflammation was minimal. This could indicate that other factors such as animal handling and/or transport conditions were different in 2003-4 vs the 2012-2013 study. Caging per se did not have an effect on inflammation-associated indices. A previously unreported intranuclear virus was detected in a low number of crabs, mostly in Louisbourg region.

Feeding behavior

The evaluation of snow crab feeding behavior by stomach content analysis and stable isotope analysis suggests that caging may have deleterious effects on snow crabs. Stomach contents analyses showed that the majority of free snow crab sampled had very little content in the stomachs, suggesting a bias in our sampling methods towards hungry crab. In terms of stomach content weight, free crabs had significantly higher weights than caged crabs for two weeks at both sites and for all crab categories. No differences were observed between free and caged crabs for 6 or 12 months.

A variety of prey species were identified but no seasonal or site trends were observed. Some prey categories were more often found in caged crabs suggesting caging may cause a shift in diet composition and caging effects may be more pronounced in larger crab due to their higher energetic demands.

The application of stable isotope analyses on snow crab muscle samples showed that all three crab categories (free and caged) share the same trophic level. Possible differences in feeding behavior (in terms of primary food sources) were observed and may be related to crab size. Stable isotope analysis results also suggest caging may induce a nutritional dietary shift and certain level of nutritional stress for mature females and large males, respectively.
The results suggest that caging may introduce a certain level of nutritional stress and dietary shift, especially in larger males. Although these effects may only be detectable at the most sensitive levels, the observed effects must be considered in future caging studies.

Larval morphometrics

In the 2003 seismic study, a slower developmental rate and smaller morphometric features were observed in zoea I hatched in the laboratory from embryos carried by females caged at the seismic site, compared to those from females caged at the control site. However, it was not possible to determine if these differences resulted from exposure to seismic energy, or to differences in temperatures between the caging sites or the slightly smaller size of females caged at the seismic site. Results of the present study support the hypothesis that these differences could have resulted from embryonic rearing at different locations within the same general geographic area rather than from differential exposure to seismic energy.

In 2013 samples of the present study, zoea I larvae from Margaree Harbor were significantly larger than those sampled in Cheticamp only 30 km away and a similar trend was observed in 2012 (despite the fact that we do not know the origin and hatching time of those zoeae). Results of the present study also demonstrated the lack of significant differences in the assemblage of morphometric features in zoea I larvae between years (2012 vs 2013), demonstrating the greater influence of local environmental conditions on size than inter-annual influence. In zoea stage II, the combination of incubation temperature and rearing temperature during the zoea I stage would also influence size. Factors such as geographic origin, size of females and primiparous vs multiparous females could also contribute to size differences in larval stages of *C. opilio*. Hence, significant differences observed in the 2003 seismic study could have been related to local conditions of the caging sites separated by 35-41 km rather than by differential exposure to seismic energy.

The results of the present study showed that the natural incidence of morphological abnormalities in field-collected zoeal stages of *C. opilio* was very low. A single occurrence of a dual dorsal spine in a zoea I larva was the only morphological abnormality observed.

Development of hemolymph biochemistry profiles for snow crab

Blood (serum, plasma) biochemistry profiles are a widely used diagnostic tool in human and veterinary medicine to evaluate health. Analysis of hemolymph constituents using biochemistry panels in crustaceans is a non-lethal sampling protocol that could be used repeatedly to evaluate the animal's response over time to a variety of conditions e.g., diet, environment, disease, tissue injury, and normal physiological processes related to reproduction and moult.
Enzyme activity in the hemolymph (blood) is useful to identify damaged tissues. To use this tool in snow crab, the tissue origins of the enzymes included in the biochemistry profiles had to be determined so that changes in hemolymph activity could be correctly interpreted. To achieve this, the distribution of eight enzymes (amylase, lipase, ALT, AST, GD, SDH, ALP and GGT) across

seven tissues (heart, hepatopancreas, muscle, ovary, testes, intestine, subcuticular epidermis) and hemocyte pellets was determined. To capture potential variability related to sex, maturity level or time of year, samples were collected from multiple crab categories in the fall of 2011 (adolescent males, large mature males, prepubescent females, and mature females) and the spring of 2012 (pygmy males, large mature males, and mature females).
The most promising indicators of hepatopancreas-specific injury were amylase, GGT, SDH, and ALP (in non-adolescent male crabs). Increases in ALT and AST could be expected to accompany muscle and possibly hepatopancreas injury with increases in AST indicating more severe cellular injury. Glutamate dehydrogenase (GD) appears more muscle-specific but, as for AST, may require more severe cellular injury for release into the circulation. Lipase was widely distributed and is commonly detected in hemolymph plasma samples.
Three sex- and/or age-related patterns in tissue enzyme profiles were noted. First, the activity of GD was higher in leg muscle tissue of immature male and female crabs in the fall samples. This may reflect muscle growth in these non-terminally moulted crabs. Second, ALP activity was generally up to 1000-fold higher in testes from adolescent male crabs than other male crabs and so is possibly related to maturation of the male reproductive system. Finally, differences in the enzyme profile of ovaries (increased AST, ALT, lipase activity) and hepatopancreas (increased ALP activity) of mature female crabs collected in the spring compared to prepubescent or mature fall-caught female crabs were noted. These are suspected to be related to spring spawning and the subsequent physiologic resorption of non-spawned oocytes.
Plasma (derived from non-clotted hemolymph with hemocytes removed) was determined to be preferred over serum (derived from clotted hemolymph) for analysis. The release of enzymes, and possible inhibitors, from hemocytes during the clotting process complicates interpretation of results in serum samples.

A refrigerated stability study concluded that nearly all 23 directly measured analytes in the hemolymph plasma biochemistry profile (calcium, magnesium, urea, glucose, creatinine, cholesterol, triglyceride, lactate, uric acid, total protein, albumin, and the enzymes amylase, lipase, ALP and GGT) were considered clinically stable for up to 1 week, with activities of GD, ALT, AST, and SDH enzymes the only exceptions. Electrolyte values, while also likely stable, are less reproducible due to a manual dilution step required for analysis. This confirmed that hemolymph plasma samples collected in the field would be suitable for analysis for up to one week if kept cool (refrigerated).

Trawling and trapping were compared to identify the crab collection method that would cause the least trauma, as identified by changes in the hemolymph plasma biochemistry profile, to three categories of free crabs: pygmy males (PM), large mature males (LM), and mature females (MF). Due to physical limitations of some vessels, alternate methods of holding the crabs (immediate sampling on trawler deck; sampling after holding in vivier tanks; and sampling after holding in coolers) were also examined.

Reference intervals were calculated for collection and holding method combinations; however, were conservative due to the low (10-36) number of crabs per group, below the recommended minimum of 120 individuals. Crab category and season were shown to be necessary factors to consider when constructing RIs e.g., mature males differed from pygmy males and mature females which were similar to each other, much higher levels of triglyceride and total protein were observed in mature females presumably associated with oocyte development in the fall.

Trawling with sampling accomplished within 15 min of the trawl net arriving on deck resulted in relatively minor changes in biochemistry panel parameters associated with emersion or tissue trauma. Free crabs held in coolers (up to eight hours) prior to hemolymph collection showed marked changes related to emersion (increased lactate, uric acid, urea) with minimal elevations in muscle enzymes suggesting little tissue damage. The MF group was an exception; however, this was likely due to an as yet unidentified stress affecting the crabs kept in the cooler which resulted in a large amount of limb autotomy. Interpretation of data from vivier-held crabs was complicated by an inadvertent exposure to lower salinity water and the unexpectedly long emersion times (up to 68 min) for some samples to be collected. Trawled crabs often had higher levels of energy-related metabolites (cholesterol, triglyceride) compared to free crabs. Whether this represents less selective collection by trawl nets vs. traps, or reflects a period of fasting experienced by free crabs held in coolers or vivier tanks remains to be determined.

The trawl vs trap study clearly illustrated the effects of prolonged emersion on hemolymph parameters and suggested potential effects that trap bias may be having on the population sampled. Hemolymph samples collected from trawled crabs within a short period e.g., 10 – 15 min, upon arrival on deck likely best represent the range of values to be expected from a crab population in its natural environment with access to a traditional diet. The 2w-6m-12m-caging study required commercial vessels with cage-hauling capacity (but lacking vivier tanks) to recover the caged crabs. Given the known differences between trapped and trawled crabs, and that the crabs selected for caging would initially be collected by commercial traps, trapping was determined to be the most suitable method to use to collect control/'free' crabs for the larger caging study.

Plasma biochemistry profiles were evaluated for all free and caged crabs as part of the caging study. As expected, the conservative reference intervals were not as sensitive to small changes in the treatment group (caged) crabs; although, were able to detect some changes associated with caging as early as 6 months and even more after 12 months. For the relatively small experimental groups in the current study, direct comparison of treatment and control animals was more sensitive to changes.

Crabs caged for a two week period had statistically significant lower median values for uric acid, urea, potassium and magnesium in all crab categories when compared to free crabs. Similar trends were noted for total protein, calcium, cholesterol, and triglyceride concentrations. These changes were presumptively attributed to decreased food intake in caged crabs and reduced

energy reserves, barring an environmental change which would have similarly affected food availability for free crabs had they been sampled at the same time as the caged crabs.

Crabs caged for six months tended to have lower levels, usually significant, of many metabolites (total protein, globulin, urea, triglyceride, cholesterol, glucose, and lactate), and increased albumin:globulin (A:G) ratios, when compared to free crabs. Hemolymph total protein, and derived parameters, were the only parameters to consistently detect a significant difference between caged and free crabs. This could reflect the order of use of energy reserves in snow crab i.e., protein reserves prior to lipid. Large mature male crabs generally had lower levels of uric acid regardless of location or collection method which may reflect dietary or metabolic differences compared to PM or MF crabs. Marginally lower levels of GD activity in caged crabs were detected.

After a 12 month caging period even lower values for protein and energy-related parameters in caged crabs compared to free crabs were detected. This presumably reflected inadequate energy intake and/or decreased tissue stores to meet metabolic requirements. Lower levels of activity of the muscle- associated enzymes, glutamate dehydrogenase (GD) and alanine aminotransferase (ALT) were more pronounced than in the six month caging samples and likely reflected lower muscle and/or hepatopancreas mass in caged crabs due to catabolism of muscle protein.

Overall, hemolymph biochemistry profiles were a sensitive tool for detecting changes in crab physiology, and presumably health, related to caging. In addition, the sampling is non-lethal and could be repeated on the same individual over time if necessary.

Biochemical analysis of hepatopancreas and correlation to hemolymph parameters

Biochemical analysis on hepatopancreas and hemolymph has been conducted for the first time in snow crab. Seasonal and sex-related fluctuations were observed for hepatopancreas lipid content with higher values noted in the fall and depletion of reserves in mature females in the spring presumably due to transfer to the ovary for oocyte development. Many hemolymph parameters, primarily metabolites, also showed similar seasonal fluctuations with higher values in the fall. Periodic collection of baseline information on the structure of tissues and hemolymph composition would better define normal physiological variations in snow crab populations and better understand the reaction of snow crabs to stress than collecting samples by bi-annual sampling as conducted in this present study. Future research should include a wider range of snow crab size classes collected by different sampling methods, areas and seasons.

Hemolymph biochemistry parameters were reasonably correlated to hepatopancreas energy stores and could estimate energy reserves using regression equations to some degree but this varied with crab category and time of year e.g., the best correlations were noted for lipid in PM and MF crabs. These correlations, and resulting regression equations, might be improved if total body energy stores were calculated i.e., hepatopancreas, muscle, and gonad levels of lipid, glycogen, and protein. Such calculations would account for the effect of transfer of lipid and

protein to the ovaries for oocyte development in mature female crabs and might allow for better correlations in mature male crabs if, for instance, this crab category is drawing on energy reserves in their abundant muscle tissue.

Tissue (hepatopancreas, muscle, ovary) moisture content is simpler to measure than lipid, energy or protein content and, as suggested by others, may be a useful proxy for energy reserves. This would still require invasive and lethal tissue sampling to determine, however. Hemolymph biochemistry analysis has shown promising correlations to hepatopancreas energy reserves in the current study and may present a non-invasive, non-lethal, alternative sampling method to assess total body energy reserves in terminally moulted crabs in future studies were a parameter(s) to be identified. This could be used to efficiently field test a large number of crabs.

GENERAL RECOMMENDATIONS

Crab Holding Conditions

While holding crabs in cages on the ocean floor following seismic shooting, or other form of experimental treatment, is expected to be beneficial by allowing the crabs to remain in their natural environment prior to sampling, caging is not recommended. The biochemical analysis of hemolymph, hepatopancreas lipid and glycogen stores, as well as the changes in morphometric relationships, feeding behavior, mortality, histologic appearance of ovary tissue, and fecundity, in caged crab showed increasingly negative effects over time. For assessment of long-term effects, alternate holding methods such as laboratory-based aquaria with appropriate non-exposed control groups could be considered.

Crab Collection: Trapping vs Trawling

It is suggested that a 5-minute trawl is preferable over 24-hour trap soak time to sample the crab population of interest, due to: 1) the capacity for immediate onboard sampling/dissection on trawl vessels; 2) the vast choice of size range and crab type available; 3) reduced sample bias (e.g. traps may attract only hungry crabs, select for crabs that are only healthy enough to be able to detect bait/are attracted to the odour of the bait and, mobile enough to move into the trap); and, 4) the more natural setting than trapping (some crabs are left in artificially high density environments for up to 24 hours in the traps and potentially forced to endure rapidly changing temperatures).

The preferred capture/collection method used for future studies will be dependent on the type of information required. Biochemistry panels collected from crabs within 15 min of the trawl net

arriving on deck showed minimal changes related to emersion or fasting (a potential effect related to delay required to transport crabs to shore prior to sampling). Ultimately, the most ideal sampling method is to collect samples from trawled crabs following seismic testing vessel for immediate impact assessment as there are fewer potential artifacts from fasting during caging, emersion, and limb loss during cooler transport.

LIST OF ABBREVIATIONS

A:G	albumin:globulin ratio
Alb	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AM	adolescent male
AMY	amylase
ANOVA	Analyse of variance
AST	aspartate aminotransferase
AVCLSC	Atlantic Veterinary College Lobster Science Center
AW	abdomen width
B	Grande-Rivière station
BW	body weight
BCG	bromocresol green
Ca	calcium
CB	citrate buffer
CFV	commercial fishing vessel
CGS	coast guard ship
CH	chela height
CHOL	cholesterol
Cl	chloride
CPD	critical point dryer
CV	co-efficient of variation,
CW	carapace width
dH2O	sterile distilled water

DFO	Department of Fisheries and Oceans
E1	Louisbourg station
EPI	epidermis
ESRF	Environmental Studies Research Fund
GD	glutamate dehydrogenase
GGT	gamma glutamyltransferase
Glob	globulin
H	heart
H & E	hematoxylin and eosin
HLS	hemocyte lysate supernatants
HP	hepatopancreas
IM	immature male
INT	proximal intestine
K	potassium
LIP	lipase
LM (or MM)	large mature male
MF	mature female
Mg	magnesium
MGS	mean grain size
Na	sodium
OERA	Offshore Environmental Research Association of Nova Scotia
PCA	Principal Coordinates Analysis
Phos	phosphorus
PM	pygmy male
PrF	prepubescent female
RI	reserve inclusions (or reference interval)

RPC	Research and Productivity Council
SD (or sd)	standard deviation
SDH	sorbitol dehydrogenase
SDS	sodium dodecyl sulfate
SEM	scanning electron microscopy
TAE	total allowable error
TNTC	too numerous to count
TP	total protein
TRIG or TG	triglyceride
W1	Margaree Harbor station
W2	Cheticamp station
Wt (or W)	weight
2W	2 weeks caging duration (16/17 days of immersion)
6M	6 months caging duration (214/222 days of immersion)
12M	12 months caging duration (345/355 days of immersion)

I. INTRODUCTION

The snow crab fishery in the southern Gulf of Saint Lawrence began in 1966 and is now one of the more important commercial fisheries in Atlantic Canada (Hebert *et al.*, 1992; Sainte-Marie *et al.*, 1995). This hard shell male-only fishery is currently regulated by quotas, a minimum legal size of 95 mm carapace width (CW) and a limited number of traps per permit (Hebert *et al.*, 1992). Yearly quotas are estimated by post-fishing season trawl surveys, which provide important information on snow crab population densities and composition (Moriyasu *et al.*, 1998). Economically, the snow crab industry is worth millions of dollars and generates thousands of jobs, either directly (fishermen, crew members) or indirectly (boat building, fish processing, tourism). Consequently, many small Atlantic coastal communities depend on and greatly benefit from this fishery.

In recent years, there has been an increasing interest in oil and gas prospecting off the western coast of Cape Breton, an area also well known for its snow crab fishery and benthic biodiversity. The use of seismic surveys to explore for oil or gas offshore Nova Scotia has been controversial, due to competing interests between the fishing and oil and gas industries and the lack of definitive scientific data on whether or not these types of surveys are harmful to marine ecosystems.

The issue of whether or not snow crabs (*Chionoecetes opilio*) are susceptible to exposure to sound energy generated by seismic exploration is technically and perceptionally challenging to resolve. Past research (DFO 2004) has provided a considerable amount of insight into the subject; however there remain many gaps to fill regarding the science of the crab species and also the interpretation or acceptance of previous findings.

Many marine experiments such as Moriyasu *et al.* (unpublished) used caged animals in their studies; however, it remains unknown if caged snow crabs are representative of the natural population. DFO (2004) noticed a lack of physiological parameters that evaluate crab health in the natural habitat. Health can be defined as *'a condition in which the organism is in complete accord with its surrounding'* (Boyd 1970), however, at present, normal tissue and blood (hemolymph) composition of healthy snow crab population have not been established. There is also little information on snow crab's physiological response to stress, hence the limited ability to distinguish healthy crabs from stressed or injured crabs.

The research outlined in this report was designed to address and resolve many of the questions raised in recent studies with snow crabs. The approach proposed taken incorporated a scientific analysis of the normal characteristics of snow crabs (i.e. what crabs "look like" in their natural habitat when not exposed to seismic energy) and includes an engagement process with stakeholders (i.e. those with social, environmental, economic and scientific interests) to ensure coverage of issues that have been identified previously. Certainly all possible questions will not

be addressed in this study and no seismic testing (shooting) in the field nor in the laboratory will be involved in this study despite multiple suggestions made during the process of consultations.

I-1. BACKGROUND

In conjunction with a seismic exploration program conducted offshore western Cape Breton by Corridor Resources Inc., a collaborative research study was conducted in 2003 to investigate possible effects of seismic exploration on mature female snow crabs. The program was the first of its kind. Crabs were caged at two sites: a test site where caged crabs were directly exposed to the seismic energy, and "control" location where caged crabs were not directly exposed to the survey. The purpose of the study was to examine the possible short- and long-term impacts of exposure to seismic energy on mortality, morphology and physiology of the crabs. Findings of the study showed that the seismic energy did not cause immediate mortality in any of the exposed crabs. In the laboratory following the field survey period, there was no evidence of external damage or abnormal feeding behavior in the specimens. Survival and locomotion of produced larvae did not appear to be different between eggs hatched from test and control sites. When observing the gills and antennules twelve days after snow crabs were exposed to seismic energy, sediment accumulation was observed in these tissues: however, sediment accumulation was not observed in snow crab caged for five months after being exposed to seismic energy.

However, the researchers noted some effects in the crabs that appeared to persist five months after exposure: the hepatopancreas of some crabs from the test site were found to be bruised; ovaries were bruised and oocytes were dilated with detached chorion, egg hatch time was approximately five days later for crabs from the test site; the weight and size of off-springs were typically lower than control site eggs; yet crabs from the test site typically exhibited faster "turnover" rates (i.e. ability to right themselves after being turned on their backs) (DFO 2004).

The research also indicated that there were certain confounding factors which could impact the interpretation of the study results (e.g. variations between the test and control sites in terms of water depth, temperature and organic concentrations in seabed sediment, as well as slightly larger average crab size at the control site). The research team concluded that further research would be required to resolve questions arising from these factors, and a third-party independent review of the study results suggested that while potential effects of seismic energy were observed in some of the crab samples, not enough was known about the natural state of crabs to discern if the observed features were not simply naturally occurring in the snow crab population (i.e. in the absence of exposure to seismic noise) (Courtenay *et al.* 2009).

An OEER workshop was organized in September 2007 regarding the possible effects of seismic surveys on marine invertebrates. This open forum concluded that there was a need for further scientific studies on the impact of seismic exploration activities on invertebrates. Results of this

workshop also pointed out that there is a total lack of basic information on the normal/healthy status of the organs, appendages and normal behavior of most marine invertebrates making it difficult to distinguish between healthy/normal and unhealthy/affected animals.

I-2. OBJECTIVES

The purpose of this new study is to address the various issues arising from the previous (2003) snow crab study, by providing baseline information about the inherent characteristics of snow crabs which can be compared with effects observed during or after handling and seismic exposure (Matthews 2012).

Based on results of earlier studies and the recognition that there are significant knowledge gaps on snow crab physiology and handling effects many questions are yet to be answered before further laboratory and/or field studies on the impact of seismic noise on snow crabs should be carried out. Therefore, the objectives of this study are to:

- Determine the normal condition of organs, appendages and tissues of snow crabs, and the natural variability of key characteristics of crabs found in their natural state within the primary habitat condition.

- Evaluate the possible effects of caging (for immersion duration of 12 days, 6 and 12 months) and of trawl sampling (compared to trap sampling) on the condition of organs, appendages and tissues, includinig hemolymph, of crabs.

I-3. REFERENCES

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II. SAMPLING STATIONS

II-1. SAMPLING STATIONS AND THEIR CHARACTERISTICS

Total of four (4) sampling stations (Figure 1) were set for the project composed of three (3) stations in the southern Gulf of Saint Lawrence and one station on the Scotian Shelf.

Grande-Rivière, Québec (station B, Figure 1) at positions between 48°10.028N/64°26.187W and 48°14.833N/ 64°25.575W with a depth range and between 78.0 to 105.0 meters. The sampling area is about 10 miles off the port of Grande-Rivière and in the inner part of the commercial snow crab fishing ground. The bottom type is mixture of mud and gravel. The fishery was opened between April 15 and July 11, 2012 and April 28 and July 15, 2013, therefore both spring sampling was conducted during the fishing season.

Margaree Harbor, Nova Scotia (station W1, Figure 1) at positions between 46°27.124N/ 61°31.283W and 46°29.411N/ 61°18.344W with a depth range between 61.0 and 66.0 meters, 10 miles off the port of Margaree Harbor. The fishery was open between July 14 and August 9, 2012 and July 15 and August 21, 2013. Therefore our trap samplings in the spring and fall were conducted out of fishing season for both years. This station is located in the regular commercial snow crab fishing ground. The bottom type is mainly muddy (typical snow crab habitat). This station was also used for the caging study.

Cheticamp, Nova Scotia (station W2, Figure 1) at positions between 46°49.084N/61°18.056W and 46°46.959N/ 61°08.113W with a depth range between 62.5 and 85.0 meters, 11 miles off the port of Cheticamp. The fishery opened between July 14 and August 9, 2012 and July 15 and August 21, 2013. Therefore our trap samplings in spring and fall were conducted out of fishing season for both years. This station is located in the regular commercial snow crab fishing ground. The bottom type is mainly muddy (typical snow crab habitat). This station was also used for the caging study.

Louisbourg, Nova Scotia (station E1, Figure 1) at positions between 45°49.36N/ 59°50.39W and 45°48.68N/59°50.57W with a depth range between 81.0 and 97.0 meters 15 miles off the shore line between the port of Louisbourg and Framboise. The bottom type is muddy mixed with gravel. The commercial fishery (Area 23) was opened between April 2nd and September 30th in both 2012 and 2013.

Station B was set as the farthest station from the possible future seismic testing areas around Cape Breton Island. Stations Margaree Harbor (W1) and Cheticamp (W2) were set based on the previous seismic study (DFO 2004; Courtenay *et al.* 2009) in which the former was set as test station and the latter as control station. Louisbourg station E1 was set as this station is physically separated from the other stations by Cape Breton Island. Originally it was supposed to set two

stations (in- and off-shore stations), but it was modified due to logistical problems. Consequently, a new station closer to the original in-shore station was selected.

The sampling was conducted at these four stations twice a year in 2012 (spring and fall/winter), but only once in 2013 (spring/summer) 2013. In the fall 2013, sampling was only amenable at Margaree Harbor (W1) and Cheticamp (W2) due to unfavorable weather conditions.

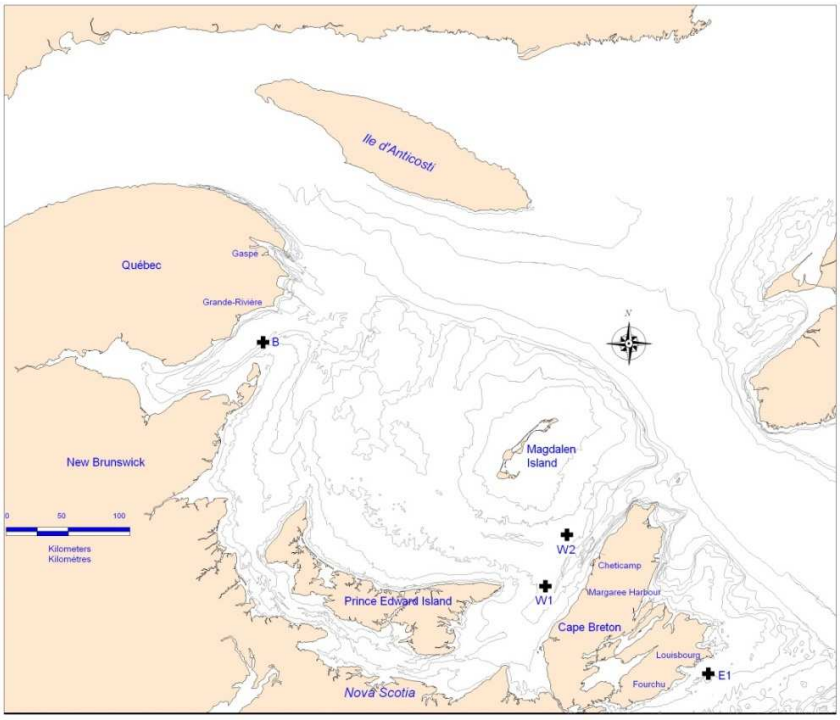

Figure 1. Geographic location of crab sampling and caging stations.
B: Gaspésie/trapping (Grande-Rivière), W1: Western Cape Breton 1/ trapping and caging (Margaree Harbor), W2: Western Cape Breton 2/ trapping and caging (Cheticamp), E1: Eastern Cape Breton 1/ trapping (Louisbourg).

II-2. BOTTOM SEDIMENT

Sediments were collected by a core sampler Van Veen (Canipex Ltd. Halifax, NS) (Figures 2A & B) in the spring 2013 after trapping at each sampling station. Grab sampling is a common technique used to examine the surface sediment (top 10-15 cm). They were divided into three large plastic bags and frozen upon arrival at DFO Moncton laboratory for later separate analysis i.e. particle analysis, SEM/EDAX X-ray analysis and collection of benthos for stable isotope analysis.

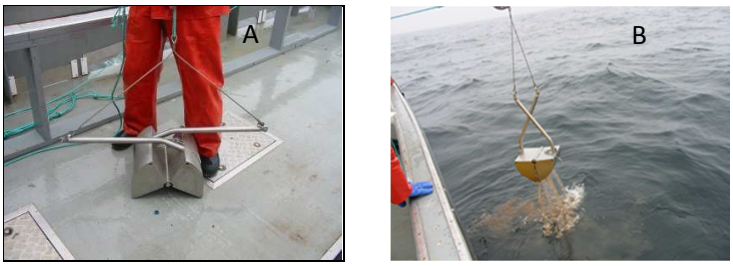

Figure 2 (A, B). Core sampler Van Veen used for sediment sampling.

The samples for particle analysis were processed and analyzed at DFO, SEM/EDAX X-ray have been observed at UNB Microscopy and Microanalysis Facility (Fredericton, New Brunswick) and the third samples were kept frozen for benthos collection for stable isotopic analysis (Section V).

II-2-1 Sediment particle analysis

The four sediment samples collected from each of the five study sites were thawed and a subsample of approximately 100-150 g was collected from each sample for analysis. The subsamples were weighed, dried at 70°C for 24h and then re-weighed to determine water content. The dry subsamples were then ashed at 500°C for 3h and weighed once more to determine organic content. Finally, the ash sediments were passed through a series of six sieves with a mechanical sieve shaker for 10 min to fraction the sediments into very coarse sand (> 2000 µm), coarse sand (> 1000 µm), medium sand (> 500µm), fine sand (>250µm), very fine sand (>125µm), coarse silt (>63µm) and silt (<63µm) (Giere *et al.*, 1988). The Mean Grain Size (MGS) which represents the median grain size at which 50% of the sample is dominant, was calculated with the following equation:

MGS = a - x

where:

a = grain size class which precedes the cumulative dry weight of 50% of the sediment sample.

and:

x = (50 - b)*(c / (d - b))

where:

b = % cumulative dry weight of the grain size class which precedes the cumulative dry weight of 50% of the sediment sample.

c = grain size class which follows the cumulative dry weight of 50% of the sediment sample.

d = % cumulative dry weight of the grain size class which follows the cumulative dry weight of 50% of the sediment sample.

These calculations are based on information provided in Giere *et al.* (1988). For sediment samples with a MGS larger than 2000 µm, a MGS of 2000 µm was used to calculate descriptive statistics and to perform statistical analyses.

It is important to note that grain size composition is influenced by numerous environmental factors (e.g., exposure, currents, nature and amount of suspended matter). Therefore, it is not surprising to see large grain sizes in channels or at highly exposed beaches and smaller grain sizes in well protected areas with low current. The grain size composition influences the distribution and abundance of macrofauna as well as the meiobenthic animals that live within the substrate. For our purpose, the MGS helps us determine if the site is mostly muddy (fine silt and sand) which usually means higher percentage organic and moisture content.

Statistical analyses

Differences among groups were tested by one-way ANOVA followed by Tukey multiple comparison tests. Prior to the analysis, data were tested for normality (probability plot) and homoscedasticity (Levene/Bartlett test). To meet these assumptions, a logarithmic transformation was applied to the MGS data. Analyses were performed with Systat version 13.0 (Systat Software Inc., San Jose, CA, USA). The level of significance was $p<0.05$. Back-transformed means are accompanied by their 95% confidence intervals.

Results

The MGS of sediment samples collected in Grande-Rivière B_1 & B_2), was medium to coarse sand (Table 1). The larger MGS of these sediments was largely due to the presence of gravel and rocks in many of the samples. Consequently, three of the four samples from Grande-Rivière and one of the four samples from B_1 had a MGS larger than 2000 µm. However, because the largest grain size class used to classify the sediments in the present study was 2000 µm, sediments with a MGS larger than 2000 µm had to be included in the 2000 µm size class for the descriptive statistics and the statistical analyses. Therefore, the MGS of sediments from Grande-Rivière were underestimated and would likely belong to a larger grain size class such as gravel. Sediment samples collected from sites in the Cap-Breton area (Chéticamp, Margaree Harbor & Louisbourg) ranged from very fine sand to fine sand. The water content in sediments from Grande-Rivière (B_2) was significantly lower than all other sites (Table 2). This may be due to the larger MGS of these sediments which is usually associated with lower porosity and consequently, lower water content (Berner 1971). Louisbourg and Grande-Rivière (B_1) also had significantly lower water content compared to Cheticamp and Margaree Harbor which may also be due to the larger MGS of Grande-Rivière (B_2) sediments but not for Louisbourg which was the site with the smallest MGS of all sites included in this study. Finally, there were no significant differences in organic content among sites (Table 2).

II-2-2 Observations of sediment samples with EDAX X-ray

One issue pointed out during review of the December 2003 caging experiment (Moriyasu *et al.* unpublished) was whether the difference in dirtiness of gill between the seismic and control site might be related to different sediment compositions in the two areas and not related to seismic noise. If this is the case, caged crabs may accumulate the same type of materials among the gill lamellae and the detailed analysis with EDAX X-ray of the sediments helps in comparison of materials which might be found among the gill lamellae with the sediment collected on-site. To help interpret observations of gill-fouling in the present study, sediment samples were collected concurrent with snow crab sampling.

Each sample (2 samples from B, one for W1, W2 and E1) was processed in the following manner: Two vials per station were taken from a bucket containing frozen sediment sample. Sub-samples were extracted from each vial and placed into conical filters. These 10 sub-samples were then well rinsed with distilled water to remove salt. The samples were then air-dried, and a portion of each sample was placed on a 12 mm aluminum stub. The material was affixed to each stub with double-sided carbon tape. The ten stubs were then carbon-coated by high-vacuum thermal evaporation, and examined in a JEOL 6400 scanning electron microscope (SEM).

For each sample, three secondary electron images were collected at a screen magnification of 50x and 150x using an accelerating voltage of 15 kV, and a beam current of 0.1 nA. For each sample, approximately 160 mineral grains were identified as to mineral species (Table 3).

Table 1.Detailed information on the sediment samples mean grain size (MGS).

Site	% humidity	% organic	> 2000 µm	% > 2000 µm	1000 µm	cum% 1000 µm	500 µm	cum% 500 µm	250 µm	cum% 250 µm	125 µm	cum% 125 µm	63 µm	cum% 63 µm	cum < 63 µm	cum% < 63 µm	x value	Mean grain size (µm)
$W2_1$	41.73	2.486526213	0.44	0.55	1.09	1.92	7.50	11.31	17.96	33.79	29.19	70.34	17.05	91.69	6.60	99.95	55.43	194.57
$W2_2$	43.19	2.871491115	0.86	1.13	1.00	2.44	3.58	7.15	20.66	34.30	24.93	67.06	18.45	91.30	6.36	99.66	59.92	190.08
$W2_3$	40.43	2.681406562	7.56	9.05	0.66	9.84	2.07	12.32	21.15	37.63	27.17	70.15	18.75	92.59	6.18	99.99	47.55	202.45
$W2_4$	42.18	2.01894317	1.41	1.78	1.16	3.24	7.05	12.14	19.23	36.41	27.61	71.25	16.80	92.45	5.92	99.92	48.76	201.24
$W1_1$	42.50	3.411022093	9.55	11.88	2.27	14.71	8.28	25.01	22.01	52.40	20.86	78.36	8.51	88.95	7.71	98.54	228.08	271.92
$W1_2$	40.61	2.629074471	14.00	16.98	3.32	21.01	9.59	32.64	27.42	65.90	15.20	84.34	5.56	91.08	6.38	98.82	130.47	369.53
$W1_3$	39.73	2.881095761	10.38	12.49	3.17	16.30	10.21	28.59	19.23	51.72	24.24	80.88	8.32	90.89	7.12	99.46	231.41	268.59
$W1_4$	38.34	2.723782097	12.63	15.46	3.56	19.82	10.66	32.88	28.61	67.91	14.01	85.06	5.80	92.16	5.90	99.39	122.20	377.80
$E1_1$	34.72	1.890756303	0.03	0.03	0.22	0.27	0.25	0.53	2.21	2.88	33.09	38.07	49.19	90.39	9.24	100.21	14.36	110.64
$E1_2$	34.42	1.804287837	0.09	0.10	0.07	0.17	0.42	0.62	2.24	3.03	52.29	59.19	27.35	88.57	10.53	99.88	104.54	145.46
$E1_3$	32.61	1.849746822	0.55	0.58	0.27	0.86	0.20	1.07	1.41	2.54	38.39	42.69	45.85	90.65	8.82	99.87	9.60	115.40
$E1_4$	36.18	1.646046261	7.11	7.72	0.04	7.77	0.22	8.01	2.72	10.96	38.95	53.28	3.35	56.92	39.54	99.88	115.31	134.69
B_1	26.06	1.497953108	42.96	40.41	2.84	43.09	9.99	52.48	10.37	62.24	9.75	71.41	20.29	90.50	9.74	99.66	367.87	632.13
B_1	24.00	3.621521336	68.44	52.34	2.77	54.46	2.78	56.58	9.99	64.22	13.35	74.43	22.40	91.56	10.33	99.46		> 2000
B_1	21.25	1.824235278	88.61	62.15	2.46	63.88	1.61	65.01	7.44	70.23	10.01	77.25	22.54	93.06	9.41	99.66		> 2000
B_1	21.64	1.631469979	71.59	59.88	3.75	63.01	7.46	69.25	7.00	75.11	6.55	80.59	15.16	93.27	7.88	99.86		> 2000
B_2	31.84	2.4600246	42.48	44.25	0.87	45.16	1.69	46.92	8.81	56.09	13.88	70.55	17.12	88.39	10.91	99.75	84.00	416.00
B_2	35.91	2.271447344	29.66	28.21	0.87	29.04	1.78	30.73	11.90	42.05	20.79	61.82	25.83	86.39	13.65	99.37	50.26	199.74
B_2	27.74	2.041472432	61.55	50.09	1.06	50.95	1.14	51.88	9.45	59.57	20.47	76.22	17.11	90.15	11.49	99.50		> 2000
B_2	28.52	2.995608532	58.83	47.12	2.20	48.88	1.82	50.34	7.44	56.30	19.68	72.06	21.36	89.17	12.82	99.44	383.24	616.76

$W2_1$-$W2_4$: Cheticamp sediment (sub-sample 1-4), $W1_1$-$W1_4$: Margaree Harbor sediment (sub-sample 1-4), $E1_1$- $E1_4$: Louisbourg sediment (sub-sample 1-4), B1-B2: Grande-Rivière sediment.

Table 2. Mean water content, organic content and mean grain size (with 95% confidence intervals) of sediment samples collected at the study sites. Sites sharing a common letter are not significantly different (one-way ANOVA, Tukey test, $p<0.05$).

Sites	Water content (%)	Organic content (%)	Mean Grain Size (µm)
Chéticamp (W2)	41.9 (40.1-43.7) A	2.5 (1.9-3.1)	197.2 (187.8-206.5) AB
Margaree Harbor (W1)	40.3 (37.5-43.1) A	2.9 (2.4-3.5)	317.4 (235.6-428.5) AB
Louisbourg (E1)	34.5 (32.2-36.8) B	1.8 (1.6-2.0)	125.8 (102.5-154.2) A
Grande-Rivière (B_1)	31.0 (25.1-36.9) B	2.4 (1.8-3.1)	566.5 (122.0-2617.2) BC
Grande-Rivière (B_2)	23.2 (19.7-26.8) C	2.1 (0.6-3.7)	1498.7 (600.2-3748.3) C
ANOVA	$F_{4, 15} = 44.65$, $p<0.0001$	$F_{4, 15} = 2.45$, $p=0.091$	$F_{4, 15} = 14.17$ $p<0.0001$

Table 3. Identification of mineral species based on approximately 160 grains in each sample and its abundances.

Formula	B_1-a	B_1-b	B_2-a	B_2-b	W_2-a	W_2-b	E_1-a	E_1-b	W_1-a	W_1-b
SiO_2	97	97	100	103	90	97	100	108	83	75
$KAlSi_3O_8$	10	10	8	8	18	13	17	16	12	17
$K_2Al_4(Si_6Al_2O_{20})(OH)_4$	3	2	1	2	3	3	3	2	9	13
$K_2(Fe,Mg)_6(Si_6Al_2O_20)(OH)_4$	10	16	18	20	19	25	15	12	41	23
$(Na,Ca)(Al,Si)_4O_8$	7	4	6	10	1	5	4	7	2	2
$(Ca,Na)(Al,Si)_4O_8$		3	4	3	1		2	3		1
$NaAlSi_3O_8$	15	11	7	8	19	14	10	10	8	11
$Ca_2(Mg,Fe)_5Si_8O_{22}(OH)_2$		1	4	2	3	1	2	2	2	3
$(Mg,Fe)_{12}(Si,Al)_8O_{20}(OH)_{16}$	3	1	4	2	6	4	3	4	3	9
$CaMg(CO_3)_2$	1	6	1	2	1		2	1	1	2
$FeO(OH).nH_{2O}$		1			1		1			
$(Mg,Fe)SiO_3$	1									
TiO_2	1								1	
SiO_2	3	2	4	4		1	1	1	3	7
$Ca_5(PO_4)_3(OH,F)$	1				1					1
$(Fe,Mg)(Cr,Al)_2O_4$										
$FeTiO_3$	1		1		1					1
$CaCO_3$	5	5		1				1		
$Ca2(Fe,Al)_3(SiO_4)_3(OH)$	1						1			
Al_2SiO_5							1			
$(Mg,Fe)Al_2O_4$										1
FeS_2										1
$BaSO_4$										1
	159	159	158	165	164	163	162	167	165	168

B1: Baie des Chaluers station sample 1, B2: Baie des Chaluers station sample 2, W1: Margaree Harbor station sample,
W2 : Cheticamp station sample, E1: Louisbourg station sample. a and b are subsamples within each station.

Sediment samples from Stations B1, B2, W2, and E1 are essentially similar, consisting predominantly of tectosilicates (quartz, K-feldspar, and plagioclase feldspar) with less abundant phyllosilicates (muscovite, biotite, chlorite), and minor occurrences of a few other minerals (Table 4). Sample from W1 is somewhat different, as it contains a higher proportion of phyllosilicates, at the expense of the tectosilicates. The amount of muscovite plus biotite plus chlorite is considerably higher in the W1 samples.

Table 4. Sediment composition at each station expressed as a percentage of average mineral grains belonging to two groups (phyllosilicates and tectosilicates) indicating the particularity of the sediment from station W1 (Margaree Harbor).

Station	Phyllosilicates	Tectosilicates
B1	11%	80%
B2	15 %	80%
W2	19%	76%
E1	12 %	84%
W1	30%	64%

Averaged percentage between two subsamples in each station

II-3. DISCUSSION

Four sampling stations were selected for different reasons, first Margaree Harbor and Cheticamp stations have been selected as these stations were also the original stations for the 2003-04 seismic testing study (test and control sites, respectively). In addition, these stations are located in areas of possible furture seismic testing. The Grande-Rivière station was selected as the farest station from these original stations by assuming that this station would be the least influenced site by seismic noise conducted in the western Cape Breton area. The Louisbourg station was selected as it sits in a possible future seismic testing area and it is physically separated from southern Gulf stations by the Cape Breton Island.
These stations were different in depth and sediment characteristics (except for organic contents). All stations were located within commercial snow crab fishing grounds with different fishing seasons:a summer fishery in Area 19 (Margaree Harbor and Cheticamp stations) and spring fisheries in Areas 12 and 23 (Grande-Rivière and Louisbourg stations). Differences in depth and sediment characteristics and commercial fishery activities may have influenced snow crab biological characteristics measured in this study e.g. biochemical parameters and feeding behavior.

In this study, there was no notable accumulation of sediment-like substance in gill lamellae or antennule. Therefore, further sediment analysis with EDAX X-ray was not required.

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III. TRAPPING AND CAGING

III-1. SAMPLING AND CRAB MEASUREMENT PROCEDURES

III-1-1. Fixed station sampling and procedures

At each station, trapping was conducted by using up to 6 large conical commercial snow crab traps, up to 6 modified shrimp traps and up to 12 commercial 'rock crab' traps with four, two and one bait bags containing 5-6 frozen mackerels or herrings, respectively. The baits were put in a nylon micromesh bait bag to prevent crabs from ingesting the bait and immersed for 24 hours (Figure 1).

Figure 1. Catching crabs with commercial conical snow crab trap commonly used in CFA 19 (bait is placed in white micromesh bait bags).

Morphometric measurements, dissections, hemolymph sampling, and crab muscle extraction were done at on site laboratories (CEGEP, Grande-Rivière, QC for station B samples, at a fisheman's shed in Bell Côte, NS for stations W1 and W2 samples, and at DFO Coast Guard station, Louisbourg, NS or a fisherman's shed, Framboise, NS for station E1 samples). All collected samples (statocysts, antennules, gills, stomach contents, muscle, eggs, ovary and hepatopancreas) were further processed for subsequent observations at the DFO laboratory in Moncton.

The following information was recorded for all snow crabs:
Morphometric measurements:

- Carapace width (CW) to the nearest 0.01mm
- Chela height (CH) to the nearest 0.01mm (males only)
- Abdomen width (AW) to the nearest 0.01mm (females only)

Weight measurements:

- Total body weight to the nearest 0.01 g

- Hepatopancreas weight to the nearest 0.0001g
- Ovary weight to the nearest 0.0001 g (females only)
- Dry egg weight after being removed form abdomen and pleopods, rinsed, dried and cleaned to the nearest 0.0001 g (females only)

Observations of external condition

- Carapace condition (Hébert *et al.* 1997): For this study only individuals with carapace conditions 3 and early-4 were selected. A condition 3 crab terminally molted 1-2 years pior to the sampling date and has a clean, hard shell that is starting to yellow in color but still has a shiny claw. In terms of the fishery, this is a high quality crab since the meat yield is high and the carapace does not have to be cleaned at processing plants. Condition 4 crabs have moss (fouling community present on the carapace such as bryozoan) on their carapace that must be removed during processing. The claw no longer shines and scars can also be present. They molted 2-3 years prior to the sampling date.
- For caged crab, mortality was also recorded. Mortality was noted if the crab was dead in a cage only the carapace was found or missing the individual without any trace (dead crab body can be rapidly disintegrated by various scavenging species.

Color observations and measurements:

- Hepatopancreas color determined by visual observation and measured with a colorimeter (Konica Minolta Chroma meter CR-400®)
- Ovary color (beige, light orange or orange) determined by visual observation and measured with a colorimeter.
- External egg mass color (light orange, dark orange, brown or black) determined by visual observation, embryonic development (according to Moriyasu & Lanteigne 1998) and measured with a colorimeter.

Dissections:

- Left-side gills (#3-8) were dissected out for gill fouling species determination and abundance estimates; right-side gill #5 was dissected out for histology study; gill #6 or 7 for SEM observations.
- A sub sample of hepatopancreas, ovary (females) and the 3rd right gill were collected for histology. Tissues were immersed in Davidson's fixative (OIE, 2014) for 24 to 48 hours and then rinsed twice in 70% ethanol prior to dehydration and paraffin embedding (Appendix A). In the laboratory, tissue samples were dehydrated through an ethanol series (70%: 2 x 60 minutes; 95%: 3 x 60 minutes; 100%: 2 x 60 minutes) cleared in xylene (3 x 90 minutes) and transferred into paraffin (4 changes x 30 minutes at 60°C). Tissues were then embedded in paraffin for sectioning. Serial 5µm sections were cut through the blocks with a rotary microtome. Sections were placed on Superfrost

Plus® treated slides (Fisher Scientific, Canada) to increase section adhesion and air dried. Slides were then stained with hematoxylin and eosin with an automatic stainer (Atlantic Veterinary College, Diagnostic Services, University of Prince Edward Island) and coverslipped

- Egg masses were dissected out with abdominal segments and preserved in 10% formalin (a small portion of eggmass was preserved in Bouins solution for embryonic stage study).
- Right antennules and statocysts were also dissected out for assessing their condition under a dissection microscope and scanning electron microscope (SEM). A part of statocyst wall containing group hairs from the statocyct was further dissected out prior to the observations.
- Stomachs were dissected out and frozen for stomach contents analysis (Section V-3).
- Muscle of the merus of the 2ndwalking leg was extracted from 10 crabs per category for stable isotope analysis.
- Hepatopancreas was sampled from every 5th crab and kept frozen for lipid and glycogen analysis (frozen samples were sent to NB Research and Productivity Council (RPC) for further analysis).
- Hemolymph was collected from caged and free crabs from Margaree Harbor and Cheticamp. The area between the coxa of the first right walking leg and body was disinfected with 70% ETOH and hemolymph samples (1.0 to 1.5ml) were taken with a chilled 22G needle and 3ml syringe. Hemolymph samples were then transferred into chilled 1.7 ml tubes and centrifuged (7,000 x g, 5 min). Plasma was then separated from the hemocyte pellet with a chilled plastic transfer pipette and placed in a new chilled tube and preserved on ice until enzyme analysis was performed at the Diagnostic Services Laboratory at the Atlantic Veterinary College (Charlottetown, UPEI).

Specimens:

Trapped crabs were sorted onboard and selected crabs were divided into 3 categories: large commercial sized adult male (LM), under sized adult males (pygmy males: PM) and mature females (MF). The selection of adult males was based on the discriminant function Y= -0.78893 $\log_e$ CW + 0.614488 $\log_e$ Ch + 1.76051 between carapace width (CW) and chela height (CH) (Conan & Comeau 1986). Commercial size adult males are adult males larger than 95 mm carapace width (CW) while pygmy males are adult males lesser than 95 mm CW. The carapace conditions 3 and 4 were selected (2-3 years after the terminal molt).

Mature females collected for this study were multiparous females carrying a full brood of external eggs (light orange in color with estimated hatching period: May-June 2014) (Moriyasu & Lanteigne 1988) with a carapace condition of 3. These females have likely mated more than once in their life (multiparous) based on their carapace condition. The carapace condition of primiparous female in spring should be either 1 or 2 and early 3 in the fall with noticeable

irridiscence on the claw and mating scars on the walking legs. We excluded any possible pre-senile and senile females (not carrying full brood and/or carapace conditions of 4 or 5). All trapped crabs that were not selected for this study were immediately returned at sea.

The collected samples of crabs were kept in coolers with ice until arrival at the local laboratory. Hemolymph sampling was performed first as soon as the morphometric measurements were made. Crabs were then kept chilled in coolers until sacrificed for dissections. The times from removal of crab out of water (from trap hauling, crab sorting, crabs enclosed in coolers) to dissections varied between 3-8 hours.

III-1-2. Ad hoc trawling and trapping for hemolymph sampling

Besides regular bi-annual sampling at predetermined stations, additional samplings were performed to compare the health condition of snow crab collected by either trawling or trapping. Trapping occurred on August 27th, 2012 near Cheticamp (between 46°34.492 61°11.813 and 46°52.469 and 61°21.059, depth range between 33 ftm and 43 ftm) and crabs were caught using the same trap types as in the spring and fall sampling. Crabs were separated by category (commercial size adult males, undersize adult males and ovigerous females) and immediately placed in either chilled coolers or the recirculation water system (water temperature 4°C) on board the CCGC *Opilio* until arrival at the wharf. At the wharf, crabs were measured and hemolymph samples were collected in the order of which crabs were sampled. For crabs in coolers, one hemolymph sample was taken while crabs placed in the recirculation system, two hemolymph samples were taken. An initial hemolymph sample was taken immediately after the crab was removed from the recirculation system. The crab was then tagged and placed in a cooler filled with ice for the same duration as the crabs originally placed in coolers (approximately 4 hours) and a second hemolymph sample was taken. A total of 72 (20 commercial size adult males, 20 undersize adult males and 32 ovigerous females) and 78 (32 commercial size adult males, 16 undersize adult males and 30 ovigerous females) crabs were kept in coolers and the recirculation system, respectively.

Trawling (using a Bigouden *Nephrops* net, mesh size of 50mm at cod end) occurred on September 4th, 2014 near Cheticamp (same area as trapping) on board the CFV *Marco-Michel*, during the annual snow crab stock assessment in the southern Gulf of St. Lawrence. Once at the bottom, the trawl was towed for 5 minutes. Contents of the trawl were placed on the deck, time of trawl on deck was recorded and species were sorted. Selected crabs were measured and hemolymph samples were immediately taken. A maximum of 20 minutes was allotted per tow for hemolymph sampling from when the trawl landed on the deck. A total of 8 tows were completed to collect 120 crabs (40 commercial size males, 40 undersize adult males and 40 ovigerous females). Only morphometric measurements and hemolymph samples were collected for this comparison study. Hemolymph samples were collected from the area between the coxa of the first right walking leg and body. The area was disinfected with 70% ETOH and

hemolymph samples (1.0 to 1.5ml) were taken with a chilled 22G needle and 3ml syringe. Hemolymph samples were then transferred into chilled 1.7 ml tubes and centrifuged (7,000 x g, 5 min). Serum was then separated from the plasma with a chilled plastic transfer pipette and placed in a new chilled tube and preserved on ice until enzyme analysis was performed at the Diagnostics Laboratory at the Atlantic Veterinary College (Charlottetown, UPEI). Hemolymph sampling times were recorded for every trapped and trawled crab and all crabs were returned to sea after sampling.

III-2. CRAB SAMPLING BY TRAPPING

II-2-1 2012 Spring Trapping

The 2012 spring trap survey began on May 22nd and was completed June 14th. Sampling was conducted on May 24th for station B, on May 30th for station W2, on June 1st for W1, and on June 4th for E1 on board the CGS *Opilio*. As an insufficient number of ovigerous females were caught at station E1, an additional sampling was conducted for this station on June 14th, 2012 on board the CFV *Britanny Madison*. The target number of (20) pygmy/under sized adult males (PM) was not met at station B, however an additional sampling was not amenable due to tight vessel schedule. As such only 3 pygmy males were collected at station B. Collected crabs were kept in coolers with ice until arrival at the local laboratory for dissections (Table 1). Crab dissections were completed on May 24th, May 30th, June 1st, for stations B, W2, W1, respectively, and on June 4th and 14th for station E1.

Table 1. Morphological characteristics (mean ±, standard deviation, maximum and minimum (in parentheses) and median (m) values) for carapace width (CW, in mm) and body weight (BW, in g)) of crabs collected in spring 2012.

Crab category		**Sampling Station**				**Total**
		Grande-Rivière (B)	Margaree Harbor (W1)	Cheticamp (W2)	Louisbourg (E)	
Commercial size adult males (LM)	CW	114.60 ± 9.45 (99.50 – 134.60) m= 112.88	120.72 ± 9.85 (103.4 – 140.0) m=117.43	119.31 ± 6.07 (110.99 – 135.74) m=118.53	119.82 ± 7.13 (109.71 – 136.12) m=119.38	82
	BW	660.69 ± 166.0 (426 – 1053.0) m= 625.2 n = 21	788.91 ± 179.31 (598 – 1127.0) m= 733.95 n = 20	726.54 ± 115.86 (543.8 – 1043) m= 719.6 n = 21	705.44 ± 143.17 (478.6 – 991.4) m= 685.2 n = 20	
Undersized adult/pygmy males (PM)	CW	74.70 ± 3.20 (75.61) (71.14 – 77.35) m=75.61	83.17 ± 10.16 (55.25 – 94.82) m=82.69	59.10 ± 5.91 (47.71 – 67.47) m=58.68	77.18 ± 7.27 (63.70 – 90.27) m=77.42	64
	BW	162.0 ±175.1 (133.0 – 178.5) m= 174.5 n = 3	237.36 ± 84.92 (59.7 – 367.2) m= 223.25 n = 20	84.87 ± 26.26 (41.1 – 121.9) m= 82.3 n = 21	176.55 ± 46.33 (95.3 – 273.6) m= 175.45 n = 20	
Mature females (MF)	CW	70.15 ± 6.03 (61.48 - 88.39) m=69.47	71.03 ± 6.23 (60.20 – 80.19) m=70.2	72.66 ± 5.87 (61.54 – 83.67) m=73.72	68.62 ± 5.27 (56.77 – 79.45) m=68.96	85
	BW	137.9 ± 38.15 (92.7 – 262.0) m= 130.0 n = 21	125.68 ± 31.55 (77.8 – 184.5) m= 115.45 n = 20	139.58 ± 30.89 (87.9 – 198.1) m= 143.9 n = 20	109.56 ± 22.54 (65.5 – 156.5) m= 108.85 n = 24	
Total		45	60	62	64	231

III-2-2 2012 Fall Trapping

The 2012 fall trap survey started September 17th and was completed November 4th. Crab samples were collected at all four stations. For station E1, setting traps was done onboard a chartered commercial snow crab fishing vessel (CFV) *BrittanyMaddison* on September 17th and sample catching and dissection was completed on September 18th. For station B, trap setting was done on September 21st onboard the CGS *Opilio* while trap retrieval and dissections were done on September 22nd. For station W1, trap setting was conducted on November 1st on board the CFV *Fishfull Thinking* and sample collection and dissection were completed on November 2nd. For station W2, trap setting was was executed on November 3rd on the same vessel and sample collection and dissection were completed on the November 4th. Due to unfavorable weather conditions, sampling dates between the four sampling stations were spread over 7 weeks.
At each station, approximately sixty crabs (20 mature females, pygmy males and large commercial males) were collected and kept in coolers for transportation to the local laboratory for dissections (Table 2). Commencing with this sampling, new sampling procedures were introduced: (i.e. sampling of hepatopancreas for lipid/glycogen analyses and sampling of leg muscle for stable isotopic analyses (feeding behavior)) for 10 individuals per category per station were performed.

Table 2. Morphological characteristics (mean ±, standard deviation, maximum and minimum (in parentheses) and median (m) values) for carapace width (CW, in mm) and body weight (BW, in g)) of crabs collected in fall 2012.

Crab category		**Sampling Station**				**Total**
		Baie des Chaleurs (B)	Margaree Harbor (W1)	Cheticamp (W2)	Louisbourg (E1)	
Commercial size adult males (LM)	CW	117.75 ± 6.96 (109.66 – 135.20) m=117.02	120.33 ± 6.06 (109.59 – 130.81) m=120.22	115.94 ± 8.80 (102.66 – 132.43) m=114.05	118.73 ± 7.89 (105.95 – 139.60) m=117.19	80
	BW	689.97 ± 149.2 (502.3 – 1064.9) m= 659.2 n = 20	784.88 ± 118.89 (568.3 – 964.7) m= 796.75 n = 20	667.53 ± 154.25 (471.5 – 985.2) m= 603.05 n = 20	705.76 ± 149.57 (502.9 – 1139.1) m= 695.75 n = 20	
Undersized adult/ pygmy males (PM)	CW	76.06 ± 7.14 (65.25 – 89.42) m=76.96	80.00 ± 9.52 (59.53 – 93.61) m=80.33	82.34 ± 3.61 (74.51 – 89.03) m=82.32	76.18 ± 5.92 (61.24 – 83.82) m=76.64	80
	BW	172.5 ± 44.2 (111.2 – 255.6) m= 161.35 n = 20	204.29 ± 71.84 (95.9 – 342.9) m= 192.4 n = 20	228.99 ± 30.70 (177.7 – 285.6) m= 222.6 n = 20	167.36 ± 33.89 (93.7 – 213.6) m= 176.35 n = 20	
Mature females (MF)	CW	68.97 ± 7.17 (58.56 – 85.35) m=66.52	69.22 ± 4.92 (62.28 – 81.16) m=68.83	71.00 ± 6.78 (59.60 – 82.78) m=69.67	72.21 ± 3.65 (63.66 – 77.39) m=72.45	81
	BW	116.96 ± 34.28 (70.0 – 194.4) m= 106.4 n = 21	123.85 ± 26.70 (90.5 – 200.7) m= 119.95 n = 20	132.86 ± 36.83 (81.4 – 201.7) m= 121.4 n = 20	132.68 ± 18.70 (88.9 – 158.6) m= 135.0 n = 20	
Total		61	60	60	60	241

III-2-3 2013 Spring Trapping

The 2013 spring sampling was done at station B on May 30th, 2013 onboard CGS *Perley*, at station W1 on June 18th and station W2 on June 11th onboard the chartered CFV *FishFull Thinking*, and at station E1 on June 24th and 26th onboard the chartered CFV *Madison & Brittany*. All categories of crab (LM, PM, and MF) were collected with the exception of females from E1; only five females were collected (Table 3). The collection of 20 females at this station was often challenging as females seem to move quite actively in this area.

Table 3. Morphological characteristics (mean ± standard deviation, maximum and minimum (in parentheses) and median (m) values) for carapace width (CW, in mm) and body weight (BW, in g)) of crabs collected in spring 2013.

		Sampling Station				Total
Crab category		Grande-Rivière (B)	Margaree Harbor (W1)	Cheticamp (W2)	Louisbourg (E)	
Commercial size adult males (LM)	CW	110.18 ± 7.81 (98.06 - 126.98) m=109.24	120.34 ± 6.09 (107.64 – 131.82) m=119.9	120.33 ± 7.12 (109.49 – 130.52) m=120.73	114.23 ± 11.44 (96.74-136.19) m=111.66	80
	BW	566.5±131.2 (377.3-831.1) m=536.4 n = 20	764.0 ± 114.5 (570.7-1039.8) m=753.9 n = 20	763.7 ± 135.8 (531.0-950.5) m=768.55 n = 20	617.5 ± 202.5 (361.0-1011.3) m=567.75 n = 20	
Undersized adult/ pygmy males (PM)	CW	72.27 ± 8.52 (57.24-89.63) m=71.71	74.93 ± 6.18 (56.28-80.73) m=76.29	75.25 ± 9.04 (58.36-89.37) m=78.27	67.78 ± 5.04 (58.83-74.17) m=68.61	80
	BW	162.1±61.82 (74.9-334.7) m=149.65 n = 20	172.55 ± 38.5 (68.8-230.1) m=179.3 n = 20	178.1 ± 62.23 (73.0-282.8) m=186.1 n = 20	122.4 ± 26.1 (75.5 – 157.4) m=126.15 n = 20	
Mature females (MF)	CW	64.48 ± 3.91 (57.88-73.24) m=64.36	69.64 ± 5.29 (62.14-81.36) m=69.39	69.08 ± 7.71 (57.94-84.22) m=67.83	69.16±7.51 (59.60 – 77.74) m=70.29	65
	BW	101.0 ± 19.82 (73.9 - 154.1) m=99.2 n = 20	121.44 ± 25.7 (78.4-178.6) m=116.3 n = 20	123.6 ± 39.06 (73.5 – 201.8) m=122.15 n=20	111.3±29.9 (71.6-145.4) m=108.9 n = 5	
Total		60	60	60	45	225

III-2-4 2013 Fall Trapping

The 2013 fall sampling was done at station W2 on October 16th and W1 on November 6th both onboard the chartered CFV FishFull Thinking. The sampling in stations B and E1 was not amenable because of continuous unfavorable weather. The time lug between sampling at two stations (W1 and W2) could not be reduced due to the same reason and resulted in a 21-day difference. All categories of crab (large adult males=LM, mature females=MF, and pygmy males= PM) necessary for the analysis were collected (Table 4).

Table 4. Morphological characteristics (mean ± standard deviation, maximum and minimum in parentheses and median (m) values for carapace width (CW, in mm) and body weight (BW, in g)) of crabs collected in fall 2013 at stations W1 and W2

Crab category		**Sampling Station**				**Total**
		Grande-Rivière (B)	Margaree Harbor (W1)	Cheticamp (W2)	Louisbourg (E)	
Commercial size adult males (LM)	CW	-	122.02 ± 8.01 (111.66 – 142.82) m=120.13	122.87 ± 8.39 (111.4– 141.37) m=121.55	-	40
	BW		778.9 ± 165.6 (567.1-1214.1) m= 713.4 n = 20	814.4 ± 162.5 (604.9-1260.2) m=793.6 n = 20		
Undersize adult/pygmy males (PM)	CW	-	78.86 ± 6.36 (62.95 – 87.01) m=79.61	66.57 ± 6.38 (51.83-79.16) m=66.33	-	40
	BW		198.7 ± 47.02 (106.1-279.3) m=197.65 n = 20	120.9 ± 33.69 (53.8-195.8) m=119.1 n = 20		
Mature females (MF)	CW	-	70.10 ± 7.90 (60.03– 95.94) m=68.28	66.69 ± 9.02 (46.56-80.36) m=66.77	-	40
	BW		117.1 ± 24.3 (83.5-168.1) m=110.65 n = 20	104.1 ± 35.7 (40.2 – 170.4) m=102.3 n = 20		
Total		0	60	60	0	120

III-3. CAGE SETTING AND RETRIEVAL

III-3-1 Cage setting

Trapping was done on November 2nd and at station W1 for collecting 20 individuals per category for crabs for regular sampling and caging, and on November 5th for station W2. The latter was

done solely for caging sampling and regular sampling was done on November 4th. Six lines of cages (3 sets of cages) per station were immersed on November 2nd and November 5th 2012 at stations W1 and W2, respectively.
Cages used for this study were wire mesh cages (48 in x 36 in x 16 in width, 1 ½ in mesh) with a rectangular top opening (42 1/8 in x 27 5/8 in) and weighted with three cement bars of 48 in x 2 ½ in each (total of 20 lbs, Figure 2). During the consultation process prior to the project, it was suggested that it is preferable to replace a top mesh panel by ether plastic panel or nylon mesh web in order to reduce the possible amplification of seismic noise in the cage (pers. comm. G. Lee-Dadswell. However, the proposed modification was not considered for this project because there was no seismic shooting and this modification may weaken the trap structure and increases the risk of losing crabs during cage hauling.

A total of 180 cages (90 at each station) were set. The cage setting plan was slightly modified by reducing the number of cages per line from the original one line of 30 cages. The original plan of one line composted with 30 cages was divided into 2 lines of 15 cages to increase chances of cage retrieval. The first line of the first 15 cages, 10 cages contained one large male, 3 cages contained 4 mature females and 2 cages contained 4 pygmy males. The other line of 15 cages was composed of 10 cages with a large male, 3 cages with 4 pygmy males and 2 cages with 4 mature females (Figures 2 and 3). Each cage door was secured by black UV temper-proof 8" tie wraps to prevent crab from escaping from the gap and to make sure that the door was not opened. The distance between each line was set at approximately 2,000 feet to reduce the risk of line tangling.

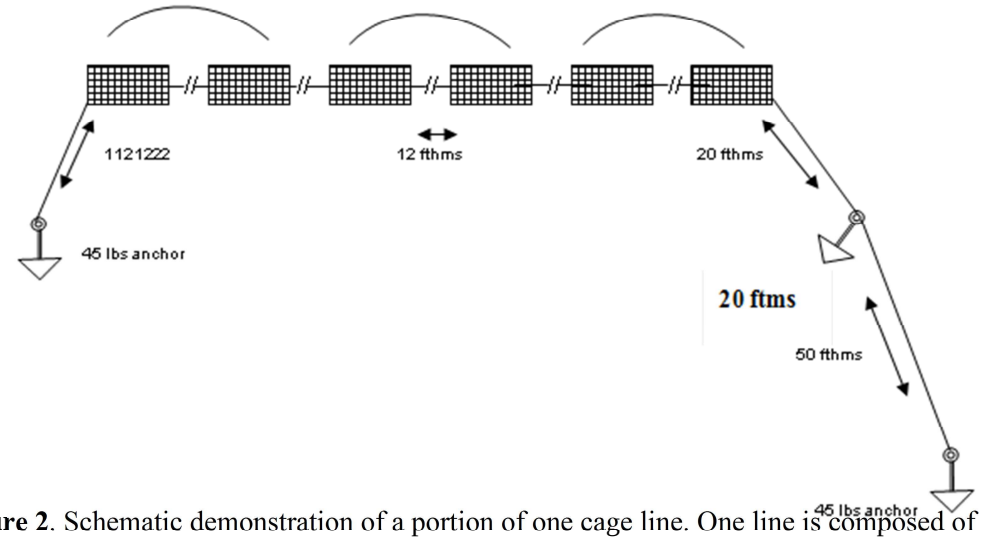

Figure 2. Schematic demonstration of a portion of one cage line. One line is composed of 10 cages with one large adult male, 3 cages with 4 undersized adult/pygmy males and 2 cages of 4 mature females; the second line includes10 cages for large males, 2 cages of 4 pygmy males and 3 cages of 4 mature females, totaling 20 crabs for each category. Two lines per caging station were lifted for each sampling.

Figure 3. A: Catching crabs to for the caging study, B: 4 females in a cage, C: 4 pygmy males in a cage, D: one large male in a cage (with individual tag on its right claw), E: all cages of one line ready to be immersed, F: mature female with individual tag. Pictures were taken onboard CFV *Fishfull Thinking* off Cheticamp, NS in November 2012.

III-3-2 Cage retrieval operation

Each cage line was retrieved by using a special galvanized hook with 6 spines (Figures 4 A & B) attached to a 12-feet lead chain with 50 lbs of weight (chains). This hook was towed with twice the length of the depth at each site with ¾" rope (variable depending on the sea condition) with the purpose of securing the end of each line (which was composed of 50-fathoms (900m) of rope with a small float anchored at both sides). Cage retrieval was facilitated with the computer program 'Nobeltec Catch® program with a DGPS' on board the CFV *Fishfull Thinking*. Each crab (360 crabs) was measured and marked with a spaghetti type tag (on the right claw for both large and pygmy males, and around the carapace for females: see Figures 3, C, D, & F). The purpose of this cage setting is to evaluate the possible effects of caging on crab health after 12 days, 6 months and 12 months of immersion.

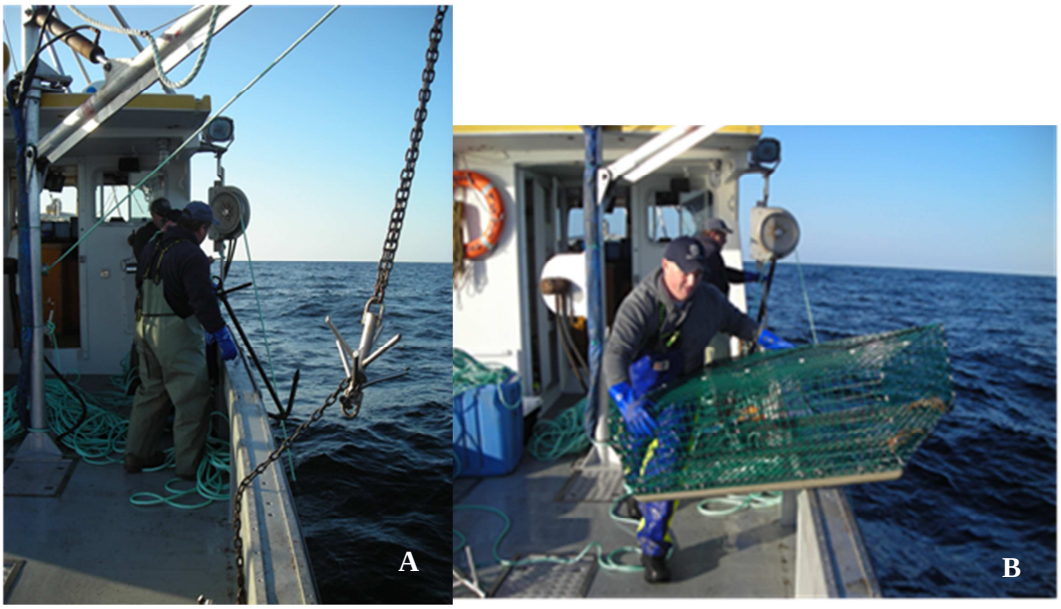

Figure 4. Galvanized hook used for cage retrieval (A) and cage retrieved (B) on board CFV Fishfull Thinking off Margaree Harbor.

III-3-3 2012 Fall cage retrieval (16/17 days of immersion[1])

After 16 and 17 days of immersion at W1 and W2 stations, respectively, two lines of 15 cages from each station were hauled and all crabs were retrieved. (The original plan was to haul the lines after 12 days of immersion to be consistent with the 2003/2004 study (DFO, 2004).

[1] In this document unless specified it is called 2 week caging

However, strong winds restricted cage hauling on the 12th day (November 14th and 16th for Margaree Harbor and Cheticamp, respectively).

At station W1, two (2) large males died leaving only the carapace in the cage while at station W2, one pygmy male disappeared (presumably dead with carapace either completely consumed by micro-organisms or passed through the cage mesh due to its smaller size). The remaining crabs had no apparent weakness (Table 5).

Retrieved crabs were measured and treated by following the regular sampling protocol as described in Section III-1. Hemolymph samples were collected as described in Section VII-1-3.

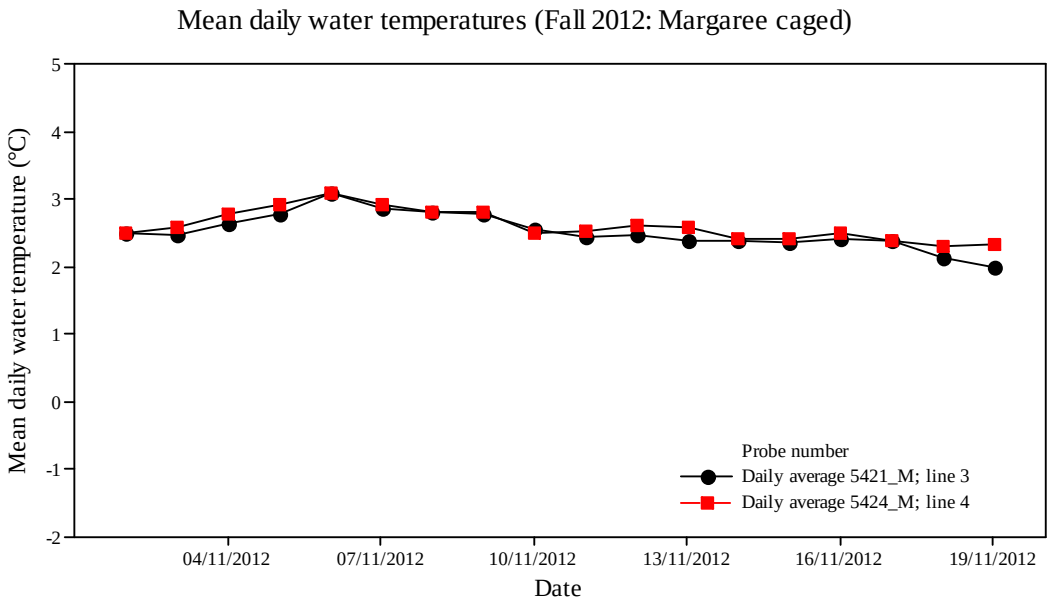

Figure 5. Mean daily bottom water temperatures recorded with VEMCO mini-log temperature recorders at station W1 for 16 days of immersion.

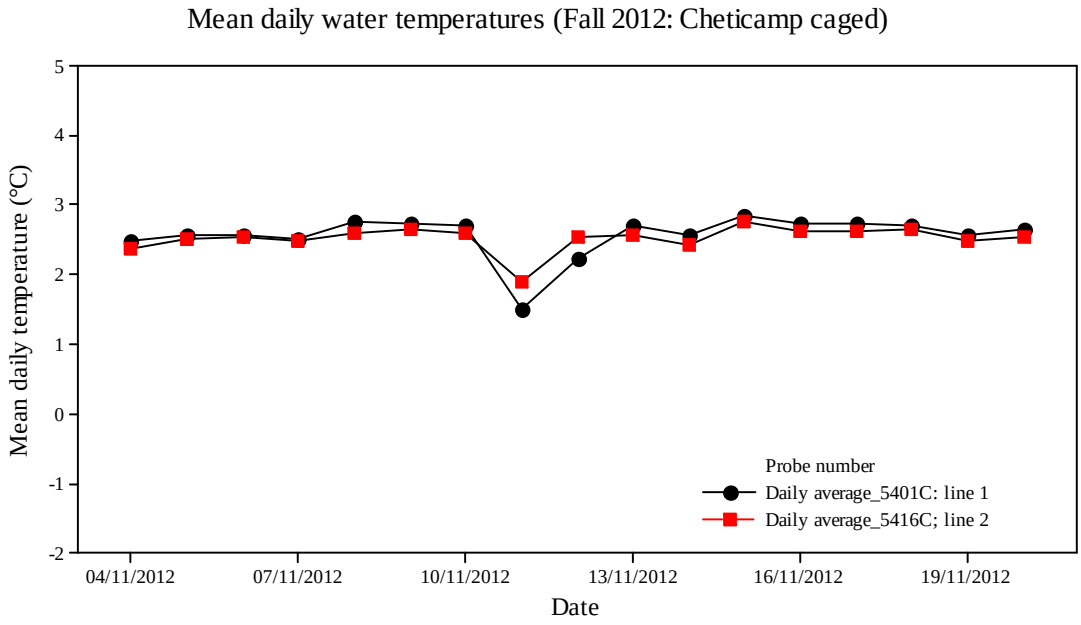

Figure 6. Mean daily bottom water temperatures recorded with VEMCO mini-log temperature recorders at station W2 for 17 days of immersion.

According to temperature probes attached to each of line (total of two per station), the mean daily bottom water temperatures varied between 1.9°C and 3.2°C, 1.0°C and 3.2°C with an average temperature between 2.54-2.62°C and 2.52-2.56 °C for station W1 and W2, respectively (Figures 5 & 6). The cumulative degree day was 45.47°C (line 1/1, probe #5421) and 46.91°C (line1/2, probe #5424) for station W1, and 43.50°C (line 1/1, probe #5401) and 42.76°C (line1/2, probe #5416) for station W2. A sudden decrease in bottom temperature was recorded on both lines of cages on November 10th at station W2 (Figures 6).

Table 5. Morphological characteristics (mean ± standard deviation, maximum and minimum (in parentheses) and median (m) values for carapace width (CW, in mm) and body weight (BW, in g)) of caged crabs retrieved in fall 2012 at stations W1 and W2.

Crab category		***Sampling Station***		***Total***
		Margaree Harbour (W1)	Cheticamp (W2)	
Commercial size adult males (LM)	CW	126.16 ± 7.95 (110.11 – 138.01) m=127.79	116.71 ± 6.02 (102.11 – 127.58) m=116.43	38
	BW	839.07 ± 162.93 (590.9 – 1161.1) m=846.15 n = 18	675.42 ± 107.12 (417.3 – 872.2) m=658.05 n = 20	
Undersize adult/pygmy males (PM)	CW	88.68 ± 5.82 (72.08 – 94.11) m=91.58	84.46 ± 8.43 (63.68 – 94.02) m=88.12	39
	BW	273.03 ± 46.85 (144.9 – 346.1) m=277.4 n = 20	251.99 ± 72.76 (96.9 – 328.3) m=268.3 n = 19	
Mature females (MF)	CW	69.29 ± 6.19 (59.43 – 79.95) m=68.75	69.23 ± 5.05 (62.67 – 82.08) m=68.72	40
	BW	121.88 ± 29.95 (81.2-186.3) m=123.2 n = 20	122.02 ± 26.42 (93.9 – 199.1) m=117.17 n = 20	
Total		58	59	117

III-3-4 2013 Spring Cage retrieval (214/222 days of immersion[2])

On June 4th, two lines of cages immersed on November 2nd 2012 were successfully retrieved at W1 (with immersion duration of 214 days). On June 11th, 2013, two lines of cages immersed on November 5th, 2012 were successfully retrieved at W2 (with immersion duration of 222 days). A total of 4 LM, 2 PM and 1 MF at W1, and 2 LM, 1 PM, and 1 MF at W2 were missing (no trace of body parts in the cage) or dead (body parts/carapace remained in the cage) (Table 6). The remaining crabs showed some weakness. Contrary to the original plan for the immersion duration of 6 months (180 days), we could not retrieve the cages earlier and ended up with an additional 34 and 42 days of immersion for stations W1 and W2, respectively.

[2] In this document unless specified it is called 6 month caging

Table 6. Morphological characteristics (mean ± standard deviation, maximum and minimum (in parentheses) and median (m) values for carapace width (CW) and body weight (BW)) of caged crabs retrieved in spring 2013 at stations W1 and W2.

Crab category		***Sampling Station***		***Total***
		Margaree Harbor (W1)	Cheticamp (W2)	
Commercial size adult males (LM)	CW	121.80 ± 5.93 (113.70 – 132.93) m=120.28	124.36 ± 6.81 (110.93 – 135.87) m=126.53	34
	BW	791.6 ± 144.8 (642.8 – 1136.1) m=744.55 n = 16	849.00 ± 147.7 (626.5 – 1066.1) m= n = 18	
Undersized adult/pygmy males (PM)	CW	84.36 ± 10.27 (59.74 -94.75) m=86.84	80.02 ± 9.60 (64.92 – 94.07) m=82.33216.7 ± 74.53 (108.5 – 351.2)	37
	BW	256.6 ± 84.03 (96.4 – 354.9) m=247.55 n = 18	m=231.4 n = 19	
Mature females (MF)	CW	69.69 ± 5.31 (59.14 – 79.46) m=69.31	71.32 ± 9.17 (58.06 – 87.58) m=70.47	38
	BW	124.3 ± 25.8 (78.8 – 176.7) m=124.1 n = 19	131.5 ± 40.59 (72.8 – 204.6) m=117.9 n = 19	
Total		53	56	109

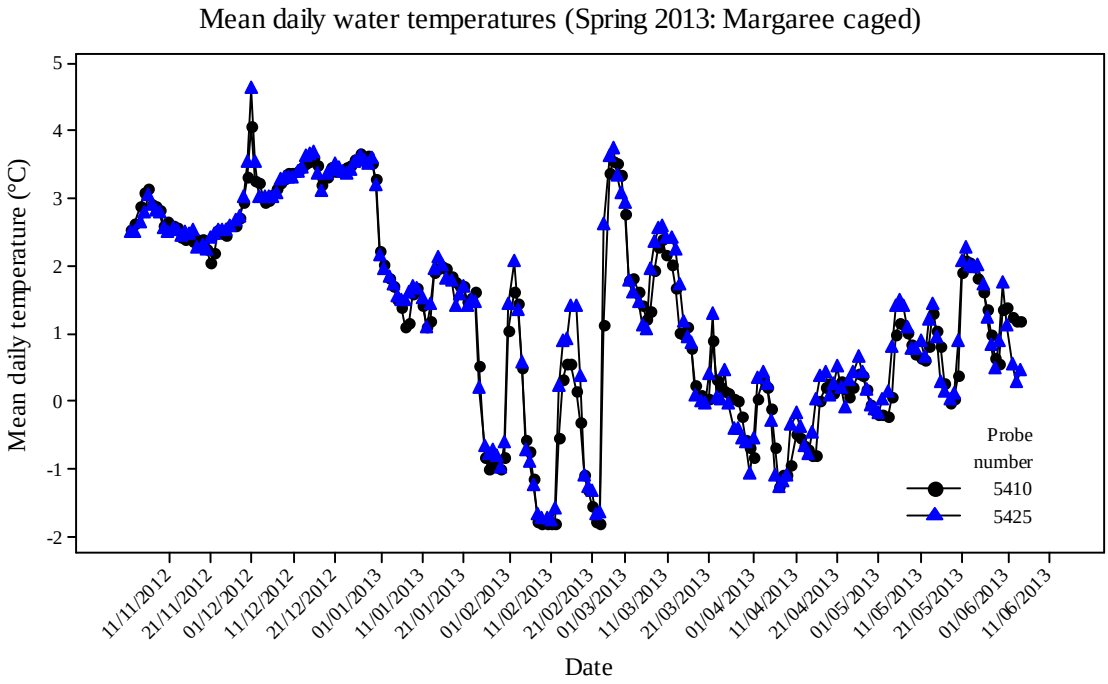

Figure 7. Mean daily bottom water temperatures recorded with VEMCO minilog temperature recorders at station W1 for 214 days of immersion.

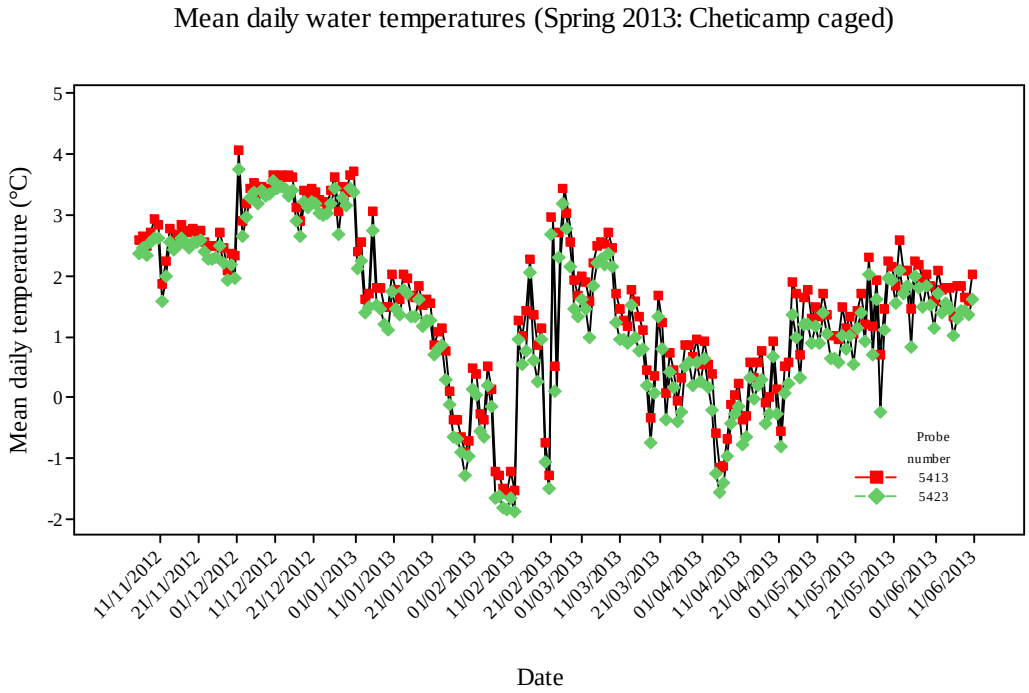

Figure 8. Mean daily bottom water temperatures recorded with VEMCO mini-log temperature recorders at station W2 for 222 days of immersion.

According to temperature probes attached to each line (total of two per station), the mean daily bottom water temperatures was between 1.26°C - 1.31°C, and 1.28°C - 1.61°C for station W1 and W2, respectively. The cumulative degree day was 270.95°C (line 1/1, probe #5410) and 282.14°C (line1/2, probe #5425) for W1 station, and 351.64°C (line 1/1, probe #5413) and 281.22°C (line1/2, probe #5423) for W2 station. Both mean daily water temperatures and cumulative degree days were generally higher in station W2 than station W1. The amplitude (lowest and highest) mean daily water temperatures was 6.4-7.9°C and 6.8-9.2°C for stations W1 and W2, respectively. Within the same station (W2), a noticeable difference was found in the mean and cumulative temperature between 2 lines which were approximately 600m apart. The period with the most variable water temperatures was between mid-January to mid-April (Figures 7 & 8).

III-3-5 2013 Fall Cage Retrieval (345/355 days of immersion[3])

On October 15th, 2013, the last set of cages immersed on November 2nd 2012 was successfully retrieved at station W1 (with 345 days of immersion duration). On October 22nd, 2013, the last two lines of cages immersed on November 5th, 2012 were successfully retrieved at station W2 (355 days of immersion). A total of 7 LM, 7 PM and 5 MF from W1, and 5 LM, 4 PM, and 4 MF from W2 were missing (no trace of body part in the cage/presumed dead) or confirmed death (body parts/i.d. tag remained in the cage). The remaining crabs had no obvious injury, but showed some conspicuous weakness in the majority of retrieved samples (Table 7). Caging snow crabs longer than 152 days has never been conducted in the past.

Retrieved crabs were measured and treated by following the regular sampling protocol as described in Section III-1 and the hemolymph samples were collected as described in Section VII-1-3.

Additional sample treatment and analyses are described in subsequent sections.

[3] In this document unless specified it is called 12 month caging

Table 7. Morphological characteristics (mean ± standard deviation, maximum and minimum (in parentheses) and median (m) values) for carapace width (CW, in mm) and body weight (BW, in g)) of caged crabs retrieved in fall 2013 at stations W1 and W2.

Crab category		***Sampling Station***		***Total***
		Margaree Harbor (W1)	Cheticamp (W2)	
Commercial size adult males (LM)	CW	121.36 ± 6.76 (113.8 – 131.61) m=123.38	127.12 ± 7.06 (117.34 – 144.22) m=126.88	24
	BW	800.96 ± 122.0 (642.2 – 1015.0) m=778.7 n = 9	877.29 ± 128.29 (655.2 – 1100.4) m=874.1 n = 15	
Undersized adult/ pygmy males (PM)	CW	85.77 ± 8.82 (68.17 – 95.53) m=89.79	68.03 ± 6.92 (61.46 – 80.56) m=65.23	29
	BW	262.51 ± 72.38 (123.6 – 342.5) m=291.7 n = 13	124.98 ± 42.48 (64.7 – 200.4) m=118.4 n = 16	
Mature females (MF)	CW	71.59 ± 6.89 (56.0 – 84.32) m=73.52	68.96 ± 5.96 (57.29- 79.50) m=68.63	33
	BW	135.06 ± 35.36 (59.6 -214.2) m=136.1 n = 17	111.97 ± 25.64 (73.5 – 166.4) m=105.7 n = 16	
Total		39	47	86

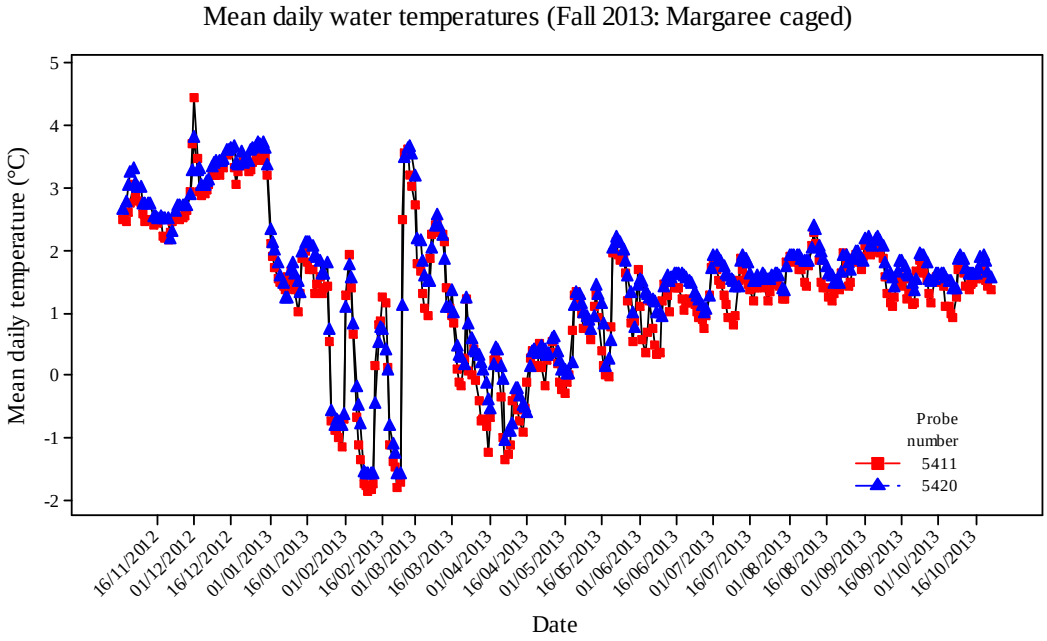

Figure 9. Mean daily bottom water temperatures recorded with VEMCO mini-log temperature recorders at station W1 for 345 days of immersion.

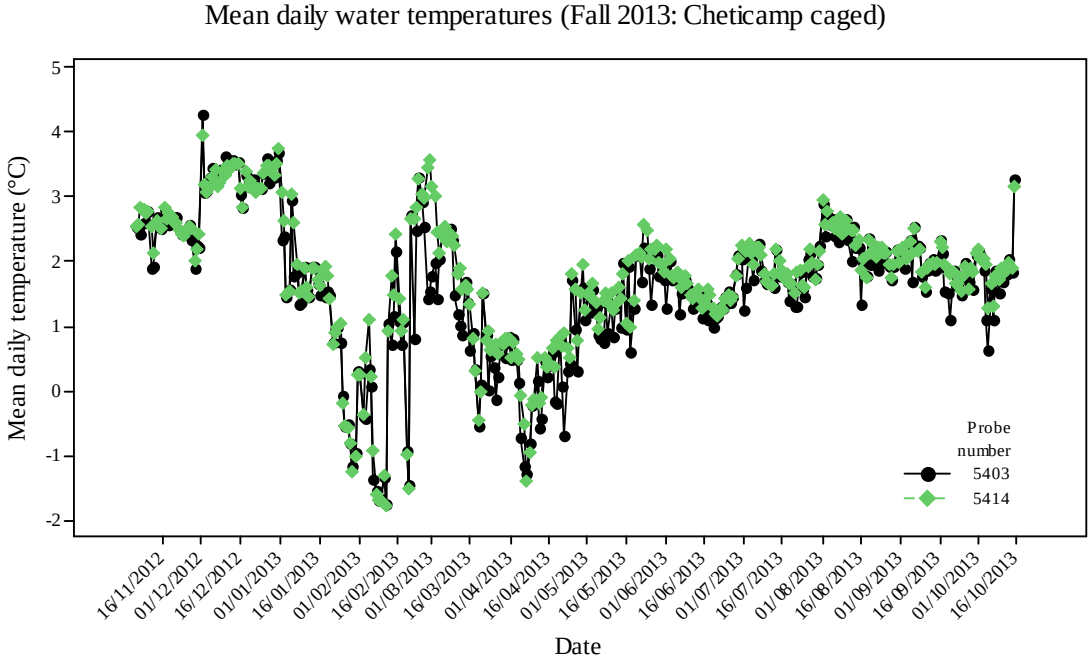

Figure 10. Mean daily bottom water temperatures recorded with VEMCO mini-log temperature recorders at station W2 for 355 days of immersion.

According to temperature probes attached to each of line (total of two per station), the mean daily bottom water temperatures were between 1.33°C and 1.51°C and 1.60°C and 1.76°C for station W1 and W2, respectively. The cumulative degree days were 473.19°C (line 1/1, probe #5411) and 537.65°C (line1/2, probe #5420) for station W1, and 551.93°C (line 1/1, probe #5403) and 606.12°C (line1/2, probe #5414) for station W2. Both mean daily water temperatures and cumulative degree days were higher in station W2 than station W1. The amplitude (lowest and highest) of mean daily water temperatures was between 5.7 and7.5°C and 10.3 and10.8°C for stations W1 and W2, respectively. At both stations, the mean bottom temperature and cumulative temperature were different between lines. The period with the most variable water temperatures was found between mid-January to mid-April (Figures 9 and 10).

Moriyasu and Lanteigne (1998) observed that at the cumulative temperature of 673.8˚C (daily average of 1.85°C), multiparous females adopted a one year (365 days) reproductive cycle. Although no cumulative degree-day information is available for apprehending the threshold between a one-year and a two-year embryonic development cycle, the cumulative degree days between 473 and 606°C is very high to maintain a two-year embryonic development cycle (especially considering that the calculated degree-days did not include periods between egg extrusion and crab capture).

III-3-6. Discussion

We encountered some difficulties in at-sea trapping due to 1) bad weather (consequently sampling period was extended) 2) commercial fishing (sampling stations were in the middle of commercial fishing ground and in some cases, traps were empty when retrieved/lifted), and 3) the biological cycle and natural abundance of different crab categories (difficultly trapping immature females and adolescent males and lack of mature females at pre-determined stations known for high concentrations of females (based on stock assessements and local fishermen's experience and knowledge)). Consequently, crab categories were modified from "immature females, mature females, adolescent males and commercial size adult males" to "mature females, undersize adult/pygmymales and commercial size adult males") after the first sampling off Grande-Rivière station in spring 2012. The second, deeper station off the Scotian shelf near Louisbourg was also abandoned due to the lack of females after the first attempt in spring 2012.

Some difficulties in trap setting were also encountered due to unfavourable weather conditions and vessel schedule. As such, four planned sampling dates were rescheduled for a later time. Delayed sampling dates can significantly affect results, especially when examining reproductive parameters such as embryonic and ovary development and fecundity. Using different vessels with different sampling teams may resolve this problem although it is not cost effective.

III-4. MORPHOMETRIC PARAMETERS OF CRAB SAMPLES

III-4-1 Mean size

Data analysis

Data analysis using parametric and non-parametric tests was performed with Minitab ® (version 16.2.3.0, MINITAB Inc. State College, PA, USA) and Microsoft Excel (version 14.0., Microsoft. Redmond, Washington, DC, USA) statistical softwares. Data was examined for normality (based on the Anderson-Darling normality test), and variance homogeneity (Bartlett's test). When data were normally distributed, one way analysis of variance (ANOVA) and Tukey multiple comparison tests were applied. When data did not follow a normal distribution, or if heteroscedasticity was detected and did not improve even after data transformation, non-parametric tests (Kruskal-Wallis, nonparametric multiple comparison test, nonparametric Tukey-type Nemenyi test) were applied. All results obtained were considered significant at the 0.05 level.

Results

Statistical information for carapace widths for free and caged crabs from different sampling areas is summarized in Table 8. While Large Mature Males were the largest regardless of season or sampling area, pygmy males were not always larger than mature females. Mean carapace widths for females from Cheticamp were greater than pygmy males in the spring 2012 and the fall 2013 (both caged and free crabs). In the spring 2013, mean carapace widths for free females from Louisbourg were also higher than pygmy males; however, only a small number of these males were collected.

Sampling area differences in terms of carapaces widths are summarized in Table 9. In the spring 2013, females caged in Cheticamp were significantly larger than those from Grande-Rivière (ANOVA, $F_{5,99} = 2.48$, $p = 0.037$; Tukey test, $p < 0.05$). No significant differences were observed among 2012 samples or fall 2013 samples. For large mature males, no significant differences were observed among sampling areas in the spring 2012 or in the fall 2013. In the fall 2012, caged crab from Margaree Harbor were significantly larger than those free in Cheticamp (Kruskal-Wallis test: $p = 0.005$, nonparametric Tukey-type Nemenyi test: $q = 4.22 > q0.05, \infty, 6 = 4.030$). In the spring 2013, caged large mature males from Cheticamp were significantly larger than samples from Louisbourg and Grande-Rivière and Grande-Rivière samples were smaller than all other sampling areas except Louisbourg (ANOVA, $F_{5,109} = 8.62$, $p < 0.0005$; Tukey test, $p < 0.05$).

For pygmy males, significant differences were noted among all sampling seasons. In the spring 2012, pygmy males free in Cheticamp were significantly smaller than all other sampling areas

(ANOVA, $F_{3,60} = 35.20$, $p < 0.0005$, Tukey test, $p < 0.05$). In the fall 2012, caged crabs from Margaree Harbor and Cheticamp were significantly larger than crabs caught in Louisbourg and Grande-Rivière (Kruskal-Wallis test: $p < 0.0005$, nonparametric Nemenyi test: qMargaree Harbor caged vs Louisbourg = $7.28 > q_{0.05}, \infty, 6 = 4.030$; qMargaree Harbor caged vs Grande-Rivière = $7.15 > q_{0.05}, \infty, 6 = 4.030$; qCheticamp caged vs Louisbourg = $4.60 > q_{0.05}, \infty, 6 = 4.030$; qcheticamp caged vs grande-rivière = $4.46 > q0.05, \infty, 6 = 4.030$). Caged crabs from Margaree Harbor were also significantly larger than free crabs from Margaree Harbor (nonparametric Nemenyi test: qMargaree Harbor caged vs Margaree Harbor freed = $4.83 > q_{0.05}, \infty, 6 = 4.030$). In the spring 2013, pygmy males caged in Margaree Harbor were significantly larger than males collected in Louisbourg and Grande-Rivière (Kruskal-Wallis test: $p < 0.0005$, nonparametric multiple comparison test: QMargaree Harbor caged vs Louisbourg = $5.20 > Q_{0.05}, \infty, 6 = 2.936$; $Q_{\text{Margaree caged vs Grande-Rivière}} = 3.66 > Q_{0.05}, \infty, 6 = 2.936$). Crabs caged in Cheticamp were also significantly larger than those from Louisbourg (Kruskal-Wallis test: $p < 0.0005$, nonparametric multiple comparison test: $Q_{\text{Cheticamp caged vs Louisbourg}} = 4.049 > Q(_{0.05}, \infty, 6 = 2.936$). In the fall 2013, crabs from Margaree (both caged and free) were significantly larger than those from Cheticamp (caged and free) (Kruskal-Wallis test: $p < 0.0005$, nonparametric multiple comparison test: $Q_{\text{Margaree caged vs Cheticamp free}} = 4.97 > Q(_{0.05}, \infty, 4) = 2.639$; QMargaree Harbor caged vs Cheticamp caged = $4.62 > Q(_{0.05}, \infty, 4) = 2.639$; QMargaree Harbor free vs Cheticamp free = $3.81 > Q(_{0.05}, \infty, 4) = 2.639$; QMargaree Harbor free vs Cheticamp caged = $3.46 > Q(_{0.05}, \infty, 4) = 2.639$).

Table 8. Summary statistics for carapace width (CW) in mm, for free and caged mature females (MF), Large Mature Males (MM), and pygmy males (PM) by season and sampling area.

Date	Area	Sampling method	Crab category	min	median	max	n	Mean	St dev
Spring 2012	Cheticamp	Free	MF	61.54	73.72	83.67	20	72.66	5.87
			MM	110.99	118.53	135.74	21	119.33	6.07
			PM	47.71	58.68	67.47	21	59.10	5.91
	Grande-Rivière	Free	MF	61.48	69.47	88.39	21	70.15	6.03
			MM	99.50	112.88	134.6	21	114.60	9.45
			PM	71.14	75.61	77.35	3	74.7	3.2
	Louisbourg	Free	MF	56.77	68.96	79.45	24	68.62	5.27
			MM	109.71	119.38	136.12	20	119.82	7.13
			PM	63.70	77.42	90.27	20	77.18	7.27
	Margaree Harbor	Free	MF	60.20	70.20	80.19	20	71.03	6.23
			MM	103.40	117.43	140.00	20	120.72	9.85
			PM	55.25	82.69	94.82	20	83.17	10.16
Fall 2012	Cheticamp	Free	MF	59.60	69.67	82.78	20	71.00	6.78
			MM	102.66	114.05	132.43	20	115.94	8.80
			PM	74.51	82.32	89.03	20	82.34	3.61
	Cheticamp	caged	MF	62.67	68.72	82.08	20	69.23	5.05
			MM	102.11	116.43	127.58	20	116.71	6.02
			PM	63.68	88.12	94.02	19	84.46	8.43
	Grande-Rivière	Free	MF	58.56	66.52	85.35	21	68.97	7.17
			MM	109.66	117.02	135.20	20	117.75	6.96
			PM	65.25	76.96	89.42	20	76.06	7.14
	Louisbourg	Free	MF	63.66	72.45	77.39	20	72.21	3.65
			MM	105.95	117.19	139.60	20	118.73	7.89
			PM	61.24	76.64	83.82	20	76.18	5.92
	Margaree Harbor	Free	MF	62.28	68.83	81.16	20	69.22	4.92
			MM	109.59	120.22	130.81	20	120.33	6.06
			PM	59.53	80.33	93.61	20	80.00	9.52
	Margaree Harbor	caged	MF	59.43	68.75	79.95	20	69.29	6.19
			MM	110.11	127.79	138.01	18	126.16	7.95
			PM	72.08	91.58	94.11	20	88.68	5.82

Table 8 (continued)

Date	Area	Sampling method	Crab category	min	median	max	n	Mean	St dev
Spring 2013			PM	58.36	78.27	89.37	20	75.25	9.04
	Cheticamp	caged	MF	58.06	70.47	87.58	19	71.32	9.17
			MM	110.93	126.53	135.87	18	124.36	6.81
			PM	64.92	82.33	94.07	19	80.02	9.60
	Grande-Rivière	Free	MF	57.88	64.36	73.24	20	64.48	3.91
			MM	98.06	109.24	126.98	20	110.18	7.81
			PM	57.24	71.72	89.63	20	72.27	8.52
	Louisbourg	Free	MF	59.60	70.29	77.74	5	69.16	7.51
			MM	96.74	111.66	136.19	20	114.23	11.44
			PM	58.83	68.61	74.17	20	67.78	5.04
	Margaree Harbor	Free	MF	62.14	69.39	81.36	20	69.64	5.29
			MM	107.64	119.90	131.82	20	120.34	6.09
			PM	56.28	76.29	80.73	20	74.93	6.18
	Margaree Harbor	caged	MF	59.14	69.31	79.46	19	69.69	5.31
			MM	113.70	120.28	132.93	16	121.80	5.93
			PM	59.74	86.84	94.75	18	84.36	10.27
Fall 2013	Cheticamp	Free	MF	46.56	66.77	80.36	20	66.69	9.02
			MM	111.40	121.55	141.37	20	122.87	8.39
			PM	51.83	66.33	79.16	20	66.57	6.38
	Cheticamp	caged	MF	57.29	68.63	79.50	16	68.96	5.96
			MM	117.34	126.88	144.22	15	127.12	7.06
			PM	61.46	65.23	80.56	16	68.03	6.92
	Margaree Harbor	Free	MF	60.03	68.28	95.94	20	70.10	7.90
			MM	111.66	120.13	142.82	20	122.02	8.01
			PM	62.95	79.61	87.01	20	78.86	6.36
	Margaree Harbor	caged	MF	56.00	73.52	84.32	17	71.59	6.89
			MM	113.79	123.38	131.61	9	121.36	6.76
			PM	68.17	89.79	95.53	13	85.77	8.82

Table 9. Summary of carapace width (CW) values (in mm) of snow crab by sampling area from different seasons. Data are separated to show the three crab categories: mature females (MF), Large Mature Males (MM) and pygmy males (PM). Median and mean values sharing the same letter are not different. For sampling areas where no subscript are shown, no differences were detected among sampling areas. No asterisks = mean CW values /One way analysis of variance (ANOVA, $p < 0.05$); asterisk = median CW values (in mm)/ Non parametric Kruskal-Wallis testing, $p < 0.05$).

Crab Category	Sampling season	Margaree Harbor Free	n	Cheticamp Free	n	Margaree Harbor caged	n	Cheticamp cage	n	Grande-Rivière Free	n	Louisbourg Free	n	P
	Spring 2012	71.03	20	72.66	20	-	-	-	-	70.15	21	68.62	24	0.150
MF	Fall 2012	69.22	20	71.00	20	69.29	20	69.23	20	68.97	21	72.21	20	0.391
	Spring 2013	69.64ab	20	69.08ab	20	69.69ab	19	71.32a	19	64.48b	20	69.16ab	5	0.037
	Fall 2013	70.10	20	66.69	20	71.59	18	68.96	16	-	-	-	-	0.178
	Spring 2012*	117.43	20	118.53	21	-	-	-	-	112.88	21	119.38	20	0.127
LM	Fall 2012*	120.22ab	20	114.05a	20	127.79b	20	116.43ab	20	117.02ab	20	117.19ab	20	0.005
	Spring 2013	120.34ab	20	120.33ab	20	121.80ab	16	124.36a	18	110.18c	20	114.23bc	20	<0.0005
	Fall 2013	122.02	20	122.87	20	121.36	13	127.12	15	-	-	-	-	0.200
	Spring 2012	83.17b	20	59.10a	21	-	-	-	-	74.70b	3	77.18b	20	<0.0005
PM	Fall 2012*	80.33bc	20	82.32abc	20	91.58a	20	88.12ab	20	76.96c	20	76.64c	20	<0.0005
	Spring 2013*	76.29abc	20	78.27abc	20	86.84c	18	82.33bc	19	71.72ab	20	68.61a	20	<0.0005
	Fall 2013*	79.61b	20	66.33a	20	89.79b	13	65.23a	16	-	-	-	-	<0.0005

III-4-2 Size weight relationship

Statistical approach

The statistical analysis was designed to answer two questions:

- What is the natural variability of the parameters across locations and years?
- What is the effect of caging?

The research project involves measurement of a large number of parameters on crabs taken in 4 locations, over 2 calendar years, in two seasons each year, and subjected to two treatments. Not all combinations of conditions were examined (for example, caged crabs were obtained only from two of the four locations).The target sample size was 20 crabs for each combination of conditions.

Since there are a large number of statistical tests and analyses, care should be taken when interpreting statistical significance (p-values) in order to prevent large number of false positives (type I errors) project-wise. While we used mitigating methods within single statistical analyses, a significance threshold at a 0.001 or less has to be set and results clearly inconsistent with a priori knowledge should not be given a high weight.

Analysis of relationships between continuous parameters (carapace width-body weight, carapace width-ovary weight, carapace width – fecundity and carapace size – hepatopancreas weight relationships) was examined for several pairs of parameters Y (the response variable) and X (the explanatory variable: carapace width).

Data preparation was carried out separately for each statistical analysis.
Records with missing values for the two parameters and the factors of interest (typically location, year, season, treatment) were removed from the analysis.

A robust linear regression of Trans(Y) on Trans(X) with the group as factor was carried out for the four possible combination of Trans = Identity or Log.
The following plots were compared:

- Residuals vs Trans(X) for trendlessness
- Normal QQ-plot of the residuals for normality
- Box-and-whiskers plot of the residuals vs the group for homogeneity of variance

The transformation combination giving the best acceptable compromise was retained (in most cases, Identity-Identity or Log-Log). Once the transformations were selected, a loess regression of Trans(Y) on Trans(X) was carried out. The residuals of that regression were analysed using the boxplot approach described for single variables. Observations giving

residuals approximately three times the interquartile range (IQR) from the 1st or 3rd quartile as appropriate were removed from the analysis.

Analysis of natural variability in free crabs

Linear regression of Trans(Y) on Trans(X) was carried out by group and the results were examined graphically and numerically for similarity of slope in order to choose between a single slope or a "split-slope" model.

A single-slope model was applicable in most cases; therefore the following applies mostly to that case. In a single-slope model, the Trans(Y) value for any single selected value of Trans(X) (e.g. the y-intercept, corresponding to Trans(X) = 0) describes the line location for each group. The linear regression Trans(Y) vs Trans(X) was computed and a box-and-whisker plot of residual vs group was created.

The following analyses were carried separately for each season:
The general approach was that of analysis of variance and covariance (ANCOVA) with statistical test applied to appropriate contrasts. Contrasts were tested using the methodology described in Differences between groups were tested using the methodology described in Hothorn *et al.* (2008).

An ANCOVA of Trans(Y) vs (Trans(X) with Location, Year and interaction Location xYear as factor was carried out. Differences between locations and between years were reported as percentage of the overall mean with a significance level. We also reported the result of a Levene-Brown-Forsythe test for homogeneity of variance test between groups.

We propose that the results be interpreted as follows.
In the rare cases where a single-slope model was not applicable, the relation between Trans(Y) and Trans(X) was weak. In these cases, further examination of Y is required independently of X.

For cases where a single-slope model was applicable, if the Location*Year interaction is statistically significant, the largest observed difference between groups should be taken as reference. If the Location*Year interaction is not statistically significant, then the largest difference between Years and between Locations can be examined, since it might be possible to modelled these differences. Finally, if the Levene-Brown-Forsythe test is highly significant, large difference is standard deviations should be explained.

The absence of statistically significant variability between the two years of observation or of statistically significant interaction for the two years of observation should not be taken as indicative of absence the same in the long term.

Analysis of the caging effect

Linear regression of Trans(Y) on Trans(X) was carried out by group and the results were examined graphically and numerically for similarity of slope in order to choose between a single slope or a "split-slope" model. A single-slope model was applicable in most cases; therefore the following applies mostly to that case. In a single-slope model, the Trans(Y) value for any single selected value of Trans(X) (e.g. the y-intercept, corresponding to Trans(X) = 0) describes the line location for each group. The linear regression 'Trans(Y) vs Trans(X)' was computed and a box-and-whisker plot of residual vs group was created.

An ANCOVA of Trans(Y) vs (Trans(X) with Location, YearSeason factor and Treatement nested was carried out.

Contrasts were tested for differences between locations, between seasons and finally, for differences between wild and free crabs at each season (the nested parameter).
Mean values of Trans(y) are also reported for free and caged crabs at each location and Year-Season.

In the rare cases where a single-slope model was not applicable, the relation between Trans(Y) and Trans(X) was weak. In these cases, further examination of Y is required independently of X. For cases where a single-slope model was applicable, we propose that the focus be on the contrasts between free and caged crabs. Unless the effect of caging is not significant, the difference observed is the most important parameter.

Carapace width-body weight relationship

The carapace width-weight relationship was established for location, season, year, categories and treatment (Tables 10, 11 & 12) in order to investigate the natural variability of the relationship and whether the relationship for caged crabs differs from that for free crabs with increasing immersion time.

Large males

Analysis of variance-covariance by location, year and year-location interaction in the spring showed that crab carapace size vs body-weight relationships were significantly different in terms of location (p=0.0000). Multiple comparison showed that the significant difference (p=0.0000) was observed between Louisbourg and Chaticamp, Louisbourg and Margaree Harbor as well as Grande-Rivière and Louisbourg (Table 13). In the fall, there was a significant difference (p=0.0000) in terms of location-year interaction. The observed difference was significant (p=0.00033) between Grande-Rivière fall 2012 and Margaree

Harbor fall 2012 (Table 14). There was no clear seasonal or annual tendancy in regression residual (Figure 11).
Comparison between caged and free crabs (Table 15), multiple comparison showed that there was no significant difference in terms of location, year/season and year/season-treatment (p= 0.28789, 0.04526 and 0.02392, respectively). In terms of treatment effect (caged vs free), there was no significant difference (p=0.0000) for all three caging duration (2 weeks, 6 months and 12 months) (Table 13). The regression residuals for caged large males continuously decreased at both stations with the duration of immersion (Figure 12).

Pygmy males

Analysis of variance-covariance by location, year and year-location interaction in the spring showed that crab carapace size vs body-weight relationships were significantly different in terms of location (p=0.00066)(Table 16) in the spring. In the fall, there was also a significant difference (at p=0.00009) for location. The observed difference was significant (p=0.00035) between Louisbourg and Cheticamp (p=0.00009) (Table 17). There was no clear seasonal or annual tendancy in regression residuals (Figure 13).

Comparison between caged and free crabs (Table 18 and Figure 14), multiple comparison showed that there was no significant difference in terms of location (p=0.43318), year/season (p=0.33539) and year/season-treatment (p=0.64991).

Mature females

Analysis of variance-covariance by location, year and year-location interaction in the spring showed that crab carapace size vs body-weight relationships were significantly different in terms of location and year/location interaction (p=0.00000 and p=0.00012)(Table 19). Multiple comparison showed that a significant difference (p=0.0000) was observed between Grande-Rivière in the spring 2012 and Louisbourg in the spring 2013 (Table 19). In the fall, there was significant difference for location and year (p=0.00035 and p=0.00000, respectively). The observed difference between Margaree Harbor in the fall 2012 and Cheticamp in the fall 2013 was significant (p=0.00000) (Table 20 & Figure 15).
Comparison between caged and free crabs, multiple comparison showed that there was significant difference in terms of year/season (p=0.0000) (Table 21). In terms of treatment effect (caged vs free), there was no significant difference for 2 week caged, 6 months or 12 month caged females vs free (p=0.46409, p=0.99252, and p=0.01671, respectively)(Table 21 & Figure 16).

Table 10. Regression coefficients (for log transformed variables) by location, season, and year for large male crabs.

Location	Year	Season	Treatment	n	y-intercept	slope
Margaree	2012	Spring	Free	18	-7.46	2.94
Margaree	2012	Fall	Free	20	-7.67	2.99
Margaree	2012	Fall	Caged	18	-7.34	2.91
Margaree	2013	Spring	Free	20	-6.69	2.78
Margaree	2013	Spring	Caged	16	-8.84	3.23
Margaree	2013	Fall	Free	20	-7.77	3.00
Margaree	2013	Fall	Caged	9	-6.24	2.69
Cheticamp	2012	Spring	Free	20	-7.64	2.97
Cheticamp	2012	Fall	Free	20	-7.14	2.87
Cheticamp	2012	Fall	Caged	20	-8.17	3.08
Cheticamp	2013	Spring	Free	20	-8.11	3.08
Cheticamp	2013	Spring	Caged	18	-8.23	3.10
Cheticamp	2013	Fall	Free	20	-6.22	2.68
Cheticamp	2013	Fall	Caged	15	-5.29	2.49
Louisbourg	2012	Spring	Free	20	-8.74	3.19
Louisbourg	2012	Fall	Free	20	-6.99	2.83
Louisbourg	2013	Spring	Free	20	-8.54	3.15
Grande-Riviere	2012	Spring	Free	21	-7.57	2.96
Grande-Riviere	2012	Fall	Free	20	-9.52	3.36
Grande-Riviere	2013	Spring	Free	20	-8.89	3.23

Table 11. Regression coefficients (for log transformed variables) by location, season, and year for pygmy male crabs.

Location	Year	Season	Treatment	n	y-intercept	slope
Cheticamp	2012	Spring	Free	21	-8.92	3.27
Cheticamp	2012	Fall	Free	20	-6.64	2.74
Cheticamp	2012	Fall	Caged	16	-5.44	2.47
Cheticamp	2013	Spring	Free	20	-8.22	3.09
Cheticamp	2013	Spring	Caged	19	-8.03	3.05
Cheticamp	2013	Fall	Free	20	-7.43	2.90
Cheticamp	2013	Fall	Caged	16	-8.46	3.14
Margaree	2012	Spring	Free	20	-8.92	3.24
Margaree	2012	Fall	Free	19	-7.12	2.84
Margaree	2012	Fall	Caged	20	-6.17	2.62
Margaree	2013	Spring	Free	20	-7.74	2.98
Margaree	2013	Spring	Caged	18	-7.91	3.03
Margaree	2013	Fall	Free	20	-7.55	2.94
Margaree	2013	Fall	Caged	13	-7.47	2.92
Louisbourg	2012	Spring	Free	20	-7.28	2.86
Louisbourg	2012	Fall	Free	20	-6.61	2.70
Louisbourg	2013	Spring	Free	20	-7.41	2.89
Grande.Riviere	2012	Spring	Free	3	-10.33	3.57
Grande.Riviere	2012	Fall	Free	17	-7.93	3.02
Grande.Riviere	2013	Spring	Free	20	-8.06	3.06

Table 12. Regression coefficients (for log transformed variables) by location, season, and year for mature female crabs.

Location	Year	Season	Treatment	n	y-intercept	slope
Cheticamp	2012	Spring	Free	19	-7.20	2.83
Cheticamp	2012	Fall	Free	20	-7.11	2.81
Cheticamp	2012	Fall	Caged	20	-6.52	2.67
Cheticamp	2013	Spring	Free	20	-6.87	2.75
Cheticamp	2013	Spring	Caged	19	-5.31	2.38
Cheticamp	2013	Fall	Free	20	-6.42	2.63
Cheticamp	2013	Fall	Caged	15	-4.99	2.29
Margaree	2012	Spring	Free	20	-6.99	2.77
Margaree	2012	Fall	Free	20	-7.10	2.81
Margaree	2012	Fall	Caged	20	-6.62	2.69
Margaree	2013	Spring	Free	20	-6.66	2.70
Margaree	2013	Spring	Caged	19	-6.38	2.64
Margaree	2013	Fall	Free	19	-6.68	2.70
Margaree	2013	Fall	Caged	15	-6.39	2.64
Louisbourg	2012	Spring	Free	24	-6.32	2.60
Louisbourg	2012	Fall	Free	40	-7.26	2.84
Louisbourg	2013	Spring	Free	5	-6.06	2.54
Grande.Riviere	2012	Spring	Free	20	-7.71	2.97
Grande.Riviere	2012	Fall	Free	21	-6.93	2.75
Grande.Riviere	2013	Spring	Free	20	-7.83	2.99

Table 13. Spatial and temporal variability of the carapace width-body weight relationship for free mature male crabs in spring (Cheticamp, Margaree Harbor, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-8.11	3.06
Cheticamp	2013	20	-8.07	3.06
Margaree	2012	21	-8.04	3.06
Margaree	2013	20	-8.03	3.06
Louisbourg	2012	20	-8.03	3.06
Louisbourg	2013	20	-8.10	3.06
Grande.Riviere	2012	20	-8.06	3.06
Grande.Riviere	2013	18	-8.05	3.06

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	8.89		8.89	4647	0.00000	***
Location	3	0.11	25.5	0.04	18	0.00000	***
Year	1	0.00	0.1	0.00	0	0.73119	
Location:Year	3	0.02	5.4	0.01	4	0.01004	*
Residuals	150	0.29	69.1	0.00			

Observed difference(s) (Multiple comparison). These differences show the effects of natural variability.

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	1.01	0.0053	0.0102	0.081	0.98191	
Location: Louisbourg - Cheticamp	0.94	-0.0592	0.0101	-0.909	0.00000	***
Location: Grande.Riviere - Cheticamp	0.99	-0.0055	0.0106	-0.085	0.98105	
Location: Louisbourg - Margaree	0.94	-0.0645	0.0104	-0.990	0.00000	***
Location: Grande.Riviere - Margaree	0.99	-0.0108	0.0109	-0.166	0.82994	
Location: Grande.Riviere - Louisbourg	1.06	0.0537	0.0102	0.824	0.00000	***
Year: 2013 - 2012	1.00	0.0024	0.0072	0.037	0.99656	

Homogeneity of variance test (Levene-Brown-Forsythe) for Weight : p.value = 0.0109

Mean, median and standard deviations for residuals of log Weight on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Male Spring 2012 Free	20	0.0021	0.0002	0.0388
Cheticamp Male Spring 2013 Free	20	0.0268	0.0337	0.0401
Grande.Riviere Male Spring 2012 Free	21	0.0257	0.0274	0.0415
Grande.Riviere Male Spring 2013 Free	20	-0.0050	-0.0110	0.0469
Louisbourg Male Spring 2012 Free	20	-0.0421	-0.0427	0.0578
Louisbourg Male Spring 2013 Free	20	-0.0460	-0.0445	0.0350
Margaree Male Spring 2012 Free	18	0.0067	0.0083	0.0277
Margaree Male Spring 2013 Free	20	0.0311	0.0386	0.0533

Table 14. Spatial and temporal variability of the carapace width-body weight relationship for free male crabs in fall (Cheticamp, Margaree Harbor, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-7.44	2.93
Cheticamp	2013	20	-7.45	2.93
Margaree	2012	20	-7.37	2.93
Margaree	2013	20	-7.45	2.93
Louisbourg	2012	20	-7.40	2.93
Grande.Riviere	2012	20	-7.43	2.93

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	4.58		4.58	1665	0.00000	***
Location	3	0.04	10.3	0.01	5	0.00315	**
Year	1	0.00	0.7	0.00	1	0.32819	
Location:Year	1	0.04	9.8	0.04	14	0.00030	***
Residuals	113	0.31	79.3	0.00			

Location-Year interaction is important - The largest between-group difference is reported

Observed difference(s) (Multiple comparison)
These differences show the effects of natural variability.

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Gr12FallFr - Ma12FallFr	0.929	-0.0732	0.0167	-1.11	0.00033	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Weight: p.value = 0.7740
Mean, median and standard deviations for residuals of log Weight on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Male Fall 2012 Free	20	-0.0110	-0.0081	0.0493
Cheticamp Male Fall 2013 Free	20	0.0190	0.0298	0.0484
Grande.Riviere Male Autumn 2012 Free	20	-0.0241	-0.0232	0.0459
Louisbourg Male Autumn 2012 Free	20	-0.0237	-0.0198	0.0662
Margaree Male Autumn 2012 Free	20	0.0476	0.0483	0.0453
Margaree Male Autumn 2013 Free	20	-0.0078	-0.0031	0.0561

Table 15. Comparison of caged and free large male crabs (Cheticamp, Margaree Harbor) for the carapace width-body weight relationship.

Location	YearSeason	y-intercept	slope	treatment.effect (Caged)	treatment.effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Fall	-7.47	2.94	-0.0006	-0.009	0.96949	0.39636	6.48	6.50
Cheticamp	2013Spr	-8.16	3.09	0.0045	0.067	0.79068	0.93193	6.62	6.73
Cheticamp	2013Fall	-5.92	2.62	-0.0096	-0.144	0.59791	0.52897	6.69	6.77
Margaree	2012Fall	-7.43	2.94	-0.0772	-1.160	0.00038	0.80451	6.65	6.71
Margaree	2013Spr	-7.58	2.97	-0.0045	-0.068	0.83308	0.31702	6.63	6.66
Margaree	2013Fall	-7.41	2.93	0.0554	0.835	0.02196	0.46898	6.64	6.68

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	7.35		7.35	2370	0.00000	***
Location	1	0.00	0.5	0.00	1	0.28789	
YearSeason	2	0.02	2.8	0.01	3	0.04526	*
YearSeason:Treatment	3	0.03	4.3	0.01	3	0.02392	*
Residuals	208	0.64	92.4	0.00			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	1.01	0.00838	0.00764	0.127	0.27407	

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr – 2012Fall	1.00	0.00237	0.0125	0.0358	0.98039	
2013Fall – 2012Fall	0.99	-0.00955	0.0127	-0.1443	0.73101	
2013Fall - 2013Spr	0.99	-0.01192	0.0125	-0.1801	0.60667	

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012Fall 2w	0.96	-0.0374	0.0127	-0.565	0.01081	*
Caged vs Free: 2013Spr 6m	1.00	0.0038	0.0131	0.058	0.98763	
Caged vs Free: 2013Fall 12m	1.01	0.0130	0.0145	0.196	0.75102	

Table 16. Spatial and temporal variability of the carapace width-body weight relationship for free pygmy male crabs in spring (Cheticamp, Margaree Harbor, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	21	-8.22	3.09
Cheticamp	2013	20	-8.26	3.09
Margaree	2012	20	-8.22	3.09
Margaree	2013	20	-8.20	3.09
Louisbourg	2012	20	-8.23	3.09
Louisbourg	2013	20	-8.25	3.09
Grande.Riviere	2012	20	-8.29	3.09
Grande.Riviere	2013	3	-8.26	3.09

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	27.4		27.4	6510	0.00000	***
Location	3	0.1	11.2	0.0	6	0.00066	***
Year	1	0.0	3.1	0.0	5	0.02728	*
Location:Year	3	0.0	2.5	0.0	1	0.26349	
Residuals	135	0.6	83.2	0.0			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	0.99	-0.0103	0.0162	-0.21	0.96072	
Location: Louisbourg - Cheticamp	0.95	-0.0461	0.0149	-0.92	0.01478	*
Location: Grande.Riviere - Cheticamp	1.01	0.0113	0.0179	0.23	0.96108	
Location: Louisbourg - Margaree	0.96	-0.0359	0.0150	-0.72	0.09789	.
Location: Grande.Riviere - Margaree	1.02	0.0216	0.0179	0.43	0.70360	
Location: Grande.Riviere - Louisbourg	1.06	0.0575	0.0175	1.15	0.00841	**
Year: 2013 - 2012	1.03	0.0252	0.0114	0.51	0.14386	

Homogeneity of variance test (Levene-Brown-Forsythe) for Weight: p.value = 0.0922

Mean, median and standard deviations for residuals of log Weight on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Pygmy male Spring 2012 Free	21	0.0101	0.0122	0.0760
Cheticamp Pygmy male Spring 2013 Free	20	0.0124	0.0246	0.0639
Grande.Riviere Pygmy male Spring 2012 Free	3	-0.0225	-0.0469	0.0588
Grande.Riviere Pygmy male Spring 2013 Free	20	0.0415	0.0456	0.0585
Louisbourg Pygmy male Spring 2012 Free	20	-0.0507	-0.0644	0.0502
Louisbourg Pygmy male Spring 2013 Free	20	-0.0167	-0.0315	0.0575
Margaree Pygmy male Spring 2012 Free	20	-0.0119	-0.0046	0.0743
Margaree Pygmy male Spring 2013 Free	20	0.0182	0.0207	0.0702

Table 17. Spatial and temporal variability of the carapace width-body weight relationship for free pygmy male crabs in the fall (Cheticamp, Margaree Harbor, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-7.25	2.86
Cheticamp	2013	19	-7.23	2.86
Margaree	2012	20	-7.30	2.86
Margaree	2013	20	-7.23	2.86
Louisbourg	2012	17	-7.24	2.86
Grande.Riviere	2012	20	-7.20	2.86

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	12.2		12.2	3201	0.00000	***
Location	3	0.1	15.7	0.0	7	0.00024	***
Year	1	0.0	1.0	0.0	1	0.25609	
Location:Year	1	0.0	2.2	0.0	3	0.09016	.
Residuals	109	0.4	81.2	0.0			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	0.99	-0.0122	0.0146	-0.24	0.88791	
Location: Louisbourg - Cheticamp	0.92	-0.0839	0.0185	-1.63	0.00009	***
Location: Grande.Riviere - Cheticamp	0.98	-0.0217	0.0197	-0.42	0.75004	
Location: Louisbourg - Margaree	0.93	-0.0717	0.0195	-1.39	0.00241	**
Location: Grande.Riviere - Margaree	0.99	-0.0094	0.0209	-0.18	0.98697	
Location: Grande.Riviere - Louisbourg	1.06	0.0622	0.0206	1.21	0.01901	*
Year: 2013 - 2012	0.98	-0.0177	0.0156	-0.34	0.73152	

Homogeneity of variance test (Levene-Brown-Forsythe) for Weight: p.value = 0.0130
Mean, median and standard deviations for residuals of log Weight on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Pygmy male Autumn 2012 Free	20	0.0369	0.0401	0.0574
Cheticamp Pygmy male Autumn 2013 Free	20	0.0020	-0.0024	0.0703
Grande.Riviere Pygmy male Autumn 2012 Free	17	0.0067	0.0024	0.0525
Louisbourg Pygmy male Autumn 2012 Free	20	-0.0561	-0.0606	0.0544
Margaree Pygmy male Autumn 2012 Free	19	0.0038	-0.0101	0.0685
Margaree Pygmy male Autumn 2013 Free	20	0.0078	0.0152	0.0645

Table 18. Comparison of caged and free pygmy male crabs (Cheticamp, Margaree Harbor) for the carapace width-body weight relationship.

Location	YearSeason	y-intercept	slope	treatment.effect (Caged)	treatment.effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Aut	-6.13	2.62	0.0381	0.703	0.13607	0.58836	5.43	5.63
Cheticamp	2013Aut	-7.86	3.01	-0.0444	-0.933	0.15988	0.47566	4.76	4.78
Cheticamp	2013Spr	-8.13	3.07	0.0083	0.163	0.74173	0.83586	5.12	5.31
Margaree	2012Aut	-6.90	2.78	0.0038	0.072	0.88475	0.43806	5.28	5.59
Margaree	2013Aut	-7.52	2.93	0.0237	0.451	0.38771	0.96055	5.26	5.53
Margaree	2013Spr	-7.87	3.01	0.0164	0.321	0.56766	0.86489	5.12	5.48

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	34.3		34.3	5906	0.00000	***
Location	1	0.0	0.3	0.0	1	0.43318	
YearSeason	2	0.0	1.0	0.0	1	0.33539	
YearSeason:Treatment	3	0.0	0.8	0.0	1	0.64991	
Residuals	213	1.2	98.0	0.0			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	0.992	-0.00781	0.0107	-0.151	0.46737	

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr - 2012Aut	0.999	-0.000959	0.0176	-0.0186	0.99836	
2013Aut - 2012Aut	0.994	-0.006264	0.0180	-0.1214	0.93530	
2013Aut - 2013Spr	0.995	-0.005305	0.0171	-0.1028	0.94840	

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012Aut 2w	0.99	-0.0076	0.0181	-0.148	0.96487	
Caged vs Free: 2013Spr 6m	1.01	0.0136	0.0179	0.263	0.83124	
Caged vs Free: 2013Aut 12m	0.98	-0.0171	0.0187	-0.331	0.73863	

Table 19. Spatial and temporal variability of the carapace width-body weight relationship for free female crabs in spring (Cheticamp, Margaree Harbor, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	24	-6.99	2.76
Cheticamp	2013	20	-6.90	2.76
Margaree	2012	20	-6.90	2.76
Margaree	2013	5	-7.00	2.76
Louisbourg	2012	20	-6.95	2.76
Louisbourg	2013	20	-6.83	2.76
Grande.Riviere	2012	19	-6.92	2.76
Grande.Riviere	2013	20	-6.93	2.76

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	8.24		8.24	3581	0.00000	***
Location	3	0.29	43.8	0.10	42	0.00000	***
Year	1	0.00	0.4	0.00	1	0.28200	
Location:Year	3	0.05	7.7	0.02	7	0.00012	***
Residuals	139	0.32	48.1	0.00			

Observed difference(s) (Multiple comparison). These differences show the effects of natural variability.

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Gr12SprFr - Lo13SprFr	1.19	0.170	0.0240	3.56	0.00000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Weight: p.value = 0.2395

Mean, median and standard deviations for residuals of log Weight on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Spring 2012 Free	19	0.0069	0.003	0.0288
Cheticamp Female Spring 2013 Free	20	0.0177	0.015	0.0531
Grande.Riviere Female Spring 2012 Free	20	0.0916	0.115	0.0478
Grande.Riviere Female Spring 2013 Free	20	0.0218	0.022	0.0476
Louisbourg Female Spring 2012 Free	24	-0.0674	-0.072	0.0496
Louisbourg Female Spring 2013 Free	5	-0.0784	-0.095	0.0556
Margaree Female Spring 2012 Free	20	-0.0315	-0.025	0.0473
Margaree Female Spring 2013 Free	20	-0.0057	-0.007	0.0534

Table 20. Spatial and temporal variability of the carapace width-body weight relationship for free female crabs in fall (Cheticamp, Margaree Harbor, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-6.83	2.72
Cheticamp	2013	21	-6.80	2.72
Margaree	2012	20	-6.75	2.72
Margaree	2013	19	-6.77	2.72
Louisbourg	2012	20	-6.74	2.72
Grande.Riviere	2012	40	-6.78	2.72

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	9.07		9.07	5175	0.00000	***
Location	3	0.03	9.8	0.01	7	0.00035	***
Year	1	0.07	20.0	0.07	40	0.00000	***
Location:Year	1	0.01	3.7	0.01	7	0.00737	**
Residuals	133	0.23	66.4	0.00			

Observed difference(s) (Multiple comparison). These differences show the effects of natural variability.

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Ma12FallFr - Ch13FallFr	1.10	0.0956	0.0134	2.00	0.00000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Weight : p.value = 0.0008
Mean, median and standard deviations for residuals of log Weight on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Fall 2012 Free	20	0.0301	0.0400	0.0413
Cheticamp Female Fall 2013 Free	20	-0.0526	-0.0501	0.0530
Grande.Riviere Female Fall 2012 Free	21	-0.0188	-0.0058	0.0438
Louisbourg Female Fall 2012 Free	40	-0.0020	-0.0032	0.0335
Margaree Female Fall 2012 Free	20	0.0405	0.0395	0.0396
Margaree Female Fall 2013 Free	19	0.0061	0.0105	0.0463

Table 21. Comparison of caged and free female crabs (Cheticamp, Margaree Harbor) for the carapace width-body weight relationship.

Regression parameters by group

Location	YearSeason	y-intercept	slope	treatment.effect (Caged)	treatment.effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Fall	-6.89	2.76	-0.0050	-0.104	0.75735	0.49602	4.85	4.78
Cheticamp	2013Fall	-6.11	2.55	0.0327	0.713	0.08387	0.06500	4.58	4.72
Cheticamp	2013Spr	-5.98	2.54	-0.0134	-0.280	0.46407	0.01541	4.77	4.83
Margaree	2012Fall	-6.79	2.74	-0.0231	-0.482	0.07065	0.48037	4.80	4.77
Margaree	2013Fall	-6.56	2.67	0.0401	0.845	0.01853	0.77608	4.74	4.92
Margaree	2013Spr	-6.53	2.67	0.0222	0.465	0.19286	0.79298	4.78	4.80

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	14.5		14.5	5296	0.00000	***
Location	1	0.0	3.1	0.0	8	0.00481	**
YearSeason	2	0.1	9.0	0.0	12	0.00002	***
YearSeason:Treatment	3	0.0	3.7	0.0	3	0.02440	*
Residuals	219	0.6	84.2	0.0			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	1.02	0.0196	0.00695	0.413	0.00514	**

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr – 2012Fall	0.979	-0.0208	0.0117	-0.437	0.18028	
2013Fall – 2012Fall	0.938	-0.0642	0.0119	-1.350	0.00000	***
2013Fall- 2013Spr	0.957	-0.0434	0.0118	-0.913	0.00084	***

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012 Fall 2w	0.98	-0.0154	0.0117	-0.325	0.46409	
Caged vs Free: 2013 Spr 6m	1.00	0.0029	0.0119	0.062	0.99252	
Caged vs Free: 2013 Fall 12m	1.04	0.0359	0.0128	0.754	0.01671	*

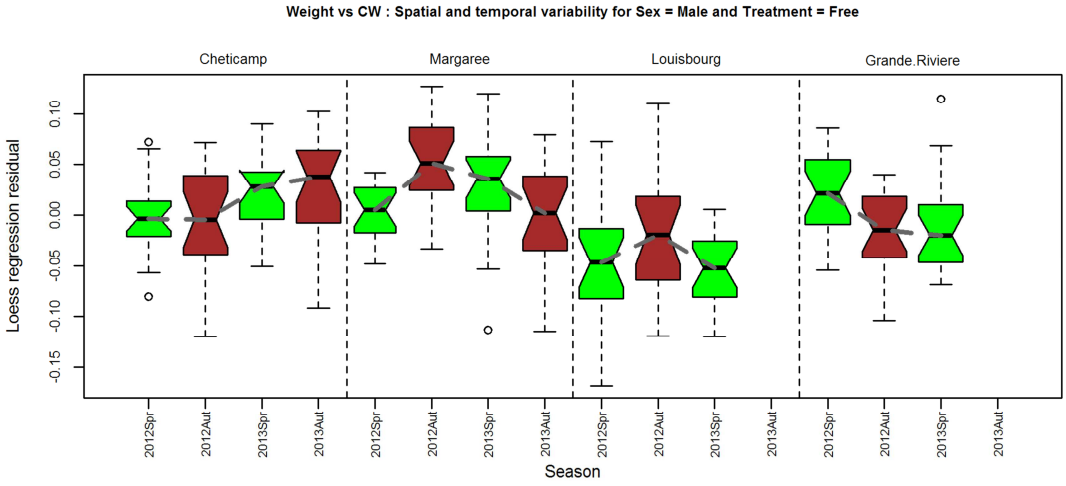

Figure 11. Spatial and temporal variability in the carapace width vs body weight relationships in large male snow crab in Grande-Rivière, Margaree Harbor, Cheticamp and Louisbourg stations.

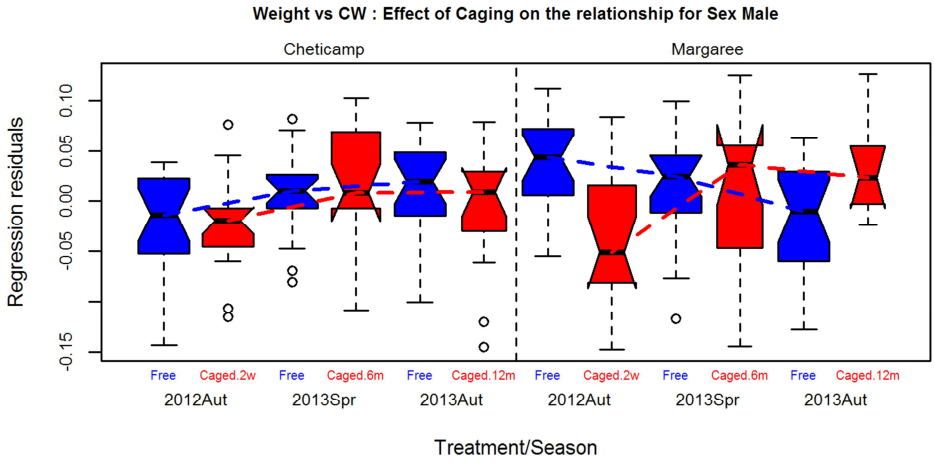

Figure 12. Effect of caging on the carapace width vs body weight relationships in large male snow crab in Margaree Harbor and Cheticamp stations.

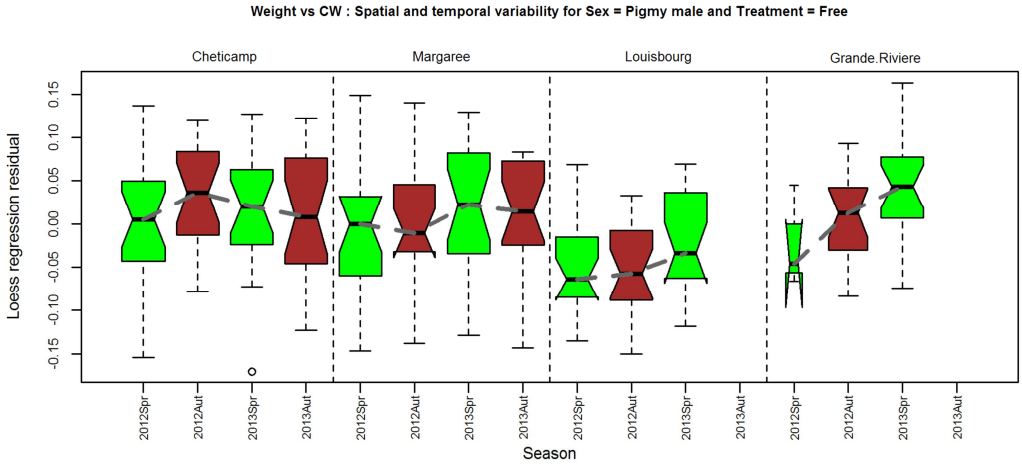

Figure 13. Spatial and temporal variability in the carapace width vs body weight relationships in pygmy male snow crab in Grande-Rivière, Margaree Harbor, Cheticamp and Louisbourg stations.

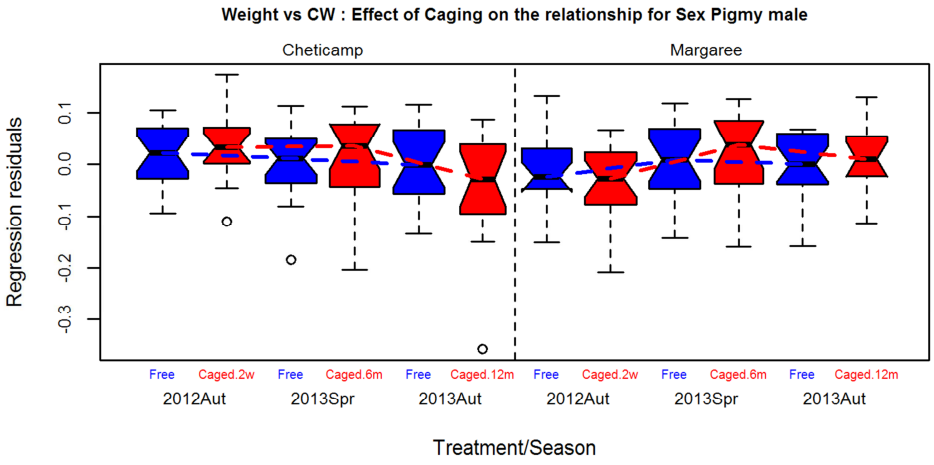

Figure 14. Effect of caging on the carapace width vs body weight relationships in pygmy male snow crab in Margaree Harbor and Cheticamp stations.

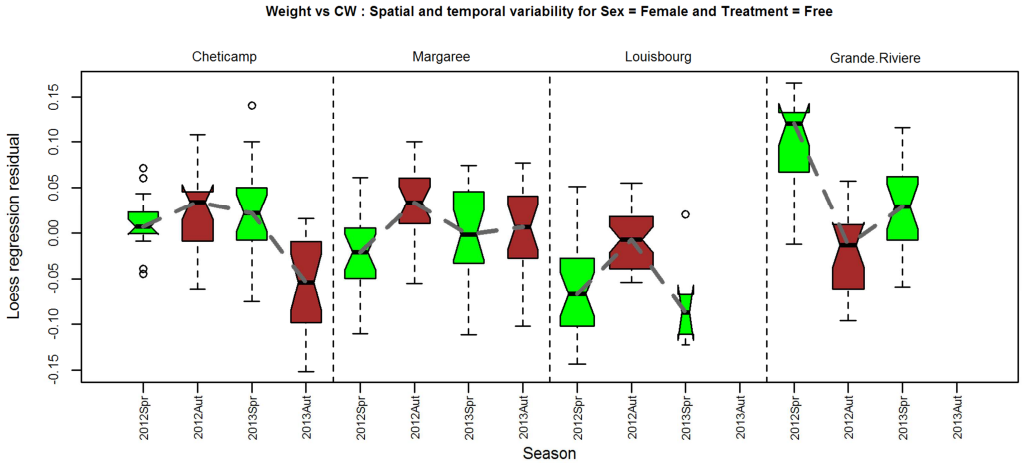

Figure 15. Spatial and temporal variability in the carapace width vs body weight relationships in mature female snow crab in Grande-Rivière, Margaree Harbor, Cheticamp and Louisbourg stations.

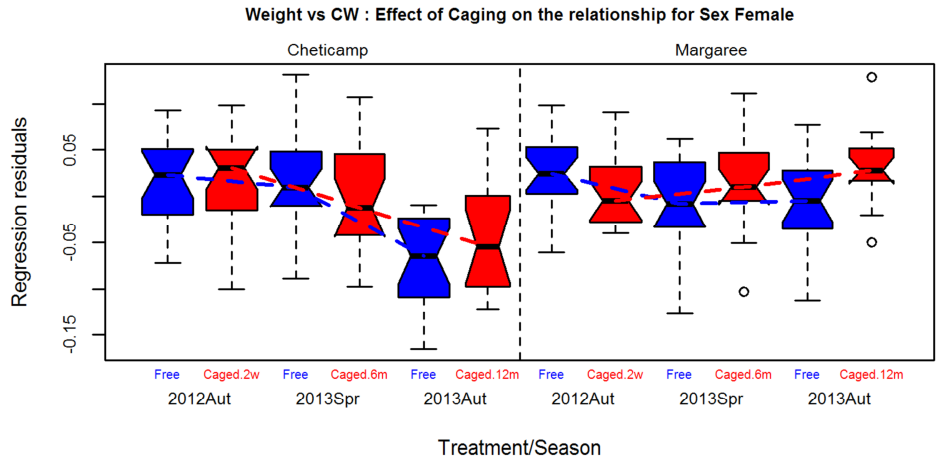

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Figure 16. Effect of caging on the carapace width vs body weight relationships in mature female snow crab in Margaree Harbor and Cheticamp stations.

III-4-3 Mortality

There was no significant difference in crab mortality between caging sites (p = 0.4027); however, crab mortality significantly increased with the length of the caging period (from 2 weeks, 6 months and 12 months) at both caging sites and for all three crab categories (Table 22). Furthermore, mature males had significantly higher mortalities than mature females and pygmy males (Table 22).

Table 22. Percentage (%) of mortality for each crab category (large males, pygmy males and mature females) between caged and free crabs in Cheticamp and Margaree Harbor stations for fall 2012 (free vs 2 week caged), spring 2013 (free vs 6-month caged), fall 2013 (free vs 12-month caged).

Large Males:
Proportion (%) of dead crab

Site	Fall 2012	Spring 2013	Fall 2013
Margaree Harbour	10	30	52.6
Cheticamp	0	10	25.0

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Location	6.5374	117	1	115.811	0.015630
YearSeason	15.5575	115	2	100.253	0.000418
Location: YearSeason	1.0317	113	2	99.222	0.59697

Model: binomial

Pygmy males:
Proportion (%) of dead crab

Site	Fall 2012	Spring 2013	Fall 2013
Margaree Harbour	0	0	35
Cheticamp	0	5	20

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Location	0.3358	117	1	77.472	0.5622
YearSeason	21.5150	115	2	55.957	2.128e-05
Location: YearSeason	2.1028	113	2	53.855	0.3494

Model: binomial

Mature Females:
Proportion (%) of dead crab

Site	Fall 2012	Spring 2013	Fall 2013
Margaree Harbour	0	0	10
Cheticamp	0	5	20

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Location	1.3556	117	1	51.889	0.2443
YearSeason	10.2718	115	2	41.618	0.00588
Location: YearSeason	0.6575	113	2	40.960	0.7198

Model: binomial

Summary of the fitted logistic modelshowing that the mortality in large male is significantly different from mature females and pygmy males.

Parameters	Estimate	std. error	z-value	Pr(>|z|)
(intercept)	-5.0539	0.8163	-6.191	5.98e-10
YearSeason 2013 spring	1.7084	0.7908	2.160	0.030749
YearSeason 2013 Fall	3.2004	0.7506	4.264	2.01e-05
Large male	1.6493	0.4757	3.467	0.000526
Pigmy male	0.6262	0.5130	1.221	0.222226

III-5 REFERENCES

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III-6 APPENDICES

Appendix A Tissue Fixative (Davidson's Fixative)

Davidson't fixative is a rapid fixative which gives good nuclear detail. Specimen exposure to Davidson's should be limited to 24-48 hours (tissues should be transferred to 70% ethanol for storage).
Storage:

- Davidson's fixative may be stored at room temperature in plastic or glass containers for an indefinite time.

Recipe (for 4L of fixative)

Stock solution:

- Glycerin 400 ml
- Formaldehyde 37% 800 ml
- Ethanol 95% 1200 ml
- Seawater (30g/L(1200 ml

- Add in order given, stirring well. Store at room temperature. Shake well before use.

- **Just before use:** add 1 part acetic acid to 9 part stock solution (400 ml of acetic acid for 3600 ml of stock solution)

- After fixation (24-48 hours), rinse in 70% ethanol for two hours (agitate specimen). Wash at least once and store in 70% ethanol.

IV. OBSERVATION OF APPENDAGES

IV-1 GILLS

IV-1-1 Introduction

The respiratory system in the snow crab is composed of a set of eight gills on each side of the cephalothorax in the branchial chamber. The two most anterior gills are very small in size compared to the remaining 6 pairs (Figure 1). Seven pairs of gills are of the phyllobranch type: (composed of a central axis (lamellar septum) bearing an anterior and posterior row of closely set and regularly spaced leaf-like lamellae) while the most anterior gill (arthrobranch) has only a posterior row of lamellae as described for blue crab (Kennedy & Cronin 2007).

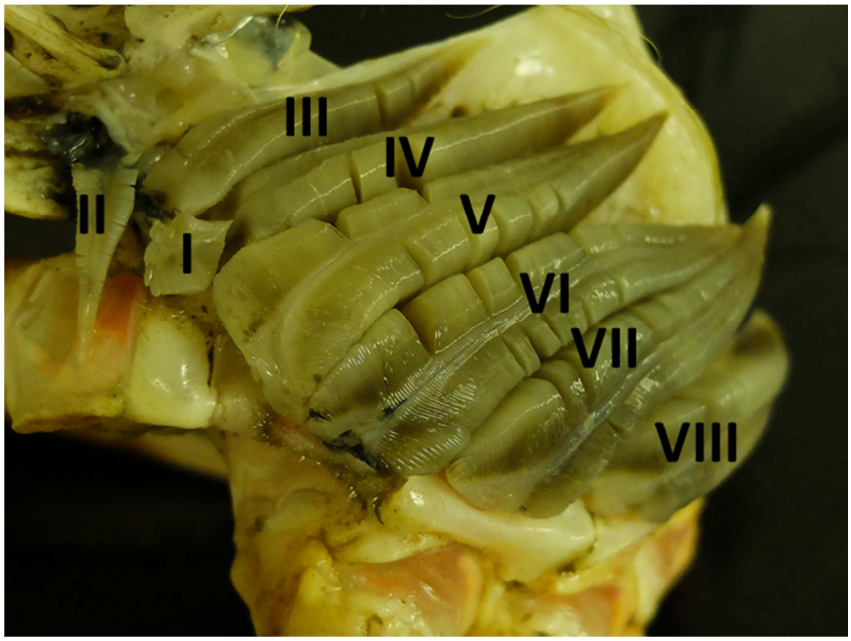

Figure 1. Gills from the left side of a snow crab (carapace removed) showing the arrangement of the 8 gill lamellae.

The number of pairs of gill varies within Brachuyran species e.g. 9 pairs for *Calappa* sp (Schoffeniels & Dandrifosse 1994), *Cancer pagurus* (Pearson 1908 in Schoffeniels & Dandrifosse 1994), *C. maenus* (Schoffeniels & Dandrifosse 1994), *Grapsus grapsus* (Schoffeniels & Dandrifosse 1994), 8 for *Callinectes sapidus* (Kennedy & Cronin 2007), 7 for *Ocypode* sp. (Schoffeniels & Dandrifosse 1994), and *Pinnotheres pisum* (Schoffeniels & Dandrifosse 1994). Water enters the branchial chamber through the inhalant apertures at the base of the pereopods and flows into the hypobranchial space under the gills, up between the lamellae to the epibranchial space and out through the exhalant aperture on each side of the epistome

(Kennedy & Cronin 2007). An epipodite on the first maxipilled is located above the gills on each side contributing to gill maintenance and cleaning (Figure 2).

Figure 2. Snow crab gills showing two sets of gill lamellae and the position of epipodites (ep) on the first maxipilled. The epipodites are located above the gills on each side and contribute to gill maintenance.

Drach (1930) described in details gill morphology and general histology of decapods.
Gills play respiratory (gas exchange) and homeostatic (ionic exchanges) roles between the hemolymph and the medium across a single epithelial and circular layer. As each gill lamella is regularly cleaned by the epipodite of the 1st maxilliped and enclosed in the cepharothorax, gill lamellae are usually free of dirt accumulation (except for some very old animals such as senile females). Therefore, the prevalence of dirty gill lamellae filled with heavily compacted sediment observed in the 2003 study had to be caused by exceptional circumstances. It is reasonable to hypothesize that gills under such conditions may significantly reduce the capability to maintain respiratory, osmotic, excretory and acid-base homeostasis possibly resulting in subsequent impacts on other organs such as antennal gland (also called urinary or green gland) and haemolymph (haemolymph solutes, serum proteins, serum enzymes and various haemocytes). In addition, a reduction in space among gill lamellae (due to the accumulation of foreign substances related to seismic noise) or a reduction in gill cleaning activities (possibly due to caging) may influence the composition and abundance of gill fouling species.

IV-1-2 Sample treatment

Gills #3-8 from the left side of each snow crab were dissected out and preserved in 10% formalin solution in 100 ml sample bag (the 1st and 2nd gills were often too small and difficult to be dissected out from smaller crabs). Gills were dissected out from 1,132 individuals and the majority of samples consisted of 6 gills (92.4%). The remaining samples had 4 gills (0.35%), 5 gills (3.4%), 7 gills (1.2%), or 8 gills (0.9%). Since 20 samples had missing information in regards to the number of gills collected, the average of 6 gills was applied to these samples when estimating gill fouling species abundances. For each sample, the largest gill i.e, the 5th or the 6th, was chosen and photographed with a digital camera (PixeLink PL-B686CU, Canimpex Enterprises Ltd) under a dissection microscope (Mz12.5, Leica) with a magnification of 8X.

The condition of gill (degree of dirtiness among gill lamellae) was classified into four categories: 1) clean (with no organic and inorganic substances), 2) relatively clean, clean with some organic and/or inorganic substances, 3) medium (abundant organic and/or inorganic substances), and 4) very dirty (filled with compacted sediment-like substance, identified 'dirty' in Moriyasu *et al.*, unpublished).

Gills and content of each sample bag and was transferred into 200cc beaker with 70% ethanol and stirred with magnetized stirring bar for 60 minutes at a speed of 350rpm. After stirring, the gills were pulled out and the content was poured into a 200 cc Erlenmeyer flask through a 70 µ filter (Falcon™ Cell Strainers, Corning Life Sciences). The filter was washed with 70% alcohol. The fouling species were sorted by species according to the preliminary determined species/group list (limited to 10 most encountered species) and counted in a 100 mm x 100 mm x 15 mm square dish with 6 x 6 grids under a stereo microscope with a magnification at x32. The optimum duration of sample stirring for 60 minutes was determined by pre-study tests for 15, 30, 45 and 60 minutes of stirring. For each set of stirring duration, the gills were put back into 200cc beaker with 70% ethanol and the remaining fouling species were counted. This pre-test showed that no more fouling species were found after 4 mixings of 15 minutes each. Throughout the study, additional unknown species occurred at low abundance but were not considered for abundance study.

The 10 groups of pre-sorted specimens preserved in 70% ethanol were transferred to glycerin for dissection (when necessary) and mounted on glass slides. Detailed illustrations were made using a camera lucida (drawing tube).

This present study also collected the 6th or 7th gill from the right side of every 5th crab sample for scanning electron microscopy (SEM). The gill was fixed with 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 for 1 hour and rinsed in cold 0.2M cacodylate buffer at pH 7.2. Dehydration was achieved through increasing concentrations (up to 100%) of ethanol within 15

minutes. The samples were then dried in a critical point dryer (CPD) at the critical point of CO_2. The posterior portion of gill lamella was cut at a length of 5-10mm and mounted on aluminum stubs with double side adhesive tabs, was sputter-coated with gold-palladium and examined with a JEOL 6400SEM at 10 kV acceleration voltage at the Microscopy and Microanalysis Facility, University of New Brunswick (UNB).

IV-1-3 Condition of gill lamellae

Out of 1,132 gill samples observed, 96.7% (1,095) of samples were classified as clean, 3.1% (35) as relatively clean and 0.2% (2) as intermediate (Tables 1 and 2, Figure 3). The two gill samples that were categorized as intermediate were from a free female in Cheticamp from fall 2013 and a caged male retrieved in fall 2013(after 12 months of immersion). None of the samples examined was classified as dirty (filled with compacted sediment-like substances observed in Moriyasu *et al.* unpublished at the seismic test site). In comparison with the results obtained by Moriyasu *et al.* (2011), the category 'relatively clean' in this study should correspond to 'clean' in Moriyasu *et al.* (2011).

There was neither a spatio-temporal nor a sex-based tendency in the occurrence of 'trace of dirtiness and intermediate' categories. In healthy snow crab, gill lamellae must be continuously cleaned/swept by the setose epipods of the first, second and third maxillipeds.

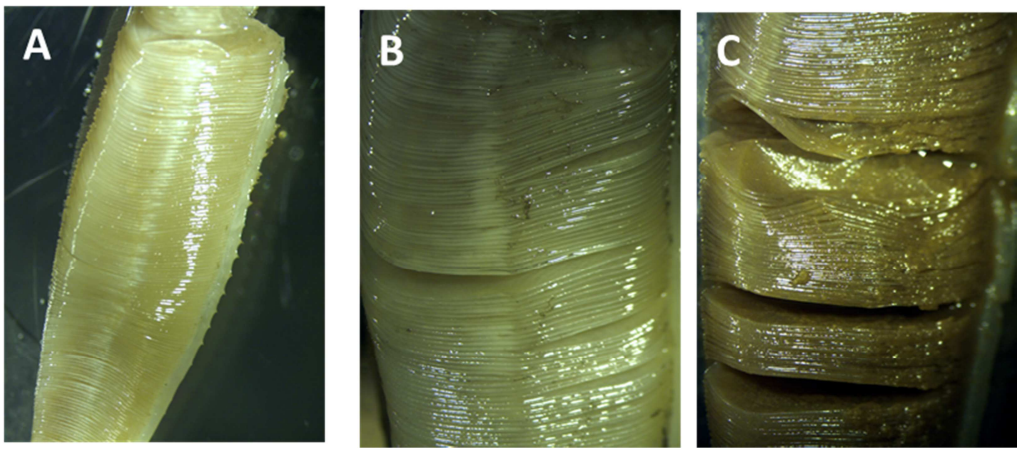

Figure 3. Condition of gill lamellae (A: clean (x8), B: Relatively clean (x16), C: Intermediate (x16))

The results of gill condition observations (Tables 1 & 2) were regrouped into two categories for analysis: 1) clean and 2) non-clean including relatively clean, intermediate and dirty conditions (single binary parameters). The percentage of clean condition was calculated by crab category, location, and season/caging duration.

These parameters were analysed using logistic regression. A range of models, from a full model with interactions to a model without treatment effects, were applied and compared using analysis of variance (chi-square tests). The simplest model not statistically significantly different from more complex models was retained. In particular, the significance or non significance of adding the cage vs free treatment variable to the model was examined. In some cases, the log-odds ratio was used to compare results between caging to free treatment. This type of analysis (Analysis of single binary parameters; McCullagh & Nelder 1989). applies to other subjects in this document such as presence/absence of species type and mortality assessment of caged crabs.

The results showed (Table 3) that there was no caging effect on the condition (% of clean gills) of gills for any of the crab categories (large males, pygmy males and mature females).

Table 1. Condition of gills by season, station, treatment and crab category (large males (LM), pygmy males (PM) and mature females (MF) in 2012 (1: clean, 2: relatively clean, 3: intermediate, 4: dirty).

	Spring 2012					Fall 2012					
Grande-Rivière free		1	2	3	4	Total		1	2	3	4	Total
	LM	21	0	0	0	21	LM	21	0	0	0	21
	PM	3	0	0	0	3	PM	18	1	0	0	19
	MF	18	2	0	0	20	MF	20	1	0	0	21
		42	2	0	0	44		59	2	0	0	61
Margaree Harbor free		1	2	3	4	Total		1	2	3	4	Total
	LM	18	2	0	0	20	LM	22	0	0	0	22
	PM	20	0	0	0	20	PM	20	0	0	0	20
	MF	18	2	0	0	20	MF	20	0	0	0	20
		56	4	0	0	60		62	0	0	0	62
Cheticamp free		1	2	3	4	Total		1	2	3	4	Total
	LM	21	0	0	0	21	LM	20	0	0	0	20
	PM	20	1	0	0	21	PM	19	0	0	0	19
	MF	20	0	0	0	20	MF	20	0	0	0	20
		61	1	0	0	62		59	0	0	0	59
Louisbourg free		1	2	3	4	Total		1	2	3	4	Total
	LM	19	0	0	0	19	LM	17	2	0	0	19
	PM	20	0	0	0	20	PM	19	1	0	0	20
	MF	24	0	0	0	24	MF	20	0	0	0	20
		63	0	0	0	63		56	3	0	0	59
Margaree Harbor caged								1	2	3	4	Total
							LM	18	0	0	0	18
							PM	20	0	0	0	20
							MF	19	1	0	0	20
								57	1	0	0	58
Cheticamp caged								1	2	3	4	Total
							LM	20	0	0	0	20
							PM	19	0	0	0	19
							MF	20	0	0	0	20
								59	0	0	0	59

Table 2. Condition of gills by season, station, treatment and crab category (large males (LM), pygmy males (PM) and mature females (MF) in 2013 (1: clean, 2: relatively clean, 3: intermediate, 4: dirty).

SPRING 2013

Grande-Rivière free

	1	2	3	4	Total
LM	19	1	0	0	20
PM	18	2	0	0	20
MF	18	2	0	0	20
	55	5	0	0	60

Margaree Harbor free

	1	2	3	4	Total
LM	19	0	0	0	19
PM	20	0	0	0	20
MF	20	1	0	0	21
	59	1	0	0	60

Cheticamp free

	1	2	3	4	Total
LM	20	0	0	0	20
PM	19	1	0	0	20
MF	21	0	0	0	21
	60	1	0	0	61

Louisbourg free

	1	2	3	4	Total
LM	19	1	0	0	20
PM	20	0	0	0	20
MF	5	0	0	0	5
	44	1	0	0	45

Margaree Harbor caged

	1	2	3	4	Total
LM	15	1	0	0	16
PM	18	0	0	0	18
MF	18	1	0	0	19
	51	2	0	0	53

Cheticamp caged

	1	2	3	4	Total
LM	17	1	0	0	18
PM	19	0	0	0	19
MF	19	0	0	0	19
	55	1	0	0	56

FALL 2013

Margaree Harbor free

	1	2	3	4	Total
LM	18	1	0	0	19
PM	18	2	0	0	20
MF	20	0	0	0	20
	56	3	0	0	59

Cheticamp free

	1	2	3	4	Total
LM	20	1	0	0	21
PM	20	0	0	0	20
MF	16	4	1	0	21
	56	5	1	0	62

Margaree Harbor caged

	1	2	3	4	Total
LM	9	0	1	0	10
PM	13	1	0	0	14
MF	17	0	0	0	17
	39	1	1	0	41

Cheticamp caged

	1	2	3	4	Total
LM	16	0	0	0	16
PM	14	2	0	0	16
MF	16	0	0	0	16
	46	2	0	0	48

Table 3. Percentage (%) of clean gill and results of deviance table analysis for each crab category (large males, pygmy males and mature females) between caged and free crabs in Cheticamp and Margaree Harbor stations for fall 2012 (free vs 2 week caged), spring 2013 (free vs 6-month caged), fall 2013 (free vs 12-month caged).

Large Males:

Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	100.0	95.2
Caged	100	94.4	100

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	95.0	94.7
Caged	100	93.8	100

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	215	42.586			
Model 2	214	42.577	1	0.0096151	0.9219

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

Pygmy Males

Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	95	100.0
Caged	100	100	87.5

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	100	90.0
Caged	100	100	92.9

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	221	46.695			
Model 2	219	46.592	1	0.10315	0.7481

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

Mature Females

Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	100	76.2
Caged	100	100	100

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	100.0	100
Caged	95	94.7	100

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	229	56.691			
Model 2	228	55.801	1	0.99957	0.3456

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

IV-1-4 Identification Commensal Organisms from the Gills of the Snow Crab

Ten pre-sorted most frequently observed species/groups collected from caged and free crabs were examined and identified as follows (Table 4 and Figure 4):

Type I: Harpacticoida, Copepoda, Laophontidae, *Laophontidae*, Laophonte sp.
Size: Females: 1055.2 - 1079.3 µm, Males: 862.0 - 893.1 µm. (Figure 4-I)

These copepods belong to the Order Harpacticoida and Family Laophontidae. The identification to species level was not amenable. This unknown type-I harpacticoid copepod belonging to the Laophontidae family have been sent to Dr. Kai George (Meiobenthonische Arthropoda, Abt. DZMB, Senckenberg am Meer Wilhelmshaven, Südstrand 44, D-26382 Wilhelmshaven, Germany). Based on the preliminary investigation, these abundant copepods, despite of their abundance, may represent an entirely new genus and a new species within the Laophontidae harpacticoid copepod family. Further evaluation is required, but not pursued here as this is out of scope of the current project.

Type II: Harpacticoida Copepoda, *Tisbe celata*
These organisms consisted of adult copepods belonging to the Order Harpacticoida and Family Tisbidae, *Tisbe celata* Humes (1954). Size: Females: 857.5 - 872.3 µm, Males: 686.0 - 721.8 µm. These were the most abundant organism in the commercial size male crabs' gill chambers but also showed high variability in number (Figure 4-II).

The observations revealed that the above mentioned two Harpacticoid copepods inhabit the narrow space between the gill lamellae. Within this space they tended to be concentrated near the blood canals that are centrally located along the length of the gills.

Type III: Bryozoa (Ectoprocta), Order Ctenostomadida
Further work on the identification of this organism continues to suggest that it belongs to the Phylum Bryozoa (Ectoprocta) and the Order Ctenostomadida. Size: 1438.5 - 1783.7 µm (Figure 4-III). The single individual recorded here represent the zooid phase since the form is erect, bears tentacles (lophophore), exhibits a collar (usually damaged) and shows bud-like projections where young are formed. Finding intact Bryozoan colonies on the additional gill samples examined later confirmed that this is a Bryozoan zooid probably belonging to the genus *Bowerbankia* sp.

The Ctenostomadida represent a group of Bryozoa (Ectoprocta) that are often found as ectosymbients or epibionts on decapod crustaceans (Abello & Corbera, 1996; Dick *et al.*, 1998; Winston & Key, 1999; Savoie et al., 2007; Gordon & Spencer-Jones, 2013). In fact, Dick *et al.* (1998) and Savoie *et al.* (2007) found several species of Bryozoan epibionts on the Tanner crab (*Chionoecetes bairdi*) and the Snow Crab (*Chionoecetes opilio*) respectively. However, only Dick *et al.* (1998) described the Bryozoan genera, *Triticella* sp. and *Alcyonidium* sp., residing in

the gill chambers of the Tanner crab from Alaska. There may be another explanation for the occurrence of the Bryozoan in the gill chambers of the current samples and the lack of attached individuals. Possibly their presence may be due to contamination from the outer shell during gill chamber removal. The literature indicates that dense colonies of Bryozoa can coat the exoskeletons of decapod crustacea and that Ctenostomadida Bryozoa are easily broken off during handling, thus potentially contaminating gill chambers during removal.

Type IV: Turbellaria Flatworms

The five flatworms found in the samples belong to the Class Turbellaria, Order Seriata, Family Monocelididae and most likely the genus *Ectocotyla* spp. Size: 1055.9 - 1550.2 µm (Figure 4-IV). They only occurred in two of the gill chambers and only in the commercial size males. Two species of Ectocotyla are known to co-occur in the branchial chambers of *Chionoecetes opilio* and include *Ectocotyla hirudo* (Levensen 1879) and *E. multitesticulata* Fleming and Burt, 1978 (Fleming & Burt, 1978). These flatworms are fairly rare in the gill chambers. As will be discussed later the attached egg capsules of these flatworms are found in all the gill chambers examined. This suggests that the flatworms find the environment in the gill chamber suitable for reproduction. But the current and previous data suggest that their numbers remain quite low.

Both species of worms have been recorded from the gills and branchial chambers of the crabs *Chionoecetes opilio* and *Hyas araneus* from off the east coast of Newfoundland and New Brunswick. *E. hirudo* has also been reported from the mouth cavity of *C. opilio* from as far away as southern Greenland (Steinbock & Reisinger, 1930).

As mentioned above, these flat worms tended to be very small and immature, while the larger adults were found outside of the gills in the gill chamber. This suggests that the newly hatched flat worms may use the gills as a refuge from predators such as the large and abundant polychaete worms, living nearby in the gill chamber. While in the gills the flat worms appear to coexist with the numerous copepods and nauplii sharing the confines of the inter-lamellar spaces. Dick *et al.* (1998) reported small flat worms from the spaces between the gill lamellae in the tanner crab, *C. bairdi*.

Type V: Nematoda

This group includes small roundworms belonging to the Phylum Nematoda. Size: L = 2285.7 - 2446.6 µm, W = 29.8 - 37.3 µm Figure 4-V). Further identification of these worms has been unsuccessful. They occurred in nearly all the samples but in low abundance but were not observed in situ in the gills. Instead, they were found in the gill extract and in the original preservative that contained the gill chambers. The nematodes were most abundant in the commercial size males. It is very difficult to detect them because of their small size and thin body. They can easily be overlooked or ignored as filamentous algae.

In only one instance was a typical nematode cyst-like structure found on a gill lamellae and this curled up nematode was fairly large in size. Most nematodes associated with the gills were found in the inter-lamellar spaces and these were usually quite small and often difficult to detect. Nematodes associated with the inner wall of the gill chamber were much less abundant and often lacking.

Nematode worms are common commensals on a wide variety of marine animals (Barnes 1980; Thorp & Covich 2001). They can be permanent commensals or use the host to complete its life cycle. Therefore it is not surprising to find them associated with the gills of snow crabs. Yet, a review of the literature did not reveal citations of nematodes associated with crab gills or the gill chamber. Rather the nematodes cited were found in the crab haemocoel or muscle bundles (Moravec *et al.* 2003). The common occurrence of nematodes in snow crab gills and gill chamber indicates that the gills provide a favorable habitat and perhaps protection from predators. The Phylum Nematoda is exceedingly large, complex and taxonomically in a state of constant revision and so no attempt at further identification was attempted.

Type VI: Harpacticoida Copepoda Nauplii
Copepod nauplii only occurred in the gill chambers of the commercial size males (3 out of the 5 samples) and then in low numbers. Size (*Tisbae celata*): 206.9 - 229.3 µm (Figure 4-VI). They are probably the naupliar stages of the three species of harpacticoid copepods that also occur in the gill chambers. No attempt was made to determine which species of copepods these early developmental stages belong to. They do attest to successful reproduction within the gill chambers as do the egg sacs carried by some of the adult female copepods.

Type VII: Kinorhyncha
This group includes the Phylum Kinorhyncha, Order Cyclorhagida, Family Echinoderidae and most probably the genus and species *Echinoderes elongatus* Nyholm (1947). Size: 346.8 - 359.4 µm (Figure 4-VII). Although there is no record that Echinoderes is a commensal or parasite on the snow crabs in the literature, it was frequently observed in the present study.

Type VIII: Polychaete Worms
This group of worms belongs to the Phylum Annelida and Class Polychaeta. Size: 821.9 - 1369.9 µm (Figure 4-VIII). Though somewhat rare, Brattey *et al.* (1985) found the polychaete *Ophryotrocha geryonicola* in the gills of *Chionoecetes opilio*, from the waters off Cheticamp, Gabarus and Igonish and from Pleasant Bay, Cape Breton Island, Nova Scotia. Brattey *et al.* (1985) reported low abundance of polychaetes from the gill chambers of *C. opilio* but did not indicate exactly where within the gill chambers the worms were observed.

In this study, polychaete worms, associated with the gills, were found in only about one-fourth of the additional samples examined and they were generally in low abundance. Only a few of the

worms were observed residing in mud-tubes constructed on the gills and these worms were small in size. By contrast the polychaeta inhabiting the inner wall of the gill chamber were much more abundant, contained more species all living in mud-tubes and were substantially larger in size. The general lack of Polychaeta on the gills suggests that the gills may not be a particularly good substrate for polychaete worms to inhabit. Because of their relatively large size they cannot reside in the inter-lamellar spaces of the gills and the gill sweeping appendages of *C. opilio* likely does an efficient job of removing polychaete worms from the gill surfaces.

Observations on the additional samples revealed that the polychaete species, *Polydora sp*. (adults and juveniles), appeared most commonly. The genera of *Polydora* include common fouling species and some species, such as *Polydora commensalis*, have been observed to live within the shells of hermit crabs (Pollock 1998). At least three other species of Polychaeta were observed in the gill chambers of *C. opilio* in the current study but these have not been further identified.

Type IX: Mites (Halacaroidea)
Only two marine mite specimens were contained in these gill chamber samples and only from the commercial size males. Size: 401.1 - 482.8 µm (Figure 4-IX). Both appear to be similar to those found in the previous samples and belong to the Phylum Arthropoda, Class Arachnida and Order Halacaroidea. Clearly, mites are rare organisms in the gill chambers and easily missed due to their small size. No documented evidence is found that mites regularly occur in gill chambers of decapod crustaceans, which suggests that their presence is mostly by chance.

Type X: Turbellaria Worm Egg Capsules
This group consists of the egg capsules of Turbellaria worms (Type IV). Size: 420.3 - 750.7 µm (Figure 4-X). Their identification was confirmed using the paper by Fleming and Burt (1978). These workers were the first to record these capsules from the gills and gill cavity of *Chiononecetes opilio* from Newfoundland. They also described the adult Turbellaria worms that released these egg capsules. The capsules occurred in all the groups examined and they were fairly abundant. Most were found free in the gill extracts with just a few seen attached to the gills and in the crevices of the inner wall of the carapace. Each rounded egg capsule is attached to the substrate by a single fine thread. Clearly, the Turbellaria worms are successfully reproducing in the gill chambers.

Table 4. Classification of commensal organisms found among the snow crab gill lamellae.

Type I:	Harpacticoida, copepoda, Family Laophontidae. *Leophonte sp.*
Type II:	Harpacticoida, copepoda, *Tisbe celata*.
Type III:	Bryozoa (Ectoprocta), Order Ctenostomadida.
Type IV:	Turbellaria flatworms, *Ectocotyla hirudo* and *E. multitesticulata*.
Type V:	Nematoda, Unknown genus and species.
Type VI:	Harpacticoida, copepoda nauplii, *Tisbe celata*?
Type VII:	Kinorhyncha, *Echinoderes elongates*.
Type VIII:	Polychaeta larvae, *Ophryotrocha geryonicola*.
Type IX:	Mites (Halacaridae), *Copidognathus sp*.?
Type X	Turbellaria, egg capsules.

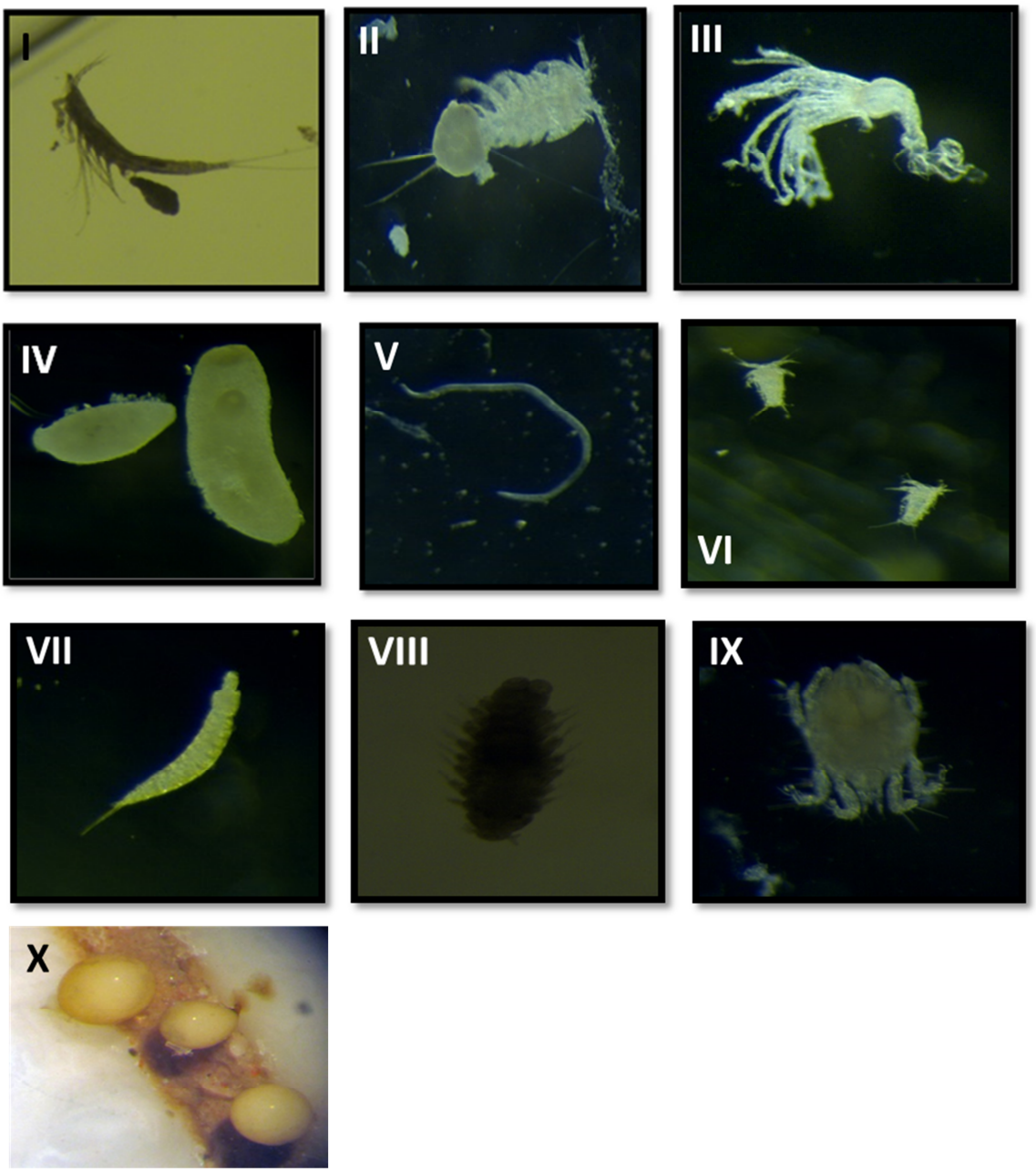

Figure 4. Commensal organisms (type I to X) found among the snow crab gill lamellae.

I:Harpacticoida, copepoda, *Laeophonte* sp., II: Harpacticoida, copepoda, *Tisbe celata*, III: Bryozoa Ctenostomadida, IV:Turbellaria flatworms, *Ectocotyla hirudo* and *E. multitesticulata.*, V: Nematoda, VI:Harpacticoida, copepoda nauplii, VII: Kinorhyncha, *Echinoderes elongates*. VIII: Polychaeta larvae, *Ophryotrocha geryonicola*. IX: Mites (Halacaroidae), X: Turbellaria, egg capsules.

IV-1-5 Abundance of gill fouling species among gill lamellae

Among 10 gill fouling species, ranking in terms of frequency of observation was as follows: Type V, II, I, VI, VII, IV, III, X, VIII and IX and in terms of average number of individual per crab, type I, V, VI, II, X, III/IV and VII/VIII/IX (Table 5). Therefore, types VIII and IX seem to be very rare species present among the gill lamellae of snow crab.

In terms of average total number of gill fouling species per season and year as well as per station (Figure 5), there seem not have any particular spatio-temporal trend. However, the average abundance of total gill fouling species in Louisbourg station seems to be higher than other stations. In terms of the total abundance of gill fouling species, type VII is the most abundant followed by types I, V, II and VI. The average number of gill fouling species per crab (carrying at least one individual of a given type of gill fouling species) was also the type VII (5,002/crab) followed by types I, VI, V, II. The frequency of prevalence was the highest for type V followed by types II, I, VI, VII, IV, III and X.

Table 5. Summary of number (and %) of crab carrying a given gill fouling species/type, the total number of gill fouling species/type (x1000) and the average gill fouling species/type per crab carrying at least one individual of gill fouling species.

	I	II	III	IV	V	VI	VII	VIII	IX	X
Number of crab carrying a given fouling species	744 (66%)	795 (71%)	271 (24%)	310 (28%)	908 (81%)	452 (40%)	450 (40%)	11 (1%)	8 (1%)	162 (15%)
Total number of gill fouling species (x1000)	393	53	1	1	65	41	2,251	0	0	3
Average number of gill fouling species per crab* (a set of 6 gills)	528	66	3	3	71	90	5,002	0	0	18

* Per number of crab carrying a given gill fouling species

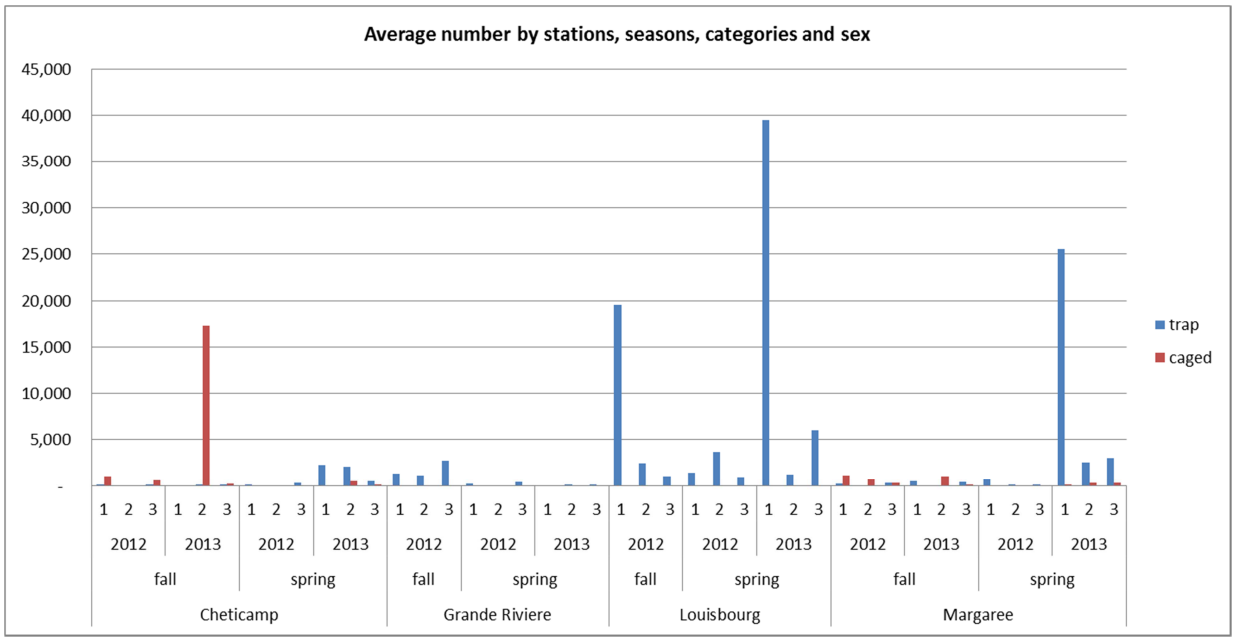

Figure 5. Average total number of commensal organisms found in gill lamellae of snow crab by season, by year, by station, by sampling method (trap or cage) and by crab category (1: LM, 2, MF and 3: PM)

The results of observations on the abundance of gill fouling species and, the percentage presence of each type of gill fouling species were calculated for caged and free snow crabs in Cheticamp and Margaree Harbor in the fall 2012 (2 week-caged), spring 2013 (6-month caged) and fall 2013 (12-month caged) for three crab categories (large males, pygmy males and mature females) (Tables 6-8). The percentage of clean condition was then compared between treatments (free and caged) for each category of crab (large male, pygmy male and female) with deviance table analysis (McCullagh & Nelder 1989).

The results (Tables 9 - 12) showed that there is a significant difference (at p=0.001) between free and caged crab for gill fouling species type VI (Harpacticoida, copepoda nauplii), VII (Kinorhyncha, Echinoderes elongates) and X (Turbellaria, egg capsules) for all crab categories (large male, pygmy male and female). Types VI and X are early life stages of harpacticoida copepod and generally higher percentage of presence in free crabs. However, type X (Turbellaria, egg capsules) showed that the percentage is higher in caged crabs. Cages might have provided a favorable environment to Turbellaria for depositing their egg capsules in gill lamellae of snow crab e.g. stagnation or reduced current flow in cages. However, it is difficult to understand the cause of difference without any information on the life cycle and habitat preference of these species.

Table 6. Summary of % presence of each type of gill fouling species in caged and free large male snow crabs in Cheticamp and Margaree Harbor in the fall 2012 (2 week-caged), spring 2013 (6-month caged) and fall 2013 (12-montn caged) for large males.

GFS Type I		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	50	60	33.3
	C	50	50	62.5
Margaree Harbor	F	52.4	80	94.7
	C	44.4	50	88.9
GFS Type II		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	100	100.0	57.1
	C	95	83.3	50
Margaree Harbor	F	100.0	100	84.2
	C	88.9	100	88.9
GFS Type III		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	20	30.0	19.0
	C	30	11.1	6.2
Margaree Harbor	F	52.4	70	26.3
	C	44.4	75	33.3
GFS Type IV		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	10	20.0	4.8
	C	25	55.6	18.8
Margaree Harbor	F	23.8	50.0	26.3
	C	16.7	43.8	33.3
GFS Type V		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	90	90.0	42.9
	C	95	61.1	43.8
Margaree Harbor	F	81	90	52.6
	C	100	75	55.6
GFS Type VI		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	55	80	0
	C	65	0	0
Margaree Harbor	F	66.7	100	0
	C	72.2	0	0
GFS Type VII		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	55	80	0
	C	65	0	0
Margaree Harbor	F	66.7	100	0
	C	72.2	0	0
GFS Type X		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	10	0.0	9.5
	C	0	72.2	18.8
Margaree Harbor	F	0	0	36.8
	C	0	81.2	66.7

F: free crabs, C: caged crabs

Table 7. Summary of % presence of each type of gill fouling species in caged and free pygmy male snow crabs in Cheticamp and Margaree Harbor in the fall 2012 (2 week-caged), spring 2013 (6-month caged) and fall 2013 (12-montn caged) for pygmy males.

GFS Type I		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	89.5	75.0	80.0
	C	52.6	68.4	87.5
Margaree Harbor	F	75	75.0	85.0
	C	60	72.2	78.6
GFS Type II		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	73.7	95.0	80.0
	C	89.5	84.2	43.8
Margaree Harbor	F	85	95.0	90.0
	C	70	83.3	85.7
GFS Type III		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	15.8	15.0	0
	C	31.6	10.5	0
Margaree Harbor	F	10	15.0	15.0
	C	25	33.3	35.7
GFS Type IV		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	21.1	45.0	5
	C	52.6	47.4	25
Margaree Harbor	F	15	35.0	40.0
	C	40	61.1	7.1
GFS Type V		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	84.2	100.0	45.0
	C	89.5	63.2	43.8
Margaree Harbor	F	85	95.0	70.0
	C	95	44.4	71.4
GFS Type VI		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	63.2	85	0
	C	73.7	0	0
Margaree Harbor	F	65	95	0
	C	45	0	0
GFS Type VII		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	63.2	85	0
	C	73.7	0	0
Margaree Harbor	F	65	95	0
	C	45	0	0
GFS Type X		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp		5.3	0.0	35.0
		0.0	63.2	68.8
Margaree Harbor		0	0.0	20.0
		0	38.9	71.4

F: free crabs, C: caged crabs

Table 8. Summary of % presence of each type of gill fouling species in caged and free female snow crabs in Cheticamp and Margaree Harbor in the fall 2012 (2 week-caged), spring 2013 (6-month caged) and fall 2013 (12-montn caged) for mature females.

GFS Type I		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	50	38.1	90.5
	C	30	63.2	100.0
Margaree Harbor	F	60	80.0	85.0
	C	55	42.1	88.2
GFS Type II		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	75	47.6	33.3
	C	55	63.2	62.5
Margaree Harbor	F	40	55.0	5.0
	C	45	42.1	52.9
GFS Type III		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	30	14.3	14.3
	C	20	0.0	0
Margaree Harbor	F	0	20.0	20.0
	C	10	10.5	11.8
GFS Type IV		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	75	85.7	42.9
	C	85	68.4	43.8
Margaree Harbor	F	90	95.0	40.0
	C	90	63.2	58.8
GFS Type V		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	75	85.7	42.9
	C	85	68.4	43.8
Margaree Harbor	F	90	95.0	40.0
	C	90	63.2	58.8
GFS Type VI		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	5	9.5	4.8
	C	20	15.8	18.8
Margaree Harbor	F	15	10.0	25.0
	C	0	31.6	11.8
GFS Type VII		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	50	52.4	0
	C	40	5.3	0
Margaree Harbor	F	40	65	0
	C	40	0	0
GFS Type X		Fall 2012 (2 weeks)	Spring 2013 (6 months)	Fall 2013 (12 months)
Cheticamp	F	0	19.0	19
	C	0	89.5	75
Margaree Harbor	F	0	0.0	5.0
	C	5	31.6	47.1

F: free crabs, C: caged crabs

Table 9. Results of deviance table analysis and comparison of % presence of each gill fouling species (type I-VII & X) in large male between caged and free crabs in Cheticamp and Margaree Harbor stations for large males.

Type I	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	214	284.99			
Model II	213	284.50	1	0.48929	0.48424
Type II	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	214	124.74			
Model II	213	121.94	1	2.8063	0.093893
Type III	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	214	247.24			
Model II	213	246.95	1	0.29284	0.58841
Type VI	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	214	236.73			
Model II	213	233.93	1	2.8028	0.0941
Type V	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	214	211.20			
Model II	213	210.93		0.26938	0.6037
Type VI	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	214	203.22			
Model II	213	177.59	1	25.627	4.1417e-07
Type VII	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	214	203.22			
Model II	213	177.59	1	25.627	4.1417e-07
Type X	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	214	188.61			
Model II	213	155.79	1	32.82	1.011e-08

Model 1: y ~ Location + YearSeason, Model 2: y ~ Treatment + Location + YearSeason

Table 10. Results of deviance table analysis and comparison of % presence of each gill fouling species (type I-VII & X) in pygmy male snow crab between caged and free crabs in Cheticamp and Margaree Harbor stations for pygmy males.

Type I	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	221	250.64			
Model II	220	247.44	1	3.2054	0.0734
Type II	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	221	206.71			
Model II	220	202.50	1	4.2064	0.04027
Type III	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	221	198.70			
Model II	220	194.12	1	4.581	0.03233
Type VI	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	221	274.30			
Model II	220	269.88	1	4.421	0.0355
Type V	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	221	234.17			
Model II	220	229.12	1	5.0451	0.0247
Type VI	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	221	210.16			
Model II	220	172.65	1	37.508	9.103e-10
Type VII	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	221	210.16			
Model II	220	172.65	1	37.508	9.103e-10
Type X	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	221	190.85			
Model II	220	154.10	1	32.752	1.342e-09

Model 1: y ~ Location + YearSeason, Model 2: y ~ Treatment + Location + YearSeason

Table 11. Results of deviance table analysis and comparison of % presence of each gill fouling species (type I-VII & X) in female snow crab between caged and free crabs in Cheticamp and Margaree Harbor stations for mature females.

Type I	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	229	264.07			
Model II	228	263.49	1	0.58744	0.4434
Type II	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	229	310.92			
Model II	228	308.43	1	2.492	0.1144
Type III	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	229	178.31			
Model II	228	175.36	1	2.9511	0.08582
Type VI	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	229	184.24			
Model II	228	183.08	1	1.1615	0.2812
Type V	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	229	250.91			
Model II	228	250.51	1	0.40102	0.5266
Type VI	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	229	208.19			
Model II	228	193.39	1	14.802	0.0001194
Type VII	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	229	207.70			
Model II	228	191.49	1	16.218	5.646e-05
Type X	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)
Model I	229	194.53			
Model II	228	142.64	1	51.895	5.854e-13

Model 1: y ~ Location + YearSeason, Model 2: y ~ Treatment + Location + YearSeason

Table 12. Summary of the results (significance p-value) of deviance table analysis and comparison of % presence of each gill fouling species (type I-VII & X) for each category of crab (large males, pygmy males and mature females) between caged and free crabs in Cheticamp and Margaree Harbor stations.

Species Type	large males	pygmy males	mature females
I	0.48424	0.073398	0.44341
II	0.093893	0.040272	0.11443
III	0.58841	0.032328	0.085821
IV	0.0941	0.0355	0.28116
V	0.60374	0.024696	0.52656
VI	**4.1417e-07**	**9.1031e-10**	**0.00011941**
VII	**4.1417e-07**	**9.1031e-10**	**5.6462e-05**
X	**1.0111e-08**	**1.3416e-09**	**5.8537e-13**

IV-1-6 Histological observations

Objective

To review histologic sections of gill tissue collected from free (collected by commercial trap) and caged snow crab (2012-2013) to look for effects of short- and long-term caging.

Methodology

Tissues were collected from crabs within 15 minutes of death/euthanasia (Section III-1) and placed into fixative for processing as described in Section III-1.
Haematoxylin and eosin (H&E) stained slides of gill tissue were provided for direct light microscopic evaluation. Slides represented crabs originating from four stations (Cheticamp, NS, Margaree Harbor, NS, Loiusbourg, NS, Grande-Rivière, QC) with samples collected at four times (Spring 2012, Fall, 2012, Spring 2013, Fall 2013) over a two year period. The fifth gill on the right side was collected for histological examination. Subgroups of crabs in Cheticamp and Margaree had been caged for a period of 2 weeks (Fall 2012), 6 months (Spring 2013), or 12 months (Fall 2013). Crabs collected by trap (i.e., no caging period) were used as the control groups. All slides had been randomised and renumbered allowing for non-biased evaluation by the observer (A. Battison).

As gill tissue was not consistently collected from crabs in spring 2012 samples, none of the histological gill sections from any spring 2012 crabs were evaluated. Half of the remaining crabs were examined for this portion of the study. To avoid potential bias associated with order of sample collection, crabs were alternately assigned a value of 0 or 1 in order of sample collection and then a coin toss used to determine which group was examined (0's). This approach kept the ratio of crabs examined within each sample group, out of those available for examination, similar to that in Section IV-6-5 (Hepatopancreas histology).

Scoring criteria for gill tissues were devised after review of literature describing gill anatomy in a variety of crabs (no snow crab-specific publications available), general crustacean reviews (Johnson 1980a, Taylor & Taylor 1992), and results from Section IV-1-4 and examination of a subset of slides. The following criteria were selected as potentially useful for general descriptive purposes and for detecting differences among crab groups:

1) Presence of Bryozoans (absent/present)
2) Presence of Copepods (absent/present)
3) Presence of Flatworms (absent/present)
4) Presence of Nematodes (absent/present)
5) Presence of Polychaetes (absent/present)
6) Presence of Amphipods (absent/present)
7) Presence of Other unidentified flora (absent/present)

8) Degree of bacterial fouling on gill surface (none seen/mild/moderate/marked/severe)
9) Degree of sediment accumulation on gill surface (none seen/mild/moderate/marked)
10) Presence and abundance of intracytoplasmic inclusions i.e., epithelial cells, endothelial cells, both epithelial and endothelial cells (none seen/mild/moderate/ marked)
11) Nephrocyte vacuolation (none seen/few small vacuoles/large vacuole)
12) Reserve Inclusion (RI) cell fullness (none, mild, moderate, marked) based on subjective average of number of RI cells and degree of fullness
13) Diffuse hemocyte infiltrates in loose connective tissue (none or few seen/mild / moderate/marked)
14) Hemocyte aggregates (non-organised, but loosely packed) in loose connective tissue (none or few seen/mild / moderate/marked)
15) Hemocyte nodules (organised, some evidence of whorling) in loose connective tissue
16) Brown-gold pigment (melanin) deposition (none/mild/moderate/marked)
17) Presence of infective agents within lesions (none seen/bacteria/fungi or yeast/protozoan/other)

Many scores were subjective and took into account overall distribution and severity of changes e.g., hemocyte nodules.

Data was analysed using STATA ® Statistics/Data Analysis 12.1 (StataCorp, TX, USA) and Excel 2010© (Microsoft Corporation, Microsoft Canada Inc. ON, Canada). Significance level was set at $p < 0.05$. Slides were examined using a Leitz Dialux 20 microscope. Images were captured with a PixeLINK® camera and associated μScope software (PixeLINK®).

Results

The plane of section of the tissue samples was inconsistent as was the amount of material available for examination. The stain intensity of some slides was quite pale which diminished the prominence of hemocyte granules. The central septum area was absent in some slides and therefore the amount of connective tissue was minimal, making it difficult to assess criteria such as RI cells, nephrocytes, and hemocyte infiltrates. In such cases, no scores were recorded.

Slides were not available for 21 crabs, presumably due to mortalities. Table 13 summarises the distribution of the 448 samples available for evaluation.

Table 13. Summary table showing number of gill samples available for histological examination from each station and the date of sample collection.

Station	Gear Type	Sampling Time											
		Spring 2012			Fall 2012			Spring 2013			Fall 2013		
		PM	LM	MF	PM	LM	MF	PM	LM	MF	PM	LM	MF
Cheticamp, NS													
	Free	n/e[1]	n/e	n/e	10	10	9	10	10	10	10	10	10
	Caged	n/c[2]	n/c	n/c	10	10	10	10	9	10	8	7	8
Margaree, NS													
	Free	n/e	n/e	n/e	10	10	10	10	10	10	10	10	10
	Caged	n/c	n/c	n/c	10	9	10	8	8	9	6	5	8
Louisbourg, NS													
	Free	n/e	n/e	n/e	10	10	10	10	10	3	n/c	n/c	n/c
Grand-Rivière, QC													
	Free	n/e	n/e	n/e	10	10	11	10	10	10	n/c	n/c	n/c

[1]not examined, [2]not collected

During examination of the slides, three additional characteristics were noted. The first was accumulation of eosinophilic, hyaline, material in the vascular spaces of gill lamella, most often at the tips but occasionally throughout the lamellae. This appeared to begin as aggregations of the eosinophilic granules usually present in the vascular spaces which became more densely packed, eventually developing into the solid hyaline material (Figure 6). Rarely, a faint golden hue was noted in the lesions interpreted as being more developed. The changes were most often noted at the distal tip or base of the gill lamellae.

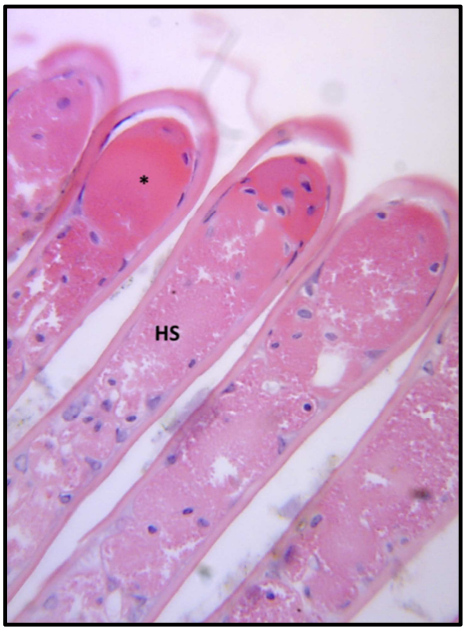

Figure 6. Histology of gill lamellae (H&E). Gill tip showing accumulation of hyaline eosinophilic material (*) and the eosinophilic granules usually present in the hemolymph spaces (HS) attributed to hemolymph protein precipitation.

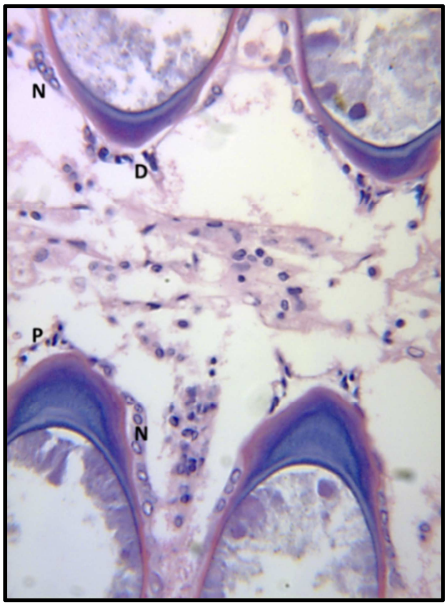

Figure 7. Histology of gill lamellae (H&E). Normal subcuticular epithelium (N) with mixture of euchromatin and heterochromatin alongside abnormal epithelium having darker, smaller nuclei (D) or pyknotic nuclei (P).

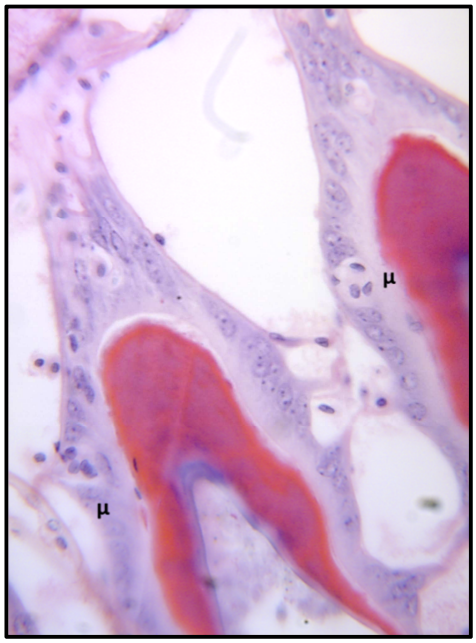

Figure 8. Histology of gill lamellae (H&E). Small accumulations of hemocytes within the subcuticular epithelium interpreted as microabscesses (µ).

The second was the occurrence of changes in subcuticular epithelial cells located most often at and near the base of the lamellae. These varied from nuclei with increased pallor and loss of chromatin detail to smaller, hyperchromic nuclei due to much reduced amounts of euchromatin (Figure 7). Occasionally, nuclei were pyknotic. Karyorrhexis was not observed. Cytoplasmic vacuolation (large clear vacuoles, 'ballooning') was occasionally seen in association with the nuclear changes. At times, cells were detached from the cuticle. There was no inflammatory response. These changes occurred in focal or multifocal groups of 5 – 20+ cells with adjacent cells normal in appearance.

Finally, tiny intraepithelial accumulations of hemocytes (micro-abscesses) were noted in six trapped crabs (fall 2012: two $LM_{Grande\ Rivière}$ and one $LM_{Cheticamp}$ and spring 2013: two $LM_{Grande\ Rivière}$ and one $PM_{Margaree}$) (Figure 8).

Comparisons of gills of free and caged crabs

Free Crabs

The fall 2012 sample collection times were separated by approximately nine weeks with Louisbourg (Sept. 18th) and Grande-Rivière (Sept. 22nd) collected before Margaree Harbor (Nov. 2nd) and Cheticamp (Nov. 4th). The spring 2013 collections by approximately four weeks with Grande-Rivière collected first (May 30th), followed by Cheticamp (June 12th), Margaree Harbor (June 18th) and finally Louisbourg (June 26th), (Table 13).

Observations of gill commensals are presented in Appendix A for informational purposes only. Detailed evaluation of gill organisms is beyond the scope of this report although some general observations included: 1) while their overall numbers were low, Byrozoans appeared more frequently in LM crabs at all stations and times; 2) Copepods were observed commonly in all sexes (PM, LM, MF), at all stations at all collection times; 3) Nematodes were also widely distributed with possibly more noted in fall samples from Grande Rivière.

Scores for gill fouling with bacteria were generally higher for PM and MF crabs at all stations and all collection times (Figure 9). A similar distribution for sediment accumulation on the gills was also noted (Figure 10). As these characteristics are best assessed by gross examination of the entire gill chamber and using a dissecting microscope, no further evaluation was conducted.

Reserve inclusion (RI) cells were seen infrequently (n = 11/293 crabs) (Figure 11). A seasonal pattern was noted with most (9/11) observations in the fall, particularly fall 2012 (7/9). Vacuolation of nephrocytes was difficult to score as the degree of vacuolation varied along the gill. Subjective averaging was attempted but as no clear pattern was evident (data not shown), this characteristic was not evaluated further.

Scores for characteristics associated with acute inflammation (diffuse hemocyte infiltrates and hemocyte aggregates) were generally low with no apparent pattern visible (data not shown). Hemocyte nodules (more chronic inflammation) were absent from most samples but, when present, higher scores were noted more often in LM and MF crabs (Figure 12). Melanin pigment deposits were also uncommon. They were only found in crabs where nodules were recorded and moderate pigment scores were only in crabs with nodules also scored as moderate.

Tiny intraepithelial accumulations of hemocytes (micro-abscesses) were noted in six free crabs (fall 2012: two $LM_{Grande\ Rivière}$ and one $LM_{Cheticamp}$ and spring 2013: two $LM_{Grande\ Rivière}$ and one $PM_{Margaree}$).

Eosinophilic hyaline deposits within vascular spaces showed a tendency to be observed more often and/or have higher scores in fall samples from Cheticamp and Margaree for all sexes (Figure 13). No pattern was noted for Louisbourg or Grande-Rivière crabs. Observations of clusters of epithelium with dark, smaller nuclei, were far more common in PM and MF crabs at all locations, especially in spring 2013 (Figure 14).

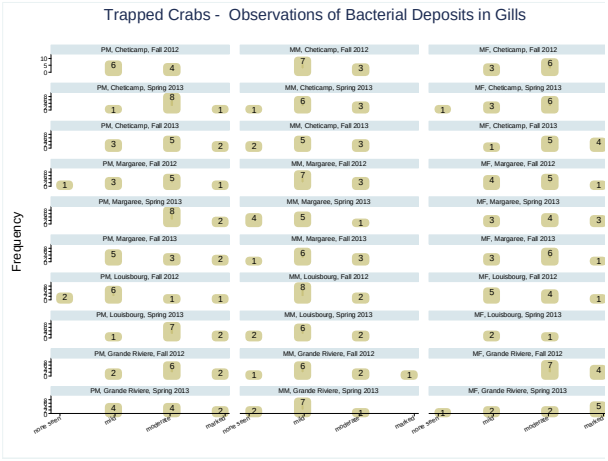

Figure 9. Frequency distribution of bacterial fouling on gill tissue by sex, station, and sample time. (Note: MM = LM)

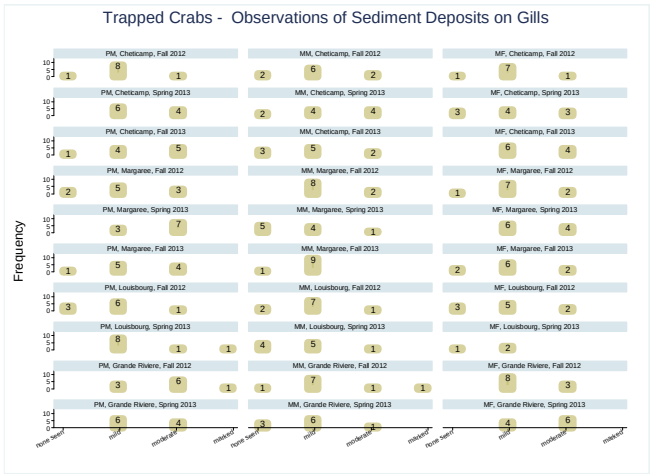

Figure 10. Frequency distribution of sediment deposits on gill tissue by sex, station, and sample time

Figure 11. Frequency distribution of reserve inclusion cells in gill tissue by sex, station, and sample time.

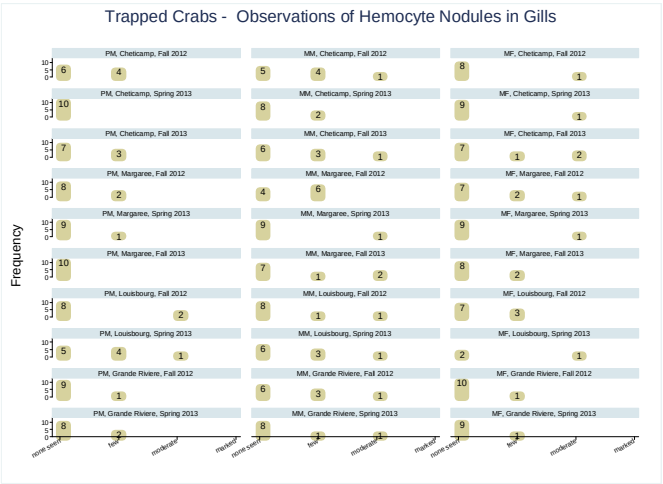

Figure 12. Frequency distribution of hemocyte nodules in gill tissue by sex, station, and sample time.

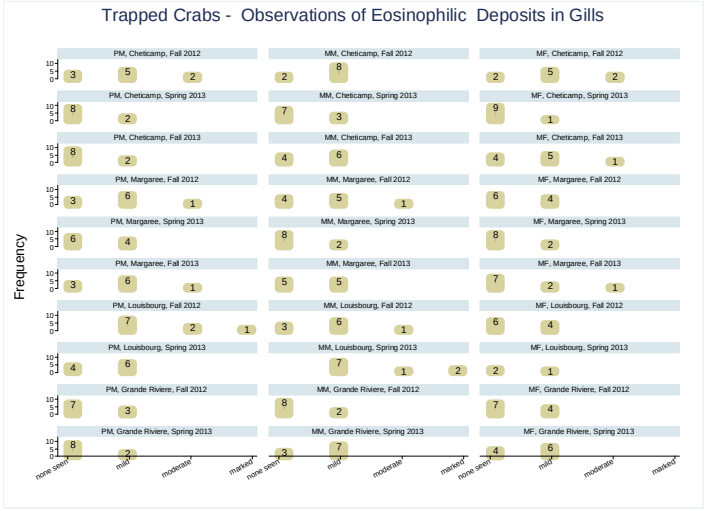

Figure 13. Frequency distribution of eosinophilic deposits in gill tissue by sex, station, and sample time.

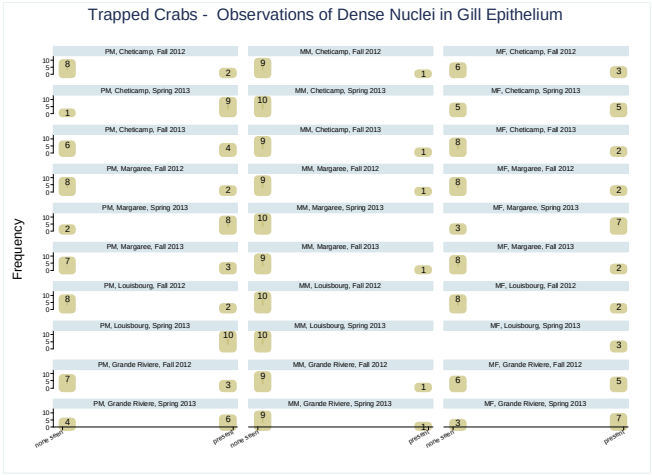

Figure 14. Frequency distribution of shrunken nuclei in subcuticular epithelium of gill tissue by sex, station, and sample time.

Caged vs Free Crabs – Cheticamp & Margaree Stations

Observations of gill commensals are presented in Appendix A for informational purposes only. Detailed evaluation of gill organisms is beyond the scope of this report although some general observations are included. There were no patterns associated with caging for Bryozoan, copepod, or flatworm scores. Nematode scores appeared lower for spring 2013 caged PM and LM at both stations and for caged fall 2013 PM, LM, and MF at Cheticamp. Scores were significantly lower (Wilcoxon signed rank test) for caged $PM_{Margaree}$ in spring 2013 ($p = 0.0178$) and caged $PM_{Cheticamp}$ in fall 2013 ($p = 0.0459$) than their free counterparts.

Bacterial fouling scores appeared less severe for caged crabs (Figure 15) and were significantly lower (Wilcoxon Rank Sum test) for spring 2013 $PM_{Cheticamp}$($p = 0.0022$), $PM_{Margaree}$($p = 0.0109$), $MF_{Margaree}$($p = 0.0067$), and fall 2013 $PM_{Cheticamp}$ ($p = 0.0134$) crabs. Scores for sediment deposits also appeared lower for caged crabs; however this was only significant for fall 2012 $PM_{Cheticamp}$ (Figure 16).

Reserve inclusion (RI) cells were infrequently observed but, identified in both caged and free crabs in fall 2012, no crabs in spring 2013, and only in two free $MF_{Cheticamp}$ crabs in fall 2013 (Figure 17). No significant differences between caged and free crabs were identified. There was no conclusive pattern (data not shown) associated with caging for nephrocyte vacuolation and this characteristic was not pursued further.

Scores for characteristics associated with acute inflammation (diffuse hemocyte infiltrates and hemocyte aggregates) were generally low with no pattern associated with caging visible (data not shown). Significant differences were not identified.

Hemocyte nodules (indicative of more chronic inflammation) were noted more often and with higher scores in caged crabs in the spring 2013 at both stations (Figure 18). Median scores were significantly higher for spring 2013 caged LM ($p = 0.0469$) and MF ($p = 0.0471$) crabs at Margaree Harbor only. Brown-gold pigment (melanin) deposits were uncommon; however when observed, usually associated with hemocyte nodules. A few crabs had some surface erosions that were pigmented unassociated with hemocyte nodules. Intraepithelial hemocyte accumulations (microabscesses) were not observed in any caged crabs.

Eosinophilic hyaline deposits within vascular spaces were found in low to moderate number of crabs in both free and caged crabs in the fall 2012 two week caging study; were uncommon in the spring 2013 samples, and present almost exclusively in free crabs in fall

2013 samples (Figure 14). Median scores were significantly lower for fall 2013 caged crabs for $PM_{Margaree}$ ($p = 0.0446$), $LM_{Cheticamp}$ ($p = 0.0134$), and $MF_{Cheticamp}$ ($p = 0.0099$).

Observations of clusters of epithelium with dark, smaller and/or pyknotic nuclei were more numerous in the spring 2013 samples for both caged and free/trapped crabs (Figure 15). The only significant difference was in median scores was for spring 2013 $PM_{Cheticamp}$ caged crabs which was less than free crabs.

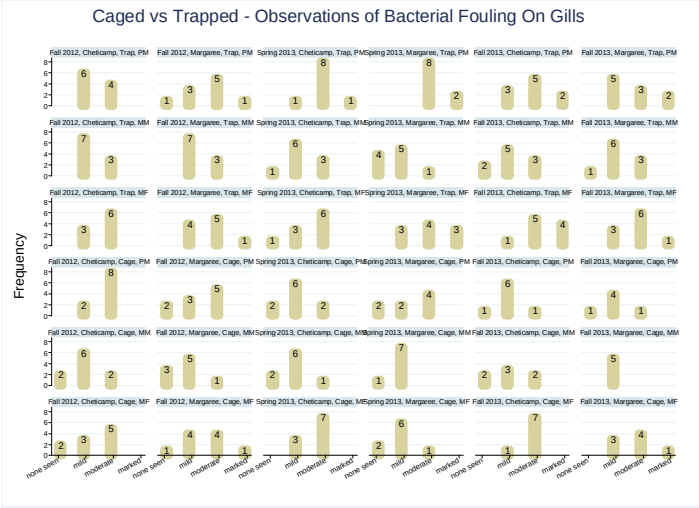

Figure 15. Frequency distribution of scores for bacterial fouling of gills in caged and free/trapped crabs at Cheticamp and Margaree Harbor by sample time.

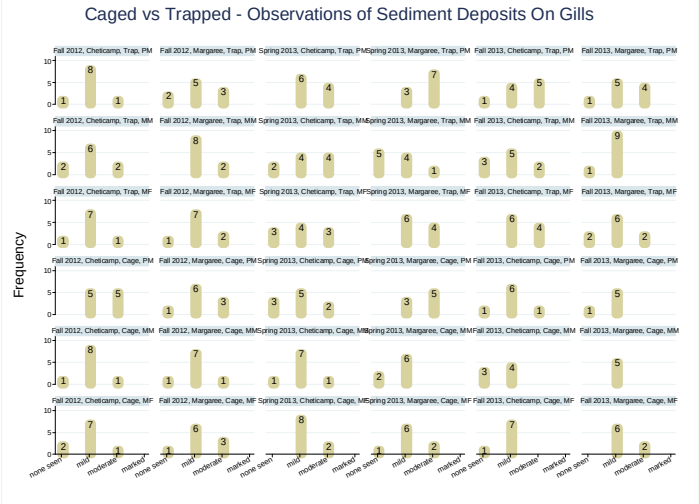

Figure 16. Frequency distribution of scores for sediment deposits on gills in caged and free/trapped crabs at Cheticamp and Margaree Harbor by sample time.

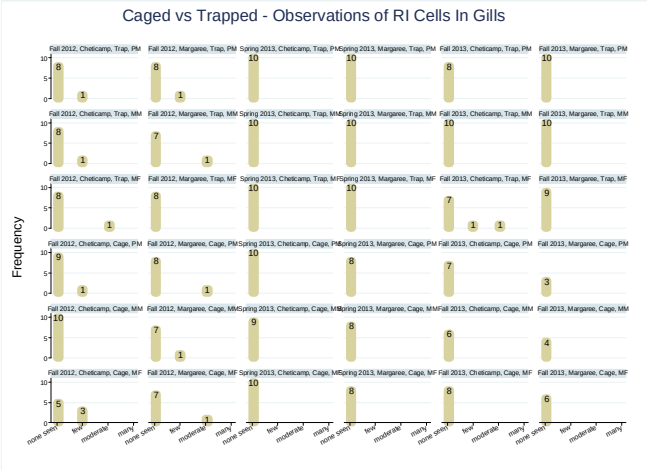

Figure 17. Frequency distribution of scores for reserve inclusion (RI) cells in gills of caged and free/trapped crabs at Cheticamp and Margaree Harbor by sample time.

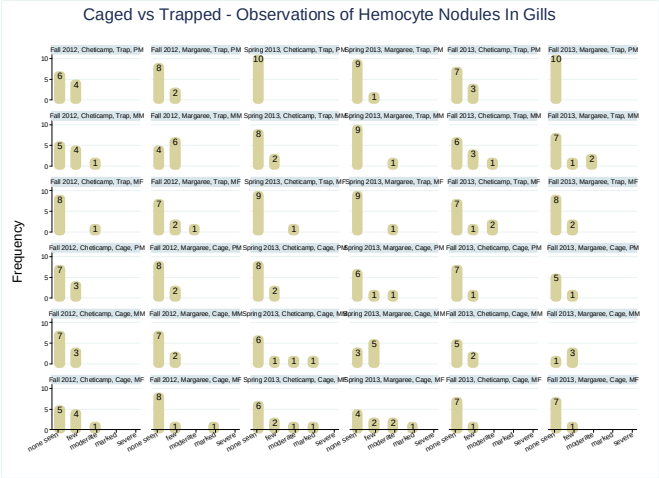

Figure 18. Frequency distribution of scores for hemocyte nodules in gills of caged and free/trapped crabs at Cheticamp and Margaree Harbor by sample time.

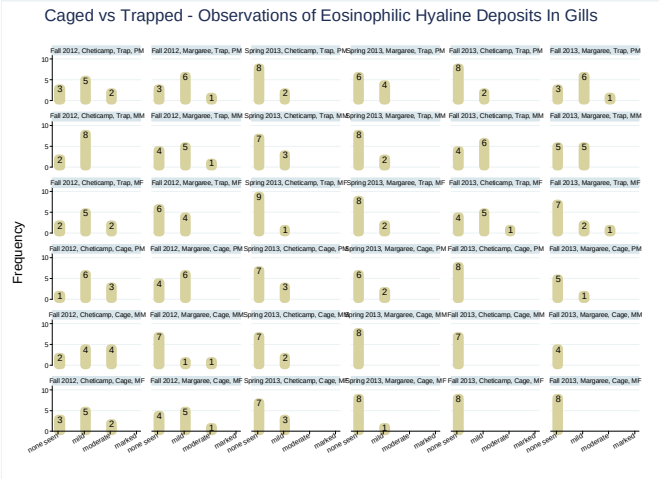

Figure 19. Frequency distribution of scores for eosinophilic hyaline deposits in gills of caged and free/trapped crabs at Cheticamp and Margaree Harbor by time.

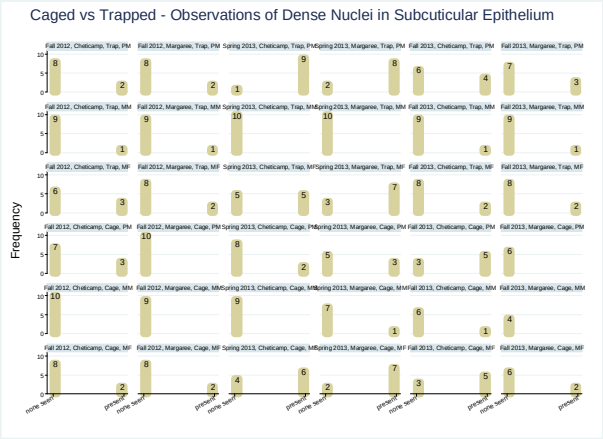

Figure 20. Frequency distribution of scores for dense nuclei of epithelium of caged and free/trapped crabs at Cheticamp and Margaree Harbor by sample time.

Inclusion Bodies

Among free crabs, the small intracytoplasmic inclusions, eosinophilic to amphophilic, (Figure 21) were observed in eight crabs (five PM, one LM and two MF) summarised in Table 14. Inclusions were noted most often in crabs from Cheticamp (n = 5), then Margaree (n = 2) and finally only a single crab in Grande Rivière. Inclusions were noted in both epithelial and endothelial cells in all but one crab (fall 2013, $PM_{Cheticamp}$) where inclusions were in epithelial cells only. Inclusion density was scored as moderate or high in all crabs but the one LM and a fall 2013 $PM_{Cheticamp}$ crab. Tissue recovered from the samples collected for scanning electron microscopy studies (see Section IV-1-3) were processed for transmission electron microscopy (Diagnostic Services, Atlantic Veterinary College). Images revealed very high numbers of viral particles (~ 70 – 80 nm in diameter) in honeycomb-like arrangements filling the cytoplasm of subcuticular epithelial cells (Figures 22 & 23). Far fewer viral particles were occasionally noted in endothelial cells.

The occurrence of viral inclusions had an odd pattern as they were only noted in free crabs in fall 2012, caged crabs in spring 2013, and more in free crabs again in the fall of 2013 (Table 14). All had inclusions in both epithelial and endothelial cells but for two crabs (one fall 2013 free $PM_{Cheticamp}$ and one fall 2013 caged $MF_{Margaree}$) which had epithelial inclusions only.

Table 14. Summary table showing number of gill samples in which intracytoplasmic inclusions were observed (in epithelium and/or endothelium) from each station.

Station	Gear Type	Sampling Time											
		Spring 2012			Fall 2012			Spring 2013			Fall 2013		
		PM	LM	MF	PM	LM	MF	PM	LM	MF	PM	LM	MF
Cheticamp, NS													
	Free	n/e[1]	n/e	n/e	1/10	1/10	1/9	0/10	0/10	0/10	1/10	0/10	1/10
	Caged	n/c[2]	n/c	n/c	0/10	0/10	0/10	0/10	4/9	1/10	0/8	0/7	0/8
Margaree, NS													
	Free	n/e	n/e	n/e	1/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10
	Caged	n/c	n/c	n/c	0/10	0/9	0/10	2/8	2/8	0/9	0/6	0/5	1/8
Louisbourg, NS													
	Free	n/e	n/e	n/e	0/10	0/10	0/10	0/10	0/10	0/3	n/c	n/c	n/c
Grand Rivière, QC													
	Free	n/e	n/e	n/e	1/10	0/10	0/11	0/10	0/10	0/10	n/c	n/c	n/c

[1]not examined, [2]not collected

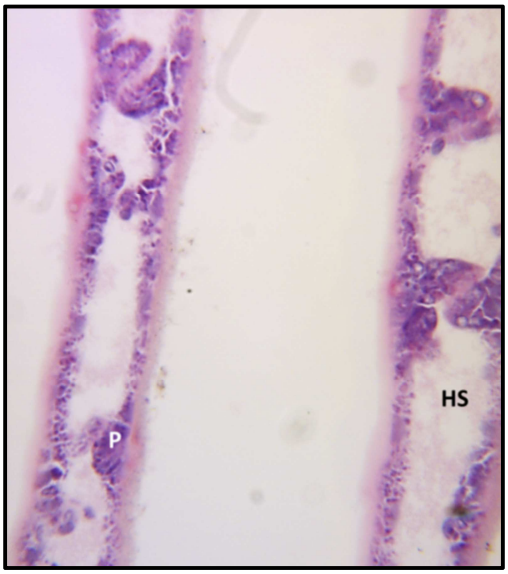

Figure 21. Histology of gill lamellae (H&E). Two gill lamella with epithelial cell cytoplasm filled with small amphophilic granules. Note the thickness of the normally attenuated epithelium. Pillar cell (P); hemolymph space (HS).

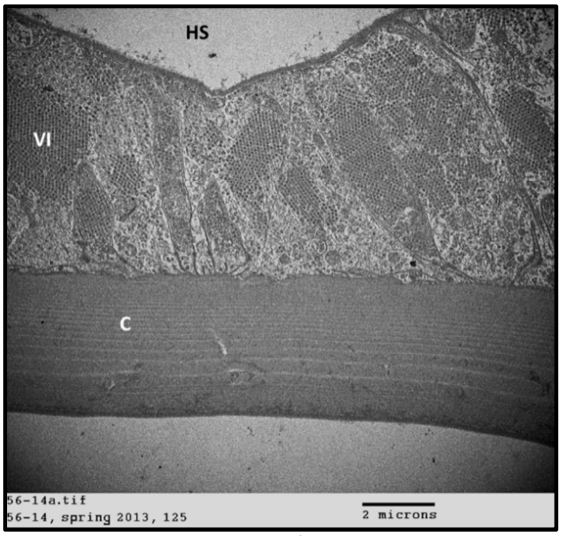

Figure 22. TEM image of gill. Viral inclusions (VI) corresponding to coarse granulation can be seen in a honeycomb -like arrangement. Cuticle (C); hemolymph space (HS).

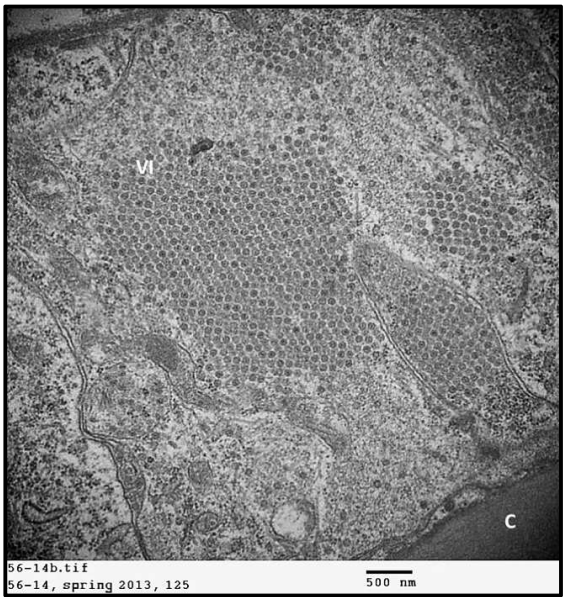

Figure 23. TEM image of gill. Viral inclusions (VI) in the cytoplasm of an epithelial cell. A small section of cuticle is (C) present in the lower right corner of image.

Conclusions: Gill Histology

Histologic examination of gill tissue of free crabs from four stations collected in fall 2012 and spring 2013 identified minor trends associated with sex, region and/or season. Minimal changes were associated with 2 weeks, six months, or 12 months of caging *per se* in samples collected from Cheticamp and Margaree stations in fall 2012, spring 2013, and fall 2013. The histology of gill tissue did not vary between free and caged crabs. Intracytoplasmic viral inclusions involving primarily subcuticular epithelial cells were more numerous than in hepatopancreatic tissue and were identified primarily in crabs from the Cheticamp and Margaree regions.

There were some differences in commensal gill organisms between caged and free crabs, however. Bryozoans, while uncommon, were observed more often on gills from free LM crabs at all stations at both sample times. The larger LM crab gill chambers may provide a more favourable environment. In contrast, bacterial fouling and sediment deposits were more prominent in the smaller free PM and MF crabs than the LM crabs.

There was a tendency for fouling scores to be lower in caged PM and MF crabs after six and 12 months of caging. This was somewhat unexpected as it was anticipated that crabs in poorer nutritional condition would be expected to expend less energy for gill cleaning. Perhaps the commensal organisms are less attracted to animals in poorer condition? Nematodes were quite small and usually found ensconced within the accumulations of bacteria and sediment, so it is not surprising that their scores tended to parallel that for bacterial fouling and sediment accumulation. It is noted that gross examination of fresh gills and wet mounts would likely provide a better indication of overall levels of commensals and fouling (see Section IV-1-4)). The effect of histological slide processing, involves multiple rinsing steps, on the type and amount of commensals and fouling observed is not known.

Reserve inclusion cells (RI) are considered to fluctuate in size and number with concert with the nutritional condition of the animal (Johnson 1980b). This was observed in the hepatopancreas samples (see Section IV-6-5). A seasonal trend was similarly noted for gill tissue with higher scores in the fall 2012 (period of high nutrition) samples for free and caged crabs, with disappearance of RI cells in the spring (period of lower nutrition). As the fall 2012 crabs had only been caged for two weeks, no differences were expected or found. Consistent with this, RI cells appeared to be returning in free crabs by spring 2013, but not caged in crabs. The RI cells are located within the loose connective tissue of the septum which was not always present, or present in only small amounts in many crabs. This would have affected the ability to detect them. It may also be possible that RI cells

'refill' later in the connective tissue of the gill than in the hepatopancreas and/or are always present in lower numbers.

Inflammatory changes in the gills were minimal, regardless of caged or free status. Small hemocyte nodules, often with dark brown-gold pigment (melanin) suggesting chronicity, showed a tendency for higher scores in free LM and/or MF crabs at all stations. Median scores for nodules were significantly higher for spring 2013 caged LM and MF crabs at Margaree Harbor than for free counterparts. Nodules found in the gills could have formed *in* situ, as a result of hemocytes attracted to bacteria which had settled in gill tissue. Alternately, the hemocyte nodules may have formed around bacteria while both were still circulating within the vaculature and became trapped/filtered out when attempting to pass through smaller vessels in the gill filaments. No infective agents were observed in the nodules; however this is not uncommon even when the animals are known to be bacteremic (A. Battison, Personal observations). Nutritional stress and subsequent decreased defenses and/or injury from conspecifics in group-caged MF crabs, may account for the slightly increased scores in caged crabs. The etiology of the tiny intraepithelial abscesses noted almost exclusively (4/6) in free $LM_{\text{Grande Rivière}}$ remains undetermined.

The eosinophilic hyaline deposits in the vascular spaces observed most often at the gill tips were an unusual finding. The cause remains speculative. The eosinophilic granules/precipitate in hemolymph spaces are interpreted as representing hemolymph proteins, of which hemocyanin is the main component in crustaceans. Development of the eosinophilic to occasionally red-gold hyaline material is typical for inflammatory reactions where prophenoloxidase has been activated, usually in association with release of activating factors from hemocytes (Sritunyalucksana & Söderhäll 2000). These lesions often proceed to form deposits of brown-gold pigment (melanin) (Sritunyalucksana & Söderhäll 2000). Hemocyte infiltrates were not part of the gill lesions in these crabs, however.

Hemocyanin is known to have phenoloxidase activity that can be activated by non-hemocyte derived factors such as detergents, sodium dodecyl sulphate (SDS) under laboratory conditions (Perdomo-Morales *et al.* 2008). The lesions observed in this study could be interpreted as concentration of hemolymph proteins secondary to gill dehydration. The change was noted more often in the gills of the smaller PM and MF crabs which may be at a greater risk of drying during transport in coolers. A possible explanation could be that slowed hemolymph flow, expected as hemolymph viscosity increased, led to local hypoxia/tissue injury triggering hemocyanin phenoloxidase activity. It is interesting that this change was uncommon in the 12 month caged crabs. Hemolymph total protein concentration, and presumably hemocyanin as well, was much lower in this group (see

Section VII). Less hemocyanin could translate into less granulation and thereby, less hemolymph concentration and hyalinization.

The etiology of the focal to multifocal hyperchromic and pyknotic nuclei and cytoplasmic vacuolation affecting epithelial cells at the base of the gill lamella and most often in the spring samples from PM and MF crabs also remains undetermined. Possible explanations to consider are tissue injury related to handling or transport of the smaller crabs, environmental (water-borne toxin/contaminant), or an infectious (viral) cause. Transmission electron microscopic examination of the tissues may be informative but has not been performed to date.

The intracytoplasmic viral inclusions noted in low to moderated numbers in the endothelial cells, and possibly fixed phagocytes, of the hepatopancreas (Section IV-6-5) were observed at much higher numbers in gill epithelium and sometimes in gill endothelium in lower amounts. The inclusions were conspicuous, at times completely filling the subcuticular cell cytoplasm. When observed, the inclusions were present in epithelium only or, in epithelium and endothelium but never only endothelium. The density of inclusions was also always greater in epithelial cells. These findings could indicate that epithelium is the preferred and first tissue infected. As with the hepatopancreas tissues, most of the infected crabs were from the Cheticamp and Margaree stations.

Unusual distributions of inclusions were noted in caged as compared to free crabs. First, inclusions were only detected in free crabs at either station in the fall 2012 samples despite the caged crabs having been collected and transferred to cages the same day that the free crabs were collected and processed. Also, the highest number of virus-positive crabs was noted for the spring 2013 caged group while no free crabs were affected. Possible explanations could include one or more of: 1) natural progression of the disease over the winter led to death, weakness and predation, or poor trapping success of free crabs, while caged crabs were protected from predation and were already caged so would be sampled; 2) caged crabs could not change location and may have been exposed to a vector that normal movement of free crabs allowed them to avoid; 3) nutritional stress compromised the immune system of caged crabs allowing the infection to progress more quickly.

The only virus reported for *C. opilio* is a baculo-like virus, *Co*BV, from the Sea of Japan (Kon *et al.* 2011). Viral particles (144 – 338 nm in diameter) were found in the nucleus of interstitial cells of various tissues but not in epithelial or endothelial cells. The viral particles in the current study ranged from about 70 nm to 80 nm in diameter. More study of this virus and its potential impact on this (or other hosts) would be warranted. Comparing the results found in other parts of the current study of these infected animals to uninfected crabs could illuminate some of the pathogenic effects, if any, the virus is having but this was beyond the scope of the current report.

Histologic evaluation of gill tissue did not reveal any particular characteristic unique to caged crabs other than perhaps a slightly lower degree of gill fouling by bacteria, sediment, and nematodes. Gill samples did provide better material for evaluation of the intracytoplasmic inclusion bodies first noted in the hepatopancreas and confirmed their viral origin. Gill would be an excellent tissue to use for screening purposes for the virus in the event that further work is to be done.

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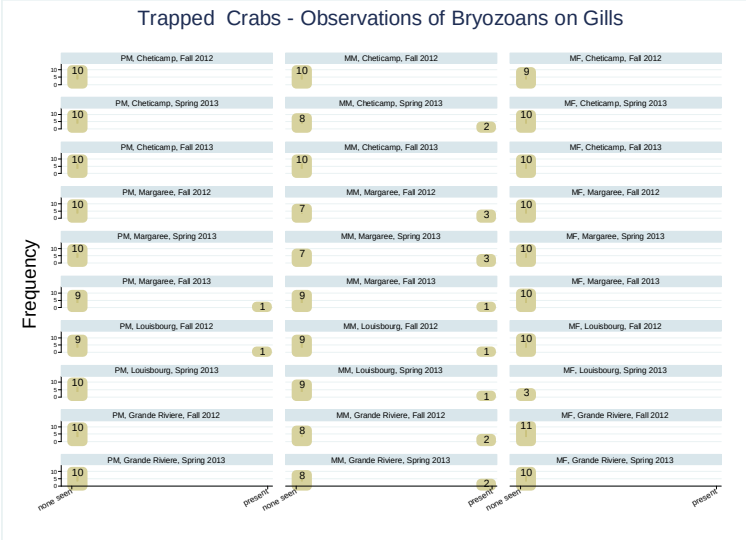
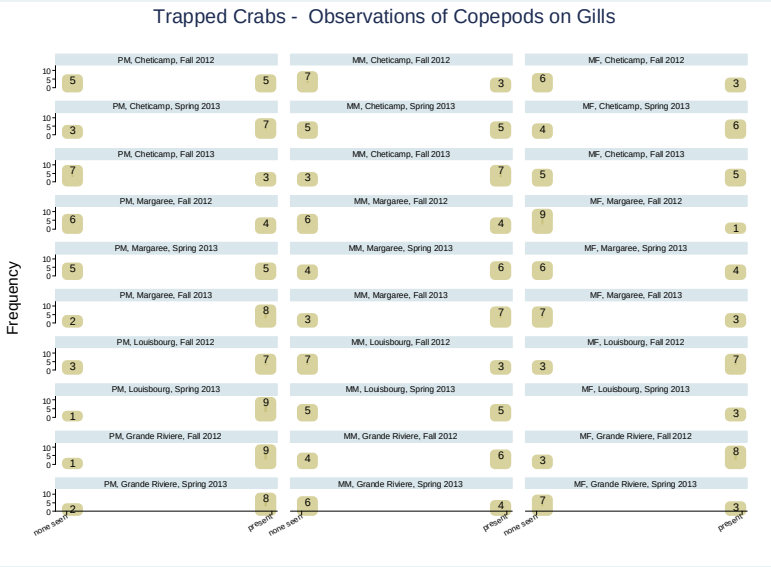
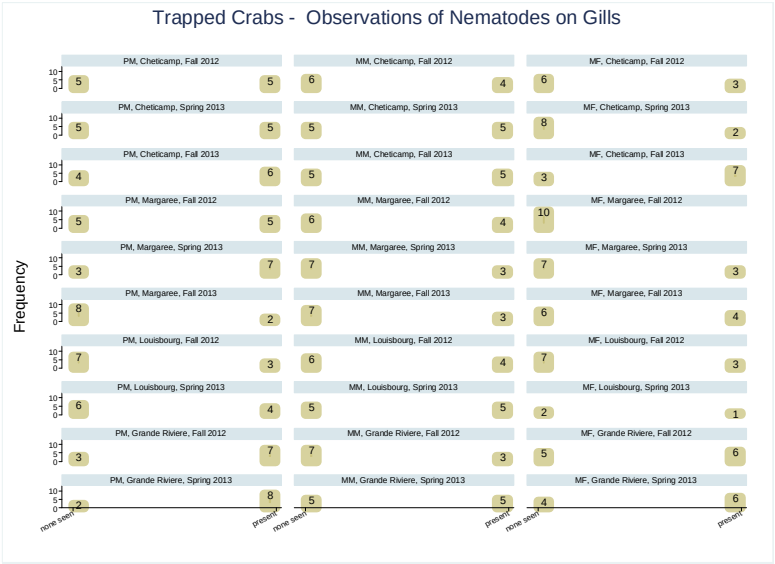
IV-1-8 Appendices

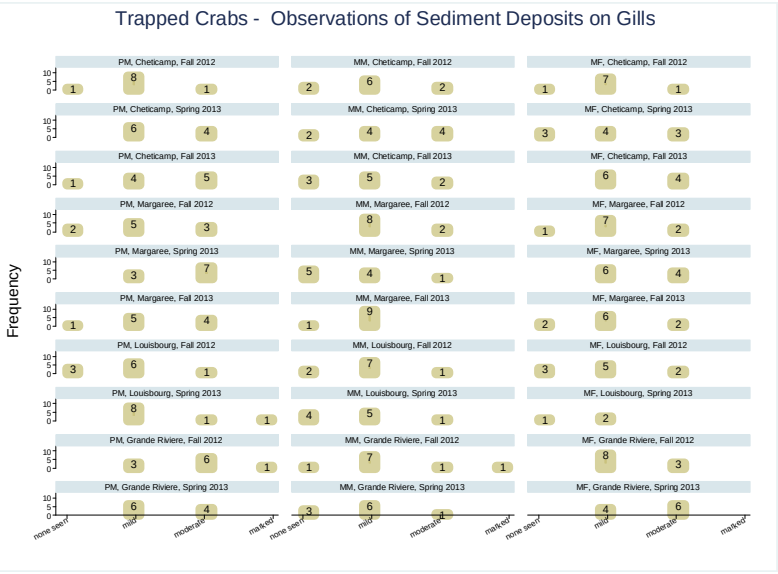
Appendix A Frequency Distribution Histograms – Gill Commensals in Free (Trapped) Crabs & Caged vs Free Crabs

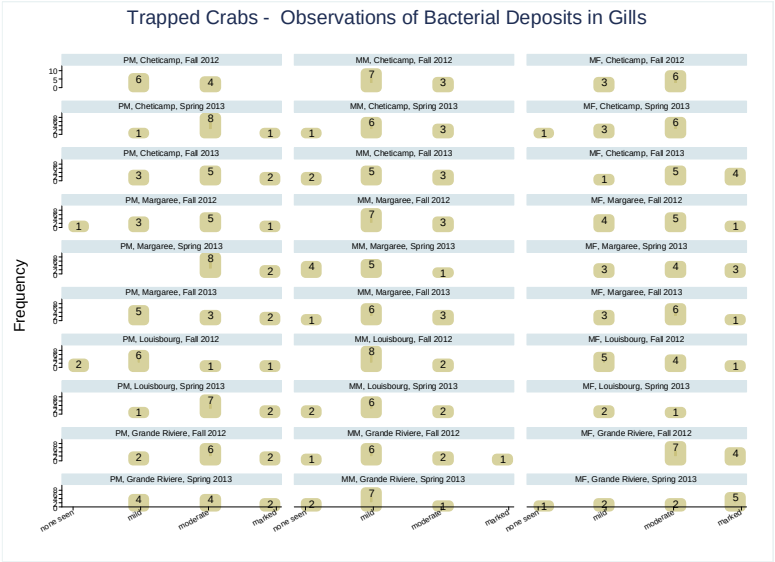


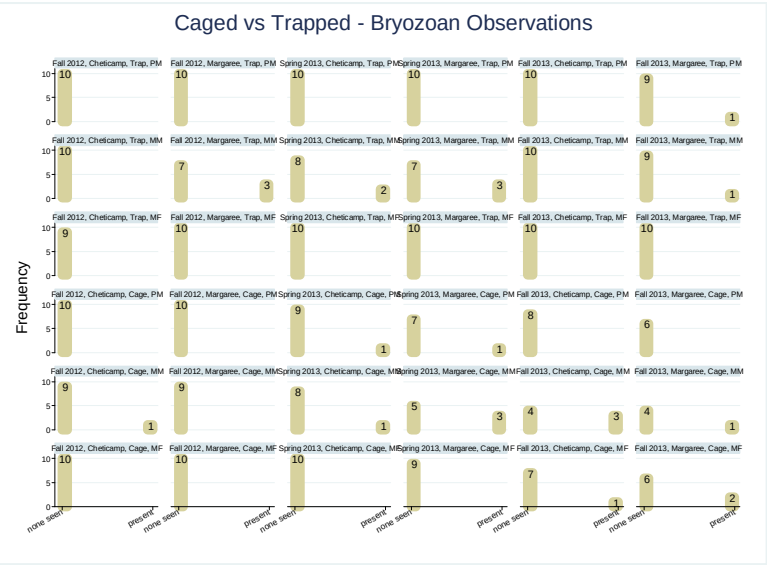


Appendix A (continued)









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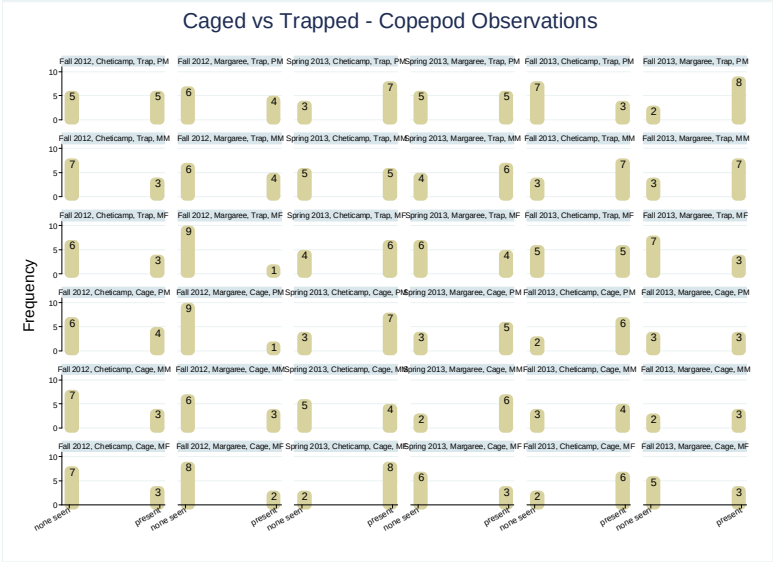

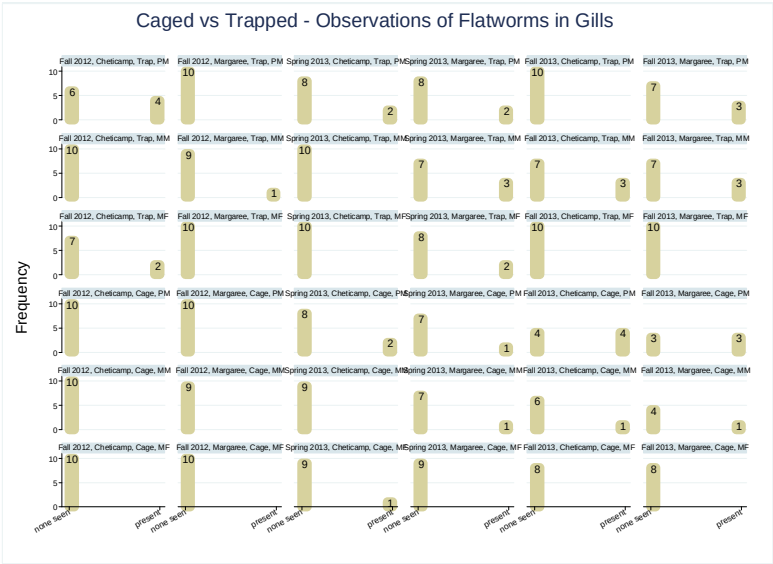

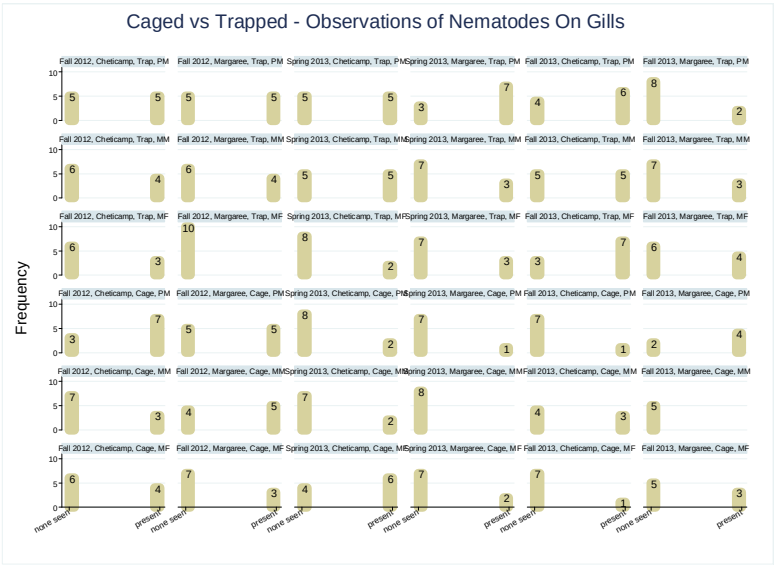

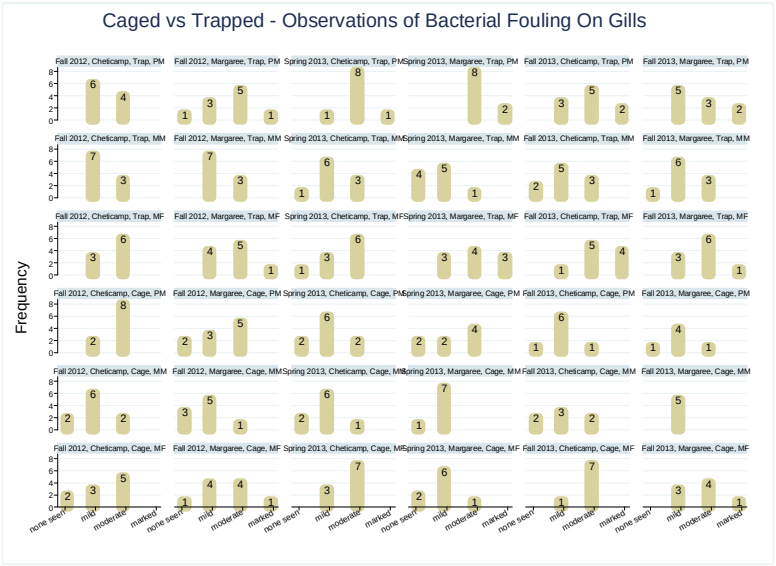

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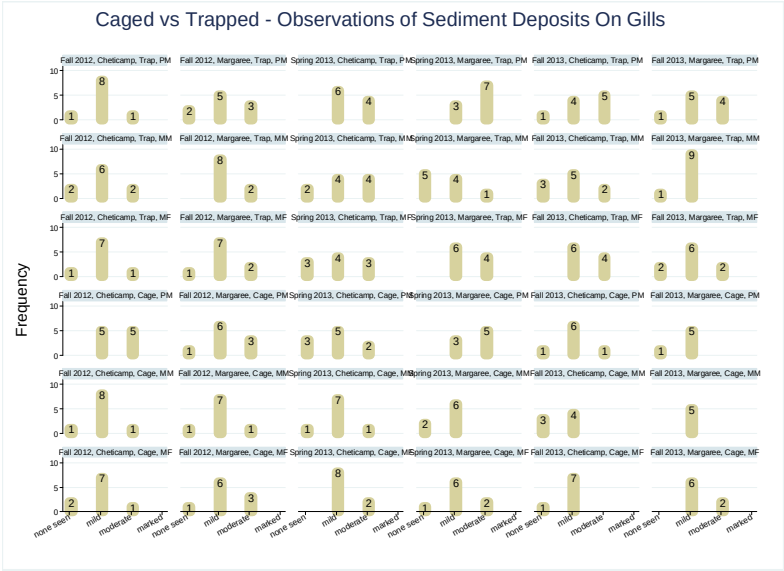

IV-2 ANTENNULES

IV-2-1 Introduction

A pair of short antennules is located close to the head in a cavitycalled the antennular fossa (Figure 1). The antennules arise below and slightly medial to the eyestalks. They consist of a basal peduncle with two antennular segments (proximal and distal). The distal antennular segment bears inner and outer flagellum. The outer flagellum bears rows of aesthetasc hairs (Figure 1). The lateral flagella of the antennules containing crustacean-specific aesthetasc sensilla with olfactory neurons are considered as chemoreceptors. Although crabs may completely conceal the outer and inner flagella into the cephalothoracic groove when necessary, the outer flagellum could still be exposed to water. Therefore, if any disturbances occurred, the aesthetasc hairs may reflect some degree of impact.

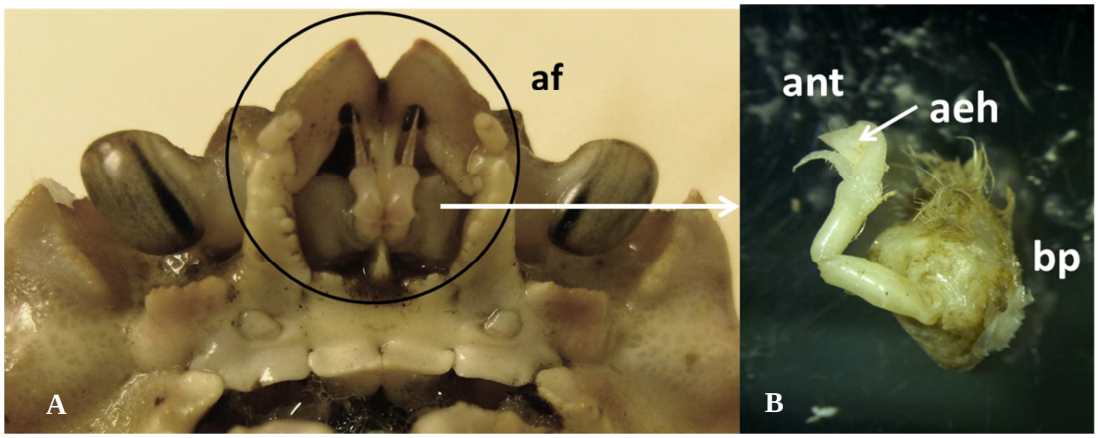

Figure 1. A: Frontal view of head region of a snow crab showing the position of pair of antennules in antennular fossa (af) and B: Enlarged view of a right antennule (ant) composed of basal peduncle (bp) and two antennular segments. The distal antennular segment bears aesthetasc hairs (aeh).

Light microscopic observations

A pair of antennules with peduncles were dissected out and preserved in 10% formalin solution in 20 ml vial. Each antennule was further dissected out at the base of peduncle for observation. The peduncle in which statocyst resides was dissected for statocyst observations (Section IV-3). Antennules were dissected out from 1125 individuals. The antennule was photographed with a digital camera (PixeLink PL-B686CU, Canimpex Enterprises Ltd) under a dissection microscope (Mz12.5, Leica) with magnification of up to 25X (Figure 1).

Scanning Electron Microscopic (SEM) observations

The right antennule with peduncle was dissected out from every 5th crab. The samples were immediately fixed at 4°C with 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 for 1 hour and rinsed in cold 0.175M cacodylate buffer at pH 7.2. Dehydration was achieved through increasing the concentration (up to 100%) of ethanol within 15 minutes. The samples were then dried in a Critical Point Dryer (CPD) at the critical point of CO2. Specimens, mounted on aluminum stubs with double side adhesive tabs, and sputter-coated with gold-palladium, were examined with a JEOL 6400SEM at 10 kV acceleration voltage at the Microscopy and Microanalysis Facility, University of New Brunswick (Fredericton, NB). A total of 237 antennules were observed and photographed with a magnification of 50X. These photographed images were not used for direct classification of antennule conditions, but rather used for additional detailed information.

IV-2-2 Morphological observations and condition of antennules

The conditions of the outer flagellum and aesthetasc hairs of the antennules were classified into five categories: 1) clean, 2) relatively clean (some dirtiness on the aesthetasc hairs), 3) intermediate, 4) dirty and 5) damaged outer flagellum and/or aesthetasc hairs (Figure 2).

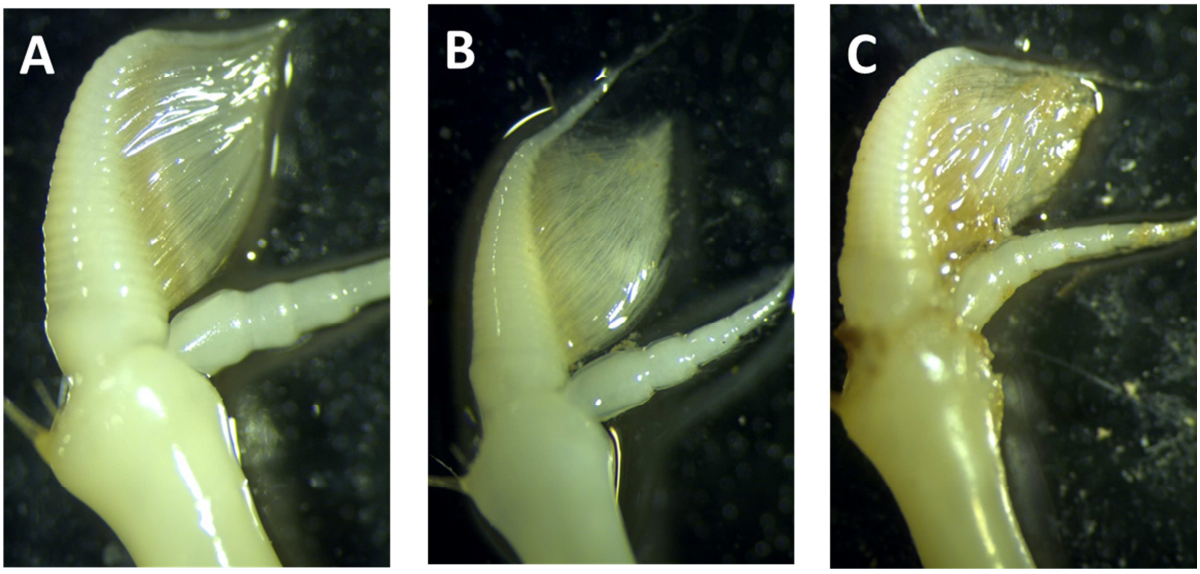

Figure 2. Condition of antennule observed under a dissection microscope at a magnification 25X (A: Clean, B: Relatively clean, C: Intermediate)

Out of 1,125 antennules observed from caged and free crabs, 865 (76.8%) were classified as clean, 230 (20.4%) as relatively clean, 20 (1.8%) as intermediate, and 11 (1%) as dirty (Tables 1 & 2). Some degrees of damage in aesthetasc hairs were previously observed in the wild (pers. obs. M. Moriyasu), which seems to be age-dependent i.e. more aesthetasc hairs are damaged in

older individuals. However, in this study, although some antennular segments (proximal and/or distal) were blackened (possibly bacterial infection), no damaged aesthetasc hair was observed.

In comparison with the results obtained by Moriyasu *et al.* (unpublished), the category 'relatively clean' in this study corresponds to 'clean' in Moriyasu *et al.* (2011). Although some aesthetasc hairs in this study were classified as'dirty', these were not comparable to the 'dirty' aesthetasc hairs observed by Moriyasu *et al.* (2011) in which sediment-like substances were tangled into the aesthetasc hairs. Some 'Intermediate' antennules were also observed in this study, but the accumulation of substances on these hairs seemed to be of organic origins.

The results of antennule condition observations (Tables 1 & 2) were regrouped into two conditions for analysis: 1) clean and 2) non-clean (relatively clean, intermediate and dirty conditions). The percentage of clean condition was calculated by crab category and by location. The percentage of clean condition was then compared between treatments (free and caged) for each category of crab (large male, pygmy male and female) with deviance table analysis (McCullagh & Nelder 1989: see Section IV-1-3 for detail of the analysis).

The results showed (Table 3) that there was no effect of caging on the condition (% of clean antennule) of antennule for three categories of crab (large male, pygmy male and female).

Table 1. Cleanliness categorization of antennules by season, station, treatment, and crab category (large males (LM), pygmy males (PM), mature females (MF) in 2012. (1: clean, 2: relatively clean, 3: intermediate, 4: dirty).

Grande-Rivière Free

	1	2	3	4	Total
LM	16	5	0	0	21
PM	3	0	0	0	3
MF	12	6	1	1	20
	31	11	1	1	44

	1	2	3	4	Total
LM	12	8	1	0	21
PM	7	11	0	0	18
MF	8	10	2	1	21
	27	29	3	1	60

Margaree free

	1	2	3	4	Total
LM	15	3	2	0	20
PM	14	4	2	0	20
MF	12	7	0	1	20
	41	14	4	1	60

	1	2	3	4	Total
LM	13	9	0	0	22
PM	10	8	1	1	20
MF	15	5	0	0	20
	38	22	1	1	62

Cheticamp free

	1	2	3	4	Total
LM	20	0	0	1	21
PM	19	2	0	0	21
MF	19	1	0	0	20
	58	3	0	1	62

	1	2	3	4	Total
LM	14	6	0	0	20
PM	14	2	1	2	19
MF	18	1	0	0	19
	46	9	1	2	58

Louisbourg free

	1	2	3	4	Total
LM	14	5	0	0	19
PM	17	3	0	0	20
MF	23	1	0	0	24
	54	9	0	0	63

	1	2	3	4	Total
LM	2	15	2	0	19
PM	15	4	1	0	20
MF	15	5	0	0	20
	32	24	3	0	59

Margaree caged

	1	2	3	4	Total
LM	11	7	0	0	18
PM	17	3	0	0	20
MF	16	3	0	0	19
	44	13	0	0	57

Cheticamp caged

	1	2	3	4	Total
LM	18	2	0	0	20
PM	18	1	0	0	19
MF	19	1	0	0	20
	55	4	0	0	59

Table 2. Cleanliness categorization of antennules by season, station, treatment, and crab category (large males (LM), pygmy males (PM), mature females (MF) in 2013. (1: clean, 2: relatively clean, 3: intermediate, 4: dirty).

SPRING 2013

Grande-Rivière free

	1	2	3	4	Total
LM	18	2	0	0	20
PM	14	4	1	0	19
MF	13	4	2	1	20
	45	10	3	1	59

Margaree free

	1	2	3	4	Total
LM	18	1	0	0	19
PM	16	3	0	0	19
MF	17	4	0	0	21
	51	8	0	0	59

Cheticamp free

	1	2	3	4	Total
LM	20	0	0	0	20
PM	20	0	0	0	20
MF	21	0	0	0	21
	61	0	0	0	61

Louisbourg free

	1	2	3	4	Total
LM	16	4	0	0	20
PM	14	5	1	0	20
MF	4	1	0	0	5
	34	10	1	0	45

Margaree caged

	1	2	3	4	Total
LM	12	4	0	0	16
PM	13	5	0	0	18
MF	16	2	0	0	18
	41	11	0	0	52

Cheticamp caged

	1	2	3	4	Total
LM	17	1	0	0	18
PM	16	3	0	0	19
MF	18	1	0	0	19
	51	5	0	0	56

FALL 2013

Margaree free

	1	2	3	4	Total
LM	12	5	1	1	19
PM	13	5	2	0	20
MF	16	4	0	0	20
	41	14	3	1	59

Cheticamp free

	1	2	3	4	Total
LM	13	7	0	0	20
PM	16	4	0	0	20
MF	19	2	0	0	21
	48	13	0	0	61

Margaree caged

	1	2	3	4	Total
LM	4	6	0	0	10
PM	12	2	0	0	14
MF	13	3	0	1	17
	29	11	0	1	41

Cheticamp caged

	1	2	3	4	Total
LM	16	0	0	0	16
PM	10	5	0	1	16
MF	11	5	0	0	16
	37	10	0	1	48

Table 3. Percentage (%) of clean antennules and results of deviance table analysis for each category of crab (large male, pygmy male and mature female) between caged and free crabs in Cheticamp and Margaree Harbor stations for fall 2012 (free vs 2 week caged), spring 2013 (free vs 6-month caged), fall 2013 (free vs 12-month caged).

Large Males:

Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	70	100	65
Caged	90	94.4	100

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	59.1	95	63.2
Caged	61.1	75	44.4

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	214	206.32			
Model 2	213	198.00	1	0.42913	0.516

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

Pygmy Males:

Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	73.7	100	80
Caged	94.7	84.2	62.5

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	50	84.2	65
Caged	85	72.2	85.7

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	220	227.87			
Model 2	219	226.93	1	0.93893	0.3326

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

Mature Females:

Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	94.7	100	90.5
Caged	95	94.7	68.8

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	75	80.0	80.0
Caged	84.2	88.9	76.5

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	226	175.86			
Model 2	225	175.72	1	0.14311	0.7052

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

IV-2-3 References

DFO 2004. Potential Impacts of Seismic Energy on Snow Crab. DFO Can. Sci. Advis. Sec. Habitat Status Report 2004/004.

McCullagh P. and Nelder J. 1989. Generalized Linear Models, Second Edition. Chapman & Hall/CRC.

Moriyasu M., Allain R., Hébert M., Ferron C., Benhalima K. and Belfry C.S. 2011. Study of possible impact of seismic energy on female snow crab, Chionoecetes opilio (Cruistacea, Decapoda), by a caging experiment off western Cape Breton Island, Gulf of St. Lawrence, Canada. Mus. Reg. Sci. Nat. Torino, IX Colloquium Crustacea Mediterranea Torino, September 2-6, 2008: 79-100.

Moriyasu M., Benhalima K., Allain R., Hébert M., Wade E., Sabean C. and Belfry S.C. (unpublished). Effects of seismic noise on female snow crab (*Chionoecetes opilio*) evaluated by caging study conducted off western Cape Breton Island in 2003-2004. Draft Working paper, Seismic Peer Review meeting September 29, 2004. Moncton NB.

IV-3. STATOCYSTS

IV-3-1 Introduction

In snow crab as well as in all decapods, a pair of statocysts (Prentiss, 1901; Cohen & Dijkgraaf, 1961; Sandeman & Okajima 1972) is located in the first segment (basal peduncle) of the antennules. Statocysts come into contact with external water through a slit-median aperture. These organs consist of a transparent membranous wall of a complex shape due to a deep invagination. The mechano-sensory sensilla, composed of four different types of sensory hairs, (group hairs, thread hairs, hook hairs, and statolith hairs) project into the lumen from the median and lateral wall and the floor of the statocyst. Moriyasu *et al.* (unpublished) provided detailed observations on each component of statocyst.

Group hairs are large (15µ m in diameter at the base), long (500 µm in length), simple and smooth. The shaft tapers to a sharp point. They form a group of approximately 100 hairs uniformly arranged on the convex surface of the lateral wall of the statocyst. Thread hairs (about 30 in number) are extremely fine (2 µm in diameter) and long (300 µ m in length). They bear delicate long thin pinnules arranged in an alternating pattern. Each hair is set into a cup-like depression. They form a single row projecting free into the lumen and located on the median wall above the sensory cushion floor of the statocyst. The tips come into contact with the statolith. Hook hairs (about 20 in number) measure 80 µm in length and 5 µm in diameter and are arranged in a crescent-shaped row along the sensory cushion floor of the statocyst. The hairs are bent into a hook-like shape towards the crescent and bear plumose hairs to where attach small fragments of bounded particles.

The base of the shaft is set into a cup-shaped depression with supra-cuticular insertion. Statolith hairs are numerous, extremely small, short and fine (less than 15 µm in length and 2 µm in diameter). They touch and penetrate into the statolith with their fine tips. The statolith hairs are implanted and arranged in the floor of the sesnsory cushion of the statocyst. The statolith (a lenticular shape and composed of compacted various fragments from ocean bottom sediment such as siliceous and the diatoms) is either in contact with or attached to the tips of statolith hairs.

It appeared from the 2003 seismic study (DFO 2004, Moriyasu *et al.* 2011, Moriyasu *et al.* unpublished) that statocysts were seemed to be an appropriate indicator for impact assessment. Statocysts possess the ability to signal the animal's position with respect to gravity (Cohen & Dijkgraff, 1961). Sandeman and Okajima (1972) demonstrated in mud crab, *Scylla serrata*, that thread hairs are most probably the receptors responsible for the detection of rotation about the vertical axis. Hook hairs are for the detection of rotation about the horizontal axis and statolith hairs are sensitive to position changes.

The effects of sound on sensory hairs of fish have been studied. McCauley *et al.* (2003) reported delayed effects of sound on caged fish. Sensory cells of ensonified fish were missing from the epithelium and there was considerable evidence of dying cells. Popper (2003) mentioned that intense sounds are able to damage the sensory hair cells of fish and even if the sounds do not kill the fish directly, permanent or even temporary loss of hearing will clearly affect the chance of survival.

IV-3-2 Materials and Methods

Moriyasu *et al.* (unpublished) observed group hairs and statolith to categorize the condition of statocyst. However, whole statocyst could not be consistently dissected out from all samples due to a very fragile membranous wall especially on the specimens preserved in 70% ETOH. In addition, the statolith is often dislodged and the thread hairs were damaged during the dissection. However, the group hairs are easily and consistently dissected out under a dissection microscope with lesser impact on its structure and condition. According to Moriyasu *et al.* (2011) statocyst conditionscan be determined either the presence/absence of stotolith or the degree of dirtiness of group hairs (Figure 4E in Moriyasu *et al.* 2011). Therefore, we chose group hairs as the best candidate for the observations of the condition of the statocyst with lesser impact of dissection-induced artefact.

Light microscopic observations

The right statocyst (basal peduncle of the antennule with two antennular segments) was dissected out from antennular fossa from frontal region of the carapace (Figures 1A, B) of each crab and immediately put in 70% ETOH for further observations.

Prior to the dissection of the basal peduncle from free and caged samples, 50 pairs of basal peduncles were colleted from additional snow crab samples on which a practice dissection was performed in order to ensure consistent results. The original objective was to dissect out main components of the statocysts i.e. group hairs and statolith. The thread hairs are extremely fine and were not useful to assess the abnormal condition of statocyst (Moriyasu *et al.* 2011, Moriyasu *et al.* unpublished). During this practice session, statoliths were often (32 out of 89 successfully dissected statocysts) dislocated from the normal position. As such, it was determined to be prudent not to use the condition of the statolith when determining statocyst conditions. This present study therefore only focused on group hairs to determine the condition of statocysts.

The basal peduncle of the antennule with two antennular segments (Figure 1B) was put on a petri dish under a dissection microscope so that the frontal region of the peduncle was up (face to objective lens). With a razor blade, the peduncle was sliced into two pieces on the sagittal plane at the position posterior to the basal segment of the antennule (Figure 1C). Group hairs

are lodged in the posterior piece of the peduncle (Figure 1D). Group hairs of the statocyst were dissected out from all 1,125 samples and photographed with a digital camera (PixeLink PL-B686CU, Canimpex Enterprises Ltd) under a dissection microscope (Mz12.5, Leica) with a magnification of 32X.

Scanning Electron Microscopic (SEM) observations

Selected samples of group hairs dissected from the statocyst were fixed at 4°C with 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 for 1 hour and rinsed in cold 0.175M cacodylate buffer at pH 7.2. Dehydration was achieved through increasing concentrations (up to 100%) of ethanol within 15 minutes. The samples were then dried in a CPD at the critical point of CO2. Specimens, mounted on aluminum stubs with double side adhesive tabs, were sputter-coated with gold-palladium and examined with a JEOL 6400SEM at 10 kV acceleration voltage at the Microscopy and Microanalysis Facility, University of New Brunswick (UNB). Each specimen was photographed with magnifications of 25X and 150X. As the condition of group hairs can easily be observed under a dissection microscope, only 24 selected specimens were photographed to show the detailed arrangement of group hairs (Figure 2).

IV-3-3 Condition of group hairs in statocyst

Based on the observations by dissection microscope and SEM (Figure 2), the condition of statocysts (group hairs) was classified into four categories: 1) clean, 2) relatively clean, 3) intermediate and 4) dirty. In total 1,125 samples of group hairs were dissected out of which 1,086 (96.5%) were clean and 39 relatively clean (3.5%) (Tables 1 & 2). There was no 'intermediate or dirty' condition observed. There was no trace of displaced statolith on group hairs comparable to that observed by Moriyasu *et al.* (unpublished).

The results of statocyst (group hairs) condition observations (Tables 1 & 2) were regrouped into two conditions for analysis: 1) clean and 2) non-clean (relatively clean, intermediate and dirty conditions). The percentage of clean condition was calculated by crab category and by location. The percentage of clean condition was then compared between treatments (free and caged) for each category of crab (large male, pygmy male and female) with deviance table analysis (McCullagh & Nelder 1989: see Section IV-1-3 for detail of the analysis).

The results showed (Table 3) that there was no effect of caging on the condition (% of clean statocyst) of statocyst for three categories of crab (large male, pygmy male and female).

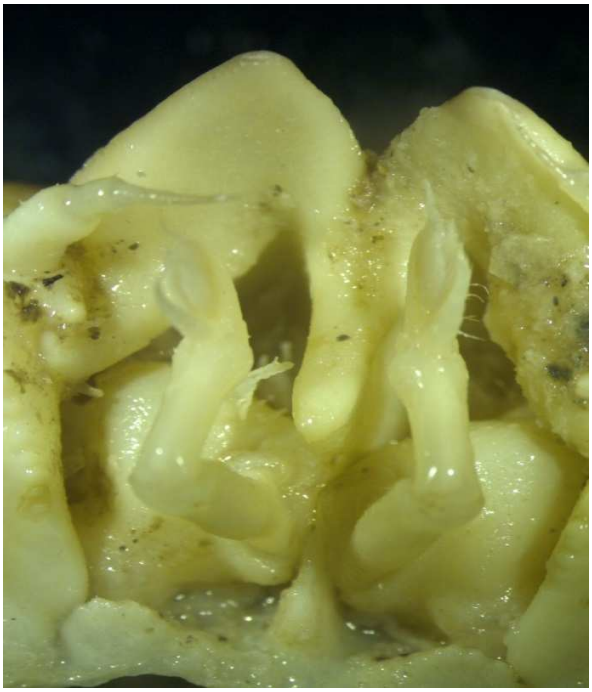

Figure 1A. Location of statocysts in an excavation (antennular fossa).

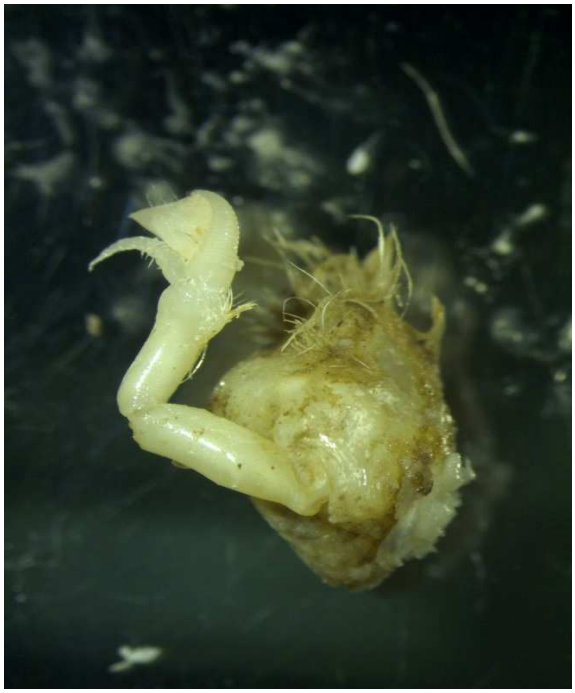

Figure 1B. Right statocyst dissected out from antennular fossa.

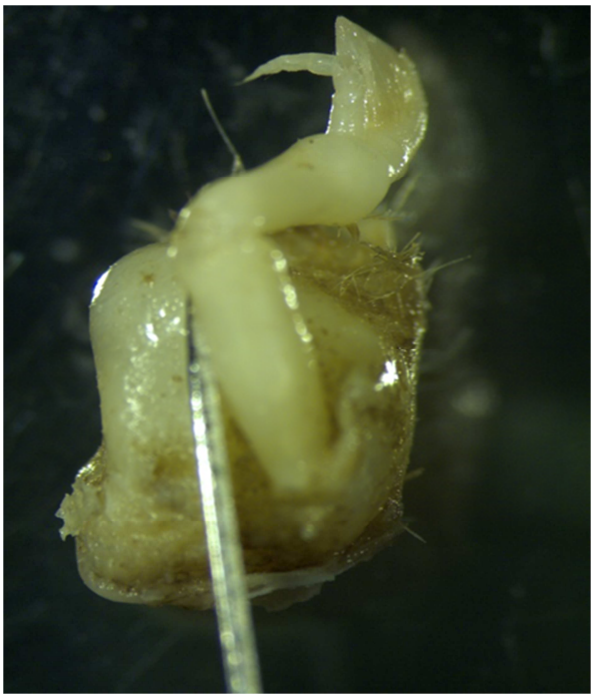

Figure 1C. The position of dissection of statocyst to extract group hairs shown with a scalpel blade.

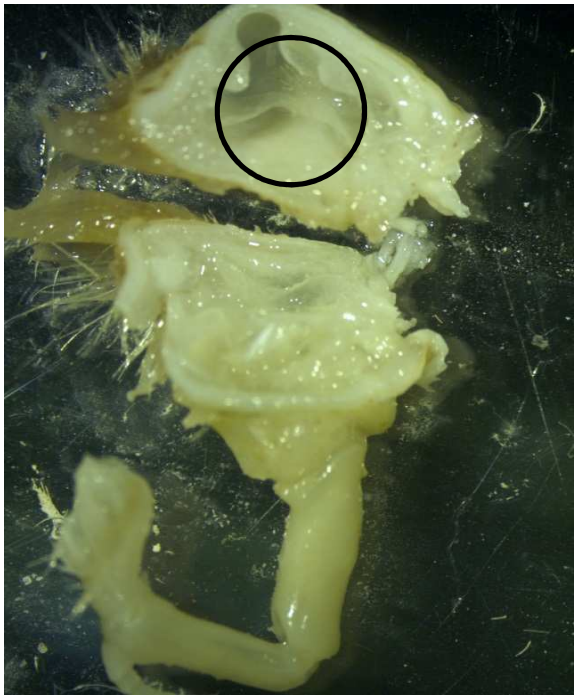

Figure 1D. Perpendicular cut of the right statocysts showing group hairs lodged in the left half of the statocyst (circle)

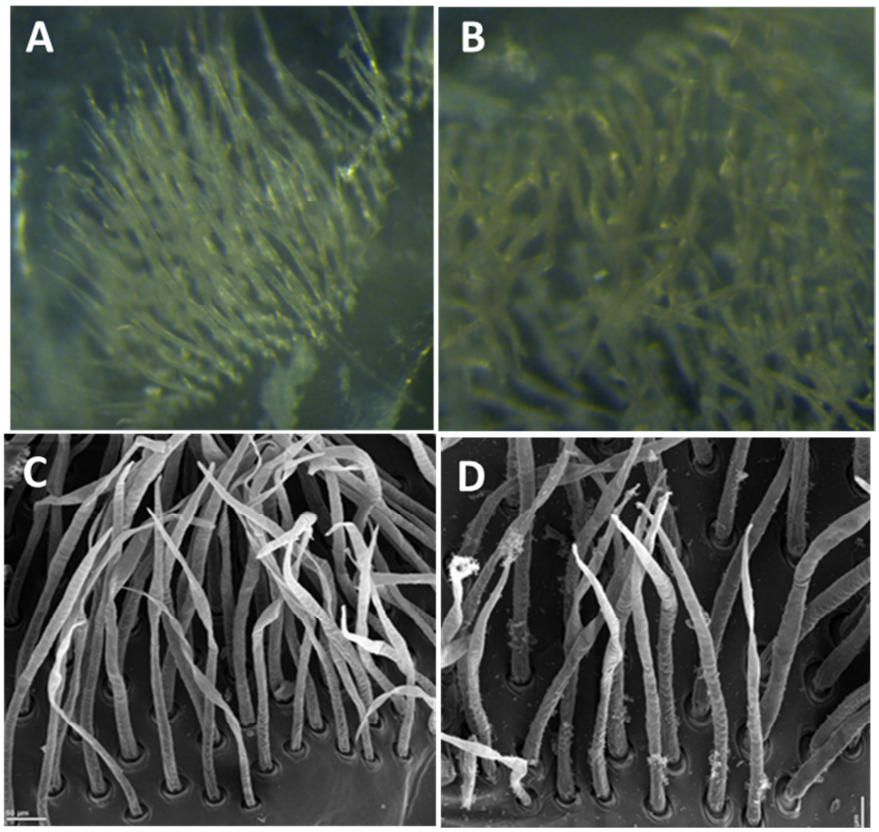

Figure 2. Condition of statocyst (group hairs). A&C: clean, B&D: relatively clean. A&B were pictured under dissection microscope (x25) and C&D were under Scanning Electron Microscope (x 150)

Table 1. Condition of statocyst (group hairs) by season, station, treatment and crab category (large males (LM), pygmy males (PM), mature females (MF) in 2012. (1: clean, 2: relatively clean, 3: intermediate, 4: dirty).

Spring 2012

		1	2	3	4	Total
Grande-Rivière free	LM	20	1	0	0	21
	PM	3	0	0	0	3
	MF	20	0	0	0	20
		43	1	0	0	44
Margaree free		1	2	3	4	Total
	LM	20	0	0	0	20
	PM	20	0	0	0	20
	MF	20	0	0	0	20
		60	0	0	0	60
Cheticamp free		1	2	3	4	Total
	LM	21	0	0	0	21
	PM	21	0	0	0	21
	MF	19	1	0	0	20
		61	1	0	0	62
Louisbourg free		1	2	3	4	Total
	LM	18	1	0	0	19
	PM	19	1	0	0	20
	MF	23	0	0	0	23
		60	2	0	0	62

Fall 2012

		1	2	3	4	Total
Grande-Rivière free	LM	19	2	0	0	21
	PM	19	0	0	0	19
	MF	20	0	0	0	20
		58	2	0	0	60
Margaree free		1	2	3	4	Total
	LM	22	0	0	0	22
	PM	20	0	0	0	20
	MF	19	1	0	0	20
		61	1	0	0	62
Cheticamp free		1	2	3	4	Total
	LM	20	0	0	0	20
	PM	17	2	0	0	19
	MF	19	0	0	0	19
		56	2	0	0	58
Louisbourg free		1	2	3	4	Total
	LM	19	0	0	0	19
	PM	19	1	0	0	20
	MF	19	1	0	0	20
		57	2	0	0	59
Margaree caged		1	2	3	4	Total
	LM	17	1	0	0	18
	PM	19	1	0	0	20
	MF	19	1	0	0	20
		55	3	0	0	58
Checticamp caged		1	2	3	4	Total
	LM	19	1	0	0	20
	PM	16	3	0	0	19
	MF	19	1	0	0	20
		54	5	0	0	59

Table 2. Condition of statocyst (group hairs) by season, station, treatment and crab category (large males (LM), pygmy males (PM) and mature females (MF) in 2013. (1: clean, 2: relatively clean, 3: intermediate, 4: dirty).

STATOCYST

Grande-Rivière free

	Spring 2013					Fall 2013				
	1	2	3	4	Total					
LM	19	1	0	0	20					
PM	19	0	0	0	19					
MF	20	0	0	0	20					
	58	1	0	0	59					

Margaree free

	Spring 2013					Fall 2013				
	1	2	3	4	Total		1	2	3	4	Total
LM	18	1	0	0	19	LM	19	0	0	0	19
PM	18	1	0	0	19	PM	19	1	0	0	20
MF	19	2	0	0	21	MF	20	0	0	0	20
	55	4	0	0	59		58	1	0	0	59

Cheticamp free

	Spring 2013					Fall 2013				
	1	2	3	4	Total		1	2	3	4	Total
LM	18	1	0	0	20	LM	20	1	0	0	21
PM	19	1	0	0	20	PM	20	0	0	0	20
MF	21	0	0	0	21	MF	21	0	0	0	21
	59	2	0	0	61		61	1	0	0	62

Louisbourg free

	Spring 2013					Fall 2013				
	1	2	3	4	Total					
LM	19	1	0	0	20					
PM	18	1	0	0	19					
MF	5	0	0	0	5					
	42	2	0	0	44					

Margaree caged

	Spring 2013					Fall 2013				
	1	2	3	4	Total		1	2	3	4	Total
LM	14	2	0	0	16	LM	9	1	0	0	10
PM	18	0	0	0	18	PM	13	1	0	0	14
MF	18	0	0	0	18	MF	17	0	0	0	17
	50	2	0	0	52		39	2	0	0	41

Checticamp caged

	Spring 2013					Fall 2013				
	1	2	3	4	Total		1	2	3	4	Total
LM	17	1	0	0	18	LM	13	3	0	0	16
PM	18	1	0	0	19	PM	16	0	0	0	16
MF	19	0	0	0	19	MF	16	0	0	0	16
	54	2	0	0	56		45	3	0	0	48

Table 3. Percentage (%) of clean statocyst (group hairs) and results of deviance table analysis for each category of crab (large male, pygmy male and mature female) between caged and free crabs in Cheticamp and Margaree Harbor stations for fall 2012 (free vs 2 week caged), spring 2013 (free vs 6-month caged), fall 2013 (free vs 12-month caged).

Large Males:
Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	90.0	95.2
Caged	95	94.4	81.2

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100.0	90.0	100.0
Caged	94.4	87.5	88.9

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	215	100.192			
Model 2	214	97.573	1	2.6184	0.1056

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

Pygmy Males:
Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	89.5	95.0	90
Caged	84.2	94.7	100

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	84.2	95.0
Caged	85	100.0	92.9

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	220	119.07			
Model 2	219	119.06	1	0.0059801	0.9384

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

Mature Females:
Proportion clean (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	100	90.5
Caged	90	100	100.0

Proportion clean (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	95	95	100
Caged	95	100	100

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	227	60.592			
Model 2	226	60.500	1	0.09189	0.7618

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

IV-3-4 References

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IV-4 OVARY

IV-4-1 Introduction

The paired ovaries in snow crab extend along the length of the abdomen. The ovarian lobe is enclosed in a thin envelope of fibrous connective tissue (Figure 1). The determination of ovarian stages was based on histological observations as well as egg and ovary color.

In the southern Gulf of St. Lawrence, the majority of multiparous females have historically followed a 2-year reproductive cycle (mating and subsequent oviposition in May and going through maturation process until the second following May when hatching occurs). Therefore in May of a given year, there should be two groups of multiparous females: 1) females carrying newly extruded eggs (bright orange in color) and 2) those carrying one year old eggs (dark orange in color). For the current project, we have chosen multiparous females (in November 2012) carrying 6-month old eggs (extruded in May 2012). These eggs were expected to hatch in May 2014. In November 2012 the eggs were orange in color and gonad color was either beige or clear orange. We excluded primiparous females, multiparous females carrying developed embryos (dark orange in color in November 2012) and senile females (carapace condition 4-5 and lacking afull clutch of eggs).

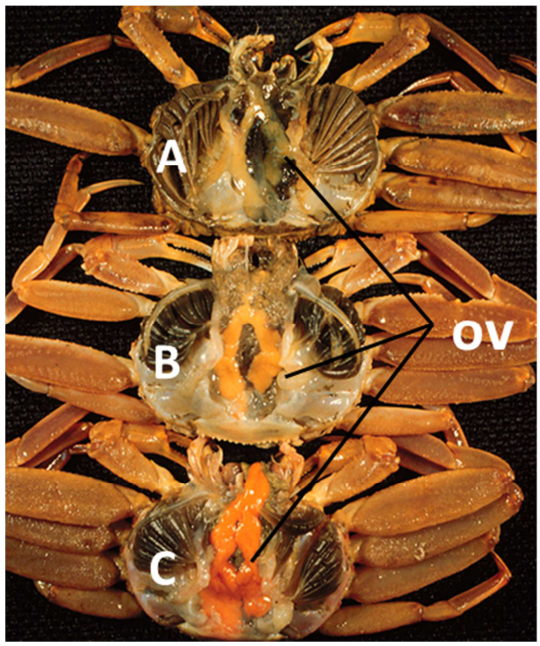


Figure 1. Position and different development stages of ovary (ov). A: ovary at developing stage in beige, B: ovary at intermediate development stage in clear orange, C: fully developed ovary in dark orange/reddish colour.

IV-4-2 Sample treatment

The abdomen with egg mass was dissected out from females and put in plastic petri dish (25 mm or 45mm in diameter for small and large sample, respectively) and the egg mass color measurement was done with a Konica Minolta Chroma meter CR-400® (Colorimeter), with a glass light-projection tube (CR-A33f®) by averaging three consecutively measured values. The colorimeter was mounted on a stand so that the color measurement can be performed at an angle of 90 degrees. The color data were printed and stored in the data processor (DP-400®). Harada and Ohtani (2006) used a colorimeter for an objective assessment of color of hepatopancreas and showed that the colorimeter parameters were related to its lipid contents. Colorimeter parameters are expressed by hue, lightness and saturation projected on different color space. We have measured the ovary color with CIELAB color space (L*, a*, b*). The parameters a* and b* are chromaticity values and L* is lightness.

For every female, ovary and egg clutch color (determined by the naked eyes) and ovary weight (to the nearest 0.0001g), were determined. Approximately 20-50 eggs per egg clutch were collected in 20ml glass vials and preserved in 3% Bouin's solution diluted with filtered seawater (Durapore ® membrane filter pore size = 0.1μm) to determine developmental stages. A sub-sample of ovaries was also collected after taking total ovary weight and preserved in Davidson's solution for histological examinations.

After color measurments and sampling of ovary and eggs for embryonic stage determination were completed, remaining eggs and abdomen were collected in sample bags and preserved in 10% formalin for fecundity estimates. In the laboratory, pleopods and eggs were separated from the abdomen, placed in small fish nets and rinsed with running water for 1 hour. They were then transferred into disposable wax free paper drinking cups and dried for at least 48 hours in a drying oven at temperatures ranging between 50°C and 60°C. Dried eggs were then cleaned by manually separating them and removing debris and dust in a plastic weighing dish. Weighing dishes were sprayed with Staticide ® anti-static spray when needed. Eggs were then returned to the drying oven for 24 hours. A sub-sample of 500 eggs was counted under a dissection microscope and weighed to the nearest 0.0001g. Fecundity was estimated as the ratio of total dry egg weight to the sub-sample egg weight.

In the laboratory, embryo development was examined under a dissection microscope and compared with embryonic stages as described in Moriyasu and Lanteigne (1998). Embryonic stages were classified into 4 color groups: 1) light orange (early stages 1-4); 2) orange (intermediate stages 5-10); 3) dark orange (advanced stages 11-12); and 4) brown (final stages 13-14).

If upon dissection, females were categorized having a carapace condition 3 (primiparous females) or 5 (senile females with disintegrating oocytes), they were excluded from the analysis.

IV-4-3 Size-ovary weight relationship

The same statistical approach explained in III-4-2 was applied for data preparation, analyses and interpretation.
Carapace width and wet ovary weight relationships (log_e transformed data) were established by season, by year and by geographic location/sampling station (Table 1).

Although there were significant differences in carapace width and wet ovary weight relationships in terms of location, year and location-year interaction, there was a tendancy of annual variation pattern in Loess regression residual being low in spring and high in fall. This was the case for Margaree Harbor, Cheticamp and Louisbourg stations. In Grande-Rivière, the values continuously increased from spring 2012 to spring 2013 (Tables 2 & 3, Figure 2). This suggests that the reproductive schedule in Grande-Rivière might be different (possibly 2 years) compared to the other three stations (possibly 1 year).

For caged crabs, there were significant differences in carapace width and wet ovary weight relationships in terms of year/season and location-treatment interaction, but there was no difference in terms of location. In terms of treatment effects, there was a significant difference between caged and free crabs for 6- and 12-months caged experiment (Table 4 and Figure 3). This suggests that caging mature females may result in desynchronization of reproductive cycle.

Table 1. Regression parameters of ovary weight (OW, in g) on carapace width (CW, in mm) for caged and free female snow crab sampled in 2012 and 2013 from four sampling areas.

Location	Year	Season	Treatment	n	y-intercept	slope
Cheticamp	2012	Spring	Free	20	-2.485	0.059
Cheticamp	2012	Fall	Free	20	-8.967	0.213
Cheticamp	2012	Fall	Caged	20	-4.357	0.152
Cheticamp	2013	Spring	Free	20	-3.687	0.081
Cheticamp	2013	Spring	Caged	17	-4.855	0.100
Cheticamp	2013	Fall	Free	20	-3.530	0.093
Cheticamp	2013	Fall	Caged	15	0.869	0.006
Margaree	2012	Spring	Free	20	-2.087	0.052
Margaree	2012	Fall	Free	20	-8.904	0.204
Margaree	2012	Fall	Caged	20	-12.824	0.275
Margaree	2013	Spring	Free	20	-1.689	0.043
Margaree	2013	Spring	Caged	18	-25.912	0.425
Margaree	2013	Fall	Free	20	-1.318	0.076
Margaree	2013	Fall	Caged	17	-2.328	0.054
Louisbourg	2012	Spring	Free	24	-1.766	0.045
Louisbourg	2012	Fall	Free	40	-15.938	0.283
Louisbourg	2013	Spring	Free	4	-4.756	0.091
Grande.Riviere	2012	Spring	Free	8	-5.447	0.101
Grande.Riviere	2012	Fall	Free	20	-1.635	0.071
Grande.Riviere	2013	Spring	Free	20	-7.444	0.187

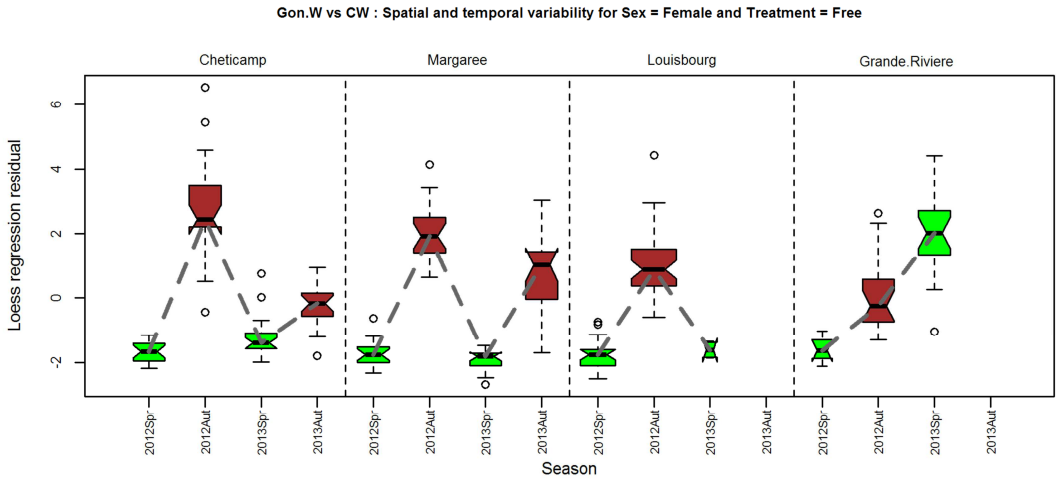


Figure 2. Spatial and temporal variability in the Loess regression residuals of the carapace width vs ovary weight relationships in female snow crab in Cheticamp, Maragree Harbor, Louisbourg and Grande-Rivière stations.

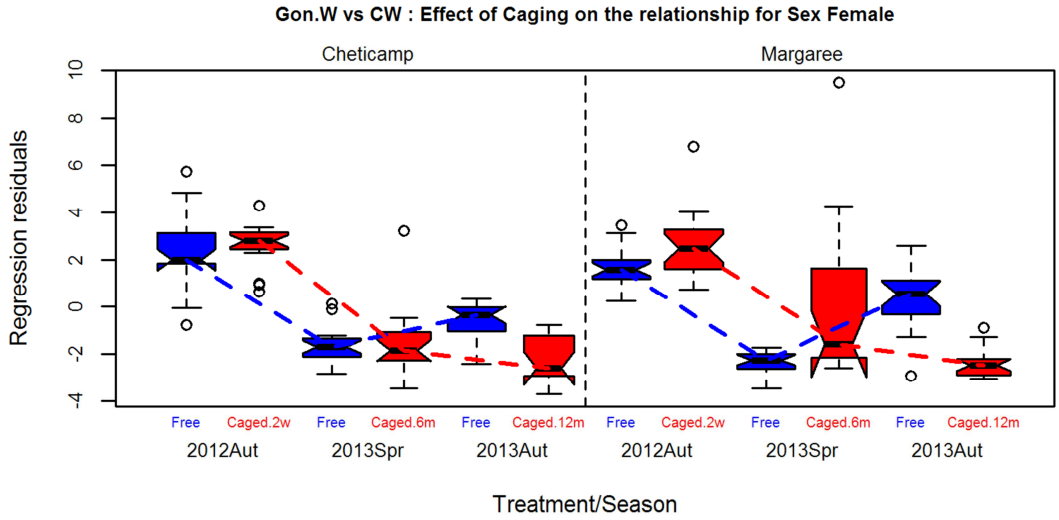


Figure 3. Comparison between caged and free crabs. Loess regression residuals of the carapace width vs ovary weight relationships in female snow crab in Cheticamp and Margaree Harbor stations.

Table 2. Spatial and temporal variability of the relationship between carapace width and ovary weight for free crabs in spring: Cheticamp, Margaree, Louisbourg, Grande-Rivière.

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	24	-3.6183	0.0721
Cheticamp	2013	20	-0.0286	0.0721
Margaree	2012	20	-3.0814	0.0721
Margaree	2013	4	-3.4972	0.0721
Louisbourg	2012	20	-3.5303	0.0721
Louisbourg	2013	20	-3.4376	0.0721
Grande.Riviere	2012	20	-3.7263	0.0721
Grande.Riviere	2013	8	-3.3856	0.0721

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	1		0.5	1	0.23560	
Location	3	126	53.7	41.9	115	0.00000	***
Year	1	12	5.3	12.4	34	0.00000	***
Location:Year	3	49	21.1	16.5	45	0.00000	***
Residuals	127	46	19.8	0.4			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Gr13SprFr - Ma13SprFr	3.70	3.70	0.197	182	0	***

Homogeneity of variance test (Levene-Brown-Forsythe) for ovary weight : p.value = 0.0000

Mean, median and standard deviations for residuals of ovary weight oncarapace width by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Spring 2012 Free	20	-0.26	-0.27	0.38
Cheticamp Female Spring 2013 Free	20	-0.12	-0.39	0.85
Grande.Riviere Female Spring 2012 Free	8	-0.28	-0.41	0.76
Grande.Riviere Female Spring 2013 Free	20	2.64	2.39	1.35
Louisbourg Female Spring 2012 Free	24	-0.69	-0.75	0.39
Louisbourg Female Spring 2013 Free	4	-0.67	-0.84	0.60
Margaree Female Spring 2012 Free	20	-0.45	-0.51	0.41
Margaree Female Spring 2013 Free	20	-0.73	-0.75	0.24

Table 3. Spatial and temporal variability of the relationship between carapace width and ovary weight for Free crabs in fall: Cheticamp, Margaree, Louisbourg, Grande-Rivière.

Regression parameters by group (single slope model not applicable)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-6.03	0.131
Cheticamp	2013	20	-5.78	0.131
Margaree	2012	20	-3.14	0.131
Margaree	2013	20	-5.14	0.131
Louisbourg	2012	20	-3.85	0.131
Grande.Riviere	2012	40	-4.94	0.131

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	132		132	103	0.00000	***
Location	3	26	8.9	9	7	0.00025	***
Year	1	86	29.1	86	67	0.00000	***
Location:Year	1	12	4.3	12	10	0.00213	**
Residuals	133	169	57.7	1			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Ch13AutFr - Ch12AutFr	-2.89	-2.89	0.363	-66.6	0.00000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for ovary weight: p.value = 0.5273
Mean, median and standard deviations for residuals ovary weight on carapace width by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Autumn 2012 Free	20	1.68	1.40	1.41
Cheticamp Female Autumn 2013 Free	20	-1.14	-1.00	0.82
Grande.Riviere Female Autumn 2012 Free	20	-0.94	-1.12	1.30
Louisbourg Female Autumn 2012 Free	40	-0.15	-0.39	1.10
Margaree Female Autumn 2012 Free	20	1.00	0.99	0.76
Margaree Female Autumn 2013 Free	20	-0.31	-0.23	1.26

Table 4. Spatial and temporal variability of the relationship between carapace width and ovary weight for caged crabs in Cheticamp, Margaree and comparison between caged and free crabs.

Location	YearSeason	y-intercept	slope	treatment.effect (Caged)	treatment.effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Fall	-7.434	0.191	0.39	6	0.30509	0.36509	6.15	6.20
Cheticamp	2013Fall	-2.035	0.071	-1.59	-59	0.00000	0.01769	2.70	1.27
Cheticamp	2013Spr	-4.371	0.091	0.16	9	0.65878	0.68446	1.90	2.29
Margaree	2012Fall	-11.901	0.247	0.98	19	0.00142	0.18888	5.21	6.20
Margaree	2013Fall	-0.701	0.068	-2.61	-65	0.00000	0.59677	4.03	1.52
Margaree	2013Spr	-14.018	0.220	2.31	178	0.00265	0.00461	1.30	3.54

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	157		157	82	0.00000	***
Location	1	0	0.0	0	0	0.62327	
YearSeason	2	665	55.1	333	173	0.00000	***
YearSeason:Treatment	3	121	10.1	40	21	0.00000	***
Residuals	219	421	34.8	2			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	0.136	0.136	0.184	3.84	0.46022	

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr – 2012Fall	-3.98	-3.98	0.310	-112.3	0.00000	***
2013Fall – 2012Fall	-2.10	-2.10	0.311	-59.1	0.00000	***
2013Fall - 2013Spr	1.89	1.89	0.310	53.2	0.00000	***

Caged vs Free effect

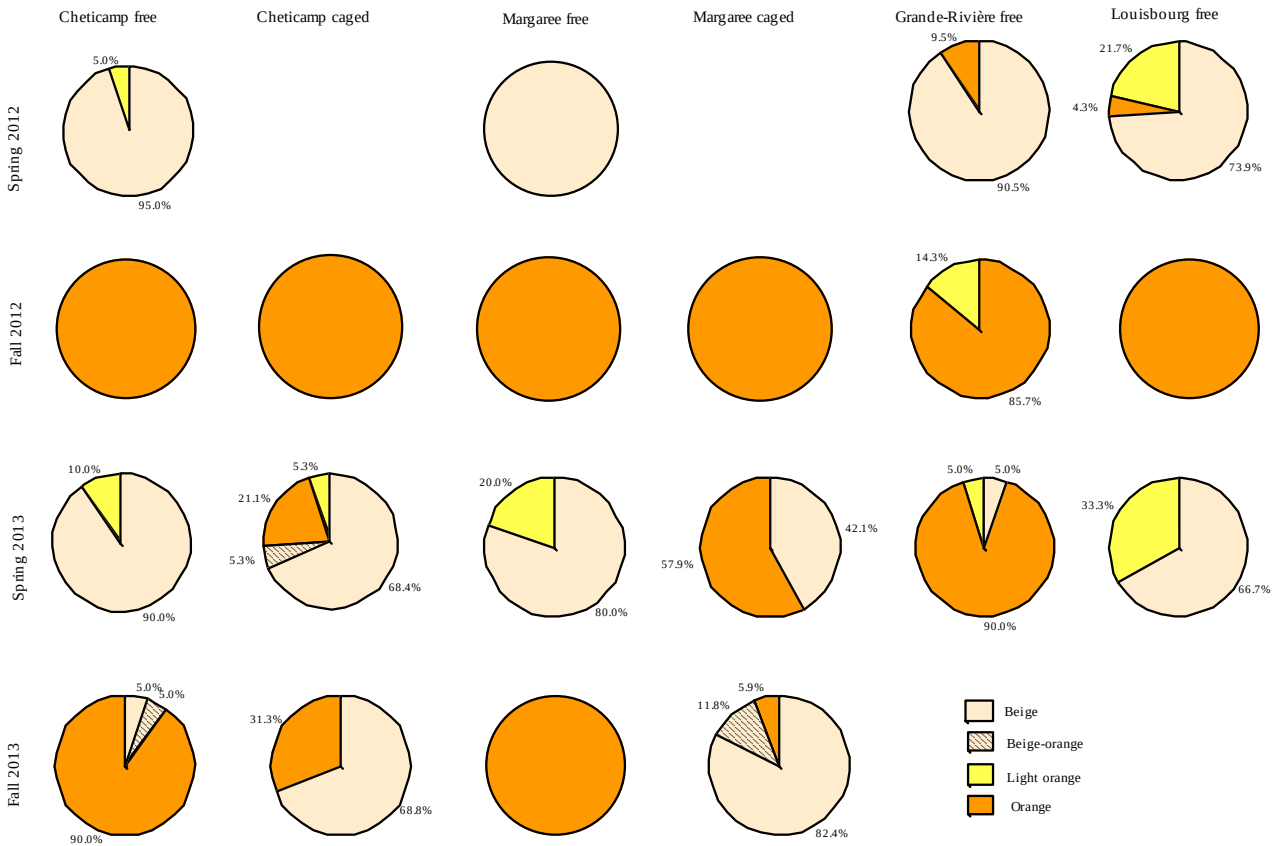
	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012Fall 2w	0.63	0.63	0.310	17.7	0.12491	
Caged vs Free: 2013Spr 6m	1.20	1.20	0.321	33.8	0.00073	***
Caged vs Free: 2013Fall 12m	-2.21	-2.21	0.330	-62.4	0.00000	***

IV-4-4 Ovary color observations and measurements

Visual Observations

The color of the ovary was observed upon dissection and classified into four categories (beige, beige-orange, clear orange and orange). In spring 2012 the majority of females bore beige ovaries (after extrusion of new eggs) (Figure 4). They became orange in fall 2012 except for in Grande-Rivière (14% of individuals had light-orange). In spring 2013 the color of ovary was again beige/light orange (10-33%) for free crabs except for Grande-Rivière (90% of individual bore orange ovaries). For caged crab the ovary color was not consistent with free crab i.e. only 42-62% of ovaries were beige. In fall 2013 the majority of free females bore orange ovaries (90-100%), whereas 69-82% of ovaries for caged females were orange. There was no sample for Grande-Rivière and Louisbourg stations in fall 2013. Under a 2-year embryonic cycle (Moriyasu & Lanteigne 1998), the ovary color should be beige in spring 2012 (i.e. after extrusion of new eggs as multiparous female), the ovary color then progresses into clear-orange towards the end of 2012 and light-orange to orange into the spring 2013 and orange in fall 2013 to be ready for hatching in spring 2014. The results of observations suggest that the reproductive cycle of the majority of females in the southeastern Gulf of St. Lawrence might be one year contrary to what has been observed in early 1990's (Moriyasu & Lanteigne 1998). For the Louisbourg station, results showed some discrepancies compared to those observed in Margaree Harbor and Cheticamp, however this might be due to the differences in sampling periods as a combination of a 1 year and 2 year cycle in a same area is highly unlikely. For the Grande-Rivière station, the slower progression of ovary color strongly suggests that the reproductive cycle is 2 years. However, the lack of samples in fall 2013 prevents us to confirm this hypothesis. For caged females, the reproductive cycle seemed to be disturbed, i.e. 20-58% of ovary showed a non-spent condition in spring 2013, whereas 80-90% of free females in the same area showed a spent ovary condition. In addition, only 6-30% of ovaries from caged females progressed to the orange color in fall 2013.

Figure 4. Ovary color showing different stages of development of female snow crab by sampling season and area.



Color measurements with a colorimeter
Statistical analysis

The statistical analysis was designed to answer two questions:

- What is the natural variability of the parameters across locations and years?
- What is the effect of caging?

The research project involves measurement of a large number of parameters on crabs taken in 4 locations, over 2 calendar years, in two seasons each year, and subjected to two treatments. Not all combinations of conditions were examined (for example, caged crabs were obtained only from two of the four locations).The target sample size was 20 crabs for each combination of conditions. As expected in such field studies, the final sample sizes were somewhat less.

The results of statistical analysis should be interpreted with caution by setting a significance threshold at a 0.001 level in order to prevent large number of false positives (type I errors) and the results clearly inconsistent with a priori knowledge should not be given a high weight.

Analysis of single continuous parameters (Hepatopancreas color: "Hep.L", "Hep.a", "Hep.b", Embryo color: "Egg.L", "Egg.a", "Egg.b", Ovary color: "Gon.L", "Gon.a", "Gon.b", Stomach content: "Stomach.Content.Weight.Shifted.Log", and "Repletion.Index", Biochemical parameter analysis: 'PCA Componants') were examined independently.

Data preparation was carried out separately for each statistical analysis. Records with missing values for the parameter and the factors of interest (typically location, year, season, treatment) were removed from the analysis.

Box and whiskers plots of the parameter were computed separately for each factor combination (Location, year, season, and treatment). Values further than approximately three times the interquartile range (IQR) from the 1^{st} or 3^{rd} quartile as appropriate were removed from the analysis. Many of the data values were removed through this procedure which appeared to be erroneous (e.g. measurement errors, data entry errors) as opposed to naturally occurring outliers.

Analysis of natural variability in free crabs

Values of the parameter were examined graphically using grouped box and whisker plots. The plots are pinched to show the confidence interval for the median and their width represents sample sizes. Within each location, a broken line shows the seasonal evolution. We analysed spring and autumn data separately given the large differences expected (and observed) due to seasonal cycles. Furthermore, the analysis was carried out with all locations (Cheticamp, Margaree Harbor, Louisbourg, Grande-Rivière) included. For each season, we report the result of an analysis of variance with Location, Year as factors and the

Location*Year interaction included. We then apply the Tukey's HSD (honest significant difference) test to the differences between Locations and Years. We report the largest differences, as a percentage of the mean value. We report the result of a Levene-Brown-Forsythe test for homogeneity of variance between groups. Finally, we report the mean, median and standard deviation of each group.

We propose that the results be interpreted as follows: If the Location*Year interaction is statistically significant, the largest observed difference between groups should be taken as reference. If the Location*Year interaction is not statistically significant, then the largest difference between Years and between Locations can be examined, since it might be possible to modelled these differences. Finally, if the Levene-Brown-Forsythe test is highly significant, large differences in standard deviations should be explained.

The absence of statistically significant variability between the two years of observation or of statistically significant interaction for the two years of observation should not be taken as indicative of absence of the same in the long term.

Analysis of the caging effect

Values of the parameter were examined graphically using grouped box and whisker plots. The plots are pinched to show the confidence interval for the median, their width represents sample sizes. Within each location, a broken line shows the seasonal evolution.
Crabs were placed in cages in the autumn 2012 and observed 2 weeks, 6 months and 12 months later, i.e. in the autumn 2012, spring 2013 and autumn 2013. As a consequence, spring and autumn data must be analysed simultaneously. We carried out an analysis of variance with the Location and Year+Season (three levels: Automn) as factor and Treatment nested within Year+Season. In this analysis, the Year+Season effect is expected to be significant due to the seasonal variation. We report the difference due to Location, YearSeason and the nested Treatment factor. The effect of Caging vs Free is computed as a percentage of the mean value of the parameter. We report the result of a Levene-Brown-Forsythe test for homogeneity of variance between groups. Finally, we report the mean, median and standard deviation of each group.

We propose that the results be interpreted as follows. The most important information is the difference between Cage vs Free and its statistical significance. If the Levene-Brown-Forsythe test is highly significant, large difference is standard deviations should be explained.

Results

The color characteristics of the ovary were measured with a chromameter (Konica-Minolta CR400) to avoid introducing the subjectivity in the determination of the color. In order to obtain standard color measurements, the color of the specimen (L*a*b* color space) was

measured with the receptor at a $0^{\circ} \pm 5^{\circ}$ angle from the normal perpendicular line. Color values (CIELAB a*, b* and L*) measured with a colorimeter were analysed by ANOVA (this method was also applied to embryo and hepatopancreas color measurement in this document: see sections IV-5-4 and IV-6-3, respectively).

Color values measured with a colorimeter showed a great variability in year, location and year-location interaction in free crabs (Tables 5 & 6), the same tendancy was observed for Margaree Harbor, Cheticamp and Louisbourg. Green-red chromaticy coordinate (a*) showed clearer seasonal trend (Figure 5).

Spatial and temporal (annual) variability of ovary color red-green (a*) measurement for free crabs in Cheticamp, Margaree, Louisbourg and Grand-Riviere was significant in the spring seasons in terms of location, year and location-year interaction. However, it was sigfificant only in terms of location and year in the fall season (Tables 5 & 6). The maximum difference was observed between Grande-Rivière and Cheticamp in terms of location, and Grande-Rivière 2013 and Grande-Rivière 2012 in terms of location-year interaction (Tables 5 & 6).

In Margaree Harbor, Cheticamp and Louisbourg, the color value increased from spring to fall and fell in the following spring and increased again for the following fall. For Grande-Rivière, it seemed that the color value did not follow the same pattern observed in other stations, i.e. the value did not decreased in fall 2012 (redness in ovary continued to increase from spring 2012 to fall 2013 and remained at the same level in fall 2013).

For comparison between caged and free females, although no significant difference was observed in the fall 2012, the difference was significant for 6 month caged vs free and 12 month caged vs free and the difference increased with the immersion duration (Table 7, Figure 6). This suggests that caging for longer periods may impact the gonadal development schedule.

The comparison between caged and free crab in a given season in Margaree Harbor showed that there was a significant difference in gonad color value (a*) in the fall 2013 (t-test $p<0.0001$), whereas there were no noticeable differences in the fall 2012 and spring 2013 (t-test, $p=0.0278$; Mann-Whitney-test, $p=0.1686$, respectively). For Cheticamp the results showed the same tendancy being no significant difference in the fall 2012 and spring 2013 (t-test, $p=0.4275$; Mann-Whitney -test, $p=0.6404$, respectively) but a significant difference was observed in the fall 2013 (t-test, $p<0.0001$).

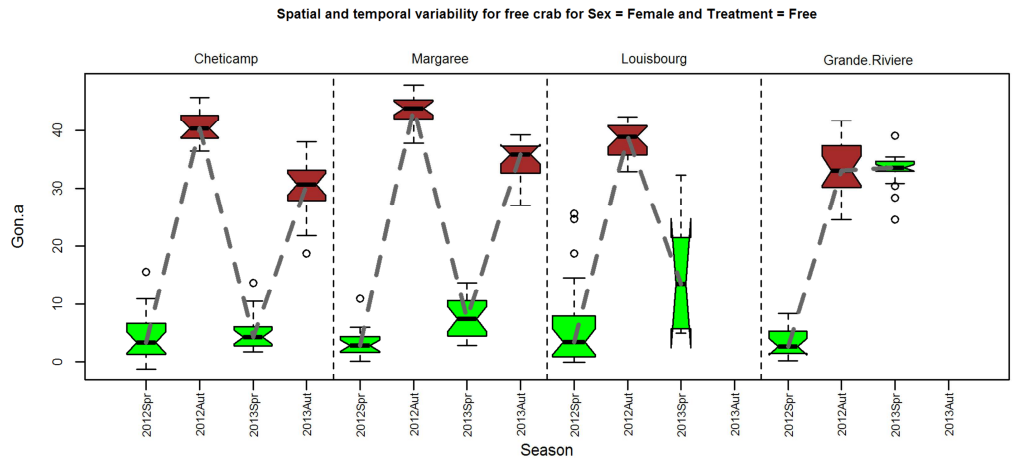


Figure 5. Spatial and temporal variability in the ovary color parameter (a*) measured with a colorimeter of free female snow crabs in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

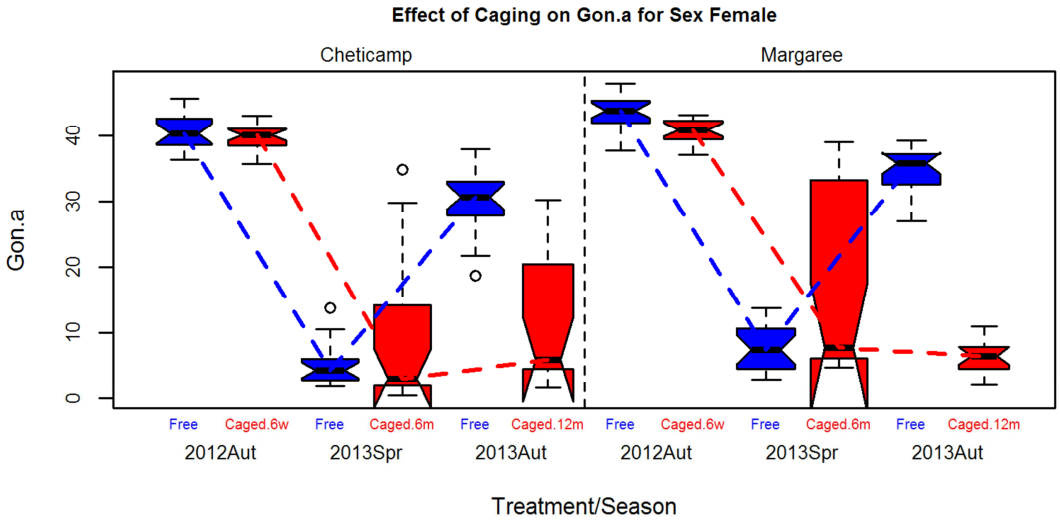


Figure 6. Effect of caging on the ovary color parameter (a*) measured with a colorimeter of caged female snow crabs in comparison with free females in Cheticamp and Margaree Harbor stations.

Table 5. Spatial and temporal variability of ovary color red-green (a*) measurement for free crabs: Cheticamp, Margaree, Louisbourg and Grand-Riviere (2012-2013 spring seasons)

Season: Spring
Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	4461	27.1	1487	70	0.00000	***
Year	1	4191	25.4	4191	196	0.00000	***
Location:Year	3	4882	29.6	1627	76	0.00000	***
Residuals	138	2945	17.9	21			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Gon.a : 8.98

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Grande.Riviere-Cheticamp	13.5	1.397	150.0	0.0000	***
Year	2013-2012	10.4	0.779	116.3	0.0000	***
Location:Year	Grande.Riviere:2013-Grande.Riviere:2012	29.9	2.354	333.3	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Gon.a : p.value = 0.0002
Mean, median and standard deviations for Gon.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Spring 2012 Free	20	4.27	3.33	4.29
Cheticamp Female Spring 2013 Free	19	5.05	4.26	3.23
Grande.Riviere Female Spring 2012 Free	19	3.16	2.63	2.21
Grande.Riviere Female Spring 2013 Free	19	33.09	33.56	3.01
Louisbourg Female Spring 2012 Free	24	6.12	3.47	7.50
Louisbourg Female Spring 2013 Free	5	15.59	13.57	11.49
Margaree Female Spring 2012 Free	20	3.27	2.81	2.44
Margaree Female Spring 2013 Free	20	7.55	7.38	3.43

Table 6. Spatial and temporal variability of ovary color red-green (a*) measurement for free crabs: Cheticamp, Margaree, Louisbourg and Grand-Riviere (2012-2013 fall seasons)

Season: Fall
Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	611	15.3	204	15	0.00000	***
Year	1	1891	47.3	1891	142	0.00000	***
Location:Year	1	10	0.3	10	1	0.38439	
Residuals	112	1490	37.2	13			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Gon.a : 8.98

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Grande.Riviere-Cheticamp	13.5	1.397	150.0	0.0000	***
Year	2013-2012	10.4	0.779	116.3	0.0000	***
Location:Year	Grande.Riviere:2013-Grande.Riviere:2012	29.9	2.354	333.3	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Gon.a: p.value = 0.0275
Mean, median and standard deviations for Gon.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Autumn 2012 Free	18	40.4	40.3	2.62
Cheticamp Female Autumn 2013 Free	19	29.9	30.7	4.95
Grande.Riviere Female Autumn 2012 Free	20	33.5	33.0	4.59
Louisbourg Female Autumn 2012 Free	20	38.4	38.9	2.81
Margaree Female Autumn 2012 Free	20	43.6	43.8	2.65
Margaree Female Autumn 2013 Free	20	34.5	35.8	3.61

Table 7. Spatial and temporal variability of ovary color red-green (a*) measurement for caged crabs: Cheticamp, Margaree.

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	1	366	0.6	366	8	0.00447	**
YearSeason	2	39579	66.5	19789	447	0.00000	***
YearSeason:Treatment	3	9980	16.8	3327	75	0.00000	***
Residuals	217	9612	16.1	44			

Differences due to Location (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
Location	Margaree-Cheticamp	2.56	0.894	10.5	0.0045	**

Differences due to YearSeason (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
YearSeason	2013Spr-2012Aut	-31.8	1.29	-130.2	0.0000	***
YearSeason	2013Aut-2012Aut	-19.0	1.32	-78.1	0.0000	***
YearSeason	2013Aut-2013Spr	12.7	1.33	52.1	0.0000	***

Differences due Treatment at 2 weeks, 6 months, 12 months (Tukey HSD)

Treatment	Values	Difference	SE	Difference (%)	HSD.p.value	Significance
2 weeks	2012Aut:Caged-2012Aut:Free	-1.76	2.21	-7.2	0.8526	
6 months	2013Spr:Caged-2013Spr:Free	6.26	2.23	25.7	0.0007	***
12 months	2013Aut:Caged-2013Aut:Free	-23.25	2.37	-95.3	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Gon.a: p.value = 0.0000
Mean, median and standard deviations for Gon.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Fall 2012 Caged	20	39.74	40.16	2.07
Cheticamp Female Fall 2012 Free	18	40.43	40.34	2.62
Cheticamp Female Fall 2013 Caged	15	11.68	5.87	10.47
Cheticamp Female Fall 2013 Free	19	29.90	30.68	4.95
Cheticamp Female Spring 2013 Caged	19	9.35	3.00	11.54
Cheticamp Female Spring 2013 Free	19	5.05	4.26	3.23
Margaree Female Fall 2012 Caged	20	40.84	40.94	1.66
Margaree Female Fall 2012 Free	20	43.63	43.84	2.65
Margaree Female Fall 2013 Caged	15	6.25	6.46	2.57
Margaree Female Fall 2013 Free	20	34.47	35.84	3.61
Margaree Female Spring 2013 Caged	19	15.78	7.71	13.79
Margaree Female Spring 2013 Free	20	7.55	7.38	3.43

IV-4-5 Histological observations of ovary collected in 2012 and 2013

Material and Methods

Haematoxylin and eosin (H&E) stained slides of ovarian tissue (n = 383) were provided for direct light microscopic evaluation (see Section III-1). Slides represented crabs originating from four stations (Cheticamp, NS, Margaree, NS, Louisbourg, NS, Grande-Rivière, QC) with samples collected at four times (Spring 2012, Fall 2012, Spring 2013, Fall 2013) over a two year period. Subgroups of crabs in Cheticamp and Margaree had been caged for a period of 2 weeks (Fall 2012), 6 months (Spring 2013), or 12 months (Fall 2013) were also provided. Crabs collected by trap (i.e., free, no caging period) were used as the control groups. All slides had been randomised and renumbered allowing for non-biased evaluation by the observer (A. Battison).
Data was analysed using STATA ® Statistics/Data Analysis 12.1 (StataCorp, TX, USA) and Excel 2010© (Microsoft Corporation, Microsoft Canada Inc. ON, Canada). Significance level was set at $p < 0.05$. Slides were examined using a Leitz Dialux 20 microscope. Images were captured with a DPI stand-alone camera (Olympus Corporation, PA).

Criteria used for histologic scoring were derived from those established for examination of Masson-trichrome stained sections from the 2003-2004 study (Supplement, Section IV-7). Some modifications were made to allow for the differences in stains used and the results of the 2003-2004 study.

Ovary stage was assessed by examining oocyte maturity. Oocytes were divided into three very general categories: basophilic oocytes (cytoplasm is primarily basophilic, low numbers of eosinophilic yolk droplets or clear vacuoles may be present); primary oocytes (cytoplasm is primarily eosinophilic with a few lipid vacuoles and eosinophilic yolk droplets (yolk plates) comprising less than 50% of the cytoplasm and present in less than 50% of oocytes); secondary oocytes (cytoplasm is eosinophilic with yolk droplets present in > 50% of oocytes and occupying >50% cytoplasmic area). An overall classification of ovary stage as early vitellogenic (majority of ovary composed of basophilic oocytes and primary oocytes) or late vitellogenic (majority of ovary composed of secondary oocytes) was also recorded for simplification. Germinal centre (areas, usually found as cords or islands of dividing oogonia – cells with high N:C ratios and deeply basophilic cytoplasm) activity was recorded either not observed, mildly active, or active.

Criteria presumptively associated with the immediate post-spawning period included: the presence of remnant follicles lacking yolk residue, previously referred to as ‘spawning scars’ (see Supplement, Section IV-7) enumerated as the number clear remnants/50 oocytes

(primary and secondary combined), diffuse hemocyte infiltrates (mild, moderate, marked, severe), karyorrhexis of follicular cells (present/absent).

Oocytes (primary or secondary, or remnants thereof) showing loss of normal structure such as coalescence of yolk droplets into larger plates, fragmentation of plates, cytoplasm containing clear vacuoles of irregular size and shape, and/or macrophage infiltrates or phagocytic follicular epithelium were classed as 'resorbing' and enumerated (number of resorbing oocytes/50 oocytes, primary and secondary combined). The presence of degenerative nodules as described by Kon *et al.* (2010), was recorded (none seen, mild, moderate, marked) as a subjective score taking into account the number and relative size of the nodules in the section. Follicle remnants containing a small amount of pale eosinophilic material in their centre were enumerated (# follicles with residue/50 oocytes).

Features associated with inflammation, separate from the diffuse infiltrates associated with recently spawned ovaries, included presence of organised hemocyte foci, necrosis, brown pigment (melanin) deposition, fibrosis were recorded (none seen, mild, moderate, marked) as was the presence of infectious agents (bacterial, protozoal, viral, other).

The occurrence of deep, homogenous, 'smooth' basophilia, negative for mineral (von Kossa stain) of yolk droplets and/or deep basophilia of cytoplasm inappropriate for oocyte stage was recorded as present or absent. The degree of separation between follicular cells and oocyte chorionic membrane was considered as a possible seasonal effect related to overall tissue water content in the 2003-2004 study results and recorded as mild, moderate, marked based on an overall assessment of the section. Sperm were noted as present/absent.

Results

The plane of section of the tissue samples was inconsistent as was the amount of material available for examination. The stain intensity of some slides was quite pale and hemocyte granules were not always distinct.

Slides were not available for evaluation for 16 crabs, presumably due to mortalities: spring 2012, Grande-Rivière free (#25, 34); fall 2012 Grande-Rivière free (#116); spring 2013, Cheticamp free (#224); spring 2013, Cheticamp, caged (#180); spring 2013, Margaree, caged (#119); fall 2013, Cheticamp, caged (#41, 57, 58, 59, 60), fall 2013, Margaree, caged (#178, 179, 180). Thirteen slides from spring 2012, Grande-Rivière free were cut but not stained (ID#'s 31, 32, 33, 35, 36, 37, 38, 39, 40, 41, 42, 44, 45). Slides from five crabs did not contain ovary tissue for evaluation: spring 2012, Grande-Rivière free (#25, 34); spring 2013, Cheticamp, free (#224); Fall 2013, Cheticamp, free (#101), fall 2013, Cheticamp caged (#41). Table 8 summarises the number and distribution of samples available for evaluation.

Table 8. Summary table showing number of samples available for histological examination from each station, the date of sample collection, and percent occurrence of degenerative nodules and sperm in the ovary samples.

Station	Gear Type	Sampling Time											
		Spring 2012			Fall 2012			Spring 2013			Fall 2013		
		n	Nodules (%)	Sperm (%)	n	Nodules (%)	Sperm (%)	n	Nodules (%)	Sperm (%)	n	Nodules (%)	Sperm (%)
Cheticamp													
	Free	20	15	0	20	20	0	19	16	5	19	0	0
	Caged	n/c[1]	--	--	20	5	0	19	11	11	15	0	20
Margaree													
	Free	20	0		20	0	0	20	15	0	20	0	0
	Caged	n/c	--	--	20	5	0	19	0	0	17	12	29
Louisbourg													
	Free	24	4	0	20	10	0	5	0	20	n/c	--	--
Grand Rivière													
	Free	6	17	17	20	0	0	20	0	0	n/c	--	--

Free crabs

Ovary stage showed a definite pattern in free (trapped) crabs, switching between ovaries dominated by basophilic and primary oocytes in the spring and secondary oocytes in the fall samples (Figure 7).

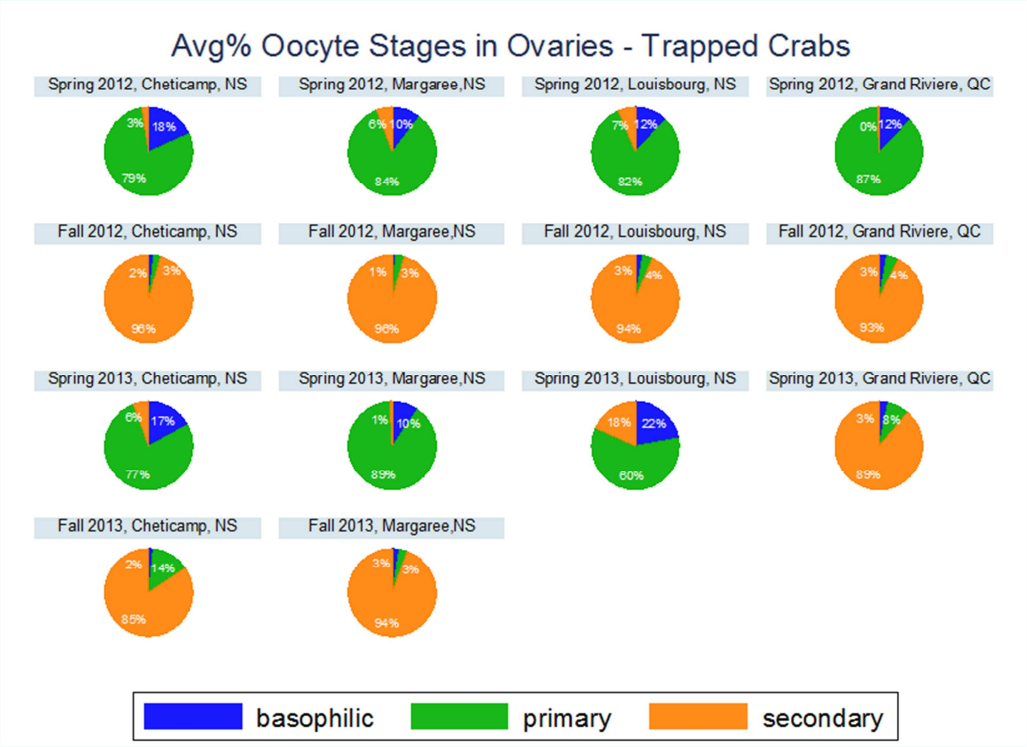


Figure 7. Average relative composition of ovaries by oocyte type for all free (trapped) snow crabs collected from four sampling stations from spring, 2012 through fall, 2013.

The spring 2012 samples were collected within a nine day window for Grande-Rivière (May 24th), Cheticamp (May 30th), and Margaree (June 1st) while, those from Louisbourg were collected 14 d later (June 14th). The fall 2012 sample collection times were separated by approximately nine weeks with Louisbourg (Sept. 18th) and Grande-Rivière (Sept. 22nd) collected before Margaree (Nov. 2nd) and Cheticamp (Nov. 4th). The spring 2013 collections by approximately four weeks with Grande-Rivière collected first (May 30th), followed by Cheticamp (June 12th), Margaree (June 18th) and finally Louisbourg (June 26th).

Criteria generally associated with recent spawning were almost exclusively observed in the spring samples for free crabs (Figures 8, 9, 10). Clear follicle remnants (no residue) were scored at $\geq$ 50, or too numerous to count (TNTC), for 100% of crabs at all four stations in spring 2012. There were differences in spring 2013 with scores of TNTC reported for 90% of crabs at Cheticamp and Margaree, 60% of crabs from Louisbourg, but 0% for crabs from Grand Rivière. Fall 2012 samples showed the majority of crabs with occurrences of 0% in Cheticamp and Margaree. More varied numbers were noted for both Louisbourg and Grand Rivière although most scores were less than 15/50 oocytes.

The pattern of degree of hemocyte infiltration were most consistent in the 2012 samples, with all four station graded as moderate, marked, or rarely severe in the spring and then as normal, mild ,or occasionally moderate, in the fall. Intensity of infiltrates increased again, although to a lesser degree, in the spring in Cheticamp, Margaree, and Louisbourg but not in Grande-Rivière. The majority of crabs in Cheticamp (95%) and Margaree Harbor (80%) had scores of normal or moderate infiltrates in the fall of 2013.

Karyorrhexis, when detected (13% -35%), occurred almost exclusively in the spring 2012 and 2013 samples in all stations. Karyorrhexis was absent only from the spring 2013 sample from Grande-Rivière. Germinal centre activity was variable over time; however, was generally decreased in the fall samples.

In general, histologic criteria presumptively associated with oocyte resorption (resorbing oocytes, follicle remnants with residual material, degenerative nodules) were also observed more frequently in spring samples (Figures 11, 12, 13). Resorbing oocytes were observed more often (35% – 84% of crabs), and at higher numbers (up to around 10 per 50 oocytes), in spring caught crabs. In contrast, oocyte resorption was generally uncommon in fall caught crabs, with none reported for 70% - 90% of crabs at all stations.

Observations of follicle remnants containing eosinophilic residue were almost entirely restricted to spring caught crabs, with the fall 2012 Grande-Rivière (10%) crabs the one exception. When observed, they were usually present at very high numbers (TNTC). The percentage of crabs showing this feature was generally lower in spring 2012 (Cheticamp and Margaree 10%; Louisbourg 12.5% versus spring 2013 (Cheticamp 58%, Margaree 55%, Louisbourg 40%). The pattern was reversed in Grande-Rivière (50% and 10%, respectively).

Degenerative nodules were observed in 5% - 15% of free crabs (Table 8). Basophilic deposits while generally uncommon were observed in the spring 2012 samples (Cheticamp 10%, Margaree 5%, Louisbourg 17%) and at 5% in the fall 2013, Cheticamp samples. Sperm was detected infrequently in low percentages of crabs in spring 2012 in Grande-Rivière (17%) and in spring 2013 in Cheticamp (5%) and Louisbourg (20%).

Chorion separation was more common in spring caught crabs and more noticeable (grades of mild or moderate) than in fall caught crabs (graded at none to mild). The exception was the spring 2013 sample from Grande-Rivière where chorion separation was not observed.

Inflammation, distinct from the diffuse infiltrates associated with recent spawning was rarely observed. Intracytoplasmic inclusion bodies, consistent with the unidentified virus as described in section IV-1-6, were noted in the endothelium of one crab (#162, fall 2013, Margaree, caged).

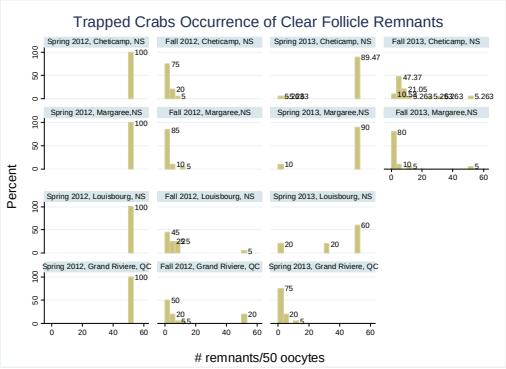


Figure 8. Percent distribution histogram of occurrence of clean follicle remnants/scars in free snow crab collected at four stations from Spring 2012 to Fall 2013.

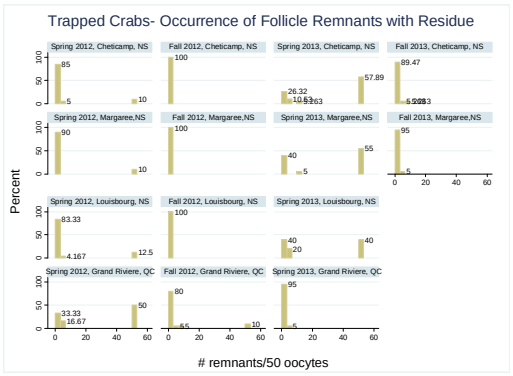


Figure 11. Percent distribution histogram of occurrence of follicle remnants with residual material in free snow crab collected at four stations from Spring 2012 to Fall 2013.

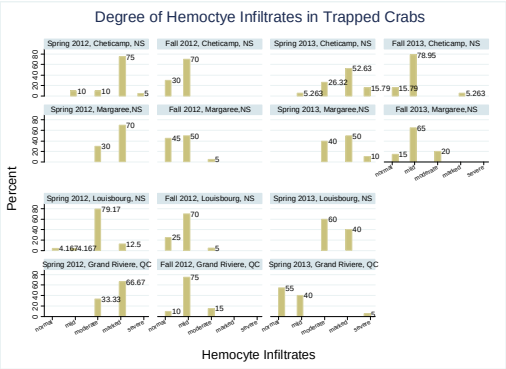


Figure 9. Percent distribution histogram of occurrence of hemocyte infiltrates in free snow crab collected at four stations from Spring 2012 to Fall 2013.

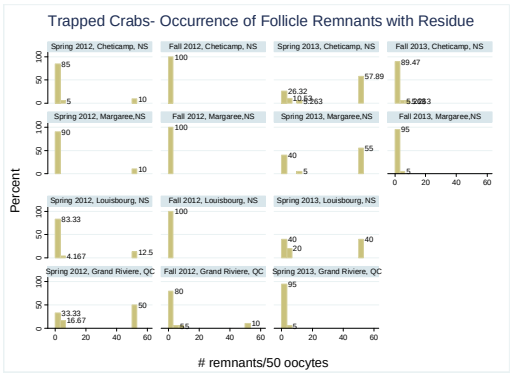


Figure 12. Percent distribution histogram of occurrence of follicle remnants with residual material in free snow crab collected at four stations from Spring 2012 to Fall 2013.

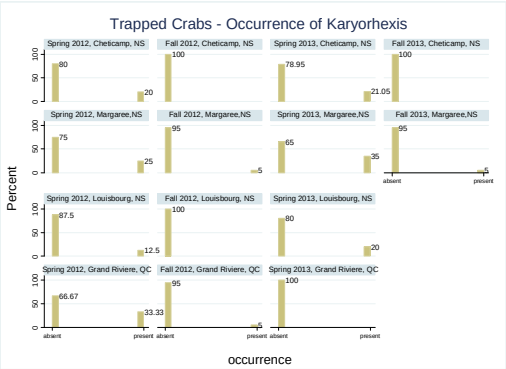


Figure 10. Percent distribution histogram of occurrence of karyorrhexis in free snow crab collected at four stations from Spring 2012 to Fall 2013.

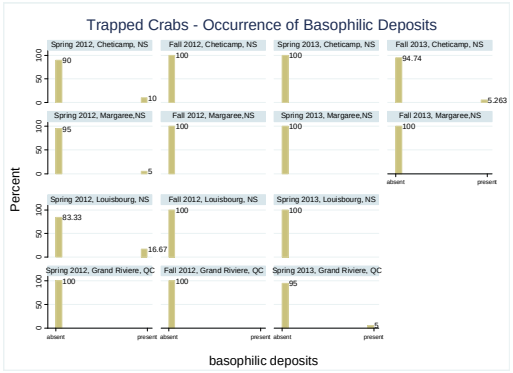


Figure 13. Percent distribution histogram of occurrence of basophilic deposits in oocytes of free snow crab collected at four stations from Spring 2012 to Fall 2013.

Caged vs free Crabs: Cheticamp & Margaree Stations

The pattern of ovary stage was similar for the crabs caged for six months (6M crabs) when compared to free crabs in spring 2013. In contrast to free crabs in the fall of 2013, the ovaries of crabs caged for 12 months (12M crabs) resembled spring ovaries in that they were dominated by basophilic and primary oocytes (Figure 14).

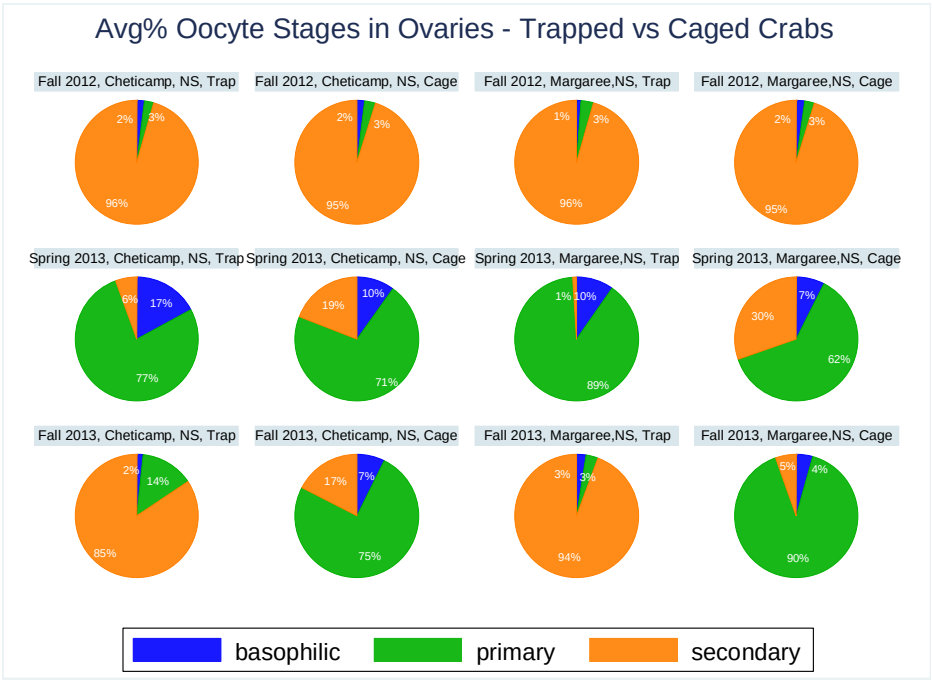


Figure 14. Average relative composition of ovaries by oocyte type for free (trapped) and caged snow crabs collected from Cheticamp, NS and Margaree, NS sampling stations from fall, 2012 through fall, 2013

Free crabs to be used for comparison to caged crabs were collected two weeks before the caged crabs in the two week caging study at both Cheticamp and Margaree. Samples for comparison of caged and freed crabs were collected on consecutive days for the 6M and 12M samples for Cheticamp, but free crabs were collected 14 – 15 days later than caged crabs for 6M and 12M samples at Margaree Harbor.

Higher scores for # of resorbing oocytes/50 oocytes observed were always higher in caged crabs at 2 weeks, six months and 12 months, and median scores were significantly greater (Wilcoxon signed rank test) for caged crabs in all but two week caged crabs held at Margaree (Figure 15). This was the only notable difference in the two week study.

The patterns for most criteria observed in 6M caged crabs held in Cheticamp and Margaree Harbor generally resembled their free counterparts while patterns for 12M

caged crabs were overall more typical of spring crabs (Figures 15-20) with some differences in degree of features observed.

Fewer 6M caged crabs (Spring 2013) had clear follicle remnants graded at TNTC than their free counterparts in both Cheticamp (79% vs 80%) and Margaree (68% vs 90%), although the overall median scores were not significantly different (Wilcoxon signed rank test). The pattern was reversed in the 12M caged group (Fall 2013) with caged crabs having more clear remnants at the TNTC level than free counterparts Cheticamp (60% vs 5%) and Margaree (71% vs 5%). The overall median scores were significantly different (Wilcoxon signed rank test).

The degree of hemocyte infiltration was similar in both groups in spring 2013, with a tendency for more crabs to have scores of moderate or severe, although less pronounced for 6M caged crab crabs held at Margaree. Scores tended to remain high in 12M caged crabs but not free crabs collected in the fall 2013. Overall scores were significantly (Wilcoxon signed rank test) higher for free crabs in spring and caged crabs in the fall at both stations.

The pattern for karyorrhexis observations appeared less affected by caging and followed a more seasonal pattern (Figure 18).

There were fewer crabs with follicle remnants with eosinophilic residue scored at TNTC observed in 6M caged crabs compared to free crabs at both stations; however, overall median score was only significantly lower for caged Cheticamp crabs. In the fall 2013 samples, remnant scores of TNTC were only observed in 12M caged crabs at Cheticamp. Median scores for caged crabs were significantly higher at both stations (Wilcoxon signed rank test).

The presence of basophilic deposits was most pronounced in the 12M caged group (Figure 20). Deposits were observed in approximately 5% of 2 week, 16% of 6M, and 53% of 12M caged crabs at Cheticamp, and 82% of 12M caged crabs at Margaree Harbor. In contrast, deposits were only noted in 5% of fall 2013 free crabs at Cheticamp.

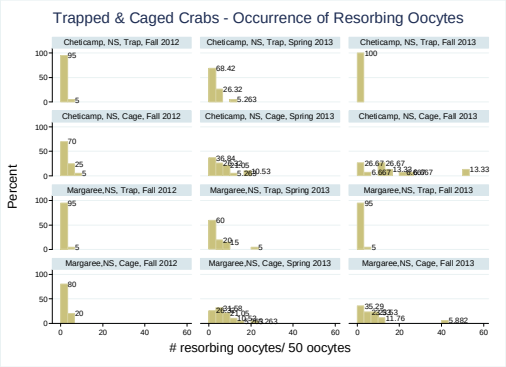


Figure 15. Percent distribution histogram of occurrence of resorbing oocytes in free and caged snow crab collected at two stations from Fall 2012 to Fall 2013

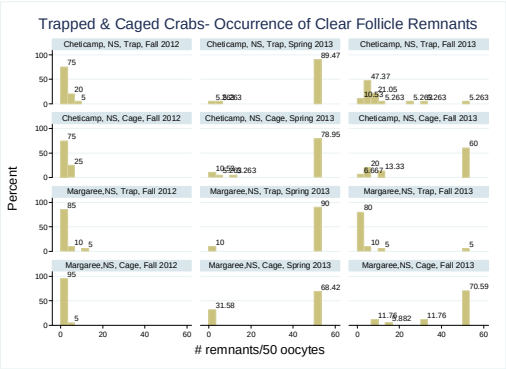


Figure 16. Percent distribution histogram of occurrence of clean follicle remnants/scars in free and caged snow crab collected at two stations from Fall 2012 to Fall 2013.

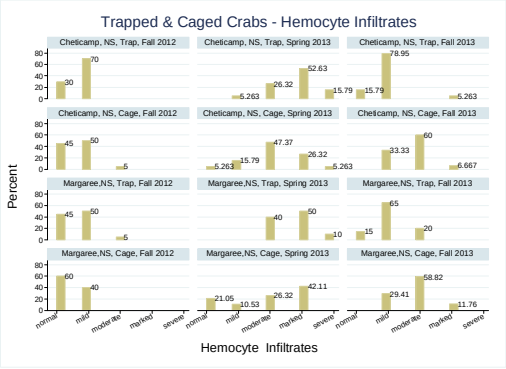


Figure 17. Percent distribution histogram of occurrence of hemocyte infiltrates in free and caged snow crab collected at two stations from Fall 2012 to Fall 2013

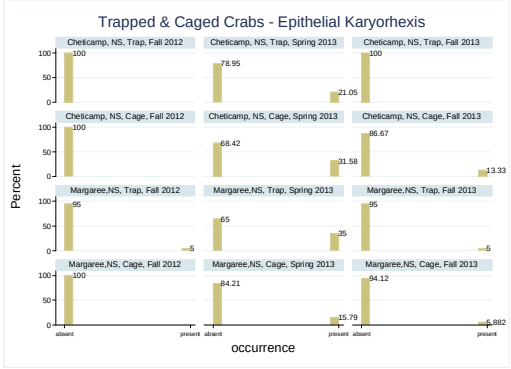


Figure 18. Percent distribution histogram of occurrence of karyorrhexis in free and caged snow crab collected at two stations from Fall 2012 to Fall 2013

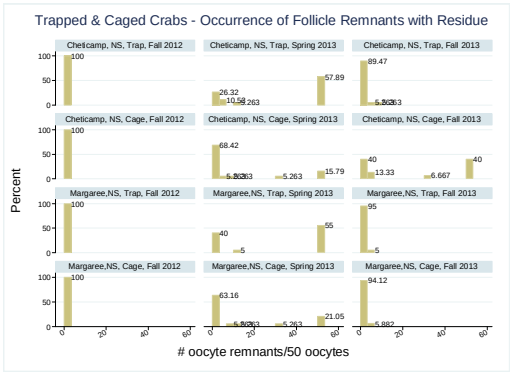


Figure 19. Percent distribution histogram of occurrence of karyorrhexis in free and caged snow crab collected at two stations from Fall 2012 to Fall 2013

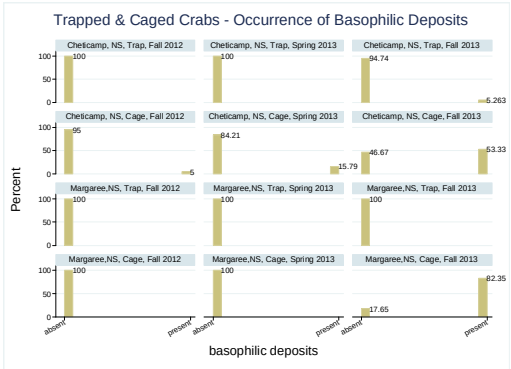


Figure 20. Percent distribution histogram of occurrence of basophilic deposits in oocytes of free and caged snow crab collected at two stations from Fall 2012 to Fall 2013.

Fewer 6M caged Cheticamp crabs had scores of ‘moderate’ for chorion membrane separation than free counterparts. Overall median scores for 6M caged crabs at Cheticamp were significantly lower while, no difference was found for Margaree crabs (Wilcoxon signed rank test). In contrast, the median score for 12M caged crabs at Margaree was significantly higher thanfree crabs while there was no significant difference between crabs collected at Cheticamp.

Overall ovary stage

Using criteria associated spawning and resorption (number of clear follicle remnants, number of follicle remnants with eosinophilic residue, number of resorbing oocytes, and hemocyte infiltrate density, follicular epithelial cell thickness, vitellogenic state), four overall ovary categories were devised to represent a proposed progression of ovary spawning stages : not spawned, recently spawned, resolving, recovering (Table 9, Figures 21,22 and 23). Recovered ovaries would continue to mature into ovaries dominated by secondary oocytes, in preparation for the next spawn when they would be classed again as ‘not spawned’.

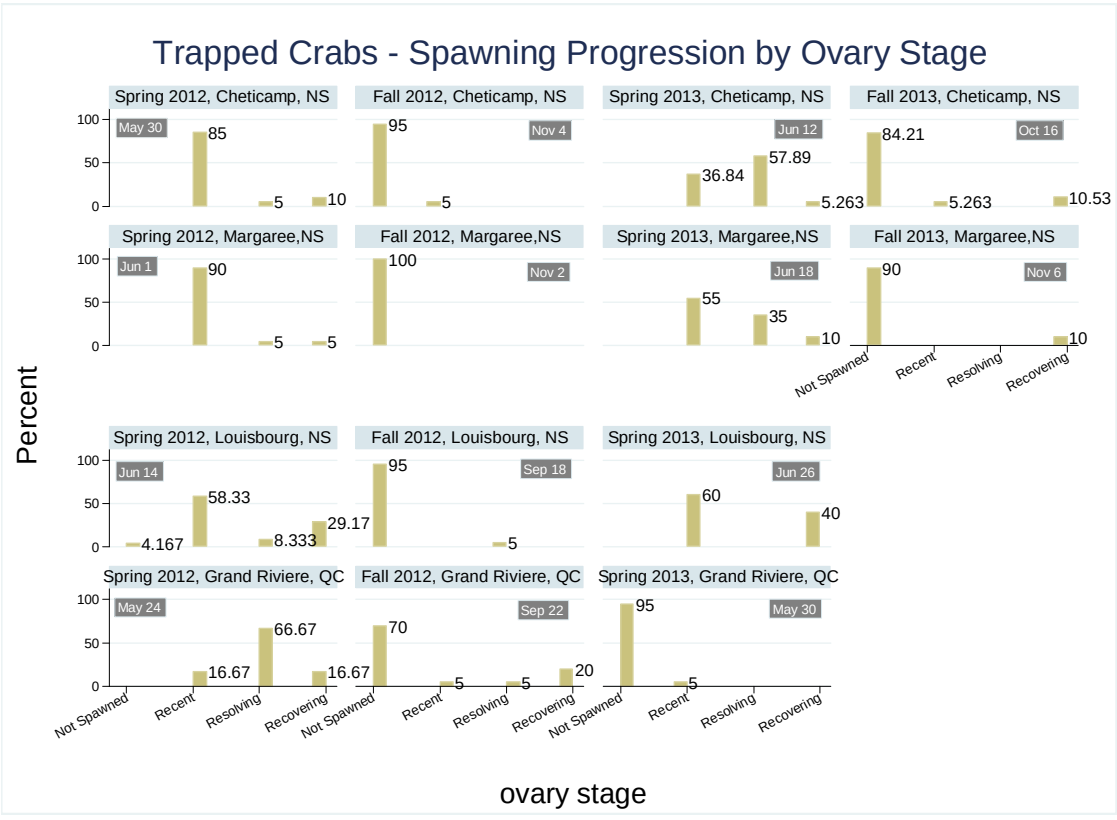


Figure 21. Percent distribution histograms demonstrating progression of ovaries through spawning using a proposed classification system for all free/trapped snow crabs collected from four sampling stations from Spring, 2012 through Fall, 2013. Bar label indicates percent (%).

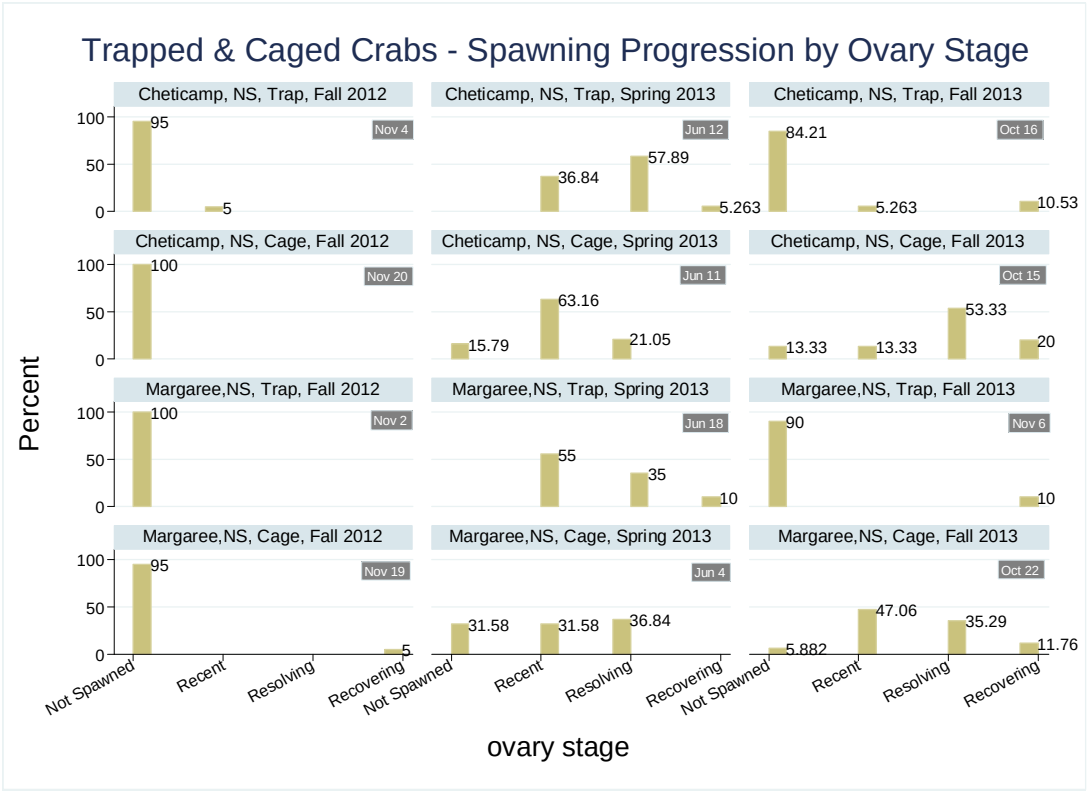


Figure 22. Percent histograms demonstrating progression of ovaries through spawning using a proposed classification system for caged and free (trapped) snow crabs collected from Cheticamp and Margaree stations from Fall, 2012 through Fall, 2013. Bar label indicates percent (%).

Table 9. Criteria used to assign ovary stage since spawning for haematoxylin and eosin-stained histologic sections of ovary from snow crab.

Stage	Clear Follicles	Follicles with Residue	Resorbing Oocytes	Follicular Thickness	Hemocyte Infiltrates	Vitellogenic Stage
Not spawned	few	few	few	thin	Normal- mild	Secondary
Recently Spawned	TNTC	few	few	thin	Moderate to marked	Primary
Resolving	TNTC	TNTC	Mild to moderate	Thin	Moderate to marked	Primary
Recovering	few	few	Mild to moderate	thick	Normal to mild (moderate)	Primary

Comparison of the percent distribution histograms of free crabs suggest that in the spring of 2012, Grand Rivière was the most progressed through the spawn and recovery sequence, followed by Louisbourg, then Cheticamp, which was only marginally ahead of Margaree. In the spring 2013 samples, Cheticamp was more progressed than Margaree, followed by Louisbourg and Grande-Rivière.

Comparison of the percent distribution histograms of caged vs free crabs at Cheticamp and Margaree Harbor would suggest that spawning in the 6M caged crabs was delayed at both stations compared to free counterparts in spring 2013, with approximately 16% and 32% of crabs still classed as 'not spawned', respectively.

By the fall 2013 sample, the majority (53%) of 12M caged crabs at Cheticamp were at the 'mixed' stage. Margaree Harbor 12M caged crabs had also progressed but the majority (47%) were at the 'recent' stage. In contrast, the vast majority of ovaries in free crabs collected at Cheticamp (84%) and Margaree (90%) were classed as 'not spawned' (Figure 23).

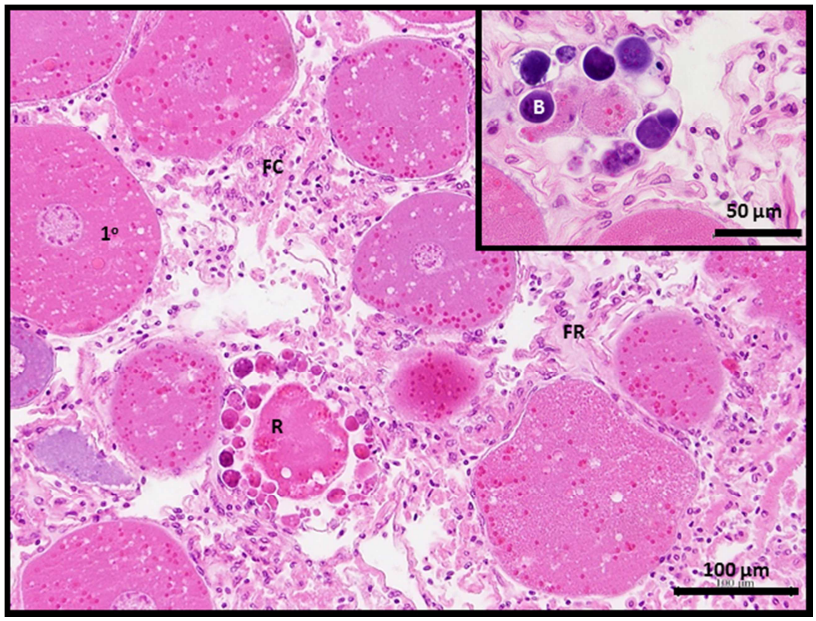


Figure 23. Ovary. Haematoxylin & eosin. Section of ovary at 'resolving' stage as discussed in the text. Inset shows higher magnification of the basophilic deposits. Resorbing oocyte (R) with broken up cytoplasm, basophilic deposits (B) Primary oocytes (1^{o}), clear follicle remnant lacking residue (FC), follicle remnant with residue (FR).

Discussion

The histologic data from the free crabs suggests that snow crabs are following a one year spring spawning cycle in three of the four sample regions. Effects associated with caging were evident, although mild, at both Margaree and Cheticamp stations beginning as early as the two week sample and became progressively more marked as caging continued. After two weeks of caging, a slightly higher number of resorbing oocytes was noted caged crabs at Cheticamp. The effects noted in the 6M and 12M samples were primarily associated with what was interpreted as delayed progression of the ovary through the spring spawning and post-spawn recovery periods with increased resorption, respectively.
The timing of sample collection needs to be considered when comparing trends across the four stations for free and caged crabs. In some cases, due to adverse weather conditions and ship and/or personnel availability, there was up to six weeks difference in collection times among stations e.g., fall 2012 free samples. In such cases one station may seem to be 'behind' another simply because samples were collected earlier. Local environmental conditions e.g., water temperature, food availability, may also be playing a role.

The conclusion that snow crab in at least three regions could be on a one year spawning cycle, vs the traditional two year cycle, is based on the observation that ovaries from nearly 100% of free crabs collected in spring 2012 and spring 2013, with the exception of Grande Rivière in 2013, had histologic features consistent with recent spawning. Crabs were selected, in part, based on the gross appearance of the clutch which may be a confounding factor. Review of the embryo staging data (see Section IV-5 of this report) showed that the spring 2013 embryos from Grande Rivière were at stage 7 or 'mixed', which would be consistent with a predominantly two year cycle (Moriyasu & Lanteigne 1998). It would be interesting to determine if bottom water temperatures have warmed or food supply changed at Cheticamp, Margaree, and Louisbourg stations compared to Grande Rivière, somehow affecting the spawning interval.

Using the proposed classification of ovary stage with respect to spawning, and assuming a one year cycle is correct, crabs collected in spring 2013 from Cheticamp, Margaree Harbor, and to a lesser extent, Louisbourg were more progressed than those collected in 2012. This is consistent with the fact that sampling was 13 - 18 days later in 2013 compared to 2012. The low number of crabs (n = 5) in the Louisbourg, spring 2013 sample may not be an accurate representation of the population. Very few of the ovaries collected from Grand Rivière crabs indicating spawning in spring 2013, despite the fact that they were collected at the same time as in 2012. This, combined with the stage 7 embryos, suggests that these crabs were on a two year spawning cycle. Examining reasons for the apparent one year cycle are beyond the scope of this report but, may include changes in bottom water temperature, food availability, etc.

An interesting observation is the intense inflammatory response present in post-spawning ovaries as noted in *Callinectes danae* (Zara *et al.* 2013). Karyorrhexis involving follicular epithelial cells was far more common in ovaries classed as recently spawned. It is possible that karyorrhexis is

one of the stimuli for the marked inflammatory response seen in ovaries after spawning as DNA-associated proteins are a recognised stimulant for inflammation in other species (Newton & Dixit 2012).

Interpretation of the caging data requires recognising that there will be some degree of resorption of non-spawned oocytes as part of the normal physiologic process of the spawning cycle (Tan-Fermin *et al.* 1989; Zara *et al.* 2013). Visual inspection of the histograms for free crab suggests that around 10% or, 5 resorbing oocytes per 50 normal-appearing oocytes (1^o and 2^o oocytes combined), could be observed after a normal spawn. Also important to consider is context i.e., whether or not the appearance or stage of the ovary observed is appropriate, given other data such as time of year, age of embryos in clutch attached to the abdomen, etc.

A slight increase in the number of resorbed oocytes was noted for caged crab compared to free counterparts after only a two week caging period. It is possible that the stress of caging alone triggered resorption. Lobsters are known to resorb oocytes when placed into holding facilities which is presumably a stressful environment. Caged crabs may not have had the same access to food as free crabs leading to resorption of oocytes to meet energy requirements, although, this seems unlikely as crabs usually have good nutritional reserves in the hepatopancreas at this time (see Section VII-3 of this report). Finally, as free crabs used for comparison were collected two weeks before the caged crabs it is possible that this is a normal finding at that time and would have been documented in free crabs had the free crabs been collected at the same time as caged crabs.

By spring 2013, more tangible differences between caged and free crabs were observed. Based on the percentage of caged crabs with ovaries classed as late vitellogenic, Cheticamp (26%) and Margaree (32%), many caged crabs had not spawned while, all free crabs had spawned (100% early vitellogenic stage at both stations). Examining the percentages of crabs with counts of clear follicles in the TNTC category, indicating recent spawning, produced similar conclusions. These observations in the caged crabs could reflect either: 1) a delayed spawn in 26% - 32% of caged crabs as oocytes had not yet matured due to e.g., decreased energy reserves or, 2) spawning had been completely aborted and would progress to resorption.

After a 12 month caging period, fall 2013, the histologic observations of ovaries were consistent with a recent spawning and/or the process of recovering from a spawning event for approximately 87% of crabs at Cheticamp and 95% of crabs at Margaree. However, as the age of the attached embryos (See Section IV of this report) were similar to free crabs, the data was interpreted to indicate that although most caged crabs had spawned in the spring, or perhaps later in the summer for some, the ovaries had not recovered to the appropriate stage for the fall i.e., fall ovaries should be at the late vitellogenic stage as observed for free crabs. Ovaries classified as 'not spawned' with fall age-appropriate embryos, would be interpreted as had recovered from a spring spawn (normal). These crabs may have had greater nutritional reserves at the start of the caging period and/or were more successful at feeding while in the caged environment.

Decreased lipid, and likely protein (unmeasured), reserves compared to free crabs (see SectionsVII-2 and VIII-3 of this report) are considered to be the main cause of the delayed spawn and recovery in caged crabs. 'Caging stress' may also be contributing, but as a 'caged with feeding' control group was not part of this study, this cannot be assessed.

Oocyte resorption was above the proposed physiologic level of 10% (5/50 oocytes) for this study in approximately 70% of 12M caged crabs from Cheticamp and about 40% of 12M caged crabs from Margaree. Resorption of primary oocytes, and occasionally basophilic oocytes, was also noted in some cases. This would be considered pathological resorption as only secondary oocytes would be expected to be resorbed as part of the normal post-spawning recovery phase. Oocytes undergoing resorption are variably referred to as atretic or degenerative oocytes (Zara *et al.* 2013). Ovaries which have just spawned are also referred to as 'spent', or ovigerous in reference to the assumption that the female is now carrying eggs/embryos (Zara *et al.* 2013).

The nature of the basophilic deposits which were most prominent in the 12M caged, although also present in spring free crabs, remains undetermined. They appear related to oocyte resorption and may represent a degenerative material. Further testing would be required. Grading of the severity of the deposits, which was not done in this study, may help discriminate between physiologic and pathologic resorption.

Chorion separation was again more noticeable in spring samples, with the exception of spring 2013 in Grand Rivière. This could be consistent with the results suggested in the 2003-2004 study that increases in tissue - including hemolymph - water as other tissue energy reserves are depleted, leads to an artifactual separation during tissue processing. Oocyte development would effectively drain other reserves. As the Grand Rivière crabs were at a less mature stage of ovary development, this could account for the different pattern in that group.

Degenerative nodules were observed in crabs from all stations during at least one sample period. There was no particular pattern apparent other than being observed most often (3 of 4 samples) in Cheticamp. The nodules are usually multilobular and appear to represent coalescence of secondary oocytes that presumably were not resorbed. Kon *et al.* (2010) had suggested that these nodules were more common in aged female crabs and indicating ovary senescence. Crabs chosen for this study were supposed to be younger females and, obviously, still active reproductively. Perhaps nodule development is related to the one-year vs two year spawning cycle and faster ovary cycling in some manner?

An interesting observation was the presence of sperm, free or enmeshed within homogenous, eosinophilic extracellular material – possibly prostatic fluid or material from sperm plug – in up to 29% of some samples (12M Margaree Harbor, caged). It could be that the spermatheca was ruptured and/or that the spermatheca was perhaps more fragile for some reason and contaminated the ovary tissue during specimen collection or, that there is a normal physiologic reason for its presence. Kon *et al.* (2010) also noted spermatids in one their samples, remarking that it was

unusual. Sainte-Marie *et al.* (2000) suggested that mixing of male and female gametes occurs, to some degree, in the ovaries in preparation for oviposition. It is unclear why sperm was only found in tissue from caged females in the fall 2013 samples – perhaps if unable to breed while caged, stored sperm was mobilised to a greater degree than usual in preparation for the spring/summer 2013 spawn.

Overall, both short and long-term caging appears to be associated with changes in the ovaries. A slight increase in oocyte resorption was noted after two weeks, evidence of delayed and/or aborted spawning at six months, and delayed recovery and/or marked resorption of oocytes at 12 months. Changes at two weeks seem more likely to be related to handling and/or caging stress while, changes noted at six and 12 months are more likely related to decreased nutritional stores. The apparent shift to a one year spawning cycle at Cheticamp, Margaree, and Louisbourg stations, with maintenance of a two year cycle at Grande Rivière, may merit further investigation.

IV-4-6 References

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IV-5 EMBRYO

IV-5-1 Introduction

As previously described for the ovary, the majority of multiparous snow crab females follow a 2-year reproductive cycle (mating with subsequent oviposition in May and go through a maturation process until the second following May when hatching occurs) in the southern Gulf of St. Lawrence. For this current project, multiparous females carrying 6-month old eggs (extruded in May 2012) were selected (Figure 1). These eggs were expected to hatch in May 2014 assuming that the embryonic development follows a 2-year cycle (Moriyasu & Lanteigne, 1998). However, the embryonic development is highly influenced by water temperature. Moriyasu and Lanteigne (1998) found that multiparous females can hatch their brood for the incubation time of 365 days at cumulative degree-days of 673.8˚C (1.85˚C/day). In general under a 2-year reproductive cycle, multiparous females that have spawned in the spring 2012 should maintain an embryonic stage of 4 or less 4 until December 2012, progress between stages 4 and 8 by spring 2013 and between stages 8 and 12 by fall 2013.

During this long embryonic development period, females may lose eggs resulting from interference by other males during copulatory embrace (newly extruded eggs are more susceptible for loss before the development of funiculus (filaments to which eggs attach), and parasites (nemertean worms) in the egg mass. In this study, we established a size-fecundity relationship for comparison among stations and between seasons. In addition, the relationships were compared between the free and caged females based on the hypothesis that caged females lose more eggs than free females.

Figure 1. Multiparous female carrying external eggs (photographed in November 2012)

IV-5-2 Sample treatments

See Section III-1-1 sample treatment.

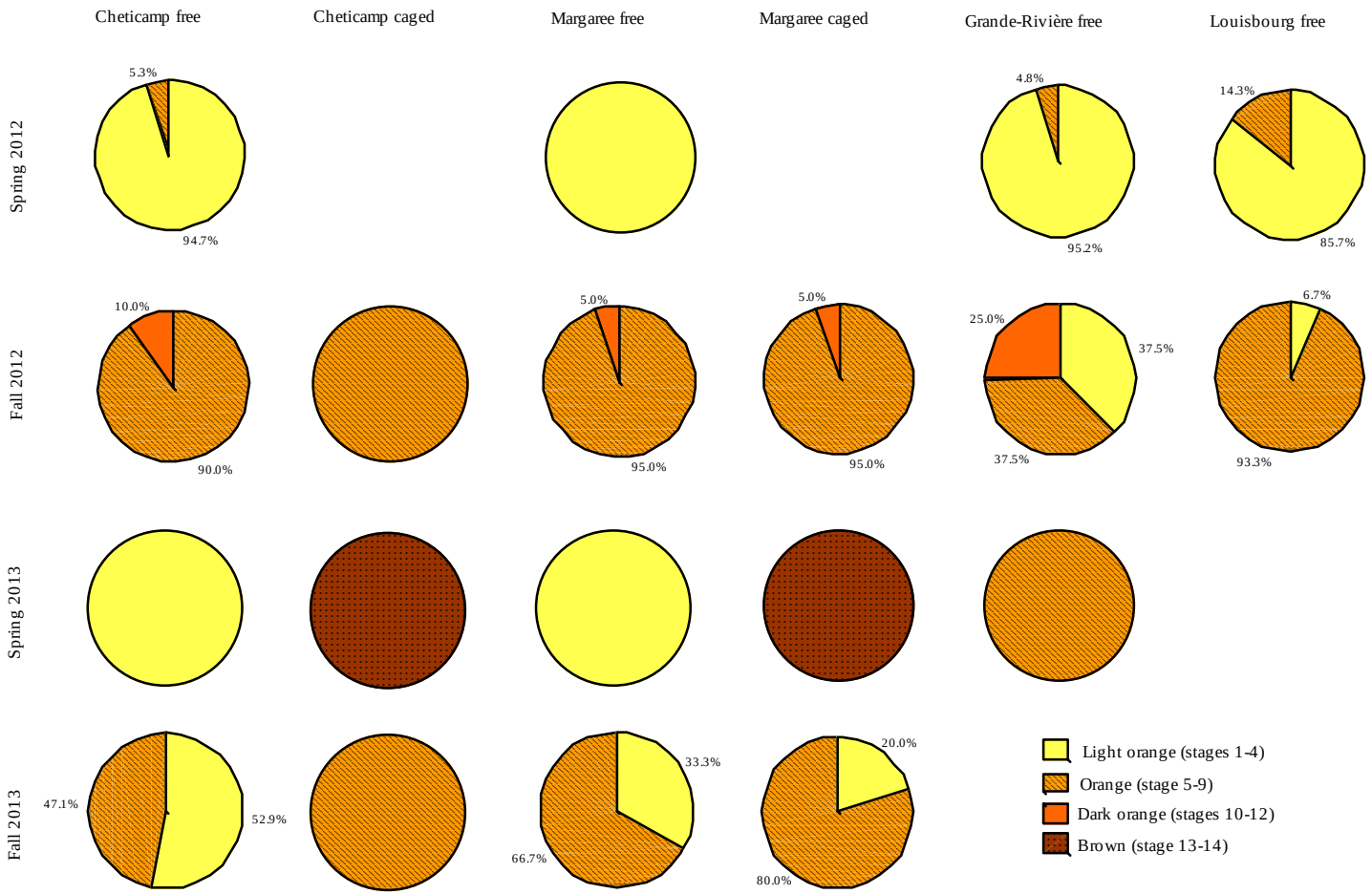
IV-5-3 Embryonic development stages

A total of 377 mature females ranging from 46.56 to 95.94 mm CW were analysed for fecundity estimates whereas only 252 were used for embryonic development stage determination.

Embryonic development was classified into four categories stages 1-4, 5-9, 10-12 and 13-14 based on Moriyasu & Lanteigne (1998), which correspond to the egg color observed by naked eyes light-orange, orange, dark-orange and brown, respectively.

In spring 2012, the majority of eggs were at stages 1-4 with small percentage of 5-9 (newly extruded eggs). In fall 2012, the majority progressed to stages 5-9 except for Grande-Rivière samples which showed a mixture of three different stages 1-4, 5-9 and 10-12. In spring 2013, 100% of free females in Margaree Harbor and Cheticamp stations carried eggs at stages 1-4 (new eggs), whereas 100% of caged crabs carried eggs at stages 13-14 (about to hatch). At the Grande-Rivière station, all females carried eggs at stages 5-9. In fall 2013, 100% of caged females in the Cheticamp station carried eggs at stages 5-9, the other females carried a mixture of stages 1-4 and 5-9 (Figure 2).

Figure 2. Egg color (based on embryonic developmental stages described by Moriyasu and Lanteigne, 1998) of female snow crab by sampling season and area.



IV-5-4 Color measurements of eggs

Among the color values (CIELAB a*, b* and L*) measured with a colorimeter, lightness coordinate (L*) showed clearer seasonal trend (Figure 3). The same statistical approach explained in IV-4-4 was applied for data preparation, analyses and interpretation. These measures showed a great variability in year and location in free crabs, but no significant difference in location-year interaction for both spring and fall seasons (Tables 1 & 2). The maximum observed difference (largest effects of natural variability) was (Tukey HSD) for location (between Grande-Rivière and Cheticamp) and for location-year interaction (between Grande-Rivière 2013 and Cheticamp 2013). Despite the high variability, the same tendancy was observed for Margaree Harbor, Cheticamp and Louisbourg (although color data for the spring 2012 samples were missing). In Margaree Harbor, Cheticamp and Louisbourg, the lightness value showed the same tendancy i.e. high (clearer) in spring and low (darker) in fall, whereas in Grande-Rivière, the values decreased from fall 2012 to spring 2013.

For caged females, the lightness value showed the same tendancy compared to that observed in free females. There were significant difference in terms of year/season and year/season-treatment (Table 3). For comparison between caged and free females, although there were no significant differences between 2 week caged vs free, and 12 month caged vs free, a significant difference was observed between 6 month caged vs free (Table 3, Figure 4). This suggests that caging females with developing embryo for up to 6 months prior to egg hatching might have impacted the embryonic development and egg hatching schedule.

The comparison between caged and free crab in a given season in Margaree Harbor showed that there was no significant difference in embryo color value (L*) in three different periods, fall 2012, spring 2013 and fall 2013(t-test p=0.0278; Mann-Whitney test p=0.3726 and t-test p=0.6751, respectively). In Cheticamp there was a significant difference in the fall 2012 (t-test p=0.0002) but no significant difference at 0.001 level for the fall 2012 and spring 2013 (t-test, p=0.0578; Mann-Whitney-test, p=0.0165, respectively).

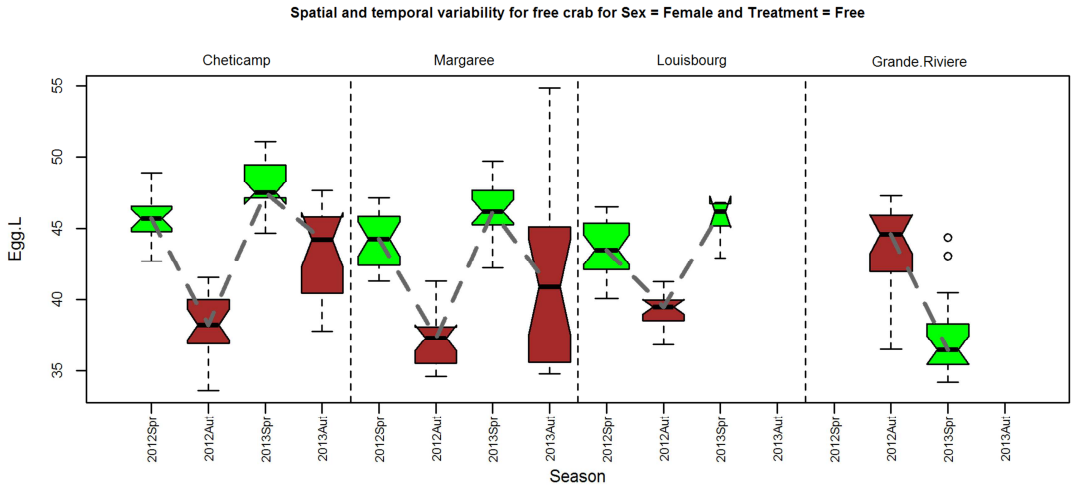


Figure 3. Spatial and temporal variability in the egg color parameter (L*) measured with a colorimeter of free female snow crabs in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

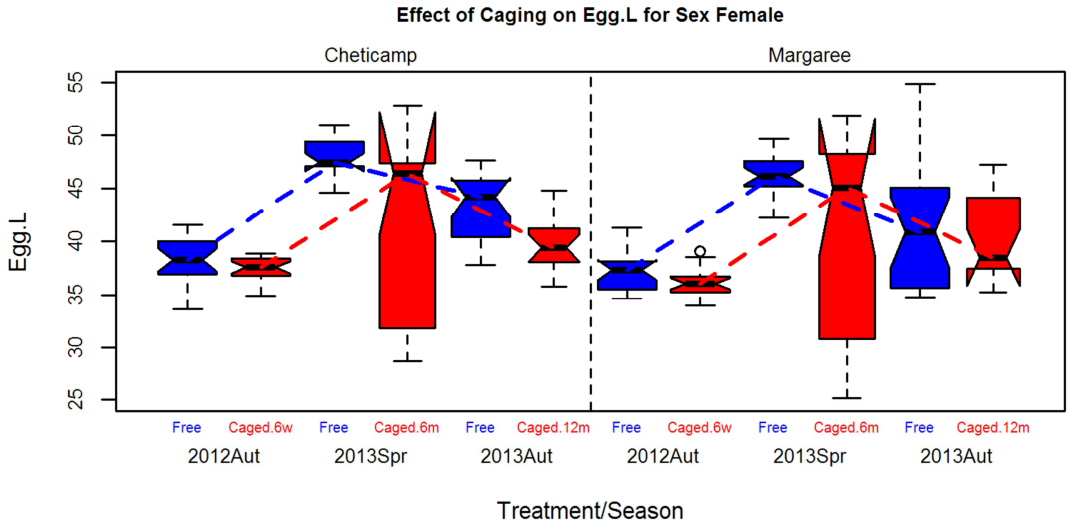


Figure 4. Effect of caging on the egg color parameter (L*) measured with a colorimeter of caged female snow crabs in comparison with free females in Cheticamp and Margaree Harbor stations.

Table 1. Spatial and temporal variability of egg color lightness (*L) measurement for free crabs in the spring: Cheticamp, Margaree Harbor, Louisbourg and Grand-Rivière in the spring.

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	1268	67.9	423	105	0.00000	***
Year	1	107	5.7	107	26	0.00000	***
Location:Year	2	0	0.0	0	0	0.94754	
Residuals	122	491	26.3	4			

Maximum observed difference(s) (Tukey HSD)
Observed mean value of Egg.L : 44.2

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Grande.Riviere-Cheticamp	-9.55	0.734	21.6	0.0000	***
Year	2013-2012	1.60	0.358	3.6	0.0000	***
Location:Year	Grande.Riviere:2013-Cheticamp:2013	-10.58	0.999	23.9	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Egg.L : p.value = 0.6622
Mean, median and standard deviations for Egg.L by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Spring 2012 Free	19	45.8	45.7	1.75
Cheticamp Female Spring 2013 Free	20	47.9	47.5	1.91
Grande.Riviere Female Spring 2013 Free	20	37.3	36.5	2.79
Louisbourg Female Spring 2012 Free	24	43.6	43.5	1.82
Louisbourg Female Spring 2013 Free	5	45.5	46.1	1.60
Margaree Female Spring 2012 Free	20	44.0	44.2	1.90
Margaree Female Spring 2013 Free	20	46.3	46.2	1.79

Table 2. Spatial and temporal variability of egg color lightness (*L) measurement for free crabs in the fall: Cheticamp, Margaree Harbor, Louisbourg and Grand-Riviere in the fall.

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	315	17.3	105	11.0	0.00000	***
Year	1	387	21.2	387	40.4	0.00000	***
Location:Year	1	10	0.5	10	1.0	0.32075	
Residuals	116	1112	61.0	10			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Egg.L : 44.2

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Grande.Riviere-Cheticamp	-9.55	0.734	21.6	0.0000	***
Year	2013-2012	1.60	0.358	3.6	0.0000	***
Location:Year	Grande.Riviere:2013-Cheticamp:2013	-10.58	0.999	23.9	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Egg.L : p.value = 0.0000
Mean, median and standard deviations for Egg.L by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Autumn 2012 Free	20	38.4	38.3	2.03
Cheticamp Female Autumn 2013 Free	20	43.4	44.2	2.95
Grande.Riviere Female Autumn 2012 Free	21	43.5	44.6	3.20
Louisbourg Female Autumn 2012 Free	20	39.2	39.5	1.16
Margaree Female Autumn 2012 Free	20	37.2	37.3	1.69
Margaree Female Autumn 2013 Free	20	40.9	40.9	5.53

Table 3. Spatial and temporal variability of egg color lightness (L*) measurement for free and caged crabs: Cheticamp and Margaree Harbor.

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	1	109	1.6	109	6.1	0.01429	*
YearSeason	2	2097	31.1	1049	58.9	0.00000	***
YearSeason:Treatment	3	630	9.3	210	11.8	0.00000	***
Residuals	220	3916	58.0	18			

Differences due to Location (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
Location	Margaree-Cheticamp	-1.38	0.563	-3.4	0.0143	*

Differences due to YearSeason (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
YearSeason	2013Spr-2012Aut	7.33	0.814	17.9	0.0000	***
YearSeason	2013Aut-2012Aut	3.87	0.828	9.4	0.0000	***
YearSeason	2013Aut-2013Spr	-3.46	0.838	-8.5	0.0000	***

Differences due Treatment at 2 weeks, 6 months, 12 months (Tukey HSD)

Treatment	Values	Difference	SE	Difference (%)	HSD.p.value	Significance
2 weeks	2012Fall:Caged-2012 Fall:Free	-1.064	1.38	-2.6	0.8695	
6 months	2013Spr:Caged-2013Spr:Free	-5.230	1.42	-12.8	0.0000	***
12 months	2013Fall:Caged-2013Fall:Free	-2.260	1.48	-5.5	0.2244	

Homogeneity of variance test (Levene-Brown-Forsythe) for Egg.L : p.value = 0.0000
Mean, median and standard deviations for Egg.L by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Fall 2012 Caged	20	37.4	37.6	1.05
Cheticamp Female Fall 2012 Free	20	38.4	38.3	2.03
Cheticamp Female Fall 2013 Caged	15	39.5	39.4	2.49
Cheticamp Female Fall 2013 Free	20	43.4	44.2	2.95
Cheticamp Female Spring 2013 Caged	18	42.8	46.4	7.91
Cheticamp Female Spring 2013 Free	20	47.9	47.5	1.91
Margaree Female Fall 2012 Caged	20	36.1	36.0	1.33
Margaree Female Fall 2012 Free	20	37.2	37.3	1.69
Margaree Female Fall 2013 Caged	16	40.2	38.5	4.06
Margaree Female Fall 2013 Free	20	40.9	40.9	5.53
Margaree Female Spring 2013 Caged	18	41.0	45.1	9.09
Margaree Female Spring 2013 Free	20	46.3	46.2	1.79

IV-5-5 Fecundity

Carapace width and fecundity relationships (log_e transformed data) were established by season, by year and by geographic location/sampling station (Table 4). The same statistical approach explained in III-4-2 was applied for data preparation, analyses and interpretation.
There was a significant difference in carapace width and fecundity relationships in terms of location, and year in the spring (Tables 4 & 5 Figures 5, 6). For location, Margaree Harbor Cheticamp (p=0.00003), Louibourg-Cheticamp (0.00000), Grande-Rivière-Margaree (p=0.00001), Grande-Rivière-Margaree Harbor (p=0.00000) showed a significant difference. For year effect, there was a significant difference between 2012 - 2013 (p=0.00006) (Tables 4, Figure 5). In the fall (Table 6, Figure 5), there was a significant difference in terms of location (Grande-Rivière-Cheticamp (p=0.00006, Louisbourg-Margaree Harbor p=0.0006, and Grande-Rivière-Margaree Harbor p=0.00000) and year (2012-2013 p=0.00000). No clear seasonal tendancy in regression residual was observed in free crabs among four stations

For caged vs free crabs in Margaree Harbor and Cheticamp (Table 7, Figure 6), there was a significant difference (p=0.00000) in terms of year/season and year/season and treatment effects. For year/season effects, 2013 spring vs 2012 fall was significantly different (p=0.00002) and for year/season-treatment effect, caged vs free in the fall 2013 (12 month caged vs free) showed a significant difference (p=0.00000).

The size-fecundity relationships showed that there is a high variability in terms of season, and geographic location. For caged crabs, there was a significant difference in the size-fecundity relationship after 12 months of retention. This means that there is a significant effect of caging on fecundity, possibly due to continuously changing oceanographic parameters (e.g. temperature) which could have impacted on reproductive schedule of females (embryonic development and ovary development: see IV-4-3, VI-4-4, IV-5-3, IV-5-4).

Table 4. Regressions of fecundity (FEC, total number of eggs per brood), on carapace width (CW in mm) for caged and free female snow crab sampled in 2012 and 2013 in Grande-Rivière, Margaree Harbor, Cheticamp and Louisbourg (log transformed).

Regression parameters by group

Location	Year	Season	Treatment	n	y-intercept	slope	Correlation	Significance
Cheticamp	2012	Spring	Free	18	1.6	2.26	0.841	0.000
Cheticamp	2012	Autumn	Free	19	0.5	2.52	0.865	0.000
Cheticamp	2012	Autumn	Caged	20	-1.9	3.06	0.776	0.000
Cheticamp	2013	Spring	Free	20	3.3	1.81	0.851	0.000
Cheticamp	2013	Spring	Caged	13	9.6	0.27	0.147	0.631
Cheticamp	2013	Autumn	Free	20	-0.1	2.59	0.932	0.000
Cheticamp	2013	Autumn	Caged	15	14.4	-0.95	-0.147	0.602
Margaree	2012	Spring	Free	20	3.2	1.83	0.609	0.004
Margaree	2012	Autumn	Free	20	0.9	2.42	0.782	0.000
Margaree	2012	Autumn	Caged	20	0.7	2.46	0.941	0.000
Margaree	2013	Spring	Free	18	0.7	2.38	0.643	0.004
Margaree	2013	Spring	Caged	9	-1.4	2.91	0.690	0.040
Margaree	2013	Autumn	Free	20	7.9	0.75	0.349	0.132
Margaree	2013	Autumn	Caged	16	1.1	2.28	0.472	0.065
Louisbourg	2012	Spring	Free	24	-0.4	2.66	0.555	0.005
Louisbourg	2012	Autumn	Free	20	-10.0	4.91	0.846	0.000
Louisbourg	2013	Spring	Free	2	2.8	1.91	1.000	NaN
Grande.Riviere	2012	Spring	Free	17	0.7	2.47	0.887	0.000
Grande.Riviere	2012	Autumn	Free	20	1.2	2.27	0.739	0.000
Grande.Riviere	2013	Spring	Free	18	0.7	2.44	0.820	0.000

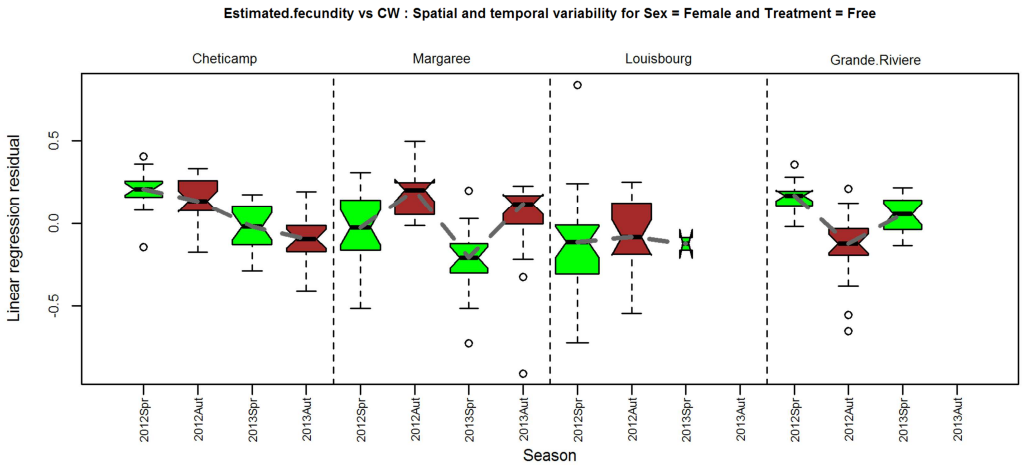


Figure 5. Spatial and temporal variability in Loess regression residuals of the carapace width vs fecundity relationships in female snow crab in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

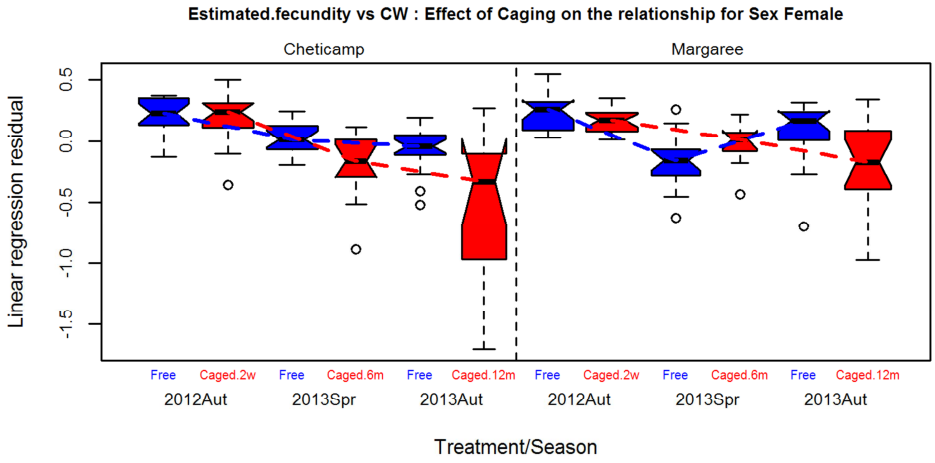


Figure 6. Effect of caging. Comparison of Loess regression residuals of the carapace width vs fecundity relationships in Cheticamp and Margaree Harbor, between free and caged female snow crab.

Table 5. Spatial and temporal variability of the carapace width-fecundity relationship for free female crabs in the spring (Cheticamp, Margaree, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	24	1.68	2.17
Cheticamp	2013	18	1.83	2.17
Margaree	2012	20	1.76	2.17
Margaree	2013	20	1.75	2.17
Louisbourg	2012	17	1.95	2.17
Louisbourg	2013	18	2.00	2.17
Grande.Riviere	2012	18	1.57	2.17
Grande.Riviere	2013	2	1.67	2.17

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	5.14		5.14	139	0.00000	***
Location	3	1.58	21.8	0.53	14	0.00000	***
Year	1	0.80	11.0	0.80	22	0.00001	***
Location:Year	3	0.11	1.5	0.04	1	0.40330	
Residuals	128	4.74	65.7	0.04			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	0.81	-0.215	0.0442	-1.96	0.00003	***
Location: Louisbourg - Cheticamp	0.76	-0.271	0.0523	-2.47	0.00000	***
Location: Grande.Riviere - Cheticamp	1.02	0.018	0.0466	0.16	0.99353	
Location: Louisbourg - Margaree	0.95	-0.056	0.0517	-0.51	0.77597	
Location: Grande.Riviere - Margaree	1.26	0.233	0.0465	2.13	0.00001	***
Location: Grande.Riviere - Louisbourg	1.34	0.289	0.0523	2.64	0.00000	***
Year: 2013 - 2012	0.85	-0.168	0.0363	-1.53	0.00006	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Estimated.fecundity : p.value = 0.1418

Mean, median and standard deviations for residuals of log Estimated.fecundity on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Spring 2012 Free	18	0.209	0.223	0.117
Cheticamp Female Spring 2013 Free	20	-0.020	-0.013	0.131
Grande.Riviere Female Spring 2012 Free	17	0.171	0.178	0.088
Grande.Riviere Female Spring 2013 Free	18	0.055	0.060	0.108
Louisbourg Female Spring 2012 Free	24	-0.105	-0.104	0.311
Louisbourg Female Spring 2013 Free	2	-0.114	-0.114	0.045
Margaree Female Spring 2012 Free	20	-0.032	-0.020	0.215
Margaree Female Spring 2013 Free	18	-0.214	-0.200	0.212

Table 6. Spatial and temporal variability of the carapace width-fecundity relationship for free female crabs in the fall (Cheticamp, Margaree, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	1.33	2.26
Cheticamp	2013	20	1.29	2.26
Margaree	2012	19	1.58	2.26
Margaree	2013	20	1.46	2.26
Louisbourg	2012	20	1.61	2.26
Grande.Riviere	2012	20	1.36	2.26

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	6.37		6.37	176	0.00000	***
Location	3	0.93	16.0	0.31	9	0.00004	***
Year	1	0.75	13.0	0.75	21	0.00001	***
Location:Year	1	0.04	0.8	0.04	1	0.27197	
Residuals	112	4.06	70.2	0.04			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	1.08	0.0773	0.0430	0.704	0.31865	
Location: Louisbourg - Cheticamp	0.83	-0.1923	0.0574	-1.749	0.00677	**
Location: Grande.Riviere - Cheticamp	0.77	-0.2637	0.0569	-2.399	0.00006	***
Location: Louisbourg - Margaree	0.76	-0.2696	0.0566	-2.453	0.00006	***
Location: Grande.Riviere - Margaree	0.71	-0.3410	0.0567	-3.102	0.00000	***
Location: Grande.Riviere - Louisbourg	0.93	-0.0714	0.0611	-0.650	0.71409	
Year: 2013 - 2012	0.82	-0.1964	0.0431	-1.787	0.00010	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Estimated.fecundity : p.value = 0.2363
Mean, median and standard deviations for residuals of log Estimated.fecundity on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Autumn 2012 Free	19	0.140	0.129	0.132
Cheticamp Female Autumn 2013 Free	20	-0.100	-0.101	0.146
Grande.Riviere Female Autumn 2012 Free	20	-0.146	-0.126	0.207
Louisbourg Female Autumn 2012 Free	20	-0.078	-0.096	0.206
Margaree Female Autumn 2012 Free	20	0.171	0.193	0.135
Margaree Female Autumn 2013 Free	20	0.020	0.111	0.269

Table 7. Spatial and temporal variability of the relationship between carapace width and fecundity for caged and free female crabs in Cheticamp, Margaree Harbor.

Regression parameters by group

Location	YearSeason	y-intercept	slope	treatment.effect (Caged)	treatment.effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Aut	-0.46	2.74	0.001	0.01	0.98893	0.41830	11.2	11.1
Cheticamp	2013Aut	3.26	1.80	-0.503	-4.66	0.00201	0.01443	10.8	10.3
Cheticamp	2013Spr	6.03	1.16	-0.214	-1.96	0.00308	0.00769	10.9	10.7
Margaree	2012Aut	0.80	2.45	-0.072	-0.65	0.04891	0.94046	11.2	11.1
Margaree	2013Aut	5.80	1.23	-0.242	-2.20	0.01955	0.17466	11.0	10.8
Margaree	2013Spr	0.13	2.51	0.176	1.64	0.06363	0.71307	10.8	10.8

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	6.4		6.38	89.0	0.00000	***
Location	1	0.3	1.2	0.27	3.7	0.05424	.
YearSeason	2	5.2	23.1	2.60	36.3	0.00000	***
YearSeason:Treatment	3	2.6	11.5	0.86	12.0	0.00000	***
Residuals	202	14.5	64.3	0.07			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	1.07	0.0663	0.0372	0.604	0.07568	.

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr - 2012Aut	0.75	-0.2820	0.0611	-2.568	0.00002	***
2013Aut - 2012Aut	0.81	-0.2065	0.0605	-1.880	0.00225	**
2013Aut - 2013Spr	1.08	0.0755	0.0608	0.688	0.43034	

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012Aut 2w	0.959	-0.0418	0.0603	-0.381	0.86576	
Caged vs Free: 2013Spr 6m	0.946	-0.0558	0.0719	-0.508	0.82197	
Caged vs Free: 2013Aut 12m	0.683	-0.3811	0.0645	-3.471	0.00000	***

IV-5-6 References

Moriyasu M. and Lanteigne C. 1998. Embryo development and reproductive cycle in the snow crab, *Chionoecetes opilio* (Crustacea: Majidae), in the southern Gulf of St. Lawrence, Canada. Can. J. Zool., 76(11): 2040-2048.

IV-6. HEPATOPANCREAS

IV-6-1 Introduction

The hepatopancreas consists of a multi-branched tubular structure and forms a large compact paired bilateral glandular mass occupying the cephalothoracic cavity (Figure 1).

The decapod hepatopancreas is a large digestive organ and its composition can be influenced by molt cycle, nutrition and stress (Gibson & Barker 1979). The hepatopancreas performs a wide variety of vital functions including the production and secretion of digestive enzymes, absorption and storage of nutrients and protection against pollutants (Gibson & Barker 1979). As a result, changes in the size, color or content of this organ may be useful to assess the status of the health of crab.

Each digestive tubule is imposed of an epithelium showing three principal types of cells: B-cells, R-cells and F-cells. B-cells are the largest cells and are characterized by a large vacuole with a thin cytoplasm and nuclei that are localized in the basal part of the cell. R-cells are the most abundant. The cytoplasm is homogeneous with a few vacuoles in the apical cytoplasm and nuclei are localized in the basal region of these columnar cells. F-cells are less numerous and have a fibrar appearance. Their cytoplasm is strongly basophilic and compressed between the R- and B-cells. The hepatopancreas is involved in diverse metabolic activities i.e. synthesis and secretion of digestive enzymes (F-cells), intracellular digestion (B-cells), and storage of lipid and glycogen (R-cells), (Gibson & Barker 1979, Al-Mohanna & Nott 1987, Icely & Nott 1992).

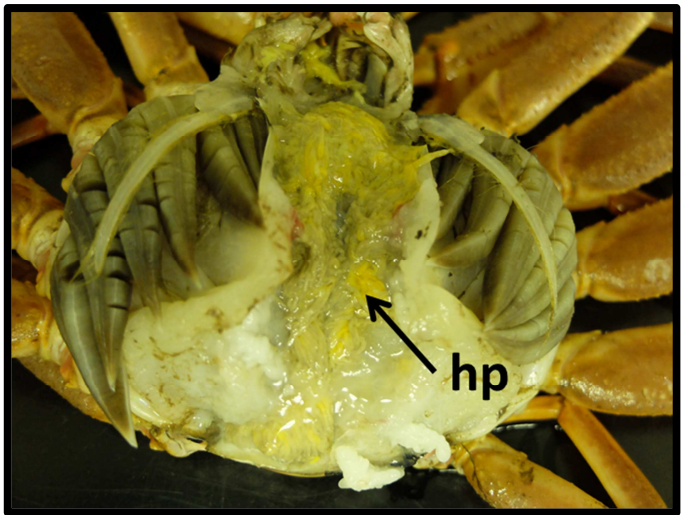


Figure 1. General view of the hepatopancreas

IV-6-2 Condition of hepatopancreas by SEM observation of the external wall

During the 2003-04 study on the possible impacts of seismic noise on snow crab Moriyasu *et al.* (unpublished) described that there were two morphological types of external wall of the hepatopancreas observed under Scanning Electron Microscope, i.e. smooth and convoluted. They hypothesized that the smooth-type may be a normal condition and the convoluted-type may be under stressful conditions. In order to verify this hypothesis, we have examined the outer wall of hepatopancreas under SEM in the current study.

A portion of hepatopancreas, about 3 - 5 mm in length depending on its size was dissected out from every 5th crab sampled. Tissue samples were immediately fixed at 4°C with 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 for 1 hour and rinsed in cold 0.175M cacodylate buffer at pH 7.2. Dehydration was achieved through increasing concentrations (up to 100%) of ethanol within 15 minutes. The samples were then dried in a Critical Point Dryer (CPD) at the critical point of CO2. Specimens, mounted on aluminum stubs with double side adhesive tabs, were sputter-coated with gold-palladium and examined with a JEOL 6400SEM at 10 kV acceleration voltage at the Microscopy and Microanalysis Facility, University of New Brunswick (UNB).

The majority of the outer hepatopancreatic walls were considered as ‘smooth’ or relatively convoluted (Tables 1 and 2, Figure 2A-C). In the 2012 spring samples, 8% (4) samples were classified as relatively convoluted, 2012 fall all samples 100% (70) were classified as smooth. In the 2013 spring samples, 20% of samples were relatively convoluted of which 17% were caged samples. In the fall 2013, 25% of samples were relatively convoluted of which 23% were caged samples. No highly convoluted hepatopancreatic wall (Figure 3) was observed such as being observed by Moriyasu *et al.* (unpublished).

In addition, the color of the hepatopancreas was measured by a chromameter to evaluate whether this parameter can be used for assessing the condition of hepatopancreas of snow crab (see Section IV-4-2, ovary sample treatment).

The result of hepatopancreas condition observations (Tables 1 &2) were regrouped into two conditions for analysis: 1) smooth and 2) non-smooth (relatively convoluted, convoluted and highly convoluted). The percentage of smooth condition was calculated by crab category and by location. The percentage of smooth condition was then compared between treatments (free and caged) for each category of crab (large male, pygmy male and female) with deviance table analysis (McCullagh & Nelder 1989: see Section IV-1-3 for detail of the analysis).

The results showed (Table 3) that there was effect of caging on the condition (% of smooth outer wall) of hepatopancreas for female crab (p=2.018e-06). No significant difference was observed in large male and pygmy male at p=0.001, the significance level was at p= 0.02504 and 0.01818 for large male and pygmy male, respectively, suggesting that the condition of the hepatopancreas outer wall shows some degree of modification by caging treatment. It is possible that decreased feeding activity and/or the quality of prey items may have impacted the morphological condition of hepatopancreas (some relationship with lower lipd content over time especially in mature females after 6-month caging see section VIII).

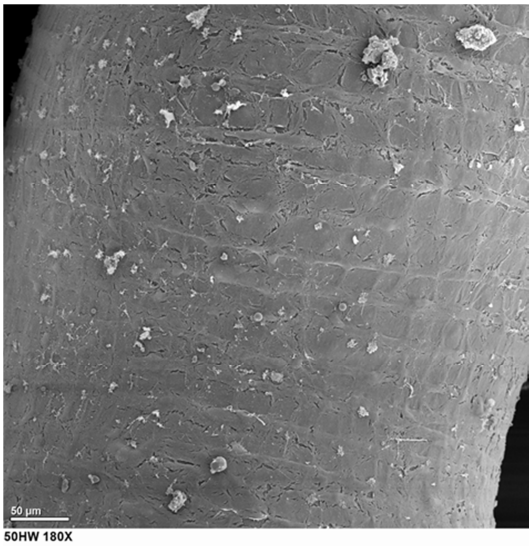


Figure 2-A. SEM view (180x) of the outer wall of hepatopancreas (smooth surface).

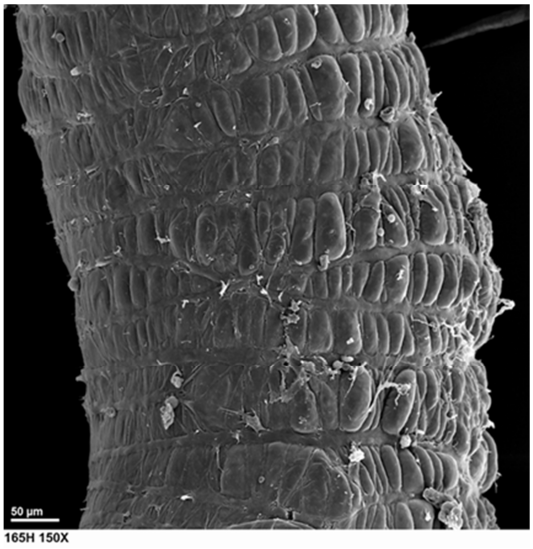


Figure 2-C. SEM view (150x) of the outer wall of hepatopancreas (convoluted).

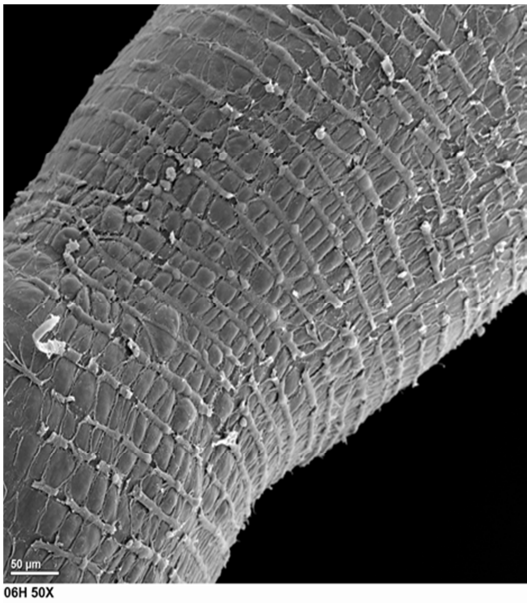


Figure 2-B. SEM view (50x) of the outer wall of hepatopancreas (partially or relatively convoluted).

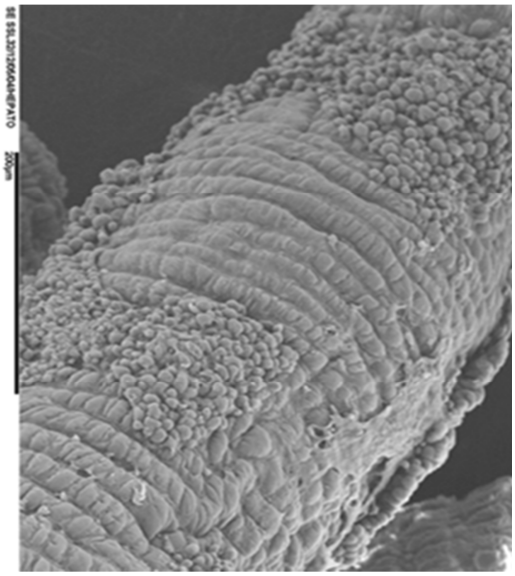


Figure 3. Highly convoluted hepatopancreatic wall observed (Figure 28-B in Moriyasu *et al.* unpublished).

Table 1. Condition of the outer wall of the hepatopancreas by season, station, treatment and crab category (large males (LM), pygmy males (PM) and mature females (MF) in 2012. (1: smooth, 2: relatively convoluted, 3: convoluted, 4: highly convoluted).

	SPRING 2012					FALL 2012				
Grande-Rivière free		1	2	3	4	Total	1	2	3	4	Total
	LM	8	5	0	0	13	4	0	0	0	4
	PM	0	0	0	0	0	2	2	0	0	4
	MF	0	0	0	0	0	1	3	0	0	4
		8	5	0	0	13	7	5	0	0	12
Margaree Harbor free		1	2	3	4	Total	1	2	3	4	Total
	LM	3	1	0	0	4	4	0	0	0	4
	PM	1	3	0	0	4	3	1	0	0	4
	MF	1	0	3	0	4	4	0	0	0	4
		5	4	3	0	12	11	1	0	0	12
Cheticamp free		1	2	3	4	Total	1	2	3	4	Total
	LM	2	1	0	0	3	4	0	0	0	4
	PM	2	3	0	0	5	3	1	0	0	4
	MF	1	2	1	0	4	4	0	0	0	4
		5	6	1	0	12	11	1	0	0	12
Louisbourg free		1	2	3	4	Total	1	2	3	4	Total
	LM	3	0	0	0	3	2	1	0	0	3
	PM	2	2	0	0	4	3	1	0	0	4
	MF	2	3	0	0	5	4	0	0	0	4
		7	5	0	0	12	9	2	0	0	11
Margaree caged							1	2	3	4	Total
							LM	2	1	0	0	3
							PM	2	2	0	0	4
							MF	1	3	0	0	4
								5	6	0	0	11
Checticamp caged							1	2	3	4	Total
							LM	3	1	0	0	4
							PM	1	3	0	0	4
							MF	2	2	0	0	4
								6	6	0	0	12

Table 2. Condition of the outer wall of the hepatopancreas by season, station, treatment and crab category (large males (LM), pygmy males (PM) and mature females (MF) in 2013. (1: smooth, 2: relatively convoluted, 3: convoluted, 4: highly convoluted).

SPRING 2013 **FALL 2013**

Grande-Rivière free

	1	2	3	4	Total
LM	2	2	0	0	4
PM	2	2	0	0	4
MF	3	0	1	0	4
	7	4	1	0	12

Margaree Harbor free

	1	2	3	4	Total
LM	2	2	0	0	4
PM	1	3	0	0	4
MF	3	1	0	0	4
	6	6	0	0	12

	1	2	3	4	Total
LM	3	0	0	0	3
PM	3	1	0	0	4
MF	4	0	0	0	4
	10	1	0	0	11

Cheticamp free

	1	2	3	4	Total
LM	1	3	0	0	4
PM	2	2	0	0	4
MF	2	2	0	0	4
	5	7	0	0	12

	1	2	3	4	Total
LM	4	0	0	0	4
PM	2	2	0	0	4
MF	2	1	1	0	4
	8	3	1	0	12

Louisbourg free

	1	2	3	4	Total
LM	5	0	0	0	5
PM	2	2	0	0	4
MF	1	0	0	0	1
	8	2	0	0	10

Margaree caged

	1	2	3	4	Total
LM	3	1	0	0	4
PM	3	1	0	0	4
MF	0	1	3	0	4
	6	3	3	0	12

	1	2	3	4	Total
LM	0	2	0	0	2
PM	0	1	1	0	2
MF	1	1	1	0	3
	1	4	2	0	7

Checticamp caged

	1	2	3	4	Total
LM	2	0	2	0	4
PM	0	2	2	0	4
MF	0	3	1	0	4
	2	5	5	0	12

	1	2	3	4	Total
LM	0	2	1	0	3
PM	0	1	3	0	4
MF	0	0	3	0	3
	0	3	7	0	10

Table 3. Percentage (%) of hepatopancreas with a smooth outer wall and results of deviance table analysis for each category of crab (large males, pygmy males and mature females) between caged and free crabs in Cheticamp and Margaree Harbor for fall 2012 (2 week caged), spring 2013 (free vs 6-month caged), fall 2013 (free vs 12-month caged).

Large Males:

Proportion smooth wall (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	25	100
Caged	75	50	0

Proportion smooth wall (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100.0	50	100
Caged	66.7	75	0

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	41	51.401			
Model 2	40	46.379	1	5.0213	0.02504

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

Pygmy Males

Proportion smooth wall (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	75	50	50
Caged	25	0	0

Proportion smooth wall (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	75	25	75
Caged	50	75	0

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	43	60.479			
Model 2	42	54.900	1	5.5789	0.01818

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

Mature Females

Proportion smooth wall (%)/ Cheticamp

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	50	50
Caged	50	0	0

Proportion smooth wall (%)/ Margaree Harbor

Treatment	Fall 2012	Spring 2013	Fall 2013
Free	100	75	100.0
Caged	25	0	33.3

Analysis of deviance table

	Resid.	Df Resid.	Dv Df	Deviance	Pr(>Chi)
Model 1	42	58.286			
Model 2	41	35.708	1	22.578	2.018e-06-06

Model 1: y ~ Location + YearSeason, Model 2: y ~ Location/Treatment + YearSeason

IV-6-3 Hepatopancreas color measurements by colorimeter

Color values (CIELAB a*, b* and L*) measured with a colorimeter showed a great variability in year and location in free crabs. Chromaticity coordinate (red-green: a*) showed clearer seasonal trend for hepatopancreas color.The same statistical approach explained in IV-4-4 was applied for data preparation, analyses and interpretation.

Large males

Analyses of variance showed that chromaticity coordinate values (red-green: a*) are highly variable especially through year and year-location interaction factors (Tables 4 & 5). The maximum significant difference was observed for location between Grande-Rivière and Margaree, and for location-year interaction between Louisbourg 2013 and Grande-Rivière 2012. Despite of high variability, the same tendancy was observed at four stations i.e. lower (redness) in spring and higher (redness) in fall (Figure 4).
For caged males, the value showed the same tendancy compared to that observed in free crabs (Table 6, Figure 5). Analysis of variance showed that no significant difference in terms of treatment for each cage immersion duration (Table 6). The comparison of chromaticity paramaeter values between treatment within the season and location showed significant difference only in Cheticamp stations (t-test, $p=0.0001$) for the fall 2013 (12 month-caged).

Pygmy males

Analyes of variance showed that there was no significant difference (at $p=0.001$) in observed in terms of location, year and year-location interaction in chromaticity coordinate (red-green: a*) for the spring season, but was significant in terms of location and location-year interaction for the fall season. The chromaticity value has slightly changed seasonally for four stations i.e. lower (redness) in spring and higher (redness) in fall (Tables 7, 8, Figure 6). For caged pygmy males, the chromaticity coordinate values showed the same tendancy compared to that observed in free crabs although significant difference was observed between cage and free crabs for each length of caging (2 weeks, 6 months and 12 months) in both stations (Table 9, Figure 7). The comparison of chromaticity paramaeter values between treatment within the season and the locations showed significant difference in both Margaree Harbor and Cheticamp stations (t-test, $p=0.0001$, Mann-Whitney test, $p<0.0005$, respectively) for the fall 2013 (12 month-caged).

Mature females

Analyses of variance showed that chromaticity coordinate values (red-green: a*) are highly variable especially in location for the spring and location, year and location-year interaction for the fall. (Tables 10, 11, Figure 8).

Despite high variability, the same tendancy was observed at three stations (except for Margaree fall 2012 where the value was comparable to that in spring 2012 and Grande-Rivière where fall 2012 data were not available) i.e. lower (redness) in spring and higher (redness) in fall.

For caged females, analyse of variance showed that chromaticity coordinate values (red-green: a*) are highly variable in terms of year/season and year/season-treatment. The chromaticity coordinate values showed the same tendancy compared to that observed in free crabs although significant difference was observed between 2 week-caged and free crabs (Table 12, Figure 9). The comparison of chromaticity paramaeter values between treatment within the season and location showed significant difference between Margaree Harbor and Cheticamp (t-test, p=0.0001 for both cases) for the fall 2013 (12 month-caged).

In summary, the chromaticity coordinate values of hepatopancreas showed a high variability in terms of location, season/year, and location-year interaction. For caged crabs the difference was significant between 12 month caged crabs and free crabs except for large males (except for Margaree Harbor).

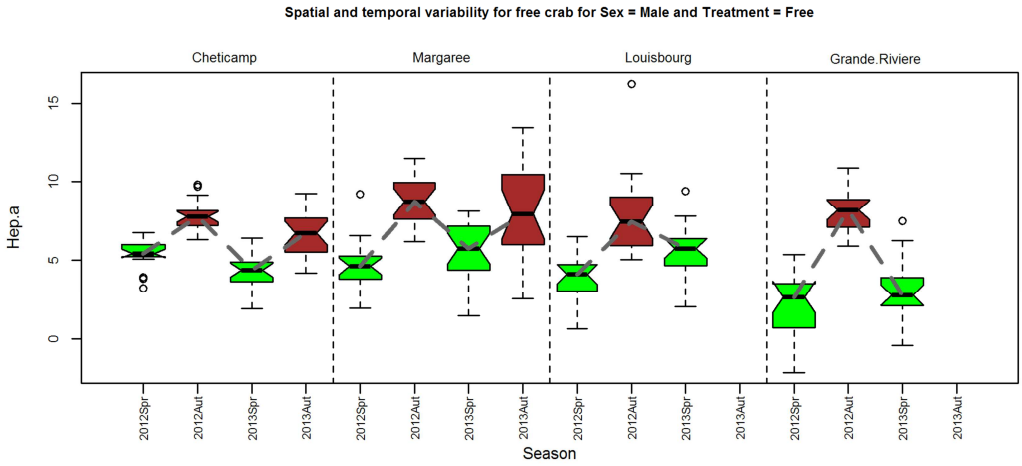


Figure 4. Spatial and temporary variability of hepatopancreas color value a* (red-green) measured with a chromameter (Konica-Minolta CR400) for male snow crab in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

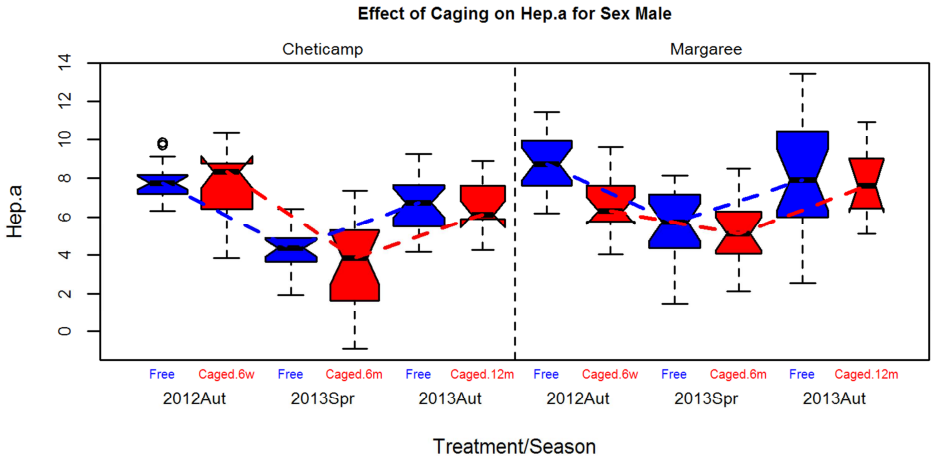


Figure 5. Effect of caging on the hepatopancreas color value a* (red-green) parameter (L*) measured with a chromameter (Konica-Minolta CR400) of caged large male snow crabs in comparison with free large males in Cheticamp and Margaree Harbor stations.

Table 4. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for large male free crabs: Cheticamp, Margaree, Louisbourg and Grand-Riviere (spring season).

Season: Spring

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	153	24.7	50.9	18.7	0.00000	***
Year	1	10	1.7	10.3	3.8	0.05356	.
Location:Year	3	44	7.2	14.7	5.4	0.00145	**
Residuals	151	411	66.5	2.7			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Hep.a : 4.34

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Grande.Riviere-Margaree	-2.397	0.489	55.3	0.0000	***
Year	2013-2012	0.509	0.264	11.7	0.0537	.
Location:Year	Louisbourg:2013-Grande.Riviere:2012	3.270	0.819	75.4	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0961
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Male Spring 2012 Free	21	5.44	5.42	0.882
Cheticamp Male Spring 2013 Free	20	4.33	4.36	1.132
Grande.Riviere Male Spring 2012 Free	20	2.34	2.65	2.092
Grande.Riviere Male Spring 2013 Free	20	2.98	2.77	1.894
Louisbourg Male Spring 2012 Free	18	3.79	4.09	1.663
Louisbourg Male Spring 2013 Free	20	5.61	5.74	1.684
Margaree Male Spring 2012 Free	20	4.65	4.60	1.575
Margaree Male Spring 2013 Free	20	5.45	5.72	1.949

Table 5. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for free large male crabs: Cheticamp, Margaree, Louisbourg and Grand-Rivière (fall season)

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	28	6.0	9.3	2.58	0.05673	.
Year	1	15	3.3	15.3	4.26	0.04134	*
Location:Year	1	4	0.8	3.5	0.97	0.32579	
Residuals	116	417	89.9	3.6			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Hep.a : 4.34

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Grande.Riviere-Margaree	-2.397	0.489	55.3	0.0000	***
Year	2013-2012	0.509	0.264	11.7	0.0537	.
Location:Year	Louisbourg:2013-Grande.Riviere:2012	3.270	0.819	75.4	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0007
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Male Autumn 2012 Free	19	7.86	7.76	0.921
Cheticamp Male Autumn 2013 Free	20	6.58	6.72	1.324
Grande.Riviere Male Autumn 2012 Free	20	8.06	8.20	1.229
Louisbourg Male Autumn 2012 Free	20	7.87	7.46	2.528
Margaree Male Autumn 2012 Free	19	8.70	8.72	1.483
Margaree Male Autumn 2013 Free	20	8.06	7.92	2.995

Table 6. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for caged and free large male crabs: Cheticamp, Margaree.

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	1	37	3.3	37	11.4	0.00088	***
YearSeason	2	398	34.8	199	60.8	0.00000	***
YearSeason:Treatment	3	30	2.7	10	3.1	0.02759	*
Residuals	207	678	59.3	3			

Differences due to Location (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
Location	Margaree-Cheticamp	0.836	0.249	12.9	0.0009	***

Differences due to YearSeason (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
YearSeason	2013Spr-2012Aut	-3.039	0.356	-46.7	0.0000	***
YearSeason	2013Aut-2012Aut	-0.427	0.370	-6.6	0.3480	
YearSeason	2013Aut-2013Spr	2.612	0.372	40.2	0.0000	***

Differences due Treatment at 2 weeks, 6 months, 12 months (Tukey HSD)

Treatment	Values	Difference	SE	Difference (%)	HSD.p.value	Significance
2 weeks	2012Aut:Caged-2012Aut:Free	-1.1619	0.609	-17.9	0.0616	.
6 months	2013Spr:Caged-2013Spr:Free	-0.5009	0.619	-7.7	0.8427	
12 months	2013Aut:Caged-2013Aut:Free	-0.1338	0.686	-2.1	0.9997	

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0003
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Male Autumn 2012 Caged	20	7.55	8.32	1.702
Cheticamp Male Autumn 2012 Free	19	7.86	7.76	0.921
Cheticamp Male Autumn 2013 Caged	15	6.70	6.13	1.350
Cheticamp Male Autumn 2013 Free	20	6.58	6.72	1.324
Cheticamp Male Spring 2013 Caged	18	3.58	3.85	2.424
Cheticamp Male Spring 2013 Free	20	4.33	4.36	1.132
Margaree Male Autumn 2012 Caged	18	6.59	6.33	1.329
Margaree Male Autumn 2012 Free	19	8.70	8.72	1.483
Margaree Male Autumn 2013 Caged	9	7.72	7.61	1.956
Margaree Male Autumn 2013 Free	20	8.06	7.92	2.995
Margaree Male Spring 2013 Caged	16	5.24	5.12	1.625
Margaree Male Spring 2013 Free	20	5.45	5.72	1.949

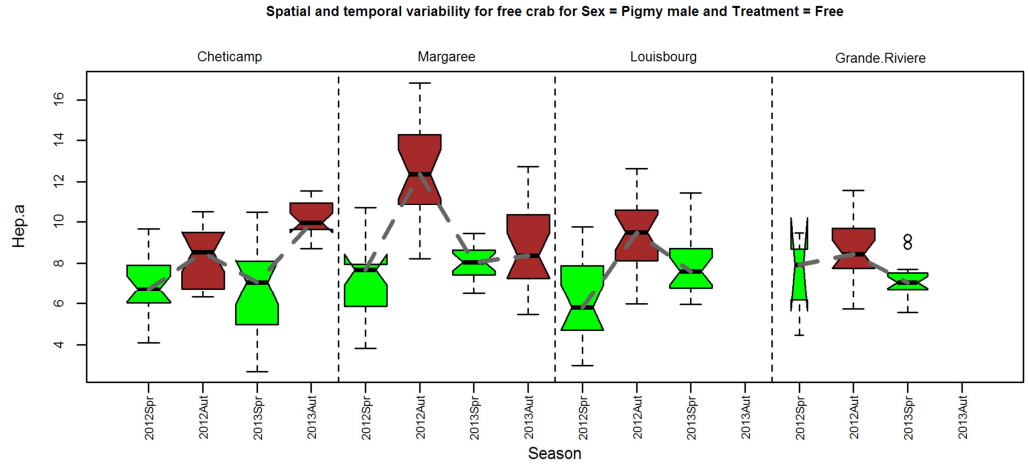


Figure 6. Spatial and temporary variability of hepatopancreas color value a* (red-green) measured with a chromameter (Konica-Minolta CR400) pygmy males in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

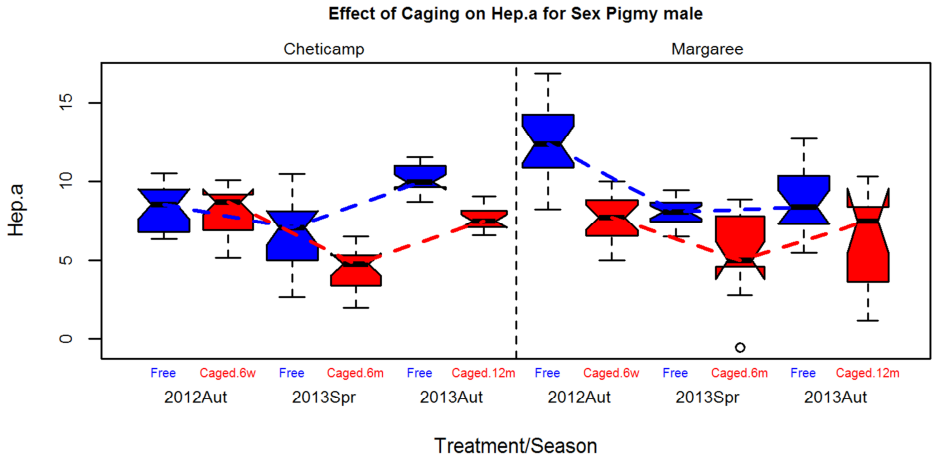


Figure 7. Effect of caging on the hepatopancreas color value a* (red-green)parameter (L*) measured with a chromameter (Konica-Minolta CR400) of caged pygmy males in comparison with free large males in Cheticamp and Margaree Harbor stations.

Table 7. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for free pygmy male crabs: Cheticamp, Margaree, Louisbourg and Grand-Riviere (spring season)

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	14	3.9	4.8	2.01	0.11555	
Year	1	13	3.5	13.0	5.44	0.02117	*
Location:Year	3	19	5.1	6.2	2.59	0.05524	.
Residuals	134	319	87.4	2.4			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Hep.a : 7.13

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Margaree-Cheticamp	0.817	0.455	11.5	0.0859	.
Year	2013-2012	0.587	0.263	8.2	0.0259	*
Location:Year	Margaree:2013-Louisbourg:2012	1.786	0.767	25.1	0.0085	**

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0118
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Pygmy male Spring 2012 Free	21	6.92	6.73	1.469
Cheticamp Pygmy male Spring 2013 Free	20	6.62	7.05	1.945
Grande.Riviere Pygmy male Spring 2012 Free	3	7.28	7.91	2.558
Grande.Riviere Pygmy male Spring 2013 Free	18	7.16	7.07	0.847
Louisbourg Pygmy male Spring 2012 Free	20	6.25	5.82	1.947
Louisbourg Pygmy male Spring 2013 Free	20	7.76	7.61	1.336
Margaree Pygmy male Spring 2012 Free	20	7.14	7.69	1.728
Margaree Pygmy male Spring 2013 Free	20	8.04	8.05	0.933

Table 8. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for free pygmy male crabs: Cheticamp, Margaree, Louisbourg and Grand-Riviere (fall season).

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	70	11.9	23	7.9	0.00008	***
Year	1	20	3.5	20	6.9	0.00970	**
Location:Year	1	160	27.4	160	54.6	0.00000	***
Residuals	114	335	57.2	3			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Hep.a : 7.13

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Margaree-Cheticamp	0.817	0.455	11.5	0.0859	.
Year	2013-2012	0.587	0.263	8.2	0.0259	*
Location:Year	Margaree:2013-Louisbourg:2012	1.786	0.767	25.1	0.0085	**

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0177
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Pygmy male Autumn 2012 Free	20	8.35	8.54	1.474
Cheticamp Pygmy male Autumn 2013 Free	20	10.17	9.96	0.881
Grande.Riviere Pygmy male Autumn 2012 Free	20	8.65	8.43	1.461
Louisbourg Pygmy male Autumn 2012 Free	20	9.38	9.50	1.756
Margaree Pygmy male Autumn 2012 Free	20	12.60	12.36	2.221
Margaree Pygmy male Autumn 2013 Free	20	8.76	8.37	2.124

Table 9. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for caged and free pygmy male crabs: Cheticamp, Margaree.

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	1	27	1.7	27	6.8	0.00977	**
YearSeason	2	367	22.8	184	46.5	0.00000	***
YearSeason:Treatment	3	352	21.9	117	29.7	0.00000	***
Residuals	218	861	53.6	4			

Differences due to Location (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
Location	Margaree-Cheticamp	0.691	0.266	8.7	0.0098	**

Differences due to YearSeason (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
YearSeason	2013Spr-2012Aut	-2.970	0.383	-37.5	0.0000	***
YearSeason	2013Aut-2012Aut	-0.766	0.394	-9.7	0.0528	.
YearSeason	2013Aut-2013Spr	2.204	0.397	27.9	0.0000	***

Differences due Treatment at 2 weeks, 6 months, 12 months (Tukey HSD)

Treatment	Values	Difference	SE	Difference (%)	HSD.p.value	Significance
2 weeks	2012Aut:Caged-2012Aut:Free	-2.643	0.656	-33.4	0.0000	***
6 months	2013Spr:Caged-2013Spr:Free	-2.358	0.665	-29.8	0.0000	***
12 months	2013Aut:Caged-2013Aut:Free	-2.528	0.711	-32.0	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0000
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Pygmy male Autumn 2012 Caged	19	8.02	8.70	1.437
Cheticamp Pygmy male Autumn 2012 Free	20	8.35	8.54	1.474
Cheticamp Pygmy male Autumn 2013 Caged	16	7.61	7.46	0.760
Cheticamp Pygmy male Autumn 2013 Free	20	10.17	9.96	0.881
Cheticamp Pygmy male Spring 2013 Caged	19	4.54	4.73	1.190
Cheticamp Pygmy male Spring 2013 Free	20	6.62	7.05	1.945
Margaree Pygmy male Autumn 2012 Caged	20	7.67	7.70	1.432
Margaree Pygmy male Autumn 2012 Free	20	12.60	12.36	2.221
Margaree Pygmy male Autumn 2013 Caged	13	6.03	7.49	3.174
Margaree Pygmy male Autumn 2013 Free	20	8.76	8.37	2.124
Margaree Pygmy male Spring 2013 Caged	18	5.41	4.97	2.204
Margaree Pygmy male Spring 2013 Free	20	8.04	8.05	0.933

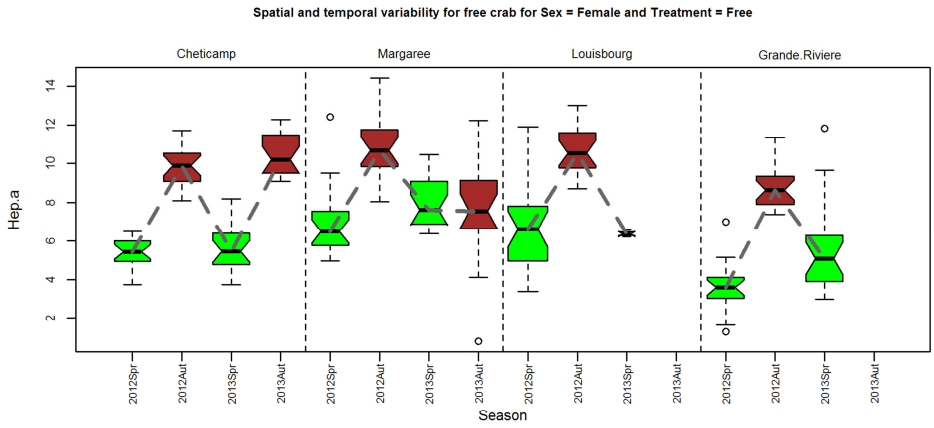


Figure 8. Spatial and temporary variability of hepatopancreas color value a* (red-green) measured with a chromameter (Konica-Minolta CR400) mature females in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

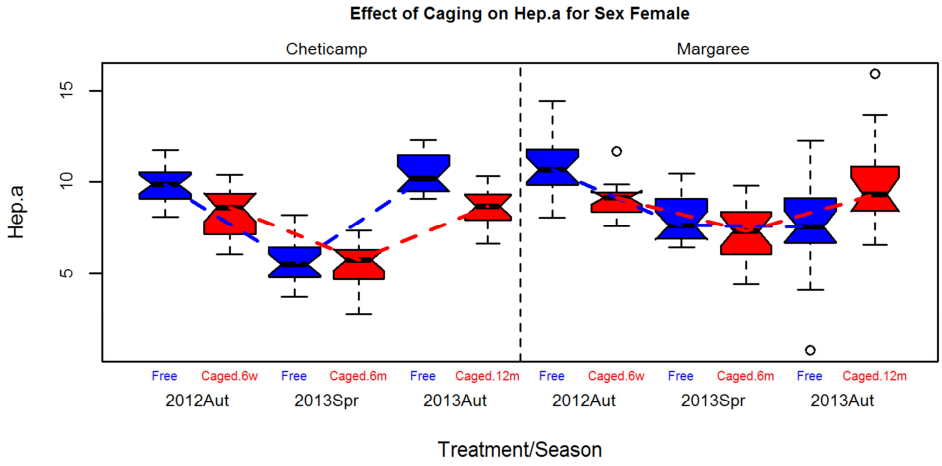


Figure 9. Effect of caging on the hepatopancreas color value a* (red-green) parameter (L*) measured with a chromameter (Konica-Minolta CR400) of caged females in comparison with free females in Cheticamp and Margaree Harbor.

Table 10. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for free female crabs: Cheticamp, Margaree Harbor, Louisbourg and Grand-Riviere (spring season).

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	183	32.6	61.0	25.4	0.00000	***
Year	1	27	4.8	27.0	11.3	0.00102	**
Location:Year	3	21	3.7	7.0	2.9	0.03634	*
Residuals	138	331	58.9	2.4			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Hep.a : 6

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Grande.Riviere-Margaree	-2.858	0.459	47.6	0.0000	***
Year	2013-2012	0.832	0.261	13.9	0.0016	**
Location:Year	Margaree:2013-Grande.Riviere:2012	4.307	0.769	71.8	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0096
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Spring 2012 Free	19	5.42	5.44	0.751
Cheticamp Female Spring 2013 Free	20	5.62	5.46	1.065
Grande.Riviere Female Spring 2012 Free	20	3.60	3.58	1.322
Grande.Riviere Female Spring 2013 Free	20	5.57	5.09	2.198
Louisbourg Female Spring 2012 Free	23	6.68	6.62	1.983
Louisbourg Female Spring 2013 Free	4	6.38	6.37	0.145
Margaree Female Spring 2012 Free	20	6.98	6.51	1.719
Margaree Female Spring 2013 Free	20	7.91	7.62	1.289

Table 11. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for free female crabs: Cheticamp, Margaree Harbor, Louisbourg and Grand-Riviere (fall season).

Season: Fall
Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	3	54	12.2	17.9	7.7	0.00010	***
Year	1	35	8.0	35.2	15.1	0.00017	***
Location:Year	1	81	18.4	81.0	34.6	0.00000	***
Residuals	116	271	61.5	2.3			

Maximum observed difference(s) (Tukey HSD) showing the largest effects of natural variability.
Observed mean value of Hep.a : 6

Factor	Values	Largest difference	SE	Largest difference (%)	HSD p-value	Significance
Location	Grande.Riviere-Margaree	-2.858	0.459	47.6	0.0000	***
Year	2013-2012	0.832	0.261	13.9	0.0016	**
Location:Year	Margaree:2013-Grande.Riviere:2012	4.307	0.769	71.8	0.0000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0193
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Autumn 2012 Free	20	9.81	9.89	1.02
Cheticamp Female Autumn 2013 Free	20	10.41	10.19	1.06
Grande.Riviere Female Autumn 2012 Free	21	8.81	8.64	1.14
Louisbourg Female Autumn 2012 Free	20	10.68	10.54	1.19
Margaree Female Autumn 2012 Free	20	10.87	10.67	1.60
Margaree Female Autumn 2013 Free	20	7.53	7.55	2.59

Table 12. Spatial and temporal variability of hepatopancreas color measurement (a*: red-green) for caged and free female crabs: Cheticamp, Margaree Harbor.

Analysis of variance: Location, Year with Interaction

Factor	Df	Sum.Sq	Sum.Sq. (%)	Mean.Sq	F.value	Pr(>F)	Significance
Location	1	25	2.2	25	8.3	0.00445	**
YearSeason	2	383	33.2	192	62.3	0.00000	***
YearSeason:Treatment	3	59	5.1	20	6.4	0.00038	***
Residuals	223	686	59.5	3			

Differences due to Location (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
Location	Margaree-Cheticamp	0.664	0.232	7.9	0.0045	**

Differences due to YearSeason (Tukey HSD)

Factor	Values	Difference	SE	Difference (%)	HSD p-value	Significance
YearSeason	2013Spr-2012Aut	-2.902	0.337	-34.5	0.0000	***
YearSeason	2013Aut-2012Aut	-0.415	0.343	-4.9	0.3138	
YearSeason	2013Aut-2013Spr	2.487	0.344	29.6	0.0000	***

Differences due Treatment at 2 weeks, 6 months, 12 months (Tukey HSD)

Treatment	Values	Difference	SE	Difference (%)	HSD.p.value	Significance
2 weeks	2012Aut:Caged-2012Aut:Free	-1.672	0.579	-19.9	0.0005	***
6 months	2013Spr:Caged-2013Spr:Free	-0.305	0.583	-3.6	0.9727	
12 months	2013Aut:Caged-2013Aut:Free	0.297	0.605	3.5	0.9793	

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.a : p.value = 0.0018
Mean, median and standard deviations for Hep.a by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Autumn 2012 Caged	20	8.33	8.60	1.336
Cheticamp Female Autumn 2012 Free	20	9.81	9.89	1.021
Cheticamp Female Autumn 2013 Caged	16	8.71	8.69	1.011
Cheticamp Female Autumn 2013 Free	20	10.41	10.19	1.063
Cheticamp Female Spring 2013 Caged	19	5.54	5.71	1.174
Cheticamp Female Spring 2013 Free	20	5.62	5.46	1.065
Margaree Female Autumn 2012 Caged	19	9.01	9.14	0.928
Margaree Female Autumn 2012 Free	20	10.87	10.67	1.599
Margaree Female Autumn 2013 Caged	17	9.82	9.33	2.657
Margaree Female Autumn 2013 Free	20	7.53	7.55	2.585
Margaree Female Spring 2013 Caged	19	7.38	7.31	1.584
Margaree Female Spring 2013 Free	20	7.91	7.62	1.289

IV-6-4 Crab size-hepatopancreas weight relationship

The same statistical approach explained in III-4-2 was applied for data preparation, analyses and interpretation. Regression parameters for large males, pygmy males and mature females are described in Tables 13, 14 and 15 respectively.

Large males

Analysis of variance-covariance by location, year and year-location interaction in the spring showed that crab carapace size vs hepatopancreas-weight relationships were significantly different in terms of location (p=0.0000). In the spring, multiple comparison showed significant difference (p=0.0000) between Louisbourg and

Cheticamp, Louisbourg and Margaree as well as Grande-Rivière and Louisbourg (Table 16). In the fall, there was a significant difference (p=0.0003) in terms of location-year interaction. The observed difference was significant (p=0.00035) between Grande-Rivière fall 2012 and Margaree Harbor fall 2012 (Table 17). There was no clear seasonal or annual tendancy in regression residual (Figure 10).
Multiple comparisons between caged and free crabs showed that there was significant difference in terms of year/season (p=0.0000) and year/season-treatment (p=0.0000). The season effect was significant between fall 2012 and fall 2012 as well as spring 2013 and fall 2013.

In terms of treatment effect (caged vs free), there was a significant difference (p=0.0000) for all three caging duration (2 weeks, 6 months and 12 months) (Table 18). The regression residual for caged large males continuously decreased in both station with the duration of immersion (Figure 11).

Pygmy males

Analysis of variance-covariance by location, year and year-location interaction in the spring showed that crab carapace size vs hepatopancreas-weight relationships were significantly different in terms of location and year (p=0.00054 and p=0.00000). Multiple comparison showed that the significant difference (p=0.0000) was observed between 2012 and 2013 (Table 19) in the spring. In the fall, there was no significant difference (at p=0.0001). The observed difference was significant (p=0.00035) between Grande-Rivière fall 2012 and Margaree Harbor fall 2012 (Table 20). There was no clear seasonal or annual tendancy in regression residual (Figure 12).
Comparison between caged and free crabs, multiple comparison showed that there was significant difference in terms of year/season (p=0.0000) and year/season-treatment (p=0.0000). The season effect was significant between fall 2012 and spring 2013 (p=0.0000) as well as spring 2013 and fall 2013 (p=0.0000).

In terms of treatment effect (caged vs free), there was a significant difference (p=0.0000) for 12 month caged vs free in the fall 2013 (Table 21 & Figure 13).

Mature females

Analysis of variance-covariance by location, year and year-location interaction in the spring showed that crab carapace size vs hepatopancreas-weight relationships were significantly different in terms of location and year/location interaction (p=0.00000 and p=0.00040)(Table 22). Multiple comparison showed that the significant difference (p=0.0000) was observed between spring 2012 and 2013 for free crabs (Table 22) in the spring. In the fall, there was no significant difference (at p=0.0001). The observed difference was significant (p=0.00035) between Grande-Rivière fall 2012 and Margaree

Harbor fall 2012 (Table 23). There was no clear seasonal or annual tendancy in regression residual (Figure 14).
Comparison between caged and free crabs, multiple comparison showed that there was significant difference in terms of year/season (p=0.0000) as well as year/season-treatment interaction (p=0.0000) (Table 24). The year/seson effect was significant between the fall 2012 and spring 2013 (p=0.0000) as well as the spring 2013 and fall 2013 (p=0.0000).

In terms of treatment effect (caged vs free), there was a significant difference (p=0.0000) for 2 week caged vs free in the fall 2012 and for 12 month caged vs free in the fall 2013 (Table 24 & Figure 15).

Table 13. Carapace width – hepatopancreas weight relationship regression parameters for large males

Location	Year	Season	Treatment	n	y-intercept	slope
Cheticamp	2012	Spring	Free	21	-27.57	0.54
Cheticamp	2012	Fall	Free	20	-57.47	0.86
Cheticamp	2012	Fall	Caged	20	-44.92	0.69
Cheticamp	2013	Spring	Free	20	-72.27	0.91
Cheticamp	2013	Spring	Caged	18	-35.90	0.53
Cheticamp	2013	Fall	Free	20	-74.92	0.96
Cheticamp	2013	Fall	Caged	15	6.43	0.14
Margaree	2012	Spring	Free	20	-50.09	0.84
Margaree	2012	Fall	Free	20	-27.89	0.63
Margaree	2012	Fall	Caged	18	-13.41	0.46
Margaree	2013	Spring	Free	20	-53.72	0.71
Margaree	2013	Spring	Caged	16	-55.49	0.70
Margaree	2013	Fall	Free	20	-27.88	0.55
Margaree	2013	Fall	Caged	9	-63.90	0.71
Louisbourg	2012	Spring	Free	20	-107.87	1.30
Louisbourg	2012	Fall	Free	20	-71.07	1.00
Louisbourg	2013	Spring	Free	20	-90.42	1.11
Grande.Riviere	2012	Spring	Free	21	-84.82	1.09
Grande.Riviere	2012	Fall	Free	20	3.22	0.30
Grande.Riviere	2013	Spring	Free	20	-82.22	1.05

Table 14. Carapace width – hepatopancreas weight relationship regression parameters for pygmy males.

Location	Year	Season	Treatment	n	y-intercept	slope
Cheticamp	2012	Spring	Free	21	-8.0075	0.195
Cheticamp	2012	Autumn	Free	20	-28.9078	0.530
Cheticamp	2012	Autumn	Caged	18	-35.1781	0.584
Cheticamp	2013	Spring	Free	20	-13.7537	0.270
Cheticamp	2013	Spring	Caged	19	-15.9243	0.288
Cheticamp	2013	Autumn	Free	20	-5.4830	0.175
Cheticamp	2013	Autumn	Caged	16	-4.1005	0.108
Margaree	2012	Spring	Free	19	-31.2376	0.549
Margaree	2012	Autumn	Free	20	-28.3391	0.512
Margaree	2012	Autumn	Caged	20	-35.1472	0.587
Margaree	2013	Spring	Free	20	-12.0241	0.255
Margaree	2013	Spring	Caged	18	-19.5364	0.341
Margaree	2013	Autumn	Free	20	-21.9183	0.425
Margaree	2013	Autumn	Caged	13	-21.3925	0.349
Louisbourg	2012	Spring	Free	20	-21.0943	0.421
Louisbourg	2012	Autumn	Free	19	-22.5654	0.450
Louisbourg	2013	Spring	Free	20	-14.4730	0.300
Grande.Riviere	2012	Spring	Free	3	-0.0560	0.122
Grande.Riviere	2012	Autumn	Free	16	-29.3204	0.514
Grande.Riviere	2013	Spring	Free	20	-18.1311	0.344

Table 15. Carapace width – hepatopancreas weight relationship regression parameters for mature females.

Location	Year	Season	Treatment	n	y-intercept	slope
Cheticamp	2012	Spring	Free	20	-2.72	0.136
Cheticamp	2012	Autumn	Free	20	-14.53	0.306
Cheticamp	2012	Autumn	Caged	20	-7.99	0.193
Cheticamp	2013	Spring	Free	20	-8.17	0.182
Cheticamp	2013	Spring	Caged	19	-0.71	0.062
Cheticamp	2013	Autumn	Free	20	-8.73	0.228
Cheticamp	2013	Autumn	Caged	16	-0.80	0.056
Margaree	2012	Spring	Free	20	-8.64	0.224
Margaree	2012	Autumn	Free	20	-15.50	0.323
Margaree	2012	Autumn	Caged	20	-9.43	0.219
Margaree	2013	Spring	Free	20	-6.82	0.160
Margaree	2013	Spring	Caged	19	-2.32	0.089
Margaree	2013	Autumn	Free	20	1.07	0.074
Margaree	2013	Autumn	Caged	17	-6.19	0.143
Louisbourg	2012	Spring	Free	24	-11.44	0.255
Louisbourg	2012	Autumn	Free	40	-20.38	0.405
Louisbourg	2013	Spring	Free	5	-8.26	0.192
Grande.Riviere	2012	Spring	Free	8	-8.44	0.206
Grande.Riviere	2012	Autumn	Free	21	-6.80	0.188
Grande.Riviere	2013	Spring	Free	20	-17.53	0.353

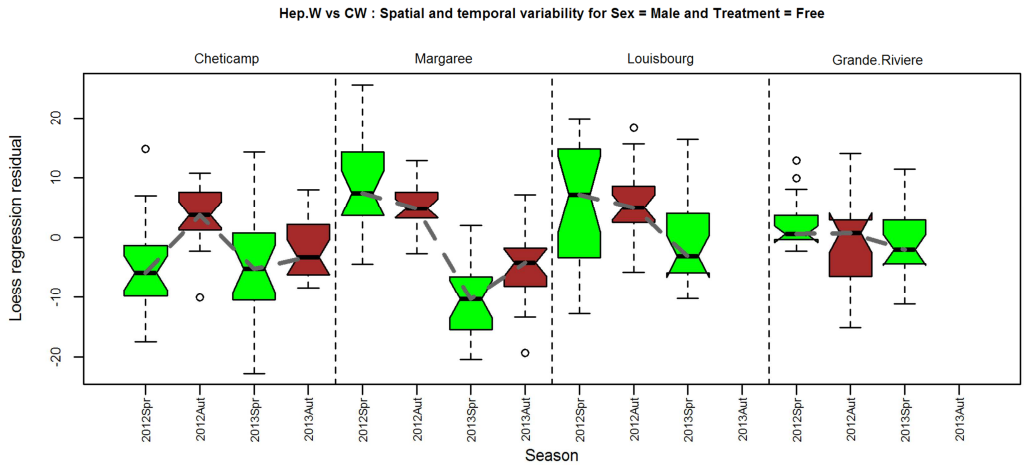


Figure 10. Spatial and temporal variability in Loess regression residuals for carapace size-hepatopancreas weight relationships for free large male snow crabs in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

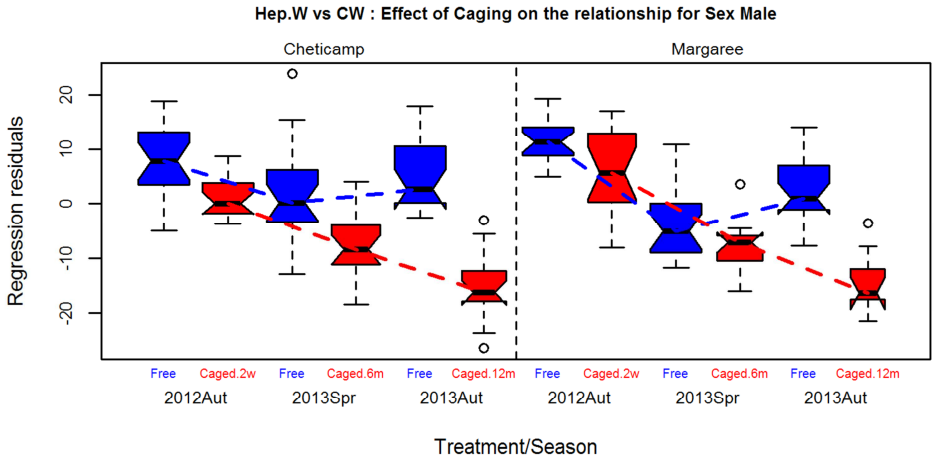


Figure 11. Effect of caging. Comparison of Loess regression residuals for carapace size-hepatopancreas weight relationships for caged and free large male crabs between Cheticamp and Margaree Harbor stations.

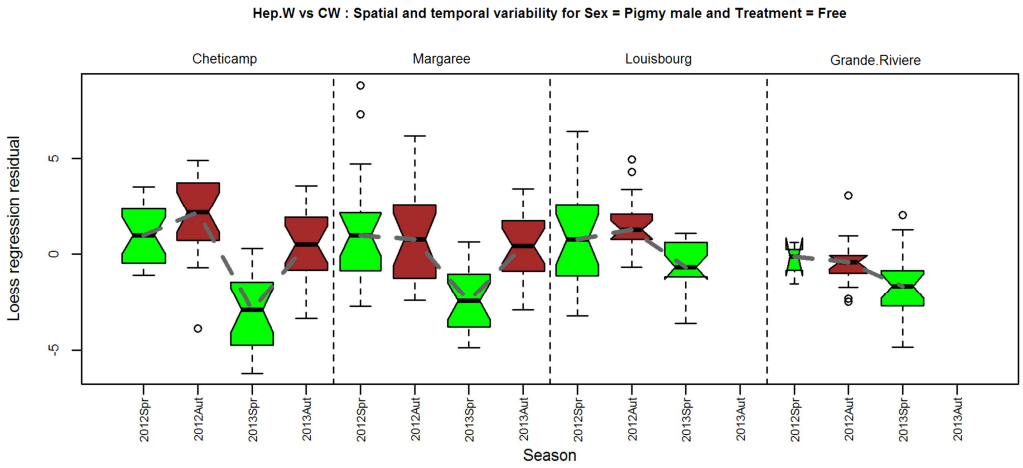


Figure 12. Spatial and temporal variability in Loess regression residuals for carapace size-hepatopancreas weight relationships for free pygmy male snow crabs in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

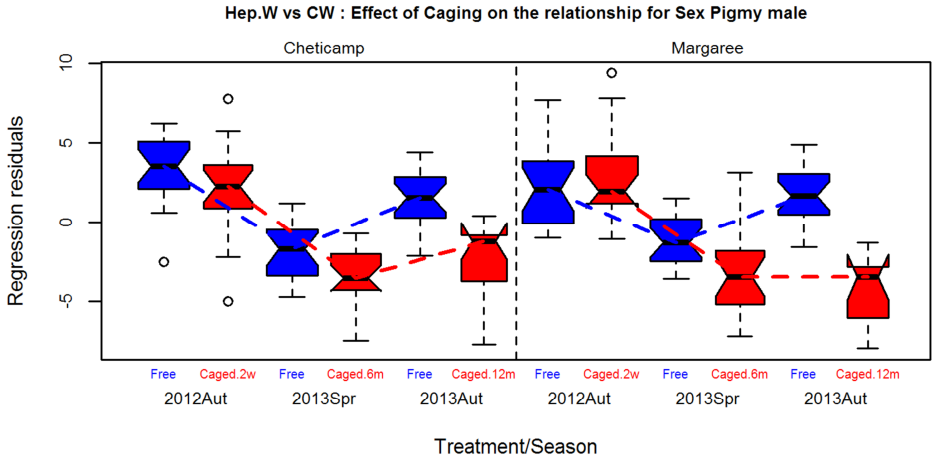


Figure 13. Effect of caging. Comparison of Loess regression residuals for carapace size-hepatopancreas weight relationships for caged and free pygmy male crabs between Cheticamp and Margaree Harbor stations.

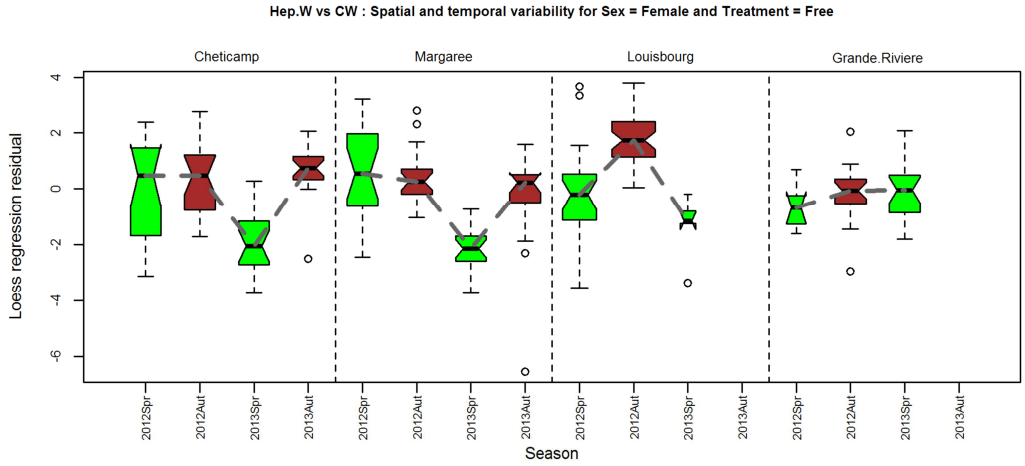


Figure 14. Spatial and temporal variability in Loess regression residuals for carapace size-hepatopancreas weight relationships for free female snow crabs in Cheticamp, Margaree Harbor, Louisbourg and Grande-Rivière stations.

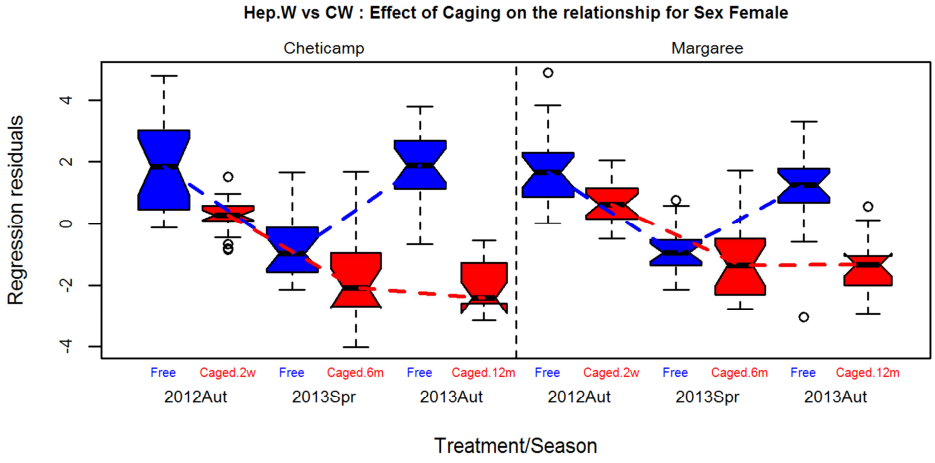


Figure 15. Effect of caging. Comparison of Loess regression residuals for carapace size-hepatopancreas weight relationships for caged and free female crabs between Cheticamp and Margaree Harbor stations.

Table 16. Spatial and temporal variability of the carapace width-hepatopancreas weight relationship for free large male crabs in spring (Cheticamp, Margaree, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-8.11	3.06
Cheticamp	2013	20	-8.07	3.06
Margaree	2012	21	-8.04	3.06
Margaree	2013	20	-8.03	3.06
Louisbourg	2012	20	-8.03	3.06
Louisbourg	2013	20	-8.10	3.06
Grande.Riviere	2012	20	-8.06	3.06
Grande.Riviere	2013	18	-8.05	3.06

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	8.89		8.89	4647	0.00000	***
Location	3	0.11	25.5	0.04	18	0.00000	***
Year	1	0.00	0.1	0.00	0	0.73119	
Location:Year	3	0.02	5.4	0.01	4	0.01004	*
Residuals	150	0.29	69.1	0.00			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	1.01	0.0053	0.0102	0.081	0.98191	
Location: Louisbourg - Cheticamp	0.94	-0.0592	0.0101	-0.909	0.00000	***
Location: Grande.Riviere - Cheticamp	0.99	-0.0055	0.0106	-0.085	0.98105	
Location: Louisbourg - Margaree	0.94	-0.0645	0.0104	-0.990	0.00000	***
Location: Grande.Riviere - Margaree	0.99	-0.0108	0.0109	-0.166	0.82994	
Location: Grande.Riviere - Louisbourg	1.06	0.0537	0.0102	0.824	0.00000	***
Year: 2013 - 2012	1.00	0.0024	0.0072	0.037	0.99656	

Homogeneity of variance test (Levene-Brown-Forsythe) for Weight : p.value = 0.0109

Mean, median and standard deviations for residuals of log Weight on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Male Spring 2012 Free	20	0.0021	0.0002	0.0388
Cheticamp Male Spring 2013 Free	20	0.0268	0.0337	0.0401
Grande.Riviere Male Spring 2012 Free	21	0.0257	0.0274	0.0415
Grande.Riviere Male Spring 2013 Free	20	-0.0050	-0.0110	0.0469
Louisbourg Male Spring 2012 Free	20	-0.0421	-0.0427	0.0578
Louisbourg Male Spring 2013 Free	20	-0.0460	-0.0445	0.0350
Margaree Male Spring 2012 Free	18	0.0067	0.0083	0.0277
Margaree Male Spring 2013 Free	20	0.0311	0.0386	0.0533

Table 17. Spatial and temporal variability of the carapace width-hepatopancreas weight relationship for free large male crabs in fall (Cheticamp, Margaree, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-7.44	2.93
Cheticamp	2013	20	-7.45	2.93
Margaree	2012	20	-7.37	2.93
Margaree	2013	20	-7.45	2.93
Louisbourg	2012	20	-7.40	2.93
Grande.Riviere	2012	20	-7.43	2.93

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	4.58		4.58	1665	0.00000	***
Location	3	0.04	10.3	0.01	5	0.00315	**
Year	1	0.00	0.7	0.00	1	0.32819	
Location:Year	1	0.04	9.8	0.04	14	0.00030	***
Residuals	113	0.31	79.3	0.00			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Gr12AutFr - Ma12AutFr	0.929	-0.0732	0.0167	-1.11	0.00035	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Weight: p.value = 0.7740
Mean, median and standard deviations for residuals of log Weight on log CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Male Autumn 2012 Free	20	-0.0110	-0.0081	0.0493
Cheticamp Male Autumn 2013 Free	20	0.0190	0.0298	0.0484
Grande.Riviere Male Autumn 2012 Free	20	-0.0241	-0.0232	0.0459
Louisbourg Male Autumn 2012 Free	20	-0.0237	-0.0198	0.0662
Margaree Male Autumn 2012 Free	20	0.0476	0.0483	0.0453
Margaree Male Autumn 2013 Free	20	-0.0078	-0.0031	0.0561

Table 18. Comparison of caged and free large male crabs (Cheticamp, Margaree) for the carapace width-hepatopancreas weight relationship.

Regression parameters by group

Location	YearSeason	y-intercept	slope	treatment.effect (Caged)	treatment.effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Aut	-51.3	0.807	-7.00	-16.6	0.00001	0.40915	42.3	35.9
Cheticamp	2013Aut	-40.5	0.676	-21.29	-50.0	0.00000	0.00343	42.6	24.2
Cheticamp	2013Spr	-51.7	0.742	-10.29	-27.4	0.00021	0.28637	37.6	30.3
Margaree	2012Aut	-15.2	0.526	-6.79	-14.1	0.00344	0.56783	48.1	44.4
Margaree	2013Aut	-32.4	0.590	-16.63	-41.9	0.00000	0.65171	39.6	22.6
Margaree	2013Spr	-52.8	0.706	-3.96	-12.3	0.02786	0.95188	32.2	29.3

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	2692		2692	75.1	0.00000	***
Location	1	53	0.3	53	1.5	0.22361	
YearSeason	2	5590	27.3	2795	77.9	0.00000	***
YearSeason:Treatment	3	7344	35.9	2448	68.3	0.00000	***
Residuals	208	7459	36.5	36			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	-0.234	-0.234	0.821	-0.578	0.77631	

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr - 2012Aut	-11.80	-11.80	1.34	-29.2	0.00000	***
2013Aut - 2012Aut	-7.09	-7.09	1.36	-17.5	0.00000	***
2013Aut - 2013Spr	4.71	4.71	1.34	11.7	0.00155	**

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012Aut 2w	-7.42	-7.42	1.37	-18.4	0.00000	***
Caged vs Free: 2013Spr 6m	-7.07	-7.07	1.41	-17.5	0.00000	***
Caged vs Free: 2013Aut 12m	-19.31	-19.31	1.56	-47.8	0.00000	***

Table 19. Spatial and temporal variability of the carapace width-hepatopancreas weight relationship for free pygmy male crabs in spring (Cheticamp, Margaree, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	21	-17.9	0.363
Cheticamp	2013	19	-15.9	0.363
Margaree	2012	20	-20.1	0.363
Margaree	2013	20	-19.5	0.363
Louisbourg	2012	20	-20.8	0.363
Louisbourg	2013	20	-18.8	0.363
Grande.Riviere	2012	20	-16.6	0.363
Grande.Riviere	2013	3	-18.1	0.363

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	2188		2188	596	0.00000	***
Location	3	69	7.9	23	6	0.00054	***
Year	1	278	32.1	278	76	0.00000	***
Location:Year	3	28	3.2	9	3	0.06100	.
Residuals	134	492	56.8	4			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	1.30	1.30	0.483	16.5	0.04726	*
Location: Louisbourg - Cheticamp	1.65	1.65	0.444	21.0	0.00197	**
Location: Grande.Riviere - Cheticamp	1.10	1.10	0.534	13.9	0.20511	
Location: Louisbourg - Margaree	0.35	0.35	0.452	4.5	0.92124	
Location: Grande.Riviere - Margaree	-0.20	-0.20	0.537	-2.6	0.99425	
Location: Grande.Riviere - Louisbourg	-0.55	-0.55	0.525	-7.0	0.79396	
Year: 2013 - 2012	-2.92	-2.92	0.341	-37.2	0.00000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.W : p.value = 0.0000

Mean, median and standard deviations for residuals of Hep.W on CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Pygmy male Spring 2012 Free	21	0.91	0.85	1.29
Cheticamp Pygmy male Spring 2013 Free	20	-2.31	-2.14	1.56
Grande.Riviere Pygmy male Spring 2012 Free	3	0.40	0.67	0.96
Grande.Riviere Pygmy male Spring 2013 Free	20	-0.98	-1.01	1.62
Louisbourg Pygmy male Spring 2012 Free	20	1.80	1.28	2.44
Louisbourg Pygmy male Spring 2013 Free	20	-0.13	-0.15	1.19
Margaree Pygmy male Spring 2012 Free	19	2.38	1.76	3.22
Margaree Pygmy male Spring 2013 Free	20	-1.65	-1.71	1.41

Table 20. Spatial and temporal variability of the carapace width-hepatopancreas weight relationship for free pygmy male crabs in fall (Cheticamp, Margaree, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-22.7	0.434
Cheticamp	2013	20	-22.1	0.434
Margaree	2012	19	-21.4	0.434
Margaree	2013	20	-22.6	0.434
Louisbourg	2012	16	-23.4	0.434
Grande.Riviere	2012	20	-21.0	0.434

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	1686		1686	460	0.00000	***
Location	3	43	9.4	14	4	0.01074	*
Year	1	15	3.3	15	4	0.04416	*
Location:Year	1	6	1.3	6	2	0.21104	
Residuals	108	396	86.1	4			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	-0.563	-0.563	0.448	-5.14	0.65049	
Location: Louisbourg - Cheticamp	-0.003	-0.003	0.581	-0.03	1.00000	
Location: Grande.Riviere - Cheticamp	-2.008	-2.008	0.617	-18.32	0.00945	**
Location: Louisbourg - Margaree	0.560	0.560	0.610	5.11	0.85137	
Location: Grande.Riviere - Margaree	-1.445	-1.445	0.653	-13.18	0.14225	
Location: Grande.Riviere - Louisbourg	-2.005	-2.005	0.652	-18.29	0.01595	*
Year: 2013 - 2012	-0.975	-0.975	0.480	-8.90	0.20661	

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.W : p.value = 0.0001
Mean, median and standard deviations for residuals of Hep.W on CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Pygmy male Autumn 2012 Free	20	0.947	1.11	2.08
Cheticamp Pygmy male Autumn 2013 Free	20	-0.222	0.02	2.09
Grande.Riviere Pygmy male Autumn 2012 Free	16	-1.154	-1.22	1.25
Louisbourg Pygmy male Autumn 2012 Free	19	0.823	0.45	1.47
Margaree Pygmy male Autumn 2012 Free	20	-0.058	-0.12	2.38
Margaree Pygmy male Autumn 2013 Free	20	-0.527	-0.41	1.87

Table 21. Comparison of caged and free caged pygmy male crabs (Cheticamp, Margaree) for the carapace width-hepatopancreas weight relationship.

Regression parameters by group

Location	YearSeason	y-intercept	slope	treatment.effect (Caged)	treatment.effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Aut	-32.34	0.571	-1.764	-12.00	0.03960	0.76268	14.7	14.8
Cheticamp	2013Aut	-3.33	0.142	-3.139	-51.00	0.00000	0.18409	6.2	3.2
Cheticamp	2013Spr	-14.44	0.279	-0.806	-12.27	0.07944	0.71718	6.6	7.1
Margaree	2012Aut	-29.97	0.532	-0.328	-2.60	0.72817	0.53568	12.6	16.9
Margaree	2013Aut	-18.63	0.383	-5.700	-49.16	0.00000	0.44697	11.6	8.5
Margaree	2013Spr	-16.58	0.316	-0.880	-12.37	0.29448	0.38578	7.1	9.2

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	3739		3739	733	0.00000	***
Location	1	0	0.0	0	0	0.78123	
YearSeason	2	1076	42.6	538	105	0.00000	***
YearSeason:Treatment	3	349	13.8	116	23	0.00000	***
Residuals	216	1103	43.6	5			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	0.200	0.200	0.317	2.04	0.52998	

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr - 2012Aut	-4.63	-4.63	0.518	-47.3	0.00000	***
2013Aut - 2012Aut	-1.74	-1.74	0.529	-17.7	0.00338	**
2013Aut - 2013Spr	2.89	2.89	0.507	29.5	0.00000	***

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012Aut 2w	0.0720	0.0720	0.523	0.736	0.99868	
Caged vs Free: 2013Spr 6m	-1.2421	-1.2421	0.532	-12.690	0.05994	.
Caged vs Free: 2013Aut 12m	-4.4305	-4.4305	0.555	-45.264	0.00000	***

Table 22. Spatial and temporal variability of the carapace width-hepatopancreas weight relationship for free mature female crabs in spring (Cheticamp, Margaree, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model applied)

Location	Year	n	y-intercept	slope
Cheticamp	2012	24	-7.86	0.203
Cheticamp	2013	20	-7.85	0.203
Margaree	2012	20	-9.65	0.203
Margaree	2013	5	-9.01	0.203
Louisbourg	2012	20	-7.14	0.203
Louisbourg	2013	20	-7.60	0.203
Grande.Riviere	2012	20	-9.83	0.203
Grande.Riviere	2013	8	-8.24	0.203

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	236		236	125	0.00000	***
Location	3	12	3.2	4	2	0.10462	
Year	1	83	22.2	83	44	0.00000	***
Location:Year	3	37	9.9	12	6	0.00040	***
Residuals	128	241	64.8	2			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Ma13SprFr - Ma12SprFr	-2.69	-2.69	0.435	-46.9	0.00000	***

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.W : p.value = 0.6426
Mean, median and standard deviations for residuals of Hep.W on CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Spring 2012 Free	20	0.71	1.12	1.73
Cheticamp Female Spring 2013 Free	20	-1.32	-1.51	1.08
Grande.Riviere Female Spring 2012 Free	8	0.07	-0.04	0.73
Grande.Riviere Female Spring 2013 Free	20	0.51	0.49	1.01
Louisbourg Female Spring 2012 Free	24	0.47	0.31	1.73
Louisbourg Female Spring 2013 Free	5	-0.68	-0.44	1.17
Margaree Female Spring 2012 Free	20	1.18	1.22	1.67
Margaree Female Spring 2013 Free	20	-1.51	-1.39	0.79

Table 23. Spatial and temporal variability of the carapace width-hepatopancreas weight relationship for free mature female crabs in fall (Cheticamp, Margaree, Louisbourg, Grande-Rivière).

Regression parameters by group (single slope model not applicable)

Location	Year	n	y-intercept	slope
Cheticamp	2012	20	-8.48	0.224
Cheticamp	2013	21	-9.27	0.224
Margaree	2012	20	-8.71	0.224
Margaree	2013	20	-9.44	0.224
Louisbourg	2012	20	-8.67	0.224
Grande.Riviere	2012	40	-7.32	0.224

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	359		359	267	0.00000	***
Location	3	78	29.5	26	19	0.00000	***
Year	1	2	0.6	2	1	0.29254	
Location:Year	1	5	1.9	5	4	0.05780	.
Residuals	134	180	68.1	1			

Observed difference(s) (Multiple comparison)

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Location: Margaree - Cheticamp	-0.45	-0.45	0.262	-6.3	0.35504	
Location: Louisbourg - Cheticamp	1.15	1.15	0.295	15.9	0.00110	**
Location: Grande.Riviere - Cheticamp	-0.81	-0.81	0.342	-11.2	0.09843	.
Location: Louisbourg - Margaree	1.60	1.60	0.294	22.1	0.00000	***
Location: Grande.Riviere - Margaree	-0.36	-0.36	0.343	-5.0	0.78139	
Location: Grande.Riviere - Louisbourg	-1.96	-1.96	0.320	-27.1	0.00000	***
Year: 2013 - 2012	-0.28	-0.28	0.263	-3.8	0.78537	

Homogeneity of variance test (Levene-Brown-Forsythe) for Hep.W : p.value = 0.1241
Mean, median and standard deviations for residuals of Hep.W on CW by group

Group	Number	Mean	Median	Standard deviation
Cheticamp Female Autumn 2012 Free	20	-0.27	-0.20	1.20
Cheticamp Female Autumn 2013 Free	20	0.05	0.09	0.93
Grande.Riviere Female Autumn 2012 Free	21	-0.79	-0.63	1.08
Louisbourg Female Autumn 2012 Free	40	1.10	1.04	0.98
Margaree Female Autumn 2012 Free	20	-0.19	-0.36	0.92
Margaree Female Autumn 2013 Free	20	-0.97	-0.41	1.79

Table 24. Comparison of caged and free caged mature female crabs (Cheticamp, Margaree) for the carapace width-hepatopancreas weight relationship.

Regression parameters by group

Location	YearSeason	y-intercept	slope	treatment.effect (Caged)	treatment.effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Aut	-11.661	0.266	-1.368	-18.98	0.00007	0.03494	7.21	5.37
Cheticamp	2013Aut	-5.785	0.184	-3.858	-59.56	0.00000	0.00023	6.48	3.04
Cheticamp	2013Spr	-3.442	0.113	-0.916	-20.97	0.04969	0.02710	4.37	3.71
Margaree	2012Aut	-11.115	0.260	-1.093	-15.94	0.00006	0.02030	6.86	5.78
Margaree	2013Aut	-0.800	0.101	-2.420	-38.51	0.00000	0.19846	6.28	4.01
Margaree	2013Spr	-4.421	0.125	-0.408	-9.48	0.21795	0.26843	4.30	3.90

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	239		239	182	0.00000	***
Location	1	0	0.0	0	0	0.76088	
YearSeason	2	202	28.1	101	77	0.00000	***
YearSeason:Treatment	3	223	31.1	74	57	0.00000	***
Residuals	223	293	40.8	1			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	0.0553	0.0553	0.151	0.934	0.71468	

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr - 2012Aut	-2.58	-2.58	0.257	-43.6	0.00000	***
2013Aut - 2012Aut	-0.38	-0.38	0.257	-6.3	0.31255	
2013Aut - 2013Spr	2.20	2.20	0.257	37.2	0.00000	***

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012Aut 2w	-1.320	-1.320	0.257	-22.3	0.00000	***
Caged vs Free: 2013Spr 6m	-0.717	-0.717	0.260	-12.1	0.01884	*
Caged vs Free: 2013Aut 12m	-3.151	-3.151	0.271	-53.3	0.00000	***

IV-6-5 Review of histological observation of hepatopancreas collected in 2012-2013

Summary

Histological examination of hepatopancreas tissue identified seasonal and caging-related differences in R-cell vacuolation similar to those seen for directly measured lipid content and RI cell abundance i.e., both having generally lower values in the spring and after 12 months of caging. Some sex and region-related changes were also identified with LM crabs tending to differ from PM and MF crabs and Grande Rivière continuing to differ from the other regions. Histology is a useful tool for rapid estimation of lipid reserves but direct measurement is recommended when accurate values are required.

In contrast to the 2003-2004 study (Supplement, Section IV-7) inflammation was minimal. This could indicate that other factors such as animal handling and/or transport conditions were different in 2003-4 vs the 2012-2013 study. Caging *per se* did not have an effect on inflammation-associated indices.

Also of interest was the observation of two viruses based on transmission electron microscopic examination of suspicious inclusions noted on the haematoxylin and eosin-stained slides. Intranuclear inclusions were noted in B-cell nuclei of six crabs, primarily from the Louisbourg region. Intracytoplasmic inclusions were observed in endothelial cells of 18 crabs primarily from Cheticamp and shown to be viral particles during examination of gill tissue (Section IV-4-5). As no other viruses have been reported for snow crab from this region, further study of these two infections would be warranted.

Objective

To review histologic sections of hepatopancreas tissue collected from free and caged snow crab (2012-2013) to look for effects of short- and long-term caging. Criteria established for hepatopancreas tissues collected during the 2003-2004 study were used as a basis for the evaluation.

Methodology

Haematoxylin and eosin (H&E) stained slides of hepatopancreas tissue were provided for direct light microscopic evaluation. Tissues were collected within 15 minutes of death and placed in Davidson's fixative for processing as described in Section III-1. Slides represented crabs originating from four stations (Cheticamp, NS, Margaree, NS, Loiusbourg, NS, Grande-Rivière, QC) with samples collected at four times (Spring 2012, Fall, 2012, Spring 2013, Fall 2013) over a two year period. Subgroups of crabs in Cheticamp and Margaree had been caged for a period of 2 weeks (Fall 2012), seven months (Spring, 2013), or 12 months (Fall 2013). Crabs collected

by trap (i.e., no caging period) were used as the control (free) groups. All slides had been randomised and renumbered allowing for non-biased evaluation by the observer (A. Battison). Scoring criteria for hepatopancreas tissue were modified slightly from those devised for the 2003-2004 study (Supplement, Section IV-7) and literature review (Icely & Nott 1992; Johnson 1980a, Johnson 1980b, Johnson 1980c, Al-Mohana *et al.*1985, Al-Mohana & Nott 1987). Observations were divided into two main functional components – epithelial (digestive) and connective (circulation, immunity/phagocytosis) tissues.

1) Degree of autolysis (<10%, 10 - 25%, 25 – 50%, 50 – 75%, > 75% of tissue affected)
2) Presence/absence of apoptotic cells within tubule wall.
3) Extent to which R-cell lipid vacuoles filled the cytoplasm (none seen, < 50%, 50 –75%, 75% – 100%, > 100%). R-cells containing only vacuoles with golden-brown, granular contents were graded as 'none'. Cells where lipid vacuoles were so abundant that the cell shape was distorted were classed as > 100%.
4) Presence/absence of organisms in tubule lumens (none seen, bacteria, fungi/yeast, protozoan, other)
5) Hemocyte infiltrates in tubular epithelium (none, mild, moderate, marked).
6) Organising hemocytic nodules in epithelium (none, mild, moderate, marked).
7) Pigment (presumptive melanin) deposits in association with inflammation (none, mild, moderate, marked)
8) Tubular fibrosis/collagen deposition (none, mild, moderate, marked)
9) Tubular necrosis (<10%, 10 - 25%, 25 – 50%, 50 – 75%, > 75% of tublues affected)
10) Estimation of circulating hemocyte numbers based on subjective assessment of hemocyte numbers in vessels (normal, mild, moderate, marked)
11) Number of hemocytes in the connective tissue (normal, mild, moderate, marked)
12) Presence of hemocyte foci in connective tissue (non-organised)
13) Presence oforganised hemocyte nodules in connective tissue (none, mild, moderate, marked, severe)
14) Presence of pigment (melanin) deposits in connective tissue (none, mild, moderate, marked, severe)
15) Presence of fibroplasia or collagen deposits in connective tissue (none, mild, moderate, severe)
16) Presence/absence of organisms in connective tissue (none seen, bacteria, fungus/yeast, protozoan, other)
17) Reserve Inclusion (RI) cell fullness (none, mild, moderate, marked) based on subjective average of number of RI cells and degree of fullness
18) Degree of activation of the fixed phagocyte cells located around small vessels (none, mild, moderate, marked). Degree of cytoplasmic vacuolation, presence of phagocytosed material was used to determine an overall tissue score.

19) Degree of fixed phagocyte hyperplasia based on a subjective assessment of phagocyte density/numbers.

Data was analysed using STATA ® Statistics/Data Analysis 12.1 (StataCorp, TX, USA) and Excel 2010© (Microsoft Corporation, Microsoft Canada Inc. ON, Canada). Significance level was set at $p < 0.05$. Slides were examined using a Leitz Dialux 20 microscope. Images were captured with a PixeLINK® camera and associate μScope software (PixeLINK®).

Results

The plane of section of the tissue samples was inconsistent as was the amount of material available for examination. The stain intensity of some slides was quite pale and hemocyte granules were not always distinct.

Slides were not available for 50 crabs, presumably due to mortalities. An additional 13 slides from spring 2012 free $MF_{Grande\ Rivière}$, and one free $LM_{Cheticamp}$ were cut but not stained. Slides from two crabs (fall 2013, caged $LM_{Margaree}$ and fall 2013 caged $LM_{Cheticamp}$) did not contain hepatopancreas tissue for evaluation. Table 25 summarises the distribution of the 1177 samples available for evaluation.

Free Crabs

The spring 2012 samples were collected within a nine day window for Grande-Rivière (May 24th), Cheticamp (May 30th), and Margaree (June 1st) while, those from Louisbourg were collected 3 and 14 d later (June 4th and 14th). The fall 2012 sample collection times were separated by approximately nine weeks with Louisbourg (Sept. 18th) and Grande-Rivière (Sept. 22nd) collected before Margaree (Nov. 2nd) and Cheticamp (Nov. 4th). The spring 2013 collections by approximately four weeks with Grande-Rivière collected first (May 30th), followed by Cheticamp (June 12th), Margaree (June 18th) and finally Louisbourg (June 24th).

Seasonal and sex-related patterns were noted for R-cell vacuolation (interpreted as lipid content). In general, lipid content tended to be higher in the fall than in the spring samples (Figures 16-18). Kruskal-Wallis testing of median score within a station by sex, across collection times, detected significant differences for all combinations except $LM_{Grande\text{-}Rivière}$. No further statistical analysis at this time. In addition, Cheticamp, Margaree, and Louisbourg stations tended to be more like each other than Grande-Rivière. The $MF_{Grande\text{-}Rivière}$ crabs had much higher lipid content in both spring samples than the other three stations. After excluding the Grande-Rivière crabs, LM crabs tended to have higher scores for vacuolation (lipid content) than either PM or MF crabs at their respective stations.

The abundance of reserve inclusion (RI) cells showed a strong seasonal pattern where RI cells were generally absent in the spring and noted again in the fall samples at all stations and for all sexes except $MF_{Grande\text{-}Rivière,}$ $LM_{Grande\text{-}Rivière}$, and $LM_{Louisbourg}$ (Figures 19-21). Kruskal-Wallis testing of median score within a station by sex, across collection times, detected significant differences for all combinations except those listed. No further statistical analysis at this time.

There were very few (<10) non-zero (abnormal) scores for epithelial-related criteria of the 801 free crabs examined. Only nine crabs (three PM, four LM, 2 MF) all at different stations or collected at different time had bacteria (rods or mixed bacteria) recorded growing in the tubule lumen. Of these, only three (2 MF, 1 LM) had associated inflammatory response consisting of hemocyte infiltrates, pigmentation, and necrosis. Secretory packets were only observed in 19 crabs (5 PM, 9 LM, 5 MF) at varied stations and collection times

Non-zero scores for criteria for connective tissue pigmentation (n = 4) and fibrosis (n = 11) were uncommon. Hemocyte nodule formation was noted in 36 crabs (11 PM, 13 LM, 12 MF) equally distributed over all stations at all times. Bacterial organisms were noted in nodules in two crabs only (spring 2012 $MF_{Cheticamp}$, spring 2013 $PM_{Louisbourg}$). There was some variation in scores for connective tissue hemocyte infiltrates, although there was no consistent pattern with respect to location or sex evident. Kruskal-Wallis testing showed differences for $PM_{Cheticamp}$ (spring 2013 scores appear higher), $PM_{Margaree}$ (spring 2012 scores appear lower), $PM_{Louisbourg}$ (fall 2012 scores appear lower), $LM_{Louisbourg}$ (fall 2012 scores appear lower), $MF_{Cheticamp}$ and $MF_{Margaree}$ (scores appear higher spring 2012 and 2012), $MF_{Louisboug}$ (spring 2013 scores lower but n = 5). No further analysis pursued.

Table 25. Summary table showing number of hepatopancreas samples available for histological examination from each station and the date of sample collection.

Station	Gear Type	Sampling Time											
		Spring 2012			Fall 2012			Spring 2013			Fall 2013		
		PM	LM	MF	PM	LM	MF	PM	LM	MF	PM	LM	MF
Cheticamp, NS													
	Trap/free	21	20	20	20	20	20	20	20	20	20	20	20
	Caged	n/c[1]	n/c	n/c	19	20	20	19	18	19	16	14	16
Margaree, NS													
	Trap/free	19	20	20	20	20	20	20	20	20	20	20	20
	Caged	n/c	n/c	n/c	20	18	20	18	16	19	13	9	17
Louisbourg, NS													
	Trap/free	20	20	24	20	20	20	20	20	5	n/c	n/c	n/c
Grande-Rivière, QC													
	Trap/free	3	21	8	20	20	21	20	20	20	n/c	n/c	n/c

[1]not collected

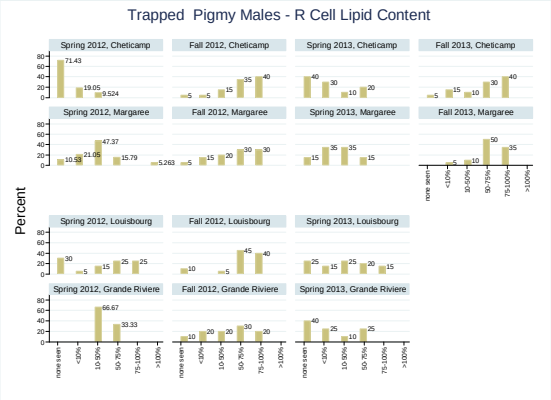


Figure 16. Percent distribution histogram of R-cell lipid content in trapped pygmy male snow crab *(C. opilio*) collected at four stations from Spring 2012 to Fall 2013.

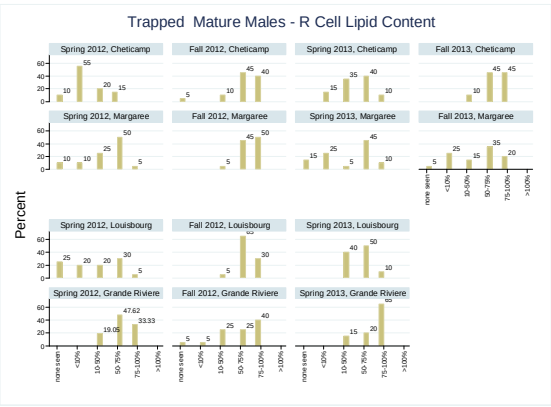


Figure 17. Percent distribution histogram of R-cell lipid content in trapped mature male snow crab *(C. opilio*) collected at four stations from Spring 2012 to Fall 2013.

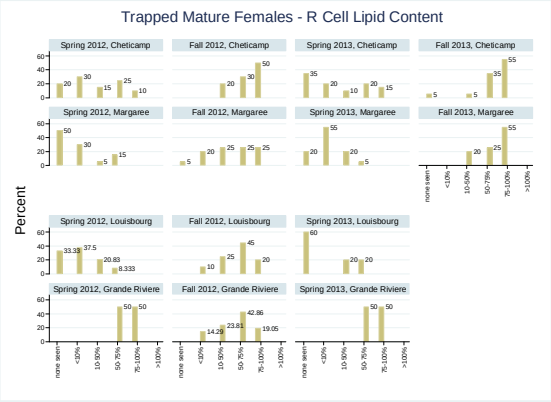


Figure 18. Percent distribution histogram of R-cell lipid content in trapped mature female snow crab *(C. opilio*) collected at four stations from Spring 2012 to Fall 2013

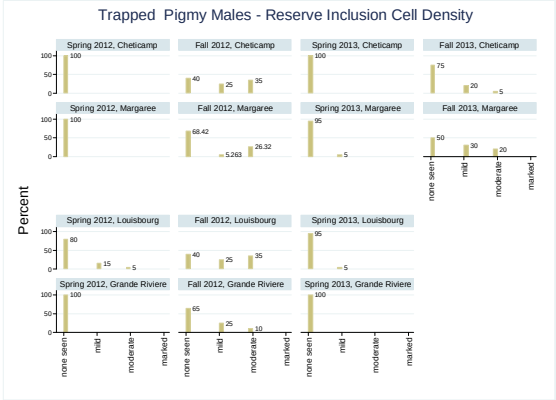


Figure 19. Percent distribution histogram of occurrence of reserve inclusion (RI) cell density in trapped pygmy male snow crab *(C. opilio*) collected at four stations from Spring 2012 to Fall 2013.

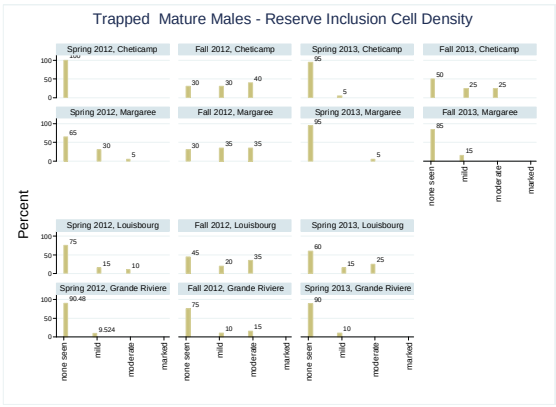


Figure 20. Percent distribution histogram of reserve inclusion (RI) cell density in trapped mature male snow crab *(C. opilio*) collected at four stations from Spring 2012 to Fall 2013.

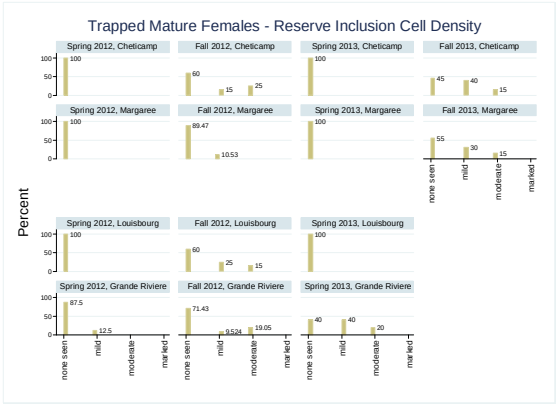


Figure 21. Percent distribution histogram of occurrence of reserve inclusion (RI) cell density in trapped mature female snow crab *(C. opilio*) collected at four stations from Spring 2012 to Fall 2013.

Caged vs Free Crabs – Cheticamp & Margaree Stations

The distribution of R cell lipid scores for fall 2012 appeared similar for PM and LM caged and free crabs at both stations with the majority of crabs having higher scores (≥50%) (Figures 16-18). A similar pattern was observed for MF crabs except for free $MF_{Margaree}$ crabs which had more crabs with lower values. By spring 2013, there was a shift towards lower scores compared to fall 2012 for all crabs, most noticeable for MF, then PM, and finally LM. The shift appeared greatest for caged MF crabs. By fall 2013, free crabs had returned to score distributions similar to the fall 2012 values while, scores in caged crabs generally continued to decrease with many having lipid scores of 'none seen'. This was most pronounced in MF, then PM, and finally LM crabs at both stations.
The pattern for RI cell distribution was most affected by collection time/season (Figures 19-21). The RI cells, when observed, were seen primarily in the fall 2012 samples, caged and free crabs, at both stations for PM and LM crabs and $MF_{Cheticamp}$. The RI cells were less common in free $MF_{Margaree}$ crabs, similar to the observations of lower R-cell lipid scores. The RI cells essentially disappeared from all crabs, both stations in the spring 2013 samples, returning only in free crabs in the fall 2013 samples.

There was essentially no effect of caging on the number of crabs with non-zero scores for epithelial-related criteria. Organisms were observed in the tubule lumen of four caged crabs (spring 2013 $PM_{Margaree}$, fall 2013 $MF_{Margaree}$, fall 2013 $MM_{Cheticamp,}$ spring 2013 $MM_{Margaree}$). High scores for tubule hemocyte infiltrates, pigmentation (melanisation), necrosis, and fibrosis ($MM_{Cheticamp}$ only) were also observed for the two LM crabs. (Data not presented.) Secretory packets were only observed in six caged crabs (0 PM, 2 LM, 4 MF) mostly (5/6) at Margaree spread over all three sample collections.

No effects of caging on connective tissue criteria scores were noted. Bacteria were noted only for two caged crabs (fall 2012 $PM_{Cheticamp}$ and spring 2013 $PM_{Margaree}$) which also had higher scores for connective tissue pigmentation and nodules. There were no significant differences (Wilcoxon signed rank test) between scores for caged and free crabs for connective tissue infiltrates at any location, time or crab category other than spring 2013 $MM_{Cheticamp}$ crabs. Hyperplasia of fixed phagocytes was scored positive most commonly in spring 2013 crabs (caged $PM_{Margaree}$, $LM_{Margaree}$, and $MF_{Margaree}$, caged $PM_{Cheticamp}$ and $MF_{Cheticamp}$, and free $PM_{Cheticamp}$ and $LM_{Margaree}$). There were no visible trends in FP activation.

Inclusion Bodies

Large basophilic to amphophilic homogenous, crystalline inclusion bodies were observed nearly completely filling the nuclei of B-cells in six crabs – four from Louisbourg (spring 2012 MF; fall 2012 LM and MF; and spring 2013 PM) one from Margaree (spring 2013 PM, caged) and one from Grande Rivière (spring 2013 MF) (Table 26, Figures 22-25). Fixed, unprocessed, tissue collected for scanning electron microscopy studies (Section IV-6-2) were processed for transmission electron microscopy (Diagnostic Services, Atlantic Veterinary College). Initial images show what is interpreted as closely packed linear aggregates of viral particles in the nucleus (Figure 24). No further analysis completed at this time.

Small eosinophilic to amphophilic cytoplasmic inclusions were also noted in the endothelial cells and possibly fixed phagocytes of 18 crabs (Table 26). The hepatopancreas was examined in all crabs whereas gill tissue was not. These inclusions were confirmed as viral particles and discussed more fully in Section IV-1-6 as they were most prominent in the gill epithelial and, to a lesser extent, endothelial cells. The hepatopancreas was examined in all crabs, whereas gill tissue was not, so may provide a better idea of the distribution of this virus. However, respiratory epithelial tissue appears to be the preferred tissue for infection.

Table 26. Summary table showing number of hepatopancreas samples in which intranuclear and intracytoplasmic inclusion bodies were observed (in B-cells or endothelium and/or fixed phagocytes, respectively) from each station.

Station	Gear Type	Sampling Time											
		Spring 2012			Fall 2012			Spring 2013			Fall 2013		
		PM	LM	MF	PM	LM	MF	PM	LM	MF	PM	LM	MF
INTRANUCLEAR INCLUSIONS													
Cheticamp, NS													
	Trap/free	0	0	0	0	0	0	0	0	0	0	0	0
	Caged	n/c[1]	n/c	n/c	0	0	0	0	0	0	0	0	0
Margaree, NS													
	Trap/free	0	0	0	0	0	0	0	0	0	0	0	0
	Caged	n/c	n/c	n/c	0	0	0	1	0	0	0	0	0
Louisbourg, NS													
	Trap/free	0	0	1	0	1	1	1	0	0	n/c	n/c	n/c
Grand Rivière, QC													
	Trap/free	0	0	0	0	0	0	0	0	1	n/c	n/c	n/c
INTRACYTOPLASMIC INCLUSIONS													
Cheticamp, NS													
	Trap/free	0	1	1	0	0	0	0	0	0	1	1	0
	Caged	n/c	n/c	n/c	0	0	0	2	0	0	3	0	0
Margaree, NS													
	Trap/free	0	0	0	0	0	0	0	0	0	0	0	1
	Caged	n/c	n/c	n/c	0	0	0	1	0	2	1	1	0
Louisbourg, NS													
	Trap/free	0	1	0	0	0	0	0	0	0	n/c	n/c	n/c
Grand Rivière, QC													
	Trap/free	0	0	0	0	0	1	0	1	0	n/c	n/c	n/c

[1]not collected

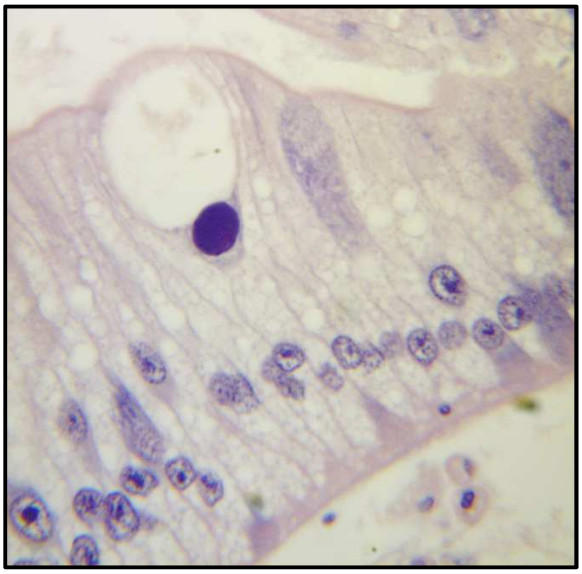


Figure 22. Haematoxylin & Eosin. Snow crab Hepatopancreas.. Large basophilic intranuclear inclusion body in a B (blister) –cell. Vacuolated (lipid) R (reserve)-cells comprise the majority of the tubule wall.

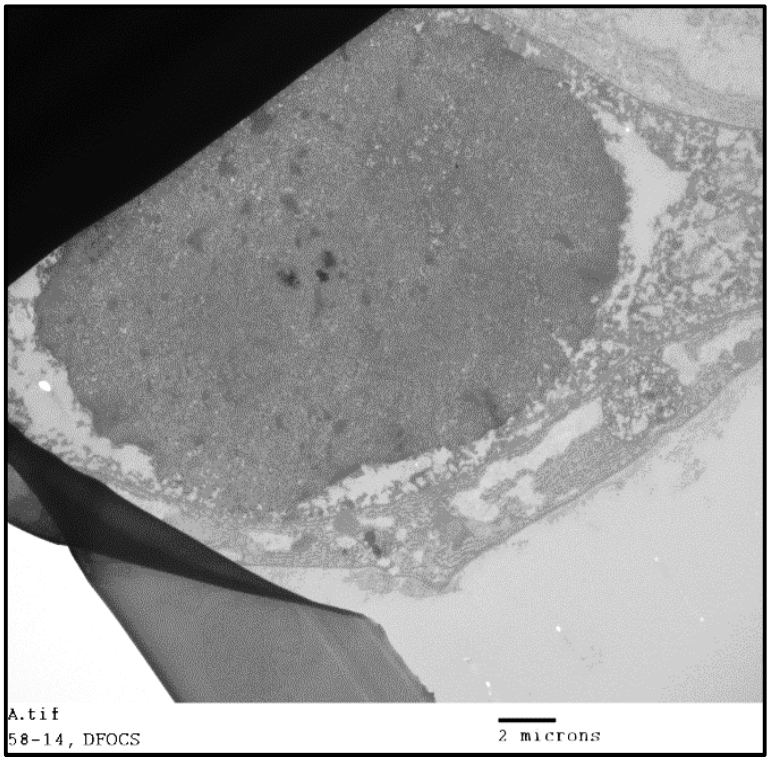


Figure 23. Transmission electron microscopy. Snow crab. Hepatopancreas. Large intranuclear inclusion body in a B (blister) –cell essentially displacing/replacing normal chromatin.

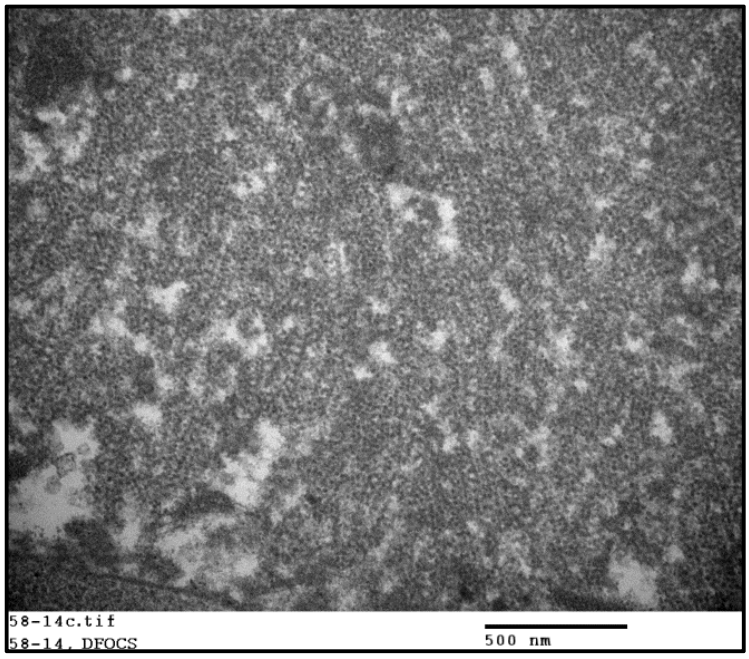


Figure 24. Transmission electron microscopy. Snow crab. Hepatopancreas B-cell nucleus. Viral particles in parallel linear arrangements. A small amount of nuclear chromatin is visible as si the nuclear membrane (at lower left).

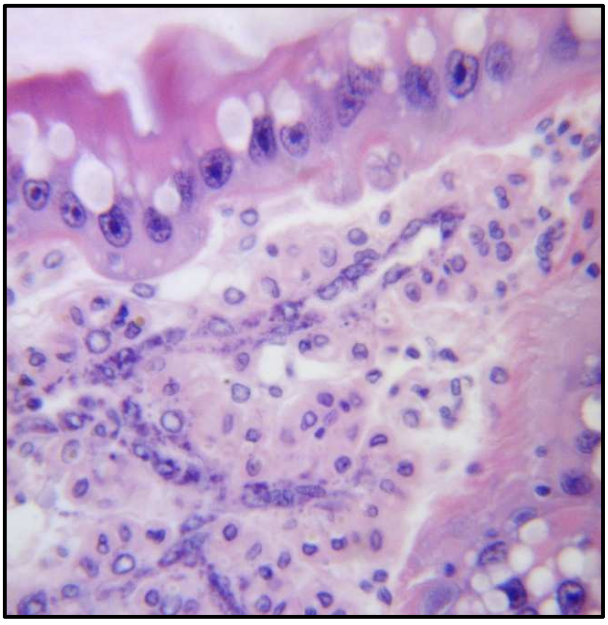


Figure 25. Haematoxylin & Eosin. Snow crab Hepatopancreas. Small eosinophilic to amphophilic intracytoplasmic inclusions located within the endothelial cells outlining small vessels. Fixed phagocytes surround the vessels. The tubule wall is composed of 'empty' R-cells lacking typical lipid vacuoles (see Figure 22).

Conclusions

Histopathological examination of hepatopancreas sections identified two criteria that clearly varied with season, sex, region and caged/free status: R-cell vacuolation (lipid content) and reserve inclusion (RI) cell abundance. Inflammation was minimal in all crabs and showed no particular pattern with respect to season, sex, or caged/free status. Two viruses were observed but not identified.

The degree of vacuolation of R-cells is considered reflective of lipid content (Icely & Knott 1992). The changes in R-cell vacuolation mirrored that of lipid content which was measured directly (see VIII -1, 2, 3). Seasonal trends in PM, LM, and MF crabs were prominent with the fall being a period of higher energy reserves, with the exception of Grande Rivière. Some differences may be due to timing of sample collection as there was up to six weeks difference between collections for some samples.

Results from Grande Rivière continue to differ as seen in other analyses. The most noticeable difference was for MF crabs where R-cell lipid scores were high in the spring. This was not surprising for the spring 2013 crabs as ovary histology suggested that they had not spawned (see IV-4-5 & IV-4-6) However, spawning had occurred in spring 2012 (n = 8) and hepatopancreas lipid scores were expected to be lower as for the other three regions due to mobilisation of lipid to oocytes prior to spawning. Grande-Rivière may provide better feeding opportunities over the winter for MF crabs compared to Cheticamp, Margaree, and Louisbourg.

The effect of caging was noticeable and significant differences detectable, by histologic scoring after 12 months in all crabs at both stations. This is similar to lipid analyses where significantly lower values were only detected after 12 months for all crabs. The only difference was that lower lipid values were detected for caged MF in the six month samples when measured directly. Vacuolation varied along the length of the tubules requiring subjective averaging of overall vacuolation in the section. It was also necessary to find a consistent plane of section through the tubules for scoring. It is unknown if vacuolation is consistent across different areas of the hepatopancreas (area of hepatopancreas collected not standardised). For these reasons, while histologic assessment should be able to provide a decent andmore rapid estimate of lipid reserves, biochemical analysis would be the preferred method when accurate values are required.

The origin (synthesised or simply stored) of the RI cell cytoplasmic material (considered proteinaceous) is undetermined although a hemocyanin reserve has been suggested by some (Johnson 1980b). The RI cells are found within spongy connective tissues and are generally more prominent during periods of good energy reserves and so fluctuate seasonally and/or with the moult in other crustaceans, e.g. American lobster (Johnson 1980b). This seasonality was clearly evident in the histologic sections of these terminally moulted PM and MF snow crabs

with RI cells essentially absent in the spring samples (exception for Grande-Rivière) – a period associated with low nutritional reserves after a winter of decreased feeding/food availability. The pattern was less clear for LM crabs and varied by area. Again, this could reflect different feeding success and/or energy needs of LM crabs compared to PM or MF crabs.

In contrast to the 2003-2004 study (Supplement, Section IV-7) inflammation was minimal in this study. This could indicate that other factors such as animal handling and/or transport conditions were different in 2003-4 versus the 2012-2013 study. Caging *per se* did not have an effect on inflammation-associated indices.

Caging appeared to be associated with hyperplasia of the fixed phagocytes. This could simply reflect the decreased tissue volume of hepatopancreas cells as lipid reserves diminished causing an apparent relative increase in fixed phagocytes density as they were more readily observed. Secretory packets were observed in an attempt to assess recent feeding but were not useful. The combined soak time and transportation from boat to dissection station (> 24 hours) may be a confounding factor as the digestive cycle is often completed in 24 hours in some species (Icely & Nott 1992)

A very interesting finding was the observation of two viruses in the tissue as there are no viruses reported for *Chionoecetes opilio* from this region. A baculo-like virus, *Co*BV, was reported in *C. opilio* from the Sea of Japan (Kon *et al.* 2011). Viral particles were found in the nucleus and cytoplasm of interstitial cells of various tissues – not hepatopancreatic B-cells. Hepatopancreatic epithelial cell infections are thought to cause minimal damage to the crab host as the cells are constantly replaced in the hepatopancreas as part of the normal digestive process (Johnson 1978, Icely & Knott 1992). This virus had a regional distribution focussed in Louisbourg area with no apparent association with sex or season in the small numbers of crabs infected. In contrast, the intracytoplasmic virus was observed most often in the Cheticamp and Margaree samples, again with no apparent sex or seasonal association. Information on this virus is presented in more detail in the section on gill histology where it was more prominent (see IV-1-6).

Histological assessment of hepatopancreas tissue should provide an expedient way to estimate tissue lipid stores; however, direct measurement is preferred when accurate values are required. Histopathology could also serve as a screening tool in any future investigations to identify viral inclusions associated with the two newly observed viruses in this study.

IV-6-6 References

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IV-7 SUPPLEMENT

Review of Histologic Samples of Ovary and Hepatopancreas: 2003-2004 Study

Summary

A seismic exposure trial and follow-up caging study utilising snow crab, *Chionoecetes opilio*, as the test subject was conducted in 2003 and 2004 with samples collected at 12 days and five months after exposure. Results of that study suggested that histological changes of ovarian and hepatopancreatic tissues were associated more with prolonged caging and/or handling than seismic exposure *per se*. Recommendations included a specific study to collect more extensive baseline data on histomorphologic features of snow crab. Such a study was conducted in 2012-2013. The histological sections of the ovarian and hepatopancreas tissues collected during the 2003-4 study were reassessed to establish scoring criteria for the caging study conducted in 2012-13.
The current review also found differences between the two sampling periods – 12 days post-exposure (December 2003) and five months post-exposure (May 2004). The more dramatic changes could be due to prolonged caging and/or represent normal seasonal variation experienced by this species, and/or related to regional environmental differences or crab handling. These include the noticeable decrease in lipid content of the hepatopancreas by May 2004 in both groups and an increased degree of chorion membrane separation in oocytes. Inflammatory changes were also more prominent in the May 2004 samples which may be indirectly related to decreased lipid reserves. Two observations may be linked to seismic testing exposure (or location) alone but would require further investigation. These included suggestion of decreased feeding by crabs at the test site at 12 d post-exposure and an apparent increased percentage of degenerative oocytes in ovaries of crabs held at the test site at five months post-exposure.

Overall, the findings in the current review have many similarities to the general findings of Lee and Wright (2009). Their report focussed on an overall score rather than comparing each characteristic separately so, it is difficult to make direct comparisons. In addition, different criteria were used. Lee and Wright (2009) did not report a particular characteristic to be associated with exposure to seismic testing. The effects of handling stress and caging were identified in both reports as having probable impact on tissue pathology. The April 2009 ESRF report also indicated that while the intended control site was located 23 km away from the test site, seismic noise was detected which may have contributed to the difficulties in finding clear histomorphologic differences between test and control crabs in either review.

The follow-up study conducted in 2012-2013 addresses concerns of regional, seasonal, and caged vs wild-sourced crabs so should prove to be very useful to discern between normal physiologic processes and those associated with short- and long-term caging in multiple sample

regions. The observations and classification criteria in the current report will be used for evaluation of the 2012 and 2013 sample.

Objective

To review and develop familiarity with the histological features of ovary and hepatopancreas tissue samples collected from caged snow crab, *Chionoecetes opilio*, in December 2003 and May 2004, as part of the Environmental Studies Research Funds study entitled "Potential Impacts of Seismic Energy on Snow Crab". Also reviewed, were tissues from crabs that had not been exposed to seismic testing but held at the same caging sites for 12 days in December 2004 in an effort to assess the effects of short-term caging alone on crab tissues. Results are compared to the ESRF summary report (particularly Chapters 3 and 4), (Courtenay *et al*. 2004). This information will be used to develop scoring criteria for evaluation of tissues (hepatopancreas, gill, and ovary) during the 2012- 2013 short and long-term caging study.

Methodology

A total of 760 Masson Trichrome - stained and unstained slides of ovarian (n = 353) and hepatopancreatic tissue (n = 407) were provided for direct light microscopic evaluation. Upon review with Fisheries and Oceans Canada staff, it was determined that crabs had been chilled in ice-packed coolers as a form of anaesthesia prior to manual separation of dorsal carapace from the body for tissue collection. Samples of hepatopancreas and ovary were placed immediately into Bouin's fixative, refrigerated, for 24 hours then rinsed twice in 70% ethanol prior to paraffin embedding in preparation for Masson-Trichrome staining.
Two unstained slides of each hepatopancreas and ovary were submitted to Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island for Masson Trichrome staining to check for changes in stain quality after a long (10 years) period of storage.

Data was analysed using STATA ® Statistics/Data Analysis 12.1 (StataCorp, TX, USA) and Excel 2010© (Microsoft Corporation, Microsoft Canada Inc. ON, Canada). Slides were examined using an Olympus BX46 microscope (Olympus Corporation, PA, USA) and a Leitz Dialux 20 microscope. Images were captured with a DP21 stand-alone camera (Olympus Corporation, PA, USA).

Ovarian Tissue

Descriptions of ovary development and pathological changes in snow and other crab species were used to help identify relevant characteristics to include in a histological scoring system (Johnson 1980a, Tan-Fermin & Pudadera 1989, Krol *et al.* 1992, Stewart *et al.* 2007).

Subsequently, 34 slides representing each of the six experimental groups: Dec 2003 Seismic Short term (SS); Dec 2003 Non-seismic short term (NS); May 2004 Seismic Long term (SL); May 2004 Non-seismic Long term (NL); December 2004 short term caging only at Margaree

(prior test/seismic site); and December 2004 short term caging only at Cheticamp (prior control/non-seismic site) were randomly chosen and scored using the initial criteria. The scoring system was assessed and then modified slightly to provide a final scoring system for use with the entire slide set. The slides were examined without knowledge of group of origin, using the number key provided to Dr. Lee. While multiple serial sections were present on most slides, only one section was evaluated per slide for scoring purposes. Multiple sections were examined if clarification of an observation was required. After all slides had been scored, data was entered into an Excel spreadsheet where group codes were revealed.

Ovary stage was split into very general categories: immature (oogonia, no oocyte development); early vitellogenesis (oocytes with predominantly basophilic granular cytoplasm with a few lipid vacuoles and yolk droplets, blue or red, making up less than 50% of the cytoplasm and present in less than 50% of oocytes); late vitellogenic (yolk droplets present in > 50% of oocytes and occupying >50% cytoplasmic area.

The degree of cytoplasmic lipid droplet accumulation was recorded as mild, moderate, or marked. Similarly, the degree of separation between follicular cells and oocyte chorionic membrane was recorded as mild, moderate, marked based on an overall assessment of the section. In some samples nuclei appeared 'glassy' (pale, blue-grey, homogenous nucleoplasm) and this was recorded as present/absent. Areas of mitotic/meiotic activity were recorded when observed.

Spawning 'scars' were represented by follicular epithelial cells found in circular to oval or coiled and collapsed shapes indicating a prior spawning episode. An important criterion was the absence of remnant oocyte cytoplasmic material within the centre of the follicular cells to differentiate from an end-stage degenerating/resorbing oocyte. Follicular epithelial cells which displayed variable degrees of cytoplasmic enlargement and vacuolation and euchromatic nuclei were considered 'active' vs non-vacuolated, heterochromatic quiescent, 'non-active', counterparts. The number of scars present were counted and grouped into four categories – none; 1 – 5; 6 – 20; 20+; and, 'too numerous to count' (TNTC). 'Spent' ovaries were those that had high numbers of spawning scars visible, few/no late vitellogenic oocytes and variable numbers of early vitellogenic oocytes.

Degenerate, or 'atretic,' oocytes were oocytes at any stage of maturation showing changes including: coalescence of cytoplasm into irregular plates of material; marked, irregular cytoplasmic vacuolation; enlargement of follicular epithelial cells; and/or phagocytosis of ooplasm by follicular or other cells.

The presence of nodules as described by Kon *et al.* (2010), commonly observed in aged female *C. opilio* in Sea of Japan was recorded. Whether or not hemocytic infiltrates, pigment (melanin) deposition, or fibrosis was associated with the nodules was recorded separately. Loss of yolk droplet definition with resultant homogenous ooplasm ('laking') was recorded as present or absent.

The abundance of hemocytes within vessels and sinuses, but not infiltrating the tissue, was recorded as mild, moderate, or marked. The presence of inflammatory infiltrates, pigment deposition, fibrosis, or necrosis distinct from the nodules was recorded separately as mild, moderate, or marked. A category for the presence of infectious agents (bacterial, protozoal, viral, other) was also included.

Hepatopancreas Tissue

Slides containing hepatopancreas tissue were examined in a similar manner as described for the ovaries. Scoring criteria for hepatopancreas tissue were devised by separating the tissue into two main functional components – epithelial (digestive) and connective (circulation, immunity/phagocytosis) tissues. Reference materials were reviewed to assist with developing a list of histologic criteria (Johnson 1980b, Johnson 1980c, Al-Mohana *et al*., 1985, Al-Mohana & Nott 1987; Icely & Nott 1992)

In addition to a general score for degree of autolysis(none, <25% tissue affected, 25 – 50% tissue affected, 50 – 75% tissue affected, > 75% tissue affected), identified as areas of tissue having pale stained cytoplasm, often less distinct intercellular borders, and/or pale, swollen nuclei, criteria for the epithelial component included:

1) Lumen shape (stellate, round, or dilated with thin walls, or a combination/mixed), as a suggested indication of recent feeding. Cross sections of smaller tubules (not ducts) were assessed and the overall impression recorded.
2) Presence/absence of sloughed blister cells (B-cells), often with a small amount of pale yellow/gold granular material in the large cytoplasmic vacuole which identifies this cell, in the tubule lumen as recognised indicators of recent feeding cycle
3) Degree of B-cell cytoplasmic vacuole filling (< 50% or > 50%), subjectively averaged over all cells, as a suggested indicator of recent feeding
4) Presence/absence of apoptotic cells (condensed cytoplasm, condensed and hyperchromatic chromatin, occasionally fragmented nuclei) within tubule wall. Extent to which R-cell lipid vacuoles filled the cytoplasm (none seen, < 50%, 50 – 75%, 75% – 100%, > 100%). R-cells containing only vacuoles with golden-brown, granular contents were graded as 'none'. Cells where lipid vacuoles were so abundant that the cell shape was distorted were classed as > 100%.
5) Degree of tubular cell basement membrane undulation/folding (mild, moderate, marked)
6) Presence/absence of organisms in tubule lumens (none seen, bacteria, fungi/yeast, protozoan, other)
7) Hemocyte infiltrates in tubular epithelium (none, mild, moderate, marked).
8) Organising hemocytic nodules in epithelium (none, mild, moderate, marked).
9) Pigment (presumptive melanin) deposits in association with inflammation (none, mild, moderate, marked)
10) Tubular fibrosis/collagen deposition (none, mild, moderate, marked)

11) Tubular necrosis (none, mild, moderate, marked)

Features assessed in the connective tissue component included:

12) Estimation of circulating hemocyte numbers based on subjective assessment of hemocyte numbers in vessels (normal, mild, moderate, marked)
13) Number of hemocytes in the connective tissue (normal, mild, moderate, marked)
14) Presence of hemocyte nodules (none, mild, moderate, marked, severe)
15) Presence of pigment (melanin) deposits (none, mild, moderate, marked, severe)
16) Presence of fibroplasia or collagen deposits (none, mild, moderate, severe)
17) Presence/absence of organisms (none seen, bacteria, fungus/yeast, protozoan, other)
18) Reserve Inclusion (RI) cell fullness (none, mild, moderate, marked) based on subjective average of number of RI cells and degree of fullness
19) Degree of activation (subjective average of phagocyte density/number, degree of cytoplasmic vacuolation, presence of phagocytosed material was used to determine an overall tissue score) of the fixed phagocyte cells located around small vessels (none, mild, moderate, marked).

Results

Ovarian Tissue

The plane of section of the tissue samples was inconsistent. Stain penetration also appeared inconsistent within a slide. Colouration of yolk droplets (blue or red) varied within an oocyte and staining often showed a distinct line across oocytes. Stain intensity and affinity also varied between ovaries which looked to be at similar stages morphologically.

The distribution of scores for each characteristic by sample collection time and treatment are summarised in Tables 1 - 3. As the number of crabs was different for each sample period and treatment, the relative percent distribution was also calculated to provide a degree of standardisation. Percent distribution histograms are shown for those characteristics that showed interesting changes over time and/or treatment based on visual inspection of the data.

No immature ovaries were observed in any of the three sample groups. In one of the December 2003 non-exposed crabs (#147, coded) there was too much pathology present to readily identify ovary stage and the sample was simply classed as ‘other’. The early vitellogenic ovary stage was far less common than the late vitellogenic stage which was the predominant stage for all combinations of treatment and sample time (Figures 1 & 2). The majority (88% – 93%) of crabs at all sample times had cytoplasmic lipid droplet density scores of mild, with the remainder being ‘moderate’. Spent ovaries were uncommon (8% - 10%) for all sample periods.

The distribution of spawning scars in December 2003 was similar between the two groups with the exception that more (12%) of Control crabs had very high numbers of scars (too numerous to count category - TNTC) than seismic-exposed crabs (2%), (Figures 3 & 4). This general pattern was repeated in May 2004. In the December 2004 caging study, spawning scars were less common in crabs held at the Cheticamp control site.

The scores for degenerative oocytes in December 2003 were similar for the 'none' and '1-5' categories for control (47%, 35%) and seismic sites (53%, 42%); however, the higher scores were found more often in the control group (Figures 5 & 6). Both groups shifted towards the '1-5' and '6-20' categories by May 2004, with only 6% of seismic-exposed crabs having scores of 'none', compared to 17% of Control crabs. The distributions for the December 2004 crabs were very similar. The relative distribution of vacuolated follicular epithelial cells was nearly identical (~50%) between control and seismic-exposed crabs in December 2003 and May 2004. Vacuolated epithelial cells were less frequently identified in crabs caged at the Cheticamp control site (83% scored 'none seen') compared to crabs held at Margaree, the previous seismic testing site (64%).

More crabs, seismic and non-seismic exposed, showed an increased degree of membrane separation in May 2004 than in December of 2003 (more scores of 'moderate' and first appearance of the 'marked' category) (Figures 7 & 8. The degree of membrane separation in the December 2004 caging study was slightly higher for the crabs held at the previous control site (Cheticamp) than at the prior seismic testing site (Margaree).

The presence of homogenous, 'laked' oocyte cytoplasm was uncommon at all sample periods. Slightly more non seismic-exposed crabs demonstrated this change than exposed crabs in December 2003 and May 2004 – 20% vs 7%, and 15% vs 11%, respectively. Slightly fewer (5% vs 12%) crabs caged at Cheticamp (previous non-seismic site) showed this change in the December 2004 samples. There was no consistent pattern of observations of homogenous, 'glassy', nuclear morphology, among the samples other than it being more often not observed than observed.

Ovary nodule formation (large, coalescing, degenerative oocytes) was generally absent (96%) in control site and seismic-exposed crabs in December 2003. Nodules were more common in control site crabs (11% affected) than in seismic site crabs (5% affected) in the May 2004 samples. Nodules were only noted in 5% of crabs caged at the prior seismic testing site in the December 2004 samples. The degree of hemocyte infiltration, pigment deposition, and fibrosis associated with the nodules was quite varied (Tables 1 – 3).

When examining the connective tissues supporting the oocytes/ovarian stroma, scores for hemocyte infiltrates tended to be slightly higher for the non-seismic exposed crabs than exposed crabs in the December 2003 samples, especially in the 'moderate' category (10% vs 2%). By the May 2004 sampling, the distribution of scores was more similar, although the non-seismic crabs

still showing slightly higher scores. In contrast, in the December 2004 caging study, the pattern was reversed with more (14%) of the crabs held at Margaree (previous seismic-exposed site) having hemocyte infiltrates while only 3% of crabs caged at the previous control site near Cheticamp had detectable infiltrates. Indicators of chronic inflammation (pigment deposition, fibrosis), necrosis, and infectious agents were absent from all samples.

The density of circulating hemocyte showed some relative differences between seismic-exposed and control crabs in December 2003, with slightly higher values noted for the control group. The relative distributions were more similar in the May 2004 samples. In December 2004, the trend was reversed, with crabs caged at the prior seismic site showing slightly higher densities of circulating hemocytes.

Table 1. Absolute and relative distribution of characteristics used to score ovary tissue collected from snow crab in December 2003 after a short-term (12 day) holding period following a seismic exposure study conducted in December 2003. Absolute and percentage values (rounded to nearest whole value in parentheses) reported.

	Non-Seismic *(n = 49)* Score					Seismic (*n = 55*) Score				
	0	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Oocyte										
Ovary stage[1]	-[2]	0	5(10)	43 (88)	1 (2)	-	0	0	55 (100)	0
Cytoplasmic vacuoles[3] **(lipid)**	0	43 (88)	6 (12)	0	-	0	55 (100)	0	0	-
Spent ovaries[3]	43 (88)	6 (12)	-	-	-	55 (100)	0	-	-	-
Spawning scars[4]	20 (41)	17 (35)	5 (10)	1 (2)	6 (12)	24 (44)	24 (44)	5 (9)	1 (2)	1 (2)
Degenerative oocytes[5]	23 (47)	17 (35)	8(16)	1 (2)	0	29 (53)	23 (42)	3 (5)	0	0
Vacuolated follicular cells[3]	24 (49)	25 (51)	-	-	-	28 (51)	27 (49)	-	-	-
Membrane separation[4]	-	30 (61)	19 (39)	-	-	-	35 (64)	20 (36)	0	-
Glassy nuclei[3]	38 (78)	11 (22)	-	-	-	45 (82)	10 (18)	-	-	-
'Laked' cytoplasm[3]	39 (80)	10 (20)	0	0	-	51 (93)	4 (7)	0	0	-
Mitotic centers[3]	47 (96)	2 (4)	-	-	-	53 (96)	2 (4)	-	-	-
Nodules										
Occurrence[3]	47 (96)	2 (4)	0	0	-	53 (96)	1(2)	0	1 (2)	-
Hemocytes-infiltrating[4]	-	0	0	0	-	-	1 (50)	0	0	-
Pigment deposition[4]	-	2 (100)	0	0	-	-	1 (50)	1 (50)	0	-
Fibrosis[4]	-	0	0	0	-	-	1 (50)	0	0	-
Stroma										
Hemocytes – circulating[4]	0	34 (70)	13 (27)	2 (4)	-	0	45 (82)	10 (18)	0	-
Hemocyte infiltrates[4]	41 (84)	3 (6)	5 (10)	0	-	49 (90)	5 (9)	1 (2)	0	-
Pigment deposition[4]	49 (100)	0	0	0	-	55 (100)	0	0	0	-
Fibrosis[4]	49 (100)	0	0	0	-	55 (100)	0	0	0	-
Necrosis[4]	49 (100)	0	0	0	-	55 (100)	0	0	0	-
Infective Agents[6]	49 (100)	0	0	0	-	55 (100)	0	0	0	-

[1] 1 = immature; 2 = early vitellogenic; 3 = late vitellogenic; 4 = other

[2] not applicable to this characteristic

[3] 0 = no and 1 = yes

[4] 0 = none, 1 = mild, 2 = moderate, 3 = marked, 4 = severe

[5] 0 = none, 1 = 1 to 5, 2 = 6 to 20, 3 = 21+, and 4 = too numerous to count (TNTC)

[6] 0 = none seen; 1 = bacteria; 2 = fungi/yeast; 3 = protozoa; 4 = other

Table 2. Absolute and relative distribution of characteristics used to score ovary tissue collected from snow crab in May 2004 after a long-term (five month) holding period following a seismic exposure study conducted in December 2003. Absolute and percentage values (rounded to nearest whole value in parentheses) reported. (no slide available for one 'seismic-exposed' crab).

	Non-Seismic (*n* = *88*) Score					Seismic (*n* = 89) Score				
	0	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Oocyte										
Ovary stage[1]	-[2]	0	7 (8)	81 (92)	0	-	0	4 (4)	85 (96)	0
Cytoplasmic vacuoles[3] **(lipid)**	0	82 (93)	6 (7)	0	-	0	85 (96)	4 (4)	0	-
Spent ovaries[3]	78 (89)	10 (11)	-	-	-	83 (93)	6 (7)	-	-	-
Spawning scars[4]	26 (30)	39 (44)	12 (14)	3 (3)	8 (9)	37 (42)	40 (45)	7 (8)	2 (2)	3 (3)
Degenerative oocytes[5]	15 (17)	36 (41)	25 (28)	4 (5)	8 (9)	5 (6)	41 (46)	36 (40)	5 (6)	2 (2)
Vacuolated follicular cells[3]	42 (48)	46 (52)	-	-	-	47 (53)	42 (47)	-	-	-
Membrane separation[4]	0	29 (33)	44 (50)	15 (17)	-	0	18 (20)	52 (58)	19 (21)	-
Glassy nuclei[3]	74 (84)	14 (16)	-	-	-	61 (69)	28 (31)	-	-	-
'Laked' cytoplasm[3]	75 (85)	13 (15)	0	0	-	79 (89)	10 (11)	0	0	-
Mitotic centers[3]	82 (93)	6 (7)	-	-	-	84 (94)	5 (6)	-	-	-
Nodules										
Occurrence[3]	78 (87)	8 (9)	2 (2)	0	-	84 (94)	4 (4)	1(1)	0	-
Hemocytes-infiltrating[4]	-	5 (45)	0	0	-	-	4 (80)	0	0	-
Pigment deposition[4]	-	8 (73)	0	0	-	-	5 (100)	0	0	-
Fibrosis[4]	-	0	0	0	-	-	1 (20)	0	0	-
Stroma										
Hemocytes – circulating[4]	0	67 (76)	15 (17)	6 (7)	-	0	73 (82)	12 (13)	4 (4)	-
Hemocyte infiltrates[4]	63 (72)	16 (18)	7 (8)	2 (2)	-	71 (80)	13 (15)	5 (6)	0	-
Pigment deposition[4]	88 (100)	0	0	0	-	89 (100)	0	0	0	-
Fibrosis[4]	88 (100)	0	0	0	-	89 (100)	0	0	0	-
Necrosis[4]	88 (100)	0	0	0	-	89 (100)	0	0	0	-
Infective Agents[6]	88 (100)	0	0	0	-	89 (100)	0	0	0	-

[1] 1 = immature; 2 = early vitellogenic; 3 = late vitellogenic; 4 = other
[2] not applicable to this characteristic
[3] 0 = no and 1 = yes
[4] 0 = none, 1 = mild, 2 = moderate, 3 = marked, 4 = severe
[5] 0 = none, 1 = 1 to 5, 2 = 6 to 20, 3 = 21+, and 4 = too numerous to count (TNTC)
[6] 0 = none seen; 1 = bacteria; 2 = fungi/yeast; 3 = protozoa; 4 = other

Table 3. Absolute and relative distribution of characteristics used to score ovary tissue collected from snow crab in December 2004 after a short-term (12 day) caging period in the sites where a seismic exposure study was conducted in December 2003. Absolute and percentage values (rounded to nearest whole value in parentheses) reported.

	Caged at Non-Seismic Site (*n* = *30*) Score					Caged at Prior Seismic Site (*n* = 42) Score				
	0	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Oocyte										
Ovary stage[1]	-[2]	0	0	30 (100)	-	-	0	2 (5)	40 (95)	0
Cytoplasmic vacuoles[3] **(lipid)**	0	27 (90)	3 (10)	-		0	41 (98)	1 (2)	0	-
Spent ovaries[3]	30 (100)	0	-	-	-	41 (98)	1 (2)	--	--	--
Spawning scars[4]	24 (80)	5 (17)	1 (3)	0	0	21 (50)	17 (40)	3 (7)	1 (2)	0
Degenerative oocytes[5]	15 (50)	13 (43)	2 (7)	0	0	21 (50)	16 (38)	3 (7)	0	2 (5)
Vacuolated follicular cells[3]	25 (83)	5 (17)	-	-	-	27 (64)	15 (36)	-	-	-
Membrane separation[4]	-	15 (50)	12 (40)	3 (10)	-	1 (2)	30 (71)	11 (26)	0	-
Glassy nuclei[3]	20 (67)	10 (33)	-	-	-	26 (62)	16 (38)	-	-	-
'Laked' cytoplasm[3]	28 (93)	2 (7)	0	0	-	37 (88)	4 (10)	1 (2)	0	-
Mitotic centers[3]	30 (100)	0	-	-	-	41 (98)	1 (2)	-	-	-
Nodules										
Occurrence[3]	30 (100)	0	-	-	-	40 (95)	2 (5)	0	0	-
Hemocytes-infiltrating[4]	-	0	0	0	-	-	2 (100)	0	0	-
Pigment deposition[4]	-	0	0	0	-	-	2 (100)	0	0	-
Fibrosis[4]	-	0	0	0	-	-	0	0	0	-
Stroma										
Hemocytes – circulating[4]	0	28 (93)	2 (7)	0	-	0	33 (79)	8 (19)	1 (2)	-
Hemocyte infiltrates[4]	29 (97)	1 (3)	0	0	-	36 (86)	5 (12)	0	1 (2)	-
Pigment deposition[4]	30 (100)	0	0	0	-	42 (100)	0	0	0	-
Fibrosis[4]	30 (100)	0	0	0	-	42 (100)	0	0	0	-
Necrosis[4]	30 (100)	0	0	0	-	42 (100)	0	0	0	-
Infective Agents[6]	30 (100)	0	0	0	-	42 (100)	0	0	0	-

[1] 1 = immature; 2 = early vitellogenic; 3 = late vitellogenic; 4 = other
[2] not applicable to this characteristic
[3] 0 = no and 1 = yes
[4] 0 = none, 1 = mild, 2 = moderate, 3 = marked, 4 = severe
[5] 0 = none, 1 = 1 to 5, 2 = 6 to 20, 3 = 21+, and 4 = too numerous to count (TNTC)
[6] 0 = none seen; 1 = bacteria; 2 = fungi/yeast; 3 = protozoa; 4 = other

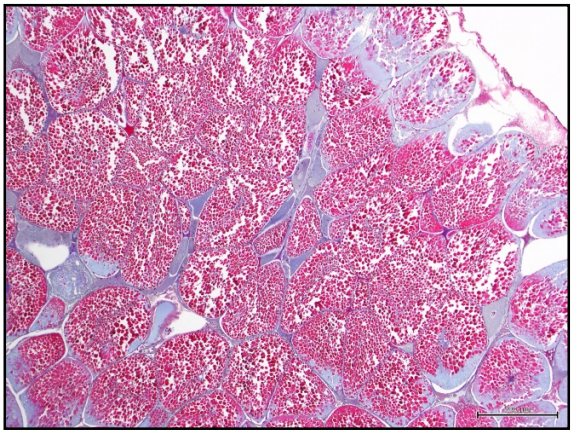

Figure 1. *Chionoecetes opilio*. Ovary. Masson's Trichrome. Low magnification image of late vitellogenic ovary. Oocytes filled with yolk droplets with individual oocyte borders poorly defined. Minimal space between oocytes Scale bar = 500µm.

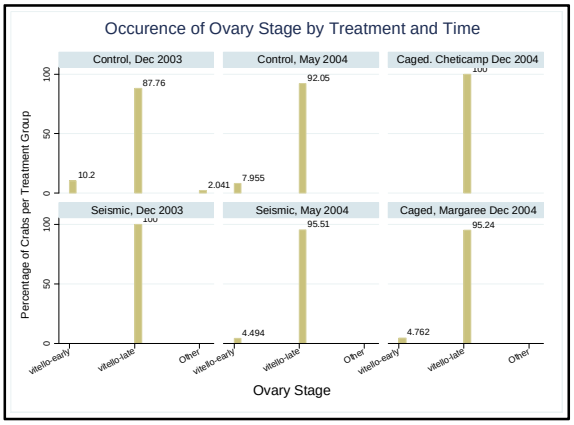

Figure 2. Histogram showing the relative percent distribution of ovary stages of *Chionoecetes opilio* sampled after different caged holding times with or without seismic exposure for the 2003-2004 seismic study and the December 2004 caging study.

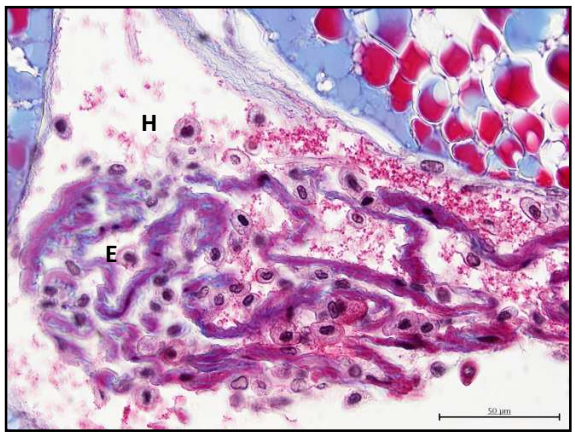

Figure 3. *Chionoecetes opilio*. Ovary. Masson's Trichrome. Collapsed group follicular epithelial cells (E) which previously surrounded a mature oocyte representing a 'spawning scar'. There are low numbers of hemocytes (H) present among the epithelial cells. Fine granular eosinophilic droplets represent hemolymph proteinaceous material. Scale bar = 50 µm.

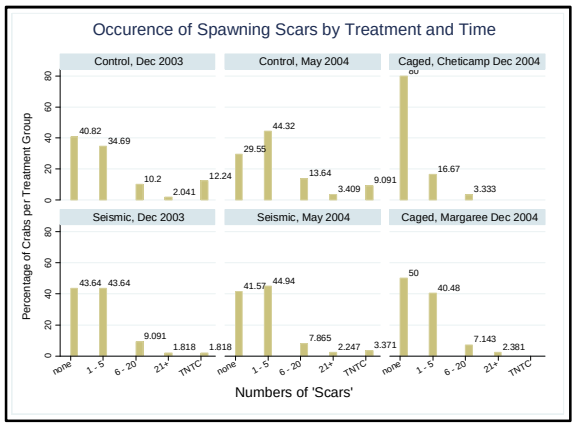

Figure 4. Histogram showing the relative percent distribution of degree of ovulation/spawning scars in ovaries of *Chionoecetes opilio* sampled after different caged holding times with or without seismic exposure for the 2003-2004 seismic study and the December 2004 caging study.

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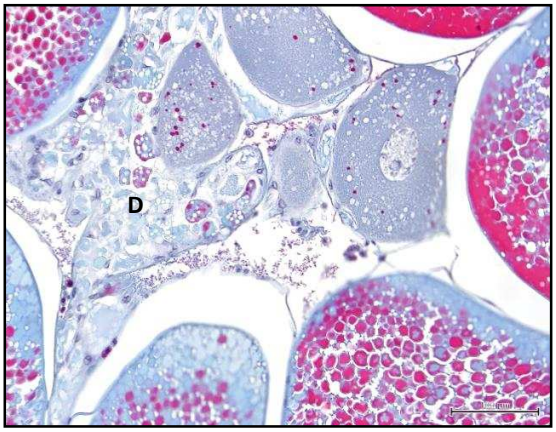

Figure 5. *Chionoecetes opilio.* Ovary. Masson's Trichrome. A degenerating oocyte (D) showing cytoplasmic fragmentation. There is no inflammatory response. There is a moderate to marked degree of membrane separation evident. Scale bar = 100 µm.

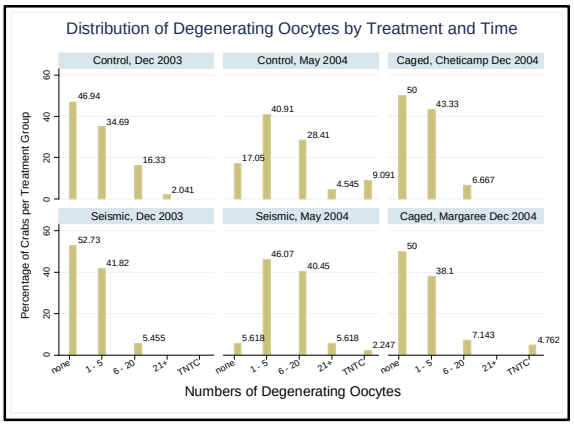

Figure 6. Histogram showing the relative percent distribution of occurrence of degenerating oocytes in ovaries of *Chionoecetes opilio* sampled after different caged holding times and with or without seismic exposure for the 2003-2004 seismic study and the December 2004 caging study.

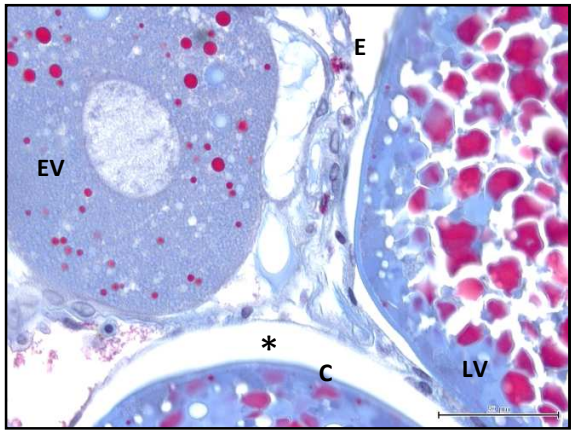

Figure 7. *Chionoecetes opilio.* Ovary. Masson Trichrome. Separation of the chorion from surrounding follicular cells and loose connective tissue is marked with an asterix (*). A early vitellogenic oocyte with a few clear lipid vacuoles, red yolk droplets, and pale nucleus is visible in the upper left of the image (EV). A late-vitellogenic oocyte with more numerous yolk droplets is occupies the upper right portion of the image. A narrow layer of follicular epithelial cells (E) with dark oval nuclei, surrounds the mature oocyte. The homogenous, acellular, chorion (C) layer is also visible in the two mature oocytes. Scale bar = 50 µm.

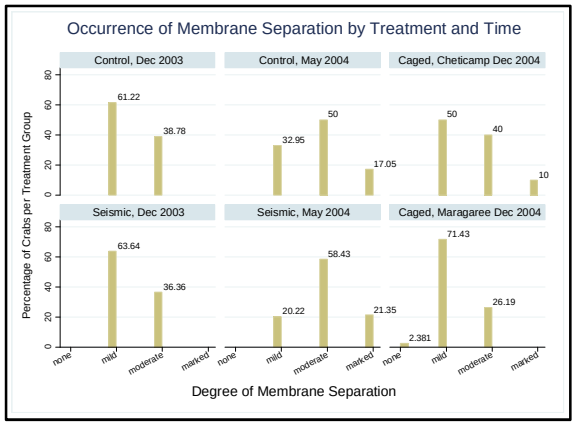

Figure 8. Histogram showing the relative percent distribution of degree of membrane separation in ovaries of *Chionoecetes opilio* sampled after different caged holding times with or without seismic exposure for the 2003-2004 seismic study and the December 2004 caging study.

Hepatopancreas Tissue

Slides corresponding to crabs numbered 35 (December 2004, Control) and 233 (group unknown) were not present in the slide boxes. The slide for crab number 387 (May 2004, Control) contained ovarian tissue only. The plane of section/area of the hepatopancreas contained within each slide was inconsistent – some contained larger duct structures while others did not. Staining quality e.g., intensity, colour, also varied among slides.

The distribution of scores for each characteristic by sample collection time and treatment are summarised in Tables 4 – 6, which include absolute and relative numbers (expressed as percentages) as the number of crabs was different for each sample period and treatment. Analysis was limited to visual inspection of the raw frequency distribution histograms for patterns and trends.

For all combinations of sample time and treatment, autolysis was noted. When detected, it was generally mild, affecting less than 25% of the tissue. Autolytic changes were seen most frequently in the May 2004 samples with 35% of all crabs showing some change. The least amount of autolytic changes were seen in the December 2004 caging only study, with only 6% of crabs caged at the prior seismic site showing changes and ~ 20% of crabs at the control site affected.

Round tubule lumens, alone or in combination with stellate lumens, were more commonly seen in Control crabs in December 2003. Sloughed cells were much more common in the May 2004 crabs. Apoptotic cells were noted more often in the seismic-exposed than no-exposed crabs in December 2003 and May 2004. There was little difference between control site and seismic site caged crabs in December 2004, which had the higher percentages of all sample groups (25%).

Only Control crabs in the short-term holding periods had B-cells with vacuoles scored as '> 50% full' (December 2003 and 2004), with the exception of one seismic-exposed crab in May 2004. Overall, the R-cell lipid content appeared higher in the seismic-exposed crabs in the December 2003 and May 2004 samples. Lipid content in the December 2004 caging study was similar between the two caging sites; however, overall higher than either of the December 2003 crab groups (Figures 9 & 10). A higher percentage of non-exposed crabs had 'moderate' basement membrane folding in December 2003. The number of crabs, seismic-exposed and non-exposed, with a moderate degree of folding increased from December 2003 to May 2004. In the December 2004 caging study a greater percentage of the seismic site crabs had a score of 'moderate'.

Changes associated with inflammation (infectious agents, nodule formation, hemocyte infiltration, pigment deposition) and cell death (necrosis) were not observed within the epithelial tissue in any seismic-exposed or non-exposed crabs in December 2003, May 2004 or the December 2004 caging study. Inflammatory-related (groups of hemocytes focussed on one area or diffusely) hemocyte infiltrates of the tubular epithelium were not observed. However,

individual cells with moderate nuclear:cytoplasmic ratios, non-granulated cytoplasm resembling semi-granular hemocytes were observed in low numbers scattered randomly amongst the tubular epithelium between the basement membrane area and the tubule lumen partway through examination of the slide set. The presence/absence of these cells was noted but not reported herein as not all tissues were examined for this criterion. Positive staining for collagen in the basement membrane area of the tubules was uncommon. Staining was detected most frequently in the December 2004 Margaree (previous seismic-site) caged group.

Within the connective tissue component, the pattern of density of circulating hemocytes was similar for all crabs in December 2003 and May 2004, regardless of seismic exposure. Higher densities were observed more frequently for both groups of crabs in the December 2004 caging study with increasing percentages of crabs with a score of 'moderate' and approximately 10% of crabs in the 'marked' category.

The density of hemocytes within the connective tissue showed variation by sample time and seismic exposure (Figure 11). In the December 2003 group, more seismic-exposed crabs had a score of 'mild' and very few (3%) crabs with a score of 'normal'. In contrast, 20% of non-exposed crabs had a score of 'normal' with only 48% scoring 'mild'. In May 2004, the relative distribution of scores for seismic-exposed and non-exposed crabs was similar with the exception of two seismic-exposed crabs with scores of 'severe' (neither of which was positive for cocci). In the December 2004 caging study, the relative distribution of scores was similar with a score of 'mild' being the most common (78%).

Hemocyte nodules, usually showing minimal organisation, (Figures 12 - 14) were most often detected in the May 2004 samples with a higher percentage (20%) of non-seismic exposed crabs having nodules than seismic-exposed (13%). Pigment deposition was very uncommon (1% - 2% of all crabs; and noted only in the May 2004 samples for both groups). All crabs with pigment also had nodules recorded. Both crabs with scores of 'marked' for nodules had pigment while only one of the four crabs with nodule scores of 'moderate' had pigment deposits. Fibrosis was also very uncommon, being noted only for the December 2003 and May 2004 samples, with similar distribution across seismic-exposed and non-exposed crabs. Only one crab (May 2004, seismic-exposed) had a score of 'moderate', while all others were 'mild'. This crab also had the highest scores for nodules ('marked') and hemocyte connective tissue density ('severe'). The only infectious agents observed were bacterial cocci (possibly diplococci) and these were found in three seismic-exposed crabs in the May 2004 samples.

The fullness of the reserve inclusion (RI) cells varied across treatments and sample time. Reserve inclusion (RI) cells were almost never observed in the May 2004 crabs, seismic-exposed or not. In December 2003, RI cells were more often observed and had more content in the seismic-exposed crabs. In December 2004, the distribution between the two groups was more similar, with a slightly higher percentage of crabs caged at the Cheticamp control area having an

RI score of ‘moderate’. Overall, RI cells were more commonly noted in the December 2004 crabs and were larger/fuller.

The relative distribution of scores for fixed phagocyte activation was quite similar for either treatment group in both the December 2003 and May 2004 samples with a score of ‘mild’ about three times more common than ‘moderate’. This decreased to about a two-fold difference in May 2004. The least amount of activation was noted for the December 2004 caging study where ‘mild’ accounted for ~ 90% and 86% of control (Cheticamp) and seismic (Margaree) areas, respectively.

Table 4. Absolute and relative distribution of characteristics used to score hepatopancreas tissue collected from snow crab in December 2003 after a short-term (12 day) holding period following a seismic exposure study conducted in December 2003. Absolute and percentage values (rounded to nearest whole value in parentheses) reported. Suboptimal sample quality precluded assessment of all characteristics for all crabs.

	Caged at Non-Seismic Site (*n = 66)* Score					Caged at Seismic Site (*n = 64)* Score				
	0	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Epithelium										
Autolysis[1]	50 (76)	10 (15)	6 (9)	0	0	43 (67)	16 (25)	5 (8)	0	0
Lumen shape[2]	52 (79)	4 (6)	2 (3)	8 (12)	0	57 (89)	1 (2)	6 (9)	--	--
Sloughed cells (lumen)[3]	51 (77)	15 (23)	--	--	--	55 (86)	9 (14)	--	--	--
Apoptotic cells (wall)[3]	56 (85)	10 (15)	--	--	--	51 (80)	13 (30)	--	--	--
B-cell vacuole fullness[4]	59 (89)	7 (11)	--	--	--	64 (100)	0	--	--	--
R-cell lipid content[5]	0	9 (14)	23 (35)	34 (52)	0	1 (2)	1 (2)	10 (16)	50 (78)	2 (3)
Basement membrane folding[6]	0	35 (53)	31 (47)	0	0	0	50 (78)	13 (20)	1 (2)	0
Nodule formation[6]	66 (100)	0	0	0	0	64 (100)	0	0	0	0
Pigment deposition[6]	66 (100)	0	0	0	0	64 (100)	0	0	0	0
Fibrosis/collagen deposition[6]	56 (85)	8 (12)	2 (3)	0	0	52 (81)	12 (19)	0	0	0
Tubule necrosis[6]	66 (100)	0	0	0	0	64 (100)	0	0	0	0
Infectious agents[7]	66 (100)	0	0	0	0	64 (100)	0	0	0	0
Connective Tissue										
Circulating hemocytes[8]	0	59 (89)	6 (9)	1 (2)	0	0	59 (92)	5 (8)	0	0
Infiltrating hemocyte s[8]	13 (20)	31 (48)	19 (29)	2 (3)	0	2 (3)	45 (70)	17 (27)	0	0
Nodule formation[6]	63 (97)	2 (3)	0	0	0	64 (100)	0	0	0	0
Pigment deposition[6]	65 (100)	0	0	0	0	64 (100)	0	0	0	0
Fibrosis/collagen deposition[6]	60 (92)	5 (8)	0	0	0	60 (94)	4 (6)	0	0	0
Infectious agents[7]	65 (100)	0	0	0	0	64 (100)	0	0	0	0
Reserve Inclusion cell fullness[6]	51 (78)	14 (22)	0	0	--	22 (34)	34 (53)	8 (13)	0	--
Fixed phagocyte activation[8]	1 (2)	48 (74)	16 (25)	0	--	1 (2)	45 (70)	18 (28)	0	--

[1] 0 = none, 1 is <25% tissue affected, 2 is25 – 50% tissue affected, 3 is 50 – 75% tissue affected, 4 is > 75% tissue affected
[2] 0 = stellate; 1 = round; 2 = dilated, thin walls; 3= mixed stellate and round; 4 = mixed stellate and dilated
[3] 0 = no and 1 = yes;
[4] 0 = none seen; 1 is < 50%; 2 is > 50%
[5] 0 = none seen; 1 is < 50%; 2 is 50 – 75%; 3 is 75 – 100%; 4 is > 100%
[6] 0 = none seen; 1 = mild, 2 = moderate, 3 = marked; 4 = severe
[7] 0 = none seen; 1 = bacteria; 2 = fungi/yeast; 3 = protozoa; 4 = other
[8] 0 = normal; 1 = mild, 2 = moderate, 3 = marked

Table 5. Absolute and relative distribution of characteristics used to score hepatopancreas tissue collected from snow crab in May 2004 after a long-term (5 month) holding period following a seismic exposure study conducted in December 2003. Absolute and percentage values (rounded to nearest whole value in parentheses) reported.

	Non-Seismic *(n = 88)* Score					Seismic (*n = 90*) Score				
	0	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Epithelium										
Autolysis[1]	57 (65)	27 (31)	3 (3)	1 (1)	0	70 (78)	19 (21)	1 (1)	0	0
Lumen shape[2]	81 (92)	1 (10	5 (6)	1 (1)	0	88 (98)	0	0	2 (2)	0
Sloughed cells (lumen) [3]	47 (53)	41 (47)	--	--	--	45 (50	45 (50)	--	--	--
Apoptotic cells (wall) [3]	77 (87)	11 (13)	--	--	--	71 (79)	19 (21)	--	--	--
B-cell vacuole fullness[4]	0	88 (100)	0	--	--	1 (1)	88 (98)	1 (1)	--	--
R-cell lipid content[5]	10 (11)	18 (20)	18 (20)	41 (47)	1 (1)	6 (7)	11 (12)	18 (20)	55 (61)	0
Basement membrane folding[6]	0	17 (19)	56 (63)	15 (17)	0	0	19 (21)	55 (61)	16 (18)	0
Nodule formation[6]	88 (100)	0	0	0	0	90 (100)	0	0	0	0
Pigment deposition [6]	88 (100)	0	0	0	0	90 (100)	0	0	0	0
Fibrosis/collagen deposition[6]	78 (89)	8 (9)	2 (2)	0	0	82 (91)	5 (6)	3 (3)	0	--
Tubule necrosis[6]	88 (100)	0	0	0	0	90 (100)	0	0	0	0
Infectious agents [7]	88 (100)	0	0	0	0	90 (100)	0	0	0	0
Connective Tissue										
Circulating hemocytes[8]	0	81 (92)	7 (8)	0	0	0	79 (88)	10 (11)	1 (1)	0
Infiltrating hemocyte s [8]	1 (1)	32 (36)	48 (55)	7 (8)	0	2 (2)	28 (31)	54 (60)	4 (4)	2(2)
Nodule formation [6]	70 (80)	14 (16)	3 (3)	1 (1)	0	78 (87)	10 (11)	1 (1)	1 (1)	0
Pigment deposition[6]	86 (98)	1 (1)	0	1 (1)	0	89 (99)	1 (1)	0	0	0
Fibrosis/collagen deposition[6]	86 (98)	2 (2)	0	0	0	87 (97)	2 (2)	1 (1)	0	0
Infectious agents[7]	88 (100)	0	0	0	0	87 (97)	3 (3)	0	0	0
Reserve Inclusion cell fullness[6]	83 (94)	5 (6)	0	0	--	88 (98)	2 (2)	0	0	--
Fixed phagocyte activation[8]	0	58 (66)	30 (34)	0	--	0	57 (63)	33 (37)	0	--

[1] 0 = none, 1 is <25% tissue affected, 2 is25 – 50% tissue affected, 3 is 50 – 75% tissue affected, 4 is > 75% tissue affected
[2] 0 = stellate; 1 = round; 2 = dilated, thin walls; 3= mixed stellate and round; 4 = mixed stellate and dilated
[3] 0 = no and 1 = yes;
[4] 0 = none seen; 1 is < 50%; 2 is > 50%
[5] 0 = none seen; 1 is < 50%; 2 is 50 – 75%; 3 is 75 – 100%; 4 is > 100%
[6] 0 = none seen; 1 = mild, 2 = moderate, 3 = marked; 4 = severe
[7] 0 = none seen; 1 = bacteria; 2 = fungi/yeast; 3 = protozoa; 4 = other
[8] 0 = normal; 1 = mild, 2 = moderate, 3 = marked

Table 6. Absolute and relative distribution of characteristics used to score hepatopancreas tissue collected from snow crab during seismic exposure study conducted in December 2004 after a short-term (12 day) holding period in December 2004. Absolute and percentage values (rounded to nearest whole value in parentheses) reported. Suboptimal sample quality precluded assessment of all characteristics for all crabs.

	Non-Seismic *(n = 49)* Score					Seismic (*n = 50*) Score				
	0	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Epithelium										
Autolysis[1]	39 (80)	8 (16)	2 (4)	0	0	47 (94)	2 (4)	1 (2)	0	0
Lumen shape[2]	49 (100)	0	0	0	0	50 (100)	0	0	0	0
Sloughed cells (lumen)[3]	46 (94)	3 (6)	--	--	--	49 (98)	1 (2)	--	--	--
Apoptotic cells (wall)[3]	37 (76)	12 (24)	--	--	--	37 (74)	13 (26)	--	--	--
B-cell vacuole fullness[4]	0	46 (94)	3 (6)	--	--	0	50 (100)	0	--	--
R-cell lipid content[5]	0	0	4 (8)	45 (92)	0	1 (2)	0	7 (14)	39 (78)	3 (6)
Basement membrane folding[6]	--	29 (59)	20 (41)	0	0	0	(24 (48)	26 (52)	0	0
Nodule formation[6]	49 (100)	0	0	0	0	50 (100)	0	0	0	0
Pigment deposition[6]	49 (100)	0	0	0	0	50 (100)	0	0	0	0
Fibrosis/collagen deposition[6]	42 (86)	6 (12)	1 (2)	0	0	24 (68)	11 (22)	5 (10)	0	0
Tubule necrosis[6]	49 (100)	0	0	0	0	50 (100)	0	0	0	0
Infectious agents[7]	49 (100)	0	0	0	0	50 (100)	0	0	0	0
Connective Tissue										
Circulating hemocytes[8]	0	37 (76)	7 (14)	5 (10)	0	0	36 (72)	9 (18)	5 (10)	0
Infiltrating hemocyte s[8]	3 (6)	38 (78)	8 (16)	0	--	5	39 (78)	6 (12)	0	--
Nodule formation[6]	47 (96)	2 (4)	0	0	0	50 (100)	0	0	0	0
Pigment deposition[6]	49 (100)	0	0	0	0	50 (100)	0	0	0	0
Fibrosis/collagen deposition[6]	49 (100)	0	0	0	0	48 (96)	2 (4)	0	0	0
Infectious agents[7]	49 (100)	0	0	0	0	50 (100)	0	0	0	0
Reserve Inclusion cell fullness[6]	16 (37)	16 (33)	14 (29)	1 (2)	0	22 (44)	18 (36)	9 (18)	1 (2)	0
Fixed phagocyte activation[8]	0	44 (90)	5 (10)	0	0	0	43 (86)	7 (14)	0	0

[1] 0 = none, 1 is <25% tissue affected, 2 is25 – 50% tissue affected, 3 is 50 – 75% tissue affected, 4 is > 75% tissue affected
[2] 0 = stellate; 1 = round; 2 = dilated, thin walls; 3= mixed stellate and round; 4 = mixed stellate and dilated
[3] 0 = no and 1 = yes;
[4] 0 = none seen; 1 is < 50%; 2 is > 50%
[5] 0 = none seen; 1 is < 50%; 2 is 50 – 75%; 3 is 75 – 100%; 4 is > 100%
[6] 0 = none seen; 1 = mild, 2 = moderate, 3 = marked; 4 = severe
[7] 0 = none seen; 1 = bacteria; 2 = fungi/yeast; 3 = protozoa; 4 = other
[8] 0 = normal; 1 = mild, 2 = moderate, 3 = marked

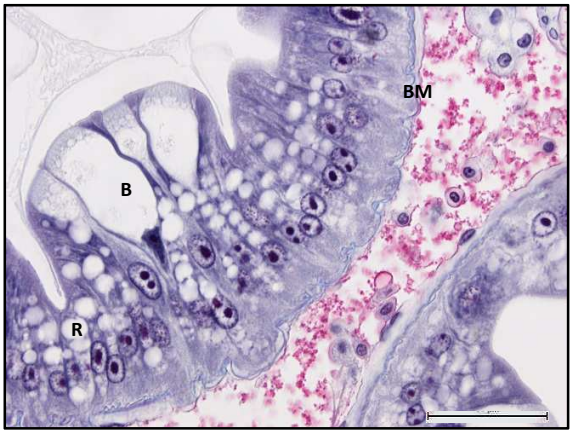

Figure 9 *Chionoecetes opilio.* Hepatopancreas. Masson's Trichrome. Rerseve **(R),** R-cell, cytoplasm shows 50% -75% filling by clear lipid vacuoles. Blister **(B),** B- cells, vacuoles are < 50% filled with material. There is moderate folding of the basement membrane **(BM)**. Red granular (proteinaceous) material is present in the connective tissue space. Scale bar = 50 µm.

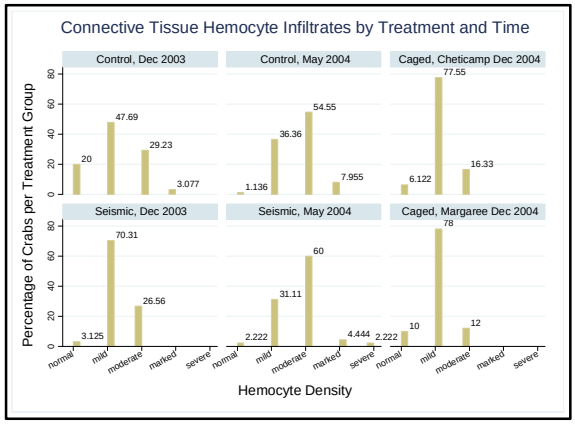

Figure 11. Percent distribution histogram showing the average degree of inflammatory cell infiltrates in the connective tissue of the hepatopancreas of *Chionoecetes opilio* by treatment group for the 2003-2004 seismic study and the December 2004 caging study.

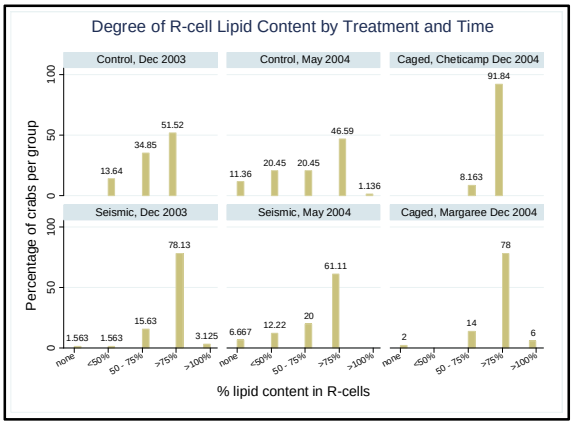

Figure 10. Percent distribution histogram showing the average amount of lipid in R-cells in hepatopancreas of *Chionoecetes opilio* by treatment group for the 2003-2004 seismic study and the December 2004 caging study.

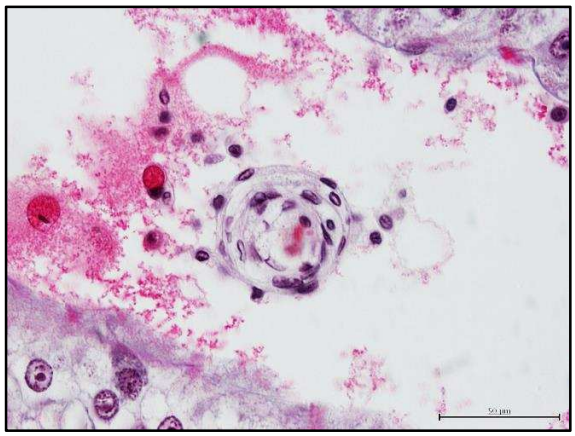

Figure 12. *Chionoecetes opilio.* Hepatopancreas. Masson's Trichrome. Early hemocytic nodule in the connective tissue compartment. Two granulated hemocytes are in the background. Scale bar = 50 µm.

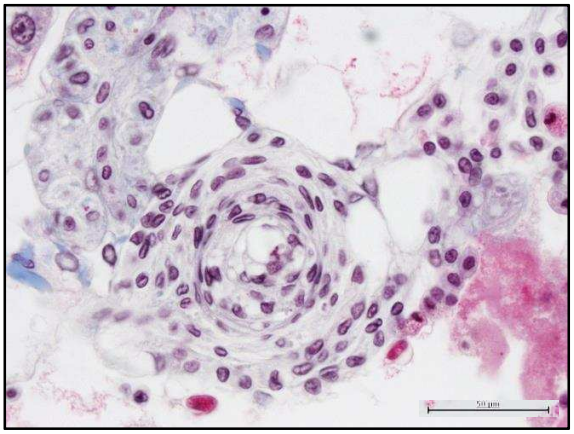

Figure 13. *Chionoecetes opilio.*
Hepatopancreas. Masson's Trichrome.
Hemocytic nodule in the connective tissue compartment demonstrating the whorling pattern of hemocytes (H) development of concentric rings.
Scale bar = 50 µm

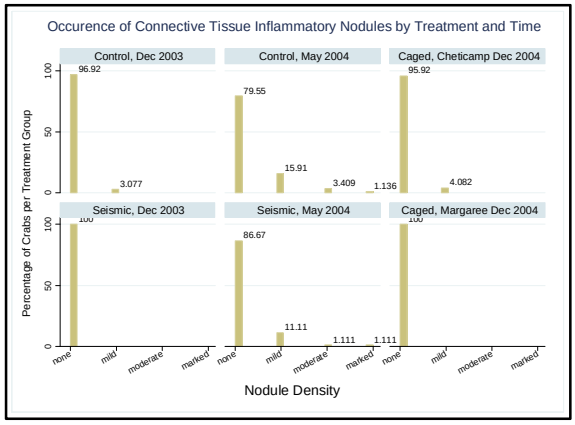

Figure 14. Percent distribution histogram showing the occurrence of inflammatory nodules in the connective tissue of the hepatopancreas of *Chionoecetes opilio* by treatment group for the 2003-2004 seismic study and the December 2004 caging study.

Conclusions

Review of the histologic samples of the ovary and hepatopancreas tissues from the 2003-2004 seismic exposure study found differences between the two sampling periods – 12 days post-exposure (December 2003) and five months post-exposure (May 2004). The more dramatic changes could be due to prolonged caging and/or represent normal seasonal variation experienced by this species, and/or possibly related to regional environmental differences or crab transportation (discussed in detail below). These include the noticeable decrease in lipid content of the hepatopancreas by May 2004 in both groups and an increased degree of chorionic membrane separation in oocytes. Inflammatory changes were also more prominent in the May 2004 samples which may be indirectly related to decreased lipid reserves. Two observations may be linked to seismic testing exposure (or location) alone but would require further investigation. These included suggestion of decreased feeding by crabs at the test site at 12 d post-exposure and an apparent increased percentage of degenerative oocytes in ovaries of crabs held at the test site at five months post-exposure.

There were no remarkable differences between groups at the end of the 12-day caging study conducted in December 2004. It was noted that these crabs appeared to be in overall better condition (greater R-cell lipid, more RI cells) than the crabs collected in December 2003.

Autolysis was noted in many of the hepatopancreas samples to varying degree – most notably in May 2004. Rapid (changes as early as two hours post-mortem) and severe autolysis of the hepatopancreas tissue in crustaceans is a well-recognised problem ascribed to the wealth of proteolytic and other digestive enzymes contained within this tissue (Lightner 1973, Lightner 1996). More severe autolysis is noted in the centre of the hepatopancreas (solid tissue in shrimp vs frond-like arrangement in snow crab). This is considered to be due to poor penetration of the fixative to the innermost part of the tissue (Lightner 1973). To minimise autolytic changes in penaeid shrimp, it is recommended to minimise the stress (e.g., handling, emersion) of the animal as much as possible prior to fixation and then immerse larval and juvenile shrimp directly into fixative (1:10 tissue:fixative volume ratio) while still alive and then cutting open the dorsal cuticle to enhance fixative penetration. For larger shrimp, injection of the fixative directly into the live shrimp is advised (Lightner 1973, Lightner 1996).

In the studies reviewed herein, tissues were collected into fixative with minutes of removal of the dorsal carapace, making delayed fixation an unlikely cause of the autolysis seen. The tissue:fixative volume ratio, estimated at 1:6 to 1:7[4], was lower than

[4] Renée Allain, personal communication

the recommended 1:10, and so may have contributed to the autolysis. Extended emersion, and potential handling trauma, may have also been contributing causes as crabs were emersed for a minimum of eight hours[1] while held, loosely packed without padding, in coolers for transport after hauling of cages until they were processed on-shore (DFO Moncton,NB). Considerations for future tissue sample collection that may help minimise the amount of autolysis observed histologically, would include: minimising stress (emersion, trauma) prior to tissue collection; ensuring adequate tissue:fixative ratio of $\geq$ 1:10; consistency in the section/region of hepatopancreas sampled; and, gentle handling of the tissue to avoid triggering a manual release of enzymes from the hepatopancreas cells.

Hepatic lipid content, as assessed by degree of vacuolation/'fullness' of the R-cells and inversely related to degree folding of the tubule basement membrane, showed a clear trend towards decreased content over time from December 2003 to May 2004 in both groups. This could be attributed to reduced access to food by crabs due to caging. It is also possible that lipid content would have decreased over the winter regardless of caging if crab foraging activity normally decreases at this time. Comparison of R-cell vacuolation of free-ranging crabs collected in May would be necessary to assess this.

There was also a notable decrease in R-cell lipid content in the crabs held at the control site after only 12 d compared to the test site. This was unexpected given that all crabs were collected from the same site. Possibilities to consider would be decreased food availability at the cage deployment site and/or increased food requirement for crabs at the control site, and/or decreased feeding activity at the control site. Review of the original report indicated that the water temperature was higher at the control site so may have increased the basal metabolic rate, and subsequent energy requirement, of the crabs held there as crabs were unable to move to more favourable temperatures. Crabs moved to the control site were also reported as being slightly larger than those at the test site – perhaps less food was available per gram of crab? Finally, crabs at the control site likely experienced greater transportation stress than those immediately redeployed at the test site i.e., two hours emersion to reach the test site. Whether or not this could have negatively impacted feeding behaviour is unknown. The data on hepatopancreatic tubule lumen shape and fullness of B-cells (see below) would suggest decreased feeding by the test-site crabs, so this needs to be evaluated more closely.

Separation in the oocyte creating a 'space' between the chorionic and follicular epithelial cells was dramatic at times and initially considered to be an artifact of sample storage or slide preparation during blinded evaluation of the slides as there was no inflammatory response accompanying the tissue disruption which would be expected if the separation

had occurred *in vivo*. However, this characteristic appeared more often in samples collected in May. This could reflect excess water in the tissues and biological/physiological, or even iatrogenic, causes should be considered. As hepatopancreatic lipid, and potentially protein, reserves were decreased in both groups after the five month caging period, it is likely that tissue water content increased. Direct measurement of tissue water content is relatively easy (wet weight – dry weight) and should be considered in future studies. Abnormally increased water content may have made the tissues more susceptible to shrinkage/rupture artifacts during slide preparation which involves dehydration steps. Variation in the tissue collection/fixation/or processing procedure in May could also be considered e.g., delayed fixation could lead to enhanced autolysis. Bouin's solution has been variably reported to exaggerate shrinkage artifacts in histological sections (Speilberg *et al*. 1993).

Inflammation was noted in a number of samples and patterns could be discerned. The majority of changes were considered to be of a fairly recent (or acute) rather than a longstanding (chronic) nature. These included increased numbers of hemocytes infiltrating the connective tissues compartments of hepatopancreas, small localised/focal aggregations of hemocytes lacking the prominent whorled pattern of organised nodules, and an almost complete absence of brown, red-brown, or gold pigment (melanin) deposits. (Sritunyalucksana & Söderhäll 2000).

A general shift towards increased general hemocyte infiltrates in the hepatopancreas was noted over time in both groups. Also apparent was the development of non-organised, non-melanised inflammatory nodules in the May 2004 samples. Three crabs at the test site also had very low numbers of bacterial cocci noted in the nodules, presumably associated with the fixed phagocytes. A role of the fixed phagocytes in the hepatopancreas is to remove circulating bacteria and other infective organisms (Johnson 1980d). The nature of these changes – more acute to subacute than chronic, would suggest a more recent stimulus rather than being directly related to the five month caging period. A greater degree of nodule organisation and pigment deposit would have been expected for the latter. While a mild degree of pigment deposition was noted for three crabs, this was not the overwhelming pattern.

Hepatopancreas lipid content was decreased in all crabs in May 2004 compared to December 2003, but apparently more so in control, non-exposed, crabs. Crabs in a poorer nutritional state may also be more susceptible to infections and may explain the greater occurrence of inflammatory nodules in the control group. While long term caging/decreased nutritional state may have predisposed to probable bacteremia and

inflammation, a more proximate cause such as local environmental change or crab transport and handling conditions should be reviewed as possible contributing factor(s).

Two characteristics appeared more related to treatment, and/or perhaps location, as this was also an uncontrolled variable. Criteria reported to be associated with the recent feeding (Johnson 1980b) such as round/distended hepatopancreatic tubule lumens and increased material (fullness) of the Blister (B-cells) were observed in a greater proportion of the control crabs than seismic-exposed crabs after 12 days of caging in the December 2003 samples. Assuming a similar amount of time between cage retrieval and crab dissection for the two groups, this could indicate more of the control group crabs were feeding and that seismic exposure had a negative impact on feeding behaviour in the short term.

The percentage of crabs with degenerative oocytes in both groups increased over time. By May 2004, degenerative oocytes were recorded in 94% of seismic-exposed crabs but only 83% of control crabs. Seismic-exposed crabs tended to have higher numbers of degenerative oocytes than control group crabs. As only one serial section per slide was usually examined for scoring purposes, subtle differences between serial sections could have resulted in slightly different scores e.g., differences of one or two in the number of degenerative oocytes were occasionally noted between sections such that a score of 'none' might be noted for one section and a score of '1-5' for another etc., although it is presumed that the trends observed are still valid.

Given that seismic-exposed crabs appeared to have more lipid reserves in the hepatopancreas at this time than control crabs, and so would be less likely to resorb oocytes for nutritional purposes, this could indicate another factor(s) e.g., exposure to seismic testing, location was having a long-term effect. It is interesting to note that after 12 days of caging, the control group had higher numbers of degenerate oocytes than seismic-exposed crabs which could be considered consistent with the need to supplement nutritional intake. Control crabs had lower hepatopancreatic lipid scores than seismic crabs after 12 d of caging (discussed earlier).

Ovaries were classified as 'late vitellogenic' for the vast majority of crabs in any group. This was a fairly generalised classification system based on systems reported by others where distinctions are made between pre-vitellogenic and vitellogenic oocytes (Tan-Fermin & Pudadera 1989, Krol *et al.* 1992, Stewart *et al.* 2007). The late vitellogenic stage would be consistent for crabs which were in the process of developing oocytes for future spawning.

The staining of the yolk droplets was inconsistent within and between slides and it often seemed that the stain had not consistently penetrated the sections. This may be related to the acidophilia of the contents of the yolk droplets which can change as the oocyte matures as has been noted in *Penaeus monodon* (Tan-Fermin & Pudadera 1989). However, it was also noted that a colour band would cross oocyte borders which appeared to be more of a potential stain penetration artifact. Comparison of ovary tissue immersed in an alternate fixative, such as 10% neutral buffered formalin (NBF) in seawater or, Davidson's followed by Masson-Trichrome or hematoxylin and eosin staining might help to identify features associated with the fixation/staining process itself, rather than the physiology of the crab (Speilberg *et al.* 1993). As oocyte maturation is reported to progress synchronously through the oocyte as yolk accumulates (Krol *et al.* 1992), it is possible this colour band reflects a combination of this progressive maturation and plane of section. The latter was inconsistent among the slides which complicated interpretation.

The degree of oocyte cytoplasmic vacuolation did not appear associated with seismic exposure or time of sample collection. These vacuoles most likely represent lipid droplet accumulation as part of normal oocyte development (Krol *et al.* 1992) and not a pathological change.

A few crabs had less developed ovaries – with oocytes primarily in the pre – to early vitellogenic stage of development. These ovaries also usually contained numerous degenerating oocytes and/or spawning scars. These were similar to ovaries described as 'atretic' or 'spent' by others (Krol *et al.* 1992, Stewart *et al.* 2007, Kon *et al.* 2010), consistent with 'recent' spawning. A reference describing the normal progression of morphological changes in snow crab ovaries post-spawning was not located. In blue crabs (*Callinectes danae*), resorption of the non-spawned oocytes (identified as degenerative or atretic) was listed as a normal occurrence as was an accompanying mild to moderate inflammatory response (hemocyte infiltrates) to effect tissue repair post-spawning (Zara *et al.* 2013). Hemocyte infiltrates, when noted, were usually in association with spent ovaries and could represent the normal physiologic response to remove the non-spawned, oocytes as summarised by Zara *et al.* (2013).

Spent ovaries were infrequently observed (7% – 12%) in the non-seismic December 2003 and both May 2004 groups. Only one crab was identified in the 2004 December caging study (seismic site). It is not known how long it would take for histological evidence of prior spawning to disappear, i.e. completion of tissue repair and development of the next batch of oocytes. It is possible that crabs with spent ovaries in December 2003 had spawned in the spring of 2003 while the others spawned in spring of 2004 while caged.

Review of crab records should help to clarify this. Following this approach, crabs with 'late' vitellogenic ovaries would have spawned in spring of 2002 so had more time for ovaries to recover and develop oocytes.

The numbers of spawning scars – as represented by collapsed rings of follicular cells lacking any ooplasm in their centres, appeared disproportionally distributed between the groups. Ovaries with higher numbers of scars were always in the control group. It may be that there was an uneven distribution of multiparous vs primiparous females at the control site. As multiparous females tend to carry more eggs than primaparous females[5], they would also be expected to have more spawning scars in the ovaries. Review of the data from the 2003 ESRF report for primi- vs multiparous female distribution should help to confirm or refute this hypothesis.

The other characteristics examined did not show any obvious patterns associated with seismic exposure or time of sample collection. The significance of the 'glassy' nuclei and homogenous 'laked' ooplasm remains unknown. They were detected to varying degrees in all groups. These could be related to the fixation/staining protocol used and/or possibly represent autolytic changes. Ultrastructural studies of the tissue samples might help to better define this. Confirming the persistence of these observations in new tissues fixed and stained using more common methods e.g. NBF and hematoxylin and eosin, could also be helpful in ascertaining their etiology. As the oocyte nuclei are not consistently in the plane of section, it is difficult to standardise nuclear observations. The mitotic centers (germinal zones) are described by others and represent preparation of the ovary for a new oogenic cycle (Krol *et al.* 1992, Stewart *et al.* 2007).

Ovarian nodules were observed in a number of crabs. Kon *et al.* (2010) associated these with older snow crabs. These structures are quite large, visible macroscopically as red or dark nodules within the ovary. Microscopically, they have a centre of degenerative ooplasm, usually with pigment (melanin) deposits, and a perimeter of vacuolated follicular cells/macrophages. They are described as representing and end-stage degenerative oocytes. The authors felt that crabs having these nodules were less likely to successfully spawn. What directs degenerative oocytes to nodule formation vs complete resorption was not discussed.

The occurrence of pigment (melanin) deposition is usually the consequence of activation of the prophenoloxidase system which occurs with inflammation (Sritunyalucksana & Söderhäll 2000). While pigment was detected in most nodules, the inflammatory

[5] Mikio Moriyasu, personal communication

response was minimal. Possibilities to consider are that inflammation occurred previously and the only remaining evidence is the pigment. Alternately, pigment development could represent auto-oxidation of a tyrosine-containing compound within the oocyte triggered by an unknown cause.

The description by Kon *et al.* (2010) is similar to what was seen in the current samples. Larger nodules appeared to represent the coalescence of multiple (3 – 5) degenerating oocytes in some cases. There was usually only a minimal inflammatory response present and adjacent ovary tissue seemed unaffected. For this reason, gross evaluation of the ovaries for the presence of nodules would be more reliable than histologic assessment as the microscopic changes are so localised. Nodules occurred in pre-/early vitellogenic and late vitellogenic ovaries suggesting that they were not impacting the ability of the ovary to progress through a maturation cycle. This contrasts somewhat with Kon *et al.* (2010).

The density of hemocytes in vessels was recorded separately as circulating hemocyte counts could be affected by a number of factors unrelated to events in the ovary e.g., emersion in other crustaceans will increase total hemocyte counts, inflammation in a different tissue. The overall pattern was similar for both hepatopancreas and ovary samples in both studies with most (70% – 93% crabs having scores of 'mild'. Values for both groups of the 2004 caging study were shifted a bit towards the higher densities but no reason was immediately apparent. This was an extremely subjective assessment so drawing too many conclusions is not advisable nor is the use of this parameter in future studies.

There are two general types of scoring systems used for evaluation of histological slides - summary/'lumping' and non-summary/'splitting' schemes (Cross 1998, Gibson-Corley *et al.* 2013). Summative schemes will usually group a series of observations into a single category, while non-summative schemes will assign a score for each observation and record it separately. Summary schemes are usually more efficient for the observer and work well when the groups being examined have overt differences and when a specific question is being addressed but, information is invariably lost when transferred to a second party (Cross 1998, Gibson-Corley *et al.* 2013, Klopfeisch 2013). Non-summary schemes tend to be more sensitive to parameter-specific or sequential changes and have more repeatability (Gibson-Corley *et al.* 2013). The choice of one system over the other depends on the question(s) being asked and the ultimate use of the information (Gibson-Corely *et al.* 2013, Klopfeisch 2013).

The 2009 report by Lee and Wright (2009) focussed on an overall score rather than listing each characteristic separately so, it is difficult to make direct comparisons to the

review herein. Overall, the findings in the current review have many similarities to the general findings of Lee and Wright (2009) where duration of caging seemed to be a major factor. Lee and Wright (2009) did not find a particular characteristic associated with exposure to seismic testing, while an increased number of degenerative oocytes after five months, and decreased feeding at 12 d, of caging were tentatively connected to seismic exposure (or location) in the current review. The effects of handling stress were identified in both reports as having probable impact on tissue pathology. The ESRF report also indicated that while the intended control site was located 23km away from the test site, seismic noise was detected (118 dBre µPa compared to 178 dB re µPa at the test site). Depending on the threshold value at which noise will impact crabs (currently unknown), this may have contributed to the difficulties in finding histomorphologic differences between crabs held at the test site and the putative control site.

The December 2004 caging study commented on the presence of 'hemorrhage' in the tissues. It is suspected that this is referring to the variable amount of red stained particulate material distributed throughout the hemolymph spaces. This varied staining has been attributed to variation in hemolymph protein content[6] in lobster tissue with animals with higher protein content showing more intense staining with haematoxylin and eosin. Hemolymph protein concentrations are part of the data for the 2012-2013 study so precipitate levels will be recorded and compared.

Unfortunately, it is not possible to thoroughly assess the significance of the findings of either the 2003-2004 seismic study or the 2004 caging only study without non-caged control groups from the same areas for comparison. The follow-up study conducted in 2012-2013 addresses these issues so should prove to be very useful to discern between normal physiologic processes and those associated with caging in the two sample regions. The observations and classification criteria in the current report will be used as a basis for evaluation of the 2012 and 2013 sample sets which will be assessed in more detail.

[6] Personal observations, A. Battison

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V. FEEDING BEHAVIOR

V-1. MATERIALS AND METHODS

V-1-1 Sample preparation for stomach analysis and prey identification

Stomachs from each crab were dissected out and immediately frozen in sample bags. In the laboratory, stomachs were thawed and weighed to the nearest 0.1mg. Stomach fullness was visually estimated on a scale of 1 (< 25%), 2 (25 < 50%), 3 (50 < 75%) to 4 (>75%). Stomachs were opened and rinsed with water to remove their content. Food items were observed under a dissection microscope, identified and classified to the lowest taxon possible. Food items were divided into 11 categories: polychaetes, fish, crab, non-crab crustaceans (amphipods, copepods, shrimp, barnacles), mollusk (bivalves, gastropods), echinoderms (brittle star, starfish, sea urchins), plant/algae, eggs, man-made (twine, rope, gloves, plastic), detritus and other (insect, foraminifers, parasites, plankton). Empty stomachs and their food items were placed on separate filters (VWR filter paper with a 7.5cm diameter and grade 413) and then weighed and dried for a minimum of 24 hours at temperatures ranging between 55°C and 60°C in a drying oven. Dried stomachs and their content were weighed to the nearest 0.1mg. Content weight percentages and frequency of occurrences were calculated for all prey items in the stomachs of each snow crab category.

V-1-2 Stable Isotope Analysis and Sediment Sampling

For stable isotope analysis, samples of merus muscle (2^{nd} walking leg) were carefully dissected from the exoskeleton and tendons and immediately frozen in sample bags at -20°C. For smaller sized crabs, merus muscle from both 2^{nd} walking legs was collected in order to have the enough tissue for analysis.
Sediment samples were collected using a bottom sampler (bottom sampler according to Van Veen, Canimpex Ltd) and immediately frozen in large sample bags at two sites in the fall of 2012 and at all four sites in the spring of 2013. No sediment samples were collected in the fall of 2013.

In the laboratory, snow crab muscle samples were thawed and transferred into 20 ml vials with top openings covered with aluminum foil. Sediment samples were rinsed with water over a 3 layer sieve (grid size 12.5mm, 4mm and 2mm) to collect possible prey items, which were sorted by taxa group, as above mentioned. Very hard shells or parts (i.e

mollusc shells) from possible prey items were removed prior to drying. Muscle and prey item samples were dried in a drying oven for a minimum of 48 hours at 60°C. Samples were then manually grinded into a fine powder with a mortar and pestle, returned in capped vials and brought to the Stable Isotopes in Nature Laboratory, Nature Laboratory (SINLAB), Fredericton, New Brunswick for stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Dried tissue samples were weighed to the nearest 0.001mg and packed into tin capsules. Samples were flash combusted at 1100ºC using either a Carlo Erba NC2500 or Costech 4010 Elemental Analyser and resultant gases via continuous-flow were analyzed using a DELTA Advantage Isotope Ratio Mass Spectrometer. Isotopic ratios were expressed in conventional delta (δ) notation in parts per thousand: δ X = [(R sample / R standard) -1] where X is 13C or 15N and R is the ratio of 13C /12C or 15N /14N. Measurements of commercially available reference material were compared across all runs for accuracy and precision. These reference materials were calibrated against the International Atomic Energy Agency (IAEA) standards which include Vienna Peedee Belemnite carbonate (VPDB) and atmospheric nitrogen (AIR) for carbon and nitrogen, respectively. Within a given analytical run, one standard deviation of sample repeats was lower than 0.1‰ for $\delta^{13}C$ or 0.2‰ for $\delta^{15}N$. Analytical precision in regards to reference materials was better than 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$.

V-1-3 Statistical Analysis

Data analysis using parametric and non-parametric tests was performed with Minitab ® (version 16.2.3.0, MINITAB Inc. State College, PA, USA) and Microsoft Excel (version 14.0., Microsoft. Redmond, Washington, DC, USA) statistical software packages. Data were examined for normality (based on the Anderson-Darling normality test), and variance homogeneity (Bartlett's test). As data did not follow a normal distribution, or heteroscedasticity was detected and did not improve even after data transformation, non-parametric tests (Kruskal-Wallis, followed by nonparametric multiple comparison tests) were applied. All results obtained were considered significant at the significance level of 0.05.

V-2. RESULTS

V-2-1 Stomach contents analysis

A total of 1127 crab stomachs (377 large males, 368 pygmy males and 382 mature females) were analyzed for this study (Table 1). 4% of stomachs were completely empty and 48% of all crab (45% of large males, 47% of mature females and 51% of pygmy

males) contained less than 10mg of food items. Visually, the majority of crab stomachs were less than 25% full regardless of crab category, season, area sampled or treatment (Figure 1). Stomachs sampled in fall 2012 had the most content (in terms of fullness and dry weight) for all free crab categories (Figures 1 & 2). Samples from the spring 2012 generally had the least amount of contents in their stomachs. The differences between stomach content weights of caged and free crabs from Cheticamp and Margaree are described in Figure 3. Caged crabs did not always have less content than free crabs and some categories (females caged for 6 months in Margaree and Cheticamp) had more content in their stomachs than free crabs from the same area. No trend was observed between the length of the caging period and the mean content weight.

Prey species

Overall, the most common identified prey categories observed in stomachs were polychaetes, fish, crab (almost all snow crab), crustacean (non-crab) and mollusk (Figures 4a, b & 5). Polychaetes were present in most crab categories regardless of area or season. Polychaetes were present in 21% of large male stomachs (27% free; 6 % caged), in 22% of pygmy male stomachs (24% free; 17% caged) and 35% in mature females (39% in free; 24% in caged). Fish were present in 25% of large male stomachs (32% free; 5% caged), in 17% of pygmy male stomachs (24% free; 1% caged) and 18% in mature females (24% in free; 3% in caged). More specifically, fish was frequently observed in the fall 2012 of free crabs. Crustaceans were present in 12% of large male stomachs (12% free; 10% caged), in 22% of pygmy male stomachs (29% free; 6% caged) and 27% in mature females (34% in free; 10% in caged). Crab were present in 10% of large male stomachs (10% free; 10% caged), in 12% of pygmy male stomachs (13% free; 8% caged) and 8% in mature females (10% in free; 5% in caged). The miscellaneous categories “other” and “man-made” were frequently observed in caged animals. Detritus was detected in all crab categories (large males: 51% free; 60% caged; pygmy males: 52% free; 70% caged mature females: 50% free; 61% caged).

The maximum number of prey items observed in a stomach was 6 and 7 for males (both categories) and females, respectively. 49% and 31% of free and caged large males, respectively, had more than a single type of prey item in their stomachs while 57% of free and 49% of caged pygmy males had 2 or more types of prey in their stomachs. The majority of female crabs (70% of free and 57% of caged females) had more than one type of prey in their stomachs.

In terms of prey content weight, categories with high frequency occurrences of fish were also the heaviest which overshadowed the weight of other prey items (Figure 6). Furthermore, when comparing prey item frequency of occurrences and dry weight percentages, crab and crustacean preys contributed more to the total content weight even when these items were not observed as frequently (Figures 4a,b & 7a,b).

Table 1. Summary statistics for total dry stomach content weights (mg) for free and caged large males (LM), pygmy males (PM) and mature females (MF) by season and sampling area.

Date	Area	Sampling treatment	Crab category	min	median	max	n	Mean	St dev	no empty stomachs	no stomachs (less than 10mg)	% of stomach content less 10mg
Spring 2012	Cheticamp	Free	LM	0.0	4.8	273.6	21	35.3	63.8	1	12	57.1
			PM	0.1	2.7	68.6	21	15.6	20.4	-	12	57.1
			MF	1.6	9.0	124.3	20	23.2	31.4	-	11	55.0
	Grande-Rivière	Free	LM	0.2	4.6	375.3	21	53.1	103.9	-	13	61.9
			PM	1.1	26.6	147.4	5	62.2	65.1	-	1	20.0
			MF	0.0	7.0	140.6	20	31.2	42.9	2	11	55.0
	Louisbourg	Free	LM	3.8	39.0	1281.6	20	212.3	347.4	-	4	20.0
			PM	0.0	11.9	154.3	20	21.8	34.5	1	7	35.0
			MF	0.4	47.1	203.7	23	69.7	64.3	-	5	21.7
	Margaree	Free	LM	0.0	13.3	241.7	20	42.3	63.5	1	9	45.0
			PM	0.8	23.8	242.0	20	45.9	64.5	-	8	40.0
			MF	1.9	16.6	116.1	20	29.5	29.8	-	10	50.0
Fall 2012	Cheticamp	Free	LM	3.0	367.0	1890	20	564.0	611.0	-	3	15.0
			PM	3.5	114.3	506.3	20	171.4	161.8	-	2	10.0
			MF	0.0	34.5	166.4	20	47.4	51.0	1	7	35.0
	Cheticamp	Caged	LM	1.2	6.1	45.4	20	9.5	10.3	-	13	65.0
			PM	0.0	1.0	183.7	19	12.5	41.7	4	16	84.2
			MF	0.0	0.9	24.3	20	2.5	5.4	4	19	95.0
	Grande-Rivière	Free	LM	1.9	121.7	1417.0	20	315.4	436.3	-	6	30.0
			PM	0.0	1.4	15.6	20	3.1	4.2	3	18	90.0
			MF	0.0	26.3	255.7	21	49.1	65.3	3	9	42.9
	Louisbourg	Free	LM	2.0	376.0	1521.0	20	502.0	488.0	-	3	15.0
			PM	11.3	135.3	739.7	20	199.2	199.9	-	0	0.0
			MF	4.7	138.8	527.3	20	169.8	143.3	-	3	15.0
	Margaree	Free	LM	9.0	270.0	1706.0	20	473.0	509.0	-	1	5.0
			PM	0.0	76.9	259.3	18	86.5	074.2	1	1	5.6
			MF	0.0	7.0	43.9	19	9.8	12.2	3	11	57.9
	Margaree	Caged	LM	0.9	5.5	61.4	18	11.3	14.6	-	13	72.2
			PM	0.0	2.9	34.0	21	4.6	7.2	2	19	90.5
			MF	11.5	185.1	345.2	21	183.1	101.3	-	0	0.0

Table 1 (continued). Summary statistics for total dry stomach content weights (mg) for free and caged large males (LM), pygmy males (PM) and mature females (MF) by season and sampling area.

Date	Area	Sampling treatment	Crab category	min	median	max	n	Mean	St dev	no empty stomachs	no stomachs (< 10mg)	% stomach content (< 10mg)
Spring 2013	Cheticamp	Free	LM	0.4	27.4	543.5	20	83.0	139.2	-	6	30.0
			PM	1.2	11.9	314.6	20	55.1	92.7	-	9	45.0
			MF	1.1	23.7	239.3	20	50.6	70.6	-	7	35.0
	Cheticamp	Caged	LM	0.2	4.1	62.8	18	8.4	14.6	-	14	77.8
			PM	0.0	2.1	207.5	19	34.6	73.7	1	13	68.4
			MF	3.9	82.8	374.2	19	104.3	107.1	-	5	26.3
	Grande-Rivière	Free	LM	0.0	190.0	2096.0	20	467.0	580.0	1	3	15.0
			PM	0.0	17.1	167.3	19	34.8	43.5	1	7	36.8
			MF	0.6	19.8	101.9	20	30.6	31.2	-	7	35.0
	Louisbourg	Free	LM	2.0	10.9	282.7	20	51.4	84.7	-	8	40.0
			PM	3.3	23.8	133.6	18	48.0	45.0	-	3	16.7
			MF	9.3	102.9	123.4	7	83.1	43.9	-	1	14.3
	Margaree	Free	LM	2.2	10.7	749.7	19	104.8	219.4	-	9	47.4
			PM	0.0	7.3	413.4	20	39.4	98.5	2	13	65.0
			MF	0.3	5.7	174.1	21	30.0	47.4	-	11	52.4
	Margaree	Caged	LM	0.2	2.6	92.1	16	10.3	22.8	-	12	75.0
			PM	0.4	1.5	12.2	18	2.9	3.2	-	16	88.9
			MF	1.3	5.9	161.0	19	32.7	46.0	-	10	52.6
Fall 2013	Cheticamp	Free	LM	0.0	10.5	151.8	20	23.8	39.6	1	10	50.0
			PM	0.0	2.9	352.1	20	23.0	77.7	1	14	70.0
			MF	0.0	7.9	599.9	20	43.5	132.2	1	11	55.0
	Cheticamp	Caged	LM	0.0	1.4	880.3	15	62.3	226.4	1	11	73.3
			PM	0.0	13.8	133.2	16	33.0	44.0	2	8	50.0
			MF	0.0	3.8	86.1	16	19.6	27.9	1	11	68.8
	Margaree	Free	LM	0.0	3.7	378.3	20	38.5	89.7	1	14	70.0
			PM	0.0	1.9	350.4	21	28.7	80.5	3	18	85.7
			MF	0.0	2.4	52.1	19	8.1	13.3	3	15	78.9
	Margaree	Caged	LM	0.0	2.3	865.7	9	104.5	286.0	1	6	66.7
			PM	0.0	3.0	29.7	13	5.3	10.4	2	11	84.6
			MF	0.0	3.7	232.7	17	22.1	56.0	2	12	70.6

Figure 1: Histogram showing stomach fullness for large males (LM), pygmy males (PM) and mature females (MF) by season and sampling area (grey bars: less than 25% full; diagonal lines: 25-50% full; black bars: 50-75% full; diagonal grid: 75-100%).

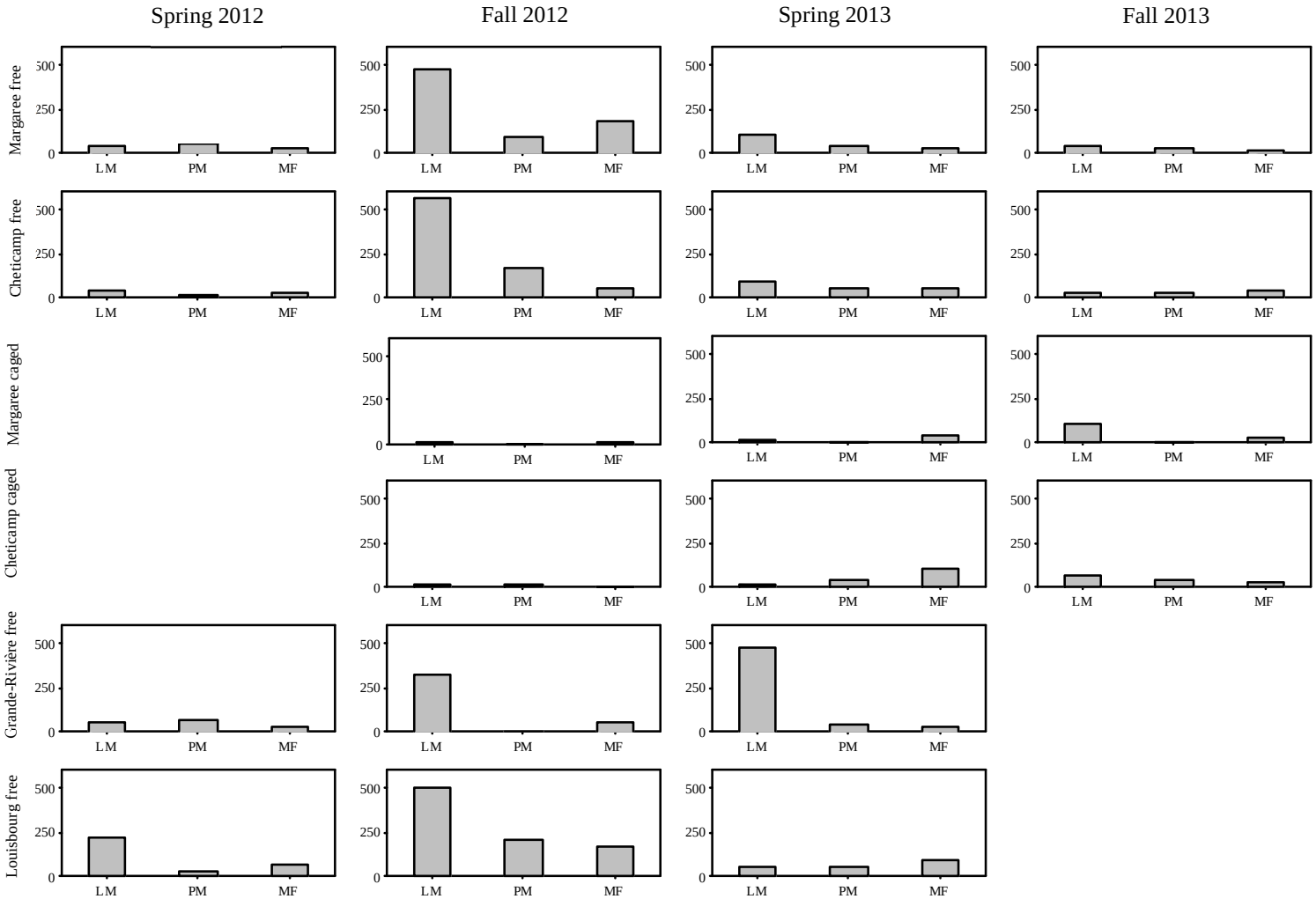

Figure 2: Histogram showing mean dry stomach content weights (mg) of large males (LM), pygmy males (PM) and mature females (MF) by sampling season and area. Stomach content weights were not standardized by size/weight.

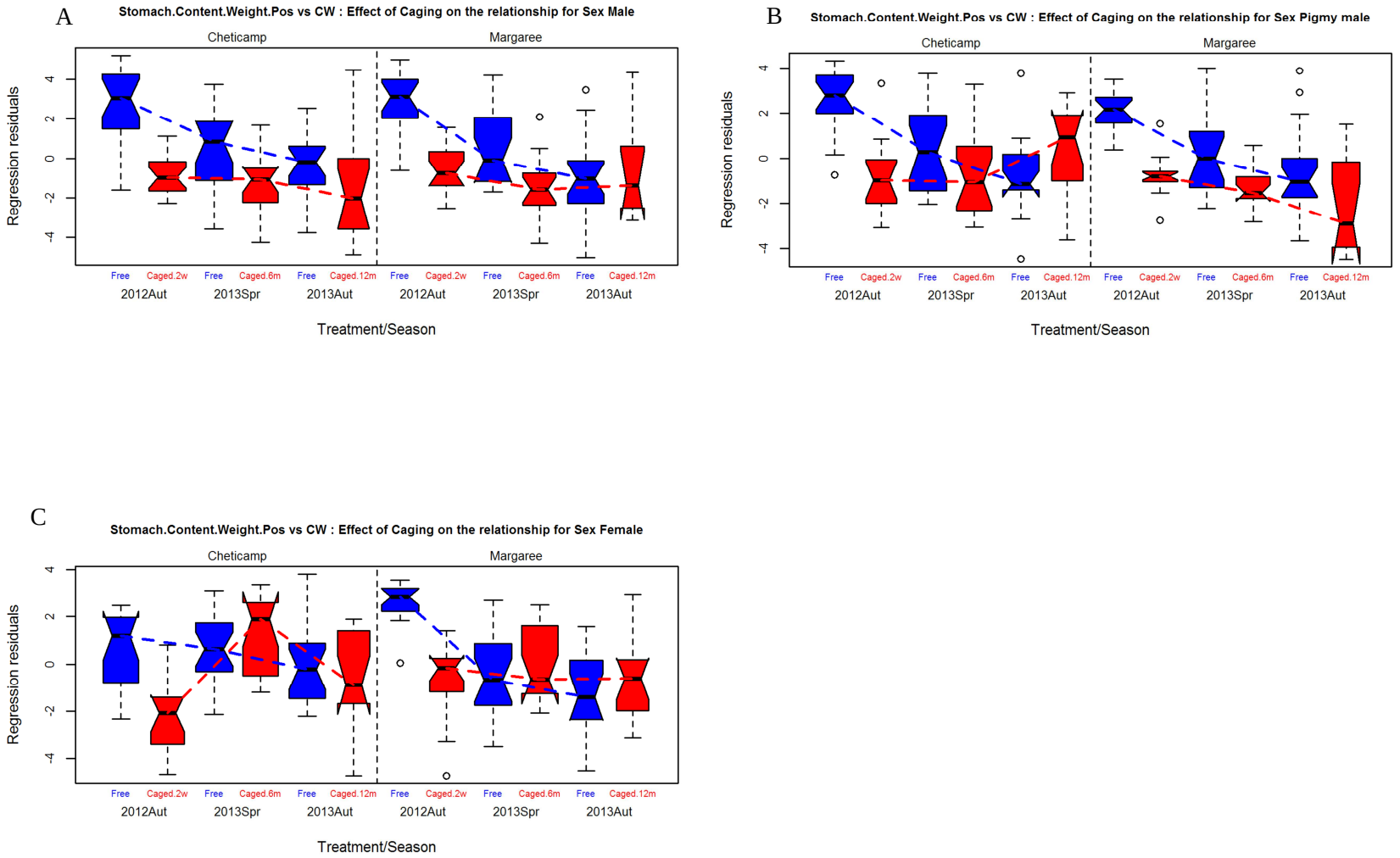

Figure 3. Comparison of Loess regression residuals in the crab size-stomach content weight relationships between caged and free crabs. A) large male, B) pygmy male and C) mature female snow crabs in Margaree and Cheticamp.

Figure 4a: Frequency of occurrence histogram showing prey categories for large males (LM), pygmy males and mature females (MF) by sampling season and area.

Figure 4b: Frequency of occurrence histogram showing prey categories for large males (LM), pygmy males (PM) and mature females (MF) by sampling season and area (detritus excluded).

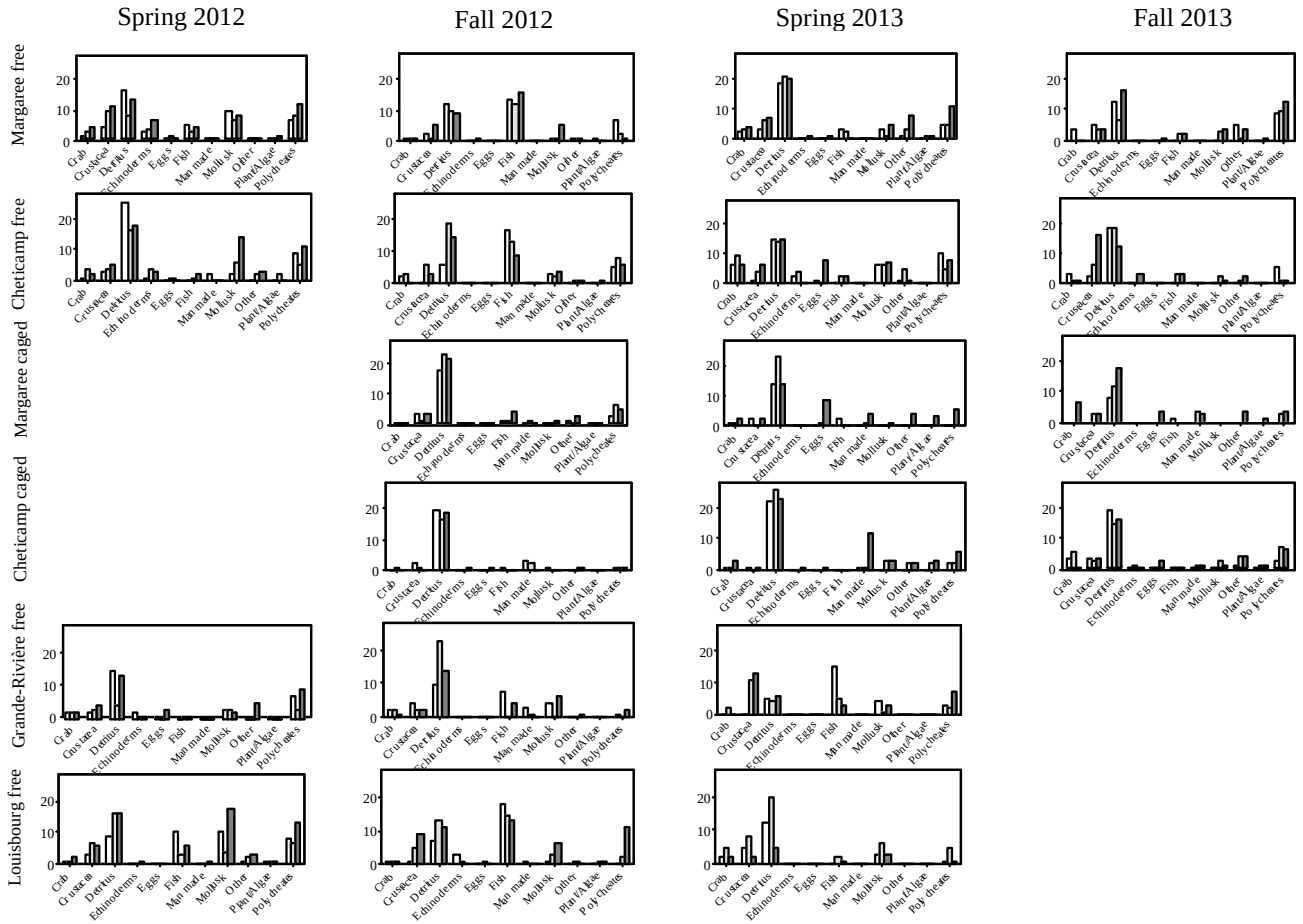

Figure 5: Frequency of occurrence histogram showing prey categories for large males (LM, white bars), pygmy males (PM, light grey bars) and mature females (MF, dark grey bars) by sampling season and area.

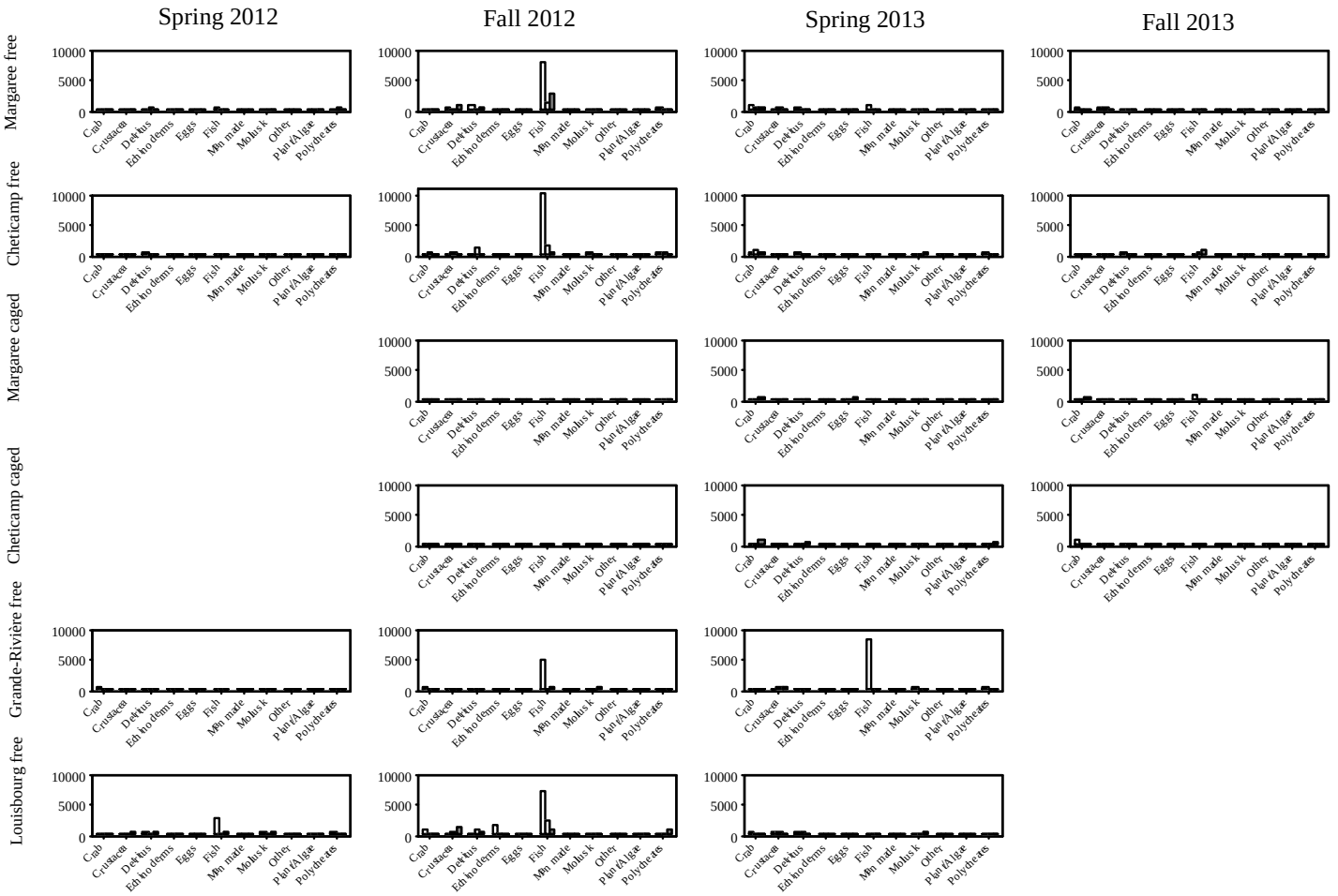

Figure 6: Histogram showing total stomach content weights (in mg) by prey category for large males (LM, white bars), pygmy males (PM, light grey bars) and mature females (MF, dark grey bars) by sampling season and area.

Figure 7a: Histogram showing the content weight percentages by prey category for large males (LM), pygmy males (PM) and mature females (MF) by season and area.

Figure 7b: Histogram showing the content weight percentages by prey category for large males (LM), pygmy males (PM) and mature females (MF) by season and area (detritus excluded).

Content weights: Seasonal comparisons

Seasonal differences in terms of content weight are summarized in Table 2. For free large males from Margaree, significantly higher median content weights were observed in fall 2012 compared to all other sampling seasons (nonparametric multiple comparison test: $Q_{\text{fall 2012 vs spring 2012}} = 3.9028 > Q_{(0.05, \infty, 4)} = 2.639$; $Q_{\text{fall 2012 vs spring 2013}} = 3.2712 > Q_{(0.05, \infty, 4)} = 2.639$ and $Q_{\text{fall 2012 vs fall 2013}} = 5.1627 > Q_{(0.05, \infty, 4)} = 2.639$). These same differences were also observed in free large males from Cheticamp (nonparametric multiple comparison test: $Q_{\text{fall 2012 vs spring 2012}} = 4.3806 > Q_{(0.05, \infty, 4)} = 2.639$; $Q_{\text{fall 2012 vs spring 2013}} = 2.8092 > Q_{(0.05, \infty, 4)} = 2.639$ and $Q_{\text{fall 2012 vs fall 2013}} = 4.355 > Q_{(0.05, \infty, 4)} = 2.639$). For free large males sampled from Grande-Rivière, significant differences were seen among samples from spring 2012 and fall 2012 and spring 2013 (nonparametric multiple comparison test: $Q_{\text{spring 2012 vs fall 2012}} = 2.698 > Q_{(0.05, \infty, 3)} = 2.394$; $Q_{\text{spring 2012 vs spring 2013}} = 3.1688 > Q_{(0.05, \infty, 3)} = 2.394$). In the fall 2012, free large males from Louisbourg were significantly higher in terms of median content weight from those in the spring 2013 (nonparametric multiple comparison test: $Q_{\text{fall 2012 vs spring 2013}} = 3.7438 > Q_{(0.05, \infty, 3) = 2.394)}$. For caged males in Cheticamp, median content weights for animals caged for 2 weeks were significantly higher than those caged for 12 months (nonparametric multiple comparison test: $Q_{\text{fall 2012 vs fall 2013}} = 2.4834 > Q_{(0.05, \infty, 3)} = 2.394$). No significant differences were found among sampling seasons and large males caged in Margaree. For free pygmy males caught in Margaree, significant differences were observed among fall 2012 samples and spring and fall 2013 samples (nonparametric multiple comparison test: $Q_{\text{fall 2012 vs spring 2013}} = 3.0847 > Q_{(0.05, \infty, 4)} = 2.639$; $Q_{\text{fall 2012 vs fall 2013}} = 4.1511 > Q_{(0.05, \infty, 4)} = 2.639$). Fall 2012 stomach median content weights were also significantly higher than other sampling seasons for free pygmy males in Cheticamp (nonparametric multiple comparison test: $Q_{\text{fall 2012 vs spring 2012}} = 4.2541 > Q_{(0.05, \infty, 4)} = 2.639$; $Q_{\text{fall 2012 vs spring 2013}} = 3.0895 > Q_{(0.05, \infty, 4)} = 2.639$ and $Q_{\text{fall 2012 vs fall 2013}} = 4.7417 > Q_{(0.05, \infty, 4)} = 2.639$). Median content weights from free samples caught in Grande-Rivière and Louisbourg in the fall 2012 also showed significant differences among spring 2012 and 2013 sampling seasons (nonparametric multiple comparison test, Grande-Rivière: $Q_{\text{fall 2012 vs spring 2012}} = 2.7403 > Q_{(0.05, \infty, 3)} = 2.394$; $Q_{\text{fall 2012 vs spring 2013}} = 3.4993 > Q_{(0.05, \infty, 3)} = 2.394$; and Louisbourg: $Q_{\text{fall 2012 vs spring 2012}} = 4.9063 > Q_{(0.05, \infty, 3)} = 2.394$; $Q_{\text{fall 2012 vs spring 2013}} = 2.971 > Q_{(0.05, \infty, 3)} = 2.394$). No significant differences were observed among caged pygmy crabs and sampling seasons.

For free females caught in Margaree, fall 2012 median stomach content weights were significantly higher than other sampling seasons (nonparametric multiple comparison test: $Q_{\text{fall 2012 vs spring 2012}} = 3.8364 > Q_{(0.05, \infty, 4)} = 2.639$; $Q_{\text{fall 2012 vs spring 2013}} = 4.8482 > Q_{(0.05, \infty, 4)} = 2.639$ and $Q_{\text{fall 2012 vs fall 2013}} = 6.4303 > Q_{(0.05, \infty, 4)} = 2.639$). Significant

differences were also notes among 6 month caged females (spring 2013) and 2 week (fall 2012) and 12 months (fall 2013) caged females from Cheticamp (nonparametric multiple comparison test: $Q_{6\text{ months vs 2 weeks}} = 2.9151 > Q_{(0.05,\ \infty,\ 3)} = 2.394$; $Q_{6\text{ months vs 12 months}} = 4.921 > Q_{(0.05,\ \infty,\ 3)} = 2.394$. For females from Louisbourg, median fall 2012 content weights were significantly higher than spring 2012 samples (nonparametric multiple comparison test: $Q_{\text{fall 2012 vs spring 2012}} = 2.5129 > Q_{(0.05,\ \infty,\ 3)} = 2.394$). No significant differences were found among sampling seasons in free females from Cheticamp, Grande-Rivière or females caged in Margaree.

Content weights: Sampling area comparisons

Sampling area differences in terms of content weight are summarized in Table 3. For large males collected in the spring 2012, content weights from Louisbourg (free) were significantly higher than those from Cheticamp (free) and Grande-Rivière (free) (nonparametric multiple comparison test: $Q_{\text{Louisbourg free vs Cheticamp free}} = 2.9521 > Q_{(0.05,\ \infty,\ 4)} = 2.639$; $Q_{\text{Louisbourg free vs Grande-Rivière free}} = 3.1587 > Q_{(0.05,\ \infty,\ 4)} = 2.639$). In fall 2012 stomach content weights from large males caged in Cheticamp were significantly lower than free large male crabs (nonparametric multiple comparison test: $Q_{\text{Cheticamp cage vs Cheticamp free}} = 4.5667 > Q_{(0.05,\ \infty,\ 6)} = 2.936$; $Q_{\text{Cheticamp cage vs Louisbourg free}} = 4.4557 > Q_{(0.05,\ \infty,\ 6)} = 2.936$; $Q_{\text{Cheticamp cage vs Grande-Rivière free}} = 2.9859 > Q_{(0.05,\ \infty,\ 6)} = 2.936$; $Q_{\text{Cheticamp cage vs Margaree free}} = 4.7053 > Q_{(0.05,\ \infty,\ 6)} = 2.936$). Margaree caged animals also had significantly lower median content weight values than free caught crab from Louisbourg, Cheticamp and Margaree (nonparametric multiple comparison test: $Q_{\text{Margaree cage vs Louisbourg free}} = 4.3009 > Q_{(0.05,\ \infty,\ 6)} = 2.936$; $Q_{\text{Margaree cage vs Cheticamp free}} = 4.4089 > Q_{(0.05,\ \infty,\ 6)} = 2.936$; $Q_{\text{Margaree cage vs Margaree free}} = 4.5438 > Q_{(0.05,\ \infty,\ 6)} = 2.936$). In the spring 2013, median content weights from Grande-Rivière were significantly higher than both caged groups (nonparametric multiple comparison test: $Q_{\text{Grande-Rivière free vs Cheticamp caged}} = 4.050 > Q_{(0.05,\ \infty,\ 6)} = 2.936$; $Q_{\text{Grande-Rivière free vs Margaree caged}} = 4.5718 > Q_{(0.05,\ \infty,\ 6)} = 2.936$). No significant differences were observed among large males sampled in fall 2013.

In spring 2012, no significant differences were observed among stomach content weights of pygmy males. For fall 2012, content weights were significantly lower in caged animals in Cheticamp than free animals from Cheticamp, Louisbourg and Margaree (nonparametric multiple comparison test: $Q_{\text{Cheticamp cage vs Cheticamp free}} = 5.3107 > Q_{(0.05,\ \infty,\ 6)} = 2.936$; $Q_{\text{Cheticamp cage vs Louisbourg free}} = 5.6027 > Q_{(0.05,\ \infty,\ 6)} = 2.936$; $Q_{\text{Cheticamp cage vs Margaree free}} = 4.2571 > Q_{(0.05,\ \infty,\ 6)} = 2.936$). Results were similar for animals caged in Margaree

(nonparametric multiple comparison test: $Q_{\text{Margaree cage vs Cheticamp free}} = 4.799 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Margaree cage vs Louisbourg free}} = 5.0992 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Margaree cage vs Margaree free}} = 3.7314 > Q_{(0.05, \infty, 6)} = 2.936$). Content weights were also significantly lower in Grande-Rivière samples compared to free samples from Cheticamp, Margaree and Louisbourg (nonparametric multiple comparison test: $Q_{\text{Grande-Rivière free vs Cheticamp free}} = 5.5558 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Grande-Rivière free vs Margaree free}} = 4.4809 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Grande-Rivière free vs Louisbourg free}} = 5.8517 > Q_{(0.05, \infty, 6)} = 2.936$). In the spring 2013, content weights for pygmy males were significantly lower in Magaree caged animals compared to free animals from Cheticamp, Louisbourg and Grande-Rivière (nonparametric multiple comparison test: $Q_{\text{Margaree cage vs Cheticamp free}} = 3.0901 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Margaree cage vs Louisbourg free}} = 4.4130 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Margaree cage vs Grande-Rivière free}} = 3.3003 > Q_{(0.05, \infty, 6)} = 2.936$). Lower values were also statistically significant between caged animals from Cheticamp and free animals from Louisbourg (nonparametric multiple comparison test: $Q_{\text{Cheticamp cage vs Louisbourg free}} = 3.5353 > Q_{(0.05, \infty, 6)} = 2.936$). No significant differences were observed among pygmy males sampled in fall 2013.

In the spring 2012, median content weights from free females caught in Louisbourg were significantly higher than free females collected in Grande-Rivière (nonparametric multiple comparison test: $Q_{\text{Louisbourg free vs Grande-Rivière free}} = 2.7510 > Q_{(0.05, \infty, 4)} = 2.639$. For fall 2012 median content weight samples, free females from Margaree were significantly higher than all other sampling areas except for Louisbourg (nonparametric multiple comparison test: $Q_{\text{Margaree free vs Cheticamp caged}} = 6.7628 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Margaree free vs Cheticamp free}} = 3.4593 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Margaree free vs Grande-Rivière free}} = 3.7634 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Margaree free vs Margaree cage}} = 5.3569 > Q_{(0.05, \infty, 6)} = 2.936$. Females caged in Cheticamp had significantly lower values than free females in Cheticamp, Grande-Rivière and Louisbourg (nonparametric multiple comparison test: $Q_{\text{Cheticamp cage vs Cheticamp free}} = 3.1766 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Cheticamp cage vs Grande-Rivière free}} = 3.0455 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Cheticamp cage vs Louisbourg free}} = 5.9727 > Q_{(0.05, \infty, 6)} = 2.936$. Content weights from Louisbourg were significantly higher than Grande-Rivière and Margaree (caged) (nonparametric multiple comparison test: $Q_{\text{Louisbourg free vs Grande-Rivière free}} = 2.9995 > Q_{(0.05, \infty, 6)} = 2.936$; $Q_{\text{Louisbourg free vs Margaree caged}} = 4.5945 > Q_{(0.05, \infty, 6)} = 2.936$). In the spring 2013, caged females from Cheticamp had significantly higher median content weights than free females from Margaree (nonparametric multiple comparison test: $Q_{\text{Cheticamp cage vs Margaree free}} = 3.1996 > Q_{(0.05, \infty, 6)} = 2.936$). No significant differences were observed among females sampled in fall 2013.

When comparing each caging site separately, significant differences were observed between free and caged crabs for two weeks at both caging sites for all three crab categories (Tables 4A-C). No significant differences were observed between free and caged crabs for 6 and 12 months at either site for any crab category.

Table 2. Summary of median dry stomach content weight values (in mg) of snow crab by season from different sampling areas. Data are separated to show the three crab categories: large males (LM), pygmy males (PM) and mature females (MF). Median values sharing the same subscript are not different. For sampling areas where no letters are shown, no differences were detected among seasons (Kruskal-Wallis testing, $p < 0.05$).

Crab category	Area	n	Spring 2012	n	Fall 2012	n	Spring 2013	n	Fall 2013	P
LM	Margaree free	20	13.3a	20	270.1b	19	10.7a	20	3.7a	< 0.0005
	Cheticamp free	21	4.8a	20	366.6b	20	27.35a	20	10.5a	<0.0005
	Margaree caged	-	-	18	5.45	16	2.6	9	2.3	0.189
	Cheticamp caged	-	-	20	6.05a	18	4.05ab	15	1.4b	0.047
	Grande-Rivière free	20	4.6a	20	121.6b	20	189.6b	-	-	0.003
	Louisbourg free	20	39.05ab	20	376.05a	19	10.9b	-	-	0.001
PM	Margaree free	20	23.85ab	18	76.85a	20	7.3b	21	1.9b	<0.0005
	Cheticamp free	21	2.7b	19	112.7a	20	11.95b	20	2.9b	<0.0005
	Margaree caged	-	-	21	2.9	19	1.5	13	0.3	0.082
	Cheticamp caged	-	-	19	1	19	2.1	16	13.75	0.11
	Grande-Rivière free	5	26.6b	20	1.4a	19	17.1b	-	-	<0.0005
	Louisbourg free	20	11.9b	20	135.35a	18	23.85b	-	-	<0.0005
MF	Margaree free	20	16.5b	21	185.1a	21	5.7b	19	2.4b	< 0.0005
	Cheticamp free	20	9	19	34.5	20	23.7	20	7.85	0.146
	Margaree caged	-	-	19	7	19	5.9	17	3.7	0.202
	Cheticamp caged	-	-	20	0.9a	18	82.8b	16	3.75a	< 0.0005
	Grande-Rivière free	20	6.95	21	26.3	20	19.8	-	-	0.563
	Louisbourg free	23	47.1a	20	138.75b	7	102.9ab	-	-	0.043

Table 3. Summary of median dry stomach content weight values (in mg) of snow crab by sampling area from different seasons. Data are separated to show the three crab categories: large males (LM), pygmy males (PM) and mature females (MF). Median values sharing the same subscript are not different. For sampling areas where no letters are shown, no differences were detected among sampling areas (Kruskal-Wallis testing, $p < 0.05$).

Crab Category	Sampling season	Margaree free	n	Cheticamp free	n	Margaree caged	n	Cheticamp caged	n	Grande-Rivière free	n	Louisbourg free	P
LM	Spring 2012	13.3ab	21	4.8b	-	-	-	-	20	4.6b	20	39.05a	0.006
	Fall 2012	270.15a	20	366.6a	18	5.45bc	20	6.05b	20	121.65ac	20	376.05a	<0.0005
	Spring 2013	10.7ab	20	27.35ab	16	2.6a	18	4.05a	20	189.65b	19	10.9ab	< 0.0005
	Fall 2013	3.7	20	10.5	9	2.3	15	1.4	-	-	-	-	0.166
PM	Spring 2012	23.85	21	2.7	-	-	-	-	5	26.6	20	11.9	0.204
	Fall 2012	76.85b	20	114.25b	21	2.9a	19	1a	20	1.4a	20	135.35b	< 0.0005
	Spring 2013	7.3abc	20	11.95bc	17	1.5a	19	2.1ac	19	17.1bc	18	23.85b	< 0.0005
	Fall 2013	1.9	20	2.9	13	0.3	16	13.75	-	-	-	-	0.074
MF	Spring 2012	16.55ab	20	9ab	-	-	-	-	20	6.95a	23	47.1b	0.042
	Fall 2012	185.1a	19	34.5d	19	7bc	20	0.9b	21	26.3c	20	138.75ad	<0.0005
	Spring 2013	5.7a	20	23.7ab	19	5.9ab	18	82.8b	20	19.8ab	7	102.9ab	0.003
	Fall 2013	2.1	20	7.85	17	3.7	16	3.75	-	-	-	-	0.169

Table 4A. Spatial and temporal variability of stomach content weights for free and caged large male crabs in Cheticamp and Margaree.

Location	Year Season	y-intercept	slope	Treatment effect (Caged)	Treatment effect % (Caged)	treatment p-value	split-slope model p-value	Mean y. free	Mean y. caged
Cheticamp	2012Aut	-40.591	8.20	-3.501	212	0.00000	0.28849	-1.65	-5.09
Cheticamp	2013Aut	-7.848	0.66	-1.470	31	0.04804	0.62247	-4.69	-6.15
Cheticamp	2013Spr	-15.081	2.36	-1.951	52	0.00161	0.75715	-3.79	-5.66
Margaree	2012Aut	0.491	-0.41	-3.579	246	0.00000	0.35589	-1.46	-5.05
Margaree	2013Aut	-29.934	5.16	0.225	-4	0.81190	0.21816	-5.19	-4.98
Margaree	2013Spr	-41.676	7.90	-2.004	52	0.00098	0.49945	-3.85	-5.76

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	5		5	1.9	0.17033	
Location	1	1	0.1	1	0.4	0.51951	
YearSeason	2	135	12.9	67	23.2	0.00000	***
YearSeason: Treatment	3	320	30.6	107	36.8	0.00000	***
Residuals	203	589	56.4	3			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.com p.p-value	Significance
Margaree - Cheticamp	1.00	0.00300	0.236	-0.0874	0.98990	

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.com p.p-value	Significance
2013Spr - 2012Aut	0.098	-2.33	0.385	68	0.00000	***
2013Aut - 2012Aut	0.030	-3.50	0.394	102	0.00000	***
2013Aut - 2013Spr	0.309	-1.17	0.387	34	0.00757	**

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.com p.p-value	Significance
Caged vs Free: 2012Aut 2w	0.027	-3.601	0.391	105	0.00000	***
Caged vs Free: 2013Spr 6m	0.140	-1.966	0.400	57	0.00001	***
Caged vs Free: 2013Aut 12m	0.431	-0.841	0.459	25	0.19104	

Since this analysis only compared two groups at a time, the significance level was reduced to p = 0.0001 in order to minimize the type I error (significant differences are highlighted in red).

Table 4B. Spatial and temporal variability of stomach content weights for free and caged pygmy male crabs in Cheticamp and Margaree.

Location	YearSeason	y-intercept	slope	Treatment effect (Caged)	Treatment effect % (Caged)	treatment p-value	split-slope model p-value	Mean y. free	Mean y. caged
Cheticamp	2012Aut	-38.56	8.20	-3.82	161	0.00000	0.06281	-2.38	-5.82
Cheticamp	2013Aut	2.44	-1.89	1.05	-19	0.11543	0.50651	-5.48	-4.45
Cheticamp	2013Spr	3.58	-1.84	-1.01	23	0.14179	0.10569	-4.36	-5.48
Margaree	2012Aut	2.63	-1.24	-2.85	101	0.00000	0.41592	-2.81	-5.79
Margaree	2013Aut	-27.19	4.98	-1.91	35	0.02992	0.04415	-5.49	-7.04
Margaree	2013Spr	-3.20	-0.36	-1.48	31	0.01445	0.02205	-4.75	-6.26

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum. Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	4		3.7	1.3	0.25517	
Location	1	17	2.0	16.9	6.0	0.01512	*
YearSeason	2	112	13.0	56.1	19.9	0.00000	***
YearSeason:Treatment	3	183	21.1	61.0	21.7	0.00000	***
Residuals	197	554	64.0	2.8			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.com p.p-value	Significance
Margaree - Cheticamp	0.583	-0.539	0.246	12.9	0.02979	*

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.com p.p-value	Significance
2013Spr - 2012Aut	0.132	-2.025	0.393	48.5	0.00000	***
2013Aut - 2012Aut	0.050	-2.995	0.407	71.7	0.00000	***
2013Aut - 2013Spr	0.379	-0.970	0.393	23.2	0.03850	*

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.com p.p-value	Significance
Caged vs Free: 2012Aut 2w	0.045	-3.104	0.409	74.4	0.00000	***
Caged vs Free: 2013Spr 6m	0.290	-1.238	0.406	29.7	0.00785	**
Caged vs Free: 2013Aut 12m	0.899	-0.106	0.436	2.5	0.99285	

Since this analysis only compared two groups at a time, the significance level was reduced to p = 0.0001 in order to minimize the type I error (significant differences are highlighted in red).

Table 4C. Spatial and temporal variability of stomach content weights for free and caged mature female crabs in Cheticamp and Margaree.

Location	Year Season	y-intercept	slope	Treatment effect (Caged)	Treatment effect % (Caged)	treatment p-value	split-slope model p-value	Mean y.free	Mean y.caged
Cheticamp	2012Aut	-17.0692	3.09	-2.818	73	0.00002	0.17482	-3.86	-6.79
Cheticamp	2013Aut	0.0781	-1.13	-0.504	11	0.43340	0.45469	-4.68	-5.22
Cheticamp	2013Spr	-0.2784	-0.85	0.828	-21	0.11060	0.33739	-3.86	-3.07
Margaree	2012Aut	-0.0777	-0.42	-3.380	180	0.00000	0.45826	-1.87	-5.25
Margaree	2013Aut	-32.2310	6.24	0.478	-8	0.42735	0.47324	-5.73	-5.24
Margaree	2013Spr	-5.7214	0.19	0.394	-8	0.46379	0.49461	-4.91	-4.52

Analysis of variance-covariance: Location, Year with Interaction

	Df	Sum.Sq	% Sum.Sq.	Mean.Sq	F.value	Pr(>F)	Significance
trans(CW)	1	3		3.4	1.2	0.28420	
Location	1	0	0.0	0.0	0.0	0.97137	
YearSeason	2	45	5.3	22.3	7.5	0.00073	***
YearSeason: Treatment	3	184	21.9	61.3	20.6	0.00000	***
Residuals	205	610	72.7	3.0			

Location effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Margaree - Cheticamp	0.970	-0.0307	0.237	0.750	0.89677	

Season effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
2013Spr - 2012Aut	0.209	-1.565	0.391	38.2	0.00027	***
2013Aut - 2012Aut	0.097	-2.334	0.406	57.0	0.00000	***
2013Aut - 2013Spr	0.464	-0.769	0.400	18.8	0.13455	

Caged vs Free effect

	Diff. untransformed	Diff. Transformed	SE Transformed	Diff. Transformed (%)	mult.comp.p-value	Significance
Caged vs Free: 2012Aut 2w	0.04	-3.178	0.411	77.6	0.00000	***
Caged vs Free: 2013Spr 6m	1.76	0.566	0.394	-13.8	0.39067	
Caged vs Free: 2013Aut 12m	0.93	-0.077	0.430	1.9	0.99715	

Since this analysis only compared two groups at a time, the significance level was reduced to p = 0.0001 in order to minimize the type I error (significant differences are highlighted in red).

Content weights: Crab category comparisons

Sampling area differences in terms of content weight by crab category are summarized in Table 5. In the spring 2012, only median content weights of free large males from Louisbourg were significantly different than free pygmy males (nonparametric multiple comparison test: $Q_{\text{large males vs pygmy males}} = 2.864 > Q_{(0.05, \infty, 3)} = 2.394$). No other differences were observed among crab categories in sampling areas for the spring 2012 sampling season.

For fall 2012 samples, significant differences were noticed between free male categories from Margaree (nonparametric multiple comparison test: $Q_{\text{large males vs pygmy males}} = 3.226 > Q_{(0.05, \infty, 3)} = 2.394$). Significant differences were also noted between free pygmy males and females and between free pygmy males and large males in Grande-Rivière (nonparametric multiple comparison test: $Q_{\text{pygmy males vs mature females}} = 2.992 > Q_{(0.05, \infty, 3)} = 2.394$; $Q_{\text{pygmy males vs large males}} = 4.791 > Q_{(0.05, \infty, 3)} = 2.394$). In free crabs from Cheticamp, median stomach content weight were significantly lower in females than in males (nonparametric multiple comparison test: $Q_{\text{mature females vs large males}} = 3.961 > Q_{(0.05, \infty, 3)} = 2.394$; $Q_{\text{mature females vs pygmy males}} = 2.520 > Q_{(0.05, \infty, 3)} = 2.394$). Median stomach content weigths of large males caged for two weeks in Cheticamp were statistically heavier than those of females and pygmy males (nonparametric multiple comparison test: $Q_{\text{large males vs mature females}} = 3.956 > Q_{(0.05, \infty, 3)} = 2.394$; $Q_{\text{large males vs pygmy males}} = 2.999 > Q_{(0.05, \infty, 3)} = 2.394$). No significant differences were observed among crab categories from Louisbourg or caged in Margaree.

In the spring 2013, free large males from Grande-Rivière had stomach content weights significantly heavier than free females and pygmy males (nonparametric multiple comparison test: $Q_{\text{large males vs mature females}} = 2.504 > Q_{(0.05, \infty, 3)} = 2.394$; $Q_{\text{large males vs pygmy males}} = 2.581 > Q_{(0.05, \infty, 3)} = 2.394$). Females at both caging sites had the heaviest median stomach content weights and results were significant in Margaree between pygmy males (nonparametric multiple comparison test: $Q_{\text{mature females vs pygmy males}} = 3.538 > Q_{(0.05, \infty, 3)} = 2.394$) and in Cheticamp between both male categories (nonparametric multiple comparison test: $Q_{\text{mature females vs large males}} = 3.408 > Q(0.05, \infty, 3) = 2.394$; $Q_{\text{mature females vs pygmy males}} = 3.719 > Q_{(0.05, \infty, 3)} = 2.394$). No significant differences were observed among free crab categories from Margaree, Cheticamp or Louisbourg.
In the fall 2013, no significant differences were observed among crab categories and sampling areas.

Table 5. Summary of median dry stomach content weight values (in mg) of snow crab category (large males (LM), pygmy males (PM) and mature females (MF)) by area. Data are separated to show the four sampling season. Median values sharing the same subscript are not different. For sampling areas where no subscript are shown, no differences were detected among sampling areas (Kruskal-Wallis testing, $p < 0.05$).

Sampling season	Sampling area	Large males	n	Pygmy males	n	Mature females	n	P
Spring 2012	Margaree free	13.30	20	23.85	20	16.55	20	0.921
	Margaree caged	-	-	-	-	-	-	-
	Cheticamp free	4.80	21	2.70	21	9.00	20	0.493
	Cheticamp caged	-	-	-	-	-	-	-
	Grande-Rivière free	4.60	20	26.60	6	6.95	20	0.532
	Louisbourg free	39.05a	20	11.90b	20	47.10ab	23	0.011
Fall 2012	Margaree free	270.15a	20	76.85b	18	185.10ab	21	0.004
	Margaree caged	5.45	18	2.9	21	7	19	0.103
	Cheticamp free	366.60b	20	114.25b	20	34.50a	19	<0.0005
	Cheticamp caged	6.05b	20	1.00a	19	0.90a	20	<0.0005
	Grande-Rivière free	121.65a	20	1.40b	20	26.30a	21	<0.0005
	Louisbourg free	376.00	20	135.3	20	138.80	20	0.139
Spring 2013	Margaree free	10.70	19	7.30	20	5.70	21	0.166
	Margaree caged	2.60ab	16	1.50b	17	5.90a	19	0.001
	Cheticamp free	27.35	20	11.95	20	23.70	20	0.566
	Cheticamp caged	4.05b	18	2.10b	19	82.80a	18	<0.0005
	Grande-Rivière free	189.65b	20	17.10a	19	19.80a	20	0.013
	Louisbourg free	10.90	19	23.85	18	102.90	7	0.055
Fall 2013	Margaree free	3.70	20	1.90	21	2.40	19	0.507
	Margaree caged	2.30	9	0.30	13	3.70	17	0.100
	Cheticamp free	10.5	20	2.90	20	7.85	20	0.215
	Cheticamp caged	1.4	15	13.75	16	3.75	16	0.230
	Grande-Rivière free	-	-	-	-	-	-	-
	Louisbourg free	-	-	-	-	-	-	-

V-2-4 Stable isotope analysis

A total of 415 crab muscle samples (140 large males, 141 pygmy males and 134 mature females) and 25 possible prey items from sediment samples were analyzed for this study. Given that very few possible prey items from sediment samples were collected per site, and that some prey categories observed in stomach contents (i.e. fish, echinoderms) were not collected, prey items from each site were combined and divided into 4 categories: plants, bivalves, worms and amphipods. Additionally, crab sampling sites and seasons were combined to assess primarily the isotopic composition of sources sampled and the possible differences among crab categories and sampling method.

Stable isotope analysis of C ($\delta^{13}C$) and N ($\delta^{15}N$) of snow crab muscle are summarized in Table 6. Overall, isotope values were depleted in 13C in females in all treatment when compared to male categories (Figures 8, 9, Table 7). $\delta^{15}N$ values were similar among free crab categories. For crab caged for 6 months, females had the highest $\delta^{15}N$ values while large males had the highest values of $\delta^{15}N$ for crabs caged for 12 months. Values of $\delta^{13}C$ and $\delta^{15}N$ for pygmy males generally overlapped both female and large male values. $\delta^{13}C$ signatures for all three crab categories were within the range of possible prey items for each sampling type (Figure 10).

When comparing $\delta^{13}C$ values of crabs by treatment (free versus 6 and 12 month caged), significant differences were observed among all three free crab categories (nonparametric multiple comparison test: $Q_{mf\ vs\ lm} = 8.654 > Q_{(0.05,\ \infty,\ 3)} = 2.394$; $Q_{mf\ vs\ pm} = 3.394 > Q_{(0.05,\ \infty,\ 3)} = 2.394$ and $Q_{lm\ vs\ pm} = 5.335 > Q_{(0.05,\ \infty,\ 3)} = 2.394$; lm= large males; pm = pygmy males; mf = mature females) (Table 6). For 6 month caged crabs, significant differences were observed between females and both male categories (Nonparametric multiple comparison test: $Q_{mf\ vs\ lm} = 5.112 > Q_{(0.05,\ \infty,\ 3)} = 2.394$; $Q_{mf\ vs\ pm} = 3.876 > Q_{(0.05,\ \infty,\ 3)} = 2.394$). For 12 month caged crabs, significant differences were noted between females and large males ($Q_{mf\ vs\ lm} = 4.798 > Q_{(0.05,\ \infty,\ 3)} = 2.394$) and between pygmy and large males ($Q_{lm\ vs\ pm} = 2.708 > Q_{(0.05,\ \infty,\ 3)} = 2.394$).

When comparing the $\delta^{13}C$ values among sampling methods for each crab category, no significant difference was observed in either male crab categories among free, 6 month and 12 month caged animals (Table 8). Significant differences were observed among females with difference noted between free females and both caged categories (nonparametric multiple comparison test: $Q_{free\ vs\ 6\ months} = 3.441 > Q_{(0.05,\ \infty,\ 3)} = 2.394$; $Q_{free\ vs\ 12\ months} = 5.041 > Q_{(0.05,\ \infty,\ 3)} = 2.394$).

In terms of possible prey items, no significant differences were observed among $\delta^{13}C$ values for any possible prey items ($p = 0.906$). Plant material had the most negative $\delta^{13}C$ values and high variability (Figure 10). Amphipod, worms and bivalves had similar $\delta^{13}C$ values suggesting a similar primary source.

For $\delta^{15}N$ values, significant differences were observed among crab categories of caged animals (Table 7). For 6 month caged crabs, significant differences were observed between females and both male categories (Nonparametric multiple comparison test: $Q_{mf \text{ vs } lm} = 2.601 > Q_{(0.05, \infty, 3)} = 2.394$; $Q_{mf \text{ vs } pm} = 3.245 > Q_{(0.05, \infty, 3)} = 2.394$). For 12 month caged crabs, significant differences were noted between females and large males ($Q_{mf \text{ vs } lm} = 2.562 > Q_{(0.05, \infty, 3)} = 2.394$) and between large and pygmy males ($Q_{lm \text{ vs } pm} = 2.526 > Q_{(0.05, \infty, 3)} = 2.394$). No significant differences were observed among crab categories of free crabs.

When comparing $\delta^{15}N$ values of sampling method by crab category, there was a trend of increasing values of $\delta^{15}N$ for free, 6 month caged and 12 month caged of both male categories (Table 8). Significant differences between sampling treatments were observed in large males but not in pygmy males. A nonparametric multiple comparison test showed significant differences in $\delta^{15}N$ values between free large males and large males caged for 12 months ($Q_{\text{free vs 12 months}} = 5.886 > Q_{(0.05, \infty, 3)} = 2.394$) as well as between large males caged for 6 months and 12 months ($Q_{\text{6 months vs 12 months}} = 3.255 > Q_{(0.05, \infty, 3)} = 2.394$). Although no increasing trend was observed in females, significant differences were nonetheless observed. Free females had significantly lower $\delta^{15}N$ values than 6 and 12 month caged females (nonparametric multiple comparison test: $Q_{\text{free vs 6 months}} = 4.341 > Q_{(0.05, \infty, 3)} = 2.394$ and $Q_{\text{free vs 12 months}} = 3.253 > Q_{(0.05, \infty, 3)} = 2.394$, respectively).

In terms of possible prey items, significant differences were observed in the $\delta^{15}N$ values of possible prey items ($p = 0.005$). Plant materials had the lowest $\delta^{15}N$ values (and highest variations) and these values were significantly lower compared to worms (nonparametric multiple comparison test: $Q_{\text{plant vs worm}} = 3.489 > Q_{(0.05, \infty, 4)} = 2.639$). Higher variations were also observed with worms which had values approximately one trophic level lower than snow crab. Bivalves and amphipods had similar $\delta^{15}N$ values and these prey items were approximately two trophic levels lower than snow crab assuming a trophic discrimination factor of ca. 3 to 3.4 per mil (Owens 1987, Peterson & Fry 1987, Post 2002).

Table 6. Summary of mean carapace width (CW), weight and δ^{13}C and δ^{15}N values (with standard deviation in parentheses) of large male (LM), pygmy male (PM) and mature female (MF) snow crab. Data are separated to show the three sampling treatment: wild, caging for 6 months and caging for 12 months.

Sampling type	crab category	n	CW (LM)	Weight (g)	δ^{13}C	δ^{15}N
Free	LM	101	119.58 (9.03)	743.2 (176.1)	-17.622 (0.360)	12.988 (0.459)
	PM	100	75.75 (7.80)	177.0 (52.4)	-17.963 (0.441)	13.017 (0.420)
	MF	94	70.23 (6.76)	123.0 (29.6)	-18.191 (0.396)	13.103 (0.423)
6 months caged	LM	20	123.78 (6.68)	837.3 (155.6)	-17.739 (0.221)	13.194 (0.380)
	PM	21	85.04 (10.49)	264.8 (83.8)	-17.957 (0.530)	13.121 (0.572)
	MF	20	73.40 (7.02)	142.9 (33.6)	-18.523 (0.285)	13.556 (0.414)
12 months caged	LM	19	123.91 (6.79)	838.3 (128.8)	-17.660 (0.554)	14.279 (0.874)
	PM	20	78.67 (11.16)	208.4 (86.1)	-18.274 (0.634)	13.556 (0.909)
	MF	20	70.75 (6.76)	126.6 (35.6)	-18.695 (0.324)	13.505 (0.561)

Table 7. Summary of median δ^{13}C and δ^{15}N values of snow crab among crab category (large males (LM), pygmy males (PM) and mature females (MF) by sampling treatment (Kruskal-Wallis testing, $p < 0.05$).

	Sampling type	crab category	n	Median	H	P
δ^{13}C	free	LM	101	-17.58	76.45	<0.0005
		PM	100	-18.02		
		MF	94	-18.21		
	6 months caged	LM	20	-17.75	28.35	<0.0005
		PM	21	-17.85		
		MF	20	-18.40		
	12 months caged	LM	19	-17.49	23.23	<0.0005
		PM	20	-18.30		
		MF	20	-18.65		
δ^{15}N	free	LM	101	13.01	4.48	0.106
		PM	100	12.96		
		MF	94	13.11		
	6 months caged	LM	20	13.15	11.67	0.003
		PM	21	12.97		
		MF	20	13.52		
	12 months caged	LM	19	14.34	8.62	0.013
		PM	20	13.37		
		MF	20	13.36		

Table 8. Summary of median δ^{13}C and δ^{15}N values of snow crab among sampling treatments, by crab category (Kruskal-Wallis testing, $p < 0.05$).

	Crab category	Sampling treatment	n	Median	H	P
δ^{13}C	Large males	12 months caged	19	-17.49	3.17	0.205
		6 months caged	20	-17.75		
		free	101	-17.58		
	Pygmy males	12 months cage	20	-18.30	5.27	0.072
		6 months cage	21	-17.85		
		free	100	-18.02		
	Mature females	12 months caged	20	-18.65	32.23	<0.0005
		6 months caged	20	-18.40		
		free	94	-18.21		
δ^{15}N	Large males	12 months caged	19	14.34	35.33	<0.0005
		6 months caged	20	13.15		
		free	101	13.01		
	Pygmy males	12 months caged	20	13.37	4.51	0.105
		6 months caged	21	12.97		
		free	100	12.96		
	Mature females	12 months caged	20	13.36	25.27	<0.0005
		6 months caged	20	13.52		
		free	94	13.11		

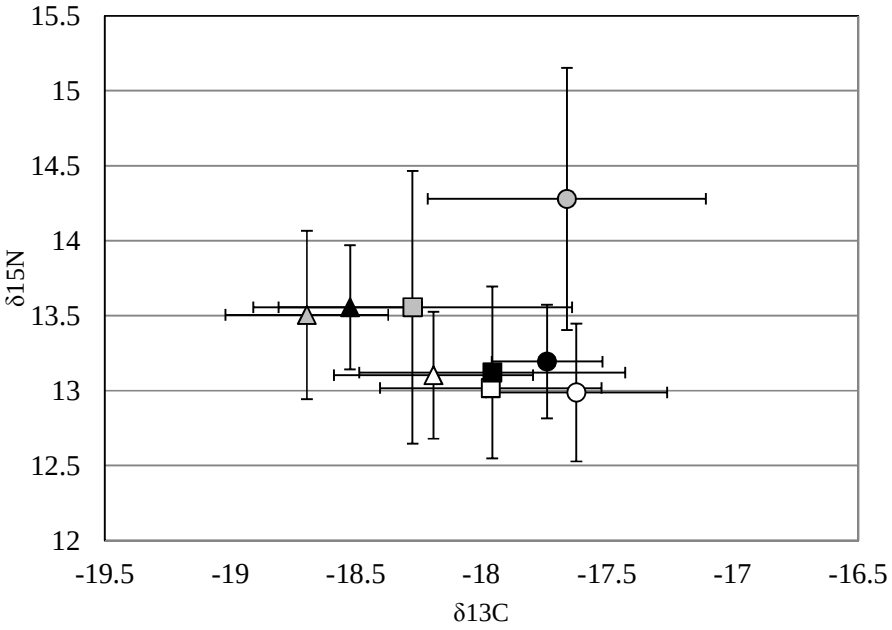

Figure 8: Mean values (± 1SD) of δ^{13}C versus δ^{15}N for large males (circles), pygmy males (squares) and mature females (triangles) by sampling treatment (free (white); 6 months-caged (black) and 12 month-caged (grey)).

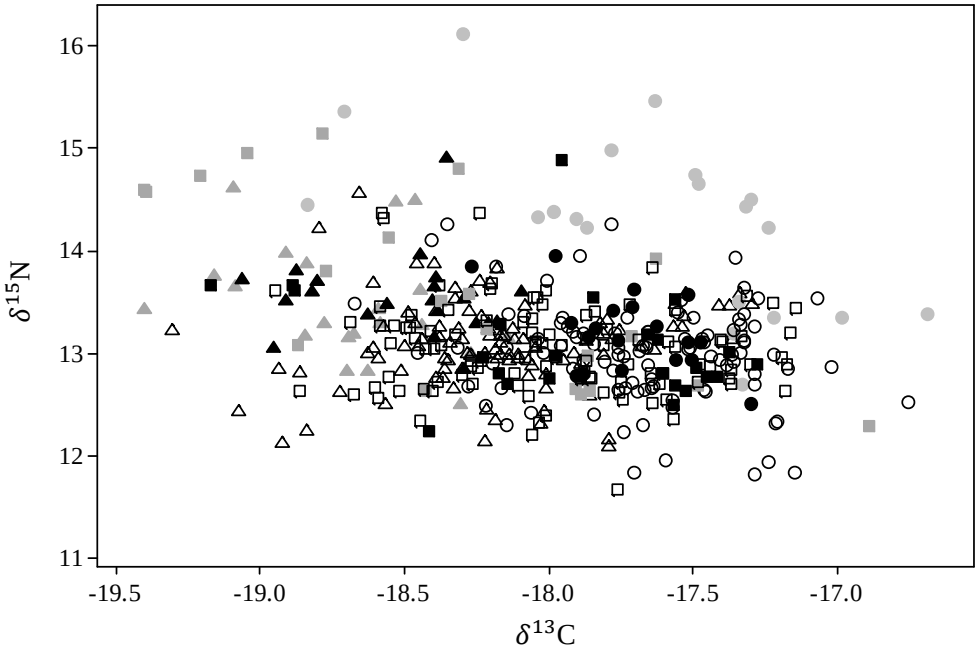

Figure 9. Scatterplot showing values of δ^{13}C versus δ^{15}N for large males (circles), pygmy males (squares) and mature females (triangles) by sampling treatment (free (white); 6 month-caged (black) and 12 month-caged (grey)).

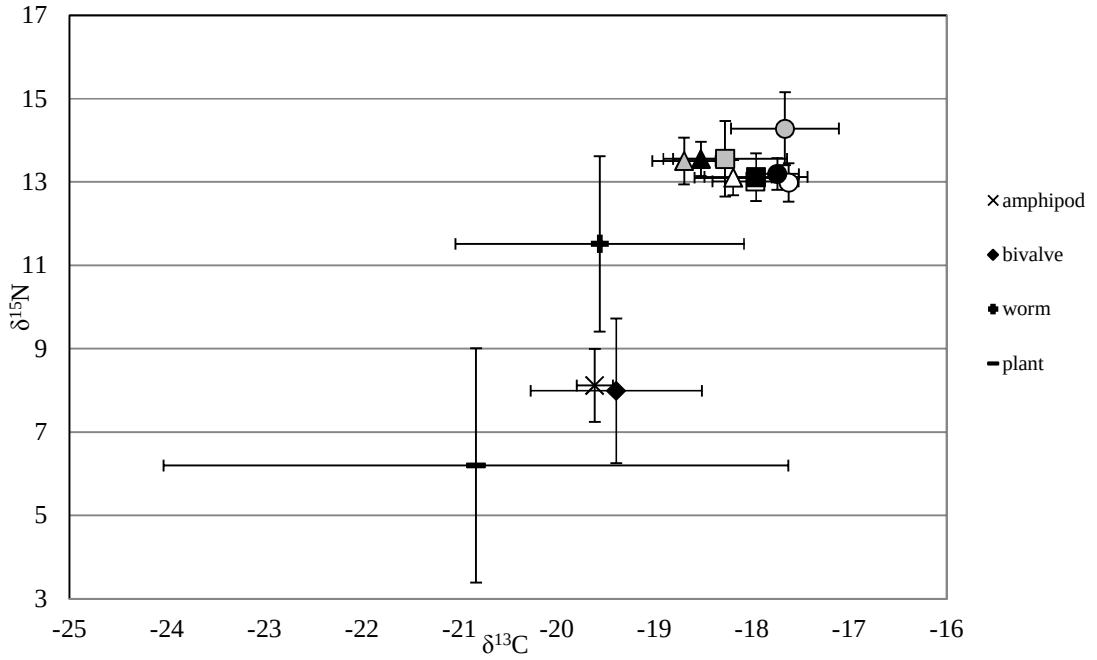

Figure 10. Mean (± 1SD) of δ^{13}C versus δ^{15}N for large males (circles), pygmy males (squares) and mature females (triangles) by sampling treatment (free (white); 6 month-caged (black) and 12 month-caged (grey)) as well as potential prey items. * Values were not corrected for the trophic discrimination factors of 0.4 per mil and 3.4 per mil for C and N, respectively (Post, 2002).

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V-3. DISCUSSION

V-3-1 Stomach content analysis

Very few crabs collected for this study had stomachs that were full (in terms of stomach fullness and total content weight) regardless of sampling area, season or crab category. Stomach fullness was usually similar among crab categories for any given sampling season and area. Furthermore, stomach content dry weights were less than 10mg for almost half of our samples, suggesting that our sampling method by trapping may be biased toward hungry crab. Results from one study in 2003 collected snow crabs by trawling and wet contents weights were generally much higher than results from this current study for both males and females of determined size (Squires & Dawe 2003). The only occasions where results of this present study showed comparable wet content weights was when fish was the dominant prey item (see free large males from fall 2012 samples and free large males from spring 2013 in Grande-Rivière). Free crabs caught by trapping may also hide any seasonal feeding patterns. Although stomachs were the most full (in terms of fullness and weight) in the fall 2012 and most empty in the spring 2012 samples, these observations were not repeated in 2013.

This study also showed the importance of examining both frequency of occurrences and biomass percentages of prey items. Dense, calcareous prey items (i.e. crab, crustaceans) that are difficult to digest can be overrepresented and overshadow lighter prey items (i.e., eggs, polychaetes) in biomass percentages as seen in this study. Furthermore, the regular presence of detritus also suggest that ingested items could not be identified due to their small size or advanced digestion and softer diet components may be significantly underestimated.

At first glance, results of this study suggest that for the majority of caged animals, stomach contents do not seem to be affected by the length of the caging period. However, content weights were initially low and remained low for all caged crab categories (and both areas) with the exception of females in Cheticamp caged for 6 months. These females had the highest median content weight values; interestingly, most of that weight was contributed by crab prey items. More specifically, one cage contained only the remaining carapace of a tagged female, suggesting a recent mortality and possibly an easy meal for other females inside that cage. For large males, the decreasing values of median stomach content weight with increasing caging period at both sites suggest that caging may have a more deleterious effect on larger crab. More specifically, the 1.5 inch wire mesh cages may restrict the ability of larger crabs to use their claws to catch prey

outside the cage, further limiting their access to food. Consequently, as larger crabs have higher energetic demands, they may have greater difficulty in maintaining metabolic homeostasis when caged.

The most common identified prey groups (polychaetes, fish, crab, crustacean (non-crab) and mollusk) observed in this study are in accordance to previous snow crab studies (Lovrich & Sainte-Marie 1997; Squires & Dawe 2003). While ophiuroidae was not a common prey item observed in stomachs of this present study, snow crab stomach analyses from the northern Bering Sea found this taxon group to be a dominant prey item while fish was rarely observed (Kolts *et al.* 2013a). The majority of crabs collected in the Bering Sea were less than 60 mm CW. Furthermore, Lovrich and Sainte-Marie (1997) found prey groups "echinoderms" and "mollusk" were less frequent in larger crabs (> 60 mm CW) and tended to be replaced by fish and crustaceans prey items. Although smaller crabs (< 60 mm CW) were not collected in this study, these results suggest that the selection of prey items may be dependent on predator size.

In terms of the frequency of occurrence of a given prey group, some variations were observed (i.e. more fish in fall 2012 samples in several crab categories) but a seasonal or regional effect was not detected. More specifically, an increase in occurrence was observed in "man-made" and "other" prey categories in caged crab. Man-made items may have been more available in cages with more than one crab as ingested tag parts from other crab occupying the cage or dead crab may explain this increase in caged mature females and pygmy males. Nonetheless, the increase in these types of prey further suggests that caged crab may have been experiencing an undetermined level of nutritional stress.

The presence of polychaetes in a lot of crab stomachs suggests that this prey item is readily available to most crab. A reduction in frequency of polychaete prey was observed in caged crab compared to free crab and this reduction was more pronounced in large males than females or pygmy males. Furthermore, frequency of occurrences for crustaceans as prey items slightly dropped in caged crabs for all categories, but the reduction was larger in females and pygmy males. As such, the effects of caging in terms of prey availability may be dependent on prey type and crab size. Kolts *et al.* (2013a) and Lovrich and Sainte-Marie (1997) did not find any differences in diets between male and female snow crab of similar size but did find differences with increasing crab size (larger crab consumed larger, harder preys that require greater claw strength while smaller, juvenile crab focused on softer, easier to handle preys).

Baited traps may also have an indirect effect on stomach contents by attracting other possible prey species. Fish was a predominant prey item but was more often identified in free animals compared to caged animals for most crab categories. Interestingly, the majority of fish discovered in stomachs were cartilaginous. This cartilaginous fish may be a type of snail fish, a slow moving fish that could easily be attracted by bait and caught and ingested by free crabs. Lumpfish, also known for their poor swimming ability and slow speed, have also previously been observed in stomachs of snow crab (Squires & Dawe 2003).

Frequency of occurrences of crabs as prey items were similar in both free and caged crab for all three categories, suggesting caging does not increase cannibalism, at least not for mature females and pygmy males of similar size. Cannibalism has been documented in free snow crab populations but mostly between different size classes and is often density dependent (Lovrich & Sainte-Marie 1997).

In summary, the majority of crabs, regardless of the sampling method used, had little in their stomach which suggest that our samples may have been biased toward hungry free crab. While frequency of occurrences, stomach fullness and content weights provide different insights on the feeding habits of snow crab, determining the importance and dominance of each prey category remains unclear. Furthermore, prey abundance was not estimated in this study and could have helped to explain some variability observed in prey categories during selected sampling periods and areas. Additionally, certain prey categories were more often found in caged crab suggesting caging may alter prey selectivity and induce an unknown level of nutritional stress. The effects of caging may be even more pronounced on larger crab as they have higher energetic requirements and are more limited in terms of accessibility and catchability of prey due to their size.

V-3-2 Stable isotope analysis

The main objective of applying stable isotope analyses on snow crab muscle samples for this study was to compare these results with those obtained from stomach content analyses. While stomach content analyses document feeding habits on a short-term scale, measurements of naturally occurring stable isotopes of nitrogen and carbon can provide dietary patterns and trophic relationships integrated over a period of time. Stable isotope analysis can also help alleviate the challenge of stomachs containing little content or unidentifiable prey items. In this study, since important prey items such as fish were not collected, it is difficult to accurately quantify the importance of each prey item by stable isotope analysis. Nonetheless, a general view of snow crab isotopic composition was

obtained and possible differences among crab categories and sampling method were observed.

$\delta^{13}C$

Carbon isotope composition of a consumer provides information of the source of carbon. Although negligible differences occur between trophic levels for δ^{13}C, the ratio of the stable carbon isotope 13C, to the more common 12C, is used to discriminate the degree to which organisms are relying on pelagic and benthic based food sources (primary producers such as phytoplankton and microphytobenthos) within the foodweb (Dennard *et al.* 2009). Values of δ^{13}C for snow crab were within the range of potential food sources and were comparable to current literature (Kolts *et al.* 2013b). Females were significantly depleted in δ^{13}C compared to males (both large and pygmy) regardless of sampling method, and values of δ^{13}C in pygmy males usually fell between females and large males. Even though the minimum of 0.4 to 1‰ in δ^{13}C values was small, the differences between males and females were consistent and are likely due to size differences. Bodin *et al.* (2007) found higher δ^{13}C values in older/larger crab compared to juvenile/smaller Maja brachydactyla suggesting a change in feeding habit. As previous work on stomach content analyses have documented differences between size classes (Lovrich & Sainte-Marie 1997, Squires & Dawe 2003), it would be interesting to apply stable isotope analysis on smaller/immature snow crab.

δ15N

δ^{15}N data have been used to delineate trophic structure in a food web since δ^{15}N in tissues of consumers typically increases by 3 to 3.4‰ relative to their prey (Owens 1987, Peterson & Fry 1987, Post 2002). In this present study, a 3‰ shift in trophic level was not observed regardless of crab category and sampling method. A trophic level shift with body size in *Maja brachdactyla* was also not observed by Bodin *et al.* (2007). Nonetheless, the significantly higher δ^{15}N values noted among 6 month caged females and 12 month large mature males may suggest that these crab categories may be experiencing a shift in diet composition or physiological stress. Physiological stress, such as limited food availability, has been documented to increase δ^{15}N values in sunfish, planarians and beetles (Colborne & Robinson 2013, Boag *et al.* 2006, Scrimgeour *et al.* 1995, respectively). The gradual increase in δ^{15}N observed among wild, 6 month caged and 12 month caged large mature males suggests that long term caging may have more deleterious effects on larger crab. Caging may not meet the energetic requirements of large mature males and thus may be more detrimental for this category of crab. Interestingly, nutritional stress may not explain the higher values of

δ^{15}N observed in females caged for 6 months as stomach contents for this category were fuller than other female crab categories. One possible explanation is the type of prey ingested. Females caged for 6 months had high levels of crab in their stomachs which could result in an increase in δ^{15}N values as they are eating from a higher trophic level (i.e. cannibalism).
In summary, stable isotope analysis, in combination with stomach content analysis, provide relevant information on taxonomic importance and trophic position of snow crab. The diet of snow crab, in terms of stomach content and stable isotope analysis, is composed of a wide variety of taxa and shows possible size differences and caging effects. Crab sampling by trapping may be biased towards hungry crab and other sampling methods (such as trawling) should be considered in future studies. Additionally, as previous stomach content studies suggest that differences in diet may be dependent on the size of the crab, especially between small/immature crab and large/mature crab, a wider range of crab size should be sampled. Seasonal and site-specific patterns were not consistently observed but differences could be attributed to prey abundance during sampling and should be examined in future studies. Values of δ^{13}C suggest a relative change in primary food source between males and females that is related to crab size. Comparable values of δ^{15}N for wild crab categories suggest large mature males and females share the same trophic level. Higher values of δ^{15}N observed in large mature males caged for 12 months and females caged for 6 months may suggest a nutritional stress and dietary shift in these crab categories, respectively.

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VI. LARVAL MORPHOMETRICS

VI-1. SUMMARY

In 2003, a study was conducted to determine if there were any effects of seismic energy on snow crab (*Chionoecetes opilio*) during a commercial seismic survey. Results of the larval component of this study showed a significant delay in hatch, differences in proportions of pre-zoea and zoea 1 and smaller zoea 1 from females caged at the seismic site compared to those caged at a control site. However, it was not possible to determine if these differences resulted from exposure to seismic energy, differences in temperatures at the caging sites, slightly smaller females in the seismic group or some other difference between caging sites. To increase our knowledge of natural variability within snow crab populations and to better assess and validate results of future studies, the present study reports morphometric data from snow crab zoeal stages sampled in the southern Gulf of St. Lawrence. In June 2012 and 2013, a total of 746 *C. opilio* zoea stage 1 and 2 were collected offshore of the Margaree and Cheticamp areas, west of Cape Breton Island. Attempts to collect zoea 2 in August were unsuccessful as only the megalopa stage was found at this time of year. Zoeae I from Margaree were larger than those sampled off Cheticamp and this difference was significant for 2013 but not 2012 samples. Sizes did not differ significantly between 2012 and 2013. Sufficient numbers of zoea stage 2 for analysis were only obtained in 2012 which revealed no significant difference in size between sites sampled. Only one zoea exhibited a morphological abnormality which was a dual dorsal spine in a zoea 1 larva. Results of the present study show that significant differences in morphometric features of zoea I can be observed in samples separated by as little as 30 km. Therefore, differences reported in the 2003 seismic study could have been related to localised conditions of the caging sites separated by 35-41 km rather than differential exposure to seismic energy.

VI-2. INTRODUCTION

Early life stages are often most sensitive to anthropogenic insult (Weis & Weis 1989). With very little information known of potential impacts of seismic energy on the early life stages of snow crab, an experimental seismic survey was funded in 2002 by the Environmental Studies Research Fund (ESRF) to assess potential impacts of seismic energy on snow crab (Christian *et al.* 2003). Results of this preliminary study showed delayed development in eggs from a single female exposed to high levels of seismic sound (221 dB) at a very close range of 2 m (Christian *et al.* 2003). To further investigate this potential impact, a study was conducted in 2003 off western Cape Breton, Nova Scotia in conjunction with a seismic exploration program led by Corridor Resources Inc.

Embryos hatched in the laboratory from females that had been caged in the ensonified area hatched later than embryos of females caged in an unensonified reference area (DFO 2004, Courtenay *et al.* 2009, Boudreau *et al.* 2009). Furthermore, more embryos and pre-zoea were released from seismic-exposed females compared to a greater proportion of zoea stage 1 from control females. Stage 1 zoeae released from seismic-exposed females were also slightly smaller, in terms of their abdominal length, and they had shorter dorsal, rostral and lateral spines and eyes relative to abdominal length. However, it was not possible to determine if these differences were due to exposure to seismic energy or other factors such as temperature at the caging site or slightly smaller size of females in the seismic exposed group (2% by carapace or abdomen width or 7% by weight).
Because of confounding factors in the 2003 seismic study, the assessment of potential impacts of exposure to seismic energy to snow crab was problematic as observed impacts could be related to manipulations during the study, environmental conditions or the natural states of these crabs. Hence, the overall purpose of the present study was to establish normal characteristics of snow crab populations to better assess and validate results of future studies. Specifically, the objective of the larval component of the present study was to collect morphometric data and monitor the incidence of naturally occurring morphological abnormalities in the two zoeal stages of the larval development of *C. opilio* from plankton tows collected in two areas off the west coast of Cape-Breton, NS, Margaree and Cheticamp. Sampling occurred in June and August of 2012 and 2013 at water depths ranging from 0 to 35 m. As such, this study compared the natural interannual and geographical variability in morphometric features and morphological abnormalities of the zoeal stages of *C. opilio*.

VI-3. LARVAL SAMPLING

Snow crab larval sampling was conducted close to stations W1 and W2 off western Cape Breton in the spring and fall of 2012 and 2013. A standard plankton net (diameter: 75 cm; mesh size: 1000 microns Nitex, Filmar Inc, Bic, QC, Canada) attached to a V-Fin towed at an average speed of 1.5 knots for 5 minutes was used with a towing cable length ratio of 1:3 (Figure 1A,B). Towing depths ranged between 0 and 35 meters while the number of tows for each sampling date ranged between 5 and 11 (Tables 1 & 2). A VEMCO minilog was attached to the wing net to measure water temperature during most tows (Figure 2). After each tow, the net was rinsed with sea water and plankton was collected with a cod end jar. Samples were then transferred into 1L mason jars and preserved in a 50-50% formalin-seawater solution with a layer of marble chips covering the bottom of each jar. All collected plankton samples were then brought to the laboratory for sorting,

identification and measurements. Plankton abundance estimates and vertical distribution within the water column were not examined in this present study.

In 2012, a total of 5 tows and 9 tows ranging between 5 and 10 meters in depth were performed off Cheticamp and off Margaree Harbor on June 19th, respectively (Table 1).

Off Cheticamp the first plankton towing was started at 07h43 at the position 46˚49.951'N/61˚06.086W and the last tow at 08h28 at the position 46˚48.072'N/61˚05.921'W. The water depths of the plankton sampling area off Cheticamp varied between 84 m and 91m. Off Margaree Harbor, the first plankton towing was started at 10h53 at the position 46˚32.421'N/61˚18.066W and the last tow at 12h01 at the position 46˚30.277'N/61˚19.557'W. The water depths of the plankton sampling area off Margaree Harbor varied between 61 m and 68 m.

A total of 17 tows ranging between 2 and 30 meters in depth were performed off Cheticamp on August 24th and 25th, 2012 (Table 2). Due to the lack of larval samples collected from the Cheticamp area at that time, sample tows from the Margaree area were postponed until spring/summer 2013.

In the spring of 2013 (June 19), a total of 10 and 6 tows at water depths ranging between 5 and 20 meters were completed off Cheticamp and Margaree, respectively (Table 1).

Off Cheticamp the first plankton towing was started at 13h15 at the position 46˚48.061'N/61˚05.666W and the last tow at 15h22 at the position 46˚50.083'N/61˚03.975'W. The water depths of the plankton sampling area off Cheticamp varied between 85 m and 90m. Off Margaree Harbor, the first plankton towing was started at 08h55 at the position 46˚30.322'N/61˚19.589W and the last tow at 10h08 at the position 46˚31.161'N/61˚19.189'W. The water depths of the plankton sampling area off Margaree Harbor varied between 64 m and 65 m.

On September 1st, 2013, a total of 10 tows at towing depths ranging between 15 and 35 meters were completed at both sampling sites (Table 2). Off Margaree Harbor the first plankton towing was started at 08h09 at the position 46˚30.343'N/61˚19.354W and the last tow at 11h29 at the position 46˚29.314'N/61˚19.870'W. The water depths of the plankton sampling area off Margaree Harbor varied between 59 and 63 m. Off Cheticamp, the first plankton tow started at 13h47 at the position 46˚48.237'N/61˚05.694W and the last tow was completed at 15h49 at the position 46˚45.720'N/61˚06.110'W. Water depths for plankton sampling off Cheticmap varied between 89 and 131m.

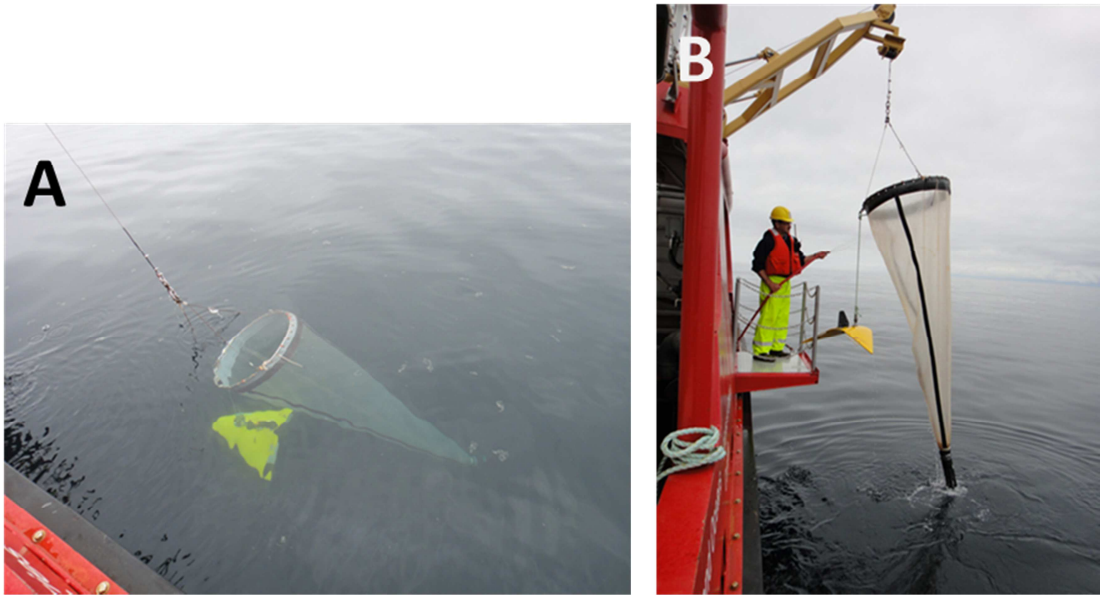

Figure 1. Picture of plankton sampling off Margaree Harbour (W1) conducted on September 1st, 2013 (A: Plankton net and V-fin in the water, B: at the end of a tow showing V-fin, plankton net and a cod end jar).

Table 1. Summary of 2012 and 2013 spring snow crab larval sampling in Cheticamp and Margaree, NS.

Date	Station	Tow	Tow depth	Start position		Depth (start)	Time	End position		Time	Depth (end)
June 19, 2012	Cheticamp	1	5-10m	46°48.951N	61°06.086W	84.0m	07:43	46°48.957N	61°06.312W	07:48	83.5m
		2	5-10m	46°48.923N	61°06.035W	84.5m	07:59	46°48.789N	61°05.922W	08:04	85.5m
		3	5-10m	46°48.615N	61°05.879W	86.5m	08:10	46°48.474N	61°05.965W	08:15	87.0m
		4	5-10m	46°48.316N	61°05.913W	88.0m	08:20	46°48.183N	61°05.911W	08:25	89.5m
		5	5-10m	46°48.072N	61°05.921W	91.0m	08:28	46°47.906N	61°05.944W	08:33	93.0m
	Margaree	1	5-10m	46°32.412N	61°18.066W	68.4m	10:53	46°32.257N	61°18.200W	10:58	67.6m
		2	5-10m	46°32.112N	61°18.311W	65.6m	11:02	46°31.959N	61°18.429W	11:07	62.0m
		3	5-10m	46°31.859N	61°18.496W	63.6m	11:10	46°31.706N	61°18.618W	11:15	65.2m
		4	5-10m	46°31.486N	61°18.775W	63.6m	11:21	46°31.341N	61°18.884W	11:26	63.2m
		5	5-10m	46°31.249N	61°18.943W	63.2m	11:29	46°31.107N	61°19.046W	11:34	63.6m
		6	5-10m	46°31.011N	61°19.110W	63.6m	11:37	46°30.867N	61°19.218W	11:42	64.0m
		7	5-10m	46°30.764N	61°19.280W	63.6m	11:45	46°30.606N	61°19.378W	11:50	64.4m
		8	5-10m	46°30.519N	61°19.420W	64.4m	11:53	46°30.364N	61°19.513W	11:58	62.8m
		9	5-10m	46°30.277N	61°19.557W	61.6m	12:01	46°30.113N	61°19.656W	12:06	60.8m
June 19, 2013	Cheticamp	1	5	46°48.061N	61°05.666W	90.00m	13:15				
		2	5	46°48.455N	61°05.590W	89.86m	13:25				
		3	10	46°48.663N	61°05.410W	89.23m	13:38				
		4	10	46°48.894N	61°05.412W	88.03m	13:47				
		5	15	46°49.318N	61°05.408W	85.64m	14:15				
		6	15	46°49.454N	61°05.374W	85.28m	14:28				
		7	15	46°49.655N	61°05.230W	85.64m	14:40				
		8	20	46°49.986N	61°04.430W	88.39m	15:10				
		9	20	46°50.040N	61°04.135W	88.86m	15:22				
		10	20	46°50.083N	61°03.975W	89.85m	15:34				
	Margaree	1	0	46°30.322N	61°19.589W	64.4m	08:55				
		2	5	46°30.632N	61°19.459W	64.5m	09:10				
		3	10	46°30.728N	61°19.379W	65.3m	09:20				
		4	15	46°31.040N	61°19.321W	65.1m	09:45				
		5	15	46°31.078N	61°19.240W	64.9m	09:55				
		6	20	46°31.161N	61°19.189W	65.1m	10:08				

Table 2. Summary of 2012 and 2013 fall snow crab larval sampling in Cheticamp and Margaree, NS.

Date	Station	Tow	Tow depth	Start position		Depth (start)	Time	End position		Time	Depth (end)
Aug 24, 2012	Cheticamp	1	5-10m	46°48.951N	61°06.086W	84.0m	07:43	46°48.957N	61°06.312W	07:48	
		2									
		3									
		4									
		5									
		6									
		7									
		8									
		9									
		10									
		11									
		12									
		13									
		14									
		15									
		16									
		17									
Sept 01, 2013	Cheticamp	1	15	46°48.237N	61°05.694W	89.0m	13:42	46°48.036N	61°05.673W	13:47	91.6m
		2	15	46°48.124N	61°05.801W	90.0m	13:50	46°47.901N	61°05.924W	13:55	91.0m
		3	20	46°47.873N	61°05.809W	92.6m	14:02	46°47.677N	61°05.758W	14:12	97.7m
		4	20	46°47.548N	61°05.808W	100.0m	14:15	46°47.437N	61°05.862W	14:20	103.0m
		5	25	46°47.290N	61°05.765W	112.0m	14:34	46°47.111N	61°05.760W	14:39	118.0m
		6	25	46°46.962N	61°05.824W	120.0m	14:43	46°46.786N	61°05.860W	14:48	125.0m
		7	30	46°46.733N	61°05.840W	127.0m	15:03	46°46.540N	61°05.946W	15:08	129.0m
		8	30	46°46.443N	61°06.031W	128.0m	15:13	46°46.267N	61°06.105W	15:18	130.0m
		9	35	46°46.180N	61°05.966W	131.0m	15:33	46°45.991N	61°05.932W	15:38	123.0m
		10	35	46°45.881N	61°05.997W	122.0m	15:44	46°45.720N	61°06.110W	15:49	120.0m
	Margaree	1	15	46°30.343N	61°19.354W	62.4m	08:09	46°30.172N	61°19.367W	08:14	59.4m
		2	15	46°30.159N	61°19.474W	59.1m	08:19	46°29.047N	61°19.581W	08:23	59.3m
		3	20	46°30.042N	61°19.610W	59.7m	08:37	46°29.893N	61°19.674W	08:42	61.5m
		4	20	46°30.099N	61°19.735W	59.9m	09:50	46°29.987N	61°19.906W	09:55	61.4m
		5	25	46°30.184N	61°19.774W	59.3m	10:11	46°30.041N	61°19.866W	10:16	61.2m
		6	25	46°29.966N	61°19.911W	62.2m	10:22	46°29.830N	61°19.918W	10:27	63.2m
		7	30	46°29.910N	61°19.832W	62.4m	10:41	46°29.746N	61°19.867W	10:46	61.9m
		8	30	46°29.715N	61°19.925W	61.8m	10:52	46°29.574N	61°20.021W	10:57	60.7m
		9	35	46°29.687N	61°19.802W	61.1m	11:12	46°29.524N	61°19.918W	11:17	59.9m
		10	35	46°29.546N	61°19.855W	60.4m	11:24	46°29.314N	61°19.918W	11:29	59.8m

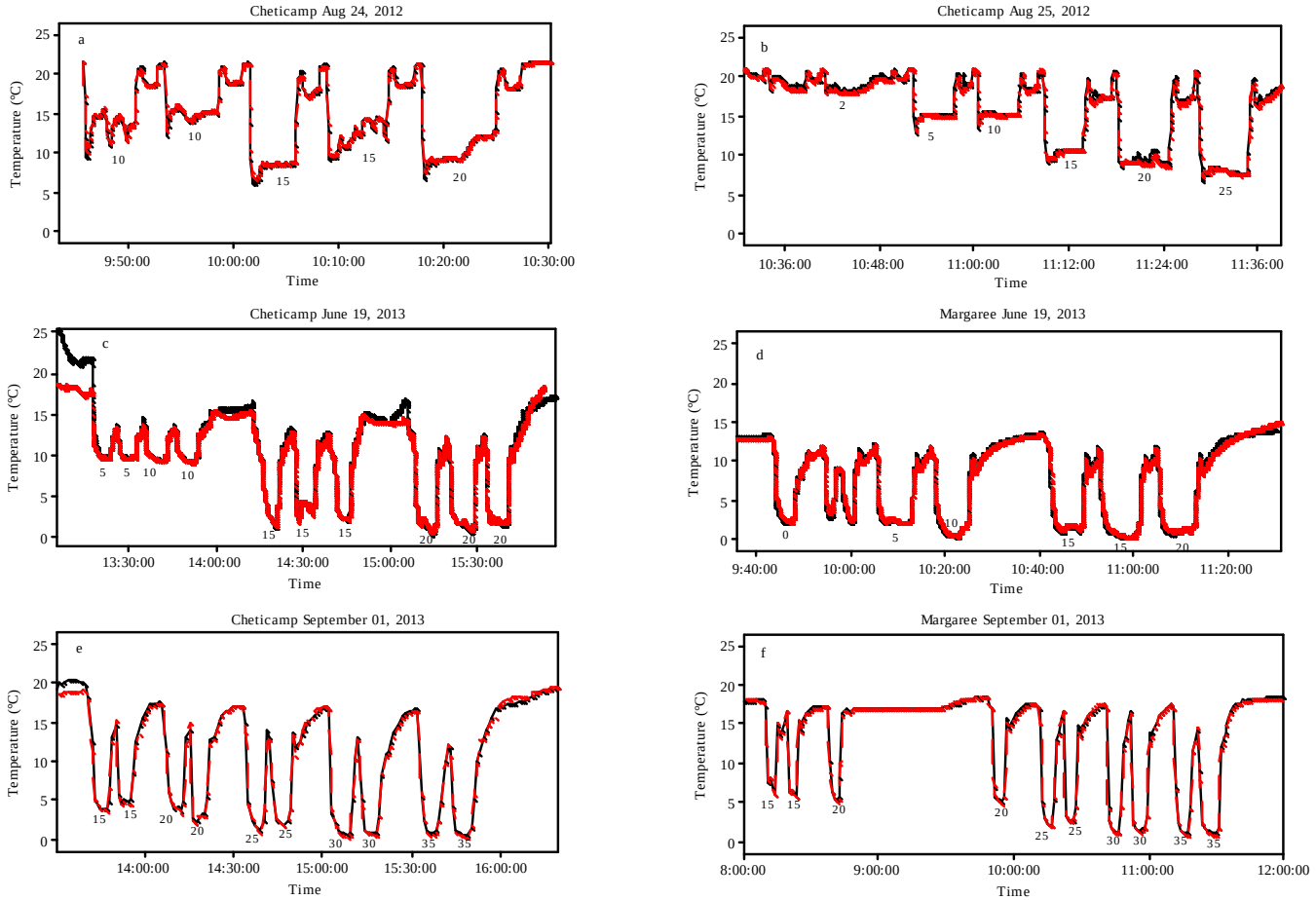

Figure 2. Water temperature recordings at varying depths during snow crab larval sampling in Cheticamp (a, b, c, e) and Margaree (d, f), NS in 2012 and 2013. For every 5 minute tow, water depth (in meters) is indicated in each valley. Two Vemco temperature probes were attached for each tow (black and red lines). Valleys without water depths were incomplete tows.

VI-4. LARVAL MORPHOMETRIC OBSERVATIONS

VI-4-1 Materials and Methods

Plankton samples collected by plankton net towing at Margaree Harbor and Cheticamp stations (Section VI-3) were brought back to the Gulf Fisheries Center in Moncton, NB were initially preserved in 5% buffered formalin, and then transferred to 70% ethanol within a month of their arrival. Whole samples were sorted by placing the contents in a large glass Pyrex pan (38 x 27 x 5 cm) and removing snow crab pre-zoea, zoea I, zoea II and megalopa as well as zoea I and zoea II stages of toadcrabs (*Hyas araneus*, *Hyas coarctatus alutaceus* and *Hyas coarctatus coarctatus*) which are very similar to snow crab (Davidson & Chin 1991, Pohle 1991). Once isolated, larval stages from each individual sample were transferred to separate 20 ml scintillation vials and kept in 70% ethanol.
To differentiate and isolate the proper zoeal stages and exclude all *Hyas* spp. from our samples, stage I and stage II zoeae (for all species) were separated and all pre-zoeae and megalopae were removed. Very few pre-zoea were found in our samples, not surprisingly as this larval stage only lasts approximately one hour (Pohle 1991). It was easily distinguished from zoea 1 and 2 as the spines on the cephalothorax and appendages are not developed. The megalopa were also easily distinguished from the zoeal stages as there are no lateral spines on the cephalothorax and the dorsal and rostral spines are much shorter than in the zoeal stages. Megalopa stage, larvae now resembles a small crab (Pohle 1991). The main feature utilised to differentiate between the zoeal stages was the presence of pleopod buds on somites 2-5 of the abdomen in the second zoeal stage (Figure 1(3); Davidson & Chin 1991). This feature was the most evident to distinguish between stages, the presence of the endopodite on the antennae was used to confirm the second zoeal stage (Davidson & Chin, 1991) if there was any uncertainty.

Once the zoeal stages were separated, the identification of *C. opilio* and *Hyas* spp. was undertaken. The separation between these groups proved problematic; as such a combination of three features was used to make this distinction (Table 3; Roff *et al.* 1984, Lanteigne 1985, Davidson & Chin 1991, Pohle 1991).

Table 3. Morphological features utilised to differentiate between *C. opilio* and *Hyas* spp. for both zoeal stages.

Chionoecetes opilio	*Hyas* spp.
Abdomen: The length of the spines on somites 3 and 4 extend as long as or longer than the next somite in zoea 1 and this is also true for somite 5 in zoea II.	Abdomen: The length of the spines on somites 3 and 4 (and 5 for zoea stage II) do not extend the length of the next somite.
Cephalothorax: Lateral spines are longer in proportion to the carapace which results in the width of the zoea (Figure 3) corresponding to approximately 1/2 of the rostrodorsal length. This criterion was used for zoea 1 only.	Cephalothorax: Lateral spines are shorter than *C. opilio* which results in the width of the zoea (Figure 3) corresponding to approximately 1/3 of the rostrodorsal length. This criterion was used for zoea 1 only.
Rostrodorsal length: zoea I $\geq$ 3.7 mm	Rostrodorsal length: zoea I < 3.7 mm

All observations for the identification of developmental stages and species, collection of morphometric data and identification of morphological abnormalities were done with a computer-based image analysis system (uScope PixeLINK, version 3.6, PixeLINK, Ottawa, ON, Canada) linked to a microscope (Leitz, Wild Photomakroskop M400, Leica Microsystems, Willowdale, ON, Canada) (16x to 90x) through a video camera (PixeLINK USB 2.0 camera, PixeLINK, Ottawa, ON, Canada). To facilitate observations and to ensure the proper positioning of the zoea during the morphological measurements, the zoeae were placed in glycerol.

Morphological characters for both zoeal stages included (Figure 3):

- Dorsal spine length
- Rostral spine length
- Rostrodorsal length (tip of dorsal spine to tip of rostral spine)
- Left lateral spine length
- Right lateral spine length
- Body width (tip of left lateral spine to tip of right lateral spine)
- Left eye diameter
- Right eye diameter
- Abdominal somite 6 length (stage 2 only)
- Abdominal somite 5 length
- Abdominal somite 4 length
- Abdominal somite 3 length
- Abdominal somite 2 length

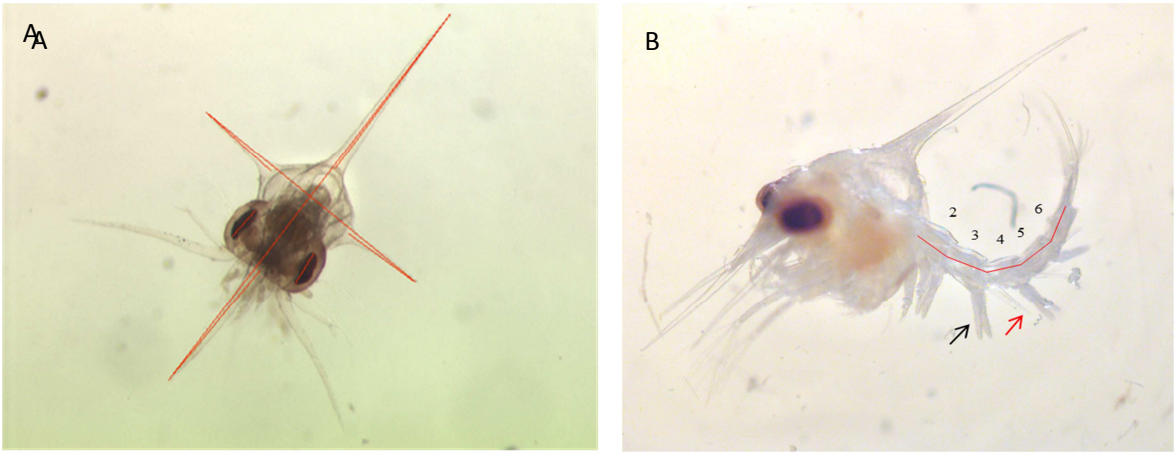

Figure 3. Morphometric data collected from zoeal stages I and II of *C. opilio* for frontal (A) and side views (B). Measurements included in frontal view (A): rostrodorsal length (tip of dorsal spine to tip of rostral spine), body width (tip of right lateral spine to tip of left lateral spine), dorsal spine, rostral spine, left lateral spine, right lateral spine, left eye diameter and side view (B) abdomen length which is the sum of the length of somites 2 to 6 (identified in picture) for zoea II as shown in figure or somites 2 to 5 for zoea I. In panel B, black arrow indicates pleopod buds and red arrow indicates lateral spines of the abdominal somites. Panel A is reprinted from Courtenay *et al.* (unpublished) and panel B was taken during the present study.

In the present study, zoea I and zoea II were selected to collect morphometric data and monitor morphological abnormalities. In the 2003 study, these data were collected for zoea I only as the zoeae were preserved after hatch and not reared to reach the second zoeal stage. The same morphometric data as were collected during the 2003 seismic study were collected for both zoeal stages in the present study with the following exceptions. The diameter of both eyes was measured in the frontal view but the additional measurement for one of the eyes from a side view, as was done in the 2003 seismic study, was not done in the present study. Somite 6 was included in the measurements of the abdominal sections for zoea II. This somite only appears in the second zoeal stage. In the 2003 seismic study, an erroneous measurement was taken and reported as somite 6 in the first zoeal stage. For both the 2003 seismic study and the present study, somite 1 was not measured because it is usually located under the cephalothorax and therefore not visible. The position utilised to take the different measurements were not pre-defined as in the 2003 study. The spines on the cephalothorax (dorsal, rostral and lateral spines) were often bent which would result in underestimating their length if the measurements were taken facing up. Therefore, the zoeae were observed from all angles to ensure that the best angle was selected for the measurements. In the present study, only 746 zoeae were collected (for both

years, locations and zoeal stages). Hence measurements were taken from all zoeae for body parts that were not damaged in order to gather as much information as possible rather than selecting zoeae with no breaks or severe bends as in the 2003 study.

While being measured, each larva was observed for morphological abnormalities present in the eyes, spines, telson and appendages. Any abnormalities, breaks and underdevelopment in the spines were noted.

VI-4-2 Statistical analysis

Morphometric data were compared between sampling locations (Cheticamp and Margaree) and years (2012 and 2013) by multivariate analyses (PERMANOVA) in individual analysis for zoea I and zoea II following methods described by Anderson *et al.* (2008) and Clarke *et al.* (2014). The multivariate analyses, which included all measures in a single analysis, enable a comparison of the zoeae as whole organisms rather than conducting individual analyses for each section of the zoea. Water depth was not recorded in 2012 and insufficient numbers of zoeae were collected per depth in 2013 which precluded this factor from being included in the analyses. No transformation or normalisation of the data was conducted prior to generating the Euclidean distance resemblance matrices. A two-way crossed factor PERMANOVA was utilised for zoea I (location and year as factors) and a one-way PERMANOVA testing the effect of location was utilised for zoea II as insufficient zoea II (n=4 at Margaree and none at Cheticamp) were collected in 2013. When a significant interaction was observed in the two-way PERMANOVA, individual one-way PERMANOVAs were utilised to test effects of individual factors. PERMDISP, which is used to test for homogeneity of multivariate dispersions on the basis of a resemblance measure (Anderson *et al.*, 2008), was tested prior to the analyses and indicated that the dispersions among groups were homogeneous. A 'similarity percentages routine' (SIMPER) was done following the PERMANOVA to determine the average contribution of each measurement to the overall difference between groups. As SIMPER cannot accommodate missing data, this analysis was performed on the subset of complete data. A Principal Coordinates Analysis (PCO) was used to provide a visual representation of the data. Power analyses cannot be performed on PERMANOVA analyses (personal communication, Robert Clarke, PRIMER-E Ltd, Plymouth, UK). Consequently, the most influential variable as identified by SIMPER (rostrodorsal length) was selected and Power Analyses were performed for one-way analyses with rostrodorsal length as the response variable and location as the factor for zoea stage I and II for 2012. All multivariate analyses were performed with PRIMER version 7.0 (PRIMER-E Ltd, Plymouth, UK). Power analyses were performed with SYSTAT version 13.0 (SYSTAT Software Inc., San Jose, CA, USA). The level of significance was $p<0.05$ and means are accompanied by their 95% confidence intervals.

VI-4-3 Results

Morphometric data

Because of the fragile nature of spines, especially the lateral spines, it was often not possible to obtain all morphological measures from a given specimen. In the present study, a total of 746 zoeae were collected with 56% of these having at least one break, severe bend or lateral compression of the carapace. In the 2003 study, only zoeae from which all measurements could be collected were included in the analysis as thousands of larvae were available. By comparison, only 746 zoeae were collected in the present study (total across years, locations and larval stages). Consequently, measurements were taken from all zoeae for body parts that were not damaged to gather as much information as possible.

Zoea I

Results of the two-way PERMANOVA showed that morphometric features of zoea 1 larvae varied significantly between locations but not between years. As the interaction of this analysis was significant, locations were then compared by testing each year separately. Locations differed significantly only in 2013 (Table 4) with larvae from Margaree being larger than those collected in Cheticamp. This same trend was also observed in 2012 but the difference in size between groups was not significant. However, the power of this analysis to detect a significant difference was low ($P = 0.095$, n = 47). The results of the PERMANOVAs can be visualised with the PCO plot as the distance between points (combination of location and year), is proportional to the results (p value) of these analyses (Figure 4 and Table 5). The SIMPER analysis showed that over 90% of the variability between Margaree and Cheticamp in 2013 was accounted for by three morphometric characters: rostrodorsal length (accounting for 63.1% of location dissimilarity), dorsal spine (16.3%) and rostral spine (11.4%). As reported in Table 5, the largest difference between morphometric characters was for the right lateral spine which was not identified by SIMPER as one of the main contributors to the variability between locations. The use of a subset of data, with no missing data, to perform the SIMPER analysis may be responsible for this discrepancy.

Table 4. Morphometric features of *Chionoecetes opilio* zoea stage I from plankton tows in Cheticamp and Margaree, NS on June 19th of 2012 and 2013. Included in the table are means with 95% confidence interval and sample size. Two-way crossed PERMANOVA were performed with location and year as factors. The significant interaction was explored with one-way PERMANOVAs ($p < 0.05$). Abdomen length is the sum of the lengths of abdominal somites 2-5. Rostrodorsal length is the tip of the dorsal spine to the tip of the rostral spine. Body width is the tip of the left lateral spine to the tip of the right lateral spine. Morphometric features are also presented as averages of both groups when groups were not significantly different.

ZOEA I

Two-way crossed design PERMANOVA:
Year: pseudo-$F_{1, 410} = 0.08$, $p = 0.853$
Location: pseudo-$F_{1, 410} = 6.30$, **$p = 0.004$**
Year x Location: pseudo-$F_{1, 410} = 4.58$, **$p = 0.016$**

One-way PERMANOVA: Location: pseudo-$F_{1, 316} = 3.15$, $p = 0.068$							One-way PERMANOVA: Location: pseudo-$F_{1,94} = 6.56$, **$p = 0.002$**				
	2012						2013				
	Margaree	n	Cheticamp	n	**Average for both locations**	**n**	Margaree	n	Cheticamp	n	% difference
Rostrodorsal Length	4.286 (4.193-4.379)	47	4.251 (4.224-4.278)	211	4.257 (4.230-4.285)	258	4.321 (4.272-4.370)	64	4.175 (3.967-4.382)	16	3.4
Body Width	2.280 (2.183-2.376)	29	2.136 (2.101-2.171)	128	2.163 (2.129-2.197)	157	2.256 (2.202-2.311)	35	2.141 (1.853-2.429)	5	5.1
Abdomen	1.275 (1.234-1.316)	51	1.220 (1.207-1.232)	254	1.229 (1.216-1.241)	305	1.247 (1.227-1.267)	74	1.265 (1.206-1.324)	21	-1.4
Dorsal Spine	1.805 (1.752-1.857)	51	1.801 (1.785-1.817)	227	1.802 (1.786-1.818)	278	1.841 (1.812-1.870)	67	1.796 (1.703-1.890)	17	2.4
Rostral Spine	1.476 (1.436-1.515)	51	1.496 (1.483-1.508)	241	1.492 (1.480-1.504)	292	1.514 (1.493-1.535)	72	1.473 (1.384-1.562)	17	2.7
Right Lateral Spine	0.678 (0.648-0.707)	39	0.670 (0.659-0.682)	197	0.671 (0.661-0.682)	236	0.713 (0.690-0.736)	64	0.615 (0.563-0.668)	15	13.7
Left Lateral Spine	0.679 (0.646-0.712)	45	0.659 (0.647-0.671)	206	0.663 (0.651-0.674)	251	0.689 (0.662-0.715)	58	0.696 (0.652-0.741)	17	-1.0
Right Eye Diameter	0.348 (0.340-0.355)	56	0.338 (0.334-0.342)	257	0.340 (0.336-0.343)	313	0.335 (0.328-0.341)	75	0.324 (0.308-0.340)	21	3.3
Left Eye Diameter	0.349 (0.338-0.360)	55	0.336 (0.333-0.340)	258	0.338 (0.335-0.342)	313	0.333 (0.327-0.339)	73	0.320 (0.307-0.333)	21	3.9

Table 5. Morphometric features of *Chionoecetes opilio* zoea stage II from plankton tows in Cheticamp and Margaree, NS on June 19th of 2012. An insufficient number of zoea-II were collected in 2013 to be included in the analyses. Included in the table are means with 95% confidence interval and sample size. One-way PERMANOVA was performed with location as the factor. Abdomen length is sum of lengths of abdominal somites 2-6. Rostrodorsal length is the tip of dorsal spine to the tip of the rostral spine. Body width is the tip of the left lateral spine to the tip of the right lateral spine. Morphometric features are also presented as averages for both groups as these groups were not significantly different.

ZOEA II

One-way PERMANOVA:
Location: pseudo-$F_{1, 326} = 3.19$, $p = 0.06$

	Margaree 2012	n	Cheticamp	N	**Average for both locations**	n
Rostrodorsal Length	5.995 (5.918-6.072)	254	6.170 (5.972-6.368)	38	6.018 (5.946-6.090)	292
Body Width	2.538 (2.490-2.586)	163	2.634 (2.476-2.791)	18	2.548 (2.502-2.594)	181
Abdomen	2.117 (2.085-2.150)	281	2.102 (2.023-2.181)	41	2.115 (2.085-2.146)	322
Dorsal Spine	2.567 (2.527-2.607)	264	2.626 (2.538-2.714)	39	2.575 (2.538-2.611)	303
Rostral Spine	2.020 (1.990-2.050)	272	2.112 (2.040-2.184)	41	2.032 (2.005-2.060)	313
Right Lateral Spine	0.624 (0.610-0.637)	215	0.696 (0.652-0.739)	31	0.633 (0.619-0.646)	246
Left Lateral Spine	0.612 (0.598-0.626)	229	0.644 (0.601-0.688)	31	0.616 (0.602-0.629)	260
Right Eye Diameter	0.380 (0.374-0.386)	282	0.372 (0.355-0.390)	42	0.379 (0.374-0.384)	324
Left Eye Diameter	0.378 (0.373-0.383)	275	0.370 (0.356-0.385)	42	0.377 (0.372-0.382)	317

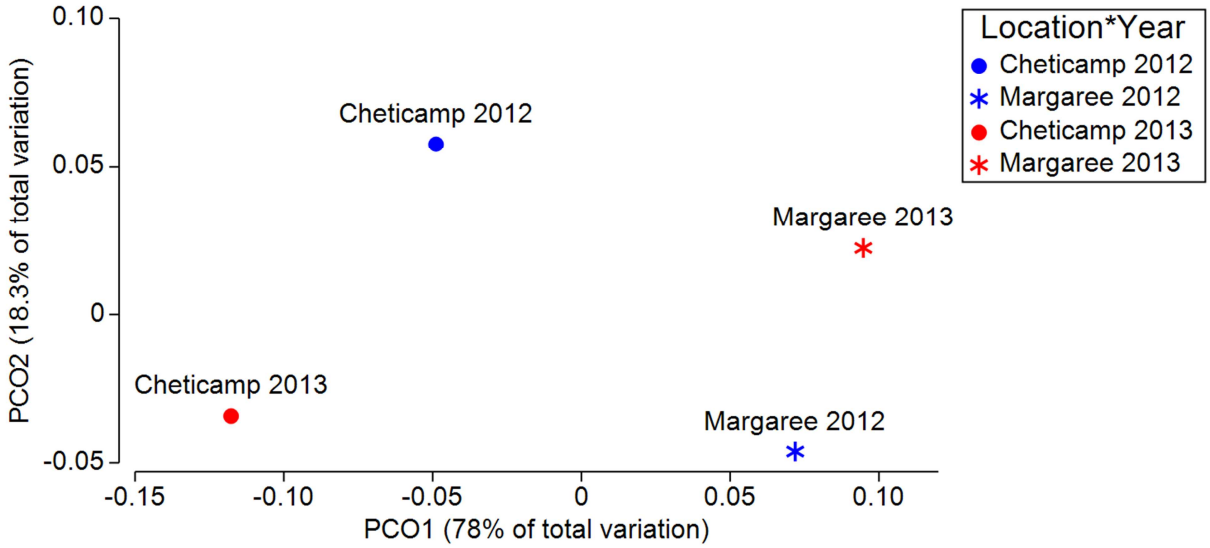

Figure 4. Principal Coordinates Analysis (PCO) showing the mean distribution of the assemblage of all morphometric data for *Chionoecetes opilio* zoea stage I from plankton tows in Margaree and Cheticamp (NS) in June of 2012 and 2013. The percentage of variation explained by each PCO axis is indicated by the axis. The n per group ranged from 16 to 211.

Zoea II

In 2013, there were only four zoea II larvae collected in Margaree and none in Cheticamp. Therefore, year was not included as a factor for the zoea 2 analysis. No significant difference in morphometric characters was Summary

Morphological abnormalities

As in the 2003 seismic study, very few morphological abnormalities were observed in these larvae. The only abnormality observed that we are confident was not an artifact of sampling, handling or preservation, was a zoea I with a dual dorsal spine (Figure 5). Two zoea I were noted to have abdominal curvatures but this may well have been the product of collection and processing rather than a natural abnormality. Spines that were incompletely grown were observed, but as this condition was present in zoea I larvae, this state may simply represent a larva transitioning from a pre-zoea to a zoea I (Kon 1967) and not an abnormal development of the spines. Observations of these zoeae also revealed many eye abnormalities but none of these

abnormalities could conclusively be attributed to natural deformity rather than commonly observed effects of handling and preservation. Close to half of the zoeae in a subsample examined more closely (44% of 297) had slight to severe eye abnormalities which ranged from one or both eyes having abnormal pigmentation, shrivelling (loss of moisture) of the eye, to the complete loss of an eye. The subsample re-examined for eye damage was selected from pictures of the zoeae, taken when they were measured, with a clear view of the eyes.

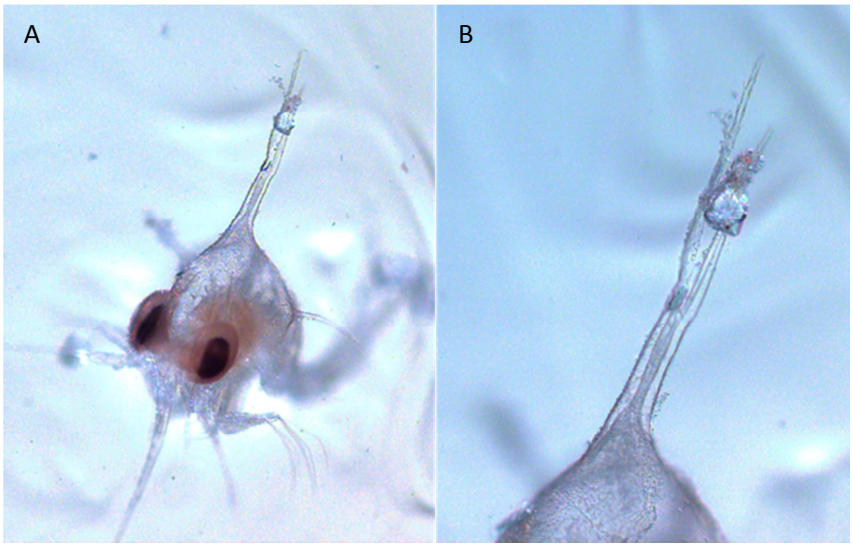

Figure 5. Dual dorsal spine observed in zoea I larva collected in Margaree Harbor in 2012. Panel A shows entire view of larva and panel B shows enlarged view of dual dorsal spines.

VI-5 DISCUSSION

In the 2003 seismic study, a slower developmental rate was observed in zoea I, hatched in the laboratory from embryos carried by *C. opilio* females caged at the seismic site, compared to zoea I from females caged at the control site. Some morphometric features of the zoea I larvae were also smaller in the seismic group compared to the control group. However, it was not possible to determine if these significant differences between groups resulted from exposure to seismic energy, differences in temperatures between the caging sites or the slightly smaller size of females caged at the seismic site (DFO 2004, Courtenay *et al.* 2009, Boudreau *et al.* 2009). Results of the present study support the hypothesis that these differences could have resulted from embryonic rearing at different locations within the same general geographic area rather

than from differential exposure to seismic energy. In 2013 samples of the present study, zoea I larvae from Margaree were significantly larger than those sampled in Cheticamp only 30 km away. The caging sites in the 2003 study were further apart than this (35-41 km). This difference in size between Margaree and Cheticamp zoea I was significant in 2013 and a similar trend was observed in 2012. The marginally significant differences in location (zoea I, $p = 0.068$ and zoea II, $p = 0.06$) in 2012 could be related to the low power of these analyses associated with the small sample sizes for some of these groups (Table 4 & 5). Results of the present study also demonstrated the lack of significant difference in the assemblage of morphometric features in zoea I larvae between years (2012 vs 2013) demonstrating the greater influence of local environmental conditions on size than interannual influence.

Morphometric features of zoea I and II from the present study were smaller than those from laboratory reared *C. opilio* zoeae in the Davidson and Chin (1991) and Pohle (1991) studies but were similar to the 2003 seismic and Webb *et al.* (2006) studies (Table 6). The size differences among studies may be related to incubation (embryogenesis) and rearing (larvae) temperatures. In *C. opilio,* embryogenesis lasts for approximately 2 years (Moriyasu & Lanteigne 1998), hence, the influence of temperature on size must be considered for months prior to hatch. This was studied by Webb *et al.* (2006) who held ovigerous female snow crabs in the laboratory at a range of temperatures, 0-3 and 6°C, from embryo stage 5 (Moriyasu & Lanteigne 1998) until hatch. These authors observed that zoea I increased in size with incubation temperature from 0 to 3°C but were smallest when reared at 6°C. In zoea stage II, the combination of incubation temperature and rearing temperature during the first zoeal stage would influence size. In mud crab (*Rhithropanopeus harrisii*) the effect of temperature was additive as the megalopa stage of these crabs was smallest if exposed to low temperature during both embryogenesis and the zoeal stages. In the present study, and the 2003 seismic study, incubation temperature would correspond to bottom temperatures off the west coast of Cape Breton of approximately 0-1°C (Chassé *et al.* 2014). Such low temperatures could explain the smaller sizes of zoea I for both these studies. In the Davidson and Chin study, the incubation temperature for the last 4-5 months of embryogenesis was 3-5°C which may explain the larger size of these zoeae I. In the present study, temperatures at the collection sites during the plankton tows ranged from approximately 0-10°C in Cheticamp and 0-3°C in Margaree at depths of 0 to 20 m (Figure 2). Because *C. opilio* zoeal stages migrate downwards during the day and upwards at night (Roff *et al.* 1984), these larvae might be exposed to a range of temperature lower than the 8.2-11°C of the Davidson and Chin study. Therefore, the combination of colder incubation (0-1°C for bottom temperature; Chassé *et al.* 2014) and rearing temperatures could also account for the smaller zoea II in the present study. However, factors such as geographic origin, size of females and primiparous vs multiparous females could contribute to size differences in larval stages of *C. opilio*. Such factors may also be responsible for the similar sizes for both zoeal stages (Table 6) in the Davidson and

Chin (1991), and Pohle (1991) studies as incubation and rearing temperatures were higher in the Davidson and Chin (1991) study.

Table 6. Comparison among studies of rostrodorsal length (tip of dorsal spine to tip of rostral spine) of snow crab zoea stage I and stage II from field collected or laboratory reared zoeae at different incubation (embryo) and rearing (zoea I) temperatures and geographic origin.

Source	Present study	2003 seismic study	Webb *et al.* (2006)	Davidson and Chin (1991)	Pohle (1991)
Origin	Western Cape-Breton	Western Cape-Breton	Eastern Bering Sea	Western Cape-Breton	Baie des Chaleurs
Collected from	Plankton tows	Laboratory	Laboratory	Laboratory	Laboratory
Incubation temperature	0-1°C (field)	0-1°C (field)	0-3°C and 6°C (lab, 240-353 d)	3-5°C (lab, 4-5 months)	0-1°C (field)
Rearing temperature	0-10°C (field)	5.1 and 6.2°C (mean per group)	0-3°C and 6°C	8.2 and 11°C (mean per group)	3-7°C
Rostrodorsal length	Range of mean ± 95%CI	Mean	Mean ± SD	Mean and range	Mean ± SD
n =	16 – 258	159 per group	15	-	50
Zoea 1	4.175 (3.967-4.382) 4.321 (4.272-4.370)	4.268 (seismic group) 4.416 (control group)	4.46 ± 0.22 to 4.66 ± 0.12 (0-3°C) 4.32 ± 0.09 (6°C)	4.84 (4.68-5.00)	4.92 ± 0.17
Zoea 2	6.018 (5.946-6.090)	-	-	6.64 (6.48-6.80)	6.63 ±0.22

One of the main challenges of the present study was the separation of *C. opilio* and *Hyas* spp. *Hyas araneus*, *Hyas coarctatus alutaceus* and *Hyas coarctatus coarctatus* overlap in geographical range and hatching periods with *C. opilio*, and could therefore be present in our samples (Lanteigne 1985, Sabean 2007). Morphological features and dimensions most appropriate to differentiate these species (Davidson & Chin 1991, Pohle 1991), and that were used in this study, are listed in Table 1. However, features such as the length of the lateral spines on the somites of the abdomen and the length of the lateral spines on the carapace, are very similar for *C. opilio* and *H. coarctatus alutaceus* (Pohle 1991). Because of the similarity of these morphometric characters, one of the features recommended by Pohle (1991) is the overall size (rostrodorsal length) of these zoeae, as *H. coarctatus alutaceus* are smaller than *C. opilio*. The absolute difference in rostrodorsal length between *C. opilio* and *H. coarctatus alutaceus* reported by Pohle was approximately 0.6 mm and 1 mm, for zoea I and zoea II respectively. However, size can vary with local environmental conditions (Table 6). To differentiate among species,

Christiansen (1973) suggests that because size differences are present between *Hyas* spp. larvae from different areas, these species can only be separated when specimens are collected from the same area. Christiansen (1973) also suggests that even though size differs among areas, the corresponding differences between species should remain the same, i.e. *H. araneus* should always be larger than *H. coarctatus coarctatus*. In the present study, the rostrodorsal lengths of *C. opilio* zoea I were similar to those observed in the 2003 seismic study, as these were from the same area, which can validate the identification of these zoeae as *C. opilio* (Table 6). Although zoeae were not reared to the second zoeal stage in the 2003 seismic study, the average rostrodorsal length of zoea II from the present study, approximately 6 mm, is larger than lengths of 5.6 and 5.65 mm for *H. coarctatus alutaceus* reported by Pohle (1991), also validating the identification of these zoeae as *C. opilio*. However, as noted by Pohle, sizes in these two species may overlap at the extreme of the ranges. Therefore, in the present study we cannot eliminate the possibility that some of the smaller zoeae of both developmental stages could be *H. coarctatus alutaceus*.

The difficulty in properly identifying larval stages of *C. opilio* and *Hyas* spp. is further complicated by the large variability in morphometric data among geographical distributions, between laboratory reared and wild caught specimens, rearing temperature etc. (Table 6). As such, this large variability results in *C. opilio* zoea I from the present study, the 2003 seismic study or those from the Webb *et al.* (2006) study, not being identified as *C. opilio* by the classification keys of the Pohle (1991) and Davidson and Chin (1991) studies as these keys classify rostrodorsal length of *C. opilio* as > 4.5 mm and ≥ 4.6 respectively. Further complicating this task are erroneous measurements such as the mean length of the lateral spines in Davidson and Chin (1991) of 1.51 mm which is at least twice the size of those in the present study or Pohle (1991). In Roff *et al.* (1984), the description of the relative length of the terminal setae on the antennal exopodites for *C. opilio* zoea I did not agree with observations of reference *C.opilio* zoea I from the 2003 seismic study, in which the shortest of the three unequal setae was approximately 1/3 of the longest setae as was described for *Hyas* spp. by Roff *et al.* (1984) not for *C. opilio* (M. Boudreau, personal observation). Our observation of the erroneous description of these setae for *C. opilio* by Roff *et al.* (1984) was also corroborated by Pohle (1991). Finally, caution should be taken when consulting earlier descriptions of *Hyas* spp. as the descriptions given for morphometric and morphological features do not include those of *H. coarctatus alutaceus* in the general description of the *Hyas* genus (Roff *et al.* 1984, Lanteigne 1985, Davidson & Chin 1991).

A discrepancy that should be noted between the morphometric data of the present study and the 2003 seismic study is the size of the abdomen in zoea I. The 2003 seismic study reported measurements for somites 2 to 6 but this is impossible because somite 6 is not present in zoea 1 (Courtenay *et al.* unpublished). In zoea II, a section of the telson separates and becomes the 6th

somite. Therefore, the measurement taken in the 2003 seismic study for somite 6 was likely a section of the telson resulting in larger abdomen size (1.599 and 1.564 mm, control and seismic groups respectively) reported in that study than the present study (1.220 - 1.265 mm, range of means from Table 2).

In the present study, only a single morphological abnormality (dual dorsal spine) was identified as a natural abnormality rather than an artefact of manipulations (during field collection, sorting of samples or during observations) or preservation. One of the most common preservation artifact we observed was eye damage, such as abnormal pigmentation and shrivelling (loss of moisture) of the eye. This type of artifact is common for samples preserved in formalin and ethanol (pers. Comm. R. Bernier). Also observed was the loss of an eye for several zoeae (n = 12), which would be an artifact of manipulations. Unfortunately, such a high prevalence of artificial eye abnormalities in our samples could mask naturally occurring abnormalities as it may be impossible to distinguish between the two. These eye abnormalities are worth mentioning as eye abnormalities such as microphthalmia (small eye, unilateral or bilateral), anophthalmia (absence of one or both eyes) or cyclopia (fusion of both optic vesicles) can be caused by exposure to toxicants such as metals (Weis & Weis 1989). Therefore, research studies examining specific impacts of toxicants on *C. opilio*, such as eye defects, should consider the effects of preservation/manipulation on these structures. As such, the use of live animal may be more appropriate for such research studies. The evaluation of live animals would also validate abnormalities such as abnormal alignment of the somites of the abdomen which was observed in two samples of zoea I stage and presumed to be an artefact of manipulation. In larval fish, slight vertebral curvatures were best evaluated on live, naturally moving animals, as these abnormalities could also be produced by preservation or misinterpreted when assessed through images (M. Boudreau, pers. obs.).

Results of the temporal distribution of zoea II in the present study corresponded to their temporal distribution in the Baie des Chaleurs which was reported to end by mid-August (Lanteigne 1985). In the present study *C. opilio* zoea II were absent from samples collected in late August or early September (24-25 August 2012 and 1 September 2013). In the Baie des Chaleurs, zoea II were present at the beginning of June, also substantiated in the present study as zoea II were present in samples collected on 19 June 2012. However, only four samples of zoea-II stage were collected on 19 June in 2013 (four zoeae II from Margaree and none from Cheticamp).

In conclusion, results of the present study showed that the natural incidence of morphological abnormalities in field-collected zoeal stages of *C. opilio* was very low. A single occurrence of a dual dorsal spine in a zoea I larva was the only morphological abnormality observed. Local environmental conditions appeared more important in influencing size in *C. opilio* zoeae than interannual variations. No significant difference in the zoeal size was observed between years;

however our results showed that significant differences in morphometric features of zoea I can be observed in samples separated by only 30 km. Hence, significant differences observed in the 2003 seismic study could have been related to local conditions of the caging sites separated by 35-41 km rather than by differential exposure to seismic energy.

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VII HEMOLYMPH BIOCHEMISTRY ANALYSIS

VII-1 DETERMINATION AND COMPARISON OF SPRING & FALL DISTRIBUTION OF TISSUE ENZYME ACTIVITY

VII-1-1 Summary

Analysis of hemolymph constituents using biochemistry panels in crustaceans is a non-lethal sampling protocol that can be used repeatedly to evaluate the animal's response over time to a variety of conditions e.g., diet, environment, disease, tissue injury, and normal physiological processes related to reproduction and moult. Enzyme activity is an important component of these panels, particularly when assessing tissue injury. Interpretation of enzyme activity requires knowledge of the tissue(s) of origin of the enzymes within the animal. To achieve this, the distribution of eight enzymes (amylase, lipase, ALT, AST, GD, SDH, ALP and GGT) commonly included in biochemistry panels across seven tissues (heart, hepatopancreas, muscle, ovary, testes, intestine, subcuticular epidermis) and hemocyte pellets was determined for snow crabs. To capture potential variability related to sex, maturity level or time of year, samples were collected from multiple crab categories in the fall of 2011 (adolescent males, large mature males, prepubescent females, and mature females) and the spring of 2012 (pygmy males, large mature males, and mature females).
Plasma is required for accurate analysis. The release of enzymes, and possible inhibitors, from hemocytes during the clotting process complicates interpretation of results in serum samples.

The most promising indicators of hepatopancreas-specific injury are amylase, GGT, SDH, and ALP (in non-adolescent male crabs). Increases in ALT and AST could be expected to accompany muscle and possibly hepatopancreas injury with increases in AST indicating more severe cellular injury. Glutamate dehydrogenase (GD) appears more muscle-specific but, as for AST, may require more severe cellular injury for release into the circulation. Lipase was widely distributed and is commonly detected in hemolymph plasma samples. Continued observation under different physiological and pathological conditions will be required to characterise the origin of any changes noted in plasma. Conversely, GGT, SDH, and ALP have rarely been detected in plasma samples from apparently healthy snow crabs; as such, their potential as diagnostic enzymes to reveal hepatopancreas injury or other pathology remains to be determined.

Three sex- and/or age-related patterns in tissue enzyme profiles were noted. First, the activity of GD was higher in leg muscle tissue of immature male and female crabs in the fall samples. This may reflect muscle growth in these non-terminally moulted crabs. Second, ALP activity was generally up to 1000-fold higher in testes from adolescent male crabs than other male crabs and so is possibly related to maturation of the male reproductive system. Finally, differences in the

enzyme profile of ovaries (increased AST, ALT, lipase activity) and hepatopancreas (increased ALP activity) of mature female crabs collected in the spring compared to prepubescent or mature fall-caught female crabs were noted. These are suspected to be related to spring spawning and the subsequent physiologic resorption of non-spawned oocytes.

While many questions remain to be answered, the results of this study continue to add information on the origin of enzyme activity detected in hemolymph plasma and the use of biochemistry profiles as a tool for evaluating the health of snow crab.

VII-1-2 Introduction

As part of normal, physiologic, cell turnover, tissue-based enzymes are released into the circulation where their activity can be detected (Moss & Henderson 1998). The amount of activity detected is proportional to the mass of the tissue releasing the enzyme, the rate of release, and the rate of clearance. The half-life (t½) of the enzyme, which is time required for the body to reduce (clear) the amount of detectable activity to 50% of its original value, will determine how long the activity of an enzyme can be detected (Moss & Henderson 1998). This will vary among enzymes. Tissue injury e.g., physical, chemical/toxic, hypoxic or inflammatory, will generally increase the rate of release, while some physiologic changes may increase the amount of enzyme produced, and therefore available for release, by the cell. Enzymes may be located on the cell membrane, within the cytosol, and/or the mitochondria. Detection of increased activity of mitochondrial-based enzymes is generally considered indicative of more severe injury (Moss & Henderson 1998).
Biochemistry profiles in crustaceans have a wide range of potential applications. The small volume required, as little as one millilitre (1.0 mL), allows for sample collection from small crabs and/or multiple collections from a single crab over relatively short time periods. Biochemistry profiles could be used to evaluate responses to a variety of conditions including changes in diet, environment, disease, and to better understand the normal physiology related to reproduction and moult. Enzyme activity is an important component of these panels; however, accurate interpretation requires knowing from which tissues the enzymes may be derived.

One of the overall goal The first step in determining which enzymes may be of diagnostic use for identifying tissue injury using hemolymph samples is to identify the tissue(s) of origin for the enzymes.

The current study was undertaken to:

1. Evaluate the suitability of serum (produced by hemolymph clotting) vs. plasma (no clotting) as samples for hemolymph biochemistry profiles.

2. Determine the tissue(s) of origin for eight enzymes which are being evaluated for inclusion in hemolymph biochemistry panels for snow crab (*Chionoecetes opilio*) using tissue samples collected from immature and mature, male and female, crabs in fall 2011 and spring 2012.

VII-1-3 Materials and methods

All animal handling and holding procedures were approved by the Animal Care Committee of the University of Prince Edward Island, Charlottetown, PE.

Sample Source & Husbandry

Fall 2011

Crabs were collected by DFO staff (Snow Crab Section, Gulf Region, Moncton, NB), from crab fishing area 19 as part of the fall trawl survey. Live crabs were kept on salt-ice and then transported (emersed) in coolers from Souris, PE to the Atlantic Veterinary College Lobster Science Centre (AVCLSC), Charlottetown, PE, by land (DFO truck). In total, 16 snow crabs were received at the AVCLSC. Four large mature males (LM) of CW > 90 mm were received on September 28th, 2011. Four adolescent males (AM), of carapace width (CW) 35 – 45 mm, four prepubescent females (PrF) of CW 35-45 mm, and four mature females (MF) were received on October 3, 2011.
Crabs were held in a recirculation system at 1.5^{o}C, salinity (Instant Ocean®, Aquarium Systems Inc., Mentor, OH, USA) at 31.6 ppt for up to two weeks. Water quality (ammonia, nitrate, nitrite, and pH) was monitored every 2 weeks. Crabs were offered thawed shrimp every four days at which point any uneaten shrimp was removed from the tank.

Spring 2012

Thirty-five snow crabs (n = 15 LM, 95-135 mm CW; n= 12 MF, 60 – 70 mm CW; n= 8 pygmy male (PM), <95 mm CW) were obtained from a trapping survey ('free' crabs) and delivered to the Atlantic Veterinary College on June 6, 2012. Transportation was in coolers on salt-water ice. Crabs were held in a recirculation system at 1.5^{o}C, salinity (Instant Ocean®, Aquarium Systems Inc., Mentor, OH, USA) at 31.6 ppt for up to four weeks. Water quality (ammonia, nitrate, nitrite, and pH) was monitored every 2 weeks. Crabs were offered thawed shrimp every four days at which point any uneaten shrimp was removed from the tank.

Gross Physical Examination

Fall 2011

Observations were not recorded.

Spring 2012

Information (carapace width, presence of injuries, lost limbs, carapace condition, activity level/responsiveness) was recorded for all crabs (n = 5 large mature males (LM), n = 5 mature females (MF), n = 4 pygmy males (PM)) from which tissue samples were collected.

Assessment of Clotting on Hemolymph Biochemistry Parameters: Plasma vs. Serum

Fall 2011

Hemolymph samples were collected from the area between the coxa of the first walking leg and body using a 22G needle and 3 mL syringe after surface disinfection with 70% alcohol. For preparation of 'plasma' samples, hemolymph was gently transferred into pre-chilled 1.8 mL microcentrifuge tubes after removing the needle from the syringe. The samples were immediately centrifuged (4,000 x g, 5 min, at 4^{o}C). The supernatant, 'plasma', was removed using a plastic transfer pipet, leaving a buffer layer above the cell pellet to avoid contaminating the plasma with hemocyte contents thereby reducing the risk of clotting. The pellets were saved for measurement of enzyme content of hemocyte lysate supernatant (HLS) (see 'Determination of Tissue Distribution of Enzyme Activity' below).
'Serum' samples were prepared by filling a second microcentrifuge tube and leaving the sample (on ice or refrigerated) for 2.5 – 3 h prior to centrifugation (4,000 x g, 5 min, 4^{o}C). Biochemistry panel analyses were performed the same day of sample collection by Diagnostic Services, AVC, UPEI, Charlotttetown, PE using the Cobas c501 (Roche Diagnostics Corporation, Indianapolis, IN, USA) automated biochemistry analyser.

Spring 2012

As for Fall 2011 with exception that centrifuge speed was 3,500 x *g*.

Determination of Tissue Distribution of Enzyme Activity

Fall 2011

After collection of hemolymph for plasma and serum samples, the dorsal carapace was either removed entirely or, partially lifted for access to tissues in the case of small crabs. Representative samples (0.02 – 1.5 g depending on size of crab) of the heart (H), hepatopancreas (HP), testes or ovary, proximal intestine (INT), subcuticular tissue from the dorsal carapace to obtain epidermis (EPI) and muscle from the first or second walking leg (M) were rinsed with sterile distilled water (dH_2O), transferred to 15 mL transport tubes or 1.8 mL microcentrifuge tubes, and placed on ice for tissue enzyme studies.
Tissues were weighed directly into new microcentrifuge tubes or 15 mL tubes. Chilled, sterile dH_2O was added to all tissue samples > 0.05 g to make a 10% w/v suspension. For samples <0.05 g, and all hemocyte pellets, 0.5 ml of dH_2O was added. All samples were homogenised (OMNI International TH homogeniser with a stainless steel EZ Coupling G5-75, 5mm diameter and 75 mm length, generator probe, Kennesaw, GA) until no large particles remained (approximately 20 - 30 s). The probe was rinsed with dH_2O and wiped dry between tissues.

All tissue homogenates were left to lyse, refrigerated, for one hour. Samples were then centrifuged (15,000 x g, 4^{o}C, for 15 min) and the supernatants transferred to microcentrifuge tubes and submitted to Diagnostic Services, AVC, UPEI, for biochemistry panel analysis. The panel included determination of the activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate dehydrogenase (GD), amylase (AMY), lipase (LIP), sorbitol dehydrogenase (SDH), gamma glutamyltransferase (GGT) and protein content using a pyrallogallol red-based microprotein assay or the biuret method for samples with protein concentrations > 5.0 g/L. Enzyme activities per gram of wet tissue and per gram of total protein in the lysate supernatants, after standardisation for the dilution factors, were calculated.

Spring 2012

As for fall 2011 samples with an additional rinsing step where the homogenisation probe was run in 1000 µL of (dH_2O) to further decrease the chance of fluid carryover between tissue homogenates.

Histology

Fall 2011

Representative samples of the tissues noted above and gill were placed into a glutaraldehyde-formaldehye fixative (Appendix A) for subsequent histological examination. Whole cross-sections of the abdomen were placed in fixative to preserve tissue architecture for smaller AM and PrF crabs.

Fixed tissues were processed routinely to produce 3- 5 µm sections stained with haematoxylin and eosin for light microscopic examination.

Spring 2012

Tissue sample collection was as for fall 2011 crabs with spermathecae also collected from MF crabs when present.
Statistical analyses were completed using Stata®/IC v12.1 (StataCorp LP) and Minitab® v16. (Minitab Inc.). Results were considered significant for *p*-values < 0.05 unless otherwise indicated.

VII-1-4 Results

Hemolymph Plasma vs Serum

Fall 2012

Plasma samples were collected from 16 crabs (n = 4 AM; n = 4 MM; n = 4 PrF; n = 4 MF) and serum was available for 14 crabs (n = 2 AM; n = 4 MM; n = 2 PrF; n = 4 MF). The small size of two of the AM and two of the PrF precluded getting enough hemolymph for both plasma and serum preparation; subsequently, only plasma was prepared for these four crabs. The weight of hemocyte pellets was unavailable.
The raw data for plasma and serum (as available) biochemistry panel results for the 16 crabs are presented in Appendix B. The difference (serum – plasma) for all measured parameters on the Cobas c501 biochemistry analyser, by crab group, are summarised in Table 1. Regarding detectable enzyme activity, the most consistent and relevant changes were increases in serum amylase activity and decreases in serum ALT and AST activity compared to plasma.

As shown in Figure 1, the relative pattern of enzyme content in the HLS (per gram total protein in the HLS) was similar across all four groups with amylase, ALT, and AST dominating the enzyme profiles. Kruskal-Wallis testing revealed significant differences across crab groups for: amylase ($p = 0.0302$), due to low values for prepubescent females; ALP ($p = 0.0330$), due to more activity in adolescent males; AST ($p = 0.0407$), due to adolescent male and mature female crabs being different from the other two groups but not from each other (Wilcoxon Signed Rank test) and ALT ($p = 0.0228$), due to higher activity in mature male crabs.

Spring 2012

Serum and plasma samples were collected from 14 crabs (n = 5 MM; n = 5 MF, n = 4 PM). Magnesium values were unavailable for serum for four MM, one MF, and one PM crab, uric acid value for one MM, and electrolytes for one MF, due to a technical error and/or lack of sufficient sample volume to rerun the tests.

The raw data for the serum and plasma biochemistry panel results for all 14 crabs are presented in Appendix C. The absolute values of the difference (serum – plasma) for all measured parameters on the Cobas c501 anlayser are shown in Table 2. The most relevant changes were the consistent increase in amylase for all crabs and variable changes recorded for AST and ALT. The changes noted for other variables were marginal with the exception of the decreases in glucose and ‘albumin’ recorded for PM 1.

Table 1. Summary of differences (serum value – plasma value) for each crab for all parameters measured on the Cobas c501 automated analyser in fall 2011 samples. Time lapse (clotting period) for production of the serum sample was approximately two hours. (Note: creatinine was not detected in any sample and so not included in the table.)

Crab Group[1]	Na[2]	K[2]	Cl[2]	Ca[2]	Phos[2]	Mg[2]	Urea[2]	Gluc[2]	Chol[2]	Trig[2]	TP[3]	Alb[3]	Lact[3]	Uric Acid[4]	AMY[5]	LIP[5]	ALP[5]	AST[5]	ALT[5]	GGT[5]	GD[5]	SDH[5]
AM3	-3	0.1	-3	0.25	0.91	-0.26	0.1	0.0	0.02	0.00	-2	1	0.00	-1	6	1	0	-5	-86	-1	1	0
AM4	-15	-0.2	-18	0.18	0.01	0.70	0	0.1	0.00	0.00	1	0	-0.01	-1	6	3	0	-5	-19	-1	-3	0
LM1	30	0.6	48	0.41	-0.01	-0.05	0	0.0	0.01	0.00	0	1	-0.02	-1	12	0	0	-147	-29	0	-1	0
LM2	12	0.2	27	0.08	0.11	0.35	0.1	0.1	0.00	0.00	1	1	0.04	1	15	1	0	-57	-16	0	2	0
LM3	33	0.8	63	-0.13	0.06	-1.84	0	0.0	-0.01	0.00	0	0	0.00	1	5	-2	0	-24	-27	0	2	0
LM4	-3	-0.4	-54	0.06	-0.02	-1.19	0	0.1	0.00	0.01	1	0	0.02	0	4	0	0	-11	-28	0	-1	0
PrF3	9	1.3	-12	0.41	0.36	0.70	0	0.0	-0.01	0.01	1	1	0.00	-2	3	-1	0	-7	-43	0	-12	0
PrF4	-12	-1.4	-27	0.39	0.11	-0.44	0	-0.1	0.00	0.05	1	0	0.00	0	1	0	0	-6	-32	1	-4	0
MF1	12	0.6	9	0.24	-1.09	-0.44	0.1	-0.1	0.03	0.00	1	3	0.03	1	58	3	0	-9	-69	1	6	2
MF2	15	0.6	24	-0.14	0.35	0.01	-0.1	-1.4	0.02	0.02	0	0	-0.01	1	57	-2	0	-13	-14	1	-45	0
MF3	-9	0.1	-3	0.45	-0.74	0.23	0	-0.1	0.03	0.05	0	0	0.01	1	26	0	0	-5	-5	0	-3	0
MF4	12	0.7	18	-0.32	0.12	0.56	0	-0.1	0.00	0.00	-2	3	0.02	-1	69	0	0	2	4	0	-4	0

1. AM ~ adolescent male; LM ~ large mature male; PrF ~ prepubescent female; MF ~ mature female
2. Units for: sodium (Na), chloride (Cl), potassium (K), calcium (Ca), phosphorus (Phos), magnesium (Mg), urea, glucose (Gluc), cholesterol (Chol), triglyceride (Trig), and lactate are mmol/L.
3. Units for total protein (TP), albumin (Alb), and lactate (Lact) are g/L.
4. Units for uric acid are µmol/L
5. Units for all enzymes: amylase (AMY), lipase (LIP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), glutamate dehydrogenase (GD), and sorbitol dehydrogenase (SDH) are U/L.

Figure 1. Box and whisker plots**.** Graphical representation of the relative amounts of eight enzyme activities per gram of total protein in hemocyte lysate supernatants of four crab groups collected in fall 2011. AM = adolescent males (n = 4); MM = large mature males (n = 4); PrF = prepubescent females (n = 4); MF = mature females (n = 4); amylase (AMY), lipase (LIP); alkaline phosphatase (ALP); aspartate aminotransferase (AST); alanine aminotransferase (ALT); gamma glutamyl transferase (GGT); glutamate dehydrogenase (GD); and sorbitol dehydrogenase (SDH).

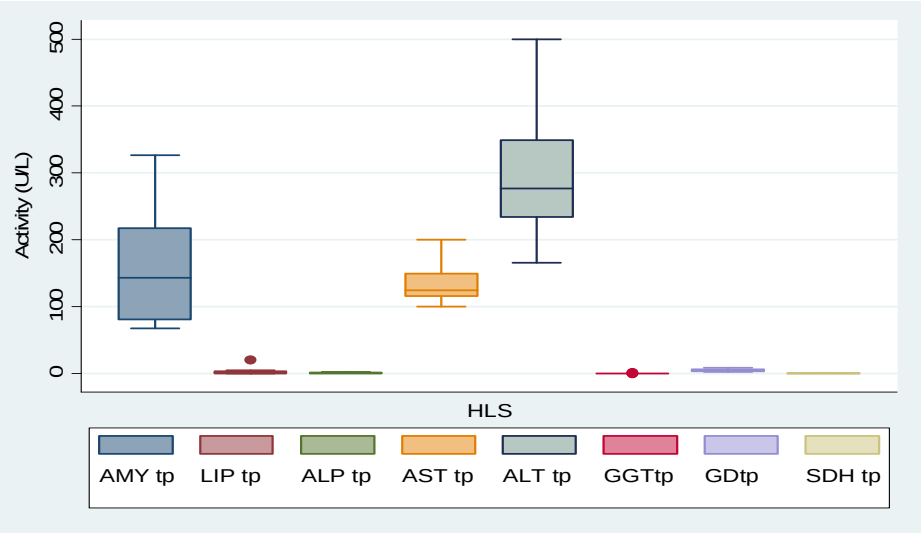

Figure 2. Box and Whisker plots. Comparison of enzyme activity as calculated per gram of total protein (tp) in the hemocyte lysate supernatants (HLS) of crabs collected in spring 2012. Results were pooled for all three categories (PM, n = 4; LM, n = 5; MF, n = 5) as no differences among categories were observed. **A**mylase (AMY), lipase (LIP); alkaline phosphatase (ALP); aspartate aminotransferase (AST); alanine aminotransferase (ALT); gamma glutamyl transferase (GGT); glutamate dehydrogenase (GD); and sorbitol dehydrogenase (SDH).

Table 2. Summary of differences (serum value – plasma value) for each crab for all parameters measured on the Cobas c501 automated analyser in spring 2012 samples. Time lapse (clotting period) for production of the serum sample was approximately two hours. (Note: creatinine was not detected in any sample and so not included in the table.)

Crab Group[1]	Na[2]	K[2]	Cl[2]	Ca[2]	Phos[2]	Mg[2]	Urea[2]	Gluc[2]	Chol[2]	Trig[2]	TP[3]	Alb[3]	Lact[3]	Uric Acid[4]	AMY[5]	LIP[5]	ALP[5]	AST[5]	ALT[5]	GGT[5]	GD[5]	SDH[5]
LM 1	0	0.3	3	0.02	-0.16	n/a[1]	0.0	-0.1	0.03	0.00	0	3	0.05	0	37	3	0	-196	-65	0	6	0
LM 2	6	0.2	6	-0.15	0.03	n/a	-0.1	0.0	0.01	0.00	0	0	0.00	-1	1	2	0	1	-5	0	1	0
LM 3	3	0.0	3	-0.01	0.98	0.00	0.0	0.0	0.01	0.01	1	0	-0.03	2	7	-1	0	-70	-46	0	-2	0
LM 4	30	0.5	30	0.08	-0.24	n/a	0.0	-0.1	0.03	0.03	0	1	0.04	0	22	-5	0	-53	-54	-1	3	0
LM 5	-18	-0.4	-27	0.40	0.07	n/a	0.0	-0.1	0.01	0.01	0	0	-0.01	0	36	0	0	-6	-9	0	-2	0
MF 1	0	0.0	0	0.01	0.04	-1.02	0.0	-0.1	0.09	0.07	0	1	0.08	2	75	0	0	-20	-11	0	0	0
MF 2	-18	0.4	-30	-0.21	0.29	1.60	0.2	-0.1	0.10	0.10	0	3	0.10	21	70	0	0	43	24	0	-3	1
MF 3	3	0.4	6	-0.08	-0.22	-0.30	0.0	-0.1	0.02	0.01	0	3	0.01	1	20	1	0	-2	1	0	-3	0
MF 4	9	1.0	12	0.01	0.24	0.92	0.1	-0.2	0.03	0.02	0	4	0.05	0	81	1	0	29	7	0	-6	1
MF 5	n/a	n/a	n/a	-0.17	0.01	n/a	0.0	0.1	0.00	0.01	0	0	0.01	0	4	2	1	-17	-9	0	-2	0
PM 1	24	0.5	27	-0.97	-0.39	0.00	-0.3	-0.6	-0.01	0.00	1	-5	0.06	2	16	1	-1	-2	-5	1	4	0
PM 2	36	1.1	42	-0.09	-0.64	n/a	0.0	0.0	0.02	0.01	1	1	0.09	2	24	-1	0	2	-8	0	5	0
PM 3	0	0.6	3	-0.21	0.28	1.53	0.0	0.0	0.04	0.03	0	4	0.10	2	145	-1	0	11	7	0	-3	1
PM 4	24	1.1	33	-0.13	-0.11	-1.16	0.0	-0.1	0.05	0.02	2	2	0.07	0	20	-1	0	-2	-8	0	2	0

1. AM ~ adolescent male; LM ~ large mature male; PrF ~ prepubescent female; MF ~ mature female
2. Units for: sodium (Na), chloride (Cl), potassium (K), calcium (Ca), phosphorus (Phos), magnesium (Mg), urea, glucose (Gluc), cholesterol (Chol), triglyceride (Trig), and lactate are mmol/L.
3. Units for total protein (TP), albumin (Alb), and lactate (Lact) are g/L.
4. Units for uric acid are µmol/L
5. Units for all enzymes: amylase (AMY), lipase (LIP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), glutamate dehydrogenase (GD), and sorbitol dehydrogenase (SDH) are U/L.

Gross Physical Examination

Fall 2011

Observations not recorded.

Spring 2012

Crabs were generally in good shape with most being very vigorous and responsive when handled. A few crabs were missing one or more walking legs without evidence (pigmentation/melanisation) of healing (n = 2 PM; n = 1 LM). Healed sites of previously lost limbs, indicated by pigmentation/melanisation at the amputation site, were noted on three MF. The duration of the lesion could not be determined. A large area of the carapaces of four MF crabs and one PM crab were covered by a thin layer of epibionts. Three of the MF crabs had moderate to severe shell disease (erosion and/or pitting with melanisation) over a large portion of the carapace. The undersides of the abdomen of all MF were filled with orange eggs.

Histological Examination

Fall 2011

Tissues from LM and MF were not collected for histological evaluation. Due to the small size of the heart in the AM and PrF crabs, all tissue was used for the enzyme studies and unavailable for histologic examination. Inadequate fixation due to poor fixative penetration in the smaller crabs fixed whole was noted in many of the tissue samples.

Ovaries – Approximately 90% - 95% of the ovarian tissue in all PrF was composed of maturing, secondary, oocytes (relatively large size, containing intensely eosinophilic droplets, see section IV-4).

Testes – Testicular tissue, and in some cases vas deferens, was present in three of four of the AMs; all showed active sperm production. Varying amounts of a homogenous eosinophilic fluid was noted in tubule lumens.

Gastrointestinal tract – Intestine was variably present for examination in the abdominal cross sections. When present, no visible lesions were detected. The stomach of AM #4 had a moderate, focal, aggregation of hemocytes with large eosinophilic granules below the epithelial

layer of a small area of the stomach which extended into the underlying muscle tissue in some areas (gastritis). What was available of the overlying tissue for examination was intact i.e., no evidence of ulceration or erosion, to account for the inflammatory infiltrate.

Hepatopancreas – Small to moderate amounts of crystalline material deposits interpreted as mineral (special stains not conducted), were found in all crabs primarily associated with the R-cells. The vacuoles of the B-cells of all crabs were from ½ to ⅔ filled with orange-pink granular material. Autolysis was noted in some areas.

Connective Tissues - The reserve inclusion (RI) cells found in the loose connective tissues were stained an intense eosinophilic colour and prominent (full).

Heart – This tissue was not available for histological examination (see above).

Gills – No significant lesions. RI cells were often prominent.

Leg and abdominal muscle – The section of the abdomen for PrF #3 showed cytoplasmic pallor, loss of the normal striated appearance of the tissue, and some pyknotic nuclei in one of the lateral muscles. Also present was a small amount of granular material which could represent mineralisation (special stains not performed) and remnants of what are suspected to be hemocytes (i.e. as part of an inflammatory response). The findings are consistent with acute necrosis and myositis. Similar, but less convincing changes were noted in muscle samples from PrF #4 and AM #3. No indication of muscle injury was noted in AM #1 however, only very small amounts of muscle tissue were available for examination from this crab.

Subcuticular tissue – the tissue was heterogeneous, being composed primarily of loose connective tissue, with smaller amounts of striated muscle fibres, and a single layer of columnar epithelial cells. The tissue samples represented primarily dermis rather than epidermis.

Spring 2012

Histologic lesions varied by tissue and were relatively minor. Due to the small size of the intestine, the proximal section was used for tissue analysis and the distal segment for histological evaluation.

Ovaries – all appeared to contain collapsed follicular spaces suggesting that they had recently spawned. Only less mature, primary oocytes, were present. Traces of hepatopancreas tissue were noted in some of the ovary sections

Testes – all LMs showed active sperm production (mitotic figures, range of developing sperm in tubules); some level of sperm production was noted in three PMs.

Intestine – mild inflammation (mixed hemocyte infiltration) at the junction of the midgut and hindgut was noted in one LM and one PM crab. Rod-shaped bacteria were present on the cuticle surface of the distal intestine.

Hepatopancreas – numerous rod-shaped bacteria were noted in the lumens of the HP tubules of three MF crabs without evidence of inflammation/host response in the surrounding tissue. The vacuole space of the B-cells of all crabs was ⅓ to ½ filled with orange-pink granular material.

Connective Tissues – The RI cells found in the loose connective tissues were only partially filled and usually stained at less than maximal intensity.

Heart – the RI cells were not prominent; when noted, contained only a minimal amount of material.

Gills – mild fouling was noted in some crabs. A very early (peracute) systemic infection was suspected in LM 2 due to the presence of low numbers of hemocyte aggregates (presumed to be early organising 'granulomas') in the vascular space of the heart and gills.

Leg muscle – no lesions noted.

Subcuticular tissue (Epidermis) – the tissue was heterogeneous, being composed primarily of loose connective tissue (made up of usually empty/vacuolated, presumably RI, cells) with smaller amounts of striated muscle fibres, and a single layer of columnar epithelial cells. The tissue samples represented dermal and epidermal tissue.

Spermathecae – inflammation and bacterial growth present in all MF.

Tissue Enzyme Distribution

Fall 2011

The mean, median, minimum, and maximum values for enzyme activity per gram wet weight ($_{ww}$) and per gram of supernatant total protein ($_{TP}$) for all eight tissues by crab group (AM, LM, PrF, and MF) are reported in Appendices D and E. The wet weight of the hemocyte pellets was not available; hence, HLS_{ww} calculations were not completed. Data are also represented graphically in Figures 3 – 20. Sample processing times from tissue collection to lysate analysis were not recorded.

The overall pattern of enzyme distribution across tissues was similar for all crab categories with the exception of extremely high ALP activity detected in testes of all four AM and one of the LM. Median values of enzyme activity per gram total protein in lysate supernatants and wet weight were compared across all crab categories – AM, LM, PrF, and MF for each tissue (Kruskal-Wallis test if n = 4 or Wilcoxon Signed Rank test if n = 2). When statistical differences were detected, pairwise comparisons between groups were made (Wilcoxon Signed Rank test with Bonferroni-adjusted levels of significance of 0.0125) in select cases where information could be of diagnostic relevance. Results for HLS were not included in these comparisons as hemocyte contents should not contribute to plasma enzyme values *in vivo*.

Amylase

This enzyme was primarily found in the hepatopancreas and intestine in all crab groups with values in the hepatopancreas 2 – 4 fold higher than other tissues (Figures 3 & 4)**.** Statistically significant differences among crab groups, within specific tissues, were found for $amylase_{ww}$ for intestine ($p = 0.0357$) and ovary ($p = 0.0421$) due to lower medians in PrF crabs. Differences for $amylase_{tp}$were found in: heart ($p = 0.0101$, LM and MF having higher values); hepatopancreas ($p = 0.0242$, LM having the higher values); ovary ($p = .00421$, higher values in MF); intestine ($p = 0.0091$) and epidermis ($p = 0.0065$). Activity detected in the tissue supernatants ranged from 43 to 436 U/L for hepatopancreas; whereas values for epidermis and heart were much lower, ranging from only 1 – 13 U/L.

Lipase

Lipase activity was detected in the supernatants of the testes, hepatopancreas, muscle and intestine (Figures 5 & 6). Statistically significant differences among crabs, within tissues, for $lipase_{ww}$ were found for heart ($p = 0.0407$) and testes ($p = 0.0433$, AM> LM). For $lipase_{tp}$, significant differences were found in heart ($p = 0.0123$), hepatopancreas ($p = 0.0492$), and muscle ($p = 0.0281$). Within the HP, the difference was due to the LM, which tended to have higher values than the other groups. The actual amount of lipase activity detected in heart supernatants was very low (0 – 4 U/L) compared to the hepatopancreas (12 – 38 U/L), testes (12 – 56 U/L) and intestine (7 – 16 U/L).

ALP

By up to 4000-fold, the testes of all AM, and one LM, contained the most ALP activity per gram of wet weight or per gram TP in supernatant (Figures 7 & 8). Data graphed without testes better shows the activity among the remaining tissues (Figures 9 & 10). Statistically significant differences among crab groups, within specific tissues, for ALP_{ww} ALP_{ww} were found for heart ($p = 0.0375$) and intestine ($p = 0.0112$). Comparisons of ALP_{tp} found significant differences for heart ($p = 0.0387$), intestine ($p = 0.0128$), and epidermis ($p = 0.0491$).

AST

This enzyme was primarily associated with muscle tissue (heart and walking leg), followed by the hepatopancreas (Figures 11 & 12). Significant differences were detected for AST_{ww} among crab groups, within specific tissues, for: heart ($p = 0.0441$, MF different from the others by having significantly lower median values); hepatopancreas ($p = 0.0230$); intestine ($p = 0.009$); testes ($p = 0.0209$, AM>LM); and epithelium ($p = 0.0202$). Comparisons of AST_{TP} showed significant differences for: heart ($p = 0.0317$, LM differing from the others having significantly higher values); hepatopancreas ($p = 0.0351$); and testes ($p = 0.0209$, AM >LM).

ALT

The relative distribution across tissues for ALT was similar to AST; however the magnitude of activity was slightly lower for heart than leg tissue (Figures 13 & 14). Significant differences across crab groups, within specific tissues, for ALT_{ww}, were found for: heart ($p = 0.0186$, MF = LM < AM = PrF); hepatopancreas ($p = 0.0116$, MF = LM < AM = PrF); intestine ($p = 0.009$), epidermis ($p = 0.0132$); and testes ($p = 0.0209$, AM > LM). Comparisons of ALT_{TP}, showed significant differences for hepatopancreas ($p = 0.0302$, MF had the lowest values), intestine ($p = 0.0273$), and testes ($p = 0.0209$, AM > LM).

GGT

Gamma GT was almost exclusively localised to the hepatopancreas, with only minor activity detected in the intestine, testes, and epidermis on a per gram wet weight or gram TP basis. Comparison of GGT_{WW} found significant differences among crab groups, within tissues, for muscle ($p = 0.0370$) and testes ($p = 0.0209$). However, detection of any substantive GGT activity in tissue supernatants was limited to the hepatopancreas (33 - 169 U/L) and epidermis (11 – 19) while most other tissue lysate supernatants had values < 9 U/L.

GD

The primary location for GD was muscle of the walking leg. Comparison of GD_{ww} by crab group, within tissue types, showed differences in testes ($p = 0.0209$) with AM having greater values than LM and in epithelium ($p = 0.0175$). Differences in GD_{TP} were found in: heart ($p = 0.0308$); hepatopancreas ($p = 0.0053$); epidermis ($p = 0.0252$); and, testes ($p = 0.0209$). Median muscle GD activity was not different across crab groups in either comparison.

SDH

Some of the highest levels for this enzyme were found in the hepatopancreas, with far lower amounts in the intestine and testes of AM crabs on a WW or TP basis; however, compared to most enzymes, relative activities were low. Comparison among crab groups by SDH_{ww}, within tissue groups, found significant differences restricted to the hepatopancreas ($p = 0.0178$) where, LM were not different from PrF and AM were similar to MF. Comparison among SDH_{TP}, found

differences for: hepatopancreas (p = 0.0167); intestine (p = 0.0327); and, epidermis (p = 0.0037). Similar to GGT, the tissue supernatants of the hepatopancreas lysates were the only ones to have reasonable amounts (9 - 59 U/L) of SDH activity detected while most other tissues had values ranging from only 1 – 9 U/L.

Spring 2012

Sample processing – from tissue collection to end of homogenisation – required approximately one hour per crab. The lysing period, centrifugation step, and completion of analysis on the biochemistry analyser, generally required an additional 2.5 h. The mean, standard error, median, minimum, and maximum values for all tissues (activity per gram wet weight and per gram of supernatant protein) by group (LM, MF, and PM) are reported in Appendices F and G. The enzymes AST and ALT had the highest relative activity in all tissues for all crab categories by 40- to 80-fold. Data were not available for EPI for LM 1(tissue not collected) nor was the wet weight for the hemocyte pellet for MF 4 (data not recorded).
The data are represented graphically in Figures 3 through 20 for comparative purposes. HLS was examined separately (see 'plasma vs serum' section) and not discussed here as it would not contribute to hemolymph plasma activity.

The general pattern of enzyme distribution across tissues appeared similar for all three crab groups. Comparison of median values of enzyme activity per gram total protein (TP) in lysate supernatants or wet weight ($_{ww)}$ across groups (Kruskal-Wallis test) revealed only the following nine differences: AST_{TP} and SDH_{TP} for heart (p = 0.0080; p = 0.0470), LIP_{TP} for intestine (p = 0.0300), GGT_{TP} and SDH_{TP} for EPI (p = 0.0350; p = 0.0410, respectively), ALP_{ww} for hepatopancreas and intestine (p = 0.0100; p = 0.0500, respectively), and SDH_{ww} for subcuticular epithelium and heart (p = 0.0100; p = 0.0400, respectively).

The MF group tended to have lower AST_{ww} and AST_{tp} activity for heart and was statistically different from the LM and PM crabs (Wilcoxon signed rank test). Marked activity of ALP in the testes supernatant of a PM and a LM crab masked the relative activity of the other tissues. Graphs of ALP activity without the testes data were generated (Figures 9 & 10) to permit better comparison of the results. The MF group with its higher ALP activity was the cause of the statistical difference (Mann-Whitney test) for hepatopancreas tissue, whereas the PM group was only different from MF for intestine.

When looking at enzyme activity per gram of wet weight or per gram of TP in the supernatant, the following generalisations can be made:

Amylase
The main source was the hepatopancreas and intestine with a smaller contribution from ovaries of MF (interpreted histologically as recently spawned).

Lipase
A broadly distributed enzyme with similar levels found in hepatopancreas, leg muscle, and intestine of all crab groups showing variation within a tissue; however some of the highest values were found in testes.

ALP
The highest levels (by 100 – 200X) were detected in the testes of a PM and a LM. The next highest activity was found in the hepatopancreas of MF followed by intestine of PM.

AST
This enzyme was broadly distributed although the highest levels were found in muscle and heart with the values overlapping to some degree.

ALT
This enzyme was also broadly distributed similar to AST although values for heart tissue were slightly lower.

GGT
The vast majority of GGT activity was only detected in hepatopancreas with some noted in intestine.

GD
The distribution pattern was very similar to ALT, being located primarily in leg muscle tissue

SDH
The enzyme was predominantly located in the hepatopancreas; however, the amounts found in tissue were the lowest of all enzymes studied.

Fall 2011 vs Spring 2012

Statistical testing was limited to Wilcoxon signed rank tests of enzyme activity for LM and MF by season (spring versus fall) as these were the only crab groups included in both samples. Kruskall-Wallis (KW) testing was used to examine differences among crab groups within a tissue where visual assessment showed a possible difference. Excepting the following observations, there was little overall variation by season or crab group for enzyme activity by tissue:

Amylase

There was no detectable difference for the hepatopancreas amylase activity/g TP despite the suggestion of higher values in the fall for MF and LM.

Lipase

Spring 2012 MF had the highest $lipase_{TP}$ activity in the intestine ($p = 0.0365$). The ovaries of spring MF also had the highest $lipase_{TP}$ activity with levels for all three groups of female crabs being significantly different.

ALP

Spring MF had significantly higher values of ALP_{ww} in the hepatopancreas ($p = 0.0066$).

AST

AST_{TP} and AST_{ww} for the ovary in spring MF were notably higher, and significantly different ($p = 0.0129$) than the other females.

ALT

Levels of ALT_{TP} and ALT_{ww} in the ovary were higher in MF in the spring ($p = 0.0106$, $p = 0.0150$, respectively).

GGT

Activity was predominantly located in the hepatopancreas with some of the highest values of GGT_{TP} observed in samples from fall crabs; although, these were not significantly different.

GD

Crabs caught in the fall generally had higher values of GD_{ww} in muscle ($p = 0.0028$) with a similar trend being present, but less marked, for GD_{TP} ($p = 0.0199$). When the AM and PrF were removed from the comparison, no differences remained.

Data were compared in more detail (Wilcoxon Signed Rank test) for trapped (free) LM in the spring (n=5) and trawled LM in the fall (n = 4) and, for trapped (free) MF in the spring (n = 5) and trawled MF in the fall (n = 4) as these groups were part of both the fall 2011 and spring 2012 sample collections. There were few differences noted for LM (Table 3). Differences were noted for MF (Table 4) however, most commonly in the ovary.

Table 3. Summary of statistically significant (Wilcoxon Signed Rank Test; $p < 0.05$) differences, and associated p-value, for tissue enzyme activity in large mature male snow crabs caught in the fall 2011 and spring 2012.

Enzyme	Heart			Hepatopancreas			Muscle			Intestine			Epidermis			Testes		
	fall	**spring**	***p***	**fall**	**spring**	***p***	**fall**	**spring**	***p***	**fall**	**spring**	***p***	**fall**	**spring**	***p***	**fall**	**spring**	***p***
AMY_{ww}			–			–			–			–			–			–
AMY_{TP}			–	129.40	28.3	*0.0275*			–			–			–			–
LIP_{ww}			–			–			–			–			–			–
LIP_{TP}			–	17.48	6.6	*0.0143*			–			–			–			–
ALP_{ww}			–			–			–			–			–			–
ALP_{TP}			–			–			–			–			–			–
AST_{ww}			–			–			–			–			–			–
AST_{TP}			–			–			–			–			–			–
ALT_{ww}			–			–			–			–			–			–
ALT_{TP}			–			–			–			–			–			–
GGT_{ww}			–			–	0.00	0.01	*0.0105*			–			–			–
GGT_{TP}			–			–	0.00	0.54	*0.0105*			–			–			–
GD_{ww}			–	0.11	0.34	*0.0143*			–	0.44	1.52	*0.0143*			–			–
GD_{TP}			–						–	19.12	8.1	*0.0143*			–			–
SDH_{ww}			–			–			–			–			–			–
SDH_{TP}			–			–			–			–			–			–

Table 4. Summary of statistically significant (Wilcoxon Signed Rank Test; $p < 0.05$) differences, and associated p-value, for tissue enzyme activity in mature female snow crabs caught in the fall 2011 (F) and spring 2012 (S).

Enzyme	Heart			Hepatopancreas			Muscle			Intestine			Epidermis			Ovary		
	F	**S**	***p***	**F**	**S**	***p***	**F**	**S**	***p***	**F**	**S**	***p***	**F**	**S**	***p***	**F**	**S**	***p***
AMY_{ww}			–			–	0.04	0.01	*0.0350*			–			–	0.31	0.12	*0.0143*
AMY_{TP}			–			–			–			–			–	7.05	0.93	*0.0143*
LIP_{ww}			–			–			–			–			–	0.10	0.00	*0.0105*
LIP_{TP}			–			–			–			–			–	2.64	0.00	*0.0105*
ALP_{ww}			–	0.29	1.51	*0.0143*			–			–	0.01	0.04	*0.0135*			–
ALP_{TP}			–	16.62	76.9	*0.0275*			–			–	0.30	2.56	*0.0143*			–
AST_{ww}			–	4.46	10.85	*0.0143*			–	5.64	11.33	*0.0275*	6.11	2.92	*0.0143*	11.09	3.29	*0.0143*
AST_{TP}	1180.16	2386	*0.0143*	232	525	*0.0143*			–	196.36	442	*0.0143*	136.42	225	*0.0143*	288	27.08	*0.0143*
ALT_{ww}			–			–			–	5.80	13.26	*0.0143*			–	17.42	5.56	*0.0275*
ALT_{TP}	576.73	1543	*0.0143*	86.57	149	*0.0143*	1307.98	1769	*0.0143*	195.89	498	*0.0143*	348.41	964	*0.0143*	407	46.33	*0.0143*
GGT_{ww}			–			–	0.01	0.00	*0.0260*	0.04	0.09	*0.0143*	0.04	0.00	*0.0289*			–
GGT_{TP}			–			–			–	1.12	3.97	*0.0143*			–			–
GD_{ww}			–			–	3.37	1.52	*0.0275*			–			–	0.41	0.05	*0.0143*
GD_{TP}	16.55	41	*0.0143*			–			–			–	3.63	23.1	*0.0143*	9.34	0.39	*0.0143*
SDH_{ww}	0.04	0.00	*0.0120*			–	0.00	0.02	*0.0318*	0.09	0.03	*0.0358*	0.05	0.00	*0.0146*			–
SDH_{TP}			–			–	0.00	0.85	*0.0318*			–			–	0.86	0.17	*0.0127*

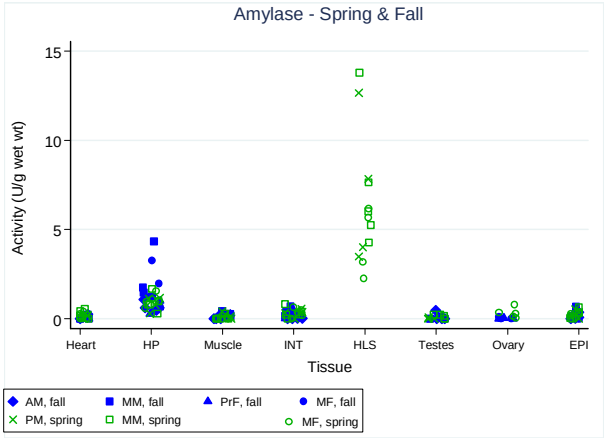

Figure 3. Comparison of amylase activity (U/g wet weight) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

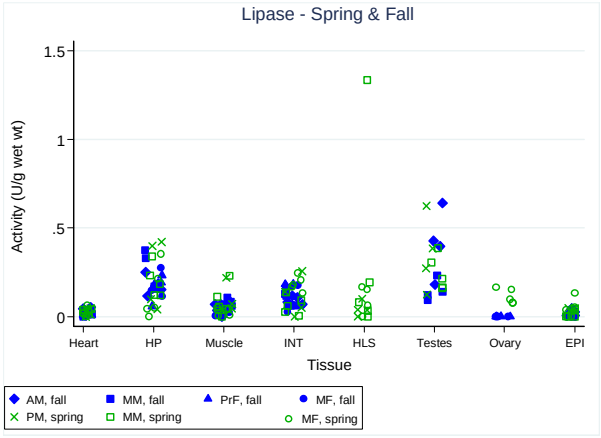

Figure 5. Comparison of lipase activity (U/g wet weight) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

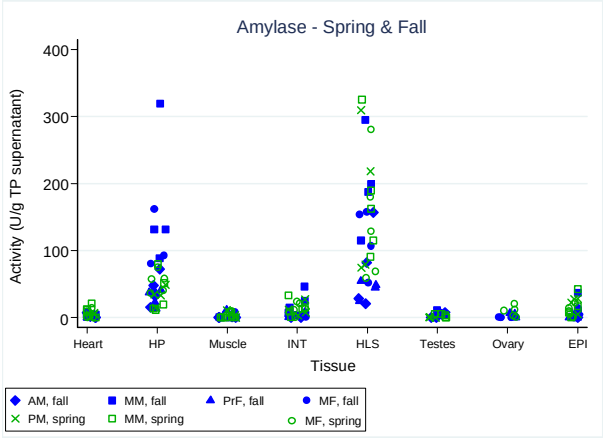

Figure 4. Comparison of amylase activity (U/g supernatant total protein (TP)) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

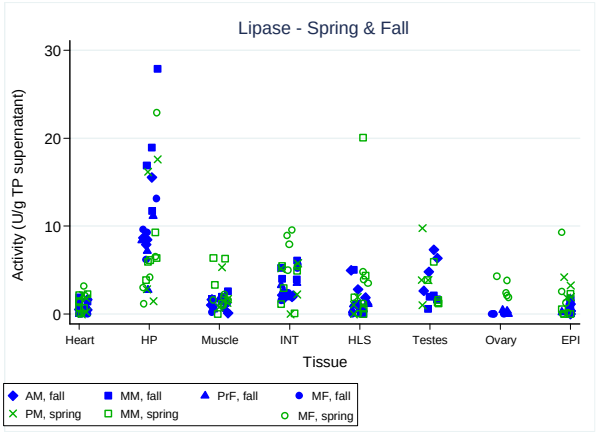

Figure 6. Comparison of lipase activity (U/g supernatant total protein (TP)) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

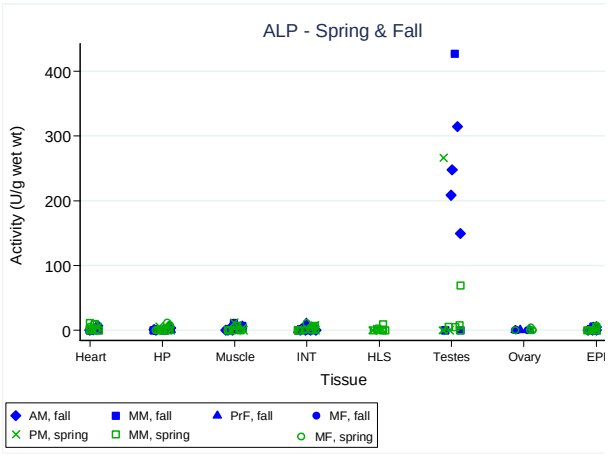

Figure 7. Comparison of ALP activity (U/g wet weight) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

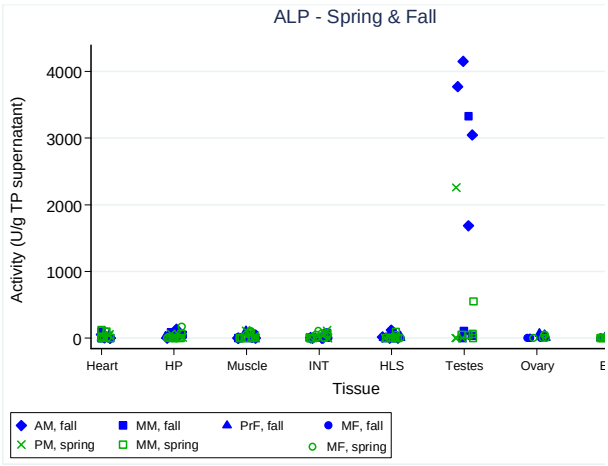

Figure 8. Comparison of ALP activity (U/g supernatant total protein (TP)) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

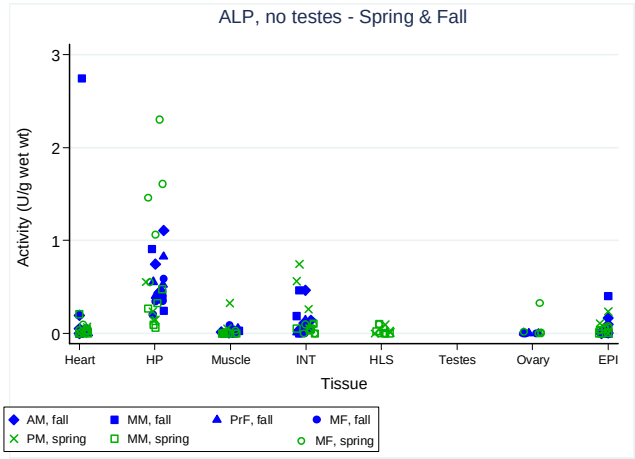

Figure 9. Comparison of ALP activity (U/g wet weight) in seven tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

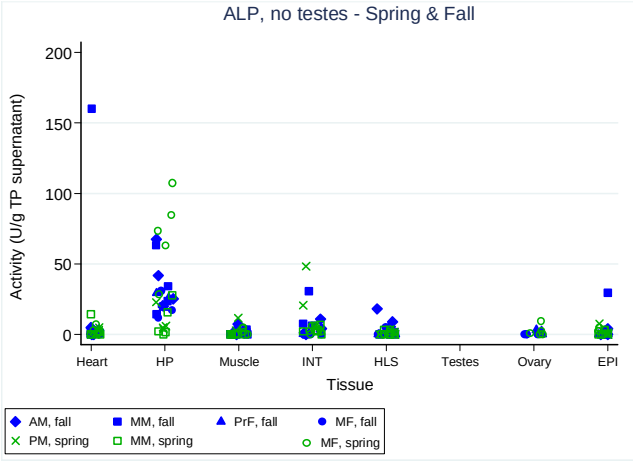

Figure 10. Comparison of ALP activity (U/g supernatant total protein (TP)) in seven tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

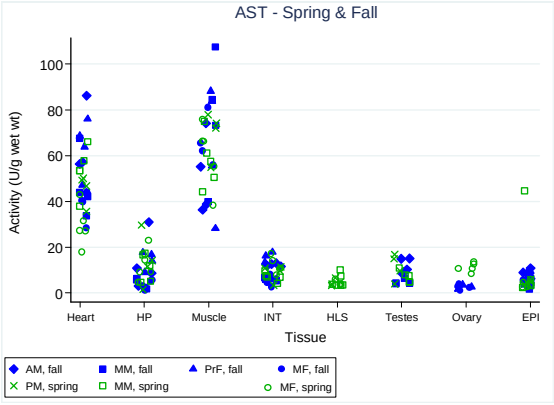

Figure 11. Comparison of AST activity (U/g wet weight) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

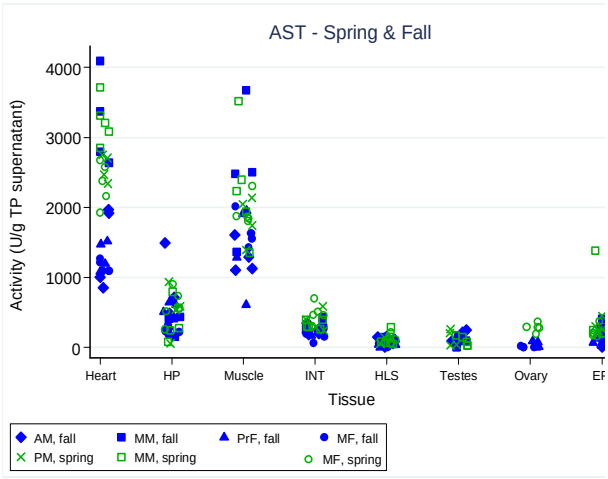

Figure 12. Comparison of AST activity (U/g supernatant total protein (TP)) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

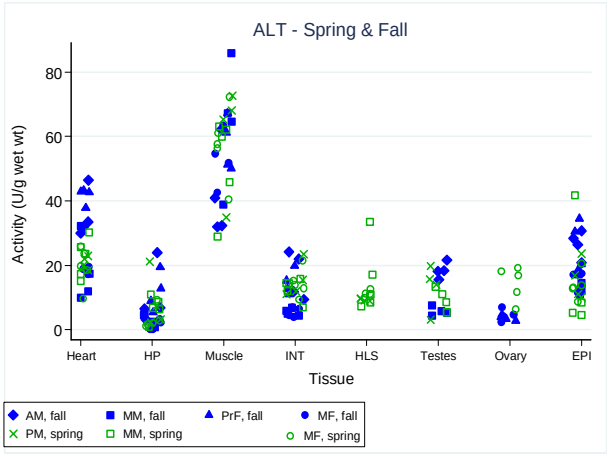

Figure 13. Comparison of ALT activity (U/g wet weight) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

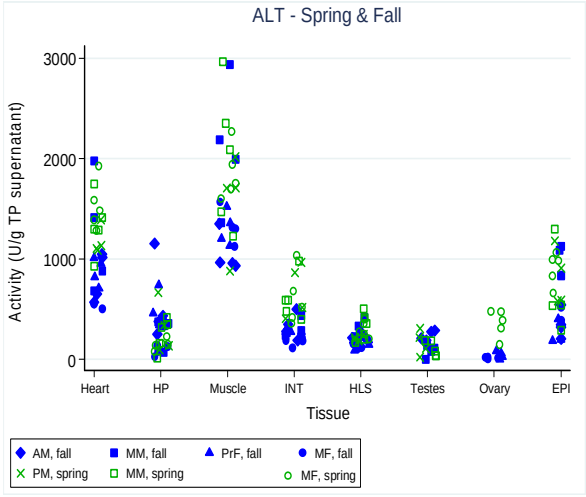

Figure 14. Comparison of ALT activity (U/g supernatant total protein (TP)) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

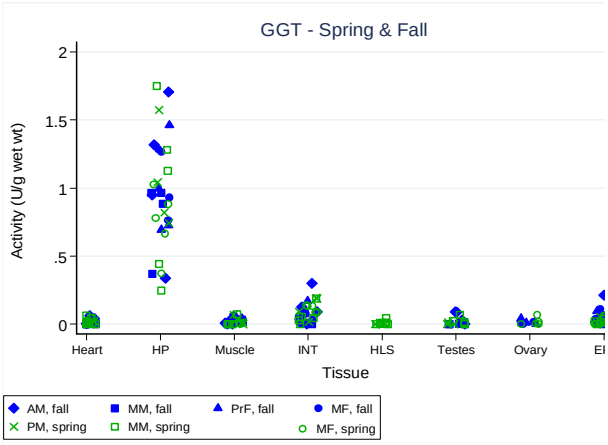

Figure 15. Comparison of GGT activity (U/g wet weight) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

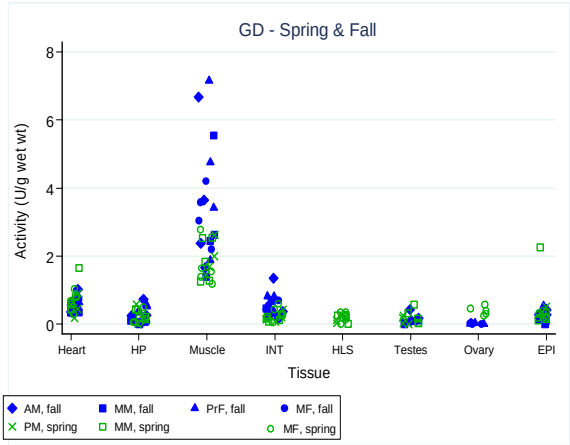

Figure 17. Comparison of GD activity (U/g wet weight) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

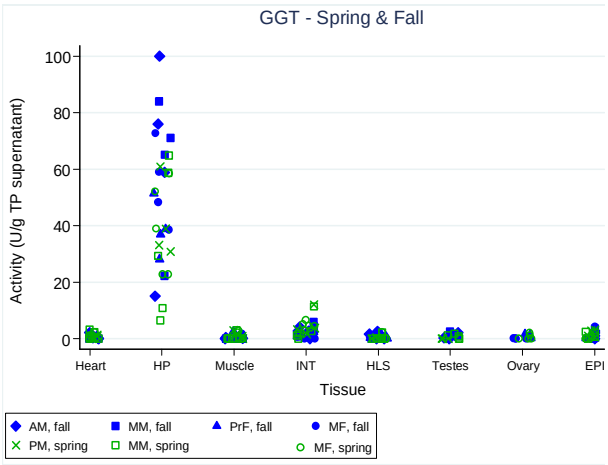

Figure 16. Comparison of GGT activity (U/g supernatant total protein (TP)) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

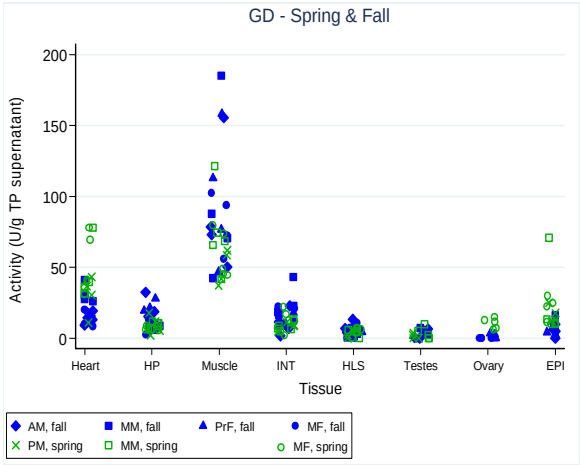

Figure 18. Comparison of GD activity (U/g supernatant total protein (TP)) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

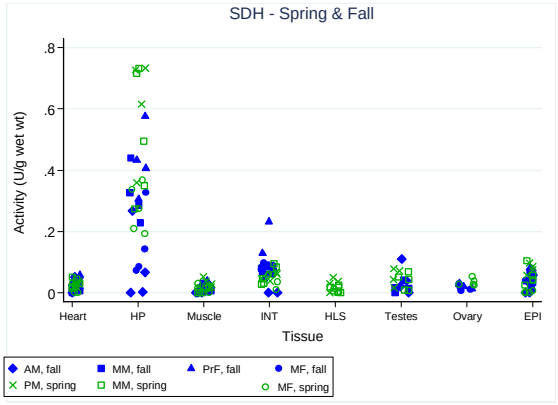

Figure 19. Comparison of SDH activity (U/g wet weight) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

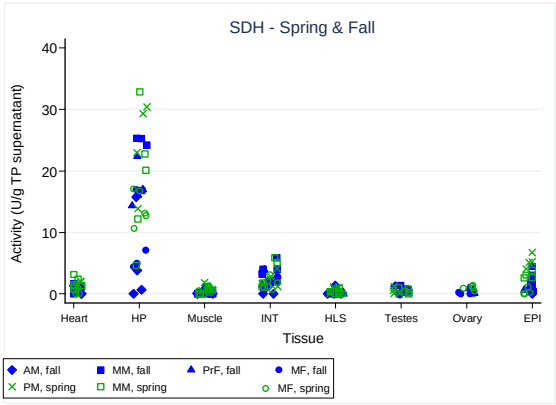

Figure 20. Comparison of SDH activity (U/g supernatant total protein (TP)) in eight tissues from immature and mature snow crab collected in fall 2011 and spring 2012.

VII-1-5 Discussion

Plasma was found to be the preferred sample when submitting hemolymph for enzyme activity analysis as part of a biochemistry profile. The distribution of eight enzymes was successfully determined in seven issues and hemocyte lysate supernatants (HLS) from snow crab (*Chionoecetes opilio*) collected in the fall of 2011 (AM, LM, PrF, MF), and the spring of 2012 (PM, LM, MF). Excluding HLS, amylase, SDH, and GGT activities were predominantly located in the hepatopancreas (tissue specific). Alkaline phosphatase (ALP) activity was primarily located in the hepatopancreas but also present at unexpectedly high levels in the testes, particularly of fall 2011 AM crabs. Muscle tissue, walking leg and heart, was the main source of GD, AST and ALT activity. Moderate levels of ALT and AST were also detected in many other tissues e.g., hepatopancreas. The latter may be important for interpretation of hemolymph levels as the hepatopancreas is a very large organ and could contribute significantly to plasma enzyme activity. Lipase activity was widely distributed among many tissues.

Multiple categories of crabs collected in two seasons were evaluated in the study in the event that sex, size, and/or reproductive physiological state might affect which enzyme activities could be released into the hemolymph. With the exception of a few sex and seasonal-related differences noted for ALP, lipase, and amylase, AST, and ALT the relative distribution of enzyme activity was similar across all crab categories. Mature female crabs captured in the spring had higher levels of AST, ALT, and lipase activity in the ovary, higher lipase activity in the intestine, and

higher ALP activity in the hepatopancreas than other crab groups. This may be related to resorption of unspawned oocytes and marked increased in relative mass of connective tissue in the post-spawn ovary. Levels of GD in walking leg muscle tissue were generally higher in non-terminally moulted fall crabs (AM and PrF).

Cuticular erosions, resembling shell disease were noted in the spring 2012 crabs. As the lesions did not penetrate the surface, it was believed that they were unlikely to affect results of the study.

Plasma vs Serum

Serum and plasma samples were compared to determine if the clotting process, which produces serum, had any significant effects on the results of the biochemical profile. During clotting, hemocytes release cell contents into the hemolymph to trigger coagulation. In American lobsters, serum has higher activities of AST, ALT, and GD compared to plasma due to the high levels of these enzymes in the HLS (Battison 2006). This can complicate interpretation of the plasma biochemistry results as it is impossible to tell if the enzyme activity detected was present as a result of the clotting process or tissue injury. However as preparation of plasma requires extra sample handling at the time of collection, a centrifuge, and a suitable work area, serum was evaluated as a possible alternate sample for snow crab.
The HLS of snow crab had high levels of amylase (the highest of any tissue), AST and ALT – although there were slight differences across crab groups, the overall patterns were similar. Thus, the anticipated changes in enzyme activity when comparing serum to plasma would be increases in amylase, AST, and ALT. This occurred consistently for amylase but, surprisingly, not for AST or ALT. The greatest differences were seen for plasma samples with the highest AST values. There was no relationship between AST levels in the HLS and the changes in serum. The results suggest instability of AST and ALT in serum. The reason for this is unknown; however, it is possible that HLS contains an inhibitor of some sort which, when released into the serum, decreases the activity of any AST and ALT (hemocyte or plasma origin). Regardless of the cause, use of serum is not recommended when evaluating hemolymph enzyme activity in snow crab.

Tissue Distribution of Enzymes

As part of normal cell turnover, enzymes are released into the circulation where their activity can be measured (Moss & Henderson 1998). The amount of activity detected is proportional to the mass of the tissue releasing the enzyme and the rate of release. The half-life (t½) of the enzyme, which is time required to clear (remove) half of the enzyme activity from the circulation, will determine how long the activity of an enzyme can be detected. This will vary among enzymes. Tissue injury e.g., physical, chemical/toxic, hypoxic or inflammatory, will generally increase the

rate of release, while some physiologic changes may increase the amount of enzyme produced, and therefore available for release, by the cell. Enzymes may be located on the cell membrane, within the cytosol, and/or the mitochondria. Detection of increased activity of mitochondrial-based enzymes is generally considered indicative of more severe cellular injury.
Amylases are secreted outside of the cells to digest complex carbohydrates such as glycogen into simple monosaccharide sugars in vertebrates (Moss & Henderson 1998). That amylase was primarily located in the hepatopancreas, the probable main site of glycogen digestion and storage in snow crab, is logical. The tendency for AM and PrF to have lower amylaseTP values for the hepatopancreas may be related to the tissue glycogen stores which could more labile in non-terminally moulted crabs. Glycogen stores were higher in fall LM and MF crabs than their spring-collected counterparts. This might account for the tendency for higher, but not statistically different, amaylase activity per gram TP in fall crabs. Amylase activity detected in the intestine could originate from the proximal intestinal tissue itself, mixed with digesta in the intestinal lumen originating from the hepatopancreas or could be due to the presence of sloughed hepatopancreas cells in the intestinal lumen, a common histological finding, which is reported as a normal part of the digestive process in the American lobster (Factor 1995). Histochemistry could be used to localise the site(s) of amylase activity.

Lipases are involved in triglyceride metabolism by hydrolysing glycerol esters into long chain fatty acids (Moss & Henderson 1998). The hepatopancreas being a major lipid storage site in snow crab (Hardy *et al.* 2000), it was not surprising to find activity in this organ. As for amylase, lipase activity detected in the intestine samples could be derived primarily from the intestinal tissue and/or secondarily from the hepatopancreas. Histochemistry could be used to localise the site(s) of activity. The presence of lipase in the testes, especially of AM crabs, may suggest a role for lipase in the developing gonad. Muscle-based activity may be related to storage of lipid in this tissue or, is perhaps utilised for extracting lipids from the circulation for use by muscle tissue.

Alkaline phosphatase is found in many tissues (liver, bone, intestinal epithelium, renal tubules) in man and other vertebrates, usually at or in cell membranes (Moss & Henderson 1998). The natural substrate is unknown; however, it appears to be associated with lipid transport in intestinal tissue and calcification of boney tissue in man (Moss & Henderson 1998). The extremely high activity detected in the testes of all AM crabs and one each PM and LM crab is suggestive of a role in the gonad, as with lipase. Histochemical evaluation of testicular tissue could help to locate the source of the ALP activity within this tissue.

When results for the testes were excluded, it was apparent that ALP activity was slightly higher in the hepatopancreas overall; however, spring MF had the highest activity in all but one instance, suggesting a sex-related bias. The hepatopancreas is an important energy reservoir for the crab

and would have an integral role in processing and transporting lipid from the hepatopancreas to the ovaries and vice versa. Resorption of the lipoprotein component of unspawned oocytes in the spring may account for the higher levels noted in these MF crabs.

The ALP_{ww} and ALP_{TP} values for the heart of LM #2 (fall 2011) were unusually high and their validity is questioned. This particular crab also had very high ALP_{ww} and ALP_{TP} activities for the testes. It is possible that there was a small amount of testicular material inadvertently mixed with the heart tissue during sample collection or, that there was cross-contamination during homogenisation (unfortunately, the order of homogenisation of tissues was not recorded).

The highest levels of alanine aminotransferase (ALT) activity were generally found for heart and walking leg muscle in both studies. Moderate activity was also found in many other tissues such as the hepatopancreas which, given its mass needs to be considered as another significant reservoir. In vertebrates, this enzyme is generally restricted to the cytosol. Injury to muscle and hepatopancreas tissue could be expected to cause an increase in hemolymph ALT activity in snow crab. Given the relatively small amount of heart muscle tissue in comparison to exoskeleton-associated muscle tissue, damage to this organ is not anticipated to have much of an effect on plasma ALT activity.

The tissue distribution pattern of aspartate aminotransferase (AST) activity resembled ALT, being found at highest levels in muscle tissue with walking leg activity tending to be higher than activity in the heart. Moderate activity was also noted in other tissues, including hepatopancreas, indicating that AST is also not tissue-specific. It is important to note that while activity was lower in non-muscle tissue, the relative activity of this enzyme and ALT is still up to 10-fold higher than most other enzymes in this study. In vertebrates, this enzyme is found in the cytosol and mitochondria with levels in the mitochondria being higher (Moss & Henderson 1998). This type of distribution can result in much higher plasma levels when cellular injury/leakage is severe i.e., enough to damage mitochondrial membranes (Moss & Henderson 1998). As with ALT, damage to exoskeleton- associated muscle and/or hepatopancreas tissue would be expected to be the main sources of increased plasma AST activity. Higher values for AST and ALT were observed in MF crabs which had experienced high frequency of limb autotomy (see Section VIII). Loss of a limb would be expected to be associated with tissue damage.

Given the tissue enzyme distribution and very high levels of AST and ALT activity in crab AM #1 from the fall 2011 group, evidence of tissue injury upon histologic examination of muscle or hepatopancreas tissue was expected but in fact, not observed. It is possible that the injury did occur but was not present in the small amount of tissue available for histologic examination. It is also possible to have cellular injury that is not visible upon light microscopic examination of tissue sections. In another case, fall 2011 PrF #3 did show indication of muscle necrosis on

histologic examination of tissues, but no marked increases in plasma enzyme activities (AST, AST or GD) were detected. It is possible that, overall, the total amount of muscle tissue damaged was very small and so did not affect plasma enzyme activity. Clearly, these two instances show a need for more complete examination of what can affect plasma enzyme activity in this species.

Glutamate dehydrogenase (GD) is a mitochondrial enzyme located primarily in the vertebrate liver with lesser amounts in heart and skeletal muscle, and kidney (Moss & Henderson 1998). The enzyme removes hydrogen from L - glutamate, generating a ketimino acid that will spontaneously hydrolyse resulting in production of ammonia (Moss & Henderson 1998). Ammonia would then be expected to be converted in the liver to a less toxic form e.g., urea or urate for excretion in vertebrates. This would not be required in aquatic crustaceans as ammonia is the usual end product of nitrogen metabolism and readily excreted by diffusion across the gills (Claybroook 1983). A role for GD in osmoregulation in some crustaceans via generation of free amino acids has been indicated (Wang & Li 2012). In this study, GD was predominantly located in the leg muscle tissue and would be expected to be released into the plasma with injury to muscle tissue. If the intracellular locations are similar to vertebrates, hemolymph GD activity would be expected to parallel changes in AST activity i.e., released with more severe cellular injury.

Glutamate dehydrogenase activity tended to be higher in muscle tissue on either a wet weight or TP basis in the fall, due to the contribution of the AM and PrF groups. Presuming these crabs (AM, PrF) had moulted earlier that year, one possible explanation could be that the muscle tissue of AM and PrF crabs is more physiologically active (growing) and so has higher enzyme activity. Unfortunately, muscle samples of AM and PrF were not ideal for examination – only a small amount was present and not all samples were preserved optimally. Peripheral (satellite) nuclei were noted in some samples and could support the concept of active/regenerating muscle tissue.

Gamma glutamyl transferase (GGT) activity was essentially restricted to the hepatopancreas, making this a tissue-specific enzyme for all crabs at both sampling times. In vertebrates, GGT is found primarily within cell membranes of many tissues where it is involved in the cleavage of peptides to form, and then transfer, amino acids to acceptor molecules (Moss & Henderson 1998). It is used as a marker of hepatobiliary disease in many vertebrate species (Duncan *et al.* 1994, Moss & Henderson 1998). It may also have a role in glutathione metabolism. Its role in snow crab physiology has not been documented, but the restricted distribution observed could indicate a similar function. Gamma glutamyl transferase activity was rarely observed in plasma samples at any appreciable levels e.g., the highest activity noted in Section V-3, was 5 U/L (two instances).

Sorbitol dehydrogenase (SDH) activity also appeared somewhat tissue-specific as most was detected in the hepatopancreas for all crabs at both sample periods. Low levels recovered from the intestine could either be intrinsic to the intestine or reflect detection of activity in the luminal contents as with lipase and amylase. Intestine would not be expected to contribute much activity to the hemolymph as the relative mass of the intestine is very low compared to the hepatopancreas. The limited activity in testes of AM would also be anticipated to contribute little to plasma enzyme activity. In vertebrates, SDH functions in carbohydrate metabolism, converting sorbitol (glucose alcohol) to fructose and has been used to detect hepatocellular injury in some species (Duncan *et al.* 1994, Moss & Henderson 1998). Its primary location in the hepatopancreas, an organ likely to function as a carbohydrate reservoir therefore, seems logical.

Collection of epidermis was attempted as enzyme activity in the epithelial tissue could potentially be quite dynamic over the moult cycle. However, histological examination of the samples showed them to be quite heterogeneous with respect to content. Samples contained a mixture of muscle and connective tissue which greatly exceeded the amount of epithelium present and would be expected to contribute to the enzyme profile of the tissue. Similarly, it was difficult to ensure that there was no contamination from hepatopancreas tissue during sample collection. Consequently, few conclusions could be drawn with respect to the enzyme activity detected in the subcuticular 'epidermal' samples.

When looking for seasonal effects, the spring 2012 MF crabs tended to have higher enzyme in ovary tissue when differences were noted ($lipase_{tp}$, AST_{ww}, AST_{tp}, $ALT_{ww,}$ ALT_{tp}). This could be explained by a combination of physiologic resorption of unspawned oocytes requiring more local lipase activity and the relative increase in the ratio of connective tissue mass:overall ovary mass in post-spawn ovaries with the loss of most of the large, presumably enzyme-poor, yolk-filled, lipoprotein-rich secondary oocytes (see Section IV-4) in the spring spawn.

While cell lysates do not represent an ideal environment for measuring enzyme activity, they do provide a means for relative comparison of enzyme activity. A potential pitfall of this technique occurs when only minimal enzyme activity is detected in the lysates e.g., 0 – 3, up to 5 U/L. Depending on the precision (within-run repeatability) of the specific assay, especially at such low values, a result of 0, 1, 2, or 3 could be obtained from the same sample. When this value is then used in calculations of enzyme activity per gram of wet weight or total protein in the lysate supernatants, statistically significant differences may be detected which, in fact, are incorrect. As such, when statistical differences were detected in these circumstances (very low enzyme activity detected in lysates), the practical significance of the results was interpreted with caution.

One of the primary goals of the overall study was to compare the effects of sampling methods (trapping versus trawling) on snow crab health by hemolymph biochemistry analysis. The first

step in determining which enzymes may prove to be of diagnostic use for identifying tissue injury was to identify the tissue(s) of origin for the enzymes. This has been accomplished. The most promising indicators of hepatopancreas-specific injury are amylase, GGT, SDH, and ALP (in non-AM crabs). Increases in ALT and AST could be expected to accompany muscle and possibly hepatopancreas injury with increases in AST indicating more severe cellular injury. Glutamate dehydrogenase appears more muscle-specific but, as for AST may require more severe cellular injury for release into the circulation. Lipase was widely distributed and is usually detected in the hemolymph plasma samples – continued observation under different physiological and pathological conditions will be required to characterise the origin of any changes noted in plasma. Histochemistry studies could provide more information on the location and possibly physiological role of enzymes such as ALP in testes, amylase and GGT in intestine, etc.

In addition to establishing the tissue of origin for the enzyme activity detectable in hemolymph, plasma, while requiring slightly more effort to prepare, was clearly the preferred hemolymph sample type for analysis as better interpretation of enyme activity is possible. While many questions remain to be answered, the results of this study continue to add information on the role of enzyme activity in hemolymph plasma biochemistry profiles as a tool for evaluating the health of snow crab *Chionoecetes opilio*. The study examining alternate collection and holding methods (Section VIII) provides some preliminary information on how different transport conditions could affect hemolymph enzyme activity. The study on feeding behavior (Section V) contains data on seasonal and regional variation that could be analysed further in relation to hemolymph biochemistry.

VII-1-6 References

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VII-1-7 Appendices

Appendix A: Tissue Fixative

For one litre of fixative:

120 ml	37 - 40% neutral buffered formalin
20 ml	50% glutaraldehyde
360 ml	tap water
500 ml	artificial seawater (Instant Ocean®, Aquarium Systems Inc., Mentor, Ohio 440060, USA)

Modified from Howard D, Smith C. Histological Techniques for Marine Bivalve Mollusks. *NOAA Technical Memorandum NMFS-F/NEC-25*. National Marine Fisheries Service, Woods Hole; 1983

Appendix B: Biochemistry profile results for plasma and serum samples by group for fall 2011 crabs

Crab Group[1]	P/S[2]	Na[3]	K[3]	Cl[3]	Ca[3]	Phos[3]	Mg[3]	Urea[3]	Gluc[3]	Chol[3]	Trig[3]	TP[4]	Alb[4]	Glob[4]	Lact[4]	Uric Acid[5]	AMY[6]	LIP[6]	ALP[6]	AST[6]	ALT[6]	GGT[6]	GD[6]	SDH[6]
AM1	P	405	11.0	441	15.73	0.30	43.31	0.1	1.1	1.10	0.27	77	18	59	0.00	4	86	7	0	7093	2426	1	41	0
AM1	S	–[3]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AM2	P	402	9.9	435	16.32	0.24	44.68	0.2	1.1	1.12	0.21	81	19	62	0.10	8	13	8	0	197	374	0	47	0
AM2	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AM3	P	390	10.2	429	14.89	0.17	45.18	0.1	1.0	0.78	0.15	67	17	50	0.00	1	2	10	0	16	181	1	16	0
AM3	S	387	10.3	426	15.14	1.08	44.92	0.2	1.0	0.80	0.15	65	18	47	0.00	0	8	11	0	11	95	0	17	0
AM4	P	411	10.0	453	14.64	0.16	43.85	0.1	0.4	0.31	0.06	26	8	18	0.11	2	2	4	0	14	56	1	7	0
AM4	S	396	9.8	435	14.82	0.17	44.55	0.1	0.5	0.31	0.06	27	8	19	0.10	1	8	7	0	9	37	0	4	0
LM1	P	438	11.2	453	15.19	0.20	33.13	0.1	0.3	0.25	0.04	21	4	17	0.60	4	11	7	0	337	79	0	4	0
LM1	S	468	11.8	501	15.60	0.19	33.08	0.1	0.3	0.26	0.04	21	5	16	0.58	3	23	7	0	190	50	0	3	0
LM2	P	444	12.1	477	15.94	0.17	35.71	0.0	0.4	0.44	0.06	24	5	19	0.38	2	27	4	0	142	51	0	3	0
LM2	S	456	12.3	504	16.02	0.28	36.06	0.1	0.5	0.44	0.06	25	6	19	0.42	3	42	5	0	85	35	0	6	0
LM3	P	438	11.5	453	15.94	0.14	34.00	0.1	0.5	0.43	0.07	28	7	21	0.01	1	13	10	0	192	91	0	7	0
LM3	S	471	12.3	516	15.81	0.20	32.16	0.1	0.5	0.42	0.07	28	7	21	0.01	2	18	8	0	168	64	0	9	0
LM4	P	423	10.9	405	15.05	0.09	38.85	0.1	0.3	0.29	0.04	20	4	16	0.19	2	7	7	0	116	59	0	5	0
LM4	S	420	10.5	351	15.11	0.07	37.66	0.1	0.4	0.29	0.05	21	4	17	0.21	2	11	7	0	105	31	0	4	0
PrF1	P	378	10.6	405	16.36	0.20	42.27	0.2	1.7	1.13	0.47	86	20	66	0.00	9	32	8	0	38	99	0	30	0
PrF1	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PrF2	P	387	9.8	426	17.06	0.93	45.15	0.2	1.2	1.14	0.48	74	20	54	1.08	21	14	3	0	83	145	2	19	0
PrF2	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PrF3	P	456	12.3	525	16.56	0.00	42.33	0.1	1.0	1.05	0.60	78	20	58	1.19	9	13	2	0	136	334	1	33	0
PrF3	S	465	13.6	513	16.97	0.36	43.03	0.1	1.0	1.04	0.61	79	21	58	1.19	7	16	1	0	129	291	1	20	0
PrF4	P	444	12.1	489	16.13	0.21	44.54	0.2	1.3	1.27	0.78	93	20	73	0.33	11	8	4	0	234	311	0	23	0
PrF4	S	432	10.7	462	16.52	0.32	44.10	0.2	1.2	1.27	0.83	94	20	74	0.33	11	9	4	0	228	279	1	20	0
MF1	P	390	10.5	417	15.01	1.32	45.91	0.2	1.6	0.69	0.20	85	15	70	0.21	0.00	4	4	0	38	167	0	29	0
MF 1	S	402	11.1	426	15.25	0.23	45.47	0.3	1.5	0.72	0.20	86	18	68	0.26	0.03	5	7	0	29	98	1	35	2
MF 2	P	405	10.6	429	15.68	0.01	16.22	0.4	1.4	0.70	0.26	83	14	69	0.2	0.04	3	3	0	23	41	0	68	0
MF 2	S	420	11.2	453	15.54	0.36	16.23	0.3	0.0	0.72	0.28	83	14	69	0.2	0.03	4	1	0	10	27	1	22	0
MF 3	S	426	11.3	477	14.61	0.76	47.15	0.1	1.0	0.60	0.25	57	11	46	0.24	0.01	9	3	0	13	39	1	18	0
MF 3	P	435	11.2	480	14.16	1.50	46.92	0.1	1.1	0.57	0.20	57	11	46	0.24	0.00	8	3	0	18	44	1	21	0
MF 4	P	423	10.9	462	14.48	0.60	44.89	0.2	1.0	0.76	0.31	62	11	51	0.22	0.02	3	6	0	3	36	0	30	1
MF 4	S	435	11.6	480	14.16	0.72	45.45	0.2	0.9	0.76	0.31	60	14	46	0.3	0.04	2	6	0	5	40	0	26	1

1: AM ~ adolescent male; LM ~ large mature male; PrF ~ prepubescent female; MF ~ mature female, P = plasma; S = serum. 2: Units for: sodium (Na), chloride (Cl), potassium (K), calcium (Ca), phosphorus (Phos), magnesium (Mg), urea, glucose (Gluc), cholesterol (Chol), triglyceride (Trig), and lactate are mmol/L. 3: Units for total protein (TP), albumin (Alb),globulin (Glob) and lactate (Lact) are g/L. 4: Units for uric acid are µmol/L. 5: Units for all enzymes: amylase

(AMY), lipase (LIP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), glutamate dehydrogenase (GD), and sorbitol dehydrogenase (SDH) are U/L.

Appendix C: Biochemistry profile results for plasma and serum samples by group for spring 2012 crabs.

Crab Group[1]	P/S[2]	Na[3]	K[3]	Cl[3]	Ca[3]	Phos[3]	Mg[3]	Urea[3]	Gluc[3]	Chol[3]	Trig[3]	TP[4]	Alb[4]	Glob[4]	Lact[4]	Uric Acid[5]	AMY[6]	LIP[6]	ALP[6]	AST[6]	ALT[6]	GGT[6]	GD[6]	SDH[6]
PM 1	P	369	7.8	384	12.47	0.41	n/a	0.3	0.7	0.13	0.03	35	7	28	0.13	1	5	4	2	11	13	0	16	0
PM 1	S	393	8.3	411	11.50	0.02	n/a	0.0	0.1	0.12	0.03	36	2	34	0.19	3	21	5	1	9	8	1	20	0
PM 2	P	321	6.5	330	12.48	0.94	37.71	0.4	1.1	0.25	0.04	58	13	45	0.07	1	1	6	0	6	19	0	12	0
PM 2	S	357	7.6	372	12.39	0.30	n/a	0.4	1.1	0.27	0.05	59	14	45	0.16	3	25	5	0	8	11	0	17	0
PM 3	P	372	7.7	366	12.89	0.37	36.57	0.3	0.9	0.39	0.06	49	11	38	0.03	1	11	13	0	6	10	0	15	0
PM 3	S	372	8.3	369	12.68	0.65	38.10	0.3	0.9	0.43	0.09	49	15	34	0.13	3	156	12	0	17	17	0	12	1
PM 4	P	360	7.5	360	12.32	0.72	35.64	0.2	0.9	0.21	0.04	41	10	31	0.02	2	1	10	0	14	15	0	9	0
PM 4	S	384	8.6	393	12.19	0.61	34.48	0.2	0.8	0.26	0.06	43	12	31	0.09	2	21	9	0	12	7	0	11	0
LM 1	P	384	8.1	387	12.97	0.76	34.33	0.2	0.7	0.41	na	37	9	28	0.32	n/a	9	10	0	313	124	0	6	0
LM 1	S	384	8.4	390	12.99	0.60	n/a	0.2	0.6	0.44	na	37	12	25	0.37	n/a	46	13	0	117	59	0	11	0
LM 2	P	393	7.7	414	11.97	0.05	35.94	0.2	0.3	0.15	0.02	18	3	15	0.00	1	3	6	0	64	41	0	5	0
LM 2	S	399	7.9	420	11.82	0.08	n/a	0.1	0.3	0.16	0.02	18	3	15	0.00	0	4	8	0	65	36	0	6	0
LM 3	P	375	7.8	378	12.58	0.27	n/a	0.2	0.6	0.31	0.03	35	9	26	0.03	0	14	11	0	266	85	0	13	0
LM 3	S	378	7.8	381	12.57	1.25	n/a	0.2	0.6	0.32	0.04	36	9	27	0.00	2	21	10	0	196	39	0	11	0
LM 4	P	375	8.1	372	12.66	0.92	33.41	0.3	0.5	0.33	0.07	32	8	24	0.00	1	5	6	1	72	74	1	6	0
LM 4	S	405	8.6	402	12.74	0.68	na	0.3	0.4	0.36	0.10	32	9	23	0.04	1	27	1	1	19	20	0	9	0
LM 5	P	402	7.8	411	12.17	0.19	34.21	0.4	0.6	0.32	0.03	33	8	25	0.02	2	10	6	0	10	15	0	18	0
LM 5	S	384	7.4	384	12.57	0.26	n/a	0.4	0.5	0.33	0.04	33	8	25	0.01	2	46	6	0	4	6	0	16	0
MF1	P	na	na	na	11.32	0.21	37.55	0.2	0.4	0.07	0.05	22	7	15	0.01	3	7	21	0	40	26	0	10	0
MF1	S	na	na	na	11.33	0.25	36.53	0.2	0.3	0.16	0.12	22	8	14	0.09	5	82	21	0	20	15	0	10	0
MF 2	P	378	7.9	396	11.46	0.13	35.70	0.1	0.6	0.22	0.11	28	6	22	0.00	5	1	6	0	12	12	0	13	0
MF 2	S	360	8.3	366	11.25	0.42	37.30	0.3	0.5	0.32	0.21	28	9	19	0.10	26	71	6	0	55	36	0	10	1
MF 3	P	360	7.6	369	11.99	0.80	37.29	0.2	0.7	0.18	0.10	37	7	30	0.00	6	1	9	0	6	10	0	18	0
MF 3	S	363	8.0	375	11.91	0.58	36.99	0.2	0.6	0.20	0.11	37	10	27	0.01	7	21	10	0	4	11	0	15	0
MF 4	P	357	7.3	363	12.01	0.22	37.65	0.2	0.8	0.27	0.15	39	9	30	0.03	12	3	9	0	11	26	0	18	0
MF 4	S	366	8.3	375	12.02	0.46	38.57	0.3	0.6	0.30	0.17	39	13	26	0.08	12	84	10	0	40	33	0	12	1
MF 5	P	na	na	na	11.48	0.03	n/a	0.1	0.2	0.07	0.04	11	4	7	0.00	5	1	3	1	440	575	1	12	1
MF 5	S	na	na	na	11.31	0.04	n/a	0.1	0.3	0.07	0.05	11	4	7	0.01	5	5	2	1	67	118	0	4	0

1. AM ~ adolescent male; LM ~ large mature male; PrF ~ prepubescent female; MF ~ mature female
2. P = plasma; S = serum
3. Units for: sodium (Na), chloride (Cl), potassium (K), calcium (Ca), phosphorus (Phos), magnesium (Mg), urea, glucose (Gluc), cholesterol (Chol), triglyceride (Trig), and lactate are mmol/L.
4. Units for total protein (TP), albumin (Alb),globulin (Glob) and lactate (Lact) are g/L.
5. Units for uric acid are µmol/L

Appendix D: Descriptive Statistics, by Tissue and Group, for Enzyme Activity per Gram of Tissue Wet Weight for Fall 2011 Crabs.

Crab Group = Adolescent Male (AM)

TissGp	stats	AMYww	LIPww	ALPww	ASTww	ALTww	GGTww	GDww	SDHww
Heart	mean	0.05	0.03	0.06	60.24	34.95	0.02	0.60	0.02
	median	0.03	0.03	0.04	56.88	32.25	0.01	0.56	0.02
	min	0.01	0.01	0.01	42.71	30.28	0.01	0.39	0.00
	max	0.13	0.05	0.16	84.48	45.03	0.06	0.90	0.05
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP	mean	0.72	0.18	0.70	13.49	10.14	1.08	0.36	0.09
	median	0.68	0.16	0.64	9.28	6.25	1.15	0.26	0.03
	min	0.43	0.14	0.40	4.70	4.43	0.33	0.20	0.00
	max	1.09	0.25	1.12	30.68	23.63	1.69	0.70	0.28
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Muscle	mean	0.03	0.05	0.03	51.81	42.39	0.01	3.64	0.00
	median	0.03	0.06	0.02	48.17	37.54	0.01	3.03	0.00
	min	0.01	0.01	0.02	38.20	33.44	0.01	1.84	0.00
	max	0.05	0.07	0.07	72.71	61.03	0.02	6.67	0.01
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INT	mean	0.13	0.11	0.19	13.58	17.87	0.15	0.63	0.04
	median	0.13	0.11	0.15	13.71	18.28	0.12	0.38	0.04
	min	0.07	0.08	0.09	12.09	9.76	0.03	0.24	0.00
	max	0.20	0.15	0.38	14.83	25.15	0.32	1.52	0.10
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Testes	mean	0.09	0.41	230.66	12.35	18.53	0.05	0.22	0.04
	median	0.08	0.40	226.31	11.56	18.06	0.04	0.22	0.02
	min	0.06	0.21	152.06	10.50	15.83	0.01	0.17	0.00
	max	0.15	0.65	317.98	15.80	22.16	0.13	0.26	0.13
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
EPI	mean	0.09	0.02	0.08	8.45	26.37	0.08	0.32	0.03
	median	0.07	0.01	0.04	8.80	27.41	0.04	0.31	0.03
	min	0.02	0.01	0.04	6.57	20.99	0.02	0.29	0.00
	max	0.19	0.04	0.19	9.62	29.65	0.19	0.38	0.06
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Appendix D (continued)

Crab Group = Large Mature Male (LM)

TissGp	stats	AMYww	LIPww	ALPww	ASTww	ALTww	GGTww	GDww	SDHww
Heart	mean	0.09	0.03	0.69	47.64	18.53	0.00	0.48	0.02
	median	0.10	0.02	0.01	43.99	14.50	0.00	0.45	0.01
	min	0.06	0.02	0.00	32.78	11.09	0.00	0.34	0.01
	max	0.12	0.04	2.75	69.79	34.01	0.00	0.68	0.03
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP	mean	2.29	0.27	0.47	4.69	3.74	0.81	0.11	0.32
	median	1.67	0.27	0.37	5.20	4.48	0.93	0.11	0.31
	min	1.47	0.16	0.26	2.06	0.89	0.37	0.08	0.23
	max	4.36	0.38	0.87	6.31	5.13	1.00	0.12	0.44
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Muscle	mean	0.03	0.06	0.01	75.03	63.17	0.00	2.88	0.00
	median	0.03	0.06	0.01	77.76	65.06	0.00	2.46	0.00
	min	0.02	0.03	0.00	37.06	36.61	0.00	1.18	0.00
	max	0.04	0.09	0.05	107.54	85.94	0.00	5.41	0.01
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INT	mean	0.39	0.10	0.18	6.39	5.76	0.05	0.47	0.08
	median	0.35	0.10	0.11	6.41	5.63	0.05	0.44	0.08
	min	0.18	0.06	0.02	6.17	5.13	0.02	0.33	0.07
	max	0.69	0.13	0.51	6.58	6.68	0.08	0.68	0.09
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Testes	mean	0.07	0.16	106.70	5.79	6.45	0.00	0.13	0.03
	median	0.07	0.15	0.00	5.87	5.83	0.00	0.13	0.03
	min	0.06	0.12	0.00	5.08	5.61	0.00	0.08	0.01
	max	0.10	0.23	426.80	6.36	8.53	0.01	0.17	0.04
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
EPI	mean	0.22	0.02	0.11	4.62	13.97	0.02	0.17	0.04
	median	0.16	0.02	0.01	4.54	13.53	0.02	0.16	0.04
	min	0.09	0.00	0.01	3.96	12.72	0.01	0.13	0.03
	max	0.49	0.03	0.40	5.45	16.09	0.04	0.22	0.06
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Appendix D (continued)

Crab Group = Prepubescent Female (PrF)

TissGp	stats	AMYww	LIPww	ALPww	ASTww	ALTww	GGTww	GDww	SDHww
Heart	mean	0.04	0.02	0.00	64.28	41.94	0.02	0.68	0.04
	median	0.04	0.02	0.00	66.69	43.18	0.02	0.71	0.04
	min	0.03	0.02	0.00	47.03	37.48	0.01	0.56	0.03
	max	0.05	0.03	0.01	76.72	43.92	0.03	0.73	0.06
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP	mean	0.72	0.18	0.60	15.35	12.39	0.99	0.52	0.44
	median	0.65	0.20	0.56	15.26	11.96	0.89	0.52	0.43
	min	0.51	0.09	0.46	10.96	6.98	0.71	0.50	0.32
	max	1.07	0.24	0.84	19.93	18.64	1.47	0.56	0.57
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Muscle	mean	0.03	0.05	0.00	63.51	57.14	0.01	4.27	0.01
	median	0.03	0.05	0.00	69.60	55.84	0.01	3.98	0.01
	min	0.01	0.04	0.00	26.46	48.70	0.00	1.97	0.00
	max	0.04	0.07	0.01	88.39	68.17	0.02	7.16	0.03
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INT	mean	0.08	0.15	0.03	13.12	14.49	0.06	0.57	0.12
	median	0.08	0.16	0.02	14.23	14.03	0.05	0.55	0.11
	min	0.03	0.09	0.01	7.56	12.37	0.04	0.36	0.06
	max	0.12	0.18	0.07	16.46	17.54	0.12	0.83	0.21
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Ovary	mean	0.04	0.00	0.00	3.11	3.89	0.02	0.03	0.03
	median	0.04	0.00	0.00	3.46	3.83	0.01	0.03	0.03
	min	0.02	0.00	0.00	2.00	3.22	0.00	0.01	0.02
	max	0.07	0.00	0.02	3.54	4.70	0.05	0.05	0.03
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
EPI	mean	0.13	0.02	0.02	7.32	26.33	0.07	0.41	0.07
	p50	0.12	0.01	0.01	7.64	25.71	0.05	0.41	0.07
	min	0.04	0.01	0.00	5.42	17.56	0.04	0.37	0.05
	max	0.22	0.03	0.08	8.57	36.32	0.13	0.47	0.07
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Appendix D (continued)

Crab Group = Mature Female (MF)

TissGp	stats	AMYww	LIPww	ALPww	ASTww	ALTww	GGTww	GDww	SDHww
Heart	mean	0.06	0.00	0.00	37.77	18.20	0.01	0.48	0.04
	median	0.06	0.00	0.00	40.22	18.75	0.01	0.46	0.04
	min	0.03	0.00	0.00	27.50	16.34	0.00	0.36	0.03
	max	0.08	0.01	0.00	43.15	18.97	0.03	0.65	0.05
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP	mean	1.02	1.02	0.34	3.86	2.21	0.98	0.40	0.17
	median	1.15	0.22	0.29	4.46	2.27	0.92	0.11	0.12
	min	0.02	0.12	0.20	0.40	1.30	0.73	0.09	0.09
	max	1.73	3.53	0.60	6.13	3.00	1.33	1.30	0.33
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Muscle	mean	0.04	0.02	0.01	65.81	52.78	0.01	3.23	0.00
	median	0.04	0.01	0.01	64.59	53.23	0.01	3.37	0.00
	min	0.02	0.01	0.00	55.72	43.56	0.01	2.18	0.00
	max	0.07	0.03	0.01	78.33	61.09	0.02	3.99	0.00
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INT	mean	0.20	0.12	0.01	5.76	5.75	0.03	0.52	0.08
	median	0.16	0.13	0.01	5.64	5.80	0.04	0.55	0.09
	min	0.07	0.05	0.00	4.90	5.49	0.01	0.30	0.05
	max	0.40	0.16	0.01	6.85	5.93	0.05	0.68	0.11
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Ovary	mean	0.11	0.00	0.00	3.40	5.15	0.02	0.05	0.02
	median	0.12	0.00	0.00	3.29	5.56	0.02	0.05	0.02
	min	0.06	0.00	0.00	2.88	2.39	0.00	0.03	0.02
	max	0.14	0.00	0.00	4.12	7.09	0.04	0.07	0.03
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
EPI	mean	0.30	0.01	0.01	6.32	15.21	0.05	0.20	0.05
	median	0.31	0.01	0.01	6.11	15.66	0.04	0.15	0.05
	min	0.17	0.00	0.01	5.00	11.51	0.02	0.11	0.03
	max	0.43	0.02	0.02	8.07	18.01	0.11	0.37	0.07
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Appendix E: Descriptive Statistics, by Tissue and Group, for Enzyme Activity per Gram of Total Protein in Lysate Supernatants for Fall 2011 Crabs.

Crab Group = Adolescent Male (AM)

TissGp	stats	AMYtp	LIPtp	ALPtp	ASTtp	ALTtp	GGTtp	GDtp	SDHtp
Heart	mean	0.98	0.75	1.87	1428.41	816.59	0.44	13.77	0.41
	median	0.77	0.54	0.86	1446.12	840.41	0.29	13.84	0.40
	min	0.23	0.17	0.20	844.07	530.50	0.20	6.77	0.00
	max	2.17	1.74	5.57	1977.35	1055.05	1.00	20.62	0.83
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP	mean	41.24	10.10	40.14	734.77	550.25	62.48	19.58	4.82
	median	38.67	8.73	34.72	554.87	373.26	66.54	15.33	1.78
	min	21.94	7.87	23.67	264.04	248.88	16.84	11.83	0.00
	max	65.66	15.06	67.47	1565.31	1205.61	100.00	35.82	15.73
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Muscle	mean	0.77	1.19	0.88	1289.27	1058.12	0.40	89.51	0.06
	median	0.74	1.37	0.51	1211.81	961.22	0.39	76.15	0.00
	min	0.23	0.28	0.45	1110.47	947.78	0.22	50.97	0.00
	max	1.39	1.74	2.03	1622.99	1362.28	0.58	154.76	0.22
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INT	mean	2.56	2.19	4.09	273.72	348.82	2.95	11.98	0.80
	median	2.90	2.14	3.59	254.19	333.25	2.91	9.44	0.60
	min	1.10	2.01	1.51	240.06	224.11	0.55	4.10	0.00
	max	3.34	2.47	7.69	346.42	504.68	5.42	24.96	2.01
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HLS	mean	72.32	2.65	9.41	96.09	193.87	0.55	7.42	0.07
	median	50.52	2.79	9.22	93.14	201.90	0.27	7.39	0.00
	min	28.33	0.00	0.86	46.43	144.94	0.00	6.83	0.00
	max	159.89	5.00	18.33	151.67	226.74	1.67	8.07	0.30
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Testes	mean	1.31	5.37	3174.10	164.35	247.07	0.79	2.98	0.62
	median	1.17	5.67	3411.04	163.85	246.41	0.58	2.89	0.30
	min	0.63	3.13	1634.63	148.18	238.25	0.13	2.30	0.00
	max	2.30	7.00	4239.70	181.51	257.20	1.88	3.84	1.88
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
EPI	mean	1.03	0.28	0.94	110.95	345.92	0.88	4.33	0.31
	median	0.94	0.12	0.70	98.60	306.69	0.70	3.41	0.25
	min	0.33	0.10	0.44	82.13	262.38	0.22	3.22	0.00
	max	1.90	0.77	1.90	164.48	507.92	1.90	7.30	0.75
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Appendix E (continued)

Crab Group = Large Mature Male (LM)

TissGp	stats	AMYtp	LIPtp	ALPtp	ASTtp	ALTtp	GGTtp	GDtp	SDHtp
Heart	mean	6.35	1.71	41.08	3249.75	1257.41	0.00	32.63	1.22
	median	6.66	1.61	0.31	3089.91	1154.78	0.00	30.55	1.21
	min	4.76	1.23	0.00	2665.04	695.68	0.00	27.56	0.60
	max	7.32	2.38	163.69	4154.17	2024.40	0.00	41.85	1.85
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP	mean	164.79	18.65	34.12	326.58	261.80	59.90	7.47	22.61
	median	129.40	17.48	28.99	355.18	310.24	68.14	7.50	24.42
	min	82.12	11.68	14.53	150.36	64.96	20.67	5.69	16.79
	max	318.25	27.94	63.97	445.59	361.76	82.64	9.19	24.79
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Muscle	mean	1.03	1.91	0.46	2523.85	2133.98	0.00	97.32	0.10
	median	0.95	1.88	0.17	2499.02	2097.72	0.00	79.52	0.00
	min	0.68	1.15	0.00	1414.50	1397.33	0.00	44.85	0.00
	max	1.53	2.73	1.52	3682.88	2943.15	0.00	185.41	0.38
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INT	mean	20.69	4.65	10.05	316.94	288.46	2.56	23.91	4.00
	median	17.08	4.68	4.36	311.76	257.09	2.22	19.12	3.69
	min	7.29	3.59	0.94	266.40	239.68	1.03	16.87	3.24
	max	41.32	5.63	30.54	377.84	400.00	4.79	40.54	5.39
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HLS	mean	203.12	1.96	0.16	133.39	334.96	0.00	5.14	0.00
	median	199.20	1.15	0.00	135.05	306.95	0.00	5.29	0.00
	min	122.22	0.00	0.00	120.41	276.39	0.00	4.17	0.00
	max	291.84	5.56	0.64	143.06	449.56	0.00	5.82	0.00
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Testes	mean	0.64	1.40	820.77	52.14	58.80	0.03	1.17	0.22
	median	0.65	1.33	0.00	53.31	53.38	0.00	1.18	0.23
	min	0.50	1.20	0.00	42.33	43.15	0.00	0.61	0.10
	max	0.77	1.77	3283.08	59.60	85.30	0.10	1.70	0.31
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
EPI	mean	15.43	1.14	7.39	324.26	984.41	1.62	11.84	3.10
	median	10.02	1.26	0.79	326.70	977.87	1.57	11.66	2.81
	min	8.11	0.00	0.58	270.35	829.65	0.58	9.24	2.68
	max	33.56	2.01	27.40	373.29	1152.25	2.74	14.79	4.11
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Appendix E (continued)

Crab Group = Prepubescent Female (PrF)

TissGp	stats	AMYtp	LIPtp	ALPtp	ASTtp	ALTtp	GGTtp	GDtp	SDHtp
Heart	mean	0.80	0.48	0.05	1346.37	894.26	0.37	14.28	0.85
	median	0.80	0.46	0.00	1331.08	901.11	0.31	14.03	0.80
	min	0.56	0.37	0.00	1172.82	699.25	0.21	13.60	0.56
	max	1.05	0.63	0.19	1550.53	1075.56	0.63	15.45	1.26
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP	mean	28.86	7.39	23.94	610.45	486.74	38.91	21.16	17.46
	median	29.13	7.68	23.51	591.39	432.34	38.36	19.81	16.71
	min	19.77	3.38	19.38	509.79	359.79	27.52	19.32	14.34
	max	37.41	10.82	29.37	749.25	722.48	51.40	25.67	22.09
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Muscle	mean	0.62	1.20	0.11	1427.98	1296.26	0.23	98.09	0.36
	median	0.67	1.20	0.11	1572.67	1301.65	0.24	96.50	0.34
	min	0.25	0.85	0.00	619.67	1140.52	0.00	41.59	0.00
	max	0.88	1.54	0.23	1946.92	1441.23	0.44	157.80	0.75
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INT	mean	1.57	2.84	0.60	254.57	286.64	1.28	11.08	2.38
	median	1.59	3.07	0.35	267.11	274.68	0.93	10.83	1.91
	min	0.60	1.90	0.30	167.41	226.92	0.77	7.88	1.27
	max	2.50	3.31	1.39	316.67	370.28	2.50	14.77	4.44
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HLS	mean	44.48	1.12	0.00	57.84	166.26	0.23	5.05	0.00
	median	45.85	1.17	0.00	51.76	164.52	0.21	5.16	0.00
	min	25.13	0.51	0.00	44.32	141.62	0.00	3.99	0.00
	max	61.08	1.62	0.00	83.51	194.36	0.51	5.90	0.00
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Ovary	mean	0.32	0.00	0.04	22.99	29.05	0.13	0.23	0.19
	median	0.29	0.00	0.00	25.25	28.51	0.07	0.23	0.19
	min	0.15	0.00	0.00	15.38	23.00	0.00	0.09	0.14
	max	0.54	0.00	0.15	26.08	36.15	0.38	0.35	0.23
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
EPI	mean	1.43	0.17	0.28	81.08	293.49	0.76	4.59	0.72
	median	1.44	0.12	0.06	80.67	290.04	0.50	4.64	0.74
	min	0.40	0.10	0.00	67.75	195.11	0.40	4.17	0.63
	max	2.44	0.33	1.00	95.22	398.75	1.63	4.93	0.78
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Appendix E (continued)

Crab Group = Mature Female (MF)

TissGp	stats	AMYtp	LIPtp	ALPtp	ASTtp	ALTtp	GGTtp	GDtp	SDHtp
Heart	mean	1.73	0.08	0.00	1192.09	583.07	0.31	15.52	1.20
	median	1.55	0.00	0.00	1180.16	576.73	0.14	16.55	1.15
	min	1.20	0.00	0.00	1104.42	522.59	0.00	9.83	0.86
	max	2.62	0.33	0.00	1303.61	656.22	0.98	19.16	1.64
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HP	mean	92.91	9.50	19.77	232.84	86.49	54.48	5.27	8.27
	median	87.88	9.47	16.62	241.84	86.57	52.99	5.29	5.86
	min	38.97	6.15	15.08	133.33	40.00	37.63	4.56	4.44
	max	156.89	12.89	30.77	314.36	132.82	74.30	5.93	16.92
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Muscle	mean	1.05	0.44	0.12	1641.22	1316.50	0.31	80.49	0.00
	median	0.98	0.38	0.12	1565.92	1307.98	0.25	81.90	0.00
	min	0.52	0.24	0.00	1414.21	1075.56	0.24	55.41	0.00
	max	1.73	0.74	0.26	2018.81	1574.48	0.51	102.76	0.00
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INT	mean	6.30	3.89	0.16	193.11	192.62	1.07	17.54	2.84
	median	5.23	4.05	0.15	196.36	195.89	1.12	18.46	2.97
	min	2.52	1.69	0.00	149.85	177.68	0.36	10.07	1.68
	max	12.23	5.76	0.34	229.87	201.02	1.68	23.15	3.73
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
HLS	mean	115.72	0.00	0.00	58.43	181.53	0.15	5.00	0.00
	median	124.74	0.00	0.00	58.25	181.95	0.00	4.37	0.00
	min	59.84	0.00	0.00	50.00	158.33	0.00	2.73	0.00
	max	153.57	0.00	0.00	67.21	203.91	0.60	8.51	0.00
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Ovary	mean	0.88	0.00	0.00	27.41	42.21	0.14	0.39	0.18
	median	0.93	0.00	0.00	27.08	46.33	0.11	0.39	0.17
	min	0.50	0.00	0.00	21.14	17.07	0.00	0.23	0.17
	max	1.17	0.00	0.00	34.33	59.08	0.33	0.57	0.21
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
EPI	mean	6.89	0.20	0.32	140.18	348.67	1.25	4.01	1.08
	median	6.93	0.24	0.30	136.42	348.41	0.79	3.63	1.11
	min	4.42	0.00	0.27	127.49	225.28	0.43	3.00	0.82
	max	9.26	0.32	0.43	160.39	472.58	3.00	5.78	1.28
	N	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

Appendix F: Descriptive Statistics, by Tissue and Group, for Enzyme Activity per Gram of Tissue Wet Weight for Spring 2012 Crabs

Results for Tissue = EPI

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMYww	MF	5	0.1638	0.0342	0.0765	0.0649	0.1587	0.2667
	LM	4	0.2670	0.1390	0.2790	0.0920	0.1470	0.6830
	PM	4	0.3300	0.0883	0.1766	0.0973	0.3541	0.5145
LIP ww	MF	5	0.03860	0.02230	0.05000	0.00000	0.03130	0.12200
	LM	4	0.00832	0.00618	0.01237	0.00000	0.00355	0.02618
	PM	4	0.04580	0.00710	0.01419	0.03488	0.04082	0.06667
ALP ww	MF	5	0.04066	0.00661	0.01477	0.02667	0.03968	0.06494
	LM	4	0.01520	0.00608	0.01217	0.00654	0.01081	0.03261
	PM	4	0.09190	0.05100	0.10210	0.00870	0.06640	0.2260
AST ww	MF	5	3.422	0.420	0.939	2.662	2.927	4.747
	LM	4	13.5	10.8	21.6	2.0	3.1	45.9
	PM	4	5.216	0.254	0.508	4.509	5.376	5.603
ALT ww	MF	5	13.37	1.69	3.78	8.57	13.09	19.18
	LM	4	15.13	9.30	18.59	3.46	7.11	42.83
	PM	4	15.68	3.02	6.04	10.74	14.35	23.28
GGTww	MF	5	0.00627	0.00627	0.01402	0.00000	0.00000	0.03135
	LM	4	0.01807	0.00349	0.00699	0.01309	0.01542	0.02837
	PM	4	0.03061	0.00810	0.01620	0.01622	0.02694	0.05233
GDww	MF	5	0.2842	0.0364	0.0815	0.1786	0.3008	0.3896
	LM	4	0.6950	0.5530	1.1070	0.1130	0.1560	2.3540
	PM	4	0.3351	0.0661	0.1322	0.2222	0.3146	0.4890
SDH ww	MF	5	0.00627	0.00627	0.01402	0.00000	0.00000	0.03135
	LM	4	0.05800	0.01910	0.03820	0.02840	0.04470	0.11410
	PM	4	0.08147	0.00598	0.01196	0.06667	0.08164	0.09593

Appendix F (continued)

Results for Tissue = H

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMYww	MF	5	0.0562	0.0157	0.0350	0.0200	0.0588	0.1064
	LM	5	0.1153	0.0429	0.0960	0.0299	0.0736	0.2256
	PM	4	0.0581	0.0214	0.0427	0.0213	0.0486	0.1139
LIP ww	MF	5	0.01697	0.00735	0.01645	0.00000	0.02000	0.03546
	LM	5	0.02200	0.01090	0.02450	0.00000	0.01470	0.05330
	PM	4	0.02000	0.01250	0.02500	0.00000	0.01420	0.05170
ALP ww	MF	5	0.01824	0.00759	0.01697	0.00000	0.02632	0.03546
	LM	5	0.02760	0.02550	0.05710	0.00000	0.00000	0.12950
	PM	4	0.01101	0.00637	0.01273	0.00000	0.01064	0.02278
AST ww	MF	5	29.16	3.71	8.30	18.21	29.18	41.52
	LM	5	52.71	4.74	10.61	37.29	53.55	66.87
	PM	4	45.19	3.73	7.47	34.62	47.47	51.19
ALT ww	MF	5	19.30	2.52	5.63	9.82	20.25	24.70
	LM	5	21.56	2.78	6.21	14.49	21.66	30.75
	PM	4	20.57	1.12	2.25	17.70	20.86	22.86
GGTww	MF	5	0.00400	0.00400	0.00894	0.00000	0.00000	0.02000
	LM	5	0.00199	0.00199	0.00445	0.00000	0.00000	0.00996
	PM	4	0.00862	0.00862	0.01724	0.00000	0.00000	0.03448
GDww	MF	5	0.636	0.122	0.273	0.316	0.592	0.965
	LM	5	0.749	0.243	0.542	0.387	0.595	1.702
	PM	4	0.528	0.125	0.251	0.269	0.520	0.805
SDH ww	MF	5	0.00748	0.00460	0.01029	0.00000	0.00000	0.02000
	LM	5	0.02467	0.00394	0.00881	0.01473	0.02649	0.03553
	PM	4	0.02672	0.00300	0.00600	0.02128	0.02555	0.03448

Appendix F (continued)

Results for Tissue = HLS

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMYww	MF	4	4.413	0.929	1.857	2.586	4.364	6.338
	LM	5	7.43	1.72	3.84	3.95	6.00	13.84
	PM	4	7.09	2.16	4.32	3.72	5.86	12.91
LIP ww	MF	4	0.1022	0.0316	0.0631	0.0259	0.1125	0.1579
	LM	5	0.3209	0.2540	0.5690	0.0000	0.0860	1.3330
	PM	4	0.0478	0.0196	0.0392	0.0000	0.0501	0.0909
ALP ww	MF	4	0.0322	0.0195	0.0391	0.0000	0.0250	0.0789
	LM	5	0.0390	0.0181	0.0404	0.0000	0.0341	0.0857
	PM	4	0.0237	0.0146	0.0292	0.0000	0.0174	0.0600
AST ww	MF	4	4.970	0.400	0.799	3.850	5.157	5.716
	LM	5	5.94	1.05	2.35	4.24	5.14	10.00
	PM	4	5.330	0.286	0.571	4.605	5.426	5.864
ALT ww	MF	4	11.722	0.972	1.943	9.316	12.017	13.538
	LM	5	15.61	4.85	10.85	7.59	9.41	33.33
	PM	4	9.823	0.706	1.412	8.455	9.898	11.043
GGTww	MF	4	0.00647	0.00647	0.01293	0.00000	0.00000	0.02586
	LM	5	0.00682	0.00682	0.01525	0.00000	0.00000	0.03409
	PM	4	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
GDww	MF	4	0.2555	0.0727	0.1455	0.1100	0.2366	0.4388
	LM	5	0.2144	0.0436	0.0976	0.0619	0.2667	0.2966
	PM	4	0.2031	0.0220	0.0439	0.1500	0.2084	0.2457
SDH ww	MF	4	0.00647	0.00647	0.01293	0.00000	0.00000	0.02586
	LM	5	0.01110	0.00709	0.01585	0.00000	0.00000	0.03409
	PM	4	0.03100	0.01060	0.02110	0.00000	0.03920	0.04550

Appendix F (continued)

Results for Tissue = HP

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMYww	MF	5	1.088	0.119	0.266	0.771	1.213	1.392
	LM	5	1.065	0.235	0.524	0.615	0.837	1.940
	PM	4	0.833	0.143	0.286	0.603	0.766	1.197
LIP ww	MF	5	0.1379	0.0551	0.1232	0.0262	0.0803	0.3342
	LM	5	0.2146	0.0454	0.1014	0.1061	0.2152	0.3723
	PM	4	0.2390	0.1090	0.2190	0.0240	0.2510	0.4310
ALP ww	MF	5	1.4030	0.2670	0.5980	0.6020	1.5100	2.2260
	LM	5	0.2636	0.0587	0.1312	0.1194	0.2766	0.4481
	PM	4	0.3181	0.0858	0.1716	0.2019	0.2522	0.5663
AST ww	MF	5	12.38	2.69	6.01	6.55	10.85	20.55
	LM	5	12.25	3.34	7.47	4.14	14.23	19.40
	PM	4	14.16	5.18	10.35	3.75	12.32	28.24
ALT ww	MF	5	3.617	0.974	2.178	1.504	3.089	7.247
	LM	5	7.58	2.08	4.65	0.73	8.39	12.71
	PM	4	8.49	3.91	7.82	3.38	5.25	20.05
GGTww	MF	5	0.751	0.109	0.243	0.415	0.812	1.057
	LM	5	0.990	0.270	0.603	0.284	1.108	1.749
	PM	4	1.043	0.199	0.399	0.746	0.906	1.615
GDww	MF	5	0.1827	0.0442	0.0989	0.0373	0.1916	0.3154
	LM	5	0.3671	0.0718	0.1605	0.1521	0.3418	0.5923
	PM	4	0.2464	0.0808	0.1616	0.1429	0.1783	0.4863
SDH ww	MF	5	0.2787	0.0338	0.0756	0.1799	0.2945	0.3484
	LM	5	0.5214	0.0973	0.2176	0.2730	0.5123	0.7463
	PM	4	0.6083	0.0822	0.1645	0.3767	0.6614	0.7336

Appendix F (continued)

Results for Tissue = INT

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMYww	MF	5	0.2307	0.0291	0.0651	0.1627	0.2566	0.3125
	LM	5	0.3100	0.1270	0.2840	0.1420	0.1920	0.8150
	PM	4	0.3188	0.0632	0.1263	0.1579	0.3254	0.4667
LIP ww	MF	5	0.1775	0.0172	0.0384	0.1382	0.1875	0.2250
	LM	5	0.0644	0.0238	0.0531	0.0000	0.0710	0.1376
	PM	4	0.1085	0.0609	0.1218	0.0000	0.0838	0.2667
ALP ww	MF	5	0.0237	0.0118	0.0265	0.0000	0.0197	0.0625
	LM	5	0.0303	0.0128	0.0286	0.0000	0.0385	0.0568
	PM	4	0.4150	0.1600	0.3200	0.0530	0.4150	0.7800
AST ww	MF	5	11.61	2.17	4.85	6.66	11.33	19.57
	LM	5	7.05	1.08	2.42	3.96	7.61	9.75
	PM	4	9.10	1.51	3.01	5.42	9.42	12.13
ALT ww	MF	5	14.13	2.09	4.66	8.40	13.26	20.38
	LM	5	12.66	1.90	4.24	5.54	14.47	15.74
	PM	4	15.66	2.86	5.72	11.02	13.81	24.00
GGTww	MF	5	0.1052	0.0203	0.0454	0.0625	0.0987	0.1807
	LM	5	0.0616	0.0274	0.0612	0.0000	0.0385	0.1563
	PM	4	0.1269	0.0331	0.0662	0.0526	0.1275	0.2000
GDww	MF	5	0.2892	0.0589	0.1316	0.1937	0.2230	0.5145
	LM	5	0.1707	0.0209	0.0468	0.0969	0.1685	0.2202
	PM	4	0.2289	0.0884	0.1769	0.0833	0.1762	0.4800
SDH ww	MF	5	0.0392	0.0115	0.0258	0.0000	0.0395	0.0723
	LM	5	0.0560	0.0114	0.0255	0.0281	0.0459	0.0923
	PM	4	0.05498	0.00418	0.00835	0.04688	0.05320	0.06667

Appendix F (continued)

Results for Tissue = M

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMYww	MF	5	0.01308	0.00588	0.01314	0.00000	0.01381	0.02928
	LM	5	0.03323	0.00875	0.01957	0.00000	0.03690	0.05091
	PM	4	0.01076	0.00369	0.00738	0.00000	0.01370	0.01565
LIP ww	MF	5	0.04562	0.00926	0.02071	0.01464	0.04464	0.07018
	LM	5	0.09690	0.04370	0.09770	0.00000	0.08230	0.24250
	PM	4	0.07750	0.04260	0.08530	0.00000	0.05700	0.19610
ALP ww	MF	5	0.01720	0.00731	0.01634	0.00000	0.02232	0.03509
	LM	5	0.00545	0.00230	0.00513	0.00000	0.00727	0.01078
	PM	4	0.06890	0.05980	0.11960	0.00000	0.01380	0.24810
AST ww	MF	5	61.45	6.66	14.89	37.99	66.60	77.47
	LM	5	58.66	5.35	11.95	46.01	55.75	76.73
	PM	4	68.49	5.17	10.33	53.21	72.87	75.01
ALT ww	MF	5	58.35	5.41	12.10	40.18	58.96	74.02
	LM	5	52.77	6.40	14.31	30.44	60.71	64.50
	PM	4	59.23	8.83	17.66	33.46	65.21	73.02
GGTww	MF	5	0.00276	0.00276	0.00618	0.00000	0.00000	0.01381
	LM	5	0.01912	0.00669	0.01495	0.00727	0.01175	0.04310
	PM	4	0.00388	0.00388	0.00775	0.00000	0.00000	0.01550
GDww	MF	5	1.787	0.277	0.619	1.355	1.522	2.877
	LM	5	1.962	0.308	0.690	1.150	2.013	2.665
	PM	4	1.843	0.307	0.613	1.233	1.741	2.657
SDH ww	MF	5	0.02013	0.00607	0.01356	0.00000	0.02232	0.03509
	LM	5	0.00865	0.00450	0.01006	0.00000	0.00727	0.02425
	PM	4	0.02468	0.00454	0.00908	0.01190	0.02776	0.03130

Appendix F (continued)

Results for Tissue = Ovary

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMYww	MF	5	0.3541	0.0879	0.1965	0.1515	0.3101	0.6667
LIP ww	MF	5	0.1158	0.0160	0.0358	0.0775	0.1070	0.1656
ALP ww	MF	5	0.0725	0.0570	0.1276	0.0000	0.0303	0.2989
AST ww	MF	5	11.436	0.939	2.099	9.174	11.093	13.857
ALT ww	MF	5	14.59	2.40	5.38	6.97	17.42	19.28
GGTww	MF	5	0.01831	0.00891	0.01993	0.00000	0.01529	0.04598
GDww	MF	5	0.4176	0.0364	0.0815	0.3165	0.4109	0.5103
SDH ww	MF	5	0.03727	0.00649	0.01452	0.02299	0.03311	0.06116

Results for Tissue = Testes

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMYww	LM	5	0.04530	0.00571	0.01276	0.02720	0.04630	0.06211
	PM	4	0.05504	0.00905	0.01810	0.03623	0.05291	0.07813
LIP ww	LM	5	0.2390	0.0404	0.0903	0.1498	0.2176	0.3704
	PM	4	0.3620	0.1060	0.2120	0.1270	0.3470	0.6250
ALP ww	LM	5	15.0	14.0	31.3	0.0	0.0	70.9
	PM	4	66.8	66.8	133.5	0.0	0.0	267.0
AST ww	LM	5	6.644	0.825	1.845	4.896	6.713	9.582
	PM	4	12.08	2.81	5.63	4.22	13.62	16.88
ALT ww	LM	5	8.29	1.27	2.85	5.56	7.87	12.24
	PM	4	13.70	3.59	7.18	3.26	15.92	19.69
GGTww	LM	5	0.01107	0.00898	0.02008	0.00000	0.00000	0.04630
	PM	4	0.01197	0.00731	0.01462	0.00000	0.00906	0.02976
GDww	LM	5	0.1843	0.0817	0.1827	0.0535	0.1024	0.4907
	PM	4	0.1661	0.0488	0.0975	0.0344	0.1862	0.2578
SDH ww	LM	5	0.0318	0.0118	0.0263	0.0000	0.0409	0.0624
	PM	4	0.0580	0.0161	0.0322	0.0181	0.0622	0.0893

Appendix G: Descriptive Statistics, by Tissue and Group, for Enzyme Activity per Gram of Total Protein in Lysate Supernatants for Spring 2012 Crabs.

Results for Tissue = EPI

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMY tp	MF	5	11.55	2.69	6.01	5.00	10.26	19.61
	LM	4	17.07	8.84	17.68	4.06	10.55	43.12
	PM	4	22.59	4.71	9.43	8.45	27.29	27.31
LIP tp	MF	5	2.75	1.73	3.87	0.00	1.82	9.38
	LM	4	0.657	0.456	0.911	0.000	0.347	1.932
	PM	4	2.943	0.664	1.327	1.852	2.686	4.545
ALP tp	MF	5	2.894	0.576	1.287	1.818	2.564	5.000
	LM	4	0.778	0.119	0.238	0.483	0.806	1.015
	PM	4	2.57	1.81	3.62	0.46	0.94	7.95
AST tp	MF	5	237.9	29.7	66.4	174.4	225.0	349.0
	LM	4	515	305	611	147	243	1429
	PM	4	329.7	58.8	117.6	239.4	296.4	486.6
ALT tp	MF	5	915.4	78.0	174.3	660.0	964.7	1112.7
	LM	4	670	232	463	255	546	1334
	PM	4	826	154	307	579	756	1214
GGTtp	MF	5	0.364	0.364	0.813	0.000	0.000	1.818
	LM	4	1.292	0.506	1.011	0.508	0.942	2.778
	PM	4	2.309	0.323	0.646	1.408	2.525	2.778
GDtp	MF	5	20.33	3.44	7.68	11.54	23.13	30.00
	LM	4	26.6	15.6	31.2	8.3	12.3	73.3
	PM	4	17.71	3.65	7.29	12.08	15.69	27.39
SDH tp	MF	5	0.364	0.364	0.813	0.000	0.000	1.818
	LM	4	3.116	0.206	0.412	2.752	3.080	3.553
	PM	4	5.443	0.548	1.097	4.545	5.093	7.042

Appendix G (continued)

Results for Tissue = H

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMY tp	MF	5	4.51	1.09	2.45	1.41	4.60	8.11
	LM	5	6.85	2.26	5.06	1.65	6.49	12.05
	PM	4	3.20	1.13	2.27	1.43	2.51	6.33
LIP tp	MF	5	1.322	0.583	1.304	0.000	1.408	2.703
	LM	5	1.202	0.529	1.182	0.000	1.299	2.479
	PM	4	0.993	0.617	1.233	0.000	0.714	2.542
ALP tp	MF	5	1.730	0.724	1.618	0.000	2.500	3.448
	LM	5	1.93	1.82	4.08	0.00	0.00	9.22
	PM	4	0.726	0.426	0.852	0.000	0.633	1.639
AST tp	MF	5	2356	133	297	1934	2386	2739
	LM	5	3195	122	274	2862	3113	3602
	PM	4	2556	103	206	2286	2592	2757
ALT tp	MF	5	1543.1	98.3	219.8	1286.2	1543.2	1857.5
	LM	5	1306	121	270	934	1278	1667
	PM	4	1173.7	63.7	127.3	1095.7	1117.6	1363.9
GGTtp	MF	5	0.282	0.282	0.630	0.000	0.000	1.408
	LM	5	0.142	0.142	0.317	0.000	0.000	0.709
	PM	4	0.424	0.424	0.847	0.000	0.000	1.695
GDtp	MF	5	51.75	8.98	20.08	31.55	41.38	73.51
	LM	5	42.27	9.26	20.70	31.06	34.16	79.17
	PM	4	29.88	6.11	12.21	13.22	32.87	40.57
SDH tp	MF	5	0.5120	0.3160	0.7060	0.0000	0.0000	1.4080
	LM	5	1.4930	0.2030	0.4550	0.8930	1.4930	2.1280
	PM	4	1.5072	0.0988	0.1976	1.2658	1.5340	1.6949

Appendix G (continued)

Results for Tissue = HLS

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMY tp	MF	5	145.1	38.8	86.8	67.6	133.1	275.9
	LM	5	177.5	41.0	91.6	90.0	153.7	326.3
	PM	4	172.7	57.9	115.8	76.8	149.0	316.0
LIP tp	MF	5	2.840	0.745	1.667	0.676	3.571	4.762
	LM	5	5.40	3.76	8.41	0.00	2.02	20.00
	PM	4	1.088	0.402	0.805	0.000	1.283	1.786
ALP tp	MF	5	0.916	0.385	0.861	0.000	1.205	1.786
	LM	5	0.907	0.416	0.931	0.000	0.847	2.020
	PM	4	0.567	0.334	0.669	0.000	0.485	1.299
AST tp	MF	5	132.8	11.2	25.0	105.5	122.2	167.5
	LM	5	138.2	17.6	39.3	100.0	128.3	200.0
	PM	4	123.19	2.85	5.69	115.18	124.72	128.16
ALT tp	MF	5	310.0	28.3	63.3	210.7	340.5	365.1
	LM	5	335.5	58.6	131.0	178.8	348.1	500.0
	PM	4	229.7	22.3	44.6	166.1	241.3	270.2
GGTtp	MF	5	0.376	0.245	0.548	0.000	0.000	1.205
	LM	5	0.169	0.169	0.379	0.000	0.000	0.847
	PM	4	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
GDtp	MF	5	5.79	1.01	2.25	3.49	4.94	9.21
	LM	5	4.816	0.913	2.043	2.407	4.000	7.373
	PM	4	4.739	0.580	1.161	3.247	4.850	6.011
SDH tp	MF	5	0.135	0.135	0.302	0.000	0.000	0.676
	LM	5	0.271	0.174	0.390	0.000	0.000	0.847
	PM	4	0.732	0.246	0.493	0.000	0.932	1.064

Appendix G (continued)

Results for Tissue = HP

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMY tp	MF	5	56.97	6.88	15.38	43.20	51.72	82.54
	LM	5	39.5	13.3	29.8	13.3	28.3	87.2
	PM	4	32.76	5.80	11.59	23.53	28.90	49.73
LIP tp	MF	5	7.83	3.77	8.43	1.27	4.76	22.41
	LM	5	6.639	0.967	2.161	3.857	6.600	9.906
	PM	4	9.51	4.47	8.94	1.13	9.63	17.65
ALP tp	MF	5	73.2	12.7	28.4	29.1	76.9	104.3
	LM	5	10.41	4.20	9.40	2.80	5.37	25.85
	PM	4	12.63	3.65	7.30	7.98	9.50	23.53
AST tp	MF	5	632	117	262	334	525	979
	LM	5	436	144	323	77	335	872
	PM	4	529	152	305	146	543	884
ALT tp	MF	5	177.7	33.8	75.7	100.9	149.4	289.9
	LM	5	283.8	79.7	178.1	12.3	321.1	483.0
	PM	4	308	113	225	141	232	628
GGTtp	MF	5	39.90	6.87	15.35	24.60	39.24	56.90
	LM	5	35.1	11.7	26.1	8.6	29.3	63.9
	PM	4	40.93	7.52	15.04	31.02	34.84	63.03
GDtp	MF	5	8.92	1.70	3.80	2.50	9.76	12.62
	LM	5	11.33	1.64	3.66	7.14	10.50	15.37
	PM	4	9.19	2.16	4.33	5.94	7.81	15.22
SDH tp	MF	5	14.39	1.41	3.14	10.76	13.92	17.75
	LM	5	18.89	4.89	10.93	4.57	19.73	33.56
	PM	4	24.11	3.58	7.16	14.71	25.63	30.48

Appendix G (continued)

Results for Tissue = INT

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMY tp	MF	5	9.78	2.00	4.48	4.62	9.63	15.15
	LM	5	14.05	4.89	10.93	6.90	9.09	33.33
	PM	4	13.57	2.52	5.04	10.45	11.39	21.05
LIP tp	MF	5	7.117	0.768	1.716	5.185	7.143	9.091
	LM	5	2.90	1.02	2.28	0.00	3.45	5.45
	PM	4	3.22	1.41	2.82	0.00	3.46	5.97
ALP tp	MF	5	1.015	0.553	1.237	0.000	1.020	3.030
	LM	5	1.655	0.712	1.591	0.000	2.299	3.571
	PM	4	20.3	10.9	21.8	3.6	13.3	50.9
AST tp	MF	5	452.6	59.1	132.1	252.3	442.4	587.8
	LM	5	325.7	25.3	56.6	247.6	326.8	398.9
	PM	4	389.7	51.1	102.2	271.6	383.9	519.3
ALT tp	MF	5	577	112	251	318	498	988
	LM	5	605	104	232	348	592	968
	PM	4	695	132	264	405	728	919
GGTtp	MF	5	3.984	0.340	0.759	3.030	3.977	5.128
	LM	5	3.36	1.72	3.84	0.00	2.40	9.82
	PM	4	5.51	1.69	3.38	3.45	4.02	10.53
GDtp	MF	5	11.55	2.20	4.91	6.21	11.11	19.49
	LM	5	8.064	0.773	1.728	6.058	8.103	10.536
	PM	4	9.00	2.40	4.80	4.83	8.09	15.00
SDH tp	MF	5	1.456	0.379	0.848	0.000	1.705	2.051
	LM	5	2.985	0.902	2.016	1.149	1.818	5.769
	PM	4	2.574	0.560	1.120	1.493	2.616	3.571

Appendix G (continued)

Results for Tissue = M

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMY tp	MF	5	0.4530	0.2130	0.4760	0.0000	0.4100	1.0750
	LM	5	1.4170	0.4080	0.9130	0.0000	1.4490	2.5450
	PM	4	0.2803	0.0940	0.1879	0.0000	0.3606	0.4000
LIP tp	MF	5	1.497	0.318	0.711	0.391	1.717	2.151
	LM	5	3.60	1.43	3.20	0.00	3.85	6.91
	PM	4	1.94	1.05	2.10	0.00	1.45	4.88
ALP tp	MF	5	0.5870	0.2420	0.5410	0.0000	0.8580	1.0750
	LM	5	0.2174	0.0888	0.1985	0.0000	0.3610	0.3636
	PM	4	1.7800	1.5400	3.0800	0.0000	0.3600	6.4000
AST tp	MF	5	1913	109	243	1707	1830	2324
	LM	5	2326	368	823	1384	2300	3588
	PM	4	1780	168	335	1324	1847	2104
ALT tp	MF	5	1834	106	237	1634	1769	2196
	LM	5	2050	315	704	1252	2041	3016
	PM	4	1543	250	499	832	1674	1994
GGTtp	MF	5	0.0820	0.0820	0.1833	0.0000	0.0000	0.4098
	LM	5	0.6750	0.2010	0.4500	0.3610	0.5490	1.4490
	PM	4	0.1000	0.1000	0.2000	0.0000	0.0000	0.4000
GDtp	MF	5	57.09	8.55	19.13	40.20	45.32	82.00
	LM	5	76.3	13.9	31.0	38.7	70.1	124.6
	PM	4	47.53	7.34	14.69	31.80	47.84	62.66
SDH tp	MF	5	0.6605	0.2040	0.4570	0.0000	0.8580	1.0750
	LM	5	0.3130	0.1360	0.3040	0.0000	0.3640	0.6540
	PM	4	0.6250	0.0992	0.1984	0.3521	0.6739	0.8000

Appendix G (continued)

Results for Tissue = Ovary

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMY tp	MF	5	8.83	2.35	5.26	3.55	7.05	17.16
LIP tp	MF	5	2.878	0.467	1.045	1.762	2.642	4.310
ALP tp	MF	5	1.85	1.47	3.29	0.00	0.71	7.69
AST tp	MF	5	278.5	17.4	39.0	226.4	288.8	315.0
ALT tp	MF	5	355.4	56.4	126.2	172.1	407.8	477.6
GGTtp	MF	5	0.454	0.225	0.504	0.000	0.377	1.183
GDtp	MF	5	10.29	1.10	2.45	7.81	9.34	13.14
SDH tp	MF	5	0.911	0.159	0.355	0.592	0.862	1.509

Results for Tissue = Testes

Variable	Group	N	Mean	SE Mean	StDev	Minimum	Median	Maximum
AMY tp	LM	5	0.4554	0.0806	0.1803	0.2143	0.5000	0.6993
	PM	4	0.7090	0.1890	0.3780	0.3080	0.6550	1.2200
LIP tp	LM	5	2.655	0.846	1.892	1.091	1.714	5.594
	PM	4	4.830	1.810	3.610	1.080	4.250	9.760
ALP tp	LM	5	120	113	252	0	0	570
	PM	4	567	567	1134	0	0	2268
AST tp	LM	5	69.7	16.4	36.7	38.6	49.3	117.0
	PM	4	162.0	50.3	100.7	35.8	174.3	263.4
ALT tp	LM	5	89.6	24.9	55.6	43.8	57.3	151.0
	PM	4	184.0	59.0	118.0	27.7	200.5	307.3
GGTtp	LM	5	0.1540	0.1370	0.3060	0.0000	0.0000	0.6990
	PM	4	0.1194	0.0771	0.1543	0.0000	0.0769	0.3236
GDtp	LM	5	2.340	1.330	2.980	0.420	0.750	7.410
	PM	4	2.278	0.819	1.637	0.292	2.398	4.024
SDH tp	LM	5	0.280	0.102	0.229	0.000	0.375	0.500
	PM	4	0.752	0.230	0.459	0.154	0.817	1.220

VII-2. DETERMINATION OF STABILITY OF 23 HEMOLYMPH PLASMA BIOCHEMISTRY PROFILE PARAMETERS

VII-2-1 Summary

Nineteen of the 23 directly measured parameters in the plasma hemolymph biochemistry profiles were shown to be stable, under refrigerated conditions for up to one week, allowing for at-sea sample collection for future studies. Hemolymph samples obtained from immature female (IF), immature male (IM), mature female (MF), and large mature male (LM) snow crabs (*Chionoecetes opilio*) in fall 2011 and from pygmy male (PM), LM and MF crabs in spring 2012 were used for evaluation. Nearly all analytes in the biochemistry profile (calcium, magnesium, urea, glucose, creatinine, cholesterol, triglyceride, lactate, uric acid, total protein, albumin, and the enzymes amylase, lipase, ALP and GGT were considered clinically stable for up to 1 week), with GD, ALT, AST, and SDH the only exceptions. Electrolyte values, while likely stable, are less reproducible due to a manual dilution step required for analysis. Microbial growth was detected in five of the 10 fall 2011 samples and had significant effects on many parameters, highlighting the need for careful selection of crabs used for hemolymph sample collection and attention to aseptic sample collection and handling techniques to minimise this possibility in future projects. Hemolymph plasma biochemistry profiles can be confidently incorporated into future studies of snow crab.

VII-2-2 Objective

To determine the refrigerated stability of 23 analytes making up the biochemistry panel in hemolymph plasma samples prepared from snow crab (*Chionoecetes opilio*) over a one week period. The results will be used to determine the maximum time interval between sample collection and analysis that will provide useful results. To capture data on analytes that may have had seasonal fluctuations with respect to presence/absence and/or degree of activity or concentrations, hemolymph samples were collected from crabs in the fall of 2011 and again in spring of 2012 – times when crabs might be sampled from the fishery.

VII-2-3 Methodology

All procedures and protocols used in this study were approved by the Animal Care Committee of the University of Prince Edward Island, Charlottetown, PE.
The samples in the fall 2011 study included immature male (IM), immature female (IF), large mature male (LM) and mature female (MF) crabs which were more readily caught with trawl nets than in commercial cage traps. Samples from the spring 2012 were from free pygmy male, large mature male, and mature female crabs held in the aquatic facility at the Atlantic Veterinary College for up to four weeks.

Fall 2011 Samples

Thirty snow crabs (n = 9 immature female (IF), 35 – 45 mm carapace width (CW); n = 9 immature male (IM); 35 – 35 mm CW; n = 4 large mature male (LM), 95-135 mm CW; and, n = 8 mature female (MF), 60 – 70 mm CW were collected as part of a DFO research trawl survey, stored in coolers on salt-water ice overnight, collected from Souris, PE, and then transported by motor vehicle to the Atlantic Veterinary College on September 19, 2011. Crabs were placed into a 31.6 ppt salt water (Instant Ocean®, Aquarium Systems Inc., Mentor, OH, USA) recirculation system at 1.5^{o}C for approximately 4 hours.
Hemolymph was collected from the area between the coxa of the first walking leg and the body using a 22G needle and 3 or 10 mL syringe (dependent on the size of crab and expected volume) after surface disinfection with 70% alcohol. Hemolymph was gently transferred to pre-chilled microcentrifuge tubes after removing the needle from the syringe. The samples were centrifuged (3,500 x g, 5 min, at room temperature). The supernatant, 'plasma', was removed using a plastic transfer pipet, leaving a buffer layer above the cell pellet to avoid contaminating the plasma with hemocyte contents thereby reducing the risk of clotting.

For each of the four groups of crab, plasma was transferred to a single 15 mL tube for mixing and reallocation into microfuge tubes (~1.0 mL per tube) for refrigerated storage. There were two pools for IF (5 x 1.0 mL; 4x 1.0 mL), two pools for IM (3 x 1.0 mL, 4 x 1.0 mL), two pools for MF (5 x 1.0 mL, 5 x 1.0 mL), and a sample from each of the four LM crabs (5 x 1.0 mL). All aliquots were refrigerated (2 – 4^{o}C).

The first aliquot (0 h) was submitted to Diagnostic Services, Atlantic Veterinary College, UPEI, (Charlottetown, PE) for evaluation using the automated biochemistry analyser (Cobas c501, Roche Diagnostics Corporation, Indianapolis, IN, USA and associated reagents) on the day of collection. Subsequent aliquots were submitted for evaluation at 24 h, 48 h, 72 h and 1w after collection.

Twenty-three parameters in three general categories were measured: ***Electrolytes and Minerals*** including sodium (Na), potassium (K), chloride(Cl), phosphorus (Phos), magnesium (Mg), calcium (Ca); ***Metabolites*** including urea, uric acid, glucose, total protein (TP), 'albumin'[7] (ALB), creatinine, cholesterol (CHOL), triglyceride (TRIG), lactate; and, ***Enzyme Activity*** of amylase (AMY), lipase (LIP), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), glutamate dehydrogenase (GD), and sorbitol dehydrogenase (SDH). A manual 1:2 dilution of an aliquot of each sample with distilled water was required to determine sodium, chloride, and potassium concentrations for analysis using the ion-selective electrode.

[7] The assay used is designed to detect the vertebrate protein albumin based on albmin's ability to bind to the dye (bromocresol green) used in the assay. The protein(s) bound by the dye in invertebrates has not been determined; however, for simplicity the term 'albumin' is used to describe this fraction.

To estimate expected variation in values due to inherent day-to-day variation, the cumulative Quality Control results (mean value, standard deviation (SD)) for all parameters on the c501 analyser using human-based control samples over a six month period were obtained from Diagnostic Services. As many of the values in the snow crabs are at much different levels than the human controls, the mean and SD results from a separate within-run precision study (20 replicates, same-day analyses) of a single sample using plasma from a LM snow crab were also obtained (A. Battison, unpublished results). The SDs from both studies were used to generate estimates of the range of Total Allowable Error (TAE_{DS} and TAE_{crab}) with lower and upper limits (value 0 hr ±2.8 SD) that could be expected from analyser variation alone (Bellamy & Olexson 2000). Ranges for TAE_{crab} for SDH and GGT were unavailable as there was no activity detected in the test LM crab.

Spring 2012 Samples

Thirty-five snow crabs (n = 15 large mature male, 95-135 mm CW; n= 12 mature female, 60 – 70 mm CW; n= 8 pygmy male; <95 mm CW) were obtained from a trapping survey conducted by DFO and delivered in coolers on salt-water ice to the Atlantic Veterinary College on June 6, 2012. Crabs were held, for three to four weeks, in a recirculation system at 1.5^{o}C, salinity (Instant Ocean®, Aquarium Systems Inc., Mentor, OH, USA) at 31.6 ppt. Water quality (ammonia, nitrate, nitrite, and pH) was monitored every 2 weeks. Crabs were offered thawed shrimp every four days at which point any uneaten shrimp was removed from the tank. Hemolymph samples were collected as described for fall 2011 crabs. All plasma samples were inspected for evidence of clotting (small strings of material) after 10 to 30 min. All non-clotted plasma was transferred to a single 15 mL tube for mixing and reallocation into microfuge tubes (~1.0 mL per tube) for refrigerated storage. Ten plasma samples from individual large mature males (LM, n = 3), individual mature females (MF, n = 2), two pooled mature females (MF_{pooled}, n = 2) and individual pygmy males (PM, n = 3) were evaluated.

The first aliquot (0 h) was submitted to Diagnostic Services for evaluation using the automated biochemistry analyser (Cobas c501, Roche Diagnostics Corporation, Indianapolis, IN, USA and associated reagents) on the day of collection. Subsequent aliquots were submitted for evaluation at 24 h, 48 h and one week after collection.

VII-2-4 Results

Fall 2011 Samples

Initial attempts to collect hemolymph from the crabs when they arrived at the AVC were met with minimal success as only small volumes of hemolymph could be obtained. Consequently, the crabs were left to (partially) rehydrate for ~4 h by placing into the aquatic holding system. Many of the IF and IM crabs had lost legs recently (no pigmentation/melanisation) and there were legs noted in the transport cooler when the crabs were removed.

Opacity was noted in five of the 10 samples after one week (IF pool #2, IM pool #2, MF pool #1, MF pool #2, LM pool #3). Portions of each of these were plated onto blood agar with 3% NaCl at 2^{o}C. Growth was noted, however, Gram stain results were not performed.

The individual results and the calculated lower and upper limits (TAE_{DS} and TAE_{crab}) are presented in Tables 1-3.

Electrolytes and Minerals

The repeatability for the electrolytes sodium, potassium, and chloride was very poor. Almost all values for all time periods failed to fall within the limits set for TAE_{crab} and TAE_{DS}. Calcium and magnesium values fell out of the TAE_{DS} range but not the TAE_{crab} range in most cases. The repeatability for phosphorus in LM was reasonably good, but less so in the other crab groups. There was no obvious correlation with presence/absence of microbial presence and repeatability.

Metabolites

Urea levels were steady over 72 h in all crabs; however, increased at 1 w in all four crabs with microbial contamination which had a 1 w aliquot available for testing. Conversely, glucose was within TAE_{DS} and only marginally (0.1 g/L) above TAE_{crab}, except for sharp decreases at 1 w for all four crabs with 1 w aliquots which contained microbes. One sample (IF pool#1) showed an increased glucose over time. Uric acid showed marginal variability outside TAE_{crab} with slight decreases noted at 1 w in microbe-positive samples. Triglyceride levels were generally stable with some values only slightly above TAE_{crab} in IM, IF, and MF and two LM. Values for triglyceride at 1 w decreased markedly (up to 50%) in three samples, all of which had evidence of microbial growth.

Creatinine was stable in the one sample in which it was detected. 'Albumin' values were stable for one week in all samples. Total protein values tended to increase slightly exceeding TAE_{crab} in seven samples (all IF, n = 1 IM, MF, and n = 2 LM). Cholesterol levels were generally very stable, straying slightly beyond TAE_{crab} in IM, IF and a 1 w value for one MF and one LM.

Enzyme Activity

In almost all instances, activities of AST and ALT increased over the storage period, exceeding both TAE_{DS} and TAE_{crab}. In the sample with the highest AST and ALT activities, values decreased on the final measurement at 1 w. Increases were not associated with presence of microbial growth.
Amylase was essentially stable with only minor deviations beyond TAE_{crab}. The behaviour of lipase was similar, with the exception of two samples (MF pools 1 & 2) which both had marked increases (3 – 11 fold) in the 1 w samples.

ALP was detected in all IM and IF pooled samples and the activity was stable for 72 h. Three samples tested at 1 w showed activity; however only one, IF pool 2, originally had activity at 0 h.

GGT activity was initially detected in three samples – both IF and IM pool 2. All samples with microbial growth showed either increased activity from 0h or, activity where none had previously been detected, in aliquots tested after one week of refrigerated storage.

The activity of GD over time was inconsistent. Nine of ten values had decreased by 24 h. Four of five samples with microbial growth showed increased activity between the second last and the last aliquot measured.

Low levels of SDH were initially detected in IF pool 1 and IF pool 2. Again, however, all three samples with microbial growth and aliquots measured at 1 w showed either increased activity from baseline (IF pool 2) or, activity where none had existed at 0 h (MF 2, LM 3).

Table 1. Electrolytes and Minerals. Change in measured values over time in 10 aliquots of snow crab plasma under refrigerated storage for fall 2011 samples. All assays performed on the Cobas c501 automated biochemistry analyser. Total Allowable Error limits were calculated using the cumulative (6 month) standard deviation values as recorded by Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, for human control samples (Between-Run Precision), and from a precision study (20 replicate analyses) of plasma from a large mature male snow crab (Within-Run Precision). 'Level' indicates the mean value of the analyte tested during the current precision study. Shading indicates samples with microbial growth.

Analyte	Group[1]	Duration of Sample Storage					Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 h	24 h	48 h	72 h	1 w	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
Na	**IF pool 1**	303	390[a, b]	405[a, b]	366[a, b]	–	**298**	**308**	1.7	**297**	**309**	2.041
(mmol/L)	**IF pool 2**	387	432[a, b]	396[a, b]	–	384	**382**	**392**	*(126)*	**381**	**393**	*(437)*
	IM pool 1	420	489[a, b]	402[a, b]	–	–	**415**	**425**		**414**	**426**	
	IM pool 2	357	330[a, b]	354	363[a]	–	**352**	**362**		**351**	**363**	
	MF pool 1	429	357[a, b]	426	387[a, b]	411[a, b]	**424**	**434**		**423**	**435**	
	MF pool 2	432	435	414[a, b]	–	432	**427**	**437**		**426**	**438**	
	LM #1	462	435[a, b]	468[a]	435[a, b]	–	**457**	**467**		**456**	**468**	
	LM #2	456	438[a, b]	417[a, b]	417[a, b]	–	**451**	**461**		**450**	**462**	
	LM #3	480	420[a, b]	426[a, b]	–	423[a, b]	**475**	**485**		**474**	**486**	
	LM #4	447	420[a, b]	420[a, b]	–	462[a, b]	**442**	**452**		**441**	**453**	
K	**IF pool 1**	9.5	11.9[a, b]	12.9[a, b]	11.9[a, b]	–	**9.3**	**9.7**	0.0567	**9.4**	**9.6**	0.0489
(mmol/L)	**IF pool 2**	17.5	17.8	18.6[a, b]	–	17.2	**17.3**	**17.7**	*(3.3)*	**17.4**	**17.6**	*(12.5)*
	IM pool 1	10.4	12.9[a, b]	9.5[a, b]	–	–	**10.2**	**10.6**		**10.3**	**10.5**	
	IM pool 2	13.9	12.7[a, b]	13.9	13.7[b]	–	**13.7**	**14.1**		**13.8**	**14.0**	
	MF pool 1	10.3	8.7[a, b]	9.8[a, b]	9.5[a, b]	10.0[a, b]	**10.1**	**10.5**		**10.2**	**10.4**	
	MF pool 2	9.1	8.7[a, b]	8.3[a, b]	–	9.5[a, b]	**8.9**	**9.3**		**9.0**	**9.2**	
	LM #1	10.8	9.0[a, b]	9.2[a, b]	9.3[a, b]	–	**10.6**	**11.0**		**10.7**	**10.9**	
	LM #2	9.3	8.5[a, b]	8.4[a, b]	8.7[a, b]	–	**9.1**	**9.5**		**9.2**	**9.4**	
	LM #3	10.1	8.9[a, b]	9.1[a, b]	–	9.0[a, b]	**9.9**	**10.3**		**10.0**	**10.2**	
	LM #4	8.7	8.1[a, b]	8.4[a, b]	–	9.2[a, b]	**8.5**	**8.9**		**8.6**	**8.8**	
Cl	**IF pool 1**	321	414[a, b]	462[a, b]	393[a, b]	–	**317**	**325**	1.46	**312**	**330**	3.2541
(mmol/L)	**IF pool 2**	435	423[a, b]	444[b]	–	408	**431**	**439**	*(96)*	**426**	**444**	*(486)*
	IM pool 1	459	528[a, b]	411[a, b]	–	–	**455**	**463**		**450**	**468**	
	IM pool 2	384	348[a, b]	375[b]	387	–	**380**	**388**		**375**	**393**	
	MF pool 1	459	384[a, b]	438[a, b]	414[a, b]	444[a, b]	**455**	**463**		**450**	**468**	
	MF pool 2	462	435[a, b]	408[a, b]	–	468[b]	**458**	**466**		**453**	**471**	
	LM #1	486	438[a, b]	450[a, b]	456[a, b]	–	**482**	**490**		**477**	**495**	
	LM #2	504	438[a, b]	426[a, b]	435[a, b]	–	**500**	**508**		**495**	**513**	
	LM #3	474	417[a, b]	441[a, b]	–	432[a, b]	**470**	**478**		**465**	**483**	
	LM #4	450	423[a, b]	438[a, b]	–	480[a, b]	**446**	**454**		**441**	**459**	

[1] Group: Immature Female (IF); Immature Male (IM); Mature Female (MF); Large Mature Male (LM) [2] Standard Deviation. [a] Indicates value is outside of limits as set by the 'between-run' SD for the analyte. [b] Indicates value is outside of limits as set by the 'within-run' SD for the analyte.

Table 1. Electrolytes and Minerals (continued)

Analyte	Group[1]	Duration of Refrigerated Storage					Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 h	24 h	48 h	72 h	1 w	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
Ca (mmol/L)	**IF pool 1**	15.16	17.83 [a, b]	17.42 [a, b]	17.00 [a, b]	–	**14.99**	**15.33**	0.0615	**14.07**	**16.25**	0.3900
	IF pool 2	18.10	18.55 [a]	18.11	–	17.73 [a]	**17.93**	**18.27**	(3.31)	**17.01**	**19.19**	(15.83)
	IM pool 1	18.57	19.58 [a]	18.67	–	–	**18.40**	**18.74**		**17.48**	**19.66**	
	IM pool 2	17.36	17.38	16.96 [a]	17.11	–	**17.19**	**17.53**		**16.27**	**18.45**	
	MF pool 1	16.83	16.77	16.41 [a]	16.27 [a]	16.99	**16.66**	**17.00**		**15.74**	**17.92**	
	MF pool 2	17.10	16.86 [a]	17.09	–	16.86 [a]	**16.93**	**17.27**		**16.01**	**18.19**	
	LM #1	16.52	16.53	16.22 [a]	15.92 [a]	–	**16.35**	**16.69**		**15.43**	**17.61**	
	LM #2	16.39	17.11 [a]	16.61 [a]	16.84 [a]	–	**16.22**	**16.56**		**15.30**	**17.48**	
	LM #3	16.70	16.55	16.02 [a]	–	11.90 [a, b]	**16.53**	**16.87**		**15.61**	**17.79**	
	LM #4	17.07	17.87 [a]	17.21	–	11.64 [a, b]	**16.90**	**17.24**		**15.98**	**18.16**	
Phos (mmol/L)	**IF pool 1**	2.12	2.18	2.42 [a, b]	2.43 [a, b]	–	**2.00**	**2.24**	0.0419	**2.09**	**2.15**	0.0107
	IF pool 2	3.76	3.82 [b]	3.78	–	6.09 [a, b]	**3.64**	**3.88**	(1.19)	**3.73**	**3.79**	(0.22)
	IM pool 1	2.06	3.08 [a, b]	1.85 [a]	–	–	**1.94**	**2.18**		**2.03**	**2.09**	
	IM pool 2	3.30	3.27	1.84 [a, b]	3.05 [a, b]	–	**3.18**	**3.42**		**3.27**	**3.33**	
	MF pool 1	1.29	1.72 [a, b]	1.39 [b]	2.84 [a, b]	1.36 [b]	**1.17**	**1.41**		**1.26**	**1.32**	
	MF pool 2	1.97	1.28 [a, b]	2.00	–	2.04 [b]	**1.85**	**2.09**		**1.94**	**2.00**	
	LM #1	0.67	0.68	0.69	0.68	–	**0.55**	**0.79**		**0.64**	**0.70**	
	LM #2	1.78	1.77	1.81	2.27 [a, b]	–	**1.66**	**1.90**		**1.75**	**1.81**	
	LM #3	1.71	1.74	1.70	–	1.71	**1.59**	**1.83**		**1.68**	**1.74**	
	LM #4	1.03	1.07 [b]	1.05	–	1.10 [b]	**0.91**	**1.15**		**1.00**	**1.06**	
Mg (mmol/L)	**IF pool 1**	37.76	39.48 [a]	38.95 [a]	39.97 [a]	–	**37.69**	**37.83**	0.0251	**35.45**	**40.07**	0.8259
	IF pool 2	35.66	36.72 [a]	36.58 [a]	–	36.78 [a]	**35.59**	**36.36**	(1.39)	**33.35**	**37.97**	(42.65)
	IM pool 1	39.27	39.88	39.87	–	–	**39.20**	**39.97**		**36.96**	**41.58**	
	IM pool 2	35.91	37.63 [a]	36.95 [a]	37.31 [a]	–	**35.84**	**36.61**		**33.60**	**38.22**	
	MF pool 1	43.41	43.01 [a]	42.94 [a]	44.14 [a]	44.71 [a]	**43.34**	**44.11**		**41.10**	**45.72**	
	MF pool 2	43.25	43.55	43.66	–	43.97 [a]	**43.18**	**43.95**		**40.94**	**45.56**	
	LM #1	34.83	35.46	35.70 [a]	36.27 [a]	–	**34.76**	**35.53**		**32.52**	**37.14**	
	LM #2	36.46	37.24 [a]	36.31 [a]	36.23 [a]	–	**36.39**	**37.16**		**34.15**	**38.77**	
	LM #3	34.59	36.01 [a]	35.35 [a]	–	35.58 [a]	**34.52**	**35.29**		**32.28**	**36.90**	
	LM #4	35.60	36.67 [a]	36.18	–	36.50 [a]	**35.53**	**36.30**		**33.29**	**37.91**	

[1] Group: Immature Female (IF); Immature Male (IM); Mature Female (MF); Large Mature Male (LM)
[2] Standard Deviation
[a] Indicates value is outside of limits as set by the 'between-run' SD for the analyte
[b] Indicates value is outside of limits as set by the 'within-run' SD for the analyte

Table 2. Metabolites. Change in measured values over time in 10 aliquots of snow crab plasma under refrigerated storage for fall 2011 samples. All assays performed on the Cobas c501 automated biochemistry analyser. Total Allowable Error limits were calculated using the cumulative (6 month) standard deviation values as recorded by Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, for human control samples (Between-Run Precision), and from a precision study (20 replicate analyses) of plasma from a large mature male snow crab (Within-Run Precision). 'Level' indicates the mean value of the analyte tested during the current precision study. Shading indicates samples with microbial growth.

Analyte	Group[1]	Duration of Refrigerated Storage					Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 h	24 h	48 h	72 h	1 w	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
urea	**IF pool 1**	0.9	0.8	1.0	1.0	–	**0.0**	**2.1**	0.4240	**0.6**	**1.2**	0.1200
(mmol/L)	**IF pool 2**	0.9	1.1	1.2	–	4.9 [a, b]	**0.0**	**2.1**	(22.84)	**0.6**	**1.2**	(0.12)
	IM pool 1	0.4	0.4	0.5	–	–	**0.0**	**1.6**		**0.1**	**0.7**	
	IM pool 2	1.2	1.2	1.3	1.3	–	**0.0**	**2.4**		**0.9**	**1.5**	
	MF pool 1	0.4	0.4	0.4	0.4	0.8 [b]	**0.0**	**1.6**		**0.1**	**0.7**	
	MF pool 2	0.3	0.3	0.3	–	0.8 [b]	**0.0**	**1.5**		**0.0**	**0.6**	
	LM #1	0.2	0.2	0.3	0.4	–	**0.0**	**1.4**		**0.0**	**0.5**	
	LM #2	0.4	0.4	0.5	0.5	–	**0.0**	**1.6**		**0.0**	**0.7**	
	LM #3	0.3	0.4	0.4	–	0.6	**0.0**	**1.5**		**0.0**	**0.6**	
	LM #4	0.3	0.4	0.4	–	0.5	**0.0**	**1.5**		**0.0**	**0.6**	
Glucose	**IF pool 1**	3.8	4.7 [a, b]	5.0 [a, b]	5.3 [a, b]	–	**3.2**	**4.4**	4.90	**3.8**	**3.0**	0.00
(mmol/L)	**IF pool 2**	5.0	6.2 [a, b]	6.6 [a, b]	–	0.4 [a, b]	**4.4**	**5.6**	(4.9)	**5.0**	**5.0**	(0.3)
	IM pool 1	3.0	3.1 [b]	3.0	–	–	**2.4**	**3.6**		**3.0**	**3.0**	
	IM pool 2	3.5	3.8 [b]	3.8 [b]	4.0 [b]	–	**2.9**	**4.1**		**3.5**	**3.5**	
	MF pool 1	1.0	1.1 [b]	1.1 [b]	1.1 [b]	0.6 [b]	**0.4**	**1.6**		**1.0**	**1.0**	
	MF pool 2	1.2	1.2	1.2	–	0.8 [a, b]	**0.6**	**1.8**		**1.2**	**1.2**	
	LM #1	1.3	1.3	1.4 [b]	1.3	–	**0.7**	**1.9**		**1.3**	**1.3**	
	LM #2	3.9	4.0 [b]	4.0 [b]	4.0 [b]	–	**3.3**	**4.5**		**3.9**	**3.9**	
	LM #3	3.5	3.5	3.5	–	0.3 [a, b]	**2.9**	**4.1**		**3.5**	**3.5**	
	LM #4	1.9	2.0 [b]	1.9	–	1.9	**1.3**	**2.5**		**1.9**	**1.9**	
Creat	**IF pool 1**	0	0	0	0	–	**0**	**9**	3.16	n/a	n/a	0
(µmol/L)	**IF pool 2**	5	3	5	–	0	**0**	**14**	(96.4)			
	IM pool 1	0	0	0	0	–	**0**	**9**				
	IM pool 2	0	0	0	0	–	**0**	**9**				
	MF pool 1	0	0	0	0	0	**0**	**9**				
	MF pool 2	0	0	0	0	0	**0**	**9**				
	LM #1	0	0	0	0	–	**0**	**9**				
	LM #2	0	0	0	0	–	**0**	**9**				
	LM #3	0	0	0	–	0	**0**	**9**				
	LM #4	0	0	0	–	0	**0**	**9**				

[1] Group: Immature Female (IF); Immature Male (IM); Mature Female (MF); Large Mature Male (LM); shaded aliquots had microbial growth., [2] Standard Deviation., [a] Indicates value is outside of limits as set by the 'between-run' SD for the analyte., [b] Indicates value is outside of limits as set by the 'within-run' SD for the analyte.

Table 2. Metabolites. (continued)

Analyte[1]	Group	Duration of Refrigerated Storage					Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 h	24 h	48 h	72 h	1 w	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
Chol (mmol/L)	**IF pool 1**	0.55	0.58 [b]	0.59 [b]	0.60 [b]	–	**0.32**	**0.78**	0.0837	**0.53**	**0.57**	0.0075
	IF pool 2	0.60	0.64 [b]	0.68 [b]	–	0.70 [b]	**0.37**	**0.83**	(4.72)	**0.58**	**0.62**	(0.20)
	IM pool 1	0.74	0.79 [b]	0.79 [b]	–	–	**0.51**	**0.97**		**0.72**	**0.76**	
	IM pool 2	0.49	0.53 [b]	0.53 [b]	0.53 [b]	–	**0.26**	**0.72**		**0.47**	**0.51**	
	MF pool 1	0.52	0.51	0.51	0.52	0.48 [b]	**0.29**	**0.75**		**0.50**	**0.54**	
	MF pool 2	0.72	0.71	0.71	–	0.74	**0.49**	**0.95**		**0.70**	**0.74**	
	LM #1	0.30	0.27 [b]	0.30	0.30	–	**0.07**	**0.53**		**0.28**	**0.32**	
	LM #2	0.39	0.39	0.40	0.40	–	**0.16**	**0.62**		**0.37**	**0.41**	
	LM #3	0.35	0.35	0.35	–	0.36	**0.12**	**0.58**		**0.33**	**0.37**	
	LM #4	0.25	0.24	0.25	–	0.31 [b]	**0.02**	**0.48**		**0.23**	**0.27**	
Trig (mmol/L)	**IF pool 1**	0.35	0.38 [b]	0.39 [b]	0.40 [b]	–	**0.23**	**0.47**	0.0424	**0.34**	**0.36**	0.0051
	IF pool 2	0.70	0.80 [b]	0.82 [b]	–	0.33 [a, b]	**0.58**	**0.82**	(2.30)	**0.69**	**0.71**	(0.04)
	IM pool 1	0.19	0.22 [b]	0.23 [b]	–	–	**0.07**	**0.31**		**0.18**	**0.20**	
	IM pool 2	0.39	0.43 [b]	0.43 [b]	0.43 [b]	–	**0.27**	**0.51**		**0.38**	**0.40**	
	MF pool 1	0.22	0.24 [b]	0.23	0.24 [b]	0.12 [b]	**0.10**	**0.34**		**0.21**	**0.23**	
	MF pool 2	0.24	0.26 [b]	0.25	–	0.17 [b]	**0.12**	**0.36**		**0.23**	**0.25**	
	LM #1	0.07	0.08	0.09 [b]	0.10 [b]	–	**-0.05**	**0.19**		**0.06**	**0.08**	
	LM #2	0.14	0.13	0.14	0.16 [b]	–	**0.02**	**0.26**		**0.13**	**0.15**	
	LM #3	0.09	0.10	0.10	–	0.09	**-0.03**	**0.21**		**0.08**	**0.10**	
	LM #4	0.05	0.06	0.06	–	0.08 [b]	**-0.07**	**0.17**		**0.04**	**0.06**	
Lactate (mmol/L)	**IF pool 1**	11.06	11.48 [a, b]	11.29 [a, b]	11.40 [a, b]	–	**10.97**	**11.15**	0.0318	**11.04**	**11.08**	0.0079
	IF pool 2	14.65	14.85 [a, b]	14.90 [a, b]	–	13.00 [a, b]	**14.56**	**14.74**	(2.93)	**14.63**	**14.67**	(0.31)
	IM pool 1	17.18	17.20	17.25 [b]	–	–	**17.09**	**17.27**		**17.16**	**17.20**	
	IM pool 2	15.13	15.93 [a, b]	15.41 [a, b]	15.73 [a, b]	–	**15.04**	**15.22**		**15.11**	**15.15**	
	MF pool 1	6.53	6.53	6.54	6.48 [b]	3.90 [a, b]	**6.44**	**6.62**		**6.51**	**6.55**	
	MF pool 2	9.30	9.44 [b]	9.29	–	8.71 [a, b]	**9.21**	**9.39**		**9.28**	**9.32**	
	LM #1	4.30	4.26 [b]	4.29	4.32	–	**4.21**	**4.39**		**4.28**	**4.32**	
	LM #2	7.46	7.52 [b]	7.39 [b]	7.50 [b]	–	**7.37**	**7.55**		**7.44**	**7.48**	
	LM #3	11.17	11.20 [b]	11.03 [a, b]	–	11.33 [a, b]	**11.08**	**11.26**		**11.15**	**11.19**	
	LM #4	7.55	7.66 [a, b]	7.50 [b]	–	7.69	**7.46**	**7.64**		**7.53**	**7.57**	

[1] Group: Immature Female (IF); Immature Male (IM); Mature Female (MF); Large Mature Male (LM); shaded aliquots had microbial growth., [2] Standard Deviation., [a] Indicates value is outside of limits as set by the 'between-run' SD for the analyte., [b] Indicates value is outside of limits as set by the 'within-run' SD for the analyte

Table 2. Metabolites. (continued)

Analyte[1]	Group	Duration of Refrigerated Storage					Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 h	24 h	48 h	72 h	1 w	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
Uric Acid (µmol/L)	**IF pool 1**	217	223[b]	222[b]	226[b]	–	**203**	**231**	4.9	**217**	**219**	0.8507
	IF pool 2	227	228	228	–	211[a, b]	**213**	**241**	(269)	**227**	**229**	(6)
	IM pool 1	250	256[b]	253[b]	–	–	**236**	**264**		**250**	**252**	
	IM pool 2	249	255[b]	254[b]	251	–	**235**	**263**		**249**	**251**	
	MF pool 1	201	204[b]	205[b]	198[b]	167[a, b]	**187**	**215**		**201**	**203**	
	MF pool 2	201	199[b]	200	–	194[b]	**187**	**215**		**201**	**203**	
	MM #1	76	78	77	78	–	**62**	**90**		**76**	**78**	
	LM #2	115	102[b]	98[b]	101[b]	–	**101**	**129**		**115**	**117**	
	LM #3	107	107	100[b]	–	89[a, b]	**93**	**121**		**107**	**109**	
	LM #4	150	153[b]	151	–	145[b]	**136**	**164**		**150**	**152**	
TP (g/L)	**IF pool 1**	51	54[b]	54[b]	54[b]	–	**48**	**54**	0.9300	**50**	**52**	0.4104
	IF pool 2	56	58[b]	59[b]	–	59[b]	**53**	**59**	(45)	**55**	**57**	(18)
	IM pool 1	58	61[b]	60[b]	–	–	**55**	**61**		**57**	**59**	
	IM pool 2	55	55	55	55	–	**52**	**58**		**54**	**56**	
	MF pool 1	44	48[b]	48[b]	46[b]	47[b]	**41**	**47**		**43**	**45**	
	MF pool 2	55	57[b]	56	–	57[b]	**52**	**58**		**54**	**56**	
	LM #1	20	21	21	20	–	**17**	**23**		**19**	**21**	
	LM #2	27	28	28	27	–	**24**	**30**		**26**	**28**	
	LM #3	23	25[b]	25[b]	–	25[b]	**20**	**26**		**22**	**24**	
	LM #4	23	25[b]	25[b]	–	25[b]	**20**	**26**		**22**	**24**	
Alb (g/L)	**IF pool 1**	16	15	15	16	–	**14**	**18**	0.6300	**14**	**18**	0.5712
	IF pool 2	16	16	16	–	16	**14**	**18**	(27)	**14**	**18**	(4)
	IM pool 1	16	16	16	–	–	**14**	**18**		**14**	**18**	
	IM pool 2	15	15	15	16	–	**13**	**17**		**13**	**17**	
	MF pool 1	10	10	10	9	10	**8**	**12**		**8**	**12**	
	MF pool 2	11	11	10	–	10	**9**	**13**		**9**	**13**	
	LM #1	5	6	5	5	–	**3**	**7**		**3**	**7**	
	LM #2	8	8	8	8	–	**6**	**10**		**6**	**10**	
	LM #3	6	6	6	–	6	**4**	**8**		**4**	**8**	
	LM #4	6	6	6	–	7	**4**	**8**		**4**	**8**	

[1] Group: Immature Female (IF); Immature Male (IM); Mature Female (MF); Large Mature Male (LM); shaded aliquots had microbial growth., [2] Standard Deviation., [a] Indicates value is outside of limits as set by the 'between-run' SD for the analyte., [b] Indicates value is outside of limits as set by the 'within-run' SD for the analyte

Table 3. **Enzyme Activities.** Change in measured values over time in 10 aliquots of snow crab plasma under refrigerated storage for fall 2011 samples. All assays performed on the Cobas c501 automated biochemistry analyser. Total Allowable Error limits were calculated using the cumulative (6 month) standard deviation values as recorded by Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, for human control samples (Between-Run Precision), and from a precision study (20 replicate analyses) of plasma from a large mature male snow crab (Within-Run Precision). 'Level' indicates the mean value of the analyte tested during the current precision study. Shading indicates samples with microbial growth.

Analyte[1]	Group	Duration of Refrigerated Storage					Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 h	24 h	48 h	72 h	1 w	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
AMY (U/L)	**IF pool 1**	49	47[b]	47[b]	47[b]	–	**38**	**60**	3.9300	**48**	**50**	0.4702
	IF pool 2	82	78[b]	78[b]	–	78[b]	**71**	**93**	(76)	**81**	**83**	(35)
	IM pool 1	20	20	20	–	–	**9**	**31**		**19**	**21**	
	IM pool 2	52	52	52	52	–	**41**	**63**		**51**	**53**	
	MF pool 1	40	42[b]	42[b]	41[b]	42[b]	**29**	**51**		**39**	**41**	
	MF pool 2	14	14	14	–	14	**3**	**25**		**13**	**15**	
	LM #1	19	19	19	19	–	**8**	**30**		**18**	**20**	
	LM #2	17	17	17	17	–	**6**	**28**		**16**	**18**	
	LM #3	16	16	17	–	20[b]	**5**	**27**		**15**	**17**	
	LM #4	39	40	40	–	40	**28**	**50**		**38**	**40**	
LIP (U/L)	**IF pool 1**	10	11	11	9	–	**0**	**24**	5.0600	**7**	**13**	0.9445
	IF pool 2	18	17	14[b]	–	12[b]	**4**	**32**	(110)	**15**	**21**	(14)
	IM pool 1	4	6	4	–	–	**0**	**18**		**1**	**7**	
	IM pool 2	11	11	11	11	–	**0**	**25**		**8**	**14**	
	MF pool 1	8	10	7	7	112[a,b]	**0**	**22**		**5**	**11**	
	MF pool 2	6	6	5	–	17[b]	**0**	**20**		**3**	**9**	
	LM #1	15	13	12	12	–	**1**	**29**		**12**	**18**	
	LM #2	5	5	4	3	–	**0**	**19**		**2**	**8**	
	LM #3	2	4	2	–	5	**0**	**16**		**0**	**5**	
	LM #4	10	7	5[b]	–	6[b]	**0**	**24**		**7**	**13**	
ALP (U/L)	**IF pool 1**	4	3	3	4	–	**0**	**17**	4.7800	**3**	**5**	0.2236
	IF pool 2	5	5	5	–	8[b]	**0**	**18**	(202)	**4**	**6**	(0)
	IM pool 1	2	1	1	–	–	**0**	**15**		**1**	**3**	
	IM pool 2	18	19	18	18	–	**0**	**31**		**17**	**19**	
	MF pool 1	0	0	0	0	4[b]	**0**	**13**		**0**	**1**	
	MF pool 2	0	0	0	–	4[b]	**0**	**13**		**0**	**1**	
	LM #1	0	0	0	0	–	**0**	**13**		**0**	**1**	
	LM #2	0	0	0	0	–	**0**	**13**		**0**	**1**	
	LM #3	0	0	0	–	0	**0**	**13**		**0**	**1**	
	LM #4	0	0	0	–	0	**0**	**13**		**0**	**1**	

[1] Group: Immature Female (IF); Immature Male (IM); Mature Female (MF); Large Mature Male (LM); shaded aliquots had microbial growth., [2] Standard Deviation., [a] Indicates value is outside of limits as set by the 'between-run' SD for the analyte., [b] Indicates value is outside of limits as set by the 'within-run' SD for the analyte.

Table 3. **Enzyme Activities.** (continued)

Analyte[1]	Group	Duration of Refrigerated Storage					Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 h	24 h	48 h	72 h	1 w	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
AST (U/L)	**IF pool 1**	2393	2542 [a, b]	2593 [a, b]	2662 [a, b]	–	**2384**	**2402**	3.2200 (131)	**2363**	**2423**	10.5431 (552)
	IF pool 2	5924	6011 [a, b]	6077 [a, b]	–	3977 [a, b]	**5915**	**5933**		**5894**	**5954**	
	IM pool 1	1437	1543 [a, b]	1580 [a, b]	–	–	**1428**	**1446**		**1407**	**1467**	
	IM pool 2	3150	3392 [a, b]	3413 [a, b]	3378 [a, b]	–	**3141**	**3159**		**3120**	**3180**	
	MF pool 1	172	201 [a]	211 [a, b]	219 [a, b]	260 [a, b]	**163**	**181**		**142**	**202**	
	MF pool 2	242	269 [a]	282 [a, b]	–	319 [a, b]	**233**	**251**		**212**	**272**	
	LM #1	722	819 [a, b]	881 [a, b]	904 [a, b]	–	**713**	**731**		**692**	**752**	
	LM #2	572	654 [a, b]	679 [a, b]	699 [a, b]		**563**	**581**		**542**	**602**	
	LM #3	348	374 [a]	384 [a, b]	–	473 [a, b]	**339**	**357**		**318**	**378**	
	LM #4	496	564 [a, b]	592 [a, b]	–	685 [a, b]	**487**	**505**		**466**	**526**	
ALT (U/L)	**IF pool 1**	823	1322 [a, b]	1571 [a, b]	1653 [a, b]	–	**815**	**831**	2.7100 (118)	**797**	**849**	9.2258 (128)
	IF pool 2	2741	3557 [a, b]	3970 [a, b]	–	938 [a, b]	**2733**	**2749**		**2715**	**2767**	
	IM pool 1	477	644 [a, b]	727 [a, b]	–	–	**469**	**485**		**451**	**503**	
	IM pool 2	1168	1816 [a, b]	2092 [a, b]	2117 [a, b]	–	**1160**	**1176**		**1142**	**1194**	
	MF pool 1	55	100 [a, b]	111 [a, b]	121 [a, b]	127 [a, b]	**47**	**63**		**29**	**81**	
	MF pool 2	69	138 [a, b]	150 [a, b]	–	134 [a, b]	**61**	**77**		**43**	**95**	
	LM #1	212	330 [a, b]	405 [a, b]	428 [a, b]	–	**204**	**220**		**186**	**238**	
	LM #2	191	349 [a, b]	450 [a, b]	500 [a, b]	–	**183**	**199**		**165**	**217**	
	LM #3	77	106 [a, b]	121 [a, b]	–	117 [a, b]	**69**	**85**		**51**	**103**	
	LM #4	180	245 [a, b]	276 [a, b]	–	275 [a, b]	**172**	**188**		**154**	**206**	
GGT (U/L)	**IF pool 1**	3	3	3	3	–	**0**	**12**	3.2600 (174)	**N/A**	**N/A**	0.0000 (0)
	IF pool 2	5	6	5	–	8	**0**	**14**				
	IM pool 1	0	0	1 [b]	–	–	**0**	**9**				
	IM pool 2	8	7	7	7	–	**0**	**17**				
	MF pool 1	0	0	0	0	3	**0**	**9**				
	MF pool 2	0	0	0	–	2	**0**	**9**				
	LM #1	0	0	0	0	–	**0**	**9**				
	LM #2	0	0	0	0	–	**0**	**9**				
	LM #3	0	0	0	–	2	**0**	**9**				
	LM #4	0	0	0	–	0	**0**	**9**				

[1] Group: Immature Female (IF); Immature Male (IM); Mature Female (MF); Large Mature Male (LM); shaded aliquots had microbial growth., [2] Standard Deviation., [a] Indicates value is outside of limits as set by the 'between-run' SD for the analyte., [b] Indicates value is outside of limits as set by the 'within-run' SD for the analyte

Table 3. **Enzyme Activities.** (continued)

Analyte[1]	Group	Duration of Refrigerated Storage					Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 h	24 h	48 h	72 h	1 w	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
GD (U/L)	**IF pool 1**	35	17 [a, b]	22 [a, b]	23 [a, b]	–	**35**	**35**	0.0820 (22)	**32**	**38**	1.0267 (5)
	IF pool 2	103	77 [a, b]	45 [a, b]	–	58 [a, b]	**103**	**103**		**100**	**106**	
	IM pool 1	28	16 [a, b]	80 [a, b]	–	–	**28**	**28**		**25**	**31**	
	IM pool 2	49	30 [a, b]	37 [a, b]	29 [a, b]	–	**48**	**49**		**46**	**51**	
	MF pool 1	18	22 [a, b]	16 [a]	6 [a, b]	19 [a]	**17**	**18**		**15**	**20**	
	MF pool 2	12	9 [a]	10 [a]	–	21 [a, b]	**11**	**12**		**9**	**14**	
	LM #1	8	5 [a]	7	4 [a]	–	**7**	**8**		**5**	**11**	
	LM #2	13	8 [a, b]	9 [a, b]	7 [a, b]	–	**12**	**13**		**10**	**15**	
	LM #3	9	7 [a]	3 [a, b]	–	13 [a, b]	**8**	**9**		**6**	**12**	
	LM #4	7	8	8	–	5 [a]	**7**	**8**		**4**	**10**	
SDH (U/L)	**IF pool 1**	3	1	1	1	–	**0**	**7**	1.2900 (7)	**N/A**	**N/A**	0.2236 (0)
	IF pool 2	3	4	2	–	9 [a]	**0**	**7**				
	IM pool 1	1	0	0	–	–	**0**	**5**				
	IM pool 2	1	2	2	1	–	**0**	**5**				
	MF pool 1	1	0	1	1	0	**0**	**5**				
	MF pool 2	0	0	0	–	2	**0**	**4**				
	LM #1	0	0	1	1	–	**0**	**4**				
	LM #2	1	0	0	1	–	**0**	**5**				
	LM #3	0	0	0	–	10 [a]	**0**	**4**				
	LM #4	0	0	0	–	0	**0**	**4**				

[1] Group: Immature Female (IF); Immature Male (IM); Mature Female (MF); Large Mature Male (LM); shaded aliquots had microbial growth., [2] Standard Deviation., [a] Indicates value is outside of limits as set by the 'between-run' SD for the analyte., [b] Indicates value is outside of limits as set by the 'within-run' SD for the analyte

Spring 2012 Samples

Hemolymph plasma was successfully collected from 12 snow crabs to create 10 samples (two pooled) for stability analysis. No clotting was observed when the samples were collected; however, a small amount of clotting was noted in sample MF pool 3 when re-examined after 21 days of storage. The individual results and the calculated lower and upper limits are presented in Tables 4 - 6.

Electrolytes and Minerals

This group showed the most variation, especially for sodium, chloride and phosphorus where values at 24 hr, 48 hr, and one week were usually above or below the limits set for TAE_{crab} and TAE_{DS}. Potassium values showed variation in the MF and PM categories. Calcium values fell out of the TAE_{DS} range but not the TAE_{crab} range. Results for magnesium were generally similar.

Metabolites

Urea, total protein, and albumin did not exceed the limits for either range over the one week time period. Marginal variability was noted for glucose, triglyceride, cholesterol, uric acid, and lactate, although only when compared to TAE_{crab}. The one week sample for LM 23 showed the greatest decrease for the group. Creatinine was not detected in any sample.

Enzyme Activity

Amylase, AST, and for the most part, lipase all remained very stable and did not exceed the estimated limits. ALT activity tended to decrease over time but only exceeded range limits in one sample (PM 21) at one week. Neither GGT, ADH, nor ALP showed any appreciable activity for evaluation.

Table 4. Electrolytes and Minerals. Summary of changes in values over time (0 h, 24 h, 48 h, and one week) for electrolytes and minerals in snow crab hemolymph plasma under refrigerated storage for spring 2012 samples. All assays performed on the Cobas c501 automated biochemistry analyser. Acceptable limits/range for each parameter were calculated using the cumulative (6 month) standard deviation values as recorded by the Diagnostic Services Laboratory, Atlantic Veterinary College, University of Prince Edward Island, for human control samples (represents between-run precision), and from a precision study (20 replicate analyses) of plasma from a large mature male snow crab (represents within-run precision). 'Level' indicates the mean value of the analyte in the tested sample during precision studies.

Analyte	Group[1]	Duration of Refrigerated Storage				Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 hr	24 hr	48 h	1 wk	lower	upper	SD[2] *(level)*	lower	upper	SD *(level)*
Na	**LM 21**	372	369	369	369	**367**	**377**	1.7	**366**	**378**	2.041
(mmol/L)	**LM 22**	378	372 [a]	360 [a,b]	381	**373**	**383**	(126)	**372**	**384**	(437)
	LM 23	375	360 [a,b]	357 [a,b]	357 [a,b]	**370**	**380**		**369**	**381**	
	MF 21	378	369 [a,b]	354 [a,b]	378	**373**	**383**		**372**	**384**	
	MF 22	375	369 [a]	366 [a,b]	375	**370**	**380**		**369**	**381**	
	MF pool 3	396	402 [a]	342 [a,b]	348 [a,b]	**391**	**401**		**390**	**402**	
	MF pool 4	378	378	369 [a,b]	348 [a,b]	**373**	**383**		**372**	**384**	
	PM 21	393	369 [a,b]	366 [a,b]	381 [a,b]	**388**	**398**		**387**	**399**	
	PM 22	363	351 [a,b]	360	372 [a,b]	**358**	**368**		**357**	**369**	
	PM 23	381	378	351 [a,b]	360 [a,b]	**376**	**386**		**375**	**387**	
K	**LM 21**	7.6	7.6	7.6	7.7	**7.4**	**7.8**	0.0567	**7.5**	**7.7**	0.0489
(mmol/L)	**LM 22**	8.5	8.3	8.0	8.3	**8.3**	**8.7**	(3.3)	**8.4**	**8.6**	(12.5)
	LM 23	7.9	7.5	7.5	7.5	**7.7**	**8.1**		**7.8**	**8.0**	
	MF 21	8.4	8.3	8.0 [a]	8.3	**8.2**	**8.6**		**8.3**	**8.5**	
	MF 22	8.2	8.0	7.9 [a,b]	8.1	**8.0**	**8.4**		**8.1**	**8.3**	
	MF pool 3	8.3	8.2	7.2 [a,b]	7.4 [a,b]	**8.1**	**8.5**		**8.2**	**8.4**	
	MF pool 4	7.9	8.0	7.7	7.4 [a,b]	**7.7**	**8.1**		**7.8**	**8.0**	
	PM 21	8.4	7.8 [a,b]	7.7 [a,b]	7.8 [a,b]	**8.2**	**8.6**		**8.3**	**8.5**	
	PM 22	7.8	7.5 [a,b]	7.6 [b]	8.0 [b]	**7.6**	**8.0**		**7.7**	**7.9**	
	PM 23	8.0	8.1	7.5 [a,b]	7.5 [a,b]	**7.8**	**8.2**		**7.9**	**8.1**	
Cl	**LM 21**	363	366	360	372 [a]	**359**	**367**	1.46	**354**	**372**	3.2541
(mmol/L)	**LM 22**	381	375 [a]	354 [a,b]	372 [a]	**377**	**385**	(96)	**372**	**390**	(486)
	LM 23	366	348 [a,b]	354 [a,b]	363	**362**	**370**		**357**	**375**	
	MF 21	396	384 [a,b]	366 [a,b]	387 [a]	**392**	**400**		**387**	**405**	
	MF 22	387	378 [a]	369 [a,b]	387	**383**	**391**		**378**	**396**	
	MF pool 3	414	408 [a]	348 [a,b]	360 [a,b]	**410**	**418**		**405**	**423**	
	MF pool 4	375	384 [a]	378	360 [a,b]	**371**	**379**		**366**	**384**	
	PM 21	396	366 [a,b]	360 [a,b]	366 [a,b]	**392**	**400**		**387**	**405**	
	PM 22	366	354 [a,b]	360 [a]	375 [a]	**362**	**370**		**357**	**375**	
	PM 23	372	384 [a,b]	360 [a,b]	357 [a,b]	**368**	**376**		**363**	**381**	

Table 4. Electrolytes and Minerals (continued)

Analyte	Group	Duration of Refrigerated Storage				Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 hr	24 hr	48 h	1 wk	lower	upper	SD^2 *(level)*	lower	upper	SD *(level)*
Ca	**LM 21**	12.12	12.46 [a]	12.61 [a]	12.56 [a]	**11.95**	**12.29**	0.0615	**11.03**	**13.21**	0.39
(mmol/L)	**LM 22**	12.10	12.19	12.02	12.41 [a]	**11.93**	**12.27**	(3.31)	**11.01**	**13.19**	(15.83)
	LM 23	12.55	12.45	12.46	13.28 [a]	**12.38**	**12.72**		**11.46**	**13.64**	
	MF 21	11.63	11.57	11.40	12.00 [a]	**11.46**	**11.80**		**10.54**	**12.72**	
	MF 22	12.36	12.17 [a]	12.46	12.54 [a]	**12.19**	**12.53**		**11.27**	**13.45**	
	MF pool 3	11.64	11.88 [a]	11.47	12.12 [a]	**11.47**	**11.81**		**10.55**	**12.73**	
	MF pool 4	12.14	12.34 [a]	12.39 [a]	12.71 [a]	**11.97**	**12.31**		**11.05**	**13.23**	
	PM 21	12.87	12.42 [a]	12.38 [a]	12.83	**12.70**	**13.04**		**11.78**	**13.96**	
	PM 22	12.40	12.18 [a]	12.16 [a]	12.24 [a]	**12.23**	**12.57**		**11.31**	**13.49**	
	PM 23	12.33	12.37	12.15 [a]	12.83 [a]	**12.16**	**12.50**		**11.24**	**13.42**	
Phos	**LM 21**	0.56	0.19 [a, b]	0.37 [a, b]	0.27 [a, b]	**0.39**	**0.73**	0.0419	**0.53**	**0.59**	0.0107
(mmol/L)	**LM 22**	0.66	0.30 [a, b]	0.3 [a, b]	0.97 [a, b]	**0.49**	**0.83**	(1.19)	**0.63**	**0.69**	(0.22)
	LM 23	0.11	0.99 [a, b]	0.55 [a, b]	0.75 [a, b]	**0.00**	**0.28**		**0.08**	**0.14**	
	MF 21	0.30	0.30	0.32	0.26 [b]	**0.13**	**0.47**		**0.27**	**0.33**	
	MF 22	0.36	0.85 [a, b]	0.58 [a, b]	0.51 [b]	**0.19**	**0.53**		**0.33**	**0.39**	
	MF pool 3	0.16	0.53 [a, b]	0.16	0.63 [a, b]	**0.00**	**0.33**		**0.13**	**0.19**	
	MF pool 4	0.62	1.40 [a, b]	0.30 [a, b]	0.61	**0.45**	**0.79**		**0.59**	**0.65**	
	PM 21	0.91	0.95 [b]	0.13 [a, b]	0.98 [b]	**0.74**	**1.08**		**0.88**	**0.94**	
	PM 22	0.70	0.76 [b]	0.84 [b]	0.94 [a, b]	**0.53**	**0.87**		**0.67**	**0.73**	
	PM 23	0.58	0.83 [a, b]	0.78 [a, b]	0.76 [a, b]	**0.41**	**0.75**		**0.55**	**0.61**	
Mg	**LM 21**	32.98	33.85 [a]	33.49 [a]	33.32 [a]	**32.91**	**33.15**	0.0251	**30.67**	**35.29**	0.8259
(mmol/L)	**LM 22**	34.05	34.61 [a]	34.35 [a]	33.92 [a]	**33.98**	**34.22**	(1.39)	**31.74**	**36.36**	(42.65)
	LM 23	34.73	35.52 [a]	34.96 [a]	36.31 [a]	**34.66**	**34.90**		**32.42**	**37.04**	
	MF 21	37.10	37.90 [a]	38.56 [a]	35.88 [a]	**37.03**	**37.27**		**34.79**	**39.41**	
	MF 22	38.13	38.61 [a]	39.03 [a]	38.78 [a]	**38.06**	**38.30**		**35.82**	**40.44**	
	MF pool 3	36.27	36.88 [a]	37.14 [a]	36.60 [a]	**36.20**	**36.44**		**33.96**	**38.58**	
	MF pool 4	36.51	37.47 [a]	37.92 [a]	36.71 [a]	**36.44**	**36.68**		**34.20**	**38.82**	
	PM 21	35.63	35.82 [a]	35.14 [a]	35.87 [a]	**35.56**	**35.80**		**33.32**	**37.94**	
	PM 22	36.58	35.91 [a]	36.68	35.79 [a]	**36.51**	**36.75**		**34.27**	**38.89**	
	PM 23	36.01	37.40 [a]	36.43 [a]	36.38 [a]	**35.94**	**36.18**		**33.70**	**38.32**	

[1] Group ~ Large Mature Male (LM); Mature Female (MF); Pygmy Male (PM)., [2] Standard Deviation
[a] indicates value is outside of limits as set by the 'between-run' SD for the analyte
[b] indicates value is outside of limits as set by the 'within-run' SD for the analyte

Table 5. Metabolites. Summary of changes in values over time (0 h, 24 h, 48 h, and one week) for electrolytes and minerals in snow crab hemolymph plasma under refrigerated storage storage for spring 2012 samples. All assays performed on the Cobas 501 automated biochemistry analyser (Roche Diagnostics Corporation). Acceptable limits/range for each parameter were calculated using the (6 month) standard deviation values as recorded by the Diagnostic Services Laboratory, for human control samples (represents between-run precision), and from a precision study (20 replicate analyses) of plasma from a large mature male snow crab (represents within-run precision). 'Level' indicates the mean value of the analyte in the tested sample during precision studies.

Analyte	Group[1]	Duration of Refrigerated Storage				Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 hr	24 hr	48 h	1 wk	lower	upper	*SD*[2] *(level)*	lower	upper	*SD (level)*
Urea (mmol/L)	**LM 21**	0.2	0.2	0.2	0.3	**0.0**	**1.4**	0.4240	**0.0**	**0.3**	0.0366
	LM 22	0.3	0.3	0.3	0.4	**0.0**	**1.5**	(22.84)	**0.0**	**0.4**	(0.12)
	LM 23	0.3	0.3	0.4	0.4	**0.0**	**1.5**		**0.0**	**0.4**	
	MF 21	0.1	0.2	0.2	0.2	**0.0**	**1.3**		**0.0**	**0.2**	
	MF 22	0.2	0.2	0.2	0.3	**0.0**	**1.4**		**0.0**	**0.3**	
	MF pool 3	0.2	0.2	0.2	0.2	**0.0**	**1.4**		**0.0**	**0.3**	
	MF pool 4	0.3	0.3	0.3	0.2	**0.0**	**1.5**		**0.0**	**0.4**	
	PM 21	0.3	0.3	0.3	0.3	**0.0**	**1.5**		**0.0**	**0.4**	
	PM 22	0.4	0.3	0.3	0.4	**0.0**	**1.6**		**0.0**	**0.5**	
	PM 23	0.3	0.4	0.3	0.3	**0.0**	**1.5**		**0.0**	**0.4**	
Glucose (mmol/L)	**LM 21**	0.8	0.7 [b]	0.7 [b]	0.7 [b]	**0.2**	**2.0**	0.2110	**0.8**	**0.8**	0.0000
	LM 22	0.8	0.8	0.8	0.8	**0.2**	**2.0**	(4.9)	**0.8**	**0.8**	(0.3)
	LM 23	0.8	0.8	0.8	0.5 [b]	**0.2**	**2.0**		**0.8**	**0.8**	
	MF 21	0.6	0.7 [b]	0.7 [b]	0.6	**0.0**	**1.8**		**0.6**	**0.6**	
	MF 22	0.8	0.8	0.9 [b]	0.8	**0.2**	**2.0**		**0.8**	**0.8**	
	MF pool 3	0.6	0.6	0.6	0.6	**0.0**	**1.8**		**0.6**	**0.6**	
	MF pool 4	0.8	0.8	0.8	0.9 [b]	**0.2**	**2.0**		**0.8**	**0.8**	
	PM 21	0.8	0.8	0.9 [b]	0.9 [b]	**0.2**	**2.0**		**0.8**	**0.8**	
	PM 22	1.1	1.1	1.1	1.0 [b]	**0.5**	**2.3**		**1.1**	**1.1**	
	PM 23	0.9	0.9	0.9	0.9	**0.3**	**2.1**		**0.9**	**0.9**	
CHOL (mmol/L)	**LM 21**	0.34	0.33	0.34	0.33	**0.11**	**0.56**	0.0837	**0.32**	**0.36**	0.0075
	LM 22	0.24	0.24	0.26	0.28 [b]	**0.01**	**0.47**	(4.72)	**0.22**	**0.26**	(0.20)
	LM 23	0.55	0.56	0.58 [b]	0.60 [b]	**0.32**	**0.79**		**0.53**	**0.57**	
	MF 21	0.11	0.08	0.11	0.09	**0.00**	**0.31**		**0.09**	**0.13**	
	MF 22	0.38	0.35 [b]	0.38	0.34 [b]	**0.15**	**0.58**		**0.36**	**0.40**	
	MF pool 3	0.18	0.18	0.19	0.15 [b]	**0.00**	**0.41**		**0.16**	**0.20**	
	MF pool 4	0.27	0.27	0.29	0.27	**0.04**	**0.50**		**0.25**	**0.29**	
	PM 21	0.39	0.40	0.43 [b]	0.39	**0.16**	**0.63**		**0.37**	**0.41**	
	PM 22	0.32	0.32	0.32	0.31	**0.09**	**0.55**		**0.30**	**0.34**	
	PM 23	0.17	0.21 [b]	0.22 [b]	0.18	**0.00**	**0.44**		**0.15**	**0.19**	

[1] Group ~ Large Mature Male (LM); Mature Female (MF); Pygmy Male (PM)., [2] Standard Deviation. [a] indicates value is outside of limits as set by the 'between-run' SD for the analyte. [b] indicates value is outside of limits as set by the 'within-run' SD for the analyte.

Table 5. Metabolites. (continued)

Analyte	Group	Duration of Refrigerated Storage				Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 hr	24 hr	48 h	1 wk	lower	upper	*SD[1] (level)*	lower	upper	*SD (level)*
TRIG (mmol/L)	**LM 21**	0.08	0.10 [b]	0.10 [b]	0.10 [b]	**0.00**	**0.20**	0.0424	**0.07**	**0.09**	0.0051
	LM 22	0.04	0.06 [b]	0.05	0.05	**0.00**	**0.16**	(2.30)	**0.03**	**0.05**	(0.04)
	LM 23	0.16	0.16	0.19 [b]	0.19 [b]	**0.04**	**0.28**		**0.15**	**0.17**	
	MF 21	0.06	0.07	0.07	0.06	**0.00**	**0.18**		**0.05**	**0.07**	
	MF 22	0.11	0.13 [b]	0.12	0.18 [b]	**0.00**	**0.23**		**0.10**	**0.12**	
	MF pool 3	0.08	0.08	0.10 [b]	0.09	**0.00**	**0.20**		**0.07**	**0.09**	
	MF pool 4	0.11	0.11	0.12	0.13 [b]	**0.00**	**0.23**		**0.10**	**0.12**	
	PM 21	0.06	0.07	0.07	0.07	**0.00**	**0.18**		**0.05**	**0.07**	
	PM 22	0.06	0.07	0.07	0.06	**0.00**	**0.18**		**0.05**	**0.07**	
	PM 23	0.03	0.04	0.06 [b]	0.05 [b]	**0.00**	**0.15**		**0.02**	**0.04**	
Lactate (mmol/L)	**LM 21**	0.02	0.01	0.00	0.01	**0.00**	**0.14**	0.0318	**0.00**	**0.04**	0.0079
	LM 22	0.68	0.68	0.69	0.65 [b]	**0.59**	**0.80**	(2.93)	**0.66**	**0.70**	(0.31)
	LM 23	0.27	0.27	0.29	0.29	**0.18**	**0.39**		**0.25**	**0.29**	
	MF 21	0.34	0.35	0.37 [b]	0.36	**0.25**	**0.46**		**0.32**	**0.36**	
	MF 22	0.50	0.51	0.52	0.51	**0.41**	**0.62**		**0.48**	**0.52**	
	MF pool 3	0.32	0.30	0.31	0.33	**0.23**	**0.44**		**0.30**	**0.34**	
	MF pool 4	0.42	0.42	0.42	0.42	**0.33**	**0.54**		**0.40**	**0.44**	
	PM 21	0.60	0.63	0.61	0.62	**0.51**	**0.72**		**0.58**	**0.62**	
	PM 22	0.78	0.79	0.80	0.78	**0.69**	**0.90**		**0.76**	**0.80**	
	PM 23	0.25	0.27	0.26	0.25	**0.16**	**0.37**		**0.23**	**0.27**	
Uric Acid (µmol/L)	**LM 21**	2	3	3	1			NA[3]	**0**	**4**	0.8507
	LM 22	3	2	2	1				**1**	**5**	(6)
	LM 23	1	2	2	3				**0**	**3**	
	MF 21	10	11	12	11				**8**	**12**	
	MF 22	10	10	11	10				**8**	**12**	
	MF pool 3	14	11	11	12				**12**	**16**	
	MF pool 4	12	14	13	15				**10**	**14**	
	PM 21	6	7	8	5				**4**	**8**	
	PM 22	7	10 [b]	9	8				**5**	**9**	
	PM 23	2	2	2	3				**0**	**4**	

[1] Group ~ Large Mature Male (LM); Mature Female (MF); Pygmy Male (PM)., [2] Standard Deviation
[a] indicates value is outside of limits as set by the 'between-run' SD for the analyte
[b] indicates value is outside of limits as set by the 'within-run' SD for the analyte

Table 5. Metabolites. (continued)

Analyte	Group	Duration of Refrigerated Storage				Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 hr	24 hr	48 h	1 wk	lower	upper	*SD (level)*	lower	upper	*SD (level)*
TP (g/L)	**LM 21**	28	29	29	29	**25**	**31**	0.9300	**27**	**29**	0.4104
	LM 22	29	29	29	30	**26**	**32**	(45)	**28**	**30**	(18)
	LM 23	43	42	42	43	**40**	**46**		**42**	**44**	
	MF 21	20	20	20	21	**17**	**23**		**19**	**21**	
	MF 22	31	31	31	31	**28**	**34**		**30**	**32**	
	MF pool 3	26	26	26	27	**23**	**29**		**25**	**27**	
	MF pool 4	42	42	42	42	**39**	**45**		**41**	**43**	
	PM 21	46	46	46	46	**43**	**49**		**45**	**47**	
	PM 22	53	54	54	52	**50**	**56**		**52**	**54**	
	PM 23	44	43	44	44	**41**	**47**		**43**	**45**	
ALB (g/L)	**LM 21**	7	7	7	8	**5**	**10**	0.6300	**5**	**9**	0.5712
	LM 22	7	7	7	7	**5**	**10**	(27)	**5**	**9**	(4)
	LM 23	12	11	13	11	**10**	**15**		**10**	**14**	
	MF 21	6	6	6	5	**4**	**9**		**4**	**8**	
	MF 22	6	7	6	7	**4**	**9**		**4**	**8**	
	MF pool 3	7	7	6	7	**5**	**10**		**5**	**9**	
	MF pool 4	8	9	9	9	**6**	**11**		**6**	**10**	
	PM 21	11	11	11	11	**9**	**14**		**9**	**13**	
	PM 22	11	11	11	12	**9**	**14**		**9**	**13**	
	PM 23	9	9	10	11	**7**	**12**		**7**	**11**	

[1] Group ~ Large Mature Male (LM); Mature Female (MF); Pygmy Male (PM)., [2] Standard Deviation
[3] Not available for this analyte., [a] indicates value is outside of limits as set by the 'between-run' SD for the analyte
[b] indicates value is outside of limits as set by the 'within-run' SD for the analyte

Table 6. Enzyme Activities. Summary of changes in values over time (0 h, 24 h, 48 h, and one week) for electrolytes and minerals in snow crab hemolymph plasma under refrigerated storage storage for spring 2012 samples. All assays performed on the Cobas 501 automated biochemistry analyser. Acceptable limits/range for each parameter were calculated using the cumulative (6 month) standard deviation values as recorded by the Diagnostic Services Laboratory, Atlantic Veterinary College, University of Prince Edward Island, for human control samples (represents between-run precision), and from a precision study (20 replicate analyses) of plasma from a large mature male snow crab (represents within-run precision). 'Level' indicates the mean value of the analyte in the tested sample during precision studies.

Analyte	Group[1]	Duration of Refrigerated Storage				Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 hr	24 hr	48 h	1 wk	lower	upper	*SD*[2] *(level)*	lower	upper	*SD (level)*
AMY (IU/L)	**LM 21**	2	2	2	2	**0**	**13**	3.93	**1**	**3**	0.4702
	LM 22	4	4	4	4	**0**	**15**	(76)	**3**	**5**	(35)
	LM 23	3	4	4	4	**0**	**14**		**2**	**4**	
	MF 21	3	2	2	2	**0**	**14**		**2**	**4**	
	MF 22	3	3	4	4	**0**	**14**		**2**	**4**	
	MF pool 3	3	3	3	3	**0**	**14**		**2**	**4**	
	MF pool 4	2	2	2	2	**0**	**13**		**1**	**3**	
	PM 21	4	5	4	5	**0**	**15**		**3**	**5**	
	PM 22	4	3	4	4	**0**	**15**		**3**	**5**	
	PM 23	5	4	4	4	**0**	**16**		**4**	**6**	
LIP (IU/L)	**LM 21**	7	6	9	7	**0**	**18**	5.06	**4**	**10**	0.9445
	LM 22	8	4	8	8	**0**	**19**	(110)	**5**	**11**	(14)
	LM 23	6	6	4	5	**0**	**17**		**3**	**9**	
	MF 21	17	17	16	19	**3**	**28**		**14**	**20**	
	MF 22	4	5	4	6	**0**	**15**		**1**	**7**	
	MF pool 3	11	12	11	8	**0**	**22**		**8**	**14**	
	MF pool 4	6	6	5	8	**0**	**17**		**3**	**9**	
	PM 21	7	6	5	14 [b]	**0**	**18**		**4**	**10**	
	PM 22	7	7	10	9	**0**	**18**		**4**	**10**	
	PM 23	6	4	4	6	**0**	**17**		**3**	**9**	
ALP (IU/L)	**LM 21**	0	0	0	0	**0**	**11**	4.78	**0**	**1**	0.2236
	LM 22	0	0	0	1	**0**	**11**	(202)	**0**	**1**	(0)
	LM 23	0	0	0	0	**0**	**11**		**0**	**1**	
	MF 21	0	0	0	1	**0**	**11**		**0**	**1**	
	MF 22	0	0	0	0	**0**	**11**		**0**	**1**	
	MF pool 3	1	0	0	1	**0**	**12**		**0**	**2**	
	MF pool 4	0	0	0	0	**0**	**11**		**0**	**1**	
	PM 21	0	0	0	0	**0**	**11**		**0**	**1**	
	PM 22	0	0	0	0	**0**	**11**		**0**	**1**	
	PM 23	0	0	0	0	**0**	**11**		**0**	**1**	

[1] Group ~ Large Mature Male (LM); Mature Female (MF); Pygmy Male (PM). [2] Standard Deviation. [3] Not available for this analyte. [a] indicates value is outside of limits as set by the 'between-run' SD for the analyte. [b] indicates value is outside of limits as set by the 'within-run' SD for the analyte.

Table 6. Enzyme Activities. (continued)

Analyte	Group	Duration of Refrigerated Storage				Total Allowable Error (TAE) Between-Run Precision (TAE_{DS})			Within-Run Precision (TAE_{crab})		
		0 hr	24 hr	48 h	1 wk	lower	upper	*SD[1] (level)*	lower	upper	*SD (level)*
AST (IU/L)	**LM 21**	7	6	6	6	**0**	**16**	3.22	**0**	**37**	10.5431
	LM 22	33	33	33	37	**24**	**42**	(131)	**3**	**63**	(552)
	LM 23	8	9	9	10	**0**	**17**		**0**	**38**	
	MF 21	16	15	16	17	**7**	**25**		**0**	**46**	
	MF 22	10	8	8	9	**1**	**19**		**0**	**40**	
	MF pool 3	15	14	13	13	**6**	**24**		**0**	**45**	
	MF pool 4	4	4	5	4	**0**	**13**		**0**	**34**	
	PM 21	16	18	17	16	**7**	**25**		**0**	**46**	
	PM 22	26	23	22	23	**17**	**35**		**0**	**56**	
	PM 23	15	15	14	13	**6**	**24**		**0**	**45**	
ALT (IU/L)	**LM 21**	15	12	12	10	**7**	**24**	2.71	**0**	**41**	9.2258
	LM 22	59	54	56	62	**51**	**68**	(118)	**33**	**85**	(128)
	LM 23	15	10	9	11	**7**	**24**		**0**	**41**	
	MF 21	11	7	7	7	**3**	**20**		**0**	**37**	
	MF 22	9	5	5	6	**1**	**18**		**0**	**35**	
	MF pool 3	12	6	6	2 [a]	**4**	**21**		**0**	**38**	
	MF pool 4	8	3	3	4	**0**	**17**		**0**	**34**	
	PM 21	25	18	17	14 [a]	**17**	**34**		**0**	**51**	
	PM 22	19	13	13	12	**11**	**28**		**0**	**45**	
	PM 23	39	16	16	12	**31**	**48**		**13**	**65**	
GGT (IU/L)	**LM 21**	0	0	0	0	**0**	**9**	3.26	**0**	**0**	0
	LM 22	1	0	0	0	**0**	**10**	(174)	**1**	**1**	(0)
	LM 23	0	0	0	0	**0**	**9**		**0**	**0**	
	MF 21	0	0	0	0	**0**	**9**		**0**	**0**	
	MF 22	0	0	0	0	**0**	**9**		**0**	**0**	
	MF pool 3	0	0	0	0	**0**	**9**		**0**	**0**	
	MF pool 4	0	0	0	0	**0**	**9**		**0**	**0**	
	PM 21	0	0	0	0	**0**	**9**		**0**	**0**	
	PM 22	0	0	0	0	**0**	**9**		**0**	**0**	
	PM 23	0	0	0	0	**0**	**9**		**0**	**0**	

[1] Group ~ Large Mature Male (LM); Mature Female (MF); Pygmy Male (PM)., [2] Standard Deviation
[3] Not available for this analyte., [a] indicates value is outside of limits as set by the 'between-run' SD for the analyte
[b] indicates value is outside of limits as set by the 'within-run' SD for the analyte.

Table 6. Enzyme Activities. (continued)

Analyte	*Group*	*Duration of Refrigerated Storage*				Total Allowable Error (TAE)					
						Between-Run Precision (TAE_{DS})			*Within-Run Precision (TAE_{crab})*		
		0 hr	**24 hr**	**48 h**	**1 wk**	**lower**	**upper**	***SD*[1] *(level)***	**lower**	**upper**	***SD (level)***
GD (IU/L)	**LM 21**	7	8	8	8	**5**	**9**	0.082	**4**	**10**	1.0267
	LM 22	10	10	10	7	**8**	**12**	(22)	**7**	**13**	(5)
	LM 23	3	4	17 [b]	19 [b]	**1**	**5**		**0**	**6**	
	MF 21	3	5	7 [b]	6	**1**	**6**		**1**	**6**	
	MF 22	8	9	7	12 [b]	**6**	**10**		**5**	**11**	
	MF pool 3	9	6	6	11	**6**	**11**		**6**	**12**	
	MF pool 4	9	12	10	18 [b]	**6**	**11**		**6**	**12**	
	PM 21	13	12	10	16	**11**	**16**		**10**	**16**	
	PM 22	12	19 [b]	16 [b]	15	**10**	**14**		**9**	**15**	
	PM 23	10	8	13	17 [b]	**8**	**13**		**7**	**13**	
SDH (IU/L)	**LM 21**	0	0	0	0	**0**	**0**	1.29	**0**	**1**	0.2236
	LM 22	0	0	0	0	**0**	**0**	(7)	**0**	**1**	(0)
	LM 23	0	0	0	1	**0**	**0**		**0**	**1**	
	MF 21	0	0	0	0	**0**	**0**		**0**	**1**	
	MF 22	0	0	0	1	**0**	**0**		**0**	**1**	
	MF pool 3	0	0	0	0	**0**	**0**		**0**	**1**	
	MF pool 4	0	0	0	1	**0**	**0**		**0**	**1**	
	PM 21	0	0	0	0	**0**	**0**		**0**	**1**	
	PM 22	0	0	0	0	**0**	**0**		**0**	**1**	
	PM 23	0	0	0	1	**0**	**0**		**0**	**1**	

[1] Group ~ Large Mature Male (LM); Mature Female (MF); Pygmy Male (PM), [2] Standard Deviation
[a] indicates value is outside of limits as set by the 'between-run' SD for the analyte
[b] indicates value is outside of limits as set by the 'within-run' SD for the analyte

VII-2-5 Conclusions

The fall 2011 and spring 2012 samples both showed that most metabolites (urea, 'albumin', total protein, uric acid, glucose, triglyceride, cholesterol, and lactate), the minerals calcium and magnesium, and the enzymes amylase and lipase can be considered stable, under refrigeration, for up to one week. This knowledge allows for at-sea sampling when same-day evaluation of the sample is unlikely to occur. Activity of other enzymes was inconsistent and unpredictable. Alkaline phosphatase (ALP) was detected only in the fall 2011 samples from immature male and female crabs, where stability was acceptable for 72 hours. Activity of GGT appeared stable in the three samples it was detected in while SDH activity was rarely observed and generally too low to allow for any conclusions. Whether this was related to the enzymes themselves and/or affected by bacterial growth within the hemolymph samples requires further investigation.
The study demonstrated the potential effects of microbial growth in the stored aliquots can have marked changes on the composition of the plasma. In particular, increases in urea and lactate, and decreases in glucose concentrations could be attributed to microbe metabolism. Indirectly, significant elevations in e.g., lactate, may be affecting the assays for the enzymes AST and ALT; however, this requires further investigation. There was a tendency for ALP, GGT, and SDH activity to be present in, or appear after a week, in samples with bacterial growth. It is possible that the enzymes were of bacterial origin. Nonetheless, maintaining aseptic technique when collecting and handling hemolymph samples is critical. When possible, it is advisable to avoid collecting samples from crabs which may be bacteremic i.e., have bacteria in the circulation secondary to recent open wounds and/or infection.

The crabs used for the fall 2011 and spring 2012 samplings had very different capturing and holding regimes, fed and fasted states, and represented different maturity stages. This generated a wide range (high and low) of values which is desirable for a stability study.

Traditional laboratory evaluation would involve comparing the day-to-day variability of the result for each analyte over time to the accepted Total Allowable Error (TAE) for the analyte. The TAE is based on a number of factors including: the repeatability of an analyser, systematic and random errors, and clinically acceptable error for a result (Bellamy & Olexson 2000). Not all of these values could be calculated for the current study. As an alternate, two ranges of TAE were calculated. The first, $TAE_{DS,}$ was based on the known repeatability of the Cobas c501 analyser for human control samples. The second, TAE_{crab}, was derived from the repeatability determined after calculating the mean and SD for aliquots of an adult male crab plasma sample ran 20 times in one day. The first provided a way of estimating between-run variation for the analyser for the parameters at the levels usually found in human sera. The second provided within-run precision estimates for the parameters at the levels to be expected in large mature male crabs sampled in the fall. In general, within-run /same-day precision is higher (less variable) when compared to day-to-day values for the same parameter.

Electrolytes and Minerals

Sodium, *chloride*, and *potassium* had poor reproducibility for both fall 2011 and spring 2012 samples. This is most likely due to the manual dilution step with distilled water that must be carried out prior to analysis. This step is required as the concentrations of sodium and chloride are far higher (3-4X) in snow crab compared to vertebrate samples for which the equipment is designed. All three electrolytes are tested as a group so the dilution step applies to all.
The most dramatic changes were noted for sodium and chloride in the samples from MF pool 3, spring 2012. This sample was pooled as it was difficult to obtain large volume of hemolymph from the smaller female crabs. Sample clotting is more likely when collection is difficult e.g., due to increased tissue trauma, prolonged time to draw hemolymph into the syringe. A small amount of clotting was detected in the remaining stored sample two weeks after the final aliquot was anlaysed. Small clots can interfere with sample analysis (for any parameter) on the c501 as they may alter the volume aspirated by the machine and this may have contributed to the marked differences noted in the last two aliquots of this sample.

The one week stability for both *calcium* and *magnesium* was quite good for both sample times as all values were within the TAE_{crab} limits. Exceeding the TAE_{DS} limits was not surprising given the low testing level (3.31 mmol/L, 1.39 mmol/L) for human controls compared to the crab values (15.16 – 18.17 mmol/L, 34.52 – 43.34 mmol/L) for calcium and magnesium, respectively in fall 2011. Values in spring 2012 (11.63 – 12.87 mmol/L, 32.98 – 38.61 mmol/L) were lower than fall 2011, but still well above human control sera.

Phosphorus showed surprisingly good repeatability in LM crabs, compared to the other three groups in fall 2011 and most spring 2012 samples. Phosphorus usually has poor repeatability in lobster samples (A. Battison, personal obs.). Phosphorus is measured directly on the analyser, without dilution. The source of the variability is not yet determined.

Metabolites

The results for *urea, uric acid, total protein, 'albumin'*, and *lactate* were essentially unchanged, in the absence of bacterial contamination, for a one week period in both sampling times and would be considered very stable.
Likewise, for any practical purpose, *glucose* would also have to be considered stable for the same period. In the crab precision study used for calculating the TAE_{crab} for glucose, the SD was 0 which allowed for no variation at all – an unlikely scenario on a day to day basis. It is interesting to note however, that one value did decrease by almost 50% (LM 23, 1 week, spring 2012) which may indicate bacterial overgrowth (not confirmed). The unusual increase in glucose measured in IF pool 1, fall 2011 samples, might be explained by an incorrect reading at 0 h (e.g., due to a small clot, air bubble), as the variation in the remaining results would fall within TAE_{DS} if the initial value had been 4.7 mmol/L.

The very high levels for *lactate* in the fall 2011 IM and IF crabs compared to most other crabs, the test level for this analyte in human controls, or the LM crab used for the precision study, is a potential cause of some values exceeding both TAEs. The absolute changes observed from initial values would be unlikely to affect any clinical interpretation of the data. Lactate levels in the spring 2012 samples were much lower with only two samples exceeding the TAE_{crab} limits. Variation in total emersion time prior to sampling and/or tissue glycogen stores (not measured in these crabs) are likely contributors to the variation seen in lactate.

Cholesterol and *triglyceride* showed minor, inconsistent (no pattern with respect to time, crab group, or analyte level) variability when compared to TAE_{crab} only in both the fall 2011 and spring 2012 samples. This most likely reflects the tighter limits of the within-run precision study as the mean value for the LM used for TAE_{crab} was lower than nearly all crabs in this study. For practical purposes, both plasma cholesterol and triglyceride levels could be considered stable for 7 days.

Creatinine is an unexpected metabolite to detect in crustacean hemolymph; therefore, its detection in IF pool #2, fall 2011, was unusual. In vertebrates, creatinine is generated spontaneously from phosphocreatine in muscle tissues where phosphocreatine functions as a repository of high-energy phosphate bonds (Newman & Price 1998). Phosphoarginine performs this role in crustaceans (Yao *et al.* 2009). There are non-creatinine chromagens e.g., acetoacetate, acetone, pyruvate, which can react in the Jaffé assay used on the Cobas c501 (Newman & Price 1998). While pyruvate is not measured in the biochemistry profile, it is a direct precursor to lactate and high levels of lactate were present in many of the fall 2011 samples. Ketones are associated with metabolism of fat. Ketones were not measured as part of the biochemistry profile; consequently, their levels - and potential effects on the assay - are unknown at this time. Ingestion, digestion, and absorption of a creatinine or creatinine chromagen is another possibility to consider.

Enzyme Activity

Enzyme activity is expected to be the least stable category as measurement requires the protein to be functional in a kinetic assay compared to the other categories where the absolute quantity of a substance is being measured (end-point assays). From a clinical standpoint, relatively small changes in enzyme activity are usually of little diagnostic consequence.
Two enzymes, a*mylase* and *lipase*, had very good stability under refrigerated conditions for at least one week in both the fall 2011 and spring 2012 samples. Activity of *ALP* and *GGT* also appear stable based on the few samples with detectable activity. Ideally, it would have been preferable to have had more samples with higher activity to evaluate. The question of the potential origin of these enzymes from bacteria in the contaminated samples was discussed earlier. Alkaline phosphatase was detected in immature, i.e., non-terminal moult, crabs only in the fall. This enzyme has been associated with cuticular epithelium in post-moult spiny lobsters (Travis DF 1957) and plasma activity was detected most often in post-moult American lobsters,

Homarus americanus sampled in the fall (Ciaramella 2011). Detection of *GGT* activity in hemolymph plasma is extremely rare and may represent inadvertent aspiration of hepatopancreas tissue would be an additional consideration.

The behaviour of *ALT* and *AST* was unusual in that both enzymes showed increased activity over 72 h in the fall 2011 samples where initial activity levels were quite high. In contrast, in the spring 2012 samples, initial activity was much (up to 250-fold) lower and was either stable (AST) or showed a tendency to decrease (ALT). The expected pattern is for detectable activity to decrease over time due to deterioration of the enzyme. An increase in activity would require addition of enzyme to the system, loss of an inhibitor from the system, addition of a promoter to the system or, some form of positive interference affecting the assay.

Incomplete separation of the plasma from the hemocyte pellet and/or rupture of the hemocytes during centrifugation, both of which could possibly result in release of hemocyte contents over time into the aliquots, was considered at the time and evaluated. Varying the centrifugal force from 2,000 x *g* to 4,000 x *g* did not affect enzyme activity (data not shown). The tissue enzyme distribution studies (see Section V-I) clearly showed that release of hemocyte contents into the plasma actually reduces the amount of AST and ALT activity detected in the serum hence, hemocyte contamination an unlikely cause of the increases in contrast to American lobster.

The assay utilised for measuring ALT activities involve the following steps (Roche Diagnostics GmBH 2008a):

L-alanine + 2-oxoglutarate → pyruvate + L-glutamate *(action of AST/P-5'-P)*

Pyruvate + NADH + H^+ → L-lactate (reduced) + NAD^+ *(action of lactate dehydrogenase)*

The amount of ALT present is directly proportional to the decrease in the amount of NADH which is measured spectrophotometrically. A similar oxidation reaction: oxaloacetate + NADH + H^+ → L-malate + NAD^+, *malate dehydrogenase,* is part of the AST assay (Roche Diagnostics GmBH 2008a b). Pyridoxal-5'-phosphate (P-5'-P), active form of vitamin $B_{6,}$ and its amino analogue pyridoxamine 5'-phosphate are recognised co-enzymes in the ALT and AST reactions (Moss & Henderson 1998, Roche Diagnostics GmBH 2008a, Roche Diagnostics GmBH 2008b). Maximal AST and ALT activity is detected when P-5'-P is present in adequate amounts. Human serum can contain both holo-enzyme (enzyme with bound co-factor) and P-5'-P-deprived enzyme (Moss & Henderson 1998, Roche Diagnostics GmBH 2008a, Roche Diagnostics GmBH 2008b). Addition of exogenous P-5'-P to the sample can increase enzyme activity and is often included in the reaction in diagnostic assays for this reason. The assays used by Diagnostic Services at the AVC do not include additional P-5'-P (Roche Diagnostics GmBH 2008a, Roche Diagnostics GmBH 2008b). An endogenous source of P-5'-P, increasing during storage, in the current study seems unlikely, but release from the bacterial contaminants could be considered.

The lactate levels in the fall 2011 crabs ranged from 4.3 to 17.18 mmol/L which is 10 to 30-fold higher than levels seen in crabs that have only experienced short emersion times. Lactate is produced during the assay of ALT activity due to the oxidation of NADH. If the plasma sample contains an extremely high level of lactic acid, the oxidation of NADH might be increased, suggesting more ALT (and AST) activity than is actually present. The effect of these unusual, and extremely high, levels of lactate on the reaction system is unknown and may also require further investigation.

The degree and direction of changes in activity of *GD* over time varied more in the fall 2011 samples than the spring 2012 samples. Initial activity levels were up to three-fold higher in the fall 2011 samples which may be a contributing factor. This could reflect the greater GD activity found in muscle tissue homogenates noted in the non-terminal moult (IF, IM) crabs (Section VII-I). Fall 2011 crabs with the higher GD activity also tended to be smaller crabs with detectable GGT activity. This could indicate partial contamination of the sample with hepatopancreas. The hepatopancreas is an enzyme-rich tissue and a likely storage site for heavy metals and other potential enzyme inhibitors or cofactors which may have affected the assay. The stability and value of GD as a diagnostic enzyme remains undetermined at this point.

Evaluation of *SDH* was difficult given only a few samples had detectable activity which was very low. This is similar to findings in American lobster where the enzyme is not used in the biochemistry panel.

Effects of Bacterial Contamination

Microbial growth was confirmed by culture in five of the fall 2011 samples and suspected in one of the spring 2012 samples. The pattern of changes associated with this growth included: increased urea concentration, decreased glucose levels and often decreased triglyceride, lactate and uric acid levels, and sometimes, the appearance of ALP, GGT and/or lipase activity at 1 w in samples previously lacking such activity. These changes are presumed to be due to microbial growth resulting in consumption of glucose and/or lactate, and/or uric acid, production of urea, and leakage of microbial enzymes into the sample.

Bacterial growth occurred most often in samples from smaller crabs. It is possible that the crabs were bacteremic (bacteria in the circulation) at the time of hemolymph collection. Disarticulated limbs were observed in the cooler once emptied and it was noted that small crabs had recently lost limbs – perhaps due to trauma of collection and/or a consequence of the stress of prolonged emersion ($\geq$ 12 h) and transport. Open wounds would be an ideal portal of entry for bacteria. Alternately, the samples may have been contaminated when the hemolymph was collected and/or handled prior to processing which may have been more difficult in the smaller crabs. Surface disinfection with 70% alcohol should have eliminated that possibility, however.

In addition to microbial growth, there were other interesting observations. The activity of AST and ALT was highest in the IM and IF crabs in fall 2011 samples – both enzymes have been shown (see Section VII-I) to be located primarily in the muscle tissue, with lower levels in the hepatopancreas. Trauma related to capture and/or transport, and/or autotomy of the limbs may be the cause(s) of these increases. Severe muscle injury would be expected to cause increases in GD as well. Plasma from IF pool #2 showed very high GD activity, while values in the other groups were not as elevated. As all crabs were presumed to have been handled similarly, rough handling during capture and/or transport seems less likely, unless small crabs would be expected to be more severely affected due to their small size?

Difficulty collecting hemolymph from these smaller crabs in fall 2011 and inadvertent contamination of the sample with tissue fluid may also need to be considered when interpreting the data. These were some of the earliest samples collected, and technique may not have been optimal. Penetration of muscle tissue during collection could contribute excess AST and ALT, and some GD to the sample. Similarly, if hepatopancreas tissue fluid was collected, this could explain the presence of low amounts of GGT, ALP, and SDH activity in the plasma samples from these smaller crabs. Trauma during collection and/or transport and resultant 'bruising' of the tissues would have a similar effect - release of enzymes into the circulation would be expected. Tissues were not examined histologically in this study to assist with differentiating among these possible explanations.

The current work shows that refrigerated plasma samples stored for up to one week will provide useful results for several parameters - primarily metabolites, minerals, and some enzymes. Electrolyte values, while likely stable, are less reproducible due to a manual dilution step required for analysis. Some unusual results were noted in this study. Microbial growth within the samples was shown to affect many metabolite parameters over time and possibly having effect on some of the enzyme activity detected by releasing the enzymes themselves into the stored hemolymph aliquots or co-enzymes. Determining which, if either, of these situations occurred would require a specific study to address the question. Care in selecting crabs to sample (i.e., no visible recent injury), ensuring adequate surface disinfection when collecting hemolymph, and expedient analysis should help to decrease this complications, making biochemistry profiles a useful tool in future studies.

VII-2-6 References

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VII-3 HEMOLYMPH BIOCHEMISTRY PROFILE REFERENCE INTERVALS TRAWL AND TRAP COLLECTION WITH ALTERNATE HOLDING METHODS

VII-3-1 Summary

Trawling and trapping were compared in an attempt to identify the method that would cause the least trauma, as identified by changes in the hemolymph plasma biochemistry profile, to three categories of free crabs: pygmy males (PM), large mature males (LM), and mature females (MF). Due to physical limitations of some vessels, alternate methods of holding the crabs (immediate sampling on trawler deck; sampling after holding in *vivier* tanks; and sampling after holding in coolers) were also examined. Trawling with sampling accomplished within 15 min of the net arriving on deck resulted in relatively minor changes in biochemistry panel parameters associated with emersion. Free crabs held in coolers (up to eight hours) prior to hemolymph collection showed marked changes related to emersion (increased lactate, uric acid, urea) with minimal elevations in muscle enzymes suggesting little tissue damage. The MF group was an exception; however, this was likely due to an as yet unidentified stress affecting the crabs kept in the cooler which resulted in a large amount of limb autotomy. Interpretation of data from *vivier*-held crabs was complicated by an inadvertent exposure to lower salinity water and the unexpectedly long emersion times (up to 68 min) for some samples to be collected. Trawled crabs often had higher levels of energy-related metabolites (cholesterol, triglyceride) compared to free crabs. Whether this represents less selective collection by trawl nets vs. traps, or reflects a period of fasting experienced by free crabs held in coolers or vivier tanks remains to be determined.

Selection of a capture method for future studies will be dependent on the type of information required. Biochemistry panels collected from crabs within 15 min of the trawl net arriving on deck showed minimal changes. If this 15 min time period could be achieved for samples collected from crabs held in *vivier* tanks, similar results may be obtainable.

VII-3-2 Objective

To examine the effects to two methods of crab collection (trawl and commercial traps) of free crabs and three holding methods (no holding/immediate sample, *vivier* tank, dry cooler) on parameters measured on hemolymph plasma biochemistry profiles.

VII-3-3 Methodology

Three categories of crabs - pygmy males (PM), large mature males (LM), and mature females (MF) were collected for all methods. Four combinations of collection and holding prior to hemolymph sampling were evaluated: trapped/ cooler-held; trapped /*vivier*-held; trapped/*vivier*-held followed by cooler; and, trawled/no holding.

All trapped crabs were collected by Fisheries and Oceans Canada staff in Crab Fishing Area (CFA) 19 on August 27, 2012, using commercial-type traps and the research vessel CGS *Opilio*. Crabs in the 'cooler-held' group were transferred to ice-filled/sea-water-moistened newspaper-containing/ transport coolers and taken to shore for hemolymph collection and plasma preparation (see Section III-1 for details) hours later. Data were also available from a group of 36 cooler-held LM crabs collected by traps on August 26, 2012.

The second group of trapped crabs were transferred to the *vivier* system on board the research vessel. This system (1.8 m in width x 3.6m in length with regulable depth up to 1m) provides a constant jet spray of seawater to keep the crabs moist while being transported to shore and are only available on some vessels. Hemolymph was collected after removal from the *vivier* tanks in the ship's hold and transfer to shore which required 40 to 60 minutes (*viver*- held group). Crabs were transferred to coolers, under similar conditions to those for the 'cooler-held' group, for 4h (LM), 6h (PM), or 8h (MF) to match holding times of the crabs placed immediately in coolers after trapping, and then a second hemolymph sample was collected (post-*vivier* group).

Trawled crabs were collected using a trawling vessel (10 minute time frame per trawl) on September 4, 2012. Hemolymph samples were collected on board over a 10 minute period after the trawl net was landed from PM, LM, and MF in no particular order, until 40 samples per category were acquired (approximately 10 – 15 crabs/trawl).

All samples were submitted to Diagnostic Services at the Atlantic Veterinary College, UPEI (Charlottetown, PE) for analysis on the Cobas c501 automated biochemistry analyser (Roche Diagnostics Corporation, Indianapolis, IN, USA) using appropriate reagent kits (Roche Diagnostics Corporation) and ion-specific electrodes. Biochemistry panels included analyses of concentration of multiple electrolytes, minerals, metabolites, and enzyme activities: sodium (Na), chloride (Cl), potassium (K), Na:K ratio, phosphorus (P), magnesium (Mg), calcium (Ca), urea, uric acid, total protein (TP), albumin (Alb), globulin (TP- Alb = glob), albumin:globulin ratio (A:G), creatinine, triglyceride (TG), cholesterol (chol), glucose, lactate, amylase activity, lipase activity, glutamate dehydrogenase (GD) activity, alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, gamma glutamyl transferase (GGT) activity, sorbitol dehydrogenase (SDH) activity, and alkaline phosphatase (ALP) activity.

The 36 samples from LM crabs collected August 26, 2012 were analysed within 24 hours. There were 228 plasma samples collected from trapped LM, PM, and MF crabs on August 27th and

received at Diagnostic Services August 28th. Of these, biochemistry profiles were completed on 186 samples. Due to the high number of samples received, analysis was staggered over three days see Table 1. Of the 120 trawl samples collected, 30 from each crab category were selected at random after ensuring there was no evidence of clotting of the sample, and analysed within one day of collection.

Table 1. Summary of number of samples collected August 27, 2012 per crab group and distribution of time of analysis after arrival at Diagnostic Services, Atlantic Veterinary College, UPEI (Charlottetown, PE).

Group & Holding Method	**Number of Samples**		
	1 day post collection	**2 days post collection**	**3 days post collection**
Pygmy Male			
Vivier-held	10	–	6
Post-*vivier*	10	6	–
Cooler	10	3	–
Large mature male			
Vivier-held	10	–	20
Post-*vivier*	10	10	–
Cooler	10	10	–
Mature Female			
Vivier-held	10	–	20
Post-*vivier*	10	–	–
Cooler	10	20	–

Data sheets with additional information collected by DFO staff (carapace condition, carapace width, abdomen width, claw height, missing limbs – recent or regenerating, ovary colour, hemolymph collection time, depth, and bottom temperature) were provided with the samples.

All graphical and statistical analyses were completed using statistical software (Stata/IC 12.1, Copyright 1985-2011 StataCorp LP) and Reference Value Advisor v2.1 freeware (http://www.biostat.envt.fr/spip/spip.php?aricle63).

VII-3-4 Results

All hemolymph plasma samples were in good condition (no clotting, etc.) upon arrival at Diagnostic Services.
The biochemistry data were examined visually with box and whisker plots (Appendix A) and frequency distribution histograms (Appendix B) to look for outliers. Data from six crabs were considered suspect and removed from the data set. The sodium and chloride values from PM_{trawl} #'s 97, 99, and 120 were all unusually low; as these required a manual dilution step, technical error was suspected. The calcium value for MF_{cooler}#132 was extremely low (50% normal) and a technical error (e.g., air bubble or small clot) was suspected. Many values for PM_{trawl} #120 were highly unusual and suggestive of inadvertent contamination with hepatopancreas fluid – all values from this crab were excluded from analysis. The creatinine value (31 µmol/L) for LM#26 obtained on August 26 was considered likely interference.

The samples from trapped crabs had been processed over a period of three days from the time of collection. Many analytes are stable/reproducible for up to one week under refrigerated conditions (see Section VII-2 Stability study), with exceptions being electrolytes, and the enzymes GD, ALT, AST, and SDH. To create larger groups for calculating the reference intervals by combining data from all days, Kruskal-Wallis testing of each variable, for each crab group by day was completed to confirm no variability related to day of testing was present. Minor variation was noted for *vivie*r-held PM for magnesium and calcium; however, due to the small sample size for each sample day (n = 10 and n = 6), all data were combined.

Large (n>120) sample sizes are preferred when calculating reference intervals for parameters on biochemistry panels as non-parametric methods (percentiles) can be readily applied and 90% confidence intervals (CI) for the upper and lower reference limits calculated (Geffré A *et al.* 2011). The smallest permitted sample size for applying percentiles is 39 (no 90% CI calculated) (Friedrichs K.R. *et al.* 2012). The number of crabs per group in the current study was <39 in all cases. Consequently, parametric statistical methods (mean, ± 2SD with 90% CI) were used for groups where $20 \leq n \geq 40$ with Gaussian distributions, or robust methods with 90% CI for non-Gaussian distributions. For groups where $10 \leq n < 20$, mean, median, and range were reported. As *vivier*-held and post-*vivier* crabs experienced rather unusual conditions – prolonged emersion and probable salinity stress the mean, median, and range, rather than upper and lower limits even when group size would have allowed it, to better represent the responses seen. Box and Whisker plots for each analyte, by sex and holding method appear in Appendix A. Histograms appear in Appendix B.

The results are presented for each type of capture/holding method in Tables 2 - 6.

Notable observations in the dataset included:

1. Lowest values for lactate, uric acid, and most enzymes were noted for trawled crabs;
2. Lactate and uric acid levels in cooler-held LM crabs were lower than PM and MF crabs;
3. A drop in sodium and chloride in all groups of *vivier*-held crabs compared to trawled crabs;
4. Unusually high levels of AST and ALT in cooler-held MF crabs;
5. Unusually high levels of AST and ALT in hemolymph from *vivier*-held crabs; and
6. Detection of significant amounts of GGT activity in the samples from cooler-held LM crabs collected on August 26th.

Table 2. Summary of mean, median, minimum (Min) and maximum (Max) or upper and lower reference limits and 90% confidence intervals (CI), for 26 hemolymph plasma parameters assayed on the Cobas c501 biochemistry analyser for three groups of snow crab collected by commercial traps. Emersion times (pygmy male ~6 h; large mature males ~ 4 h; mature female ~8 h.

	TRAPPED, COOLER-HELD																			
Analyte	**Pygmy Male**							**Large Mature Male**							**Mature Female**					
	n	**mean**	**median**	**Min**	**90% CI**	**Max**	***90% CI***	***n***	**mean**	**median**	**Lower Limit**	***90% CI***	**Upper Limit**	***90% CI***	***n***	**mean**	**median**	**Lower Limit**	**90% CI**	**Upper Limit**	**90% CI**
Sodium(mmol/L)	*13*	444	444	423	-	462	-	*20*	465	465	441	*434-448*	490	*482-497*	*29*	436	444	385	*367-404*	496	*483-510*
Potassium (mmol/L)	*13*	10.9	1.07	9.9	-	13.2	-	*20*	11.0	11.3	9.2	*n/a*	12.7	*n/a*	*29*	10.9	10.7	7.8	*6.8-8.8*	13.5	*12.8-14.1*
Na:K	*13*	41	41	34	-	46	-	*20*	43	42	36	*35-38*	49	*47-51*	*29*	41	41	33	*31-35*	51	*48-55*
Chloride (mmol/L)	*13*	469	471	444	-	498	-	*20*	494	498	459	*n/a*	513	*n/a*	*29*	464	477	396	*n/a*	507	*n/a*
Calcium (mmol/L)	*13*	13.64	13.49	12.38	-	15.53	-	*20*	13.09	13.02	12.15	*11.87-12.43*	14.03	*13.74-14.33*	*30*	14.59	14.43	12.51	*11.95-13.08*	16.67	*16.10-17.23*
Phosphorus (mmol/L)	*13*	2.12	1.86	1.28	-	5.62	-	*20*	1.50	1.40	0	*0-0.55*	3.02	*2.13-4.00*	*30*	1.86	1.81	0.72	*0.42-1.03*	3.0	*2.71-3.29*
Magnesium (mmol/L)	*13*	43.43	43.01	41.59	-	46.26	-	*20*	41.32	41.48	38.53	*37.61-39.41*	44.12	*43.25-44.89*	*30*	43.59	43.48	40.05	*39.43-40.83*	46.67	*45.84-47.71*
Urea (mmol/L)	*13*	0.5	0.4	0.3	-	1.1	-	*20*	0.3	0.3	0.2	*n/a*[1]	0.6	*n/a*	*30*	0.6	0.5	0.2	*n/a*	2.2	*n/a*
Creatinine (µmol/L)	*13*	0	0	0	-	0	-	*20*	0	0	0	*n/a*	0	*n/a*	*30*	0	0	0	*n/a*	0	*n/a*
Glucose (mmol/L)	*13*	1.7	1.5	0.7	-	2.6	-	*20*	1.0	1.0	0.5	*0.4-0.6*	1.7	*1.4-2.0*	*30*	1.2	1.2	0.3	*0.1-0.6*	2.1	*1.9-2.3*
Cholesterol (mmol/L)	*13*	0.53	0.48	0.22	-	1.07	-	*20*	0.43	0.41	0.14	*0.09-0.21*	0.89	*0.71-1.11*	*30*	0.43	0.45	0.05	*0-0.14*	0.81	*0.70-0.90*
Triglyceride (mmol/L)	*13*	0.11	0.12	0.05	-	0.2	-	*20*	0.08	0.08	0.03	*0.02-0.04*	0.16	*0.13-0.20*	*30*	0.22	0.22	0	*0-0.05*	0.44	*0.38-0.49*
Total Protein (g/L)	*13*	61	57	36	-	97	-	*20*	41	38	14	*7-22*	65	*54-72*	*30*	45	48	9	*1-19*	81	*72-90*
Albumin (g/L)	*13*	13	13	8	-	22	-	*20*	9	9	6	*5-6*	16	*13-19*	*30*	9	9	3	*2-5*	15	*13-16*
Globulin (g/L)	*13*	48	44	28	-	78	-	*20*	32	29	12	*6-18*	52	*45-57*	*30*	36	39	11	*7-15*	71	*60-81*
A:G	*13*	0.28	0.29	0.23	-	0.33	-	*20*	0.30	0.29	0.28	*0.21-0.25*	0.46	*0.38-0.62*	*30*	0.27	0.25	0.19	*0.19-0.20*	0.46	*0.37-0.57*
Lactate (mmol/L)	*13*	5.74	3.96	2.15	-	13.05	-	*20*	1.50	1.26	0.0	*0-0*	3.47	*2.58-4.20*	*30*	5.94	5.20	0.98	*0.46-1.75*	14.46	*11.41-17.64*
Uric Acid (µmol/L)	*13*	113	96	65	-	199	-	*20*	41	40	6	*0-18*	75	*64-86*	*30*	142	146	62	*43-83*	222	*204-243*

1. n/a 90% CI unavailable due to incompatible data distribution

Table 2. (continued)

Analyte	TRAPPED, COOLER-HELD																			
	Pygmy Male							Large mature male							Mature Female						
	n	mean	median	Min	90% *CI*	Max	*90% CI*	*n*	mean	median	Lower Limit	*90% CI*	Upper Limit	*90% CI*	*n*	mean	median	Lower Limit	90% CI	Upper Limit	90% CI
Amylase (U/L)	*13*	7	6	3	-	18	-	*20*	8	7	1	*n/a*	19	*n/a*	*30*	11	8	1	*1-2*	45	*27-66*
Lipase (U/L)	*13*	10	10	3	-	13	-	*20*	8	7	4	*3-5*	15	*12-18*	*20*	11	11	1	*0-4*	21	*18-23*
AST (U/L)	*13*	54	33	13	-	203	-	*20*	18	15	4	*3-6*	46	*33-60*	*30*	141	95	16	*8-26*	486	*346-697*
ALT (U/L)	*13*	53	45	23	-	105	-	*20*	20	19	6	*4-9*	45	*35-57*	*30*	85	70	0	*0-0*	188	*155-239*
GD (U/L)	*13*	19	20	9	-	37	-	*20*	12	12	4	*1-6*	21	*19-24*	*30*	14	12	4	*3-5*	39	*28-53*
SDH (U/L)	*13*	0	0	0	-	1	-	*20*	0	0	0	*n/a*	0	*n/a*	*30*	1	1	0	*n/a*	2	*n/a*
GGT (U/L)	*13*	0	0	0	-	1	-	*20*	0	0	0	*n/a*	0	*n/a*	*30*	0	0	0	*n/a*	4	*n/a*
ALP (U/L)	*13*	0	0	0	-	1	-	*20*	0	0	0	*n/a*	0	*n/a*	*30*	1	1	0	*n/a*	13	*n/a*

1. n/a 90% CI unavailable due to incompatible data distribution

Table 3. Summary of mean, median, minimum (Min) and maximum (Max) values for 26 hemolymph plasma parameters assayed on the Cobas c501 biochemistry analyser for three groups of *vivier*-held snow crab collected by commercial traps. Emersion times estimated from 40 – 68 minutes prior to sampling.

	TRAPPED, *VIVIER*-HELD																			
Analyte	**Pygmy Male**							**Large Mature Male**							**Mature Female**					
	n	**mean**	**median**	**Min**	**90% CI**	**Max**	***90% CI***	***n***	**mean**	**median**	**Min**	***90% CI***	**Max**	***90% CI***	***n***	**mean**	**median**	**Min**	**90% CI**	**Max**	**90% CI**
Sodium (mmol/L)	*16*	369	369	351	-	384	-	*30*	378	378	321	-	441	-	*30*	362	365	321	-	387	-
Potassium (mmol/L)	*16*	7.5	7.5	6.4	-	8.4	-	*30*	7.9	8.0	6.6	-	9.1	-	*30*	7.3	7.3	6.4	-	9.1	-
Na:K	*16*	46	49	1	-	55	-	*30*	48	48	43	-	53	-	*30*	50	50	43	-	54	-
Chloride (mmol/L)	*16*	377	378	354	-	399	-	*30*	385	386	312	-	462	-	*30*	376	377	339	-	411	-
Calcium (mmol/L)	*16*	11.81	11.95	10.26	-	12.74	-	*30*	11.84	12.05	10.34	-	12.97	-	*30*	11.84	12.06	10.38	-	12.89	-
Phosphorus (mmol/L)	*16*	1.52	1.38	0.64	-	3.03	-	*30*	0.88	0.84	0.13	-	1.87	-	*30*	0.91	0.89	0.06	-	1.98	-
Magnesium (mmol/L)	*16*	39.51	39.41	37.02	-	42.55	-	*30*	37.30	37.60	34.41	-	40.39	-	*30*	39.09	39.08	37.38	-	40.65	-
Urea (mmol/L)	*16*	0.2	0.2	0.1	-	0.4	-	*30*	0.2	0.2	0.1	-	0.3	-	*30*	0.2	0.2	0.1	-	0.3	-
Creatinine (µmol/L)	*16*	0	0	0	-	0	-	*30*	0	0	0	-	0	-	*30*	0	0	0	-	0	-
Glucose (mmol/L)	*16*	1.5	1.5	0.7	-	2.4	-	*30*	0.9	0.9	0.5	-	1.4	-	*30*	1.1	1.1	0.4	-	1.7	-
Cholesterol (mmol/L)	*16*	0.47	0.46	0.28	-	0.78	-	*30*	0.41	0.40	0.16	-	0.66	-	*30*	0.39	0.38	0.09	-	0.67	-
Triglyceride (mmol/L)	*16*	0.09	0.08	0.04	-	0.12	-	*30*	0.07	0.07	0.01	-	0.14	-	*30*	0.18	0.20	0.02	-	0.32	-
Total Protein (g/L)	*16*	57	55	29	-	94	-	*30*	43	44	23	-	59	-	*30*	44	43	18	-	71	-
Albumin (g/L)	*16*	12	12	7	-	19	-	*30*	10	10	6	-	13	-	*30*	9	10	5	-	14	-
Globulin (g/L)	*16*	45	44	22	-	76	-	*30*	34	34	17	-	48	-	*30*	35	34	12	-	59	-
A:G	*16*	0.27	0.26	0.22	-	0.35	-	*30*	0.30	0.29	0.21	-	0.43	-	*30*	0.29	0.27	0.19	-	0.5	-
Lactate (mmol/L)	*16*	1.97	1.4	0.59	-	4.86	-	*30*	0.27	0.13	0	-	1.49	-	*30*	1.13	0.91	0.12	-	4.23	-
Uric Acid (µmol/L)	*16*	51	48	27	-	116	-	*30*	9	5	0	-	60	-	*30*	45	46	14	-	87	-

Table 3. (continued)

Analyte	TRAPPED, *VIVIER*-HELD																		
	Pygmy Male							Large mature male							Mature Female						
	n	mean	median	Min	90% C*I*	Max	*90% CI*	*n*	mean	median	Min	*90% CI*	Max	*90% CI*	*n*	mean	median	Min	90% CI	Max	90% CI
Amylase (U/L)	*16*	10	8	1	-	32	-	*30*	9	7	2	-	31	-	*30*	11	10	2	-	26	-
Lipase (U/L)	*16*	9	9	4	-	17	-	*30*	8	8	4	-	11	-	*30*	9	10	4	-	16	-
AST (U/L)	*16*	46	38	11	-	113	-	*30*	32	21	2	-	183	-	*30*	88	70	21	-	256	-
ALT (U/L)	*16*	76	60	18	-	189	-	*30*	35	28	9	-	140	-	*30*	75	73	24	-	147	-
GD (U/L)	*16*	15	12	5	-	35	-	*30*	13	12	0	-	22	-	*30*	13	11	5	-	45	-
SDH (U/L)	*16*	0	0	0	-	3	-	*30*	1	1	0	-	4	-	*30*	1	1	0	-	4	-
GGT (U/L)	*16*	0	0	0	-	1	-	*30*	0	0	0	-	1	-	*30*	0	0	0	-	1	-
ALP (U/L)	*16*	0	0	0	-	2	-	*30*	1	1	0	-	2	-	*30*	1	1	0	-	2	-

Table 4. Summary of mean, median, minimum (Min) and maximum (Max) values, for 26 hemolymph plasma parameters assayed on the Cobas c501 biochemistry analyser for three groups of trap-collected, *vivier*-held snow crabs after a four to eight hour emersion period (*similar to cooler-held groups).*

	TRAPPED, POST-*VIVIER*																			
Analyte	**Pygmy Male**							**Large Mature Male**							**Mature Female**						
	n	**mean**	**median**	**Min**	**90% CI**	**Max**	***90% CI***	***n***	**mean**	**median**	**Min**	***90% CI***	**Max**	***90% CI***	***n***	**mean**	**median**	**Min**	**90% CI**	**Max**	**90% CI**
Sodium (mmol/L)	*16*	367	366	339	-	393	-	*20*	380	381	321	-	453	-	*10*	369	372	351	-	375	*n/a*
Potassium (mmol/L)	*16*	9.0	9.0	7.9	-	9.9	-	*20*	9.1	9.3	7.4	-	10	-	*10*	8.9	8.8	6.3	-	11.4	*n/a*
Na:K	*16*	41	41	38	-	46	-	*20*	42	41	39	-	50	-	*10*	42	42	33	-	59	*n/a*
Chloride (mmol/L)	*16*	373	371	342	-	402	-	*20*	383	371	312	-	471	-	*10*	383	384	357	-	396	*n/a*
Calcium (mmol/L)	*16*	12.83	12.80	11.21	-	14.92	-	*20*	12.10	12.00	10.48	-	14.01	-	*10*	11.83	11.67	11.28	-	13.22	*n/a*
Phosphorus (mmol/L)	*16*	1.89	1.79	1.14	-	3.23	-	*20*	1.54	1.42	0.91	-	2.58	-	*10*	1.34	1.35	0.87	-	2.08	*n/a*
Magnesium (mmol/L)	*16*	39.90	40.29	35.43	-	41.54	-	*20*	38.36	38.41	35.84	-	40.18	-	*10*	40.16	40.28	38.93	-	41.91	*n/a*
Urea (mmol/L)	*16*	0.4	0.4	0.0	-	0.6	-	*20*	0.4	0.4	0.2	-	0.6	-	*10*	0.4	0.4	0.2	-	0.5	*n/a*
Creatinine (µmol/L)	*16*	0	0	0	-	1	-	*20*	0	0	0	-	0	-	*10*	0	0	0	-	0	*n/a*
Glucose (mmol/L)	*16*	1.7	1.6	0.9	-	2.8	-	*20*	1.2	1.3	0.7	-	1.7	-	*10*	1.2	1.3	0.6	-	1.8	*n/a*
Cholesterol (mmol/L)	*16*	0.48	0.47	0.27	-	0.88	-	*20*	0.41	0.39	0.19	-	0.71	-	*10*	0.43	0.44	0.23	-	0.65	*n/a*
Triglyceride (mmol/L)	*16*	0.11	0.11	0.05	-	0.20	-	*20*	0.07	0.07	0.0	-	0.15	-	*10*	0.24	0.24	0.13	-	0.33	*n/a*
Total Protein (g/L)	*16*	63	63	32	-	103	-	*20*	45	44	24	-	64	-	*10*	48	49	23	-	72	*n/a*
Albumin (g/L)	*16*	13	13	6	-	20	-	*20*	10	10	6	-	14	-	*10*	9	9	5	-	14	*n/a*
Globulin (g/L)	*16*	50	49	26	-	84	-	*20*	35	35	18	-	52	-	*10*	38	40	18	-	58	*n/a*
A:G	*16*	0.26	0.26	0.21	-	0.30	-	*20*	0.30	0.30	0.22	-	0.39	-	*10*	0.25	0.26	0.20	-	0.31	*n/a*
Lactate (mmol/L)	*16*	6.12	5.26	3.24	-	9.42	-	*20*	2.16	2.08	0.72	-	4.38	-	*10*	4.04	3.66	2.37	-	7.47	*n/a*
Uric Acid (µmol/L)	*16*	110	104	45	-	198	-	*20*	52	46	13	-	185	-	*10*	114	123	56	-	154	*n/a*

Table 4 (continued)

TRAPPED, POST-*VIVIER*

Analyte	Pygmy Male							Large Mature Male							Mature Female						
	n	mean	median	Min	90% CI	Max	90% CI	*n*	mean	median	Min	90% CI	Max	90% CI	*n*	mean	median	Min	90% CI	Max	90% CI
Amylase (U/L)	*16*	13	10	0	-	50	-	*20*	13	11	3	-	38	-	*10*	19	12	4	-	74	*n/a*
Lipase (U/L)	*16*	10	10	3	-	25	-	*20*	9	10	0	-	12	-	*10*	10	10	4	-	16	*n/a*
AST (U/L)	*16*	147	128	10	-	451	-	*20*	114	64	10	-	801	-	*10*	246	244	103	-	467	*n/a*
ALT (U/L)	*16*	146	133	51	-	419	-	*20*	78	57	16	-	286	-	*10*	170	163	71	-	326	*n/a*
GD (U/L)	*16*	16	15	0	-	58	-	*20*	15	15	0	-	27	-	*10*	15	14	8	-	29	*n/a*
SDH (U/L)	*16*	1	0	0	-	4	-	*20*	1	0	0	-	6	-	*10*	0	0	0	-	0	*n/a*
GGT (U/L)	*16*	1	0	0	-	5	-	*20*	0	0	0	-	3	-	*10*	0	0	0	-	0	*n/a*
ALP (U/L)	*16*	2	0	0	-	12	-	*20*	1	1	0	-	1	-	*10*	0	0	0	-	0	*n/a*

Table 5. Summary of mean, median, minimum, maximum, upper and lower reference limits and respective 90% confidence intervals (CI), for 26 hemolymph plasma parameters assayed on the Cobas c501 biochemistry analyser for three groups of cooler-held, trapped, large male snow crab. Emersion time ~3h.

TRAPPED, COOLER-HELD MALES (AUG 26, 2012)

Analyte	Large Mature Male						
	n	mean	median	Min	90% *CI*	Max	*90% CI*
Sodium (mmol/L)	***36***	445	444	426	*n/a*	468	*n/a*
Potassium (mmol/L)	***36***	10.0	9.9	9.0	8.8-9.2	11.6	11.1-12.2
Na:K	***36***	45	45	39	38-41	50	49-51
Chloride (mmol/L)	***36***	479	480	450	443-456	507	500-513
Calcium (mmol/L)	***36***	12.96	12.85	12.03	11.80-12.25	13.88	13.67-14.12
Phosphorus (mmol/L)	***36***	1.04	0.96	0.22	*n/a*	3.05	*n/a*
Magnesium (mmol/L)	***36***	41.91	42.04	39.48	38.95-40.10	44.33	43.75-44.84
Urea (mmol/L)	***36***	0.2	0.2	0	*n/a*	0.6	*n/a*
Creatinine (µmol/L)	***36***	0	0	0	*n/a*	0	*n/a*
Glucose (mmol/L)	***36***	0.9	0.9	0.3	0.2-0.4	1.4	1.3-1.6
Cholesterol (mmol/L)	***36***	0.42	0.39	0.13	0.09-0.17	0.84	0.72-0.96
Triglyceride (mmol/L)	***36***	0.08	0.07	0.02	0.01-0.03	0.25	0.18-0.33
Total Protein (g/L)	***36***	43	41	20	15-26	67	61-72
Albumin (g/L)	***36***	10	9	4	3-5	15	14-17
Globulin (g/L)	***36***	34	32	15	11-20	52	48-56
A:G	***36***	0.29	0.29	0.21	0.19-0.23	0.37	0.35-0.39

TRAPPED, COOLER-HELD MALES (AUG 26, 2012)

Analyte	Large Mature Male						
	n	mean	median	Min	90% *CI*	Max	*90% CI*
Lactate (mmol/L)	***36***	0.80	0.65	0.00	0-0	1.88-2.13	*n/a*
Uric Acid (µmol/L)	***36***	19	16	2	1-3	57	45-72
Amylase (U/L)	***36***	11	10	1	*n/a*	48	*n/a*
Lipase (U/L)	***36***	15	11	5	*n/a*	83	*n/a*
AST (U/L)	***36***	22	17	0	*n/a*	116	*n/a*
ALT (U/L)	***36***	23	19	2	0-4	70	53-93
GD (U/L)	***36***	14	12	4	3-5	35	27-46
SDH (U/L)	***36***	0	0	0	*n/a*	3	*n/a*
GGT (U/L)	***36***	2	1	0	*n/a*	17	*n/a*
ALP (U/L)	***36***	0	0	0	*n/a*	0	*n/a*

Table 6. Summary of mean, median, and 90% confidence intervals (CI), for 26 hemolymph plasma parameters assayed on the Cobas c501 biochemistry analyser for three groups of snow crab collected by trawl with hemolymph.

	TRAWLED SAMPLES																			
Analyte	**Pygmy Male**							**Large Mature Male**							**Mature Female**					
	n	**Mean**	**Med**	**Lower Limit**	**90% *CI***	**Upper Limit**	***90% CI***	***n***	**Mean**	**Med**	**Lower Limit**	***90% CI***	**Upper Limit**	***90% CI***	***n***	**Mean**	**Med**	**Lower Limit**	**90% CI**	**Upper Limit**	**90% CI**
Sodium (mmol/L)	*27*	417	414	389	*382-396*	444	*437-451*	*30*	435	435	390	*380-401*	481	*469-492*	*30*	403	396	346	*332-360*	460	*445-474*
Potassium (mmol/L)	*27*	9.2	9.3	7.4	*n/a*[1]*-8.3*	10.2	*10.0-10.3*	*30*	9.7	9.7	8.1	*7.6-8.5*	11.5	*11.0-11.9*	*30*	8.7	8.8	7.0	*6.4-7.8*	10.6	*9.8-11.3*
Na:K	*27*	46	45	42	*n/a*	58	*n/a*	*30*	43	45	4	*n/a*	50	*n/a*	*30*	46	46	41	*n/a*	73	*n/a*
Chloride (mmol/L)	*27*	438	435	405	*n/a*	477	*n/a*	*30*	452	453	385	*367-402*	520	*501-537*	*30*	422	414	330	*309-352*	514	*491-539*
Calcium (mmol/L)	*29*	12.46	12.57	10.52	*n/a-11.50*	13.35	*13.17-13.50*	*30*	12.42	12.49	11.48	*11.22-11.75*	13.37	*13.12-13.61*	*30*	13.03	13.02	12.03	*11.75-12.31*	14.03	*13.76-14.29*
Phosphorus (mmol/L)	*29*	0.95	0.78	0	*0-0*	2.75	*1.67-3.77*	*30*	0.99	0.91	0.21	*0.11-0.36*	2.32	*1.87-2.77*	*30*	0.88	0.67	0.16	*0.12-0.23*	3.40	*2.16-5.55*
Magnesium (mmol/L)	*29*	41.86	41.89	37.67	*36.65-38.76*	46.04	*44.79-47.10*	*30*	39.89	40.11	35.68	*33.53-37.27*	42.54	*42.00-43.07*	*30*	45.11	45.11	41.38	*39.85-42.66*	47.72	*47.11-48.23*
Urea (mmol/L)	*29*	0.3	0.3	*0.1*	*n/a*	*0.5*	*n/a*	*30*	0.3	0.2	0	*n/a*	0.7	*n/a*	*30*	0.3	0.3	0.1	*n/a*	0.5	*n/a*
Creatinine (µmol/L)	*29*	0	0	*0*	*n/a*	*0*	*n/a*	*30*	0	0	0	*n/a*	0	*n/a*	*30*	0	0	0	*n/a*	0	*n/a*
Glucose (mmol/L)	*29*	1.1	1.2	*0.5*	*0.3-0.8*	*1.9*	*1.6-2.1*	*30*	0.9	0.9	0.2	*0.1-0.4*	1.8	*1.5-.1*	*30*	1.2	1.2	0.8	*0.7-0.8*	2.0	*1.7-2.3*
Cholesterol (mmol/L)	*29*	0.55	0.55	*0.10*	*0-0.22*	*0.99*	*0.88-1.11*	*30*	0.51	0.50	0.11	*0.01-0.22*	0.92	*0.81-1.04*	*30*	0.65	0.64	0.18	*0.05-0.28*	1.12	*1.01-1.20*
Triglyceride (mmol/L)	*29*	0.09	0.10	*0.02*	*0-0.04*	*0.17*	*0.15-0.19*	*30*	0.08	0.08	0	*0.00-0.03*	0.16	*0.14-0.18*	*30*	0.33	0.31	0.12	*0.06-0.17*	0.54	*0.48-0.60*
Total Protein (g/L)	*29*	56	62	*26*	*12-40*	*98*	*86-107*	*30*	44	44	16	*9-24*	73	*65-79*	*30*	62	58	31	*20-38*	92	*81-98*
Albumin (g/L)	*29*	13	13	*0*	*0-7*	*17*	*16-18*	*30*	10	10	4	*2-6*	16	*14-18*	*30*	12	11	8	*n/a*	16	*n/a*
Globulin (g/L)	*29*	44	48	*0*	*n/a-21*	*61*	*59-64*	*30*	34	34	8	*6-18*	57	*51-63*	*30*	51	48	25	*16-31*	75	*66-80*
A:G	*29*	0.30	0.28	*0.24*	*0.23-0.24*	*0.45*	*0.36-n/a*	*30*	0.29	0.29	0.24	*0.23-0.25*	0.44	*0.38-0.57*	*30*	0.23	0.23	0.19	*0.19-0.20*	0.31	*0.28-0.35*
Lactate (mmol/L)	*29*	0.50	0.48	*0.02*	*0-0.16*	*0.97*	*0.84-1.09*	*30*	0.37	0.34	0.04	*n/a*	0.98	*n/a*	*30*	0.29	0.30	0.02	*0.0-0.08*	0.57	*0.50-0.64*
Uric Acid (µmol/L)	*29*	18	18	*0*	*0-4*	*36*	*32-41*	*30*	12	12	0	*0-0.2*	27	*22-31*	*30*	17	17	3	*0-6*	32	*27-35*

1. n/a 90% CI unavailable due to incompatible data distribution

Table 6. (continued)

Analyte	TRAWLED SAMPLES																		
	Pygmy Male							Large mature male							Mature Female						
	n	mean	median	Lower Limit	90% *CI*	Upper Limit	*90% CI*	*n*	mean	median	Lower Limit	*90% CI*	Upper Limit	*90% CI*	*n*	mean	median	Lower Limit	90% CI	Upper Limit	90% CI
Amylase (U/L)	*29*	10	7	1	*1-1*	35	*25-48*	*30*	8	7	0	*0-2*	16	*14-18*	*30*	7	5	1	*1-1*	27	*18-39*
Lipase (U/L)	*29*	11	11	7	*n/a*	17	*n/a*	*30*	11	11	8	*7-9*	16	*15-17*	*30*	8	8	1	*0-3*	15	*14-17*
AST (U/L)	*29*	20	16	3	*n/a*	104	*n/a*	*30*	21	12	2	*1-3*	104	*60-76*	*30*	16	13	5	*4-6*	72	*36-183*
ALT (U/L)	*29*	36	31	8	*6-11*	110	*80-147*	*30*	33	24	5	*4-7*	114	*78-52*	*30*	29	23	7	*5-9*	82	*62-106*
GD (U/L)	*29*	16	17	4	*2-7*	27	*24-30*	*30*	14	14	3	*0-6*	25	*22-28*	*30*	18	18	2	*0-6*	34	*30-37*
SDH (U/L)	*29*	1	1	0	*n/a*	4	*n/a*	*30*	1	0	0	*n/a*	3	*n/a*	*30*	1	1	2	*n/a*	4	*n/a*
GGT (U/L)	*29*	2	2	2	*n/a*	3	*n/a*	*30*	1	2	0	*n/a*	2	*n/a*	*30*	0	0	0	*n/a*	0	*n/a*
ALP (U/L)	*29*	0	0	0	*n/a*	0	*n/a*	*30*	0	0	0	*n/a*	0	*n/a*	*30*	0	0	0	*n/a*	0	*n/a*

1. n/a 90% CI unavailable due to incompatible data distribution

VII-3-5 Conclusions

Reference intervals were calculated for 26 parameters (23 measured, 3 calculated) contained within the hemolymph biochemistry profile for three crab groups (PM, LM, and MF) for four combinations of collection and holding (trapped /cooler-held, trapped/*vivier*-held, trapped/*vivier*-held followed by cooler-holding, trawled/no holding). Trawled crabs had the least changes related to emersion (increased lactate, uric acid, and urea). There were some unexpected patterns detected in trapped crabs with regard to:

1) tendency for decreased levels of cholesterol and triglyceride (MF) possibly related to short term fasting during holding; and
2) possible muscle injury (increased AST, ALT) related to repeated sampling and/or potential salinity stress in *vivier*-held crabs; and
3) muted physiologic response to emersion of LM crabs in the hemolymph biochemistry parameters compared to PM and MF crabs.

Further investigation of these observations to determine their cause would be warranted as selection of capture and holding method for future studies will need to take into account such changes. General comments based on visual inspection of the data for each group of biochemical parameters are provided below.

Electrolytes & Minerals

The box and whisker plots and frequency distribution histograms clearly showed an unusual downward shift in *sodium* and *chloride* concentration for all groups of *vivier*-held crabs. Given that snow crab are osmoconformers, this would suggest the crabs were exposed to lower salinity water than that found in the area they were captured (Hardy at al. 1994). Further investigation (M. Moriyasu, personal communication) revealed that the water used to fill the *vivier* tanks was collected inshore to allow for thorough chilling prior to use. While the salinity was not recorded, it is presumed to have been lower and could have accounted for the shift seen in the hemolymph concentrations. The tolerance of snow crab for such a salinity shift is unknown and brings into question the possibility of cell-swelling in other tissues causing enzyme leakage (See Enzyme Activity comments below).

Cooler-held crabs showed the highest values for sodium and chloride concentrations which could be attributed to dehydration during holding. Similar increases would have been expected in the post-*vivier*, cooler-held group as well; however, the degree of this response was probably compromised by the presumed lower salinity water of the *vivier* tanks.

The higher potassium concentrations in cooler-held crabs could represent a decreased ability to excrete potassium and/or be part of an acid-base adjustment to the lactic acidosis expected with emersion (and confirmed by higher lactate levels). The values in the post-*vivier* cooler-held crabs also showed an increase over the *vivier* samples which could reflect the effects of emersion.

Calcium levels in all cooler-held groups were higher than those of trawled crabs. During the lactic acidosis induced by emersion, carbonate (as calcium carbonate) can be released from the cuticle as a base to help titrate the acid load and is considered a likely cause for the increases noted similar to changes observed in *Nephrops norvegicus* (Berlasconi CJ & Uglow RF. 2008). The MF crabs were held in coolers for the longest period and would be anticipated to have the lower hemolymph pH values; this could account for the higher calcium levels in this group.

Shifts in *magnesium* concentrations showed a similar pattern to calcium and may be for the same reasons in PM and LM crabs. Similar patterns have been observed in lobsters, *Homarus americanus,* emersed for 48 hours (Battison *et al.* 2012). However, trawled MF crabs had the highest magnesium levels of all groups yet total emersion time was less than any of the cooler-held crabs. The cause is uncertain although, comparison to ovary stage may help if reproductive stage is a factor.

The factors affecting *phosphorus* concentration are not well understood. The data would suggest that emersion tends to cause an increase.

Metabolites

Urea and *uric acid* were consistently higher in all cooler-held crabs than trawled counterparts which would be compatible with changes induced by loss of the normal excretory route through the gills for ammonia (Claybrook 1983). As ammonia accumulates due to protein metabolism, it is probable that some will be converted to water-soluble urea for excretion by the antennal glands and to non-water soluble uric acid for storage in tissues (Claybrook 1983). The pattern was less pronounced in the LM crabs. Baseline uric acid levels will reflect protein turnover (dietary, catabolic). Lower uric acid levels are observed in fasted vs fed American lobsters (A Battison, personal observation). It is speculated that lower protein reserves or lower rate of nitrogen metabolism may be dampening the rise in plasma uric acid levels in the LM crabs.

Detection of *creatinine* was not expected in these panels given that crustaceans use phosphoarginine to store energy in muscle rather than phosphocreatine. Detection of creatinine (31 µmol/L) in one of the LM crabs was very unusual. Possible explanations include the presence of a substance that reacts with the assay (a non-creatinine chromagen). Recognised chromagens include acetoacetate, acetone, pyruvate however, these were not measured in this study[6]. Alternately, ingestion of a substance (creatinine or non-creatinine chromagens) causing a reaction might be possible.

Hemolymph *glucose* levels would be anticipated to be defined, in part, by tissue glycogen reserves as glycogen is made from glucose and glucose is released from glycogen as required. Increased hemolymph glucose levels would be expected with stress (handling, emersion, exposure to low salinity, hauling of trawl nets or traps). This pattern was somewhat evident in the PM group only. How long the elevated glucose levels can be maintained may also be a function of glycogen reserves.

Cholesterol levels showed marked variation in all groups; however, levels tended to be higher in trawled crabs overall with apparently little change in response to emersion. As trawled and trapped crabs were collected in the same area, the question arises if traps are attracting 'hungrier' crabs or, if the period of fasting during transport could cause the hemolymph cholesterol levels to decrease?

Levels of plasma *triglyceride* were remarkably higher in MF crabs than the other two groups. This pattern has been observed in female American lobsters, associated with ovary maturation, and is assumed to be related to the transfer of lipoprotein from the hepatopancreas to the developing oocytes (Battison *et al.* 2011). Comparison of ovary stage to plasma triglyceride levels would help determine if a similar process is responsible in snow crabs. The median triglyceride level in trawled MF crabs was notably higher in than trapped MF crabs. Differences in PM and LM were inconsistent. Again, this raises the question of trawling being a less selective collection technique and/or if a period of fasting will decrease plasma triglyceride levels.

Values for *total protein*, '*albumin'*, and *globulin* again showed higher levels for trawled PM and MF crabs compared to their cooler-held free counterparts (NB: *vivier*-held animals may have experienced dilution of the total protein concentrations complicating interpretation). Less difference was noted for LM crabs but, could be related to poorer nutritional reserves suspected in these crabs. The effect of hemolymph loss (hemorrhage) associated with loss of appendages on the total protein, albumin, and globulin values is unknown. In vertebrates, hemorrhage will decrease the plasma total protein concentration secondary to dilution by extracellular fluid. Given the open circulation system in crabs, it is uncertain if a similar situation would be observed.

An increase in *lactate* concentration was expected with emersion. The increase would be proportional to the length of emersion given similar glycogen reserves in all crabs as lactate is produced from glucose during anaerobic metabolism resulting from emersion. Overall, lower levels were seen in the LM crabs which, is believed to be due to lower glycogen reserves in this group but would require tissue glycogen analysis to confirm (no sample was taken in this study).

Enzyme Activity

Baseline levels of plasma enzyme activity are going to be determined by the amount of enzyme in a tissue, its subcellular location (cytosolic vs mitochondrial), and the amount of that tissue in the body. Cellular injury/damage that results in leakage of the enzyme into the circulation will increase detectable activity as can increased production of the enzyme over normal levels (physiologic and pathologic induction are both possible) (Moss & Henderson 1998).

One of the goals of the current study was to compare tissue injury, as assessed by increases enzyme activity, in trawled and trapped crabs. The assumption was that trawling would be more

damaging to muscle and possibly hepatopancreas tissue via exposure to physical trauma during trawling.

Unfortunately, it was discovered after sample collection that the crabs placed in the *vivier* tanks were likely exposed to low salinity stress. It is possible that, in addition to the changes in electrolytes that were noted, cell swelling with some leakage of cytosolic enzymes may have occurred. This is a potential cause of the increases noted in *vivier*-held crabs for *AST* and *ALT* in MF and PM crabs. This pattern was not noted for the LM crabs. Possibilities to consider would be that the muscle tissue in LM crabs was less hyperosmotic compared to the low salinity hemolymph than muscle in MF or PM crabs because the LM crabs were in poor nutritional condition. This may have made the tissue more resilient. A less likely explanation was that muscle swelling was great enough to cause macroscopic enlargement of the muscles in PM and MF crabs so that it was more difficult to avoid muscle tissue when collecting hemolymph in these smaller crabs.

The process of collecting the first hemolymph sample in the *vivier*-held crabs may have induced some muscle tissue trauma at the site of collection. Muscle-associated enzymes (See Section VII-3) could then be released into the circulation and would be detected when the crabs were sampled a second time for the post-*vivier* cooler-held samples and is considered the most likely explanation for the increases in *AST* and *ALT* observed.

Trapped, cooler-held crabs showed very low muscle enzyme activity overall suggesting minimal trauma associated with trapping. The exception was the cooler-held MF crabs where *AST* and *ALT* activity was higher than trawled or other categories of trapped crabs. Review of the data sheets showed more lost appendages in this group than others (presumed to be due to recent autotomy). Autotomization of a limb would be expected to cause muscle tissue damage and is the presumed explanation for the increases seen in this group. The cause for the increased autotomy in this group remains undetermined but, dense packing and/or prolonged emersion (this group was held in coolers for the longest amount of time) could be considered.

Gamma glutamyl transferase (*GGT*) and *ALP* are enzymes specific to the hepatopancreas for MF and LM crabs, while ALP is also often found in the testes of PM crabs (see Section VII-3 tissue distribution). Activity of these enzymes is rarely detected in plasma from apparently healthy crabs. The observation of activity in some crabs in this study was, therefore, unusual. Review of the dataset showed that high ALP and GGT activity often occurred in the same crab. It is suspected that these samples were inadvertently contaminated with hepatopancreas tissue/fluid during sample collection. It is possible, but considered unlikely, that the enzymes were released *in vivo* and represent hepatopancreas injury as other enzymes (SDH, AST, ALT) associated with the hepatopancreas were not similarly elevated in these crabs.

The amount of activity of *SDH* detected in any sample was extremely low (0 – 4 U/L) - typical for crustacean plasma. As such, little can be made of its significance.

The reference intervals constructed for this dataset should be regarded as a starting point rather than a finished product. The intervals were constructed very conservatively to be able to include most of the samples that were collected, apart from marked outliers. This approach was taken as sex, size, sexual maturity, and collection method were the only factors used to assign crabs into categories. More refined reference intervals can be developed as the understanding of which factors are contributing to the range of values observed increases e.g., crab fishing area, reproductive stage of females, time of year, time elapsed since terminal moult, etc. As snow crab are exothermic, ambient temperature may also have a significant effect on the baseline levels of parameters associated with metabolic rate. The latter is believed to occur in American lobsters based on available data (A. Battison, personal observation) and ambient temperature may need to be taken into account when comparing hemolymph biochemistry data collected from crabs residing in different areas.

As seen in this study, capture method may also create its own bias. Traps may only attract crabs which are hungry, able to detect the bait, and able to enter the trap etc. Different fishing vessels are required to collect crabs by trapping vs. trawling. Lack of usable deck space on board the former resulted in the longer interval (hours) between crab capture and hemolymph sampling on shore. Use of the *vivier* tanks, with appropriate salinity, may be able to remove the effects of prolonged emersion on the biochemistry panels if hemolymph could be collected within the 10 min period as on the trawl vessels (not achieved in the current study). Such a study would help to determine if e.g., the lower values for cholesterol are due to the collection method itself (traps collect hungrier crabs) or if the prolonged period between capture and hemolymph collection is the cause.

This study clearly illustrated the effects of prolonged emersion on hemolymph parameters and suggested potential effects that trap bias may be having on the population sampled. Hemolymph samples collected from trawled crabs within a short period e.g., 10 min, upon arrival on deck likely best represent the range of values to be expected from a crab population in its natural environment with access to a traditional diet. The 2w-6m-12m caging study required commercial vessels with cage-hauling capacity, vessels which lack *vivier* tanks, to recover the caged crabs, however. Given the known difference between trapped and trawled crabs, and that the crabs selected for caging were collected by commercial traps, trapping was determined to be the most suitable method to use to collect control ‘free’ crabs fro the larger study.

VII-3-6. References

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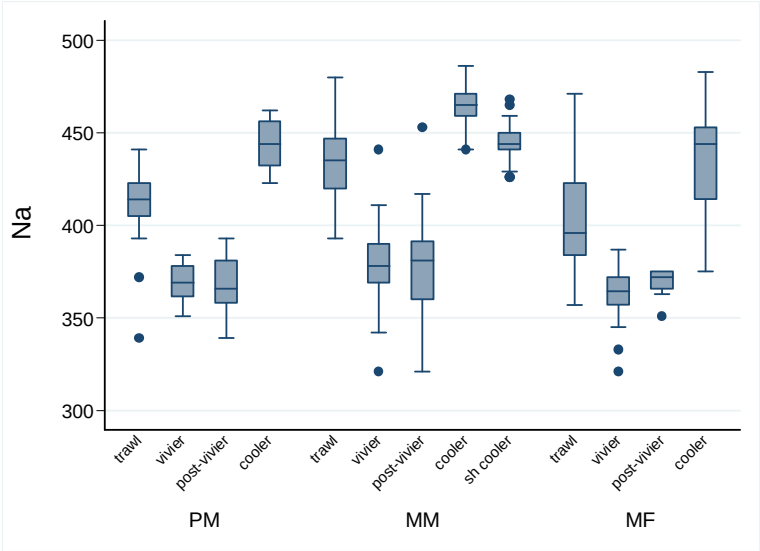
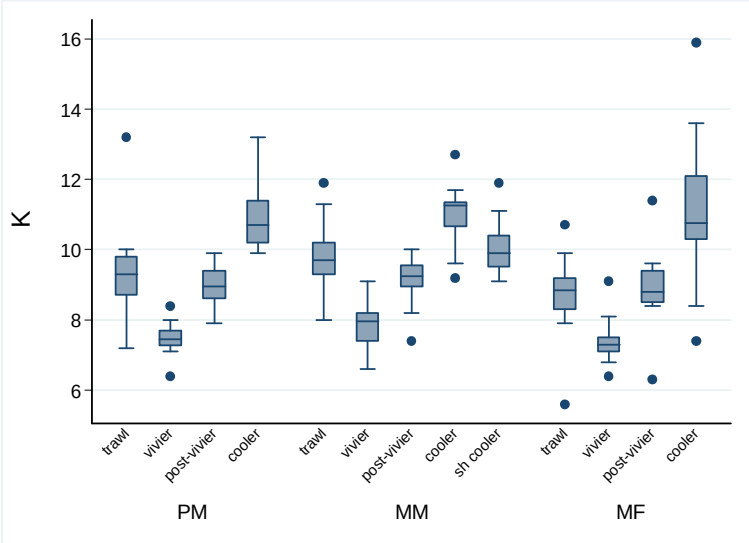
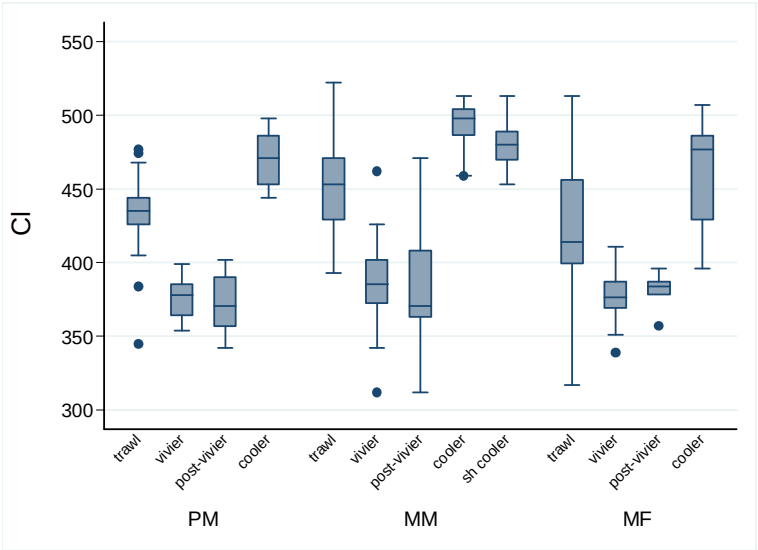
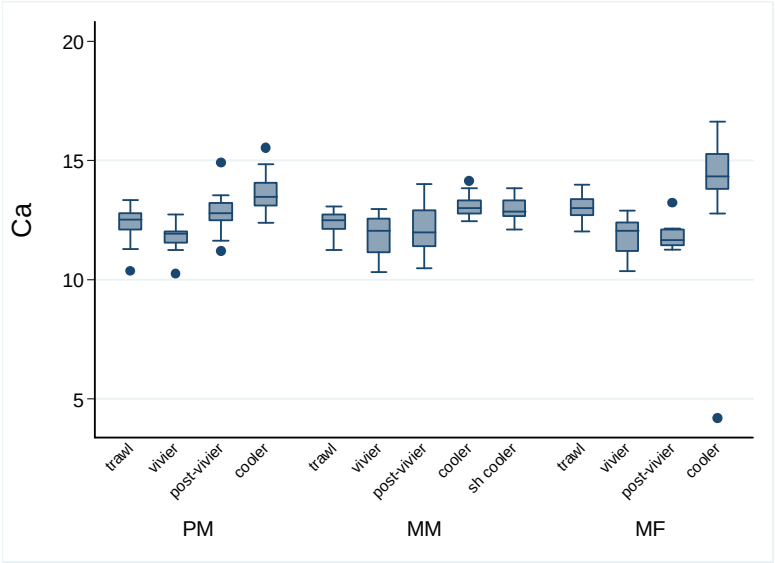
APPENDIX A: Box & Whisker Plots of Hemolymph Plasma Biochemistry Profile Parameters for Three Crab Groups and Four Holding Methods. Groups are as follows: trawl/not held (trawl); *vivier*-held (vivier); post-*vivier* cooler-held (post –vivier); cooler-held (cooler); short-emersion, cooler-held LM (sh cooler).





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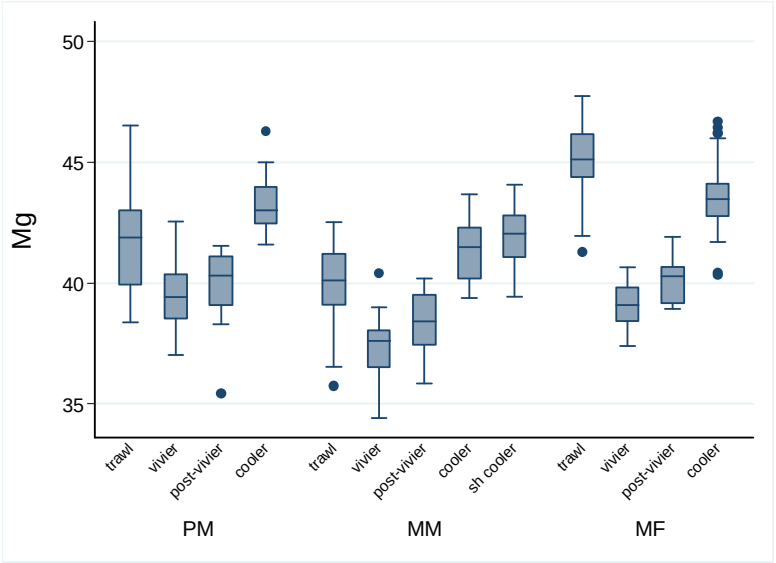
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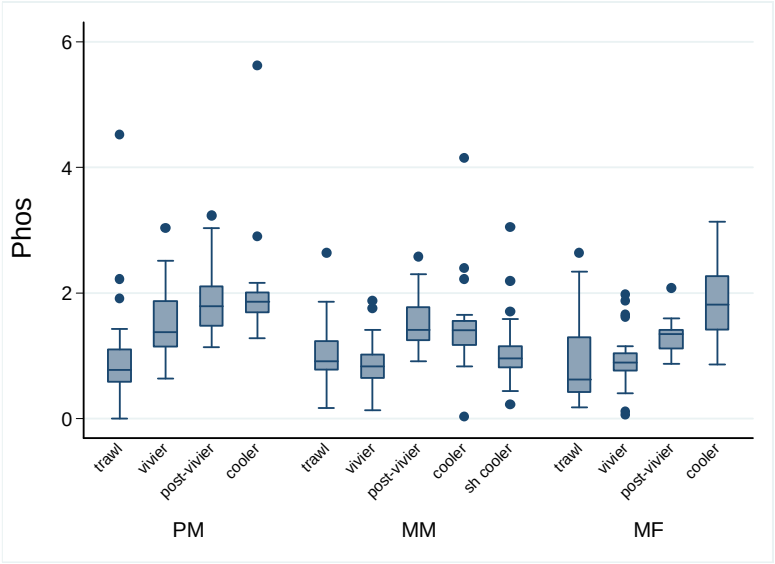
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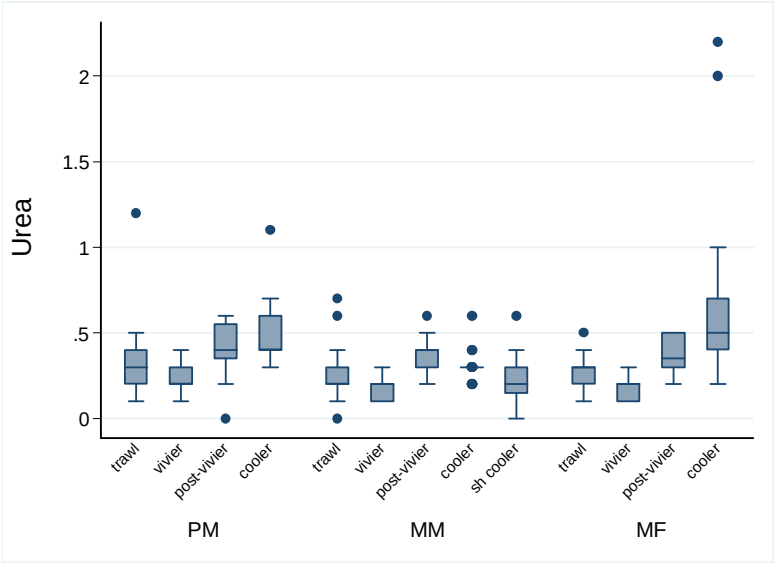
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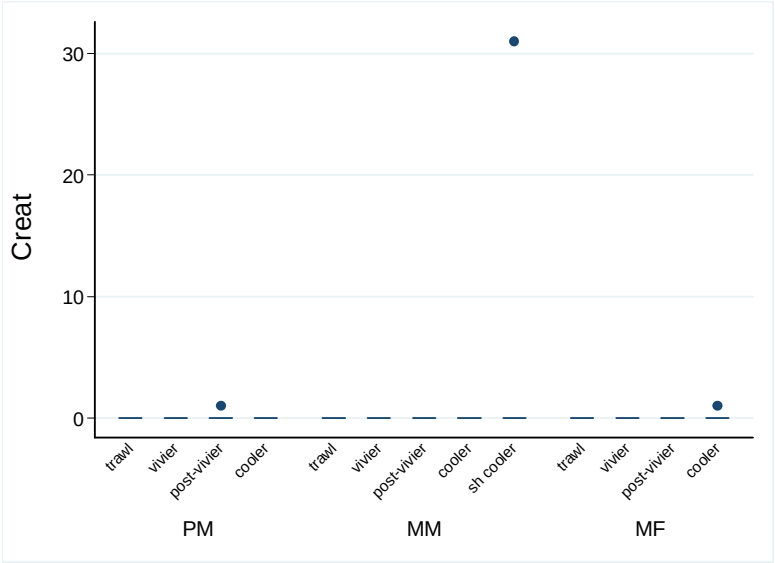
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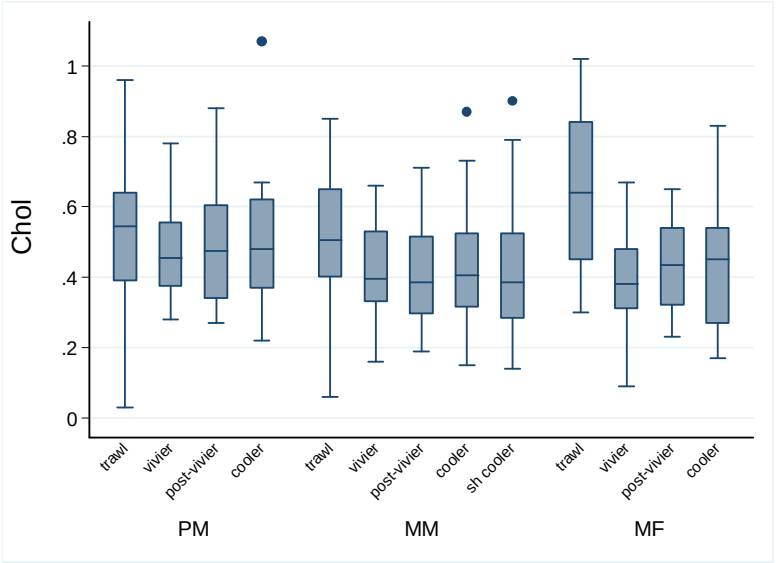
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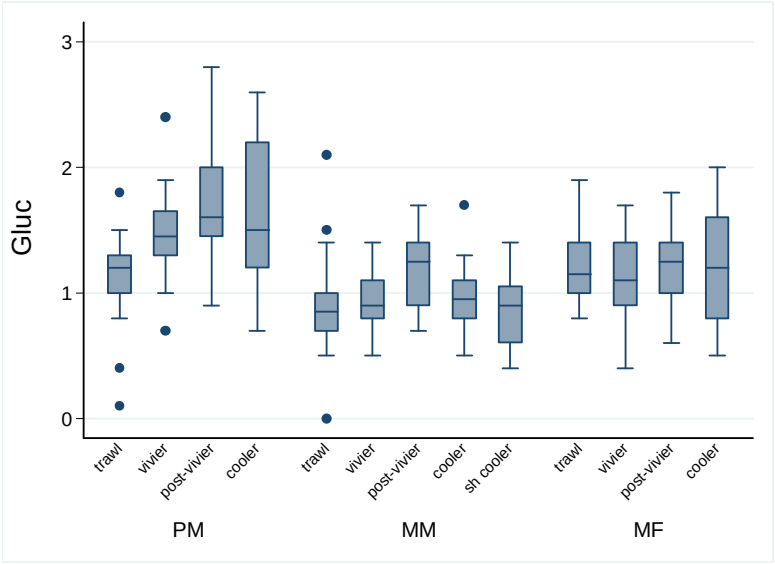
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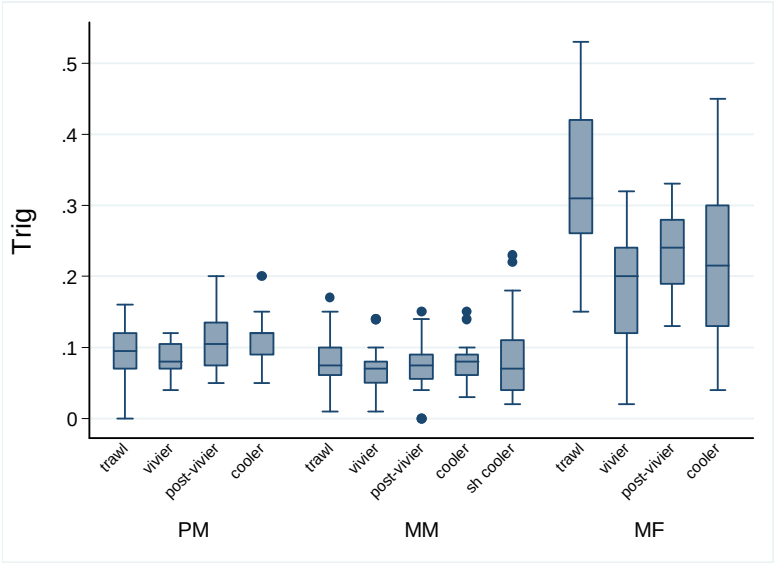
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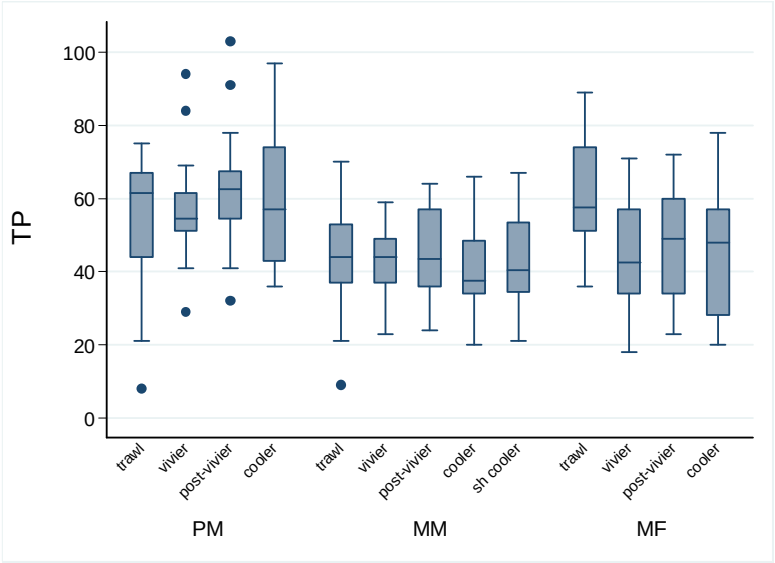
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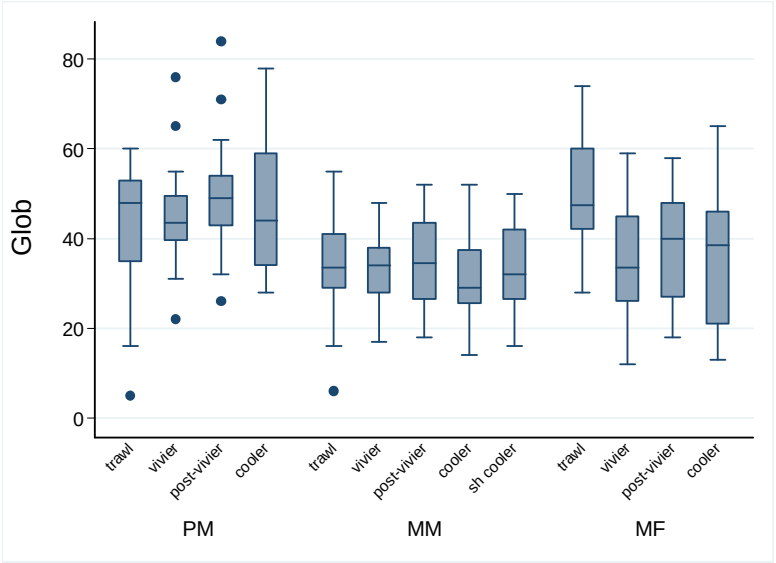
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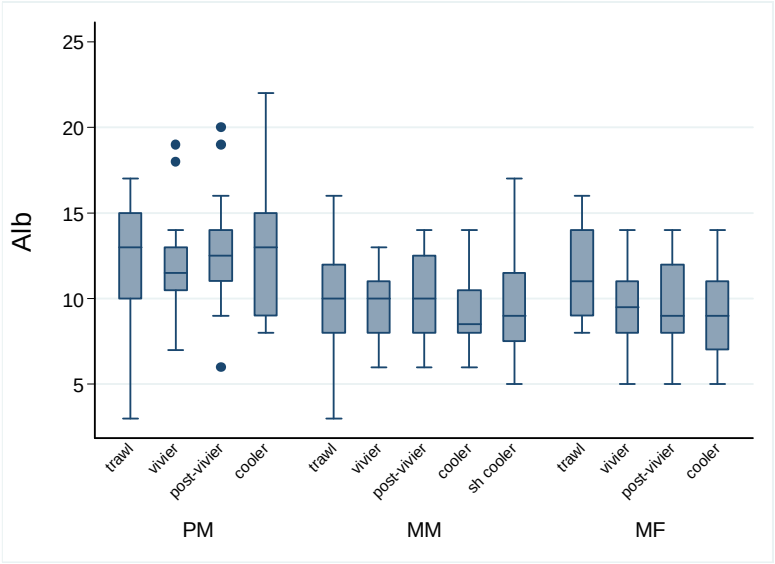
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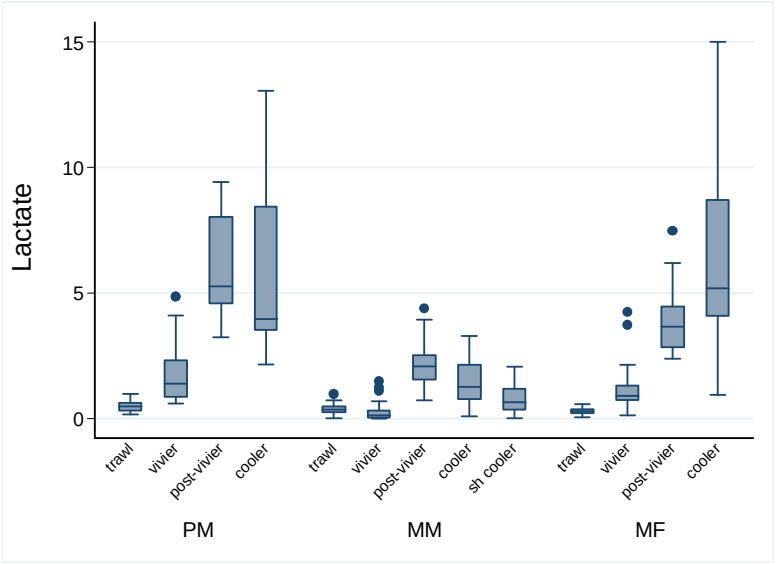
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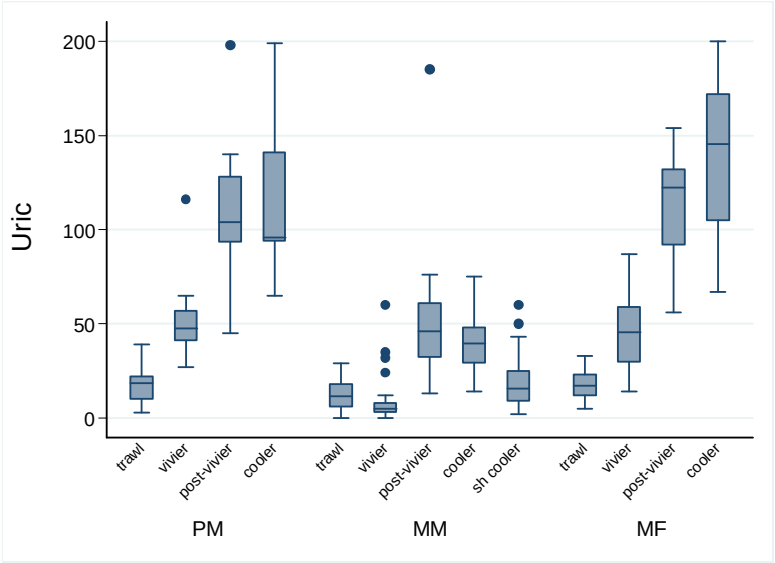
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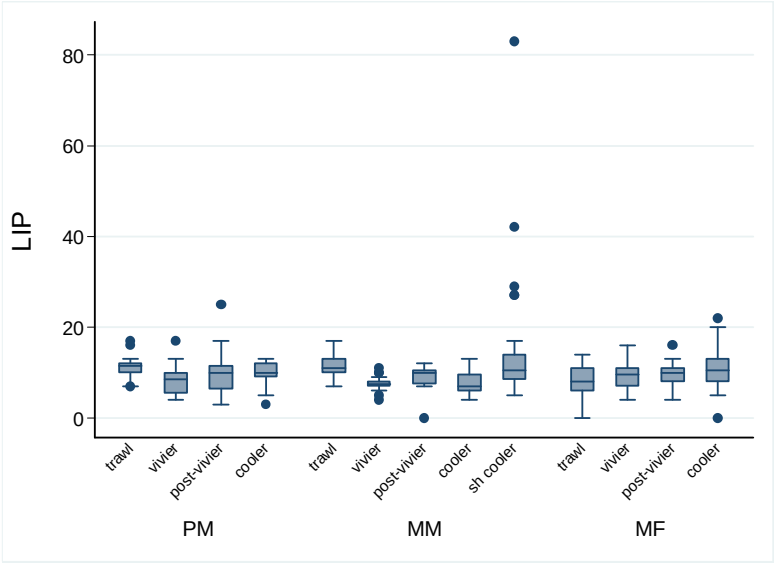
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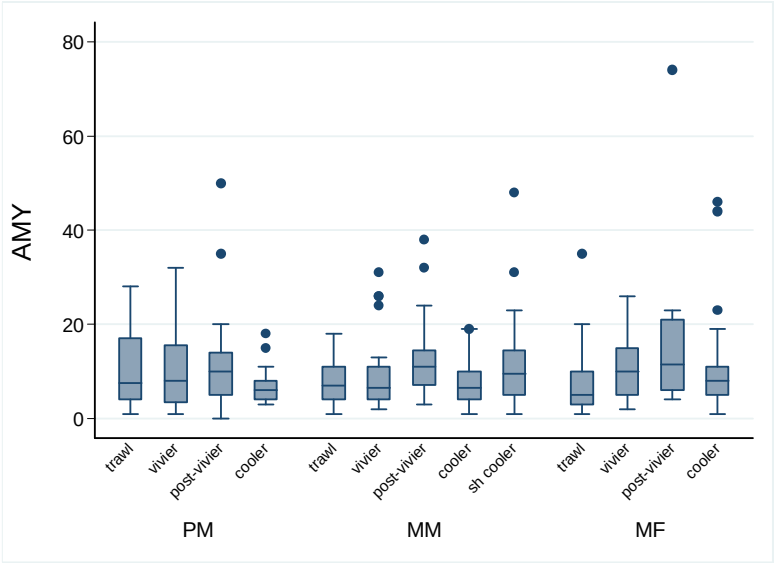
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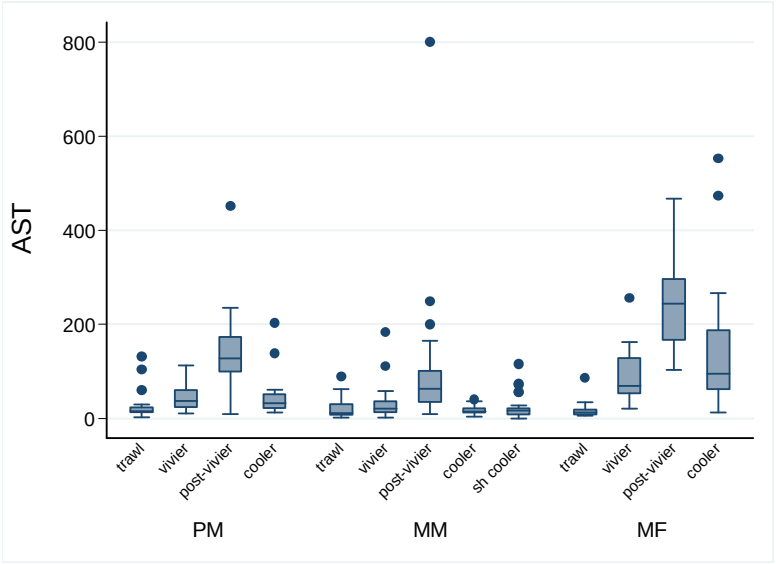
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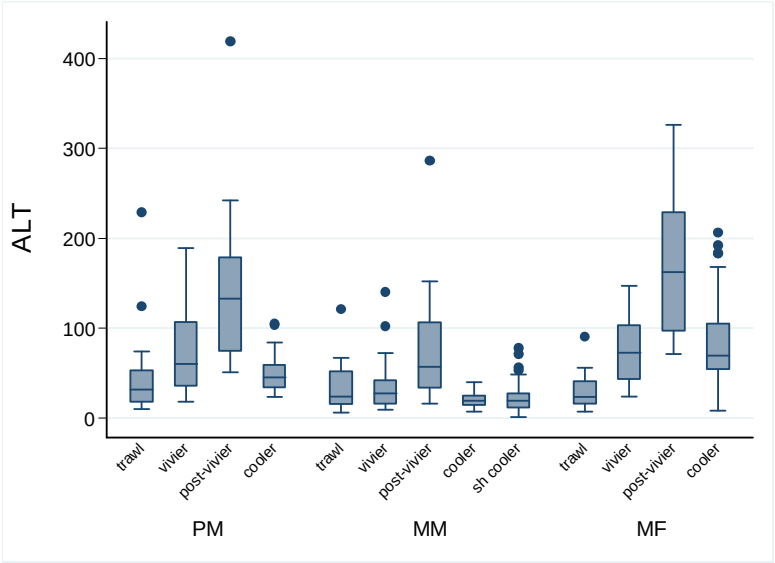
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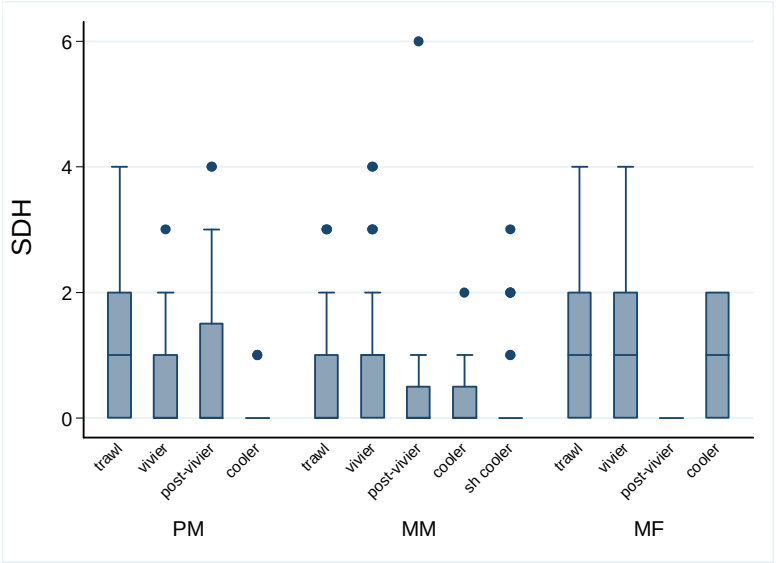
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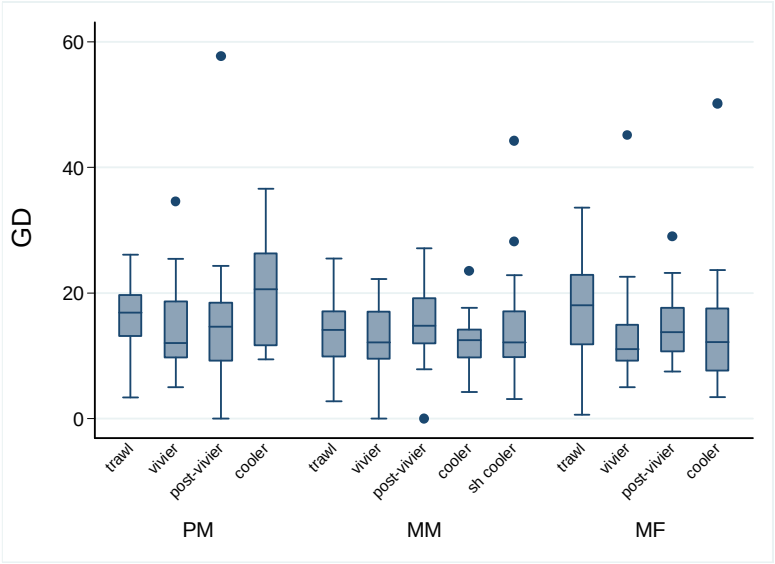
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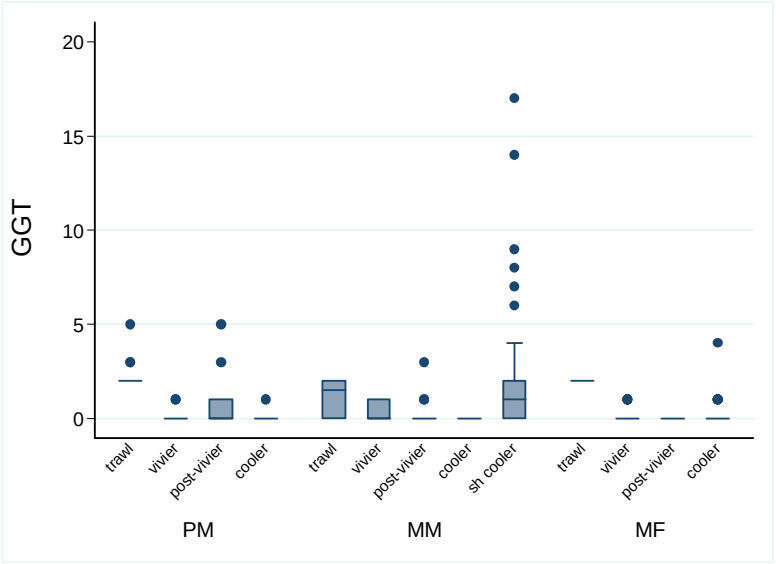
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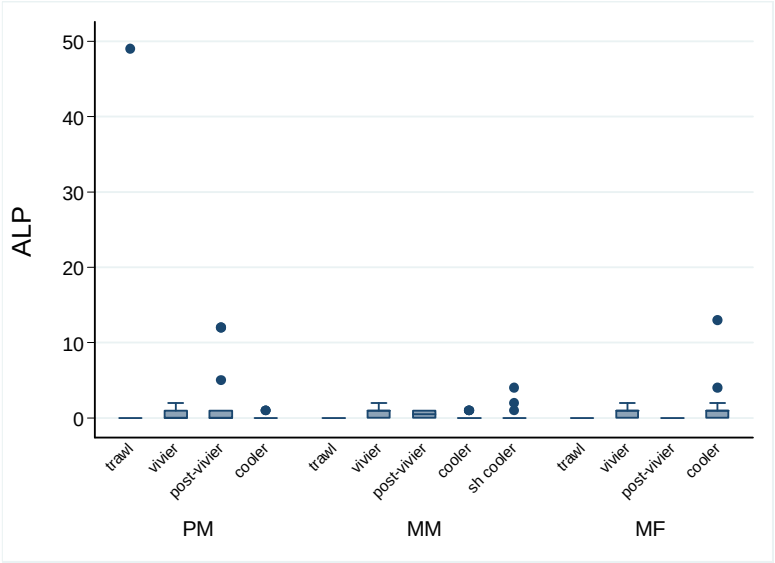
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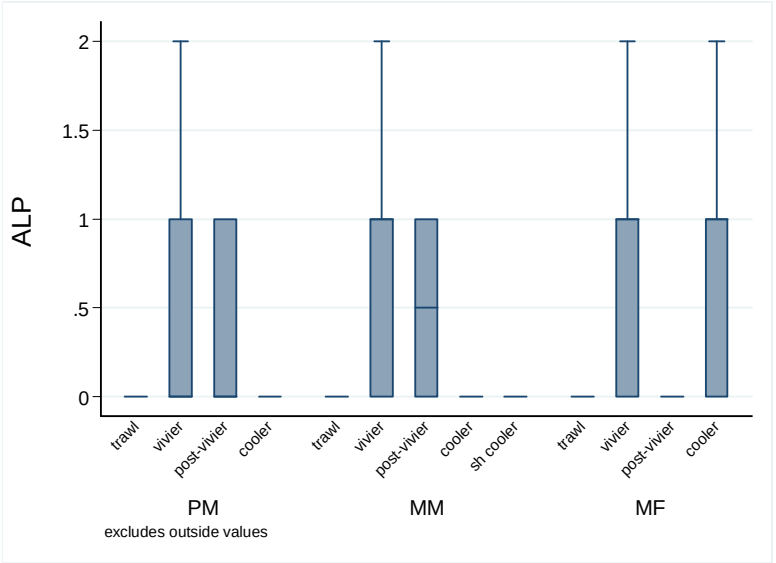
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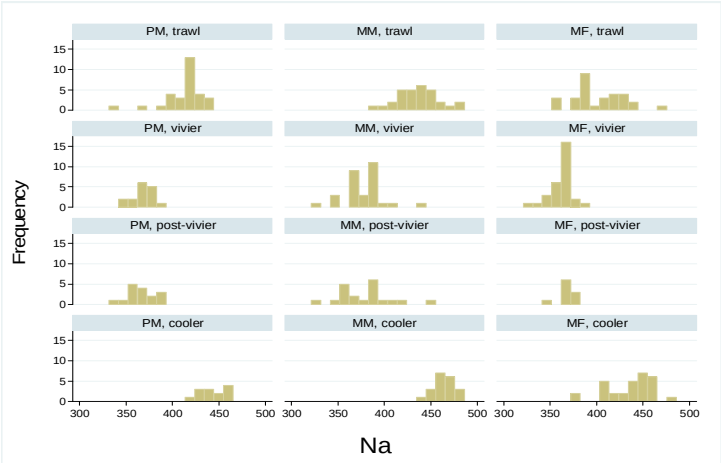
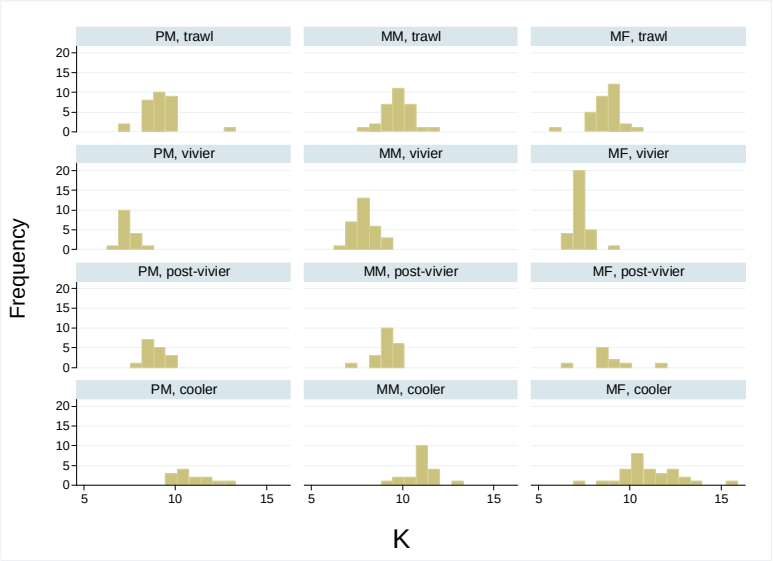
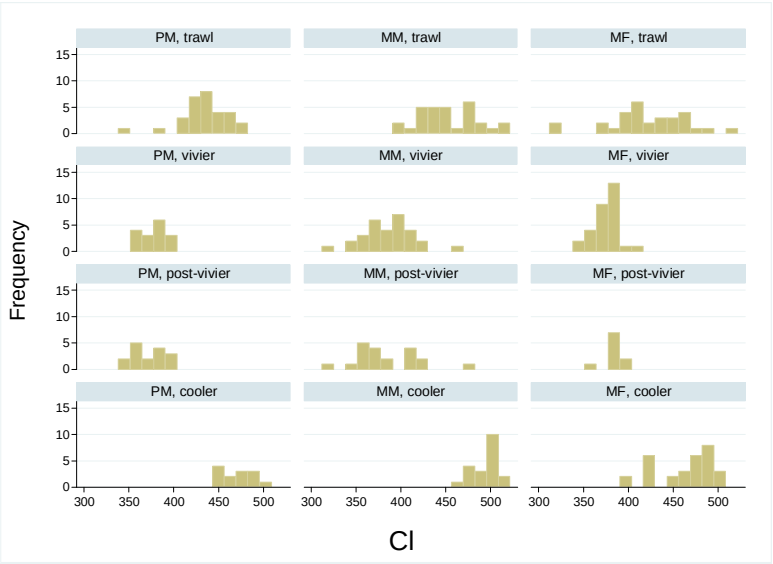
1.5
1
.5
0
trawl
vivier
post-vivier
cooler
trawl
vivier
post-vivier
cooler
sh cooler
trawl
vivier
post-vivier
cooler
PM
MM
MF
excludes outside values

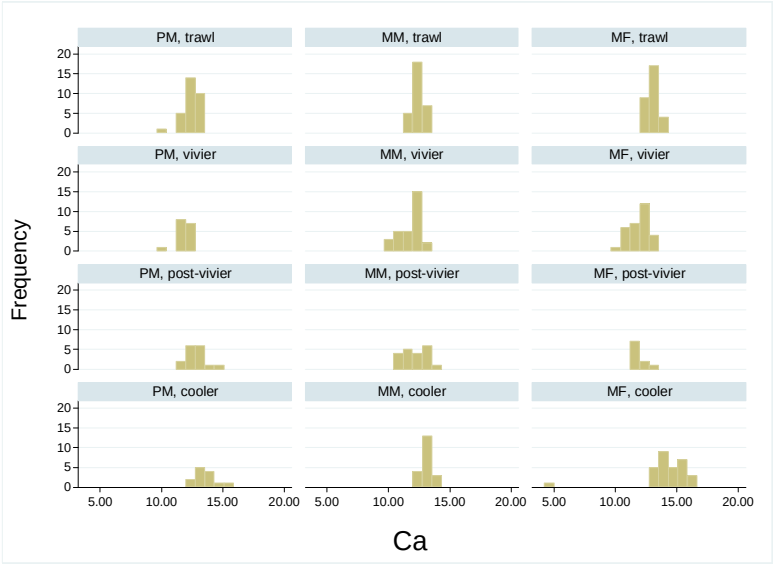
APPENDIX B Frequency Distribution Histograms of Hemolymph Plasma Biochemistry Profile Parameters for Three Crab Groups and Four Holding Methods.

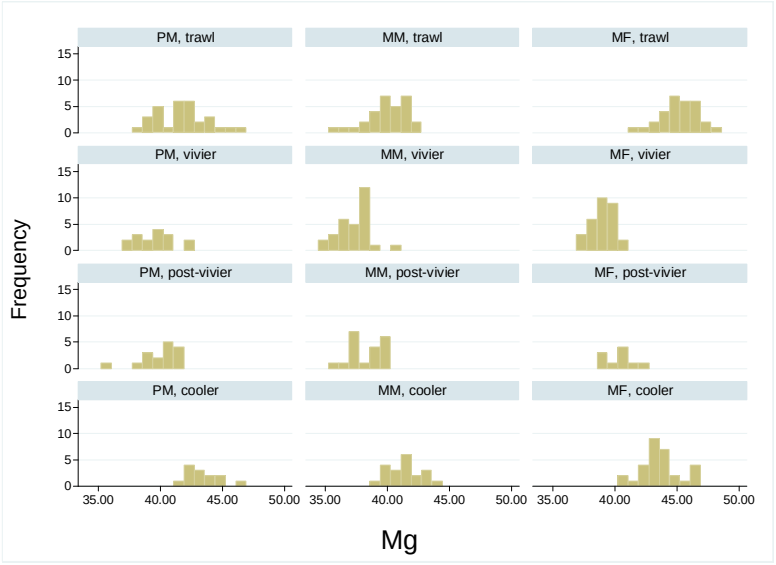
Groups are as follows: trawl/not held (trawl); vivier-held (*vivier*); post-*vivier* cooler-held (post –*vivier*); cooler-held (cooler).

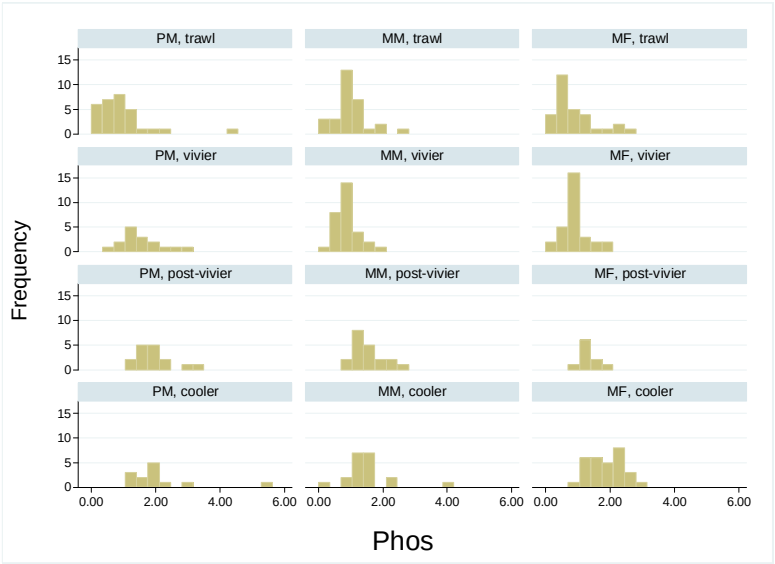


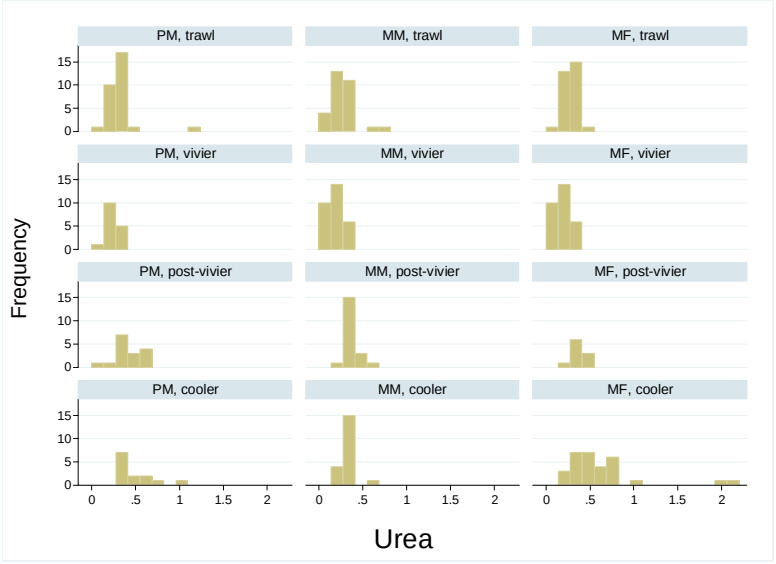


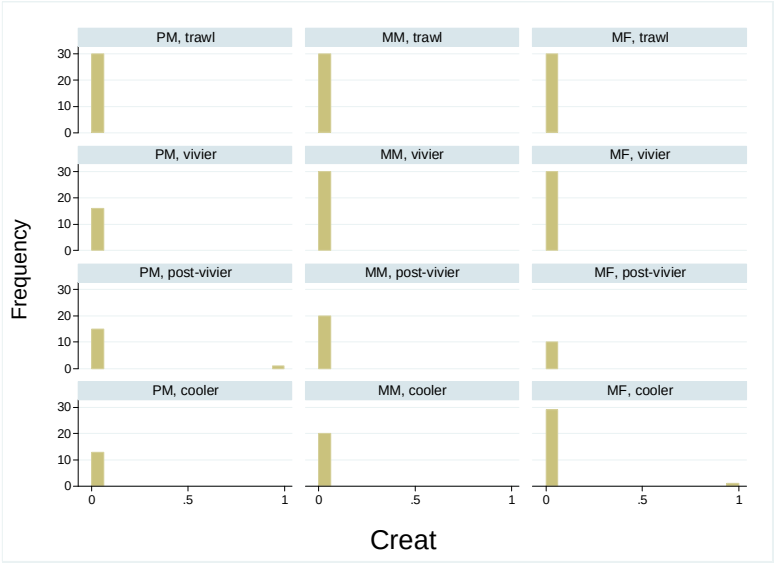


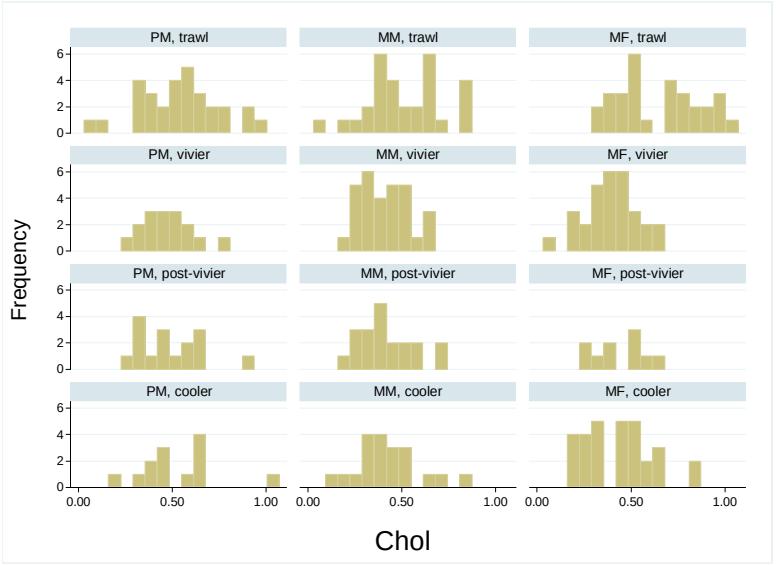

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Ca

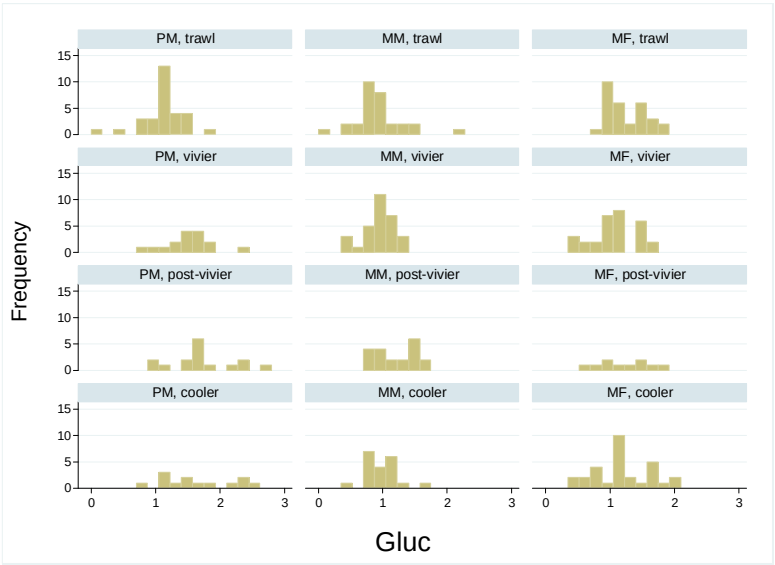

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Mg

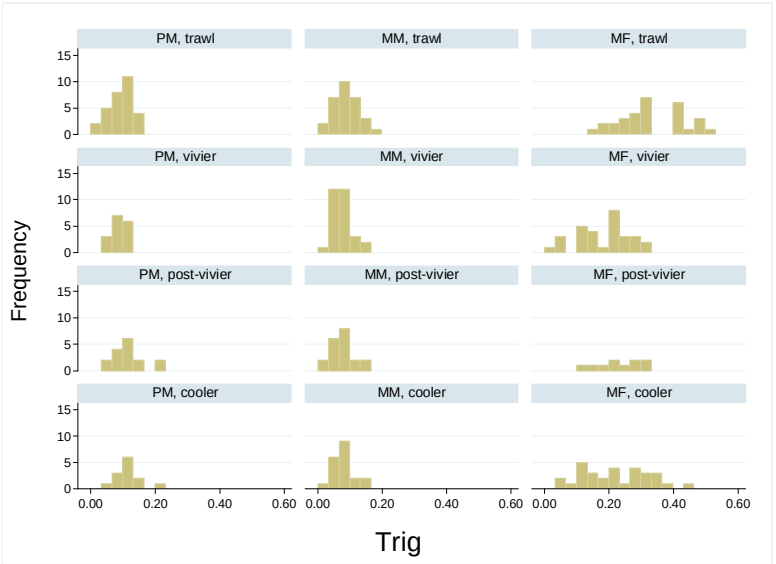

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Phos

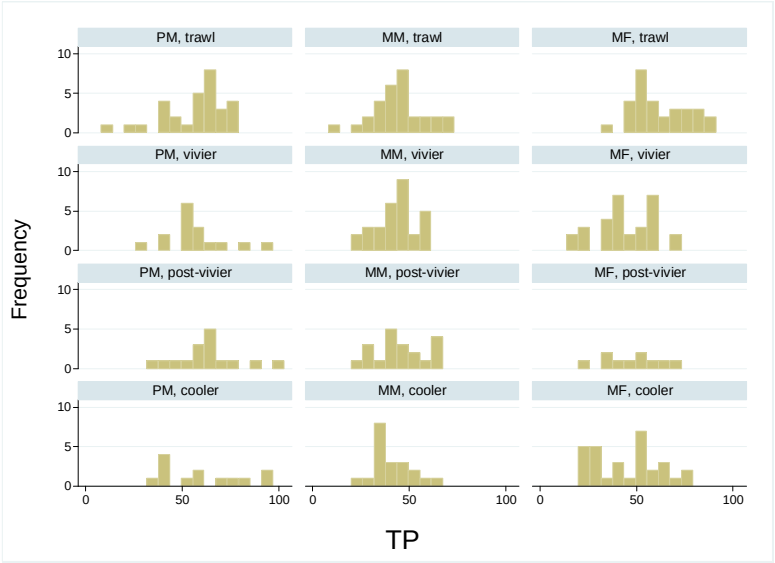

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Urea

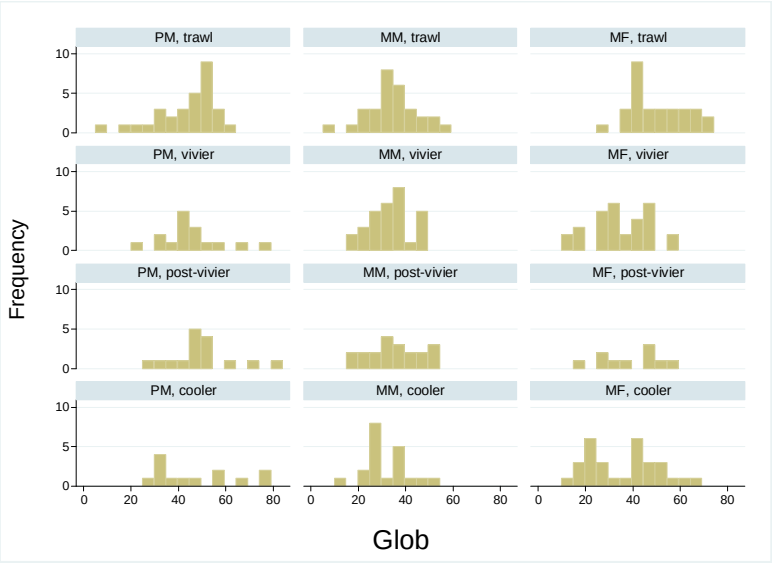

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Creat

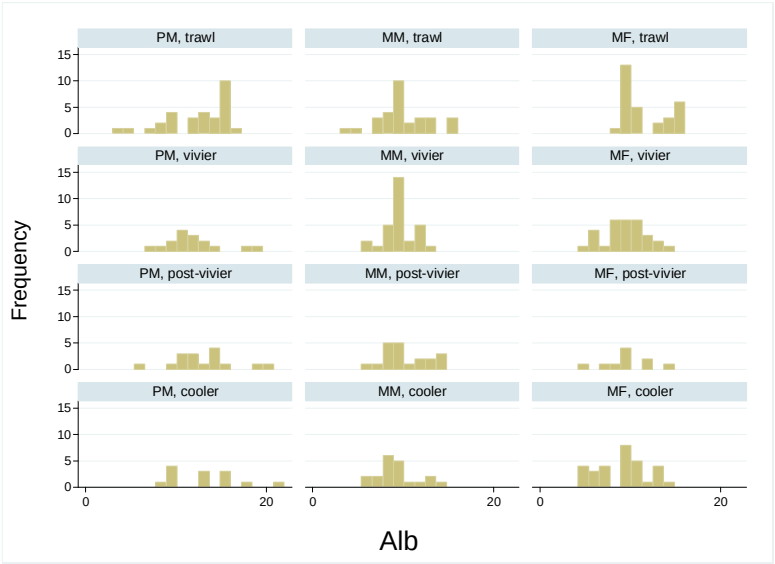

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Chol


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Gluc


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Trig


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
TP


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Glob


PM, trawl
MM, trawl
MF, trawl
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MM, vivier
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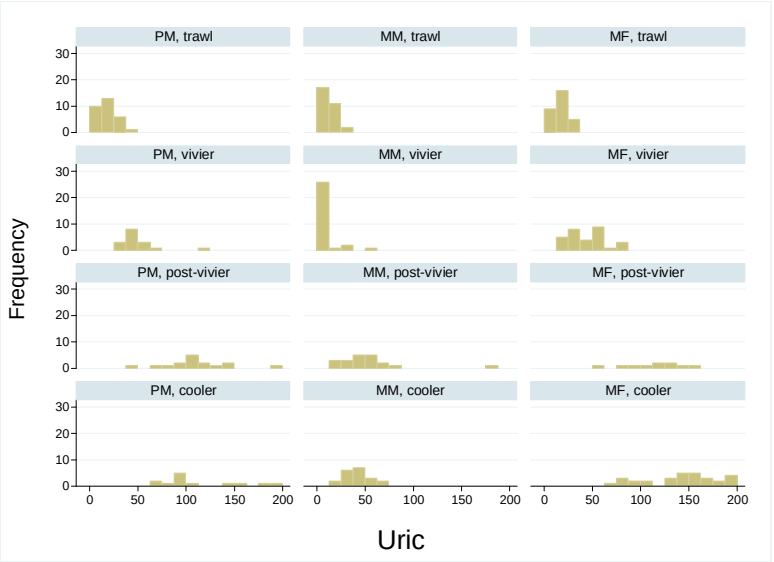
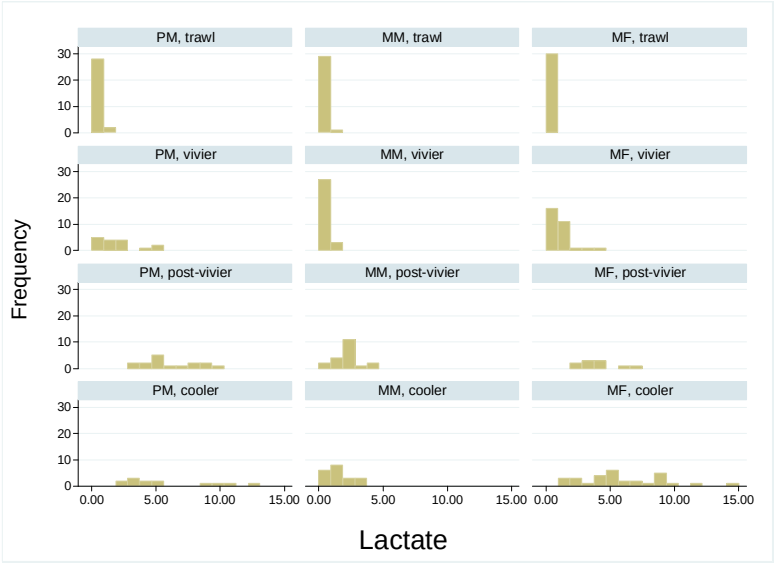
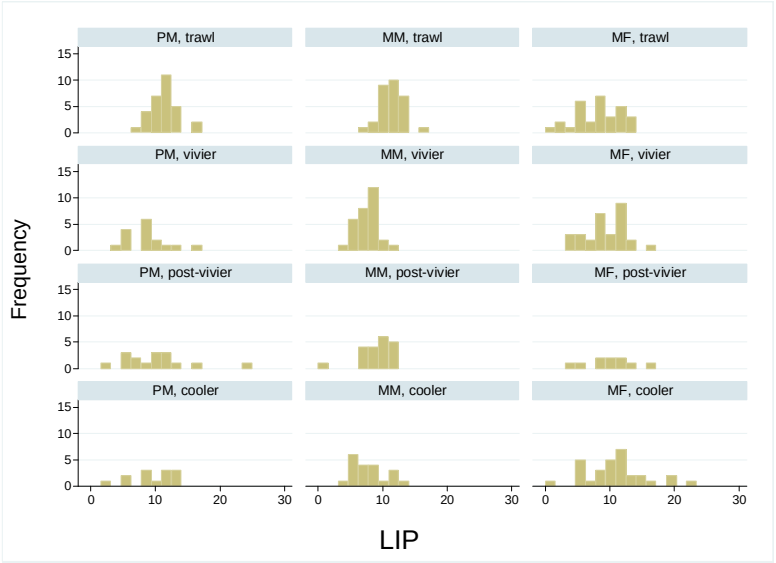
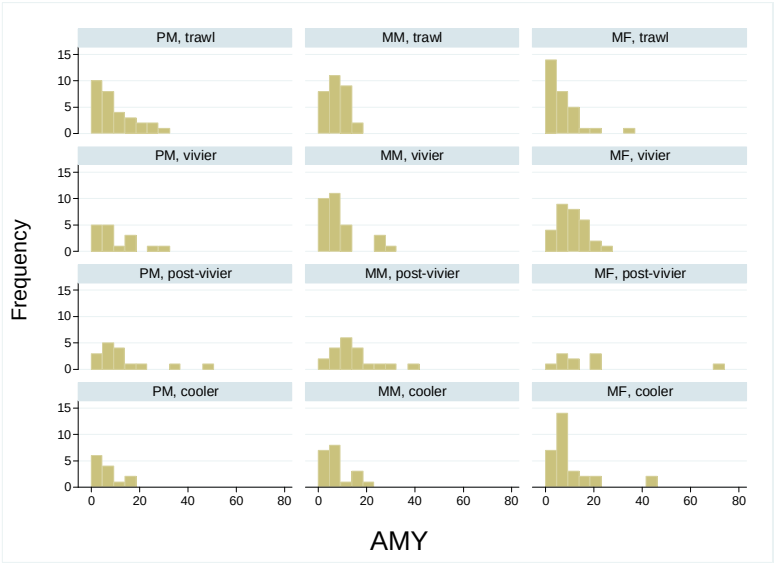
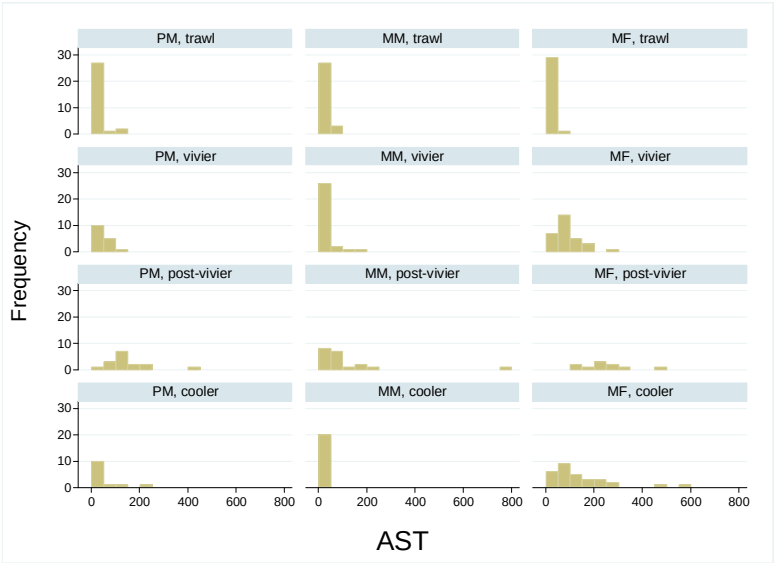
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Alb

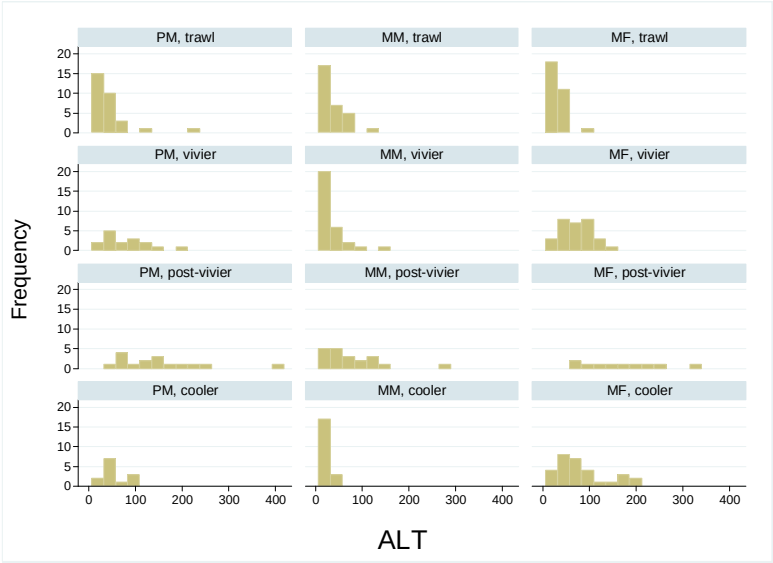

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Uric

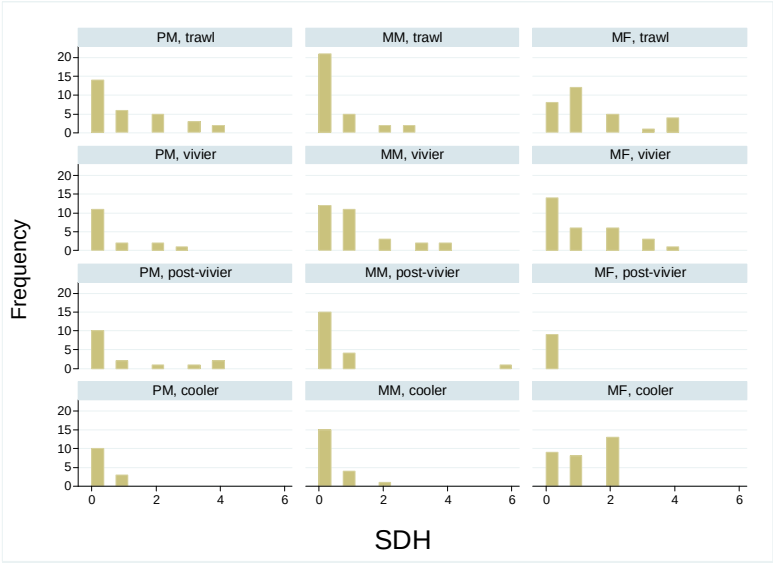

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
Lactate

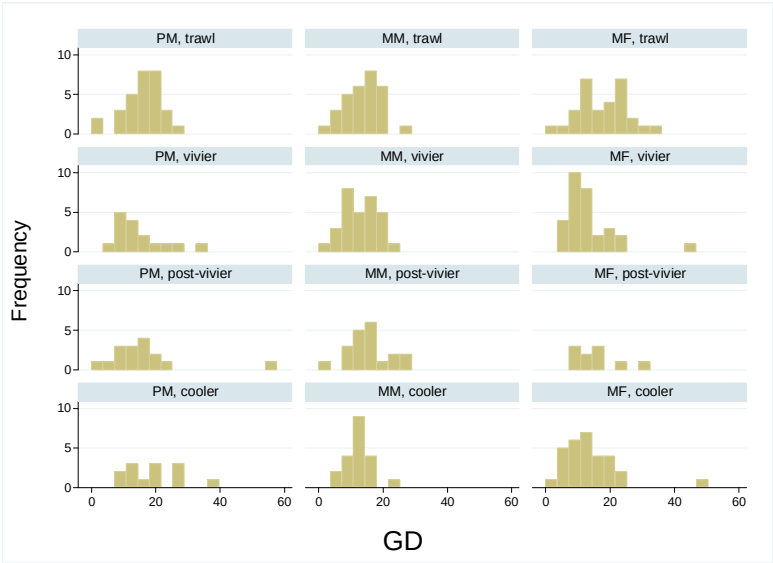

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
LIP

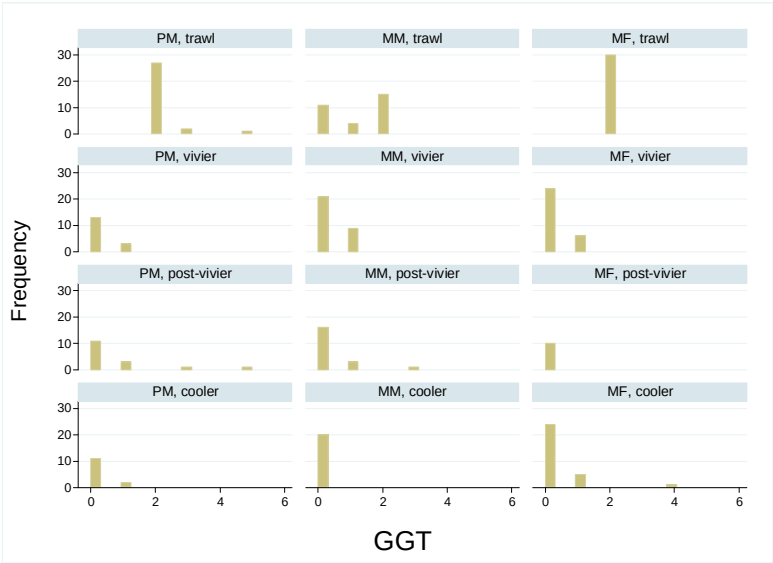

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
AMY

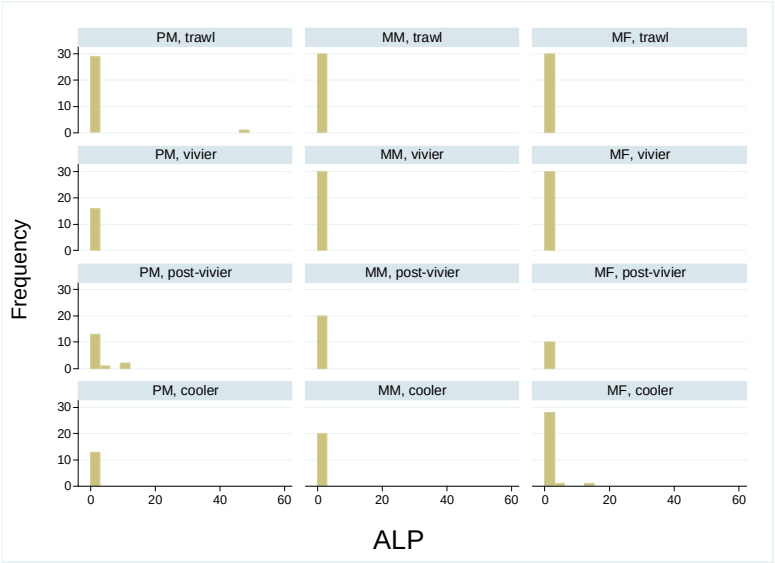

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
AST

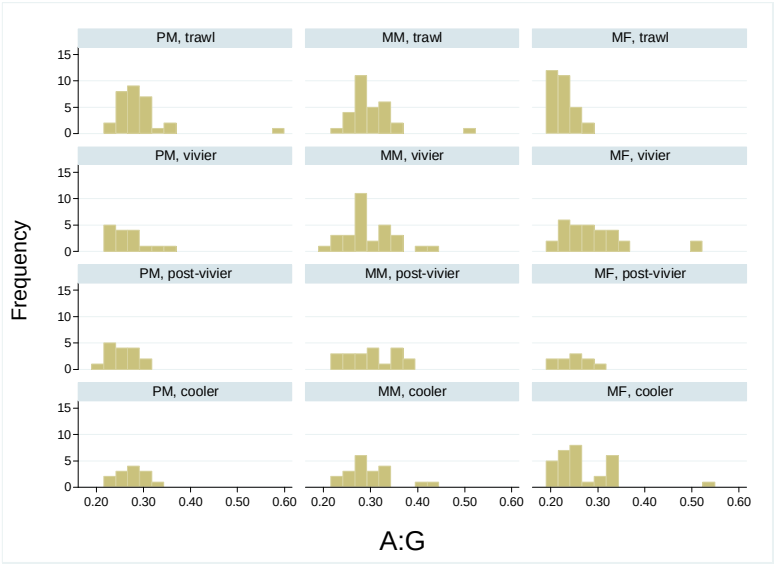

PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
ALT


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
SDH


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
GD


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
GGT


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
0
10
20
30
0
20
40
60
ALP


PM, trawl
MM, trawl
MF, trawl
PM, vivier
MM, vivier
MF, vivier
PM, post-vivier
MM, post-vivier
MF, post-vivier
PM, cooler
MM, cooler
MF, cooler
Frequency
0
5
10
15
0.20
0.30
0.40
0.50
0.60
A:G

VIII HEPATOPANCREAS ENERGY RESERVES: RELATIONSHIP TO HEMOLYMPH PARAMETERS

VIII-1. TWO WEEK (16-17 DAYS) CAGING

VIII-1-1 Summary

The study identified significant differences in some hemolymph biochemistry parameters among free pygmy male (PM), large mature male (LM) and mature female (MF) crabs, and crabs caged for a two week period at Cheticamp and Margaree Harbor fishing stations in Crab Fishing Area 19, Cape Breton, NS, in November 2012. Reference levels for hepatopancreas energy stores for the fall season, in the form of lipid and glycogen content in free (non-caged) crabs, were established for use in subsequent studies. Hepatopancreas lipid content could be predicted using hemolymph biochemistry parameters in PM and MF crabs better than in LM crabs. The reverse was true for hepatopancreas glycogen stores which could be better estimated for LM than for PM or MF crabs.

Crabs caged for a two week period had statistically significant lower median values for the hemolymph biochemistry parameters uric acid, urea, potassium and magnesium in all crab groups when compared to free crabs collected by traps two weeks previously. Similar trends were noted for total protein, calcium, cholesterol, and triglyceride concentrations. These changes are presumptively attributed to decreased food intake in caged crabs and reduced energy reserves, barring an environmental change which would have similarly affected food availability for free crabs had they been sampled at the same time as caged crabs. These results suggest that even a two week caging period is affecting crab physiology.

Hepatopancreas lipid content was more consistent for crabs from Cheticamp; while in Margaree, lipid content was significantly higher for LM compared to MF crabs. A tendency for lipid content to increase with increased carapace width (CW) was also noted for $PM_{Margaree}$ crabs. Lipid content was correlated to hemolymph cholesterol, triglyceride, total protein, and glucose concentrations in PM and MF but not LM crabs. Hepatopancrea lipid content was best predicted by simple linear regression using cholesterol $PM_{Margaree}$ ($R^2 = 0.6765$), $PM_{Cheticamp}$ ($R^2 = 0.6238$), $MF_{Margaree}$ ($R^2 = 0.6196$), and $MF_{Cheticamp}$ ($R^2 = 0.6749$).

Conversely, hepatopancreas glycogen stores were significantly correlated with hemolymph cholesterol, total protein, triglyceride, and glucose in LM crabs, but not PM or MF crabs, especially in Margaree. The adjusted R^2 values improved using multiple linear regression. The best R^2 value of 0.6535 was obtained for $LM_{Cheticamp}$. Inclusion of carapace width improved the R^2 value from 0.4570 to 0.6088 for $LM_{Margaree}$, but not for $LM_{Cheticamp}$ ($R^2 = 0.5347$). There were no detectable differences in hepatopancreas glycogen content among crab categories within a

station but comparison across stations revealed that the median value for $MF_{Margaree}$ was greater than $MF_{Cheticamp}$.

Hemolymph analysis could prove to be a non-lethal means to estimate energy stores in crab populations in future studies. Determination of total body (muscle, hepatopancreas, and gonad) energy stores is anticipated to improve the correlation to hemolymph parameters and the subsequent predictive capacity.

VIII-1-2 Objective

The objectives of this segment of the project were four-fold:

A. To examine differences in hemolymph biochemistry profiles between free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 16 – 17 days at Margaree and Cheticamp stations in fall 2012.

B. To measure hepatopancreas lipid content in free PM, LM, and MF crabs at Margaree and Cheticamp stations in fall 2012 and assess the value of hemolymph biochemistry profiles to predict lipid content.

C. To measure hepatopancreas glycogen content in free PM, LM, and MF crabs at Margaree and Cheticamp stations in fall 2012 and assess the value of hemolymph biochemistry profiles to predict glycogen content.

D. To calculate hepatopancreas lipid:glycogen ratios to compare the two forms of energy storage in the hepatopancreas.

VIII-1-3 Methodology

Crabs were collected and sampled as per criteria outlined in the main study, "Establishment of baseline biological data on snow crab (*Chionoecetes opilio*) offshore Cape Breton for future assessment of potential impacts of seismic noise on snow crab" (see Sections II & III). These included three categories based on sex and maturity: pygmy males (PM), large mature males (LM), and mature females (MF) which were carrying eggs presumably spawned in the spring 2012.
Hemolymph plasma samples were collected as described in Section III-1 and delivered to Diagnostic Services at the Atlantic Veterinary College, University of Prince Edward Island, (Charlottetown, PE) for analysis of biochemistry parameters (see Section VII-3 trawl vs trap for details).

Data on hepatopancreas moisture, lipid and glycogen content were received from RPC Science and Engineering, (Fredericton, NB). Sample processing was completed as per M. Ciaramella et al 2011 (Appendices A and B).

Data analysis was completed with STATA statistical software (STATA I/C 12.1, StataCorp LP) and Microsoft Excel (Excel 2010©, Microsoft Corporation). Bonferroni adjustments of significance were made where required for multiple comparisons.

VIII-1-4 Results

A. Hemolymph Plasma Biochemistry Profiles

All samples were processed within 24 – 48 hours of collection, well within previously established time frame for sample stability (see Section VII-2). Measurement of electrolytes (sodium, chloride, potassium) and minerals (calcium and magnesium) requires manual or programmed analyser dilution, respectively. Review of the results showed that this was a source of error (laboratory error) of some of the outliers noted in the boxplots in a few instances (Appendix C). These values were deleted from the dataset prior to statistical analyses. These deletions are indicated in Tables 1 –17. Examination of the frequency histograms (Appendix D) showed skewing of enzyme values while most other distributions were visually normal. Occasional biological (non-laboratory) outliers were noted on boxplots for many of the variables. As the sample sizes were small (maximum of 20 animals per group) and no pattern consistent with a physiological reason e.g., trauma, hemorrhage, as a cause of the marginally high or low values could be identified in crabs that had one or two outlier results on a panel with 27 values, neither the crab, nor these values were deleted from the dataset for statistical evaluation.

Values for all 26 biochemistry parameters were compared within a station across crab categories for crabs collected by traps and after the two week caging period (Tables 1 & 2). Enzyme activity showed the least difference across categories for either free or caged crabs, while numerous differences were noted for other parameters.

Hemolymph biochemistry results from crabs after a two week caging period for each category (sex) of crab in Margaree and Cheticamp, respectively, are summarised in Tables 3 and 4. Uric acid, urea, magnesium showed consistent, statistically significant lower values in all three categories. Both stations showed very little change in enzyme activity. Mineral and electrolyte changes were very common in Cheticamp crabs.

Comparison of crab category across stations for free crabs (Table 5) showed crabs from Cheticamp had higher (although not always significant), or equal, values for almost all metabolites. The trend was less consistent for caged crabs except LM crabs from Cheticamp (Table 6).

Data (count, minimum, maximum, SD, mean, and median values) from free crabs were compared to the reference intervals (RI) previously calculated for free, cooler-held, crabs collected in August near the Cheticamp station (see Section VII-3). These data are presented in Tables 7 - 18. With the exception of slightly decreased uric acid concentration in PM and MF crabs from Margaree, the medians of all other parameters fell within the limits calculated using data from the Cheticamp crabs. Other than a marginally increased median lactate in $MF_{Cheticamp}$ crabs, medians of all other parameters from the free Cheticamp crabs fell within the previously established reference interval for each crab category. Minimum and maximum values did not always fall within the RI, however.

Comparison of values from crabs caged for two weeks to the RIs for free crabs found median magnesium levels below, or very close to, the lower limit of the RI for PM and MF at both stations. Median levels of sodium and chloride were equal to or lower than the RI in PM and LM crabs from Cheticamp only. Median uric acid levels were below the RI for PM and MF at both stations. Median urea concentrations were lower than the RI in PM and LM crabs in Margaree and Cheticamp while median values were at the lower limit of the RI for MF crabs at both stations. Median enzyme activity in caged crabs fell within the RI for all crabs but $PM_{Cheticamp}$ which was below the lower limit.

Table 1. Summary of median values of hemolymph plasma biochemistry parameters of snow crab collected from Margaree, NS in November 2012 by traps (free) or after two weeks of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Median values sharing the same superscript are not different (Wilcoxon rank sum, Bonferroni-adjusted $p > 0.017$ from each other; for analytes where no superscripts are shown, medians were not different (Kruskall-Wallis testing, $p < 0.05$)

Analyte	Margaree											
	Free						Caged					
	n[1]	PM	*n*	LM	*n*	MF	*n*	PM	*n*	LM	*n*	MF
Sodium (mmol/L)		[a,b]454.5		[a]457.5		[b] 444	*18*	[a]451.5	*15*	[a]459		423
Potassium (mmol/L)		11.25		10.95		11.4		10.2	*18*	10.2		10.2
Na:K		[a,b]40		[a]42		[b]38		43	*18*	43		42
Chloride (mmol/L)		474		480		478.5	*18*	472.5	*15*	474		451.5
Calcium (mmol/L)		13.665		13.365		13.305		[a]13.28	*17*	[c]12.49		[b]13.82
Phosphorus (mmol/L)		1.77		1.31		1.605		1.43	*18*	1.485		1.59
Magnesium (mmol/L)		[ab]42.12		[c]39.51		[b]46.515		[a]38.885	*18*	[c]37.22		[b]40.82
Urea (mmol/L)		[b]0.4		0.25		[b]0.35		[a,b]0.2	*18*	[a]0.1		[b]0.2
Creatinine (mmol/L)		.[2]		.		.		.		.		.
Glucose (mmol/L)		[a]1.65		[c]1.0		[b]1.3		[b]1.5	*18*	0.95		[b]1.85
Cholesterol (mmol/L)		[a]0.65		[a]0.505		0.325		0.46	*18*	0.37		0.335
Triglyceride mmol/L)		[a]0.11		[a]0.85		0.21		[a]0.08	*18*	[a]0.06		0.155
Total Protein (g/L)		[b]65		46.5		[b]58.5		[b]51	*18*	33		[b]61
Albumin (g/L)		14		[c]11		[c]11.5		[b]13	*18*	8.5		[b] 12.5
Globulin (g/L)		[b]50.5		35		[b]45.5		[b]38	*18*	25		[b]48
A:G		[b]0.28		0.315		[b]0.255		[a]0.31	*18*	[a]0.305		[b,c]0.265
Lactate (mmol/L)		2.69		[c]1.285		[c]1.475		1.22	*18*	1.435		1.095
Uric Acid (µmol/L)		[a]44		[c]19		[b]59.5		[a]22.5	*18*	[c]11.5		[b]44.5
Amylase (U/L)		4		5.5		5.5		5	*18*	7		5
Lipase (U/L)		[a]8.5		[a]10		12.5		9.5	*18*	10.5		10
AST (U/L)		[,b]20		12.5		[b]22		[a]8	*18*	[a,c]19		[c]15.5
ALT (U/L)		[b]31		[c]17		[b,c]29.5		22.5	*18*	26		24
GD (U/L)		12		10		10		[b]14	*18*	9		[b]15
SDH (U/L)		[a,b]0		[a]1		[b]0		0	*18*	0.5		0.5
GGT (U/L)		0		0		0		0	*18*	0		0
ALP (U/L)		0		0		0		0	*18*	0		0

[1] number of crabs per sample = 20 unless indicated otherwise
[2] analyte not detected

Table 2. Summary of median values of hemolymph plasma biochemistry parameters of snow crab collected from Cheticamp, NS in November 2012 by traps (free) or after two weeks of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Median values sharing the same superscript are not different (Wilcoxon rank sum, Bonferroni-adjusted $p > 0.017$ from each other; for analytes where no superscripts are shown, medians were not different (Kruskall-Wallis testing, $p < 0.05$).

Analyte	Cheticamp										
	Free						Caged				
	n[1]	PM	*n*	LM	*n*	MF	*n*	PM	*n*	LM	*n*	MF
Sodium (mmol/L)	***17***	[a]459		[a]456		429	*19*	[a]423		[a]423		396
Potassium (mmol/L)		[a]12.3		[a,c]11.55		[c]10.8	*19*	9.3		9.6		9.3
Na:K		37		38		38	*19*	[a]45		[a]44		43
Chloride (mmol/L)	***17***	[a]471		[a]471		453	*19*	[a,b]429		[a]432		[b]408
Calcium (mmol/L)		[b]14.435	*19*	[c]13.82	*19*	[b,c]13.70	*19*	14.15		13.955		14.19
Phosphorus (mmol/L)	*17*	1.81		2.225		1.795	*19*	[a]1.40		[a,c]1.475		[c]1.91
Magnesium (mmol/L)		[a]42.19	*19*	[c]40.27		[b]45.09	*19*	[a]39.38		[a]38.66		41.84
Urea (mmol/L)		[b]0.6		[c]0.4		[b,c]0.4	*19*	[a,b]0.1		[a]0.1		[b]0.2
Creatinine (mmol/L)		.[2]		.		.		.		.		.
Glucose (mmol/L)		1.95		1.5		1.6	*19*	[b]1.7		1.3		[b]1.75
Cholesterol (mmol/L)		0.67		0.49		0.56	*19*	0.51		0.445		0.47
Triglyceride (mmol/L)		[a]0.11		[c]0.08		[b]0.285	*19*	[a]0.08		[a]0.07		0.19
Total Protein (g/L)		[,b]73.5		[c]54.5		[b]69.5	*19*	[b]58		47		[b]62
Albumin (g/L)		16		[c]11		[c]13	*19*	12		10.5		11.5
Globulin (g/L)		[b]58.5		43.5		[b]55.5	*19*	[b]46		35		[b]51
A:G		[a]0.275		[a]0.29		0.23	*19*	[a]0.27		[a]0.29		0.22
Lactate (mmol/L)		[a]4.535		[a]3.54		1.925	*19*	1.42		1.105		1.55
Uric Acid (µmol/L)		[b]68		43.5		[b]74	*19*	[a]18		[c]11.5		[b]40
Amylase (U/L)		4		4		3.5	*19*	4		5		4
Lipase (U/L)		11		10		11	*19*	[b]7		10		[b]7.5
AST (U/L)		32.5		20		22.5	*19*	7		11		11
ALT (U/L)		49.5		30.5		33	*19*	20		21.5		18
GD (U/L)		[b]17		11		[b,c]12	19	14		12		14
SDH (U/L)		0		0		0	19	0		0		0
GGT (U/L)		0		0		0	19	0		0		0
ALP (U/L)		0		0		0	19	0		0		0

[1] number of crabs per sample = 20 unless indicated otherwise

[2] analyte not detected

Table 3. Comparison of median values of hemolymph plasma biochemistry parameters of snow crab (free or after a two week period of caging) snow crab collected from Margaree, NS in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) changes were noted are shown.

Analyte	Margaree												
	PM					LM					MF		
	n[1]	Trap	*n*	Cage	*p*	*n*	Trap	*n*	Cage	*p*	*n*	Trap	*n*	Cage	*p*
Sodium (mmol/L)		454.5	*18*	451.5	----	*15*	457.5		459	----		444		423	----
Potassium (mmol/L)		11.25		10.2	*0.0000*	*18*	10.95		10.2	*0.0004*		11.4		10.2	*0.0001*
Na:K		40		43	*0.0000*	*18*	42		43	*0.0568*		38		42	*0.0000*
Chloride (mmol/L)		474	*18*	472.5	----	*15*	480		474	----		478.5		451.5	*0.0383*
Calcium (mmol/L)		13.665		13.28	----	*17*	13.365		12.49	*0.0000*		13.305		13.82	*0.0098*
Phosphorus (mmol/L)		1.77		1.43	----	*18*	1.31		1.485	----		1.605		1.59	----
Magnesium (mmol/L)		42.12		38.885	*0.0000*	*18*	39.51		37.22	*0.0001*		46.515		40.82	*0.0000*
Urea (mmol/L)		0.4		0.2	*0.0000*	*18*	0.25		0.1	*0.0001*		0.35		0.2	*0.0002*
Creatinine (mmol/L)		.		.	.[2]		.		.	.		.		.	.
Glucose (mmol/L)		1.65		1.5	----	*18*	1.0		0.95	----		1.3		1.85	*0.0001*
Cholesterol (mmol/L)		.65		0.46	*0.0222*	*18*	0.505		0.37	*0.0088*		0.325		0.335	----
Triglyceride (mmol/L)		.11		0.08	----	*18*	0.085		0.06	*0.0286*		0.21		0.155	*0.0226*
Total Protein (g/L)		65		51	*0.0358*	*18*	46.5		33	*0.0006*		58.5		61	----
Albumin (g/L)		14		13	----	*18*	11		8.5	*0.0010*		11.5		12.5	----
Globulin (g/L)		50.5		38	*0.0371*	*18*	35		25	*0.0007*		45.5		48	----
A:G		0.28		0.31	----	*18*	0.315		0.305	----		0.255		0.265	----
Lactate (mmol/L)		2.69		1.22	*0.0002*	*18*	1.285		1.435	----		1.475		1.095	----
Uric Acid (µmol/L)		44		22.5	*0.0001*	*18*	19		11.5	*0.0027*		59.5		44.5	*0.0005*
Amylase U/L)		4		5	----	*18*	5.5		7	----		5.5		5	----
Lipase (U/L)		8.5		9.5	----	*18*	10		10.5	----		12.5		10	*0.0163*
AST (U/L)		20		8	*0.0006*	*18*	12.5		19	----		22		15.5	----
ALT (U/L)		31		22.5	----	*18*	17		26	*0.0164*		29.5		24	----
GD (U/L)		12		14	----	*18*	10		9	----		10		15	*0.0012*
SDH (U/L)		0		0	----	*18*	1		0.5	----		0		0.5	*0.0025*
GGT (U/L)		0		0	.	*18*	0		0	.		0		0	----
ALP (U/L)		0		0	----	*18*	0		0	.		0		0	.

[1] numbers of crabs per sample presented as free/caged

[2] analyte not detected

Table 4. Comparison of median values of hemolymph plasma biochemistry parameters of snow crab (free or after a two week period of caging) collected from Cheticamp, NS in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) changes were noted are shown.

Analyte	Cheticamp													
	PM					LM					MF			
	n[1]	Trap	*n*	Cage	*p*	*n*	Trap	n	Cage	*p*	n	Trap	*n*	Cage	*p*
Sodium (ol/L)	*17*	459	*19*	423	*0.0000*		456		423	*0.0000*		429		396	*0.0007*
Potassium (mmol/L)		12.3	*19*	9.3	*0.0000*		11.55		9.6	*0.0000*		10.8		9.3	*0.0000*
Na:K		37	*19*	45	*0.0000*		38		44	*0.0000*		38		43	*0.0000*
Chloride (mmol/L)	*17*	471	*19*	429	*0.0000*		471		432	*0.0000*		453		408	*0.0001*
Calcium (mmol/L)		14.435	*19*	14.15	----	*19*	13.82		13.955	-----	*19*	13.70		14.19	----
Phosphorus (mmol/L)		1.81	*19*	1.40	*0.0350*		2.225		1.475	*0.0045*		1.795		1.91	----
Magnesium (mmol/L)		42.19	*19*	39.38	*0.0000*	*19*	40.27		38.66	*0.0000*	*19*	45.09		41.84	*0.0000*
Urea (mmol/L)		0.6	*19*	0.1	*0.0000*		0.4		0.1	*0.0000*		0.4		0.2	*0.0000*
Creatinine (mmol/L)		.[2]		.	.		.		.	.		.		.	.
Glucose (mmol/L)		1.95	*19*	1.7	*0.0350*		1.5		1.3	*0.0018*		1.6		1.75	----
Cholesterol (mmol/L)		0.67	*19*	0.51	*0.0128*		0.49		0.445	----		0.56		0.47	----
Triglyceride (mmol/L)		0.11	*19*	0.08	*0.0015*		0.08		0.07	----		0.285		0.19	*0.0108*
Total Protein (g/L)		73.5	*19*	58	*0.0032*		54.5		47	*0.0113*		69.5		62	----
Albumin (g/L)		16	*19*	12	*0.0011*		11		10.5	----		13		11.5	----
Globulin (g/L)		58.5	*19*	46	*0.0047*		43.5		35	*0.0089*		55.5		51	----
A:G		0.275	*19*	0.27	----		0.29		0.29	----		0.23		0.22	----
Lactate (mmol/L)		4.535	*19*	1.42	*0.0000*		3.54		1.105	*0.0000*		1.925		1.55	*0.0315*
Uric Acid (µmol/L)		68	*19*	18	*0.0000*		43.5		11.5	*0.0000*		74		40	*0.0000*
Amylase(U/L)		4	*19*	4	----		4		5	----		3.5		4	----
Lipase (U/L)		11	*19*	7	*0.0001*		10		10	----		11		7.5	*0.0000*
AST (U/L)		32.5	*19*	7	*0.0004*		20		11	*0.0008*		22.5		11	*0.0055*
ALT (U/L)		49.5	*19*	20	*0.0005*		30.5		21.5	*0.0410*		33		18	*0.0371*
GD (U/L)		17	*19*	14	----		11		12	----		12		14	----
SDH (U/L)		0	*19*	0	----		0		0	*0.0080*		0		0	----
GGT (U/L)		0	*19*	0	----		0		0	----		0		0	----
ALP (U/L)		0	*19*	0	----		0		0	.		0		0	.

[1] numbers crabs per sample = 20 unless indicated otherwise

[2] analyte not detected

Table 5. Comparison between Margaree Harbor and Cheticamp median values of hemolymph plasma biochemistry parameters for snow crab collected by traps (free) in November 2012. Only p-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) differences were detected are shown.

Analyte	Free Crabs													
	PM					LM					MF			
	n[1]	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*
Sodium (mmol/L)		454.5	*17*	459	----		457.5		456	----		444		429	*0.0005*
Potassium (mmol/L)		11.25		12.3	*0.0133*		10.95		11.55	----		11.4		10.8	----
Na:K		40		37	*0.0360*		42		38	*0.0087*		38		38	----
Chloride (mmol/L)		474	*17*	471	----		480		471	----		478.5		453	*0.0000*
Calcium (mmol/L)		13.665		14.435	*0.0058*		13.365	*19*	13.82	*0.0150*		13.305	*19*	13.70	*0.0228*
Phosphorus (mmol/L)		1.77		1.81	----		1.31		2.225	*0.0005*		1.605		1.795	----
Magnesium (mmol/L)		42.12		42.19	----		39.51	*19*	40.27	----		46.515		45.09	*0.0097*
Urea (mmol/L)		0.4		0.6	*0.0008*		0.25		0.4	*0.0000*		0.35		0.4	----
Creatinine (mmol/L)		[2].		.	.		.		.	.		.		.	.
Glucose (mmol/L)		1.65		1.95	----		1.0		1.5	*0.0000*		1.3		1.6	*0.0129*
Cholesterol (mmol/L)		0.65		0.67	----		0.505		0.49	----		0.325		0.56	*0.0038*
Triglyceride (mmol/L)		0.11		0.11	----		0.085		0.08	----		0.21		0.285	----
Total Protein (g/L)		65		73.5	----		46.5		54.5	----		58.5		69.5	*0.0424*
Albumin (g/L)		14		16	----		11		11	----		11.5		13	----
Globulin (g/L)		50.5		58.5	----		35		43.5	*0.0291*		45.5		55.5	*0.0324*
A:G		0.28		0.275	----		0.315		0.29	*0.0209*		0.255		0.23	*0.0352*
Lactate (mmol/L)		2.69		4.535	*0.0161*		1.285		3.54	*0.0000*		1.475		1.925	*0.0193*
Uric Acid (µmol/L)		44		68	*0.0001*		19		43.5	*0.0000*		59.5		74	----
Amylase(U/L)		4		4	----		5.5		4	----		5.5		3.5	*0.0471*
Lipase U/L)		8.5		11	*0.0116*		10		10	----		12.5		11	----
AST (U/L)		20		32.5	----		12.5		20	*0.0011*		22		22.5	----
ALT (U/L)		31		49.5	----		17		30.5	*0.0000*		29.5		33	----
GD (U/L)		12		17			10		11			10		12	
SDH (U/L)		0		0			1		0			0		0	
GGT (U/L)		0		0			0		0			0		0	
ALP (U/L)		0		0			0		0			0		0	

[1] numbers crabs per sample = 20 unless indicated otherwise

[2] analyte not detected

Table 6. Comparison between Margaree Harbor and Cheticamp of median values of hemolymph plasma biochemistry parameters for snow crab held in cages for two weeks in November 2012. Only p-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) differences were detected are shown.

Analyte	Caged Crabs													
	PM					LM					MF			
	n[1]	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*
Sodium (mmol/L)	*18*	451.5	*19*	423	*0.0000*	*15*	459		423	*0.0000*		423		396	*0.0002*
Potassium (mmol/L)		10.2	*19*	9.3	*0.0005*	*18*	10.2		9.6	*0.0202*		10.2		9.3	*0.0002*
Na:K		43	*19*	45	*0.0244*	*18*	43		44	----		42		43	*0.1233*
Chloride (mmol/L)	*18*	472.5	*19*	429	*0.0000*	*15*	474		432	*0.0000*		451.5		408	*0.0001*
Calcium (mmol/L)		13.28	*19*	14.15	*0.0024*	*17*	12.49		13.955	*0.0000*		13.82		14.19	*0.1074*
Phosphorus (mmol/L)		1.43	*19*	1.40	----	*18*	1.485		1.475	----		1.59		1.91	*0.0337*
Magnesium (mmol/L)		38.885	*19*	39.38	----	*18*	37.22		38.66	*0.0068*		40.82		41.84	*0.0658*
Urea (mmol/L)		0.2	*19*	0.1	----	*18*	0.1		0.1	----		0.2		0.2	----
Creatinine (mmol/L)		[2].		.	.		.		.	.		.		.	.
Glucose (mmol/L)		1.5	*19*	1.7	----	*18*	0.95		1.3	*0.0257*		1.85		1.75	----
Cholesterol (mmol/L)		0.46	*19*	0.51	----	*18*	0.37		0.445	*0.0378*		0.335		0.47	*0.0192*
Triglyceride (mmol/L)		0.08	*19*	0.08	----	*18*	0.06		0.07	----		0.155		0.19	----
Total Protein (g/L)		51	*19*	58	----	*18*	33		47	*0.0036*		61		62	----
Albumin (g/L)		13	*19*	12	----	*18*	8.5		10.5	*0.0399*		12.5		11.5	----
Globulin (g/L)		38	*19*	46	----	*18*	25		35	*0.0017*		48		51	----
A:G		0.31	*19*	0.27	*0.0345*	*18*	0.305		0.29	----		0.265		0.22	*0.0000*
Lactate (mmol/L)		1.22	*19*	1.42	----	*18*	1.435		1.105	----		1.095		1.55	----
Uric Acid (µmol/L)		22.5	*19*	18	----	*18*	11.5		11.5	----		44.5		40	----
AmylaseU/L)		5	*19*	4	----	*18*	7		5	*0.0437*		5		4	----
Lipase (U/L)		9.5	*19*	7	*0.0091*	*18*	10.5		10	----		10		7.5	*0.0001*
AST (U/L)		8	*19*	7	----	*18*	19		11	----		15.5		11	*0.0237*
ALT (U/L)		22.5	*19*	20	----	*18*	26		21.5	----		24		18	----
GD (U/L)		14	*19*	14	----	*18*	9		12	----		15		14	----
SDH (U/L)		0	*19*	0	----	*18*	0.5		0	----		0.5		0	----
GGT (U/L)		0	*19*	0	----	*18*	0		0	----		0		0	.
ALP (U/L)		0	*19*	0	----	*18*	0		0	.		0		0	.

[1] numbers crabs per sample = 20 unless indicated otherwise

[2] analyte not detected

Table 7. Summary statistics for mineral and electrolyte concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Na[2]	20	408	477	450	18.1311	454.5	423	462
	K	20	10.2	14.1	11.58	1.059096	11.25	9.9	13.2
	Na:K	20	32	43	39	3.305759	40	34	46
	Cl	20	411	501	465.1	27.90944	474	444	498
	Mg	20	38.29	46.21	42.219	2.028011	42.12	41.59	46.26
	Ca	20	11.33	14.86	13.4915	.9641264	13.665	12.38	15.53
	Phos	20	.69	3.62	1.806	.7290686	1.77	1.28	5.62
LM	Na	20	438	495	460.8	14.86996	457.5	441	490
	K	20	9.9	12.3	11.165	.6899847	10.95	9.2	12.7
	Na:K	20	36	45	41	2.894231	42	36	49
	Cl	20	429	504	477.15	17.233	480	459	513
	Mg	20	37.33	42.71	39.7225	1.386157	39.51	38.53	44.12
	Ca	20	12.32	15.03	13.4545	.6491977	13.365	12.15	14.03
	Phos	20	.48	2.9	1.4235	.6321915	1.31	0	3.02
MF	Na	20	420	462	442.05	10.9375	444	385	496
	K	20	10.5	12.6	11.38	.5434781	11.4	7.8	13.5
	Na:K	20	35	42	38	1.574233	38	33	51
	Cl	20	447	504	476.7	13.27086	478.5	396	507
	Mg	20	42.87	51.8	46.599	2.075722	46.515	40.05	46.67
	Ca	20	12.79	15.53	13.483	.6695254	13.305	12.51	16.67
	Phos	20	.85	3.03	1.627	.5712231	1.605	0.72	3.00

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits for the sampled populations.[2] Units for all parameters are mmol/L.

Table 8. Summary statistics for mineral and electrolyte concentrations for two week caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Na[2]	18	417	474	449.6667	17.67018	451.5	423	462
	K	20	7.7	11.1	10.14	.932117	10.2	9.9	13.2
	Na:K	20	41	46	43.4	1.698296	43	34	46
	Cl	18	423	504	468.8333	24.54108	472.5	444	498
	Mg	20	34.64	42.68	38.924	1.964668	38.885	41.59	46.26
	Ca	20	12.02	14.21	13.1695	.5824221	13.28	12.38	15.53
	Phos	20	0.04	3.82	1.472	.8079317	1.43	1.28	5.62
LM	Na	15	402	480	456	19.07504	459	441	490
	K	18	6.6	12	9.961111	1.253296	10.2	9.2	12.7
	Na:K	18	38	48	43.83333	2.431412	43	36	49
	Cl	15	405	501	469.2667	23.73204	474	459	513
	Mg	18	32.98	40.89	37.23167	1.819658	37.22	38.53	44.12
	Ca	17	11.69	13.74	12.42824	.5094757	12.49	12.15	14.03
	Phos	18	.54	2.93	1.492778	.5878456	1.485	0	3.02
MF	Na	20	393	477	430.2	24.61194	423	385	496
	K	20	8.4	12	10.32	.8166685	10.2	7.8	13.5
	Na:K	20	38	47	42.3	2.319256	42	33	51
	Cl	20	405	522	456.15	33.63938	451.5	396	507
	Mg	20	39.95	43.83	41.3015	1.250155	40.82	40.05	46.67
	Ca	20	12.45	15.14	13.8255	.5567148	13.82	12.51	16.67
	Phos	20	0.14	2.63	1.526	.6121438	1.59	0.72	3.00

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.
[2] Units for all parameters are mmol/L.

Table 9. Summary statistics for mineral and electrolyte concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Na[2]	17	441	468	456	8.551316	459	423	462
	K	20	11.1	14.7	12.32	.9105233	12.3	9.9	13.2
	Na:K	20	31	41	37	2.465929	37	34	46
	Cl	17	456	504	472.2353	11.7182	471	444	498
	Mg	20	40.51	45	42.5325	1.41102	42.19	41.59	46.26
	Ca	20	12.52	15.68	14.3595	.8674978	14.435	12.38	15.53
	Phos	20	0.84	4.48	1.945	.8006478	1.81	1.28	5.62
LM	Na	20	426	483	455.4	15.9519	456	441	490
	K	20	10.2	14.7	11.845	1.146379	11.55	9.2	12.7
	Na:K	20	31	43	38	2.927186	38	36	49
	Cl	20	429	495	469.35	18.40273	471	459	513
	Mg	19	37.91	42.34	40.33263	1.065118	40.27	38.53	44.12
	Ca	19	13.18	14.62	13.84316	.3843472	13.82	12.15	14.03
	Phos	20	1.29	5.65	2.613	1.314923	2.225	0	3.02
MF	Na	20	390	456	423.3	17.29953	429	385	496
	K	20	9.9	12.9	11.055	.8828632	10.8	7.8	13.5
	Na:K	20	32	42	38	2.489602	38	33	51
	Cl	20	408	483	450	20.83014	453	396	507
	Mg	19	41.88	47.67	45.00684	1.487929	45.09	40.05	46.67
	Ca	19	12.59	15.65	13.94632	.8250469	13.70	12.51	16.67
	Phos	20	0.25	3.66	1.947	.9390482	1.795	0.72	3.0

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.

[2] Units for all parameters are mmol/L.

Table 10. Summary statistics for mineral and electrolyte concentrations for two week caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	Na[2]	19	390	444	419.3684	12.27106	423	423	462
	K	19	8.1	10.8	9.347368	.6185722	9.3	9.9	13.2
	Na:K	19	39	50	44.89474	2.514287	45	34	46
	Cl	19	402	465	428.8421	15.63565	429	444	498
	Mg	19	36.97	41.56	39.48632	1.346754	39.38	41.59	46.26
	Ca	19	11.62	15.2	13.92526	.8613708	14.15	12.38	15.53
	Phos	19	.53	3.85	1.517895	.8068428	1.4	1.28	5.62
LM	Na	20	402	459	424.2	11.93668	423	441	490
	K	20	9	10.5	9.6	.4129483	9.6	9.2	12.7
	Na:K	20	41	46	44.15	1.348488	44	36	49
	Cl	20	408	468	432.15	13.84985	432	459	513
	Mg	20	36.07	40.22	38.536	.9378104	38.66	38.53	44.12
	Ca	20	13.12	14.8	13.9145	.4992676	13.955	12.15	14.03
	Phos	20	.51	3.79	1.6425	.7460413	1.475	0	3.02
MF	Na	20	378	459	401.55	21.41931	396	385	496
	K	20	8.4	11.1	9.375	.6447154	9.3	7.8	13.5
	Na:K	20	40	46	43.1	1.518309	43	33	51
	Cl	20	381	480	413.7	25.91504	408	396	507
	Mg	20	40.37	43.29	41.821	.8220123	41.84	40.05	46.67
	Ca	20	13.4	14.66	14.0675	.4008921	14.19	12.51	16.67
	Phos	20	1.08	4.24	2.0195	.6749306	1.91	0.72	3.0

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.

[2] Units for all parameters are mmol/L.

Table 11. Summary statistics for metabolite concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Urea[2]	20	.1	2.6	.47	.5141165	.4	0.3	1.1
	Creat	0	.	.	.	.	.	0	0
	Gluc	20	.7	2.2	1.615	.4404244	1.65	0.7	2.6
	Chol	20	.16	1.17	.6205	.2632484	.65	0.22	1.07
	Trig	20	.03	.25	.1105	.0526633	.11	0.05	0.2
	TPb	20	18	91	62.05	20.16635	65	36	97
	Alb	20	5	20	13.55	3.88621	14	8	22
	Glob	20	13	73	48.5	16.3948	50.5	28	78
	A:G	20	.24	.38	.2885	.0368889	.28	0.23	0.33
	Lactate	20	.9	12.34	3.4835	2.508494	2.69	2.15	13.05
	Uric	20	16	128	44.85	23.38527	44	65	199
LM	Urea	20	.1	.4	.245	.0759155	.25	0.2	0.6
	Creat	0	.	.	.	.	.	0	0
	Gluc	20	.6	1.5	1.045	.237254	1.0	0.5	1.7
	Chol	20	.24	.88	.541	.1811339	.505	0.14	0.89
	Trig	20	.04	.16	.0945	.0353144	.085	0.03	0.16
	TPb	20	29	63	47	9.392046	46.5	14	65
	Alb	20	7	16	11.2	2.117595	11	6	16
	Glob	20	22	50	35.8	7.681831	35	12	52
	A:G	20	.22	.39	.316	.0418519	.315	0.28	0.46
	Lactate	20	.16	2.45	1.34	.6108924	1.285	0.0	3.47
	Uric	20	13	31	20.4	5.825534	19	6	75
MF	Urea	20	.2	.6	.375	.1019546	.35	0.2	2.2
	Creat	0	.	.	.	.	.	0	0
	Gluc	20	.8	1.8	1.315	.2870448	1.3	0.3	2.1
	Chol	20	.1	.82	.377	.1803535	.325	0.05	0.81
	Trig	20	.11	.59	.249	.1261953	.21	0	0.44
	TPb	20	36	77	56.1	11.16338	58.5	9	81
	Alb	20	8	15	11.4	1.846761	11.5	3	15
	Glob	20	28	63	44.7	9.712174	45.5	11	71
	A:G	20	.19	.32	.26	.0350939	.255	0.19	0.46
	Lactate	20	.35	3.38	1.536	.6469556	1.475	0.98	14.46
	Uric	20	47	130	63.5	17.96341	59.5	62	222

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.

[2] Units for urea, creatinine, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid (µmol/L); for total protein, albumin, and globulin (g/L)

Table 12. Summary statistics for metabolite concentrations for two week caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Urea[2]	20	.1	.3	.175	.0786398	0.2	0.3	1.1
	Creat	20	0	0	0	0	0	0	0
	Gluc	20	1	2.1	1.525	.3507511	1.5	0.7	2.6
	Chol	20	.25	.87	.461	.1561342	0.46	0.22	1.07
	Trig	20	.04	.14	.0885	.0296071	0.08	0.05	0.2
	TPb	20	33	73	51.8	12.47144	51	36	97
	Alb	20	8	16	12.05	2.139233	13	8	22
	Glob	20	23	59	39.75	10.74526	38	28	78
	A:G	20	.23	.43	.3135	.0559393	0.31	0.23	0.33
	Lactate	20	.27	3.41	1.447	.8636526	1.22	2.15	13.05
	Uric	20	12	38	23.35	7.77496	22.5	65	199
LM	Urea	18	0	.3	.1333333	.0685994	.1	0.2	0.6
	Creat	18	0	0	0	0	0	0	0
	Gluc	18	.4	1.6	.9833333	.3807887	.95	0.5	1.7
	Chol	18	.07	.59	.3644444	.1490449	.37	0.14	0.89
	Trig	18	.01	.11	.0672222	.0273981	.06	0.03	0.16
	TPb	18	12	52	33.44444	10.6783	33	14	65
	Alb	18	3	12	8.277778	2.539235	8.5	6	16
	Glob	18	9	41	25.16667	8.542282	25	12	52
	A:G	18	.26	.6	.3394444	.0881491	.305	0.28	0.46
	Lactate	18	.34	5.1	1.616667	1.192299	1.435	0.0	3.47
	Uric	18	5	26	13.44444	6.57287	11.5	6	75
MF	Urea	20	0	.4	.22	.1151658	.2	0.2	2.2
	Creat	20	0	0	0	0	0	0	0
	Gluc	20	1.1	2.4	1.81	.3754296	1.85	0.3	2.1
	Chol	20	.14	.66	.3665	.1425067	.335	0.05	0.81
	Trig	20	.08	.28	.165	.0498946	.155	0	0.44
	TPb	20	30	87	58.25	12.94472	61	9	81
	Alb	20	7	16	12	2.294157	12.5	3	15
	Glob	20	23	71	46.25	10.84763	48	11	71
	A:G	20	.21	.32	.2645	.0278104	.265	0.19	0.46
	Lactate	20	.07	3.86	1.369	.9571168	1.095	0.98	14.46
	Uric	20	24	96	46.45	16.62109	44.5	62	222

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, creatinine, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid (µmol/L); for total protein, albumin, and globulin (g/L)

Table 13. Summary statistics for metabolite concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Urea[2]	20	.2	.8	.555	.1503505	.6	0.3	1.1
	Creat	0	.	.	.	.	.	0	0
	Gluc	20	.8	2.5	1.825	.4540751	1.95	0.7	2.6
	Chol	20	.11	1.1	.677	.2512097	.67	0.22	1.07
	Trig	20	.06	.21	.117	.0405359	.11	0.05	0.2
	TPb	20	24	91	69.75	17.05988	73.5	36	97
	Alb	20	6	19	14.9	3.322966	16	8	22
	Glob	20	18	73	54.85	13.92187	58.5	28	78
	A:G	20	.23	.33	.2755	.0292853	.275	0.23	0.33
	Lactate	20	1.13	11.85	5.1185	2.751706	4.535	2.15	13.05
	Uric	20	24	143	73.1	25.93494	68	65	199
LM	Urea	20	.3	.6	.46	.0994723	.4	0.2	0.6
	Creat	0	.	.	.	.	.	0	0
	Gluc	20	1.1	2.5	1.58	.3778053	1.5	0.5	1.7
	Chol	20	.25	.79	.5115	.1280327	.49	0.14	0.89
	Trig	20	.04	.13	.0825	.027886	.08	0.03	0.16
	TPb	20	32	71	53.2	10.11305	54.5	14	65
	Alb	20	8	16	11.75	2.314144	11	6	16
	Glob	20	24	55	41.45	8.127115	43.5	12	52
	A:G	20	.22	.33	.286	.0333088	.29	0.28	0.46
	Lactate	20	1.58	8.87	4.208	2.144917	3.54	0.0	3.47
	Uric	20	16	82	48.35	14.98868	43.5	6	75
MF	Urea	20	0	1	.47	.2792848	.4	0.2	2.2
	Creat	0	.	.	.	.	.	0	0
	Gluc	20	.4	2.5	1.615	.4837083	1.6	0.3	2.1
	Chol	20	.22	1.18	.579	.2397565	.56	0.05	0.81
	Trig	20	.1	.49	.2915	.1033785	.285	0	0.44
	TPb	20	38	110	66.65	16.6742	69.5	9	81
	Alb	20	5	17	12.15	2.960708	13	3	15
	Glob	20	30	94	54.55	15.15699	55.5	11	71
	A:G	20	.07	.3	.2305	.0479556	.23	0.19	0.46
	Lactate	20	.76	6.28	2.4105	1.511171	1.925	0.98	14.46
	Uric	20	18	102	68.55	21.99874	74	62	222

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, creatinine, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid (µmol/L); for total protein, albumin, and globulin (g/L)

Table 14. Summary statistics for metabolite concentrations for two week caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Urea[2]	19	0	.2	.1368421	.0597265	0.1	0.3	1.1
	Creat	19	0	0	0	0	0	0	0
	Gluc	19	.6	2.3	1.542105	.4426132	1.7	0.7	2.6
	Chol	19	.17	.73	.4731579	.1692484	.51	0.22	1.07
	Trig	19	.02	.14	.0726316	.0297848	.08	0.05	0.2
	TPb	19	14	78	54.84211	16.95833	58	36	97
	Alb	19	4	16	11.52632	3.133483	12	8	22
	Glob	19	10	62	43.31579	13.95251	46	28	78
	A:G	19	.21	.43	.28	.0523874	.27	0.23	0.33
	Lactate	19	.07	5.16	1.379474	1.213125	1.42	2.15	13.05
	Uric	19	11	50	23.42105	10.94083	18	65	199
LM	Urea	20	0	.3	.115	.0587143	0.1	0.2	0.6
	Creat	20	0	0	0	0	0	0	0
	Gluc	20	.8	1.6	1.24	.2257152	1.3	0.5	1.7
	Chol	20	.25	.83	.49	.1678031	.445	0.14	0.89
	Trig	20	.03	.15	.075	.0325253	.07	0.03	0.16
	TPb	20	30	61	44.6	8.928959	47	14	65
	Alb	20	7	16	10.15	2.390221	10.5	6	16
	Glob	20	23	50	34.45	7.074602	35	12	52
	A:G	20	.22	.4	.2955	.045477	.29	0.28	0.46
	Lactate	20	.44	2.34	1.228	.5315281	1.105	0.0	3.47
	Uric	20	8	25	12.95	4.817457	11.5	6	75
MF	Urea	20	.1	.3	.17	.0656947	0.2	0.2	2.2
	Creat	20	0	0	0	0	0	0	0
	Gluc	20	.8	2.4	1.7	.393366	1.75	0.3	2.1
	Chol	20	.2	.86	.5	.1847901	.47	0.05	0.81
	Trig	20	.1	.43	.2105	.089294	.19	0	0.44
	TPb	20	32	79	59.3	14.26479	62	9	81
	Alb	20	6	14	10.75	2.510504	11.5	3	15
	Glob	20	26	65	48.55	11.88309	51	11	71
	A:G	20	.19	.28	.223	.0207998	.22	0.19	0.46
	Lactate	20	.12	2.9	1.492	.8337777	1.55	0.98	14.46
	Uric	20	24	53	39.2	8.023649	40	62	222

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, creatinine, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid (µmol/L); for total protein, albumin, and globulin (g/L)

Table 15. Summary statistics for enzyme activity for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	AMY[2]	20	1	13	5.15	3.5729245	4	3	18
	LIP	20	5	17	9.15	3.133436	8.5	3	13
	AST	20	8	134	37.3	38.547442	20	13	203
	ALT	20	5	120	39.75	30.487055	31	23	105
	GD	20	3	28	12	6	12	9	37
	SDH	20	0	2	.35	.67082039	0	0	1
	GGT	20	0	0	0	0	0	0	1
	ALP	20	0	0	0	0	0	0	1
LM	AMY	20	2	30	8.95	7.6603903	5.5	1	19
	LIP	20	5	13	9.3	2.2734162	10	4	15
	AST	20	4	56	15.35	13.107953	12.5	4	46
	ALT	20	5	69	19.05	13.124806	17	6	45
	GD	20	5	19	10	4	10	4	21
	SDH	20	0	3	.85	.87509398	1	0	0
	GGT	20	0	0	0	0	0	0	0
	ALP	20	0	0	0	0	0	0	0
MF	AMY	20	1	40	9	9.2849624	5.5	1	45
	LIP	20	6	17	12.15	2.8704483	12.5	1	21
	AST	20	7	107	31	25.515733	22	16	486
	ALT	20	6	73	30.4	17.285223	29.5	0	188
	GD	20	5	18	10	3	10	4	39
	SDH	20	0	2	.1	.4472136	0	0	2
	GGT	20	0	1	.05	.2236068	0	0	4
	ALP	20	0	0	0	0	0	0	13

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units are U/L

Table 16. Summary statistics for enzyme activity for two week caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	AMY[2]	20	1	16	6	3.7975061	5	3	18
	LIP	20	3	17	10	3.3403435	9.5	3	13
	AST	20	0	74	14.2	18.254343	8	13	203
	ALT	20	8	172	33.15	37.662315	22.5	23	105
	GD	20	6	22	15	5	14	9	37
	SDH	20	0	2	.3	.57124057	0	0	1
	GGT	20	0	0	0	0	0	0	1
	ALP	20	0	1	.1	.30779351	0	0	1
LM	AMY	18	4	21	9.4444444	5.6069518	7	1	19
	LIP	18	5	13	9.7222222	2.6525361	10.5	4	15
	AST	18	4	50	21.055556	13.622957	19	4	46
	ALT	18	7	53	26.444444	11.932271	26	6	45
	GD	18	4	22	10	5	9	4	21
	SDH	18	0	2	.61111111	.69780234	.5	0	0
	GGT	18	0	1	.05555556	.23570226	0	0	0
	ALP	18	0	0	0	0	0	0	0
MF	AMY	20	1	14	5.7	2.8302873	5	1	45
	LIP	20	7	13	10.25	1.7733406	10	1	21
	AST	20	4	89	20.95	18.514504	15.5	16	486
	ALT	20	5	62	29.1	14.505534	24	0	188
	GD	20	5	27	16	6	15	4	39
	SDH	20	0	3	.75	.91046547	.5	0	2
	GGT	20	0	0	0	0	0	0	4
	ALP	20	0	0	0	0	0	0	13

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units are U/L

Table 17. Summary statistics for enzyme activity for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	AMY[2]	20	1	18	5.45	4.0714022	4	3	18
	LIP	20	6	55	14.3	10.503633	11	3	13
	AST	20	1	151	45.85	39.138452	32.5	13	203
	ALT	20	19	175	59.2	39.647924	49.5	23	105
	GD	20	4	37	17	8	17	9	37
	SDH	20	0	2	.4	.68055705	0	0	1
	GGT	20	0	0	0	0	0	0	1
	ALP	20	0	2	.1	.4472136	0	0	1
LM	AMY	20	0	28	6	6.6173615	4	1	19
	LIP	20	5	17	10.55	3.316228	10	4	15
	AST	20	8	125	35.7	33.182272	20	4	46
	ALT	20	17	117	38.2	23.377002	30.5	6	45
	GD	20	4	22	11	4	11	4	21
	SDH	20	0	1	.05	.2236068	0	0	0
	GGT	20	0	0	0	0	0	0	0
	ALP	20	0	0	0	0	0	0	0
MF	AMY	20	1	14	4.5	3.4868173	3.5	1	45
	LIP	20	7	43	12.7	7.6578133	11	1	21
	AST	20	7	71	28.05	20.38698	22.5	16	486
	ALT	20	12	101	36.55	22.502573	33	0	188
	GD	20	0	22	11	5	12	4	39
	SDH	20	0	3	.25	.7163504	0	0	2
	GGT	20	0	1	.15	.36634755	0	0	4
	ALP	20	0	0	0	0	0	0	13

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units are U/L

Table 18. Summary statistics for enzyme activity for two week caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2012. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	AMY	19	2	13	5	3.3829639	4	3	18
	LIP	19	5	12	7.5263158	2.0647416	7	3	13
	AST	19	0	36	9.8421053	8.5066194	7	13	203
	ALT	19	7	63	24.105263	15.036603	20	23	105
	GD	19	4	31	15	7	14	9	37
	SDH	19	0	4	.78947368	1.2283208	0	0	1
	GGT	19	0	1	.05263158	.22941573	0	0	1
	ALP	19	0	2	.10526316	.45883147	0	0	1
LM	AMY	20	0	17	6.4	4.488582	5	1	19
	LIP	20	7	13	10.15	1.6944181	10	4	15
	AST	20	3	61	15.25	13.730009	11	4	46
	ALT	20	9	96	28.2	21.276624	21.5	6	45
	GD	20	6	18	12	4	12	4	21
	SDH	20	0	4	.65	1.0399899	0	0	0
	GGT	20	0	1	.05	.2236068	0	0	0
	ALP	20	0	0	0	0	0	0	0
MF	AMY	20	2	11	4.95	2.5021044	4	1	45
	LIP	20	5	11	7.5	1.7917942	7.5	1	21
	AST	20	0	30	11.5	7.5148975	11	16	486
	ALT	20	6	46	22.65	12.650255	18	0	188
	GD	20	6	22	14	4	14	4	39
	SDH	20	0	2	.3	.57124057	0	0	2
	GGT	20	0	0	0	0	0	0	4
	ALP	20	0	0	0	0	0	0	13

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units are U/L

B. Hepatopancreas Lipid Content

The average HP lipid content was not corrected for percent moisture in the tissue as percent moisture data was not recorded for all samples. Consequently, all correlations and evaluations are based on lipid content on a dry weight basis (g lipid/g HP dry weight).
Box plots (Figure 1) indicated crab #91 (PM, Cheticamp) as an outlier; this crab was also identifiable in the frequency distribution histogram (Figure 2). Examination of the original data showed all three repeats of lipid determination to be similar. Data from crab #91 was included in all Spearman rank correlations as it represented a real value with corresponding hemolymph parameters. As variation in carapace width was greater than anticipated for PM, scatterplots showing HP lipid vs. carapace width for each category of crab were generated (Figure 3). A pattern of increasing lipid content with increasing CW was noted for PM from Margaree, but not for Cheticamp. Summary statistics for average HP lipid by category and station are provided in Table 19.

Kruskall-Wallis testing across crab categories for Margaree indicated a significant difference ($p = 0.001$) in HP lipid content. Subsequent Wilcoxon signed rank (Bonferroni significance adjusted to $p < 0.0167$) testing showed a difference between LM and MF crabs only ($p = 0.000$). There was no significant difference across categories for Cheticamp crabs without ($p = 0.5103$) or with ($p = 0.7196$) the outlier (crab #91).

Comparison (Wilcoxon signed rank testing) of average HP lipid by category across stations (Margaree and Cheticamp), found significant differences between LM (Margaree> Cheticamp) and MF (Cheticamp > Margaree) crabs. Spearman rank correlation co-efficients were calculated for average HP Lipid, all 24 directly measured hemolymph biochemistry parameters, three ratios automatically calculated as part of the biochemistry profiles and four additional calculated ratios (Tables 19-22). The highest correlation co-efficients were found for PM and MF crabs for the metabolites cholesterol, triglyceride, total protein, and glucose, whereas no significant correlations were noted for LM crabs. These relationships are also presented as scatterplots (Figures 4-7).

Regression analysis was completed for average HP lipid for each of the four hemolymph biochemistry parameters individually (Table 23) and grouped (Table 24). As carapace width was considered a possible factor, it was added into the multiple regression equations separately (Table 24). On simple regression, plasma cholesterol showed the best R^2 values for PM ($0.6765_{Margaree}$, $0.6238_{Cheticamp}$) and MF ($0.6196_{Margaree}$, $0.6749_{Cheticamp}$) crabs at both stations. Results for LM crabs were poor with the highest R^2 of 0.2401 for cholesterol and much lower for other biochemistry parameters. Addition of CW to the multiple linear regression equation improved the adjusted R^2 value for PM in Margaree only (from 0.6278 to 0.7118). Otherwise, adjusted R^2 values were much the same as for simple regressions using plasma cholesterol only. Prediction of average HP lipid in LM crabs worsened (adjusted R^2 values of -0.1109 to 0.1038) using multiple regression.

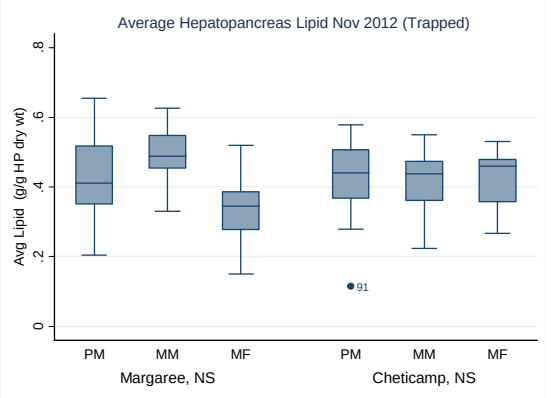


Figure 1 Box and Whisker plot showing distribution of average HP lipid (dry weight) for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab (*Chionoecetes opilio*) collected by trapping, at two stations in CFA 19, November 2012.

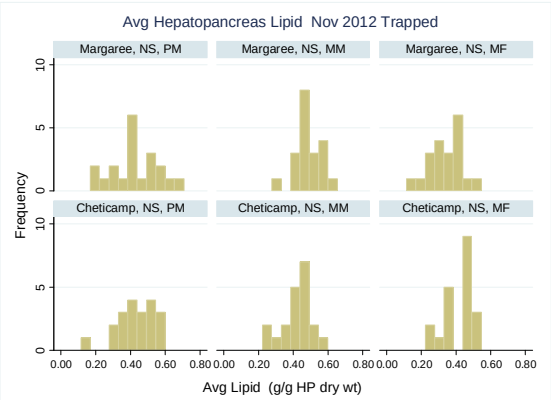


Figure 2. Frequency distribution histogram showing average HP lipid (g/g HP dry wt) for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab (*Chionoecetes opilio*) collected by trapping, at two stations in CFA 19, November 2012.

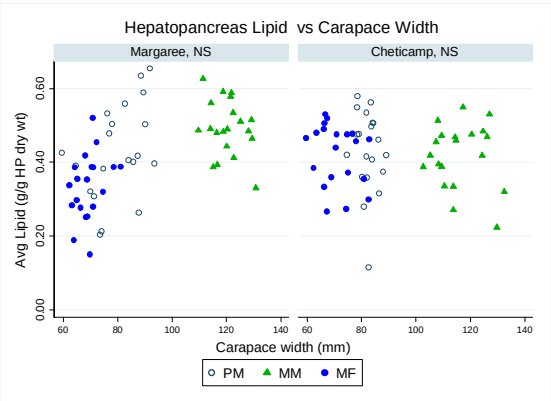


Figure 3. Scatterplot showing average HP lipid (g/g HP dry wt) by carapace width for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab (*Chionoecetes opilio*) collected by trapping, at two stations in CFA 19, November 2012.

Table 19. Summary statistics for average hepatopancreas lipid (g/g HP dry wt) for snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Different superscripts represent significant differences in median values within (letters) or between (numbers) locations.

LOCATION	GROUP	N	MEAN	SD	MEDIAN	MIN	MAX	KURTOSIS	SKEWNESS
	PM	20	0.4288	0.1290	[1;ab]0.4118	0.2040	0.6537	2.2589	-0.0268
Margaree	**LM**	20	0.4922	0.0756	[ac]0.4879	0.3300	0.6264	2.6081	-0.2229
	MF	20	0.3334	0.0887	[ab]0.3452	0.1501	0.5202	2.8653	-0.0896
	PM	20	0.4306	0.4306	[1]0.4404	0.1150	0.5787	4.2969	-1.0861
Cheticamp	**LM**	20	0.4182	0.0867	[2]0.4369	0.2230	0.5492	2.6457	-0.6078
	MF	20	0.4208	0.0832	[3]0.4594	0.2661	0.5302	1.9960	-0.5636

Table 20. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals *vs* hepatopancreas lipid content (g/g HP dry wt) in hemolymph plasma of free snow crab collected in November 2012. Results are shown for all crabs and crabs grouped by sex, and region of capture. Samples with correlation co-efficients > ±0.5 (bold) or p-values ≤ 0.05 (highlighted) are indicated.

Analyte	ALL CRABS	PYGMY MALE			LARGE MATURE MALE			MATURE FEMALE		
		ALL	MARG[1]	CHET[2]	ALL	MARG	CHET	ALL	MARG	CHET
Sodium	0.0491	-0.2495	-0.4747	0.1077	0.0164	-0.3047	0.1184	-0.4007	0.0121	-0.2688
n	117	37	20	17	40	20	20	40	20	20
p	*0.5989*	*0.1364*	*0.0344*	*0.6808*	*0.9201*	*0.1914*	*0.6189*	*0.0104*	*0.9596*	*0.2518*
Potassium	-0.1921	-0.2436	**-0.5213**	0.0779	-0.2911	-0.0925	-0.2677	-0.2546	-0.0929	-0.2618
n	120	40	20	20	40	20	20	40	20	20
p	*0.0356*	*0.1298*	*0.0184*	*0.7440*	*0.0684*	*0.6981*	*0.2538*	*0.1128*	*0.6968*	*0.2648*
Na:K	0.2444	0.1949	0.2137	0.1211	0.3879	0.0617	0.4722	0.0268	0.1346	0.0677
n	120	40	20	20	40	20	20	40	20	20
p	*0.0071*	*0.2281*	*0.3657*	*0.6111*	*0.0134*	*0.7962*	*0.0355*	*0.8698*	*0.5714*	*0.7767*
Chloride	-0.2964	**-0.5576**	**-0.7125**	-0.1369	-0.0051	-0.2798	0.0415	**-0.5497**	-0.2139	-0.4352
n	117	37	20	17	40	20	20	40	20	20
p	*0.0012*	*0.0003*	*0.0004*	*0.6004*	*0.9752*	*0.2322*	*0.8621*	*0.0002*	*0.3652*	*0.0551*
Calcium	0.3754	0.4781	0.4271	0.5102	0.0635	0.2181	0.3740	0.4544	0.2244	0.3063
n	118	40	20	20	39	20	19	39	20	19
p	*0.0000*	*0.0018*	*0.0604*	*0.0216*	*0.7010*	*0.3556*	*0.1147*	*0.0037*	*0.3415*	*0.2022*
Phosphorus	-0.1600	-0.2763	-0.1940	-0.3340	-0.2208	-0.2752	0.3624	-0.1160	-0.1490	-0.2467
n	120	40	20	20	40	20	20	40	20	20
p	*0.0809*	*0.0844*	*0.4125*	*0.1501*	*0.1709*	*0.2403*	*0.1163*	*0.4760*	*0.5307*	*0.2944*
Magnesium	-0.4103	-0.3836	-0.3287	**-0.5654**	-0.1277	-0.2647	0.3456	-0.2985	**-0.5218**	0.0448
n	118	40	20	20	39	20	19	39	20	19
p	*0.0000*	*0.0145*	*0.1571*	*0.0094*	*0.4384*	*0.2595*	*0.1472*	*0.0649*	*0.0183*	*0.8556*

[1]. Margaree, NS
[2]. Cheticamp, NS

Table 21. Summary of Spearman's rank coefficients, sample size, and significance level for the level of metabolites *vs* hepatopancreas lipid content (g/g HP dry wt) in hemolymph plasma of free snow crab collected in November 2012). Results are shown for all crabs and crabs grouped by sex, and region of capture. Samples with correlation co-efficients > ±0.5 (bold) or p-values ≤ 0.05 (highlighted) are indicated.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Glucose	0.2964	**0.5746**	**0.5664**	**0.5929**	-0.2365	0.1336	0.1112	**0.5943**	0.4778	**0.5394**
n	120	40	20	20	40	20	20	40	20	20
p	*0.0010*	*0.0001*	*0.0092*	*0.0059*	*0.1417*	*0.5745*	*0.6405*	*0.0001*	*0.0331*	*0.0141*
Cholesterol	**0.6909**	**0.7992**	**0.8158**	**0.7174**	0.3153	0.4154	0.2071	**0.8491**	**0.7833**	**0.8977**
n	120	40	20	20	40	20	20	40	20	20
p	*0.0000*	*0.0000*	*0.0000*	*0.0004*	*0.0475*	*0.0686*	*0.3810*	*0.0000*	*0.0000*	*0.0000*
Triglyceride	0.1515	**0.7113**	**0.7485**	**0.6538**	0.4506	0.3827	0.3415	**0.8172**	**0.8024**	**0.8234**
n	120	40	20	20	40	20	20	40	20	20
p	*0.0987*	*0.0000*	*0.0001*	*0.0018*	*0.0035*	*0.0959*	*0.1405*	*0.0000*	*0.0000*	*0.0000*
Total Protein	0.3628	**0.5994**	**0.6332**	**0.5367**	-0.0097	0.3377	0.0203	**0.6882**	0.4494	**0.7470**
n	120	40	20	20	40	20	20	40	20	20
p	*0.0000*	*0.0000*	*0.0027*	*0.0147*	*0.9527*	*0.1453*	*0.9322*	*0.0000*	*0.0468*	*0.0002*
Albumin	0.4638	**0.6547**	**0.6699**	**0.6578**	0.2107	0.3984	0.2258	**0.5722**	**0.5517**	**0.5632**
n	120	40	20	20	40	20	20	40	20	20
p	*0.0000*	*0.0000*	*0.0012*	*0.0016*	*0.1919*	*0.0819*	*0.3385*	*0.0001*	*0.0117*	*0.0097*
Globulin	0.3220	**0.5771**	**0.5976**	**0.5357**	-0.0921	0.2939	-0.1146	**0.6709**	0.3698	**0.7588**
n	120	40	20	20	40	20	20	40	20	20
p	*0.0003*	*0.0001*	*0.0054*	*0.0149*	*0.5718*	*0.2085*	*0.6305*	*0.0000*	*0.1085*	*0.0001*
A:G	0.0975	-0.2005	-0.2692	-0.0696	0.3192	-0.0256	0.4035	-0.3813	-0.1088	-0.4163
n	120	40	20	20	40	20	20	40	20	20
p	*0.2892*	*0.2148*	*0.2512*	*0.7705*	*0.0447*	*0.9145*	*0.0777*	*0.0152*	*0.6481*	*0.0679*
Uric Acid	-0.2519	0.0131	-0.3075	0.3227	-0.3675	0.2314	-0.4416	0.1065	-0.2170	0.0542
n	120	40	20	20	40	20	20	40	20	20
p	*.0055*	*0.9359*	*0.1873*	*0.1653*	*0.0197*	*0.3262*	*0.0513*	*0.5130*	*0.3580*	*0.8205*
Urea	0.0961	0.2125	0.1424	0.3751	-0.2887	0.5011	-0.1709	0.3266	0.2961	0.2564
n	120	40	20	20	40	20	20	40	20	20
p	*0.2964*	*0.1880*	*0.5492*	*0.1031*	*0.0709*	*0.0244*	*0.4713*	*0.0397*	*0.2049*	*0.2752*
Creatinine	.[3]	.	.	.	.	.	.	.	.	.
n	.	.	.	.	.	.	.	.	.	.
p	.	.	.	.	.	.	.	.	.	.
Lactate	0.0724	-0.0253	0.0331	-0.1368	-0.2581	0.1955	0.1203	0.4128	0.1166	0.3859
n	120	40	20	20	40	20	20	40	20	20
p	*0.4321*	*0.8767*	*0.8899*	*0.5651*	*0.1079*	*0.4088*	*0.6134*	*0.0081*	*0.6244*	*0.0929*

[1.] Margaree, NS
[2.] Cheticamp, NS
[3.] creatinine not detected in any plasma sample

Table 22. Summary of Spearman's rank coefficients, sample size, and significance level for the activity of eight enzymes *vs* hepatopancreas lipid content (g/g dry wt) in hemolymph plasma of free snow crab collected in November 2012. Results are shown for all crabs and crabs grouped by sex, and region of capture. Samples with correlation co-efficients > ±0.5 (bold) or p-values ≤ 0.05 (highlighted) are indicated.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Amylase	-0.1018	-0.1507	-0.2266	-0.1084	-0.0054	-0.2612	-0.0955	-0.2163	0.0370	-0.1522
n	120	40	20	20	40	20	20	40	20	20
p	*0.2684*	*0.3532*	*0.3367*	*0.6492*	*0.9737*	*0.2659*	*0.6887*	*0.1801*	*0.8769*	*0.5219*
Lipase	-0.0648	0.1094	0.3026	-0.2577	0.0532	0.2631	0.0900	-0.1873	-0.5572	0.1872
n	120	40	20	20	40	20	20	40	20	20
p	*0.4817*	*0.5018*	*0.1947*	*0.2727*	*0.7442*	*0.2624*	*0.7060*	*0.2473*	*0.0107*	*0.4293*
AST	-0.0710	-0.0828	-0.1543	0.0301	-0.1101	0.2257	-0.0263	0.0785	0.0422	0.0444
n	120	40	20	20	40	20	20	40	20	20
p	*0.4407*	*0.6116*	*0.5160*	*0.8998*	*.4988*	*20*	*0.9122*	*0.6303*	*0.8599*	*0.8526*
ALT	0.1140	0.1833	0.1091	0.2687	-0.1918	0.2281	-0.0083	0.3521	0.2295	0.2953
n	120	40	20	20	40	20	20	40	20	20
p	*0.2149*	*0.2576*	*0.6470*	*0.2520*	*0.2357*	*0.3335*	*0.9724*	*0.0259*	*0.3304*	*0.2062*
GD	0.2326	0.3286	0.3069	0.2745	-0.0692	0.0700	0.0594	0.4645	**0.5882**	0.2468
n	120	40	20	20	40	20	20	40	20	20
p	*0.0106*	*0.0384*	*0.1881*	*0.2414*	*0.6712*	*0.7695*	*0.8035*	*0.0025*	*0.0064*	*0.2942*
ALP	-0.1575	-0.2705	.[3]	-0.3780	.	.	.	.	.	.
n	120	40	20	20	40	20	20	40	20	20.
p	*0.0859*	*0.6116*	.	*0.1004*	.	.	.	.	.	.
SDH	0.1340	-0.0391	-0.0605	0.0392	0.0036	-0.3425	-0.0995	0.2611	0.3780	-0.0508
n	120	40	20	20	40	20	20	40	20	20
p	*0.1446*	*0.8109*	*0.8001*	*0.8697*	*0.9822*	*0.1394*	*0.6765*	*0.1037*	*0.1004*	*0.8314*
GGT	.	.	.	.	.	.	.	0.1660	0.3780	-0.1821
n	120	40	20	20	40	20	20	40	20	20
p	.	.	.	.	.	.	.	*0.3059*	0.1004	0.4422

[1.] Margaree Harbor, NS, [2.] Cheticamp, NS, [3.] creatinine not detected in any plasma sample

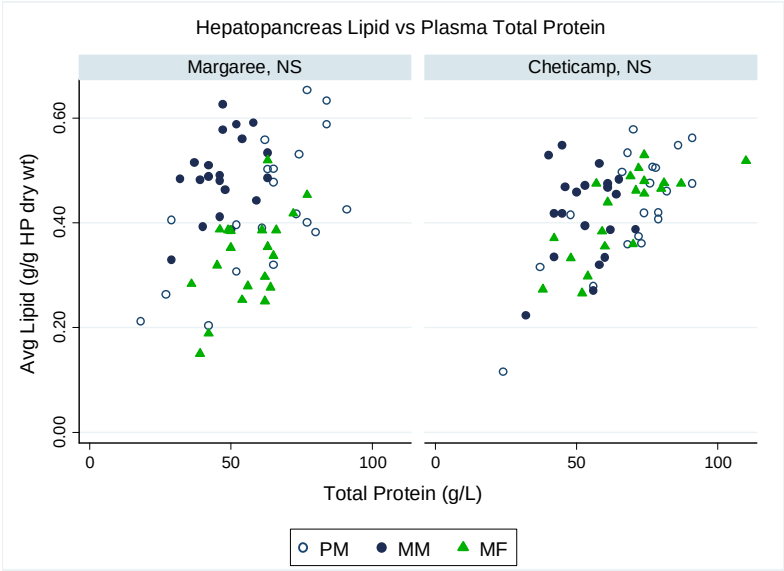


Figure 4. Scatterplot showing the average lipid (g lipid/g HP dry wt) in hepatopancreas vs. hemolymph plasma total protein concentration of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

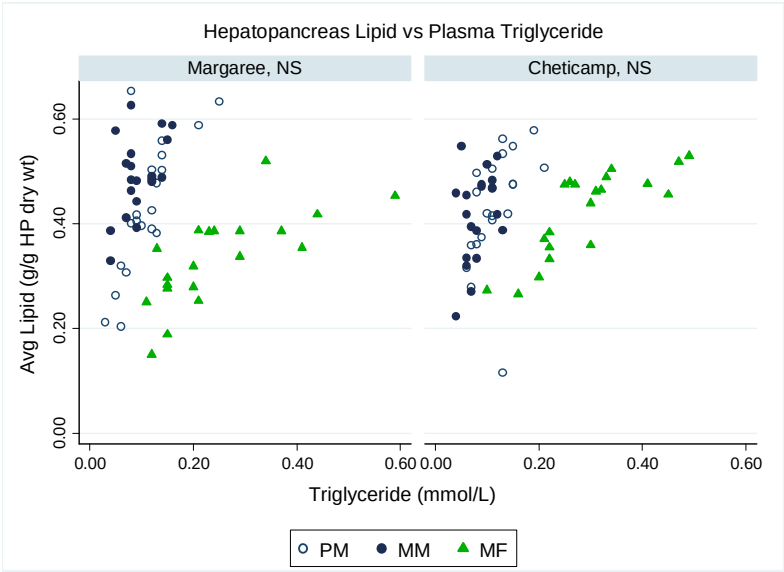


Figure 5. Scatterplot showing the average lipid (g lipid/g HP dry wt) in hepatopancreas vs. hemolymph plasma triglyceride concentration of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

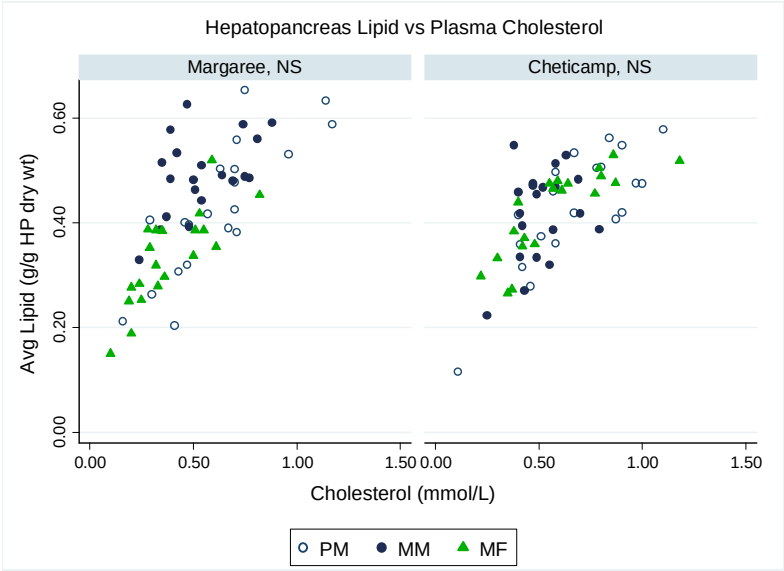


Figure 6. Scatterplot showing the average lipid (g lipid/g HP dry wt) in hepatopancreas vs. hemolymph plasma cholesterol concentration of snow crab collected from at two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

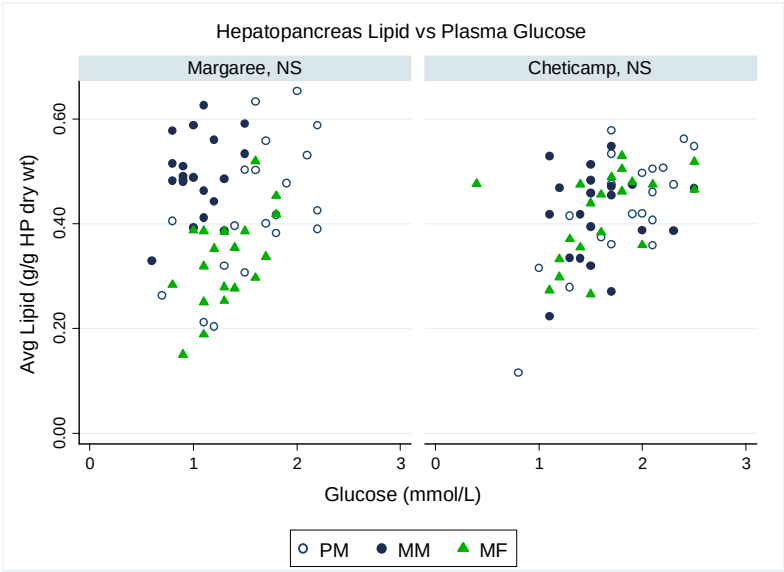


Figure 7. Scatterplot showing the average lipid (g lipid/g HP dry wt) in hepatopancreas vs. hemolymph plasma glucose concentration of snow crab collected from at two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

Table 23. Summary of equations and associated R^2 values for simple linear regression models for average hepatopancreas lipid (g lipid/g HP dry wt) for four hemolymph plasma biochemistry parameters of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree	PM	Avg Lipid = 0.0043*TP + 0.16	0.4574
		LM	Avg Lipid = 0.0031*TP + 0.34	0.1529
		MF	Avg Lipid = 0.0042*TP + 0.09	0.2498
	Cheticamp	PM	Avg Lipid = 0.0049*TP + 0.14	0.5662
		LM	Avg Lipid = 0.0011*TP + 0.35	0.0174
		MF	Avg Lipid = 0.0037*TP + 0.17	0.5659
Triglyceride (TG)	Margaree	PM	Avg Lipid = 1.862*TG + 0.22	0.5776
		LM	Avg Lipid = 1.0378*TG + 0.39	0.2345
		MF	Avg Lipid = 0.4979 *TG + 0.20	0.5015
	Cheticamp	PM	Avg Lipid = 1.3164*TG + 0.27	0.2302
		LM	Avg Lipid = 1.2000*TG + 0.31	0.1490
		MF	Avg Lipid = 0.6505*TG + 0.23	0.6527
Cholesterol (Chol)	Margaree	PM	Avg Lipid = 0.4032*Chol + 0.17	0.6765
		LM	Avg Lipid = 0.2047*Chol + 0.38	0.2401
		MF	Avg Lipid = 0.3872*Chol + 0.18	0.6196
	Cheticamp	PM	Avg Lipid = 0.3497*Chol + 0.19	0.6238
		LM	Avg Lipid = 0.2224*Chol + 0.30	0.1079
		MF	Avg Lipid = 0.2852*Chol + 0.25	0.6749
Glucose(Gluc)	Margaree	PM	Avg Lipid = 0.1722*Gluc+ 0.15	0.3457
		LM	Avg Lipid = 0.0828*Gluc+ 0.40	0.0675
		MF	Avg Lipid = 0.1029*Gluc+ 0.17	0.3213
	Cheticamp	PM	Avg Lipid = 0.1805*Gluc+ 0.10	0.5431
		LM	Avg Lipid = 0.0333*Gluc+ 0.36	0.0211
		MF	Avg Lipid = 0.0729*Gluc+ 0.30	0.1796

Table 24. Summary of equations and associated adjusted R^2 values for simple linear regression models for average hepatopancreas lipid (g lipid/g HP dry wt) for four plasma biochemistry parameters and carapace width of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

LOCATION	GROUP	SIMPLIFIED REGRESSION EQUATION	ADJUSTED R^2
Margaree	PM	Avg Lipid = -0.02*Gluc + 0.34*Chol + 0.17*Trig +0.00*TP + 0.17	0.5962
	LM	Avg Lipid = -0.06*Gluc + 0.00*Chol + 0.81*Trig +0.00*TP + 0.32	0.1038
	MF	Avg Lipid = -0.01*Gluc + 0.52*Chol - 0.00*Trig -0.00*TP + 0.22	0.5318
Cheticamp	PM	Avg Lipid = 0.10*Gluc + 0.22*Chol + 0.19*Trig – 0.00*TP + 0.09	0.6278
	LM	Avg Lipid = 0.04*Gluc + 0.07*Chol + 0.99*Trig – 0.00*TP + 0.30	-0.0552
	MF	Avg Lipid = 0.02*Gluc + 0.15*Chol + 0.30*Trig + 0.00*TP + 0.20	0.6613
Margaree	PM	Avg Lipid = 0.06*Gluc + 0.18*Chol + 0.35*Trig + 0.00*TP + 0.00*CW -0.28	0.7118
	LM	Avg Lipid = -0.06*Gluc + 0.01*Chol + 0.78*Trig +0.00*TP + 0.00*CW + 0.26	0.0409
	MF	Avg Lipid = 0.07*Gluc + 0.51*Chol - 0.23*Trig – 0.00*TP + 0.00*CW – 0.16	0.5878
Cheticamp	PM	Avg Lipid = 0.11*Gluc + 0.30*Chol + 0.10*Trig – 0.00*TP + 0.00*CW – 0.34	0.6295
	LM	Avg Lipid = 0.03*Gluc + 0.09*Chol + 1.02*Trig – 0.00*TP – 0.00*CW + 0.48	-0.1109
	MF	Avg Lipid = 0.01*Gluc + 0.14*Chol + 0.30*Trig +0.00*TP – 0.00*CW + 0.29	0.6460

C. Hepatopancreas Glycogen Content

The average HP glycogen content was not corrected for percent moisture in the tissue as percent moisture data was not recorded for all samples. Consequently, all correlations and evaluations are based on glycogen on a dry weight basis (mg glycogen/g HP dry wt).
The distribution of the data is presented in Figures 8 – 10 as boxplots, frequency distribution histograms and scatterplot vs. carapace width, respectively. Crab #55 (MF, Margaree) was identified as an outlier on the boxplots. Frequency distribution histogram suggests possible bimodal distribution for average HP glycogen for PM in Cheticamp. As opposed to HP lipid concentrations, no increase in glycogen content was apparent for increasing CW in any crab category. Summary statistics for average HP glycogen by category and station are provided in Table 25.

Kruskall-Wallis testing across crab categories showed no differences for Margaree (excluding or including outlier crab #5) or Cheticamp. Comparison (Wilcoxon signed rank testing) of average HP glycogen by crab category across stations (Margaree and Cheticamp), detected a significant difference between MF crabs (Margaree > Cheticamp) only.

Spearman rank correlation co-efficients were calculated for average HP glycogen, for all 23 directly measured hemolymph biochemistry parameters, three ratios automatically included in the biochemistry profiles, and four additional calculated ratios (Tables 26-29). In contrast to the results for HP lipid, the highest correlation co-efficients were found for $LM_{Margaree}$ crabs for the metabolites cholesterol, triglyceride, total protein, albumin, globulin and glucose with no significant correlations for PM or MF crabs. Large mature male crabs from Cheticamp showed significant correlations for glucose, total protein, albumin and globulin only. Very few significant correlations were noted for PM or MF crabs. These relationships are also presented as scatterplots in Figures 11-14.

Regression analysis was completed for average HP glycogen for each of the four parameters individually (Table 30) and combined (Table 31). As carapace width was considered a possible factor, it was added into the multiple regression equations separately (Table 31). On simple linear regression, the R^2 values were very poor with most below 0.4000. The highest R^2 values were seen for $LM_{Cheticamp}$ crabs for glucose (0.4792) and total protein (0.4471). Regression with multiple variables saw slight improvements, again primarily for LM crabs where adjusted R^2 values rose to 0.6535 and 0.6088 without and with carapace width, respectively. Addition of carapace width doubled the adjusted R^2 for $PM_{Cheticamp}$ from 0.2135 to 0.4131, whereas the adjusted R^2 value decreased in $PM_{Margaree}$ crabs in contrast to what was noted for HP lipid.

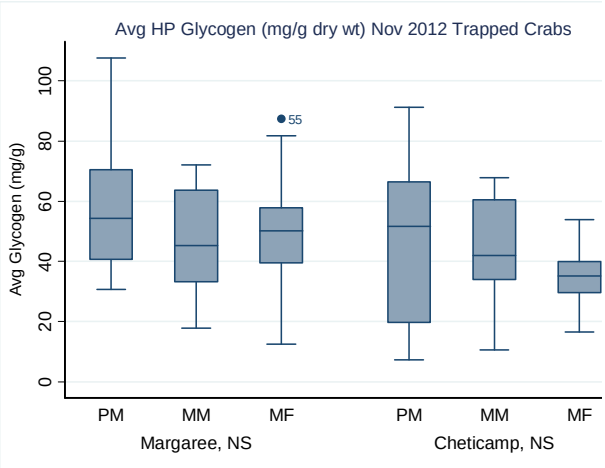


Figure 8. Box and Whisker plot showing distribution of average HP glycogen (mg/g HP) for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping, at two stations in CFA 19, November 2012.

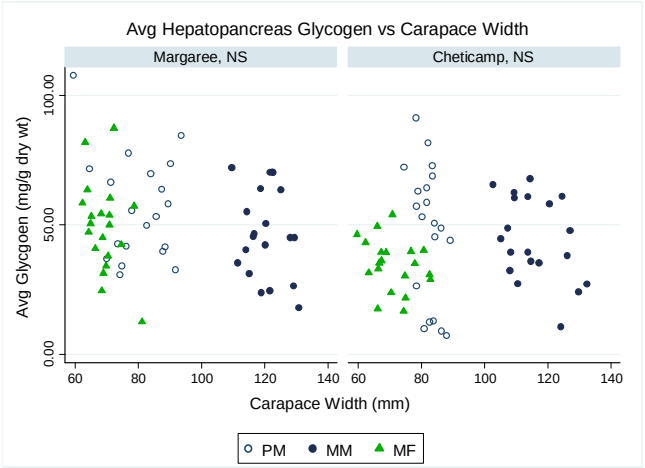


Figure 10. Scatterplot showing average HP glycogen (mg/g HP) by carapace width for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping, at two stations in CFA 19, November 2012.

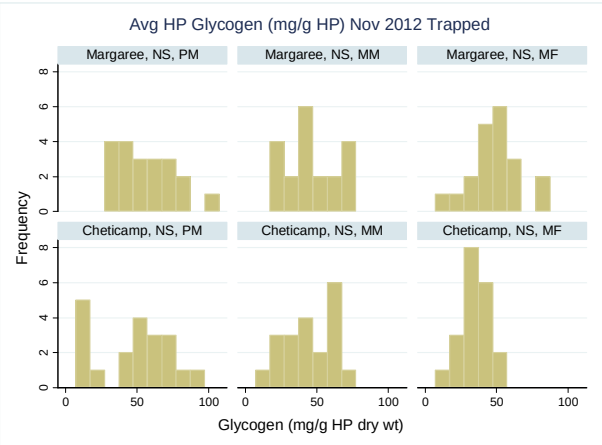


Figure 9. Frequency distribution histogram showing average HP glycogen (mg/g HP) for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping, at two stations in CFA 19, November 2012.

Table 25. Summary statistics for average hepatopancreas glycogen content (mg/g dry wt) for snow crabs collected by traps at two stations in CFA 19, November 2012. Median values by crab category within a station were not significantly different nor were median values by category across stations with the exception of MF crabs (Margaree > Cheticamp).

LOCATION	GROUP	N	MEAN	SD	MEDIAN	MIN	MAX	KURTOSIS	SKEWNESS
Margaree	**PM**	20	56.4719	20.2407	[1]54.2230	30.6183	107.5516	3.0313	0.73016
	LM	20	46.8897	17.2581	[2]45.2533	17.8423	71.9914	1.8420	0.00370
	MF	20	49.2471	17.5117	[3]50.1452	12.4830	87.3086	3.3375	0.18506
Cheticamp	**PM**	20	47.3782	26.1202	[1]51.6644	7.15166	91.2231	1.9188	-0.28004
	LM	20	44.2566	16.0196	[2]41.9709	10.6002	67.8196	2.1298	-0.20494
	MF	20	34.5386	9.95723	35.0610	16.5066	53.8990	2.5219	-0.06639

Table 26. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas glycogen content (mg/g dry wt) of free snow crab collected from two stations in CFA 19, November 2012. Results are shown for all crabs and crabs grouped by sex, and region of capture where $p \leq 0.05$.

Analyte	ALL CRABS	PYGMY MALE			LARGE MATURE MALE			MATURE FEMALE		
		ALL	MARG[1]	CHET[2]	ALL	MARG	CHET	ALL	MARG	CHET
Sodium	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Potassium	---	---	---	---	---	---	---	---	**-0.5118**	---
n	---	---	---	---	---	---	---	---	20	---
p	---	---	---	---	---	---	---	---	0.0211	---
Na:K	---	---	---	---	0.3941	---	---	---	---	---
n	---	---	---	---	40	---	---	---	---	---
p	---	---	---	---	0.0119	---	---	---	---	---
Chloride	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Calcium	---	---	---	---	0.4181	**0.5543**	---	---	---	---
n	---	---	---	---	39	20	---	---	---	---
p	---	---	---	---	0.0081	0.0112	---	---	---	---
Phosphorus	---	---	---	---	-0.3570	---	---	---	---	---
n	---	---	---	---	40	---	---	---	---	---
p	---	---	---	---	0.0237	---	---	---	---	---
Magnesium	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

[1.] Margaree, NS, [2]. Cheticamp, NS

Table 27. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas glycogen content (mg/g dry wt) of free snow crab collected from two stations in CFA 19, November 2012. Results are shown for all crabs and crabs grouped by sex, and region of capture where $p \leq 0.05$.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Glucose	0.3170	0.3848	---	**0.5413**	0.3514	**0.6217**	**0.6225**	---	0.4528	---
n	120	40	---	20	40	20	20	---	20	---
p	*0.0004*	*0.0142*	---	*0.0137*	*0.0262*	*0.0034*	*0.0034*	---	*0.0450*	---
Cholesterol	0.3004	---	---	---	0.4298	**0.5583**	---	---	**0.5568**	---
n	120	---	---	---	40	20	---	---	20	---
p	*0.0009*	---	---	---	*0.0056*	*0.0105*	---	---	*0.0108*	---
Triglyceride	---	---	---	---	0.4925	**0.5280**	---	---	0.4669	---
n	---	---	---	---	40	20	---	---	20	---
p	---	---	---	---	*0.0012*	*0.0167*	---	---	*0.0379*	---
Total Protein	0.2669	---	---	---	**0.5502**	**0.5337**	**0.6629**	---	---	---
n	120	---	---	---	40	20	20	---	---	---
p	*0.0032*	---	---	---	*0.0002*	*0.0154*	*0.0014*	---	---	---
Albumin	0.3722	---	---	---	**0.6928**	**0.5888**	**0.7596**	---	---	---
n	120	---	---	---	40	20	20	---	---	---
p	*0.0000*	---	---	---	*0.0000*	*0.0063*	*0.0001*	---	---	---
Globulin	0.2367	---	---	---	---	**0.5561**	**0.5782**	---	---	---
n	120	---	---	---	---	20	20	---	---	---
p	*0.0093*	---	---	---	---	*0.0109*	*0.0076*	---	---	---
A:G	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Uric Acid	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Urea	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Creatinine	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Lactate	0.2012	---	---	---	---	---	---	---	---	**0.5340**
n	120	---	---	---	---	---	---	---	---	20
p	*0.0276*	---	---	---	---	---	---	---	---	*0.0153*

[1.] Margaree Harbor, NS, [2]. Cheticamp, NS

Table 28. Summary of Spearman's rank coefficients, sample size, and significance level for the activity of eight enzymes in hemolymph plasma *vs* hepatopancreas glycogen content (mg/g dry wt) of free snow crab collected from two stations in CFA 19, November 2012. Results are shown for all crabs and crabs grouped by sex, and region of capture where $p \leq 0.05$.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Amylase	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Lipase	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
AST	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
ALT	---	---	---	0.4705	---	---	---	---	---	---
n	---	---	---	20	---	---	---	---	---	---
p	---	---	---	*0.0363*	---	---	---	---	---	---
GD	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
ALP	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
SDH	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

[1]. Margaree Harbor, NS, [2]. Cheticamp, NS

Table 29. Summary of Spearman's rank coefficients, sample size, and significance level for an assortment of calculated ratios in hemolymph plasma *vs* hepatopancreas glycogen content (mg/g dry wt) of free snow crab. Results are shown for all crabs and crabs grouped by sex, and region of capture where $p \leq 0.05$.

Analyte	ALL CRABS	PYGMY MALE			LARGE MATURE MALE			MATURE FEMALE		
		ALL	MARG[1]	CHET[2]	ALL	MARG	CHET	ALL	MARG	CHET
Ca:Mg	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
TP:TG	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
TP:Chol	---	---	---	---	---	---	---	---	**-0.6316**	---
n	---	---	---	---	---	---	---	---	20	---
p	---	---	---	---	---	---	---	---	*0.0028*	---
TG:Chol	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
TP:Ca	0.2615	---	---	0.4662	**0.5251**	0.4692	**0.6193**	---	---	---
n	118	---	---	20	39	20	19	---	---	---
p	*0.0042*	---	---	*0.0383*	*0.0006*	*0.0369*	*0.0047*	---	---	---

[1.] Margaree Harbor, NS, [2] Cheticamp, NS

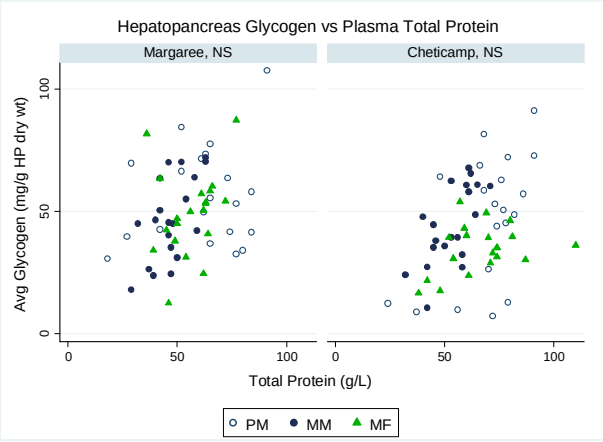


Figure 11. Scatterplot showing the average glycogen (mg/g HP dry wt) in hepatopancreas vs. hemolymph plasma total protein concentration of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

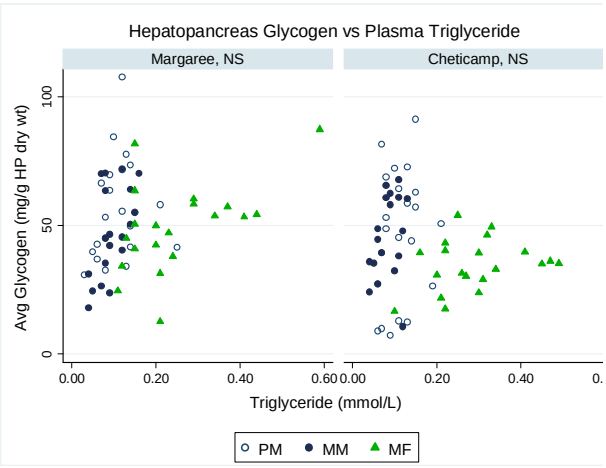


Figure 12. Scatterplot showing the average glycogen (mg/g HP dry wt) in hepatopancreas vs. hemolymph plasma triglyceride concentration of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

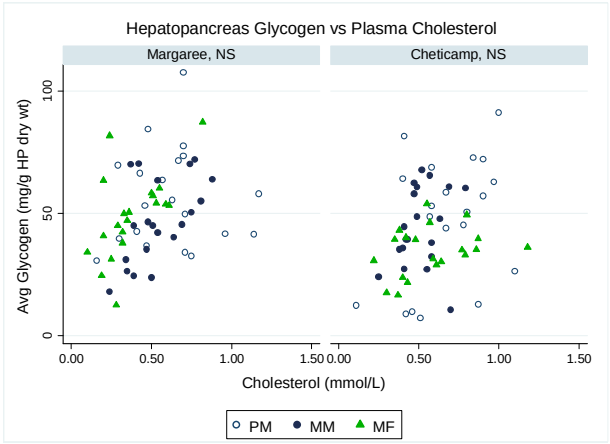


Figure 13. Scatterplot showing the average glycogen (mg/g HP dry wt) in hepatopancreas vs. hemolymph plasma cholesterol concentration of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

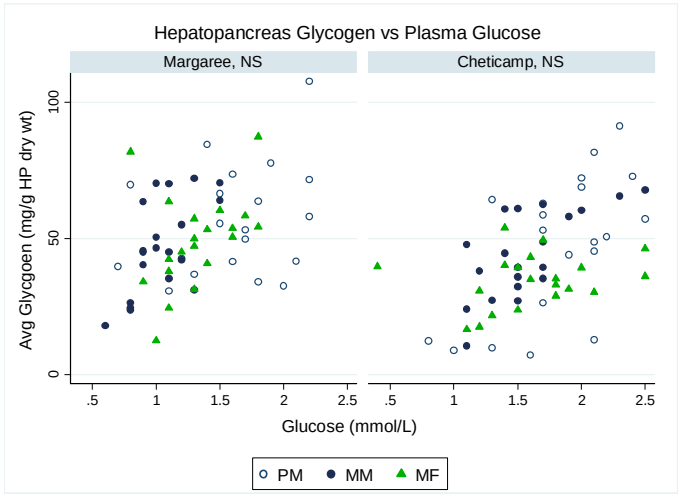


Figure 14. Scatterplot showing the average glycogen (mg/g HP dry wt)) in hepatopancreas vs. hemolymph plasma glucose concentration of snow crab collected from at two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

Table 30. Summary of equations and associated R^2 values for simple linear regression models for average hepatopancreas glycogen (mg/g HP dry weight) *vs.* four hemolymph plasma biochemistry parameters of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree	PM	Avg Glycogen = 0.1896*TP + 44.70	0.0357
		LM	Avg Glycogen = 1.1092*TP – 5.24	0.3644
		MF	Avg Glycogen = 0.4078*TP + 26.36	0.0676
	Cheticamp	PM	Avg Glycogen = 0.7968*TP – 8.20	0.2709
		LM	Avg Glycogen = 1.0591*TP – 12.09	0.4471
		MF	Avg Glycogen = 0.2100*TP + 20.53	0.1238
Triglyceride (TG)	Margaree	PM	Avg Glycogen = 57.2302*TG +50.14	0.0222
		LM	Avg Glycogen = 274.0782*TG + 20.98	0.3145
		MF	Avg Glycogen = 70.5204*TG + 31.68	0.2583
	Cheticamp	PM	Avg Glycogen = 98.7982*TG + 35.81	0.0235
		LM	Avg Glycogen = 189.4688*TG + 28.62	0.1088
		MF	Avg Glycogen = 27.3816*TG + 26.55	0.0808
Cholesterol (Chol)	Margaree	PM	Avg Glycogen = 2.1976*Chol + 54.92	0.0011
		LM	Avg Glycogen = 52.5727*Chol + 18.44	0.3045
		MF	Avg Glycogen = 55.3444*Chol + 28.38	0.3249
	Cheticamp	PM	Avg Glycogen = 35.8929*Chol + 23.07	0.1192
		LM	Avg Glycogen = 29.7031*Chol + 29.06	0.0564
		MF	Avg Glycogen = 12.8644*Chol + 27.09	0.0960
Glucose(Gluc)	Margaree	PM	Avg Glycogen = 13.1347*Gluc + 35.25	0.0817
		LM	Avg Glycogen = 45.6073*Gluc – 0.76	0.3931
		MF	Avg Glycogen = 22.7430*Gluc + 19.34	0.1390
	Cheticamp	PM	Avg Glycogen = 34.8633*Gluc – 16.24	0.3673
		LM	Avg Glycogen = 29.3538*Gluc – 2.12	0.4792
		MF	Avg Glycogen = 4.8212*Gluc + 26.75	0.0549

Table 31. Summary of equations and associated adjusted R^2 values for multiple linear regressions using four plasma biochemistry parameters and carapace width for average glycogen (mg/g HP dry weight) in the hepatopancreas of snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

LOCATION	GROUP	SIMPLIFIED REGRESSION EQUATION	ADJUSTED R^2
Margaree	PM	Avg Glycogen = 43.47*Gluc – 166.89*Chol +587.04*Trig + 0.15*TP +15.33	0.3115
	LM	Avg Glycogen = 35.10*Gluc – 44.55*Chol 397.68*Trig + 0.21*TP -13.33	0.4570
	MF	Avg Glycogen = 20.35*Gluc +86.12*Chol – 16.03*Trig -0.97*TP + 48.96	0.4089
Cheticamp	PM	Avg Glycogen = 48.42*Gluc – 4.89*Chol – 2.27*Trig -0.33*TP – 13.95	0.2135
	LM	Avg Glycogen = 11.84*Gluc -87.43*Chol + 407.37*Trig + 0.92*TP – 12.76	0.6535
	MF	Avg Glycogen = 1.17*Gluc + 3.33*Chol + 0.25*Trig + 0.14*TP + 20.70	-0.1061
Margaree	PM	Avg Glycogen = 44.25*Gluc – 168.32*Chol + 588.59*Trig + 0.15*TP + 0.04*CW + 11.2	0.2628
	LM	Avg Glycogen = 34.34*Gluc – 28.25*Chol +352.81*Trig + 0.36*TP + 0.69*CW - 108.03	0.6088
	MF	Avg Glycogen = -12.44*Gluc + 89.33*Chol + 18.43*Trig – 0.51*TP – 1.87*CW +186.04	0.4778
Cheticamp	PM	Avg Glycogen = 41.94*Gluc – 57.90*Chol + 65.07*Trig + 0.24*TP – 3.72*CW + 292.35	0.4131
	LM	Avg Glycogen = 10.81*Gluc – 85.72*Chol + 410.26*Trig + 0.90*TP – 0.12*CW + 4.33	0.5347
	MF	Avg Glycogen = -2.03*Gluc + 0.13* Chol + 0.87*Trig +0.21*TP – 0.377*CW + 50.11	-0.1178

D. Hepatopancreas Glycogen vs. Hepatopancreas Lipid Content

Spearman rank correlation co-efficients were calculated using absolute values of hepatopancreas lipid and glycogen content for all crabs (Table 32). While the correlation for all crabs was found to be statistically significant (p = 0.0104), the actual co-efficient was quite low (0.2333). Scatterplots of the data showed a tendency for tighter agreement for crabs caught in Cheticamp (Figure 15).

Ratios for average hepatopancreas lipid to glycogen content (standardised to mg/ g HP dry wt) were calculated for all crabs. Boxplots identified multiple outliers (Figure 16), which is also evident in the frequency distribution histograms (Figure 17). Kruskall-Wallis testing (outliers included) showed a significant difference among crab categories for Margaree (p = 0.0012) but not Cheticamp (p =0.0956). No further analysis completed at this time.

Table 32. Summary of Spearman's rank coefficients, sample size, and significance level for average hepatopancreas lipid (g/g HP dry wt) *vs* hepatopancreas glycogen content (mg/g HP dry wt) of free snow crab, November 2012. Results are shown for all crabs and crabs grouped by sex, and region of capture.

	ALL	PYGMY MALE			LARGE MATURE MALE			MATURE FEMALE		
	CRABS	ALL	MARG[1]	CHET[2]	ALL	MARG	CHET	ALL	MARG	CHET
rho	0.2333	0.2186	0.1098	0.3910	0.2418	0.2602	0.2195	0.0043	0.2977	0.3098
n	120	40	20	20	40	20	20	40	20	20
p	*0.0104*	*0.1754*	*0.6450*	*0.0883*	*0.1327*	*0.2680*	*0.3523*	*0.9789*	*0.2023*	*0.1838*

[1.] Margaree Harbor, NS, [2] Cheticamp, NS

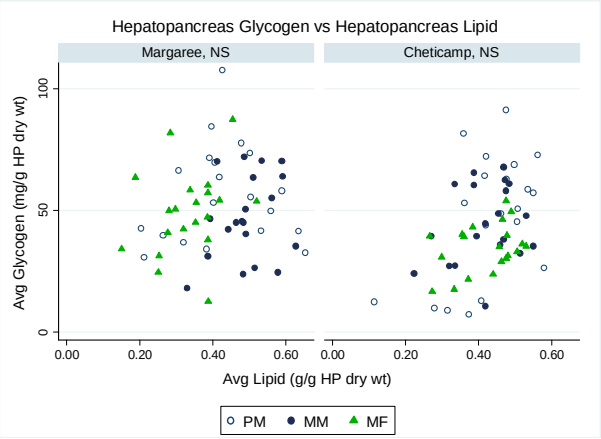


Figure 15. Scatterplot demonstrating relationship between absolute hepatopancreas lipid and glycogen concentrations for snow crab collected from at two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

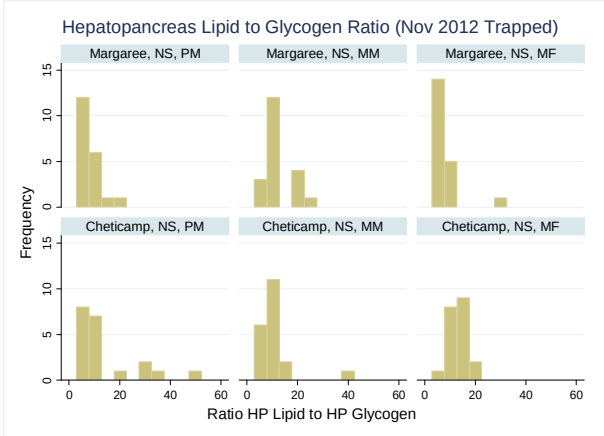


Figure 17. Frequency distribution histogram demonstrating relationship between hepatopancreas lipid and glycogen ratios for snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

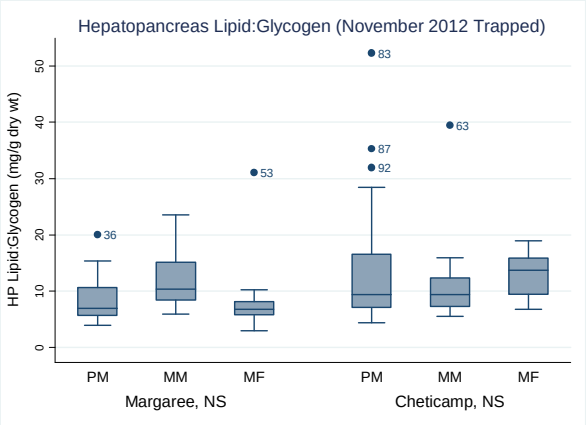


Figure 16. Box and Whisker plots demonstrating relationship between hepatopancreas lipid and glycogen ratios for snow crab collected from two stations in CFA 19 in November 2012. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

VIII-1-5 Conclusions

Caged crabs had significantly lower levels of hemolymph plasma uric acid, urea, potassium and magnesium compared to free crabs collected two weeks previously from the same area. Similar trends of lower total protein, calcium, triglyceride, and cholesterol were also observed. These findings would suggest that caging, for as little as two weeks, has a detectable effect on crab physiology with the most likely cause being decreased energy intake. Pygmy male and MF crabs tended to be more similar to each other than to LM crabs with respect to levels of many hemolymph parameters. This could indicate similarities in physiology and/or access to similar environments or food sources compared to LM crabs.

Hepatopancreas energy stores, in the form of lipid and glycogen, in free crabs collected in the fall showed few differences between fishing stations or crab category. Hepatopancreas lipid content could be predicted from plasma biochemistry parameters with moderate R^2 values for PM and MF crabs but not for LM crabs; conversely, glycogen content was predicted for LM but not PM or MF crabs using hemolymph biochemistry parameters. Hemolymph biochemistry analyses could prove useful in non-lethal assessments of hepatopancreas energy stores. Correlations may have been better had whole-body (hepatopancreas and muscle) lipid and glycogen stores been determined and could prove useful as a further study.

Hemolymph Biochemistry Profiles

Reference intervals (RI) generated from cooler-held crabs (PM, LM, and MF) collected from Cheticamp in August 2012 (see Section VII-3), were used for initial comparison in this study. Other than a marginally higher median lactate level for LM crabs, all median values for free crabs from Cheticamp fell within the previously established intervals. Except for slightly decreased median uric acid levels in PM and MF crabs, median values for all other biochemistry profile parameters for free crabs from Margaree also fell within the established RIs for Cheticamp crabs. The minimum and maximum levels exceeded the RI in some cases, however. This will require further specific evaluation as it may reflect seasonal or regional variation or, that the original RI was calculated using fairly low number of animals (n =13 PM; n =20 LM; n =29 MF). This general consistency over a two month period strengthens the potential to use RIs in future studies to evaluate snow crab populations using non-lethal sampling methods. Previously established reference intervals are most useful when they are constructed using data from a large number of animals e.g., > 120. Samples from individual animals can then be compared to the RI generated from the general population. When the goal is to compare groups of animals subjected to a treatment e.g., caging, especially those sharing characteristics such as time of collection and location as in the current study, it is advantageous to compare the two groups directly to each other as most of the difference seen between the groups would be expected to reflect treatment effects.

The protein-related metabolites uric acid, urea, and total protein for all but MF crabs, were significantly decreased in caged crabs compared to free crabs at both stations as were potassium and magnesium. Median triglyceride concentrations were also lower in caged crabs, although these decreases were not always statistically significant. Similar, but less consistent, decreases were noted for cholesterol. These changes most likely indicate decreased energy intake by caged crabs compared to free-ranging crabs collected by trapping if one assumes the two week interval had no effect on food availability for free-ranging crabs.

Hemolymph total protein, uric acid and urea are probable indicators of feeding and protein turnover (Claybrook 1983). It was interesting to see the trend for higher values for crabs in Cheticamp for all categories. This could support the suspicion of a better snow crab environment, subsequently better feeding and ultimately, better condition/muscle mass for Cheticamp crabs (M. Moriyasu, pers. comm.).

Magnesium trended with total protein with median values being significantly lower for all categories of caged crabs at both stations. A similar, though less consistent, pattern was noted for calcium. In vertebrates, the majority of calcium and magnesium in the circulation is present in its physiologically inactive form bound to plasma proteins, specifically albumin (Duncan *et al.* 1994a). The physiologically active portions of calcium and magnesium circulate as non-protein-bound ions. The assay used in the biochemistry profiles measures total (bound and unbound) calcium and magnesium. This may account for the apparent co-association of higher total protein with higher calcium and magnesium in hemolymph samples from Cheticamp crabs. Potassium levels in vertebrates can be low when dietary intake is decreased (Duncan *et al.* 1994b).

Median hemolymph triglyceride content tended to be lower in caged crabs. This could be an indication of lower hepatopancreas lipid reserves (not measured in this study) secondary to decreased dietary intake. Hemolymph triglyceride levels were correlated to hepatopancreas lipid content in intermoult, male American lobsters (Ciaramella *et al.* 2014). Diet composition for each group, as determined by stomach contents (see Section V), may also help explain some of the differences. Plasma triglyceride levels increase in vertebrates after feeding for up to 12 hours in some species (Duncan *et al.* 1994c). While free crabs did not have access to bait bags, they may have had access to other animals entering the cages attracted by the bait bags. Such feeding may have contributed to a post-prandial increase in triglyceride in free crabs. The highest triglyceride values were noted for MF crabs and are presumed to be related to oocyte development (transfer of lipid from the hepatopancreas to ovary) which is occurring in the fall (Moriyasu & Lanteigne 1998; Battison *et al.* 2011).

Caging was associated with an unexpected decrease in sodium and chloride levels in Cheticamp crabs only. Review the temperature loggers showed a notable drop in the water temperature for those cages midway during the caging period. This could reflect an influx of lower salinity water (M. Moriyasu, pers. comm.) in which case the crabs would be expected to acclimate as

they are osmoconformers (Hardy *et al.* 1994). Whether the lowered temperature alone could perhaps affect the crabs in another way e.g., lowered metabolic rate, and that this would drive the electrolyte shift indirectly is unknown. Also of interest was a tendency for lower sodium values in MF crabs regardless of location or free/caged status. The reason for this is unknown at present but is suspected to be related to physiological rather than environmental factors. Following hemolymph electrolyte concentrations while PM, LM, and MF crabs are exposed to different salinities under laboratory conditions may provide additional information on this question.

Most hemolymph parameters that had been different for free crabs when compared by category, across stations, i.e., urea, uric acid, magnesium were not different for caged crabs. It is possible that restricted/modified feeding opportunities while caged, brings these values to basal levels which are consistent between the two regions. Median total protein values remained higher (although not always significant) for Cheticamp crabs, however. This could reflect better condition and/or protein stores in Cheticamp crabs.

Free or caged status had minimal effect on hemolymph enzyme activity. Activity of three enzymes – ALP, GGT, SDH was very low or zero in most instances and is consistent with previous studies (see Section VII-3 trap vs trawl). Differences of 1-3 units of activity are not considered clinically relevant and may even be within the allowable precision error for a particular assay i.e., representing no real difference. While statistical comparisons were made, the results are of questionable value. As expected, creatinine was not detected in any of the samples. Creatinine is not a recognised component of normal crustacean physiology; however is included in the biochemistry panels to collect data as the assay occasionally will react with an as yet unknown substance (A. Battison, unpublished observations).

Overall, free LM crabs tended to have lower values for metabolites (total protein, cholesterol, triglyceride, uric acid, urea) than free PM or MF crabs at both stations, although the differences were not always statistically significant. This was initially suspected to predict lower hepatopancreas energy reserves but proved not to be the case. In addition, free $LM_{Cheticamp}$ crabs had higher values for plasma glucose and cholesterol than $LM_{Margaree}$ crabs yet, there was no significant difference in hepatopancreas glycogen content, and hepatopancreas lipid content was greater in $LM_{Margaree}$ than $LM_{Cheticamp}$. Muscle tissue is another source of glycogen, and to a lesser extent lipid, in snow crab which was not accounted for in this study (Hardy *et al.* 2000) but should be included in future studies in order to better determine total body lipid and glycogen reserves. Factors other than tissue energy stores such as stress responses, diet, etc. could also be expected to affect plasma metabolite levels. Further evaluation might better reveal these relationships.

Hepatopancreas Lipid Content

Similar to protein (not measured in this study), lipid is recognised as a major energy reserve in snow crab as (Hardy *et al.* 2000). Crabs (PM, LM, and MF) for this portion of the study were collected by trapping and so the hepatopancreas lipid content is considered representative of crabs with *ad libitum* feeding practices in their respective stations (Margaree, NS and Cheticamp, NS) in November.
Looking at results within each station, the distribution of hepatopancreas lipid (g lipid/g HP dry wt) for crabs in Cheticamp was consistent and there was no significant difference in median lipid content among the three crab categories. This could suggest that food of equal lipid content was equally available to all crabs. The size of the crab did not appear to affect hepatopancreas lipid content; however, it is noted that there was minimal overlap in carapace width among crab categories in Cheticamp.

The results for Margaree crabs were considerably different. Hepatopancreas lipid in LM crabs was higher than MF, but not PM crabs. There was no difference between PM and MF crabs. The distribution of hepatopancreas lipid content by carapace width showed a trend of increasing content with width in the 60 – 80 mm range which included both MF and some smaller PM crabs. As similarly small PM crabs were not collected from Cheticamp, it is not possible to determine if this pattern of increasing lipid with increasing carapace width is restricted to Margaree or a general phenomenon. Possible causes could include: better foraging ability of larger PM crabs, less lipid required for growth allowing for increased lipoid stores in larger PM crabs, etc. Comparison of lipid content of free crabs from the spring and fall of 2013 could provide some insights on this point.

Examination of hepatopancreas lipid content by crab category between stations, showed no significant differences in PM crabs; however, MF crabs from Cheticamp and LM crabs from Margaree had higher levels than their counterparts. Factors that may be worth considering here for MF crabs could include degree of oocyte development for MF at each station as lipid will have to be transported to the oocytes thereby potentially depleting hepatopancreas reserves. Examination of histologic sections of ovaries (see Section IV-4) showed that while all (20/20) $MF_{Margaree}$ crabs had ovaries with developing secondary (more mature) oocytes, only 19/20 $MF_{Cheticamp}$ had maturing ovaries, while the ovary of the remaining crab was composed primarily of follicle remnants. It is possible that ovary development overall was less progressed in Cheticamp crabs and so less lipid had been transferred from the hepatopancreas to the ovary. Comparison of ovary lipid content might have provided more information on this but was not part of the current study.

Hepatopancreas Glycogen Content

Glycogen is found in the hepatopancreas and muscle of adult male snow crab (Hardy *et al.* 2000). The latter was not measured in the current study but could be a significant source of total body glycogen in all crab categories. Glycogen is a source of hemolymph glucose (directly) and lactate (indirectly). Glycogenolysis releases glucose from glycogen in tissue stores e.g., hepatopancreas, muscle for local or systemic consumption. Lactate (lactic acid) is generated in tissues from glucose during anaerobic glycolysis e.g., emersion. It is probable that the degree of these responses will be moderated, in part, by total body glycogen stores.
Within Margaree station, there was no significant difference in median hepatopancreas glycogen content across crab category nor, in contrast to hepatopancreas lipid content, was any pattern in comparison to carapace width evident. Similarly, no differences in median glycogen content were detected across crab categories in Cheticamp (as for lipid), despite six of the $PM_{Cheticamp}$ crabs having much lower glycogen content evident in the frequency distribution histograms and scatterplots. The reason for the lower values in these PM crabs is unknown. The only detectable difference across stations was in MF crabs where those from Margaree had higher values. Diet is suspected to be the major contributor to hepatopancreas glycogen content in terminally moulted crabs. Variations in tissue water content will also affect the relative amount of lipid, glycogen, and all non-measured constituents such as protein and mineral/ash on a wet weight basis; unfortunately, tissue water content was not recorded for all crabs.

Preliminary evaluation of hepatopancreas lipid:glycogen found the ratio to be <20 for most crabs for both areas. Further evaluation of these ratios over time (fall/spring), relationship to diet, and the effect of caging could prove interesting but was beyond the scope of the current study.

Prediction of Hepatopancreas Energy Content Using Hemolymph Parameters

Hemolymph collection from snow crabs is a readily accomplished, non-lethal, procedure while direct analysis of hepatopancreas lipid and glycogen content requires lethal sampling. One of the goals of the current project was to determine if a hemolymph biochemical parameter(s) could prove a useful substitute for direct analysis of tissue energy content. While it was not surprising to find that metabolites such as cholesterol, triglyceride, total protein and glucose tended to have the best correlations, the pattern of correlations was unexpected.
Hepatopancreas lipid content had good correlations with hemolymph metabolites of PM and MF crabs but not LM crabs, while the opposite was true for hepatopancreas glycogen content. It is unclear from the information is this report if this is related to basic differences in physiology, metabolism, diet and/or other factors among the three categories of crabs. This needs to be investigated further.

Hepatopancreas lipid content was more readily predicted with simple regression than was glycogen. Simple regression using cholesterol provided R^2 values of 0.6196 – 0.6765 for PM and MF crabs in either station for hepatopancreas lipid content. Multiple regression improved

this somewhat to a maximum of 0.7118 for $PM_{Margaree}$ when carapace width was included otherwise, adjusted R^2 values differed little from simple regression. Cholesterol is considered more of a structural lipid, often found in cell membranes, and used as the base for many sterol hormones; levels are not expected to be as drastically affected by dietary intake or oocyte development as are triglyceride levels (Gurr *et al.* 2002). The latter are fatty acid composites and therefore, considered direct sources of tissue energy via lipid metabolism (Gurr *et al.* 2002).

Conversely, the adjusted R^2 values for hepatopancreas glycogen content in LM crabs did improve using multiple linear regression compared to simple regression. The best value of 0.6535 was obtained for LM crabs from Cheticamp. Inclusion of carapace width did not improve this value for $LM_{Cheticamp}$ (0.5347) but did improve the value from 0.4570 to 0.6088 for $LM_{Margaree}$, presumably due to the wider range of CW in PM crabs from Margaree.

Free crabs did not have access to the bait bags while in the traps used for crab collection so, were essentially fasted prior to sampling, although access to other fauna attracted into the cage by the bait bags is a possibility. It is not possible to determine how long each crab was in the trap prior to hauling and sampling. Nor is it known if this variation in fasting period would have affected hemolymph levels of e.g., glucose, triglyceride, cholesterol, or total protein and therefore, the regression analyses.

Analysis of the fall 2012 data showed that free PM and MF crabs were found to be more similar to each other than to LM crabs with respect to hemolymph metabolite levels; while, few differences in hepatopancreas lipid and glycogen stores were detected. Sex-specific simple or multiple linear regression equations incorporating levels of metabolites measured in the hemolymph had some success (R^2 0.6 – 0.7) in predicting hepatopancreatic lipid (PM and MF) and glycogen (LM) content. Hemolymph analysis could prove to be a non-lethal means to estimate energy stores in crab populations in future studies. Determination of total body (muscle, hepatopancreas, and gonad) energy stores is anticipated to improve the correlation to hemolymph parameters, however. Crabs caged for a two week caging period tended to have lower levels of hemolymph parameters associated with energy stores and feeding (urea, uric acid, total protein, triglyceride and cholesterol) compared to crabs collected by trap two weeks earlier, suggesting that even such short-term caging period is affecting crab physiology.

VIII-1-6 References

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VIII-1-7 Appendices

Appendix A: Folch centrifugation method of total lipid extraction from hepatopancreas

1. Pre-label 12 ~10 mL round bottom glass centrifuge tubes with the sample name and date for four hepatopancreas tissues in triplicate. Remove samples from storage and place on ice.
2. Zero the balance with a 10 mL round bottom glass centrifuge tube on it then weigh a ~100 mg aliquot of lyophilized hepatopancreas into it (four tissues in triplicate for a total of 12 tubes). Store on ice.

3. Pre-label and weigh twelve 10 ml glass beakers and write the mass on the label (place label on beaker before weighing).
NOTE: Perform steps 9- 19 in the fume hood.
a. To each of the hepatopancreas tissue aliquots, add 3.75 ml chloroform:methanol (2:1 v/v) and cap the tube. ***Plastic sheath's found at central services can be used as caps.*
4. Add 0.75 mL of 100% methanol to one of the samples, and then homogenize using the designated stainless steel adaptor and electric homogenizer (OMNI International) until a uniform consistency is achieved. Re-cap and store at room temperature. Repeat this for each sample.
5. Vortex each tube vigorously for 1 min, taking care not to spill the contents.
6. Let the homogenates stand in the fume hood at room temperature for 15 min.
7. Centrifuge each tube at 1000 x g for 20 min to pellet cell debris.
8. Decant supernatant into a 15 mL round bottom glass centrifuge tube. Transfer label from the 10 mL tube to the new 15 mL tube. (Discard pellet)
9. Add 1.5 mL chloroform and 1.5 ml dH_2O to each supernatant. Cap all tubes when finished.
10. Vortex each tube vigorously for 1 min, taking care not to spill the contents.
11. Centrifuge samples at 1000 x g for 20 min. The supernatant will divide into an upper water-soluble layer and a lower organic layer, divided by a thin layer of tissue debris.
12. CAREFULLY remove the bottom organic layer using a Pasteur pipette and place into its respective pre-labelled and weighed beaker. If any debris or water droplets are transferred, try to remove them from the beaker with the pipette. Repeat for all tissue aliquots.
13. Dry each beaker in the incubator in a fume hood at 50 ℃ until only lipid residue remains (mass no longer decreases) or for 96 hours. ***Keep samples in a capped container during transfer.*
14. Weigh each beaker using the analytical balance and calculate the % total lipid per mg dry weight:

$$\frac{(Beaker\ with\ lipid\ residue\ (mg)) - (Beaker\ (mg))}{Lyophilized\ tissue\ aliquot\ (mg)} * 100$$

Appendix B*:* Hexokinase method for glucose determination in hepatopancreas

1. Weigh out three aliquots, 10 - 20 mg each, of lyophilised tissue directly into a pre-labelled 2 mL microfuge tube. ***10 mg is the suggested minimum amount but closer to 20 mg would be ideal. May be prepared ahead and stored at -80℃.*
2. Prepare a 2 mg/mL stock solution of oyster glycogen (OG) in a 100 mM sodium citrate buffer pH 5.
3. Prepare a serial dilution of OG stock with the concentrations 2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.063 mg/mL and 0.031 mg/mL as described below.
    a) Label five 2 mL microfuge tubes accordingly
    b) Add 1mL of buffer to each of the 1, 0.5, 0.25, 0.125, 0.063, and 0.031 mg/mL tubes.
    c) Place one mL of 2 mg/mL stock solution into the 2 and 1 mg/mL tubes and vortex to mix.
    d) Pipette 1 mL from the 1 mg/mL tube into the 0.5 mg/mL tube and vortex to mix.
    e) Pipette 1 mL from the 0.5 mg/mL tube into the 0.25 mg/mL and vortex to mix.
    f) Pipette 1 mL from the 0.25 mg/mL tube into the 0.125 mg/mL tube and vortex to mix.
    g) Pipette 1 mL from the 0.125 mg/mL tube into the 0.063 mg/mL tube and vortex to mix.
    h) Pipette 1 mL from the 0.063 mg/mL tube into the 0.031 mg/mL tube and vortex to mix
    i) Pipette 1 mL from the 0.031 mg/mL tube and discard.

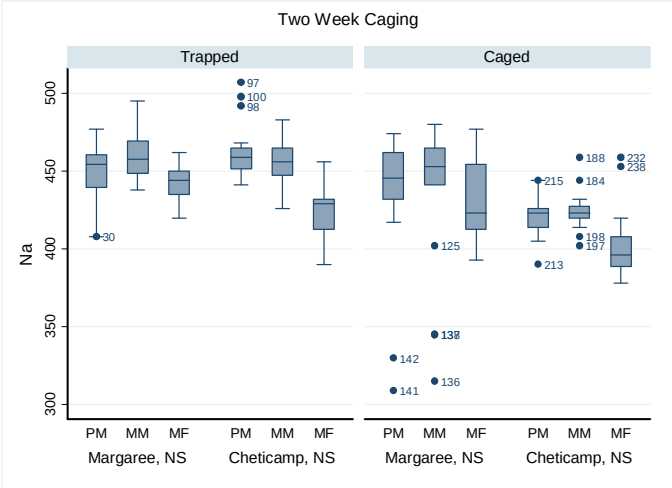
    *** The serial dilutions should be prepared in Replicates of four. Three will be digested to generate a standard curve and the third will not be digested and will act as blanks to ensure that there is no background glucose in the OG samples.*
4. To each tissue aliquot add 1 ml 100 mM sodium citrate pH 5.0.
5. Also prepare three microfuge tubes with controls and blanks: Add 1 ml of Na Citrate buffer to a negative control (buffer + enzyme) and buffer blank (Buffer only) and add 1 ml of OG (2 mg/mL working solution) to the positive control sample tube.
6. Vortex each tube prepared in steps 8-10 vigorously until a homogenous mixture is achieved.
7. Add 100 µl 0.5% amyloglucosidase to each sample (except the buffer blank and the third serial dilution that will serve as the OG blanks and vortex gently to mix.
8. Incubate for 2.5 h at 50℃ in the shaking incubator with shaker set at 150 rpm.
9. Centrifuge samples at 10,000x g for 30 min at room temperature.
10. Transfer liquid to a new 1.5 mL microfuge tube with a 1 mL pipette being careful not to transfer any of the upper lipid layer or the cell pellet. ***Or transfer liquid to a pre-labelled sample vial provided by Diagnostic Services.*
11. Submit samples to Diagnostic Services for glucose determination.
12. The equation of the regression line generated with the standard curves was then used to determine glycogen concentrations, which are then standardized as follows:

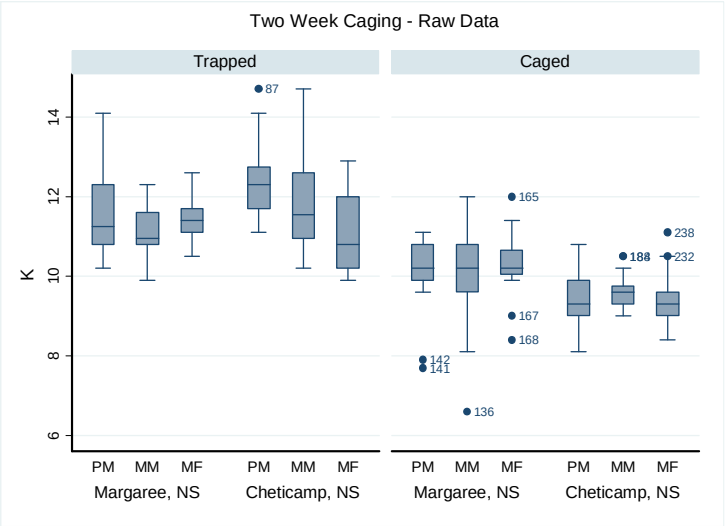
$$\frac{Mass\ of\ Glycogen\ (mg)}{Mass\ of\ Lyophilised\ Tissue\ \ (mg)} = \frac{Glycogen}{mg}\ dry\ tissue$$

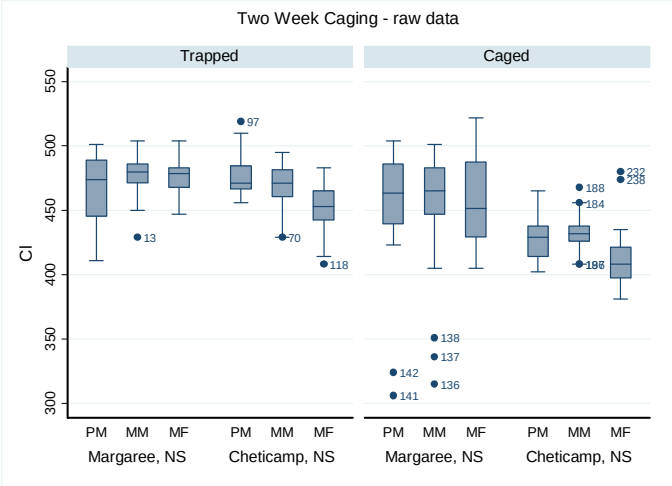
Appendix B (continued): Sodium Citrate Buffer 100 mM pH 5 (CB)

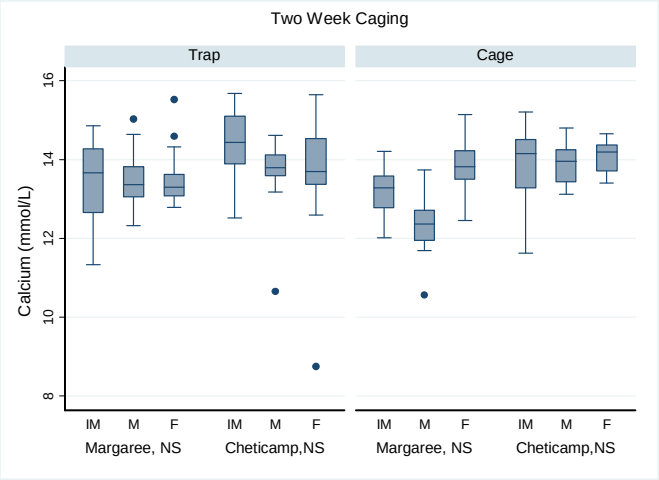
1. 7.35 g Na Citrate (S279-500 Fisher Scientific) weighed into a plastic beaker.
2. 200 mL distilled water was added to the beaker.
3. Solution was mixed and pH was adjusted to 5 with HCl acid (Accumet® Basic AB15 Fisher Scientific).
4. Solution was transferred to a graduated cylinder and distilled water was added to 250 mL.
5. Solution was then transferred to a glass bottle and stored at 4℃.

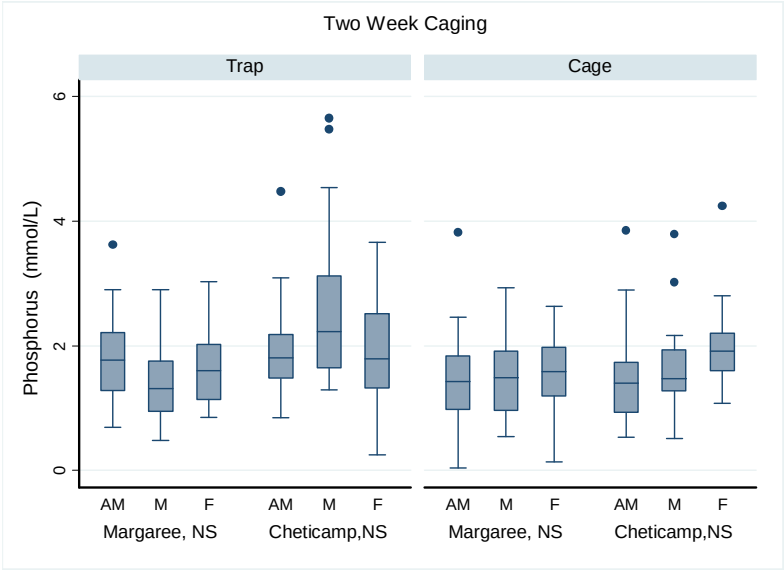
Appendix C – Boxplots of Hemolymph Biochemistry Parameters







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Cage
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2
4
6
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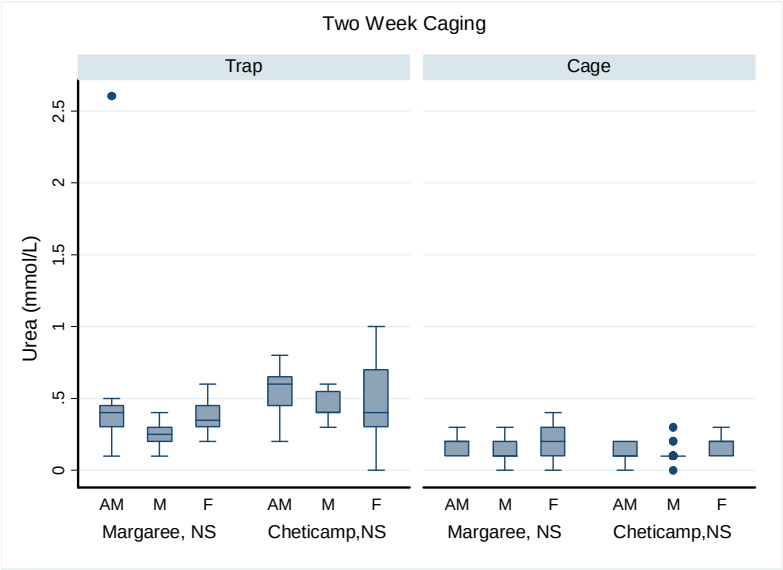
Cheticamp,NS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Trap
Cage
Urea (mmol/L)
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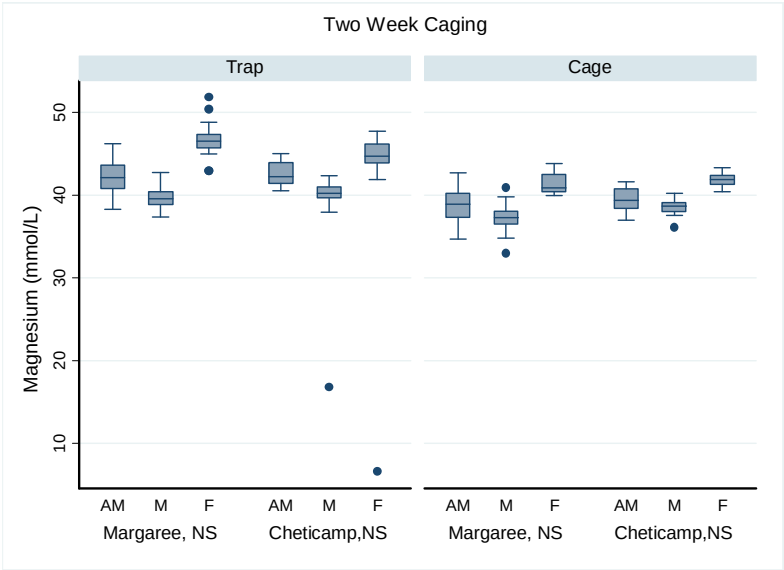
1
1.5
2
2.5
AM M F AM M F AM M F AM M F
Margaree, NS
Cheticamp,NS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Trap
Cage
Magnesium (mmol/L)
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20
30
40
50
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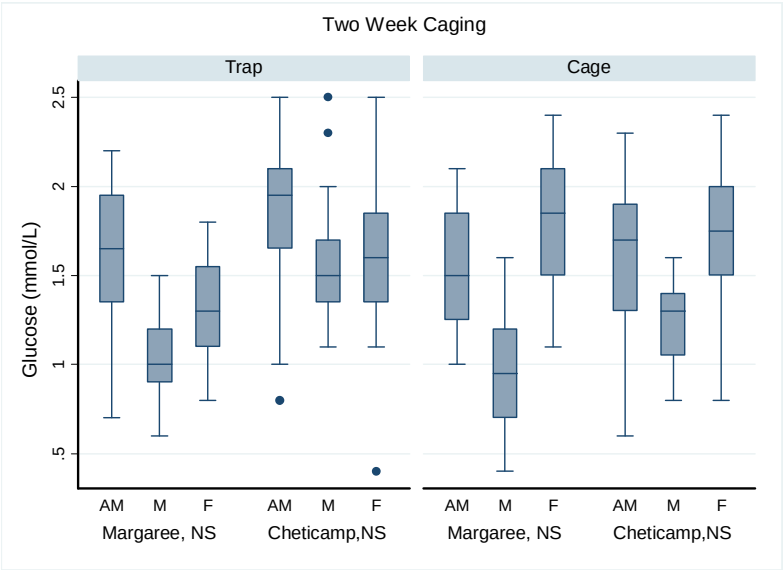
Cheticamp,NS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Trap
Cage
Glucose (mmol/L)
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1
1.5
2
2.5
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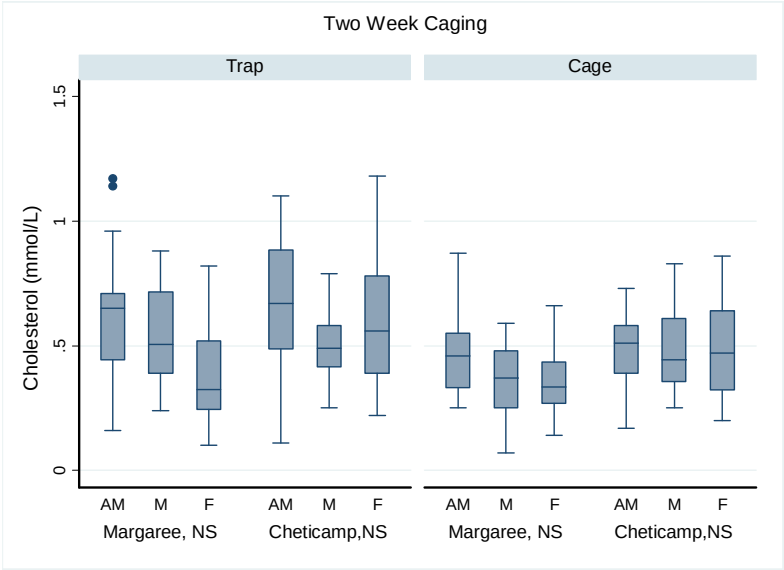
Margaree, NS
Cheticamp,NS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Trap
Cage
Cholesterol (mmol/L)
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1
1.5
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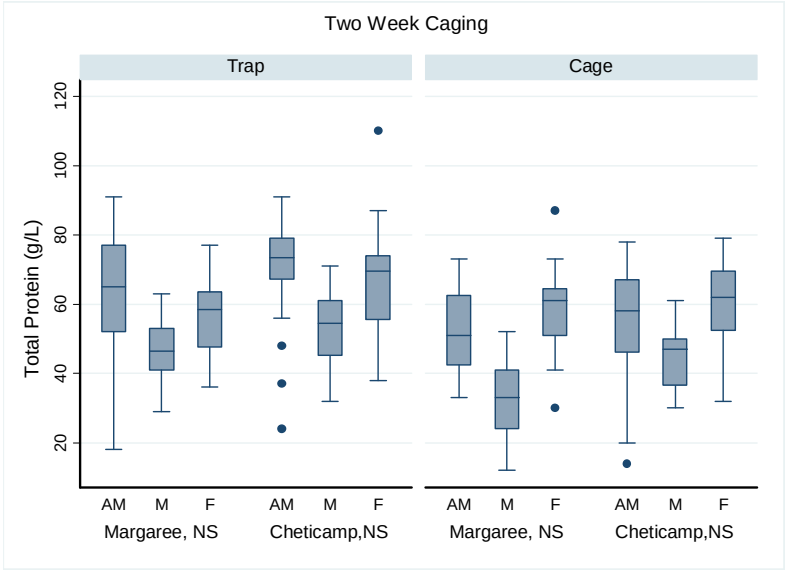
Cheticamp,NS
Margaree, NS
Cheticamp,NS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Trap
Cage
Total Protein (g/L)
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40
60
80
100
120
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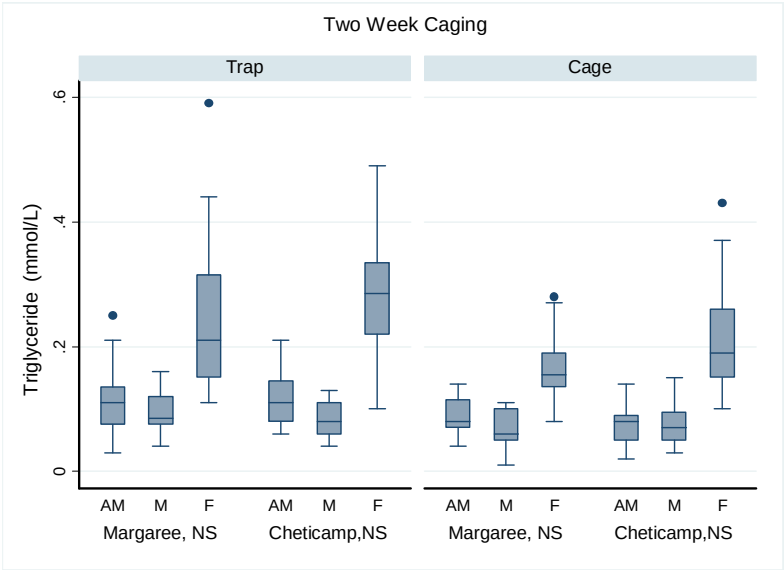
Cheticamp,NS
Margaree, NS
Cheticamp,NS


Two Week Caging
Trap
Cage
Triglyceride (mmol/L)
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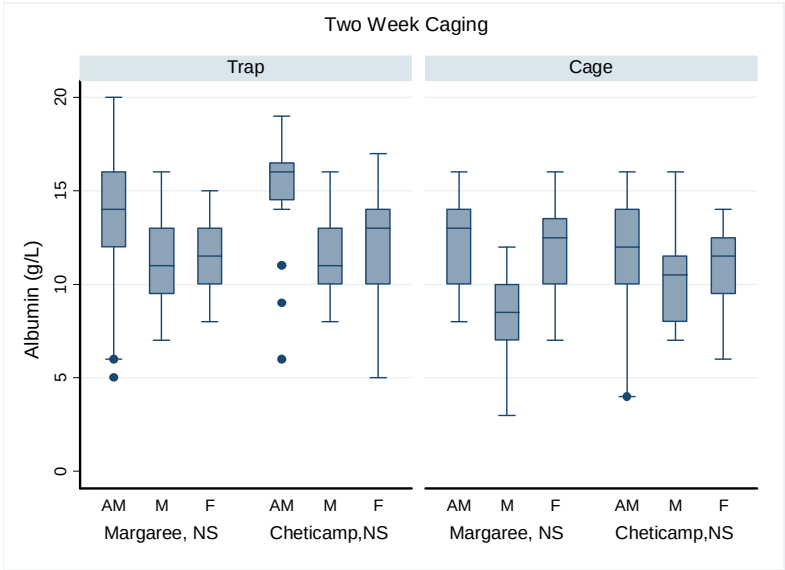
.2
.4
.6
AM M F AM M F AM M F AM M F
Margaree, NS
Cheticamp,NS
Margaree, NS
Cheticamp,NS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Trap
Cage
Albumin (g/L)
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5
10
15
20
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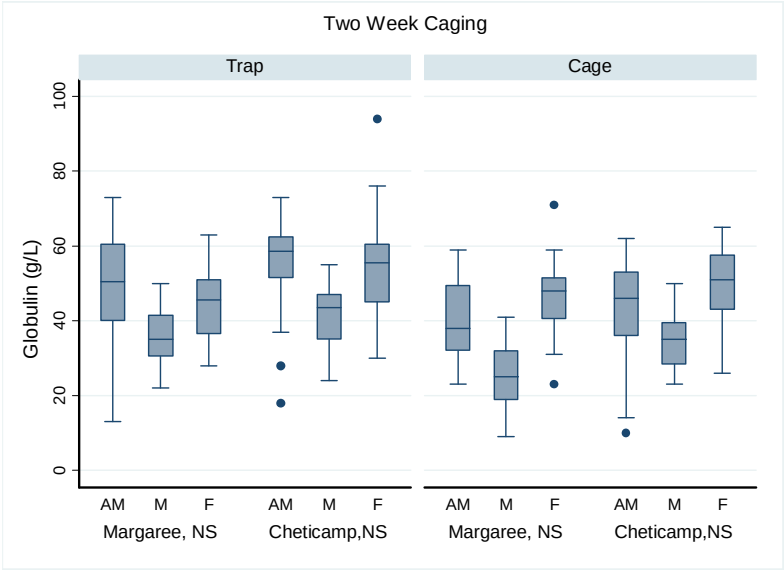
Cheticamp,NS
Margaree, NS
Cheticamp,NS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Trap
Cage
Globulin (g/L)
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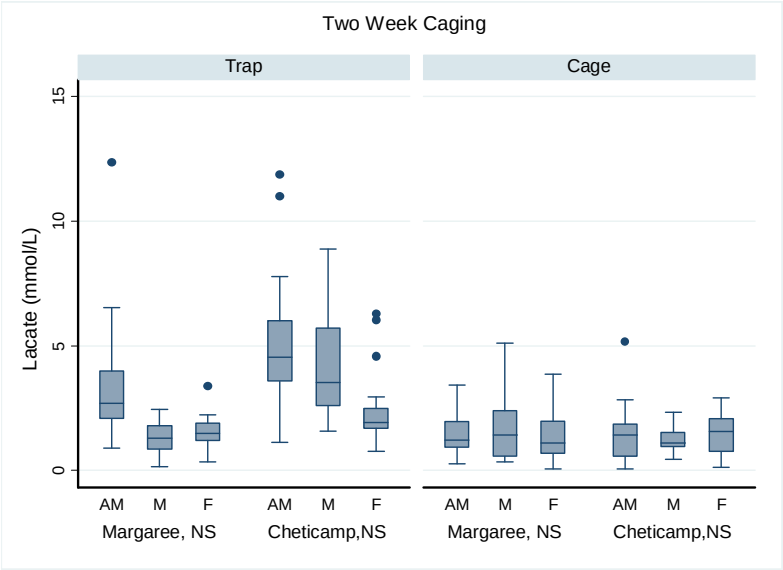
20
40
60
80
100
AM M F AM M F
Margaree, NS
Cheticamp,NS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Trap
Cage
Lacate (mmol/L)
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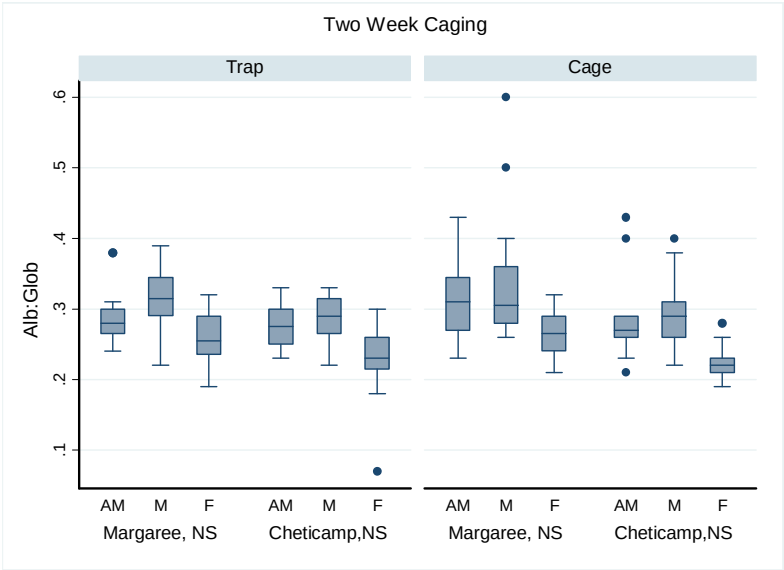
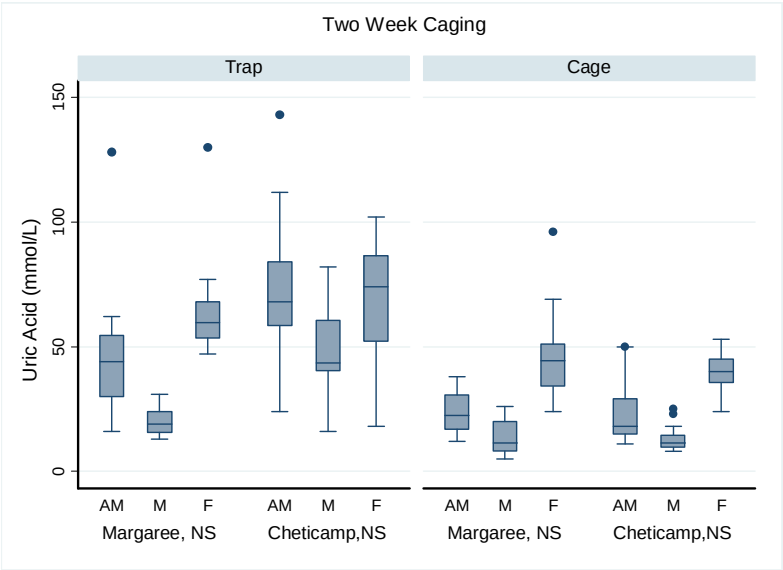
5
10
15
AM M F AM M F
Margaree, NS
Cheticamp,NS


Two Week Caging
Trap
Cage
Alb:Glob
.1
.2
.3
.4
.5
.6
AM M F AM M F
Margaree, NS
Cheticamp,NS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Trap
Cage
Uric Acid (mmol/L)
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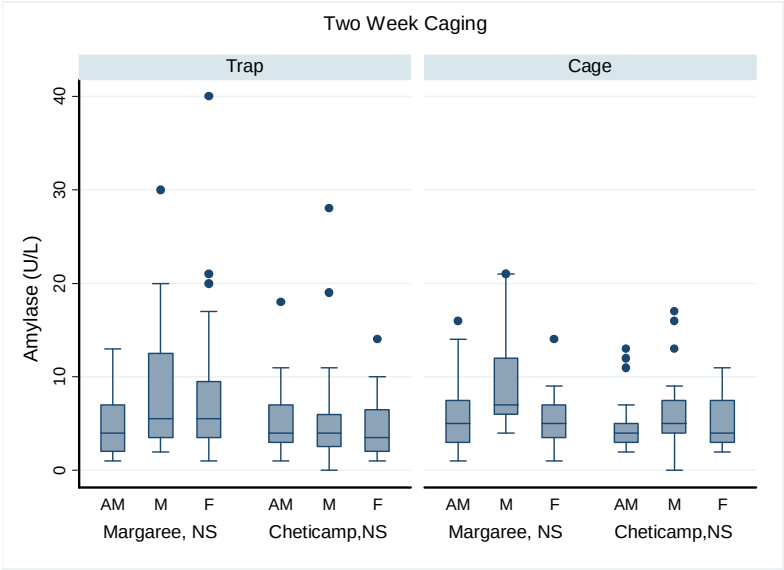
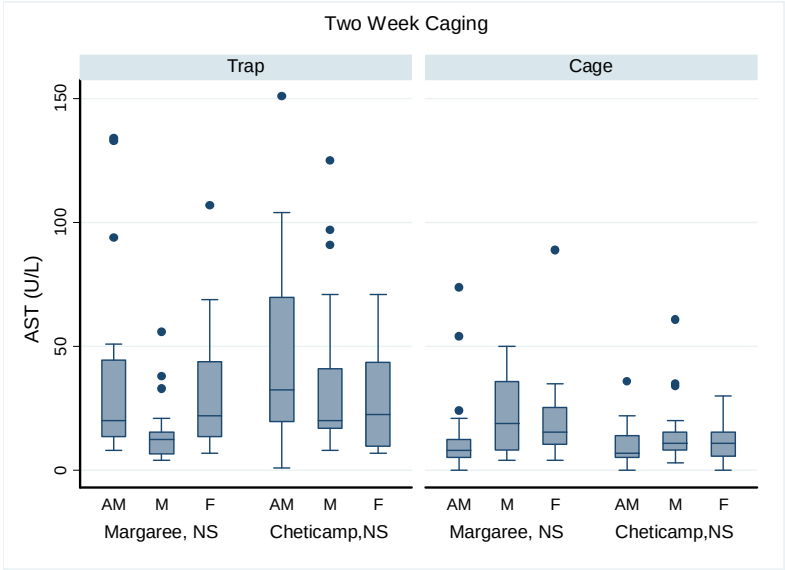
50
100
150
AM M F AM M F
Margaree, NS
Cheticamp,NS


Two Week Caging
Trap
Cage
Amylase (U/L)
0
10
20
30
40
AM M F AM M F AM M F AM M F
Margaree, NS
Cheticamp,NS
Margaree, NS
Cheticamp,NS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Trap
Cage
AST (U/L)
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50
100
150
AM M F AM M F AM M F AM M F
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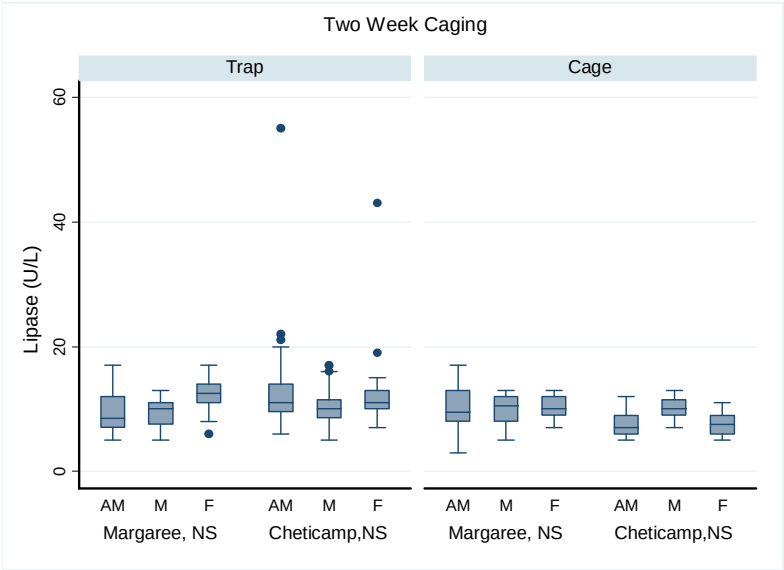
Cheticamp,NS
Margaree, NS
Cheticamp,NS


Two Week Caging
Trap
Cage
Lipase (U/L)
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20
40
60
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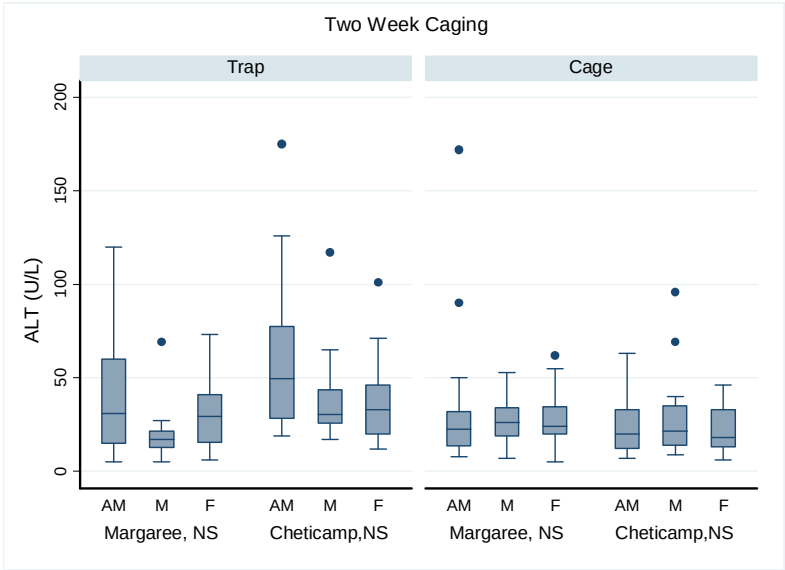
Cheticamp,NS
Margaree, NS
Cheticamp,NS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Trap
Cage
ALT (U/L)
0
50
100
150
200
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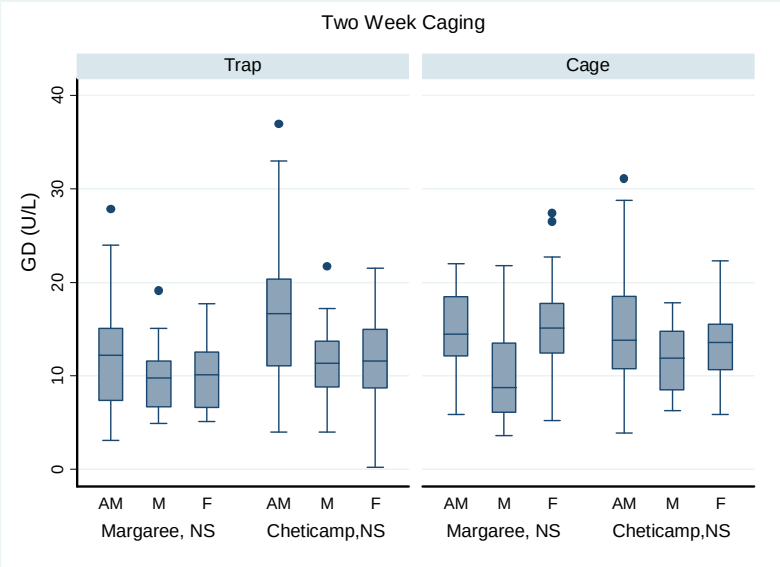
Cheticamp,NS
Margaree, NS
Cheticamp,NS


Two Week Caging
Trap
Cage
GD (U/L)
0
10
20
30
40
AM M F AM M F AM M F AM M F
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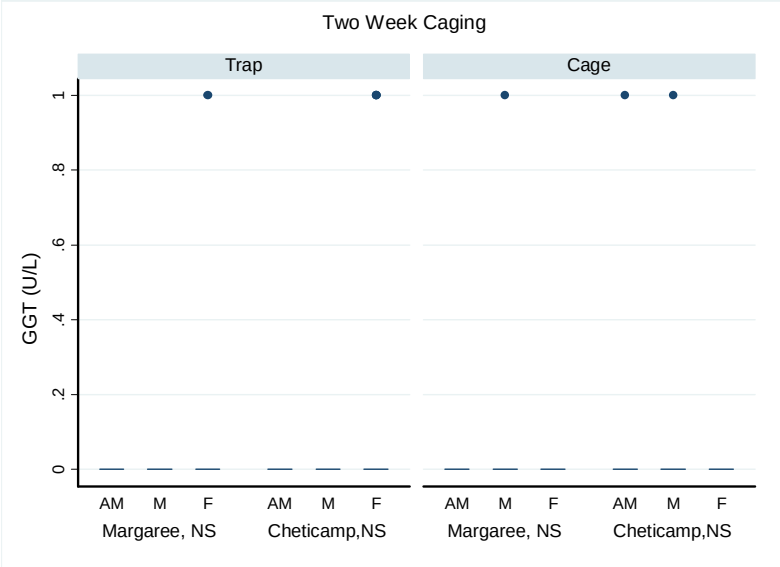
Cheticamp,NS
Margaree, NS
Cheticamp,NS


Two Week Caging
Trap
Cage
GGT (U/L)
0
.2
.4
.6
.8
1
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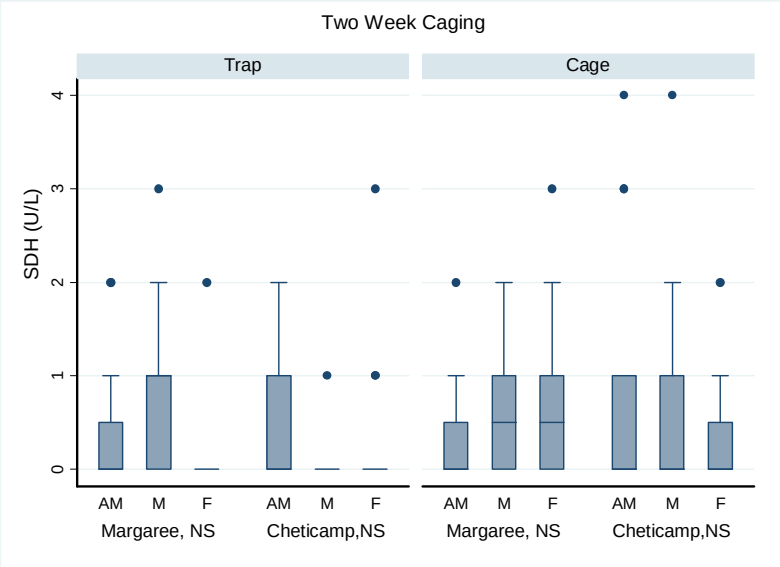
Cheticamp,NS
Margaree, NS
Cheticamp,NS


Two Week Caging
Trap
Cage
SDH (U/L)
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1
2
3
4
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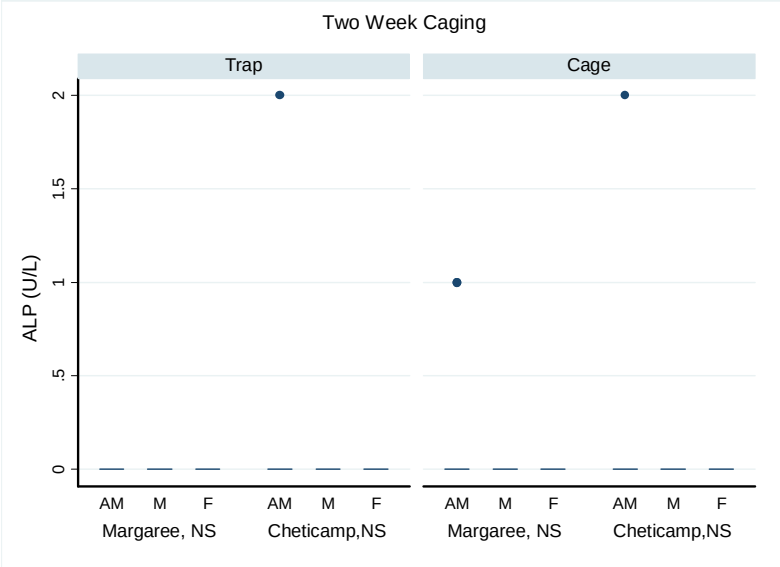
Cheticamp,NS
Margaree, NS
Cheticamp,NS


Two Week Caging
Trap
Cage
ALP (U/L)
0
.5
1
1.5
2
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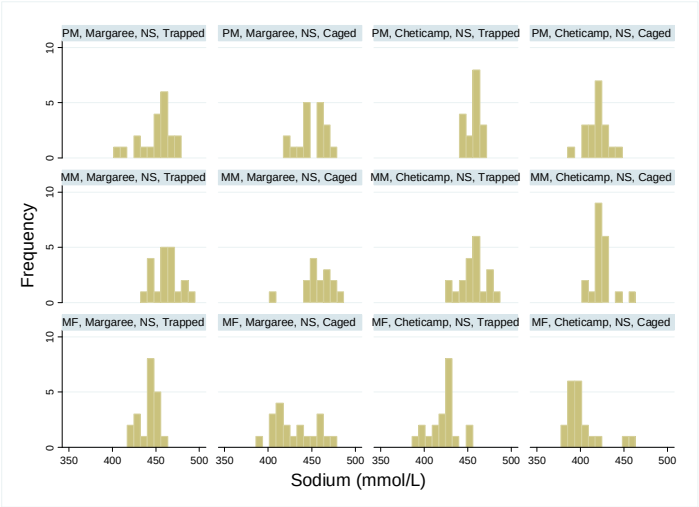
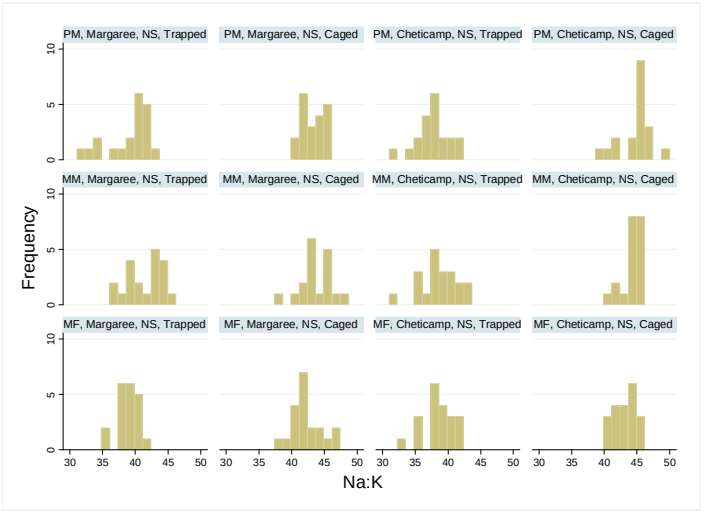
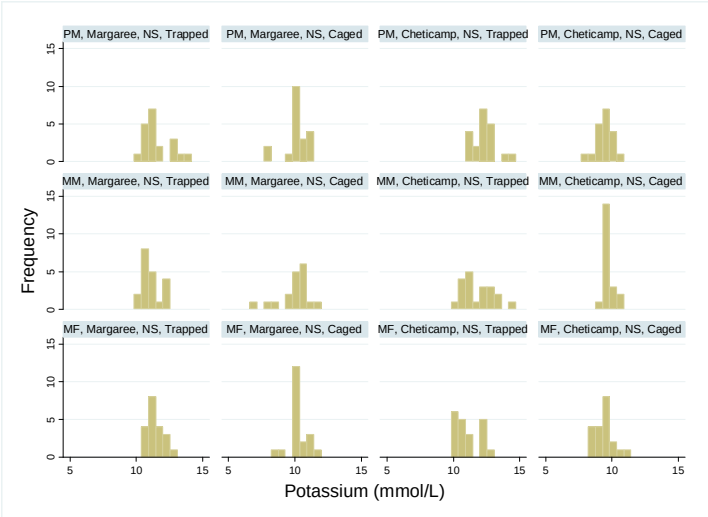
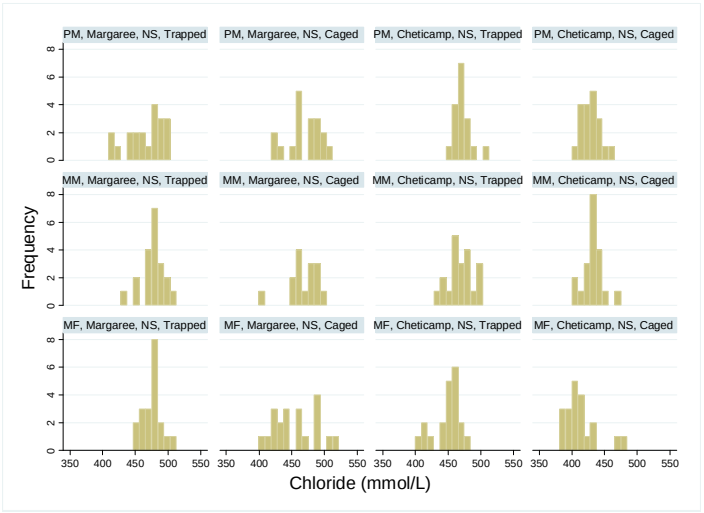
Cheticamp,NS
Margaree, NS
Cheticamp,NS

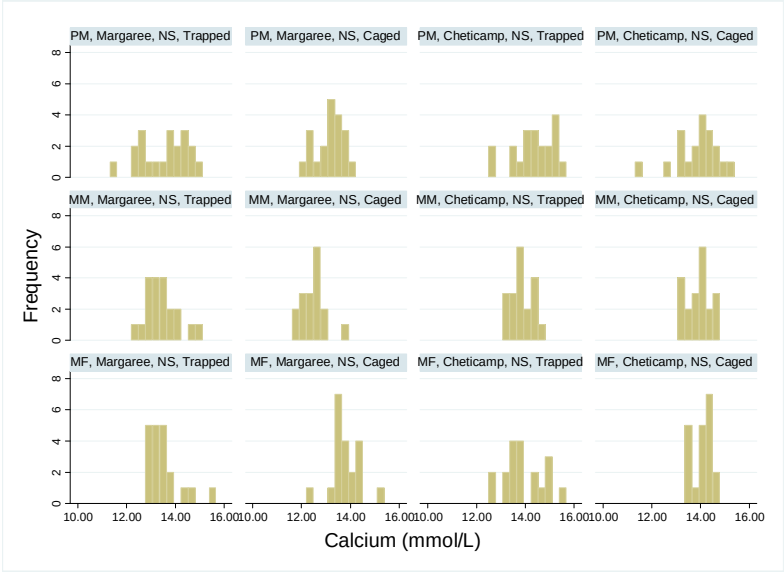
Appendix D – Frequency Distribution Histograms of Hemolymph Biochemistry Parameters







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PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
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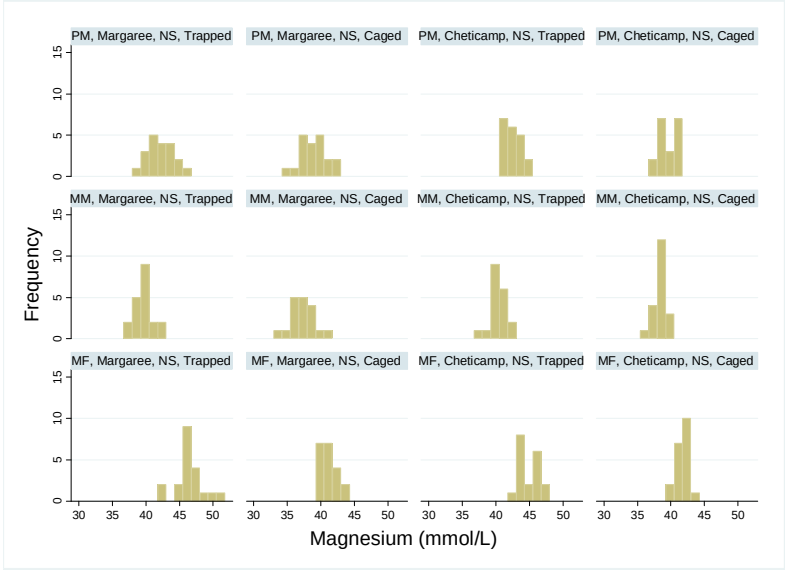
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
2
4
6
8
10.00
12.00
14.00
16.00
Calcium (mmol/L)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PM, Margaree, NS, Caged
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PM, Cheticamp, NS, Caged
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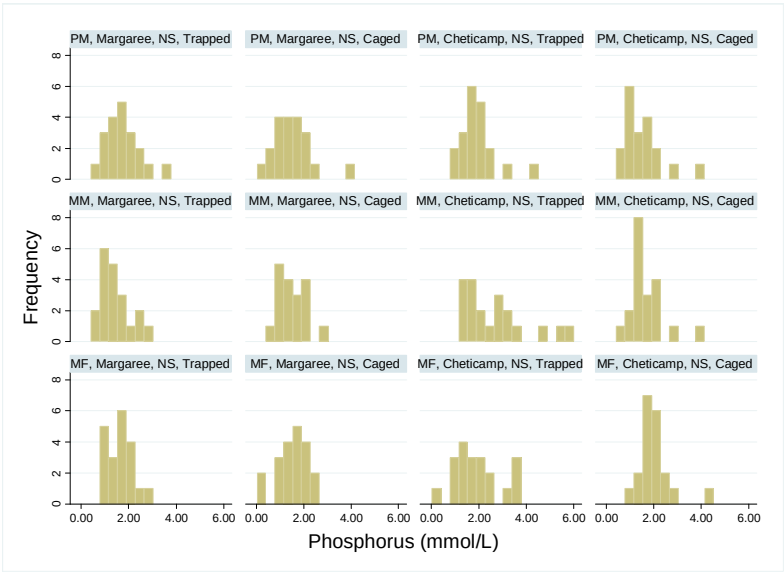
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
30
35
40
45
50
Magnesium (mmol/L)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PM, Margaree, NS, Caged
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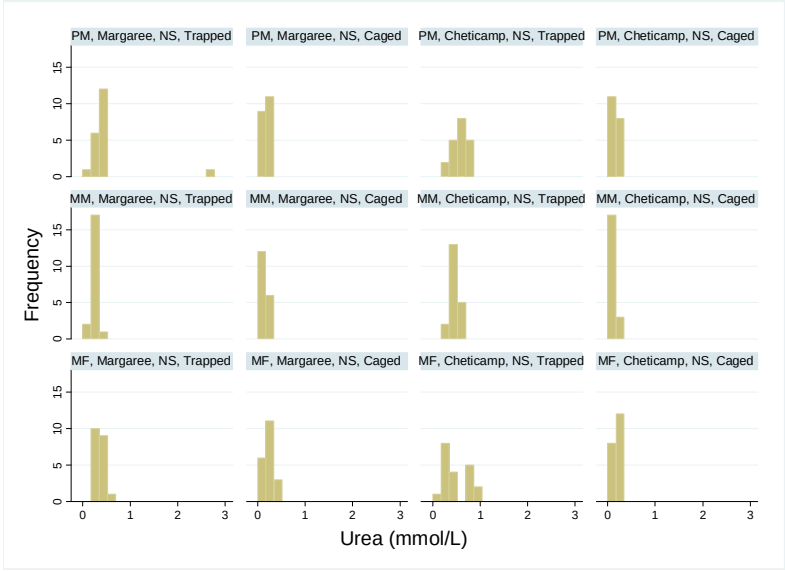
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
2
4
6
8
0.00
2.00
4.00
6.00
Phosphorus (mmol/L)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PM, Margaree, NS, Caged
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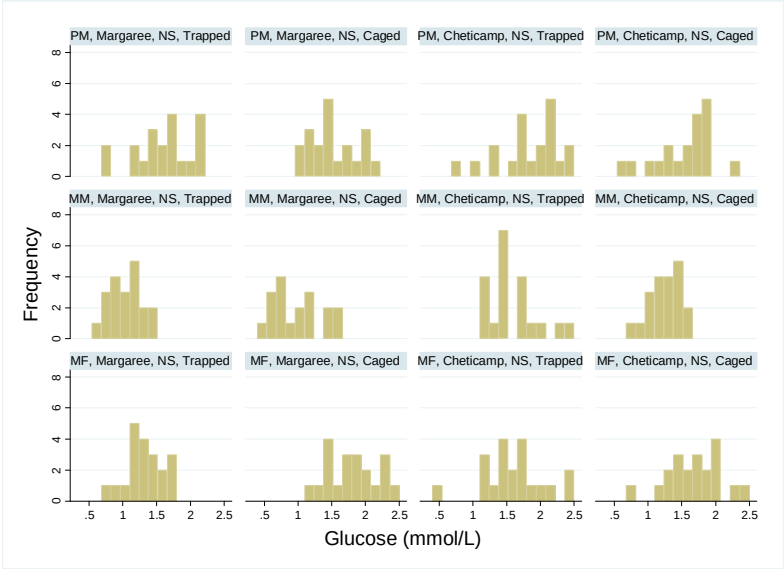
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
0
1
2
3
Urea (mmol/L)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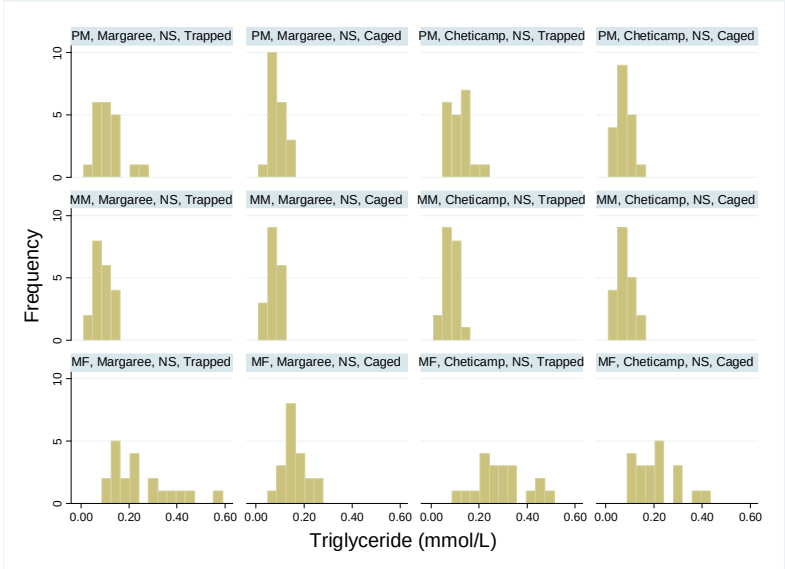
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Glucose (mmol/L)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PM, Margaree, NS, Caged
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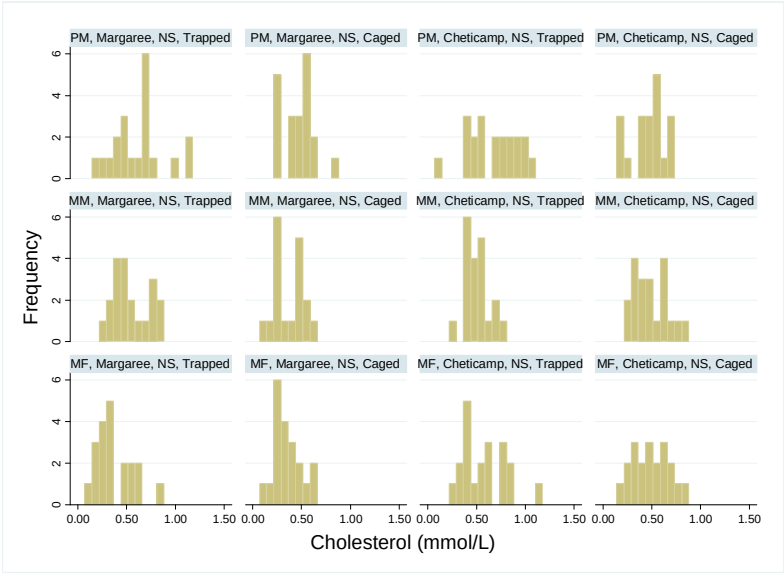
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Triglyceride (mmol/L)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PM, Cheticamp, NS, Caged
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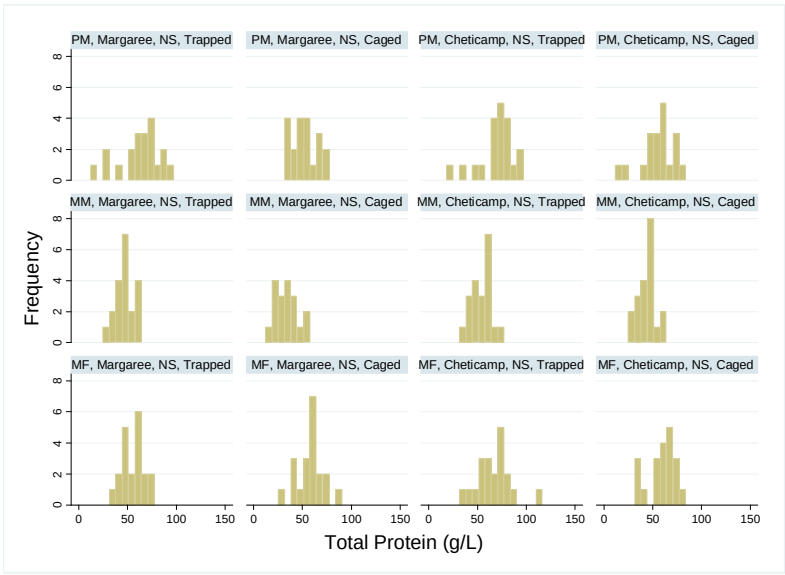
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Cholesterol (mmol/L)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PM, Margaree, NS, Caged
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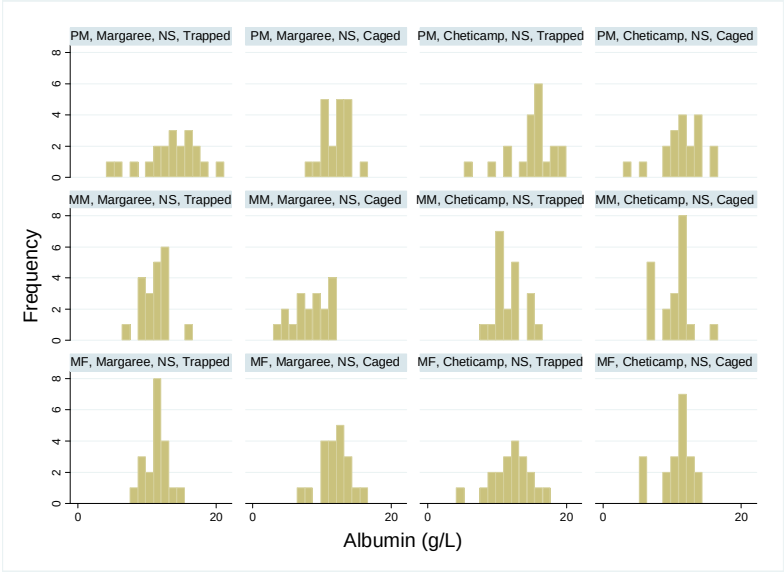
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Total Protein (g/L)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PM, Margaree, NS, Caged
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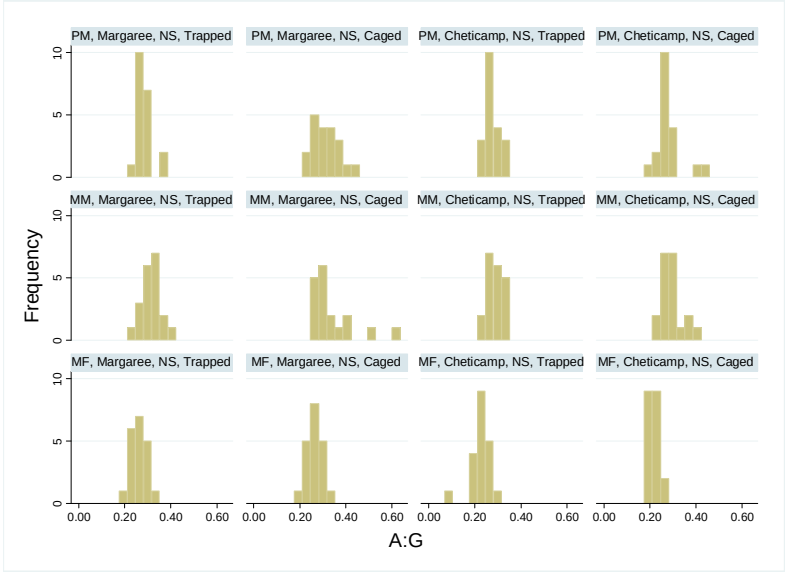
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Albumin (g/L)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PM, Margaree, NS, Caged
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PM, Cheticamp, NS, Caged
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MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
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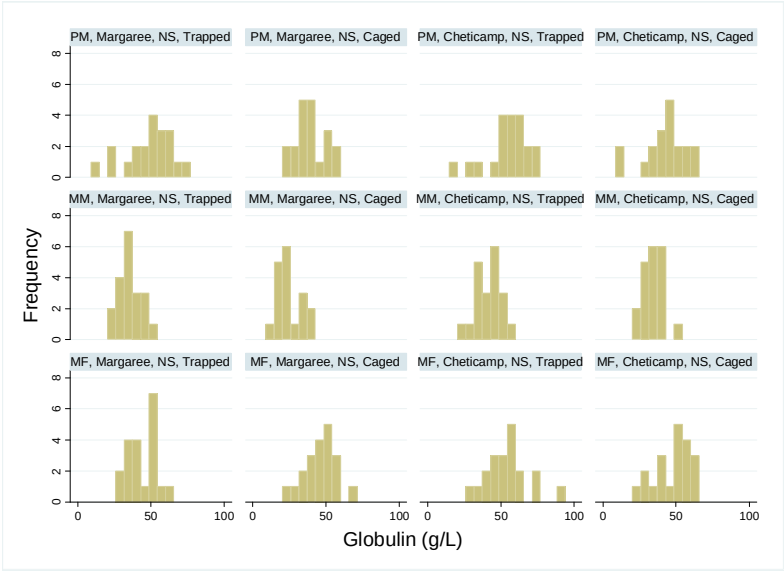
MF, Cheticamp, NS, Caged
Frequency
A:G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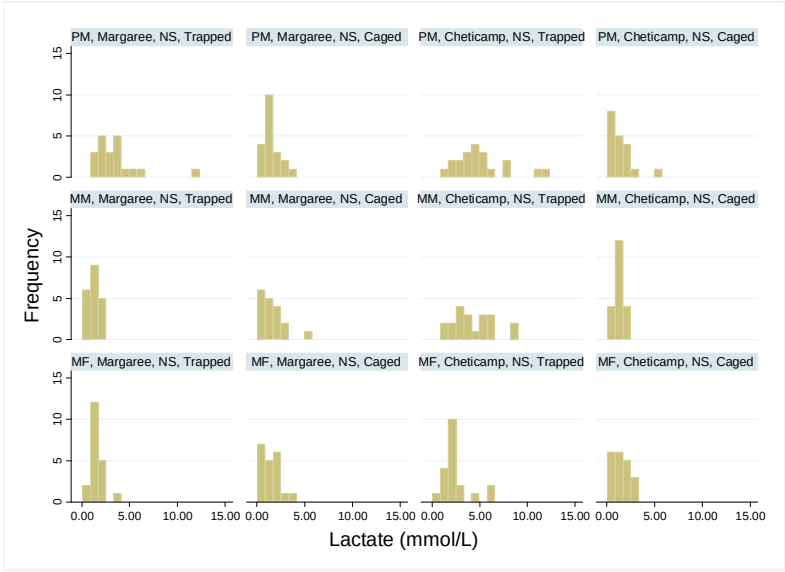
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Globulin (g/L)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PM, Margaree, NS, Caged
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PM, Cheticamp, NS, Caged
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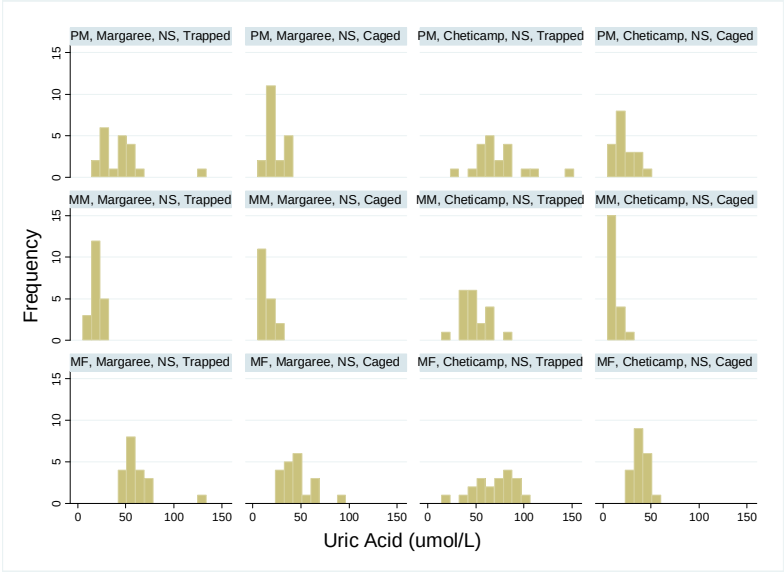
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Lactate (mmol/L)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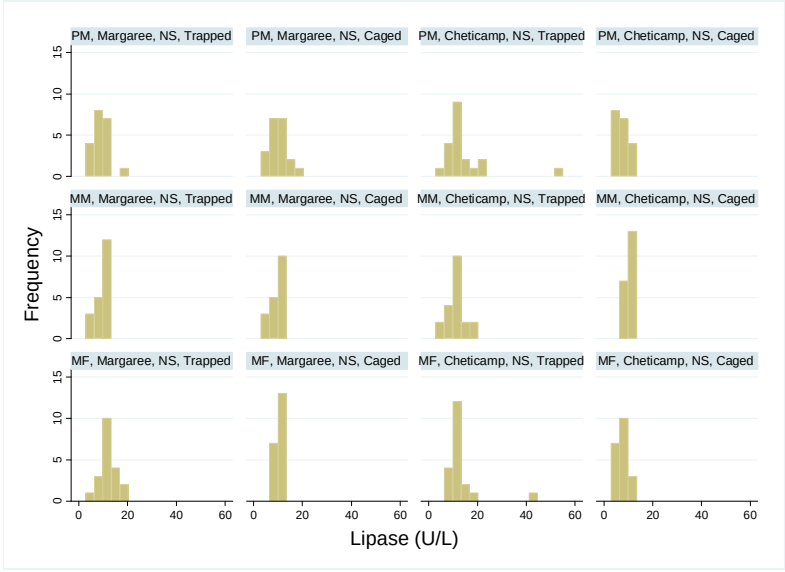
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
0
50
100
150
Uric Acid (umol/L)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PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
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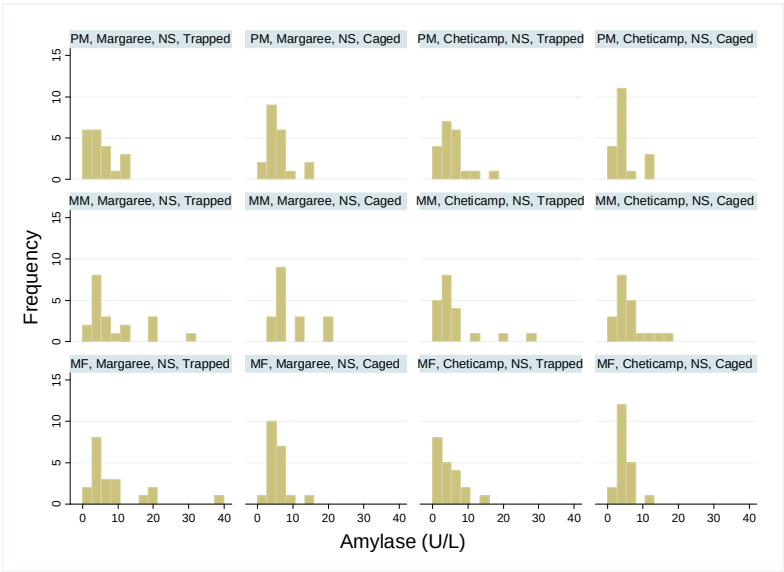
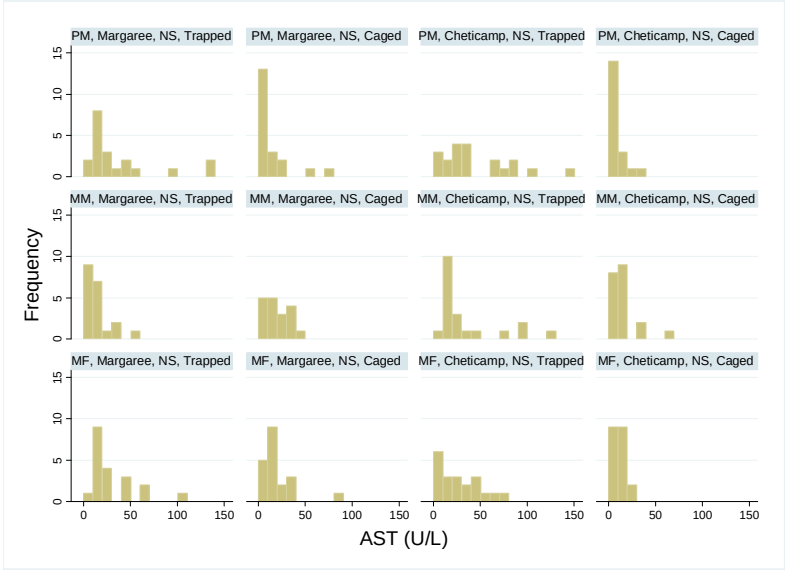
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
0
20
40
60
Lipase (U/L)


PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
0
10
20
30
40
Amylase (U/L)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PM, Margaree, NS, Caged
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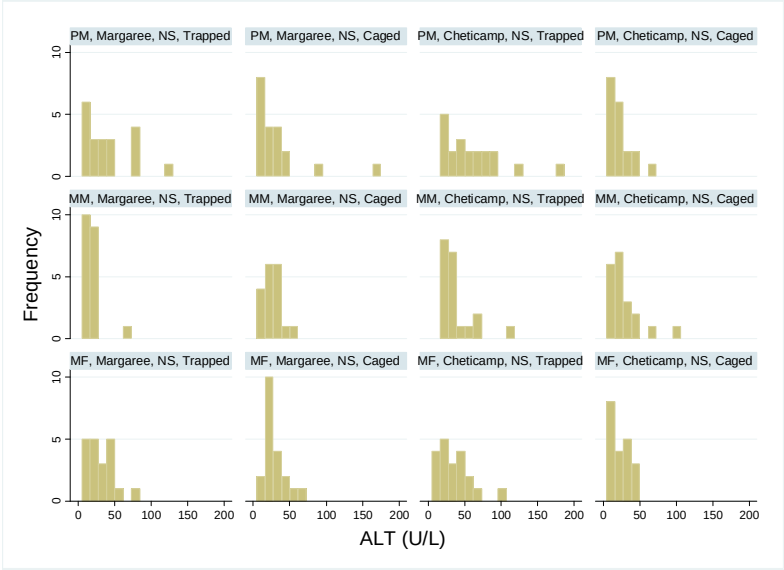
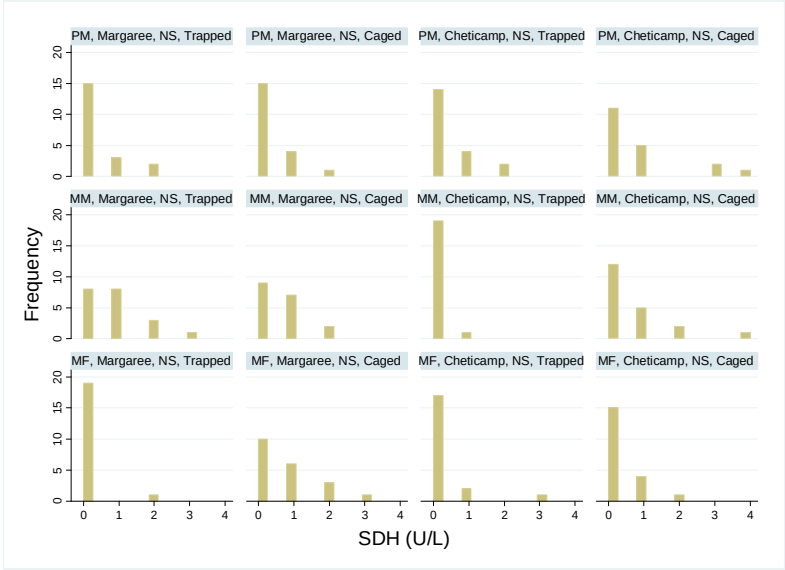
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
0
50
100
150
AST (U/L)


PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
ALT (U/L)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PM, Cheticamp, NS, Caged
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MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
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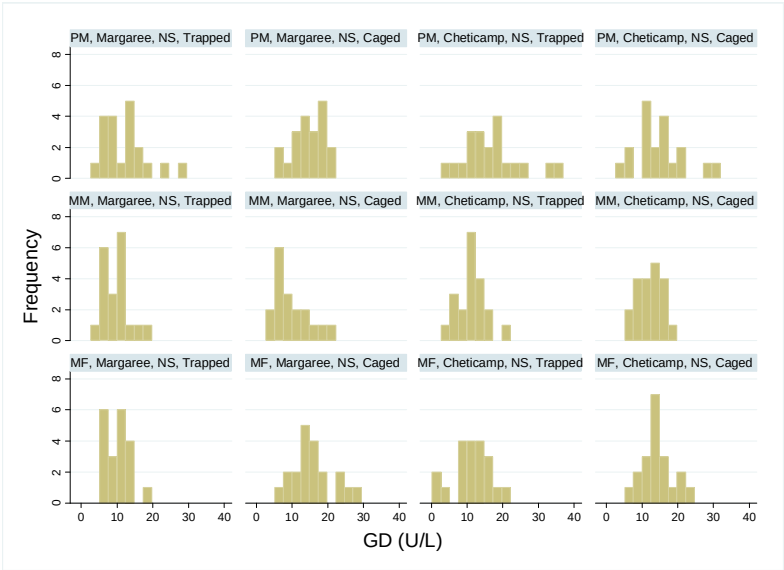
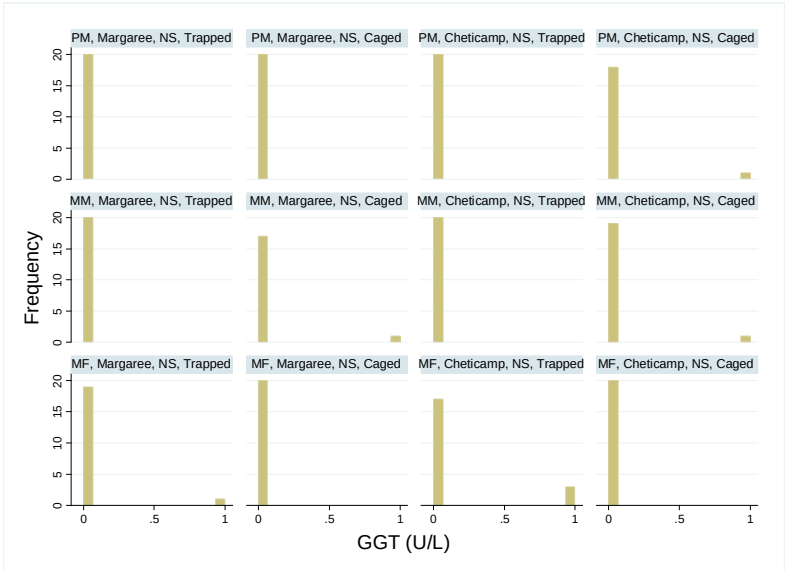
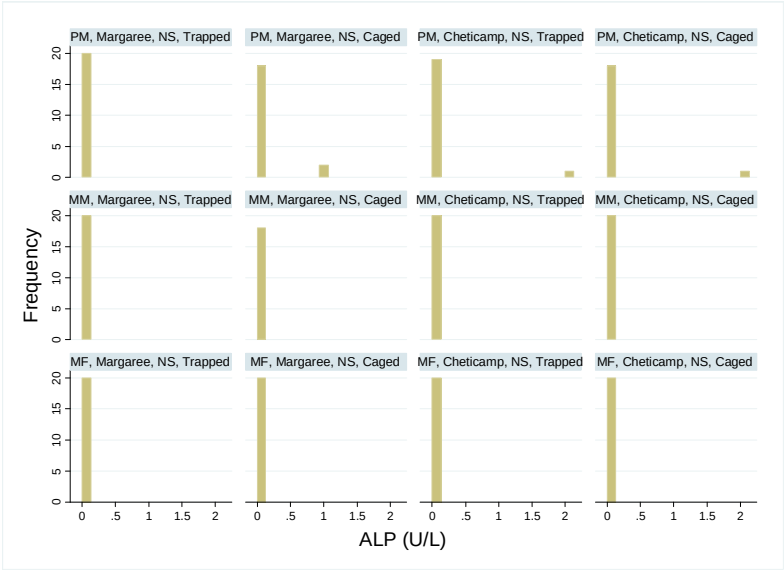
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
SDH (U/L)


PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
GD (U/L)


PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
GGT (U/L)


PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
20
0
.5
1
1.5
2
ALP (U/L)

VIII-2. SIX MONTH (214-222 DAYS) CAGING

VIII-2-1 Summary

Crabs caged for six months had lower values for many hemolymph biochemistry parameters in all (PM, LM, and MF) crabs and decreased hepatopancreas lipid and glycogen content in MF crabs only when compared to free-ranging crabs collected from the same areas. Moderate to good correlations of hemolymph parameters, particularly total protein, to hepatopancreas lipid stores were observed in many groups. Determination of total body moisture content, and its correlations to hemolymph parameters, as a proxy for tissue energy reserves in terminally moulted crabs (all crab categories) may be worth investigation in future studies.
Crabs caged for six months tended to have lower levels, usually significant, of hemolymph parameters considered 'metabolites' (total protein, globulin, urea, triglyceride, cholesterol, glucose, and lactate), and increased A:G ratios, when compared to free counterparts collected by commercial traps. These observations are a continuation of the trend noted in the fall 2012(two week caging) study and were anticipated given the presumption that caged crabs would have restricted access to food sources (quantity and quality). Hemolymph total protein, and derived parameters, were the only directly measured parameter to consistently detect a significant difference between caged and free crabs. This could reflect the order of use of energy reserves in snow crab i.e., protein reserves prior to lipid. Large mature male crabs generally had lower levels of uric acid regardless of location or collection method which may reflect dietary or metabolic differences compared to PM or MF crabs. There were few patterns of enzyme activity associated with caging, station, or crab category. Higher values for the muscle-associated enzymes AST and ALT were more common in the smaller PM and MF crabs which could represent a collection artifact i.e., associated with inadvertent muscle tissue fluid contamination during collection. Marginally lower levels of GD activity in caged crabs may prove to be an early indication of decreased muscle mass.

Significantly lower hepatopancreas lipid and glycogen contents were detected only for caged MF crabs, presumably related to redistribution of hepatopancreas energy reserves to oocytes in preparation for spawning. One possibility for a lack of difference between caged and free PM and LM crabs is that caged crabs expended less energy e.g., acquiring food, evading predators, than free crabs so required less energy intake under the winter environmental conditions and/or that caged crabs were able to acquire sufficient energy reserves (e.g., vegetation, algae, molluscs, small fish adhering to or passing by cages) to meet their metabolic requirements. An additional consideration is the role of hepatopancreas and muscle protein and muscle glycogen and lipid reserves as energy sources. It is possible that preferential consumption of these reserves decreased/delayed the need for consumption of hepatopancreas lipid and glycogen stores. It is also possible, that spring is a time of reduced energy reserves in free crabs in general.

Hemolymph total protein, and occasionally cholesterol, triglyceride, or glucose, were well correlated to hepatopancreas lipid reserves, particularly in free PM crabs and multiple regression

equations had R^2 values of 0.7275 to 0.8719. Free PM crabs may be diverting fewer reserves to reproduction than LM and MF crabs, resulting in better correlations to hepatopancreas lipid. Correlations of hemolymph parameters to hepatopancreas glycogen content were generally poor. Determination of total body (hepatopancreas, muscle, and gonad) protein, lipid, glycogen, and moisture content may have improved correlations for all crab categories and should be included in future work. The tendency for PM and MF crabs to be more similar to other than LM crabs was noted again as in the fall 2012 samples.

Following increases in tissue moisture content as a proxy for decreasing tissue energy reserves in snow crab has been suggested by others (Hardy *et al.* 2000). Determination of tissue (hepatopancreas or muscle) moisture content is simpler and less expensive than measuring tissue lipid, glycogen, and protein content yet, still requires sacrifice of the crab or loss of a leg. Hemolymph is an even simpler tissue to sample than hepatopancreas or muscle and does not require sacrificing the crab allowing for serial sampling. Identifying an indicator e.g., TP or A:G ratio that correlates well to total body moisture content may provide a way to indirectly assess tissue energy reserves in terminally moulted crabs of any sex, regardless of the order of energy reserved utilised.

VIII-2-2 Objective

The objectives of this segment of the project were five-fold:

A. To examine differences in hemolymph biochemistry profiles between free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 214 and 222 days at Margaree and Cheticamp stations in spring 2013

B. To measure hepatopancreas lipid content in free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 214 and 222 at Margaree and Cheticamp stations in spring 2013 and assess the value of hemolymph biochemistry profiles to predict lipid content.

C. To measure hepatopancreas glycogen content in free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 214 and 222 crabs at Margaree and Cheticamp stations in spring 2013 and assess the value of hemolymph biochemistry profiles to predict glycogen content.

D. Calculation of hepatopancreas lipid:glycogen ratios to compare to values obtained in the two week caging study (Section VIII-1).

E. Evaluation of hepatopancreas moisture content in free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 214 and 222 crabs at Margaree and Cheticamp stations in spring 2013.

VIII-2-3 Methodology

Crabs were collected and sampled as per criteria outlined in Sections III-1 through III-3. Data presented are from PM, LM, and MF crabs caged since the fall of 2012 (~ six months) at Margaree Harbor and Cheticamp stations and 'free' crabs collected using commercial traps from the same areas in spring 2013.
Hemolymph plasma samples were collected and delivered to Diagnostic Services at the Atlantic Veterinary College (Charlottetown, PE) for analysis of biochemistry parameters (detailed in Section VII-3 trap vs trawl).

Hepatopancreas moisture, lipid and glycogen content were determined by RPC Science and Engineering (Fredericton, NB). Sample processing was completed as per Ciaramella 2011 (VIII-1-7, Appendices A and B).

Data analysis was completed with STATA statistical software (STATA I/C 12.1, StataCorp LP) and Microsoft Excel (Excel 2010©, Microsoft Corporation). Bonferroni adjustments of significance for multiple comparisons were made where required.

VIII-2-4 Results

A. Hemolyphm Plasma Biochemistry Profiles

All hemolymph plasma samples were processed within 24 – 48 hours of collection, well within previously established time frame for sample stability (see Section VII-2). Measurement of electrolytes (sodium, chloride, potassium) and minerals (calcium and magnesium) required manual or programmed analyser dilution, respectively. The remaining analyses are fully automated.
Sample sizes were small (maximum of 20 animals per group). Records of crabs with marginal outlier values for only one or two of the 26 parameters on the biochemistry panel were reviewed. If a pathologic or physiologic reason (e.g., trauma, hemorrhage, oocyte development) for the outlier value(s) could be identified, neither the crab, nor the values were deleted from the dataset.

Biochemistry data were unavailable from 10 crabs due to mortalities or crabs recorded as 'missing' (Cheticamp caged: two LM, one PM, one MF; Margaree caged: four LM, two PM).

Electrolyte data from three Margaree caged group (PM #75, #78, #81), one Margaree free (PM #279), three Cheticamp caged (PM #159, MF #173, MF #175), and five Cheticamp free crabs (PM #212, PM #214, PM #215, PM #216, LM #190) were identified as outliers on examination of the boxplots (Appendix A) that could be attributed to probable lab error during sample processing and were deleted from the data set. Similarly, calcium and magnesium values for crab #67 (PM, Margaree, caged) were excluded as the automatic dilution was not accurately

programmed on the Cobas c501 analyser. All results from crab #152 (PM, Cheticamp, caged) were excluded as extremely high values for potassium, lactate, phosphorus, urea, ALT and AST were suggestive of a moribound crab at the time of sample collection. These deletions are indicated in Tables 1 –18. Examination of the frequency histograms (Appendix B) showed skewing of most enzyme activity towards lower values with the exception of GD which presented a more normal distribution. Other parameters tended to have a visually normal distribution.

Plasma activity of three enzymes – ALP, GGT, and SDH was very low or zero in most instances and is consistent with previous studies (see sections trap vs trawl, 2 wk caging) . Differences of 1-3 units of activity are not considered clinically relevant and may even be within the allowable precision error for a particular assay i.e., representing no real difference. Statistical comparisons were made; however the results are of questionable value. Creatinine was not detected in any of the samples.

Summary statistics (count, minimum, maximum, SD, mean, and median values) are presented in Tables 1 - 12 and compared to the reference intervals (RI) previously calculated for free, cooler-held, crabs collected in August 2012 near the Cheticamp station (see Section VII-3).

Median electrolyte (sodium, chloride, potassium) values were within the previously generated Cheticamp RI for all sexes, all sites, with or without caging but for free $LM_{Cheticamp}$ where median chloride was slightly below the RI and caged $LM_{Cheticamp}$ where sodium was also slightly below the RI. Median calcium values were below the RI for all free and Margaree caged crab categories. Median magnesium values were at, or below, the lower limit of the RI for all but caged $LM_{Cheticamp}$ and all MF crabs.

Median values for most metabolite parameters for caged and free Margaree LM and MF crabs fell with the RI except for uric acid for all MF (Tables 5 & 6) and urea in caged LM crabs. Free PM crabs had median values for uric acid and triglyceride below the RI whereas all values were at or below the lower limit of the RI for caged PM crabs. Median values for caged and free LM and MF from Cheticamp for all metabolites were within the RI other than slightly lower values for uric acid in MF (Tables 7 & 8). In Cheticamp crabs, only median values for total protein and globulin were below the RI in free PM crabs while, only lactate was within the RI for caged PM.

Enzyme activities (Tables 9 – 12) had median values within the RI for all crabs except a slightly decreased ALT for caged $PM_{Margaree}$ crabs.

Table 1. Summary statistics for mineral and electrolyte concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree, NS, June 2013. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Na[2]	19	420	471	16.55295	444	441	423	462
	K	19	9.3	12.6	.7735768	11.02105	10.8	9.9	13.2
	NaK	19	37	45	1.980903	40.42105	40	34	46
	Cl	19	423	492	18.00877	454.7368	453	444	498
	Ca	20	10.93	13.35	.5722293	12.094	12.11	12.38	15.53
	Phos	20	.27	2.18	.4539102	1.6535	1.675	1.28	5.62
	Mg	20	36.47	39.88	.9288131	38.271	38.375	41.59	46.26
LM	Na	20	426	498	18.55461	457.8	456	441	490
	K	20	9.0	11.4	.734202	10.47	10.5	9.2	12.7
	NaK	20	38	47	2.539685	43.65	44.5	36	49
	Cl	20	423	510	24.43956	466.65	462	459	513
	Ca	20	10.57	13.07	.4762007	11.9475	11.99	12.15	14.03
	Phos	20	0.57	2.05	.3962386	1.1495	1.11	0	3.02
	Mg	20	35.09	37.5	.7969579	36.1855	36.135	38.53	44.12
MF	Na	20	417	492	19.60961	452.7	454.5	385	496
	K	20	9.8	11.1	.405586	10.335	10.2	7.8	13.5
	NaK	20	40	46	1.625455	43.7	43.5	33	51
	Cl	20	450	513	17.45151	478.35	480	396	507
	Ca	20	11.00	13.60	.6319885	11.821	11.655	12.51	16.67
	Phos	20	0.76	2.01	.3484492	1.208	1.15	0.72	3.00
	Mg	20	38.83	43.1	1.071263	41.5345	41.9	40.05	46.67

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits for the sampled populations.[2] Units for all parameters are mmol/L.

Table 2. Summary statistics for mineral and electrolyte concentrations for caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, June 2013. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	Na[2]	16	417	480	15.33501	445.3125	445.5	423	462
	K	16	9.0	13.2	1.245525	11.225	10.8	9.9	13.2
	NaK	16	33	48	3.932768	40	40.5	34	46
	Cl	16	426	504	19.70945	465.9375	463.5	444	498
	Ca	17	10.75	12.42	.5431397	11.57412	11.63	12.38	15.53
	Phos	18	0.54	2.71	.5783445	1.683333	1.69	1.28	5.62
	Mg	17	34.82	42.15	1.845302	37.46176	37.06	41.59	46.26
LM	Na	15	432	480	14.18349	462.2	468	441	490
	K	15	9.3	12.6	.9341153	10.64	10.5	9.2	12.7
	NaK	15	38	48	3.376389	43.6	44	36	49
	Cl	15	447	507	16.18994	476.6	480	459	513
	Ca	16	11.00	12.83	.4955788	11.88625	11.885	12.15	14.03
	Phos	16	0.96	1.72	.2424863	1.415625	1.46	0	3.02
	Mg	16	32.94	37.72	1.067627	35.605	35.765	38.53	44.12
MF	Na	19	429	471	11.35318	450.3158	453	385	496
	K	19	10.2	12.6	.7156153	11.28947	11.4	7.8	13.5
	NaK	19	37	44	2.078855	40.10526	40	33	51
	Cl	19	444	513	18.25838	481.5789	480	396	507
	Ca	19	10.99	12.22	.3643106	11.56	11.57	12.51	16.67
	Phos	19	0.72	2.23	.4064667	1.525263	1.5	0.72	3.00
	Mg	19	38.51	42.33	1.176309	40.33368	40.25	40.05	46.67

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for all parameters are mmol/L.

Table 3. Summary statistics for mineral and electrolyte concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, June 2013. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	Na[2]	16	405	459	15.0947	439.875	441	423	462
	K	16	10.5	14.1	1.09476	11.8875	11.4	9.9	13.2
	NaK	16	32	43	3.376389	37.25	37.5	34	46
	Cl	16	405	489	20.85096	453.3125	458.5	444	498
	Ca	20	10.99	12.48	.3686519	11.791	11.76	12.38	15.53
	Phos	20	.88	2.91	.4840139	1.962	1.765	1.28	5.62
	Mg	20	35.73	39.96	1.100151	38.482	38.635	41.59	46.26
LM	Na	19	432	474	12.01534	454.4211	456	441	490
	K	19	9.9	12.3	.8265337	10.97368	11.1	9.2	12.7
	NaK	19	37	46	2.773095	41.63158	41	36	49
	Cl	19	438	474	11.69795	458.2105	456	459	513
	Ca	20	11.81	13.42	.4489508	12.459	12.275	12.15	14.03
	Phos	20	0.87	2.38	.3953626	1.512	1.505	0	3.02
	Mg	20	33.9	39.86	1.386748	37.358	37.04	38.53	44.12
MF	Na	20	393	477	19.8306	434.1	439.5	385	496
	K	20	9.3	13.2	.9967711	10.575	10.35	7.8	13.5
	NaK	20	32	46	3.446967	41.25	42	33	51
	Cl	20	402	483	23.4893	454.8	465	396	507
	Ca	20	11.16	13.39	.604369	11.8325	11.66	12.51	16.67
	Phos	20	1.11	2.85	.4161082	1.7375	1.59	0.72	3.00
	Mg	20	38.92	42.64	.9030561	40.6155	40.845	40.05	46.67

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units for all parameters are mmol/L.

Table 4. Summary statistics for mineral and electrolyte concentrations for six month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, June 2013. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	Na[2]	17	441	486	14.33194	462.8235	462	423	462
	K	17	10.5	14.7	1.076145	12.19412	12.3	9.9	13.2
	NaK	17	30	44	3.77102	38.29412	38	34	46
	Cl	17	462	525	17.29693	490.0588	489	444	498
	Ca	18	11.54	14.02	.6727577	12.75167	12.73	12.38	15.53
	Phos	18	1.32	3.39	.58789	2.071667	1.91	1.28	5.62
	Mg	18	38.62	44.79	1.721023	41.29611	41.16	41.59	46.26
LM	Na	18	414	447	7.851564	436.6667	438	441	490
	K	18	8.7	12.0	.9930642	10.68333	10.8	9.2	12.7
	NaK	18	37	50	4.062019	41.16667	41	36	49
	Cl	18	429	465	9.438656	451.1667	453	459	513
	Ca	18	12.42	13.76	.3156672	13.11889	13.14	12.15	14.03
	Phos	18	.97	3.08	.5684255	1.793889	1.815	0	3.02
	Mg	18	38.15	41.64	1.011872	40.13833	40.21	38.53	44.12
MF	Na	17	441	474	9.319887	460.8824	459	385	496
	K	17	11.4	13.8	.8016068	12.15882	11.7	7.8	13.5
	NaK	17	32	41	2.44949	38	38	33	51
	Cl	17	474	522	12.3907	500.8235	501	396	507
	Ca	19	11.96	13.49	.3665008	12.56684	12.55	12.51	16.67
	Phos	19	1.19	2.59	.3667105	1.638947	1.57	0.72	3.00
	Mg	19	39.85	44.63	1.205469	42.61105	42.83	40.05	46.67

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for all parameters are mmol/L.

Table 5. Summary statistics for metabolite concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, June 2013. Highlighted values are outside the reference interval.

Sex		N	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Urea[2]	20	0.1	0.6	.1182103	.335	.3	0.3	1.1
	Creat							0	0
	Uric	20	33	97	17.87648	59.9	57	65	199
	TPb	20	17	61	12.64495	40	39	36	97
	Alb	20	4	14	2.643761	8.6	8.5	8	22
	Glob	20	12	49	10.09638	31.4	31	28	78
	AG	20	0.22	0.42	.0382891	0.2785	0.27	0.23	0.33
	Chol	20	0.1	0.5	.1131964	0.3165	0.33	0.22	1.07
	Trig	20	0.01	0.08	.020995	0.0425	0.04	0.05	0.20
	Gluc	20	0.4	1.7	.4073018	1.22	1.4	0.7	2.6
	Lactate	20	1.86	5.66	1.023993	3.9215	3.985	2.15	13.05
LM	Urea	20	0.0	0.4	.1020836	0.19	0.2	0.2	0.6
	Creat							0	0
	Uric	20	15	45	6.844898	22.3	20	6	75
	TPb	20	12	44	8.24286	29.55	30.5	14	65
	Alb	20	3	10	2.058998	6.65	6	6	16
	Glob	20	9	34	6.373465	22.9	24	12	52
	AG	20	0.24	0.40	.0438418	0.292	0.29	0.28	0.46
	Chol	20	0.08	0.48	0.09885	0.2915	0.305	0.14	0.89
	Trig	20	0	0.10	0.0260111	0.0315	0.03	0.03	0.16
	Gluc	20	0.3	1.3	0.2665076	0.795	0.8	0.5	1.7
	Lactate	20	0.55	2.63	0.7022513	1.361	1.035	0.00	3.47
MF	Urea	20	0.1	0.6	0.1118034	0.275	0.3	0.2	2.2
	Creat							0	0
	Uric	20	14	86	16.01282	42.9	41	62	222
	TP	20	17	50	9.185801	30.8	28	9	81
	Alb	20	5	10	1.46539	6.6	6	3	15
	Glob	20	12	41	7.844542	24.2	22	11	71
	AG	20	0.21	0.42	0.0523651	0.285	0.27	0.19	0.46
	Chol	20	0.10	0.44	0.0949501	0.2205	0.20	0.05	0.81
	Trig	20	0.03	0.32	0.0699078	0.1235	0.10	0.00	0.44
	Gluc	20	0.4	1.4	0.248098	0.895	0.9	0.3	2.1
	Lactate	20	1.00	2.95	0.5091321	1.7295	1.725	0.98	14.46

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid and creatinine (µmol/L); for total protein, albumin, and globulin (g/L).

Table 6. Summary statistics for metabolite concentrations for six month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, June 2013. Highlighted values are outside the reference interval.

Sex		N	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Urea	18	0.1	0.3	.0639137	.1944444	0.2	0.3	1.1
	Creat							0	0
	Uric	18	10	102	22.83244	35.55556	29.5	65	199
	TP	18	10	50	12.24171	29.72222	30	36	97
	Alb	18	3	10	2.332633	7.166667	7.5	8	22
	Glob	18	6	40	10.13568	22.55556	23.5	28	78
	AG	18	0.23	0.67	0.1028118	0.3494444	0.33	0.23	0.33
	Chol	18	0.02	0.30	0.091296	0.1605556	0.165	0.22	1.07
	Trig	18	0.00	0.06	0.0197782	0.025	0.02	0.05	0.20
	Gluc	18	0.1	1.7	.4566144	0.8444444	0.7	0.7	2.6
	Lactate	18	0.79	4.84	1.120201	2.255	1.98	2.15	13.05
LM	Urea	16	0.1	0.3	0.0619139	0.1375	0.1	0.2	0.6
	Creat							0	0
	Uric	16	4	31	8.342412	18.4375	19.5	6	75
	TPb	16	16	39	7.033254	24.5	24	14	65
	Alb	16	4	9	1.454877	6.375	6	6	16
	Glob	16	11	30	5.714018	18.125	18	12	52
	AG	16	0.29	0.55	0.0696629	0.365625	0.36	0.28	0.46
	Chol	16	0.03	0.33	0.0933452	0.1825	0.17	0.14	0.89
	Trig	16	0.00	0.06	0.0166833	0.02375	0.02	0.03	0.16
	Gluc	16	0.4	1.4	0.310309	0.81875	0.75	0.5	1.7
	Lactate	16	0.91	3.4	0.6410873	1.719375	1.5	0.00	3.47
MF	Urea	19	0.1	0.3	0.060214	0.1842105	0.2	0.2	2.2
	Creat							0	0
	Uric	19	27	73	13.14027	43	38	62	222
	TPb	19	9	42	8.952441	22.57895	21	9	81
	Alb	19	4	9	1.492672	5.684211	5	3	15
	Glob	19	5	33	7.556415	16.89474	15	11	71
	AG	19	0.24	0.80	0.1295742	0.3768421	0.33	0.19	0.46
	Chol	19	0.01	0.36	0.0732256	0.0921053	0.09	0.05	0.81
	Trig	19	0.00	0.13	.0351188	0.04	0.03	0.00	0.44
	Gluc	19	0.1	1.1	.2685242	0.4894737	0.5	0.3	2.1
	Lactate	19	0.53	2.29	.5357467	1.366316	1.29	0.98	14.46

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid and creatinine (µmol/L); for total protein, albumin, and globulin (g/L).

Table 7. Summary statistics for metabolite concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, June 2013. Highlighted values are outside the reference interval.

Sex		N	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Urea[2]	20	0.3	0.5	0.0812728	0.365	0.3	0.3	1.1
	Creat							0	0
	Uric	20	63	251	46.50059	115.1	101.5	65	199
	TPb	20	13	50	11.02091	33.25	33	36	97
	Alb	20	4	11	2.187885	8.05	8	8	22
	Glob	20	8	39	8.994735	25.2	24.5	28	78
	AG	20	0.23	0.63	0.080451	0.3375	0.33	0.23	0.33
	Chol	20	0.03	0.59	0.1224003	0.2515	0.255	0.22	1.07
	Trig	20	0.01	0.52	0.1102437	0.078	0.05	0.05	0.20
	Gluc	20	0.3	1.6	0.384023	0.93	0.95	0.7	2.6
	Lactate	20	2.45	7.77	1.488961	4.355	4.255	2.15	13.05
LM	Urea	20	0.0	0.4	0.0940325	0.26	0.3	0.2	0.6
	Creat							0	0
	Uric	20	28	65	10.15148	44	45.5	6	75
	TPb	20	24	47	6.26099	33.6	32.5	14	65
	Alb	20	5	11	1.559352	8.3	9	6	16
	Glob	20	18	37	5.048189	25.3	23.5	12	52
	AG	20	0.26	0.43	0.0489334	0.3305	0.34	0.28	0.46
	Chol	20	0.17	0.41	0.0713701	0.279	0.25	0.14	0.89
	Trig	20	0.01	0.11	.0295359	0.0525	0.045	0.03	0.16
	Gluc	20	0.1	1.5	.3216323	0.865	0.85	0.5	1.7
	Lactate	20	0.48	3.84	.8287433	2.2305	2.26	0.00	3.47
MF	Urea	20	0.1	0.8	0.1380313	0.33	0.3	0.2	2.2
	Creat							0	0
	Uric	20	42	119	20.51822	66.45	59	62	222
	TPb	20	21	49	7.796592	30.45	28	9	81
	Alb	20	5	11	1.750188	7.3	7	3	15
	Glob	20	16	38	6.310184	23.15	21.5	11	71
	AG	20	0.24	0.44	0.0549545	0.321	0.31	0.19	0.46
	Chol	20	0.07	0.30	0.0757055	0.1805	0.17	0.05	0.81
	Trig	20	0.07	0.31	0.0612394	0.1285	0.12	0.00	0.44
	Gluc	20	0.6	1.7	0.2800376	0.95	0.9	0.3	2.1
	Lactate	20	1.46	4.46	0.983452	2.839	2.895	0.98	14.46

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units for urea, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid and creatinine (µmol/L); for total protein, albumin, and globulin (g/L).

Table 8. Summary statistics for metabolite concentrations for six month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2012. Highlighted values are outside the reference interval.

Sex		N	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	Urea[2]	18	0.1	0.8	0.1539247	0.2611111	0.2	0.3	1.1
	Creat							0	0
	Uric	18	33	118	25.07297	62.77778	57	65	199
	TP	18	7	47	12.8521	21.33333	17	36	97
	Alb	18	4	12	2.304443	6.611111	6	8	22
	Glob	18	2	35	10.64842	14.72222	11.5	28	78
	AG	18	0.31	2.5	0.5506787	0.7033333	0.495	0.23	0.33
	Chol	18	0.02	0.31	0.0924061	0.1427778	0.145	0.22	1.07
	Trig	18	0.00	0.09	0.0216629	0.0288889	0.03	0.05	0.20
	Gluc	18	0.1	2.3	0.7012594	0.8	0.5	0.7	2.6
	Lactate	18	0.66	6.68	1.596933	3.089444	3.225	2.15	13.05
LM	Urea	18	0.1	0.4	0.0783823	0.2444444	0.25	0.2	0.6
	Creat							0	0
	Uric	18	17	123	26.03122	42.27778	33.5	6	75
	TPb	18	15	44	7.376212	28.05556	28	14	65
	Alb	18	5	11	1.661757	7.055556	6.5	6	16
	Glob	18	10	33	5.940885	21	21.5	12	52
	AG	18	0.23	0.50	0.0629477	0.3472222	0.33	0.28	0.46
	Chol	18	0.10	0.47	0.0960885	0.2527778	0.24	0.14	0.89
	Trig	18	0.00	0.09	0.0215495	0.0405556	0.035	0.03	0.16
	Gluc	18	0.2	2.0	0.5041008	1.0	0.95	0.5	1.7
	Lactate	18	1.07	5.39	1.290147	3.047222	2.865	0.00	3.47
MF	Urea	19	0.1	0.4	0.0837708	0.2421053	0.2	0.2	2.2
	Creat							0	0
	Uric	19	32	100	16.07348	61.36842	56	62	222
	TPb	19	7	27	6.472163	17	16	9	81
	Alb	19	4	7	1.097578	5.263158	6	3	15
	Glob	19	3	20	5.347022	11.57895	11	11	71
	AG	19	0.35	1.33	0.2654236	0.5505263	0.43	0.19	0.46
	Chol	19	0.01	0.15	0.0430762	0.07	0.06	0.05	0.81
	Trig	19	0.01	0.12	0.0269285	0.0415789	0.03	0.00	0.44
	Gluc	19	0.1	0.9	0.2192491	0.3842105	0.4	0.3	2.1
	Lactate	19	0.69	3.750	.8640091	1.596842	1.25	0.98	14.46

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid and creatinine (µmol/L); for total protein, albumin, and globulin (g/L).

Table 9. Summary statistics for enzyme activity for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, June 2013. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	AMY[2]	20	1	41	10.36644	8.9	5	3	18
	LIP	20	1	257	55.24119	22.7	11	3	13
	AST	20	4	72	20.74761	31.4	28	13	203
	ALT	20	7	37	7.85661	18.4	18	23	105
	GD	20	7	26	5.296225	14.95	14.5	9	37
	SDH	20	0	1	.2236068	.05	0	0	1
	ALP	20	0	20	4.472136	1	0	0	1
	GGT	20	0	1	.5026247	.6	1	0	1
LM	AMY	20	0	15	3.910512	5.85	5.5	1	19
	LIP	20	4	40	7.461304	10.75	9.5	4	15
	AST	20	2	59	15.95685	15.1	9.5	4	46
	ALT	20	3	34	7.337539	10.95	8.5	6	45
	GD	20	4	23	5.077038	13.25	14.5	4	21
	SDH	20	0	1	.2236068	.05	0	0	0
	ALP	20	0	0	0	0	0	0	0
	GGT	20	0	1	.5026247	.4	0	0	0
MF	AMY	20	3	40	8.457946	8.8	6	1	45
	LIP	20	6	179	49.85292	31.95	13.5	1	21
	AST	20	7	48	11.33822	20.35	18.5	16	486
	ALT	20	5	34	8.043304	16.8	16	0	188
	GD	20	4	29	6.444908	12.8	12	4	39
	SDH	20	0	2	.680557	.4	0	0	2
	ALP	20	0	0	0	0	0	0	13
	GGT	20	0	2	.6048053	.55	.5	0	4

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units are U/L

Table 10. Summary statistics for enzyme activity for six month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, June 2013. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	AMY[2]	18	1	12	2.901431	5.777778	5.5	3	18
	LIP	18	7	19	3.13373	10.94444	11	3	13
	AST	18	5	194	60.70288	56.61111	34	13	203
	ALT	18	0	54	13.46455	20	17	23	105
	GD	18	4	30	8.224783	14.66667	12.5	9	37
	SDH	18	0	3	.9633818	.8888889	1	0	1
	ALP	18	0	3	.9164438	.3888889	0	0	1
	GGT	18	0	1	.5016313	.3888889	0	0	1
LM	AMY	16	2	10	2.362908	5.125	5	1	19
	LIP	16	5	11	1.825742	9	9	4	15
	AST	16	2	26	7.032069	11.625	9.5	4	46
	ALT	16	3	21	5.289928	10.625	10.5	6	45
	GD	16	4	19	4.333974	10.625	9.5	4	21
	SDH	16	0	1	.5123475	.4375	0	0	0
	ALP	16	0	0	0	0	0	0	0
	GGT	16	0	1	.4472136	.25	0	0	0
MF	AMY	19	2	32	7.572264	8.315789	5	1	45
	LIP	19	4	19	4.357557	10.89474	11	1	21
	AST	19	4	1129	284.4496	108.3684	16	16	486
	ALT	19	4	135	34.63114	24.73684	13	0	188
	GD	19	3	27	6.621337	11.21053	10	4	39
	SDH	19	0	1	.3153018	.1052632	0	0	2
	ALP	19	0	0	0	0	0	0	4
	GGT	19	0	1	.4775669	.3157895	0	0	13

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units are U/L

Table 11. Summary statistics for enzyme activity for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, June 2013. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	AMY[2]	20	1	27	7.408388	10.4	7	3	18
	LIP	20	3	262	83.78255	40.4	7.5	3	13
	AST	20	15	313	91.23935	88.25	64.5	13	203
	ALT	20	11	136	31.60013	37.4	26.5	23	105
	GD	20	3	27	5.489464	13.35	13	9	37
	SDH	20	0	2	.6708204	.35	0	0	1
	ALP	20	0	3	.7163504	.25	0	0	1
	GGT	20	0	0	0	0	0	0	1
LM	AMY	20	1	75	15.94159	7.85	4	1	19
	LIP	20	3	12	2.645254	5.45	4.5	4	15
	AST	20	1	54	13.80799	20.15	17.5	4	46
	ALT	20	6	22	4.321306	12.4	11	6	45
	GD	20	5	28	6.563656	14.85	14	4	21
	SDH	20	0	1	.3077935	.1	0	0	0
	ALP	20	0	0	0	0	0	0	0
	GGT	20	0	0	0	0	0	0	0
MF	AMY	20	2	21	5.623962	7.95	6	1	45
	LIP	20	2	135	35.3254	17.1	6	1	21
	AST	20	8	267	61.26611	50.8	28.5	16	486
	ALT	20	7	78	19.32894	28.15	22.5	0	188
	GD	20	6	26	4.749238	13.15	12.5	4	39
	SDH	20	0	1	.3663475	.15	0	0	2
	ALP	20	0	0	0	0	0	0	13
	GGT	20	0	1	.2236068	.05	0	0	4

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units are U/L

Table 12. Summary statistics for enzyme activity for six month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, June 2013. Highlighted values are outside the reference interval.

Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	AMY[2]	18	3	15	3.644317	6.888889	6	3	18
	LIP	18	5	22	4.570436	9.222222	8	3	13
	AST	18	8	190	52.3686	62.33333	39.5	13	203
	ALT	18	10	67	15.1861	27.83333	24	23	105
	GD	18	3	23	5.348325	9.388889	8.5	9	37
	SDH	18	0	2	.4714045	.1111111	0	0	1
	ALP	18	0	3	1.150447	.5	0	0	1
	GGT	18	0	1	.2357023	.0555556	0	0	1
LM	AMY	18	2	19	3.988955	5.833333	5	1	19
	LIP	18	5	17	3.014128	8.555556	8	4	15
	AST	18	3	116	32.19837	36.5	24.5	4	46
	ALT	18	4	64	15.66896	20.88889	14.5	6	45
	GD	18	5	16	3.462214	11.11111	10.5	4	21
	SDH	18	0	1	.3233808	.1111111	0	0	0
	ALP	18	0	0	0	0	0	0	0
	GGT	18	0	0	0	0	0	0	0
MF	AMY	19	1	27	8.157442	10.10526	6	1	45
	LIP	19	4	21	4.712805	9.894737	9	1	21
	AST	19	6	95	26.02113	30.10526	20	16	486
	ALT	19	5	124	34.44973	24.68421	12	0	188
	GD	19	2	12	3.005842	6.421053	6	4	39
	SDH	19	0	1	.3746343	.1578947	0	0	2
	ALP	19	0	0	0	0	0	0	13
	GGT	19	0	0	0	0	0	0	4

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units are U/L

Effect of Treatment (Caged vs Free) within a Station by Sex

The effects of the six month caging period for each crab category (PM, LM, MF) in Margaree Harbor and Cheticamp were compared (Wilcoxon signed rank test) and are summarised in Tables 13 & 14. In Margaree Harbor crabs, median values for calcium and magnesium were always lower in caged crabs compared to free crabs, although differences were only significant for calcium for PM and magnesium for MF crabs. Caged PM and LM crabs in Cheticamp had significantly higher sodium and chloride concentrations compared to free counterparts. Calcium and magnesium levels were significantly higher for all caged Cheticamp crabs compared to free crabs.

Median levels of metabolites were nearly always lower, and usually statistically significant, in caged crabs than free counterparts at both stations with few exceptions. Median protein (total protein, globulin, albumin, uric acid, urea) and energy (triglyceride, cholesterol, glucose) related parameters were lower, most statistically significantly so, in caged PM and MF crabs at both

stations. The median values for A:G ratios were significantly higher in caged crabs for all but caged LM crabs in Cheticamp.Lactate levels were higher in caged LM crabs at both stations.

Slightly decreased median GD activity was noted for all caged crabs; however, the decrease was only significant for caged PM and MF crabs from Cheticamp. Slight increases in lipase activity were detected for caged LM and MF crabs from Cheticamp.

Effect of Sex within a Station by Treatment (Caged or Free)

Values for all 27 biochemistry parameters were compared (Kruskal-Wallis testing) within a station across crab categories (sex) for crabs collected by traps and after the 6 month caging period (Tables 15 & 16).
Minor, inconsistent, differences were detected among crab types for sodium, chloride, and phosphorus concentrations. Potassium and phosphorus concentrations tended to be higher in PM crabs, free or caged, both sites, but this was not always statistically significant. Magnesium concentrations were greater in MF crabs at all times.

Among free crabs, PM tended to have higher median values for total protein, albumin, globulin, and uric acid although this was not always statistically significant. The trend was not present for caged crabs. Cholesterol concentrations were always lower for caged MF crabs compared to caged PM or LM crabs. In contrast, free MF crabs had median triglyceride levels that were significantly higher than free LM or PM crabs while, there was no difference among sexes for caged crabs at either station. There was no consistent pattern for glucose concentrations, while lactate levels were often highest for PM crabs, especially when collected by trapping (free).

There were no consistent patterns evident with respect to enzyme activity.

Effect of Station within a Treatment by Sex

There were few differences in electrolyte and mineral concentrations when comparing free PM, LM, and MF crabs from Margaree Harbor to those from Cheticamp. In contrast, among caged crabs, median values for potassium, calcium, and magnesium were usually higher, often significantly, in crabs (PM, LM, MF) from Cheticamp compared to Margaree Harbor.
For metabolites in free crabs (PM, LM, MF) from Cheticamp had higher values, usually significant, for uric acid, triglyceride, lactate, and A:G ratio than Margaree Harbor counterparts while, values for cholesterol tended to be lower. Few differences, none significant, were noted for median total protein, albumin, or globulin levels. Lipase activity was slightly, yet significantly lower in all free crabs from Cheticamp. The activity of AST and ALT was higher in $PM_{Cheticamp}$ only.

Comparison of metabolites in caged crabs by station found uric acid to be consistently higher in all (PM, LM, MF) crabs from Cheticamp compared to Margaree Harbor. Cheticamp PM and MF crabs tended to have lower median values for total protein, globulin, and cholesterol

although this was only significant for globulin and total protein for PM and globulin for MF. The A:G ratio was significantly higher for Cheticamp PM and MF crabs only. Conversely, LM crabs from Cheticamp had significantly higher median levels of urea, uric acid, cholesterol, triglyceride, and lactate and a trend to lower A:G ratio compared to caged LM from Margaree Harbor. Median total protein, albumin, and globulin levels were higher but not significantly so. Caged Cheticamp PM and MF crabs had significantly lower (marginal) GD activity than Margaree Harbor counterparts.

Table 13. Effect of six months of **caging** on median values of hemolymph plasma biochemistry parameters of snow crab collected from **Margaree Harbor, NS** in June 2013. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) changes were noted are shown.

Analyte	PM					LM					MF				
	n	Trap	*n*	Cage	*p*	*n*	Trap	*n*	Cage	*p*	*n*	Trap	*n*	Cage	*p*
Sodium (mmol/L)	*19*	441	*16*	445.5	---	*20*	456	*15*	468	---	*20*	454.5	*19*	453	---
Potassium (mmol/L)	*19*	10.8	*16*	10.8	---	*20*	10.5	*15*	10.5	---	*20*	10.2	*19*	11.4	*0.000*
Na:K	*19*	40	*16*	40.5	---	*20*	44.5	*15*	44	---	*20*	43.5	*19*	40	*0.000*
Chloride (mmol/L)	*19*	453	*16*	463.5	---	*20*	462	*15*	480	----	*20*	480	*19*	480	----
Calcium (mmol/L)	*20*	12.11	*17*	11.63	*0.0084*	*20*	11.99	*16*	11.885	----	*20*	11.655	*19*	11.57	----
Phosphorus (mmol/L)	*20*	1.675	*18*	1.69	----	*20*	1.11	*16*	1.46	*0.0185*	*20*	1.15	*19*	1.5	*0.0119*
Magnesium (mmol/L)	*20*	38.375	*17*	37.06	----	*20*	36.135	*16*	35.765	----	*20*	41.90	*19*	40.25	*0.0024*
Urea (mmol/L)	*20*	0.3	*18*	0.2	*0.0001*	*20*	0.2	*16*	0.1	----	*20*	0.3	*19*	0.2	*0.0026*
Uric Acid (µmol/L)	*20*	57	*18*	29.5	*0.0005*	*20*	20	*16*	19.5	----	*20*	41	*19*	38	----
Total Protein (g/L)	*20*	39	*18*	30	*0.0242*	*20*	30.5	*16*	24	*0.0365*	*20*	28	*19*	21	*0.0089*
Albumin (g/L)	*20*	8.5	*18*	7.5	----	*20*	6	*16*	6	----	*20*	6	*19*	5	*0.0423*
Globulin (g/L)	*20*	31	*18*	23.5	*0.0113*	*20*	24	*16*	18	*0.0190*	*20*	22	*19*	15	*0.0069*
A:G	*20*	0.27	*18*	0.33	*0.0058*	*20*	0.29	*16*	0.36	*0.0004*	*20*	0.27	*19*	0.33	*0.0019*
Cholesterol (mmol/L)	*20*	0.33	*18*	0.165	*0.0002*	*20*	0.305	*16*	0.17	*0.0043*	*20*	0.20	*19*	0.09	*0.0000*
Triglyceride mmol/L)	*20*	0.04	*18*	0.02	*0.0172*	*20*	0.03	*16*	0.02	----	*20*	0.10	*19*	0.03	*0.0000*
Glucose (mmol/L)	*20*	1.4	*18*	0.7	*0.0106*	*20*	0.8	*16*	0.75	----	*20*	0.9	*19*	0.5	*0.0001*
Lactate (mmol/L)	*20*	3.985	*18*	1.98	*0.0001*	*20*	1.035	*16*	1.50	----	*20*	1.725	*19*	1.29	*0.0316*
Creatinine (µmol/L)	*20*	[1].	*18*	.		*20*	.	*16*	.		*20*	.	*19*	.	
Amylase (U/L)	*20*	5	*18*	5.5	----	*20*	5.5	*16*	5	----	*20*	6	*19*	5	----
Lipase (U/L)	*20*	11	*18*	11	----	*20*	9.5	*16*	9	----	*20*	13.5	*19*	11	----
AST (U/L)	*20*	28	*18*	34	----	*20*	5.5	*16*	9.5	----	*20*	18.5	*19*	16	----
ALT (U/L)	*20*	18	*18*	17	----	*20*	8.5	*16*	10.5	----	*20*	16	*19*	13	----
GD (U/L)	*20*	14.5	*18*	12.5	----	*20*	14.5	*16*	9.5	----	*20*	12	*19*	10	----
SDH (U/L)	*20*	0	*18*	1	*0.0006*	*20*	0	*16*	0	*0.0061*	*20*	0	*19*	0	----
ALP (U/L)	*20*	0	*18*	0	----	*20*	0	*16*	0	.	*20*	0	*19*	0	.
GGT (U/L)	*20*	1	*18*	0	----	*20*	0	*16*	0	----	*20*	0.5	*19*	0	----

[1] analyte not detected

Table 14. Effect of six months of **caging** on median values of hemolymph plasma biochemistry parameters of snow crab collected from **Cheticamp, NS** in June 2013. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) changes were noted are shown.

Analyte	PM					LM					MF				
	n	Trap	*n*	Cage	*p*	*n*	Trap	n	Cage	*p*	n	Trap	*n*	Cage	*p*
Sodium (mmol/L)	*16*	441	*17*	462	*0.0003*	*19*	456	*18*	438	*0.0000*	*20*	439.5	*17*	459	*0.0000*
Potassium (mmol/L)	*16*	11.4	*17*	12.3	---	*19*	11.1	*18*	10.8	----	*20*	1.035	*17*	11.7	*0.000*
Na:K	*16*	37.5	*17*	38	---	*19*	41	*18*	41	---	*20*	42	*17*	38	*0.0011*
Chloride (mmol/L)	*16*	458.5	*17*	489	*0.0000*	*19*	456	*18*	453	----	*20*	465	*17*	501	*0.0000*
Calcium (mmol/L)	*20*	11.76	*18*	12.73	*0.0000*	*20*	12.275	*18*	13.14	*0.0001*	*20*	11.66	*19*	12.55	*0.0002*
Phosphorus (mmol/L)	*20*	1.765	*18*	1.91	----	*20*	1.505	*18*	1.815	----	*20*	1.59	*19*	1.57	----
Magnesium (mmol/L)	*20*	38.635	*18*	41.16	*0.0000*	*20*	37.04	*18*	40.21	*0.0000*	*20*	40.845	*19*	42.83	*0.0000*
Urea (mmol/L)	*20*	0.3	*18*	0.2	*0.0003*	*20*	0.3	*18*	0.25	----	*20*	0.3	*19*	0.2	*0.0117*
Uric Acid (µmol/L)	*20*	101.5	*18*	57	*0.0001*	*20*	45.5	*18*	33.5	----	*20*	59	*19*	56	----
Total Protein (g/L)	*20*	33	*18*	17	*0.0045*	*20*	32.5	*18*	28	*0.0222*	*20*	28	*19*	16	*0.0000*
Albumin (g/L)	*20*	8	*18*	6	----	*20*	9	*18*	6.5	*0.0140*	*20*	7	*19*	6	*0.0001*
Globulin (g/L)	*20*	24.5	*18*	11.5	*0.0038*	*20*	23.5	*18*	21.5	*0.0385*	*20*	21.5	*19*	11	*0.0000*
A:G	*20*	0.33	*18*	0.495	*0.0006*	*20*	0.34	*18*	0.33	----	*20*	0.31	*19*	0.43	*0.0000*
Cholesterol (mmol/L)	*20*	0.255	*18*	0.145	*0.0054*	*20*	0.25	*18*	0.24	----	*20*	0.17	*19*	0.06	*0.0000*
Triglyceride (mmol/L)	*20*	0.05	*18*	0.03	*0.0040*	*20*	0.045	*18*	0.035	----	*20*	0.12	*19*	0.03	*0.0000*
Glucose (mmol/L)	*20*	0.95	*18*	0.5	----	*20*	0.85	*18*	0.95	----	*20*	0.9	*19*	0.4	*0.0000*
Lactate (mmol/L)	*20*	4.255	*18*	3.225	*0.0193*	*20*	2.26	*18*	2.865	*0.0537*	*20*	2.895	*19*	1.25	*0.0004*
Creatinine (µmol/L)	*20*	[1].	*18*	.		*20*	.	*18*	.		*20*	.	*19*	.	
Amylase (U/L)	*20*	7	*18*	6	----	*20*	4	*18*	5	----	*20*	6	*19*	6	----
Lipase (U/L)	*20*	7.5	*18*	8	----	*20*	4.5	*18*	8	*0.0012*	*20*	6	*19*	9	*0.0258*
AST (U/L)	*20*	64.5	*18*	39.5	----	*20*	17.5	*18*	24.5	----	*20*	28.5	*19*	20	----
ALT (U/L)	*20*	26.5	*18*	24	----	*20*	11	*18*	14.5	----	*20*	22.5	*19*	12	*0.0387*
GD (U/L)	*20*	13	*18*	8.5	*0.0206*	*20*	14	*18*	10.5	----	*20*	12.5	*19*	6	*0.0000*
SDH (U/L)	*20*	0	*18*	0	----	*20*	0	*18*	0	----	*20*	0	*19*	0	----
ALP (U/L)	*20*	0	*18*	0	----	*20*	0	*18*	0	.	*20*	0	*19*	0	.
GGT (U/L)	*20*	0	*18*	0	----	*20*	0	*18*	0	----	*20*	0	*19*	0	----

[1] analyte not detected

Table 15. Summary of median values of hemolymph plasma biochemistry parameters of snow crab collected from Margaree Harbor, NS in November 2012 by traps and after 6 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Median values sharing the same superscript are not different (Wilcoxon rank sum, Bonferroni-adjusted $p > 0.017$ from each other; for analytes where no superscripts are shown, no differences were detected among three crab types (Kruskall-Wallis testing, $p < 0.05$).

Analyte	Margaree Harbor											
	Free						Caged					
	n	PM	*n*	LM	*n*	MF	*n*	PM	*n*	LM	*n*	MF
Sodium (mmol/L)	*19*	441	*20*	456	*20*	454.5	*16*	[b] 445.5	*15*	468	*19*	[b]453
Potassium (mmol/L)	*19*	[a]10.8	*20*	[ac]10.5	*20*	[c]10.2	*16*	10.8	*15*	10.5	*19*	11.4
Na:K	*19*	40	*20*	[c]44.5	*20*	[c]43.5	*16*	40.5	*15*	[c]44	*19*	[c]40
Chloride (mmol/L)	*19*	[a]453	*20*	[ac]462	*20*	[c]480	*16*	463.5	*15*	480	*19*	480
Calcium (mmol/L)	*20*	12.11	*20*	11.99	*20*	11.655	*17*	11.63	*16*	11.885	*19*	11.57
Phosphorus (mmol/L)	*20*	1.675	*20*	[c]1.11	*20*	[c]1.15	*18*	1.69	*16*	1.46	*19*	1.5
Magnesium (mmol/L)	*20*	[a]38.375	*20*	[c]36.135	*20*	[b]41.90	*17*	[a]37.06	*16*	[c]35.765	*19*	[b]40.25
Urea (mmol/L)	*20*	[b]0.3	*20*	[c]0.2	*20*	[bc]0.3	*18*	[b]0.2	*16*	[c]0.1	*19*	[bc]0.2
Uric Acid (µmol/L)	*20*	[a]57	*20*	[c]20	*20*	[b]41	*18*	[b]29.5	*16*	19.5	*19*	[b]38
Total Protein (g/L)	*20*	[b]39	*20*	[c]30.5	*20*	[bc]28	*18*	30	*16*	24	*19*	21
Albumin (g/L)	*20*	[a]8.5	*20*	[ac]6	*20*	[c]6	*18*	7.5	*16*	6	*19*	5
Globulin (g/L)	*20*	[b]31	*20*	[c]24	*20*	[bc]22	*18*	23.5	*16*	18	*19*	15
A:G	*20*	0.27	*20*	0.29	*20*	0.27	*18*	0.33	*16*	0.36	*19*	0.33
Cholesterol (mmol/L)	*20*	[a]0.33	*20*	[ac]0.305	*20*	[c]0.20	*18*	[a]0.165	*16*	[a]0.17	*19*	0.09
Triglyceride mmol/L)	*20*	[a]0.04	*20*	[a]0.03	*20*	0.10	*18*	0.02	*16*	0.02	*19*	0.03
Glucose (mmol/L)	*20*	1.4	*20*	[c]0.8	*20*	[c]0.9	*18*	[a]0.7	*16*	[a]0.75	*19*	0.5
Lactate (mmol/L)	*20*	3.985	*20*	[c]1.035	*20*	[c]1.725	*18*	[a]1.98	*16*	[ac]1.50	*19*	[c]1.29
Creatinine (mmol/L)	*20*	[1] .	*20*	.	*20*	.	*18*	.	*16*	.	*19*	.
Amylase (U/L)	*20*	5	*20*	5.5	*20*	6	*18*	5.5	*16*	5	*19*	5
Lipase (U/L)	*20*	[ab]11	*20*	[a]9.5	*20*	[b]13.5	*18*	11	*16*	9	*19*	11
AST (U/L)	*20*	[b]28	*20*	[c]5.5	*20*	[bc]18.5	*18*	[b]34	*16*	[c]9.5	*19*	[bc]16
ALT (U/L)	*20*	[ab]18	*20*	8.5	*20*	[b]16	*18*	[b]17	*16*	[c]10.5	*19*	[bc]13
GD (U/L)	*20*	14.5	*20*	14.5	*20*	12	*18*	12.5	*16*	9.5	*19*	10
SDH (U/L)	*20*	0	*20*	0	*20*	0	*18*	[a]1	*16*	[ac]0	*19*	[c]0
ALP (U/L)	*20*	0	*20*	0	*20*	0	*18*	[a]0	*16*	[a]0	*19*	0
GGT (U/L)	*20*	1	*20*	0	*20*	0.5	*18*	0	*16*	0	*19*	0

[1] analyte not detected

Table 16. Summary of median values of hemolymph plasma biochemistry parameters of snow crab collected from Cheticamp, NS in November 2012 by traps and after 6 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Median values sharing the same superscript are not different (Wilcoxon rank sum, Bonferroni-adjusted $p > 0.017$ from each other; for analytes where no superscripts are shown, no differences were detected among three crab types (Kruskall-Wallis testing, $p < 0.05$)

Analyte	Cheticamp											
	Free						Caged					
	n	PM	*n*	LM	*n*	MF	*n*	PM	*n*	LM	*n*	MF
Sodium (mmol/L)	*16*	^{ab}441	*19*	^{c}456	*20*	^{b}439.5	*17*	^{ab}462	*18*	^{c}438	*17*	^{b}459
Potassium (mmol/L)	*16*	^{ab}11.4	*19*	^{ac}11.1	*20*	^{c}10.35	*17*	^{ab}12.3	*18*	^{c}10.8	*17*	^{b}11.7
Na:K	*16*	^{ab}37.5	*19*	^{a}41	*20*	^{c}42	*17*	38	*18*	41	*17*	38
Chloride (mmol/L)	*16*	458.5	*19*	456	*20*	465	*17*	^{ab}489	*18*	^{c}453	*17*	^{b}501
Calcium (mmol/L)	*20*	^{ab}11.76	*20*	^{c}12.275	*20*	^{b}11.66	*18*	^{ab}12.73	*18*	^{ac}13.14	*19*	^{b}12.55
Phosphorus (mmol/L)	*20*	^{ab}1.765	*20*	^{c}1.505	*20*	^{bc}1.59	*18*	^{ab}1.91	*18*	^{ac}1.815	*19*	^{c}1.57
Magnesium (mmol/L)	*20*	^{ab}38.635	*20*	^{c}37.04	*20*	40.845	*18*	^{ab}41.16	*18*	^{ac}40.21	*19*	42.83
Urea (mmol/L)	*20*	^{ab}0.3	*20*	^{c}0.3	*20*	^{bc}0.3	*18*	0.2	*18*	0.25	*19*	0.2
Uric Acid (µmol/L)	*20*	^{ab}101.5	*20*	^{c}45.5	*20*	59	*18*	^{ab}57	*18*	^{c}33.5	*19*	^{b}56
Total Protein (g/L)	*20*	33	*20*	32.5	*20*	28	*18*	^{ab}17	*18*	^{ac}28	*19*	^{b}16
Albumin (g/L)	*20*	8	*20*	9	*20*	7	*18*	^{ab}6	*18*	^{ac}6.5	*19*	^{b}6
Globulin (g/L)	*20*	24.5	*20*	23.5	*20*	21.5	*18*	^{ab}11.5	*18*	^{ac}21.5	*19*	^{b}11
A:G	*20*	0.33	*20*	0.34	*20*	0.31	*18*	^{ab}0.495	*18*	^{c}0.33	*19*	^{b}0.43
Cholesterol (mmol/L)	*20*	^{ab}0.255	*20*	^{ac}0.25	*20*	^{b}0.17	*18*	^{ab}0.145	*18*	^{c}0.24	*19*	0.06
Triglyceride mmol/L)	*20*	^{ab}0.05	*20*	^{ac}0.045	*20*	0.12	*18*	0.03	*18*	0.035	*19*	0.03
Glucose (mmol/L)	*20*	0.95	*20*	0.85	*20*	0.9	*18*	^{ab}0.5	*18*	^{ac}0.95	*19*	^{b}0.4
Lactate (mmol/L)	*20*	^{ab}4.255	*20*	^{c}2.26	*20*	^{c}2.895	*18*	^{ab}3.225	*18*	^{ac}2.865	*19*	1.25
Creatinine (mmol/L)	*20*	[1].	*20*	.	*20*	.	*18*	.	*18*	.	*19*	.
Amylase (U/L)	*20*	^{ab}7	*20*	^{b}4	*20*	^{bc}6	*18*	6	*18*	5	*19*	6
Lipase (U/L)	*20*	7.5	*20*	4.5	*20*	6	*18*	8	*18*	8	*19*	9
AST (U/L)	*20*	^{ab}64.5	*20*	^{c}17.5	*20*	^{bc}28.5	*18*	^{ab}39.5	*18*	^{ac}24.5	*19*	^{c}20
ALT (U/L)	*20*	^{ab}26.5	*20*	^{c}11	*20*	^{b}22.5	*18*	^{ab}24	*18*	^{ac}14.5	*19*	^{c}12
GD (U/L)	*20*	13	*20*	14	*20*	12.5	*18*	^{ab}8.5	*18*	^{ac}10.5	*19*	^{b}6
SDH (U/L)	*20*	0	*20*	0	*20*	0	*18*	0	*18*	0	*19*	0
ALP (U/L)	*20*	0	*20*	0	*20*	0	*18*	0	*18*	0	*19*	0
GGT (U/L)	*20*	0	*20*	0	*20*	0	*18*	0	*18*	0	*19*	0

[1] analyte not detected

Table 17. Comparison between Margaree Harbor, NS and Cheticamp, NS, of median values of hemolymph plasma biochemistry parameters for snow crab collected by trapsg (free) in June 2013. Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) differences were detected are shown.

Analyte	Free Crabs													
	PM					LM					MF			
	n	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*
Sodium (mmol/L)	*19*	441	*16*	441	----	*20*	456	*19*	456	----	*20*	454.5	*20*	439.5	*0.0054*
Potassium (mmol/L)	*19*	10.8	*16*	11.4	*0.0154*	*20*	10.5	*19*	11.1	----	*20*	10.2	*20*	10.35	----
Na:K	*19*	40	*16*	37.5	*0.0065*	*20*	44.5	*19*	41	*0.0282*	*20*	43.5	*20*	42	*0.0083*
Chloride (mmol/L)	*19*	453	*16*	458.5	----	*20*	462	*19*	456	----	*20*	480	*20*	465	*0.0040*
Calcium (mmol/L)	*20*	12.11	*20*	11.76	*0.0360*	*20*	11.99	*20*	12.275	*0.0006*	*20*	11.655	*20*	11.66	----
Phosphorus (mmol/L)	*20*	1.675	*20*	1.765	----	*20*	1.11	*20*	1.505	*0.0077*	*20*	1.15	*20*	1.59	*0.0001*
Magnesium (mmol/L)	*20*	38.375	*20*	38.635	----	*20*	36.135	*20*	37.04	*0.0021*	*20*	41.90	*20*	40.845	*0.0035*
Urea (mmol/L)	*20*	0.3	*20*	0.3	----	*20*	0.2	*20*	0.3	*0.0230*	*20*	0.3	*20*	0.3	----
Uric Acid (µmol/L)	*20*	57	*20*	101.5	*0.0000*	*20*	20	*20*	45.5	*0.0000*	*20*	41	*20*	59	*0.0004*
Total Protein (g/L)	*20*	39	*20*	33	----	*20*	30.5	*20*	32.5	----	*20*	28	*20*	28	----
Albumin (g/L)	*20*	8.5	*20*	8	----	*20*	6	*20*	9	*0.0147*	*20*	6	*20*	7	----
Globulin (g/L)	*20*	31	*20*	24.5	----	*20*	24	*20*	23.5	----	*20*	22	*20*	21.5	----
A:G	*20*	0.27	*20*	0.33	*0.0004*	*20*	0.29	*20*	0.34	*0.0134*	*20*	0.27	*20*	0.31	*0.0208*
Cholesterol (mmol/L)	*20*	0.33	*20*	0.255	*0.0436*	*20*	0.305	*20*	0.25	----	*20*	0.20	*20*	0.17	----
Triglyceride mmol/L)	*20*	0.04	*20*	0.05	----	*20*	0.03	*20*	0.045	*0.0303*	*20*	0.10	*20*	0.12	----
Glucose (mmol/L)	*20*	1.4	*20*	0.95	*0.0242*	*20*	0.8	*20*	0.85	----	*20*	0.9	*20*	0.9	----
Lactate (mmol/L)	*20*	3.985	*20*	4.255	----	*20*	1.035	*20*	2.26	*0.0023*	*20*	1.725	*20*	2.895	*0.0004*
Creatinine (µmol/L)	*20*	[1].	*20*	.		*20*	.	*20*	.		*20*	.	*20*	.	
Amylase (U/L)	*20*	5	*20*	7	----	*20*	5.5	*20*	4	----	*20*	6	*20*	6	----
Lipase (U/L)	*20*	11	*20*	7.5	*0.0151*	*20*	9.5	*20*	4.5	*0.0001*	*20*	13.5	*20*	6	*0.0001*
AST (U/L)	*20*	28	*20*	64.5	*0.0068*	*20*	5.5	*20*	17.5	----	*20*	18.5	*20*	28.5	----
ALT (U/L)	*20*	18	*20*	26.5	*0.0114*	*20*	8.5	*20*	11	----	*20*	16	*20*	22.5	----
GD (U/L)	*20*	14.5	*20*	13	----	*20*	14.5	*20*	14	----	*20*	12	*20*	12.5	----
SDH (U/L)	*20*	0	*20*	0	----	*20*	0	*20*	0	----	*20*	0	*20*	0	----
ALP (U/L)	*20*	0	*20*	0	----	*20*	0	*20*	0	.	*20*	0	*20*	0	.
GGT (U/L)	*20*	1	*20*	0	*0.0000*	*20*	0	*20*	0	*0.0018*	*20*	0.5	*20*	0	*0.0016*

[1] analyte not detected

Table 18. Comparison between Margaree Harbor, NS and Cheticamp, NS, of median values of hemolymph plasma biochemistry parameters for snow crab held in cages from November 2012 – June 2013. Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) differences were detected are shown.

Analyte	Caged Crabs													
	PM					LM					MF			
	n	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*
Sodium (mmol/L)	*16*	445.5	*17*	462	*0.0022*	*15*	468	*18*	438	*0.0000*	*19*	453	*17*	459	*0.0062*
Potassium (mmol/L)	*16*	10.8	*17*	12.3	*0.0214*	*15*	10.5	*18*	10.8	----	*19*	11.4	*17*	11.7	*0.0029*
Na:K	*16*	40.5	*17*	38	----	*15*	44	*18*	41	*0.0437*	*19*	40	*17*	38	*0.0185*
Chloride (mmol/L)	*16*	463.5	*17*	489	*0.0015*	*15*	480	*18*	453	*0.0001*	*19*	480	*17*	501	*0.0019*
Calcium (mmol/L)	*17*	11.63	*18*	12.73	*0.0000*	*16*	11.885	*18*	13.14	*0.0000*	*19*	11.57	*19*	12.55	*0.0000*
Phosphorus (mmol/L)	*18*	1.69	*18*	1.91	----	*16*	1.46	*18*	1.815	*0.0101*	*19*	1.50	*19*	1.57	----
Magnesium (mmol/L)	*17*	37.06	*18*	41.16	*0.0000*	*16*	35.765	*18*	40.21	*0.0000*	*19*	40.25	*19*	42.83	*0.0000*
Urea (mmol/L)	*18*	0.2	*18*	0.2	----	*16*	0.1	*18*	0.25	*0.0003*	*19*	0.2	*19*	0.2	*0.0247*
Uric Acid (µmol/L)	*18*	29.5	*18*	57	*0.0007*	*16*	19.5	*18*	33.5	*0.0003*	*19*	38	*19*	56	*0.0009*
Total Protein (g/L)	*18*	30	*18*	17	*0.0425*	*16*	24	*18*	28	----	*19*	21	*19*	16	----
Albumin (g/L)	*18*	7.5	*18*	6	----	*16*	6	*18*	6.5	----	*19*	5	*19*	6	----
Globulin (g/L)	*18*	23.5	*18*	11.5	*0.0267*	*16*	18	*18*	21.5	----	*19*	15	*19*	11	*0.0365*
A:G	*18*	0.33	*18*	0.495	*0.0018*	*16*	0.36	*18*	0.33	----	*19*	0.33	*19*	0.43	*0.0026*
Cholesterol (mmol/L)	*18*	0.165	*18*	0.145	----	*16*	0.17	*18*	0.24	*0.0469*	*19*	0.09	*19*	0.06	----
Triglyceride mmol/L)	*18*	0.02	*18*	0.03	----	*16*	0.02	*18*	0.035	*0.0199*	*19*	0.03	*19*	0.03	----
Glucose (mmol/L)	*18*	0.7	*18*	0.5	----	*16*	0.75	*18*	0.95	----	*19*	0.5	*19*	0.4	----
Lactate (mmol/L)	*18*	1.98	*18*	3.225	----	*16*	1.50	*18*	2.865	*0.0009*	*19*	1.29	*19*	1.25	----
Creatinine (µmol/L)	*18*	.[1]	*18*	.		*16*	.	*18*	.		*19*	.	*19*	.	
Amylase (U/L)	*18*	5.5	*18*	6	----	*16*	5	*18*	5	----	*19*	5	*19*	6	----
Lipase (U/L)	*18*	11	*18*	8	*0.0389*	*16*	9	*18*	8	----	*19*	11	*19*	9	----
AST (U/L)	*18*	34	*18*	39.5	----	*16*	9.5	*18*	24.5	*0.0057*	*19*	16	*19*	20	----
ALT (U/L)	*18*	17	*18*	24	----	*16*	10.5	*18*	14.5	*0.0179*	*19*	13	*19*	12	----
GD (U/L)	*18*	12.5	*18*	8.5	*0.0456*	*16*	9.5	*18*	10.5	----	*19*	10	*19*	6	*0.0112*
SDH (U/L)	*18*	1	*18*	0	*0.0021*	*16*	0	*18*	0	*0.0339*	*19*	0	*19*	0	----
ALP (U/L)	*18*	0	*18*	0	----	*16*	0	*18*	0	.	*19*	0	*19*	0	.
GGT (U/L)	*18*	0	*18*	0	*0.0177*	*16*	0	*18*	0	*0.0261*	*19*	0	*19*	0	*0.0084*

[1] analyte not detected

B. Hepatopancreas Lipid Content

The average HP lipid content was calculated as gram per gram of dry hepatopancreas weight and converted to percent dry weight to standardise comparison to other components and data from fall 2012 study (see Section VIII-1). Average lipid content was also converted to percent lipid as a proportion of total wet weight using percent moisture data which was available for all hepatopancreas tissue.
Box plots (Figure 1) and frequency distribution histograms (Figure 2) show four outliers (#287B, 226B, 104B, and 165B) which were all MF crabs – one from each combination of station and treatment. Crab #287B also had high values for cholesterol and triglyceride. Examination of the original data showed all three replicates of lipid determination to be similar; except for #165B where only one value was available due to limited amounts of tissue (B. Forward, pers. Comm.). Outliers were excluded from regression analyses.

As variation in carapace width was greater than anticipated for PM in the November 2012 samples, scatterplots showing HP lipid vs. carapace width for each category of crab were generated for these spring 2013 crabs of which caged crabs would have been collected in November 2012 (Figure 3). No pattern of percent HP lipid content to CW was noted. The distribution of CW for PM in free crabs was similar to MF crabs at both sites; while the range of CW for caged PM crabs was larger than for free PM crabs. Summary statistics for average %HP $lipid_{dry}$ by category and station are provided in Table 19.

Effect of Caging (Treatment) across Stations by Sex

Comparison by treatment across stations found differences in median %HP $lipid_{dry\ wt}$ for MF crabs only where $MF_{caged} < MF_{Free}$ at both Margaree Harbor ($p = 0.0193$) and Cheticamp ($p = 0.0031$).

Effect of Sex within a Station by Treatment (Caged or Free)

Significant differences among median %HP $lipid_{dry\ wt}$ were detected (Kruskal-Wallis testing) across crab categories for both free and caged crabs in both Margaree Harbor and Cheticamp ($p = 0.0204$, Margaree Harbor free; $p = 0.0470$, Margaree Harbor caged; $p = 0.0018$, Cheticamp free; $p = 0.0018$, Cheticamp caged). Subsequent Wilcoxon testing, at Bonferroni-adjusted p-value of 0.0167, identified median %HP $lipid_{dry\ wt}$ in free $PM_{Margaree} <$ free $LM_{Margaree}$ ($p = 0.0156$); free $PM_{Cheticamp}$ ($p = 0.0041$) and $MF_{Cheticamp}$ ($p = 0.0015$) < $LM_{Cheticamp}$; caged $MF_{Margaree} < LM_{Margaree}$ ($p = 0.0090$); and, caged $MF_{Cheticamp} < LM_{Cheticamp}$ ($p = 0.0006$). Summary statistics are presented in Tables 19 and 20

Effect of Station within a Treatment by Sex

Comparison (Wilcoxon signed rank testing) of average %HP lipid$_{\text{dry wt}}$ by sex and collection method combinations across stations only found a significant difference for free $MF_{\text{Margaree}} > MF_{\text{Cheticamp}}$ ($p = 0.0380$).

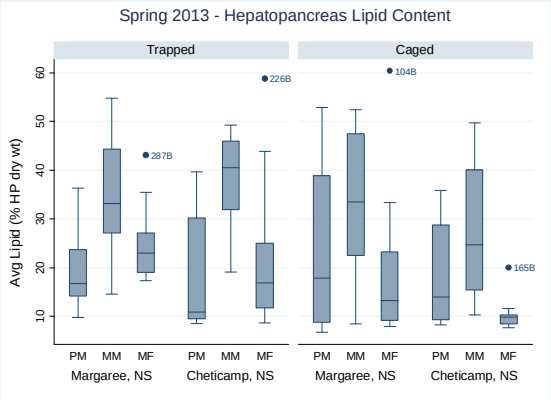


Figure 1. Box and Whisker plot showing distribution of average hepatopancreas lipid (as % HP dry weight) for Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF) snow crab collected by trapping or after six months of caging, at two stations in CFA 19, June 2013.

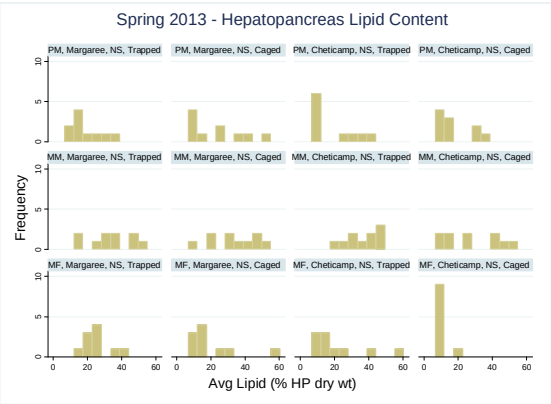


Figure 2. Frequency distribution histogram showing average HP lipid (%HP dry wt) for Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF) snow crab collected by trapping or after six months of caging, at two stations in CFA 19, June 2013.

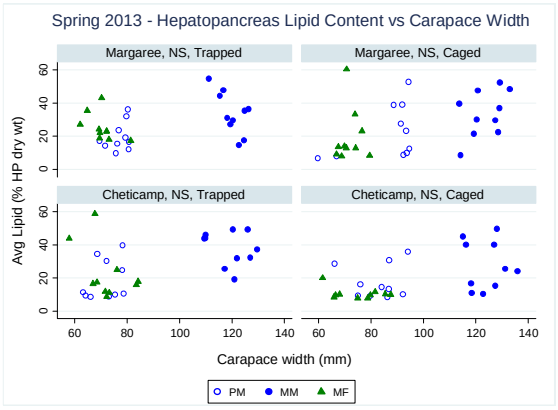


Figure 3. Scatterplot showing average HP lipid (% HP dry wt) by carapace width for Pygmy Male (PM), LargeMature Male (MM), and Mature Female (MF) snow crab collected by trapping or after six months of caging, at two stations in CFA 19, June 2013.

Table 19. Summary statistics for average hepatopancreas lipid content (% HP dry wt) for free-ranging snow crabs collected by traps at two stations in CFA 19, Spring (June) 2013. Different superscripts represent significant differences in median values within (letters) or between (numbers) stations, by crab category.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	PM	10	19.5795	8.6372	16.7488$^{a,b;1}$	9.7427	36.2959	2.5854	0.9036
	LM	10	33.8380	12.7538	33.1617$^{c;2}$	14.5426	54.7619	2.1212	0.0588
	MF	10	25.2319	8.1799	23.0160$^{b,c;3}$	17.3138	43.1084	3.3470	1.2025
Cheticamp	PM	10	18.7117	12.2722	10.8415$^{a,b;1}$	8.5388	39.6890	1.7375	0.6597
	LM	10	37.8069	10.4075	40.4918$^{c;2}$	19.0971	49.2880	2.0046	-0.5035
	MF	10	22.6631	16.1379	16.8691^{b}	8.6684	58.8031	3.6070	1.4010

Table 20. Summary statistics for average hepatopancreas lipid content (% HP dry wt) for snow crabs at two stations in CFA 19, collected in Spring (June) 2013 after a six month caging period. Different superscripts represent significant differences in median values within (letters) or between (numbers) stations, by crab category.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	PM	10	22.7444	16.3207	17.8801^{a,b}	6.7194	52.8516	2.0015	0.6083
	LM	10	33.6760	13.9106	33.4573^{a,c}	8.4695	52.4103	2.1364	-0.2942
	MF	10	19.5476	16.3325	13.2322^{b}	7.9075	60.4136	4.9928	1.7684
Cheticamp	PM	10	17.6070	10.2535	13.9302^{a,b}	8.2443	35.8463	1.9816	0.7782
	LM	10	27.7731	14.7828	24.7157^{a,c}	10.3213	49.7117	1.4985	0.2192
	MF	10	10.5162	3.5345	9.8079^{b}	7.7055	19.9612	6.4112	2.0919

Spearman rank correlation coefficients (*rho*) were calculated for average HP Lipid and all 24 directly measured hemolymph biochemistry parameters for all crabs, crabs by station, sex, and collection method (Tables 21 – 29). Similar to free fall (November) 2012 crabs, the best correlations with hepatopancreas lipid were observed for the 'metabolites' category. Correlations were better for free than caged crabs overall.

Separating crabs by treatment (caged, free) often caused correlation coefficients to increase or decrease in significance compared to the combined values. Hemolymph total protein concentration consistently had good values for *rho* for all crabs, caged or free, at either station except for free LM crabs from Margaree Harbor, while correlations for other protein indices albumin and globulin were inconsistent. Cholesterol concentrations were well-correlated for free PM and MF crabs from Margaree Harbor and free PM from Cheticamp. Hemolymph glucose for all Margaree Harbor free crabs was well-correlated to %HP lipid $_{\text{dry wt}}$. Remaining biochemistry parameters were variably correlated to %HP lipid $_{\text{dry wt}}$; overall, correlations tended to be higher for Margaree Harbor. Relationships are shown graphically as scatterplots in Figures 4 – 9 for total protein, albumin, globulin, cholesterol, triglyceride, and glucose, respectively.

Regression analysis, simple and multiple, was completed for %HP lipid $_{\text{dry wt}}$ (average of triplicate values) for each of the six hemolymph biochemistry parameters, by sex, station, and collection method (Tables 30-33). Carapace width was considered a possible factor in the fall 2012 samples (see Section VII-1). As caged crabs would have been collected in the fall 2012 study, CW was added into the multiple regression equations separately (Tables 32 & 33).

Results of the simple regressions were inconsistent, with no one biochemistry parameter having an R^2 value greater than 0.5 for all sexes at both sites or either treatment (caged or free), nor was any pattern visible. Good fit was most often noted for PM crabs, followed by MF crabs. The highest R^2 values were seen for free $PM_{Margaree}$ for triglyceride (0.8323), free $PM_{Cheticamp}$ for cholesterol (0.7786), and caged $LM_{Cheticamp}$ for total protein (0.7859) and globulin (0.7188).

The use of multiple linear regression resulted in adjusted R^2 values for free $PM_{Margaree}$ (0.8719) and $MF_{Margaree}$ (0.8819). For consistency and ease of comparison to the fall 2012 study, the same four variables were used for the multiple regression analyses. Inclusion of CW in the regression equations had a minimal negative effect on adjusted R^2 values. Caged $MF_{Margaree}$ had an adjusted R^2 of 0.7215 which exceeded that for any parameter in simple regression. The addition of CW to multiple regression equation for caged Margaree crabs improved adjusted R^2 values for all sexes, despite very low co-efficients for CW. There was no improvement in adjusted R^2 for any caged crabs from Cheticamp, with or without the addition of CW when compared to simple regression.

Table 21. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, June, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Sodium	---	---	---	---	---	---	---	---	---	-0.4502
n	---	---	---	---	---	---	---	---	---	20
p	---	---	---	---	---	---	---	---	---	*0.0464*
Potassium	-0.4791	-0.5811	-0.5334	-0.5613	---	---	---	-0.5852	---	-0.7153
n	118	40	20	20	---	---	---	40	---	20
p	*0.0000*	*0.0001*	*0.0154*	*0.0100*	---	---	---	*0.0001*	---	*0.0004*
Na:K	0.5211	0.4791	0.4578	0.4638	---	---	0.5755	0.6461	0.5427	0.7099
n	118	40	20	20	---	---	19	40	20	20
p	*0.0000*	*0.0018*	*0.0424*	*0.0394*	---	---	*0.0099*	*0.0000*	*0.0134*	*0.0005*
Chloride	-0.2952	-0.4205	---	---	---	---	---	-0.3567	---	-0.5865
n	118	40	---	---	---	---	---	40	---	20
p	*0.0012*	*0.0069*	---	---	---	---	---	*0.0238*	---	*0.0066*
Calcium	0.2792	0.3831	0.5055	---	---	---	---	---	---	---
n	119	39	19	---	---	---	---	---	---	---
p	*0.0021*	*0.0161*	*0.0273*	---	---	---	---	---	---	---
Phosphorus	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Magnesium	-0.3097	---	---	---	---	---	---	---	---	---
n	119	---	---	---	---	---	---	---	---	---
p	*0.0006*	---	---	---	---	---	---	---	---	---

[1.] Margaree Harbor, NS, [2] Cheticamp, NS

Table 22. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Margaree Harbor - HP Lipid, Spring 2013

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Trap	Cage	All	Trap	Cage	All	Trap	Cage
Sodium	---	---	---	- 0.9133	---	---	---	---	---	---
n	---	---	---	10	---	---	---	---	---	---
p	---	---	---	*0.0002*	---	---	---	---	---	---
Potassium	- 0.4791	- 0.5334	---	---	---	---	---	---	---	---
n	118	20	---	---	---	---	---	---	---	---
p	*0.0000*	*0.0154*	---	---	---	---	---	---	---	---
Na:K	0.5211	0.4578	---	---	---	---	---	0.5427	---	---
n	118	20	---	---	---	---	---	20	---	---
p	*0.0000*	*0.0424*	---	---	---	---	---	*0.0134*	---	---
Chloride	- 0.2952	---	---	- 0.7012	---	---	---	---	---	---
n	118	---	---	10	---	---	---	---	---	---
p	*0.0012*	---	---	*0.0239*	---	---	---	---	---	---
Calcium	0.2792	0.5055	0.6687	---	---	---	---	---	---	---
n	119	19	10	---	---	---	---	---	---	---
p	*0.0021*	*0.0273*	*0.0345*	---	---	---	---	---	---	---
Phosphorus	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Magnesium	- 0.3097	---	---	---	---	---	---	---	---	---
n	119	---	---	---	---	---	---	---	---	---
p	*0.0006*	---	---	---	---	---	---	---	---	---

Table 23. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

	Cheticamp- HP Lipid, Spring 2013									
Analyte	**All Crabs**	**Pygmy Male**			**Large Mature Male**			**Mature Female**		
		All	**Trap**	**Cage**	**All**	**Trap**	**Cage**	**All**	**Trap**	**Cage**
Sodium	---	---	---	---	---	---	---	-0.4502	---	---
n	---	---	---	---	---	---	---	20	---	---
p	---	---	---	---	---	---	---	*0.0464*	---	---
Potassium	-0.4791	-0.5613	-0.6647	---	---	---	-0.7230	-0.7153	---	---
n	118	20	10	---	---	---	10	20	---	---
p	*0.0000*	*0.0100*	*0.0360*	---	---	---	*0.0079*	*0.0004*	---	---
Na:K	0.5211	0.4638	---	---	0.5755	---	0.7792	0.7099	---	---
n	118	20	---	---	19	---	10	20	---	---
p	*0.0000*	*0.0394*	---	---	*0.0099*	---	*0.0079*	*0.0005*	---	---
Chloride	-0.2952	---	---	---	---	---	---	-0.5865	---	---
n	118	---	---	---	---	---	---	20	---	---
p	*0.0012*	---	---	---	---	---	---	*0.0066*	---	---
Calcium	0.2792	---	---	0.7212	---	---	---	---	---	---
n	119	---	---	10	---	---	---	---	---	---
p	*0.0021*	---	---	*0.0010*	---	---	---	---	---	---
Phosphorus	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Magnesium	-0.3097	---	---	---	---	---	0.8182	---	---	---
n	119	---	---	---	---	---	10	---	---	---
p	*0.0006*	---	---	---	---	---	*0.0038*	---	---	---

Table 24. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas lipid content (%dry wt) of free and caged snow crab collected from two stations in CFA 19, June, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Urea	---	---	---	---	---	---	---	---	0.4919	---
n	---	---	---	---	---	---	---	---	20	---
p	---	---	---	---	---	---	---	---	*0.0276*	---
Uric Acid	-0.3036	-0.2638	---	---	---	-0.5234	---	---	---	---
n	120	40	---	---	---	20	---	---	---	---
p	*0.0007*	*0.1000*	---	---	---	*0.0179*	---	---	---	---
Total Protein	0.6290	0.7331	0.7366	0.6353	0.4349	0.4112	0.4970	0.7548	0.7568	0.7420
n	120	40	20	20	40	20	20	40	20	20
p	*0.0000*	*0.0000*	*0.0002*	*0.0026*	*0.0050*	*0.0717*	*0.0258*	*0.0000*	*0.0001*	*0.0002*
Albumin	0.6070	0.7191	0.7772	0.6184	0.4897	0.4519	0.5472	0.6456	0.7082	0.7103
n	120	40	20	20	40	20	20	40	20	20
p	*0.0000*	*0.0000*	*0.0001*	*0.0037*	*0.0013*	*0.0455*	*0.0125*	*0.0000*	*0.0005*	*0.0004*
Globulin	0.6126	0.7168	0.6950	0.6266	0.3502	---	---	0.7632	0.7585	0.7380
n	120	40	20	20	40	---	---	40	20	20
p	*0.0000*	*0.0000*	*0.0007*	*0.0031*	*0.0267*	---	---	*0.0000*	*0.0001*	*0.0002*
A:G	-0.3806	-0.4506	---	---	---	---	---	-0.7335	-0.7722	-0.7144
n	120	40	---	---	---	---	---	40	20	200
p	*0.0000*	*0.0035*	---	---	---	---	---	*0.0000*	*0.0001*	*0.0004*
Cholesterol	0.6370	0.6448	0.5004	0.6533	0.3769	---	0.5059	0.6954	0.7475	0.6007
n	120	40	20	20	40	---	20	40	20	20
p	*0.0000*	*0.0000*	*0.0246*	*0.0018*	*0.0165*	---	*0.0229*	*0.0000*	*0.0002*	0.0051
Triglyceride	0.3299	0.3534	0.6601	---	0.5351	0.5370	0.5617	0.6625	0.7026	0.7262
n	120	40	20	---	40	20	20	40	20	20
p	*0.0002*	*0.0253*	*0.0015*	---	*0.0004*	*0.0146*	*0.0100*	*0.0000*	*0.0006*	0.0003
Glucose	0.5625	0.5698	0.5865	0.4673	0.4208	0.5144	---	0.7219	0.7487	0.7377
n	120	40	20	20	40	20	---	40	20	20
p	*0.0000*	*0.0001*	*0.0066*	*0.0378*	*0.0069*	*0.0203*	---	*0.0000*	*0.0001*	0.0002
Lactate	0.1855	0.5160	---	---	---	0.5100	---	0.4559	0.5820	0.4932
n	120	40	---	---	---	20	---	40	20	20
p	*0.0426*	*0.0000*	---	---	---	*0.0216*	---	*0.0031*	*0.0071*	*0.0271*
Creatinine	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

[1.] Margaree Harbor, NS, [2]. Cheticamp, NS

Table 25. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Margaree Harbor - HP Lipid, Spring 2013

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Trap	Cage	All	Trap	Cage	All	Trap	Cage
Urea	---	---	0.8128	---	---	---	---	0.4919	---	---
n	---	---	10	---	---	---	---	20	---	---
p	---	---	*0.0043*	---	---	---	---	*0.0276*	---	---
Uric Acid	-0.3036	---	---	-0.7378	-0.5234	---	-0.7805	---	---	-0.6079
n	120	---	---	10	20	---	10	---	---	10
p	*0.0007*	---	---	*0.0149*	*0.0179*	---	*0.0077*	---	---	*0.0251*
Total Protein	0.6290	0.7366	0.7781	0.7660	0.4112	0.6794	---	0.7568	0.7903	---
n	120	20	10	10	20	10	---	20	10	---
p	*0.0000*	*0.0002*	0.0080	0.0098	*0.0717*	*0.0307*	---	*0.0001*	*0.0065*	---
Albumin	0.6070	0.7772	0.7693	0.7604	0.4519	0.8299	---	0.7082	---	---
n	120	20	10	10	20	10	---	20	---	---
p	*0.0000*	*0.0001*	*0.0093*	*0.0107*	*0.0455*	*0.0030*	---	*0.0005*	---	---
Globulin	0.6126	0.6950	0.7052	0.7455	---	---	---	0.7585	0.8182	---
n	120	20	10	10	---	---	---	20	10	---
p	*0.0000*	*0.0007*	*0.0227*	*0.0133*	---	---	---	*0.0001*	*0.0038*	---
A:G	-0.3806	---	---	---	---	0.6606	---	-0.7722	-0.8537	---
n	120	---	---	---	---	10	---	20	10	---
p	*0.0000*	---	---	---	---	*0.0376*	---	*0.0001*	*0.0017*	---
Cholesterol	0.6370	0.5004	0.6606	---	---	---	---	0.7475	0.7697	---
n	120	20	10	---	---	---	---	20	10	---
p	*0.0000*	*0.0246*	*0.0376*	---	---	---	---	*0.0002*	*0.0092*	---
Triglyceride	0.3299	0.6601	0.8370	---	0.5370	---	---	0.7026	---	---
n	120	20	10	---	20	---	---	20	---	---
p	0.0002	0.0015	*0.0025*	---	*0.0146*	---	---	*0.0006*	---	---
Glucose	0.5625	0.5865	0.7622	---	0.5144	0.7594	---	0.7487	0.7195	---
n	120	20	10	---	20	10	---	20	10	---
p	*0.0000*	*0.0066*	*0.0104*	---	*0.0203*	*0.0108*	---	*0.0001*	*0.0190*	---
Lactate	0.1855	---	---	---	0.5100	0.78185	---	0.5820	0.6485	---
n	120	---	---	---	20	10	---	20	10	---
p	*0.0426*	---	---	---	*0.0216*	*0.0075*	---	*0.0071*	*0.0425*	---
Creatinine	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

Table 26. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

	Cheticamp- HP Lipid, Spring 2013									
Analyte	**All Crabs**	**Pygmy Male**			**Large Mature Male**			**Mature Female**		
		All	**Trap**	**Cage**	**All**	**Trap**	**Cage**	**All**	**Trap**	**Cage**
Urea	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Uric Acid	-0.3036	---	---	---	---	---	---	---	---	---
n	120	---	---	---	---	---	---	---	---	---
p	*0.0007*	---	---	---	---	---	---	---	---	---
Total Protein	0.6290	0.6353	0.6505	0.6809	0.4970	---	0.8781	0.7420	0.7781	---
n	120	20	10	10	20	---	10	20	10	---
p	*0.0000*	*0.0026*	*0.0417*	0.0302	*0.0258*	---	*0.0008*	*0.0002*	*0.0080*	---
Albumin	0.6070	0.6184	---	0.6791	0.5472	---	0.7691	0.7103	---	---
n	120	20	---	10	20	---	10	20	---	---
p	*0.0000*	*0.0037*	---	*0.0308*	*0.0125*	---	*0.0093*	*0.0004*	---	---
Globulin	0.6126	0.6266	0.6364	0.6687	---	---	0.8110	0.7380	0.7622	---
n	120	20	10	10	---	---	10	20	10	---
p	*0.0000*	*0.0031*	*0.0479*	*0.0345*	---	---	*0.0044*	*0.0002*	*0.0104*	---
A:G	-0.3806	---	---	---	---	---	---	-0.7144	-0.7439	---
n	120	---	---	---	---	---	---	200	10	---
p	*0.0000*	---	---	---	---	---	---	*0.0004*	*0.0136*	---
Cholesterol	0.6370	0.6533	0.6442	---	0.5059	---	---	0.6007	---	---
n	120	20	10	---	20	---	---	20	---	---
p	*0.0000*	*0.0018*	*0.0444*	---	*0.0229*	---	---	*0.0051*	---	---
Triglyceride	0.3299	---	---	---	0.5617	---	---	0.7262	---	---
n	120	---	---	---	20	---	---	20	---	---
p	*0.0002*	---	---	---	*0.0100*	---	---	*0.0003*	---	---
Glucose	0.5625	0.4673	---	0.6850	---	---	0.7523	0.7377	---	---
n	120	20	---	10	---	---	10	20	---	---
p	*0.0000*	*0.0378*	---	*0.0288*	---	---	*0.0121*	*0.0002*	---	---
Lactate	0.1855	---	---	---	---	---	---	0.4932	---	---
n	120	---	---	---	---	---	---	20	---	---
p	*0.0426*	---	---	---	---	---	---	*0.0271*	---	---
Creatinine	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

Table 27. Summary of Spearman's rank coefficients, sample size, and significance level for the level for the activity of eight enzymes in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, June, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Amylase	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Lipase	---	---	---	---	- 0.3769	---	- 0.5687	---	---	---
n	---	---	---	---	40	---	20	---	---	---
p	---	---	---	---	*0.0165*	---	*0.0089*	---	---	---
AST	- 0.3078	- 0.5689	- 0.5043	- 0.4099	---	---	- 0.4432	---	---	---
n	120	40	20	20	---	---	20	---	---	---
p	*0.0006*	*0.0001*	*0.0234*	*0.0726*	---	---	*0.0503*	---	---	---
ALT	---	- 0.3822	---	---	---	---	---	0.3916	---	---
n	---	40	---	---	---	---	---	40	---	---
p	---	*0.0149*	---	---	---	---	---	*0.0125*	---	---
GD	0.3709	0.3346	---	---	---	---	---	0.5243	0.4911	0.5530
n	120	40	---	---	---	---	---	40	20	20
p	*0.0000*	*0.0348*	---	---	---	---	---	*0.0005*	*0.0279*	*0.0114*
SDH	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
ALP	- 0.2388	- 0.3296	---	---	---	---	---	---	---	---
n	120	40	---	---	---	---	---	---	---	---
p	*0.0086*	*0.0378*	---	---	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

[1.] Margaree Harbor, NS [2]. Cheticamp, NS

Table 28. Summary of Spearman's rank coefficients, sample size, and significance level for the activity levels of eight enzymes in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

		Margaree Harbor - HP Lipid, Spring 2013								
Analyte	**All Crabs**	**Pygmy Male**			**Large Mature Male**			**Mature Female**		
		All	**Trap**	**Cage**	**All**	**Trap**	**Cage**	**All**	**Trap**	**Cage**
Amylase	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Lipase	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
AST	-0.3078	-0.5043	---	-0.7262	---	---	---	---	0.6606	---
n	120	20	---	10	---	---	---	---	10	---
p	*0.0006*	*0.0234*	---	0.0174	---	---	---	---	*0.0376*	---
ALT	---	---	---	---	---	---	---	---	0.7584	---
n	---	---	---	---	---	---	---	---	10	---
p	---	---	---	---	---	---	---	---	*0.0110*	---
GD	0.3709	---	---	---	---	---	---	0.4911	0.8085	---
n	120	---	---	---	---	---	---	20	10	---
p	*0.0000*	---	---	---	---	---	---	*0.0279*	*0.0046*	---
SDH	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
ALP	-0.2388	---	---	---	---	---	---	---	---	---
n	120	---	---	---	---	---	---	---	---	---
p	*0.0086*	---	---	---	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

Table 29. Summary of Spearman's rank coefficients, sample size, and significance level for the activity levels of eight enzymes in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

	Cheticamp- HP Lipid, Spring 2013									
Analyte	**All Crabs**	**Pygmy Male**			**Large Mature Male**			**Mature Female**		
		All	**Trap**	**Cage**	**All**	**Trap**	**Cage**	**All**	**Trap**	**Cage**
Amylase	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Lipase	---	---	---	0.7669	- 0.5687	- 0.6772	---	---	---	---
n	---	---	---	10	20	10	---	---	---	---
p	---	---	---	*0.0096*	*0.0089*	*0.0315*	---	---	---	---
AST	- 0.3078	- 0.4099	---	---	- 0.4432	---	---	---	---	---
n	120	20	---	---	20	---	---	---	---	---
p	*0.0006*	*0.0726*	---	---	*0.0503*	---	---	---	---	---
ALT	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
GD	0.3709	---	---	0.6342	---	---	0.8659	0.5530	---	---
n	120	---	---	10	---	---	10	20	---	---
p	*0.0000*	---	---	*0.0489*	---	---	*0.0012*	*0.0114*	---	---
SDH	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
ALP	- 0.2388	---	---	---	---	---	---	---	---	---
n	120	---	---	---	---	---	---	---	---	---
p	*0.0086*	---	---	---	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

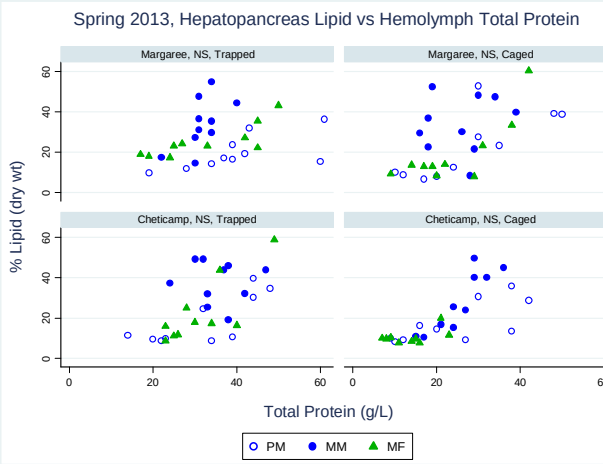


Figure 4. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma total protein concentration of snow crab collected from two stations in CFA 19 in June 2013, after trapping or a six month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

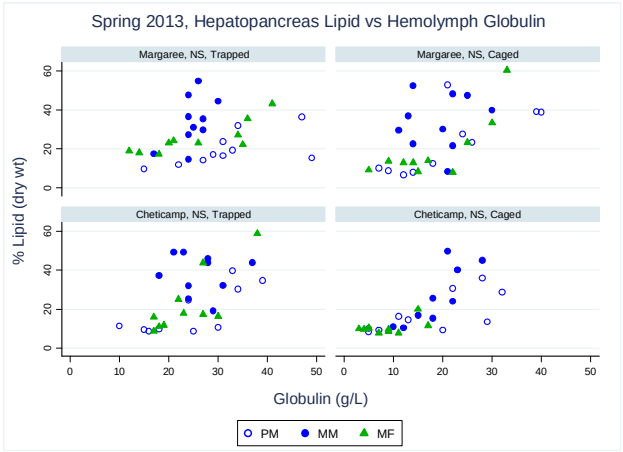


Figure 6. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma globulin concentration of snow crab collected from two stations in CFA 19 in June 2013, after trapping or a six month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

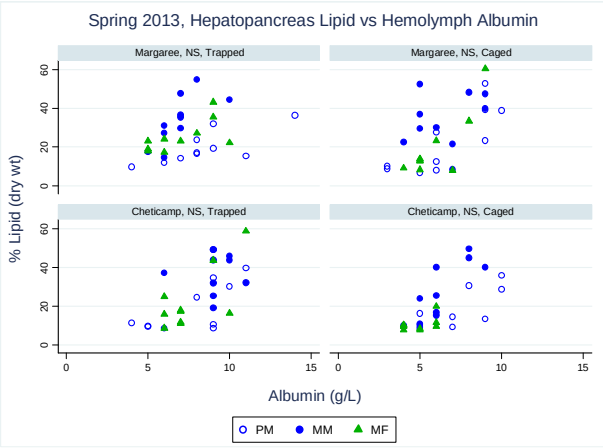


Figure 5. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma albumin concentration of snow crab collected from two stations in CFA 19 in June 2013, after trapping or a six month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

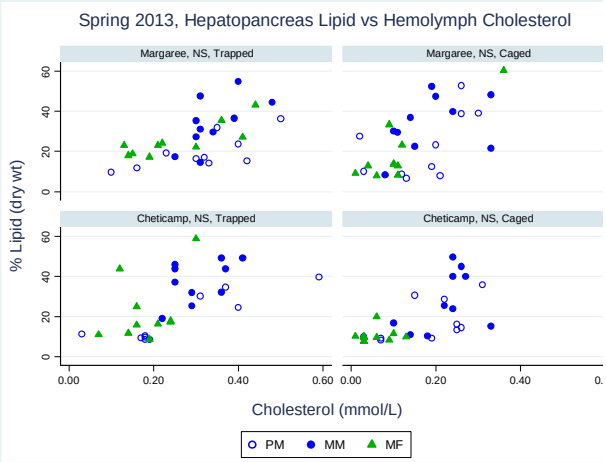


Figure 7. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma cholesterol concentration of snow crab collected from two stations in CFA 19 in June 2013, after trapping or a six month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

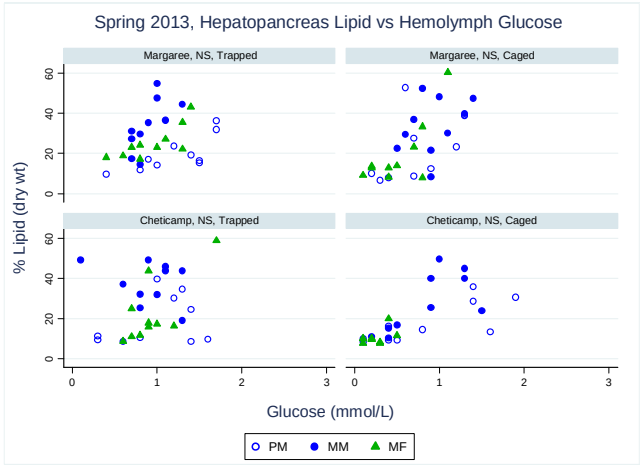


Figure 9. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma glucose concentration of snow crab collected from two stations in CFA 19 in June 2013, after trapping or a six month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

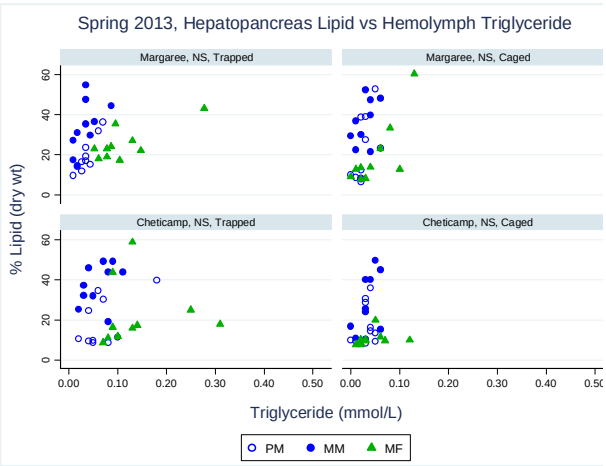


Figure 8. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma triglyceride concentration of snow crab collected from two stations in CFA 19 in June 2013, after trapping or a six month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

Table 30. Summary of equations and associated R^2 (values > 0.5 in bold) for simple linear regression models for average hepatopancreas lipid (g/g HP dry wt) for four plasma biochemistry parameters of free snow crab collected from two stations in CFA 19 in spring (June) 2013. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). To obtain values for coefficients and constants as percentage of hepatopancreas dry weight, multiply by 100.

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree Harbor	PM	Avg Lipid = 0.0043*TP + 0.02	0.4113
		LM	Avg Lipid = 0.0173*TP – 0.21	0.3827
		MF	Avg Lipid = 0.0037 *TP + 0.11	**0.5484**
	Cheticamp	PM	Avg Lipid = 0.0079*TP – 0.06	**0.5867**
		LM	Avg Lipid = -0.0008*TP + 0.40	0.0031
		MF	Avg Lipid = 0.0090 *TP -0.08	0.2700
Albumin (Alb)	Margaree Harbor	PM	Avg Lipid = 0.0240*Alb – 0.00	**0.5727**
		LM	Avg Lipid = 0.06756*Alb – 0.12+	**0.5270**
		MF	Avg Lipid = 0.0179*Alb + 0.11	0.3611
	Cheticamp	PM	Avg Lipid = 0.0359*Alb – 0.08	**0.5009**
		LM	Avg Lipid = 0.0033*Alb + 0.34	0.0017
		MF	Avg Lipid = 0.0322*Alb – 0.04	0.1807
Globulin (Glob)	Margaree Harbor	PM	Avg Lipid = 0.0050*Glob + 0.03	0.3648
		LM	Avg Lipid = 0.0205*Glob + 0.10	0.2928
		MF	Avg Lipid = 0.0046*Glob + 0.01	**0.5793**
	Cheticamp	PM	Avg Lipid = 0.0098*Glob – 0.05	**0.5868**
		LM	Avg Lipid = -0.0014*Glob + 0.41	0.0058
		MF	Avg Lipid = 0.0113*Glob – 0.06	0.2740
Triglyceride (TG)	Margaree Harbor	PM	Avg Lipid = 3.696*TG + 0.04	**0.8323**
		LM	Avg Lipid = 2.66*TG + 0.23	0.3236
		MF	Avg Lipid = 0.3258*TG + 0.19+	0.0438
	Cheticamp	PM	Avg Lipid = 1.4869 *TG + 0.08	0.2983
		LM	Avg Lipid = 1.3285 *TG + 0.29	0.1485
		MF	Avg Lipid = 0.1562*TG + 0.16	0.0154
Cholesterol (Chol)	Margaree Harbor	PM	Avg Lipid = 0.5103*Chol + 0.03	**0.5101**
		LM	Avg Lipid = 1.2079*Chol – 0.07	0.3956
		MF	Avg Lipid = 0.4069*Chol + 0.13	**0.5402**
	Cheticamp	PM	Avg Lipid = 0.6800*Chol + 0.01	**0.7786**
		LM	Avg Lipid = 0.7974*Chol + 0.13	0.2482
		MF	Avg Lipid = -0.3011*Chol + 0.23	0.0258
Glucose(Gluc)	Margaree Harbor	PM	Avg Lipid = 0.1522*Gluc+ 0.02	**0.5693**
		LM	Avg Lipid = 0.4383*Gluc – 0.05	0.4725
		MF	Avg Lipid = 0.1254*Gluc+ 0.12	0.4900
	Cheticamp	PM	Avg Lipid = 0.0876*Gluc+ 0.10	0.1127
		LM	Avg Lipid = -0.0858*Gluc+ 0.45	0.0878
		MF	Avg Lipid = 0.1193*Gluc+ 0.08	0.0418

Table 31. Summary of equations and associated R^2 (values > 0.5 in bold) for simple linear regression models for average hepatopancreas lipid (g/g HP dry wt) for four plasma biochemistry parameters of caged snow crab collected from two stations in CFA 19 in spring (June) 2013. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). To obtain values for coefficients and constants as percentage of hepatopancreas dry weight, multiply by 100.

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree Harbor	PM	Avg Lipid = 0.0089*TP - 0.02	0.5780
		LM	Avg Lipid = 0.0025*TP + 0.27	0.0194
		MF	Avg Lipid = 0.0066*TP + 0.00	0.5299
	Cheticamp	PM	Avg Lipid = 0.0058*TP + 0.03	0.5063
		LM	Avg Lipid = 0.0200*TP – 0.23	0.7859
		MF	Avg Lipid = 0.0004*TP + 0.08	0.0255
Albumin (Alb)	Margaree Harbor	PM	Avg Lipid = 0.0516*Alb – 0.11	0.6503
		LM	Avg Lipid = 0.0196*Alb + 0.20	0.0632
		MF	Avg Lipid = 0.0448*Alb – 0.09	0.4511
	Cheticamp	PM	Avg Lipid = 0.0350*Alb - 0.06	0.5699
		LM	Avg Lipid = 0.0834*Alb +- 0.25	0.6509
		MF	Avg Lipid = 0.0030*Alb + 0.08	0.0409
Globulin (Glob)	Margaree Harbor	PM	Avg Lipid = 0.0103*Glob + 0.00	0.5373
		LM	Avg Lipid = 0.0023*Glob + 0.29	0.0108
		MF	Avg Lipid = 0.0075*Glob + 0.02	0.5296
	Cheticamp	PM	Avg Lipid = 0.0069*Glob + 0.05	0.4873
		LM	Avg Lipid = 0.0226*Glob – 0.15	0.7118
		MF	Avg Lipid = 0.0004*Glob + 0.09	0.0216
Triglyceride (TG)	Margaree Harbor	PM	Avg Lipid = 5.405*TG + 0.08	0.3461
		LM	Avg Lipid = 3.6157*TG + 0.23	0.2260
		MF	Avg Lipid = 1.447*TG + 0.09	0.3458
	Cheticamp	PM	Avg Lipid = 1.1813*TG +0.13	0.0348
		LM	Avg Lipid = 4.0065*TG + 0.14	0.2808
		MF	Avg Lipid = 0.1687*TG + 0.08	0.2129
Cholesterol (Chol)	Margaree Harbor	PM	Avg Lipid = 0.9601*Chol + 0.06	0.3161
		LM	Avg Lipid = 0.6228*Chol + 0.22	0.1613
		MF	Avg Lipid = 0.7469*Chol + 0.08	0.1142
	Cheticamp	PM	Avg Lipid = 0.5806*Chol + 0.07	0.2936
		LM	Avg Lipid = 0.9867*Chol +0.05	0.1968
		MF	Avg Lipid = 0.0876*Chol + 0.08	0.0774
Glucose(Gluc)	Margaree Harbor	PM	Avg Lipid = 0.2147*Gluc+ 0.06	0.2856
		LM	Avg Lipid = 0.1752*Gluc+ 0.17	0.1334
		MF	Avg Lipid = 0.1654*Gluc+ 0.03	0.2830
	Cheticamp	PM	Avg Lipid = 0.1198*Gluc+ 0.07	0.5955
		LM	Avg Lipid = 0.2376*Gluc+ 0.07	0.5181
		MF	Avg Lipid = 0.0297*Gluc+ 0.08	0.0903

Table 32. Summary of equations and associated adjusted R^2 (values > 0.5 in bold) for multiple linear regression models for average hepatopancreas lipid (g/g HP dry wt) for four plasma biochemistry parameters and carapace width of free snow crab collected from two stations in CFA 19 in spring (June) 2013. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). To obtain values for coefficients and constants as percentage of hepatopancreas dry weight, multiply by 100.

LOCATION	GROUP	SIMPLIFIED MULTIPLE REGRESSION EQUATION	ADJUSTED R^2
Margaree Harbor	PM	Avg Lipid = 0.05*Gluc + 0.31*Chol + 4.05*Trig – 0.00*TP + 0.07	0.8719
	LM	Avg Lipid = 0.76*Gluc + 0.00*Chol – 4.69 *Trig +0.01*TP – 0.69	0.3111
	MF	Avg Lipid = 0.13*Gluc + 0.56*Chol -1.56*Trig -0.00*TP +0.17	0.8819
Cheticamp	PM	Avg Lipid = 0.00*Gluc + 0.35*Chol + 0.70*Trig +0.00*TP – 0.08	0.7275
	LM	Avg Lipid = -0.06*Gluc + 0.46*Chol + 1.52*Trig – 0.00*TP + 0.28	-0.0965
	MF	Avg Lipid = -0.21*Gluc – 0.92 *Chol + 0.44*Trig +0.01*TP – 0.04	0.1202
Margaree Harbor	PM	Avg Lipid = 0.03*Gluc + 0.40*Chol + 3.88*Trig – 0.00*TP + 0.00*CW -0.12	0.8540
	LM	Avg Lipid = 0.64*Gluc – 0.94*Chol – 1.17*Trig + 0.00*TP – 0.14*CW + 1.7	0.4913
	MF	Avg Lipid = 0.14*Gluc + 0.47*Chol – 1.44*Trig -0.00 *TP -0.00*CW + 0.29	0.8640
Cheticamp	PM	Avg Lipid = 0.01*Gluc + 0.47*Chol + 0.47*Trig + 0.00*TP – 0.00*CW + 0.30	0.7620
	LM	Avg Lipid = -0.11*Gluc + 0.72*Chol + 0.89*Trig – 0.00*TP – 0.01*CW + 1.69	0.2295
	MF	Avg Lipid = 0.56*Gluc – 0.76*Chol + 1.74*Trig – 0.02*TP – 0.02*CW + 1.89	0.3227

Table 33. Summary of equations and associated adjusted R^2 (values > 0.5 in bold) for multiple linear regression models for average hepatopancreas lipid (g/g HP dry wt) for four plasma biochemistry parameters and carapace width (CW) of caged snow crab collected from two stations in CFA 19 in spring (June) 2013. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). To obtain values for coefficients and constants as percentage of hepatopancreas dry weight, multiply by 100.

STATION	GROUP	SIMPLIFIED MULTIPLE REGRESSION EQUATION	ADJUSTED R^2
Margaree Harbor	PM	Avg Lipid = -0.21*Gluc + 0.10*Chol + 2.60*Trig +.12*TP -0.03	0.4686
	LM	Avg Lipid = 0.76*Gluc + 0.72*Chol + 2.86*Trig – 0.03*TP +0.25	0.4584
	MF	Avg Lipid = -0.54*Gluc – 0.17 *Chol + 0.62*Trig +0.02*TP – 0.07	0.7215
Cheticamp	PM	Avg Lipid = 0.12*Gluc + 0.80*Chol – 4.43*Trig – 0.00*TP +0.10	0.5619
	LM	Avg Lipid = 0.03*Gluc – 0.91*Chol + 2.43*Trig +0.01*TP – 0.12	0.6721
	MF	Avg Lipid = 0.03*Gluc – 0.11*Chol + 0.27*Trig + 0.00*TP + 0.07	-0.2617
Margaree Harbor	PM	Avg Lipid = -0.44*Gluc + 0.48*Chol + 1.98*Trig + 0.01*TP + 0.00*CW – 0.64	0.8263
	LM	Avg Lipid = 0.65*Gluc + 0.51*Chol – 1.59*Trig – 0.01*TP + 0.01*CW – 2.04	0.5792
	MF	Avg Lipid = -0.69*Gluc + 0.23*Chol + 0.34*Trig + 0.02*TP – 0.00*CW +	0.8066
Cheticamp	PM	Avg Lipid = 0.12*Gluc + 0.81*Chol – 4.45*Trig – 0.00*TP – 0.00*CW + 0.11	0.4525
	LM	Avg Lipid = -0.04*Gluc – 1.27*Chol + 2.61*Trig + 0.02*TP + 0.00*CW – 0.67	0.6123
	MF	Avg Lipid = 0.04*Gluc - 0.10*Chol + 0.23*Trig -0.00*TP + 0.00*CW + 0.05	-0.5906

C. Hepatopancreas Glycogen Content

The average HP glycogen content was calculated as milligram per gram of dry hepatopancreas weight (mg glycogen/g HP dry wt) and converted to percent dry weight to standardise comparison to other components and data from the fall 2012 study (see Section VIII-1). Average glycogen content was also converted to percent glycogen as a proportion of total wet weight using percent moisture data which was available for all hepatopancreas tissue.

The distribution of the data is presented in Figures 10-12 as boxplots, frequency distribution histograms and scatterplot vs. carapace width, respectively. Numerous outliers were identified on the boxplots (#'s 261B, 248B, 204B, 206B, 223B, 66B, 64B, 88B, 106B, 145B) and are also evident on the histograms. No pattern for glycogen content and CW was apparent. Summary statistics for average HP glycogen by category and station are provided in Tables 34 &35 for free and caged crabs, respectively.

Effect of Treatment (Caged vs Free) within a Station by Sex

Comparison (Wilcoxon rank-sum) of treatment method (caged vs free) within a station by category, found that hepatopancreas glycogen content in Margaree Harbor MF_{free} crabs > Margaree Harbor MF_{caged} ($p = 0.0002$), Cheticamp $MF_{free} > MF_{caged}$ ($p = 0.0043$), and Cheticamp $PM_{caged} > PM_{free}$ ($p = 0.0124$).

Effect of Sex within a Station by Treatment (Caged or Free)

Kruskall-Wallis testing across crab categories within station by treatment showed statistically significant differences for free crabs at both stations and caged crabs from Cheticamp. Subsequent Wilcoxon testing, at Bonferroni-adjusted p-value of 0.0167, identified free $MF_{Margaree} > PM_{Margaree}$ and ($p = 0.0003$) and $LM_{Margaree}$ ($p = 0.0041$); free $PM_{Cheticamp} < LM_{Cheticamp}$ ($p = 0.0004$) and $MF_{Cheticamp}$ ($p = 0.0015$); and, caged $LM_{Cheticamp} > MF_{Cheticamp}$ ($p = 0.0065$).

Effect of Station within a Treatment by Sex

Comparison (Wilcoxon rank-sum) of station within a treatment by sex, found differences for free crabs only, with values for free $PMM_{argaree}$ > free $PM_{Cheticamp}$ ($p = 0.0094$) and values for free $MF_{Margaree}$ > free $MF_{Cheticamp}$ ($p = 0.0025$).

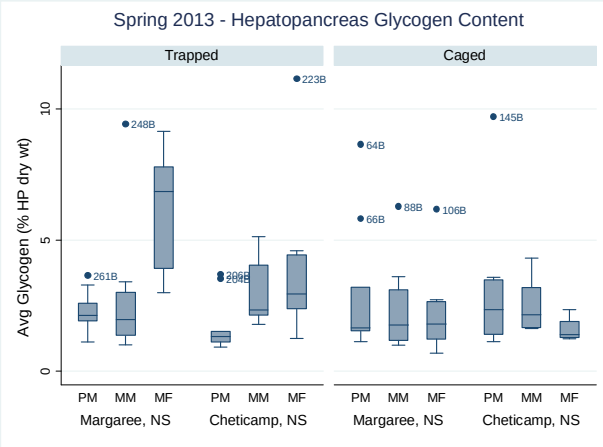


Figure 10. Box and Whisker plot showing distribution of average hepatopancreas glycogen (% HP dry weight) for Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF) snow crab collected by trapping or after six months of caging, at two stations in CFA 19, June 2013.

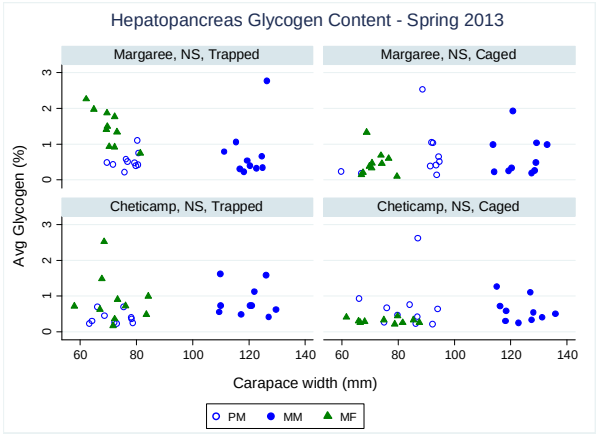


Figure 12. Scatterplot showing average HP glycogen (% HP dry wt) by carapace width for Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF) snow crab collected by trapping or after six months of caging, at two stations in CFA 19, June 2013.

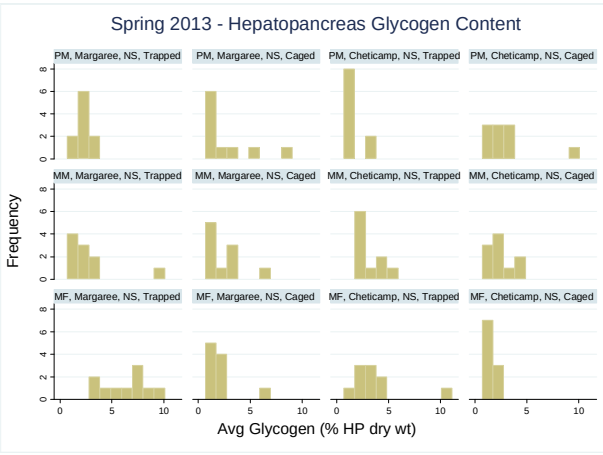


Figure 11. Frequency distribution histogram showing average HP glycogen (%HP dry wt) for Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF) snow crab collected by trapping or after six months of caging, at two stations in CFA 19, June 2013.

Table 34. Summary statistics for average hepatopancreas glycogen content (% dry wt) for free-ranging snow crabs collected by traps at two stations in CFA 19, June 2013. Different superscripts represent significant differences in median values within (letters) or between (numbers) stations.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	PM	10	2.9060	2.4636	1.6580	1.1264	8.6566	4.0065	1.5289
	LM	10	2.3946	1.6540	1.7662	1.0003	6.2821	3.9913	1.3454
	MF	10	2.1680	1.5780	1.7962	0.6832	6.1756	5.3179	1.7200
Cheticamp	PM	10	3.0163	2.5111	2.3515[a,b;]	1.1322	9.7110	6.3326	2.0871
	LM	10	2.5002	1.0168	2.1598[a,c]	1.6231	4.3243	2.1666	0.8150
	MF	10	1.5787	0.3824	1.4016[b]	1.2476	2.3485	2.5314	0.9710

Table 35. Summary statistics for average hepatopancreas glycogen content (% dry wt) for snow crabs at two stations in CFA 19, collected in June 2013 after a six month caging period. Different superscripts represent significant differences in median values within (letters) or between (numbers) stations.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	PM	10	2.2508	0.7759	2.1302[a;1]	1.1075	3.6431	2.4844	0.4191
	LM	10	2.7164	2.4840	1.9631[a,c;2]	1.0205	9.4267	6.6453	2.2033
	MF	10	6.2216	2.1232	6.8602[b;3]	3.0017	9.1524	1.7663	-0.3282
Cheticamp	PM	10	1.7157	1.0205	1.3257[a]	0.9316	3.6876	3.0768	1.3617
	LM	10	2.9548	1.2065	2.3328[c;2]	1.7782	5.1252	1.9735	0.7049
	MF	10	3.8282	2.7639	2.9541[b,c]	1.2528	11.1512	6.2066	2.0322

Spearman rank correlation co-efficients were calculated for average HP glycogen, for all 23 directly measured hemolymph biochemistry parameters and three ratios automatically included in the biochemistry profiles (Tables II.C.4 – II.C.11). There was minimal correlation between hepatopancreas glycogen and any of the mineral, electrolyte, metabolite, or enzyme parameters. Exceptions included: glucose for caged $PM_{Margaree}$ (0.7622), caged (0.6850) and free (0.6890) $PM_{Cheticamp}$; uric acid for caged $PM_{Margaree}$ (-0.6464) and caged $LM_{Margaree}$ (-0.8659); urea for caged $MF_{Cheticamp}$ (0.7677); triglyceride for caged $LM_{Margaree}$ (0.6791); hemolymph calcium in free $LM_{Margaree}$ (0.8545) and caged $MF_{Margaree}$ (0.7333); and, an unusually high correlation (0.9478) with the Na:K ratio for free $LM_{Margaree}$ sample only. Hemolymph GD activity was well correlated to free $LM_{Cheticamp}$ (0.8964) and free $PM_{Margaree}$ (0.7720). The relationships of hemolymph total protein, triglyceride, cholesterol, and glucose are presented as scatterplots in Figures 13-16, respectively, for comparative purposes to the two week caging study (SectionVIII-1) where these correlations were much higher.

As there were no consistently strong correlations, other than glucose, simple regression analyses were completed for average (%dry weight) hepatopancreas glycogen for the four parameters used in the fall 2012 samples (see Section VIII-1) i.e., total protein, triglyceride, cholesterol, and glucose to facilitate comparison between the two sampling periods. Equations are presented for caged and free crabs at both locations in Table 45 & 46, respectively. Values for R^2 were very low for all four biochemistry parameters for all categories of free crabs at either location. The highest value was 0.2156 for total protein for free $PM_{Margaree}$. The values for R^2 were also generally low for caged crabs. The highest values were found for glucose (0.5679) for $PM_{Margaree}$, cholesterol (0.5302) for $PM_{Cheticamp}$, and for total protein (0.5078) in $PM_{Margaree}$ crabs.

There was marginal improvement with multiple linear regression for free crabs (Table 47) where the adjusted R^2 increased to 0.5787 for $LM_{Margaree}$ when CW was included as a factor. Again, the variables used in the two week caging study were selected to aid comparison. The highest adjusted R^2 value for caged crabs was for $PM_{Margaree}$ crabs (0.6602) using multiple linear regression (Table 48). Addition of CW as a factor improved adjusted R^2 to 0.8073 for caged $PM_{Cheticamp}$ and caged $LM_{Cheticamp}$ (0.6054), but decreased fit for caged $PM_{Margaree}$ (0.5049).

Table 36. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, June, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Sodium	---	---	---	---	---	---	---	---	---	-0.6118
n	---	---	---	---	---	---	---	---	---	20
p	---	---	---	---	---	---	---	---	---	*0.0041*
Potassium	-0.4143	---	---	---	---	-0.6084	---	-0.5972	---	-0.6707
n	118	---	---	---	---	19	---	40	---	20
p	*0.0000*	---	---	---	---	*0.0057*	---	*0.0000*	---	*0.0012*
Na:K	0.3944	0.3371	---	---	---	0.5711	---	0.5783	0.4886	0.5606
n	118	40	---	---	---	19	---	40	20	20
p	*0.0000*	*0.0334*	---	---	---	*0.0106*	---	*0.0001*	*0.0288*	*0.0101*
Chloride	-0.1952	---	---	---	-0.3699	---	---	-0.3727	---	-0.7569
n	118	---	---	---	38	---	---	40	---	20
p	*0.0342*	---	---	---	*0.0223*	---	---	*0.0179*	---	*0.0001*
Calcium	---	---	---	0.5077	0.3400	0.4821	---	---	---	---
n	---	---	---	20	40	20	---	---	---	---
p	---	---	---	0.0223	*0.0318*	0.0313	---	---	---	---
Phosphorus	---	---	---	---	---	-0.4737	---	---	---	---
n	---	---	---	---	---	20	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Magnesium	---	---	---	---	---	---	---	---	0.4617	---
n	---	---	---	---	---	---	---	---	20	---
p	---	---	---	---	---	---	---	---	*0.0405*	---

[1.] Margaree Harbor, NS [2]. Cheticamp, NS

Table 37. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Coefficients are shown only where $p \leq 0.05$; coefficients ≤ 0.5000 are indicated in grey.

Margaree Harbor - HP Glycogen, Spring 2013

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Trap	Cage	All	Trap	Cage	All	Trap	Cage
Sodium	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Potassium	-0.4143	---	---	---	-0.6084	-0.7666	---	---	---	---
n	118	---	---	---	19	10	---	---	---	---
p	*0.0000*	---	---	---	*0.0057*	*0.0097*	---	---	---	---
Na:K	0.3944	---	---	---	0.5711	0.9478	---	0.4886	---	---
n	118	---	---	---	19	10	---	20	---	---
p	*0.0000*	---	---	---	*0.0106*	*0.0000*	---	*0.0288*	---	---
Chloride	-0.1952	---	---	---	---	---	---	---	---	---
n	118	---	---	---	---	---	---	---	---	---
p	*0.0342*	---	---	---	---	---	---	---	---	---
Calcium	---	---	---	---	0.4821	0.8545	---	---	---	0.7333
n	---	---	---	---	20	10	---	---	---	10
p	---	---	---	---	0.0313	*0.0000*	---	---	---	*0.0158*
Phosphorus	---	---	---	---	-0.4737	-0.6603	---	---	---	0.7333
n	---	---	---	---	20	10	---	---	---	10
p	---	---	---	---	---	*0.0376*	---	---	---	*0.0158*
Magnesium	---	---	---	---	---	---	---	0.4617	---	---
n	---	---	---	---	---	---	---	20	---	---
p	---	---	---	---	---	---	---	*0.0405*	---	---

Table 38. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Cheticamp- HP Glycogen, Spring 2013

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Trap	Cage	All	Trap	Cage	All	Trap	Cage
Sodium	---	---	---	---	---	---	---	-0.6118	---	---
n	---	---	---	---	---	---	---	20	---	---
p	---	---	---	---	---	---	---	*0.0041*	---	---
Potassium	-0.4143	---	---	---	---	---	---	-0.6707	-0.7038	---
n	118	---	---	---	---	---	---	20	10	---
p	*0.0000*	---	---	---	---	---	---	*0.0012*	*0.0231*	---
Na:K	0.3944	---	---	---	---	---	---	0.5606	---	---
n	118	---	---	---	---	---	---	20	---	---
p	*0.0000*	---	---	---	---	---	---	*0.0101*	---	---
Chloride	-0.1952	---	---	---	---	---	---	-0.7569	---	---
n	118	---	---	---	---	---	---	20	---	---
p	*0.0342*	---	---	---	---	---	---	*0.0001*	---	---
Calcium	---	0.5077	---	---	---	---	---	---	---	---
n	---	20	---	---	---	---	---	---	---	---
p	---	0.0223	---	---	---	---	---	---	---	---
Phosphorus	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Magnesium	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

Table 39. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, spring (June), 2013, grouped by category, and region of capture. Coefficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey font.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Urea	---	-0.3161	---	-0.5422	---	---	---	---	---	---
n	---	40	---	20	---	---	---	---	---	---
p	---	*0.0469*	---	*0.0135*	---	---	---	---	---	---
Uric Acid	---	---	---	---	---	-0.5008	---	---	---	---
n	---	---	---	---	---	20	---	---	---	---
p	---	---	---	---	---	*0.0245*	---	---	---	---
Total Protein	0.2898	---	0.4951	---	0.3142	---	---	0.5882	---	0.7013
n	120	---	20	---	40	---	---	40	---	20
p	*0.0013*	---	*0.0264*	---	*0.0483*	---	---	*0.0001*	---	*0.0006*
Albumin	0.2746	---	0.4168	---	0.3245	---	---	0.5199	---	0.6695
n	120	---	20	---	40	---	---	40	---	20
p	. *0024*	---	*0.0675*	---	*0.0410*	---	---	*0.0006*	---	*0.0012*
Globulin	0.2866	---	0.4942	---	---	---	---	0.5941	---	0.7041
n	120	---	20	---	---	---	---	40	---	20
p	*0.0015*	---	*0.0268*	---	---	---	---	*0.0001*	---	*0.0005*
A:G	-0.2378	---	---	---	---	---	---	-0.5910	---	-0.7257
n	120	---	---	---	---	---	---	40	---	20
p	*0.0089*	---	---	---	---	---	---	*0.0001*	---	*0.0003*
Cholesterol	0.2643	---	---	---	---	---	---	0.5706	0.5269	0.6347
n	120	---	---	---	---	---	---	40	20	20
p	*0.0035*	---	---	---	---	---	---	*0.0001*	*0.0170*	0.0026
Triglyceride	0.3548	---	---	---	0.4426	---	---	0.4857	0.4586	0.5897
n	120	---	---	---	40	---	---	40	20	20
p	0.0001	---	---	---	*0.0042*	---	---	*0.0015*	*0.0420*	0.0062
Glucose	0.4164	0.5400	0.5526	0.5724	0.3645	---	---	0.6239	0.5466	0.7052
n	120	40	20	20	40	---	---	40	20	20
p	*0.0000*	*0.0003*	*0.0115*	*0.0084*	*0.0208*	---	---	*0.0000*	*0.0126*	0.0005
Lactate	---	---	---	---	---	---	---	0.3582	---	0.5805
n	---	---	---	---	---	---	---	40	---	20
p	---	---	---	---	---	---	---	*0.0233*	---	*0.0073*
Creatinine	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

[1.] Margaree Harbor, NS [2]. Cheticamp, NS

Table 40. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas glycogen content (%dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Margaree Harbor - HP Glycogen, Spring 2013

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Trap	Cage	All	Trap	Cage	All	Trap	Cage
Urea	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Uric Acid	---	---	---	-0.6464	-0.5008	---	-0.8659	---	---	---
n	---	---	---	10	20	---	10	---	---	---
p	---	---	---	*0.0435*	*0.0245*	---	*0.0012*	---	---	---
Total Protein	0.2898	0.4951	---	---	---	---	---	---	---	---
n	120	20	---	---	---	---	---	---	---	---
p	*0.0013*	*0.0264*	---	---	---	---	---	---	---	---
Albumin	0.2746	0.4168	---	---	---	---	---	---	---	---
n	120	20	---	---	---	---	---	---	---	---
p	*0. 0024*	*0.0675*	---	---	---	---	---	---	---	---
Globulin	0.2866	0.4942	---	---	---	---	---	---	---	---
n	120	20	---	---	---	---	---	---	---	---
p	*0.0015*	*0.0268*	---	---	---	---	---	---	---	---
A:G	-0.2378	---	---	---	---	---	---	---	---	---
n	120	---	---	---	---	---	---	---	---	---
p	*0.0089*	---	---	---	---	---	---	---	---	---
Cholesterol	0.2643	---	---	---	---	---	---	0.5269	---	---
n	120	---	---	---	---	---	---	20	---	---
p	*0.0035*	---	---	---	---	---	---	*0.0170*	---	---
Triglyceride	0.3548	---	---	---	---	---	0.6791	0.4586	---	---
n	120	---	---	---	---	---	10	20	---	---
p	0.0001	---	---	---	---	---	*0.0308*	*0.0420*	---	---
Glucose	0.4164	0.5526	---	0.7622	---	---	---	0.5466	---	---
n	120	20	---	10	---	---	---	20	---	---
p	*0.0000*	*0.0115*	---	0.0104	---	---	---	*0.0126*	---	---
Lactate	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Creatinine	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

Table 41. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Cheticamp- HP Glycogen, Spring 2013

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Trap	Cage	All	Trap	Cage	All	Trap	Cage
Urea	---	-0.5422	---	---	---	---	---	---	---	-0.7677
n	---	20	---	---	---	---	---	---	---	10
p	---	*0.0135*	---	---	---	---	---	---	---	*0.0095*
Uric Acid	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Total Protein	0.2898	---	---	---	---	---	---	0.7013	---	---
n	120	---	---	---	---	---	---	20	---	---
p	*0.0013*	---	---	---	---	---	---	*0.0006*	---	---
Albumin	0.2746	---	---	---	---	---	---	0.6695	---	---
n	120	---	---	---	---	---	---	20	---	---
p	*0.0024*	---	---	---	---	---	---	*0.0012*	---	---
Globulin	0.2866	---	---	---	---	---	---	0.7041	---	---
n	120	---	---	---	---	---	---	20	---	---
p	*0.0015*	---	---	---	---	---	---	*0.0005*	---	---
A:G	-0.2378	---	---	---	---	---	---	-0.7257	---	---
n	120	---	---	---	---	---	---	20	---	---
p	*0.0089*	---	---	---	---	---	---	*0.0003*	---	---
Cholesterol	0.2643	---	---	---	---	---	---	0.6347	---	---
n	120	---	---	---	---	---	---	20	---	---
p	*0.0035*	---	---	---	---	---	---	0.0026	---	---
Triglyceride	0.3548	---	---	---	---	---	---	0.5897	---	---
n	120	---	---	---	---	---	---	20	---	---
p	0.0001	---	---	---	---	---	---	0.0062	---	---
Glucose	0.4164	0.5724	0.6890	0.6850	---	---	---	0.7052	---	---
n	120	20	10	10	---	---	---	20	---	---
p	*0.0000*	*0.0084*	0.0275	0.0288	---	---	---	0.0005	---	---
Lactate	---	---	---	---	---	---	---	0.5805	---	---
n	---	---	---	---	---	---	---	20	---	---
p	---	---	---	---	---	---	---	*0.0073*	---	---
Creatinine	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

Table 42. Summary of Spearman's rank coefficients, sample size, and significance level for the activity of eight enzymes in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, June, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Marg[1]	Chet[2]	All	Marg	Chet	All	Marg	Chet
Amylase	---	---	0.5418	---	---	---	---	---	---	---
n	---	---	20	---	---	---	---	---	---	---
p	---	---	*0.0136*	---	---	---	---	---	---	---
Lipase	---	0.4630	---	0.5814	---	---	---	---	---	---
n	---	40	---	20	---	---	---	---	---	---
p	---	*0.0026*	---	*0.0072*	---	---	---	---	---	---
AST	-0.2494	---	---	---	-0.4758	-0.5965	-0.5839	---	---	---
n	120	---	---	---	40	20	20	---	---	---
p	*0.0060*	---	---	---	*0.0019*	*0.0055*	*0.0069*	---	---	---
ALT	---	---	---	---	---	---	-0.4697	---	---	---
n	---	---	---	---	---	---	20	---	---	---
p	---	---	---	---	---	---	*0.0367*	---	---	---
GD	0.2591	---	---	---	0.3671	---	0.6503	0.3573	---	0.5802
n	120	---	---	---	40	---	20	40	---	20
p	*0.0043*	---	---	---	*0.0198*	---	*0.0019*	*0.0236*	---	*0.0073*
SDH	0.1824	---	0.5407	---	---	---	---	0.4745	0.4953	---
N	120	---	20	---	---	---	---	40	20	---
P	*0.0462*	---	0.0138	---	---	---	---	*0.0020*	*0.0264*	---
ALP	-0.2177	-0.3334	-0.5210	---	---	---	---	---	---	---
n	120	40	20	---	---	---	---	---	---	---
p	*0.0169*	*0.0355*	*0.0185*	---	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---	0.3337	---	---
n	---	---	---	---	---	---	---	40	---	---
p	---	---	---	---	---	---	---	*0.0354*	---	---

[1.] Margaree Harbor, NS[2]. Cheticamp, NS

Table 43. Summary of Spearman's rank coefficients, sample size, and significance level for the activity level of eight enzymes in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Margaree Harbor - HP Glycogen, Spring 2013

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Trap	Cage	All	Trap	Cage	All	Trap	Cage
Amylase	---	0.5418	---	---	---	---	---	---	---	---
n	---	20	---	---	---	---	---	---	---	---
p	---	*0.0136*	---	---	---	---	---	---	---	---
Lipase	---	---	---	---	---	---	---	---	---	-0.6893
n	---	---	---	---	---	---	---	---	---	10
p	---	---	---	---	---	---	---	---	---	*0.0274*
AST	-0.2494	---	---	---	-0.5965	-0.8528	---	---	---	---
n	120	---	---	---	20	10	---	---	---	---
p	*0.0060*	---	---	---	*0.0055*	*0.0017*	---	---	---	---
ALT	---	---	---	---	---	-0.8476	---	---	---	---
n	---	---	---	---	---	10	---	---	---	---
p	---	---	---	---	---	*0.0020*	---	---	---	---
GD	0.2591	---	0.7720	---	---	---	---	---	---	---
n	120	---	10	---	---	---	---	---	---	---
p	*0.0043*	---	*0.0089*	---	---	---	---	---	---	---
SDH	0.1824	0.5407	---	0.7922	---	---	---	0.4953	---	---
N	120	20	---	10	---	---	---	20	---	---
P	*0.0462*	0.0138	---	*0.0062*	---	---	---	0.0264	---	---
ALP	-0.2177	-0.5210	---	---	---	---	---	---	---	---
n	120	20	---	---	---	---	---	---	---	---
p	0.0169	0.0185	---	---	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

Table 44. Summary of Spearman's rank coefficients, sample size, and significance level for the activity level of eight enzymes in hemolymph plasma *vs* hepatopancreas glycogen content (% wt) in snow crab after six months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, June, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Cheticamp- HP Glycogen, Spring 2013

Analyte	All Crabs	Pygmy Male			Large Mature Male			Mature Female		
		All	Trap	Cage	All	Trap	Cage	All	Trap	Cage
Amylase	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---
Lipase	---	0.5814	---	---	---	---	---	---	---	---
n	---	20	---	---	---	---	---	---	---	---
p	---	*0.0072*	---	---	---	---	---	---	---	---
AST	-0.2494	---	---	---	-0.5839	---	---	---	---	---
n	120	---	---	---	20	---	---	---	---	---
p	*0.0060*	---	---	---	*0.0069*	---	---	---	---	---
ALT	---	---	---	---	-0.4697	---	---	---	---	---
n	---	---	---	---	20	---	---	---	---	---
p	---	---	---	---	*0.0367*	---	---	---	---	---
GD	0.2591	---	---	---	0.6503	0.8964	---	0.5802	---	---
n	120	---	---	---	20	10	---	20	---	---
p	*0.0043*	---	---	---	*0.0019*	*0.0004*	---	*0.0073*	---	---
SDH	0.1824	---	---	---	---	---	---	---	---	---
N	120	---	---	---	---	---	---	---	---	---
P	*0.0462*	---	---	---	---	---	---	---	---	---
ALP	-0.2177	---	---	---	---	---	---	---	---	---
n	120	---	---	---	---	---	---	---	---	---
p	*0.0169*	---	---	---	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---	---	---	---

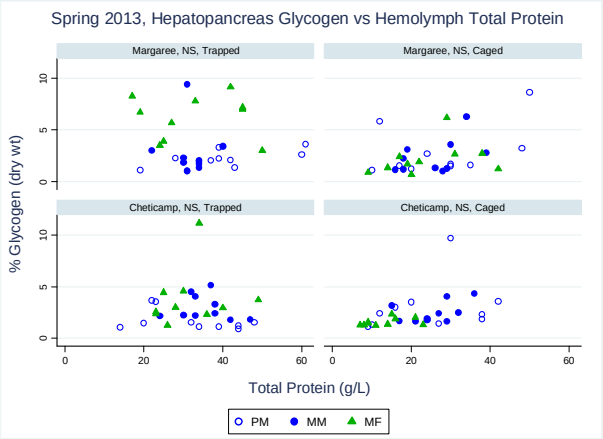


Figure 13. Scatterplot showing the average glycogen (% HP dry wt) in hepatopancreas vs. hemolymph plasma total protein concentration of snow crab collected from two stations in CFA 19 in June 2013 by trapping or, after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

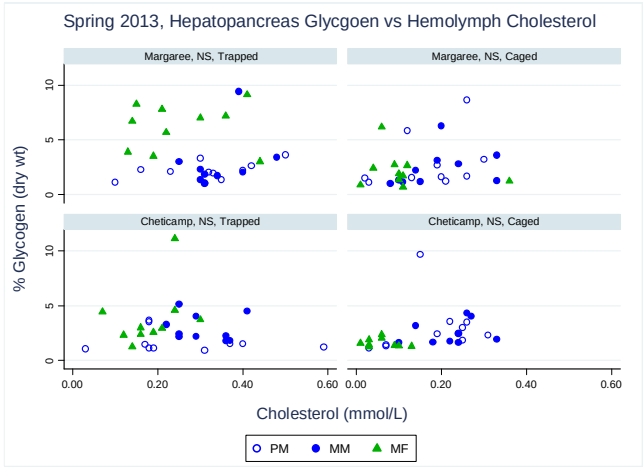


Figure 15. Scatterplot showing the average glycogen (% HP dry wt) in hepatopancreas vs. hemolymph plasma cholesterol concentration of snow crab collected from two stations in CFA 19 in June 2013 by trapping or, after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

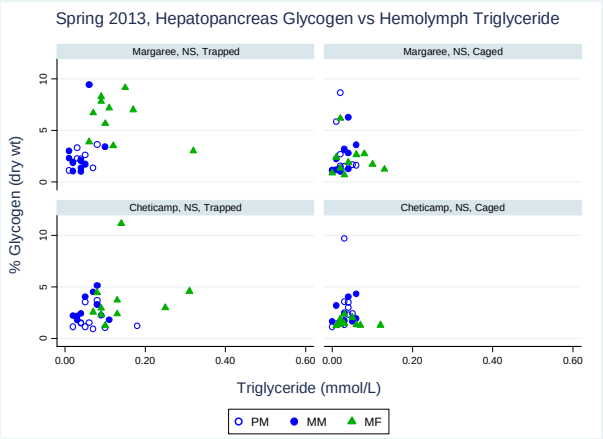


Figure 14. Scatterplot showing the average glycogen (% HP dry wt) in hepatopancreas vs. hemolymph plasma triglyceride concentration of snow crab collected from two stations in CFA 19 in June 2013 by trapping or, after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

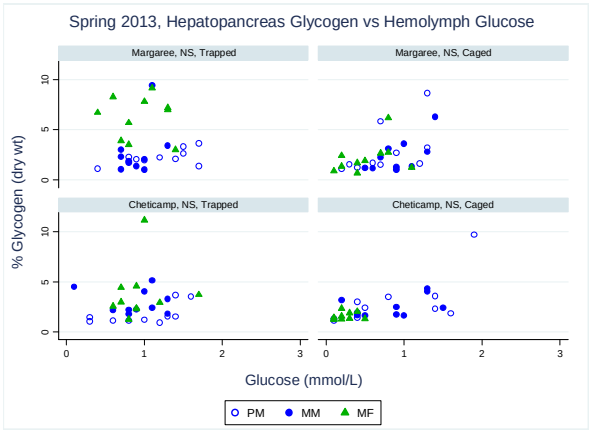


Figure 16. Scatterplot showing the average glycogen (% HP dry wt) in hepatopancreas vs. hemolymph plasma glucose concentration of snow crab collected from two stations in CFA 19 in June 2013 by trapping or, after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

Table 45. Summary of equations and associated R^2 values for simple linear regression models for average hepatopancreas glycogen (% HP dry wt) for four plasma biochemistry parameters of snow crab collected from two stations in CFA 19 in June 2013, by trapping. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree Harbor	PM	Avg Glycogen = 0.0264*TP + 1.09	0.2156
		LM	Avg Glycogen = 0.0055*TP + 1.79	0.0010
		MF	Avg Glycogen = -0.0005 *TP + 6.23	0.0000
	Cheticamp	PM	Avg Glycogen = -0.0005*TP + 1.26	0.0010
		LM	Avg Glycogen = -0.02868*TP + 3.97	0.0236
		MF	Avg Glycogen = 0.0248*TP + 2.24	0.0425
Triglyceride (TG)	Margaree Harbor	PM	Avg Glycogen = 1.1947*TG + 2.05	0.0010
		LM	Avg Glycogen = 10.3734*TG + 1.59	0.1213
		MF	Avg Glycogen = -9.9480*TG + 7.49	0.1248
	Cheticamp	PM	Avg Glycogen = -1.1856*TG + 1.32	0.0683
		LM	Avg Glycogen = 9.5472*TG + 2.38	0.0571
		MF	Avg Glycogen = 5.6890*TG + 2.22	0.1987
Cholesterol (Chol)	Margaree Harbor	PM	Avg Glycogen = 2.2672*Chol + 1.43	0.1446
		LM	Avg Glycogen = 5.0426*Chol + 0.28	0.1701
		MF	Avg Glycogen = 1.1674*Chol + 5.92	0.0040
	Cheticamp	PM	Avg Glycogen = 0.3473 *Chol + 1.14	0.0699
		LM	Avg Glycogen = -2.5226*Chol + 3.72	0.0185
		MF	Avg Glycogen = 3.8035*Chol + 2.34	0.0584
Glucose(Gluc)	Margaree Harbor	PM	Avg Glycogen = 0.6653*Gluc + 1.32	0.1875
		LM	Avg Glycogen = 1.4108*Gluc + 0.73	0.1142
		MF	Avg Glycogen = -0.2305*Gluc + 6.43	0.0013
	Cheticamp	PM	Avg Glycogen = 0.1297*Gluc + 1.12	0.0606
		LM	Avg Glycogen = -0.5742*Gluc + 3.47	0.0292
		MF	Avg Glycogen = 0.6207*Gluc + 2.43	0.0378

Table 46. Summary of equations and associated R^2 values for simple linear regression models for average hepatopancreas glycogen (% HP dry wt) for four plasma biochemistry parameters of snow crab collected from two stations in CFA 19 in June 2013, after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree Harbor	PM	Avg %Glycogen = 0.0441*TP + 0.64	0.5078
		LM	Avg %Glycogen = 0.0377*TP + 1.02	0.0838
		MF	Avg %Glycogen = 0.0278*TP + 1.06	0.1678
	Cheticamp	PM	Avg %Glycogen = 0.0243*TP + 1.69	0.1169
		LM	Avg %Glycogen = 0.0699*TP + 0.72	0.2032
		MF	Avg %Glycogen = 0.0282*TP + 1.20	0.1662
Triglyceride (TG)	Margaree Harbor	PM	Avg %Glycogen = 5.2154*TG + 1.67	0.0182
		LM	Avg %Glycogen = 35.8671*TG + 1.04	0.4636
		MF	Avg %Glycogen = 2.9125*TG +1.57	0.0285
	Cheticamp	PM	Avg %Glycogen = 27.7098*TG + 1.37	0.2603
		LM	Avg %Glycogen = 13.5087*TG + 2.04	0.0675
		MF	Avg %Glycogen = -3.0704*TG + 1.71	0.0717
Cholesterol (Chol)	Margaree Harbor	PM	Avg %Glycogen = 4.3142*Chol + 1.10	0.3578
		LM	Avg %Glycogen = 5.8324*Chol + 0.88	0.3137
		MF	Avg %Glycogen = -1.2085*Chol + 1.86	0.0249
	Cheticamp	PM	Avg %Glycogen = 6.7276*Chol + 1.03	0.5302
		LM	Avg %Glycogen = 3.6684*Chol + 1.68	0.0575
		MF	Avg %Glycogen = -1.7837*Chol + 1.68	0.0324
Glucose(Gluc)	Margaree Harbor	PM	Avg %Glycogen = 1.3498*Gluc + 0.87	0.5679
		LM	Avg %Glycogen = 1.4997*Gluc + 0.66	0.1436
		MF	Avg %Glycogen = 0.6476*Gluc + 1.40	0.0780
	Cheticamp	PM	Avg %Glycogen = 0.7102*Gluc + 1.74	0.1976
		LM	Avg %Glycogen = 0.9901*Gluc + 1.66	0.1901
		MF	Avg %Glycogen = 0.5899*Gluc + 1.43	0.0434

Table 47. Summary of equations and associated adjusted R^2 values for multiple linear regression models for average hepatopancreas glycogen (% HP dry wt) for four plasma biochemistry parameters and carapace width of snow crab collected from two stations in CFA 19 in June 2013, by trapping (free). Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

LOCATION	GROUP	SIMPLIFIED MULTIPLE REGRESSION EQUATION	ADJUSTED R^2
Margaree Harbor	PM	Avg %Glycogen = 1.14*Gluc + 0.36*Chol – 34.31*Trig + 0.02*TP + 0.91	0.0671
	LM	Avg %Glycogen = -1.77*Gluc + 16.08*Chol + 25.59*Trig – 0.25*TP + 5.46	0.2994
	MF	Avg %Glycogen = 0.13*Gluc + 22.85*Chol – 29.65*Trig – 0.06*TP + 6.34	0.0107
Cheticamp	PM	Avg %Glycogen = -0.07*Gluc + 1.82*Chol – 3.98*Trig – 0.01*TP + 1.58	0.0316
	LM	Avg %Glycogen = -4.07*Gluc – 19.25*Chol + 32.08*Trig – 0.07*TP + 7.75	0.0540
	MF	Avg %Glycogen = 0.75*Gluc – 3.15*Chol + 6.73*Trig + 0.01*TP + 1.43	-0.4791
Margaree Harbor	PM	Avg %Glycogen = 0.35*Gluc + 2.70*Chol – 33.90*Trig + 0.03*TP +0.07*CW – 4.39	-0.0715
	LM	Avg %Glycogen = -0.01*Gluc + 29.13*Chol – 4.93*Trig – 0.24*TP + 0.15*CW – 17.83	0.5787
	MF	Avg %Glycogen = 3.04*Gluc + 6.73*Chol – 14.62 *Trig – 0.13*TP – 0.27*CW + 27.55	0.1241
Cheticamp	PM	Avg %Glycogen = -0.09*Gluc + 2.31*Chol – 4.74*Trig – 0.01*TP – 0.02*CW + 2.97	0.1669
	LM	Avg %Glycogen = -4.25*Gluc – 18.43*Chol + 30.05*Trig + 0.06*TP – 0.03*CW + 12.28	-0.1364
	MF	Avg %Glycogen = -10.45*Gluc – 8.80*Chol – 13.37*Trig + 0.61*TP + 0.37*CW – 29.85	0.1012

Table 48. Summary of equations and associated adjusted R^2 values for multiple linear regression models for average hepatopancreas glycogen (% HP dry wt) for four plasma biochemistry parameters and carapace width of snow crab collected from two stations in CFA 19 in June 2013, after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

LOCATION	GROUP	SIMPLIFIED MULTIPLE REGRESSION EQUATION	ADJUSTED R^2
Margaree Harbor	PM	Avg %Glycogen = 1.26*Gluc + 2.56*Chol – 25.42*Trig + 0.01*TP + 0.77	0.6602
	LM	Avg %Glycogen = 2.58*Gluc + 0.03*Chol + 52.66*Trig – 0.13*TP + 1.69	0.2635
	MF	Avg %Glycogen = -3.76*Gluc – 5.96*Chol + 5.40*Trig + 0.15*TP + 0.25	0.3469
Cheticamp	PM	Avg %Glycogen = -0.83*Gluc + 10.52*Chol – 9.72*Trig + 0.01*TP + 0.90	0.1625
	LM	Avg %Glycogen = 0.52*Gluc – 2.13*Chol + 6.16*Trig + 0.04*TP + 1.22	-0.3981
	MF	Avg %Glycogen = -2.73*Gluc – 4.18*Chol + 2.60*Trig + 0.09*TP + 1.05	-0.0909
Margaree Harbor	PM	Avg %Glycogen = 1.14*Gluc + 2.77*Chol – 25.60*Trig + 0.01*TP + 0.00*CW + 0.44	0.5049
	LM	Avg %Glycogen = 2.33*Gluc – 1.14*Chol + 17.06*Trig + 0.01*TP + 0.12*CW – 15.70	0.3767
	MF	Avg %Glycogen = -3.96*Gluc – 6.13*Chol + 4.65*Trig + 0.16*TP – 0.02*CW + 1.82	0.1631
Cheticamp	PM	Avg %Glycogen = 1.69*Gluc + 11.02*Chol – 37.42*Trig – 0.09*TP – 0.07*CW + 8.71	0.8073
	LM	Avg %Glycogen = 4.62*Gluc + 17.20*Chol – 3.75*Trig – 0.29*TP – 0.23*CW + 31.14	0.6054
	MF	Avg %Glycogen = -2.72*Gluc – 4.18*Chol + 2.59*Trig + 0.09*TP + 0.00*CW + 1.04	-0.3637

D. Hepatopancreas Glycogen vs. Hepatopancreas Lipid Content

Spearman rank correlation co-efficients were calculated using absolute values of hepatopancreas lipid and glycogen content for all crabs (Table 49). While the correlation for all crabs was found to be statistically significant (p = 0.0010), the actual value for rho was quite low (0.2974). A remarkably high correlation (0.9030) was found for caged $LM_{Margaree}$. Scatterplots of the data showed a tendency for tighter agreement for crabs caught in Cheticamp (Figure 17).

Ratios for average hepatopancreas lipid to glycogen content (% HP dry wt) were calculated for all crabs. Boxplots (Figure 18) identified multiple outliers (#s 262,287, 224, 68, 104, 143, and 128) which were also evident in the frequency distribution histograms (Figure 19). Kruskall-Wallis testing showed a difference among sex within a treatment method and station for all combinations Margaree Harbor free (p = 0.0003), Margaree Harbor caged (p = 0.0078), Cheticamp free (p= 0.0287), and Cheticamp caged (p = 0.0258). No further analysis completed at this time.

Table 49. Summary of Spearman's rank coefficients, sample size, and significance level for average hepatopancreas lipid (%HP dry wt) *vs* hepatopancreas glycogen content (%HP dry wt) of snow crab collected in traps or after six months of caging in June 2013. Results are shown for all crabs and crabs grouped by sex, region, and treatment group.

	All Crabs	Pygmy Male					Large Mature Male					Mature Female				
		Marg[1]			Chet[2]		Marg			Chet		Marg			Chet	
		All	Trap	Cage	Trap	Cage	All	Trap	Cage	Trap	Cage	All	Trap	Cage	Trap	Cage
rho	0.2974	---	---	---	---	---	0.3578	---	0.9030	---	---	0.5099	---	---	---	0.6485
n	120	40	10	10	10	10	40	10	10	10	10	40	10	10	10	10
p	0.0010	---	---	---	---	---	0.0234	---	0.0003	---	---	0.0008	---	---	---	0.0425

[1.] Margaree Harbor, NS [2.] Cheticamp, NS

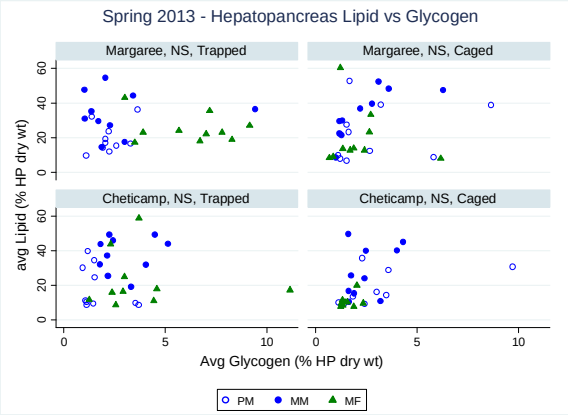


Figure 17. Scatterplot demonstrating relationship between absolute hepatopancreas lipid and glycogen concentrations for snow crab collected from at two stations in CFA 19 in June 2013 by traps or after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF).

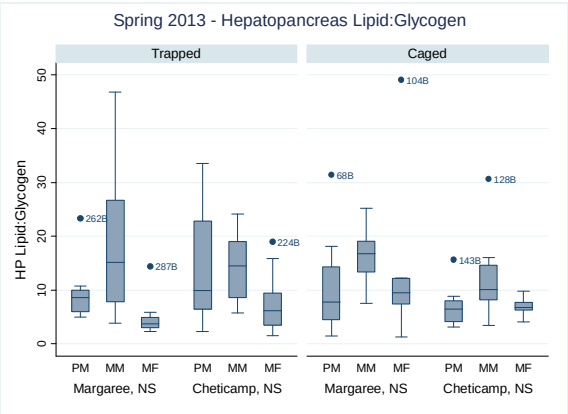


Figure 18. Box and Whisker plots demonstrating relationship between hepatopancreas lipid and glycogen ratios for snow crab collected from two stations in CFA 19 in June 2013 by traps or after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF).

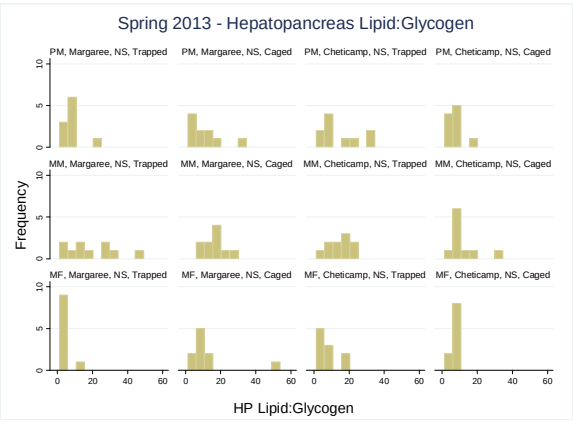


Figure 19. Frequency distribution histogram demonstrating relationship between hepatopancreas lipid and glycogen ratios for snow crab collected from two stations in CFA 19 in June 2013 by trapping or after six months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF).

E. Hepatopancreas Moisture Content

The average HP moisture content as a percentage of wet weight is presented in Figures 20 – 22 as boxplots, frequency distribution histograms and scatterplot vs. carapace width, respectively. Numerous outliers were identified on the boxplots (#'s 261B, 262B, 267B, 269B, 221B, 22B8) and are also evident on the histograms. No pattern for moisture content and CW was apparent. Summary statistics for average HP percent moisture content by category and station are provided in Tables 50 & 51, for free and caged crabs, respectively.

Effect of Treatment (Caged vs Free) within a Station by Sex

Comparison (Wilcoxon rank-sum) of treatment method (caged vs free) within a station by sex, found hepatopancreas moisture content in Margaree Harbor $MF_{caged} > MF_{free}$ crabs ($p = 0.0305$), Cheticamp $MF_{caged} > MF_{free}$ ($p = 0.0301$), and Cheticamp $LM_{caged} > LM_{free}$ ($p = 0.0494$). No differences were detected for PM crabs.

Effect of Sex within a Station by Treatment (Caged or Free)

Kruskall-Wallis testing across crab sex categories within station and treatment showed statistically significant differences for free crabs collected in Cheticamp only ($p = 0.0118$). Subsequent Wilcoxon testing, at Bonferroni-adjusted p-value of 0.0167, failed to identify a significant difference although there was a trend for LM crabs to have slightly lower percent moisture content compared to both MF ($p = 0.0343$) and PM ($p = 0.0191$) crabs.

Effect of Station within a Treatment by Sex

Comparison (Wilcoxon rank-sum) between stations within a treatment, by sex, found no differences.

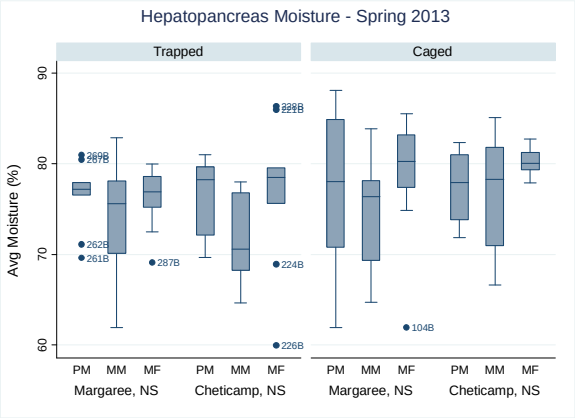


Figure 20. Box and Whisker plot showing distribution of average hepatopancreas percent moisture content for Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF) snow crab collected by trapping (free) or after six months of caging, at two stations in CFA 19, spring (June) 2013.

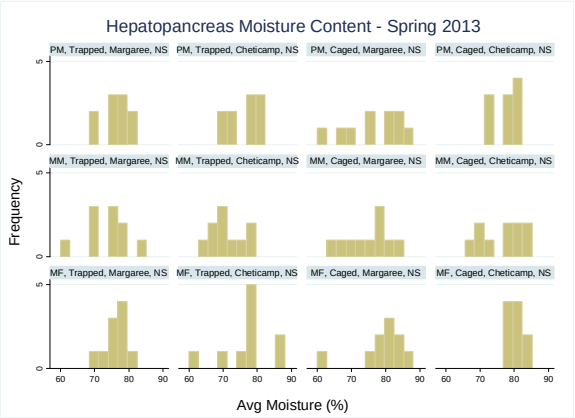


Figure 21. Frequency distribution histogram showing average hepatopancreas percent moisture content for Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF) snow crab collected by trapping (free) or after six months of caging, at two stations in CFA 19, spring (June) 2013.

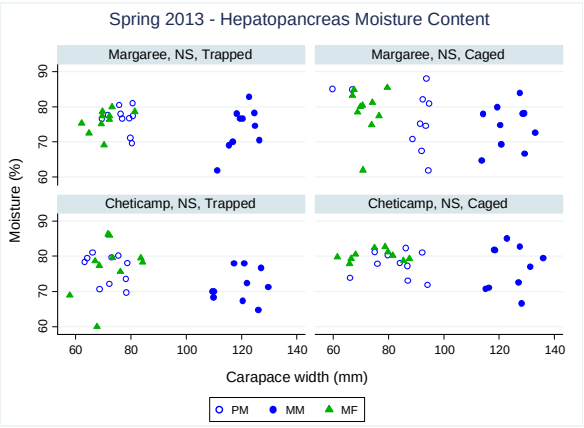


Figure 22. Scatterplot showing average hepatopancreas percent moisture content by carapace width for Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF) snow crab collected by trapping (free) or after six months of caging, at two stations in CFA 19, spring (June) 2013.

Table 50. Summary statistics for average hepatopancreas moisture content ((% wet wt) for snow crab collected by traps at two stations in CFA 19, June 2013. Median values by crab category within a station were significantly different (Kruskall-Wallis test) for Cheticamp only; however no differences were noted with pairwise comparisons ($p < 0.0167$).

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	PM	10	76.5355	3.6021	77.1651	69.6246	80.9727	2.7786	-0.8486
	LM	10	73.8713	6.0132	75.5963	61.9337	82.8335	2.6879	-0.5274
	MF	10	76.0545	3.2446	76.9036	69.1059	79.9586	3.1442	-0.9717
Cheticamp	PM	10	76.2652	4.3113	78.2245	69.6575	81.0151	1.5300	-0.4518
	LM	10	71.6380	4.6159	70.5769	64.6714	77.9888	1.8460	0.2124
	MF	10	77.0142	7.7601	78.4730	59.9717	86.3371	3.4971	-0.9831

Table 51. Summary statistics for average hepatopancreas moisture content ((% wet wt) for snow crabs at two stations in CFA 19, collected in June 2013 after a six month caging period. Median values by crab category within a station nor across stations were not significantly different.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	PM	10	77.0966	8.5370	78.0099	61.9412	88.0708	1.9990	-0.4072
	LM	10	74.5941	6.1657	76.3446	64.7418	83.8556	1.9507	-0.2775
	MF	10	78.8017	6.7712	80.2545	61.9550	85.4988	4.9680	-1.5898
Cheticamp	PM	10	77.6582	3.7003	77.9359	71.8176	82.3321	1.7449	-0.3523
	LM	10	76.8772	6.2849	78.2841	66.6092	85.0902	1.6737	-0.2816
	MF	10	80.2418	1.5484	80.0383	77.8982	82.7292	2.1386	0.3131

F. Hepatopancreas Composition by Wet Weight

The relative contribution of each category (lipid, glycogen, and moisture) to hepatopancreas composition by wet weight was determined by calculating a fourth category 'Other' (Other = 100% - %moisture - % lipid - %glycogen) to represent all non-directly measured components. The 'Other' category would include protein, mineral/ash, etc. The summarised results are presented by station, crab sex and treatment in Figure 23. All outliers for any of the three directly measured categories were excluded from the calculation.
Moisture was the largest category for any treatment, crab category, or station combination followed by the 'other', lipid, and glycogen categories. The general trend was for relative decreases or no changes in the lipid, other, and glycogen categories with concomitant increase in moisture content with caging.

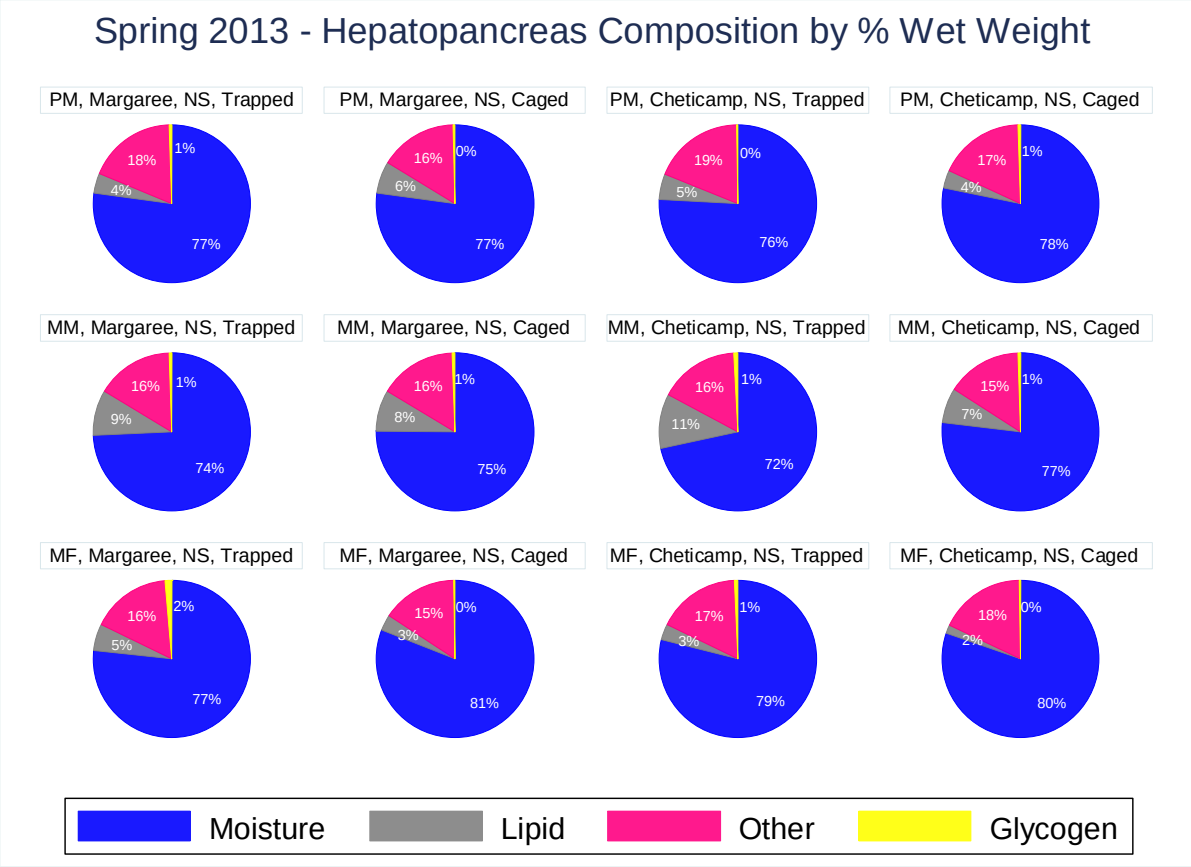


Figure 23. Pie chart indicating the relative composition of the hepatopancreas on a percent wet weight basis for snow crab collected in spring (June) 2013 by trapping (free) or after a six month caging period at two sites, Cheticamp and Margaree Harbor, NS. All outliers as identified in the percent lipid, percent glycogen, and percent moisture have been removed. Graphs show data for three crab categories Pygmy Male (PM), Large Mature Male (MM), and Mature Female (MF).

VIII-2-5 Conclusions

The study met all of its objectives in that hemolymph biochemistry parameters, hepatopancreas lipid, glycogen, lipid:glycogen ratios, and moisture data were collected and compared among free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 214 and 222 days at Margaree and Cheticamp stations in spring 2013. The caging period was associated with lower values for many hemolymph biochemistry parameters, primarily metabolites, in all crabs and were anticipated given the presumption that caged crabs would have restricted access to food sources (quantity and quality). In contrast, significantly lower levels of hepatopancreas lipid and glycogen were observed in MF crabs only presumably related to spring spawning. Moderate to good correlations for hemolymph total protein, and occasionally cholesterol, triglyceride, or glucose, to hepatopancreas lipid stores were observed, particularly in free PM crabs. Measurement of total body (hepatopancreas, muscle, and gonad) lipid, glycogen, and moisture content may improve correlations of hemolymph parameters to total body energy reserves in all crab categories. The tendency for PM and MF crabs to be more similar to other than LM crabs was noted again as in the fall 2012 study.

Electrolytes and Minerals

The most consistent and relevant changes in this category were seen for calcium and magnesium concentrations. Magnesium levels were higher in MF (free and caged) at both stations while calcium and magnesium were higher for caged crabs collected from Cheticamp. Given the tendency for calcium and mineral levels to trend with total protein to which they are bound in other species (Duncan *et al.* 1994) and supported by results in (Section VIII-1), this was unexpected.

Elevated calcium and magnesium levels have been associated with prolonged emersion in the lobster, *Homarus americanus* (Lorenzon *et al.* 2007, A. Battison, pers. Obs.). Emersion with attendant anaerobic glycolysis and increased lactate should result in a decreased hemolymph pH (metabolic acidosis) (Lorenzon *et al.* 2007). It is anticipated that under these conditions, crustaceans will first consume the supply of readily available buffers in the circulation and then have to draw upon the cuticle-based carbonates stored as calcium and magnesium carbonate to offset the acidosis (Kunkel 2013). This would result in a concurrent rise in hemolymph calcium and magnesium levels. Mature female crabs were always sampled last (longest emersion times) at all stations, either treatment group, which could explain the higher magnesium values in MF crabs. Magensium levels were also higher in MF crabs during the trawl study (Section VII-3) when there was little difference in emersion time among crab categories, suggesting a sex-related factor may also need to be considered. Oocytes in spring have high/sig levels of magnesium. In the fall, transport of magnesium to the developing oocyte in the fall and resorption of unspawned oocytes in the spring may be contributing to hemolymph magnesium levels. Review of the sample collection data with DFO staff revealed that the total emersion times for the four

collection trips were quite different: Margaree Harbor free at 3h:53m; Margaree Harbor caged at 5h:32m, Cheticamp free at 6h:24m; and, Cheticamp caged at 7h:23m. The longest emersion times for caged Cheticamp crabs could explain the higher values for calcium and magnesium in that group.

Free and caged crabs were presumably located within reasonably close proximity within each station such that crabs would be expected to be exposed to similar salinities. As snow crabs are osmoconformers, their internal sodium and chloride concentrations should reflect their surroundings in the absence of other factors (Hardy *et al.* 1994). Slight increases in median sodium and chloride concentrations were associated with caging for $PM_{Margaree}$, $LM_{Margaree}$, $PM_{Cheticamp}$, and $MF_{Cheticamp}$ crabs but were only statistically significant for Cheticamp crabs. It is possible that the longer emersion time for caged Cheticamp crabs may have caused a 'dehydration' effect which enhanced the increase. Conversely, the significant decrease for caged $LM_{Cheticamp}$ remains unexplained. If caging is having an effect on sodium and chloride concentrations, this may become more evident in the 12 month caging study results.

Phosphorus levels tended to be slightly higher in hemolymph from Cheticamp crabs, although not always significantly so. The within-run precision (co-efficient of variation) for the phosphorus assay is 4.9% (see Section VII-2 plasma stability study) which is not ideal; however, it is likely that results from Cheticamp crabs are truly higher. In vertebrates, phosphorus levels are often determined by dietary intake. Stomach content analysis results may provide support for a dietary cause for the difference. Whether the mineral/phosphorus content of the water at each station could affect hemolymph phosphorus levels requires further investigation.

Metabolites

Total protein concentrations are a reflection of the balance of protein intake, loss, production, stores, and metabolism (Claybrook 1983, Duncan *et al.* 1994). Determination of hemolymph total protein content is via the biuret reaction which measures peptide bonds (Duncan *et al.* 1994). The 'albumin' assay employs the dye bromocresol green (BCG) which binds preferentially to specific proteins (Duncan *et al.* 1994). In vertebrate species, this protein is albumin. The protein(s) that are differentially bound by BCG in snow crab is (are) not known; however, the oxygen-carrying protein hemocyanin is suspected to be a component of this fraction in American lobsters (Summerfield & Battison 2009). The term 'globulins' refers to the non-BCG binding protein and is a calculated value (total protein – 'albumin' = globulin). Decreased intake associated with caging is the probable cause of lower total protein values in all crabs at both stations. The increased A:G ratio noted in caged crabs is most likely due to a disproportionate decrease in non BCG-binding (globulin) compared to BCG-binding (albumin) protein. This might indicate a relative protection of the BCG-binding fraction, which could make sense if this fraction proves to be rich in hemocyanin-like proteins.

Ammonia is the primary means of removing nitrogenous waste produced from protein catabolism in aquatic crustacean as ammonia easily diffuses across the gills (Claybrook 1983). Ammonia is labile and was not measured in this study. Urea is also produced, but in lower amounts (Claybrook 1983). Urea levels tended to be lower in caged crabs which could support decreased production due to decreased protein intake.

Hemolymph uric acid levels in crustaceans are understood to be related to purine metabolism – e.g., turnover of endogenous and exogenous nucleic acids and dietary intake (Claybrook 1983). Caged crabs generally had lower, often statistically significant, uric acid concentrations compared to free crabs. Lower protein intake by caged crabs is a probable cause. Large mature male crabs, caged or free, always had the lowest levels. This may be related to an underlying difference in diet i.e., lower purine content in MM crabs compared to PM or MF crabs. It would also be interesting to determine if the larger male crab has a lower metabolic rate compared to smaller PM and MF crabs at a given temperature, as this could possibly be associated lower nucleic acid turnover.

Hemolymph glucose concentrations are expected to be determined by numerous factors including diet, stress induced by handling during sample collection, emersion, and total body glycogen stores (Lorenzon *et al.* 2007). The breakdown of glycogen in tissue stores via glycogenolysis generates glucose. Hepatopancreas glycogen was measured in this study but glycogen is also found in muscle tissue in adult male snow crab (Hardy *et al.* 2000, Mayrand *et al.* 2000). It is presumed that the primary role of muscle glycogen is to provide glucose to muscle tissue directly via glycogenolysis occurring in the muscle. Whether muscle glycogen would be converted to glucose and released into hemolymph for use by other tissues under conditions of nutritional stress e.g., caging, is unknown. While median glucose values were generally lower in caged crabs, there was a lot of variation with some of highest values seen in caged Cheticamp crabs as a group. This group had the longest emersion times i.e., greatest emersion stress, which might account in part for the high values.

Lactate, the product of anaerobic glycolysis, is similarly expected to be related to duration of emersion and total tissue glycogen stores (Lorenzon *et al.* 2007). Lactate levels were significantly lower in caged MF and PM crabs at both stations compared to free crabs, while hepatopancreas glycogen stores were lower in caged MF crabs only. Muscle glycogen was not measured but may be a relevant source of lactate and therefore should be evaluated in future studies.

Hemolymph triglyceride levels reflect the movement of lipids through the body and would be expected to be determined by: dietary lipid content, transfer of lipid from intestinal tract to hepatopancreas via hemolymph for storage (if applicable in this species); transport of stored lipid reserves in the hepatopancreas to peripheral tissues for use as energy; and, for females, transport of lipid to the ovary for oocyte development (Battison *et al.* 2011) and possibly, transfer of lipid from the ovary to the hepatopancreas as part of the physiological post-spawning resorption of

unspawned oocytes in the spring, or if oocyte development was aborted and the oocytes reabsorbed part of a pathological process. The significantly higher plasma triglyceride values for free MF crabs at both stations compared to PM and MM crabs may be explained by physiologic resorption as all free MF had spawned based on histological assessment of ovaries (see Section IV-4-5). Caged MF crabs had lower median hemolymph triglyceride levels than free MF crabs at each station, but not different from caged PM or LM. While most (84% at Cheticamp, 60% at Margaree Harbor) had also spawned (See Section IV-4-5) and physiologic resorption would be expected, the amount of ovarian lipid available for resorption was unknown and may have been low in caged MF crabs.

Lower triglyceride values were noted for all caged compared to free crabs, although not significantly so for LM crabs, at both stations. It is possible that the diet of LM crabs contains less lipid than PM or MF crabs so was not as affected by caging. Alternately, LM crabs may have different metabolic requirements for lipid than the other crab types.

In vertebrates, cholesterol has roles as a precursor for steroid hormones; in cell membrane structure; and, in the lipoproteins used for lipid transport (Gurr *et al*. 2002). Similar roles may occur in crustaceans. The lower median cholesterol values noted for all groups of caged crabs could represent a reduced level of lipid transport as a result of decreased dietary intake. That MF crabs at either station, whether free or caged, had statistically lower cholesterol levels is an intriguing observation. Cholesterol stores may have been consumed for oocyte production prior to spawning.

Enzymes

Hemolymph enzyme activity will be directly related to the amount of enzyme present in a particular tissue and its rate of release (Moss & Henderson 1998). The former will be related to total tissue mass and intracellular enzyme levels. The latter can be increased with tissue injury e.g., trauma, toxicity, hypoxia and physiological states in vertebrates e.g., stress (Moss & Henderson 1998).

Amylase and lipase are digestive enzymes that break down carbohydrates and lipids, respectively (Moss & Henderson 1998). In snow crab, both enzymes are found in the hepatopancreas with high levels of amylase also in hemocytes while lipase is also detected in muscle and some other tissues (see Section VII-1). Recent (within days) feeding has been associated with higher hemolymph activity of these enzymes in American lobsters (A. Battison, pers. Obs.). As the much higher lipase values were only detected in free crabs in the current study, it is possible that this is also an indication of recent feeding in snow crab; however this would require more specific investigation. Crabs with higher lipase activity did not have high amylase activity, possibly indicating a non-feeding cause for the amylase activity detected. Hemolymph clotting, which involves lysis of hemocytes, can result in increased amylase activity and may have been a factor in some samples (see Section VII-1tissue distribution).

Caging, *per se*, did not affect the amount of hemolymph AST or ALT enzyme activity detected. Higher levels were most often seen in the smaller PM or MF crabs. As both enzymes are found predominantly in muscle tissue, with moderate levels in the hepatopancreas (see Section VII-1), muscle was considered the most likely source of the hemolymph activity detected in this case. Possibilities include inadvertent contamination of the hemolymph sample with muscle tissue fluid during difficult collection in smaller crabs or recent autotomy of limbs. Autotomy would be expected to cause a degree of muscle injury and hence, increased release of enzyme into the circulation in the short term. The data sheets provided did indicate which legs, if any, were missing but not if these were losses were recent. Previous injuries which had healed to the point of melanisation would not be expected to be associated with high muscle enzyme activity unless there was ongoing muscle damage or inflammation. For future studies, recording of more data (e.g., degree, duration) of any injuries noted on the crabs is recommended to assist with interpretation of hemolymph biochemistry data.

Glutamate dehydrogenase (GD) activity was mildly decreased in all caged crab groups compared to free crabs; although, this was only statistically significant for PM and MF crabs from Cheticamp. This could reflect decreases in muscle mass and overall enzyme content; however, the changes are subtle at this point and would not usually be considered of diagnostic significance.

Hepatopancreas Lipid Content

Hardy *et al.* (2000) determined the lipid content of the hepatopancreas to be approximately 30% of tissue weight wet and only 2% of leg (merus) muscle wet weight indicating that the hepatopancreas is the main lipid reserve in spring caught, adult male crabs. Crabs collected by trapping are considered representative of crabs with *ad libitum* feeding practices in their respective stations (Margaree Harbor, NS and Cheticamp, NS). The short soak time (two days) was assumed to have had little effect on lipid reserves for free crabs. By comparison, the food source for crabs which were caged for seven months would have been limited to that which entered, attached to, or grew adjacent to, the cages. In retrospect, determination of pre-spawning/pre-breeding gonad lipid content may have provided a better indication of total body lipid reserves and should be included in the methodology for future studies.
Surprisingly, the only significant difference detected was for lower hepatopancreas lipid content in caged MF crabs than free crabs. This is attributed to redistribution of hepatopancreas lipid reserves to the ovary for oocyte development and spawning, with caged crabs unable to recoup lipid reserves as effectively as free crabs.

Possibilities to consider for the lack of difference for caged PM and LM crabs compared to free PM and LM crabs, presuming hepatopancreas lipid stores reflect the net result of energy intake and expenditure, include: 1) decreased energy expenditure under caged conditions e.g., decreased locomotion to search for food or evade predators, etc. was balanced by decreased energy intake ; 2) the energy content of the diet available to caged crabs over the winter was

adequate and energy expenditure is the same for caged and free-ranging crabs; 3) lipid was not the primary energy reserve for crabs, e.g., protein from muscle may be important (Sánchez-Paz *et al.* 2006); 4) seasonal decreases in energy reserves in free crabs minimised the differences between caged and free crabs; and although unlikely, 5) increased dietary energy availability and increased energy expenditure in caged crabs compared to free-ranging, free crabs.

The hepatopancreas lipid content of LM crabs tended to be significantly higher than PM or MF crabs, whether caged or free, at both stations. This could indicate that LM crabs have access to more lipid in their diet (quality and/or quantity of food with a higher lipid) and/or are expending less lipid-derived energy than free PM crabs over the winter as there was minimal difference across crab categories in the fall 2012 samples. General comparisons to MF crabs are difficult due to spawning.

The only effect of station was for free MF crabs where, median hepatopancreas lipid was higher for Margaree Harbor crabs. This could indicate better foraging in that area; however, findings were opposite in the fall 2012 samples (see Section VIII-1). Alternately, the transfer of lipid to the developing oocytes may have been less progressed in Margaree Harbor crabs. Recording of ovary lipid content, in addition to wet weight, moisture and glycogen content would be advisable for future studies to help address such questions.

Hepatopancreas Glycogen Content

Glycogen was a very small percentage of the hepatopancreas mass on a wet weight basis, so it was unlikely that it was a major energy reserve of this tissue. Glycogen levels were reported for the hepatopancreas and muscle tissue of adult male snow crab (Hardy *et al.* 2000). Hardy *et al.* (2000) found relatively more (~1.74-fold) glycogen per gram of wet weight of merus muscle compared to hepatopancreas tissue. Muscle glycogen was not measured in this study but could be a relevant reserve when assessing total body stores.
Dietary glycogen would come from complex (starch, glycogen) or simple (glucose) carbohydrates. Vegetation, including algae, could be a source of dietary carbohydrate and lipid. Bivalves can also be rich in glycogen and if attached to the cages, may have provided an additional source of carbohydrate. Review of cage fouling (amount, species) records may provide useful information. Animal species entering the cages would be an additional food source.

It was expected that caged crabs would have less hepatopancreas glycogen content due to restricted access to food. Surprisingly, while glycogen levels were generally lower in caged crabs, this was only significant for caged MF crabs at both stations. This could be related to oocyte production for spring spawning as glycogen is found in oocytes (Zara *et al.* 2013). Conversly, caged PM from Cheticamp had higher median HP glycogen than free counterparts. The latter is a result of the unusually very low levels in free PM crabs from Margaree Harbor. The median HP lipid content also tended to be lower in this group, although a broad range of

values were obtained, which might reflect a decreased diet quality for some reason. There were no other consistent patterns evident.

Hepatopancreas Lipid:Glycogen Ratio

Limited analyses were completed for this parameter. Visual inspection of the data suggests a much higher value for LM, caged or free, at both stations. This is reflecting the tendency for higher HP lipid, and lower HP glycogen, content in LM crabs compared to PM or MF. If the contribution of extrahepatopancreatic (muscle) glycogen in LM crabs is greater than in PM or MF crabs, this may account for some of the differences.

Hepatopancreas Moisture Content

The finding of increased moisture content in caged crabs was expected as other hepatopancreas components e.g., lipid, glycogen, 'other' category, are utilised and replaced by water in the hepatopancreas tissue. Similar results were noted by Hardy *et al.* (2000) who suggested the use of tissue (hepatopancreas or muscle) moisture content as an indication of condition for fasted adult male snow crabs.

Hemolymph Biochemistry Parameters as Predictors of Hepatopancreas Energy Reserves

Hemolymph metabolites, particularly total protein and occasionally cholesterol, triglyceride and glucose were well-correlated to hepatopancreas lipid content, most often in free PM crabs. Correlations to hepatopancreas glycogen content were poor and generally limited to hemolymph glucose levels in PM crabs. Hardy *et al.* (2000) found the hepatopancreas of spring caught, adult male snow crab was composed of approximately 57% water, 30% lipid, 2.5 – 5.4% protein, and only 0.2% glycogen – relative proportions similar to the results of the current study with protein being included in the 'other' category. Data on hepatopancreas composition of PM and MF crabs were not included in the Hardy *et al.* (2000) report.
Which energy reserve i.e., lipid, protein, or glycogen from hepatopancreas or muscle, is utilised and in what order during starvation varies among crustacean species (Sánchez-Paz *et al.*, 2006; Hardy *et al.* 2000, Maynard 2000). Hardy *et al.* (2000) concluded that during a 154 day period of fasting in adult male snow crabs, energy was primarily derived from muscle protein and hepatopancreas lipid. Unfortunately, neither hepatopancreas nor muscle protein content was determined in this study limiting direct comparisons to Hardy *et al.* (2000). Examination of scatterplots of HP lipid vs. biochemistry parameters, suggests that there may be a threshold value for HP lipid of approximately 20% dry weight below which there is little correlation with hemolymph parameters. Above 20%, the relationships tend to adopt a more linear appearance.

Moderate to strong correlations were observed more often for free than caged crabs. It is assumed that free crabs had more access to food (amount and/or type/quality of food) than caged crabs and were in an energy neutral or even excess state. If the latter, excess energy would be

stored in tissues. In contrast, caged crabs were in a relatively energy deficient state as suggested by generally lower hepatopancreas lipid content and would be mobilising reserves to meet body demands. Transportation of nutrients, in any direction, would occur via the hemolymph and so be subject to detection by plasma biochemistry profiles. Correlations of biochemistry parameters may be better for free crabs as they are more representative of the normal flow of nutrients than caged crabs.

Simple and multiple linear regression equations had better fit when predicting hepatopancreas lipid than glycogen content, and worked better for free than for caged crabs – much as for the correlations and likely for similar reasons. Pygmy male crabs were more likely to have better fit than MF or LM crabs. Inclusion of ovary composition (lipid, glycogen, protein) in the analyses in future studies may improve the results for MF crabs. The larger size of LM crabs, potential to access a different diet with different physiology, and diversion of nutrients towards sperm production may be contributing to the poorer correlations and fit of regression equations in this group. Pygmy male crabs may be a useful indicator of environmental food resources as they are less affected by seasonal reproductive stresses associated with breeding compared to large adult males.

Hardy *et al.* (2000) suggested that the increase in hepatopancreas, and possibly muscle, moisture content during starvation may be a useful indicator of nutritional status given the inverse relationship to tissue lipid and protein stores. The presumption is that as energy reserves and nutrients are being utilised, they are replaced by water in the tissue. Significantly increased hepatopancreas moisture content was noted only for caged MF crabs and $LM_{Cheticamp}$. The former has been attributed to redistribution to ovaries for oocyte development with failure to recoup losses. Changes in muscle moisture content may have occurred but were not measured in this study. Determination of tissue (hepatopancreas or muscle) moisture content is simpler and less expensive than measuring tissue lipid, glycogen, and protein content; however, still requires sacrifice of the crab or loss of a leg. Hemolymph is an even simpler tissue to sample than hepatopancreas or muscle and does not require sacrificing the crab allowing for serial sampling. Identifying an indicator e.g., TP or A:G ratio that correlates well to total body moisture content may provide a way to assess tissue energy reserves in terminally moulted crabs of any sex, regardless of the primary energy reserved utilised.

VIII-2-6 References

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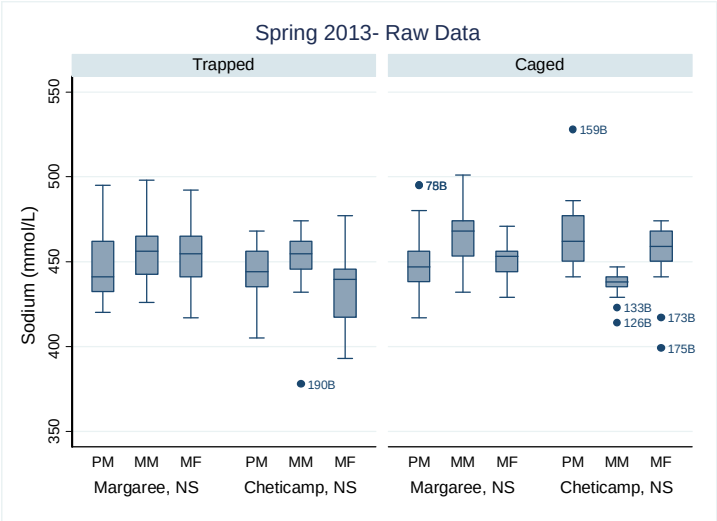
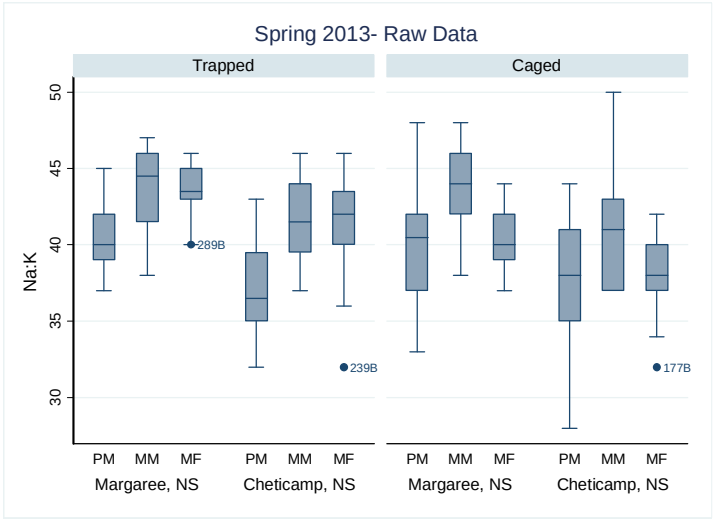
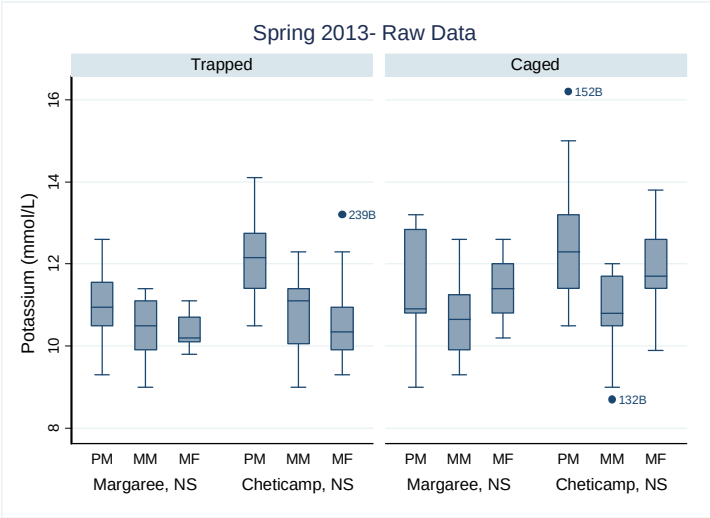
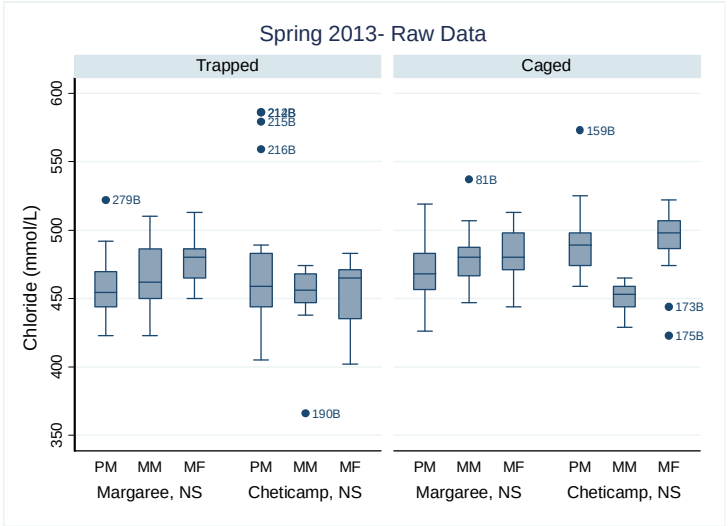
VIII-2-7 Appendices

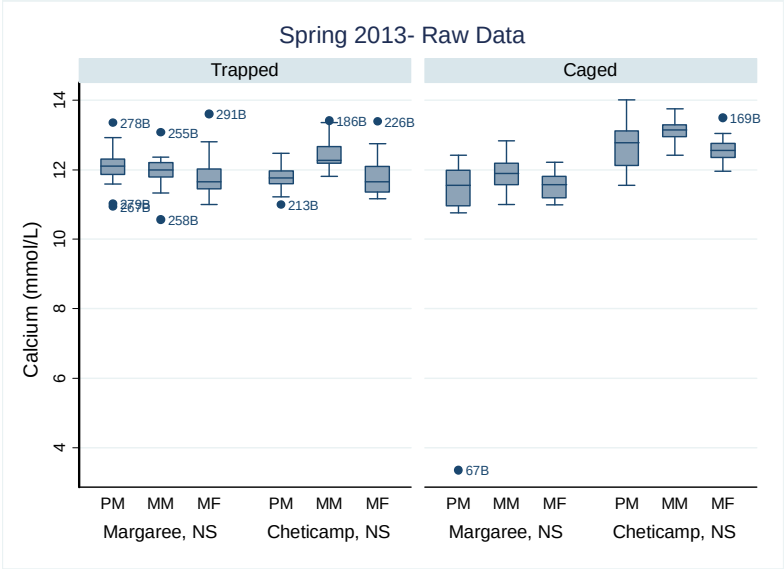
Appendix A: Box & Whisker Plots Biochemistry Profiles







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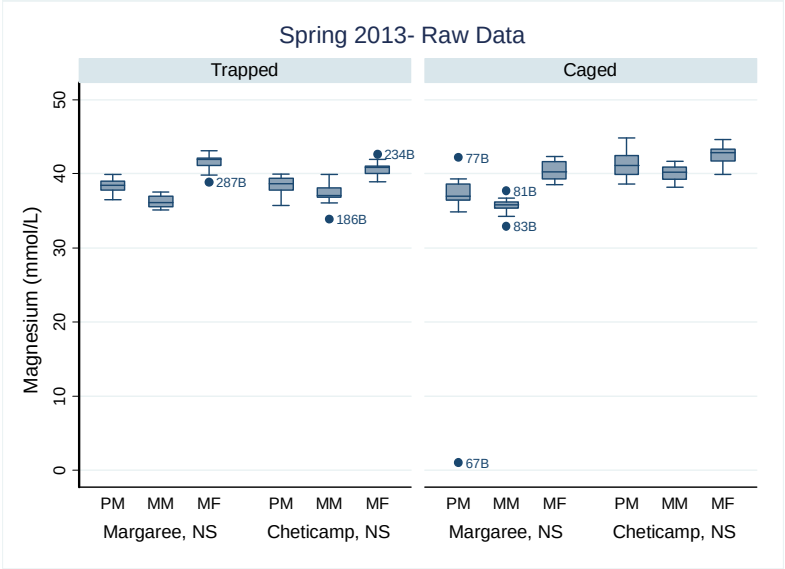
Caged
Calcium (mmol/L)
4
6
8
10
12
14
278B
255B
291B
279B
267B
258B
213B
186B
226B
67B
169B
PM MM MF PM MM MF PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS
Margaree, NS
Cheticamp, NS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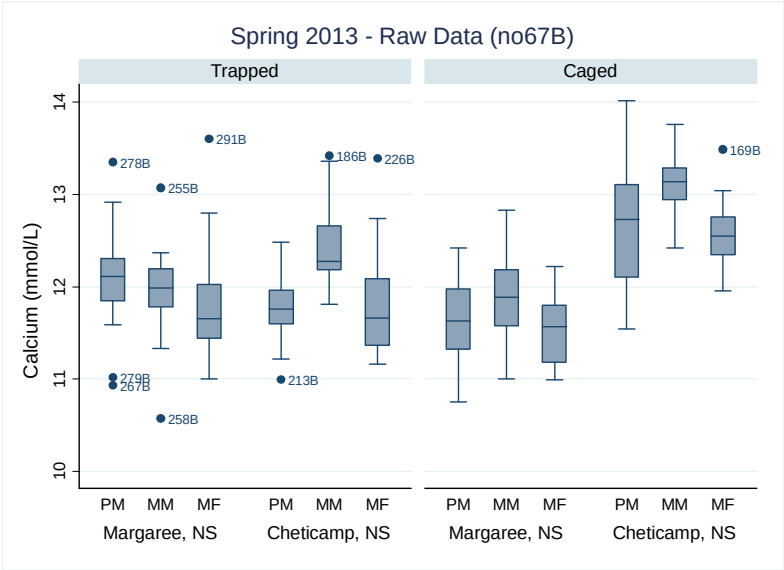
Trapped
Caged
Magnesium (mmol/L)
0
10
20
30
40
50
234B
287B
186B
77B
81B
83B
67B
PM MM MF PM MM MF PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS
Margaree, NS
Cheticamp, NS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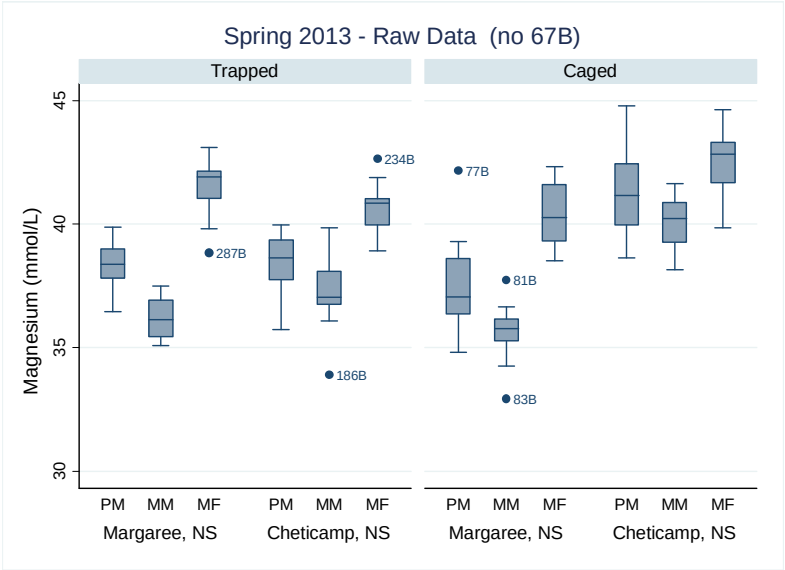
Caged
Calcium (mmol/L)
10
11
12
13
14
278B
255B
291B
279B
267B
258B
213B
186B
226B
169B
PM MM MF PM MM MF PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS
Margaree, NS
Cheticamp, NS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Caged
Magnesium (mmol/L)
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35
40
45
234B
287B
186B
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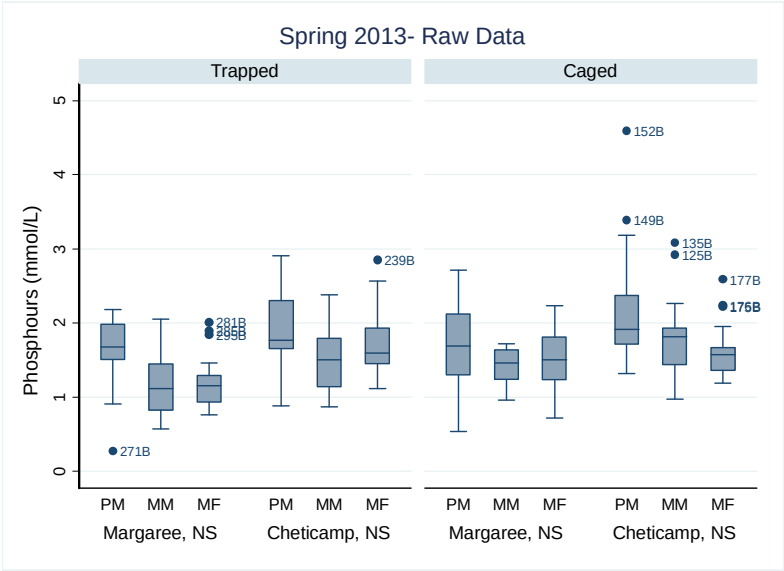
81B
83B
PM MM MF PM MM MF PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS
Margaree, NS
Cheticamp, NS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Caged
Phosphours (mmol/L)
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1
2
3
4
5
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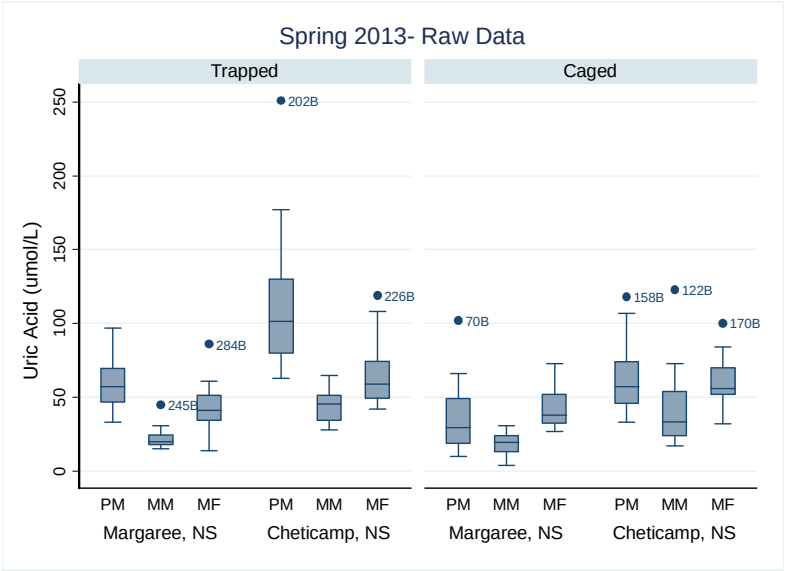
239B
281B
152B
149B
135B
125B
177B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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Caged
Uric Acid (umol/L)
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50
100
150
200
250
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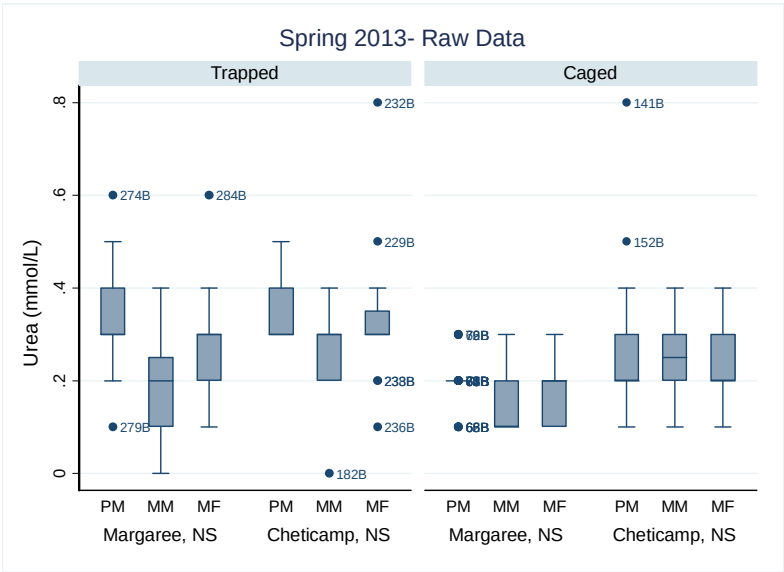
70B
158B
122B
170B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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Caged
Urea (mmol/L)
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.2
.4
.6
.8
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274B
284B
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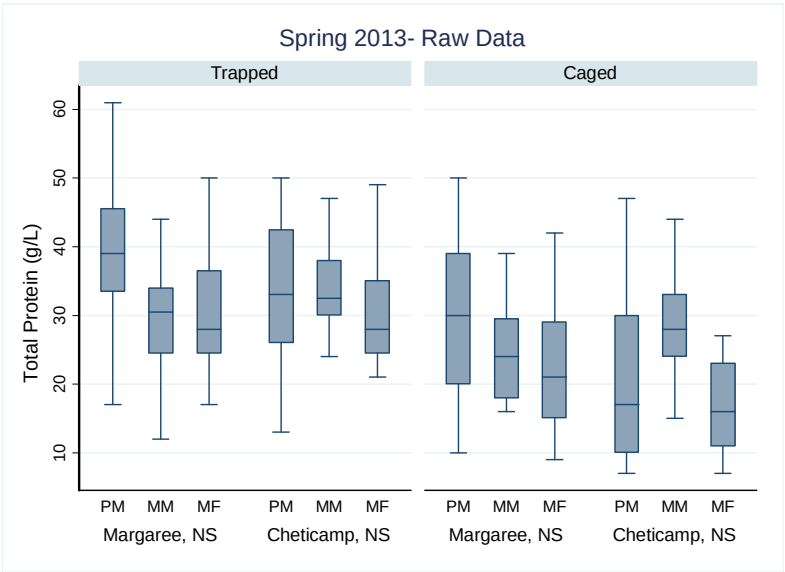
141B
152B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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Caged
Total Protein (g/L)
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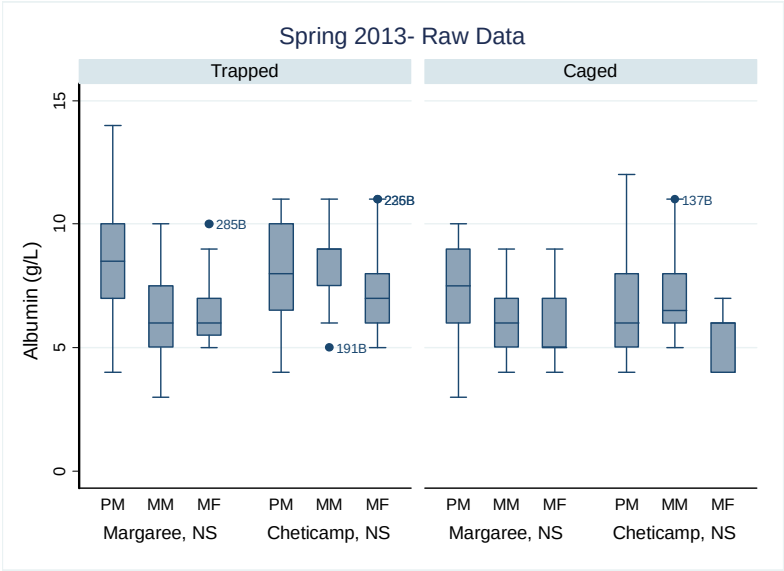
30
40
50
60
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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Albumin (g/L)
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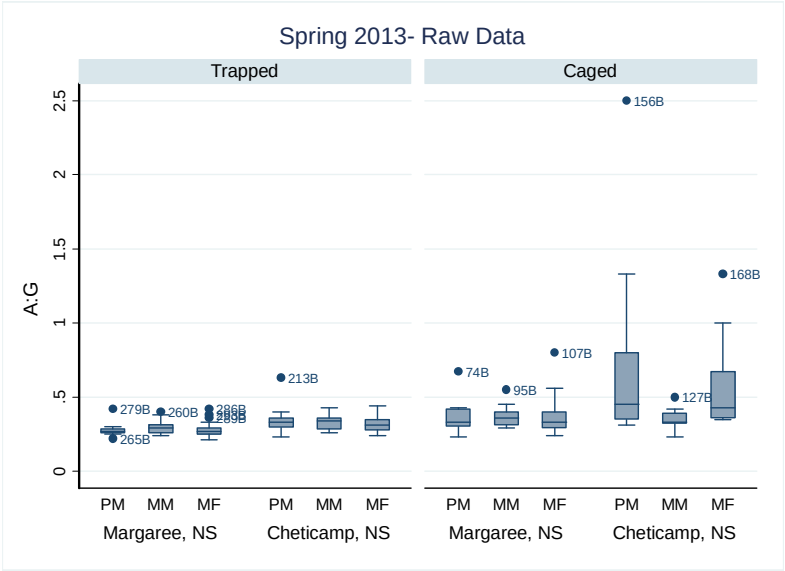
10
15
285B
226B
191B
137B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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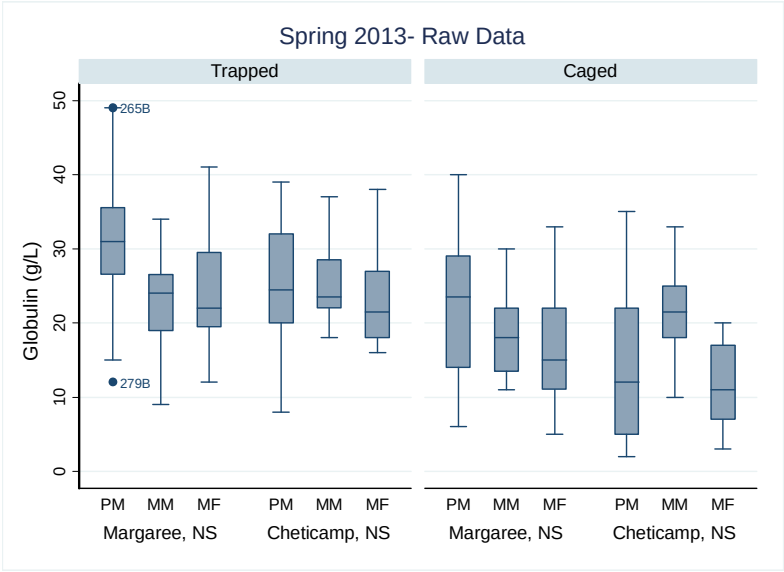
Trapped
Caged
A:G
0
.5
1
1.5
2
2.5
156B
168B
107B
74B
95B
127B
213B
279B
260B
265B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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Caged
Globulin (g/L)
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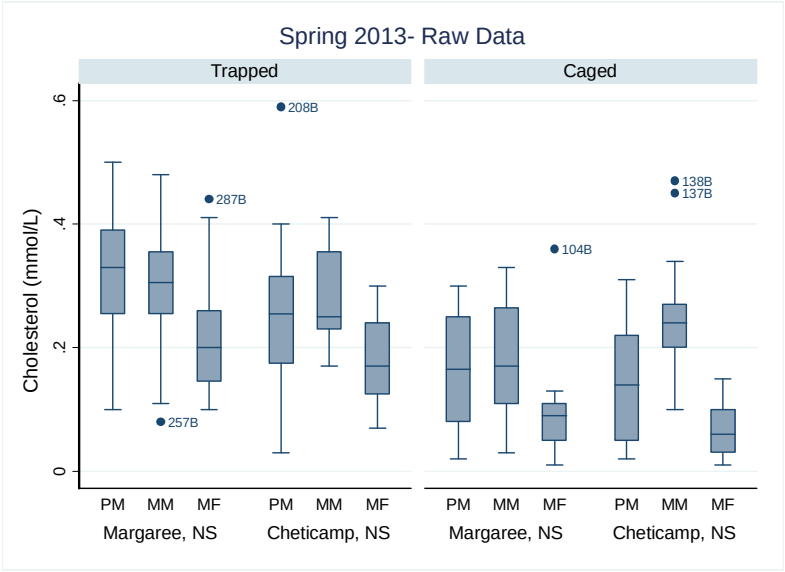
10
20
30
40
50
265B
279B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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Caged
Cholesterol (mmol/L)
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.4
.6
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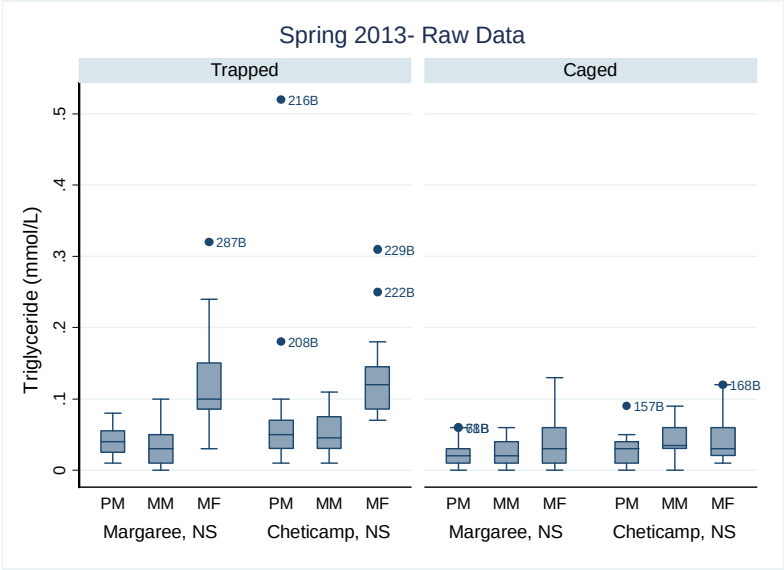
257B
138B
137B
104B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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Trapped
Caged
Triglyceride (mmol/L)
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.1
.2
.3
.4
.5
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287B
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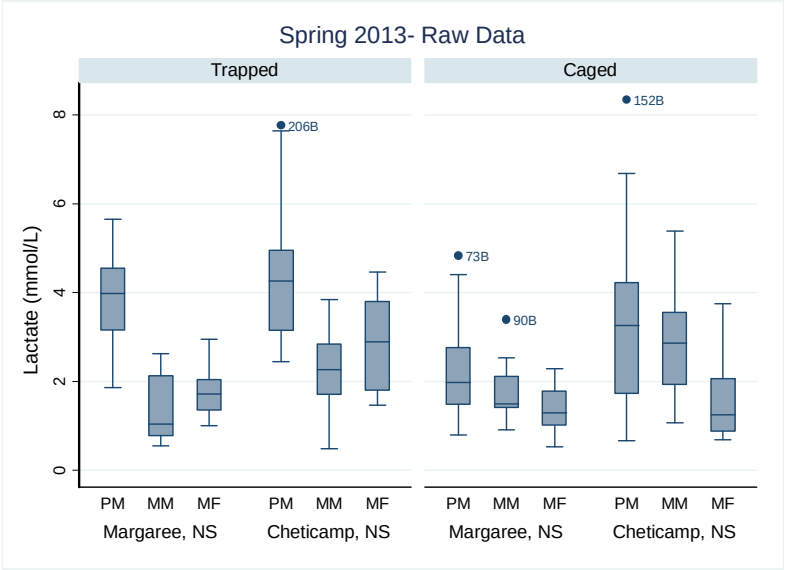
168B
157B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS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Caged
Lactate (mmol/L)
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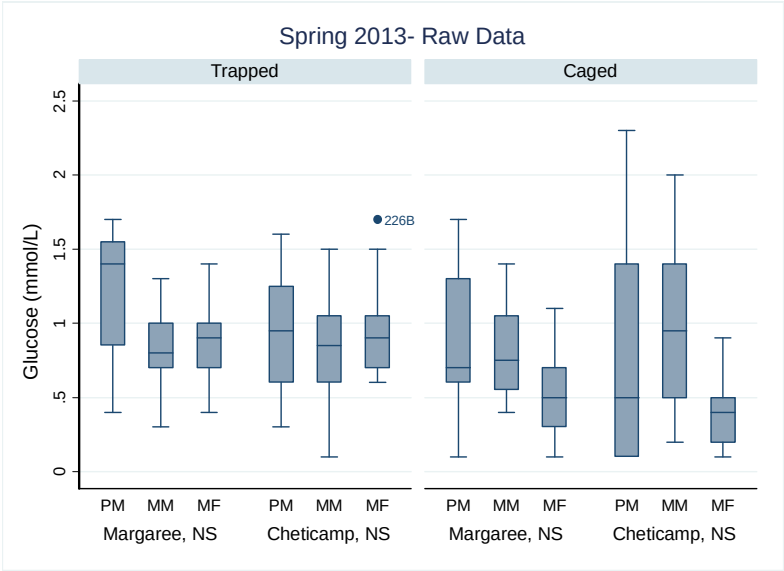
4
6
8
206B
152B
73B
90B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS


Spring 2013- Raw Data
Trapped
Caged
Glucose (mmol/L)
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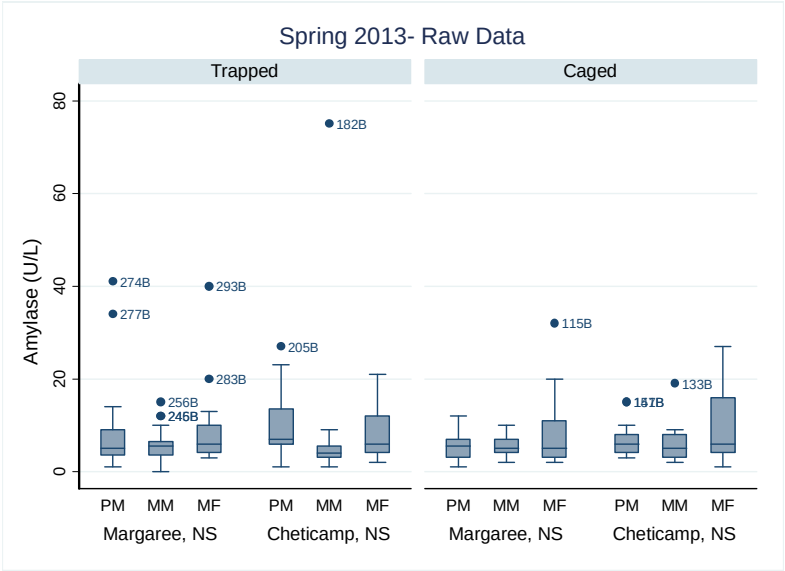
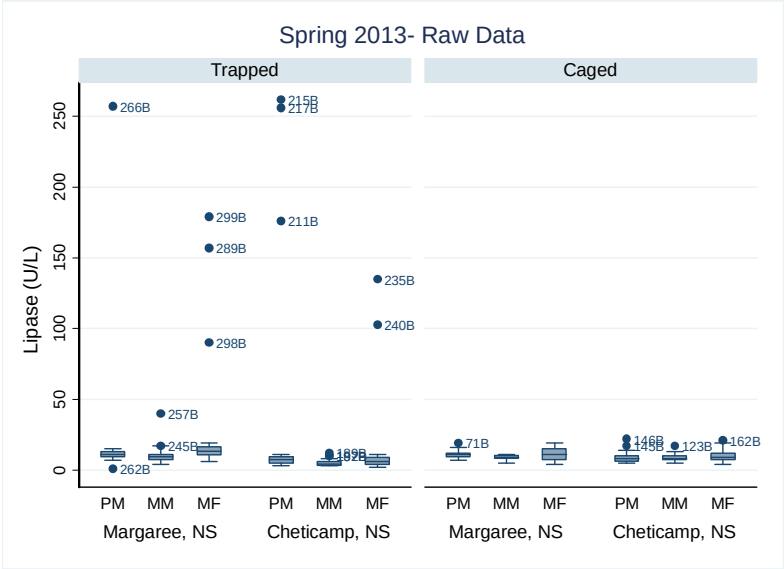
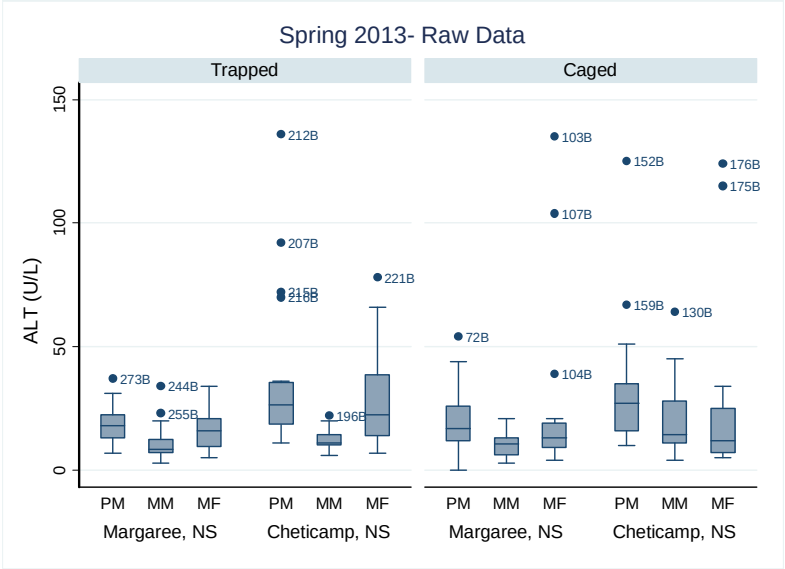
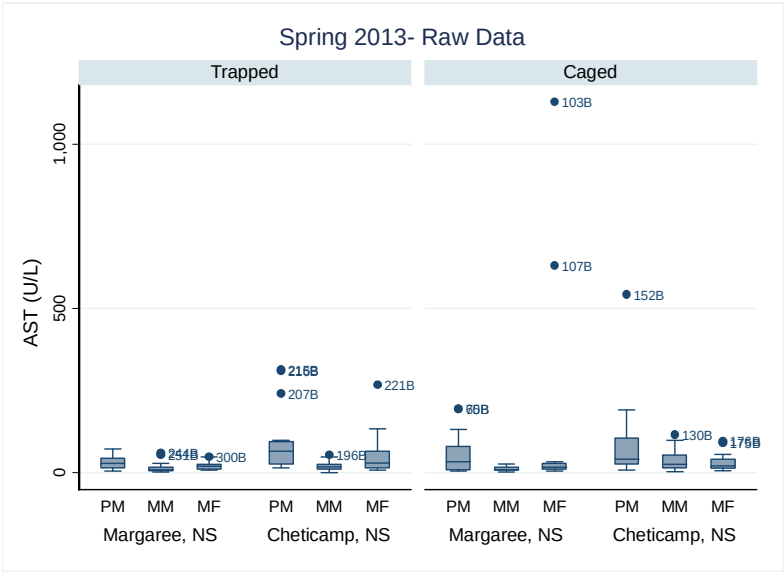
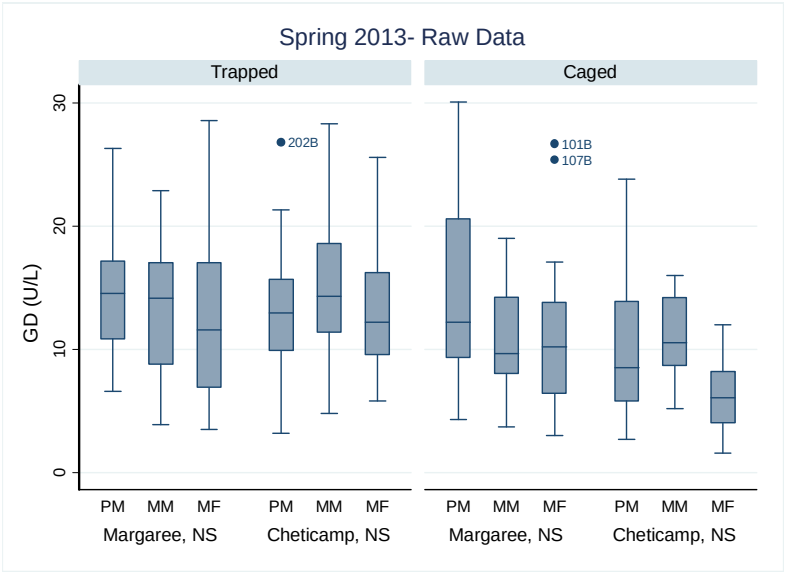
.5
1
1.5
2
2.5
226B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

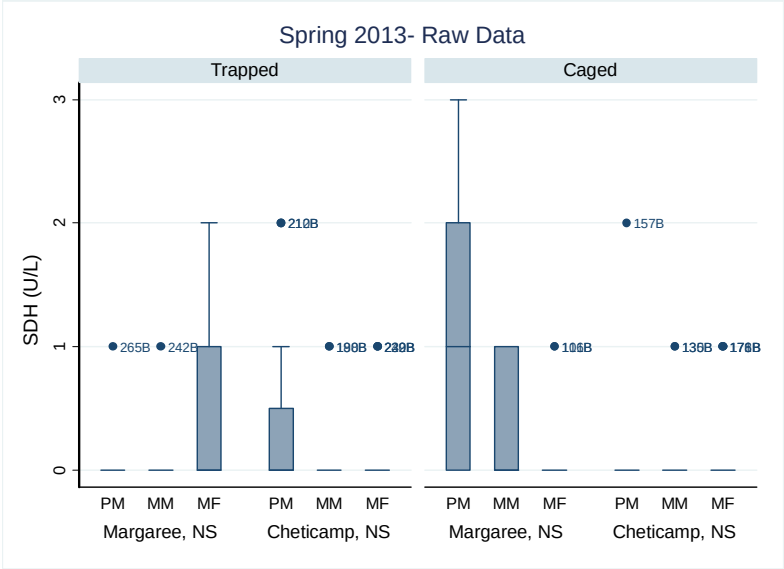

Spring 2013- Raw Data
Trapped
Caged
Amylase (U/L)
0
20
40
60
80
182B
274B
277B
293B
205B
283B
256B
246B
115B
133B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

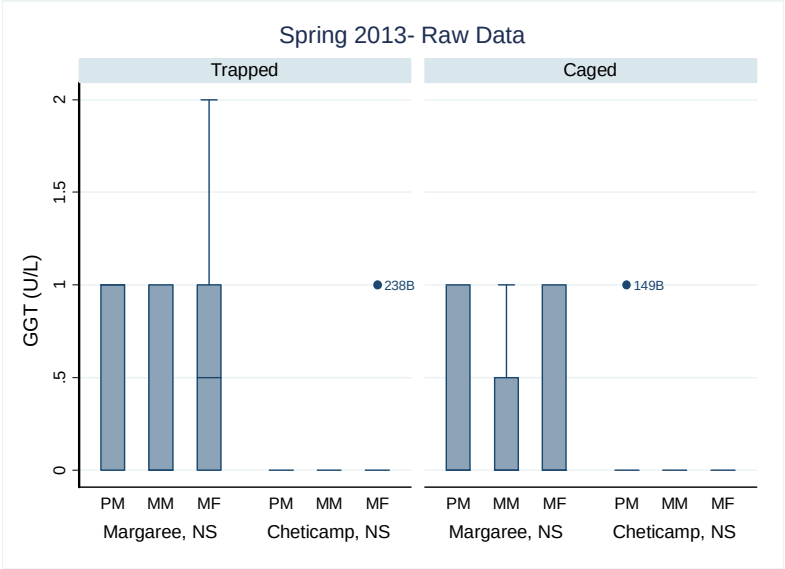

Spring 2013- Raw Data
Trapped
Caged
Lipase (U/L)
0
50
100
150
200
250
266B
215B
217B
299B
289B
211B
235B
240B
298B
257B
245B
262B
71B
123B
162B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

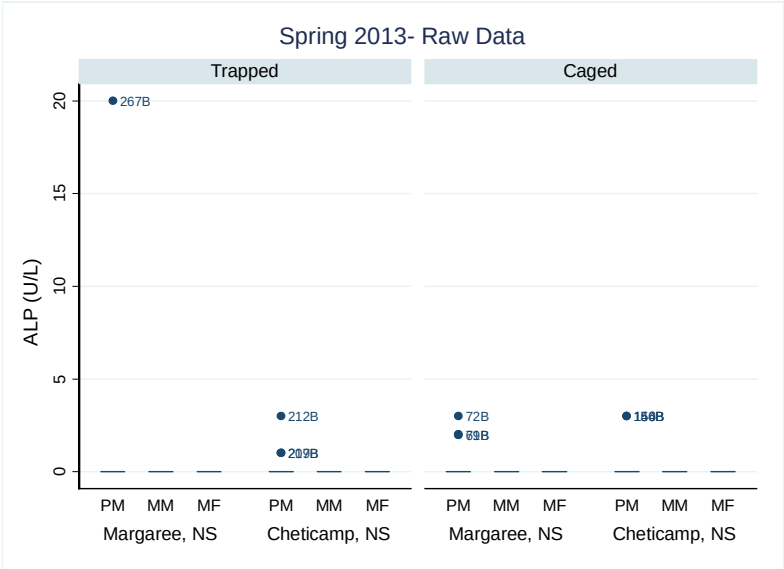

Spring 2013- Raw Data
Trapped
Caged
ALT (U/L)
0
50
100
150
212B
207B
221B
103B
152B
176B
175B
107B
159B
130B
72B
104B
273B
244B
255B
196B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS


Spring 2013- Raw Data
Trapped
Caged
AST (U/L)
0
500
1,000
103B
107B
152B
221B
207B
130B
196B
300B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

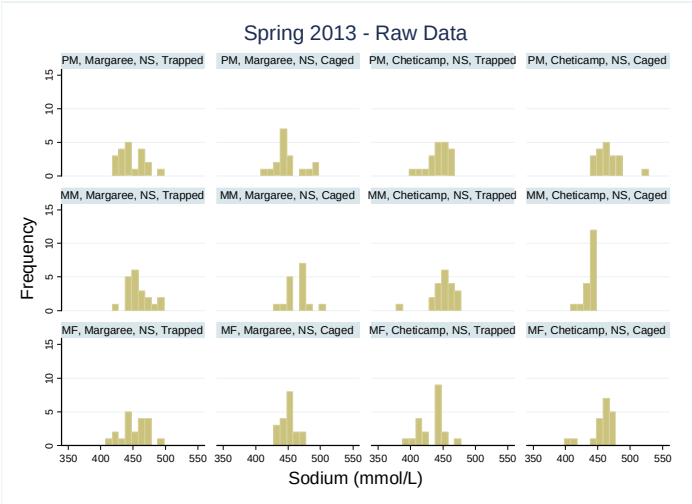

Spring 2013- Raw Data
Trapped
Caged
GD (U/L)
0
10
20
30
202B
101B
107B
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

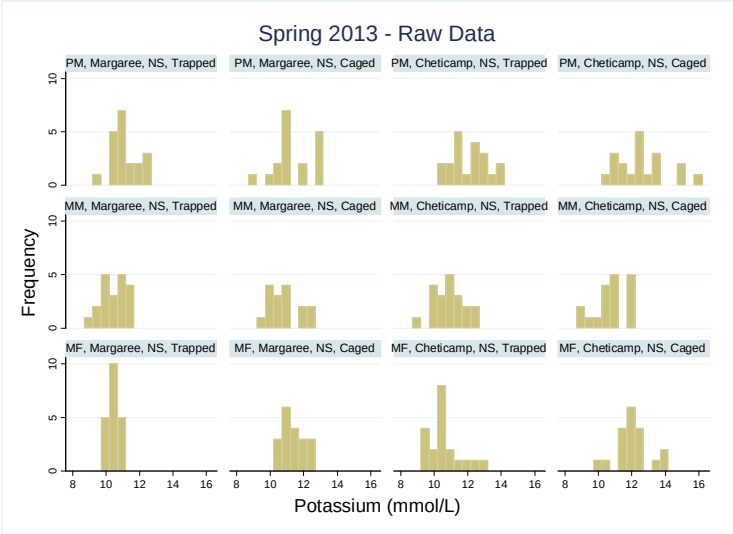

Spring 2013- Raw Data
Trapped
Caged
SDH (U/L)
0
1
2
3
265B
242B
157B
PM
MM
MF
Margaree, NS
Cheticamp, NS

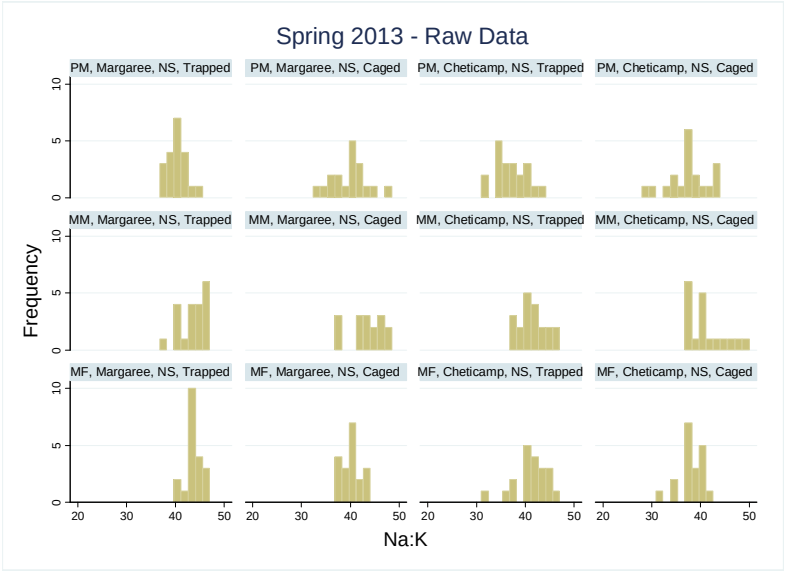

Spring 2013- Raw Data
Trapped
Caged
GGT (U/L)
0
.5
1
1.5
2
238B
149B
PM
MM
MF
Margaree, NS
Cheticamp, NS

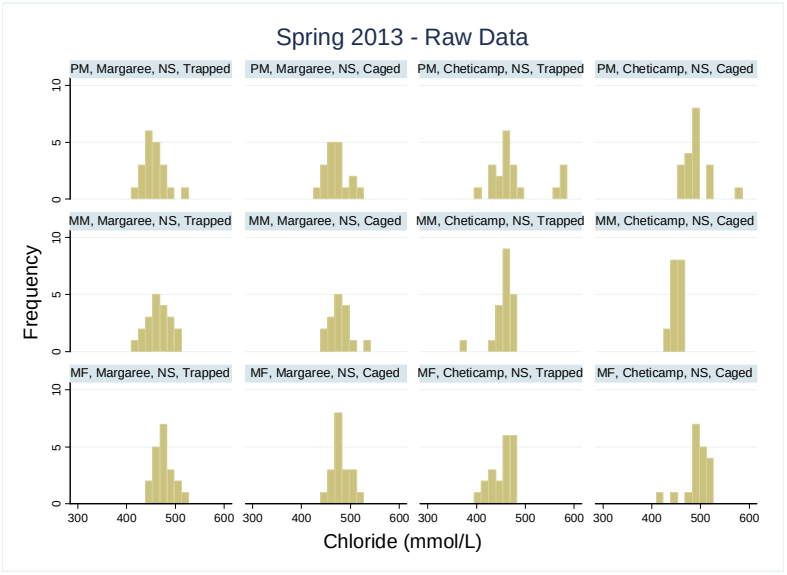

Spring 2013- Raw Data
Trapped
Caged
ALP (U/L)
0
5
10
15
20
267B
212B
72B
PM
MM
MF
Margaree, NS
Cheticamp, NS

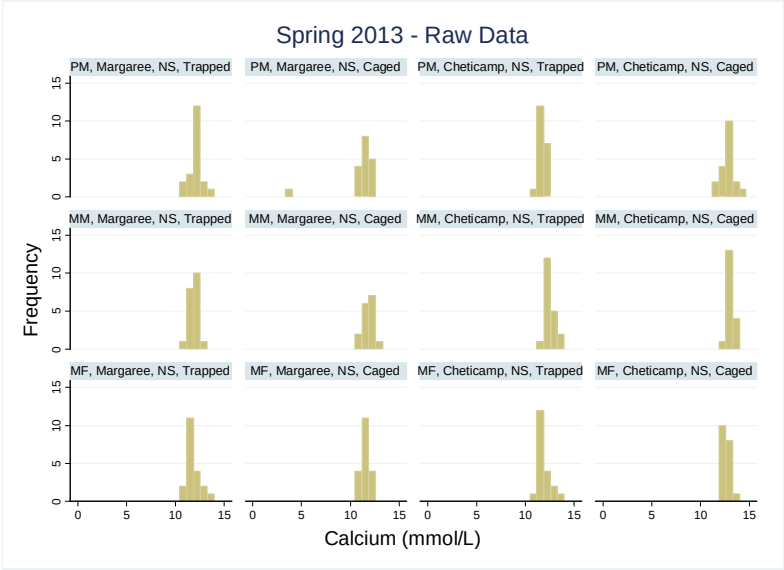
Appendix B: Frequency Distribution Histograms, Biochemistry Profiles

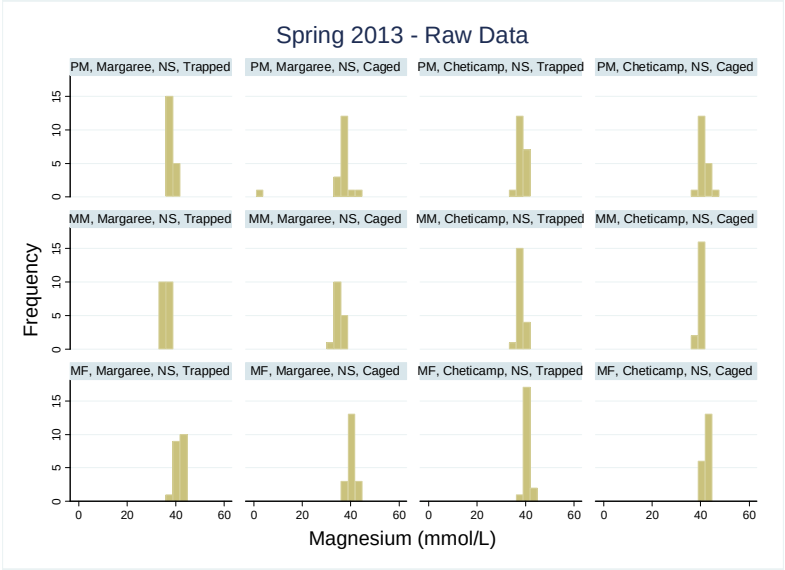


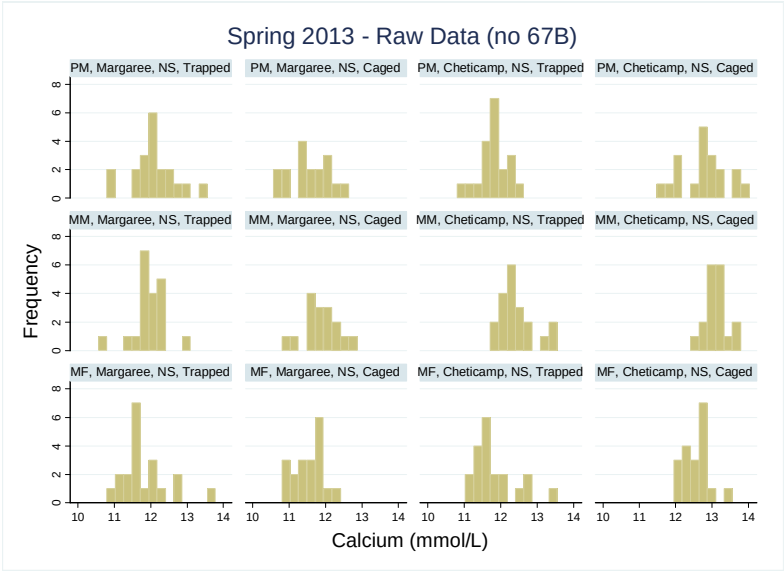


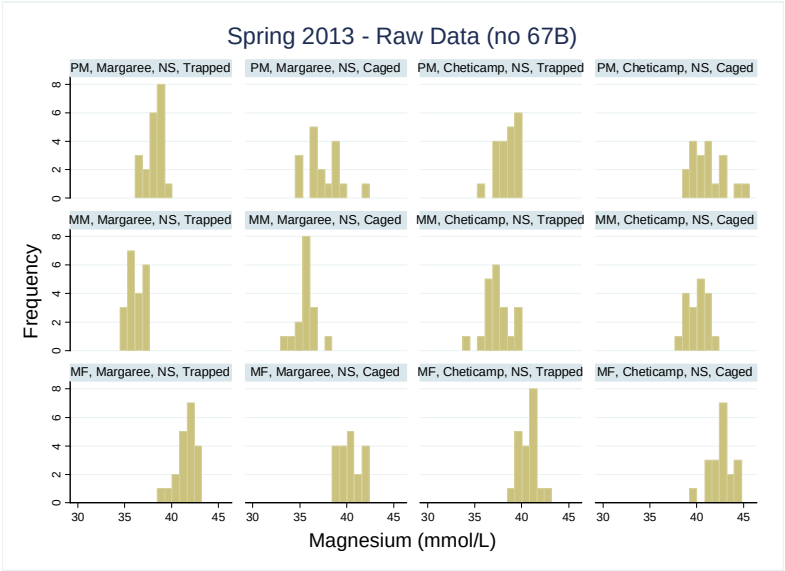


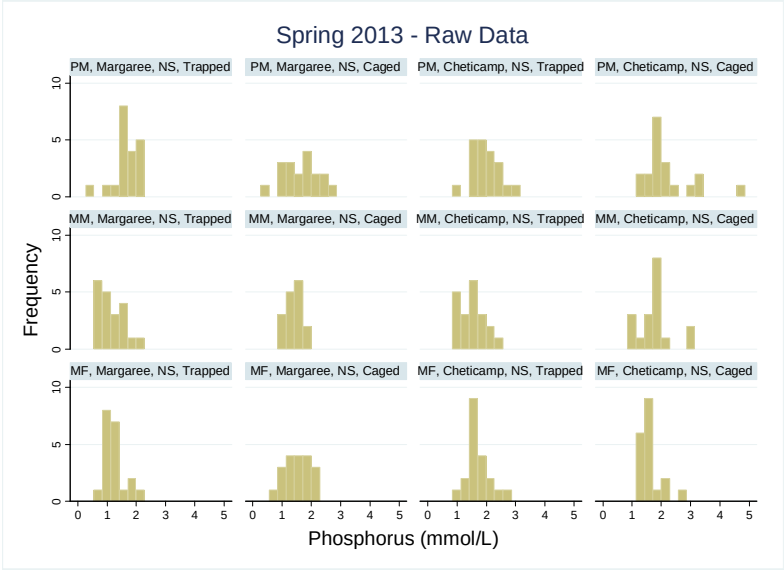


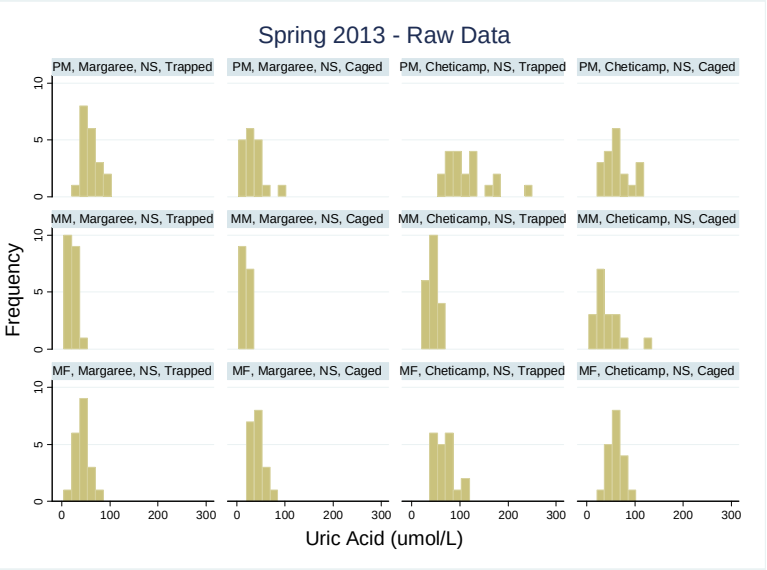

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 5 10 15
Calcium (mmol/L)

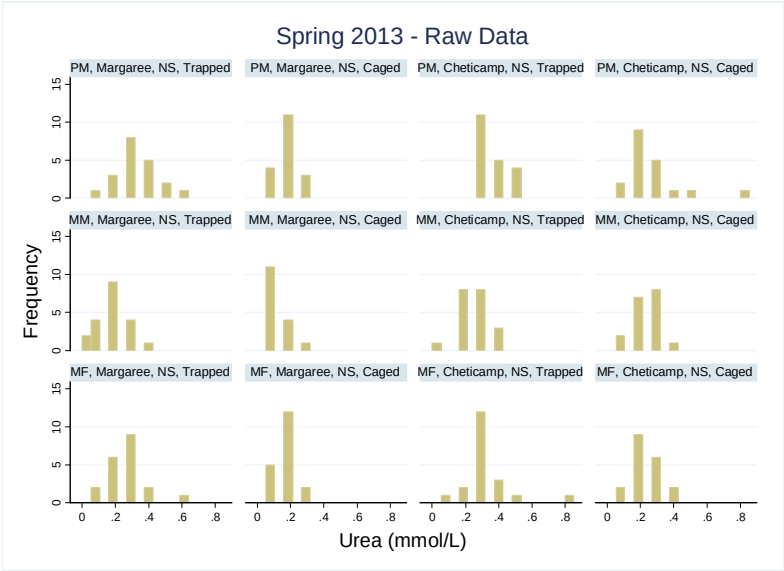

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 5 10 15
0 20 40 60
Magnesium (mmol/L)

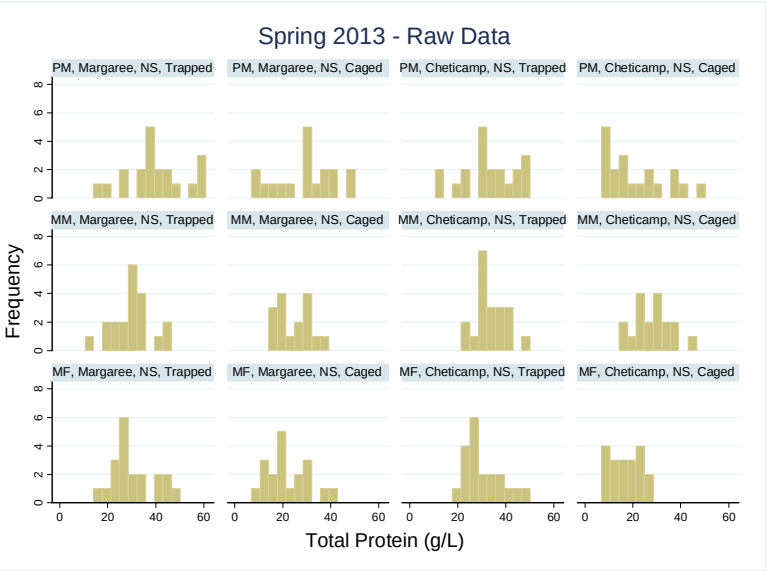

Spring 2013 - Raw Data (no 67B)
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 2 4 6 8
10 11 12 13 14
Calcium (mmol/L)

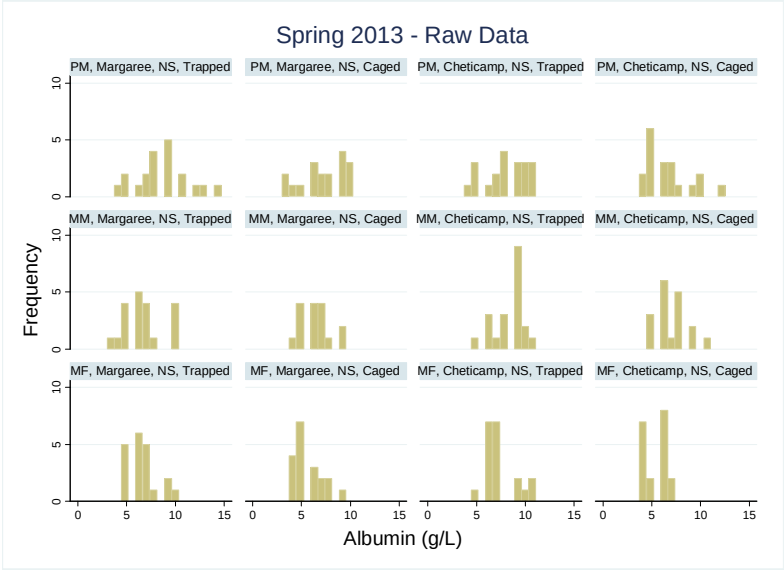

Spring 2013 - Raw Data (no 67B)
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 2 4 6 8
30 35 40 45
Magnesium (mmol/L)

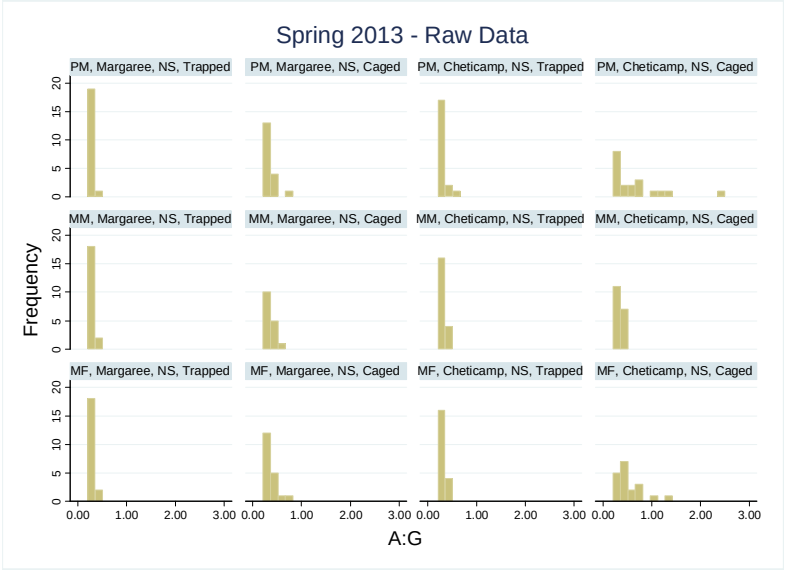

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Phosphorus (mmol/L)

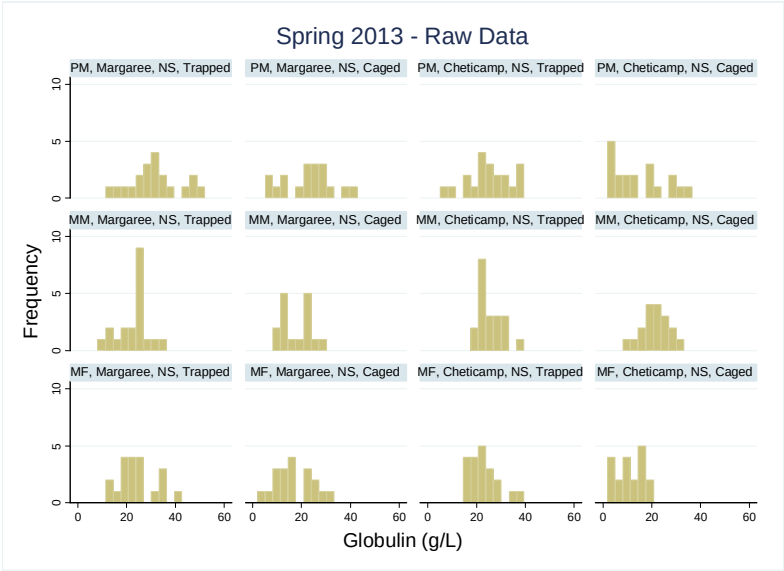

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Uric Acid (umol/L)

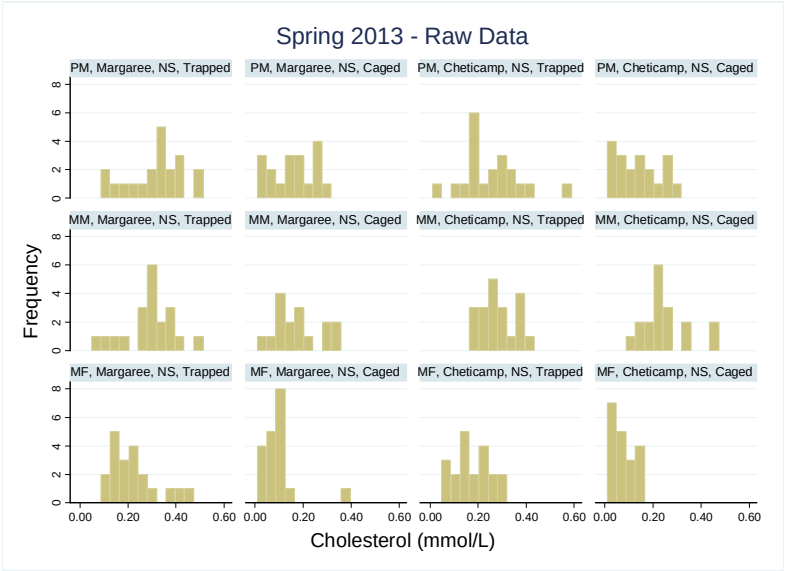

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Urea (mmol/L)

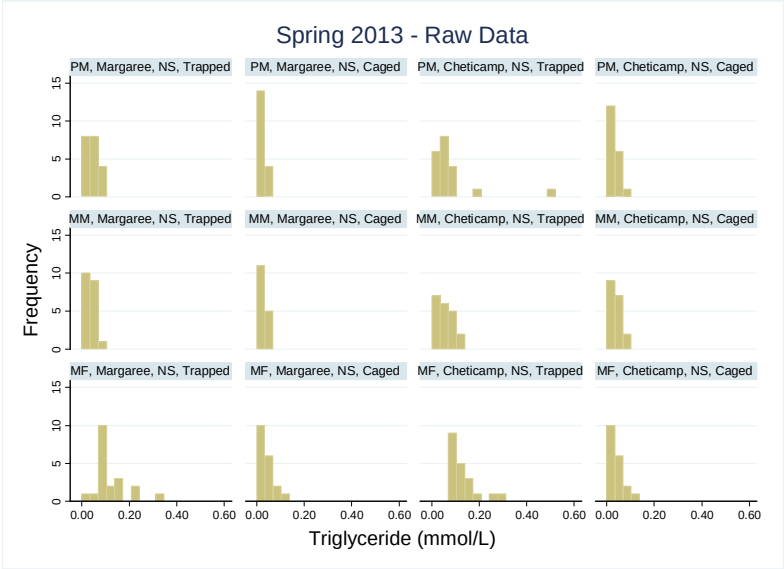

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Total Protein (g/L)

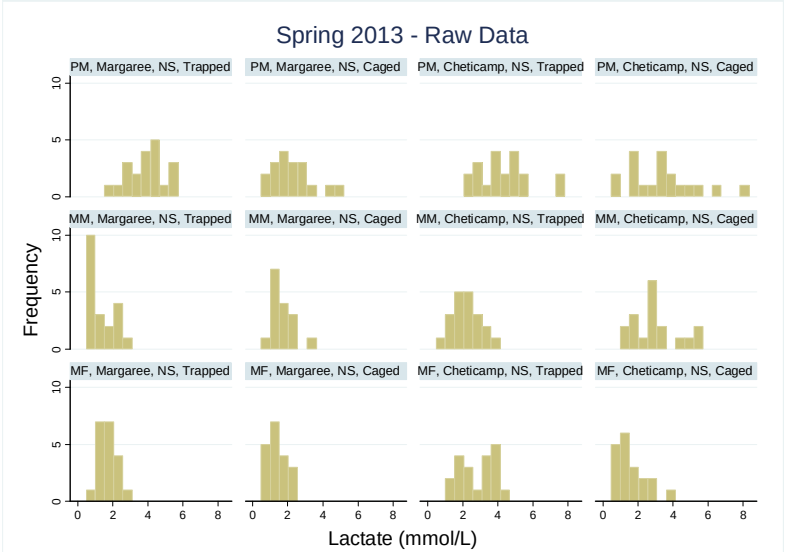

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Albumin (g/L)

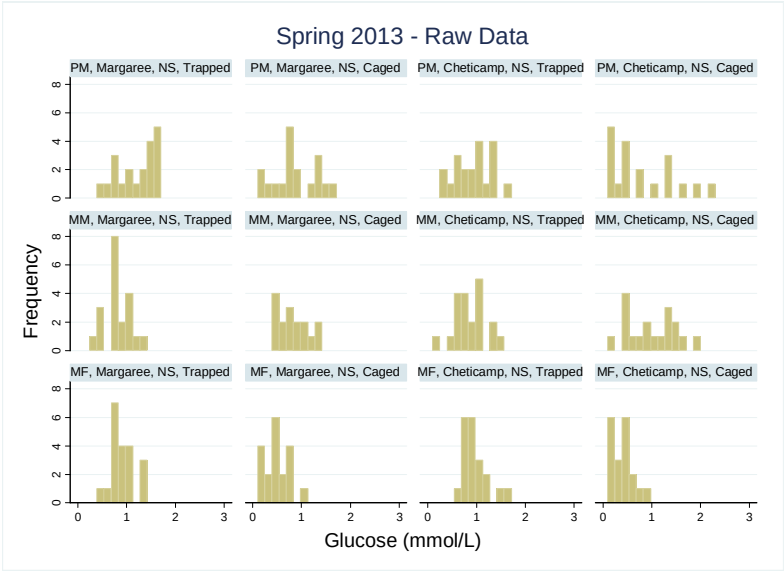

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
A:G

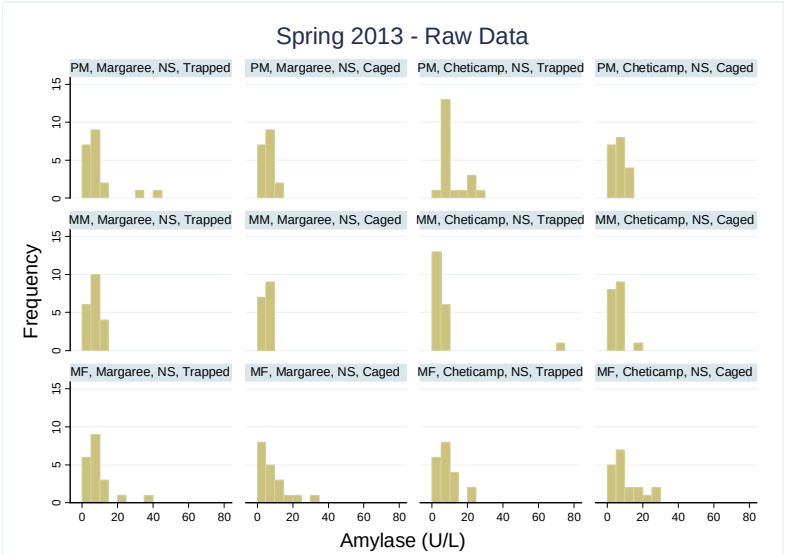

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Globulin (g/L)

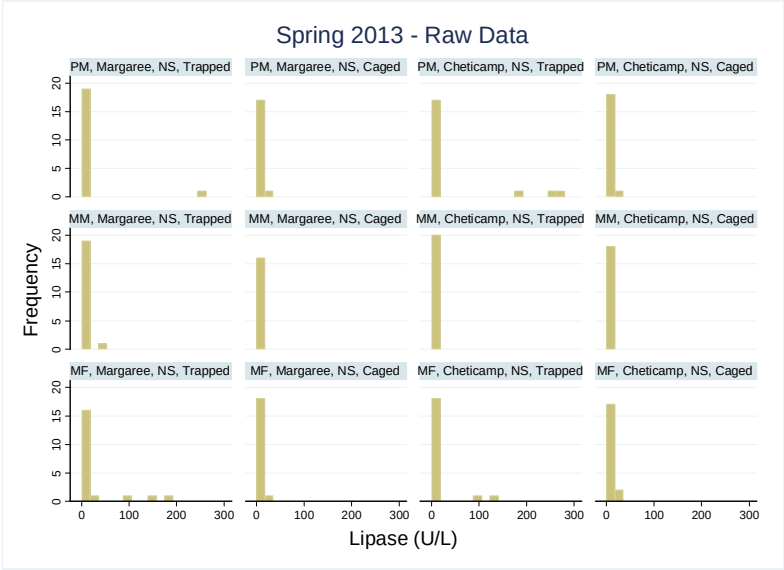

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Cholesterol (mmol/L)

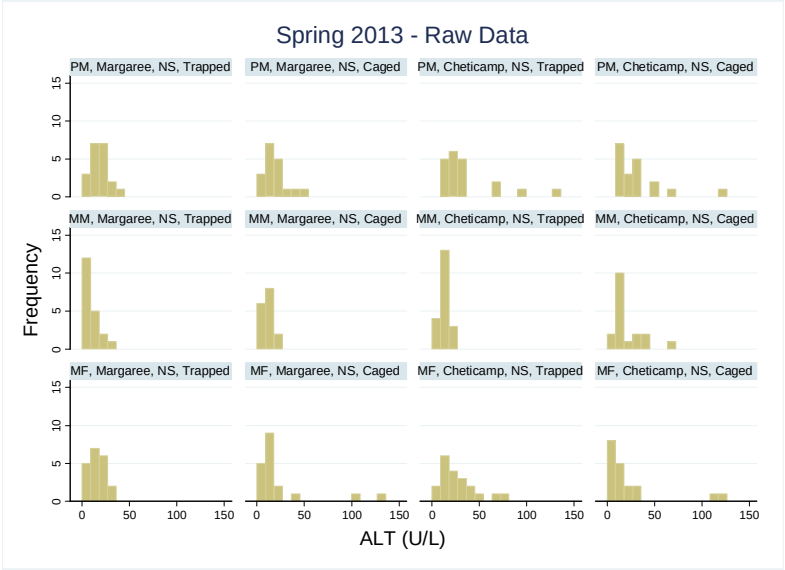

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Triglyceride (mmol/L)

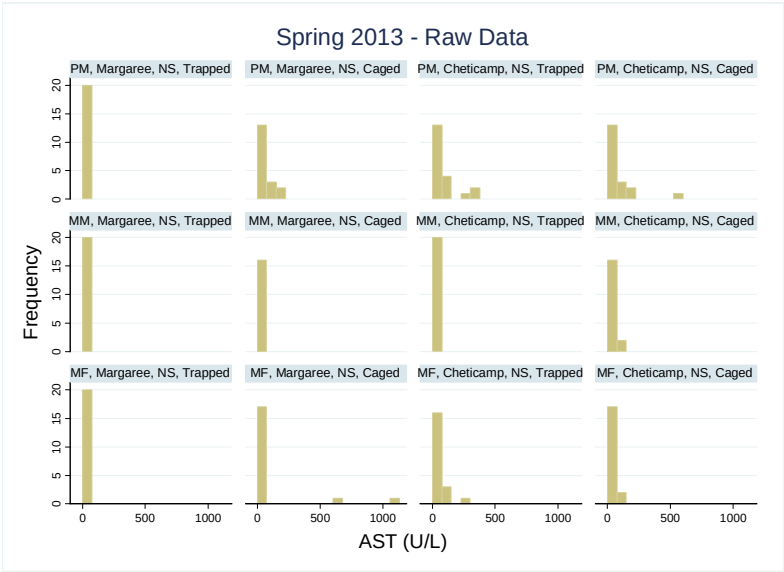

Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Lactate (mmol/L)


Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Glucose (mmol/L)


Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Amylase (U/L)


Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 5 10 15 20
0 100 200 300
Lipase (U/L)


Spring 2013 - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 5 10 15
0 50 100 150
ALT (U/L)


Spring 2013 - Raw Data
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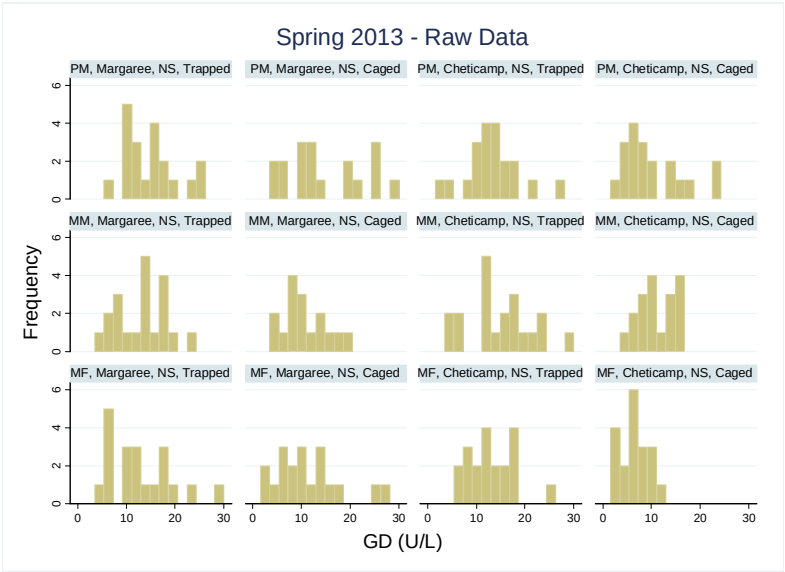
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 5 10 15 20
0 500 1000
AST (U/L)


Spring 2013 - Raw Data
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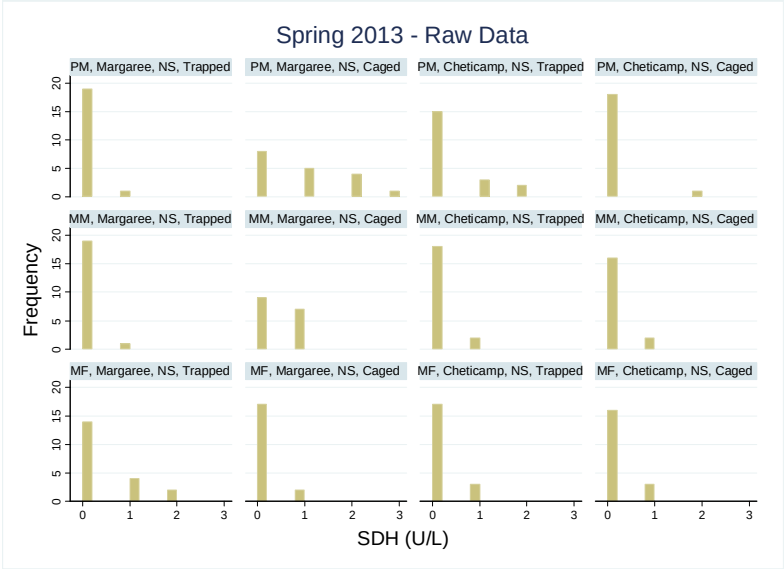
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 2 4 6
0 10 20 30
GD (U/L)


Spring 2013 - Raw Data
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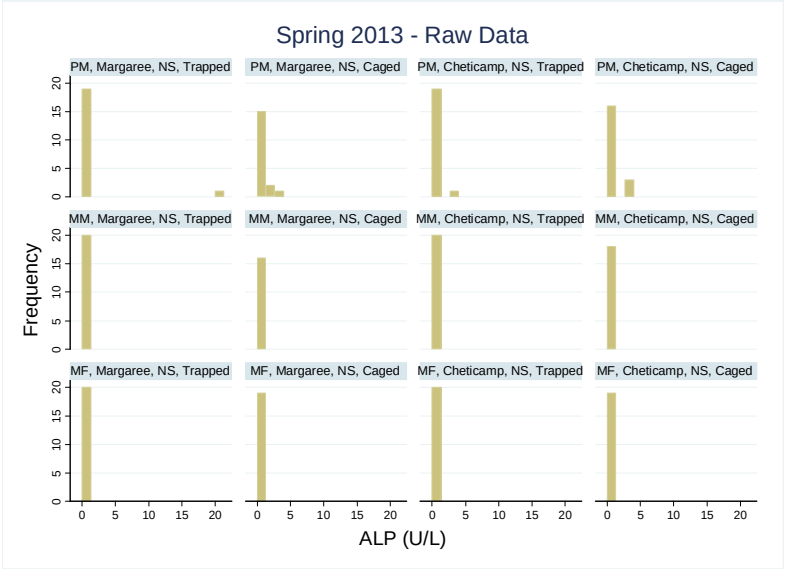
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
SDH (U/L)


Spring 2013 - Raw Data
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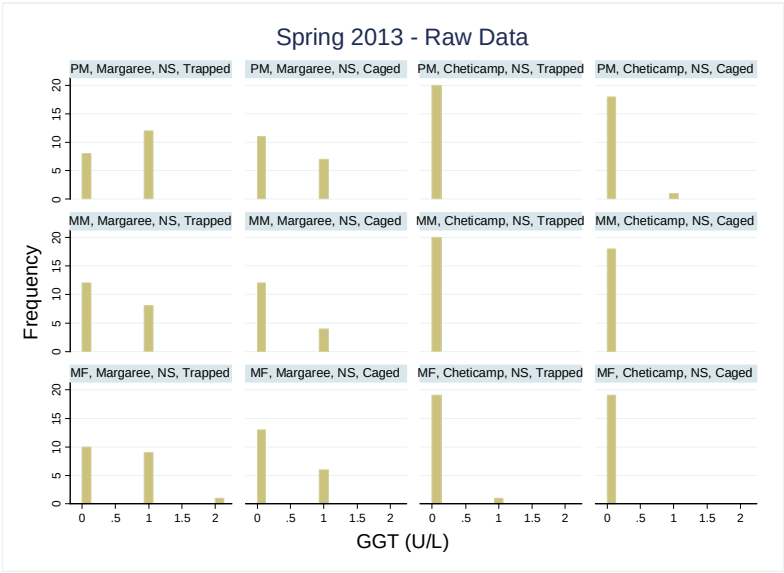
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
ALP (U/L)


Spring 2013 - Raw Data
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PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
GGT (U/L)

VIII-3. TWELVE MONTH (345-355 DAYS) CAGING

VIII-3-1 Summary

The 12 month caging period was primarily associated with lower values in caged vs free crabs for hepatopancreas lipid and glycogen stores and many metabolites in the hemolymph biochemistry profiles, especially for protein and energy-related parameters as noted in the fall 2012 (2 week caging study) and spring 2013 (6 month caging study) samples. This presumably reflected inadequate energy intake and/or decreased tissue stores to meet metabolic requirements. Lower levels of activity of the muscle- associated enzymes, glutamate dehydrogenase (GD) and alanine aminotransferase (ALT) were more pronounced than in the spring 2013 samples and likely reflected lower muscle and/or hepatopancreas mass in caged crabs.
Hepatopancreas lipid and glycogen levels were lower, and moisture contents higher, in all caged crabs compared to free crabs – in contrast to samples from crabs caged for six months (spring 2013) when only MF crabs had significantly lower hepatopancreas energy levels. These were expected findings given the anticipated even greater reduced access to food sources for caged crabs. Competition among crabs caged communally would be expected to factor in to how well each individual could compete for food sources which may explain the few outliers with higher values observed for the caged crabs at both stations.

Correlations of hemolymph biochemistry parameters to hepatopancreas energy reserves in free crabs were seen for parameters in the metabolite category as in previous samples; however, the correlations were inconsistent, with differences noted for crab category, treatment and sometimes location. Better results were usually obtained for free PM and MF crabs. Regression analyses could not consistently estimate hepatopancreas lipid or glycogen levels. Inclusion of hepatopancreas total protein and inclusion of ovary and muscle energy (lipid, glycogen, and protein) content for calculation of total body energy reserves may have improved the results and should be considered for future studies.

VIII-3-2 Objectives

The objectives of this segment of the project were five-fold:

A. To examine differences in hemolymph biochemistry profiles between free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 345-355 days at Margaree and Cheticamp stations in spring 2013.

B. To measure hepatopancreas lipid content in free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 345-355 at Margaree and Cheticamp stations in spring 2013 and assess the value of hemolymph biochemistry profiles to predict lipid content.

C. To measure hepatopancreas glycogen content in free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 345-355 crabs at Margaree and Cheticamp

stations in spring 2013 and assess the value of hemolymph biochemistry profiles to predict glycogen content.

D. Calculation of hepatopancreas lipid:glycogen ratios to compare to values obtained in the two week caging study (section ___).

E. Evaluation of hepatopancreas moisture content in free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 345-355 crabs at Margaree and Cheticamp stations in spring 2013.

VIII-3-3 Methodology

Crabs were collected and sampled as per criteria outlined in Section III-1 through III-4. Hemolymph plasma samples were delivered to Diagnostic Services at the Atlantic Veterinary College, UPEI (Charlottetown, PE) for analysis of biochemistry parameters.

Hepatopancreas moisture, lipid and glycogen content were determined by RPC Science and Engineering, Fredericton, NB. Sample processing was completed as per Ciaramella 2011.

Data analysis was completed with STATA statistical software (STATA I/C 12.1, StataCorp LP) and Microsoft Excel (Excel 2010©, Microsoft Corporation). Bonferroni adjustments of significance for multiple comparisons were made where required.

VIII-3-4 Results

A. Hemolymph Plasma Biochemistry Profiles

All hemolymph plasma samples were processed within 24 – 48 hours of collection, well within previously established time frame for sample stability (see Section VII-2). Measurement of electrolytes (sodium, chloride, potassium) and minerals (calcium and magnesium) required manual or programmed analyser dilution, respectively. As the sample sizes were small (maximum of 20 animals per group) when no pathological or physiological reason e.g., trauma, hemorrhage, oocyte development, as a cause of the marginally high or low values could be identified in crabs that had one or two outlier results on a panel with 27 values, neither the crab, nor these values were deleted from the dataset for statistical evaluation.
Biochemistry data were unavailable from 34 crabs due to mortalities or 'missing' crabs (Cheticamp caged: five LM, four PM, four MF; Margaree Harbor caged: nine LM, seven PM, three MF). Laboratory accidents resulted in the loss of all data from one LM crab from Cheticamp trap group (#71) and the non-electrolyte data from one MF crab from Cheticamp trap group (#107).

Electrolyte data from three PM from the Margaree Harbor caged group, (#141, #142, #145) were identified as outliers on examination of the boxplots (Appendix A) that could be attributed to probable laboratory error during sample processing e.g. manual dilutions, and deleted from the data set. All results from three MF crabs, two (#120, #109) Cheticamp free one (#48) Cheticamp, caged, were excluded as their biochemistry parameters (very high triglyceride and/or total protein concentrations) suggested that they were more progressed in oocyte development than the other females in their groups (this was supported by ovary colour differences) (Battison *et al.* 2011). Results from a LM crab (#123) from the Margaree Harbor, caged, group were excluded as lab data suggested that the hepatopancreas may have been inadvertently sampled during hemolymph collection (extremely high value for AST, a moderate elevation in ALT, and detectable GGT and ALP). These deletions are indicated in Tables 1-18.

Summary statistics (count, minimum, maximum, SD, mean, and median values) are presented in Tables 1-12 and compared to the reference intervals (RI) previously calculated for free, cooler-held, crabs collected in August 2012 near the Cheticamp station (see Section VII-3).

Other than slightly lower values for sodium, chloride, and magnesium in Margaree Harbor crabs, median vales for minerals and electrolytes were within the RIs for free crabs. Median calcium values were below the lower limit of the RI for caged $PM_{Margaree}$, $MF_{Margaree}$, $LM_{Cheticamp}$, and $PM_{Cheticamp}$ crabs. Median values for metabolites fell within the RI for all free crabs with the exception of uric acid in free PM crabs for both sites which was below the lower limit of the RI. Caged PM crabs, both sites, had median values below or at the lower limit of the RI for all metabolites. Other caged crabs sporadically had median values below the lower limit of the RI for albumin ($LM_{Margaree}$, $LM_{Cheticamp}$), uric acid ($MF_{Margaree}$, $MF_{Cheticamp}$), and globulin ($LM_{Cneticamp}$,

$MF_{Cneticamp}$). Median enzyme activity levels were with the RI in all cases but for mildly increased levels of amylase for $LM_{Cheticamp}$. Minimum and maximum values occasionally fell outside the upper and lower limits of the RI (see Tables). Creatinine was not detected in any of the samples.

Plasma activity of three enzymes – ALP, GGT, SDH was very low or undetectable in most instances and is consistent with previous studies. Differences of 1-3 units of activity are not considered clinically relevant and may even be within the allowable precision error for a particular assay i.e., representing no real difference.

Table 1. Summary statistics for mineral and electrolyte concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, November 2013. Highlighted values are outside the reference interval.

Crab Category		*n*	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	Na[2]	20	381	444	17.68816	407.35	406.5	423	462
	K	20	9.3	12.6	.7923815	11.145	11.1	9.9	13.2
	NaK	20	33	41	2.381397	36.75	37	34	46
	Cl	20	378	453	20.34932	408.9	409.5	444	498
	Ca	20	11.62	15.23	1.002663	13.648	13.5	12.38	15.53
	Phos	20	.8	3.23	.6951128	2.3285	2.345	1.28	5.62
	Mg	20	36.71	42.03	1.659673	39.5075	39.595	41.59	46.26
LM	Na	20	375	423	13.68211	398.4	399	441	490
	K	20	9.0	12.3	.894589	10.335	10.35	9.2	12.7
	NaK	20	33	43	2.605157	38.95	39	36	49
	Cl	20	375	432	18.14351	403.35	405	459	513
	Ca	20	10.94	13.87	.7464193	12.6755	12.68	12.15	14.03
	Phos	20	.99	3.61	.6595762	2.0825	1.965	0	3.02
	Mg	20	35.54	41.47	1.31629	38.151	38.27	38.53	44.12
MF	Na	20	393	441	16.24249	415.65	415.5	385	496
	K	20	9.0	11.1	.6630075	10.32	10.35	7.8	13.5
	NaK	20	37	44	1.846761	40.4	40	33	51
	Cl	20	387	459	21.81646	424.8	426	396	507
	Ca	20	12.3	14.79	.6402012	13.4995	13.53	12.51	16.67
	Phos	20	1.1	2.86	.4284056	1.8555	1.795	0.72	3.00
	Mg	20	39.25	43.86	1.215467	41.8765	42.2	40.05	46.67

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits for the sampled populations. [2] Units for all parameters are mmol/L.

Table 2. Summary statistics for mineral and electrolyte concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2013. Highlighted values are outside the reference interval.

Crab Category		n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2] Na	20	393	447	15.53061	419.4	421.5	423	462
	K	20	8.7	12	.8455923	10.365	10.5	9.9	13.2
	NaK	20	36	47	2.792848	40.7	40.5	34	46
	Cl	20	405	474	19.14453	434.25	426	444	498
	Ca	20	12.36	14.75	.7737061	13.29	13.065	12.38	15.53
	Phos	20	1.26	2.99	.4506635	1.9285	1.85	1.28	5.62
	Mg	20	40.47	44.76	1.178527	42.0325	42.02	41.59	46.26
LM	Na	19	414	453	11.69795	438.7895	444	441	490
	K	19	9.9	11.7	.5482028	10.40526	10.2	9.2	12.7
	NaK	19	37	45	2.225582	42.21053	43	36	49
	Cl	19	432	477	13.99624	455.6842	459	459	513
	Ca	19	12.62	14.09	.3746476	13.31053	13.33	12.15	14.03
	Phos	19	1.2	2.24	.3278086	1.648421	1.67	0	3.02
	Mg	19	38.53	43.05	.9444343	40.93684	40.91	38.53	44.12
MF	Na	18	402	459	14.66589	433.8333	433.5	385	496
	K	18	9	10.8	.4949747	10.01667	9.9	7.8	13.5
	NaK	18	39	46	1.940285	43.33333	44	33	51
	Cl	18	417	483	18.95195	457.3333	459	396	507
	Ca	17	12.48	14.71	.5486628	13.87059	13.78	12.51	16.67
	Phos	17	.76	2.91	.5095889	1.400588	1.26	0.72	3.00
	Mg	17	41.65	48.78	1.979195	44.00647	43.68	40.05	46.67

[1] Reference Interval is from August, 2012 Trap vs. Trawl study (see Section VII-3). Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits for the sampled populations.[2] Units for all parameters are mmol/L.

Table 3. Summary statistics for mineral and electrolyte concentrations for 12 month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, November 2013. Highlighted values are outside the reference interval.

Crab Category		n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2] Na	10	426	447	8.656404	439.4	442.5	423	462
	K	10	10.5	13.5	1.0005	11.79	11.4	9.9	13.2
	NaK	10	33	44	3.541814	38.1	39	34	46
	Cl	10	447	480	10.6066	463.5	462	444	498
	Ca	13	10.83	12.82	.5369644	11.95154	11.88	12.38	15.53
	Phos	13	1.12	3.21	.5978208	1.926923	1.73	1.28	5.62
	Mg	13	36.72	40.69	1.476129	39.14692	39.96	41.59	46.26
LM	Na	8	423	465	12.18606	446.25	448.5	441	490
	K	8	9.0	11.1	.6611678	9.9	9.6	9.2	12.7
	NaK	8	41	47	2	45.5	46	36	49
	Cl	8	435	492	17.00368	468.375	469.5	459	513
	Ca	8	11.68	12.25	.1999598	12.05125	12.11	12.15	14.03
	Phos	8	.59	1.35	.2707002	.9225	.855	0	3.02
	Mg	8	37.2	40.05	1.135856	38.74625	39.155	38.53	44.12
MF	Na	17	429	468	11.21318	450.8824	453	385	496
	K	17	10.5	11.7	.4677072	11.2	11.4	7.8	13.5
	NaK	17	38	43	1.649421	40.29412	40	33	51
	Cl	17	450	507	16.1514	486.3529	489	396	507
	Ca	17	10.77	12.79	.5909644	11.64529	11.53	12.51	16.67
	Phos	17	1.03	2.34	.371804	1.644118	1.58	0.72	3.00
	Mg	17	39.95	43.9	1.087975	42.17471	42.32	40.05	46.67

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits for the sampled populations.
[2] Units for all parameters are mmol/L.

Table 4. Summary statistics for mineral and electrolyte concentrations for 12 month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2013. Highlighted values are outside the reference interval.

Crab Category		n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2] Na	16	417	486	18.41195	452.25	459	423	462
	K	16	9.6	14.7	1.056232	11.86875	11.85	9.9	13.2
	NaK	16	31	43	3.255124	38.0625	39	34	46
	Cl	16	453	513	17.97394	488.4375	498	444	498
	Ca	16	11.08	12.65	.442003	12.0225	12.085	12.38	15.53
	Phos	16	.73	3.28	.5915066	1.425	1.27	1.28	5.62
	Mg	16	38.6	43.88	1.635533	41.49688	42.005	41.59	46.26
LM	Na	15	417	468	16.05437	438.2	435	441	490
	K	15	9.9	12.6	.8625543	11.36	11.4	9.2	12.7
	NaK	15	33	44	3.058166	38.73333	38	36	49
	Cl	15	444	504	20.94483	470.4	465	459	513
	Ca	15	11.15	13.23	.5034292	12.11133	12.11	12.15	14.03
	Phos	15	.72	2.26	.4080418	1.171333	1.12	0	3.02
	Mg	15	36	42.15	1.796873	39.416	39.81	38.53	44.12
MF	Na	15	447	495	11.44427	468.4	468	385	496
	K	15	10.2	13.2	.8743978	11.68	12	7.8	13.5
	NaK	15	35	45	2.782599	40.2	39	33	51
	Cl	15	486	528	11.76557	511	507	396	507
	Ca	15	11.57	13.4	.4761902	12.19	12.01	12.51	16.67
	Phos	15	.72	2.52	.4991965	1.354	1.24	0.72	3.00
	Mg	15	43.2	45.34	.8116843	44.112	43.68	40.05	46.67

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits for the sampled populations.
[2] Units for all parameters are mmol/L.

Table 5. Summary statistics for metabolite concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, November 2013. Highlighted values are outside the reference interval.

Crab	Category	n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2]Urea	20	.3	.6	.1151658	.42	.4	0.3	1.1
	Creat	20	0	0	0	0	0	0	0
	Uric	20	32	108	21.73882	53.95	48.5	65	199
	TPb	20	36	104	17.13253	68.55	67.5	36	97
	Alb	20	8	19	3.051747	13.55	14	8	22
	Glob	20	28	85	14.23487	55	52.5	28	78
	AG	20	.22	.3	.0228208	.2495	.24	0.23	0.33
	Chol	20	.24	.95	.1804935	.609	.6	0.22	1.07
	Trig	20	.04	.19	.0413076	.137	.15	0.05	0.20
	Gluc	20	1.4	3	.3797506	1.9	1.9	0.7	2.6
	Lactate	20	2.73	9.53	1.960667	5.974	6.075	2.15	13.05
LM	Urea	20	.2	.4	.0812728	.285	.3	0.2	0.6
	Creat	20	0	0	0	0	0	0	0
	Uric	20	15	59	12.47777	35.3	36	6	75
	TPb	20	11	79	16.46647	42.25	42	14	65
	Alb	20	3	15	3.103055	8.95	8.5	6	16
	Glob	20	8	64	13.5223	33.3	32	12	52
	AG	20	.20	.38	.0418361	.2785	.28	0.28	0.46
	Chol	20	.13	.82	.1454937	.46	.43	0.14	0.89
	Trig	20	.01	.13	.0324281	.079	.075	0.03	0.16
	Gluc	20	.2	1.9	.39216	1.13	1.1	0.5	1.7
	Lactate	20	1.11	5.82	1.626627	2.9425	2.49	0.00	3.47
MF	Urea	20	.2	.5	.0910465	.325	.3	0.2	2.2
	Creat	20	0	0	0	0	0	0	0
	Uric	20	37	116	20.71003	65.2	64	62	222
	TPb	20	36	75	13.14884	57.55	60.5	9	81
	Alb	20	8	15	2.473012	11.7	12	3	15
	Glob	20	28	60	10.9028	45.85	48	11	71
	AG	20	.18	.31	.0278341	.258	.255	0.19	0.46
	Chol	20	.19	.82	.1550755	.432	.425	0.05	0.81
	Trig	20	.11	.43	.0896352	.2285	.21	0.00	0.44
	Gluc	20	1	2.3	.3503382	1.48	1.5	0.3	2.1
	Lactate	20	.42	4.46	1.139048	2.529	2.48	0.98	14.46

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid and creatinine (µmol/L); for total protein, albumin, and globulin (g/L)

Table 6. Summary statistics for metabolite concentrations for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2013. Highlighted values are outside the reference interval.

Crab	Category	n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2]Urea	20	.1	.4	.0887041	.295	.3	0.3	1.1
	Creat	20	0	0	0	0	0	0	0
	Uric	20	38	92	16.91962	62.2	59.5	65	199
	TPb	20	27	103	19.65083	58.55	61	36	97
	Alb	20	5	20	3.803046	11.6	12	8	22
	Glob	20	22	83	16.018	46.95	49	28	78
	AG	20	.19	.31	.0305175	.2495	.24	0.23	0.33
	Chol	20	.27	.98	.2036276	.597	.65	0.22	1.07
	Trig	20	.04	.17	.0350038	.096	.095	0.05	0.20
	Gluc	20	.8	2.3	.4076893	1.61	1.7	0.7	2.6
	Lactate	20	1.5	8.89	2.433826	4.5255	3.885	2.15	13.05
LM	Urea	19	.2	.3	.0512989	.2526316	.3	0.2	0.6
	Creat	19	0	0	0	0	0	0	0
	Uric	19	13	50	11.89329	27.68421	24	6	75
	TPb	19	32	64	8.160309	46.42105	46	14	65
	Alb	19	6	16	2.52936	10.21053	10	6	16
	Glob	19	24	48	6.142556	36.21053	36	12	52
	AG	19	.20	.38	.0477934	.2821053	.28	0.28	0.46
	Chol	19	.32	.78	.1206924	.57	.55	0.14	0.89
	Trig	19	.04	.13	.0311945	.0878947	.08	0.03	0.16
	Gluc	19	1	1.7	.2010208	1.247368	1.1	0.5	1.7
	Lactate	19	.46	5.3	1.154605	1.956842	1.83	0.00	3.47
MF	Urea	17	.1	.5	.120049	.2764706	.3	0.2	2.2
	Creat	17	0	0	0	0	0	0	0
	Uric	17	37	95	18.05812	71.29412	71	62	222
	TPb	17	16	66	11.73168	50.41176	53	9	81
	Alb	17	5	13	2.014652	10.05882	10	3	15
	Glob	17	11	54	9.955785	40.35294	43	11	71
	AG	17	.21	.45	.0576054	.2594118	.25	0.19	0.46
	Chol	17	.11	.75	.1610718	.5023529	.51	0.05	0.81
	Trig	17	.04	.33	.0698001	.2070588	.20	0.00	0.44
	Gluc	17	.6	2.2	.3655174	1.511765	1.6	0.3	2.1
	Lactate	17	1.27	6.04	1.24368	3.634706	3.71	0.98	14.46

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units for urea, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid and creatinine (µmol/L); for total protein, albumin, and globulin (g/L)

Table 7. Summary statistics for metabolite concentrations for 12 month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, November 2013. Highlighted values are outside the reference interval.

Crab	Category	n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2]Urea	13	.1	.5	.1265924	.2461538	.2	0.3	1.1
	Creat	13	0	0	0	0	0	0	0
	Uric	13	18	51	9.636336	38.23077	41	65	199
	TPb	13	7	37	10.03711	20.07692	21	36	97
	Alb	13	3	10	2.213015	5.692308	6	8	22
	Glob	13	4	29	7.942905	14.38462	15	28	78
	AG	13	.28	.75	.1233299	.4453846	.44	0.23	0.33
	Chol	13	.1	.44	.1098776	.2569231	.26	0.22	1.07
	Trig	13	.02	.06	.0130089	.0376923	.04	0.05	0.20
	Gluc	13	.2	.9	.2531848	.5076923	.5	0.7	2.6
	Lactate	13	.81	3.11	.5891454	1.843846	2.00	2.15	13.05
LM	Urea	8	.1	.2	.0353553	.1875	.2	0.2	0.6
	Creat	8	0	0	0	0	0	0	0
	Uric	8	11	63	16.73107	26.75	23.5	6	75
	TPb	8	10	31	8.518887	19.5	18	14	65
	Alb	8	3	8	1.927248	5.5	5	6	16
	Glob	8	6	23	6.676184	14	13	12	52
	AG	8	.32	.67	.107695	.42625	.42	0.28	0.46
	Chol	8	.10	.58	.1498034	.30125	.275	0.14	0.89
	Trig	8	.02	.08	.0225198	.0475	.05	0.03	0.16
	Gluc	8	.2	.8	.203101	.5125	.55	0.5	1.7
	Lactate	8	.33	1.15	.2666961	.85125	.865	0.00	3.47
MF	Urea	17	.1	.5	.0931476	.2647059	.3	0.2	2.2
	Creat	17	0	0	0	0	0	0	0
	Uric	17	17	73	14.72043	48.23529	50	62	222
	TPb	17	6	27	6.163221	15.11765	13	9	81
	Alb	17	3	7	1.064121	4.588235	4	3	15
	Glob	17	3	20	5.209578	10.52941	9	11	71
	AG	17	.28	1.00	.2018208	.5123529	.45	0.19	0.46
	Chol	17	.11	.28	.0506066	.1611765	.15	0.05	0.81
	Trig	17	.03	.09	.017636	.0411765	.03	0.00	0.44
	Gluc	17	.1	1.4	.3804023	.5294118	.4	0.3	2.1
	Lactate	17	.38	2.71	.6298109	1.343529	1.38	0.98	14.46

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid and creatinine (µmol/L); for total protein, albumin, and globulin (g/L)

Table 8. Summary statistics for metabolite concentrations for 12 month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2013. Highlighted values are outside the reference interval.

Crab	Category	n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2]Urea	16	.1	.6	.1436141	.30625	.3	0.3	1.1
	Creat	16	0	0	0	0	0	0	0
	Uric	16	14	53	12.0499	41	43.5	65	199
	TPb	16	6	23	5.205366	10.8125	8	36	97
	Alb	16	2	6	1.152895	3.5625	3	8	22
	Glob	16	3	17	4.203173	7.25	5	28	78
	AG	16	.29	1.00	.2021705	.569375	.6	0.23	0.33
	Chol	16	.10	.31	.058935	.1675	.145	0.22	1.07
	Trig	16	.01	.05	.0110868	.021875	.02	0.05	0.20
	Gluc	16	.1	1.3	.3614784	.4	.3	0.7	2.6
	Lactate	16	.36	7.68	1.650033	1.896875	1.485	2.15	13.05
LM	Urea	15	.1	.8	.1934647	.38	.4	0.2	0.6
	Creat	15	0	0	0	0	0	0	0
	Uric	15	9	38	8.817191	20.8	20	6	75
	TPb	15	7	26	5.799836	14.93333	14	14	65
	Alb	15	2	7	1.387015	4.066667	4	6	16
	Glob	15	4	19	4.703595	10.86667	10	12	52
	AG	15	.23	.75	.137061	.41	.40	0.28	0.46
	Chol	15	.11	.50	.1032242	.2046667	.17	0.14	0.89
	Trig	15	.01	.10	.0235028	.0333333	.03	0.03	0.16
	Gluc	15	.2	.9	.2042408	.52	.5	0.5	1.7
	Lactate	15	.49	2.51	.5932461	1.238667	1.18	0.00	3.47
MF	Urea	15	.1	.6	.1334523	.2733333	.2	0.2	2.2
	Creat	15	0	0	0	0	0	0	0
	Uric	15	28	64	10.69846	43.8	41	62	222
	TPb	15	5	29	8.280787	13	9	9	81
	Alb	15	2	7	1.579632	4.066667	3	3	15
	Glob	15	2	22	6.776711	8.933333	6	11	71
	AG	15	.29	1.50	.3828428	.636	.57	0.19	0.46
	Chol	15	.12	.34	.0822598	.1866667	.15	0.05	0.81
	Trig	15	.03	.12	.0309839	.058	.05	0.00	0.44
	Gluc	15	.1	.9	.2737743	.4733333	.4	0.3	2.1
	Lactate	15	.54	3.12	.7285504	1.34	1.13	0.98	14.46

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units for urea, glucose, cholesterol, triglyceride, and lactate (mmol/L); for uric acid and creatinine (µmol/L); for total protein, albumin, and globulin (g/L)

Table 9. Summary statistics for enzyme activity for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, November 2013. Highlighted values are outside the reference interval.

Crab Category		n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2] AMY	20	1	58	12.41434	8.3	4.5	3	18
	LIP	20	3	11	2.087557	6.6	6	3	13
	AST	20	6	120	31.89522	38.4	29.5	13	203
	ALT	20	11	145	35.25173	50.5	44	23	105
	GD	20	9	44	9.052479	24.5	27	9	37
	SDH	20	0	0	0	0	0	0	1
	ALP	20	0	18	4.404244	1.35	0	0	1
	GGT	20	0	2	.5871429	.35	0	0	1
LM	AMY	20	1	52	13.09319	10.2	5	1	19
	LIP	20	4	11	1.949359	7.3	7	4	15
	AST	20	5	85	18.08016	22.45	17.5	4	46
	ALT	20	10	49	11.01721	23.7	20	6	45
	GD	20	5	31	7.445239	15.8	13.5	4	21
	SDH	20	0	2	.6569467	.3	0	0	0
	ALP	20	0	0	0	0	0	0	0
	GGT	20	0	1	.3663475	.15	0	0	0
MF	AMY	20	0	32	8.641394	9.4	6.5	1	45
	LIP	20	4	13	1.986136	6.55	6	1	21
	AST	20	6	74	18.58692	20	15	16	486
	ALT	20	10	51	10.54115	22.8	20.5	0	188
	GD	20	6	31	7.458658	16.5	16.5	4	39
	SDH	20	0	0	0	0	0	0	2
	ALP	20	0	0	0	0	0	0	13
	GGT	20	0	0	0	0	0	0	4

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations.[2] Units are U/L

Table 10. Summary statistics for enzyme activity for free Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2013. Highlighted values are outside the reference interval.

Crab Category		n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2] AMY	20	2	69	16.23665	14.05	9	3	18
	LIP	20	5	12	1.90498	8.45	8.5	3	13
	AST	20	8	97	24.87712	36.85	29	13	203
	ALT	20	17	120	30.16703	51.45	44.5	23	105
	GD	20	8	45	10.94712	23.55	19.5	9	37
	SDH	20	0	4	1.050063	.55	0	0	1
	ALP	20	0	8	1.79106	.45	0	0	1
	GGT	20	0	1	.3077935	.1	0	0	1
LM	AMY	19	0	37	9.611762	8.947368	5	1	19
	LIP	19	3	12	2.219004	7.421053	7	4	15
	AST	19	5	87	18.88082	17.52632	11	4	46
	ALT	19	9	107	21.61113	23.52632	18	6	45
	GD	19	5	23	4.415549	14.05263	15	4	21
	SDH	19	0	2	.854982	.7894737	1	0	0
	ALP	19	0	0	0	0	0	0	0
	GGT	19	0	1	.3746343	.1578947	0	0	0
MF	AMY	17	2	31	8.390734	9.176471	6	1	45
	LIP	17	4	15	3.172724	8.235294	7	1	21
	AST	17	10	47	9.341164	24.41176	22	16	486
	ALT	17	21	69	15.35391	40.35294	41	0	188
	GD	17	3	30	6.123724	16	17	4	39
	SDH	17	0	0	0	0	0	0	2
	ALP	17	0	0	0	0	0	0	13
	GGT	17	0	1	.4372373	.2352941	0	0	4

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units are U/L

Table 11. Summary statistics for enzyme activity for 12 month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Margaree Harbor, NS, November 2013. Highlighted values are outside the reference interval.

Crab Category		n	min	max	sd	mean	median	Reference Interval[1] Min	Reference Interval[1] Max
PM	[2] AMY	13	2	19	4.336784	8.153846	8	3	18
	LIP	13	6	18	3.819652	9.384615	9	3	13
	AST	13	5	178	48.64985	39.84615	19	13	203
	ALT	13	9	87	22.02272	24	14	23	105
	GD	13	3	17	3.797773	8.384615	8	9	37
	SDH	13	0	1	.2773501	.0769231	0	0	1
	ALP	13	0	1	.4803845	.3076923	0	0	1
	GGT	13	0	1	.3755338	.1538462	0	0	1
LM	AMY	8	2	11	3.226564	6.875	6.5	1	19
	LIP	8	4	11	2.199838	6.625	6.5	4	15
	AST	8	4	36	10.64945	12.625	10	4	46
	ALT	8	5	27	7.395703	9.875	7	6	45
	GD	8	3	13	3.399054	6.125	4.5	4	21
	SDH	8	0	0	0	0	0	0	0
	ALP	8	0	3	1.06066	.375	0	0	0
	GGT	8	0	1	.3535534	.125	0	0	0
MF	AMY	17	0	31	9.192788	9.411765	5	1	45
	LIP	17	4	18	4.558444	9.823529	9	1	21
	AST	17	6	117	26.4846	25.94118	16	16	486
	ALT	17	4	40	8.90555	13.94118	11	0	188
	GD	17	1	16	4.084584	5.941176	5	4	39
	SDH	17	0	0	0	0	0	0	2
	ALP	17	0	1	.3321056	.1176471	0	0	13
	GGT	17	0	1	.2425356	.0588235	0	0	4

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units are U/L

Table 12. Summary statistics for enzyme activity for 12 month caged Pygmy Male, (PM), Large Mature Male (LM), and Mature Female (MF) snow crab from Cheticamp, NS, November 2013. Highlighted values are outside the reference interval.

Crab Category		n	min	max	sd	mean	median	Reference Interval[1] Min	Max
PM	AMY[2]	16	3	25	5.982962	7.9375	5.5	3	18
	LIP	16	2	13	3.4809	7.625	8	3	13
	AST	16	5	176	50.9846	59.3125	38	13	203
	ALT	16	5	52	13.97856	26.25	25	23	105
	GD	16	3	40	10.13821	9.125	6	9	37
	SDH	16	0	1	.25	.0625	0	0	1
	ALP	16	0	28	8.144272	4.0625	1	0	1
	GGT	16	0	1	.4031129	.1875	0	0	1
LM	AMY	15	0	91	26.66851	30.73333	27	1	19
	LIP	15	4	14	3.090693	9.133333	10	4	15
	AST	15	11	104	27.383	44.4	34	4	46
	ALT	15	8	106	27.53958	37	28	6	45
	GD	15	2	12	2.88345	6.2	6	4	21
	SDH	15	0	2	.6399405	.4666667	0	0	0
	ALP	15	0	2	.6399405	.4666667	0	0	0
	GGT	15	0	1	.4140393	.2	0	0	0
MF	AMY	15	2	51	13.31952	13.13333	7	1	45
	LIP	15	2	32	9.255629	12.66667	8	1	21
	AST	15	10	63	17.63465	29.53333	28	16	486
	ALT	15	6	47	10.8619	19.46667	16	0	188
	GD	15	2	14	3.018988	5.4	6	4	39
	SDH	15	0	0	0	0	0	0	2
	ALP	15	0	1	.3518658	.1333333	0	0	13
	GGT	15	0	1	.3518658	.1333333	0	0	4

[1] Reference Interval is from August, 2012 Trap vs. Trawl study. Values are from free, cooler-held crabs captured in Cheticamp, NS. Note, values for Pygmy Males (n = 13) represent min and max of sampled crabs, while values for Large Mature Male (n = 20) and Mature Female (n = 29) are calculated upper and lower limits of the sampled populations. [2] Units are U/L

Effect of Treatment (Caged vs Free) within a Station by Sex

The effects of the 12 month caging period for each category of crab in Margaree Harbor and Cheticamp were compared (Wilcoxon signed rank test) and are summarised in Tables 13 and 14.. Sodium and chloride concentrations were consistently and significantly higher in caged crabs than free crabs for all categories and both sites (except $LM_{Cheticamp}$), while calcium concentrations were consistently and significantly lower than free crabs. Most metabolites (total protein, albumin, globulin, cholesterol, triglyceride, and glucose) were significantly lower in all categories of caged crabs compared to free crabs at both sites; while, urea was significantly lower in caged crabs at Margaree Harbor only. Lactate and uric acid concentrations were also lower for all caged crab categories compared to free crab, but decreases were not statistically significant for $LM_{Cheticamp}$. Median activities for GD were lower in all caged crabs compared to free counterparts as was ALT activity, with the exception of $MM_{Cheticamp}$.

Effect of Sex within Station by Treatment (Caged or Free)

Median values were also compared (Kruskal-Wallis and Wilcoxon signed rank test) within a station across crab categories for free crabs and caged crabs (Tables 15 & 16). Notably, MF crabs had the highest plasma magnesium levels in all cases (caged or free for either location). Free LM crabs at either site tended to have lower values for many metabolites (uric acid, total protein, albumin, globulin, glucose, lactate, and triglyceride) when compared to free PM or MF crabs although the differences were not always statistically significant. These differences often disappeared in association with caging at either site. Triglyceride levels were significantly higher in free MF compared to PM or LM crabs at both stations but only caged $MF_{Cheticamp}$. Enzyme activity showed the least differences across categories for either free or caged crabs.

Effect of Station within a Treatment by Sex

For free crabs, comparison (Wilcoxon signed rank test) of crab category between stations (Table 17) indicated that sodium, chloride, and magnesium values were significantly higher for all categories of Cheticamp crabs, while phosphorus concentrations were lower when compared to Margaree Harbor crabs. Other than $PM_{Cheticamp}$ having lower median values for urea, triglyceride, glucose, and lactate, than $PM_{Margaree}$ there were few differences observed for either metabolites or enzyme activity.

For caged crabs, comparison (Wilcoxon signed rank test) between the two sample sites (Table 18) showed higher values for PM and MF crabs from Cheticamp for sodium and chloride compared to Margaree Harbor counterparts. Magnesium values were also higher for all crab categories from Cheticamp, but only statistically higher for PM and MF crabs. Margaree Harbor caged crabs usually had slightly higher median values for many metabolites (uric acid, total protein, albumin, globulin, cholesterol, triglyceride, and lactate) compared to Cheticamp crabs; however, values were only statistically higher for PM crabs. Plasma enzyme activity was essentially equal for both sites. The caged LM crabs from Cheticamp showed slightly higher values for amylase, AST, and ALT.

Table 13. Effect of twelve months of caging on median values of hemolymph plasma biochemistry parameters of snow crab collected from Margaree Harbor, NS in November 2013. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) changes were noted are shown.

Analyte	Margaree Harbor													
	PM					LM					MF			
	n	Trap	*n*	Cage	*p*	*n*	Trap	*n*	Cage	*p*	*n*	Trap	*n*	Cage	*p*
Sodium (mmol/L)	*20*	406.5	*10*	442.5	*0.001*	*20*	399	*8*	448.5	*0.001*	*20*	415.5	*17*	453	*0.0000*
Potassium (mmol/L)	*20*	11.1	*10*	11.4	--	*20*	10.35	*8*	9.6	--	*20*	10.35	*17*	11.4	*0.0001*
Na:K	*20*	37	*10*	39	--	*20*	39	*8*	46	*0.0001*	*20*	40	*17*	40	--
Chloride (mmol/L)	*20*	409.5	*10*	462	*0.0000*	*20*	405	*8*	469.5	*0.0000*	*20*	426	*17*	489	*0.0000*
Calcium (mmol/L)	*20*	13.50	*13*	11.88	*0.0000*	*20*	12.68	*8*	12.11	*0.0146*	*20*	13.53	*17*	11.53	*0.0000*
Phosphorus (mmol/L)	*20*	2.345	*13*	1.73	--	*20*	1.965	*8*	0.855	*0.0001*	*20*	1.795	*17*	1.58	--
Magnesium (mmol/L)	*20*	39.595	*13*	39.96	--	*20*	38.27	*8*	39.155	--	*20*	42.2	*17*	42.32	--
Urea (mmol/L)	*20*	0.4	*13*	0.2	*0.0009*	*20*	0.3	*8*	0.2	*0.0036*	*20*	0.3	*17*	0.3	*0.0474*
Uric Acid (µmol/L)	*20*	48.5	*13*	41	*0.0115*	*20*	36	*8*	23.5	--	*20*	64	*17*	50	*0.0129*
Total Protein (g/L)	*20*	67.5	*13*	21	*0.0000*	*20*	42	*8*	18	*0.0010*	*20*	60.5	*17*	13	*0.0000*
Albumin (g/L)	*20*	14	*13*	6	*0.0000*	*20*	8.5	*8*	5	*0.0067*	*20*	12	*17*	4	*0.0000*
Globulin (g/L)	*20*	52.5	*13*	15	*0.0000*	*20*	32	*8*	13	*0.0007*	*20*	48	*17*	9	*0.0000*
A:G	*20*	0.24	*13*	0.44	*0.0000*	*20*	0.28	*8*	0.42	*0.0001*	*20*	0.255	*17*	0.45	*0.0000*
Cholesterol (mmol/L)	*20*	0.6	*13*	0.26	*0.0000*	*20*	0.43	*8*	0.275	*0.0156*	*20*	0.425	*17*	0.15	*0.0000*
Triglyceride mmol/L)	*20*	0.15	*13*	0.04	*0.0000*	*20*	0.075	*8*	0.05	*0.0229*	*20*	0.21	*17*	0.03	*0.0000*
Glucose (mmol/L)	*20*	1.9	*13*	0.5	*0.0000*	*20*	1.1	*8*	0.55	*0.0003*	*20*	1.5	*17*	0.4	*0.0000*
Lactate (mmol/L)	*20*	6.075	*13*	2	*0.0000*	*20*	2.49	*8*	0.865	*0.0001*	*20*	2.48	*17*	1.38	*0.0014*
Creatinine[1] (µmol/L)	*20*	0	*13*	0		*20*	0	*8*	0		*20*	0	*17*	0	
Amylase (U/L)	*20*	4.5	*13*	8	--	*20*	5	*8*	6.5	--	*20*	6.5	*17*	5	--
Lipase (U/L)	*20*	6	*13*	9	*0.0146*	*20*	7	*8*	6.5	--	*20*	6	*17*	9	*0.0184*
AST (U/L)	*20*	29.5	*13*	19	--	*20*	17.5	*8*	10	--	*20*	15	*17*	16	--
ALT (U/L)	*20*	44	*13*	14	*0.0051*	*20*	20	*8*	7	*0.0014*	*20*	20.5	*17*	11	*0.0029*
GD (U/L)	*20*	27	*13*	8	*0.0000*	*20*	13.5	*8*	4.5	*0.0008*	*20*	16.5	*17*	5	*0.0000*
SDH (U/L)	*20*	0	*13*	0	--	*20*	0	*8*	0	--	*20*	0	*17*	0	--
ALP (U/L)	*20*	0	*13*	0	--	*20*	0	*8*	0	--	*20*	0	*17*	0	--
GGT (U/L)	*20*	0	*13*	0	--	*20*	0	*8*	0	--	*20*	0	*17*	0	--

[1] analyte not detected

Table 14. Effect of 12 months of caging on median values of hemolymph plasma biochemistry parameters of snow crab collected from Cheticamp, NS in November 2013. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) changes were noted are shown.

Analyte	Cheticamp													
	PM					LM					MF			
	n	Trap	*n*	Cage	*p*	*n*	Trap	n	Cage	*p*	n	Trap	*n*	Cage	*p*
Sodium (mmol/L)	*20*	421.5	*16*	459	*0.0000*	*19*	444	*15*	435	--	*18*	433.5	*15*	468	*0.0000*
Potassium (mmol/L)	*20*	10.5	*16*	11.85	*0.0001*	*19*	10.2	*15*	11.4	*0.0018*	*18*	9.9	*15*	12	*0.0000*
Na:K	*20*	40.5	*16*	39	*0.0275*	*19*	43	*15*	38	*0.0015*	*18*	44	*15*	39	*0.0015*
Chloride (mmol/L)	*20*	426	*16*	498	*0.0000*	*19*	459	*15*	465	--	*18*	459	*15*	507	*0.0000*
Calcium (mmol/L)	*20*	13.065	*16*	12.085	*0.0000*	*19*	13.33	*15*	12.11	*0.0000*	*17*	13.78	*15*	12.01	*0.0000*
Phosphorus (mmol/L)	*20*	1.85	*16*	1.27	*0.0010*	*19*	1.67	*15*	1.12	*0.0007*	*17*	1.26	*15*	1.24	--
Magnesium (mmol/L)	*20*	42.02	*16*	42.005	--	*19*	40.91	*15*	39.81	*0.0065*	*17*	43.68	*15*	43.68	--
Urea (mmol/L)	*20*	0.3	*16*	0.3	--	*19*	0.3	*15*	0.4	*0.0277*	*17*	0.3	*15*	0.2	--
Uric Acid (µmol/L)	*20*	59.5	*16*	43.5	*0.0006*	*19*	24	*15*	20	--	*17*	71	*15*	41	*0.001*
Total Protein (g/L)	*20*	61	*16*	8	*0.0000*	*19*	46	*15*	14	*0.0000*	*17*	53	*15*	9	*0.0000*
Albumin (g/L)	*20*	12	*16*	3	*0.0000*	*19*	10	*15*	4	*0.0000*	*17*	10	*15*	3	*0.0000*
Globulin (g/L)	*20*	49	*16*	5	*0.0000*	*19*	36	*15*	10	*0.0000*	*17*	43	*15*	6	*0.0000*
A:G	*20*	0.24	*16*	0.6	*0.0000*	*19*	0.28	*15*	0.4	*0.0024*	*17*	0.25	*15*	0.57	*0.0000*
Cholesterol (mmol/L)	*20*	0.65	*16*	0.145	*0.0000*	*19*	0.55	*15*	0.17	*0.0000*	*17*	0.51	*15*	0.15	*0.0000*
Triglyceride (mmol/L)	*20*	0.095	*16*	0.02	*0.0000*	*19*	0.08	*15*	0.03	*0.0000*	*17*	0.2	*15*	0.05	*0.0000*
Glucose (mmol/L)	*20*	1.7	*16*	0.3	*0.0000*	*19*	1.1	*15*	0.5	*0.0000*	*17*	1.6	*15*	0.4	*0.0000*
Lactate (mmol/L)	*20*	3.885	*16*	1.485	*0.0002*	*19*	1.83	*15*	1.18	--	*17*	3.71	*15*	1.13	*0.0000*
Creatinine[1] (µmol/L)	*20*	0	*16*	0		*19*	0	*15*	0		*17*	0	*15*	0	
Amylase (U/L)	*20*	9	*16*	5.5	--	*19*	5	*15*	27	*0.0030*	*17*	6	*15*	7	--
Lipase (U/L)	*20*	8.5	*16*	8	--	*19*	7	*15*	10	--	*17*	7	*15*	8	--
AST (U/L)	*20*	29	*16*	38	--	*19*	11	*15*	34	*0.0005*	*17*	22	*15*	28	--
ALT (U/L)	*20*	44.5	*16*	25	*0.0051*	*19*	18	*15*	28	--	*17*	41	*15*	16	*0.0001*
GD (U/L)	*20*	19.5	*16*	6	*0.0001*	*19*	15	*15*	6	*0.0000*	*17*	17	*15*	6	*0.0000*
SDH (U/L)	*20*	0	*16*	0	--	*19*	1	*15*	0	--	*17*	0	*15*	0	--
ALP (U/L)	*20*	0	*16*	0	*0.0035*	*19*	0	*15*	0	*0.0028*	*17*	0	*15*	0	--
GGT (U/L)	*20*	0	*16*	0	--	*19*	0	*15*	0	--	*17*	0	*15*	0	--

[1] analyte not detected

Table 15. Summary of median values of hemolymph plasma biochemistry parameters of snow crab collected from Margaree Harbor, NS in November 2013 by traps and after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). Median values sharing the same superscript are not different (Wilcoxon rank sum, Bonferroni-adjusted $p > 0.017$ from each other; for analytes where no superscripts are shown, medians were not different (Kruskall-Wallis testing, $p < 0.05$)

Analyte	Margaree Harbor											
	Free						Caged					
	n	PM	*n*	LM	*n*	MF	*n*	PM	*n*	LM	*n*	MF
Sodium (mmol/L)	*20*	^{a,b}406.5	*20*	^{a,c}399	*20*	^{b}415.5	*10*	^{a,b}442.5	*8*	^{a,c}448.5	*17*	^{c}453
Potassium (mmol/L)	*20*	^{a}11.1	*20*	^{c}10.35	*20*	^{b,c}10.35	*10*	^{a,b}11.4	*8*	^{c}9.6	*17*	^{b}11.4
Na:K	*20*	^{a}37	*20*	^{c}39	*20*	^{b,c}40	*10*	^{a,b}39	*8*	^{c}46	*17*	^{b}40
Chloride (mmol/L)	*20*	^{a}409.5	*20*	^{a,c}405	*20*	^{b,c}426	*10*	^{a}462	*8*	^{a,c}469.5	*17*	^{b}489
Calcium (mmol/L)	*20*	^{a,b}13.50	*20*	^{c}12.68	*20*	^{b}13.53	*13*	^{a,b}11.88	*8*	^{a,c}12.11	*17*	^{b,c}11.53
Phosphorus (mmol/L)	*20*	2.345	*20*	1.965	*20*	1.795	*13*	^{a,b}1.73	*8*	^{c}0.855	*17*	^{b}1.58
Magnesium (mmol/L)	*20*	^{a}39.595	*20*	^{c}38.27	*20*	^{b}42.2	*13*	^{a}39.96	*8*	^{a,c}39.155	*17*	^{b}42.32
Urea (mmol/L)	*20*	^{a}0.4	*20*	^{c}0.3	*20*	^{b,c}0.3	*13*	0.2	*8*	0.2	*17*	0.3
Uric Acid (µmol/L)	*20*	^{a,b}48.5	*20*	^{c}36	*20*	^{b}64	*13*	^{a,b}41	*8*	^{a,c}23.5	*17*	^{b}50
Total Protein (g/L)	*20*	^{a,b}67.5	*20*	^{c}42	*20*	^{b}60.5	*13*	21	*8*	18	*17*	13
Albumin (g/L)	*20*	^{a,b}14	*20*	^{c}8.5	*20*	^{b,c}12	*13*	6	*8*	5	*17*	4
Globulin (g/L)	*20*	^{a,b}52.5	*20*	^{c}32	*20*	^{b}48	*13*	15	*8*	13	*17*	9
A:G	*20*	^{a,b}0.24	*20*	^{c}0.28	*20*	^{b,c}0.255	*13*	0.44	*8*	0.42	*17*	0.45
Cholesterol (mmol/L)	*20*	^{a}0.6	*20*	^{c}0.43	*20*	^{b,c}0.425	*13*	^{a}0.26	*8*	^{a,c}0.275	*17*	^{b}0.15
Triglyceride mmol/L)	*20*	^{a}0.15	*20*	^{c}0.075	*20*	^{b}0.21	*13*	0.04	*8*	0.05	*17*	0.03
Glucose (mmol/L)	*20*	^{a}1.9	*20*	^{c}1.1	*20*	^{b}1.5	*13*	0.5	*8*	0.55	*17*	0.4
Lactate (mmol/L)	*20*	^{a}6.075	*20*	^{c}2.49	*20*	^{b,c}2.48	*13*	^{a,b}2.00	*8*	^{c}0.865	*17*	^{b,c}1.38
Creatinine[1] (mmol/L)	*20*	0	*20*	0	*20*	0	*13*	0	*8*	0	*17*	0
Amylase (U/L)	*20*	4.5	*20*	5	*20*	6.5	*13*	8	*8*	6.5	*17*	5
Lipase (U/L)	*20*	6	*20*	7	*20*	6	*13*	9	*8*	6.5	*17*	9
AST (U/L)	*20*	29.5	*20*	17.5	*20*	15	*13*	19	*8*	10	*17*	16
ALT (U/L)	*20*	^{a}44	*20*	^{c}20	*20*	^{b,c}20.5	*13*	^{a,b}14	*8*	^{c}7	*17*	^{b,c}11
GD (U/L)	*20*	^{a}27	*20*	^{c}13.5	*20*	^{b,c}16.5	*13*	8	*8*	4.5	*17*	5
SDH (U/L)	*20*	0	*20*	0	*20*	0	*13*	0	*8*	0	*17*	0
ALP (U/L)	*20*	^{a}0	*20*	^{a,c}0	*20*	^{b}0	*13*	0	*8*	0	*17*	0
GGT (U/L)	*20*	0	*20*	0	*20*	0	*13*	0	*8*	0	*17*	0

[1] analyte not detected

Table 16. Summary of median values of hemolymph plasma biochemistry parameters of snow crab collected from Cheticamp, NS in November 2013 by traps and after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). All groups have 20 crabs unless indicated. Median values sharing the same superscript are not different (Wilcoxon rank sum, Bonferroni-adjusted $p > 0.017$ from each other; for analytes where no superscripts are shown, medians were not different (Kruskall-Wallis testing, $p < 0.05$)

Analyte	Cheticamp											
	Free						Caged					
	n	PM	*n*	LM	*n*	MF	*n*	PM	*n*	LM	*n*	MF
Sodium (mmol/L)	*20*	[a,b]421.5	*19*	[c]444	*18*	[c]433.5	*16*	[a,b]459	*15*	[a]435	*15*	[c]468
Potassium (mmol/L)	*20*	10.5	*19*	10.2	*18*	9.9	*16*	11.85	*15*	11.4	*15*	12
Na:K	*20*	[a]40.5	*19*	[a,c]43	*18*	[b,c]44	*16*	39	*15*	38	*15*	39
Chloride (mmol/L)	*20*	[a,b]426	*19*	[a,c]459	*18*	[b]459	*16*	[a]498	*15*	[a,c]465	*15*	[b,c]507
Calcium (mmol/L)	*20*	[a,b]13.065	*19*	[a,c]13.33	*17*	[b]13.78	*16*	12.085	*15*	12.11	*15*	12.01
Phosphorus (mmol/L)	*20*	[a]1.85	*19*	[a,c]1.67	*17*	[b]1.26	*16*	1.27	*15*	1.12	*15*	1.24
Magnesium (mmol/L)	*20*	[a]42.02	*19*	[c]40.91	*17*	[b]43.68	*16*	[a]42.005	*15*	[c]39.81	*15*	[b]43.68
Urea (mmol/L)	*20*	0.3	*19*	0.3	*17*	0.3	*16*	0.3	*15*	0.4	*15*	0.2
Uric Acid (µmol/L)	*20*	[a,b]59.5	*19*	[c]24	*17*	[b]71	*16*	[a,b]43.5	*15*	[c]20	*15*	[b]41
Total Protein (g/L)	*20*	[a,b]61	*19*	[c]46	*17*	[b,c]53	*16*	8	*15*	14	*15*	9
Albumin (g/L)	*20*	12	*19*	10	*17*	10	*16*	3	*15*	4	*15*	3
Globulin (g/L)	*20*	[a,b]49	*19*	[c]36	*17*	[b,c]43	*16*	5	*15*	10	*15*	6
A:G	*20*	0.24	*19*	0.28	*17*	0.25	*16*	[a,b]0.60	*15*	[c]0.40	*15*	[b,c]0.57
Cholesterol (mmol/L)	*20*	0.65	*19*	0.55	*17*	0.51	*16*	0.145	*15*	0.17	*15*	0.15
Triglyceride mmol/L)	*20*	[a]0.095	*19*	[a,c]0.08	*17*	[b]0.20	*16*	[a]0.02	*15*	[a,c]0.03	*15*	[b]0.05
Glucose (mmol/L)	*20*	[a,b]1.7	*19*	[c]1.1	*17*	[b]1.6	*16*	0.3	*15*	0.5	*15*	0.4
Lactate (mmol/L)	*20*	[a,b]3.885	*19*	[c]1.83	*17*	[b]3.71	*16*	1.485	*15*	1.18	*15*	1.13
Creatinine[1] (mmol/L)	*20*	0	*19*	0	*17*	0	*16*	0	*15*	0	*15*	0
Amylase (U/L)	*20*	9	*19*	5	*17*	6	*16*	[a,b]5.5	*15*	[c]27	*15*	[b,c]7
Lipase (U/L)	*20*	8.5	*19*	7	*17*	7	*16*	8	*15*	10	*15*	8
AST (U/L)	*20*	[a,b]29	*19*	[c]11	*17*	[b]22	*16*	38	*15*	34	*15*	28
ALT (U/L)	*20*	[a,b]44.5	*19*	[c]18	*17*	[b]41	*16*	25	*15*	28	*15*	16
GD (U/L)	*20*	[a,b]19.5	*19*	[c]15	*17*	[b,c]17	*16*	6	*15*	6	*15*	6
SDH (U/L)	*20*	[a]0	*19*	[a,c]1	*17*	[b]0	*16*	[a,b]0	*15*	[a,c]0	*15*	[b]0
ALP (U/L)	*20*	0	*19*	0	*17*	0	*16*	[a]0	*15*	[a,c]0	*15*	[b,c]0
GGT (U/L)	*20*	0	*19*	0	*17*	0	*16*	0	*15*	0	*15*	0

[1] analyte not detected

Table 17. Comparison between Margaree Harbor, NS and Cheticamp, NS, of median values of hemolymph plasma biochemistry parameters for snow crab collected by traps in November 2013. Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) differences were detected are shown.

Analyte	Free Crabs													
	PM					LM					MF			
	n	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*
Sodium (mmol/L)	*20*	406.5	*20*	421.5	*0.0244*	*20*	399	*19*	444	*0.0000*	*20*	415.5	*18*	433.5	*0.0020*
Potassium (mmol/L)	*20*	11.1	*20*	10.5	*0.0053*	*20*	10.35	*19*	10.2	--	*20*	10.35	*18*	9.9	--
Na:K	*20*	37	*20*	40.5	*0.0001*	*20*	39	*19*	43	*0.0003*	*20*	40	*18*	44	*0..0001*
Chloride (mmol/L)	*20*	409.5	*20*	426	*0.0007*	*20*	405	*19*	459	*0.0000*	*20*	426	*18*	459	*0.0001*
Calcium (mmol/L)	*20*	13.50	*20*	13.065	--	*20*	12.68	*19*	13.33	*0.0036*	*20*	13.53	*17*	13.78	--
Phosphorus (mmol/L)	*20*	2.345	*20*	1.85	*0.337*	*20*	1.965	*19*	1.67	*0.0305*	*20*	1.795	*17*	1.26	*0.0013*
Magnesium (mmol/L)	*20*	39.595	*20*	42.02	*0.0000*	*20*	38.27	*19*	40.91	*0.0000*	*20*	42.20	*17*	43.68	*0.0012*
Urea (mmol/L)	*20*	0.4	*20*	0.3	*0.0016*	*20*	0.3	*19*	0.3	--	*20*	0.3	*17*	0.3	--
Uric Acid (µmol/L)	*20*	48.5	*20*	59.5	--	*20*	36	*19*	24	--	*20*	64	*17*	71	--
Total Protein (g/L)	*20*	67.5	*20*	61	--	*20*	42	*19*	46	--	*20*	60.5	*17*	53	--
Albumin (g/L)	*20*	14	*20*	12	--	*20*	8.5	*19*	10	--	*20*	12	*17*	10	--
Globulin (g/L)	*20*	52.5	*20*	49	--	*20*	32	*19*	36	--	*20*	48	*17*	43	--
A:G	*20*	0.24	*20*	0.24	--	*20*	0.28	*19*	0.28	--	*20*	0.255	*17*	0.25	--
Cholesterol (mmol/L)	*20*	0.60	*20*	0.65	--	*20*	0.43	*19*	0.55	*0.0109*	*20*	0.425	*17*	0.51	--
Triglyceride mmol/L)	*20*	0.15	*20*	0.095	*0.0028*	*20*	0.075	*19*	0.08	--	*20*	0.21	*17*	0.20	--
Glucose (mmol/L)	*20*	1.9	*20*	1.7	*0.0486*	*20*	1.1	*19*	1.1	--	*20*	1.5	*17*	1.6	--
Lactate (mmol/L)	*20*	6.075	*20*	3.885	*0.0398*	*20*	2.49	*19*	1.83	--	*20*	2.48	*17*	3.71	*0.0167*
Creatinine[1] (µmol/L)	*20*	0	*20*	0		*20*	0	*19*	0		*20*	0	*17*	0	
Amylase (U/L)	*20*	4.5	*20*	9	--	*20*	5	*19*	5	--	*20*	6.5	*17*	6	--
Lipase (U/L)	*20*	6	*20*	8.5	*0.0066*	*20*	7	*19*	7	--	*20*	6	*17*	7	--
AST (U/L)	*20*	29.5	*20*	29	--	*20*	17.5	*19*	11	--	*20*	15	*17*	22	*0.0123*
ALT (U/L)	*20*	44	*20*	44.5	--	*20*	20	*19*	18	--	*20*	20.5	*17*	41	*0.0004*
GD (U/L)	*20*	27	*20*	19.5	--	*20*	13.5	*19*	15	--	*20*	16.5	*17*	17	--
SDH (U/L)	*20*	0	*20*	0	*0.0090*	*20*	0	*19*	1	*0.0408*	*20*	0	*17*	0	--
ALP (U/L)	*20*	0	*20*	0	--	*20*	0	*19*	0	--	*20*	0	*17*	0	--
GGT (U/L)	*20*	0	*20*	0	--	*20*	0	*19*	0	--	*20*	0	*17*	0	*0.0235*

[1] analyte not detected

Table 18. Comparison between Margaree Harbor, NS and Cheticamp, NS, of median values of hemolymph plasma biochemistry parameters for snow crab held in cages from November 2012 – November 2013. Only *p*-values for parameters where significant ($p < 0.05$, Wilcoxon signed rank test) differences were detected are shown.

Analyte	Caged Crabs													
	PM					LM					MF			
	n	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*	*n*	Marg	*n*	Chet	*p*
Sodium (mmol/L)	*10*	442.5	*16*	459	*0.0387*	*8*	448.5	*15*	435	--	*17*	453	*15*	468	*0.0003*
Potassium (mmol/L)	*10*	11.4	*16*	11.85	--	*8*	9.6	*15*	11.4	*0.0011*	*17*	11.4	*15*	12	--
Na:K	*10*	39	*16*	39	--	*8*	46	*15*	38	*0.0002*	*17*	40	*15*	39	--
Chloride (mmol/L)	*10*	462	*16*	498	*0.0028*	*8*	469.5	*15*	465	--	*17*	489	*15*	507	*0.0001*
Calcium (mmol/L)	*13*	11.88	*16*	12.085	--	*8*	12.11	*15*	12.11	--	*17*	11.53	*15*	12.01	*0.0062*
Phosphorus (mmol/L)	*13*	1.73	*16*	1.27	*0.0124*	*8*	0.855	*15*	1.12	--	*17*	1.58	*15*	1.24	*0.0344*
Magnesium (mmol/L)	*13*	39.96	*16*	42.005	*0.0015*	*8*	39.155	*15*	39.81	--	*17*	42.32	*15*	43.68	*0.0000*
Urea (mmol/L)	*13*	0.2	*16*	0.3	--	*8*	0.2	*15*	0.4	*0.0110*	*17*	0.3	*15*	0.2	--
Uric Acid (µmol/L)	*13*	41	*16*	43.5	--	*8*	23.5	*15*	20	--	*17*	50	*15*	41	--
Total Protein (g/L)	*13*	21	*16*	8	*0.0045*	*8*	18	*15*	14	--	*17*	13	*15*	9	--
Albumin (g/L)	*13*	6	*16*	3	*0.0050*	*8*	5	*15*	4	--	*17*	4	*15*	3	--
Globulin (g/L)	*13*	15	*16*	5	*0.0054*	*8*	13	*15*	10	--	*17*	9	*15*	6	--
A:G	*13*	0.44	*16*	0.6	--	*8*	0.42	*15*	0.4	--	*17*	0.45	*15*	0.57	--
Cholesterol (mmol/L)	*13*	0.26	*16*	0.145	*0.0281*	*8*	0.275	*15*	0.17	--	*17*	0.15	*15*	0.15	--
Triglyceride mmol/L)	*13*	0.04	*16*	0.02	*0.0025*	*8*	0.05	*15*	0.03	--	*17*	0.03	*15*	0.05	--
Glucose (mmol/L)	*13*	0.5	*16*	0.3	--	*8*	0.55	*15*	0.5	--	*17*	0.4	*15*	0.4	--
Lactate (mmol/L)	*13*	2.00	*16*	1.485	--	*8*	0.865	*15*	1.18	--	*17*	1.38	*15*	1.13	--
Creatinine[1] (µmol/L)	*13*	0	*16*	0		*8*	0	*15*	0		*17*	0	*15*	0	
Amylase (U/L)	*13*	8	*16*	5.5	--	*8*	6.5	*15*	27	*0.0107*	*17*	5	*15*	7	--
Lipase (U/L)	*13*	9	*16*	8	--	*8*	6.5	*15*	10	--	*17*	9	*15*	8	--
AST (U/L)	*13*	19	*16*	38	--	*8*	10	*15*	34	*0.0019*	*17*	16	*15*	28	--
ALT (U/L)	*13*	14	*16*	25	--	*8*	7	*15*	28	*0.0011*	*17*	11	*15*	16	*0.0490*
GD (U/L)	*13*	8	*16*	6	--	*8*	4.5	*15*	6	--	*17*	5	*15*	6	--
SDH (U/L)	*13*	0	*16*	0	--	*8*	0	*15*	0	*0.0432*	*17*	0	*15*	0	--
ALP (U/L)	*13*	0	*16*	0	--	*8*	0	*15*	0	--	*17*	0	*15*	0	--
GGT (U/L)	*13*	0	*16*	0	--	*8*	0	*15*	0	--	*17*	0	*15*	0	--

[1] analyte not detected

B. Hepatopancreas Lipid Content

The average HP lipid content was calculated as gram per gram of dry hepatopancreas weight and converted to percent dry weight to standardise comparison to other components and data from fall 2012. Average lipid content was also converted to percent lipid as a proportion of total wet weight using percent moisture data which was available for all hepatopancreas tissue.
Box plots (Figure 1) and frequency distribution histograms (Figure 2) identified four outliers (#105, 165, 169, and 46) which were all MF crabs with all but #105 having higher lipid content than others in theirgroup. Examination of the original data showed all three replicates of lipid determination to be similar; except for #165B where only two values were available due to limited amounts of tissue. Outliers were excluded from regression analyses and comparisons.

As variation in carapace width (CW) was greater than anticipated for PM in the November 2012 samples, scatterplots showing HP lipid vs. carapace width for each category of crab were generated for these fall 2013 crabs of which caged crabs would have been collected in November 2012 (Figure 3). No pattern of percent HP lipid content to CW was noted. Summary statistics for average %HP lipid by category and station are provided in Table 19.

Effect of Treatment (Caged vs Free) within Station by Sex

Comparison (Wilcoxon signed rank testing, $p < 0.05$) of treatment (cage vs trap) on median %HP found free crabs to have significantly higher % lipid than caged crabs for all but $LM_{Margaree}$ ($PM_{Margaree}$, $p = 0.0233$; $MF_{Margaree}$ $p = 0.0004$; $PM_{Cheticamp}$ $p = 0.0000$; $LM_{Cheticamp}$ $p = 0.0002$; $MF_{Cheticamp}$ $p = 0.0003$).

Effect of Sex within Station by Treatment (Caged or Free)

Significant differences among median %HP lipid were detected by Kruskal-Wallis testing across crab categories for all but Margaree Harbor free crabs (Margaree Harbor caged, $p = 0.0063$; Cheticamp free, $p= 0.0049$; Cheticamp caged, $p= 0.0002$). Subsequent Wilcoxon testing, at Bonferroni-adjusted p-value of 0.0167, identified median values for caged $LM_{Margaree}$ > caged $MF_{Margaree}$ ($p = 0.0005$); caged $LM_{Cheticamp}$ > than caged $MF_{Cheticamp}$ ($p = 0.0032$) or caged $PM_{Cheticamp}$ ($p = 0.0002$); and, free $PM_{Cheticamp}$ lower than free $MF_{Cheticamp}$ ($p = 0.0025$) or free $LM_{Cheticamp}$ ($p = 0.0102$). Results, with medians, are summarised in Tables 19 and 20.

Effect of Station within Treatment by Sex

No significant differences were found when comparing between stations for sex and treatment combinations.

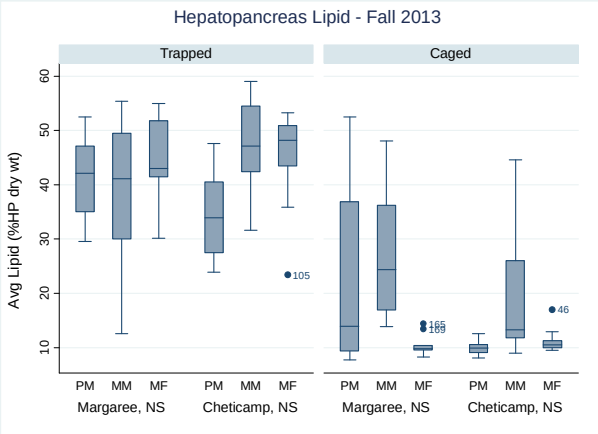

Figure 1. Box and Whisker plot showing distribution of average hepatopancreas lipid (as % HP dry weight) for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013

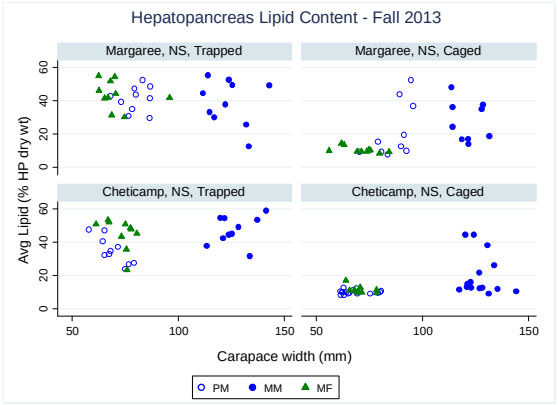

Figure 3. Scatterplot showing average HP lipid (% HP dry wt) by carapace width for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013.

.

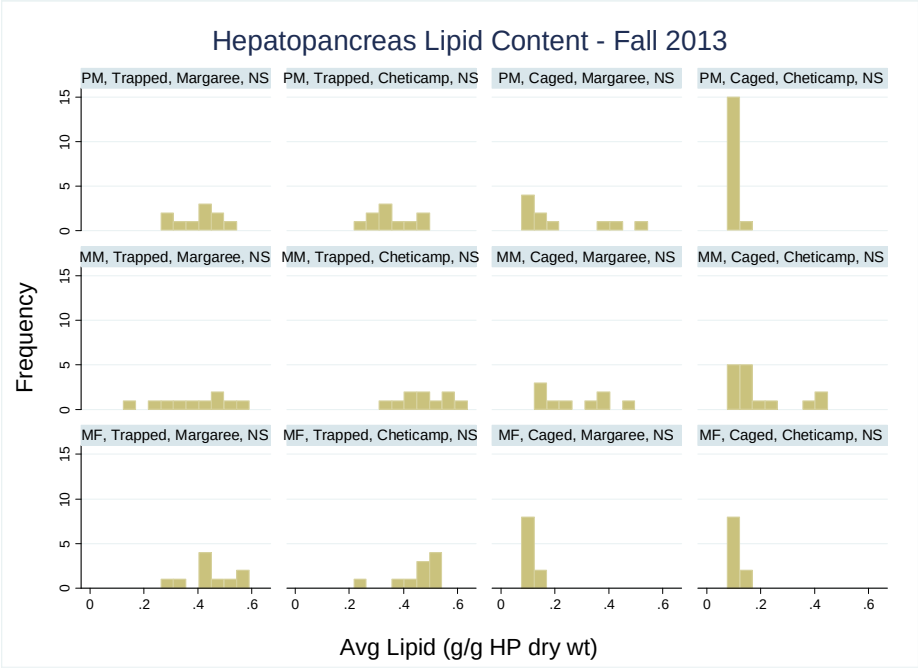

Figure 2. Frequency distribution histogram showing average HP lipid (%HP dry wt) for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013.

Table 19. Summary statistics for average hepatopancreas lipid content ((% HP dry wt)) for free-ranging snow crabs collected by traps at two stations in CFA 19, November 2013. Different superscripts indicate significant differences (p > 0.017) in median values within stations. There were no differences in medians by crab category across stations.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	PM	10	41.09	7.53	42.13	29.56	52.46	1.98	-0.20
	LM	10	39.01	13.74	41.14	12.55	55.32	2.29	-0.57
	MF	10	43.80	8.57	43.00	30.17	54.93	2.08	-0.26
Cheticamp	PM	10	35.06	8.21	33.87[a]	23.86	47.63	1.96	0.32
	LM	10	47.18	8.53	47.11[c]	31.62	59.02	2.20	-0.39
	MF	9	47.54	5.41	48.56[b,c]	35.84	53.31	3.41	-1.12

Table 20. Summary statistics for average hepatopancreas lipid content (% HP dry wt) for snow crabs at two stations in CFA 19, collected in November 2013 after a 12 month caging period. Different superscripts indicate significant differences (p > 0.017) in median values within stations. There were no differences in medians by crab category across stations.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	PM	10	21.65	16.49	13.88[a,b]	7.68	52.51	2.20	0.90
	LM	9	27.47	12.03	24.31[a]	13.81	48.04	1.78	0.39
	MF	8	9.58	0.68	9.51[b,c]	8.25	10.40	3.03	-0.73
Cheticamp	PM	16	9.93	1.26	9.87[a,b]	8.11	12.57	2.79	0.60
	LM	15	19.94	12.49	13.27[c]	8.95	44.60	2.79	1.17
	MF	9	10.62	1.09	9.95[b]	9.52	12.91	3.02	1.00

Spearman rank correlation co-efficients were calculated for average HP Lipid and all 24 directly measured hemolymph biochemistry parameters for all crabs, crab category by station, sex, and collection method (Tables 21 – 29). Overall, 'metabolites' had the highest correlations with HP lipid. The most correlations were found for free PM and MF crabs at both stations and caged LM crabs at Cheticamp. Total protein, cholesterol, glucose and triglyceride were the most common parameters showing good correlations (*rho* > 0.5). Remaining biochemistry parameters were variably correlated to %HP lipid. Relationships are shown graphically as scatterplots in Figures 4 –9 for total protein, albumin, globulin, cholesterol, triglyceride, and glucose, respectively.

Regression analyses, simple and multiple, were completed for median %HP lipid for each of the six hemolymph biochemistry parameters, by sex, station, and collection method (Tables 30 - 33). Carapace width was considered a possible factor in fall 2012 samples. As caged crabs would have been collected in November 2012, CW was added into the multiple regression equations separately (Tables 32 & 33).

Results of the simple regressions (Tables 30-31) were inconsistent, with no one biochemistry parameter having an R^2 value greater than 0.5 for all sexes at both sites or either treatment (caged or free), with values ranging from 0.5213 to 0.7889. Simple regression using protein-related indices – total protein, albumin, and globulin – showed good fit for free and caged MF crabs from Margaree Harbor but not Cheticamp. Using multiple regression (Tables 32-33)equations for MF crabs resulted in little or no improvement except for caged Cheticamp crabs. Multiple regressions improved the fit for LM crabs for all but free crabs from Cheticamp for which R^2 and adjusted R^2 values were always below 0.5. The inclusion of CW provided a small improvement. For caged and free PM crabs from Margaree Harbor, multiple linear regressions resulted in improved fit over simple regression, especially for caged PM where the adjusted R^2 was 0.8433 without using CW and 0.9436 with CW. Conversely, simple regression for free Cheticamp PM has as good or better fit than multiple regressions. Neither simple nor multiple linear regressions resulted in good fit for caged PM crabs from Cheticamp.

Table 21. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, November, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Marg[1]	Chet[2]	Marg	Chet	Marg	Chet
Sodium	-0.5557	-0.5963	-0.5199	---	---	-0.7480	-0.8783
n	126	17	26	---	---	20	20
p	*0.0000*	*0.0115*	*0.0065*	---	---	*0.0001*	*0.0000*
Potassium	-0.5806	---	-0.6142	---	-0.6348	-0.5705	-0.7778
n	126	---	26	---	25	20	20
p	*0.0000*	---	*0.0008*	---	*0.0007*	*0.0086*	*0.0001*
Na:K	0.2863	---	---	---	0.7458	---	---
n	126	---	---	---	25	---	---
p	*0.0012*	---	---	---	*0.0000*	---	---
Chloride	-0.6607	-0.7255	-0.5946	-0.5018	---	-0.8215	-0.8584
n	126	17	26	18	---	20	20
p	*0.0000*	*0.0010*	*0.0014*	*0.0338*	---	*0.0000*	*0.0000*
Calcium	0.7187	0.5972	0.7352	0.5459	0.7746	0.7838	0.8486
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0054*	*0.0000*	*0.0191*	*0.0000*	*0.0000*	*0.0000*
Phosphorus	0.2649	---	0.5255	---	0.4913	0.5433	---
n	128	---	26	---	25	20	---
p	*0.0025*	---	*0.0058*	---	*0.0126*	*0.0133*	---
Magnesium	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

[1.] Margaree Harbor, NS [2]. Cheticamp, NS

Table 22. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

	Margaree Harbor - HP Lipid, Fall 2013						
Analyte	**All Crabs**	**Pygmy Male**		**Large Mature Male**		**Mature Female**	
		Trap	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**
Sodium	-0.5557	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Potassium	-0.5806	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Na:K	0.2863	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0012*	---	---	---	---	---	---
Chloride	-0.6607	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Calcium	0.7187	---	0.7599	---	---	---	---
n	128	---	10	---	---	---	---
p	*0.0000*	---	*0.0108*	---	---	---	---
Phosphorus	0.2649	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	0.0025	---	---	---	---	---	---
Magnesium	---	-0.6848	---	---	---	---	---
n	---	10	---	---	---	---	---
p	---	*0.0289*	---	---	---	---	---

Table 23. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

	Cheticamp- HP Lipid, Fall 2013						
Analyte	**All Crabs**	**Pygmy Male**		**Large Mature Male**		**Mature Female**	
		Trap	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**
Sodium	-0.5557	-0.6912	---	---	0.5643	---	---
n	126	10	---	---	15	---	---
p	*0.0000*	*0.0269*	---	---	*0.0284*	---	---
Potassium	-0.5806	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Na:K	0.2863	---	---	---	0.5386	---	---
n	126	---	---	---	15	---	---
p	*0.0012*	---	---	---	*0.0383*	---	---
Chloride	-0.6607	-0.7509	---	---	0.5157	---	---
n	126	10	---	---	15	---	---
p	*0.0000*	*0.0123*	---	---	*0.0491*	---	---
Calcium	0.7187	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Phosphorus	0.2649	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	0.0025	---	---	---	---	---	---
Magnesium	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

Table 24. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas lipid content (%dry wt) of free and caged snow crab collected from two stations in CFA 19, November, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Marg[1]	Chet[2]	Marg	Chet	Marg	Chet
Urea	---	---	---	---	-0.5890	0.4822	---
n	---	---	---	---	25	20	---
p	---	---	---	---	0.0019	*0.0313*	---
Uric Acid	---	---	0.5216	---	---	0.5629	0.6345
n	---	---	26	---	---	20	19
p	---	---	*0.0063*	---	---	*0.0098*	*0.0035*
Total Protein	0.7929	0.6629	0.7380	0.6333	0.7848	0.8618	0.8806
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0014*	*0.0000*	*0.0048*	*0.0000*	*0.0000*	*0.0000*
Albumin	0.7753	0.6720	0.6883	0.5936	0.7607	0.8480	0.9156
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0012*	*0.0001*	*0.0094*	*0.0000*	*0.0000*	*0.0000*
Globulin	0.7923	0.6581	0.7416	0.6333	0.7934	0.8713	0.8656
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0016*	*0.0000*	*0.0048*	*0.0000*	*0.0000*	*0.0000*
A:G	-0.6811	---	-0.6895	-0.6277	-0.5214	-0.7999	-0.7466
n	128	---	26	18	25	20	19
p	*0.0000*	---	*0.0001*	*0.0053*	*0.0075*	*0.0000*	*0.0002*
Cholesterol	0.8172	0.6298	0.7604	0.6732	0.8585	0.9089	0.8171
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0029*	*0.0000*	*0.0022*	*0.0000*	*0.0000*	*0.0000*
Triglyceride	0.7473	0.6205	0.7481	0.6719	0.8174	0.8583	0.8256
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0035*	*0.0000*	*0.0023*	*0.0000*	*0.0000*	*0.0000*
Glucose	0.7532	0.6793	0.7038	0.7429	0.8198	0.8021	0.8808
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0010*	*0.0001*	*0.0004*	*0.0000*	*0.0000*	*0.0000*
Lactate	0.4420	---	0.5510	0.5191	---	0.5429	0.8404
n	128	---	26	18	---	20	19
p	*0.0000*	---	*0.0035*	*0.0273*	---	*0.0134*	*0.0000*
Creatinine	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

[1]. Margaree Harbor, NS [2]. Cheticamp, NS

Table 25. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

	Margaree Harbor - HP Lipid, Fall 2013						
Analyte	**All Crabs**	**Pygmy Male**		**Large Mature Male**		**Mature Female**	
		Trap	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**
Urea	---	---	---	---	---	0.6483	---
n	---	---	---	---	---	10	---
p	---	---	---	---	---	*0.0425*	---
Uric Acid	---	---	---	---	---	---	0.6727
n	---	---	---	---	---	---	10
p	---	---	---	---	---	---	*0.0330*
Total Protein	0.7929	0.6444	---	---	---	0.7842	---
n	128	10	---	---	---	10	---
p	*0.0000*	0.0443	---	---	---	*0.0072*	---
Albumin	0.7753	0.8030	---	---	---	0.7034	---
n	128	10	---	---	---	10	---
p	*0.0000*	*0.0052*	---	---	---	0.0232	---
Globulin	0.7923	---	---	---	---	0.8268	---
n	128	---	---	---	---	10	---
p	*0.0000*	---	---	---	---	*0.0032*	---
A:G	-0.6811	0.7632	---	---	---	---	---
n	128	10	---	---	---	---	---
p	*0.0000*	*0.0102*	---	---	---	---	---
Cholesterol	0.8172	---	0.7112	---	0.7619	0.6444	0.6789
n	128	---	10	---	8	10	10
p	*0.0000*	---	*0.0211*	---	*0.0280*	*0.0443*	*0.0309*
Triglyceride	0.7473	---	0.8602	---	---	---	---
n	128	---	10	---	---	---	---
p	*0.0000*	---	*0.0014*	---	---	---	---
Glucose	0.7532	---	---	0.6403	0.7785	0.7424	---
n	128	---	---	10	8	10	---
p	*0.0000*	---	---	*0.0217*	*0.0229*	*0.0139*	---
Lactate	0.4420	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Creatinine	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---		---

Table 26. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

		Cheticamp- HP Lipid, Fall 2013					
Analyte	**All Crabs**	**Pygmy Male**		**Large Mature Male**		**Mature Female**	
		Trap	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**
Urea	---	0.9211	---	---	---	---	---
n	---	10	---	---	---	---	---
p	---	*0.0022*	---	---	---	---	---
Uric Acid	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
Total Protein	0.7929	0.7697	---	---	0.5336	0.8703	---
n	128	10	---	---	15	9	---
p	*0.0000*	*0.0092*	---	---	*0.0405*	*0.0023*	---
Albumin	0.7753	---	---	---	---	0.9328	---
n	128	---	---	---	---	9	---
p	*0.0000*	---	---	---	---	*0.0002*	---
Globulin	0.7923	0.7964	---	---	0.5776	0.8703	---
n	128	10	---	---	15	9	---
p	*0.0000*	*0.0058*	---	---	*0.0241*	*0.0023*	---
A:G	-0.6811	---	---	0.7594	-0.5398	---	---
n	128	---	---	10	15	---	---
p	*0.0000*	---	---	*0.0108*	*0.0378*	---	---
Cholesterol	0.8172	0.8061	---	---	0.7343	0.8667	0.6791
n	128	10	---	---	15	9	10
p	*0.0000*	*0.0049*	---	---	*0.0018*	*0.0025*	*0.0308*
Triglyceride	0.7473	0.6585	---	---	---	0.8656	0.8218
n	128	10	---	---	---	9	10
p	*0.0000*	*0.0384*	---	---	---	*0.0026*	*0.0035*
Glucose	0.7532	0.8232	---	---	0.6384	0.8368	---
n	128	10	---	---	15	9	---
p	*0.0000*	*0.0034*	---	---	*0.0104*	*0.0049*	---
Lactate	0.4420	0.6364	---	---	---	---	---
n	128	10	---	---	---	---	---
p	*0.0000*	*0.0479*	---	---	---	---	---
Creatinine	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

Table 27. Summary of Spearman's rank coefficients, sample size, and significance level for the level for the activity of eight enzymes in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, November, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5are indicated in grey.

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Marg[1]	Chet[2]	Marg	Chet	Marg	Chet
Amylase	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
Lipase	-0.1942	-0.5083	---	---	---	---	---
n	128	20	---	---	---	---	---
p	0.0280	*0.0221*	---	---	---	---	---
AST	-0.2755	---	---	---	-0.6069	---	---
n	128	---	---	---	25	---	---
p	*0.0016*	---	---	---	*0.0013*	---	---
ALT	0.1760	---	---	---	---	---	0.6848
n	128	---	---	---	---	---	19
p	0.0470	---	---	---	---	---	*0.0015*
GD	0.6074	0.5257	0.5794	0.5645	0.7230	0.5358	0.7167
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0173*	*0.0019*	*0.0147*	*0.0000*	*0.0149*	*0.0006*
SDH	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
ALP	-0.3594	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
GGT	---	0.4769	---	---	-0.4097	---	---
n	---	20	---	---	25	---	---
p	---	*0.0335*	---	---	*0.0420*	---	---

[1.] Margaree Harbor, NS [2]. Cheticamp, NS

Table 28. Summary of Spearman's rank coefficients, sample size, and significance level for the activity levels of eight enzymes in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

		Margaree Harbor - HP Lipid, Fall 2013					
Analyte	**All Crabs**	**Pygmy Male**		**Large Mature Male**		**Mature Female**	
		Trap	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**
Amylase	---	---	---	---	---	---	-0.7818
n	---	---	---	---	---	---	10
p	---	---	---	---	---	---	*0.0075*
Lipase	-0.1942	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0280*	---	---	---	---	---	---
AST	-0.2755	---	---	---	-0.7470	---	---
n	128	---	---	---	8	---	---
p	*0.0016*	---	---	---	*0.0332*	---	---
ALT	0.1760	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0470*	---	---	---	---	---	---
GD	0.6074	---	---	---	0.8051	---	---
n	128	---	---	---	8	---	---
p	*0.0000*	---	---	---	*0.0159*	---	---
SDH	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
ALP	-0.3594	---	-0.6963	---	---	---	---
n	128	---	10	---	---	---	---
p	*0.0000*	---	0.0253	---	---	---	---
GGT	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

Table 29. Summary of Spearman's rank coefficients, sample size, and significance level for the activity levels of eight enzymes in hemolymph plasma *vs* hepatopancreas lipid content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients $\leq 0.5$are indicated in grey.

Cheticamp- HP Lipid, Fall 2013

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Trap	Cage	Trap	Cage	Trap	Cage
Amylase	---	---	---	---	0.5469	---	---
n	---	---	---	---	15	---	---
p	---	---	---	---	*0.0349*	---	---
Lipase	-0.1942	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0280*	---	---	---	---	---	---
AST	-0.2755	0.7091	---	---	---	---	---
n	128	10	---	---	---	---	---
p	*0.0016*	*0.0217*	---	---	---	---	---
ALT	0.1760	0.6485	---	---	---	---	---
n	128	10	---	---	---	---	---
p	*0.0470*	*0.0425*	---	---	---	---	---
GD	0.6074	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
SDH	---	---	---	---	-0.5980	---	---
n	---	---	---	---	15	---	---
p	---	---	---	---	*0.0185*	---	---
ALP	-0.3594	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

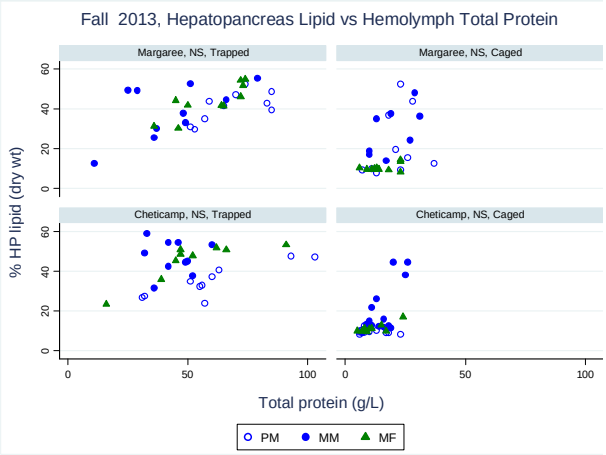

Figure 4. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma total protein concentration of snow crab collected from two stations in CFA 19 in November 2013, after trapping or a 12 month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

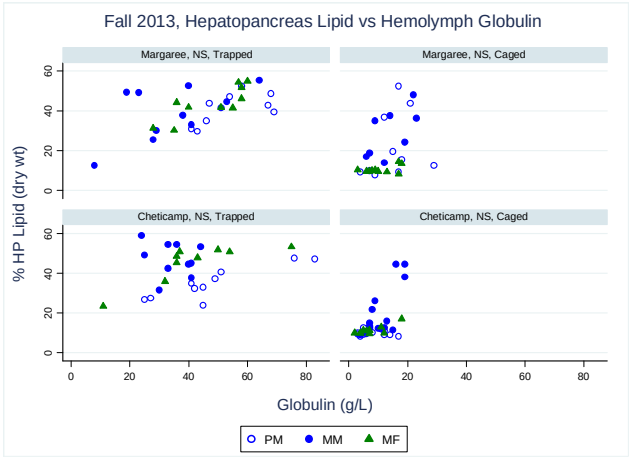

Figure 6. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma globulin concentration of snow crab collected from two stations in CFA 19 in November 2013, after trapping or a 12 month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

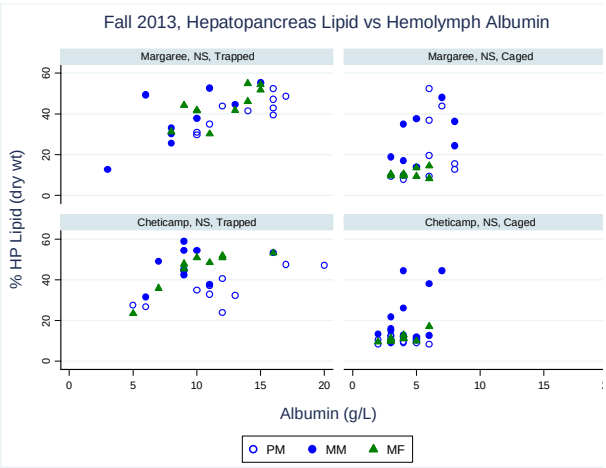

Figure 5. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma albumin concentration of snow crab collected from two stations in CFA 19 in November 2013, after trapping or a 12 month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

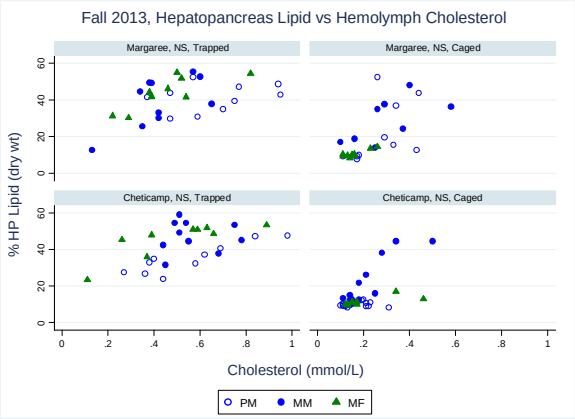

Figure 7. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma cholesterol concentration of snow crab collected from two stations in CFA 19 in November 2013, after trapping or a 12 month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

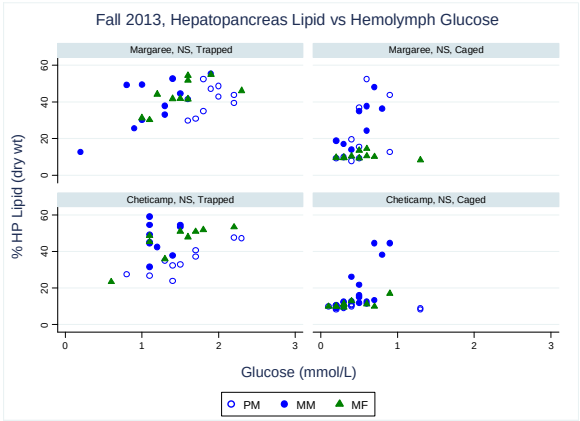

Figure 9. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma glucose concentration of snow crab collected from two stations in CFA 19 in November 2013, after trapping or a 12 month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

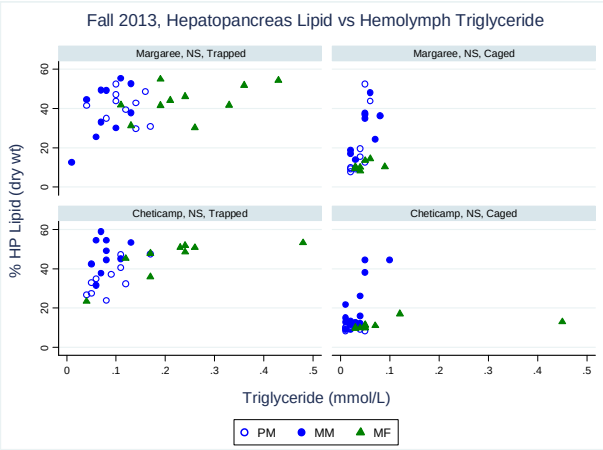

Figure 8. Scatterplot showing the average lipid (%HP dry wt) in hepatopancreas vs. hemolymph plasma triglyceride concentration of snow crab collected from two stations in CFA 19 in November 2013, after trapping or a 12 month caging period. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

Table 30. Summary of equations and associated R^2 values (shown in grey font when <0.5) for simple linear regression models for average hepatopancreas lipid (g/g HP dry wt) for four plasma biochemistry parameters of snow crab collected from two stations in CFA 19 in November 2013, by trapping. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree Harbor	PM	Avg % Lipid = 0.38*TP + 15.13	0.4445
		LM	Avg % Lipid = 0.40*TP +21.59	0.3440
		MF	Avg % Lipid = 0.50*TP + 13.77	0.6847
	Cheticamp	PM	Avg % Lipid = 0.31*TP + 16.29	0.7570
		LM	Avg % Lipid = -0.03*TP + 48.58	0.0011
		MF	Avg % Lipid = 0.22*TP + 35.27	0.4697
Albumin (Alb)	Margaree Harbor	PM	Avg %Lipid = 2.26*Alb + 9.77	0.7014
		LM	Avg %Lipid = 2.38*Alb + 18.00	0.3808
		MF	Avg %Lipid = 2.49*Alb + 14.13	0.5721
	Cheticamp	PM	Avg %Lipid = 1.45*Alb + 18.08	0.6262
		LM	Avg %Lipid = 1.14*Alb + 36.28	0.1295
		MF	Avg %Lipid = 1.64*Alb + 30.32	0.6509
Globulin (Glob)	Margaree Harbor	PM	Avg %Lipid = 0.43*Glob + 17.61	0.3706
		LM	Avg %Lipid = 0.48*Glob + 22.51	0.3326
		MF	Avg %Lipid = 0.59*Glob + 15.39	0.6726
	Cheticamp	PM	Avg %Lipid = 0.39*Glob + 16.15	0.7784
		LM	Avg %Lipid = -0.22*Glob + 54.96	0.0333
		MF	Avg %Lipid = 0.25*Glob + 36.45	0.4263
Triglyceride (TG)	Margaree Harbor	PM	Avg %Lipid = -42.70*TG + 45.99	0.0494
		LM	Avg %Lipid = 208.10*TG + 23.36	0.3416
		MF	Avg %Lipid = 39.45*TG + 34.12	0.2204
	Cheticamp	PM	Avg %Lipid = 149.15*TG + 21.93	0.5419
		LM	Avg %Lipid = 100.16*TG + 39.26	0.0810
		MF	Avg %Lipid = 30.27*TG + 40.82	0.3505
Cholesterol (Chol)	Margaree Harbor	PM	Avg %Lipid = 11.80*Chol + 33.32	0.0964
		LM	Avg %Lipid = 58.82*Chol +14.00	0.4185
		MF	Avg %Lipid = 41.60*Chol +25.03	0.6364
	Cheticamp	PM	Avg %Lipid = 32.00*Chol + 17.26	0.7982
		LM	Avg %Lipid = 3.92*Chol + 44.93	0.0032
		MF	Avg %Lipid = 19.25*Chol + 37.55	0.4792
Glucose(Gluc)	Margaree Harbor	PM	Avg %Lipid = 13.97*Gluc + 14.80	0.1668
		LM	Avg %Lipid = 21.47*Gluc + 14.74	0.5213
		MF	Avg %Lipid = 14.78*Gluc + 21.32	0.4416
	Cheticamp	PM	Avg %Lipid = 15.84*Gluc + 10.66	0.7889
		LM	Avg %Lipid = 7.35 *Gluc + 38.20	0.0228
		MF	Avg %Lipid = 8.89*Gluc + 34.37	0.3602

Table 31. Summary of equations and associated R^2 values (shown in grey font when <0.5) for simple linear regression models for percent average hepatopancreas lipid (% HP dry wt) for four plasma biochemistry parameters of snow crab collected from two stations in CFA 19 in November 2013, after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree Harbor	PM	Avg % Lipid = 0.52*TP +10.73	0.0801
		LM	Avg % Lipid = 0.85*TP + 12.06	0.3652
		MF	Avg % Lipid = -0.10*TP + 10.93	0.6282
	Cheticamp	PM	Avg % Lipid = -0.06*TP + 10.60	0.0660
		LM	Avg % Lipid = 1.55*TP – 3.33	0.5244
		MF	Avg % Lipid = 0.11*TP + 9.46	0.1747
Albumin (Alb)	Margaree Harbor	PM	Avg %Lipid = 3.12*Alb + 3.50	0.1023
		LM	Avg %Lipid = 2.81*Alb + 13.34	0.2004
		MF	Avg %Lipid = -0.52*Alb + 11.74	0.5884
	Cheticamp	PM	Avg %Lipid = -0.23*Alb + 10.75	0.0453
		LM	Avg %Lipid = 4.76*Alb + 0.58	0.2797
		MF	Avg %Lipid = 0.46*Alb + 9.00	0.1435
Globulin (Glob)	Margaree Harbor	PM	Avg %Lipid = 0.60*Glob + 12.55	0.0728
		LM	Avg %Lipid = 1.16*Glob + 12.51	0.4121
		MF	Avg %Lipid = -0.12*Glob + 10.71	0.6286
	Cheticamp	PM	Avg %Lipid = -0.77*Glob + 10.49	0.0675
		LM	Avg %Lipid = 1.95*Glob – 1.31	0.5431
		MF	Avg %Lipid = 0.13*Glob + 9.76	0.1578
Triglyceride (TG)	Margaree Harbor	PM	Avg %Lipid = 867.67*TG – 11.32	0.6029
		LM	Avg %Lipid = 361.71*TG + 11.63	0.4522
		MF	Avg %Lipid = 10.79*TG + 9.15	0.1085
	Cheticamp	PM	Avg %Lipid = -5.36*TG + 10.05	0.0022
		LM	Avg %Lipid = 388.96*TG + 6.97	0.5359
		MF	Avg %Lipid = 6.62*TG + 10.01	0.6728
Cholesterol (Chol)	Margaree Harbor	PM	Avg %Lipid = 71.03*Chol +2.32	0.2415
		LM	Avg %Lipid = 50.25*Chol + 13.67	0.3863
		MF	Avg %Lipid = 2.65*Chol + 9.21	0.0063
	Cheticamp	PM	Avg %Lipid = 0.86*Chol + 9.78	0.0016
		LM	Avg %Lipid = 107.31*Chol – 2.01	0.7868
		MF	Avg %Lipid = 8.51*Chol + 9.09	0.6970
Glucose(Gluc)	Margaree Harbor	PM	Avg %Lipid = 33.59*Gluc + 4.17	0.2195
		LM	Avg %Lipid = 46.39*Gluc + 5.03	0.6053
		MF	Avg %Lipid = -1.08*Gluc + 10.16	0.3120
	Cheticamp	PM	Avg %Lipid = -1.17*Gluc + 10.40	0.1153
		LM	Avg %Lipid = 43.02*Gluc – 2.42	0.4951
		MF	Avg %Lipid = 1.34*Gluc + 10.15	0.0576

Table 32. Summary of equations and associated adjusted R^2 values (shown in grey font when <0.5) for multiple linear regression models for average hepatopancreas lipid (% HP dry wt) for four plasma biochemistry parameters and carapace width of snow crab collected from two stations in CFA 19 in November 2013, by trapping. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

STATION	GROUP	SIMPLIFIED MULTIPLE REGRESSION EQUATION FREE	ADJ. R^2
Margaree Harbor	PM	Avg %Lipid = 8.86*Gluc – 4.72*Chol + 7.06*Trig -*0.69TP + 5.26*Alb + 1.73	0.7081
	LM	Avg %Lipid = 62.72*Gluc -+40.15 *Chol + 100.06*Trig -*1.37TP + 2.79*Alb +11.74	0.2784
	MF	Avg %Lipid = -4.69*Gluc + 32.58*Chol – 45.88*Trig +0.14*TP + 2.21*Alb + 12.55	0.5622
Cheticamp	PM	Avg %Lipid = -3.30*Gluc + 39.90*Chol - *84.71Trig +0.36*TP – 1.23*Alb + 17.84	0.7261
	LM	Avg %Lipid = -9.74*Gluc – 14.58*Chol + 125.34*Trig -*0.90TP + 3.76*Alb + 61.77	-0.0881
	MF	Avg %Lipid = 15.61*Gluc – 14.06*Chol – 30.75*Trig -*0.75TP + 6.40*Alb + 12.43	0.6571
Margaree Harbor	PM	Avg %Lipid = 10.98*Gluc – 0.82*Chol – 0.16*Trig -*0.65TP + 4.86*Alb + 0.20*CW – 18.19	0.6581
	LM	Avg %Lipid = 87.85*Gluc – 89.21*Chol + 171.47*Trig -*1.20TP + 1.69*Alb + 0.99*CW - 121.62	0.6207
	MF	Avg %Lipid = -4.95*Gluc + 31.30*Chol – 43.58*Trig +0.15*TP+ 2.15 *Alb – 0.02*CW +14.52	0.4173
Cheticamp	PM	Avg %Lipid = -19.32*Gluc + 63.55*Chol – 216.92*Trig +0.48*TP – 1.30*Alb – 0.77*CW + 87.77	0.8970
	LM	Avg %Lipid = -26.06*Gluc – 39.57*Chol + 361.93*Trig -*1.55TP + 5.80*Alb – 0.72*CW + 177.75	-0.0258
	MF	Avg %Lipid = 37.09*Gluc – 42.71*Chol + 25.13*Trig -1.83*TP + 10.73*Alb + 0.42*CW – 35.20	0.3871

Table 33. Summary of equations and associated adjusted R^2 values (shown in grey font when <0.5) for multiple linear regression models for average hepatopancreas lipid (%HP dry wt) for four plasma biochemistry parameters and carapace width (CW) of snow crab collected from two stations in CFA 19 in November 2013, after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

STATION	GROUP	SIMPLIFIED MULTIPLE REGRESSION EQUATION CAGED	ADJ. R^2
Margaree Harbor	PM	Avg %Lipid = 34.84*Gluc – 125.23*Chol + 1902.99*Trig – 1.89*TP + 2.90*Alb – 12.24	0.8433
	LM	Avg %Lipid = 49.13*Gluc -*95.11Chol + 522.38*Trig +3.47*TP – 15.42*Alb +24.57	0.7926
	MF	Avg %Lipid = 0.04 *Gluc + 7.30*Chol + 2.11*Trig -*0.12TP + 0.10*Alb + 9.66	-0.1680
Cheticamp	PM	Avg %Lipid = -3.19*Gluc + 11.14*Chol + 65.38*Trig -*0.03TP – 0.30 *Alb + 9.37	0.1037
	LM	Avg %Lipid = 9.66*Gluc + 114.43*Chol – 80.62*Trig +0.11*TP – 1.05*Alb – 3.26	0.7142
	MF	Avg %Lipid = 2.47*Gluc + 19.15*Chol - 5.14*Trig -*0.27TP + 0.45*Alb + 7.99	0.5387
Margaree Harbor	PM	Avg %Lipid = 71.83*Gluc - 211.96*Chol + 1545.39*Trig – 2.83*TP+ 10.81 *Alb + 0.82*CW – 92.53	0.9436
	LM	Avg %Lipid = 33.17*Gluc – 100.18*Chol + 857.44*Trig +4.00*TP – 23.11*Alb – 1.11*CW + 186.59	0.8755
	MF	Avg %Lipid = 0.23*Gluc + 8.52*Chol + 0.19*Trig -*0.14TP + 0.10*Alb + 0.00*CW + 9.01	-1.2986
Cheticamp	PM	Avg %Lipid = -3.25*Gluc + 10.75*Chol + 68.41*Trig-+0.02*TP – 0.35*Alb + 0.00*CW + 8.99	0.0055
	LM	Avg %Lipid = 19.35*Gluc + 126.87*Chol – 187.12*Trig +0.40*TP – 2.23*Alb + 0.49*CW – 69.31	0.7383
	MF	Avg %Lipid = 8.25*Gluc – 3.73*Chol + 12.84*Trig -*0.30TP – 0.41*Alb – 0.13*CW + 21.35	0.3954

C. Hepatopancreas Glycogen Content

The average HP glycogen content was calculated as milligram per gram of dry hepatopancreas weight (mg glycogen/g HP dry wt) and converted to percent dry weight to standardise comparison to other components. Average glycogen content was also converted to percent glycogen as a proportion of total wet weight using percent moisture data which was available for all hepatopancreas tissue.
The distribution of the data is presented in Figures 9-11 as boxplots, frequency distribution histograms and scatterplot vs. carapace width, respectively. Numerous outliers were identified on the boxplots (#'s141, 143, 145, 122, 127, 163, 24, 5, 12, and 46) and were also evident on the histograms. No pattern for glycogen content and CW was apparent. Summary statistics for average HP glycogen by category and station are provided in Tables 34 & 35 for free and caged crabs, respectively.

Effect of Treatment (Caged vs Free) within a Station by Sex

Comparison (Wilcoxon rank-sum, $p < 0.05$) of treatment method (caged vs free) within a station by sex, found that hepatopancreas glycogen content in free crabs exceeded caged crabs in all cases ($PM_{Margaree}$, $p = 0.0006$; $LM_{Margaree}$, $p = 0.0025$; $MF_{Margaree}$, $p = 0.0284$; $PM_{Cheticamp}$, $p = 0.0003$; $LM_{Cheticamp}$, $p = 0.0002$; $MF_{Cheticamp}$, $p = 0.0019$).

Effect of Sex within a Station by Treatment (Caged or Free)

Kruskall-Wallis testing across crab sex categories within station by treatment showed statistically significant differences for free and caged crabs at both locations (Margaree Harbor free, $p = 0.0057$; Margaree Harbor caged, $p = 0.0350$; Cheticamp free, $p = 0.0007$; Cheticamp caged, $p = 0.0068$). Subsequent Wilcoxon testing of median hepatopancreas glycogen values, at Bonferroni-adjusted p-value of 0.0167, found free $PM_{Margaree}$ > free $LM_{Margaree}$ ($p = 0.0041$) or free $MF_{Margaree}$ ($p = 0.0102$) crabs; caged $MF_{Margaree}$ crabs higher but not significantly different from caged $PM_{Margaree}$ (0.0248) or caged $LM_{Margaree}$ ($p = 0.0510$) crabs; free $LM_{Cheticamp}$ < free $PM_{Cheticamp}$ ($p = 0.0002$) or free $MF_{Cheticamp}$ ($p = 0.0156$) crabs; and, caged $MF_{Cheticamp}$ > caged $LM_{Cheticamp}$ ($p = 0.0068$) crabs.

Effect of Station within a Treatment by Sex

There were no significant differences between stations for any combination of sex or treatment.

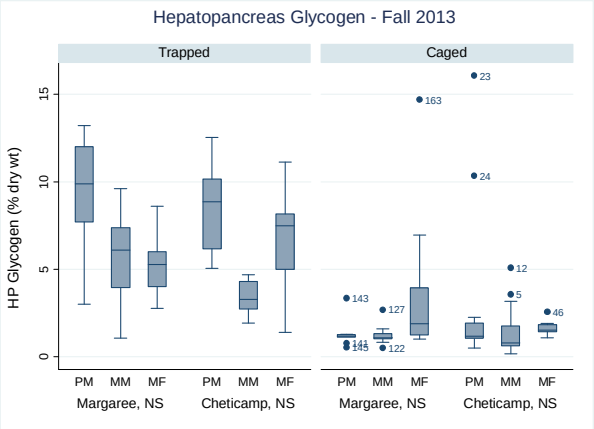

Figure 9. Box and Whisker plot showing distribution of average hepatopancreas glycogen (as % HP dry weight) for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013.

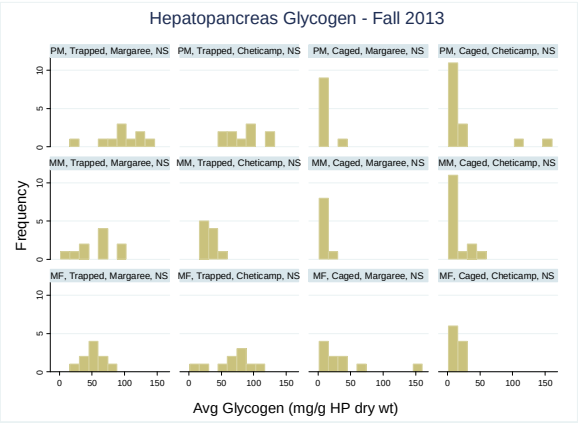

Figure 10. Frequency distribution histogram showing average HP glycogen (%HP dry wt) for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013.

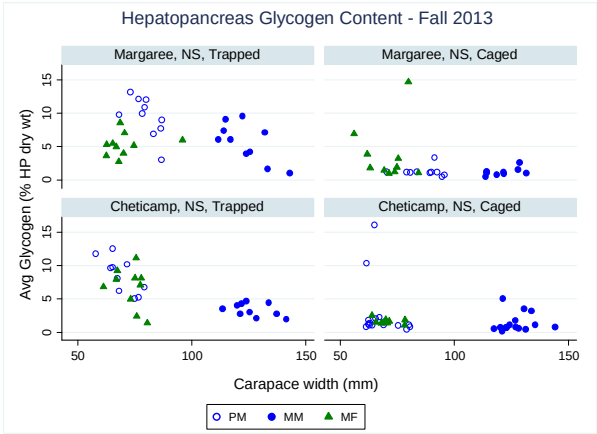

Figure 11. Scatterplot showing average HP glycogen (% HP dry wt) by carapace width for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013.

Table 34. Summary statistics for average hepatopancreas glycogen content (% dry wt) for free-ranging snow crabs collected by traps at two stations in CFA 19, November 2013. Different superscripts indicate significant differences (p > 0.017) in median values within stations. There were no differences between stations by sex.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	**PM**	10	9.47	3.01	9.89[a]	3.01	13.22	3.12	-0.85
	LM	10	5.62	2.89	6.08[c]	1.06	9.60	1.96	-0.24
	MF	10	5.30	1.68	5.27[b,c]	2.75	8.59	2.75	0.43
Cheticamp	**PM**	10	8.52	2.66	8.86[a,b]	5.07	12.54	1.68	0.08
	LM	10	3.35	1.00	3.27[c]	1.92	4.69	1.58	-0.04
	MF	10	6.72	3.01	7.49[b]	1.38	11.12	2.41	-0.55

Table 35. Summary statistics for average hepatopancreas glycogen content (% dry wt) for snow crabs at two stations in CFA 19, collected in November 2013 after a 12 month caging period. Different superscripts indicate significant differences (p > 0.017) in median values within stations. There were no differences between stations by sex.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	**PM**	7	1.19	0.06	1.19[a,b]	1.10	1.27	1.78	0.01
	LM	7	1.15	0.25	1.07[a,c]	0.82	1.60	2.52	0.62
	MF	10	3.73	4.26	1.86[b,c]	1.00	14.69	5.51	1.92
Cheticamp	**PM**	15	2.26	3.85	1.15[a,b]	0.50	16.09	12.70	3.39
	LM	13	0.98	0.76	0.77[a,c]	0.17	3.17	6.45	1.99
	MF	9	1.53	0.26	1.53[b]	1.07	1.90	2.46	-0.15

Spearman rank correlation co-efficients were calculated for average HP glycogen, for all 23 directly measured hemolymph biochemistry parameters and three calculated (globulin, A:G and Na:K) values automatically included in the biochemistry profiles (Tables 37-44). There was minimal correlation between hepatopancreas glycogen and any of the mineral, electrolyte, or enzyme parameters. The metabolite category had some good correlation values (*rho*) between hepatopancreas glycogen and biochemistry parameters, particularly for free $PM_{Cheticamp}$ crabs for urea (0.8685), total protein (0.7333), albumin (0.6768), globulin (0.7599), cholesterol (0.7697), triglyceride (0.7317), and glucose (0.8110). Caged $PM_{Cheticamp}$ and free $MF_{Margaree}$ also had multiple strong correlations with some biochemistry parameters. The relationships of hemolymph total protein, triglyceride, cholesterol, and glucose are presented as scatterplots in Figures 37-40, respectively, for comparative purposes to fall 2012 (2 week) and spring 2013(six month) cageing studies presented in sections VIII-2 and VIII-3.

Simple and multiple regression analyses were completed for average (%dry weight) hepatopancreas glycogen for the four parameters used in fall 2012 and spring 2013 samples (total protein, triglyceride, cholesterol, and glucose) to facilitate comparison between the sampling periods (Tables 45-48). Globulin was added as it had good correlations for free and caged $PM_{Cheticamp}$. Equations are presented for caged and free crabs at both locations in Tables 45-48. On simple regression, values for R^2 were very low for all but free $PM_{Cheticamp}$; interestingly, multiple regression resulted in a slightly decreased fit. In contrast, multiple regression improved the fit for free $PM_{Margaree}$ to 0.6427 from values below 0.5. Other than glucose for $PM_{Cheticamp}$ (R2 = 0.9247) and $MF_{Margaree}$ (0.8642), simple regression resulted in poor fit for caged crabs at either station. Use of multiple regression created a marked improvement of fit for caged PM and MF crabs at both stations but, did not improve results for LM crabs. Inclusion of CW in the multiple regression equations usually offered little improvement except for caged LM and MF crabs from Cheticamp.

Table 36. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, November, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Marg[1]	Chet[2]	Marg	Chet	Marg	Chet
Sodium	-0.4735	-0.5926	-0.5336	-0.6350	---	-0.4405	-0.5861
n	126	17	26	18	---	20	20
p	*0.0000*	*0.0122*	*0.0050*	*0.0046*	---	*0.0519*	*0.0066*
Potassium	-0.2957	---	-0.6063	---	---	---	-0.7188
n	126	---	26	---	---	---	20
p	*0.0000*	---	*0.0010*	---	---	---	*0.0004*
Na:K	---	---	---	-0.6309	---	---	0.5929
n	---	---	---	18	---	---	20
p	---	---	---	*0.0050*	---	---	*0.0059*
Chloride	-0.5208	-0.5637	-0.6737	-0.6340	---	-0.4255	-0.5911
n	126	17	26	18	---	20	20
p	*0.0000*	*0.0184*	*0.0002*	*0.0047*	---	*0.0615*	*0.0061*
Calcium	0.5636	0.5205	0.7071	---	0.5062	---	0.7538
n	128	20	26	---	25	---	19
p	*0.0000*	*0.0186*	*0.0001*	---	*0.0098*	---	*0.0002*
Phosphorus	0.4760	0.4632	0.4974	0.7259	---	---	---
n	128	20	26	18	---	---	---
p	*0.0000*	*0.0397*	*0.0097*	*0.0006*	---	---	---
Magnesium	0.1895	---	---	---	0.4960	---	---
n	128	---	---	---	25	---	---
p	*0.0321*	---	---	---	*0.0117*	---	---

[1.] Margaree Harbor, NS, [2]. Cheticamp, NS

Table 37. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Margaree, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

Margaree Harbor - HP Glycogen, Fall 2013

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Trap	Cage	Trap	Cage	Trap	Cage
Sodium	-0.4735	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Potassium	-0.2957	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Na:K	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
Chloride	-0.5208	---	0.8214	---	---	---	---
n	126	---	7	---	---	---	---
p	*0.0000*	---	*0.0234*	---	---	---	---
Calcium	0.5636	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Phosphorus	0.4760	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Magnesium	0.1895	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0321*	---	---	---	---	---	---

Table 38. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of electrolytes and minerals in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

Cheticamp- HP Glycogen, Fall 2013

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Trap	Cage	Trap	Cage	Trap	Cage
Sodium	-0.4735	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Potassium	-0.2957	---	---	---	---	-0.6791	---
n	126	---	---	---	---	10	---
p	*0.0000*	---	---	---	---	*0.0308*	---
Na:K	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
Chloride	-0.5208	---	---	---	---	---	---
n	126	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Calcium	0.5636	---	---	---	---	---	0.6930
n	128	---	---	---	---	---	10
p	*0.0000*	---	---	---	---	---	*0.0263*
Phosphorus	0.4760	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Magnesium	0.1895	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0321*	---	---	---	---	---	---

Table 39. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, November, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Marg[1]	Chet[2]	Marg	Chet	Marg	Chet
Urea	---	0.6722	---	0.5095	---	---	---
n	---	20	---	18	---	---	---
p	---	*0.0012*	---	*0.0308*	---	---	---
Uric Acid	0.3363	---	0.4336	---	---	---	---
n	128	---	26	---	---	---	---
p	*0.0001*	---	*0.0269*	---	---	---	---
Total Protein	0.7013	0.7223	0.8517	0.6581	0.5789	---	0.7550
n	128	20	26	18	25	---	19
p	*0.0000*	*0.0003*	*0.0000*	*0.0030*	*0.0024*	---	*0.0002*
Albumin	0.6827	0.7228	0.8073	0.6134	0.5535	---	0.7901
n	128	20	26	18	25	---	19
p	*0.0000*	*0.0003*	*0.0000*	*0.0068*	*0.0041*	---	*0.0001*
Globulin	0.7029	0.7266	0.8493	0.6746	0.5808	---	0.7241
n	128	20	26	18	25	---	19
p	*0.0000*	*0.0003*	*0.0000*	*0.0021*	*0.0023*	---	*0.0005*
A:G	-0.6246	-0.7594	-0.7691	-0.6629	---	---	-0.6324
n	128	20	26	18	---	---	19
p	*0.0000*	*0.0001*	*0.0000*	*0.0027*	---	---	*0.0037*
Cholesterol	0.6103	0.7269	0.8196	---	0.5789	---	0.6341
n	128	20	26	---	25	---	19
p	*0.0000*	*0.0003*	*0.0000*	---	*0.0024*	---	*0.0035*
Triglyceride	0.6385	0.6750	0.7930	---	0.5194	0.5057	0.6022
n	128	20	26	---	25	20	19
p	*0.0000*	*0.0011*	*0.0000*	---	*0.0078*	*0.0229*	*0.0064*
Glucose	0.7732	0.7660	0.8569	0.7253	0.5484	0.5714	0.7857
n	128	20	26	18	25	20	19
p	*0.0000*	*0.0001*	*0.0000*	*0.0007*	*0.0045*	*0.0085*	*0.0001*
Lactate	0.6813	0.8135	0.7527	0.8019	---	0.4842	0.6684
n	128	20	26	18	---	20	19
p	*0.0000*	*0.0000*	*0.0000*	*0.0001*	---	*0.0305*	*0.0018*
Creatinine	---	---	---		---	---	---
n	---	---	---		---	---	---
p	---	---	---		---	---	---

[1.] Margaree Harbor, NS, [2]. Cheticamp, NS

Table 40. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas glycogen content (%dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

Margaree Harbor - HP Glycogen, Fall 2013

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Trap	Cage	Trap	Cage	Trap	Cage
Urea	---	---	---	---	---	-0.7364	---
n	---	---	---	---	---	10	---
p	---	---	---	---	---	*0.0152*	---
Uric Acid	0.3363	---	---	---	---		---
n	128	---	---	---	---		---
p	*0.0001*	---	---	---	---		---
Total Protein	0.7013	---	---	---	---	-0.7903	---
n	128	---	---	---	---	10	---
p	*0.0000*	---	---	---	---	*0.0065*	---
Albumin	0.6827	---	---	---	---	-0.7951	---
n	128	---	---	---	---	10	---
p	*0.0000*	---	---	---	---	*0.0060*	---
Globulin	0.7029	---	---	---	---	-0.6930	---
n	128	---	---	---	---	10	---
p	*0.0000*	---	---	---	---	*0.0263*	---
A:G	-0.6246	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Cholesterol	0.6103	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Triglyceride	0.6385	---	---	---	---	---	0.6617
n	128	---	---	---	---	---	10
p	*0.0000*	---	---	---	---	---	*0.0372*
Glucose	0.7732	---	---	---	---	---	0.8013
n	128	---	---	---	---	---	10
p	*0.0000*	---	---	---	---	---	*0.0053*
Lactate	0.6813	---	---	0.7091	---	---	---
n	128	---	---	10	---	---	---
p	*0.0000*	---	---	*0.0217*	---	---	---
Creatinine	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

Table 41. Summary of Spearman's rank coefficients, sample size, and significance level for the concentration of metabolites in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5 are indicated in grey.

Cheticamp- HP Glycogen, Fall 2013

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Trap	Cage	Trap	Cage	Trap	Cage
Urea	---	0.8685		---	---	---	---
n	---	10		---	---	---	---
p	---	*0.0011*		---	---	---	---
Uric Acid	0.3363	---		---	---	---	---
n	128	---		---	---	---	---
p	*0.0001*	---		---	---	---	---
Total Protein	0.7013	0.7333	0.7213	---	---	---	---
n	128	10	16	---	---	---	---
p	*0.0000*	*0.0158*	*0.0016*	---	---	---	---
Albumin	0.6827	0.6768	---	---	---	---	0.7792
n	128	10	---	---	---	---	10
p	*0.0000*	*0.0316*	---	---	---	---	*0.0079*
Globulin	0.7029	0.7599	-0.5481	---	---	---	---
n	128	10	16	---	---	---	---
p	*0.0000*	*0.0108*	*0.0280*	---	---	---	---
A:G	-0.6246	---	0.5387	-0.7100	---	---	---
n	128	---	16	10	---	---	---
p	*0.0000*	---	*0.0313*	*0.0214*	---	---	---
Cholesterol	0.6103	0.7697	---	---	---	---	---
n	128	10	---	---	---	---	---
p	*0.0000*	*0.0092*	---	---	---	---	---
Triglyceride	0.6385	0.7317	---	---	---	---	---
n	128	10	---	---	---	---	---
p	*0.0000*	*0.0162*	---	---	---	---	---
Glucose	0.7732	0.8110	-0.6303	---	---	---	---
n	128	10	16	---	---	---	---
p	*0.0000*	*0.0044*	*0.0089*	---	---	---	---
Lactate	0.6813	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
Creatinine	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

Table 42. Summary of Spearman's rank coefficients, sample size, and significance level for the activity of eight enzymes in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) of free and caged snow crab collected from two stations in CFA 19, November, 2013, grouped by sex, and region of capture. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

Analyte	All Crabs	Pygmy Male		Large Mature Male		Mature Female	
		Marg[1]	Chet[2]	Marg	Chet	Marg	Chet
Amylase	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
Lipase	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
AST	---	---	---	0.5933	-0.4200	---	---
n	---	---	---	18	25	---	---
p	---	---	---	*0.0094*	*0.0366*	---	---
ALT	0.3199	0.4503	---	0.7291	---	---	0.6462
n	128	20	---	18	---	---	19
p	*0.0002*	*0.0463*	---	*0.0006*	---	---	*0.0028*
GD	0.5957	0.6944	0.4690	0.6732	0.5413	---	0.5684
n	128	20	26	18	25	---	19
p	*0.0000*	*0.0007*	*0.0157*	*0.0022*	*0.0052*	---	*0.0111*
SDH	---	---	0.4184	---	---	---	---
N	---	---	26	---	---	---	---
P	---	---	*0.0334*	---	---	---	---
ALP	-0.3307	---	-0.4382	---	---	---	---
n	128	---	26	---	---	---	---
p	*0.0001*	---	*0.0251*	---	---	---	---
GGT	---	---	-0.5056	---	---	---	---
n	---	---	26	---	---	---	---
p	---	---	*0.0084*	---	---	---	---

[1.] Margaree Harbor, NS [2]. Cheticamp, NS

Table 43. Summary of Spearman's rank coefficients, sample size, and significance level for the activity level of eight enzymes in hemolymph plasma *vs* hepatopancreas glycogen content (% dry wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Margaree Harbor, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

	Margaree Harbor - HP Glycogen, Fall 2013						
Analyte	**All Crabs**	**Pygmy Male**		**Large Mature Male**		**Mature Female**	
		Trap	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**
Amylase	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
Lipase	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
AST	---	---	---	---	---	0.6991	---
n	---	---	---	---	---	10	---
p	---	---	---	---	---	*0.0245*	---
ALT	0.3199	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0002*	---	---	---	---	---	---
GD	0.5957	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0000*	---	---	---	---	---	---
SDH	---	---	---	---	---	---	---
N	---	---	---	---	---	---	---
P	---	---	---	---	---	---	---
ALP	-0.3307	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0001*	---	---	---	---	---	---
GGT	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---

Table 44. Summary of Spearman's rank coefficients, sample size, and significance level for the activity level of eight enzymes in hemolymph plasma *vs* hepatopancreas glycogen content (% wt) in snow crab after 12 months of caging on ocean floor (caged) or free-ranging (trap), from Cheticamp, NS, CFA 19, November, 2013. Results are grouped by sex and treatment group. Co-efficients are shown only where $p \leq 0.05$; co-efficients ≤ 0.5000 are indicated in grey.

	Cheticamp- HP Glycogen, Fall 2013						
Analyte	**All Crabs**	**Pygmy Male**		**Large Mature Male**		**Mature Female**	
		Trap	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**
Amylase	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
Lipase	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
AST	---	---	---	---	---	---	---
n	---	---	---	---	---	---	---
p	---	---	---	---	---	---	---
ALT	0.3199	---	---	---	---	0.7333	---
n	128	---	---	---	---	9	---
p	*0.0002*	---	---	---	---	*0.0246*	---
GD	0.5957	0.7173	---	---	---	---	---
n	128	10	---	---	---	---	---
p	*0.0000*	*0.0195*	---	---	---	---	---
SDH	---	---	---	---	---	---	---
N	---	---	---	---	---	---	---
P	---	---	---	---	---	---	---
ALP	-0.3307	---	---	---	---	---	---
n	128	---	---	---	---	---	---
p	*0.0001*	---	---	---	---	---	---
GGT	---	---	-0.5732	---	---	---	---
n	---	---	16	---	---	---	---
p	---	---	*0.0203*	---	---	---	---

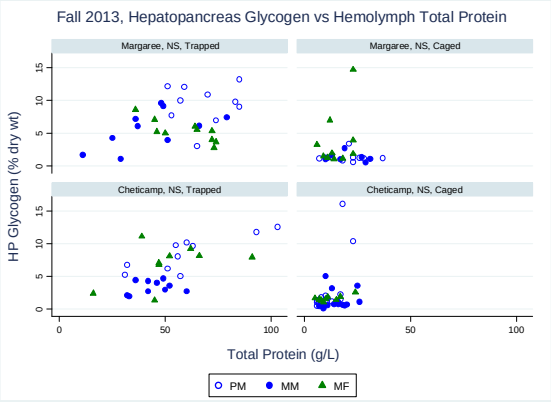

Figure 9. Scatterplot showing the average glycogen (% HP dry wt) in hepatopancreas vs. hemolymph plasma total protein concentration of snow crab collected from two stations in CFA 19 in November 2013 by trapping or, after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

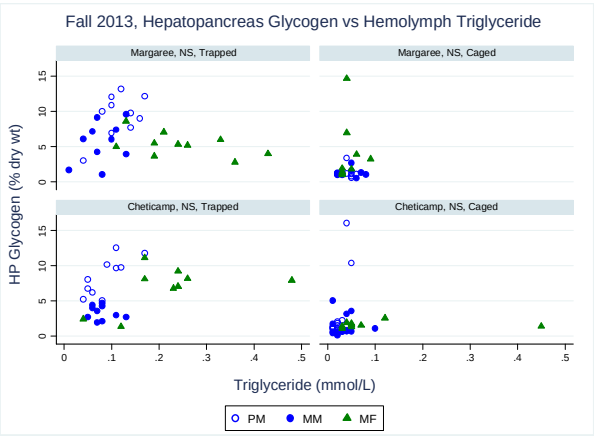

Figure 10. Scatterplot showing the average glycogen (% HP dry wt) in hepatopancreas vs. hemolymph plasma triglyceride concentration of snow crab collected from two stations in CFA 19 in November 2013 by trapping or, after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF)

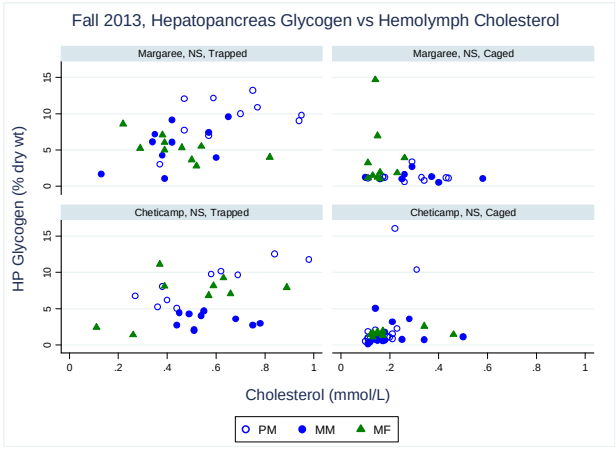

Figure 11. Scatterplot showing the average glycogen (% HP dry wt) in hepatopancreas vs. hemolymph plasma cholesterol concentration of snow crab collected from two stations in CFA 19 in November 2013 by trapping or, after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

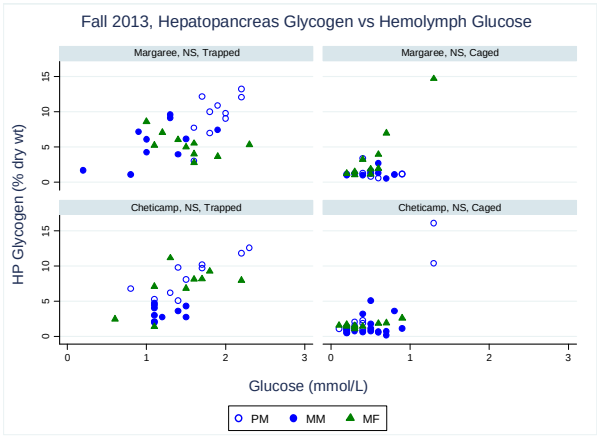

Figure 12. Scatterplot showing the average glycogen (% HP dry wt) in hepatopancreas vs. hemolymph plasma glucose concentration of snow crab collected from two stations in CFA 19 in November 2013 by trapping or, after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

Table 45. Summary of equations and associated R^2 values (values < 0.05 in grey font)for simple linear regression models for average hepatopancreas glycogen (% HP dry wt) for four plasma biochemistry parameters of snow crab collected from two stations in CFA 19 in November 2013, by trapping. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree Harbor	PM	Avg %Glycogen = 0.01*TP + 8.58	0.0032
		LM	Avg %Glycogen = 0.08*TP + 1.96	0.3436
		MF	Avg %Glycogen = -0.09*TP + 10.93	0.6242
	Cheticamp	PM	Avg %Glycogen = 0.09*TP + 2.70	0.6926
		LM	Avg %Glycogen = 0.02*TP + 2.21	0.0524
		MF	Avg %Glycogen = 0.06*TP + 3.42	0.1929
Triglyceride (TG)	Margaree Harbor	PM	Avg %Glycogen = 40.01*TG + 4.86	0.2717
		LM	Avg %Glycogen = 28.49*TG + 3.34	0.1449
		MF	Avg %Glycogen = -8.44*TG + 7.37	0.2619
	Cheticamp	PM	Avg %Glycogen = 51.26*TG + 4.00	0.6102
		LM	Avg %Glycogen = -8.92*TG + 4.05	0.0469
		MF	Avg %Glycogen = 12.37*TG + 4.22	0.2304
Cholesterol (Chol)	Margaree Harbor	PM	Avg %Glycogen = 6.35*Chol + 5.28	0.1748
		LM	Avg %Glycogen = 10.05*Chol + 1.34	0.2768
		MF	Avg %Glycogen = -6.63*Chol + 8.29	0.4206
	Cheticamp	PM	Avg %Glycogen = 10.16*Chol + 2.86	0.7678
		LM	Avg %Glycogen = -1.36*Chol + 4.12	0.0278
		MF	Avg %Glycogen = 7.23*Chol + 3.31	0.2990
Glucose(Gluc)	Margaree Harbor	PM	Avg %Glycogen = 9.20*Gluc – 7.84	0.4527
		LM	Avg %Glycogen = 3.79*Gluc + 1.33	0.3686
		MF	Avg %Glycogen = -2.41*Gluc + 8.97	0.3051
	Cheticamp	PM	Avg %Glycogen = 4.88*Gluc + 1.00	0.7134
		LM	Avg %Glycogen = 0.45*Gluc + 2.78	0.0065
		MF	Avg %Glycogen = 4.05*Gluc + 1.10	0.3703
Globulin (Glob)	Margaree Harbor	PM	Avg %Glycogen = 0.02*Glob + 8.06	0.0082
		LM	Avg %Glycogen = 0.10*Glob + 2.04	0.3535
		MF	Avg %Glycogen = -0.10*Glob + 10.48	0.5807
	Cheticamp	PM	Avg %Glycogen = 0.11*Glob + 2.72	0.6962
		LM	Avg %Glycogen = 0.05*Glob + 1.53	0.1320
		MF	Avg %Glycogen = 0.08*Glob + 3.55	0.2049

Table 46. Summary of equations and associated R^2 values (values < 0.05 in grey font)for simple linear regression models for average hepatopancreas glycogen (% HP dry wt) for four plasma biochemistry parameters of snow crab collected from two stations in CFA 19 in November 2013, after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

PARAMETER	LOCATION	GROUP	REGRESSION EQUATION	R^2
Total Protein (TP)	Margaree Harbor	PM	Avg %Glycogen = -0.00*TP + 1.2	0.0084
		LM	Avg %Glycogen = -0.00*TP + 1.23	0.0053
		MF	Avg %Glycogen = 0.26*TP - 0.24	0.1452
	Cheticamp	PM	Avg %Glycogen = 0.53*TP – 3.06	0.3389
		LM	Avg %Glycogen = 0.00*TP + 0.90	0.0012
		MF	Avg %Glycogen = 0.02*TP + 1.30	0.1229
Triglyceride (TG)	Margaree Harbor	PM	Avg %Glycogen = -1.90*TG + 1.25	0.2307
		LM	Avg %Glycogen = 2.51*TG + 1.08	0.0851
		MF	Avg %Glycogen = 23.18*TG + 2.73	0.0112
	Cheticamp	PM	Avg %Glycogen = 307.02*TG – 3.87	0.4538
		LM	Avg %Glycogen = 5.19*TG + 0.80	0.0268
		MF	Avg %Glycogen = -0.21*TG + 1.54	0.0132
Cholesterol (Chol)	Margaree Harbor	PM	Avg %Glycogen = -0.20*Chol + 1.24	0.1885
		LM	Avg %Glycogen = -0.03*Chol + 1.21	0.0007
		MF	Avg %Glycogen = -5.46*Chol + 4.60	0.0039
	Cheticamp	PM	Avg %Glycogen = 33.15*Chol - 2.97	0.1610
		LM	Avg %Glycogen = 1.20*Chol + 0.73	0.0292
		MF	Avg %Glycogen = -0.18*Chol + 1.56	0.0063
Glucose(Gluc)	Margaree Harbor	PM	Avg %Glycogen = -0.06*Gluc + 1.22	0.0880
		LM	Avg %Glycogen = 0.22*Gluc + 1.09	0.0435
		MF	Avg %Glycogen = 12.78*Gluc – 3.17	0.8642
	Cheticamp	PM	Avg %Glycogen = 13.23*Gluc – 2.23	0.9247
		LM	Avg %Glycogen = -0.33*Gluc + 1.14	0.0082
		MF	Avg %Glycogen = 0.74*Gluc + 1.27	0.3239
Globulin (Glob)	Margaree Harbor	PM	Avg %Glycogen = -0.00*Glob + 1.19	0.0092
		LM	Avg %Glycogen = -0.00*Glob + 1.24	0.0086
		MF	Avg %Glycogen = 0.28*Glob + 0.61	0.1241
	Cheticamp	PM	Avg %Glycogen = 0.73*Glob + - 2.57	0.4238
		LM	Avg %Glycogen = 0.00*Glob + 0.97	0.0000
		MF	Avg %Glycogen = 0.01*Glob + 1.42	0.0429

Table 47. Summary of equations and associated adjusted R^2 values (values < 0.05 in grey font)for multiple linear regression models for average hepatopancreas glycogen (% HP dry wt) for four plasma biochemistry parameters and carapace width of snow crab collected from two stations in CFA 19 in November 2013, by trapping. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

STATION	GROUP	SIMPLIFIED MULTIPLE REGRESSION EQUATION	ADJ. R^2
Margaree Harbor	PM	Avg %Glycogen = 11.83*Gluc + 5.80*Chol + 21.23*Trig -0.14*TP – 0.02*Glob -7.57	0.6427
	LM	Avg %Glycogen = -0.74*Gluc + 31.75*Chol – 86.53*Trig – 0.76*TP + 0.95*Glob – 0.05	-0.0790
	MF	Avg %Glycogen = 3.02*Gluc – 3.66*Chol + 13.74*Trig – 0.91*TP + 0.87*Glob + 11.87	0.5576
Cheticamp	PM	Avg %Glycogen = -1.14*Gluc + 11.41*Chol – 13.01*Trig + 0.04*TP – 0.00*Glob + 3.06	0.5027
	LM	Avg %Glycogen = 1.08*Gluc – 7.27*Chol + 7.37*Trig – 0.27*TP + 0.47*Glob 1.47	0.4224
	MF	Avg %Glycogen = -1.21*Gluc + 44.03*Chol – 33.52*Trig – 4.05*TP+ 4.56*Glob + 13.67	0.8972
Margaree Harbor	PM	Avg %Glycogen = 10.40*Gluc + 3.16*Chol + 26.13*Trig + 0.08*TP -0.29*Glob – 0.14*CW + 5.9	0.6596
	LM	Avg %Glycogen = -6.11*Gluc + 42.23*Chol – 101.79*Trig – 0.56*TP +0.72*Glob – 0.21*CW + 28	0.1633
	MF	Avg %Glycogen = 3.51*Gluc – 1.38*Chol + 9.61*Trig – 0.83*TP + 0.76*Glob +0.04*CW + 8.35	0.4933
Cheticamp	PM	Avg %Glycogen = -2.75*Gluc + 13.79*Chol – 26.33*Trig + 0.04*TP - 0.02*Glob – 0.07*CW + 10.11	0.3624
	LM	Avg %Glycogen = 1.88*Gluc – 6.05*Chol – 4.12*Trig – 0.34*TP + 0.56*Glob + 0.03*CW – 4.16	0.3030
	MF	Avg %Glycogen = -1.55*Gluc + 44.41*Chol – 34.30*Trig – 4.10*TP + 4.63*Glob -0.00*CW + 14.45	0.8458

Table 48. Summary of equations and associated adjusted R^2 values (values < 0.05 in grey font)for multiple linear regression models for average hepatopancreas glycogen (% HP dry wt) for four plasma biochemistry parameters and carapace width of snow crab collected from two stations in CFA 19 in November 2013, after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

STATION	GROUP	SIMPLIFIED MULTIPLE REGRESSION EQUATION	ADJ. R^2
Margaree Harbor	PM	Avg %Glycogen = 0.88*Gluc – 0.79*Chol – 11.22*Trig + 0.09*TP -0.10*Glob + 1.05	0.9751
	LM	Avg %Glycogen = 2.44*Gluc – 6.91*Chol + 21.55*Trig – 0.35*TP +0.47*Glob + 1.4	……..
	MF	Avg %Glycogen = 14.15*Gluc + 4.80*Chol + 6.41*Trig + 0.75*TP -1.09*Glob -4.60	0.7851
Cheticamp	PM	Avg %Glycogen = 16.10*Gluc + 11.58*Chol – 56.41*Trig + 0.43*TP – 0.76*Glob – 3.22	0.8966
	LM	Avg %Glycogen = -1.12*Gluc + 4.12*Chol – 3.06*Trig + 0.09*TP – 0.14*Glob + 0.94	-0.4857
	MF	Avg %Glycogen = 0.74*Gluc – 2.45*Chol + 1.79*Trig + 0.26*TP – 0.32*Glob + 0.98	0.6595
Margaree Harbor	PM	Avg %Glycogen = 0.86*Gluc 0.43*Chol – 12.50*Trig + 0.08*TP – 0.09*Glob -0.00*CW + 1.23	…..
	LM	Avg %Glycogen = 2.86*Gluc – 2.18*Chol – 0*Trig + 0.39*TP – 0.48*Glob +0.07*CW -9.40	…..
	MF	Avg %Glycogen = 12.75*Gluc – 17.60*Chol + 11.47*Trig + 1.34*TP – 1.62*Glob – 0.10*CW + 3.61	0.7539
Cheticamp	PM	Avg %Glycogen = 16.27*Gluc + 12.53*Chol – 61.74*Trig + 0.50*TP – 0.86*Glob – 0.01*CW – 2.61	0.8841
	LM	Avg %Glycogen = 0.07*Gluc + 4.39*Chol – 15.74*Trig + 0.00*TP + 0.02*Glob + 0.06*CW – 7.72	-0.5370
	MF	Avg %Glycogen = -2.11*Gluc + 8.88*Chol – 7.36*Trig + 0.70*TP – 0.75*Glob + 0.06*CW – 5.63	0.8812

D. Hepatopancreas Glycogen vs. Hepatopancreas Lipid Content

Spearman rank correlation co-efficients were calculated using absolute values of hepatopancreas lipid and glycogen content for all crabs (Table 49). While the correlation for all crabs was found to be statistically significant (p = 0.0010), the actual value for *rho* was low (0.4268). A high correlation (0.8424) was found for caged $PM_{Cheticamp}$. Scatterplots of the data showed a tendency for tighter negative association for free $LM_{Cheticamp}$, and positive association for free $PM_{Cheticamp}$ (Figure 13).

Ratios for average hepatopancreas lipid to glycogen content (% HP dry wt) were calculated for all crabs. Boxplots (Figure 14) identified multiple outliers (#s201, 185, 102, 121, 1, 2, 14) which were also evident in the frequency distribution histograms (Figure 15). Kruskall-Wallis testing showed a difference among sex within a treatment method and station for all combinations: Margaree Harbor free (p = 0.0100), Margaree Harbor caged (p = 0.0012), Cheticamp free (= 0.0001), and Cheticamp caged (p = 0.0031). No further analysis completed at this time.

Table 49. Summary of Spearman's rank coefficients, sample size, and significance level for average hepatopancreas lipid (%HP dry wt) *vs* hepatopancreas glycogen content (%HP dry wt) of snow crab (*Chionoecetes opilio*), collected in traps or after 12 months of caging in November 2013. Results are shown for all crabs and crabs grouped by sex, region, and treatment group.

	All Crabs	**Pygmy Male**				**Large Mature Male**				**Mature Female**			
		Marg[1]		**Chet**[2]		**Marg**		**Chet**		**Marg**		**Chet**	
		Trap	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**	**Trap**	**Cage**
rho	0.4268	---	---	0.8424	---	---	---	---	---	-0.6485	---	---	---
n	130	10	10	10	16	10	10	10	15	10	10	10	10
p	0.0000	---	---	*0.0022*	---	---	---	---	---	*0.0425*	---	---	---

[1.] Margaree Harbor, NS, [2.] Cheticamp, NS

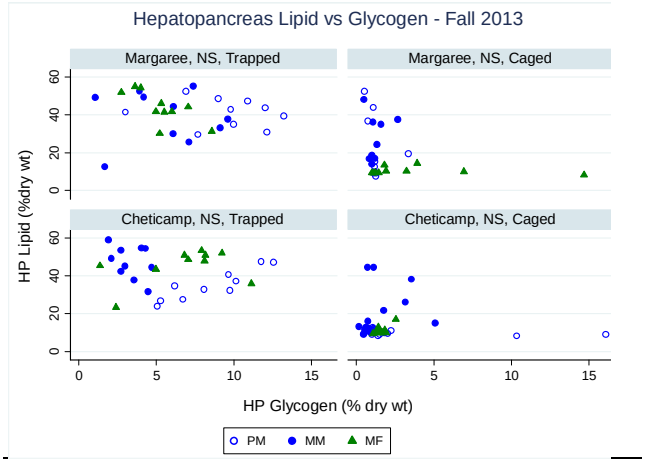

Figure 13. Scatterplot demonstrating relationship between hepatopancreas lipid and glycogen concentrations for snow crab collected from at two stations in CFA 19 in November 2013 by traps or after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

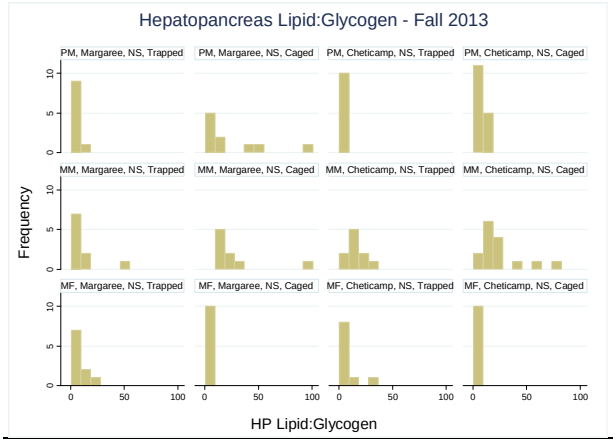

Figure 15. Frequency distribution histogram demonstrating relationship between hepatopancreas lipid and glycogen ratios for snow crab collected from two stations in CFA 19 in November 2013 by trapping or after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

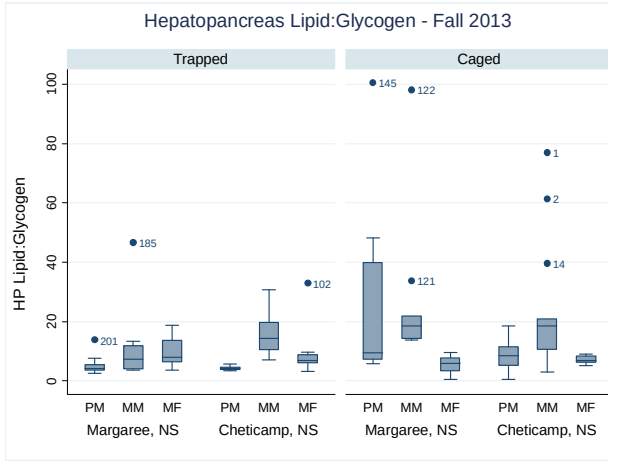

Figure 14. Box and Whisker plots demonstrating relationship between hepatopancreas lipid and glycogen ratios for snow crab collected from two stations in CFA 19 in November 2013 by traps or after 12 months of caging. Data are separated to show three categories of crab: Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF).

E. Hepatopancreas Moisture Content

The average HP moisture content as a percentage of wet weight is presented in Figures 16-18 as boxplots, frequency distribution histograms and scatterplot vs. carapace width, respectively. Numerous outliers were identified on the boxplots (#'s 122, 24, 14, and 2) and were also evident on the histograms. No pattern for moisture content and CW was apparent. Summary statistics for average HP percent moisture content by category and station are provided in Tables 50 and 51, for free and caged crabs, respectively.

Effect of Treatment (Caged vs Free) within Station by Crab Category

Comparison (Wilcoxon rank-sum) of treatment method (caged vs free) within a station by sex, found significant differences for all crab categories at both stations ($PM_{Margaree}$, $p = 0.0041$; $LM_{Margaree}$, $p = 0.0045$; $MF_{Margaree}$, $p = 0.0002$; $PM_{Cheticamp}$, $p = 0.0000$; $LM_{Cheticamp}$, $p = 0.0001$; $MF_{Cheticamp}$, $p = 0.0004$).

Effect of Sex within a Station by Treatment (Caged or Free)

Kruskall-Wallis testing across crab sex categories within station and treatment detected no statistically significant differences among sex for any station/treatment combination.

Effect of Station within a Treatment by Sex

Comparison (Wilcoxon rank-sum) between stations within a treatment, by sex, found a significant difference for free $LM_{Margaree} > LM_{Cheticamp}$ ($p = 0.0494$) crabs only.

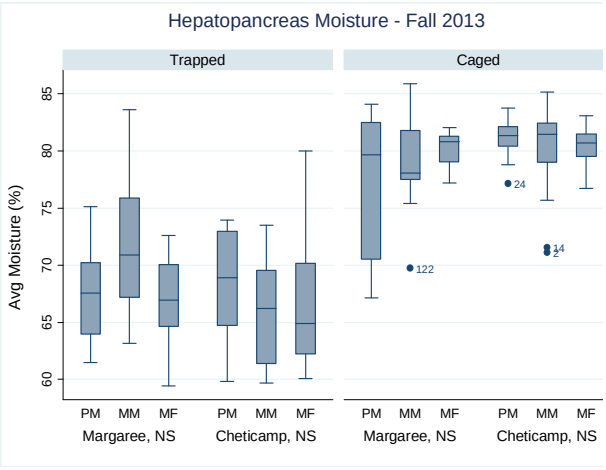

Figure 16. Box and Whisker plot showing distribution of average hepatopancreas percent moisture content for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013

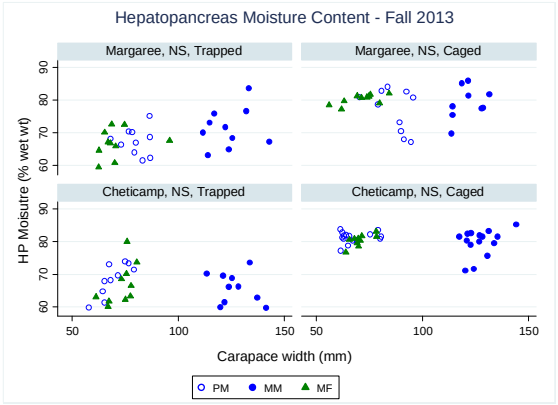

Figure 18. Scatterplot showing average hepatopancreas percent moisture content by carapace width for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013.

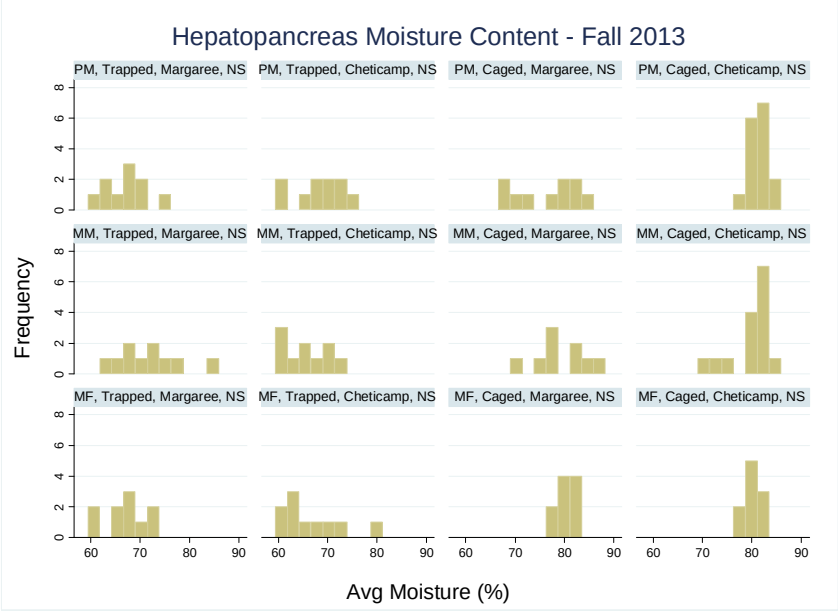

Figure 17. Frequency distribution histogram showing average hepatopancreas percent moisture content for Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF) snow crab collected by trapping or after 12 months of caging, at two stations in CFA 19, November 2013

Table 50. Summary statistics for average hepatopancreas moisture content ((% wet wt) for snow crabs collected by traps at two stations in CFA 19, November 2013. There were no significant differences in median values by crab category within a station nor between stations with the exception of LM crabs for which median values were higher in Margaree Harbor.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	**PM**	10	67.35	4.13	67.55	61.46	75.12	2.47	0.25
	LM	10	71.47	6.14	70.92	63.15	83.62	2.62	0.54
	MF	10	66.73	4.39	66.95	59.43	72.61	2.17	-0.25
Cheticamp	**PM**	10	68.33	4.99	68.92	59.80	73.96	1.99	-0.54
	LM	10	65.84	4.74	66.20	59.68	73.52	1.76	0.07
	MF	10	66.92	6.27	64.87	60.08	80.00	2.82	0.91

Table 51. Summary statistics for average hepatopancreas moisture content (% wet wt) for snow crabs at two stations in CFA 19, collected in November 2013 after a 12 month caging period. There were no significant differences in median values by crab category within a station nor between stations.

Location	Group	n	Mean	SD	Median	Min	Max	Kurtosis	Skewness
Margaree Harbor	**PM**	10	76.82	6.51	79.67	67.15	84.09	1.54	-0.43
	LM	8	80.35	3.81	79.71	75.42	85.91	1.72	0.30
	MF	10	80.23	1.56	80.80	77.21	82.04	2.32	-0.70
Cheticamp	**PM**	15	81.46	1.34	81.51	78.78	83.76	2.60	-0.01
	LM	13	81.10	2.32	81.50	75.66	85.17	3.72	-0.64
	MF	10	80.38	1.78	80.72	76.71	83.10	3.09	-0.66

F. Hepatopancreas Composition by Wet Weight

The relative contribution of each category (lipid, glycogen, and moisture) to hepatopancreas composition by wet weight was determined by calculating a fourth category 'Other' (Other = 100% - %moisture - % lipid - %glycogen) to represent all non-directly measured components. The 'Other' category would include protein, mineral/ash, etc. The summarised results are presented by station, crab category and treatment in Figure 19. All outliers for any of the three directly measured categories were excluded from the calculation.
Moisture was the largest category for any treatment, sex, or station combination followed by the 'other', lipid, and glycogen categories. The general trend was for relative decreases or no changes in the lipid, other, and glycogen categories with concomitant increase in moisture content with caging.

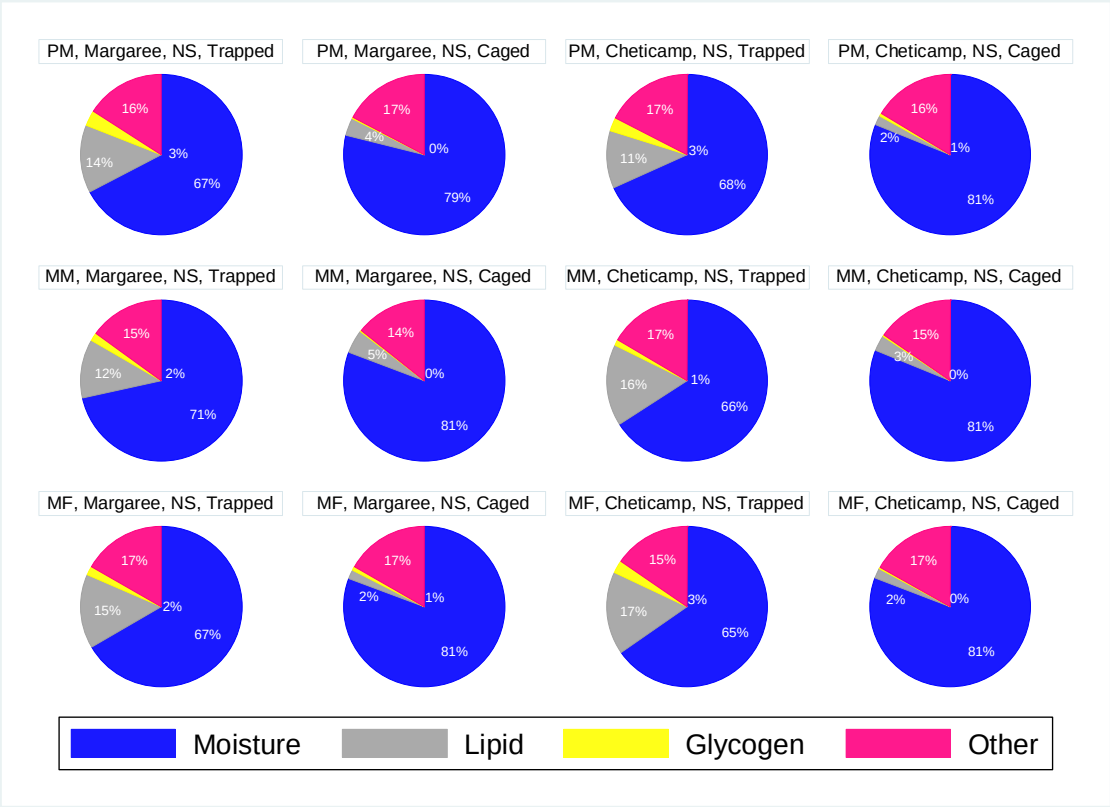

Figure 19. Pie chart indicating the relative composition of the hepatopancreas on a percent wet weight basis for snow crab collected in the Fall 2013 by trapping or after a 12 month caging period at two sites, Cheticamp and Margaree Harbor, NS. All outliers as identified in the percent lipid, percent glycogen, and percent moisture have been removed. Graphs show data for three sexes Pygmy Male (PM), Large Mature Male (LM), and Mature Female (MF). (Note: the percent value for glycogen is offset when pie 'slice' was too small to contain the label)

VIII-3-5 Conclusions

The study met all of its objectives in that hemolymph biochemistry parameters, hepatopancreas lipid, glycogen, lipid:glycogen ratios, and moisture data were collected and compared among free PM, LM, and MF crabs and immersed PM, LM, and MF crabs caged for 345-355 days at Margaree and Cheticamp stations in fall 2013. All caged crabs had lower median values than free crabs for the hemolymph activity of GD and ALT (except $LM_{Cheticamp}$) and all metabolites but urea; while, values for the electrolytes sodium and chloride (except $LM_{Cheticamp}$). The latter are attributed to the lower energy content that was documented for the hepatopancreas. The lower enzyme activities are interpreted as reflections of the documented decrease in hepatopancreas mass (section IV-6-4) and/or presumed decrease in muscle mass (not recorded in this study) given their location in these tissues (see section VII- Tissue Distribution Study) . Hepatopancreas energy, in the form of lipid and glycogen content, was significantly lower, and moisture content significantly higher, in all caged crabs compared to free crabs (exception of lipid in $LM_{Margaree}$). These changes are a continuation of the trends noted in the six month caged study (spring 2013, section VIII-2) Restricted access to food sources (quantity and quality) for caged crabs compared to free crabs is the likely cause of these changes.
Hemolymph metabolites tended to be better correlated with hepatopancreas lipid in free PM and MF crabs than in MM crabs, either station, a pattern continued with simple and multiple regression analyses. In contrast , the better correlations and R^2 values for simple regression of hemolymph metabolites to hepatopancreas glycogen was limited to free $PM_{Cheticamp}$ crabs; while, multiple regression analyses showed good adjusted R^2 values for PM and MF, free or caged, at both stations. This similarity between PM and MF crabs compared to LM crabs was also noted in the two week and six month caging studies (see sections VII-1 and VII-2). Determination of total body energy reserves (hepatopancreas, muscle, and gonad lipid and glycogen content) may improve correlations to hemolymph biochemistry parameters and regression results.

Hemolymph Biochemistry Profiles

Reference intervals (RI) established for Cheticamp 2012 fall free crabs were able to detect some of the changes observed in caged crabs regardless of station as minimum, mean, and median values were occasionally below the lower limits of the RIs. Downward trends for electrolytes, more so for free crabs, at both stations compared to the RI were identified. Lower levels for most metabolites were noted for caged crabs, especially PM crabs, at both stations. Median enzyme activity was only outside of the RI one time (mildly increased amylase, caged $LM_{Cheticamp}$, suspected artifact – see below). This was not surprising as the most common/diagnostically useful change in enzyme activity are marked increases associated with tissue injury which was an unlikely component of a caging study. Reference intervals may have been more sensitive to changes if they had been constructed using larger numbers of crabs per category (≥ 120). Season and region are additional factors that should be accounted for when constructing RI for use in future studies.

Electrolytes and Minerals

There was an unusual trend for median sodium, chloride and potassium concentrations to be higher at Cheticamp when comparing between stations and in caged vs. free crabs. Higher values were seen for crabs with the shorter emersion times making dehydration of the crabs during transport an unlikely factor. Different salinities between the two stations (data not available) and/or changes in plasma osmolality may be an overall factor. Hemolymph osmolality will be determined by the concentration of electrolytes such as sodium and chloride and proteins (George 1994). Given the marked decreases in total protein concentrations observed in the 12 month caged crabs, it is possible that sodium and chloride are being retained at higher than normal concentrations to make up for the loss in osmolality. Plasma osmolality can be measured directly e.g., freezing point depression, but was not done in this study. Potassium changes may be related to acid-base balance (not evaluated in this study) or possibly release from muscle as tissue is being catabolised (George 1994).
Magnesium was consistently higher in MF crabs compared to PM or LM crabs. This may reflect the longer emersion times for MF crabs as this was noted in fall 2012 and spring 2013 samples (see Sections VIII-1, VIII-2). A sex-associated effect could also be contributing as higher magnesium levels were noted in trawled MF crabs (section VII-3) where emersion times were not longer for MF crabs. The lower median values for total calcium levels in caged crabs is consistent with the decreased total protein levels as discussed in Section VIII-2 (Duncan 1994) and most likely reflect the lowered total protein concentrations which were also observed.

Metabolites

The lower median values for most metabolites (total protein, albumin, globulin, cholesterol, triglyceride, glucose, and lactate) in caged crabs compared to free crabs were similar to trends observed in the two week (fall 2012) and six month (spring 2013) caging studies (see sections VIII-1 and VIII-2). Notably, the 'albumin' fraction was now lower in all caged crabs when compared to free crabs. Significantly lower values were also observed for cholesterol, triglyceride, glucose, in caged LM crabs at both stations in contrast to the spring 2013 samples when there were no significant differences. These lower values are attributed to continued consumption of protein, lipid, and carbohydrate/glycogen reserves in caged crabs to make up for decreased ability to forage, as discussed in Section VIII-2. The A:G ratio was significantly higher in all caged crabs compared to free crabs, suggesting preferential consumption of the 'globulin' fraction over the BCG-binding 'albumin' fraction as both fractions were lower in caged crabs. The albumin fraction may contain hemocyanin proteins and therefore somewhat more protected from catabolism (Summerfield & Battison 2009).
Patterns in triglyceride concentrations in MF crabs were interesting. Levels in free MF were higher than PM or LM crabs at both stations. This could be related to transfer of lipid from the

hepatopancreas to the developing oocytes in the ovary in preparation for spring spawning (Battison *et al.* 2011). Conversely, plasma triglyceride levels in caged MF crabs were approximately half that of free MF crabs. This could suggest that oocytes in caged crabs were not developing at the same rate as in free due to low hepatopancreas lipid reserves (~20% of that in free crabs) which was supported by histology (mostly primary oocytes in ovaries of 12 month caged crabs – see Section IV-4-6).

Enzymes

Enzyme activities were generally unaffected by caging with the exception of GD and ALT which were significantly lower in all caged crabs, and ALT which was lower in all caged crabs except $LM_{Cheticamp}$ crabs. Hemolymph enzyme activity is proportional to the rate of release of an enzyme from its source tissue and, the amount of the source tissue (Moss & Henderson 1998). It is anticipated that there was a marked decrease in the amount of muscle tissue, which contains both GD and ALT, in the caged crabs compared to free crabs. As such, even if the rate of enzyme release remained constant, there would be less in the circulation given the decrease in muscle mass. The lower amount of hepatopancreas tissue, and additional source of ALT activity, in caged crabs (see section VII-1) may be contributing to the lower hemolymph ALT activity.

Caged $LM_{Cheticamp}$ had statically significant higher values for amylase activity than trapped counterparts or, indeed any crab group. The highest concentrations of this enzyme are found in the hemocyte lysate supernatants (HLS) and the hepatopancreas of snow crab (see Section VII-1). It is suspected that plasma collection/separation was somehow delayed for this group, which would provide an opportunity for the hemocytes to lyse and release amylase into the plasma, producing and artifactual increase.

Hepatopancreas Lipid Content

The 12 month caging period was anticipated to further stress the nutritional reserves of the caged crabs due to reduced access to food sources (quantity and/or quantity) compared to free crabs. Caged MF crabs at both stations and $PM_{Cheticamp}$ had much lower median lipid than their free counterparts. Continued inability to recoup losses related to spring 2013 spawning is the proposed cause of low lipid in caged MF crabs. Differences were not detected for $LM_{Margaree}$ crabs where a similarly wide range of values was found for both free and caged crabs. There was no non-lethal way to determine hepatopancreatic lipid reserves at the start of the caging period, so this could not be standardised. Caged $LM_{Margaree}$ crabs may have had high lipid reserves when placed into the cages in the fall 2012. Alternatively, they may have been better able to acquire food than $LM_{Cheticamp}$.

Free PM and MF crabs generally had better correlations between hepatopancreas lipid and hemolymph metabolites, particularly total protein, despite a similar range of hepatopancreas lipid

content as free LM crabs (exception lower lipid in $PM_{Cheticamp}$). This may indicate that tissue energy reserves i.e., hepatopancreas and muscle protein, lipid, and/or glycogen, are used or distributed relatively differently in LM crabs compared to PM or MF crabs. This would require further, directed study to confirm.

Cholesterol was well correlated to hepatopancreas lipid in all caged crabs but for $PM_{Cheticamp}$ while other parameters were generally poorly correlated. The exception was caged $LM_{Cheticamp}$ crabs which had multiple good correlations. Cholesterol is considered an essential nutrient for crustaceans as it cannot be synthesised (Sánchez-Paz *et al.* 2006). As a structural lipid required for cell membrane integrity, it is believed to be the last lipid scavenged for energy in crustaceans (Sánchez-Paz *et al.* 2006).

Neither simple nor multiple linear regressions were able to consistently predict hepatopancreas lipid content for all three sex categories at both sites for both treatment groups. Metabolism of lipid from extrahepatopancreatic sites (Hardy *et al.* 2000), e.g., muscle or gonad/ovary not accounted for in this study, or other unknown factors, may also be affecting hemolymph metabolite concentrations.

Hepatopancreas Glycogen

Caged crabs had lower hepatopancreatic glycogen compared to free crabs in all cases. This is presumed to reflect decreased quality and/or quantity of nutrients available to caged crabs. Interestingly, while not statistically significant, median values for free Cheticamp crabs tended to be lower than free Margaree Harbor crabs. This could be a reflection of better dietary source of carbohydrate in the Margaree Harbor area.

Correlations of hepatopancreas glycogen content with hemolymph biochemistry parameters were essentially non-existent for free or caged crabs with the exception of metabolites for free $PM_{Cheticamp}$ (total protein, urea, albumin, globulin, cholesterol, triglyceride, glucose) and $MF_{Margaree,}$ (total protein, urea, albumin, globulin) which had strong positive and negative correlations, respectively. Simple regression equations tended to have the better results for free $PM_{Cheticamp}$. Simple regression equations for caged crabs had poor fit, possibly the result of the much lower levels of hepatopancreas glycogen in caged crabs. The only two exceptions were caged $MF_{Margaree}$ and $PM_{Cheticamp}$, where both had a few individual crabs with glycogen levels near values for free crabs.

Surprisingly, multiple regression created little improvement (higher adjusted R^2) over simple regression for free crabs with the exception of $MF_{Cheticamp}$ with or without CW as a factor. In contrast, multiple regression resulted in a remarkably improved fit for caged PM and MF crabs at both stations with adjusted R^2 values as high as 0.9751 for $PM_{Margaree}$.

It is notable that neither simple nor multiple regression could not predict hepatopancreas glycogen content for free or caged LM crabs. Again, this raises the question of different physiology in LM crabs compared to PM and MF crabs. As in other sampling periods, the PM crabs are most consistent, possibly because of lower investment in reproduction that MF or LM crabs.

It was interesting to observe that for free crabs with free access to food sources, hepatopancreas lipid content had a maximum value of approximately 60% with varying levels of glycogen. Competition among crabs caged communally would be expected to factor in to how well each individual could compete for food sources which may explain outliers with higher values observed for the caged crabs at both stations. The increase in hepatopancreas moisture content in caged crabs was an expected result as tissue lipid and glycogen reserves are consumed and replaced by water.

Overall, lower hepatopancreas lipid and glycogen reserves and were observed for caged crabs compared to free crabs and is attributed to limited access to food sources. Caged crabs would have had to rely upon vegetation growing adjacent to the cages, cage fouling organisms, any animals attracted to the cages that could be caught and consumed, and potentially other crabs in cages holding multiple animals. Energy expenditure of caged crabs for foraging and predator evasion would be less than for free crabs and could consequently affect their net energy balance. Variation in habitat quality could also be impacting results.

Correlations of hemolymph biochemistry parameters to hepatopancreas energy reserves were generally seen for parameters in the 'metabolite' category which was expected; however, the correlations were inconsistent with differences noted for treatment, crab category, and sometimes location. Correlations may be improved by including determination of hepatopancreas total protein and ovary and muscle energy (lipid, glycogen and protein) content for calculation of total body energy reserves in future studies.

Crustaceans will vary as to which energy source (carbohydrate, lipid, or protein) and in which order they use to meet energy needs during periods of reduced energy intake e.g., starvation trials (Sánchez-Paz *et al.* 2006). Ovary lipid and protein levels would vary considerably depending on the state of oocyte development in MF crabs. The large amount of muscle tissue in LM crabs, containing protein, glycogen, and some lipid stores (Hardy *et al.* 2000) may account for the poorer correlations of biochemistry parameters to hepatopancreas energy reserves generally seen in this group. Assessing each crab category separately in future studies is recommended given the differences noted in the current study. Determination of total tissue moisture levels as a proxy for total tissue energy reserves was suggested by Hardy *et al.* (2000). This approach, combined with identification of a hemolymph parameter(s) that correlates well with total tissue moisture content, may prove to be a simple, non-lethal, way to assess tissue energy changes in crabs regardless of location or category.

VIII-3-6 References

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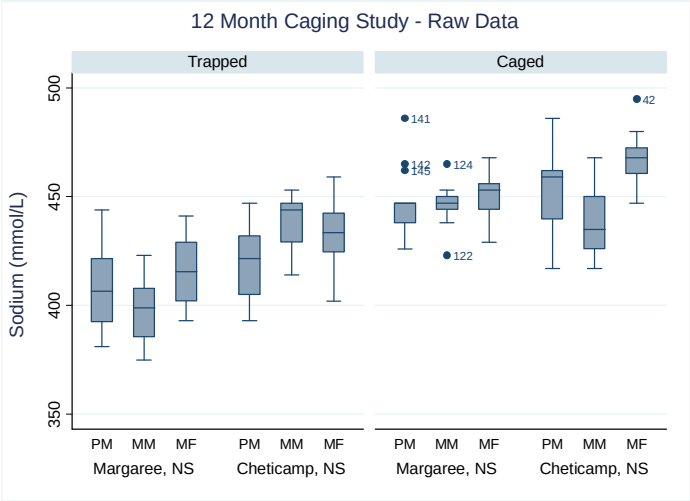
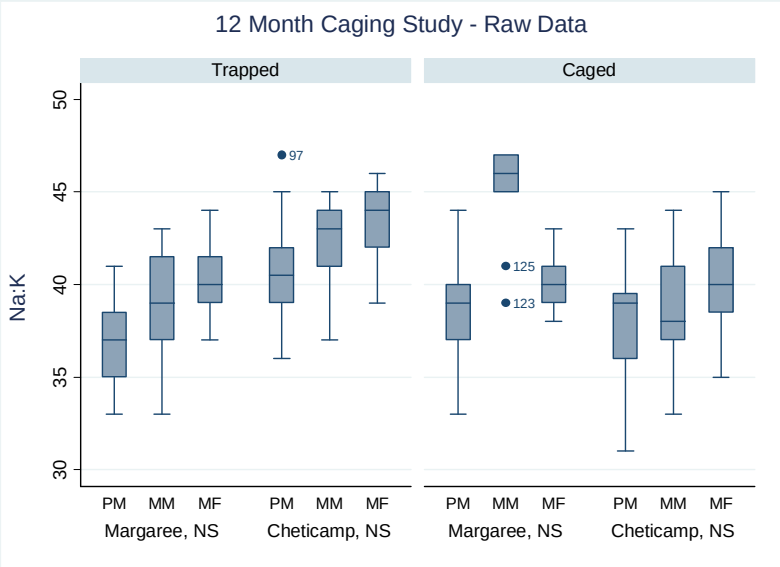
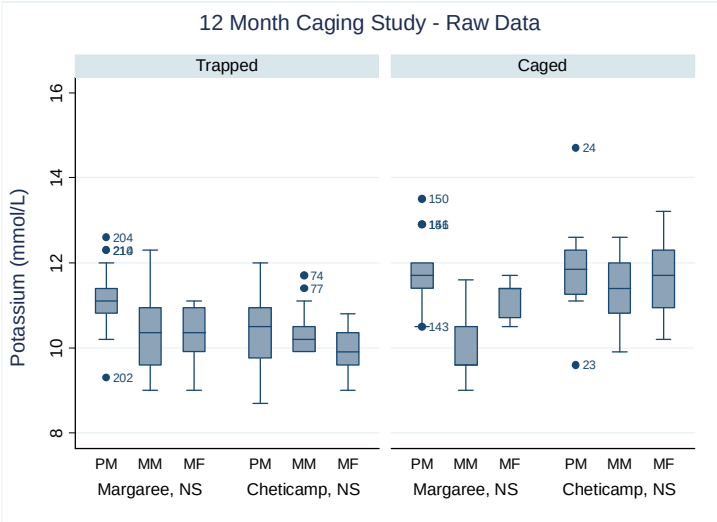
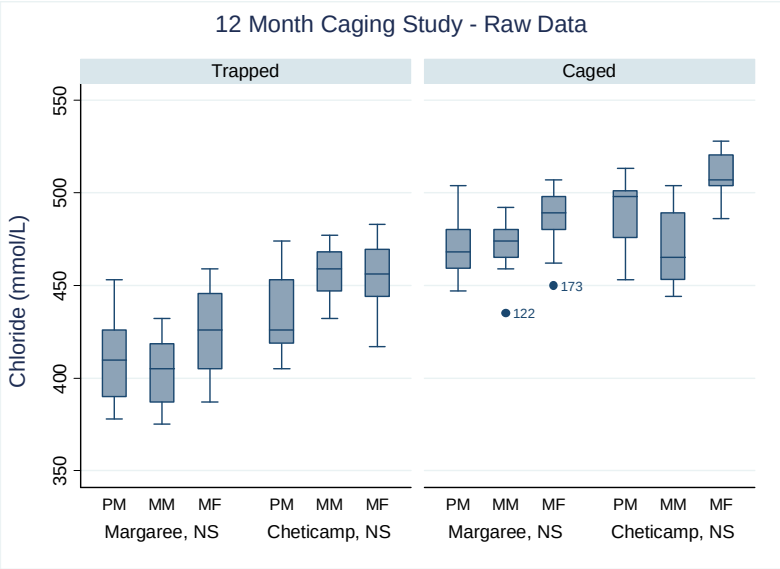
VIII-3-7 Appendices

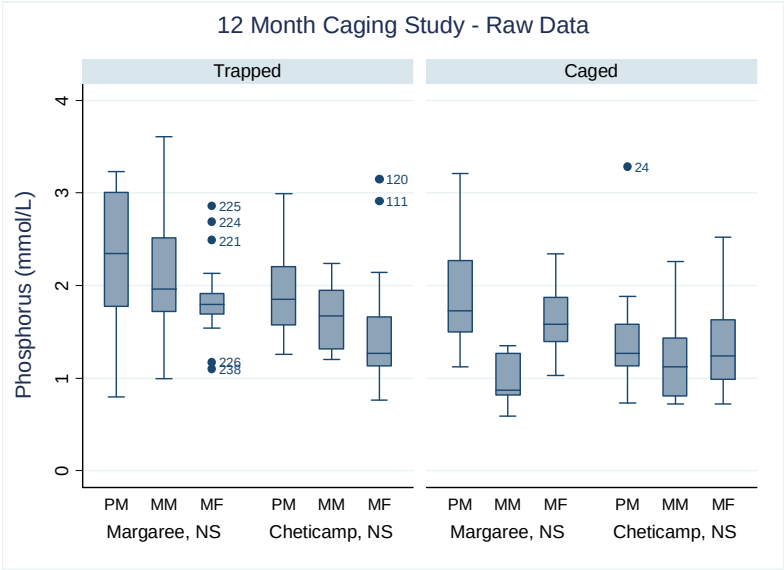
Appendix A*:* Box & Whisker Plots Biochemistry Profiles

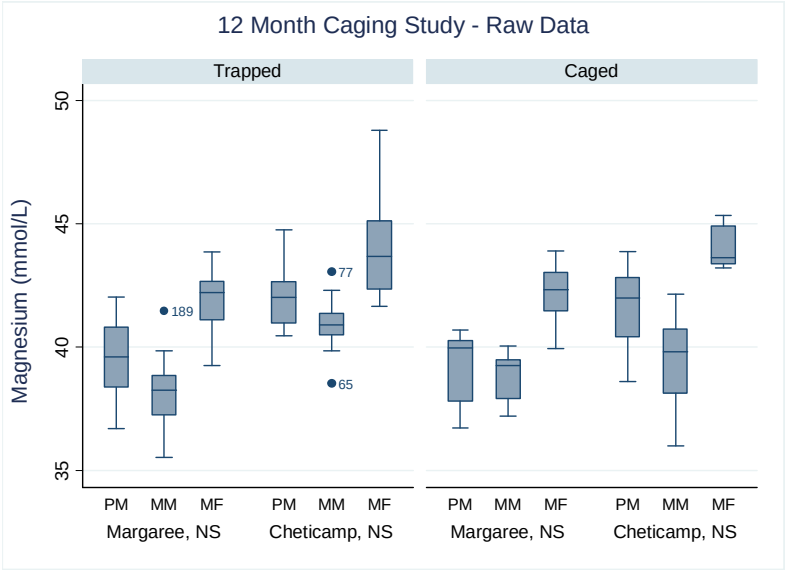


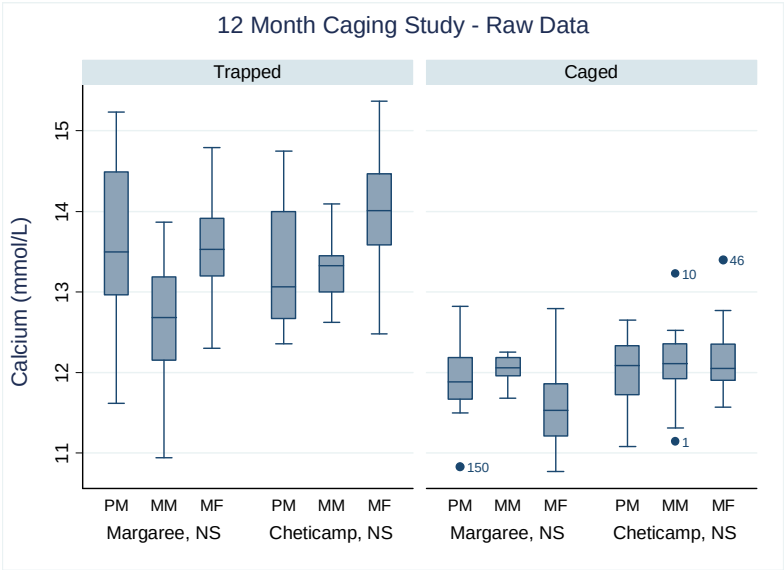


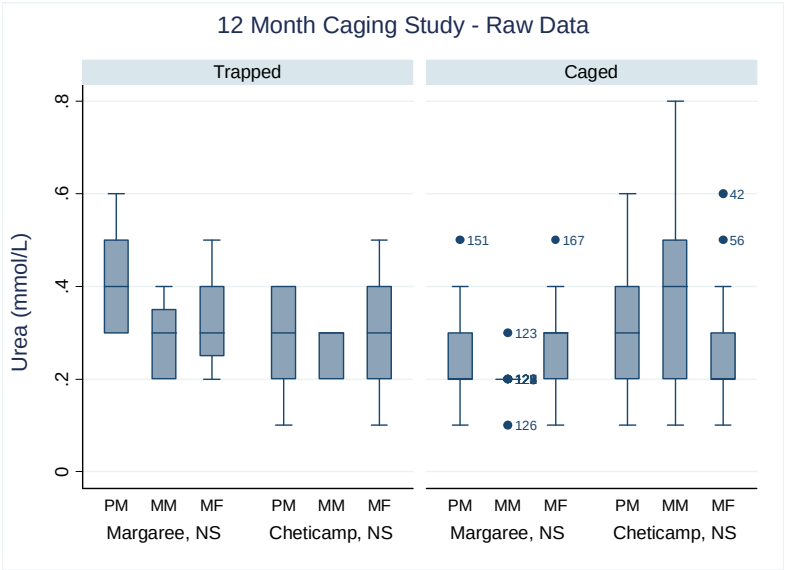


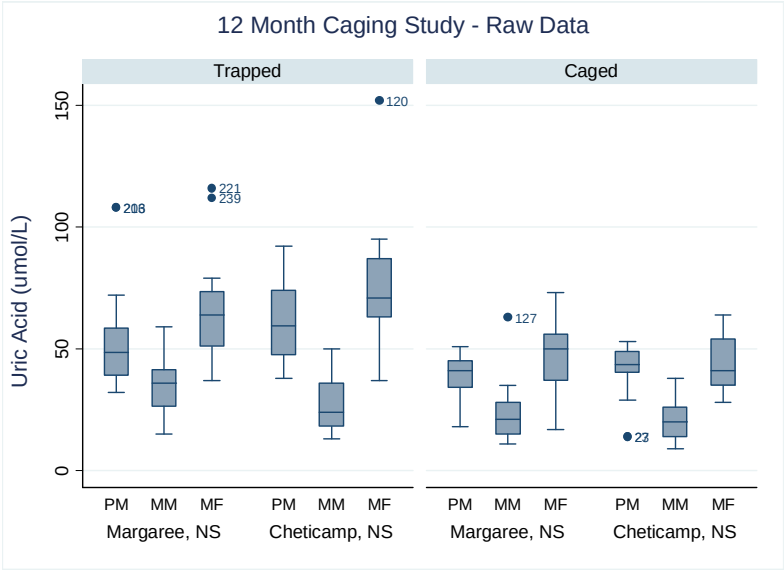


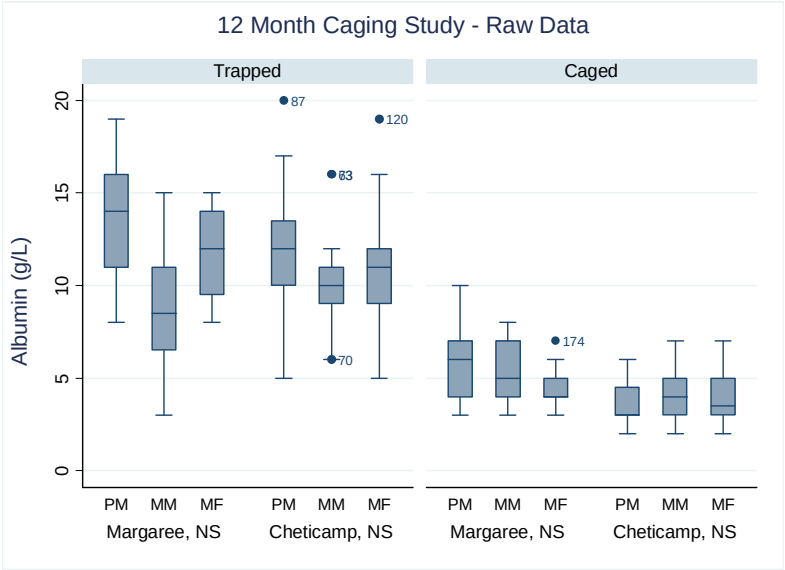

12 Month Caging Study - Raw Data
Trapped
Caged
Phosphorus (mmol/L)
0
1
2
3
4
225
224
221
226
238
120
111
24
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

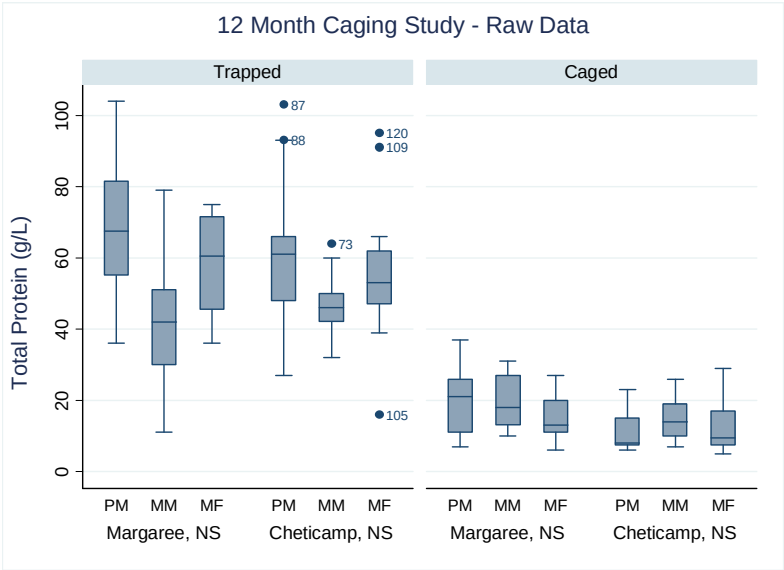

12 Month Caging Study - Raw Data
Trapped
Caged
Magnesium (mmol/L)
35
40
45
50
189
77
65
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

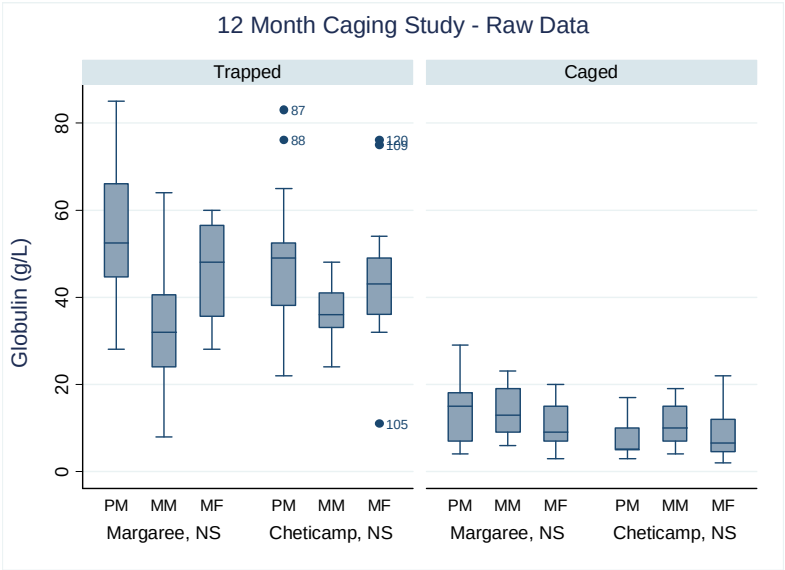

12 Month Caging Study - Raw Data
Trapped
Caged
Calcium (mmol/L)
11
12
13
14
15
150
10
46
1
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

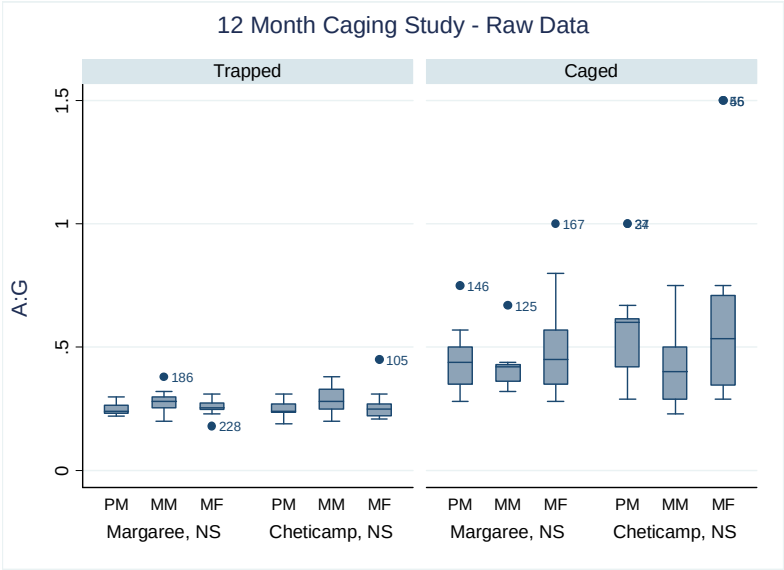

12 Month Caging Study - Raw Data
Trapped
Caged
Urea (mmol/L)
0
.2
.4
.6
.8
151
167
123
126
42
56
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

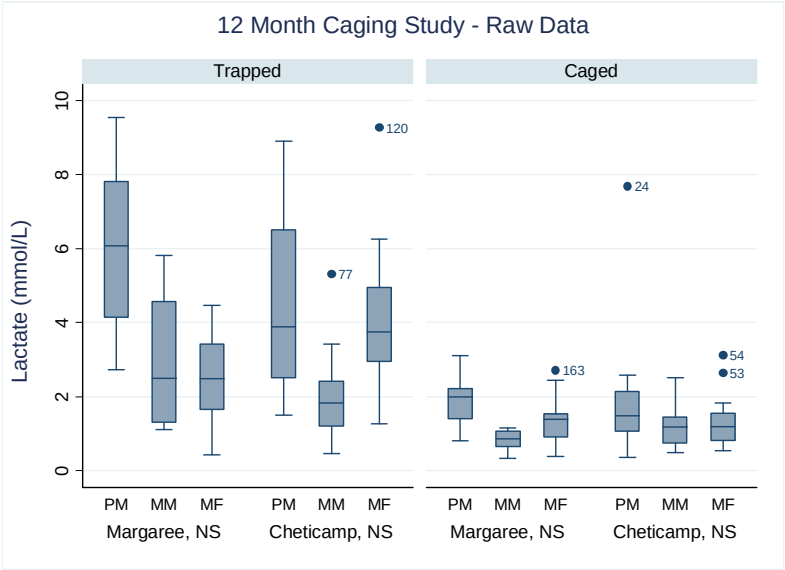

12 Month Caging Study - Raw Data
Trapped
Caged
Uric Acid (umol/L)
0
50
100
150
120
221
239
127
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

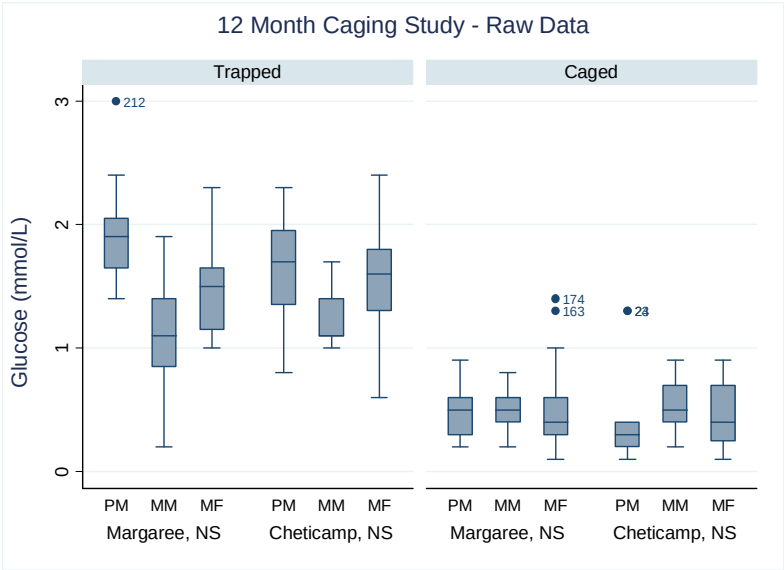

12 Month Caging Study - Raw Data
Trapped
Caged
Albumin (g/L)
0
5
10
15
20
87
120
70
174
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

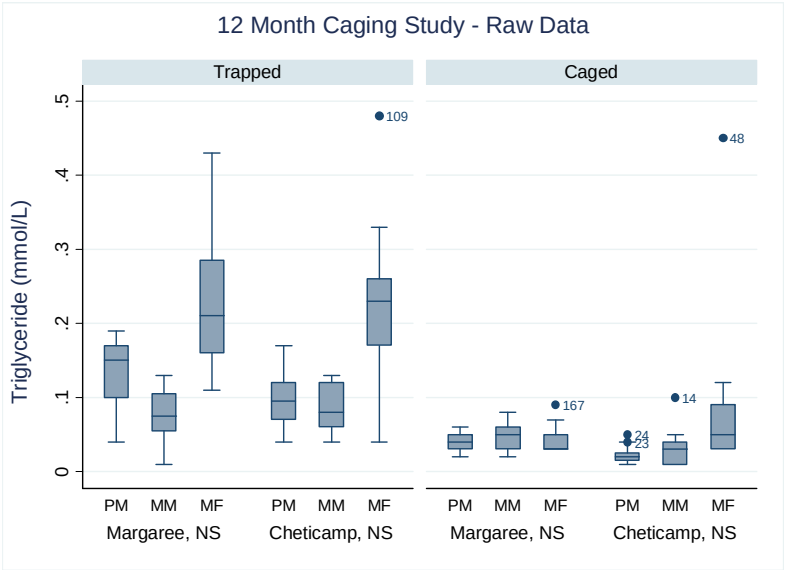

12 Month Caging Study - Raw Data
Trapped
Caged
Total Protein (g/L)
0
20
40
60
80
100
87
88
120
109
73
105
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

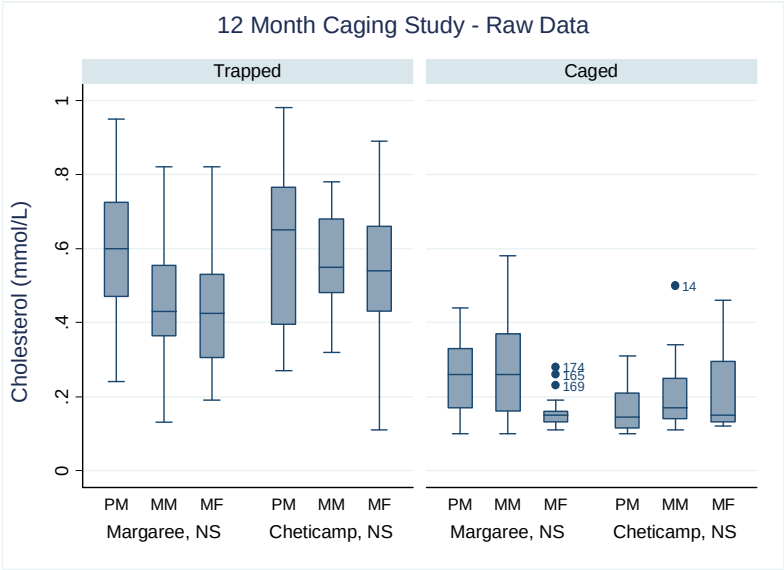

12 Month Caging Study - Raw Data
Trapped
Caged
Globulin (g/L)
0
20
40
60
80
87
88
105
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

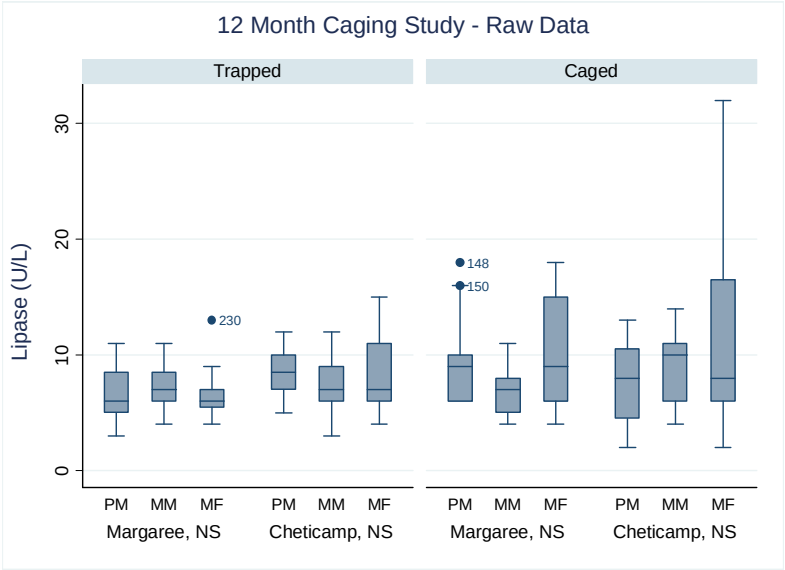

12 Month Caging Study - Raw Data
Trapped
Caged
A:G
0
.5
1
1.5
186
228
105
146
125
167
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

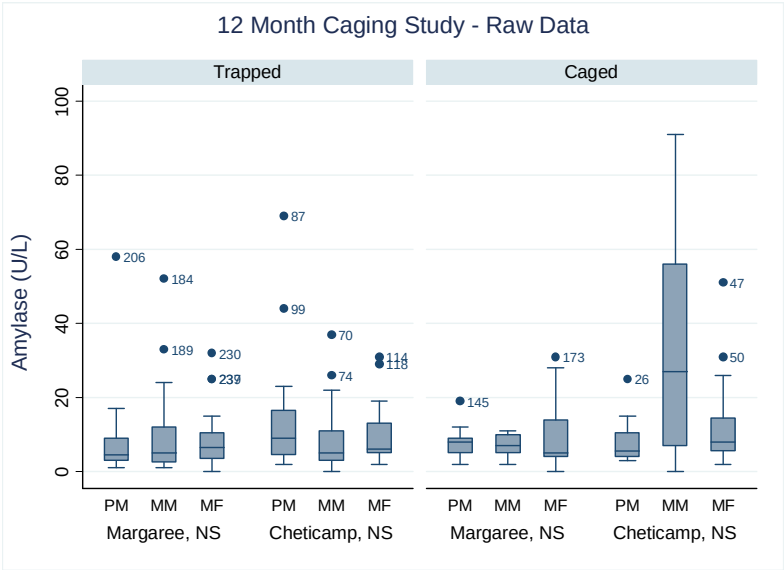

12 Month Caging Study - Raw Data
Trapped
Caged
Lactate (mmol/L)
0
2
4
6
8
10
120
77
24
163
54
53
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

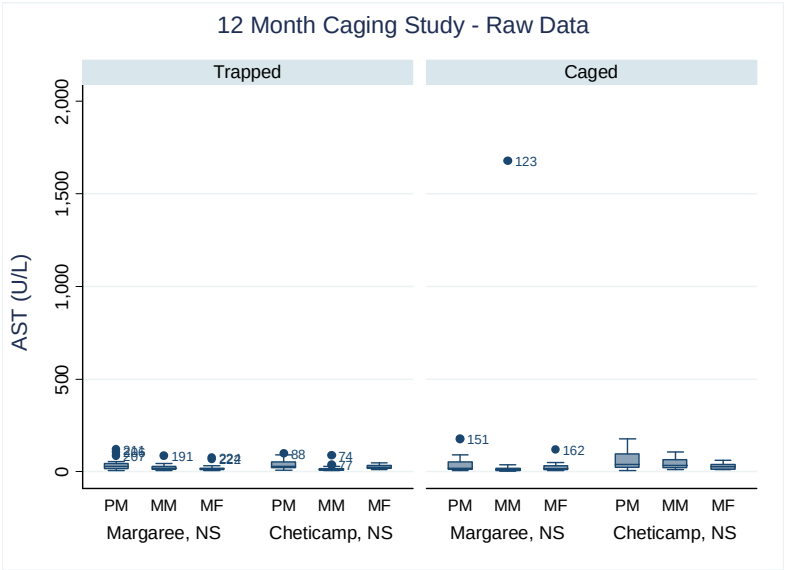

12 Month Caging Study - Raw Data
Trapped
Caged
Glucose (mmol/L)
0
1
2
3
212
174
163
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

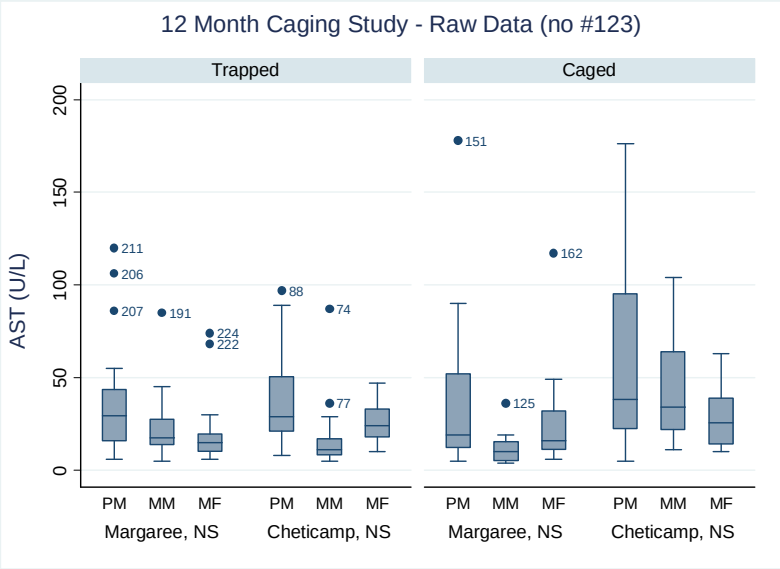

12 Month Caging Study - Raw Data
Trapped
Caged
Triglyceride (mmol/L)
0
.1
.2
.3
.4
.5
109
48
167
14
24
23
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

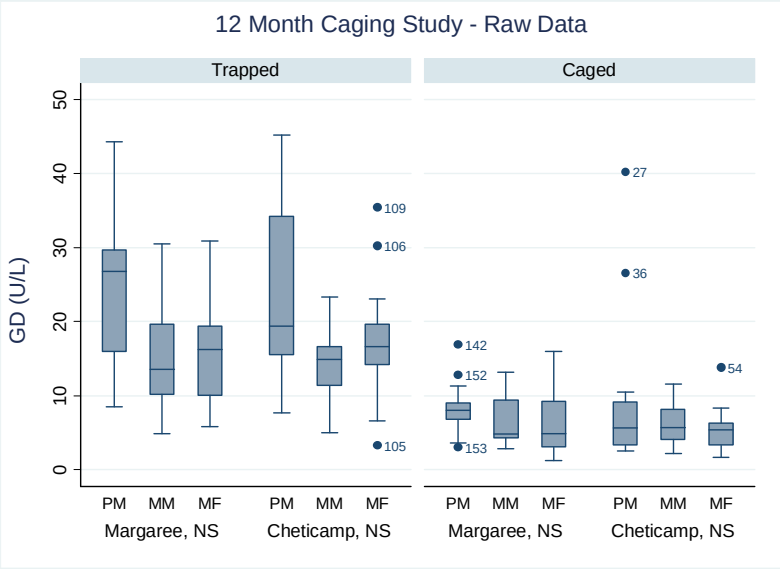

12 Month Caging Study - Raw Data
Trapped
Caged
Cholesterol (mmol/L)
0
.2
.4
.6
.8
1
174
165
169
14
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

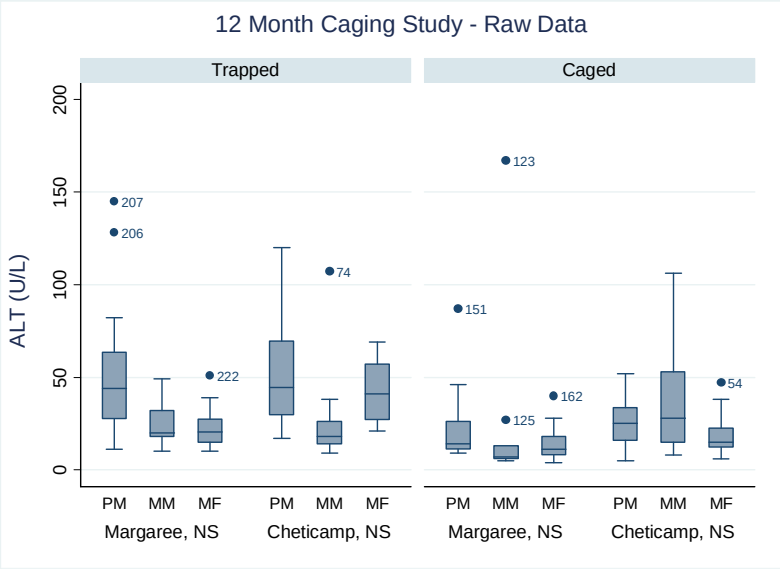

12 Month Caging Study - Raw Data
Trapped
Caged
Lipase (U/L)
0
10
20
30
230
148
150
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

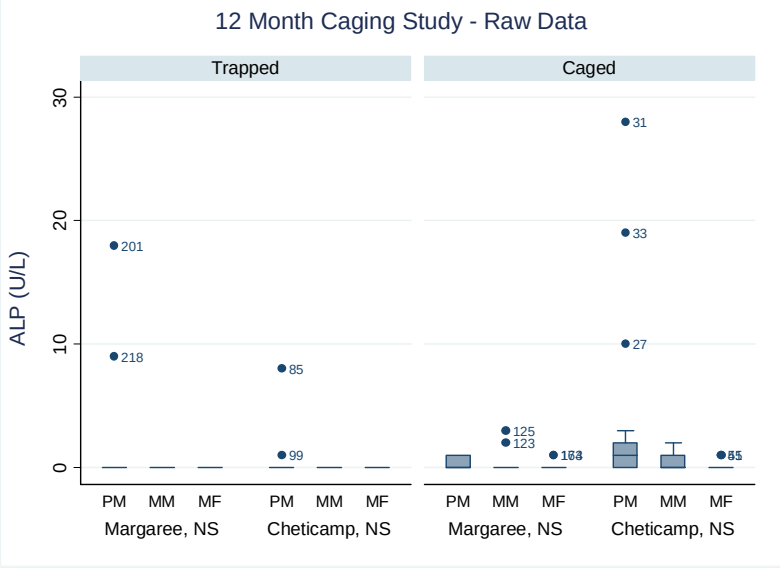

12 Month Caging Study - Raw Data
Trapped
Caged
Amylase (U/L)
0
20
40
60
80
100
206
184
189
230
87
99
70
74
114
145
173
26
47
50
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

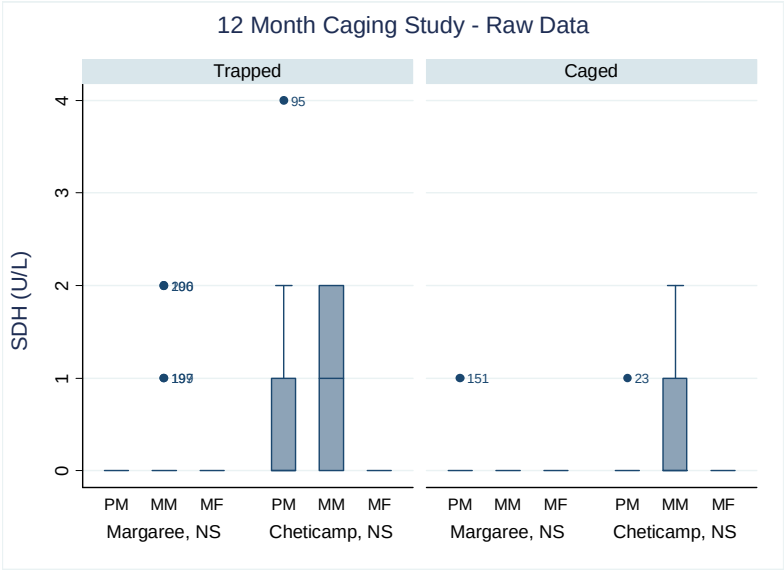

12 Month Caging Study - Raw Data
Trapped
Caged
AST (U/L)
0
500
1,000
1,500
2,000
123
191
88
74
151
162
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

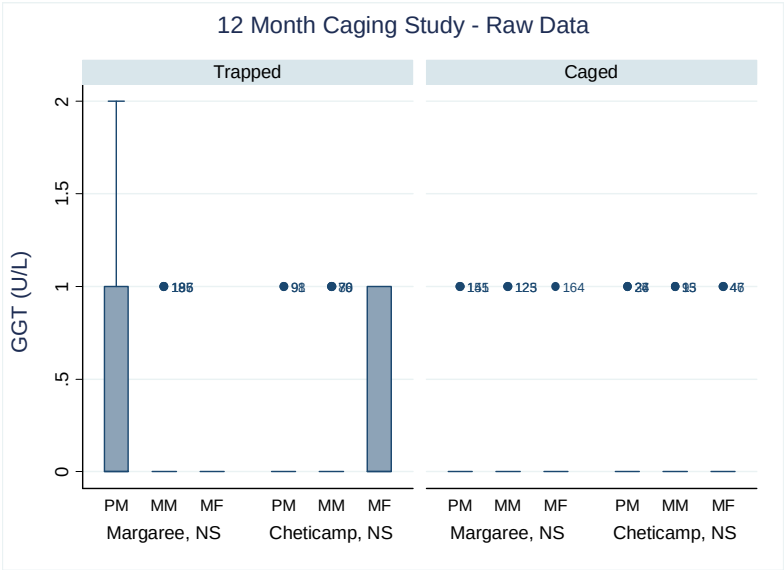

12 Month Caging Study - Raw Data (no #123)
Trapped
Caged
AST (U/L)
0
50
100
150
200
211
206
207
191
224
222
88
74
77
151
125
162
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS


12 Month Caging Study - Raw Data
Trapped
Caged
GD (U/L)
0
10
20
30
40
50
109
106
105
142
152
153
27
36
54
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

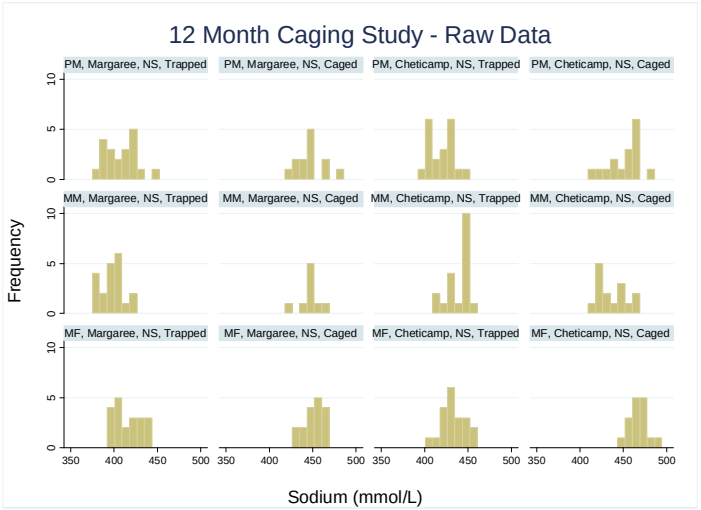

12 Month Caging Study - Raw Data
Trapped
Caged
ALT (U/L)
0
50
100
150
200
207
206
74
222
123
151
125
162
54
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

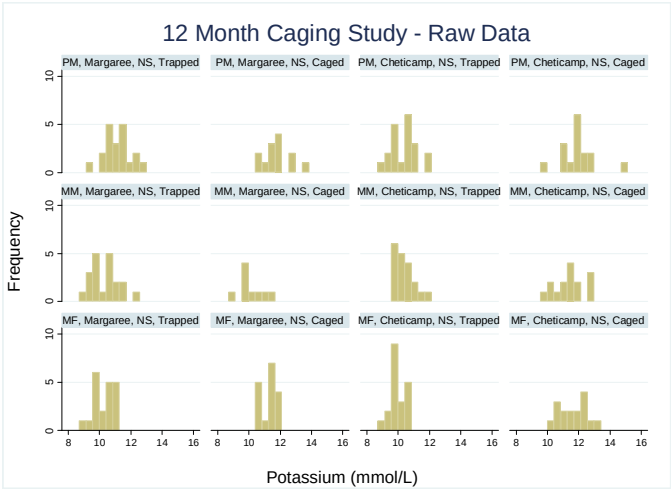

12 Month Caging Study - Raw Data
Trapped
Caged
ALP (U/L)
0
10
20
30
201
218
85
99
31
33
27
125
123
PM MM MF PM MM MF
Margaree, NS
Cheticamp, NS

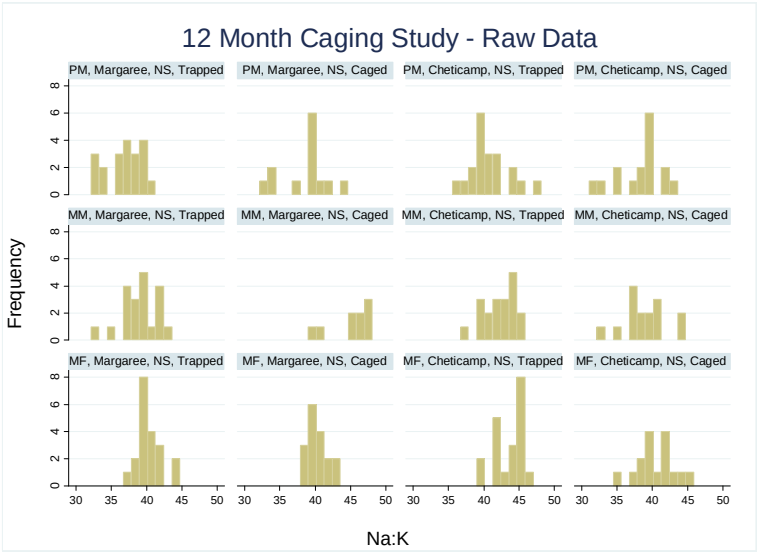

12 Month Caging Study - Raw Data
Trapped
Caged
SDH (U/L)
0
1
2
3
4
95
151
23
PM
MM
MF
Margaree, NS
Cheticamp, NS

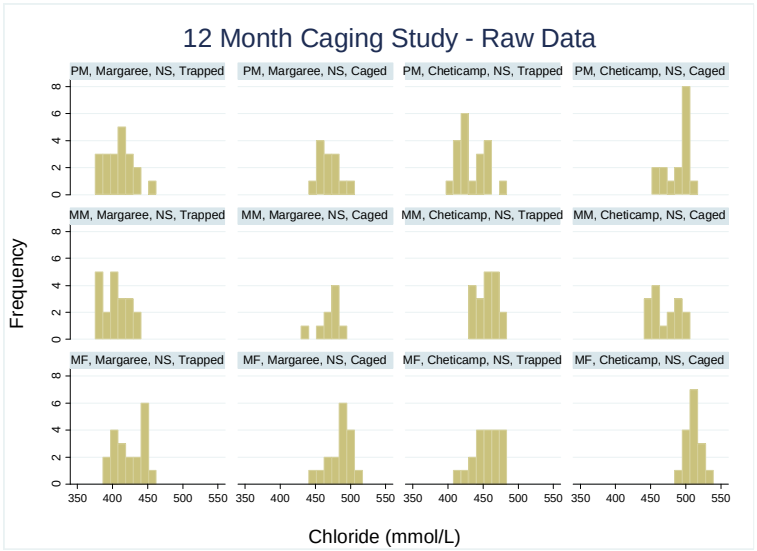

12 Month Caging Study - Raw Data
Trapped
Caged
GGT (U/L)
0
.5
1
1.5
2
164
PM
MM
MF
Margaree, NS
Cheticamp, NS

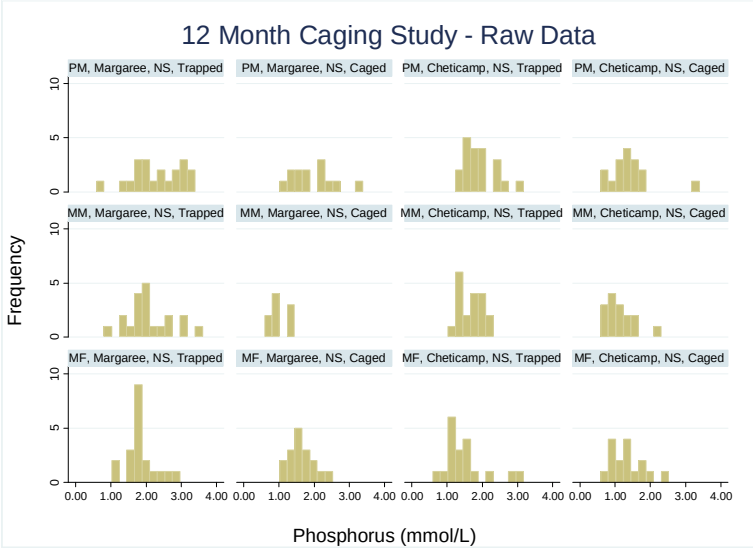
Appendix B: Frequency Distribution Histograms, Biochemistry Profiles

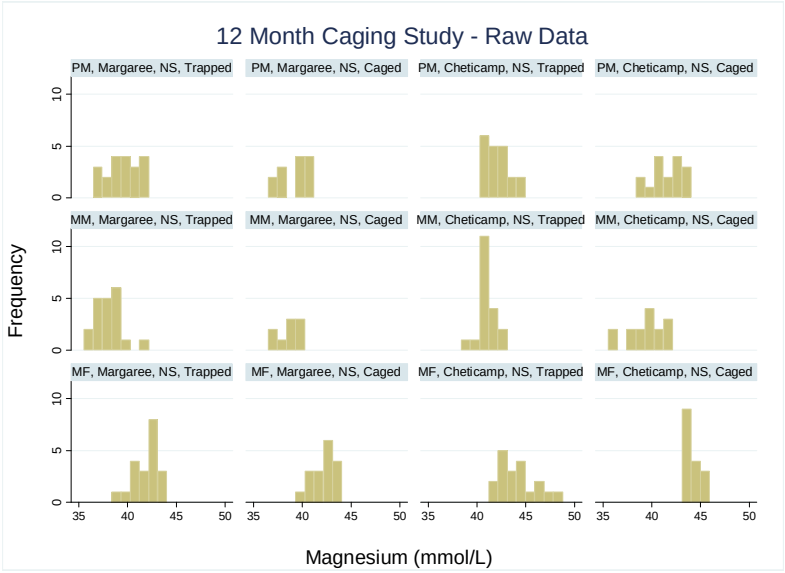


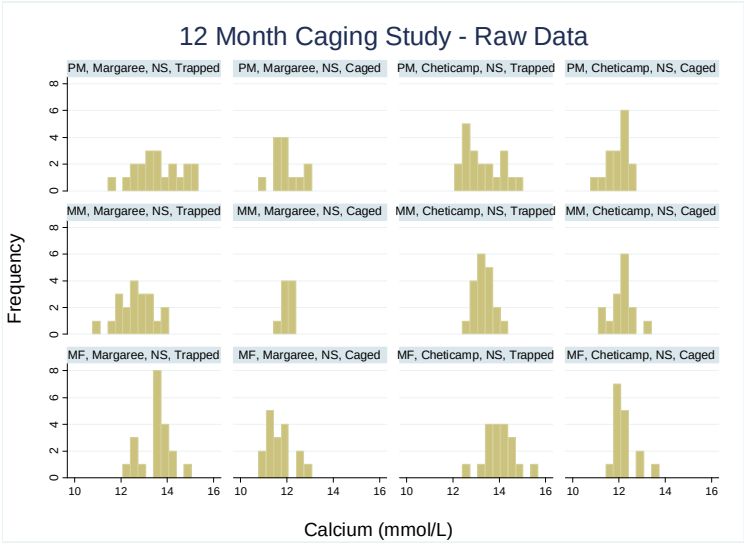


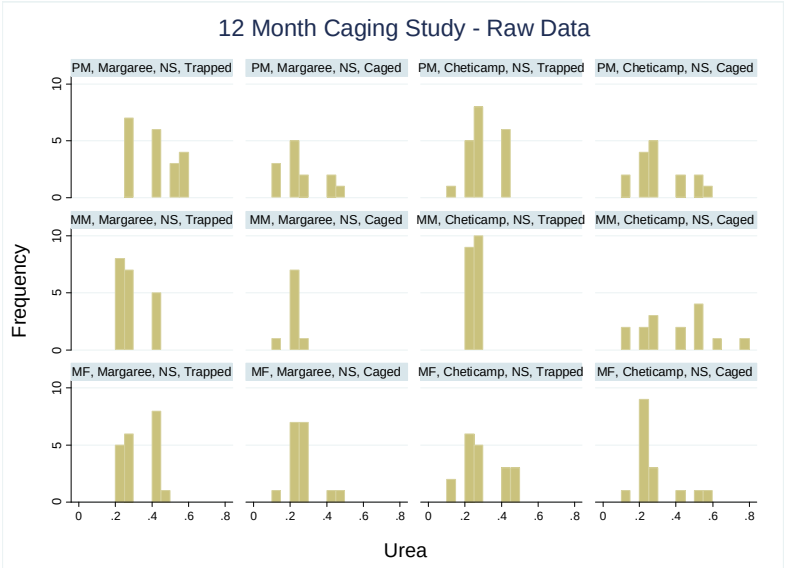


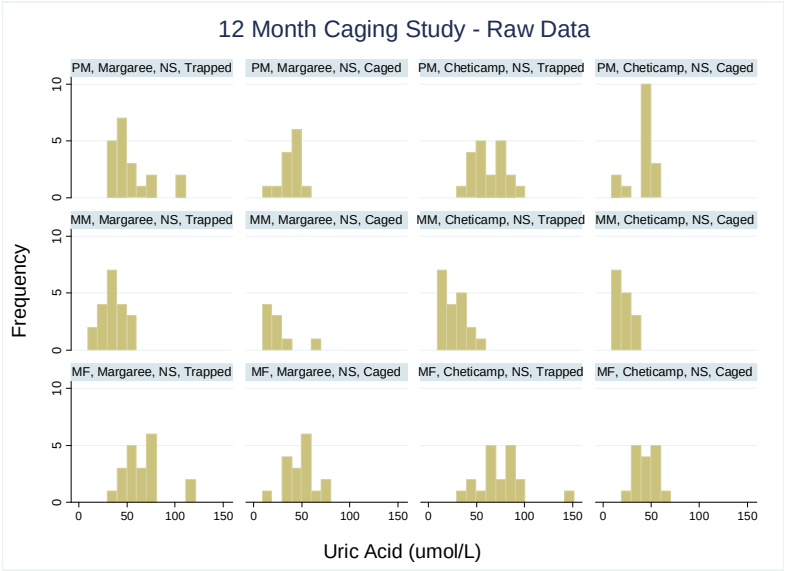


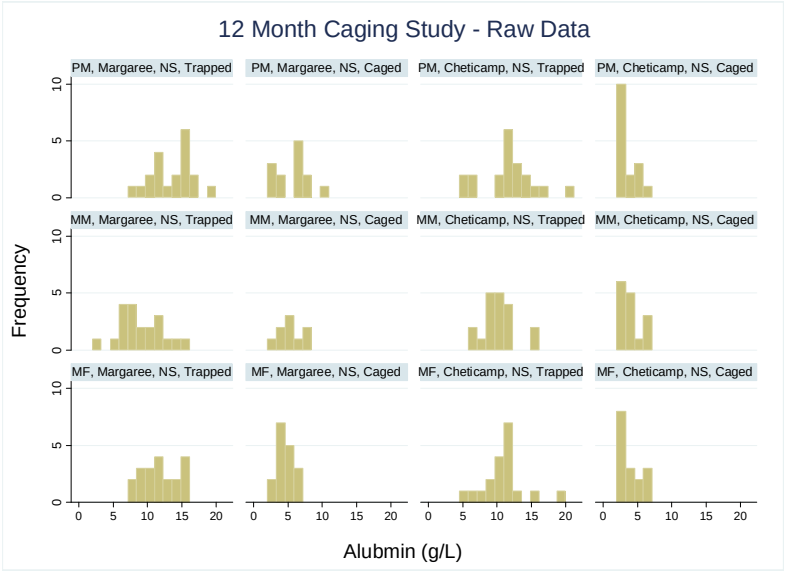

12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 5 10
0.00 1.00 2.00 3.00 4.00
Phosphorus (mmol/L)

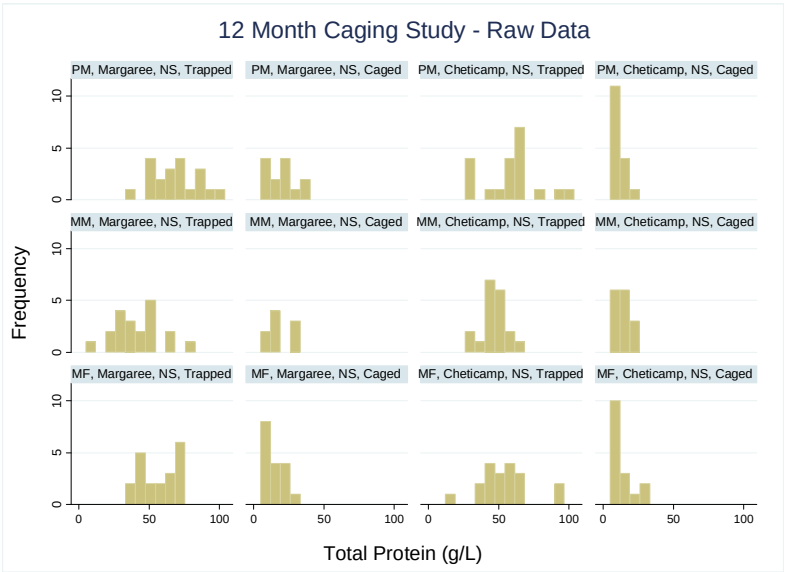

12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 5 10
35 40 45 50
Magnesium (mmol/L)

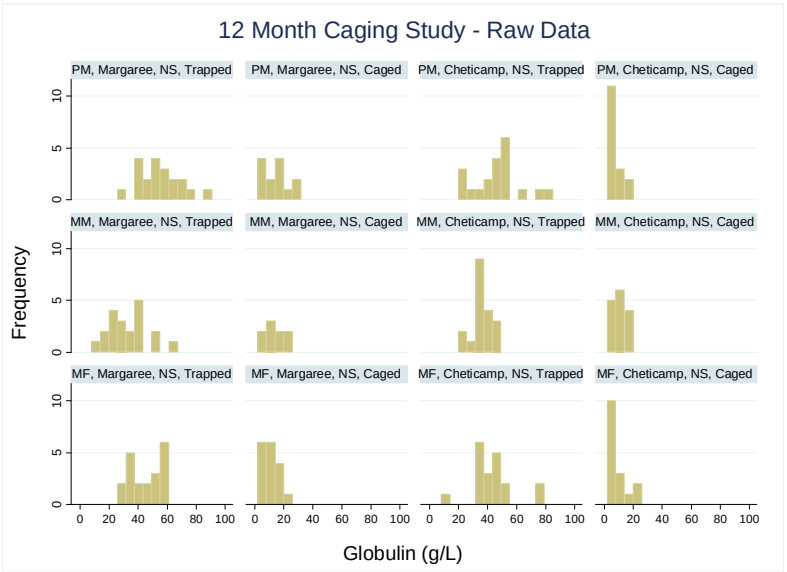

12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 2 4 6 8
10 12 14 16
Calcium (mmol/L)

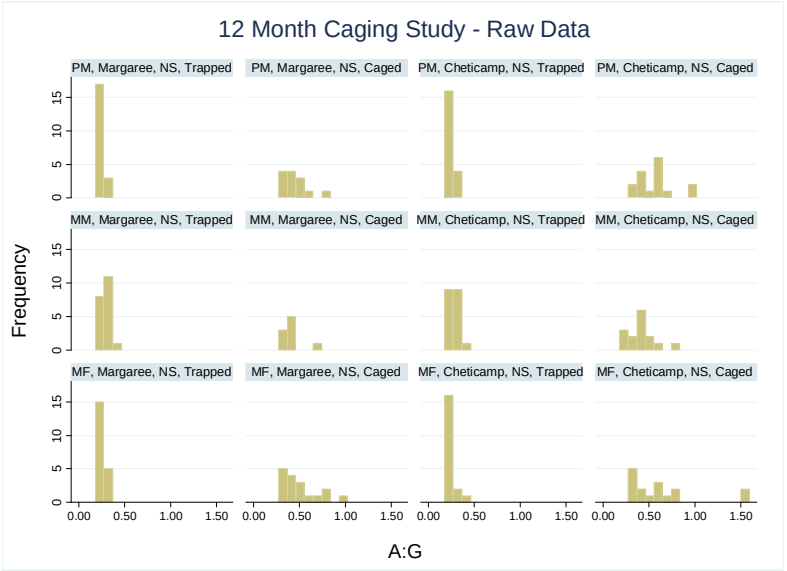

12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0 5 10
0 .2 .4 .6 .8
Urea

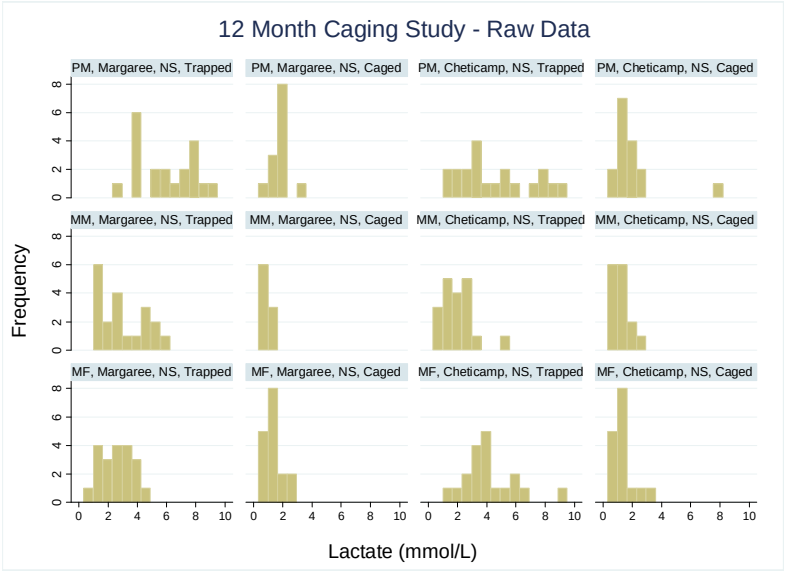

12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Uric Acid (umol/L)


12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Alubmin (g/L)


12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Total Protein (g/L)


12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Globulin (g/L)


12 Month Caging Study - Raw Data
PM, Margaree, NS, Trapped
PM, Margaree, NS, Caged
PM, Cheticamp, NS, Trapped
PM, Cheticamp, NS, Caged
MM, Margaree, NS, Trapped
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
A:G


12 Month Caging Study - Raw Data
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PM, Margaree, NS, Caged
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PM, Cheticamp, NS, Caged
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MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
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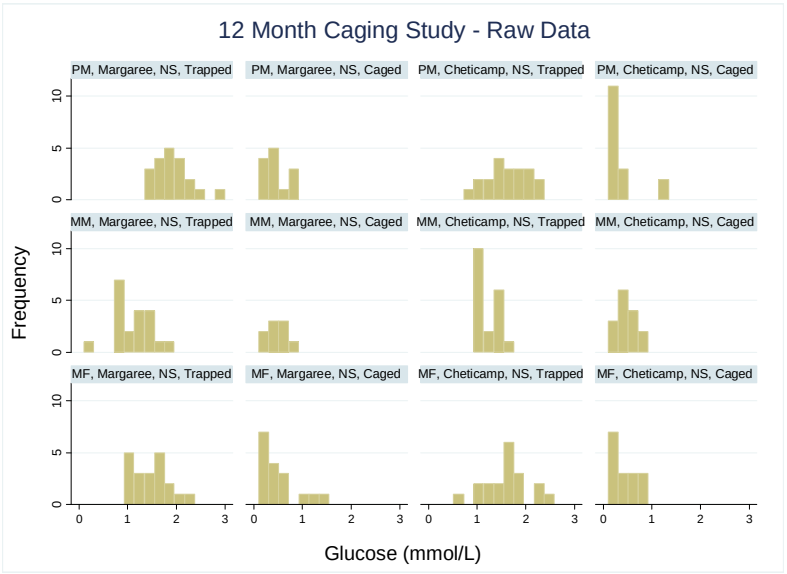
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
Lactate (mmol/L)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MM, Cheticamp, NS, Caged
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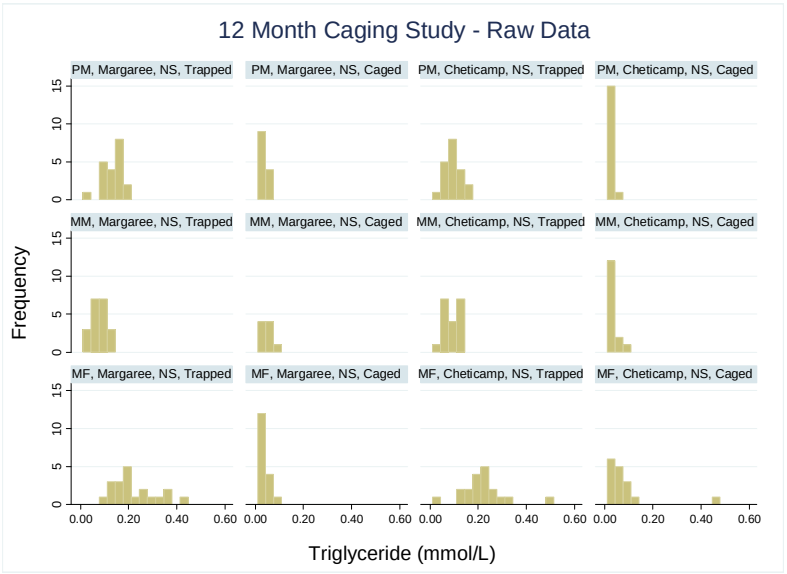
Frequency
Glucose (mmol/L)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MM, Cheticamp, NS, Caged
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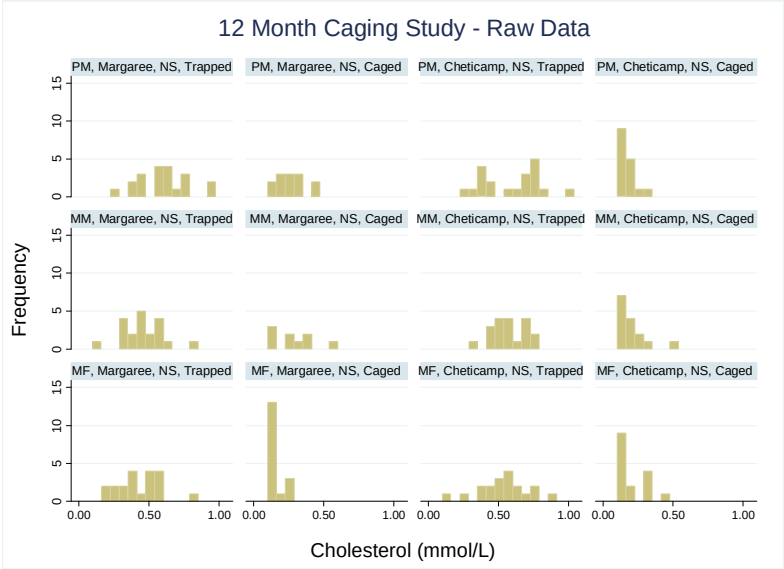
MF, Cheticamp, NS, Caged
Frequency
Triglyceride (mmol/L)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MM, Cheticamp, NS, Caged
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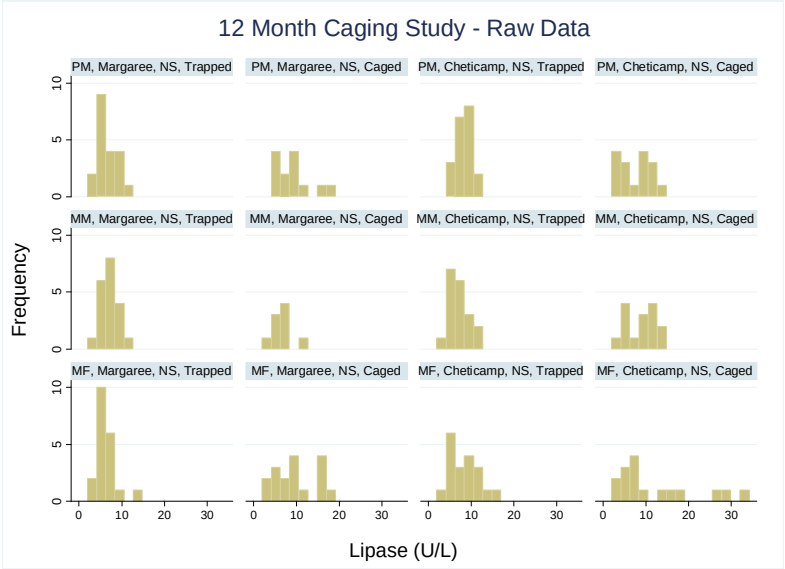
Frequency
Cholesterol (mmol/L)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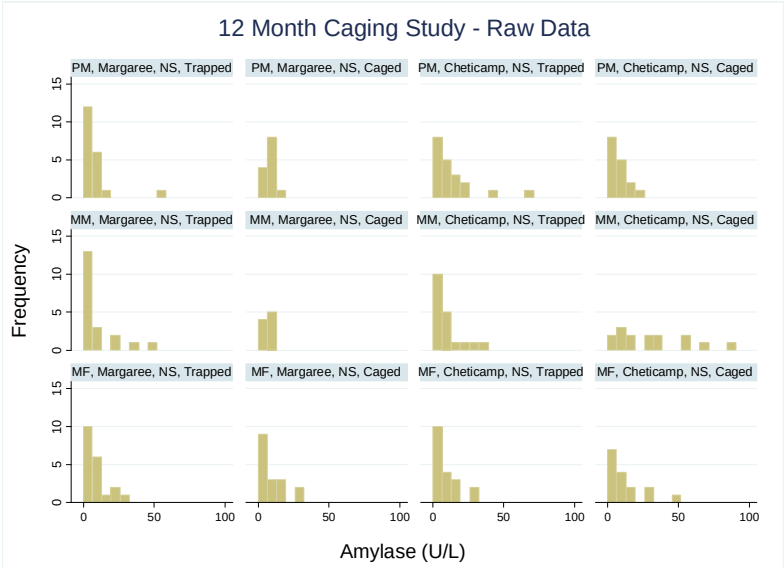
Frequency
Lipase (U/L)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MM, Cheticamp, NS, Caged
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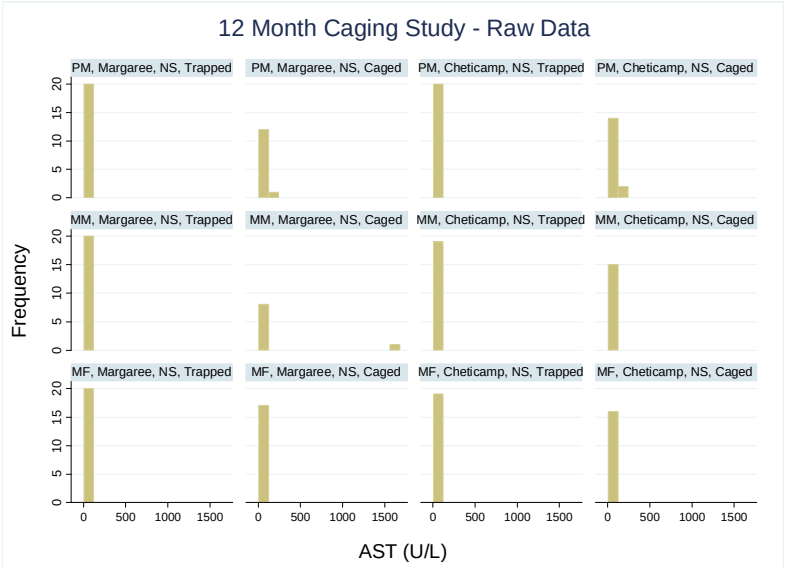
Frequency
Amylase (U/L)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MM, Cheticamp, NS, Caged
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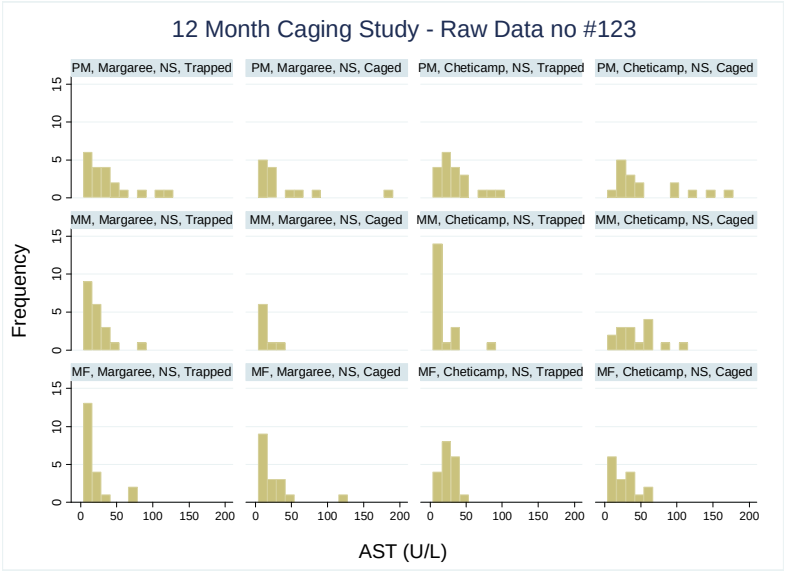
Frequency
AST (U/L)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MM, Cheticamp, NS, Caged
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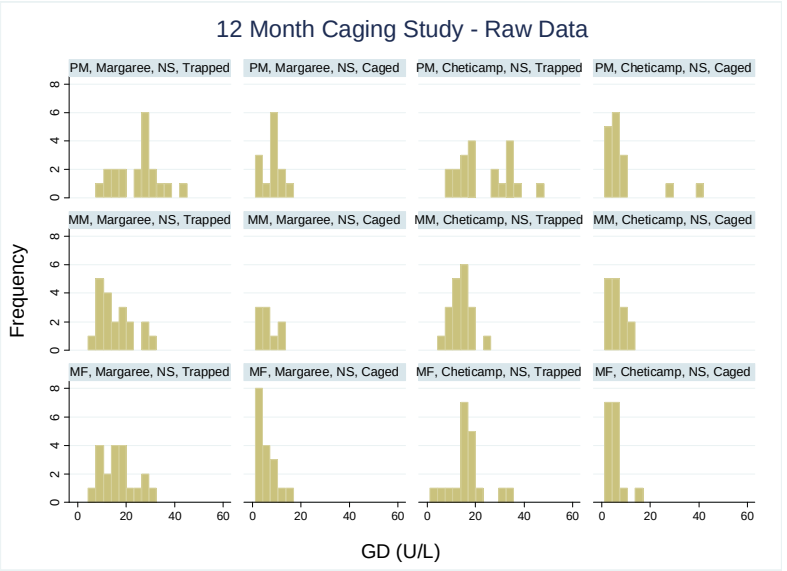
Frequency
AST (U/L)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MM, Cheticamp, NS, Caged
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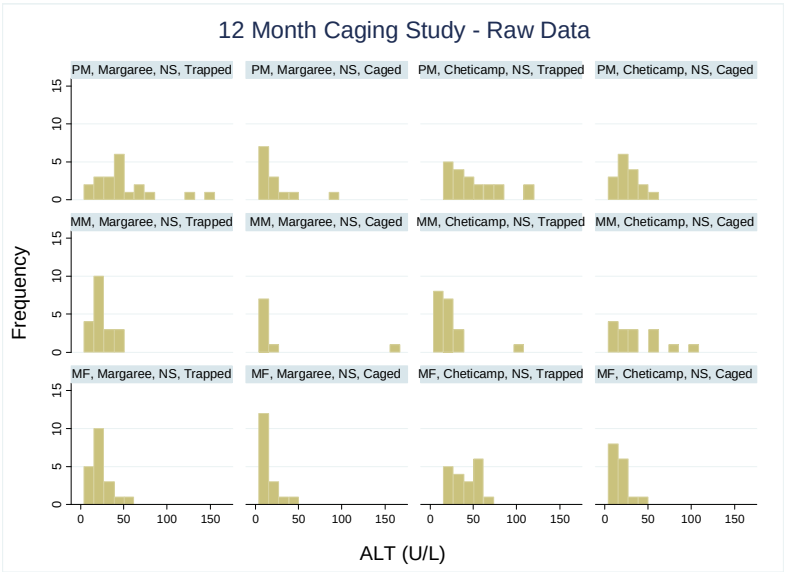
MF, Cheticamp, NS, Caged
Frequency
GD (U/L)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MM, Cheticamp, NS, Caged
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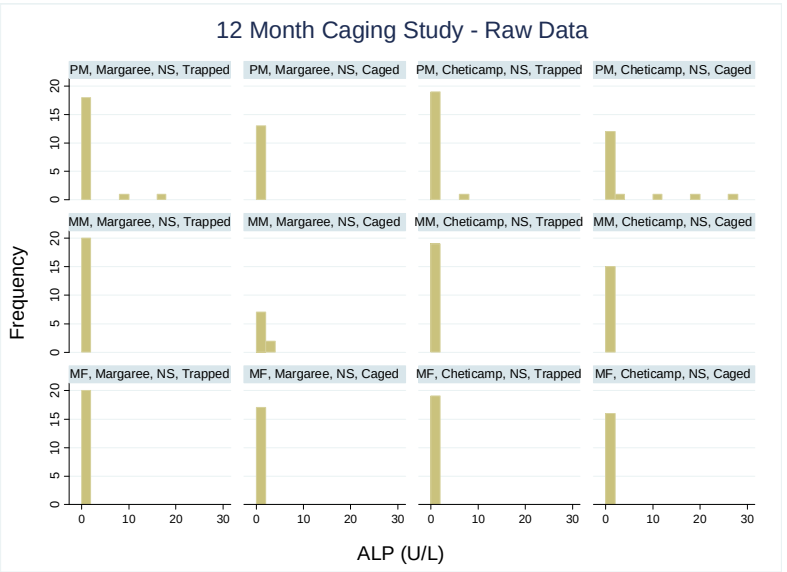
Frequency
ALT (U/L)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MM, Cheticamp, NS, Caged
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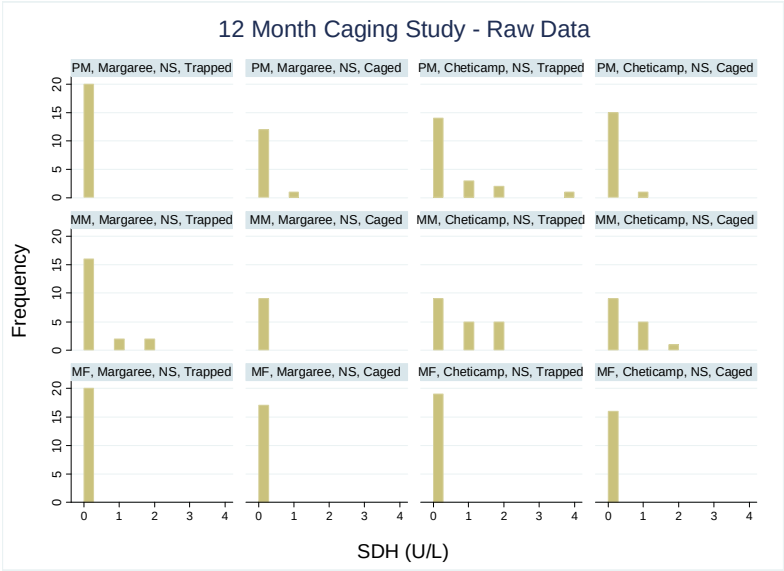
MF, Cheticamp, NS, Caged
Frequency
ALP (U/L)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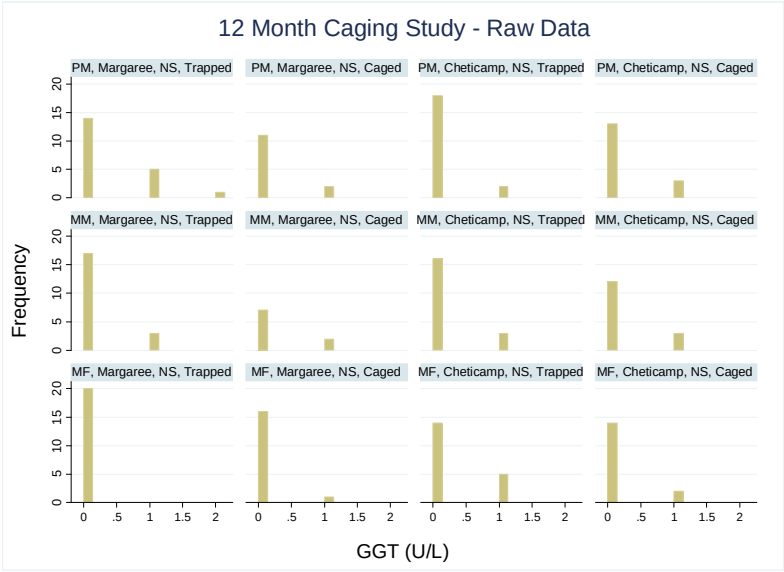
MM, Margaree, NS, Caged
MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
20
0
1
2
3
4
SDH (U/L)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MM, Cheticamp, NS, Trapped
MM, Cheticamp, NS, Caged
MF, Margaree, NS, Trapped
MF, Margaree, NS, Caged
MF, Cheticamp, NS, Trapped
MF, Cheticamp, NS, Caged
Frequency
0
5
10
15
20
0
.5
1
1.5
2
GGT (U/L)

IX. ACKNOWLEDGMENTS

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*Current address: Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australia

** Current address: Canadian Association of Petroleum Producers (CAPP).

Scientific assistance:

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Review of various versions of the report:

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