Progress Report #3 (9/2023 to 4/2024)

Project Title: NA22NMF4270121-T1-01 “An examination of softshell clam, *Mya arenaria* L., fecundity along the Maine coast: the influence of clam size, tidal height, season, and geographic region.”

Major Activities

The work reported here covers two independent sources of data. The first is the collection (irregularly) of commercial size-frequencies of soft-shell clams from a buying station in eastern Maine (A.C. Inc. – Town of Beals) and southern Maine (Cantrell’s Seafood – Topsham). Table 1 gives the sampling frequency (since September 2022) from these two buying stations. The second is the collection of data associated with a comparative field experiment initiated in March 2023 at three tidal heights (upper; mid; lower) at an intertidal flat in Brunswick (southern), Bremen (midcoast), and Jonesport (eastern).

Size-frequency measurements of commercial-size soft-shell clams

Data were collected from two commercial soft-shell clam buying stations – one in eastern Maine (A.C. Inc. – town of Beals) and the other in southern Maine (Cantrell’s Seafood – Topsham). Data were collected monthly at irregular intervals that coincided with the astronomically high/low tides of a given month. The reason for this is that clammers typically can access the lowest tidal levels each month when the largest clams are accessible. The ultimate purpose for this exercise is to provide the state of Maine with size-frequency data from the commercial harvest to assess the percent frequency of the largest clams in the event that an upper size limit is established. It may be reasonable to discuss/debate an upper size limit for soft-shell clams if reproductive senility does not occur with increasing size/age and if viable offspring are produced from the largest animals in the population. Recently, commercial landings data for soft-shell clams harvested in 2023 in Maine were released by Maine’s Department of Marine Resources that show clam landings in 2023 were at historic lows (5.2 million pounds, worth $13.9 million). Since 1977 when 38.4 million pounds were landed, soft-shell clam landings have decreased by ~85%, and, using 2023 dollars to estimate the value of the fishery in 1977, its value has declined by 71.5%.

Beginning in September 2022 through February 2024, ten visits to A.C. Inc. and five visits to Cantrell’s Seafood occurred (Table 1). On each date, researchers measured the shell length (SL; greatest linear anterior-posterior distance) of 25 clams sampled randomly from nine bushel collected at the buying station over a 3-day period (Tables 2-3; Figs. 1-4). The nine bushel of clams were not processed prior to pouring each bushel separately onto a table, after which clams were mixed before 25 clams were haphazardly removed for measuring. Each bushel was in the condition it had been when it first reached the buying station – that is, no effort was made to wash clams or remove broken/cracked individuals prior to measuring.

Comparative Field Experiment to investigate soft-shell clam reproductive cycle and fecundity

To examine potential regional and local factors affecting soft-shell clam reproductive cycle and fecundity, in March 2023, 24 clams ranging in size from 30-90 mm SL were placed in 1 ft^2 × 6-inch deep field cages (wooden boxes; 0.5 ft^3, or 0.014 m^3) designed to exclude large predators. Boxes were established along a tidal gradient at a flat in southern (Brunswick, near Middle Bay: 43º51'16.31"N; 69º57'46.52"W), midcoast (Bremen, near the mouth of the Medomak River: 43º59'26.35"N; 69º24'32.99"W), and eastern Maine (Jonesport, near Flake Point Bar: 44o36'48.23"N; 67o33'44.92"W) (Figs. 5-7).

Boxes (n = 42 per each of three tidal heights: upper, mid, and lower intertidal) were dug into the sediments at each study site, and filled with ambient sediments by clammers and researchers who carefully inspected sediments for and removed predators such as green crabs (*Carcinus maenas*) and milky ribbon worms (*Cerebratulus lacteus*). All clams > 50.8 mm SL transplanted to boxes were accessed from local clam buyers. Clams < 50.8 mm SL were obtained from two sources. Clams used at the Brunswick and Bremen sites were collected near the upper intertidal at Thomas Point flat in Brunswick (43º53'35.72"N; 69º53'30.86"W) whereas those used at Jonesport were collected near the upper intertidal at Flake Point Bar. Four clams from each of six size classes (I: 30-38 mm; II: 38.1-50.7 mm; III: 50.8-61.5 mm; IV: 61.6-74.5 mm; V: 74.6-87.5 mm; VI: > 87.5 mm) were added to each box at each site and tidal height (3 sites x 3 tidal heights x 42 boxes/tidal height x 6 size classes x 4 individuals/size class = 9,072 clams). Clams were placed on top of the sediments and allowed to burrow *in sua*.

To protect clams from predators, a predator exclusion top was screwed tightly to the box opening (four 3-inch screws; Fig. 6A). Each protective top was constructed of a 1-inch thick wooden frame (1 ft^2). A piece of vinyl-coated trap wire (aperture = ½-inch; 12 mm) was stapled to the frame top, and a piece of 3.2 mm polyethylene netting (https://www.industrialnetting.com/xv1672-square-black-polyethylene-mesh.html) was stapled to the frame bottom. While this configuration was sufficient to exclude large predators, it was insufficient to exclude *C. lacteus*. Therefore, before a box top was affixed to the box, a piece of PetScreen® (a densely-woven vinyl-coated polyester; aperture = 0.9 mm x 1.7 mm; https://www.phifer.com/product/petscreen/; 1.17 ft^2, or 0.109m^2; Fig. 6B) was placed over the sediments to exclude ribbon worms. Temperature was recorded every 30 minutes in one box at each site and tidal height using a HOBO Pendant. Because temperature probes recorded temperatures 24/7 (i.e., daily during both low and high tides), to estimate seawater temperatures that clams experienced, we took three readings around each high tide (at 1 hr before and after high tide and at high tide) and averaged them to obtain an estimate of "high tide temperature."

All live and dead clams from boxes were removed by hand from two randomly chosen cages at each site and tidal height (Fig. 8) beginning the first week of May when seawater temperatures typically are below 10ºC, which may be a spawning threshold temperature for individuals north of Cape Cod, Massachusetts (Snelgrove et al. 2023). Clams from Jonesport were collected on Saturdays and driven to the Downeast Institute (DEI) where they were washed and stored in a walk-in cooler (8-10ºC) until Monday mornings, when they were induced to spawn. Sampling occurred in Bremen on Mondays. Clams were held in chilled, Styrofoam containers that were delivered via Fed-Ex to DEI on Tuesday, stored in the cooler, and induced to spawn on

Wednesdays. Clams from Brunswick were collected on Tuesdays and treated similarly to those collected from Bremen except they were induced to spawn on Thursdays after delivery to DEI occurred on Wednesdays.

Spawning induction at DEI (Fig. 9) proceeded each time (3 days/week for 19 weeks) by placing animals from each tidal height into one of three "spawning tanks" (125-liters, and measuring 36-inches x 36-inches x 6-inches deep) and then filling with 5µm-filtered ambient seawater (Fig. 9A). Approximately 500 ml of cultured microalgae was added to each tank at that time. After an hour, seawater in each tank was drained and replaced immediately with filtered, warm (24ºC) seawater. Within a spawning tank, animals from both boxes at a given tidal height were physically separated (Fig. 9B). This cycle of ambient/warm seawater was repeated 2-3 times for most attempts to induce spawning that occurred over 6-7 hrs each time.

When a clam began to release its gametes, it was immediately removed from the spawning tank and placed into a glass fingerbowl (Fig. 10) with 24ºC seawater where it finished releasing – a period that took between 1-15 minutes. Sometimes, the density of eggs/bowl were so great that spawning females would be moved to fingerbowls with clean seawater to prohibit egg ingestion. Once completed, the contents of all fingerbowls containing eggs from a single female were added to a single bucket and its volume brought to 10 L. Next, six 1 ml samples were taken from the bucket and the eggs from three samples were counted using a Sedgwick Rafter Cell and a microscope (4X). The other three 1 ml samples were counted using a FlowCam (https://www.fluidimaging.com/). To estimate viability, when males and females from the same tidal height spawned, eggs from at least one female were fertilized by at least one male. Within 30 minutes of fertilization, eggs were inspected microscopically for polar body formation/ extrusion. When polar bodies formed, embryos were presumed viable (Arellano and Young 2009). Percent viability was estimated for each Male:Female pairing by counting the number of eggs with polar body formation from 10 randomly observed eggs per female.

Additional data

Two additional data sets were collected at the same time collection of the data described above occurred. This portion of the research was funded by the Maine Economic Improvement Fund – Small Campus Initiative that used the NOAA-SK grant NA22NMF4270121-T1-01 to leverage additional funds to examine gonadal and condition indices as well as histology to generate visual information about development and gonadal cycles.

Clams that did not spawn on a given date from a given location were the population used for these data.

On 11 dates between and including 9 May and 5 September between 2-6 clams were chosen for histology from each location and tidal height. Animals (30-90 mm SL) were fixed in 10% buffered formalin for two weeks prior to removing the animal from its shell and then dissecting a portion of the visceral mass adjacent to the foot containing a portion of the gonad. Individual gonad tissue samples were placed in an embedding cassette before being sent to the Animal Diagnostic Laboratory at the University of New Hampshire where they were transferred to alcohol, then xylene, and then embedded in paraffin. Once firm, the paraffin with gonadal tissue was cut into 7µm ribbons using a microtome. Ribbons from a single individual were placed on a heated (42ºC) slide to melt the paraffin and isolate the tissue for staining (Mason's Trichrome) to reveal the general gonadal condition and developmental stage of the clam. Individuals were ranked as belonging to one of 10 gonadal stages (Table 4). This method also allowed us to determine if sex ratios were different from 1:1.

On all sampling dates (n = 18), between 2-6 clams were selected from each tidal height and location to determine condition index (dry tissue mass/length[cm]) and gonad index (dry gonad tissue mass/[dry gonad tissue mass + dry other tissue] x 100%).

Significant Results

Size-frequency measurements of commercial-size soft-shell clams

Approximately 2/3's of clams from eastern Maine were < 2.75-inches (< 69.9 mm) in shell length (Table 2; Fig. 3) as opposed to clams from southern Maine where only ~45% of clams were smaller than 2.75 inches (Table 3; Fig. 4; G = 146.52, df = 8, $P < 0.0001$). In eastern Maine, 4.27 ± 1.79% of clams (n = 10 sampling dates) were greater than 3.5 inches in shell length compared to 3.91 ± 3.77% (n = 5 sampling dates). The mean difference of 0.36 ± 3.48% was not significantly different from zero ($t_{obs} = 0.22$, df = 13, P = 0.828).

Comparative Field Experiment to investigate soft-shell clam reproductive cycle and fecundity

Brunswick (*Seawater Temperatures, Spawning, Egg Counts, and Viability*)

Unexpectedly, seawater temperatures (Fig. 11) approached 10ºC (a temperature used by some to predict when spawning occurs in soft-shell clams that inhabit intertidal flats at latitudes north of Cape Cod, Massachusetts; see: Nelson [1928]; Battle [1932]; Sullivan [1948]; MacLean [2003]; Beal [pers. obs.]) as early as 12 April at all intertidal heights (Fig. 11B).

A pilot-scale study conducted in nearby Freeport (Harraseeket River) in 2020 from 10 May to 10 July demonstrated that clams (40-80 mm SL) spawned when seawater temperatures approached 10ºC (25 May), and ceased when temperatures were warmer than 14ºC (26 June; Fig. 12). This was not what we observed in 2023 in Brunswick (Fig. 13) where no spawning was detected in clams from the upper intertidal (Fig. 13A) until 28 August when high tide seawater temperatures were above 20ºC. Because seawater temperatures during high tide were at/above 10ºC prior to our first collection (9 May) one hypothesis is that clams at this site may have spawned at least twice – once, prior to 9 May, and at least once after this date depending on tidal height. In addition, the pattern of spawning for clams in boxes at the mid and lower tidal heights did not follow results from the pilot-scale study. Initially, clams from both the two lower tidal heights commenced spawning on 20 June (~ 20%) when seawater temperatures were approaching 15ºC. Highest spawning percentage observed at both the two lower tidal heights (35-58%) occurred on 8 August when seawater temperatures were ~20ºC (Fig. 13B-C).

Forty-four clams (of 2,736 initially seeded into the 38 boxes sampled between 9 May and 4 October 2023, or 1.61%) released gametes during spring/summer 2023. Sex ratio was not different from 1:1 (χ^2_{obs} = 0.82, df = 1, P = 0.366; ♂ = 56.8%, ♀ = 43.2%). Clams within an experimental unit apparently did not develop gametes at the same rate as there was no significant linear (r^2 = 0.019; P = 0.5639) or curvilinear (r^2 = 0.023; P = 0.5334) relationship between size of clam and number of eggs it released (Fig. 14). Egg diameter, measured microscopically for clams collected on six dates (20 June; 11 July; and 1, 8, 15, and 29 August), was found to be independent of clam size (Fig. 15). Mean diameter ± 95% CI was 70.17 ± 1.41 µm (n = 18).

Egg viability was observed on three sampling dates (20 June; 1 and 8 August) across 17 mating pairs (Table 5). Mean viability was 51.03%. Viability appeared to decrease with increasing size of females as opposed to males where viability appeared to increase with increasing size.

Brunswick (*Gonad-Somatic Index*)

Gonad-Somatic Index (GSI) was found to be size dependent (Fig. 16). Adjusting the GSI equation to control for the effect due to size follows:

$$GSI^* = \left[\left(\frac{dry\ gonad\ mass\ (G)}{G + dry\ other\ tissue\ mass}\right)/\text{Shell length (mm)}\right] \times 100\%.$$

The equation produced an index that was independent of clam size (Fig. 17). A two-way ANOVA demonstrated significant variability in GSI* across sampling dates (P = 0.005; Fig. 18) and tidal heights (P < 0.0001; Fig. 19). Mean GSI decreased significantly over time (r = -0.868, P < 0.0001, n = 19), and increased by 12% from upper to lower intertidal. To determine if experimental clams housed within the boxes (experimental units) were behaving differently than those in ambient sediments, clams were collected from experimental units and ambient sediments from the upper and mid intertidal on four occasions (30 May; 14 June; 5 September; and, 3 October) when GSI* data was also recorded (Fig. 20). (No ambient clams were observed at the low tide mark). No effect of tidal height on mean GSI* was observed; however, ambient clams had significantly higher mean GSI* for each of the first three sampling dates than those in the wooden boxes. No significant tidal height effect was observed on 3 October.

Brunswick (*Condition Index*)

Body condition index (CI) is a physiological concept used to evaluate bivalve health, growth, meat yield, etc., and can be used to infer the presence or absence of environmental stresses (Lucas and Beninger 1985; Cataldo et al. 2001; Filgueira et al. 2013; Zeng and Yang 2020). Typically, condition index is a ratio of body mass to body size; however, 19 different equations have been used to calculate the ratio, and there is no agreed standard across or even within disciplines (Zeng and Yang 2020). To compare CI between dates or tidal heights, CI must be independent of size; however, for the data collected from Brunswick (May-October 2023), CI (Condition Index = Dry Body Mass [g]/Shell Length [cm]) was size dependent (Fig. 21). Nakagawa et al. (2017) recommended the following equation to account for allometry and control for the effect of size on dry body mass:

Equation 1: $\hat{Y}_{i\ Ln[dry\ tissue\ mass]} = b_0 + b_1X_{i[Group]} + b_2X_{i[Ln(Size)]} + e_i$; where:

Group = A fixed factor such as geographic location, tidal height, or sampling date;

Size = Shell length (cm);
e_i = residual (difference between Y_{obs} and $\hat{Y}$).

A third (interaction) term was added to the equation so that a test for equality of regression slopes could proceed. The new equation (2) is as follows:

Equation 2: $\hat{Y}_{i\ Ln[dry\ tissue\ mass]} = b_0 + b_1 X_{i[Group]} + b_2 X_{i[Ln(Size)]} + b_3 X_{i[Group\ x\ Ln(Size)]}$

This allows for a test of the null hypothesis Ho: $\beta_3 = 0$. If the null hypothesis is rejected, then the relationship between dry tissue mass and ln-transformed shell length differs between the groups. That is, suppose groups referred to the three tidal heights in the present study (upper; mid; lower). If the null hypothesis is rejected, it would mean that the dry mass vs. shell length relationship differs across the three tidal heights. If the null hypothesis is not rejected, then the slopes of the group variable are considered statistically equivalent, and an analysis of covariance (ANCOVA) can proceed. In this case, the slopes of the three lines (tidal heights) were all parallel. ANCOVA tests whether the least-square means between group(s) are equal. A common shell length (65.7 mm) was used in the analyses to estimate the least-square means so that comparisons were independent of size. In addition, there was no difference in slopes between sampling dates.

The results (Fig. 22) suggest that dry tissue mass decreased significantly through time (a least-squares regression line had an $r^2 = 0.592$ $P < .0001$, $\hat{Y} = 0.75 - 0.0193X$, $n = 19$; ANOVA: $F_{obs} = 4.50$, $df = 18, 280$, $P < 0.0001$). In addition, the effect due to tidal height as demonstrated by their least-square means (Fig. 23) shows increasing values with decreasing tidal height. That is, animals at the mid and low tidal mark apparently encountered less environmental or other stressors than those at the upper intertidal.

On four occasions (30 May; 14 June; 5 September; and, 3 October), size-adjusted dry tissue mass was compared for clams collected from experimental units vs. those collected outside the units in the ambient sediments. No ambient clams were collected from the low intertidal due to low natural densities at this tidal height. Significant differences in least-square means was observed for the first two collections ($P < 0.0001$) when values were higher for clams sampled from ambient sediments; however, no statistical differences were observed between least-square means from the last two collections ($P > 0.0703$; Fig. 24).

Bremen (*Seawater Temperatures, Spawning, Egg Counts, and Viability*)

Seawater temperatures in Bremen reached 10ºC as early as 14 April 2023 according to the temperature recorder that was placed within an upper intertidal experimental unit (Fig. 25). The first collection of clams from units occurred on 8 May 2023, so it may be possible that, as with the Brunswick site, clams may have spawned prior to the first collection occurred.

Of 2,592 experimental clams established in the boxes across 18 sampling dates, 67 individuals (or 2.58%) were induced to release gametes. The sex ratio (M:F, 34:33) was not significantly different from 1:1 ($df = 1$, $G = 0.015$, $P = 0.903$). The first spawning occurred on 19 June (2 females from the mid intertidal) when average seawater temperatures were ~15ºC (Fig. 26B).

Except for results on 4 September when ~38% of all live clams spawned in the upper intertidal boxes, the percent spawning through time was similar between clams in the upper and mid intertidal boxes (Fig. 26A & B). Clams held in low tide boxes first spawned on 24 July (15.2%), and only two other spawning events occurred at that tidal height (3.6% and 5.6%) on 7 and 14 August, respectively. No significant linear ($r^2 = 0.0379$; P = 0.2774) or curvilinear ($r^2 = 0.0277$; P = 0.3549) relationship existed between size of clam and number of eggs it released (Fig. 27). Egg diameter, measured microscopically for clams collected on six dates (26 June; 3 & 24 July; 7 & 28 29 August; and 4 September), was found to be independent of clam size (Fig. 28). Mean diameter ± 95% CI was 71.54 ± 1.09 µm (n = 33).

Egg viability was observed on five sampling dates (26 June; 24 July; 7 & 28 August; and, 4 September) across 44 mating pairs (Table 6). Mean viability was 57.5%. No pairing occurred with small (< 50 mm SL) females, as none was observed to spawn. Viability appeared to decrease among medium size males compared with large and small males. No large differences in viability were observed between medium and large females.

Bremen (*Gonad-Somatic Index*)

Gonad-Somatic Index (GSI) was found to be size dependent (Fig. 29A); however, adjusting the GSI equation as explained above did not produce an index that was independent of clam size (Fig. 29B). In an attempt to resolve this statistical problem, a regression approach (Nakagawa et al. 2017) was used instead where dry gonad weight was adjusted for clam size; unfortunately, tests of model assumptions (equal slopes) demonstrated that violations existed. Hence, we took a different approach that allowed us to examine how GSI* varied across sampling dates and tidal height for discrete size classes of clams (I: $\leq$ 50 mm; II: 50-59.9 mm; III; 60-69.9 mm; IV: $\geq$ 70 mm). First, for each size class, no significant linear relationship between shell length and GSI* was detected (P > 0.081). Clams in all size classes demonstrated a significant sampling date effect (P < 0.027), but no tidal height effect (P > 0.084; Figure 30).

Bremen (*Condition Index*)

Condition index was size dependent (Fig. 31); therefore, Equation 2 (Nakagawa et al. 2017) was used (as described above). ANCOVA tested whether the least-square means differed between sampling dates and tidal heights for a common shell length (65.3 mm) so that comparisons were independent of size. Results (Fig. 32) suggest that dry tissue mass decreased significantly through time (a least-squares regression line had an $r^2 = 0.790$, P < .0001, $\hat{Y} = 0.60 - 0.0258X$, n = 18; ANOVA: $F_{obs} = 6.01$, df = 17, 293, P < 0.0001). Tidal height did not play a significant role in dry tissue mass ($F_{obs} = 0.32$, df = 2, 293, P = 0.724).

Jonesport (*Seawater Temperatures, Spawning, Egg Counts, and Viability*)

Seawater temperatures at Flake Point Bar did not vary widely between tidal heights (Fig. 33A). In fact, the date when average daily temperatures at high tide first reached 10ºC was identical at the mid and low intertidal (29 April) as was the last day when average daily temperature at high tide was 10ºC (27 May; Fig. 33B; Fig. 34A). Conditions were similar at the upper intertidal where the first time the average daily temperature at high tide first reached 10ºC was 28 April and the last day when the average temperature at high tide was 10ºC was 27 May (Fig. 34B).

On 16 July, a HOBO Pendant temperature recorder was deployed adjacent to the box at the low intertidal that contained another HOBO temperature recorder that had been deployed on 17 March. The test was carried out to determine if thermal conditions within the box were similar to those outside the box. During the period from 16 July to 14 September 2023 there were 117 high tides. For each temperature recorder, we compared the average of three recordings taken 1 hour before, 1 hour after, and during high tide. That is, for each high tide, we calculated the difference in average temperature around the high tide between the seawater temperature recorded inside vs. outside the box (i.e., Inside average temperature – Outside average temperature = x). If no statistical difference were observed in average temperatures between inside and outside the box, we would expect about as many of the differences to be above zero as those to be below zero. Instead, we saw for a majority of the differences values greater than zero (Fig, 35) that suggested the thermal climate within the boxes was different than that of the overlying seawater. The average difference between inside vs. outside the box was 0.24 ± 0.03ºC (n = 117). Because the data were not normal (Shapiro-Wilk; W = 0.96, P = 0.001), a Sign Test was used to examine whether the median difference in seawater temperature between the inside vs. outside of the low intertidal box was equal to zero. It was not (M = 53.5, P < 0.0001).

Seventy experimental clams (or 2.70%) were induced to spawn on seven dates beginning on 24 June and ending on 12 August (Fig. 36). The sex ratio (M:F, 34:36) was not significantly different from 1:1 (df = 1, G = 0.057, P = 0.811). Experimental clams from all three tidal heights spawned on 24 June when average weekly seawater temperatures ranged between 12.4-13.0ºC (Fig. 36). No spawning was observed in experimental clams after 12 August when seawater temperatures were ~15ºC. Tidal height did not play a significant role in explaining percent spawning (P = 0.7458). The 2023 data from Jonesport did not conform with results obtained from the pilot study conducted at the same flat in 2020. That study showed spawning commenced in early June when seawater temperatures approached 10ºC and ceased in early August when temperatures exceeded 13ºC (Fig. 37). As we did at the Brunswick site, we decided to collect clams from the ambient flat adjacent to the boxes containing the experimental clams on six dates (6/3; 6/17; 6/24; 7/15; 7/29 & 8/12; Table 7) to compare percent spawning as well as GSI and condition indices between experimental (boxed) vs. control (unboxed) animals. Across the six sampling dates, 23 control clams of 314 collected (7.32%) were induced to spawn (M:F, 13:10, G = 0.391, P = 0.532). The control clams spawned a week earlier than experimental clams (i.e., 17 June vs. 24 June); however, the majority of clams that spawned (13 of 14 individuals) were collected from the upper intertidal (Fig. 38). The other clam that spawned was from the lower intertidal. Control clams did not spawn on the last collection date (8/12), and this was similar to experimental clams from the lower intertidal (Fig. 36C; Table 7). No significant linear (r^2 = 0.080; P = 0.0565) or curvilinear (r^2 = 0.064; P = 0.0923) relationship existed between size of clam and number of eggs it released (Fig. 39). Egg diameter, measured microscopically for clams collected on six dates (24 June; 17, 22 & 29 July; and, 5 August), was independent of clam size (Fig. 40). Mean diameter ± 95% CI was 74.00 ± 0.90 µm (n = 33).

Egg viability was observed on four sampling dates (24 June; 15 and 29 July; and, 12 August) across 56 mating pairs (Table 8). Mean viability was 58.04%. Except for small females, of which there were only two, viability appeared to be independent of clam size.

Jonesport (*Gonad-Somatic Index*)

Both GSI indices (GSI & GSI*) were found to be size dependent (Fig. 41). Therefore, we examined GSI across five size classes (I: < 50 mm, n = 56; II: 50-59.9 mm, n = 49; III: 60-69.9 mm, n = 64; IV: 70-79.9 mm, n = 68; and, V: ≥ 80 mm, n = 68). For each size class, GSI was independent of shell length (P > 0.0911), and a two-way ANOVA was used to test whether mean GSI varied by date, tidal height, or the interaction of those two main factors. No significant interaction (sampling date × tidal height) occurred for GSI data associated with clams from any size class (P > 0.1404; Table 9). Significant differences in mean GSI were observed across sampling dates for clams in size classes I and IV (Table 9; Fig. 42). For the smallest clams, there was no significant linear ($r^2 = 0.181$; P = 0.079) or curvilinear ($r^2 = 0.191$; P = 0.7232) relationship between mean GSI and sampling date. Similar results occurred for clams from size class IV ($r^2_{lin} = 0.175$; P = 0.084; $r^2_{curv} = 0.421$; P = 0.199). The mean GSI for size class II clams varied significantly with tidal height (Table 9), with a 19.1% difference in means between the upper and mid intertidal (Fig. 43). A lack-of-fit test on a composite of mean GSI for clams within each size class (Fig. 44) demonstrated a curvilinear relationship (i.e., significantly greater mean GSI with increasing size).

Clams were sampled both from experimental units and ambient sediments from each tidal height on seven occasions (3, 17 & 24 June; 15 & 29 July; 12 August; and, 5 September). GSI of these clams (n = 132) increased with increasing size ($r^2 = 0.200$; F = 32.28; df = 1, 130, P < 0.0001). When the data was sorted into size classes (as described above), each class except for the largest (V) contained at least 13 individuals. To compare mean GSI within a given size class between experimental and ambient individuals, we used the following linear model:

$Y_{ijkl} = \mu + A_i + B_j + AB_{ij} + C_k + AC_{ik} + BC_{jk} + ABC_{ijk} + e_{l(ijk)}$, where:

Y_{ijkl} = dependent variable (mean GSI);
A_i = Clam Origin (a = 2; factor is fixed; Experimental vs. Ambient);
B_j = Sampling Date (b = 7; factor is fixed);
C_k = Tidal Height (c = 3; factor is fixed; Upper vs. Mid vs. Lower); and,
e_l = Experimental error.

The mean GSI of clams in the smallest size classes (I: < 50 mm SL) varied significantly across all three main factors, but also with two interaction sources of variation (Table 10; Fig. 45). One significant interaction was Origin × Sampling Date (P = 0.003). On each sampling date, mean GSI was greater for control than experimental clams (P = 0.016, Sign Test). In addition, mean SGI decreased linearly with sampling date for experimental clams (F = 9.14, df = 1, 5, P = 0.029; $r^2 = 0.646$), but not for control clams (F = 0.39, df = 1, 5, P = 0.560; $r^2 = 0.072$); Fig. 45A). The second significant interaction involved Origin and Tidal Height (P = 0.017). Mean GSI varied significantly along the tidal gradient; however, the pattern varied differently depending on clam origin (Table 10; Fig. 45B). Mean GSI was greater in control vs. experimental clams across each tidal height, but it did not vary significantly across the tidal gradient for experimental clams, whereas mean GSI in control clams was similar between animals sampled from the upper and lower intertidal, but significantly lower (~40%) than clams from the mid intertidal zone.

Mean GSI in clams ranging in size between 50.0-59.9 mm SL (size class II) varied significantly by origin ($P < 0.0001$), but the pattern between experimental and control clams differed across tidal height ($P = 0.0025$; Table 10; Fig. 46). Clams collected from the mudflat (controls) demonstrated an increasing mean GSI with decreasing tidal height. The opposite was true for experimental clams (Fig. 46). No differences due to origin, sampling date, or tidal height were observed for clams greater than 60 mm (Table 10).

Jonesport (*Condition Index*)

Condition Index (CI) was not independent of size (Fig. 47). A lack-of-fit test showed that the inclusion of a quadratic term to a linear model explained significantly more of the variation in CI than the linear model ($F = 19.21$, df = 1, 306, $P < 0.0001$). In addition, the inclusion of a cubic term to a model already containing both linear and quadratic terms did not explain any more significant variation ($F = 0.449$, df = 1, 305, $P = 0.4485$). Therefore, a quadratic model explained the most variation in the dependent variable: $\hat{Y} = 0.042 - 0.0023X + 0.0000815X^2$, $r^2 = 0.7348$. Therefore, we used Equation 2 (see above) to complete the analysis whether sampling date or tidal height had an effect on dry tissue mass. The lines relating the log-transformed tissue mass and shell length were parallel ($P > 0.064$), and least-square means were used to determine how dry tissue mass varied over sampling date (Fig. 48). Clams lost significant dry tissue mass through time ($r^2 = 0.68$; $F = 34.34$, df = 1, 16, $P < 0.0001$). No significant effect due to tidal height was observed ($F = 1.54$, df = 2, 288, $P = 0.2169$).

On seven dates (see above), size-adjusted dry tissue mass was compared for clams collected from experimental units vs. those collected outside the units in the ambient sediments at all three tidal heights. We asked if the relationship between ln dry tissue mass and ln SL for each of the five size classes referred to above varied by clam origin (experimental clams in boxes at each tidal height vs. ambient [control] clams dug on each sampling date in the sediments adjacent to the boxes at each tidal height). We chose this method (separating the data by size class) because there were too many instances where interactions were significant when all the data was examined together. Therefore, pooling the data across tidal heights and sampling dates, we examined for each size class whether the two lines (one for each origin) had different slopes. Both lines for each size class had similar slopes ($P > 0.629$). For each of the smallest size classes (i.e., clams < 69.9 mm SL) a significant difference in the least-square means from ANCOVA was observed. In each instance, control clams had significantly higher dry tissue mass than the experimental clams by 37-42%. No differences in least-square means was observed for clams $\geq$ 70 mm SL.

Histology

We have initiated a review of the 736 slides that were prepared to examine gametogenesis in both male and female clams (39-95 mm SL) from each tidal height and site (Table 11). These data will be presented in the next report.

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Table 1. Dates when commercial-size soft-shell clams were measured from the commercial fishery at either A.C. Inc. (Town of Beals – eastern Maine) or Cantrell's Seafood (Topsham – southern Maine). On each date, nine unprocessed bushels were sampled separately by dumping all clams from a 50 lb bushel on a table. Twenty-five clams were chosen randomly and then the greatest anterior-posterior length (shell length) was measured to the nearest 0.1 mm using Vernier calipers.

Date	A.C. Inc.	Cantrell's Seafood
09/14/2022	×	
10/12/2022	×	
11/09/2022	×	
01/26/2023	×	
02/22/2023		×
03/24/2023	×	×
05/23/2023		×
07/06/2023		×
08/04/2023	×	×
09/01/2023	×	
10/30/2023	×	
01/17/2024	×	
02/15/2024	×	

Table 2. Cumulative statistics for ten monthly samplings of the commercial catch at A.C. Inc., a shellfish buying and processing station located in the eastern Maine town of Beals. Sampling occurred irregularly and during astronomically high tides from September 2022 to February 2024. On each sampling event, nine bushels of unprocessed, recently dug clams were poured individually onto a table where the clams were mixed by hand followed by a random sample of 25 individuals that were measured (shell length to the nearest 0.1 mm using Vernier calipers). Legal size is ≥ 2-inches. No upper size limit exists.

size (inches)	Frequency	Percent	Cumulative Frequency	Cumulative Percent
<2	6	0.24	6	0.27
2.00-2.24	153	6.18	159	6.42
2.25-2.49	526	21.25	685	27.68
2.50-2.74	632	25.54	1,317	53.21
2.75-2.99	534	21.58	1,851	74.79
3.00-3.24	323	13.05	2,174	87.84
3.25-3.49	180	7.27	2,354	95.11
3.50-3.74	81	3.27	2,435	98.38
3.75-3.99	28	1.13	2,463	99.52
4.00-4.24	11	0.44	2,474	99.96
>4.25	1	0.04	2,250	100.00

Table 3. Cumulative statistics for five monthly samplings of the commercial catch at Cantrell's Seafood., a shellfish buying and processing station located in the southern Maine town of Topsham. Sampling occurred irregularly and during astronomically high tides from February to August 2024. On each sampling event, six or nine bushels of unprocessed, recently dug clams were poured individually onto a table where the clams were mixed by hand followed by a random sample of 25 individuals that were measured (shell length to the nearest 0.1 mm using Vernier calipers). Legal size is ≥ 2-inches. No upper size limit exists.

size (inches)	Frequency	Percent	Cumulative Frequency	Cumulative Percent
<2	2	0.19	2	0.19
2.00-2.24	46	4.38	48	4.57
2.25-2.49	169	16.10	217	20.67
2.50-2.74	270	25.71	487	46.38
2.75-2.99	252	24.00	739	70.38
3.00-3.24	181	17.24	920	87.62
3.25-3.49	87	8.29	1007	95.90
3.50-3.74	36	3.43	1043	99.33
3.75-3.99	7	0.67	1050	100.00
4.00-4.24	0	0.00	1050	100.00
>4.25	0	0.00	1050	100.00

Table 4. A summary of gonad conditions detected in processed animals is presented below. Clams are ranked as belonging to one of the following 10 gonadal stages (modified from Kim and Powell, 2004):

I: Inactive (no gonadal activity, resting)

G1: Gametogenic I stage; gametogenesis has begun; no ripe gametes visible

G2: Gametogenic II stage; first ripe gametes appeared; gonad developed to about one-third of its final size.

D1: Developing I stage; Gonad increased in mass to about half the full ripe condition; each follicle contains about equal proportions of ripe and developing gametes.

D2: Developing II stage; Gametogenesis still progressing; follicles mainly contain ripe gametes.

R: Ripe stage; Gonad fully ripe, early stages of gametogenesis rare; follicles distended with ripe gametes; ova compacted into polygonal configurations; sperm with visible tails.

S1: Spawning stage I; Active emission of gametes has begun; gamete density reduced.

S2: Spawning stage II; Gonad about half empty.

S3: Spawning stage III; Gonadal area reduced; follicles about one-third full of ripe gametes.

S4: Spawning stage IV; Only residual gametes remain; some may be undergoing cytolysis.

Table 5. Percent viability of eggs from 17 male:female pairings from clams from Brunswick that were induced to spawn on 20 June and 1 & 8 August 2023. Clam sizes (shell lengths, SL) were divided into three groups: Small = < 50 mm; Medium = 50-70 mm; Large = > 70 mm. X = no data. Marginal totals are means from respective rows and column.

	FEMALES			
MALES	SMALL	MEDIUM	LARGE	**TOTALS**
SMALL	X	60	20; 37.5	**39.17%**
MEDIUM	80; 20; 80	0; 30; 70; 60; 80	30	**50.00%**
LARGE	40; 70; 100	10; 80	X	**60.00%**
TOTALS	**51.67%**	**48.75%**	**29.17%**	**51.03%**

Table 6. Percent viability of eggs from 44 male:female pairings from clams from Keene Narrows Flat, Bremen that were induced to spawn on five occasions: 26 June; 24 July; 7 & 28 August; and, 4 September 2023. Clam sizes (shell lengths, SL) were divided into three groups: Small = < 50 mm; Medium = 50-70 mm; Large = > 70 mm. X = no data. Marginal totals are means from respective rows and column.

	FEMALES			
MALES	SMALL	MEDIUM	LARGE	**TOTALS**
SMALL	X	60; 50; 50	80; 50; 80; 100	**67.14%**
MEDIUM	X	50; 50; 0; 50; 0; 40; 20; 60; 60; 80; 90	70; 60; 0; 30; 70; 30 20; 50; 70; 100; 90	**49.55%**
LARGE	X	60; 50; 90; 70; 100; 80	60; 40; 50; 80; 0 80; 40; 70; 100	**64.67%**
TOTALS	**X**	**55.50%**	**59.17%**	**57.50%**

Table 7. Number of live soft-shell clams collected from two origins at Flake Point Bar, Jonesport, Maine – experimental boxes (**E**) and the ambient, control, flat (**C**) – at each tidal height (**U**pper, **M**id, **L**ower) during 2023 along with the number and percent that were induced to spawn. 24 experimental clams were established in each box at the beginning of the study, and the number of live clams reflects sampling from n = 2 boxes at each tidal height. Extra clams were collected on six dates (highlighted in red and boldfaced).

Date	Tidal Height	Origin	Live Clams	Number Spawned	Percent Spawned
5_6	U	E	48	0	0.00
	M	E	44	0	0.00
	L	E	41	0	0.00
5_13	U	E	44	0	0.00
	M	E	49	0	0.00
	L	E	44	0	0.00
5_20	U	E	48	0	0.00
	M	E	44	0	0.00
	L	E	41	0	0.00
5_27	U	E	46	0	0.00
	M	E	45	0	0.00
	L	E	43	0	0.00
6_3	U	E	41	0	0.00
	M	E	43	0	0.00
	L	E	47	0	0.00
6_3	U	C	23	0	0.00
	M	C	25	0	0.00
	L	C	21	0	0.00
6_10	U	E	44	0	0.00
	M	E	41	0	0.00
	L	E	38	0	0.00
6_17	U	E	42	0	0.00
	M	E	43	0	0.00
	L	E	45	0	0.00
6_17	U	E	24	13	54.17
	M	E	24	0	0.00
	L	E	19	1	5.26

Table 7 (cont.)

Date	Tidal Height	Origin	Live Clams	Number Spawned	Percent Spawned
6_24	U	E	43	2	4.65
	M	E	41	1	2.44
	L	E	42	7	16.67
6_24	U	E	37	0	0.00
	M	E	29	0	0.00
	L	E	39	0	0.00
7_1	U	E	46	1	2.17
	M	E	40	0	0.00
	L	E	42	0	0.00
7_8	U	E	44	0	0.00
	M	E	43	0	0.00
	L	E	40	0	0.00
7_15	U	E	42	0	0.00
	M	E	31	0	0.00
	L	E	42	8	19.05
7_15	U	E	23	2	8.70
	M	E	23	2	8.70
	L	E	25	1	4.00
7_22	U	E	44	5	11.36
	M	E	32	6	18.75
	L	E	40	5	12.50
7_29	U	E	38	9	23.68
	M	E	43	0	0.00
	L	E	43	6	13.95
7_29	U	E	23	1	4.35
	M	E	22	1	4.55
	L	E	24	2	8.33
8_5	U	E	40	6	15.00
	M	E	36	7	19.44
	L	E	44	4	9.09

Table 7 (cont.)

Date	Tidal Height	Origin	Live Clams	Number Spawned	Percent Spawned
8_12	U	E	41	2	4.88
	M	E	36	1	2.78
	L	E	38	0	0.00
8_12	U	E	23	0	0.00
	M	E	23	0	0.00
	L	E	24	0	0.00
8_19	U	E	17	0	0.00
	M	E	41	0	0.00
	L	E	13	0	0.00
8_26	U	E	44	0	0.00
	M	E	36	0	0.00
	L	E	41	0	0.00
9_2	U	E	21	0	0.00
	M	E	37	0	0.00
	L	E	31	0	0.00

Table 8. Percent viability of eggs from 56 male:female pairings from clams from Flake Point Bar, Jonesport, Maine that were induced to spawn on five occasions (number of pairings in parentheses): 24 June (n = 10); 15 July (n = 20); 29 July (n = 25); and, 12 August (n = 1). Clam sizes (shell lengths, SL) were divided into three groups: Small = < 50 mm; Medium = 50-70 mm; Large = > 70 mm. X = no data. Marginal totals are means from respective rows and column.

	FEMALES			
MALES	SMALL	MEDIUM	LARGE	**TOTALS**
SMALL	X	40; 10; 100	80; 50; 80; 60	**60.00%**
MEDIUM	100	70; 80; 20; 70; 0; 70; 80; 50; 60; 80; 80; 40	90; 80; 80; 40; 40; 10 10; 80; 90; 50; 90; 70; 10	**59.23%**
LARGE	100	70; 0; 20; 50; 80; 20; 80; 70; 40; 50; 60; 90	60; 50; 50; 10; 20 100; 100; 80; 60; 30	**56.09%**
TOTALS	**100.0%**	**54.81%**	**58.15%**	**58.04%**

Table 9. ANOVA results for the interactive effects of sampling date and tidal height on mean Gonad-Somatic Index for experimental soft-shell clams in each of five shell length classes (I: < 50 mm; II: 50.0-59.9 mm; III: 60.0-69.9 mm; IV: 70.0-79.9 mm; V: ≥ 80 mm) from Flake Point Bar, Jonesport, Maine (6 May to 2 September 2023). Because replication was not consistent between sampling dates, the data set was unbalanced. Therefore Type III sums of squares were used throughout the analyses. All factors are fixed. Significant P-values are bold-faced.

SIZE CLASS	Source of Variation	df	MS	F	Pr > F
I	Sampling Date	17	6.171	3.12	**0.0216**
	Tidal Height	2	6.175	3.12	0.0783
	Date × Tidal Height	21	54.013	2.70	0.2866
II	Sampling Date	17	11.941	2.65	0.0967
	Tidal Height	2	32.585	7.24	**0.0197**
	Date × Tidal Height	21	10.019	2.23	0.1404
III	Sampling Date	17	41.049	1.77	0.1202
	Tidal Height	2	0.449	0.02	0.9808
	Date × Tidal Height	26	30.599	1.32	0.2756
IV	Sampling Date	17	127.913	2.42	**0.0306**
	Tidal Height	2	46.408	0.88	0.4313
	Date × Tidal Height	28	42.720	0.81	0.7042
V	Sampling Date	17	131.000	1.03	0.4723
	Tidal Height	2	123.424	0.97	0.3969
	Date × Tidal Height	27	144.387	1.13	0.3914

Table 10. ANOVA results on the interactive effects of clam origin (experimental clams in wooden boxes vs. ambient clams in the flat adjacent to the boxes), sampling date (7 dates from 3 June to 2 September 2023), and tidal height (upper, mid, lower) at Flake Point Bar, Jonesport, Maine for clams in four size (shell length) classes (I: < 50 mm; II: 50.0-59.9 mm; III: 60.0-69.9 mm; IV: 70.0-79.9 mm). An additional size class (> 80 mm SL) contained too few ambient clams (n = 3) to warrant a meaningful comparison. Because replication was not consistent between sampling dates, the data set was unbalanced. Therefore Type III sums of squares were used throughout the analyses. All factors are fixed. Significant P-values are bold-faced.

SIZE CLASS	Source of Variation	df	MS	F	Pr>F
I	Origin	1	98.469	57.71	**<.0001**
	Sampling Date	6	15.195	8.91	**0.0002**
	Origin × Date	6	9.185	5.38	**0.0033**
	Tidal Height	2	13.991	8.20	**0.0035**
	Origin × Tide Height	2	9.119	5.34	**0.0167**
	Date × Tide Height	12	3.879	2.27	0.0632
	Origin × Date × Tide	12	5.381	3.15	0.0701
II	Origin	1	576.561	53.59	**<.0001**
	Sampling Date	6	7.702	0.72	0.6387
	Origin × Date	6	16.828	1.56	0.1793
	Tidal Height	2	15.234	1.42	0.2531
	Origin × Tide Height	2	73.699	6.85	**0.0025**
	Date × Tide Height	12	13.911	1.29	0.2551
	Origin × Date × Tide	12	9.371	0.87	0.5079
III	Origin	1	62.466	2.39	0.1364
	Sampling Date	6	58.578	2.24	0.0772
	Origin × Date	6	46.365	1.77	0.1511
	Tidal Height	2	30.732	1.18	0.3272
	Origin × Tide Height	2	28.919	1.11	0.3484
	Date × Tide Height	12	23.827	0.91	0.5456
	Origin × Date × Tide	12	48.784	1.87	0.1414
IV	Origin	1	35.669	0.52	0.4897
	Sampling Date	6	150.829	2.19	0.1393
	Origin × Date	6	19.547	0.28	0.9302
	Tidal Height	2	22.493	0.33	0.7292
	Origin × Tide Height	2	5.378	0.08	0.7861
	Date × Tide Height	12	26.986	0.39	0.9333
	Origin × Date × Tide	12	27.648	0.40	0.5418

Table 11. Number of soft-shell clams dissected to provide details on male and female gametogenesis from each site, sampling date, and tidal height. (n_{exp} = experimental clams; n_{cnt} = control clams; - = no sample taken due to the lack of wild clams at that tidal height; X = no sample taken)

Week	Dates	Town	Tidal Height	n_{exp}	n_{cnt}
0	3/5-3/17	Jonesport	N/A	7	
		Bremen	N/A	7	
		Brunswick	N/A	7	
I	5/6-5/9	Jonesport	Upper	6	X
			Mid	6	X
			Low	6	X
		Bremen	Upper	6	X
			Mid	6	X
			Lower	6	X
		Brunswick	Upper	6	X
			Mid	6	X
			Lower	6	X
II	5/13-5/16	Jonesport	Upper	6	X
			Mid	6	X
			Low	6	X
		Bremen	Upper	6	X
			Mid	6	X
			Lower	6	X
		Brunswick	Upper	6	X
			Mid	6	X
			Lower	6	X
IV	5/27-5/30	Jonesport	Upper	6	X
			Mid	6	X
			Low	6	X
		Bremen	Upper	6	X
			Mid	6	X
			Lower	6	X
		Brunswick	Upper	6	5
			Mid	6	6
			Lower	6	-

Week	Dates	Town	Tidal Height	n_{exp}	n_{cnt}
V	6/3	Jonesport	Upper	X	6
			Mid	X	6
			Low	X	6
VI	6/14	Brunswick	Upper	X	6
			Mid	X	6
			Low	X	-
VII	6/17-6/20	Jonesport	Upper	6	5
			Mid	6	6
			Low	6	6
		Bremen	Upper	6	X
			Mid	6	X
			Lower	6	X
		Brunswick	Upper	6	X
			Mid	6	X
			Lower	6	X
VIII	6/24-6/27	Jonesport	Upper	6	6
			Mid	6	6
			Low	6	6
		Bremen	Upper	6	X
			Mid	6	X
			Low	6	X
		Brunswick	Upper	6	X
			Mid	6	X
			Low	X	-
X	7/8-7/11	Jonesport	Upper	6	X
			Mid	6	X
			Low	6	X
		Bremen	Upper	6	X
			Mid	6	X
			Low	6	X
		Brunswick	Upper	6	X
			Mid	6	X
			Low	6	X

Week	Dates	Town	Tidal Height	n_{exp}	n_{cnt}
XI	7/15-7/18	Jonesport	Upper	6	6
			Mid	6	6
			Low	6	6
		Bremen	Upper	6	X
			Mid	6	X
			Low	6	X
		Brunswick	Upper	6	X
			Mid	6	X
			Low	6	X
XIII	7/29-8/1	Jonesport	Upper	6	6
			Mid	6	6
			Low	6	6
		Bremen	Upper	6	X
			Mid	6	X
			Low	6	X
		Brunswick	Upper	6	X
			Mid	6	X
			Low	6	X
XVII	8/26-8/29	Jonesport	Upper	6	6
			Mid	6	6
			Low	6	6
		Bremen	Upper	6	X
			Mid	6	X
			Low	6	X
		Brunswick	Upper	4	X
			Mid	6	X
			Low	5	X
XVIII	9/2-9/4	Jonesport	Upper	6	6
			Mid	6	6
			Low	6	6
		Bremen	Upper	6	X
			Mid	6	X
			Low	6	X

Week	Dates	Town	Tidal Height	n_{exp}	n_{cnt}	
XVIII	9/5	Brunswick	Upper	X	6	
			Mid	5	6	
			Low	2	-	
XXII	10/3	Brunswick	Upper	12	6	
			Mid	12	6	
			Low	9	6	
			TOTAL	574	162	(736)

Figure Legends

Figure 1. Size-frequency distribution of commercial size soft-shell clams at A.C. Inc. buying station (Black Duck Cove Road, Beals, ME) from September 2022 to February 2024 (N = 10; n = 225). (x-axis bins are in inches.)

Figure 2. Cumulative size-frequency distribution of all commercial size soft-shell clams measured at A.C. Inc. buying station (Black Duck Cove Road, Beals, ME) from September 2022 to February 2024 (n = 2,250). (x-axis bins are in inches.)

Figure 3. Size-frequency distribution of commercial size soft-shell clams at Cantrell's Seafood buying station (Lewiston Road #1, Topsham, ME) from February to August 2023 (n = 150-225). (x-axis bins are in inches.)

Figure 4. Cumulative size-frequency distribution of all commercial size soft-shell clams measured at Cantrell's Seafood buying station (Lewiston Road #1, Topsham, ME) from February to August 2023 (n = 1,050). (x-axis bins are in inches.)

Figure 5. A) Experimental unit. 1-ft x 1-ft x 6-inch deep wooden box used to house 24 clams ranging from 30-90 mm SL and protective top. B) Wooden frame (1-inch thick) used to protect clams inside of box. C) Protective frame. The bottom of the frame (portion that is closest to the clams) is covered with a piece of 3.2 mm extruded polyethylene netting (Industrial Netting - https://www.industrialnetting.com/xb1130-black-polyethylene-mesh.html), and a piece of 12.7 mm vinyl-coated trap wire is affixed to the top.

Figure 6. A) Protective tops were screwed to the top of all boxes. B) A piece of PetScreen® was placed between the clams in the box and the protective top to discourage milky ribbon worms, *Cerebratulus lacteus*, from entering the boxes.

Figure 7. A schematic of the experimental units arrayed along the tidal gradient at one of the three study sites.

Figure 8. A) Clammer removing protective top and milky ribbon worm excluder from box to access experimental clams. B) The sediments directly under the excluder mesh. C) & D) Often, the largest live clams in a box would be exposed and resting on top of the sediments with siphons fully or partially extended.

Figure 9. A) Student intern adding warm (24ºC) seawater to clams from two boxes from a single tidal height. B) Intern waiting for clams to spawn. Each tank contains experimental clams (30-90 mm SL) from two boxes that are separated at each of three tidal heights from a study site.

Figure 10. Clams that spawned in the spawning tank were placed into fingerbowls where gametes (eggs) were contained for subsequent counts.

Figure 11. A) Average weekly seawater temperatures at high tide in experimental units at each of three tidal heights at Barnes Flat, Pennellville, Brunswick, Maine (March-September 2023).

The temperature recorder failed around 16 May 2023 at both the mid and low water tidal heights. The dashed line marks 10ºC. B) Daily high tide temperatures from the high (upper) intertidal at Barnes Flat from 5 March to 14 May 2023. The drawn box suggests a high tide temperature of 10ºC may have occurred at this site as early as 12-13 April. The line of best fit (solid black) and the 95% confidence interval around $\hat{Y}$ indicates that average seawater temperature on 22 April was 10ºC. The first sampling at this site (i.e., collection of clams from boxes at each tidal height) did not commence until 9 May 2023.

Figure 12. Pilot-scale study results from Freeport, Maine (2020). Results are combined from three tidal heights (hi, mid, low) as percent of individuals that spawned on a given day was independent of location along the tidal gradient. Spawning commenced on 26 May 2020, and, by 10 July 2020, less than 5% of individuals reacted positively to thermal shock spawning cues.

Figure 13. Relationship between seawater temperature and percent spawning for experimental soft-shell clams at Barnes Flat, Pennellville, Brunswick at the upper (A), mid (B), and lower (C) tidal height. The dashed line in each graph is the 10ºC line when the commencement of spawning has been noted from other studies north of Cape Cod, Massachusetts (Snelgrove et al. 2023).

Figure 14. Relationship between number of eggs released by female clams from Brunswick on six dates between 9 May and 5 September 2023. Data is pooled across sampling date and tidal height. No significant linear ($F = 0.35$, $df = 1, 17$, $r^2 = 0.199$, $P = 0.5639$) or curvilinear (both dependent and independent variables ln-transformed; $F = 0.40$, $df = 1, 17$, $r^2 = 0.023$, $P = 0.5334$) relationship was observed.

Figure 15. Relationship between egg diameter (µm) and shell length (mm) for n = 11 clams collected from Barnes Flat, Brunswick on 20 June, n = 2 clams on 11 July, n = 1 on 1 August, n = 2 on 8 August, n = 1 on 15 August, and n = 1 on 29 August. No linear relationship was discovered ($F = 0.40$, $df = 1, 16$, $r^2 = 0.024$, $P = 0.5359$). Blue, dashed line represents mean ± 95% CI = 70.2 ± 1.4 µm, n = 18. Symbols refer to individual eggs for a given size clam (n = 10 eggs/female for 17 individuals; n = 3 eggs for 1 individual; N = 173).

Figure 16. Relationship between gonad-somatic index (GSI) and shell length for soft-shell clams at Barnes Flat, Pennellville, Brunswick. $\hat{Y} = -1.072 + 0.249X$, $r^2 = 0.1612$, $df = 1, 299$, $F = 57.44$, $P < 0.0001$, $n = 301$.

Figure 17. Gonad-somatic index that is independent of clam size for soft-shell clams at Barnes Flat, Pennellville, Brunswick. $\hat{Y} = 20.15 + 0.045X$, $r^2 = 0.0027$, $df = 1, 299$, $F = 0.81$, $P = 0.3687$, $n = 301$.

Figure 18. Mean GSI* + 95% CI for data pooled across tidal heights for each sampling date for experimental clams at Barnes Flat, Pennellville, Brunswick. ANOVA showed significant differences in means across sampling dates ($P = 0.005$). Significant loss of GSI* occurred over time (a linear regression of GSI* on date [1 = 5_9 … 19 = 10_3] revealed a negative slope and relatively high coefficient of determination: $\hat{Y} = 43.36 – 0.383X$, $r^2 = 0.753$, $df = 1, 17$, $F = 51.82$, $P < 0.0001$). n = 5-18 clams/sampling date.

Figure 19. Mean GSI* + 95% CI for tidal height data pooled across sampling dates for experimental clams at Barnes Flat, Pennellville, Brunswick. ANOVA showed a significant tidal height effect on mean GSI* ($P < 0.0001$), with mean GSI* increasing significantly with decreasing tidal height. ($n = 92$-108).

Figure 20. Mean GSI* + 95% CI for experimental and ambient clams collected from Barnes Flat, Pennellville, Brunswick on 30 May, 14 June, 5 September and 3 October 2023 for clams from the upper and mid intertidal (pooled) comparing clams held within experimental units (wooden boxes – 1-ft x 1-ft x 6-inches deep) vs. clams collected from the mudflat (control = ambient). For each sampling date, there was no significant effect due to tidal height on mean GSI* ($P > 0.062$). For the first three sampling dates, mean GSI* was significantly greater for clams from ambient sediments vs. experimental units. No significant effect due to tidal height was observed on 3 October ($P = 0.064$). n = 3-11

Figure 21. Condition Index vs. Shell Length for experimental soft-shell clams collected between 9 May and 5 October 2023 from Barnes Flat, Brunswick, Maine. Condition Index = Dry Body Mass (g)/Shell Length (cm). The least-squares regression line ($\widehat{Y}$) is shown in red along with the 95% CI for $\widehat{Y}$ in dashed blue lines. $\widehat{Y} = -0.1587 + 0.00684$ ($r^2 = 0.56825$n = 301, F = 393.97, df = 1, 299, $P < 0.0001$).

Figure 22. Size-adjusted least-square means ± 95% CI for dry body weight pooled across tidal heights on each sampling date for Barnes Flat, Pennellville, Brunswick, Maine.

Figure 23. Size-adjusted least-square means ± 95% CI for dry body weight pooled across sampling dates for each of the three tidal heights for Barnes Flat, Pennellville, Brunswick, Maine.

Figure 24. Size-adjusted least-square means ± 95% CI for dry body weight on 30 May, 14 June, 5 September and 3 October 2023 for clams from the upper and mid intertidal (pooled) comparing clams held within experimental units (wooden boxes – 1-ft x 1-ft x 6-inches deep) vs. clams collected from the mudflat (control = ambient) at Barnes Flat, Pennellville, Brunswick, Maine. Significant differences ($P < 0.05$) in least-square means was observed for the May and June collection.

Figure 25. A) Average weekly seawater temperatures at high tide in experimental units at each of three tidal heights at Keene Narrows Flat, Bremen, Maine (March-September 2023). The temperature recorder failed around 26 July 2023 at the upper tidal height. The dashed line marks 10ºC. B) Daily high tide temperatures from the high (upper) intertidal at Keene Narrows Flat from 15 March to 14 May 2023. The drawn box suggests a high tide temperature of 10ºC may have occurred at this site as early as 14 April. The line of best fit (solid black) and the 95% confidence interval around $\widehat{Y}$ indicates that average seawater temperature on 3 May was 10ºC. The first sampling at this site (i.e., collection of clams from boxes at each tidal height) did not commence until 8 May 2023.

Figure 26. Average weekly seawater temperatures (red dots) and percent of live clams that were induced to spawn (blue dots connected by black lines) from boxes placed at the A) upper; B) mid; and, C) lower intertidal at Keene Narrows Flat, Bremen, Maine. The dashed line in each graph is the 10ºC line when the commencement of spawning has been noted from other studies north of Cape Cod, Massachusetts (Snelgrove et al. 2023).

Figure 27. Relationship between mean number of eggs and clam size for animals from intertidal boxes at Keene Narrows Flat, Bremen, Maine. No significant linear relationship was observed ($r^2 = 0.0379$; $P = 0.2774$). Nor was a significant curvilinear relationship detected ($r^2 = 0.0277$; $P = 0.3549$; $n = 33$).

Figure 28. Relationship between size of clam eggs (µm) and clam size (mm) from animals collected from intertidal boxes at Keene Narrow Flat, Bremen, Maine on 26 June, 3 & 24 July, 7 & 28 August, and, 4 September. Ten eggs were measured for each of 33 females using 4X magnification and an ocular micrometer. No linear relationship was observed ($r^2 = 0.054$, $P = 0.1928$). The dashed line represents the mean egg diameter (71.54 ± 1.09 µm).

Figure 29. A) GSI vs. shell length for clams pooled across sampling date and tidal height at Keene Narrows Flat, Bremen, Maine. B) GSI* vs. shell length. In both instances, the dependent variable varied significantly ($P < 0.0001$) with clam size ($n = 314$).

Figure 30. GSI* vs. sampling date for four sizes (I-IV) of soft-shell clams at Keene Narrows Flat, Bremen, Maine pooled across tidal height (because there was no significant difference in mean GSI* due to tidal height for any size class – $P > 0.084$). The P-value in each panel is associated with the null hypothesis that there is no significant relationship between GSI* and sampling date (coded as 1 = 5_8, 18 = 9_4) that was tested using regression analysis.

Figure 31. Condition Index vs. Shell Length for experimental soft-shell clams collected between 8 May and 4 September 2023 from Keene Narrows Flat, Bremen, Maine. Condition Index = Dry Body Mass (g)/Shell Length (cm). The least-squares regression line ($\hat{Y}$) is shown in red along with the 95% CI for $\hat{Y}$ in dashed blue lines. $\hat{Y} = -0.1649 + 0.00646$ ($r^2 = 0.5671$, $n = 314$, $F = 408.71$, $df = 1, 312$, $P < 0.0001$).

Figure 32. Size-adjusted least-square means ± 95% CI for dry body weight pooled across tidal heights on each sampling date for Keene Narrows Flat, Bremen, Maine.

Figure 33. A) Mean weekly seawater temperatures (ºC) around the high water (1 hr before; during; 1 hr after) at each of the three tidal heights at Flake Point Bar, Jonesport, Maine. B) Mean seawater temperatures on each high tide between 17 March and 6 September 2023 at the low intertidal at Flake Point Bar, Jonesport, Maine. The horizontal blue dashed line represents 10ºC. The first time average temperature at high tide reached 10ºC occurred on the evening tide of 29 April 2023. The last day when average temperature was 10ºC at high tide occurred on the early morning high tide of 27 May 2023. The red box highlights those dates when seawater temperatures at high tide varied between 8ºC and 12ºC, inclusive.

Figure 34. A) Mean seawater temperatures on each high tide between 17 March and 6 September 2023 at the mid intertidal at Flake Point Bar, Jonesport, Maine. All information pertaining to the blue dashed line and red box is exactly the same as described in the Figure Legend for Figure 33B. B) Mean seawater temperatures on each high tide between 17 March and 6 September at the upper intertidal at Flake Point Bar, Jonesport, Maine. The horizontal blue line represents 10ºC. The first time average temperature at high tide reached 10ºC occurred on the afternoon tide of 28 April 2023. The last day when average temperature was 10ºC at high tide occurred on the early morning high tide of 27 May 2023. The red box is as described in the Figure Legend for Fig. 33B.

Figure 35. Difference in mean seawater temperature at high tide inside experimental unit vs. immediately outside experimental unit at Flake Point Bar, Jonesport, Maine from 16 June to 14 September 2023. A Sign test revealed that mean seawater temperatures inside experimental units was significantly warmer than outside units ($P < 0.0001$). The mean difference was +0.24ºC ($n = 118$).

Figure 36. Average weekly seawater temperatures (red dots) and percent of live clams that were induced to spawn (blue dots connected by black lines) from boxes placed at the A) upper; B) mid; and, C) lower intertidal at Flake Point Bar, Jonesport, Maine. The dashed line in each graph is the 10ºC line when the commencement of spawning has been noted from other studies north of Cape Cod, Massachusetts (Snelgrove et al. 2023).

Figure 37. Relationship between average weekly seawater temperature at Flake Point Bar in 2020 and the combined percent spawning results from each of three tidal heights (upper, mid, and lower). Clams were collected regularly through the late spring and early summer. Spawning commenced in early June when seawater temperatures were approaching 10ºC, and ceased in early August when seawater temperatures climbed over 13ºC.

Figure 38. Average weekly seawater temperature (red dots) and percent of live clams that were induced to spawn (red stars) from clams collected on six dates from the ambient mudflat at each tidal height from Flake Point Bar, Jonesport, Maine. Here, each star represents the pooled data across tidal heights on a particular sampling date.

Figure 39. Relationship between shell length and number of eggs released for experimental ($n = 36$) and control clams ($n = 10$) from Flake Point Bar (17 June to 12 August 2023). No significant linear association was discovered ($P = 0.057$), and there was no curvilinear response ($P = 0.656$).

Figure 40. Relationship between size of clam eggs (µm) and clam size (mm) from animals collected from intertidal boxes at Flake Point Bar, Jonesport, Maine on five dates (24 June, 17, 22 & 29 July, and, 5 August). Ten eggs were measured for each of 33 females using 4X magnification and an ocular micrometer. No linear relationship was observed ($r^2 = 0.003$, $P = 0.7818$). The dashed line represents the mean egg diameter (74.00 ± 0.90 µm).

Figure 41. A) GSI vs. Shell length for experimental clams from Flake Point Bar, Jonesport, Maine for each of 18 sampling dates from 6 May to 2 September 2023. A quadratic model

($\hat{Y} = b_0 + b_1X + b_2X^2$; $b_0 = 13.68$; $b_1 = -0.05457$; $b_2 = -0.007444$) was fit ($r^2 = 0.454$, $P < 0.0001$, $n = 306$). The red line is the line of best fit, and the two dashed lines are the 95% CI around $\hat{Y}$. B) GSI* vs. Shell length for the same experimental clams as described above. ($b_0 = 1.764$; $b_1 = -0.0465$; $b_2 = -0.00065$; $r^2 = 0.2779$, $P < 0.0001$).

Figure 42. Mean GSI (+95% CI) across sampling dates for A) clams less than 50 mm SL; and, B) clams between 70.0-79.9 mm SL at Flake Point Bar, Jonesport, Maine. ANOVA demonstrated a significant sampling date effect in both instances; however, no linear or curvilinear trend was detected for either size class. (n = 1-4).

Figure 43. Mean GSI (+95% CI) across tidal heights for clams from size class II (50.0-59.9 mm SL) at Flake Point Bar, Jonesport, Maine. ANOVA demonstrated a significant effect ($P = 0.0197$; Table 9).

Figure 44. Mean GSI (+95% CI) for each size class pooled over tidal height and sampling date for clams at Flake Point Bar, Jonesport, Maine. A lack-of-fit test indicated that the addition of a quadratic term to a linear model improved the fit significantly ($F = 55.13$, $df = 1, 2$, $P = 0.018$; $r^2 = 0.9978$; $\hat{Y} = 4.765 - 1.264X + 0.953X^2$).

Figure 45. A) Interaction plot (Origin × Sampling Date; Table 10) for mean GSI for ambient and experimental clams within size class I (< 50.0 mm SL) across seven sampling dates when clams from both origins were sampled (n = 1-6). B) Interaction plot (Origin × Tidal Height; Table 10) where mean GSI for is presented ambient (dark blue bars) and experimental (light blue bars) clams within size class I for each of three tidal heights (n = 5-11).

Figure 46. Interaction plot (Origin × Tidal Height; Table 10). Mean GSI for ambient (dark blue bars) clams increased with decreasing tidal height, whereas mean GSI decreased with decreasing tidal height for experimental (light blue bars) clams.

Figure 47. Condition Index (total tissue dry weight [g]/SL [cm]) for experimental clams (N = 309) collected from three tidal heights (upper, mid, lower) on 18 weekly sampling dates from Flake Point Bar, Jonesport, Maine (6 May to 2 September 2023). A lack-of-fit test showed that the inclusion of a quadratic term to a linear model explained significantly more of the variation in CI than the linear model ($F = 19.21$, $df = 1, 306$, $P < 0.0001$). In addition, the inclusion of a cubic term to a model already containing both linear and quadratic terms did not explain any more significant variation ($F = 0.449$, $df = 1, 305$, $P = 0.4485$). Therefore, a quadratic model explained the most variation in the dependent variable: ; $\hat{Y} = 0.042 - 0.0023X + 0.0000815X^2$, $r^2 = 0.7348$.

Figure 48. Least square means (+ 95% CI) of log (dry tissue weight) across sampling dates for clams from Flake Point Bar, Jonesport, Maine. A negative, linear relationship exists for this relationship ($r^2 = 0.68$; $F = 34.34$, $df = 1, 16$, $P < 0.0001$).

Figure 49. Back-calculated least-square mean of dry tissue mass (g) and 95% confidence interval for ambient (control) and experimental soft-shell clams for each size class (I: < 50 mm [n = 48]; II: 50.0-59.9 mm [n = 81]; III: 60.0-69.9 mm [n = 56]; IV: 70-79.9 mm [n = 39]; V: ≥

80 mm [n = 31]). Significant differences in least-square means was noted for size classes (I & II [$P < 0.0001$] and III [$P = 0.0055$]). No significant differences were observed for size classes IV ($P = 0.075$) and V ($P = 0.564$).

Figure 1(a)

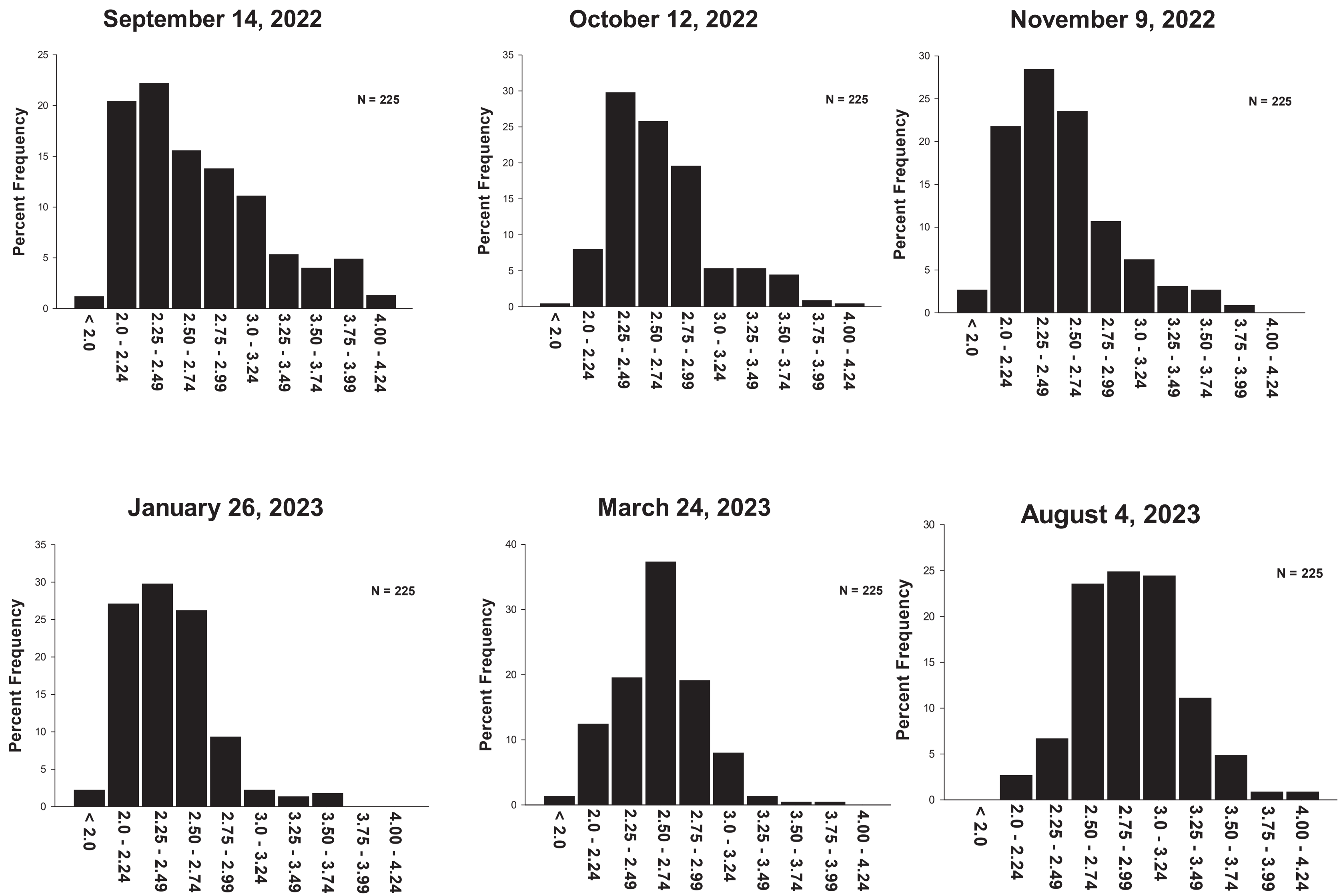
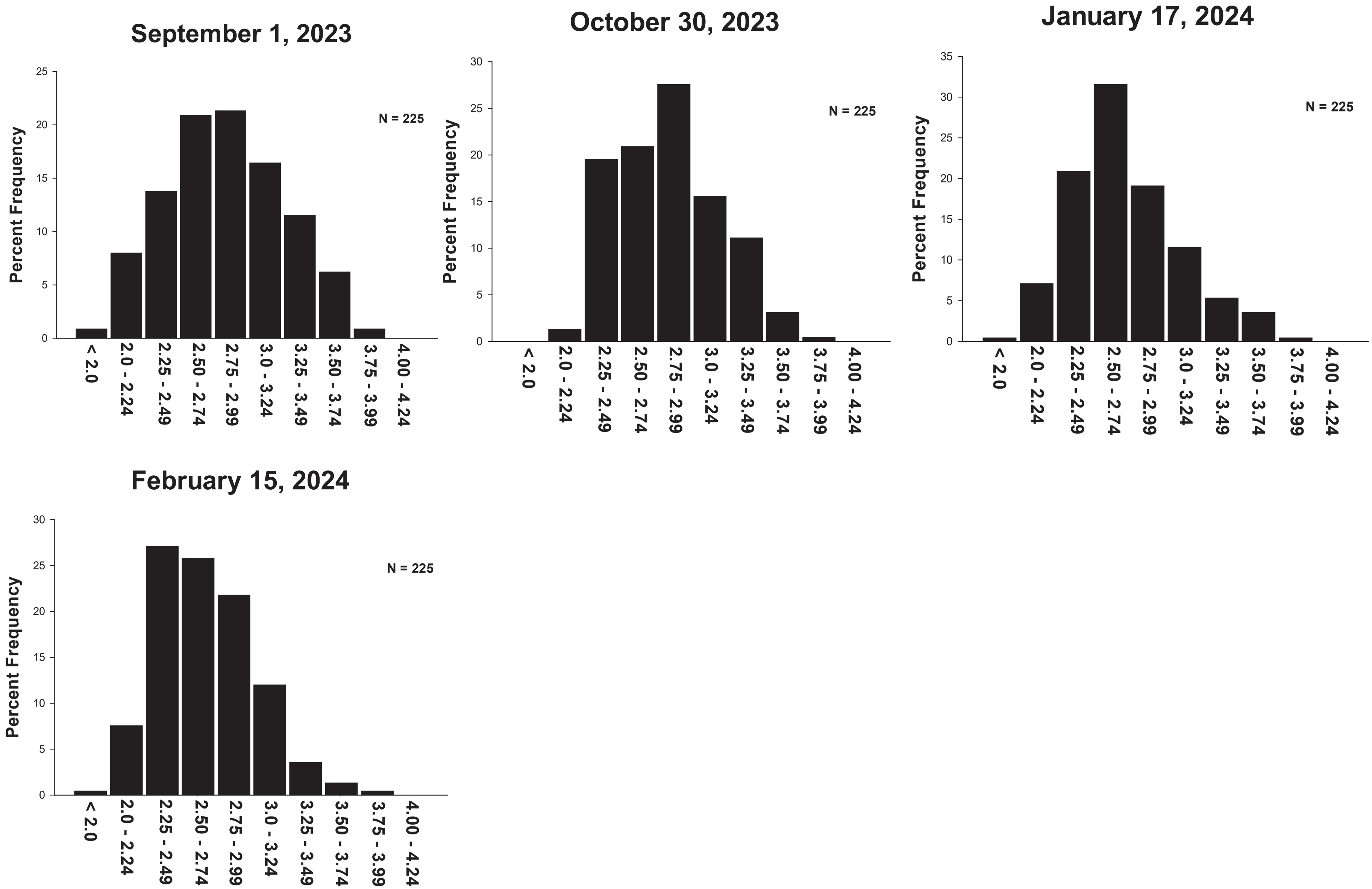
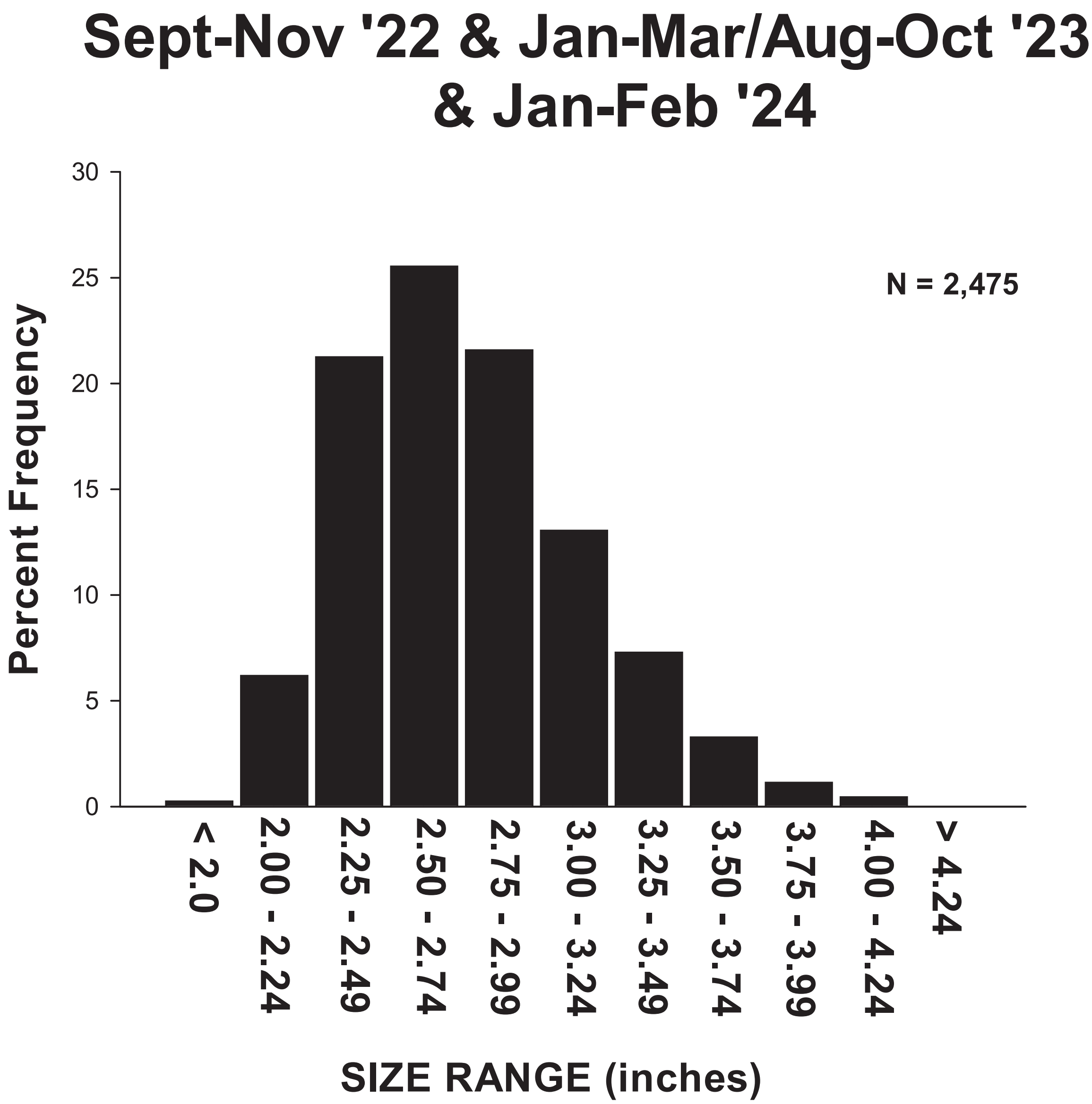

September 14, 2022
October 12, 2022
November 9, 2022
January 26, 2023
March 24, 2023
August 4, 2023
Percent Frequency
N = 225
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24

Figure 1(b)



September 1, 2023
Percent Frequency
N = 225
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24
October 30, 2023
Percent Frequency
N = 225
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24
January 17, 2024
Percent Frequency
N = 225
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24
February 15, 2024
Percent Frequency
N = 225
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24

Figure 2.



Sept-Nov '22 & Jan-Mar/Aug-Oct '23 & Jan-Feb '24
N = 2,475
Percent Frequency
30
25
20
15
10
5
0
< 2.0
2.00 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.00 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24
> 4.24
SIZE RANGE (inches)

Figure 3.

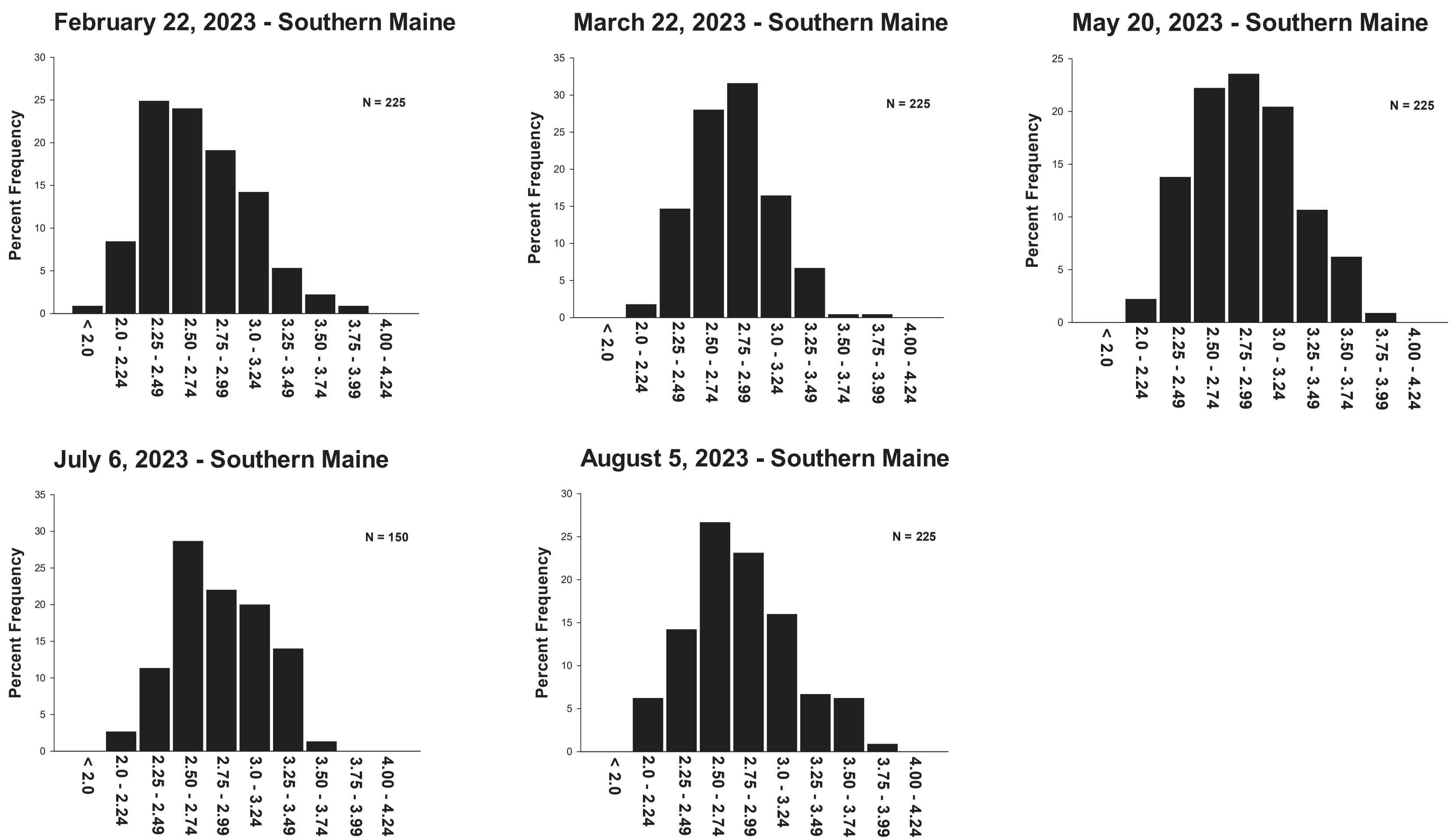

February 22, 2023 - Southern Maine
N = 225
March 22, 2023 - Southern Maine
N = 225
May 20, 2023 - Southern Maine
N = 225
July 6, 2023 - Southern Maine
N = 150
August 5, 2023 - Southern Maine
N = 225
Percent Frequency
0
5
10
15
20
25
30
35
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24

Figure 4.

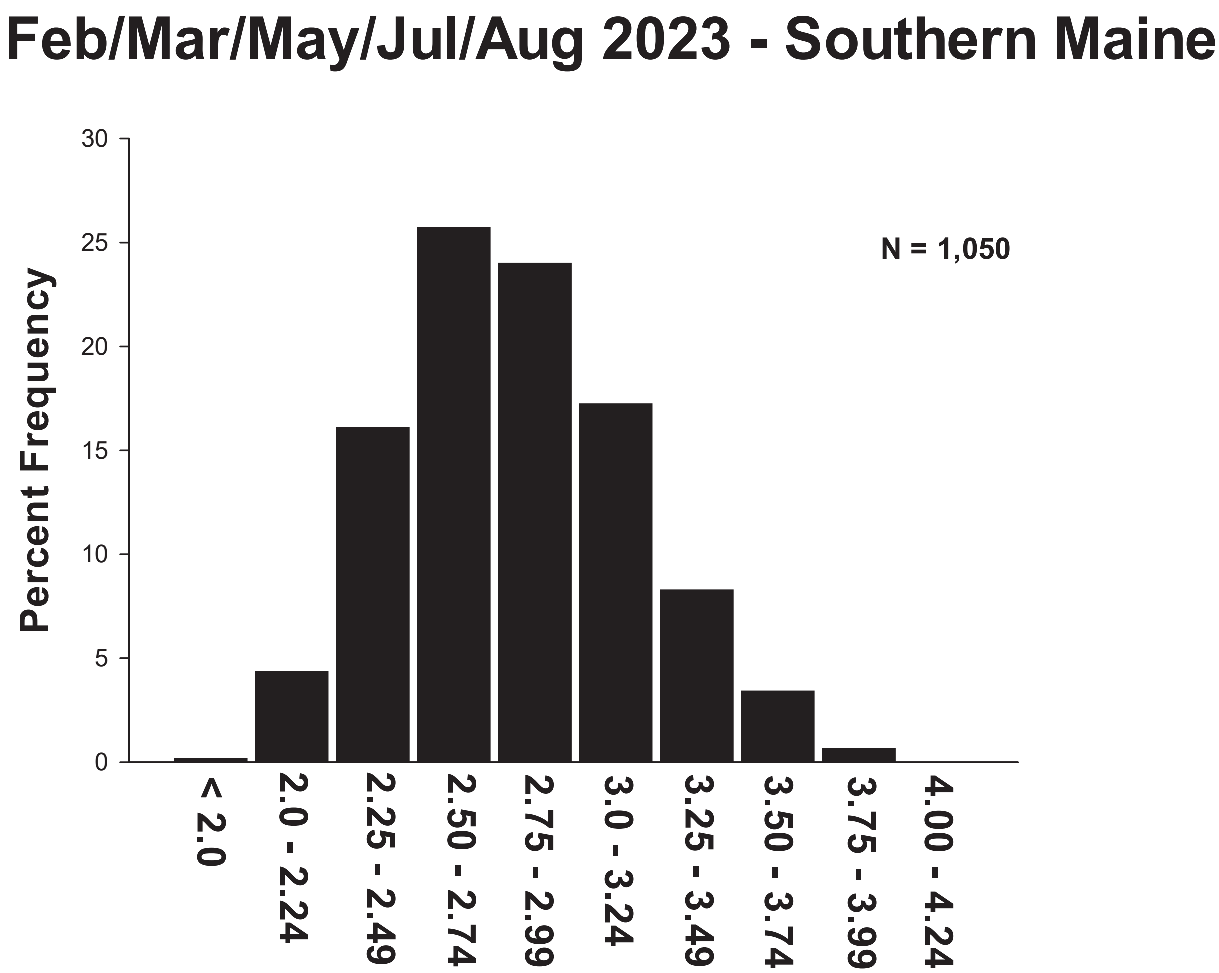

Feb/Mar/May/Jul/Aug 2023 - Southern Maine
Percent Frequency
30
25
20
15
10
5
0
N = 1,050
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24

Figure 5.

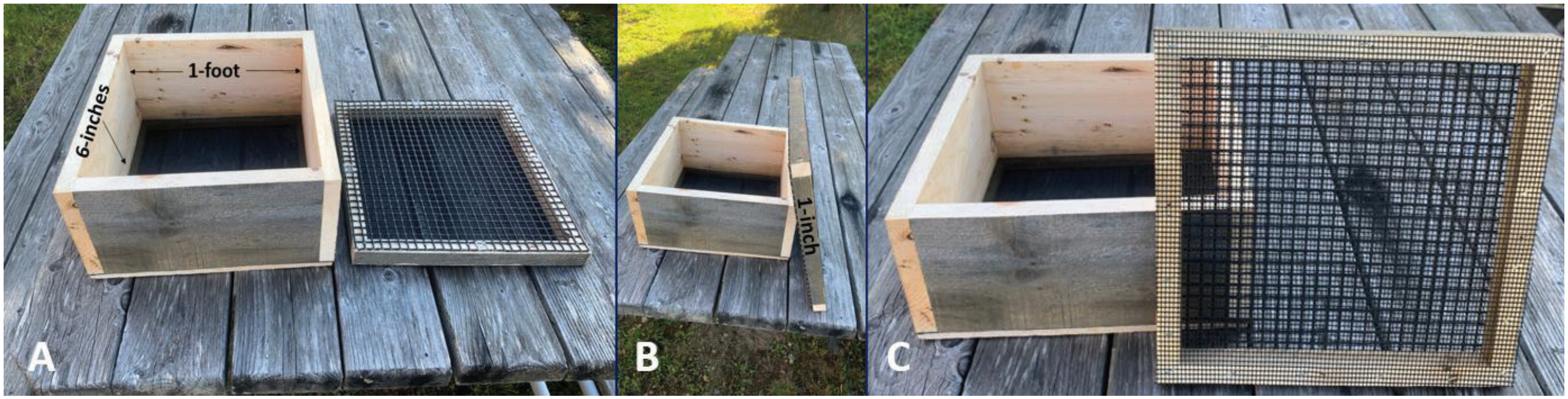


Figure 6.

Figure 7.

Figure 8.

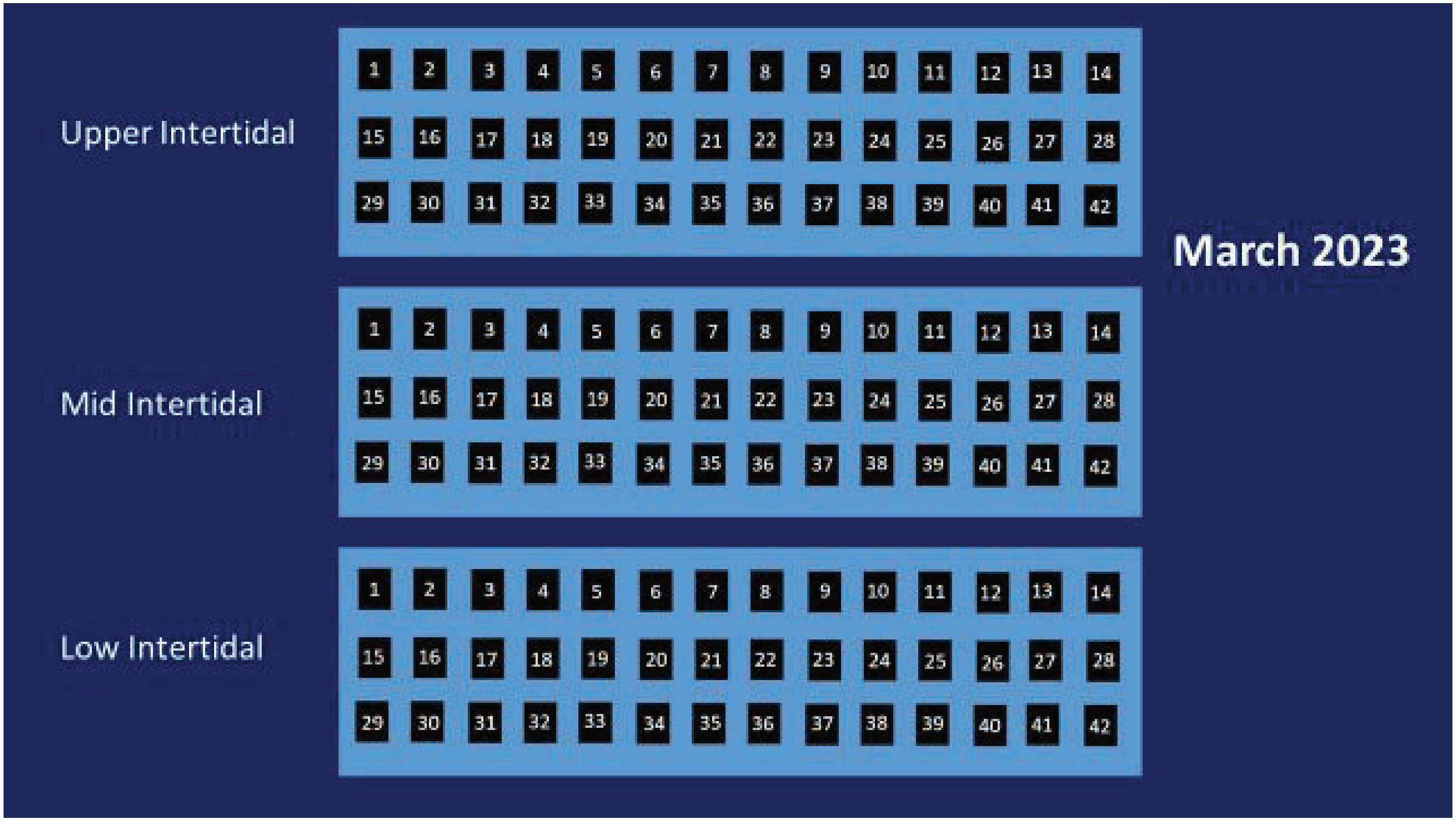

Upper Intertidal
1 2 3 4 5 6 7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22 23 24 25 26 27 28
29 30 31 32 33 34 35 36 37 38 39 40 41 42
Mid Intertidal
1 2 3 4 5 6 7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22 23 24 25 26 27 28
29 30 31 32 33 34 35 36 37 38 39 40 41 42
Low Intertidal
1 2 3 4 5 6 7 8 9 10 11 12 13 14
15 16 17 18 19 20 21 22 23 24 25 26 27 28
29 30 31 32 33 34 35 36 37 38 39 40 41 42
March 2023

Figure 9.

Figure 10.

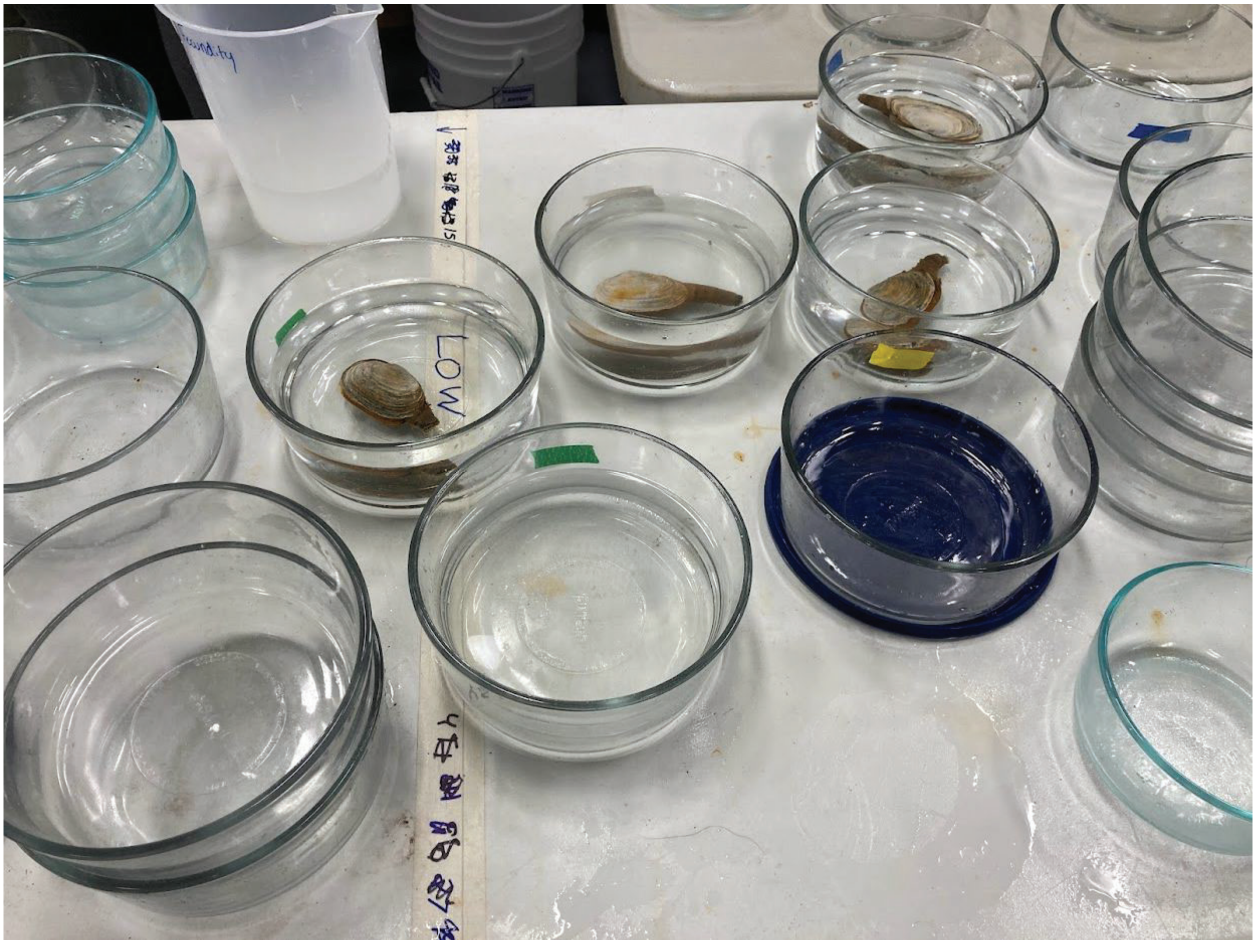
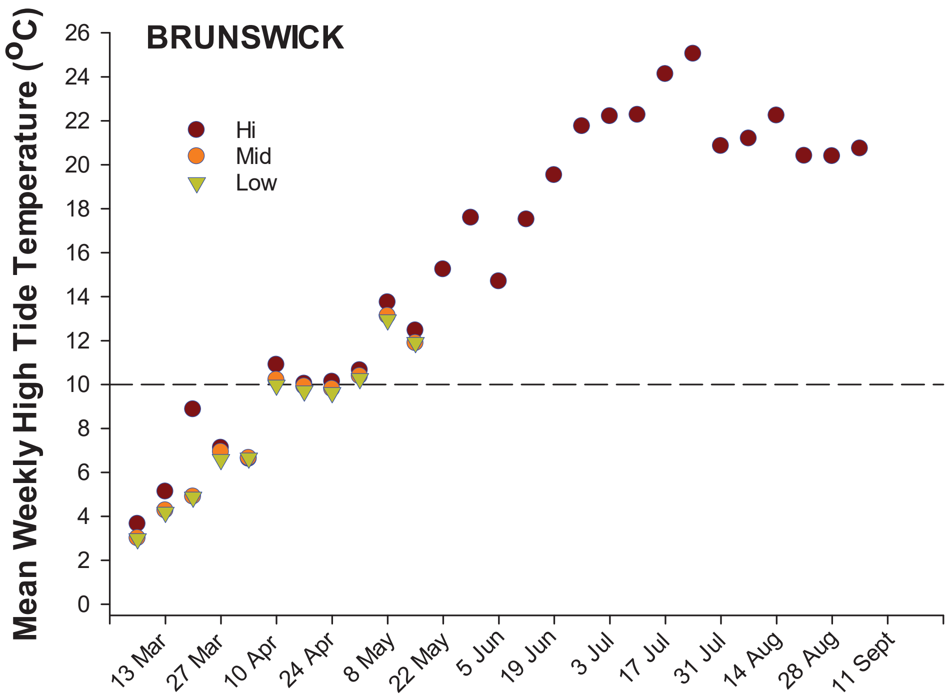


Figure 11.

A)


BRUNSWICK
Hi
Mid
Low
Mean Weekly High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
13 Mar
27 Mar
10 Apr
24 Apr
8 May
22 May
5 Jun
19 Jun
3 Jul
17 Jul
31 Jul
14 Aug
28 Aug
11 Sept

B)

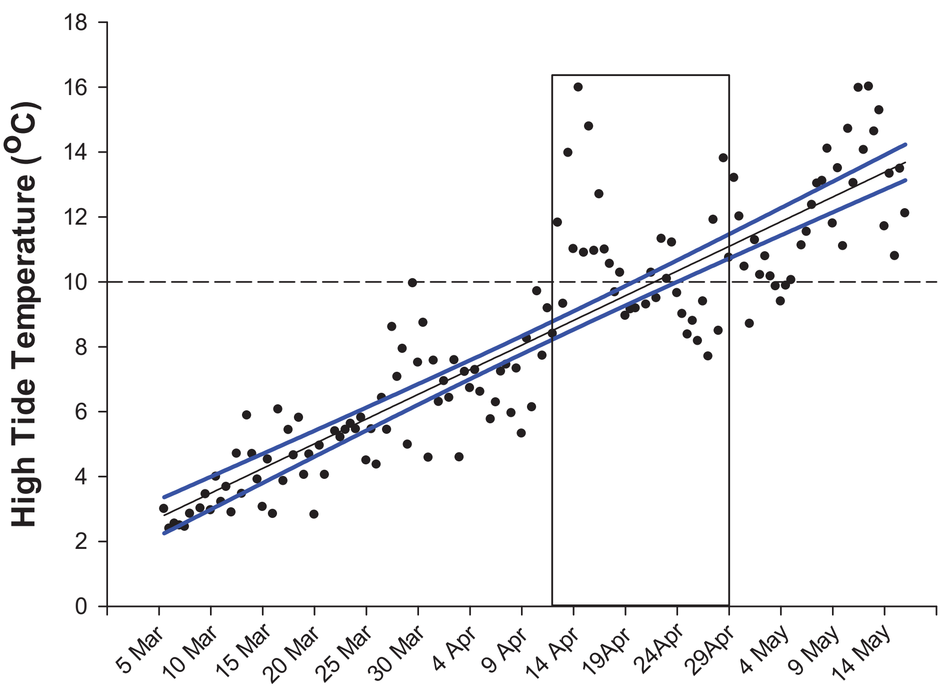

High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
5 Mar
10 Mar
15 Mar
20 Mar
25 Mar
30 Mar
4 Apr
9 Apr
14 Apr
19Apr
24Apr
29Apr
4 May
9 May
14 May

Figure 12.

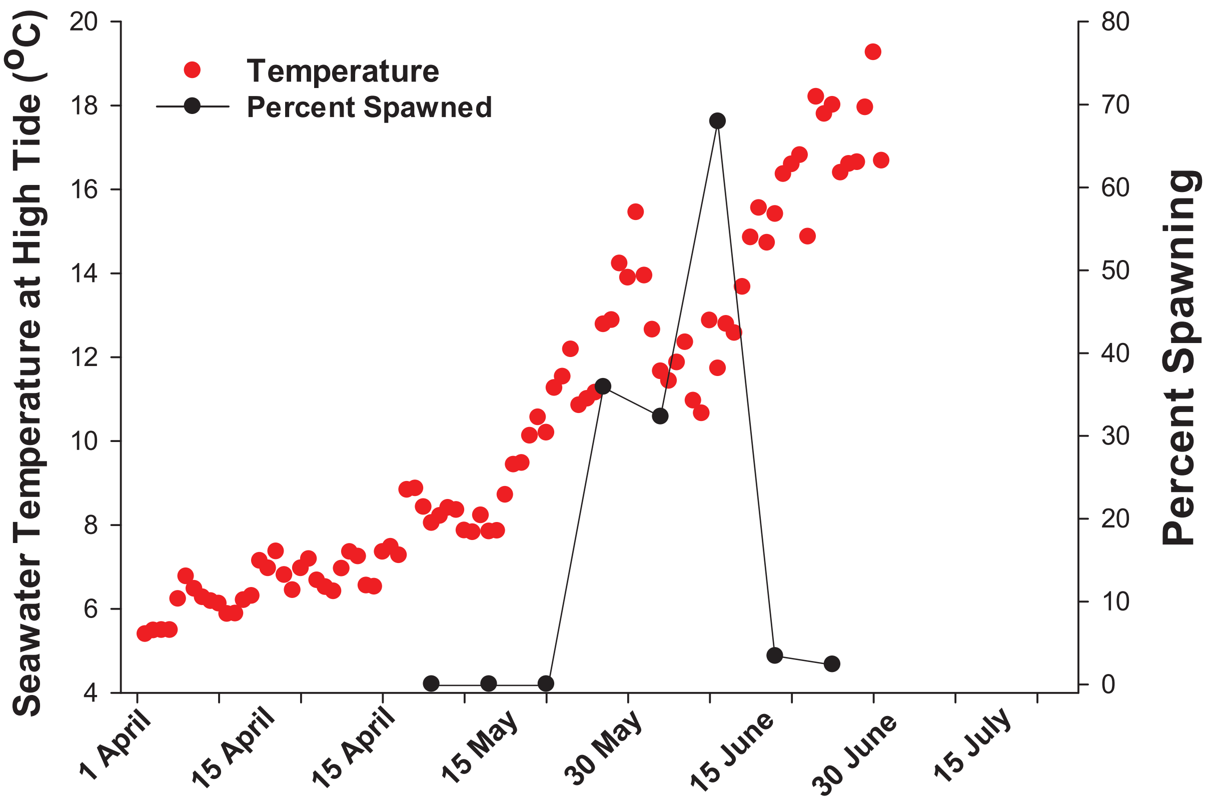
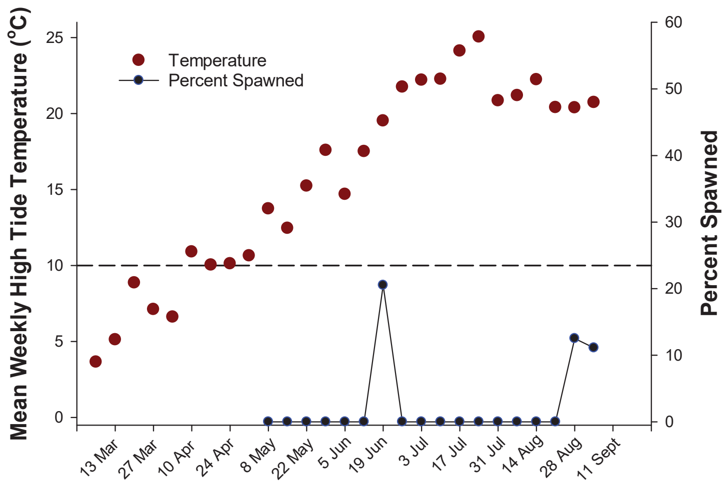
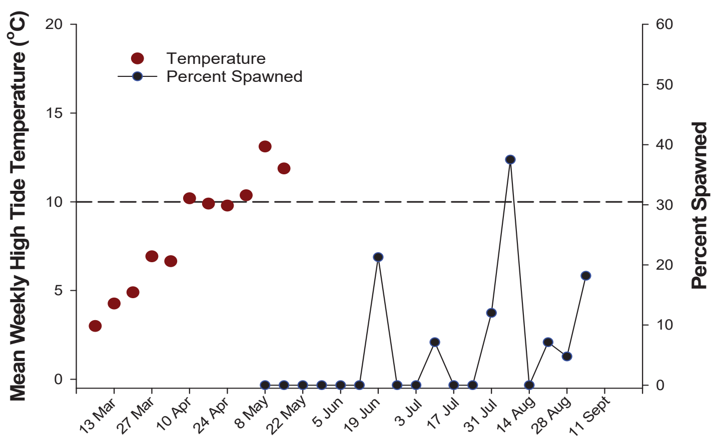

Seawater Temperature at High Tide (°C)
Percent Spawning
Temperature
Percent Spawned
20
18
16
14
12
10
8
6
4
80
70
60
50
40
30
20
10
0
1 April
15 April
15 April
15 May
30 May
15 June
30 June
15 July

Figure 13.

A)



B)



C)

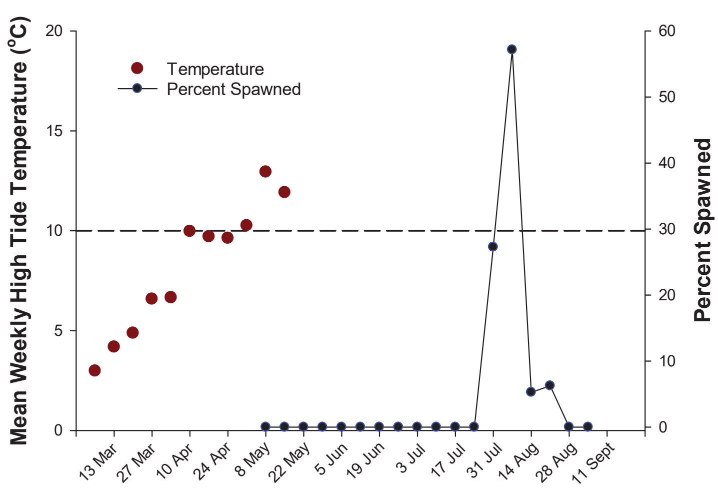
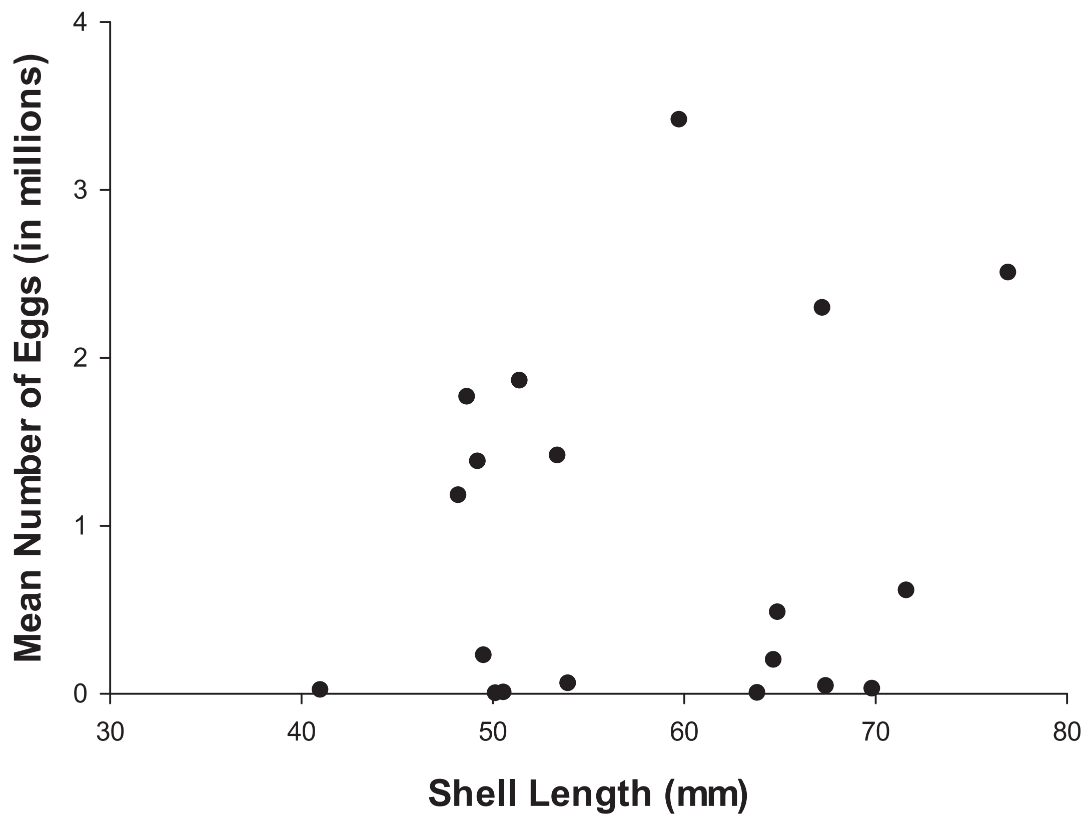
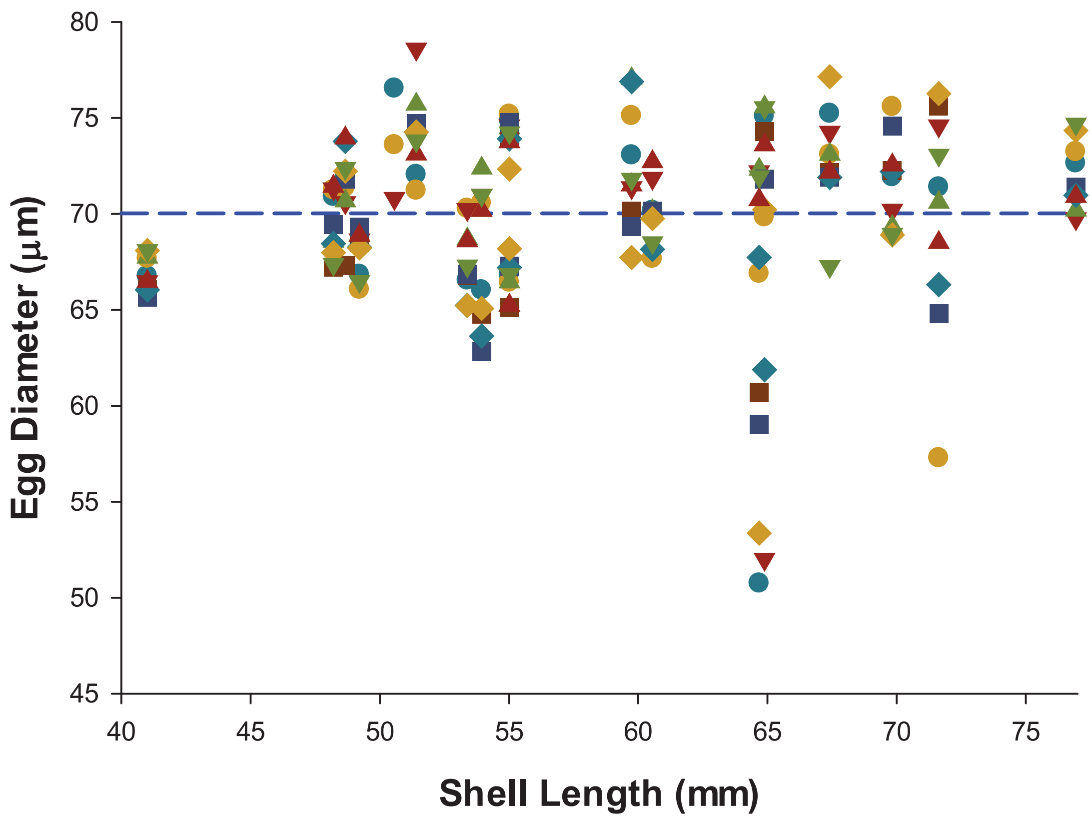


Figure 14.



Mean Number of Eggs (in millions)
4
3
2
1
0
30
40
50
60
70
80
Shell Length (mm)

Figure 15.



80
75
70
65
60
55
50
45
Egg Diameter (µm)
40
45
50
55
60
65
70
75
Shell Length (mm)

Figure 16.

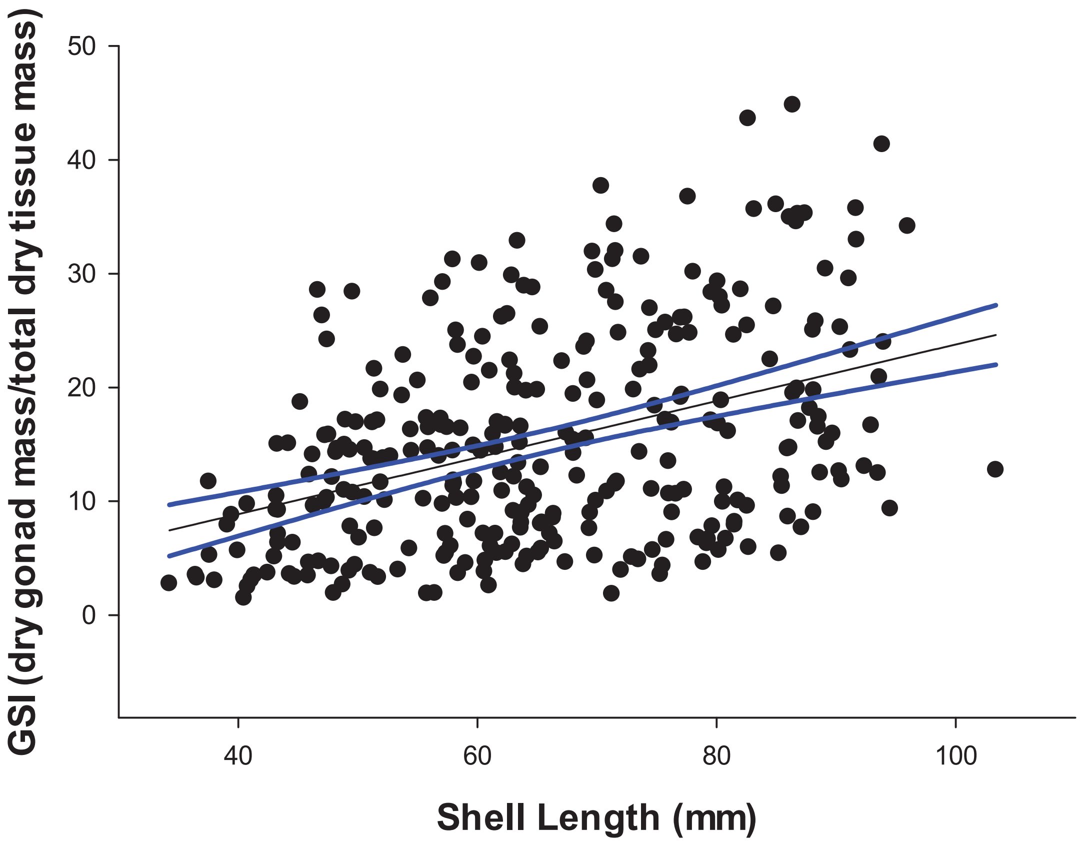
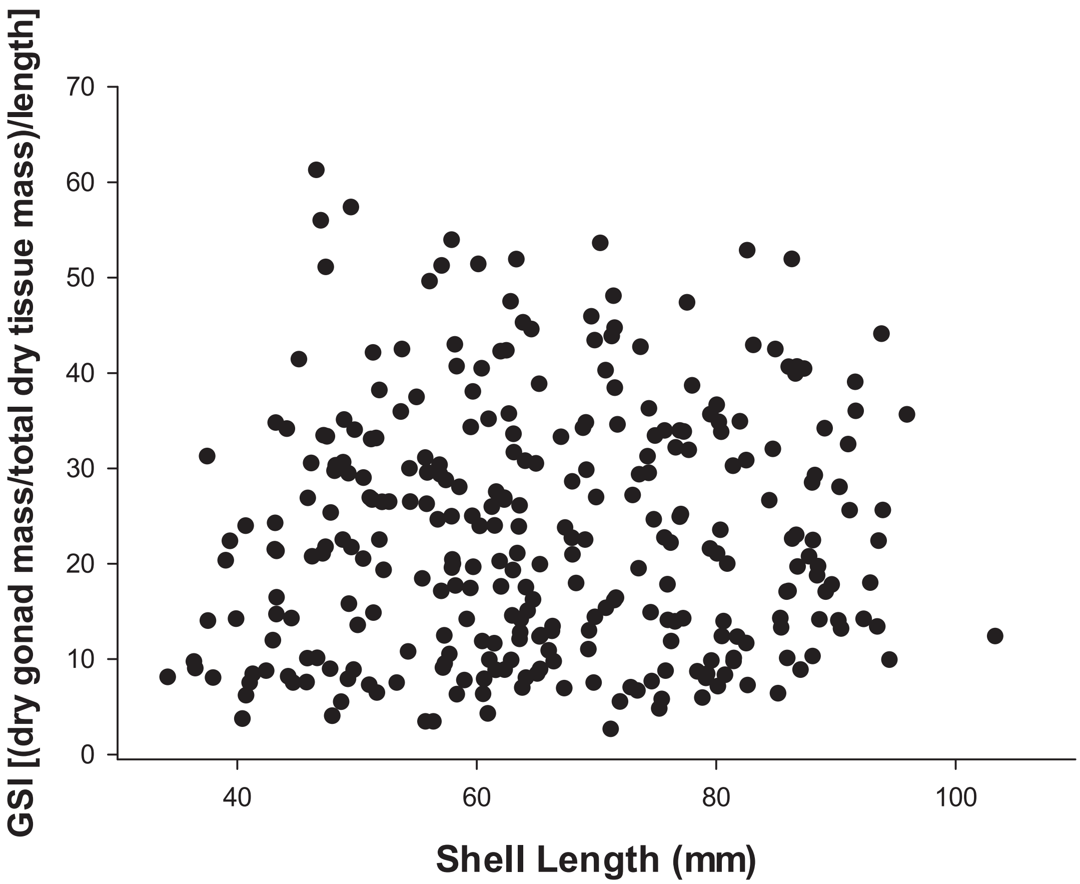

GSI (dry gonad mass/total dry tissue mass)
50
40
30
20
10
0
40
60
80
100
Shell Length (mm)

Figure 17.



GSI [(dry gonad mass/total dry tissue mass)/length]
70
60
50
40
30
20
10
0
40
60
80
100
Shell Length (mm)

Figure 18.

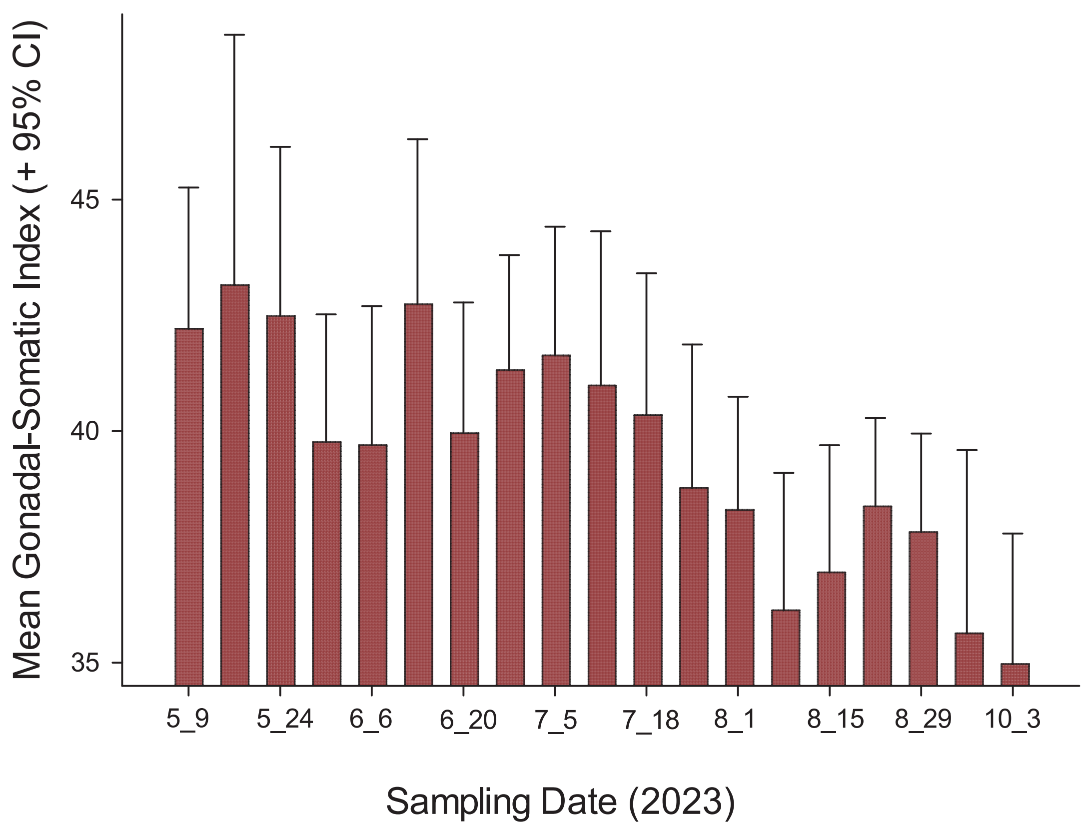

Mean Gonadal-Somatic Index (+ 95% CI)
45
40
35
5_9
5_24
6_6
6_20
7_5
7_18
8_1
8_15
8_29
10_3
Sampling Date (2023)

Figure 19.

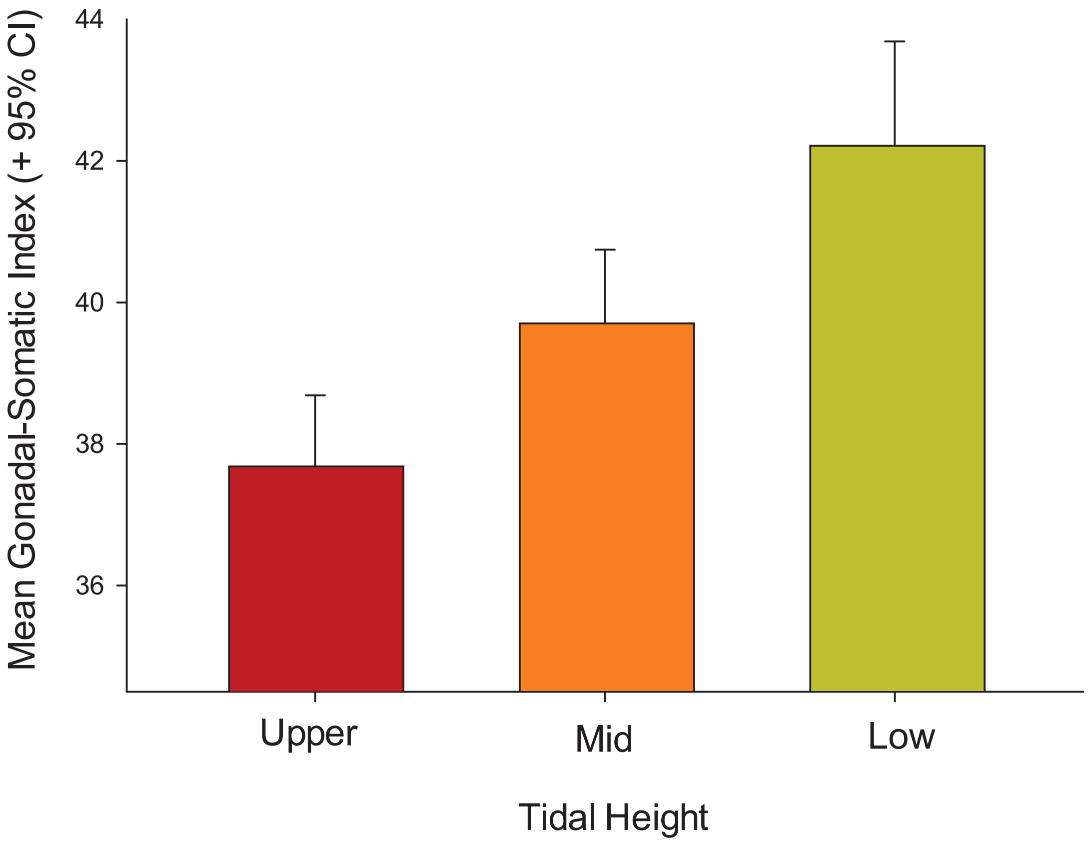

Mean Gonadal-Somatic Index (+ 95% CI)
44
42
40
38
36
Upper
Mid
Low
Tidal Height

Figure 20.

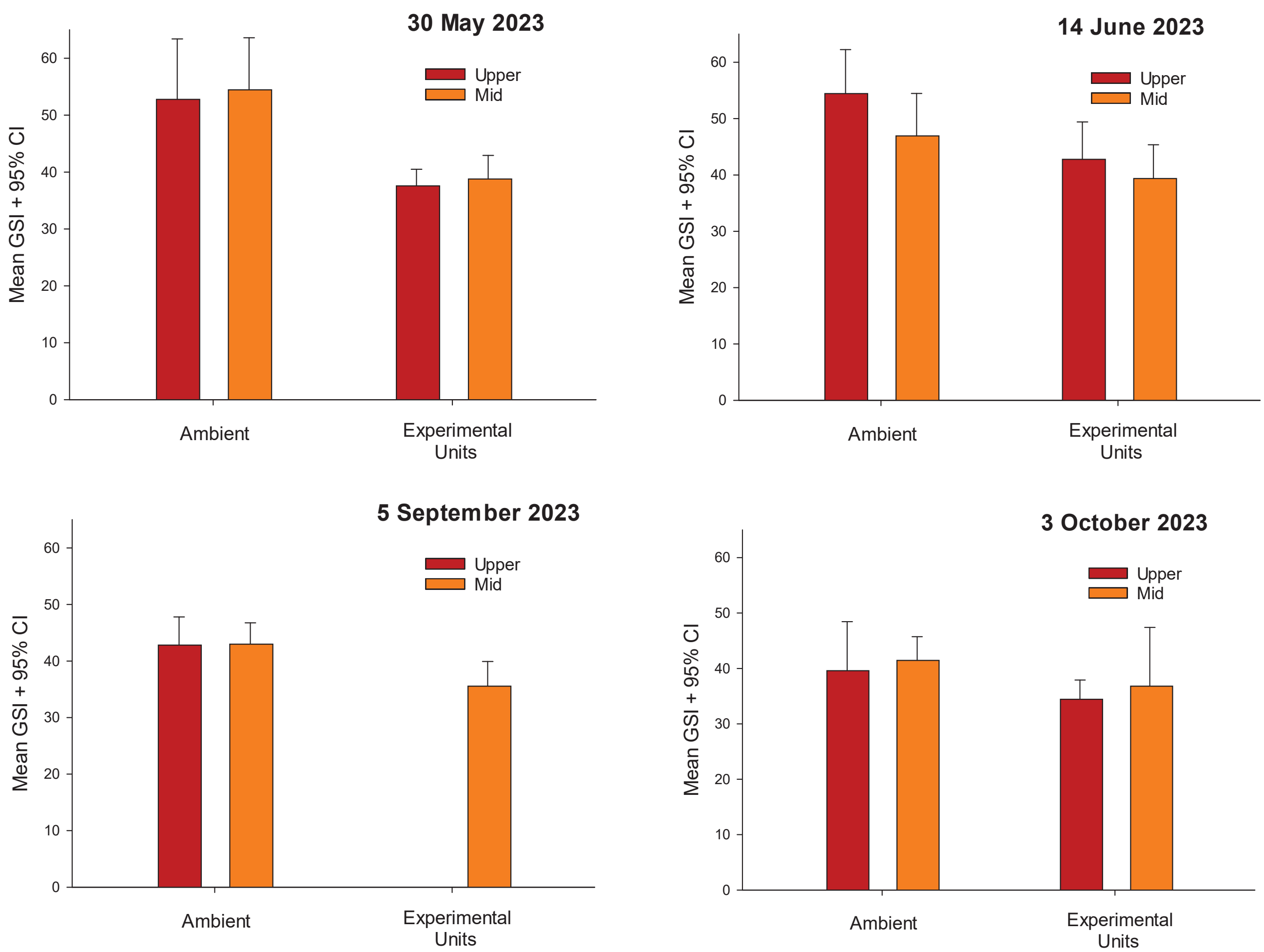

30 May 2023
14 June 2023
5 September 2023
3 October 2023
Mean GSI + 95% CI
Upper
Mid
Ambient
Experimental Units
0
10
20
30
40
50
60

Figure 21.

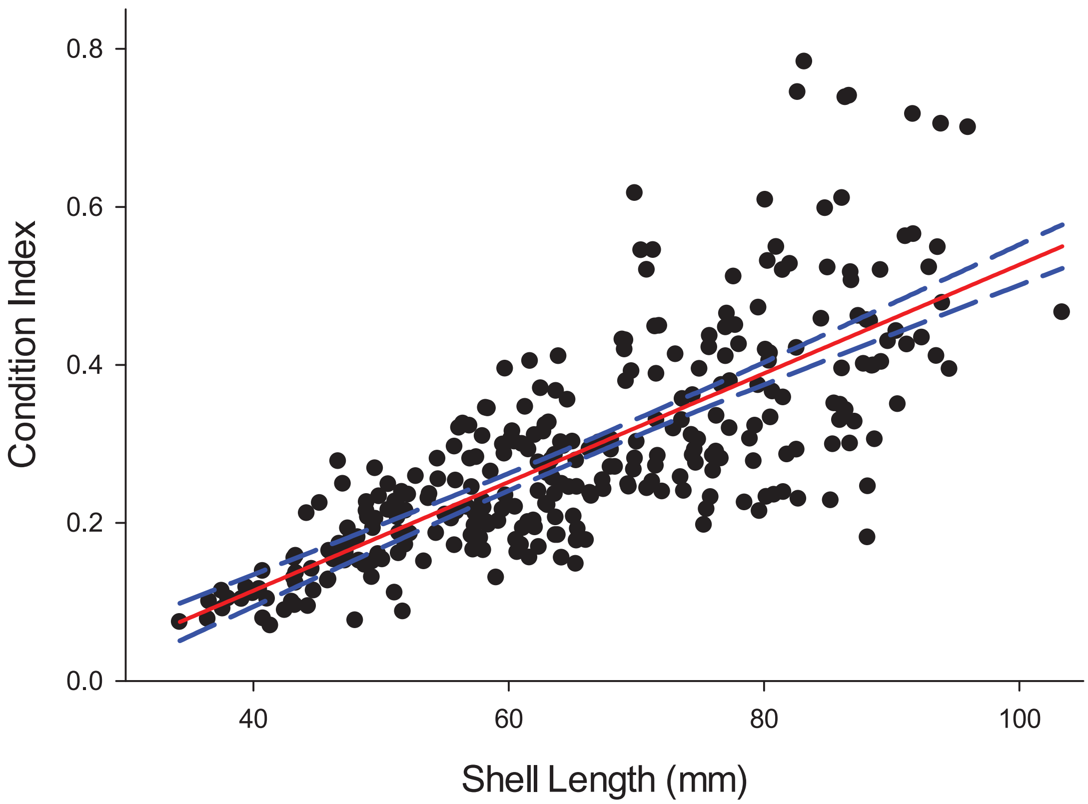


Figure 22.

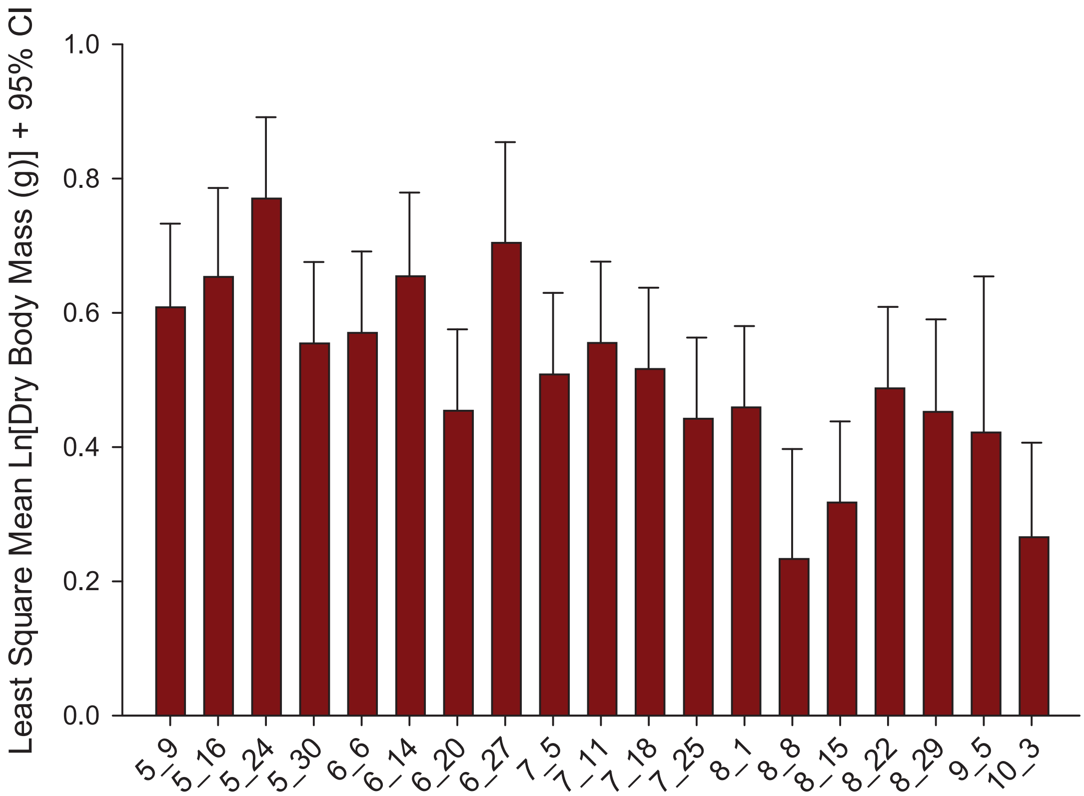

Least Square Mean Ln[Dry Body Mass (g)] + 95% CI
1.0
0.8
0.6
0.4
0.2
0.0
5_9
5_16
5_24
5_30
6_6
6_14
6_20
6_27
7_5
7_11
7_18
7_25
8_1
8_8
8_15
8_22
8_29
9_5
10_3

Figure 23.

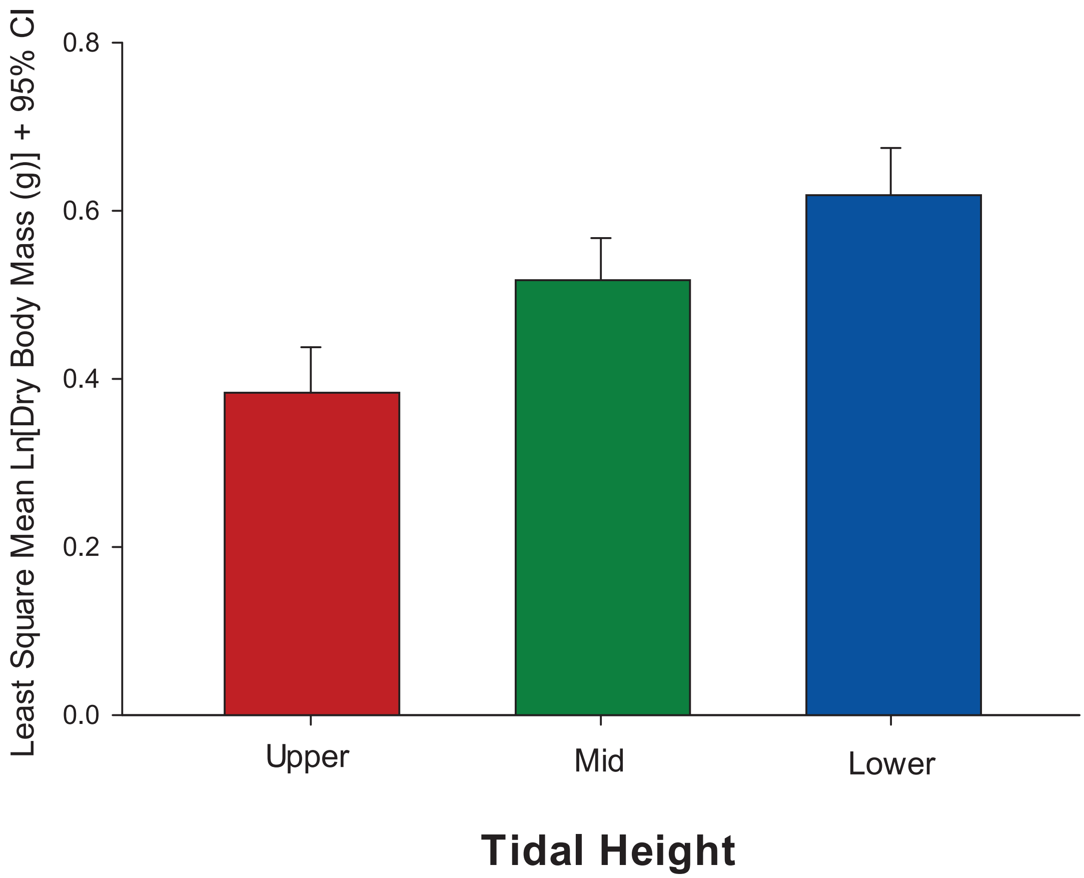

Least Square Mean Ln[Dry Body Mass (g)] + 95% CI
0.8
0.6
0.4
0.2
0.0
Upper
Mid
Lower
Tidal Height

Figure 24.

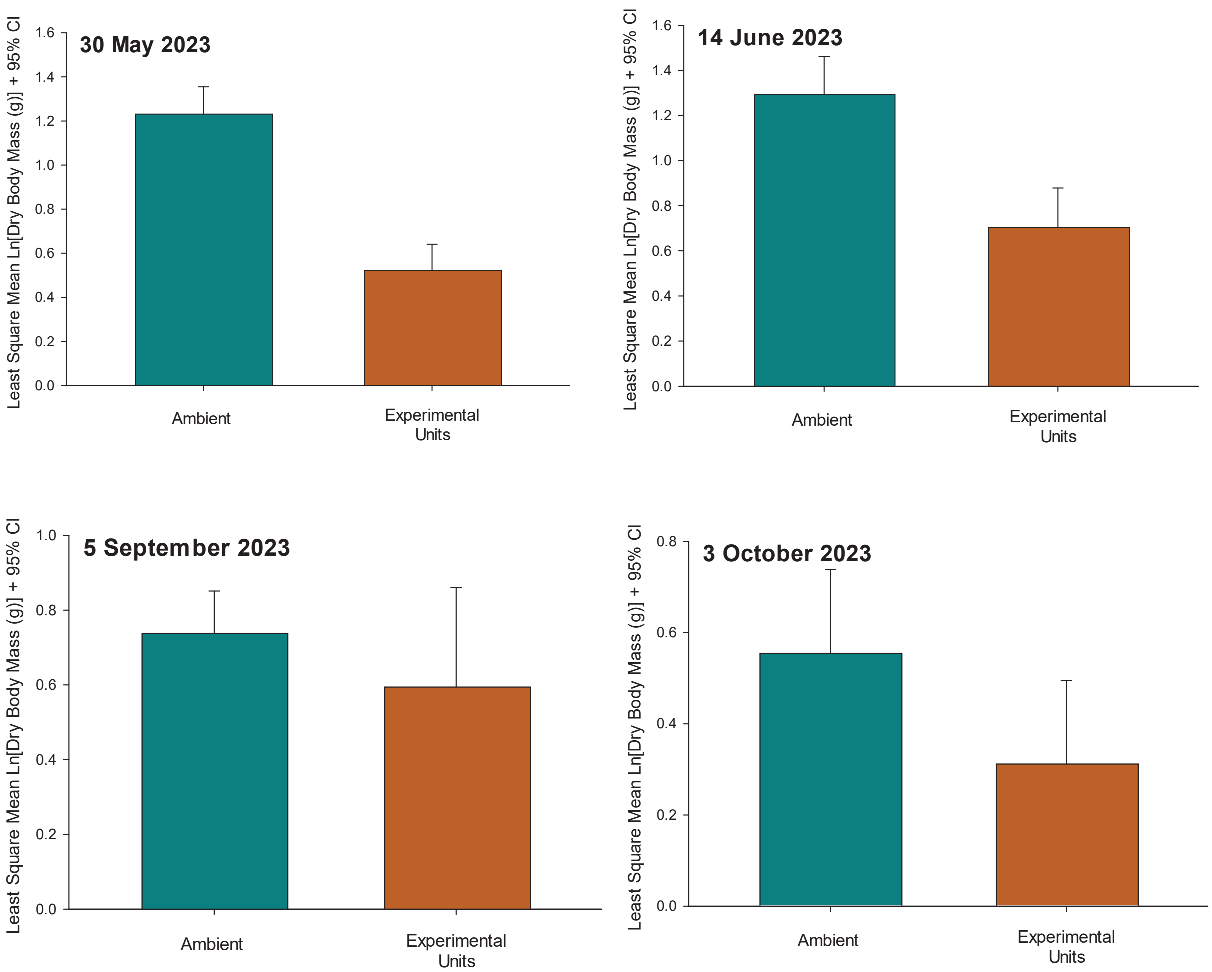
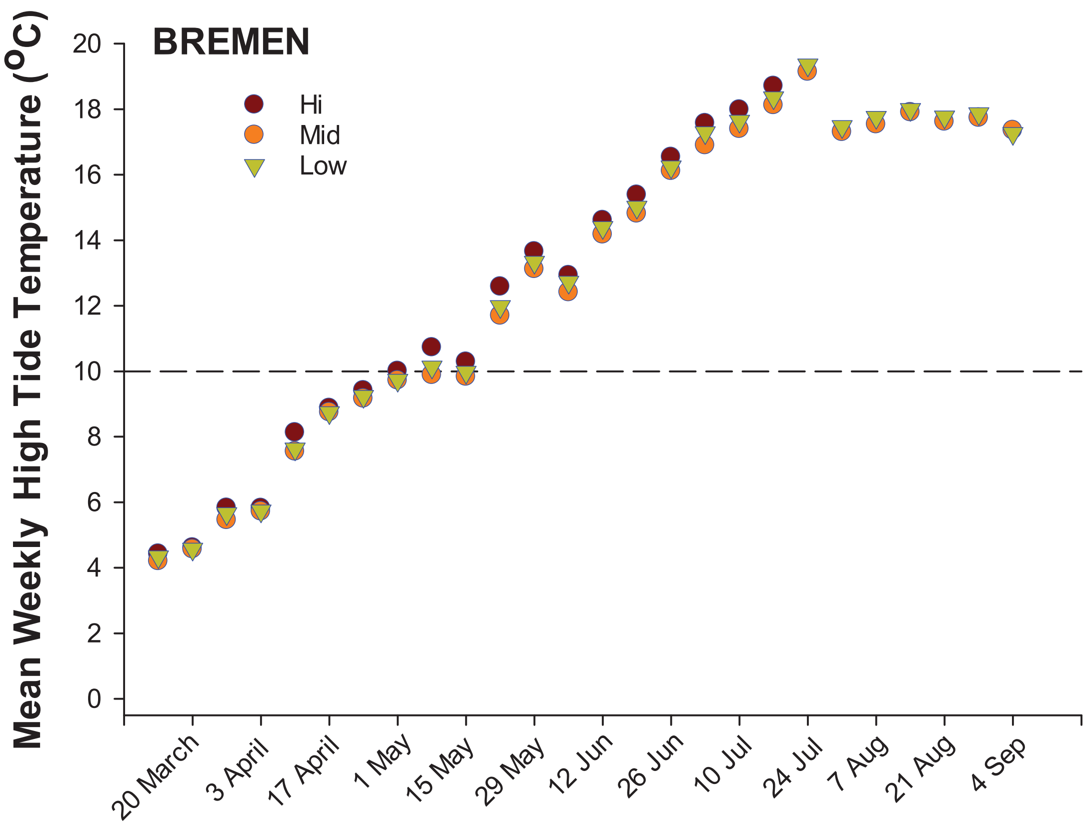

30 May 2023
14 June 2023
5 September 2023
3 October 2023
Least Square Mean Ln[Dry Body Mass (g)] + 95% CI
Ambient
Experimental Units
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0

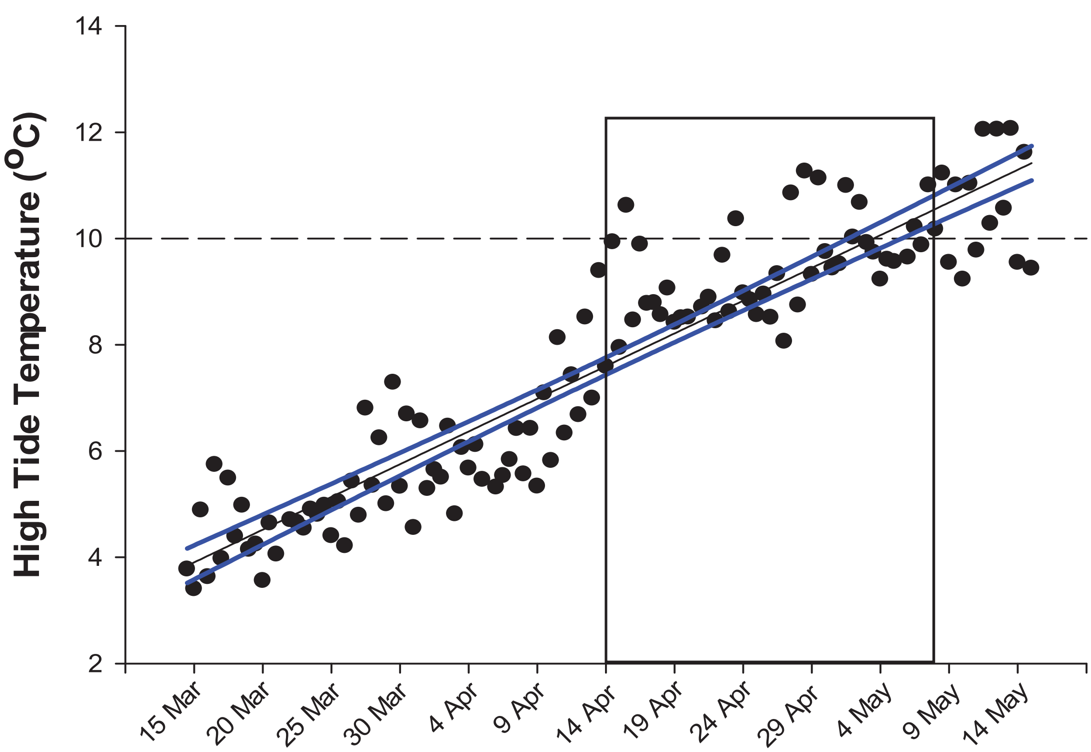
Figure 25.

A)



BREMEN
Hi
Mid
Low
Mean Weekly High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
20 March
3 April
17 April
1 May
15 May
29 May
12 Jun
26 Jun
10 Jul
24 Jul
7 Aug
21 Aug
4 Sep

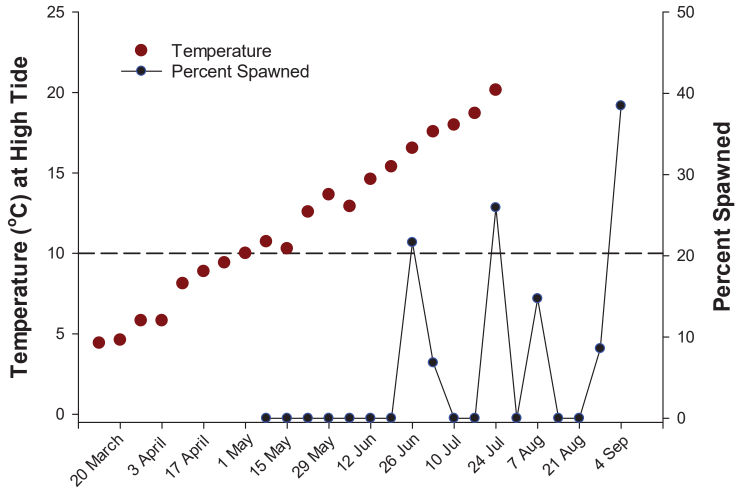
B)



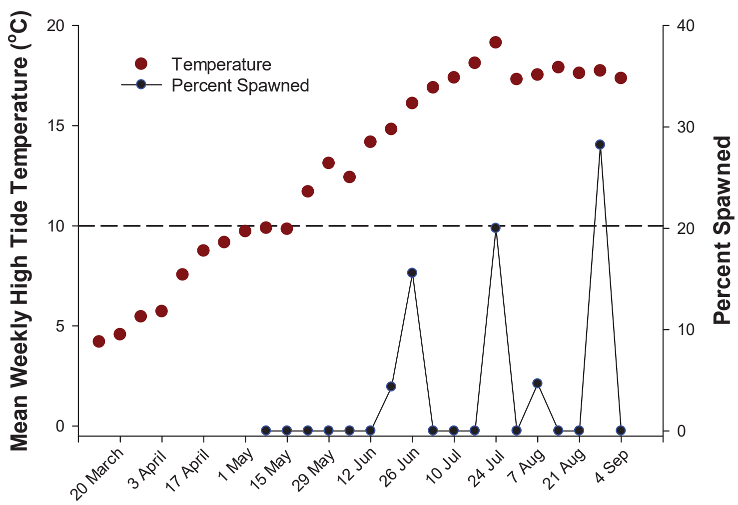
High Tide Temperature (°C)
2
4
6
8
10
12
14
15 Mar
20 Mar
25 Mar
30 Mar
4 Apr
9 Apr
14 Apr
19 Apr
24 Apr
29 Apr
4 May
9 May
14 May

Figure 26.

A)



B)



C)

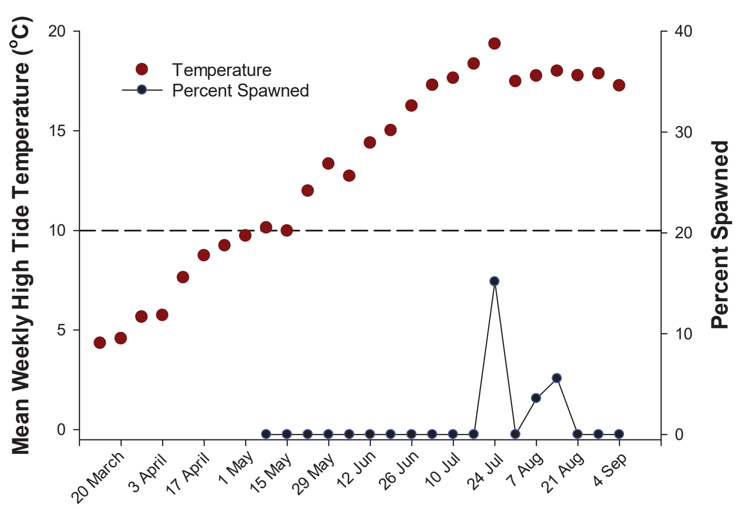


Figure 27.

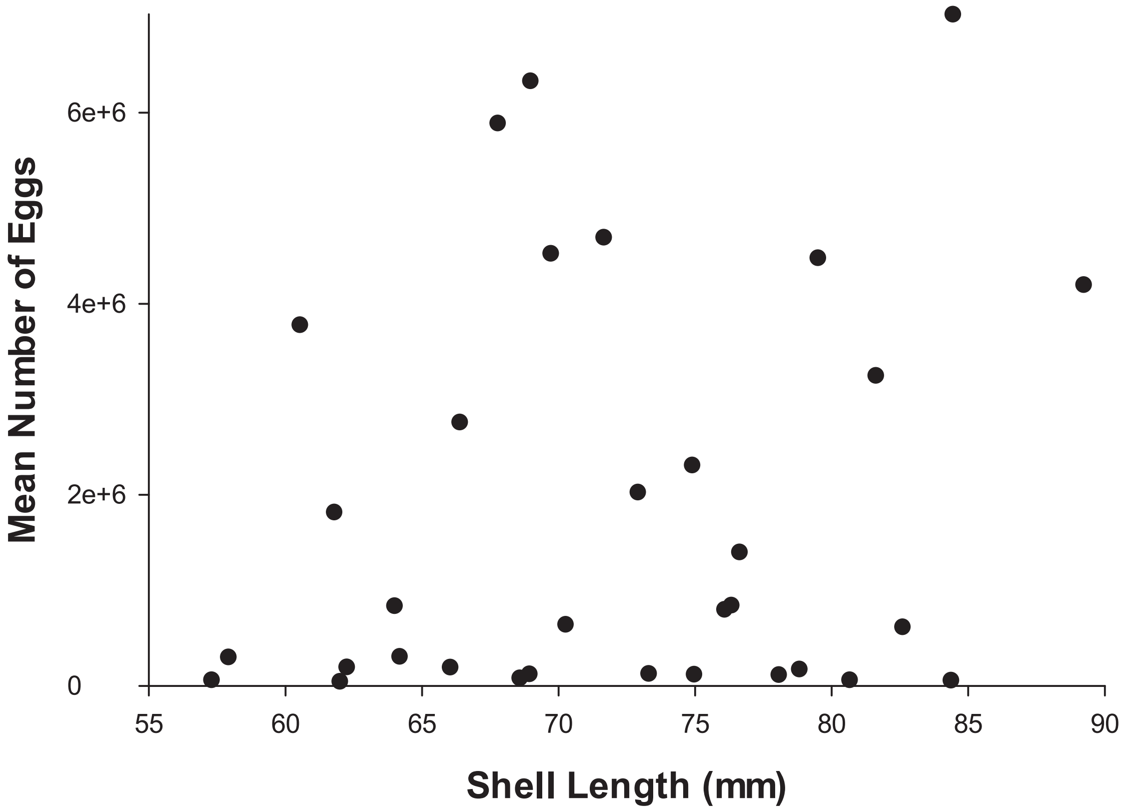
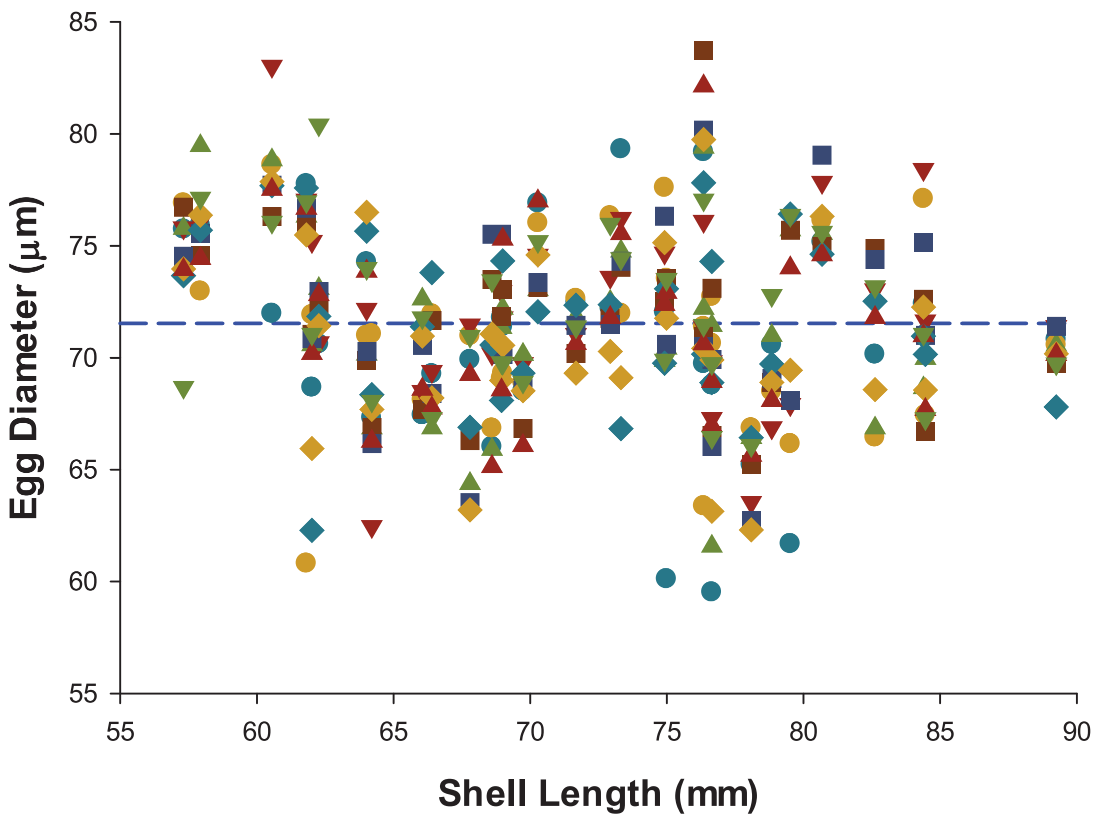

Mean Number of Eggs
6e+6
4e+6
2e+6
0
55
60
65
70
75
80
85
90
Shell Length (mm)

Figure 28.



85
80
75
70
65
60
55
55
60
65
70
75
80
85
90
Egg Diameter (µm)
Shell Length (mm)

Figure 29.

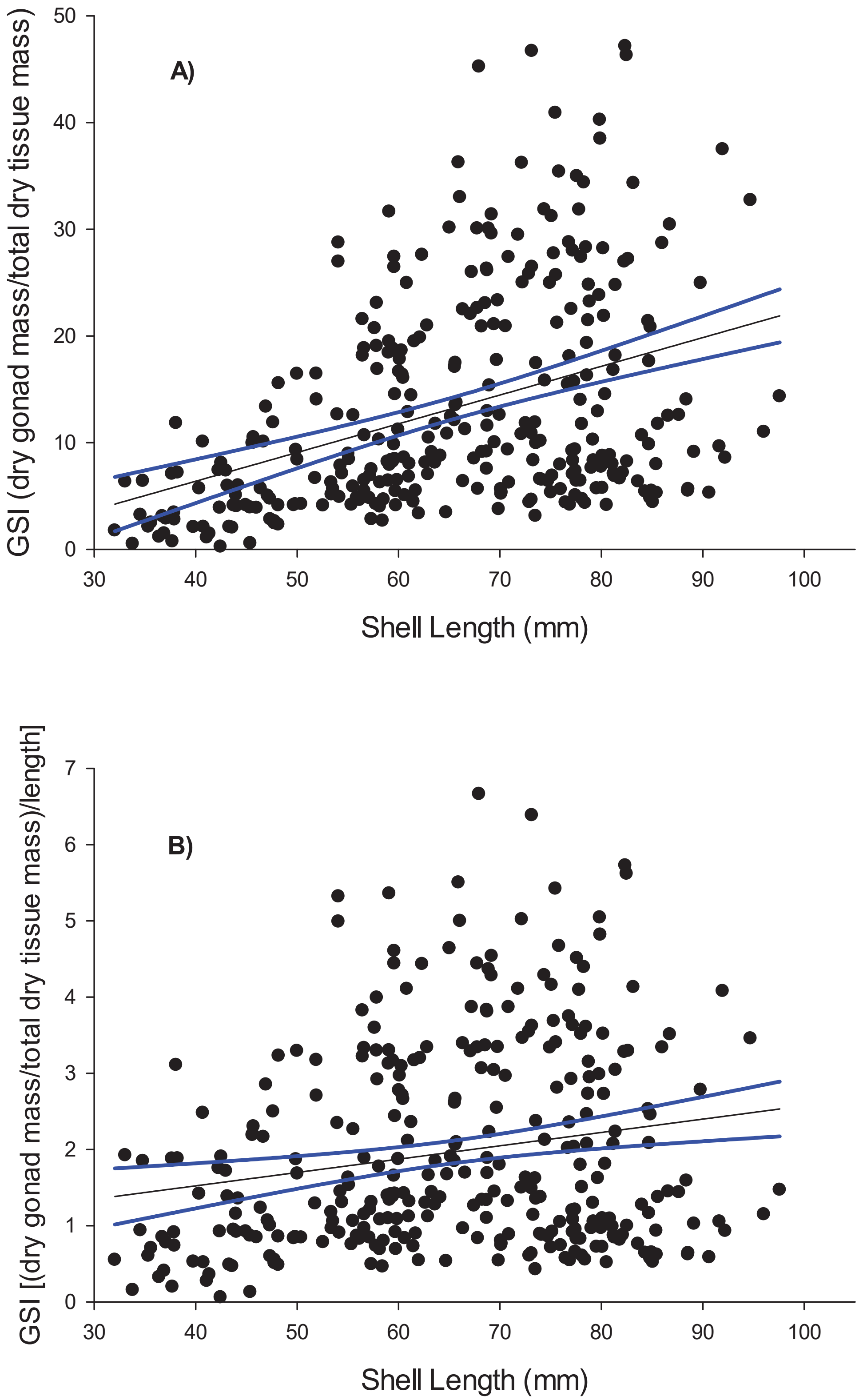

A)
GSI (dry gonad mass/total dry tissue mass)
Shell Length (mm)
B)
GSI [(dry gonad mass/total dry tissue mass)/length]
Shell Length (mm)

Figure 30.

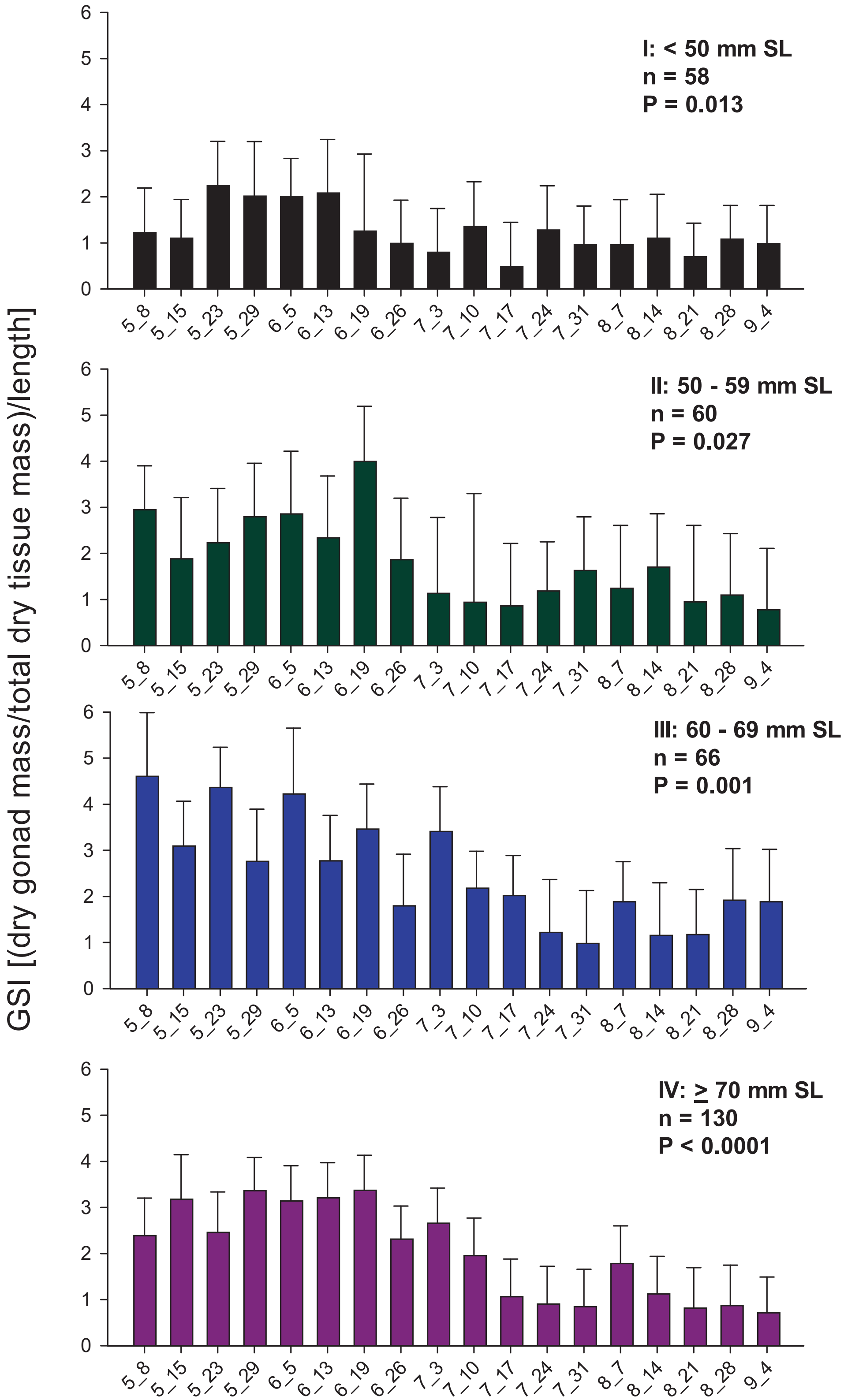

GSI [(dry gonad mass/total dry tissue mass)/length]
I: < 50 mm SL
n = 58
P = 0.013
II: 50 - 59 mm SL
n = 60
P = 0.027
III: 60 - 69 mm SL
n = 66
P = 0.001
IV: ≥ 70 mm SL
n = 130
P < 0.0001
0
1
2
3
4
5
6
5_8
5_15
5_23
5_29
6_5
6_13
6_19
6_26
7_3
7_10
7_17
7_24
7_31
8_7
8_14
8_21
8_28
9_4

Figure 31.

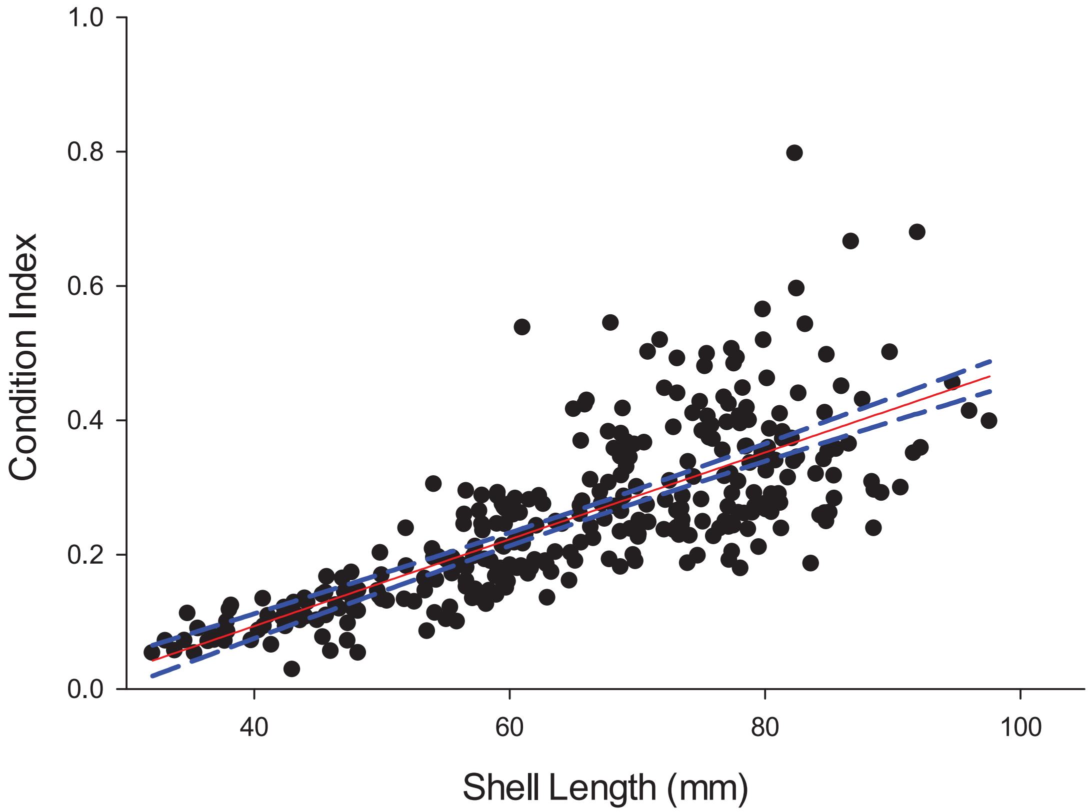
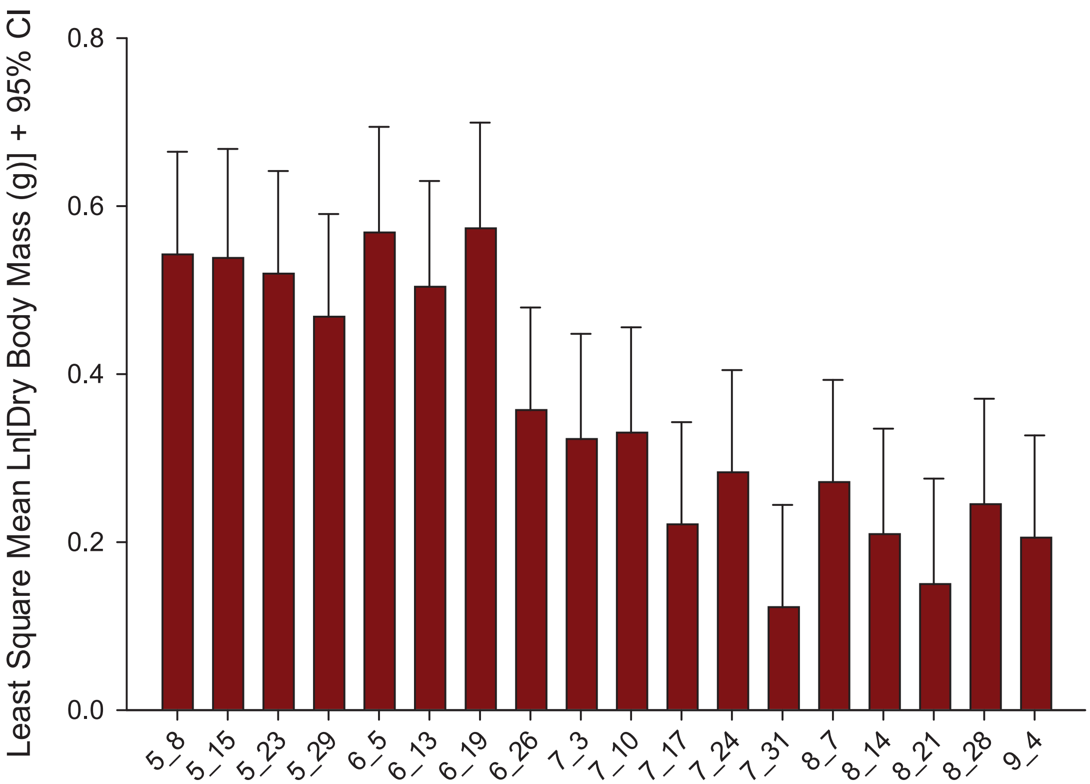


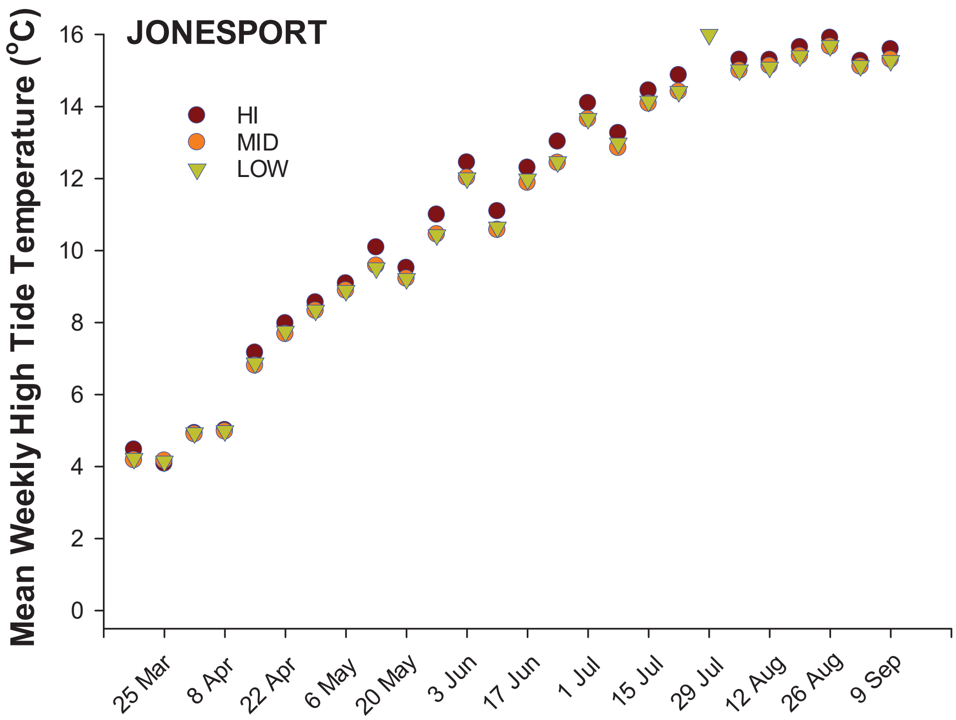
Figure 32.



Least Square Mean Ln[Dry Body Mass (g)] + 95% CI
0.8
0.6
0.4
0.2
0.0
5_8
5_15
5_23
5_29
6_5
6_13
6_19
6_26
7_3
7_10
7_17
7_24
7_31
8_7
8_14
8_21
8_28
9_4

Figure 33.

A)


JONESPORT
HI
MID
LOW
Mean Weekly High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
25 Mar
8 Apr
22 Apr
6 May
20 May
3 Jun
17 Jun
1 Jul
15 Jul
29 Jul
12 Aug
26 Aug
9 Sep

B)

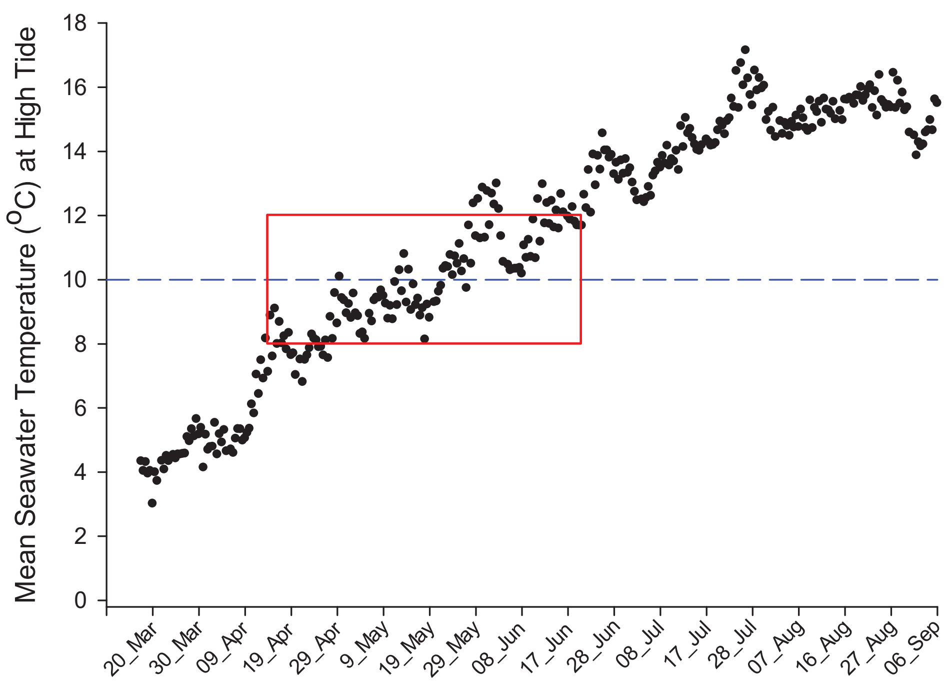
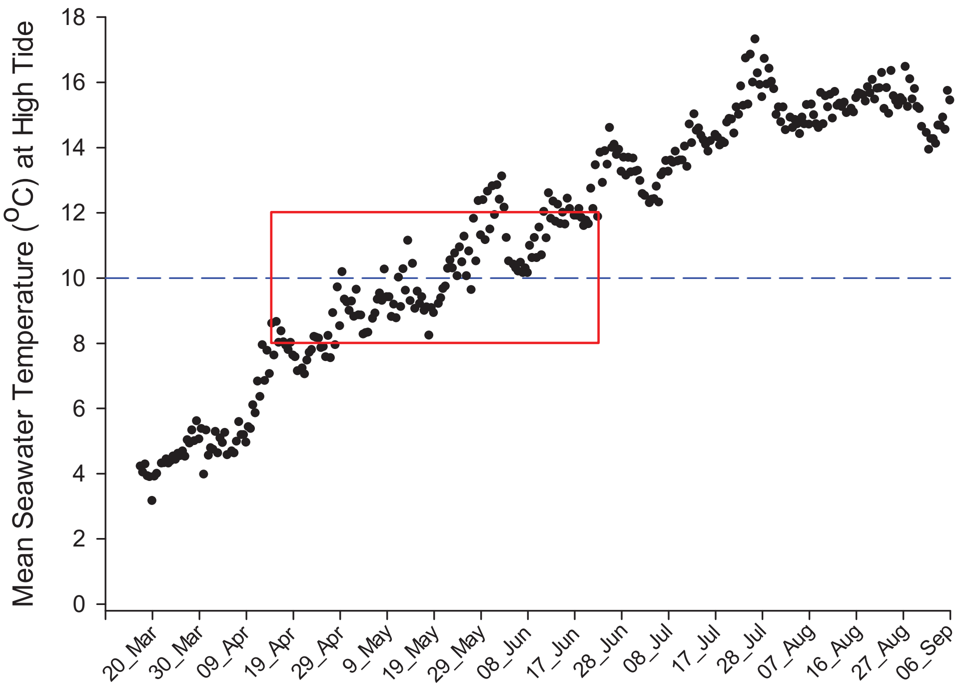

Mean Seawater Temperature (°C) at High Tide
0
2
4
6
8
10
12
14
16
18
20_Mar
30_Mar
09_Apr
19_Apr
29_Apr
9_May
19_May
29_May
08_Jun
17_Jun
28_Jun
08_Jul
17_Jul
28_Jul
07_Aug
16_Aug
27_Aug
06_Sep

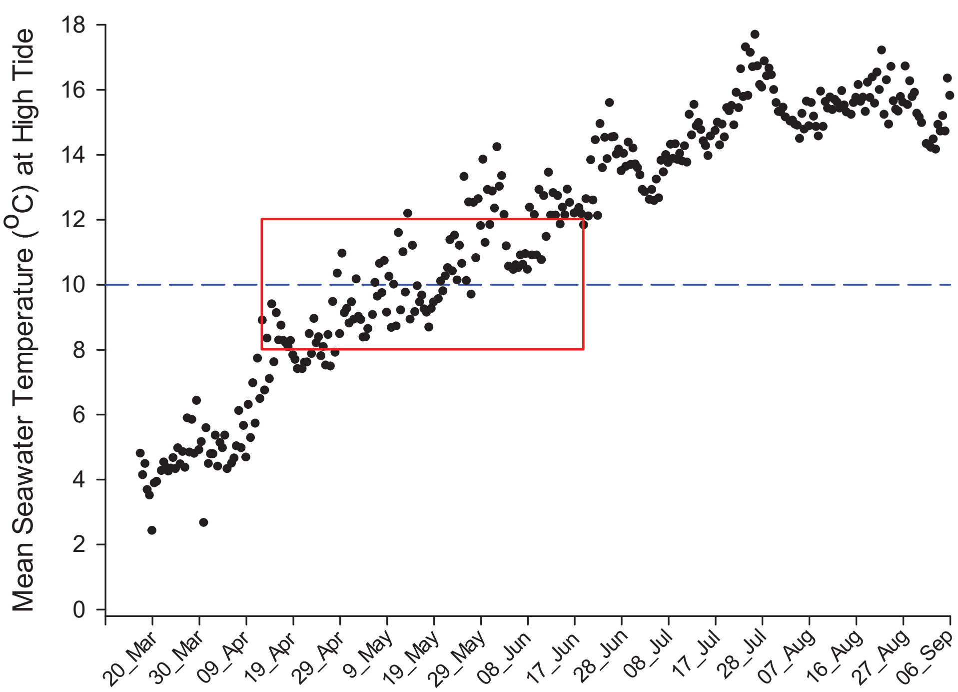
Figure 34.

A)



Mean Seawater Temperature (^{O}C) at High Tide
0
2
4
6
8
10
12
14
16
18
20_Mar
30_Mar
09_Apr
19_Apr
29_Apr
9_May
19_May
29_May
08_Jun
17_Jun
28_Jun
08_Jul
17_Jul
28_Jul
07_Aug
16_Aug
27_Aug
06_Sep

B)



Mean Seawater Temperature (^{O}C) at High Tide
0
2
4
6
8
10
12
14
16
18
20_Mar
30_Mar
09_Apr
19_Apr
29_Apr
9_May
19_May
29_May
08_Jun
17_Jun
28_Jun
08_Jul
17_Jul
28_Jul
07_Aug
16_Aug
27_Aug
06_Sep

Figure 35.

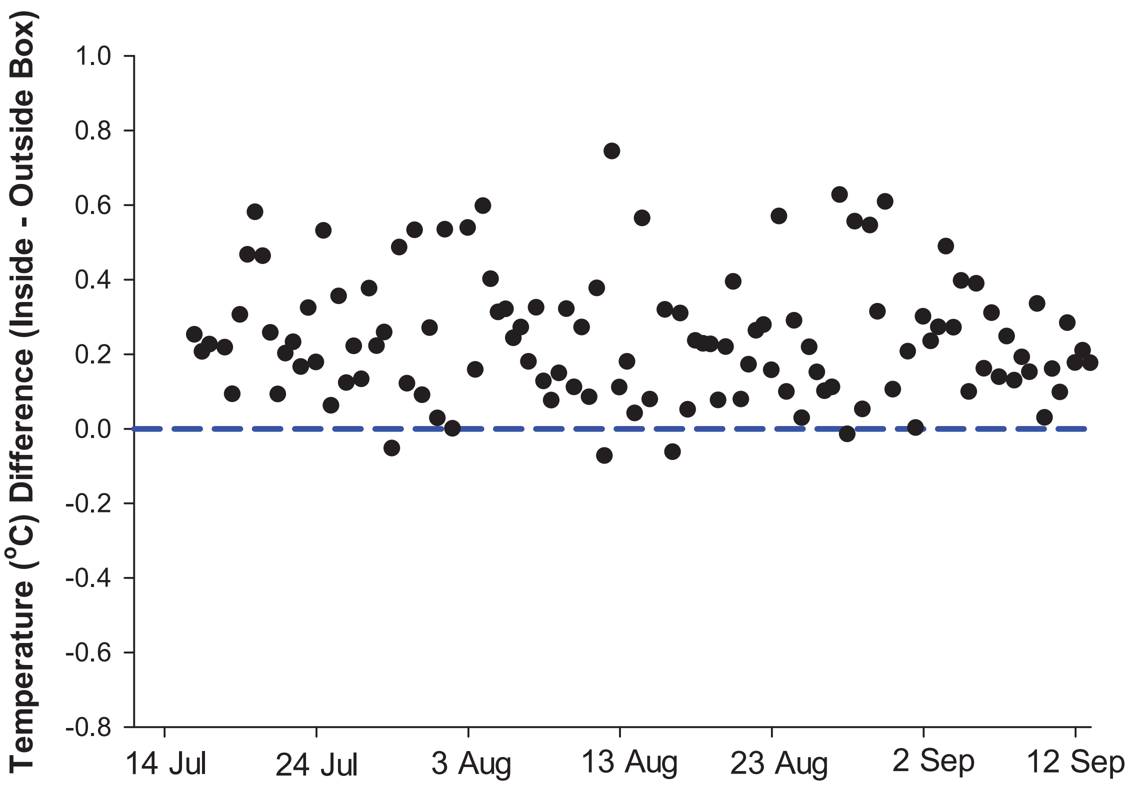
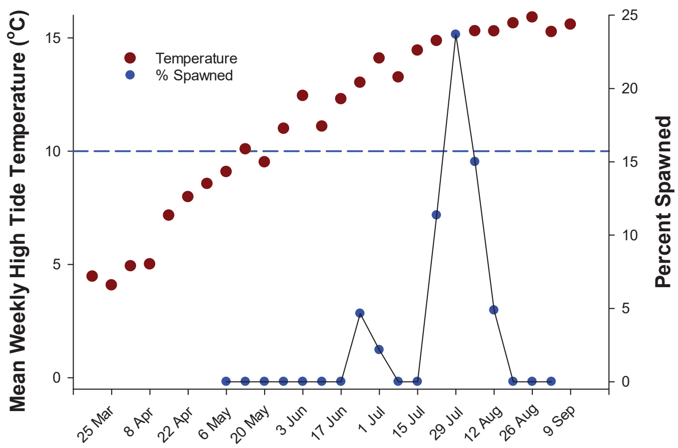
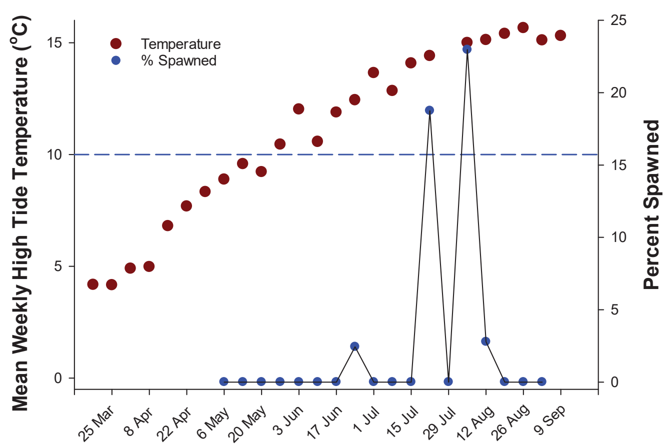

Temperature (°C) Difference (Inside - Outside Box)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
14 Jul
24 Jul
3 Aug
13 Aug
23 Aug
2 Sep
12 Sep

Figure 36.

A)



B)



C)

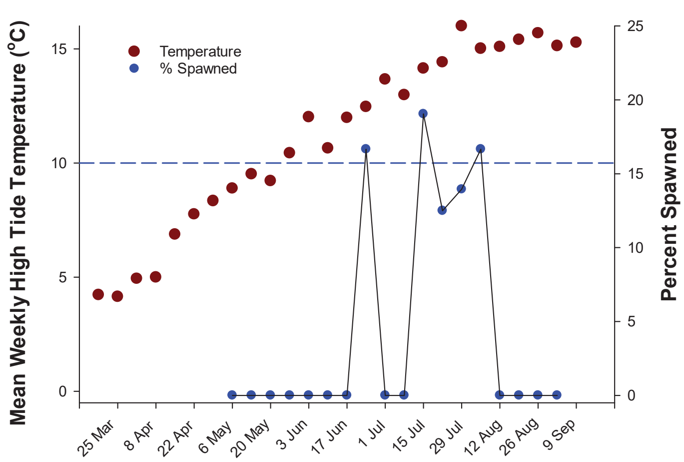
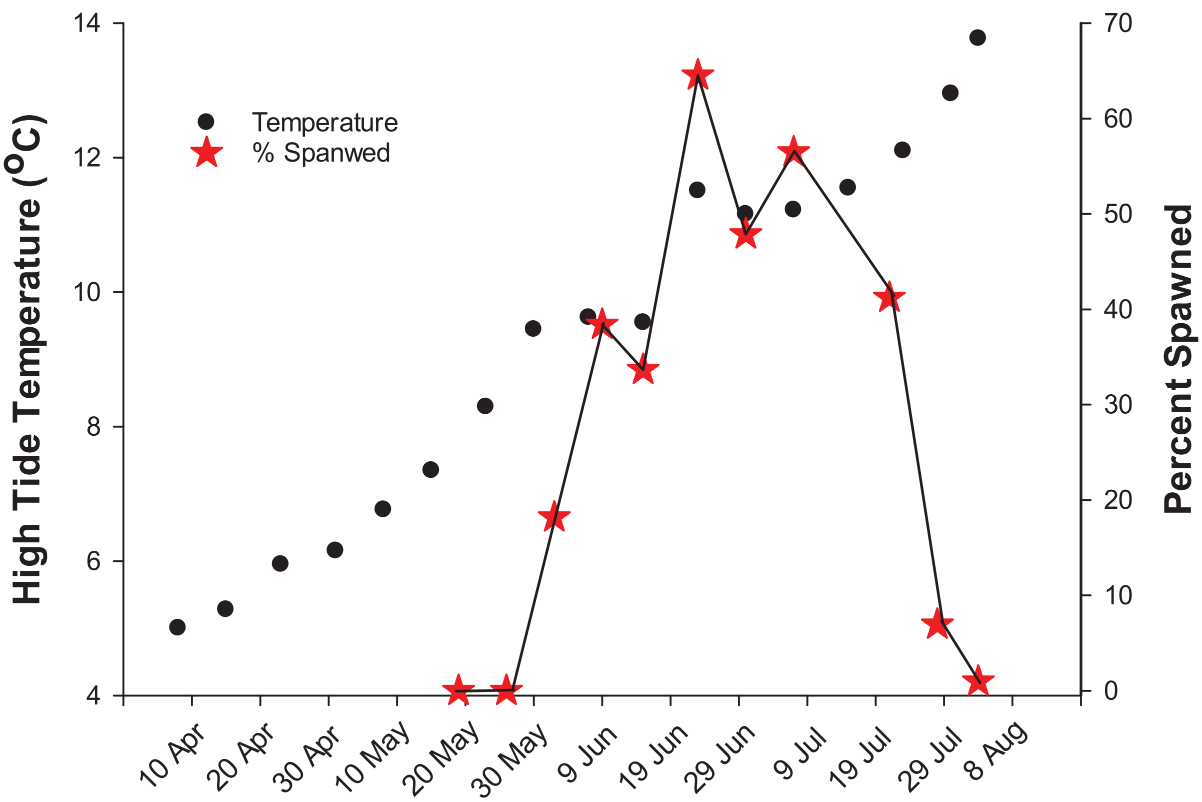
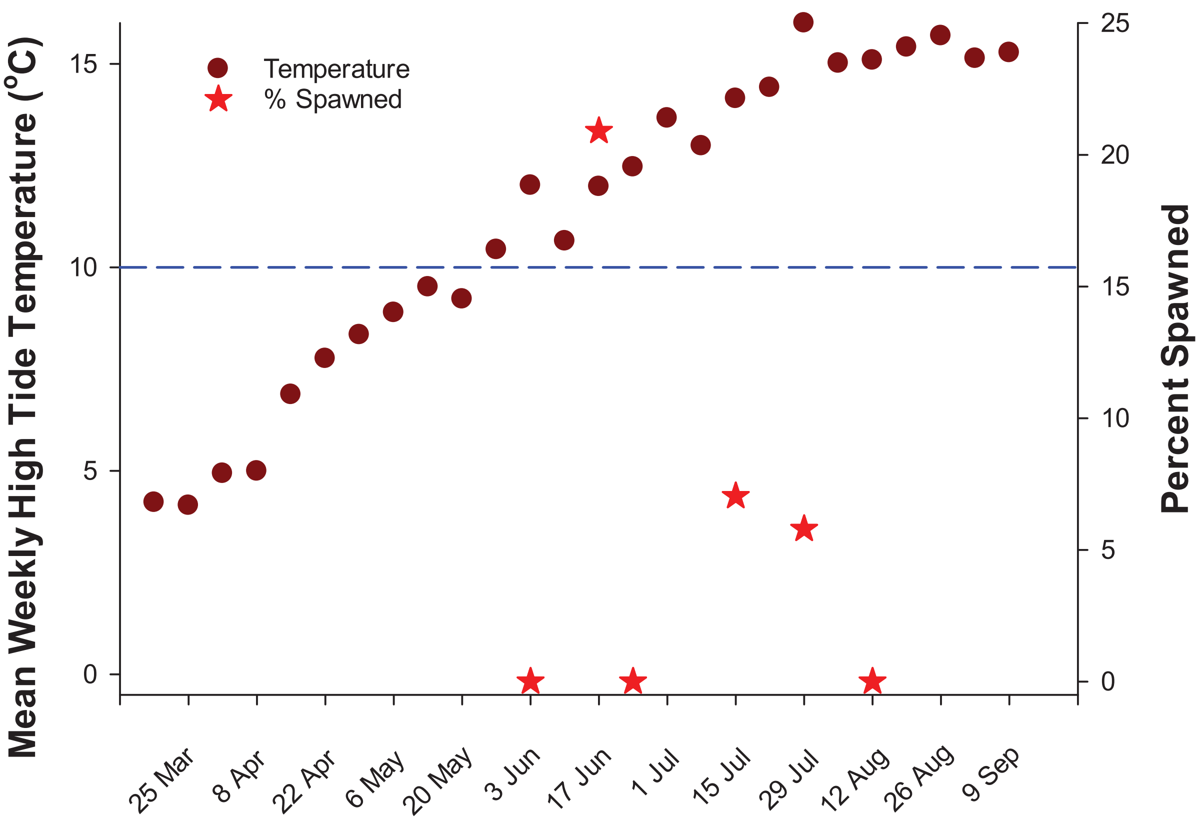


Figure 37.



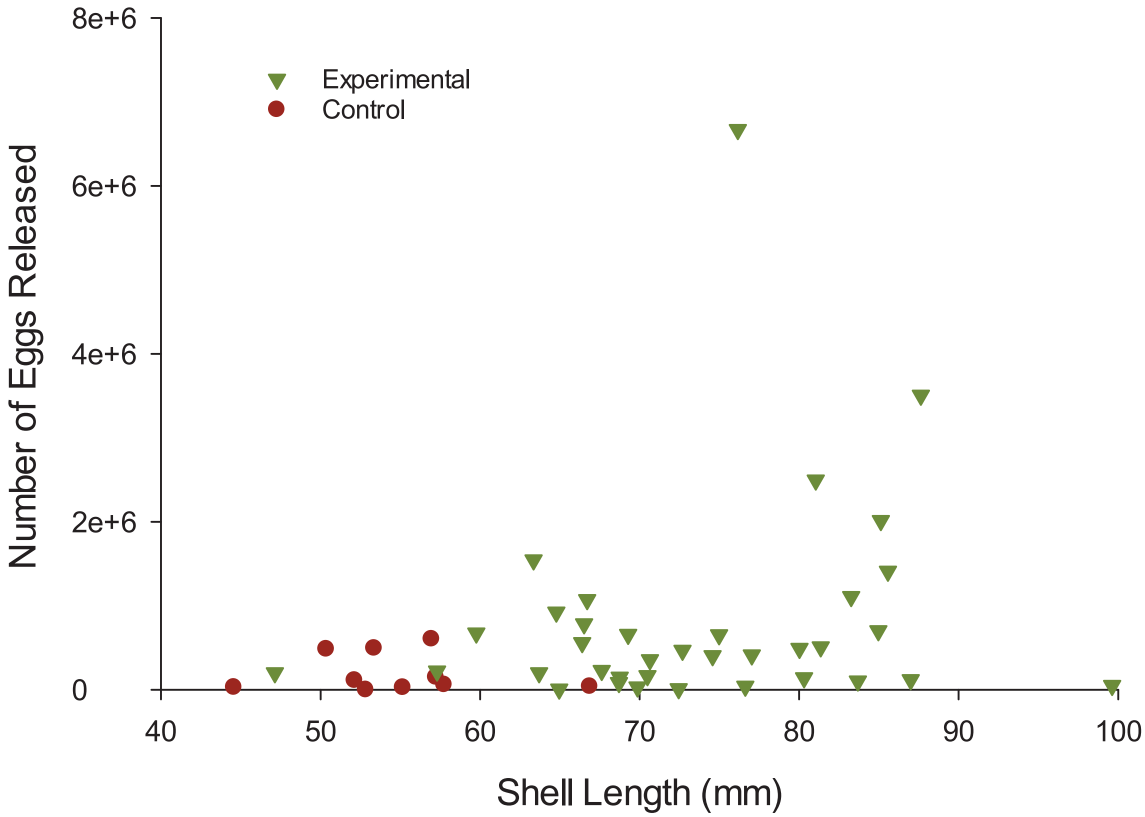
Temperature
% Spanwed
High Tide Temperature (°C)
Percent Spawned
14
12
10
8
6
4
70
60
50
40
30
20
10
0
10 Apr
20 Apr
30 Apr
10 May
20 May
30 May
9 Jun
19 Jun
29 Jun
9 Jul
19 Jul
29 Jul
8 Aug

Figure 38.



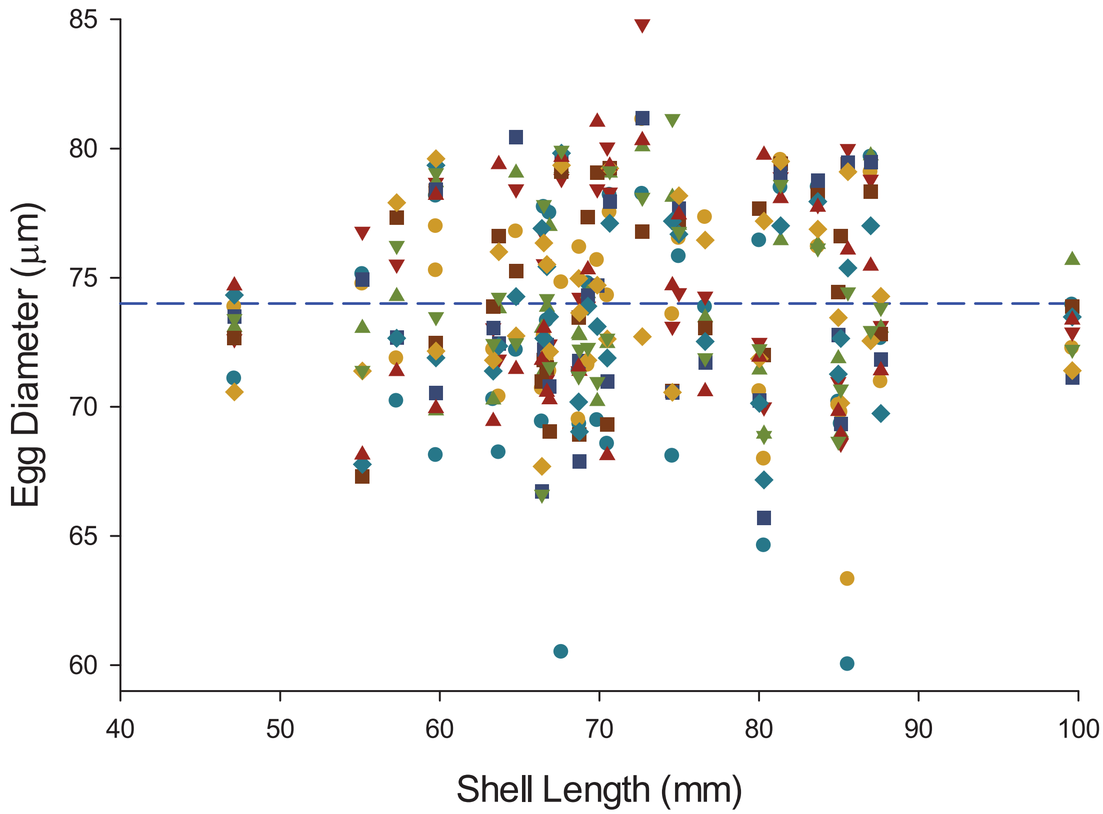
Temperature
% Spawned
Mean Weekly High Tide Temperature (°C)
Percent Spawned
0
5
10
15
20
25
25 Mar
8 Apr
22 Apr
6 May
20 May
3 Jun
17 Jun
1 Jul
15 Jul
29 Jul
12 Aug
26 Aug
9 Sep

Figure 39.



8e+6
6e+6
4e+6
2e+6
0
Number of Eggs Released
Experimental
Control
40
50
60
70
80
90
100
Shell Length (mm)

Figure 40.



85
80
75
70
65
60
Egg Diameter (µm)
40
50
60
70
80
90
100
Shell Length (mm)

Figure 41.

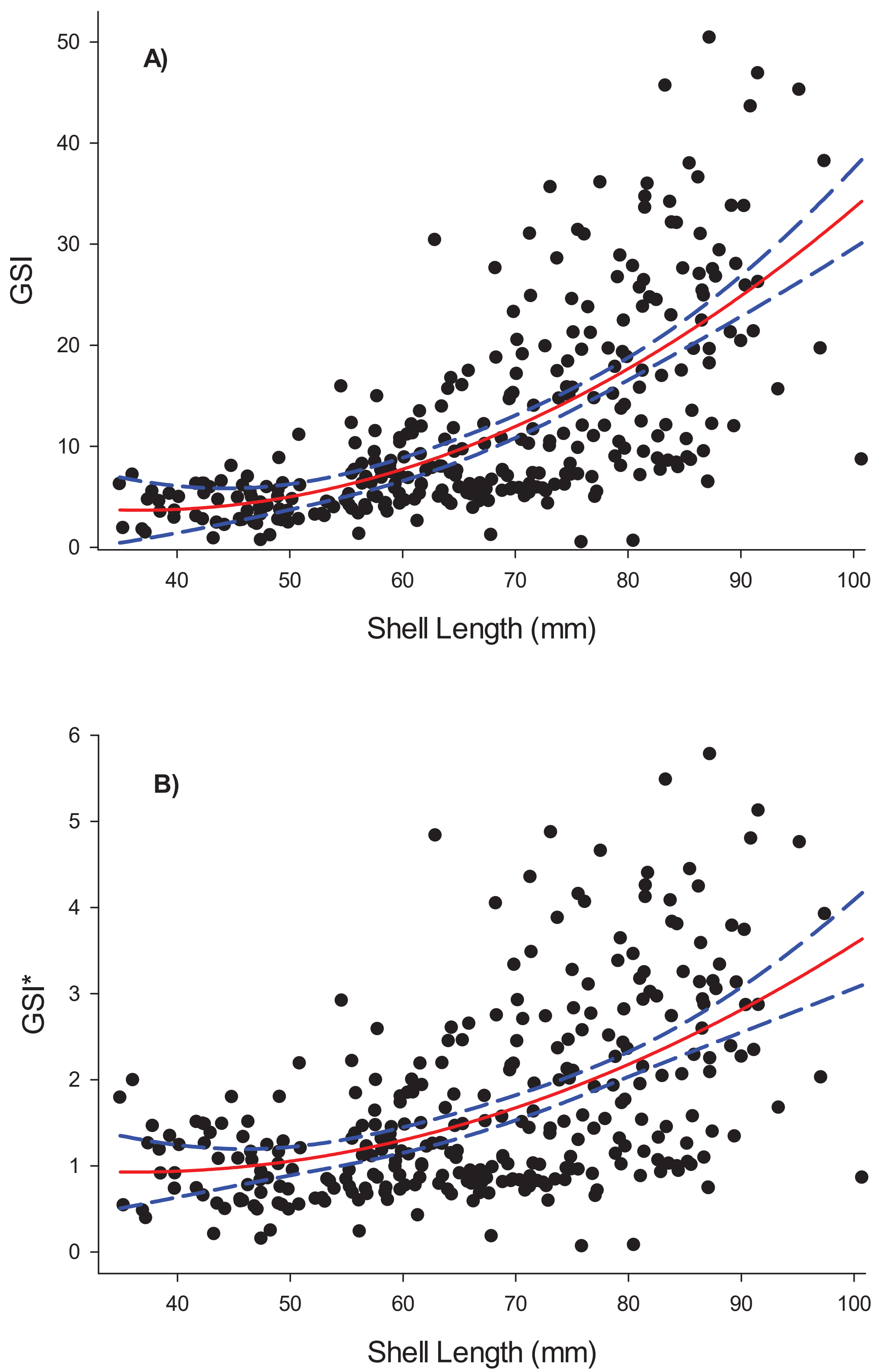

A)
GSI
50
40
30
20
10
0
40
50
60
70
80
90
100
Shell Length (mm)
B)
GSI*
6
5
4
3
2
1
0
40
50
60
70
80
90
100
Shell Length (mm)

Figure 42.

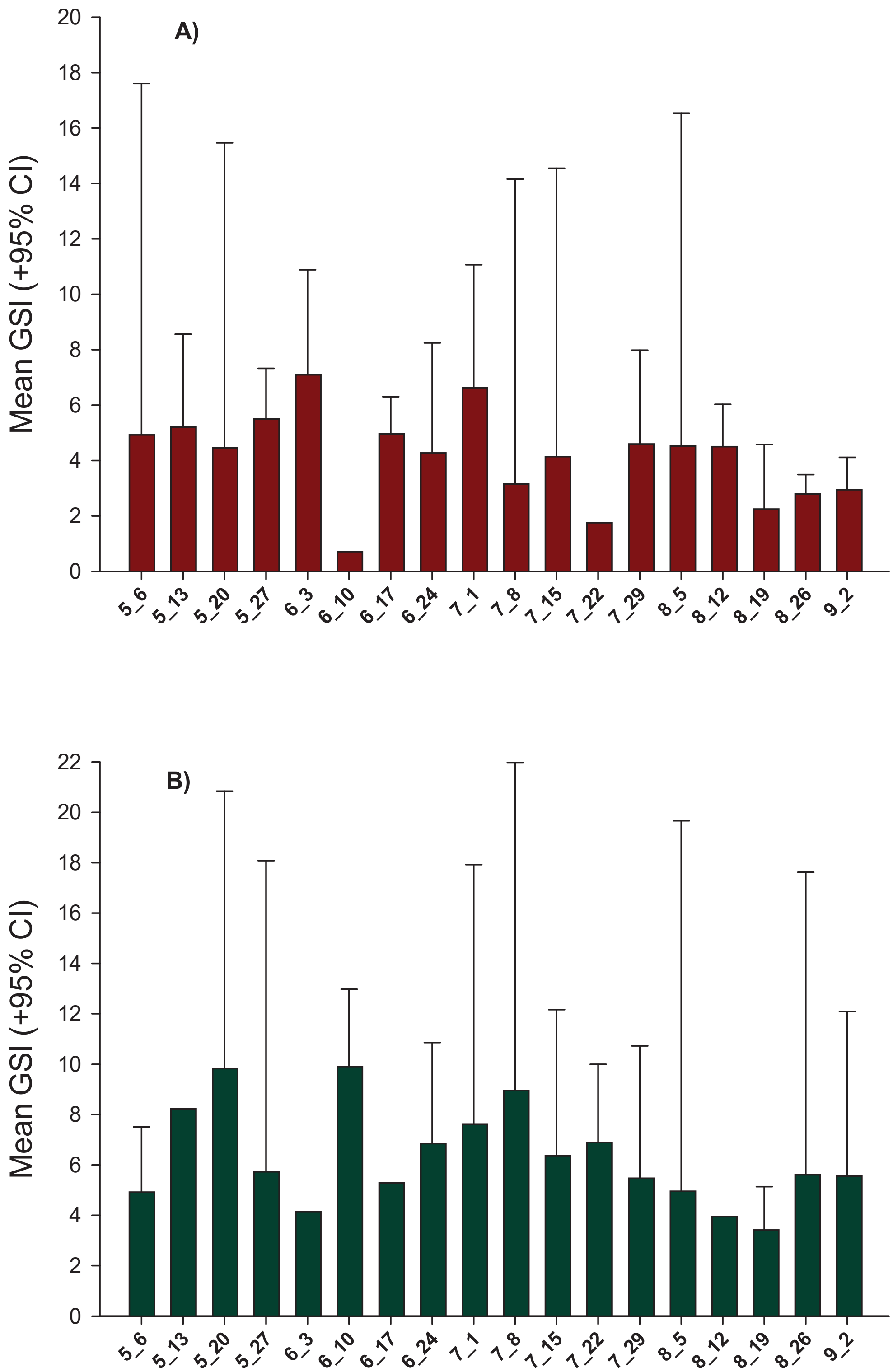
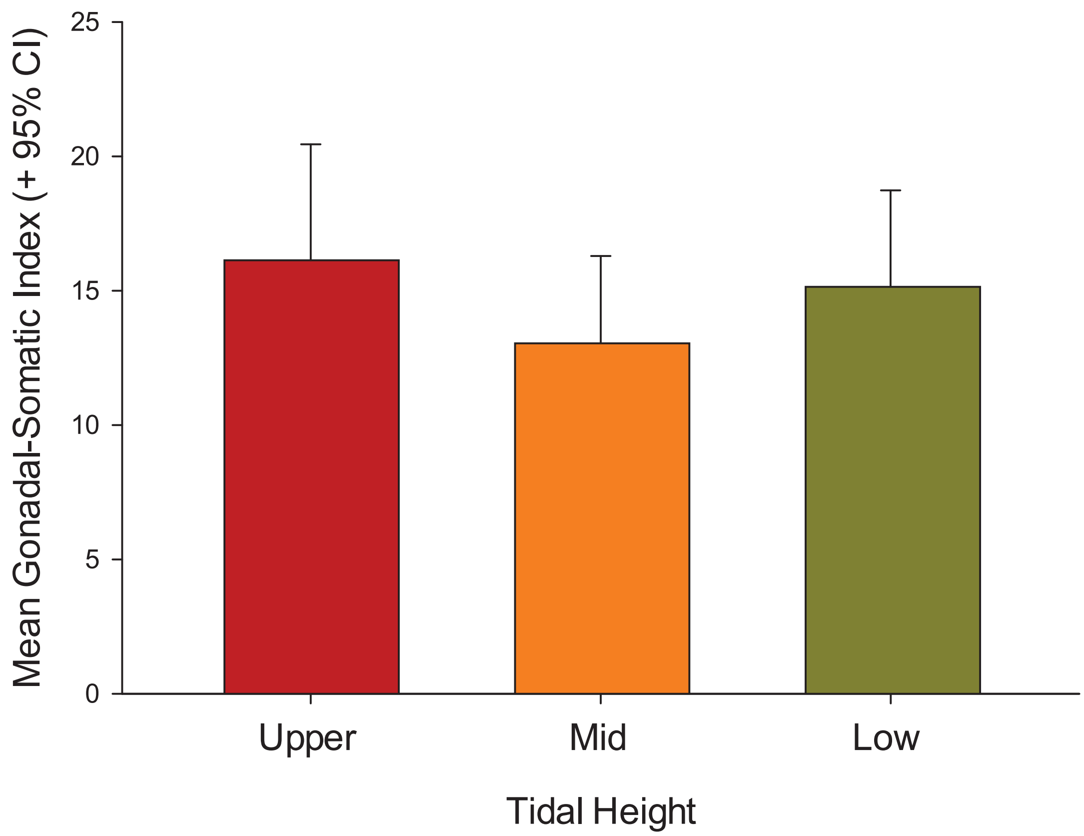

A)
B)
Mean GSI (+95% CI)
0
2
4
6
8
10
12
14
16
18
20
22
5_6
5_13
5_20
5_27
6_3
6_10
6_17
6_24
7_1
7_8
7_15
7_22
7_29
8_5
8_12
8_19
8_26
9_2

Figure 43.



Mean Gonadal-Somatic Index (+ 95% CI)
25
20
15
10
5
0
Upper
Mid
Low
Tidal Height

Figure 44.

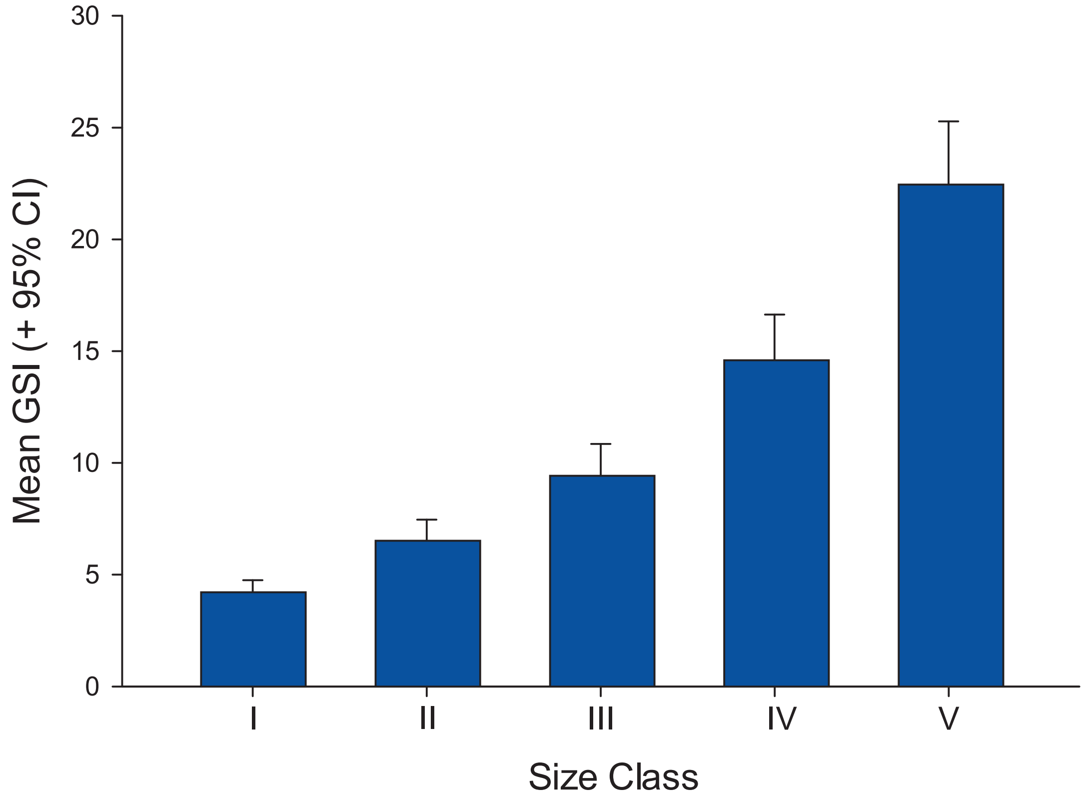


Figure 45.

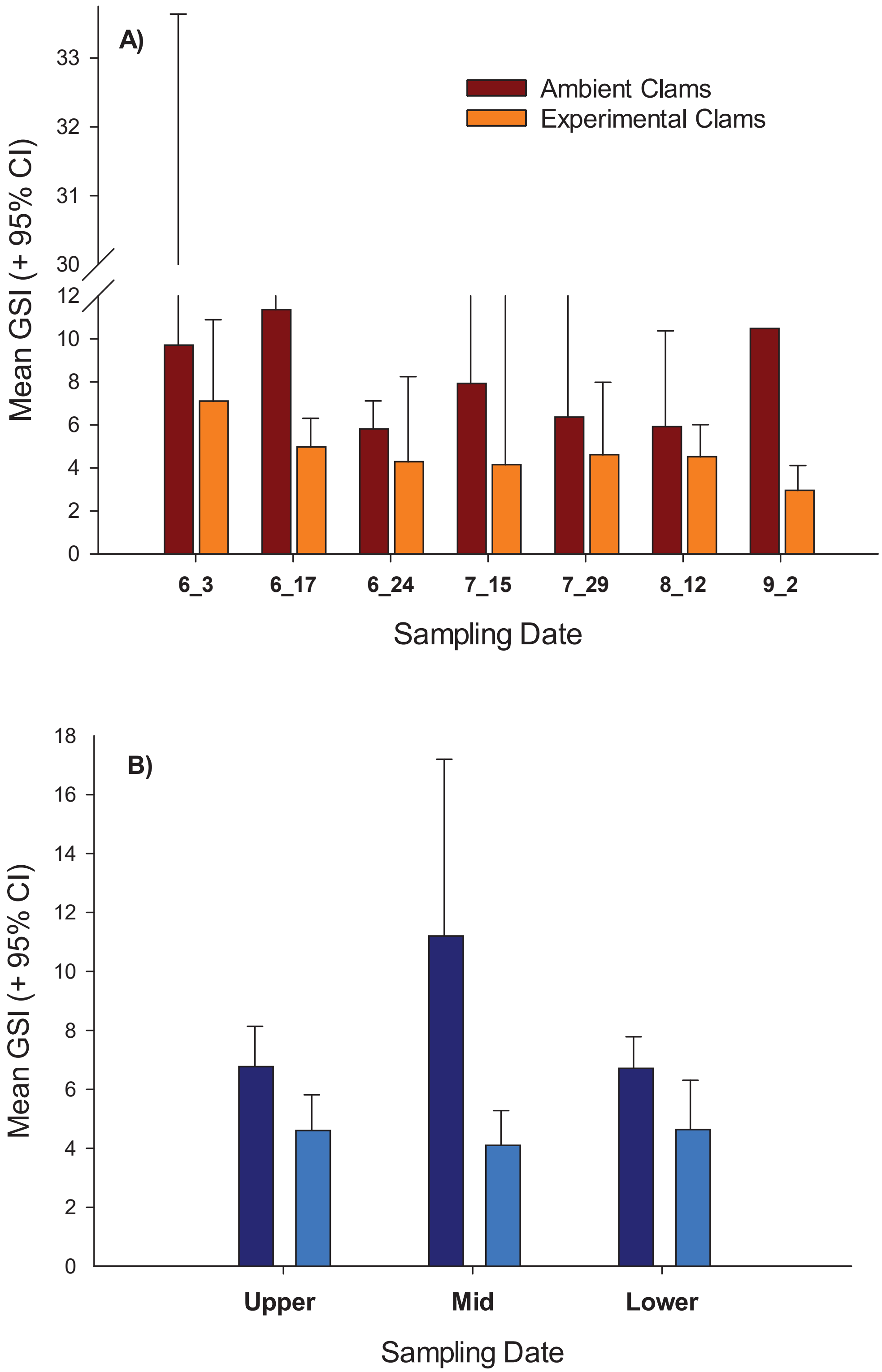
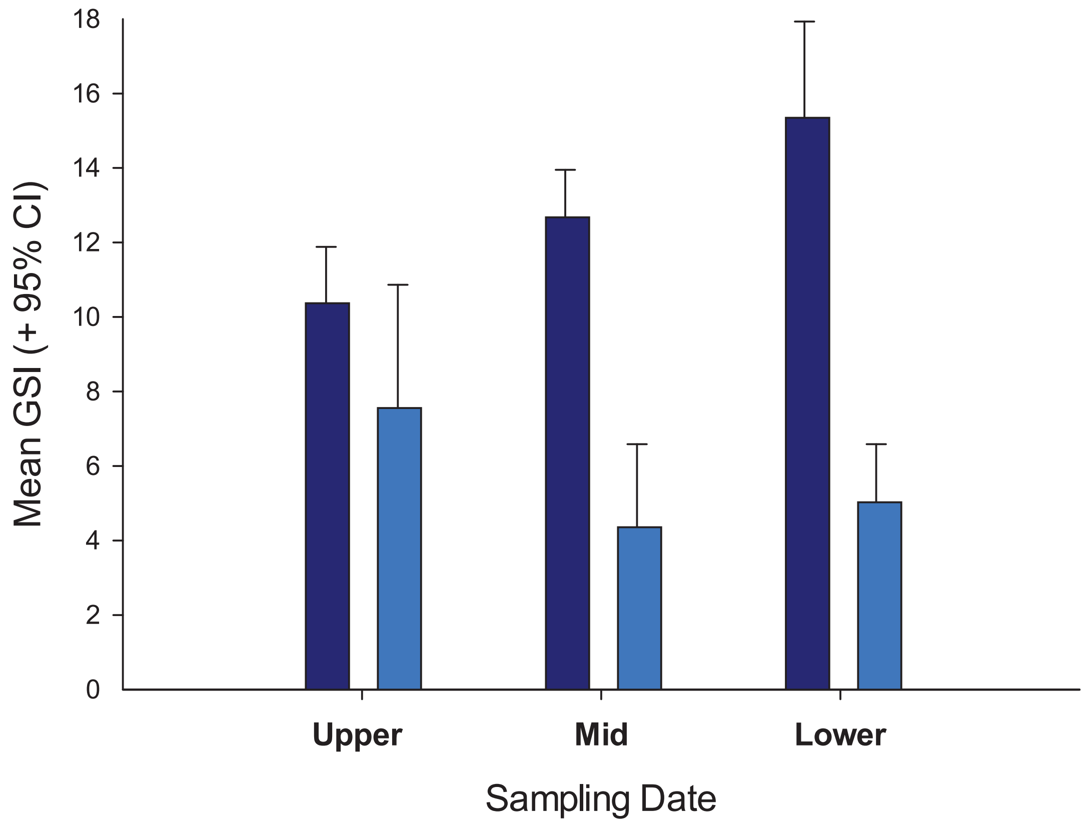
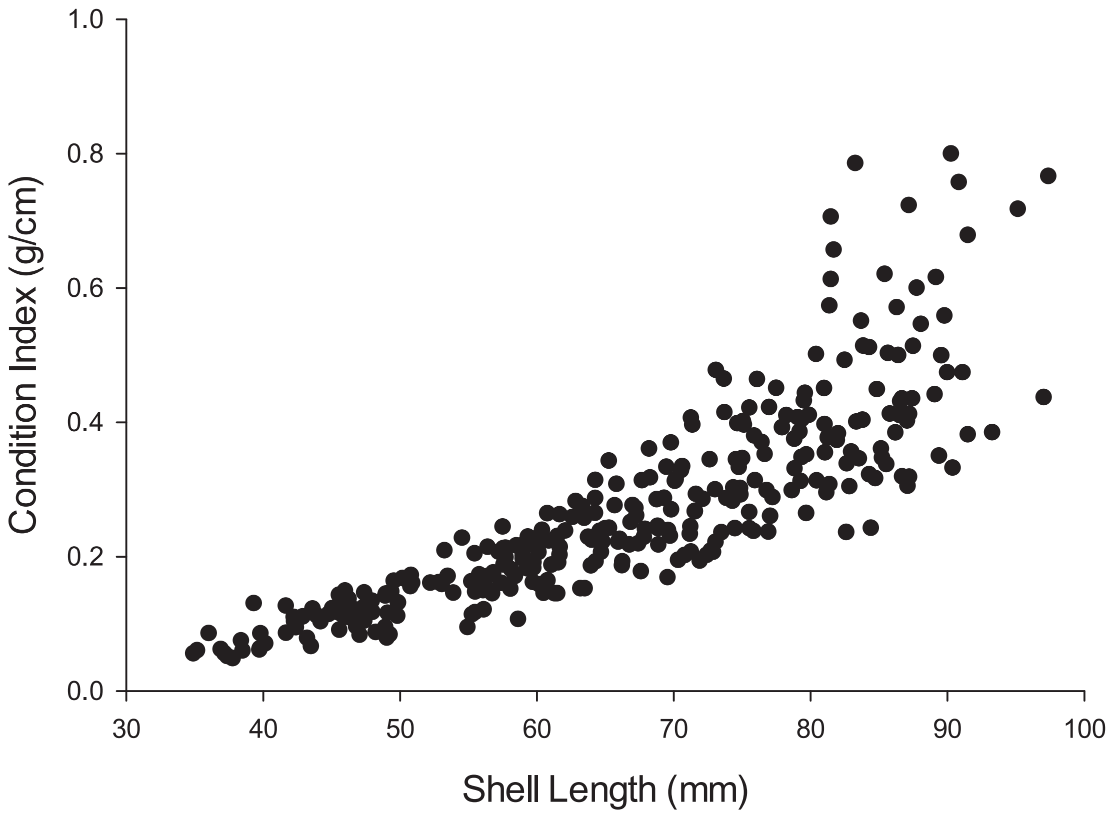

A)
Ambient Clams
Experimental Clams
Mean GSI (+ 95% CI)
0
2
4
6
8
10
12
30
31
32
33
6_3
6_17
6_24
7_15
7_29
8_12
9_2
Sampling Date
B)
Mean GSI (+ 95% CI)
0
2
4
6
8
10
12
14
16
18
Upper
Mid
Lower
Sampling Date

Figure 46.



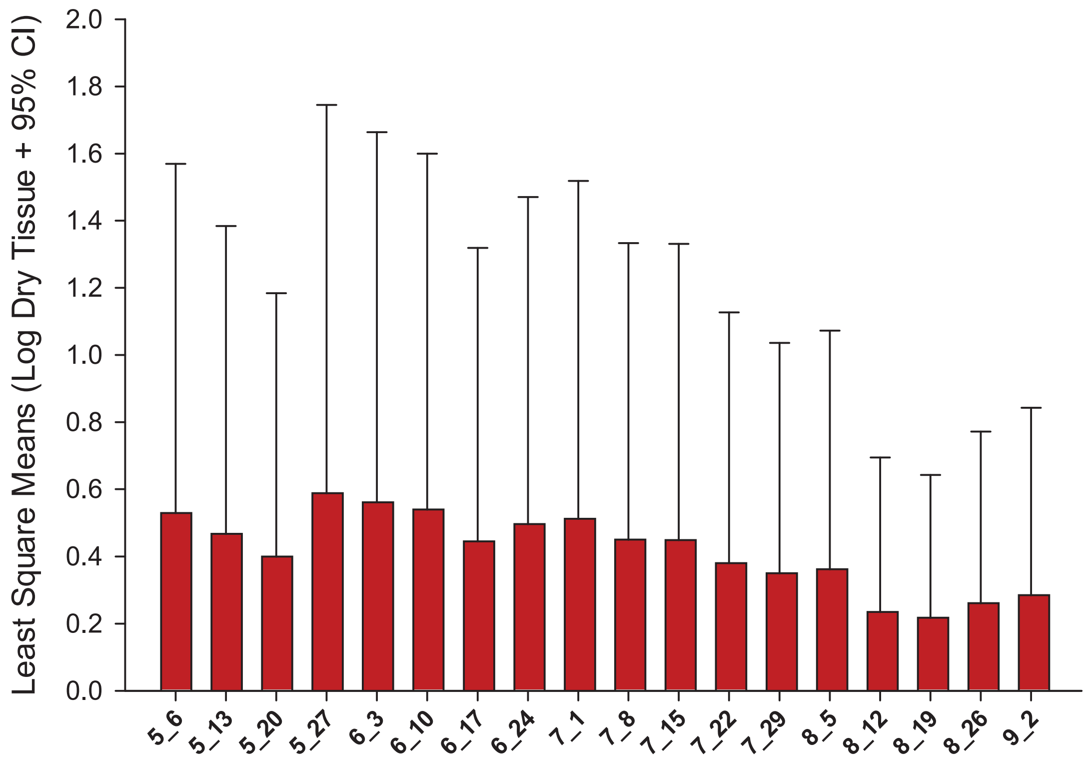
Mean GSI (+ 95% CI)
18
16
14
12
10
8
6
4
2
0
Upper
Mid
Lower
Sampling Date

Figure 47.



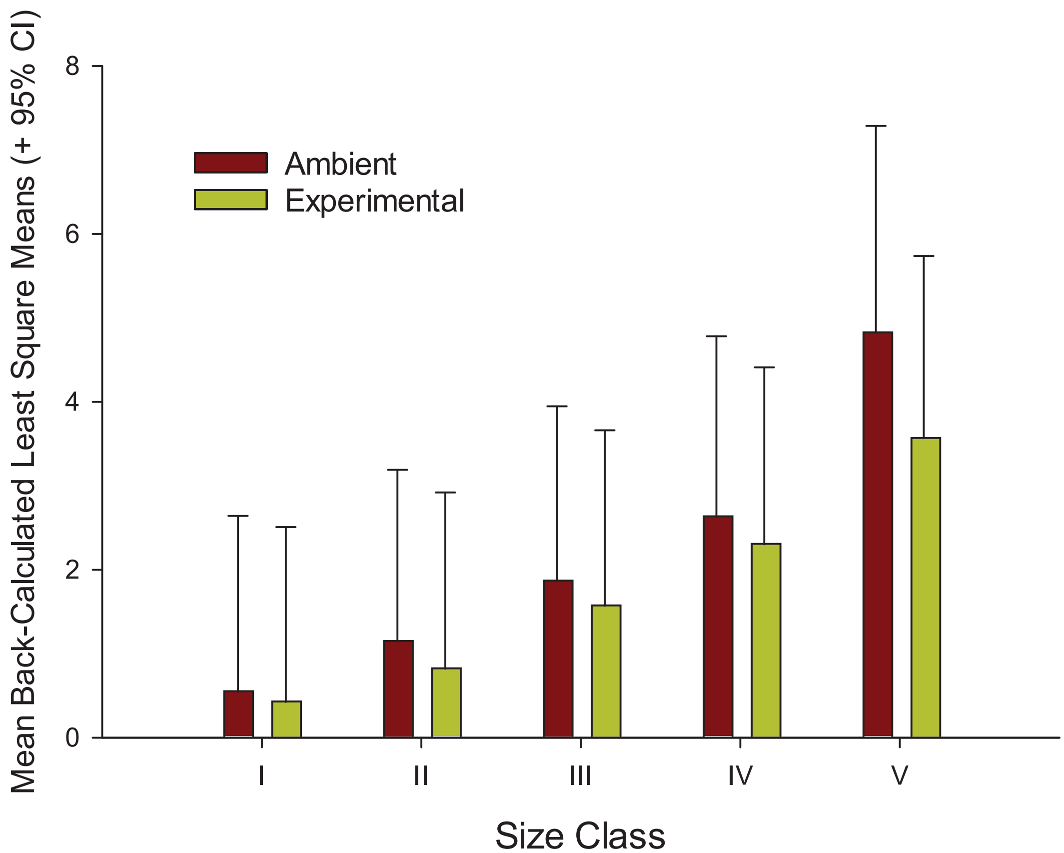
1.0
0.8
0.6
0.4
0.2
0.0
Condition Index (g/cm)
30
40
50
60
70
80
90
100
Shell Length (mm)

Figure 48.



Least Square Means (Log Dry Tissue + 95% CI)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
5_6
5_13
5_20
5_27
6_3
6_10
6_17
6_24
7_1
7_8
7_15
7_22
7_29
8_5
8_12
8_19
8_26
9_2

Figure 49.



Mean Back-Calculated Least Square Means (+ 95% CI)
8
6
4
2
0
Ambient
Experimental
I
II
III
IV
V
Size Class