Final Report (8/31/2024)

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."

Summary

Soft-shell clams, *Mya arenaria* L., have been harvested commercially for bait and consumption since before Maine became a state in 1820. For the past half century, clam landings in Maine have declined by over 80%, with 2023 representing the lowest statewide landings ever recorded (5.2 million pounds worth $13.9 million). The value of the fishery has also declined by over 70% during the same time. Since the mid-1970s, the drop in production has meant the gradual loss of jobs for over 2,500 harvesters as well as dozens of clam buyers, shippers, and others along the transportation, marketing, and sales chain. This decline has occurred at the same time ocean warming in the Gulf of Maine has become so severe that summer seawater temperatures along the southwest coast are similar to those observed in southern New England. This warming has both direct and indirect effects on intertidal and subtidal ecosystems. A major effect of ocean warming has been an explosion of the invasive green crab, *Carcinus maenas* (L.). While green crabs are consumed by many species from ducks, shorebirds, fish, lobsters, and other predators, collectively these predators cannot keep crab populations in check, and in many areas along Maine's extensive soft-bottom intertidal, the distribution of clams has become restricted to the upper shore, where crab consumption is somewhat limited by tidal inundation. As clam populations dwindle towards commercial extinction, questions concerning reproduction and fecundity have become pressing issues for managers to consider; however, the most recent investigation of clam reproductive cycles in Maine occurred during the late 1950s and early 1960s. While we understand that clam spawning occurs in the late spring/early summer in most places, we do not know if reproductive cycles and the timing of spawning events vary from the southwest to the downeast coast. Similarly, there is a lack of information about how tidal height or clam size affects reproductive cycle or fecundity – the amount of eggs produced by individuals. For example, if clams do not undergo reproductive senility with increasing size/age, then it might be prudent to initiate conversations with industry representatives about creating an upper size limit using the same logic managers have applied to Maine's iconic lobster and sea urchin fishery.

Soft-shell clam reproductive cycle and fecundity was investigated along a tidal gradient at three intertidal locations along the Maine coast (Brunswick, southern coast; Bremen, midcoast; Jonesport, downeast coast) over 18 weeks from April/May to August/September in 2023 and 2024. In both years, clams ranged in size from 30-110 mm in shell length (SL), and were added to wooden field boxes (experimental units [EU]; 1-ft x 1-ft x 6-inches deep filled with ambient sediments) with a protective cover to exclude large predators such as birds, crustaceans, and infaunal nemertean worms. At each site, 42 EU were installed in the sediments at three tidal heights (Upper; Mid; and, Lower) so that the protective top was approximately 1-inch above the sediment surface. In March during both years, clams of six size classes were distributed equally to each of the 126 EU at each site. In 2023 and 2024, 24 clams (4 from each size class) and 18 clams (3 from each size class), respectively, were added to each EU. Beginning in early May (2023) and mid-April (2024), clams from two EU per tidal height were collected weekly and transported to the Downeast Institute where attempts were made to induce spawning over 6-7 hr using a thermal shock technique. When individual clams spawned, they were isolated to retain gametes that were counted and measured (eggs, using a FlowCam) or used to estimate viability (sperm and eggs). A subset of animals over the range of sizes available on a particular day that were not induced to release gametes during the 6-7 hr period were taken to examine gonadal and condition indices as well as for histological analyses.

In both years, at all three study sites, spawning was a protracted, not discrete, event over the 18-weeks. In 2023, induced spawning of clams from Jonesport and Bremen occurred between mid-June through mid-August, and in Brunswick from mid-June through late August. In 2024, spawning occurred earlier at each site, commencing in mid-May and ceasing in mid-July in Jonesport, from early May to late August in Bremen, and from late April to mid-June in Brunswick. In 2023, all spawning was observed at each site after seawater temperatures exceeded 10^{o}C, but in 2024, spawning commenced at each site when seawater temperatures were approaching or were slightly warmer than 10^{o}C. Tidal height influenced spawning in the laboratory. In 2023, a higher percentage of clams from the upper and mid intertidal in Brunswick and Bremen spawned compared with those in the low, but the same pattern was not observed in Jonesport where tidal height did not seem important in terms of percent of animals spawning. In

2024, the pattern at the midcoast (Bremen) and southern (Brunswick) sites was similar to that observed in 2023; however, in Jonesport, clams in mid intertidal EU spawned more frequently and at a higher percentage than those at the upper and lower tidal heights.

In both years and at all three sites, the relationship between gonad dry weight and shell length followed a power exponential function ($Y = aX^b$) suggesting that gamete production does not decline with increasing size/age. Gonadal (GSI) and condition indices essentially were mirror images of each other in both years. In 2023, mean GSI declined over time in Brunswick, but showed significant decreases twice – once in late May that was not observed in the lab; another in mid-June that was – followed by a steady decline from early July to late August. At Bremen, mean GSI dropped between 13 and 19 June coinciding with induced spawning events in the lab, but that was the only time when GSI and results from induced spawning matched. Clams from Jonesport showed no relationship with tidal height or sampling date; however, mean GSI increased with increasing clam size. In 2024, mean GSI of clams in Brunswick varied through time, but depended on tidal height. For example, mean GSI of lower intertidal clams increased for a month beginning in late April declining abruptly over a week (-33%) at the end of May followed by a steady decrease through mid-August when sampling ceased. For mid intertidal and upper intertidal clams, peak spawning occurred in the laboratory on 7 May, but this pattern was not observed in terms of the gonad index that declined precipitously twice for mid intertidal clams (14-21 May; 9-16 July). The pattern of mean GSI for upper intertidal clams from Brunswick more closely resembled what was occurring at the mid intertidal. In Bremen, mean GSI decreased sharply on two occasions in the mid and lower intertidal (once in late May and once in late June) that coincided with spawning events in the laboratory. At the upper intertidal in Bremen, mean GSI peaked on 20 May and 1 July, but there was not good concordance with spawning events in the lab that occurred on 27 May and 24 June. In Jonesport, peak spawning occurred in the upper and mid intertidal on 15 June, and this coincided with the greatest value of mean GSI. For clams in low tide EU, peak spawning occurred on 13 July that coincided with the lowest mean GSI.

The histological slides helped to interpret results from spawning events in the laboratory and gonad index. In 2023, clams from the mid intertidal in Brunswick appeared to spawn from the first (9 May) to last (3 October) sampling date (at least 33% of animals were in a spawning condition on each date). Upper intertidal clams showed a delay in spawning compared with animals lower on the shore, as they began to spawn on 30 May and were still spawning on the last collection date (3 October). The pattern in the low intertidal was more closely aligned with the mid intertidal where ≥ 66% of animals were in a spawning condition during the month of May, and, for the most part spawned continuously from 11 July to the last sampling date. Clams from the low intertidal in Bremen commenced spawning as early as the first sampling date (8 May), and from 29 May to the final sampling date 33-100% of clams were in a spawning condition. Upper and mid intertidal clams from Bremen behaved similarly commencing spawning one week after clams from the low first started to spawn, and continuing (16-100%) to the final sampling date (4 September). In Jonesport, spawning was first observed in lower intertidal clams as early as 13 May followed two weeks later in the mid intertidal and four weeks later by upper intertidal animals. After 17 June, clam spawning was observed on each sampling date until 2 September, except once for upper intertidal animals in mid-July. In 2024, the histology slides associated with Brunswick clams showed spawning activity (≥ 50%) on every sampling date at all three tidal heights. In Bremen, spawning (≥ 50% of animals) occurred on every sampling date in the mid and low (29 April through 26 August). Clams in upper intertidal EU commenced spawning after 29 April, with ≥ 50% in a spawning condition from 13 May until the final sampling date. Jonesport clams also showed a delay in spawning as a function of tidal height with lower and mid intertidal clams spawning first (18 May), and continuing through mid-August with ≥ 50% of animals in a spawning condition. Upper intertidal clams did not appear in a spawning condition until 1 June (83%), and, except for 8/17 (33%), remained ≥ 50% until the final sampling date on 24 August.

The relationship between clam size and number of eggs released during an induced spawning event provide valuable information for shellfish managers. The most recent quantitative assessment of fecundity was in a 1978 publication authored by D. J. Brousseau. Clams were collected from the intertidal in the Annisquam River system, Gloucester, Massachusetts, and, using histology, estimated number of eggs for different sized clams. Brousseau (1978) reported, “Average oocyte production by a 60-mm clam during a single breeding season (two spawning periods) is about 120,000; lifetime production would be in the order of 1.5×10^6 oocytes.” The data we collected showed that the relationship between eggs released per spawning and clam size followed a power exponential function, with animals at 60 mm SL capable of releasing between 3-4 million eggs, and animals around 90 mm SL capable of releasing 10-11 million eggs. These results were consistent between years, and indicate that, like lobsters and sea urchins, soft-shell clams do not undergo reproductive senility.

Lastly, mean egg diameter and egg viability were estimated in both years. Mean diameter varied between years ($\bar{x}_{2023}$ = 74.0 ± 0.9µm, n = 33; $\bar{x}_{2024}$ = 69.1 ± 0.65 µm, n = 61). Differences may be due to how egg diameter was estimated (by hand using an ocular micrometer in 2023 vs. FlowCam in 2024). Viability was assessed across 56 mating pairs in 2023 (58.0% ± 6.2%), and was independent of clam size and location. In 2024, viability was tested across 129 pairs. Overall, mean viability was 63.3 ± 4.6%. While viability was independent of size in female clams, the size of male clam in the pairing mattered. Sperm from small clams (i.e., < 50 mm SL) resulted in 25% less successful fertilization than sperm from larger animals (51.9 ± 8.9%, n = 32 vs. 67.0 ± 5.2%, n = 97).

The study also examined the size-frequency distribution of commercial size clams at a clam buying station in eastern (n = 15 samplings) and southern Maine (n = 5 samplings) conducted between September 2022 and August 2024. The cumulative data from eastern Maine showed that 12.8% of commercially-harvested soft-shell clams are ≥ 3.25 inches, 5.72% are ≥ 3.50 inches, and 1.75% ≥ 3.75 inches. In southern Maine, cumulative data across five sampling dates indicated no clams larger than 3.75 inches, and that 4.1% of clams were ≥ 3.25 inches, while 0.67% were ≥ 3.5 inches.

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) not reported previously (i.e., April – August 2024). Table 1 gives the sampling frequency (since September 2022) from this buying station. The second is the collection of data associated with a comparative field experiment initiated in March 2024 at three tidal heights (upper; mid; lower) at an intertidal flat in Brunswick (southern Maine, Bremen (midcoast Maine), and Jonesport (eastern Maine). This was the second year the comparative experiment was carried out.

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

Data were collected monthly from a commercial soft-shell clam buying station – eastern Maine (A.C. Inc. – town of Beals) – from April to August 2024 coinciding with the astronomically highest/lowest tides in 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 was 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 (sensu Peterson 1983) does not occur with increasing size/age, and if viable offspring are produced from the largest animals in the population. Commercial landings data for soft-shell clams harvested in 2023 in Maine were released by Maine's Department of Marine Resources in February 2024 that show clam landings in 2023 were at historic lows (5.2 million pounds, worth $13.9 million; Fig. 1). Since 1977 when 38.4 million pounds were landed, soft-shell clam landings have decreased by ~85%, and, using January 2024 dollars to estimate the value of the fishery since 1977, its value has declined by 71.4%.

Beginning September 2022 and continuing through August 2024, we visited A.C. Inc. 15 times, and visited Cantrell's Seafood five times (Table 1). On each date, researchers measured the shell length (SL; greatest linear anterior-posterior distance) from a representative sample of 25 clams collected from each of nine bushels sold at the buying station over a 3-day period. Results from the last five visits to A.C. Inc. (April-August 2024) are presented in Table 2 and Figs. 2-3), and cumulative results from all 15 visits to A.C. Inc. and all five visits to Cantrell's Seafood are presented in Tables 3-4 and Figs. 3a-5). 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 selected 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. This was the closest estimate to what the commercial fishery looks like coming from the harvesting location.

The cumulative data taken across 15 sampling dates from eastern Maine shows that 12.8% of commercially-harvested soft-shell clams are ≥ 3.25 inches, 5.72% are ≥ 3.50 inches, and 1.75% ≥ 3.75 inches (Table 3; Fig. 3a). In southern Maine, cumulative data across five sampling dates indicated no clams larger than 3.75 inches, and that 4.1% of clams were ≥ 3.25 inches, while 0.67% were ≥ 3.5 inches.

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 2024, we initiated a comparative field experiment along a tidal gradient at an intertidal flat in southern (Barnes Flat; Brunswick, near Middle Bay: 43°51'16.31"N; 69°57'46.52"W), midcoast (Narrows Flat; Bremen, near the mouth of the Medomak River: 43°59'26.35"N; 69°24'32.99"W), and eastern Maine (Jonesport, near Flake Point Bar: 44o36'48.23"N; 67o33'44.92"W). Soft-shell clams ranging in size from 30-100 mm in shell length (SL) were added to 1 ft^2 × 6-inch deep field cages (wooden boxes – 0.5 ft^3, or 0.014 m^3, with a protective top) designed to exclude large predators.

Changes to the experimental and sampling design (2024)

While the overarching questions were the same for both years (2023 & 2024), we changed four items in our methods in 2024 regarding the experimental and sampling design that may have influenced results observed in 2023. First, we lowered the density of clams per box by 25% from 24 (2023) to 18 (2024). Second, we abandoned using a piece of screening designed to exclude milky ribbon worms, *Cerebratulus lacteus.* Third, we adjusted the way in which the mesh was configured on the top (protective) frame. Lastly, we commenced sampling at the southernmost site (Brunswick) two weeks earlier in 2024 vs. 2023, and at the midcoast site (Bremen) one week earlier. This decision was made because seawater temperatures (typically a proxy for when clam clam spawning commences in spring) in 2023 tended to be warmer earlier at those two locations than we had anticipated. No change was made to the sampling schedule in Jonesport, where the first sampling occurred on 6 May (2023) and 4 May (2024). Collectively, these four changes were introduced to the experimental and sampling design in 2024 because our 2023 data did not conform to that expected from the literature (e.g., Snelgrove et al. 2023; Ropes and Stickney 1965), from data collected in 2020 from the same site in Jonesport (eastern Maine) and an intertidal site in southwestern Maine near Brunswick – Freeport (Beal et al., unpubl.), and from 36 years of experience inducing soft-shell clams to spawn in a hatchery (Beal, pers. obs.).

Our reasoning in lowering the density/box by 25% was to decrease the possibility that intraspecific competition for food (sensu Peterson 1982, Ruiz et al. 1992) might have affected the ability of clams to condition (producing gametes for eventual spawning; see Barros et al. 2024) properly or somehow influenced how the spawning stimulus (thermal shock) affected the clams' ability to release gametes. In 2023, we used a 1.25-ft x 1.25-ft piece of PetScreen® (aperture = 0.9 mm x 1.7 mm) placed on top of the box over which the top frame held in place once screwed to the top periphery of each box. The exclusion mesh likely was in contact with the sediment surface in most boxes (Fig. 6). It may have interfered with clam feeding, as the siphons would have encountered the mesh and likely not permitted them full extension above the sediment-water interface. In addition, the presence of the mesh also may have reduced water flow within each box. We tested whether the presence of the PetScreen® mesh influenced the number of clams on the surface sediments of boxes by comparing photographs taken on five random dates in both years at our study site in Bremen, and did not find a significant difference between years (Fig. 7). No ribbon worm exclusion mesh was used in 2024 because we observed few individuals at any of the study sites in 2023. The change in the configuration of the protective top frame was done to allow clams to feed without interference from the mesh netting. In 2023, a piece of 14-gauge, vinyl-coated trap wire (aperture = ½-inch; 12.7 mm) was stapled to the top of the 1-inch thick, 1ft^2 frame. This large mesh was used to protect clams from large (≥18 mm) predators such as crabs, fish, and birds. Another piece of protective netting (polyethylene; 3.2 mm aperture) was stapled to the bottom of the 1-inch thick frame (Fig. 8). That mesh excluded smaller (≥ 4.5 mm) predators. That is, in 2023, clams were protected by three layers of exclusion materials: 1) PetScreen® directly in contact with the clams; 2) a 3.2 mm piece of mesh affixed to the bottom of the wooden top frame; and, 3) a piece of 12.7 mm aperture trap wire mesh. In 2024, the vinyl-coated trap wire and 3.2 mm polyethylene netting were both stapled to the top of each frame that provided a 1-inch gap between the sediment surface in each box and the protective netting (Fig. 9). Finally, the change of dates for the 2024 sampling (described above) were made to ensure that the sampling bracketed the major spring spawning period.

Unless otherwise specified, all means and least-square means are presented with their 95% confidence interval. Statistical significance was set using the Type I error rate (α) of 0.05.

2024 deployment and sampling

Boxes (n = 42 per each of three tidal heights: upper, mid, and lower intertidal) were mostly re-used in 2024. All boxes from Jonesport and Bremen established in 2023 were intact and remained in the same position they were left at the end of the 2023 sampling. Those boxes were reused. At the Brunswick intertidal site, however, all 42 boxes at the low tide mark plus 14 from the mid tide and 7 from the upper intertidal zone required replacements due to degradation at the end of the summer and through the fall in 2023 from shipworms (*Teredo navalis*).

All clams > 50.8 mm SL transplanted to boxes at each study site were accessed from local clam buyers, since this is the minimum legal size clammers can harvest. Clams < 50.8 mm SL used at all three study sites were obtained from Jonesport, and were collected (dug) near the upper intertidal at Flake Point Bar. Three clams from each of six size classes (I: 31.5-37.5 mm; II: 37.6-50.7 mm; III: 50.8-61.6 mm; IV: 61.7-74.5 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 3 individuals/size class = 6,804 clams). Clams were placed on top of the sediments within each box at each site, and allowed to burrow *sponte sua*. A protective top with two types of exclusion material (as described above) was screwed to the top periphery of the wooden box holding the clams.

Temperature was recorded hourly in one box at each site and tidal height using a HOBO Pendant data logger. Because loggers recorded temperatures 24/7 (i.e., daily during both low and high tide cycles), 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 "mean high tide temperature." In addition, to determine if thermal conditions were similar between inside vs. outside boxes, we placed a data logger immediately outside of the box at the mid intertidal at each site, and for each day that both loggers functioned properly, examined the difference in mean temperature between inside vs. outside the box (Table 5).

All live and dead clams from boxes were removed by hand from two randomly chosen boxes at each site and tidal height beginning 23 April (Brunswick), 29 April (Bremen), and 4 May (Jonesport) when seawater temperatures typically are below 10°C, 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 (8-10°C) until Monday mornings, when attempts to induce spawning occurred. Sampling in Bremen occurred on Mondays. Clams were held in chilled, Styrofoam containers and delivered via Fed-Ex to DEI on Tuesdays, stored in the walk-in cooler, and attempts to induce spawning were made on Wednesdays. Similarly, clams from Brunswick were collected on Tuesdays and treated in like fashion to those collected from Bremen except attempts to spawn those animals occurred on Thursdays after delivery to DEI was made on Wednesdays.

To better understand whether clam density and/or the piece PetScreen® used in 2023 to exclude the nemertean predator *Cerebratulus lacteus* may have influenced results during 2023, four of the 42 boxes at each site and tidal height were re-purposed for a small experiment during 2024. All four boxes were "seeded" with 24 clams representing four animals in each of the six size classes, as in 2023. Two of the boxes received a piece of PetScreen, similar to that used for all boxes in 2023. Clams in those boxes were protected by a top similar to that used in 2023 (i.e., vinyl-coated trap wire affixed to the top of the wooden frame and a piece of 3.2 mm extruded netting affixed to the bottom of the wooden frame). This treatment attempted to recreate 2023 conditions. Clams in the remaining two boxes at each site and tidal height were protected by a type of top used on all 2024 boxes (see above), and no piece of PetScreen was used. The idea was to collect the animals from these boxes (N = 12/site) in May or June once clams seeded at 18 animals/box at a particular site and tidal height demonstrated a propensity for spawning (Table 6). There was no significant difference between treatments in terms of spawning (binomial test, P = 0.219). Of eight occasions where at least one clam spawned, three instances were associated with animals from boxes under the 2023 conditions. While there was no significant treatment (P = 0.0691), tidal height (P = 0.354), or site (P = 0.437) effect on clam survival in the experimental boxes, ANOVA demonstrated that there was a significant site x treatment interaction (Table 6a; P = 0.042; Fig. 9a). At Brunswick, survival in boxes under the 2023 conditions was 61.1 ± 39.4% vs. 88.9 ± 8.1% in the other treatment. The same pattern was observed at Bremen (74.3 ± 23.7% vs. 96.5 ± 5.1%), but not at Jonesport where survival of clams in boxes under the 2023 conditions was 86.8 ± 5.8% vs. 74.3 ± 33.0% in the other treatment. Two-sample t-tests were conducted for each site comparing the two treatments, and only one (Bremen) was statistically significant (P = 0.04).

Attempts to induce spawning of experimental soft-shell clams at DEI (Fig. 10) proceeded each time (3 days/week for 18 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) filled with 5 µm-filtered ambient seawater (Fig. 10A). Approximately 500 ml of cultured microalgae (a mixture of *Tisochrysis lutea* and *Chaetoceros muelleri*) 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. 10 B). 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 (Fig. 11) in the spawning tank, it was immediately removed and placed into a glass fingerbowl (Fig. 12) 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 additional 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, three 1 ml samples were taken from the bucket and eggs counted and measured 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. Clams of both sexes were divided into three size classes: 1) < 50 mm; 2) 50-69.9 mm; and, 3) ≥ 70 mm. 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 (9 possibilities) by counting the number of eggs with polar body I 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 to collect these data.

On 11 dates between 9 May and 5 September 2023, 2-6 clams (30.3-101.3 mm SL) were chosen for histology from each location and tidal height (N = 775; Fig. 13). On 15 dates between 16 April and 26 August 2024, 5-6 clams (32.4-107.9 mm SL) were chosen for histology from each location and tidal height (N = 932; Fig. 14). In both years, some animals were taken from the ambient flat to compare stage of development between experimental and ambient populations. Animals were fixed in 10% buffered formalin for a minimum of 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 prior to sending off 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 after Kim and Powell (2004) and Porter (1974) (Table 7; Figs. 15-24). This method also allowed us to determine if sex ratios were different from 1:1. In 2023, the sex of 706 of 736 animals could be determined, and the sex ratio (M:F) of 46.2% vs. 53.8% (0.86:1.00) was significantly different from 1:1 (χ^2= 4.13, df = 1, P = 0.042). In 2024, the sex of 880 of 932 clams was determined, and the sex ratio of 43.6% vs. 56.4% (0.77:1.00) was significantly different from 1:1 (χ^2= 14.25, df = 1, P = 0.0002).

Gonadal and Condition Indices

On all sampling dates (n = 18), 3-6 clams were selected from each tidal height and location to determine condition index and gonad index. A series of measurements were taken on each clam: 1) shell length; 2) shell weight (live and dry); 3) whole body weight (live and dry); 4) whole tissue weight (live and dry); and 5) gonad weight (live and dry). All shells and tissues were dried to a constant weight at 60ºC. 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). CI integrates physiological responses to stress with changes in somatic growth (Sasikumar and Krishnakumar 2011), provides a useful measure of energy reserves (Norkko et al. 2005), and may be a proxy for following gametogenic activities (Okumuş and Stirling 1998). 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. We began by using the following formula to generate condition index data:

Equation 1: CI = dry tissue (including gonad) weight (g)/dry shell weight (g) x 100 %
(Lucas and Beninger 1985; Rainer and Mann 1992; Filgueira et al. 2013).

This index compares metabolism directed towards either shell growth or soft tissues, and does not account for possible variation in shell shape or thickness (Norkko et al. 2005). That is, this index does not account for possible variation in internal cavity capacity (volume) that may change over time as shells thicken or variation due to differences in sediment composition that may affect shell thickness independent of size.

Typically, the gonadosomatic index (GSI) is a ratio of gonad and body size used to describe and analyze the reproductive cycle of bivalves and other marine invertebrates (Ouréns et al. 2012). The formula used to generate GSI was:

Equation 2: GSI = (dry gonad tissue mass/[dry gonad tissue mass + dry other tissue]) x 100%.

As with CI, the GSI must be independent of size to make unbiased comparisons among locations, tidal heights, and time; however both CI and GSI typically increase with increasing size. Attempts were made to create a relative GSI that was independent of clam size (Erikson et al. 1985), but these efforts were unsuccessful. Another approach is to examine GSI based on restricted size categories (e.g., <50 mm, 50-60 mm, 60-70 mm, > 70 mm). A third approach is to use least-square means (LSM) calculated from an analysis of log-transformed variables (e.g., log10 dry gonad weight vs. log10 dry body weight). If slopes are equal, the LSM are calculated using a model without an interaction term; otherwise, the LSM are calculated with an interaction term.

Temperature data (2024 – Appendix A)

Of 12 temperature loggers deployed in 2024, nine were placed inside boxes and three outside boxes at the mid intertidal. Of the nine loggers inside boxes, seven failed at some point during the experimental period (Table 5). These were HOBO Pendant MX 2201Temp units. Of the three loggers placed outside the boxes in the mid intertidal, none failed. These were HOBO Pendant UA-001-64 temperature loggers.

<u>Jonesport</u> (Figs. A-1 to A-4)

All temperature loggers placed inside boxes failed at some point during the experimental period

(Table 5). Lowest temperatures recorded occurred between 22-24 March, and ranged between 2.04ºC at the upper intertidal on 22 March and 2.87ºC at the low intertidal on the same date. The logger inside the mid intertidal boxes failed to record after 22 May. Mean temperature at high tide was compared between inside and outside boxes (Fig. A-3) using one-sample t-test (Ho: $\mu_{diff(Inside\text{-}Outside)} = 0$) that revealed thermal conditions within the box were warmer than outside the box with a mean difference of +0.128 ± 0.046ºC (n = 132; P < 0.001). Highest temperatures (Table 5) ranged from 15.8ºC (27 August; outside mid intertidal boxes) to 16.5ºC (12 August; upper intertidal boxes). There was no linear relationship between mean difference in temperature inside vs. outside the box (i.e., ΔT) and date (P = 0.114). Over the interval when loggers at the upper and mid intertidal were both working, there was little difference in mean high tide temperatures. Temperatures in upper boxes appeared greater than those recorded in lower boxes (Fig A-1 vs. Fig. A-4). For example, the first date a mean high tide temperature ≥ 10ºC occurred was 29 April, and the last day when a mean temperature ≤ 10ºC was 15 May. For the low tide boxes, the first time a mean temperature ≥ 10ºC was recorded was 13 May. After 15 May, all mean temperatures were > 10ºC.

Bremen (Figs. A-5 to A-8)

The temperature logger deployed within the low intertidal box on 9 March failed to record any useable data. From the working loggers, lowest temperatures ranged from 2.91ºC on 22 March inside the mid intertidal box to 4.38ºC on 23 March outside the mid intertidal box. Highest temperatures ranged from 19.58ºC on 17 July outside boxes at the mid intertidal (Fig. A-7) and 21.69ºC on 14 July in the upper intertidal boxes (Table 5; Fig. A-5). A one-sample t-test (as described above) was used to determine whether thermal conditions within the boxes was similar to those outside the boxes. The mean difference (Inside-Outside boxes at the mid intertidal) was +0.491 ± 0.062ºC (n = 337). This mean was significantly different from zero ($T_{obs} = 13.3$, P < 0.0001; Fig. A-8) indicating that conditions within the box were warmer than those outside the box. In addition, the mean difference increased over time as the slope of the line relating the temperature difference inside vs. outside the mid intertidal box vs. time was significantly different from zero (P = 0.009; Fig. A-8). For example, the mean difference increased by 0.05ºC over each month (1 July = 0.463ºC; 1 August = 0.513ºC). The first date when mean temperature in upper intertidal boxes was ≥ 10ºC occurred on 17 April vs. 7 May in mid intertidal boxes.

Brunswick (Figs. A-9 to A-12)

The temperature logger deployed within the mid intertidal box on 9 March failed to record any useful data after 8 April (Table 5). The lowest temperature recordings across all tidal heights occurred between 9 March and 8 April (range = 2.94ºC on 22 March in upper intertidal boxes to 4.21ºC on 13 March outside of boxes at the mid intertidal; Table 5). While the logger from the mid intertidal boxes failed within a month of deployment, temperatures near the beginning of April were as high as 10ºC (Table 5; Fig. A-10). During that period, no significant difference in mean high tide temperatures were noted between inside and outside the mid intertidal box ($\bar{x}_{\text{diff}} \pm$ 95% CI = 0.016 ± 0.061ºC, n = 58; T_{obs} = 0.53, df = 57, P = 0.601). Maximum mean high tide temperatures occurred during 17-18 July inside and outside boxes, with temperatures exceeding 27ºC (Table 5). While mean seawater temperature during high tide inside upper intertidal boxes exceeded 10ºC on two dates in early April (1 April: 10.1ºC; 2 April: 11.8ºC), mean temperatures fell below 10ºC until 14 April, after which mean temperatures general exceeded that value (Fig. A-9). Mean temperature exceeded 10ºC in low intertidal boxes after 15 April (Fig. A-12).

Gametogenic stages – Histology (2023)

Jonesport

Tidal height played a role in gonadal development as clams nearest the shore took longer to condition and spawn than animals occupying experimental units at the mid and low tidal height (Fig. 25). Spawning was not observed prior to 17 June in animals from the upper intertidal whereas spawning commenced in about 15% of animals at the low intertidal as early as 13 May, and 50% were actively spawning in the mid intertidal by 27 May. Clams in a spawning condition were observed from both mid and lower intertidal zones on each sampling date from 27 May to 2 September, appearing to peak in both zones on 29 July (Fig. 25). High tide seawater temperatures were significantly higher (by an average of 0.33ºC) than the combined mean of both mid and low temperatures (T = 7.67, df = 9, P < 0.001; Fig. 26). In the laboratory, animals from all three tidal heights were first induced to release gametes using a thermal shock technique on 24 June, but spawning had apparently begun at least one month earlier at the two lowest tidal heights, and a week prior at the upper tidal height. Extra clams were collected outside the boxes from the ambient flat at each tidal height on 6 dates from 3 June to 5 September to compare gonadal development rates between experimental and wild animals. Highest concordance between the two populations occurred on 17 June and 29 July (Figs. 27 & 28). The greatest differences between the two groups occurred on: 27 June (mid intertidal, 100% of ambient clams were in a spawning condition whereas only 33% of experimental clams were observed spawning [Fig. 27]), 15 July (upper intertidal, 50% of ambient clams were spawning whereas no experimental clams were observed spawning [Fig. 28]) and, 5 September (upper intertidal, 83% of ambient clams were observed spawning, and only 16% of the experimental clams were in that same condition [Fig. 28]).

Bremen

Tidal height did not play a strong role in gonadal development at the Narrows Flat during 2023. Clam spawning was observed as early as 8 May in the lower intertidal and on 15 May at the mid and upper tidal heights (Fig. 29). Spawning was detected from clams occupying all three tidal heights on each collection date from 29 May to 4 September. At the lower tidal height, at least 50% of clams were observed in a spawning condition on 7 collection dates from 29 May to 28 August, and strongest spawning signals from clams at the upper and mid intertidal occurred from 26 June to 28 August. First spawning was observed in the laboratory (i.e., due to thermal shock) on 19 June even though spawning as observed histologically had been occurring at least a month prior to when clams were induced to spawn. As was the case in Jonesport, high tide seawater temperatures were significantly higher (by an average of 0.55°C) than the combined mean of both mid and low temperatures ($T = 6.22$, $df = 7$, $P < 0.001$; Fig. 30).

Brunswick

Tidal height influenced the rate of gonadal development at Barnes Flat (Fig. 31). Animals in boxes arrayed near the upper shore were delayed in spawning compared to experimental clams growing at the two lower tidal heights. That is, spawning was observed in ≥ 50% of individuals on the first three sampling dates (9, 16 & 30 May) at both the low and mid intertidal zone. From 20 June to 3 October, at least 33% of clams at the mid and upper intertidal zone were observed in a spawning condition. For clams in the lower intertidal, the spawning response after 30 May was more variable being observed on 5 of 6 collection dates from 11 July to 3 October (excluding 5 September when 50% of animals were ripe and the other half were inactive). The observation of active spawning into early October may be a response to warming water, as two discreet size classes of clam recruits have begun showing up in samples from southern Maine sites (B. Beal, pers. obs. – see: https://downeastinstitute.org/wp-content/uploads/2022/09/technical-report-5-clam-recruitment-monitoring-network-results.pdf). Once again, mean high tide seawater temperatures were significantly greater (by an average of 0.59ºC) than the combined mean of both mid and low temperatures ($T = 11.39$, $df = 6$, $P < 0.001$; Fig. 32).

Additional clams were collected from the upper and mid intertidal on three dates (30 May, 14 June, and 5 September) to compare gonadal development of experimental clams inside boxes vs. ambient animals from the adjacent mudflat (Fig. 33). An additional collection date occurred in which ambient clams were collected from all three tidal heights. Spawning was delayed in clams residing in boxes on 30 May and 14 June when > 80% of ambient clams from both tidal heights were observed in a spawning condition compared with experimental clams from the same tidal heights where ≤ 50% were observed in a spawning condition. On 3 October, 83% of ambient clams from the upper intertidal were observed in a spawning conditioned compared with 33% of experimental clams. Similar rates of gonadal development were observed between the two clam populations residing at the mid and low intertidal.

Gametogenic stages – Histology (2024; Appendix B)

<u>Jonesport</u>

Tidal height played a role in gonadal development (Fig. B-1). While some (16-50%) clams at the two lower tidal heights began spawning as early as 18 May, animals nearest the shore took 1-2 weeks longer to condition and spawn than animals occupying experimental units at the mid and low tidal height (Fig. B-1). Individuals in a spawning condition were observed on each collection date from 1 June to 24 August at all three tidal heights; however, no significant difference was detected in frequency of spawning clams across tidal height during that period ($\chi^2 = 2.8$, $df = 6$, $P = 0.834$). Mean percent spawning (1 June to 24 August) did not vary significantly across tidal heights ($\bar{x}_{Upper} = 66.7 \pm 13.5\%$; $\bar{x}_{Mid} = 69.7 \pm 13.5\%$; $\bar{x}_{Lower} = 78.8 \pm 13.5\%$; $n = 11$; $P = 0.412$). Mean temperature at high tide is displayed along with gametogenic stage in Fig. B-2. The temperature for a given sampling date is the mean of seawater temperature at high tide for the week preceding the collection date. For example, mean seawater temperature for the upper intertidal boxes on 4 May was 8.402ºC that was the average temperature of 15 high tide readings from the temperature logger taken between 27 April to 4 May. The histological slides did not reveal a spawning condition upon the first sampling (4 May) at any tidal height (Fig. B-1) that coincided with seawater temperatures below 10ºC (Fig. B-2). By 18 May, mean temperature around high tide at all three tidal heights averaged over 10ºC, and spawning commenced at both the two lower tidal heights (Fig. B-2). By 1 June, mean seawater temperature was 11.5ºC and 12.1ºC at the upper and lower tidal height, respectively, when 83-100% of clams were releasing gametes. We tested whether the mean difference in weekly seawater temperatures between the upper and lower intertidal (Ho: $\mu_{diff(Low-Upper)}$) was statistically significant. Average weekly seawater temperatures were 0.72 ± 0.23ºC higher along the lower vs. upper intertidal (T_{obs} = 7.22, df = 8, P < 0.001). This may explain why spawning was delayed for clams at the upper compared to mid and lower intertidal heights.

At each tidal height, a series of four extra boxes were used in an attempt to create conditions similar to those in 2023. That is, all four boxes were seeded with 24 clams, the density used in every box during 2023. Two of the boxes were protected with the same top (vinyl-coated trap wire affixed to the top of the frame; a piece of 3.2 mm extruded polypropylene netting affixed to the bottom of the frame) plus a piece of PetScreen® between the top frame and the wooden box to exclude the nemertean *Cerebratulus lacteus*. This treatment is referred to as Treatment A. The remaining two boxes were protected with a top that was the same as those used on the remaining 2024 boxes (both vinyl-coated trap wire and 3.2 mm extruded netting were affixed to the top of the frame), and no PetScreen® insert was used. This treatment is referred to as Treatment B. The logic of these treatments is as follows. Each collection date sampled two "control" boxes at each tidal height. Control boxes contained 18 individuals protected with a 2024 top (as described above). Treatment A differed from the control boxes in two ways: 1) density = 24 vs. 18 animals/ft^2; and, 2) PetScreen® insert with 2023 top. Treatment B differed from the control boxes in one way: 1) density = 24 vs. 18 animals/ft^2. If Treatment A and B differ in terms of the frequency of the five gonadal stages (Appendix B), then that difference must be due to the difference in how boxes were protected. If the Control differs from Treatment B, then the difference must stem from the two different densities (18 clams vs. 24 clams) as the tops were identical for both treatments. If the Control differs from Treatment A, then the difference either is due to the difference in density (which is tested unambiguously by Treatment B), or to the difference in predator deterrence.

Extra boxes were sampled at Flake Point Bar on 22 and 29 June (Figs. B-3, B-4). On 22 June, there was no strong or consistent difference between control clams vs. those in boxes protected with PetScreen® and 2023 tops. For example, the frequency of clams in a spawning condition in upper and lower intertidal control boxes varied between 66% and 100% compared to 50% and 83% in boxes without PetScreen® and 2024 protective tops. A Kruskal-Wallis test was conducted on the frequency of spawning between the three treatments, and found no significant difference (H_{adj} = 2.75, df = 2, P = 0.252). Results from the 29 June sampling revealed a slightly different response (Fig. B-4). Fewer clams from the two experimental treatments (A & B) appeared to be in a spawning condition than in the control; however, a Kruskall-Wallis test demonstrated this difference was not statistically significant (H_{adj} = 5.78, df = 2, P = 0.056).

<u>Bremen</u>

Except for the first sampling on 29 April, tidal height did not play an important role in the percent frequency of individuals that were spawning. On the first collection, 83% of clams in mid and lower intertidal boxes were in a spawning condition (Fig. B-5) vs. 0% of clams in the upper boxes. A closer inspection of the subcategories within the category "spawning" (see Table 7) demonstrated that 50% of the animals at the mid and lower intertidal had gonads that were half empty (spawning stage II – with 50% of the follicles within the gonad empty – indicating active gamete release). Another 25% of clams were at spawning stage IV, suggesting that spawning was nearly finished. A majority of clams (50-100% on each collection date across each tidal height) continued spawning from the second collection (13 May) until the final collection (26 August) – 15 weeks. On that date, 50% of clams in the spawning condition were at stage I suggesting that a second (fall) spawning may be imminent. Thermal conditions in upper intertidal boxes were significantly warmer than those in the mid tide boxes (Fig. B-6; a logger was positioned in a lower intertidal box; however, that instrument failed to collect any useable data) by an average of 0.481ºC (T_{obs} = 4.07, df = 11, P = 0.002). A majority of clams at mid tide were spawning on 29 April, and the mean weekly temperature at high tide during the week preceding that collection was 7.91 ± 0.32ºC (n = 15) with the minimum and maximum of 7.019ºC and 9.149ºC, respectively.

Extra boxes, each containing 24 clams as opposed to the control boxes that contained 18 clams, were sampled on 3 and 10 June from each tidal height. On 3 June, it appears that spawning was delayed at each tidal height in the treatment that best represented 2023 conditions - PetScreen® present and with 2023 protective top (i.e., Treatment A; Fig. B-7), but that delay was only a week as there was little difference in gonadal condition between control and that same treatment for clams collected on 10 June (Fig. B-8). No overarching differences existed across tidal heights for either sampling date comparing controls to Treatment B (24 clams protected using a 2024 top), suggesting little differences due to changing intraspecific densities from $24/ft^2$ to $18/ft^2$.

Brunswick

Clams were actively spawning on every sampling date across all three tidal heights (Fig. B-9). We initiated our collection in 2024 on 23 April, two weeks earlier than in 2023. On 23 April, mean seawater temperature at high tide exceeded 10ºC at all tidal heights (Fig. B-10), and between 83-100% of animals were in a spawning condition. Since most clams on all sampling dates were in a spawning condition, we asked whether there was a difference in the percent frequency of clams within each of the four spawning stages (I, II, III, IV; Table 7) over time. Specifically, we compared the frequency of clams in each stage from the three earliest sampling dates (4/23, 4/30, and 5/14) to the last three sampling dates (7/30, 8/13, and 8/20). Our expectation was that during the early spring clams would tend to display greater frequencies within the first two spawning stages (I and II), and that over time, clams would tend to display greater frequencies within the final two spawning stages (III and IV). This was not the case, however (Fig. B-11), as there was no significant change in the proportion of clams within each of the four spawning stages over time ($\chi^2 = 6.19$, df = 3, P = 0.111).

Extra clams were sampled on 14 May, 4 June, and 11 June, and there was no indication on any sampling date or tidal height that control clams were behaving differently than the clams contained in the experimental boxes (Fig. B-12).

Clam survival & Spawning induction - 2024 (thermal shock)

Jonesport

Mean clam survival was > 50% for all collection dates and tidal heights (Fig. 34); however, mortality rates across all three tidal heights increased significantly with time. Spawning induction using thermal shock was variable across tidal heights (Fig. 35). Clams from upper intertidal boxes released gametes only once, on 15 June, and this date coincided with peak spawning of clams from the mid intertidal that spawned on six dates between 25 May and 20 July. Clams in low tide boxes spawned on two occasions: 29 June and 13 July.

Bremen

Mean clam survival was > 70% for all collection dates and tidal heights except on week 13 (22 July) when only 10 live clams (55.6%) were recovered from the mid intertidal boxes (Fig. 36). Survival rate did not vary significantly across time at any tidal height. Spawning occurred most frequently at this site (Fig 37). Spawning was observed on 8 dates from clams from the upper and mid intertidal, and on 11 dates from animals in low intertidal boxes. A peak in spawning occurred at each tidal height on 27 May, followed by a higher peak across all tidal heights about a month later on 17 June (low intertidal) and 24 June (upper and mid intertidal). Highest percentage of spawning individuals occurred at the upper intertidal (58% on 24 June) followed by the mid intertidal (38% on 24 June). In the lower intertidal, the highest percentage of spawning individuals was 23% on 17 June.

Brunswick

Mean clam survival decreased significantly with time across all three tidal heights (Fig. 38), but was generally > 60%. As with experimental clams in Jonesport, survival decreased significantly across time at all three tidal heights. Most spawning was observed prior to 21 May (Fig. 39), with only minor peaks (i.e., < 5% of animals spawning) occurring at the two lower tidal heights on 18 June and on 25 June at the upper intertidal. The highest percentage of spawning individuals occurred on 7 May from animals in upper (27.7%) and mid (28.1%) intertidal boxes. No spawning was observed after 25 June from clams at any tidal height (Fig. 39)

Egg production using thermal shock

A total of 193 animals were induced to spawn (68 ♀ : 124 ♂; $\chi^2 = 16.3$, $P < 0.001$), and this ratio did not vary significantly between study sites ($\chi^2 = 1.7$, $P = 0.427$). Number of eggs per female was assessed from three 1 ml samples once the female had ceased releasing eggs into the fingerbowl it was placed. Sometimes (n = 8) a female released its eggs, but the number was too few to count efficiently, so no quantitative estimate was made of egg number. The smallest and largest individual that released eggs that were too few to count were from Jonesport (43.9 mm; 15 June) and Brunswick (81.39 mm; 14 May). Half of those that did not release enough eggs to count came from Brunswick. The earliest occurred on 30 April for clams collected from upper intertidal boxes (59.3 mm & 80.9 mm). The latest occurred on 2 July from mid tide boxes in Bremen (61.8 mm & 49.1 mm), and on 8 July from a 48.8 mm female from mid tide boxes in Jonesport.

Spawning of female clams commenced in April in Brunswick (Fig. 40). At that site, most clams induced to spawn did so in May (71.4%); however, only 14 females were induced across all collection dates. The highest percentage of spawning clams occurred in the upper intertidal boxes (64.2%). A Fishers Exact Test was conducted that showed the frequency of spawning did not vary significantly across months and tidal heights ($P = 0.108$). The most clams induced to spawn in 2024 were from Bremen (n = 48), and clams spawned there in every month from May to August (Fig. 40), although less than 10% (6.25%) spawned in August. The highest percentage of clams spawned in July (20/48, or 41.7%). Tidal height played a role, but was more complicated than at the other two sites. Only 25% of animals from mid intertidal boxes were induced to spawn, while 40% and 35% of those animals that spawned were collected from upper and lower boxes, respectively. Tidal height was not independent of date with respect to the frequency of individuals induced to spawn in Bremen ($P < 0.001$). At Jonesport, only six female clams were induced to spawn, with half spawning in June. Five of the six clams (83%) that spawned were collected from mid intertidal boxes. The other clam that released eggs was from the lower intertidal boxes. Spawning was independent of date and tidal height ($P = 0.500$). Mean size of spawning female across all sites did not vary significantly over time ($F = 0.81$; df = 1, 66; $P = 0.372$; $r^2 = 0.012$). Mean number of eggs released increased significantly over time ($F = 7.61$; df = 1, 58; $P = 0.008$; $r^2 = 0.116$; Fig. 41)

Male clams, similar to females, commenced spawning in April in Brunswick (n = 22; Fig. 42); however, most induced to spawn did so in May (59%). Highest percentage of clams induced to spawn occurred in upper intertidal boxes (45%), and lowest from clams in mid intertidal boxes (23%). The frequency of spawning across tidal heights was not independent of date ($P = 0.001$). For example, 100% of spawning induced by thermal shock in April was observed in animals at the two highest tidal heights. In May and June, 69% and 40% of spawning occurred at the two highest tidal heights. In Bremen, where the highest number of male clams were induced to spawn (n = 83), similar number of individuals spawned during May, June, and July, with a sharp drop in August (Fig. 42). The proportion of individuals induced to spawn in a particular month was not independent of tidal height ($P < 0.001$). For example, the proportion of clams induced to spawn from the two highest tidal heights varied significantly over time. In May and June, 85% and 73% of individuals induced to spawn occurred in boxes at the upper and mid tide levels whereas these percentages dropped to 48% and 63% in July and August, respectively. At Jonesport, 19 male clams were induced to spawn over three months (May to July), with 84% (16/19) of individuals originating from low intertidal boxes, and a Fisher's Exact Test indicated that this proportion was similar across months ($P = 0.065$). There was no evidence of a change in mean size of male spawners over time ($F = 0.32$; df = 1, 122; $P = 0.574$; $r^2 = 0.012$).

<u>Relationship between clam size and eggs released</u>

Number of eggs increased with increasing clam size according to a power exponential function ($Y = aX^b$; Fig. 43). A lack-of-fit test confirmed that this an appropriate model ($F = 1.66$; df = 4, 41; $P = 0.1750$) suggesting that, as was the case in 2023, reproductive senility is not occurring in this species. That is, like American lobsters (Herrick 1896; Estrella and Cadrin 1995), European lobsters (Agnault 2008), and green sea urchins (Stien et al. 1998), egg production increases with clam size (and, by deduction, age). This may be an important finding in terms of management of soft-shell clams in Maine and elsewhere. Because sufficient numbers of animals were induced to spawn in Bremen (Fig. 43), it is possible to ask whether the relationship between clam size and eggs released varied by tidal height (Fig. 44). To answer this, the lines relating number of eggs released vs. clam size were log-transformed to create a linear relationship and then analysis of regression lines used to examine whether slopes were equal. Results demonstrated homogeneous slopes ($F = 0.65$; df = 2, 40; $P = 0.5274$); hence, an analysis of covariance (ANCOVA) was performed that demonstrated the lack of a significant tidal height effect ($F = 1.54$; df = 2, 42; $P = 0.226$).

Relationship between clam size and egg size

Egg size was measured using a FlowCam. A sample of 10 eggs was taken per female, and the width of each measured to the nearest 1µm. This allowed us to determine the mean egg size across all females (n = 61) as well as determine if egg size varied geographically (i.e., from site-to-site), by clam size, and over time.

Mean egg diameter (± 95% CI) was 69.1 ± 0.65 µm (n = 61), which was significantly different from the same measurement taken on 33 clams in 2023 (74.00 ± 0.90 µm) in a two-sample t-test ($T_{obs} = -839$, df = 92, $P < 0.001$). Mean diameter of eggs varied significantly with site (Fig. 45) with the greatest difference for spawning clams between the easternmost and westernmost sites (Jonesport: 64.2 ± 23.8 µm [n = 4 females] vs. Brunswick: 71.3 ± 3.1 µm [n = 10 females). Mean size of eggs from Bremen clams was intermediate and not significantly different from either of the two other sites (68.1 ± 1.1 µm, n = 47). A significant negative relationship was observed between mean egg diameter and sampling date (Fig. 46).

Gonadosomatic Index 2024 (GSI)

Jonesport

Neither GSI nor dry gonad weight were independent of clam length (Fig. 47). Two independent analyses were conducted using dry gonad weight to examine variation due to tidal height and sampling date. Data were log-transformed and for each week, the following linear model was used to assess differences between tidal heights:

Equation 3: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \varepsilon$, where:

Y = dependent variable (log-transformed dry gonad weight; log-transformed GSI);
X_1 = shell length (mm);
X_2 = tidal height (factor is fixed; 3 levels: Upper, Middle, and Lower)

β_0 = y-intercept;

β_{1-3} = coefficients associated with shell length, tidal height and their interaction; and,

ε = residual error (difference between an observation and the fitted line)

When β_3 was not significantly different from zero (15 of 18 weeks), slopes were assumed equal and the least-square mean for each tidal height was calculated without the interaction term in the model. When β_3 was not significantly different from zero (weeks 9 [15 June], 14 [20 July], and 17 [10 August] for both variables), slopes were considered significantly different, and the least-square mean for each tidal height was calculated with the interaction term present in the model.

While not quite a mirror image, the two figures that relate the least-square mean for the dry gonad weight (Fig. 48) and GSI (Fig. 49) show very similar patterns. Using the least-square means estimation of GSI (Fig. 49), for clams at the upper and mid intertidal zone from the first to the seventh sampling date (4 May to 15 June), that there was a steady increase in mean GSI to a seasonal high (upper: 14.74 ± 6.39%; mid: 17.09 ± 6.24%). This was followed by a steady decline for 3-4 weeks (6-13 July), and may signal that spawning was occurring, although there was not good concordance with the histological data (see Figs. B-1, B-2). After a brief period (1-2 weeks), mean GSI decreased to 5.79 ± 2.34% at the mid intertidal and 8.99 ± 3.37 at the upper intertidal. Examining Fig. 49 more closely, it is possible to determine the frequency of the least-square means within three GSI categories (5-9.9%, 10-14.9%, ≥ 15%). That is, for upper intertidal clams, a mean GSI between 5-9.9% and 10-14.99% occurred 7 and 11 times, respectively, while for mid intertidal clams, the frequency of occurrence in those two categories was 6 and 9 times, respectively, and an additional 3 times mean GSI was > 15%. A Fisher's Exact Test was conducted to determine whether the frequencies within each GSI level was independent of tidal height ($P = 0.349$). This suggests that clams responded similarly to tidal elevation over the upper two intertidal heights with respect to GSI. The pattern of least-square means for GSI were somewhat different for clams residing near the low water mark (Fig. 49). There, least-square mean GSI increased steadily from 4 May to 8 June when the mean peaked (18.22 ± 8.66%), and then decreased to a seasonal low of 8.11 ± 4.49% on 13 July that was followed by two additional peaks on 20 July (16.38 ± 5.32%) and 17 August (14.64 ± 6.62%). The frequency when least-square mean GSI occurred within each of the three categories referred to above was 4, 8, and 5 times. Pooling data from the upper and mid intertidal and comparing the pooled frequencies over the three GSI categories to those from the lower intertidal revealed no significant difference ($\chi^2 = 4.127$, df = 2, P = 0.127). In summary, the data showed at least one spawning response at each tidal height that occurred gradually suggesting, as the histological data indicate, that spawning is a protracted event that likely is loosely related to temperature after mean seawater temperature reaches 10ºC.

To better understand potential interactive effects of clam size and sampling date on the gonadosomatic index, animals from six size (SL) categories (< 50 mm, 50-59.9 mm, 60-69.9 mm, 70-79.9 mm, 80-89.9 mm, ≥ 90 mm) were examined. All data for a given month and size category were pooled across tidal heights. Within each size category and month, the relationship between mean GSI and shell length was examined to determine if the two variables were independent (23 separate analyses). The two variables were independent within each size category and time except in three instances. In those cases, the least-square mean of GSI was used instead of the mean GSI. The data (Fig. 50) indicated that mean GSI was similar in clams across dates within each size category, but that mean GSI was greater in larger animals. To test this, a two-way ANOVA was conducted to determine if mean GSI was similar across months for animals ≤ 69.9 mm (F = 0.62, df = 6, 210, P = 0.7146) and another to determine if mean GSI was similar for animals ≥ 70 mm (F = 1.49, df = 6, 173, P = 0.1845). Next, all data for mean GSI and time was pooled into two groups (≤ 69.9 mm and ≥ 70 mm), and a two-sample t-test performed to test the null hypothesis that the two group means were equal. The test revealed a significant difference between the two groups ($T_{obs} = 13.81$, df = 405, P < 0.001) as mean GSI for clams ≥ 70 mm SL (19.5 ± 1.45%, n = 185) was more than double that of those ≤ 69.9 mm (9.2 ± 0.59%, n = 222).

<u>Bremen</u>

As was the case for clams in Jonesport, both GSI and dry gonad weight were not independent of clam length (Fig. 51). Two independent analyses were conducted using dry gonad weight to examine variation due to tidal height and sampling date. Data were log-transformed and for each week, the linear model (Equation 3) was used to assess differences between tidal heights. For both variables, the interaction term in Equation 3 was not significantly different from zero, which means that slopes were equal for each combination of tide and sampling date, and that least-square means for each tidal height and sampling date were calculated without the interaction term in the model.

The tidal height- and time-specific least-square means for both dry gonad weight (Fig. 52) and GSI (Fig. 53) show very similar patterns. Focusing on GSI (Fig. 53), clams at each tidal height appeared to have at least one major spawning event (decrease in mean GSI by ~10% from one week to the next), but they were not synchronous. That is, in the upper intertidal, the decrease occurred from 1-8 July; in the mid intertidal zone from 15-22 July; and, in the lower intertidal zone from 24 June to 1 July. In the upper intertidal preceding 1 July, minor spawning events may have occurred between 20 May and 24 June. After 8 July, a steady decrease in the least-square means GSI ensued until 12 August, after which a precipitous increase (nearly doubling) in GSI occurred from 12 to 26 August (9.3 ± 4.7% to 17.0 ± 4.7%). In the mid intertidal, a spawning event may have occurred between 27 May (24.2 ± 5.9%) and 3 June (15.4 ± 4.7%; Fig. 53). After 22 July, means remained around 15% until the final sampling on 26 August. Clam behavior in the lower intertidal more closely resembled that in the mid intertidal. For example, two spawning events may have occurred – one from 24 June (23.2 ± 8.0%) to 1 July (10.7 ± 3.9%), and a previous event between 27 May (31.7 ± 7.6%) and 3 June (21.3 ± 6.3%). After 1 July to the final sampling on 26 August, least-square mean GSI nearly doubled, finishing at 20.2 ± 5.6%. The steady increases in GSI seen mostly for clams in the upper and lower intertidal may be an indication of a fall (second season) spawning. There was some concordance between GSI and the percent of clams induced to spawn via thermal shock (see Fig. 37). Upper intertidal clams were induced to spawn six times between 20 May and 24 July when least-square mean GSI averaged 20.4 ± 2.6% (n = 6). Only two minor spawnings occurred in the lab occurred after 24 June (Fig. 37), when mean GSI averaged 12.4 ± 2.1% (n = 8). For mid intertidal clams, three spawning events were induced via thermal shock: 27 May; 24 June; and, 27 July. The first two dates matched reasonably well the least-square means GSI (Fig.53), and the third date was one week after the peak mean GSI at the mid intertidal (24.9 ± 10.6%). Lower intertidal clams were induced to spawn 11 times in the laboratory between 13 May and 19 August (Fig. 37); however, only two instances aligned somewhat with GSI. For example, peak least-square mean GSI occurred on 27 May (31.7 ± 7.6%) when 15% of clams from the lower intertidal were induced to spawn, the second highest percentage for that site. Approximately 20% of clams spawned on 17 June, but mean GSI stayed constant from 3 June until 24 June, when it decreased by ~ 50% to 1 July. As with the Jonesport clams, there was not good agreement between the GSI and gonad weight data with the histological data (see Figs. B-5, B-6).

The frequency of the least-square means within four GSI categories (5-9.9%, 10-14.9%, 15-19.9%, ≥ 20.0%) was examined across each tidal height. A Fisher's Exact Test was conducted to determine whether the frequencies within each GSI level was independent of tidal height ($P = 0.423$). This suggests that clams responded similarly to tidal elevation with respect to GSI.

The interactive effects of clam size and sampling date on the gonadosomatic index was examined. As with clams from Jonesport, animals from six size (SL) categories (< 50 mm, 50-59.9 mm, 60-69.9 mm, 70-79.9 mm, 80-89.9 mm, ≥ 90 mm) were examined. All data for a given month and size category were pooled across tidal heights (Fig. 54). A general trend, albeit not surprising given the least-square means analysis (Fig. 53), was decreasing mean GSI over time within most size classes. Within each size category and month, the relationship between mean GSI and shell length was examined (24 separate analyses), and the two variables were independent in each instance. Analysis of variance on mean GSI was performed using the following linear model:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_k$$

Where:

Y_{ijk} = dependent variable (mean GSI);
μ = theoretical mean;
A_i = Month (factor is fixed, i = 1 to 4);
B_j = Size Category (factor is fixed, j = 1 to 6); and,
e_k = experimental error (k = 1 to n = (10 to 31)

Several a priori, orthogonal contrasts were used for both main effects. For example, three contrasts were used for month:

A) May & June vs. June & August;

B) May vs. June; and,

C) July vs. August.

Five contrasts were used for the size categories:

D) ≤ 69.9 mm vs. ≥ 70.0 mm;

E) < 50 vs. 50-59.9 mm & 60-69.9 mm;

F) 50-59.9 mm vs. 60-69.9 mm;

G) 70-79.9 mm v. ≥ 80.0 mm; and,

H) 80-89.9 mm v. ≥ 90 mm.

Four contrasts associated with main effects were statistically significant (Table 8) – one contrast was associated with the change in mean GSI from the first two to the last two months (i.e., mean GSI for May & June = 22.2 ± 1.09% [n = 229] fell ~25% during July & August = 17.0 ± 1.2% [n = 162]), and three contrasts associated with clam size. In addition, there was a significant interaction between the two main factors (P = 0.0481), and 2 of 15 single degree-of-freedom contrasts were statistically significant (Table 8). The first (Fig. 55-A) showed that mean GSI in May-June were not different among two size classes (50-59.9 & 60-69.9) when the first two sampling months (May & June) are pooled (21.5 ± 1.89%, n = 82). During July and August, however, mean GSI dropped in the smaller size class by nearly 40% from 21.9% to 12.9% while in the larger size class the difference between the two sampling dates was only 7%. The second significant interaction occurred in clams >70 mm SL, and involved the first two sampling dates (May and June) for contrast G. In that instance (Fig 55-B), mean GSI between the two sizes (70-79.9 mm vs. ≥ 80 mm) was similar in May ($\bar{x}_{70\text{-}79}$ = 22.3 ± 3.9%, n = 12; $\bar{x}_{\geq 80\text{mm}}$ = 25.3 ± 2.7%, n = 31), but not in June as mean GSI increased by nearly 25% in the 70-79 mm size class while decreasing by ~15% in the largest size classes.

<u>Brunswick</u>

Neither GSI nor dry gonad weight was independent of clam length (Fig. 56). Both variables were used to examine variation due to tidal height and sampling date. Data were log-transformed and for each week, the linear model (Equation 3) was used to assess differences between tidal heights. The interaction term in Equation 3 was not significantly different from zero for both variables, and that least-square means for each tidal height and sampling date were calculated without the interaction term in the model.

Least-square means of both dry gonad weight (Fig. 57) and GSI (Fig. 58) varied significantly across tidal heights on 2 of 18 sampling dates, but there was not good concordance with the histological data that indicated a high preponderance of spawning at all tidal heights on most sampling dates (Figs. B-9, B-10). Because the pattern between dependent variable and sampling date was remarkably similar for both dry gonad weight and GSI, the information that follows pertains specifically to the gonadosomatic index data. The least-square mean GSI varied significantly across tidal heights on 14 ($P = 0.046$) & 28 May ($P = 0.021$). A posteriori testing (Tukey-Kramer multiple comparison test) demonstrated that in both instances, animals at the upper intertidal had significantly lower least-square means than those from those at lower tidal heights (Fig. 58).

During the first month of sampling (23 April to 14 May), least-square mean GSI of upper intertidal clams reached a peak by 7 May (28.6 ± 10.3%) and then declined a week later by ~35% to 18.5 ± 4.8%, suggesting that a spawning event may have occurred (Fig. 58). Nearly 30% of clams from the same tidal height were induced to spawn on this same date (Fig. 39). Another similar decrease (~37%) occurred from week 9 (18 June) to week 10 (25 June), and was also associated with a minor spawning in the laboratory (Fig. 39). A third decrease of ~ 50% occurred from 6 August (18.3 ± 8.2%) to 13 August (8.8 ± 3.8%). No comparable event occurred in the laboratory at that time, however. At the mid intertidal, three events occurred where the difference between consecutive weeks varied between 35-50%. The first occurred between 23 and 30 April (~38%), the second between 14 and 21 May (~35%), and the last between 9 and 16 July (~50%). After 16 July, there was little variation in least-square mean GSI (Fig. 58). There was little concordance with lab-based spawning induction at the mid intertidal (Fig. 39). Clams deployed in lower intertidal boxes showed a gradual increase in least-square mean GSI from the first two sampling dates in late April (~20%) to a peak on 28 May (35.6 ± 13.7%) that was followed by a 33% loss in least-square mean GSI (23.5 ± 3.4%). Although a gap in the lower intertidal data exists due to high clam mortality, least-square mean GSI fell by ~30% between 9 and 23 July. None of these events coincided with spawning induction trials of clams from the same tidal height (Fig.39).

The frequency of the least-square means within five GSI categories (≤ 15%, 15-19.9%, 20-24.9%, 25-29.9%, ≥ 30.0%) was examined across each tidal height. A Fisher's Exact Test was conducted to determine whether the frequencies within the GSI categories was independent of tidal height ($P = 0.039$). For mid and lower intertidal clams, 27.8% and 23.5%, respectively, of frequencies occurred in the ≥ 30.0% category, whereas no clams from the upper intertidal occurred in that category.

The interactive effects of clam size (six size categories, as described above) and sampling date (April, n = 2; May & June, n = 4; July, n = 5; August, n = 3) on the gonadosomatic index was investigated. All data for a given month and size category were pooled across tidal heights (Fig. 59). A general trend for all but the smallest ($P = 0.950$) and largest ($P = 0.387$) size classes was decreasing mean GSI over time ($P < 0.028$). Mean GSI of clams in the smallest size category (≤ 50 mm SL) increased by 75% from 11.2 ± 5.1% in April to 19.7 ± 2.6% in June, and then declined to an average of 11.5 ± 2.9% over the three sampling dates in August. Mean GSI of the largest clams (≥ 90 mm SL) increased ~45% between April and May (Fig. 59), and then maintained a relatively high mean GSI (ranging from 29.1 ± 6.5% to 35.0 ± 4.5%; $\bar{x} = 32.1 \pm 3.13\%$, n = 29) until the end of August.

Analysis of variance on mean GSI used the two-factor factorial model described above for the clams in Bremen (Table 9A). A significant interaction between size and month occurred ($F = 2.38$, df = 20, 347, $P = 0.0009$). When a subset of the data was examined (the four size classes from 50-59.9 mm to 80-89.9 mm), there was no significant interaction ($F = 0.88$, df = 12, 254, $P = 0.566$), and both main factors were statistically significant ($P \leq 0.0012$; Table 9B). Two orthogonal, a priori contrasts were developed to examine the effect of sampling date (month) on GSI: A) April & May vs. June- August; B) April vs. May; C) June vs. July & August; D) July vs August. Another set was developed for size category: E) 50-59.9 mm vs. rest; F) 60-69.9 mm vs. rest; G) 70-79.9 mm vs. 80-89.9 mm (Table 9B). The analysis showed that three of the four contrasts associated with sampling month were statistically significant, with only the contrast comparing mean GSI between April and May not significant ($P = 0.216$). More than half of the variation associated with sampling month was explained by contrast A (Table 9B) that was due to the significant drop (~25%) in mean GSI between the first two (31.99 ± 2.12%, n = 77) and last two months (24.21 ± 1.42%, n = 197). A similar pattern (earlier sampling months had higher mean GSI than later months) was observed for the remaining two contrasts (e.g., contrast C [~30%]: June = 29.16 ± 1.97, n = 82; July/August = 20.68 ± 1.75, n = 115; contrast D [~30%]: July = 22.97 ± 2.21%, n = 75; August = 16.39 ± 2.46, n = 40). Two of three contrasts associated with clam size were statistically significant (Table 9B), and the pattern was similar for both: smaller clams had a relatively lower mean GSI compared with larger clams. For example, there was a ~23% increase in mean GSI comparing clams between 50-59.9 mm (22.54 ± 2.33%, n = 67) vs. those between 60-89.9 mm (27.65 ± 1.44%, n = 207). A similar result (~12% increase) was observed for animals between 60-69.9 mm (25.63 ±2.32%, n = 69) vs. those between 70-89.9 mm (28.65 ± 1.82%, n = 138).

Condition Index 2024 (CI)

<u>Jonesport</u>

Condition index was independent of clam size over the range of sizes considered (33.8-100.8 mm SL; $F = 0.02$, $df = 1, 405$, $P = 0.8888$, $r^2 = 4.8 \times 10^{-5}$; Fig. 60). A linear model explained < 0.001% of the variation in the dependent variable, and a lack-of-fit test from a linear model was conducted to determine if the addition of higher order terms to the model would improve the fit. None did ($F = 1.10$, $df = 3, 402$, $P = 0.349$). Attempts to normalize the data using a log-transform to both variables did not improve the fit ($F = 0.24$, $df = 1, 405$, $P = 0.625$). Given the lack of a statistically significant relationship between CI and clam size, mean CI was used in all subsequent analyses.

Mean CI (Fig. 61) was highest in week 11 (13 July; 17.1 ± 9.19%, n = 18), and lowest in week 17 (24 August; 11.7 ± 5.60%, n = 18). An initial drop (~18%) in mean CI occurred between the first two weeks (4 May vs. 11 May) followed by a period of relative stasis to week 11. After that, mean CI continued to fall through the final sampling in week 18 (1 September; Fig. 61). A two-way, model I ANOVA assessed the interactive effects of tidal height and week on mean condition index (Table 10). No significant tide x week interaction occurred ($P = 0.256$), which allowed an independent interpretation of both main effects. A series of a priori, orthogonal contrasts was developed to provide additional information about each main factor. For example, the first of two single degree-of-freedom contrasts associated with tidal height compared mean CI between for clams at the upper intertidal vs. mid & lower, while the second compared mean CI between mid and lower intertidal animals. For the weekly sampling, CI was pooled by month, and four orthogonal contrasts examined: 1) May vs. June, July, August, September; 2) June vs. July, August, September; 3) July vs. August, September; and, 4) August vs. September. The overall effect due to tidal height on CI was statistically significant ($P = 0.0005$; Table 10). Mean CI for mid and lower intertidal clams (14.7 ± 0.53%, n = 271) was 8.3% greater than that for upper intertidal clams (13.6 ± 0.81%, n = 136; $P = 0.0004$; Table 10; Fig. 62). There was no significant difference in mean CI between mid and lower intertidal animals ($P = 0.092$; Table 10). Mean CI varied significantly over the weekly sampling dates (Fig. 61; $P = 0.013$), but of the four contrasts, only one (July [15.9 ± 10.15%, n = 87] vs. August, September [12.7 ± 6.63%, n = 72]) was statistically significant ($P < 0.0001$; Table 10). There was no apparent match between mean CI across sampling dates (pooled by tidal height – Fig. 61, or when each tidal height is examined separately – Fig. 63) and changes observed over time in gonadal stage (Figs. B-1, B-2), dry gonad mass (Fig. 48), or GSI (Fig. 49).

Bremen

Condition index (CI) was not independent of clam size over the range of sizes considered (34.7-109.5 mm SL; Fig. 64). A lack-of-fit test from a linear model was conducted to determine if the addition of higher order terms to the model (e.g., quadratic, cubic, quartic) would improve the fit. A linear model provided the best estimate for the relationship ($F = 2.16$, df = 3, 384, $P = 0.0927$).

The following linear model was used to assess whether slopes of the relationship between CI and shell length were equal.

Equation 4: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_2 X_3 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 X_1 X_2 X_3 + \varepsilon$, where:
Y = dependent variable (untransformed condition index);
X_1 = shell length (mm);

X_2 = tidal height (factor is fixed; 3 levels: Upper, Middle, and Lower)

X_3 = week (factor is fixed; 18 sampling dates)

β_0 = y-intercept;

β_{1-7} = coefficients associated with main and interaction terms; and,

ε = residual error (difference between an observation and the fitted line)

The first step assessed three null hypotheses: 1) Ho_1: $\beta_3 = 0$ vs. Ha_1: $\beta_3 \neq 0$; 2) Ho_2: $\beta_5 = 0$ vs. Ha_2: $\beta_5 \neq 0$; and Ho_3: $\beta_7 = 0$ vs. Ha_3: $\beta_7 \neq 0$. Each test includes an interaction between one or more fixed factors. If the specific null hypothesis is retained, then the slopes of the various lines (tidal height = three lines; weekly sampling = 18 lines; tidal height x weekly sampling = 54 lines) are considered parallel, and the terms in Equation 4 can be reduced to include a more simplified equation that pools the variation associated with those terms into residual error. Results indicated that for the analyses associated with Equation 4, each of the three null hypotheses were retained (i.e., $\beta_3 = \beta_5 = \beta_7 = 0$), therefore, Equation 5 was used:

Equation 5: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_2X_3 + \varepsilon$, where Y, β_{0-4} are the same as described for Equation 4.

In this instance, the null hypothesis (Ho: $\beta_4 = 0$) was not rejected (F = 0.78, df = 34, 334, P = 0.806). Together, the analyses indicated that an analysis of covariance (ANCOVA) would be appropriate. Equation 5 simplifies to:

Equation 6: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \varepsilon$, and this yields tests that include both main effects.

Results demonstrated a highly significant effect due to tidal height (F = 10.54, df = 2, 334, P <0.0001) and sampling date (F = 6.10, df = 17, 334, P < 0.0001). The least-square mean of condition index increased from upper to lower intertidal (Fig. 65), and each mean was significantly different from another (P ≤ 0.038; Scheffe a posteriori tests). Lowest least-square mean occurred in the upper intertidal (16.4 ± 0.78%) and the highest in the lower intertidal (18.9 ± 0.78%). Condition index varied over time (Fig. 66) showing a decrease of ~16% from week 1 (4 May) to week 8 (22 June) that did not coincide with changes observed in dry gonad mass or gonadosomatic index over that same period (Figs. 52-53). Between weeks 8 and 9, condition index increased sharply over a week (~32%) followed by a steady decrease (~25%) that, again, was not an observed pattern either in GSI or dry gonad mass over the same interval (Figs. 52-53). Because condition index is largely dependent on temperature, salinity, and reproductive stage, and has been used to evaluate growth, meat yield, effects of environmental stress (Zeng and Yang 2020), and other fitness-associated traits (Wu et al. 2022), we had intended to use condition index to help interpret GSI and changes to gonad mass over time (Yi et al. 2009). Since data in Figure 61 are a composite of all tidal heights taken together, condition index was examined over time separately by tidal height (Fig. 67), but that granularity did not enhance the interpretation of previous analyses.

Brunswick

As with clams from Flake Point Bar, Jonesport, condition index was independent of clam size over the range of sizes considered (33.8-101.1 mm SL; F = 2.69, df = 1, 372, P = 0.1020, r^2 = 0.0072; Fig. 68). A lack-of-fit test from the linear model demonstrated that no additional terms to the model would improve the fit (F = 1.56, df = 3, 370, P = 0.198). Attempts to normalize the data using a log-transform to both variables failed to improve the fit (F = 1.98, df = 1, 372, P = 0.160, r^2 = 0.0053). Given the lack of a statistically significant relationship between CI and clam size, mean CI was used in all subsequent analyses.

ANOVA on the untransformed mean condition index data (Table 11) indicated a significant effect due to week (P < 0.0001), but no significant effect due to tidal height (P = 0.064), and no significant tide x week interaction (P = 0.256). Because a priori contrasts are examined regardless whether the main effect was statistically significant, those associated with tidal height suggested a pattern similar to those observed for clams in Bremen and Jonesport (Fig. 69). That is, upper intertidal mean CI (17.4 ± 1.08%, n = 117) was ~7% lower than the combined mean of mid and lower intertidal clams (18.7 ± 0.77%, n = 257). Mean CI varied significantly across weeks (P < 0.0001; Table 11) with the general pattern showing a steady decrease from late April to mid August (Fig. 70). Four orthogonal, a priori contrasts similar in scope to those used previously for Jonesport and Bremen were as follows: 1) April vs. May, June, July, August; 2) May vs. June, July, August; 3) June vs. July, August; and, 4) July vs. August. All contrasts, except #4 were statistically significant (Table 11), and in each instance, the earlier mean was significantly greater than the later means. For example, mean CI decreased by ~20% between April (22.6 ± 2.57%, n = 34) vs. all other months (contrast #1; 17.83 ± 0.63%, n = 340). Decreases in mean CI also were observed for contrast #2 (~16%) and contrast #3 (~15%). The decrease in mean CI between July and August was ~4%, which was not significantly different from zero (P = 0.342; Table 11). Two of the largest week-to-week drops in mean GI occurred between weeks 9 and 10 (18-25 June; ~18%) and weeks 12 and 13 (9-16 July; ~20%). Expanding Fig. 70 to examine changes in weekly mean CI along each tidal height (Fig. 71) demonstrated good concordance with tidal height-specific least-square means of both dry gonad weight and dry GSI (Table 12). Each of the six Pearson correlation coefficients were positive, and five were statistically significant indicating that as condition index increased, so too did both gonadal variables. This suggests condition index may be used as a surrogate for gonad index, at this location (see Louis et al. 2024).

Viability

Viability (proportion of eggs that produced polar body I within 30 minutes upon adding sperm) of soft-shell clam eggs was tested across 129 pairings during 2024 (Fig. 72). A two-factor, fixed effects analysis of variance was performed on mean viability (Table 13) demonstrated that size of female clam did not affect viability (P = 0.333; $\bar{x}_{\text{All females}}$ = 63.3 ± 4.6%, n = 129; however, size of male clam did (P = 0.007; Table 13). A priori contrasts indicated the lack of a significant difference in mean percent viability between medium and large male clams (i.e., clams ≥ 50 mm; P = 0.200; overall mean = 67.0 ± 5.2%, n = 97). Conversely, mean percent viability in small male clams (<50 mm SL) was 51.88 ± 8.9%, n = 32), and this ~25% difference between small and large clams was significant (P = 0.004; Table 13).

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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) of each 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	×	
04/10/2024	×	
05/09/2024	×	
06/27/2024	×	
07/26/2024	×	
08/22/2024	×	

Table 2. Cumulative statistics (April to August 2024) for data collected from 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 April 2024 to August 2024 (see Table 1). 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 haphazard collection 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	0	0.00	0	0.00
2.00-2.24	23	2.04	23	2.04
2.25-2.49	103	9.16	126	11.20
2.50-2.74	210	18.67	336	29.87
2.75-2.99	264	23.47	600	53.33
3.00-3.24	255	22.67	855	76.00
3.25-3.49	135	12.00	990	88.00
3.50-3.74	94	8.36	1,084	96.36
3.75-3.99	30	2.67	1,114	99.02
4.00-4.24	10	0.89	1,124	99.91
>4.25	1	0.09	1,125	100.00

Table 3. Cumulative statistics for all (15) 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 August 2024 (see Fig. 3a). 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 haphazard collection 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 currently exists.

size (inches)	Frequency	Percent	Cumulative Frequency	Cumulative Percent
<2	40	1.11	40	1.11
2.00-2.24	385	10.69	425	11.81
2.25-2.49	687	19.08	1,112	30.89
2.50-2.74	837	23.25	1,949	54.14
2.75-2.99	675	18.75	2,624	72.89
3.00-3.24	516	14.33	3,140	87.22
3.25-3.49	254	7.06	3,394	94.28
3.50-3.74	143	3.97	3,537	98.25
3.75-3.99	47	1.31	3,584	99.56
4.00-4.24	15	0.44	3,599	99.99
>4.25	1	0.00	3,600	100.00

Table 4. 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 2023. 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 currently 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 5. Minimum and maximum mean high tide temperatures (ºC) observed at each of the study sites during 2024.

Site	Tidal Height	Minimum (date)	Maximum (date)
Brunswick	Upper	2.94 (22 March)	28.58 (17 July)
	Mid (boxes)*	3.80 (22 March)	10.11 (2 April)
	Mid (outside boxes)	4.21 (13 March)	27.40 (17 July)
	Low**	3.74 (22 March)	27.13 (18 July)
Bremen	Upper	3.10 (22 March)	21.69 (14 July)
	Mid (boxes)***	2.91 (22 March)	19.96 (11 August)
	Mid (outside boxes)	4.38 (23 March)	19.58 (17 July)
	Low*		
Jonesport	Upper****	2.04 (22 March)	16.54 (12 August)
	Mid (boxes)*****	2.41 (24 March)	12.75 (22 May)
	Mid (outside boxes)	2.77 (24 March)	15.86 (27 August)
	Low*****	2.87 (22 March)	14.11 (6 June)

* Logger failed to record after 8 April
** Logger failed to record after 5 August
*** Logger failed to record after 30 August
**** Logger failed to record after 13 August
***** Logger failed to record after 22 May

Table 6. Fate (mean percent survival – in parentheses) of soft-shell clams in boxes seeded with 24 animals in 2024 at each site and tidal height. At each tidal height, two boxes were protected from nemertean predation using a piece of PetScreen® added between the top of the box and the bottom of the protective top. The protective top on those boxes was the same used in 2023 (vinyl-coated trap wire affixed to the top of the frame; 3.2 mm extruded netting affixed to the bottom of the frame so that the bottom piece of netting and the PetScreen® were in contact with each other). The other two boxes used a protective top that was used in 2024 across all remaining boxes at each site and tidal height (i.e., the vinyl-coated trap wire and the 3.2 mm extruded netting were both affixed to the top of the wooden frame) without the additional piece of screening to exclude ribbon worms. Date is when boxes were sampled. * = spawning observed in laboratory using thermal shock.

Box	Treatment	Tide Hgt	Brunswick	Bremen	Jonesport
1)	Pet & 2023 top	Upper	5/14 (87.5%)	6/03 (37.5%)	6/22 (83.3%)
2)	Pet & 2023 top		5/14 (91.7%)	6/10 (100.0%)*	6/29 (83.3%)
3)	No Pet & 2024 top		5/14 (79.2%)	6/03 (100.0%)*	6/22 (95.8%)
4)	No Pet & 2024 top		5/14 (87.5%)	6/10 (87.5%)	6/29 (95.8%)
1)	Pet & 2023 top	Mid	6/04 (00.0%)	6/03 (62.5%)	6/22 (83.3%)
2)	Pet & 2023 top		6/11 (29.2%)	6/10 (83.3%)	6/29 (91.7%)
3)	No Pet & 2024 top		6/04 (95.8%)	6/03 (95.8%)*	6/22 (87.5%)
4)	No Pet & 2024 top		6/11 (100.0%)*	6/10 (100.0%)	6/29 (75.0%)
1)	Pet & 2023 top	Lower	6/04 (79.2%)	6/03 (70.8%)*	6/22 (83.3%)
2)	Pet & 2023 top		6/11 (79.2%)	6/10 (91.7%)	6/29 (95.8%)*
3)	No Pet & 2024 top		6/04 (87.5%)	6/03 (100.0%)*	6/22 (79.2%)
4)	No Pet & 2024 top		6/11 (83.3%)*	6/11 (95.8%)	6/29 (95.8%)

Table 6a. Analysis of variance on mean percent survival of clams seeded at a density of 24/box at each site. Treatment refers to: 1) clams protected from milky ribbon worms with PetScreen and 2023-type protective box top; and, 2) clams not protected from milky ribbon worms and 2024-type protective box top. (All factors are fixed; n = 2; statistically significant sources of variation are boldfaced)

Sourceof variation	**df**	**SS**	**MS**	**F**	**Pr>F**
Site	2	37.56	18.78	0.87	0.4372
Treatment	1	81.00	81.00	3.74	0.0691
Site x Treatment	2	164.67	82.33	3.80	**0.0420**
Tide Height	2	47.72	23.86	1.10	0.3538
Site x Tide Height	4	133.94	33.49	1.55	0.2315
Treatment x Tide Hgt	2	129.50	64.75	2.99	0.0757
Site x Trt x Tide Hgt	4	168.83	42.21	1.95	0.1460
Error	18	390.00	21.67		
Total	35	1153.22			

Table 7. 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: Active; Gametogenic I stage; gametogenesis has begun; no ripe gametes visible.

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

D1: Early - 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: Late - 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 8. Analysis of variance on mean gonadosomatic index (GSI) for six size classes of soft-shell clams across sampling time that has been pooled by month (**M**ay: 29 April – 27 May [n = 5]; **J**une: 3 June – 24 June [n = 4]; **J**uly: 1 July – 29 July [n = 5]; **A**ugust: 5 August – 26 August [n = 4]). (See Fig. 54). A priori contrasts are presented for both variables, and are indented below each main source of variation (see text for more extensive contrast description). Number of replicates for each group varied between 10 and 31; hence, Type III sums of squares are presented given an unbalanced data set. P-values for statistically significant sources of variation are boldfaced.

Sourceof variation	**df**	**SS**	**MS**	**F**	**Pr>F**
Month	3	2732.90	910.97	15.29	**<.0001**
A) M & J v. J & A	1	2577.47	2577.47	43.25	**<.0001**
B) May vs. June	1	137.22	137.22	2.31	0.1309
C) July vs. August	1	18.21	18.21	0.31	0.5817
Size	5	2380.40	476.08	7.98	**<.0001**
D) Smallest vs. Largest	1	1438.43	1438.43	24.14	**<.0001**
E) Small (50 v. rest)	1	662.55	662.55	11.12	**<.0001**
F) 50-59 v. 60-69	1	237.46	237.46	3.98	**0.0465**
G) Large (70 v. rest)	1	25.28	25.28	0.42	0.5173
H) 80-89 v. >90	1	16.68	16.68	0.28	0.5971
Month x Size	15	1523.01	101.53	1.70	**0.0481**
A x D	1	44.17	44.17	0.74	0.3902
A x E	1	24.73	24.73	0.42	0.5173
A x F	1	551.24	551.24	9.25	**0.0025**
A x G	1	4.22	4.22	0.07	0.7915
A x H	1	6.45	6.45	0.11	0.7403
B x D	1	101.55	101.55	1.70	0.1931
B x E	1	2.59	2.59	0.04	0.8416
B x F	1	18.69	18.69	0.31	0.5780
B x G	1	360.76	360.76	6.05	**0.0144**
B x H	1	143.70	143.70	2.41	0.1214
C x D	1	0.00	0.00	0.00	0.9143
C x E	1	53.01	53.01	0.89	0.3461
C x F	1	55.29	55.29	0.93	0.3355
C x G	1	113.05	113.05	1.89	0.1700
C x H	1	2.50	2.50	0.04	0.8416
Error	367	21868.88	59.59		
Total	390	28505.21			

Table 9. A) Model I analysis of variance on the full data set of mean gonadosomatic index (GSI) associated with soft-shell clams from Brunswick (N = 377). Two orthogonal factors were size class (a = 6; < 50 mm, 50-59.9 mm, 60-69.9 mm, 70-79.9 mm, 80-89.9 mm, and ≥ 90 mm) and month (b = 5; April, May, June, July, and August). Prior to the analysis, it was determined that within a size category, GSI was independent of clam size. (n varied between 3 and 23; hence, Type III sums of squares are presented – see Shaw and Mitchell-Olds 1993). All statistically significant probabilities are boldfaced. B) ANOVA on a subset of the data that excluded the two extreme size classes. A priori contrasts are presented and occur indented beneath each main factor.

A)

Sourceof variation	**df**	**SS**	**MS**	**F**	**Pr>F**
Month	4	4612.89	1153.22	16.37	**<.0001**
Size	5	9309.82	1861.96	26.43	**<0.001**
Month x Size	20	3353.82	167.69	2.38	**0.0009**
Error	347	24445.80	70.45		
Total	376	43689.43			

B)

Sourceof variation	**df**	**SS**	**MS**	**F**	**Pr>F**
Month	4	7690.99	1922.75	24.52	**<.0001**
April/May v. Jun-August	1	4466.48	4466.48	56.95	**<.0001**
April v. May	1	120.41	120.41	1.54	0.2164
June v. July/August	1	3717.17	3717.17	47.40	**<.0001**
July v. August	1	1212.70	1212.70	15.46	**0.0001**
Size	3	1278.84	426.28	5.44	**0.0012**
50-59.9 vs. rest	1	646.53	646.53	8.24	**0.0044**
60-60.9 vs. rest	1	629.05	629.05	8.02	**0.0050**
70-79.9 vs. 80-89.9	1	9.57	9.57	0.12	0.7272
Month x Size	12	829.76	69.15	0.88	0.5662
Error	254	19919.18	78.42		
Total	273	30208.10			

Table 10. Analysis of variance on the untransformed mean Condition Index for soft-shell clams at Flake Point Bar, Jonesport, Maine (4 May to 1 September 2024). A priori contrasts are indented beneath their specific main source of variation. Number of clams per week varied between 3 and 6; therefore, Type III SS are used (Shaw and Mitchell-Olds 1993). Significant P-values are boldfaced.

Source of variation	**df**	**SS**	**MS**	**F**	**Pr>F**
Tidal Height	2	306.72	153.36	7.76	**0.0005**
Upper vs. Mid & Lower	1	250.42	250.42	12.68	**0.0004**
Mid vs. Lower	1	56.52	56.52	2.86	0.0916
Week	17	656.44	38.61	1.95	**0.0133**
May vs. Jun, Jul, Aug, Sep	1	38.25	38.25	1.94	0.1650
Jun vs. Jul, Aug, Sep	1	1.03	1.03	0.05	0.8192
Jul vs. Aug, Sep	1	359.66	359.66	18.21	**<.0001**
Aug vs. Sep	1	0.97	0.97	0.05	0.8250
Tide x Week	34	775.52	22.87	1.16	0.2557
Error	353	6973.29	19.75		
Total	406	8553.31			

Table 11. Analysis of variance on mean Condition Index for experimental soft-shell clams deployed at three tidal heights and sampled on 18 dates from 23 April to 20 August 2024 at Barnes Flat, Brunswick, Maine. A priori contrasts are indented beneath their specific main source of variation. Number of clams per week varied between 3 and 6; therefore, Type III SS are used (Shaw and Mitchell-Olds 1993). Significant P-values are boldfaced.

Source of variation	**df**	**SS**	**MS**	**F**	**Pr>F**
Tidal Height	2	180.56	90.28	2.77	0.0639
Upper vs. Mid & Lower	1	172.27	172.27	5.29	**0.0220**
Mid vs. Lower	1	8.29	8.29	0.25	0.6141
Week	17	2596.77	152.75	4.69	**<.0001**
April vs. May – August	1	728.18	728.18	22.38	**<.0001**
May vs. June – August	1	794.88	794.88	24.43	**<.0001**
June vs. July – August	1	362.31	362.31	11.14	**0.0009**
July vs. August	1	29.53	29.53	0.91	0.3415
Tide x Week	33	775.52	22.87	1.16	0.2557
Error	321	10443.80	32.54		
Total	373	14245.58			

Table 12. Pearson correlation coefficient (± 95% confidence intervals) matrix for Condition Index (CI), least-square means of dry gonad weight (DGW) and mean dry gonad index (GSI) for soft-shell clams at each tidal height across sampling dates. n = 18 for all data from upper and mid intertidal, and n = 17 for all lower intertidal data. Significant P-values appear boldfaced.

	CI-Upper	CI-Mid	CI-Lower
DGW-Upper	r = 0.147 (-.10 – 0.72) P = 0.384		
DGW-Mid		r = 0.767 (0.47 – 0.91) P < **0.001**	
DGW-Lower			r = 0.565 (0.12 – 0.82) P = **0.018**
GSI-Upper	r = 0.561 (0.13 – 0.81) P = **0.016**		
GSI-Mid		r = 0.770 (0.47 – 0.91) P < **0.001**	
GSI-Lower			r = 0.597 (0.16 – 0.84) P = **0.011**

Table 13. Analysis of variance on mean percent viability of soft-shell clam eggs. Three sizes of clams (independent of sex) were: 1) Small <50 mm; 2) Medium 50.0-69.9 mml 3) Large ≥ 70 mm. Viability was assessed 30 minutes after attempts to fertilize eggs that had been released by females that responded to thermal shock in the laboratory. Data were pooled across sampling date and tidal height. Sample size (n) varied between 8-21 for each combination of male and female clam size. A priori contrasts were conducted for both main factors: 1) Small vs. Medium & Large. Type III sums of squares was used due to unbalanced data (Shaw and Mitchell-Olds 1993). Significant P-values are boldfaced.

Source of variation	**df**	**SS**	**MS**	**F**	**Pr>F**
FEMALES	2	1458.51	729.28	1.11	0.3326
Small vs. Medium & Large	1	928.00	928.00	1.41	0.2368
Medium vs. Large	1	530.51	530.51	0.81	0.3705
MALES	2	6784.65	3392.33	5.17	**0.0070**
Small vs. Medium & Large	1	5695.17	5695.17	8.67	**0.0039**
Medium vs. Large	1	1089.48	1089.48	1.66	0.2002
FEMALES x MALES	4	1147.00	286.75	0.44	0.7818
Error	120	78778.62	656.49		
Total	128	88353.50			

Figure Legends

Figure 1. Maine soft-shell clam landings (1950-2023; black bars), and annual dockside value (corrected using the Consumer Price Index for January 2024.

Figure 2. Size-frequency distribution of commercial size soft-shell clams at A.C. Inc. buying station (Black Duck Cove Road, Beals, ME) across five sampling dates from April 2024 to August 2024 (N = 5; n = 225). (x-axis bins are in inches.)

Figure 3. Cumulative size-frequency distribution of commercial size soft-shell clams at A.C. Inc. buying station (Black Duck Cove Road, Beals, ME) across 5 sampling dates from April-August 2024 (n = 1,125). (x-axis bins are in inches.)

Figure 3a. Size-frequency distribution of commercial size soft-shell clams at A.C. Inc. buying station (Black Duck Cove Road, Beals, ME) across all 15 sampling dates from September 2022 to August 2024 (n = 3,600). (x-axis bins are in inches.)

Figure 4. 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 5. 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 6. Bremen, Maine (5 June 2023). A) Piece of PetScreen® mesh used to exclude milky ribbon worms from entering the box from the mudflat surface. It is possible to observe the points of contact the mesh made with the sediment surface in the box. B) Five clams that did not burrow in the sediments after they had been placed on the sediment surface within the box (15 March 2023; 82 days). Note the impression of the PetScreen® mesh on the sediment surface.

Figure 7. Mean number of clams that did not burrow in boxes sampled from Bremen in 2023 and 2024. Photographs were taken of the surface sediments of each box on each sampling date. Five sampling dates were chosen at random in each year and photos from four of six boxes sampled on each date. The number of clams in each photo that were observed on the sediment surface in each box constituted the data. A nested ANOVA revealed the lack of a statistically significant effect due to year ($F = 2.17$, $df = 1, 8$; $P = 0.1793$). Since no PetScreen® was used in 2024, the results suggest that the difference in mean number of unburrowed clams between years (1.85 ± 0.67 v. 1.15 ± 0.63) could have occurred due to random chance alone. (n = 20)

Figure 8. Wooden top frame used to protect clams from predators in 2023. The 1-inch thick wooden frame has a piece of vinyl-coated trap wire (12.7 mm aperture) stapled to its top, and a piece of polyethylene mesh (3.2 mm aperture) stapled to its bottom.

This photo was taken immediately after the top frame was unscrewed from the box holding the experimental clams. The piece of PetScreen® intended to exclude milky ribbon worms can be seen covering the box immediately below the frame.

Figure 9. Wooden frame (1-inch thick; 1 ft^2) used to protect clams from predators in 2024. A) Photo is looking at the protective top frame as if it were screwed to the box containing clams. Both pieces of protective mesh (vinyl-coated trap wire and 3.2 mm mesh) are affixed to the top of the wooden frame. B) Photo is looking at the bottom of the protective top frame. Note that no mesh was affixed to the bottom of the frame. This resulted in a 1-inch gap between the sediment surface and the piece of 3.2 mm mesh. (Meter stick is used for scale.)

Figure 9a. Mean percent survival (+95% CI) of experimental clams in extra boxes at each site. One treatment attempted to recreate conditions that clams experienced in 2023 (24/box; PetScreen® present; 2023 protective top). The other treatment attempted to create a new condition (24/box; PetScreen® absent; 2024-type protective top). A significant site x treatment interaction occurred (P = 0.042; Table 6a).

Figure 10. A) Student intern adding warm (24°C) seawater to clams from two boxes from a single tidal height. B) Intern waiting for clams to spawn. Each tank contains experimental clams (30-100 mm SL) from two boxes that represent one of three tidal heights. A total of 18 weekly samplings occurred at each of the three study sites.

Figure 11. Soft-shell clams releasing gametes in response to thermal shock. A) Male; B) Female.

Figure 12. Clams that spawned in the spawning tank were placed into fingerbowls where gametes (eggs) were contained for subsequent counts. Sperm was used to assess percent viability as indicated by polar body I formation approximately 20 minutes after fertilization occurs.

Figure 13. Size-frequency distribution of clams from each study site that contributed to the population of animals that were selected for histology in 2023.

Figure 14. Size-frequency distribution of clams from each study site that contributed to the population of animals that were selected for histology in 2024.

Figure 15. Gonadal stages of the soft-shell clam. Upper photo: ♂ (63.14 mm) Inactive (I, Table 7); Jonesport (2 September 2023); Upper intertidal. Lower photo: ♂ (69.93 mm) Inactive; Brunswick (3 October 2023); Upper intertidal.

Figure 16. Gonadal stages of the soft-shell clam. Upper photo: ♂ (80.38 mm) Early Active (G1, Table 7); Brunswick (30 May 2023); Upper intertidal. Lower photo: ♀ (36.51 mm) Early Active; Jonesport (6 May 2023); Upper intertidal.

Figure 17. Gonadal stage of the soft-shell clam. Upper photo: ♀ (54.02 mm) Late Active (G2, Table 7); Jonesport (6 May 2023); Upper intertidal. Lower photo: ♂ (70.98 mm) Late Active; Brunswick (9 May 2023); Upper intertidal.

Figure 18. Gonadal stages of the soft-shell clam. Upper photo: ♀ (69.9 mm) Early Developing (D1, Table 7); Brunswick (8 May 2023); Upper intertidal. Lower photo: ♂ (65.5 mm) Early Developing; Brunswick (5 March 2023); Upper intertidal.

Figure 19. Gonadal stages of the soft-shell clam. Upper photo: ♀ (54.78 mm) Late Developing (D2, Table 7); Brunswick (16 May 2023); Upper intertidal. Lower photo: ♂ (86.32 mm) Late Developing; Jonesport (6 May 2023); Low intertidal.

Figure 20. Gonadal stages of the soft-shell clam. Upper photo: ♀ (71.8 mm) Ripe (R, Table 7); Brunswick (1 August 2023); Upper Intertidal. Lower photo: ♂ (38.6 mm) Ripe; Brunswick (11 July 2023); Upper intertidal.

Figure 21. Gonadal stages of the soft-shell clam. Upper photo: ♀ (75.35 mm) Spawning (S1, Table 7); Brunswick (30 May 2023); Upper Intertidal. Lower photo: ♂ (73.95 mm) Spawning (S1); Brunswick (20 June 2023); Upper intertidal.

Figure 22. Gonadal stages of the soft-shell clam. Upper photo: ♀ (68.71 mm) Spawning (S2, Table 7); Brunswick (30 May 2023); Upper Intertidal. Lower photo: ♂ (72.71 mm) Spawning (S2); Brunswick (3 June 2023); Upper intertidal.

Figure 23. Gonadal stages of the soft-shell clam. Upper photo: ♀ (67.46 mm) Spawning (S3, Table 7); Jonesport (26 August 2023); Upper Intertidal. Lower photo: ♂ (78.78 mm) Spawning (S3); Brunswick (5 September 2023); Upper intertidal.

Figure 24. Gonadal stages of the soft-shell clam. Upper photo: ♀ (83.62 mm) Spawning (S4, Table 7); Jonesport (18 July 2023); Upper Intertidal. Lower photo: ♂ (64.41 mm) Spawning (S4); Brunswick (3 October 2023); Upper intertidal.

Figure 25. Gonadal stages and the proportion of soft-shell clams within each stage across tidal height and time from Flake Point Bar, Jonesport, Maine in 2023.

Figure 26. Mean seawater temperature (ºC) at high tide superimposed on the gonadal stages and proportion of soft-shell clams within each stage across tidal height and time from Flake Point Bar, Jonesport, Maine in 2023.

Figure 27. Gonadal stages and the proportion of ambient and experimental soft-shell clams within each stage across tidal height (U, M, L = Upper, Mid, and Lower intertidal, respectively) on 3, 17, and 27 June 2023 at Flake Point Bar, Jonesport, Maine.

Figure 28. Gonadal stages and the proportion of ambient and experimental soft-shell clams within each stage across tidal height (U, M, L = Upper, Mid, and Lower intertidal, respectively) on 15 and 29 July and 2-5 September 2023 at Flake Point Bar, Jonesport, Maine.

Figure 29. Gonadal stages and the proportion of soft-shell clams within each stage across tidal height and time from Narrows Flat, Bremen, Maine in 2023.

Figure 30. Mean seawater temperature (°C) at high tide superimposed on the gonadal stages and proportion of soft-shell clams within each stage across tidal height and time from Narrows Flat, Bremen, Maine in 2023.

Figure 31. Gonadal stages and the proportion of soft-shell clams within each stage across tidal height and time from Barnes Flat, Brunswick, Maine in 2023.

Figure 32. Mean seawater temperature (°C) at high tide superimposed on the gonadal stages and proportion of soft-shell clams within each stage across tidal height and time from Barnes Flat, Brunswick, Maine in 2023.

Figure 33. Gonadal stages and the proportion of ambient and experimental soft-shell clams within each stage across tidal height (U, M, L = Upper, Mid, and Lower intertidal, respectively) on 30 May, 14 & 20 June, 5 September and 3 October 2023 at Barnes Flat, Brunswick, Maine. (There were too few ambient clams at the low intertidal to sample except on the final collection date.)

Figure 34. Mean survival of experimental clams collected from boxes arrayed at each of three tidal heights (upper, mid, and lower) at Flake Point Bar, Jonesport during 2024 (week 1 = 4 May; week 18 = 1 September). $\hat{Y}_{Upper}$ = 104.3 – 2.182X (r^2 = 0.62; F = 26.12, df = 1, 16; P < 0.001); $\hat{Y}_{Mid}$ = 98.9 – 1.794X (r^2 = 0.647; F = 29.33, df = 1, 16; P < 0.001); $\hat{Y}_{Lower}$ = 99.66 – 1.088X (r^2 = 0.51; F = 16.67, df = 1, 16; P = 0.001). N = 2; No confidence intervals are displayed due to small sample sizes.

Figure 35. Percent of living clams that were induced to release gametes (spawn) from Flake Point Bar, Jonesport, Maine in 2024 (5/4 to 9/1).

Figure 36. Mean survival of experimental clams collected from boxes arrayed at each of three tidal heights (upper, mid, and lower) at Narrows Flat, Bremen during 2024 (week 1 = 29 April; week 18 = 26 August). $\hat{Y}_{Upper}$ = 92.45 – 0.001X (r^2 = 0.00; F = 0.00, df = 1, 16; P = 0.997); $\hat{Y}_{Mid}$ = 95.64 – 0.597X (r^2 = 0.112; F = 2.01, df = 1, 16; P = 0.176); $\hat{Y}_{Lower}$ = 91.67 – 0.0447 (r^2 = 0.17; F = 0.03, df = 1, 16; P = 0.871). N = 2; No confidence intervals are displayed due to small sample sizes.

Figure 37. Percent of living clams that were induced to release gametes (spawn) from Narrows Flat, Bremen, Maine in 2024 (4/29 to 8/26).

Figure 38. Mean survival of experimental clams collected from boxes arrayed at each of three tidal heights (upper, mid, and lower) at Barnes Flat, Brunswick during 2024 (week 1 = 23 April; week 19 = 27 August).). $\hat{Y}_{Upper}$ = 100.6 – 1.103X (r^2 =0.458; F = 13.54, df = 1, 16; P = 0.002); $\hat{Y}_{Mid}$ = 101.1 – 1.858X (r^2 = 0.314; F = 7.33, df = 1, 16; P = 0.016); $\hat{Y}_{Lower}$ = 103.0 – 1.873 (r^2 = 0.445; F = 12.02, df = 1, 15; P = 0.003). N = 2; No confidence intervals are displayed due to small sample sizes.

Figure 39. Percent of living clams that were induced to release gametes (spawn) across three tidal heights from Barnes Flat, Brunswick, Maine in 2024 (4/23 to 8/20).

Figure 40. Percent of female clams induced to spawn using thermal shock by month and tidal height from each study site. (Top/green: Jonesport; Middle/blue: Bremen; Lower/orange: Brunswick). (n = number of individuals that released eggs that includes eight individuals that did not release enough eggs to count efficiently.)

Figure 41. Relationship between mean number of eggs released and sampling date (Day 1 = 30 April; Day 112 = 19 August). Least squares regression line (black) and 95% confidence interval of $\hat{Y}$. $\hat{Y}$ = 75999 + 29424X (P = 0.008; r^2 = 0.116; n = 60).

Figure 42. Percent of male clams induced to spawn using thermal shock by month and tidal height from each study site. Top, middle, and lower graphs are similar to that in Figure 40. (n = number of individuals that released sperm.)

Figure 43. Relationship between eggs released via thermal shock and shell length for soft-shell clams at the three study sites. Data is pooled across sampling dates and tidal heights. The regression equation is: $Y = 0.0399X^{3.995}$ (r^2 = 0.434, P < 0.001, n = 60).

Figure 44. Relationship between eggs released via thermal shock and shell length for soft-shell clams at Bremen across tidal heights. Regression equations for each tidal height are: Upper – $Y = 0.000849X^{5.007}$ (r^2 = 0.585, P < 0.001); Middle – $Y = 0.6237X^{3.441}$ (r^2 = 0.495, P = 0.003); Lower – $Y = 1.7539X^{2.881}$ (r^2 = 0.169, P = 0.183).

Figure 45. Boxplot of mean egg diameter taken from a sample of ten eggs from each of 61 clams that were induced to release eggs after receiving a thermal shock. Red lines are means (Jonesport, n = 4; Bremen, n = 47; Brunswick, n = 10). ANOVA revealed a significant difference in mean egg size between locations (P = 0.049), and an a posteriori test indicated that size difference between the two extreme sites (Jonesport [64.2 ± 23.8 µm] vs. Brunswick [71.3 ± 3.1 µm]) was significantly different. Bremen: 68.06 ± 1.1 µm.

Figure 46. Relationship between mean egg diameter and sampling date. A) Overall data (n = 61) with all sites and tidal heights combined. Black line is the least square regression line: $\hat{Y}$ = 71.37 - 0.259X (F = 11.22; df = 1, 59; P = 0.001, r^2 = 0.159. B) Same relationship, but with study sites defined.

gonadosomatic index (GSI) and shell length. The relationship followed a linear model with $\hat{Y} = 4.491 + 0.3009X$ ($r^2 = 0.204$, $P < 0.001$, $n = 377$).

Figure 57. Least square means ± 95% CI for dry gonad weight through time and across tidal heights of experimental clams at Barnes Flat, Brunswick, Maine from 23 April to 20 August 2024. No live clams occurred in low intertidal boxes on 16 July; hence the dotted line from 9-23 July. (n = 6)

Figure 58. Least square means ± 95% CI for gonadosomatic index (GSI) through time and across tidal heights of experimental clams at Barnes Flat, Brunswick, Maine from 23 April to 20 August 2024. No live clams occurred in low intertidal boxes on 16 July; hence the dotted line from 9-23 July. (n = 6)

Figure 59. Mean gonadosomatic (GSI) index (+ 95% CI) for clams from Barnes Flat, Brunswick, Maine within each of six size categories (< 50 mm SL to ≥ 90 mm SL) pooled across tidal heights across sampling dates in 2024 pooled by months. Sample size (n) is variable for each bar and ranged from 3 to 23.

Figure 60. Relationship between Condition Index and shell length for clams pooled across three tidal heights (upper, mid, and lower) and 18 sampling dates from Flake Point Bar, Jonesport, Maine (4 May to 1 September 2024). A lack-of-fit test indicated a linear model best described the relationship (P = 0.349). The black line represents the grand mean ($\bar{\bar{Y}} = 14.4 \pm 0.45\%$, n = 407), and the blue dashed lines represent the 95% confidence interval around $\bar{\bar{Y}}$.

Figure 61. Mean Condition Index (± 95% CI) for soft-shell clams at Flake Point Bar, Jonesport, Maine (4 May to 1 September 2024). Means are pooled across tidal heights because there was no statistically significant tidal height x sampling date interaction (Table 10).

Figure 62. Mean Condition Index (± 95% CI) across tidal heights at Flake Point Bar, Jonesport, Maine (4 May to 1 September 2024). Analysis of variance (Table 10) indicated that mean CI for clams at the upper intertidal was significantly lower than the pooled mean for mid and lower intertidal clams (P = 0.0004). No significant difference was observed between mid and lower intertidal mean CI (P = 0.092). $n_{upper} = 136$; $n_{mid} = 136$; $n_{low} = 135$.

Figure 63. Mean Condition Index (± 95% CI) for soft-shell clams at Flake Point Bar, Jonesport, Maine (4 May to 1 September 2024) by week across each tidal height. This demonstrates the lack of a significant tidal height x week interaction (P = 0.256; Table 10), since the pattern across weeks is similar between tidal heights.

Figure 64. Relationship between Condition Index (CI) and shell length for clams pooled across three tidal heights (upper, mid, and lower) and 18 sampling dates from Narrows Flat, Bremen, Maine from 29 April to 26 August 2024. A lack-of-fit test indicated a linear model best described the relationship (P = 0.0927). The least-

squares equation (black line) for the line of best fit is: $\hat{Y} = 28.56 - 0.154X$ ($r^2 = 0.223$, n = 389). The blue dashed lines represent the 95% confidence interval around $\hat{Y}$.

Figure 65. Least-square means of Condition Index (± 95% CI) for soft-shell clams at Narrows Flat, Bremen, Maine (29 April to 26 August 2024). Means of tidal height are pooled across sampling dates. An a posteriori Scheffe test showed that each least-square mean was significantly different from another ($P \leq 0.038$).

Figure 66. Least-square means of Condition Index (± 95% CI) for soft-shell clams at Narrows Flat, Bremen, Maine (29 April to 26 August 2024). Means are pooled across tidal heights because there was no statistically significant tidal height x sampling date interaction.

Figure 67. Least-square means of Condition Index (± 95% CI) for soft-shell clams at Narrows Flat, Bremen, Maine (29 April to 26 August 2024) by week across each tidal height. This demonstrates the lack of a significant tidal height x week interaction ($P = 0.806$), since the pattern across weeks is similar between tidal heights.

Figure 68. Relationship between Condition Index and shell length for clams pooled across three tidal heights (upper, mid, and lower) and 18 sampling dates from Barnes Flat, Brunswick, Maine (23 April to 20 August 2024). A lack-of-fit test indicated a linear model best described the relationship ($P = 0.198$). The black line represents the grand mean ($\bar{\bar{Y}} = 18.3 \pm 0.63\%$, n = 374), and the blue dashed lines represent the 95% confidence interval around $\bar{\bar{Y}}$.

Figure 69. Mean Condition Index (± 95% CI) across tidal heights at Barnes Flat, Brunswick, Maine (23 April to 20 August). Analysis of variance (Table 11) indicated that mean CI for clams at the upper intertidal was significantly lower than the pooled mean for mid and lower intertidal clams ($P = 0.022$). No significant difference was observed between mid and lower intertidal mean CI ($P = 0.614$). $n_{upper} = 117$; $n_{mid} = 122$; $n_{low} = 135$.

Figure 70. Mean Condition Index (± 95% CI) for soft-shell clams at Barnes Flat, Brunswick, Maine (23 April to 20 August 2024). Means are pooled across tidal heights because there was no statistically significant tidal height x sampling date interaction (Table 11).

Figure 71. Mean Condition Index (± 95% CI) for soft-shell clams at Barnes Flat, Brunswick, Maine (23 April to 20 August 2024) by week across each tidal height. This demonstrates the lack of a significant tidal height x week interaction ($P = 0.256$; Table 11), since the pattern across weeks is similar between tidal heights.

Figure 72. Mean viability of eggs (+95% CI) for Large (≥ 70 mm), Medium (50-69.9 mm), and Small (< 50 mm) Females fertilized by Males within each size category. Data is pooled over time, study site, and sampling date.

Figure 1.

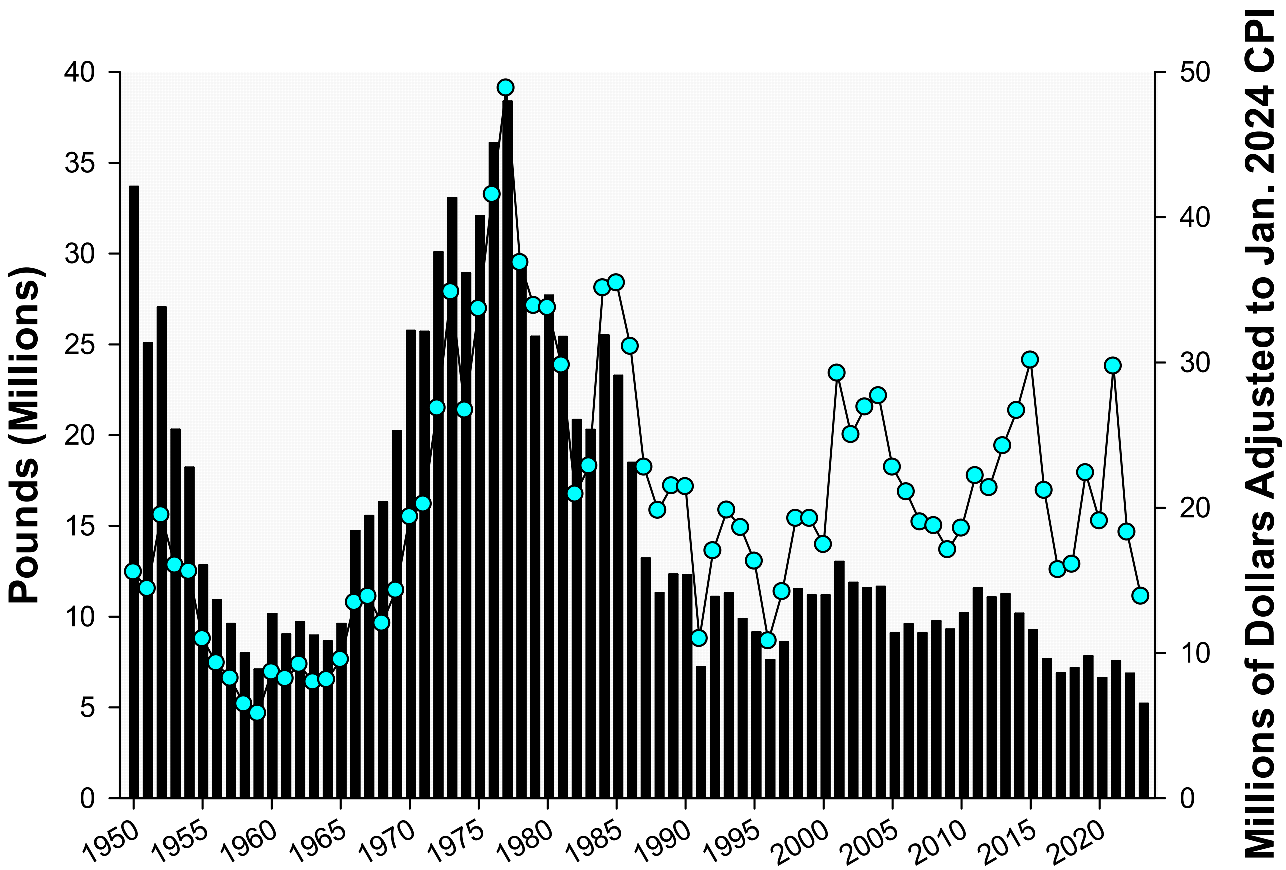

Pounds (Millions)
Millions of Dollars Adjusted to Jan. 2024 CPI
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50
1950
1955
1960
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015
2020

Figure 2.

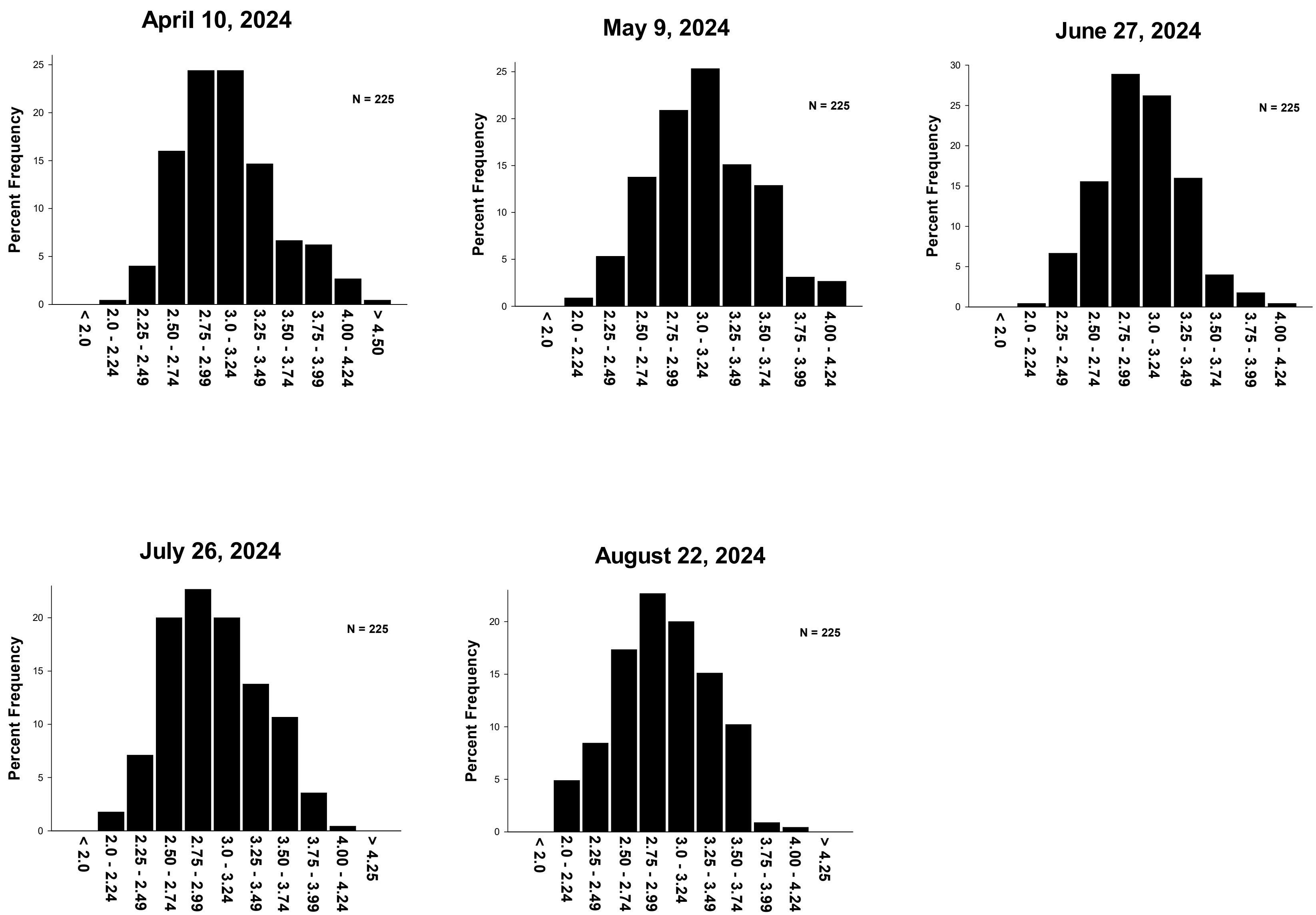

April 10, 2024
May 9, 2024
June 27, 2024
July 26, 2024
August 22, 2024
Percent Frequency
N = 225
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24
> 4.50
> 4.25

Figure 3.

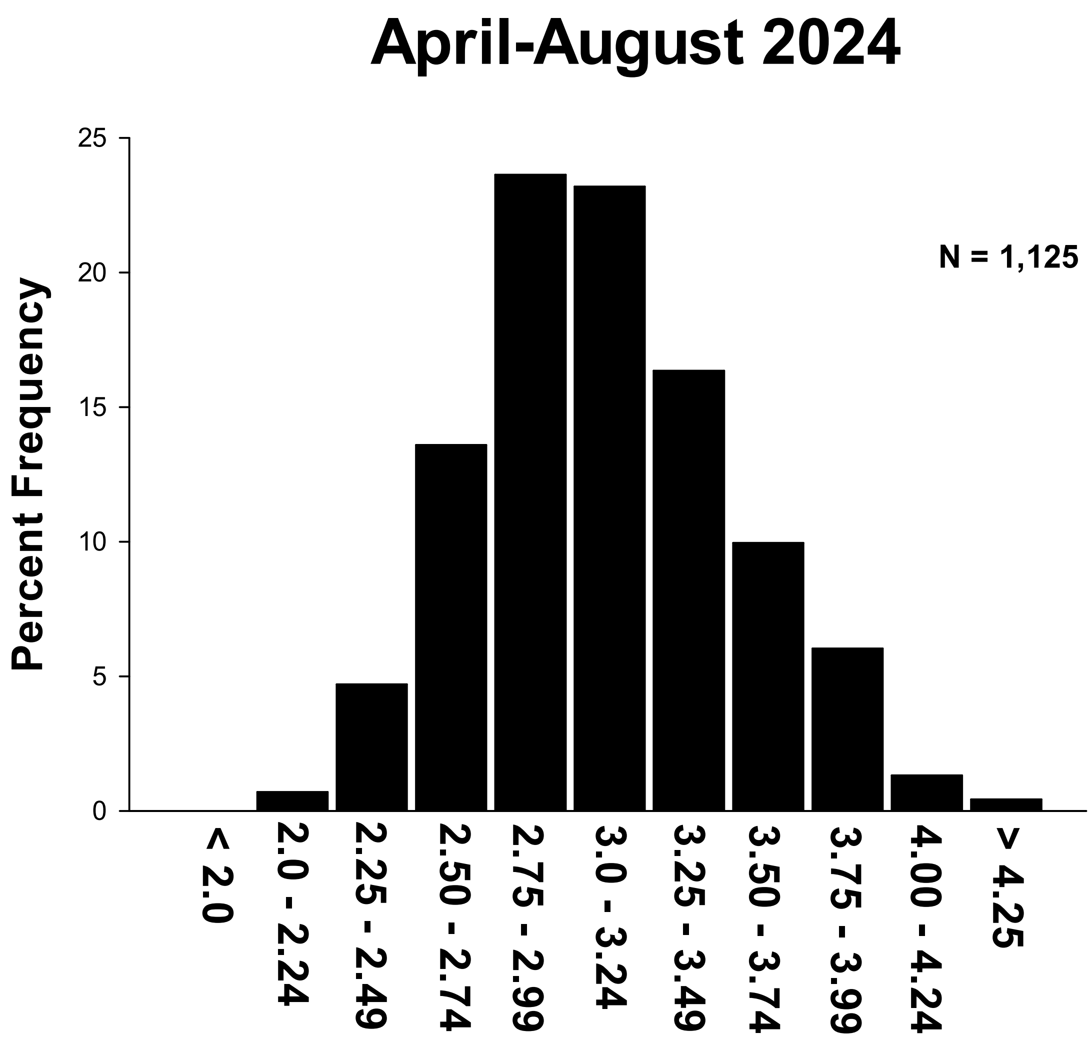

April-August 2024
Percent Frequency
0
5
10
15
20
25
N = 1,125
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24
> 4.25

Figure 3a.

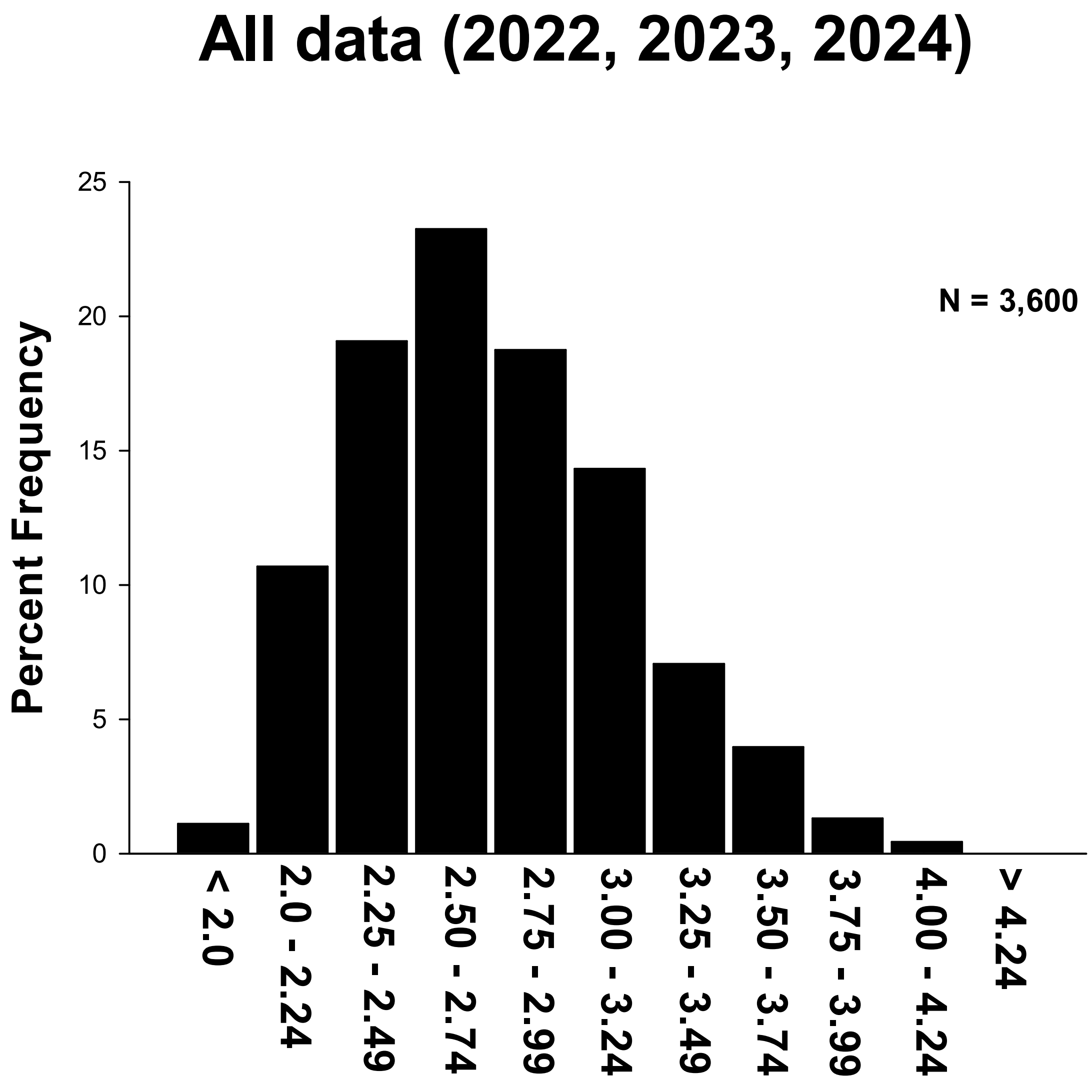

All data (2022, 2023, 2024)
N = 3,600
Percent Frequency
25
20
15
10
5
0
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.00 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24
> 4.24

Figure 4.

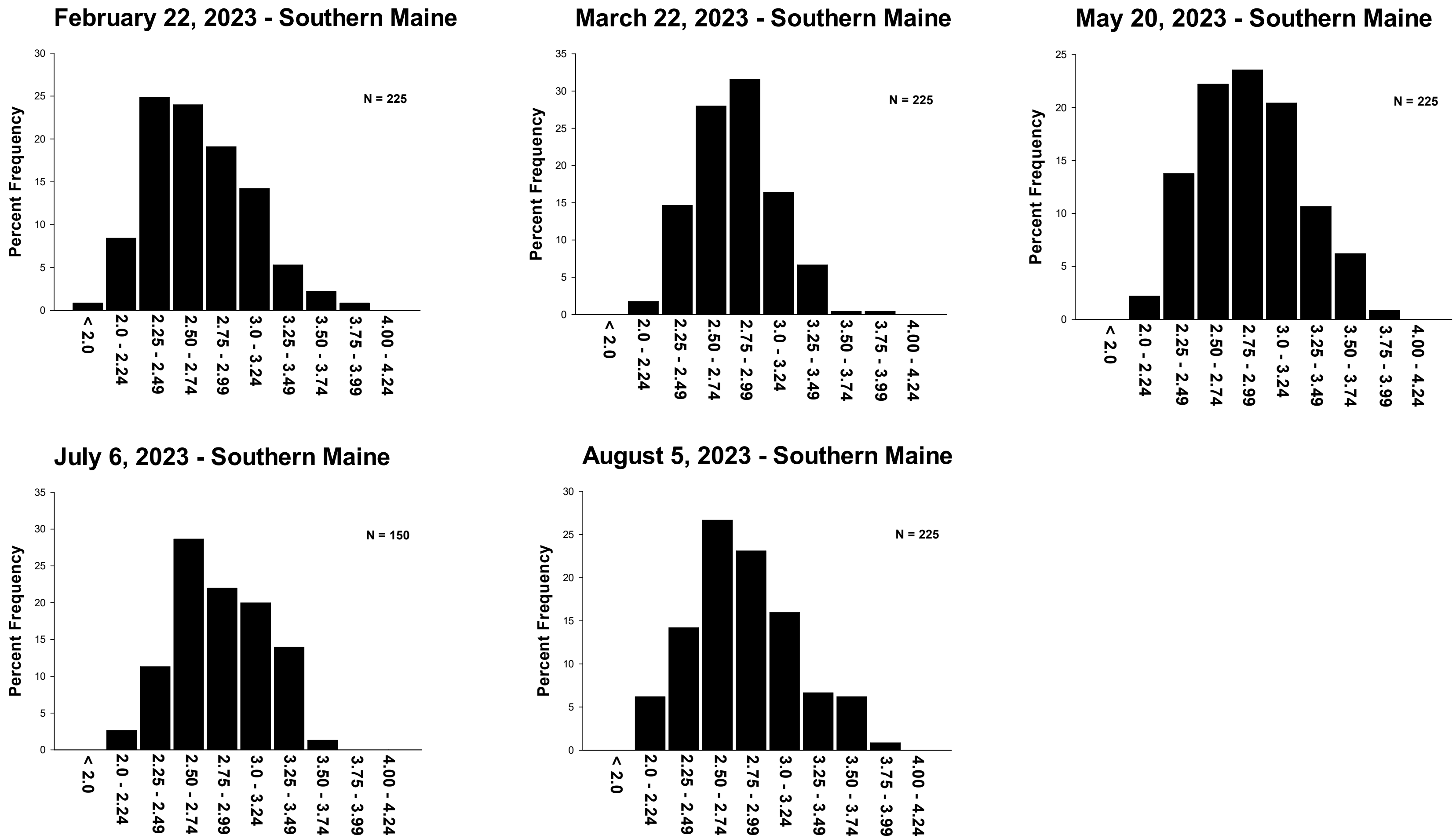

February 22, 2023 - Southern Maine
N = 225
March 22, 2023 - Southern Maine
N = 225
May 20, 2023 - Southern Maine
N = 225
July 6, 2023 - Southern Maine
N = 150
August 5, 2023 - Southern Maine
N = 225
Percent Frequency
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74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

Figure 5.

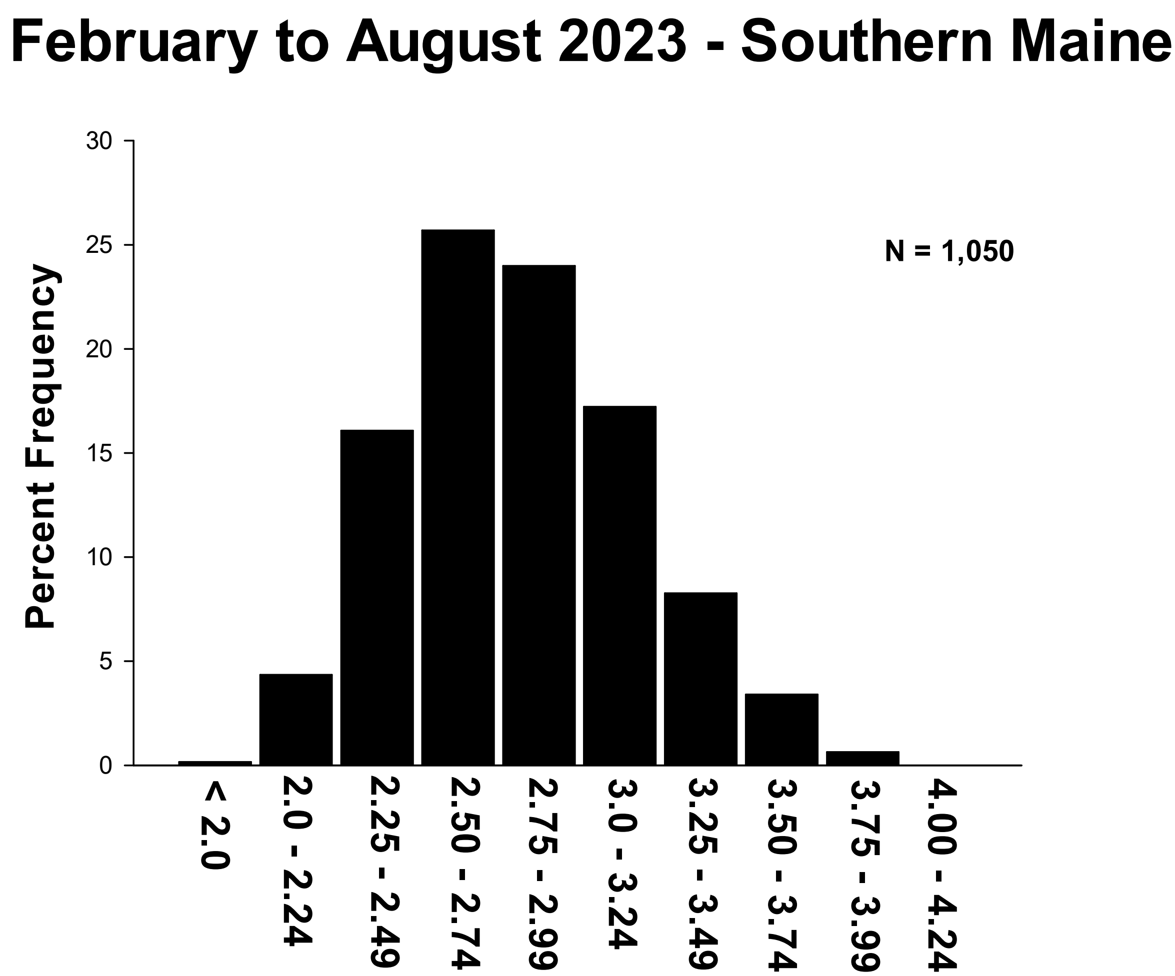

February to August 2023 - Southern Maine
N = 1,050
Percent Frequency
0
5
10
15
20
25
30
< 2.0
2.0 - 2.24
2.25 - 2.49
2.50 - 2.74
2.75 - 2.99
3.0 - 3.24
3.25 - 3.49
3.50 - 3.74
3.75 - 3.99
4.00 - 4.24

Figure 6.

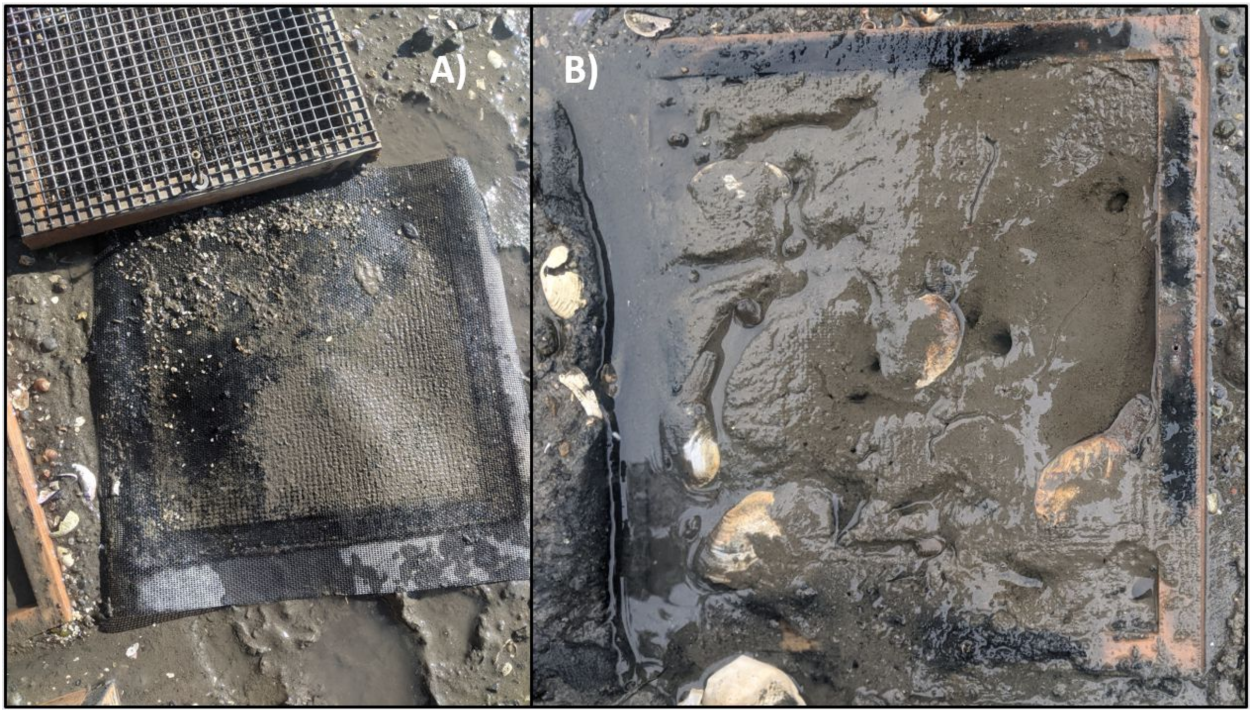


Figure 7.

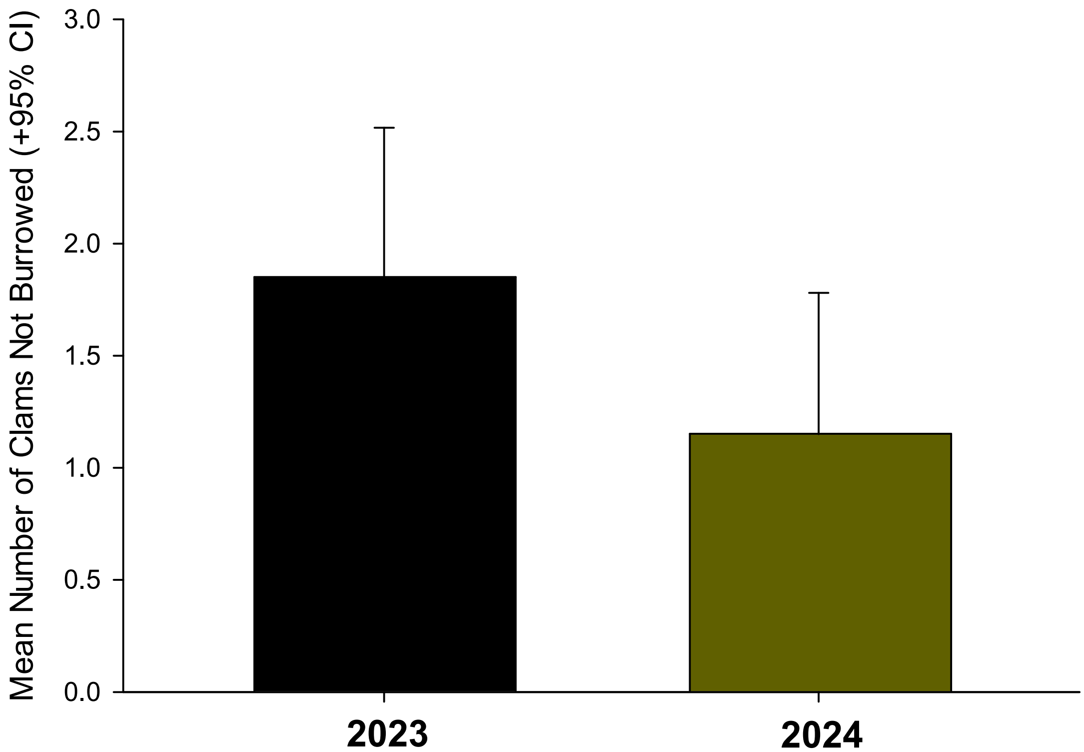

Mean Number of Clams Not Burrowed (+95% CI)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2023
2024

Figure 8.

Figure 9.

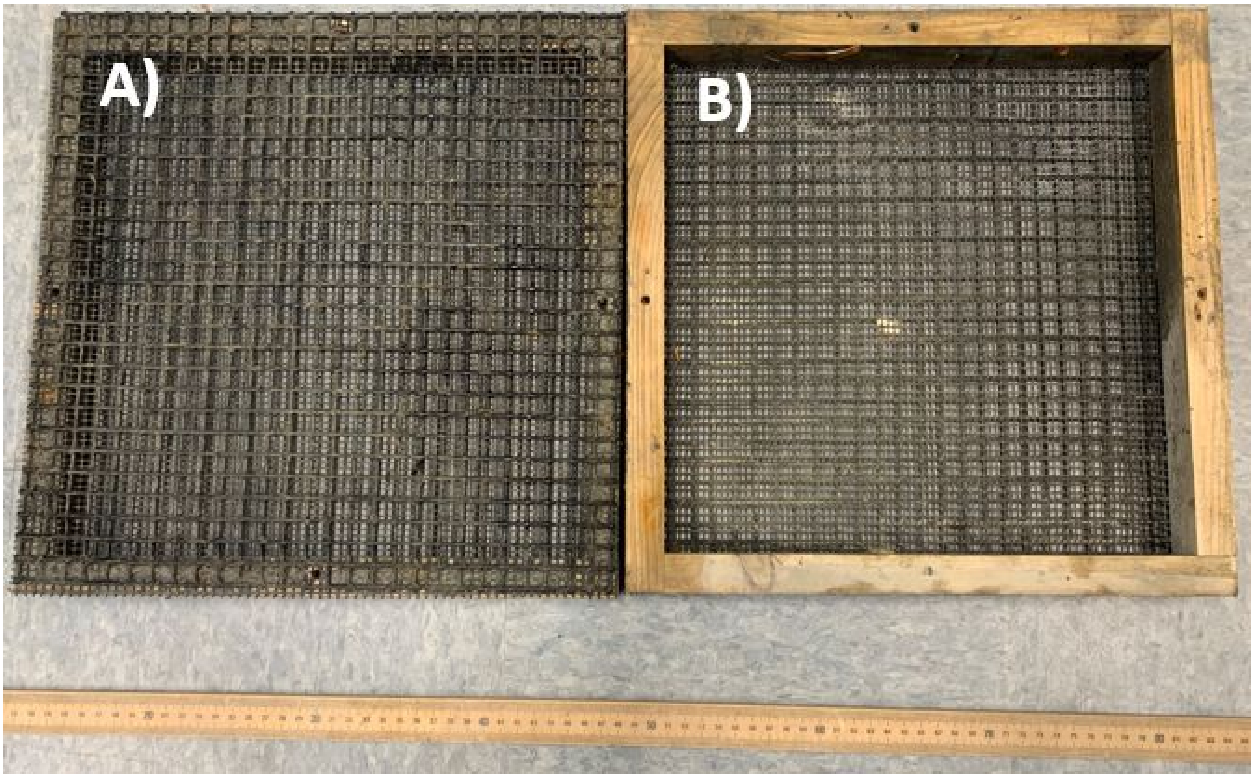


Figure 9a.

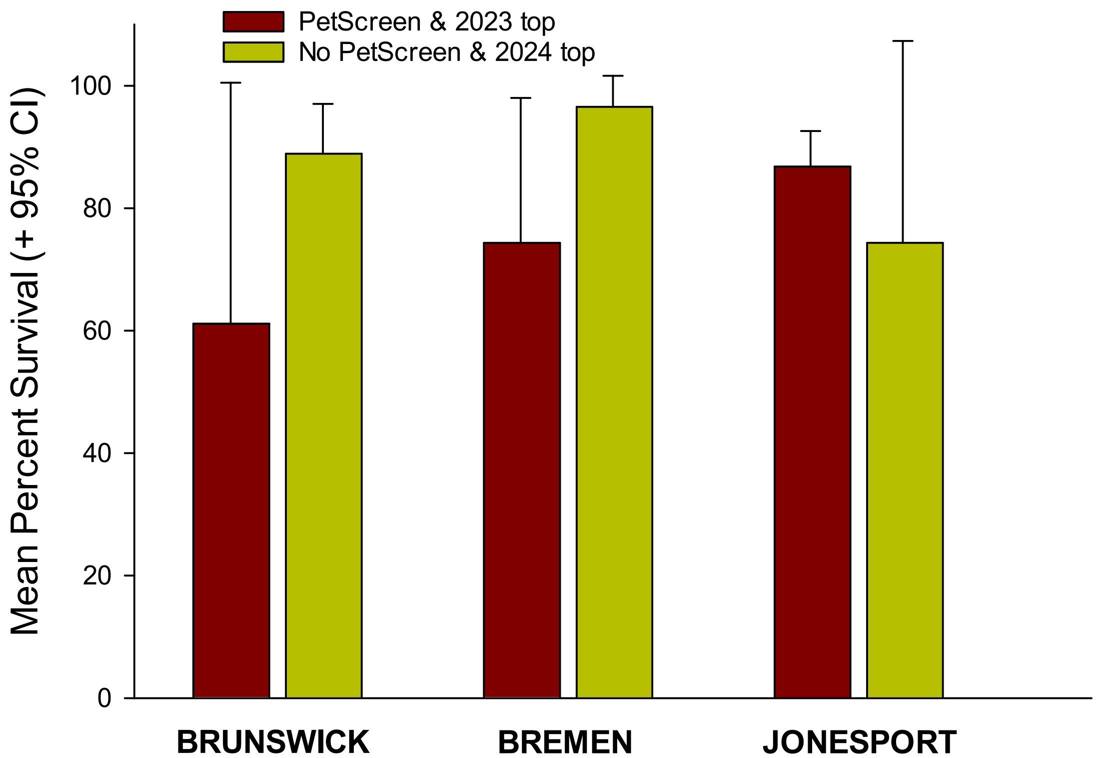

PetScreen & 2023 top
No PetScreen & 2024 top
Mean Percent Survival (+ 95% CI)
0
20
40
60
80
100
BRUNSWICK
BREMEN
JONESPORT

Figure 10.

Figure 11.

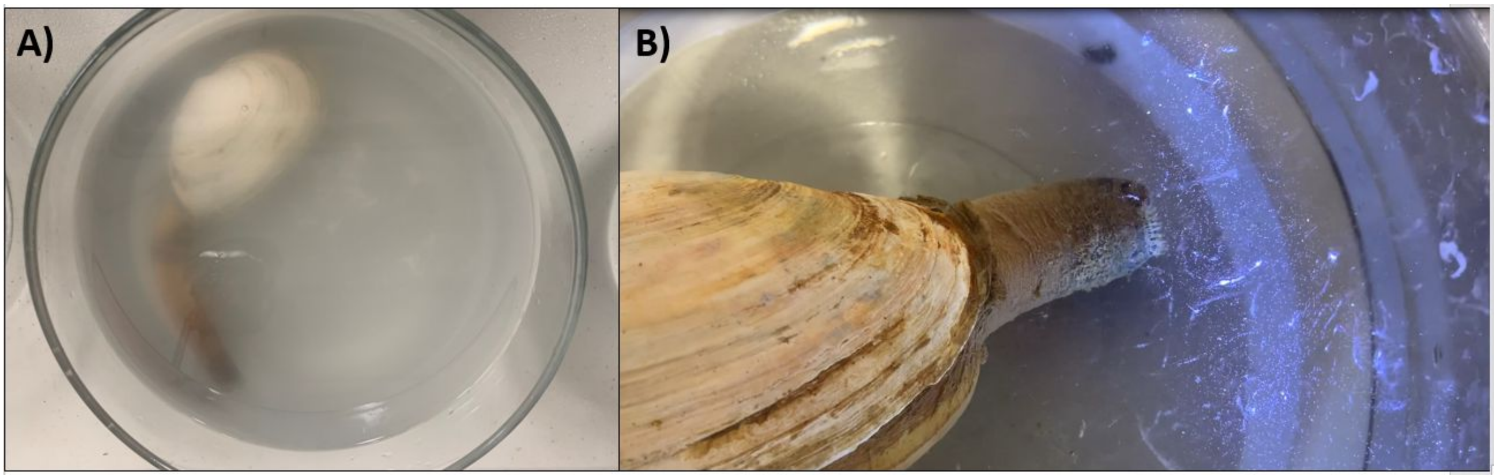


Figure 12.

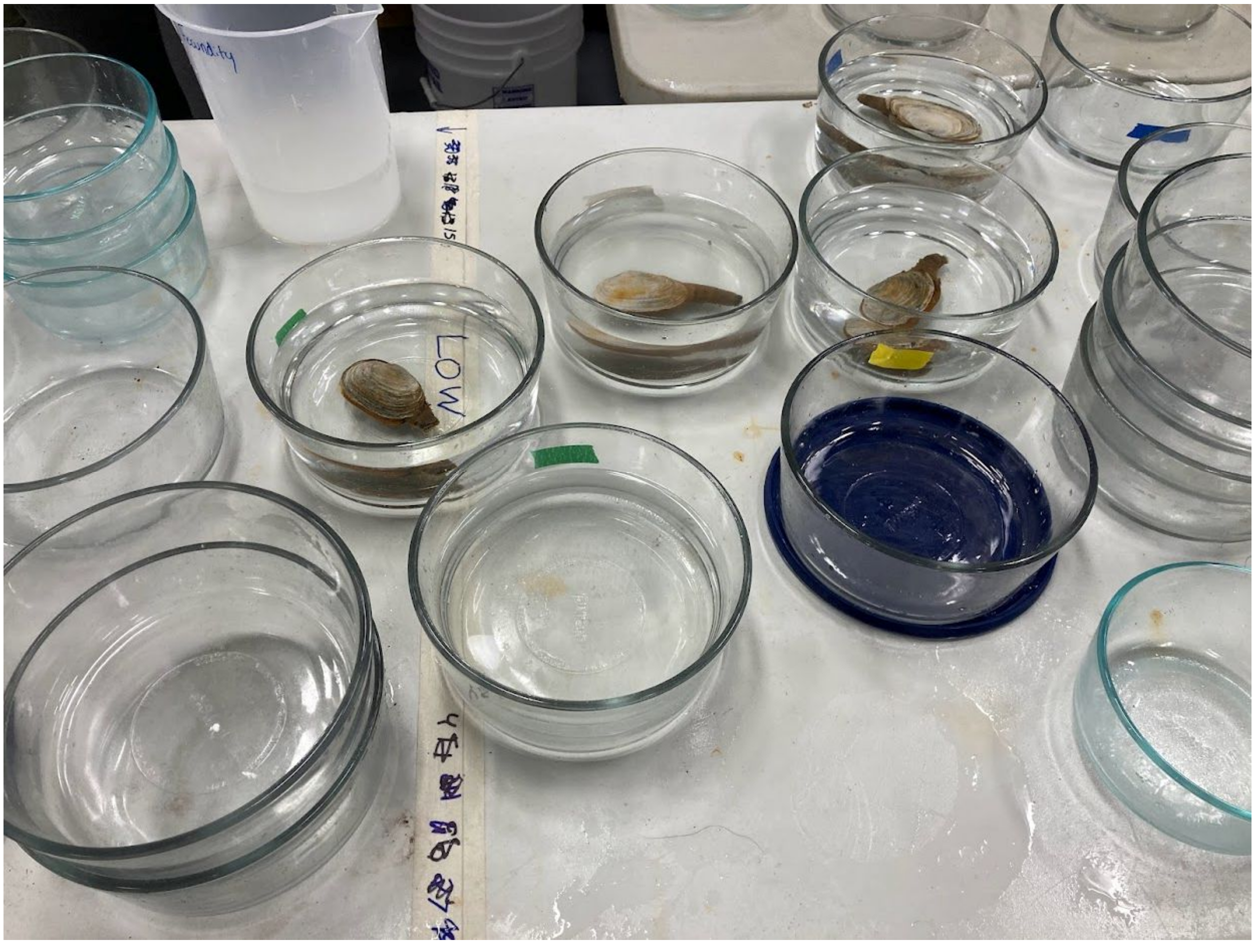


Figure 13.

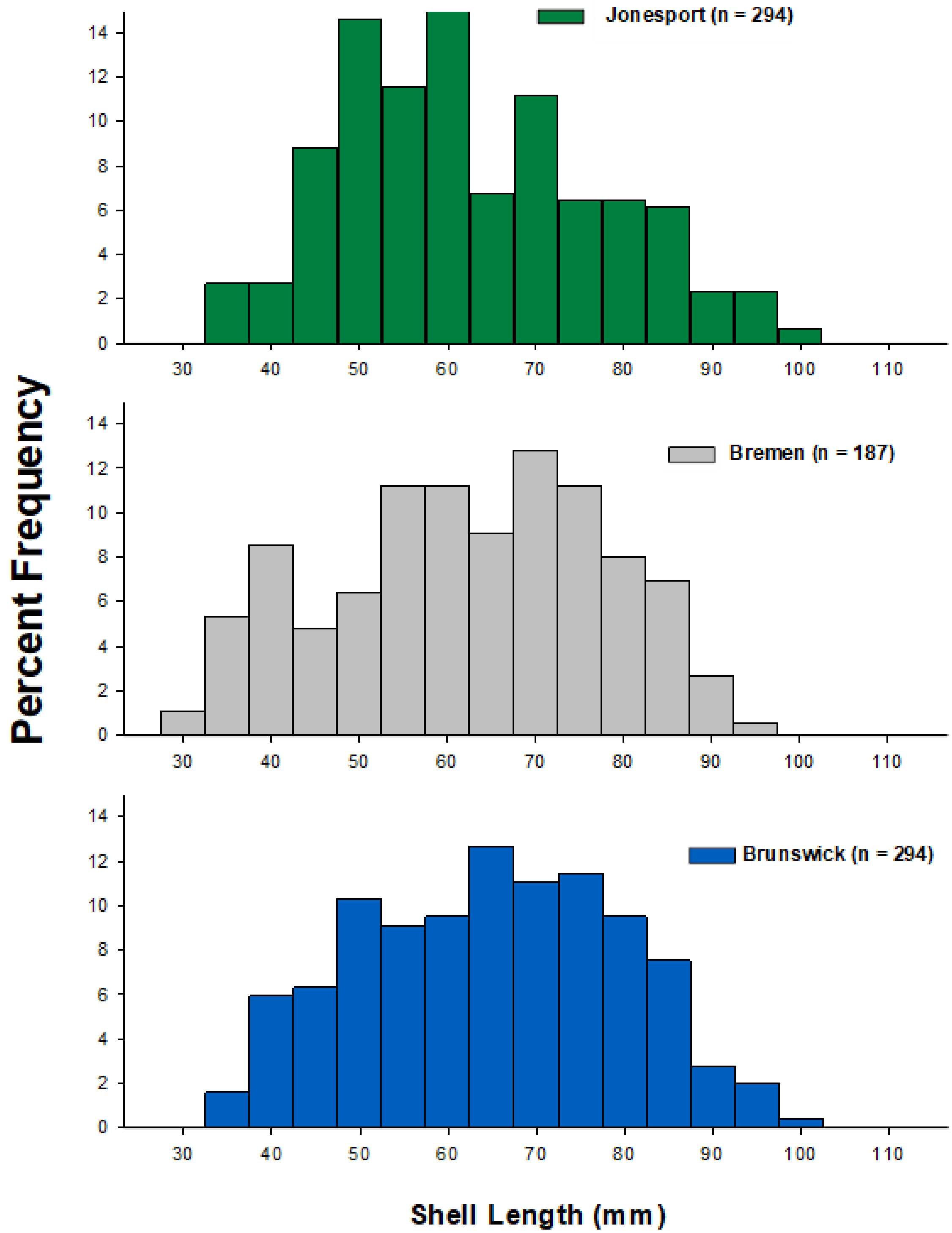

Jonesport (n = 294)
Bremen (n = 187)
Brunswick (n = 294)
Percent Frequency
Shell Length (mm)
0
2
4
6
8
10
12
14
30
40
50
60
70
80
90
100
110

Figure 14.

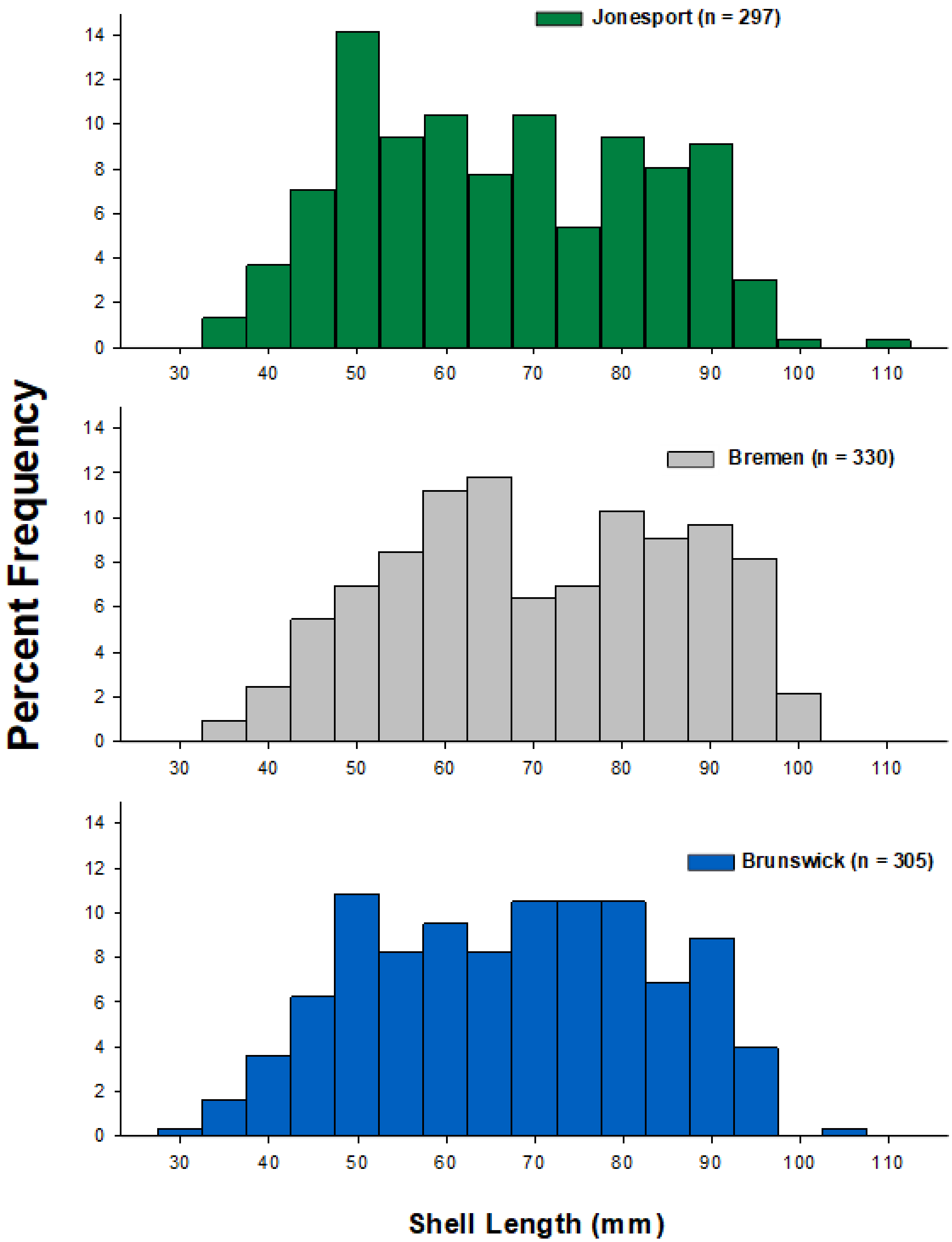
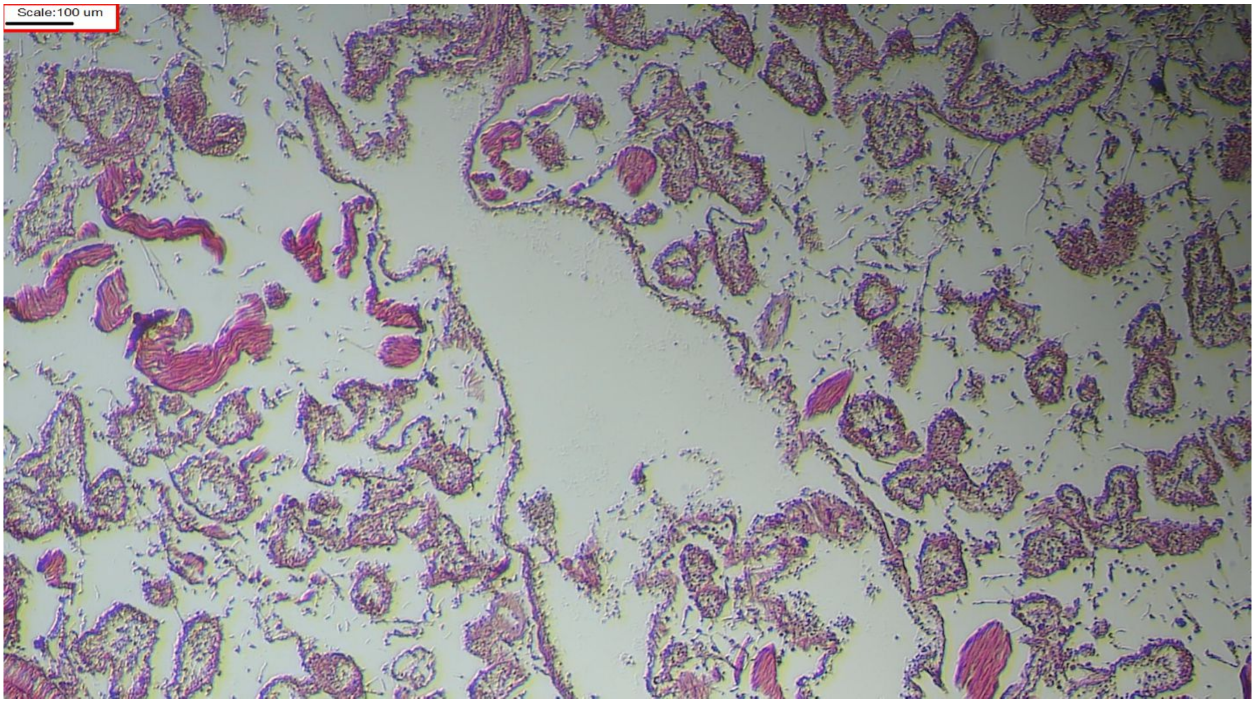

Jonesport (n = 297)
Bremen (n = 330)
Brunswick (n = 305)
Percent Frequency
Shell Length (mm)
0
2
4
6
8
10
12
14
30
40
50
60
70
80
90
100
110

Figure 15.



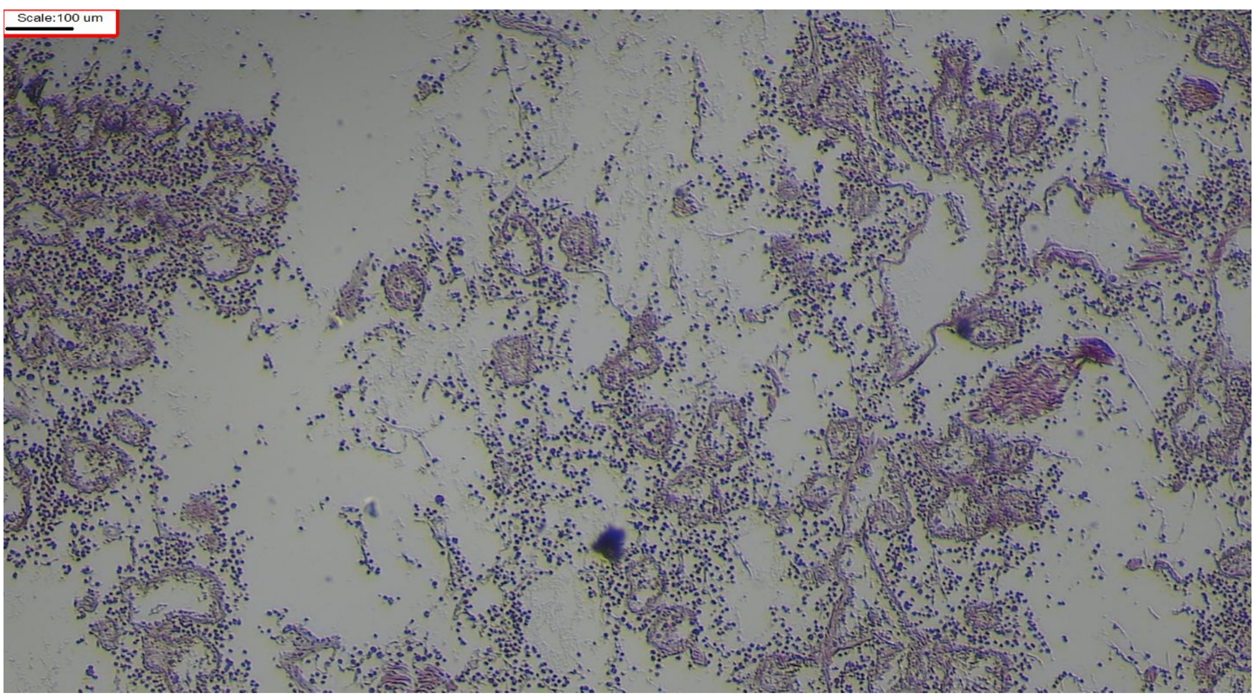
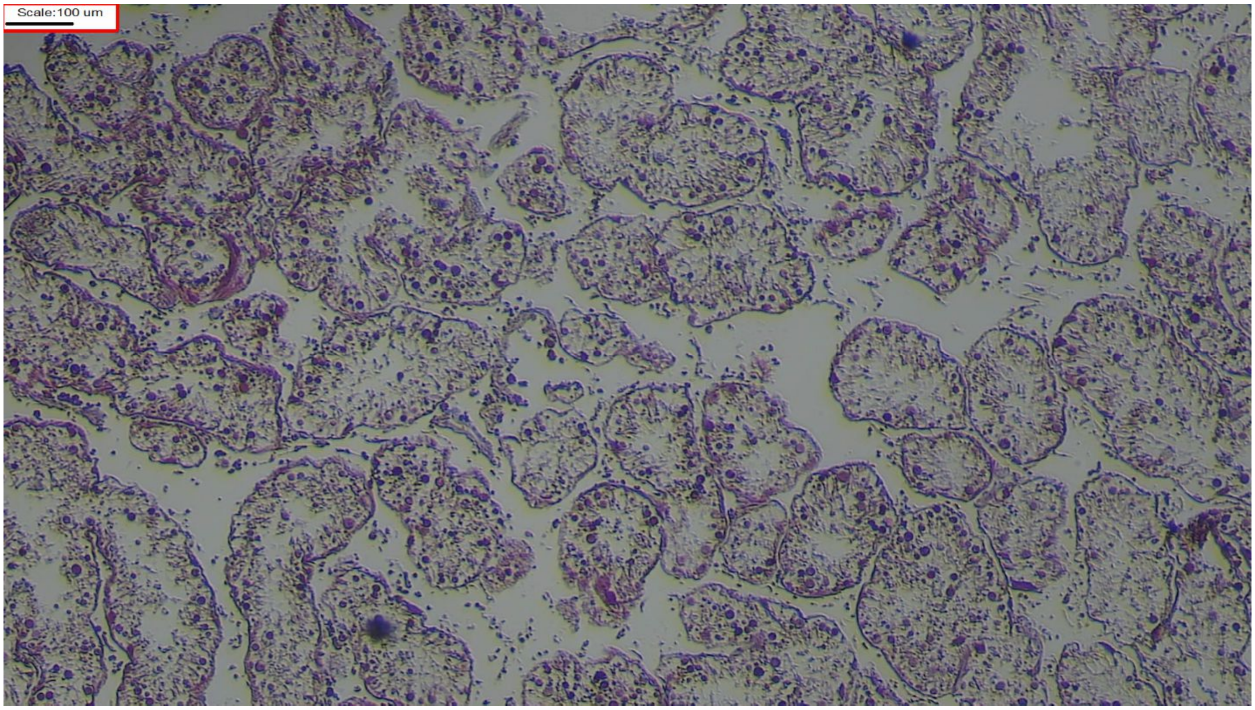


Figure 16.



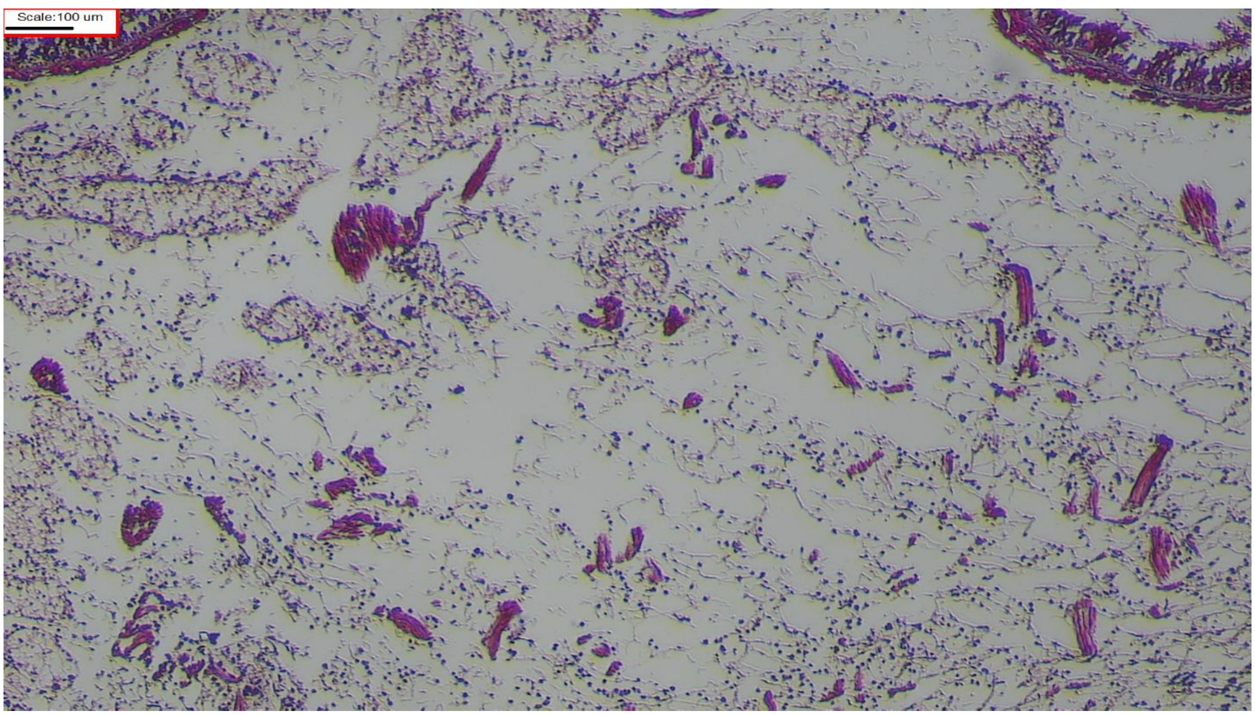


Figure 17.

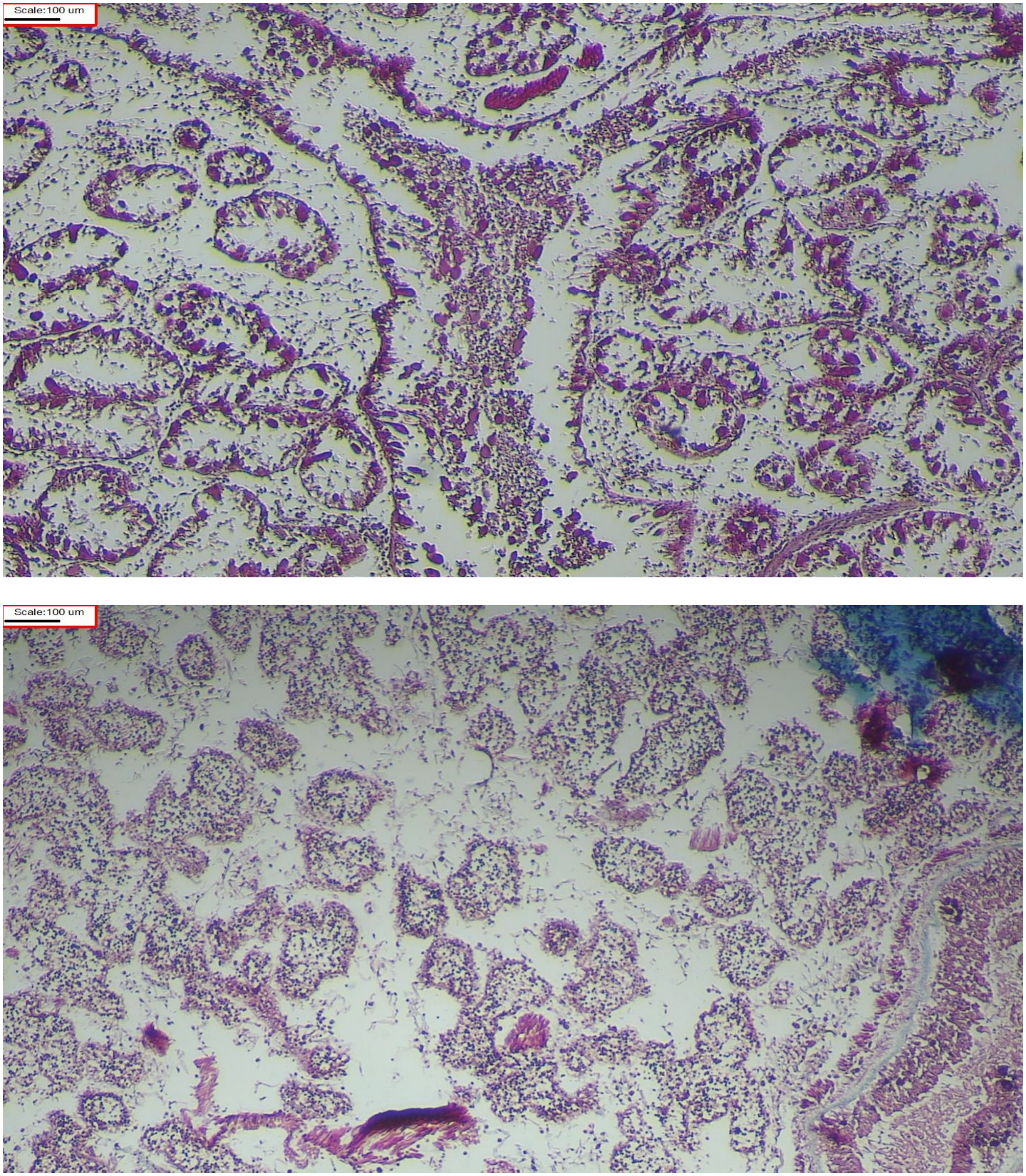
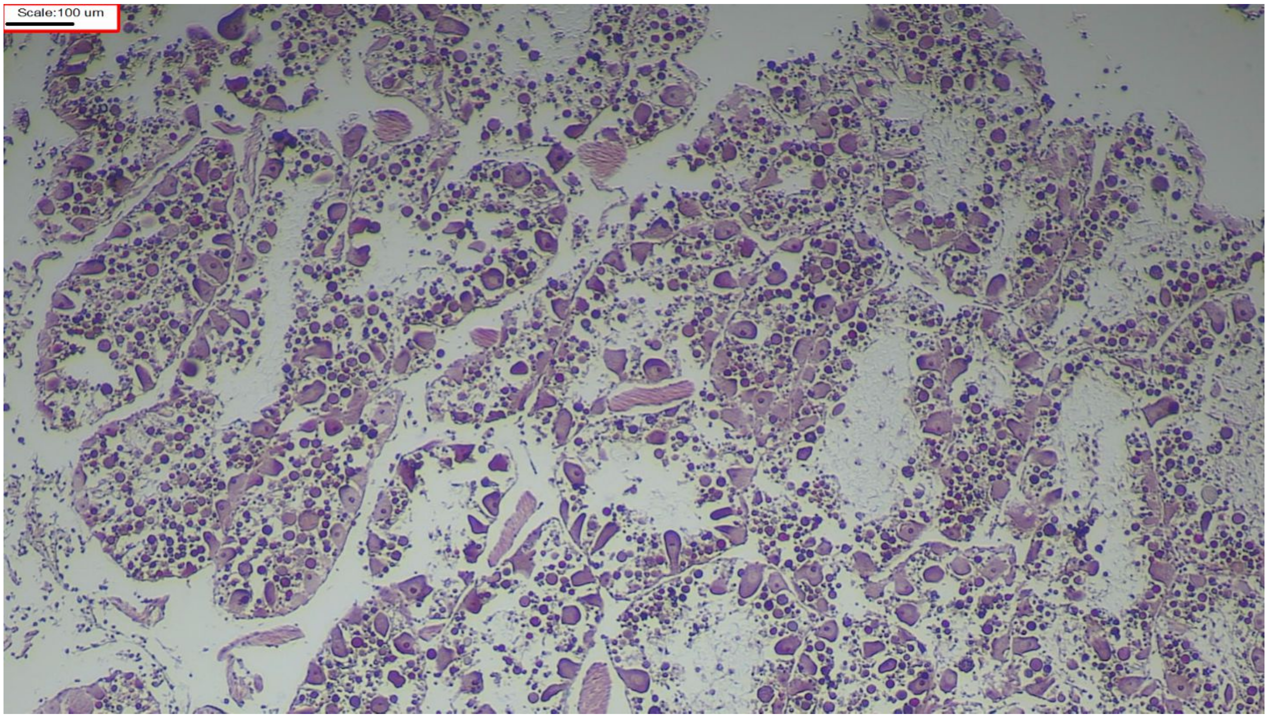


Figure 18.



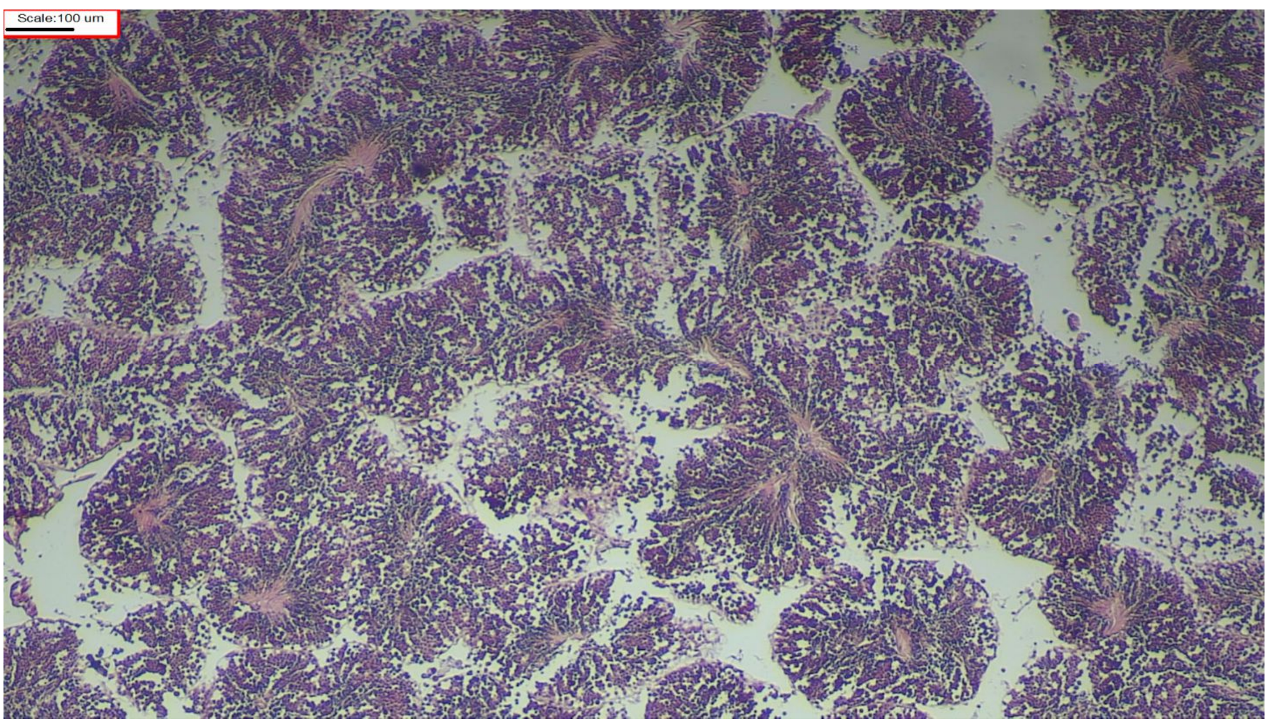


Figure 19.

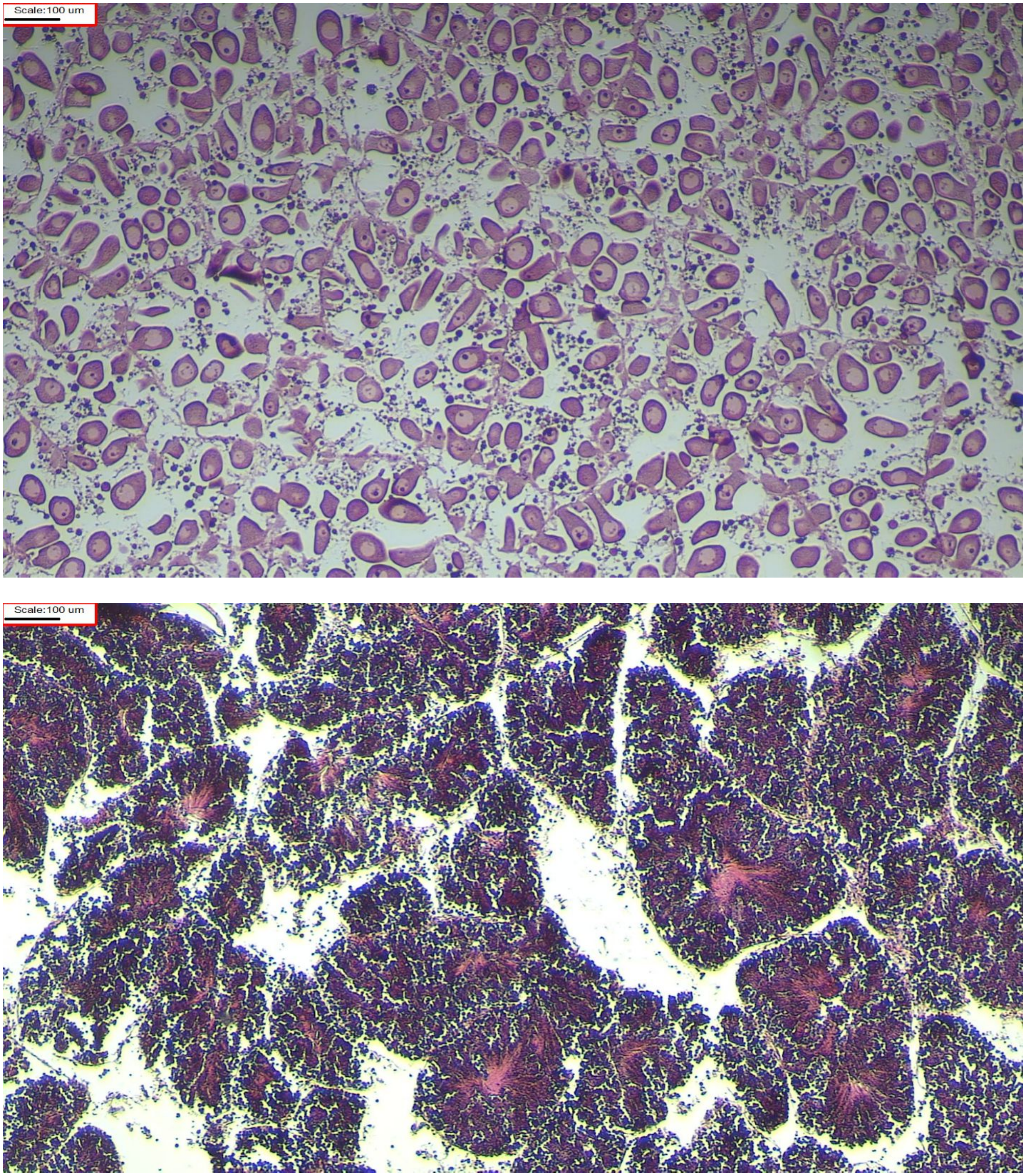
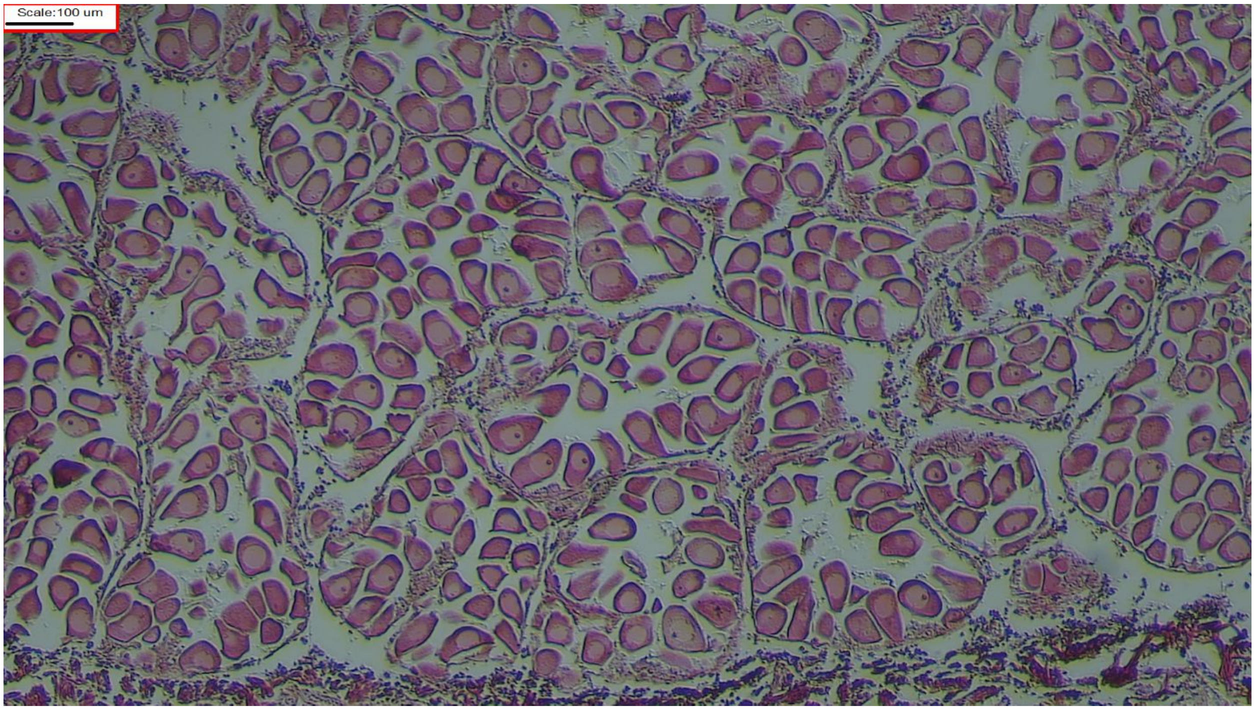


Figure 20.



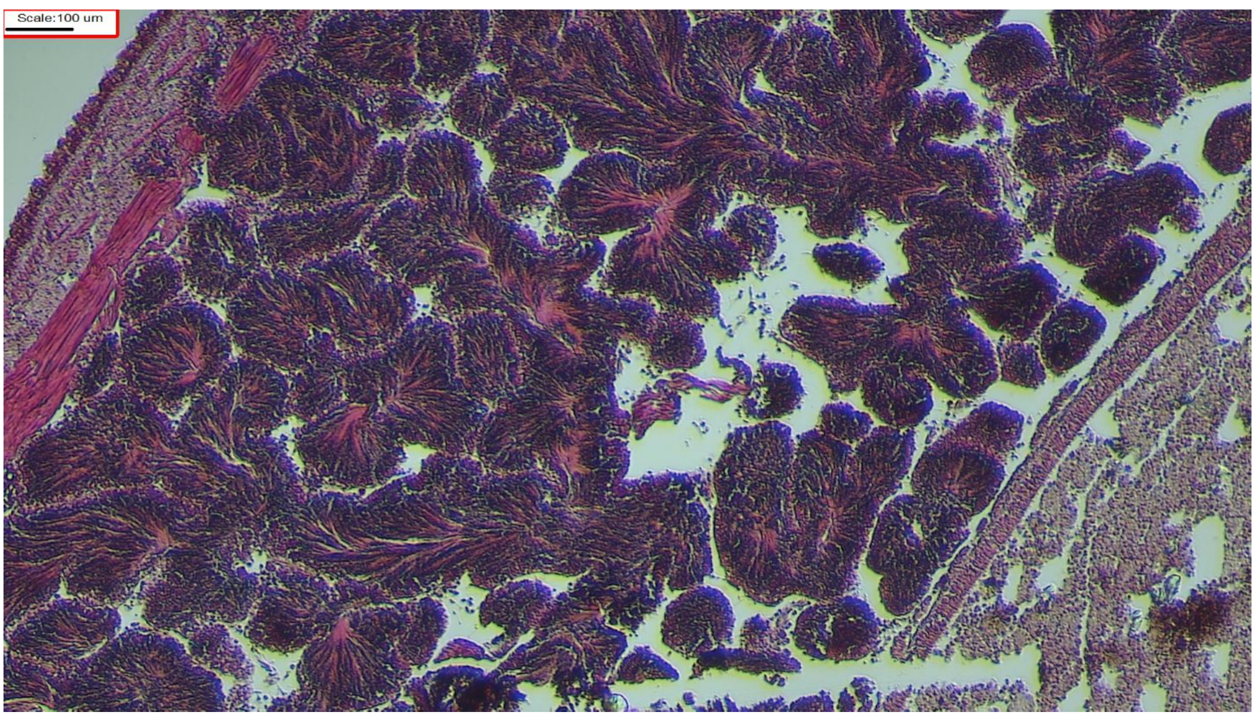
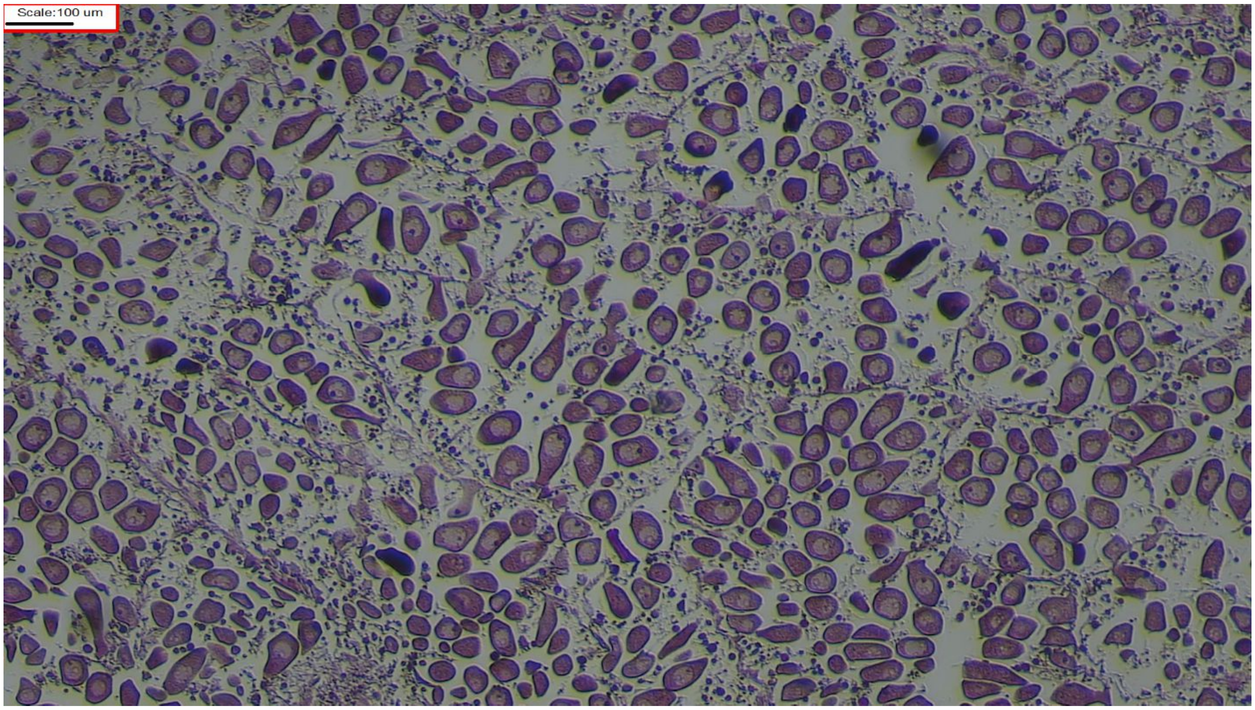


Figure 21.



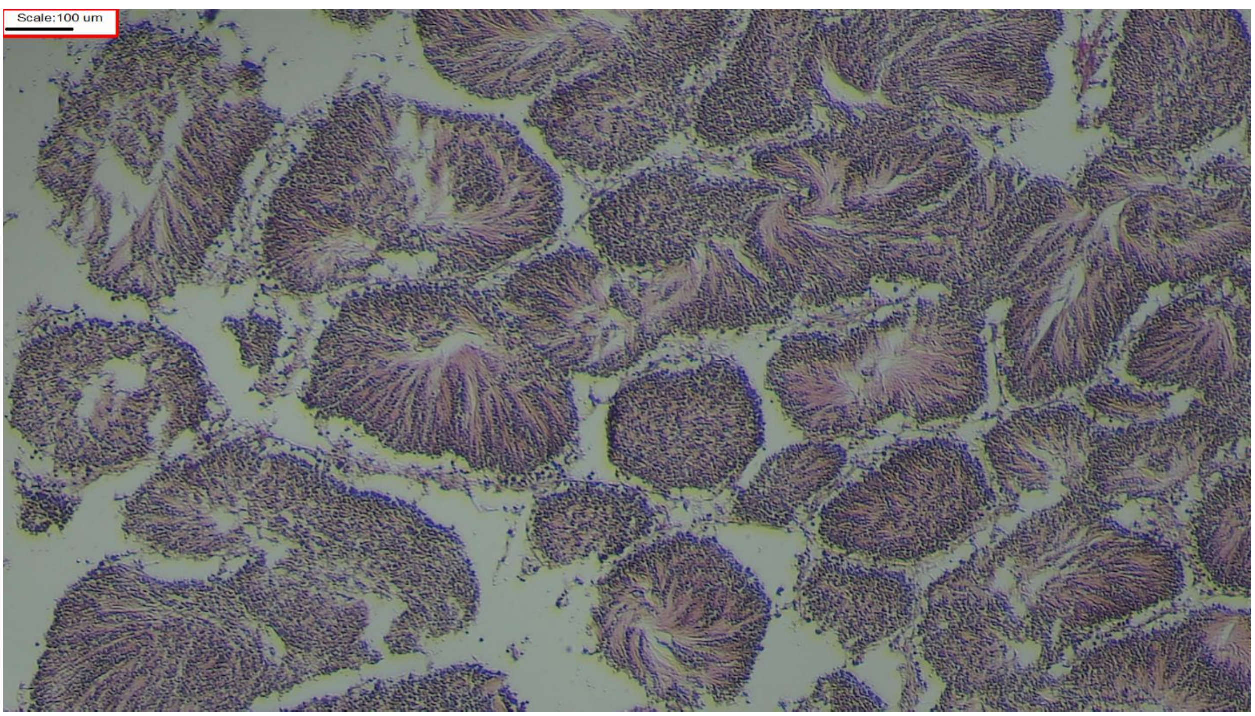


Figure 22.

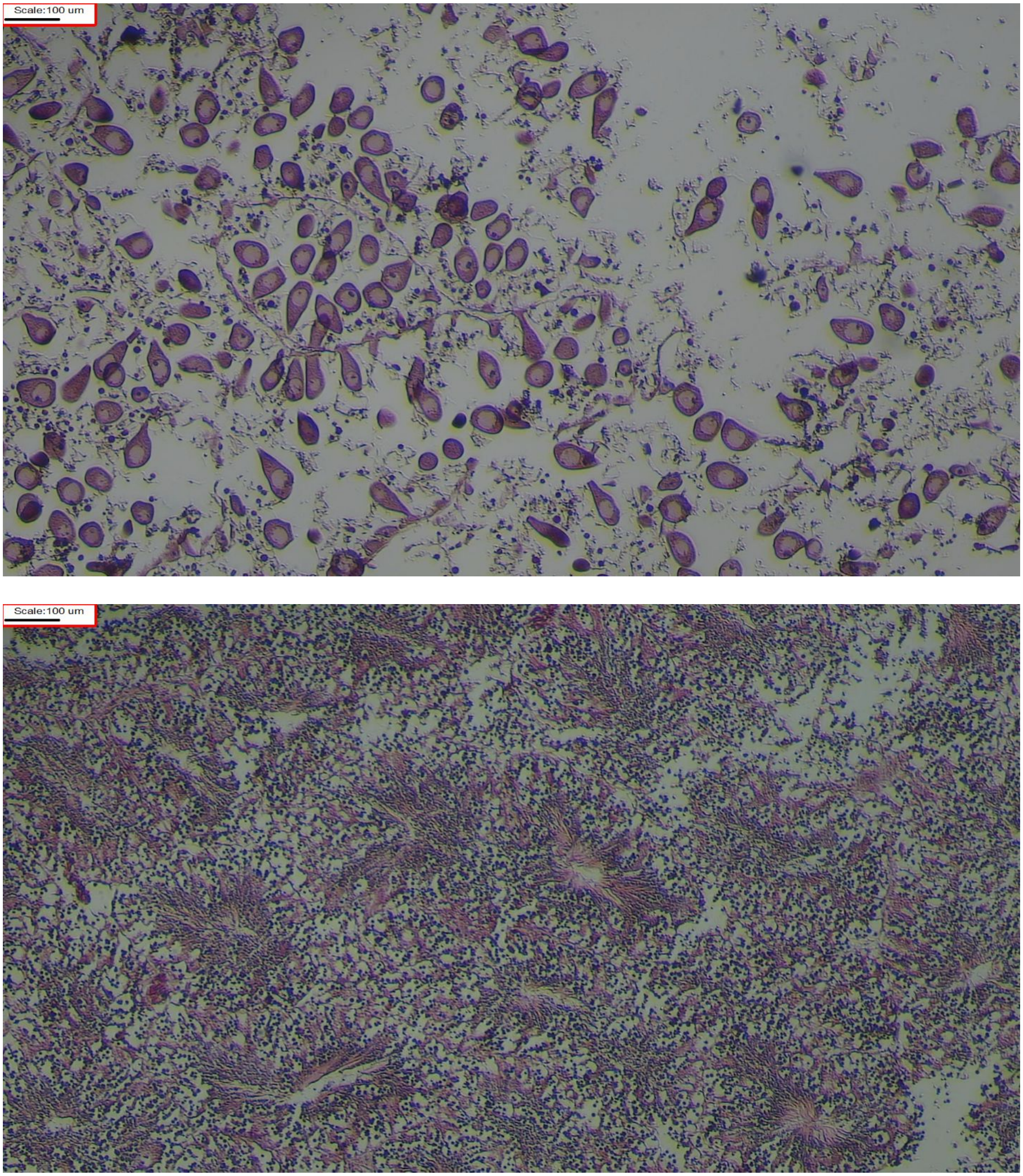
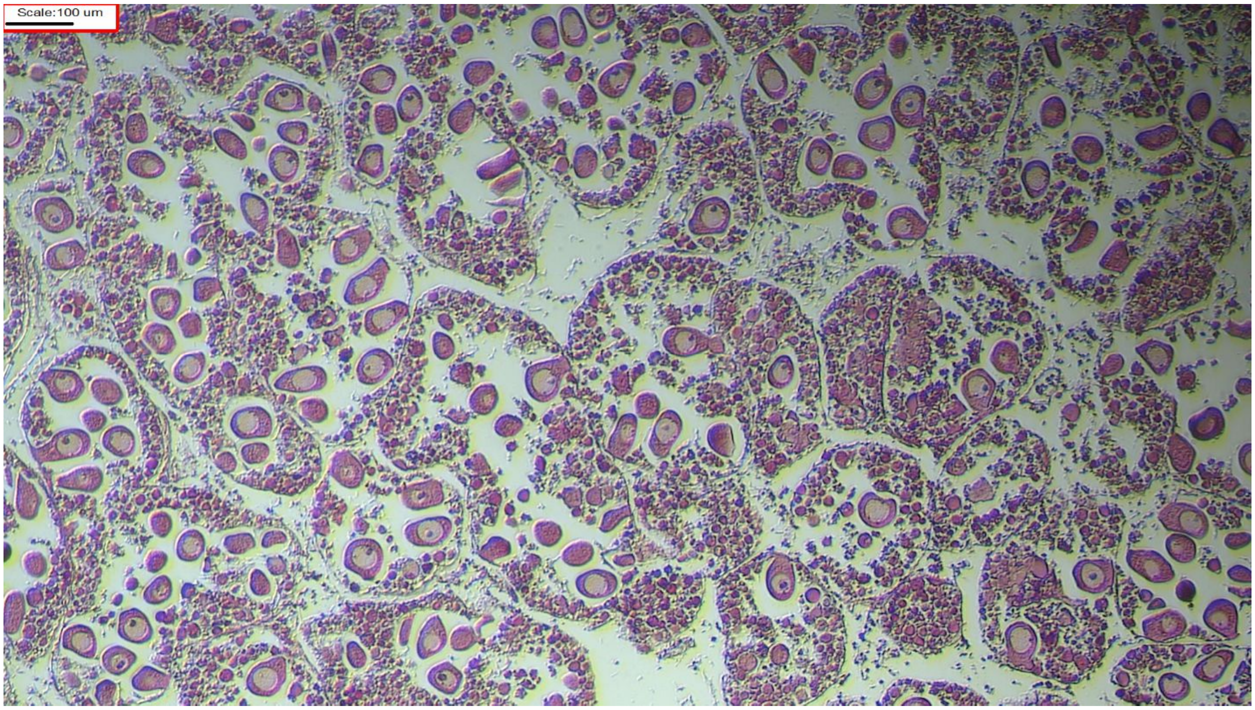


Figure 23.



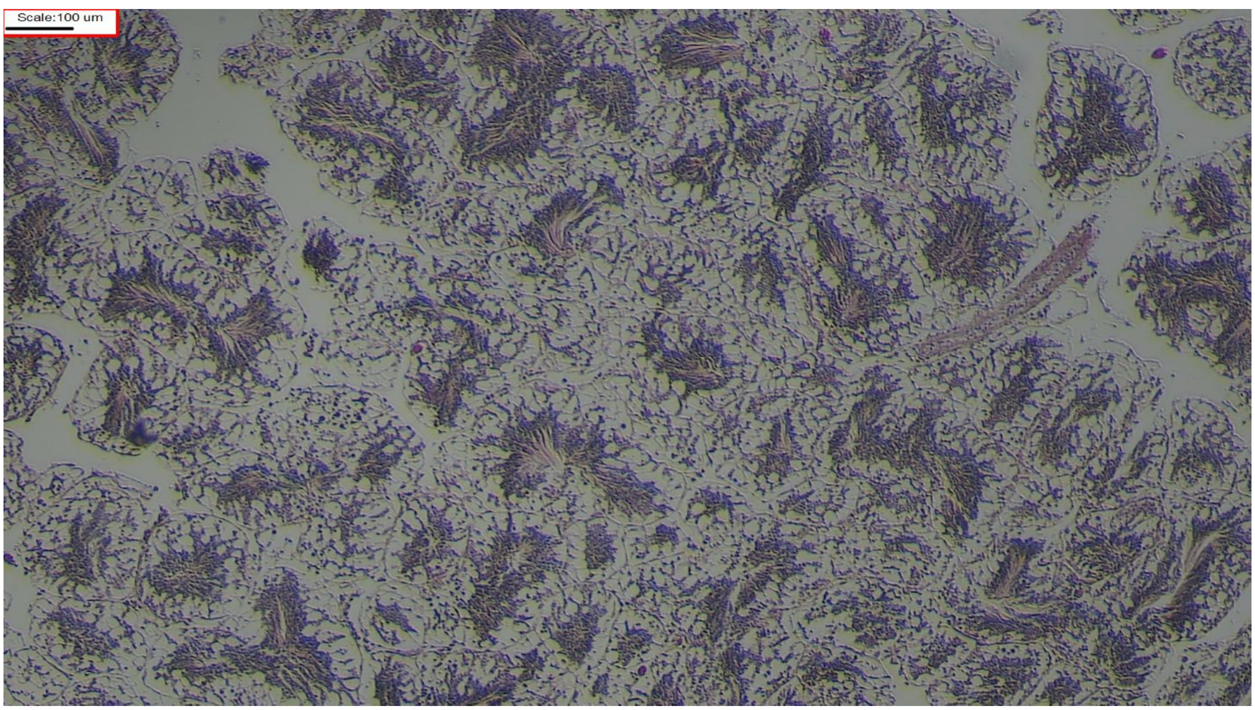
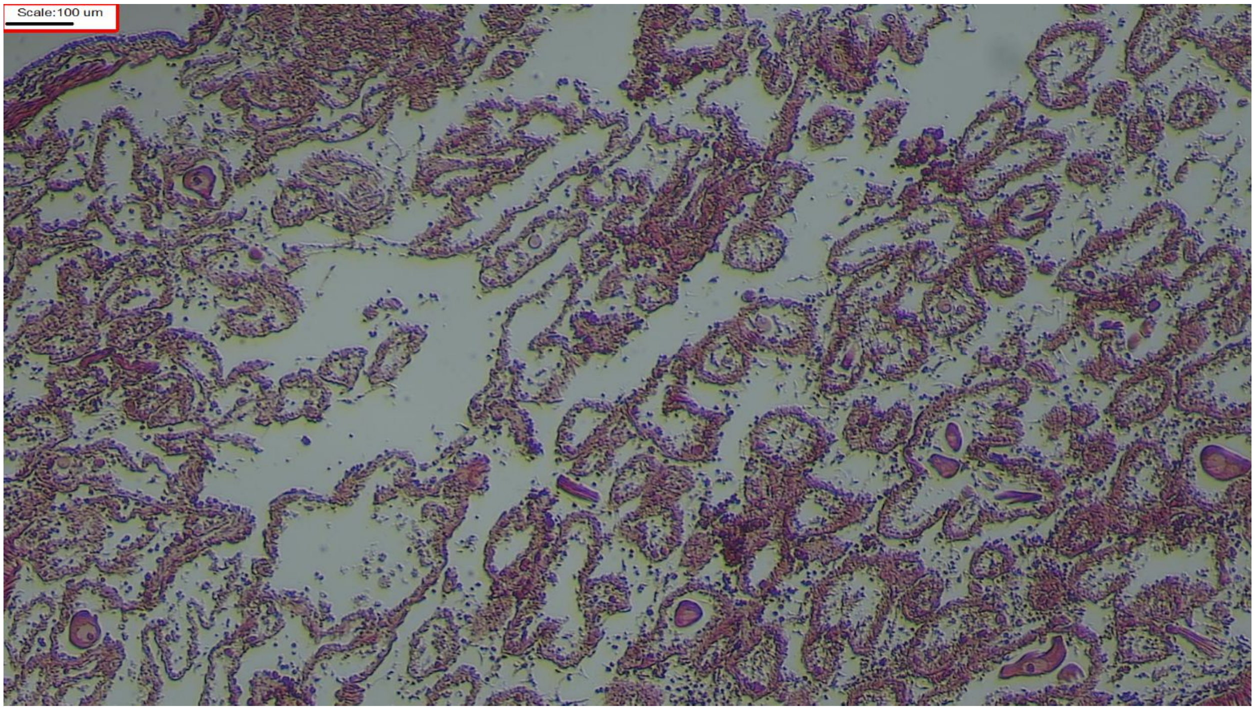


Figure 24.



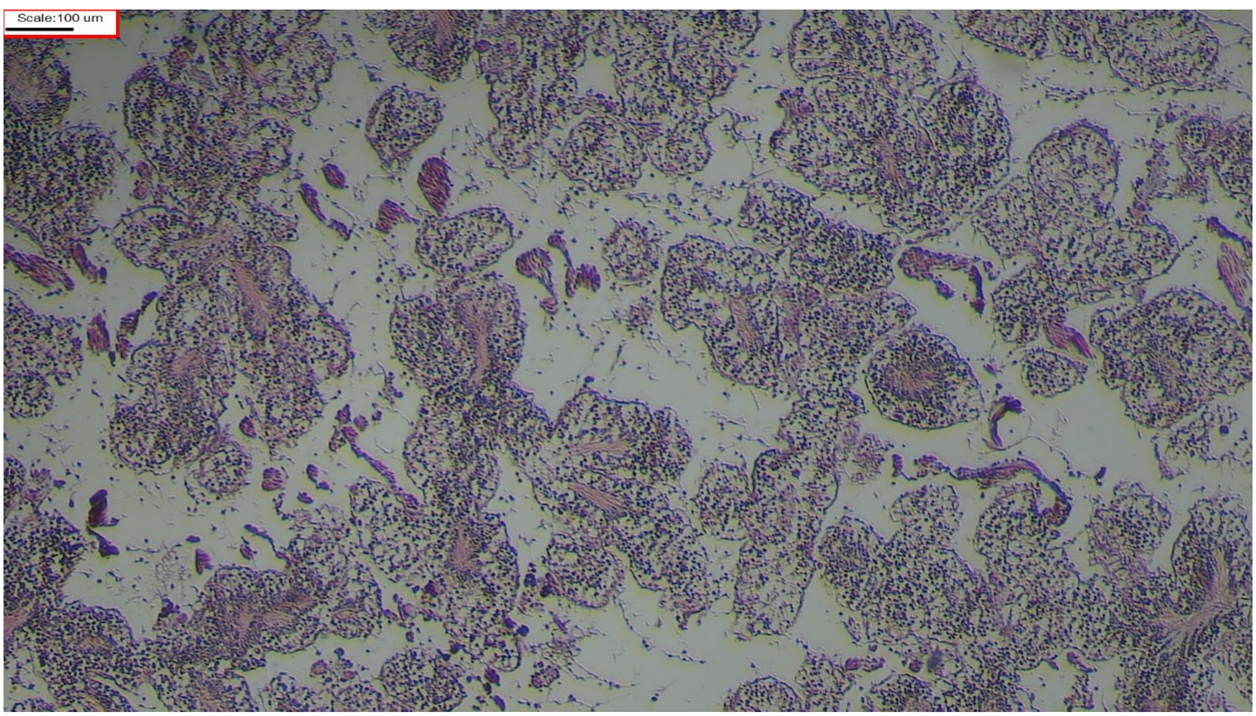


Figure 25.

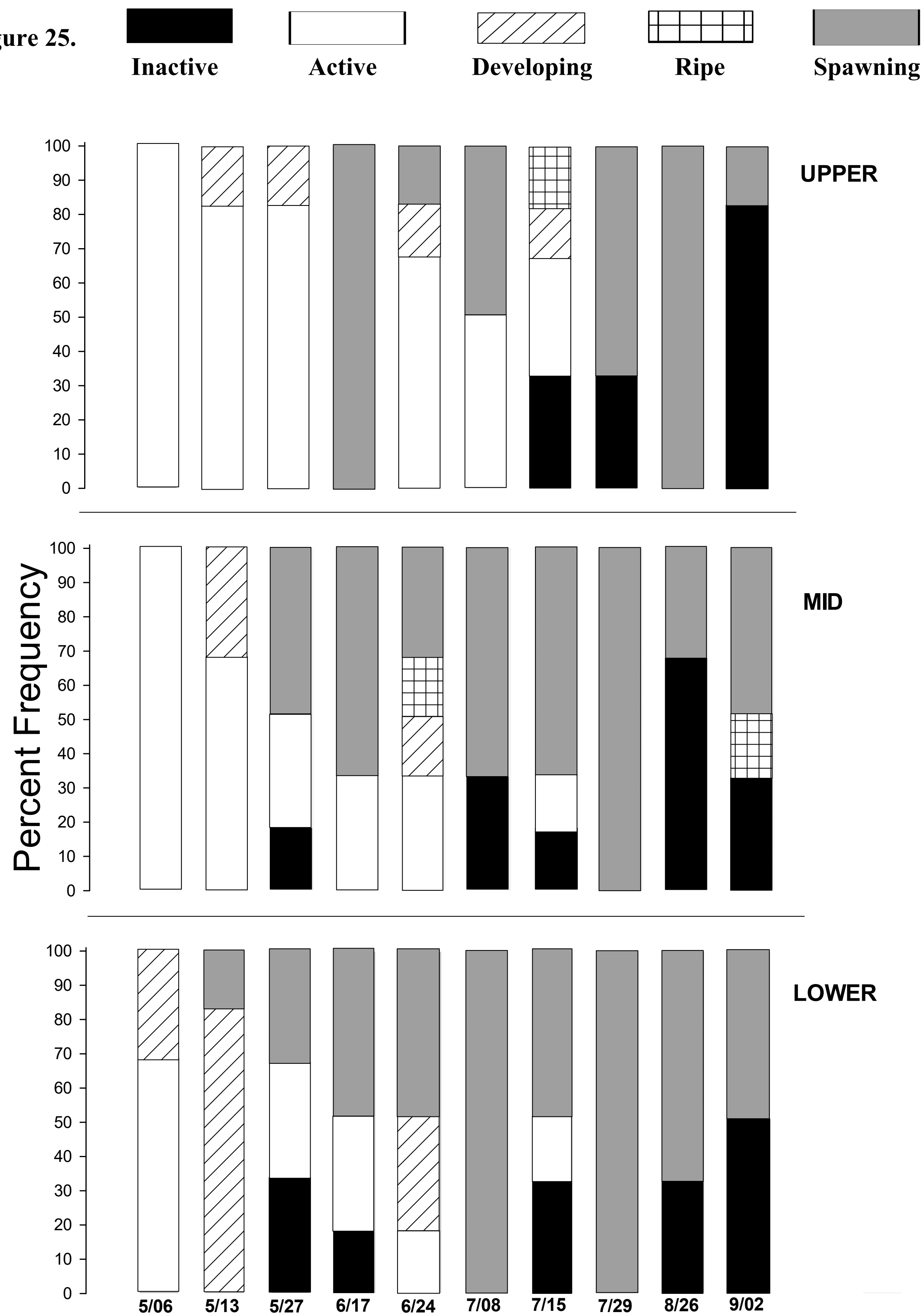

Inactive
Active
Developing
Ripe
Spawning
Percent Frequency
UPPER
MID
LOWER
0
10
20
30
40
50
60
70
80
90
100
5/06
5/13
5/27
6/17
6/24
7/08
7/15
7/29
8/26
9/02

Figure 26.

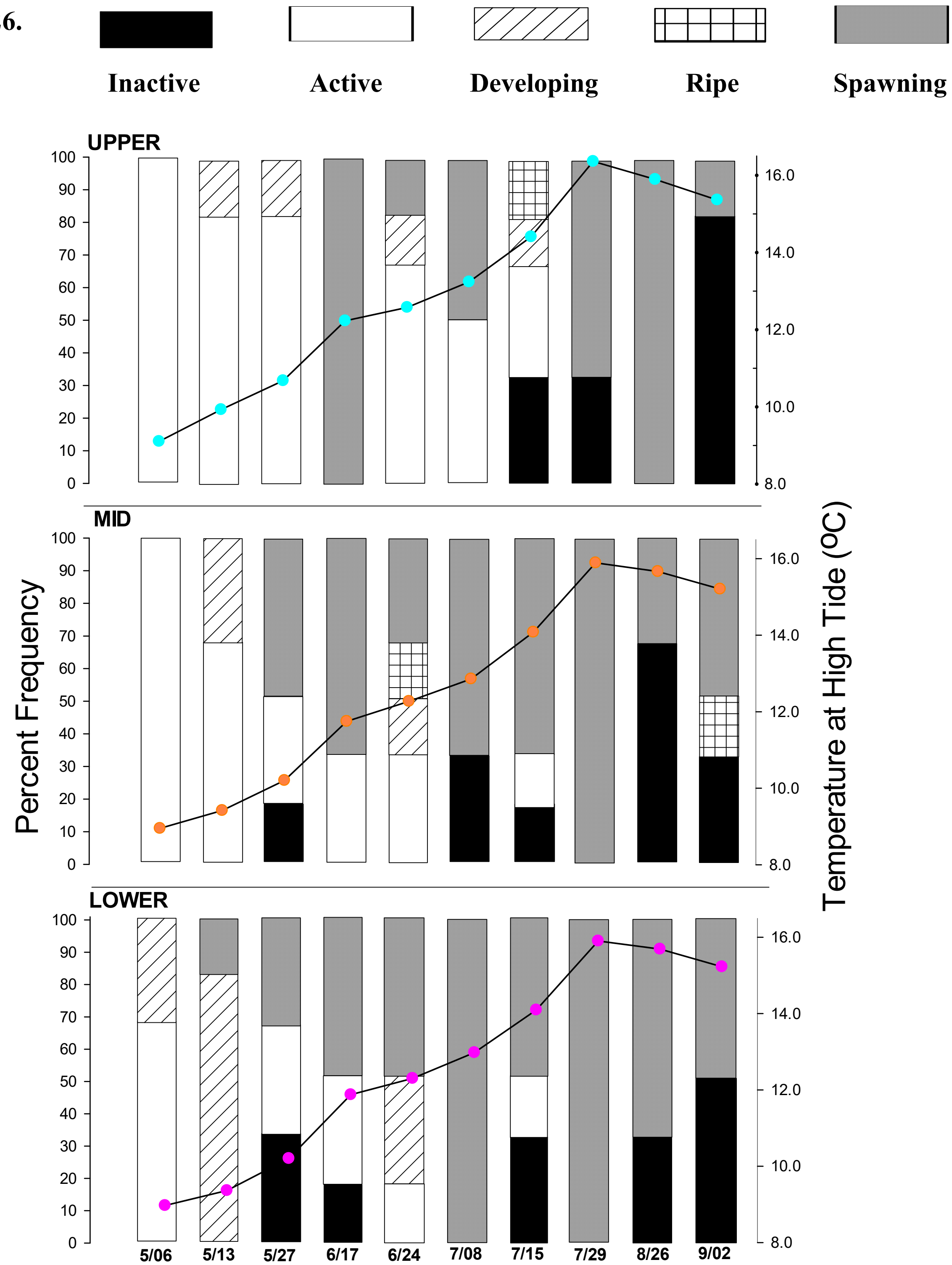

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
Temperature at High Tide (oC)
100
90
80
70
60
50
40
30
20
10
0
16.0
14.0
12.0
10.0
8.0
5/06
5/13
5/27
6/17
6/24
7/08
7/15
7/29
8/26
9/02

Figure 27.

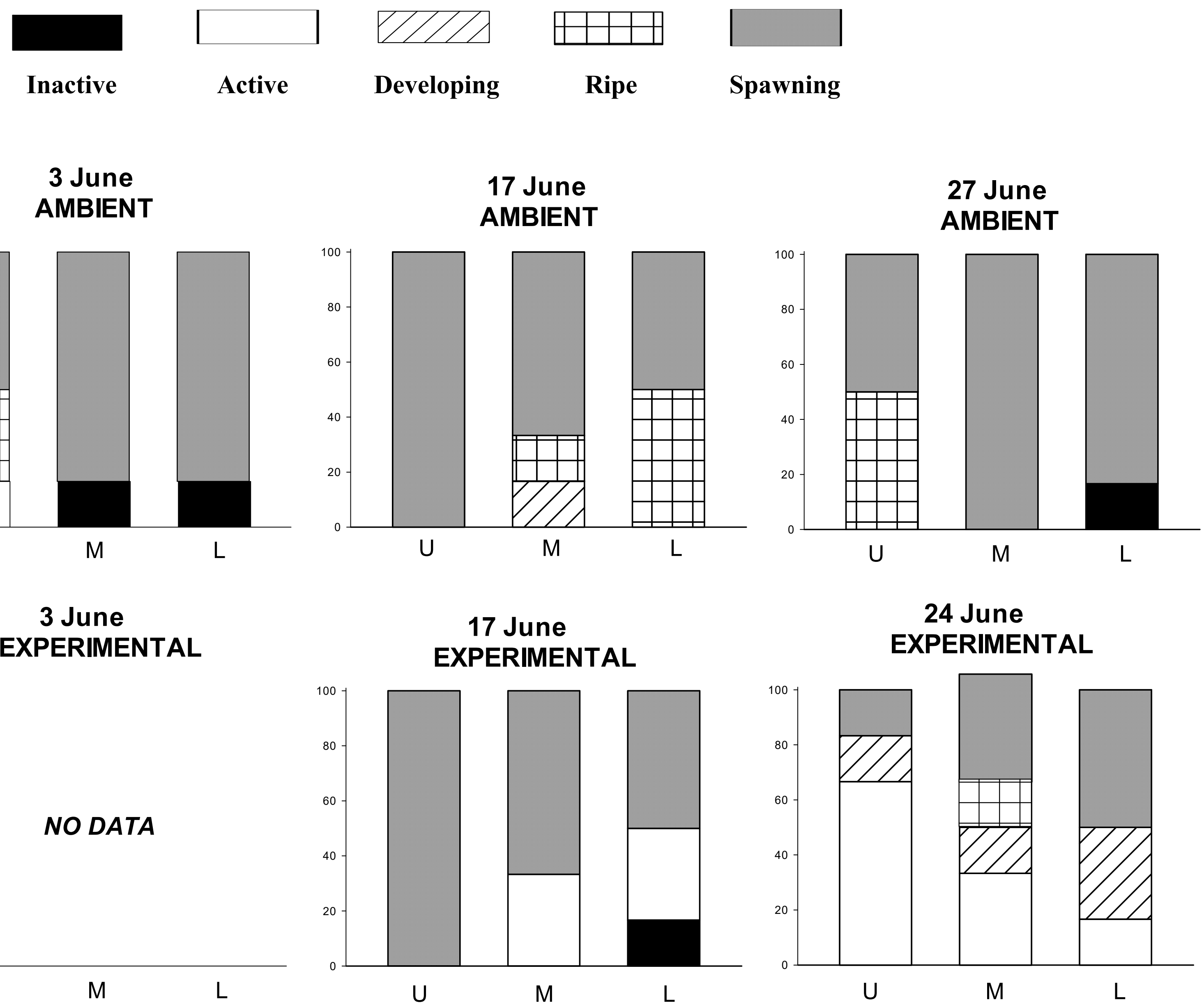

Inactive
Active
Developing
Ripe
Spawning
3 June
AMBIENT
17 June
AMBIENT
27 June
AMBIENT
Percent Frequency
0
20
40
60
80
100
U
M
L
3 June
EXPERIMENTAL
NO DATA
17 June
EXPERIMENTAL
24 June
EXPERIMENTAL

Figure 28.

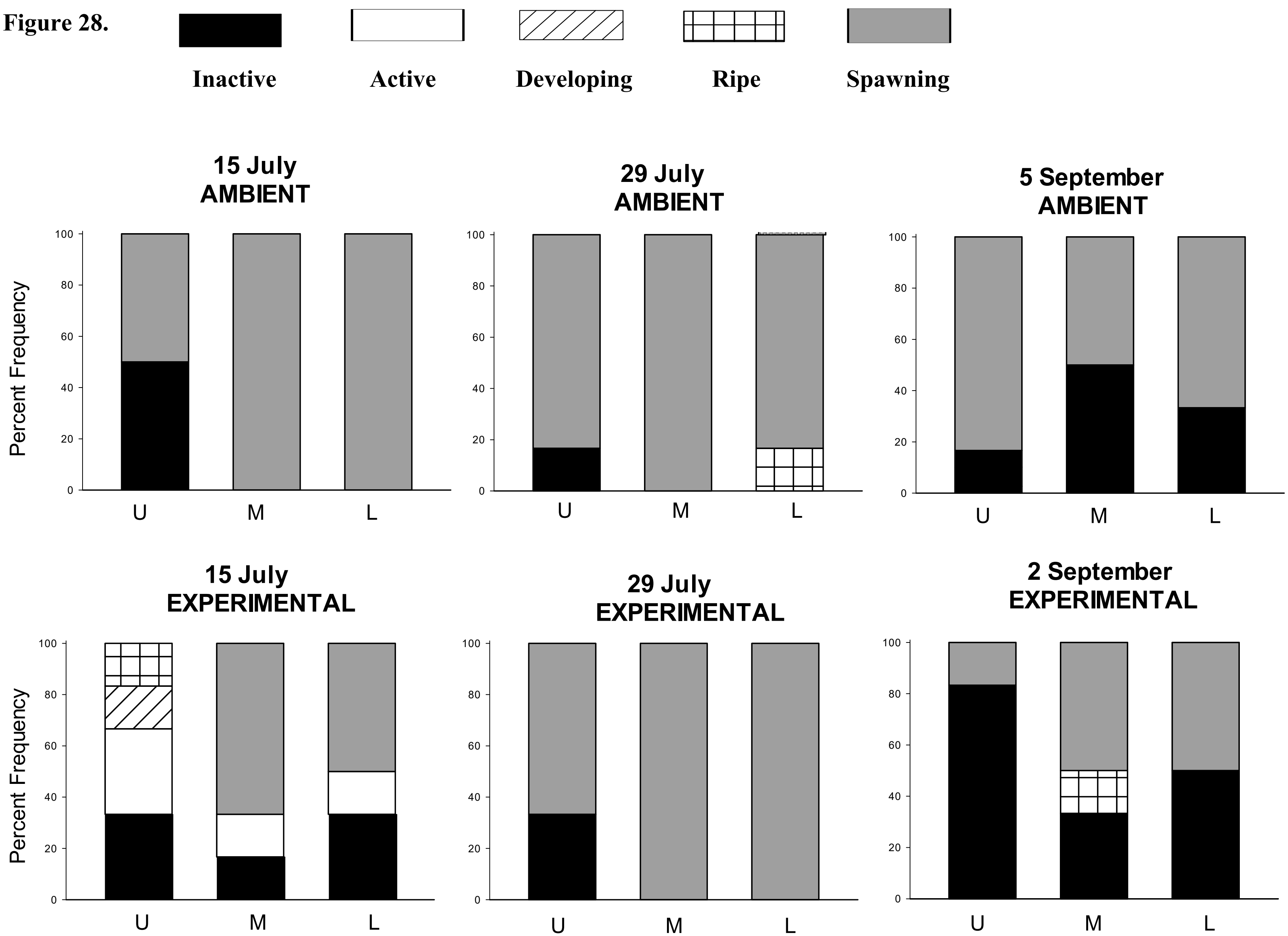

Inactive
Active
Developing
Ripe
Spawning
15 July
AMBIENT
29 July
AMBIENT
5 September
AMBIENT
15 July
EXPERIMENTAL
29 July
EXPERIMENTAL
2 September
EXPERIMENTAL
Percent Frequency
0
20
40
60
80
100
U
M
L

Figure 29.

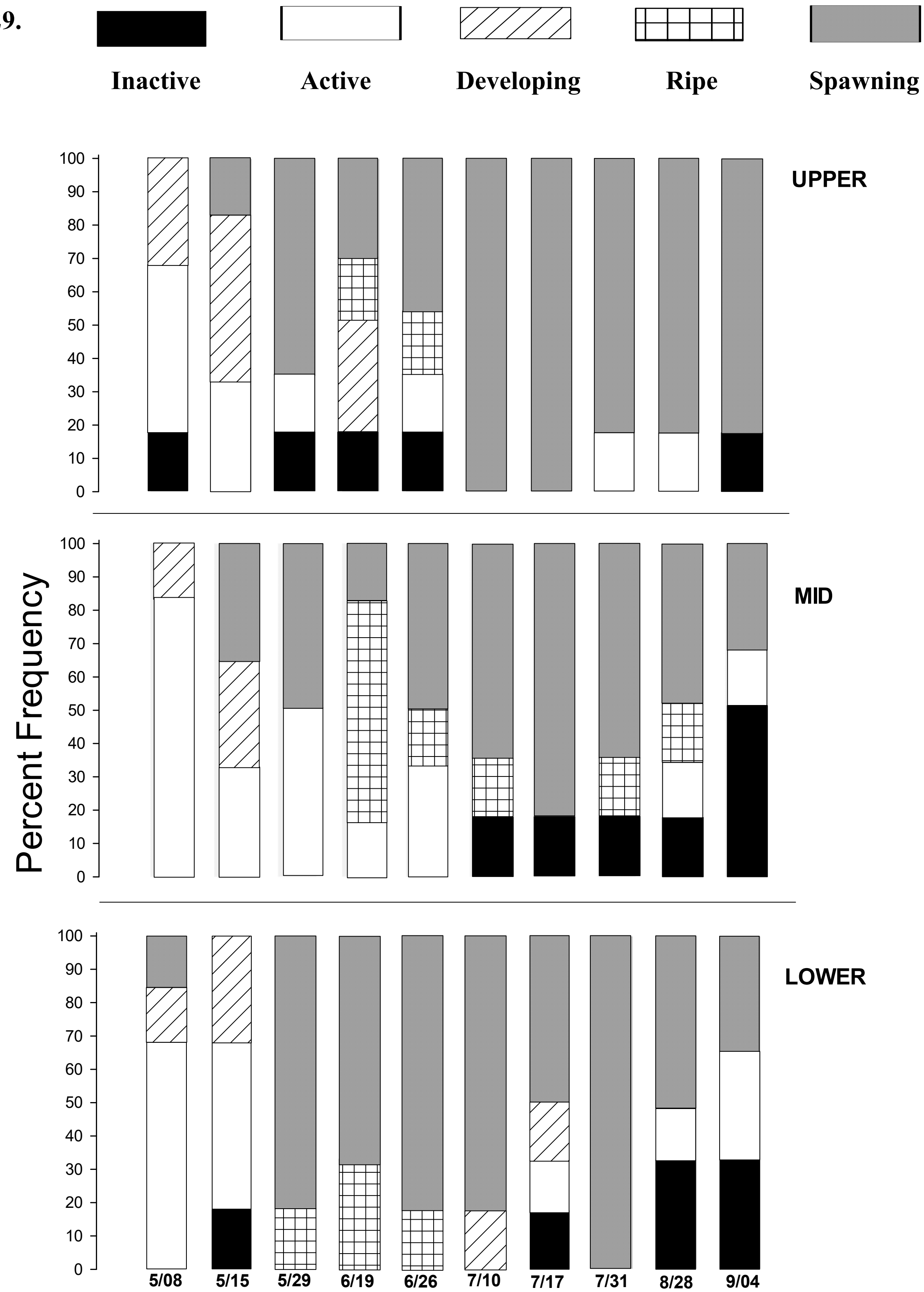

Inactive
Active
Developing
Ripe
Spawning
Percent Frequency
UPPER
MID
LOWER
0
10
20
30
40
50
60
70
80
90
100
5/08
5/15
5/29
6/19
6/26
7/10
7/17
7/31
8/28
9/04

Figure 30.

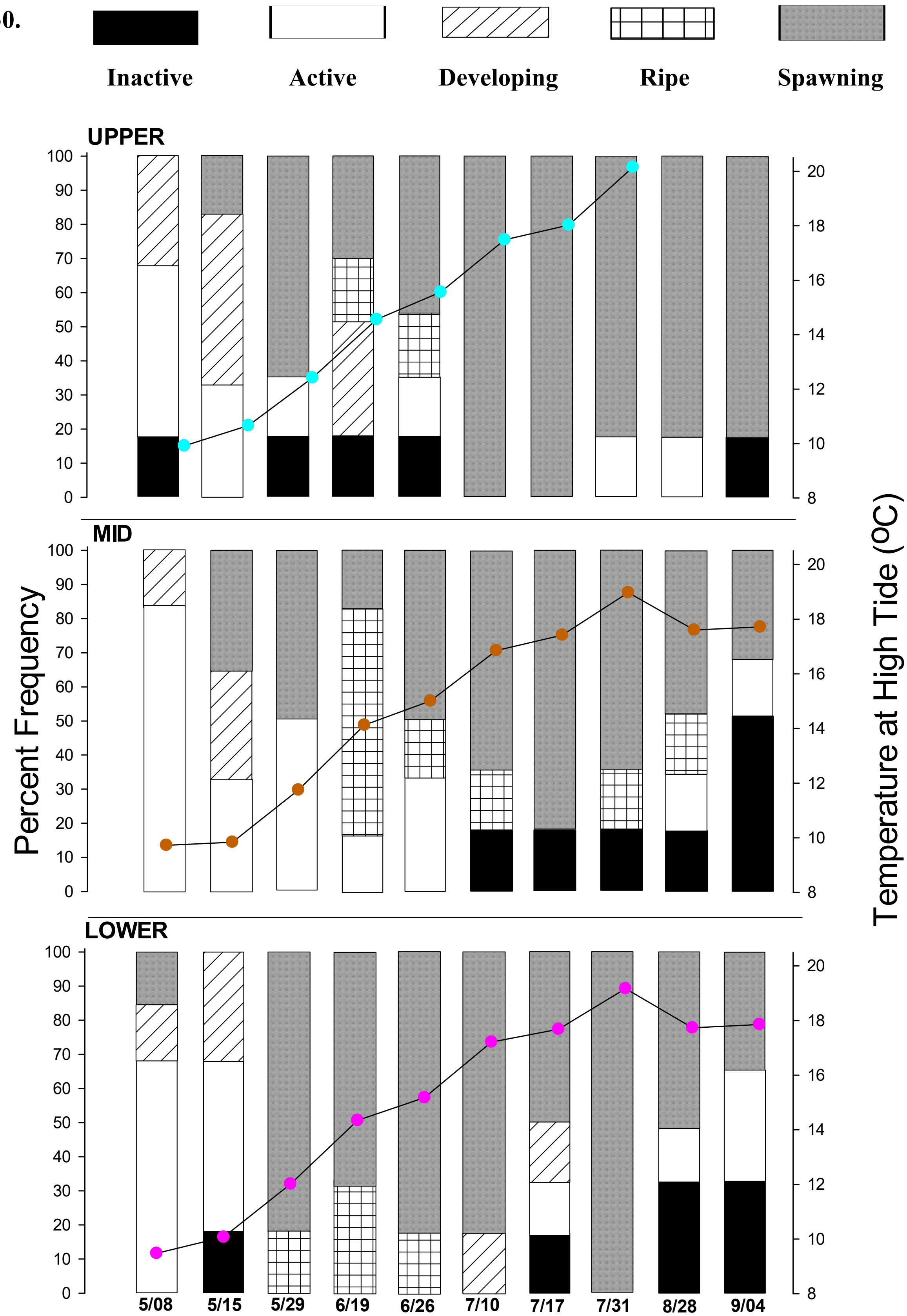

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
Temperature at High Tide (oC)
0
10
20
30
40
50
60
70
80
90
100
8
10
12
14
16
18
20
5/08
5/15
5/29
6/19
6/26
7/10
7/17
7/31
8/28
9/04

Figure 31.

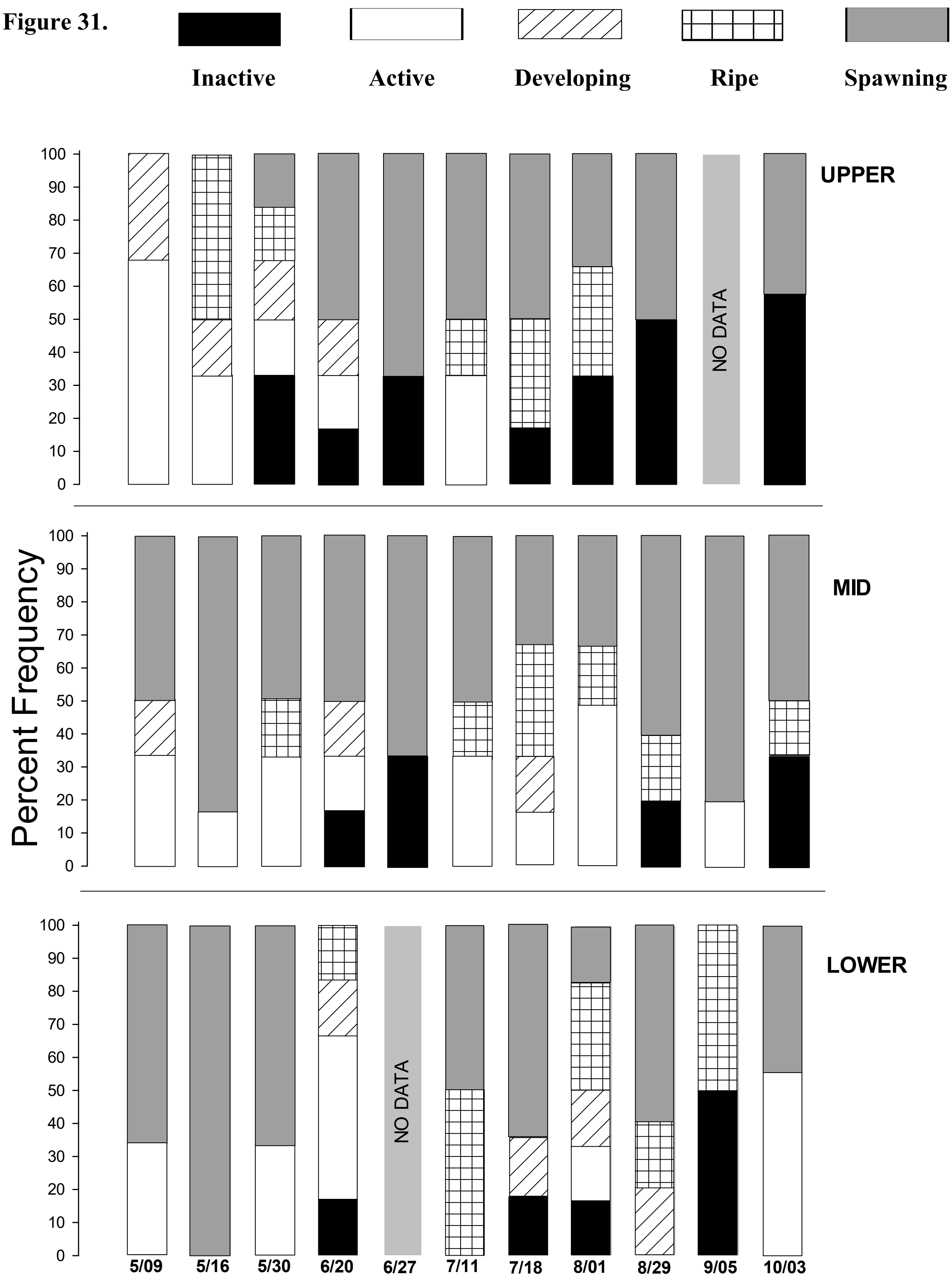

Inactive
Active
Developing
Ripe
Spawning
Percent Frequency
UPPER
MID
LOWER
NO DATA
NO DATA
5/09
5/16
5/30
6/20
6/27
7/11
7/18
8/01
8/29
9/05
10/03

Figure 32.

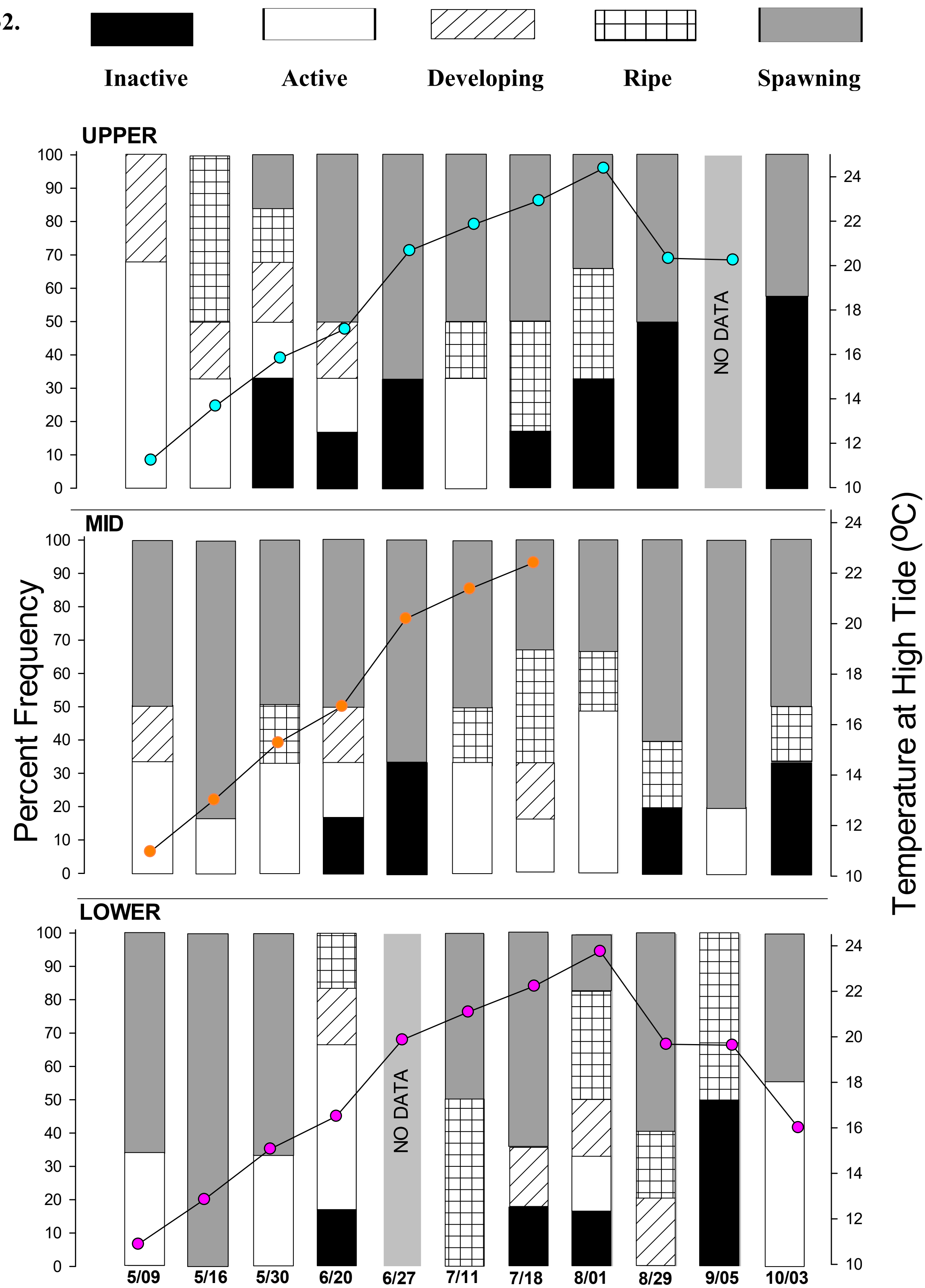

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
Temperature at High Tide (ºC)
NO DATA
NO DATA
0
10
20
30
40
50
60
70
80
90
100
10
12
14
16
18
20
22
24
5/09
5/16
5/30
6/20
6/27
7/11
7/18
8/01
8/29
9/05
10/03

Figure 33.

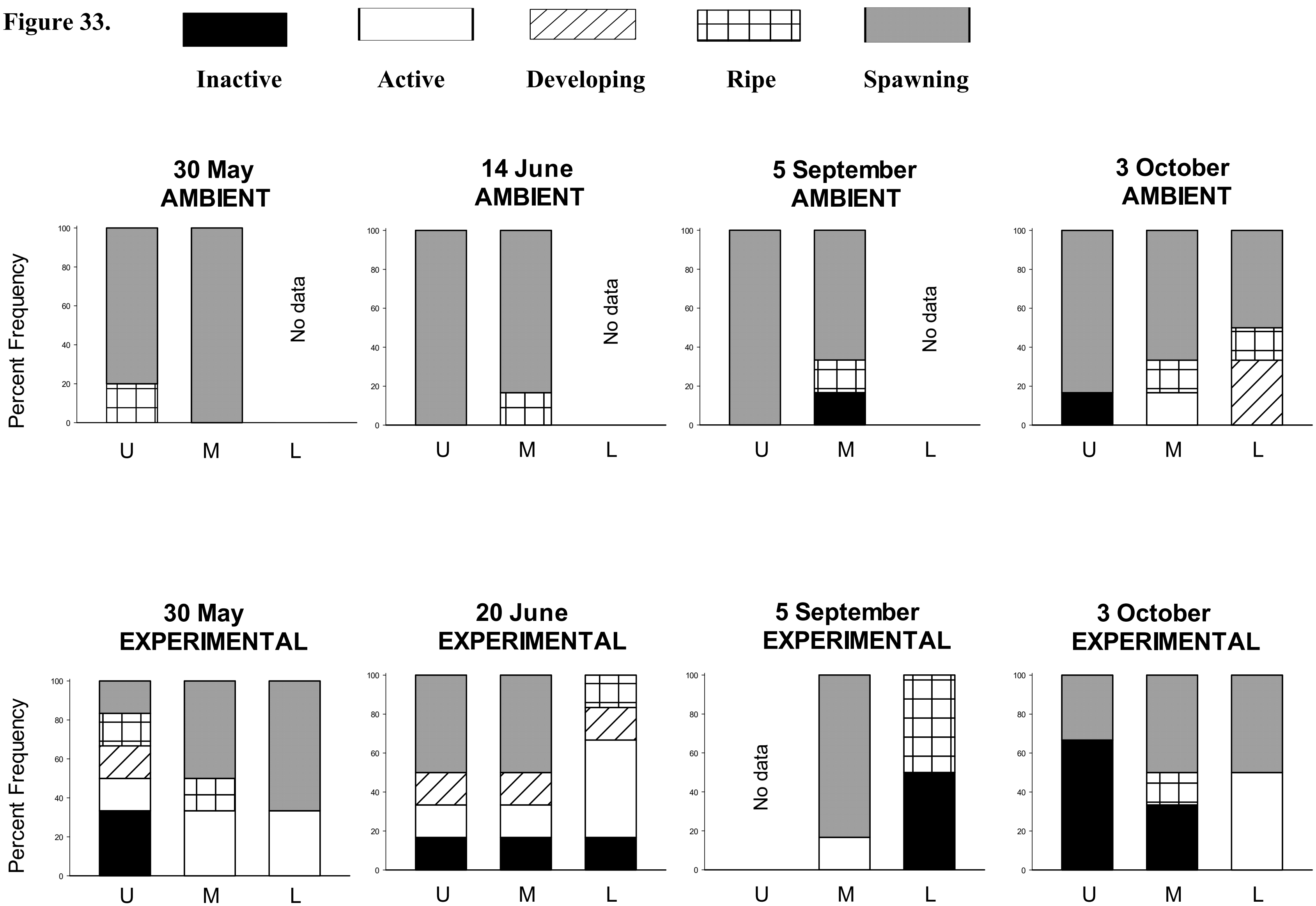

Inactive
Active
Developing
Ripe
Spawning
30 May AMBIENT
14 June AMBIENT
5 September AMBIENT
3 October AMBIENT
30 May EXPERIMENTAL
20 June EXPERIMENTAL
5 September EXPERIMENTAL
3 October EXPERIMENTAL
Percent Frequency
No data
U
M
L
0
20
40
60
80
100

Figure 34.

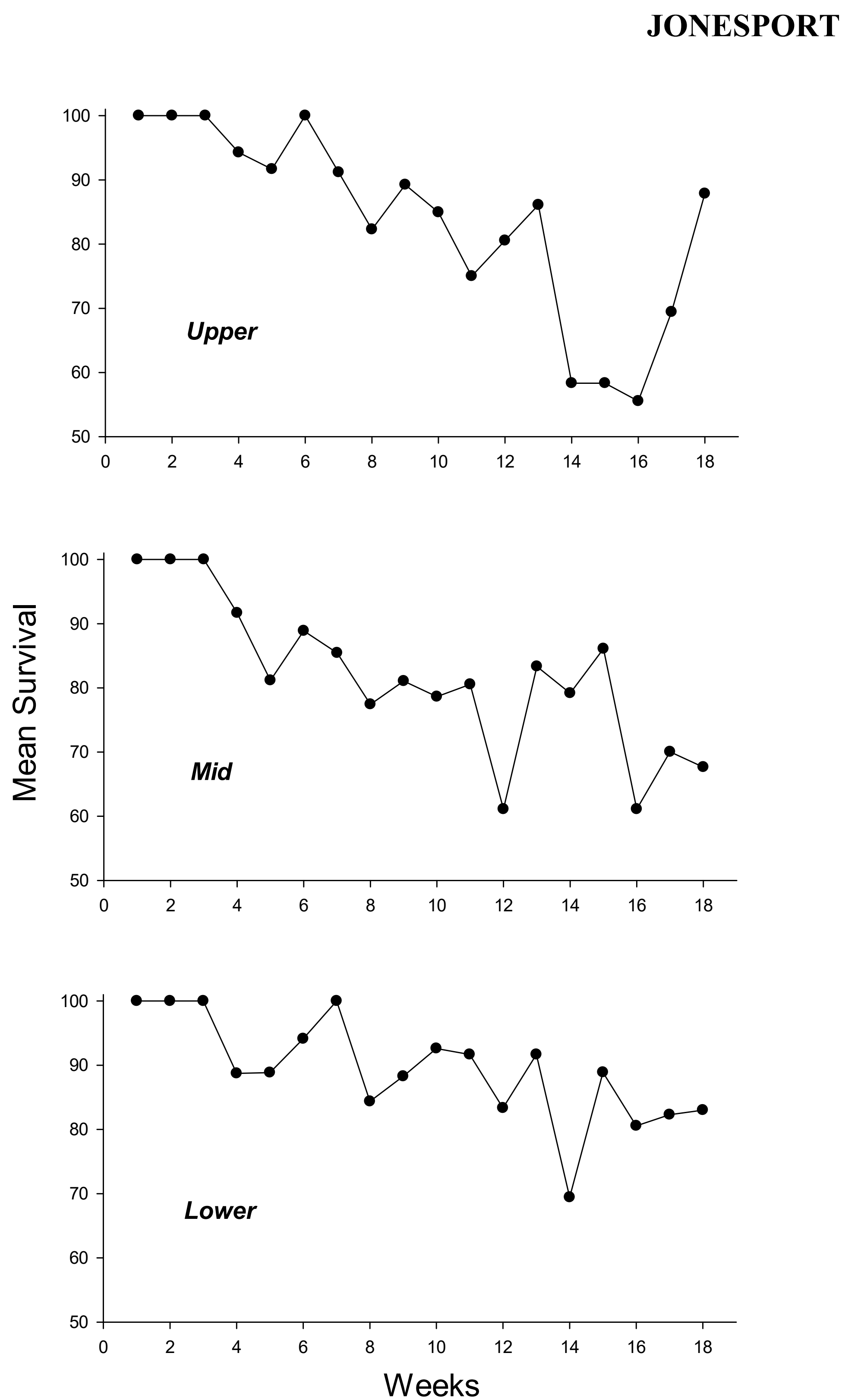

JONESPORT
Upper
Mid
Lower
Mean Survival
Weeks
100
90
80
70
60
50
0
2
4
6
8
10
12
14
16
18

Figure 35.

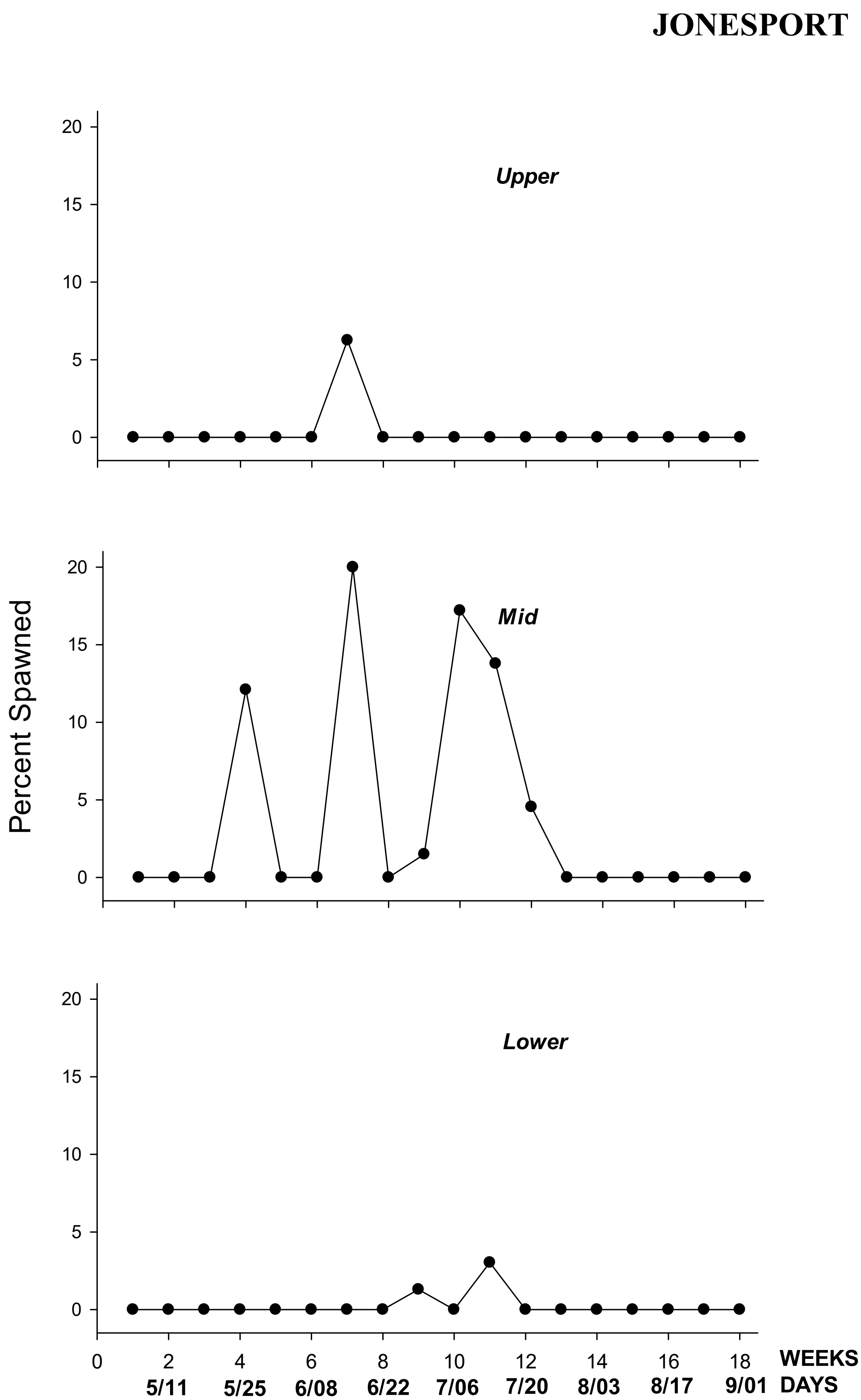

JONESPORT
Upper
Mid
Lower
Percent Spawned
20
15
10
5
0
0
2
4
6
8
10
12
14
16
18
WEEKS
5/11
5/25
6/08
6/22
7/06
7/20
8/03
8/17
9/01
DAYS

Figure 36.

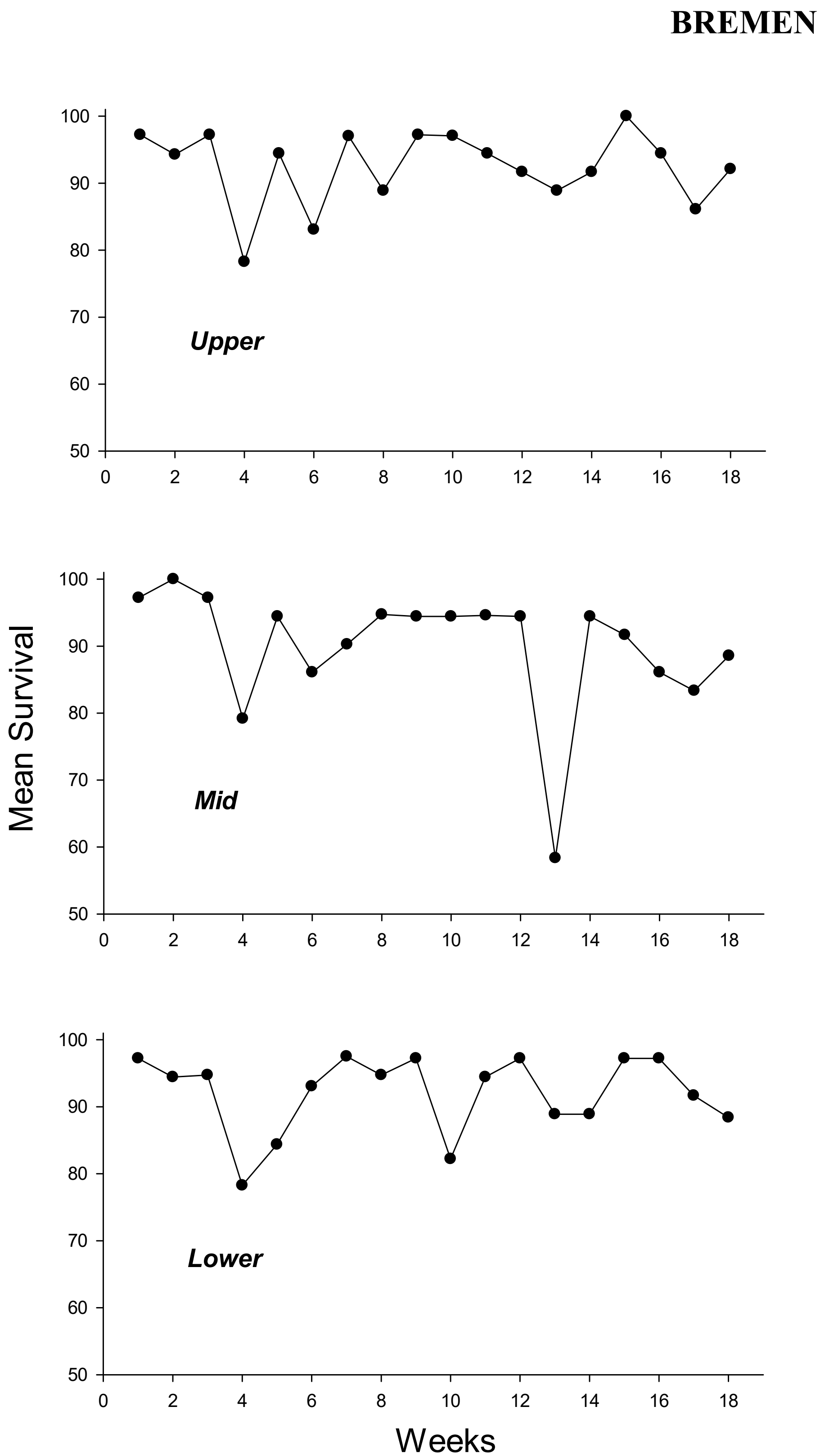

BREMEN
Upper
Mid
Lower
Mean Survival
Weeks
100
90
80
70
60
50
0
2
4
6
8
10
12
14
16
18

Figure 37.

BREMEN

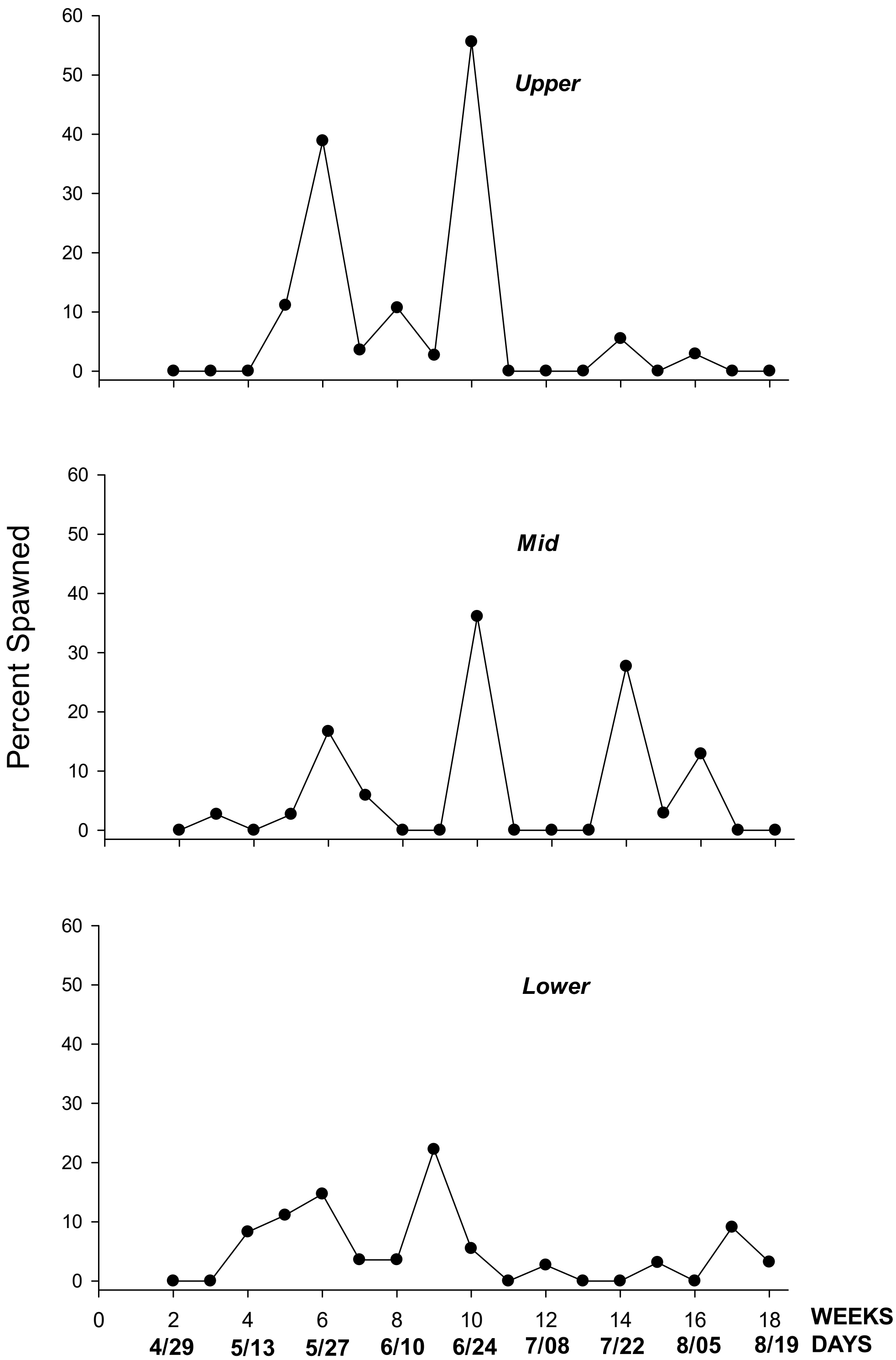


Figure 38.

BRUNSWICK

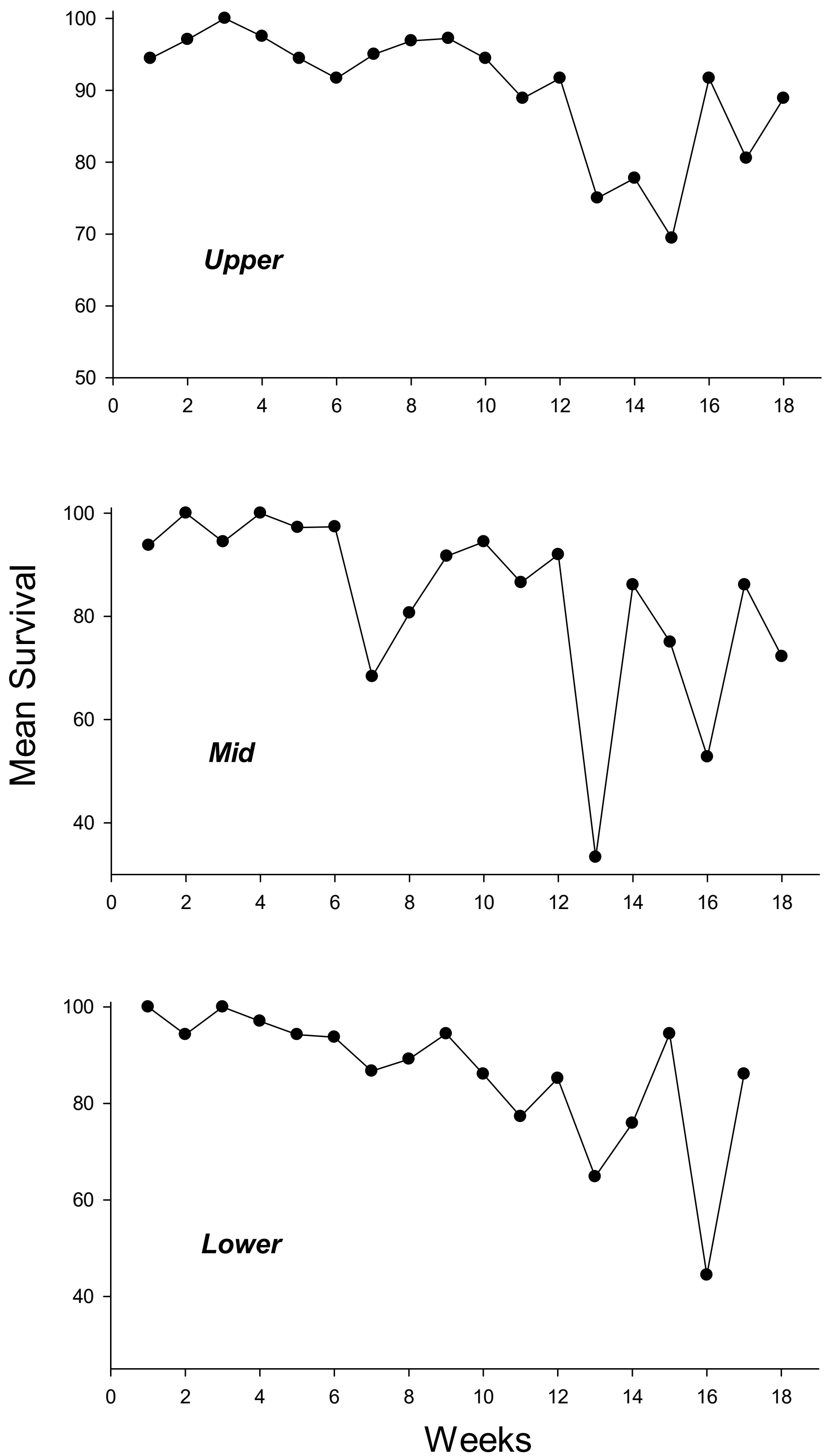


Figure 39.

BRUNSWICK

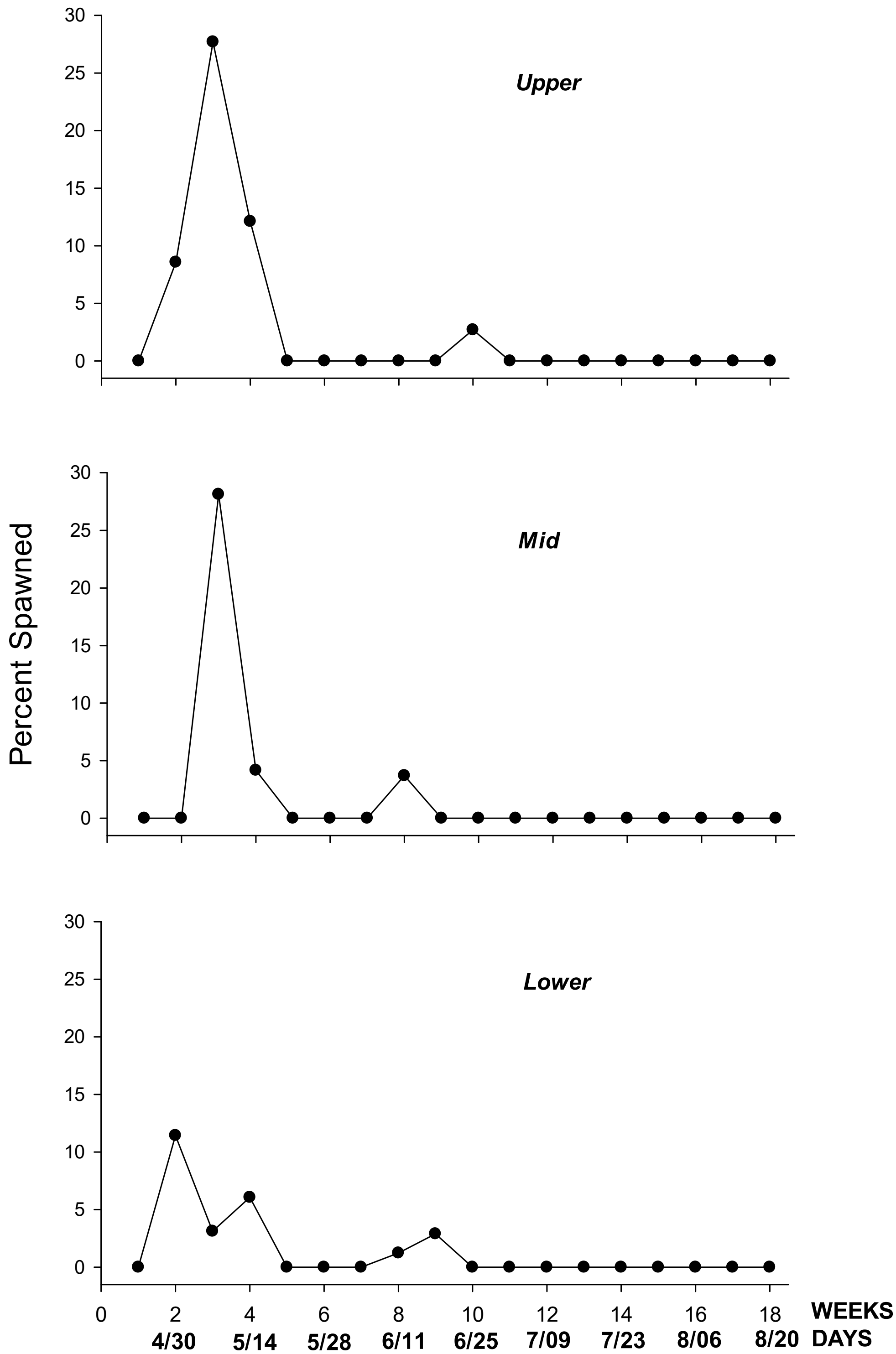


Figure 40.

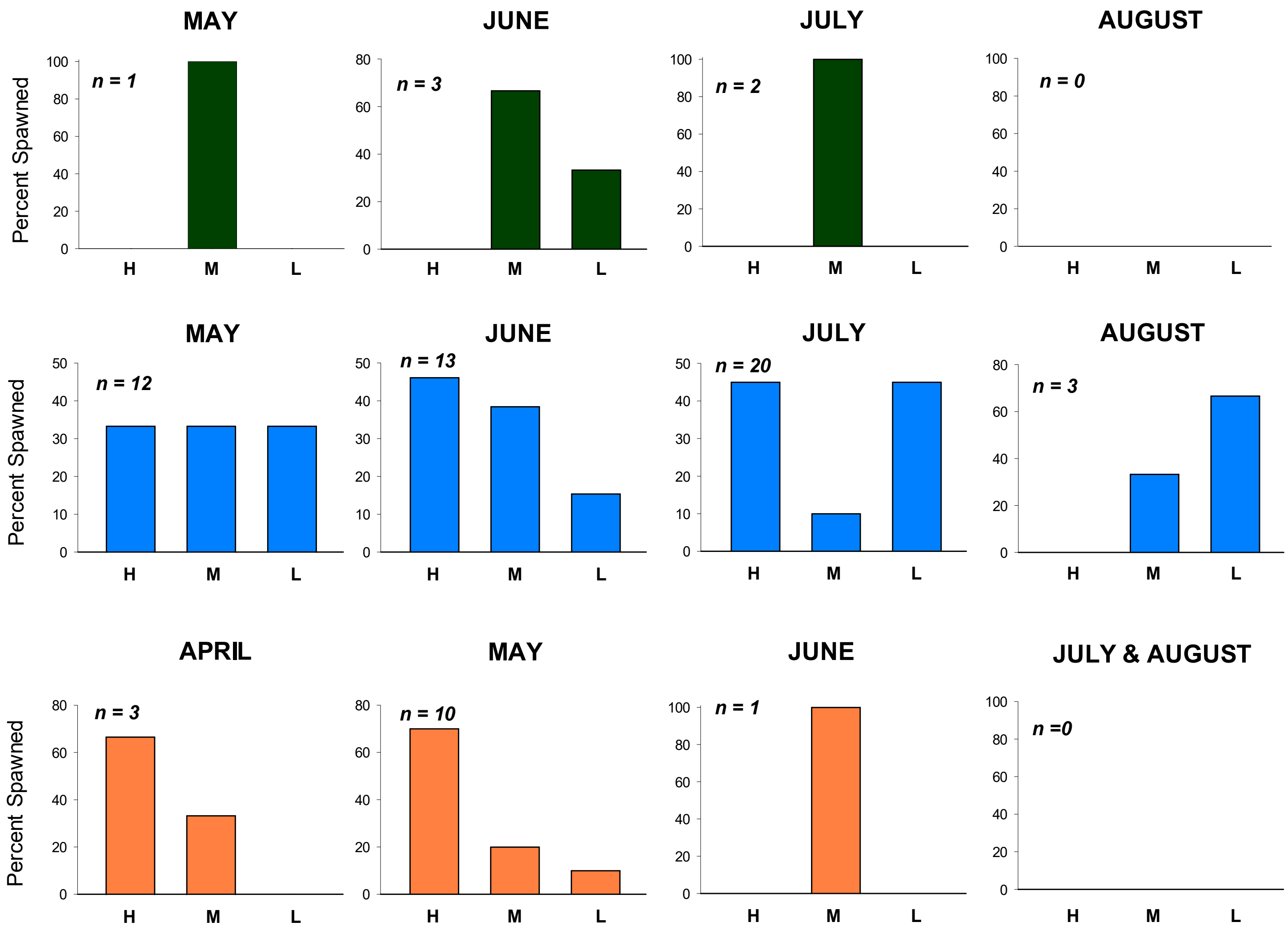

MAY
JUNE
JULY
AUGUST
Percent Spawned
n = 1
n = 3
n = 2
n = 0
MAY
JUNE
JULY
AUGUST
Percent Spawned
n = 12
n = 13
n = 20
n = 3
APRIL
MAY
JUNE
JULY & AUGUST
Percent Spawned
n = 3
n = 10
n = 1
n =0
H
M
L

Figure 41.

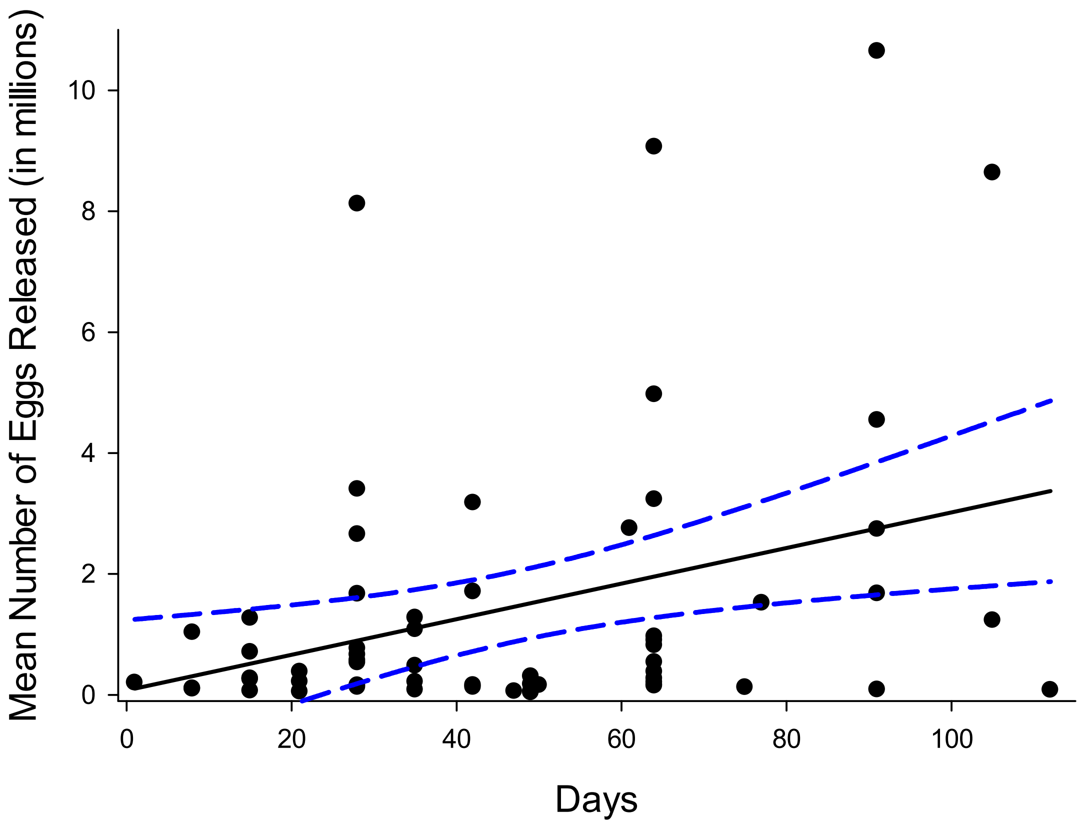

Mean Number of Eggs Released (in millions)
0
2
4
6
8
10
0
20
40
60
80
100
Days

Figure 42.

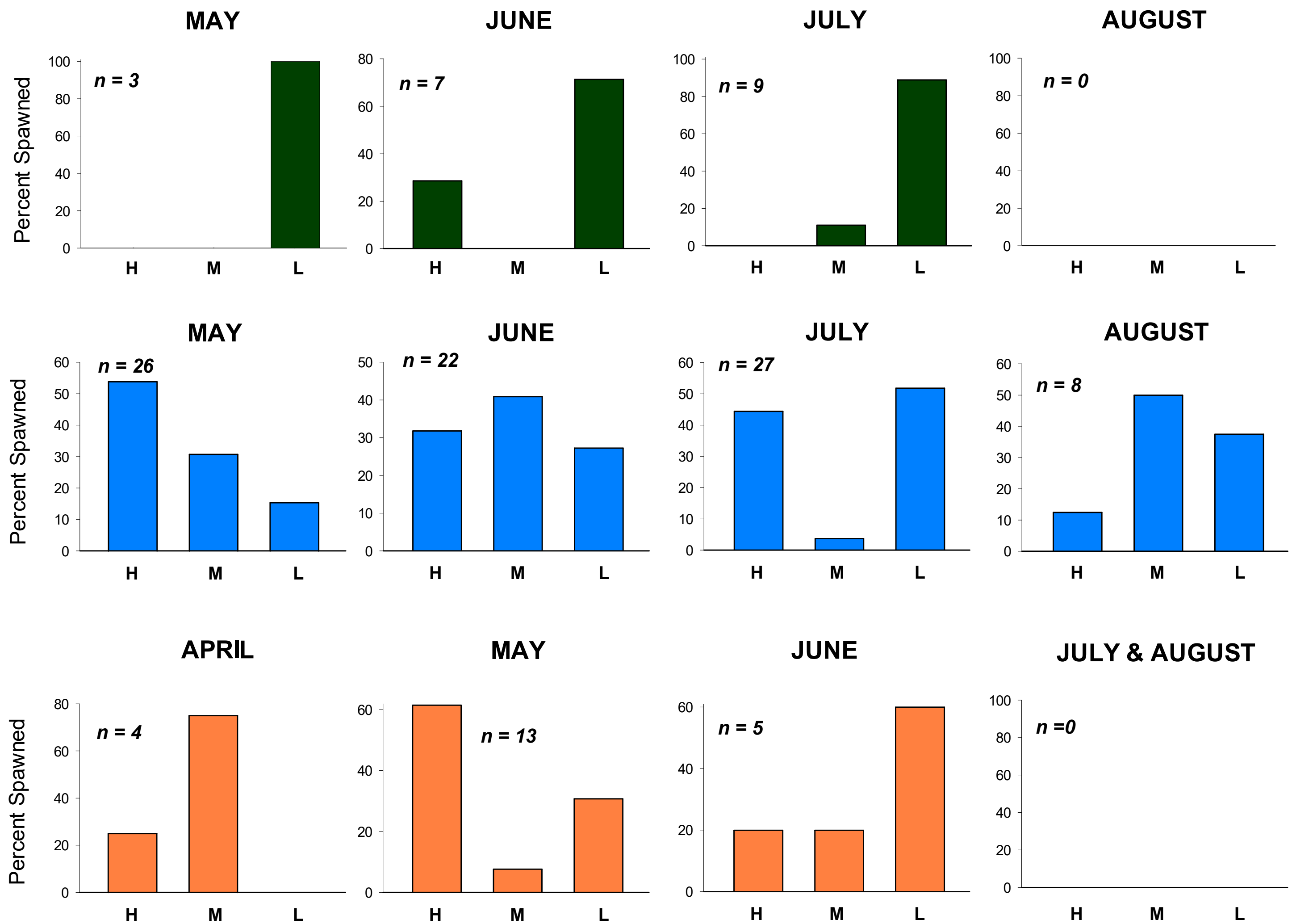

MAY
JUNE
JULY
AUGUST
Percent Spawned
n = 3
n = 7
n = 9
n = 0
MAY
JUNE
JULY
AUGUST
Percent Spawned
n = 26
n = 22
n = 27
n = 8
APRIL
MAY
JUNE
JULY & AUGUST
Percent Spawned
n = 4
n = 13
n = 5
n =0
H
M
L

Figure 43.

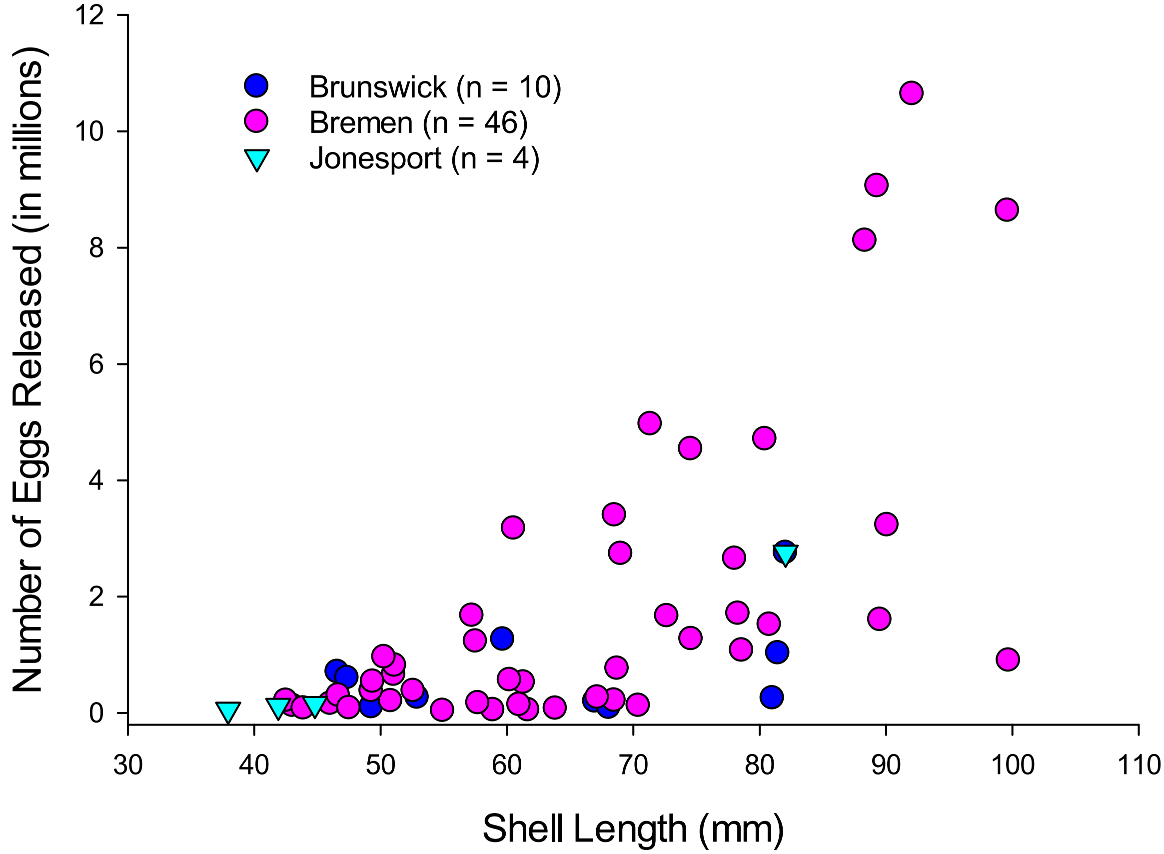

Number of Eggs Released (in millions)
Shell Length (mm)
Brunswick (n = 10)
Bremen (n = 46)
Jonesport (n = 4)
0
2
4
6
8
10
12
30
40
50
60
70
80
90
100
110

Figure 44.

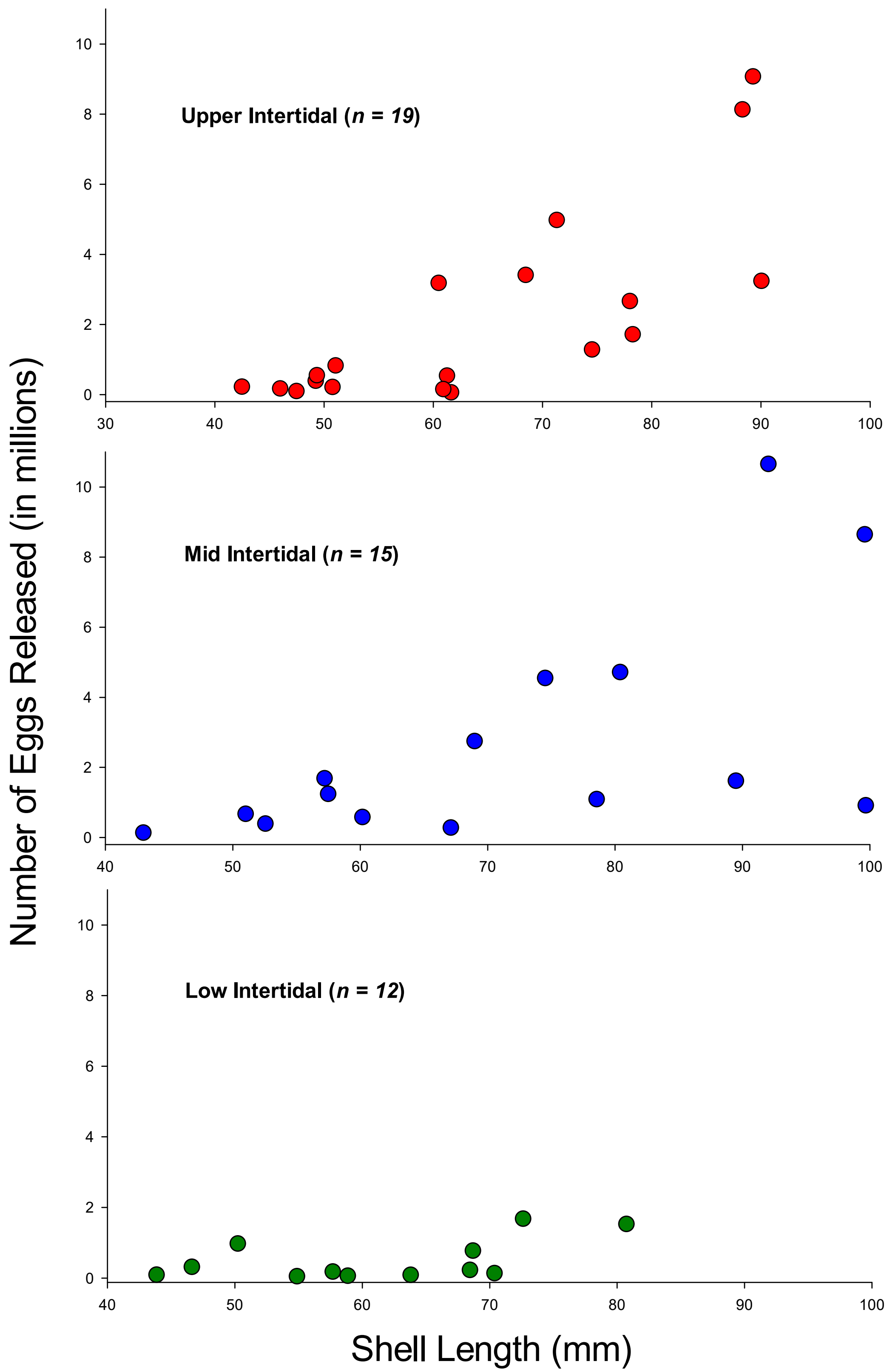

Number of Eggs Released (in millions)
Upper Intertidal (n = 19)
Mid Intertidal (n = 15)
Low Intertidal (n = 12)
0
2
4
6
8
10
30
40
50
60
70
80
90
100
Shell Length (mm)

Figure 45.

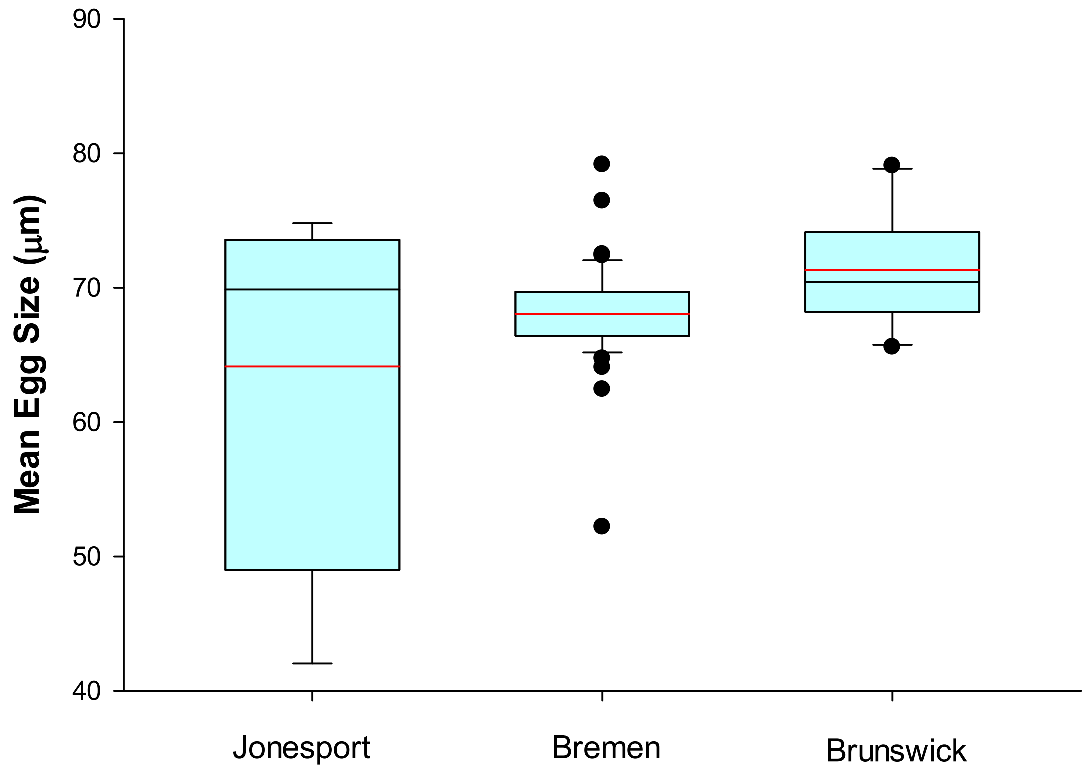
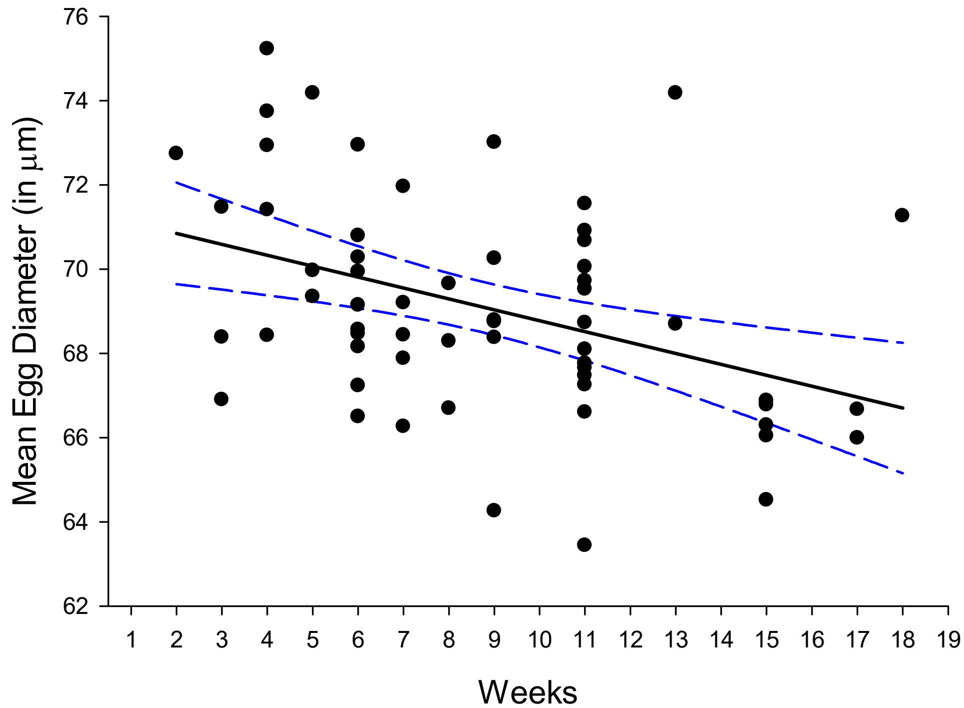


Figure 46.

A)


76
74
72
70
68
66
64
62
Mean Egg Diameter (in µm)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Weeks

B)

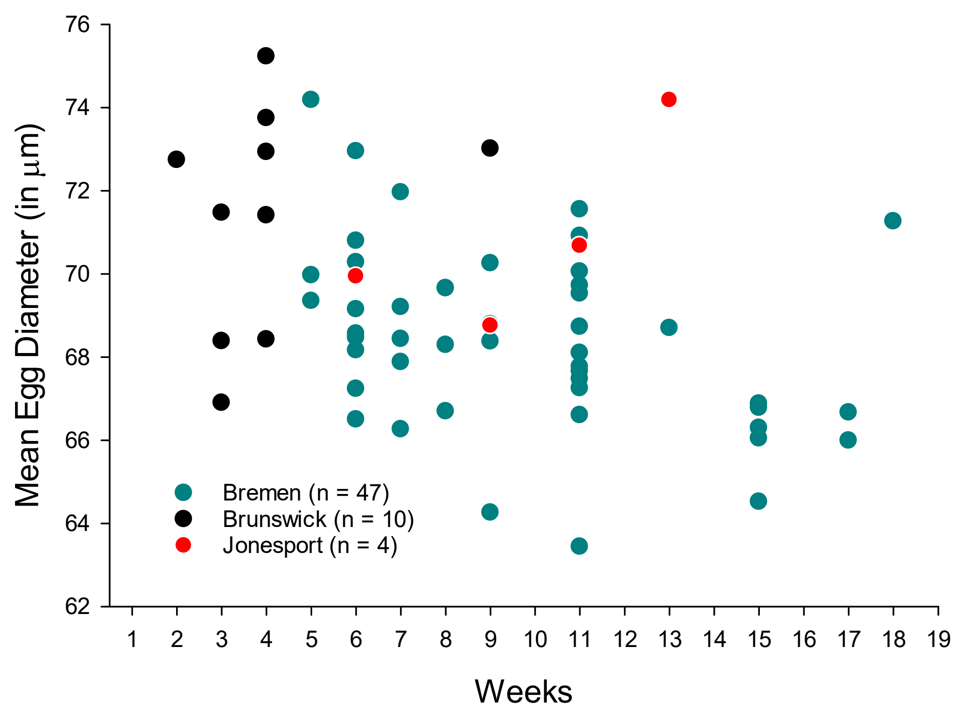

76
74
72
70
68
66
64
62
Mean Egg Diameter (in µm)
Bremen (n = 47)
Brunswick (n = 10)
Jonesport (n = 4)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Weeks

Figure 47.

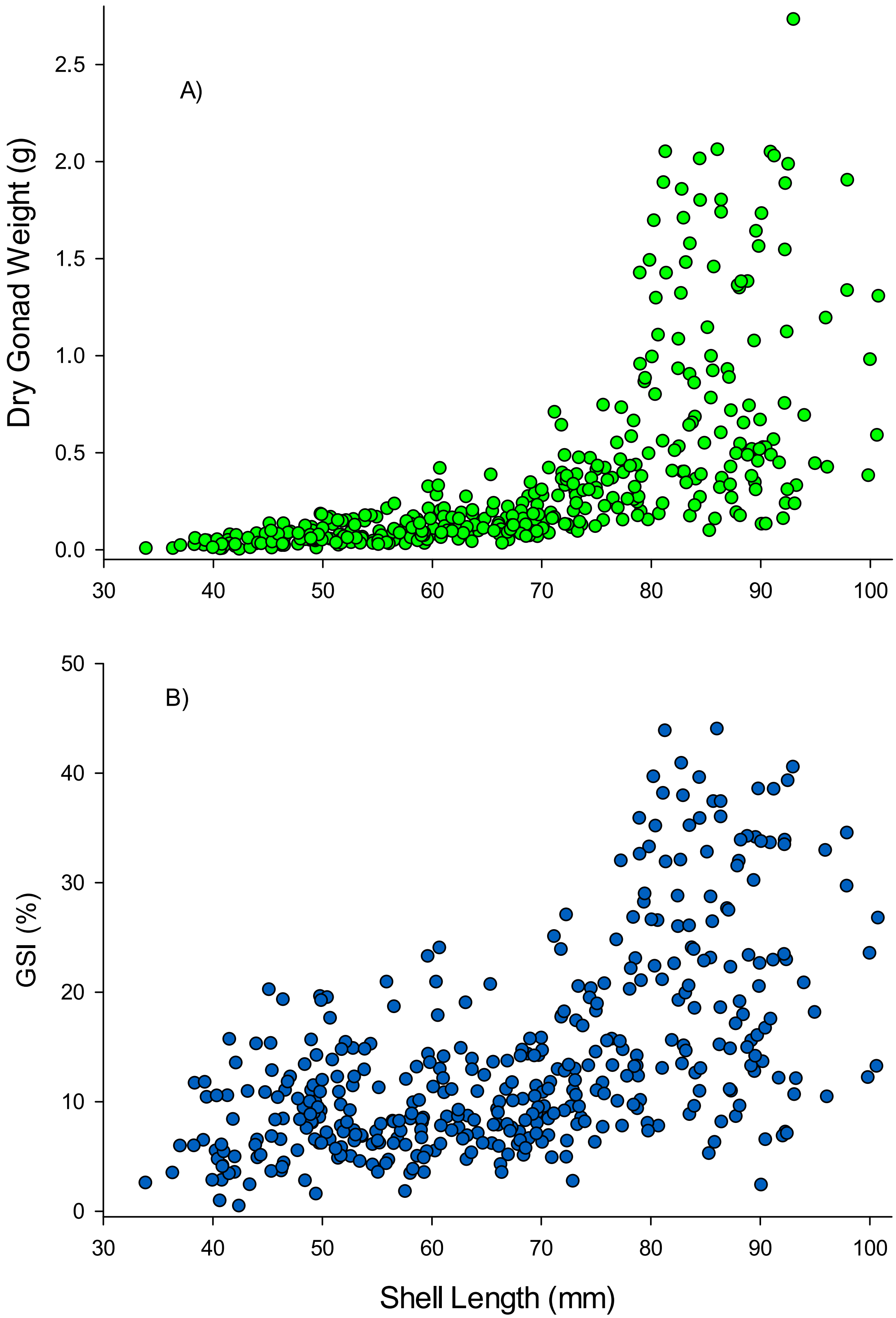

A)
Dry Gonad Weight (g)
0.0
0.5
1.0
1.5
2.0
2.5
30
40
50
60
70
80
90
100
B)
GSI (%)
0
10
20
30
40
50
30
40
50
60
70
80
90
100
Shell Length (mm)

Figure 48.

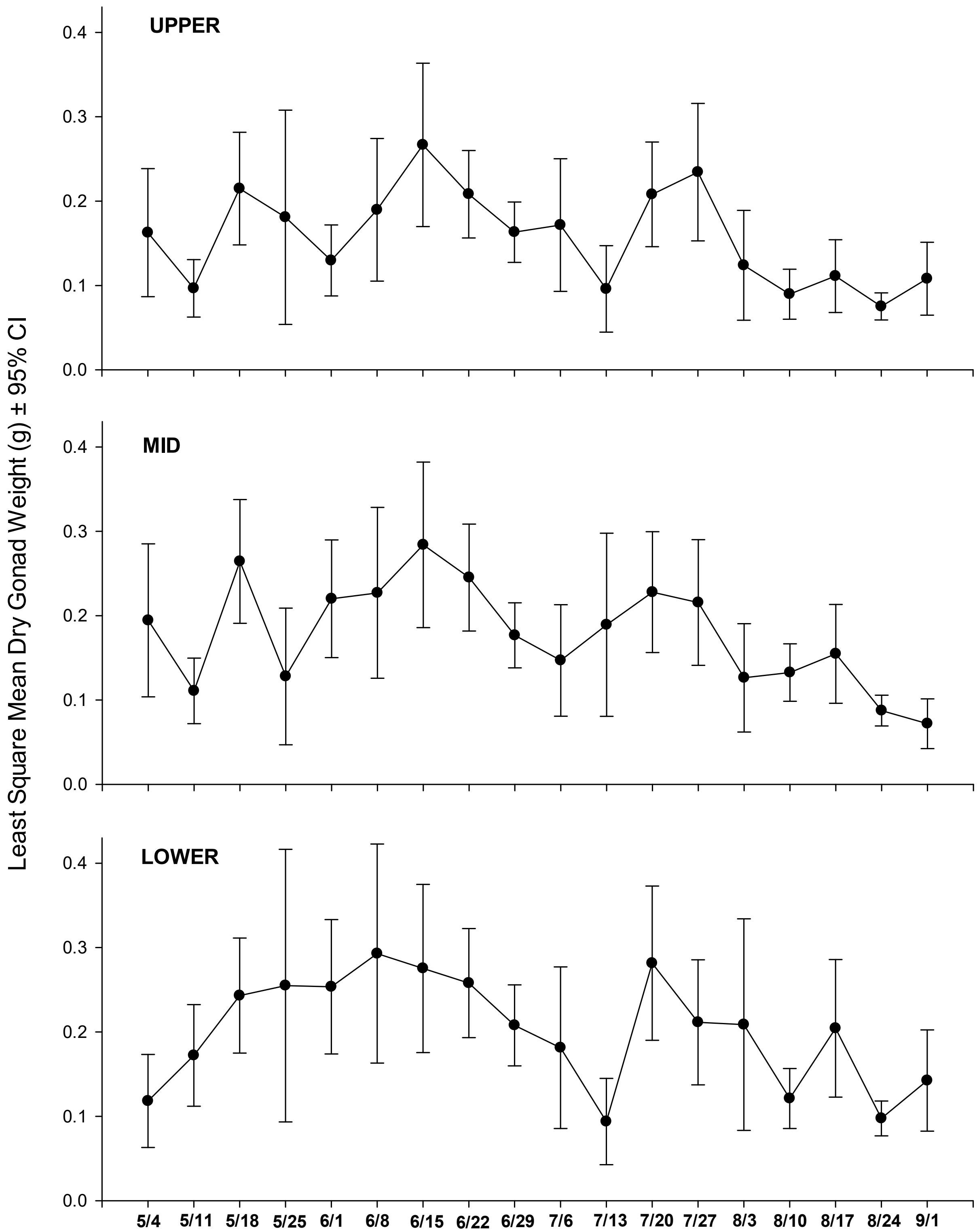

UPPER
MID
LOWER
Least Square Mean Dry Gonad Weight (g) ± 95% CI
0.0
0.1
0.2
0.3
0.4
5/4
5/11
5/18
5/25
6/1
6/8
6/15
6/22
6/29
7/6
7/13
7/20
7/27
8/3
8/10
8/17
8/24
9/1

Figure 49.

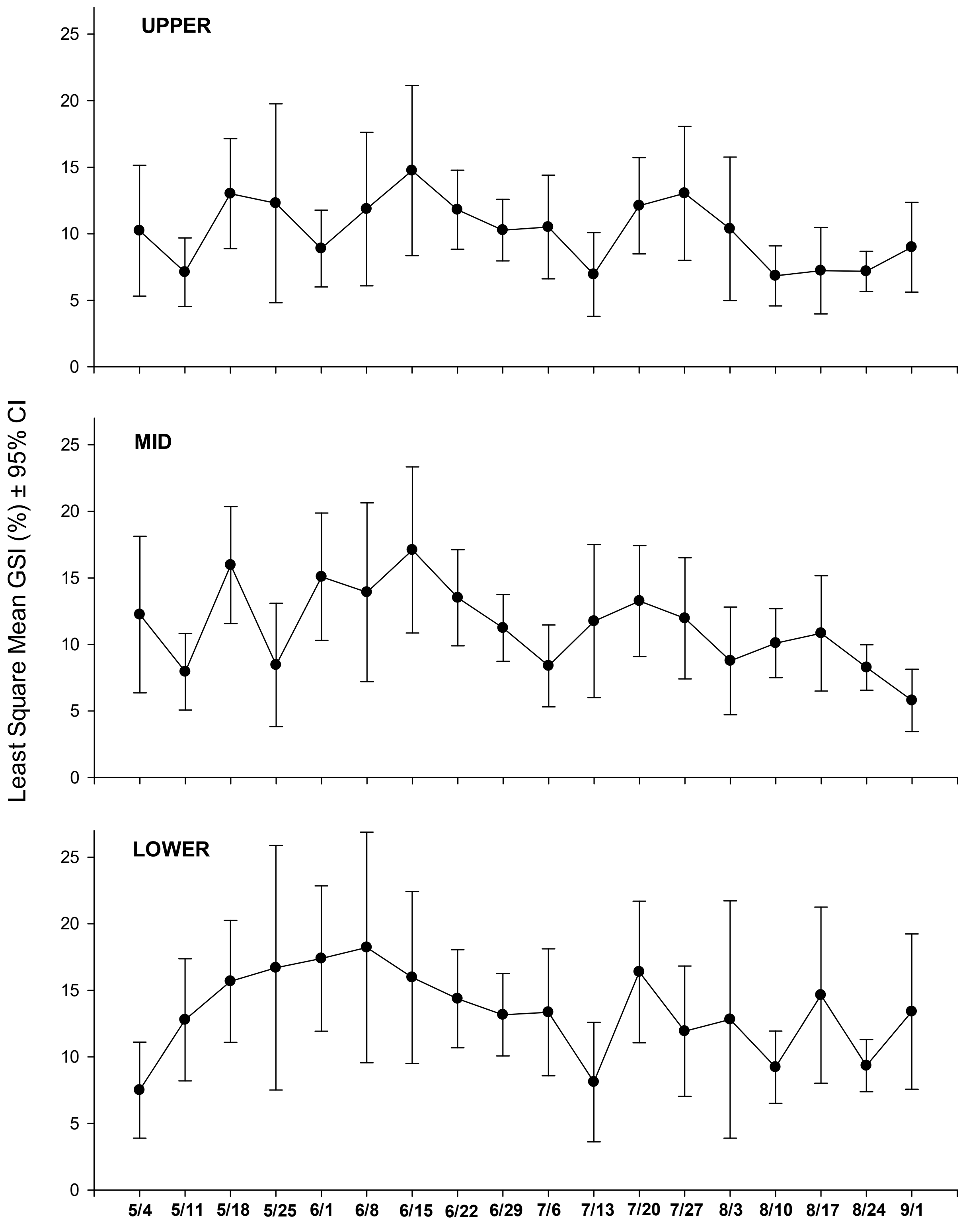

UPPER
MID
LOWER
Least Square Mean GSI (%) ± 95% CI
0
5
10
15
20
25
5/4
5/11
5/18
5/25
6/1
6/8
6/15
6/22
6/29
7/6
7/13
7/20
7/27
8/3
8/10
8/17
8/24
9/1

Figure 50.

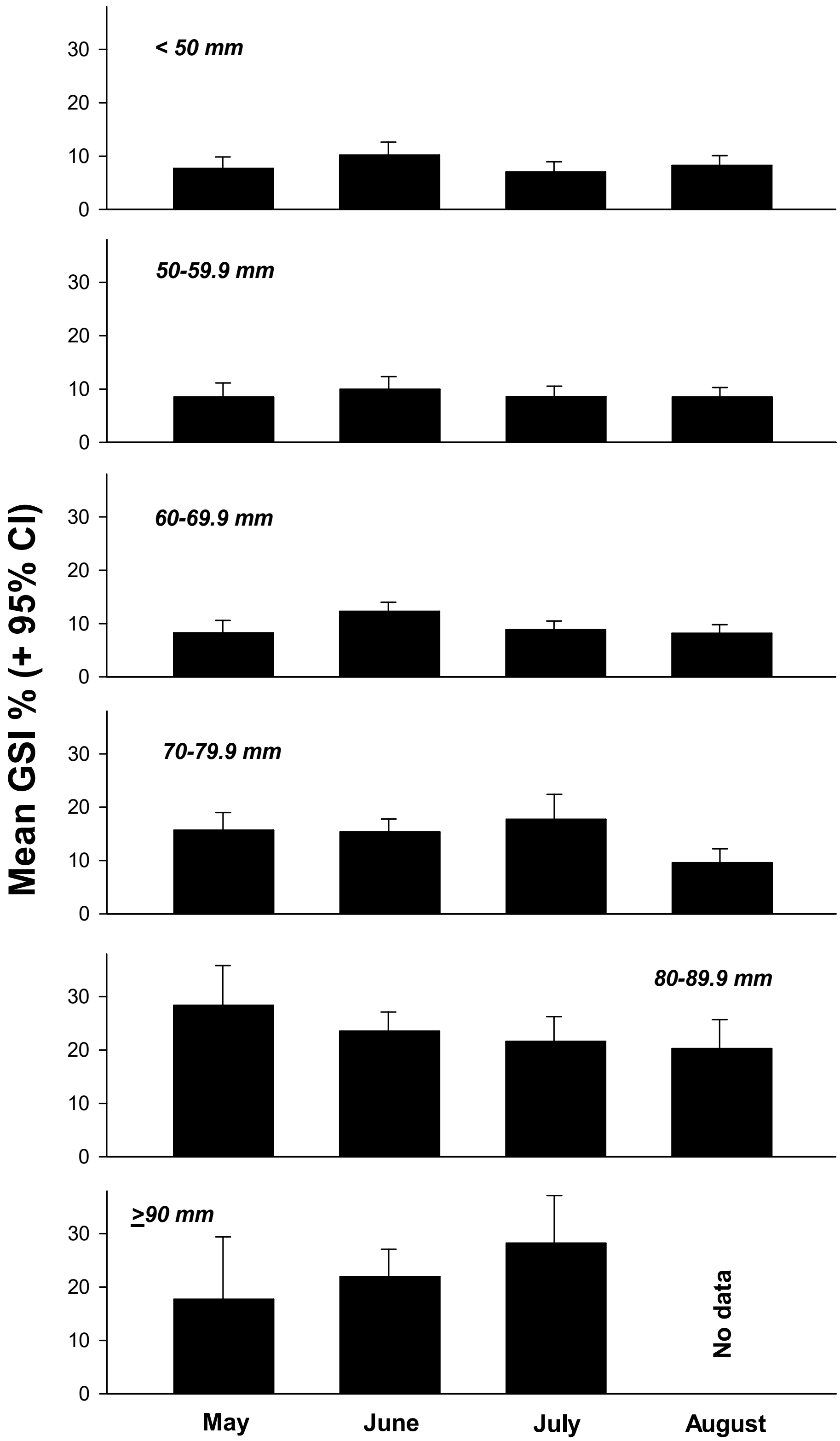

Mean GSI % (+ 95% CI)
< 50 mm
50-59.9 mm
60-69.9 mm
70-79.9 mm
80-89.9 mm
≥90 mm
No data
0
10
20
30
May
June
July
August

Figure 51.

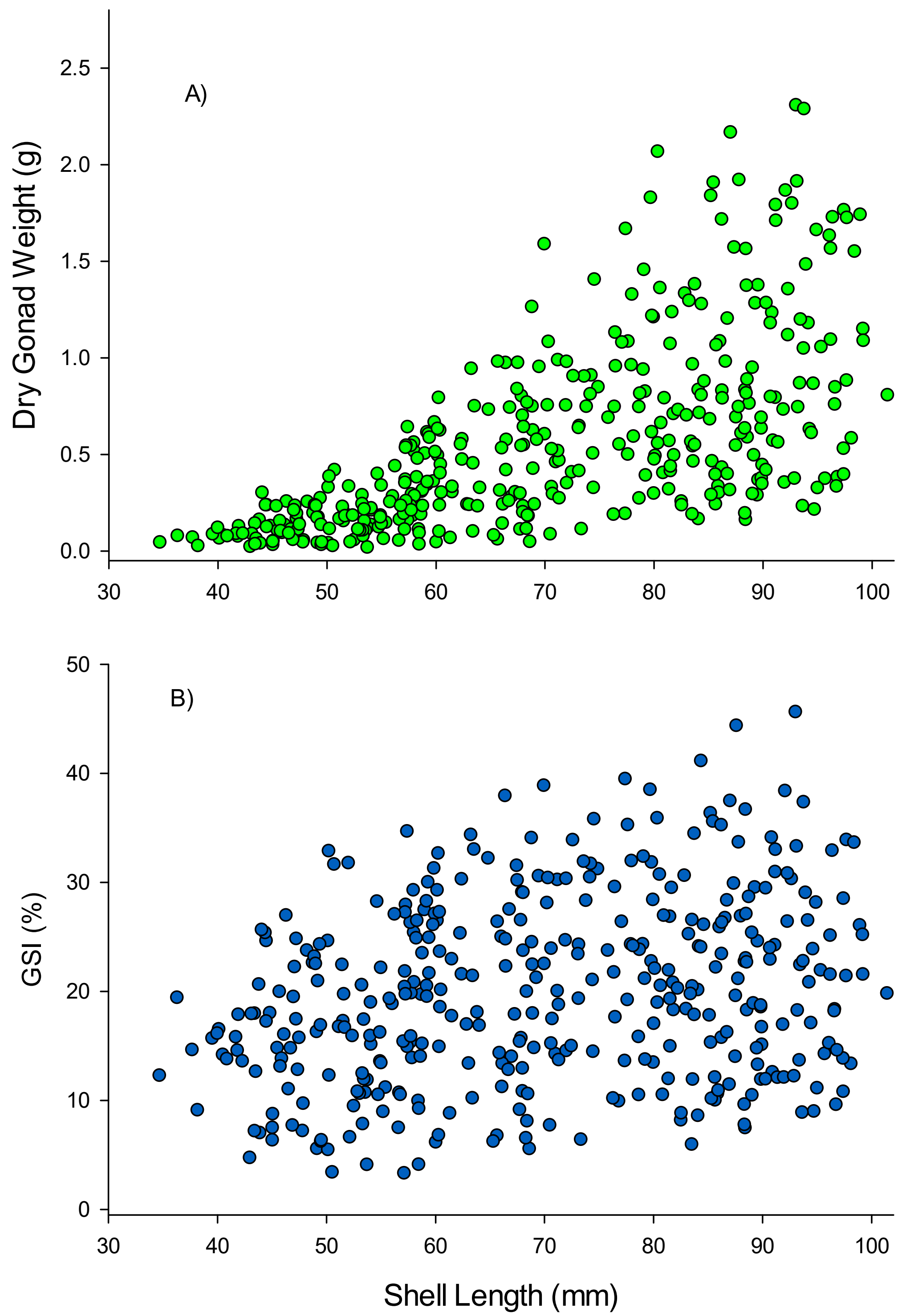

A)
Dry Gonad Weight (g)
0.0
0.5
1.0
1.5
2.0
2.5
30
40
50
60
70
80
90
100
B)
GSI (%)
0
10
20
30
40
50
Shell Length (mm)

Figure 52.

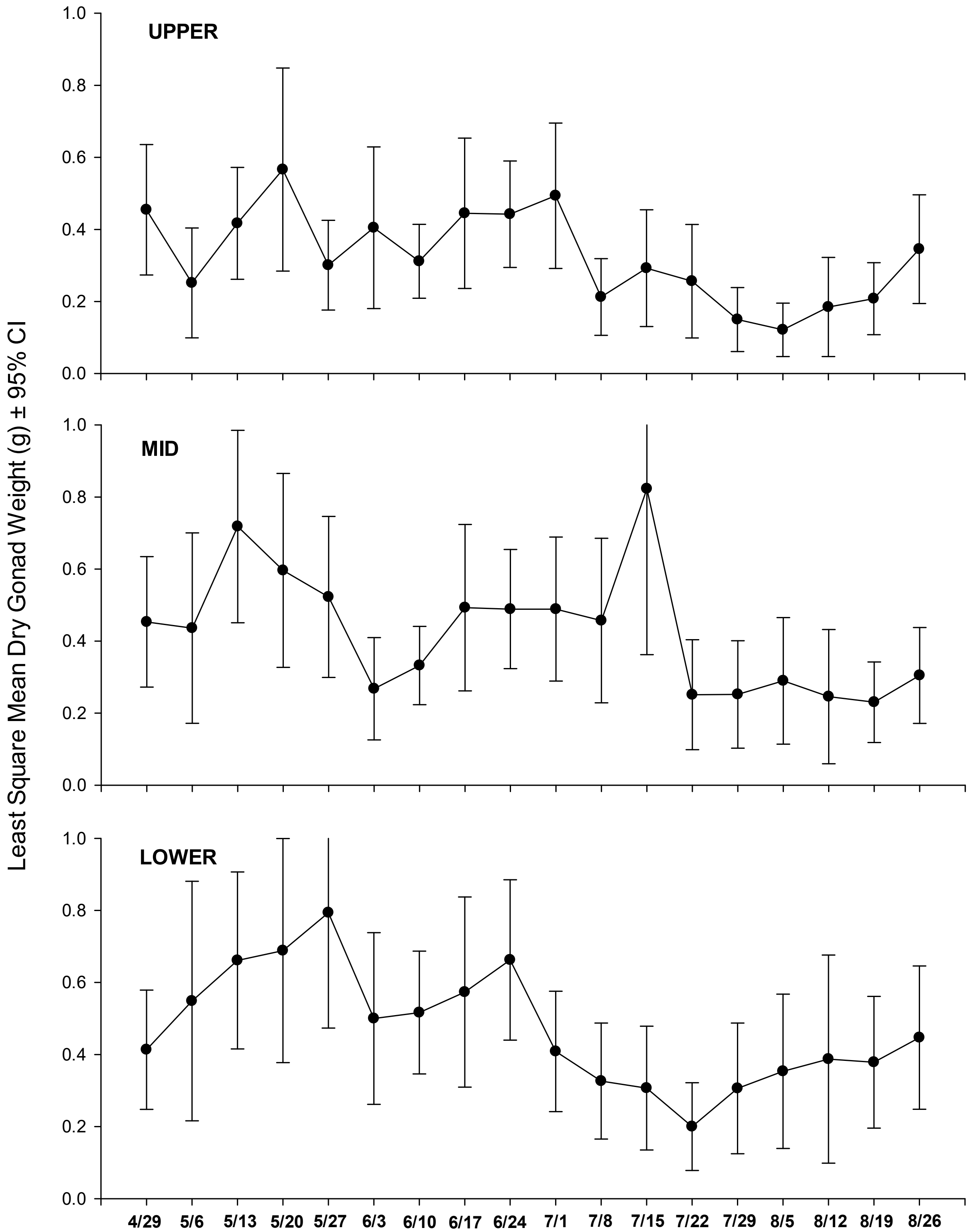

Least Square Mean Dry Gonad Weight (g) ± 95% CI
UPPER
MID
LOWER
1.0
0.8
0.6
0.4
0.2
0.0
4/29
5/6
5/13
5/20
5/27
6/3
6/10
6/17
6/24
7/1
7/8
7/15
7/22
7/29
8/5
8/12
8/19
8/26

Figure 53.

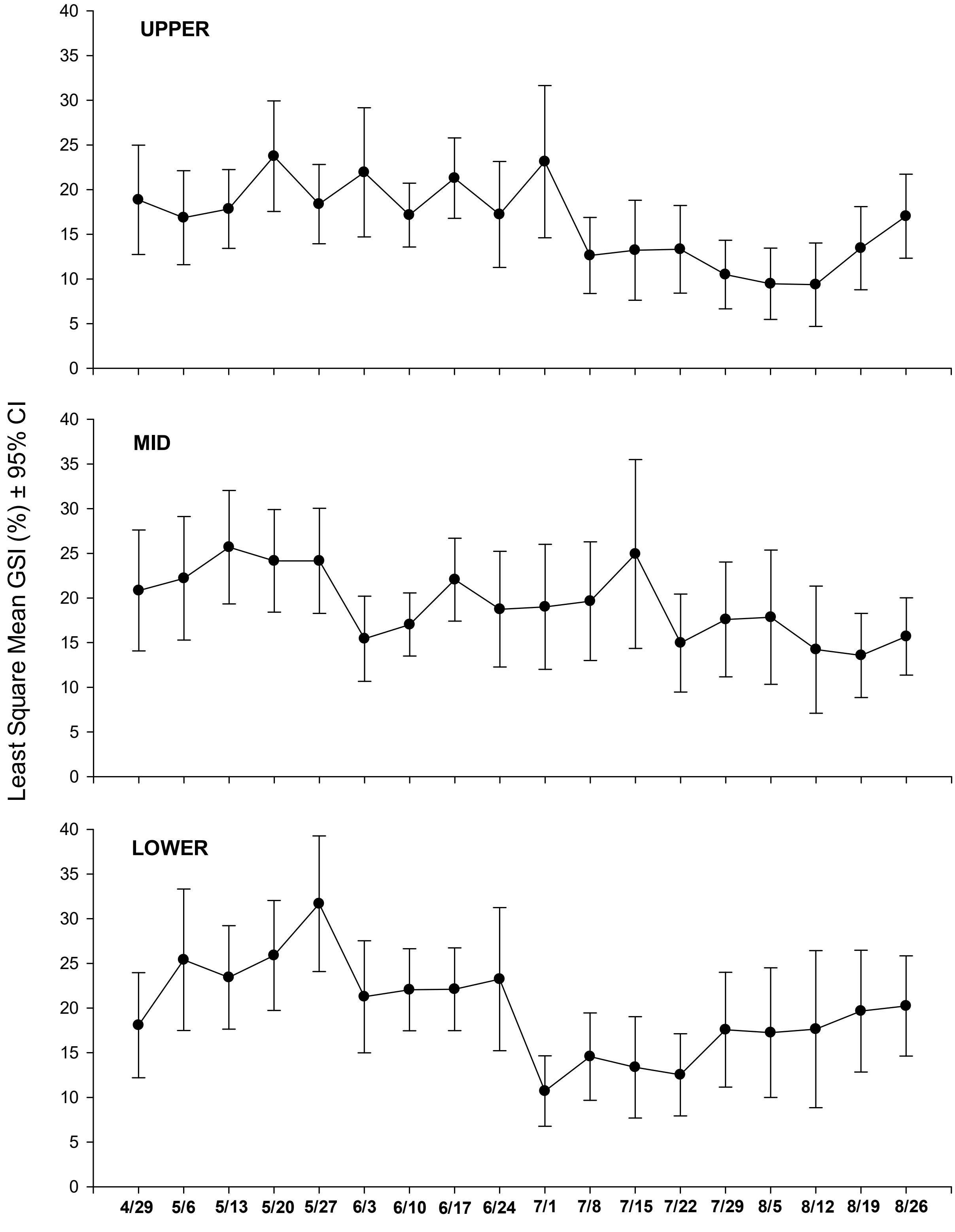

UPPER
MID
LOWER
Least Square Mean GSI (%) ± 95% CI
0
5
10
15
20
25
30
35
40
4/29
5/6
5/13
5/20
5/27
6/3
6/10
6/17
6/24
7/1
7/8
7/15
7/22
7/29
8/5
8/12
8/19
8/26

Figure 54.

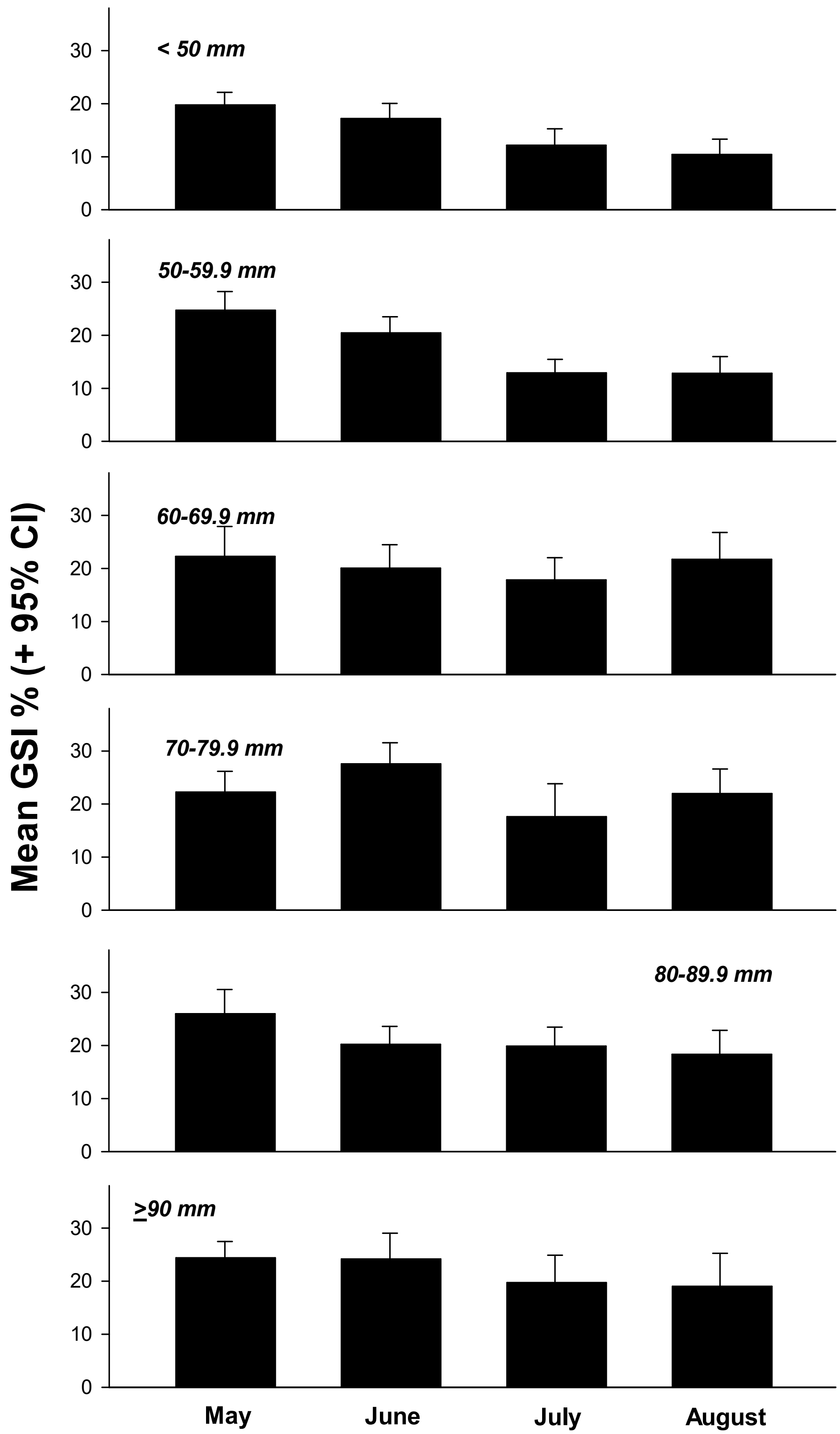

< 50 mm
50-59.9 mm
60-69.9 mm
70-79.9 mm
80-89.9 mm
≥90 mm
Mean GSI % (+ 95% CI)
0
10
20
30
May
June
July
August

Figure 55.

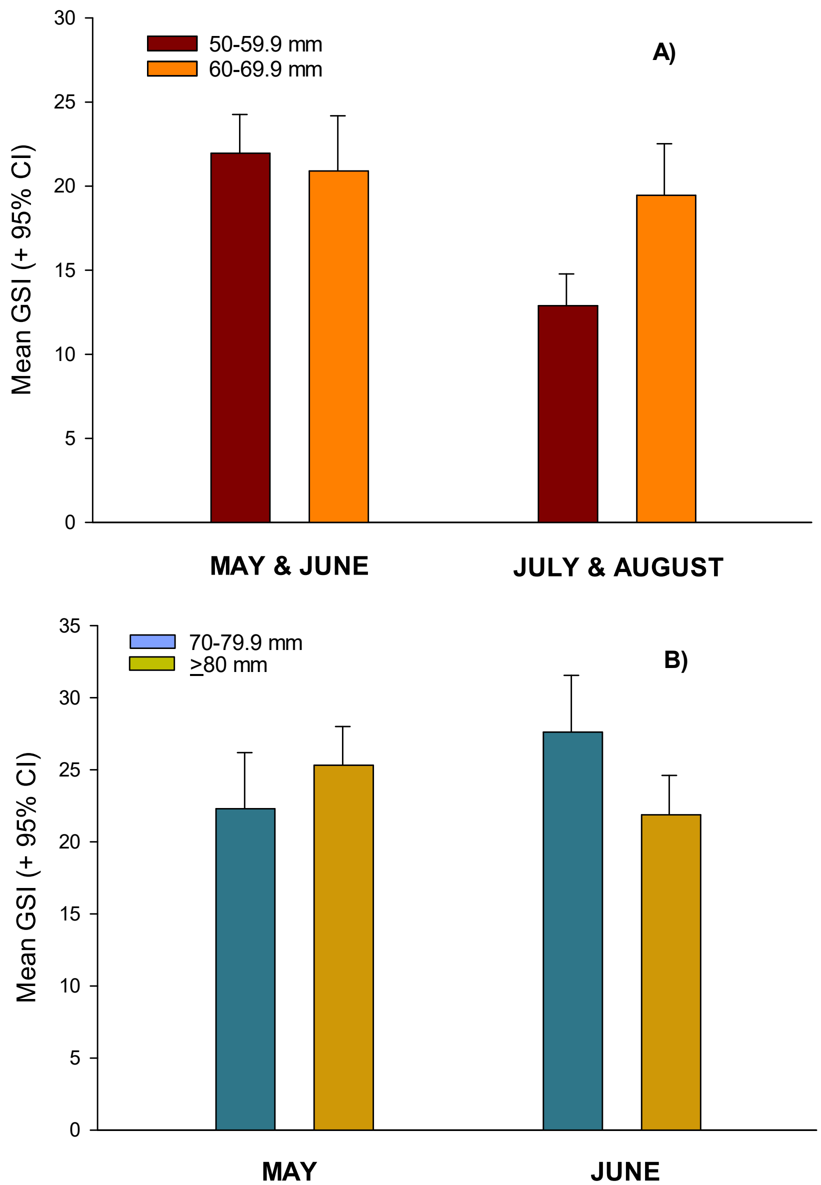

30
25
20
15
10
5
0
Mean GSI (+ 95% CI)
50-59.9 mm
60-69.9 mm
A)
MAY & JUNE
JULY & AUGUST
35
30
25
20
15
10
5
0
Mean GSI (+ 95% CI)
70-79.9 mm
>80 mm
B)
MAY
JUNE

Figure 56.

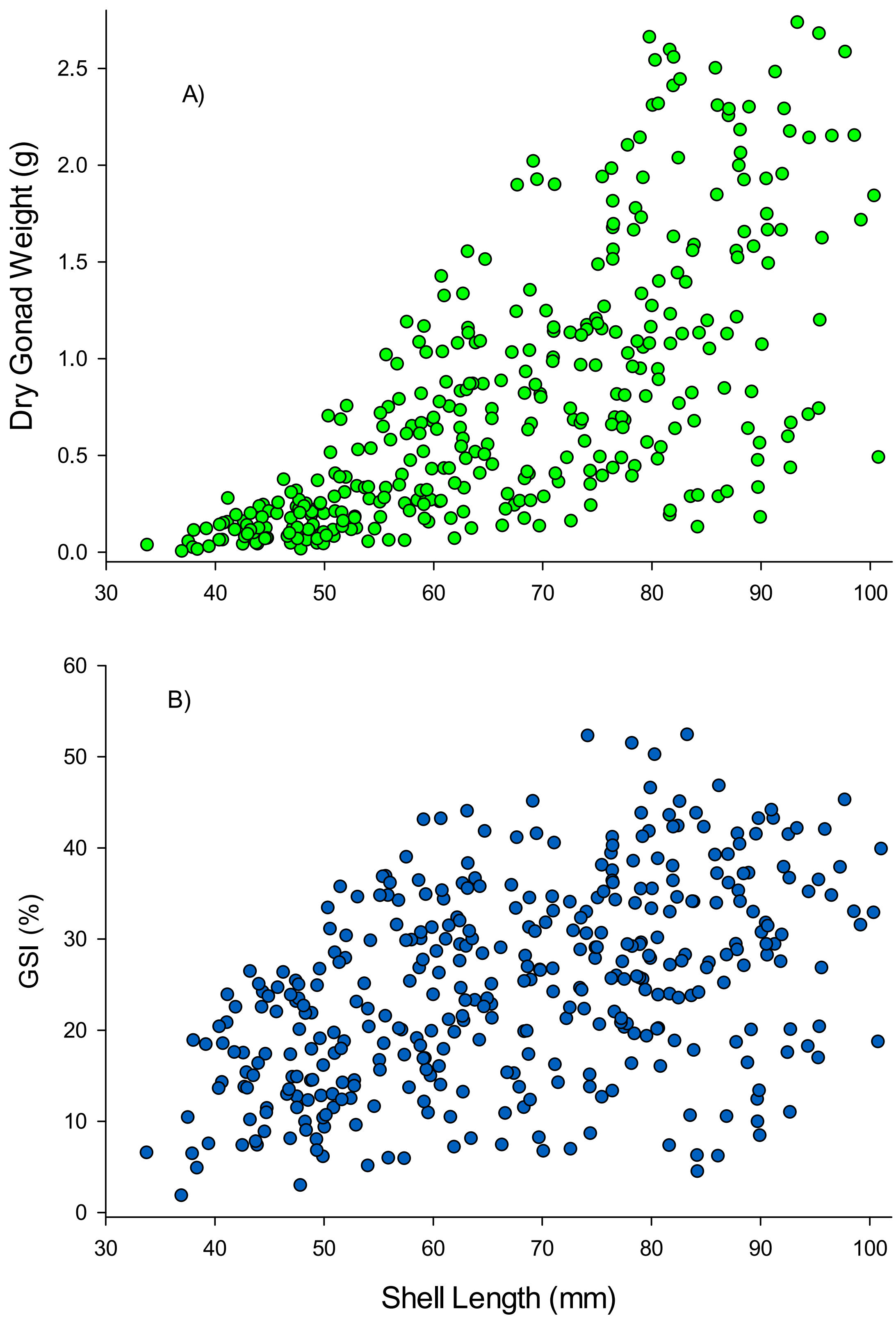

A)
Dry Gonad Weight (g)
0.0
0.5
1.0
1.5
2.0
2.5
30
40
50
60
70
80
90
100
B)
GSI (%)
0
10
20
30
40
50
60
30
40
50
60
70
80
90
100
Shell Length (mm)

Figure 57.

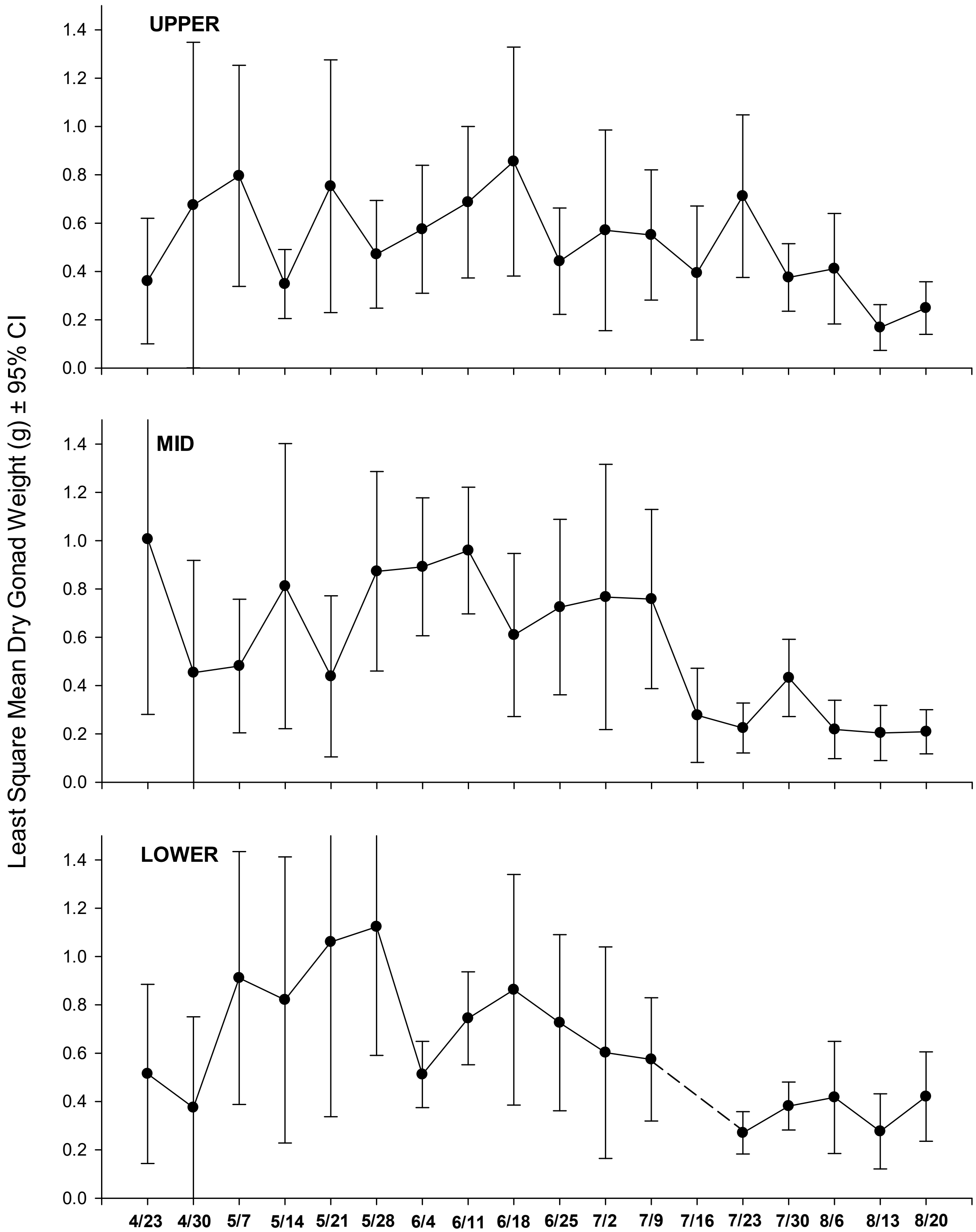

Least Square Mean Dry Gonad Weight (g) ± 95% CI
UPPER
MID
LOWER
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9
7/16
7/23
7/30
8/6
8/13
8/20

Figure 58.

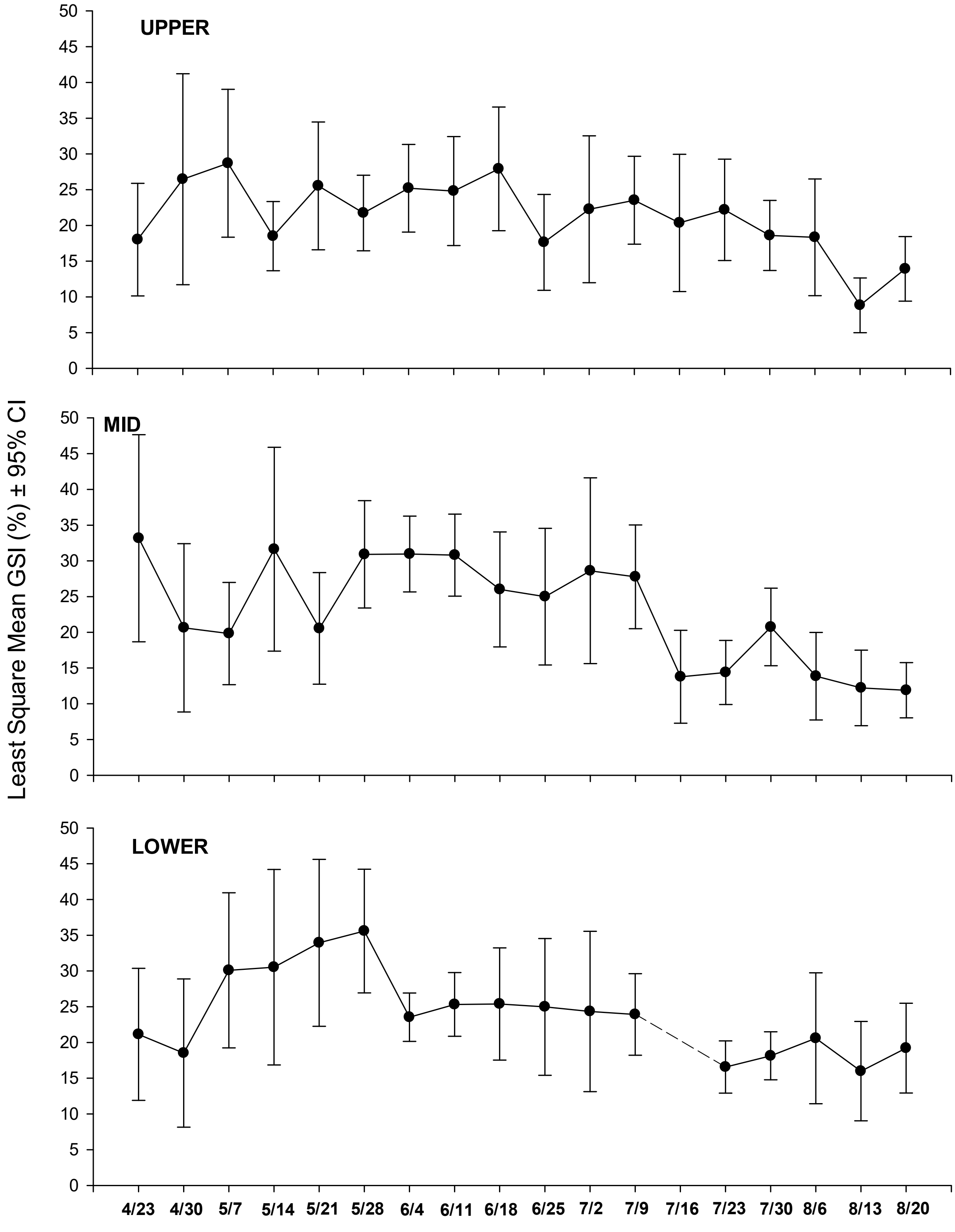

UPPER
MID
LOWER
Least Square Mean GSI (%) ± 95% CI
0
5
10
15
20
25
30
35
40
45
50
4/23
4/30
5/7
5/14
5/21
5/28
6/4
6/11
6/18
6/25
7/2
7/9
7/16
7/23
7/30
8/6
8/13
8/20

Figure 59.

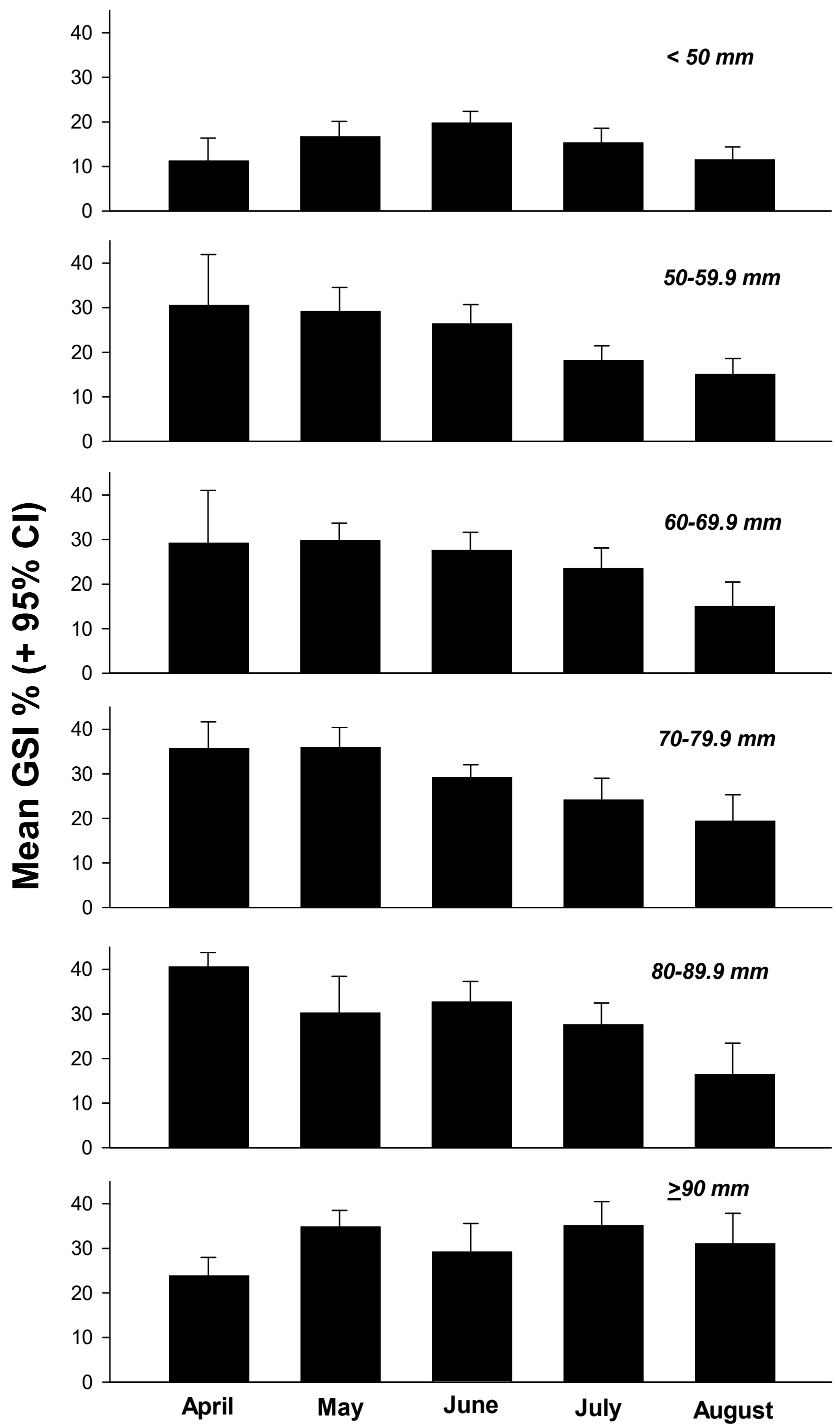

Mean GSI % (+ 95% CI)
< 50 mm
50-59.9 mm
60-69.9 mm
70-79.9 mm
80-89.9 mm
≥90 mm
0
10
20
30
40
April
May
June
July
August

Figure 60.

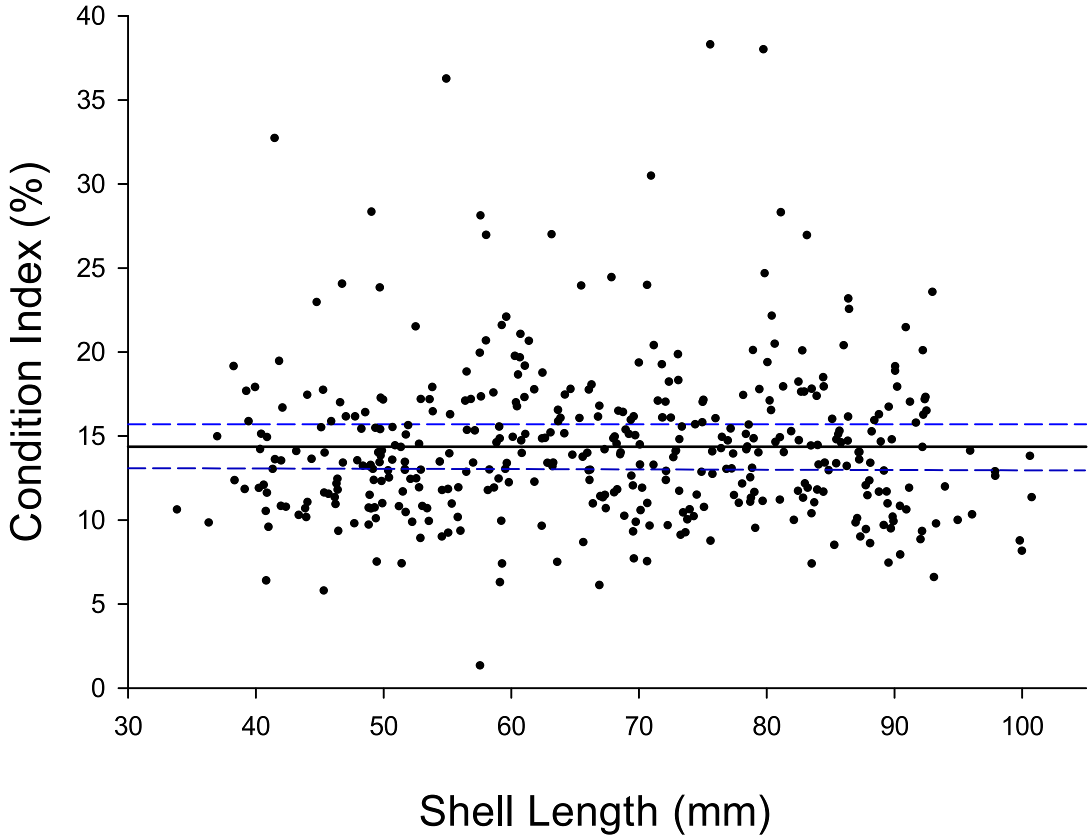

Condition Index (%)
0
5
10
15
20
25
30
35
40
30
40
50
60
70
80
90
100
Shell Length (mm)

Figure 61.

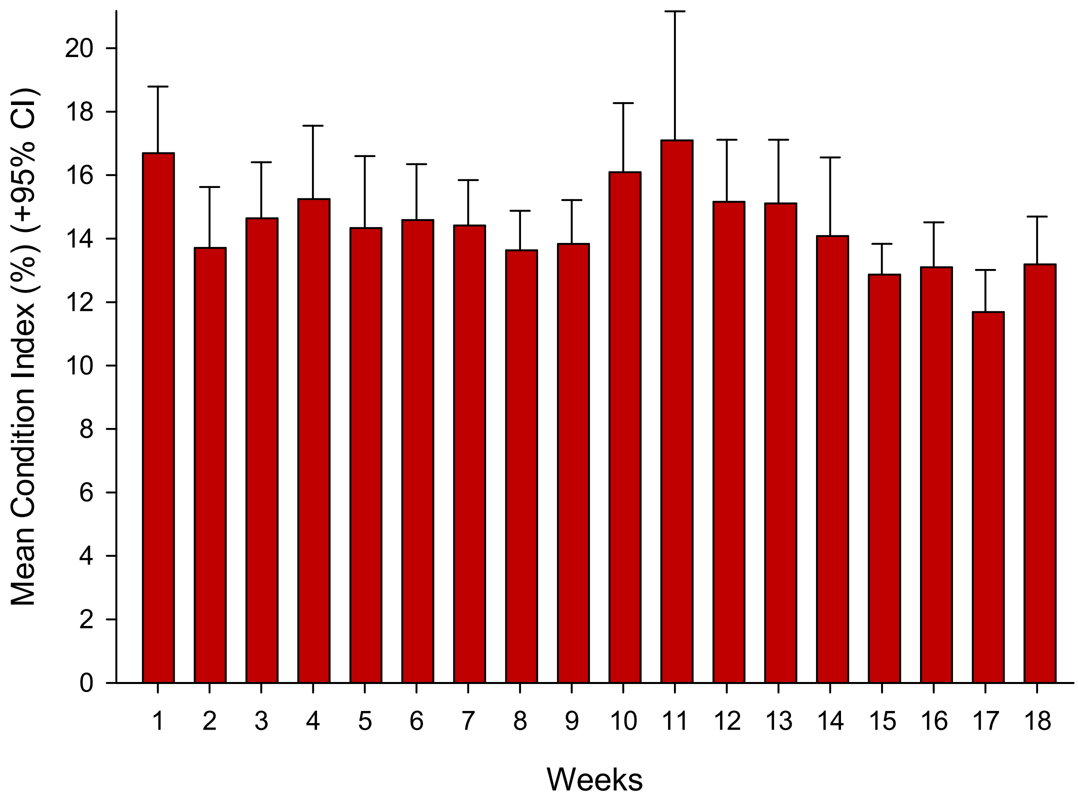

Mean Condition Index (%) (+95% CI)
0
2
4
6
8
10
12
14
16
18
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Weeks

Figure 62.

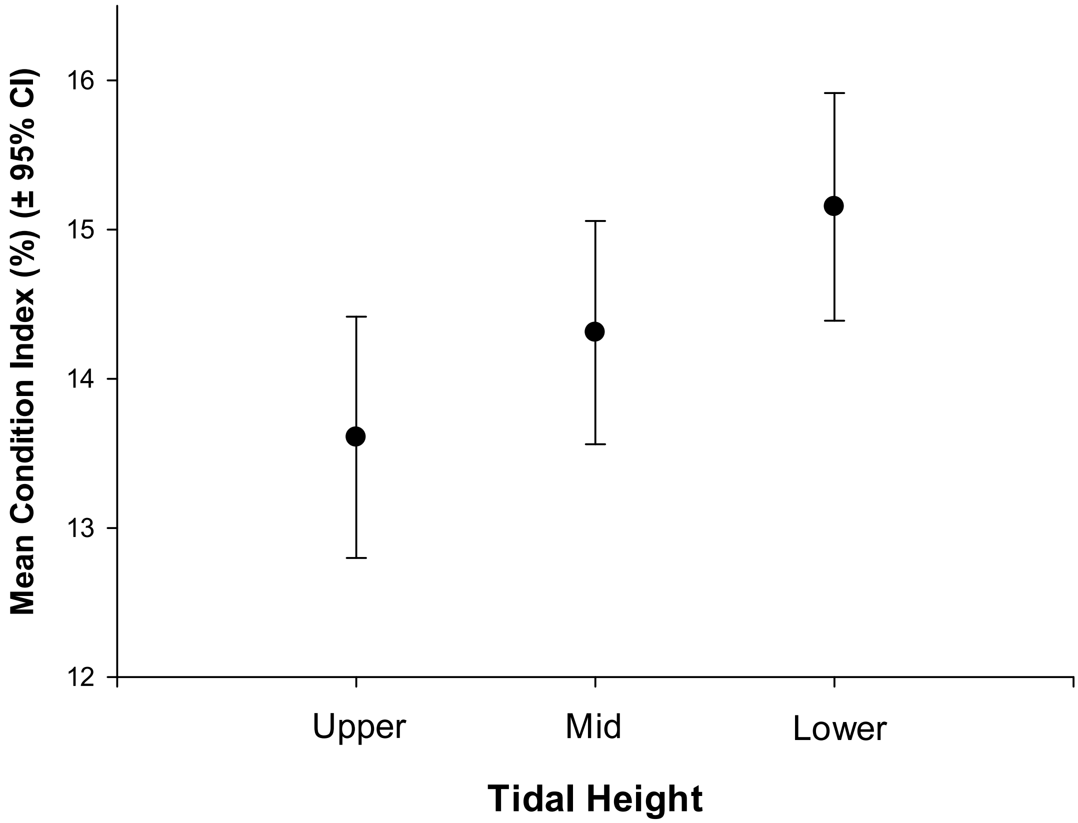

Mean Condition Index (%) (± 95% CI)
16
15
14
13
12
Upper
Mid
Lower
Tidal Height

Figure 63.

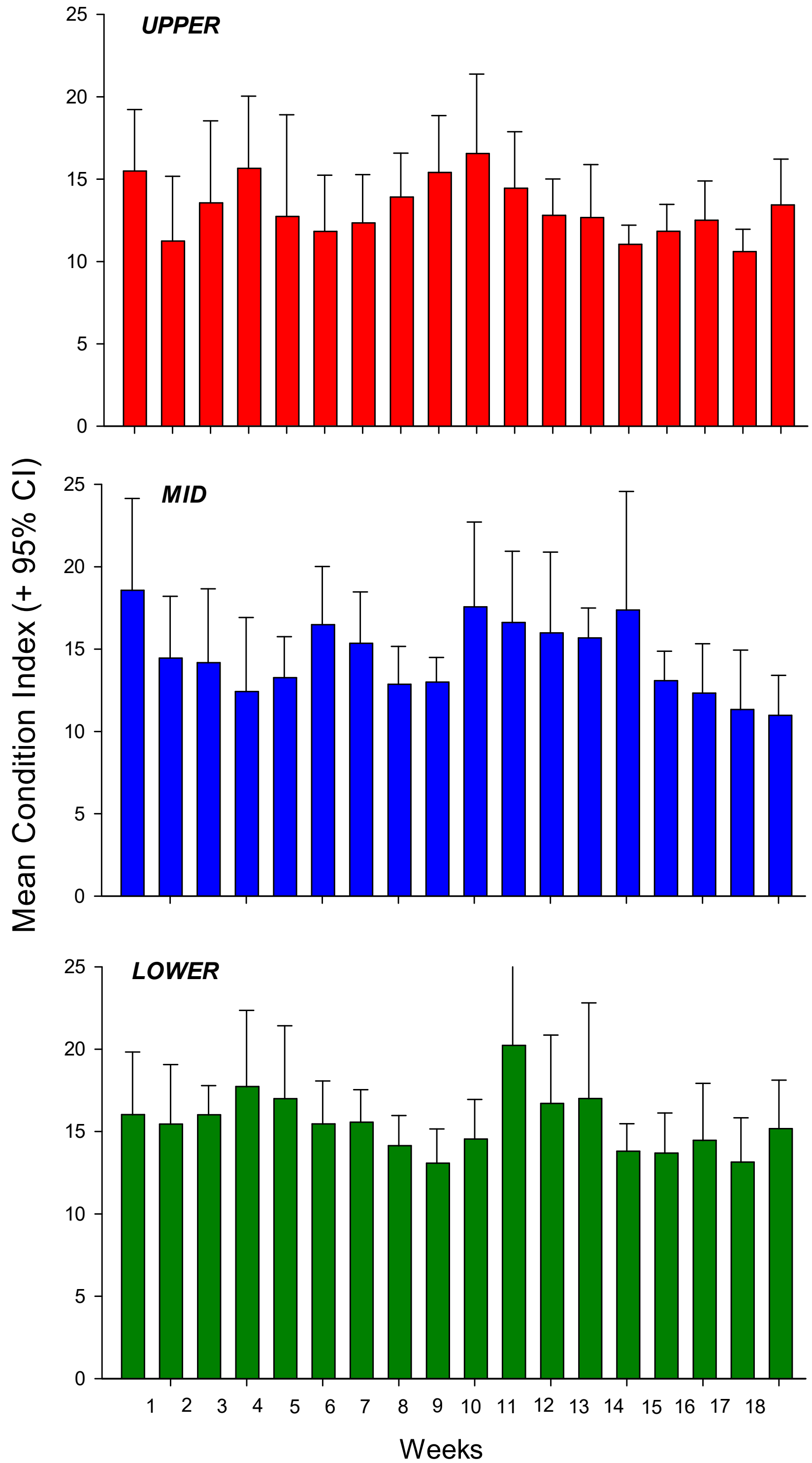

UPPER
MID
LOWER
Mean Condition Index (+ 95% CI)
Weeks
0
5
10
15
20
25
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Figure 64.

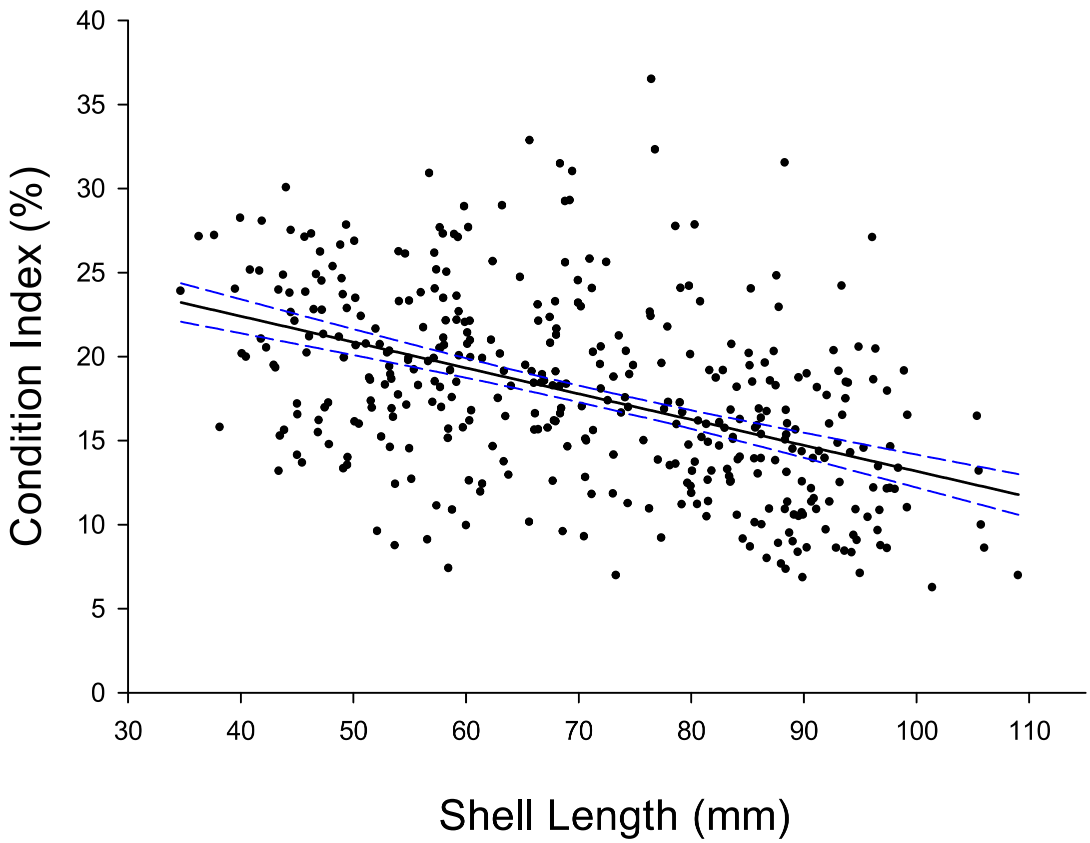

Condition Index (%)
Shell Length (mm)
0
5
10
15
20
25
30
35
40
30
40
50
60
70
80
90
100
110

Figure 65.

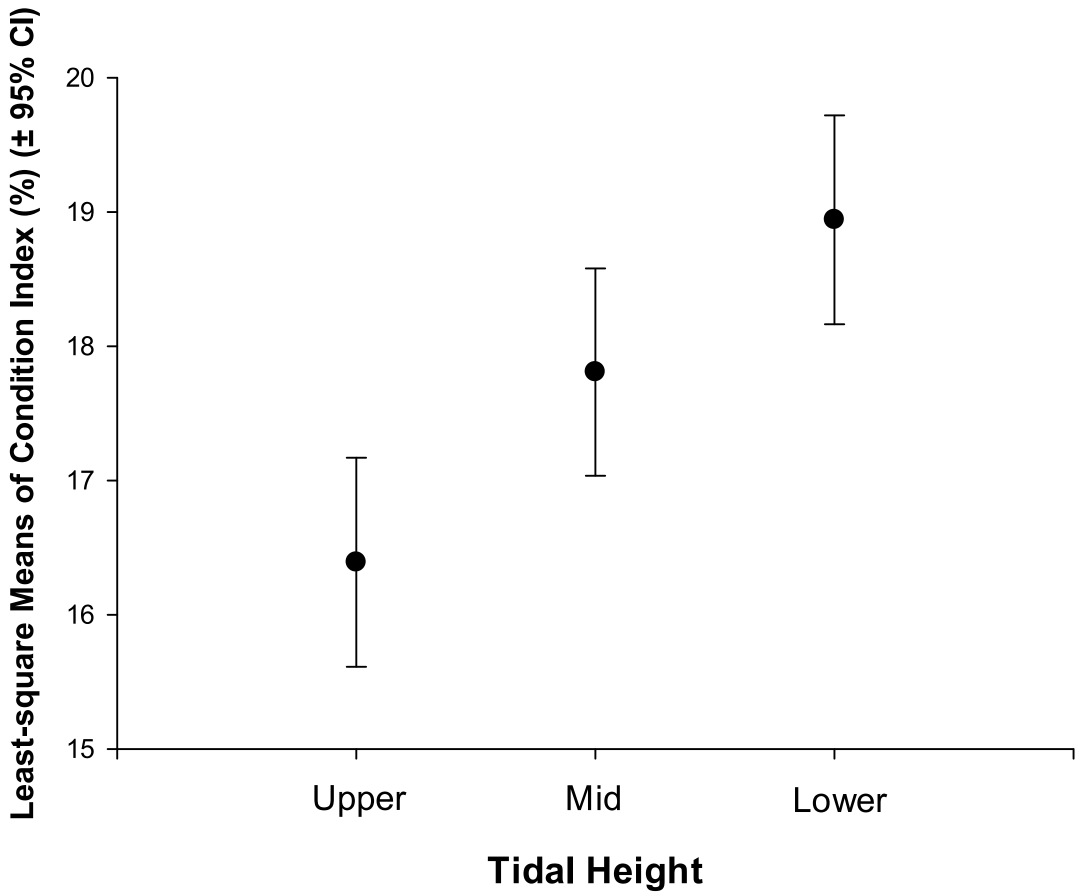

Least-square Means of Condition Index (%) (± 95% CI)
20
19
18
17
16
15
Upper
Mid
Lower
Tidal Height

Figure 66.

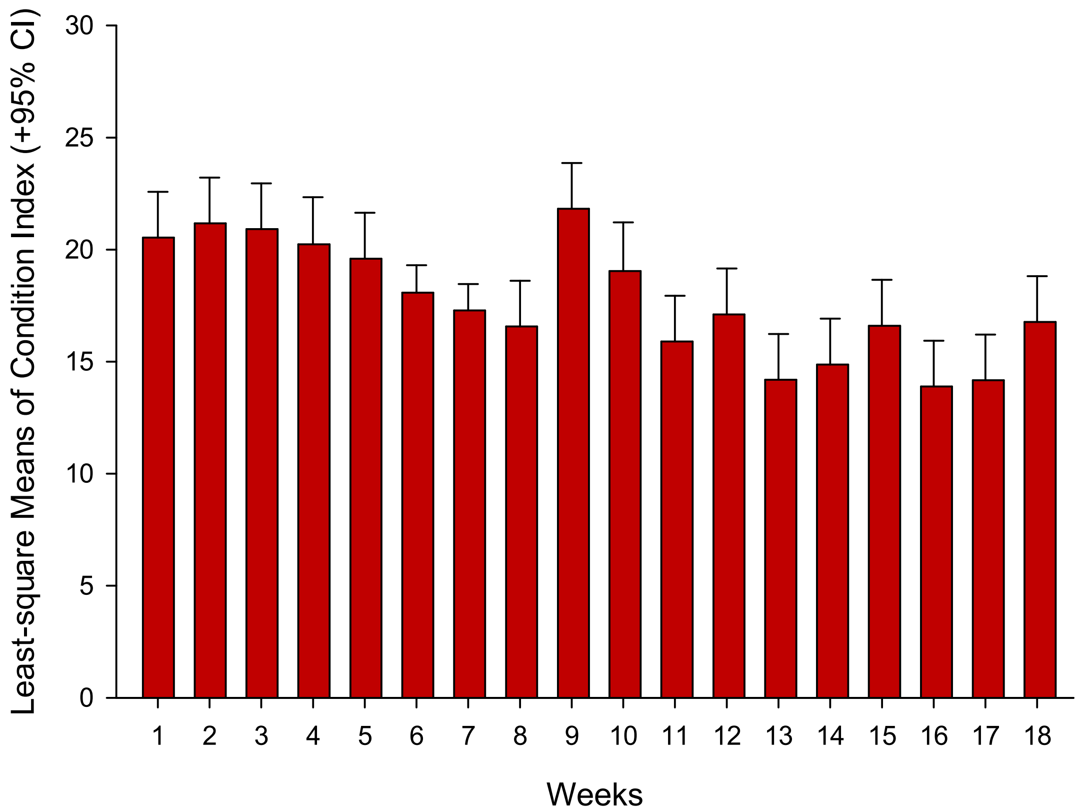

Least-square Means of Condition Index (+95% CI)
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks

Figure 67.

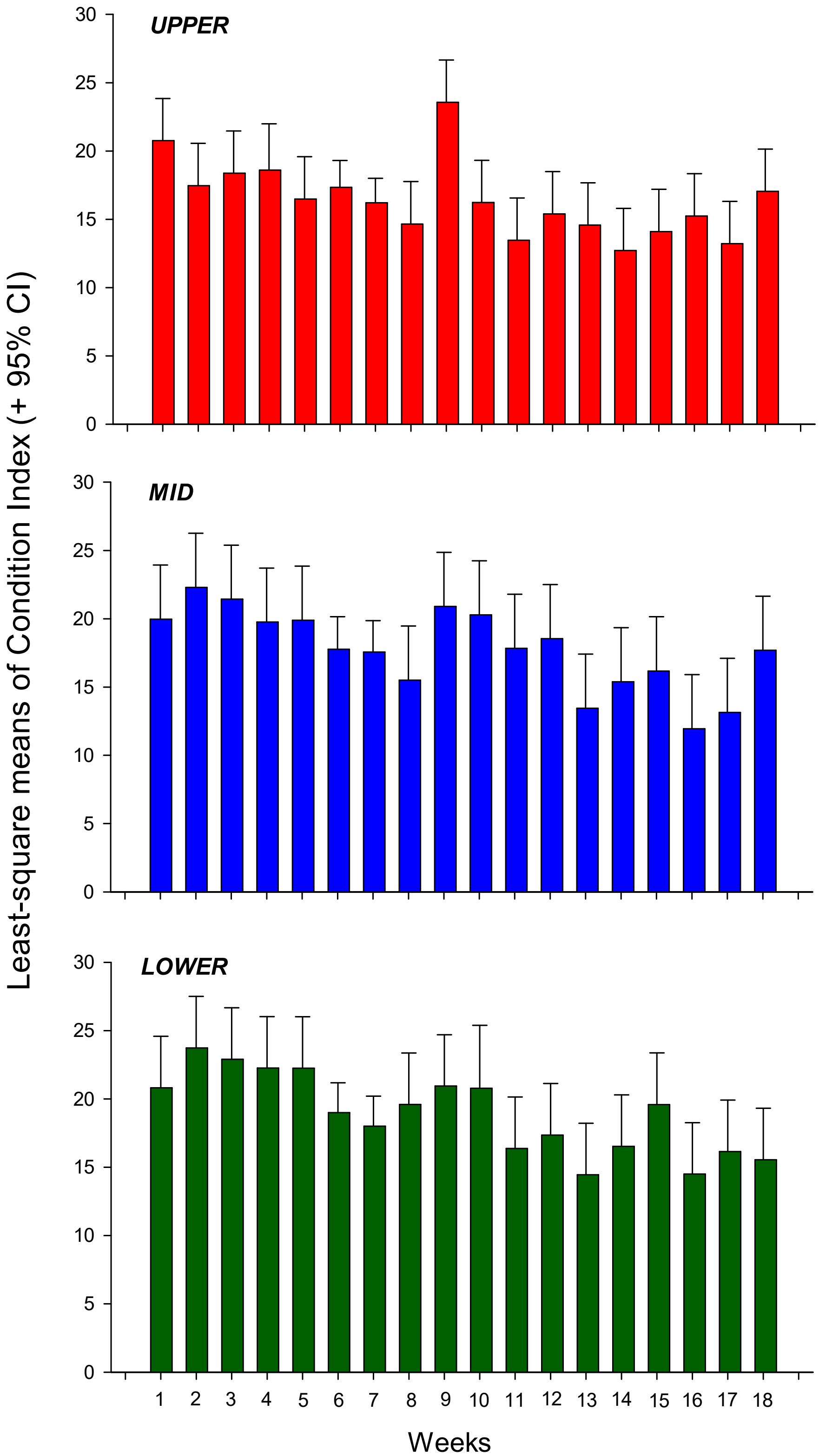

UPPER
MID
LOWER
Least-square means of Condition Index (+ 95% CI)
0
5
10
15
20
25
30
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Weeks

Figure 68.

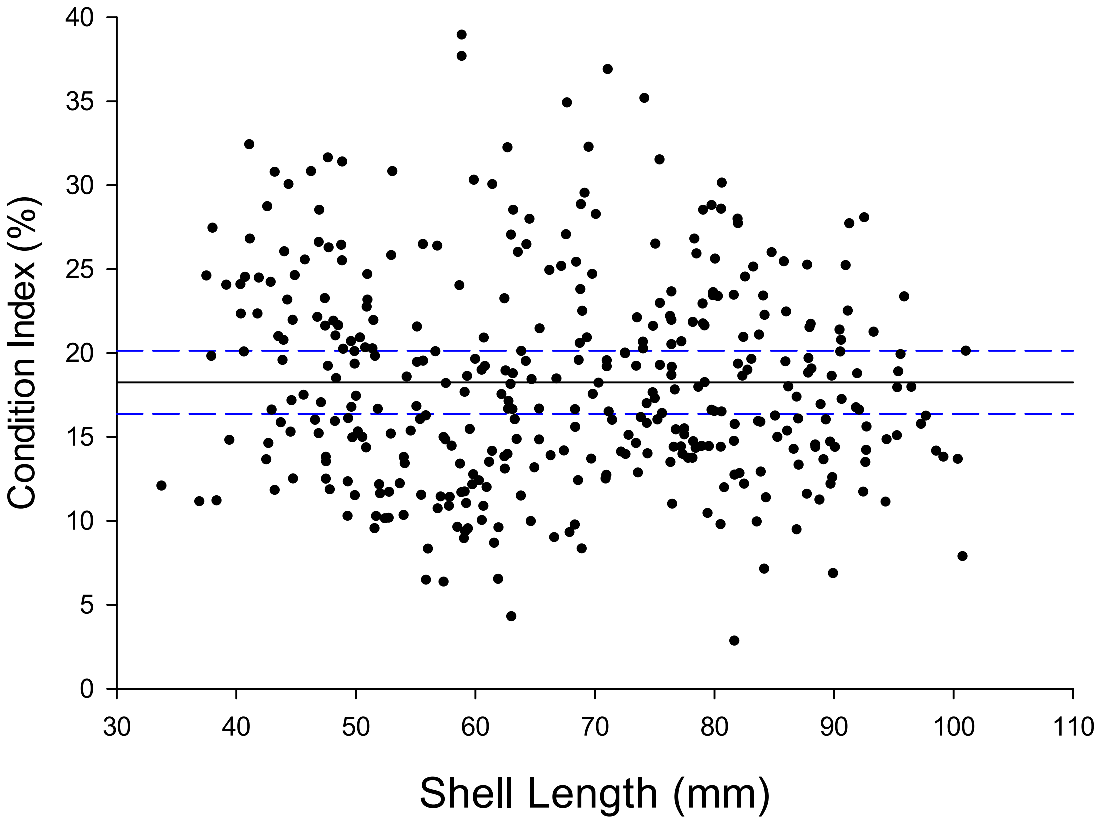

Condition Index (%)
40
35
30
25
20
15
10
5
0
30
40
50
60
70
80
90
100
110
Shell Length (mm)

Figure 69.

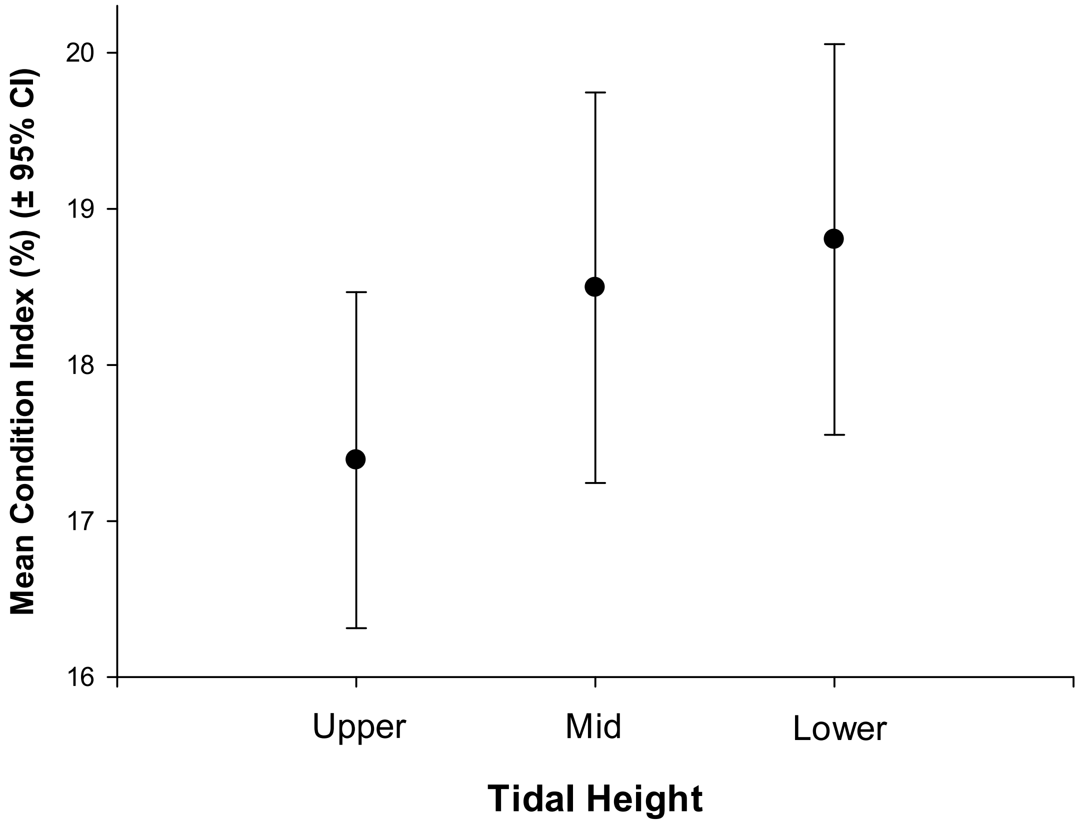

Mean Condition Index (%) (± 95% CI)
20
19
18
17
16
Upper
Mid
Lower
Tidal Height

Figure 70.

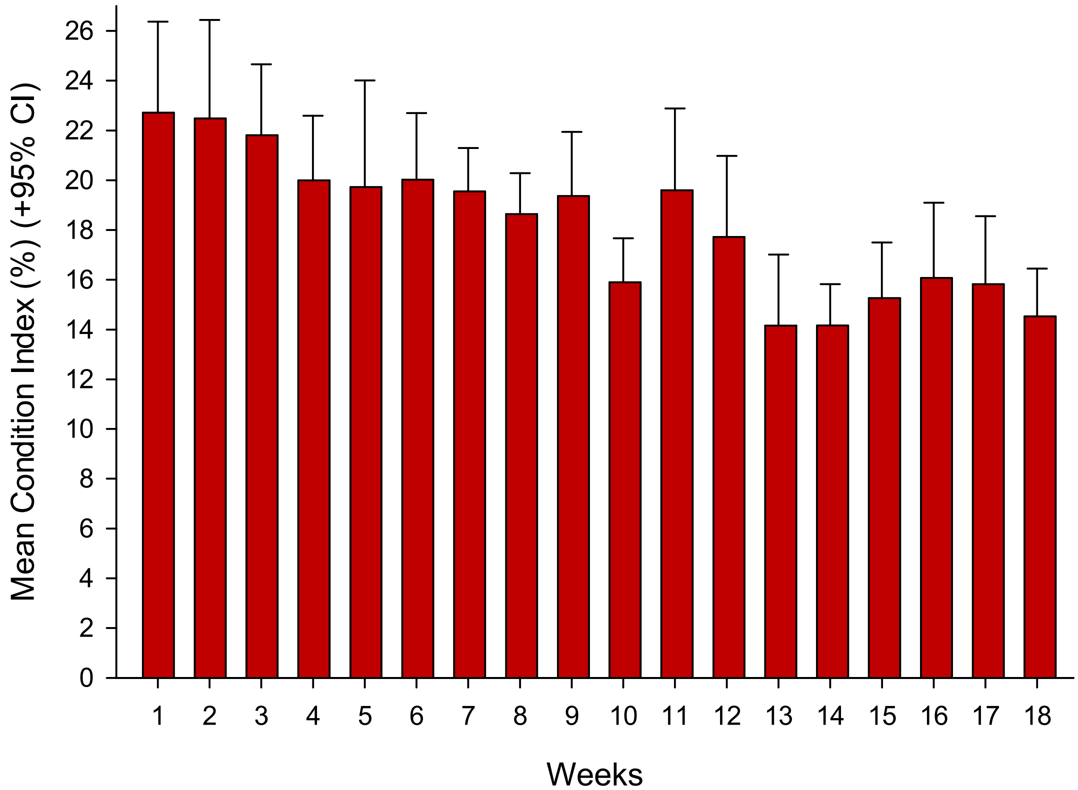

Mean Condition Index (%) (+95% CI)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks

Figure 71.

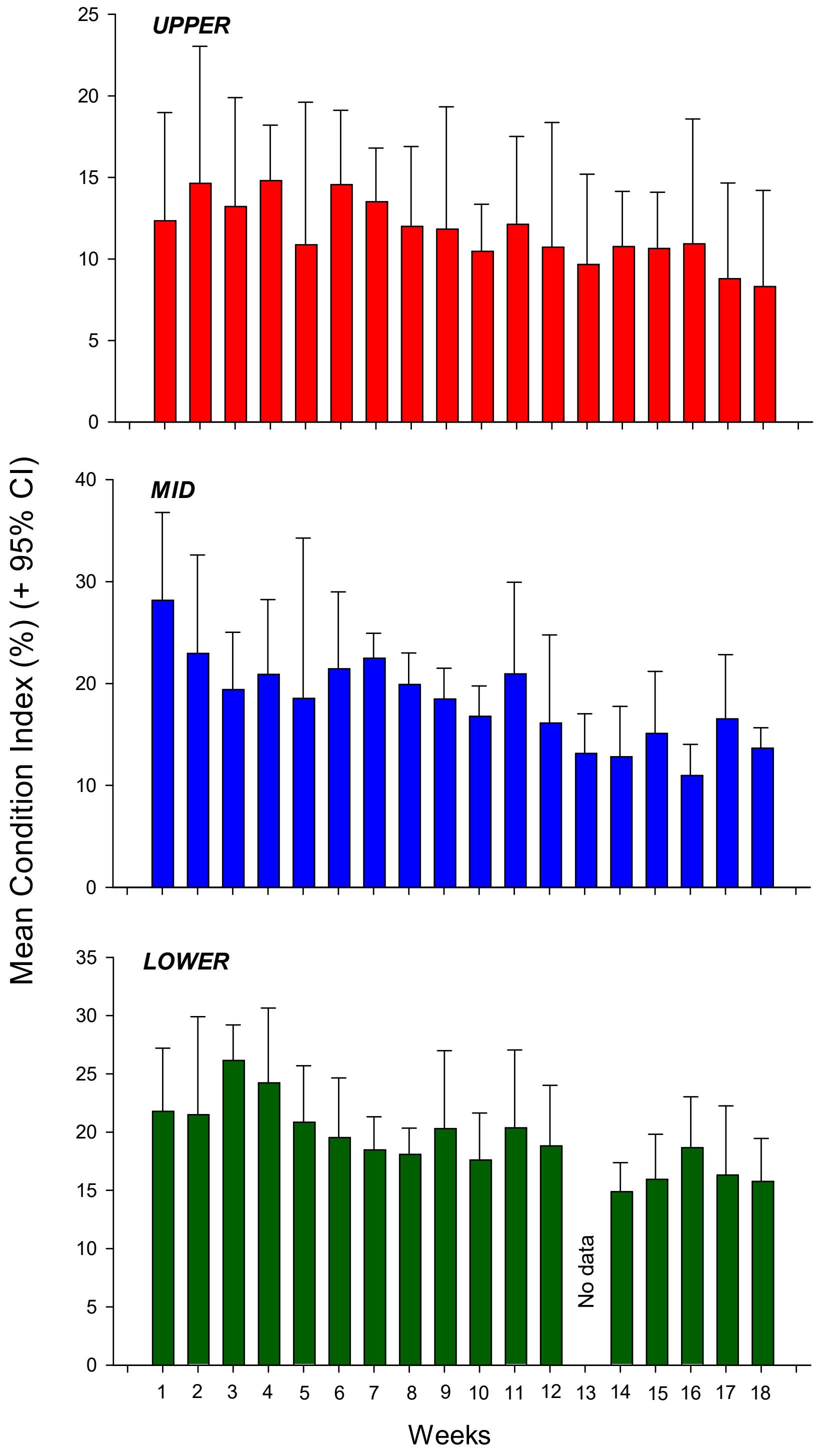
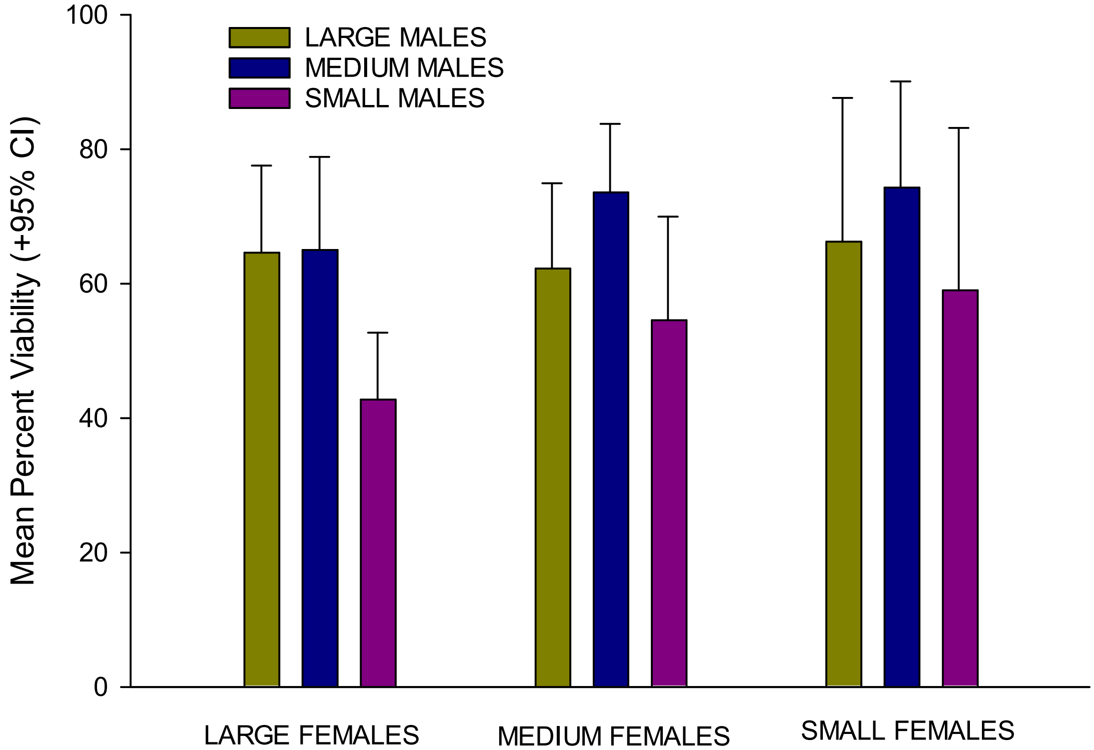

UPPER
MID
LOWER
Mean Condition Index (%) (+ 95% CI)
No data
Weeks
25
20
15
10
5
0
40
30
20
10
0
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Figure 72.


LARGE MALES
MEDIUM MALES
SMALL MALES
Mean Percent Viability (+95% CI)
100
80
60
40
20
0
LARGE FEMALES
MEDIUM FEMALES
SMALL FEMALES

Appendix A – Seawater temperatures (2024)

Figure Legends

Figure A-1. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) in an upper intertidal box at Flake Point Bar, Jonesport, Maine from 15 March to 13 August 2024 when the temperature logger failed to record additional data. (See Table 5 for dates and temperatures for minimum and maximum temperatures.)

Figure A-2. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) in a mid intertidal box at Flake Point Bar, Jonesport, Maine from 15 March to 22 May 2024 when the temperature logger failed to record additional data. (See Table 5 for dates and temperatures for minimum and maximum temperatures.)

Figure A-3. Difference in seawater temperature on each date and high tide (temperature inside box - outside mid intertidal box) at Flake Point Bar, Jonesport, Maine from 15 March to 22 May 2024, when the temperature logger inside the mid intertidal box failed to record additional data beyond that date. Mean difference was 0.128 ± 0.046°C, n = 132 (red dashed line). A one-sample t-test was performed to test the null hypothesis that the mean difference was equal to zero ($T_{obs} = 5.49$, df = 131, $P < 0.001$). This suggests that thermal conditions within the boxes were significantly warmer than outside the boxes. There was no significant relationship between date and mean difference (F = 2.53; df = 1, 130; P = 0.114).

Figure A-4. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) in a lower intertidal box at Flake Point Bar, Jonesport, Maine from 15 March to 6 July 2024 when the temperature logger failed to record additional data. (See Table 5 for dates and temperatures for minimum and maximum temperatures.)

Figure A-5. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) in an upper intertidal box at Narrows Flat, Bremen, Maine from 9 March to 25 September 2024). (See Table 5 for dates and temperatures for minimum and maximum temperatures.)

Figure A-6. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) in a mid-intertidal box at Narrows Flat, Bremen, Maine from 9 March to 25 September 2024). (See Table 5 for dates and temperatures for minimum and maximum temperatures.)

Figure A-7. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) outside of the mid intertidal box referred to in Fig. A-6 at Narrows Flat, Bremen, Maine from 9 March to 30 August 2024, when the data logger failed). (See Table 5 for dates and temperatures for minimum and maximum temperatures.)

Figure A-8. Difference in seawater temperature on each date and high tide (temperature inside box - outside mid intertidal box) at Narrows Flat, Bremen, Maine from 9 March to 25 September 2024). Mean difference was 0.491 ± 0.062ºC, n = 337. A one-sample t-test was performed to test the null hypothesis that the mean difference was equal to zero (T_{obs} = 13.26, df = 336, P < 0.001). This suggests that thermal conditions within the boxes were significantly warmer than outside the boxes. The red line represents the least-squares regression line ($\hat{Y}$ = 0.275 + 0.00162X, r^2 = 0.020, P = 0.009) and shows the rate of the mean difference increasing through time (blue lines represent the 95% confidence interval of $\hat{Y}$).

Figure A-9. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) in an upper intertidal box at Barnes Flat, Brunswick, Maine from 9 March to 30 September 2024. (See Table 5 for dates and temperatures for minimum and maximum temperatures.)

Figure A-10. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) in a mid intertidal box at Barnes Flat, Brunswick, Maine from 9 March to 8 April 2024, when the temperature logger failed to record additional data.

Figure A-11. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) outside and adjacent to the mid intertidal box referred to in Figure A-10 at Barnes Flat, Brunswick, Maine from 9 March to 23 September 2024. (See Table 5 for dates and temperatures for minimum and maximum temperatures.) Mean temperature inside vs. outside mid intertidal boxes was examined for the period in common (9 March to 8 April). The mean difference in temperature (ΔT = Inside temp - Outside temp) over that interval was 0.016 ± 0.0614 (n = 58), and this was not significantly different from zero (T_{obs} = 0.53, df = 57, P = 0.601).

Figure A-12. Mean seawater temperature during high tide recorded across three hourly intervals (1 hr before; 1 hr after; and, during high tide) in a lower intertidal box at Barnes Flat, Brunswick, Maine from 9 March to 5 August 2024 when the temperature logger failed to record additional data. (See Table 5 for dates and temperatures for minimum and maximum temperatures.)

Figure A-1.

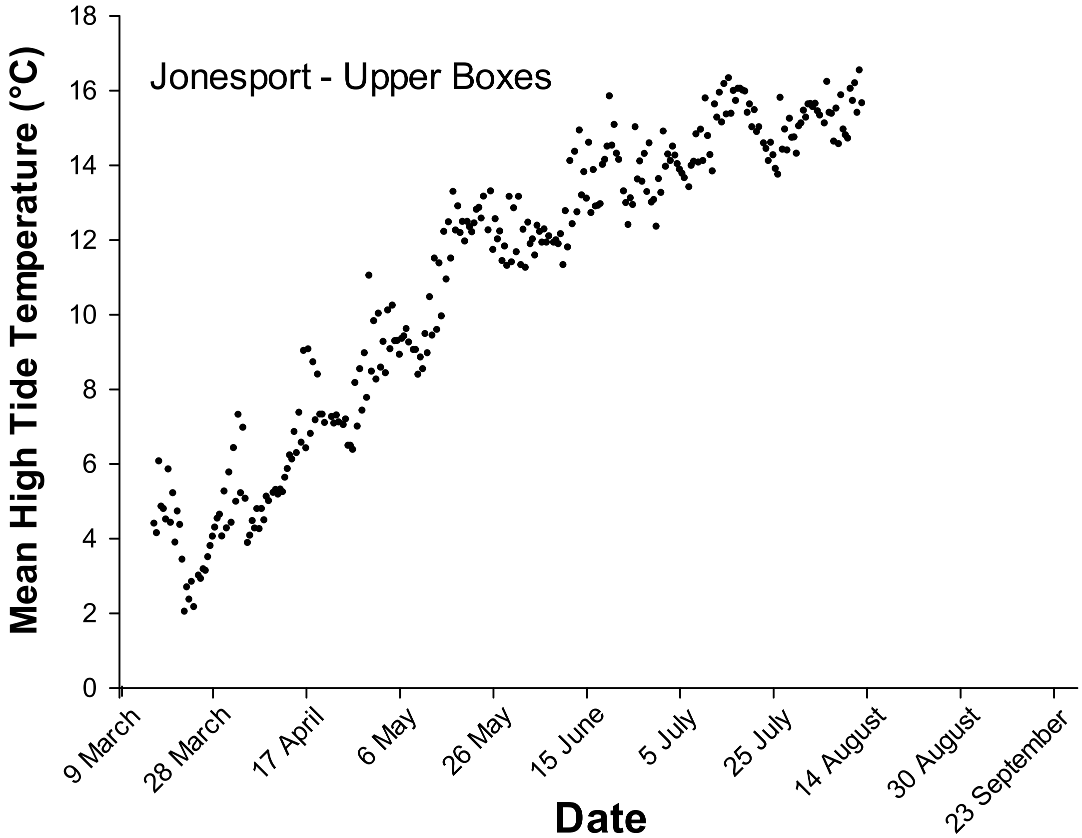

Jonesport - Upper Boxes
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

Figure A-2.

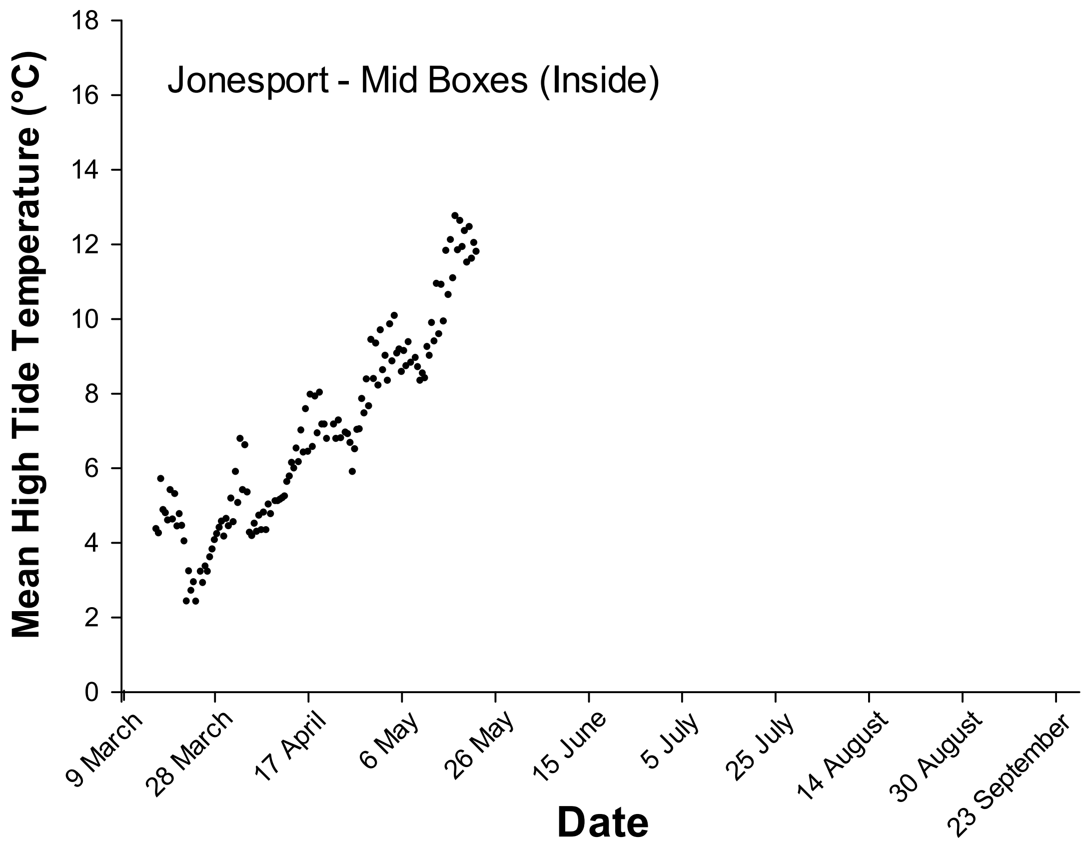

Jonesport - Mid Boxes (Inside)
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

Figure A-3.

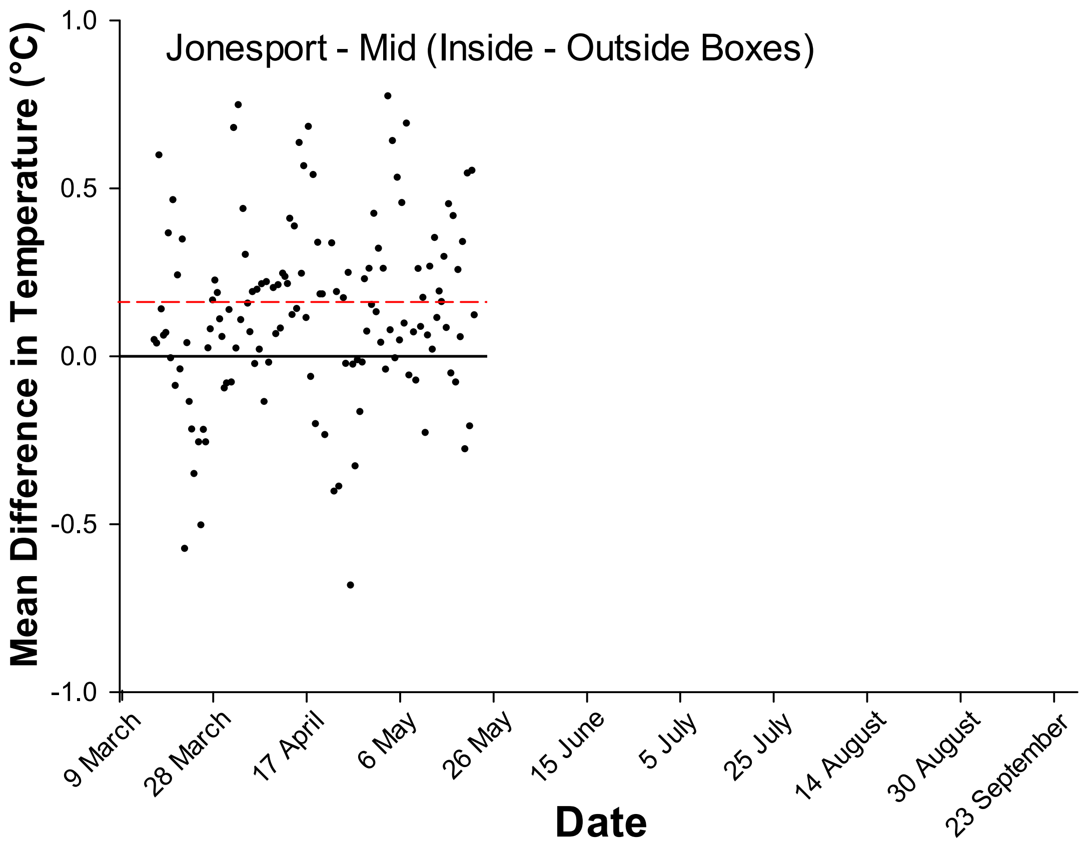

Jonesport - Mid (Inside - Outside Boxes)
Mean Difference in Temperature (°C)
1.0
0.5
0.0
-0.5
-1.0
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

Figure A-4.

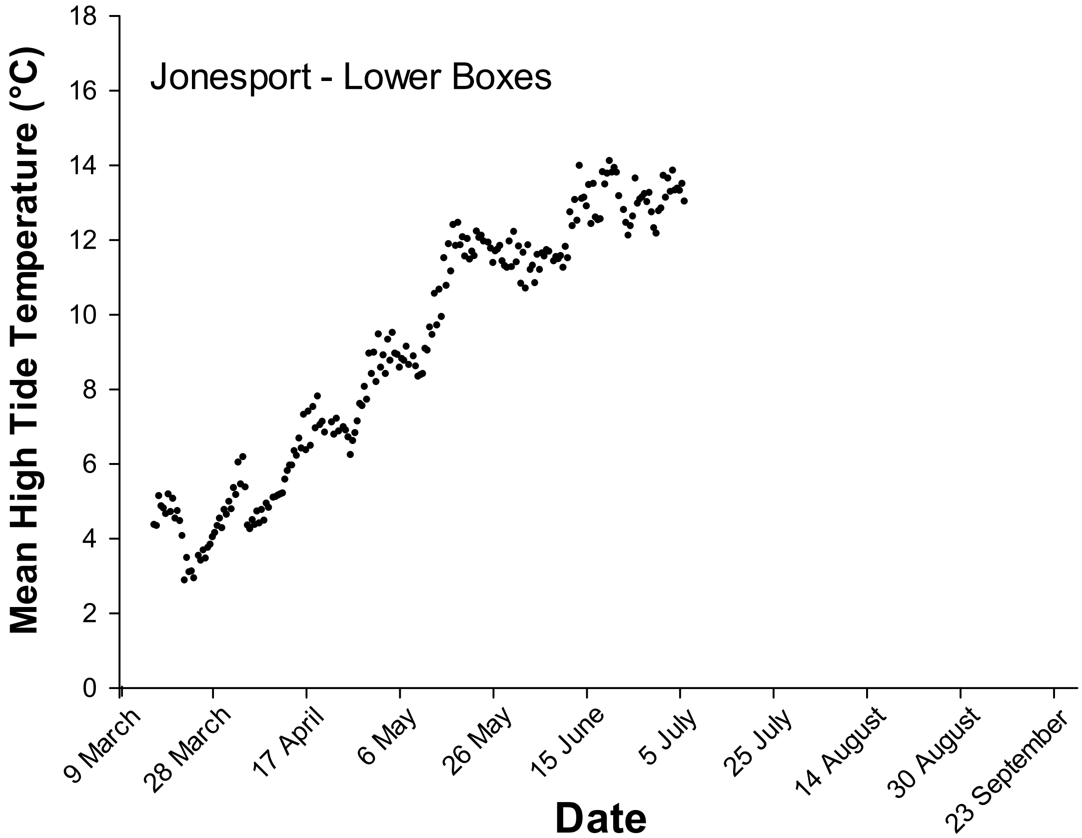

Jonesport - Lower Boxes
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

Figure A-5.

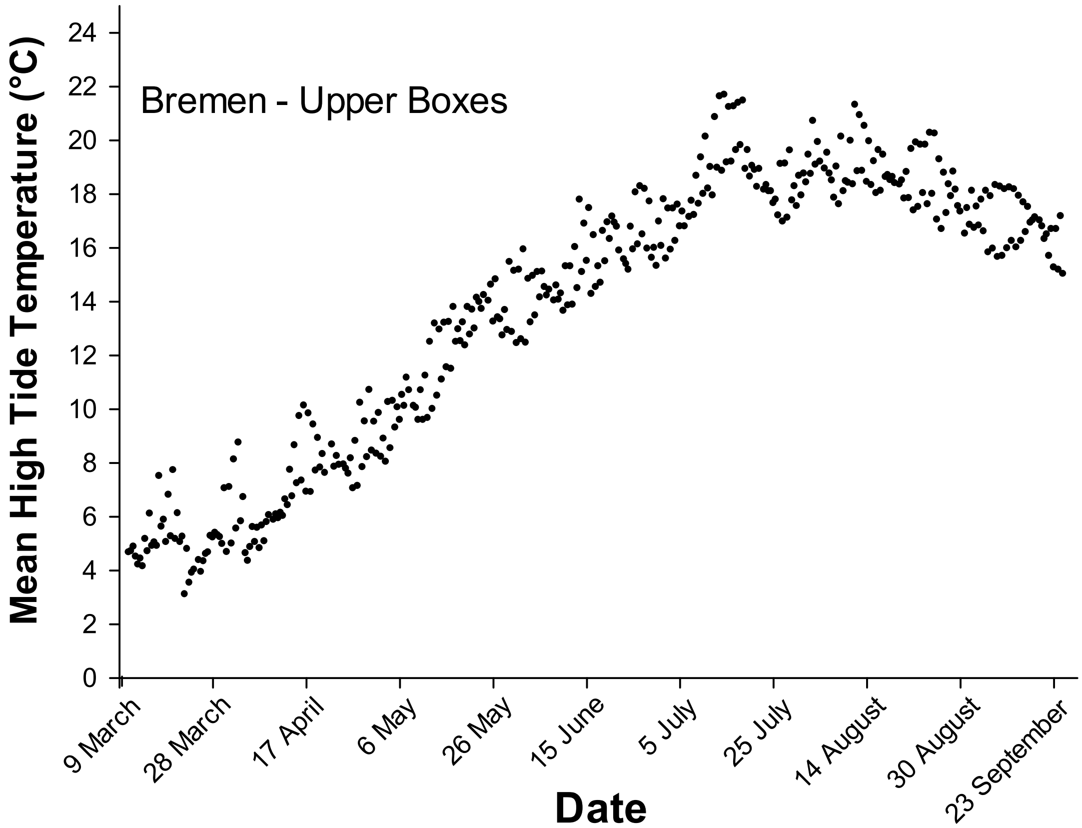

Bremen - Upper Boxes
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

Figure A-6.

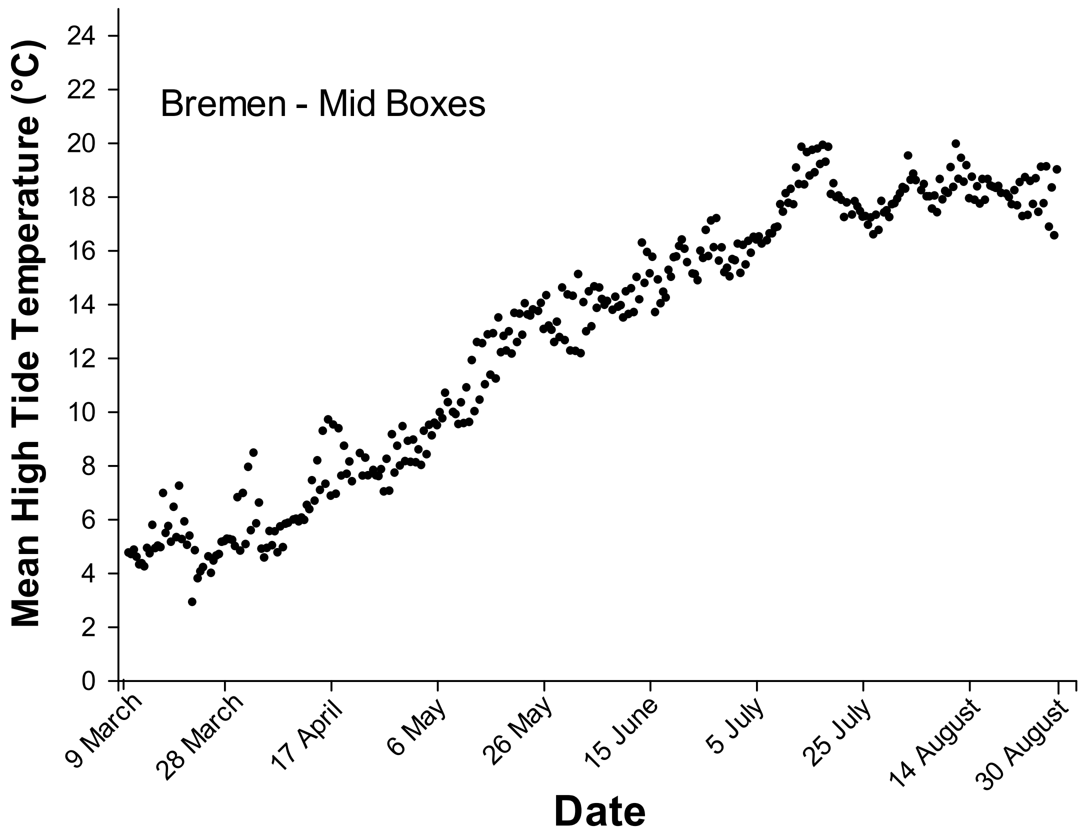

Bremen - Mid Boxes
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
Date

Figure A-7.

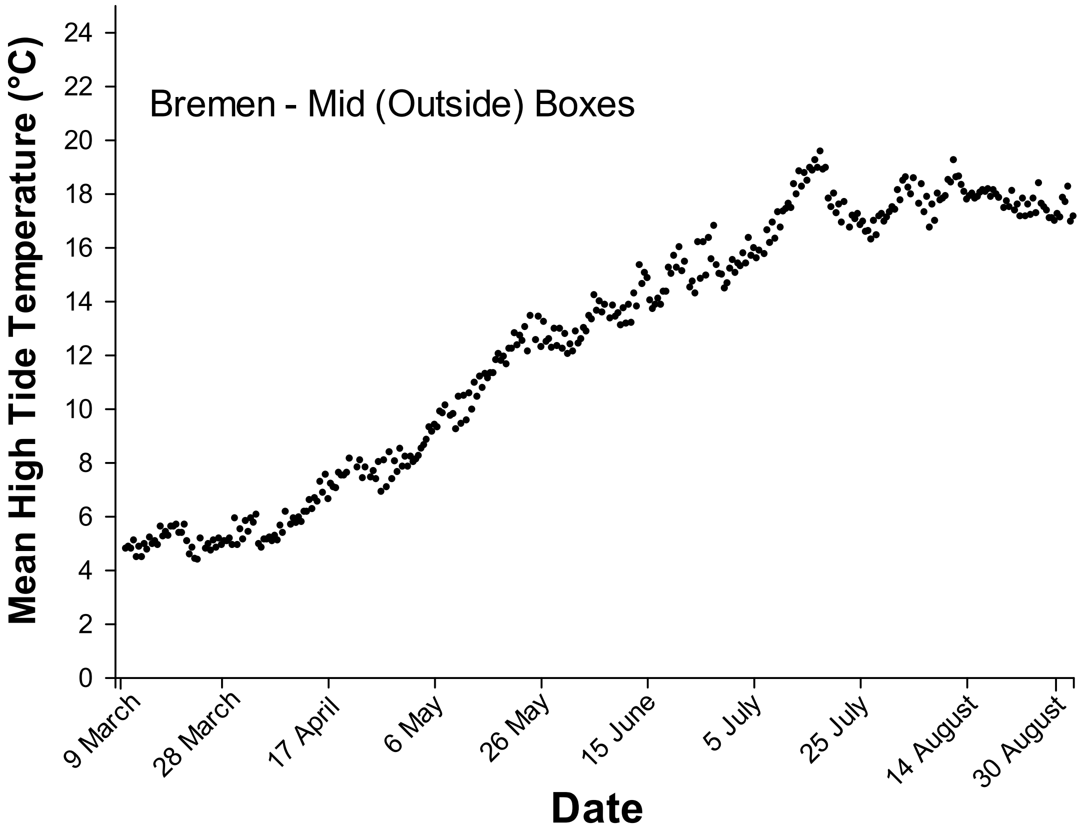

Bremen - Mid (Outside) Boxes
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
Date

Figure A-8.

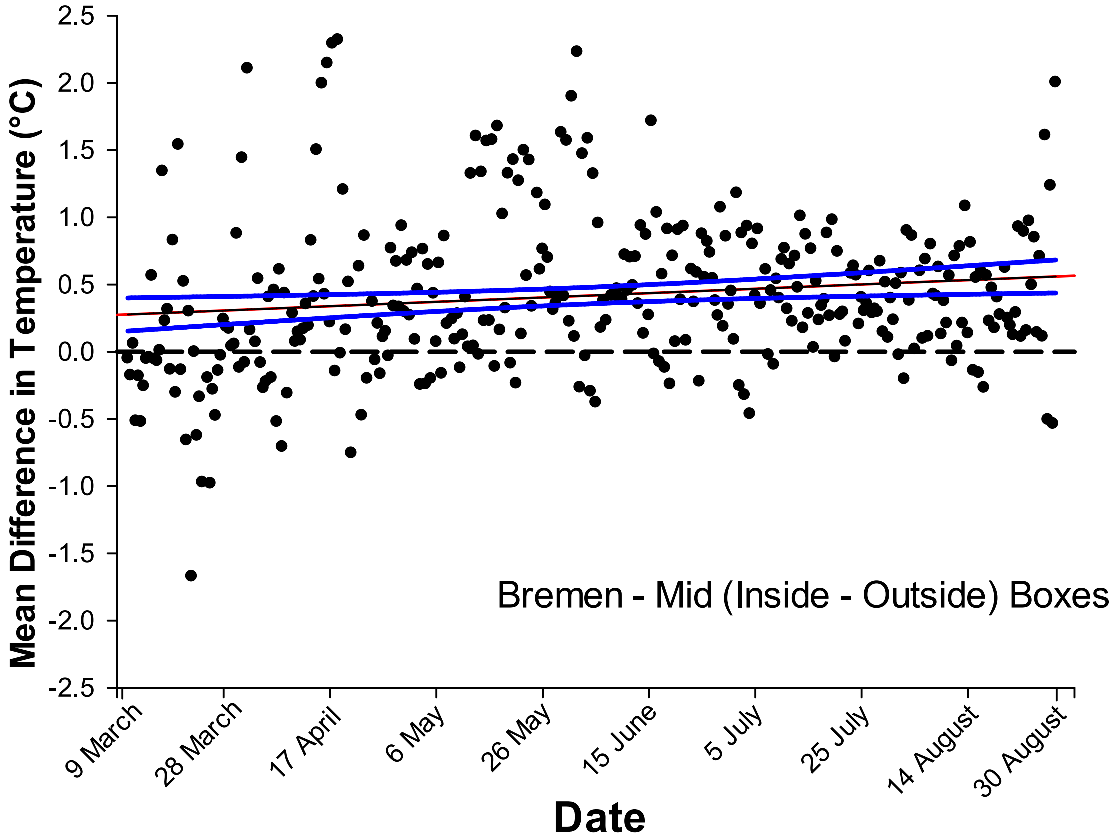

Mean Difference in Temperature (°C)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Bremen - Mid (Inside - Outside) Boxes
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
Date

Figure A-9.

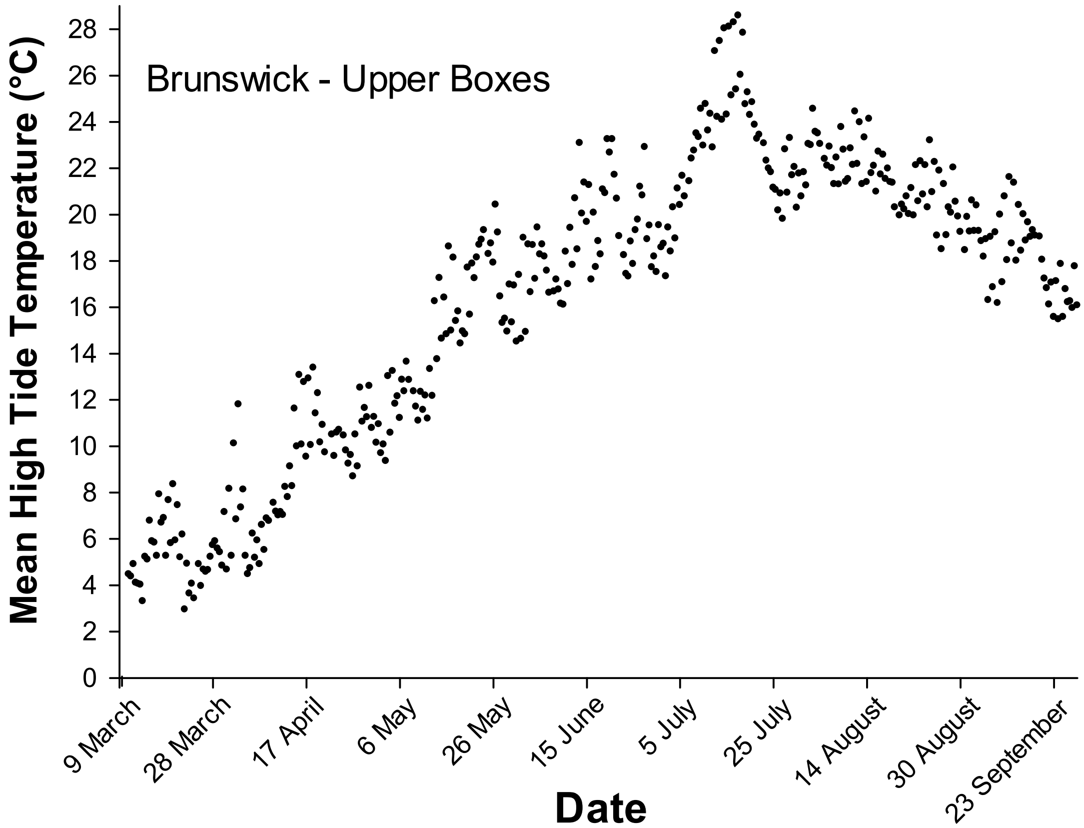

Brunswick - Upper Boxes
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

Figure A-10.

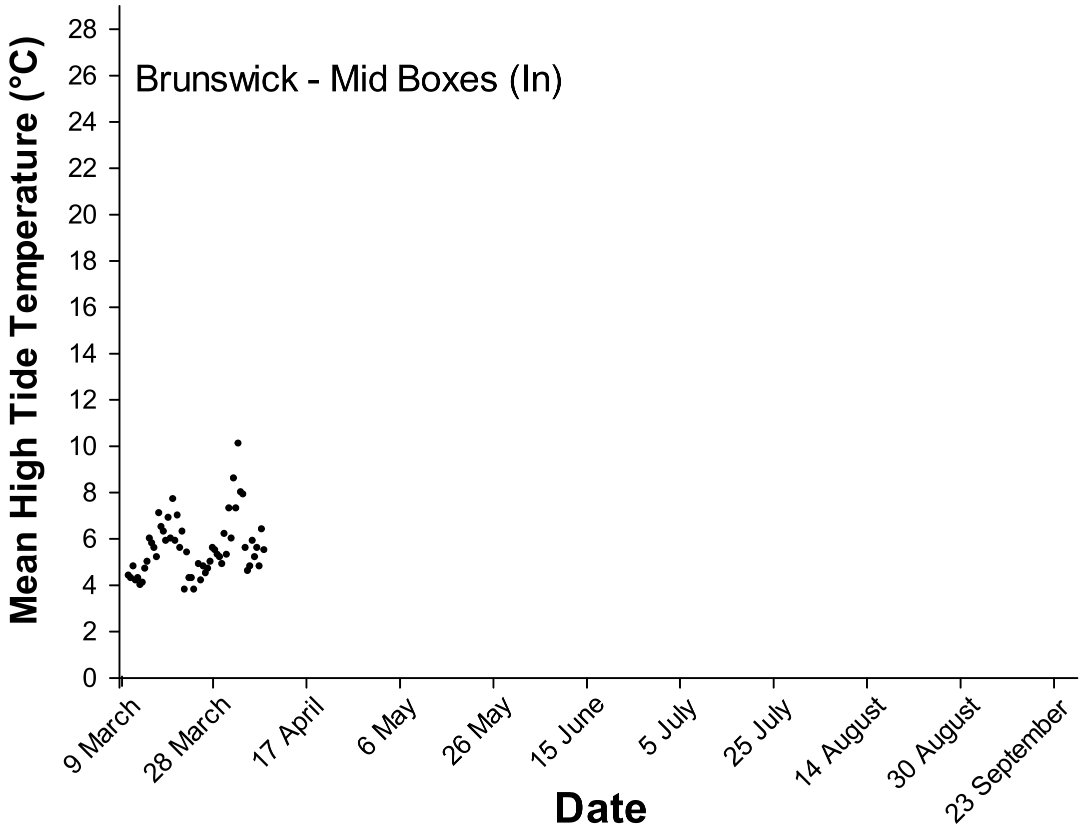

Brunswick - Mid Boxes (In)
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

Figure A-11.

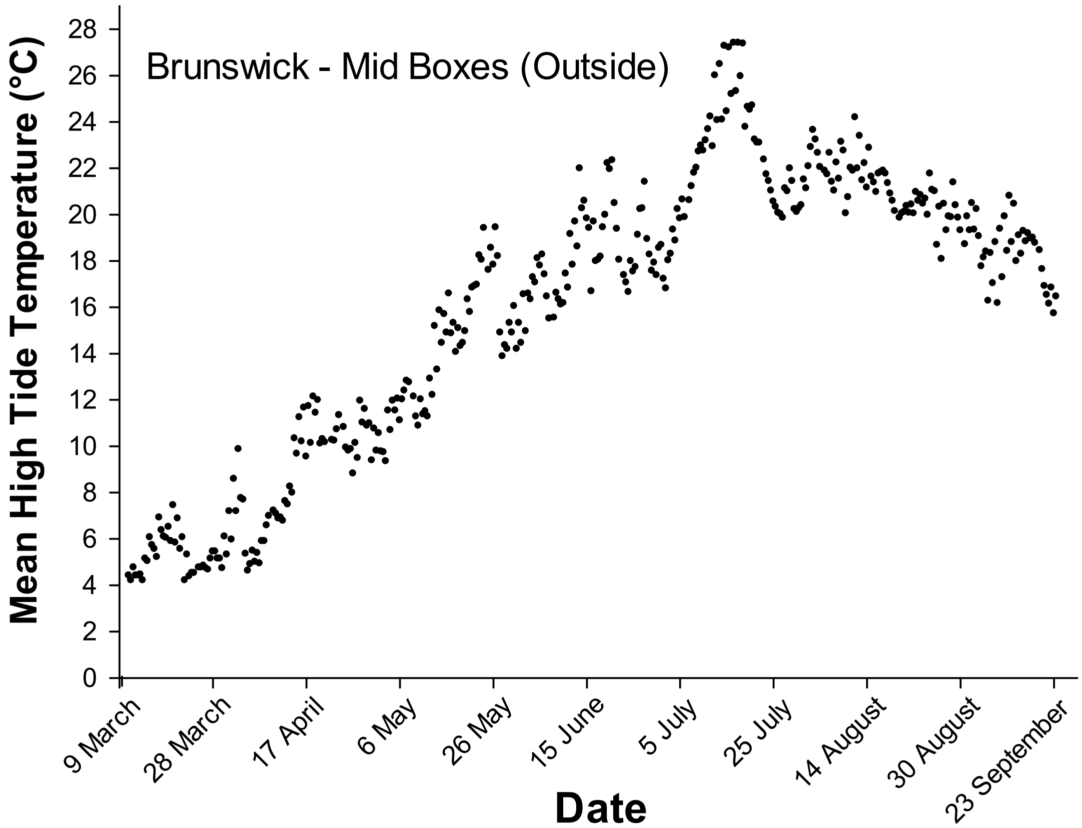
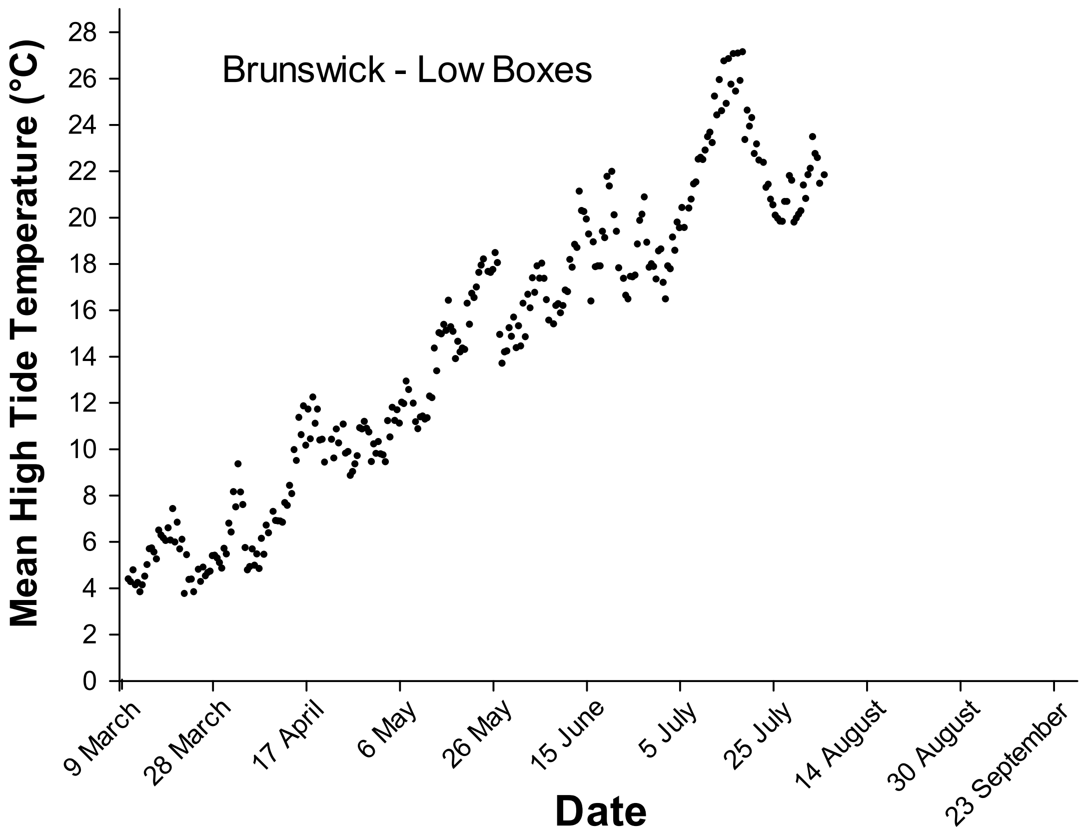

Brunswick - Mid Boxes (Outside)
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

Figure A-12.


Brunswick - Low Boxes
Mean High Tide Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
9 March
28 March
17 April
6 May
26 May
15 June
5 July
25 July
14 August
30 August
23 September
Date

APPENDIX B – Gametogenic cycle (2024)

Figure Legends

Figure B-1. Gonadal stages and the proportion of soft-shell clams within each stage across tidal height and time from Flake Point Bar, Jonesport, Maine in 2024.

Figure B-2. Mean seawater temperature (ºC) at high tide superimposed on the gonadal stages and proportion of soft-shell clams within each stage across tidal height and time from Flake Point Bar, Jonesport, Maine in 2024. (See Table 7 for dates when temperature loggers failed.)

Figure B-3. Jonesport, Maine (Flake Point Bar) 22 June 2024. Gonadal stages and the proportion of soft-shell clams within each stage in relation to three treatments: 1) Control (18 clams; 2024 tops; No PetScreen); 2) Treatment A (24 clams; PetScreen® insert [as in 2023] and a protective top that was used in 2023); 3) Treatment B (24 clams; No PetScreen® insert; 2024 top). Upper, Mid, and Lower refer to the tidal position where clams were held in 1-ft x 1-ft x 6-inch deep wooden boxes protruding 1-inch above the sediment surface.

Figure B-4. Jonesport, Maine (Flake Point Bar) 29 June 2024. Gonadal stages and the proportion of soft-shell clams within each stage in relation to three treatments described in the Figure B-4 legend. Upper, Mid, and Lower refer to the tidal position where clams were held in protected wooden boxes.

Figure B-5. Gonadal stages and the proportion of soft-shell clams within each stage across tidal height and time from Narrows Flat, Bremen, Maine in 2024.

Figure B-6. Mean seawater temperature (ºC) at high tide superimposed on the gonadal stages and proportion of soft-shell clams within each stage across tidal height and time from Narrows Flat, Bremen, Maine in 2024.

Figure B-7. Bremen, Maine (Narrows Flat) 3 June 2024. Gonadal stages and the proportion of soft-shell clams within each stage in relation to three treatments described in the Figure B-3 legend. Upper, Mid, and Lower refer to the tidal position where clams were held in protected wooden boxes.

Figure B-8. Bremen, Maine (Narrows Flat) 10 June 2024. Gonadal stages and the proportion of soft-shell clams within each stage in relation to three treatments described in the Figure B-4 legend. Upper, Mid, and Lower refer to the tidal position where clams were held in protected wooden boxes.

Figure B-9. Gonadal stages and the proportion of soft-shell clams within each stage across tidal height and time from Barnes Flat, Brunswick, Maine in 2024.

Figure B-10. Mean seawater temperature (ºC) at high tide superimposed on the gonadal stages and proportion of soft-shell clams within each stage across tidal height and time from Barnes Flat, Brunswick, Maine in 2024.

Figure B-11. Spawning stages (I, II, III, IV – see Table 7) and the proportion of soft-shell clams within each stage (pooling across tidal heights) for the first three and last three sampling dates at Barnes Flat, Brunswick, Maine during 2024. A Fisher's Exact Test using the first three sampling dates showed that spawning stage and date were independent (P = 0.375). A similar result was observed for the data across the last three sampling dates (P = 0.269). All data from the first three sampling dates were combined and compared with the data from the combined final sampling dates. This yielded a P-value of 0.111 suggesting that there was no significant change in the frequencies of clams within the spawning conditions over time.

Figure B-12. Brunswick, Maine (Barnes Flat) 14 May (upper intertidal only), 4 June, and 11 June 2024. Gonadal stages and the proportion of soft-shell clams within each stage in relation to three treatments described in the Figure B-4 legend. **M** and **L** refer to the mid and lower tidal position where clams were held in protected wooden boxes.

Figure B-1.

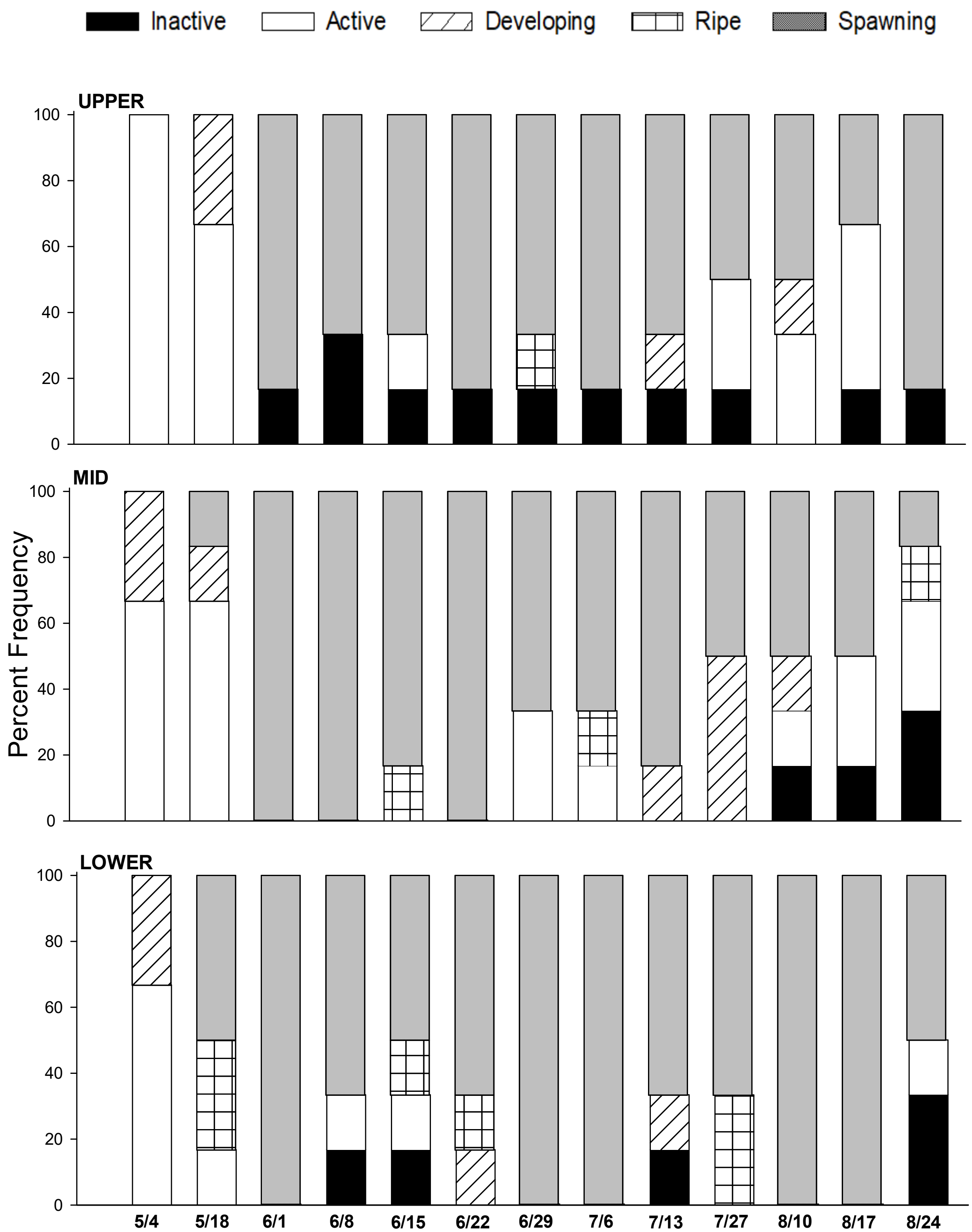

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
0
20
40
60
80
100
5/4
5/18
6/1
6/8
6/15
6/22
6/29
7/6
7/13
7/27
8/10
8/17
8/24

Figure B-2.

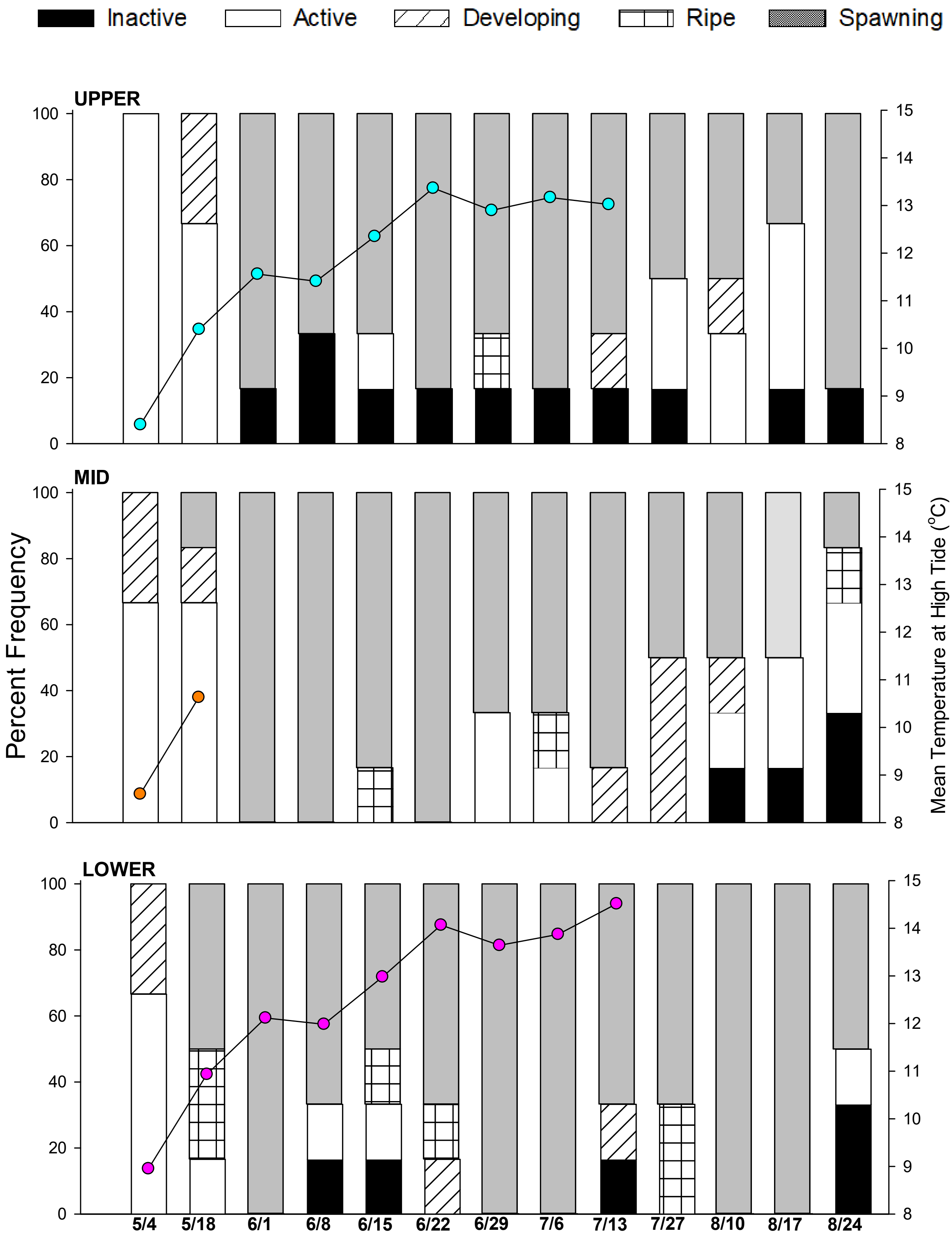

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
Mean Temperature at High Tide (°C)
100
80
60
40
20
0
15
14
13
12
11
10
9
8
5/4
5/18
6/1
6/8
6/15
6/22
6/29
7/6
7/13
7/27
8/10
8/17
8/24

Figure B-3.

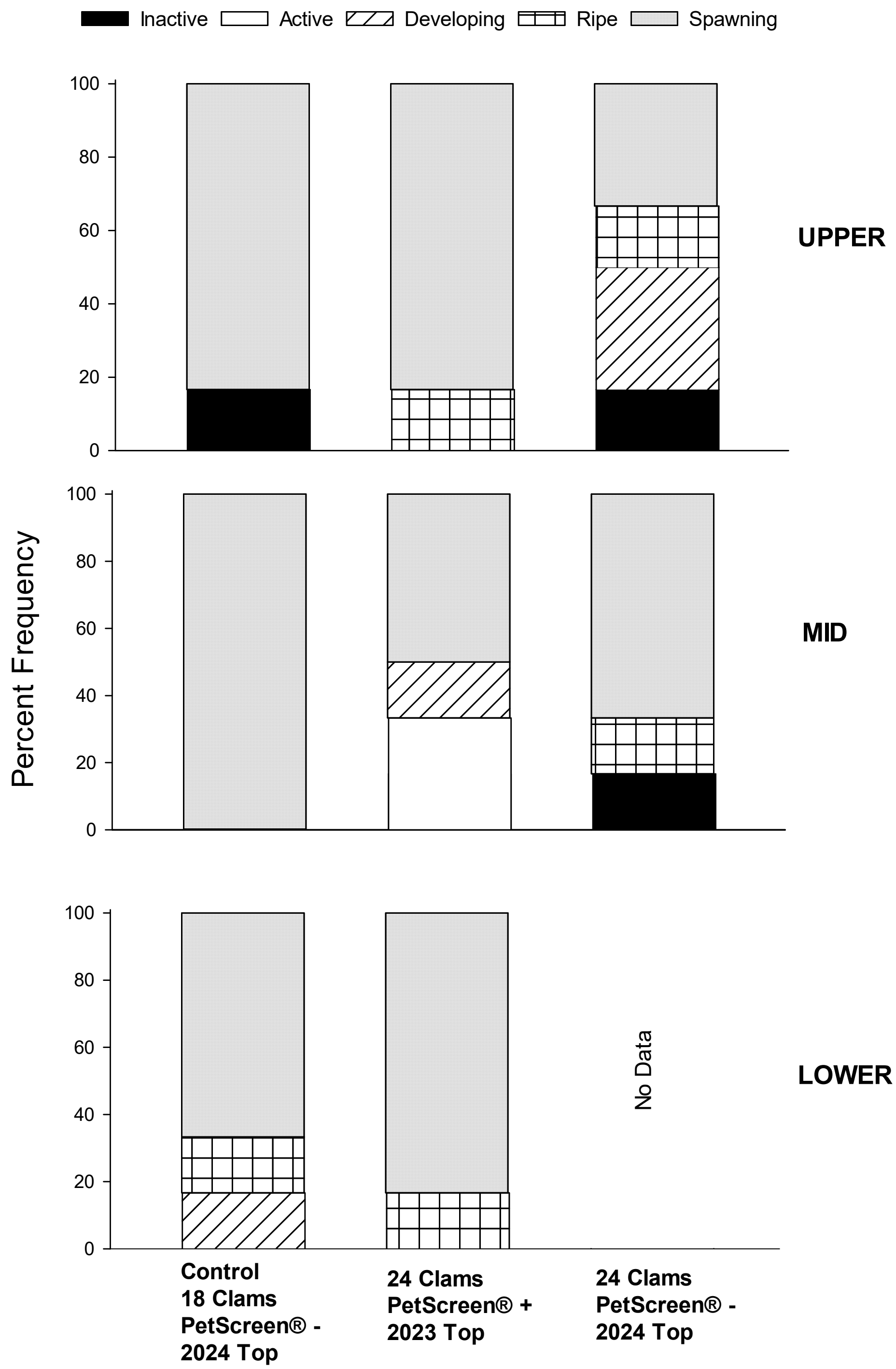

Inactive
Active
Developing
Ripe
Spawning
Percent Frequency
100
80
60
40
20
0
UPPER
MID
LOWER
No Data
Control
18 Clams
PetScreen® -
2024 Top
24 Clams
PetScreen® +
2023 Top
24 Clams
PetScreen® -
2024 Top

Figure B-4.

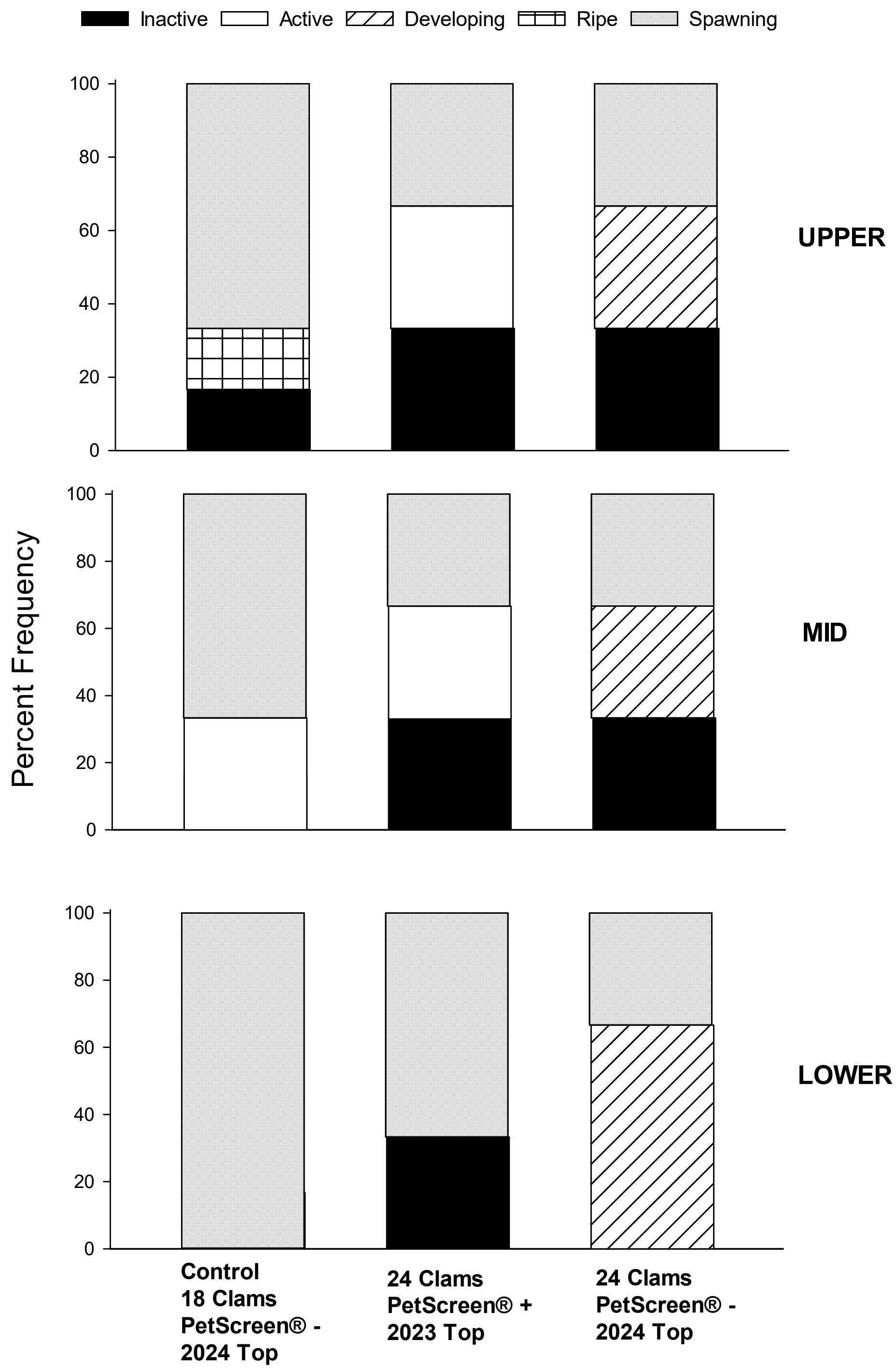

Inactive
Active
Developing
Ripe
Spawning
Percent Frequency
0
20
40
60
80
100
UPPER
MID
LOWER
Control
18 Clams
PetScreen® -
2024 Top
24 Clams
PetScreen® +
2023 Top
24 Clams
PetScreen® -
2024 Top

Figure B-5.

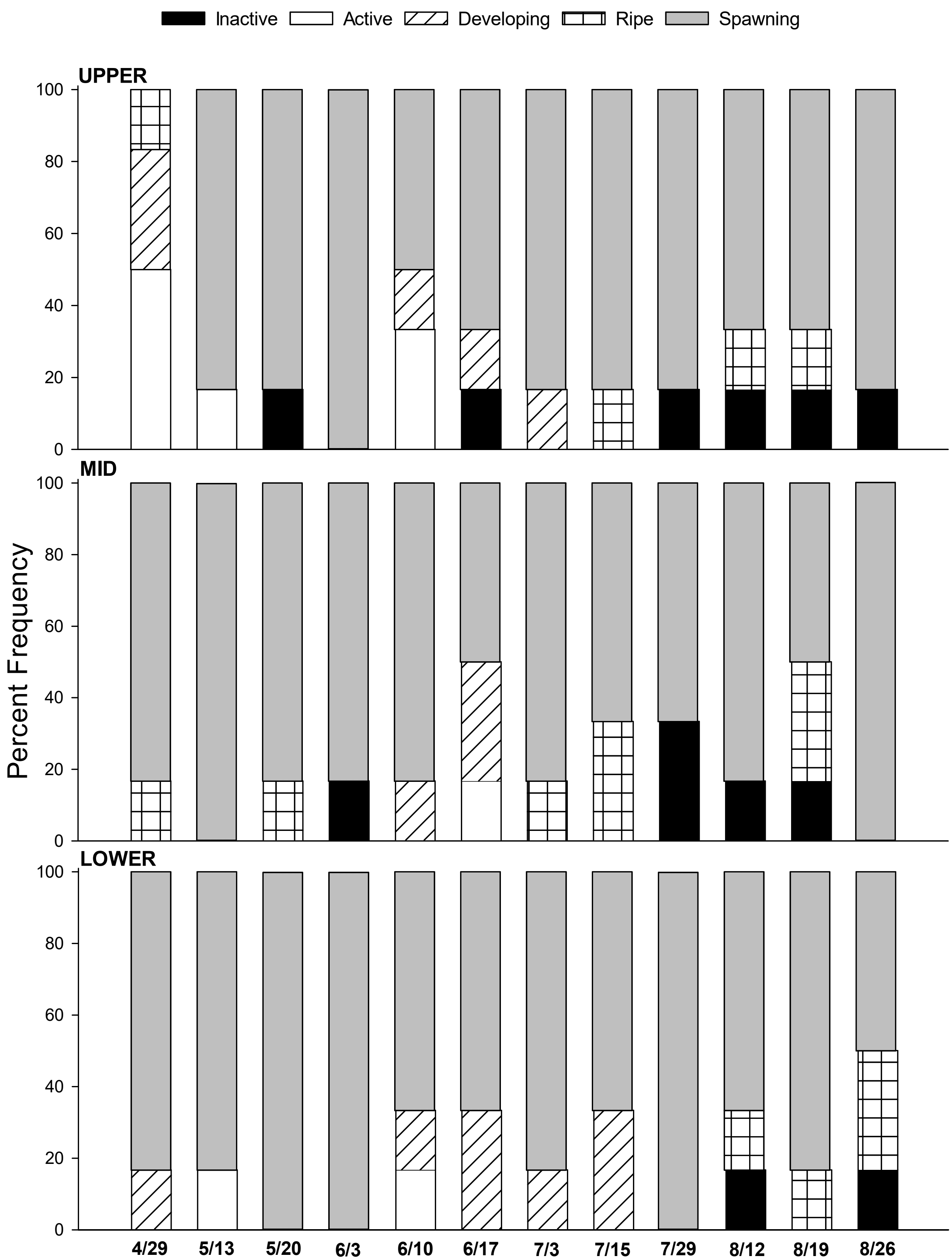

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
0
20
40
60
80
100
4/29
5/13
5/20
6/3
6/10
6/17
7/3
7/15
7/29
8/12
8/19
8/26

Figure B-6.

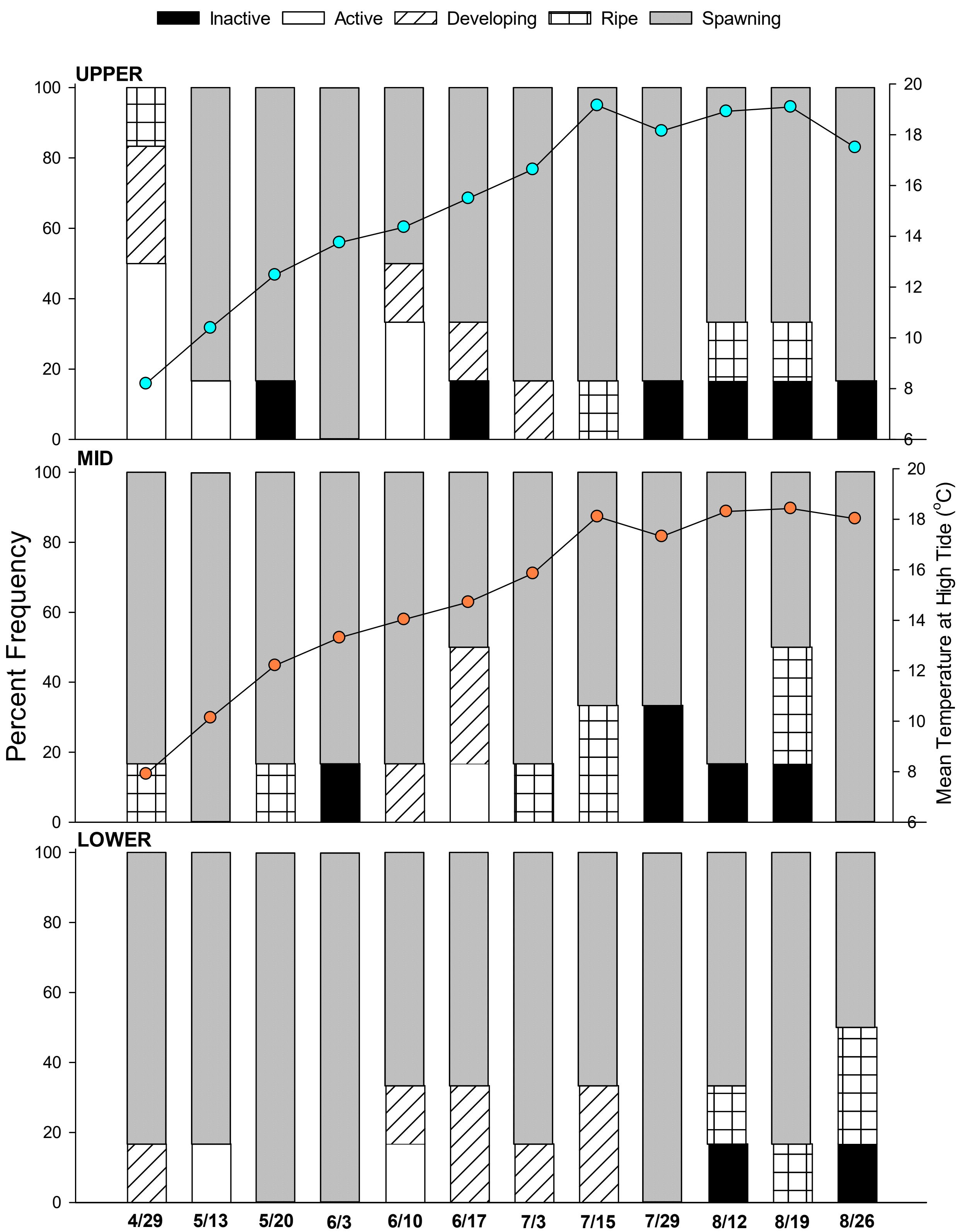

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
Mean Temperature at High Tide (°C)
0
20
40
60
80
100
6
8
10
12
14
16
18
20
4/29
5/13
5/20
6/3
6/10
6/17
7/3
7/15
7/29
8/12
8/19
8/26

Figure B-7.

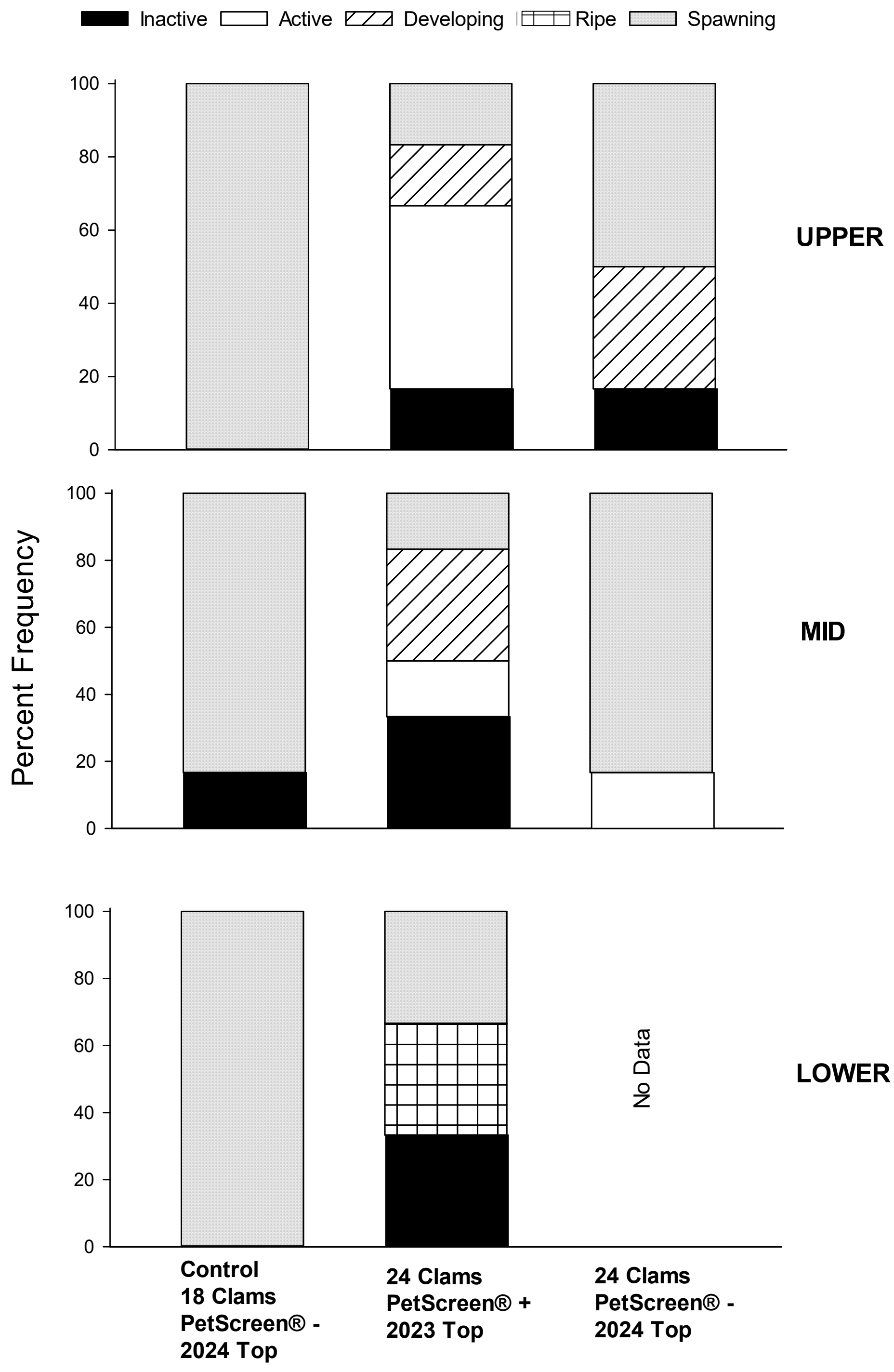

Inactive
Active
Developing
Ripe
Spawning
Percent Frequency
100
80
60
40
20
0
UPPER
MID
LOWER
No Data
Control
18 Clams
PetScreen® -
2024 Top
24 Clams
PetScreen® +
2023 Top
24 Clams
PetScreen® -
2024 Top

Figure B-8

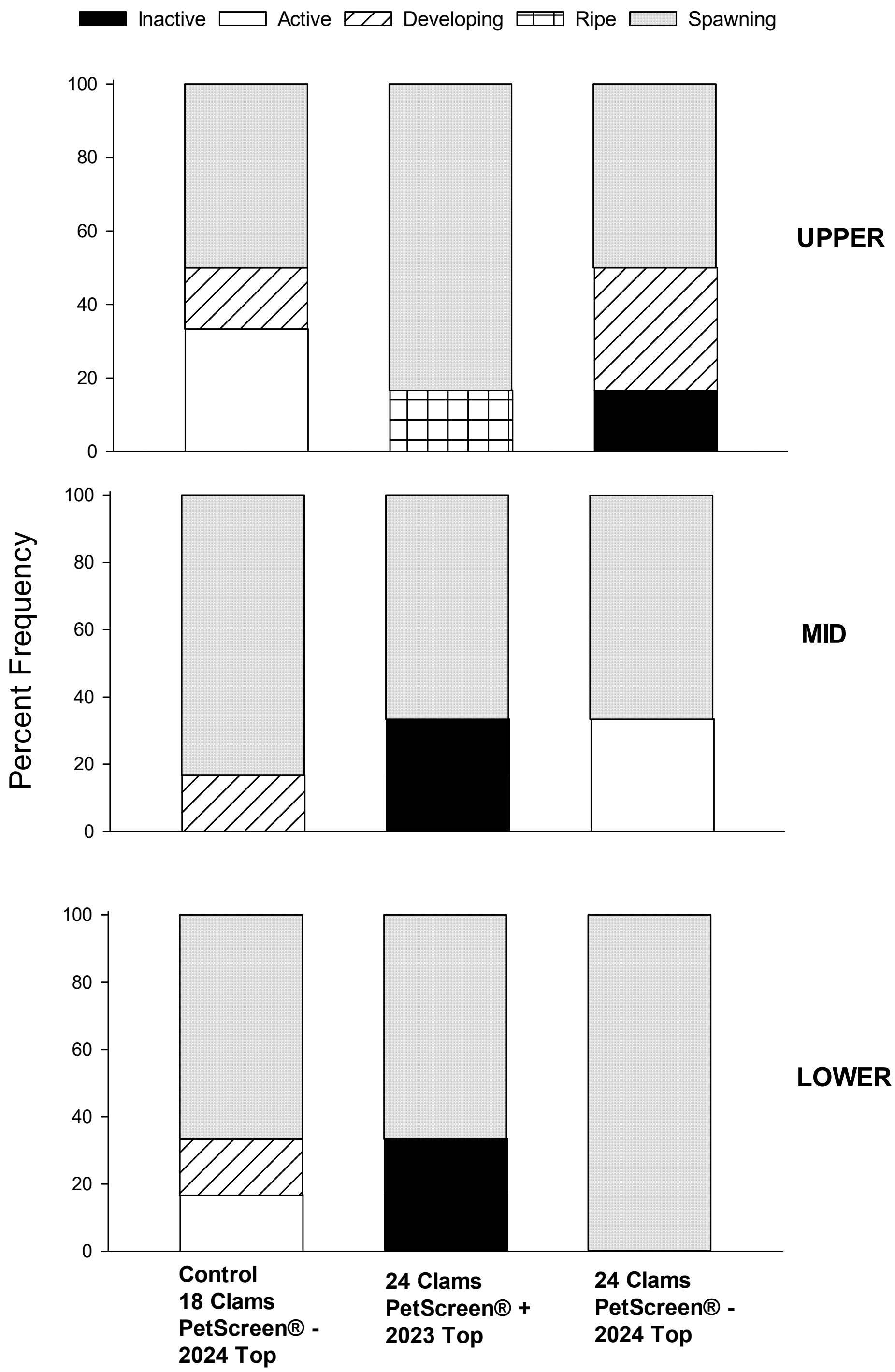

Inactive
Active
Developing
Ripe
Spawning
Percent Frequency
100
80
60
40
20
0
UPPER
MID
LOWER
Control
18 Clams
PetScreen® -
2024 Top
24 Clams
PetScreen® +
2023 Top
24 Clams
PetScreen® -
2024 Top

Figure B-9.

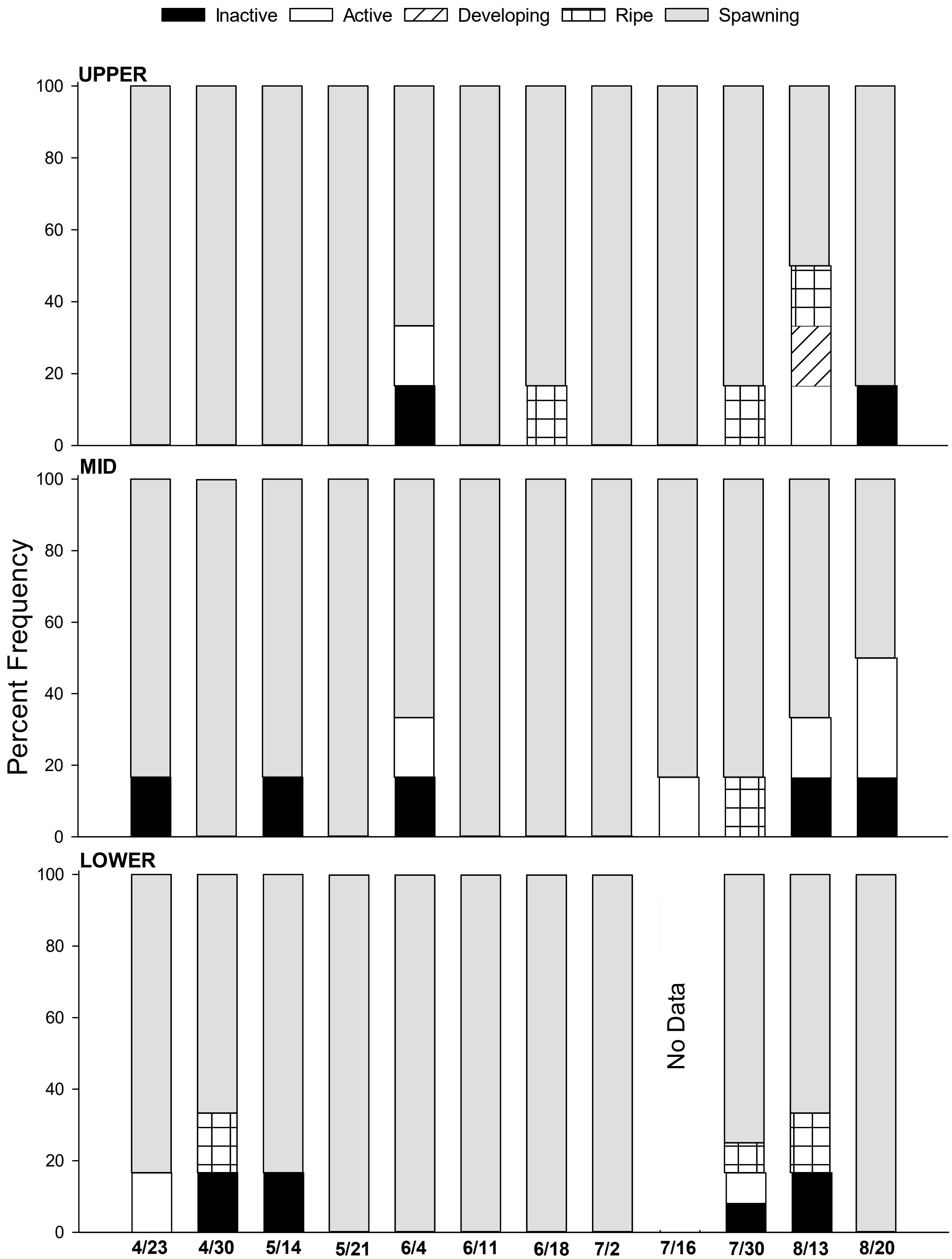

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
0
20
40
60
80
100
No Data
4/23
4/30
5/14
5/21
6/4
6/11
6/18
7/2
7/16
7/30
8/13
8/20

Figure B-10.

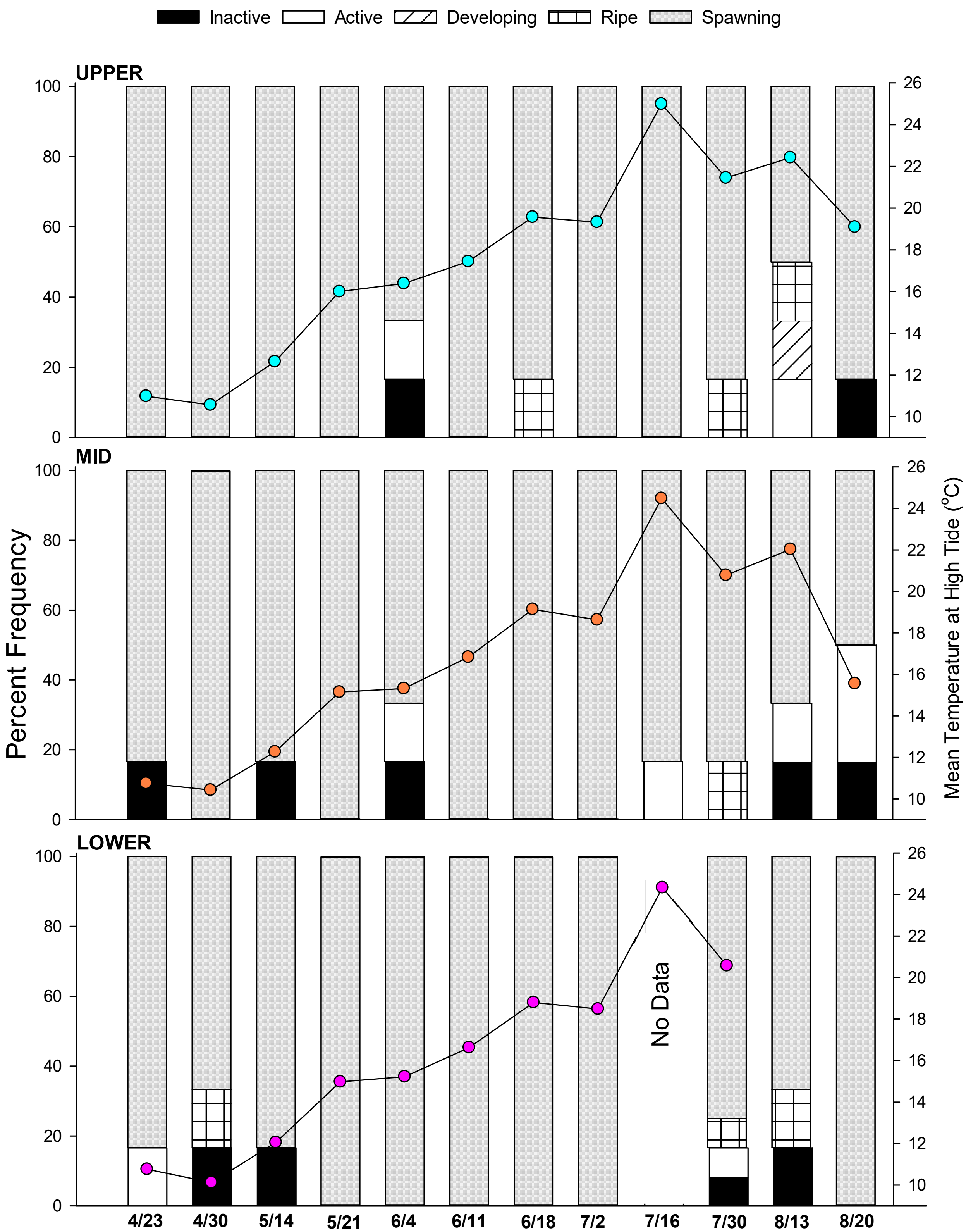

Inactive
Active
Developing
Ripe
Spawning
UPPER
MID
LOWER
Percent Frequency
Mean Temperature at High Tide (°C)
No Data
0
20
40
60
80
100
10
12
14
16
18
20
22
24
26
4/23
4/30
5/14
5/21
6/4
6/11
6/18
7/2
7/16
7/30
8/13
8/20

Figure B-11.

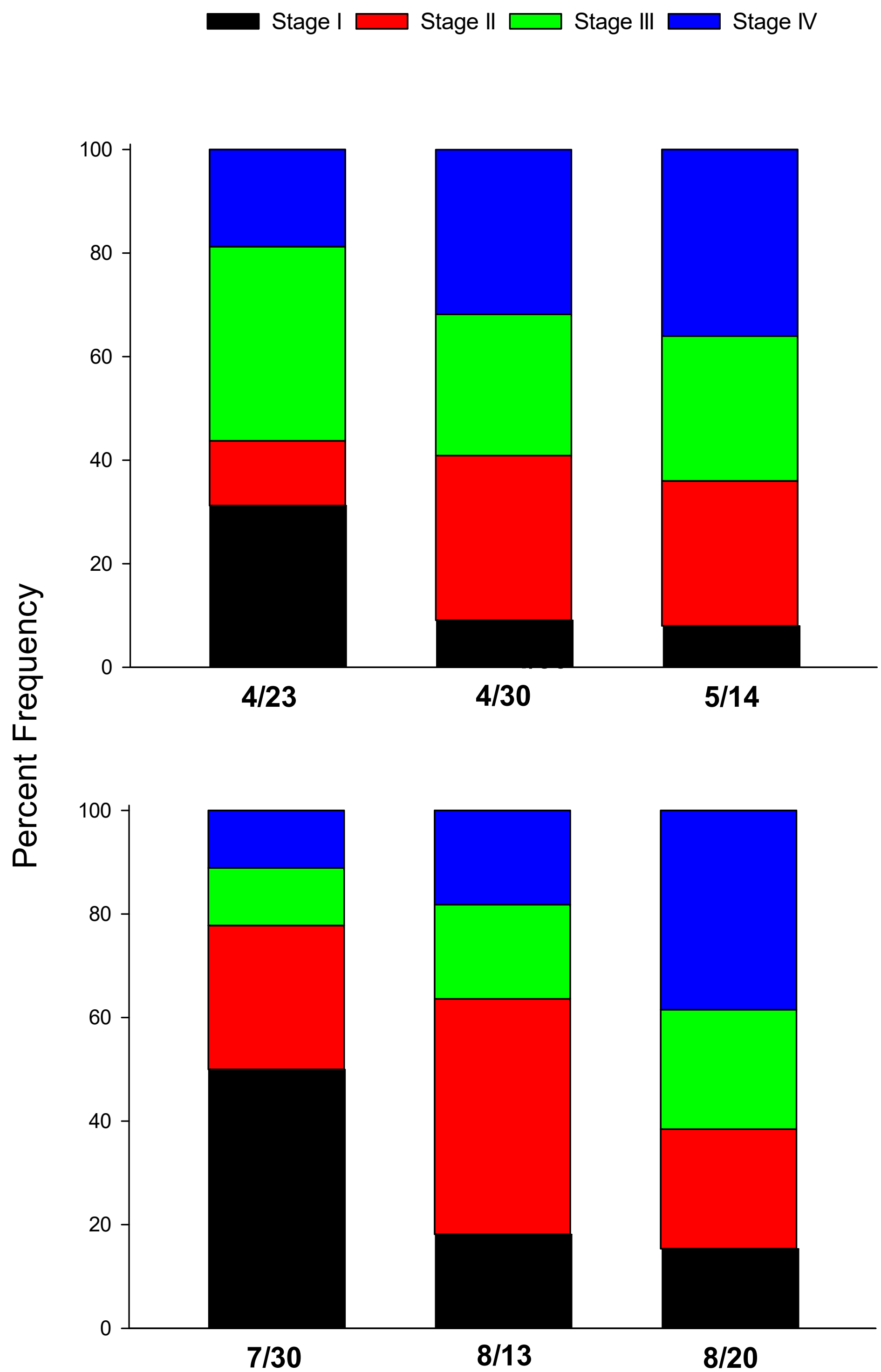
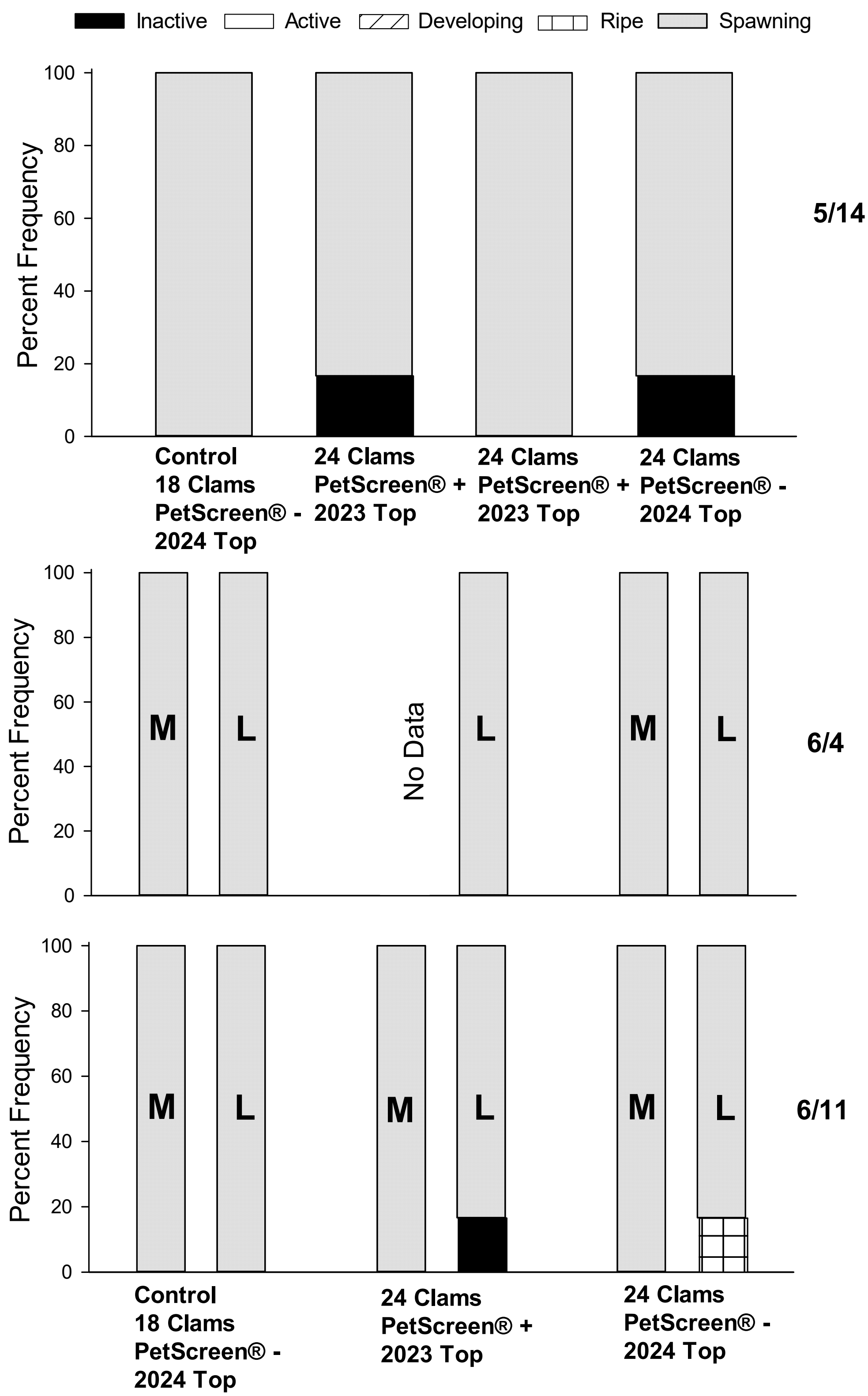

Stage I
Stage II
Stage III
Stage IV
Percent Frequency
100
80
60
40
20
0
4/23
4/30
5/14
7/30
8/13
8/20

Figure B-12.


Inactive
Active
Developing
Ripe
Spawning
Percent Frequency
0
20
40
60
80
100
5/14
Control
18 Clams
PetScreen® -
2024 Top
24 Clams
PetScreen® +
2023 Top
24 Clams
PetScreen® +
2023 Top
24 Clams
PetScreen® -
2024 Top
M
L
No Data
L
M
L
6/4
M
L
M
L
M
L
6/11
Control
18 Clams
PetScreen® -
2024 Top
24 Clams
PetScreen® +
2023 Top
24 Clams
PetScreen® -
2024 Top