

The University of Maine

DigitalCommons@UMaine

---

Miscellaneous Publications

ARI Publications

---

Spring 3-19-2019

## How acidic sediments and seawater affect interactive effects of predation on survival, growth, and recruitment of wild and cultured soft-shell clams, *Mya arenaria* L., along a tidal gradient at two intertidal sites in eastern Maine

Brian F. Beal

William Otto

Follow this and additional works at: [https://digitalcommons.library.umaine.edu/ari\\_miscpubs](https://digitalcommons.library.umaine.edu/ari_miscpubs)



Part of the [Aquaculture and Fisheries Commons](#), [Biology Commons](#), [Climate Commons](#), [Environmental Monitoring Commons](#), [Natural Resources Management and Policy Commons](#), [Sedimentology Commons](#), and the [Sustainability Commons](#)

---

This Report is brought to you for free and open access by DigitalCommons@UMaine. It has been accepted for inclusion in Miscellaneous Publications by an authorized administrator of DigitalCommons@UMaine. For more information, please contact [um.library.technical.services@maine.edu](mailto:um.library.technical.services@maine.edu).

**How acidic sediments and seawater affect interactive effects  
of predation on survival, growth, and recruitment of wild  
and cultured soft-shell clams, *Mya arenaria* L., along a tidal  
gradient at two intertidal sites in eastern Maine**

**Final Report to SEANET  
Sustainable Ecological Aquaculture Network**

**Brian F. Beal & William Otto  
University of Maine at Machias**

**19 February 2019**

## Abstract

We investigated how cultured and wild individuals of the soft-shell clam, *Mya arenaria*, react to acidic seawater and sediments along a tidal gradient at two intertidal sites in eastern Maine – one in the town of Cutler (Duck Brook Flat), one in Machiasport (Larrabee Cove). In June 2018, we initiated two comparative experiments at each intertidal flat near the upper, mid, and lower intertidal to assess the importance of predation in regulating clam populations. For the first, experimental units (EU; plastic plant pots 15 cm diameter x 15 cm deep) were filled with ambient sediments and arrayed in a 2 x 5 matrix at three blocks (25 m apart) at each tidal height. One-half of the EU in each block deterred predators with a fine mesh screening (aperture = 1.7mm x 0.9 mm), while the other half served as controls without predator exclusion screening. Twenty-four cultured individuals of *Mya* (mean shell length, SL = 10.6 mm) were added to each EU. Survival and absolute shell growth were measured for the cultured clams in each EU, as were number of wild, 0-year class recruits of *Mya*. Water and sediment pH, total alkalinity, and temperature were used to calculate aragonite saturation state ( $\Omega_{\text{aragonite}}$ ) at both sites on several occasions during the 8-month study. The second study also examined wild recruitment but used empty wooden boxes lined with the same fine mesh screening used to deter predators from the smaller EU. Boxes were arrayed in three blocks of five at each tidal height at each site. Upper and mid intertidal EU and boxes were collected in late October or mid-November 2018, and the contents of each washed through a 1 mm sieve that was followed by enumerating and measuring both cultured and wild recruits. EU and boxes were collected in mid-January (DBF) or mid-February (LC) 2019, and the contents of each processed similarly.

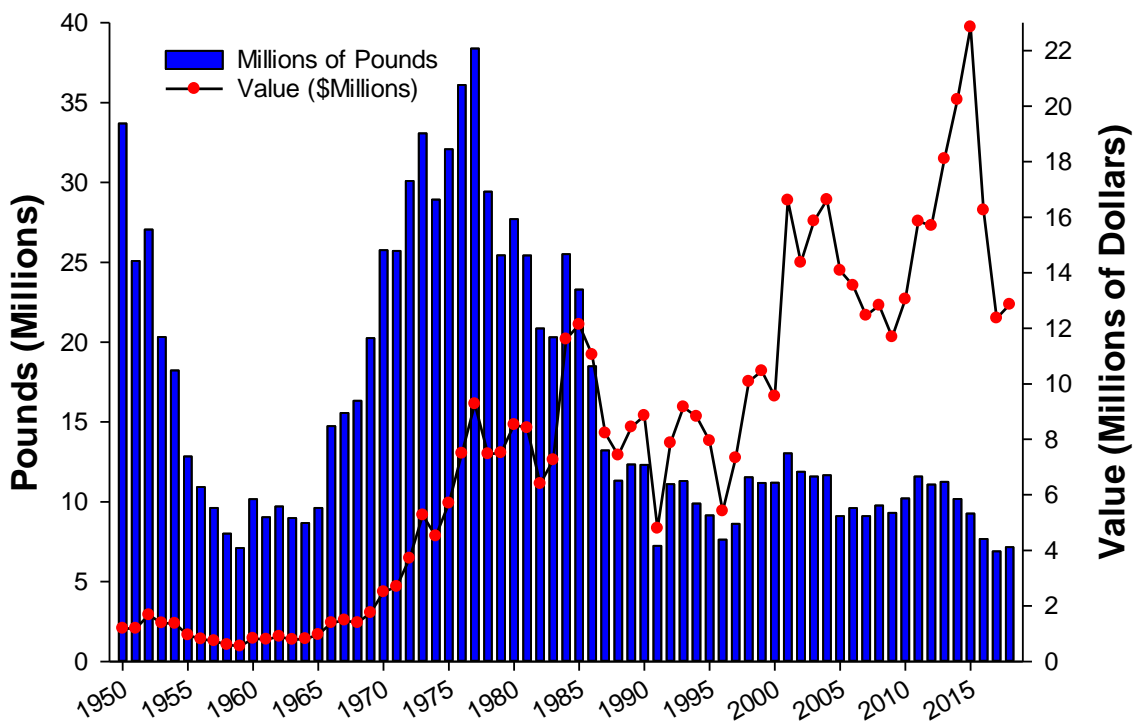
Both seawater and sediments samples showed that a highly acidic environment occurred across both sites, tidal heights, and sampling dates. Combining all samples, mean pH at DBF and LC was 7.11 and 7.43, respectively, while mean  $\Omega_{\text{aragonite}}$  was 0.33 at both sites. Despite this corrosive environment, cultured clams responded to predation threats (mostly from green crabs, *Carcinus maenas*) as expected based on similar studies conducted in eastern Maine in 2003. Mean survival of clams in protected EU, regardless of tidal height and site, was 60% vs. 10% in controls. Growth rate did not vary significantly along the tidal gradient at DBF, but did at LC where clams grew faster at the low intertidal, adding 50% and 100% more shell compared to clams at the mid and upper intertidal, respectively. Mean number of wild recruits in open EU at DBF (170 ind. m<sup>-2</sup>) did not differ across tidal heights, but did so in protected EU, ranging from a low of 300 to 1,890 ind. m<sup>-2</sup> in the upper and lower intertidal, respectively. Recruitment rates at LC were much lower than at DBF, with highest mean densities of 310 ind. m<sup>-2</sup> occurring at the mid and low compared to 84 ind. m<sup>-2</sup> in upper EU. Results from initially empty recruitment boxes at DBF suggested that numbers of 0-year class individuals reflect similar densities to those from EU, and a similar pattern along the tidal gradient. Mean densities of recruits varied from 238 to 1,069 ind. m<sup>-2</sup> at the upper and lower intertidal, respectively. Boxes scoured leaving a 2-5 cm gap between the bottom of the box and the mudflat surface at the upper and mid intertidal at LC. No recruits occurred in any of the upper intertidal boxes, and only 38 ind. m<sup>-2</sup> at the mid intertidal. Mean density at the lower intertidal was 402 ind. m<sup>-2</sup>.

This study suggests that at this time, effects of predation, rather than ocean acidification, is paramount in regulating population dynamics soft-shell clams at these two eastern Maine intertidal locations. Presently, *Mya* may be able to tolerate high levels of acidification by active

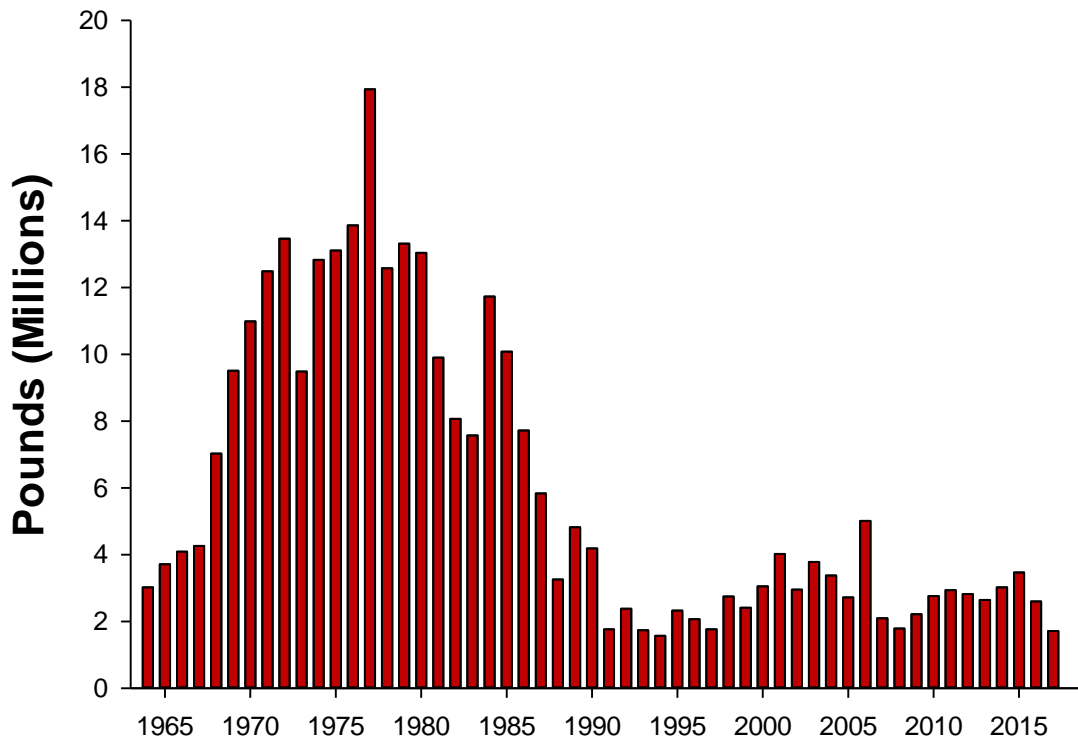
ion transport ( $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$ ) across the outer mantle controlling/maintaining pH at the site of shell accretion (calcification) through active removal of excessive  $\text{H}^+$  ions generated during  $\text{CaCO}_3$  precipitation (Zhao et al., 2018). This biogeochemical compensatory mechanism that modifies the chemistry of shell accretion in acidic settings may explain how *Mya* is able to persist in what is presumably a highly corrosive environment.

### Introduction

The soft-shell clam, *Mya arenaria* L., fishery in the state of Maine historically ranks in the top four of landings of commercially-important marine species. In 2018, for example, 7.1 million pounds were landed with a dockside value of \$12.8 million. Unfortunately, statewide landings have declined by 75% over the past four decades (Plate 1), and an even more precipitous decline (86%) has been observed in the easternmost county (Washington) over that same period (Plate 2).



**Plate 1.** Maine soft-shell clam landings and dockside value (1950-2018). Data from <https://www.maine.gov/dmr/commercial-fishing/landings/documents/softshellclam.table.pdf>.



**Plate 2.** Soft-shell clam landings (1964-2017) from Washington County, Maine. Data from <https://www.maine.gov/dmr/commercial-fishing/landings/historical-data.html>.

The major question facing the clamming industry is what can explain the dramatic declines across the state, and in Washington County? Research in Washington County since 1990 (Beal et al., 2001; Beal and Kraus, 2002; Beal, 2006a, b; Beal et al., 2016) suggest that predators play an important and disproportionate role in regulating soft-shell clam populations.

Over the past two decades, seawater temperatures in the Gulf of Maine have been warming (Pershing et al., 2016), and this has contributed to environmental conditions that are similar to that which occurred during the early 1950's when populations of the invasive green crab, *Carcinus maenas*, exploded. The consequences of that explosion can be seen in Figure 1 over the period from 1953-1956 when the clamming industry was devastated by this predator (Glude, 1955). Cold winter temperatures during the late 1950's into the late 1960's helped reverse this trend when green crab populations became scarce (Welch, 1968; Dow, 1972).

While predators play a key role in regulating populations of soft-shell clam and other bivalve mollusks, it is possible that both seawater and shallow-water sediments can be significantly undersaturated with respect to aragonite, a mineral of calcium carbonate that comprises > 97% of the shell matrix of *M. arenaria* (Ries, 2011). Dissolution of shell may occur in surface sediments where bivalves reside immediately following settlement (Green et al., 1993; 2009; Green and Aller, 2001). Saturation state of aragonite ( $\Omega_{\text{aragonite}}$ ) has been used as a measure to

determine the acceptability of the geochemical environment in which bivalve larvae settle (Green et al., 2013; Greiner et al., 2018). Sediments where  $\Omega_{\text{aragonite}} < 1$  may result in post-settlement mortality by dissolution of shell, and play a limiting role in the early life-history of bivalves.

Here, a short-term field experiment was conducted at two intertidal flats in Machias Bay (eastern Maine, USA) to determine the relative effects of sediment chemistry and predation on growth and survival of large ( $> 10$  mm shell length, SL) cultured juveniles of the soft-shell clam, *Mya arenaria*, as well as recruitment of wild clams.

## Methods and Materials

### *Study sites*

Two intertidal study sites located in Machias Bay (eastern Maine) were chosen for this experiment based on previous work (Beal, 2006b). Sediments at each site were sandy mud (sensu Folk, 1980), and the flat was extensive reaching 700-1500 m from the upper to lower intertidal at Duck Brook Flat (DBF), Holmes Bay in Cutler, ME (44.68717°N; -67.311276°W) and Larrabee Cove (LC) in Machiasport, ME (44.671696°N; -67.385320°W), respectively.

### *Experimental design – Effects of predator exclusion on survival and growth of cultured and recruitment of wild soft-shell clams*

To determine effects of predators on survival and growth of soft-shell clam juveniles, 24 cultured individuals of *M. arenaria* ( $\bar{x}_{SL} \pm 95\% \text{ CI} = 10.6 \pm 0.1$  mm,  $n = 805$ ) that had been reared the year before at the Downeast Institute (Beals, ME; 44.480733°N; -67.598687°W) and overwintered (see Beal et al., 1995) were added to 15 cm diameter x 15 cm deep plastic horticultural pots (experimental units = EU; surface area = 0.0182 m<sup>2</sup>) filled with sediments (sensu Beal, 2006a) at each of three tidal heights (upper; mid; lower) at each site. One-half of the EU at each tidal height were covered with an 18 cm x 18 cm piece of Pet Screen® (<https://www.phifer.com/product/petscreen/>) that surrounded the EU, and was held in place by a large rubber band. The aperture of the protective screening was approximately 1.7 mm x 0.9 mm (1.53 mm<sup>2</sup>) to deter predators  $> 1.9$  mm (Beal et al., 2018). The remaining EU had a strip of Pet Screen® that surrounded the periphery (2.5 cm wide x 50 cm long) that was held in place by a rubber band. The purpose of the strip was to corral the clams within the EU, yet allow predators to access the clams. The strip has no significant effect on clam survival and growth (Beal, 2006a).

At each tidal height, a total of five EU representing both protected and open treatments were arrayed randomly in three 2 x 5 matrices (blocks with 1 m spacing between rows and columns) that were approximately 20 m apart. The experiment (a generalized randomized complete block design, GRCBD – see Winer et al., 1991) was initiated on 12 June 2019 at DBF, and at LC the next day. Units from the upper and mid intertidal were collected on 31 October from DBF and 13 November from LC. EU from the lower intertidal at DBF were collected on 18 January 2019, and at LC on 16 February 2019. The contents of each EU were washed separately through a 1 mm sieve, and all cultured and wild clams were retained. Both cultured and wild clams from each EU were enumerated and, the cultured individuals were separated into two categories:

living and dead (with either crushed or chipped valves, or undamaged or intact valves). Cultured clams leave a distinct mark in their valves that denote the date they were deployed (Beal et al., 1999). This “hatchery mark” allows one to determine an individual growth rate can be estimated for each live clam. The initial and final SL of all live cultured clams, and the SL of all wild clams were measured to the nearest 0.01 mm using digital calipers.

A separate technique was used to estimate soft-shell clam recruitment at both sites across the three tidal heights. On the same day horticultural plant pots were established, a series of recruitment boxes (Beal et al., 2018) were deployed in three blocks of five boxes (separated by 1 m within a block and 20 m between blocks). Boxes were constructed of white spruce (*Picea glauca*) strapping with external dimensions of 30.5 cm x 60.9 cm x 7.6 cm. Top and bottom of each box was lined with PetScreen®. Empty boxes serve as passive settlement traps for clams and other invertebrates with planktonic larvae (including green crabs, *Carcinus maenas*). Boxes were held in place by driving a wooden lath (50.8 cm) into the mud at both short ends of each to a depth of 43 cm. Several galvanized nails were then driven through the laths into the wooden box. Boxes were removed from both sites on the same dates that horticultural pots were collected. The contents of each box was washed through a 1 mm sieve, and all clams and green crabs were enumerated. The SL of a representative sample of 25 clams from each box was measured to the nearest 0.01 mm using digital calipers. The carapace width (CW) of each green crab was measured similarly.

#### *Measures of seawater and sediment carbonate chemistry*

On several dates between July 2018 and February 2019 (Table 1), samples of seawater and/or surface sediments were taken from the three tidal heights at each site to estimate  $\Omega_{\text{aragonite}}$ . pH and temperature measurements were taken simultaneously using a Hanna HI99121 portable pH meter with 0.01 pH resolution and an accuracy of  $\pm 0.05$  pH. pH was measured from 2 cm below the sediment surface. Following the methodology of Green et al. (2013), the upper 2 mm of sediment from the mudflat surface adjacent to EU and recruitment boxes, as well as from individual EU (Table 1) was sampled by scraping with a clean, stainless steel spatula. The sediment was placed into a 60-cm<sup>3</sup> syringe that had been plugged at its base with a 30- $\mu\text{m}$  nylon mesh and fitted at the end with a 0.45- $\mu\text{m}$  Acrodisc filter, and returned to the lab where pore water from each sample was squeeze filtered into a washed 20 ml scintillation vials. Total alkalinity was determined on a 2-ml aliquot of pore water using a Hanna 902 automatic titrator with 0.01 N HCl. pH, temperature, and alkalinity from each sample were used to calculate carbonate ion ( $\text{CO}_3^{2-}$ ) concentration and pore water (and seawater) saturation state with respect to  $\Omega_{\text{aragonite}}$  using the CO2SYS program (Pelletier et al., 2007).

#### *Statistical analyses*

Univariate statistical analyses were performed using ANOVA on the arcsine-transformed survival data of cultured clams in the horticultural pots from both sites separately, and on the untransformed absolute growth (final SL - initial SL) of live cultured clams using the following linear model:

$$Y_{ijkl} = \mu + A_i + B(A)_{j(i)} + C_k + AC_{ik} + CB(A)_{kj(i)} + e_{l(ijk)}, \text{ where:}$$

$Y_{ijkl}$  = dependent variable (percent survival; absolute growth);  
 $\mu$  = theoretical mean;  
 $A_i$  = tidal height ( $a = 3$  [upper, mid, low]; factor is fixed);  
 $B_j$  = block ( $b = 3$  [I, II, III]; factor is random);  
 $C_k$  = predator exclusion ( $c = 2$  [open, protected EU]; factor is fixed); and,  
 $e_l$  = experimental error ( $n = 5$ ).

ANOVA also was used to analyze the square root-transformed counts and untransformed SL of wild juveniles of *M. arenaria* in the recruitment boxes for each site using the following linear model:

$Y_{ijk} = \mu + A_i + B(A)_{j(i)} + e_{k(ij)}$ , where:

$Y_{ijk}$  = dependent variable (number and size of soft-shell clam recruits);  
 $\mu$  = theoretical mean  
 $A_i$  = tidal height ( $a = 3$  [upper, mid, low]; factor is fixed);  
 $B_j$  = block ( $b = 3$  [I, II, III]; factor is random);  
 $e_k$  = experimental error ( $n = 5$ ).

To better understand effects due to tidal height, two pre-planned, orthogonal contrasts were conducted to test the following null hypotheses:

- 1)  $H_0: \mu_{\text{Low}} \text{ VS. } \mu_{(\text{Upper \& Mid})/2}$ ; and,
- 2)  $H_0: \mu_{\text{Upper}} \text{ VS. } \mu_{\text{Mid}}$ .

These contrasts were chosen based on previous studies that have shown predation on juveniles of *M. arenaria* to be more intense at lower vs. upper shore levels (Beal et al., 2001; Beal, 2006a).

All means are untransformed, and presented with their respective 95% confidence interval.

Differences in size-frequency distribution of wild recruits was investigated using G-tests of independence to test for tidal height and predator exclusion effects. Five discrete size classes were used: I =  $\leq 3.99$  mm; II = 4.0-5.99 mm; III = 6.0-7.99 mm; IV = 8.0-9.99 mm; V =  $\geq 10.0$  mm.

A type I error rate ( $\alpha$ ) of 0.05 was used as the decision rule for each statistical test.

## Results

### ***Duck Brook Flat***

#### *Cultured and wild clams in the predator exclusion study*

Clam survival varied significantly across only one of the five sources of variation (Table 2;  $P < 0.001$ ) with  $9.1 \pm 4.2\%$  vs.  $63.5 \pm 5.9\%$  occurring in open vs. protected EU ( $n = 45$ ), and this



trend was similar across each tidal height ( $P = 0.91$ ). Most (86%) of the clams in the open EU at each tidal height were missing, whereas approximately 30% of clams either were missing or recovered with chipped or crushed shells in the protected EU. Not surprisingly, a significantly greater number of green crabs per EU were associated with protected ( $0.6 \pm 0.3 \text{ EU}^{-1}$ ,  $n = 45$ ) vs. open ( $0.04 \pm 0.1 \text{ EU}^{-1}$ ,  $n = 45$ ) ( $P = 0.0161$ ), and that trend occurred across tidal heights ( $P = 0.98$ ). Crabs ranged in CW from 3.4 mm to 16.9 mm, with a mean of  $7.06 \pm 1.3$  mm ( $n = 29$ ). Mean absolute growth varied significantly only with predator exclusion treatment (Table 3), as clams experienced an approximate 33% growth penalty in protected ( $12.4 \pm 0.9$  mm,  $n = 43$ ) vs. open ( $18.5 \pm 2.2$  mm,  $n = 28$ ) EU. This pattern was similar across tidal heights ( $P = 0.83$ ; Table 3). Wild clam recruitment varied significantly across tidal heights, predator exclusion treatments, and the interactive effect of both main factors (Table 4). In open EU ( $n = 15$ ), recruit density was similar across tidal heights with mean number of clam recruits ranging from  $1.6 \pm 0.8$  ind. (upper intertidal) to  $4.4 \pm 1.9$  ind. (low intertidal). Conversely, in protected EU ( $n = 15$ ), clam recruitment varied directly with decreasing tidal height (Fig. 1), with approximately 6x and 2x more recruits in the low intertidal ( $34.5 \pm 10.6$  ind.  $\text{EU}^{-1}$ ) compared to the upper ( $5.5 \pm 2.5$  ind.  $\text{EU}^{-1}$ ) and mid intertidal ( $14.6 \pm 5.6$  ind.  $\text{EU}^{-1}$ ), respectively. A 3 x 5 G-test of independence on size frequencies of wild recruits demonstrated a significant difference across tidal heights ( $G = 55.69$ ,  $df = 8$ ,  $P < 0.0001$ ; Fig. 2). In addition, a 2 x 5 G-test of independence on size frequencies of wild recruits across predator exclusion treatments was not significant ( $G = 8.31$ ,  $df = 4$ ,  $P = 0.0808$ ). Mean SL of wild recruits varied significantly across tidal heights ( $P = 0.0344$ ; Table 5) but not by predator exclusion treatment ( $P = 0.9782$ ) with clams in EU at the upper and mid intertidal ( $6.3 \pm 0.7$  mm,  $n = 50$ ) approximately 25% larger than those at the lower intertidal ( $5.0 \pm 0.7$  mm,  $n = 30$ ).

#### *Wild clam juveniles in recruitment boxes*

Mean number of wild clams per box varied significantly across tidal heights (Table 6; Fig. 3), with approximately 4x more 0-year class individuals occurring in low and mid boxes ( $137.4 \pm 31.8$  ind.,  $n = 30$ ) compared to those arrayed in the upper intertidal ( $35.8 \pm 15.1$  ind.,  $n = 15$ ). Mean number of green crabs recruitment box<sup>-1</sup> ( $2.8 \pm 0.9$  ind.,  $n = 45$ ) did not vary significantly across tidal heights ( $P = 0.0566$ ) or between blocks within a tidal height ( $P = 0.9065$ ), and there was no relationship between number of clam recruits and number of crabs recruitment box<sup>-1</sup> ( $r = 0.12$ ,  $P = 0.4463$ ). Boxes containing crabs ( $n = 32$ ) had as many wild recruits of *Mya* ( $96.3 \pm 25.7$  ind) than those without ( $n = 12$ ;  $121.1 \pm 64.2$  ind.) ( $P = 0.5320$ ). Only a single recruitment box contained a green crab greater than 11 mm CW (22.3 mm), and that box (from the upper intertidal) contained no recruits. Mean crab size ( $6.9 \pm 1.2$  mm,  $n = 32$ ) did not vary significantly across tidal heights ( $P = 0.5449$ ) or between blocks within a tidal height ( $P = 0.4191$ ).

#### *Seawater and sediment chemistry*

Seawater samples were taken during July across all three tidal heights, and in September at the mid intertidal (Table 1). In July, when larvae were likely still in the water column, neither mean pH ( $7.57 \pm 0.35$ ,  $n = 9$ ) nor  $\Omega_{\text{aragonite}}$  ( $0.45 \pm 0.27$ ,  $n = 9$ ) varied significantly across tidal height ( $P > 0.3111$ ). Water samples from the mid intertidal in September had a mean pH of  $7.92 \pm 0.39$  ( $n = 4$ ), and mean  $\Omega_{\text{aragonite}}$  ( $0.94 \pm 1.05$ ).

For sediments, both mean pH ( $6.58 \pm 0.28$ ,  $n = 20$ ) and  $\Omega_{\text{aragonite}}$  ( $0.17 \pm 0.17$ ,  $n = 20$ ) did not differ significantly between August and September ( $P = 0.9358$  and  $0.7894$ , respectively). In January 2019, when low tide EU and boxes were collected, mean pH and  $\Omega_{\text{aragonite}}$  taken from EU was  $7.37 \pm 0.26$  and  $0.30 \pm 0.09$  ( $n = 12$ ), respectively. Mean pH in open units at that time was significantly lower ( $P < 0.0001$ ) than in protected units ( $7.01 \pm 0.26$  vs.  $7.73 \pm 0.11$ ;  $n = 6$ ), but no similar difference was observed for mean  $\Omega_{\text{aragonite}}$  between predator exclusion treatments ( $P = 0.1913$ ). Over the entire data set ( $N = 45$ ), there was a significant ( $r^2 = 0.822$ ;  $P < 0.0001$ ) linear relationship between pH and  $\log_{10}(\Omega_{\text{aragonite}})$  (Fig. 4). Analysis of covariance (ANCOVA) demonstrated that the relationship between pH and  $\log_{10}(\Omega_{\text{aragonite}})$  did not differ significantly between water and sediment samples ( $F = 0.01$ ,  $df = 1,42$ ,  $P = 0.9392$ ).

## *Larrabee Cove*

### *Cultured and wild clams in the predator exclusion study*

Cultured clam survival varied significantly by tidal height, but only in open EU (Table 7; Fig. 5) where mean survival in the upper and mid intertidal ( $15.4 \pm 3.8\%$ ,  $n = 30$ ) was approximately 6x higher than in the low intertidal ( $2.5 \pm 1.7\%$ ,  $n = 15$ ). Mean survival was independent of tidal height among clams in protected EU ( $56.1 \pm 4.9\%$ ,  $n = 45$ ; Fig. 5). Netting was not 100% effective in keeping green crabs or other crushing predators from clams, as between 11% (low intertidal) and 20% (upper intertidal) of clams in protected EU were recovered dead with chipped or crushed valves. Green crabs were found at each tidal height; however, fewer occurred in the low intertidal (where none was recovered from open and  $0.2 \pm 0.2$  ind. EU<sup>-1</sup> [ $n = 15$ ] from protected units) compared to the two higher tidal heights. At the mid intertidal, approximately 2.5x more green crabs occurred in netted vs. open EU ( $0.5 \pm 0.5$  ind. vs.  $0.2 \pm 0.2$  ind.,  $n = 15$ ). Similar densities of crabs occurred in upper EU regardless of treatment ( $0.4 \pm 0.3$  ind.,  $n = 30$ ). Mean absolute growth of cultured juveniles varied directly with tidal height ( $P = 0.0002$ ; Table 8; Fig. 6). Clams in both open and netted EU added shell at a faster rate at the low intertidal ( $14.4 \pm 1.5$  mm,  $n = 22$ ) than at the mid ( $9.6 \pm 1.5$ ,  $n = 30$ ) or upper intertidal ( $7.1 \pm 1.4$  mm,  $n = 30$ ). Excluding predators with PetScreen® netting resulted in a growth penalty ( $P = 0.0034$ ; Table 8), as clams grew approximately 55% faster in open ( $12.4 \pm 1.3$  mm,  $n = 37$ ) vs. netted ( $7.9 \pm 1.3$  mm,  $n = 45$ ) EU regardless of tidal height. Wild clam recruitment varied significantly across tidal heights ( $P = 0.0088$ ) and exclusion treatments ( $P = 0.0009$ ); however, the relationship between number of recruits EU<sup>-1</sup> and exclusion treatment was not the same across tidal heights ( $P = 0.0419$ ; Table 9). Significantly higher numbers of wild recruits were found in protected EU at the mid and low intertidal, but similar density of recruits occurred in both predator exclusion treatments at the upper intertidal (Fig. 7). Size-frequency distribution of wild recruits varied significantly across both tidal height ( $G = 36.9$ ,  $df = 8$ ,  $P < 0.0001$ ; Fig. 8) and predator exclusion treatment ( $G = 64.2$ ,  $df = 4$ ,  $P < 0.0001$ ). Disproportionately more recruits  $> 10$  mm occurred in EU protected from predators compared to open controls (Fig. 9). Mean SL of wild recruits varied significantly with tidal height ( $P = 0.0387$ ) and predator exclusion treatment ( $P = 0.0323$ ; Table 10). Mean SL varied inversely with decreasing tidal height as recruits at the upper ( $11.2 \pm 1.9$  mm,  $n = 17$ ) and mid ( $9.9 \pm 1.4$  mm,  $n = 26$ ) intertidal had significantly larger SL than those in the low intertidal ( $8.3 \pm 1.9$  mm,  $n = 13$ ). In addition, recruits in EU protected

with predator exclusion netting were approximately 40% larger in size than those in open controls ( $11.1 \pm 1.0$  mm,  $n = 36$  vs.  $7.8 \pm 1.6$  mm,  $n = 20$ ).

### *Wild clam juveniles in recruitment boxes*

Mean number of wild clams per box varied significantly across tidal heights (Table 11; Fig. 10). Boxes at the upper intertidal scoured considerably with a gap of 2-5 cm underneath each when sampled in November 2018. No clams or sediment occurred in any. It is likely that some clam recruitment occurred at this level, but that many larvae escaped through the aperture in the netting because there was not a secure seal between the bottom of the boxes and the mudflat surface. Similar observations were made on boxes at the mid intertidal; however, some boxes had collected sediment and a few clam recruits were found ( $5.6 \pm 5.8$  ind. EU<sup>-1</sup>,  $n = 15$ ). Relatively minor scouring occurred around the low intertidal boxes, and these contained an average of  $60.4 \pm 20.2$  ind. EU<sup>-1</sup> ( $402.7 \pm 134.7$  ind. m<sup>-2</sup>;  $n = 15$ ). Mean number of green crabs recruitment box<sup>-1</sup> varied between  $1.8 \pm 0.2$  and  $1.9 \pm 0.9$  ( $n = 15$ ) in the low and upper, respectively, to  $4.4 \pm 2.2$  ( $n = 15$ ) in the mid intertidal, but the differences were not statistically significant ( $P = 0.2335$ ). No significant difference occurred between blocks within a tidal height ( $P = 0.2448$ ), and there was no relationship between number of clam recruits and number of crabs recruitment box<sup>-1</sup> ( $r = 0.24$ ,  $P = 0.1162$ ). Excluding the upper intertidal where no juvenile clams were found in any recruitment box due, presumably, to scouring, boxes containing crabs ( $n = 20$ ) had fewer wild recruits of *Mya* ( $29.4 \pm 18.9$  ind.) than those without ( $40.4 \pm 25.7$  ind.;  $n = 10$ ); however, this difference was not statistically significant ( $P = 0.4663$ ). Mean crab size varied significantly across tidal heights ( $P = 0.0095$ ), but not between blocks within a tidal height ( $P = 0.5093$ ). Mean CW of green crabs in mid intertidal boxes ( $8.3 \pm 0.7$  mm,  $n = 11$ ) was approximately 40% greater than those in low intertidal boxes ( $5.8 \pm 0.9$  mm,  $n = 9$ ).

### *Seawater and sediment chemistry*

Seawater samples were taken during July across all three tidal heights, and in September at the upper and mid intertidal (Table 1). In July, neither mean pH ( $8.02 \pm 0.17$ ,  $n = 6$ ) nor  $\Omega_{\text{aragonite}}$  ( $0.85 \pm 0.27$ ,  $n = 6$ ) varied significantly across tidal height ( $P > 0.0693$ ). No significant difference was detected between tidal heights in September for mean seawater pH ( $7.59 \pm 0.12$ ,  $n = 11$ ) or  $\Omega_{\text{aragonite}}$  ( $0.31 \pm 0.08$ ,  $n = 11$ ) ( $P > 0.7706$ ).

For sediments, both mean pH and  $\Omega_{\text{aragonite}}$  differed significantly between dates ( $P < 0.0001$  and  $P = 0.0141$ , respectively), but not by tidal height within sampling date ( $P = 0.1965$  and  $P = 0.4684$ , respectively). The relationship between sampling date (8 = August; 9 = September; 11 = November; 14 = February) and pH was not linear, as a lack-of-fit test to a linear model was statistically significant ( $F = 17.51$ ,  $df = 2, 30$ ,  $P < 0.0001$ ), as well as to a quadratic model ( $F = 17.1$ ,  $df = 1, 30$ ,  $P = 0.0003$ ). A cubic model best explained the relationship ( $Y = 76.95 - 20.11X + 1.89X^2 - 0.06X^3$  ( $r^2 = 0.588$ ;  $P < 0.0001$ ; Fig. 11). Similar lack-of-fit tests indicated that a cubic model best explained the relationship between  $\Omega_{\text{aragonite}}$  and sampling date (Fig. 12).

In November 2018, when mid and low intertidal EU were removed from the flat, both pH and  $\Omega_{\text{aragonite}}$  measurements were taken directly from open and protected units in each block. Similar

measurements were recorded from EU sampled in February 2019. Neither variable was found to vary significantly between the type of unit sampled on each date and tidal height ( $P > 0.22$ ). Mean pH and  $\Omega_{\text{aragonite}}$  for all units sampled across the upper and mid intertidal in November 2018 was  $7.65 \pm 0.13$  and  $0.40 \pm 0.15$  ( $n = 9$ ), respectively. In February 2019, when low intertidal EU were sampled, mean pH and  $\Omega_{\text{aragonite}}$  were  $7.32 \pm 0.27$  and  $0.26 \pm 0.17$  ( $n = 8$ ), respectively. Over the entire data set ( $N = 52$ ), there was a significant ( $r^2 = 0.837$ ;  $P < 0.0001$ ) linear relationship between pH and  $\log_{10}(\Omega_{\text{aragonite}})$  (Fig. 13). ANCOVA demonstrated that the relationship between pH and  $\log_{10}(\Omega_{\text{aragonite}})$  did not differ significantly between water and sediment samples ( $F = 0.29$ ,  $df = 1, 49$ ,  $P = 0.5957$ ).

Sediment and water sample data from both Duck Brook Flat and Larrabee Cove were combined (Fig. 14). Analysis of regression lines indicated that the slopes were similar ( $F = 0.65$ ,  $df = 1, 93$ ,  $P = 0.4236$ ), and ANCOVA indicated that the lines were congruent ( $F = 0.03$ ,  $df = 1, 94$ ,  $P = 0.8666$ ).

## Discussion

This study was conducted to examine how sediment (and seawater) chemistry affects cultured clam survival and growth, as well as recruitment of 0-year class individuals. As expected (Beal, 2001; Whitlow, 2010), predation was a major regulating factor in clam survival at both study sites. Clam survival in EU with predator exclusion netting was independent of tidal height, and varied between 56% (LC) and 64% (DBF). Conversely, in EU that permitted predators unhindered access to clams (control units), survival across all tidal heights was 9% at DBF, and varied across tidal heights at LC from approximately 3% at the low intertidal to 15% at the mid and upper intertidal. Green crabs, *Carcinus maenas*, appeared to be the main predator, as individuals were found in EU and recruitment boxes at each site and tidal height. These results support those observed recently in southern Maine (Beal et al., 2018), at DBF in 2011 (Tan and Beal, 2015), and in far eastern Maine during 2003 (Beal, 2006a).

The experimental design examined recruitment using two different methods: small plastic plant pots (EU) filled with ambient sediments and either with or without protected with polypropylene exclusion netting (4.2 mm aperture), or initially empty wooden structures with small aperture PetScreen® excluding both infaunal and epifaunal predators (Beal et al., 2018). Wild, 0-year class individuals were observed in EU at both sites, but, not surprisingly, significantly higher densities of recruits occurred in EU protected from predators vs. controls ( $P < 0.001$ ; Tables 4 & 9; Figs. 1 & 7). At DBF, no difference was observed among open controls along the tidal gradient ( $3.1 \pm 0.9$  ind. EU<sup>-1</sup>, or  $170.5 \pm 49.4$  ind. m<sup>-2</sup>;  $n = 45$ ), which was approximately 6x lower than densities of recruits in protected EU ( $18.2 \pm 5.3$  ind. EU<sup>-1</sup>, or  $998.4 \pm 288.5$  ind. m<sup>-2</sup>;  $n = 45$ ). Recruitment in protected EU, densities decreased sharply from the low intertidal where densities were highest at the low intertidal ( $34.5 \pm 10.6$  ind. EU<sup>-1</sup>,  $n = 30$ ), and lowest at the upper intertidal ( $5.5 \pm 2.5$  ind. EU<sup>-1</sup>). At LC, no difference in mean recruit density occurred between predator exclusion treatments at the upper intertidal where  $1.5 \pm 0.8$  ind. EU<sup>-1</sup>, or  $84.1 \pm 43.3$  ind. m<sup>-2</sup> ( $n = 30$ ), but the typical pattern of higher densities in protected vs. control EU occurred at the mid and low intertidal (Fig. 7). Recruitment boxes at both sites showed a similar pattern with highest densities of 0-year class individuals in the low or mid intertidal vs. the upper shore. Recruit densities at each tidal height generally were one to two orders of magnitude

higher at DBF than at LC (compare Figs. 3 & 10). Highest densities in recruitment boxes occurred at the low at DBF ( $160.4 \pm 49.1$  ind. box<sup>-1</sup>, or  $1,069.3 \pm 327.4$  ind. m<sup>-2</sup>; n = 15) and LC ( $60.4 \pm 20.2$  ind. box<sup>-1</sup>, or  $402.7 \pm 134.7$  ind. m<sup>-2</sup>; n = 15).

Relatively high survival of cultured clams in EU, and enhanced recruitment of wild clams in recruitment boxes occurred in highly corrosive sediments and acidic seawater. Green et al. (2009, 2013) showed that the valves of hard clams and soft-shell clams (both of which are composed of > 97% the mineral aragonite [Ries, 2011]) are susceptible to dissolution in undersaturated sediments (i.e.,  $\Omega_{\text{aragonite}}$  values of 0.4). Green et al. (2009) conducted a 16-day field experiment at an intertidal flat in West Bath, Maine in which crushed shells of *Mya arenaria* ( $\approx 5$  mm pieces) were added to field plots to buffer sediments. Sediment sampled from the buffered and control plots showed that over time  $\Omega_{\text{aragonite}}$  doubled from 0.25 to 0.53, and pH increased from 7.04 to 7.31. At the same time, the number of live *Mya* g<sup>-1</sup> sediment was approximately 3x higher in buffered vs. control plots (0.3 vs 0.1 ind. g<sup>-1</sup>). In another field trial in southern Maine, Green et al. (2013) added crushed shells of *Mya* ( $\approx 1$  mm pieces) to 10 cm plots, while similar size plots without the biogenic calcium carbonate served as controls. Sediment were sampled on 13 occasions over a 35-day period. Buffering resulted in a 2-fold increase in  $\Omega_{\text{aragonite}}$  from 0.68 to 1.30, and increased recruitment by a factor of approximately two. While data from both studies suggest the mechanism resulting in enhanced clam recruitment was from buffering with crushed shell material, other explanations are plausible. For example, it is possible that the addition of shell to intertidal plots and cores acted as a deterrent to small predators by increasing habitat complexity/heterogeneity making it more difficult for predators to physically manipulate their prey and providing a spatial refuge for the settling clams (Grabowski, 2004; Glaspie and Seitz, 2018), or that shell increased attachment surfaces for settlers of benthic species that were easier to consume or capture by small predators (Calloway, 2018). In addition, adding shell to an otherwise homogeneous benthic environment may somehow help to mask or hide recently-settled bivalves from visual predators, which could have resulted in enhanced survival in the buffered plots. Similar studies with Manila clams on the U.S. west coast (Ruesink et al., 2014; Greiner et al., 2018) either found no effect of added shell hash on recruitment, or similar enhancement effects with both shell hash and gravel addition, suggesting habitat complexity may be the mechanism acting to enhance post-settlement survival.

In this study, no shell or other abiotic material was added to field units or recruitment boxes, and average pH (sediment and water samples combined) varied between  $7.11 \pm 0.21$  (n = 45) at DBF and  $7.43 \pm 0.10$  (n = 52) at LC. Mean aragonite saturation state was  $0.33 \pm 0.12$  at DBF and  $0.33 \pm 0.07$  at LC. While these measurements suggest a highly acidic environment, the fact that so many wild clams were recovered in EU or recruitment boxes is perplexing because of the importance of both pH and  $\Omega_{\text{aragonite}}$  to larval and settling bivalves (Waldbusser et al., 2014;

It may be possible for soft-shell clams, as it is with corals (Gagnon et al., 2012), to modify carbonate chemistry at the site of calcification that creates conditions that are more favorable thermodynamically for inorganic CaCO<sub>3</sub> precipitation than in the surrounding seawater or pore water (Cryonak et al., 2016). Recently, Zhao et al. (2018), investigated shell formation in juveniles (5-10 mm SL) of *Mya arenaria* collected from Kiel Fjord, Norway where surface seawater *p*CO<sub>2</sub> can range from 2,500-3,350  $\mu\text{atm}$  during summer and autumn as a result of strong upwelling of hypoxic bottom water. Clams were kept in sediments in the laboratory for three

months in a mesocosm experiment under seawater  $p\text{CO}_2$  levels ranging from 900-6,600  $\mu\text{atm}$ , corresponding to pH values ranging from 7.8 to 7.0. Clams grew significantly faster at 900  $\mu\text{atm}$   $p\text{CO}_2$  than at higher concentrations showing a 35% reduction in growth rate at 1,500 and 2,500  $\mu\text{atm}$ , and 60% reduction at 6,600  $\mu\text{atm}$ . No differences in growth rate occurred at the two intermediate  $p\text{CO}_2$  treatments, however, indicating that clams tolerate high  $p\text{CO}_2$  levels to a certain degree. Subsequent examination of concentrations of Ca, Cl, and Na in the valves of clams from each of the four  $p\text{CO}_2$  treatments suggested that *Mya* may have evolved efficient acid-base regulatory mechanisms to tightly control pH at the site of calcification through the removal of excessive bicarbonate ions ( $\text{HCO}_3^-$ ), and thereby partially mitigate the impact of high  $p\text{CO}_2$  on shell formation (Zhao et al., 2018).

If resilience to high levels of acidification that results in shell accretion through the active removal of protons is occurring in local populations of *Mya*, this biogeochemical mechanism may help explain why, at this point in time, predation apparently is more important than ocean acidification in regulating populations of wild clams in eastern Maine.

### Acknowledgments

This work was funded by SEANET, the Sustainable Ecological Aquaculture Network, through an EPSCoR grant to the University of Maine. Other funding sources include the University of Maine at Machias, and Downeast Institute. We thank K. Chandler and H. Wegner for assistance in the field and laboratory. In addition, students in UMM's BIO 360 (Marine Ecology) course assisted with the collection of EU and recruitment boxes on 31 October (DBF) and 13 November (LC). We also thank B. Ellis, C. Jourdet, B. Kadis, and E. Montgomery for assistance in the field.

### References

- Beal, B.F. 2006a. Relative importance of predation and intraspecific competition in regulating growth and survival of juveniles of the soft-shell clam, *Mya arenaria* L., at several spatial scales. *Journal of Experimental Marine Biology and Ecology* 336:1-17.
- Beal, B.F. 2006b. Biotic and abiotic factors influencing growth and survival of wild and cultured individuals of the soft-shell clam (*Mya arenaria* L.) in eastern Maine. *J. Shellfish Res.* 25:461-474.
- Beal, B.F., Bayer, R.C., Kraus, M.G., Chapman, S.R. 1999. A unique shell marker of juvenile, hatchery-reared individuals of the soft-shell clam, *Mya arenaria* L. *Fish. Bull.* 97:380-386.
- Beal, B.F., Coffin, C.R., Randall, S.F., Goodenow, C.A., Jr., Pepperman, K.E., Ellis, B.W., Jourdet, C.B., Protopopescu, G.C. 2018. Spatial variability in recruitment of an infaunal bivalve: experimental effects of predator exclusion on the softshell clam (*Mya arenaria* L.) along three tidal estuaries in southern Maine, USA. *J. Shellfish Res.* 37:1-27.
- Beal, B.F., Kraus, M.G. 2002. Interactive effects of initial size, stocking density, and type of

- predator deterrent netting on survival and growth of cultured juveniles of the soft-shell clam, *Mya arenaria* L. in eastern Maine. *Aquaculture* 208:81-111.
- Beal, B.F., Lithgow, C., Shaw, D., Renshaw, S., Ouellette, D. 1995. Overwintering hatchery-reared individuals of the soft-shell clam, *Mya arenaria* L.: a field test of site, clam size, and intraspecific density. *Aquaculture* 130:145-158.
- Beal, B.F., Nault, D.-M., Annis, H., Thayer, P., Leighton, H., Ellis, B. 2016. Comparative, large-scale field trials along the Maine coast to assess management options to enhance populations of the commercially important softshell clam, *Mya arenaria* L. *Journal of Shellfish Research* 35(4):711-727.
- Calloway, R. 2018. Interstitial space and trapped sediment drive benthic communities in artificial shell and rock reefs. *Front. Mar. Sci.* 5:1-10.
- Cryonak, T., Schulz, K.G., Jokiel, P.L. 2016. The omega myth: what really drives lower calcification rates in an acidifying ocean. *ICES J. Mar. Sci.* 73(3):558-562.
- Dow, R.L., 1972. Fluctuations in Gulf of Maine sea temperature and specific molluscan abundance. *J. Cons. int. Explor. Mer.* 34(3):532-534.
- Folk, R.L. 1980. Petrology of sedimentary rocks. Hemphill Publishing Company. Austin, TX.
- Gagnon, A.C., Adkins, J.F., Erez, J. 2012. Seawater transport during coral biomineralization. *Earth Planet. Sc. Lett.* 329-330:150-161.
- Glaspie, C.N., Seitz, R.D. 2018. Habitat complexity and benthic predator-prey interactions in Chesapeake Bay. *PLoS One* 13(10): e0205162.  
<https://doi.org/10.1371/journal.pone.0205162>.
- Glude, J.B. 1955. The effects of temperature and predators on the abundance of the soft-shell clam, *Mya arenaria*, in New England. *Trans. Am. Fish. Soc.* 84:13-26.
- Grabowski, J.H. 2004. Habitat complexity disrupts predator-prey interactions but not the trophic cascade on oyster reefs. *Ecology* 85(4):995-1004.
- Green, M.A., Waldbusser, G.G., Reilly, S.L., Emerson, K., O'Donnell, S. 2009. Death by dissolution: Sediment saturation state as a mortality factor for juvenile bivalves. *Limnol. Oceanogr.* 54(4):1037-1047.
- Green, M.A., Aller, R.C. 2001. Early diagenesis of calcium carbonate in Long Island Sound sediment: Benthic fluxes of  $\text{Ca}^{2+}$  and minor elements during seasonal periods of net dissolution. *J. Mar. Res.* 59:769-794.
- Green, M.A., Aller, R.C., Aller, J.Y. 1993. Carbonate dissolution and temporal abundances of Foraminifera in Long Island Sound sediments. *Limnol. Oceanogr.* 38:331-345.

- Greiner, C.M., Klinger, T., Ruesink, J.L., Barber, J.S., Horwith, M. 2018. Habitat effects of macrophytes and shell on carbonate chemistry and juvenile clam recruitment, survival, and growth. *J. Exp. Mar. Biol. Ecol.* 509:8-15.
- Pelletier, G., Lewis, E., Wallace, D. 2007. CO2SYS.xls: A calculator for the CO2 system in seawater for Microsoft Excel/VBA (Washington State Department of Ecology; Olympia Washington; Brookhaven National Laboratory, Upton, New York).
- Pershing, A. J., Alexander, M. A., Hernandez, C. M., Kerr, L. A., Le Bris, A., Mills, K. E., Nye, J. A., Record, N. R., Scannell, H. A., Scott, J. D., Sherwood, G. D., Thomas, A. C. 2015. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. *Science* 350:809–812.
- Ries, J.B. 2011. Skeletal mineralogy in a high-CO<sub>2</sub> world. *J. Mar. Biol. Ecol.* 403:54-64.
- Ruesink, J.L., Freshly, N., Herrold, S., Trimble, A.C., Patten, K. 2014. Influence of substratum on non-native clam recruitment in Willapa Bay, Washington, USA. *J. Exp. Mar. Biol. Ecol.* 459:23-30.
- Shaw, R. G., Mitchell-Olds, T. 1993. ANOVA for unbalanced data: an overview. *Ecology* 74:1638–1645.
- Tan, E.B.P., Beal, B.F. 2015. Interactions between the invasive European green crab, *Carcinus maenas* (L.), and juveniles of the soft-shell clam, *Mya arenaria* L., in eastern Maine, USA. *J. Exp. Mar. Biol. Ecol.* 462:62-73.
- Waldbusser, G.G., Hales, B., Langdon, C.J., Haley, B.A., Schrader, P., Brunner, E.L., Gray, M.W., Miller, C.A., Gimenez, I. 2014. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. *Nature Clim. Change*.  
<http://dx.doi.org/10.1038/nclimate2479>.
- Welch, W.R. 1968. Changes in abundance of the green crab, *Carcinus maenas* (L.), in relation to recent temperature changes. *Fish. Bull.* 67(2):337-345.
- Whitlow, W.L. 2010. Changes in survivorship, behavior, and morphology in native soft-shell clams induced by invasive green crab predators. *Mar. Ecol.* 31:418-430.
- Winer, B.J., Brown, D.R., Michels, K.M. 1991. *Statistical principles in experimental design*, 3<sup>rd</sup> ed. New York, NY: McGraw-Hill.
- Zhao, L., Milano, S., Walliser, E.O., Schöne, B.R. 2018. Bivalve shell formation in a naturally CO<sub>2</sub>-enriched habitat: Unraveling the resilience mechanisms from elemental signatures. *Chemosphere* 203:132-138.



Table 1. Locations, tidal heights, dates, and sample and sample size (n) for mean ( $\pm$  SE) pH and temperature ( $^{\circ}$ C) used to estimate  $\Omega_{\text{aragonite}}$ .

Location	Tidal Height	Date	Sample	n	pH	Temp	$\Omega_{\text{aragonite}}$
DBF	Upper	07-17-18	Seawater	3	7.26(0.23)	15.4(0.2)	0.197(0.09)
		08-04-18	Seawater	1	7.67(-)	19.8(-)	0.520(-)
			Sed (Box) <sup>1</sup>	1	6.12(-)	20.1(-)	0.020(-)
	Sed (Pot) <sup>2</sup>		2	6.35(0.05)	19.7(0.4)	0.025(0.01)	
	09-09-18	Sed(Box)	2	6.86(0.00)	17.3(0.0)	0.07(0.01)	
		Sed(Pot)	2	6.71(0.41)	17.3(0.04)	0.01(0.06)	
	Mid	07-17-18	Seawater	3	7.84(0.11)	15.2(0.06)	0.60(0.14)
		08-04-18	Seawater	4	7.92(0.12)	19.6(0.28)	0.94(0.33)
		09-09-18	Sed(Box)	3	6.18(0.16)	17.3(0.02)	0.02(0.01)
	Sed(Pot)		3	6.15(0.14)	17.3(0.02)	0.02(0.01)	
	Low	07-17-18	Seawater	3	7.63(0.36)	14.6(0.18)	0.54(0.29)
		09-09-18	Sed(Box)	3	6.74(0.45)	17.4(0.04)	0.24(0.21)
Sed(Pot)			3	6.96(0.57)	17.4(0.04)	0.50(0.47)	
01-18-19		Open(Sed) <sup>3</sup>	6	7.01(0.09)	2.8(0.19)	0.25(0.08)	
		Net(Sed) <sup>4</sup>	6	7.73(0.04)	1.7(0.08)	0.36(0.03)	
LC	Upper	07-15-18	Seawater	1	7.94(-)	17.7(-)	0.77(-)
		08-05-18	Seawater	5	7.59(0.13)	23.2(0.84)	0.33(0.08)
	Sed(Box)		1	7.58(-)	20.2(-)	0.28(-)	
	09-14-18	Sed(Box)	3	7.15(0.06)	13.9(0.16)	0.17(0.03)	
		Sed(Pot)	3	7.08(0.00)	13.9(0.16)	0.13(0.01)	
	11-14-18	Open(Sed)	3	7.81(0.09)	0.8(0.27)	0.37(0.09)	
		Net(Sed)	3	7.67(0.04)	0.4(0.09)	0.54(0.16)	
	Mid	07-15-18	Seawater	2	7.85(0.03)	16.2(0.00)	0.60(0.04)
08-05-18		Seawater	6	7.59(0.01)	20.6(1.13)	0.30(0.03)	
09-14-18		Sed(Box)	3	7.13(0.07)	14.9(0.36)	0.16(0.02)	

Table 1. (cont.)

Location	Tidal Height	Date	Sample	n	pH	Temp	$\Omega_{\text{aragonite}}$
LC	Mid	09-14-18	Sed(Pot)	3	7.08(0.11)	14.9(0.36)	0.15(0.03)
		11-14-18	Open(Sed)	3	7.49(0.03)	2.5(0.25)	0.55(0.39)
			Net(Sed)	3	7.48(0.04)	1.6(0.28)	0.20(0.09)
	Low	07-15-18	Seawater	3	8.15(0.06)	14.3(0.20)	1.04(0.12)
		09-14-18	Sed(Box)	2	6.87(0.05)	16.5(0.55)	0.09(0.01)
			Sed(Pot)	3	7.02(0.20)	16.8(0.43)	0.16(0.06)
		02-17-19	Open(Sed)	4	7.32(0.15)	5.3(0.06)	0.28(0.10)
			Net(Sed)	4	7.34(0.19)	5.0(0.21)	0.24(0.11)

---

<sup>1</sup> Sediment sample taken adjacent to recruitment boxes

<sup>2</sup> Sediment sample taken adjacent to plant pots

<sup>3</sup> Sediment taken directly from open pots

<sup>4</sup> Sediment taken directly from netted pots

Table 2. Analysis of variance on the mean arcsine-transformed percent survival data of cultured clam juveniles at Duck Brook Flat, Cutler, Maine (June 2018 to January 2019). Cultured clam juveniles were added to plastic horticultural pots (EU; density = 1,360 m<sup>-2</sup>) that were either open (exposed to predators) or protected with a piece of PetScreen® (fixed factor). EU were arrayed in three blocks (random factor) at each of three tidal heights (upper; mid; low; fixed factor) on 12 June 2018. Upper and mid intertidal EU were sampled on 31 October 2018. Low EU were sampled on 18 January 2019. (n = 5)

Source of Variation	DF	Type I SS	Mean Square	F Value	Pr > F
Tidal Height	2	56.59522719	28.29761360	0.09	0.9109
Block(Tidal Height)	6	1791.26640	298.54440	1.48	0.1969
Predator Exclusion Treatment	1	36281.72648	36281.72648	174.63	<.0001
Tidal Height x Treatment	2	1163.26342	581.63171	2.80	0.1384
Treatment x Block(Tidal Hgt)	6	1246.59250	207.76542	1.03	0.4126
Error	72	14513.78306	201.58032		
Total	89	55053.22709			

Table 3. Analysis of variance on the mean absolute growth of cultured juveniles in EU at Duck Brook Flat, Cutler, Maine (June 2018 to January 2019). (See Table 2 for details on sources of variation.) Sample size (n) is variable due to survival rate; hence, Type III sums of squares are used (Shaw and Mitchell-Olds, 1993).

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Tidal Height	2	11.79702667	5.89851334	0.19	0.8324
Block(Tidal Height)	6	187.0930660	31.1821777	1.88	0.1024
Predator Exclusion Treatment	1	599.7614971	599.7614971	38.58	0.0008
Tidal Height x Treatment	2	126.8124115	63.4062057	4.08	0.0761
Treatment x Block(Tidal Hgt)	6	93.2732708	15.5455451	0.93	0.4780
Error	53	881.325226	16.628778		
Total	70	1924.509652			

Table 4. Analysis of variance on the square root-transformed mean number of wild recruits (0-year class individuals) of *Mya arenaria* in EU occurring at Duck Brook Flat, Cutler, Maine (June 2018 to January 2019). (See Table 2 for details on sources of variation.) A priori contrasts are indented and occur directly beneath the tidal height and tide height x treatment interaction source of variation. (n = 5)

Source of Variation	DF	Type I SS	Mean Square	F Value	Pr > F
Tidal Height	2	76.59562804	38.29781402	19.28	0.0024
High and Mid vs. Low	1	60.41870059	60.41870059	30.41	0.0015
High vs. Mid	1	16.17692745	16.17692745	8.14	0.0290
Block(Tidal Height)	6	11.92077810	1.98679631	1.48	0.1969
Predator Exclusion Treatment	1	113.77839672	113.77839671	132.39	<.0001
Tidal Height x Treatment	2	27.28270113	13.64135058	15.87	0.0040
Low v Rest x Open vs. Net1		22.84763241	22.84763241	26.59	0.0021
High v. Mid x Open vs. Nt1		4.43506864	4.43506864	5.16	0.0635
Treatment x Block(Tidal Hgt)	6	5.15638596	0.85939761	0.64	0.6974
Error	72	96.59908382	1.34165391		
Total	89	331.33297364			

Table 5. Analysis of variance on mean SL of wild recruits in EU at Duck Brook Flat, Cutler, Maine (June 2018 to January 2019). (See Table 2 for details on sources of variation.) A priori contrasts are indented and occur directly beneath the tidal height source of variation. Sample size (n) is variable due to some EU without any recruits; hence, Type III sums of squares are used (Shaw and Mitchell-Olds, 1993).

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Tidal Height	2	40.74789219	20.37394610	6.22	0.0344
High and Mid vs. Low	1	29.85607695	29.85607695	9.12	0.0234
High vs. Mid	1	16.31493910	16.31493910	4.98	0.0671
Block(Tidal Height)	6	19.64740050	3.27456678	0.63	0.7053
Predator Exclusion Treatment	1	0.00232851	0.00232851	0.00	0.9782
Tidal Height x Treatment	2	8.86513932	4.43256966	1.55	0.2867
Treatment x Block(Tidal Hgt)	6	17.16150843	2.86025140	0.55	0.7675
Error	62	322.01480960	5.19378731		
Total	79	416.90296761			

Table 6. Analysis of variance on the square root-transformed mean number of wild 0-year class juveniles of *Mya arenaria* in recruitment boxes at Duck Brook Flat, Cutler, Maine. Planned contrasts are indented and appear below the Tidal Height source of variation. Five boxes were placed in each of three blocks at three tidal heights (upper, mid, lower) on 12 June 2018. Boxes from the upper and mid heights were retrieved on 31 October 2018. Boxes from the lower intertidal were retrieved on 18 January 2019.

Source of Variation	DF	Type I SS	Mean Square	F Value	Pr > F
Tidal Height	2	342.6380822	171.3190411	15.12	0.0045
Low vs. Upper & Mid	1	170.4112852	170.4112852	15.04	0.0082
Upper vs. Mid	1	172.2267970	172.2267970	15.20	0.0080
Block(Tidal Height)	6	67.9985761	11.3330960	1.09	0.3857
Error	36	373.5542846	10.3765079		
Total	44	784.1909429			

Table 7. Analysis of variance on the arcsine-transformed mean percent survival data from Larrabee Cove, Machiasport, Maine Flat (June 2018 to February 2019). Cultured clam juveniles were added to plastic horticultural pots (EU; density = 1,360 m<sup>-2</sup>) that were either open (exposed to predators) or protected with a piece of PetScreen® (fixed factor). EU were arrayed in three blocks (random factor) at each of three tidal heights (upper; mid; low; fixed factor) on 13 June 2018. Upper and mid intertidal EU were sampled on 13 November 2018. Low EU were sampled on 16 February 2019. Planned comparisons appear indented and below the Tidal Height and Tidal Height x Treatment source of variation. (n = 5)

Source of Variation	DF	Type I SS	Mean Square	F Value	Pr > F
Tidal Height	2	1675.064688	837.532344	33.87	0.0005
Upper and Mid v. Low	1	1674.433441	1674.433441	67.72	0.0002
Upper vs. Mid	1	0.631247	0.631247	0.03	0.8783
Block(Tidal Height)	6	148.34822	24.72470	0.30	0.9365
Treatment	1	22880.50071	22880.50071	260.45	<.0001
Tidal Height x Treatment	2	953.60503	476.80252	5.43	0.0451
Up/Mid v. Low x Treatment	1	935.468098	935.468098	10.65	0.0172
Up v. Mid x Treatment	1	18.136934	18.136934	0.21	0.6655
Treatment x Block(Tidal Hgt)	6	527.10143	87.85024	1.05	0.3979
Error	72	5997.82336	83.30310		
Total	89	32182.44344			



Table 8. Analysis of variance on the mean absolute growth of cultured soft-shell clams in EU at Larrabee Cove (June 2018 to February 2019). (See Table 7 for details on sources of variation.) Sample size (n) is variable due to survival rate; hence, Type III sums of squares are used (Shaw and Mitchell-Olds, 1993). Planned orthogonal contrasts are indented and appear directly beneath the Tidal Height source of variation.

Source of Variation	DF	Type III SS	Mean Square	F Value	Pr > F
Tidal Height	2	727.8735576	363.9367788	50.26	0.0002
Upper & Mid vs. Low	1	632.5442746	632.5442746	87.36	<.0001
Upper vs. Mid	1	95.3292830	95.3292830	13.17	0.0110
Block(Tidal Height)	6	43.4442661	7.2407110	1.32	0.2613
Treatment	1	511.4695980	511.4695980	21.89	0.0034
Tidal Height x Treatment	2	22.2911129	11.1455564	0.48	0.6423
Treatment x Block(Tidal Hgt)	6	140.1650495	23.3608416	4.26	0.0011
Error	64	351.098361	5.485912		
Total	81	1807.077230			

Table 9. Analysis of variance on the square root-transformed mean number of wild recruits of *Mya arenaria* at Larrabee Cove, Machiasport, Maine from June 2018 to February 2019. Pre-planned, orthogonal contrasts appear indented and below the Tidal Height and Tidal Height x Treatment source of variation. (n = 5).

Source of variation	DF	Type I SS	Mean Square	F Value	Pr > F
Tidal Height	2	27.65618206	13.82809103	11.53	0.0088
Upper & Mid vs. Low	1	7.49470881	7.49470881	6.25	0.0465
High vs. Mid	1	20.16147325	20.16147325	16.81	0.0064
Block(Tidal Height)	6	7.19680994	1.19946832	1.61	0.1562
Treatment	1	22.04189379	22.04189379	36.97	0.0009
Tidal Height x Treatment	2	6.72294882	3.36147441	5.64	0.0419
Up/Mid v. Low x Treatment	1	3.49277604	3.49277604	5.86	0.0518
Upper v. Mid x Treatment	1	3.23017278	3.23017278	5.42	0.0588
Treatment x Block(Tide)	6	3.57764849	0.59627475	0.80	0.5721
Error	72	53.5722282	0.7440587		
Total	89	120.7677113			

Table 10. Analysis of variance on mean SL of wild recruits in EU at Larrabee Cove, Machiasport, Maine (June 2018 to February 2019). (See Table 7 for details on sources of variation.) A priori contrasts are indented and occur directly beneath the tidal height source of variation. Sample size (n) is variable due to some EU without any recruits; hence, Type III sums of squares are used (Shaw and Mitchell-Olds, 1993).

Source of variation	DF	Type III SS	Mean Square	F Value	Pr > F
Tidal Height	2	79.84329179	39.92164589	5.87	0.0387
Upper & Mid vs. Low	1	64.28408709	64.28408709	9.46	0.0218
Upper vs. Mid	1	15.55920471	15.55920471	2.29	0.1811
Block(Tidal Height)	6	40.7910429	6.7985071	0.83	0.5561
Treatment	1	106.5428520	106.5428520	10.36	0.0323
tidehgt*Treatment	2	12.4083550	6.2041775	0.60	0.5901
Treatment x Block(Tide Hgt)	4	41.1226948	10.2806737	1.25	0.3054
Error	40	328.8739023	8.2218476		
Total	55	690.3836907			

Table 11. Analysis of variance on the square root-transformed mean number of wild 0-year class juveniles of *Mya arenaria* in recruitment boxes at Larrabee Cove, Machiasport, Maine. Planned contrasts are indented and appear below the Tidal Height source of variation. Five boxes were placed in each of three blocks at three tidal heights (upper, mid, lower) on 13 June 2018. Boxes from the upper and mid intertidal heights were retrieved on 13 November 2018. Boxes from the lower intertidal were retrieved on 16 February 2019.

Source of variation	DF	Type I SS	Mean Square	F Value	Pr > F
Tidal Height	2	462.4501848	231.2250924	176.13	<.0001
Upper & Mid vs. Low	1	445.4137047	445.4137047	339.29	<.0001
Upper vs. Mid	1	17.0364801	17.0364801	12.98	0.0113
Block(Tidal Height)	6	7.8766656	1.3127776	0.39	0.8813
Error	36	121.5313425	3.3758706		
Total	44	591.8581929			

## Figure Legends

Figure 1. Mean number (+95% CI) of wild soft-shell clam recruits in protected and control EU at Duck Brook Flat, Cutler, Maine. EU were sampled on 31 October 2018 from the upper and mid intertidal, and on 18 January 2019 from the low intertidal. See Table 4 for ANOVA results. (n = 10).

Figure 2. Size-frequency distribution of wild recruits of *Mya arenaria* in all EU at the upper, mid, and lower intertidal at Duck Brook Flat, Cutler, Maine. See legend from Fig. 1 for sampling dates across tidal heights. A 3 x 5 G-test of independence on the size-frequencies demonstrated a significant difference across tidal heights ( $G = 55.7$ ,  $df = 8$ ,  $P < 0.0001$ ).

Figure 3. Mean (+95% CI) of wild soft-shell clam juveniles in recruitment boxes at Duck Brook Flat, Cutler, Maine. See legend from Fig. 1 for sampling dates across tidal heights. See Table 6 for ANOVA results. (n = 15).

Figure 4. Relationship between pH and  $\log_{10}(\Omega_{\text{aragonite}})$  of water and sediment samples taken at Duck Brook Flat, Cutler, Maine (July 2018 to January 2019). The 95% confidence interval is shown along with the least-squares regression line. ( $Y = -6.79 + 0.8343X$ ;  $r^2 = 0.822$ ,  $P < 0.0001$ ,  $n = 45$ ).

Figure 5. Mean (+ 95% CI) of cultured clam survival in open and protected EU at Larrabee Cove, Machiasport, Maine across three tidal heights. EU were deployed on 13 June 2018, and collected from the upper and mid intertidal on 13 November 2018, and from the low intertidal on 16 February 2019. See Table 7 for ANOVA results. (n = 15).

Figure 6. Mean (+ 95% CI) of absolute growth of live cultured clams in open and protected EU at Larrabee Cove, Machiasport, Maine across three tidal heights. See legend from Fig. 5 for sampling dates across tidal heights. See Table 8 for ANOVA results. (n = 15).

Figure 7. Mean number (+95% CI) of wild soft-shell clam recruits in protected and control EU at Larrabee Cove, Machiasport, Maine. See legend from Fig. 5 for sampling dates across tidal heights. See Table 9 for ANOVA results. (n = 10).

Figure 8. Size-frequency distribution of wild recruits of *Mya arenaria* at three tidal heights at Larrabee Cove, Machiasport, Maine. See legend from Fig. 5 for sampling dates across tidal heights. A 3 x 5 G-test of independence indicated that the distribution of sizes varied significantly across tidal heights ( $G = 36.9$ ,  $df = 8$ ,  $P < 0.0001$ ). Data is pooled across predator exclusion treatments.

Figure 9. Size-frequency distribution of wild recruits of *Mya arenaria* in open and protected EU at Larrabee Cove, Machiasport, Maine. See legend from Fig. 5 for sampling dates across tidal heights. A 2 x 5 G-test of independence indicated that the distribution of sizes varied significantly across the two treatments ( $G = 64.2$ ,  $df = 4$ ,  $P < 0.0001$ ), with a disproportionate number of individuals > 10 mm SL in protected vs. open EU.

Figure 10. Mean (+95% CI) of wild soft-shell clam juveniles in recruitment boxes at Larrabee Cove, Machiasport, Maine. See legend from Fig. 5 for sampling dates across tidal heights. See Table 11 for ANOVA results. (n = 15).

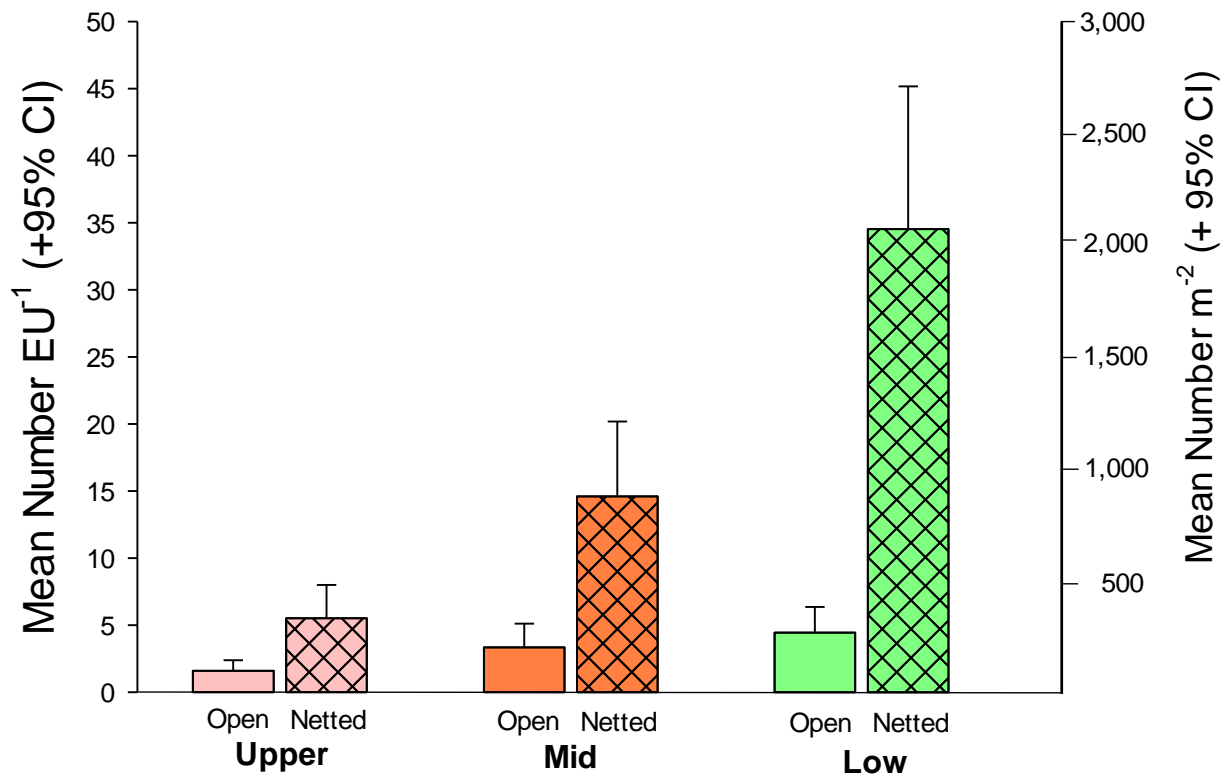
Figure 11. Relationship between pH and sampling date for sediment samples taken from Larrabee Cove, Machiasport, Maine. Date was converted to a numeric value with August, September, November, and February considered as 8, 9, 11, and 14, respectively. A cubic model best fit the data ( $Y = 76.95 - 20.11X + 1.89X^2 - 0.06X^3$ ;  $r^2 = 0.588$ ;  $P < 0.0001$ ; n = 34).

Figure 12. Relationship between  $\Omega_{\text{aragonite}}$  and sampling date for sediment samples taken from Larrabee Cove, Machiasport, Maine. Date was converted to a numeric value with August, September, November, and February considered as 8, 9, 11, and 14, respectively. A cubic model best fit the data ( $Y = 23.49 - 6.82X + 0.65X^2 - 0.02X^3$ ;  $r^2 = 0.369$ ;  $P < 0.0001$ ; n = 34).

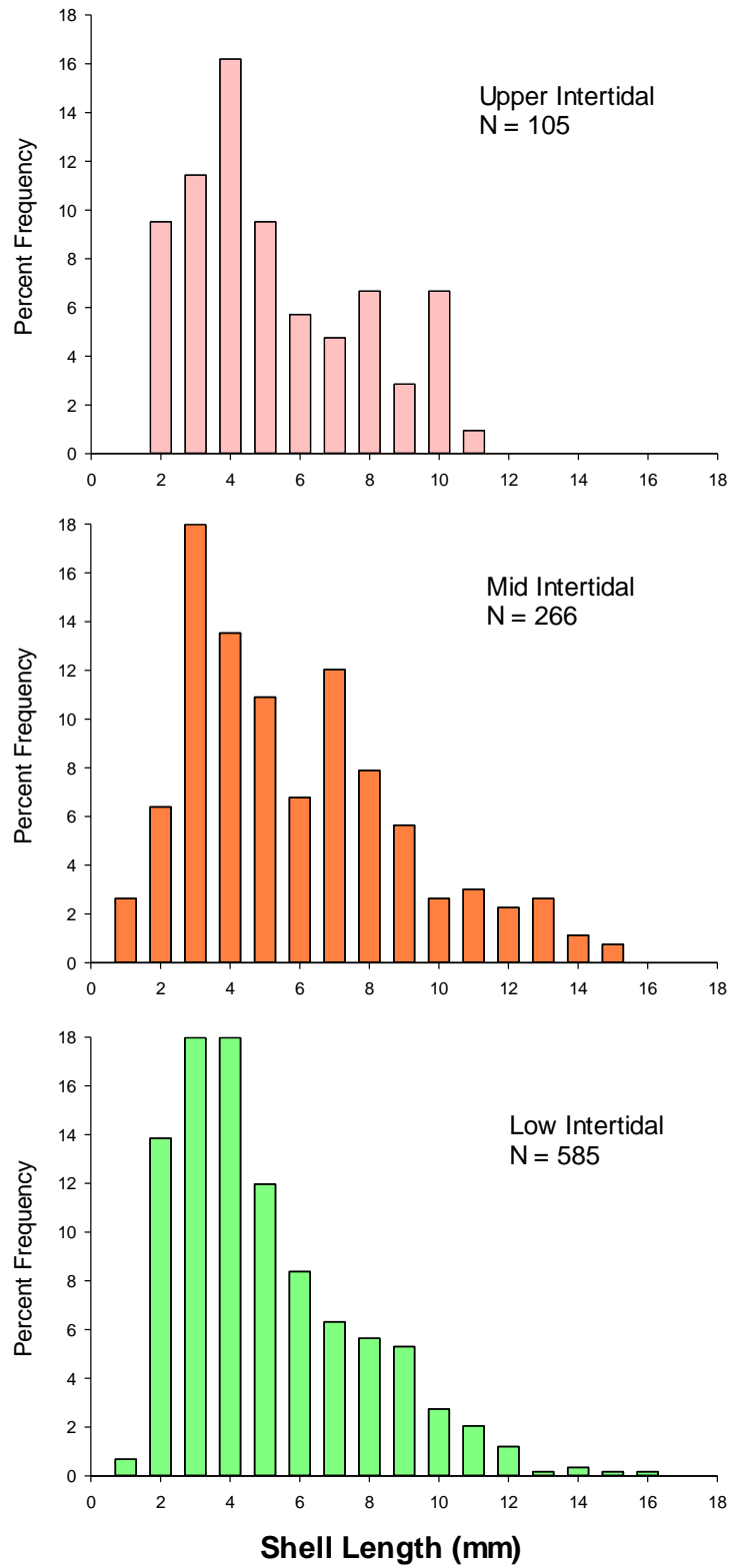
Figure 13. Relationship between pH and  $\log_{10}(\Omega_{\text{aragonite}})$  of water and sediment samples taken at Larrabee Cove, Machiasport, Maine (July 2018 to February 2019). The 95% confidence interval is shown along with the least-squares regression line. ( $Y = -6.26 + 0.7615X$ ;  $r^2 = 0.834$ ,  $P < 0.0001$ , n = 52).

Figure 14. Relationship between pH and  $\log_{10}(\Omega_{\text{aragonite}})$  of water and sediment samples taken at Duck Brook Flat (N = 45) and Larrabee Cove (N = 52). Analysis of regression lines indicated that the slopes of the two regression lines were similar ( $F = 0.65$ ,  $df = 1, 93$ ,  $P = 0.4236$ ), and ANCOVA indicated that the lines were congruent ( $F = 0.03$ ,  $df = 1, 94$ ,  $P = 0.8666$ ). The least-squares regression line ( $Y = -6.65 + 0.81X$ ;  $r^2 = 0.835$ ,  $P < 0.0001$ ) and 95% CI are shown.

**Figure 1.**



**Figure 2.**





**Figure 3.**

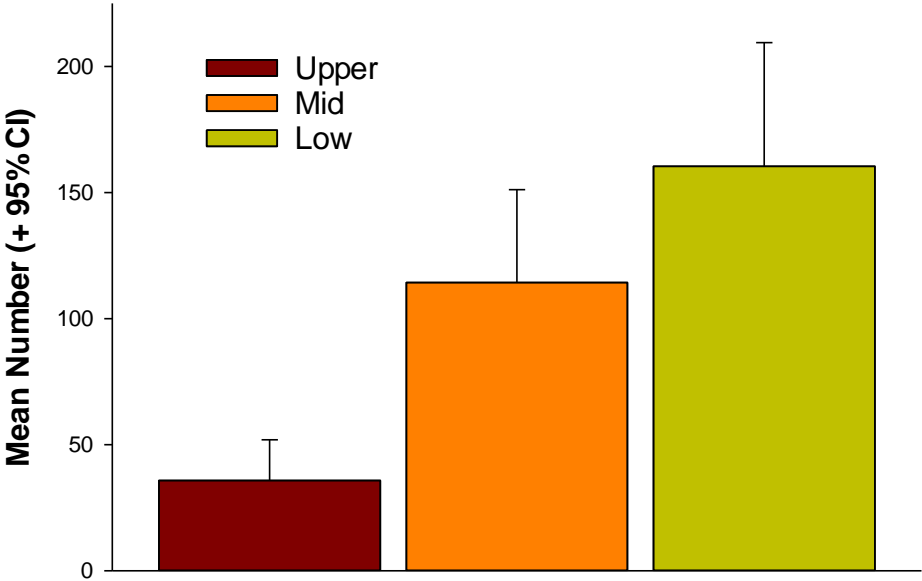


Figure 4.

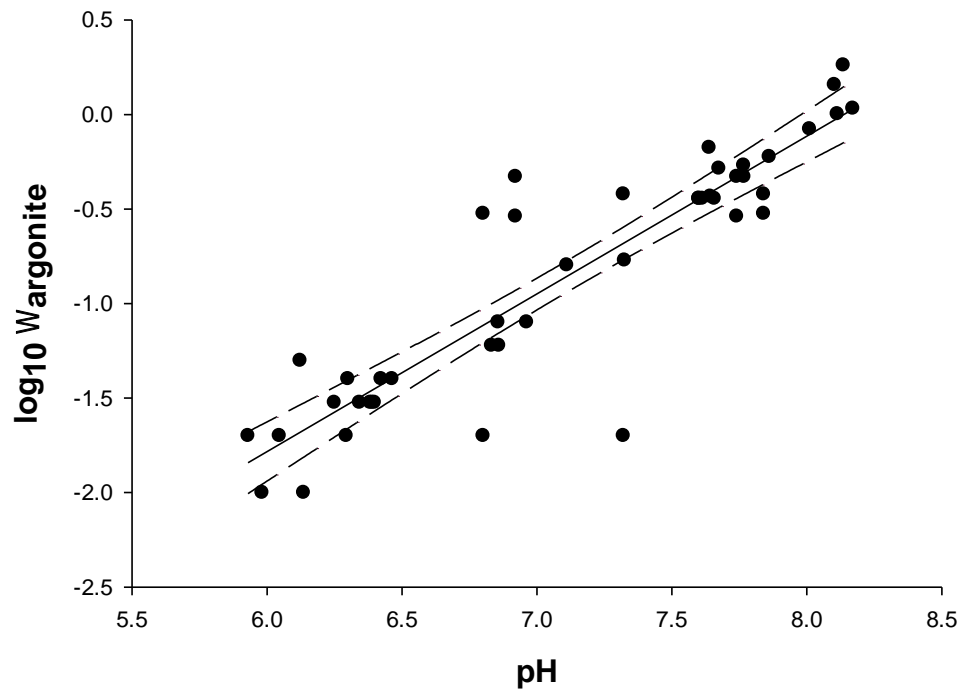
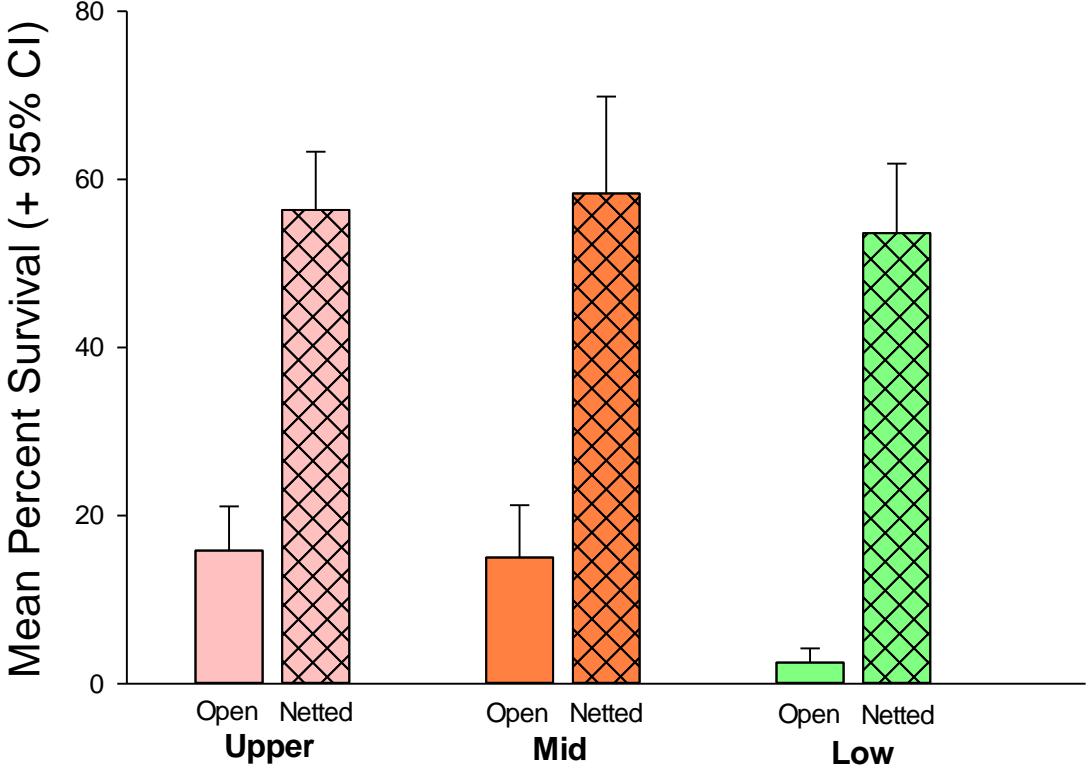


Figure 5.



**Figure 6.**

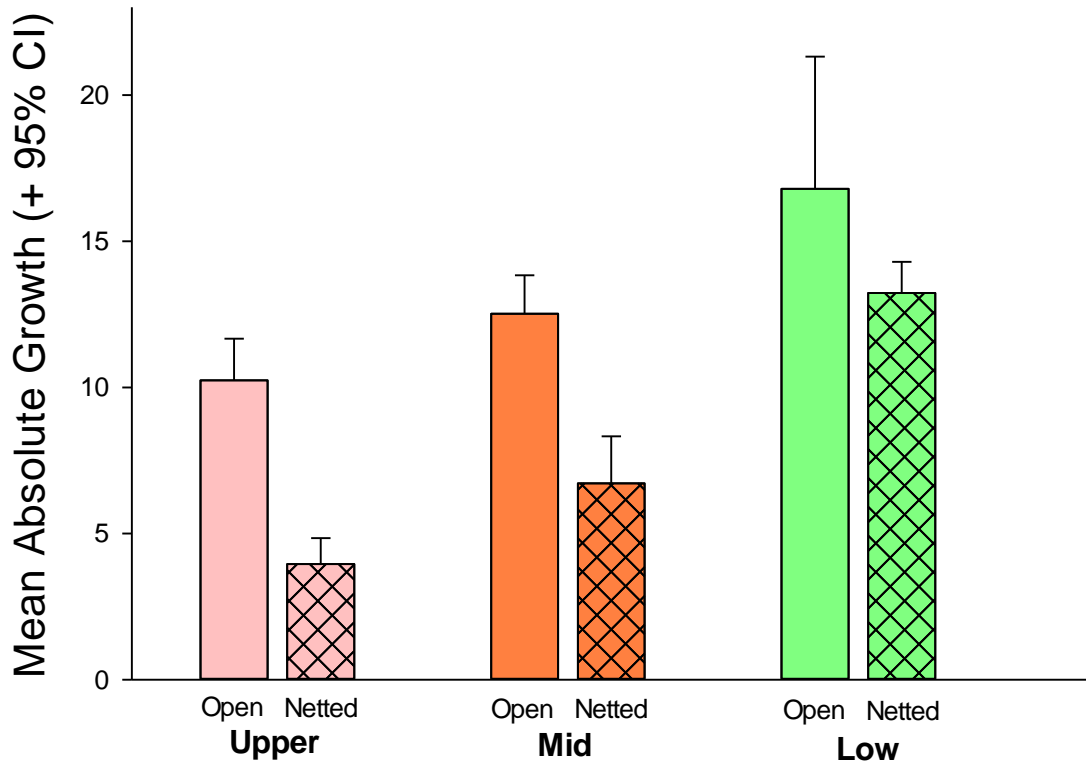
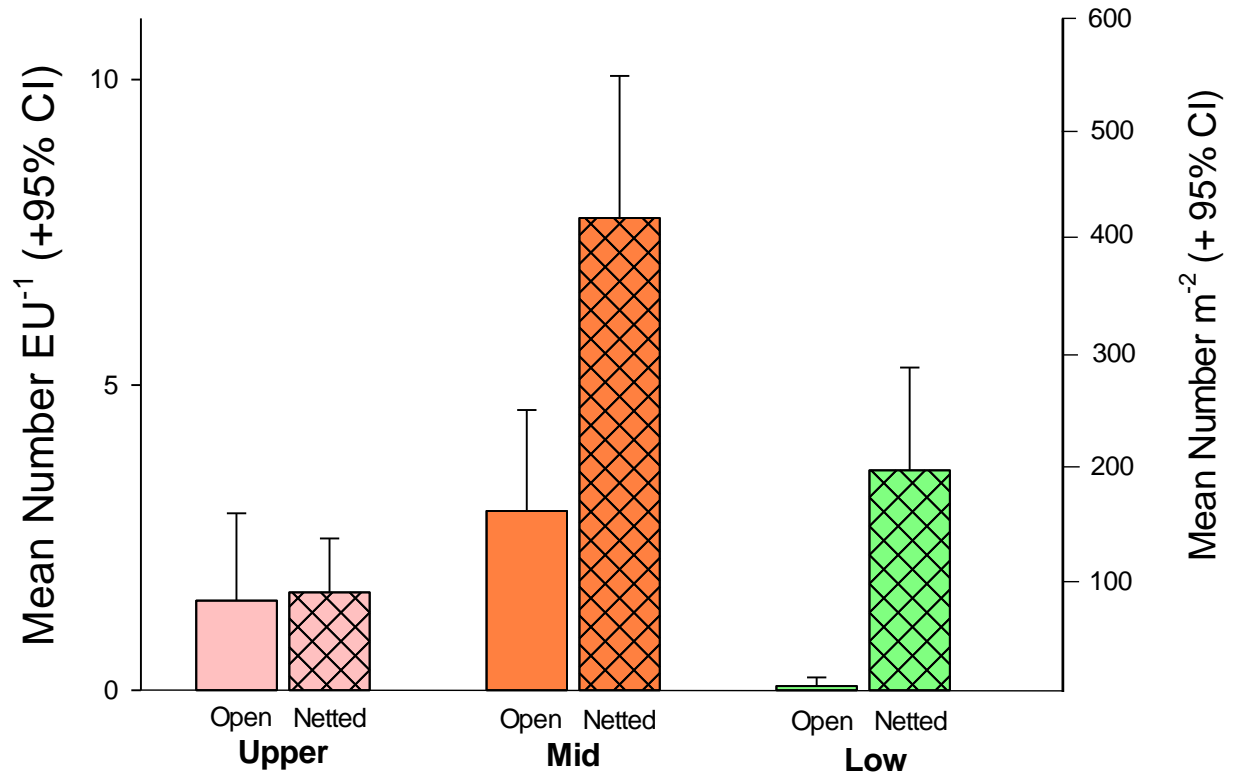
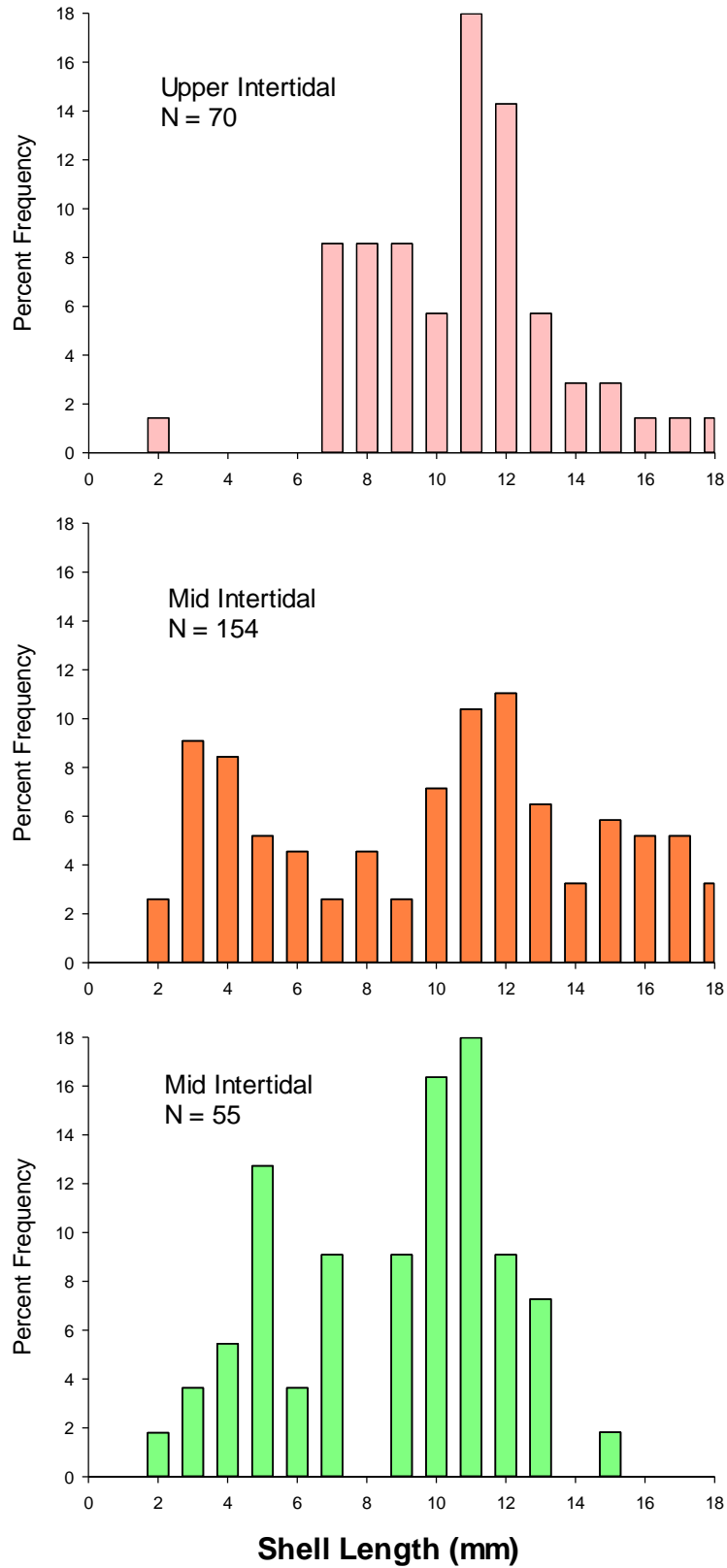


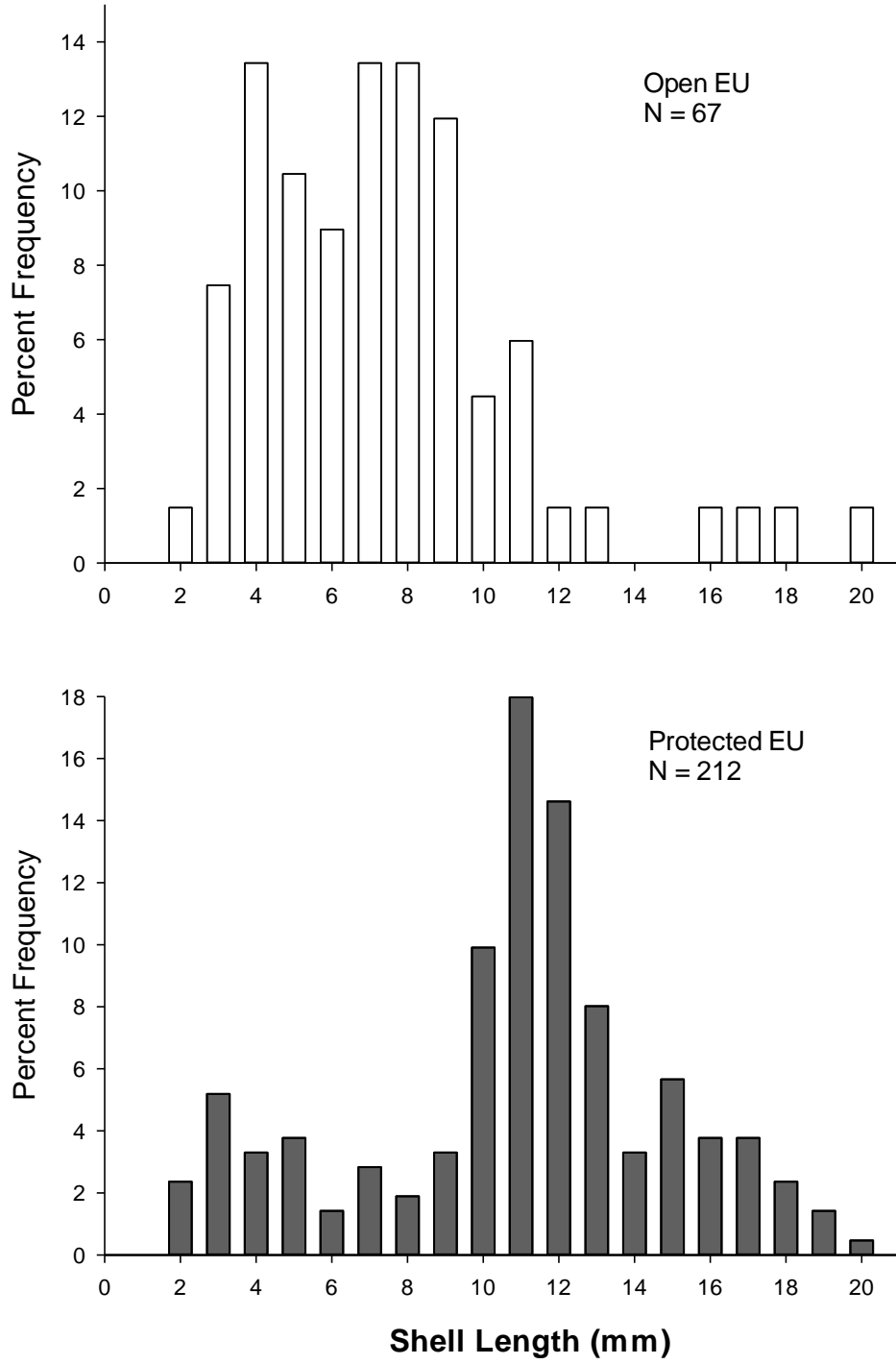
Figure 7.



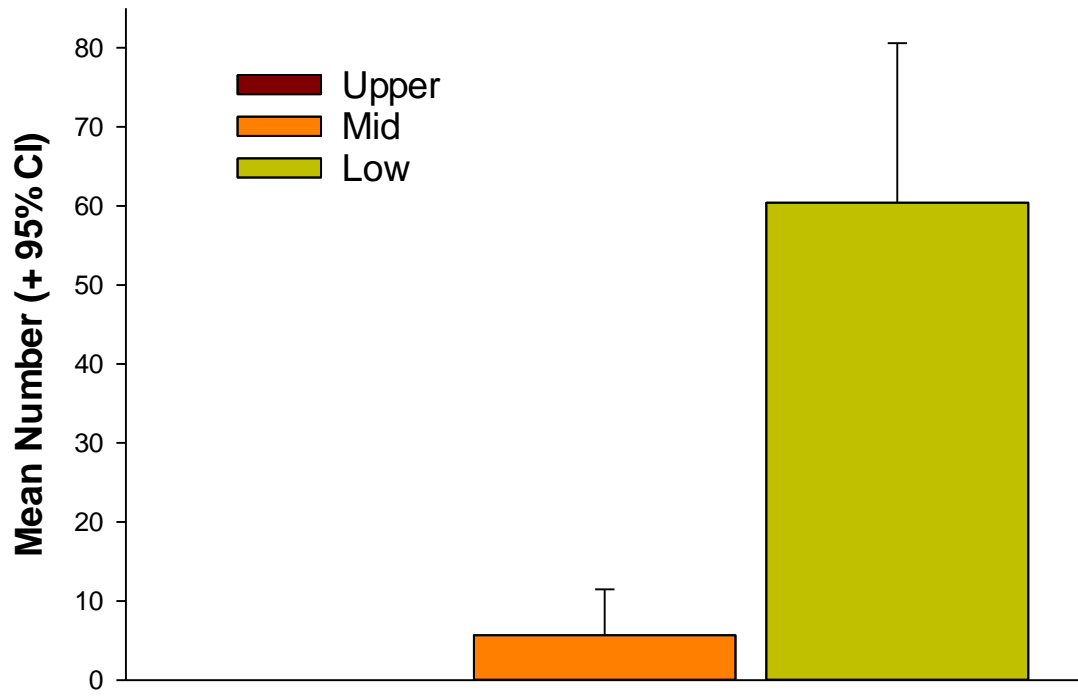
**Figure 8.**



**Figure 9.**

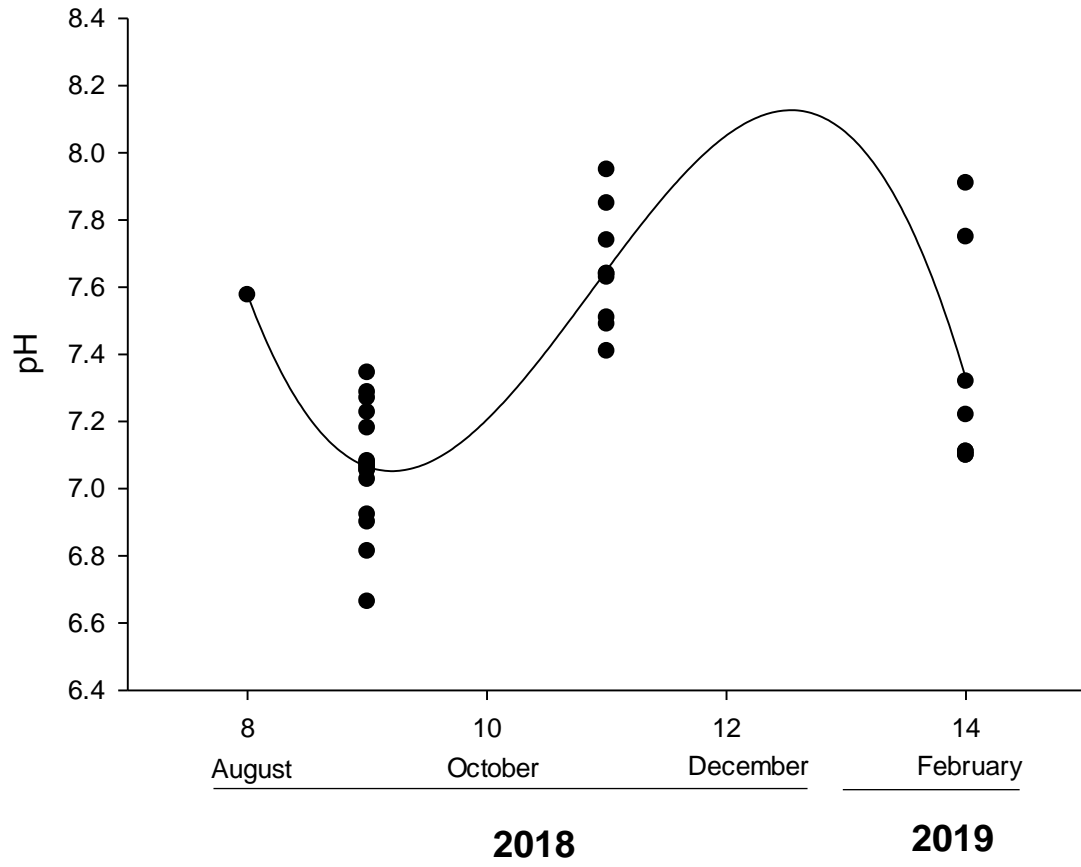


**Figure 10.**





**Figure 11.**



**Figure 12.**

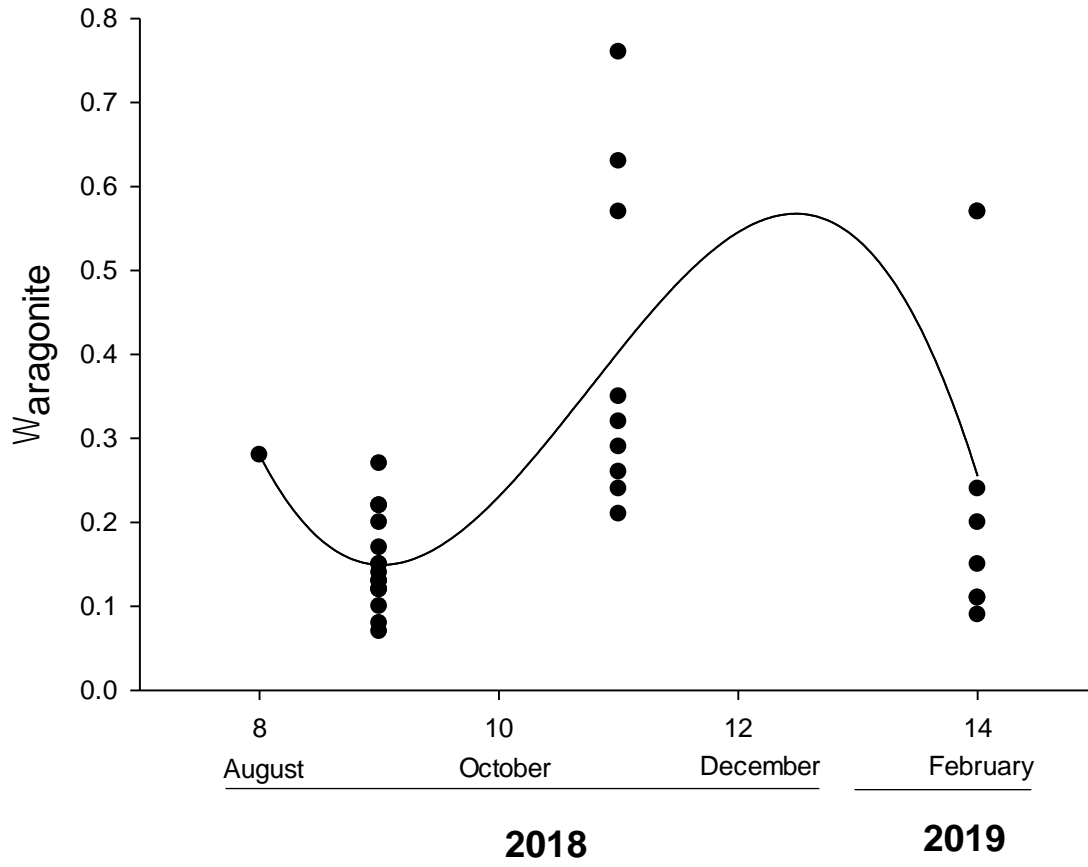


Figure 13.

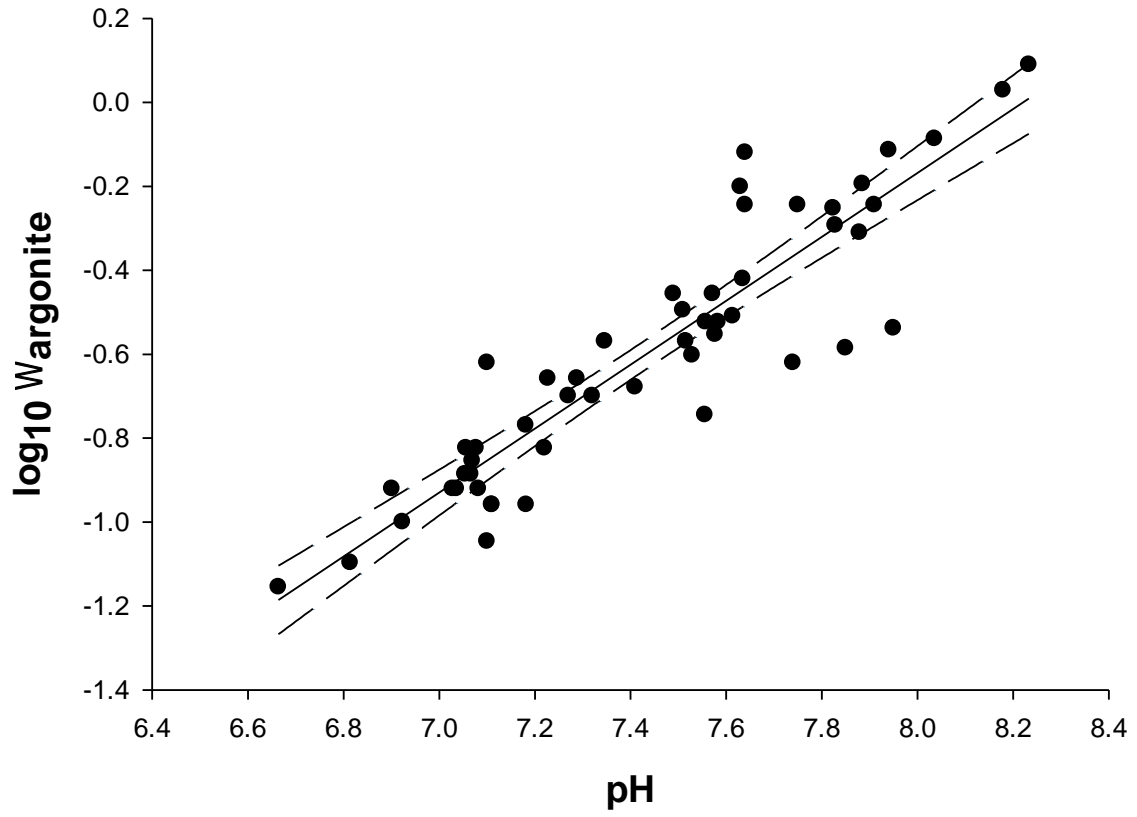
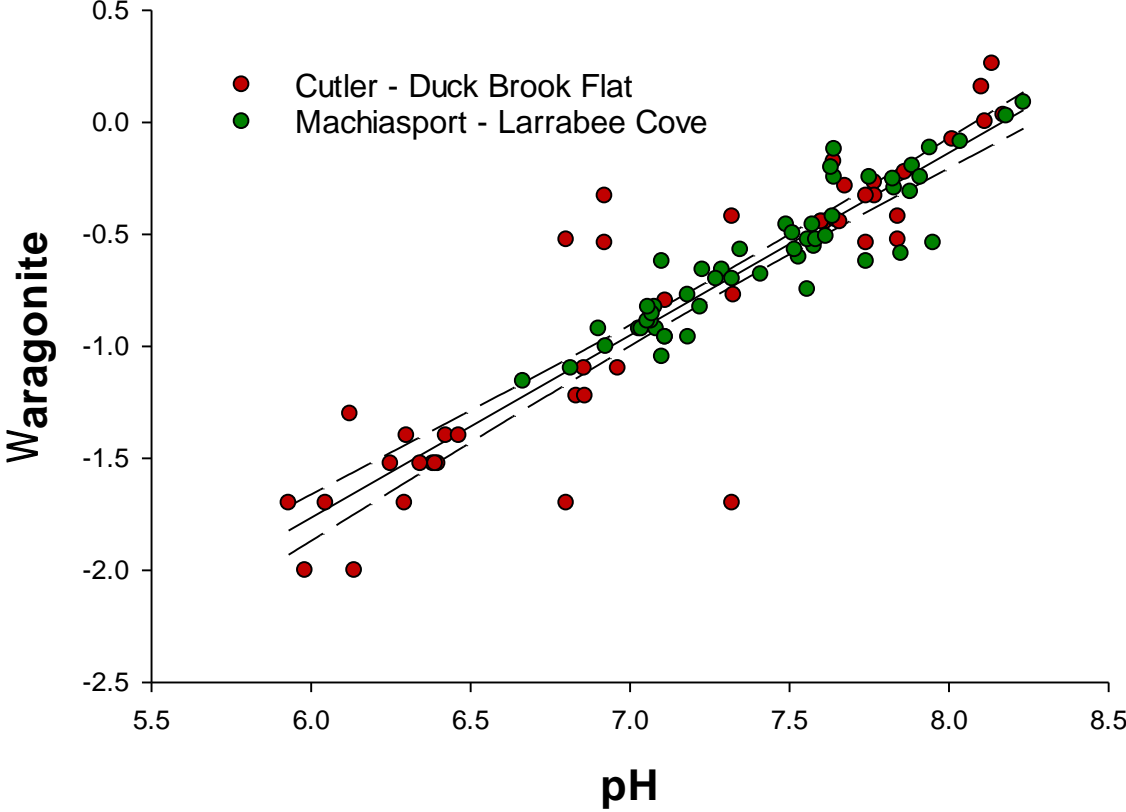


Figure 14.



# **APPENDIX**

## **Selected Photos of Field Sites and Activities**

**Photos taken by Hailey Wegner**



**Duck Brook Flat, Cutler, Maine**



**Recruitment boxes and plastic plant pots (EU – lower left hand corner) at Duck Brook Flat, Cutler, Maine (boxes in this block were adjacent to an intertidal eelgrass bed)**





**Sunrise at the upper intertidal of Larrabee Cove, Machiasport, Maine**





**Measuring pH in an EU with the protective netting removed**



**Open and protected plant pots (foreground) and recruitment boxes (background) at Duck Brook Flat, Cutler, Maine**





**Sediment sample from which pH and total alkalinity are determined**