Downeast Institute

Shell Hash for Buffering Mudflat Acidification: Experiment on the Interactive Effects of Crush Size and Density on Sediment Porewater Carbonate Chemistry in South Portland

Introduction

Out of concern that ocean acidification will reduce mudflat porewater pH and aragonite saturation below natural minima, several studies (Dethier et al. 2019, Green et al. 2009, Green et al. 2013, Greiner et al. 2018, Ruesink et al. 2014, Beal et al. 2020) have tested hypotheses that buffering marine sediments with crushed bivalve shell recycled from the seafood industry, i.e. "shell hash", will result in desirable increases in those parameters and concomitant benefits to bivalve abundance. These experiments have varied in length (i.e. 16 days to 7 months), presence of predator exclusion, species of shell, shell crush size, and shell density. Results have been mixed, with many field studies reporting no impact of shell hash addition – perhaps due in part to the lack of standardization of shell crush size and density treatments (see chart below).

The following chart from Beal et al. 2020 summarizes previous shell hash sediment buffering experiments and results.

Authors	Shell species	Size of Shell	Location	Date(s)	Results
Beal et al. (2020)	Soft-shell clam (*Mya arenaria),* Olympus oyster (*Ostrea lurida)*	1mm, 5-10mm, 15-20mm *Mya*; 1mm *Ostrea.* Larger plots avg. 19mm *Mya.* Also, marble (avg. 15 mm) and granite (avg. 22mm) chips.	Freeport, ME	May-Oct./ Nov. in 2014, 2015, 2016	Density and size of both bivalve species at the end of most field trials were significantly greater in predator-exclusion treatments vs. controls independent of shell treatment. In all 8 trials, neither *Mya* nor *Mercenaria* responded positively to the presence of shell additions.
Dethier et al. (2019)	Pacific oyster (*Crassostrea gigas*)	< 10mm	Saltish Sea, WA	July-Sep. 2007 (64-75 days)	No differences in survival or growth of *R. philippinarum* between crushed shell plots and pebble-sand (non-buffering substrate).
Green et al. (2009)	*M. arenaria*	5 mm	West Bath, ME	July 2007 (16 days)	Ω increased in buffered plots, *Mya* recruits increased 3.5xs in buffered v. control plots.
Green et al. (2013)	*M. arenaria*	1mm	South Portland, ME	June-July (35 days)	Ω increased in buffered plots. *Mya* recruits increased 2xs in buffered vs. control plots.
Grenier et al. (2018)	70% *C. gigas,* 30% mixed species clam	< 50 mm	Fidalgo Bay, WA	July-Aug. (55 days)	pH and Ω increased significantly in crushed shell treatment. No increase in *R. philippinarum* recruits.
Ruesink et al. (2014)	*Mya a., C. gigas, R. philippinarum*	10-20mm	Willapa Bay, WA	July-Aug. (49 days)	No increase in recruitment of *R. philippinarum* or *M. arenaria*

In 2021 the following lab and field experiment were undertaken to continue the effort to find sediment buffering solutions to ocean acidification and minimize effects on commercial important shellfish. The laboratory experiment tested the interactive effects of three shell crush sizes and three shell density treatments on porewater carbonate chemistry in mud cores. The field experiment was informed by the results of the laboratory trial, and examined the effects of shell hash buffering on porewater carbonate chemistry and abundance of two commercially important bivalve species: soft-shell clams (*Mya arenaria*) and quahogs (*Mercenaria mercenaria*) in experimental units on a Maine mudflat.

With the understanding that responses in the natural environment don't usually happen in isolation, the design of the field experiment integrated biological and ecological knowledge (i.e. clam lifecycle, green crab population dynamics) and findings from previous field research studies into the design and execution. The purpose of the field experiment was to determine if 1) adding shell hash decreases acidity of porewater (i.e. water surrounding sediments), and if so, what density and size works best?, along with if 2) applying shell hash increases the survival of young-of-the year soft-shell clams and quahogs.

Below details the results of the lab experiment and both chemistry and recruitment aspects of the field experiment.

1. Lab Experiment

1.1 Methods & Materials

A preliminary laboratory experiment was conducted with the aim of informing treatment selection (i.e., shell hash size and density) for the field experiment. Samples of oyster shell were crushed using the modified glass bottle crusher, and the resulting shell hash was passed through a series of graded mesh sieves (6,000 – 3,500 µm; 1,950 – 1,650 µm; 300 – 125 µm) to produce three shell crush sizes. On April 1st, 2021, 55 benthic sediment cores (area = 0.018 m^2, depth = 15 cm) were collected at low tide from the tidal mudflat at Mud Hole Cove in Beals, Maine, using a custom corer. The cores were transported to Downeast Institute and carefully transferred right-side-up into plastic buckets of equivalent dimensions with drainage holes so as not to disturb the natural stratification.

Fifty of the cores were treated with a factorial combination of shell crush sizes (small, medium, large) and densities (5, 10, 15 g shell/100 cm^2 sediment) scattered evenly on the surface, while five cores were left untreated as controls in a 5x-replicated design. The remaining five cores were reserved for sampling initial carbonate chemistry conditions. The experimental cores were interspersed randomly on the floor of an indoor flow-through seawater basin in an arrangement of five rows and 11 columns, placed atop a sheet of plastic mesh to prevent sediment from spilling out of the bucket drainage holes. The shell hash added to each core was wetted with seawater to prevent it from drifting away when the basin was filled with seawater. Untreated seawater pumped in continuously from Black Duck Cove submerged the experimental cores to a depth of 0.75 m. After an initial submersion for 24 hrs, the basin was drained of seawater for 3 hrs and refilled twice daily using actuating ball valves fitted to the seawater inflow and outflow to simulate tidal cycling. The valves were controlled using an Apex aquarium controller system

(Neptune Systems), programmed to sync with the natural tidal cycle outside. The experiment was left undisturbed for 2 weeks before the basin was drained and experimental cores were sampled for porewater carbonate chemistry.

Prior to sampling, the temperature (°C) of each experimental core was measured using a handheld meter and probe (Oakton pH 450, Oakton 35618-05). Next, the upper 0.5 cm of each core was sampled using a custom mud scraping tool consisting of a steel auger housed in a cylindrical capsule. The samples were transferred into 50 ml centrifuge tubes and centrifuged (Beckman TJ-6) at 1,500 rpm for 5 min to separate porewater from sediment. Porewater was filtered to 0.45 µm using syringe filters, and salinity was measured using a handheld meter and probe (Oakton SALT 6+, Oakton EC-CONSEN91B). Porewater electromotive force (emf) was measured using a handheld meter and probes (Oakton pH 450, Oakton 35805-67, Oakton 35618-05). Emf measurements were converted to pH measurements on the total hydrogen ion scale (pH_T) in a 1-point calibration to TRIS-HCl synthetic seawater buffer formulated in the chemistry lab at Downeast Institute (Dickson et al. 2007; Paulsen & Dickson 2020) and pH-calibrated near sample temperature. Porewater total alkalinity (TA) was measured using the open-cell titration method for seawater (Dickson et al. 2007), and titrations were performed using an autotitrator (Hanna Instruments HI901C). Approximately 10 g of sample was titrated with HCl (approx. 0.01000 mol kg^{-1}) in a 0.6 mol kg^{-1} NaCl background. Sample TA was calculated from titration data using the seacarb package (Gattuso et al. 2021) in R (R Core Team 2021). Remaining seawater carbonate chemistry parameters (partial pressure of CO_2, pCO_2; dissolved inorganic carbon, DIC; saturation state of aragonite, Ω_{Ar}) were calculated for each experimental plot per sampling event from pH_T, temperature, salinity, and TA using CO2Sys v2.1 (Pierrot et al., 2006) (K_1, K_2 from Lueker et al. 2000; K_{HSO4} from Dickson 1990; B_T from Uppstrom 1974).

1.2 Results

In a 2-way ANOVA, neither shell crush size, shell density, nor the interaction of shell crush size and density predicted porewater pH_T in the experimental cores. However, shell size nearly predicted pH_T ($F(3) = 2.98$, $p = 0.056$), and cores with the smallest shell exhibited the highest pH_T. Similarly, in another model, shell size predicted porewater Ω_{Ar} ($F(3) = 4.26$, $p = 0.018$), with the smallest shell increasing Ω_{Ar} relative to control.

2. Field Experiment

2.1 Study site

The study site was a sheltered intertidal flat located in Casco Bay at Mill Cove, South Portland, Maine adjacent to Portland Harbor (Fig. 1; Lat. 43°38'20.73"N; 70°14'58.61"W), which was the same location used by Greene et al. (1999). While no quantitative analysis was conducted on sediment grain size, the flat consisted of a sandy mud (sensu Folk 1980).

2.2 Chemistry Methods and Materials

In the field, porewater carbonate chemistry was sampled on four dates: preliminary background sampling occurred on April 22nd, 2021 after experimental plots were delineated but before shell hash and nets were placed (20 plots), subsequent sampling of non-netted plots occurred on April 24th (one day after shell hash and nets were placed) and one month later on May 24th (60 plots each), and final sampling occurred between October 10th-11th after nets were removed (120 plots). Carbonate chemistry sampling consisted of measuring porewater electromotive force (emf) and temperature using a handheld meter and probes (Oakton pH 450, Thermo Scientific Orion 8135BN, Oakton 35618-05), as well as collecting sediment samples for later porewater extraction, preservation, and analysis.

Porewater emf (mV) and temperature (°C) were measured by inserting probes into the upper 0.5 cm of sediment at low tide (using a custom PVC probe holder to ensure consistency of depth), waiting approximately 30 s for the values to settle, and recording the values. For each sampling event, one emf and temperature measurement were collected per experimental plot. TRIS-HCl synthetic seawater buffer was formulated in the chemistry lab at Downeast Institute (Dickson et al. 2007; Paulsen & Dickson 2020), pH-calibrated to the average *in situ* porewater temperature during a sampling event using a UV/Vis spectrophotometer (Agilent Cary 60) and thermostatted cell holder (Quantum Northwest Cyl 100), cooled to that temperature using a dry bath (BT Lab Systems BT1105), and measured using the same meter and probes used to measure porewater emf. Porewater emf measurements were then converted to pH measurements on the total hydrogen ion scale (pH_T) by calculating a 1-point TRIS calibration from porewater emf, porewater temperature, TRIS emf, and TRIS pH (Dickson et al. 2007). Minor differences in porewater temperature between plots (i.e., within ±3°C of the average) were adjusted for by manually calculating temperature compensation for each pH_T measurement.

Sediment samples were collected by scraping sediment with a spatula into a 50 ml centrifuge tube until the tube was full. Immediately following each porewater emf/temperature measurement, samples were scraped from the upper 0.5 cm of sediment and from within a 15 cm diameter area adjacent to that measurement. For each sampling event, one sediment sample was collected per experimental plot. Immediately following each sampling event, sediment samples were taken to Southern Maine Community College, located 1.5 miles from the field site, and centrifuged (OHAUS Frontier 5000 Series) at 6,000 rpm for 5 mins to separate porewater from sediment. Following centrifugation, porewater was decanted into new tubes and preserved using saturated mercuric chloride solution (0.05% of sample volume). Preserved porewater samples were transported back to Downeast Institute for later total alkalinity (TA) analysis.

Porewater was filtered to 0.45 μm using syringe filters, and salinity was measured using a digital refractometer (Sper Scientific 300035). Porewater (TA) was measured using the spectrophotometrically-monitored single-step acid addition titration method for seawater (Yao & Byrne 1998; Liu et al. 2015), albeit adapted to the Cary 60 spectrophotometer and for smaller sample sizes. Changes implemented and solutions created for adapting the method included a custom ADL (Applications Development Language) script to guide the titration, a smaller 25-mm path length optical cell (Hellma 402-013-10), a custom insert for the Cary 60 consisting of a frame for holding the cell over a magnetic stir plate, a custom hook-shaped diffuser for aerating the sample, and lower-concentration HCl titrant (approx. 0.01000 mol kg^{-1}) administered using a stepper pipette. Porewater samples (ranging from 8-25 ml) and titrant were measured gravimetrically. Bromocresol purple solution (*R* ratio adjusted to 0.3) was used as the pH indicator. TA method accuracy was verified using CO_2 in seawater certified reference material

(CRM, batch #162) supplied by the Dickson lab (Scripps Institution of Oceanography, UC San Diego). Remaining seawater carbonate chemistry parameters (partial pressure of CO_2, pCO_2; dissolved inorganic carbon, DIC; saturation state of aragonite, Ω_{Ar}) were calculated for each experimental plot per sampling event from pH_T, temperature, salinity, and TA using CO2Sys v2.1 (Pierrot et al., 2006) (K_1, K_2 from Lueker et al. 2000; K_{HSO4} from Dickson 1990; B_T from Uppstrom 1974).

2.3 Chemistry Results

Porewater carbonate chemistry varied considerably with sampling date, shell crush size, and shell density (pH_T: Fig. 1; Ω_{Ar}: Fig. 2). In a linear mixed-effects model in which plot was specified as a random factor, presence of shell hash ($t(54) = 2.1$, $p = 0.036$) and date (October: $t(114) = -6.1$, $p < 0.0001$) predicted pH_T: presence of shell hash increased pH_T, and pH_T was lower during the October sampling date versus April and May. However, neither shell crush size, shell density, nor the interaction of shell crush size and density predicted pH_T, suggesting that smaller crush sizes and higher densities of shell were no more effective at increasing pH_T. In a similar model with porewater Ω_{Ar} as response, only date (May: $t(109) = 3.4$, $p = 0.014$; October: $t(109) = -2.5$, $p = 0.014$) predicted Ω_{Ar}: Ω_{Ar} was higher during the May sampling date and lower during October relative to April. In linear models testing for an effect of predator-deterrent netting on porewater pH_T and Ω_{Ar} during the October sampling date, when netting was removed, the netting factor failed to predict either parameter.

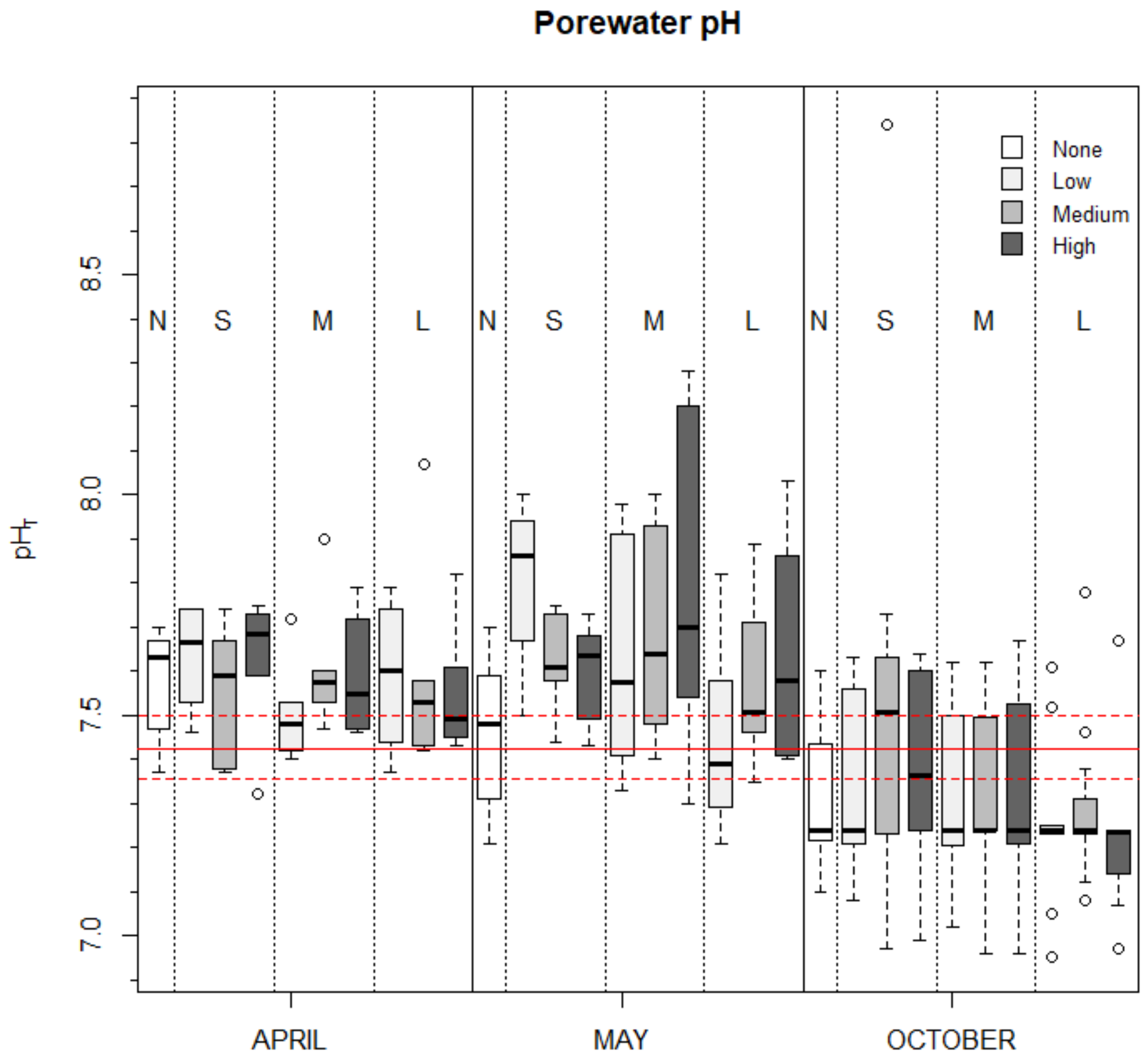

Figure 1: South Portland tidal mudflat porewater pH (total hydrogen ion scale) measured over time in experimental plots treated with different shell crush sizes and shell densities. Boxes are grouped by sampling date (x-axis) and shell crush size (N: no shell/control; S: small; M: medium; L: large), and ordered by shell density (legend). Boxes in April and May represent measurements collected from plots without predator-deterrent netting, whereas boxes in October represent measurements from both netted and non-netted plots (i.e., twice the number of measurements). Red lines represent the median pH (solid) and interquartile range (dashed) from background measurements collected 1 day prior to shell deployment/2 days prior to the April sampling date.

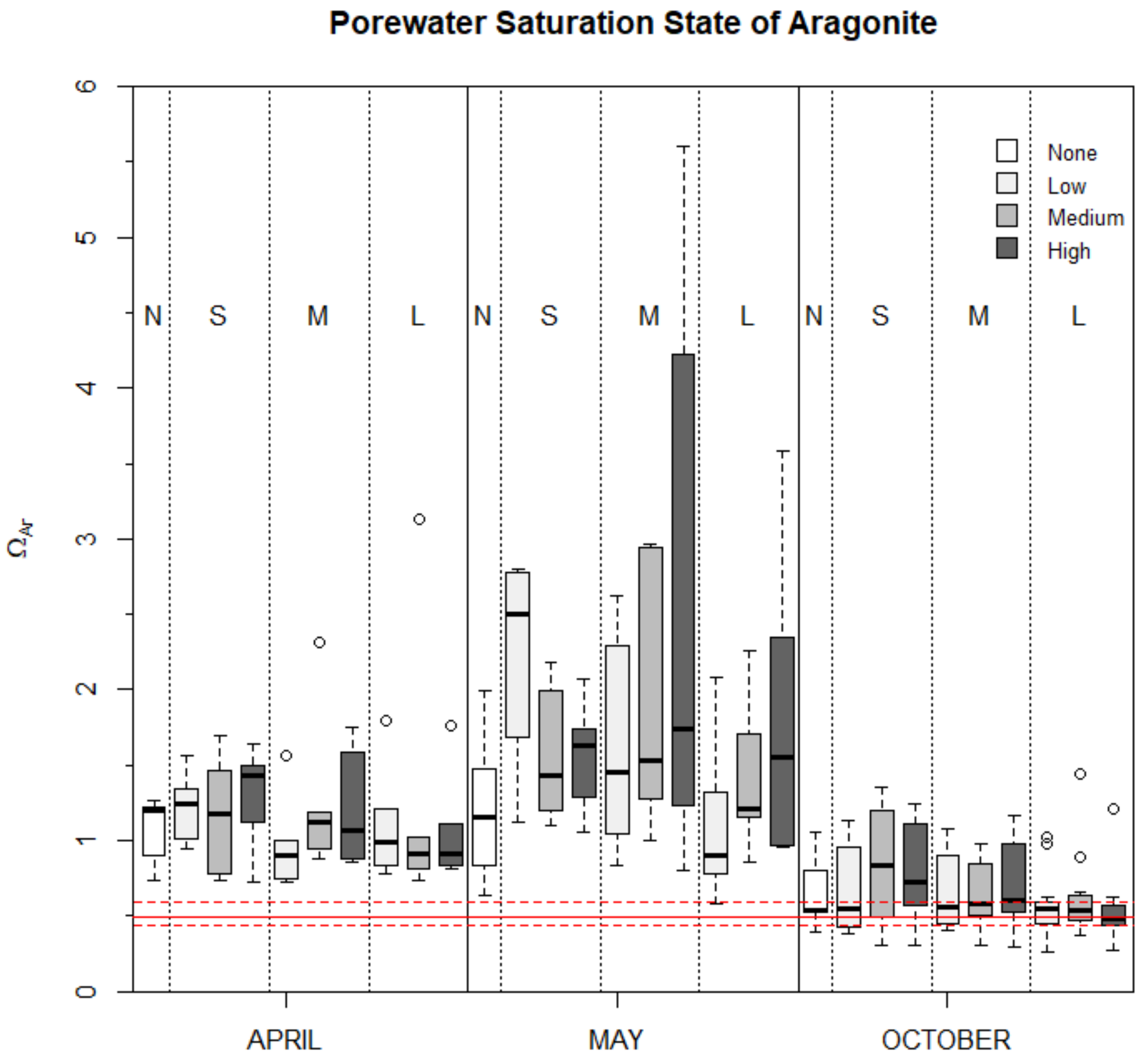

Figure 2: South Portland tidal mudflat porewater saturation state of aragonite (Ω_{Ar}) calculated over time in experimental plots treated with different shell crush sizes and shell densities. Boxes are grouped by sampling date (x-axis) and shell crush size (N: no shell/control; S: small; M: medium; L: large), and ordered by shell density (legend). Boxes in April and May represent observations from plots without predator-deterrent netting, whereas boxes in October represent observations from both netted and non-netted plots (i.e., twice the number of observations). Red lines represent the median Ω_{Ar} (solid) and interquartile range (dashed) from background observations collected 1 day prior to shell deployment/2 days prior to the April sampling date.

2.4 Clam Recruitment Methods & Materials

To assess the interactive effects of different sizes and masses of crushed oyster (*Crassostrea virginica*) shells on early recruits of the soft-shell clam, *Mya arenaria*, and other commercially important bivalve species, we initiated a field experiment on 22 April 2021 in protected (4.2 mm flexible, polypropylene netting; OV7100, https://www.industrialnetting.com/ov7100-168-polypropylene-netting.html) and unprotected 1-m^2 plots. Shells were accessed from restaurants in the greater Portland, Maine area, and weathered in covered trailers for approximately one year prior to their use in the study. Shells were crushed using a machine designed to pulverize glass bottles. Three sizes of shell were obtained by differentially sieving the residue obtained from the machine (Table 1; Fig. 2). Three masses of each shell size (1.27, 1.91, and 2.55 kg/m^2) were chosen based on results from Beal et al. (2020) who spread crushed shells of *M. arenaria* into protected and unprotected field plots at 0.63, and 1.27 kg/m^2 at another intertidal site in Casco Bay, and found no significant effect on abundance or size of recruits of two bivalve species at either experimental mass or compared to control plots without crushed shells.

A fully factorial design involving shell size (a = 3), shell mass (b = 3), and presence or absence of netting (c = 2) was deployed in 6 replicate plots per treatment. In addition, two additional controls were added to the design (plots with and without protective netting and no shell). Nets were affixed to one-half of the 120 1-m^2 plots (arrayed in a 10 x 12 matrix with 5 m spacing between rows and columns) by digging a furrow (20 cm deep) around the plot periphery, and then placing the edge of the net in the furrow and back-filling the furrow with the excavated sediments. On 10-11 October 2021 (171-172 days), five benthic cores (area = 0.0077 m^2) from each plot (N = 600) were taken to a depth of 11.5 cm (which would sample most animals <30 mm SL, shell length; Zwarts and Wanink, 1989). Core samples were washed through a 1 mm mesh and all *Mya arenaria* counted and measured (SL) to the nearest 0.01 mm using digital calipers.

To determine if bivalve larvae competent to settle occurred at the site, we deployed recruitment boxes (passive collectors) at the study site on 22 April 2022 that have been used to assess density and size of 0-year class recruits of *Mya arenaria* and other bivalves in Casco Bay (Beal et al. 2018). These are empty, wooden boxes (57 cm x 26.5 cm x 7.6 cm, or 0.15 m^2) with a piece of PetScreen® on top (rectangular aperture measuring 1.7 mm x 0.9 mm, or 1.53 m^2, that can deter predators > 1.9 mm in length or width) that are placed on the mudflat surface. Boxes are anchored by pounding two wooden laths (~Twenty boxes were deployed inside the 10 x 12 matrix in 4 rows of 5. To determine if the experimental matrix affected bivalve recruitment, an equal number of boxes was deployed outside (approximately 5 m) and adjacent to the matrix in 4 rows of 5. Boxes were removed from the flat on 10 October and the contents of each washed as described above. All bivalves in each box were identified, enumerated, and measured (SL) as described above.

Because soft-shell clams begin to spawn when seawater temperatures reach 9.5-10°C and continue until temperatures climb above 13°C (B. Beal, pers. obs.), it was imperative that the study be initiated prior to clam spawning. To assess seawater temperatures, we deployed two loggers (HOBO Pendant® Temperature/Light 64K data loggers) at the site. Loggers recorded temperature every 30 minutes both during low tide (exposed to air) and periods of tidal inundation. To obtain seawater temperatures, an average temperature was taken from five half-hour recordings – two before, one at, and two after high tide on each of the 322 days of the experiment (Fig. 3).

2.4.1. Additional benthic sampling

Additional benthic samples (using a coring device with an area of $0.0182 m^2$ to a depth of 15 cm) were taken at the study site on 6 March (N = 20) and 13 August (N = 20). Samples from each core were processed and organisms identified, enumerated, and measured as described above.

2.5 Statistics

Abundance and SL data for 0-year class individuals of *Mya arenaria* and three other commercially-harvested bivalves and the green crab, *Carcinus maenas*, from the recruitment boxes was analyzed using analysis of variance (ANOVA). The linear model was:

$Y_{ij} = \mu + A_i + e_{j(i)}$ where:

Y_{ij} = dependent variable (number of recruits and size [SL for bivalves; carapace width, CW, for green crabs] of *M. arenaria*, *Mytilus edulis*, *Ensis leei*, *Spisula solidissima*, and *C. maenas*);

Ai = Position (a = 2; inside vs. outside experimental matrix; factor is fixed); and,

e_j = Sampling error.

Because there was a preponderance of zeros in the core data from the $1\text{-}m^2$ plots, several analyses were conducted. The first was a mixed model analysis of variance that ignored assumptions of variance homogeneity and normality (Underwood, 1981) that used the full data set (N = 600). The linear model was:

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

Y_{ijklm} = dependent variable (e.g., number of *Mya arenaria*, *Mercenaria mercenaria*, *Carcinus maenas per core)*;
A_i = Shell size (a = 4; factor is fixed; None, Small, Medium, Large);
Bj = Shell mass (b = 4; factor is fixed; None, Low, Medium, High);
C_k = Netting (c = 2; factor is fixed; Present v. Absent);
D_l = Plot (d = 6; factor is random); and,
e_m = Experimental error (n = 5).

Because this analysis was not fully factorial (e.g., there are no treatments where shell size None occurs with shell mass low, medium, high), Type III SS were used in all hypothesis tests.

Unless otherwise noted, all means are presented with their 95% confidence interval.

2.6 Clam Recruitment Results

2.6.1 Additional benthic sampling

One individual of *Mya arenaria* (5.1 mm SL; 2.74 ± 5.74 individuals/m^2) was sampled from the 20 cores taken on 6 March 2021. In addition, three northern quahogs, *Mercenaria mercenaria* (18.7 mm, 45.3 mm, and 42.1 mm SL; 8.22 ± 9.40 ind./m^2) and one individual of *Ensis leei* (54.2 mm SL; 2.74 ± 5.74 ind./m^2) were discovered in the core samples on that day. No live bivalves or crabs occurred in any of the benthic cores taken on 13 August 2021.

2.6.2 Recruitment boxes

No significant difference was observed in mean abundance of any bivalve species or green crabs in recruitment boxes between inside and outside the experimental matrix (P-values ranged from 0.373 to 1.00; Table 2). Similarly, no significant difference was observed in size-frequency of any bivalve species or green crabs due to position of recruitment boxes (Figs. 4-8). Densities of soft-shell clams (86.2 ± 17.5 individuals/m^2, n = 40) and green crabs (80.6 ± 12.9 individuals/m^2, n = 40) pooled across position were highest among all five invertebrate species in recruitment boxes (Table 2).

2.6.3 Field experiment

ANOVA (Table 3) revealed no significant main or interactive sources of variation with respect to the mean number of soft-shell clam recruits, except the one that examined spatial variability of treatments between plots within the matrix. Eleven soft-shell clams (mean SL ± 95% CI = 11.78 ± 5.47 mm; minimum SL = 3.09 mm, maximum SL = 27.54 mm) were sampled from the 600 cores resulting in a mean density (± 95 CI) in the 120 plots of 2.37 ± 1.82 individuals/m^2. Given the size-frequency distribution of soft-shell clams in recruitment boxes (Fig. 4), it appears that core samples contained 0-year class individuals. Netting did not enhance the density of recruits of *Mya arenaria* (P = 0.2246; Table 3). Mean density of soft-shell clam recruits in unprotected plots was ~4.5x greater than in protected plots (3.88 ± 3.44 vs. 0.86 ± 1.21 individuals/m^2 [n = 60], respectively; Fig. 9). Results of all hypothesis tests using ANOVA on the count data were similar to those obtained using Poisson regression.

Green crabs were discovered in 14 core samples. ANOVA demonstrated that netting was the only source of variation that was statistically significant (0.0169, Table 4). Approximately 6x more green crabs occurred in netted vs. open plots (5.18 ± 2.69 vs. 0.86 ± 1.21 individuals/m^2 [n = 60], respectively).

3. Discussion

This study was conducted following the advice of Bentley and Schneider (2015) who summarized a Maine legislative commission report that suggested the addition of crushed bivalve shells to the intertidal to ameliorate effects of ocean acidification on young-of-the-year soft-shell clams and other commercially important species that must manufacture their shells. Other blue-ribbon panels in the state of Washington have suggested similar activities (WABRPOA, 2012). The advice stems from encouraging laboratory and field trials in Maine by Green et al. (2009) who increased mean sediment saturation state in the field over a two-week period (sediment buffering) by adding 1.2 kg/m^2 of crushed shells of *Mya arenaria* to field plots with a 3-fold enhancement of soft-shell clam recruits compared to controls (without crushed shells). In addition, similar results (Green et al., 2013) occurred over 5-weeks at the same location where the present study was conducted, again, with crushed shells of *Mya arenaria.* Longer-term studies (170-204 days) using crushed soft-shell clam shells and other calcified substrates at intertidal flats in the same region of southern Maine were conducted by Beal et al. (2020) were unable to replicate the results of Green et al. (2009, 2013), but showed the relative importance of predators on soft-shell clam recruitment.

Here, we showed that soft-shell clams and other commercially-important bivalve species settle to flats and recruit at densities that are much higher than that obtained in benthic core samples from the large field experiment. For soft-shell clams, the difference in density between recruitment boxes (mean = 86.23 individuals/m^2, n = 40) and core samples (mean = 2.37/m^2, n = 120) was nearly 36-fold. The addition of crushed shells to intertidal flats may have a temporary effect to enhance densities of infaunal bivalves; however, results from the present study along with those of Beal et al. (2020) suggest that over longer periods of time, the encouraging short-term effects of sediment buffering are not sustainable. It is likely that ocean warming will continue to play a disproportionate role in regulating densities of predators, especially green crabs (Young and Elliot, 2020). While warming in the Gulf of Maine continues its 40-year progression (Pershing et al., 2015), the addition of crushed bivalve shells to intertidal sediments is not warranted if the goal is to mitigate effects due to ocean and coastal acidification. Rather, it appears that some species of clams, including *Mya arenaria*, may have the ability to modify their calcifying fluid chemistry and maintain pH homeostasis at relatively high $p\text{CO}_2$ levels (Zhao et al., 2018).

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Table 1. Mean shell length (SL in mm) and mass (g) of crushed shells of Crassostrea virginica used in the design of a field experiment (22 April to 10 October 2021) at Mill Cove, South Portland, Maine. (N = 75)

Size Category	Mean	Min	Max	Lower 95% CI	Upper 95% CI
Large (SL)	9.39	6.19	15.36	8.93	9.86
Medium (SL)	5.60	2.49	9.28	5.29	5.91
Small (SL)	3.91	1.75	6.52	3.72	4.10
Large (Mass)	0.111	0.022	0.312	0.096	0.126
Medium (Mass)	0.022	0.001	0.111	0.018	0.027
Small (Mass)	0.0050	0.0007	0.0134	0.0044	0.0057

Table 2. Mean density (individuals/m^2) ± 95% CI and SL (mm) of four species of bivalves and CW (mm) green crabs from recruitment boxes located inside and outside the intertidal experimental matrix at Mill Cove, South Portland, Maine (10 October 2021). (N = 20 boxes) (n = number of individuals of each species). A t-test was performed to test the null hypothesis of no difference in density due to the position of the recruitment boxes. A chi square test of size frequencies was conducted to determine if position affected size-frequency distribution of each species.

Species	Position	Mean Density	P-value	Mean Size	df	n	P-value
Mya arenaria	Inside	84.74 (28.16)	0.8655	14.11			
	Outside	87.72 (23.33)		13.92			
Mytilus edulis	Inside	18.87 (7.96)	0.7660				
	Outside	20.52 (7.65)					
Ensis leei	Inside	20.85 (10.89)	1.0000				
	Outside	20.85 (12.88)					
Spisula solidissma	Inside	27.81 (11.04)	0.8088				
	Outside	29.79 (13.01)					
Carcinus maenas	Inside	86.39 (19.17)	0.3732				
	Outside	74.81 (18.89)					

Table 3. Analysis of variance on 0-year class recruits of Mya arenaria from core samples taken in 1-m^2 plots from the experimental matrix at Mill Cove on 10-11 October 2021.

Source of Variation	df	SS	MS	F	Pr > F
Shell Size	2	0.0333	0.0167	0.71	

Figure Legends

Figure 1. Map of Maine and the intertidal study site (Mill Cove) in South Portland.

Figure 2. Size frequency distribution of size (shell length in mm) and mass (g) of crushed oysters used in the field experiment. In addition, size-mass relationships (with 95% CI for $\hat{y}$) are presented for each of the three size categories.

Figure 3. Mean daily seawater temperature taken five times around high tide (one hour and 30 minutes before and after high tide, and once at high tide) at the intertidal study site at Mill Cove, South Portland, Maine. A mean low of 6.95°C was recorded on 23 April, and a mean high of 21.70°C was recorded on 14 August.

Mean number of soft-shell clams per square meter from all experimental treatments (n = 6)

Figure 1.

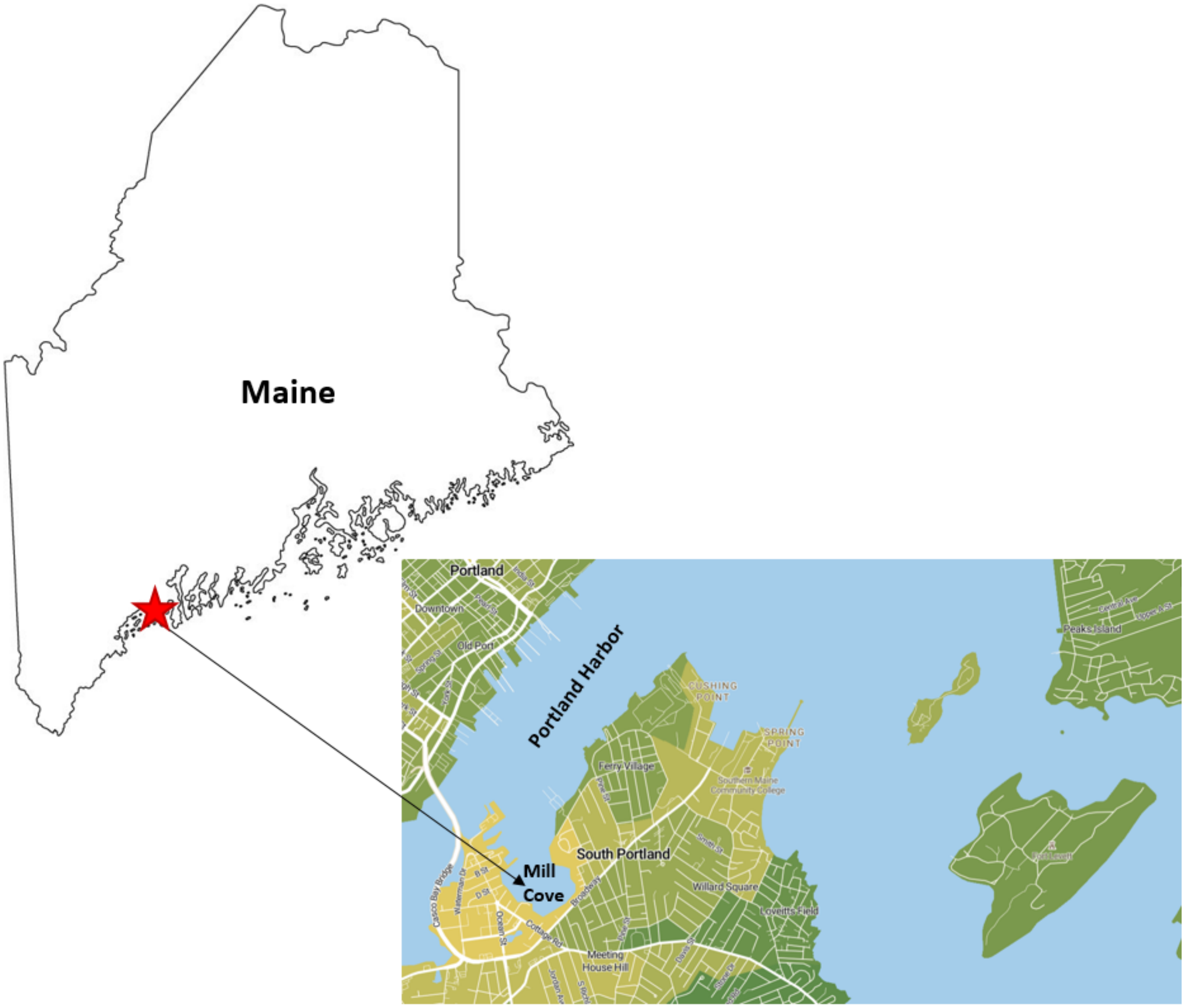

Maine
Portland
Downtown
Old Port
Portland Harbor
Peaks Island
Central Ave
Upper A St
Cushing Point
Spring Point
Ferry Village
Southern Maine Community College
South Portland
Mill Cove
Willard Square
Loveitts Field
Meeting House Hill
Casco Bay Bridge
Fort Levett
Broadway
Cottage Rd
Ocean St
Waterman Dr
B St
D St
India St
Pearl St
Spring St
York St
Pine St
Smith St
Davis St
Jordan Ave

Figure 2.

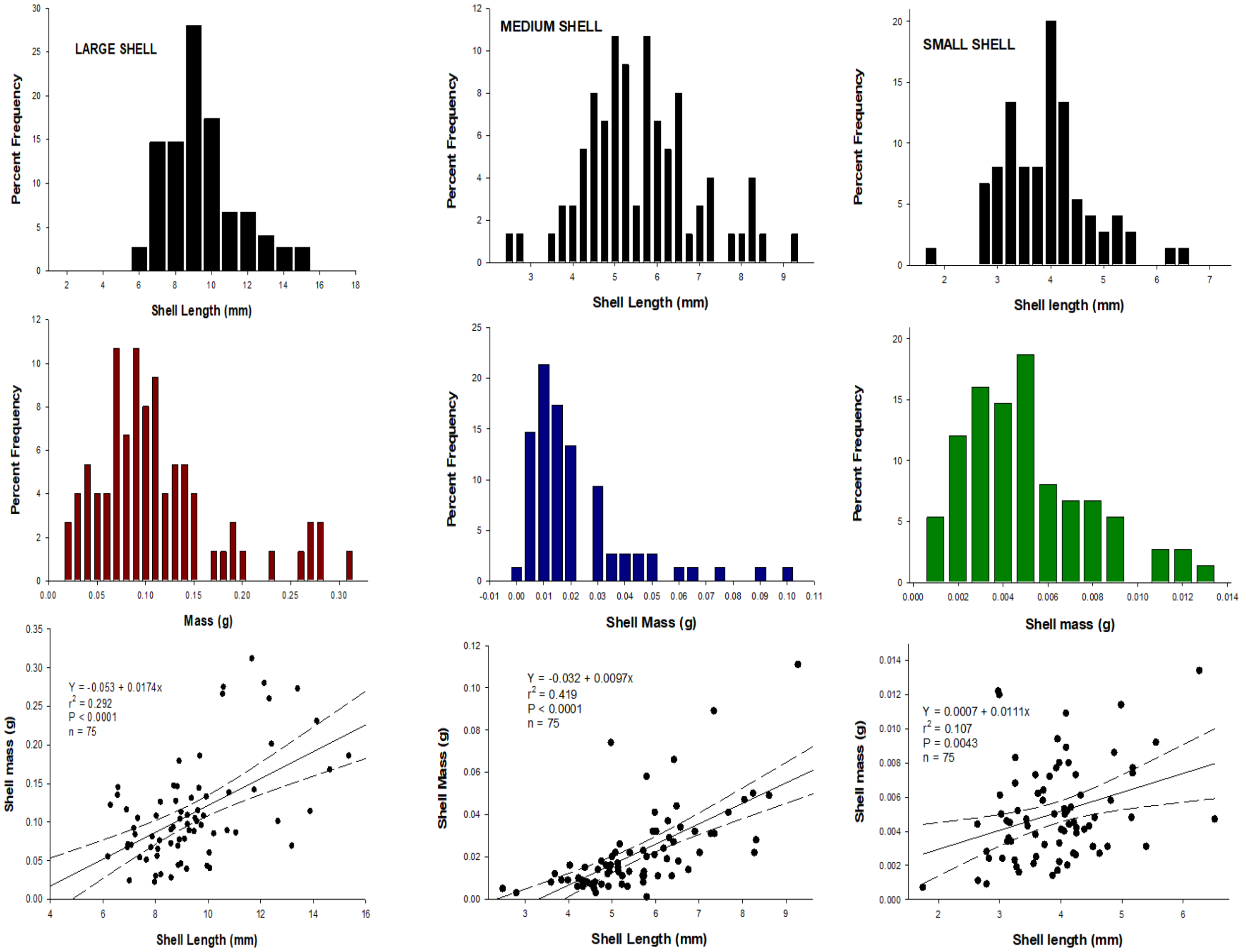
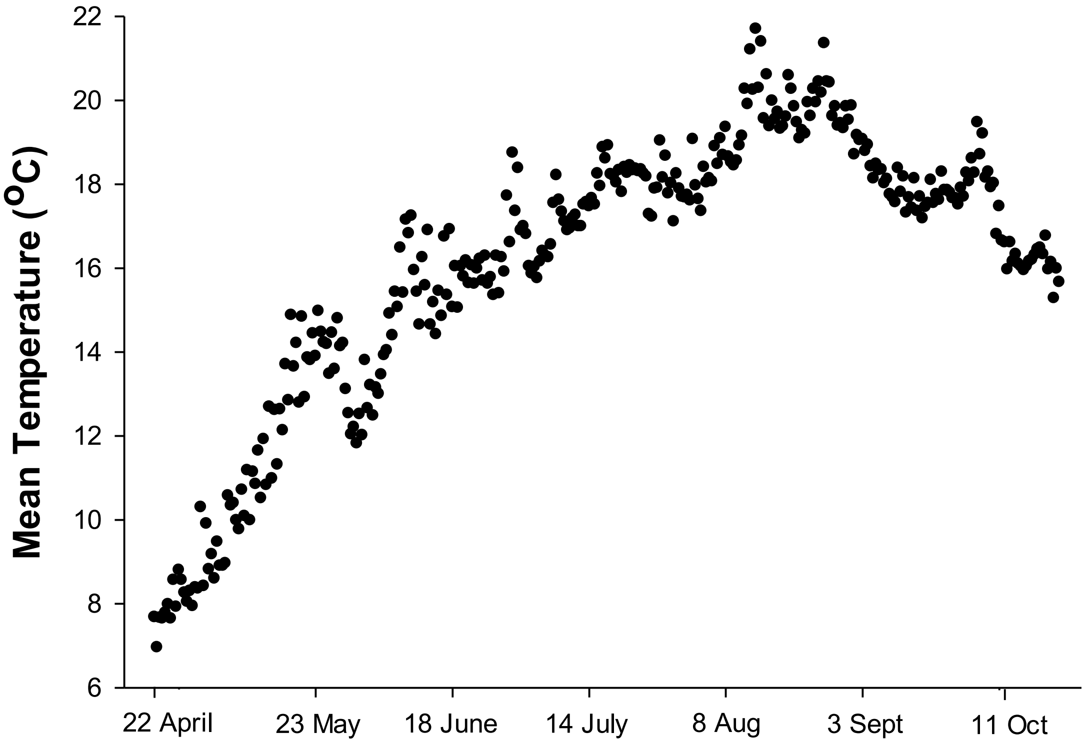

LARGE SHELL
Percent Frequency
Shell Length (mm)
MEDIUM SHELL
Percent Frequency
Shell Length (mm)
SMALL SHELL
Percent Frequency
Shell length (mm)
Percent Frequency
Mass (g)
Percent Frequency
Shell Mass (g)
Percent Frequency
Shell mass (g)
Shell mass (g)
Y = -0.053 + 0.0174x
r2 = 0.292
P < 0.0001
n = 75
Shell Length (mm)
Shell Mass (g)
Y = -0.032 + 0.0097x
r2 = 0.419
P < 0.0001
n = 75
Shell Length (mm)
Shell mass (g)
Y = 0.0007 + 0.0111x
r2 = 0.107
P = 0.0043
n = 75
Shell length (mm)

Figure 3.


Mean Temperature (^{o}C)
22
20
18
16
14
12
10
8
6
22 April
23 May
18 June
14 July
8 Aug
3 Sept
11 Oct

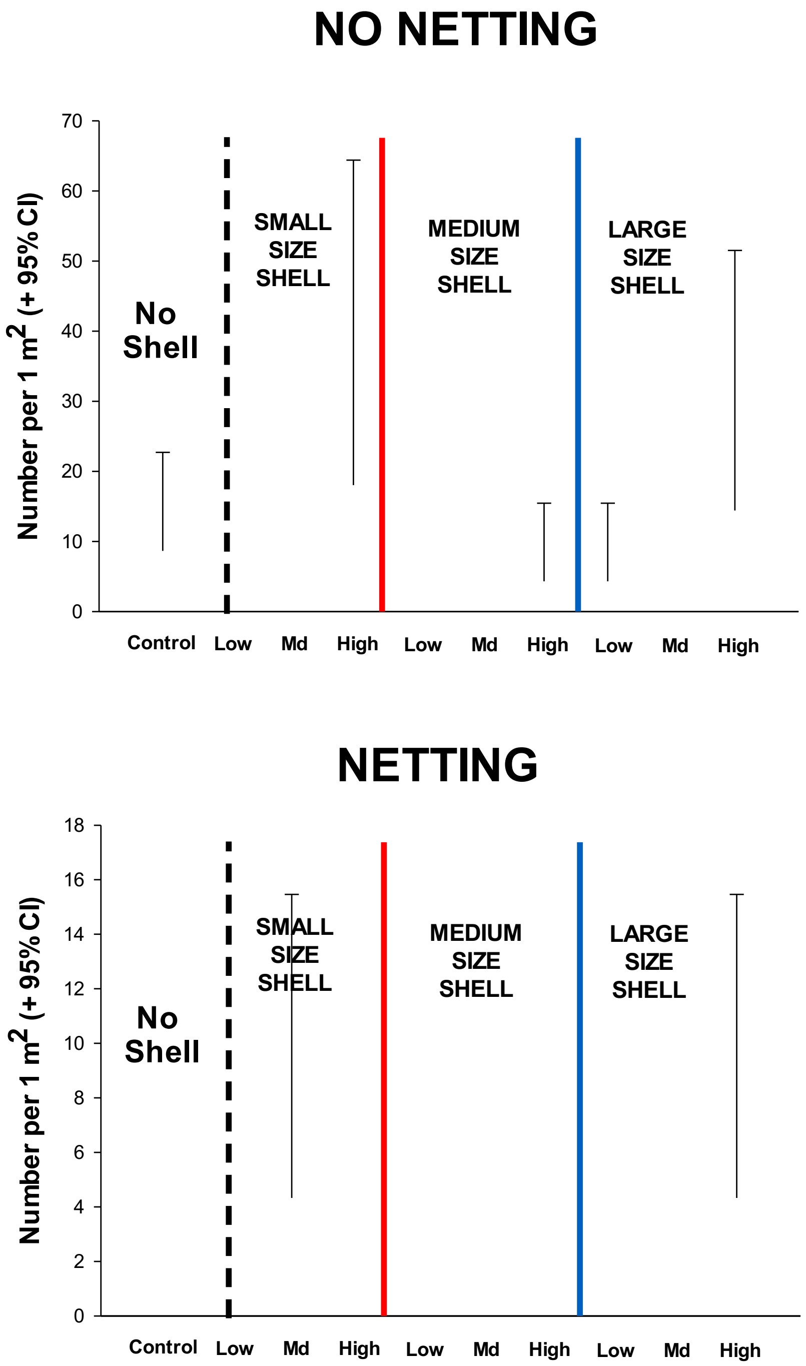

NO NETTING
Number per 1 m^2 (+ 95% CI)
70
60
50
40
30
20
10
0
No Shell
SMALL SIZE SHELL
MEDIUM SIZE SHELL
LARGE SIZE SHELL
Control
Low
Md
High
Low
Md
High
Low
Md
High
NETTING
Number per 1 m^2 (+ 95% CI)
18
16
14
12
10
8
6
4
2
0
No Shell
SMALL SIZE SHELL
MEDIUM SIZE SHELL
LARGE SIZE SHELL
Control
Low
Md
High
Low
Md
High
Low
Md
High

Figure 3.