Pilot study to investigate factors affecting growth and survival of cultured soft-shell clam juveniles and recruitment of 0-year class wild clams at an intertidal flat in West Jonesport, Maine (April-October 2022)

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Introduction

A pilot study was initiated at an intertidal flat in West Jonesport, Maine adjacent to the Bill Nunan property on 28 April 2022, and was completed after 180 days on 25 October 2022. The study was designed to examine effects of: 1) tidal height; 2) stocking density; and, 3) predator exclusion using netting on the fate and growth of hatchery-reared (cultured) soft-shell clam, *Mya arenaria*, juveniles. In addition, densities of wild, juvenile clams (i.e., recruits) were estimated using experimental units containing planted hatchery seed as well as units that were initially empty. This effort was designed to assist Mr. Nunan with information about the potential to farm clams in this location.

Methods

Soft-shell clams used in the study ($\bar{x}_{\text{Shell length}} \pm 95\%$ CI = 12.5 ± 0.6 mm, n = 96; Fig. 1) were reared at DEI during the spring/summer of 2021, and then overwintered (sensu Beal et al. 1995). On 28 April 2022, cultured clam seed at two stocking densities (60 and 120/ft^2) and protected from predators using two types of flexible netting (4.2 mm square aperture; and a rectangular aperture of 0.9 mm x 1.7 mm) were deployed at two tidal heights (upper vs. lower) in 6-inch diameter x 6-inch deep plastic plant pots. Pots were filled with ambient sediments and seed were distributed evenly on the sediment surface prior to affixing a piece of mesh netting to the top of each pot using a rubber band. Due to difficult weather conditions on the day of deployment, an unequal number of experimental units (plant pots) were distributed at each tidal height (Fig. 2).

At the end of the study, the contents of each experimental unit found (one unit containing 12 clams and protected with a 4.2 mm net was missing from the lower intertidal) was washed through a 1 mm sieve. All cultured and wild clam recruits (individuals not in the sediments at the beginning of the trial, but had settled as 0-year class clams into the pots sometime during the trial period) were counted and measured. It was possible to discern a growth rate on each live, cultured clam as these animals serendipitously mark themselves when they are transferred from the hatchery to field conditions (Beal et al. 1999). That is, clams leave a distinct disturbance line in their valves (Fig. 3) that coincides with deployment in sediments. Therefore, it was possible to measure (to the nearest 0.01 mm using digital calipers) each live clam twice – the length of the disturbance line and the final shell length (SL) – the difference between these two measurements is the clam's absolute shell growth during the study.

Another estimate of wild clam recruitment was made using "clam recruitment boxes" (sensu Beal et al. 2018). These are wooden frames (1-ft x 2-ft x 3-inches deep) lined with PetScreen® on the top and agricultural ground cover (weed barrier made of woven polypropylene strands) on the bottom (Fig. 4). Empty boxes were arrayed in groups of two (~1 m apart) on the surface of the flat in three upper (N = 6) and one lower intertidal (N = 2) locations. Boxes were anchored using a wooden lath pushed into the sediments ~ 20 inches at both short ends of each box. Laths were affixed to each box using galvanized trap nails (2/lath) that were driven through each lath and into the side of the box. At the end of the trial, the contents of each box was washed through a 1 mm sieve (as described above). All clams that had settled into each box and survived (the definition of a "clam recruit") were counted and measured (as described above).

In addition, data was recorded on number and size of all green crabs, *Carcinus maenas* (carapace width – CW – measured to the nearest 0.01 mm using digital calipers), within plant pots and recruitment boxes.

Analysis of variance (ANOVA) was used to assess mean survival and growth of cultured clams as well as mean number and size of green crabs. The following linear model was used:

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

Where:

Y_{ijklm}	=	dependent variable (percent survival; absolute growth; number of wild recruits and green crabs; and, green crab carapace width);
μ	=	theoretical mean (estimated by the overall sample mean);
A_i	=	tidal height (a = 2 levels – upper vs. lower);
B_j	=	stocking density (b = 2 levels – 60 vs. 120 individuals/ft^2);
C_k	=	netting (c = 2 levels – 4.2 mm mesh vs. PetScreen®);
D_l	=	block (d = 1.5 levels since 2 blocks occurred at the upper and one at the lower tide height); and,
e_m	=	experimental error (n = 2 replicates)

All factors (tidal height, stocking density, netting, and block) were considered fixed factors in the analyses.

<u>Results</u>

Fate and Growth of Cultured Clams and Green Crab Demographics

The fate of clams in each experimental unit is given in Table 1 and the mean survival in Table 2. ANOVA (Table 3) revealed the lack of a statistically significant result on mean survival for any of the eight hypothesis tests. For example, a difference of approximately 10% was observed in mean clam survival between the lower (47.0 ± 24.8%, n = 7) and upper shore (57.8 ± 8.1%, n = 16); however, this difference was not statistically significant (P = 0.2346[a]; Table 3). Surprisingly, very little difference was observed in mean survival between clams protected with netting having an aperture size of 4.2 mm (17.6 mm^2) (54.9 ± 11.3%.n = 11) vs. PetScreen® netting with an area of 1.5 mm^2 (54.2 ± 14.3%, n = 12). In both instances, large variation in survival between experimental units treated similarly was one reason for the lack of statistical significance.

Wild clam recruits were recovered in only two experimental units – both protected with PetScreen®: one was 4.32 mm (at the lower intertidal with 12 clams/unit); another had two clams – 6.83 mm and 3.05 mm – from an experimental unit in the upper intertidal stocked with 24 clams. No analyses were conducted since 91% of experimental units contained no wild soft-shell clam recruits. Additionally, wild clam recruitment was measured using "recruitment boxes." All six boxes were recovered from the upper intertidal while only one box from the lower shore was recovered from the two deployed (ground cover bottom and containing zero live clams and one

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[a] Statistical significance occurs when the P-value (probability value) is equal to or less than 0.05.

green crab measuring 27.3 mm CW). No significant difference was observed in mean recruit number between boxes at the upper intertidal in terms of bottom type (ground cover vs. PetScreen®; P = 0.603). Mean ± 95% CI for number per box and per square feet (n = 3) for boxes with mesh bottoms was 2.7 ± 11.5/box and 1.6 ± 7.1/ft^2. Means for wild recruits in boxes with ground cover bottoms were 4.6 ± 10.0/box and 2.9 ± 6.17/ft^2.

Neither tidal height nor stocking density had a significant effect (positive or negative) on clam growth; however, the type of netting used to protect clams from predators explained nearly 50% of the variability in observed shell growth (P = 0.0004; Table 4). The smaller aperture netting (PetScreen®) resulted in a growth penalty for the surviving clams. That is, neither position within the intertidal zone (upper vs. lower) nor density at which clams were initially stocked (60 or 120 per ft^2) played a significant role in growth. Netting did. Animals in experimental units protected with PetScreen® added approximately 35% less shell than clams in units protected with the larger aperture (4.2 mm) netting (Fig. 5). The mechanism relates to the differences in size of the netting aperture between the two types of protective mesh. Clams in the smaller aperture netting likely did not receive as much flow (hence access to food – phytoplankton) as clams in the experimental units with the larger aperture netting.

No significant difference in mean number of green crabs was observed in the plant pots between tidal heights, stocking densities, or types/sizes of netting (Table 5). Overall, mean number per unit was 1.7 ± 0.5, n = 23. Mean green crab size varied by netting (P = 0.0003), but the effect varied differently across tidal heights (P = 0.0111; Table 6; Fig. 6). That is, at the upper intertidal, larger crabs (13-20 mm CW) were observed in units protected with the larger aperture mesh whereas smaller crabs (mostly between 4-10 mm CW) were observed units protected with PetScreen®. The same clear-cut result was not observed between the two netting treatments at the low intertidal (Fig. 6). Green crabs also settled and recruited into the clam recruitment boxes. While approximately 3.5x more crabs were observed in boxes with ground cover (7.3 ± 13.7/box; 4.5 ± 8.4/ft^2) than those with PetScreen® bottoms (2.0 ± 4.3/box; 1.2 ± 2.6/ft^2), there was no statistically significant difference (P = 0.185). Green crabs ranged in size from 5.7 mm to 33.2 mm CW.

Discussion/Summary

This short-term study (180 days) demonstrated that cultured soft-shell clam seed approximately a half-inch in shell length can survive and grow along the shore adjacent to the Bill Nunan property in West Jonesport, Maine. While attempts to demonstrate the effects of stocking density (60 vs. 120 individuals per ft^2), tidal height (upper vs. lower), and type of predator deterrent netting failed to show anything statistically significant in terms of survival (overall mean percent survival ± 95% CI was 52.4 ± 9.5%, n = 23), the two types of protective netting did yield differences in clam growth. Using the smaller aperture netting (PetScreen®) resulted in a 35% reduction in shell growth compared with the larger aperture netting (Flexible – polypropylene).

Survival results are not too surprising given that: 1) the difference in actual vertical height between the upper and lower shore was likely not more than several inches (weather conditions on 28 April 2022 were windy with some rain, and this affected the tide from going as low as the tide charts for that day predicted); and, 2) stocking density typically plays a stronger role when clams are larger (Beal 2006). Between 16-29% of clams were recovered dead with undamaged valves, and between 4-25% of clams were recovered dead with crushed or chipped valves. Typically, the latter is from green crab attack or from other crushing predators such as juvenile rock crabs that make a rare appearance in the soft-bottom intertidal. Dead clams with undamaged valves can also occur due to green crab attack (see Tan and Beal 2015), but they may also result from the overwintering process (Beal et al. 1995) when the density of clams has been kept too high during the winter and clams die either due to starvation or anoxia.

Clams protected with the larger aperture netting (4.2 mm) grew relatively rapidly, adding an average of nearly one inch in shell length (mean absolute shell growth = 24.5 ± 2.7 mm), which resulted in an average final shell length (SL) of 37.2 ± 2.1 mm that was not significantly different from 38 mm, or 1.5-inches ($P = 0.514$). That is, clams grew nearly an inch in length, attaining a final mean shell length that was not significantly different from 1.5-inches in 180 days. It is likely that a two-inch clam is attainable at this site within 2 to 2.5 years of planting clams that are at least a half-inch in shell length.

The majority of intertidal flats along the Maine coast harbor green crabs at densities as high or higher than that of soft-shell clam recruits. This worrisome trend is associated indirectly with the seawater temperatures in the Gulf of Maine (Pershing et al. 2015; Beal et al. 2020). Over the past 40+ years, winter temperatures have warmed, and now few winters are considered cold enough to have negative effects on green crab populations – as was the case during the 1960s (Welch 1968). Without a reversal in the trend toward increasing seawater temperatures, the outlook is bleak for the wild clam fishery in Maine. If individuals and communities were more amenable to enhancing flats with cultured clam seed or allowing the farming clams by individuals (sensu Beal 2023), then intertidal areas that currently contain no commercial quantities of soft-shell clams, such as the Nunan Flat in West Jonesport, would be viable targets for entrepreneurial activities that could diversify the existing wild clam market.

<u>Acknowledgments</u>

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<u>References</u>

Beal, B.F. 2006. Biotic and abiotic factors influencing growth and survival of wild and cultured indivi-duals 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. 2020. Interactive effects of shell hash and predator exclusion of 0-year class recruits of two infaunal intertidal bivalve species in Maine, USA. J. Exp. Mar. Biol. Ecol. 530-53.

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., 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.

Tan, E.B.P., Beal, B.F. 2015. Interactions between 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.

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.

Welch, W.R., 1968. Changes in abundance of the green crab, *Carcinus maenas* (L.), in relation to recent temperature changes. Fish. Bull. 67, 337–345.

Table 1. Percent survival of cultured soft-shell clam seed ($\bar{x}_{\text{Shell Length}}$ = 12.48 ± 0.57 mm, n = 96) at Nunan Flat, Jonesport, Maine (28 April to 25 October 2022). Twelve (61.1 clams/ft^2) or 24 clams (122.2 clams/ft^2) were added to 6-inch diameter plastic plant pots (experimental units). To protect clams from predators, plant pots were covered completely either with an oriented, plastic (polypropylene) flexible netting (4.2 mm aperture) or a vinyl-coated polyester netting (PetScreen® = 0.9 mm x 1.7 mm aperture). Pots with clams were established near the upper and lower intertidal. A single pot (planted with 12 clams and protected with 4.2 mm netting) was missing from the lower intertidal on 25 October 2023 (n = number of replicate experimental units.)

Tidal Height	Density/Unit	Netting	Percent Survival
Upper	12	4.2 mm	41.67
			75.00
			16.67
			58.33
		PetScreen	66.67
			58.33
			66.67
			50.00
	24	4.2 mm	20.83
			58.33
			58.33
			50.00
		PetScreen	79.17
			45.83
			66.67
			58.33
Lower	12	4.2 mm	50.00
		PetScreen	83.33
			41.67
	24	4.2 mm	50.00
			70.83
		PetScreen	33.33
			0.00

Table 2. Fate (mean ± 95% confidence interval in parentheses) of cultured soft-shell clam seed at Nunan Flat, Jonesport, Maine (28 April to 25 October 2022). Density = number of clams per experimental unit (12 = $60/ft^2$; 24 = $120/ft^2$); Netting = 4.2 mm or Pet (= PetScreen®); n = number of experimental units recovered on 25 October 2022. A, DU, DC, and M = mean percent of clams recovered alive, dead with undamaged valves, dead with chipped or crushed valves, and missing, respectively.

Tidal Height	Density	Netting	n	A	DU	DC	M
Upper	12	4.2 mm	4	47.9(39.6)	20.8(22.9)	16.7(21.7)	14.6(22.6)
		Pet	4	60.4(12.7)	20.8(17.1)	10.4(12.7)	8.4(6.6)
Upper	24	4.2 mm	4	48.9(49.1)	28.1(46.7)	15.6(11.3)	7.4(11.3)
		Pet	4	61.5(22.3)	29.2(19.5)	4.2(9.4)	5.1(9.9)
Lower	12	4.2 mm	1	50.0(-)	25.0(-)	25.0(-)	0.0(-)
		Pet	2	62.5(264.7)	16.7(0.0)	16.7(211.8)	4.1(52.9)
Lower	24	4.2 mm	2	60.4(132.4)	22.9(79.4)	4.2(52.9)	12.5(158.8)
		Pet	2	16.7(211.8)	25(0.0)	18.8(132.4)	39.5(344.1)

Table 3. Analysis of variance on mean survival rate of cultured clams in experimental units at the Nunan Flat (West Jonesport, Maine) from 28 April to 25 October 2022 (180 days). All factors were considered fixed; hence, all hypothesis tests use the mean square error in all F-tests. Type III sums of squares were used because the data were unbalanced due to the loss of a single replicate from the low intertidal (a unit with 12 clams protected with 4.2 mm netting).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tide height	1	496.031746	496.031746	1.54	0.2346
Density	1	124.007937	124.007937	0.39	0.5445
Tide height x density	1	714.285714	714.285714	2.22	0.1583
Netting	1	79.365079	79.365079	0.25	0.6270
Tide height x Netting	1	600.198413	600.198413	1.87	0.1934
Density x Netting	1	1269.841270	1269.841270	3.95	0.0668
Tide height x Density x Net	1	600.198413	600.198413	1.87	0.1934
Block(Tide height)	1	108.506944	108.506944	0.34	0.5705
Error	14	4500.868056	321.490575		
Total	22	8399.758454			

Table 4. Analysis of variance on mean absolute growth (Final SL – Initial SL) of cultured clams in experimental units at the Nunan Flat (West Jonesport, Maine) from 28 April to 25 October 2022 (180 days). All factors were considered fixed; hence, all hypothesis tests use the mean square error in all F-tests. Type III sums of squares were used because the data were unbalanced due to the loss of a single replicate from the low intertidal (a unit with 12 clams protected with 4.2 mm netting), as well as one experimental unit having zero live clams.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tide height	1	36.3243389	36.3243389	2.61	0.1300
Density	1	6.8832344	6.8832344	0.50	0.4940
Tide height x Density	1	24.4197912	24.4197912	1.76	0.2078
Netting	1	316.3653917	316.3653917	22.76	0.0004
Tide height x Netting	1	11.9098640	11.9098640	0.86	0.3715
Density x Netting	1	9.8580526	9.8580526	0.71	0.4149
Tide height x Density x Net	1	2.8967250	2.8967250	0.21	0.6556
Block(Tide height)	1	9.5253126	9.5253126	0.69	0.4227
Error	13	180.6946407	13.8995877		
Total	21	671.4793976			

Table 5. Analysis of variance on mean number of green crabs occurring in experimental units (plant pots) at the Nunan Flat (West Jonesport, Maine) from 28 April to 25 October 2022 (180 days). All factors were considered fixed; hence, all hypothesis tests use the mean square error in all F-tests. Type III sums of squares were used because the data were unbalanced due to the loss of a single replicate from the low intertidal (a unit with 12 clams protected with 4.2 mm netting).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Tide height	1	0.01785714	0.01785714	0.01	0.9188
Density	1	0.44642857	0.44642857	0.27	0.6117
Tide height x Density	1	0.01785714	0.01785714	0.01	0.9188
Netting	1	0.87500000	0.87500000	0.53	0.4793
Tide height x Netting	1	3.01785714	3.01785714	1.82	0.1985
Density x Netting	1	0.01785714	0.01785714	0.01	0.9188
Tide height x Density x Net	1	0.44642857	0.44642857	0.27	0.6117
Block(Tide height)	1	3.06250000	3.06250000	1.85	0.1954
Error	14	23.18750000	1.65625000		
Total	22	31.21739130			

Table 6. Analysis of variance on mean carapace width of green crabs occurring in experimental units (plant pots) at the Nunan Flat (West Jonesport, Maine) from 28 April to 25 October 2022 (180 days). All factors were considered fixed; hence, all hypothesis tests use the mean square error in all F-tests. Type III sums of squares were used because the data were unbalanced due to the loss of a single replicate from the low intertidal (a unit with 12 clams protected with 4.2 mm netting).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
tidehgt	1	15.6197511	15.6197511	3.70	0.0832
density	1	0.0134490	0.0134490	0.00	0.9561
tidehgt*density	1	0.3028632	0.3028632	0.07	0.7941
netting	1	128.5327501	128.5327501	30.48	0.0003
tidehgt*netting	1	40.6889590	40.6889590	9.65	0.0111
density*netting	1	14.1146755	14.1146755	3.35	0.0972
tidehg*densit*nettin	1	6.1508900	6.1508900	1.46	0.2549
block(tidehgt)	1	0.6818600	0.6818600	0.16	0.6961
Error	10	42.1670113	4.2167011		
Total	18	411.2418296			

Figure Legends

Figure 1. Size-frequency distribution of soft-shell clam seed used in the intertidal pilot study in West Jonesport, Maine (28 April to 25 October 2022). Mean SL ± 95% CI = 12.5 ± 0.6 mm, n = 96). Minimum SL = 7.9 mm; Maximum = 19.43 mm.

Figure 2. Schematic (not to scale) of the experimental setup on 28 April 2022 that represents a single "block." Two such blocks were deployed in the upper and one block in the lower on 28 April 2022. The experimental units were separated by ~1 m in both row and columns. Adjacent blocks were 10 m apart.

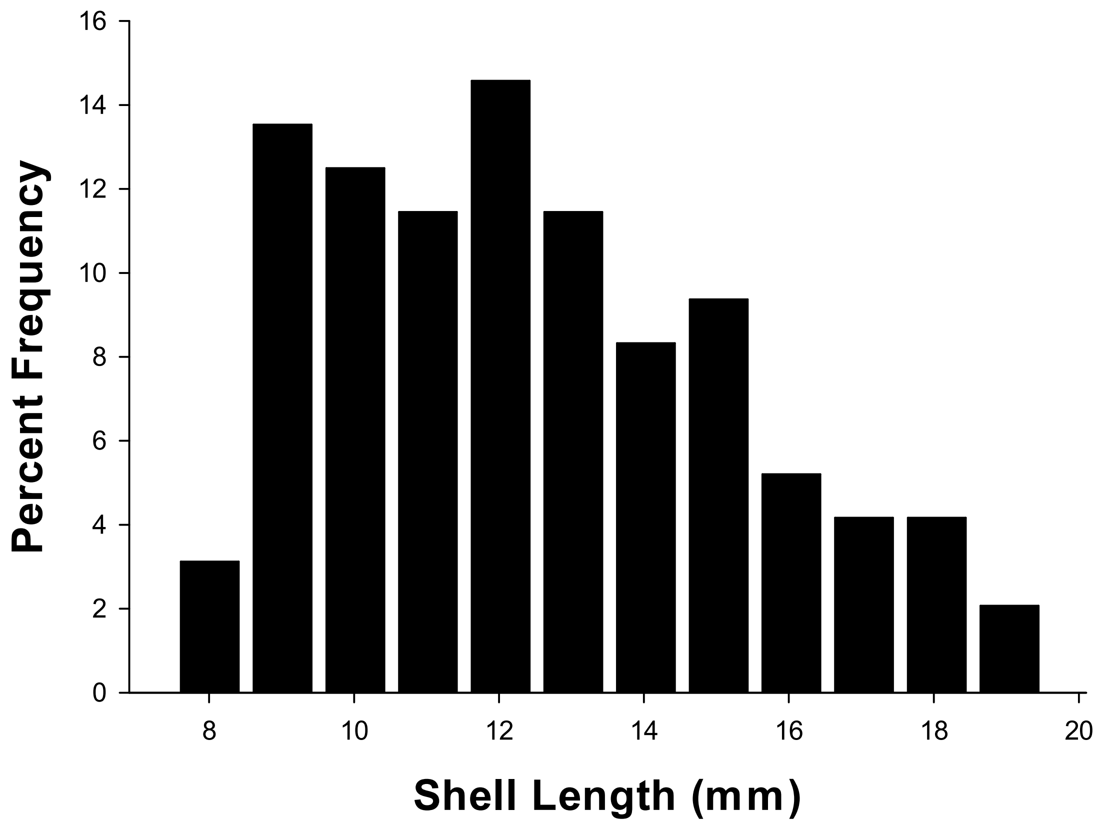
Figure 3. Photo taken on 1 September 1984 showing the disturbance line that this cultured clam incorporated into its valves during a field experiment. The pink ink dots were applied at the beginning of the field trial (26 May 1984) that took place in the upper Chandler River, Jonesboro, Maine (Beal et al. 1999).

Figure 4. Soft-shell clam recruitment box. Wooden frame: 1-ft x 2-ft x 3-inches. Lined on top with PetScreen® and on bottom with an agricultural ground cloth/weed barrier polypropylene material. Boxes were deployed empty, and act as passive collectors.

Figure 5. Mean absolute shell growth (Final SL – Initial SL) + 95% CI for live clams at the Nunan Flat, West Jonesport, Maine (28 April to 25 October 2022). Means are pooled across stocking density (60 vs. 120/ft^2) and tidal height (upper vs. lower) as no statistically significant differences were observed for these factors and their interaction terms (Table 4). Clams in units protected with the smaller aperture netting (PetScreen®; blue bar) added approximately 35% less shell than animals in units protected with the larger aperture netting (4.2 mm; pink bar; see Table 4). (Insert shows mean final SL.) (n = 11)

Figure 6. Size-frequency distribution of green crabs in experimental units at the upper and lower intertidal zone at the Nunan Flat, West Jonesport, Maine (28 April to 25 October 2022). Within each tidal height, crab sizes are displayed for both smaller aperture netting (PetScreen – 0.9 mm x 1.7 mm) and larger aperture netting (Flexible – 4.2 mm x 4.2 mm). ANOVA on mean carapace width (Table 6) demonstrated that the differences in crab sizes at the upper intertidal between animals recovered from experimental units with large vs. small aperture netting was not observed at the lower intertidal.

Figure 1.



Percent Frequency
16
14
12
10
8
6
4
2
0
8
10
12
14
16
18
20
Shell Length (mm)

Figure 2.

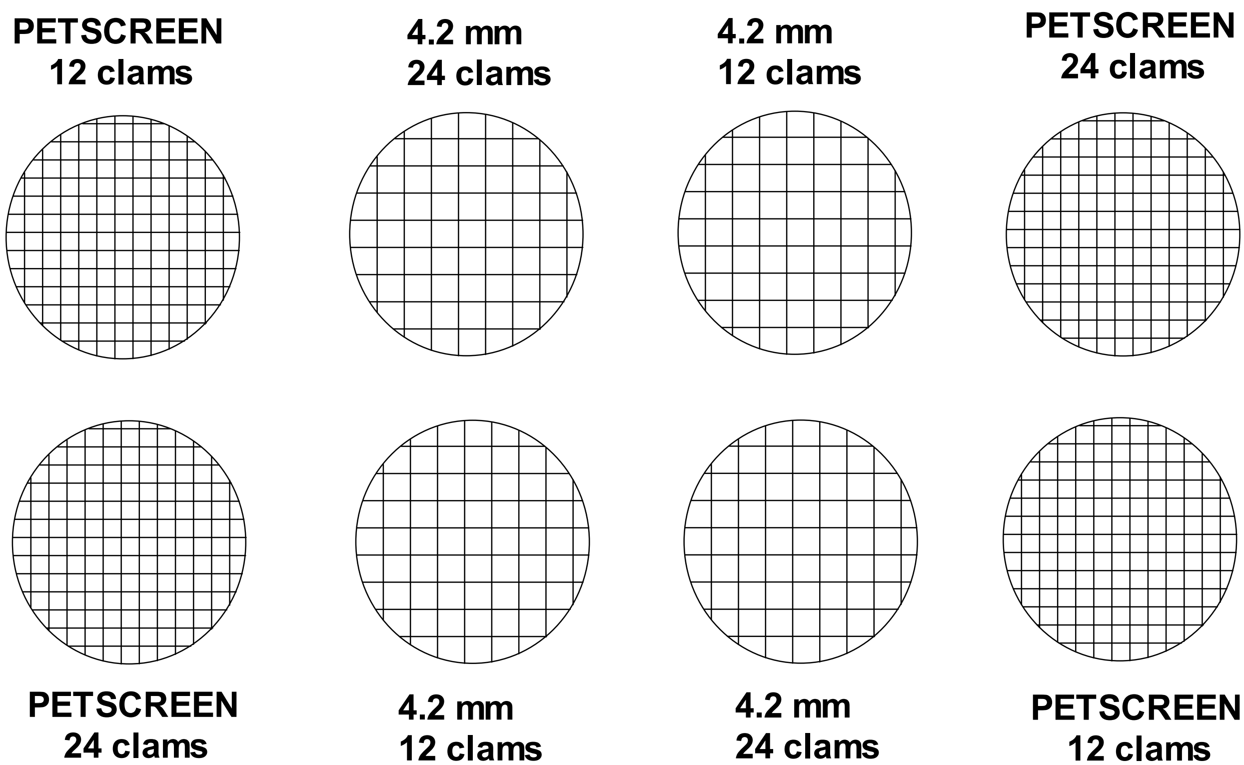

PETSCREEN
12 clams
4.2 mm
24 clams
4.2 mm
12 clams
PETSCREEN
24 clams
PETSCREEN
24 clams
4.2 mm
12 clams
4.2 mm
24 clams
PETSCREEN
12 clams

Figure 3.

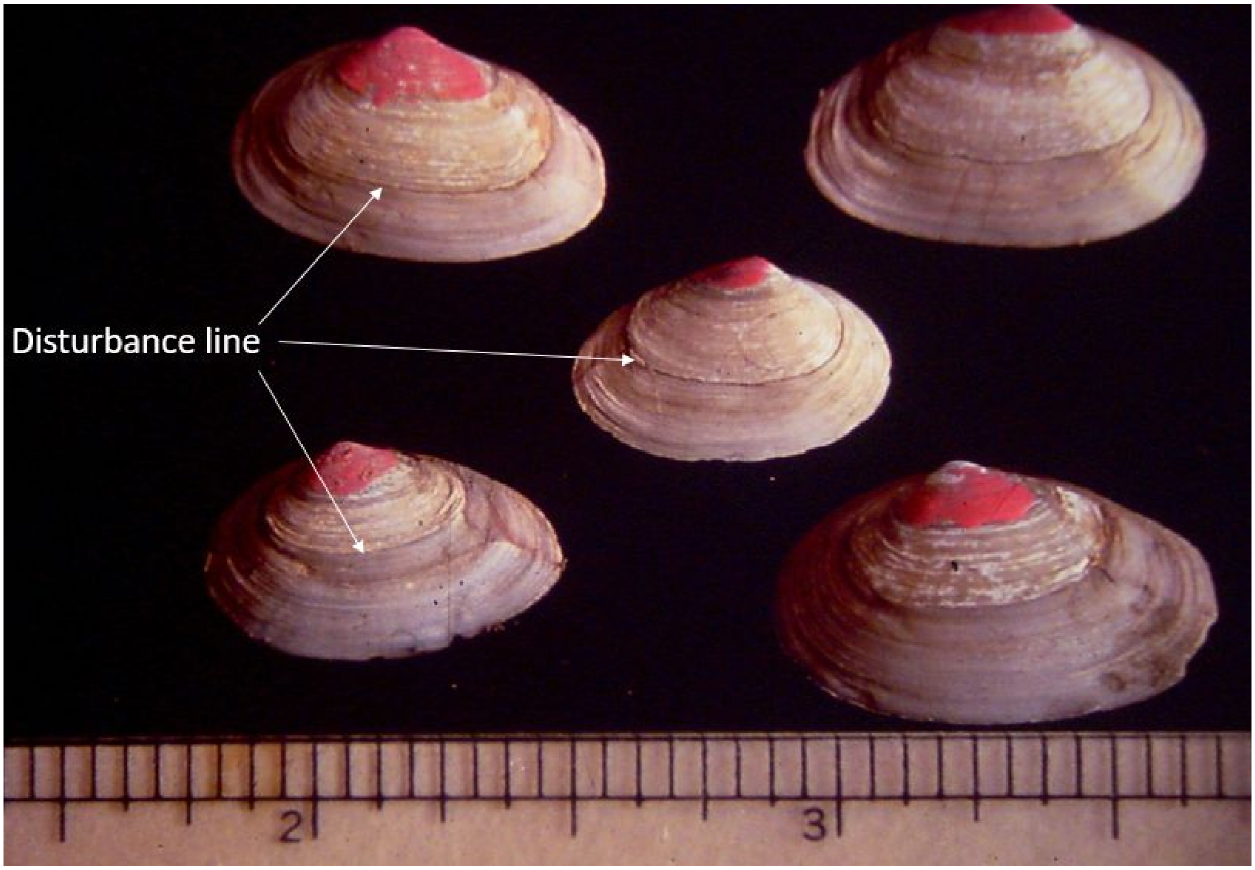
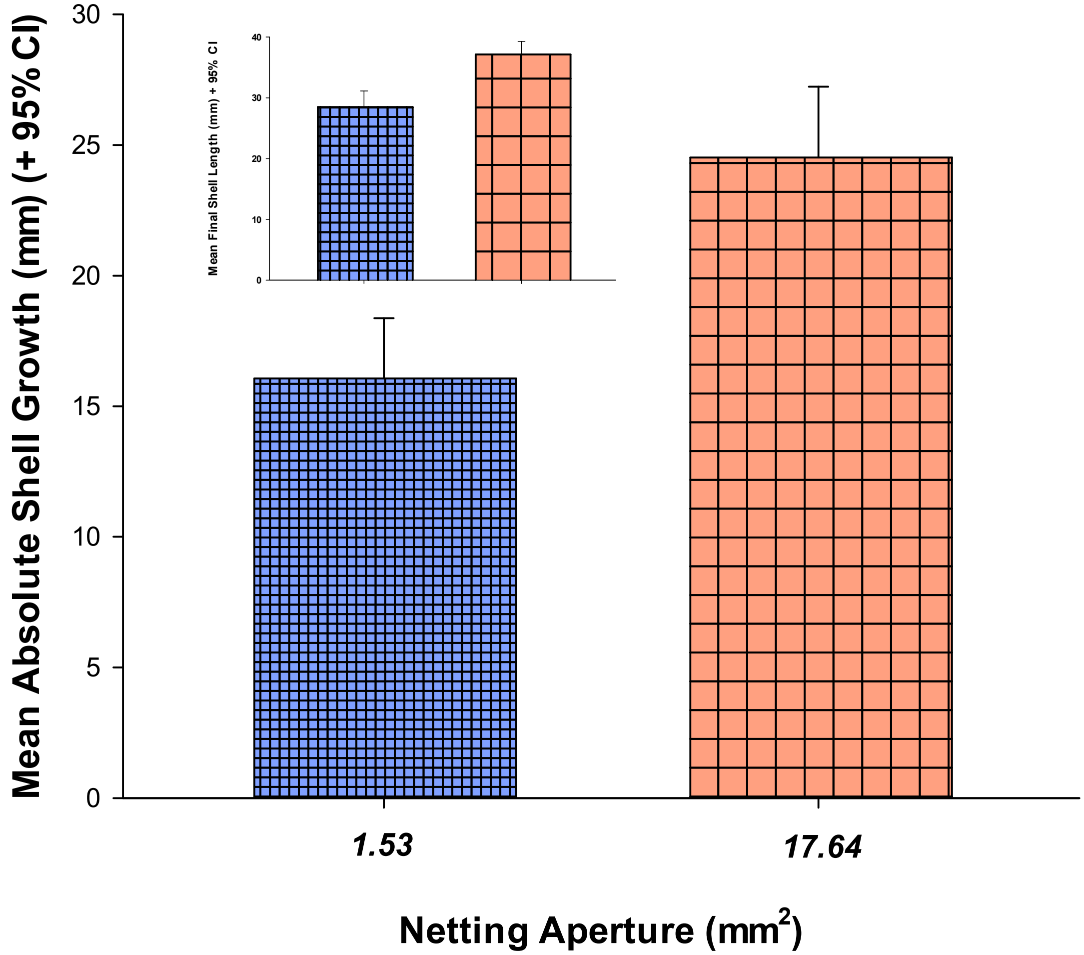


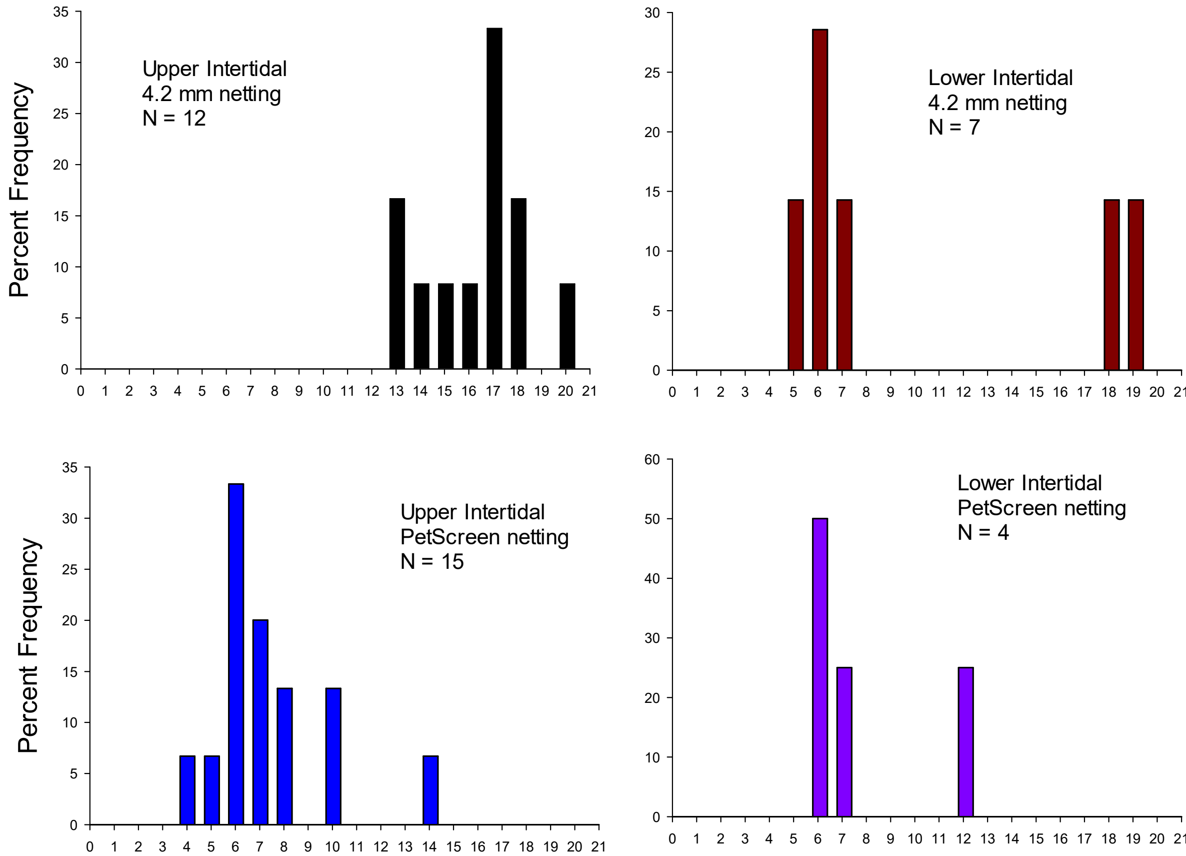
Figure 4.

Figure 5.



Mean Absolute Shell Growth (mm) (+ 95% CI)
30
25
20
15
10
5
0
1.53
17.64
Netting Aperture (mm2)
Mean Final Shell Length (mm) + 95% CI
40
30
20
10
0

Figure 6.


Upper Intertidal
4.2 mm netting
N = 12
Lower Intertidal
4.2 mm netting
N = 7
Upper Intertidal
PetScreen netting
N = 15
Lower Intertidal
PetScreen netting
N = 4
Percent Frequency
Percent Frequency