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Large-scale, manipulative field tests involving cultured and wild juveniles of the soft-shell clam, *Mya arenaria* L.: Interactive effects of predator exclusion netting, aperture size, and planting area on seasonal growth and survival at the Willows Flat within the Hampton-Seabrook Estuary.

An Interim Report to

The New Hampshire Estuaries Project

Submitted by

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Executive Summary

A series of field experiments to assess the efficacy of enhancing intertidal areas with cultured clam (*Mya arenaria* L.) seed (mean shell length [SL] = 7-10 mm) was conducted at Willows Flat, Hampton, New Hampshire from November 2004 to May 2005 and from June - October 2005. The first trial examined the interactive effects of size of planting area (4, 8, 12, 18 m^2) and predator deterrent netting (none, 4.2 mm, and 6.4 mm aperture [flexible, plastic netting]) on clam growth and survival at one intertidal location. The second trial examined the effect of predator deterrent netting on clam growth and survival at two intertidal locations.

From November 2004 to May 2005, clam survival was nearly 90% in plots protected with the smallest aperture netting, and this was three times greater than survival in plots protected with 6.4 mm mesh netting. Few animals were recovered from plots that were not covered fully with plastic netting. Overall, enhancement due to the predator deterrent netting was greater than 100-fold. Clams survival in the smallest size plots was significantly greater (by 30%) than those in the three larger sized plots. Clams reached a mean shell length of 14.6 ± 0.57 mm during this period, an average increase in shell of 4.2 mm. Growth rate of clams was 30% faster in plots protected with the smaller aperture netting. Plot size affected growth rate, but the effects were complex. For example, no differences in clam growth rate were detected between the smallest vs. the other three plot sizes; however, clams grew more slowly in the 8 m^2 plots compared to the mean of the two largest plot sizes. This study indicates that 1) it is possible to seed flats in the Hampton River area with cultured soft-shell clam seed in the late fall and be successful (i.e., attain survival rates > 75%); 2) protecting clams with plastic, flexible netting is warranted and necessary to deter predators and retain clams in the seeded areas; 3) if clam sizes are $\leq$ 10 mm

SL, using 4.2 mm mesh netting rather than 6.4 mm netting will yield higher recovery rates; and, 4) seeding small areas (< 8 m^2), rather than larger ones will result in higher clam yields.

The experiment initiated in June 2005 must be repeated in 2006 due to mass mortality shortly after seeding. Animals were seeded on an extremely hot day (11 June 2005) when pre-noon temperatures reached > 32^oC. Animals were exposed to the air and heat for several hours before the tide covered the seeded plots and observations made within a week after the seeding event suggested that a massive die-off occurred soon after the seeding event. By 8 October, losses of greater than 1,200 individuals m^{-2} had occurred in all three treatments at both intertidal locations. Although results were more than disappointing, the study yielded several pieces of valuable information that can be used in future. First, clam numbers were enhanced by using protective netting. In fact, no clams were recovered in benthic cores from plots that were seeded but not covered with netting. Second, plots covered with the smaller aperture netting at both sites produced the highest number of clams – a result similar to the first experiment – suggesting the patterns observed here and in previous trials in this region (see Beal 2002) are generalizable.

Clam populations in this region are exposed to intense predation (due mostly to green crabs and bottom feeding fish) that can eliminate entire year classes. Experimental results to date indicate that enhancement can be effective if carried out properly; however, it is unknown whether these activities are cost effective. Only after the field trials of 2006 can this important question be assessed.

Introduction

Resource managers are responsible for the stewardship of commercially or recreationally important populations of marine and terrestrial organisms. Managers must make decisions concerning the status and health of these populations for a variety of applications, the most common being whether the population is abundant enough to be harvested and what level of harvesting will have minimal impacts on future populations. Because of logistical constraints imposed by working in marine environments, managers of marine resources often have limited information about important population characteristics such as survival, growth, recruitment rate and how these parameters change spatially and temporally. Rather, decisions about harvest levels, for example, usually are limited to estimates of changes in standing stocks and size frequencies through time or between locations.

It is rare that adaptive management strategies and experimental approaches are considered by fisheries managers (but see Botsford et al., 1997; Lenihan and Micheli, 2000; Beal and Vencile, 2001); however, manipulative field experiments are the strongest and most efficient means available to managers to base decisions about the dynamics of a population (Underwood, 1990, 1991). Soft-shell clams, *Mya arenaria* L., represent an important recreational fishery along the New Hampshire coast, but specifically in the Hampton-Seabrook Estuary. During the Fall 1998, over 900 clammers easily harvested their 9.5-liter limit when one flat (Middle Ground) was opened after a 10-year hiatus due to fecal contamination (Varney, 1999). Since that time, clam abundance on that and two other flats in the same vicinity has dwindled. Recent surveys of these flats suggested to managers that the limiting factor for a sustainable fishery was poor juvenile survival (NHEP, 2001). Despite apparent successful reproduction and larval settlement, the

population of yearling clams (i.e., age 7-12 months and 26-50 mm shell length) was very low (NHEP, 2001).

During the winter of 2001 and spring/early summer of 2002, the New Hampshire Estuaries Project commissioned a study to evaluate factors contributing to the mortalities of juvenile soft-shell clams in the Hampton-Seabrook Estuary. Results from two short-term field experiments at three intertidal sites demonstrated that mortality due to disease (specifically neoplasia), interspecific competition, and winterkill due to ice and storms was minimal. Clam losses associated with sediment scouring and predation exceeded 95% in some instances over the winter (November 2001 to March 2002; Beal [2002]). Similar losses at the same sites occurred during the period from March to July 2002, but in most cases, survival was enhanced by using protective mesh netting (6.4 mm aperture).

Among the limitations of those field tests were: 1) the use of small experimental units (6-inch plastic plant pots), 2) experiments were conducted once, 3) the use of a single mesh netting aperture size, and 4) no data were collected during times when seawater temperatures were seasonally greatest (i.e., July through September).

Project Objectives

1) To determine the interactive effects of predator exclusion netting, mesh netting aperture size, and planting area on survival and growth of cultured and wild juveniles of the soft-

shell clam, *Mya arenaria* L., during the fall and winter at the Willows Flat in the Hampton-Seabrook Estuary.

2) To determine the interactive effects of predator exclusion netting, mesh netting aperture size, and intertidal location on the survival and growth of cultured and wild juveniles of the soft-shell clam, *Mya arenaria* L., during the spring through early fall at the Willows Flat in the Hampton-Seabrook Estuary.

In addition, the following questions were considered:

1) What are the costs and benefits associated with enhancing intertidal areas with hatchery-reared individuals (ca. 8 mm shell length, SL)?

2) Does the use of netting across several planting areas and aperture sizes enhance clam survival compared with similar size areas that are planted but receive no netting?

3) Is it efficacious to use netting to create spatial refuges that protect small clams already in the sediments (or that are somehow attracted to netted areas)?

4) Does growth or survival of cultured and/or wild juveniles of the soft-shell clam vary with mesh aperture size?

5) What effects on growth and survival, if any, can be attributed to the actual size of the area seeded? Do clams respond "better" (i.e., faster growth and/or higher survival) when "edge effects" due to the size of the netted area are relatively minimal or maximal?

6) What time of year (spring vs. fall) is better to initiate clam enhancement programs?

7) Is the effectiveness of netted plots similar at different intertidal sites at the same tidal height?

Methods and Materials

Experiment I.

Study site and experimental animals

An intertidal field experiment was initiated on 20-21 November 2004 at the Willows Flat (WF) in the Hampton River, Hampton, New Hampshire (42°54.49' N; 70°49.45' W) to assess the interactive effects of size of planting area and predator exclusion on the growth and survival of hatchery-reared individuals of the soft-shell clam, *Mya arenaria* L. Clams (mean shell length [SL] ± 95% CI = 10.4 ± 0.47 mm, n = 174; range = 4.2-18.3 mm) were reared in 2004 at the Downeast Institute for Applied Marine Research & Education (DEI; Beals, Maine).

Experimental design

A completely random design of 96 plots (four replicates of 24 treatments) was established in three rows of 32 plots arrayed parallel to the water at low tide (5 m spacing between plots within a row and between rows). Clams were added to one-half the plots that varied in area as follows: 4 m^2, 8m^2, 12m^2, and 18m^2. Two-thirds of the plots were protected with flexible plastic netting (InterNet, Inc., Minneapolis, MN) (aperture = 4.2 mm or 6.4 mm), while the remaining plots received no netting. Each level of each treatment (Plot size [a=4]; Clams [b=2]; Netting [c=3]) was orthogonal, or fully factorial.

Nets were established around the plots by digging a 15-20 cm deep furrow around the periphery of the plot with clam hoes (Robinson and Rowell 1990) and shovels. The edge of the netting was secured by placing it within the furrow and then back-filling sediments into the furrow. No flotation was added to the nets because sediments were sandy and rippled indicative of a high-

energy site. Previous experience has shown that in similar environments floats designed to raise the netting above the surface of the flat (*sensu* Beal and Kraus 2002) resulted in significant sediment deposition and high clam mortality due to suffocation (B. Beal, pers. obs.). After establishing each plot and before clams and/or nets were added, a garden rake was used to loosen sediments. To establish initial densities of wild clams, a benthic core (A = 0.182 m^2) sample was taken from each plot (N = 96) prior to raking and the contents washed through a 2 mm sieve.

Assessing the fate of the netting and Spring sampling

The fate of the netting was assessed nine times through the fall and winter from 3 December 2004 to 2 April 2005. On each visit, all plots were inspected and qualitatively assessed for degree of scouring and erosion. In addition, torn or ripped nets were recorded.

On 14-15 May 2005, four benthic core (A = 0.182 m^2) samples were taken from each plot. Because small clams tend to have contagious distributions (B. Beal, pers. obs.), plots were divided into fourths (parallel to the shore) and a core taken randomly from the middle of each section. Core samples were washed through a 2 mm sieve. It was possible to discern wild from cultured clams based on a discrete shell mark that occurs once cultured clams are added to sediments (Beal et al. 1999). The final SL of all live clams was measured using a Vernier caliper (to the nearest 0.1 mm). For cultured clams, initial SL was measured similarly and that allowed an estimate of an individual's growth rate during the experimental period. Because absolute growth (final SL - initial SL) was positively correlated with initial clam size ($P < 0.0001$, $r^2 = 0.209$, $n = 1790$), I used relative growth rate ([final SL - initial SL]/initial SL) instead to compare potential treatment effects.

I returned to the Willows Flat on 11 June, 26 June, and 26 July 2005 and collected experimental clams using a clam hoe in the areas that had been seeded and protected with netting. The final and initial SL of these individuals was recorded as described above.

Statistical analyses

Analysis of variance (ANOVA) was performed on the square root-transformed number of wild and cultured clams per core. Data transformation was necessary to meet both variance homogeneity and normality assumptions of ANOVA. ANOVA was performed on the untransformed relative growth rate data. The linear model I used for the ANOVA was as follows:

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

Where,

μ = theoretical mean;

A_i = Plot size (i = 4 levels: 4 m^2, 8 m^2, 12 m^2, and 18 m^2; factor is fixed);

B_j = Netting (j = 3 levels: none, 6.4 mm, and 4.2 mm aperture; factor is fixed);

C_k = Clams (k = 2 levels: present or absent; factor is fixed);

D_l = Core (l = 4 levels: a,b,c,d; factor is random); and,

e_m = Experimental error (m = 4 replicates randomly assigned per treatment).

In addition, I incorporated two sets of orthogonal, a priori, single degree-of-freedom contrasts to help discern potential main and interactive effects. These were as follows:

A) Plot size:

1) 4 m^2 vs. (8 m^2, 12 m^2, and 18 m^2);

2) 8 m^2 vs. (12 m^2 and 18 m^2);

3) 12 m^2 vs. 18 m^2;

B) Netting:

1) No netting vs. netting;

2) Small mesh vs. Large mesh

To reduce the potential for excessive type I errors, the alpha level for each set of contrasts was adjusted using the suggestion of Winer et al. (1991): $\alpha' = 1 - (1 - \alpha)^{1/r}$, where $\alpha = 0.05$ and r, the number of contrasts, equals three or two. Therefore, the adjusted alpha level was 0.0170 for the contrasts involving plot size and 0.0253 for the netting contrasts.

Experiment II.

Study site and experimental animals

A field experiment to test the effects of excluding predators using flexible netting on growth and survival of cultured clams was initiated on 11 June 2005 at two intertidal sites located approximately 400 m apart at WF in the Hampton River, Hampton, New Hampshire (site 1 = 42°54.53'N, 70 ° 49.53'W; site 2 = 42°54.41'N, 70°49.35'W). Initial clam size was 7.3 ± 0.5 mm (n = 100; range = 3.9-15.6 mm). Animals were reared at DEI in 2004 and overwintered according to Beal et al. (1995). Clam seeding occurred from 0700 to 1030, and the animals did not burrow into the sediments until plots were completely covered with seawater. Unfortunately, the tide did not cover all plots until 1230 and it was a sunny day with air temperatures at 1200 approximately 32°C. As the tide approached the plots, water was kicked onto the clams to keep them from drifting away (when the valves of small clams dry, they are highly susceptible to floating and drifting along with the tide); however, this action was not 100% effective in keeping clams from moving out of the plots. Many clams in the netted plots drifted to the shoreward limit of the plot leaving "windrows" of animals.

Experimental design

Fifteen 18m^2 plots were established at two intertidal locations and each seeded with cultured clams at a density of 1,272 m^{-2}. The sediment surface of each plot was raked (as described above). At each location, five plots were covered with a 6.4 mm or a 4.2 mm flexible netting while no netting was applied to the other five, that served as predator controls. On 8 October 2005, each plot was divided into thirds (parallel to the shore) and a single benthic core sampled (A = 0.0182 m^2) was taken (N = 45 per location). Core samples were sieved on site through a 2

mm mesh and all live clams (both wild and cultured) were retained. The length of all wild clams was recorded, as was both the initial and final length of the cultured clams (as described above). To establish initial densities of wild clams, a benthic core (A = 0.182 m^2) sample was taken from each plot (N = 30) prior to raking and the contents washed through a 2 mm sieve.

Statistical analyses

Analysis of variance (ANOVA) was performed on the square root-transformed number of wild and cultured clams per core. Data transformation was necessary to meet both variance homogeneity and normality assumptions of ANOVA. ANOVA was performed on the untransformed relative growth rate data. Mean square error terms for each source of variation were calculated using Underwood (1997). The linear model I used for the ANOVA was as follows:

$Y_{ijklm} = \mu + A_i + B_j + AB_{ij} + C(AB)_{k(ij)} + e_{l(ijk)}$

Where,

μ = theoretical mean;

A_i = Location (i = 2 levels; factor is random);

B_j = Netting (j = 3 levels: none, 6.4 mm, and 4.2 mm aperture; factor is fixed);

C_k = Core (l = 4 levels: a,b,c,d; factor is random); and,

e_m = Experimental error (m = 4 replicates randomly assigned per treatment).

In addition, a set of orthogonal, a priori, single degree-of-freedom contrasts two were conducted for the main effect due to netting as described above.

Results

Experiment I.

20-21 November 2004 sampling

Two clams were found in the cores (17.1 and 20.5 mm). This equates to a density of 1.14 ± 1.59 individuals · m^{-2} (n = 96). Also, two male green crabs were found in the cores (6.8 and 14.6 mm carapace width [CW]).

May 2005 sampling

Wild clams

Wild clams were found in 29 of the 96 plots (30.2%). ANOVA indicated that initial presence or absence of cultured clams in the plot was the only significant source of variation (P = 0.0039, Table 1). Of the 40 clams from the 384 samples, 32 occurred in plots initially seeded with cultured clams. There was an approximate 4-fold enhancement in wild clams in plots with (0.17 ± 0.07 $core^{-1}$, or 9.16 ± 3.62 m^{-2}) vs. without (0.04 ± 0.03 $core^{-1}$, or 2.29 m^{-2}) cultured clam seed. Although 80% of the wild clams were found in netted vs. unnetted plots, this was not statistically significant (P = 0.1179, Table 1). The size frequency distribution of wild seed (Fig. 1) shows that 95% of individuals were < 15 mm SL.

Cultured clams – survival

Each main factor (Netting, Plot size, Clam presence) and one interaction term (Net x Clams) was statistically significant (Table 2, P < 0.035). Although one-third of the plots received no hatchery seed clams, some dispersal apparently occurred (Table 3). In each case (5 of 12 treatments) however, the mean number per plot was not significantly different from zero (one-

sample t-test; P = 0.3910). In plots that initially received hatchery-reared individuals, the presence of plastic netting enhanced number of clams per core by a mean of 104.8 times over control plots where no netting was applied (Table 3). To determine whether this enhancement was significantly different from zero, I examined a reduced linear model (without the "clams" source of variation) and compared the twelve treatments in which hatchery seed were employed (Table 4). Both main factors (netting and plot size) were statistically significant ($P < 0.05$). Both a priori contrasts associated with the netting source of variation were highly significant ($P < 0.0001$, Table 4). Approximately three times as many clams were sampled in cores from plots protected with small versus large netting (Fig. 2).

Two nets developed tears between 18 December 2004 and 5 January 2005, and both occurred in the row nearest the low water mark. One of the nets had small mesh and protected clams in a 12 m^2 plot. That net had extensive damage as approximately one-quarter of the net was missing. I asked whether the mean number of hatchery-reared clam individuals (ind.) per core from that plot (3.75 ± 3.76 ind., n = 4) differed significantly from the mean of the other three replicates of that treatment (replicate 1: 21.5 ± 14.02 ind.; replicate 2: 17.00 ± 16.59 ind.; replicate 3: 20.75 ± 14.95 ind.; P = 0.0119). The damage to the other net that had large mesh and protected clams in an 18 m^2 plot was not extensive, as the ripping exposed less than $1/25^{th}$ of the seeded area. Although the mean number per core in that plot (5.25 ± 5.72 ind.) was less than two of the other three replicates, it was not significantly different from the mean of the other three undamaged replicates (P = 0.2548).

Significantly more clams (34.4%) were sampled from the smallest plots (657.1 ± 459.2 ind. m^{-2}, n = 12) compared to the mean of the other three plot sizes (488.8 ± 346.9 ind. m^{-2}, P = 0.0078, Table 4), and this did not vary across netting treatments (P = 0.1478). It is unclear whether this difference (Fig. 3) is due to the difference in the size of the plots or clam behavior. In general, juvenile soft-shell clams are contagiously distributed (see Commito 1982, B. Beal, pers. obs.). Although initial densities of clams was similar between plots (ca. 1310 ind. m^{-2}), animals in the smallest plots may not be able to aggregate as much as those in the larger area plots. I used Morisita's Index of Spatial Dispersion (I_d) to determine, for netted plots initially seeded with clams, the type of dispersion clams exhibited (random, uniform, contagious). The I_d value was 1.929 ($P < 0.0001$) indicating a contagious distribution. When I examined only the 4 m^2 netted plots and recalculated the Index, the I_d value was similar and the distribution, once again, was contagious ($P < 0.0001$). Because clams were not randomly distributed, it makes it difficult to assess why significantly more animals were found in cores sampled from the smallest plots. In addition, a contagious distribution makes it difficult to estimate clam survival. If animals were randomly or uniformly distributed, then fewer assumptions would be required to use the core samples to estimate survivorship.

Although animals were not randomly distributed, survivorship estimates can still be calculated, but should be interpreted cautiously. Using means from Table 2 and an initial stocking density of 1310 m^{-2}, clams under the small netting exhibited an overwinter survival of 89.7% whereas clams under the larger netting was substantially lower at 30.9%.

Cultured clams – growth

Relative growth rate varied significantly due to netting ($P < 0.0001$) and size of plot ($P = 0.0044$; Table 5). Relative growth of clams was approximately 30% faster under the small (20.7 ± 1.9%, n = 16) vs. large aperture netting (15.7 ± 3.0%, n = 16; Fig. 4); however, this difference did not translate to mean final length as clams under both types of nets had similar final SL's in May 2005 (ca. 14.5 mm SL; Fig. 5). Mean relative growth of clams in 8 m^2 plots (14.9 ± 2.8%, n = 10) was significantly slower than mean growth in the two larger plots (12 m^2 and 18 m^2: 19.8 ± 3.9%, n = 19).

Clams were sampled on three dates after the experiment concluded (11 June [n = 16], 26 June [n = 16], and 26 July 2005 [n = 10]). ANOVA on mean relative growth was significant ($P = 0.0006$) and an a posteriori Student-Newman-Keuls test indicated that the June and July means were not significantly different ($P > 0.05$; Fig. 6).

Experiment II.

11 June 2005 sampling

Wild clams

A total of sixteen wild clams were recovered from samples at site 1 (1.06 ± 0.94 ind. $core^{-1}$; 58.1 ± 52.03 ind. m^{-2}) and five from site 2 (0.2 ± 0.23 ind. $core^{-1}$; 10.9 ± 12.59 ind. m^{-2}). ANOVA on the square root-transformed density data indicated that these differences were not statistically significant ($P = 0.0638$). Mean SL (4.4 ± 0.56 mm; range = 3.4-5.6 mm) did not vary between sites ($P = 0.8325$). The value of Morisita's Index of Spatial Dispersion (I_d) was 3.684 ($P < 0.0001$) indicating a contagious, or clumped, distribution.

8 October 2005 sampling

Wild clams

A total of 111 wild clams occurred in the core samples and mean number varied significantly between sites (e.g., site 1 = 84.2 ± 26.9 ind. m^{-2}; site 2 = 50.1 ± 24.4 ind. m^{-2}; n = 15; P = 0.0096, Table 6). Significant effects were observed due to predator exclusion (0.0117). The a priori, orthogonal contrasts demonstrated that a 3-fold enhancement of wild clams occurred due to the presence of the netting ($0_{netting}$ = 87.9 ± 32.3 ind. m^{-2}, n = 20 vs. $0_{no\ netting}$ = 27.5 ± 21.6 ind. m^{-2}; n = 10; P = 0.0108). In addition, significantly more wild clams were sampled from plots protected with the small vs. large aperture netting (126.3 ± 76.2 vs. 49.4 ± 40.5 ind. m^{-2}, n = 10, P = 0.0128, Table 6). The size distribution of wild clams was bimodal (Fig. 7) with the recruits from the 2005 summer ranging in SL from 4-14 mm, while the 2004 year class ranged from 16-28 mm. ANOVA on the untransformed mean final length data indicated no differences between locations (P = 0.0872), but that clams were nearly double the size under nets than in control plots at both sites (12.1 ± 1.9 mm vs. 6.1 ± 0.8 mm; P < 0.0055).

Cultured clams – survival

Clam survival at both sites was extremely poor, presumably due to the conditions at the study site on the day when the experiment was initiated. Mean number of individuals (individuals m^{-2}) did not differ between sites (50.7 ± 32.4 m^{-2}; P = 0.6657, Table 7). The data suggests losses of greater than 1,200 individuals m^{-2} over the 119 day trial. Observations made on 26 June 2005 (15 days after the experiment was initiated) suggested that most of the mortality had occurred by that date. Many dead, undamaged individuals were observed on the sediment surface on the shoreward end of most netted plots at both sites. Few siphon holes were observed in any of the

plots, and, by the next observation date (28 July), many of the nets had silted over with the sandy sediments typical of the Willows Flat. One net at site 2 (nearest the parking area) had been completely torn, while small rips were discovered in seven of the remaining nine nets. No damage to nets was observed at site 2.

ANOVA demonstrated significant clam enhancement due to the presence of netting at both sites ($P = 0.0062$). No cultured clams were recovered from any core taken from control plots ($n = 10$) whereas a mean of 76.0 ± 45.6 individuals m^{-2} occurred in cores taken from plots protected with netting. A 9-fold difference in enhancement occurred between plots covered with the 6.4 mm netting (i.e., large net; 14.7 ± 13.5 ind. m^{-2}) vs. the 4.2 mm netting (i.e., small net; 137.4 ± 75.9 ind. m^{-2}; $P = 0.0045$, Table 7, Fig. 8).

Cultured clams – growth

A total of 82 cultured clams was sampled from the 90 cores (mean SL = 17.4 ± 0.6 mm, range = 11.6-22.8 mm; Fig. 9). No significant differences in mean relative growth occurred between sites ($P = 0.6508$) or among netting treatments (0.8734). There was a significant Location x Netting Treatment interaction ($P = 0.0454$, Fig. 10) indicating that the pattern of relative growth between the two treatments was different at the two sites.

Discussion

The work completed to date addressed two broad objectives:

1) To determine the interactive effects of predator exclusion netting, mesh netting aperture size, and planting area on survival and growth of cultured and wild juveniles of the soft-shell clam, *Mya arenaria* L., during the fall and winter at the Willows Flat in the Hampton-Seabrook Estuary; and,
2) To determine the interactive effects of predator exclusion netting, mesh netting aperture size, and intertidal location on the survival and growth of cultured and wild juveniles of the soft-shell clam, *Mya arenaria* L., during the spring through early fall at the Willows Flat in the Hampton-Seabrook Estuary.

The first objective was met. The second was not. From November 2004 to May 2005, the numbers of cultured clams were enhanced more than 100-fold by using flexible, plastic netting to deter predators. However, there was a significant difference in the effectiveness of the netting depending on its aperture size (Fig. 2). Nearly 90% of seeded clams survived in plots protected with the smaller aperture netting (4.2 mm) whereas only 30% were recovered from plots covered with the larger aperture netting (6.4 mm). This difference likely is due to small clams escaping through the apertures of the larger netting. For example, although aperture size is referred to as 6.4 mm, this measurement is the length of two sides of a right triangle, and not the hypotenuse. That is, the length of the 6.4 mm mesh along the diagonal is 9.1 mm vs. 5.9 mm for the 4.2 mm mesh. It may be possible for clams to escape through the aperture of the protective netting by crawling though, in which case clam width (measured from the umbo to the ventral margin), not

clam length, would be important. Therefore, I examined the relationship between clam length and width (Fig. 11) and it suggests that clams with SL's as large as 14 mm may be able to crawl through 6.4 mm netting whereas animals as large as 9 mm may be able to crawl through 4.2 mm netting. Past studies in eastern Maine (Beal et al. 2001; Beal and Kraus 2002) have used plastic, flexible netting (6.4 mm aperture) to protect clams from predators with excellent success (survival > 80% over an 8-month growing season – April to November). Those studies, however, were conducted in soft, muddy sediments with high water content at low tide when seeding occurred so that when clams were placed on the surface of the flat they were able to burrow rapidly below the sediment surface (typically within 30 minutes). At the Willows Flat, sediments were sandy and, since clams were seeded at low tide, animals remained on the sand flat surface until the tide covered them. It may have been likely that as the tide covered the clams, many were physically moved to the periphery of the netted plot where their momentum was hindered. For clams seeded into plots that were not covered with netting, it may have been likely that at least some were moved out of the plot area by tidal currents before they were able to burrow into the sediments. The conclusion, then, is that if clams with shell lengths < 14 mm are to be used to enhance sandy flats in this area, small aperture netting (4.2 mm) should be used to maximize survival. Another reason for using the small aperture netting is that clams grew approximately 30% faster in plots with the small vs. large aperture mesh.

This study also examined the relationship between clam numbers and plot size. Four different plot sizes were used: 4, 8, 12, and 18 m^2. For plots not protected with the mesh netting, no differences were observed in mean clam number per core or per square meter (3-27 m^{-2}) across any of the plot sizes. However, when netting was applied to the plots, approximately 30% more

clams occurred in the core samples from the smallest size plots compared to the other plots (928.91 ± 344.75 m^{-2} vs. 706.8 ± 139.86 m^{-2}). One reason for this result may be that clams in the smaller netted areas were not able to spread out as much as they might have in the larger plots, which may have concentrated them more. Nonetheless, the data suggest that smaller, rather than larger plots should be used during enhancement projects.

Although the second objective was not fully met due to poor survival as a result of planting clams on a day in June that was too hot, several important themes are worth noting. First, clam numbers were enhanced in protected vs. unprotected plots. No clams were sampled from cores taken from control plots lacking the protective netting. Second, nearly 9 times the number of clams were recovered from core samples taken in plots protected with 4.2 mm mesh vs. 6.4 mm mesh (Fig. 8). Third, the effects due to protecting clams were similar across both intertidal locations suggesting that patterns may be generalizable in these sandy sediments.

Another series of experiments are planned for the Willows Flat and possibly Middle Ground beginning in late April or early May 2006. Clams will be seeded at densities of 1,320 m-2 in 12 m^2 plots at two intertidal locations. The design of these trials will be similar to those initiated in June 2005, with one addition. An extra treatment will be added so that nets will not contact directly the sediment surface while clams are feeding (sensu Beal and Kraus 2002). Small, Styrofoam floats will be attached to the underside of some nets so that during periods of tidal inundation, nets will float approximately 15-30 cm from the sand flat surface. The experiment will be terminated in late October or early November 2006.

Acknowledgments

These investigations were funded by the New Hampshire Estuaries Program and the University of Maine at Machias. I thank the following people who helped in the field in November 2004: Jane April, Caleb Beal, Nathan Clark, Shawn Dubois, Jeffrey Enderson, Stephen Engstrom, Jennifer Hunter, David Kellam, Frank Marenghi, Chris Nash, Neil Savage, Phil Trowbridge and son, and Dan Williams. D. Kellam and Matthew Wood helped me check on the status of the netting through the winter months. J. April, N. Clark, S. Dubois, J. Enderson, N. Savage, and D. Williams, assisted with the May sampling. This effort could not have been completed without everyone's assistance for which I am grateful.

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Table 1. ANOVA results on the square-root transformed number of wild clams per core sampled from Willows Flat, Hampton, New Hampshire on 14-15 May 2005 (n = 4). To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ($\alpha'_{netting} = 0.0253$; $\alpha'_{Plot\ size} = 0.0170$). Boldface P-values indicate statistical significance

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Netting	2	0.50447315	0.25223658	2.20	0.1179
Clams	1	1.02125913	1.02125913	8.92	**0.0039**
Plot size	3	0.06153175	0.02051058	0.18	0.9102
Net * Clams	2	0.32155151	0.16077575	1.40	0.2522
Net * Plot size	6	0.66872911	0.11145485	0.97	0.4496
Clams * Plot size	3	0.00662187	0.00220729	0.02	0.9963
Net * Clams * Plot size	6	0.33336771	0.05556128	0.49	0.8173
Core(Net * Clam * Plot size)	72	8.24428009	0.11450389	1.29	0.0736
Error	288	25.50000000	0.08854167		
Corrected Total	383	36.66181432			

Table 2. ANOVA results on the square-root transformed mean number of cultured clams per core sampled from Willows Flat, Hampton, New Hampshire on 14-15 May 2005 (n = 4). To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ($\alpha'_{netting}$ = 0.0253; $\alpha'_{Plot\ size}$ = 0.0170). Boldface P-values indicate statistical significance.

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Netting	2	276.8412389	138.4206194	361.86	**<.0001**
No netting vs. net	1	224.8023888	224.8023888	587.67	**<.0001**
Large net vs. Small net	1	52.0388501	52.0388501	136.04	**<.0001**
Clams	1	501.2050637	501.2050637	1310.24	**<.0001**
Plot size	3	3.4952705	1.1650902	3.05	**0.0342**
Sm vs. rest	1	3.1371166	3.1371166	8.20	**0.0055**
8 vs. 12 & 18	1	0.0964663	0.0964663	0.25	0.6171
12 vs. 18	1	0.2616876	0.2616876	0.68	0.4109
Net * Clams	2	275.9467481	137.9733740	360.69	**<.0001**
No net v. net x clams	1	218.3569261	218.3569261	570.82	**<.0001**
Lg net v. Sm net x clams	1	57.5898220	57.5898220	150.55	**<.0001**
Net * Plot size	6	3.9191904	0.6531984	1.71	0.1316
Clams * Plot size	3	3.0159334	1.0053111	2.63	0.0567
Net * Clams * Plot size	6	3.8258411	0.6376402	1.67	0.1416
Core(Net*Clam*Plot size)	72	27.5422014	0.3825306	0.47	0.9999
Error	288	236.209339	0.820171		
Total	383	1332.000826			

Table 3. Mean number of cultured clams per core (A = 0.0182 m^2) and per m^2 on 14-15 May 2005 at the Willows Flat, Hampton, New Hampshire. Four plot sizes were employed: 4 m^2, 8 m^2, 12 m^2, and 18 m^2. Three levels of netting occurred: None, Small mesh (S = 4.2 mm aperture), and Large mesh (L = 6.4 mm aperture). Initial stocking density was approximately 1,310 m^{-2}. (n = 4)

	Plot Size	Netting	Mean number of cultured clams (± 95% CI) **Per Core**	**Per 1 m^{-2}**
	4	None	0.00 (0.00)	0.00 (0.00)
		S	0.00 (0.00)	0.00 (0.00)
		L	0.13 (0.23)	6.86 (12.62)
Plots not seeded with cultured clams	8	None	0.00 (0.00)	0.00 (0.00)
		S	0.06 (0.19)	3.43 (10.93)
		L	0.06 (0.19)	3.43 (10.93)
	12	None	0.00 (0.00)	0.00 (0.00)
		S	0.00 (0.00)	0.00 (0.00)
		L	0.06 (0.19)	3.43 (10.93)
	18	None	0.06 (0.19)	3.43 (10.93)
		S	0.00 (0.00)	0.00 (0.00)
		L	0.00 (0.00)	0.00 (0.00)
**********				
	4	None	0.50 (1.59)	27.47 (16.33)
		S	27.06 (16.33)	1486.95 (897.02)
		L	8.31 (8.87)	456.73 (487.24)
Plots seeded with cultured clams	8	None	0.19 (0.20)	10.30 (10.93)
		S	20.44 (10.07)	1122.94 (553.35)
		L	6.13 (1.51)	336.54 (82.75)
	12	None	0.06 (0.19)	3.43 (10.93)
		S	15.69 (13.02)	861.95 (715.28)
		L	8.75 (9.53)	480.77 (523.67)
	18	None	0.13 (0.23)	6.86 (12.62)
		S	22.31 (19.68)	1225.96 (1081.35)
		L	6.38 (12.13)	350.27 (261.37)

Table 4. ANOVA results on the square-root transformed mean number of cultured clams per core from plots initially seeded with cultured clams and sampled from Willows Flat, Hampton, New Hampshire on 14-15 May 2005 (n = 4). To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ($\alpha'_{netting} = 0.0253$; $\alpha'_{Plot\ size} = 0.0170$). Boldface P-values indicate statistical significance.

Source of variation	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Netting	2	552.6942369	276.3471185	374.82	**<.0001**
No netting vs. net	1	443.1358774	443.1358774	601.04	**<.0001**
Lg vs. Small net	1	109.5583596	109.5583596	148.60	**<.0001**
Plot size	3	6.4903705	2.1634568	2.93	**0.0464**
Sm vs. rest	1	5.8637039	5.8637039	7.95	**0.0078**
8 vs. 12 & 18	1	0.1032915	0.1032915	0.14	0.7104
12 vs. 18	1	0.5233751	0.5233751	0.71	0.4051
Net*Plot size	6	7.5471149	1.2578525	1.71	0.1478
core(Net * Plot size)	36	26.5422014	0.7372834	0.46	0.9962
Error	144	231.7093388	1.6090926		
Total	191	824.9832626			

Table 5. ANOVA results on the untransformed mean relative growth rate of cultured clams planted on 19-20 November 2004 at the Willows Flat, Hampton, New Hampshire and sampled on 14-15 May 2005 (n = varied from 2 to 4, depending on survival). To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ($\alpha'_{netting} = 0.0253$; $\alpha'_{Plot\ size} = 0.0170$). Boldface P-values indicate statistical significance.

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Net	2	0.10286151	0.05143075	7.62	**0.0023**
No netting vs. net	1	0.02785768	0.02785768	4.13	0.0517
Large vs. Small net	1	0.07500383	0.07500383	11.11	**0.0024**
Plot size	3	0.11076759	0.03692253	5.47	**0.0044**
4 vs. Rest	1	0.01542203	0.01542203	2.28	0.1423
8 vs. 12 & 18	1	0.05387070	0.05387070	7.98	**0.0086**
12 vs. 18	1	0.04147552	0.04147552	6.14	0.0195
Net* Plot size	6	0.07192613	0.01198769	1.78	0.1407
Core(Net*Plot size)	28	0.18908866	0.00675317	0.95	0.5507
Error	93	0.66430048	0.00714302		
Corrected Total	132	1.14716610			

Table 6. ANOVA results on the square root-transformed number of wild clams per core in samples taken at Willows Flat, Hampton, New Hampshire, on 8 October 2005. To reduce the potential for excessive type I errors, the decision rule for both a priori contrasts was adjusted ($\alpha'_{netting} = 0.0253$). Boldface P-values indicate statistical significance. (n = 5)

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Location	1	2.92562851	2.92562851	9.46	**0.0096**
Netting treatment	2	12.77604519	6.38802259	84.13	**0.0117**
No netting vs. net	1	6.94103288	6.94103288	91.42	**0.0108**
Large vs. Small net	1	5.83501231	5.83501231	76.85	**0.0128**
Location*Treatment	2	0.15185197	0.07592598	0.25	0.7861
Core(Location*Treatment)	12	3.70992921	0.30916077	0.60	0.8364
Error	72	37.19215206	0.51655767		
Corrected Total	89	56.75560694			

Table 7. ANOVA results on the square root-transformed mean number of cultured clams per core at Willows Flat on 8 October 2005. Clams (1,272 m^{-2}) were seeded into fifteen 18 m^2 plots at two intertidal locations on 11 June 2005. To reduce the potential for excessive type I errors, the decision rule for both a priori contrasts was adjusted ($\alpha'_{netting} = 0.0253$). Boldface P-values indicate statistical significance. (n = 5)

Source of variation	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Location	1	0.08002073	0.08002073	0.47	0.5045
Netting treatment	2	27.15202790	13.57601395	189.64	**0.0052**
No netting vs. net	1	11.49197468	11.49197468	160.53	**0.0062**
Large vs. Small net	1	15.66005322	15.66005322	218.75	**0.0045**
Location*Treatment	2	0.14317476	0.07158738	0.42	0.6642
Core(location*Treatment)	12	2.02839074	0.16903256	0.40	0.9602
Error	72	30.61243651	0.42517273		
Corrected Total	89	60.01605064			

Figure Legends

Figure 1. Size-frequency distribution of wild soft-shell clams sampled from benthic cores during 14-15 May 2005. Four cores (A = 0.0182 m^2) were taken from each of 96 intertidal plots at the Willows Flat, Hampton, New Hampshire. Eighty percent of the clams were found in plots in which cultured clams had been planted in November 2004.

Figure 2. Mean number of cultured clams per m^2 from core samples taken on 14-15 May 2005. Samples were taken from plots initially seeded at a density of approximately 1310 m^2. ANOVA indicated that netting enhances clam numbers by nearly 105 times compared to numbers of clams in control plots ($P < 0.0001$, Table 2). Additionally, approximately three times more clams were sampled in plots protected with small vs. large netting ($P < 0.0001$, Table 2). (n = 16)

Figure 3. Mean number of cultured clams per m^2 from core samples taken from the four different seeding areas on 14-15 May 2005. Samples were taken from plots initially seeded at a density of approximately 1310 m^2. ANOVA revealed significant differences in cultured clam density among plot sizes as the mean of the smallest area was significantly different from the mean of the other three ($P = 0.0078$, Table 4). Solid line above bars indicates that means below are equal. (n = 12)

Figure 4. Mean relative growth of cultured clams in protected and unprotected plots for each planting area size. No difference in relative growth was observed between protected and unprotected areas, but clams under small netting (aperture = 4.2 mm) grew approximately 30% faster than those under large netting (aperture = 6.4 mm) (Table 5). Size of plot also influenced growth rate (see Table 5). (n = 4)

Figure 5. Initial and final size frequency distribution of cultured clams in protected and unprotected plots at Willows Flat, Hampton, New Hampshire.

Figure 6. Mean relative growth of clams in all seeded and netted plots at Willows Flat, Hampton, New Hampshire on 14-15 May 2005 (n = 124), and on three dates after the experiment was concluded. None of the clams sampled after this date came from protected plots. (See text for number of clams sampled from the post-May samples.) A relative growth of 100 represents a doubling of shell length. Lines above bars indicate equal means ($P > 0.05$).

Figure 7. Size frequency distribution of wild clams sampled from benthic cores taken from fifteen $18m^2$ plots at two intertidal locations on 8 October 2005 at Willows Flat, Hampton, New Hampshire. (n = 111)

Figure 8. Mean number of cultured clams in control and netted plots on 8 October 2005. Clams (7.3 ± 0.5 mm SL) were seeded into 18m2 plots on 11 June 2005 at a density of 1,272 m^{-2}. ANOVA indicated no differences in mean abundance between locations, a

significant enhancement due to the presence of netting, and a significant difference in mean number per m^2 between large and small protective netting (Table 7). (n = 5)

Figure 9. Size frequency distribution of cultured clams sampled from benthic cores taken from fifteen 18 m^2 plots at two intertidal locations on 8 October 2005 at Willows Flat, Hampton, New Hampshire. (n = 82)

Figure 10. Interaction plot of mean relative growth of cultured clams from benthic cores taken from fifteen 18m^2 plots at two intertidal locations on 8 October 2005 at Willows Flat, Hampton, New Hampshire. ANOVA demonstrated that neither main effects due to Site or Netting treatment were statistically significant; however, the interaction term was significant (P = 0.0454). The dashed line indicates the value for relative growth associated with a doubling of shell length.

Figure 11. Linear relationship (± 95% CI) between clam length and width for cultured individuals of *Mya arenaria* (Y = 0.214 + 0.617, n = 16, r^2= 0.938, P < 0.0001). The inset graph shows the initial size frequency distribution of clams seeded into plots in November 2004. The arrow pointing to the 14 mm bar indicates that animals as large as 14 mm are capable of escaping through 6.4 mm aperture netting.

Figure 1.

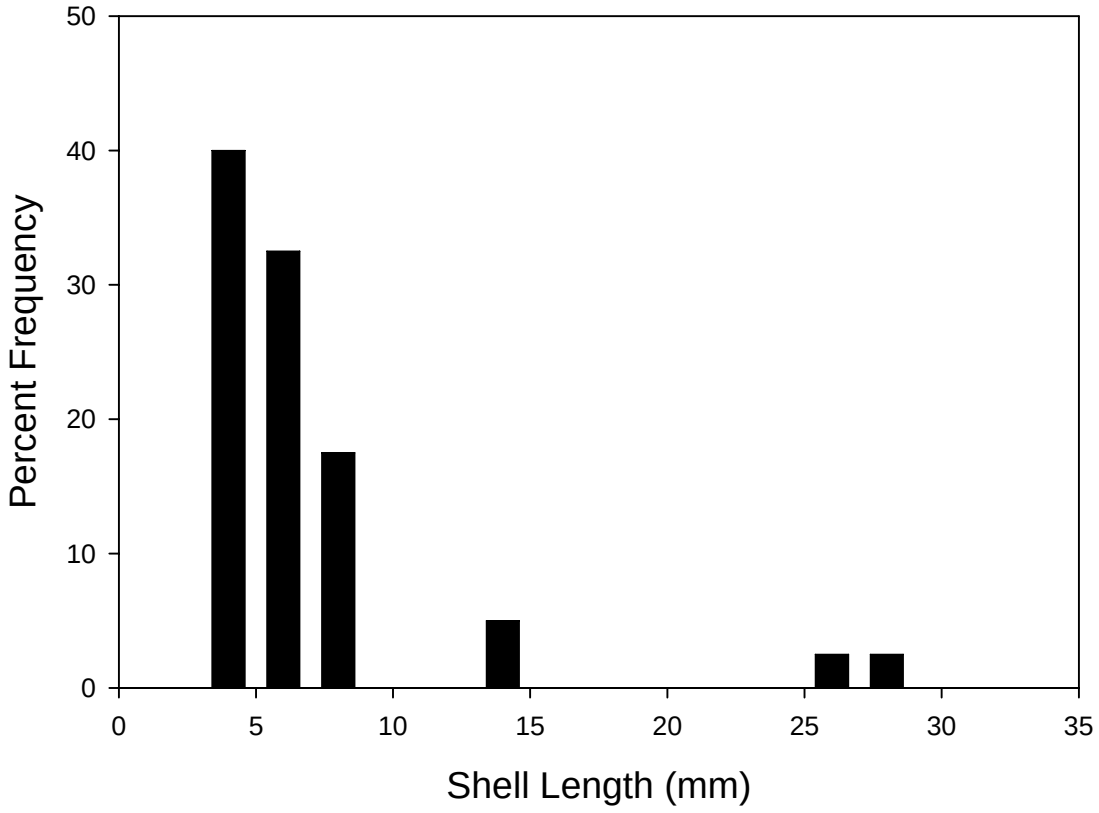

Percent Frequency
Shell Length (mm)
0
10
20
30
40
50
0
5
10
15
20
25
30
35

Figure 2.

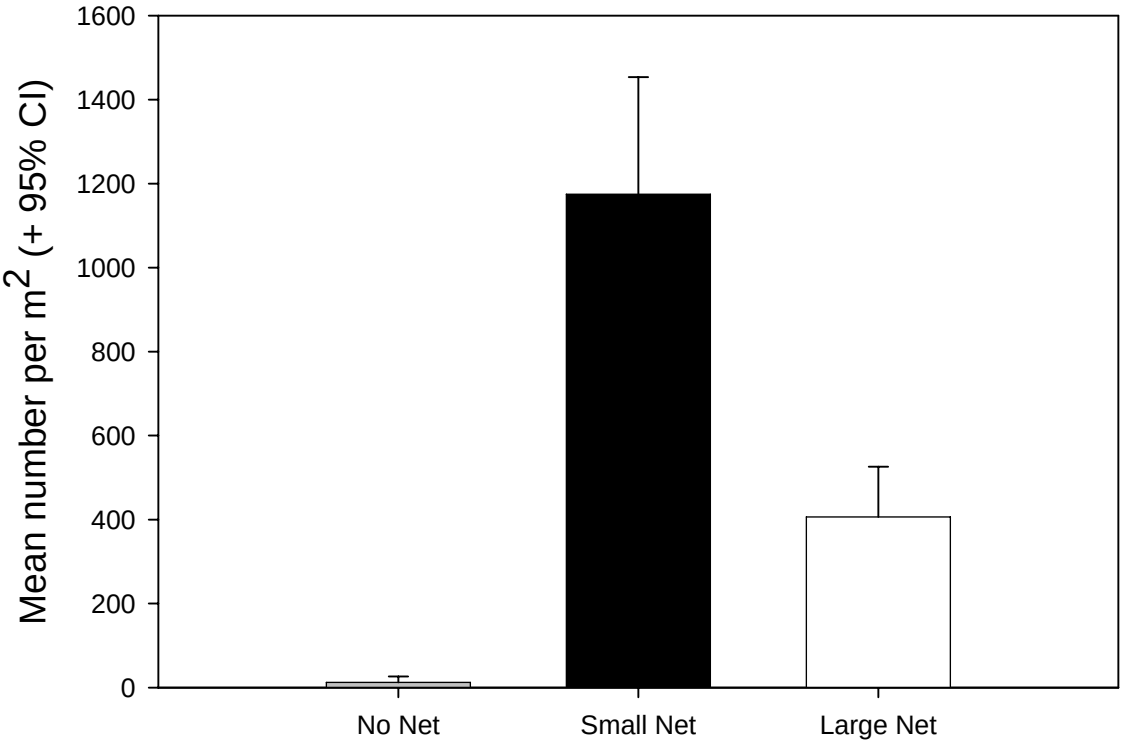

1600
1400
1200
1000
800
600
400
200
0
Mean number per m2 (+ 95% CI)
No Net
Small Net
Large Net

Figure 3.

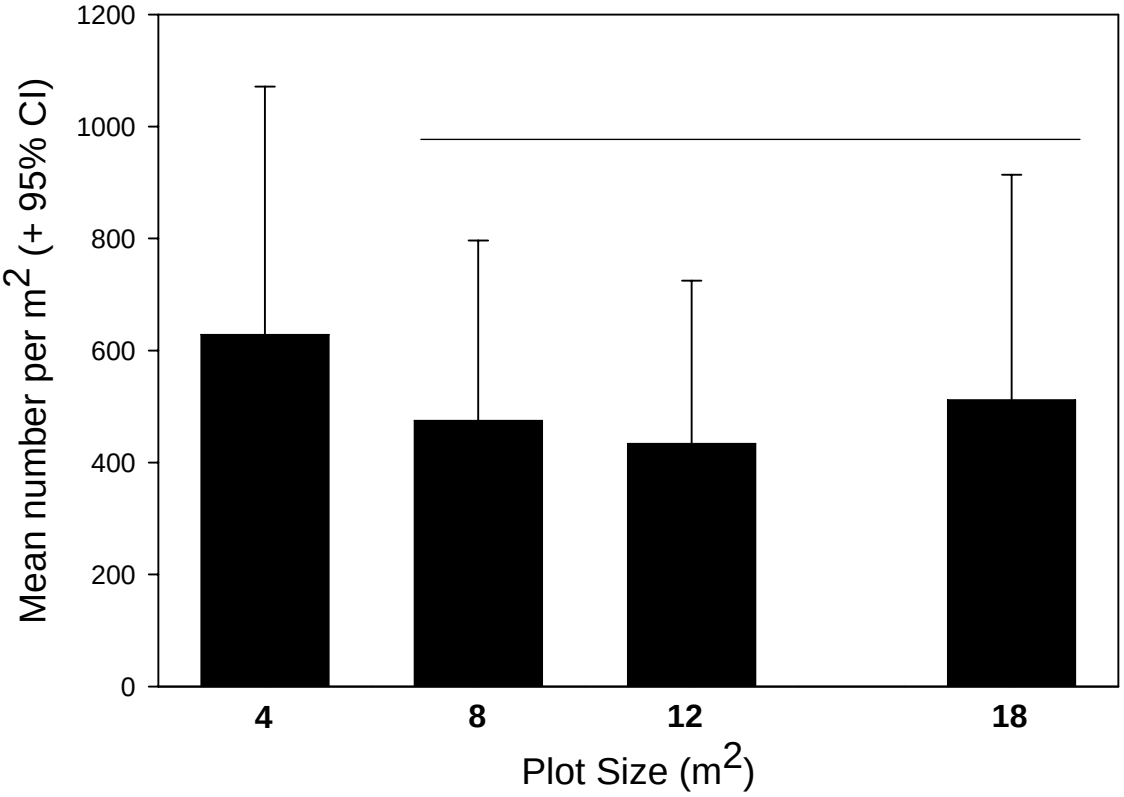

1200
1000
800
600
400
200
0
Mean number per m2 (+ 95% CI)
4
8
12
18
Plot Size (m2)

Figure 4.

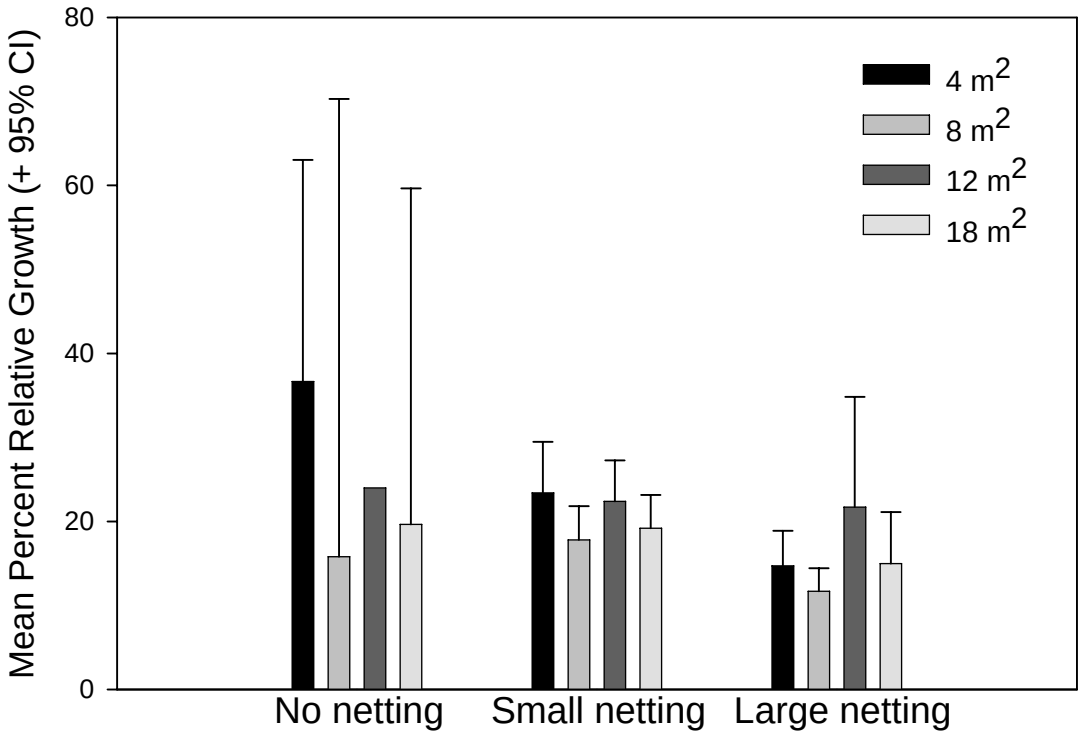

Mean Percent Relative Growth (+ 95% CI)
80
60
40
20
0
4 m2
8 m2
12 m2
18 m2
No netting
Small netting
Large netting

Figure 5.

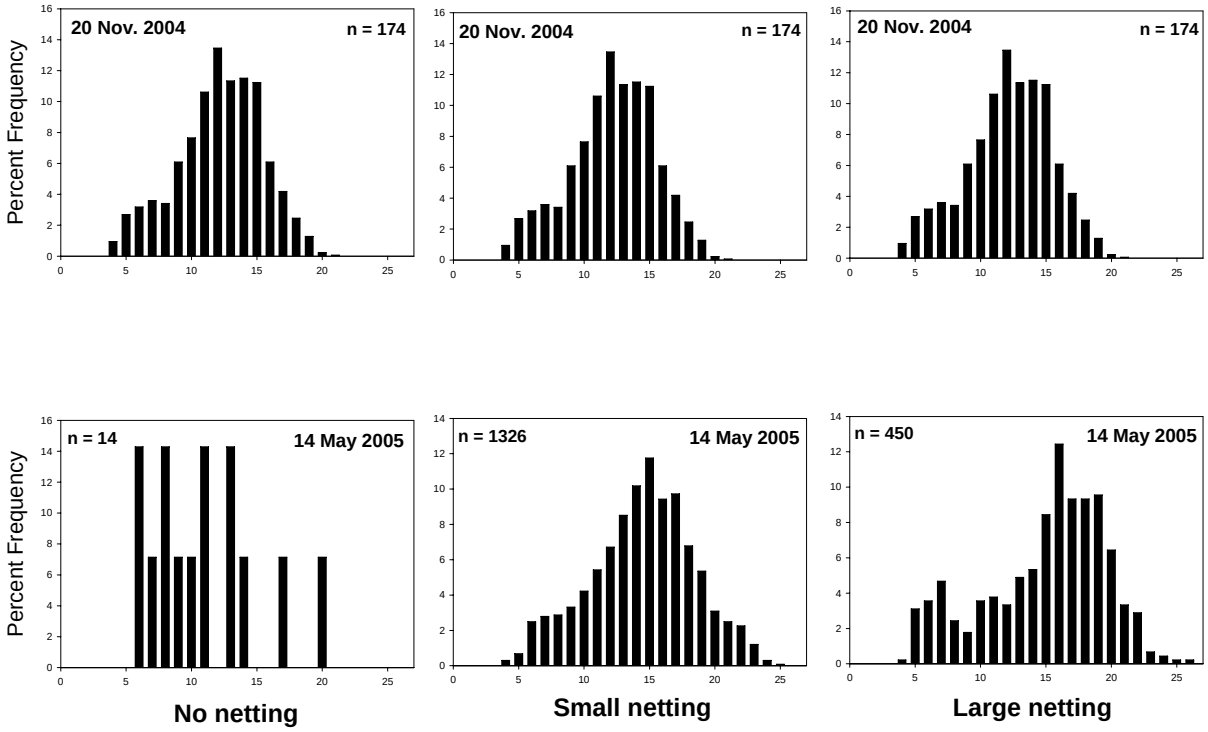

20 Nov. 2004
n = 174
20 Nov. 2004
n = 174
20 Nov. 2004
n = 174
Percent Frequency
n = 14
14 May 2005
n = 1326
14 May 2005
n = 450
14 May 2005
Percent Frequency
No netting
Small netting
Large netting

Figure 6.

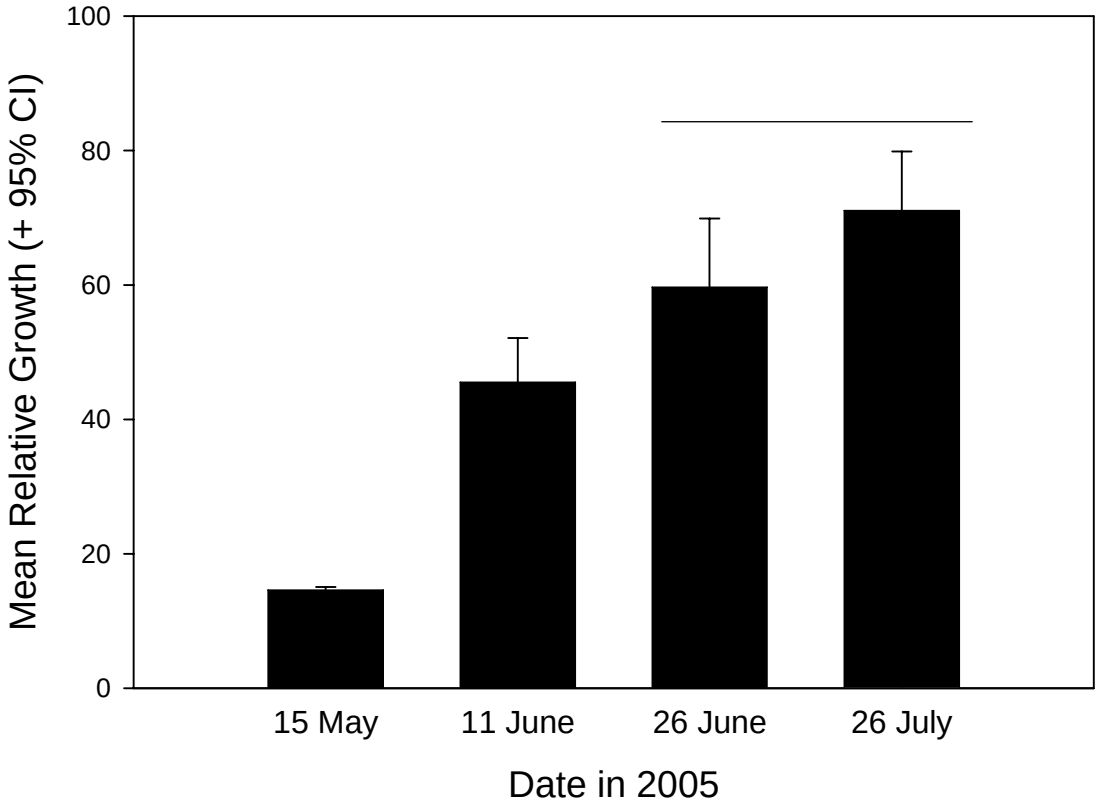

100
80
60
40
20
0
Mean Relative Growth (+ 95% CI)
15 May
11 June
26 June
26 July
Date in 2005

Figure 7.

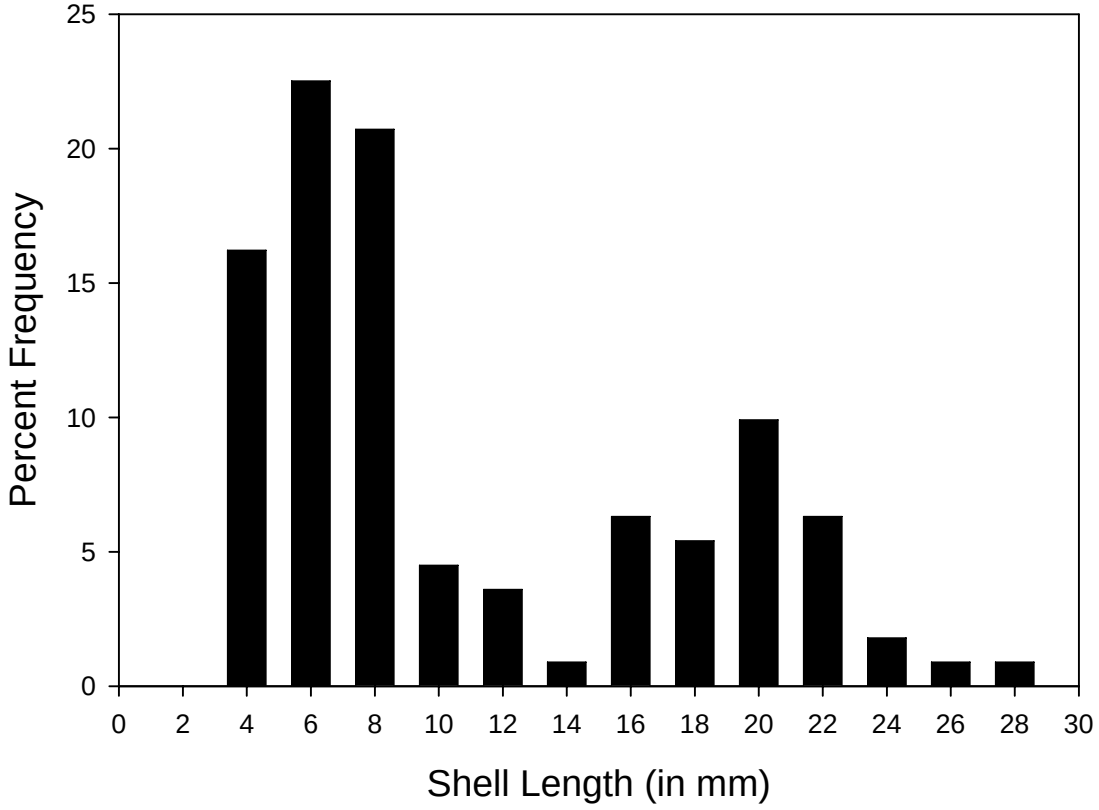

Percent Frequency
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Shell Length (in mm)

Figure 8.

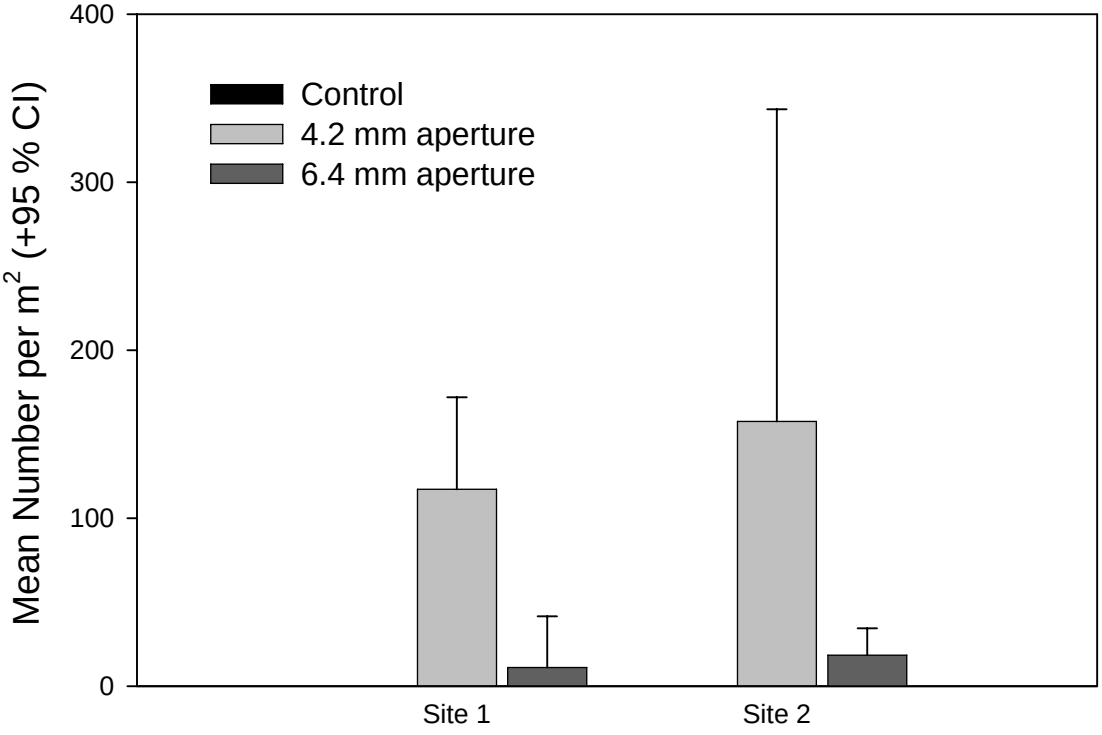

400
300
200
100
0
Mean Number per m2 (+95 % CI)
Control
4.2 mm aperture
6.4 mm aperture
Site 1
Site 2

Figure 9.

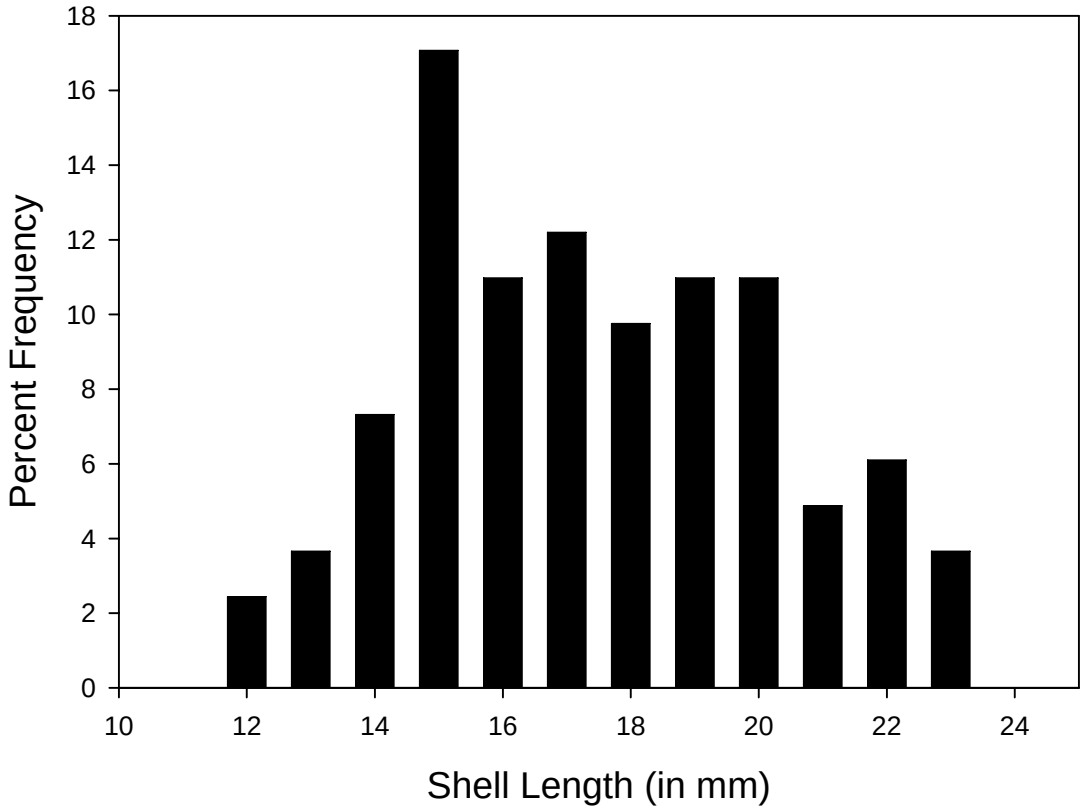

Percent Frequency
0
2
4
6
8
10
12
14
16
18
10
12
14
16
18
20
22
24
Shell Length (in mm)

Figure 10.

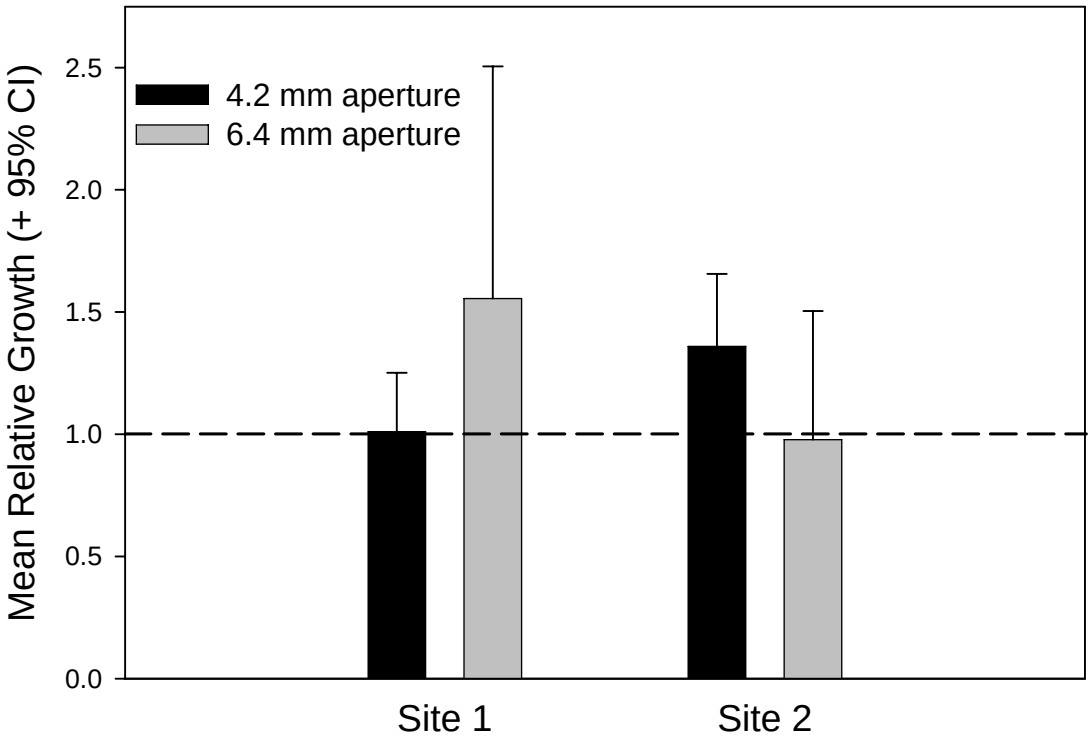
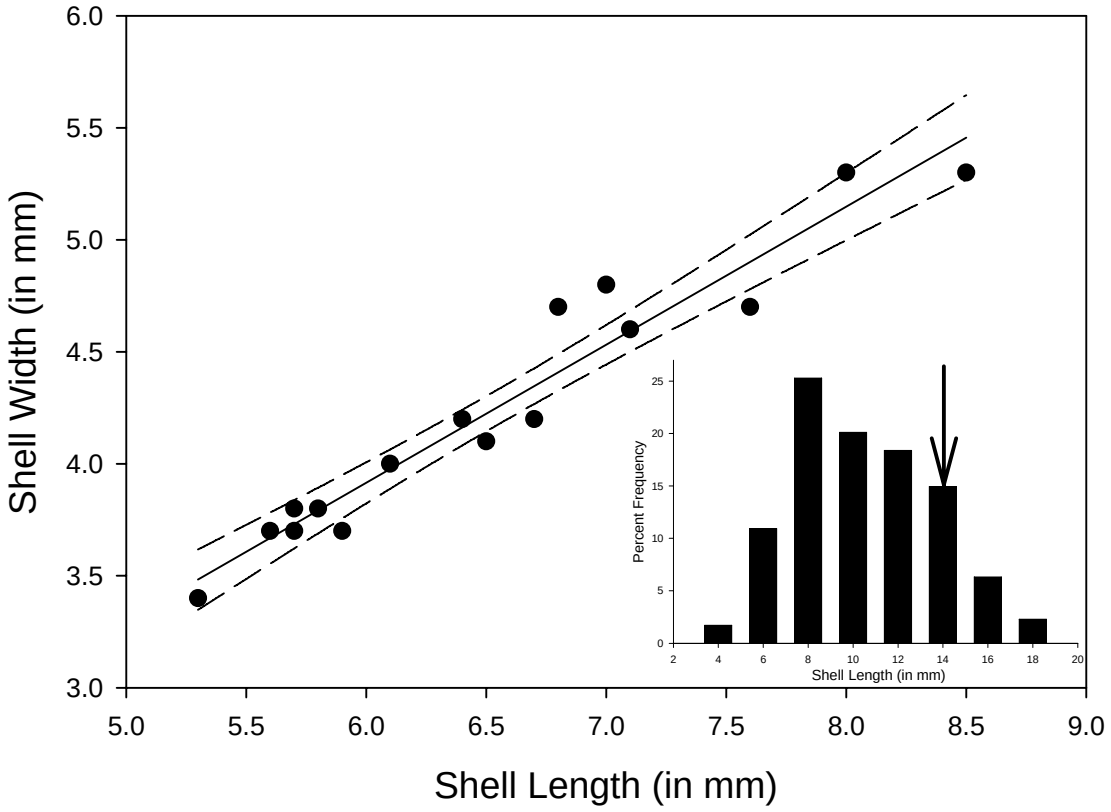

Mean Relative Growth (+ 95% CI)
2.5
2.0
1.5
1.0
0.5
0.0
4.2 mm aperture
6.4 mm aperture
Site 1
Site 2

Figure 11.


Shell Width (in mm)
Shell Length (in mm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
Percent Frequency
Shell Length (in mm)
25
20
15
10
5
0
2
4
6
8
10
12
14
16
18
20