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## Juvenile soft-shell clam, *Mya arenaria* L. research in the Hampton-Seabrook Estuary

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

A Final Report to  
The New Hampshire Estuaries Project



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## Executive Summary

A series of field experiments was conducted at two intertidal sites in the Hampton-Seabrook Estuary from November 2004-2006 to assess the efficacy of enhancing intertidal areas with cultured clam (*Mya arenaria* L.) seed (mean shell length [SL] = 7-10 mm). Measurement variables in each experiment included survival and growth of both cultured and wild seed clams. The first of three trials (November 2004 - May 2005) examined the interactive effects of size of planting area (4 m<sup>2</sup>, 8 m<sup>2</sup>, 12 m<sup>2</sup>, and 18 m<sup>2</sup>) and predator deterrent netting (none, 4.2 mm, and 6.4 mm aperture [flexible, plastic netting]) at the Willows Flat in the Hampton River. The second trial (June - October 2005) examined the effect of predator deterrent netting at two discrete intertidal locations at the Willows Flat. The third trial (April - November 2006) replicated trial two except at two intertidal sites within the estuary approximately 3 km apart.

In the first trial, clam survival was unaffected by size of planted area, and enhancement due to the presence of predator deterrent netting was greater than 100-fold. Less than 1% of seed clams were recovered from plots that were not covered with plastic netting, but approximately 90% of animals seeded in plots protected with the smallest aperture netting were recovered. This recovery rate was three times greater than in plots covered with the larger aperture (6.4 mm) mesh netting. Clams reached a mean SL of  $14.6 \pm 0.57$  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<sup>2</sup> plots compared to the mean of the two largest plot sizes.

The second field experiment suffered from a decision to seed clams at low tide on an extremely hot day (11 June 2005) when pre-noon temperatures reached > 32°C. Animals were exposed to the air and heat for several hours before the tide covered the seeded plots and observations made within weeks after initiating suggested that a massive die-off occurred soon after the seeding event. Plots initially seeded at densities of 1,275 m<sup>-2</sup> showed losses of greater than 1,200 individuals m<sup>-2</sup> in all three treatments at both intertidal locations. However dire these results, several themes could be discerned. First, mortality due to predators was controlled by the use of protective netting. Second, clam densities were enhanced 9-fold in netted plots with the smaller vs. larger aperture. Third, the effect of the netting was similar at both intertidal locations.

The third experiment was initiated at the Willows Flat and at a flat near the mouth of the Blackwater River. The design was the same at both sites and included control plots along with the two netting treatments used in the second trial. In addition, a third netting treatment was employed by affixing three evenly-spaced 100 mm-diameter x 75 mm wide Styrofoam floats to the underside of the small aperture netting. The floats lifted the netting off the flat during tidal inundation so that they would not physically interfere with clams while feeding. Unfortunately, three weeks after the experiment was initiated, > 380 mm of rain fell in this region over a 4-day period (13-16 May) resulting in some of

the worst coastal flooding in years. As a result of the increased water flow and bedload transport of sediments across these flats, many of the nets, especially at the Blackwater River site, became all or partially eroded and had to be repositioned and reburied. Many clams were lost due to predators gaining access to these plots. By November 2006, approximately 2.5 times as many clams occurred in samples from netted plots at the Willows Flat vs. Blackwater River (240 vs. 99 individuals  $m^{-2}$ ), but this was not statistically significant ( $P = 0.07$ ). Across both sites, nearly 18 times more clams were sampled from plots covered by netting with the smaller (248 individuals  $m^{-2}$ ) vs. larger apertures (14.0 individuals  $m^{-2}$ ;  $P = 0.0008$ ). Crushed clams and broken shell fragments were found in 71% of samples taken in November 2006. This implies that predation due to green crabs, that were abundant at both sites, and bottom feeding fish such as winter flounder, is intense in these sites. In addition, the presence of the Styrofoam floats had no significant effect on final clam numbers at either site. Clam growth was highly seasonal as most (70-80%) of shell growth occurred between 13 May and 13 August. Growth rate and final mean size were similar at both sites and unaffected by netting treatment. Final mean SL pooled across both sites and netting treatments was  $27.7 \pm 0.08$  mm ( $n = 117$ ), which represented an approximate tripling in linear shell growth.

A strategy for enhancing flats in the Hampton-Seabrook Estuary is presented and a demonstration enhancement project with clambers and other interested persons is recommended. Because present management approaches in this region depend on the vagaries of successful clam recruitment (high enough to swamp out factors such as

predation and bedload transport of small juveniles), it is impossible to predict how long clambers will have to wait for standing stocks of harvestable clams to return to levels of the late 1990's. Current standing stocks of adult clams in the estuary are estimated at or around 3,000 bushels, or approximately 13% of 1997 stocks. Results from the present study, together with those from a previous, smaller-scale investigation in the same estuary (Beal, 2002) suggest that clam enhancement can be successful as long as netting is properly deployed and maintained through regular inspections. Seeding should occur in early spring (late March or April of Year I) when seawater temperatures are below 10°C. Animals should be seeded at densities between 500-1000 individuals m<sup>-2</sup> (ca. 50-100 individuals ft<sup>-2</sup>) and then covered with a plastic, flexible netting with an aperture size of 4.2 mm. Because predation in the estuary is so intense and affects all but the largest sizes of clams, nets should be maintained in situ as long as possible, perhaps as long as it takes the shellfish to attain harvestable sizes (50.8 mm SL, or 2-inches). A large proportion of these animals will be ready for harvest by October or November in Year II.

A pilot-scale demonstration enhancement project should be designed and conducted with volunteers from the clamming community. The project should be conducted at a minimum of two flats in the Hampton-Seabrook Estuary that are currently open to the recreational fishery. At the very least, 10 plots with a planting area of 12 m<sup>2</sup> should be deployed and each plot covered with a plastic, flexible netting with 4.2 mm apertures. The project should be initiated in the spring, with the coordinator(s) and the volunteers agreeing to make regular visits to the seeded plots during the entire project.

## 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 incomplete information about important population parameters such as survival, growth, and recruitment rates and how these parameters may 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. Clamming is one of the oldest activities conducted in this area. Shell middens along marsh creeks in Hampton, Seabrook, and Hampton Falls attest to the importance of this resource prior to European settlers (Randall, 1989). Clam populations in this region have gone through boom-and-bust



cycles (Lindsay and Savage, 1978) related both to variable harvesting pressure and predator abundance. For example, as early as 1902, the *Hampton Union* reported, “The scarcity of clams in Hampton River has become quite a serious thing, and those who fully realize the condition see the importance of a three months’ law to protect them, either in the spring or autumn, as clams do not grow much in the winter season and in the summer they are needed for food. The continual raid that is brought to bear upon the clam bank year after year for food would soon bring this most relishable bivalve to become extinct if a remedy was not occasionally applied by law,” (Randall, 1989).

In 1997, researchers estimated that 25,000 bushels of harvestable soft-shell clams occurred in the Hampton-Seabrook Estuary (Nash, 2006). During the Fall 1998, over 900 clambers 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 have shown that the abundance of harvestable clams has fallen below 3,500 bushels, which 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 several potential 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 using cultured juveniles of the soft-shell clam demonstrated that disease-related mortalities (specifically from neoplasia), interspecific competition, and winterkill due to ice and storms was minimal. However, clam losses between November 2001 and March 2002 associated with sediment scouring and predation exceeded, in some instances, 95% (Beal, 2002). Similar losses at the same sites occurred from March to July 2002, but in most cases, survival was enhanced by using predator-deterrent, flexible mesh netting (6.4 mm aperture).

Among the limitations of those earlier field tests were: 1) the use of small experimental units (6-inch plastic plant pots); 2) the 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).

In an attempt to overcome these limitations, three large-scale field experiments were conducted at two intertidal sites within the Hampton-Seabrook Estuary from 2004 to 2006. In the first experiment, conducted from November 2004 to May 2005, the interactive effects of plot size, predator deterrent netting, aperture mesh size, and enhancement using hatchery-reared individuals of the soft-shell clam on survival and growth were tested. In the second experiment, conducted from June to October 2005 and again from April to November 2006, the interactive effects of location within the

estuary, predator deterrent netting, and aperture mesh size on cultured and wild clam survival and growth were assessed.

### 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 two locations 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 receive cultured clams but have no protective 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; Fig. 1) 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]  $\pm$  95% CI = 10.4  $\pm$  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 (1,320 m<sup>-2</sup>) were added to one-half the plots that varied in area as follows: 4 m<sup>2</sup>, 8m<sup>2</sup>, 12m<sup>2</sup>, and 18m<sup>2</sup>. 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 (*sensu* Beal and Kraus, 2002) was added to the nets to keep them from interacting with the clams during feeding. 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 ( $A = 0.182 \text{ m}^2$ ) was taken to a depth of 20 cm from each plot ( $N = 96$ ) prior to raking, and the contents of each were washed through a 2 mm sieve.

#### *Assessing the fate of the plastic 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, the number and nature of torn or ripped nets was recorded.

On 14-15 May 2005, four benthic core samples ( $A = 0.182 \text{ m}^2$ ) were taken from each plot. Because small clams tend to have contagious distributions (B. Beal, pers. obs.), plots were divided into halves (parallel to the shore; e.g., an upper [shoreward] and lower [towards water] section) and two cores taken randomly from 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 marker ("hatchery mark") that is deposited in each valve at the time 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, the initial SL, or hatchery mark, was measured similarly, which

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 ( $[(\text{final SL} - \text{initial SL})/\text{initial SL}]$ ) 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 mean 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 mean relative growth rate data (mean per sample).

The linear model used for the ANOVA was as follows:

$$Y_{ijklm} = \mu + A_i + B_j + AB_{ij} + C_k + AC_{ik} + BC_{jk} + ABC_{ijk} + D_l + AD_{il} + BD_{jl} + CD_{kl} + ABD_{ijl} + ACD_{ikl} + BCD_{jkl} + ABCD_{ijkl} + E(ABC)_{m(ijk)} + DE(ABC)_{lm(ijk)} + e_{n(ijklm)}$$

Where,

$\mu$  = theoretical mean;

$A_i$  = Plot size ( $i = 4$  levels: 4 m<sup>2</sup>, 8 m<sup>2</sup>, 12 m<sup>2</sup>, and 18 m<sup>2</sup>; 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);

$D_l$  = Section ( $l = 2$  levels: upper or lower in each plot; factor is fixed);

$E_m$  = Plot ( $m = 4$  levels: 1, 2, 3, 4; factor is random); and,

$e_n$  = Experimental error ( $n = 2$  replicate cores randomly assigned per section of plot).

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<sup>2</sup> vs. (8 m<sup>2</sup>, 12 m<sup>2</sup>, and 18 m<sup>2</sup>) = "Small vs. rest";
- 2) 8 m<sup>2</sup> vs. (12 m<sup>2</sup> and 18 m<sup>2</sup>);
- 3) 12 m<sup>2</sup> vs. 18 m<sup>2</sup>;

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; Fig. 1). Initial clam size ( $\pm$  95% CI) was  $7.3 \pm 0.5$  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 activity 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<sup>2</sup> plots were established near the lower middle intertidal at each of the two sites (two rows with ca. 5 m spacing between all plots), and each seeded with cultured clams at an approximate density of 1,275 m<sup>-2</sup>. The sediment surface of each plot was raked (as described above). At each site, five plots were covered with plastic, flexible netting either with 6.4 mm or 4.2 mm apertures 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 sample ( $A = 0.0182 \text{ m}^2$ ) was taken ( $N = 45$  per site). 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 (11 June 2005), a benthic core ( $A = 0.182 \text{ m}^2$ ) sample was taken from each plot at both sites ( $N = 30$ ) prior to raking, and the contents of each were washed through a 2 mm sieve.

### *Statistical analyses*

Analysis of variance (ANOVA) was performed on the square root-transformed mean 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 mean relative growth rate data (mean per sample). Mean square error terms for each source of variation were calculated using Underwood (1997). The linear model used for the ANOVA was as follows:

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

Where,

$\mu$  = theoretical mean;

$A_i$  = Site ( $i = 2$  levels; factor is random);

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

$C_k$  = Plot ( $k = 5$  levels: 1, 2, 3, 4, 5; factor is random); and,

$e_i$  = Experimental error ( $l = 3$  replicate cores randomly assigned per treatment).

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

## **Experiment III.**

### *Study site and experimental animals*

Because of high mortalities associated with the initiation of Experiment II, another similar field experiment was conducted in 2006 to test the effects of excluding predators using flexible netting on growth and survival of cultured clams. Two intertidal study sites were chosen specifically to reduce potential interaction between people, pets, and the netted plots. One was at WF in the Hampton River, Hampton, New Hampshire (referred to in Experiment II as site 2 = 42° 54.41'N, 70°49.35'W; Fig. 1). The second site was approximately 2.7 km southwest of WF in the Blackwater River (BR), Seabrook, New Hampshire (42° 53.01'N, 70°49.94'W; Fig. 1). Initial clam size ( $\pm$  95% CI) was  $8.9 \pm 0.2$  mm ( $n = 124$ ; range = 6.3-12.8 mm). Animals were reared at DEI in 2005 and overwintered according to Beal et al. (1995). The experiment was initiated at BR on the morning of 21 April 2006 and at WF approximately 24 hours later on 22 April 2006.

### *Experimental design*

Twenty 12m<sup>2</sup> plots were established near the lower middle intertidal at both sites (2 x 10 matrices with ca. 5 m spacing between rows and columns), and each seeded with cultured clams at an approximate density of 1,320 m<sup>-2</sup>. Prior to distributing clams on the surface within the plots, the sediment surface of each plot was raked (as described above). After seeding the plots at both sites, fifteen were covered with flexible, plastic mesh netting. Five plots were protected with netting having a 6.4 mm aperture and five with netting having a 4.2 mm aperture. The remaining five plots were protected with netting having a 4.2 mm aperture to which three evenly-spaced 100 mm diameter x 75

mm wide Styrofoam floats were affixed to the middle underside of each net. Floats were designed to keep the netting raised above the surface of the plot during tidal inundation (see Beal and Kraus, 2002). The remaining five plots at each site were not covered with predator deterrent netting and acted as controls. The status of the nets at both sites was assessed on 13 and 20 May, 20 June, and 13 August 2006. All nets were removed and samples taken from each plot on 11 & 12 November 2006 at BR and WF, respectively. Each plot was divided into three sections (upper, middle, and lower -- parallel to the shore) and three benthic cores ( $A = 0.0182 \text{ m}^2$ ) were taken from each section (20 plots x 3 sections per plot x 3 cores per section;  $N = 180$  cores per site). 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 at both sites (21-22 April 2006), a benthic core ( $A = 0.182 \text{ m}^2$ ) sample was taken from each plot ( $N = 40$ ) prior to raking, and the contents of each were washed through a 2 mm sieve.

#### *Assessing the fate of the plastic netting and interim sampling*

I returned to both sites on 13 May, 20 May, 20 June, and 13 August 2006 to observe the status of nets. Each time, I recorded relative amounts of detritus under each, whether nets were ripped or torn, and I reburied corners, sides, or ends of nets that had become exposed through erosion or other causes. On 13 May and 13 August, I took 10-15 live individuals from three netted plots from each site and measured initial and final SL (as

described above). In addition, I recorded the status of nets prior to sampling on 11-12 November.

### *Statistical analyses*

Analysis of variance (ANOVA) was performed on the square root-transformed mean 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 mean relative growth rate data and mean final length data (per sample). Mean square error terms for each source of variation were calculated using Underwood (1997). The linear model used for the ANOVA was as follows:

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

Where,

$\mu$  = theoretical mean;

$A_i$  = Site ( $i = 2$  levels: BR, WF; factor is fixed);

$B_j$  = Treatment ( $j = 4$  levels: no netting; 6.4 mm, 4.2 mm, and 4.2 mm aperture with flotation; factor is fixed);

$C_k$  = Section ( $k = 3$  levels: upper, middle, or lower in each plot; factor is fixed);

$D_l$  = Plot ( $l = 5$  levels: 1, 2, 3, 4, 5; factor is random); and,

$e_m$  = Experimental error ( $n = 3$  replicate cores randomly assigned per section of plot).

In addition, a set of orthogonal, a priori, single degree-of-freedom contrasts were conducted for the main effect due to netting as follows:

- a) No netting vs. Netting;
- b) Small vs. Large aperture mesh (4.2 mm vs. 6.4 mm); and,
- c) Floats vs. No floats (4.2 mm mesh -- with floats vs. without floats).

## Results

### *Experiment I.*

#### *20-21 November 2004 sampling*

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

#### *14-15 May 2005 sampling*

##### Wild clams

Wild clams were found in 29 of the 96 plots (30.2%), and 95% the individuals were < 15 mm SL (Fig. 2). Of the plots with clams, 24 (ca. 83%) had been covered with protective netting. The nearly three-fold difference in density of wild clams between plots with netting vs. those without ( $7.3 \pm 2.8$  vs.  $2.6 \pm 2.3$  individuals  $m^{-2}$ ,  $n = 64$  and  $32$ , respectively) was statistically significant ( $P = 0.0184$ ; Table 1). In addition, wild clam density was enhanced nearly four times in the presence of cultured clams ( $9.2 \pm 3.6$  vs.  $2.3 \pm 1.5$  individuals  $m^{-2}$ ,  $n = 48$ ;  $P = 0.0009$ ; Table 1). For example, of the 40 clams from the 384 samples, 32 (80%) occurred in plots initially seeded with cultured clams. Wild clams were distributed differently within the plots depending whether or not cultured clams had been seeded initially. That is, in plots that received no cultured clams, the difference in mean number of individuals per square meter between the upper vs. lower portion of the plots was  $1.1 \pm 1.6$  vs.  $3.4 \pm 2.7$  ( $n = 48$ ) compared to  $12.1 \pm 5.9$  vs.  $6.3 \pm 4.4$  ( $n = 48$ ) in plots initially seeded with cultured clams.



### Cultured clams – number alive in plots

The main effects of netting and clam presence, as well as the interaction of these two factors, were highly significant ( $P < 0.0001$ , Table 2). Although one-third of the plots received no cultured 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 initially seeded with hatchery-reared individuals, the presence of plastic netting enhanced their numbers by 104.8 times over control plots, where no netting was applied (Table 3; Fig. 3a). In addition, 2.9 times more individuals occurred in plots protected by small vs. large aperture nets ( $1138.4 \pm 249.0$  vs.  $386.3 \pm 72.2$  individuals  $m^{-2}$ ; Table 3; Fig. 3b). Another source of variation, plot-to-plot variability within a given treatment, was highly significant ( $P < 0.0001$ ; Table 2). By partitioning this source with its 72 df into 24 separate tests, each with three df, and adjusting for potential type I errors by reducing the decision rule to 0.0021 (Winer et al. 1991), it was possible to determine where the significant variability existed. No significant variation in mean number  $m^{-2}$  from plot-to-plot occurred in any of the eight treatments without netting or in the eight treatments where netting was applied, but no clams were seeded in the plots. Two significant sources occurred in treatments in which clams were seeded and protected with the large netting (Plot size =  $4m^2$  and  $12 m^2$ ) and three in treatments associated with the small netting (Plot size =  $4m^2$ ,  $12 m^2$ , and  $18m^2$ ; Fig. 4).

Because dispersal of cultured clams into plots (twelve treatments) that initially received no clams was minimal, I reexamined the data creating a reduced linear model (without

the “clams” source of variation) and compared the remaining twelve treatments in which hatchery seed were employed (Table 4). The results confirmed those obtained from the full model analysis (Table 2) as netting and both a priori contrasts associated with that source of variation were highly significant ( $P < 0.0001$ ; Table 4; Fig. 5). Notably, neither plot size nor any of the three contrasts associated with that source of variation was significant (Table 4; Fig. 6).

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<sup>2</sup> 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 ( $3.75 \pm 3.76$  ind.,  $n = 4$ ) differed significantly from the mean of the other three replicates of that treatment (replicate 1:  $21.5 \pm 14.02$  ind.; replicate 2:  $17.00 \pm 16.59$  ind.; replicate 3:  $20.75 \pm 14.95$  ind.;  $P = 0.0119$ ). The damage to the other net that had large mesh and protected clams in an 18 m<sup>2</sup> plot was not extensive, as the ripping exposed less than 1/25<sup>th</sup> of the seeded area. Although the mean number per core in that plot ( $5.25 \pm 5.72$  ind.) was less than two of the other three replicates, it was not significantly different from the mean of the other three undamaged replicates ( $P = 0.2548$ ).

Similar numbers of cultured clams m<sup>-2</sup> were sampled from the upper and lower sections of the plots, and this was consistent among netting and plot size treatments ( $P = 0.2368$ , Table 4). 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). Although there was an attempt to seed clams uniformly within each plot in November 2004, the  $I_d$  value was 1.929 ( $P < 0.0001$ ) indicating a contagious distribution. Fewer assumptions would be required to use the core samples to estimate survivorship had animals been randomly or uniformly distributed. However, using means from Table 2 and an initial stocking density of  $1320 \text{ m}^{-2}$ , clams under the smaller aperture netting exhibited a survival of 89.7% from November 2004 to May 2005 whereas survival under the larger aperture netting was substantially lower at 30.9%.

#### Cultured clams – relative growth

Relative growth varied significantly due to netting ( $P < 0.0120$ ) and size of plot ( $P = 0.0190$ ; Table 5). Growth was approximately 30% faster under the small ( $20.7 \pm 1.9\%$ ,  $n = 16$ ) vs. large aperture netting ( $16.0 \pm 3.8\%$ ,  $n = 16$ ; Fig. 7); 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. 14.7 mm SL; Fig. 8). Mean relative growth of clams in  $8 \text{ m}^2$  plots ( $14.9 \pm 2.8\%$ ,  $n = 10$ ) appeared slower than mean growth in the two larger plots ( $12 \text{ m}^2$  and  $18 \text{ m}^2$ :  $19.8 \pm 3.9\%$ ,  $n = 19$ ), but this was not statistically significant given the decision rule associated with this a priori contrast (Table 5).

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. 9).

## ***Experiment II.***

### *11 June 2005 sampling*

#### Wild clams

Sixteen wild clams were recovered from samples at site 1 ( $1.06 \pm 0.94$  ind. core<sup>-1</sup>;  $58.1 \pm 52.03$  ind. m<sup>-2</sup>) and five from site 2 ( $0.2 \pm 0.23$  ind. core<sup>-1</sup>;  $10.9 \pm 12.59$  ind. m<sup>-2</sup>).

ANOVA on the square root-transformed density data indicated that these differences were not statistically significant ( $P = 0.0638$ ). Mean SL ( $4.4 \pm 0.56$  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 was sampled in the 180 benthic cores. Mean number varied significantly between sites (e.g., site 1 =  $84.2 \pm 26.9$  ind. m<sup>-2</sup>; site 2 =  $50.1 \pm 24.4$  ind. m<sup>-2</sup>;  $n = 15$ ;  $P = 0.0426$ , Table 6). Significant effects were observed due to predator exclusion ( $P = 0.0089$ ). The a priori, orthogonal contrasts demonstrated that a 3-fold enhancement of wild clams occurred due to the presence of the netting ( $\bar{x}_{\text{netting}} = 87.9 \pm 32.3$  ind. m<sup>-2</sup>,  $n = 20$  vs.  $\bar{x}_{\text{no netting}} = 27.5 \pm 21.6$  ind. m<sup>-2</sup>;  $n = 10$ ;  $P = 0.0077$ ). In addition, significantly more wild clams were sampled from plots protected with the small vs. large aperture netting ( $126.3 \pm 76.2$  vs.  $49.4 \pm 40.5$  ind. m<sup>-2</sup>,  $n = 10$ ,  $P = 0.0105$ , Table 6).

The size distribution of wild clams was bimodal (Fig. 10) 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

sites ( $P = 0.5430$ ), but that clams were nearly double the size under nets than in control plots at both sites ( $12.1 \pm 1.9$  mm vs.  $6.1 \pm 0.8$  mm;  $P < 0.0055$ ).

#### Cultured clams – number alive in plots

Number of clams in plots at both sites was extremely low, presumably related to the weather 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 ( $50.7 \pm 32.4$   $m^{-2}$ ;  $P = 0.6657$ , 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 ( $P = 0.0047$ , Table 7). No cultured clams were recovered from any core taken from control plots ( $n = 10$ ) whereas a mean of  $76.0 \pm 45.6$  individuals  $m^{-2}$  occurred in cores taken from plots protected with netting. A 9-fold difference in enhancement occurred between plots covered with 4.2 mm netting (i.e., small net;  $137.4 \pm 75.9$  ind.  $m^{-2}$ ) vs. the 6.4 mm netting (i.e., large net;  $14.7 \pm 13.5$  ind.  $m^{-2}$ ;  $P =$

0.0045, Table 7, Fig. 11). The significant source of variation associated with plot-to-plot variability within a particular site and treatment ( $P = 0.0263$ , Table 7) was due to a single treatment and location (small mesh at site 2) where densities  $m^{-2} \pm 95\%$  CI's for plots 1-5 varied as follows:  $0 \pm 0$ ,  $146.5 \pm 284.1$ ,  $91.6 \pm 284.1$ ,  $146.5 \pm 343.5$ ,  $402.9 \pm 1024.5$  ( $n = 3$ ).

#### Cultured clams – relative growth

A total of 82 cultured clams was sampled from the 180 cores ( $n = 48$  from site 1;  $n = 34$  from site 2; mean SL =  $17.4 \pm 0.6$  mm, range = 11.6-22.8 mm; Fig. 12). All clams were sampled from netted plots (7 of 10 plots at site 1 and 9 of 10 plots at site 2). No significant differences in mean relative growth occurred between sites ( $P = 0.7203$ ) or among netting treatments (0.9778). In addition, there was no significant plot-to-plot variation ( $P = 0.9778$ ). There was a significant Location x Netting Treatment interaction ( $P = 0.0331$ , Fig. 13) indicating that the pattern of relative growth between the two treatments differed between the two sites.

### ***Experiment III.***

*21-22 April 2006 sampling*

#### Wild clams

Five of 20 samples at WF ( $16.5 \pm 14.7$  ind.  $m^{-2}$ ) and three of 20 samples at BR ( $8.2 \pm 12.6$  ind.  $m^{-2}$ ) contained clams. This difference in mean density between the two sites was not statistically significant using a two-sample t-test ( $P = 0.378$ ). Clams ranged in SL from 4.2-7.0 mm at WF and 6.4-8.0 mm at BR.

*11-12 November 2006 sampling*

#### Wild clams

A total of 250 wild clams ( $n_{BR} = 133$ ;  $n_{WF} = 117$ ) were sampled in the 360 benthic cores. Wild clams occurred in 16 plots at BR (61 cores of 180 total = 33.9%) and 14 plots at WF (66 cores of 180 total = 36.6%). At least one wild clam occurred in each of three control plots at BR; however, no wild clams occurred in control plots at WF. Size range of clams varied from 1.6 -72.1 mm SL at BR and 2.4-75.0 mm SL at WF (Fig. 14). Wild clam density was enhanced approximately 14 times due to the presence of protective netting ( $\bar{x}_{Control} = 3.6 \pm 5.5$  ind.  $m^{-2}$  [ $n = 10$ ] vs.  $\bar{x}_{Netting} = 50.1 \pm 16.0$  ind.  $m^{-2}$  [ $n = 30$ ]; Table 8). In addition, size of net aperture made a significant difference ( $P < 0.0001$ ) in number of wild clams. For example, a mean of  $17.7 \pm 6.3$  ind.  $m^{-2}$  ( $n = 10$ ) occurred under netting with the larger (6.4 mm) apertures, whereas 3.7 times as many wild clams ( $66.2 \pm 20.6$  ind.  $m^{-2}$ ,  $n = 20$ ) occurred in plots protected with netting having the smaller (4.2 mm) apertures. The effect due to netting was not the same at both sites, but only with respect to the presence or absence of floats on netting with the smaller aperture ( $P$

0.0030, Table 8; Fig. 15). Wild clams were not distributed evenly between the three sections of the plots as individuals were more likely to occur in the middle and upper third of plots (towards the shore) than in the lower third of plots (towards the water) ( $P = 0.0204$ , Table 8; Fig. 16).

The analysis described above (Table 8) incorporated post-settled individuals that represented production prior to 2006 as well as new recruits to the flats (i.e., 0-year class individuals that settled to the flats during the experiment). To determine if the netting treatments had any effect on new recruits, I reanalyzed the data after eliminating all clams greater than 10 mm SL because I found no clams larger than this in the initial samples (see above). That is, clams less than 10 mm SL during November are likely to represent those 0-year class individuals that settled to the flat during the experiment and their presence may have been affected by the experimental treatments. The results were very similar to the initial analysis of numbers of wild clams. Significantly more new recruits occurred in plots that had received protective netting ( $\bar{x} = 29.9 \pm 14.1$  ind.  $m^{-2}$ ,  $n = 30$ ) than in the control plots ( $\bar{x} = 2.4 \pm 4.2$  ind.  $m^{-2}$ ,  $n = 10$ ) ( $P = 0.0073$ ). In addition, aperture size was important. Approximately 4.4 times more recruits occurred in netted plots with the small vs. large apertures ( $\bar{x}_{\text{Small}} = 40.3 \pm 19.8$  ind.  $m^{-2}$ ,  $n = 20$  vs. ( $\bar{x}_{\text{Large}} = 9.2 \pm 6.3$  ind.  $m^{-2}$ ,  $n = 10$ ;  $P = 0.0091$ ). Netting treatments had no effect on the size of new recruits ( $P = 0.5580$ ), but mean SL of individuals was significantly ( $P = 0.0216$ ) larger at WF ( $6.4 \pm 0.82$  mm,  $n = 19$ ) than at BR ( $4.1 \pm 0.6$  mm,  $n = 36$ ).



### Cultured clams – number alive in plots

The effect of netting on number of live clams was highly significant ( $P < 0.0001$ , Table 9). No live clams were sampled from control plots ( $N = 90$ ) at either site (Table 10); therefore, the a priori contrast that examined the effect due to the presence of netting was statistically significant ( $P = 0.0023$ ; Fig. 17). Because there was no significant Site x Netting interaction ( $P = 0.4874$ ), the effect of the netting on number of live clams was similar across both sites. In general, nearly 18 times more clams were sampled from plots covered by netting with the smaller vs. larger aperture ( $247.6 \pm 86.2$  ind.  $m^{-2}$  [ $n = 20$ ] vs.  $14.0 \pm 12.9$  ind.  $m^{-2}$  [ $n = 10$ ];  $P = 0.0008$ , Table 9). No significant effect due to the presence of the Sytrofoam floats was detected. ANOVA was unable to detect a significant difference in mean number alive between sites ( $P = 0.0738$ ), although approximately 2.5 times as many clams were sampled from the twenty plots at WF compared to BR ( $180.1 \pm 120.9$  ind.  $m^{-2}$  vs.  $74.5 \pm 61.9$  ind.  $m^{-2}$ ). Significant plot-to-plot variation was observed at both sites, but only for the two treatments associated with plots covered by the smaller aperture netting (Table 9). The significant Section x Plot(Net x Site) source of variation in Table 9 was due to a single netting treatment (4.2 mm aperture) at WF (Fig. 18). Using these data to assess percent survival assumes that clams were distributed uniformly or randomly in the plots. Ignoring data from control plots because no live clams occurred in any of the 90 samples, I used Morisita's  $I_d$  to assess the distribution of clams in samples from plots covered with netting ( $N = 270$ ). The  $I_d$  value (4.41) was significantly different from unity indicating an extremely clumped or contagious distribution ( $P < 0.0001$ ). This makes interpreting estimates of

survival difficult; nonetheless, the highest estimate for survival is from the small mesh treatment with floats at WF ( $379.67/1,320 = 28.8\%$ ; Table 10).

This experiment, and the others, demonstrates the critical role of protective netting in deterring predation and enhancing clam survival. The fate of the plots was assessed several times during the experiment as well as on the sampling dates in November 2006. The earliest examination occurred three weeks after the experiment was initiated. On that date (13 May), two things were evident: 1) Significant amounts of shell debris and crushed valves were noted on the surface of control plots at both sites; and, 2) the corners of some of the nets, especially at BR, had lifted and crushed valves were noted in these plots. On that first examination date, six of the fifteen nets at BR required attention; that is, the lower right hand corner of each had to be repositioned (reburied) in the sediments. At WF, three plots required similar attention.

Unfortunately, 13 May represented the beginning of an unusual weather event from the northern coast of Massachusetts to the southern coast of Maine. The Hampton-Seabrook region, in particular, received greater than 15 inches (ca. 380 mm) of rain that fell steadily over four consecutive days (13-16 May). This resulted in flooding of the low-lying areas in both rivers, and, on 14 May 2006, New Hampshire Governor John Lynch declared a state of emergency due to washed out roads and forced evacuations, especially along the southern New Hampshire coast. Plots were re-examined on 20 May. Eight plots required reburying of corners at BR and two at WF. In addition, every net at BR was completely filled with detritus that included local plant material (i.e., dead

pieces of *Spartina* spp.), but especially massive amounts of imported red macroalgae (e.g., *Chondrus crispus*; *Gracilaria* sp.), brown macroalgae (e.g., *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Laminaria* sp.), and the exoskeletons and pieces of dead crustaceans (e.g., *Carcinus maenas*; juveniles of *Homarus americanus*). In addition, many of the clams under the netting were scattered on the surface of the detritus rather than being buried. Curiously, few of the netted plots at WF on 20 May had much detritus, and the amount was miniscule compared to those at BR. On 13 August, nine nets at BR and two at WF required reburying at least one corner. Three of the nets at BR had been ripped or torn. In one instance, a lead sinker, fish hook, and fishing line were attached to the ripped net. In November during the final sampling, 10 of the 15 nets at BR either were torn or the corners had lifted up. Only one net at BR (at position 2-10; treatment = 4.2 mm mesh with floats) remained relatively intact during the experiment. Its lower corner had to be reburied once (13 August). The nine core samples from that plot yielded a mean density of 390.7 m<sup>-2</sup>, which was the highest of any of the plots at BR.

#### Cultured clams – relative growth and final mean length

Live cultured clams were found in 117 of the 360 core samples taken in November 2006. ANOVA detected no significant difference in mean relative growth due to site ( $P = 0.3739$ ) or netting treatment ( $P = 0.6287$ ) (Table 11). Although there was a significant Plot(Site x Netting) source of variation, and although three of the six decomposed sources of variation were statistically significant (Table 11), there was no discernible pattern such as faster growth in plots from row two (closest to the water) vs. row one

(closest to the shore). In addition, ANOVA indicated no significant difference in mean final SL due either to site ( $P = 0.1149$ ) or netting treatment ( $P = 0.8805$ ) (Table 12). Most shell growth (ca. 70%) occurred prior to August 13 (Fig. 19a). Pooling data from both sites, final mean SL was  $27.7 \pm 0.8$  mm ( $n = 117$ ), which represented nearly a tripling of relative growth over the 182-day experiment (Fig. 19b).

Because there were no significant site or treatment effects on mean relative growth or mean final SL, I asked whether each of these two dependent variables varied significantly with number per core to determine potential effects due to varying intra-specific clam densities (Fig. 20). I used a lack-of-fit regression analysis and examined for both variables a linear, quadratic, cubic, and quartic model. None of the models explained a significant amount of the variation ( $P > 0.05$ ) indicating that during the first growing season at these sites, no significant depression in growth occurs over a range of densities from 1 to 47 ind. per core, or approximately 55 to 2,582 individuals  $m^{-2}$ .

## Discussion

This work 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 sites in the Hampton-Seabrook Estuary.

Both objectives were met, and the efforts reported here, together with results from a previous study in the same estuary (Beal, 2002), provide compelling evidence about the dynamics of recent declines in soft-shell clam populations in this region of the New Hampshire coast (Nash, 2006). Both studies were conducted at different spatial scales (one where experimental units were small, 6-inch [15 cm diameter x 15 cm deep,  $A = 0.0182 \text{ m}^2$ ] plastic plant pots, the other in larger plots ranging from  $4 \text{ m}^2$  to  $18 \text{ m}^2$ ) and in different intertidal locations within the Hampton-Seabrook Estuary. However, collective evidence from both studies suggests that predation on juvenile soft-shell clams by green crabs, *Carcinus maenas* (Lindsay and Savage 1978), and other benthic feeders such as young-of-the-year winter flounder, *Pseudopleuronectes americanus* (Fairchild

et al. 2006 a,b), mummichogs, *Fundulus heteroclitus* (Kelso, 1979), and, perhaps horseshoe crabs, *Limulus polyphemus* (Smith, 1953; Grizzle et al. 2006) is intense during most of the year. Although no direct sampling of these or other predators such as the milky ribbon worm, *Cerebratulus lacteus* (Borque et al. 2001), occurred during these studies, comparison of juvenile clam survival in protected vs. unprotected experimental areas strongly suggests that predators account for most of the losses of small clams in the estuary (Table 13). For example, the surface of control plots in all three trials from the present study was littered with broken fragments of shell within weeks after each trial was initiated (Exp. I: November 2004; Exp. II: June 2005; Exp. III: April 2006) indicating that predators are present and voracious throughout much of the year. In addition, I counted all broken clams and shell fragments with intact umbos from samples collected in November 2006 (Exp. III). Many of the valves were disarticulated, but because the valves of *Mya* are dissimilar (e.g., the left valve bears the chondrophore, the right valve does not), it was possible to conservatively count for each sample the number of crushed individuals typical of crustacean predation (Beal and Vencile, 2001). Of the 360 benthic cores, 255 (70.8%) contained crushed individuals. Because there was no significant difference in number of crushed clams between sites ( $P = 0.4997$ ), I examined numbers in each of the four treatments (Fig. 21). Not surprisingly, control plots contained the fewest crushed clams, probably because they were consumed or drifted outside of the plots. Between 72% and 88% of core samples from netted plots contained crushed or broken clams (Fig. 21). This, too, was not surprising given that netting frequently had to be reburied due to sediment erosion near one end or one corner of the plot.

Quantitative estimates exist of green crab abundance in the Hampton River adjacent to the Willows Flat (Fairchild et al. 2006a). These investigators released juvenile winter flounder into the Estuary in 2004 and conducted both trawl and SCUBA surveys of green crab densities near the enhancement sites before and after releasing fish. *C. maenas* densities increased more than 600%, from 0.6 crabs/50m<sup>2</sup> before releasing fish to 4.3 crabs/50m<sup>2</sup> after releasing fish. Green crabs are not a recent threat in this region. Lindsay and Savage (1978) blamed green crabs for successive stock recruitment failures in the Hampton-Seabrook Estuary prior to 1976, concluding that *C. maenas* consumed most of the young seed clams before they could grow to harvestable size. Only an overwhelming spatfall, resulting in seed clam densities of over 18,000 individuals per square meter, was apparently able to swamp out some of the predation and return flats to harvestable densities (Lindsay and Savage, 1978).

*Interactive effects of predator exclusion netting, mesh netting aperture size, and planting area*

Experiment I, conducted through the winter of 2004-2005, demonstrated that planting area (4 m<sup>2</sup> to 18 m<sup>2</sup>) had no significant effect on clam survival (Table 4; Fig. 6). Clams appeared to grow more slowly in the 8m<sup>2</sup> netted plots vs. larger area netted plots; however, mean final length was similar between the these treatments. Certainly, any effect of plot size on clam growth was minimal. Of the 64 plots with netting, only two were damaged and/or required reburial at that time. Number of clams in core samples at the end of the field test (a poor surrogate for percent survival since clams were not distributed evenly or randomly in the plots), especially in plots with the smaller mesh

size (4.2 mm), was high (1,174.5 ind. m<sup>-2</sup>) compared to initial seeding densities of 1,320 ind. m<sup>-2</sup>. Significantly fewer clams were sampled in plots with the larger aperture (6.4 mm) netting. In fact, on average, only 30% of clams initially seeded 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 have been possible for clams to escape through the aperture of the protective netting by crawling through, 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. 22), which 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.

*Interactive effects of predator exclusion netting, netting aperture size, and intertidal location*

Experiments II and III suffered from at least two critical aspects. First, Experiment II demonstrated the futility of planting seed clams at low tide on a hot, sunny day. Second, Experiment III demonstrated how difficult it is to maintain protective netting under flood conditions within the estuary, or near the mouth of a river. However, for all the logistical difficulties associated with these two trials, several important themes are worth noting. First, clam numbers were significantly enhanced in protected vs. unprotected plots in both experiments (Tables 7 & 9). In fact, not a single live clam was sampled from cores taken from control plots either in 2005 or 2006. Second, smaller aperture netting resulted in 9 times (Exp. II) and 18 times (Exp. III) more clams per sample than larger aperture netting (Figs. 11 & 17). Third, the effects due to protecting clams were similar across both intertidal locations in both years suggesting that patterns may be generalizable in these sandy sediments. Fourth, adding flotation to the small aperture nets, which is necessary in soft sediments (Beal and Kraus, 2002), is not necessary in sandy sediments. Clams survived and grew equally as well in netted plots without the Styrofoam floats as they did in netted plots with the floats. Fifth, no Site x

Netting interaction was statistically significant in any of the analyses on cultured clam numbers, and in only one instance with relative growth (Exp. II) was this interaction term statistically significant, indicating the generality of the netting treatments results across different sites or locations within the estuary.

Clam growth in 2006 was seasonal, with 70-80% of new shell added prior to mid-August at both sites. Neither mean relative growth nor final mean SL differed between sites or treatments, suggesting that the estuary is well-mixed and that phytoplankton abundance, integrated over the 182-day study, was similar between sites. Similar seasonal growth rates have been observed in eastern Maine (Beal et al., 2001) and Long Island Sound (Cerrato et al. 1991), which may be related to a combination of temporal variation in food quality or quantity, as well as siphonal activity (sensu Thorin, 2000). Clams attained final mean SL's between 25.1 mm and 30.6 mm (Table 12). This result is surprisingly similar to growth of cultured clams at an intertidal flat in eastern Maine from April to December 1996 (Beal et al., 2001). There, growth rate and mean final SL were related to tidal position as animals initially 12 mm SL attained final lengths of  $24.1 \pm 1.02$  mm and  $28.2 \pm 1.2$  mm at the mid and low tide level, respectively.

#### *A strategy for enhancing flats in the Hampton-Seabrook Estuary*

Presently, the essence of clam flat management in the Hampton-Seabrook Estuary is based on two primarily unrelated factors: water quality and natural recruitment. Flats are open to recreational harvesting from the first Saturday after Labor Day until the following May with clamming on Saturday's only. Individuals must obtain an annual \$30

permit from the State of New Hampshire to harvest clams, and there is a 10-quart (9.5 liters) limit per day (Nash, 2006). That is, flats are open to harvesting if water quality, which is monitored regularly for bacterial indicators such as fecal coliforms, Enterococci, and *Escherichia coli*, exceeds minimum standards set by the National Shellfish Sanitation Program (Trowbridge, 2006). In addition, PSP toxins are monitored regularly in this area by the New Hampshire Department of Environmental Services, and, when toxin levels exceed 80  $\mu\text{g}$  STX eq/100 g of tissue in mussels or clams, the flats are closed to harvesting. However, water quality has little to do with successful bivalve recruitment. This important phase in the life-history of the soft-shell clam is poorly understood (but see Emerson and Grant, 1991; Guenther, 1992; Hunt and Mullineaux, 2002). Recruitment, likely, is influenced by a multitude of biotic and abiotic factors that encompass, but are not restricted to, size of spawning stock in the region, the abundance and voracity of larval and post-larval predators, local competition with other settling organisms, as well as hydrodynamic forces in both the water column and bedload transport that are affected by tides, wind, and storm run-off. It is unlikely that management activities can regulate the recruitment of large numbers of soft-shell clam juveniles over 10's or 100's of hectares of the intertidal. Therefore, there are two strategies that managers can opt for: 1) be satisfied with the vagaries of natural recruitment in hopes that at some point in time a massive spatfall (as described in Lindsay and Savage, 1978) occurs that will essentially swamp out the negative effects of most biotic and abiotic factors eventually to produce high biomass yields; or, 2) adopt tools, such as enhancement techniques, that can be successful on some limited scale.

Results from field studies described here can be used by managers to enhance local stocks of soft-shell clams in the Hampton-Seabrook Estuary. Cultured individuals of *Mya* are available from several sources in the Northeast U.S., and 8-10 mm seed clams can be purchased for ca. \$20-\$25 per 1,000 individuals. Seeding should occur early in the spring (late March through April) when seawater temperatures are below 10°C. Although clams in this estuary add new shell year-round (Beal, 2002; this study), growth is highly seasonal, so establishing populations of cultured clams in the flats early in the Spring will ensure that animals are in place when annual growth rates are maximal (sometime between mid-May and early August). Animals should be seeded into plots (small size plots 4 m<sup>2</sup>-12 m<sup>2</sup> may be easier to manage) at densities between 500-1,000 m<sup>-2</sup> (ca. 50-100 ft<sup>-2</sup>), which are then covered with plastic, flexible netting with an aperture size of 4.2 mm. I recommend a lower density seeding than was used in these studies because if a net becomes ripped, torn, or a portion eroded, the chances of losing significant numbers of clams to predators will be reduced. Fewer clams per plot, however, would require proportionately more netting. The nets should be maintained in situ as long as possible, or as long as they do not interfere with clam growth or survival (nets may need to be excavated and then re-established if sediment loading or detritus builds up under the nets to a point that may suffocate the clams). That is, nets may be used to protect clams until clams have reached the 2-inch legal size. For example, if 8-10 mm cultured clams are planted in the spring of Year I, they will attain SL's of at least 25 mm by the following November (Table 12) (the lower in the intertidal that plots and clams are placed, the faster they will grow). These animals will likely add an average of

5 mm of new shell over the winter, and, it is very likely that a large proportion will attain legal size for a harvest by October or November of Year II.

*Additional questions*

The field efforts of this study were designed to answer specific questions about clam enhancement.

Does the use of netting across several planting areas and aperture sizes enhance clam survival compared with similar size areas that receive cultured clams but have no protective netting?

Every field experiment showed that predation and other factors that remove unprotected clams from these flats is intense and continues throughout the year (Table 13). The use of plastic, flexible netting, regardless of plot size or intertidal location within the estuary, is highly recommended. During the 2005 and 2006 experiments (II & III), samples from every control plot (i.e., those that received cultured clams but no netting) contained no cultured clams. Most had been consumed by predators soon after planting.

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)?

Netting will significantly enhance numbers of wild spat (Tables 1, 6, & 8) compared to control areas, and, using netting with a 4.2 mm aperture instead of netting with a 6.4

mm aperture generally will result in higher numbers of wild spat. However, it makes no sense to apply netting arbitrarily to areas of the intertidal in hopes that spat will “fall, drift, or otherwise move into” the netted plots. This would be akin to buying a lottery ticket. Instead, netting could be used at times when benthic sampling provides evidence of a “large” spatfall. Then, the use of netting would be appropriate and likely result in an enhancement compared to unprotected areas.

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

Survival, yes. Growth, no. It is possible that clams less than 14 mm SL “can escape” plots covered with large aperture netting (6.4 mm; Fig. 22). The same may be true of animals less than 9 mm SL protected with netting that has an aperture of 4.2 mm. Therefore, if using cultured seed to enhance areas, it makes sense to start with cultured animals > 9 mm in SL. In these trials, final mean number of cultured individuals  $m^{-2}$  or number of wild spat  $m^{-2}$  was always higher in plots covered with the smaller aperture netting.

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

Yes. The effect of the netting to enhance cultured clam survival was similar between sites (Exp. II and III). In addition, the behavior of different mesh sizes was similar

between sites.

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?

Results from Experiment I (Tables 2, 3 & 4; Fig. 6) showed conclusively that clam survival did not depend on the size of the seeded plot. Size of plot may have affected growth rate, but not final mean size (Table 5).

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

Clam growth slows down considerably in the fall and winter compared to rates in the spring and summer (Fig. 19). Enhancement programs using cultured individuals should begin in the spring so that the time it takes shellfish to attain a potential refuge size from most predators will be shortened or reduced. On the other hand, sampling to determine the density of 0-year class animals in the estuary should occur no later than mid-October, when the majority of individuals have attained sizes  $> 2$  mm. If spatfall is considered high or substantial, a decision to enhance survival of that year class using protective netting should come soon thereafter, and nets should be deployed in the fall.

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

Perhaps a better question might be, “What are the costs to the State of New Hampshire not to have a viable recreational soft-shell clam fishery?” Standing stock, measured by the number of harvestable bushels of clams, and number of clam licenses sold by the State of New Hampshire (at \$30 each) are directly related (Anon., 2000). The cost to protect areas after an intense spatfall (which could occur once in 5 or 10 years) would be related to number of nets deployed and the cost to manage these nets. A net with a total area of 180 ft<sup>2</sup> (16.7 m<sup>2</sup>) that would have a “protected area” of approximately 130 ft<sup>2</sup> (12.0 m<sup>2</sup>) (the difference is that 30% of the net – a foot around the periphery – is used to secure the netting in the sediments) costs approximately \$18.00 (prices are based on 2006 prices from InterNet, Inc. (<http://www.internetplastic.com/>)). Therefore, to protect one-quarter, one-half, three-quarters, or one acre (43,560 ft<sup>2</sup>) of flats with netting, for example, would cost \$1,089, \$2,178, \$3,267, or \$4,560, respectively. This, of course, does not include the labor to position the nets properly on the flat, nor does it account for the time spent to regularly check and inspect the nets. Given the high loss rates of wild spat encountered in this and previous study (Beal, 2002) it is likely that very few clams would be alive outside the protected areas after two years. Because netting, if properly maintained through regular inspections, can deter predators, significant enhancement of wild clams would occur after two years.



If enhancement using cultured clams is an option, costs for netting, deploying nets on the flats, and regular inspections would be similar to costs described above for wild spat. Additional costs would be the juvenile clams. Using a hatchery cost of \$25 per 1,000 animals, a planting density of 75 ft<sup>2</sup>, and a planting area of 130 ft<sup>2</sup> would result in 9,750 animals per netted plot. Therefore, the costs for a single net (\$18.00) and the clams within the netted plot (\$243.75) would be \$261.75. At \$30 per license, this would be equivalent to the license fee paid by nearly nine license holders. If 100% of the shellfish survived to be harvested, approximately 10.2 bushels of 2-inch clams (960 count of 50 mm clams = one bushel [Erkan and Gibson, 2006]), or 378.2 quarts of clams (using 1 US bushel = 37.24 US quarts). Given that each license holder is permitted 10 quarts per tide, the number of 10-quart groups per net would be 37.8. That is, at 100% survival, a single plot would enable approximately 38 people to reach their daily quota. Clearly, this is unrealistic given that some mortality will occur. The relationship between the number of people reaching their daily quota from a single plot seeded initially with 9,750 cultured juveniles is a linear function of percent survival to harvestable size ( $Y = 0.0026 + 0.378 \times \% \text{ survival}$ ). For example, with a 50% survival to harvest size, the number of people reaching their daily quota from a single plot would be approximately 19 people. The “break-even” percent survival (based entirely on material costs of nets and clams) under this scenario would be approximately 23%. That is, if clam survival to harvest size were as low as 23%, then the amount of harvestable clams surviving per net (87.3 quarts) would be enough for 8.73 people to reach their daily quota. If 8.73 people paid \$30 for their license, then their collective cost would be equal to the initial material costs for that plot (\$261.75). This, too, is simplistic given that most

permit holders go clamming more than once per season. If clamming season runs from the first Saturday after Labor Day until the end of May (Nash, 2006), then there are approximately 38 Saturdays during that interval. If most people clammed on half these Saturdays (19), and each reached his/her 10-quart limit each time, then a single individual would harvest approximately 190 quarts of clams (ca. 5 bushels, and a maximum number of 2-inch clams = 4,898) per clamming season. This number of quarts and/or clams would require a 50% survival rate per plot. In other words, if an individual clammer went to the flats on half the Saturdays from Labor Day to the end of May and harvested his/her daily limit, he/she could harvest all these clams from a single plot seeded initially with 9,750 cultured juveniles if the survival rate in that plot equaled 50%. These calculations assume that no wild spat will enter the plots and/or that wild spat enhancement is negligible. If this assumption were false, then the percent survival of cultured clams per plot would be a value less than 50%.

### *Recommendations*

The results presented are unequivocal in terms of whether or not clam stocks can be enhanced in discrete areas within the Hampton-Seabrook Estuary. Both small-scale and large-scale studies have been conducted without involving many recreational clammers. I recommend that a pilot-scale, demonstration enhancement project be designed and conducted with volunteers from the clamming community. The project should be conducted at a minimum of two flats in the Hampton-Seabrook Estuary that are currently open to the recreational fishery. At the very least, 10 plots similar in size to those used in Experiment III (ca. a planting area of 12 m<sup>2</sup>) should be deployed and

each plot covered with a plastic, flexible netting with 4.2 mm apertures. The project should be initiated in the spring, with the coordinator(s) and the volunteers agreeing to make regular visits to the seeded plots. The two most important aspects affecting the success of this project are regular visits to each site to inspect and manage the nets, and a willing and well-educated clamming population. Since 2001, we have attempted not to draw too much attention to our experimental activities in hopes that experimental units and netted plots would remain relatively undisturbed so that trials could proceed without too much unintended interference from people and pets. This was important, and we were successful in managing to stay clear of highly trafficked areas. To begin the demonstration enhancement project, however, will require excellent communication and multiple meetings with clammers and other interested parties. I recommend that results of the present study along with those from 2001-2002 (Beal, 2002) be presented to the clamming industry in a series of focused meetings designed as dialogue between scientists and clammers. It is not important to bring to these meetings the statistics, ANOVA tables, and interaction plots that cram these pages. Rather, what is needed is a low-key, well-crafted presentation with many photographs from these and/or other efforts that tells a story in 20-30 minutes of how clammers and scientists can work together to achieve a common goal of enhancing clam stocks in the Hampton-Seabrook Estuary.

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Table 1. ANOVA results on the square-root transformed mean number of wild clams per core sampled from Willows Flat, Hampton, New Hampshire on 14-15 May 2005 ( $n = 4$ ) (Exp. I). To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ( $\alpha'_{\text{netting}} = 0.0253$ ;  $\alpha'_{\text{Plot size}} = 0.0170$ ). 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	2.95	0.0588
No netting vs. net	1	0.49819309	0.49819309	5.82	<b>0.0184</b>
Lg vs. Small net	1	0.00628006	0.00628006	0.07	0.7872
Clams	1	1.02125913	1.02125913	11.94	<b>0.0009</b>
Plot size	3	0.06153175	0.02051058	0.24	0.8683
Small vs. rest	1	0.04406843	0.04406843	0.52	0.4752
8 vs. 12 & 18	1	0.01225499	0.01225499	0.14	0.7062
12 vs. 18	1	0.00520833	0.00520833	0.06	0.8058
Net x Clams	2	0.32155151	0.16077575	1.88	0.1601
Net x Plot size	6	0.66872911	0.11145485	1.30	0.2669
Clams x Plot size	3	0.00662187	0.00220729	0.03	0.9943
Net x Clams x Plot size	6	0.33336771	0.05556128	0.65	0.6903
Section	1	0.04902882	0.04902882	0.51	0.4775
Section x Net	2	0.29785862	0.14892931	1.54	0.2173
Section x Clams	1	0.39648787	0.39648787	4.10	<b>0.0444</b>
Section x Net x Clams	2	0.02193486	0.01096743	0.11	0.8929
Section x Plot size	3	0.47589102	0.15863034	1.64	0.1818
Section x Net x Plot size	6	0.78415098	0.13069183	1.35	0.2369
Section x Clams x Plot size	3	0.59374215	0.19791405	2.04	0.1090
Sect x Net x Clms x Plot sz	6	0.61885864	0.10314311	1.07	0.3847
Plot(Net x Clam x Plot size)	72	6.15849365	0.08553463	0.88	0.7245
Section x Plo(NetxClaxPlo)	72	5.76204704	0.08002843	0.83	0.8232
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) (Exp. I). To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ( $\alpha'_{\text{netting}} = 0.0253$ ;  $\alpha'_{\text{Plot size}} = 0.0170$ ). Boldface P-values indicate statistical significance.

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Netting	2	267.7813563	133.8906782	103.61	<b>&lt;.0001</b>
No netting vs. Net	1	215.6554755	215.6554755	166.88	<b>&lt;.0001</b>
Large net vs. Small net	1	52.1258808	52.1258808	40.34	<b>&lt;.0001</b>
Clams	1	481.8793646	481.8793646	372.90	<b>&lt;.0001</b>
Plot size	3	3.1382458	1.0460819	0.81	0.4927
Small vs. rest	1	2.7946823	2.7946823	2.16	0.1458
8 vs. 12 & 18	1	0.0940171	0.0940171	0.07	0.7881
12 vs. 18	1	0.2495464	0.2495464	0.19	0.6617
Netting x Clams	2	267.0248418	133.5124209	103.32	<b>&lt;.0001</b>
No net vs. net x clams	1	209.3434669	209.3434669	162.00	<b>&lt;.0001</b>
Lg net vs. Sm net x clams	1	57.6813749	57.6813749	44.64	<b>&lt;.0001</b>
Netting x Plot size	6	3.9034548	0.6505758	0.50	0.8038
Clams x Plot size	3	2.6836745	0.8945582	0.69	0.5597
Netting x Clams x Plot size	6	3.8546949	0.6424492	0.50	0.8085
Section	1	0.9536932	0.9536932	1.77	0.1852
Section x Netting	2	0.2397919	0.1198960	0.22	0.8009
Section x Clams	1	0.5966760	0.5966760	1.11	0.2943
Section x Netting x Clams	2	0.1495698	0.0747849	0.14	0.8706
Section x Plot size	3	0.6786179	0.2262060	0.42	0.7393
Section x Net x Plot size	6	5.3377407	0.8896235	1.65	0.1357
Section x Clams x Plot size	3	1.4352064	0.4784021	0.89	0.4490
Sect x Net x Clms x Plot siz	6	3.2626273	0.5437712	1.01	0.4214
Plot(Net x Clam x Plot size)	72	93.0415646	1.2922440	2.40	<b>&lt;.0001</b>
Section x Plot(NetxClaxPlo)	72	47.3216580	0.6572452	1.22	0.1463
Error	192	103.577563	0.539466		
Total	383	1286.860341			



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) (Exp. I). To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ( $\alpha'_{\text{netting}} = 0.0253$ ;  $\alpha'_{\text{Plot size}} = 0.0170$ ). Boldface P-values indicate statistical significance.

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Netting	2	534.7124481	267.3562241	105.14	<.0001
No netting vs. net	1	424.9755049	424.9755049	167.13	<.0001
Lg vs. Small net	1	109.7369432	109.7369432	43.16	<.0001
Plot size	3	5.8010870	1.9336957	0.76	0.5237
Small vs. rest	1	5.2022785	5.2022785	2.05	0.1612
8 vs. 12 & 18	1	0.0997157	0.0997157	0.04	0.8441
12 vs. 18	1	0.4990928	0.4990928	0.20	0.6604
NetxPlot size	6	7.5602331	1.2600388	0.50	0.8074
Section	1	1.5295359	1.5295359	0.60	0.4431
Section x Net	2	0.3789451	0.1894725	0.07	0.7865
Section x Plot size	3	1.9263243	0.6421081	0.25	0.6183
Section x Net x Plot size	6	8.3191180	1.3865197	0.54	0.4651
Plot(Net x Plot size)	36	91.5415646	2.5428212	2.40	<b>0.0004</b>
Section x Plot(NetxPlot sz)	36	45.8216580	1.2728238	1.20	0.2368
Error	96	101.5775626	1.0580996		
Total	191	799.1684766			

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) (Exp. I). To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ( $\alpha'_{\text{netting}} = 0.0253$ ;  $\alpha'_{\text{Plot size}} = 0.0170$ ). Boldface P-values indicate statistical significance.

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Net	2	0.11465511	0.05732755	5.27	<b>0.0120</b>
No netting vs. net	1	0.04493457	0.04493457	4.13	0.0525
Large vs. Small net	1	0.06972054	0.06972054	6.41	<b>0.0178</b>
Plot size	3	0.12901147	0.04300382	3.95	<b>0.0190</b>
4 vs. Rest	1	0.01881888	0.01881888	1.72	0.2012
8 vs. 12 & 18	1	0.06225914	0.06225914	5.72	0.0243
12 vs. 18	1	0.04793345	0.04793345	4.40	0.0451
Net x Plot size	6	0.08196811	0.01366135	1.26	0.3118
Section	1	0.03100436	0.03100436	2.85	0.1034
Section x Net	2	0.01235286	0.00617643	0.57	0.5738
Section x Plot size	3	0.01654147	0.00551382	0.51	0.6812
Section x Net x Plot size	3	0.00247069	0.00082356	0.08	0.9726
Plot(Net x Plot size)	26	0.28300555	0.01088483	1.66	0.0545
SectionxPlot(Net x Plot sz)	24	0.07989563	0.00332898	0.51	0.9663
Error	61	0.40106169	0.00657478		
Corrected Total	132	1.15196694			

Table 6. ANOVA results on the square root-transformed mean number of wild clams per core in samples taken at Willows Flat, Hampton, New Hampshire, on 8 October 2005 (Exp. II). To reduce the potential for excessive type I errors, the decision rule for all a priori contrasts was adjusted ( $\alpha'_{\text{netting}} = 0.0253$ ;  $\alpha'_{\text{plot}} = 0.0085$ ). Boldface P-values indicate statistical significance. (n = 5)

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Site	1	3.40070649	3.40070649	4.58	<b>0.0426</b>
Netting treatment	2	12.83530401	6.41765201	111.23	<b>0.0089</b>
No netting vs. net	1	7.44793964	7.44793964	129.08	<b>0.0077</b>
Large vs. Small net	1	5.38736437	5.38736437	93.37	<b>0.0105</b>
Site x Treatment	2	0.11539840	0.05769920	0.08	0.9254
Plot(Site x Treatment)	24	17.80603814	0.74191826	1.97	0.0179
Site 1: No netting	4	0.24255845	0.06063961	0.16	0.9577
Site 1: Large netting	4	4.19547666	1.04886917	2.78	0.0347
Site 1: Small netting	4	2.00846052	0.50211513	1.33	0.2692
Site 2: No netting	4	2.63972786	0.65993197	1.75	0.1509
Site 2: Large netting	4	2.16905989	0.54226497	1.44	0.2319
Site 2: Small netting	4	6.55075474	1.63768869	4.34	<b>0.0038</b>
Error	60	22.62978953	0.37716316		
Corrected Total	89	56.78723657			



Table 7. ANOVA results on the square root-transformed mean number of cultured clams per core at Willows Flat on 8 October 2005 (Exp. II). Clams (1,275 m<sup>-2</sup>) were seeded into fifteen 18 m<sup>2</sup> plots at two intertidal sites on 11 June 2005. To reduce the potential for excessive type I errors, the decision rule for all a priori contrasts was adjusted ( $\alpha'_{\text{netting}} = 0.0253$ ;  $\alpha'_{\text{plot}} = 0.0085$ ). Boldface P-values indicate statistical significance. (n = 5)

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Site	1	0.10662922	0.10662922	0.18	0.6726
Netting treatment	2	26.52328950	13.26164475	212.21	<b>0.0047</b>
No netting vs. net	1	11.28360561	11.28360561	180.56	<b>0.0055</b>
Large vs. Small net	1	15.23968388	15.23968388	243.86	<b>0.0041</b>
Site x Treatment	2	0.12498722	0.06249361	0.11	0.8987
Plot(Site x Treatment)	24	13.97840911	0.58243371	1.87	<b>0.0263</b>
Site 1: No netting	4	0.00000000	0.00000000	0.00	1.0000
Site 1: Large netting	4	1.55424723	0.38856181	1.25	0.3036
Site 1: Small netting	4	2.00565456	0.50141364	1.61	0.1834
Site 2: No netting	4	0.00000000	0.00000000	0.00	1.0000
Site 2: Large netting	4	0.66666667	0.16666667	0.53	0.7141
Site 2: Small netting	4	9.75184065	2.43796016	7.82	<b>&lt;0.0001</b>
Error	60	18.69947372	0.31165790		
Corrected Total	89	59.43278877			

Table 8. ANOVA results on the square root-transformed mean number of wild clams per core at two sites (Willows Flat [WF] and Blackwater River [BR]) on 11-12 November 2006 (see Fig. 13 for size frequency distribution at both sites) (Exp. III). Cultured clams (1,320 m<sup>-2</sup>) were seeded into twenty 12 m<sup>2</sup> plots at both sites on 21-22 April 2006. To reduce the potential for excessive type I errors, the decision rule for all a priori contrasts was adjusted ( $\alpha'_{\text{netting}} = 0.0170$ ;  $\alpha'_{\text{plot}} = 0.0064$ ). Boldface P-values indicate statistical significance. (n = 5)

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Site	1	0.00036803	0.00036803	0.00	0.9816
Netting treatment	3	33.55236239	11.18412080	16.45	< <b>0.0001</b>
Control vs. Netting	1	19.07006091	19.07006091	28.05	< <b>0.0001</b>
Small vs. Large aperture	1	14.14315691	14.14315691	20.80	< <b>0.0001</b>
Floats v. No floats	1	0.33914457	0.33914457	0.50	0.4851
Site x Netting	3	7.96443727	2.65481242	3.91	0.0175
BR v. WF x Control v. Net	1	0.54963354	0.54963354	0.81	0.3753
BR v. WF x Small v. Large	1	0.38861869	0.38861869	0.57	0.4551
BR v. WF x Floats v. None	1	7.02618504	7.02618504	10.34	<b>0.0030</b>
Section	2	2.61613997	1.30806999	4.14	<b>0.0204</b>
Netting x Section	6	2.87651066	0.47941844	1.52	0.1867
Site x Section	2	1.71710061	0.85855031	2.72	0.0736
Site x Netting x Section	6	4.17743598	0.69623933	2.20	0.0539
Plot(Site x Netting)	32	21.75429495	0.67982172	2.06	<b>0.0012</b>
BR: No Net	4	1.20000000	0.30000000	0.91	0.4587
BR: 6.4 mm	4	0.88797048	0.22199262	0.67	0.6134
BR: 4.2 mm	4	4.48492386	1.12123097	3.40	0.0099
BR: 4.2 mm & Floats	4	4.79547157	1.19886789	3.63	0.0068
WF: No Net	4	0.00000000	0.00000000	0.00	1.0000
WF: 6.4 mm	4	0.02287638	0.00571910	0.02	0.9983
WF: 4.2 mm	4	6.21490684	1.55372671	4.71	<b>0.0011</b>
WF: 4.2 mm & Floats	4	4.14814582	1.03703646	3.14	0.0153
Section x Plot(Net x Site)	64	20.21797902	0.31590592	0.96	0.5707
Error	240	79.17826820	0.32990950		
Corrected Total	359	174.05489710			

Table 9. ANOVA results on the square root-transformed mean number of cultured clams per core at two sites (Willows Flat [WF] and Blackwater River [BR]) on 11-12 November 2006 (Exp. III). Clams (1,320 m<sup>-2</sup>) were seeded into twenty 12 m<sup>2</sup> plots at both sites on 21-22 April 2006. To reduce the potential for excessive type I errors, the decision rule for all a priori contrasts was adjusted ( $\alpha'_{\text{netting}} = 0.0170$ ;  $\alpha'_{\text{plot}} = 0.0064$ ). Boldface P-values indicate statistical significance. (n = 5)

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Site	1	21.4779650	21.4779650	3.42	0.0738
Netting treatment	3	159.7476191	53.2492064	8.47	<b>0.0003</b>
Control vs. Netting	1	69.34561873	69.34561873	11.03	<b>0.0023</b>
Small vs. Large aperture	1	86.58213717	86.58213717	13.77	<b>0.0008</b>
Floats v. No floats	1	3.81986323	3.81986323	0.61	0.4415
Site x Netting	3	15.6495837	5.2165279	0.83	0.4874
Section	2	1.49620260	0.74810130	0.84	0.4380
Netting x Section	6	1.24904541	0.20817424	0.23	0.9644
Site x Section	2	3.58058892	1.79029446	2.00	0.1435
Site x Netting x Section	6	8.91157263	1.48526211	1.66	0.1453
Plot(Site x Netting)	32	201.21003185	6.28781353	9.71	<b>&lt;0.0001</b>
BR: No Net	4	0.00000000	0.00000000	0.00	1.0000
BR: 6.4 mm	4	0.31111111	0.07777778	0.12	0.9753
BR: 4.2 mm	4	12.25865984	3.06466496	4.73	<b>0.0011</b>
BR: 4.2 mm & Floats	4	43.82868470	10.95717118	16.92	<b>&lt;0.0001</b>
WF: No Net	4	0.00000000	0.00000000	0.00	1.0000
WF: 6.4 mm	4	2.69869445	0.67467361	1.04	0.3872
WF: 4.2 mm	4	39.36260962	9.84065240	15.20	<b>&lt;0.0001</b>
WF: 4.2 mm & Floats	4	102.75027215	25.68756804	39.68	<b>&lt;0.0001</b>
Section x Plot(Net x Site)	64	57.2546368	0.8946037	1.38	<b>0.0436</b>
Error	240	155.3858979	0.6474412		
Corrected Total	359	625.9631438			



Table 11. ANOVA results on the untransformed mean relative growth of cultured clams per core at two sites (Willows Flat [WF] and Blackwater River [BR]) on 11-12 November 2006 (Exp. III). Clams (1,320 m<sup>-2</sup>) were seeded into twenty 12 m<sup>2</sup> plots at both sites on 21-22 April 2006. No live clams were sampled from cores taken in control plots; therefore, Netting treatment refers to three levels: Large (6.4 mm) mesh; Small mesh (4.2 mm); and, Small mesh with floats. To reduce the potential for excessive type I errors, the decision rule for the a priori contrasts was adjusted ( $\alpha'_{\text{plot}} = 0.0085$ ). Boldface P-values indicate statistical significance. (n = 5)

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Site	1	0.70369658	0.70369658	0.84	0.3739
Netting treatment	2	0.80361024	0.40180512	0.48	0.6287
Site x Netting	2	1.34512895	0.67256448	0.80	0.4666
Section	2	0.39008692	0.19504346	0.88	0.4314
Netting x Section	4	1.43174193	0.35793548	1.61	0.2099
Site x Section	2	0.51566680	0.25783340	1.16	0.3337
Site x Netting x Section	2	0.30850075	0.15425038	0.69	0.5116
Plot(Site x Netting)	17	13.45523956	0.84095247	3.27	<b>0.0004</b>
BR: 6.4 mm	2	3.66823573	1.83411787	7.12	<b>0.0016</b>
BR: 4.2 mm	3	0.15745697	0.05248566	0.20	0.8960
BR: 4.2 mm & Floats	3	5.61186186	1.87062062	7.26	<b>0.0003</b>
WF: 6.4 mm	3	5.99582364	1.99860788	7.76	<b>0.0002</b>
WF: 4.2 mm	3	0.08460497	0.02820166	0.11	0.9540
WF: 4.2 mm & Floats	3	1.06146032	0.35382011	1.37	0.2601
Section x Plot(Net x Site)	21	4.68011088	0.22286242	0.87	0.6323
Error	63	16.22215234	0.25749448		
Corrected Total	116	48.87787762			



Table 13. Effect of protective netting in field experiments in the Hampton-Seabrook Estuary from November 2001 to November 2006. Values are mean percent survival in experimental units or plots that were covered with plastic predator deterrent netting vs. controls without netting.

<u>Site</u>	<u>Time</u>	<u>Controls</u>	<u>Protective Netting</u>	<u>Percent Enhancement due to Predator Nets</u>
Brown's Flat <sup>1</sup>	Nov. 2001- March 2002	3.6	40.7	1031
Common Island <sup>1</sup>	Nov. 2001- March 2002	13.9	68.8	395
Middle Ground <sup>1</sup>	Nov. 2001- March 2002	31.9	77.1	142
Brown's Flat <sup>1</sup>	March 2002- July 2002	2.5	25.3	912
Common Island <sup>1</sup>	March 2002- July 2002	9.0	16.6	84
Middle Ground <sup>1</sup>	March 2002- July 2002	25.2	57.9	130
Willows Flat <sup>2</sup>	Nov. 2004- May 2005	0.0	59.8	+++
Willows Flat <sup>3</sup>	June 2005- October 2005	0.0	6.0	+++
Willows Flat <sup>3</sup>	April 2006- Nov. 2006	0.0	18.2	+++
Blackwater River <sup>3</sup>	April 2006 Nov. 2006	0.0	6.0	+++

<sup>1</sup> Experimental units (plastic plant pots 15 cm diameter x 15 cm deep:  $A = 0.0182\text{m}^2$ ); from Beal, 2002.

<sup>2</sup> Core samples ( $A = 0.0182\text{ m}^2$ ) taken within larger plots varying in size from  $4\text{ m}^2$  to  $18\text{ m}^2$

<sup>3</sup> Core samples ( $A = 0.0182\text{ m}^2$ ) taken within larger plots ca.  $12\text{ m}^2$

## Figure Legends

- Figure 1. Sites within the Hampton-Seabrook Estuary used for the three studies. Experiment I (November 2004 to May 2005) was conducted between the green and blue stars at the Willows Flat in the Hampton River. Experiment II was conducted at the Willows Flat. Site 1 = green star; Site 2 = blue star. Experiment III was conducted at the Willows Flat (blue star) and at a flat near the mouth of the Blackwater River (red star). Map created using MapSend BlueNav North America v. 1.01b (2003, Thales Navigation, Inc.).
- Figure 2. Size-frequency distribution of wild soft-shell clams sampled from benthic cores during 14-15 May 2005 (Exp. I). Four cores ( $A = 0.0182 \text{ m}^2$ ) 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 3. Mean number of cultured clams  $\text{m}^{-2}$  from core samples taken on 14-15 May 2005 (Exp. I). Interaction plot demonstrating the nature of the significant Net x Clam interaction for the a priori contrast “No net vs. net x clams” (Table 2).  $n = 16$  for bars labeled “Netting Absent,” and  $n = 32$  for bars labeled “Netting Present.” b) Interaction plot demonstrating the nature of the significant Net x Clam interaction for the a priori contrast “Lg net vs. Sm net x clams” (Table 2).  $n = 16$ .
- Figure 4. Mean number of cultured clams  $\text{m}^{-2}$  from core samples taken on 14-15 May 2005 (Exp. I). Each plot demonstrates the highly significant plot-to-plot variability for a given combination of netting aperture size and plot size. The five sources of variation represented by these plots accounted for 80% of the total variation associated with the source of variation labeled as Plot(Net x Clam x Plot size) in Table 2. **a, b**) Large aperture



nets at 4 m<sup>2</sup> and 12 m<sup>2</sup>, respectively; **c, d, e**) Small aperture nets at 4 m<sup>2</sup>, 12 m<sup>2</sup>, and 18 m<sup>2</sup>, respectively.

- Figure 5. Mean number of cultured clams m<sup>-2</sup> from core samples taken on 14-15 May 2005 (Exp. I). Samples were taken from plots initially seeded at a density of approximately 1320 individuals m<sup>-2</sup>. ANOVA indicated that netting enhances clam numbers by nearly 105 times compared to numbers of clams in control plots ( $P < 0.0001$ , Table 4). Additionally, approximately three times more clams were sampled in plots protected with small vs. large netting ( $P < 0.0001$ , Tables 3 & 4). ( $n = 16$ )
- Figure 6. Mean number of cultured clams m<sup>-2</sup> from core samples taken on 14-15 May 2005 (Exp. I) from each combination of netting and plot size from plots initially stocked with hatchery-reared individuals in November 2004 at a density of approximately 1320 m<sup>-2</sup>. ANOVA (Table 4) revealed no significant differences in density among plot sizes ( $P = 0.5237$ ), but did demonstrate a significant difference among netting treatments ( $P < 0.0001$ ). ( $n = 4$ )
- Figure 7. Mean relative growth of cultured clams in protected and unprotected plots for each plot size on 14-15 May 2005 (Exp. I). No difference in relative growth was observed between protected and unprotected areas, but clams under netting with the smaller aperture (4.2 mm) grew approximately 30% faster than those under netting with the larger aperture (6.4 mm) (Table 5). Size of plot also influenced growth rate (see Table 5). ( $n = 4$ )
- Figure 8. Initial (19-20 November 2004) and final (14-15 May 2005) size frequency distribution of cultured clams in protected and unprotected plots at Willows Flat, Hampton, New Hampshire (Exp. I).

- Figure 9. Mean relative growth of clams in all seeded and netted plots at Willows Flat, Hampton, New Hampshire on 14-15 May 2005 ( $n = 124$ ) (Exp. I), 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 10. Size frequency distribution of wild clams sampled from benthic cores taken from fifteen  $18\text{m}^2$  plots at each of two intertidal sites on 8 October 2005 at Willows Flat, Hampton, New Hampshire (Exp. II). ( $n = 111$ )
- Figure 11. Mean number of cultured clams in control and netted plots on 8 October 2005 (Exp. II). Clams ( $7.3 \pm 0.5$  mm SL) were seeded into  $18\text{m}^2$  plots on 11 June 2005 at an approximate density of  $1,275\text{ m}^{-2}$ . ANOVA indicated no differences in mean abundance between sites, a significant enhancement due to the presence of netting, and a significant difference in mean number  $\text{m}^{-2}$  between large and small protective netting (Table 7). ( $n = 5$ )
- Figure 12. Size frequency distribution of cultured clams sampled from benthic cores taken from fifteen  $18\text{ m}^2$  plots at two intertidal sites on 8 October 2005 at Willows Flat, Hampton, New Hampshire (Exp. II). ( $n = 82$ )
- Figure 13. Interaction plot of mean relative growth of cultured clams from benthic cores taken from fifteen  $18\text{m}^2$  plots at two intertidal sites on 8 October 2005 at Willows Flat, Hampton, New Hampshire (Exp. II). ANOVA demonstrated that neither main effects due to Site or Netting treatment were statistically significant; however, the interaction term was significant ( $P = 0.0331$ ). The dashed line indicates the value for relative growth associated with a doubling of shell length.

- Figure 14. Size-frequency distribution of wild clams from benthic core samples at two sites on 11-12 November 2006 (Exp. III).
- Figure 15. Mean number of live wild clams  $m^{-2}$  at two sites on 11-12 November 2006 ( $n = 5$ ) (Exp. III). Interaction plot demonstrating how the effect due to the presence of flotation with the small aperture netting varies between sites ( $P = 0.0030$ , Table 8).
- Figure 16. Mean number of live wild clams  $m^{-2}$  in each section of plots at two sites on 11-12 November 2006 ( $n = 40$ ) (Exp. III). ANOVA indicated a significant difference between sections ( $P = 0.0204$ , Table 8).
- Figure 17. Mean number of live cultured clams  $m^{-2}$  in control and netted plots at the two sites on 11-12 November 2006 (Exp. III). No clams occurred in any benthic cores taken from plots without protective netting. Clams were seeded initially (21-22 April 2006) at a density of approximately  $1,320 m^{-2}$ . ANOVA (Table 8) demonstrated a significant difference in mean number between plots protected with a 6.4 mm vs. 4.2 mm aperture, but no difference between plots protected with a 4.2 mm aperture with vs. without flotation. ( $n = 5$ )
- Figure 18. Mean number of live cultured clams  $m^{-2}$  in plots at WF that were covered with protective netting (4.2 mm aperture) (Exp. III). Interaction plot showing the single treatment responsible for the significant Section x Plot(Netting x Site) source of variation in Table 9. (No live clams occurred in any of the nine samples from plot 1.) ( $n = 3$ )
- Figure 19. Relationship between SL and date (a) and relative growth and date (b) for data combined from both BR and WF (Exp. III). Experiment was initiated

on 21-22 April. Samples were taken on 13 May (n = 27), 13 August (n = 47), and 11-12 November 2006 (n = 117).

- Figure 20. Tests of effects on varying intraspecific clam densities on a) mean final SL, and b) mean relative growth (Exp. III). Neither a linear, quadratic, cubic, or quartic model fit either data set ( $P > 0.05$ ).
- Figure 21. Number of core samples containing broken and crushed clams in plots at both sites (WF & BR) (Exp. III). Ninety samples were taken from each treatment. Controls (n = 37 samples containing broken and crushed clams); 6.4 mm netting (n = 65); 4.2 mm netting (n = 79); 4.2 mm netting with floats (n = 74).
- Figure 22. Linear relationship ( $\pm 95\%$  CI) between clam length and width for cultured Individuals of *Mya arenaria* ( $Y = 0.214 + 0.617X$ , 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.



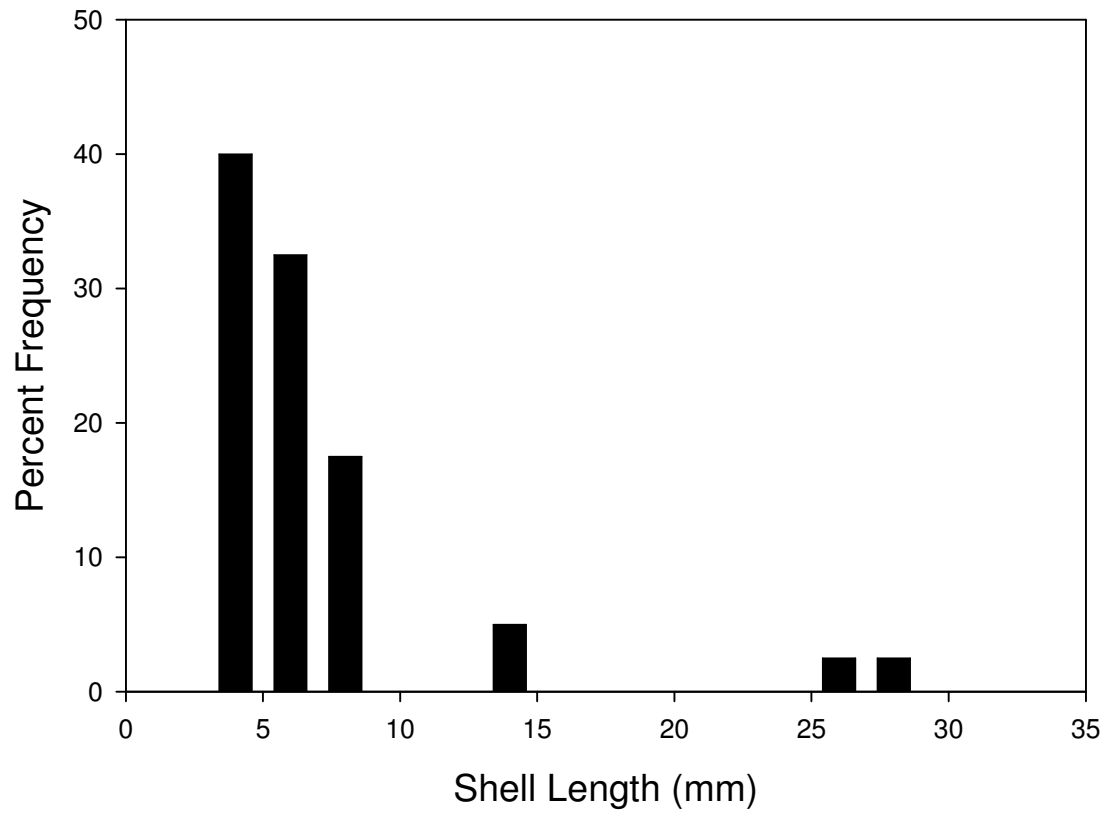
**Figure 2.**

Figure 3.

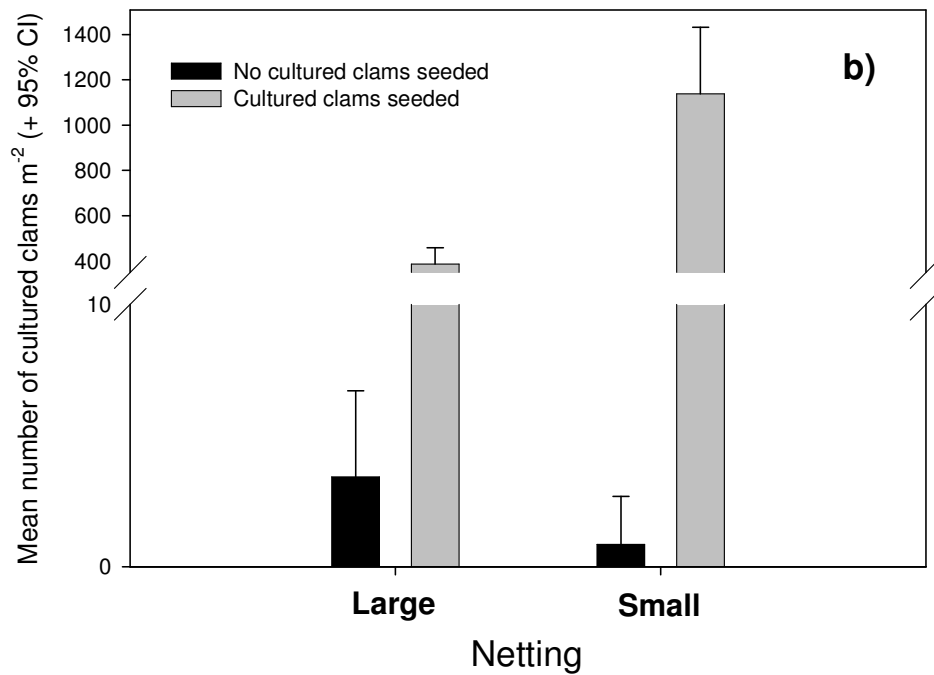
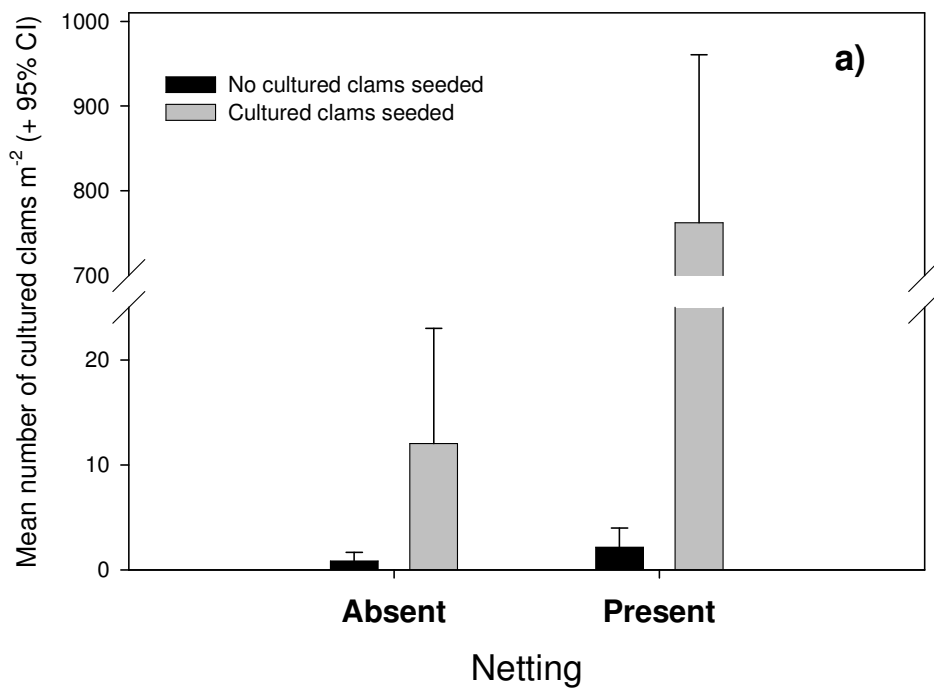
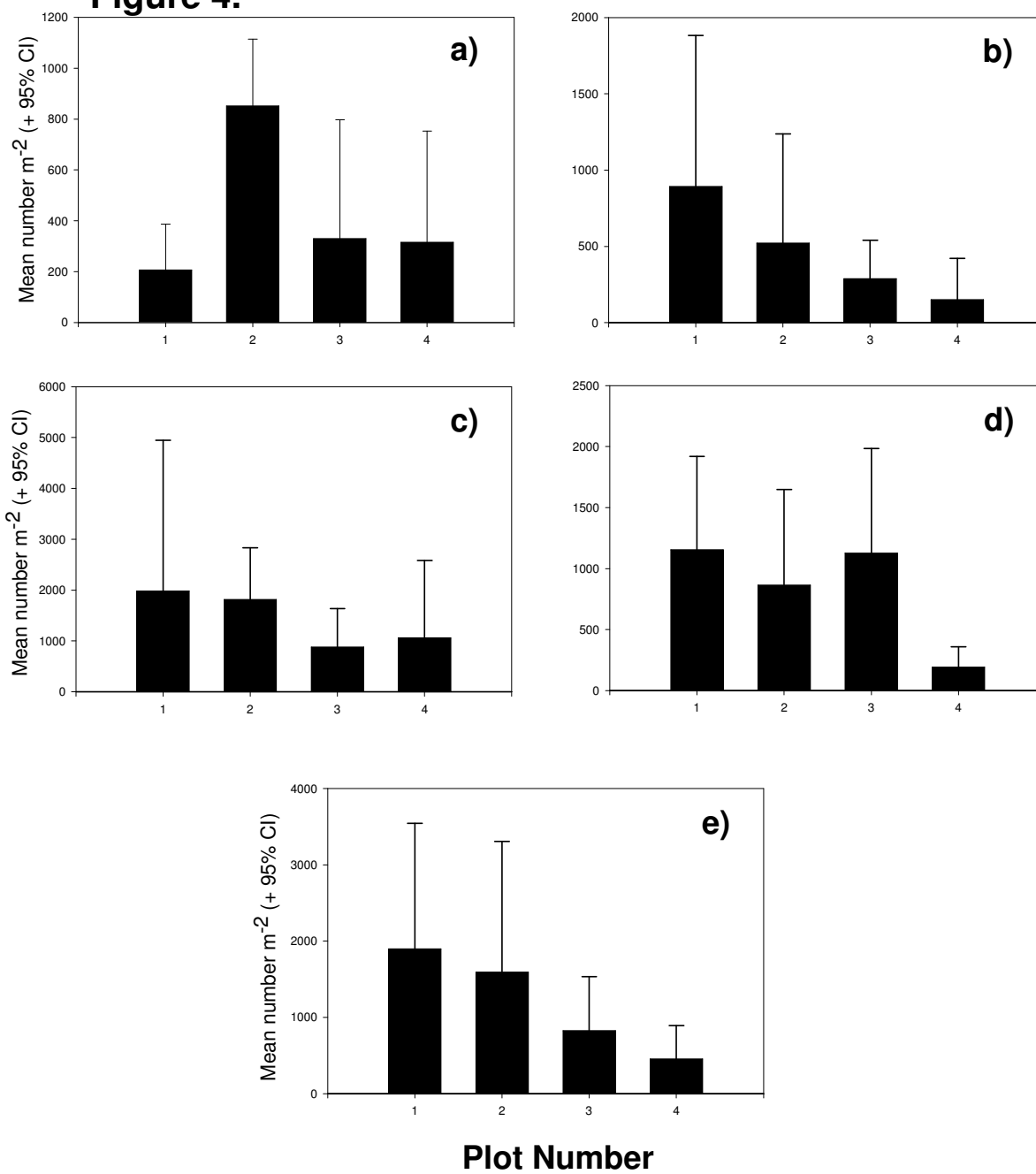
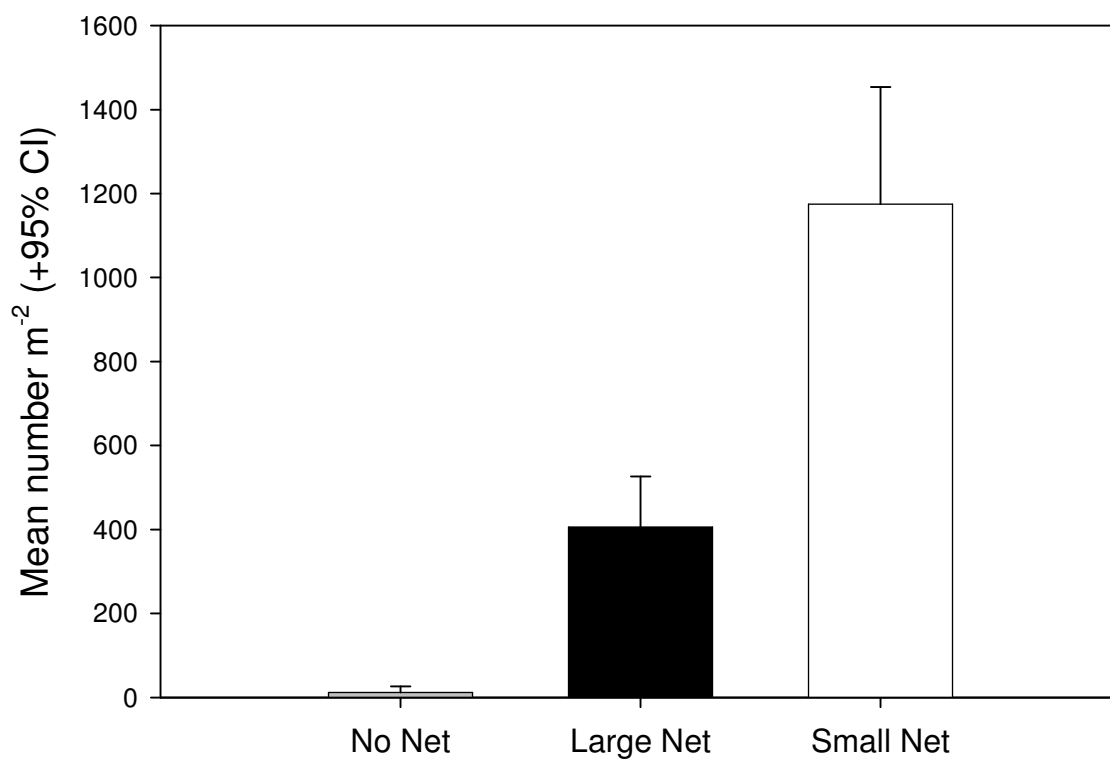
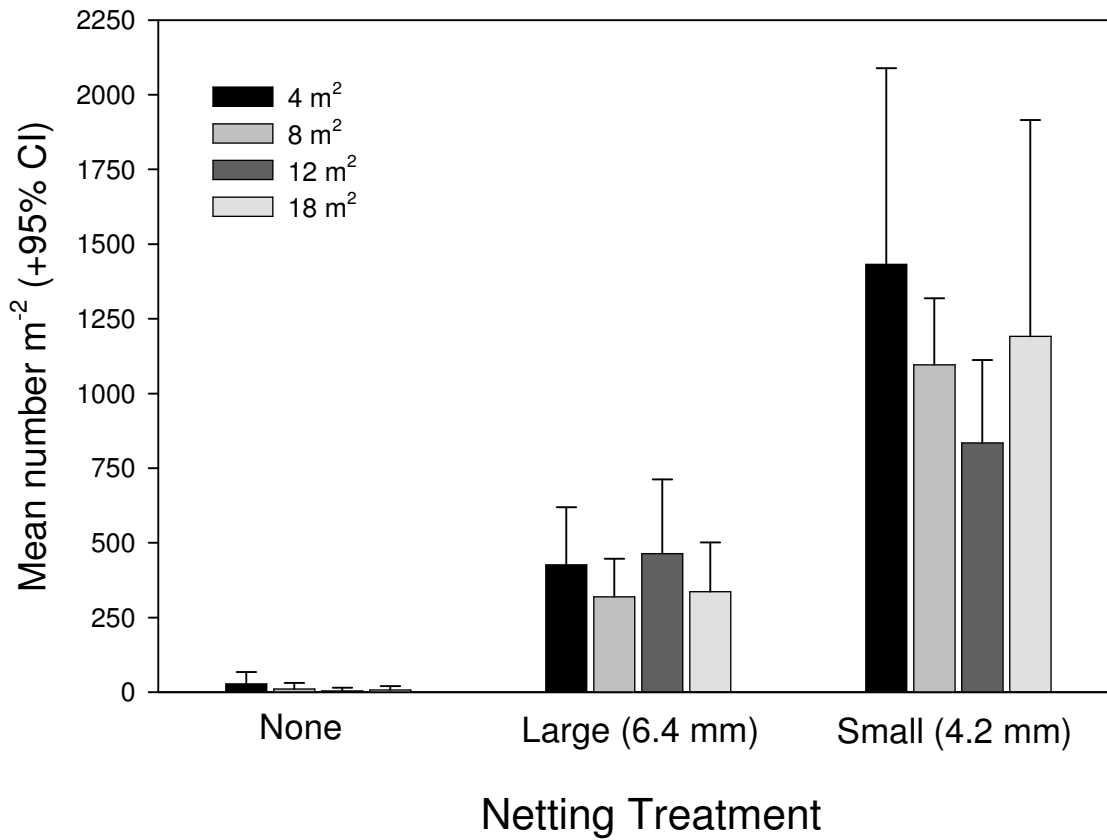


Figure 4.





**Figure 5.**

**Figure 6.**

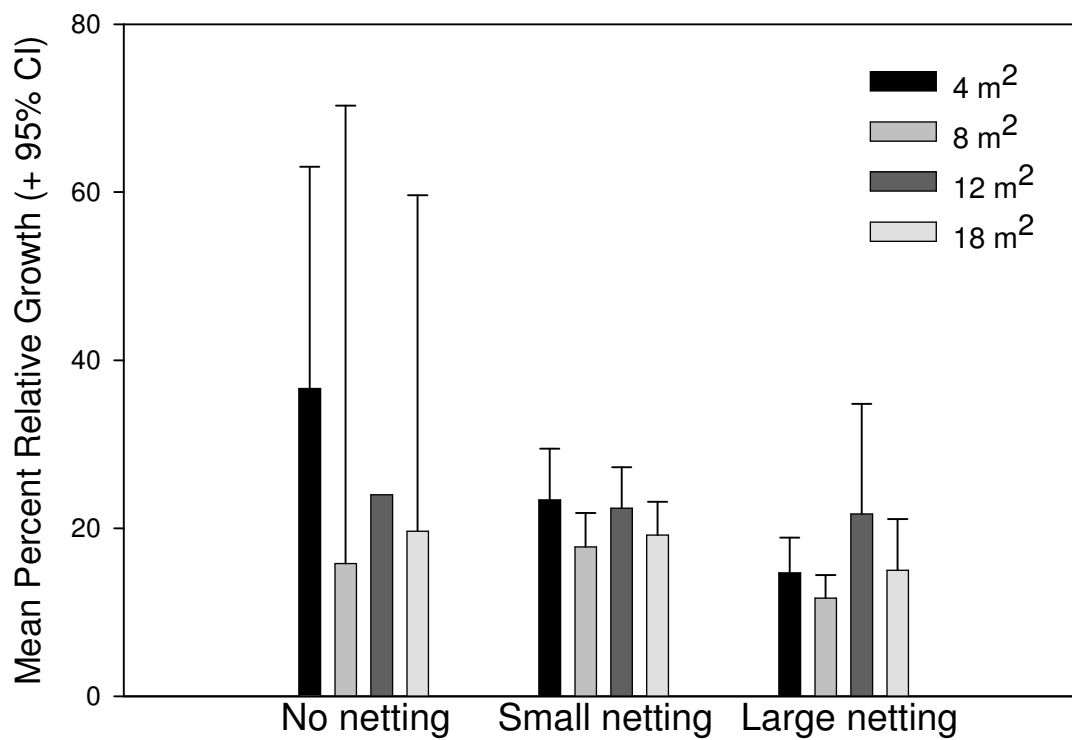
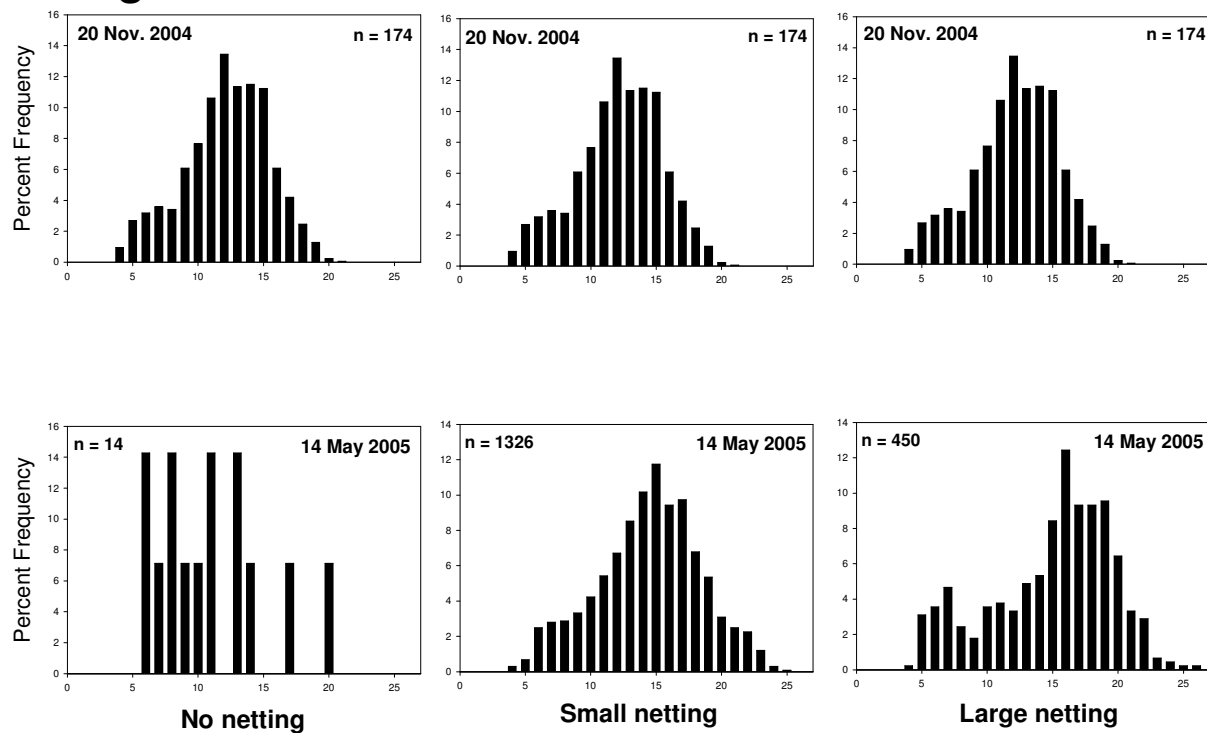
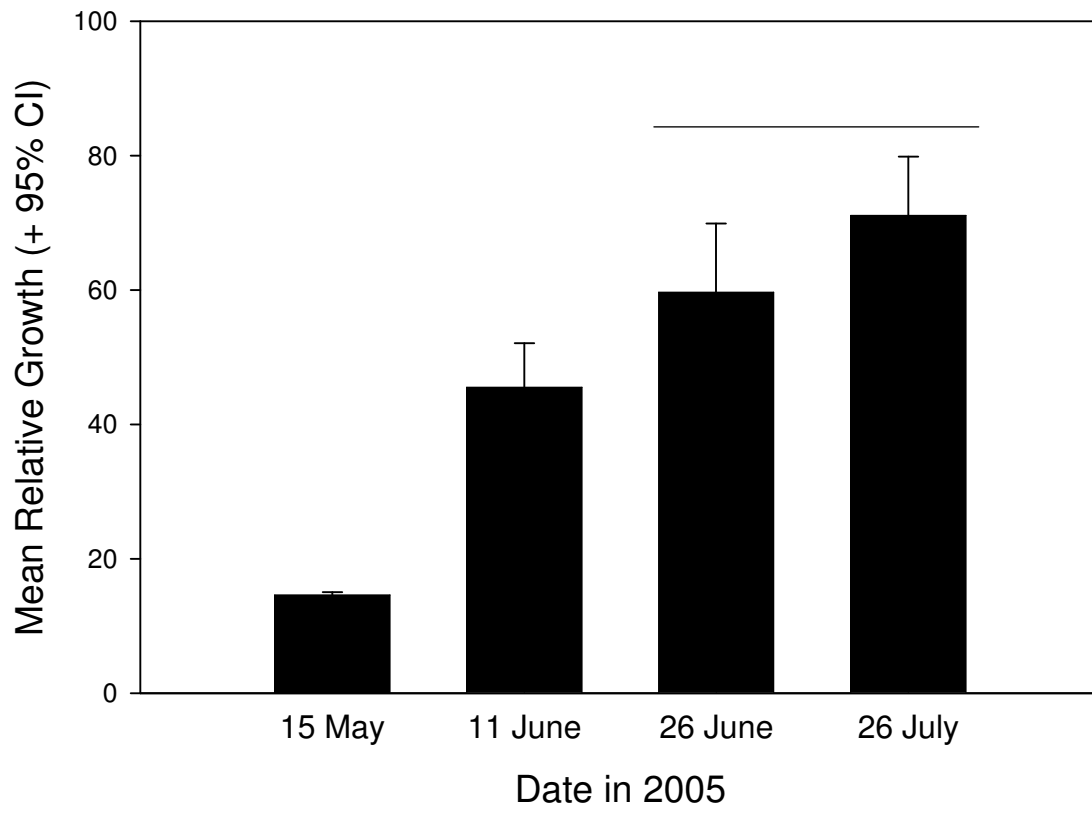
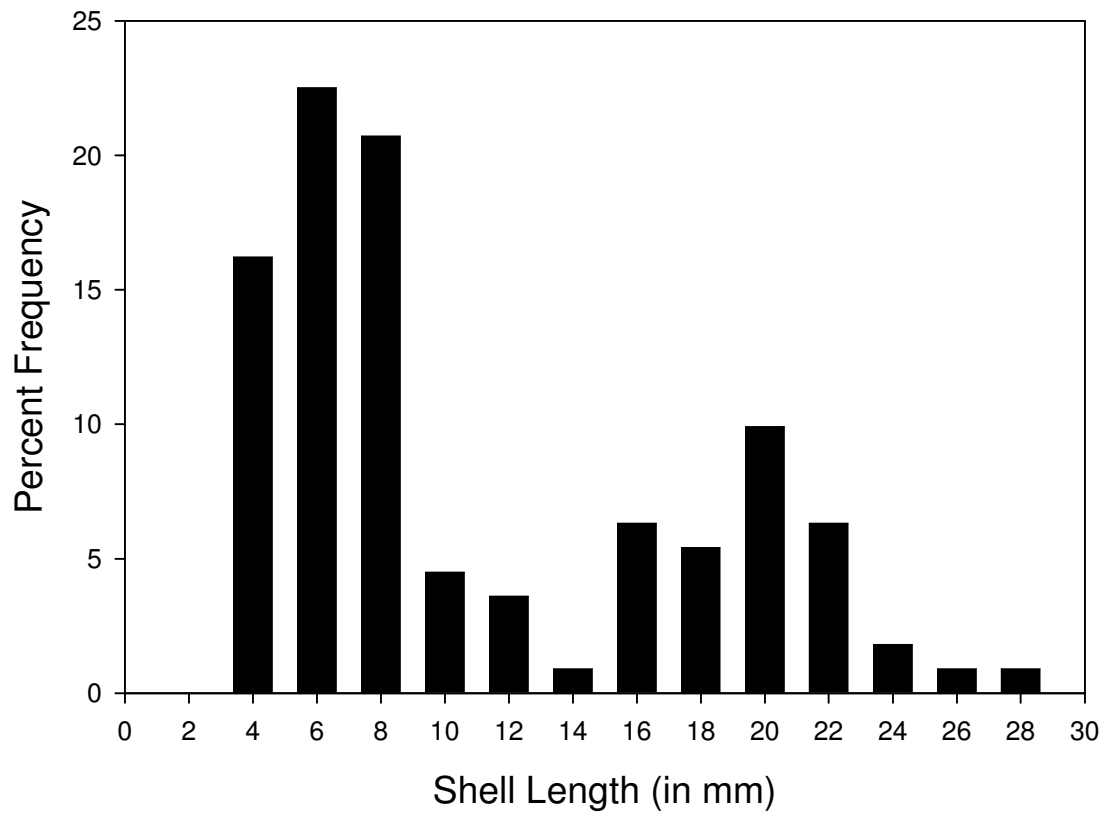
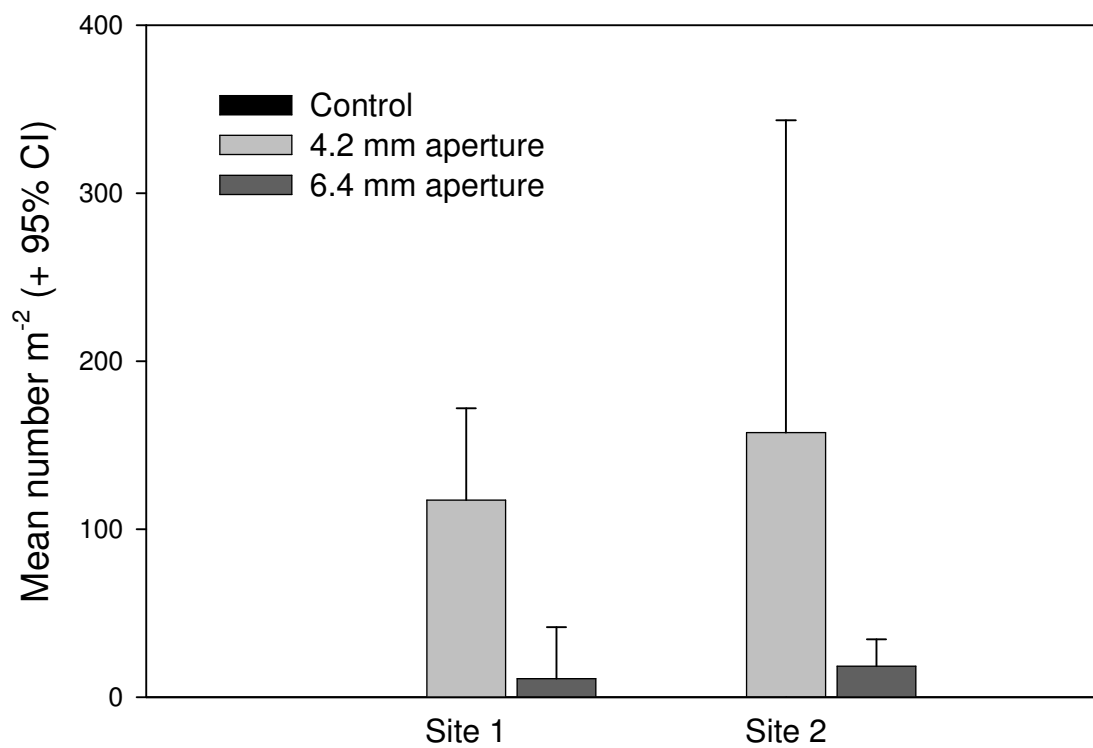
**Figure 7.**

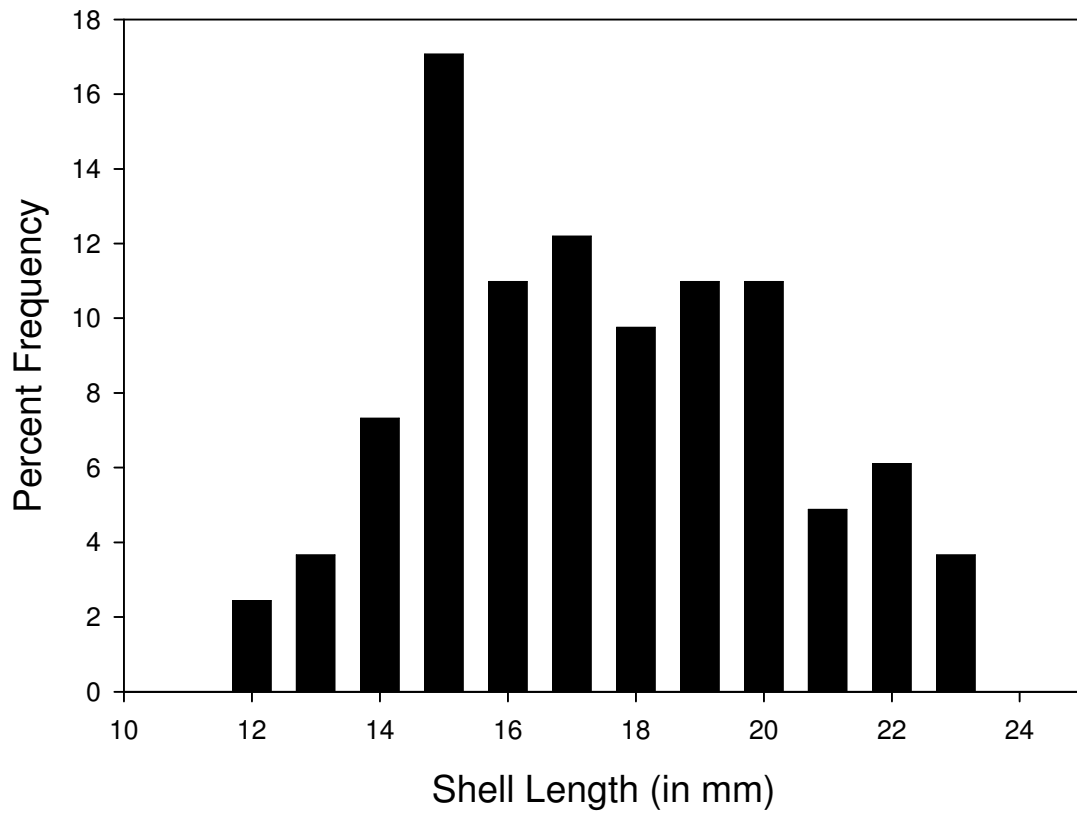
Figure 8.



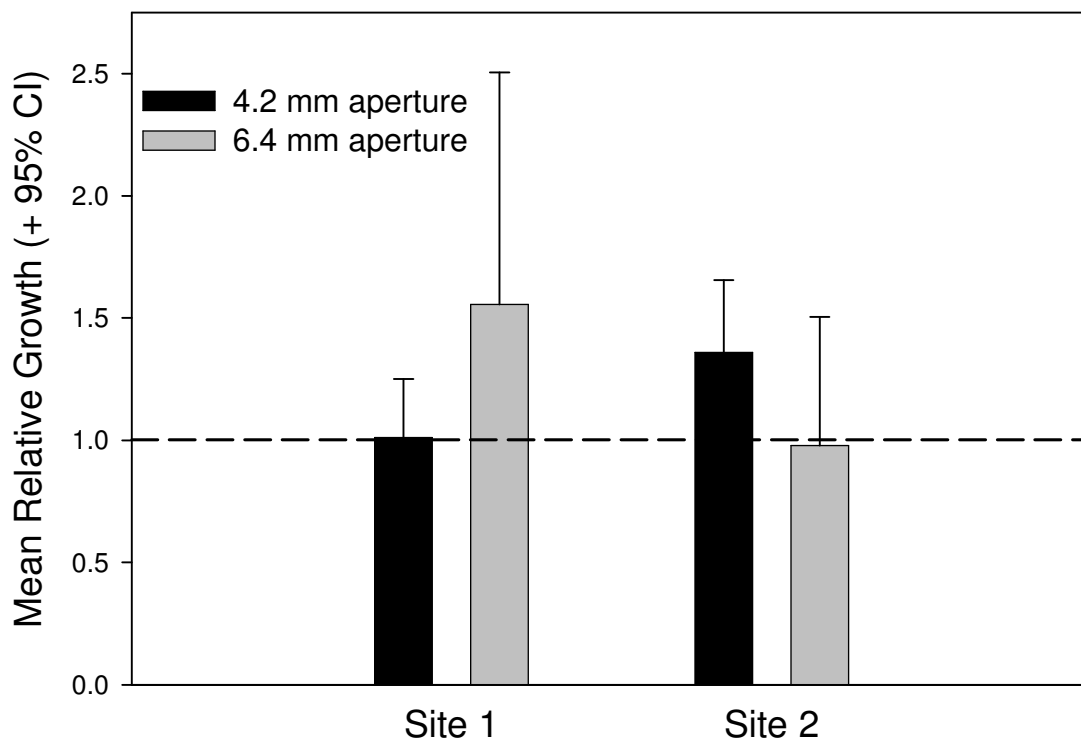
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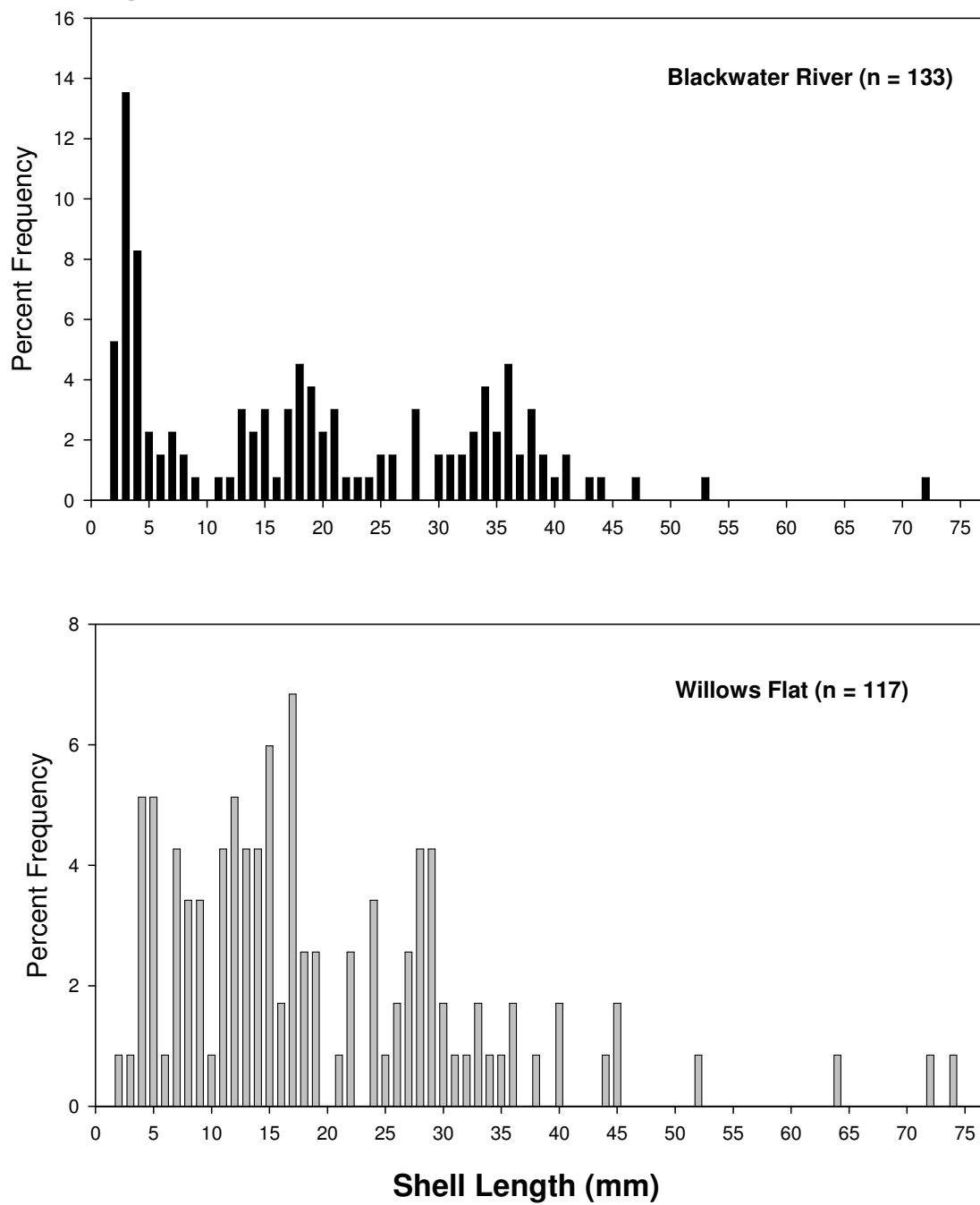
**Figure 10.**

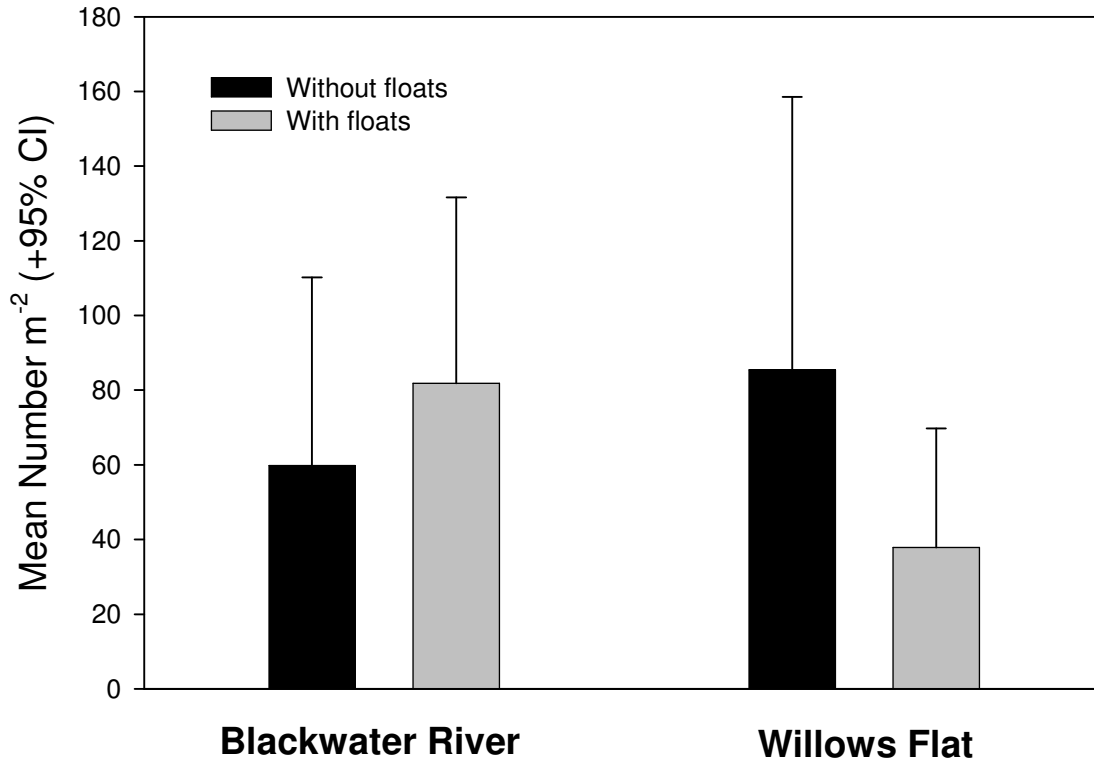
**Figure 11.**

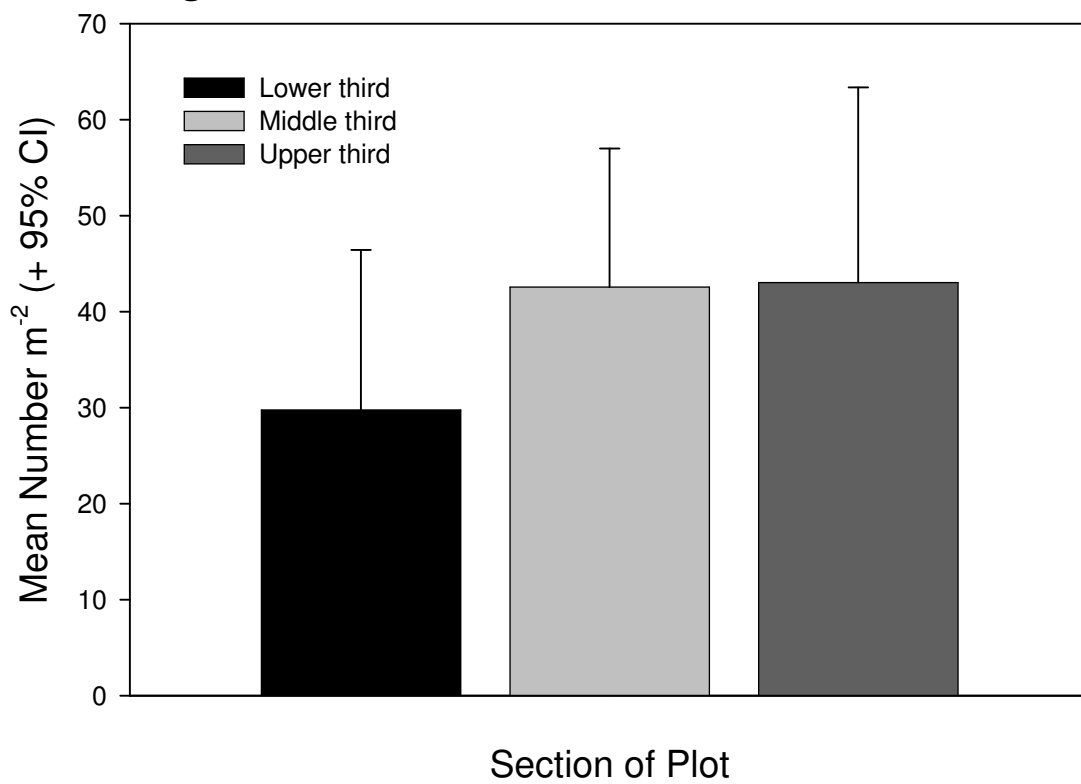
**Figure 12.**

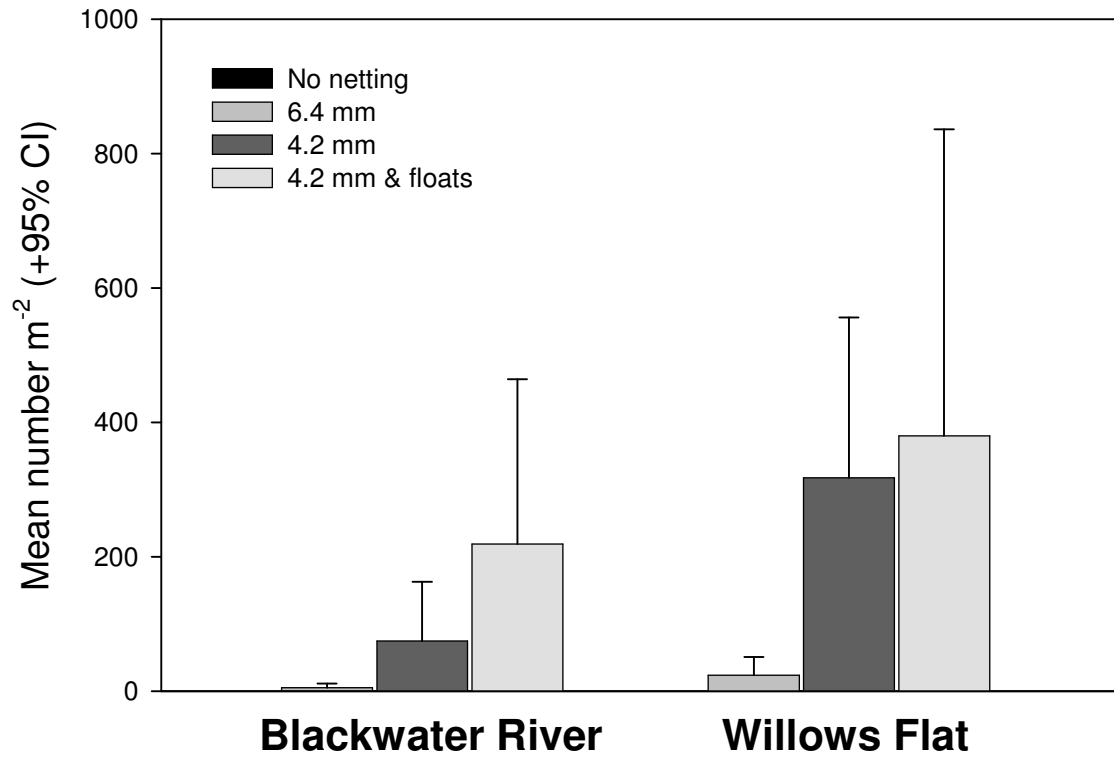


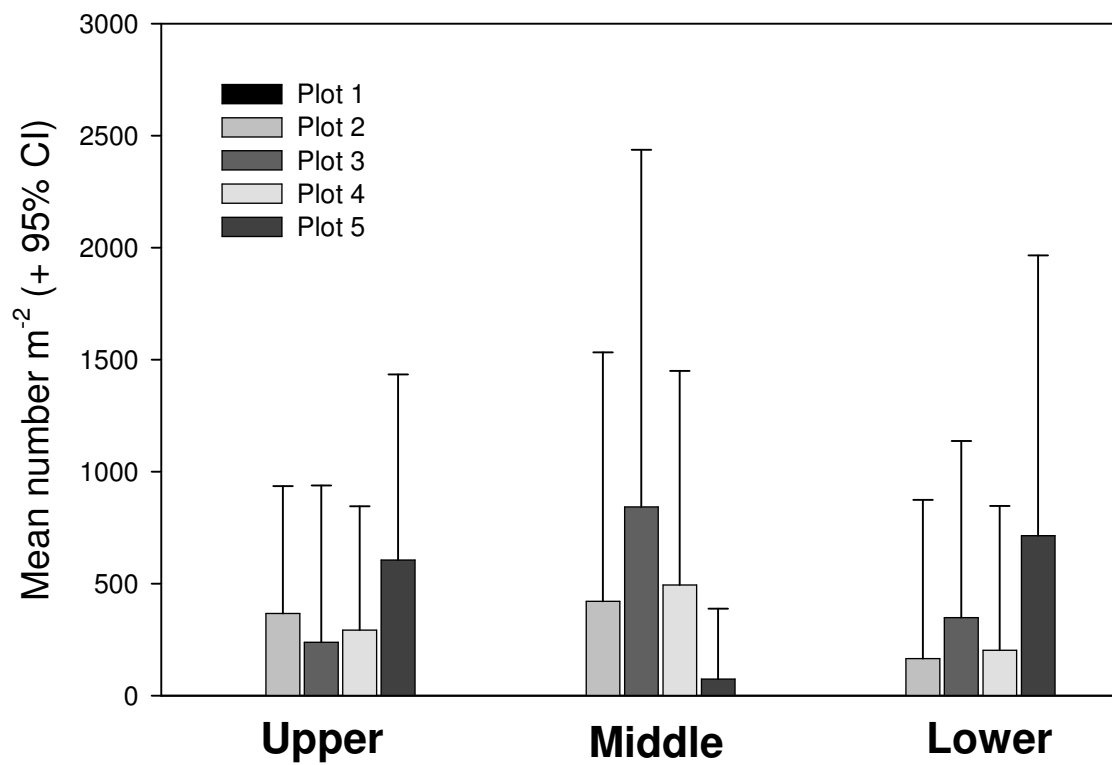
**Figure 13.**

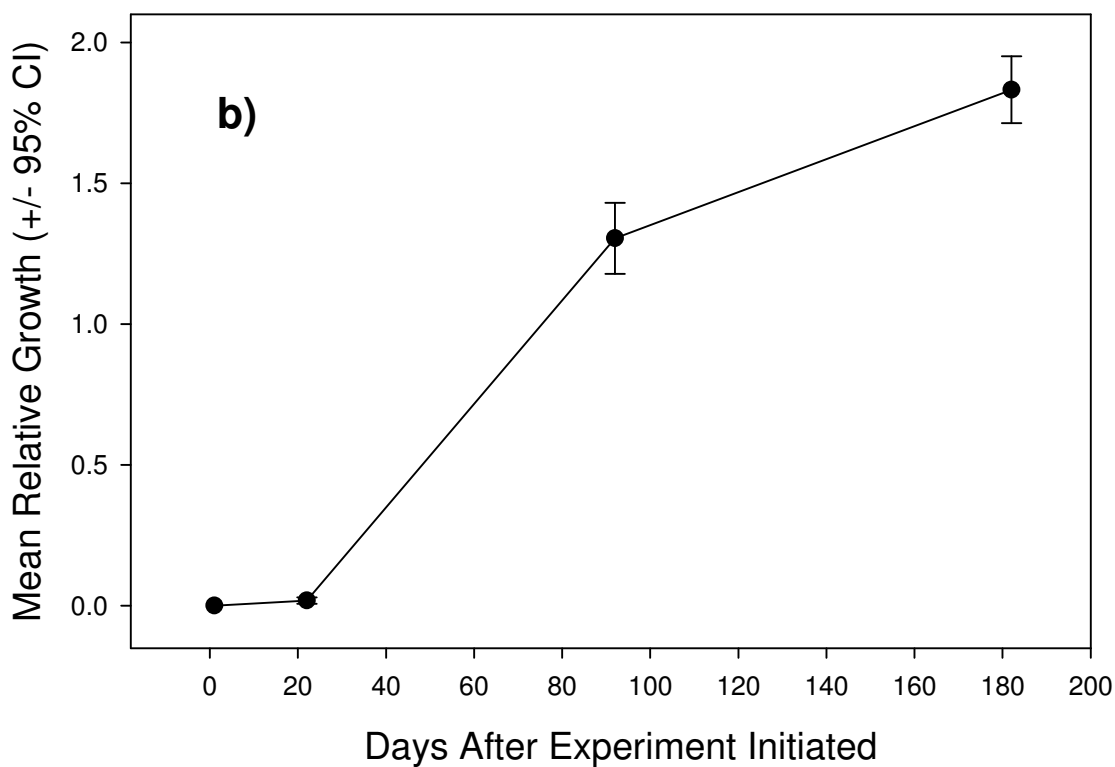
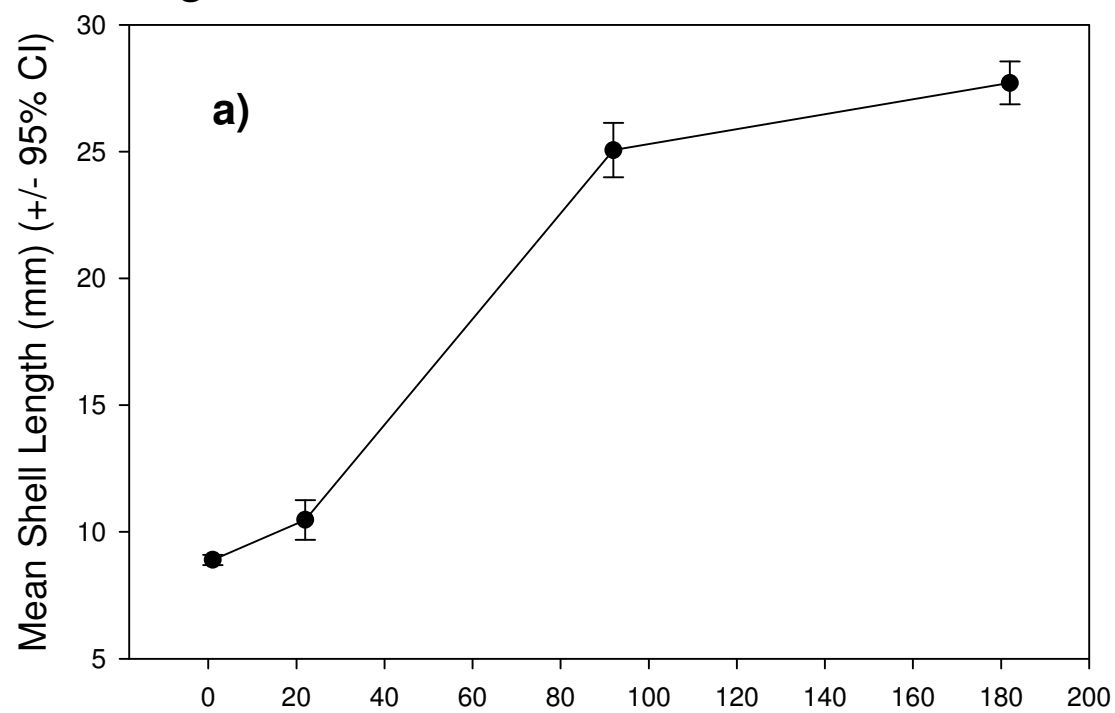
**Figure 14.**

**Figure 15.**

**Figure 16.**

**Figure 17.**

**Figure 18.**

**Figure 19.**

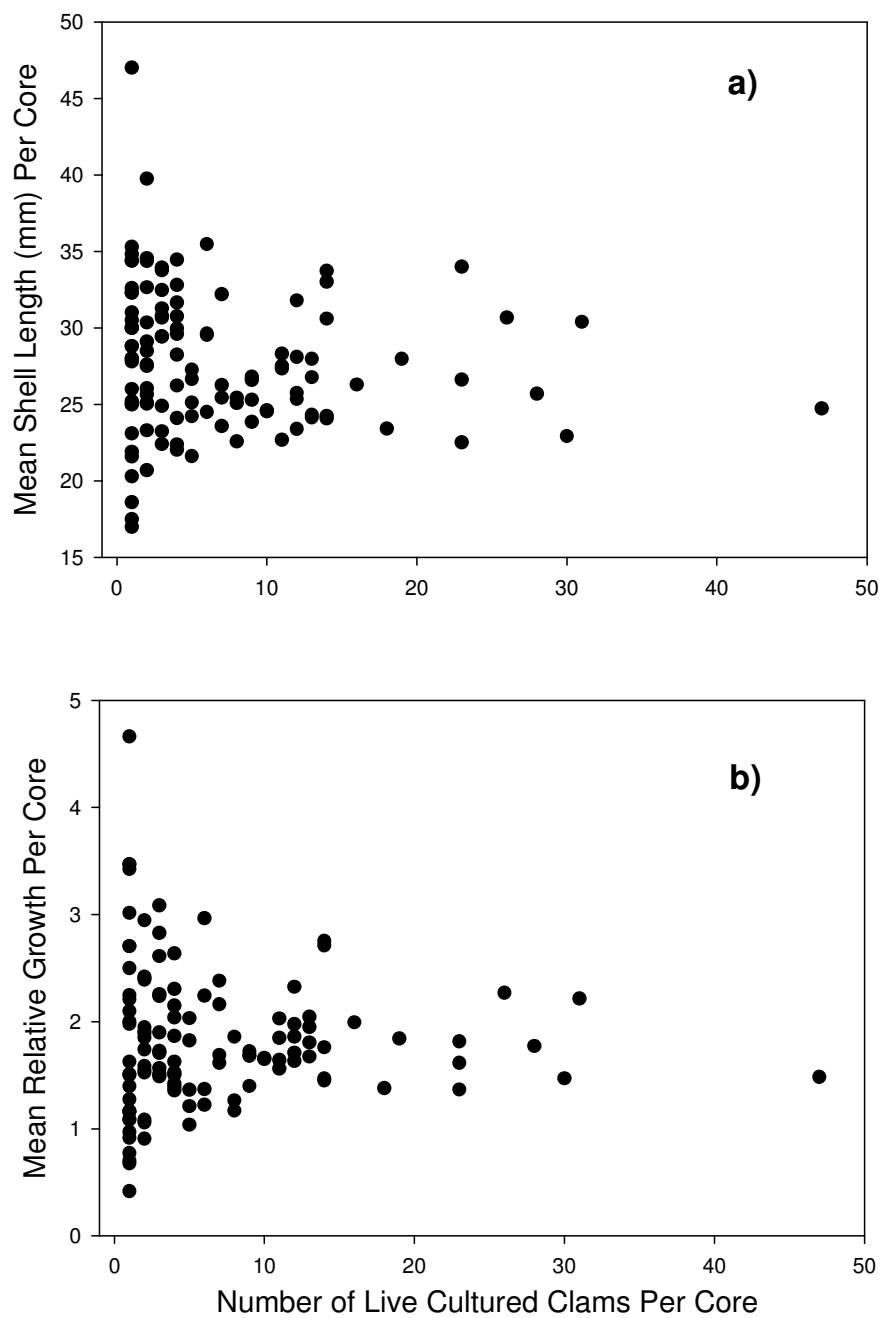
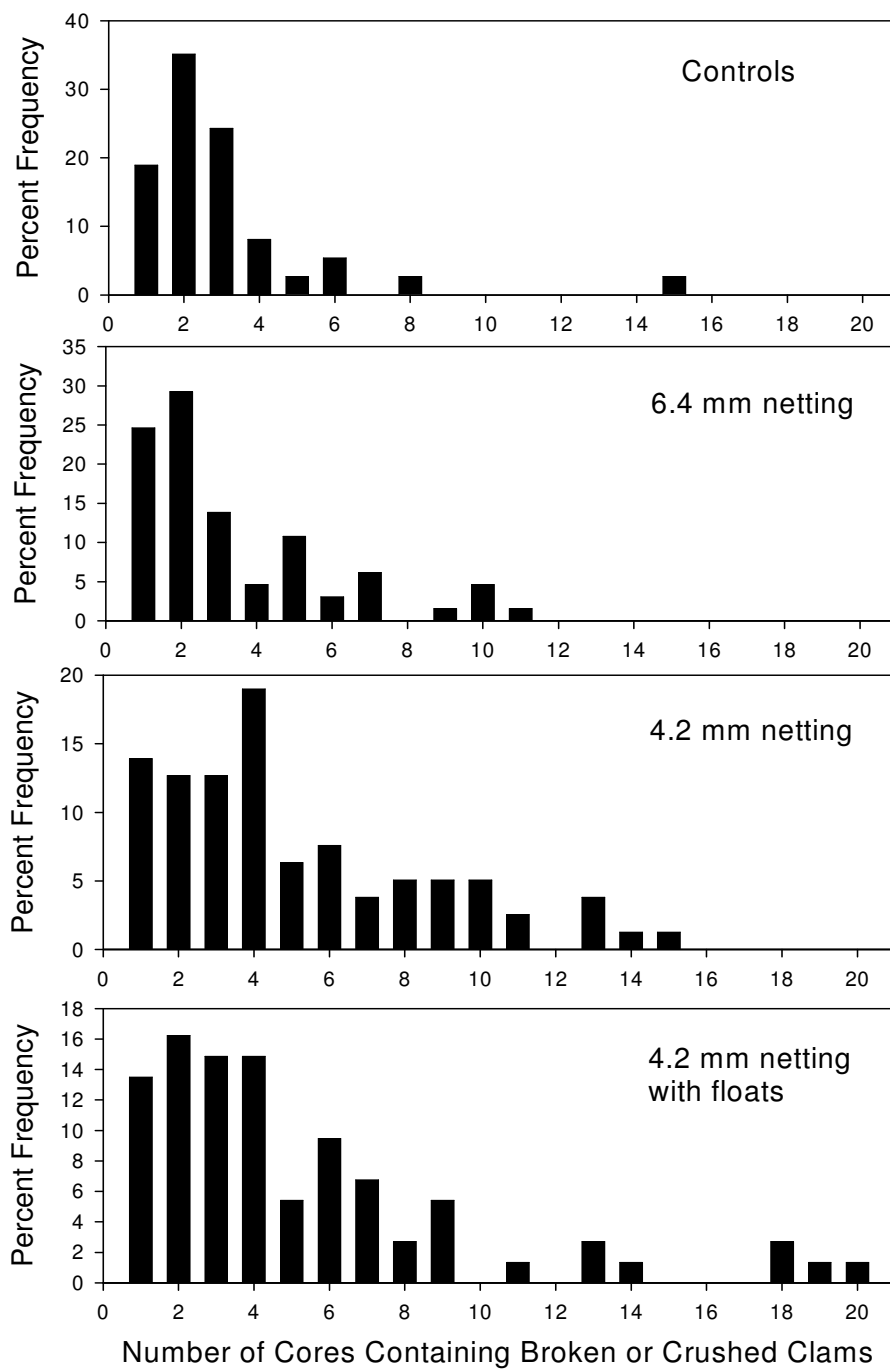
**Figure 20.**



Figure 21.



**Figure 22.**