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Juvenile Clam Mortality Study at Three Intertidal Flats in Hampton Harbor, New Hampshire

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Evaluating Factors Contributing to Mortalities of Juveniles of the Soft-shell Clam (*Mya arenaria* L.) in Hampton/Seabrook Harbor, New Hampshire

A Final Report to

The New Hampshire Estuaries Project

Submitted by

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New Hampshire Estuaries Project

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Executive Summary

Soft-shell clams, *Mya arenaria* L., represent an important recreational fishery along the New Hampshire coast. Intertidal flats in the Hampton-Seabrook Estuary are among the most heavily harvested. As recently as the fall of 1998, the sandy flats in this area supported more than 900 clammers, who, on weekends, easily harvested their 9.5-liter limit (Varney, 1999). Recently, however, quantitative benthic surveys have suggested that the abundance of adult clams (> 50 mm shell length, SL) has dwindled on the three estuarine flats (Brown's Flat, Common Island, and Middle Ground). Clammers support this contention and many have been disappointed at the relative paucity of harvestable clams and the effort required to obtain them. Surveys have shown that clams in the area are reproducing and that spat (< 25 mm SL) are abundant, but that populations of yearling clams (i.e., age 7-12 months and 26-50 mm SL) are very low. Staff at the New Hampshire Estuaries Project asked whether the limiting factor for a sustainable fishery could be poor juvenile survival.

Several factors may explain the lack of small clams along these shores. These include: 1) predation by crustaceans such as green crabs, boring gastropods, fish, or nemertean worms; 2) competition with conspecifics or other bivalves such as mussels; 3) poor recruitment or slow growth at upper intertidal levels; 4) disease such as hematopoietic neoplasia; 5) winterkill due to ice scour or excessive riverine run-off; 6) inorganic toxins such as heavy metals; 7) commercial or recreational shellfish harvesting.

Two manipulative field experiments were conducted at each of the three intertidal sites to address some of these factors. A generalized completely randomized block design was employed from November 2001 to March 2002 and from March 2002 to July 2002 to determine the influence of tidal height, stocking density, predator exclusion, and spatial variation on the growth and survival of cultured (hatchery-reared) juveniles of *Mya*. In addition, information on wild spat was collected. Clams (ca. 11 mm SL) were added to experimental units (plastic plant pots, 15 cm wide x 15 cm deep with a surface area of 0.0182 m^2) at two stocking densities (12 or 24 individuals per unit representing 660 or 1,320 individuals/m^2) at the upper and lower shore. Experimental units were either covered with a piece of flexible, plastic netting (aperture = 6.4 mm), completely open, or the periphery was rimmed with a small piece of netting that protruded 2 cm above the sediments in an attempt to discourage passive or active clam movement. Discrete blocks of units with replicated treatments were placed at 10 m intervals along the shore at both tidal heights. Samples of wild and cultured clams were inspected for hematopoietic neoplasia at the end of each experimental interval.

Results from the first experimental period suggest that winterkill due to ice and storms is minimal, but that clam losses due to physical scouring of the sediments and predators was relatively high. For example, survival in protected units at Middle Ground and Common Island varied from 65-78% even though netting sustained damage from birds and other

sources that enabled small crustaceans such as green crabs to prey on these supposedly "protected" populations. Clams recovered from both types of unprotected units (open and rimmed) was poor. For example, at Brown's Flat, less than 5% of animals initially added to unprotected units in November 2001 were recovered four months later in March 2002. Clam survival and numbers of wild spat were generally higher at Middle Ground than the other two sites. Clams grew less than 4 mm SL during the interval, which was significantly different from growth observed in the same population of cultured animals planted at a control site in eastern Maine, where growth was negligible over the same time interval.

Survival was generally lower at all three sites during the second experimental interval, but growth rates were nearly twice that observed during the first interval. Again, clam losses were caused mainly by predators and sediment scouring. Highest survival among protected units was 65% at the upper intertidal at Middle Ground and resulted in a 33% enhancement of survival. Conversely, netting did not enhance survival at the upper intertidal at Common Island where only 8% of animals were recovered from both protected and unprotected units.

The physical environment at each flat was rigorous as measured by the loss of experimental units during both experimental periods. For example, 32% of units (38 of 120) were lost at Brown's Flat over both experiments. The number of missing units increased from 22 (18%) to 46 (38%) at Common Island and from 14 (12%) to 8 (7%) at Middle Ground from the first to the second experiment. The frequency of missing experimental units explained 76.2% of the variation in mean percent survival of cultured animals across both experimental intervals. In addition, 42% of the variation in mean percent survival among units protected with netting could be explained by the rate of missing or damaged nets over both intervals.

Stocking density was unimportant in terms of attracting wild spat and had no effect on clam growth or survival. Similarly, no diseased organisms were sampled during either experimental interval. It appears that hematopoietic neoplasia does not occur in clams < 20 mm SL at these sites.

The results suggest several next steps that may help resource managers continue to explain clam losses and provide valuable information about stock enhancement. Among these are repeating portions of the current study with the most promising results. For example, the effect of netting is unambiguous in that its presence deters predators and enhances clam survival, even when nets become damaged. Nets can be used to protect wild and cultured seed and the size of the seeded areas as well as net aperture can be varied to provide information about the efficacy of larger-scale efforts. In addition, it may be possible to collect wild spat during the mid-fall using hydraulic pumps that fluidize the upper few centimeters of the sandy flats. If spat densities are as high as was observed at Middle Ground from November to March 2002 (ca. 280 individuals/m^2),

these animals could be protected in overwintering cages suspended in the water column beginning in November (see Beal et al., 1995) and then added to protected areas on the flats the following April. Other recommendations include increasing the scope of the present study to include months when seawater temperatures are at yearly peaks (i.e., August - September), determining the direct and indirect effects of recreational harvesting on wild or cultured juvenile populations, transplanting local or cosmopolitan stock (< 30 mm SL), and determining the effect of mussel beds on clam growth and survival. Clam growth and numbers of wild spat at Middle Ground were higher near populations of blue mussels that appear to decrease erosion and increase the deposition of more fine (muddy) sediments on that flat.

Although continued benthic sampling of clam populations in these areas is important, it is not possible to understand the mechanisms that control how clam abundance or size frequency distributions vary spatially and temporally using quantitative observations. Sampling enables us to develop models (theories, explanations) of how things work that can lead to hypotheses (predictions) that can be tested critically with careful, well-thought experimental studies designed to falsify various predictions (Underwood, 1991). I recommend that future investigations be experimental in nature and that managers continue working together with clammers and scientists to generate the most appropriate and sensible hypotheses to test.

Introduction

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

It is rare that adaptive management strategies and experimental approaches are considered by fisheries managers (but see Botsford et al., 1997; Lenihan and Micheli, 2000; Beal and Vencile, 2001); however, manipulative field experiments are the strongest and most efficient means available to managers to base decisions about the dynamics of a population (Underwood, 1990, 1991). Soft-shell clams, *Mya arenaria* L., represent an important recreational fishery along the New Hampshire coast, but specifically in the Hampton-Seabrook Estuary. During the Fall 1998, over 900 clammers easily harvested their 9.5-liter limit when one flat (Middle Ground) was opened after a 10-year hiatus due to fecal contamination (Varney, 1999). Since that time, clam abundance on that and two other flats in the same vicinity has dwindled. Recent surveys of these flats suggested to managers that the limiting factor for a sustainable fishery was poor juvenile survival (NHEP, 2001). Despite apparent successful reproduction and larval settlement, the population of yearling clams (i.e., age 7-12 months and 26-50 mm shell length) was very low (NHEP, 2001).

Several factors may help to explain the paucity of small clams along these shores. These include: 1) predation by crustaceans such as the invasive green crab (*Carcinus maenas* L.) or native rock crab (*Cancer irroratus* Say), boring gastropods, fish, or nemerteans (Beal et al., 2001), 2) competition for food or space with conspecifics or other bivalves such as mussels (*Mytilus edulis* L.), 3) poor recruitment or slow growth at various tidal heights (Beal and Fegley, 1996), 4) disease (e.g., Brousseau and Baglivo, 1991), 5) winterkill due to ice scour or sea birds (Beal et al., 1995), 6) inorganic toxins such as heavy metals (White and Robertson, 1996), or 7) commercial or recreational shellfish harvesting (Ambrose et al., 1998; Beal and Vencile, 2001).

A series of manipulative field experiments were conducted to examine factors that affect the mortality of juvenile soft-shell clams (< 25 mm shell length, SL). The study sites were within the Hampton-Seabrook Estuary and are the same as those that have been sampled at approximately yearly intervals since 1974 by Normandeau Associates, Inc. (Middle Ground, Common Island, and Brown's/Confluence Flat). That sampling has shown the density of small clams to vary significantly from flat-to-flat and from year-to-year, but that generally, samples taken at Middle Ground and Brown's/Confluence Flats yielded higher clam densities than those at Common Island (NAI, 2000). Historically, juvenile clam densities as high as 2,000 m^{-2} and as low as 0 m^{-2} have been reported on these flats. In 1999, however, densities of small clams at all flats combined was higher than historic means and was the third highest since 1990, when the Seabrook nuclear power plant became operational (NAI, 2000). At the same time, however, densities of larger clams (25-50 mm SL) was the lowest observed since 1990, "continuing a trend of decreasing density that began in 1996" (NAI, 2000). What happens to soft-shell clams during the time between settlement (summer and fall) and the following year was the question posed by the New Hampshire Estuaries Program. The effort described here examines the fate of small clams (8-13 mm SL) within Hampton-Seabrook Estuary as a function of: 1) site (three tidal flats); 2) tidal height (high vs. low); 3) predator deterrent

netting (present vs. absent); 4) stocking density (660 vs. 1,320 individuals/m^2); and, 5) time of year (November 2001 to March 2002 and March 2002 to July 2002).

Methods

Study sites

Three intertidal sand flats within the Hampton-Seabrook estuary (located at the southern end of the New Hampshire coast) were chosen based on previous work by Normandeau Associates (NAI, 2000) and the wishes of the New Hampshire Estuaries Program (NHEP, 2001). These flats were Middle Ground (42° 53' 22" N; 70° 49' 24 W), Common Island (42° 53' 45" N; 70° 49' 34 W), and Brown's Flat (42° 54' 03" N; 70° 49' 27 W). The latter flat is also known as Confluence Flat. The study sites are located near Hampton-Seabrook Harbor, which is characterized by a major tidal inlet, large shoals, and two major tributaries. The Hampton and Taylor Rivers lie to the north and the Blackwater River lies to the south. Historic aerial photographs show the estuarine shoals and tidal channels frequently shift position (Anon., 2002).

Benthic sampling

13 November 2001

Sampling was done at three spatial scales: 1) between flats within the estuary (100's of meters); 2) between blocks (spaced 10 meters apart); 3) within blocks (spaced 1 meter apart). Two benthic cores (surface area = 0.0182 m^2 to a depth of 9 cm) were taken 1 meter apart within each of fifteen 1-m^2 blocks separated by 10 m at each of three tidal heights (high, mid, low) at Middle Ground (northeastern shore) and Common Island (eastern shore) (Figure 1). The sites could be characterized typically as high-energy locations as indicated by sediment composition (sandy gravel) and the height of sediment ripples (ca. 2-4 cm). Samples were placed in labeled plastic bags and taken to the University of Maine at Machias (UMM) were they were washed through a series of sieves, the smallest being 0.5 mm. All clams were measured (shell length, greatest anterior-posterior measurement, SL) to the nearest 0.1 mm using vernier calipers.

24 November 2001

Sampling occurred at the same spatial scales as on 13 November; however, an additional site (Brown's Flat) was added. Two benthic cores (0.0182 m^2) were taken 1 m apart in six 1m^2 blocks at the high and two 1m^2 blocks at the low tide level at each site. The samples at each site were taken from relatively lower energy environments than samples taken on 13 November. At Middle Ground, high tide samples were taken near the middle of the flat to the south of a mussel bed. Low tide samples at Middle Ground were taken along the southeast shore. At Common Island, high tide samples were taken along the western shore near a bed of *Spartina* spp. Low tide samples at Common Island were taken along the southeastern shore. At Brown's Flat, high tide samples were taken near the western shore and low tide samples were taken along the eastern shore. Samples were taken to UMM and processed as above.

24 March 2002 & 13 July 2002

Two benthic cores (as described above) were taken haphazardly and adjacent to the six upper intertidal and two lower intertidal blocks (see **Manipulative Experiments** section) at each site. Number of samples per site was sixteen. Samples were processed as described above.

Manipulative Experiments

24 November 2001

Hatchery-reared clams (mean SL ± 95% CI = 10.8 ± 0.18 mm; n = 115; range = 8.5 to 13.5 mm; Figure 2a) originating from the Beals Island Regional Shellfish Hatchery (BIRSH; Beals, Maine; 44^o 31' 20" N; 67^o 36' 41" W) were added to plastic horticultural pots (15 cm diameter x 15 cm deep) at two densities (12 or 24 clams representing a stocking density of 660 or 1,320/m^2) within six or two arrays (3 x 5 matrix) of 15 pots at the high and low intertidal, respectively, at each of the three intertidal sites (Fig. 3). Arrays, or blocks, were located approximately 10 m apart. Pots (experimental units) in each array were buried so that a 0.5 cm lip extended above the sediment. Within a block,

three pots from each density treatment (n = 6) were covered with plastic netting (aperture = 6.4 mm; InterNet, Inc. N. Minneapolis, MN) to deter predators (green crabs, *Carcinus maenas* [L.], are abundant at these sites [NAI, 2001]). Netting was affixed around the periphery of each pot with two rubber bands. Another three replicates of each density were not covered with netting (n = 6). Finally, three replicate pots each received 12 clams (660/m^2), but a rim of netting was affixed to the circumference of each that extended above the sediment surface approximately 2 cm (as described in Beal et al., 2001). This netting was not designed to deter predators; rather, it was designed to retain clams that might otherwise migrate (sensu Baptist, 1955; Emerson and Grant, 1991) or be washed from the pots. This experimental design permits several comparisons: 1) between sites; 2) within sites; 3) between tidal heights within a site; 3) density (660 vs. 1,320/m^2); 4) predation (protected vs. unprotected experimental units); and, 5) clam movement (unprotected units: units with netting around circumference vs. units with no netting around circumference).

The experiment was concluded on 24 March 2002. Contents from each recovered experimental unit were added to labeled bags and transported to UMM where each was washed through a 0.5 mm mesh. The status of each experimental unit (plastic plant pot) and protective netting was recorded. Experimental units were either missing or present. Those present were placed into one of three categories: 1) full of sediment; 2) lacking 1/3-1/2 of the sediment; 3) partially eroded from the sediment and tipped on its side. The netting for the six units in each block was either present (whole or torn) or absent. It was possible to visually sort living and dead clams into two categories: hatchery-reared and wild. Cultured animals had a distinctive disturbance or check mark in both valves that coincided with clam size on the date each was placed in the field (Beal et al., 1999). All hatchery-reared clams recovered alive were measured twice: an initial SL characterized by the unique and distinct disturbance line and final SL. In addition, clams were recovered that had died during the experiment. These were placed into two categories: dead, with undamaged valves, that could be the result of disease, handling stress,

suffocation, or nemertean predation (Rowell and Woo, 1991; Beal and Vencile, 2001) and dead, with chipped or crushed valves typical of crustacean predation. For each live clam, relative growth was estimated using the following equation:

Relative growth (rg) = [(Final SL - Initial SL) / Initial SL] * 100%.

For example, an rg value of 100% represents a doubling in size, or 100% increase in SL, growth over a given time interval.

26 November 2001

Hatchery-reared clams (660/m^2; Fig. 2a) were added to protected pots (n = 6), unprotected pots with a rim of netting (n = 3), and unprotected pots without a rim of netting (n = 3) (as described above) in a 2 x 6 array at the upper intertidal of Duck Brook Flat, Cutler, Maine (44^o 41' 13" N; 67^o 18' 35" W). This alternate site was chosen to allow comparison of clam survival between the higher energy New Hampshire sites and a low-energy mudflat environment. Also, it was expected that the environmental conditions (especially air and seawater temperature as well as the occurrence of ice) at the eastern Maine site would be more severe than those at the New Hampshire site. This experiment was terminated on 30 March 2002. Samples were taken to UMM and processed as described above.

8 December 2002

I inspected each study site and noted the number of experimental units that were missing, the degree of sediment scour from the remaining units, and whether or not netting was present on remaining units.

23 March 2002

The same field design described above (24 November 2001) was initiated using clams (mean SL ± 95% CI = 10.9 ± 0.20 mm; n = 78; range = 9.5 to 14.5 mm from BIRSH

(Fig. 2b). The field test was concluded on 13 July 2002 and samples were treated as described above.

Disease Testing

Neoplasia is generally a lethal form of leukemia in soft-shell clams (Barber, 1996). Between November 2001 and July 2002, soft-shell clam juveniles (both cultured and wild) were tested for hematopoietic neoplasia at Micro Technologies, Inc., Richmond, Maine through examination of histological sections. Protocols for testing for the presence of diseased organisms are described in Thoesen (1994). Between 60-70 animals were transported from UMM to the laboratory on three dates (see below). Animals were stored at 5-10^{o}C prior to fixing in Bouin's solution. Sections from each individual were examined for the presence of abnormal/transformed hemocytes in connective tissue vascular spaces, particularly of the digestive organs (pers. comm., C. Giray, Micro Technologies, Inc., 10 July 2002; Quality Assurance Project Plan [QAPP], see Appendix).

27 November 2001

Cultured animals (0_{SL} = 10.8 mm) were examined from a group used in the manipulative field experiment.

28 March 2002

Three groups of animals were examined: 1) cultured animals (0_{SL} = 10.9 mm) that were from a group used in the manipulative experiment initiated on 23 March 2002; 2) cultured animals (0_{SL} = 14.4 mm) that survived the first experimental period (24 November 2001 to 24 March 2002) at sites within the Hampton-Seabrook estuary; 3) wild animals (0_{SL} = 8.9 mm) that were collected from experimental units on 24 March 2002 at sites within the Hampton-Seabrook estuary.

15 July 2002

Two groups of animals were examined: 1) cultured animals (0_{SL} = 18.1 mm) that survived the second experimental interval (23 March 2002 to 13 July 2002); 2) wild animals (0_{SL} = 14.5 mm) that were collected from experimental units on 13 July 2002 at sites within the Hampton-Seabrook estuary.

Statistical Analyses

Analysis of variance (ANOVA) was used to determine whether means (numbers per core, percent survival of cultured clams per experimental unit, rg of cultured clams per experimental unit, number of wild clams per experimental unit) differed by treatment.

I used the following linear model for the benthic core data:

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

Where:

Y_{ijkl} = square root-transformed mean number of clams per core;

μ = theoretical mean;

A_i = Site (Brown's Flat; Common Island; Middle Ground) (a fixed factor);

B_j = Tidal Height (Upper vs. Lower) (a fixed factor);

C_k = Block (a random factor); and,

$e_{l(ijk)}$ = experimental error, a measure of variation that exists among observations on experimental units treated alike.

The following linear model was used for the experimental data for each site:

$Y_{ijkl} = \mu + A_i + B_j + AB_{ij} + C(B)_{k(j)} + AC(B)_{ik(j)} + D(A)_{l(i)} + BD(A)_{jl(i)} + CD(AB)_{kl(ij)} + e_{l(ijk)}$

Where:

Y_{ijkl} = arcsine square root-transformed mean percent survival, untransformed relative growth, square root-transformed mean number of wild clams (< 25 mm) per experimental unit;

μ = theoretical mean;

A_i = Tidal Height (upper vs. lower) (fixed factor);

B_j = Density (12 vs. 24 clams per unit) (fixed factor);

C_k = Treatment (Unprotected vs. Open with Rim vs. Netting) (fixed factor);

D_l = Block (random factor); and,

$e_{l(ijk)}$ = experimental error.

I excluded from all statistical analyses experimental units that were missing and used a type I error rate (a) of 0.05 as the decision rule for all hypothesis tests. Three non-orthogonal, a priori contrasts associated with the Treatment(Density) (i.e., $C(B)_{k(j)}$) source of variation were examined: 1) density = 12 and open vs. rimmed units; 2) density = 12 and open vs. netted units; 3) density = 24 and open vs. netted units. The first contrast examines the effect of adding a mesh rim around the circumference of the experimental units. The rim of netting is designed to reduce the number of clams that are washed away or migrating from the units. If this contrast is not statistically significant, then it implies that the rim had no effect on keeping clams within the experimental units. The second and third contrast examines predator effects at each stocking density. If these contrasts are not statistically significant, then the implication is that netting had little effect on deterring predators. In some instances, a significant Block(Tidal Height) (i.e., $D(A)_{l(i)}$) source of variation occurred. I decomposed the six degrees of freedom into two contrasts with five degrees of freedom associated with the upper intertidal blocks and one degree of freedom for the lower intertidal blocks. I adjusted a for each a priori contrast using $a' = 1 - (1 - a)^{1/r}$ where r = number of contrasts (Winer et al., 1991). In all cases, the a'-value for the three contrasts is 0.0169 and for two contrasts is 0.0253.

Means, presented graphically or in the text, represent untransformed data ± 95% confidence intervals.

Results

Benthic sampling

13 November 2001

Seven clams were sampled from the 90 benthic cores at Common Island and five from Middle Ground. Clams ranged in size from 3.4 to 18.6 mm SL. No clams were found in samples taken at the mid and low tide levels at Middle Ground. Mean density (± 95% confidence interval) at the high tide level at Middle Ground was 9.15 ± 9.5 clams/m^2 (n = 30). Clams were found in samples at all three tidal heights at Common Island (0_{high} = 3.7 ± 5.2/m^2; 0_{mid} = 1.8 ± 3.8/ m^2; 0_{low} = 7.3 ± 7.1/ m^2; n = 30; Figure 4). At Middle Ground, the difference in mean density between tidal heights was statistically significant (P = 0.012), but there was no significant block-to-block variation within a tidal height (P = 0.775; Table 1a). At Common Island, neither source of variation was statistically significant (Table 1b).

24 November 2001

Forty-one clams were sampled from the sixteen cores taken at each of the three sites ($n_{Middle\ Ground}$ = 18; $n_{Common\ Island}$ = 14; $n_{Brown's\ Flat}$ = 9. None of the four sources of variation (dependent variable = number per core) was significant (Table 2). Mean density (± 95 % CI) was 46.9 ± 20.6 individuals/m^2. Mean SL (5.2 ± 1.4 mm; n = 41) did not vary significantly between site, tidal height, or the interaction of site and tidal height (P > 0.05).

24 March 2002

Fifty-four clams were sampled from the sixteen cores taken at each of the three sites (Middle Ground = 35 [0_{SL} = 8.7 ± 1.84 mm; minimum = 3.6 mm, maximum = 23.6 mm]; Common Island = 11 [0_{SL} = 8.2 ± 3.11 mm; minimum = 3.9 mm, maximum = 19.7 mm]; Brown's Flat = 8 [(0_{SL} = 5.8 ± 2.1 mm; minimum = 3.4 mm, maximum = 10.6 mm]). Mean density (± 95 % CI) was 61.8 ± 27.2 individuals/m^2. ANOVA (Table 3a) detected

no significant difference ($P > 0.05$) in mean number per core between sites, tidal heights, or the interaction of these two main factors. Significant block-to-block variability occurred, but this was due to one site and tidal height (Middle Ground, upper intertidal; $F = 5.60$, $P = 0.0015$, df = 5, 24). There was no difference in mean SL between sites ($P = 0.2923$), tidal heights (0.6267) or the other two higher-order interaction terms (Table 3b).

13 July 2002

A total of 24 clams was sampled from the sixteen cores (27.5 ± 18.7 individuals/m^2) taken at each of the three sites (Middle Ground = 22 [0_{SL} = 17.9 ± 3.91 mm; minimum = 6.6 mm, maximum = 39.9 mm]; Common Island = 2 [0_{SL} = 11.3 ± 18.37 mm; minimum = 9.8 mm, maximum = 12.7 mm]; Brown's Flat = 0). Clams were found only in the upper intertidal blocks at Middle Ground and lower intertidal blocks at Common Island; however, ANOVA was unable to detect difference in mean number between sites or tidal heights (Table 4.) Significant block-to-block variability was due to the upper intertidal samples at Middle Ground where clams were sampled from only three of the six blocks ($F = 42.61$, $P < 0.0001$, df = 5, 24). There was no significant difference in mean SL for any source of variation.

Manipulative Field Experiment

8 December 2001

Many (ca. 25%) of the pots at each site and tidal height showed extensive erosion. Two pots at Common Island and four pots at Brown's Flat were completely eroded and were removed from the flat. A small number of pots at each site were completely covered with sediment and it did not appear that the degree of erosion or sedimentation was related to whether pots were covered with netting or had a rim of netting around the circumference. All nets were in place, however, a large percent (especially at Brown's Flat= 45%) had been ripped or torn and the type of damage was similar to what I have observed on other

sandy flats in Maine when seagulls, *Larus* spp., are abundant and peck their beaks through the netting to prey on clams.

24 November 2001 - 24 March 2002

Brown's Flat – Condition of experimental units

Approximately 28% (n = 25) and 43% (n = 13) of the experimental units were missing from the upper and lower intertidal, respectively (Table 5). Of those units remaining in the sediments at the upper intertidal location during the experiment, nearly 34% (22 of 65) showed some degree of sediment scour. In many cases, scour had removed 25-40% of the sediment. Similar scouring (35%, or 6 of 17 units) occurred within the lower intertidal blocks. Finally, I observed sea gulls, *Larus* spp., attempting to prey on clams in protected units while setting up the experiment. Apparently, they were able to force their beaks through the netting of some protected units and prey on clams. They also preyed on clams in unprotected units. No attempt was made to quantify their predation rate; however, it was possible to count the number of nets that had been damaged (torn, ripped, etc.) at the end of the experiment. In the upper intertidal blocks, 25 of 27 (93%) nets protecting clams in experimental units had been damaged. In some cases, the torn netting had allowed small green crabs to enter the units. All nets protecting clams in the lower intertidal blocks had been damaged.

Brown's Flat – Survival of cultured animals

Only 21.3 ± 6.6% (n = 65) and 9.1 ± 9.1% (n = 17) of clams were recovered from experimental units at the upper and lower intertidal sites, respectively, at the end of the experiment (P = 0.1073; Table 6). In spite of damage to plastic netting caused by birds or other sources, significantly more clams survived in units that received netting than those that did not (Table 6; Fig. 5). This enhancement, pooled across densities (P = 0.2982; Table 6) and tidal heights, averaged 36.4% when mean percent survival in protected units (40.7 ± 9.5%, n = 33) was compared to the mean of open and rimmed units (3.6 ± 2.7%, n = 32 and 4.9 ± 5.5%, n = 17, respectively). The experimental units

with rims to discourage clams from emigrating, apparently failed to do so (P = 0.9984; Table 6).

Brown's Flat – Growth of cultured animals

Live clams were found in 46 of the 82 remaining experimental units (56.1%). Clams grew to a mean SL of 13.2 ± 0.43 mm (rg = 14.1 ± 3.32%), representing an average increase of 2.4 mm over the four-month period (Fig. 6a). It was not possible to determine when this growth occurred, however. None of the fixed factors (tidal height, intraspecific density, and predator exclusion) explained a significant amount of the variation in growth during the experiment (Table 7).

Brown's Flat – Number of wild clams within experimental units

A total of 41 wild clams (< 25 mm SL) was found in the 82 experimental units that remained on the flat until the end of the experiment (0 = 7.5 ± 1.01 mm; SL range = 3.8 - 15.8 mm; Figure 7a). None of the eight sources of variation associated with mean number of wild clams per experimental unit was significant (Table 8). Mean number pooled across all four main sources of variation (tidal height, density, predator exclusion, and blocks) was 27.4 ± 9.126 individuals/m^2.

Common Island – Condition of experimental units

Approximately 19% (17 of 90) and 17% (5 of 30) of the experimental units were missing from the upper and lower intertidal, respectively (Table 5). Of those units remaining in the sediments at the upper intertidal location during the experiment, approximately 25% (18 of 73) showed some degree of sediment scour. In most instances, scouring had removed no more than 25% of the sediment. Similar scouring (24%, or 6 of 25 units) occurred within the lower intertidal blocks. Nearly 63% of the protected units at the upper intertidal location at the end of the experiment had nets that were damaged whereas 66.7% were damaged at the lower intertidal location.

Common Island – Survival of cultured animals

Mean clam survival did not differ between tidal heights (0_{upper} = 40.9 ± 7.1%, n = 73; 0_{lower} = 48.2 ± 14.4%, n = 25; P = 0.1315; Table 9) or stocking densities ($0_{660/m}{}^2$ = 43.1 ± 8.53%, n = 60; $0_{1320/m}{}^2$ = 42.4 ± 10.0%, n = 38; P = 0.8684; Table 9). However, the addition of plastic netting to deter predators enhanced percent survival significantly (P< 0.001; Table 9; Fig. 8). At the lower stocking density, mean survival increased from 15.6 ± 10.3% (n = 15) in unprotected units to 73.1 ± 8.8% (n = 22) in units with protective netting. A similar increase was observed at the higher stocking density (12.2 ± 7.9%, n = 16 vs. 64.4 ± 7.4%, n = 22). There was nearly a 17% difference in mean survival between unprotected units and those with a rim of netting around their circumference (32.245 ± 11.9%) (Fig. 8), but ANOVA was not powerful enough to detect a significant difference for this comparison (Table 9).

Common Island – Growth of cultured animals

Live clams were found in 85 of the 98 remaining experimental units (86.7%). Clams increased in SL an average of 1.9 mm (12.7 ± 0.24 mm; Fig. 6b; rg = 11.4 ± 20.5%); however, tidal height differences in rg were significant (P < 0.0001; Table 10). For example, clams attained a mean SL of 12.3 ± 0.23 mm (n = 63) (rg = 7.7 ± 1.59%) at the upper intertidal location compared to 13.8 ± 0.41 mm (n = 22) (rg = 21.9 ± 4.1%) at the lower intertidal. In addition, animals at the lower stocking density appeared to grow more slowly in units protected with netting than in unprotected units (Table 10; Fig. 9). This may due to a "hidden tidal height effect." That is, the comparison was biased due to a disproportionate number of protected experimental units at the upper intertidal where clams grew more slowly. For example, the number of protected and unprotected units from the upper intertidal location was 16 and 6, respectively, whereas the number of protected and unprotected units from the lower intertidal location was 8 and 2, respectively. In addition, the effect of density and treatment varied between blocks

within a given tidal height making comparison of main effects even more ambiguous (Table 10).

Common Island – Number of wild clams within experimental units

A total of 128 wild clams (< 25 mm SL) was found in the 98 experimental units that remained on the flat until the end of the experiment (0 = 7.5 ± 0.49 mm; SL range = 2.9 - 17.6 mm; Figure 7b). None of the eight sources of variation associated with mean number of wild clams per experimental unit was significant (Table 11). Mean number pooled across all four main sources of variation (tidal height, density, predator exclusion, and blocks) was 71.6 ± 18.9 individuals/m^2.

Middle Ground – Condition of experimental units

Exactly 10% (9 of 90) and 17% (5 of 30) of the experimental units were missing from the upper and lower intertidal, respectively (Table 5). Of those units remaining in the sediments at the upper intertidal location during the experiment, nearly one-half (39 of 81) showed some degree of sediment scour. In the lower intertidal, 28% (7 of 25 units) were scoured. In most instances, scouring had removed no more than 25% of the sediment. Damage to nets from birds and other sources was lower at Middle Ground than either Brown's Flat or Common Island (7 of 36, or 19.4% at the upper intertidal; 6 of 25, or 24% at the lower intertidal).

Middle Ground – Survival of cultured animals

Mean percent survival was significantly higher at the upper (55.1 ± 6.4%, n = 81) than at the lower intertidal location (41.7 ± 14.0%, n = 25; P = 0.0312; Table 12); however, the effect of the treatments (open vs. rimmed vs. protected units) differed between tidal heights (P = 0.0236; Table 12; Fig. 10). In every combination of stocking density and tidal height, clams protected from predators with netting survived better than those without. Figure 10 demonstrates that the enhancement effect of the netting was greater at

the lower intertidal location than at the upper intertidal one (Upper: $0_{protected}$ = 77.7 ± 4.8%, n = 36, vs. $0_{unprotected}$ = 37.1 ± 7.5%, n = 45; Lower: $0_{protected}$ = 75.0 ± 4.7%, n = 11, vs. $0_{unprotected}$ = 15.5 ± 11.3%, n = 14). In addition, there was a significant difference in clam survival between unprotected (20.2 ± 8.1%, n = 14) and rimmed (53.7 ± 10.2%, n = 18) units stocked with 660 individuals/m^2 at the upper intertidal, but not at the lower intertidal where mean survival was only 13.5% in both treatments.

Middle Ground – Growth of cultured animals

Live clams were found in 97 of the 106 remaining experimental units (91.5%). Mean SL was 14.4 ± 0.25 mm (Figure 6c; rg = 26.5 ± 2.28%), representing an increase of approximately 3.6 mm during the experiment. Neither tidal height, stocking density, nor predator exclusion had a significant effect on growth; however, there was significant spatial variation from block-to-block within the upper intertidal location (Table 13; Fig. 11). No attempt was made to quantify potential differences among the blocks. The only qualitative difference between blocks I-III and IV-VI (Fig. 11) was that sediments in the latter blocks were perceptibly muddier than the former. In addition, there was some variation in tidal height among the upper intertidal blocks with blocks IV-VI noticeably lower than the others. This difference in tidal height may have enabled clams to feed longer in blocks IV-VI compared to those in blocks I-III.

Middle Ground – Number of wild clams within experimental units

A total of 504 wild clams (< 25 mm SL) was found in the 106 experimental units that remained on the flat until the end of the experiment (0 = 8.4 ± 0.26 mm; SL range = 3.3 - 20.3 mm; Figure 7c). Neither tidal height nor stocking density affected mean number of wild clams (Table 14); however, open units contained significantly more wild clams at the end of the experiment than units with rims (303.1 ± 163.4 individuals/m^2 vs. 171.6 ± 81.9 individuals/m^2; Table 14; Fig. 12). Netting appeared to enhance numbers of wild clams at the lower intertidal location compared with unprotected units, but this same

effect was not observed at the upper intertidal (Fig. 12). In addition, there was a highly significant difference in wild clam numbers between blocks in the upper intertidal ($P < 0.0001$; Table 14; Fig. 13). Although number of wild clams varied by a factor of approximately 1.75 between blocks in the lower intertidal, the variances were too large to detect a significant difference ($P = 0.0666$; Table 14).

Cutler, Maine (26 November 2001 - 30 March 2002)

Seven of the 12 experimental units were recovered at the end of the experiment (netted units = 3; open units = 3; rimmed units = 1). No treatment effect was observed ($P = 0.1580$) and mean percent survival was 59.5 ± 27.94%). Several units were found adjacent to the upper intertidal site that had been tipped over and sediments removed. This movement was likely due to ice that formed in late January in the area and persisted until late February. No shell growth was detected and no other clams besides cultured animals were found.

23 March - 13 July 2002

Brown's Flat – Condition of experimental units

Approximately 36% (n = 32) and 20% (n = 6) of the experimental units were missing from the upper and lower intertidal, respectively (Table 15). Of those units remaining in the sediments at the upper intertidal location during the experiment, nearly 17% (10 of 58) showed some degree of sediment scour. In most cases, scouring was not as severe as observed during the first experimental interval. At most, scouring had removed 25% of the sediment in some experimental units. Scouring was more severe in the lower intertidal blocks (37.5%, or 9 of 24 remaining units) as more than 50% of the sediment was missing in some units. Although sea gulls, *Larus* spp., were observed attempting to prey on clams in protected units while initiating the experiment, the number of gulls was much less than the number observed in November 2001 (ca. 10-20 vs. 30-40). Of the 22 units protected with netting in the upper intertidal blocks, five were missing (23%) and 12 were ripped or torn (55%). In the lower intertidal blocks, nine of 12 (75%) nets

protecting clams in experimental units had been damaged. In addition, five of the remaining 12 (42%) units rimmed with netting to decrease emigration were missing.

Brown's Flat – Survival of cultured animals

Mean survival was relatively low at both tidal heights; however, survival was significantly lower in the upper (8.6 ± 3.6%, n = 58) vs. lower (20.3 ± 9.5%, n = 24) intertidal blocks (P = 0.0079; Table 16). Although the main effect due to density was not significant, there was a significant tidal height x density interaction (Table 16). No effect due to density was observed at the upper intertidal location, but significantly more animals survived that were initially stocked at 1,320 compared to 660/m^2 at the lower intertidal (Fig. 14). The enhancement was approximately 20%. In addition, there was a significant enhancement due to the presence of netting (Fig. 15). At the low stocking density, clams in protected units had a 22.2% higher survival rate than animals in unprotected units. A similar result (20.8%) occurred in the high-density treatments.

Brown's Flat – Growth of cultured animals

Live clams were found in only 42 of the 82 (51.2%) experimental units remaining at the end of the experiment. Animals grew to a mean SL of 20.1 ± 1.14 mm (rg = 69.7 ± 9.3%; Fig. 16a); however, relative growth and mean SL varied significantly from the upper to lower tidal levels (P = 0.0002; Table 17). Clams attained a mean SL of 19.1 ± 1.55 mm (n = 27) and 21.8 ± 1.09 mm (n = 15) in the upper and lower intertidal units, respectively.

Brown's Flat – Number of wild clams within experimental units

Eighteen wild clams (< 30 mm SL) were found in the 82 experimental units that remained on the flat until the end of the experiment (0 = 15.8 ± 2.24 mm; SL range = 7.9-25.2 mm; Figure 17a). Significantly more clams were found in lower than upper intertidal units (25.1 ± 15.23 individuals/m^2, n = 24 vs. 6.6 ± 4.74 individuals/m^2, n = 58; P = 0.0013; Table 18). The Treatment(Density) source of variation was significant

(Table 18); however, none of the three contrasts were statistically significant at the a' level of 0.0169. Figure 18 suggests that more wild clams were found in the protected vs. unprotected units at both stocking densities.

Common Island – Condition of experimental units

Approximately 42% (n = 38) and 27% (n = 8) of the experimental units were missing from the upper and lower intertidal, respectively (Table 15). Of those units remaining in the sediments at the upper intertidal location during the experiment, nearly 65% (34 of 52) showed some degree of sediment scour. In most cases, scour had removed 10-25% of the sediment. Much less scouring was observed in the lower intertidal units (36%, or 8 of 22 units). Of the 22 units remaining in the upper intertidal that had been initially protected with netting, 86% were either missing (64%) or torn (22%). All nets from the lower intertidal units that were recovered were either missing (38%) or torn (62%).

Common Island – Survival of cultured animals

Live clams were found in 42 of 74 (56.8%) experimental units recovered on 13 July 2002. Survivorship pooled across both tidal heights was 12.4 ± 4.2%; however, there was a significant Tidal height x Treatment(Density) interaction (P = 0.0107; Table 19). In the upper intertidal, where mean survival was 8.4 ± 3.5% (n = 52), no significant differences were observed between protected and unprotected units at either stocking density (Fig. 19). Conversely, clam survival pooled across stocking density in the lower intertidal was enhanced in units protected with netting (37.9 ± 19.1%, n = 10) compared with those without netting (11.3 ± 14.8%, n = 7).

Common Island – Growth of cultured animals

Animals grew to a mean SL of 19.6 ± 0.90 mm (rg = 66.3 ± 7.3%, n = 42; Fig. 16b); however, relative growth and mean SL varied significantly from the upper to lower tidal levels (P = 0.0091; Table 20). Clams attained a mean SL of 18.9 ± 1.14 mm (n = 26) and

20.7 ± 1.25 mm (n = 16) in the upper and lower intertidal units, respectively. No other source of variation was significant (Table 20).

Common Island – Number of wild clams within experimental units

Seventy wild clams (< 30 mm SL) were found in the 74 experimental units that remained on the flat until the end of the experiment (51.9 ± 20.58 individuals/m^2; 0_{SL} = 13.2 ± 1.09 mm; SL range = 6.2-24.9 mm; Figure 17b). There was no significant effect due to tidal height, stocking density, or predator exclusion; however, ANOVA demonstrated a significant spatial variability component (P = 0.0096; Table 21). Block-to-block variability occurred in the lower, but not upper, intertidal location (Fig. 20).

Middle Ground – Condition of experimental units

Two (2.2%) and six (16.7%) units were missing from the upper and lower intertidal locations, respectively (Table 15). Of those units remaining in the sediments at the upper intertidal location during the experiment, 20% (18 of 88) showed some degree of sediment scour. More severe scouring 63% (15 of 24) occurred in the lower intertidal blocks; however, in most cases, scour had removed less than 25% of sediments at either tidal height. Damage to nets in the upper intertidal was less severe at this site compared to Brown's Flat or Common Island (19%, or 7 of 36) whereas all twelve nets in the lower intertidal were torn.

Middle Ground – Survival of cultured animals

At least one live clam was found in all but one experimental unit at the upper intertidal (87/88 = 98.8%) and in 15 of 24 units at the lower intertidal (62.5%). This difference also is reflected in the mean percent survival between the two tidal heights (0_{upper} = 48.3 ± 9.4%, n = 88; 0_{lower} = 23.1 ± 10.7%, n = 24; P = 0.0019; Table 22). In addition, netting resulted in significant enhancement of clam survival (ca. 32.8%) compared with unprotected treatments for both stocking densities (P < 0.0001; Table 22; Fig. 21). For

example, the mean percent survival, pooled over both stocking densities, for protected vs. unprotected units was 57.9 ± 6.4% (n = 48) vs. 25.2 ± 6.9% (n = 41).

Middle Ground – Growth of cultured animals

Clams attained a mean SL of 20.5 ± 0.53 mm (n = 102; rg = 78.6 ± 3.7%; Fig. 16c). There was no tidal height or predator exclusion effect on mean rg; however, rg was 10% greater at the lower (82.6 ± 5.0%, n = 61) vs. higher stocking density (72.6 ± 5.3%, n = 41) (P = 0.0264; Table 23). This difference amounted to less than 1.5 mm in mean SL between the two densities (0_{low} = 20.9 ± 0.6 mm vs. 0_{high} = 19.7 ± 0.5 mm). The effect due to stocking density was ambiguous due to a significant Tidal height x Treatment (Density) interaction (P = 0.0034; Table 23). This effect was due to disproportionately faster growth of clams in protected units in the lower stocking density (Fig. 22.)

Middle Ground – Number of wild clams within experimental units

A total of 183 wild clams was found in the 112 experimental units recovered at the end of the experiment. Mean SL was 17.0 ± 0.8 mm (range = 4.2-29.7 mm; Fig. 17c). The percent of units containing wild clams varied between tidal heights (low: 10/24 = 41.7% ; upper: 54/84 = 61.4%) but this did not translate to a significant difference (P = 0.4000; Table 24) as the variability associated with mean number of wild clams per unit (or m^2) from upper intertidal plots (102.8 ± 23.8 individuals/ m^2, n = 88) was relatively low compared with variability associated with lower intertidal units (41.1 ± 38.2, n = 24). Overall, number of wild clams was 89.6 ± 20.7 individuals/m^2, n = 112). In addition, there was significant spatial variation among blocks at the two intertidal heights (P < 0.0001; Table 24). Separating the Tidal height(Block) source of variation into an upper and lower intertidal component demonstrated that block-to-block variation was limited to the upper intertidal experimental units (Table 24; Fig. 23).

Discussion

The field studies from November 2001 to July 2002 examined multiple hypotheses concerning mortality agents of soft-shell clams. These included the interactive effects of site, time of year, location within the intertidal zone, intraspecific competition for food or space, disease, migration, and predation. Because the three sites were located in a relatively high-energy, sandy environment where sediments typically were rippled (1-4 cm), an unanticipated outcome of the study was an ability to measure the relative energy at each site and tidal height based on numbers of missing experimental units (Tables 5 & 15). Although the frequency of missing experimental units was independent of tidal height (upper vs. lower intertidal) and sampling date (March vs. July 2002) for each site (Fisher's Exact Test: Brown's Flat [P = 0.1106]; Common Island [P = 0.7431]; Middle Ground [P = 0.1827]), the frequencies did depend on sampling date and site (G-test of independence, df = 2; G = 8.36; P = 0.0153). For example, no change in number of missing units occurred between dates at Brown's Flat (38), numbers more than doubled from the March to July 2002 sampling at Common Island (22 vs. 46), and missing pots decreased over the same time interval at Middle Ground (14 vs. 8). Since experimental units were placed similarly in the sediments at each site and on each initiation date, these data suggest that there is wide variation in the cumulative effects of physical energy (e.g., sediment scour from wind waves, tidal currents, freshwater flow, deposition, etc.) at each site. Additional qualitative data on the degree of sediment scour observed in experimental units at each site supports the results of the analyses of frequency of missing units. The areas where blocks of experimental units (Fig. 3) were located at Common Island and Brown's Flat appear to be different from Middle Ground with respect to physical energy (Brown's Flat . Common Island < Middle Ground).

Survival of Cultured Individuals

The frequency of missing experimental units explained 76.2% of the variation in mean percent survival of cultured animals across both experimental intervals (Fig. 24).

Greatest losses of experimental units during an experimental interval (> 25% at both Brown's Flat and Common Island) coincided with mean survival rates < 30%. The effects of several of the experimental factors were not as straightforward. For example, during the first experimental period (November 2001 to March 2002), significant variation in mean survival was influenced by tidal height only once and this occurred at Middle Ground (Table 12; Fig. 12) where 55.1% of clams survived in the upper intertidal compared to 41.7% in the lower intertidal blocks. During the second experimental period (March to July 2002), tidal height effects were observed at each of the three study sites; however, the direction and magnitude of the effect differed between sites. Although survival rates were generally lower during the second experimental interval, again, more clams at Middle Ground survived in the upper vs. lower intertidal blocks (48.3% vs. 23.1%), but the magnitude of the effect was nearly 12% greater during the warmer vs. colder weather period. At Brown's Flat, the opposite situation occurred where mean percent survival of clams was greater in the lower compared to the upper intertidal blocks (20.3% vs. 8.6%; Table 16). This comparison, however, was influenced by density, as more animals survived in the higher stocking density (24 clams/unit, or 1,320 individuals/ m^2) at the lower intertidal location (Table 16; Fig. 14). At Common Island, mean percent survival was higher in the lower intertidal blocks and Figure 19 indicates that this is because proportionately more clams survived in protected vs. unprotected units at the lower vs. upper intertidal location. Other studies that have examined the role of tidal height on juvenile and adult clam survival have demonstrated that mortality generally increases from high to low shore areas (Beal, 1994; Beal et al., 2001; Zacklan and Ydenberg, 1997). Typically, more waterborne predators (e.g., fish, crustaceans, polychaetes) than terrestrial ones (birds and mammals such as raccoons) exist in marine systems and these organisms have a longer time to forage on infaunal bivalves at lower intertidal levels. In addition, since predators must excavate sediments to obtain their clam prey, doing so is easier during tidal inundation when sediments have higher water content than when the same sediments are exposed to air.

The effects of stocking density on clam survival were unimportant in these studies. In no instance was the density source of variation significant at any site over either experimental period and only once was there a significant two-factor interaction involving density (Table 16; Fig. 14). In that case, clam survival was greater in the highest stocking density at the lower intertidal blocks (Brown's Flat, March to July 2002). Higher-order interactions involving density were observed at Middle Ground (Fig. 10, first experimental period) and Common Island (Fig. 19, second experimental period). In both instances, the interaction involved a disproportionate enhancement of survival in protected experimental units in lower tidal vs. upper intertidal blocks for clams stocked at both densities. It was unlikely that competition for space would occur since less than 10% of the available space within experimental units containing animals at the higher density was occupied. Beal et al. (2001) observed a small (4.5%), but significant overall reduction in mean percent survival across experimental densities from 660 to 1,320 individuals/m^2. In that study, density-dependent mortality was independent of other factors (season, tidal height, and predator exclusion). In the few studies that have demonstrated strong density-dependent morality (Boulding and Hay, 1984; Summerson et al., 1995), predators concentrated their activities in high density rather than low density patches. As Beal et al. (2001) showed in eastern Maine, it is likely that density-dependent responses are not important in regulating populations of juvenile *Mya* at these sites.

Predator netting enhanced survival for both stocking densities in 11 of 12 (ca. 92%) instances (Tables 6, 9, 12, 16, 19, 22). The only time when netting did not produce higher mean survival was at Common Island for the low density units in the upper intertidal blocks from March to July 2002 (Table 19; Fig. 19). Overall, these results are somewhat surprising given that most nets at each site were either damaged or missing at the end of each experimental period. Highest damage and loss rates occurred at Brown's Flat and Common Island (> 55% for each combination of tidal height and sampling date). To determine if rate of missing or damaged nets (independent variable) could explain any

of the variation associated with mean percent survival among clams assigned initially to "protected units" (dependent variable), I conducted a regression analysis and found that approximately 42% of the variation in mean percent survival was accounted for by the independent variable (Fig. 25). This relationship demonstrates that at least over short periods of time (i.e., four months), percent survival increases with decreasing damage to nets. Netting played a significant role in enhancing survival in both experiments. During the first experimental period, survival enhancement due at least to the initial presence of netting varied between sites (36.4% at Brown's Flat; 54.8% at Common Island; 44.6% at Middle Ground). During the interval from March to July 2002, the degree of enhancement was not as great (21.5% at Brown's Flat; 26.6% in the lower tidal blocks at Common Island and 0% in the upper intertidal blocks; 32.8% at Middle Ground). The effect of netting was the most important source of variation in each of the six ANOVA's that tested main and interactive effects (see Methods) based on the ratio of the sum of squares attributed to the Treatment(Density) source vs. total variation. Collectively, this information suggests that the cumulative effect of predators is one of the most important sources of clam mortality at these sites. The valves of a majority of dead clams in the experimental units at the end of each experimental period were crushed or chipped – a condition typical of crustacean predation (Beal, 1994).

Another source of clam loss was due to animals either actively or passively emigrating from experimental units. The loss of surface sediments from experimental units due to scouring was relatively high ($\leq$ 25% in most instances). Since juvenile clams burrow in the sediments to a depth that is directly proportional to their SL (Zwarts and Wanink, 1989). An attempt was made within the experimental design to quantify the difference between active and passive clam movement by comparing survival in open vs. rimmed units stocked with 12 animals. A significant difference in survival between open (20%) and rimmed (54%) units was detected once (Middle Ground – first experimental period; Table 12; Fig. 10) and this occurred only in the upper intertidal. Besides predators, physical scouring was primarily responsible for clam losses at these sites. It is unclear as

to the fate of these animals. That is, are they swept away and deposited elsewhere on the flat (extreme upper or lower intertidal), are they preyed on, or do they remain in the same general area where they were transplanted? When clams were not allowed to migrate (experimental units protected with netting), survival rates were as high as 78% (Common Island – first experimental period, lower intertidal blocks) compared to rates less than 10% in open and rimmed units.

The study also was designed to examine potential effects of winterkill and disease on clam survival. Although clam losses were relatively high during late fall and winter (November 2001 to March 2002), mean survival at each site pooled across all main factors was higher during this interval than survival during the March to July 2002 interval (Fig. 26). Further, clams planted in late November at an intertidal flat in eastern Maine (Duck Brook Flat, Cutler, Me.) that experienced minor icing during late January to February (B. Beal, pers. obs.) had survival rates similar to those observed at Middle Ground by late March 2002. If clams experience significant winterkill, mortality rates should have been significantly higher at the eastern Maine location. Lastly, no icing was observed at any of the New Hampshire sites during the first experimental period (B. Brindamor, pers. comm.). Winterkill could also have occurred due to excess freshwater, or riverine loads, during this period. Had events of this type contributed significantly to clam mortality, the number of dead and undamaged clams likely would have been dramatically higher than what was observed (i.e., < 10% of animals with undamaged valves).

None of the cultured clams developed neoplastic cells (hematopoietic neoplasia) during either experimental interval. In addition, none of the wild, juvenile clams found in experimental units in March or July tested positively for the presence of neoplastic cells. Weinberg et al. (1997) conducted a manipulative field experiment with juveniles of *Mya* at an intertidal flat in southeastern Massachusetts from 1991 to 1992 to determine effects of induced hematopoietic neoplasia on animals of three size groups (20-20 mm SL; 30-39

mm; 40-49 mm) . Although clam survival was positively size dependent (larger clams had higher survival rates than smaller clams), mortality due to disease was more prevalent among larger clams. It does not appear that disease (neoplasia) is an important factor influencing mortality in juvenile clams at these intertidal flats.

Growth of Cultured Individuals

As expected, clam growth was highly seasonal. Mean SL increased less than 4 mm (mean rg = 11.4 - 26.5%) during the first experimental period and approximately 9 mm (mean rg = 66.3 - 78.6%) from March to July 2002. In eastern Maine, juvenile clams do not growth in length from mid-fall (late October/early November) through the following April (Beal, 1994; Beal et al., 2001). Most (ca. 65%) shell growth occurs between June and August. Clams transplanted to Duck Brook Flat in Cutler, Maine added no shell between November and March, which was different from that observed at the three New Hampshire flats. It was not possible, given the sampling scheme, to determine if clams ceased growing in length for a period of time and then began to grow sometime in late February or March or whether they grew slowly, but steadily, from November to March. Brousseau and Baglivo (1987) and Cerrato et al. (1991) examined growth of *Mya* in Long Island Sound and found that shell growth begins as early as January.

Clams grew significantly faster at lower tidal levels at Common Island during the first experimental interval (12.2%; Table 10), and at Brown's Flat (14.1%; Table 17) and Common Island (9.5%; Table 20) from March to July 2002. This is not too surprising as clams have longer periods to feed at lower tidal levels. It is not clear why clams at Middle Ground during the second experimental interval did not display a significant tidal height effect on growth rate. Peterson and Black (1993) found that the venerid bivalves, *Katelysia scalarina* and *K. rhytiphora*, suffered a 50% reduction in growth in protected cages vs. open enclosures during a 10-week study in Western Australia due to increased disturbance in protected cages that failed to keep out a predatory seastar. Beal et al. (2001) found that the presence of predators in experimental units designed to inhibit

predators resulted in a nearly 7% decrease in shell length compared to similar units that successfully excluded predators. In the present study, all nets in the lower intertidal at Middle Ground were torn or otherwise damaged. Perhaps the presence of predators at this site and tidal height was sufficient to suppress growth rate. I tested whether the presence of damaged nets resulted in significantly lower relative growth and final SL at the lower intertidal at Brown's Flat (P = 0.5381 and 0.2041, respectively) and Common Island (0.0219 and 0.1217, respectively). At Common Island, relative growth was suppressed by 26%, but this only translated to a difference in final length of 2.1 mm, which was not significantly different from zero.

No other main effects (density and predator protection) had consistent effects on relative growth. Clam growth was suppressed in protected experimental units initially stocked with 1,320 individuals/m^2 in lower intertidal blocks at Middle Ground from March to July 2002 (Table 23; Fig. 22). Density-dependent regulation of growth rates has been observed in populations of suspension-feeding bivalves (Peterson and Beal, 1989; Montaudouin and Bachelet, 1996; Beal et al., 2001). Ecological theory would predict that competition might be sporadic and limited to occasions when and where resources are in short supply (Weins, 1977). For example, Beal et al. (2001), examining the growth of *Mya* juveniles at three tidal heights at an intertidal mud flat in eastern Maine, found that density-dependent processes occurred only at high intertidal levels (vs. mid and low), but the suppression was less than 10%. Density-dependent growth may have occurred at these intertidal flats, but predation and other agents that removed clams from experimental units may have masked these effects. To determine whether density effects occurred in protected units with undamaged nets, I performed a series of ANOVA's for each site (a = 3) and tidal height (b = 2) for the second experimental interval. None of the six analyses (a x b) was significant.

Spatial variation in growth and survival

The experimental design employed in these field studies was a generalized randomized complete block design (Underwood, 1997). The arrangement of experimental units in blocks with replicated treatments enables one to answer the question whether or not spatial differences in mean percent survival or growth are important at the scale of 10's of meters for each sampling date and tidal height. Typically, field experiments are conducted in one small area within a particular tidal zone (Peterson and Black, 1987; Rosenberry et al., 1991; Stiven and Gardner, 1992). Subsequent analyses to detect patterns of differences in means or frequencies between tidal heights assume that the area chosen is representative of that tidal height. Nested experimental designs, such as the one employed here, permit tests of within-tidal height variability. In the present study, block effects were observed four times (Middle Ground, relative growth and mean number of wild clams, November 2001 to March 2002, Tables 13 & 14, Fig. 11 & 13; Common Island, mean number of wild clams, March to July 2002, Table 21, Fig. 20; Middle Ground, mean number of wild clams, March to July 2002, Table 24, Fig. 23). In the first two instances, the observed differences occurred among upper intertidal blocks. Blocks closest to a relatively muddy area (dominated by mussels, *Mytilus edulis*) had highest number of wild clams per square meter and fastest growth rates. The pattern for growth rate observed at Middle Ground during the first experimental period was not repeated during the second (Table 23), but was for mean number of wild spat.

Wild spat

No overall pattern emerged with respect to wild spat sampled from experimental units in March and July 2002 except that: 1) overall densities were greater at Middle Ground than the other two sites, and 2) more spat were recovered at Middle Ground during the first experimental period (Fig. 27).

Recommendations for increasing information and enhancing local stocks

These short-term field trials only can begin to answer some questions about factors affecting the mortality of juveniles of *Mya arenaria* on the three intertidal flats within the Hampton-Seabrook Estuary. Although the tests were replicated in time, the results are somewhat ambiguous with respect to a number of variables (e.g., effect of tidal height; spatial variation among blocks). Others, however, are less ambiguous. For example, predation is clearly important in this system and predator netting (even if it is damaged somewhat) acts as a significant deterrent and enhances clam survival. Stocking density (at least at the two experimental levels reported here – 660 and 1,320/m^2) is unimportant in terms of attracting wild spat, and does not affect clam growth or survival. Disease (hematopoietic neoplasia) is not important in clams < 20 mm SL at these sites. Mortality in the winter is not as severe as mortality between March and July.

1) Repeat those portions of the experiment that seem the most promising. For example, although there is a small possibility that wild and cultured clam spat suffer some overwinter mortality due to abiotic factors that scour upper portions of the sediment, much clam loss can be attributed to predators. Flexible, plastic nets (6.4 mm aperture) can help deter predators and reduce clam emigration. A study should be conducted during the fall and winter (when clam spat are abundant, especially at the upper intertidal at Middle Ground) by adding protective netting (area of netting, aperture size, net location could be independent factors) to selected areas. Areas under netting could be "seeded" with hatchery-reared juveniles (densities to at least 1,320 indviduals/m^2) and these could be compared to two types of controls: 1) netted areas without cultured seed, and 2) unnetted areas. Benthic cores should be taken in both "controls" and "treatment" areas at the beginning (late October/early November) and end of the study (late April/early May) to compare changes in density through time and as a function of the experimental manipulations.

2) Conduct longer-term studies to determine juvenile clam growth and survival during the period between March/April and October/November. This would likely bracket the

period of greatest growth and most intense mortality due to predators. The present study failed to assess survival and growth during the warmest period of the year (August to September).

3) Determine the direct effects of commercial harvesting on wild and/or cultured juveniles. Winter digging, especially, may exacerbate physical scouring that normally occurs on flats during this time. Beal and Vencile (2001) examined effects of commercial harvesting of soft-shell clams and blood worms (*Glycera dibranchiata*) on the survival of juveniles of *Mya arenaria* at a mudflat in Brunswick, Maine. They found that both types of commercial harvesting reduced wild clam numbers significantly compared to controls, but the effects due to worming were more benign than effects due to clamming. The likely reason is that clammers excavate larger volumes of sediment than wormers and these sediments (muddy) can suffocate clams if they become too deeply burrowed. Because clams tend to position themselves deeper in most sediments during winter months (Beal, pers. obs.; Zwarts and Wanink, 1989), clammers must excavate more sediments to capture clams than they would in warmer months. In addition, since clam burial depth is a direct function of its size (Zacklan and Ydenberg, 1997), the larger the clam, the more sediment must be excavated to capture it. Therefore, if clam populations tend to become older with larger individuals dominating the size frequency distribution, recreational or commercial digging may be detrimental to the smaller individuals in the population because of the volume of sediments that must be turned over during the harvesting process.

4) Determine if it is feasible to collect wild spat from the benthos prior to the recreational harvesting season (by using hand-held devices that pump jets of water onto the flat fluidizing the upper few centimeters of sediments that small mesh screens can easily be pulled through). If it is, then these animals can be overwintered using the techniques described in Beal et al. (1995) and transplanted to sites protected with netting during April of the following year. Managing marine resources by enhancing juvenile survival

may be a more effective way of boosting numbers of legal or commercial size individuals in a population than traditional attempts to limit catch, effort, or rotate flats between open and closed times.

5) Conduct transplanting experiments in the spring of the year using local or cosmopolitan stock (< 30 mm individuals). Animals could be planted under netting or in unnetted areas and the fate of each group followed through time.

6) Monitor the growth of mussel beds on the Middle Ground. In areas where mussels began to monopolize surface space, more muddy sediments existed and these also were the areas of fastest clam growth and highest abundance of wild spat. Too many mussels will be detrimental for two reasons: 1) exploitative competition for food, and 2) interference competition for space. However, it could be that maintaining a certain density of mussels in an area might help increase surface roughness and provide enough drag on the water flowing over the flats to enable clams to feed more efficiently and their spat to settle out of the water column. Experimental manipulation of mussels together with clams may show interesting results not only in terms of enhancing clam populations but in answering basic ecological questions about the relative role epibenthic suspension feeders may play in the dynamics of infaunal suspension feeding populations.

No matter what route managers of soft-shell clam populations at these sites decide to take, it should be apparent that answers about the mechanisms controlling clam distribution and abundance can only be attained by manipulative field investigations. Benthic sampling will give a picture, a quantitative observation, of how population numbers and clam sizes vary between sites through time, but cannot be used to answer why numbers or sizes change. Sampling is important because it allows us to create models that attempt to explain the observed patterns, or lack of a pattern. Models yield hypotheses that can be tested (falsified) by critical tests (Underwood, 1991). The outcome of these tests, or experiments, ultimately will become the manager's keystone.

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Table 1. **a)** ANOVA on the square root-transformed number of juvenile (< 25 mm SL) soft-shell clams within benthic cores (0.0182 m^2) at Middle Ground within the Hampton-Seabrook Estuary on 13 November 2001. Two benthic cores (depth = 9 cm) were taken 1 m apart within 1 m^2 blocks (random factor) located 10 m apart at three tidal heights (fixed factor) (high, mid, and low). A total of five clams (4.1 to 12.3 mm SL) was sampled (all at the upper tidal height). **b)** ANOVA on the square root-transformed number of juvenile (< 25 mm SL) soft-shell clams within benthic cores (0.0182 m^2) at Common Island within the Hampton-Seabrook Estuary on 13 November 2001. A total of seven clams (3.4 to 18.6 mm SL) was sampled.

a)

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	2	0.4330	0.2165	4.91	***0.0121***
Block (Tidal Height)	42	1.8504	0.0441	0.79	0.7747
Error	45	2.5000	0.5556		
Total	89	4.7834			

b)

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	2	0.1556	0.0778	1.17	0.3213
Block (Tidal Height)	42	2.8000	0.0667	0.86	0.6918
Error	45	3.5000	0.0778		
Total	89	6.4556			

Table 2. ANOVA on the square root-transformed number of juvenile (< 25 mm SL) soft-shell clams within benthic cores (0.0182 m^2) at Middle Ground, Common Island, and Brown's Flat within the Hampton-Seabrook Estuary on 24 November 2001. Two benthic cores (depth = 9 cm) were taken 1 m apart within 1 m^2 blocks (random factor) located 10 m apart at two tidal heights (fixed factor) (high, mid, and low) at each site (fixed factor). A total of 41 clams (2.1 to 25.4 mm SL) was sampled.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Site	2	0.6265	0.3132	0.46	0.6410
Tidal Height	1	0.4608	0.4608	0.67	0.4235
Site x Tidal Height	2	0.4332	0.2166	0.32	0.7336
Block (Site x Tidal Height)	18	12.3677	12.3677	1.73	0.1053
Error	24	9.5572	0.3982		
Total	47	23.4454			

Table 3. **a)** ANOVA on the square root-transformed number of juvenile (< 25 mm SL) soft-shell clams within benthic cores (0.0182 m^2) at Middle Ground, Common Island, and Brown's Flat within the Hampton-Seabrook Estuary on 24 March 2002. Two benthic cores (depth = 9 cm) were taken 1 m apart within 1 m^2 blocks (random factor) located 10 m apart at two tidal heights (fixed factor) (high, mid, and low) at each site (fixed factor). A total of 54 clams (3.4 to 23.6 mm SL) was sampled. **b)** ANOVA on the untransformed SL of juvenile (< 25 mm SL) soft-shell clams within benthic cores (0.0182 m^2) at Middle Ground, Common Island, and Brown's Flat within the Hampton-Seabrook Estuary on 24 March 2002. Animals were sampled in 26 of the 48 benthic cores. Mean SL of clams in the cores = 8.2 ± 1.36 mm (n = 54).

a)

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Site	2	3.0702	1.5350	1.77	0.1988
Tidal Height	1	0.1672	0.1672	0.19	0.6659
Site x Tidal Height	2	0.7795	0.3898	0.45	0.6451
Block (Site x Tidal Height)	18	15.6153	0.8675	2.42	***0.0222***
Error	24	8.6093	0.3587		
Total	47	29.2614			

b)

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Site	2	88.559	44.279	1.35	0.2923
Tidal Height	1	8.116	8.116	0.25	0.6267
Site x Tidal Height	2	83.163	41.581	1.27	0.3131
Block (Site x Tidal Height)	13	425.098	32.699	3.07	0.0715
Error	7	74.605	10.658		
Total	25	679.541			

Table 4. ANOVA on the square root-transformed number of juvenile (< 40 mm SL) soft-shell clams within benthic cores (0.0182 m^2) at Middle Ground and Common Island within the Hampton-Seabrook Estuary on 13 July 2002 (no live clams were sampled from the cores at Brown's Flat). Two benthic cores (depth = 9 cm) were taken 1 m apart within 1 m^2 blocks (random factor) located 10 m apart at two tidal heights (fixed factor) (high, mid, and low) at each site (fixed factor). A total of 24 clams (6.6 to 39.9 mm SL) was sampled.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Site	2	1.9385	0.9693	1.59	0.2311
Tidal Height	1	0.2012	0.2012	0.33	0.5727
Site x Tidal Height	2	3.2479	1.6240	2.67	0.0969
Block (Site x Tidal Height)	18	10.9682	0.6093	11.84	***<0.0001***
Error	24	1.2353	0.0515		
Total	47	17.5911			

Table 5. Fate of experimental units during the first experimental interval (24 November 2001 to 24 March 2002. Tidal Height (U = upper intertidal; L = lower intertidal); Netting and Rim (+ = present; - = absent); Density (12 or 24 clams/unit = 660 or 1,320 individuals/m^2, respectively).

Site	Tidal Height	Netting	Rim	Density	Initial Number	Final Number	Percent Missing
Brown's Flat	U	+	-	12	18	15	16.7
		+	-	24	18	12	33.3
		-	-	12	18	12	33.3
		-	-	24	18	13	27.8
		-	+	12	18	13	27.8
	L	+	-	12	6	4	33.3
		+	-	24	6	2	66.7
		-	-	12	6	3	50.0
		-	-	24	6	4	33.3
		-	+	12	6	4	33.3
Common Island	U	+	-	12	18	16	11.1
		+	-	24	18	16	11.1
		-	-	12	18	11	38.9
		-	-	24	18	13	27.8
		-	+	12	18	17	5.6
	L	+	-	12	6	6	00.0
		+	-	24	6	6	00.0
		-	-	12	6	4	33.3
		-	-	24	6	3	50.0
		-	+	12	6	6	00.0
Middle Ground	U	+	-	12	18	18	00.0
		+	-	24	18	18	00.0
		-	-	12	18	14	22.2
		-	-	24	18	13	27.8
		-	+	12	18	18	00.0
	L	+	-	12	6	6	00.0
		+	-	24	6	6	00.0
		-	-	12	6	2	66.7
		-	-	24	6	6	00.0
		-	+	12	6	5	16.7

Table 6. ANOVA on the arcsine-transformed percent survival data from Brown's Flat for the first experimental period (24 November 2001 to 24 March 2002). A total of 90 and 30 experimental units was established at the upper and lower intertidal, respectively; however, only 65 and 17 were recovered, resulting in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0169 was applied to each of the three single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	531.795	531.795	3.58	0.1073
Density	1	232.544	232.544	1.30	0.2982
Tidal Height x Density	1	116.888	116.888	0.65	0.4503
Treatment (Density)	3	9886.477	3295.492	11.58	***0.0003***
12: Open vs. Rimmed units	1	0.001	0.001	0.00	0.9984
12: Open vs. Netted units	1	4862.740	4862.740	17.08	***0.0008***
24: Open vs. Netted units	1	2974.359	2974.359	10.45	***0.0052***
Tide x Treatment (Density)	3	2262.282	754.094	2.65	0.0842
Block (Tidal Height)	6	890.883	148.481	0.92	0.4882
Block x Density (Tide)	6	1075.907	179.318	1.11	0.3698
Block x Treatment (Tide, Density)	16	4554.481	284.655	1.77	0.0684
Error	44	7082.251	160.960		
Total	81	26633.508			

Table 7. ANOVA on the untransformed mean relative growth data from Brown's Flat for the first experimental period (24 November 2001 to 24 March 2002). Live clams were found in only 46 of the 82 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). There are no degrees of freedom (df) for the Tide x Treatment (Density) source of variation because live clams were found in only one experimental unit from the following treatments: low intertidal, 12 individuals, unprotected; low intertidal, 24 individuals, unprotected.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	1.435	1.435	0.01	0.9312
Density	1	56.702	56.702	0.72	0.4343
Tidal Height x Density	1	0.002	0.002	0.00	0.9959
Treatment (Density)	3	570.231	190.077	0.75	0.5545
Tide x Treatment (Density)	0	0.000	-	-	-
Block (Tidal Height)	5	870.507	174.101	1.87	0.1473
Block x Density (Tide)	5	392.772	78.554	0.84	0.5356
Block x Treatment (Tide, Density)	8	2039.154	254.894	2.74	0.0341
Error	19	1769.029	93.107		
Total	43	5699.832			

Table 8. ANOVA on the square root-transformed mean number of wild clams (< 25 mm SL) found within experimental units from Brown's Flat for the first experimental period (24 November 2001 to 24 March 2002). A total of 41 live clams was found in the 82 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993).

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	0.002	0.002	0.01	0.9439
Density	1	0.438	0.438	3.70	0.1028
Tidal Height x Density	1	0.049	0.049	0.41	0.5441
Treatment (Density)	3	3.310	1.103	2.36	0.1098
Tide x Treatment (Density)	3	1.169	0.390	0.83	0.4948
Block (Tidal Height)	6	2.332	0.389	1.48	0.2342
Block x Density (Tide)	6	0.711	0.118	0.45	0.8420
Block x Treatment (Tide, Density)	16	7.477	0.467	1.77	0.0682
Error	44	11.619	0.264		
Total	81	27.107			

Table 9. ANOVA on the arcsine-transformed percent survival data from Common Island for the first experimental period (24 November 2001 to 24 March 2002). A total of 90 and 30 experimental units was established at the upper and lower intertidal, respectively; however, only 73 and 25 were recovered, resulting in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0169 was applied to each of the three single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	165.149	165.149	3.05	0.1315
Density	1	7.195	7.195	0.03	0.8684
Tidal Height x Density	1	76.109	76.109	0.32	0.5942
Treatment (Density)	3	30342.446	10114.149	34.18	<***0.0001***
12: Open vs. Rimmed units	1	1319.973	1319.973	4.46	0.0508
12: Open vs. Netted units	1	16208.121	16208.121	54.78	<***0.0001***
24: Open vs. Netted units	1	11629.370	11629.370	39.30	<***0.0001***
Tide x Treatment (Density)	3	739.551	246.517	0.83	0.4951
Block (Tidal Height)	6	325.170	54.159	0.19	0.9783
Block x Density (Tide)	6	1443.270	240.545	0.85	0.5394
Block x Treatment (Tide, Density)	16	4734.099	295.881	1.04	0.4293
Error	60	17051.042	284.184		
Total	97	54884.031			

Table 10. ANOVA on the untransformed mean relative growth data from Common Island for the first experimental period (24 November 2001 to 24 March 2002). Live clams were found in only 85 of the 98 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0169 was applied to each of the three single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	2990.759	2990.759	118.73	***<0.0001***
Density	1	196.175	196.175	2.00	0.2073
Tidal Height x Density	1	3.751	3.751	0.04	0.8515
Treatment (Density)	3	949.317	316.439	4.39	***0.0210***
12: Open vs. Rimmed units	1	473.64	473.64	6.56	0.0217
12: Open vs. Netted units	1	864.796	864.796	11.98	***0.0035***
24: Open vs. Netted units	1	74.675	74.675	1.03	0.3253
Tide x Treatment (Density)	3	321.383	107.128	1.48	0.2589
Block (Tidal Height)	6	151.138	25.189	0.81	0.5707
Block x Density (Tide)	6	589.448	98.241	3.14	***0.0112***
Block x Treatment (Tide, Density)	15	1082.349	72.157	2.31	***0.0145***
Error	48	1501.109	31.273		
Total	84	7785.429			

Table 11. ANOVA on the square root-transformed mean number of wild clams (< 25 mm SL) found within experimental units from Common Island for the first experimental period (24 November 2001 to 24 March 2002). A total of 128 live clams was found in the 98 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993).

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	0.043	0.043	0.07	0.8046
Density	1	0.130	0.130	1.60	0.2523
Tidal Height x Density	1	0.352	0.352	4.33	0.0825
Treatment (Density)	3	1.524	0.508	0.95	0.4393
Tide x Treatment (Density)	3	0.254	0.098	0.18	0.9059
Block (Tidal Height)	6	3.823	0.637	0.84	0.5477
Block x Density (Tide)	6	0.487	0.081	0.11	0.9954
Block x Treatment (Tide, Density)	16	8.543	0.534	0.70	0.7829
Error	60	45.780	0.763		
Total	97	60.936			

Table 12. ANOVA on the arcsine-transformed percent survival data from Middle Ground for the first experimental period (24 November 2001 to 24 March 2002). A total of 90 and 30 experimental units was established at the upper and lower intertidal, respectively; however, only 81 and 25 were recovered, resulting in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0169 was applied to each of the three single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	2722.415	2722.415	7.84	***0.0312***
Density	1	331.694	331.694	1.22	0.3110
Tidal Height x Density	1	730.817	730.817	2.70	0.1517
Treatment (Density)	3	24548.510	8182.837	66.85	<***0.0001***
12: Open vs. Rimmed units	1	2487.911	2487.911	20.33	<***0.0001***
12: Open vs. Netted units	1	15964.735	15964.735	130.42	<***0.0001***
24: Open vs. Netted units	1	7335.949	7335.949	59.93	<***0.0001***
Tide x Treatment (Density)	3	1477.398	492.466	4.02	***0.0236***
Block (Tidal Height)	6	2084.674	347.446	2.11	0.0637
Block x Density (Tide)	6	1626.331	271.055	1.65	0.1485
Block x Treatment (Tide, Density)	18	2203.332	122.407	0.74	0.7545
Error	66	10868.445	164.673		
Total	105	46593.616			

Table 13. ANOVA on the untransformed mean relative growth data from Middle Ground for the first experimental period (24 November 2001 to 24 March 2002). Live clams were found in 97 of the 106 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0253 was applied to each of the two single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	422.685	422.685	0.63	0.4590
Density	1	88.611	88.611	1.19	0.3170
Tidal Height x Density	1	1.942	1.942	0.03	0.8770
Treatment (Density)	3	502.557	167.519	1.83	0.1803
Tide x Treatment (Density)	3	585.055	195.018	2.13	0.1344
Block (Tidal Height)	6	4051.284	675.214	8.75	***<0.0001***
Upper Intertidal Blocks	5	4000.451	800.090	10.38	***<0.0001***
Lower Intertidal Blocks	1	50.833	50.833	0.70	0.4062
Block x Density (Tide)	6	446.384	74.397	0.96	0.4570
Block x Treatment (Tide, Density)	17	1557.867	91.639	1.19	0.3023
Error	58	4471.747	77.099		
Total	96	12128.133			

Table 14. ANOVA on the square root-transformed mean number of wild clams (< 25 mm SL) found within experimental units from Middle Ground for the first experimental period (24 November 2001 to 24 March 2002). A total of 504 live clams was found in the 106 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993).

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	2.246	2.246	0.16	0.7068
Density	1	0.196	0.196	0.52	0.4985
Tidal Height x Density	1	0.582	0.582	1.54	0.2614
Treatment (Density)	3	6.566	2.189	7.55	***0.0018***
12: Open vs. Rimmed Units	1	3.223	3.233	11.15	***0.0037***
12: Open vs. Protected Units	1	0.052	0.052	0.18	0.6764
24: Open vs. Protected Units	1	1.867	1.867	6.44	***0.0206***
Tide x Treatment (Density)	3	2.001	0.669	2.31	0.1110
Block (Tidal Height)	6	86.562	14.427	23.69	***<0.0001***
Upper Intertidal Blocks	5	84.445	16.889	27.73	***<0.0001***
Lower Intertidal Blocks	1	2.118	2.118	3.48	0.0666
Block x Density (Tide)	6	2.271	0.378	0.62	0.7123
Block x Treatment (Tide, Density)	18	5.220	0.289	0.48	0.9595
Error	65	39.579	0.609		
Total	104	145.223			

Table 15. Fate of experimental units during the first experimental interval (24 November 2001 - 24 March 2002. Tidal Height (U = upper intertidal; L = lower intertidal); Netting and Rim (+ = present; - = absent); Density (12 and 24 = 12 and 24 clams/unit, respectively, or 660 and 1,320 individuals/m^2, respectively).

Site	Tidal Height	Netting	Rim	Density	Initial Number	Final Number	Percent Missing
Brown's Flat	U	+	-	12	18	10	44.4
		+	-	24	18	12	33.3
		-	-	12	18	14	22.2
		-	-	24	18	8	55.6
		-	+	12	18	12	33.3
	L	+	-	12	6	5	16.7
		+	-	24	6	6	00.0
		-	-	12	6	5	16.7
		-	-	24	6	2	66.7
		-	+	12	6	6	00.0
Common Island	U	+	-	12	18	11	38.9
		+	-	24	18	11	38.9
		-	-	12	18	15	16.7
		-	-	24	18	5	72.2
		-	+	12	18	10	44.4
	L	+	-	12	6	3	50.0
		+	-	24	6	5	16.7
		-	-	12	6	6	00.0
		-	-	24	6	3	50.0
		-	+	12	6	5	16.7
Middle Ground	U	+	-	12	18	18	00.0
		+	-	24	18	18	00.0
		-	-	12	18	18	22.2
		-	-	24	18	17	5.6
		-	+	12	18	17	5.6
	L	+	-	12	6	6	00.0
		+	-	24	6	6	00.0
		-	-	12	6	4	33.3
		-	-	24	6	2	66.7
		-	+	12	6	6	00.0

Table 16. ANOVA on the arcsine-transformed percent survival data from Brown's Flat for the second experimental period (23 March to 13 July 2002). A total of 90 and 30 experimental units was established at the upper and lower intertidal, respectively; however, only 58 and 24 were recovered, resulting in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993).

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	1170.844	1170.844	15.25	***0.0079***
Density	1	17.806	17.806	0.24	0.6436
Tidal Height x Density	1	497.093	497.093	6.76	***0.0483***
Treatment (Density)	3	7320.815	2440.272	19.48	<***0.0001***
12: Open vs. Rimmed units	1	0.482	0.482	0.00	0.9513
12: Open vs. Netted units	1	3694.009	3694.009	29.49	<***0.0001***
24: Open vs. Netted units	1	2494.146	2494.146	19.91	***0.0003***
Tide x Treatment (Density)	3	1033.957	344.652	2.75	0.0747
Block (Tidal Height)	6	460.555	76.759	0.47	0.8247
Block x Density (Tide)	6	367.921	73.584	0.45	0.8085
Block x Treatment (Tide, Density)	17	2129.591	125.270	0.77	0.7132
Error	44	7140.691	162.288		
Total	81	20139.273			

Table 17. ANOVA on the untransformed mean relative growth data from Brown's Flat for the second experimental period (23 March to 13 July 2002). Live clams were found in only 42 of the 82 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993).

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	5586.313	5586.313	64.44	***0.0002***
Density	1	3.364	3.364	0.01	0.9421
Tidal Height x Density	1	494.916	494.916	0.86	0.3970
Treatment (Density)	3	5374.079	1791.359	3.35	0.1365
Tide x Treatment (Density)	3	54.151	18.050	0.03	0.9904
Block (Tidal Height)	6	520.145	86.691	0.09	0.9962
Block x Density (Tide)	5	2886.151	577.230	0.62	0.6873
Block x Treatment (Tide, Density)	4	2137.313	534.255	0.57	0.6861
Error	17	15857.660	932.804		
Total	41	32914.092			

Table 18. ANOVA on the square root-transformed mean number of wild clams (< 30 mm SL) found within experimental units from Brown's Flat for the second experimental period (23 March to 13 July 2002). A total of 18 live clams was found in the 82 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0169 was applied to each of the three single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	0.937	0.937	32.36	***0.0013***
Density	1	0.024	0.024	0.24	0.6429
Tidal Height x Density	1	0.123	0.123	1.27	0.3113
Treatment (Density)	3	2.002	0.667	4.25	***0.0207***
12: Open vs. Rimmed Units	1	0.000	0.000	0.00	0.9674
12: Open vs. Protected Units	1	1.095	1.095	6.97	0.0172
24: Open vs. Protected Units	1	0.575	0.575	3.66	0.0728
Tide x Treatment (Density)	3	0.750	0.250	1.59	0.2286
Block (Tidal Height)	6	0.174	0.029	0.20	0.9749
Block x Density (Tide)	5	0.485	0.097	0.67	0.6482
Block x Treatment (Tide, Density)	17	2.672	0.157	1.09	0.3954
Error	44	6.364	0.145		
Total	81	13.531			

Table 19. ANOVA on the arcsine-transformed percent survival data from Common Island for the second experimental period (23 March to 13 July 2002). A total of 90 and 30 experimental units was established at the upper and lower intertidal, respectively; however, only 52 and 22 were recovered, resulting in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0169 was applied to each of the three single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	918.515	918.515	6.92	***0.0390***
Density	1	349.203	349.203	2.38	0.1738
Tidal Height x Density	1	602.923	602.923	4.11	0.0890
Treatment (Density)	3	2413.410	804.470	5.03	***0.0131***
12: Open vs. Rimmed units	1	221.987	221.987	1.39	0.2567
12: Open vs. Netted units	1	339.763	339.763	2.13	0.1617
24: Open vs. Netted units	1	1436.896	1436.896	8.99	***0.0077***
Tide x Treatment (Density)	3	2549.929	849.976	5.32	***0.0107***
Block (Tidal Height)	6	796.247	132.707	0.66	0.6783
Block x Density (Tide)	6	880.009	146.668	0.73	0.6248
Block x Treatment (Tide, Density)	15	2396.992	159.799	0.80	0.6697
Error	37	7385.893	199.619		
Total	73	18293.121			

Table 20. ANOVA on the untransformed mean relative growth data from Common Island for the second experimental period (23 March to 13 July 2002). Live clams were found in only 42 of the 74 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993).

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	4623.682	4623.682	14.32	***0.0091***
Density	1	120.887	120.887	0.71	0.4461
Tidal Height x Density	1	108.061	108.061	0.64	0.4695
Treatment (Density)	3	3597.424	3597.424	2.86	0.1679
Tide x Treatment (Density)	3	1023.698	341.233	0.81	0.5490
Block (Tidal Height)	6	1936.931	322.827	0.87	0.5371
Block x Density (Tide)	4	678.462	169.616	0.46	0.7670
Block x Treatment (Tide, Density)	4	1675.831	418.958	1.13	0.3754
Error	18	6697.517	372.084		
Total	41	20462.493			

Table 21. ANOVA on the square root-transformed mean number of wild clams (< 30 mm SL) found within experimental units from Common Island for the second experimental period (23 March to 13 July 2002). A total of 70 live clams was found in the 74 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0253 was applied to each of the two single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	3.943	3.943	3.12	0.1278
Density	1	0.199	0.199	0.47	0.5177
Tidal Height x Density	1	0.094	0.094	0.22	0.6526
Treatment (Density)	3	1.716	0.572	1.59	0.2324
Tide x Treatment (Density)	3	1.972	0.657	1.83	0.1844
Block (Tidal Height)	6	7.586	1.264	3.36	***0.0096***
Upper Intertidal Blocks	5	2.677	0.535	1.42	0.2399
Lower Intertidal Blocks	1	4.909	4.909	13.06	***0.0009***
Block x Density (Tide)	6	2.524	0.421	1.12	0.3707
Block x Treatment (Tide, Density)	15	5.379	0.359	0.95	0.5189
Error	37	13.919	0.376		
Total	73	37.332			

Table 22. ANOVA on the arcsine-transformed percent survival data from Middle Ground for the second experimental period (23 March to 13 July 2002). A total of 90 and 30 experimental units was established at the upper and lower intertidal, respectively; however, only 88 and 24 were recovered, resulting in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0169 was applied to each of the three single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	7634.536	7634.536	27.59	***0.0019***
Density	1	160.398	160.398	0.72	0.4298
Tidal Height x Density	1	6.802	6.802	0.03	0.8674
Treatment (Density)	3	13701.780	4567.260	29.75	***<0.0001***
12: Open vs. Rimmed units	1	221.237	221.237	1.44	0.2457
12: Open vs. Netted units	1	7792.979	7792.979	50.76	***<0.0001***
24: Open vs. Netted units	1	4462.478	4462.478	29.07	***<0.0001***
Tide x Treatment (Density)	3	742.549	247.517	1.61	0.2216
Block (Tidal Height)	6	1660.812	276.697	1.66	0.1445
Block x Density (Tide)	6	1343.636	223.939	1.34	0.2509
Block x Treatment (Tide, Density)	18	2763.364	153.520	0.92	0.5595
Error	72	12034.186	167.141		
Total	111	40048.063			

Table 23. ANOVA on the untransformed mean relative growth data from Middle Ground for the second experimental period (23 March to 13 July 2002). Live clams were found in only 102 of the 112 experimental units that were recovered. No live clams were found in unprotected units at either density in low intertidal experimental units. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993).

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	177.734	177.734	0.51	0.5003
Density	1	711.875	711.875	8.57	***0.0264***
Tidal Height x Density	1	111.128	111.128	1.34	0.2914
Treatment (Density)	3	1879.608	626.536	2.72	0.0791
Tide x Treatment (Density)	1	2724.186	2724.186	11.82	***0.0034***
Block (Tidal Height)	6	2074.022	345.670	0.91	0.4960
Block x Density (Tide)	6	498.412	83.069	0.22	0.9698
Block x Treatment (Tide, Density)	16	3687.491	230.468	0.60	0.8693
Error	66	25173.883	381.442		
Total	41	37038.339			

Table 24. ANOVA on the square root-transformed mean number of wild clams (< 30 mm SL) found within experimental units from Middle Ground for the second experimental period (23 March to 13 July 2002). A total of 70 live clams was found in the 74 experimental units that were recovered. This resulted in unbalanced data. Type III sums of squares were used in all hypothesis tests (Shaw and Mitchell-Olds, 1993). An adjusted type I error rate (a') of 0.0253 was applied to each of the two single-degree-of-freedom contrasts.

Source of Variation	**df**	**SS**	**MS**	**F**	**Pr > F**
Tidal Height	1	4.299	4.299	0.82	0.4000
Density	1	0.109	0.109	1.28	0.3012
Tidal Height x Density	1	0.196	0.196	2.30	0.1803
Treatment (Density)	3	2.376	0.792	1.13	0.3625
Tide x Treatment (Density)	3	2.572	0.857	1.23	0.3293
Block (Tidal Height)	6	31.436	5.239	12.32	***<0.0001***
Upper Intertidal Blocks	5	31.002	6.200	14.58	***<0.0001***
Lower Intertidal Blocks	1	0.434	0.434	1.02	0.3159
Block x Density (Tide)	6	0.512	0.085	0.20	0.9755
Block x Treatment (Tide, Density)	18	12.589	0.669	1.64	0.0716
Error	72	30.625	0.425		
Total	73	84.714			

Figure Legends

Figure 1. Sampling schematic associated with 13 November 2001 sampling that occurred at Middle Ground and Common Island. This is a generalized randomized complete block design (Underwood, 1997).

Figure 2. Initial size frequency distribution of hatchery-reared soft-shell clams used in field experiments at three intertidal flats in the Hampton-Seabrook Estuary. **a**) Animals transplanted on 24 November 2001: 0 ± 95% confidence interval = 10.8 ± 0.18 mm (n = 115). **b**) Animals transplanted on 23 March 2002: 10.9 ± 0.20 mm (n = 78). ANOVA indicated no significant difference in mean shell length between dates/groups ($F = 1.46$; $df = 1, 191$; $P = 0.2283$). G-test of independence indicated that the frequency distributions were similar ($G = 4.496$; $df = 6$; $P = 0.6099$).

Figure 3. Schematic of field experiments initiated at three intertidal flats in the Hampton-Seabrook Estuary on 24 November 2001 and 23 March 2002. Open circles represent 15 cm wide x 15 cm deep plastic horticultural plant pots (experimental units). Circles with cross-hatching represent units protected with plastic mesh netting (6.4 mm aperture). Circles with short lines around the circumference represent units with a rim of 6.4 mm netting designed to retain small clams from migrating or being washed out of the unit. The numbers within each circle (12 or 24) represent the number of clams transplanted to each unit (i.e., stocking densities of 660 or 1,320/m^2, respectively).

Figure 4. Mean number of juvenile soft-shell clams within benthic cores taken on 13 November 2001 at Common Island and Middle Ground. * = $P < 0.05$ and indicates that the density of clams at the upper intertidal at Middle Ground differs significantly from zero (the density of clams at the lower and middle intertidal at that site). No density differences existed between tidal heights at Common Island (n = 30).

Figure 5. Mean percent survival (+ 95% CI) of cultured clams at Brown's Flat from 24 November 2001 to 24 March 2002. Data are pooled across upper and lower intertidal locations ($P = 0.1073$; Table 6). ANOVA detected no differences between open and rimmed units with 12 clams ($P = 0.9984$; Table 6); however, there was a significant enhancement ($P < 0.01$; Table 6) in survival at both stocking densities due to the presence of plastic netting.

Figure 6. **a**) Initial (0 = 10.8 ± 0.18 mm, n = 115) and final (0 = 13.2 ± 0.25 mm, n = 245) size distribution of cultured clams from 24 November 2001 to 24

March 2002 at Brown's Flat. **b**) Initial and final (0 = 12.7 ± 0.13 mm, n = 697) size distribution of cultured clams at Common Island. **c**) Initial and final (0 = 14.4 ± 0.14 mm, n = 929) size distribution of cultured clams at Middle Ground.

Figure 7. Size-frequency distribution of wild clams sampled from experimental units on 24 March 2002 from **a**) Brown's Flat (0 = 7.5 ± 1.01 mm, n = 41); **b**) Common Island (0 = 7.5 ± 0.49 mm, n = 128); **c**) Middle Ground (0 = 8.4 ± 0.24 mm, n = 540).

Figure 8. Mean percent survival (+ 95% CI) of cultured clams at Common Island from 24 November 2001 to 24 March 2002. Data are pooled across upper and lower intertidal locations (P = 0.1315; Table 9). ANOVA detected no differences between open and rimmed units with 12 clams (P = 0.0508; Table 9); however, there was a significant enhancement (P < 0.0001; Table 9) in survival at both stocking densities due to the presence of plastic netting.

Figure 9. Mean relative growth (%) of cultured clams at Common Island from 24 November 2001 to 24 March 2002. ANOVA indicated that for the lower stocking density, clams grew significantly slower in the protected (12.4 ± 0.48 mm, n = 22) vs. unprotected (13.1 ± 1.11 mm, n = 10) units.

Figure 10. Mean percent survival (+ 95% CI) of cultured clams at Middle Ground from 24 November 2001 to 24 March 2002. Tidal height effects (P = 0.0312; Table 12) indicate that survival was higher in the upper vs. lower intertidal, but that most of the difference was due to poorer survival in unprotected units at the lower compared to the upper shore. In addition, enhanced survival of clams in rimmed units was observed only at the upper intertidal.

Figure 11. Spatial variation in mean percent relative growth within upper and lower intertidal blocks at Middle Ground from 24 November 2001 to 24 March 2002. ANOVA indicated that the variability between upper intertidal blocks was significant (P < 0.0001), but not for the lower intertidal blocks (P = 0.4062; Table 10).

Figure 12. Mean number of wild clams per square meter recovered in experimental units at Middle Ground on 24 March 2002. ANOVA detected significant differences between open and rimmed units for treatments at the lower stocking density (P = 0.0037; Table 14) and for the comparison at the upper stocking density (P = 0.0206; Table 14).

Figure 13. Spatial variation in mean number of wild clams per square meter in experimental units at Middle Ground on 24 March 2002. Significant block-to-block differences were detected among the upper intertidal blocks ($P < 0.0001$), but not the lower intertidal blocks (Table 14).

Figure 14. Mean percent survival (+ 95% CI) of cultured clams at two tidal heights at Brown's Flat from 23 March to 13 July 2002. ANOVA indicated a significant tidal height ($P = 0.0079$) and tidal height x stocking density interaction ($P = 0.0483$; Table 16).

Figure 15. Mean percent survival (+ 95% CI) of cultured clams at two stocking densities and three levels of netting treatments at Brown's Flat from 23 March to 13 July 2002. ANOVA (Table 16) revealed that there was no significant difference between open experimental units and those rimmed with netting; however, there was a significant survival enhancement due to the presence of netting at both stocking densities ($P < 0.001$; Table 16).

Figure 16. **a**) Initial ($0 = 10.9 \pm 0.20$ mm, $n = 78$) and final ($0 = 20.1 \pm 0.57$ mm, $n = 167$) size distribution of cultured clams from 23 March to 13 July 2002 at Brown's Flat. **b**) Initial and final ($0 = 19.6 \pm 0.48$ mm, $n = 160$) size distribution of cultured clams at Common Island. **c**) Initial and final ($0 = 20.5 \pm 0.25$ mm, $n = 759$) size distribution of cultured clams at Middle Ground.

Figure 17. Size-frequency distribution of wild clams sampled from experimental units on 13 July 2002 from **a**) Brown's Flat ($0 = 15.8 \pm 2.24$ mm, $n = 18$); **b**) Common Island ($0 = 7.5 \pm 0.49$ mm, $n = 128$); **c**) Middle Ground ($0 = 8.4 \pm 0.24$ mm, $n = 540$).

Figure 18. Mean number of wild clams per square meter at Brown's Flat on 13 July 2002. ANOVA indicated an overall Treatment(Density) effect ($P = 0.0207$); however, none of the three a priori contrasts were significant at $a' = 0.0169$ (Table 18).

Figure 19. Mean percent survival (+ 95% CI) of cultured clams at Common Island on 13 July 2002. ANOVA indicated a significant Tidal Height x Treatment (Density) interaction ($P = 0.0107$; Table 19). Protective netting did not enhance survival of clams at either stocking density in the upper intertidal, but did so in the lower intertidal.

Figure 20. Spatial variability among wild clams at Common Island on 13 July 2002. ANOVA indicated that block-to-block variation existed in the lower intertidal, but not in the upper intertidal ($P = 0.0096$; Table 21).

Figure 21. Mean percent survival (+ 95% CI) of cultured clams at Middle Ground from 23 March to 13 July 2002. Data are pooled across upper and lower intertidal locations. ANOVA detected no differences between open and rimmed units with 12 clams ($P = 0.2457$; Table 22); however, there was a significant enhancement in survival at both stocking densities due to the presence of plastic netting (ca. 33%; $P < 0.0001$; Table 22).

Figure 22. Mean relative growth (+ 95% CI) of cultured clams at Middle Ground from 23 March to 13 July 2002. No live clams were found in unprotected units at either density in low intertidal experimental units. ANOVA indicated a significant Tidal height x Treatment (Density) interaction ($P = 0.0034$; Table 23).

Figure 23. Spatial variability among wild clams at Middle Ground on 13 July 2002. ANOVA indicated that block-to-block variability existed in the upper intertidal, but not in the lower intertidal ($P < 0.0001$; Table 24).

Figure 24. Relationship between frequency of missing experimental units (pooled across tidal heights, stocking densities, and predator exclusion treatments) and mean percent survival for all sites (BF = Brown's Flat; CI = Common Island; MG = Middle Ground) and sampling dates (March = 24 March 2002; July = 13 July 2002). A trend analysis indicated no significant quadratic or cubic trend. $Y = 56.57 – 1.185X$; $r^2 = 0.762$; $P = 0.0002$. 95% confidence limits are presented on either side of the least-squares regression line.

Figure 25. Relationship between percent of damaged or missing nets associated with "protected units" and mean percent survival. (See legend for Figure 24 for explanation of abbreviations.) A trend analysis indicated no significant quadratic or cubic trend. $Y = 80.13 – 0.491X$; $r^2 = 0.416$; $P = 0.0236$. 95% confidence limits are presented on either side of the least-squares regression line.

Figure 26. Mean percent survival (+ 95% confidence interval) for each site and sampling date (March & July 2002) pooled over tidal height, stocking density, and level of protection. (Brown's Flat: $n_{\text{November to March}} = 82$, $n_{\text{March to June}} = 82$; Common Island: $n_{\text{November to March}} = 98$, $n_{\text{March to June}} = 74$; Middle Ground: $n_{\text{November to March}} = 106$, $n_{\text{March to June}} = 112$).

Figure 27. Mean number of wild spat per m^2 (+ 95% confidence interval) for each site and sampling date pooled over tidal height, stocking density, and level of protection. (See legend for Figure 26 to obtain number of samples associated with each mean.)

Figure 1.

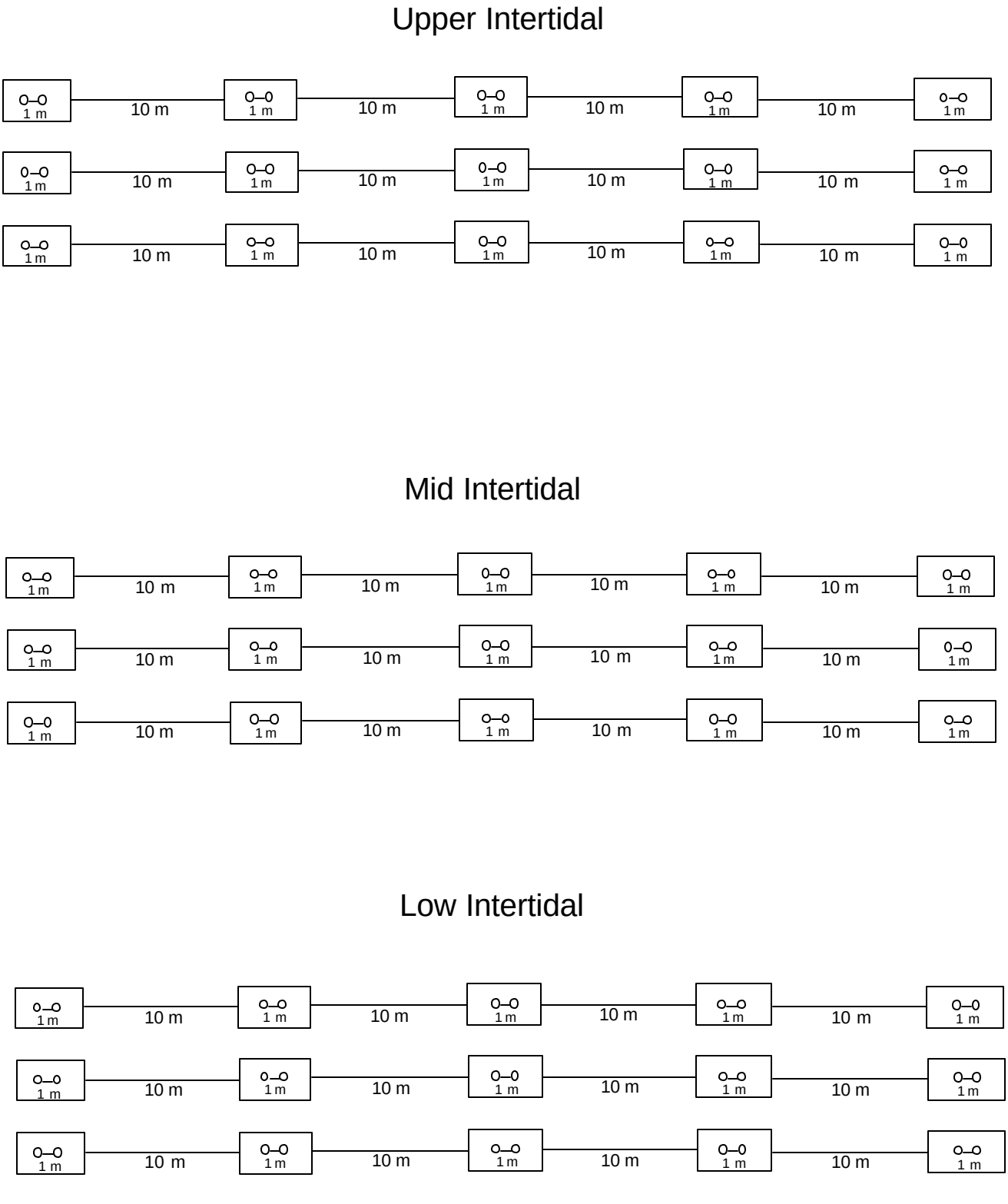


Figure 2.

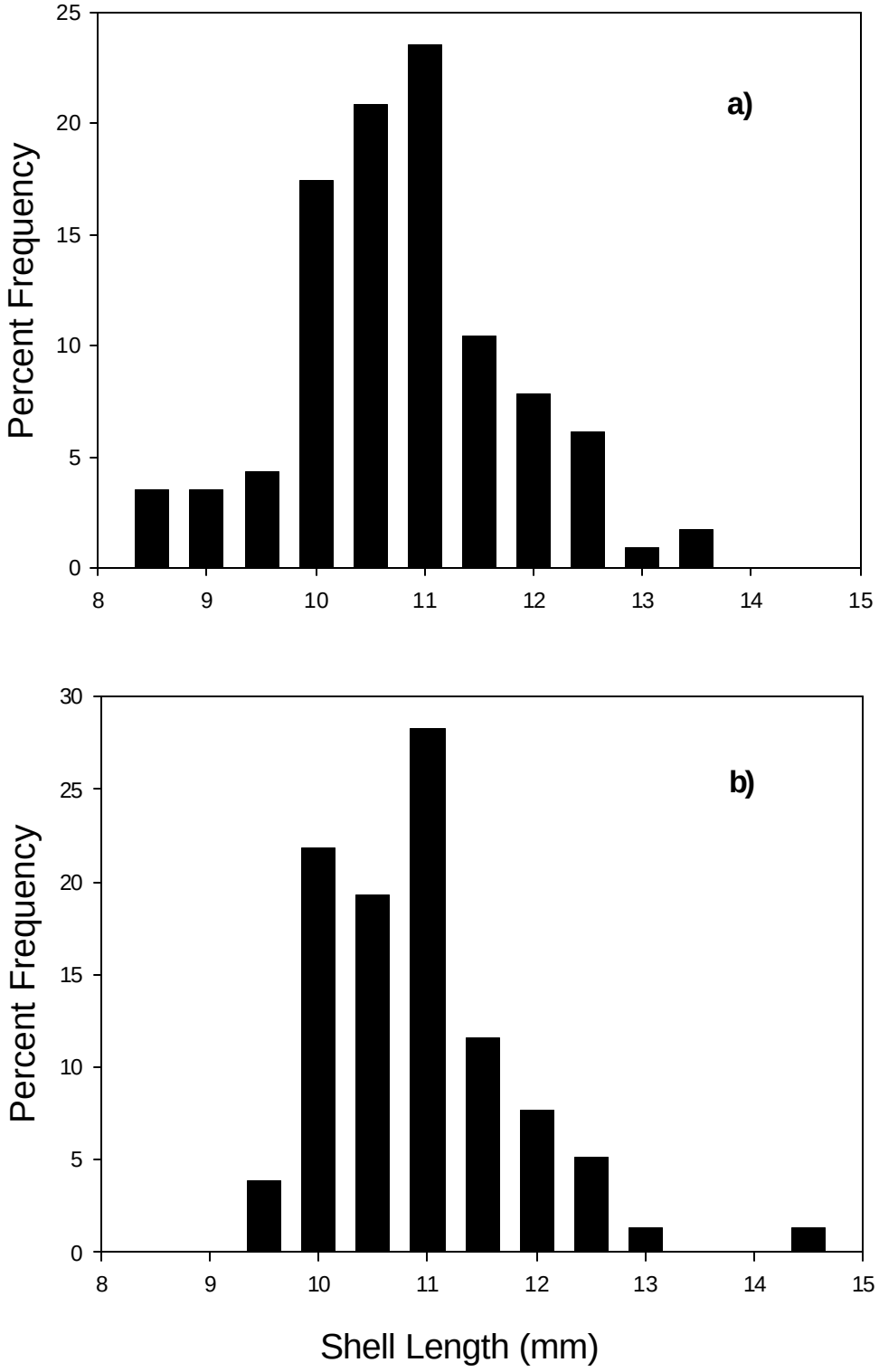
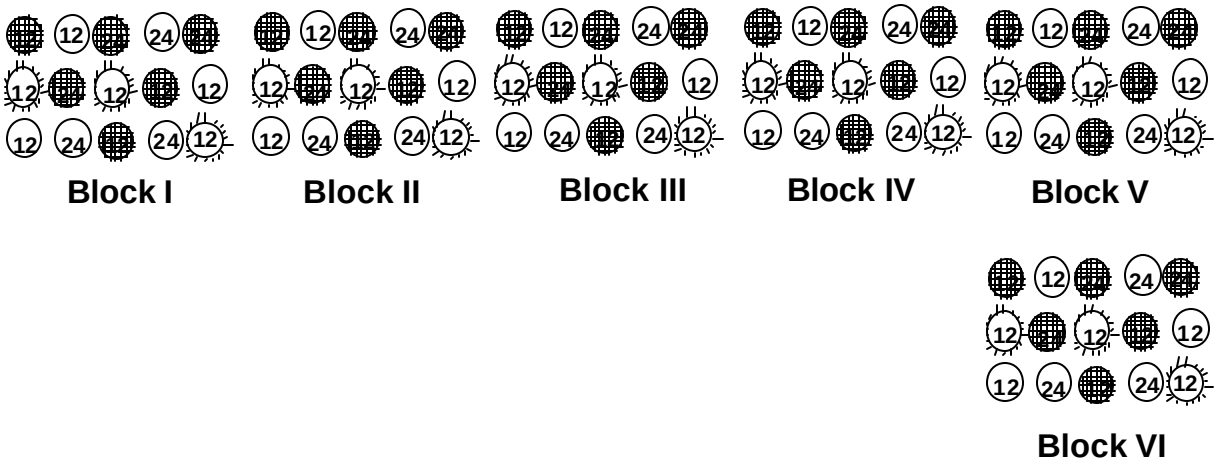

a)
b)
Percent Frequency
Percent Frequency
Shell Length (mm)
0
5
10
15
20
25
30
8
9
10
11
12
13
14
15

Figure 3.

Upper intertidal region



Lower intertidal region

Figure 4.

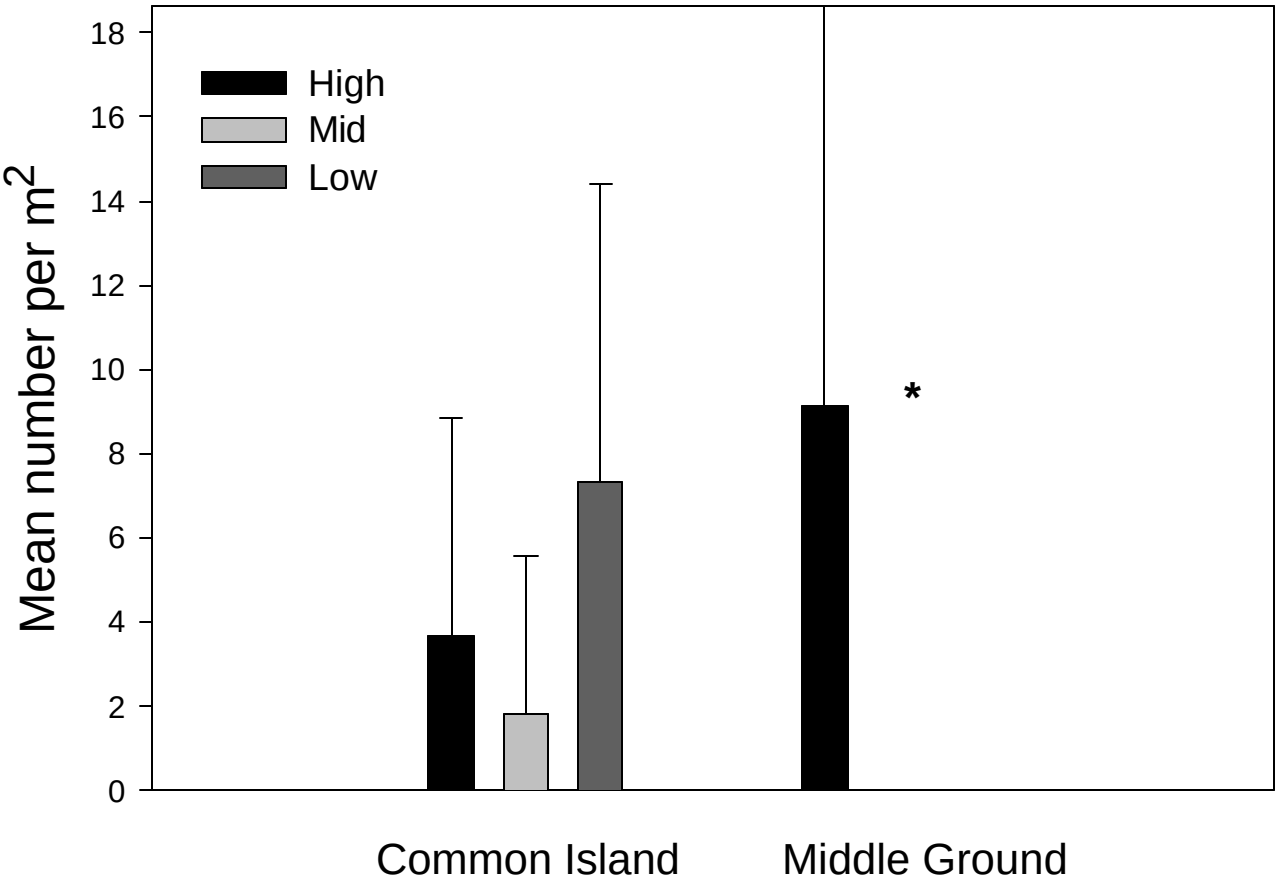

18
16
14
12
10
8
6
4
2
0
Mean number per m2
High
Mid
Low
*
Common Island
Middle Ground

Figure 5.

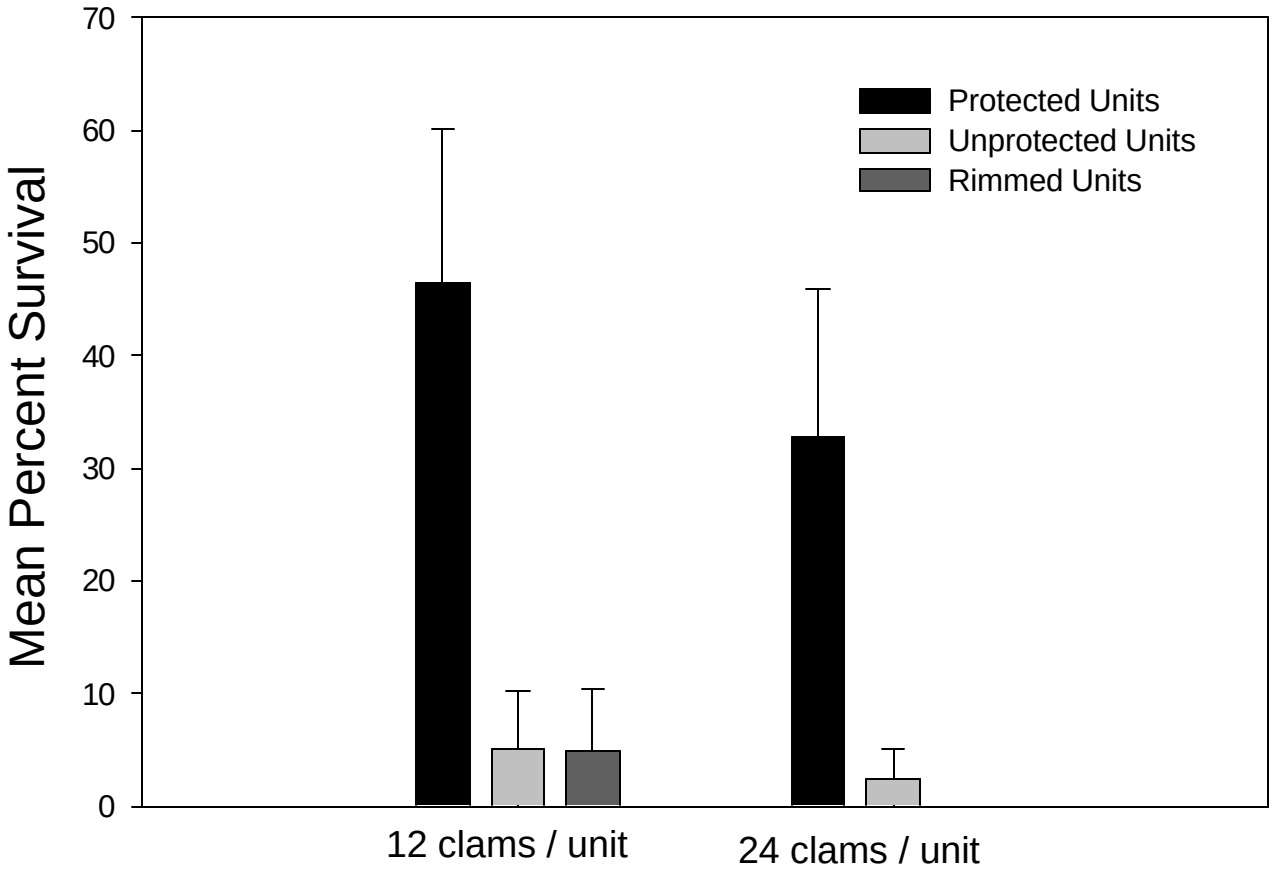

70
60
50
40
30
20
10
0
Mean Percent Survival
Protected Units
Unprotected Units
Rimmed Units
12 clams / unit
24 clams / unit

Figure 6.

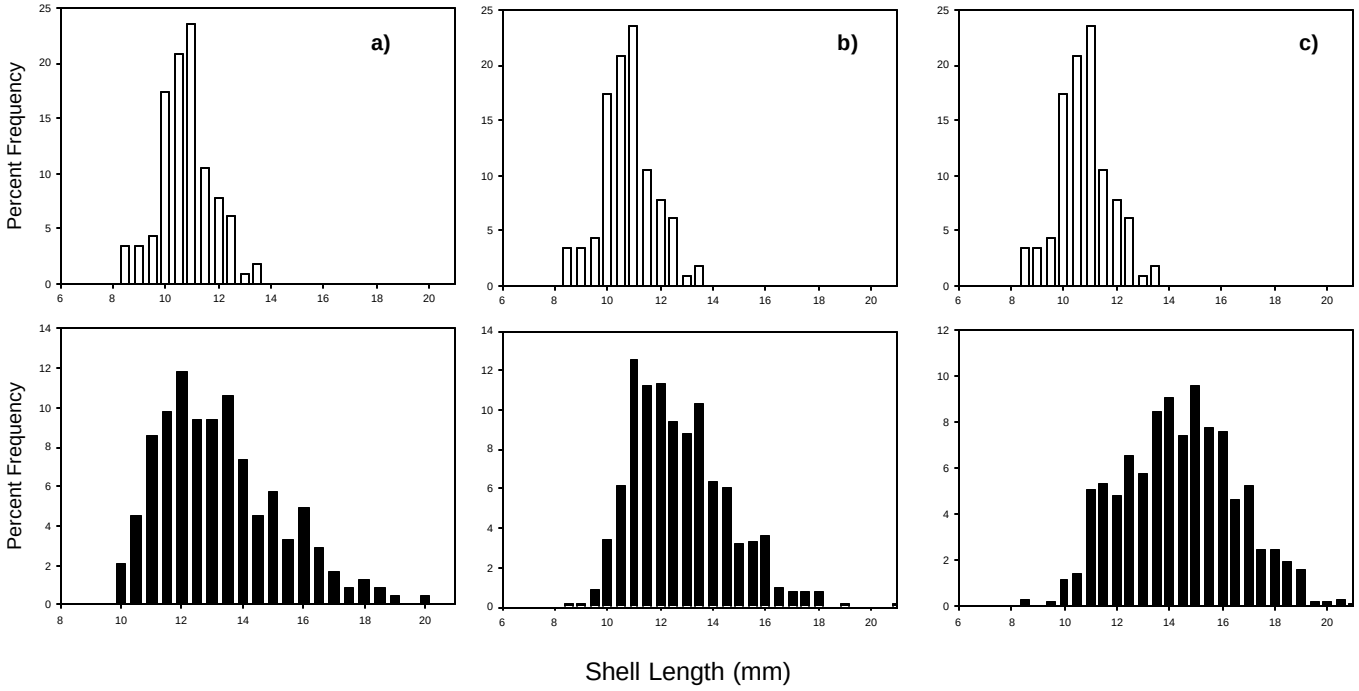


Figure 7.

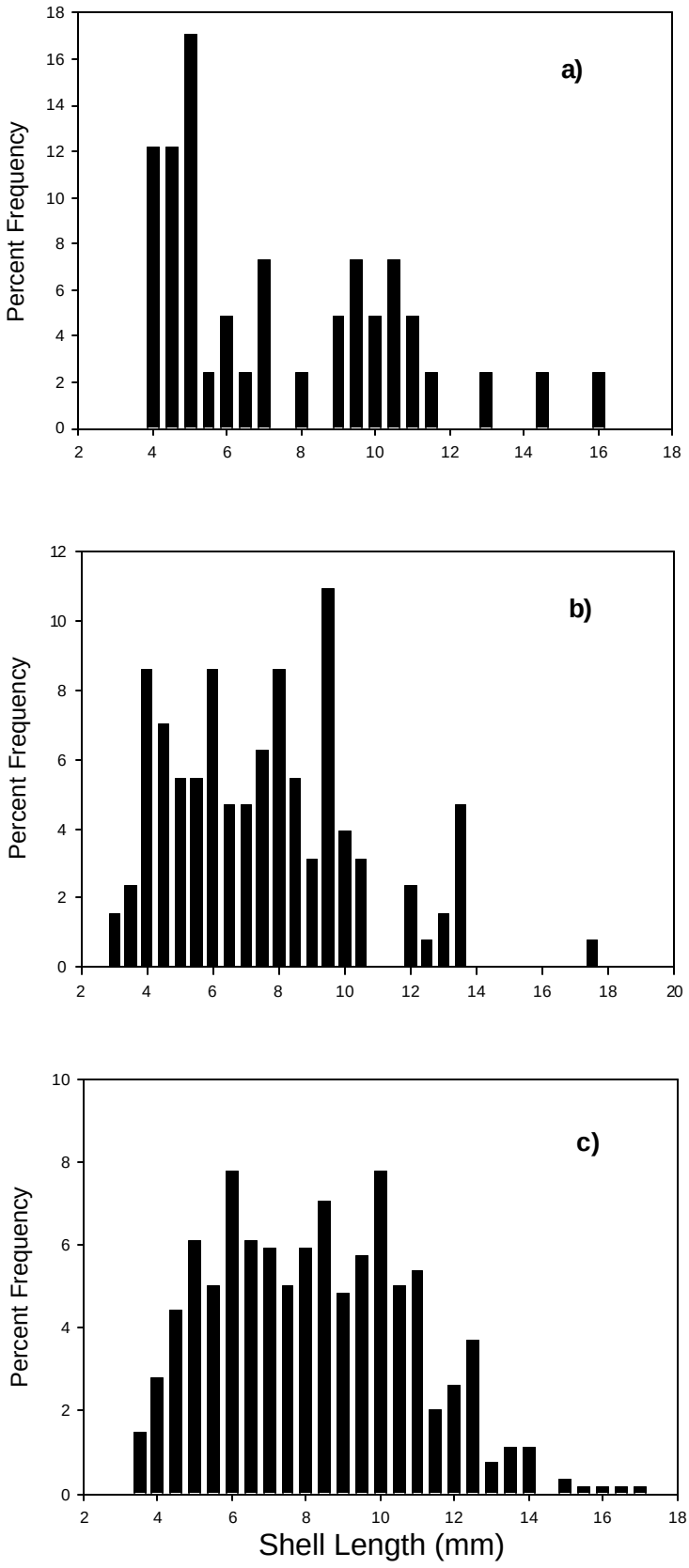

a)
b)
c)
Percent Frequency
Percent Frequency
Percent Frequency
Shell Length (mm)

Figure 8.

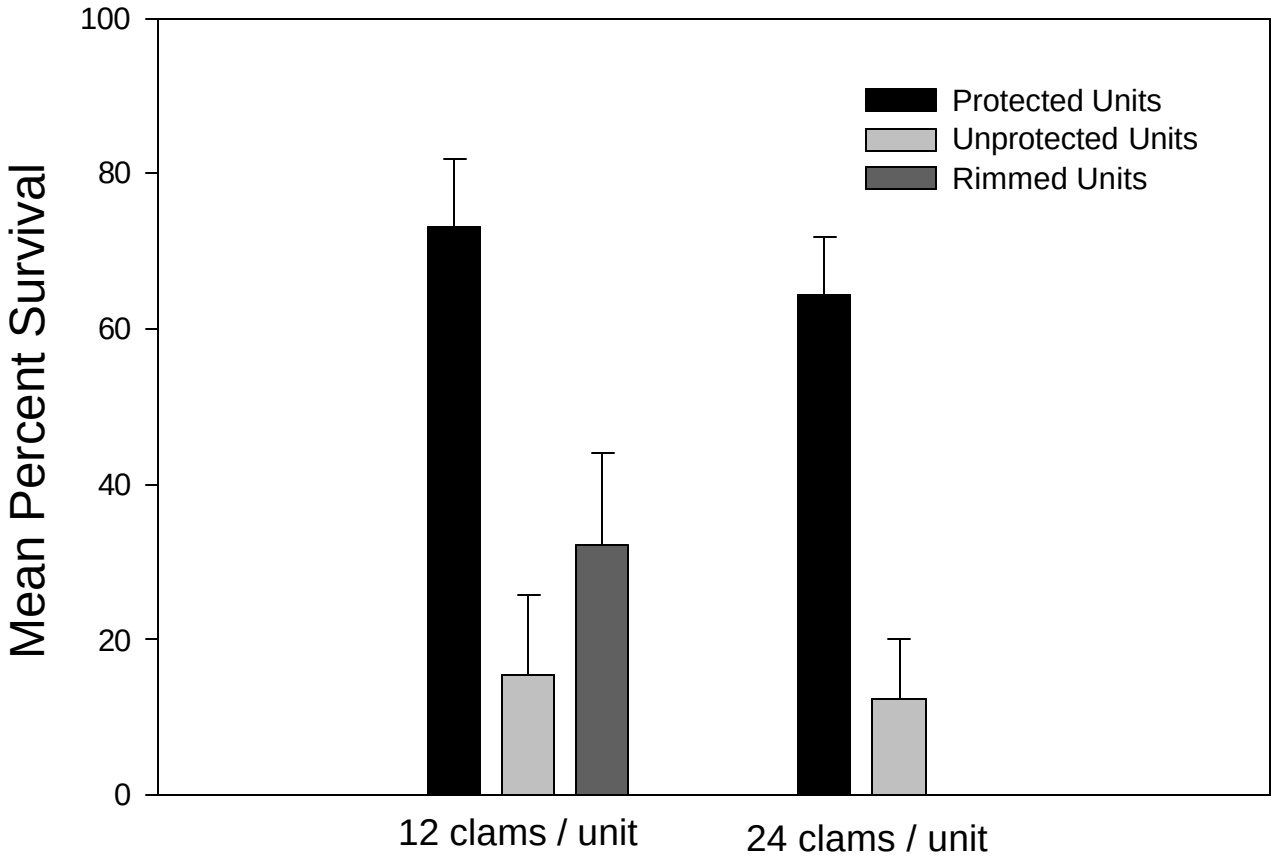

100
80
60
40
20
0
Mean Percent Survival
Protected Units
Unprotected Units
Rimmed Units
12 clams / unit
24 clams / unit

Figure 9.

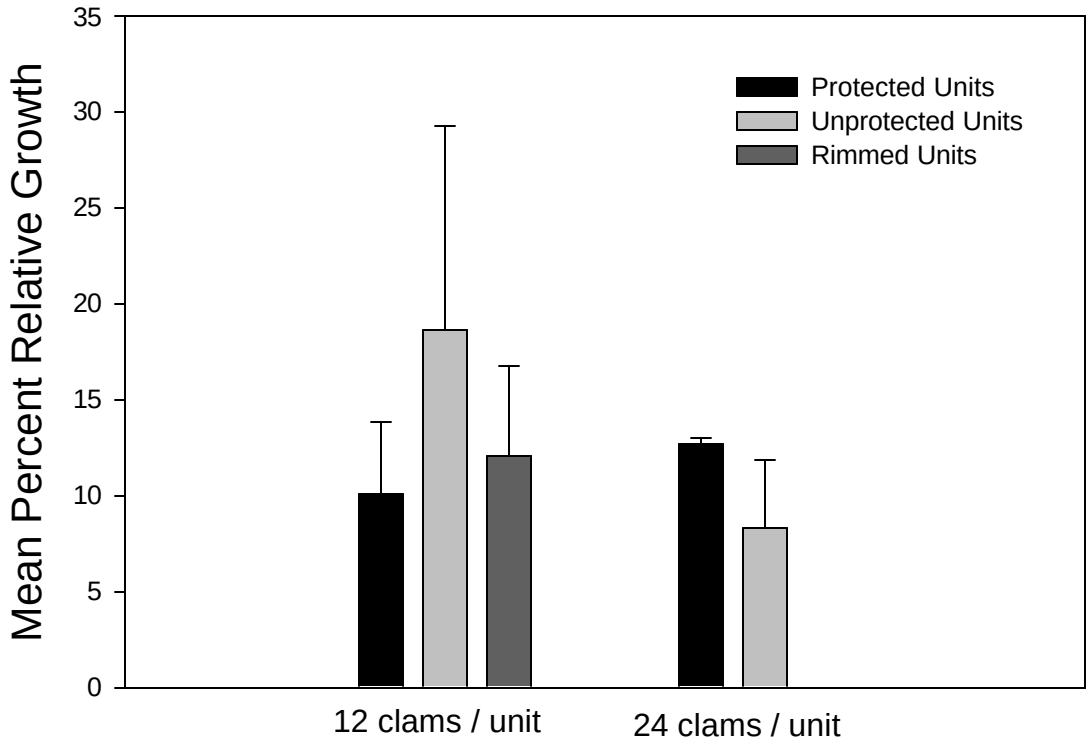

Mean Percent Relative Growth
35
30
25
20
15
10
5
0
Protected Units
Unprotected Units
Rimmed Units
12 clams / unit
24 clams / unit

Figure 10.

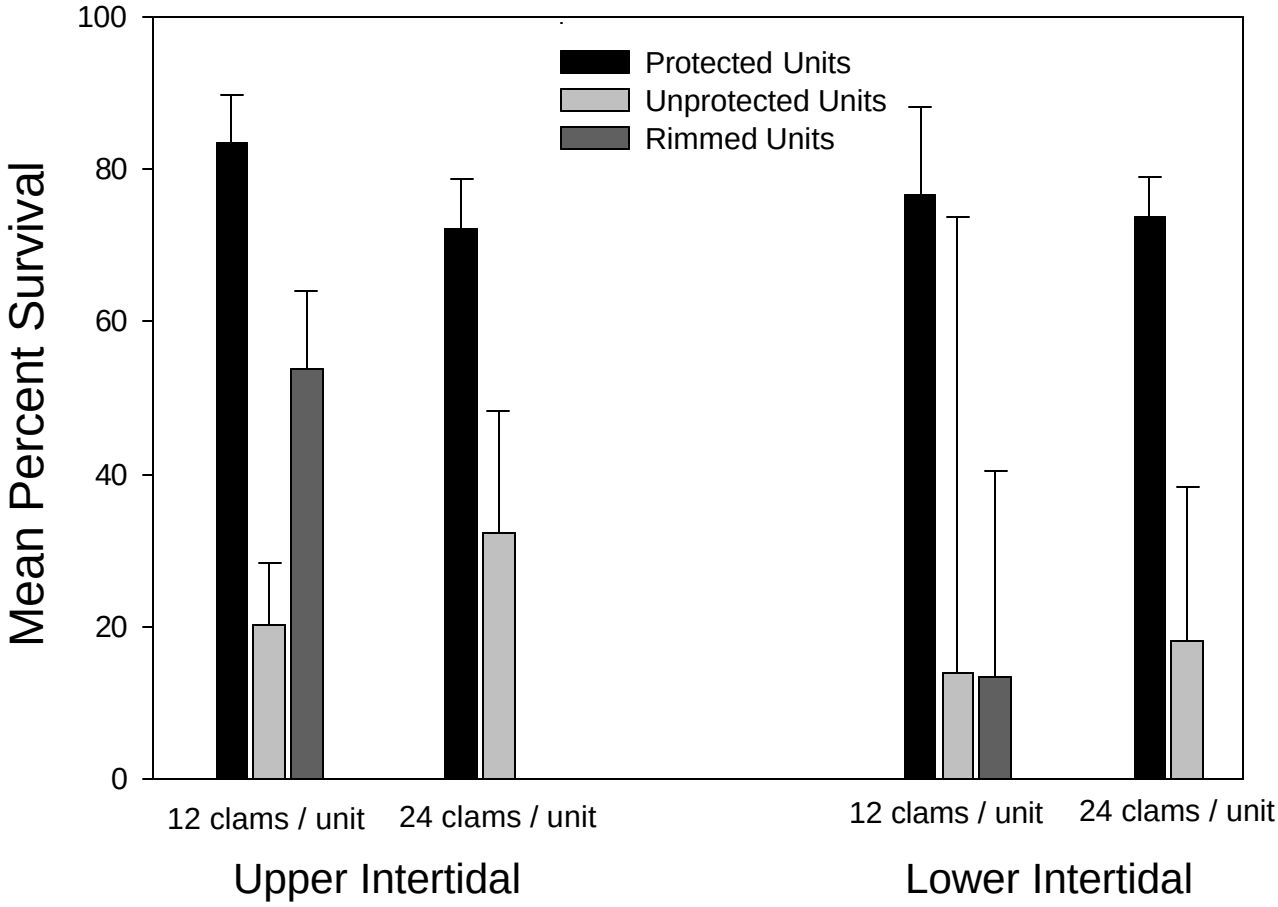

Mean Percent Survival
100
80
60
40
20
0
Protected Units
Unprotected Units
Rimmed Units
12 clams / unit
24 clams / unit
12 clams / unit
24 clams / unit
Upper Intertidal
Lower Intertidal

Figure 11.

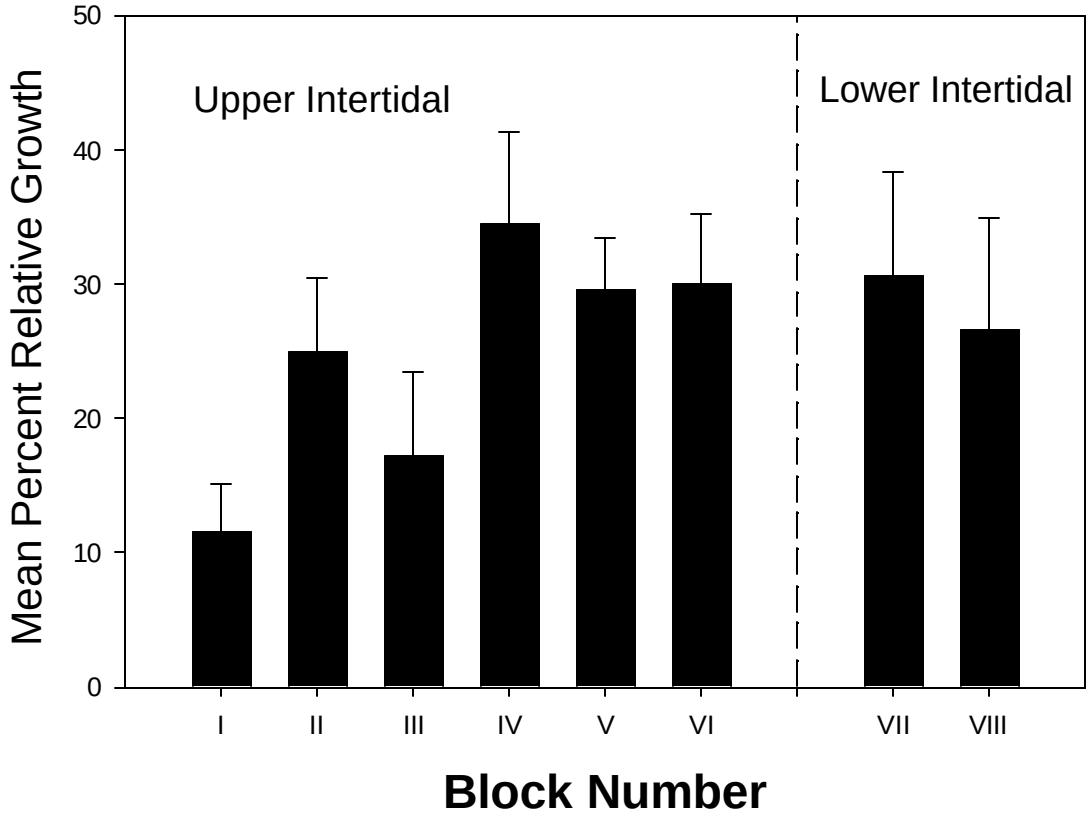

Mean Percent Relative Growth
50
40
30
20
10
0
Upper Intertidal
Lower Intertidal
I
II
III
IV
V
VI
VII
VIII
Block Number

Figure 12.

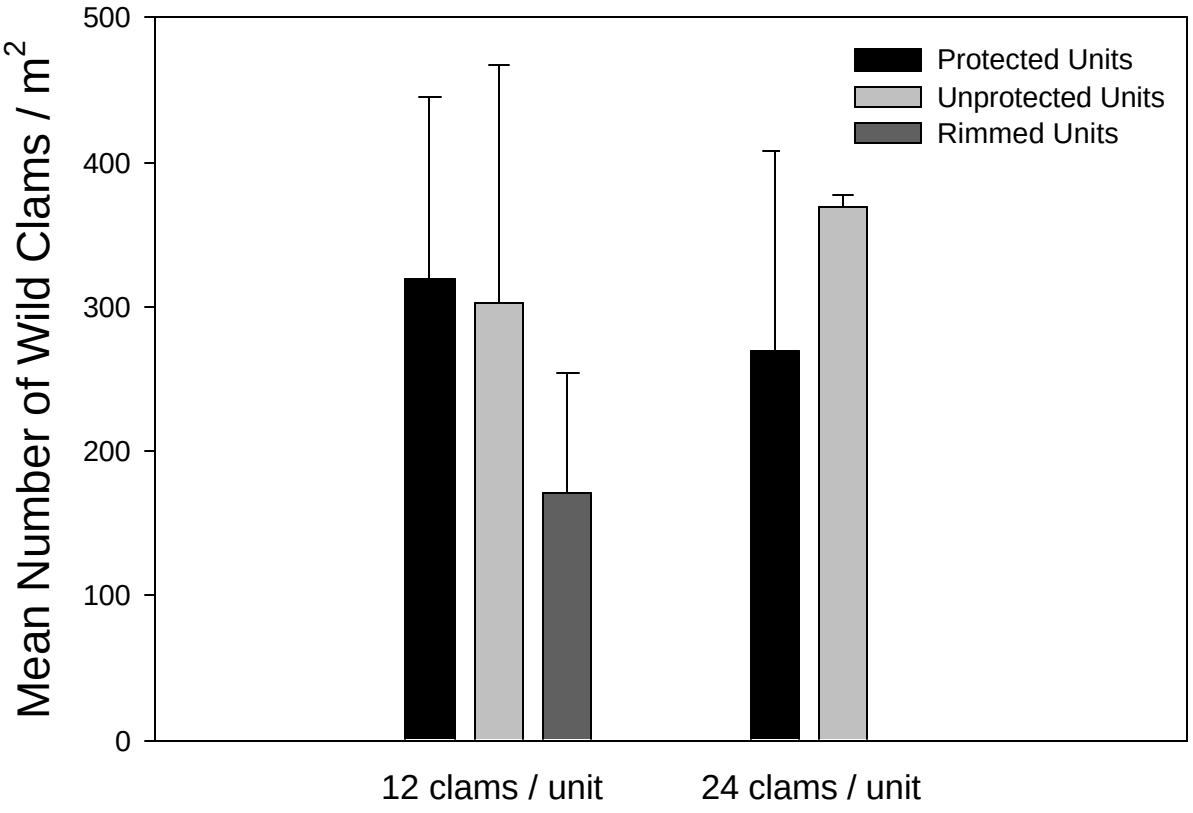

Mean Number of Wild Clams / m2
500
400
300
200
100
0
Protected Units
Unprotected Units
Rimmed Units
12 clams / unit
24 clams / unit

Figure 13.

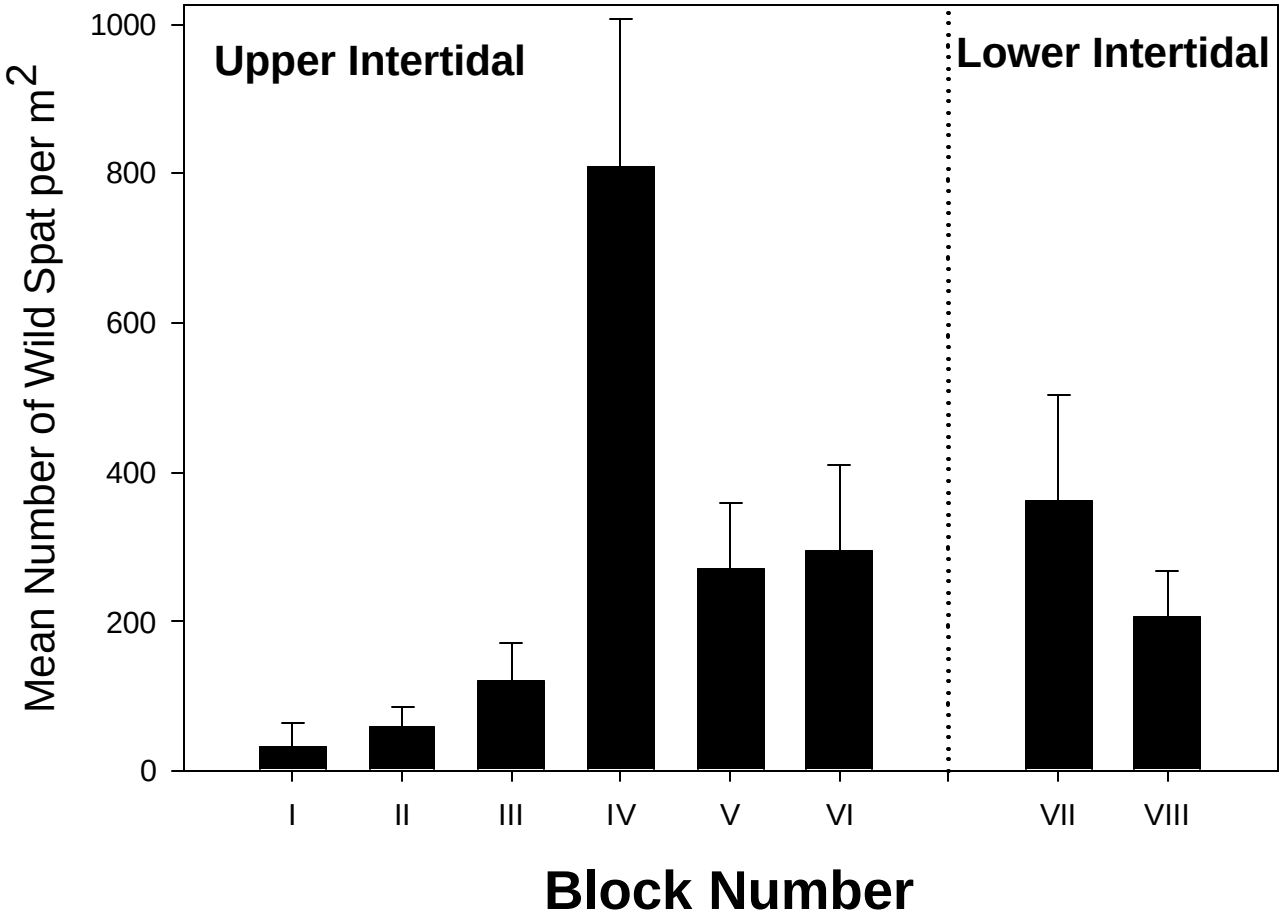

Upper Intertidal
Lower Intertidal
Mean Number of Wild Spat per m2
1000
800
600
400
200
0
I
II
III
IV
V
VI
VII
VIII
Block Number

Figure 14.

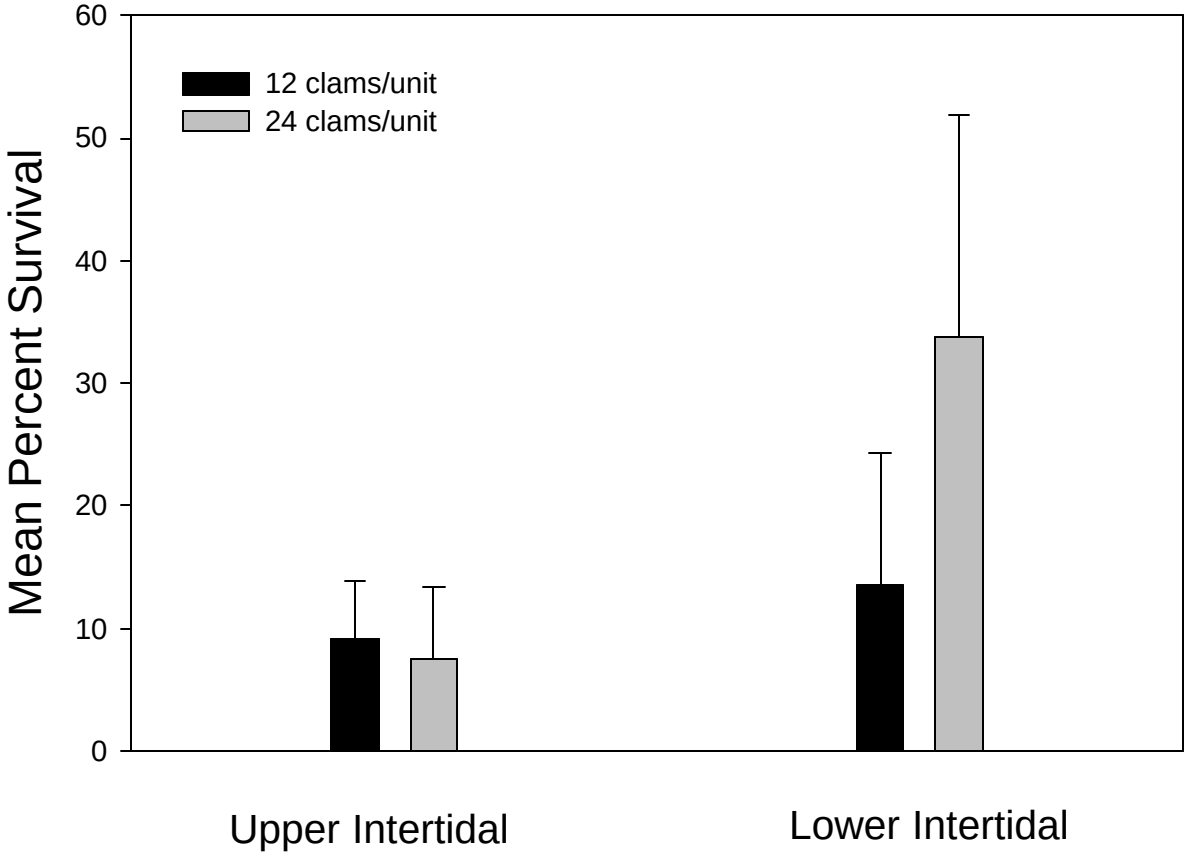

60
50
40
30
20
10
0
Mean Percent Survival
12 clams/unit
24 clams/unit
Upper Intertidal
Lower Intertidal

Figure 15.

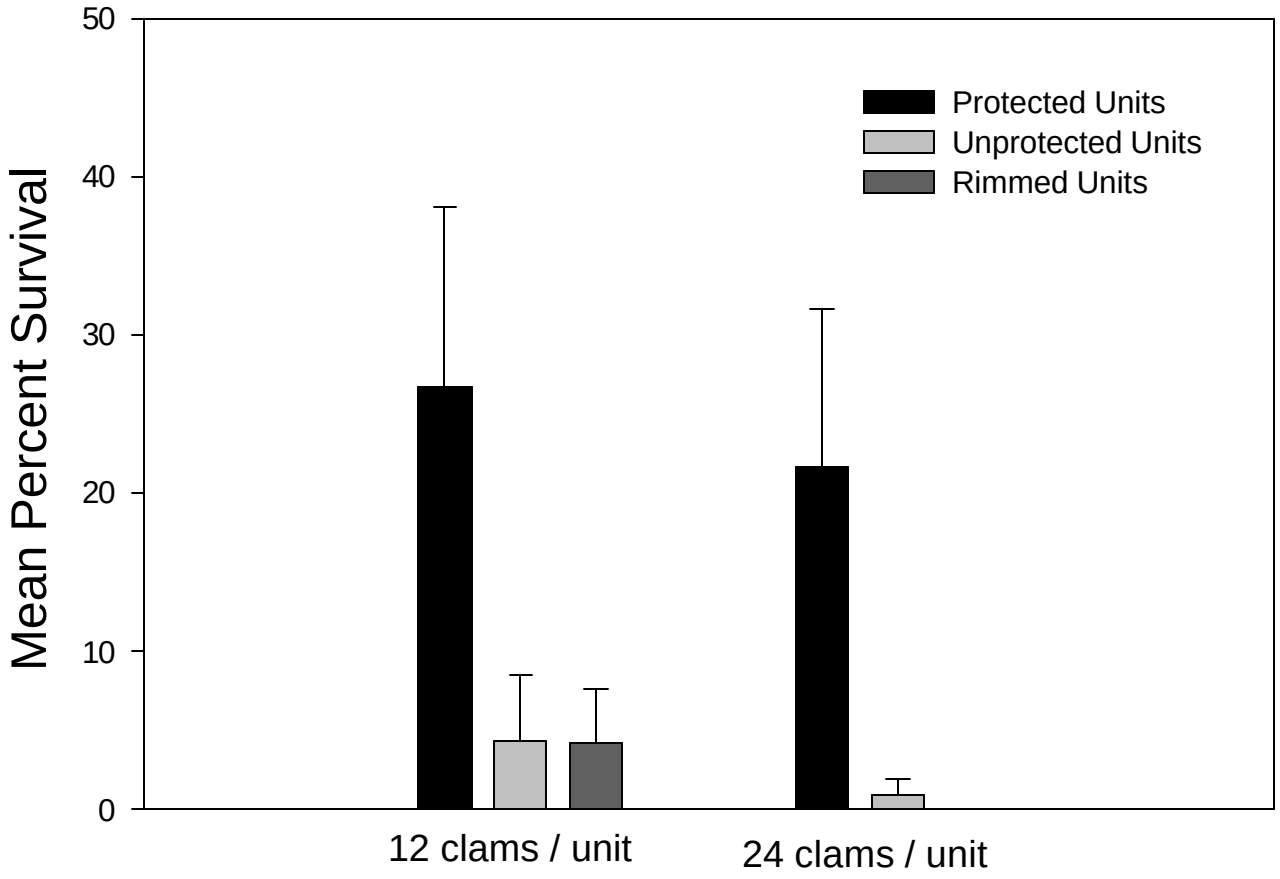

Mean Percent Survival
50
40
30
20
10
0
Protected Units
Unprotected Units
Rimmed Units
12 clams / unit
24 clams / unit

Figure 16.

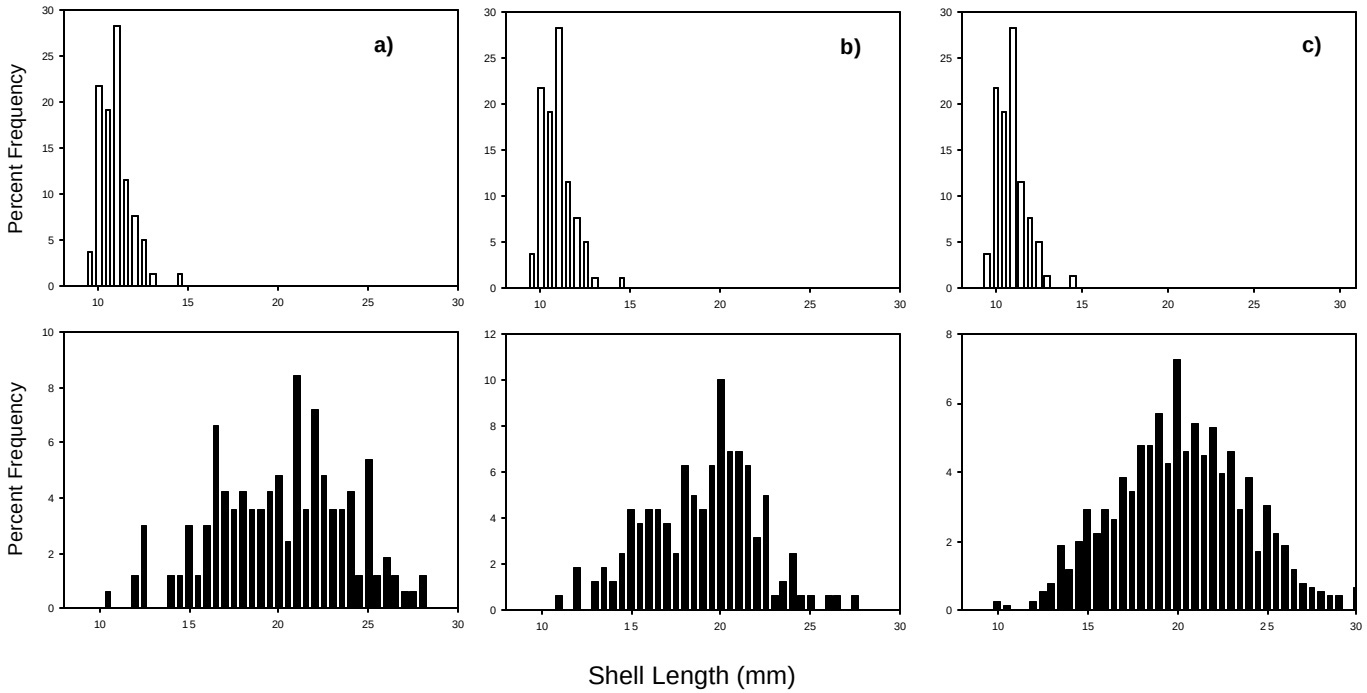


Figure 17.

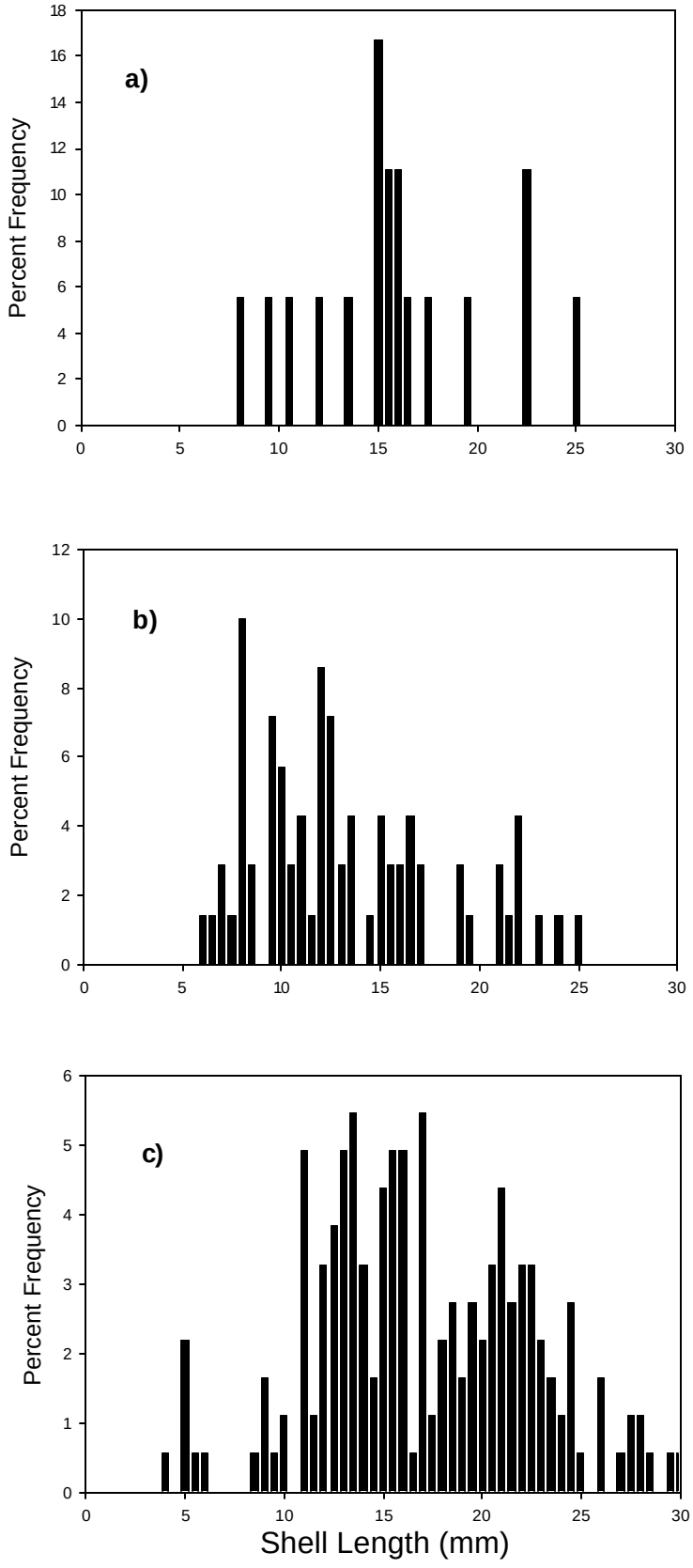


Figure 18.

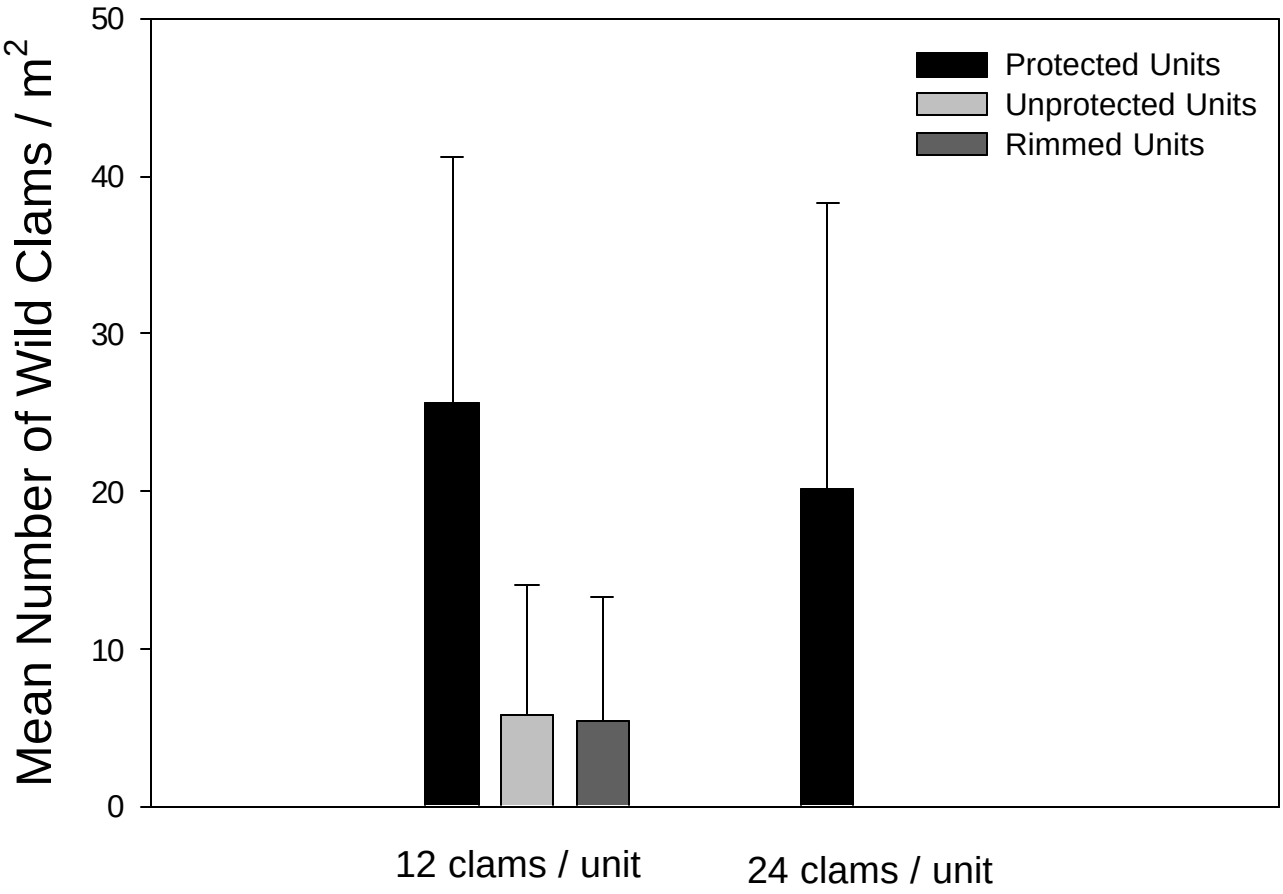

Mean Number of Wild Clams / m2
50
40
30
20
10
0
Protected Units
Unprotected Units
Rimmed Units
12 clams / unit
24 clams / unit

Figure 19.

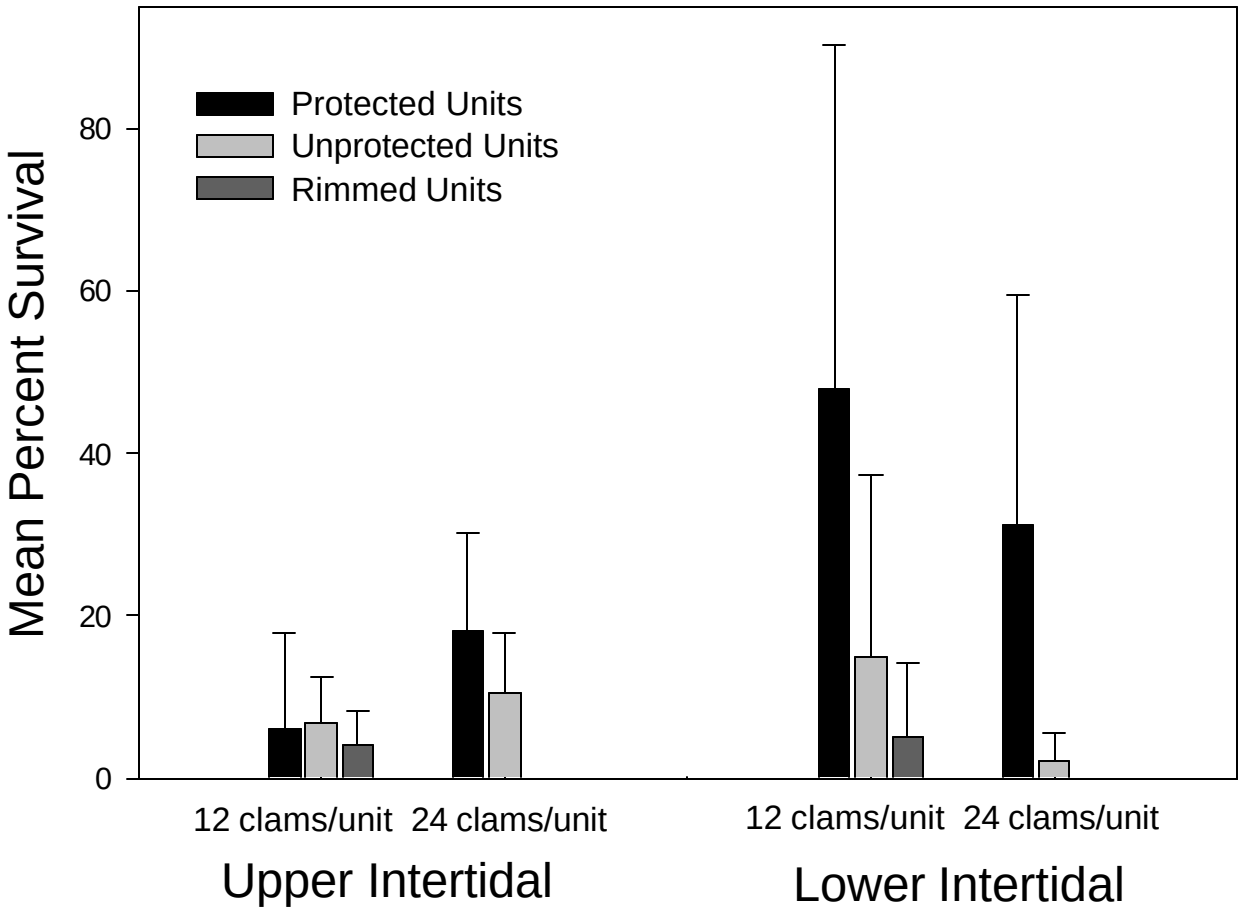

Protected Units
Unprotected Units
Rimmed Units
Mean Percent Survival
0
20
40
60
80
12 clams/unit
24 clams/unit
12 clams/unit
24 clams/unit
Upper Intertidal
Lower Intertidal

Figure 20.

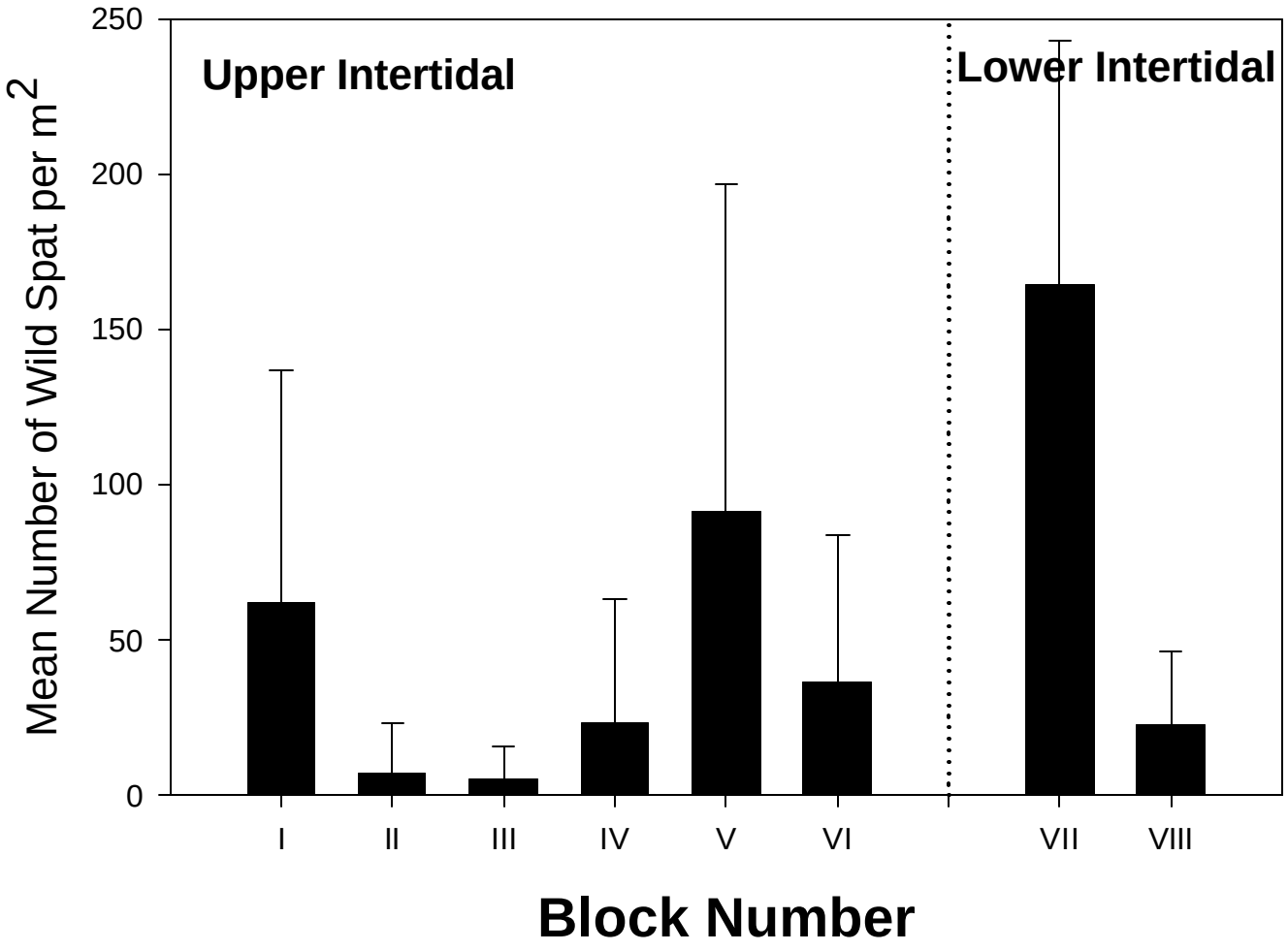

Upper Intertidal
Lower Intertidal
Mean Number of Wild Spat per m2
250
200
150
100
50
0
I
II
III
IV
V
VI
VII
VIII
Block Number

Figure 21.

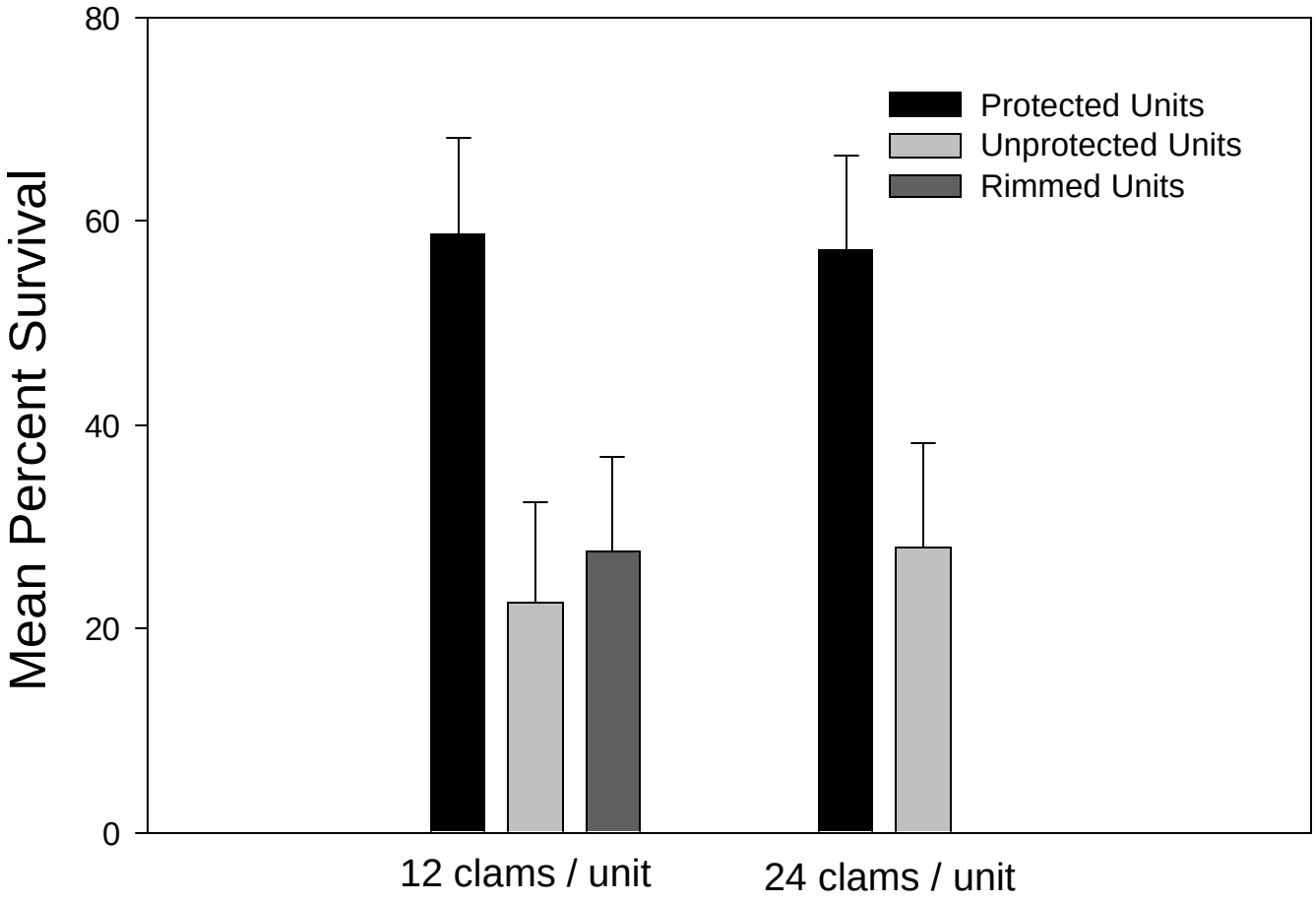

80
60
40
20
0
Mean Percent Survival
Protected Units
Unprotected Units
Rimmed Units
12 clams / unit
24 clams / unit

Figure 22.

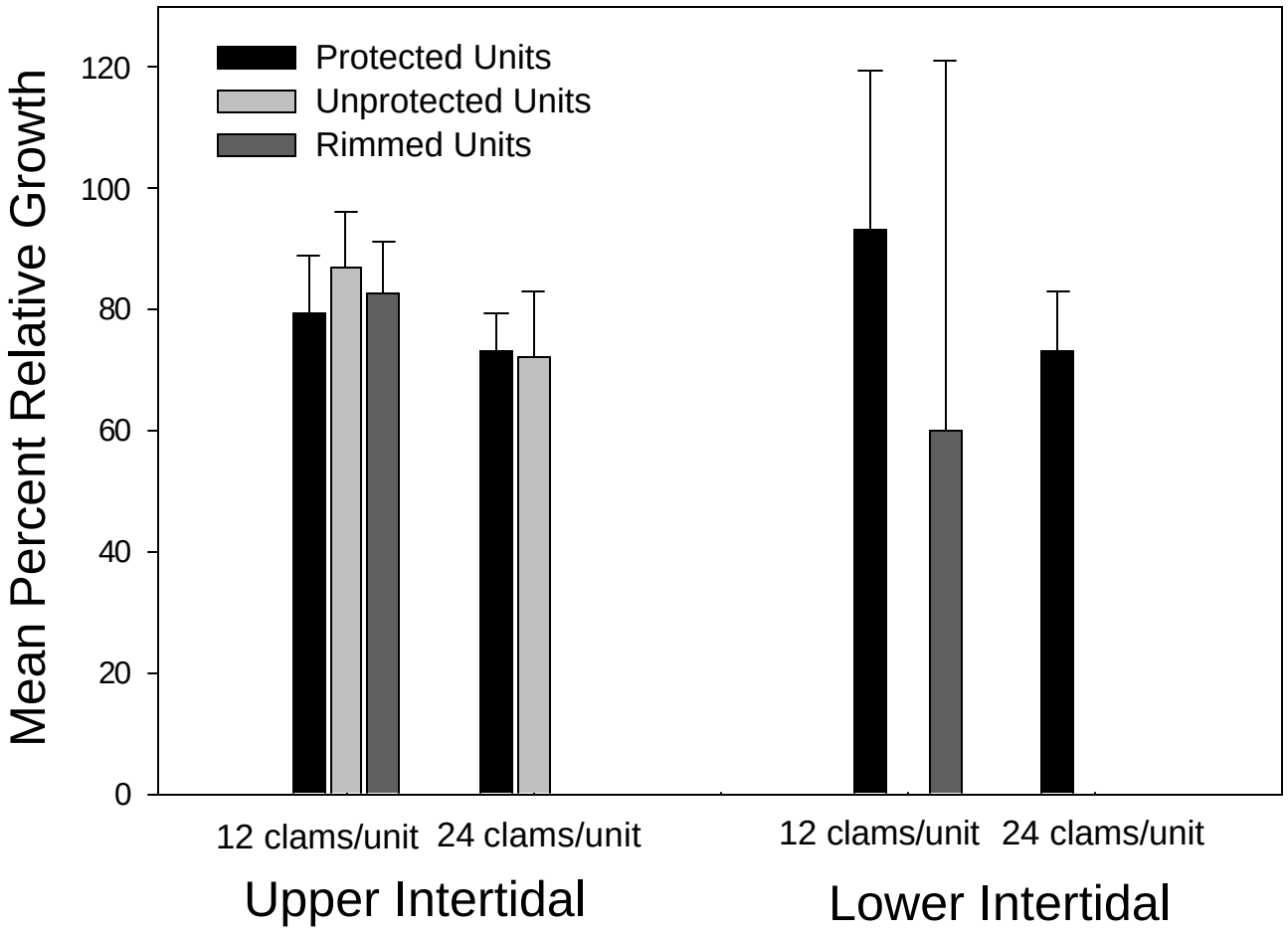

Protected Units
Unprotected Units
Rimmed Units
Mean Percent Relative Growth
120
100
80
60
40
20
0
12 clams/unit
24 clams/unit
12 clams/unit
24 clams/unit
Upper Intertidal
Lower Intertidal

Figure 23.

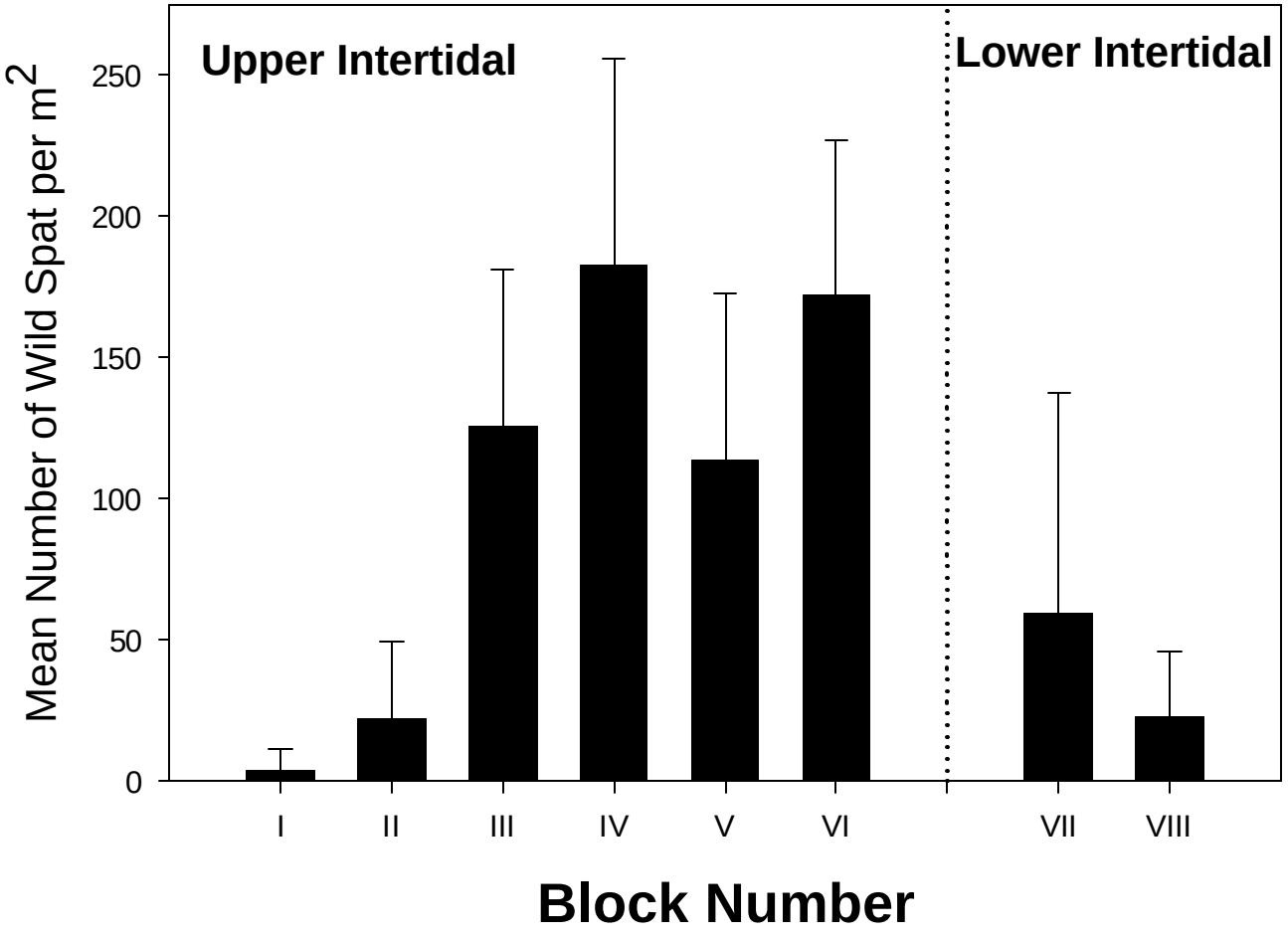

Upper Intertidal
Lower Intertidal
Mean Number of Wild Spat per m2
250
200
150
100
50
0
I
II
III
IV
V
VI
VII
VIII
Block Number

Figure 24.

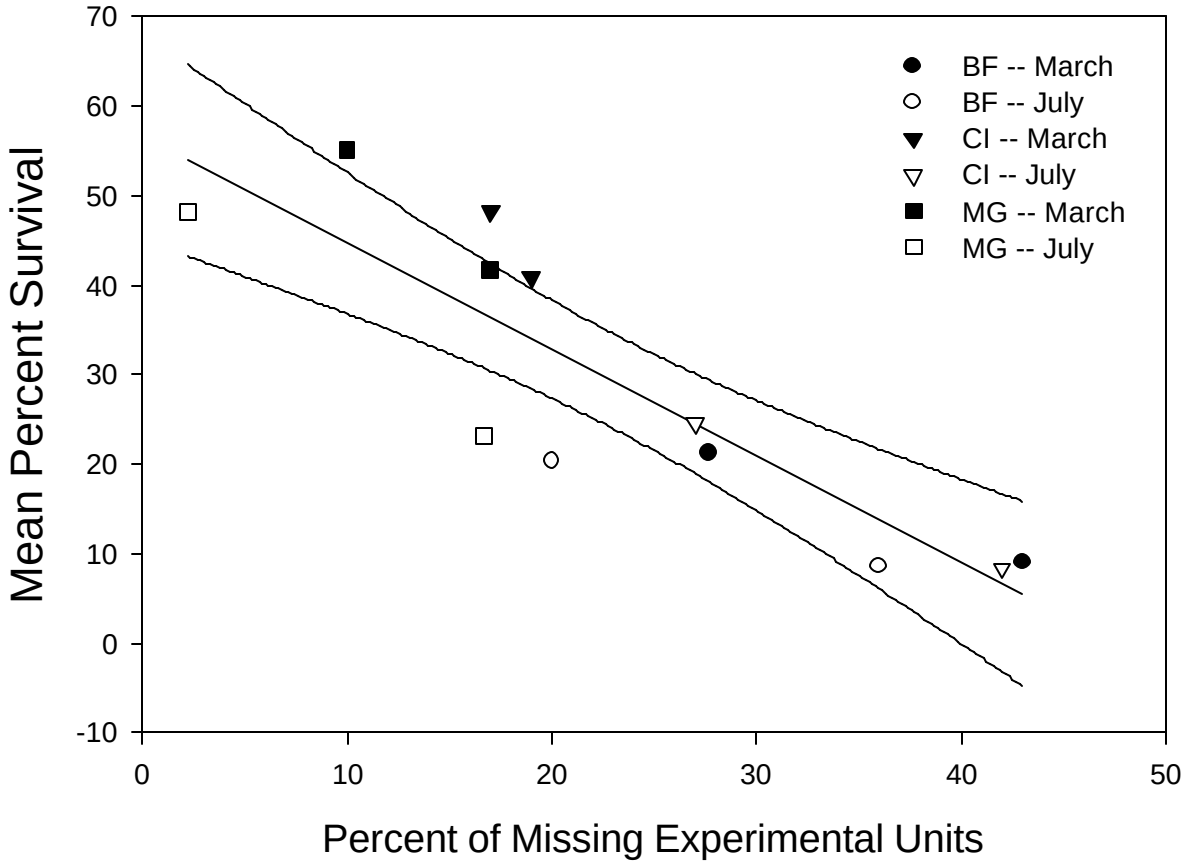

Mean Percent Survival
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
Percent of Missing Experimental Units
BF -- March
BF -- July
CI -- March
CI -- July
MG -- March
MG -- July

Figure 25.

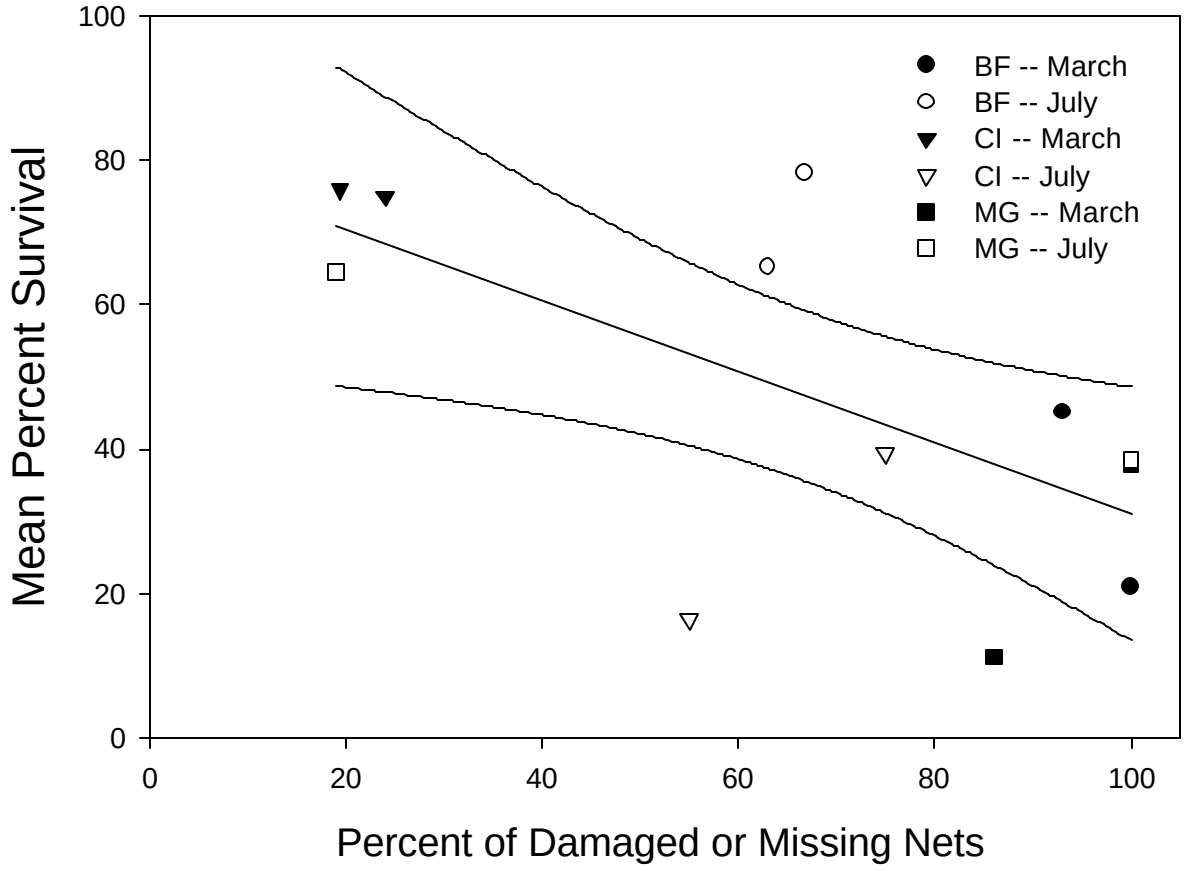

Mean Percent Survival
Percent of Damaged or Missing Nets
0
20
40
60
80
100
BF -- March
BF -- July
CI -- March
CI -- July
MG -- March
MG -- July

Figure 26.

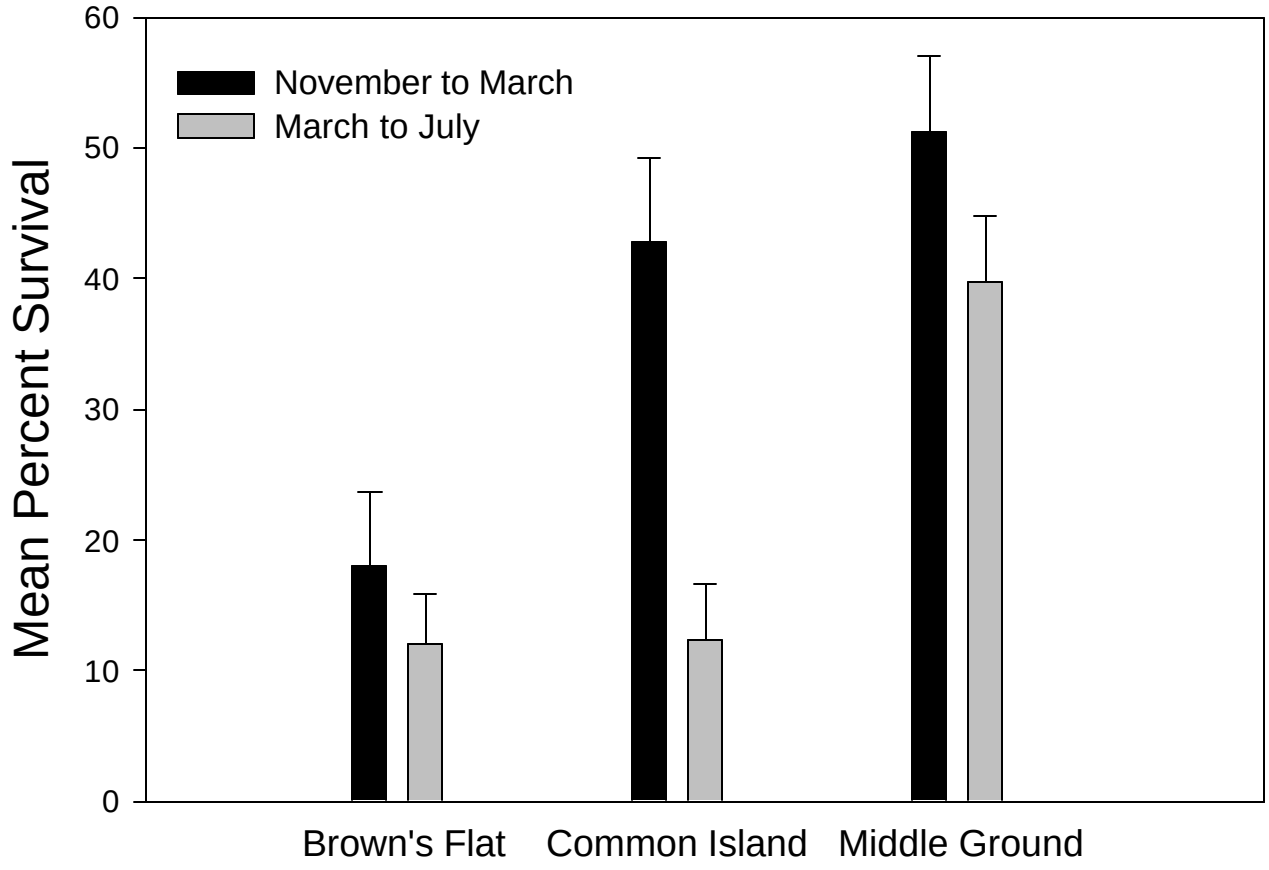
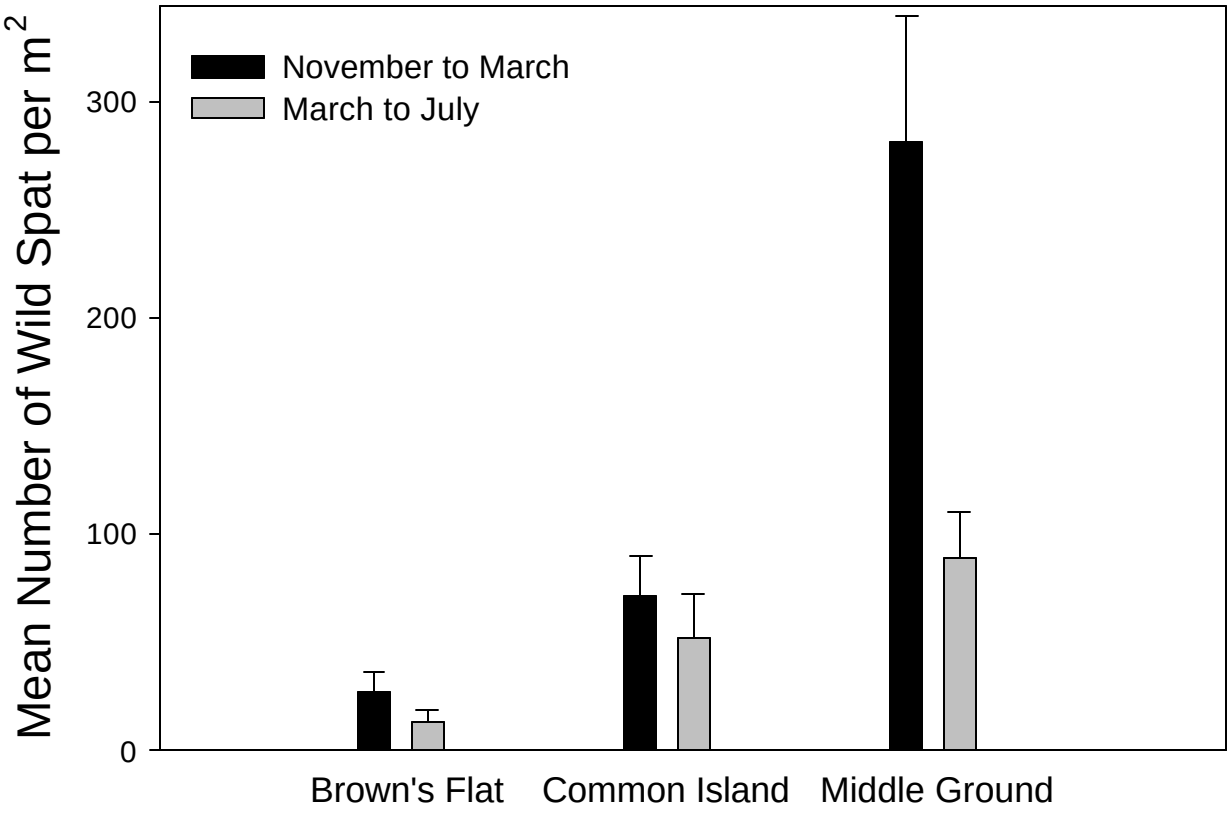

Mean Percent Survival
60
50
40
30
20
10
0
November to March
March to July
Brown's Flat
Common Island
Middle Ground

Figure 27.


Mean Number of Wild Spat per m2
November to March
March to July
300
200
100
0
Brown's Flat
Common Island
Middle Ground

Appendix

Evaluating factors contributing to mortalities of juveniles of the soft-shell clam (*Mya arenaria* L.) in Hampton/Seabrook Harbor, New Hampshire

Revision: 2

November 26, 2002

Prepared by

Phil Trowbridge
Coastal Scientist
NH Estuaries Project/ NH DES

and

Dr. Brian F. Beal
Associate Professor of Marine Ecology
University of Maine and Machias

Project Manager: _____
Signature / Date
Dr. Brian F. Beal, UMM

Laboratory Quality Assurance Officer: _____
Signature / Date
Cem Giray, Ph.D., Micro Technologies

NHEP Program Manager: _____
Signature / Date
Phil Trowbridge, NHEP

USEPA Project Manager: _____
Signature / Date
Jean Brochi, US EPA Region I

USEPA Quality Assurance Manager: _____
Signature / Date
Arthur Clark, US EPA Region I

A2 - Table of Contents

Appendices

A3 - Distribution List

Table 1. QAPP Distribution List

QAPP Recipient Name	Project Role	Organization	Telephone number and Email address
Brian Beal	Project Manager	Univ. of Maine at Machias	207-255-1314 bbeal@maine.edu
Thomas Potter	Chief Financial Officer	University of Maine at Machias	207-255-1200 tpotter@maine.edu
Dr. Cem Giray	Laboratory Liaison	MicroTechnologies	207-737-2637 microtech@wiscasset.net
Jennifer Hunter	NHEP Director	NH Estuaries Project	603-433-7187 jennifer.hunter@rscs.net
Phil Trowbridge	NHEP Coastal Scientist	NHDES Watershed Management Bureau	603-271-8872 603-661-7561 (mobile) ptrowbridge@des.state.nh.us
Jean Brochi	EPA Project Officer (National Estuary Program)	USEPA New England	617-918-1536 brochi.jean@epa.gov
Arthur Clark	EPA Quality Assurance Officer	USEPA New England	617-918-8374 Clark.Arthur@epamail.epa.gov
Bruce Smith	NHF&G Biologist	NHF&G Region 3 225 Main Street Durham NH	603-868-1095

Based on EPA-NE Worksheet #3

A4 - Project/Task Organization

The project manager for this study is Dr. Brian Beal of the University of Maine at Machias. Dr. Beal is responsible for maintaining and distributing the approved QA Project Plan, experimental and sampling designs, fieldwork, enumeration, clam measurement, data analysis, quality assurance, and filing interim and final reports with NHEP.

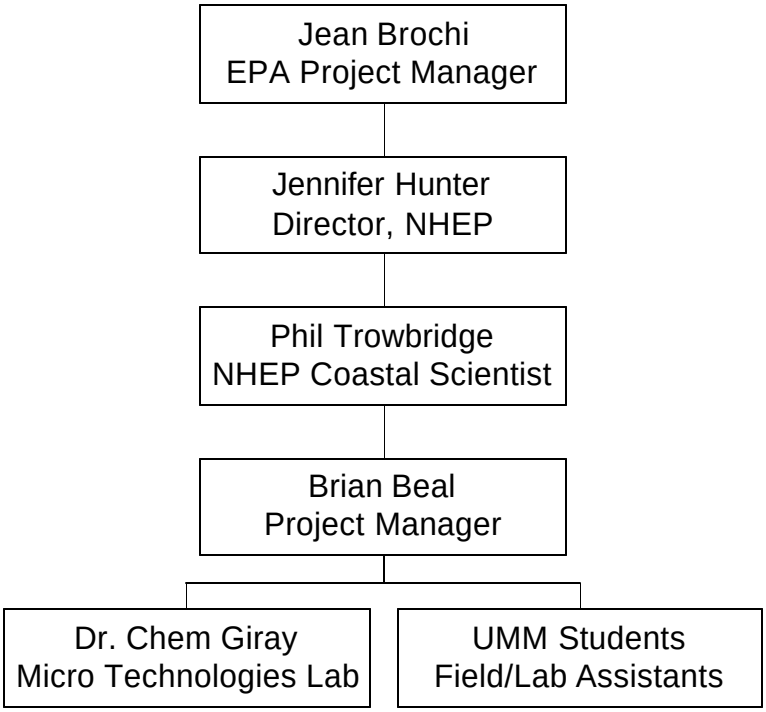
Micro Technologies of Richmond, Maine (Dr. Cem Giray, is the laboratory contact) is being subcontracted to perform disease testing for hematopoetic neoplasia on cultured and wild juveniles of the soft-shell clam, *Mya arenaria* .

Dr. Beal is assisted with field and lab work by students from the University of Maine at Machias.

The principal data users will be personnel at the New Hampshire Estuaries Program and the New Hampshire Fish & Game Department.

The New Hampshire Estuaries Project is funding the study.

Figure 1: Organizational Summary



A5 - Problem Definition/Background

Soft-shell clams, *Mya arenaria* L., represent an important recreational fishery along the New Hampshire coast. During the fall 1998, over 900 clammers easily harvested their 9.5-liter limit when the Middle Ground flats in Hampton/Seabrook Harbor were opened (Varney, 1999). Since that time, clam abundance has dwindled and recent surveys of several Hampton/Seabrook clam flats suggest that the limiting factor for a sustainable fishery is poor juvenile survival. Despite apparent successful reproduction and larval settlement, the population of yearling clams (i.e., age 7-12 months and 26-50 mm shell length) is very low (NHEP, 2001).

Several factors may help to explain the paucity of small clams along these shores. These include: 1) predation by crustaceans such as green crabs and other invasive crustaceans, boring gastropods, fish, or nemerteans (Beal et al., 2001), 2) competition with other bivalves such as mussels (*Mytilus edulis* L.), 3) poor recruitment or slow growth at various tidal heights (Beal and Fegley, 1996), 4) disease (e.g., Brousseau and Baglivo, 1991), 5) winterkill due to ice scour or sea birds (Beal et al., 1995), 6) inorganic toxins such as heavy metals (White and Robertson, 1996), or 7) commercial or recreational shellfish harvesting (Beal and Vencile, 2001).

The New Hampshire Estuaries Project (NHEP) is seeking to fund projects that will "determine the cause(s) of juvenile soft-shell clam mortality in the Hampton/Seabrook Estuary." In addition, the request for proposal (RFP) indicates that the study "should address all possible relevant mortality factors." Any assessment of the causes of mortality among juveniles of *M. arenaria* requires, at the very least, experimental manipulation. Although one can observe that mortalities are more likely to occur in winter, for example, than spring, to ascertain the cause(s) of those winter mortalities requires that various factors (e.g., clam density, excluding predators, etc.) be manipulated over a shore-level gradient and at several sites simultaneously. That is, this project requires two levels of investigation. The first is to quantify

observations about the distribution, abundance, and health of juvenile clams at several sites within the Hampton/Seabrook Estuary (HSE) through time. The second is to use those observations to formulate testable hypotheses to help determine the source(s) of clam mortality.

A6 - Project/Task Description

The following work tasks have been specified for this project in the contract between the NHEP and UMM.

1. REVIEW RECENT SURVEYS OF CLAM FLATS IN THE HAMPTON-SEABROOK ESTUARY (HSE)

   Before initiating field sampling or experiments, the project director will review recent assessments of the clam flats in the HSE by Seabrook Station, NH Fish & Game Department (NHF&G), and NHEP.

2. MEET WITH NHEP AND NHF&G STAFF

   Before initiating field sampling or experiments, the project director will meet with NHEP, NHF&G, and other agency staff in Portsmouth NH to discuss the project and which locations on the clam flats should be used for field sampling and manipulative experiments.

3. OBTAIN PERMIT FROM NHF&G

   Before initiating field sampling or experiments, the project director will obtain a scientific permit from NHF&G Region 3 to harvest soft-shell clams from the HSE.

4. PREPARE QUALITY ASSURANCE PROJECT PLAN

   Before initiating field sampling or experiments, the project director will prepare a Quality Assurance Project Plan. This plan must be approved by Quality Assurance staff from EPA Region I.

5. CONDUCT TWO BENTHIC SAMPLING EVENTS

   In November 2001 and March 2002, the project director will organize intensive benthic sampling at a minimum of two intertidal sites in HSE (to be chosen in consultation with NHEP and other state agency staff) using coring devices (0.02 m^2) followed by washing samples through a 0.5 mm mesh. The sampling design will be a generalized randomized complete block design where multiple samples are taken within blocks along each tidal height.

6. CONDUCT MANIPULATIVE FIELD EXPERIMENT

   A manipulative field experiment using hatchery-reared soft-shell clam juveniles will be initiated at three intertidal sites within the estuary (to be chosen in consultation with NHEP and other agency staff). Animals (8-12 mm) will be planted in 6-inch diameter x 6-inch deep plastic plant pots at two stocking densities (660 or 1320 individuals per m^2). At each site and tidal height (high and mean low), five blocks containing three replicates of each of the four treatments (660 per m^2 unprotected; 660 per m^2 protected [6.4 mm flexible netting]; 1320 per m^2 unprotected; 1320 per

m^2 protected) will be deployed. The experiment will be initiated in November 2001 and will be terminated in March 2002.

7. CONDUCT SECOND MANIPULATIVE FIELD EXPERIMENT

A second manipulative field experiment using hatchery-reared soft-shell clam juveniles will be initiated at three intertidal sites within the estuary (to be chosen in consultation with NHEP and other agency staff). Animals (8-12 mm) will be planted in 6-inch diameter x 6-inch deep plastic plant pots at two stocking densities (660 or 1320 individuals per m^2). At each site and tidal height (high and mean low), five blocks containing three replicates of each of the four treatments (660 per m^2 unprotected; 660 per m^2 protected [6.4 mm flexible netting]; 1320 per m^2 unprotected; 1320 per m^2 protected) will be deployed. The experiment will be initiated in March 2002 and will be terminated in July 2002.

8. CONDUCT DISEASE TESTING OF SOFT-SHELL CLAMS

A subsample of the clams found within the benthic cores taken in March 2002 will be tested for disease.

9. PERFORM STATISTICAL TESTS OF HYPOTHESES

The project director will test hypotheses concerning the relative importance of several factors influencing the fate of soft-shell clam juveniles.

Major milestones for this project are summarized in the following table.

Table 2. Project Schedule Timeline

Activity	Dates (MM/DD/YYYY)		Product	Due Date
	Anticipated Date(s) of Initiation	Anticipated Date(s) of Completion		
Conduct first benthic coring study	11/01/01	11/30/01	Data for study	11/30/01
Conduct first manipulative experiment	11/01/01	03/31/02	Data for study	03/31/02
Present interim findings to NHEP	06/01/02	06/30/02	Presentation to NHEP Management Committee and quarterly report	6/30/02
Conduct second benthic coring study	04/01/02	04/30/02	Data for study	04/30/02
Conduct second manipulative experiment	04/01/02	07/31/02	Data for study	07/31/02
Present final report to NHEP	08/01/02	12/31/02	Final report to NHEP	12/31/02

A7 - Quality Objectives and Criteria

The majority of this study involves statistical tests for differences in clam density and survival at three different sites with different 'treatments. The statistical tests that will be applied are Analysis of Variance (ANOVA). A false rejection rate of 0.05 will be used as a decision rule. Therefore, the data quality objective for the statistical component of this study is to be able to detect differences between treatment blocks.

The only component of this study that involves laboratory analysis is the clam disease testing for neoplasia. The data quality objectives for this test are:

Precision
It is not possible to measure precision for this project because the analytical methods only determine the presence/absence of neoplasia Therefore, laboratory or field duplicates will not be analyzed for this project, and analytical or sampling precision will not be measured.

Accuracy
It is not possible to measure accuracy for this project because the analytical methods only determine the presence/absence of neoplasia.

Representativeness
The sampling design should provide representative data for the each shellfish growing area. As discussed in Section B-1, specimens will be collected from each bed using a randomized sample design.

Comparability
The project should follow standard methods so that the results are comparable to other studies. Therefore, tests for the presence of neoplasia follow the protocols of the Fish Health Section, American Fisheries Society in "Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens" 4th ed, version 1.

Sensitivity/Quantitation Limits
The study should be capable of detecting the presence of neoplasia within a group of 60 clam samples.

Completeness
A total of 6 neoplasia samples are scheduled to be collected/analyzed:
- Cultured clams before they are released into the field (November 2001, March 2002);
- Wild clams from the experimental unit sites (March 2002, July 2002);
- Cultured clams from experimental unit sites (March 2002, July 2002)

For each sample, 60 clams in the 15-50 mm length range must be collected. It is important for the integrity and utility of the data set that 100% of the scheduled neoplasia samples (6 samples) be collected and analyzed.

A8 - Special Training/Certification

Field assistants will be trained by the Project Manager prior to their conducting work on this project.

A9 - Documents and Records

The Project Manager will be responsible for maintaining the approved QA Project Plan and for distributing the latest version of the plan to all parties on the distribution list in section A3. A copy of the approved plan will be on file with the NHEP Coastal Scientist.

Quarterly interim reports and one final report will be produced for the NHEP. This report will be available to the public in hardcopy from the NHEP and its abstract (in text form only) will be included in the NHEP tracking database. See section C2 for the reporting schedule and details. The final report will be in the form of a manuscript that could be submitted to a marine ecology scientific journal for publication.

GROUP B: DATA GENERATION AND ACQUISITION

B1- Sampling Process Design (Experimental Design)

The work will begin in mid-October 2001 with a review of recent surveys of the Hampton/ Seabrook Estuary (HSE) clam flats that have suggested to some that the limiting factor for a sustainable fishery is juvenile survival. This review will be followed by meetings with members of the New Hampshire Estuaries Project and other natural resource agency people who are knowledgeable about the status of soft-shell clams in the HSE.

These meetings will be followed approximately one month later by intensive benthic sampling of a minimum of two intertidal sites within the Estuary (to be chosen in consultation with Estuary Project members and other agency members) using coring devices (0.02 m^2) followed by washing samples through a 0.5 mm mesh. The sampling will allow a pre-winter assessment of the distribution and abundance of small clams (and other infaunal residents) within and between tidal gradients at the study sites. A post-winter sampling of the same sites and tidal heights will be conducted in March 2002. The sampling protocol on both dates will examine horizontal (i.e., within a tidal height) and vertical (i.e., between tidal heights) variability in soft-shell clam numbers. The sampling design will be a generalized randomized complete block design (GRCBD, *sensu* Underwood, 1997) where multiple samples are taken within blocks along each tidal height. Several studies have shown that clam recruitment varies along shore-level gradients due to hydrodynamic and other physical factors (Matthiessen, 1963; Emerson and Grant, 1991). A subsample of clams found within the cores will be set aside for disease testing (i.e., hematopoetic and gonadal neoplasms). Samples will be analyzed through subcontracts with laboratories agreed to by the investigator and NHEP Project Managers.

A series of manipulative field experiments using hatchery-reared soft-shell clam juveniles produced at the Beals Island Regional Shellfish Hatchery (BIRSH) will be initiated at Middle Ground, Common Island, and the Confluence Flat near the time when benthic sampling occurs (winter and spring). These experiments will test simultaneously the effects of crowding, predators, and tidal height on clam survival and growth. Animals (8 - 12 mm) will be planted in 6-inch diameter x 6-inch deep (15.2 cm x 15.2 cm) plastic plant pots (Beal, 1994; Beal and Kraus, 2001; Beal et al., 2001) at two stocking densities (12 or 24 individuals $unit^{-1}$ representing approximate stocking densities of 660, and 1,320 m^{-2}). At each site and tidal height (high and mean low), five "blocks" containing three replicates of each of the four treatments (660 m^{-2} unprotected; 660 m^{-2} protected [6.4 mm flexible netting]; 1,320 m^{-2} unprotected; 1,320 m^{-2} protected) will be deployed. This completely factorial GRCBD will result in 120 experimental units at each intertidal site (Blocks = 5; Tidal height = 2; Stocking density = 2; Predator exclusion = 2; n = 3 – 5 x 2 x 2 x 2 x 3 = 120 units). The experiment initiated in November 2001, will be terminated in March 2002. The experiment initiated in March 2002 will be terminated in July 2002. Should additional studies be warranted, similar experiments could be designed to begin in July 2002 and end in November 2002.

Samples will be placed into labeled plastic bags and transported to the University of Maine at Machias. The samples will be stored until they can be individually washed through a 0.5 mm mesh. At that time, clams retained on the sieve will be enumerated and measured (initial length and final length of cultured individuals and the final length of all wild individuals).

Cultured juveniles of *Mya arenaria* will be used for five reasons. First, hatchery-reared animals have been shown to model the behavior (survival and growth characteristics) of similarly sized wild individuals (Beal and Vencile, 2001). Second, animals may be obtained in sufficient quantities and within a narrow size range for large-scale manipulative studies. Third, once added to sediments, cultured individuals develop a unique mark so that they are easily distinguished from wild clams (Beal et al., 1999). Fourth,

small clams are more susceptible to mortality agents and generally grow faster than large clams allowing treatment effects to be detected more easily. Fifth, New Hampshire has begun efforts to enhance intertidal stocks of soft-shell clams. Planting flats with cultured juveniles can be economically efficacious (Beal, 1994). Results from experimental field manipulations, as opposed to correlative studies, have the potential to provide local stewards and fisheries managers greater insights about which factor(s) are important in regulating populations and can be used to design and test new management strategies on larger temporal or spatial scales (Botsford et al., 1997; Lenihan and Micheli, 2000).

The activities described above will enable the applicant to test hypotheses concerning the relative importance of several factors influencing the fate of soft-shell clam juveniles. Combined with systematic sampling to quantify distribution and abundance of small clams within the Estuary before and after winter, and tissue samples to estimate levels of disease, these efforts should help to identify causes of mortality of young clams so that appropriate restoration/enhancement projects can be pursued.

B2 - Sampling Methods

Three populations of clams are collected for neoplasia testing.

- The first population is wild clams taken from benthic cores or those that occur in the experimental units. Benthic core samples will be collected in November 2001 and March 2002. Experimental units will be sampled in March 2002 and July 2002.
- The second population of clams sampled for neoplasia are those cultured clams from the experimental units. The wild and cultured clams in the experimental units can be distinguished by the presence of a marking on the shell. Experimental units will be sampled in March 2002 and July 2002.
- The third population of clams sampled for neoplasia are those cultured clams that are to be placed in the experimental units at the beginning of a particular test. These populations will be tested in November 2001 and March 2002 prior to seeding them on the flats.

In each case, the clams chosen for the neoplasia testing are a representative sample from the sizes of available clams in either the experimental pots or in the samples provided by the hatchery. A total of 60 clams in the 5-15 mm size range will be taken for each neoplasia measurement.

B3 - Sample Handling and Custody

Once clams have been removed from the sediments by sieving (a process that takes 1-2 days) the clams are placed in a cold room and are then driven by the Project Manager to the MicroTechnologies Laboratory in Richmond, Maine (a 3.5 hour trip one-way) the next day. So, for example, if clams were sampled on a Monday from a flat in NH, they would be taken to Richmond on a Wednesday or Thursday. During that time, the animals are kept in a chilled (5oC) environment. The lab will only test live animals. If too many of the animals are dead, the laboratory will contact the Project Manager before conducting any analyses.

The lab prepares the clams for histology on the day they are delivered. See Appendix A for the laboratory SOPs for neoplasia testing.

B4 - Analytical Methods

Tests for the presence of neoplasia follow the protocols of the Fish Health Section, American Fisheries Society in "Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens" 4th ed, version 1. See Appendix A for additional information.

B5 - Quality Control

Any QC procedures specified for neoplasia tests in the protocols of the Fish Health Section, American Fisheries Society in "Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens" 4th ed, version 1 will be followed.

B6 - Instrument/Equipment Testing, Inspection, and Maintenance
There will not be any equipment used that requires inspection or maintenance.

B7 - Instrument/Equipment Calibration and Frequency
There will not be any instruments or equipment used that require calibration.

B8 - Inspection/Acceptance of Supplies and Consumables

Dr. Cem Giray is the Laboratory Liaison and will be responsible for supplies. Dr. Giray will ensure that all fluids and equipment used for analysis are inspected and deemed acceptable for use. Any fluid that is beyond its expiration date will be replaced prior to analysis.

B9 - Non-direct Measurements

No other data than those collected here are needed for project implementation.

B10 - Data Management

Data Recording Procedures: Field data will be recorded on standardized field data sheets. When completing these forms, the field staff will be sure that all entries are legible.

Data Entry Procedures: Data entry will be checked using two methods. First, the entire data set will be printed and checked against the entries in each data sheet by the Project Manager. Second, the Project Manager will use box-plots and other graphical tools (such as residual plots) to determine if there are outliers in the data set. If a potential outlier is discovered, the Project Manager will go back to the data sheet and then to the entered data and determine whether the outlier is a data-entry error or whether it was recorded as such on the data sheet.

Data Management: All data from the experiment will be maintained by the Project Manager. Data include mean estimates and 95% confidence intervals for natural densities, survival of experimental clams, growth rates of experimental clams, and percent of organisms tested for neoplasia. Data will be stored electronically in spreadsheets or SAS datafiles. Management of hardcopy data and documents is described in Section A9.

Data Analysis: Data will be analyzed using ANOVA and a type I error rate of 0.05 will be used as a decision rule. Each hypothesis tested using ANOVA will be in the form of a rejection or a failure to reject a null hypothesis. This is consistent with data analysis in the field of marine ecology.

GROUP C: ASSESSMENT AND OVERSIGHT

C1 - Assessments and Response Actions

The Project Manager will evaluate the sample collection methodology both during and after the project. Unanticipated problems with the procedures will then be addressed to avoid difficulties during subsequent sampling efforts.

C2 - Reports to Management

Reports will be submitted to the NHEP according to the following schedule from the NHEP-UMM contract:

1. Interim report #1 on the project status plus an invoice for approved project costs - December 31, 2001 (two copies)

2. Interim report #2 on the project status plus an invoice for approved project costs - March 31, 2002 (two copies)

3. Interim report #3 on the project status plus an invoice for approved project costs - June 30, 2002 (two copies)

4. Interim report #4 on the project status plus an invoice for approved project costs - September 30, 2002 (two copies)

5. Final Report upon the completion of all tasks in Section 2-E (Work Tasks) plus a final invoice for approved project costs. The final report shall describe the mortality factors investigated, methodologies employed, a presentation and discussion of results, and conclusions. The final report should also present quantitative estimates of the contribution of each factor to overall juvenile clam mortality - December 31, 2002 (five copies and one unbound original)

For information about where the data will be stored and the format of graphics and hardcopy reports, please see section A9.

GROUP D: DATA VALIDATION AND USABILITY

D1 - Data Review, Verification, and Validation

The data from MicroTechnologies and field data sheets will be verified by the Project Manager to ensure that the data quality objectives from Section A-7 are met.

D2 - Verification and Validation Methods

The process by which data will be verified will involve one or more of the following:

1. The project manager will check documents along the chain-of-custody for completeness and consistency.
2. At the end of each field season, the project manager will evaluate whether the data quality objectives stated in section A-7 of this plan are being met.
3. The project manager will discuss discrepancies or anomalies in the data with field assistances or the contract laboratory.
4. If discrepancies cannot be resolved, appropriate measures will be taken. These measures could include but are not limited to:
    a. rejection and exclusion of data from reports with an explanation.

b. re-sampling the appropriate station.

D3 - Reconciliation with User Requirements

Any problems with the data analysis and interpretation will be reconciled by the Project Manager after consultation with New Hampshire Estuaries Program staff.

Data will be generated based on the quality objectives defined in section A7 and verified according to section D2. Limitations in the data will be clearly defined for potential end users in all reports produced.

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