USDA SBIR FINAL REPORT

PHASE I

Project Title: Evaluating subtidal and intertidal grow-out methods for cultured hard clams in eastern Maine: A series of manipulative field experiments

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Acknowledgments

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Because of the nature of the field site at Goose Cove (shallow subtidal) in Trenton, Maine where 80% of the work was conducted for the Phase I studies, we were limited to only a few days each month (both during the initiation of the experiments in Spring and Summer and the sampling during the Fall and early Winter) that we could work to set up experiments or sample them. This required us to enlist the help of many individuals to help us complete the work at both the beginning and end of the experiments. Therefore, we acknowledge and thank the following for their diligence, patience, and hard work to help us initiate and sample the field experiments: Cody Jourdet, Kyle Pepperman, Jennifer Reynolds, Ariel Harris-Porada, George Protopopescu, and students in the Fall BIO 360 marine ecology course at the University of Maine at Machias – Ashley Couture, Satan Soule, Kevin Flanagan, Summer Meredith, Stephen Diegleman, Stephanie Mathias, Janyne Pringle, and Michael Oram. In addition, we received assistance in the laboratory measuring clams and with data entry. We gratefully acknowledge those who assisted us with this portion of the studies: Cody Jourdet, Kyle Pepperman, Ariel Harris-Porada, Emily Zimmerman, Michelle Block, and George Protopopescu.

Executive Summary

Hard clams, *Mercenaria mercenaria* (L.), occur at very low densities from the low intertidal to shallow subtidal zone in Maine's easternmost counties of Washington and Hancock where they support a small, commercial fishery (ca. 5-10 seasonal harvesters). The potential exists here to develop farming techniques on leased grounds to produce commercial quantities of littlenecks and top necks to fill existing orders locally, and expand business into the mid-coast and southern Maine. Hard clams (also known as quahogs, top necks, little necks, and cherrystones) are popular among out-of-state tourists visiting coastal Maine, but supplies from wild stocks cannot meet existing demands. Preliminary growth studies from a subtidal population of wild hard clams near Trenton, Maine (near Bar Harbor) indicate that 3 to 4+ years are required for animals to reach market size. Growth rates may be faster with selective breeding; however, no information exists concerning field grow-out of cultured juveniles in cold-water environments.

Bagaduce River Oyster Company, Egypt Bay Aquafarms, and the Downeast Institute for Applied Marine Research and Education (DEI) investigated the efficacy of several strategies for growing cultured hard clam seed (6-10 mm SL) at four field sites in eastern Maine from May to December 2009. Grow-out techniques complimented those used successfully to culture individuals of *Mercenaria mercenaria* in other coastal states in the Mid-Atlantic and New England. We followed an experimental approach to examine field grow-out techniques during the first full growing season that would enable us to determine the most efficient methodologies to eventually grow animals to market size. Because Maine law does not restrict the size of cultured shellfish, it may be possible to develop, as has been reported from South Carolina (see MacKenzie et al. 2002), local markets for hard clams that are smaller than little necks (i.e., 25 mm thick) such as “pasta necks” (16-19 mm thick) or “petite necks” (20-22 mm thick).

Since January 2006, Egypt Bay Aquafarms has worked with the state of Maine’s Department of Marine Resources (DMR) to provide a foundation for a follow-on Phase II R&D effort from the same group of businesses. On 22 June 2007, DMR Commissioner George LaPointe approved Egypt Bay’s application for three contiguous 2-acre subtidal leases at Goose Cove, Trenton, Maine for the purpose of conducting research and developing culture techniques to farm hard clams. Portions of each lease site were used during the Phase I field trials, as well as several public areas east of Goose Cove. We anticipate the most promising results will be used to scale the activities to commercial levels during the Phase II effort. In addition, in November 2009, Egypt Bay Aquafarms received permission from the state of Maine for an additional 2-acre subtidal lease within five miles of the site in Trenton. Other applications for sites west of Trenton (Blue Hill Bay) are in the process of being reviewed by officials with the Maine DMR.

The specific objectives of our field investigations during the 2009 growing season were to determine the interactive effects of a) planting date, b) size of seed, c) predator exclusion, and d) stocking density on growth and survival of cultured clams. Generally, we designed our trials with one overarching question in mind: “Is it biologically and economically feasible to grow hatchery-reared juvenile hard clams in eastern Maine during their first growing season?” Results from these field tests provided us with an unambiguous answer: “Yes.” The next question, that will provide the focus of our Phase II effort, is: “Can cultured hard clams be reared to market sizes efficiently and economically?”

The first experiment examined the interactive effects of planting date and predator exclusion on juvenile growth and survival. We placed 500 clams (ca. 9 mm SL) inside 1-m^2 mesh bags constructed of 4.2 mm or 6.4 mm flexible netting. One third of the bags were placed directly onto the benthos of the shallow subtidal of Goose Cove. Another third were covered with a piece of 4.2 mm flexible netting, and the remaining third were covered with a piece of 6.4 mm flexible netting. In addition, 250 clams were added to 0.5 m^2 growout cages constructed of 4.2 mm extruded netting and 6.4 mm flexible netting. Finally, 500 clams were seeded directly onto the surface of the soft sediments within 1-m^2 plots that were protected with flexible netting (4.2 mm or 6.4 mm aperture) or that were left unprotected to serve as controls where predators could have unrestricted access to the small clams. These eleven "treatments" were replicated twice in four "blocks" on each of four planting dates (May, June, July, and August 2009). Blocks were used to assess spatial variability, and whether treatment effects on either clam growth or survival would vary from one location within the site to another. Bags, cages, and plots were sampled in mid-December 2009. Overall, bags (4.2 mm aperture) provided the best survival (92%) and growth. Clams seeded in May averaged 19.3 mm SL in December, while those planted on the other three dates did not differ significantly and averaged 13.3 mm. Clams seeded into sediments that remained unprotected during the experiment grew significantly more slowly than clams in the other ten treatments. Presumably, the presence of predators interferes with hard clam growth, as is the case with cultured individuals of the soft-shell clam, *Mya arenaria* (Beal et al, 2001).

The second experiment investigated how initial clam size, stocking density, and predator exclusion affect juvenile hard clam survival and growth at Goose Cove from May to November 2009. Clams, averaging 5.1 mm, 6.5 mm, and 7.9 mm, were added to 1-m^2 field plots at densities of 400, 500, or 600 individuals m^{-2}. One-third of the plots were covered with a piece of plastic, flexible netting (4.2 mm aperture), one-third were covered with a piece of 6.4 mm flexible netting, and the remaining one-third were left as controls, without netting. We found that predation was intense, particularly in the control plots, and on the smallest clams, where less than half of the animals survived the 162-day trial. Approximately 2.5x more clams were sampled from plots seeded with the two larger two sizes of clams, and no significant differences were observed in numbers from plots seeded with "medium" vs. "large" seed. The presence of netting enhanced hard clam survival by a factor of 2.4. Small clams grew to an average size of only 11.4 mm, whereas large clams attained a mean final SL of 16.7 mm. Clams seeded at the lowest stocking density had a final mean SL that was 5% smaller than the average of the two higher densities, and, although this difference was statistically significant, it is likely not biologically meaningful.

Predators were not excluded from field plots in the third experiment. We asked how the combined effects of planting date (May vs. June), initial clam size (6.3 mm vs. 8.4 mm), and stocking density (400 vs. 500 vs. 600 individuals m^{-2}) influenced juvenile clam growth and survival at Goose Cove. Approximately 75% of the animals had disappeared from the plots by the end of the trial, and this estimate was unaffected by planting date or stocking density. Approximately 45% more clams were sampled from plots initially seeded with the larger vs. smaller clams. Growth was surprisingly slow, regardless of planting date, compared to growth in other field experiments conducted at this shallow subtidal location. Mean final SL for clams planted in June (13.8 mm) was remarkably larger than the mean final SL for clams planted in May (11.1 mm). Stocking density did not play an important role either in survival or growth. This study, in the context of the experiments described above, demonstrates the importance of predator deterrence, and its effect on both clam survival and growth.

To determine how hard clams grow and survive at sites east of Goose Cove, we designed a series of comparative field experiments using smaller experimental units (0.018 m^2 – plastic horticultural pots – See Beal, 2006) that were initiated in May and June 2009. The studies were set up at Goose Cove in Trenton, Egypt Bay in Franklin, East Machias along the Machias River Estuary, and Tide Mill Cove, in Edmunds and within Cobscook Bay. Hard clams do not occur naturally at the two latter sites. For this study, we counted and manipulated a total of 22,320 individuals. In addition to planting date, at each site we tested the interactive effects of five stocking densities from 165 to 2,640 individuals m^{-2}, predator exclusion (no netting; 4.2 mm netting, and 6.4 mm netting), and clam size (6.4 mm vs. 7.7 mm individuals). Three major results occurred: 1) clam survival was low and growth negligible at the two easternmost sites; 2) a low-density refuge occurred (increasing survival with decreasing stocking densities) at each site except in Cobscook Bay, where moon snails consumed approximately 45% of clams planted on both dates; and 3) predators are deterred by flexible netting, and, not surprisingly, highest survival rates were observed for clams in experimental units protected with the smaller aperture netting. The small experimental units are easy to manipulate and can answer very effectively questions about growth and survival from one geographic location to another.

A final field test was undertaken to study the potential interspecific effects on hard clam survival and growth due to the presence juveniles of *Mya arenaria*, a common, commercial bivalve species that co-occurs with Mercenaria at Goose Cove and sites west of that site. This trial occurred east of Trenton in Cutler, Maine, where hard clams do not occur naturally. Cultured soft-shell clams and hard clams of the same approximate sizes were established in small experimental units (0.018 m^2, as described above) at three intraspecific densities (330, 660, and 1,320 individuals m^{-2}), and every factorial combination creating nine interspecific density treatments. Units were covered with a piece of flexible netting (6.4 mm aperture) to deter predators. The experiment ran for 191 days from 24 May to 30 November 2009. No significant effects of either intra- or intraspecific density on hard clam growth or survival were detected; however, growth was negligible, with animals increasing in shell length only 1 mm over that period.

Results of these field experiments provide a refinement of questions for the next phase of investigation, and a clearer direction for future work leading to commercialization. Several overarching results became apparent during the Phase I effort. First, although it is possible to rear hard clams in the colder waters of eastern Maine (i.e., locations east of Goose Cove, in Trenton), it does not appear that this area is amenable for hard clam farming because of poor growth as well as poor survival. Survival rates in protected experimental units was especially poor at the Machias River and Edmunds site, the latter where 45% of clams were found dead with countersunk drill holes due to predation by two species of moon snails. This result encourages us to find and test locations to the west of Goose Cove that are suitable sites for farming hard clams. Second, planting the largest hard clam seed in field plots resulted in the highest survival and largest final SL at all sites. This result will become the primary focus of our

Phase II work, which will examine several different nursery grow-out scenarios at multiple locations. Third, protective netting is essential (at least during the first growing season) to deter predators. The primary predator at Goose Cove was green crabs. This invasive species also was important, and played a large role in hard clam mortality at two of the eastern locations. Netting can be a double-edged sword. It deters predators, but also can entrap them, increase sedimentation, and enhance fouling by blue mussels. Larger aperture netting will reduce these unwanted, indirect effects, but will require the use of larger seed, as we found that small seed does not achieve the same survival success beneath large aperture netting as it does beneath small aperture netting.

Lastly, the results presented here are not, for the most part, ambiguous because adequate replication of treatments was incorporated into our experimental design, and many of the field trials allowed us to determine the extent to which spatial variation played a role in treatment effects. One important aspect of our work to consider in terms of its scope and our ability to scale results to larger, commercial levels is that our effort was confined to a single growing season, and over 80% of the work occurred at a single site (Goose Cove). We cannot know if the 2009 growing season was representative, nor can we be assured that our results at Goose Cove are typical of results at other sites (except for our comparative small-scale study conducted at three other locations east of Goose Cove). Therefore, we intend to continue our experimental approach during Phase II, but will begin with the most promising results from the Phase I effort.

Technical Objectives

The technical questions we addressed were:

1) What time of year (May and June vs. July and August) is the best to plant cultured hard clam juveniles to optimize survival and growth?

2) What size of hatchery seed (6 mm, 8 mm, 10 mm) should be planted to optimize clam survival?

3) Is it possible to exclude or deter predators (e.g., green crabs, *Carcinus maenas*; winter flounder, *Pseudopleuronectes americanus*; lobsters, *Homarus americanus*; rock crabs, *Cancer irroratus*) from seeded areas, and, if so, is the procedure/process cost-effective?

4) What stocking density (400, 500, 600 m^{-2}) will optimize growth and survival?

The USDA SBIR focus area of this work was under the topic of Aquaculture (section 8.7). Specifically, our effort focused on the priorities listed in sub-sections 3 & 4 (Integrated Aquatic Animal Health Management; Improved Production Systems and Management Strategies) by examining methods to control predation in aquaculture production systems and develop management strategies for farming cultured hard clams in areas of coastal U.S. where, heretofore, no grow-out methods or aquaculture production systems have been investigated.

Background

Hard clams are not presently farmed in Maine, but hard clam farming activities in other states along the eastern US coast generate millions of dollars annually in sales that contribute positively to the aquaculture economy. For example, in Virginia, the growth of the cultured hard clam industry has added significant value to that state's seafood marketplace. During 2004, hard clam farmers from Virginia's Eastern Shore sold 150 million market clams valued at $23.9 million (Murray and Kirkley, 2005). In Florida, the cultured hard clam industry supports more than ten hatcheries and 75 land-based nurseries. In 2005, thirty producers sold 478 million clam seed with total sales of $3.3 mil-
lion. "Spin-off" businesses include seamstresses making clam bags, boat builders specializing in clam work skiffs and manufacturers producing harvesting and processing equipment (Woods, 2006). Fifteen hard clam farms exist on Cape Cod, Massachusetts (MAA, 2006), and a recent study of Massachusetts and Rhode Island restaurants (Barnes, 2004) showed that consumers are willing to pay more for farm-raised than wild hard clams. The annual value of hard clam farming in Wellfleet Harbor alone is estimated at $2-3 million (Anon., 2007).

Hard clam culture consists of three phases: hatchery, nursery, and grow-out. The effort reported here
concentrated on the initial stages of field grow-out (May through December 2009) at several intertidal and subtidal locations in eastern Maine by examining four variables:

- seeding date,
- seed size,
- predator exclusion, and
- stocking density.

Presently, the only applied research conducted on cultured bivalves living in soft bottoms in eastern Maine has focused on the soft-shell clam, *Mya arenaria* (Beal et al. 1995, 1999, 2001). A mariculture strategy for intertidal grow-out of cultured individuals of *Mya* in eastern Maine was proposed by Beal and Kraus (2002). Animals produced in the spring and summer of Year I (8-12 mm SL) are held over the winter in flow-through nylon bags (2-3 mm apertures) placed in the water column of a protected
embayment. During April-May of Year II, the cultured juveniles are seeded in protected plots (6.4 mm aperture flexible netting) arrayed near the low tide mark at densities between 300-600 individuals m^{-2}. Netting is removed in late fall when animals have attained sizes between 25-30 mm SL, and, since *Mya* burrows deeper in the sediments with increasing size (Zaklan and Ydenberg 1997), animals are insulated from effects of ice during most winters as well as protected by a depth refuge from many burrowing predators (Commito 1982). Whether similar grow-out strategies exist for cultured hard
clam juveniles remains to be seen and is the focus of the proposed effort.

Bagaduce River Oyster Company, Egypt Bay Aquafarms, and the Downeast Institute are aware of successful grow-out strategies for cultured hard clams in areas south of Maine. For example, in the southeastern U.S., Lorio and Malone (1995) suggest seeding cultured juveniles (7-15 mm SL) at densities of 500-800 m^{-2} and planting in pens, trays, or under netting. Grow-out to 45-50

mm SL may require 18-36 months. Whetstone et al. (2005) describe the suite of predators that prey on hard clams in the southeastern U.S. (blue crabs, stone crabs, mud crabs, conchs, sting rays, horseshoe crabs, and snails). These animals are deterred from preying on hard clam juveniles when farmers use cages, soft bags, and nets. Using information from Eldridge et al. (1979) working in South Carolina and
Peterson et al. (1995) from North Carolina, Grabowski et al. (2003) planted cultured juveniles (> 10 mm SL, with 0 = 13.7 mm) in nylon bags (1.44 m_2, with 9.4 mm aperture) at each of three densities (489, 729, and 972 m^{-2}) near Smyrna, North Carolina. Bags were staked to the bottom in October 2000 and collected one year later. Stocking density had no effect on survival rate (65% to 78%), but did affect growth as animals stocked at the lowest density doubled in size (to 26.5 mm SL) whereas clams at the two upper densities reached an average of 23.8 mm SL, or an approximate 12% suppression in growth due to intraspecific clam density. After evaluating the economic feasibility
of clam culture, Grabowski et al. (2003) demonstrated that clams planted at the intermediate density (i.e., 729 m^{-2}) resulted in the greatest return on initial investment.

Many field strategies to grow hard clams in the Northeast U.S. follow a two-step process which begins with planting seed in net-covered boxes in late summer or early fall. Because seed prices increase dramatically with size, most farmers begin by purchasing small (1-2 mm seed) and then use a variety of techniques (upwellers, downwellers, FLUPSY's, raceways, etc.) to grow animals to field transplant sizes. Once seed has attained a size > 6 mm SL, they are transferred to boxes filled with sand that has been sieved to remove small crabs and other infaunal predators (e.g., snails, worms). Seed at densities of ca. 3000 m^{-2} are then added to the boxes where they remain until they reach ca. 20-25 mm SL. Then, animals are transferred to narrow, net-covered plots for grow-out to 50 mm SL (MacKenzie et al. 2002). Other methods include the use of two sets of soft bags (as described above and in Powers et al. 2007) that are staked to the bottom in which naturally occurring sediments serve as the bottom substrate. Initially, seed (5-8 mm SL) is placed in bags with apertures ca. 6.4 mm (1/4-inch). Once clams reach a size of 15-20 mm SL, they are transferred to bags with a larger mesh size, where they remain until they reach market size (Kraeuter et al. 1998).

We undertook some applied research at Goose Cove prior to this Phase I project. During the spring of 2006, we received a Seed Grant from the Maine Technology Institute (MTI) to examine the hatchery and nursery phases of hard clam culture in eastern Maine. Broodstock were harvested from Goose Cove in Trenton, Maine (45^o 25.80' N, 68^o 23.10' W) in December 2005 and successfully conditioned for two months at temperatures of 20^{o}C at the shellfish production and research center at the Downeast Institute for Applied Marine Research & Education (DEI) on Great Wass Island in the town of Beals. Approximately 1 million juveniles were cultured during Spring and Summer 2006, and 500,000 were used to investigate effects of crowding in a nursery system located at Mud Hole Cove, Great Wass Island, Beals, Maine (44° 29.15' N, 67° 35.17' W; see Beal et al. 1995 for a detailed description of this site). On 5-6 July 2006, animals (ca. 2.5 mm SL) were placed into 4-foot x 3-foot wooden trays lined with window screening at one of four densities/tray: 2500, 5000, 7500, or 10000 (n = 20 replicates per density treatment). The eighty trays (four rows of 20 trays each with 10 m spacing between rows and 0.6 m between adjacent trays) remained floating on the surface of the cove until 16 November 2006 when they were retrieved and returned to DEI. A random sample (14 g) was taken from each tray, all live

and dead animals from each sample were counted, and the SL of each live clam measured to the nearest 0.1 mm using Vernier calipers. No dead animals or empty valves were recovered in any of the 80 trays; however, stocking density had a highly significant effect on final mean SL. Mean SL ± 95 % CI of animals in trays at the lowest density (8.4 ± 0.13 mm) was approximately 11% greater than those at the highest density (7.6 ± 0.22 mm). We conducted overwintering trials at DEI using the same individuals (16 November 2006 to 13 May 2007). Animals were sorted into two sizes (Small: 0 = 5.1 ± 0.2 mm, minimum = 2.5 mm, maximum = 7.5 mm, n = 100; Large: 8.7 " 0.2 mm, minimum = 6.5 mm, maximum = 11.3 mm, n = 110). Large clams were added to 45 cm x 45 cm bags constructed of nylon window screening at each of three masses (0.6 kg, 1.2 kg, and 1.6 kg, representing approximate densities per bag of 3360, 6720, and 8960 individuals, respectively). Seven bags from each density treatment were added to overwintering containers. Containers were modified commercial lobster traps constructed of vinyl-coated 14-gauge wire mesh (0.96 m x 0.45 m x 0.45 m), except instead of opening like a chest freezer, each container opened like a refrigerator. Each cage had a series of eight horizontal shelves that were spaced equidistant. One bag containing clams from a single density was placed onto one shelf within each container except the bottommost. Two containers were used for each density treatment. Small clams were placed in similar sized bags at three different masses (0.51 kg [n = 2], 0.72 kg [n = 13], and
0.99 kg [n = 1], representing approximate densities per bag of 7990, 11302, and 15510 individuals, respectively. Two overwintering containers were used to hold the small clams. On 16 November 2006, all containers were placed into a 15 m long x 1.5 m wide x 1.5 m deep cement tank at DEI that received ambient, flowing seawater. Containers and bags of clams were removed from the tank and cleaned (sprayed with freshwater to remove silt) four times during the 177-day experiment. On 13 May 2007, a 15g random sample was taken from each bag, and the number of live and dead hard clams was recorded. Seawater temperature in the tank varied from 9^{o}C on 16 November 2006 to 1^{o}C on 26 January 2007. On 13 May 2007, seawater temperature was 7^{o}C. Overall mean percent survival for the “large” clams for the 177-day experiment was 99.4 ± 0.28% (n = 42). No significant effect due to mass treatments was observed (P = 0.9842). “Small” clam survival was 99.7 ± 0.24% (n = 16). No significant shell growth occurred for any size clam or in any mass treatment. This information subsequently has been published (Beal et al. 2009).

These initial efforts indicated that it is possible to culture hard clams using local broodstock through the hatchery and nursery phases in eastern Maine, and hold them over the winter for planting the following spring/summer. What the Phase I effort enabled us to learn was what field conditions are necessary to optimize growth and survival of these cultured juveniles during the first, and most critical, growing season (May to December). The Phase I work grew directly from the work we conducted prior to 2009. We are hopeful that with additional research and development, that our work will lead to the development of a new culture industry in eastern Maine.

Results and Accomplishments

Experiment I.

Work Plan from Phase I grant proposal:

Clams (8-10 mm SL) will be planted in field plots at a density of 500 m^{-2}, ca 50 ft^{-2}, in May, June, July, and August 2009 in the shallow subtidal at Goose Cove, Trenton, Maine. On each date, we will compare the effects on clam growth and survival using ten different types of predator protection: 1) flexible plastic netting (4.2 mm aperture), 2) flexible plastic netting (6.4 mm aperture), 3) extruded plastic clam growing cage (4.2 mm aperture), 4) extruded plastic clam growing cage (6.4 mm aperture), 5) soft bags (1 m^2, 4.2 mm aperture), 6) soft bags (6.4 mm aperture), 7) soft bags (4.2 mm aperture) covered with 4.2 mm flexible netting, 8) soft bags (4.2 mm aperture) covered with 6.4 mm flexible netting, 9) soft bags (6.4 mm aperture) covered with 4.2 mm flexible netting, and 10) soft bags (6.4 mm aperture) covered with 6.4 mm flexible netting). Control (unprotected) plots will be used on each seeding date to determine overall effects due to predator exclusion/ deterrent methods. All treatments will be 1-m^2 in size, except growing cages, which will be stocked with 250 clams each. Flexible netting used to cover clams directly or protect soft bags will be anchored in place by stepping the periphery of each piece into the soft mud. A 4-inch diameter Styrofoam float will be affixed to the middle of each piece of flexible netting that will raise it off the bottom approximately 10 cm during tidal inundation. This will keep the netting from interfering with clam feeding (sensu Beal and Kraus 2002). Grow-out cages and soft bags (nylon) will be placed directly on top of the soft sediments, and these are expected to work slowly down into the sediments over time (Sturmer et al. 1997; Grabowski et al. 2000). The arrangement of the treatments will be a generalized completely randomized block design with two replicates of each treatment within each block and three blocks established during each of the four months (see above). At the end of the field trial (December 2009), all experimental units (22 per block x 3 blocks per planting date x 4 planting dates = 264) will be sampled. This will involve removing all soft bags and grow-out cages and estimating all live and dead clams within each. Two benthic cores (A = 0.0077 m^2) will be taken from the area below the soft bags and growout cages and later sieved using a 2 mm mesh. To obtain estimates of growth from each living clam at the end of the experiment, we will measure its initial shell length (SL), that is demarcated by a disturbance line laid down in the shell at the time of its planting, and its final SL. This disturbance line is similar to one that appears when cultured individuals of *Mya arenaria* are introduced to sediments (Beal et al. 1999).

<u>Methods</u>

The experiments were established on 27 May, 26 June, 23 July, and 20 August at Goose Cove, Trenton, Maine (44^o 25.80'N; 68^o 23.11'W) where three 2-acre bottom leases exist in the shallow subtidal. A block design was used with two replicates for each of eleven treatments. Three blocks were established on each planting date. One-meter spacing was used between each row and column within a given block (i.e., 22 plots per block), and 5 m spacing occurred between adjacent blocks. Clams used (0_{SL} ± 95% CI = 8.7 ± 0.5 mm, range = 5.8-11.6 mm, n = 42) were produced at the Downeast Institute (DEI), Beals, Maine the previous year from Goose Cove broodstock and overwintered at the facility according to Beal et al. (2009). Because of time constraints associated with tidal conditions, only two of the three blocks from each initiation date were sampled in December. Sampling occurred over three days (13-15 December 2009). Three separate data sets were collected from the experiment: a) the number of live/dead clams per soft bag and growout cage; b) the final SL of live clams growing in the soft bags and growout cages; and, c) the number and size of live clams from the benthic cores taken within each of the 22 plots per block for each planting date.

Estimates of live and dead clams were taken from the soft bags. After removing bags from the field, each was sprayed with freshwater and the contents poured into a 2 mm sieve. Animals were placed into a labeled plastic bag and then taken to the laboratory at the University of Maine at Machias (UMM). In the lab, separate samples were washed again through a 2 mm sieve and larger pieces of dead fauna and detritus removed. Because of the extensive amount of time that would have been required to count individuals, an estimate of clam numbers was made for most of the soft bag samples. This was accomplished by placing the sample in a 1-l beaker and recording the mass of the sample to the nearest 0.01 g using an electronic balance. Next, a 30g sample was randomly taken from the larger sample and the number of live and dead clams counted. Percent alive was estimated using these counts. To estimate number per sample, we assumed a linear relationship between number per 30 g sample and number per larger sample. To test this assumption, we counted each clam in three separate samples and found that our estimates were within ca. 1-2% of the actual counts (Table I-a). Some soft bags and many of the largest aperture grow-out cages contained very few live clams. For those samples, a complete count of hard clam numbers was made. To estimate growth of clams within the soft bags and grow-out cages, no more than twelve clams, chosen randomly, were measured (both initial and final SL). Clams from each subsample were spread on a piece of acetate containing numbers from 1 to 48 (Fig. 1-a-a). One clam from the sample was then placed over each number. Then, a random number table was consulted and a sample of 12 drawn. The initial and final SL of each clam from the sample was measured to the nearest 0.1 mm using Vernier calipers (Fig. 1-a-b,c,d).

To assess number of clams that may have gone through the soft bags and growout cages, two benthic cores ($A = 0.0077\ m^2$) were taken under each bag and cage. In addition, two cores were taken from plots with netting only and from control plots where no structures existed. Samples were placed into separately labeled plastic bags and taken to UMM where each was washed through a 2 mm sieve. All clams from each sample were counted and the initial and final SL of each measured as described above.

Analysis of variance was used to assess treatment differences. When raw data were not normal or variances heterogeneous, a square root-transformation was applied. The following linear model was used:

$$Y_{ijklmn} = \mu + A_i + B(A)_{j(i)} + C_k + AC_{ik} + CB(A)_{jk(i)} + e_{l(ijk)}.$$

Where:

μ = theoretical mean;

Y = dependent variable (i.e., count of living/dead clams; final shell length; relative growth);
A_i = Planting Date – (May, June, July, August) – factor is fixed;
B_j = Block (1 vs. 2) – factor is random;
C_k = Treatment (eleven separate planting treatments from control plots without netting, netted plots, soft bags with and without additional protective netting, and grow-out cages) – factor is fixed;
e_l = Experimental error associated with the n = 2 replicates per combination of treatments.

A series of orthogonal contrasts were developed to answer specific technical questions regarding project objectives. For the source of variation related to date, we chose three contrasts to better understand how time of year affects clam survival and growth. Specifically, we tested the following contrasts:

1) O_{May} vs. $O_{\text{(June. July, August)}}$;
2) O_{June} vs. $O_{\text{(July, August)}}$; and,
3) O_{July} vs. O_{August}

For the source of variation related to treatment (i.e., type of container clams were placed within), we chose the following contrasts when considering effects of soft bags and grow-out cages alone:

1) $O_{\text{Soft bags}}$ vs. $O_{\text{Grow-out cages}}$;
2) $O_{\text{4.2 mm Cages}}$ vs. $O_{\text{6.4 mm Cages}}$;
3) $O_{\text{4.2 mm Soft bags}}$ vs. $O_{\text{6.4 mm Soft bags}}$;
4) 4.2 mm Soft bags: $O_{\text{No Netting}}$ vs. O_{Netting};
5) 4.2 mm Soft bags: $O_{\text{4.2 mm Netting}}$ vs. $O_{\text{6.4 mm Netting}}$;
6) 6.4 mm Soft bags: $O_{\text{No Netting}}$ vs. O_{Netting}; and,
7) 6.4 mm Soft bags: $O_{\text{4.2 mm Netting}}$ vs. $O_{\text{6.4 mm Netting}}$

When all plots within blocks were considered (benthic cores), the following orthogonal contrasts associated with treatment effects were considered:

1) $O_{\text{Control Plots}}$ vs. $O_{\text{All other plots}}$;
2) $O_{\text{Structures}}$ vs. $O_{\text{Netted plots with no additional structures}}$;
3) Netted plots with no structures: $O_{\text{4.2 mm}}$ vs. $O_{\text{6.4 mm}}$;
4) $O_{\text{Soft bags}}$ vs. $O_{\text{Grow-out cages}}$;
5) Grow-out cages: $O_{\text{4.2 mm}}$ vs. $O_{\text{6.4 mm}}$;
6) Soft bags: $O_{\text{4.2 mm}}$ vs. $O_{\text{6.4 mm}}$;
7) 4.2 mm Soft bags: $O_{\text{No Netting}}$ vs. O_{Netting};

8) 4.2 mm Soft bags: $O_{\text{4.2 mm Netting}}$ vs. $O_{\text{6.4 mm Netting}}$;

9) 6.4 mm Soft bags: $O_{\text{No Netting}}$ vs. O_{Netting}; and,

10) 6.4 mm Soft bags: $O_{\text{4.2 mm Netting}}$ vs. $O_{\text{6.4 mm Netting}}$.

Underwood (1997) was used to determine appropriate mean square estimates for each source of variation. To avoid excessive type I errors associated with orthogonal contrasts, an adjusted alpha ($\alpha' = 1 - [1-\alpha]^{1/n}$; where α=0.05 and n=number of contrasts) was used as a decision rule following the advice of Winer et al. (1991).

Results

Numbers and percent survival of hard clam juveniles in soft bags and growout cages

Both mean number of live clams and percent survival in soft bags and growout cages varied significantly over the planting dates, with highest values observed during June and August plantings (Fig. 1-a). Although mean percent survival from bags and cages established in May was high (O = 77.7 ± 7.0%, n = 32), this was significantly lower than the mean from the other three planting dates (O = 86.0 ± 2.5%, n = 96). Numbers per container varied through time with each orthogonal contrast yielding a significant difference (Table I-b). Although no statistical differences in mean percent survival were observed between treatments, such was not the case for mean number (P < 0.0001). Mean number in growout cages (that had been stocked with half the number of juvenile clams as the soft bags) was significantly less at the end of the trial than in the soft bags (Table I-b). This was due apparently to the fact that the smaller aperture soft bags contained significantly more animals than the larger aperture soft bags (Fig. 1-b). A significant difference in mean number per cage was detected between the larger vs. smaller aperture grow-out cages (P < 0.0001, Table I-b). This was not too surprising given the fact that many of the clams placed into the 6.4 mm cages (and soft bags) on each planting date were observed to go through, rather than be retained on, the mesh netting. This observation also explains much of the observed variation in the contrast comparing the smaller vs. larger soft bags. That is, approximately 2/3[rds] of the variability associated with the treatments was due to differences in mean number between 4.2 mm soft bags vs. 6.4 mm soft bags. The smaller soft bags contained nearly 4x the mean number of hard clams at the end of the trial than the larger soft bags (428.3 ± 51.3 ind. vs. 112.2 ± 32.9 ind., n = 48).

Green crabs, *Carcinus maenas,* were attracted to the structures that housed the hard clam juveniles. Crabs within the bags and grow-out cages were mostly 0-year class individuals ranging in size from 4-15 mm carapace width (CW); however, a few animals within the structures were as large as 30 mm CW. We asked whether number of crabs within the structures varied across the different container/structure types by performing an ANOVA on the square root-transformed number of crabs per bag or cage. A significant treatment effect was observed (P < 0.0001), but no other differences (e.g., due to planting date, block within date, etc.) were detected. Three orthogonal contrasts were highly significant (P < 0.0001): a) small aperture vs. large aperture grow-out cages (10x more green crabs were sampled from the small vs. large aperture cages); b) 4.2 mm vs. 6.4 mm soft bags (4.3x more crabs were found in the 4.2 mm vs. 6.4 mm bags); and c) for the 4.2 mm soft bags, 5x more crabs were found in bags with extra netting than in bags without additional netting (Fig. I-c).

Growth of hard clams in cages and bags

A significant ($F = 17.19$, df = 1, 1454, $P < 0.0001$), positive relationship existed between absolute growth (final SL - initial SL) and initial SL; hence, mean relative growth estimates were used in all growth analyses. Approximately 60% of the variation in relative growth was due to the date when experiments were established (Table I-c). Mean relative growth decreased through time (Fig. I-e), and mean final SL did not vary significantly across the June-August planting dates. Fig I-e shows clearly that a growth penalty exists when clams are planted at this site after May. Clams planted in late May added 45% more shell compared to the mean of the other three planting dates (19.3 ± 0.5 mm, n = 32 vs. 13.3 ± 0.4 mm, n = 96; Fig. I-f). Relative growth varied across treatments ($P = 0.0004$, Table I-c); however, these effects varied according to planting date ($P = 0.0153$). To better understand these interactive effects, we performed separate single-factor ANOVA's for treatments on the untransformed mean relative growth data for each date. Significant treatment effects on mean relative growth were observed only for clams planted in July ($F = 10.96$, df = 7, 24, $P < 0.0001$). Two orthogonal contrasts were significant ($\alpha' = 0.0073$). Relative growth of clams in the smaller aperture growout cages was 65% faster than in the larger aperture growout cages; however, final mean SL differed by only 20% between these two treatments (13.6 ± 0.5 mm vs. 11.3 ± 1.9 mm, n = 4). In addition, clams apparently grew faster in the smaller aperture compared to the larger aperture soft bags. Relative growth differed by 61% and final mean SL differed by 27% (14.9 ± 0.5 mm vs. 11.7 ± 0.9 mm, n = 12).

Numbers of hard clams from benthic cores

Mean number of hard clam juveniles in benthic cores did not vary significantly across planting dates ($P = 0.7393$); however, treatment effects were highly significant (Table I-e), with three of the orthogonal contrasts demonstrating significant differences. As expected, significantly more clams were sampled from plots protected with netting than from plots with structures (grow-out cages and soft bags). Bags and structures were supposed to contain all clams planted within each; however, cultured animals generally were smaller than the greatest diagonal aperture width, and many were not retained inside the structures. For plots with protective netting only, mean number of individuals per $1 m^2$ was 739.6 ± 118.3 (n = 64). This was approximately 100% higher than the combined mean from plots with structures (376.8 ± 85.5 ind. m^{-2}, n = 256; Fig. I-g). Another significant contrast included the grow-out cages, where essentially no live clams were found in cores beneath the smaller aperture structures (fabricated from extruded plastic; 8.1 ± 11.5 ind. m^{-2}, n = 32) compared to the larger aperture structures (fabricated from flexible plastic; 331.4 ± 147.9 ind. m^{-2}, n = 32). Adult green crabs, *Carcinus maenas*, appeared to be attracted to the extruded plastic grow-out cages (189.9 ± 83.7 ind. m^{-2}, n = 32) compared the flexible grow-out cages (4.0 ± 8.2 ind. m^{-2}, n = 32). None of the other structures harbored significant numbers of green crab adults (0's ranged from 8.1 to 24.3 ind. m^{-2}). Finally, approximately 20 x more hard clam juveniles were sampled from plots underneath the larger (848.7 ± 183.4 ind. m^{-2}, n = 96) vs. smaller aperture soft bags (43.1 ± 39.4 ind. m^{-2}, n = 96; Fig. I-g).

Growth of hard clams from benthic cores

As with juvenile hard clams growing within the structures, relative growth in sediments under the structures and within control and netted plots varied significantly over planting date ($P < 0.0001$; Table I-e). Estimates of relative growth and final mean SL were remarkably similar to estimates of the same variables from the structures (compare Figs. I-e & I-h). Once again, a growth penalty was observed for clams planted after May. Clams planted in June, July, or August were approximately 35%, on average, smaller than those planted in May with respect to final SL (Fig. I-i). Mean percent relative growth varied across treatments (Table I-e; Fig. I-k). Lowest relative growth was observed in control plots (Fig. I-k), and this was associated with the smallest final mean SL (12.8 ± 1.7 mm, n = 20). All other mean percent relative growth estimates fell within the 95% confidence interval of 100%, or a doubling in size.

Summary: This study was designed to assess if hard clams could grow and survive as well in structures vs. seeding them directly into protected or unprotected bottom plots. Soft bags and grow-out cages potentially provide the farmer with greater seed management options because, if survival is high and growth reasonable during the first growing season removed from hatchery production, these structures enable one to move 1-2 yr individuals without having to dig up large portions of sediment. The idea was that the structures would completely contain the animals, and that growth and survival inside the structures could be compared to those variables measured in plots without structures. Assessing which treatment yielded the "best" results requires combining survival information from Figs. I-c & I-g, and growth information from Figs. I-e, -f, -h, -i, -k, and understanding the intent of the experiment. Unfortunately, the cultured clams from our hatchery supplier were smaller than anticipated, and many of them were not retained in the bag, and fell through the apertures of the structures to the benthos beneath. Regardless, it appears that soft bags with a 4.2 mm aperture provide adequate protection for juveniles during the first growing season (for the size animals used in this study), yielding a mean survival rate of nearly 90%, and that growth is actually better in these bags compared to bags with the larger aperture netting. It is not important to cover the bag with additional netting, as this did not provide better protection or growth. In fact, we observed that the additional netting (especially the small aperture material) enhanced deposition of sediments and increased fouling by mussels, *Mytilus edulis*, especially in the plots established in May 2009. In addition, by assessing planting date effects, we determined that although there might be a slight improvement in survival by postponing planting until June, July, or August, there would be a significant growth penalty for choosing this strategy. As with other trials (see below) predators played an important role in limiting hard clam numbers in this experiment. It is possible to deter predators, but netting or other material must be managed as most invertebrate predators can settle into caged or protected plots at sizes much smaller than the smallest practical size mesh netting. We found that if soft bags or grow-out cages were properly installed, only the smallest green crabs (0-year class individuals < 20 mm CW) were found inside with clams by December, and these individuals are too small to do extensive damage to the clams. If structures are not managed, crabs (and sometimes sea stars, *Asterias forbseii*) may reach sizes where they could destroy most or all of the hard clams within.

Soft bags can be used to hold cultured seed during the first growing season without worrying too much about negative effects of sediment build-up, predators, or growth penalties. Bags should be deployed during or before May to take advantage of intense seasonal growth. Most shell growth will occur before the first of October (Beal et al., 2009), so removing clams from bags and seeding them into commercial plots should be considered at that time.

Table I-a. Relationship between number of clams estimated from small (30 g) samples and actual counts.

Treatment	Sample Weight (g)	Subsample Weight (g)	Estimated number of clams	Actual count	% Δ	$\frac{(Obs - Exp)^2}{Exp}$
6.4 mm Soft Bag + 6.4 mm Net	152.16	30.21	197	201	2.03	0.08122
4.2 mm Soft Bag	406.75	31.76	563	574	1.95	0.21492
4.2 mm Soft Bag + 6.4 mm Net	403.06	32.16	488	495	1.43	0.10040
						$\chi^2 =$ 0.39654

df = 2; P = 0.8202

Table I-b. Analysis of variance on **a**) the arcsine-transformed mean percent alive and **b**) square root-transformed mean number across soft bags and growout cages (i.e., Treatment) for experiments initiated during the spring and summer of 2009 in the shallow subtidal of Goose Cove, Trenton, Maine. Orthogonal contrasts are underlined and use α' as the decision rule (e.g., for the contrasts associated with Date, α' = 0.0170; for Treatment, α' = 0.0073). (n = 2)

a)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	3	1029.75282	343.2509	10.19	0.0241
May vs. Jun, Jul, Aug	1	600.30607	600.3060	17.83	0.0135
June vs. July, August	1	195.42590	195.4259	5.80	0.0736
July vs. August	1	234.02085	234.0208	6.95	0.0578
Block(Date)	4	134.70041	33.6751	0.37	0.8283
Treatment	7	1021.69885	145.9569	0.60	0.7479
Date*treatment	21	3857.27489	183.6797	0.76	0.7397
Treatment x Block(Date)	28	6772.67271	241.8811	2.67	0.0006
Error	64	5805.45494	90.7102		
Corrected Total	127	18621.55463			

b)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	3	557.85509	185.9516	52.84	0.0011
May vs. Jun, Jul, Aug	1	55.42307	55.4230	15.75	0.0166
June vs. July, August	1	115.49076	115.4907	32.81	0.0046
July vs. August	1	386.94126	386.9412	109.94	0.0005
Block(Date)	4	14.07781	3.5194	0.38	0.8188
Treatment	7	4218.56661	602.6523	30.82	<.0001
soft bags vs. cages	1	652.61247	652.6124	33.38	<.0001
4 GC vs. 6 GC	1	636.08047	636.0804	32.53	<.0001
4 soft vs. 6 soft	1	2806.48565	2806.4856	143.53	<.0001
4 soft: Netting vs. no net	1	2.99386	2.9938	0.15	0.6985
4 soft: 4.2 mm vs. 6.4 mm	1	3.08156	3.0815	0.16	0.6944
6 soft: Netting vs. no net	1	110.80150	110.8015	5.67	0.0243
6 soft: 4.2 mm vs. 6.4 mm	1	6.51108	6.5110	0.33	0.5685
Date*treatment	21	653.51756	31.1198	1.59	0.1243
Treatment x Block(Date)	28	547.47484	19.5526	2.14	0.0063
Error	64	585.461151	9.147830		
Corrected Total	127	6576.953085			

Table I-c. Analysis of variance on the untransformed relative growth data for hard clam juveniles in soft bags and grow-out cages (i.e., Treatment) established in May, June, July, and August 2009 at Goose Cove, Trenton, Maine. Orthogonal contrasts are underlined, and use α' as a decision rule.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	3	7.97100885	2.65700295	252.53	<.0001
May vs. Jun, Jul, Aug	1	4.92666455	4.92666455	468.25	<.0001
June vs. July, August	1	1.05772473	1.05772473	100.53	0.0006
July vs. August	1	1.98661957	1.98661957	188.82	0.0002
Block(date)	4	0.04208566	0.01052142	0.32	0.8631
Treatment	7	1.11926492	0.15989499	5.72	0.0004
soft bags vs. cages	1	0.04075120	0.04075120	1.46	0.2374
4 GC vs. 6 GC	1	0.11379789	0.11379789	4.07	0.0533
4 soft vs. 6 soft	1	0.81059842	0.81059842	28.99	<.0001
4 soft: Netting v. No net	1	0.03765728	0.03765728	1.35	0.2556
4 soft: 4.2 mm v. 6.4 mm	1	0.00290732	0.00290732	0.10	0.7495
6 soft: Netting v. No net	1	0.09600353	0.09600353	3.43	0.0744
6 soft: 4.2 mm v. 6.4 mm	1	0.01754928	0.01754928	0.63	0.4349
Date x Treatment	21	1.41392297	0.06732967	2.41	0.0153
Block x Treatment(Date)	28	0.78282557	0.02795806	0.85	0.6732
Error	64	2.09954418	0.03280538		
Corrected Total	127	13.42865215			

Table I-d. Analysis of variance on the square root-transformed number of hard clam juveniles within benthic cores (A = 0.0077 m^2; n = 2) taken 13-15 December 2009 from underneath 1-m^2 soft bags and netted plots, 0.5 m^2 grow-out cages, and within 1-m^2 control plots. Clams were placed within the structures (bags and cages), and on the bottom of control and netted plots on each of four dates: 27 May, 26 June, 23 July, and 20 August 2009. Orthogonal contrasts are underlined and use α' as a decision rule (e.g., for contrasts associated with Date and Treatment, α' = 0.0169 and 0.0051, respectively).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	3	1.76028409	0.58676136	0.44	0.7393
May vs. Rest	1	0.05876098	0.05876098	0.04	0.8446
June vs. Rest	1	1.60841414	1.60841414	1.20	0.3356
July vs. August	1	0.09310897	0.09310897	0.07	0.8055
Block(Date)	4	5.3801040	1.3450260	1.62	0.1702
Treatment	10	297.3587415	29.7358741	22.07	<.0001
Control Plots vs. Rest	1	2.9920748	2.9920748	2.22	0.1440
Structure vs. Netting alone	1	76.7608959	76.7608959	56.98	<.0001
Soft bags vs. Grow-out Cages	1	11.7209380	11.7209380	8.70	0.0053
Netting alone: 4.2 v. 6.4 mm	1	0.1264154	0.1264154	0.09	0.7609
Grow-out Cages: 4.2 vs. 6.4mm	1	21.4354228	21.4354228	15.91	0.0003
Bags: 4.2 mm vs. 6.4 mm	1	178.9498414	178.9498414	132.85	<.0001
Bags 4.2: No Net v. Netting	1	0.0090287	0.0090287	0.01	0.9352
Bags 4.2: 4.2 mm v. 6.4 mm	1	0.1718750	0.1718750	0.13	0.7228
Bags 6.4: No Net v. Netting	1	0.0000596	0.0000596	0.00	0.9947
Bags 6.4: 4.2 mm v. 6.4 mm	1	5.1921900	5.1921900	3.85	0.0566
Date*Treatment	30	52.6116023	1.7537201	1.30	0.2157
Treatment x Block(Date)	40	53.8820544	1.3470514	1.62	0.0145
Error	264	219.5637251	0.8316808		
Corrected Total	351	630.5565114			

Table I-e. Analysis of variance on the untransformed relative growth data of living hard clam juveniles sampled within benthic cores ($A = 0.0077 m^2$) at Goose Cove, Trenton, Maine (13-15 December 2009). Approximately 500 clams were placed directly into soft bags ($A = 1 m^2$) and 250 into grow-out cages ($A = 0.5 m^2$) that were placed on top of the sediments. Also, 500 clams were seeded into 1-m^2 plots that either received no protective netting or plots that received one of two kinds of flexible netting (4.2 mm or 6.4 mm aperture). Orthogonal contrasts (underlined) use α' (see Table I-d). (n = 2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	3	10.21048844	3.40349615	212.90	<.0001
<u>May vs. Jun, Jul, Aug</u>	1	4.75981695	4.75981695	95.79	0.0006
<u>June vs. July & August</u>	1	0.62695591	0.62695591	12.62	0.0238
<u>July vs. August</u>	1	4.82371526	4.82371526	97.08	0.0006
Block(Date)	4	0.06394610	0.01598652	0.26	0.9008
Treatment	10	5.64350377	0.56435038	7.21	<.0001
<u>Control Plots vs. Rest</u>	1	2.87838208	2.87838208	36.79	<.0001
<u>Structure vs. Netting alone</u>	1	0.00152773	0.00152773	0.02	0.8899
<u>Soft bags vs. Grow-out Cages</u>	1	0.02747043	0.02747043	0.35	0.5588
<u>Netting alone: 4.2 v. 6.4 mm</u>	1	0.05534125	0.05534125	0.71	0.4083
<u>Grow-out Cages: 4.2 vs. 6.4mm</u>	1	0.01132888	0.01132888	0.14	0.7068
<u>Bags: 4.2 mm vs. 6.4 mm</u>	1	0.46743612	0.46743612	5.98	0.0219
<u>Bags 4.2: No Net v. Netting</u>	1	1.06649310	1.06649310	13.63	0.0011
<u>Bags 4.2: 4.2 mm v. 6.4 mm</u>	1	0.99079099	0.99079099	12.67	0.0015
<u>Bags 6.4: No Net v. Netting</u>	1	0.14403780	0.14403780	1.84	0.1869
<u>Bags 6.4: 4.2 mm v. 6.4 mm</u>	1	0.00069537	0.00069537	0.01	0.9256
Date x Treatment	23	3.36739655	0.14640855	1.87	0.0640
Treatment x Block(Date)	25	1.95571716	0.07822869	1.29	0.1806
Error	127	7.69870937	0.06061976		
Corrected Total	192	31.97156249			

Figure Legends

Figure I-a. a) Hard clam juveniles on a numbered piece of acetate. A random number table was used to select twelve clams to estimate mean shell lengths for samples containing > 12 individuals. b) Close-up of hard clam juvenile showing distinct disturbance line that coincides with planting date (final SL = 12.9 mm; initial SL = 10.0 mm). c) Another example of the disturbance line, or "hatchery mark," associated with planting date. d) clam #9 final SL = 28.9 mm, initial SL = 12.2 mm.

Figure I-b. Effects of planting date on mean percent survival and mean numbers of juvenile hard clams added to soft bags and growout cages at Goose Cove, Trenton, Maine during the spring and summer of 2009. Both variables were statistically significant (Table I-a). Orthogonal contrasts demonstrated that mean percent survival was lowest for clams planted in May, but that no significant difference existed between the other planting dates. Each of the three contrasts associated with mean numbers was statistically significant. (n = 32)

Figure I-c. Effects of different types of containers used to hold juvenile hard clams on mean number alive in December 2009 at Goose Cove, Trenton, Maine. (Means are pooled over four monthly planting dates from May to August 2009.) Soft bags were constructed of flexible, plastic netting of two aperture sizes (4.2 mm and 6.4 mm), and were approximately 1-m^2. Bags were each stocked with 500 animals. Grow-out cages were approximately 0.5 m^2, and stocked with 250 animals. Cages with the smaller aperture size were constructed from extruded, plastic netting whereas cages with the larger aperture were constructed from the same material as the soft bags. Clams ranged in size from 5.8-11.6 mm SL. (n = 16)

Figure I-d. Mean number of green crabs, *Carcinus maenas*, within soft bags and grow-out cages at Goose Cove, Trenton, Maine on 13-15 December 2009. ANOVA on the square root-transformed numbers demonstrated a significant "container" effect. Orthogonal contrasts showed significant differences between a) types of grow-out cages; b) 4.2 mm vs. 6.4 mm soft bags; and, c) netted vs. unnetted 4.2 mm soft bags ($P < 0.0001$ in each instance). (n = 16)

Figure I-e. Mean relative growth and mean final shell length associated with date of planting at Goose Cove, Trenton, Maine during 2009. Dashed line indicates a doubling in size. Experiments were initiated on 27 May, 26 June, 23 July, and 20 August. Growth was measured during 13-15 December. (n = 32)

Figure I-f. Size-frequency distributions for final shell length (13-15 December 2009) of juvenile hard clams planted at Goose Cove, Trenton, Maine on 27 May (n = 358), 26 June (n = 382), 23 July (n = 332), and 20 August (n = 384) 2009 that were growing in soft bags and grow-out cages. Final mean SL's were not significantly different for the June-August plantings.

Figure I-g. Mean number of hard clam juveniles (per square meter) in benthic cores taken from control plots and plots protected with netting, and from underneath soft bags and grow-out cages. (n = 32)

Figure I-h. Effects of planting date on mean relative growth and mean final SL of living hard clam juveniles in benthic cores taken from control plots and plots protected with netting, and from underneath soft bags and grow-out cages. (May, n = 55; June, n = 49; July, n = 46; August, n = 43. Values of "n" refer to the number of core samples containing live clams out of 88 total taken per month.)

Figure I-i. Size-frequency distribution of final SL's of juvenile hard clams (13-15 December 2009) at planted at Goose Cove, Trenton, Maine on 27 May (n = 232), 26 June (n = 352), 23 July (n = 284), and 20 August (n = 293) 2009 that were sampled in benthic cores taken underneath grow-out cages and soft bags, and from plots with and without (controls) protective netting.

Figure I-k. Effects of planting treatment on mean relative growth of hard clam juveniles in December 2009 (see Table I-e for results from orthogonal contrasts). Dashed line indicates a doubling in size. ($3 \leq n \leq 32$)

Figure I-a.

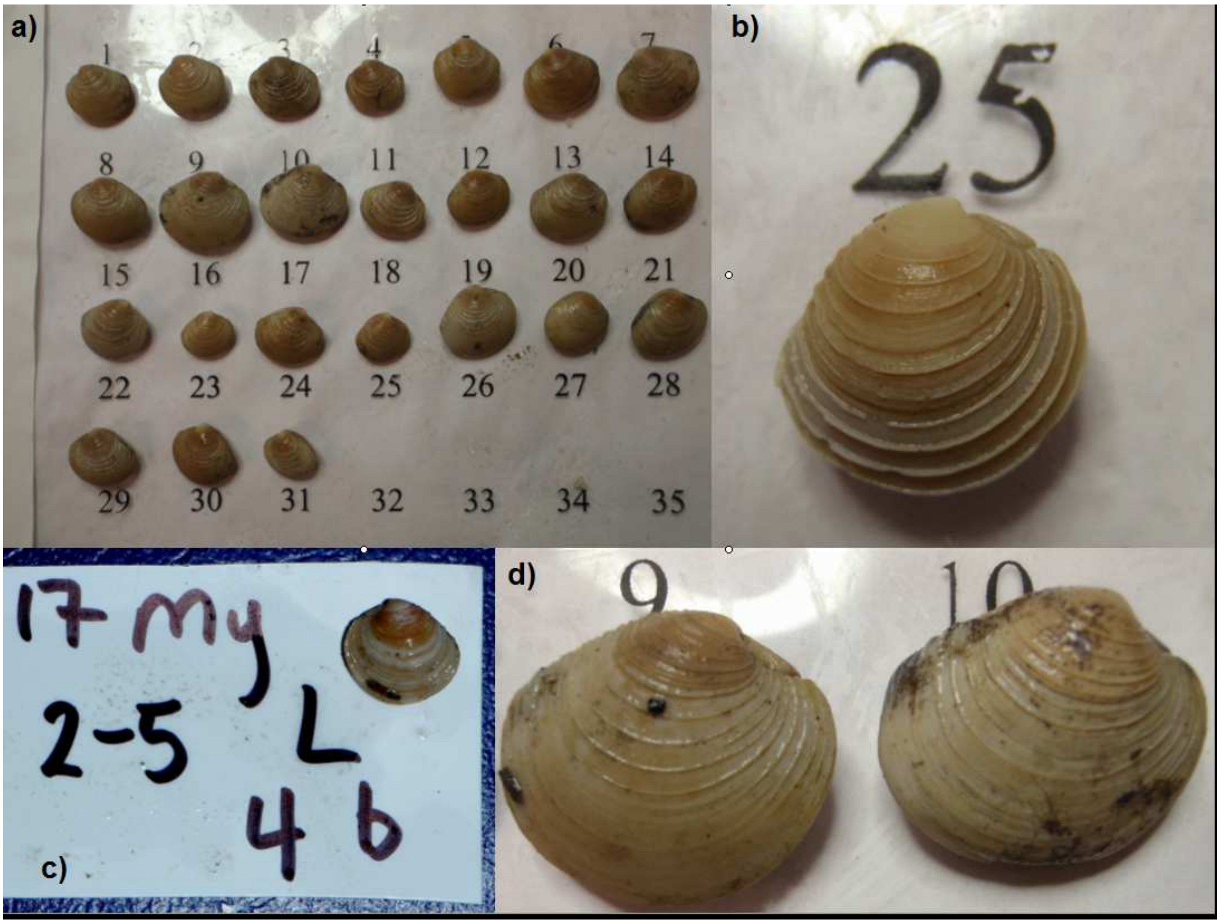

a)
1 2 3 4 5 6 7
8 9 10 11 12 13 14
15 16 17 18 19 20 21
22 23 24 25 26 27 28
29 30 31 32 33 34 35
b)
25
c)
17 My
2-5 L
4 b
d)
9
10

Figure I-b.

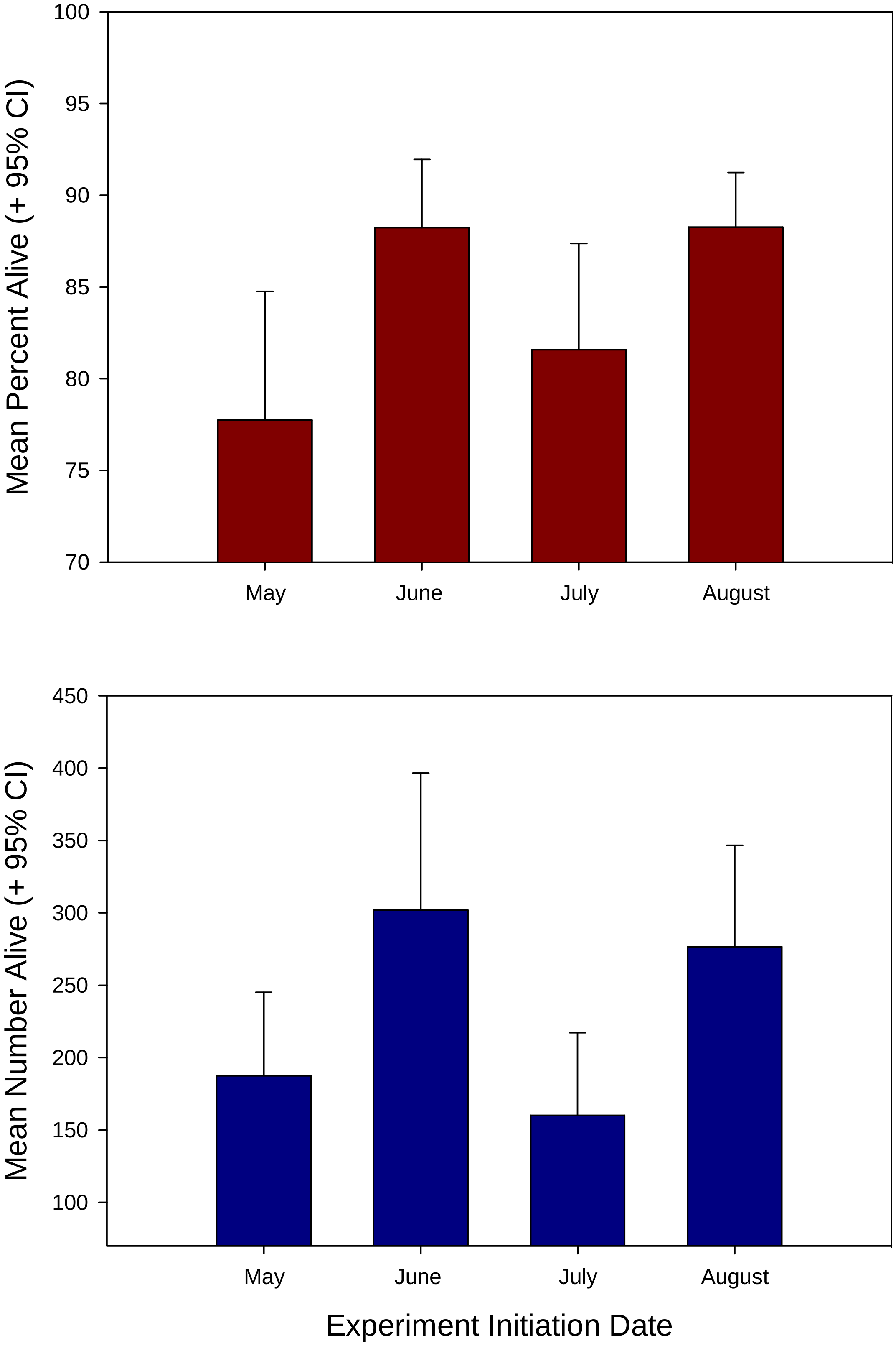
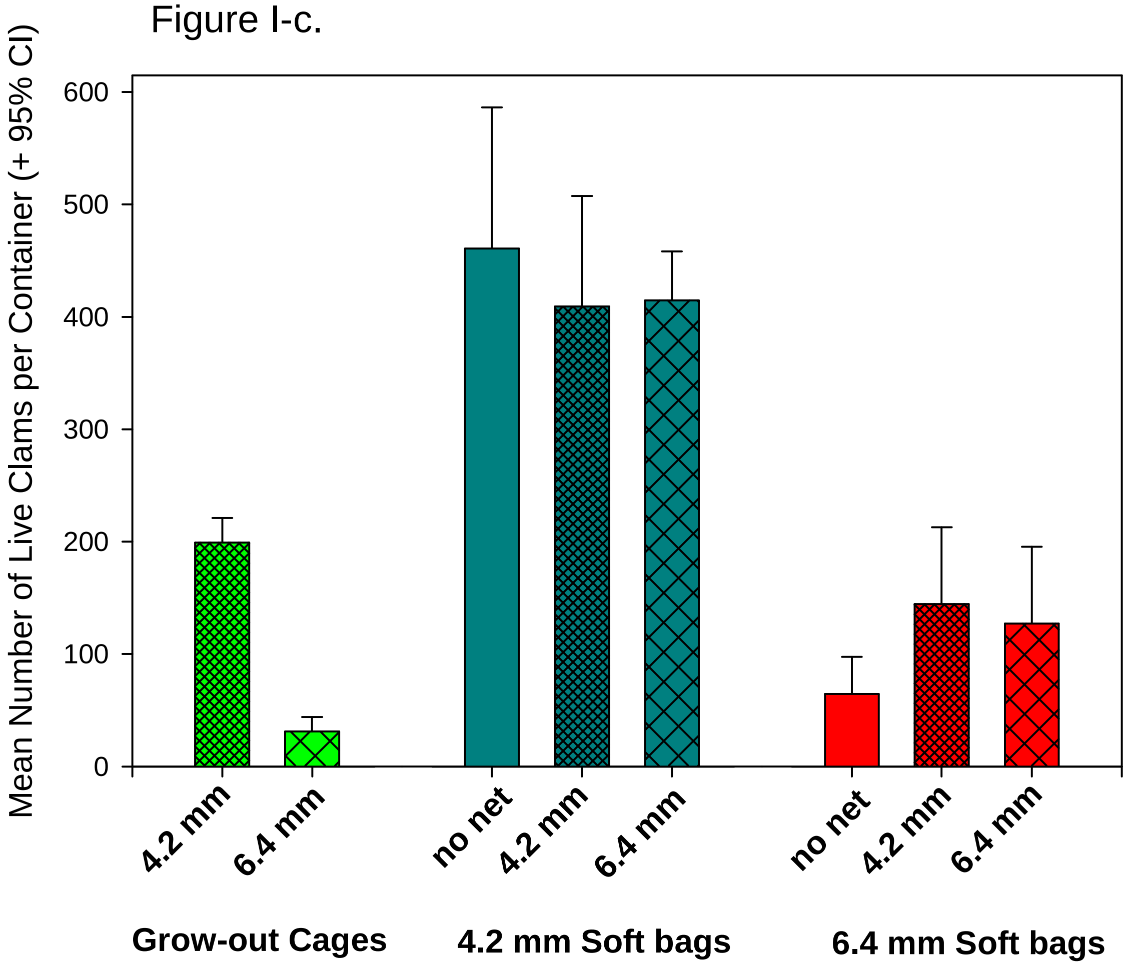

100
95
90
85
80
75
70
Mean Percent Alive (+ 95% CI)
May
June
July
August
450
400
350
300
250
200
150
100
Mean Number Alive (+ 95% CI)
May
June
July
August
Experiment Initiation Date

Figure I-c.



Mean Number of Live Clams per Container (+ 95% CI)
600
500
400
300
200
100
0
4.2 mm
6.4 mm
no net
4.2 mm
6.4 mm
no net
4.2 mm
6.4 mm
Grow-out Cages
4.2 mm Soft bags
6.4 mm Soft bags

Figure I-d.

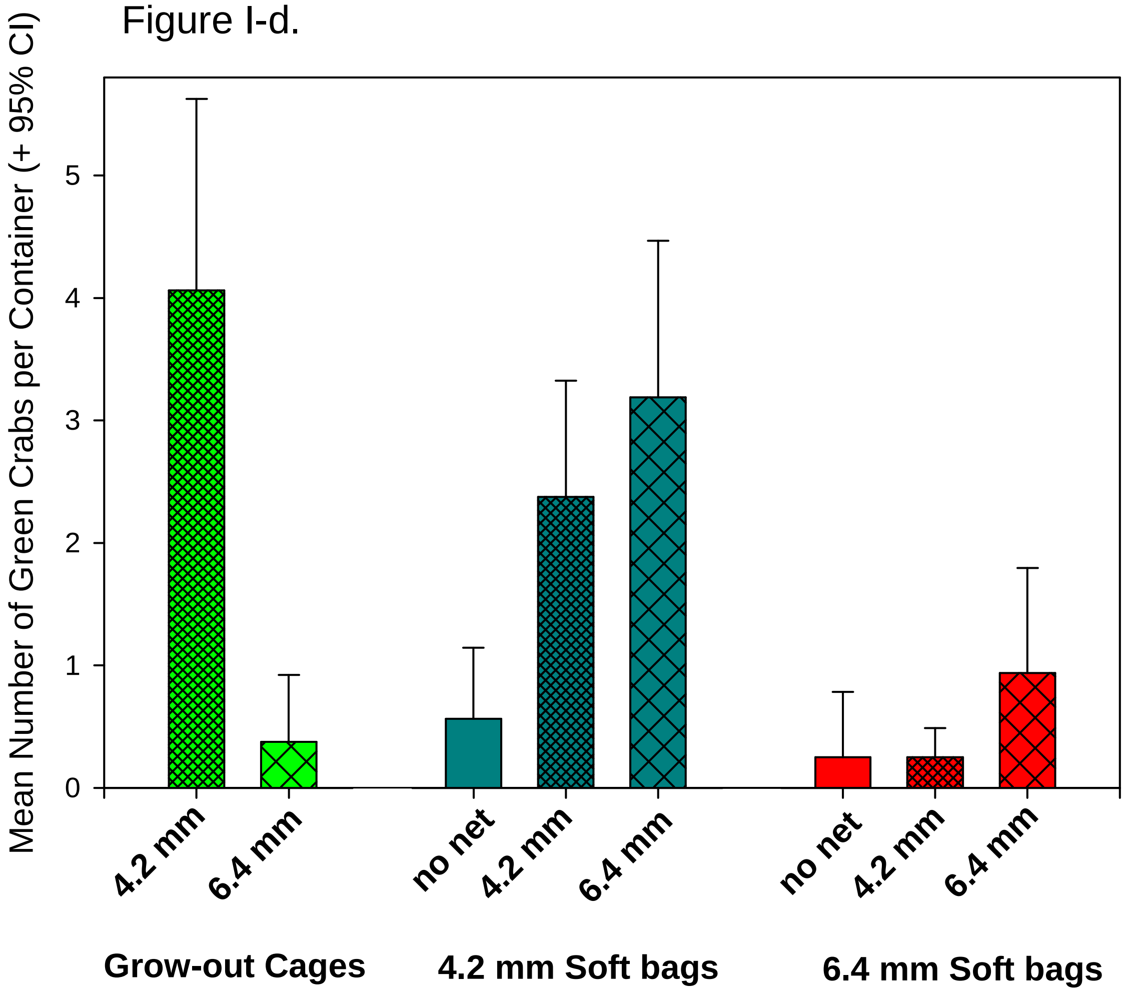
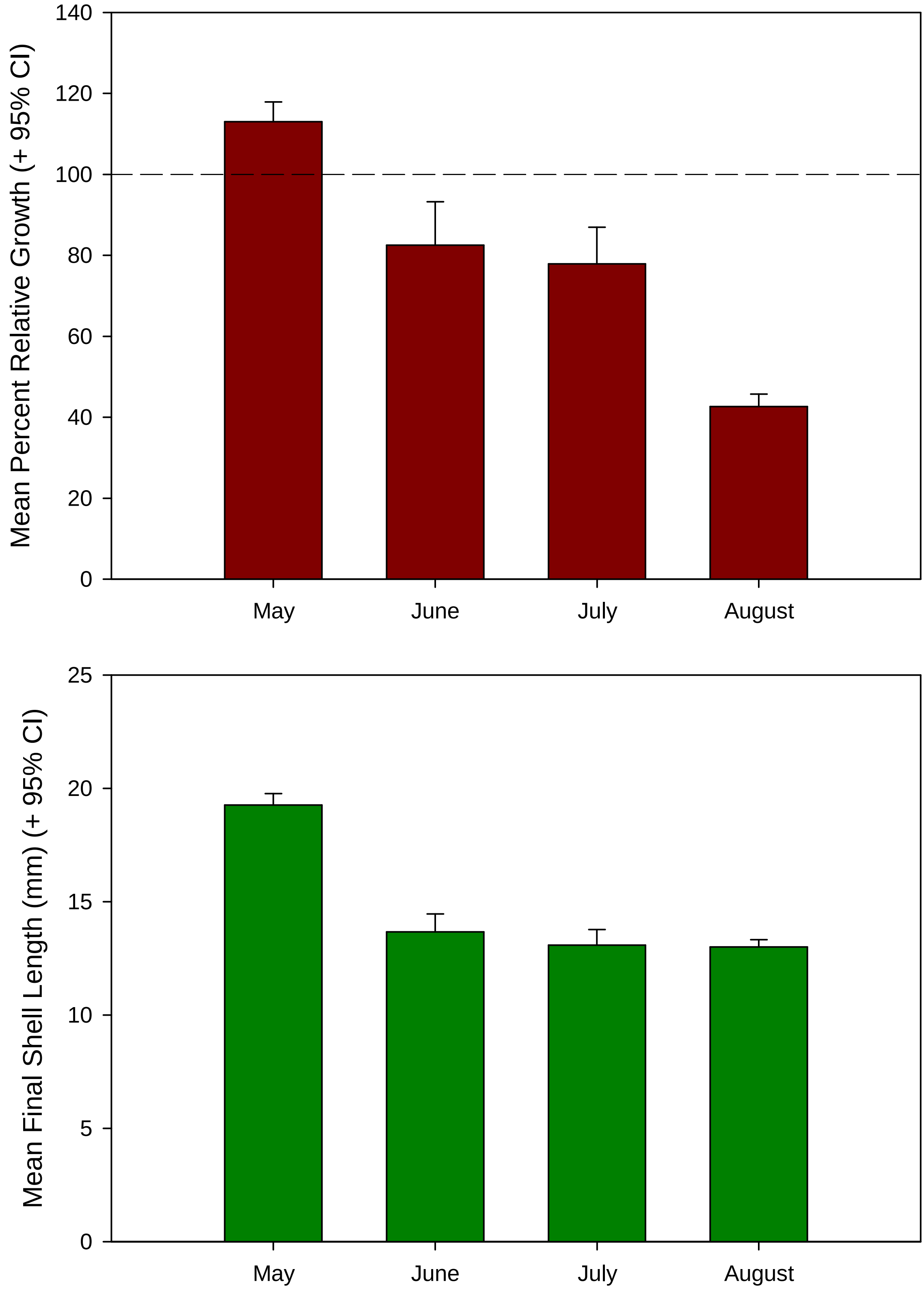

Mean Number of Green Crabs per Container (+ 95% CI)
0
1
2
3
4
5
4.2 mm
6.4 mm
no net
4.2 mm
6.4 mm
no net
4.2 mm
6.4 mm
Grow-out Cages
4.2 mm Soft bags
6.4 mm Soft bags

Figure I-e.



Mean Percent Relative Growth (+ 95% CI)
140
120
100
80
60
40
20
0
May
June
July
August
Mean Final Shell Length (mm) (+ 95% CI)
25
20
15
10
5
0
May
June
July
August

Figure I-f.

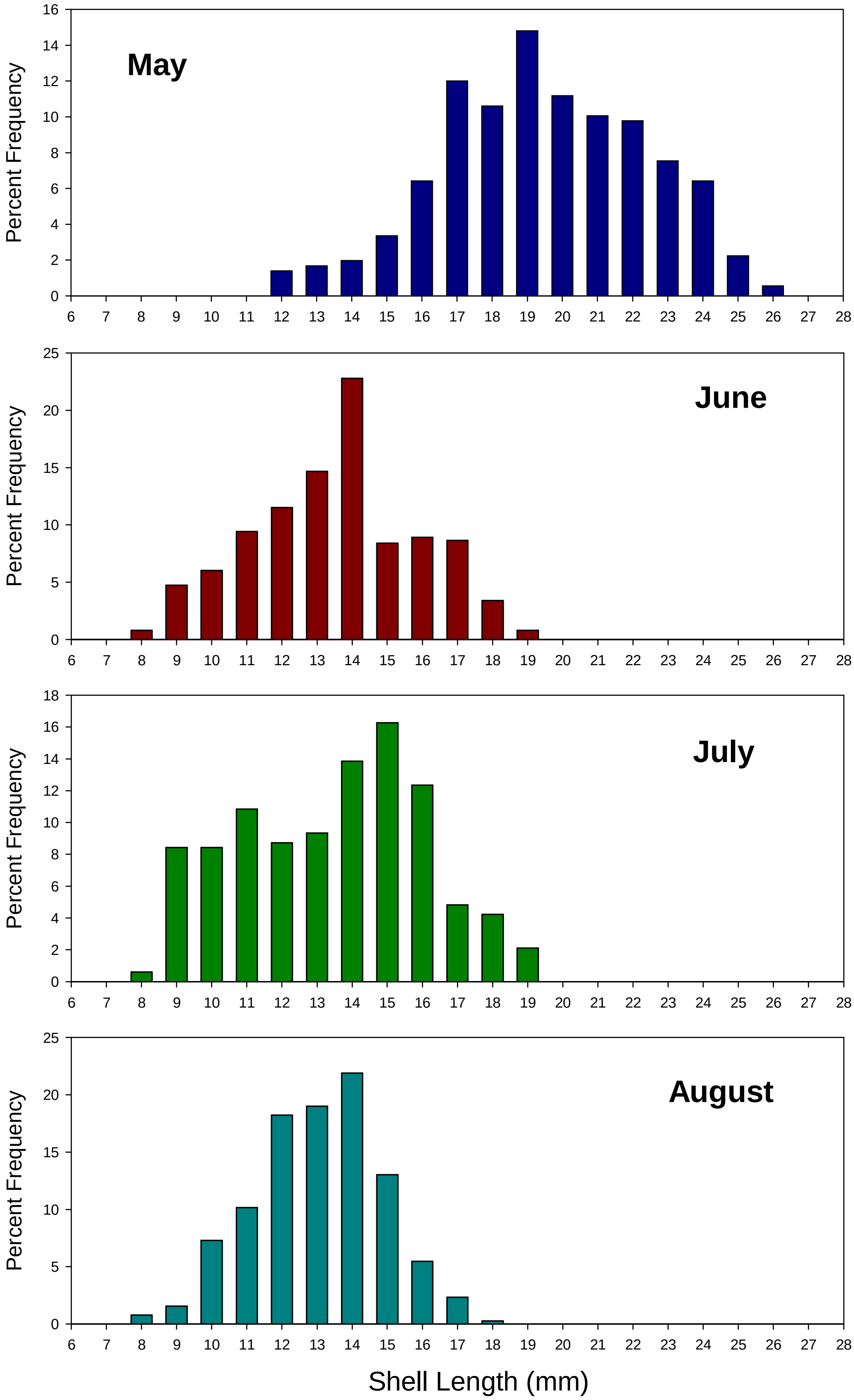
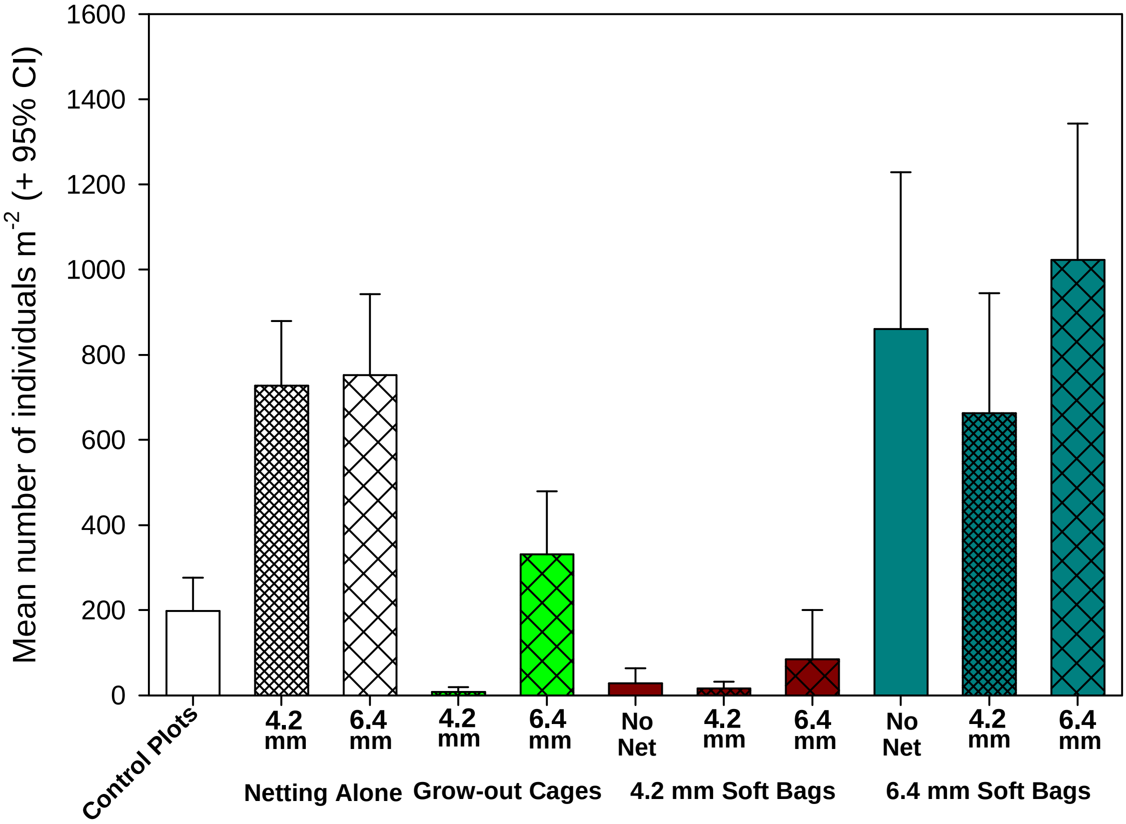
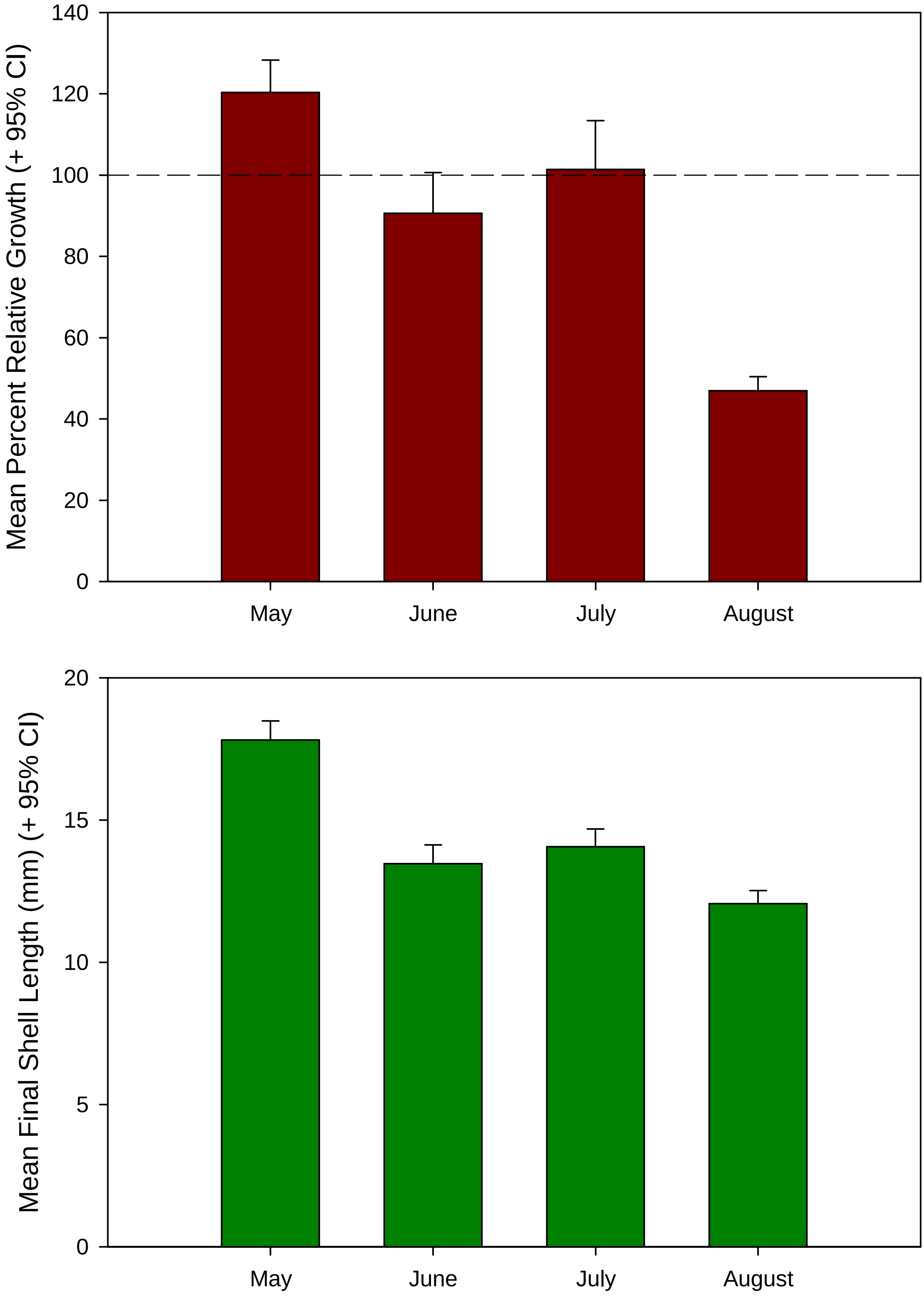


Figure I-g.



Mean number of individuals m^{-2} (+ 95% CI)
1600
1400
1200
1000
800
600
400
200
0
Control Plots
4.2 mm
6.4 mm
4.2 mm
6.4 mm
No Net
4.2 mm
6.4 mm
No Net
4.2 mm
6.4 mm
Netting Alone
Grow-out Cages
4.2 mm Soft Bags
6.4 mm Soft Bags

Figure I-h.



Mean Percent Relative Growth (+ 95% CI)
140
120
100
80
60
40
20
0
May
June
July
August
Mean Final Shell Length (mm) (+ 95% CI)
20
15
10
5
0
May
June
July
August

Figure I-i.

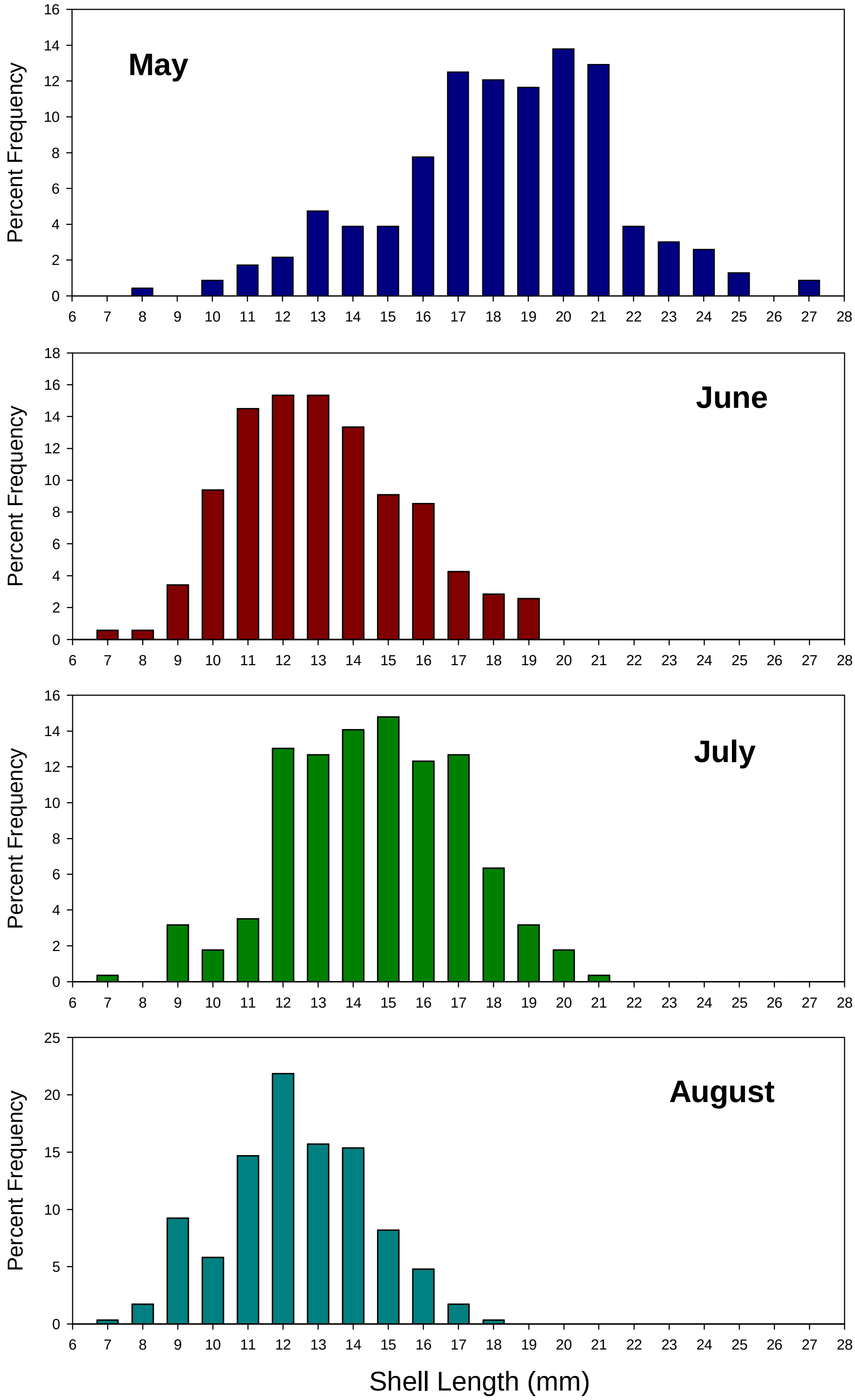
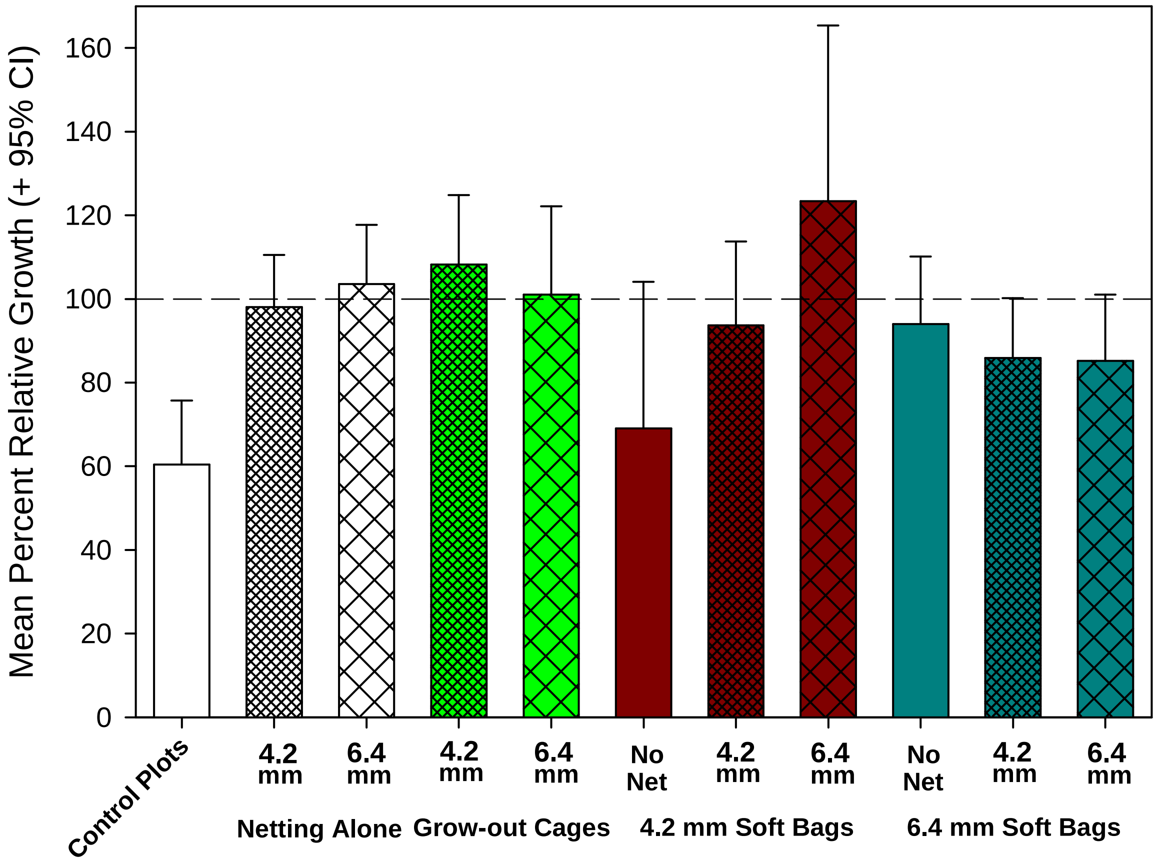


Figure I-k.


Mean Percent Relative Growth (+ 95% CI)
160
140
120
100
80
60
40
20
0
Control Plots
4.2 mm
6.4 mm
4.2 mm
6.4 mm
No Net
4.2 mm
6.4 mm
No Net
4.2 mm
6.4 mm
Netting Alone
Grow-out Cages
4.2 mm Soft Bags
6.4 mm Soft Bags

Experiment II.

Work Plan from Phase I grant proposal:

Clams of each size class (6, 8, and 10 mm SL) will be added to 1-m^2 plots at each of three stocking densities (400, 500, and 600 m^{-2}) in May 2009. One third of all plots will be protected with plastic flexible netting (6.4 mm aperture), one third will be protected with flexible netting (4.2 mm aperture), and the remaining plots will serve as controls without netting. The design will be similar to Experiment I except four blocks, instead of three, will be used (3 sizes x 3 densities x 3 netting treatments x 4 blocks x 2 replicates per block = 216 experimental units). In November 2009, two benthic cores (0.0077 m^2) will be randomly taken from each plot. Each sample will be washed through a 2 mm sieve. Mean number of clams per plot (adjusted for differences in stocking density) will be estimated. Absolute growth of each live clam will be estimated by subtracting initial SL from final SL using Vernier calipers to the nearest 0.1 mm. Analysis of variance with orthogonal contrasts will be used to analyze both main and interactive effects (e.g., size – a. 6 mm vs. 8 & 10 mm, b. 8 mm vs. 10 mm; density – a. 400 m^{-2} vs. 500 & 600 m^{-2}, b. 500 m^{-2} vs. 600 m^{-2}; predator exclusion – a. netting vs. no netting, b. 4.2 mm vs. 6.4 mm aperture).

<u>Methods</u>

This experiment was established on 25 May 2009 in the soft sediments of the shallow subtidal at Goose Cove, Trenton, Maine (44^{o} 25.80'N; 68^{o} 23.11'W). Three size classes of hatchery-reared hard clam juveniles produced at the Downeast Institute during 2008 and overwintered according to Beal et al. (2009) were used in the field trial (Fig. II-a). Clams were smaller than originally intended (O_{Small} = 5.1 ± 0.14 mm, n = 67; O_{Medium} = 6.5 ± 0.12, n = 85; O_{Large} = 7.9 ± 0.10, n = 94). Clams for each stocking density and added to 1-m^2 plots were established by using size-specific relationships relating mass-to-number of individual hard clams. That is, actual number of clams added to individual plots was not counted, but a mass of clams representing a specific density (400, 500, or 600) was used. Netting (1.25 m x 1.25 m) used to cover two-thirds of the plots in each of the four blocks was piece of flexible, plastic mesh material (Industrial Netting, Minneapolis, MN; http://www.industrialnetting.com/). A small (10 cm diameter) polyethylene foam float was permanently affixed to the bottom side of each piece of predator exclusion netting to ensure that nets would float above the benthos during tidal inundation and not interfere with hard clam feeding and growth. Control plots were delineated at each corner using wooden laths. Approximately one meter spacing was used between rows and columns within each block, and blocks were arrayed 5-7 m apart.

Plots were sampled on 2-3 November 2009 by taking two benthic cores (area = 0.0077 m^2) to a depth of 12 cm from each. Each sample (N = 432) was placed into a separately labeled plastic bag, taken to the University of Maine at Machias, and washed through a 2 mm sieve. All live clams were counted, and both initial and final shell lengths were measured to the nearest 0.1 mm using Vernier calipers. Although none of the hatchery-reared clams were marked uniquely at the beginning of the field trial, a distinct line forms in the shell, distinguishing initial clam size, when animals are added to sediments (Beal et al., 2009; Fig. I-a), similar to the line that forms in the shell of cultured soft-shell clams, *Mya arenaria*, when added to sediments (Beal et al, 1999).

Analysis of variance (ANOVA) was performed on the square root-transformed counts of living and dead clams per core as well as the untransformed final shell length and absolute growth (Final shell length - initial shell length). The linear model was:

$Y_{ijklmn} = \mu + A_i + B_j + AB_{ij} + C_k + AC_{ik} + BC_{jk} + ABC_{ijk} + D_l + AD_{il} + BD_{jl} + CD_{kl} + ABD_{ijl} + BCD_{jkl} + ACD_{ikl} + ABCD_{ijkl} + E(A)_{m(i)} + BE(A)_{jm(i)} + CE(A)_{km(i)} + DE(A)_{lm(i)} + BCE(A)_{jkm(i)} + + BDE(A)_{jlm(i)} + CDE(A)_{klm(i)} + BCDE(A)_{jklm(i)} + e_{n(ijklm)}.$

Where:

μ = theoretical mean;
Y = dependent variable (i.e., count of living/dead clams; final shell length; absolute growth);
A_i = Block – factor is random;
B_j = Clam Size ("Small" vs. "Medium" vs. "Large") – factor is fixed;
C_k = Predator Exclusion (none vs. 4.2 mm flexible vs. 6.4 mm flexible) – factor is fixed;
D_l = Density (400 vs. 500 vs. 600 m^{-2}) – factor is fixed;
E_m = Plot (a vs. b) nested within block; and,
e_n = Experimental error associated with the n = 4 replicate cores per combination of treatments.

Results

Survival

Number of live hard clams sampled in November varied significantly with both initial size and level of predator exclusion (Table II-a). The largest source of variation in mean hard clam number was explained by initial size (Fig. II-b). Approximately 2.5x more clams occurred from plots in which medium and large clams were planted vs. small clams (490.8 ± 65.6 ind. m^{-2}, n = 144 vs. 187.7 ± 64.8 ind. m^{-2}, n = 72). This contrast (small clams vs. the mean of the two larger sizes) was highly significant ($P < 0.0001$) whereas the contrast comparing mean number of live clams from the two larger size categories was not significant ($P = 0.3878$; Table II-a). The presence of netting enhanced hard clam numbers by a factor of 2.4 (482.3 ± 70.5 ind. m^{-2}, n = 144 vs. 204.8 ± 45.3 ind. m^{-2}, n = 72); however, the effect of netting was influenced by stocking density (i.e., a significant density x netting interaction; $P = 0.0048$, Table II-a; Fig. II-c). Decomposing this source of variation into four single degree-of-freedom contrasts showed that at a stocking density of 500 clams m^{-2}, mean number of living clams in plots protected by 4.2 mm vs. 6.4 mm netting increased by approximately 115% whereas at a stocking density of 600 clams m^{-2}, the opposite occurred (Fig. II-c). Not all nets remained in place to protect clams seeded in plots during the 162-day field experiment. For example, nine of the smaller mesh nets (4.2 mm aperture) and eleven of the larger mesh nets (6.4 mm aperture) were missing when plots were sampled in November. Although there is no way to determine how long nets were in place prior to their disappearance, we asked whether mean number of living clams in these plots was similar to that in plots where netting remained intact for the entire experimental period. ANOVA on the square root-transformed number of living clams demonstrated that plots with lost netting contained significantly fewer clams (34%) than those plots retaining netting during the entire experimental interval (333.0 ± 149.3 ind m^{-2}, n = 20 vs. 506.3 ± 77.8 ind. m^{-2}, n = 124; $P = 0.0067$). Mean number of live clams in plots with lost nets did not differ significantly from mean number in control plots ($P = 0.1342$). Dead clams with undamaged valves, and those with chipped or crushed valves were present in some samples, but at relatively low densities (Table II-b). Significantly more dead small clams with undamaged valves were found in samples than the two other size classes (e.g., small vs. medium vs. large = 70.0 ± 24.2 vs. 26.0 ± 11.9 vs. 29.6 ± 15 ind. m^{-2}, respectively, $P = 0.0034$; $n = 72$). In addition, 120% more dead clams with undamaged valves were found in samples from netted vs. control plots ($P = 0.0020$; Table II-b). For dead clams with crushed or chipped valves, no significant effects due to size or netting occurred ($P > 0.20$).

Growth

Final mean shell length of hard clams sampled on 2-3 November varied by initial size ($P < 0.0001$) and density ($P = 0.0089$; Table II-c). Small clams had a final mean SL of 11.4 ± 0.70 mm ($n = 45$) that was approximately 32% smaller than large clams (16.7 ± 0.47 mm, $n = 69$) (Fig. II-e). Both orthogonal contrasts based on clam size were highly significant ($P < 0.0001$, Table II-c). Clams seeded at the lowest density (400 ind. m^{-2}) had a significantly smaller final mean SL (by 5%) than clams seeded at the two higher densities (500 & 600 ind. m^{-2}). Although statistically significant, this difference is small enough so that it is unlikely to be biologically meaningful (Fig. III-e). No differences were observed for final mean shell length due to the presence/absence of predator netting ($P = 0.3025$, Table II-c). Clams attained a final mean shell length of 14.1 ± 0.89 mm ($n = 58$) in control plots vs. 14.7 ± 0.68 mm ($n = 124$) in plots protected with predator netting. When absolute shell growth (final shell length - initial shell length) was examined (Table II-d), slightly different results appeared in terms of treatment effects. Not surprisingly, initial size was statistically significant ($P = 0.0011$), but there was no effect due to density ($P = 0.5829$) or any main or interactive effect. Finally, several of the nets ($n = 6$) were fouled heavily with small (< 40 mm SL) mussels, *Mytilus edulis*. We asked whether the growth of hard clams in the netted plots with heavy mussel fouling grew differently than animals in netted plots without fouling, and found no significant effect of biofouling on final mean SL ($P = 0.9544$) or absolute shell growth ($P = 0.7198$).

Summary: This study was designed to assess the interactive effects of initial clam size, stocking density, and predator exclusion on clam growth and survival. Results showed that predation was intense, particularly in control plots without netting, and on small clams. Surprisingly, although there was only about a 3 mm difference in initial mean SL between “small” and “large” clams, this difference appeared to be important both in terms of survival (Fig. II-b) and growth (Fig. II-e). Clams smaller than 6 mm should remain in nursery trays or other systems designed to increase shell growth, rather than used in field plots to produce market size animals. Although it is possible to protect animals of such small sizes with flexible netting (4.2 mm or 6.4 mm aperture), the animals may be able to migrate through the netting or may get washed out of the plot through the apertures during inclement weather. One management practice that seems appropriate from results presented here is that clams should be washed through the particular size of net used to protect them in the field prior to their introduction to the field. This would ensure that clams are unable to migrate outside protected plots and that only the complete loss of the netting would allow predators undeterred access to clams. Stocking densities (400-600 ind. m^{-2}) did not appear to have a significant effect on either growth (final SL; Fig. II-e) or survival, suggesting that seeding plots with at least 600 ind. m^{-2} during the first growing season would be appropriate. Netting did not interfere with clam growth, and the presence of netting enhanced clam survival (across all clam sizes; Fig. II-c). Netting, or other material that can deter predators, is necessary for successful farming based on results of these tests. Mesh size should be a function of the size of hard clam juveniles, with the goal of the protective netting to retain all seed within the netted area. Netting with a larger aperture size than the mean or median clam size will enable clams to migrate outside the protected space or allow them to be washed outside the space when abiotic factors such as inclement weather events (wind, high tidal currents, etc.) are severe.

Table II-a. Analysis of variance on the square root-transformed number of hard clam juveniles in benthic core samples taken on 2-3 November 2009 associated with Experiment II. Orthogonal contrasts are underlined. For contrasts with df = 2, α' = 0.0253, for contrasts with df = 4, α' = 0.0127. (n = 2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Block	3	0.75675555	0.25225185	1.22	0.4122
Size	2	81.46834775	40.73417387	47.04	0.0002
Small vs. Larger	1	80.71798382	80.71798382	93.21	<.0001
Medium vs. Large	1	0.75036393	0.75036393	0.87	0.3878
Size x Block	6	5.19588344	0.86598057	0.81	0.5903
Netting	2	41.79774906	20.89887453	11.84	0.0083
Control vs. Netting	1	39.72449115	39.72449115	22.50	0.0032
4.2mm vs. 6.4mm	1	2.07325791	2.07325791	1.17	0.3201
Netting x Block	6	10.59130173	1.76521696	2.74	0.0940
Size x Net	4	5.74088424	1.43522106	2.89	0.0689
Size x Netting x Block	12	5.95956315	0.49663026	0.36	0.9584
Density	2	8.95625392	4.47812696	2.37	0.1747
400 vs. 500 & 600	1	6.84510825	6.84510825	3.62	0.1059
500 vs. 600	1	2.11114567	2.11114567	1.12	0.3315
Density x Block	6	11.35495884	1.89249314	2.35	0.1314
Size x Density	4	5.57851839	1.39462960	2.25	0.1243
Density x Netting	4	25.42385576	6.35596394	6.59	0.0048
400 v Rest x Net v No Net	1	0.10027281	0.10027281	0.10	0.7526
400 v Rest x 4.2mm v 6.4mm	1	0.31676284	0.31676284	0.33	0.5771
500 v 600 x Net v No Net	1	0.40822645	0.40822645	0.42	0.5275
500 v 600 x 4.2mm v 6.4mm	1	24.59859366	24.59859366	25.51	0.0003
Size x Density x Block	12	7.43788923	0.61982410	1.27	0.3244
Size x Density x Netting	8	10.34376443	1.29297055	1.07	0.4129
Density x Netting x Block	12	11.57107033	0.96425586	0.60	0.8088
Size x Density x Net x Blck	24	28.88000124	1.20333338	0.97	0.5263
Plot(Block)	4	0.82998466	0.20749617	0.37	0.8269
Size x Plot(Block)	8	8.55834485	1.06979311	1.93	0.0570
Netting x Plot(Block)	8	5.14584021	0.64323003	1.16	0.3247
Density x Plot(Block)	8	6.45382362	0.80672795	1.45	0.1753
Size x Netting x Plot(Block)	16	21.78004635	1.36125290	2.45	0.0019
Size x Density x Plot(Block)	16	7.83903807	0.48993988	0.88	0.5888
Density x Net x Plot(Block)	16	25.51056106	1.59441007	2.88	0.0003
Size x Den x Net x Plot(Blk)	32	39.77718845	1.24303714	2.24	0.0004
Error	216	119.7690794	0.5544865		
Corrected Total	431	496.7207038			

Table II-b. Mean number per square meter (± 95% CI) of living clams, dead clams with undamaged valves, and dead clams with crushed or chipped valves sampled from benthic cores taken from plots seeded with Small, Medium, or Large cultured seed (see Fig. II-a) at one of three stocking densities (400, 500, or 600 m^{-2}) with no netting, or with 4.2 mm or 6.4 mm flexible netting. (n = 8)

Clam Size	**Density**	**Netting**	**Alive**	**Dead Undamaged**	**Dead Chipped**
Small	400	Control	40.4 (27.9)	32.3 (57.8)	16.2 (38.2)
		4.2	218.2 (275.5)	24.2 (57.3)	32.3 (40.9)
		6.4	282.9 (218.0)	16.2 (25.0)	16.2 (38.2)
	500	Control	105.1 (119.9)	8.1 (19.1)	32.3 (57.8)
		4.2	16.2 (38.2)	0.0 (0.0)	16.2 (25.0)
		6.4	379.9 (328.7)	96.9 (108.1)	48.5 (75.1)
	600	Control	96.9 (147.3)	16.2 (38.2)	0.0 (0.0)
		4.2	412.2 (349.1)	56.6 (45.1)	40.4 (49.5)
		6.4	137.4 (124.1)	16.2 (25.0)	56.6 (78.8)
Medium	400	Control	177.8 (125.1)	32.3 (57.8)	32.3 (57.8)
		4.2	476.9 (312.5)	40.4 (57.3)	40.4 (76.1)
		6.4	396.0 (179.1)	24.2 (40.3)	0.0 (0.0)
	500	Control	177.8 (114.7)	0.0 (0.0)	24.2 (57.3)
		4.2	509.2 (372.8)	56.6 (60.9)	32.3 (40.9)
		6.4	808.3 (320.5)	40.4 (49.5)	8.1 (19.1)
	600	Control	379.9 (226.5)	0.0 (0.0)	40.4 (57.3)
		4.2	1012.3 (458.6)	32.3 (57.7)	16.2 (25.0)
		6.4	323.3 (182.7)	8.1 (19.1)	56.6 (78.8)
Large	400	Control	185.9 (127.4)	24.3 (40.2)	56.6 (78.8)
		4.2	387.9 (256.8)	32.2 (40.9)	48.5 (62.9)
		6.4	476.9 (232.8)	24.2 (28.0)	24.2 (57.3)
	500	Control	315.2 (127.4)	48.5 (38.2)	0.0 (0.0)
		4.2	493.0 (253.4)	129.3 (150.2)	48.5 (47.9)
		6.4	986.1 (548.1)	177.8 (154.9)	48.5 (75.1)
	600	Control	363.7 (141.4)	48.5 (38.2)	24.2 (27.9)
		4.2	711.3 (366.6)	64.7 (91.4)	72.7 (60.9)
		6.4	654.7 (124.1)	121.2 (105.9)	0.0 (0.0)

Table II-c. Analysis of variance on the untransformed final shell length data of juvenile hard clams from benthic cores taken 2-3 November 2009. Hatchery-reared reared clams of three sizes (Fig. II-a) at three densities (400, 500, or 600 ind. m^{-2}) were added to square meter plots that were either protected with flexible netting (4.2 mm or 6.4 mm aperture) or unprotected (controls) on 25 May 2009 at Goose Cove, Trenton, Maine. Orthogonal contrasts are underlined (α' = 0.0253). Number of replicates varies between 1 and 4 depending on number of living animals sampled in benthic cores from the $1m^2$ plots.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Block	3	20.91471772	6.97157257	0.74	0.5790
Size	2	820.4716419	410.2358210	98.90	<.0001
<u>Small vs. Larger</u>	1	565.3179890	565.3179890	136.30	<.0001
<u>Medium vs. Large</u>	1	255.1536529	255.1536529	61.52	<.0001
Size x Block	6	24.88679728	4.14779955	0.93	0.5205
Netting	2	27.36405608	13.68202804	1.47	0.3025
Neting x Block	6	55.87360706	9.31226784	1.08	0.4476
Size x Netting	4	16.53350979	4.13337745	0.71	0.5985
Size x Netting x Block	12	69.52515026	5.79376252	2.20	0.0937
Density	2	18.08376097	9.04188049	11.46	0.0089
<u>400 vs. (500 & 600)</u>	1	12.70321671	12.70321671	16.11	0.0070
<u>500 vs. 600</u>	1	5.38054426	5.38054426	6.82	0.0400
Density x Block	6	4.73248779	0.78874796	0.08	0.9973
Size x Density	4	10.39811030	2.59952757	0.27	0.8926
Density x Netting	4	29.03878288	7.25969572	1.25	0.3407
Size x Density x Block	12	116.1718045	9.6809837	1.65	0.1908
Size x Density x Netting	7	24.50513987	3.50073427	0.71	0.6639
Density x Netting x Block	12	69.49127507	5.79093959	0.95	0.5275
Size x Density x Net x Block	17	83.71377542	4.92433973	0.66	0.7810
Plot(Block)	4	37.4452496	9.3613124	2.54	0.0428
Size x Plot(Block)	8	35.5931540	4.4491443	1.21	0.2990
Netting x Plot(Block)	8	69.1259665	8.6407458	2.35	0.0218
Density x Plot(Block)	8	83.9204462	10.4900558	2.85	0.0060
Size x Netting x Plot(Block)	12	31.6622578	2.6385215	0.72	0.7328
Size x Density x Plot(Block)	13	76.1852503	5.8604039	1.59	0.0954
Density x Net x Plot (Block)	16	97.6339245	6.1021203	1.66	0.0633
Size x Densty x Net x Plot(Blk)	9	67.3051342	7.4783482	2.03	0.0408
Error	128	471.134463	3.680738		
Corrected Total	309	2848.082959			

Table II-d. Mean final shell length (mm) and absolute shell growth (mm) (± 95% CI) for hard clam juveniles from Experiment II (25 May to 2-3 November 2009) at Goose Cove, Trenton, Maine. Small, Medium, and Large initial clam sizes were 5.1 ± 0.14 mm, 6.5 ± 0.12, and 7.9 ± 0.10, respectively. Stocking densities are numbers of individuals per square meter. Netting refers to flexible, plastic mesh with an aperture size of either 4.2 mm or 6.4 mm whereas Control refers to square meter plots seeded with clams that received no predator netting. Number of replicate plots (n) containing live hard clams varies according to survival.

Clam Size	**Density**	**Netting**	**n**	**Final Length**	**Absolute Growth**
Small	400	Control	5	8.9 (2.28)	3.9 (2.70)
		4.2	6	10.3 (2.25)	5.1 (2.02)
		6.4	7	12.7 (1.66)	7.5 (1.69)
	500	Control	4	10.7 (6.19)	5.4 (5.43)
		4.2	1	12.3 (-)	6.4 (-)
		6.4	5	12.1 (1.29)	7.2 (1.45)
	600	Control	4	12.0 (5.25)	6.5 (5.79)
		4.2	7	12.1 (1.18)	6.1 (1.43)
		6.4	6	11.8 (2.44)	6.6 (2.84)
Medium	400	Control	7	14.7 (3.47)	8.0 (3.04)
		4.2	6	15.1 (0.73)	8.6 (0.65)
		6.5	8	14.1 (1.66)	7.5 (1.78)
	500	Control	7	13.6 (1.43)	7.4 (1.59)
		4.2	8	14.4 (1.27)	8.0 (1.23)
		6.4	8	14.1 (0.95)	7.8 (1.03)
	600	Control	8	14.1 (0.95)	7.8 (1.03)
		4.2	8	15.2 (0.04)	8.6 (1.00)
		6.4	8	14.3 (2.38)	7.4 (1.97)
Large	400	Control	7	15.4 (1.90)	7.9 (1.94)
		4.2	7	16.8 (1.73)	9.2 (1.36)
		6.4	8	16.7 (1.26)	8.8 (0.96)
	500	Control	8	15.8 (2.17)	7.9 (1.67)
		4.2	7	15.9 (1.52)	7.8 (1.22)
		6.4	8	17.3 (1.51)	9.5 (1.29)
	600	Control	8	17.0 (1.79)	8.9 (1.78)
		4.2	8	17.7 (1.31)	9.3 (1.43)
		6.4	8	17.5 (1.47)	9.5 (0.98)

Figure Legends

Figure II-a. Initial size frequency distribution for cultured hard clam juveniles used in Experiment I (25 May 2009). $0_{SMALL} \pm 95\%$ CI = 5.1 ± 0.14 mm, n = 67; 0_{MEDIUM} = 6.5 ± 0.12, n = 85; 0_{LARGE} = 7.9 ± 0.10, n = 94.

Figure II-b. Mean number of living clams per m^2 on 2-3 November 2009 at Goose Cove, Trenton, Maine as a function of initial planting size (25 May 2009). ANOVA demonstrated that significantly fewer clams were sampled from benthic cores taken from plots seeded with SMALL clams vs. plots seeded with larger clams (Table II-a). Underlined bars represent means that are not significantly different. (n = 72)

Figure II-c. Interactive effects of netting and stocking density on mean number of live clams per m^2 on 2-3 November 2009 at Goose Cove, Trenton, Maine.

Figure II-d. Effects of missing netting on mean number of live clams per m^2 on 2-3 November 2009 at Goose Cove, Trenton, Maine. Control (n = 72); 4.2 mm netting (present, n = 63; absent, n = 9); 6.4 mm netting (present, n = 61; absent, n = 11). ANOVA demonstrated a significant difference in means between plots with netting vs. those with netting absent (P = 0.0067).

Figure II-e. Effects of initial size (see Fig. II-a) and stocking density on final mean shell length of hard clams on 2-3 November 2009 at Goose Cove, Trenton, Maine at the end of a 162-day field experiment. Both initial size and stocking density had significant effects on final mean shell length (see Table II-c).

Figure II-a.

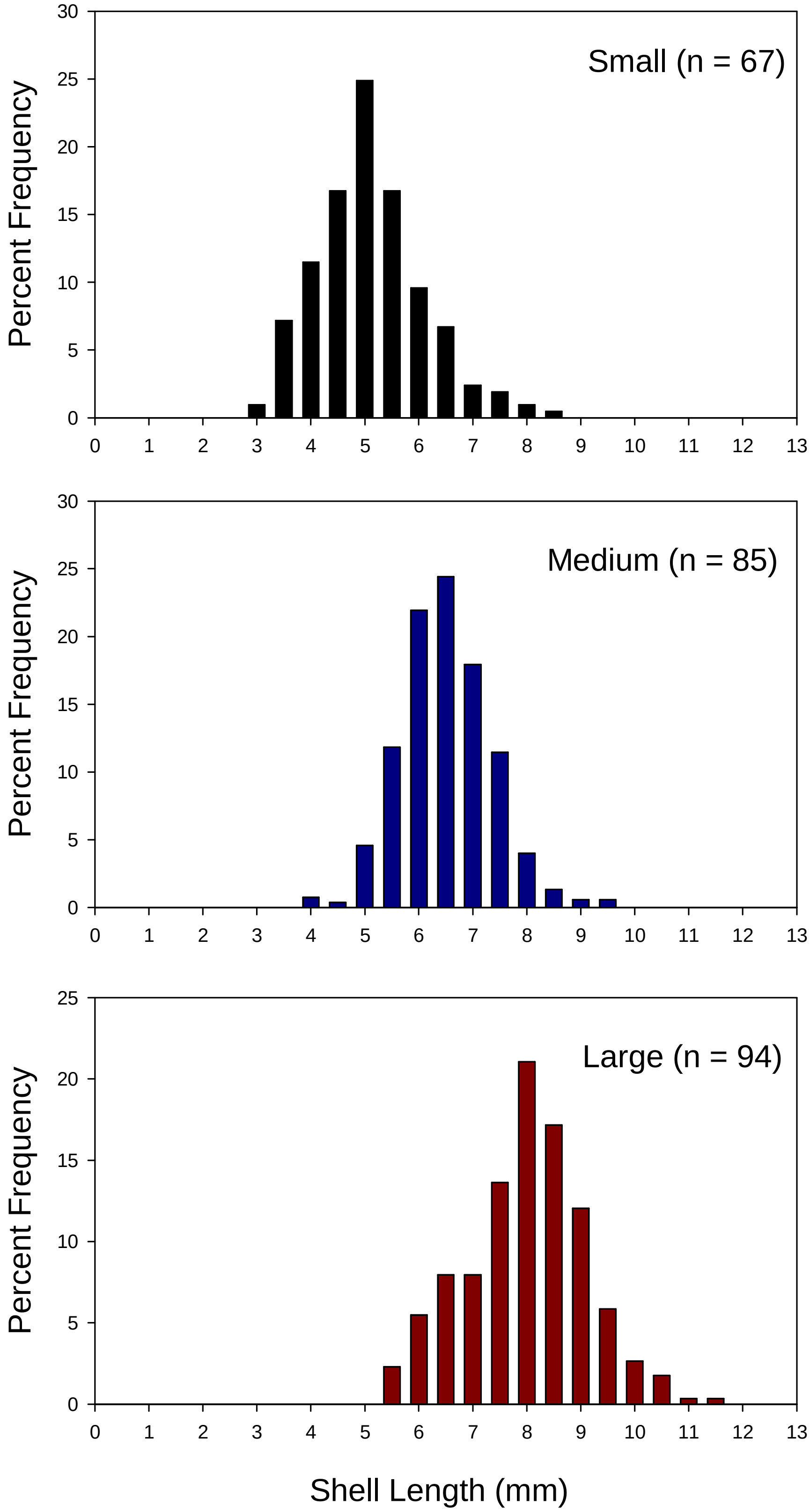

Small (n = 67)
Percent Frequency
Medium (n = 85)
Percent Frequency
Large (n = 94)
Percent Frequency
Shell Length (mm)

Figure II-b.

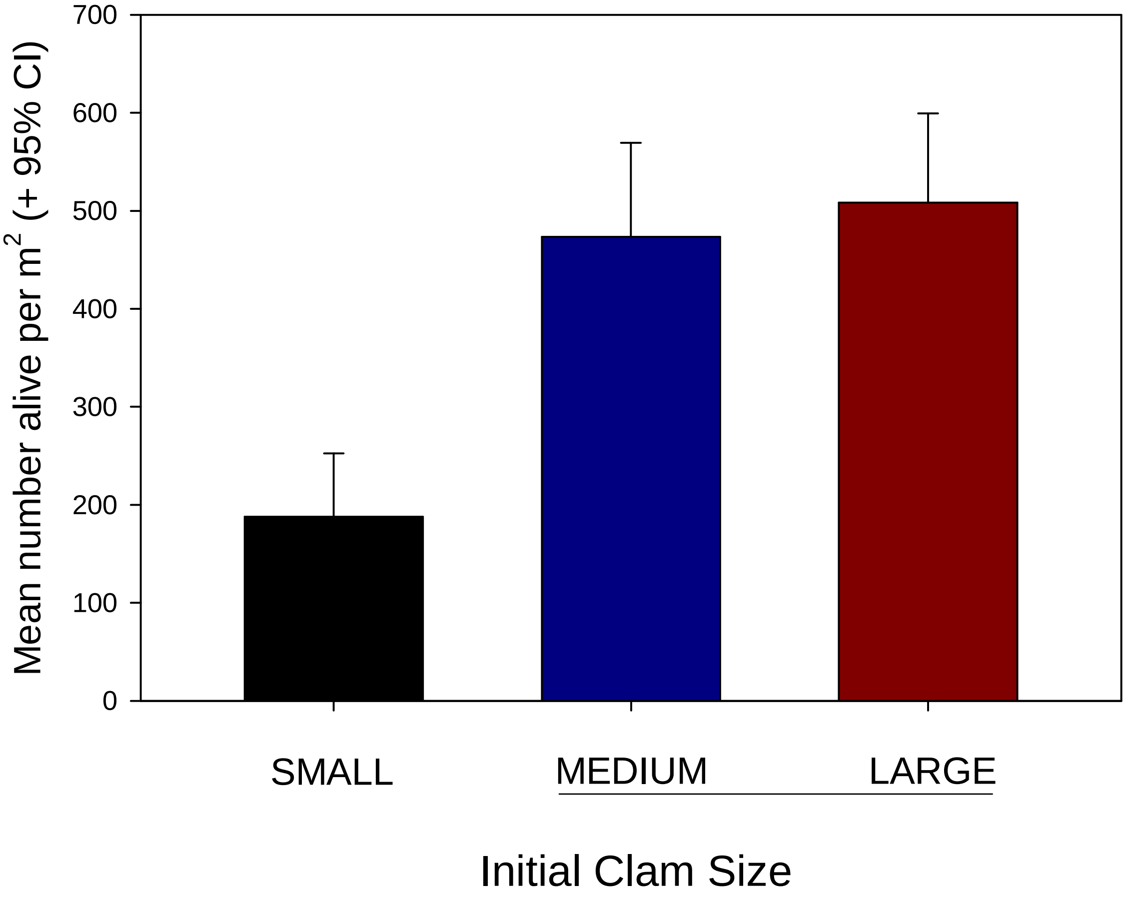
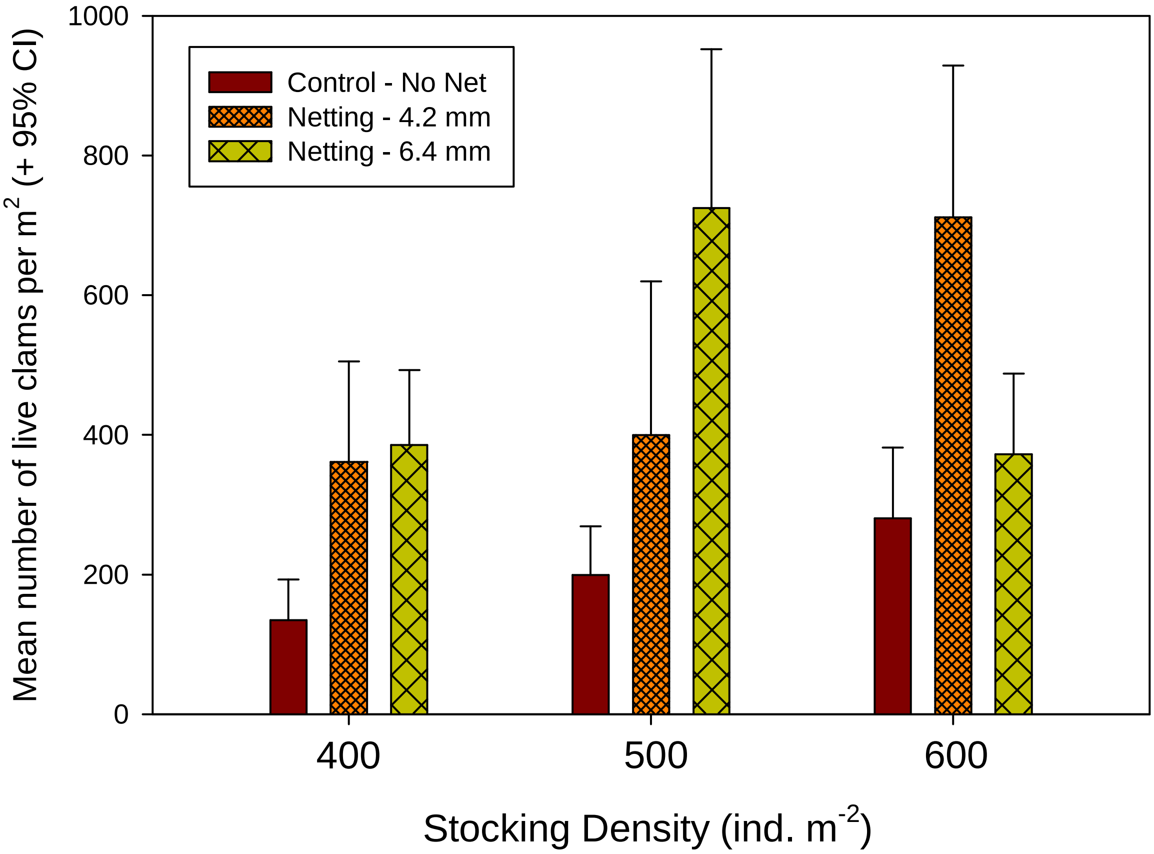
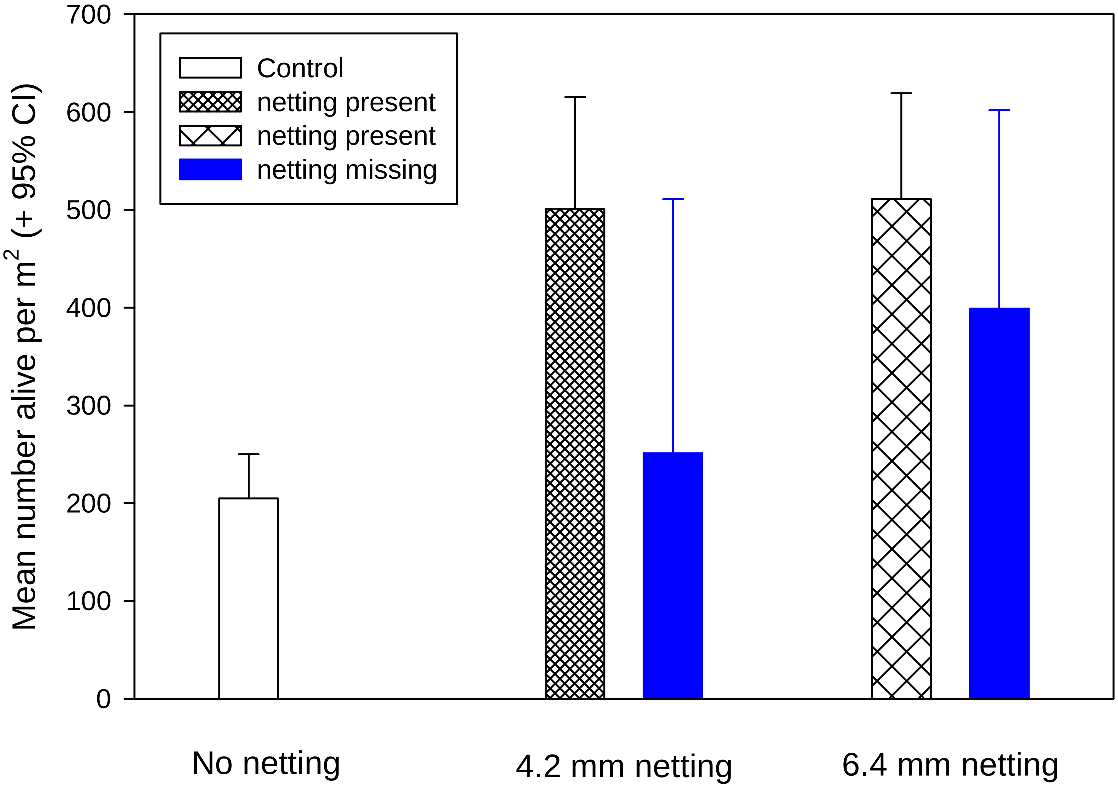

Mean number alive per m2 (+ 95% CI)
700
600
500
400
300
200
100
0
SMALL
MEDIUM
LARGE
Initial Clam Size

Figure II-c.



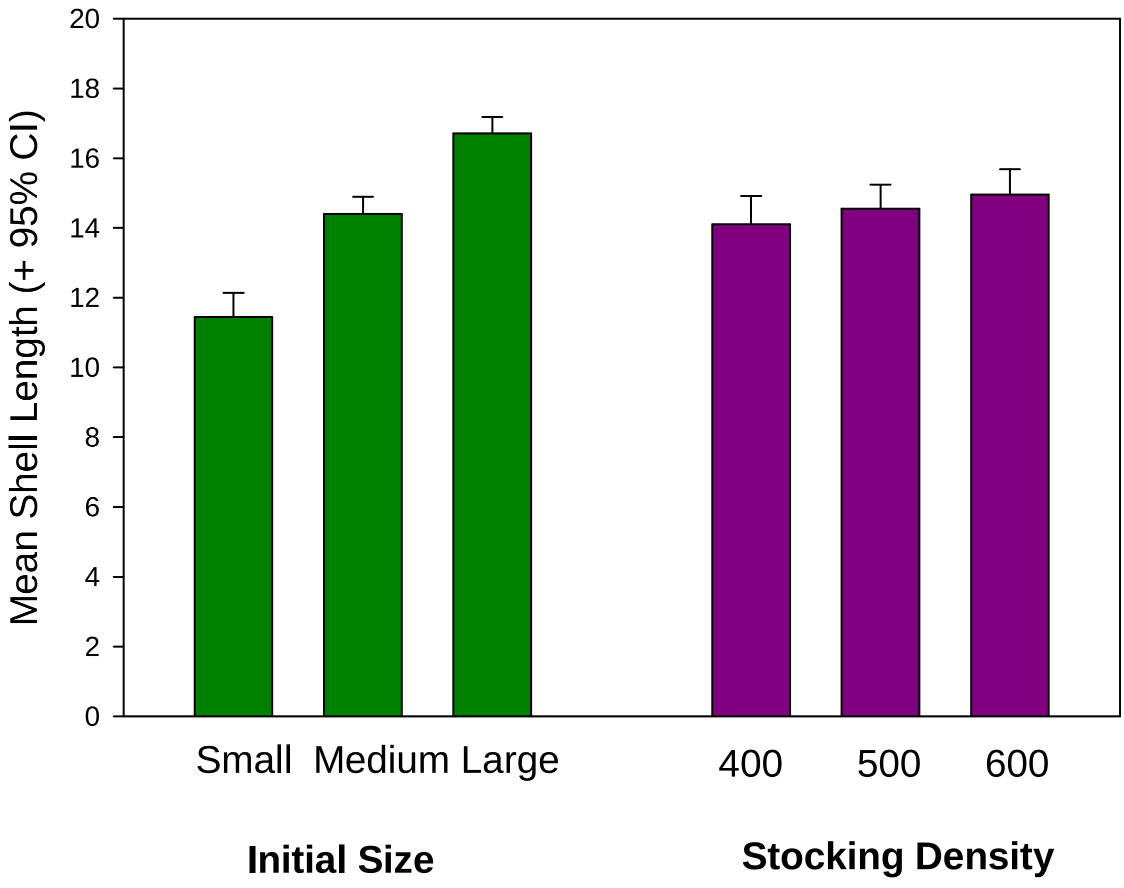
Mean number of live clams per m2 (+ 95% CI)
1000
800
600
400
200
0
Control - No Net
Netting - 4.2 mm
Netting - 6.4 mm
400
500
600
Stocking Density (ind. m-2)

Figure II-d.



Control
netting present
netting present
netting missing
Mean number alive per m2 (+ 95% CI)
700
600
500
400
300
200
100
0
No netting
4.2 mm netting
6.4 mm netting

Figure II-e.



20
18
16
14
12
10
8
6
4
2
0
Mean Shell Length (+ 95% CI)
Small
Medium
Large
400
500
600
Initial Size
Stocking Density

Experiment III.

Work Plan from Phase I grant proposal:

Clams (8 and 10 mm SL, referred to as "Medium" and "Large," respectively) will be planted in 1-m^2 plots at each of three stocking densities (400, 500, and 600 m^{-2}) in May and again in June 2009. On each initiation date, ten blocks with n = 2 replicates per treatment will be established (2 sizes x 3 densities x 10 blocks x 2 initiation dates, approximately 28 days apart, or on each set of spring tides x 2 replicates per block = 240 experimental units). In October 2009, four randomly placed benthic cores (0.0077 m^2) will be taken from each plot. ANOVA will be used to examine variation in mean survival and growth. In addition, we will examine the relationship, if one exists, between stocking density and each measurement variable using lack-of-fit tests (Winer et al. 1991) to determine whether the relationship is linear or quadratic.

Methods

This experiment was established on 25-26 May 2009 in soft sediments in the shallow subtidal of Goose Cove, Trenton, Maine (44^o 25.80'N; 68^o 23.11'W). Two sizes classes of hatchery-reared clams (produced at the Downeast Institute – Beals, Maine – the previous year, and overwintered according to Beal et al. [2009]) at the three stocking densities were added (spread uniformly) to 120 unprotected square-meter plots delineated by wooden laths at each corner. Planting densities for each size class were estimated by creating a regression plot of clam number vs. mass (g), and then size-specific regression equations (Table III-a) were used to establish actual plot densities per plot during field planting. Plots were marked at each corner using wooden laths, and one meter spacing was left between each row and column. Five meter spacing occurred between adjacent blocks (n = 10). Clam sizes were smaller than anticipated with the mean of the smaller size class at 6.3 ± 0.10 mm (n = 191) and the larger size class at 8.4 ± 0.20 mm (n = 67) (Fig. III-a). The same experimental design was initiated approximately one month later on 23 June 2009 adjacent to the plots established in May.

On 17-18 October 2009, we took four benthic cores to a depth of 12 cm from each plot and block established in May (N = 480) and from four of the ten blocks (48 plots; N = 192) established in June. We were unable to sample the remaining six blocks due to inclement weather (snow/sleet/gusting wind), and present results from the 14 blocks we sampled. Each core was bagged separately, removed from the tidal flat, and taken to the University of Maine at Machias where each was washed through a 2 mm sieve. All live and dead clams (separated into individuals with undamaged vs. crushed or chipped valves) were counted and measurements taken on the initial and final shell length (to the nearest 0.1 mm) of all living individuals using Vernier calipers. Initial size was estimated from the distinct mark that appears in the shell of these animals when placed into sediments for the first time, similar to the line that appears in the shells of cultured soft-shell clams, *Mya arenaria*, when added to soft sediments (sensu Beal et al., 1999).

Analysis of variance (ANOVA) was performed on the square root-transformed counts of living and dead clams per core as well as the untransformed final shell length and absolute growth (Final shell length - Initial shell length). The linear model was:

$$Y_{ijklmn} = \mu + A_i + B(A)_{j(i)} + C_k + AC_{ik} + CB(A)_{kj(i)} + D_l + AD_{il} + CD_{kl} + ACD_{ikl} + DB(A)_{lj(i)} + CDB(A)_{klj(i)} + E(ABCD)_{m(ijkl)} + e_{n(ijklm)}.$$

Where:

μ = theoretical mean;
Y = dependent variable (i.e., count of living/dead clams; final shell length; absolute growth);
A_i = Date (May vs. June) – factor is fixed;
B_j = Block (nested within date) – factor is random;
C_k = Size (“Medium” vs. “Large”) – factor is fixed;
D_l = Density (400 vs. 500 vs. 600 m^{-2}) – factor is fixed;
E_m = Plot (a vs. b) nested within each combination of date, block, clam size, and density); and,
e_n = Experimental error associated with the n = 4 replicate cores per combination of treatments.

Underwood (1997) was used to determine appropriate mean square estimates for each source of variation. In addition, several times the block(date) source of variation was decomposed to elucidate differences in spatial variability according to planting date. To avoid excessive type I errors, an adjusted alpha ($\alpha' = 1 - [1-\alpha]^{1/n}$; where α=0.05 and n = number of contrasts) was used as a decision rule following the advice of Winer et al. (1991).

Results

Survival

Number of hard clams sampled in October was independent of planting date ($\bar{O}_{May}$ = 97.3 ± 18.0 ind. m^{-2}, n = 120 vs. $\bar{O}_{June}$ = 146.8 ± 28.8 ind. m^{-2}, n = 48; P = 0.1423; Table III-b), and stocking density (P = 0.1756, Table III-b), suggesting clam losses occurred in a density-independent fashion. Overall clam losses, based on initial stocking densities and pooled across planting date, averaged 77.5% (Fig. III-b). Individuals that died with valves intact as well as those with chipped or crushed shells were common among the samples (Table III-c). Number of crushed individuals per sample did not vary significantly with date (P = 0.3317), initial clam size (P = 0.9435), or stocking density (P = 0.0840). Initial clam size played an important role in number of live individuals in core samples (P = 0.0349; Table III-c) as approximately 45% more clams were sampled in cores from plots seeded with “Large” vs. “Medium” size clams (Fig. III-c). Significant spatial (block-to-block) variability in number of living individuals was observed (P < 0.0001; Table III-c); however, this variation was due to blocks established in May rather than June (Fig. III-d). In addition, significant variation was observed between replicate plots within blocks, planting dates, and treatments (P = 0.0025; Table III-b); however, when a single block (block #10; Fig. III-e) was removed from the analysis, this variability became nonsignificant (P = 0.2126; df = 78, 468).

Growth

Remarkably, hard clams planted during June 2009 attained a larger final mean shell length (SL) (+ 25%) and added more shell (i.e., faster absolute growth; +37%) than those planted the prior month, in May (Figs. III-f & III-g; Table III-d). Large clams (initial O_{SL} = 8.4 mm) attained an 18% greater final mean SL than medium clams (initial O_{SL} = 6.3 mm) (e.g., 12.8 ± 0.55 mm [n = 79] vs. 10.9 ± 0.57 mm [n = 69]); however, this difference was not statistically significant (P = 0.1195). Conversely, large clams added significantly more new shell than medium clams (i.e., absolute growth was 22.4% more in large vs. medium clams; P = 0.0087; Tables III-d & III-e). Significant block-to-block variation occurred for both mean SL and mean absolute growth (Table III-d; Fig. III-h); however, as with number of live individuals (see above), the variation was restricted to those blocks established in May (Table III-d).

Summary: This field experiment clearly showed that seeding clams in the shallow subtidal at this site without protecting them with flexible netting or other predator deterrent is not warranted. Clam mortality was estimated to be 77.5% during the period from late May to mid-October and this was unrelated to stocking density (Fig. III-b). Not surprisingly, survival was related to initial clam size, with 45% more clams found in cores that had been seeded with "Large" vs "Medium" sizes. Absolute increase in shell length (but not final SL) was influenced by clam size. Clams seeded in plots in May attained a mean final SL that was smaller than those planted in June. This could be explained logically if clams were smaller initially in May vs. June, but this did not occur. This result is surprising, and we have no reasonable explanation for it at this time. Once again, stocking density (400-600 ind. m^{-2}) did not influence growth, but this may have been due to the poor survival across each density treatment.

Table III-a. Relationship between counts of hatchery-reared clams of two sizes and mass (g) used to establish stocking densities in field plots of Experiment III. Size "M" and "L" have mean shell lengths of 6.3 ± 0.10 mm and 8.4 ± 0.20 mm, respectively.

Size	**Count (no. individuals)**	**Mass (g)**
M[1]	400	11.0
M	500	12.7
M	600	14.5
L[2]	430	80.4
L	425	82.0
L	515	100.3
L	523	102.1
L	555	110.3
L	603	117.4

[1] $Y_M = -227.4 + 57.1X$ ($r^2 = 0.999$; $P < 0.0001$)

[2] $Y_L = 46.6 + 4.7X$ ($r^2 = 0.992$; $P < 0.0001$)

Table III-b. Analysis of variance on the square root-transformed number of living juvenile hard clams in 1-m^2 plots at Goose Cove, Trenton, Maine from samples taken on 17-18 October 2009. Block(Date) source of variation is decomposed into two orthogonal contrasts (α' = 0.0253). (n = 4)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Planting Date (May v. June)	1	6.65116662	6.65116662	2.47	0.1423
Block(Date)	12	32.36871228	2.69739269	4.41	<.0001
May	9	31.75863150	3.52873683	5.77	<.0001
June	3	0.61008078	0.20336026	0.33	0.8018
Clam Size ('MD' v. 'LG')	1	4.33283469	4.33283469	331.95	0.0349
Date x Size	1	0.01305254	0.01305254	0.02	0.8771
Block x Size(Date)	12	6.28027618	0.52335635	0.86	0.5937
Density (400 v. 500 v. 600)	2	1.66064741	0.83032370	1.87	0.1756
Date x Density	2	0.24554695	0.12277348	0.28	0.7606
Density x Size	2	0.55721926	0.27860963	1.05	0.3647
Date x Density x Size	2	0.67232059	0.33616030	1.27	0.2991
Density x Block(Date)	24	10.64715557	0.44363148	0.73	0.8118
Density x Size x Block(Date)	24	6.35465197	0.26477717	0.43	0.9887
Plot(Date x Den x Siz x Blk)	84	51.36355798	0.61147093	1.55	0.0025
Error	504	198.87371290	0.39459070		
Corrected Total	671	321.29577620			

Table III-c. Fate of cultured hard clams in Experiment III at Goose Cove, Trenton, Maine related to planting date (25-26 May 2009 vs. 23 June 2009), initial clam size ("M" = 6.3 + 0.10 mm vs. "L" = 8.4 ± 0.20 mm), and stocking density (400 vs. 500 vs. 600 ind. m^{-2}). Mean number per square meter (± 95% confidence intervals) is given for individuals Alive (A), Dead with Undamaged valves (DU), and Dead with Crushed or Chipped valves (DC) within benthic cores taken on 17-18 October 2009. Experimental interval = 147 days.

Planting Date	**Clam Size**	**Stocking Density**	**n**	**A**	**DU**	**DC**
May	M	400	20	63.0(42.0)	40.4(18.9)	46.1(22.93)
		500	20	75.9(43.4)	54.9(31.9)	67.9(23.9)
		600	20	93.8(31.4)	61.4(22.4)	54.9(24.6)
	L	400	20	98.6(39.1)	33.9(21.1)	35.6(17.6)
		500	20	129.3(68.6)	46.9(28.8)	59.8(26.1)
		600	20	122.8(44.9)	52.3(18.5)	49.6(22.3)
June	M	400	8	137.4(95.6)	20.2(20.1)	24.2(27.9)
		500	8	117.2(66.1)	12.1(13.9)	24.3(19.1)
		600	8	98.6(39.1)	33.9(21.1)	35.6(17.6)
	L	400	8	133.4(66.9)	44.5(28.8)	20.2(28.7)
		500	8	185.9(106.9)	68.7(46.7)	52.5(61.1)
		600	8	189.9(96.3)	24.2(19.1)	64.7(50.0)

Table III-d. Analysis of variance on the untransformed **a)** final mean shell length, and **b)** absolute growth data from Experiment III at Goose Cove, Trenton, Maine from May/June to October 2009. Number of replicates varies from 1 to 4 depending on the number of living individuals sampled in each benthic core. Only cores containing live clams are included in these analyses. Orthogonal contrasts (underlined sources of variation) used α' = 0.0253.

a)

Source	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Date	1	490.8168811	490.8168811	34.37	<.0001
Block(Date)	12	171.3400854	14.2783405	2.02	0.0366
May	9	163.1686358	18.1298484	2.56	0.0139
June	3	8.1714496	2.7238165	0.39	0.7641
Clam Size	1	277.2732419	277.2732419	27.71	0.1195
Date x Size	1	10.00667448	10.00667448	1.20	0.2946
Size x Block(Date)	12	99.9779340	8.3314945	1.18	0.3179
Density	2	30.38148118	15.19074059	2.78	0.0818
Date x Density	2	22.10194614	11.05097307	2.02	0.1540
Density x Size	2	27.82250178	13.91125089	2.57	0.0978
Date x Density x Size	2	1.25586820	0.62793410	0.12	0.8911
Density x Block(Date)	24	130.9951814	5.4581326	0.77	0.7562
Density x Size x Block(Date)	24	130.1385305	5.4224388	0.77	0.7619
Plot(Date x Block x Den x Size)	64	452.6870951	7.0732359	1.52	0.0164
Error	177	821.128021	4.639141		
Corrected Total	324	2674.348536			

b)

Source	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Date	1	176.2366717	176.2366717	11.81	0.0049
Block(Date)	12	179.0052763	14.9171064	2.94	0.0026
May	9	164.0735025	18.2303892	3.59	0.0011
June	3	14.9317738	4.9772579	0.98	0.4073
Clam Size	1	58.42299266	58.42299266	5373.50	0.0087
Date x Size	1	0.01087243	0.01087243	0.00	0.9698
Size x Block(Date)	12	87.4510507	7.2875876	1.44	0.1731
Density	2	28.15338842	14.07669421	2.90	0.0742
Date x Density	2	21.87188615	10.93594308	2.26	0.1266
Density x Size	2	14.80676906	7.40338453	1.59	0.2243
Date x Density x Size	2	3.31826938	1.65913469	0.36	0.7036
Density x Block(Date)	24	116.3520200	4.8480008	0.96	0.5325
Density x Size x Block(Date)	24	111.6147288	4.6506137	0.92	0.5801
Plot(Date x Block x Den x Size)	64	324.6949876	5.0733592	1.48	0.0235
Error	177	606.416325	3.426081		
Corrected Total	324	1726.148624			

Table III-e. Final mean shell length (SL) and mean absolute growth (AG) (in mm) of cultured hard clams (± 95% confidence intervals) in Experiment III at Goose Cove, Trenton, Maine related to planting date (25-26 May 2009 vs. 23 June 2009), initial clam size ("M" = 6.3 + 0.10 mm vs. "L" = 8.4 ± 0.20 mm), and stocking density (400 vs. 500 vs. 600 ind. m^{-2}). Clams were sampled using benthic cores taken from unprotected square-meter plots on 17-18 October 2009. Experimental interval = 147 days. Number of replicates (= number of square meter plots) varies by treatment according to number of living clams within each sample.

Planting Date	**Clam Size**	**Stocking Density**	**n**	**SL**	**AG**
May	M	400	15	10.0 (1.22)	3.7 (1.25)
		500	14	9.4 (1.09)	3.2 (1.04)
		600	17	10.8 (0.84)	4.6 (0.82)
	L	400	18	11.7 (1.19)	4.7 (1.12)
		500	18	12.0 (1.04)	4.9 (0.93)
		600	19	11.9 (0.82)	4.9 (0.72)
June	M	400	8	13.3 (1.95)	6.1 (1.64)
		500	8	11.3 (1.45)	4.6 (1.37)
		600	7	12.9 (2.58)	5.8 (2.00)
	L	400	8	15.8 (1.74)	7.3 (1.47)
		500	8	14.6 (1.14)	5.9 (1.15)
		600	8	13.3 (1.95)	6.1 (1.64)

Figure Legends

Figure III-a. Size frequency distribution for **a)** “Medium” (Ο = 6.3 ± 0.10 mm, n = 191) and **b)** “Large” (Ο = 8.4 ± 0.20 mm, n = 67) hard clam juveniles used in Experiment III (25-26 May to 17-18 October 2009) at Goose Cove, Trenton, Maine.

Figure III-b. Mean number of living, cultured juveniles of *Mercenaria mercenaria* on 17-18 October 2009 at Goose Cove, Trenton, Maine as a function of initial (25-26 May 2009) stocking density. ANOVA on the square root-transformed number showed no significant effect due to density (see Table III-b).

Figure III-c. Effects of initial clam size on number of living, cultured hard clam juveniles at Goose Cove, Trenton, Maine. Clams were seeded into 1-m^2 unprotected plots on 25-26 May and 23 June 2009 and sampled on 17-18 October 2009. Means are pooled over planting date and stocking density. (n = 84)

Figure III-d. Spatial variability of living hard clams from block-to-block across both planting dates. Significant variation is observed for blocks established in May 2009 ($P < 0.0001$), but not for blocks established in June 2009 ($P = 0.8018$; Table III-b).

Figure III-e. Plot-to-plot variation in number of living individuals of cultured hard clams between replicates of the six treatments within a single block (#10) from benthic cores taken on 17 October 2009 at Goose Cove, Trenton, Maine. When data from this block was removed from the overall ANOVA (Table III-b), the variation due to the source titled Plot(Date x Density x Size x Block) became nonsignificant ($P = 0.2126$) suggesting that this block was unique compared to the other thirteen blocks used in this experiment.

Figure III-f. Final mean shell length and absolute mean shell growth (+ 95% CI) of juvenile hard clams in October 2009 as a function of planting date (25-26 May 2009 vs. 23 June 2009). n = number of plots containing at least one living hard clam in October 2009. ANOVA indicated significant difference for both variables between planting dates ($P < 0.005$; Table III-d).

Figure III-g. Final size-frequency distribution of cultured hard clams planted in May and June 2009 at Goose Cove, Trenton, Maine. Mean shell length was significantly larger in June- vs. May-planted clams (see Table III-d). A 2 x 7 G-test of independence also indicated that proportionately more clams planted in June attained sizes greater than 16 mm than did clams planted in May ($G = 71.379$, $df = 6$, $P < 0.0001$).

Figure III-h. Spatial variability in growth of hard clams from block-to-block in final mean shell length and absolute shell growth across both planting dates. Significant variation was observed for blocks established in May 2009 for both variables, but not for blocks established in June 2009 (Table III-d).

Figure III-a.

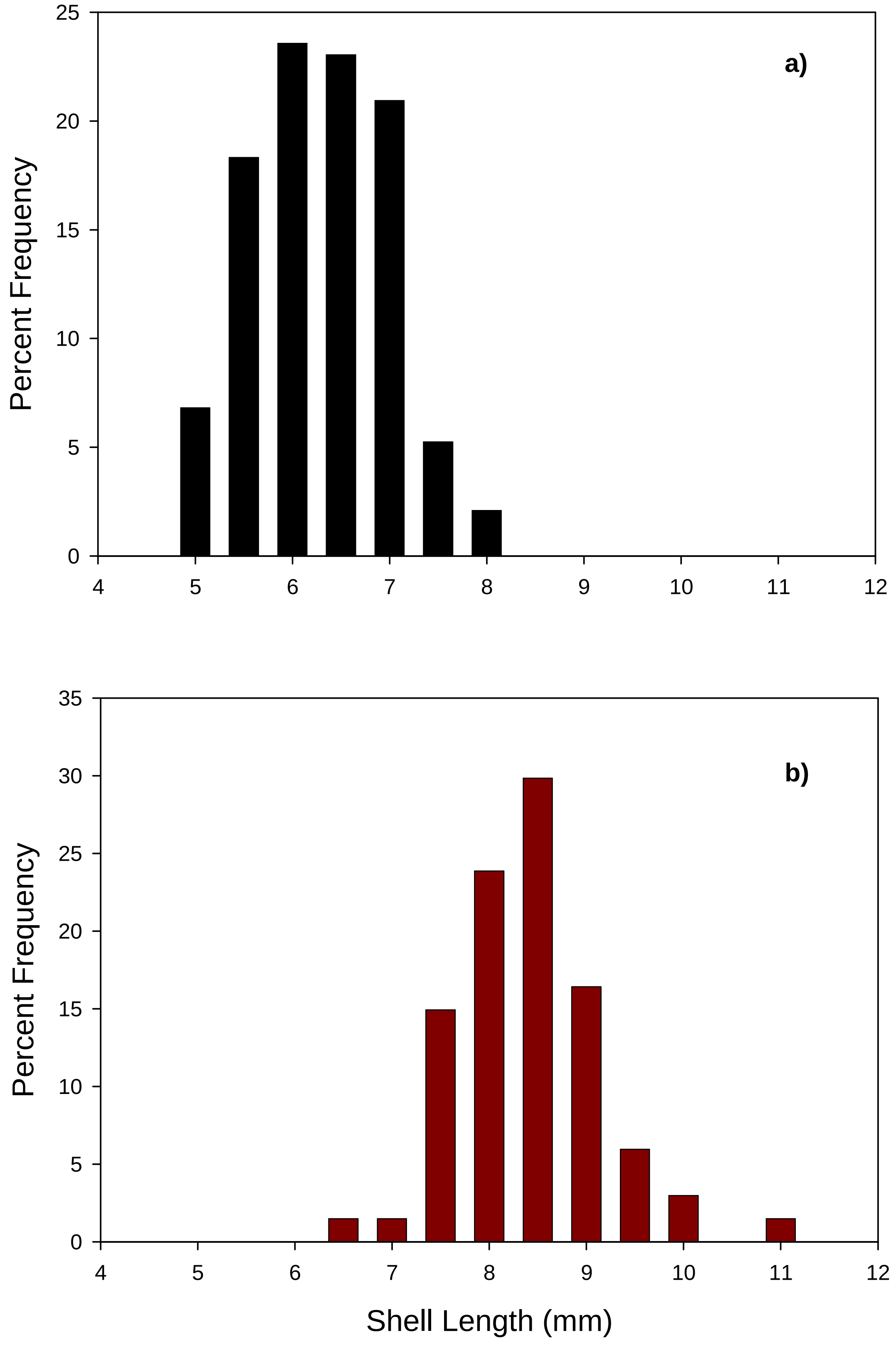
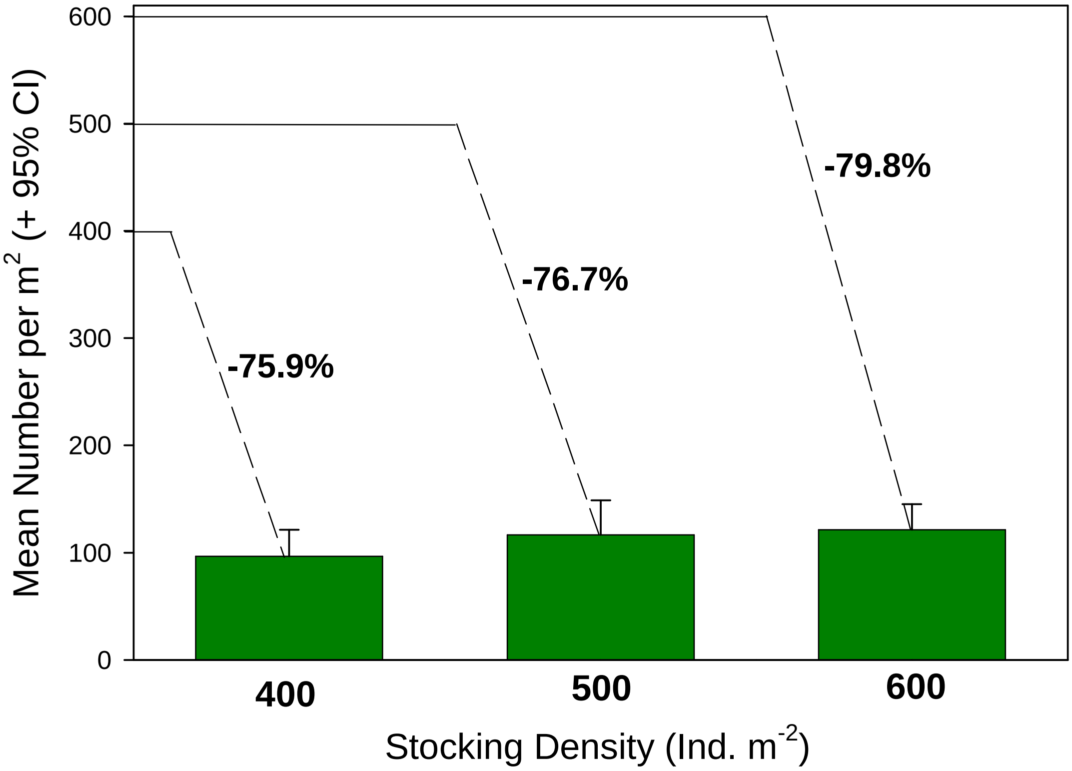
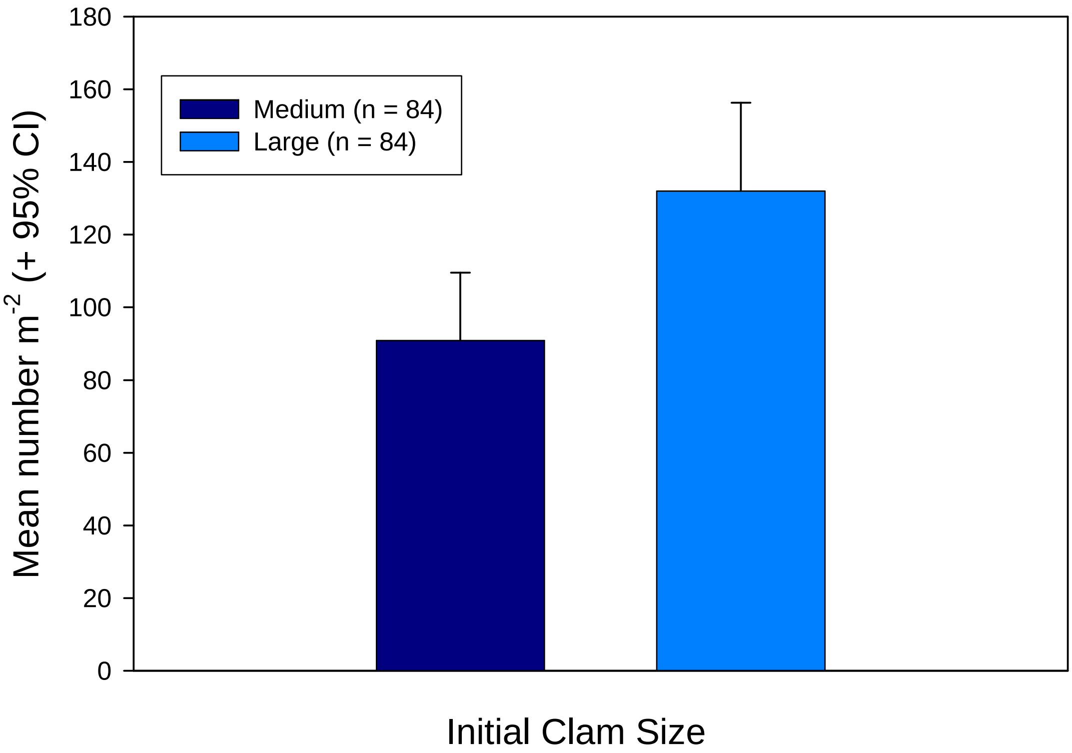

a)
b)
Percent Frequency
Percent Frequency
Shell Length (mm)
0
5
10
15
20
25
30
35
4
5
6
7
8
9
10
11
12

Figure III-b.



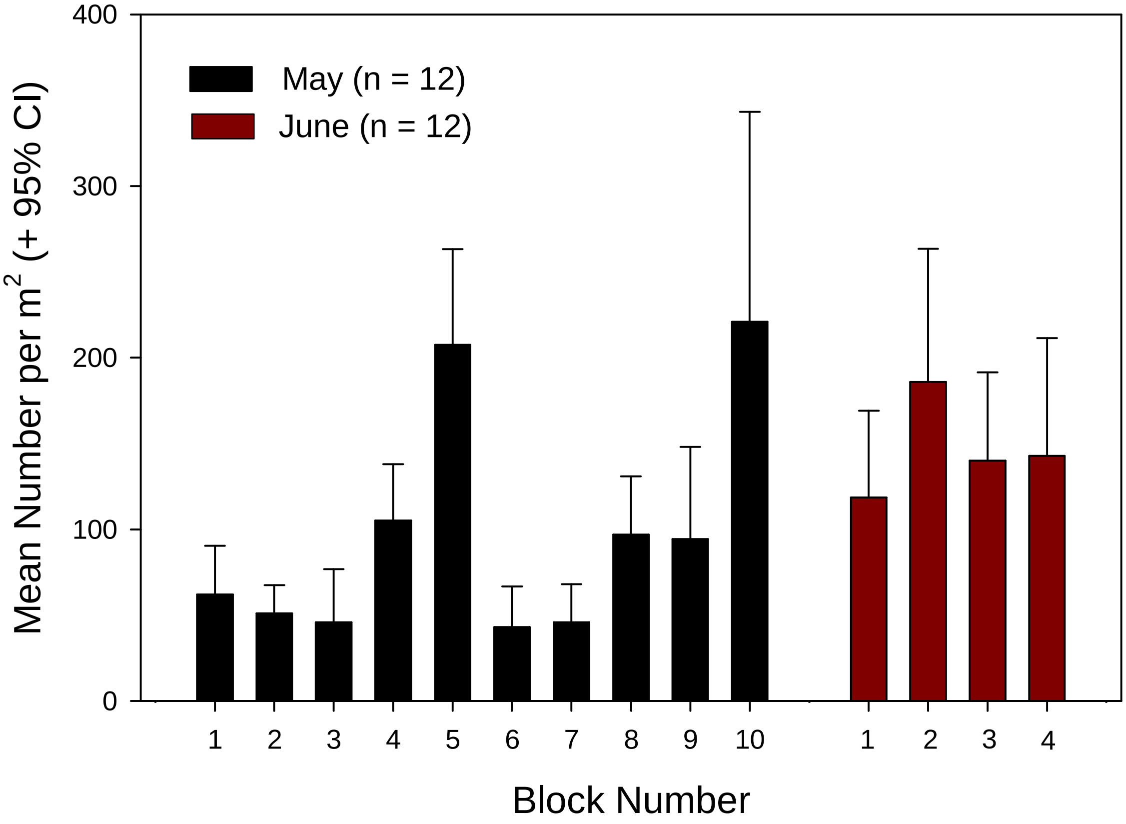
600
500
400
300
200
100
0
Mean Number per m2 (+ 95% CI)
-79.8%
-76.7%
-75.9%
400
500
600
Stocking Density (Ind. m-2)

Figure III-c.



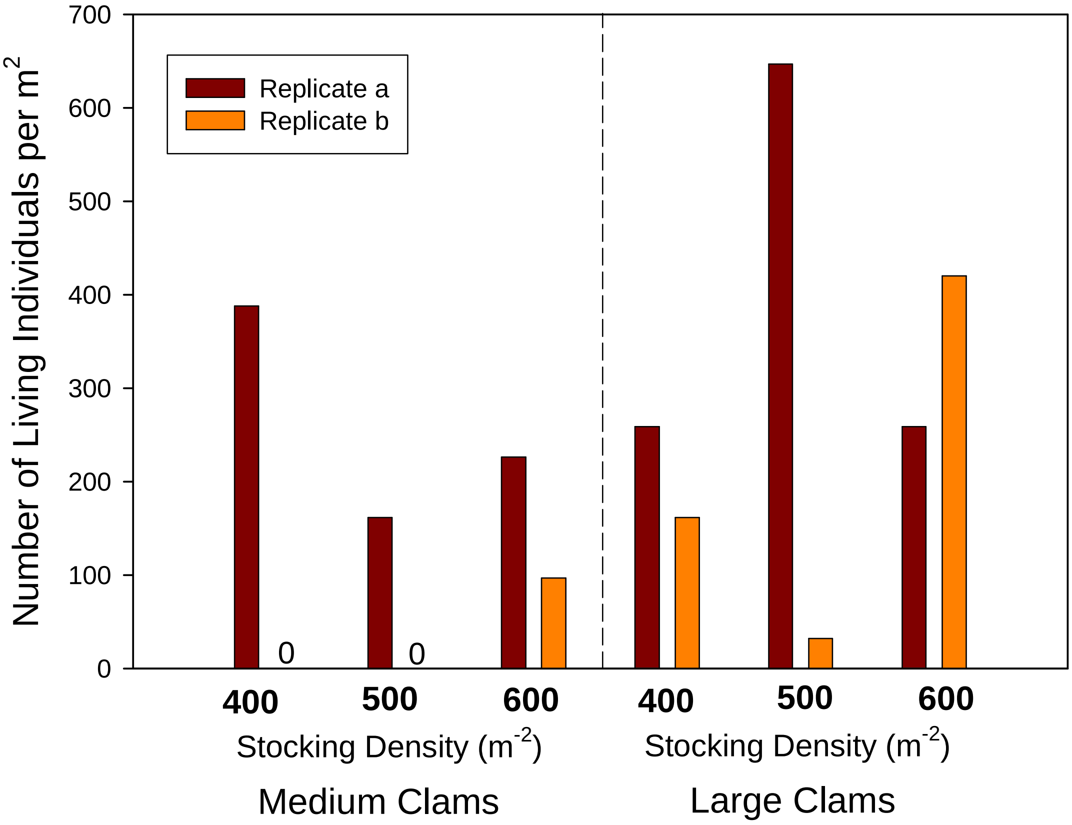
Mean number m-2 (+ 95% CI)
180
160
140
120
100
80
60
40
20
0
Medium (n = 84)
Large (n = 84)
Initial Clam Size

Fig. III-d.



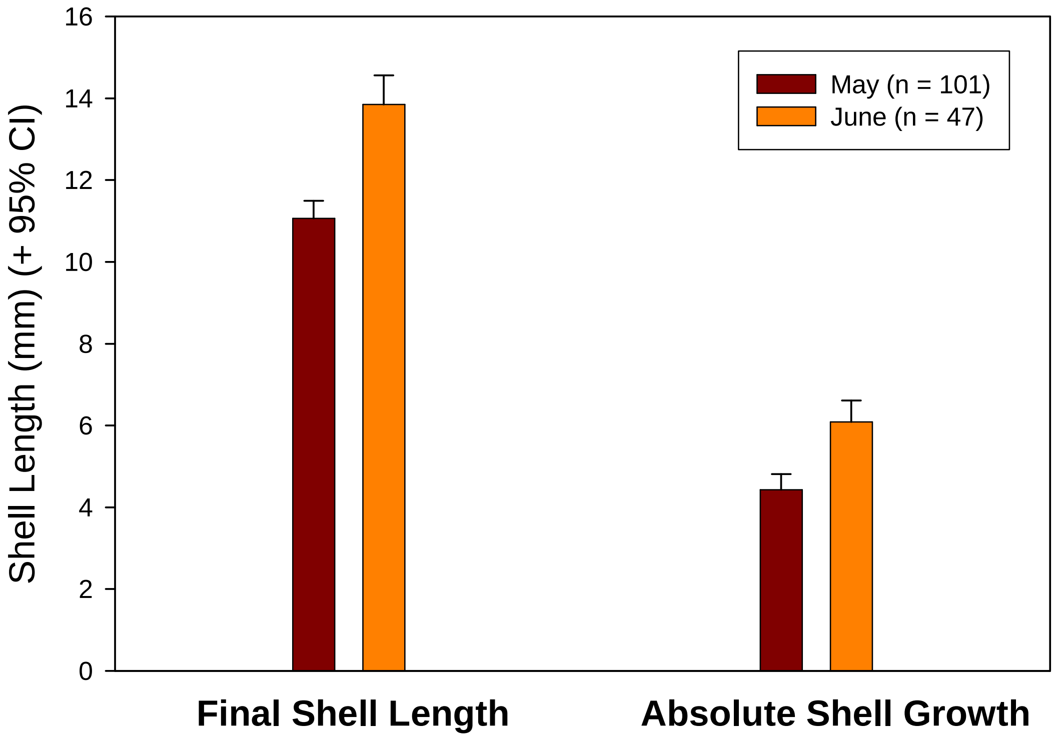
May (n = 12)
June (n = 12)
Mean Number per m2 (+ 95% CI)
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 1 2 3 4
Block Number

Figure III-e.



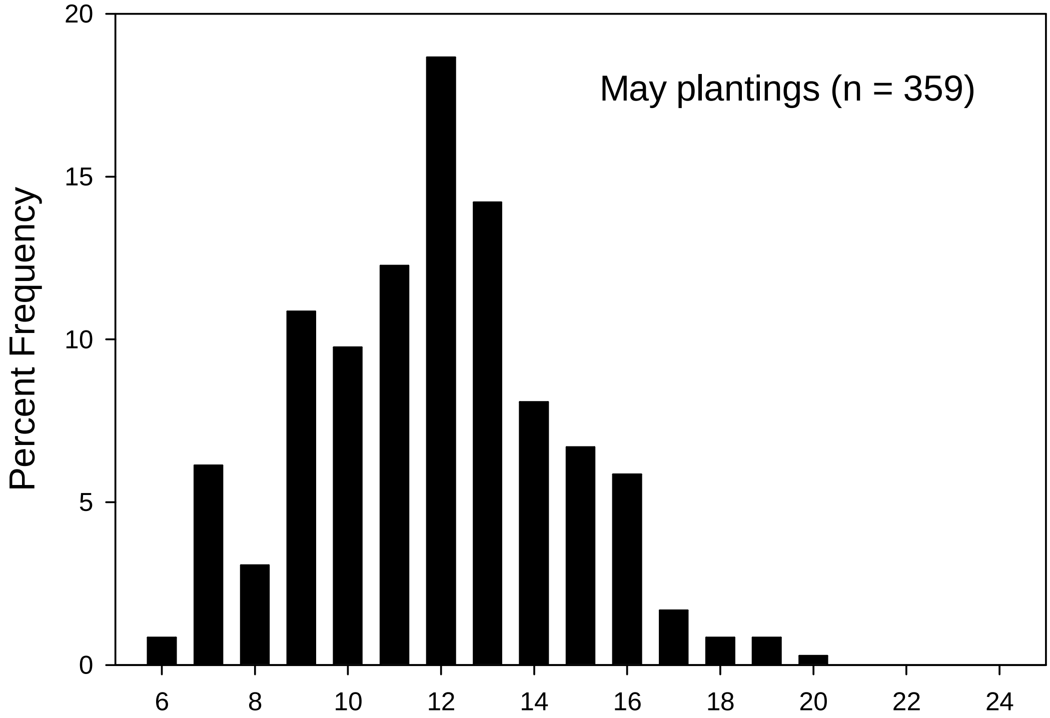
Number of Living Individuals per m2
700
600
500
400
300
200
100
0
Replicate a
Replicate b
0
0
400
500
600
400
500
600
Stocking Density (m-2)
Stocking Density (m-2)
Medium Clams
Large Clams

Figure III-f.



16
14
12
10
8
6
4
2
0
Shell Length (mm) (+ 95% CI)
May (n = 101)
June (n = 47)
Final Shell Length
Absolute Shell Growth

Figure III-g.


May plantings (n = 359)
Percent Frequency
20
15
10
5
0
6
8
10
12
14
16
18
20
22
24

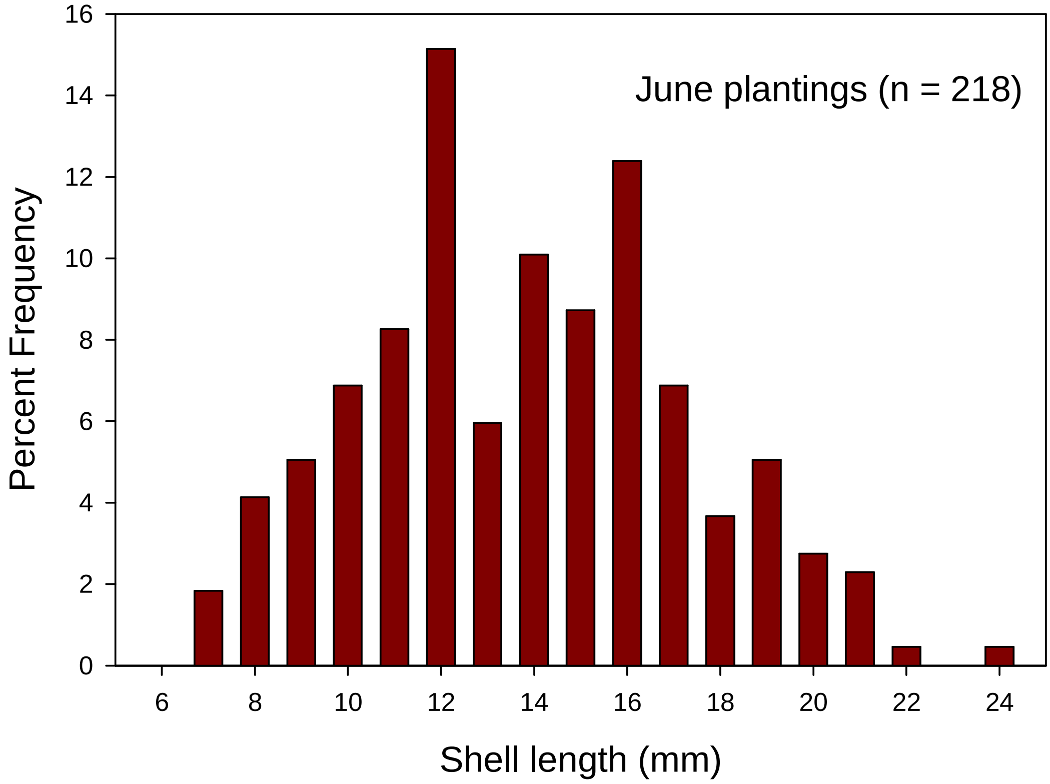
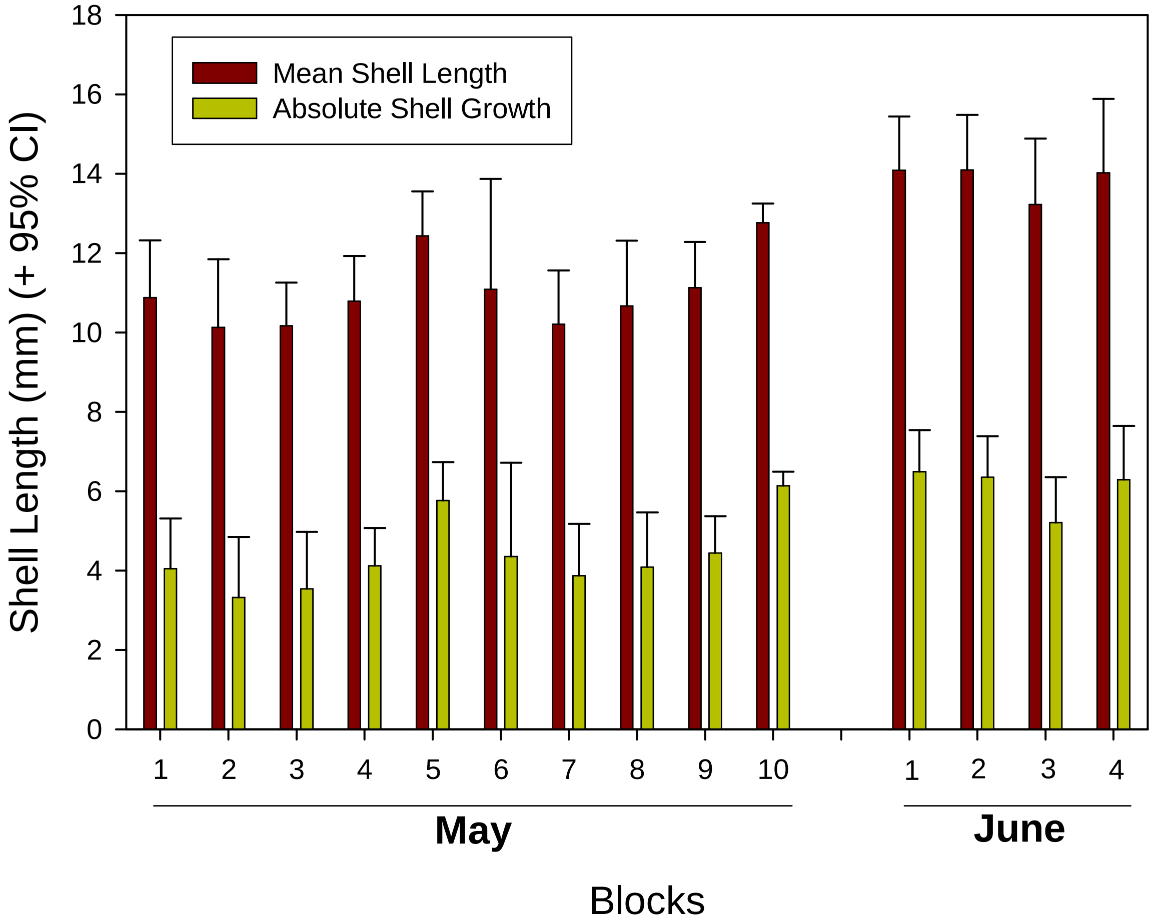

June plantings (n = 218)
Percent Frequency
16
14
12
10
8
6
4
2
0
6
8
10
12
14
16
18
20
22
24
Shell length (mm)

Figure III-h.


Mean Shell Length
Absolute Shell Growth
Shell Length (mm) (+ 95% CI)
0
2
4
6
8
10
12
14
16
18
1
2
3
4
5
6
7
8
9
10
1
2
3
4
May
June
Blocks

Other Experiments (A)

Work Plan from Phase I grant proposal:

To assess spatial variation in hard clam growth and survival, an experiment will be conducted to examine the interactive effects of stocking density, predator exclusion, transplant date, and clam size at each of four low intertidal locations. Two low intertidal study sites will be located in Hancock County near Bar Harbor. One site will be at Goose Cove in Trenton, Maine where Experiments I-III will occur. Another site will be Egypt Bay in Franklin, Maine where low densities of hard clams currently exist (J. Porada, pers. obs.). The other two study sites will be located in far eastern Maine, in Washington County, in the towns of Edmunds and Lubec where soft-shell clam farming demonstration projects are underway through the Downeast Institute for Applied Marine Research & Education (B. Beal, pers. obs.).

Beginning in May 2009, and again in June 2009, a completely randomized design (CRD) will be established at each of the four sites (this assumes that the low intertidal area we choose to set up the experiment at each site is representative of the low intertidal area in general at each site). Plastic horticultural pots (15 cm diameter x 15 cm deep; see Beal et al., 2001, Beal, 2006) will be used as the experimental units. Units will be seeded with 3, 6, 12, 24, and 48 clams (6 mm SL vs. 10 mm SL) per unit, representing densities of 165, 330, 660, 1320, and 2640 individuals m^{-2}, respectively. One-third of the units will be covered with a piece of 4.2 mm flexible netting, one-third covered with a 6.4 mm flexible netting, and the remaining units will have no netting, serving as open controls. Five replicates of each treatment (5 densities x 3 netting x 2 clam sizes) will be deployed (150 experimental units) at each site on each date (total units for the four sites = 1200). All units will be excavated and the contents of each sieved using a 2 mm mesh during November 2009. Survival and growth will be estimated as described above. Analysis of variance on mean percent survival and absolute growth will be conducted, and, for the quantitative main factor associated with stocking density, lack-of-fit tests (as described above) will be employed to examine possible linear, quadratic, cubic, or quartic models.

<u>Methods</u>

These small-scale experiments were established as follows: 28 May and 24 June 2009 at Goose Cove; 28-30 May and 24-27 June at Egypt Bay (44^o 33.52’N, 68^o 16.35’W); 29 May and 25 June at the two sites in eastern Maine (Machias River near the Rim River bridge, 44^o 42.92’N, 67^o 23.02’W; Cobscook Bay – Edmunds, at Tide Mill Farm cove, 44^o 49.04’N, 67^o 09.47’W). The experiments were collected from the field as follows: Goose Cove: 6-7 November; Egypt Bay: 5 and 10 November; Rim River bridge: 14 November; Tide Mill Farm cove: 12 and 13 November). Experimental units (plastic horticultural pots) were secured in the sediments in a 10 x 15 array (with 1 m spacing between rows and columns) on each initiation date by placing each in a small hole dug either by hand or with a small garden trowel so that a 1 cm lip extended above the sediment surface. A piece of flexible netting (ca. 48 cm x 48 cm) was secured around the top of the units (N = 100) using a rubber band. Nets (two sizes: 4.2 mm or 6.4 mm aperature) were used to deter predators. The remaining units were treated as controls, with no complete piece of netting to deter predators. Instead, a small strip of 4.2 mm flexible netting (10 cm wide x 35 cm long) was secured around the periphery using staples or a rubber band. This strip extended above the top of each unit approximately 1 cm and was designed to corral the clams so that any movement would confine them to the area of the unit. The strip of netting does not deter predators from entering the units (see Beal, 2006). Two sizes of hatchery-reared clams with somewhat overlapping size-frequency distributions were used (Fig. IV-a) in these experiments (Small = 6.4 ± 0.05, n = 100; Large = 7.7 ± 0.06, n = 114). During the fall sampling, units were taken to the University of Maine at Machias where they were processed as described above. Percent survival was assessed by counting living individuals per experimental unit and dividing by the treatment-specific stocking density. The initial and final sizes of all living clams in density treatments of 3, 6, and 12 were measured and recorded. For the two higher stocking density treatments (24 and 48 per unit), a maximum of twelve live clams was measured for each unit. These clams were chosen randomly by placing all individuals on a numbered piece of acetate, then using a random number table, twelve clams was chosen and the initial and final shell lengths recorded to the nearest 0.1 mm using Vernier calipers. Relative growth was estimated as: ([Final SL - Initial SL] / Initial SL) x 100%, where values = 100% represent a doubling of growth. Because each experiment was a completely randomized design with four fixed factors, statistical analyses were relatively straightforward (i.e., each source of variation tested using the Mean Square Error – Underwood, 1997). Percentage data was arcsine-transformed prior to statistical analyses. Orthogonal contrasts for netting are as described above. Relative growth analyses used Type III sums of squares (Shaw and Mitchell-Olds, 1993) because data were unbalanced due to the fact that one or more experimental units from each location contained no live clams at the end of the experiment.

Results

Goose Cove – Survival
Planting date significantly influenced clam survival (P = 0.0237, Table IV-a). Survival of juvenile clams planted in May was 8.5% lower than clams planted in June ($\bar{x}_{May}$ = 72.6 ± 4.3% vs. $\bar{x}_{June}$ = 78.7 ± 3.9%; n = 150). Initial clam size also played an important role with respect to juvenile clam survival. Large clams had nearly a 15% higher survival than small clams (80.8 ± 3.4% vs. 70.5 ± 4.5%; n = 150). Clam survival was enhanced due to the presence of protective netting, but only during the period from June to November 2009 (Fig. IV-b; Table IV-a). For both planting dates, no significant difference in mean percent survival was observed between clams in units protected with 4.2 mm vs. 6.4 mm netting (P = 0.8611, Table IV-a). Clams appeared to reach a low density refuge (Fig. IV-c), as survival decreased linearly with increasing intraspecific density (P = 0.0060. Table IV-a); however, the difference in mean percent survival between the lowest and highest density was 13% (80.6 ± 7.5% vs. 71.3 ± 5.6%, n = 60).

Goose Cove – Growth
There was a significant linear (positive) relationship between absolute growth and initial shell length (Y = 1.49 + 1.079X; F = 113.94, P < 0.0001, df = 1, 291, r^2 = 0.2814); hence, analyses of growth used relative growth ([final SL – initial SL]/initial SL) as the dependent variable. Not surprisingly, clams planted in May (pooled over both clam sizes) grew to a larger final mean size than those planted in June. The difference was approximately 17% (14.9 ± 0.26 mm, n = 147 vs. 17.4 ± 0.46 mm, n = 146), but only 7% (125.8 ± 3.4% vs. 134.3 ± 4.4%) when relative growth is

considered (P = 0.0011. Table IV-b). Pooling data from each date, stocking densities, and predator netting treatments, large clams grew, on average, 2.4 mm larger than small clams (17.4 ± 0.39 mm, n = 150 vs. 14.9 ± 0.34 mm, n = 143). For relative growth measurements, this difference was statistically significant (P = 0.0009, Table IV-b). Large clams planted in May reached an average shell length of 19.1 ± 0.45 mm (n = 75) vs. 15.7 ± 0.57 (n = 71) for small clams planted in May. The difference in final mean shell length between large clams planted in May vs. June was 3.4 mm (that is, clams planted in June were, on average, 3.4 mm smaller than those planted in May). The difference averaged only 1.5 mm between small clams planted in May vs. June (e.g., the final average SL for small clams planted in June was 14.2 ± 0.31, n = 72). Effects of netting were complicated by the interactive effects with both planting date and initial clam size (Fig. IV-d). From May to November 2009, there was no difference in mean relative growth between the two netting treatments and the control. Conversely, from June to November 2009, relative growth of clams in the control units was slower than those in the netted units. Similarly, both large and small clams grew relatively slower in unprotected units vs. netted units; however, small clams grew similarly in the netted units, but larger clams appeared to have a lower mean relative growth rate in units protected by the larger (6.4 mm) vs. smaller mesh (4.2 mm) (Table IV-b; Fig. IV-d).

Egypt Bay – Survival

Each one of the four experimental factors (planting date, initial clam size, stocking density, and predator exclusion) significantly affected juvenile hard clam survival (Table IV-c). For example, clams seeded in June had nearly a 22% higher survival rate than those seeded in May (83.4 ± 3.4% vs. 68.5 ± 4.7%, n = 150). Overall, small clam survival was approximately 11% less than that of the large clams (72.1 ± 4.4% vs. 79.8 ± 3.9%, n = 150). Protective netting enhanced hard clam survival by approximately 25% compared to clams in units without netting (81.7 ± 3.3%, n = 200 vs. 64.5 ± 5.5%, n = 100). The orthogonal contrast associated with the Netting source of variation (Table IV-c) demonstrated a significant difference in the protective nature the smaller vs. larger mesh. Survival of clams in units protected with the 4.2 mm mesh (86.7 ± 3.8, n = 100) was 13% higher than clams in units protected with the 6.4 mm netting (76.7 ± 5.3%, n = 100). Finally, there was a statistically significant low-density refuge similar to that observed at Goose Cove. That is, survival was a negative linear function of stocking density (Fig. IV-e; Table IV-c).

Egypt Bay – Growth

There was no significant relationship between absolute growth and initial SL (F = 2.33, df = 1, 289, P = 0.1282). Planting date had a significant effect on mean final SL (Fig. IV-f) and relative growth (P < 0.0001, Table IV-e). Clams planted in May attained a final mean SL of 12.0 ± 0.21 mm (n = 142), only 6% larger than those planted in June (11.4 ± 0.19 mm, n = 149). Differences observed for relative growth between planting dates was approximately 10%, but in the opposite direction (0_{June} = 72.4 ± 2.1%; 0_{May} = 65.9 ± 2.4%). Clam size also was a significant source of variation (P = 0.0046, Table IV-e). Mean final SL of large clams (pooled across both planting dates) was 15% greater than that of small clams (12.5 ± 0.2 mm, n = 146 vs. 10.9 ± 0.2 mm, n = 145). Although the overall effect due to netting was not statistically significant, one of the two orthogonal contrasts detected a significant (P = 0.0205) difference of 7% in relative growth between clams protected with 4.2 mm (66.8 ± 3.1%, n = 97) vs. 6.4 mm (71.3 ± 2.9%, n = 99) netting; however, the difference in mean final SL was only 0.1 mm (11.7 ± 0.3 mm vs. 11.6 ± 0.2

mm). Stocking density had a significant effect on mean relative growth, but the pattern was different according to initial clam size ($P = 0.0450$, Table IV-e). Relative growth increased linearly with stocking density for small clams and increased according to a cubic model for large clams (Fig. IV-g).

Machias River – Survival

Clam survival varied significantly with each of the factors manipulated in the study (Table IV-f). Survival was approximately 40% higher between June and November (59.2 ± 5.3%, n = 150) than between May and November (42.3 ± 5.3%, n = 150). The effect of clam size on survival varied significantly with planting date ($P = 0.0064$, Table IV-f). For the portion of the experiment initiated in May, small clam survival was significantly depressed compared to large clams, but no significant difference was observed between the two sizes when planted in June (Fig. IV-h). Predators played an important role in clam survival at this site. Only 22.1 ± 4.1% (n = 100) of clams in control units were recovered alive in November, whereas 65.0 ± 4.1% (n = 200) were recovered alive from units protected with netting, nearly a three-fold difference; however, effects of netting varied with clam size. (Table IV-f; Fig. IV-i). Significantly more large clams survived in experiment units protected with the larger aperture netting than small clams protected with the same size netting. Presumably, smaller clams were more susceptible to predators in that treatment due to consumers such as green crabs, *Carcinus maenas*, that may have been able to reach clams through the netting. A low-density refuge apparently exists for hard clam juveniles at this site. The source of variation due to stocking density suggested a significant non-linear (cubic) relationship and a lack-of-fit test (Fig. IV-j) confirmed this effect. A significant 3-way interaction (Date x Netting x Density, $P = 0.0075$; Table IV-f)) complicates this relationship somewhat. Of the eight single degree-of-freedom contrasts, only one was significant at $\alpha' = 0.0064$. For both planting dates, clam survival in netted units decreased linearly with stocking density; however, in units without netting, a low-density refuge was observed for clams planted in June, but not in May (Fig. IV-k).

Machias River – Growth

Shell growth was negligible over both planting intervals, although ANOVA demonstrated a significant difference in final mean SL between hard clams planted in May vs. June ($0_{May} = 7.4 \pm 0.2$ mm, n = 132; $0_{June} = 6.3 \pm 0.087$). Compared with mean relative growth rates at Goose Cove (> 100%; Fig. IV-d) and Egypt Bay (> 60%; Fig. IV-g), those observed at the Machias River site were essentially zero ($0_{May} = 0.3 \pm 0.15\%$; $0_{June} = 0.9 \pm 0.52$ %; Table IV-g).

Cobscook Bay – Survival

Mean percent survival at this site was extremely low across both planting dates (e.g., $0_{May} = 9.9 \pm 2.9\%$ vs. $0_{June} = 24.5 \pm 5.5\%$, n = 150). The difference between dates (nearly 150%) was statistically significant ($P < 0.0001$; Table IV-h). Survival rate of large clams was significantly higher than that of small clams ($P = 0.0241$; $0_{Small} = 14.6 \pm 4.2\%$ vs. $0_{Large} = 19.6 \pm 4.9\%$, n = 150). Netting enhanced survival compared to controls, but the effect of the netting varied significantly with planting date (Table IV-h). For example, for clams planted in May, there was no significant difference in mean percent clam survival between control and 6.4 mm netting treatments (pooled $0 = 4.75 \pm 2.4\%$, n = 100; Fig. IV-l). Large netting provided essentially no protection for clams of either size class; however, the smaller aperture netting did provide some protection (0 = 20.1 ± 6.9%, n = 50). The general pattern was the same for clams planted in June (Fig. IV-l; pooled mean survival from control and 6.4 mm netting treatments = 9.9 ± 4.3%, n = 100); however, percent survival of clams protected with the smaller aperture netting was 52.9 ± 10.5% (n = 50). The majority of mortality could be explained by predation from moon snails, *Euspira heros* and *E. triseriata*. Many of the dead valves were recovered with countersunk holes drilled near the umbo. Analysis of variance on the arcsine-transformed percent mortality due to moon snail attack demonstrated no significant effect due to planting date (P = 0.5143). Overall, 44.5 ± 3.3% (n = 300) of clams were found dead in November with shell damage typical of moon snail predation. Both stocking density and predator exclusion played a significant role in the percent of clams found dead with drilled holes in their valves (Table IV-i). Percent mortality due to moon snail predation increased with stocking density (165 to 660 ind. hard clams m-2), then leveled off. The relationship was not linear (Table IV-i), as lack-of-fit testing demonstrated a significant curvilinear model (Fig. IV-i). Netting reduced mortality due to moon snails by approximately 60% ($0_{Control}$ = 59.1 ± 4.7%, n = 100; x$0_{Netting}$ = 37.1 ± 4.1%, n = 200); however, significantly higher mortality was observed in units covered with the larger vs. smaller aperture netting ($0_{4.2\ mm}$ = 32.8 ± 5.9% vs. $0_{6.4.mm}$ = 41.4 ± 5.5%, n = 100). Living moon snails were found in both control and netted experimental units at the end of the field experiment. Snail density per unit averaged 0.35 ± 0.08 individuals (n = 300), or approximately 19.1 ± 4.19 ind m^{-2}. No significant difference in snail density was observed between netted vs. control units or between the two types of netted units (F = 0.77, df = 2, 297, P = 0.4635). Snail sizes varied from 3.2-17.6 mm. It is theoretically possible that snails as large as 5.9 mm were able to fit through the aperture of the smaller flexible netting, and snails as large as 9.0 mm were able to fit in units covered with the larger aperture netting because the dimensions of the netting (4.2 mm and 6.4 mm) are not measured along the diagonal. It is also possible that snails larger than 10 mm could have been in the sediments at the beginning of the trial, and inadvertently added to units, as no effort was made to remove fauna from the ambient sediments placed into in each experimental unit.

Cobscook Bay – growth
No source of variation associated with relative growth or absolute growth of hard clam juveniles was statistically significant (P > 0.0654). As was the case in the Machias River Estuary, shell growth was negligible. Mean final SL differed significantly according to planting date (P = 0.0006), initial size (P < 0.0001), and stocking density (P = 0.0365). Animals planted in May attained a final SL of 8.1 ± 0.5 mm (n = 62), whereas those planted in June attained a final SL of 7.1 ± 0.2 mm (n = 83). Large clams attained a mean final SL that was approximately 18% larger than smaller clams (0_{Large} = 8.1 ± 0.4 mm, n = 79 vs. 0_{Small} = 6.8 ± 0.2 mm, n = 66). The relationship between stocking density and final mean SL was curvilinear, and shows a decrease in shell length from low to intermediate densities with an increase in length at the highest density (Fig. IV-n).

Summary: These small-scale experiments were designed to examine how predator exclusion, stocking density, planting date, and clam size affect clam growth and survival, and were invaluable because they allowed us to follow the fate of individual hard clam juveniles through the growing season at multiple sites. Overall, survival decreased from west to east, with highest survival occurring at Goose Cove, in Trenton, and poorest survival occurring in Cobscook Bay where moon snails consumed nearly 45% of all juveniles. Although snails were not observed in

large numbers at either the Rim River bridge (Machias River Estuary) or Egypt Bay (Franklin), most mortality at these sites was related to the presence of crushing predators that appeared to forage (as they did at Goose Cove) in a density-dependent fashion resulting in a low-density refuge for hard clams. It appears that survival in the small experimental units is elevated compared to larger (1-m^2) plots. For example, survival at Goose Cove from May to December was approximately 70% in control (unnetted) experimental units (0.0182 m^2) whereas estimated survival at the same site over the period from May to October in 1-m^2 plots was approximately 30%. In addition, hard clams appeared to grow faster in the small experimental units at Goose Cove than in the larger plots, soft bags, and grow-out cages. This may, however, be more related to spatial variation in growth rate at this site (see results from Experiment II). Further investigation with both types of experimental units in the same general area will provide clear resolution to this situation.
Netting was important in deterring predators, and the smaller aperture netting (4.2 mm mesh size) appeared to be more effective in enhancing clam survival than the larger aperture netting (6.4 mm mesh). This was especially true at sites east of Goose Cove where grow rates were slower and clams may have been more susceptible to being washed out of the protected pots during various weather events.

The experiment demonstrated that growth rates were highly site-specific, and that final mean SL's decreased significantly in an easterly direction. For example, final mean SL of clams planted in May at Goose Cove, Egypt Bay, Machias River, and Cobscook Bay was 17.4 ± 0.46 mm, 12.0 ± 0.21, 7.4 ± 0.2 mm, and 8.1 ± 0.5 mm, respectively. These values suggest that consideration of grow-out sites in Egypt Bay and areas east should be abandoned. This information is of great use to us as we pursue additional grow-out sites. In future, we will be examining how hard clam juveniles grow and survival in the shallow subtidal of sites west of Goose Cove.

The use of small-scale experimental units has its advantages in that results are comparable between locations and planting times, units are relatively easy to manipulate, and multiple factors can be examined simultaneously using completely random designs.

Table IV-a. Analysis of variance on the arcsine-transformed survival data from the small-scale field experiment in the shallow subtidal at Goose Cove, Trenton, Maine (28 May and 24 June to 6-7 November 2009). Clams of two different sizes (Fig. IV-a) were seeded into experimental units (horticultural plant pots – 15 cm diameter x 15 cm deep) at each of five densities (165, 330, 660, 1320, and 2640) on each of the two initiation dates. Predator exclusion netting was applied to two-thirds of the units (100 units were protected using 4.2 mm flexible, plastic netting; 100 units were protected with 6.4 mm netting). The remaining 100 units served as controls without netting. Each factor was considered fixed. Orthogonal contrasts (underlined) for factors with more than two df used α' (Winer et al., 1991). Lack-of-fit tests (underlined) were conducted to determine the relationship between survival and stocking density. (n = 5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	1	1894.70237	1894.70237	5.18	0.0237
Clam Size	1	5682.57827	5682.57827	15.55	0.0001
Date x Size	1	263.81785	263.81785	0.72	0.3964
Density	4	5421.55646	1355.38911	3.71	0.0060
Linear	1	3282.29033	3282.29033	8.98	0.0030
Quadratic	1	110.477343	110.477343	0.30	0.5829
Cubic	1	1113.868919	1113.868919	3.05	0.0821
Quartic	1	914.892081	914.892081	2.50	0.1149
Date x Density	4	1413.43819	353.35955	0.97	0.4263
Size x Density	4	1180.47174	295.11794	0.81	0.5214
Date x Size x Density	4	972.95740	243.23935	0.67	0.6165
Netting	2	13051.91927	6525.95964	17.86	<.0001
Date x Netting	2	5468.95291	2734.47645	7.48	0.0007
May v. June x Control vs. net	1	5457.742335	5457.742335	14.93	0.0001
May v. June x 4.2 mm vs. 6.4 mm	1	11.210573	11.210573	0.03	0.8611
Size x Netting	2	209.02989	104.51494	0.29	0.7515
Date x Size x Netting	2	859.22039	429.61019	1.18	0.3104
Density x Netting	8	3564.93236	445.61654	1.22	0.2882
Date x Density x Netting	8	2065.58064	258.19758	0.71	0.6857
Size x Density x Netting	8	1201.84226	150.23028	0.41	0.9136
Date x Size x Density x Netting	8	1568.26564	196.03320	0.54	0.8285
Error	240	87706.3659	365.4432		
Corrected Total	299	132525.6316			

Table IV-b. Analysis of variance on the untransformed relative growth data from the small-scale field experiment in the shallow subtidal at Goose Cove, Trenton, Maine (28 May and 24 June to 6-7 November 2009). (See Table IV-a) for descriptions of each factor. Orthogonal contrasts (underlined) for factors with more than two df used α' (Winer et al., 1991). (n is variable depending on number of living individuals)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	1	0.53061821	0.53061821	10.89	0.0011
Clam Size	1	0.55140327	0.55140327	11.32	0.0009
Date x Size	1	0.04405562	0.04405562	0.90	0.3426
Density	4	0.30655431	0.07663858	1.57	0.1822
Date x Density	4	0.43928792	0.10982198	2.25	0.0640
Size x Density	4	0.41066249	0.10266562	2.11	0.0806
Date x Size x Density	4	0.07315101	0.01828775	0.38	0.8260
Netting	2	1.01864112	0.50932056	10.46	<.0001
Control vs. (4.2 mm & 6.4 mm)	1	0.84206896	0.84206896	17.29	<.0001
4.2 mm vs. 6.4 mm	1	0.17657216	0.17657216	3.62	0.0582
Date x Netting	2	0.34933397	0.17466699	3.59	0.0293
May v. June x Control v. net	1	0.21663544	0.21663544	4.45	0.0360
May v. June x 4.2 mm v. 6.4 mm	1	0.13269853	0.13269853	2.72	0.1002
Size x Netting	2	0.30530867	0.15265433	3.13	0.0454
Larg v. Sml x Control v. net	1	0.08151742	0.08151742	1.67	0.1971
Larg v. Sml x 4.2 mm v. 6.4 mm	1	0.22379125	0.22379125	4.59	0.0331
Date x Size x Netting	2	0.09135223	0.04567611	0.94	0.3930
Density x Netting	8	0.45739571	0.05717446	1.17	0.3157
Date x Density x Netting	8	0.50129783	0.06266223	1.29	0.2513
Size x Density x Netting	8	0.46532150	0.05816519	1.19	0.3033
Date x Size x Density x Netting	8	0.67017545	0.08377193	1.72	0.0946
Error	233	11.34947921	0.04871021		
Corrected Total	292	17.56403850			

Table IV-d. Analysis of variance on the arcsine-transformed percent survival data from Egypt Bay (28-30 May and 24-27 June to 5-10 November 2009). Orthogonal contrasts (α' = 0.0253) and lack-of-fit tests (α' = 0.0127) are underlined. (n = 5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	1	11250.80393	11250.80393	29.28	<.0001
Clam Size	1	3140.09304	3140.09304	8.17	0.0046
Date x Size	1	1281.51612	1281.51612	3.33	0.0691
Density	4	3806.14446	951.53612	2.48	0.0449
<u>Linear</u>	1	1581.77831	1581.77831	4.12	0.0436
<u>Quadratic</u>	1	195.75345	195.75345	0.51	0.4761
<u>Cubic</u>	1	580.58044	580.58044	1.51	0.2202
<u>Quartic</u>	1	1448.01304	1448.01304	3.77	0.0534
Date x Density	4	754.28580	188.57145	0.49	0.7426
Size x Density	4	1275.11632	318.77908	0.83	0.5075
Date x Size x Density	4	1616.70726	404.17682	1.05	0.3812
Netting	2	16555.07194	8277.53597	21.54	<.0001
<u>Control vs. (4.2 mm & 6.4 mm)</u>	1	13573.27319	13573.27319	35.32	<.0001
<u>4.2 mm vs. 6.4 mm</u>	1	2981.79875	2981.79875	7.76	0.0058
Date x Netting	2	849.05391	424.52696	1.10	0.3330
Size x Netting	2	435.58314	217.79157	0.57	0.5681
Date x Size x Netting	2	93.68435	46.84218	0.12	0.8853
Density x Netting	8	2561.60678	320.20085	0.83	0.5741
Date x Density x Netting	8	2791.33630	348.91704	0.91	0.5105
Size x Density x Netting	8	5501.43231	687.67904	1.79	0.0798
Date x Size x Density x Netting	8	1904.04073	238.00509	0.62	0.7613
Error	240	92234.4559	384.3102		
Corrected Total	299	146050.9323			

Table IV-e. Analysis of variance on the untransformed relative growth data for hard clam juveniles at Egypt Bay, Franklin, Maine (May/June to November 2009). Orthogonal contrasts ($\alpha' = 0.0253$) and lack-of-fit tests ($\alpha' = 0.0127$) are underlined.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	1	0.31493479	0.31493479	18.44	<.0001
Clam Size	1	0.43758641	0.43758641	25.62	<.0001
Date x Size	1	0.03962351	0.03962351	2.32	0.1291
Density	4	0.18521923	0.04630481	2.71	0.0309
Linear	1	0.14075365	0.14075365	8.24	0.0045
Quadratic	1	0.01528268	0.01528268	0.89	0.3452
Cubic	1	0.00449876	0.00449876	0.26	0.6083
Quartic	1	0.02591611	0.02591611	1.52	0.2193
Date x Density	4	0.04212076	0.01053019	0.62	0.6512
Size x Density	4	0.16925637	0.04231409	2.48	0.0450
Small vs. Large x Linear	1	0.00411234	0.00411234	0.24	0.6241
Small vs. Large x Quadratic	1	0.01844139	0.01844139	1.08	0.2999
Small vs. Large x Cubic	1	0.12515048	0.12515048	7.33	0.0073
Small vs. Large x Quartic	1	0.01839440	0.01839440	1.08	0.3005
Date x Size x Density	4	0.05162234	0.01290558	0.76	0.5552
Netting	2	0.09500839	0.04750420	2.78	0.0640
Control vs. Netting	1	0.00222289	0.00222289	0.13	0.7186
4.2 mm vs. 6.4 mm	1	0.09298042	0.09298042	5.44	0.0205
Date x Netting	2	0.01841996	0.00920998	0.54	0.5839
Size x Netting	2	0.03063986	0.01531993	0.90	0.4092
Date x Size x Netting	2	0.00401709	0.00200855	0.12	0.8891
Density x Netting	8	0.18330977	0.02291372	1.34	0.2238
Date x Density x Netting	8	0.09827089	0.01228386	0.72	0.6745
Size x Density x Netting	8	0.17156171	0.02144521	1.26	0.2679
Date x Size x Density x Netting	8	0.01962217	0.00245277	0.14	0.9970
Error	231	3.94549407	0.01708006		
Corrected Total	290	5.80209946			

Table IV-f. Analysis of variance on the arcsine-transformed percent survival data from the Machias River Estuary (29 May and 25 June to 14 November 2009). Orthogonal contrasts and lack-of-fit tests (underlined) use α' (as described above).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	1	12506.47893	12506.47893	49.83	<.0001
Clam Size	1	4407.91628	4407.91628	17.56	<.0001
Date x Size	1	1899.84538	1899.84538	7.57	0.0064
Density	4	4939.54323	1234.88581	4.92	0.0008
Linear	1	2884.52244	2884.52244	11.49	0.0008
Quadratic	1	18.78613	18.78613	0.07	0.7846
Cubic	1	1834.87067	1834.87067	7.31	0.0073
Quartic	1	201.34362	201.34362	0.80	0.3713
Date x Density	4	2190.65141	547.66285	2.18	0.0717
Size x Density	4	2392.65111	598.16278	2.38	0.0521
Date x Size x Density	4	1386.71516	346.67879	1.38	0.2411
Netting	2	91180.78945	45590.39472	181.64	<.0001
Control vs. Rest	1	67092.50047	67092.50047	267.31	<.0001
4.2 mm vs. 6.4 mm	1	24088.28898	24088.28898	95.97	<.0001
Date x Netting	2	757.95007	378.97503	1.51	0.2230
Size x Netting	2	1547.46329	773.73165	3.08	0.0477
Lg v Sm x 0 vs. Rest	1	155.10265	155.10265	0.62	0.4326
Lg v Sm x 4.2 v. 6.4	1	1392.36064	1392.36064	5.55	0.0193
Date x Size x Netting	2	3675.41933	1837.70966	7.32	0.0008
Date x Size x No net vs. net	1	1917.28913	1917.28913	7.64	0.0062
Date x Size x 4.2 vs. 6.4 mm	1	1758.13020	1758.13020	7.00	0.0087
Density x Netting	8	1934.62779	241.82847	0.96	0.4652
Date x Density x Netting	8	5399.80054	674.97507	2.69	0.0075
M v. June x Linear x 0 v Rest	1	1259.164607	1259.164607	5.02	0.0260
M v June x Linear x 4 v 6mm	1	489.495401	489.495401	1.95	0.1639
M v June x Quad x 0 v Rest	1	0.746964	0.746964	0.00	0.9565
M v June x Quad x 4 v 6mm	1	35.155411	35.155411	0.14	0.7085
M v June x Cubic x 0 v Rest	1	1567.951223	1567.951223	6.25	0.0131
M v June x Cubic x 4 v 6mm	1	63.339506	63.339506	0.25	0.6159
M v June x Quartic x 0 v Rest	1	1959.052268	1959.052268	7.81	0.0056
M v June x Quartic x 4 v 6mm	1	24.869773	24.869773	0.10	0.7532
Size x Density x Netting	8	3309.54115	413.69264	1.65	0.1120
Date x Size x Density x Netting	8	5140.61797	642.57725	2.56	0.0107
Error	240	60239.0483	250.9960		
Corrected Total	299	202909.0594			

Table IV-g. Analysis of variance on mean relative growth of hard clam juveniles at the Rim River bridge site in the Machias Estuary. Clams were planted on two dates (29 May and 25 June 2009) and were removed from the site on 14 November 2009. Number of replicate units contributing to mean relative growth (n) depended on survival, and varied from 1 to 5.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	1	0.00264127	0.00264127	4.57	0.0336
Clam Size	1	0.00105865	0.00105865	1.83	0.1772
Date x Size	1	0.00035036	0.00035036	0.61	0.4369
Density	4	0.00203118	0.00050780	0.88	0.4771
Date x Density	4	0.00183026	0.00045756	0.79	0.5313
Size x Density	4	0.00074709	0.00018677	0.32	0.8621
Date x Size x Density	4	0.00169820	0.00042455	0.74	0.5689
Netting	2	0.00028706	0.00014353	0.25	0.7802
Date x Netting	2	0.00051996	0.00025998	0.45	0.6381
Size x Netting	2	0.00071811	0.00035905	0.62	0.5380
Date x Size x Netting	2	0.00076885	0.00038443	0.67	0.5150
Density x Netting	8	0.00398228	0.00049779	0.86	0.5494
Date x Density x Netting	8	0.00212288	0.00026536	0.46	0.8835
Size x Density x Netting	8	0.00524554	0.00065569	1.14	0.3406
Date x Size x Density x Netting	8	0.00193297	0.00024162	0.42	0.9092
Error	215	0.12415537	0.00057747		
Corrected Total	274	0.15462712			

Table IV-h. Analysis of variance on the arcsine-transformed percent survival data of hard clam juveniles added to experimental units at a low intertidal location in Cobscook Bay (Edmunds) from 29 May and 25 June to 12-13 November 2009. Orthogonal contrasts (underlined sources of variation) used an $\alpha' = 0.0253$. (n = 5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	1	10756.68554	10756.68554	29.08	<.0001
Clam Size	1	1905.75321	1905.75321	5.15	0.0241
Date x Size	1	131.50422	131.50422	0.36	0.5516
Density	4	1772.61844	443.15461	1.20	0.3123
Date x Density	4	2965.75056	741.43764	2.00	0.0946
Size x Density	4	510.15801	127.53950	0.34	0.8475
Date x Size x Density	4	1286.36275	321.59069	0.87	0.4829
Netting	2	41982.75688	20991.37844	56.75	<.0001
Control vs. Rest	1	16910.10765	16910.10765	45.72	<.0001
4.2 mm vs. 6.4 mm	1	25072.64924	25072.64924	67.79	<.0001
Date x Netting	2	7815.31460	3907.65730	10.56	<.0001
May v. June x 0 vs. netting	1	3946.78268	3946.78268	10.67	0.0012
May v. June x 4.2 vs. 6.4mm	1	3868.53192	3868.53192	10.46	0.0014
Size x Netting	2	967.98921	483.99460	1.31	0.2721
Date x Size x Netting	2	796.60048	398.30024	1.08	0.3423
Density x Netting	8	2045.07691	255.63461	0.69	0.6992
Date x Density x Netting	8	2095.36226	261.92028	0.71	0.6843
Size x Density x Netting	8	4649.61850	581.20231	1.57	0.1340
Date x Size x Density x Netting	8	4465.82114	558.22764	1.51	0.1545
Error	240	88769.3899	369.8725		
Corrected Total	299	172916.7626			

Table IV-i. Analysis of variance on the arcsine-transformed mean percent of hard clam juveniles with countersunk holes in their valves due to moon snail (*Euspira heros, E. triseriata*) predation in the lower intertidal at Tide Mill Farm cove, Edmunds, in Cobscook Bay. Experiment was initiated on 29 May and 25 June 2009 and ended on 12-13 November 2009. Orthogonal contrasts and lack-of-fit tests (underlined) used α' as a decision rule (see above). (n = 5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Date	1	186.38088	186.38088	0.43	0.5143
Clam Size	1	466.32451	466.32451	1.07	0.3026
Date x Size	1	935.30607	935.30607	2.14	0.1448
Density	4	6510.92733	1627.73183	3.73	0.0058
<u>Linear</u>	1	33.77297	33.77297	0.08	0.7812
<u>Quadratic</u>	1	3905.22543	3905.22543	8.94	0.0031
<u>Cubic</u>	1	2340.07380	2340.07380	5.36	0.0215
<u>Quadratic</u>	1	231.84482	231.84482	0.53	0.4671
Date x Density	4	1314.78661	328.69665	0.75	0.5573
Size x Density	4	893.79420	223.44855	0.51	0.7274
Date x Size x Density	4	3924.82490	981.20623	2.25	0.0648
Netting	2	18521.88737	9260.94368	21.20	<.0001
<u>Control vs. Rest</u>	1	15703.42003	15703.42003	35.94	<.0001
<u>4.2 mm vs. 6.4 mm</u>	1	2818.46734	2818.46734	6.45	0.0117
Date x Netting	2	1869.20504	934.60252	2.14	0.1200
Size x Netting	2	1617.92355	808.96177	1.85	0.1592
Date x Size x Netting	2	1850.20959	925.10480	2.12	0.1226
Density x Netting	8	2375.12821	296.89103	0.68	0.7094
Date x Density x Netting	8	1177.09362	147.13670	0.34	0.9511
Size x Density x Netting	8	2112.68957	264.08620	0.60	0.7738
Date x Size x Density x Netting	8	4833.06672	604.13334	1.38	0.2046
Error	240	104863.3936	436.9308		
Corrected Total	299	153452.9418			

Figure Legends

Figure IV-a. Initial size-frequency distribution of hatchery-reared hard clams used in small-scale experiments (May/June to November 2009) at four locations in eastern Maine. (0_{SMALL} = 6.4 ± 0.05, n = 100; 0_{LARGE} = 7.7 ± 0.06, n = 114)

Figure IV-b. Interaction plot demonstrating the significant Date x Netting source of variation (see Table IV-a) with respect to survival of cultured hard clams at Goose Cove, Trenton, Maine. Bars represent means pooled across planting dates and clam sizes. Lines below bars indicate means that are not significantly different. (n = 50)

Figure IV-c. Relationship between mean percent survival and stocking density in small-scale field experiments initiated in May and June 2009 at Goose Cove, Trenton, Maine. Lack-of-fit tests demonstrated that a significant negative, linear trend (P = 0.0030; Table IV-a), and there was no significant deviation in this relationship from a linear model (F = 1.95, df = 3, 240, P = 0.1219).

Figure IV-d. Interactive effects of date and netting as well as initial size and netting treatments on relative growth of hard clams at Goose Cove, Trenton, Maine. The dashed line represents a doubling of growth. Both Date x Netting and Size x Netting were statistically significant (Table IV-b). Number of replicates for the Date x Netting plot and the Size x Netting plot = 71-75 and 47-50, respectively.

Figure IV-e. Effect of stocking density on hard clam survival at Egypt Bay, Franklin, Maine. Experiment was conducted from May/June to November 2009. A lack-of-fit test demonstrated no significant departure from a negative, linear relationship (F = 1.93, df = 3, 240, P = 0.1255).

Figure IV-f. Final size-frequency distribution of small and large hard clam juveniles planted in May and June at Egypt Bay, Franklin, Maine.

Figure IV-g. Interactive effects of initial clam size and stocking density on mean relative growth of hard clam juveniles from May/June to November at Egypt Bay, Franklin, Maine.

Figure IV-h. Effects of planting date and initial clam size on mean percent survival of hard clam juveniles near the Rim River Bridge in the Machias River Estuary. The interaction of the two factors was statistically significant (Table IV-f).

Figure IV-i. Interactive effects of initial clam size and predator exclusion on survival of hard clam juveniles near the Rim River Bridge in the Machias River Estuary. Con = control units without netting. 4.2 mm and 6.4 mm refer to the size of the aperture of the flexible netting used to deter predators. Significantly fewer small clams were found alive in units protected with the larger aperture netting than larger clams protected with the same netting. (See Table IV-f.)

Figure IV-j. Relationship between stocking density and mean percent survival for hard clam juveniles in the Machias River Estuary near the Rim River bridge. ANOVA (Table IV-f) indicated that the relationship was non-linear, and a lack-of-fit test confirmed this ($F = 2.79$, df = 3, 240, $P = 0.0446$) suggesting that a low-density refuge for these animals occurs.

Figure IV-k. Interactive effects of planting date, stocking density, and predator exclusion on mean percent survival of hard clam juveniles in the Machias River Estuary near the Rim River bridge. ANOVA (Table IV-f) demonstrated that the source of variation due to the three-way interaction of these factors was highly significant. A statistically significant single degree-of-freedom orthogonal contrast (May vs. June x Quartic x Control vs. Netting) helped to understand the interaction. The figure shows a negative, linear relationship between survival and density for clams in netted units planted in June, but a negative curvilinear relationship between these two variables for clams in netted units planted in May. In addition, a low-density refuge exists for unprotected clams planted in June, but not for those planted in May.

Figure IV-l. Interactive effects of planting date and predator exclusion on mean survival of juvenile hard clams in Cobscook Bay-Edmunds from May/June to November 2009. Both orthogonal contrasts associated with the two-way interaction source of variation were highly significant (Table IV-h; $n = 50$).

Figure IV-m. Relationship between stocking density and mean percent dead drilled hard clam individuals in Cobscook Bay-Edmunds. ANOVA (Table IV-i) indicated that the relationship was non-linear, and a lack-of-fit test confirmed this ($F = 4.94$, df = 3, 240, $P = 0.0024$) indicating that moon snails prey in a density-dependent fashion.

Figure IV-n. Effects of initial stocking density and final mean shell length of juvenile hard clams in Cobscook Bay-Edmunds (12-13 November 2009). ANOVA demonstrated a significant effect due to density ($P = 0.0370$). ($22 \leq n \leq 37$)

Figure IV-a.

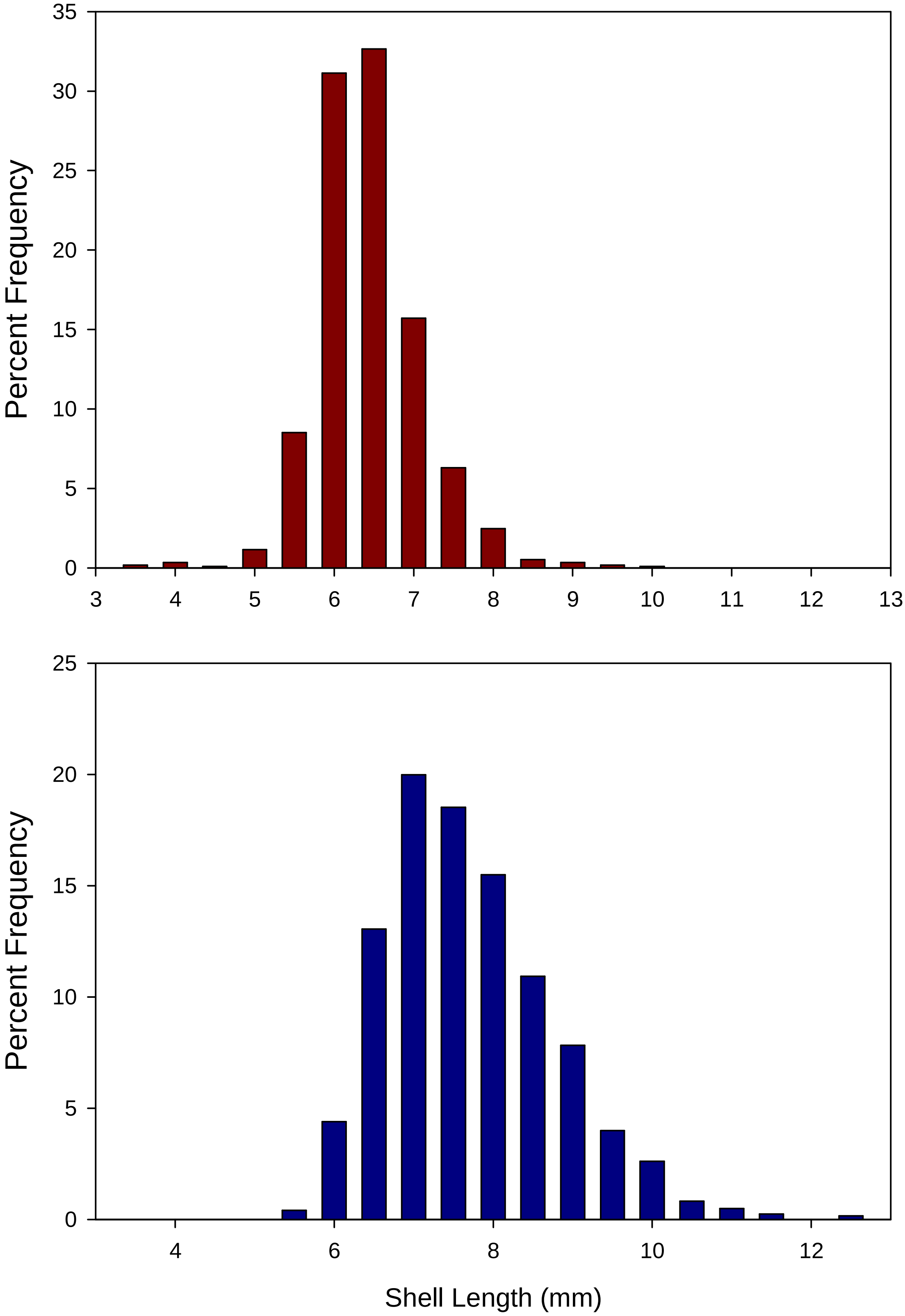
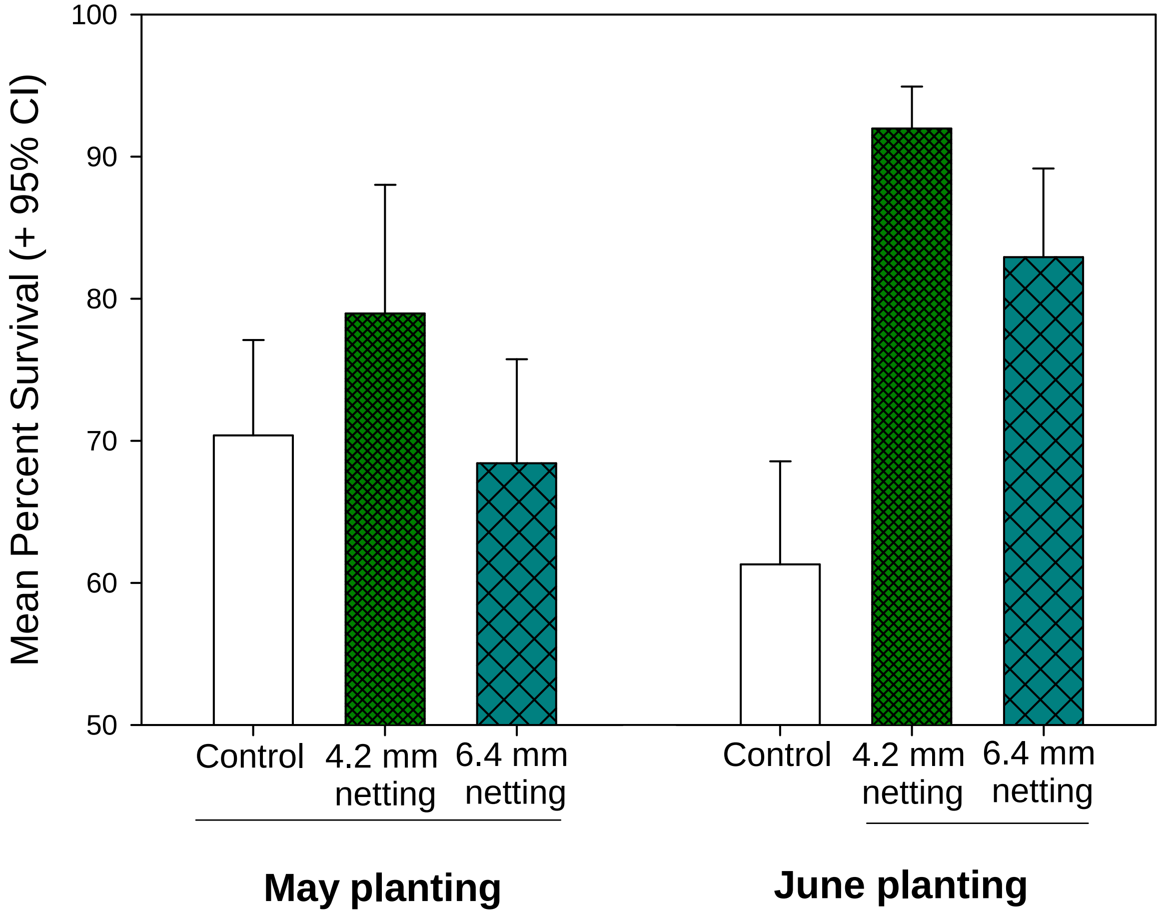
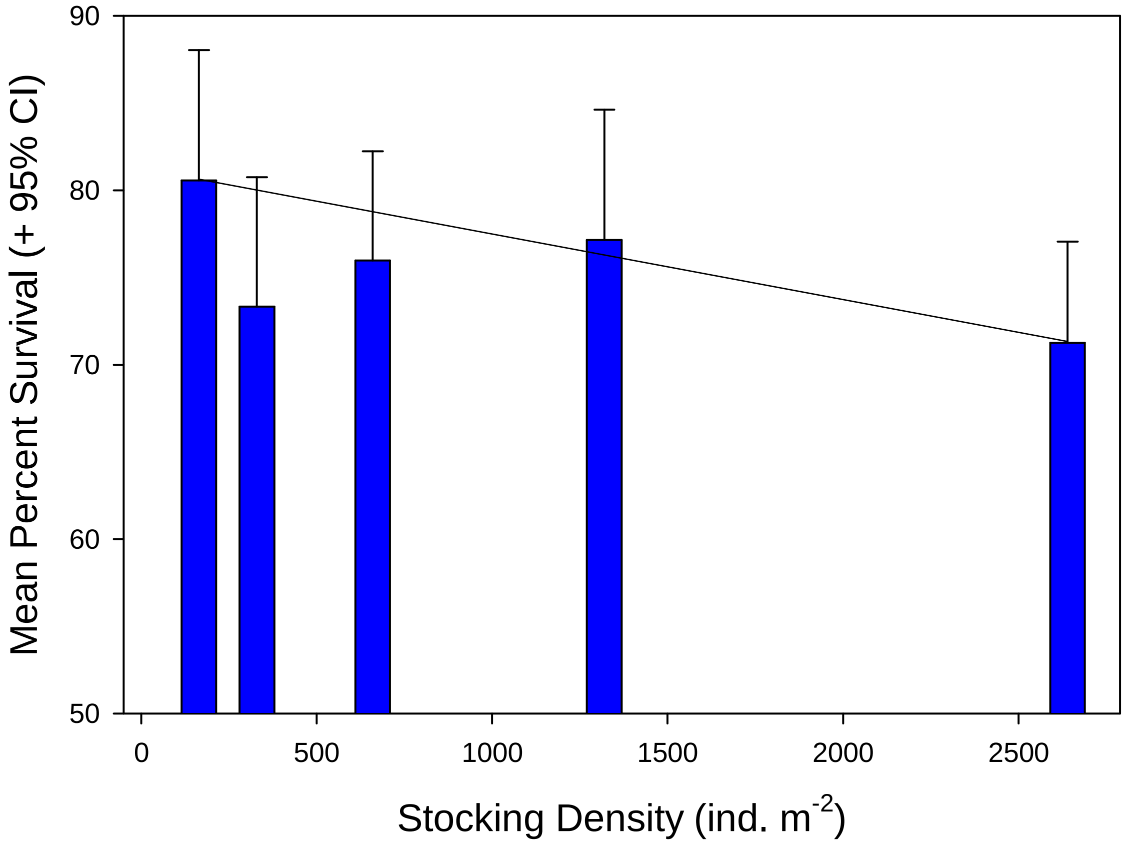

Percent Frequency
35
30
25
20
15
10
5
0
3
4
5
6
7
8
9
10
11
12
13
Percent Frequency
25
20
15
10
5
0
4
6
8
10
12
Shell Length (mm)

Figure IV-b.



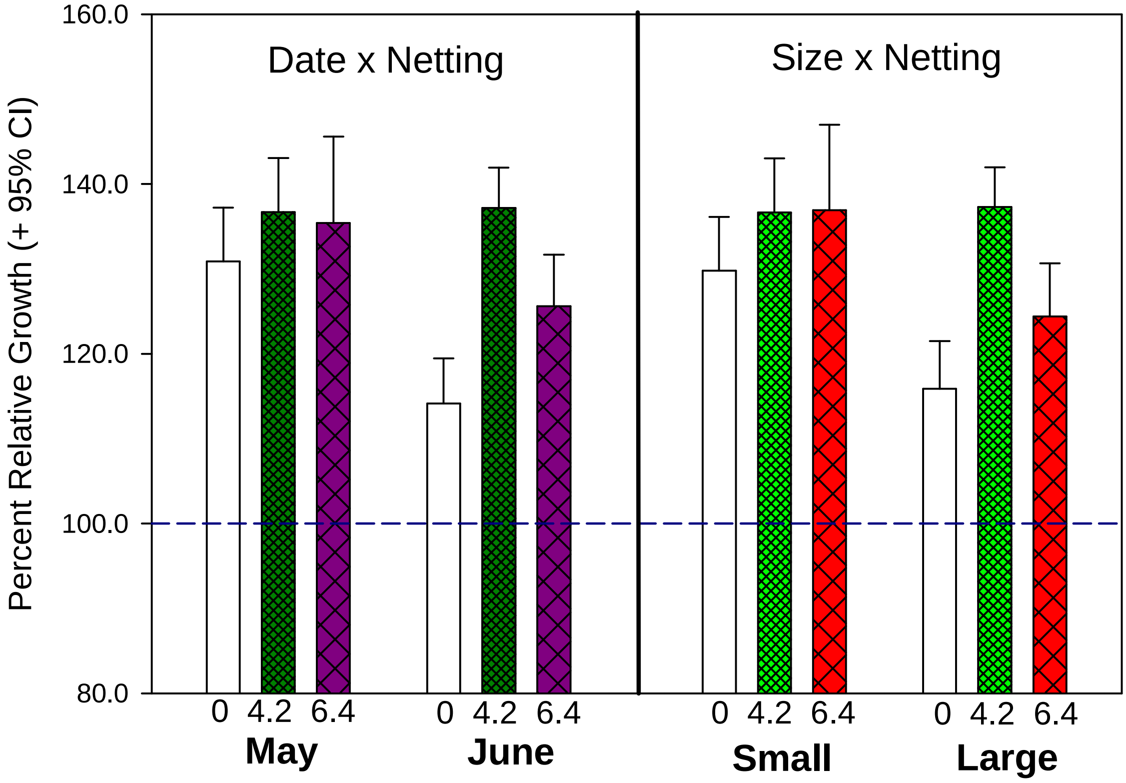
100
90
80
70
60
50
Mean Percent Survival (+ 95% CI)
Control
4.2 mm netting
6.4 mm netting
Control
4.2 mm netting
6.4 mm netting
May planting
June planting

Figure IV-c.



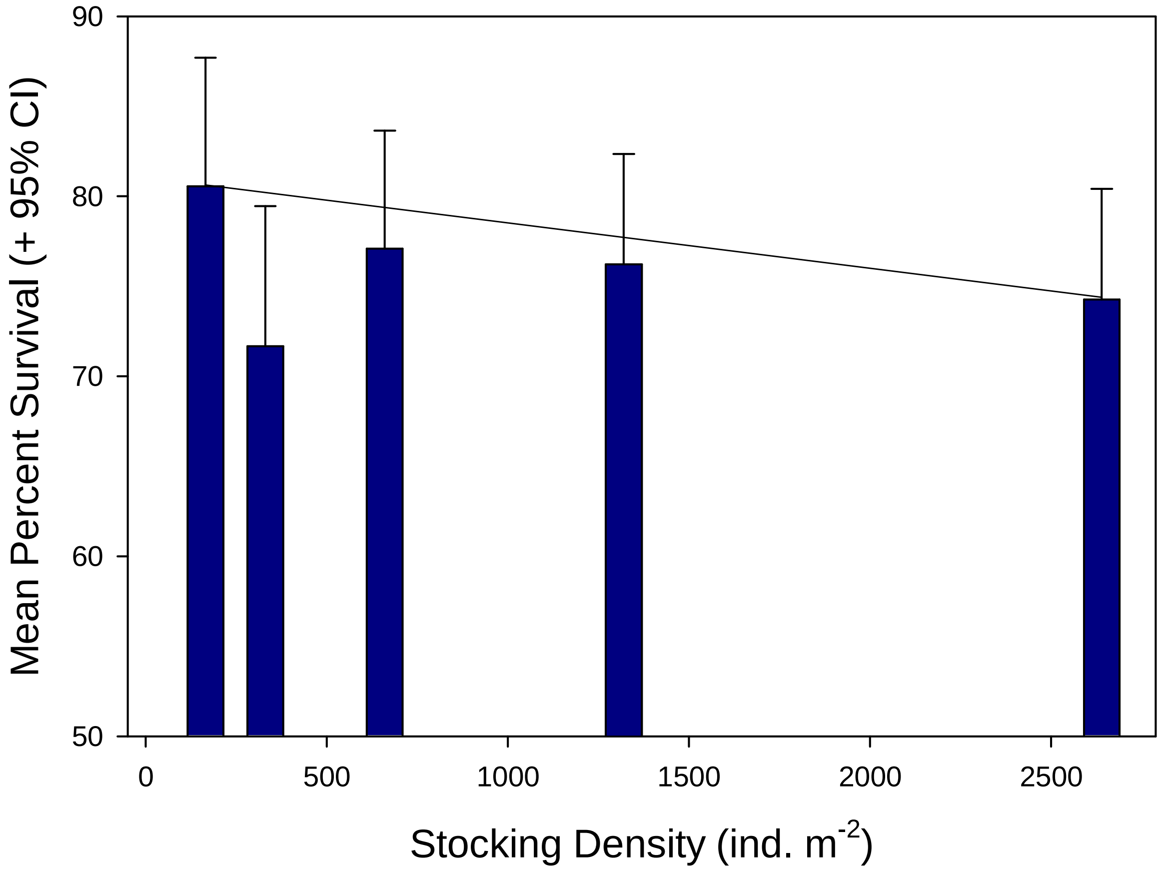
90
80
70
60
50
Mean Percent Survival (+ 95% CI)
0
500
1000
1500
2000
2500
Stocking Density (ind. m-2)

Figure IV-d.



Date x Netting
Size x Netting
Percent Relative Growth (+ 95% CI)
160.0
140.0
120.0
100.0
80.0
0
4.2
6.4
May
June
Small
Large

Figure IV-e.



Mean Percent Survival (+ 95% CI)
90
80
70
60
50
0
500
1000
1500
2000
2500
Stocking Density (ind. m-2)

Figure IV-f.

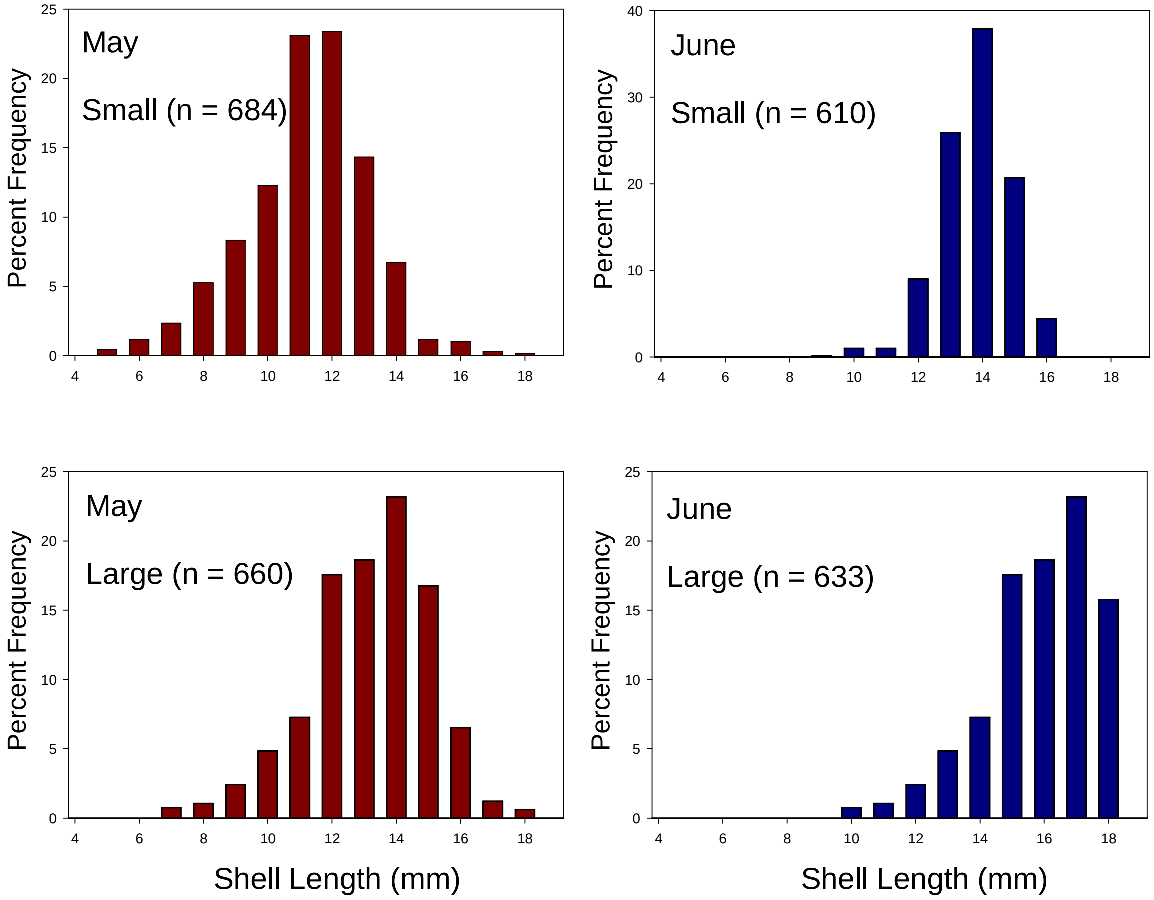

May
Small (n = 684)
June
Small (n = 610)
May
Large (n = 660)
June
Large (n = 633)
Percent Frequency
Shell Length (mm)

Figure IV-g.

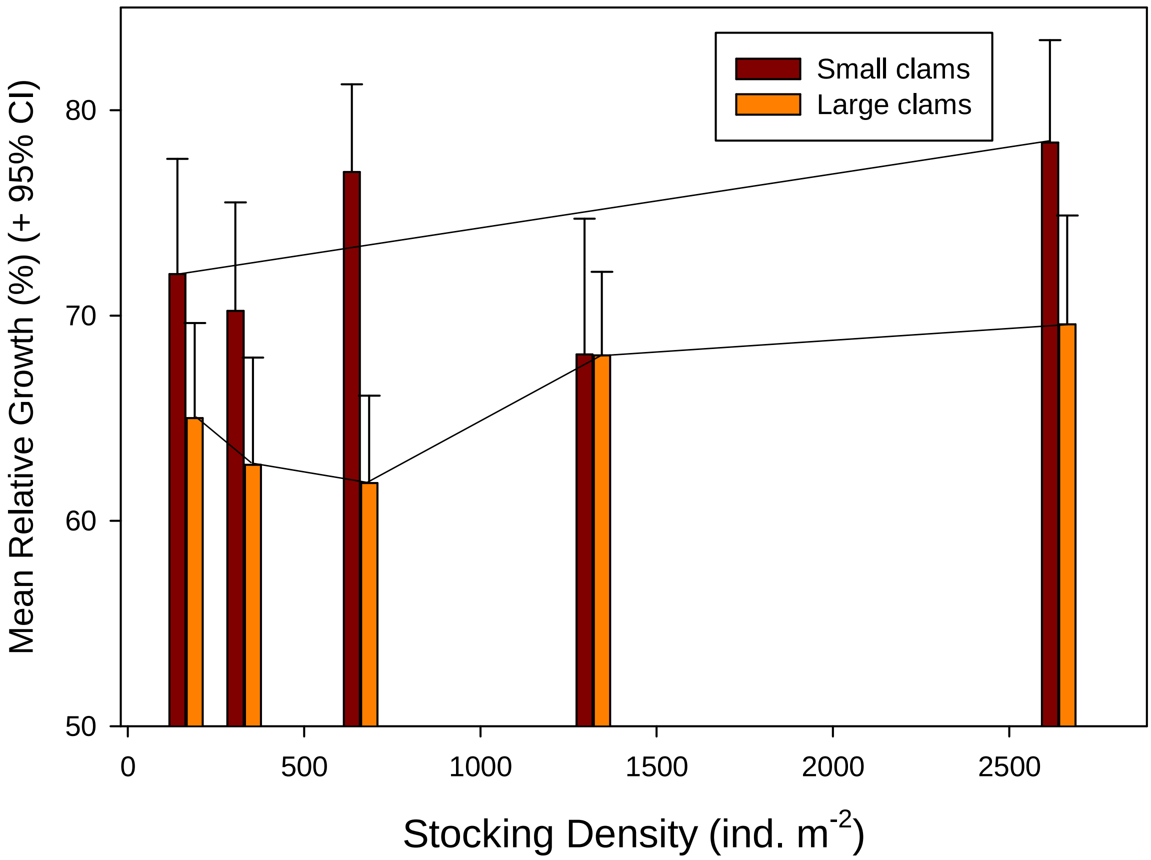
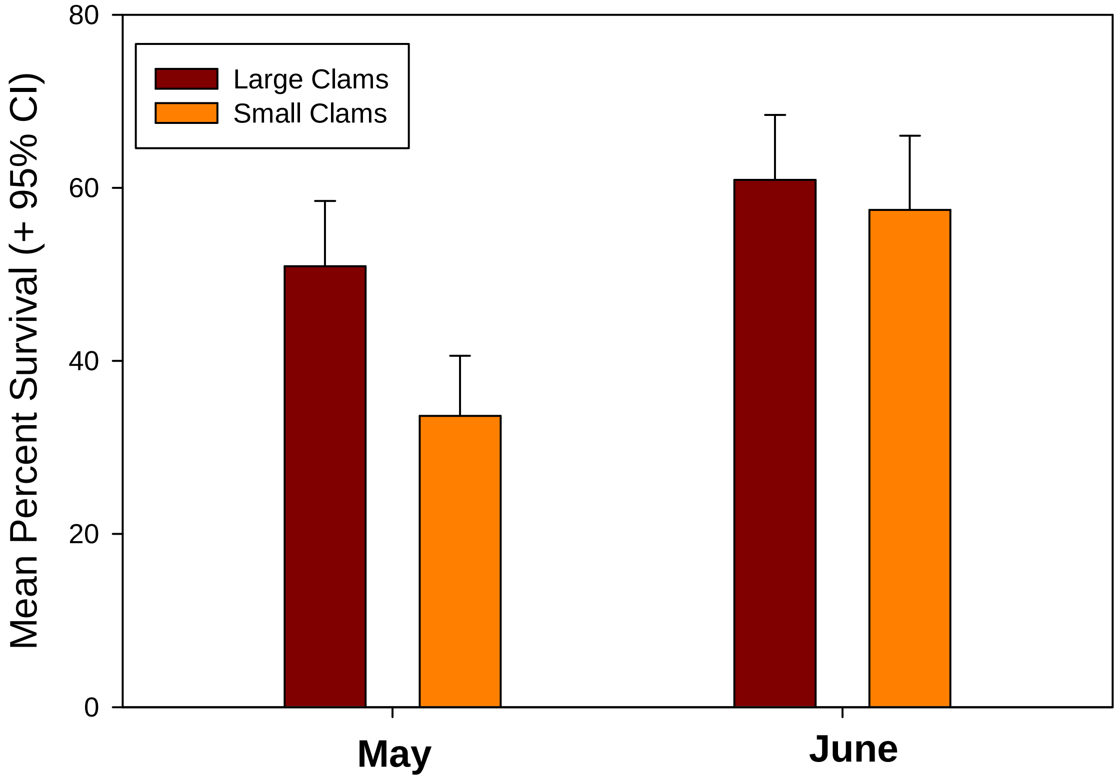
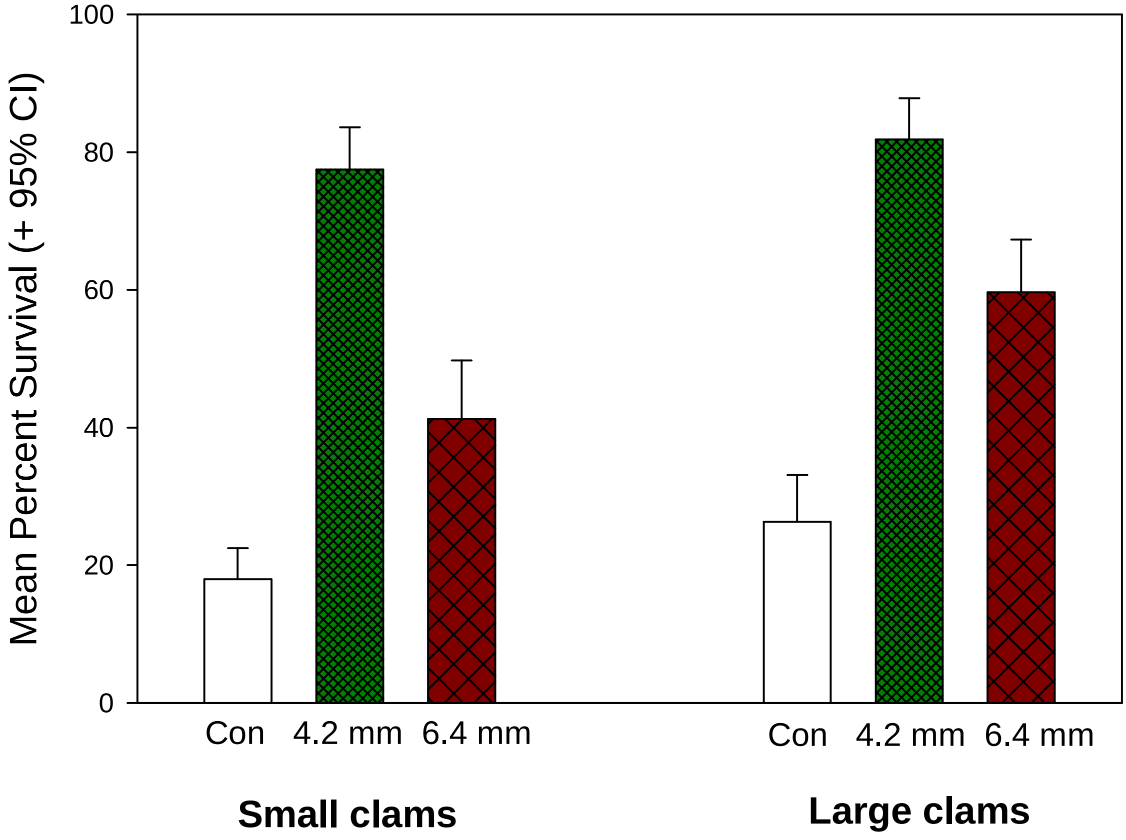

Small clams
Large clams
Mean Relative Growth (%) (+ 95% CI)
50
60
70
80
0
500
1000
1500
2000
2500
Stocking Density (ind. m-2)

Figure IV-h.



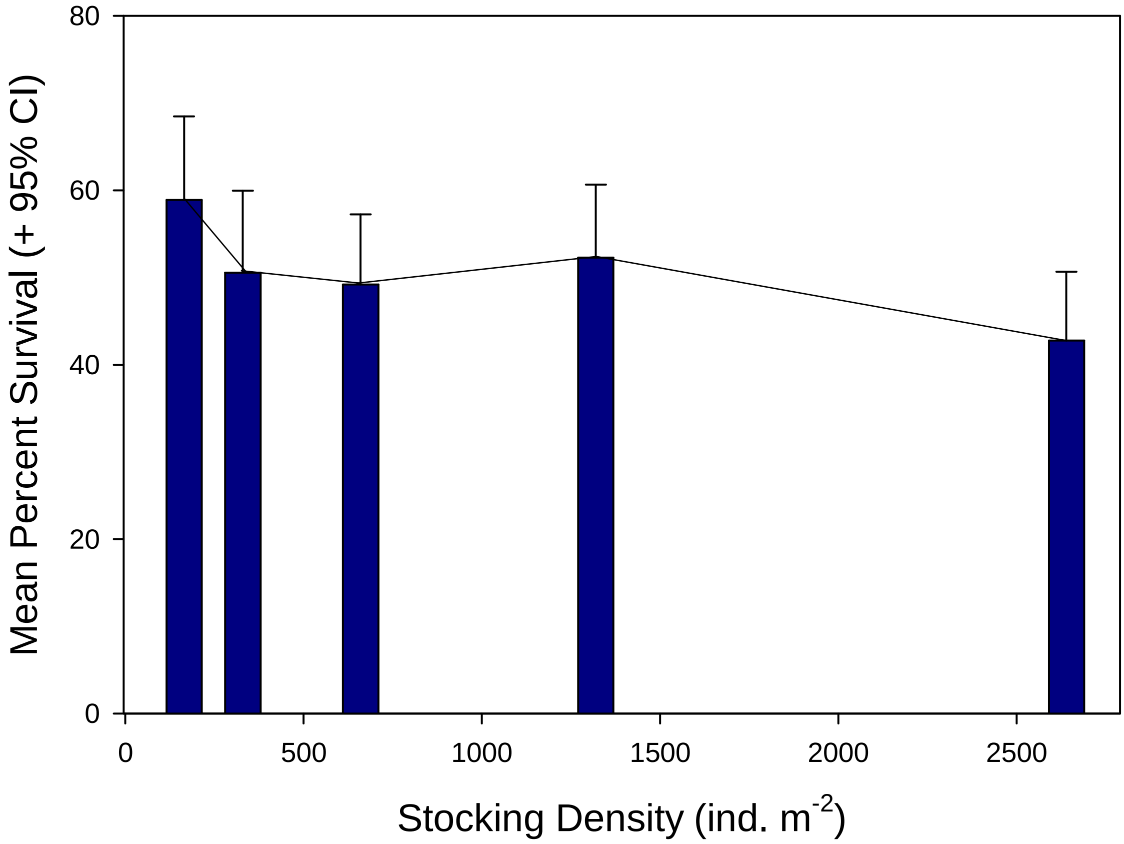
Mean Percent Survival (+ 95% CI)
80
60
40
20
0
Large Clams
Small Clams
May
June

Figure IV-i.



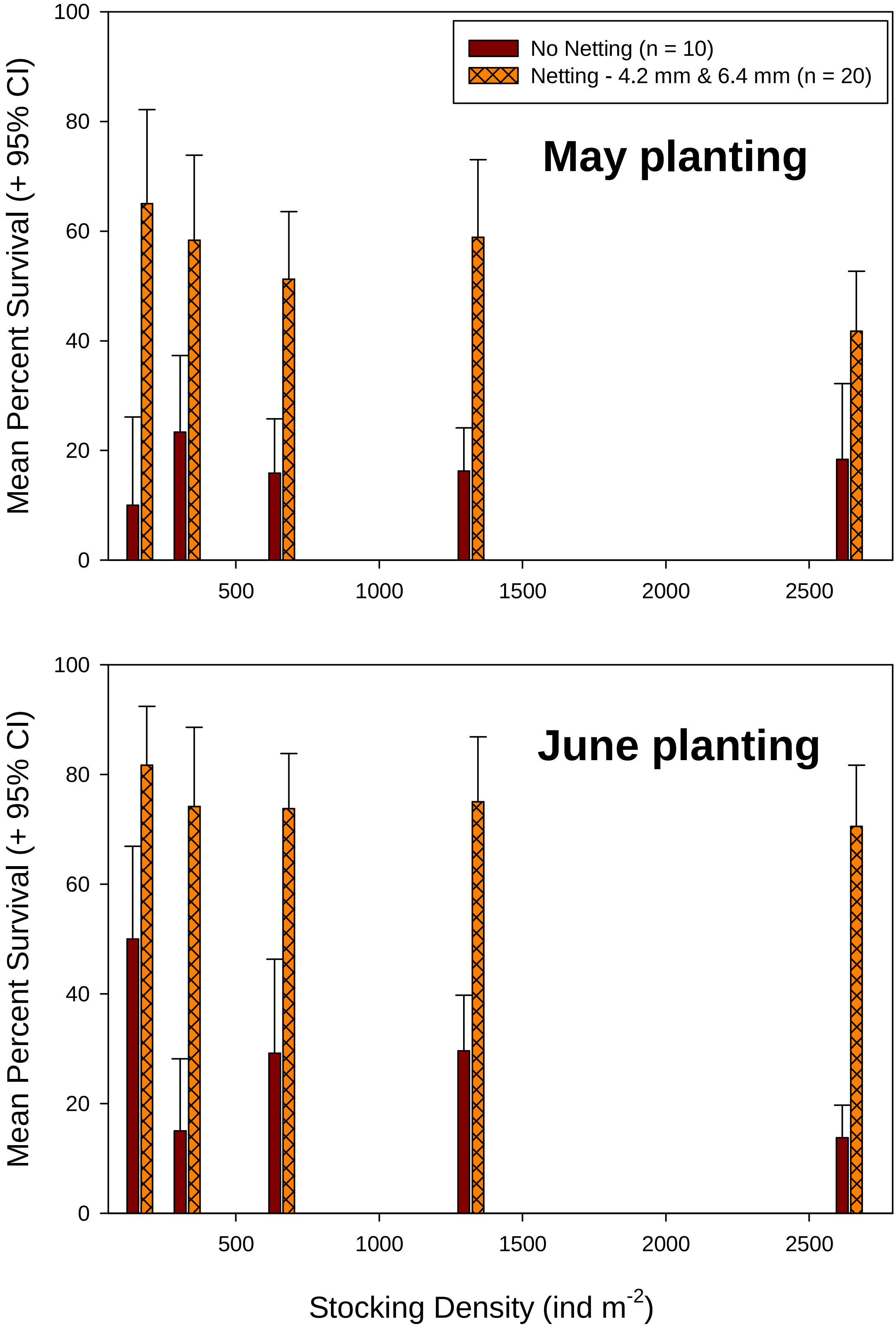
Mean Percent Survival (+ 95% CI)
100
80
60
40
20
0
Con
4.2 mm
6.4 mm
Con
4.2 mm
6.4 mm
Small clams
Large clams

Figure IV-j.



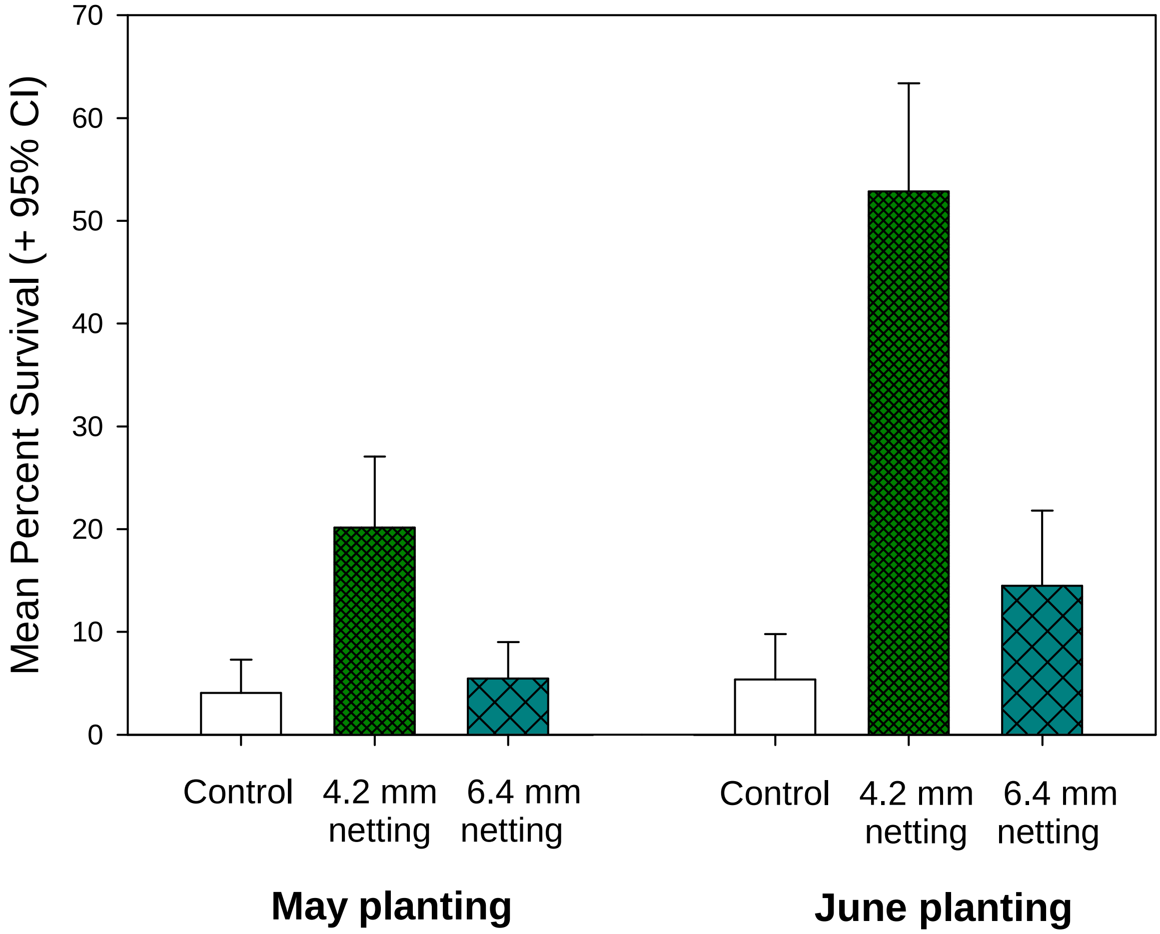
80
60
40
20
0
Mean Percent Survival (+ 95% CI)
0
500
1000
1500
2000
2500
Stocking Density (ind. m-2)

Figure IV-k.



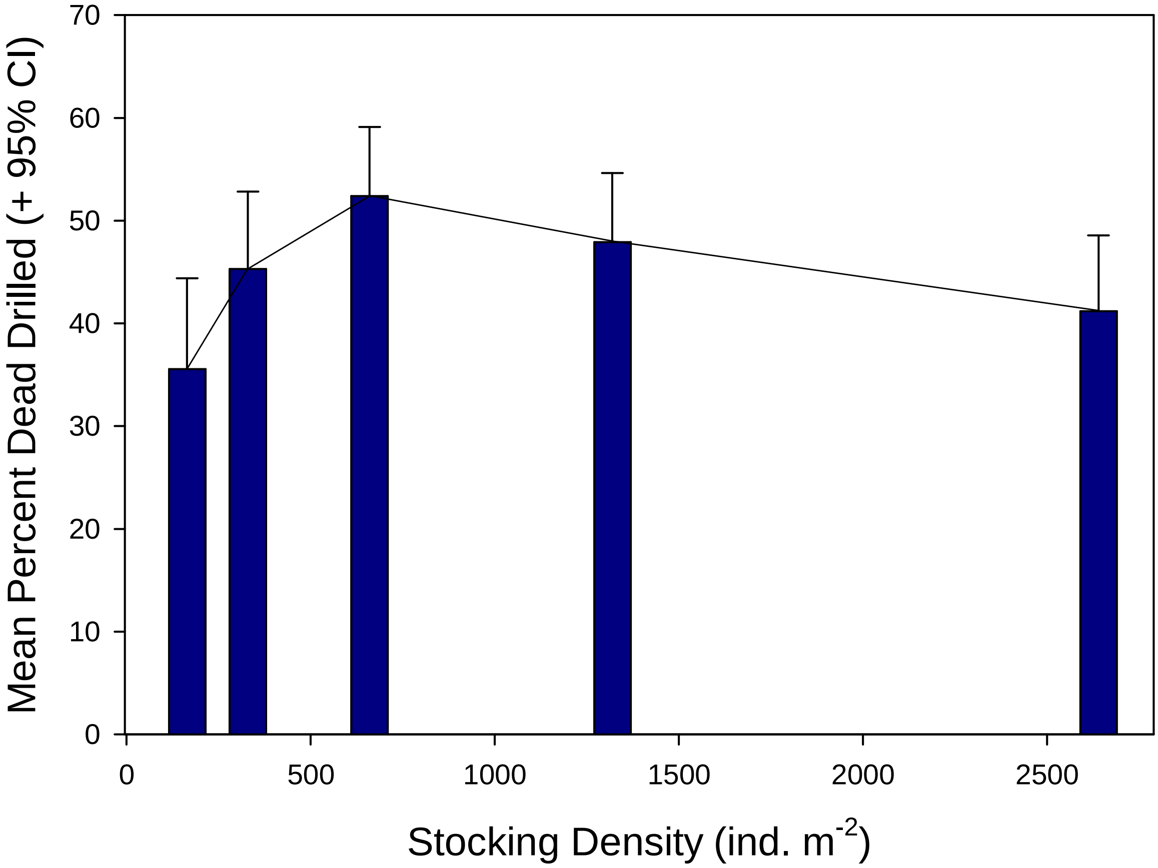
No Netting (n = 10)
Netting - 4.2 mm & 6.4 mm (n = 20)
May planting
June planting
Mean Percent Survival (+ 95% CI)
100
80
60
40
20
0
500
1000
1500
2000
2500
Stocking Density (ind m^{-2})

Figure IV-I.



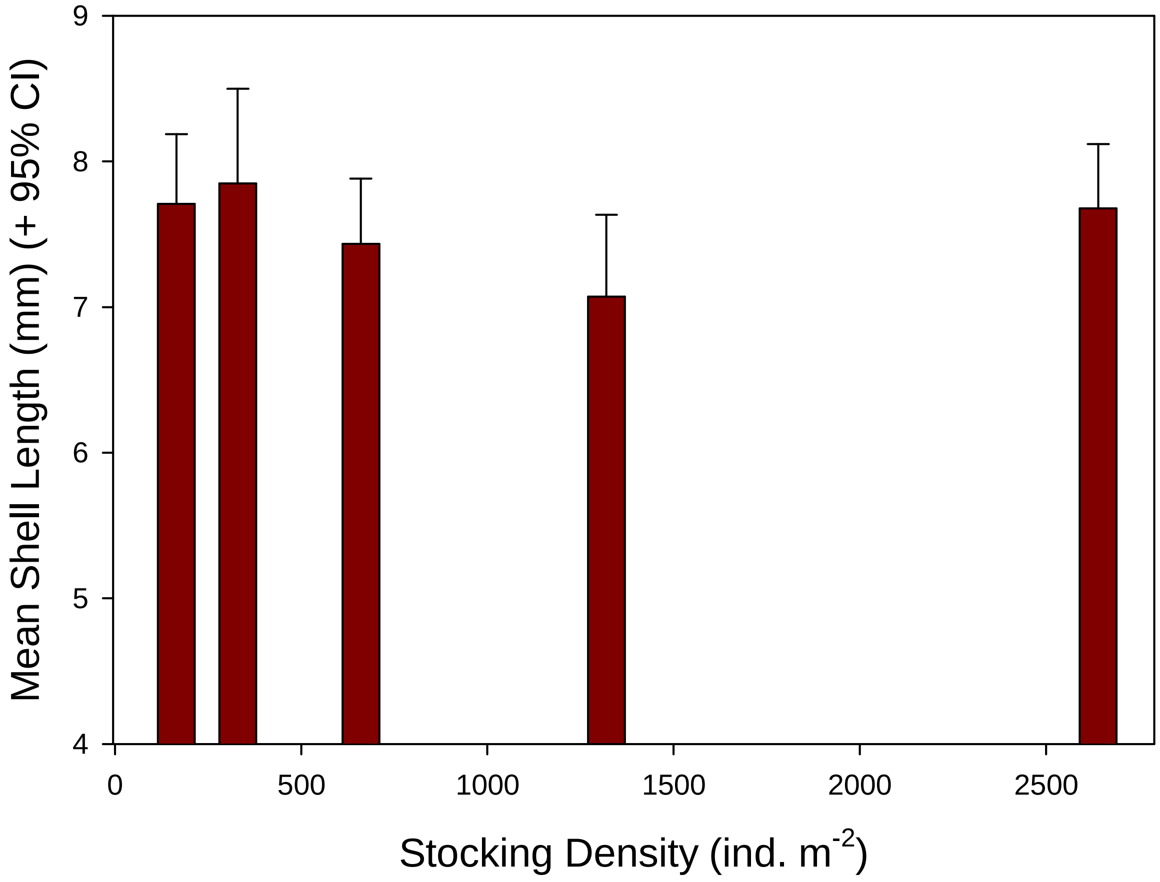
Mean Percent Survival (+ 95% CI)
0
10
20
30
40
50
60
70
Control
4.2 mm netting
6.4 mm netting
Control
4.2 mm netting
6.4 mm netting
May planting
June planting

Figure IV-m.



Mean Percent Dead Drilled (+ 95% CI)
0
10
20
30
40
50
60
70
0
500
1000
1500
2000
2500
Stocking Density (ind. m-2)

Figure IV-n.



9
8
7
6
5
4
Mean Shell Length (mm) (+ 95% CI)
0
500
1000
1500
2000
2500
Stocking Density (ind. m-2)

Other Experiments (B)

The experiment described below was not, officially, part of the USDA-SBIR study by Bagaduce River Oyster, Egypt Bay Sea Farms, and the Downeast Institute for Applied Marine Research & Education. It does, however, provide some insights about hard clam growth and survival in eastern Maine, and is therefore included in this Final Report.

The study was part of a class research project for Marine Ecology (BIO 360) students from the University of Maine at Machias (UMM). The field trial was conducted from 24 May to 30 November 2009 at the lower intertidal at Duck Brook Flat, within Holmes Bay, Cutler, Maine (44^{o} 41.28’N; 67^{o}18.62’W), and was designed to examine whether or not cultured hard clam juveniles and cultured soft-shell clam juveniles compete with each other to limit each other’s growth and/or survival.

A complete factorial design was used to test both potential intra- and intraspecific competition in both *Mya arenaria* and *Mercenaria mercenaria* at three densities: 330, 660, and 1,320 ind. m^{-2}. Clams were added to experimental units (A = 0.0182 m^2), and a piece of 6.4 mm flexible netting was added to the top of each unit to deter predators. A total of six intraspecific treatments (*Mya* @ 330, 660, and 1,320 ind. m^{-2}; *Mercenaria* @ 330, 660, and 1,320 ind, m^{-2}) and nine inter-specific treatments (all possible combinations of densities, e.g., *Mya* @ 330 + *Mercenaria* @ 330 …… *Mya* @ 660 + *Mercenaria* @ 330 ….. *Mya* @ 1,320 + *Mercenaria* @ 1,320). Individuals of *Mya* ranged in size from 7.7 mm to 11.0 mm, with a mean SL = 9.4 ± 0.10 mm (n = 162). Individuals of *Mercenaria* ranged in size from 7.0 mm to 12.0 mm, with a mean SL = 9.2 ± 0.25 mm (n = 82). Five replicates of each treatment were deployed. The field layout was a 5 x 15 matrix with 1 m spacing between rows and columns.

In November, all experimental units were removed from the tidal flat and taken to UMM, where the contents of each was sieved through a 1 mm mesh. All live and dead clams were collected, and the initial and final SL’s of all live clams were measured to the nearest 0.1 mm using Vernier calipers (as described above). Dead clams were categorized by shell damage: none vs. crushed or chipped. In addition, missing clams were enumerated.

Analysis of variance was conducted on the arcsine-transformed mean percent survival and the untransformed mean relative growth and final SL. Two orthogonal contrasts for Intraspecific densities were examined based on the assumption of limiting resources:

1) O_{330} vs. $O_{660 \text{ \& } 1,320}$; and,

2) O_{660} vs. $O_{1,320}$.

Eight interspecific orthogonal contrasts were examined:

1) Overall interspecific effects (compares the mean of all three intraspecific treatments vs. the mean of all nine interspecific treatments);

2) Interspecific 330 vs. Rest (compares the mean of all three interspecific treatments with 330 *Mercenaria* to the mean of the remaining six interspecific treatments);

3) Interspecific 660 vs. 1,320 (compares the mean of the three interspecific treatments with 660 *Mercenaria* to the mean of the remaining three interspecific treatments);

4) Interspecific 330 *Mercenaria* w *Mya* (330 vs. Rest). Compares the mean of the 330 *Mercenaria* | 330 *Mya* treatment vs. the pooled mean of the 330 *Mercenaria* | 660 *Mya* & 330 *Mercenaria* | 1,320 *Mya* treatments.

5) Interspecific 330 *Mercenaria* w *Mya* (660 vs. 1,320). Compares the mean of the 330 *Mercenaria* | 660 *Mya* treatment vs. the mean of the 330 *Mercenaria* | 1,320 *Mya* treatment.

6) Interspecific 660 *Mercenaria* w *Mya* (330 vs. Rest). Compares the mean of the 660 *Mercenaria* | 330 *Mya* treatment vs. the pooled mean of the 660 *Mercenaria* | 660 *Mya* & 660 *Mercenaria* | 1,320 *Mya* treatments.

7) Interspecific 660 *Mercenaria* w *Mya* (660 vs. 1,320). Compares the mean of the 660 *Mercenaria* |660 *Mya* treatment vs. the mean of the 660 *Mercenaria* | 1,320 *Mya* treatment.

8) Interspecific 1,320 *Mercenaria* w *Mya* (330 vs. Rest). Compares the mean of the 1,320 *Mercenaria* | 330 *Mya* treatment vs. the pooled mean of the 1,320 *Mercenaria* | 660 *Mya* & 1,320 *Mercenaria* | 1,320 *Mya* treatments.

9) Interspecific 1,320 *Mercenaria* w *Mya* (660 vs. 1,320). Compares the mean of the 1,320 *Mercenaria* | 600 *Mya* with the mean of the 1,320 *Mercenaria* | 1,320 *Mya*.

To avoid excessive errors, an adjusted decision rule (α’) was used as described above (Winer et al., 1991).

Results

Survival

Overall mean survival of juveniles of *Mercenaria* was 83.9 ± 4.1% (n = 60) (Table V-a), which was more than three times higher than that of *Mya* (Table V-b). One reason for such low survivorship of *Mya* was due to the accidental presence of green crab juveniles, *Carcinus maenas* (CW range = 17.8 to 22.4 mm), within the experimental units. For example, of the sixty experimental units containing *M. arenaria* individuals, 36 (60%) contained one or more *C. maenas* at the end of the experiment. In units with *C. maenas*, *Mya* survival was 7.5 ± 5.6% vs. 52.6 ± 11.0% (n = 24) in units without *C. maenas*. Two plausible scenarios exist for green crab presence in these units protected with 6.4 mm flexible netting. First, small crabs (< 10 mm CW) may have been in sediments used to fill the units at the beginning of the trial, as no effort was made to remove any fauna prior to placing ambient sediments in the units. Second, small crabs (< 9 mm CW) could have entered units through the netting, then molted several times, and became entrapped with their prey. Interestingly, 38 units with individuals of *Mercenaria* were found to contain individuals of *C. maenas*; however, these crabs must have not been able to prey as intensely on the hard clams because mean percent clam survival in the units with *C. maenas* was 85.4 ± 4.1% vs. 81.3 ± 9.1% (n = 22) in units without the predator (P = 0.4638). The apparent difference in vulnerability between the two species likely relates to differences in shell strength and/or thickness. Although soft-shell clams of a given shell length can bury deeper in the sediments than *M. mercenaria* due to differences in the morphology of the siphons, apparently for the sizes of *C. maenas* trapped in the experimental units, this behavioral mechanism gave *Mya* no survival advantage over *Mercenaria* in this field test. Hard clams have a much thicker shell, more dense shell structure (composite prisms) than soft-shell clams (Taylor and Layman, 1972) that takes predators more energy and power to crush. It appears from the evidence provided by the accidental inclusion of green crabs in the experimental units of this study that for the sizes of hard clams (initially ca. 9 mm SL and growing to an average size of 10.2 mm [see below]), green crabs are not important predators. The converse is true for soft-shell clams that grew from 9.4 mm SL to a mean SL of 17.7 mm (see below).

No significant intraspecific effects of *Mercenaria* density on hard clam survival were detected, nor did the presence of *Mya* have a significant effect on hard clam survival (Table V-c). Because it may be possible for *C. maenas* to have influenced possible interactions (positive or negative) between the two bivalve species, we tested for both inter- and interspecific effects on *Mercenaria* survival in only those experimental units that did not contain green crabs at the end of the experiment (N = 22; Table V-d). No significant effects occurred.

Growth
As occurred at the Machias River site approximately 8 km away (see *Growth*, p. 66), shell growth of hard clam juveniles at Duck Brook over the 191-day trial was negligible (Table V-e; Fig. V-b). Final mean SL's varied from 9.5 mm to 11.6 mm (Fig. V-c), with relative growth values ranging between 5.5% to 26.0%. No statistically significant effects of intraspecific density were observed for either relative growth or final SL (Table V-f). The presence of green crabs in experimental units had the effect of reducing relative growth by 60% (P < 0.0001).

Summary: This study was designed to assess potential interactions between juveniles of *M. arenaria* and *M. mercenaria*. The location, Cutler, Maine, is an area where hard clams do not exist naturally. Soft-shell clams are prevalent at this site, and are harvested commercially from time-to-time. Results showed that neither growth nor survival of hard clams was affected by the presence of soft-shell clams. Green crabs accidentally became included in the protected experimental units, but their presence did not affect the interspecific interactions. Growth of hard clams was poor, with an overall mean increase in shell length during the 191-day experiment of only 1 mm.

Table V-a. Untransformed means (± 95% confidence intervals) for the fate of hatchery-reared juveniles of *Mya arenaria* and *Mercenaria mercenaria* in protected (6.4 mm flexible netting) experimental units (A = 0.0182m^2) from 24 May to 30 November 2009 near the low intertidal at Duck Brook Flat, Cutler, Maine. %**A**, % **DU**, %**DC**, and %**M** = percent **A**live, **D**ead with **U**ndamaged valves, **D**ead with **C**rushed or chipped valves; and, **M**issing, respectively. Intraspecific densities for both species were 6, 12, or 24 individuals per unit, representing approximately 330, 660 or 1,320 individuals m^{-2}. Nine interspecific treatments were established using each factorial combination of each density. (n = 5). Totals (N = 60).

Species	Density of *Mya arenaria*	Density of *Mercenaria mercenaria*	**%A**	**%DU**	**%DC**	**%M**
Mya						
	6	0	53.3(44.8)	0.0(0.0)	26.7(37.6)	20.0(26.9)
	12	0	23.3(40.3)	15.0(17.0)	35.0(41.6)	26.7(24.7)
	24	0	2.5(6.9)	5.0(8.5)	57.5(21.1)	35.0(16.2)
	6	6	10.0(27.8)	13.3(17.3)	46.7(39.8)	30.0(22.7)
	6	12	23.3(40.3)	20.0(17.3)	46.7(37.0)	10.0(11.3)
	6	24	46.7(53.6)	6.7(15.3)	43.3(55.9)	0.0(0.0)
	12	6	0.0(0.0)	23.3(26.8)	55.0(17.3)	21.7(15.7)
	12	12	35.0(35.4)	5.0(9.3)	21.7(23.8)	38.3(32.4)
	12	24	23.3(26.8)	13.3(21.5)	41.7(28.3)	16.7(12.7)
	24	6	17.5(31.1)	2.5(2.8)	38.3(12.9)	41.7(28.6)
	24	12	44.2(32.2)	19.2(59.9)	11.7(19.8)	25.0(7.3)
	24	24	27.5(44.6)	8.3(17.5)	35.0(37.9)	29.2(16.0)
		TOTALS	25.6(7.8)	10.9(4.1)	38.3(7.0)	25.2(4.8)
Mercenaria						
	0	6	90.0(18.5)	3.3(9.3)	0.0(0.0)	6.7(11.3)
	0	12	80.0(20.2)	11.7(11.8)	0.0(0.0)	8.3(12.7)
	0	24	86.7(14.4)	5.0(11.2)	1.7(2.8)	6.6(5.9)
	6	6	83.3(22.7)	3.3(18.5)	3.3(9.3)	10.1(0.0)
	6	12	80.0(18.8)	13.3(13.9)	3.3(5.7)	3.4(9.3)
	6	24	88.3(9.9)	6.7(7.8)	0.8(2.3)	4.2(5.2)
	12	6	90.0(18.5)	3.3(9.3)	6.7(18.5)	0.0(0.0)
	12	12	85.0(15.4)	1.7(4.6)	8.3(17.9)	5.0(9.3)
	12	24	90.0(7.9)	6.7(5.9)	0.8(2.3)	2.5(4.6)
	24	6	70.0(39.8)	20.0(34.0)	0.0(0.0)	10.0(18.5)
	24	12	73.3(19.8)	11.7(15.7)	5.0(9.3)	10.0(13.5)
	24	24	90.0(8.7)	6.7(8.7)	0.8(2.3)	2.5(2.8)
		TOTALS	83.9(4.1)	7.8(2.9)	2.6(1.8)	5.7(2.2)

Table V-b. Analysis of variance on mean arcsine-transformed percent survival for both *Mya arenaria* and *Mercenaria mercenaria* at Duck Brook Flat, Cutler, Maine from 24 May to 30 November 2009. See Table V-a for description of intra- and interspecific density treatments. (n = 5).

Source of variation	**DF**	**Sum of Squares**	**Mean Square**	**F-Value**	**Pr > F**
Treatment	14	24564.25378	1754.58956	4.54	<.0001
Species	1	48908.39790	48908.39790	126.45	<.0001
Treatment*Species	8	5365.54387	670.69298	1.73	0.1002
Error	96	37132.1649	386.7934		
Corrected Total	119	115970.3604			

Table V-c. **a)** Analysis of variance on the arcsine-transformed mean percent alive cultured individuals of *Mercenaria mercenaria* at Duck Brook, Cutler, Maine from 24 May to 30 November 2009. Clams were placed in experimental units (0.0182 m^2) at one of three interspecific densities (6, 12, or 24, representing approximately 330, 660, and 1320 individuals m^{-2}) and at similar densities (factorially) with the same densities of cultured individuals of *Mya arenaria*. Total number of interspecific treatments = 9. (See Table 1 for a complete description of the twelve treatments; see Figure 1 for initial size-frequency distributions of these bivalves.) *A priori* contrasts (underlined) test specific hypotheses concerning both intraspecific and interspecific effects on survival of *Mercenaria mercenaria*. (n = 5) **b)** Effects of accidental inclusion of *Carcinus maenas* within the experimental units on survival of *Mercenaria mercenaria*. ANOVA performed on the arcsine-transformed mean percent alive. α' = 0.0047.

a)

Source of variation	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Treatment	11	1958.16325	178.01484	0.89	0.5571
<u>Intra 330 vs. Rest (660 & 1,320)</u>	1	391.6101751	391.6101751	1.96	0.1684
<u>Intra 660 vs. 1,320</u>	1	60.0180313	60.0180313	0.30	0.5866
<u>Overall Interspecific</u>	1	33.5448612	33.5448612	0.17	0.6841
<u>Interspecific 330 vs. Rest (660 & 1,320)</u>	1	10.9428947	10.9428947	0.05	0.8162
<u>Interspecific 660 vs. 1,320</u>	1	347.8705775	347.8705775	1.74	0.1937
<u>Interspecific 330 Merc (Mya 330 vs. Rest)</u>	1	16.6345047	16.6345047	0.08	0.7744
<u>Interspecific 330 Merc (Mya 660 vs. 1,320)</u>	1	810.0000147	810.0000147	4.05	0.0499
<u>Interspecific 660 Merc (Mya 330 vs. Rest)</u>	1	9.3585035	9.3585035	0.05	0.8298
<u>Interspecific 660 Merc (Mya 660 vs. 1,320)</u>	1	259.1335102	259.1335102	1.29	0.2609
<u>Interspecific 1,320 Merc (Mya 330 v. Rest)</u>	1	117.5459367	117.5459367	0.59	0.4473
<u>Interspecific 1,320 Merc (Mya 660 vs 1,320)</u>	1	396.1522250	396.1522250	1.98	0.1660
Error	48	9611.33540	200.23615		
Corrected Total	59	11569.49864			

b)

Carcinus	<u>n</u>	Mean (± 95% CI)
Present within experimental units	38	85.4 (4.1)
Absent within experimental units	22	81.3 (9.1)

Source of variation	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Carcinus	1	107.47019	107.47019	0.54	0.4638
Error	58	11462.02845	197.62118		
Corrected Total	59	11569.49864			

Table V-d. Analysis of variance on the arcsine-transformed mean percent alive cultured individuals of *Mercenaria mercenaria* at Duck Brook, Cutler, Maine from 24 May to 30 November 2009. Data include only those experimental units that did not accidentally include a green crab, *Carcinus maenas*. Only a priori contrasts that are mathematically estimable are presented.

Source of variation	**DF**	**Sum of Squares**	**Mean Square**	**F-Value**	**Pr > F**
Treatment	10	4167.216559	416.721656	3.04	0.0410
Intra 330 vs. Rest (660 vs. 1,320)	1	677.6539997	677.6539997	4.94	0.0481
Intra 660 vs. 1,320	1	146.3709976	146.3709976	1.07	0.3237
Interspecific 330 Merc (Mya 330 vs. Rest)	1	262.2927207	262.2927207	1.91	0.1941
Interspecific 330 Merc (Mya 660 vs. 1,320)	1	377.6061037	377.6061037	2.75	0.1252
Interspecific 1,320 Merc (Mya 330 vs. Rest)	1	12.8544550	12.8544550	0.09	0.7652
Interspecific 1,320 Merc (Mya 660 vs 1,320)	1	16.6694853	16.6694853	0.12	0.7339
Error	11	1508.243188	137.113017		
Corrected Total	21	5675.459747			

Table V-e. Mean relative growth (± 95% CI) and mean final shell length (mm) (± 95% CI) for cultured individuals of *Mya arenaria* and *Mercenaria mercenaria* at Duck Brook, Cutler, Maine from 24 May to 30 November 2009. Intraspecific and interspecific density treatments are the same as in Table V-a. A relative growth equal to 1 means a doubling of shell growth. Sample size (n) is variable in experimental units containing *Mya* due to poor survival due to accidental inclusion of *Carcinus maenas* at the end of the experiment.

Species	Density of *Mya arenaria*	Density of *Mercenaria mercenaria*	n	**Relative Growth**	**Final Length**
Mya					
	6	0	4	0.939 (0.434)	17.6 (2.23)
	12	0	2	0.842 (2.167)	16.5 (10.03)
	24	0	1	0.882 ( )	19.0 ( )
	6	6	1	0.853 ( )	17.6 ( )
	6	12	2	0.731 (1.087)	16.8 (9.63)
	6	24	3	0.928 (0.253)	17.7 (2.23)
	12	6	0		
	12	12	4	0.788 (0.137)	17.4 (2.00)
	12	24	4	0.882 (0.299)	19.7 (4.76)
	24	6	2	0.909 (2.867)	17.6 (0.94)
	24	12	4	0.743 (0.287)	16.5 (3.78)
	24	24	3	1.003 (0.302)	18.7 (0.97)
Mercenaria					
	0	6	5	0.100 (0.100)	9.6 (1.06)
	0	12	5	0.088 (0.076)	10.5 (0.94)
	0	24	5	0.128 (0.164)	9.8 (1.59)
	6	6	5	0.055 (0.107)	10.2 (1.55)
	6	12	5	0.151 (0.228)	10.1 (1.29)
	6	24	5	0.241 (0.165)	10.6 (1.70)
	12	6	5	0.084 (0.144)	9.5 (1.51)
	12	12	5	0.114 (0.124)	9.7 (1.04)
	12	24	5	0.228 (0.191)	11.6 (1.69)
	24	6	5	0.171 (0.169)	10.3 (1.04)
	24	12	5	0.262 (0.133)	10.2 (1.71)
	24	24	5	0.168 (0.193)	10.7 (1.00)

Table V-f. **a)** Analysis of variance on the untransformed a) mean relative growth and b) final mean shell length of cultured individuals of *Mercenaria mercenaria* at Duck Brook, Cutler, Maine from 24 May to 30 November 2009. Clams were placed in experimental units (0.0182 m^2) at one of three interspecific densities (6, 12, or 24, representing approximately 330, 660, and 1320 individuals m^{-2}) and at similar densities (factorially) with the same densities of cultured individuals of *Mya arenaria*. Total number of interspecific treatments = 9. (See Table 1 for a complete description of the twelve treatments.) *A priori* contrasts (underlined) test specific hypotheses concerning both intraspecific and interspecific effects on growth of *Mercenaria mercenaria*, and use α' as a decision rule (n = 5.) c) Effects of the accidental inclusion of *Carcinus maenas* in the experimental units on mean relative growth and mean final SL of cultured *M. mercenaria*. Mean relative growth of juvenile hard clams was depressed by nearly 60% in experimental units with vs. without *Carcinus maenas*. Final shell length was approximately 10% greater in units with vs. without green crabs.

a)

Source of variation	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Treatment	11	0.24536310	0.02230574	1.42	0.1929
Intra 330 vs. Rest (660 & 1,320)	1	0.00019210	0.00019210	0.01	0.9123
Intra 660 vs. 1,320	1	0.00406460	0.00406460	0.26	0.6127
Overall Interspecific	1	0.03830700	0.03830700	2.45	0.1243
Interspecific 330 vs. Rest (660 & 1,320)	1	0.08186251	0.08186251	5.23	0.0267
Interspecific 660 vs. 1,320	1	0.01016407	0.01016407	0.65	0.4243
Interspecific 330 Merc (Mya 330 vs. Rest)	1	0.01744721	0.01744721	1.11	0.2964
Interspecific 330 Merc (Mya 660 vs. 1,320)	1	0.01857580	0.01857580	1.19	0.2815
Interspecific 660 Merc (Mya 330 vs. Rest)	1	0.00456077	0.00456077	0.29	0.5919
Interspecific 660 Merc (Mya 660 vs. 1,320)	1	0.05499928	0.05499928	3.51	0.0670
Interspecific 1,320 Merc (Mya 330 vs. Rest)	1	0.00653624	0.00653624	0.42	0.5213
Interspecific 1.320 Merc (Mya 660 vs 1,320)	1	0.02215159	0.02215159	1.42	0.2401
Error	48	0.75142125	0.01565461		
Corrected Total	59	0.99678435			

b)

Source of variation	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Treatment	11	18.54758776	1.68614434	1.37	0.2174
Intra 330 vs. Rest (660 & 1,320)	1	0.87055699	0.87055699	0.71	0.4043
Intra 660 vs. 1,320	1	1.09412262	1.09412262	0.89	0.3503
Overall Interspecific	1	1.47166809	1.47166809	1.20	0.2795
Interspecific 330 vs. Rest (660 & 1,320)	1	2.41575847	2.41575847	1.96	0.1675
Interspecific 660 vs. 1,320	1	7.06756000	7.06756000	5.75	0.0205
Interspecific 330 Merc (Mya 330 vs. Rest)	1	0.32642424	0.32642424	0.27	0.6088
Interspecific 330 Merc (Mya 660 vs. 1,320)	1	1.72723360	1.72723360	1.40	0.2418
Interspecific 660 Merc (Mya 330 vs. Rest)	1	0.11839324	0.11839324	0.10	0.7577
Interspecific 660 Merc (Mya 660 vs. 1,320)	1	0.73584782	0.73584782	0.60	0.4430
Interspecific 1,320 Merc (Mya 330 vs. Rest)	1	0.07830165	0.07830165	0.06	0.8019
Interspecific 1,320 Merc (Mya 660 vs 1,320)	1	0.62489107	0.62489107	0.51	0.4794
Error	48	59.03714463	1.22994051		
Corrected Total	59	77.58473239			

c)

Carcinus	n	Mean Relative Growth	Final Mean Length
Present within experimental units	38	0.098 (0.035)	10.8 (0.44)
Absent within experimental units	22	0.238 (0.054)	9.9 (0.37)

Dependent Variable: Relative Growth

Source of variation	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Carcinus	1	0.27407403	0.27407403	22.00	<.0001
Error	58	0.72271033	0.01246052		
Corrected Total	59	0.99678435			

Dependent Variable: Final Shell Length

Source of variation	DF	Sum of Squares	Mean Square	F-Value	Pr > F
Carcinus	1	9.25369192	9.25369192	7.85	0.0069
Error	58	68.33104047	1.17812139		
Corrected Total	59	77.58473239			

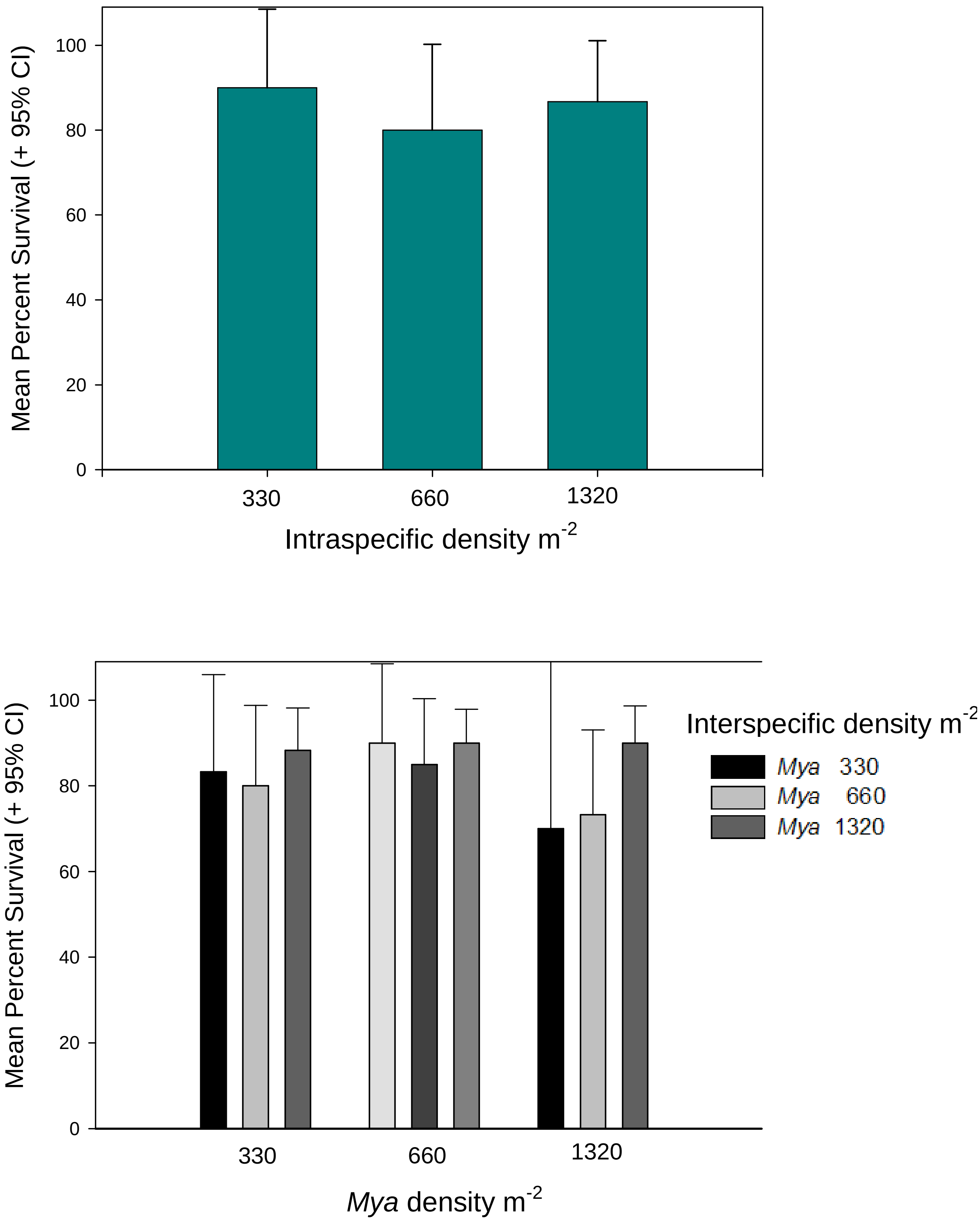
Figure Legends

Figure V-a. Mean percent survival (+ 95% CI) of *Mercenaria mercenaria* at Duck Brook Flat, Cutler, Maine from 24 May to 30 November 2009. **a**) Effects of intraspecific Density on mean percent survival. **b**) Effects of interspecific density (*Mya arenaria*) on mean percent survival.

Figure V-b. Mean relative growth for living individuals of *Mercenaria mercenaria* at Duck Brook, Cutler, Maine from 24 May to 30 November 2009. **a**) Effects of intraspecific density on mean relative growth. **b**) Effects of interspecific density (*Mya arenaria*) on mean percent survival.

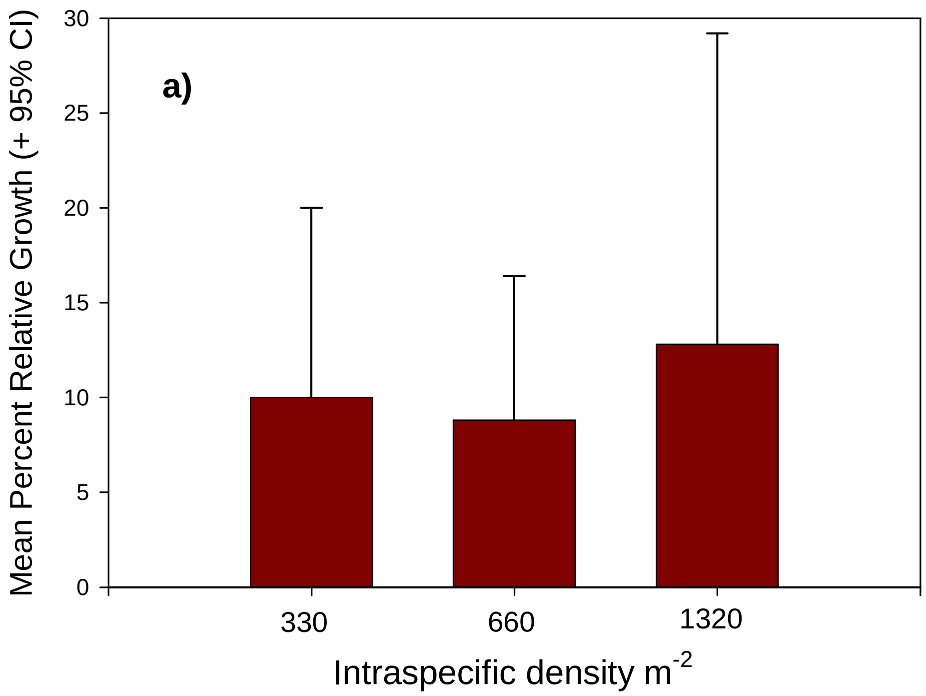
Figure V-c. Initial and final size frequency distribution of *Mercenaria mercenaria* at Duck Brook, Cutler, Maine from 24 May to 30 November 2009. Final mean length ± 95% CI = 10.5 ± 0.15 mm (n = 717).

Figure V-a.



Mean Percent Survival (+ 95% CI)
100
80
60
40
20
0
330
660
1320
Intraspecific density m-2
Mean Percent Survival (+ 95% CI)
100
80
60
40
20
0
330
660
1320
Mya density m-2
Interspecific density m-2
Mya 330
Mya 660
Mya 1320

Figure V-b.


a)
Mean Percent Relative Growth (+ 95% CI)
0
5
10
15
20
25
30
330
660
1320
Intraspecific density m-2

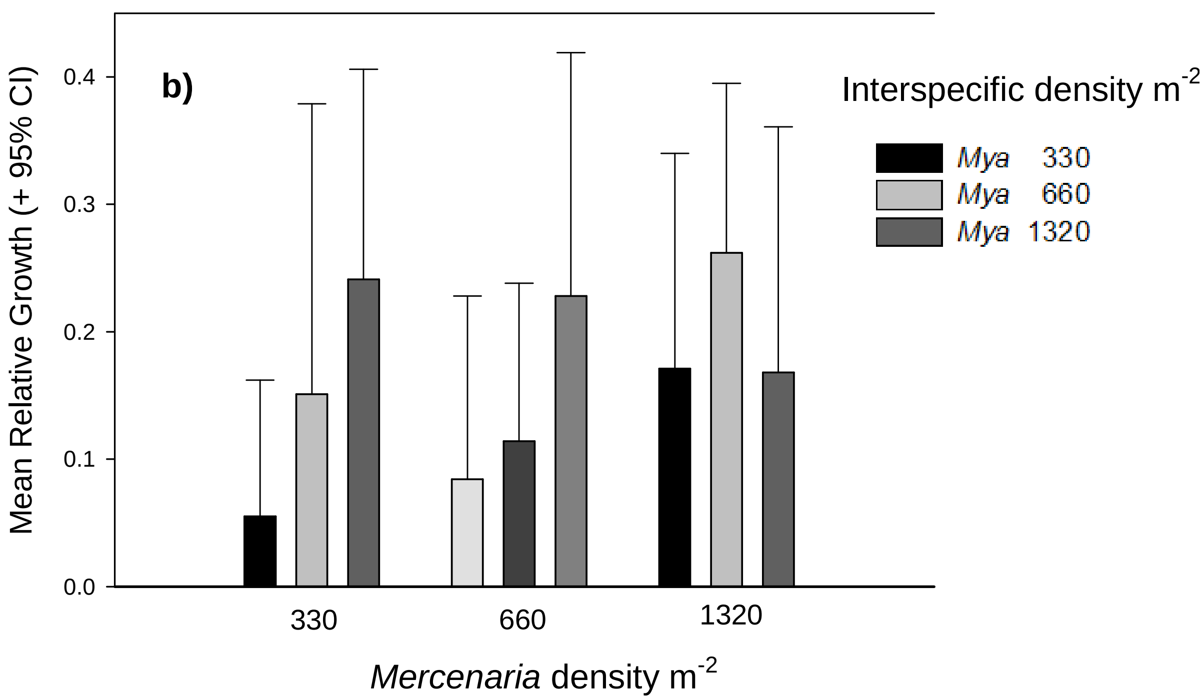
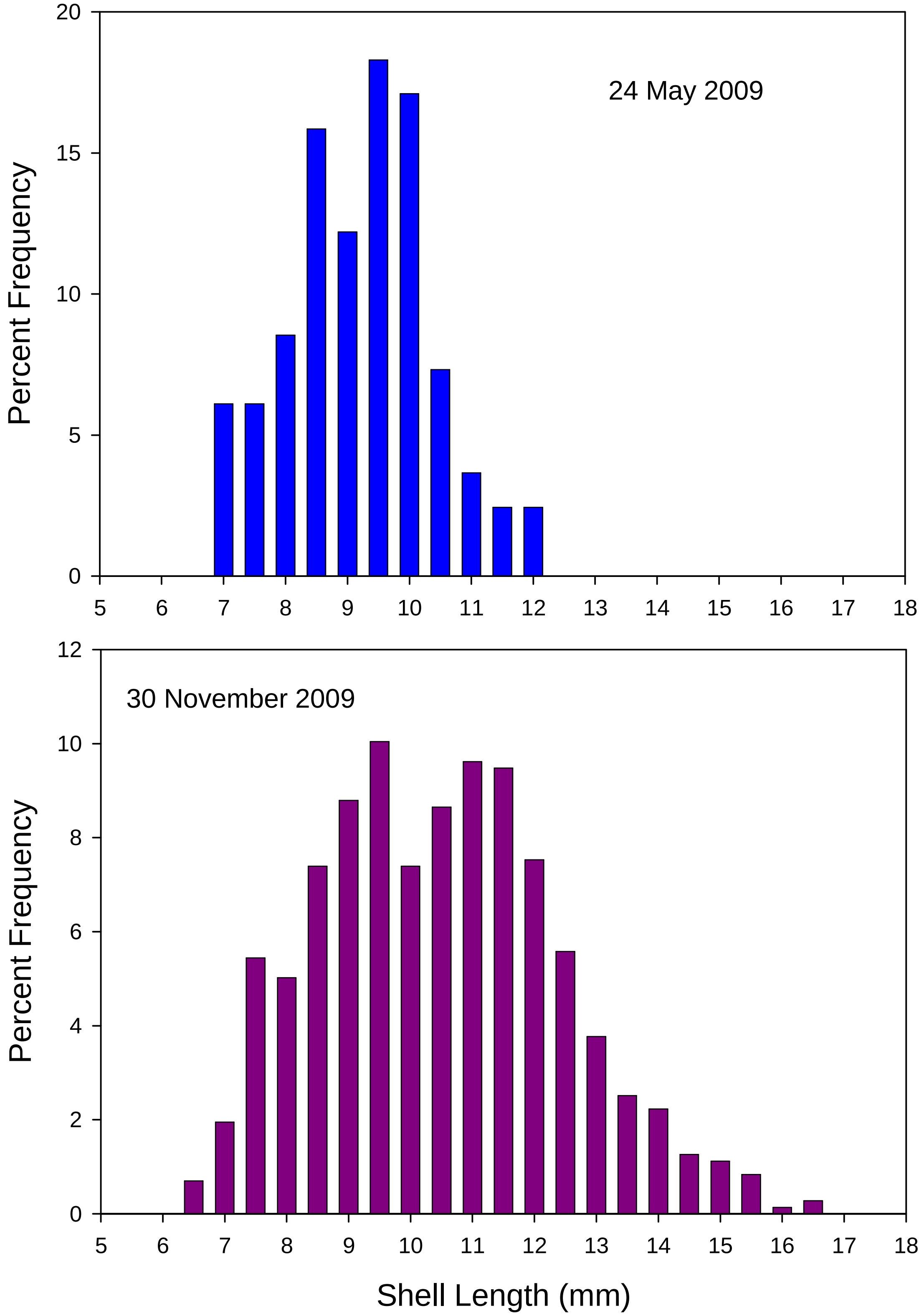

b)
Mean Relative Growth (+ 95% CI)
0.0
0.1
0.2
0.3
0.4
330
660
1320
Mercenaria density m-2
Interspecific density m-2
Mya 330
Mya 660
Mya 1320

Figure V-c.



24 May 2009
Percent Frequency
0
5
10
15
20
5
6
7
8
9
10
11
12
13
14
15
16
17
18
30 November 2009
Percent Frequency
0
2
4
6
8
10
12
Shell Length (mm)

Commercialization Plan

No commercialization activities occurred during the project.

Our future commercialization plans are tied directly to our proposed Phase II research and development work. Although we have answered the questions we posed in our technical objectives, we must now focus attention in the direction that the Phase I work has led us toward.

Specifically, our commercialization plan will address the potential for production of Maine- and farm-grown hard clams to sell to markets in Maine and Greater New England. Currently, hard clams grown and harvested in Maine constitute only a small percentage of those sold in this region. Most hard clams sold in Maine and elsewhere are grown and harvested from Massachusetts to Florida. In Maine, no other hard clam sea farms exist, nor is there a substantial wild harvest. Most hard clams purchased by the consumer in the State of Maine are harvested from areas outside the state that require the clams to be sanitized through depuration before sale. Depuration is a process that involves passing seawater holding the animals under ultraviolet light to kill pathogens (bacteria and viruses) present in and on the shellfish at the time of their harvest. While this depuration process results in a safe product for sale and human consumption, the flavor is altered, and most agree that it becomes more bland as a result. In addition, shelf life of depurated clams is dramatically shortened. Both effects are undesirable in the seafood trade because the consumer is presented with a diminished quality product whose flavor has been essentially neutralized.

Besides low levels of fecal contamination that depuration can counteract, many hard clams on the market come from areas prone to red tide or paralytic shellfish poisoning (PSP), and these harvesting areas are often closed during months of peak demand because of the presence of these dangerous algal blooms seems to coincide with late spring and summer weather events. The areas in and around Trenton, Maine that are being farmed by Bagaduce River Oyster Company and Egypt Bay Sea Farms have historically been clean pockets, free of blooms of the dinoflagellates that are associated with red tide/paralytic shellfish poisoning (PSP). This means that successful completion of the upcoming Phase II effort has the potential of providing a steady, safe supply of hard clams to local and New England markets when large portions of the East Coast of the United States are closed due to the presence of red tides causing paralytic shellfish poisoning.

Another positive attribute of hard clams grown in Downeast Maine is their hardiness and shelf life. Hard clams that are harvested from the cold waters of eastern Maine, and particularly from the area where we work, have been shown to have extended shelf life compared to hard clams from along the southern Maine coast, and particularly areas to the south of Maine. Extended shelf life is a tremendous management and marketing asset that means clams are more healthy, they will keep longer under refrigerated conditions, will be a more stable inventory in retail businesses, and be a safer product overall. An analogy would be purchasing wilted, browning lettuce from a market instead of choosing the newest, most crisp heads. This information about shelf life is based on comments from local (Ellsworth, Bar Harbor, Blue Hill) individuals, wholesalers, restaurateurs, and retailers as well as personal experience. We have seen and been told that our clams can be stored cold/moist for up to and beyond a month with zero mortality.

Under the same storage conditions, clams originating south of our cold, eastern Maine waters or those having gone through a depuration process often begin to succumb within 7 to 10 days. To produce a steady crop of marketable hard clams using sustainable farming techniques, we must continue the research and development initiative that we started during the Phase I process. We will ask four major questions:

1) Can our best growth and survival results be repeated in another year and at larger scales?

2) How can we work to produce larger seed clams for field planting?

3) Is it possible to overwinter first year seed (sizes = 6 to 12 mm SL) at volumes greater than what we have investigated to date?

4) Are there other sites west of Trenton, such as Mt. Desert, Surry, and Blue Hill that will yield as good or better growth and survival as we have observed at Goose Cove in Trenton?

Hatchery and Field Nursery Applications: In Phase I, we established that conditioning broodstock hard clams in the shellfish research and production center at the Downeast Institute in the winter was feasible and effective. Conditioning was followed by spawn-ing, larval rearing, and growing post-settlement individuals to 2-3 mm using cultured algae at DEI. We then determined that it was possible to produce 5-12 mm clams for seeding by floating 2-3 mm individuals in field-based nursery trays in a cove on Beals Island from late spring to early fall. Next, we learned to store these 5-12 mm animals over the winter using techniques that we transferred from previous efforts using cultured soft-shell clam seed. This technology transfer resulted in approximately 99% survival of the hard clam seed from mid-November to late April/early May. It is thought that plant-ing small seed in the fall would not be efficacious due to the extended period of time when clams would not be adding to their shell (October to May), and the fact that this period of time is associated with inclement weather (high winds, storms, and ice events). Overwintering enables us to plant our seed stock early the following spring when rising seawater temperatures and increasing densities of phytoplankton (naturally occurring, single-celled floating plants that is the primary source of food for clams) become avail-able to optimized clam survival and growth. Here in Maine, hard clams generally have been reared from hatchery stock in spring, then field planted on ocean bottoms in the fall. Survival using this method is low, resulting in costly seed being lost. We have achieved survival rates in field plots at our leased sites in Goose Cove during the first growing season of approximately 90% from specific treatments used in the Phase I experiments. We found that plots protected with flexible, plastic netting (4.2 mm aperture) yielded highest survival (and growth) rates but that clams plots that permit predators unrestricted access (control plots without protective netting) experience > 70% mortality. To date, however, all experiments have been conducted on a small scale, in confined areas, and over a short span of time. In our Phase II project, we will work at larger, commercial scales and in a variety of different geographic areas to determine the limits to actual survival from hatchery-reared seed to a marketable product.

We must focus on research to produce larger plantable seed, as our field trials during Phase I continued to point to the seed size as a critical variable in the survival equation. We will examine the response of clam (2-3 mm SL) to different field nursery locations in eastern Maine to determine how geographic location and nursery techniques affect growth over the first spring, summer, and early fall. One method, that we will not incorporate into our business and commercialization plan is to send seed from the hatchery to a southern (e.g., Massachusetts, New Jersey, Virginia) nursery site. Although this methodology may result in larger seed to plant in farming plots the next spring, at least three reasons exist for not incorporating this scenario into our plans: 1) it runs the risk of introducing disease (e.g., neoplasia; QPX; Perkinsus) not only to the cultured stocks, but to wild stocks at the field growout sites; 2) it is unclear whether seed reared in the warmer waters will respond to overwintering in the same way that clams that have been in colder waters respond; and, 3) it takes jobs away from eastern Maine. Instead, we will examine the following three scenarios:

a) Upwellers. Currently, Bagaduce River Oyster farm uses an upweller on its leased nursery farm site, and has had great success using this technology to grow cultured oyster seed, *Crassostrea virginica*, to field plantable sizes. We will build an upweller large enough to rear 1 million hard clams to at least a 12 mm SL. Provided it is placed in nutrient-rich, relatively warm waters, and is managed well, an upweller will allow large quantities of tiny (ca. 1 mm) clams to be grown to field plantable sizes in a small area. Large amounts of water are pumped through containers holding clam seed, which greatly increases food availability to the small clams. In addition, an upweller can accept clams that are smaller than those that can be reared using floating trays or boxes; hence, the initial cost per individual seed is approximately 90% less than the cost of seed used in trays.

b) Floating Trays. We have used floating trays lined with nylon window screening material with some success to rear 2-3 mm seed from the hatchery to field plantable sizes. In Phase II, we will compare growth and survival in these trays from three different water bodies (Beals, in far eastern Maine), Morgan Bay (Surry, in Upper Blue Hill Bay), and the Bagaduce River (Penobscot, off upper Penobscot Bay). All the water bodies have significant differences in water flow, water temperature, salinity and, presumably, food availability.

c) Nursery bags. One of us (PI Porada) has had excellent preliminary success with "nursery mats." These are essentially sealed bags constructed of nylon window screening (as described above for use with the floating trays) on the bottom and 1/6- or 1/4-inch plastic flexible netting on top. The mats, or bags, (8-ft x 8-ft) sink slowly into the mud over time allowing the small clams to live in sediments as they would normally in the wild. Three bags were deployed in the shallow subtidal at Goose Cove during the summer of 2009, using a stocking density of 4,000 m^{-2} (i.e., 375 ft^{-2}), and survival was similar to that from floating trays at the Beals Island nursery site – nearly 100%. In addition, growth was, on average, significantly greater than that observed from the floating trays. In Phase II, we will use these mats at multiple sites, and compare growth and survival results to that from the floating trays and upweller.

All trials will compare growth and survival in year one. In year two, the same experiments will be refined and repeated to estimate temporal variation at each site.

Predator Control: Once seed clams are planted in the shallow, soft-bottom subtidal areas, predators such as the exotic European green crabs can wreak havoc if not deterred. Clams planted in plots without any crab deterrent netting had poor survival – as low as 20% – because crabs consumed many of them. Thus far, we have seen excel-lent survival of hard clam seed during the first field growing season by using protective netting (4.2 mm or 6.4 mm plastic, flexible mesh) coverings. Netting placed directly over clams larger than the aperture in the nets resulted in excellent survival (≥ 80%). Clams placed in soft bags (made from the same flexible netting material) and hard bags (made from extruded plastic, with similar aperture openings) also had excellent survival (in some cases ≥ 90%). Clearly, nets and mesh bags are effective against green crab predation. We do not know how effective any of these methods will be at larger scales, however. We will expand our planting scenarios using the most successful netting applications and continue an experimental approach to determine the most effective grow-out methods. In addition, we will examine the efficacy of using traps to limit green crab predation by comparing numbers of green crabs using baited traps and unbaited traps. Unbaited traps as a means of control will take advantage of green crab behavior, as they tend to be found hiding under objects. We found large numbers (ca. 190 m^{-2}) of adult green crabs underneath our hard bag grow-out cages (see Results of Experiment I, p. 16) as we took benthic cores during our December 2009 sampling. This lead us to the idea of using habitat, a hiding place, as another form of "bait".

Grow-Out from Seed to Market Size, Quantity and Market Production: Our effort toward commercialization began with our Phase I work, and will continue through our Phase II project. During the next two years, we will continue to refine our methods and technologies using an experimental approach that will examine expanded and commercial- scale treatments, such as floating nurseries, bottom nurseries, large covered clam seed beds, crab trapping variations, etc. Through this applied research and new innovation we will determine the best practices to plant and grow commercial quantities of hard clams to optimum market sizes. As we enter Phase III, what we learn from our research and development activities will allow us to provide a steady supply of product to the Maine and New England markets. The most promising Phase I results related to overwintering, planting on mixed mud substrates, predator control with netting, and predator trapping will all be tested together experimentally with new design innovations specific to Phase II. Our focus will be to establish our planned field trials at commercial scales, examine the effects of nursery and field grow-out over a wider geographic region, and determine temporal variability both within and between sites by replicating our work over the additional year allowed by the Phase II grant.

The Market, Customer, and Competition: The primary end market for our product will be individuals who enjoy the best in fresh, quality seafood. Some of these individuals will be served our product in restaurants, catered events, and fairs. Others will enjoy our clams in dishes they prepare themselves at home. Some of the most common favorite recipes and serving methods for this clam species include raw on the half shell, traditional garlic and herb clam linguini, bouillabaisse, paella and steamed clams with butter. The FAO reports that the prime market for cultured hard clams is live in the shell. The product is used either fresh on the half-shell, or steamed. The primary market for the half shell is as an appetizer in up-scale restaurants (FAO, 2010).

Seafood wholesalers such as J.P.'s Shellfish (Elliot, Maine) and Maine Shellfish Company (Ellsworth) will be our main vehicle for distribution to the end consumer though restaurants, local supermarket chains, and seafood markets. These are large shellfish companies that have built their reputations on quality and customer satisfaction, and we are fortunate to work closely with them to see that our product reaches consumers in a timely and reliably.

Initially, most of our sales will be through Maine Shellfish Company, a member of the Ipswich Shellfish Company, which is a major seafood wholesale delivery company with hubs throughout the Northeast from Maryland to Maine. J.P.'s Shellfish is the wholesale distributor of many of our American oysters. We will work with these companies, and others, to further develop our hard clams as the product reaches market size in sufficient numbers – two to three summers depending on consumer and market preferences regarding size. One reason for relying on Maine Shellfish Company at the outset is that Mr. Porada currently sells wild harvested stock to this company, that has a significant client base. In Maine, the Maine Shellfish Company is the largest, most comprehensive seafood company delivery system with offices and facilities in Ellsworth (Downeast) and Kennebunk (Southern) Maine. With Maine Shellfish Company as the primary vehicle for our product, we will first target upper-end establishments that focus their food purchasing on locally available, sustainable, and clean foods and shellfish. For the past several years, we have supplied a select, local clientele with approximately 60,000 wild hard clams annually through this company. Given our current customer base and sales, and projecting that into the available market in Maine and Greater New England, we will have the capacity to sell 5 million hard clams annually throughout this region. Two million of those are expected to be sold to specialty markets, including middle- and high-end restaurants and caterers. Another 3 million clams are expected to be sold across the market spectrum. Maine markets will be our initial focus; however, while we are approaching our sales capacity in Maine and Greater New England, we will be testing markets in New York State and Pennsylvania. Our focus at the point of market saturation in Maine will be the large wholesale seafood hub in Boston, MA, Fulton Fish Market in New York City, and select seafood wholesalers across the Northeast. We expect to be able to achieve sales outside Maine in numbers greater than 4 million as we begin Phase III.

Our primary competition in Maine is from one company, Spinney Creek Shellfish. They are primarily an oyster company in southern Maine that also sells hard clams. Most of the hard clams available through Spinney Creek are harvested south of Maine, and most of these clams are depurated at their plant in Elliot, Maine. Many Maine customers who have purchased this product have complained about poor shelf life, and how quickly clams die. Other competition is indirect, through small local wholesalers whose product is harvested largely from outside Maine and New England. Hard clams marketed and competing in Maine come from as far away as Florida. Very few hard clams are Maine-grown, and those that are harvested from wild stocks, in areas prone to pollution and red tide/PSP.

We have a significant advantage over competitors outside Maine in several key areas. One is the name recognition of "Maine," which, for most people easily relates to a pristine and high quality seafood producing region. Another is the location of our farm site(s), that will be marketed as the northernmost hard clam growing area in the United States. A third advantage is that our hard clams are grown in areas that are historically clean and free of harmful bacteria and red tide/PSP (paralytic shellfish poisoning) organisms. Coupling these advantages with Bagaduce River Oyster Company's existing market recognition, Mr Porada's established markets for hard shell clams, and the innovations developed in Phase I and Phase II for growing hard clams efficiently in Downeast Maine, we will gain substantial market leverage in our targeted markets.

Additional factors contributing to our competitive advantage are that:

- Our grow-out sites are centered in a high-traffic tourist area with a high density of restaurants and seafood related businesses, many of whom currently purchase our wild harvest product. For instance, downtown Bar Harbor, Maine and Acadia National Park, with over 2 million summer tourists annually, are within minutes of our main grow-out site in Trenton. Some restaurants include this fact on menus.

- Maine Shellfish Company and J.P.'s Shellfish, our major wholesale customers and distributors, are located within ten miles of our grow-out sites. These two companies have the largest, most comprehensive networks for delivering Maine seafood to customers in northern New England. These companies regularly truck product to and from states as far away as Florida.

- Our product is delivered to the wholesale dealer and trucking facilities within 1 hour of its harvest, and is delivered to end users within 48 hours. This assures greatest freshness and highest quality to our buyers and consumers. Our competitors cannot boast of such rapidity from the ocean to the plate. More often, our product will be on the plate or in the kitchen before our competitor's product has reached a distributor.

- Our farm-raised products will allow individuals, markets and restaurants the ability to special order specific sizes of hard clams daily. Basic sizes include: little necks, 2-2.5 inches, top necks, 2.5-3 inches, and cherrystones, 3-3.5 inches. In addition, we will pursue markets for smaller animals (e.g., pasta necks and petite necks) that will allow us to bring product to market more quickly.

- The PI has established a friendly, reliable business relationship with many local restaurant owners and chefs, many of whom have been recommending our product to others along the coast of New England, and as far as New York City.

- Our sites are the northernmost viable commercial habitat for hard clams in the eastern United States. As such, our clams come from the cleanest and coldest waters, and are hardier than clams growing in and harvested from more southern areas. The extended shelf life of our product compared to clams from more southern areas is due primarily to the colder water temperatures, high rate of water flow associated with astronomically large tides in eastern Maine, and that hard clams here are better adapted to this cooler, more extreme environment.

- Our company has moved ahead in a systematic way to find the best growing sites and methods of bringing clams to market based on scientific investigation and experimental rigor. The fact that we are working with scientists from the University of Maine at Machias and the Downeast Institute and participate actively in the pursuit of the research and development is unique in Maine and presents us with another marketing advantage.

Mr. Porada has been harvesting wild hard clams commercially for over twenty years. During this time, he has established and cultivated an excellent rapport with local seafood purveyors, restaurant owners and chefs. This trust, working together with the large distributors (Maine Shellfish Company and J.P.'s Seafood), and the fact that our shellfish grow in what are unarguably the cleanest waters on the east coast of the United States will allow Bagaduce River Oyster Company to capture a significant portion of the hard clam market.

Hard clam aquaculture is the largest, most valuable of the shellfish aquaculture industries on the east coast of the United States, and accounts for more than $50 million in economic value annually (Whetstone et al., 2005). According to industry estimates, the current market supports annual sales over 210 million cultivated hard clams compared to 7 million just 25 years ago. Wild harvested clams contribute only a small potion to the market.

Some disadvantages and hurdles are that:

- Established shellfish companies selling hard clams in Maine have their own clientele which will take us more time to enter certain markets.

- Our products will be more expensive to the end consumer than those from southern areas; however, the difference in the quality of our product (as described above) can easily be demonstrated, but it will take time for consumers to make these comparisons.

- Our grow-out sites often freeze over during winter months. Clams will have to be harvested before ice forms, and stored in cages at alternative sites for easy access when this occurs. Overwinter storage is an important feature of the research investigations to be undertaken in Phase II. Bagaduce River Oyster Company's aquaculture nursery site, as well as other sites in Blue Hill Bay, will be used for winter inventory storage and experimentation.

- Depending on rainfall amounts, shellfish growing areas are required to be closed to all harvesting by officials within the Maine Department of Marine Resources. These areas remain closed until impurities (i.e., fecal coliform bacteria) have dissipated to their normal background levels, which in our areas is below detectable levels. During times when we are unable to harvest from our grow-out sites, we will work with local companies that have the capability of using clean seawater that is recirculated within indoor tanks for shellfish storage as a means of keeping our product on the market during these extreme weather events. There are several such companies in our area.

- Our company is far from major market distribution hubs such as Boston and New York. We are fortunate in this area to have a large and varied seafood shipping infrastructure covering the coast of Maine, the Northeast, New Brunswick, Canada, and the southeast coast of the United States. We will be exploring alternate companies and various transportation methods and as we make market connections further away from eastern Maine. In addition, we intend to develop a mail-order business using media and on-line marketing.

Revenue Stream: The infrastructure for an efficient revenue stream already exists in Bagaduce River Oyster Company's oyster marketing, and through the wild product harvested and sold to wholesalers by Mr. Porada. The plan for generating a revenue stream for our cultured product will be developed as marketable stock becomes from our Phase II project becomes available. A large proportion of the cultured stock from our Phase I plantings will be reaching market size beginning in the fall of 2011, and continuously thereafter. Clams planted during our Phase II work will begin reaching a marketable size during the summer of 2013.

We will use what we learn and discover during our Phase II research to develop full-scale planting strategies for commercial amounts of seed clams in Phase III. We plan to seed plots over five successive springs so that we can sell and market five million pieces annually, which is our expected market share. Clams will be planted in blocks so that the animals within a given block will grow to market size and can be harvested without interfering with animals growing elsewhere at the farm sites. After harvesting and turning over the sediments, a block will be replanted the following spring. We anticipate harvests to begin during the third growing season in each block. Once this rotation is established, we will have a continuous harvestable product available for our customers.

A substantial positive factor of our research and development activities, beginning in Phase I, is that none of our seed stock is wasted. It will become the beginning our revenue stream. That is, all clams planted for research during Phase I and Phase II will continue to grow in their ocean farms plots, and will add to the revenue stream fully commercialized during Phase III.

Between 30,000-40,000 juvenile clams were removed from the site during our Phase I sampling. We intend to plant these clams this spring, as these animals presently are being overwintered at the Downeast Institute's (DEI) shellfish production and research center in Beals, Maine. An additional 200,000 clams remain growing in plots at Goose Cove that we seeded during the Phase I research. Approximately 1.5 million juvenile clams (5-12 mm SL) were produced during 2009 and are being stored over the winter at DEI (see Beal et al., 2009 for overwintering methodologies) to be used for experimentation during the Phase II grant. Another 2-3 million juveniles will be produced in 2010 for field experiments planned for 2011 (year 2 of Phase II). In total, we will manipulate 3million seed clams during our Phase II research. We will use the methods and techniques that resulted in fastest growth and highest survival during Phase I in our Phase II investigations. We will refine and modify these techniques during the Phase II project, and these clams will become the foundation of our revenue stream as we proceed to Phase III.

Conclusions

The research we completed and present in this Report from our Phase I effort has offered us a valuable roadmap to the future.

We used an experimental approach to determine how cultured hard clam seed (sizes between 6-12 mm SL) growth and survival was affected by planting location, planting date, initial seed size, stocking density, and predator exclusion netting. Most experiments used a combination of blocking (to assess spatial variation in treatment effects) and the factorial combination of the factors listed above. The replication was considerable, and allowed us high statistical power to detect differences between means if they truly existed. All the work was conducted in the field at a total of five locations in eastern Maine between the last week of May and mid-December 2009.

We discovered that cultured hard clam juveniles seeded at shell lengths ranging between 6-12 mm can double, at least, in size under some growing conditions. These conditions, generally were unrelated to the stocking densities we varied (400-600 individuals m^{-2}), but were related strongly to planting time of year and, most importantly, grow-out location. We had hoped that hard clam farming could become a viable alternative to wild soft-shell clam harvesting in far eastern Maine where native stocks have been extremely low for over a decade, but our field trials in this region (e.g., Machias River, Holmes Bay, and Cobscook Bay) have discouraged us from further attempts to grow clams there. Seawater temperatures, even during summer months, rarely exceed 16^{o}C in eastern Maine, and clams cease growth at temperatures ca. 8^{o}C (Rice and Pechenik, 1992). Therefore, the hard clam growing season is too short given the temperature regimes observed in this part of Maine. Instead, the Phase I work has encouraged us to examine growth and survival rates west of our leased site at Goose Cove in Trenton (near Bar Harbor).

Besides some of our earlier work from 2006 and 2007 (Beal et al., 2009), this effort represents the first detailed applied research on juvenile hard clam growth and survival in Maine. The only prior published work on hard clams of any size in this state was from a 1968 review of geographic variation in hard clam growth along the east coast of North America (Ansell, 1968), and the work cited in that publication from Maine was conducted in the late 1950's with 3-4 year-old hard clam individuals in Casco Bay (near Brunswick, about 100 miles southeast of our field site in Trenton).

Growth and survival of hard clam juveniles was excellent and comparable in both small aperture (4.2 mm) soft bags and in plots where seed was added directly to soft sediments and then covered with the smallest aperture (4.2 mm) flexible predator netting. We estimate survival over the first growing season of > 85% in some of the treatments, and conservatively > 75% in most protected plots (see Experiment I and II). In addition, clams planted in May attained final mean SL's of ca. 19 mm, with approximately 35% of animals ≥ 20 mm SL. Finally, in most of our trials, we found that the larger the seed, the better it performed both in percent survival and growth. Taken together, these are encouraging results, and gives us very specific directions for further research and development.

Several unresolved issues remain to be addressed. First, we are uncertain whether treatment effects on growth and survival observed during May-December 2009 are similar (i.e., representative) of what we can expect in future. That is, was the 2009 growing season unique? To answer this, we must re-examine some of the same factors at our grow-out site during 2010 and compare results to those obtained during the Phase I work. Second, we need to develop a nursery system (post hatchery sizes [ca. 1 to 3 mm SL] – to plantable sizes ≥ 12 mm SL) that is economical and effective. Currently, we rely on a floating nursery system in far eastern Maine where seed attain an average size of 7-8 mm SL during the period between early July and mid-November. Ultimately, since survival and growth during the first growing season in field plots is related directly to the size of seed planted in the spring, we need to produce the largest possible seed. We will explore how stocking density, nursery location, and type of nursery infrastructure influence size of seed in both of the years funded by the Phase II grant. Third, overwintering hard clam seed grown in the nursery during the first year prior to planting in field plots the following spring is critical to the success of our commercialization plans. We have developed a technique, to store 5-12 mm SL seed from November through early May that is similar to that used to store cultured soft-shell clam seed over the winter; however, we have not fully examined the limits of this methodology with respect to clam volumes and sizes, and must do so in Phase II. Fourth, we must determine how hard clams grow and survive in areas west of our leased site at Goose Cove in Trenton. We cannot and should not rely on a single site for our commercialization plan, as this risks the chance that some change occurs at the site that prohibits us from harvesting our product (i.e., disease, pollution, incursion of blue mussels, higher than expected predation from green crabs or other predators, or some other unanticipated disaster). We plan to expand our efforts in Phase II to one additional grow-out site, and three additional nursery sites. Mr. Porada is in the process of working with Maine's Department of Marine Resources to obtain a small subtidal lease in Morgan Bay (Upper Blue Hill Bay), near Surry. This site will serve as both a grow-out and nursery site.

Provide a discussion of the utility, economic feasibility, and general attractiveness of the research and/or technology. Tie together all of the results from published and unpublished information. Address any unresolved issues with regard to the problem statement or background and current transition status.

Retail market prices of little necks (2-2.5 inches) from Maine and Massachusetts to Virginia range from $0.48 to $0.75 per individual. Wholesale prices range from $.20 to $.30. If it costs a hard clam farmer $25.00 per 1,000 animals for seed (10-12 mm SL), then break-even survival rates would be around 25% and 10% for product sold wholesale and retail, respectively. With first-year survival rates in protected plots or soft bags > 70%, and with survival rates typically increasing with increasing bivalve size (e.g., soft-shell clams, *Mya arenaria*, Brousseau, 1978; cockles, *Clinocardium nuttallii*, Gallucci and Gallucci, 1982; blue mussels, *Mytilus edulis*, Thompson, 1984; and others, see Juanes, 1992) it would appear that farming hard clams in the waters of eastern Maine may be profitable. Further research and development to refine and improve nursery and field grow-out techniques are needed to evaluate critically any commercialization program.

References

Anonymous 2007. A sea change for conservation. USDA. NRCS. 2 p. http://www.ma.nrcs.usda.gov/news/PDF/Showcase_Shellfish_EQIP.pdf.

Ansell, A.D. 1968. The rate of growth of the hard clam *Mercenaria mercenaria* (L.) throughout the geographical range. Journal du Conseil 31, 364-409.

Barnes, N.G., 2004. Marketing opportunities for Cape Cod hard clam producers based on purchase and serving practices of restaurants. South Eastern Massachusetts Aquaculture Center, University of Massachusetts, Dartmouth. 31 p. http://www.umassd.edu/cbr/studies/ semac2.pdf.

Beal, B.F. 2006. Relative importance of predation and intraspecific competition in regulating growth and survival of juveniles of the soft-shell clam, *Mya arenaria* L., at several spatial scales. Journal of Experimental Marine Biology and Ecology 336, 1-17.

Beal, B.F., Protopopescu, G., Yeatts, K., and J. Porada. 2009. Experimental trials on the nursery culture, overwintering, and field grow-out of hatchery-reared northern quahogs (hard clams), *Mercenaria mercenaria* (L.) in eastern Maine. Journal of Shellfish Research 28, 763-776.

Beal, B.F., Bayer, R.C., Kraus, M.G., and S.R. Chapman. 1999. A unique shell marker of juvenile, hatchery-reared individuals of the soft-shell clam, *Mya arenaria* L. Fishery Bulletin 97, 380-386.

Beal, B.F., and M.G. Kraus. 2002. Interactive effects of initial size, stocking density, and type of predator deterrent netting on survival and growth of cultured juveniles of the soft-shell clam, *Mya arenaria* L. in eastern Maine. Aquaculture 208, 81-111.

Beal, B.F., Lithgow, C., Shaw, D., Renshaw, S., and D. Ouellette. 1995. Overwintering hatchery-reared individuals of the soft-shell clam, *Mya arenaria* L.: a field test of site, clam size, and intraspecific density. Aquaculture 130, 145-158.

Beal, B.F., Parker, M.R., and K.W. Vencile. 2001. Seasonal effects of intraspecific density and predator exclusion along a shore-level gradient on survival and growth of juveniles of the soft-shell clam, *Mya arenaria* L., in Maine, USA. Journal of Experimental Marine Biology and Ecology 264:133-169.

Brousseau, D.J. 1978. Population dynamics of the soft-shell clam, *Mya arenaria*. Marine Biology 50, 63-71.

Commito, J.A. 1982. Effects of *Lunatia heros* predation on the population dynamics of *Mya arenaria* and *Macoma balthica* in Maine, USA. Marine Biology 69, 187-193.

Eldridge, P.J., Eversole, A.G., and J.M. Whetstone. 1979. Comparative survival and growth rates of hard clams, *Mercenaria mercenaria*, planted in trays subtidally and intertidally at varying densities in a South Carolina estuary. Proceedings of the National Shellfisheries Association 69, 30-39.

FAO. 2010. Cultured Aquatic Species Information Programme. Text by Kraeuter, J. N. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 15 March 2005. http://www.fao.org/fishery/culturedspecies/Mercenaria_mercenaria/en#tcN900FE.

Gallucci, V.F., and B.B. Gallucci. 1982. Reproduction and ecology of the hermaphroditic Cockle *Clinocardium nuttallii* (Bivalvia: Cardiidae) in Garrison Bay. Marine Ecology Progress Series 7, 137-145.

Grabowski, J.H., Powers, S.P., and M. Hooper. 2000. Balancing tradeoffs between predator protection and associated growth penalties in aquaculture of northern quahogs, *Mercenaria mercenaria* (Linnaeus, 1758): A comparison of two common grow-out methods. Journal of Shellfish Research 19, 957-962.

Juanes, F. 1992. Why do decapod crustaceans prefer small-sized molluscan prey? Marine Ecology Progress Series 87, 239-249.

Lorio, W.J., and S. Malone. 1995. Biology and culture of the northern quahog clam (*Mercenaria mercenaria*). Southern Regional Aquaculture Center Publication No. 433. 4 p.

MacKenzie, Jr., C.L., Morrison, A., Taylor, D.L., Burrell, Jr., V.G., Arnold, W.S., and A.T. Wakida-Kusunoki. 2002. Quahogs in eastern North America: Part II, History by province and state. Marine Fisheries Review 64, 1-64.

Massachusetts Aquaculture Association. 2006. http://www.massaqua.org.

Murray, T.J., and Kirkley, J.E., 2005. Economic activity associated with clam aquaculture in Virginia – 2004. VIMS Marine Resource Report No. 2005-5. Sea Grant Virginia. 21 p.

Peterson, C.H., Summerson, H.C., and J. Huber. 1995. Replenishment of hard clam stocks using hatchery seed: Combined importance of bottom type, seed size, planting season, and density. Journal of Shellfish Research 14, 293-300.

Powers, M.J, Peterson, C.H., Summerson, H.C., and S.P. Powers. 2007. Macroalgal growth on bivalve aquaculture netting enhances nursery habitat for mobile invertebrates and juvenile fishes. Marine Ecology Progress Series 339, 109-122.

Rice, M.A. and J.A. Pechenik. 1992. A review of the factors influencing the growth of the northern quahog, *Mercenaria mercenaria* (Linnaeus, 1758). Journal of Shellfish Research 11, 279-287.

Shaw, R.G., and T. Mitchell-Olds. 1993. Anova for unbalanced data: An overview. Ecology 74, 1638-1645.

Sturmer, L.N., Quesenberry, E., and D.E. Vaughan. 1997. Development of hard clam aquaculture on Florida's west coast: from training to production to a sustainable industry (Abstract). World Aquaculture Society Book of Abstracts, p. 443-444.

Taylor, J.D., and M. Layman. 1972. The mechanical properties of bivalve (Mollusca) shell structures. Paleontology 15, 73-87.

Thompson, R.J. 1984. Production, reproductive effort, reproductive value and reproductive cost in a population of the blue mussel *Mytilus edulis* from a subarctic environment. Marine Ecology Progress Series 16, 249-257.

Underwood, A.J. 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, U.K. 504 p.

Whetstone, J.M., Sturmer, L.N., and M.J. Osterling. 2005. Biology and culture of the hard clam (*Mercenaria mercenaria*). Southern Regional Aquaculture Center Publication No. 433. 6 p.

Winer, B.J., Brown, D.R., and K.M. Michels. 1991. Statistical principles in experimental design. 3rd edition. McGraw-Hill, New York.

Woods, C., 2006. University of Florida, Institute of Food and Agricultural Sciences. http://news.ifas.ufl.edu/story.aspx?id=1090.

Zaklan, S.D., and R. Ydenberg. 1997. The body size-burial depth relationship in the infaunal clam *Mya arenaria*. Journal of Experimental Marine Biology and Ecology 215, 1-17.