Pictorial Manual for the Culture and Grow-out of Soft-Shell Clams: A Practical Guide to Farming Clams in Maine's Intertidal Zone

By

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Introduction

This pictorial manual is designed for those who are interested in culturing soft-shell clams both in a hatchery environment and in a field setting to grow to commercial sizes. The effort comprises a period of time from May 1987 when the Beals Island Regional Shellfish Hatchery (BIRSH) opened its doors for business – to produce cultured soft-shell clam seed for communities to enhance their wild stocks – to 2010, a time when hatchery production of soft-shell clam seed continues at the Downeast Institute for Applied Marine Research and Education (DEI).

During this 23-year period, between 1-10 million clams have been produced each year for a variety of field grow-out scenarios ranging from public stock enhancement to private clam farming. Cultured clam seed from BIRSH and DEI has been seeded in 60 of Maine's 77 towns with a state-approved municipal shellfish conservation ordinance. In addition, applied research has been conducted in every coastal county over this time, but most extensively in Washington County. This research effort has focused on a number of factors related to the goal of rearing clams under field conditions that will result in optimal growth and survival. Most of the research has been published in peer-reviewed scientific journals that are available online or through the Downeast Institute. Research has been graciously supported by a number of organizations, including: Davis Conservation Foundation; Dolphin Trust; Eastern Maine Conservation Initiative; Economic Development Administration; Jessie B. Cox Charitable Trust; Maine Sea Grant; Maine Technology Institute; National Coastal Resources and Research Institute; National Marine Fisheries Service; Robert & Patricia Switzer Foundation; Passamaquoddy Tribal Council; and, the University of Maine at Machias. Unless otherwise stated, all photos were taken by Brian F. Beal.

I. Hatchery culture

Hatchery culture of soft-shell clams, *Mya arenaria*, begins by obtaining broodstock (adults). Several different methods can be employed. Clams can be obtained directly from the flats, from clammers, or from a buying station. Typically, clams ranging in size from 2 ½-3 ½ inches in length perform best (i.e., can be induced to spawn, and produce millions of eggs and sperm per individual). It is possible to obtain clams that are "ripe" during early May through early June depending on location along the coast. Ripeness can been discerned by removing one shell and examining the visceral mass, the largest part of the soft body that contains the digestive and reproductive system of the clam and ends ventrally as the muscular foot. This region will appear creamy in color and have a firm texture when clams are ready to spawn. After spawning, and for a few months prior to spawning, the visceral mass region will appear yellowish-brown and be flaccid to the touch. It is also possible to collect clams in the winter that are not naturally ripe, and "condition" them over a 7-8 week period in the hatchery by gradually increasing the seawater temperature and feeding with increasingly greater volumes of cultured microalgae.

Figure A-4.1. Soft-shell clams, *Mya arenaria*. Adults (broodstock) can be stimulated to spawn by collecting them from intertidal flats during early May to early June when they are naturally "ripe," or they can be collected early in the winter and "conditioned" over a 7-8 week period, and then induced to spawn.

Figure A-4.2. Soft shell clam in a "ripe" (left) and "unripe" (right) condition. Animals that are ripe can be induced to spawn.

If broodstock are collected in May-June, they can be induced to spawn immediately; however, if broodstock are obtained at any other time of year and require a period of conditioning, they must be held in the hatchery in such a way that they are easily cleaned and will remain healthy for up to 3-4 months. Large clams require a certain amount of external pressure on their valves (shells) to help them shut or close. In the wild, this pressure is exerted on the shells by the sediment that surrounds the animals at various depths in the flats. Once exposed, however, large clams typically gape and die within a few weeks if some force is not applied to their valves. We have had success inserting clams into

sand that is contained in plastic fish totes, and then submerging the totes into a large tank filled with seawater where it is possible to feed clams daily. Although this method works well, totes are heavy, difficult to clean, and animals must be dug out of the sand each time a spawning scenario is attempted. For the past twenty years, we have used "clam sandwiches" to hold clams prior to attempting to spawn them. The sandwich is composed of two pieces of 14-gauge lobster trap wire and two pieces of 1/4-inch VEXAR netting. Each piece is approximately 18-inches x 18-inches. Clams rest between the two pieces of VEXAR with the heavier trap wire on the top and bottom of this arrangement. Approximately 15-25 clams can be held in a single sandwich. Pressure is applied to the valves using nylon cable ties that are carefully cinched around the periphery and middle of each sandwich. Several sandwiches can be placed inside 100-gallon "broodstock" tanks, and cultured microalgae delivered to each.

Figure 4-A.3. Clam sandwich (left) holding 15 soft-shell clam adults. Broodstock tank (right) with four sandwiches. Cultured microalgae is added to the tank daily. Conditioning at DEI occurs in January-February and is the process of gradually increasing seawater temperature from 1-2^{o}C to 15-16^{o}C and microalgal density over a 7-8 week period. At that time, clams are ready for spawning.

Microalgal (unicellular algae) production is a critical phase in broodstock conditioning as well as larval and juvenile rearing. A stepwise process is used to produce microalgae. First, seawater is added to small, 125 ml flasks. A mixture of nutrients including nitrate, phosphate, and iron along with micronutrients including vitamins, zinc, cobalt, manganese, and molybdenum are added next. Then, flasks are autoclaved for 20 minutes at 15 psi. Once cooled to room temperature, a 5 ml aliquot of week-old microalgae from a similar 125 ml flask is transferred to the flask using a sterile technique (algal transfers occur under UV light, and glass pipettes used in all transfers are flamed to kill bacteria). Microalgae in the small flasks are kept at room temperature and are placed in front of fluorescent lights. These are referred to as stock cultures. Stock cultures serve a dual role. First, they are used each week to perpetuate a given strain of microalgae (some strains at DEI are 15 years old). Second, once the 5 ml of stock culture is transferred to another 125 ml flask, the remaining volume of microalgae is transferred to a larger 500 ml flask. Prior to that

transfer, the larger flasks are treated similarly to the smaller flasks in that they are filled with seawater containing nutrients, autoclaved, and then cooled to room temperature. After a week under fluorescent lighting, the entire volume of microalgae in the 500 ml flask is transferred to a 3.5 L glass jug containing "sterilized seawater." Seawater in the jug is sterilized using a small volume of Clorox bleach (sodium hypochlorite, NaOCl). After sitting for 24 hours, the bleach is neutralized by adding an aliquot of sodium thiosulfate ($Na_2S_2O_3$). Then, nutrients and micronutrients are added along with the entire volume of microalgae from the 500 ml flask. After a week in front of fluorescent lighting, the contents of the 3.5 L jug are transferred to a 90L "algal tube." Tubes are constructed of fiberglass and are 87% transparent. Microalgae grow in the tubes for 7-10 days and, depending on the particular algal species, can reach densities of 10-12 million cells per 1 ml. A combination of diatoms and flagellates are reared at the Downeast Institute. Diatoms include *Chaetoceros gracilis*, *Chaetoceros muelleri*, *Thalassiosira pseudonanna*, and *Thalassiosira weissfloggii*. Flagellates include *Isochrysis galbana*, clone T-Iso and clone C-Iso; *Pavlova pinguis*, *Pavlova lutheri*, *Tetraselmis maculata*, *Tetraselmis chuii*, and *Rhodomonas salina*. Diatoms require silicates in the form of sodium metasilicate (Na_2SiO_3) that are added with the other nutrients at each step in the microalgae rearing process.

Figure 4-A.4. Stock microalgal cultures in 125 ml flasks (left) and cultures recently transferred to 500 ml flasks (right). Sterile techniques are used for transferring algae from flask-to-flask. In addition, seawater and nutrients are sterilized using an autoclave prior to algal transfer.

Figure A-4.5. Jug culture at the Downeast Institute. The green microalgae is *Tetraselmis maculata*.

Figure A-4.6. Mass microalgal culture in fiberglass tubes. Each tube contains ca. 90 L. The light brown microalgae in the foreground is *Isochrysis galbana* (clone T. iso). Microalgae are used to condition broodstock, and to feed clam larvae and juveniles.

Once broodstock are conditioned (when gametes in both males and females have developed to a mature stage, the gonad is fully distended, and where ooyctes are mature in females and attached to the follicle wall by a narrow stalk or are free in the lumen, and where males have follicles filled with spermatozoa or sperm), they are ready to be induced to spawn. Typically, spawning occurs via thermal shock. Broodstock are removed from the conditioning tanks and from their sandwiches and are placed in the bottom of a shallow

tank. Initially, seawater temperature is the same as it was in the conditioning tank (ca. 15-16°C). Animals are fed and allowed to clear the water. Then, the entire volume of the tank is drained and the tank filled with 23-24°C seawater. Time to spawning is related to degree of ripeness. Sometimes spawning occurs within 30 minutes of the thermal shock. Sometimes, repeated heating and cooling of seawater can occur over a 6-8 hour period followed by spawning. Sometimes, spawning does not occur at all. When clams do not respond to thermal shock stimulus over a normal working day, 20-30 individuals are placed into shallow wooden trays lined with window screening and then added to the surface of a 400-gallon tank that will become a larval rearing tank. Seawater temperature in the larval tank is 23-24°C. Many times, clams will spawn during the evening and trocophore larvae will appear in the tank the next day.

Figure A-4.7. Clams in shallow spawning tray (left). Close-up of clam siphon (right). The largest aperture is the incurrent siphon that is used for ingesting food. The smaller aperture is the excurrent siphon that expels feces and gametes. Typically, male clams will spawn first followed by the females. Once the first male spawns, it remains in the spawning tank so that it will stimulate others into a mass spawning event. Subsequent individuals that spawn are removed from the tank and are placed separately into glass bowls to complete their spawning.

When thermal shocking is effective, male clams typically will spawn first. Also, it is normal for 40% or fewer of the broodstock to spawn during a thermal shocking event. The first male to spawn will be left in the spawning tray. The released gametes coincides with a release of pheromones ("external hormones") that helps stimulate other clams to release their gametes. Once the second individual in the tank begins to spawn, it and subsequent individuals are quickly removed from the tank and placed into buckets or bowls or other containers filled with warm seawater. The purpose behind this management technique is to reduce the likelihood of polyspermy (multiple fertilization of a single egg) that results in the improper development of larvae. Eggs from all females are collected and combined in

one or more large buckets. Sperm from all spent males similarly are collected and combined in other buckets. Fertilization of eggs occurs by transferring a small volume of "sperm water" to a bucket/container with eggs and then observing the development of polar bodies using a compound microscope at 40x magnification. Polar bodies are the by-products of the egg's division during meiosis, and since polar body formation cannot occur without proper fertilization, this is an excellent method to determine whether additional sperm is required to complete the fertilization process.

Figure A-4.8. Close-up of male clam spawning (left). Male clam in fingerbowl (right) ca. two minutes after removing from larger spawning tray.

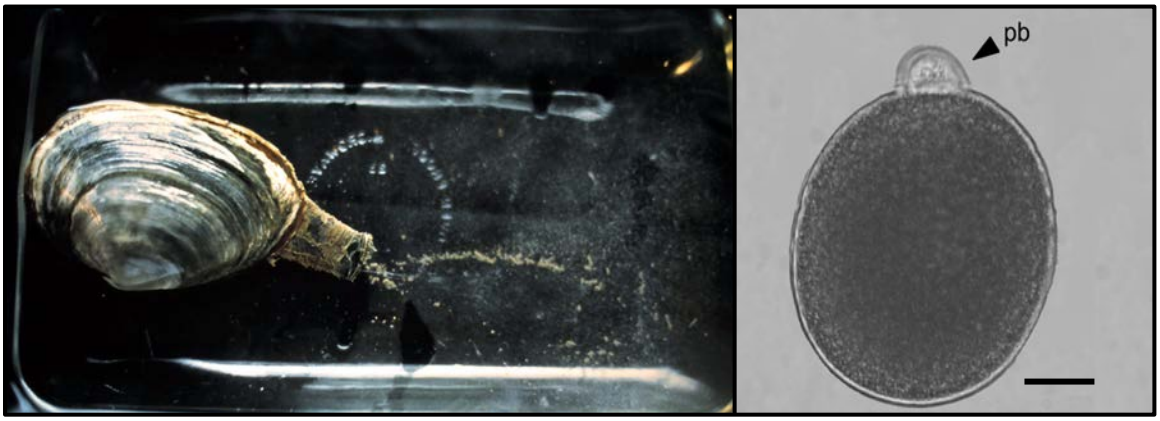

Figure A-4.9. Female clam spawning (left) with many eggs visible as small white spheres in the righthand portion of the spawning dish. A fertilized egg of a razor clam (*Ensis arcuatus*) (right) just after spawning with a polar body (photo from Da Costa et al. 2008 -- http://mollus.oxfordjournals.org/content/74/2/103.full.pdf+html). Scale bar = 20 μm

After successful fertilization, zygotes are placed into 400-gallon tanks filled with seawater at a temperature of 23-24°C. Over a period of 14-18 days, clam larvae develop through several stages (trocophore; veliger; pediveliger), increasing size and volume along the way. Every two days, the entire content of a larval tank is drained completely, larvae caught on small mesh screens, and transferred quickly to an adjacent clean tank containing warm seawater and microalgae.

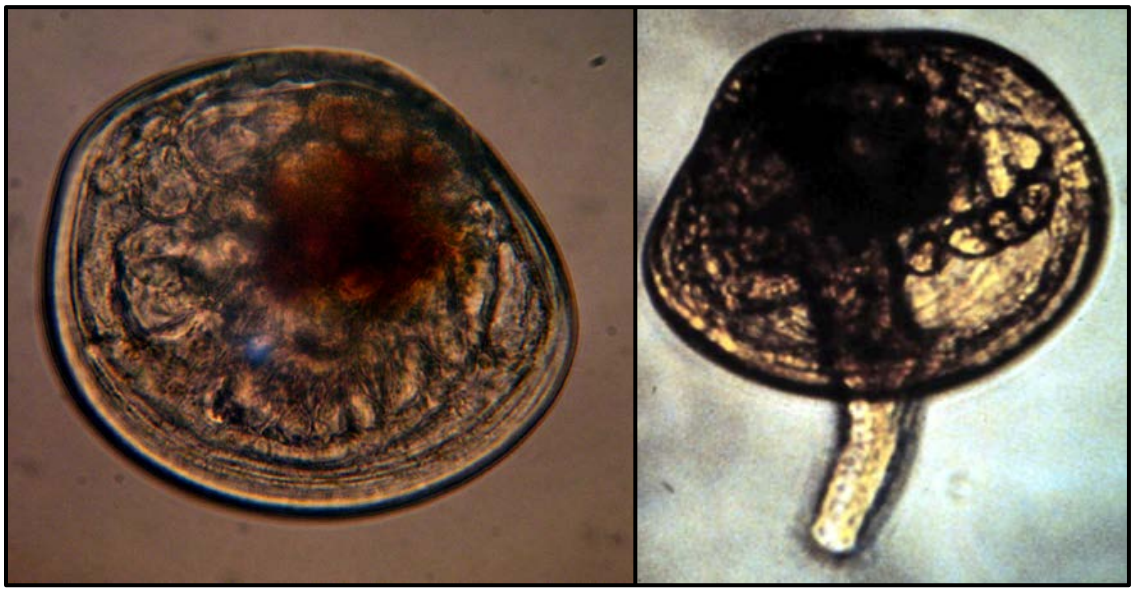

Figure A-4.10. Soft-shell clam larvae. 12-day old veliger stage (left – ca. 100 microns). The brownish area near the hinge is digested microalgae. 18-day old pediveliger (right – ca. 180 microns). A mixture of diatoms and flagellates are fed to clam larvae daily.

Figure A-4.11. Larval rearing tanks (400-gallons) at the Downeast Institute.

Once pediveligers appear in the larval tanks, this is a signal that animals are ready to metamorphose, cease swimming, and settle to the bottom. At this time, when animals are 150-175 µm, they are removed from the larval tanks and placed into 800-gallon "setting" tanks where juveniles will remain until they attain sizes between 3-4 mm in length. Clams are placed onto setting trays (initially with 125 µm mesh), and then as animals grow, they are graded and placed on other trays with larger mesh (170, 250, 500, and 1,000 µm). It may take 2-4 months for all animals from a single spawning to attain sizes 3-4 mm in length. Grading occurs every two weeks. Approximately 800 liters (ca. 210 gallons) of microalgae are produced each day to feed clams at the Downeast Institute.

Figure A-4.12. An 800-gallon setting tank (left) with wooden trays containing recently settled soft-shell clam juveniles. Seawater flows across each tray by pumping water from the bottom of the tank through a 3-inch pipe with holes that sits in the middle of the tank. The flow oxygenates the water and brings microalgae (food) to the clams in the trays. A tray (right) containing 150,000-200,000 soft-shell clams, that about 250 µm in length.

Figure A-4.13. Clams in the setting tanks are graded every two weeks by removing them from trays (left). Then, animals are washed through a series of graded sieves. Clams of similar sizes are reintroduced to a tray and placed back into the setting tank. With daily feeding, animals eventually will reach a size where they are large enough to be retained on window screening (right).

II. Nusery culture

Once animals are large enough to be retained on window screening (3-4 mm in length), they are once again removed from the setting tanks and trays and then are transferred 15,000 at a time to field nursery trays. These trays are 4-ft x 3-ft and are lined (top and bottom) with window screening. The trays are made of wooden strapping material and built as a 4-ft x 3-ft wooden frame with a center piece of strapping to give the tray additional strength. This center piece creates a tray that has two 2-ft x 3-ft compartments or sections, and 7,500 clams are placed into each section. A handful of periwinkles (*Littorina littorea*) is added to both sections of each tray along with the clams. Periwinkles graze on diatoms and other small organisms that might attach to the window screening and reduce flow in-and-out of each tray. The top of each tray is covered with a piece of thin, black plastic film that inhibits gulls from pecking holes through the window screening and eating clams. The film also reduces sunlight, and therefore green macroalgae growth inside the sections containing the clams. Nursery trays are taken to a small cove on the eastern side of Great Wass Island, Mud Hole Cove, where they float on the surface of the water from June/July through mid-November. During this time, clams attain sizes that are appropriate for seeding on the flats (8-15 mm in length).

Figure A-4.14. A 4-ft x 3-ft nursery tray (left) that holds 15,000 soft-shell clams (3-4 mm in length). Trays are deployed during June/July at a subtidal location on the eastern side of Great Wass Island (Mud Hole Cove – right) where they remain until November each year.

Seeding of flats in the fall is strongly discouraged. Clam growth is seasonal (see below), and after October, no significant shell is added to clams growing in Maine flats. In fact, clams add no new shell until the following mid-April. This means that if clams are seeded in the fall, they spend approximately 6 months at very shallow depths in the flats, and become susceptible to mortality by waterfowl, especially Eider ducks that use their webbed

feet in shallow water to stir up small clams to consume (a process called puddling). In addition, ice may form on flats during the winter, and given the right conditions may scrape

Figure A-4.15. Nursery tray containing 15,000 clams (8-15 mm in length) in November 2008 (left) and a close-up (right) of these cultured individuals.

or scour large intertidal areas moving or crushing clams. Because of our inability to predict precisely the winter conditions of a given flat, we encourage clam seeding to be conducted in the spring – sometime in late April or in May.

Figure A-4.16. An intertidal flat in Edmunds (Washington County) during February 1989 (left). An ice floe (ca. 5-ft x 7-ft) in the Chandler River in January 1986 (middle). Notice that the surface of the floe appears muddy. Closer inspection (right) showed that it was mud, and that small soft-shell clams were in the mud. This suggests that ice can transport clams from one area to another, and it may be one way for clams to be transported from the intertidal zone to subtidal areas where clams are not accessible to commercial harvesting. (Lens cap in rightmost photo is from a 35 mm SLR camera.)

Spring planting requires that clams somehow be stored over the winter. It is not reasonable to store them in the hatchery setting tanks that they resided in as smaller

juveniles because it would take too much cultured microalgae to keep them alive for six months. They cannot remain in the nursery trays over the winter because it is possible that winter storms and/or ice will destroy the relatively fragile trays. And, we have shown that fall seeding can result in 80-100% mortality by the beginning of the next spring; therefore, planting clams in the fall should not be considered a viable option. Instead, we have developed a simple and effective overwintering method that results in 90-95% survival from mid-November through the end of the following May (ca. 6 months). The method involves removing all clams from nursery trays, and then placing up to 2.5 kg (5.5 pounds) of seed into a mesh bag (18-inches x 18-inches) constructed of fiberglass window screening. Bags are fashioned so that one end is open and the other sewn shut. Clams are added to the bags, and then the open end is secured using an 18-inch wooden lath that is rolled down over the bag 2-3 times. Then, the ends of the bag are secured around the ends of the lath using rubber lobster bands.

Figure A-4.17. Removing seed clams from nursery trays at the Downeast Institute during November 2008 (left). An overwintering cage (middle) that is the size of a standard lobster trap, but with eight equally-spaced shelves. A single bag of soft-shell clam seed (2.5 kg) is added to each shelf (right). Bags are constructed of fiberglass window screening and are 18-inches x 18-inches.

Bags are then added to a cage constructed of PCV-coated, 14-gauge lobster wire mesh that is the same size as a standard lobster trap. At the Downeast Institute, cages are placed into the bottom of a cement tank (50-ft long x 5-ft wide x 5-ft deep ≈ 9,350 gallons) that receives ambient seawater that flows continuously through the winter and spring. Air is added to the seawater from several hoses connected to an air pump. Cages are removed bi-weekly and the eight bags in each cage are sprayed with freshwater to remove mud/silt that has

built up during the two-week interval. We also have deployed cages in the field using one of two methods. The first is to suspend the cages from a float or dock. The second is to attach large floats to the top of the cage and then an anchor to the bottom of the cage that allows the cage to reside in the water column 6-8 feet off the bottom. A buoy (surface float) is attached to the anchor that allows relatively easy retrieval of the cage. The latter method should be used over as hard a bottom as possible to reduce potential negative effects of resuspended mud/silt settling in the bags. It may not be possible to clean cages that are deployed in the field on a regular (bi-weekly) basis, but some cleaning (at least monthly) is advised.

Figure A-4.18. Cement tank at the Downeast Institute (left) used to overwinter cultured soft-shell clam juveniles for planting during the spring. Tank holds approximately 75 cages (up to 12 million clam seed). Seed clams (right) that have been cleaned after two weeks in the overwintering tank.

III. Field Grow-out Methods

Clams are planted in the spring (late April through May) onto intertidal flats. Methods used to enhance wild seed (public stock enhancement) and those used for private farming are identical. The process begins with producing predator deterrent nets. These are flexible,

plastic nets that are either 1/4- or 1/6-inch aperture. The netting we have used since 1987 is produced by Industrial Netting (Minneapolis, MN; http://www.industrialnetting.com/predator.html). Netting can be cut to any length required. Typically, we have purchased rolls (3,000 ft long x 14-ft wide), and then cut the nets ourselves. Nets are lightweight and are easy to handle on land. Nets are cut to produce a 22-ft long x 14-ft wide piece that allows for a 20-ft x 12-ft (240 ft^2) planting area. Once nets are cut to the desired length, Styrofoam floats, or toggles, are secured to the underside of each in a quincunx pattern (same as the number five appears on a die). To do this, floats are placed on the underside of each net, and a 3-inch long wooden lath is placed on top of the float so that the netting is sandwiched between the lath and the float. Two galvanized trap nails are pounded through the lath and into the float to secure the float to the net. The floats lift the nets off the sediment during tidal inundation when clams are feeding. It also helps in muddy areas to baffle sediments that tend to build up on the surface of the nets if no floats are used.

Figure A-4.19. Rolls of 1/4-inch netting (left) are cut into 22-ft x 14-ft pieces. Then, Styrofoam floats are secured to the underside of the nets (right). Five floats are used and are centered in the middle of the net similar to the pattern of the number five on a die.

The number of nets to use depends on the amount of seed clams that are planted. Clams should be planted at densities of 40-60 per square foot to optimize growth to commercial size. For example, if one planted 500,000 clams at 60 per square foot (into 240 ft^2 planting areas), then about 35 nets would be needed. If the same number of clams were seeded at 40 per square foot, 52 nets would be needed.

Before reaching the flats, clams should be divided into as many groups as there are nets. Each group is measured/counted so that they can be easily planted at the chosen density. To do this, one estimates clam numbers using a volume displacement technique. Clams are dropped into a 50 ml or 100 ml graduated cylinder, and the volume of water the clams displaces is measured. Next, the clams in the cylinder must be counted, and that number (along with the volume displaced) recorded. The process is repeated at least two more

times so a minimum of three counts is taken. An average of the count per volume is used to estimate the number of clams to plant in one planting area. For example, suppose the three counts result in an average of 192 clams per 50 ml, and the desired planting density is 60 clams per square foot. This would require about 3,750 ml (3.75 liters) of clams per planting area ([50 ml x 14,400 clams/planting area]/192 clams/50 ml).

Once at the flat, a series of 20-ft x 12-ft planting areas should be staked off. Then, clams are spread as evenly as possible within that planting area. Planting clam seed is simple. A handful of seed is broadcast within the planting area as if one were throwing grass seed onto a lawn. One tries not to get too many in a small area. Once all the seed is spread into the plot, nets are used to cover and protect them from predators such as green crabs, bottom-feeding fish, ducks, and other predators that crawl on the surface of the flat or prey on clams from the water column. Depending on the consistency of the sediments, there are two different approaches to securing nets around the planted seed. If sediments are sandy or gravelly, then a furrow must be dug around the periphery of the planting area.

Figure A-4.20. From left to right (a-d). a) Measuring seawater into a graduated cylinder. b) Measuring the volume displaced by clams. c) Counting clams in the cylinder. d) Using a large measure to estimate number needed for a 240 square foot planting area.

Figure A-4.21. A bag containing soft-shell clam seed (left). Seed has been carefully measured to yield a number that is appropriate for the chosen seeding density. Typically, seed is then placed into a five-gallon bucket (middle) to make it easier to pick up to spread into the planting area by hand. Broadcasting seed (right) within the planting area.

The net is then spread over the planting area and the edges should fall into the furrow. Then, the sediment that was removed from the furrow is placed back into the furrow to secure the edge of the netting. If sediments are muddy, then after the netting is spread over the planting area, one carefully walks on the edge of the net around the entire periphery to secure the net in place. The person's weight forces the nets down into the mud. Any depression around the periphery should be filled in by pushing mud into the depression with boots.

Nets should remain in place from spring through the fall (when nearly 100% of the yearly shell growth occurs). Around the end of October or beginning of November, nets should be removed from the flats because they may be lost due to winter storms or ice scour. Careful removal of nets will ensure that they can be re-used. It is possible to re-use nets up to three times if they are not ripped or torn during their removal.

Figure A-4. 22. Sediments at this site in the town of Beals were too sandy and a furrow had to be dug (left) around the periphery of the planting area. Once clams are seeded and the furrow dug, the netting is placed over the planting area and furrow (right).

Figure A-4.23. In more muddy sediments, such as at this site in Edmunds, it is possible to walk the edge of the net into the mud to secure the nets in place.

Figure A-4.24. Deploying nets (left – Beals; and, middle - Georgetown), and nets that have been secured and in place for several months at a mud flat in Edmunds.

Figure A-4.25. Netting secured around 12-ft x 20-ft planting areas at low tide, and the same nets as the tide inundates the plots. The Styrofoam floats are used to lift the netting off the flat during periods of high tide so that it does not interfere with clam feeding.

IV. Site Selection

The most difficult task in planting clams for public stock enhancement or private clam farming is site selection. Careful planning and thought should go into this step prior to seeding clams because once the clams are seeded onto a flat, it is all but impossible to move them elsewhere in a cost-effective manner.

Most clammers know where the clamming is the best in their community, and these are the places that most times either do not need to be enhanced with cultured seed or these are places where clammers elect not to plant hatchery seed. Instead, cultured seed is planted in areas that "used to be productive," or "should be productive" but are not. Caution should tell us that places where no native clams exist are problematic for a reason. Rather than planting all cultured clams in these areas, it is always prudent to find out why no native clams exist in those areas well in advance of the enhancement date.

Several methods exist to make a reasonable determination of survival and growth of a particular site, but these take time (at least a year prior to the enhancement date), and some effort that may or may not pay dividends. The best method in selecting a site in advance of the actual planting day for a large volume of cultured clams is to set up a series of small scale field tests using cultured seed. These are simple and straightforward to

deploy and can yield an enormous amount of information that allows a community or a farmer to make an educated decision whether or not it would be prudent to carry out the enhancement at site A or B, or neither.

Cultured seed similar in size to that used in a large-scale planting can be obtained from the Downeast Institute in late April or early May. Seed can be planted or placed into 6-inch plant pots at a known density (e.g., 12 clams/pot is equivalent to a planting density of 60 per square feet). Pots are filled with ambient sediments at the field location of interest, then clams are placed on the surface of the sediments in each pot. Pots can be covered with predator deterrent netting to get an understanding of the importance of predators at that particular location. Several variations on this theme can be incorporated into the design of this field trial. For example, one may wish to locate the pots with clams at several tidal heights to understand how growth varies along the tidal gradient. Rather than using a single planting density, some pots may be seeded with 12 clams, while others may be seeded with 6 clams (30 per square feet) or 18 clams (90 per square feet) to get an idea of how stocking density might affect clam growth and survival. In addition, there may be an interest in using two different types of netting – some pots receiving netting with a 1/4-inch aperture, while others receive netting with a 1/6-inch aperture. The only word of caution is that a minimum of five replicates should be used for each "treatment" tested. A single replicate for a particular treatment is not considered an experiment, since it is not possible, under those conditions, to determine whether the results observed occurred due to random chance alone or whether it was due to the particular treatment. A minimum of two replicates is required to be able to discern anything from an experiment. Clearly, the more replicates of a particular treatment you can put out, the better (more accurate) your estimate of growth and survival is going to be. Five replicates per treatment is an adequate number for a first-time (pilot study) field trial.

For example, suppose that one wished to learn how tidal height and predation (together) affect clam survival and growth. Three tidal heights (Upper, Middle, and Lower shore) are chosen along with three types of netting (none, 1/4 –inch aperture, and 1/6-inch aperture netting). Twelve clams are to be placed into each pot. Three tidal heights x three netting types x 5 replicate pots per treatment = 45 pots. That is, a total of 15 pots are required for each of the three tidal heights. At each tidal height, five pots will be unprotected, five pots will be covered with a piece of protective netting with a 1/4-inch aperture, and five pots will be covered with a piece of netting with a 1/6-inch aperture. To ensure that no bias of the results occurs, treatments should be randomized for each pot.

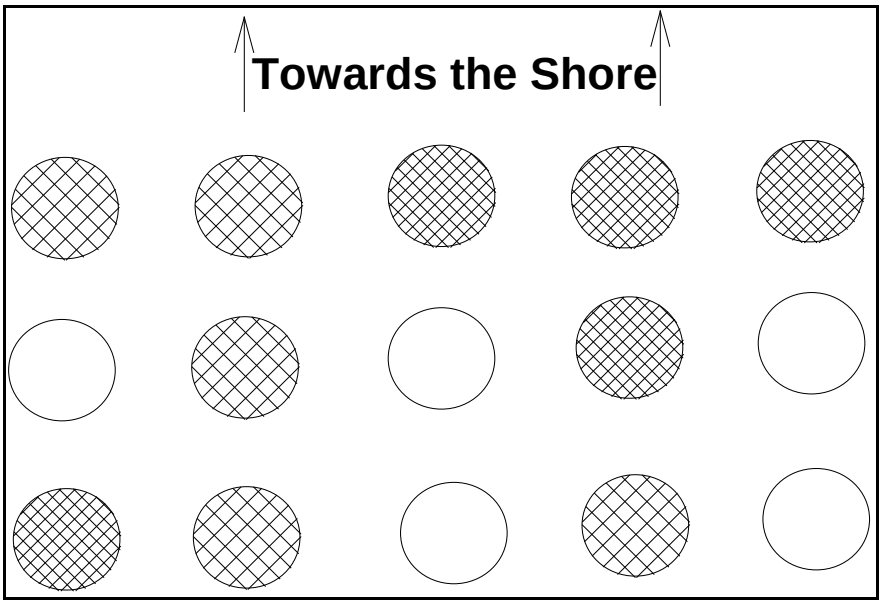

Figure A-4.26. A field layout that shows a random allocation of three netting treatments (none, 1/6-inch aperture netting, 1/4-inch aperture netting) to a total of fifteen plant pots. This design could be used at each of three tidal heights to better understand the interactive effects of tidal height and predator exclusion on growth and survival of cultured clam seed.

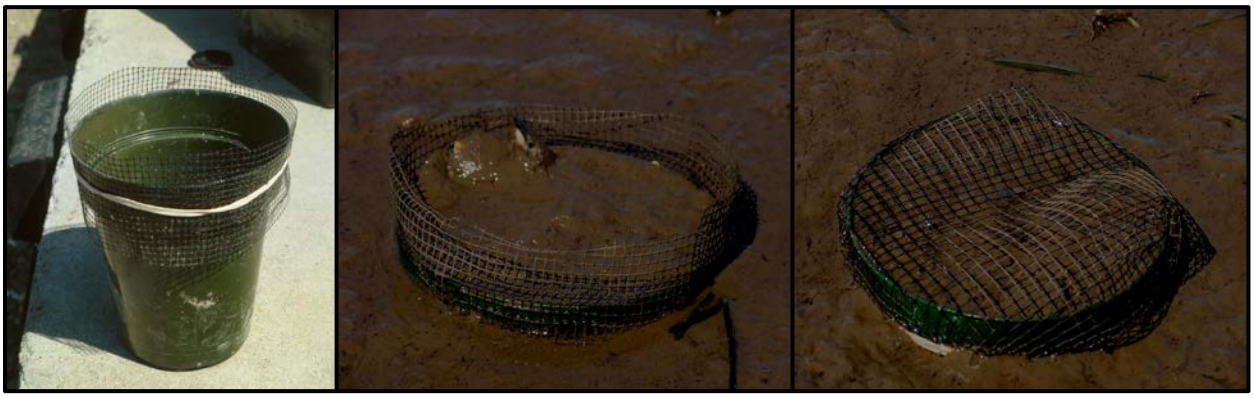

Figure A-4.27. A pot with a rim of netting (left) around the periphery (to ensure that clams remain in the pot and do not crawl away). Pot with rim of netting deployed in the field (middle), and a pot with a 1/4-inch piece of flexible netting to deter predators (right).

At the end of the field study (as late into the fall as the weather will permit), all the pots from the experiment should be removed and each labeled with a waterproof tag that indicates what the particular treatment was. Each pot can be washed through a sieve using a window screening mesh or something similar. Then, the clams can be counted and measured to provide good estimates of survival and growth. For reasons that are still poorly understood even after 20 years of practice, cultured clams mark themselves when they are first planted on the flats. That is, a disturbance line is laid down in the shell that

defines the exact size of the clam on the day it was seeded. This mark can be used not only to discern wild clams (that do not have similar disturbance marks) from cultured clams, but can be used to estimate a growth rate from each individual that survives.

Figure A-4.28. Removing plant pots containing mud flat sediments and clams from a field site along the Machias River Estuary (left). Processing samples using a box sieve (middle). The contents of a sample showing cultured clams that have grown from May-October 1998 from a flat in Holmes Bay, Cutler, Maine (right).

Figure A-4.29. Initially (1984) it was unknown that cultured clams will mark themselves when seeded onto flats by laying down a disturbance line that delineates the exact size clams were on the date they were planted. Clams marked with pink ink (left) from a study conducted in 1984 along the Chandler River, Jonesboro, Maine. Disturbance lines are laid down independent of initial clam size (middle) or location (right), and are used to discern growth rates of individual clams.

Careful site selection will also avoid places where environmental conditions may deteriorate through time. For example, in the spring, the surface of most Maine mud flats appears "normal." That is, surface muds are light brown and nothing appears unusual. Some flats, especially those along the downeast coast, begin to develop a growth of green macroalgae (clammers call it "green slime") during late June/early July that is comprised of about six different species with the most abundant being *Ulva lactuca*, *Cladophora* spp. and *Enteromorpha prolifera*. A localized bloom may develop, typically in the middle and upper intertidal, and, by late August/early September, the mass of green algae may be so widespread and thick that it actually creates anoxic (without oxygen) patches between the mud flat surface and the bottom of the algae. Lifting the green algae exposes numerous infaunal organisms (clams, worms, amphipods, etc.) that have squirmed their way out of the mud in an effort to obtain oxygenated seawater. Knowledge of whether these blooms occur regularly will help communities and clam farmers choose grow-out sites wisely.

Figure A-4.30. Typical mud flat in early spring (left). An intense bloom of green algae may develop during the summer (right) so that by late August, the mass of algae may be so thick that it creates anoxic conditions at the mud flat surface that results in heavy clam mortality.

Figure A-4.31. A flat in Whiting Bay (left) on 1 September 1984 with green algal "ropes." Meter stick in foreground for scale. An algal bloom on a flat in Machiasport in 2003 shows several small juvenile clams at the sediment surface. Vernier caliper (total length = 15 cm) for scale.

V. Predators

A number of predators are responsible for reducing both cultured and wild clam populations in Maine. Some predators can be deterred by the netting described above. These include green crabs, horseshoe crabs, fish such as mummichogs, ducks, seals, and other organisms that attack clams by digging or somehow removing sediments around clams to expose them. Netting must be secured around the planting area thoroughly, and should be checked periodically during the first six months. Other predators exist within the mud flat itself, and these are referred to as infaunal predators. For cultured seed that is 8-15 mm in shell length, the biggest threats are imposed by two groups of infaunal invertebrates: moon snails and milky ribbon worms. In addition, another bottom-feeder – a benthic fish, the wrymouth eel, has recently been discovered to consume small clams, but its relative importance compared to snails and ribbon worms has yet to be discerned. Netting does little to protect small clams from these infaunal, burrowing predators, so care should be taken to avoid areas with high densities of these organisms.

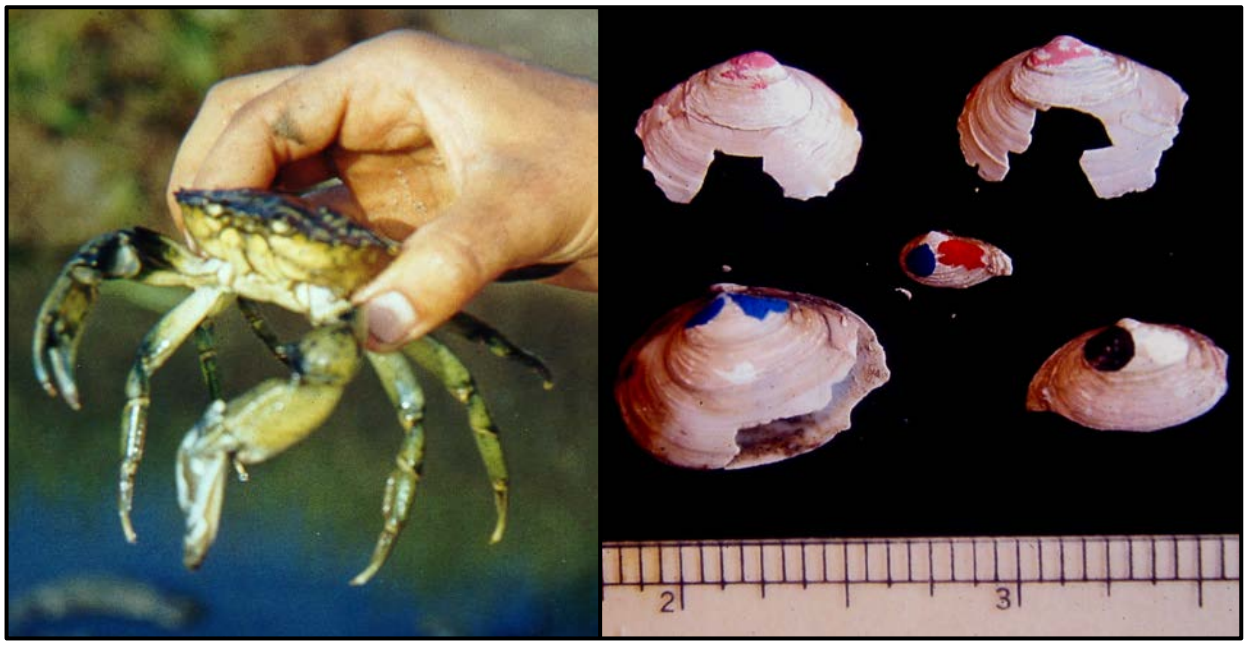

Figure A-4.32. Green crabs (left), *Carcinus maenas*, pose the greatest threat by an invertebrate predator on small (8-15 mm), cultured clams coast wide (Photo by Dana Wallace, Brunswick, ME, September 1956). Crabs typically leave crushed or chipped valves in their wake (right). These dead clams were taken from an experiment conducted in Jonesboro along the Chandler River during the summer of 1984. Green crabs are an invasive species, originating from the British Isles during the time of the U.S. Civil War in the Long Island Sound region. They had reached Portland, Maine by 1900, and arrived in the Jonesport area (Washington County) by 1954. Crabs were responsible for decimating clam populations in eastern Maine through the early 1960's. They have no natural predators, although they are eaten by a variety of organisms (gulls, lobsters, other crabs, etc.). The only natural check on their population numbers is severe winter weather.

Figure A-4.33. Moon snail, *Euspira heros* (left), from 1984 from Edmunds. Size is approximately 60 mm from the spire to apex. Snails appear to increase in size from the upper intertidal to the lower intertidal and into the subtidal. They leave a characteristic countersunk hole in their soft-shell clam prey (middle). Photo was taken in 1998 from an experiment conducted at a mud flat near Addison. Notice the "hatchery mark" (the disturbance line) on most of the clams that indicates that the clams grew several millimeters, and then were preyed on by the moon snail. The dead clams with bore holes in the photo on the right were from an experiment conducted in Lubec in 2008. Notice the size of the bore hole is not the same from clam-to-clam, and notice that these cultured clams did not grow very much before they were consumed by moon snails. Studies have shown that bore hole diameter increases linearly with snail size, so it is possible to estimate the size of the snail predator by measuring the diameter of the bore hole.

Figure A-4.34. Wrymouth eel (*Crypacanthodes maculatus*) burrows (left). This is considered a benthic (bottom) fish. It creates an extensive network of burrows in the sediment near and below the low intertidal mark that extends into the subtidal zone. The animal in the middle photo was collected in August 2009 from a mud flat in Perry. The gut contents of the animal included small fish, amphipods, and bivalve shells.

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