November 29, 2020

LARGE-SCALE CULTURE METHODS FOR BLUE MUSSELS IN MAINE AND THE NORTHEAST:

EXPERIMENTAL LABORATORY & FIELD TRIALS

By Dr. Brian Beal and Kyle Pepperman

A. Broodstock conditioning / Cryopreservation of gametes and larvae

Objective #1: To evaluate the toxicity of cryoprotectants on sperm, eggs, and trochophore larvae from naturally- and artificially-conditioned mussel broodstock.

Several trials were conducted in 2018 to test the viability of preserving mussel gametes, embryos and larvae for future use. DEI staff and collaborator Mr. Chris Maloney (Aqualine, LLC; Providence, RI) used several different types of cryo-preservatives and methods for preserving early stage mussels. Specifically, the study evaluated the effects of several cyroprotectants and their concentrations, loading and unloading strategies, as well as freezing and thawing method to develop a protocol for cryopreserving gametes and early larval stages (trocophores). We fol-lowed published guidelines that have been successful with other species of mussels (Wang et al., 2011; Paredes et al., 2012), but none of the methods produced viable gametes, embryos or larvae after being placed in suspended animation.

The first trial was conducted in February of 2018 using 10% Ethylene Glycol (EG) as the cryo-preservative. Both fresh distilled water and 1-micron filtered seawater were used to dilute the EG. Eggs, sperm, embryos, trochophores and D-stage veligers were placed in the EG solutions for 15 minutes. Next, all but the sperm were removed from the solution using a sieve and placed into a cryopreservation vial that was housed in a "Mr. Frosty" freezing container (https://www.custombiogenics.com/). The sperm and EG solution were added to the cryovials as it was unable to be separated from the EG solution due to its small size. The freezing containers were placed into a -80^{o}C freezer that cooled the cryo-vials at a rate of 1^{o}C per minute until reaching -80^{o}C. Reanimation was attempted one week after the samples were frozen by removing the vessels from the freezer, hand warming the cryovials, and rinsing the contents into warm filtered seawater. Samples were observed using a compound microscope but no signs of life were seen in any life stage. All treatments using distilled water resulted in lysed cells.

The second round of trials were conducted in March 2018 using both 10% EG and 10% Glycerol solutions as the cryopreservative treatments. All solutions were made using 1-micron filtered seawater based on results from the first trail. Three appli-cation treatments were used to expose the life stages to the cryopreservatives: 1) soaking life stage in solution for 15 minutes then removing/rinsing using a sieve and placing the samples in cryovials; 2) quickly adding life stage to solution and pouring solution and life stage into cryovials; and, 3) a combination of both methods (i.e., soaking for 15 minutes then putting life stage and solution into cryovials). Vials were immediately placed into freezing containers, and frozen as described above. Reanimation was attempted five days after preservation by removing vessels from freezer, hand warming vials and rinsing into warm seawater. None of the treatment combinations yielded any viable animals. In the Gycerol treatments, all of the sperm had lost their tails, the eggs and trochophores were lysed and the D-stage veligers had lost all of the cilia from their velums. The treatment that showed the most promise was the EG soak and rinse into the vial. Any treatment where the cryopreservative was left in the vial resulted in the formation of a gelatinous pellet of protein and no signs of life.

The third round of trials took place in April of 2018 using only 10% EG solution to soak then rinse life stages into vials. Extra care was taken to use as little rinse water as possible because we thought that the seawater was causing an adverse reaction during the freezing process. Most of the liquid was removed from life stages by decanting from the cryovial. Vials were placed into the freezing containers and frozen. Reanimation occurred seven days later with two warming methods: 1) 15°C seawater added to the vials; and, 2) 28°Ce seawater added to the vials. No live animals were observed from any treatment or life stage. Many eggs began to lyse when they were exposed to seawater (Figure 1), and the velum of most veligers were lacking any cilia (Figure 2). Warming temperature did not result in any visible difference in cell structure or animal condition.

The fourth round of trials took place in September of 2018 using only 10% EG solution to soak the life stages then a seawater rinse was used to remove preservative solution. Three treatments were tested for removing moisture from life stages: 1) control; that is, rinsing life stages into vials using minimal liquid; 2) rinsing life stages into vials with small cotton plugs to separate animals from liquid; and, 3) rinsing life stages into vials with small cotton plugs and placing a small amount of desiccant in vial to absorb moisture. Sperm was mixed 1:1 with full strength EG and placed into vials. All vials were placed in a freezing container and frozen as described above. Reanimation occurred two weeks after freezing by hand warming vials and adding to 15°C filtered seawater. The only treatment that showed signs of life was the sperm that was treated with full strength EG. There was evident movement of the tails though swimming activity was minimal. It is not likely that if alive the sperm would have been viable. The cotton plug and desiccant treatment produced the best looking animals with minimal morphological damage to the cells but no animals successfully reanimated.

No further attempts at cryopreservation were made. Instead, we focused on developing methods for year round broodstock conditioning and spawning.

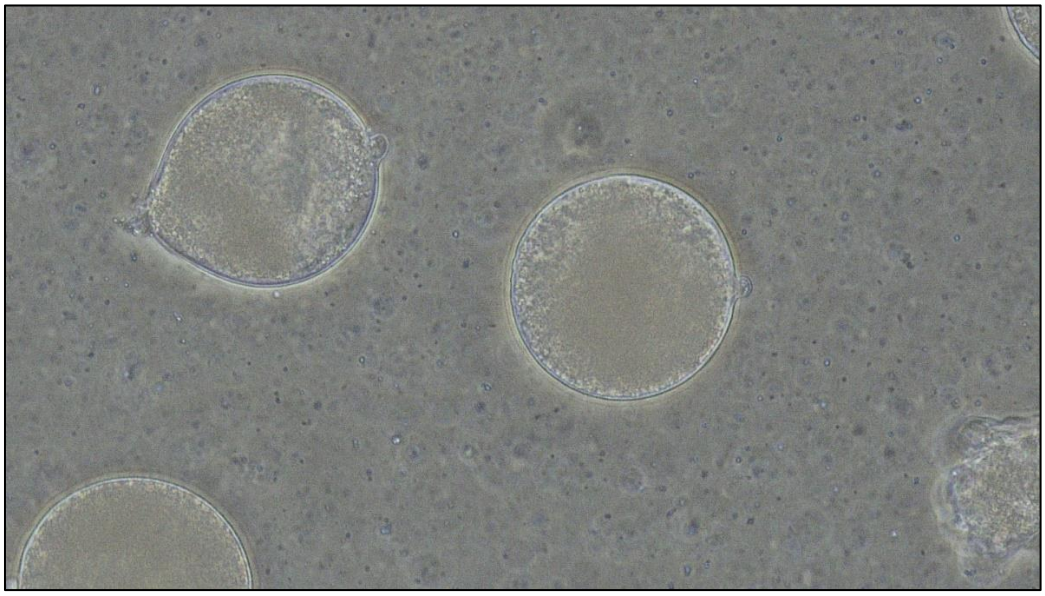

Figure 1. Photomicrograph of an intact fertilized blue mussel egg (right) and a lysed egg (left) in cryopreservative. Eggs are approximately 65-microns in diameter.

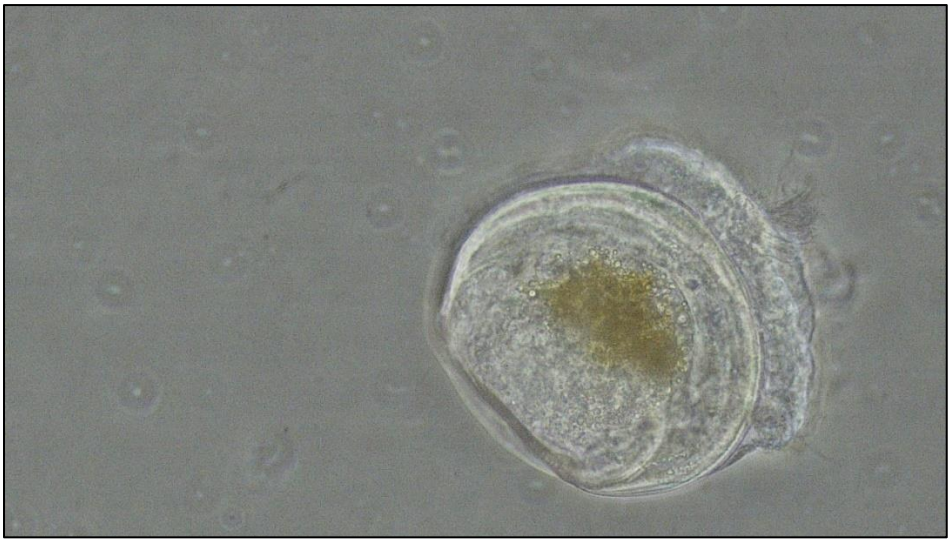

Figure 2. Photomicrograph of a late stage blue mussel veliger with missing cilia after attempted reanimation. The animal is approximately 100 microns diameter.

Broodstock conditioning has become an essential part of the hatchery process as it allows for year round production of mussel seed. Manipulating seawater temperature of tanks within which broodstock reside to initial gametogenesis while providing adequate quantities of high quality cultured microalgae results in better egg quality and lower bacterial loads compared to spawning wild conditioned animals, which we have abandoned due to the difference in the quality of the ensuing larvae. The ability to spawn broodstock outside of the spawning season and keep brood-stock from spawning during the spawning season is paramount to year round production as well as the ability to grow multiple cohorts of seeded ropes economically using the same hatchery infrastructure.

***Question #1*:** Is it possible to obtain a consistent supply of viable blue mussel seed using cryogenic techniques vs. standard hatchery methods to produce larvae and juveniles?

It may be possible to cryopreserve mussel gametes, embryos or larvae but we were unsuccessful.

B. Testing the efficacy of various materials for settlement substrates in the hatchery

Objective #2: To compare the efficacy of different ropes used for settling larvae in the hatchery at the Downeast Institute versus a new material called “spat tape.”

The product "spat tape" was never made available for use in the hatchery. The inventor of the "spat tape" dissolved his business shortly after the project was funded, and no product ever went into production. Instead, after using information from Protopopescu and Beal (2015), we commenced settlement trials with two types of ropes of extreme morphological difference (used lobster pot warp vs. a "fuzzy" rope that is used exclusively in New Zealand by aquaculturists producing the green-lipped mussel, *Perna canaliculus* – see Alfaro and Jeffs, 2005*)*.

Question #2: Which substrate surface (polypropylene rope with long loops, polypropylene rope with short loops, or spat tape) has qualities that attract mussel larvae and performs best (measured by density of individuals/linear unit area) under hatchery conditions?

Methods and Materials

A trial was initiated to test the attractive settlement qualities of New Zealand fuzzy rope produced by Quality Equipment New Zealand (https://musselrope.co.nz/MUSSEL_SPAT_CATCHING_ROPE.php), compared to used lobster rope (pot warp). Fifty meters of each rope type was wound onto PVC racks (Figure 3) with three replicates of each rope type (i.e. three racks of lobster rope and three racks of NZ rope). Three days prior to initiation, ropes were placed into 3000 L tanks with filtered seawater and sterilized overnight in a light bleach solution, after which the bleach water was neutralized using a solution of sodium thiosulfate. Ropes were then sprayed vigorously with freshwater, and the tank filled with filtered seawater and aerated for two days to promote biofilm formation.

Conditioned mussel broodstock were spawned on 22 November 2018, and the larvae reared for 14 days until metamorphosis to the pediveliger stage. On 6 December 2018, the pre-pared racks were moved to a clean 3000 L tank with filtered seawater and arrayed in alternating order (i.e., Lob-NZ-Lob-NZ-Lob-NZ) with an air stone between each rack. Competent pediveliger larvae (~4,600,000) were added to the tank and allowed to settle. Larvae were fed a mixture of cultured phytoplankton once daily. Every three days, racks were transferred to an adjacent 3000 L tank with clean, filtered seawater and cultured microalgae. After three weeks, the tank was put on flow-through ambient seawater, and the racks remained in the hatchery until spring when they were deployed in the field (Figs. 4-5).

Initial density and size frequency measurements were taken at the time of deployment by randomly removing a one-meter section of rope from each rack and taking three sub-samples. One 3-4 cm section was cut randomly from within the: 1) top 10 cm; 2) middle 45 cm; and, 3) bottom 45 cm. This sampling method was used because the mussel spat are not evenly distributed along the section of rope and the top 10 cm has a greater density than the bottom 90 cm. The sample ropes were measured and all mussel juveniles were removed and counted from each sample section of rope to establish initial mussel density. Twenty mussels were selected at random and measured to establish a size frequency distribution.

Results

Analysis of variance on the mean number of cultured blue mussels per 1 mm length of rope from the two rope types (Table 1; Fig. 6) indicated that while 15% more mussels were ob-served to settle on the New Zealand fuzzy rope vs. the used lobster pot warp, this difference was not statistically significant (P=0.6679). In addition, no difference in size-frequency distribution was detected in mussels between rope types (G = 4.24, df = 6, P = 0.6444; Fig. 7).

These results suggested that costs associated with setting cultured blue mussel larvae in the hatchery can be minimized by using locally-provisioned, used lobster pot warp.

Table 1. Analysis of variance on mean number of cultured blue mussels per millimeter of rope from two rope types (used lobster warp vs. New Zealand fuzzy). Racks were considered a random factor. (n = 3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Rack	2	6.41227311	3.20613655	3.05	0.0849
Type	1	0.72068393	0.72068393	0.25	0.6679
Rack*Type	2	5.81386257	2.90693129	2.77	0.1028
Error	12	12.61113592	1.05092799		
Corrected Total	17	25.55795553			

Figure 3. Cultured blue mussels racks across two types of rope (left = New Zealand fuzzy; right = used lobster warp). Ropes are wrapped around a PVC frame that is approximately 1-m tall and 1-m wide. Tanks hold 3000 L. Photo does not represent the design used in 2018, and was taken in spring 2019.

Figure 4. Mass of cultured blue mussels on the New Zealand fuzzy rope.

Figure 5. Cultured mussel spat on used lobster warp.

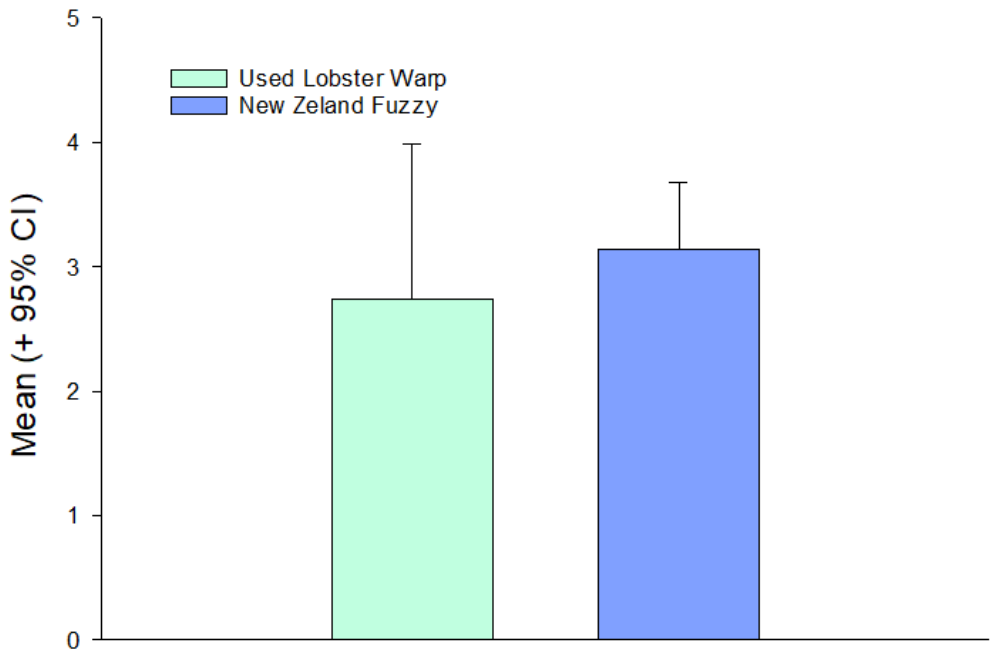

Figure 6. Mean number ± 95% CI of cultured blue mussels per 1 mm of rope of two types. Means are pooled across racks (n = 9). Approximately 15% more mussels settled and grew on the New Zealand fuzzy rope than the used lobster warp; however, this difference was not significantly different (P = 0.668; Table 1).

The mussel ropes were deployed on Blue Hill Bay Mussel's lease site in South Blue Hill on 17 April 2019. The ropes were removed from the racks and hung in shallow loops off a horizontal mainline where they remained until June of 2020. At that time, and without informing Downeast Institute staff, the farmer stripped the seed from the ropes and transferred them to his grow-out rafts. Without having had an opportunity to sample the ropes to determine final density it was not possible to evaluate which rope type yielded better retention rates. We did learn from the farmer, however, that the mussels on each rope type were of equal quality and size, and it was easier and less time-consuming to strip the mussel seed from the local lobster pot warp.

Additional trial

Methods and Materials

A second settlement trial was initiated on 19 March 2019 to test settlement rates of blue mussel larvae given a choice between two settlement substrates: New Zealand fuzzy rope vs. local, used lobster pot warp. Fifty meters of each rope type was wound onto PVC racks with nine replicate racks of each rope type. Three days prior to initiation, ropes were placed into 3000 L tanks with filtered seawater and sterilized overnight in a light bleach solution. The following day, the bleach water was neutralized, and a single tank filled with filtered seawater. Ropes were sprayed vigorously with freshwater, and then the 18 racks

of rope were placed into the tank that was filled with filtered seawater and aerated where they remained for two days to allow for biofilm formation.

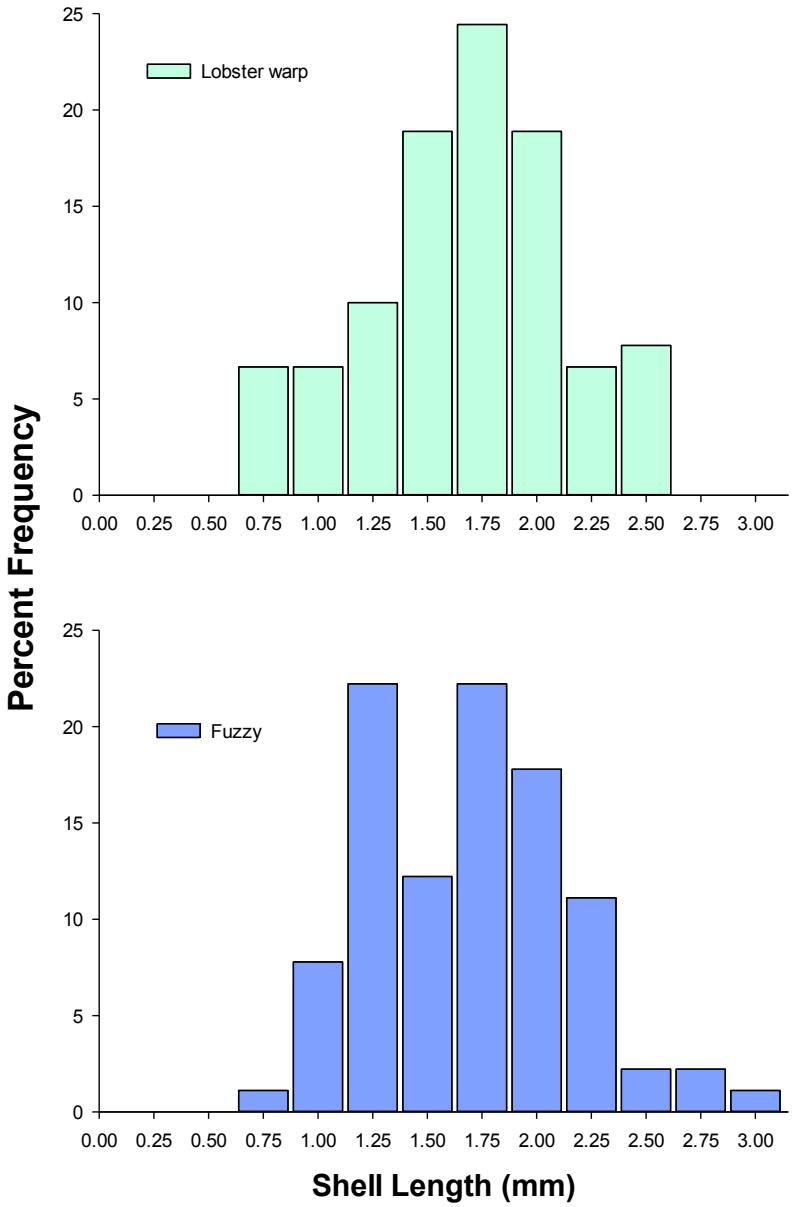

Figure 7. Size-frequency distribution of cultured blue mussels settling on two different types of rope substrates. A 2 x 7 G-test of independence demonstrated no significant different in the size distributions between the two rope types (G = 4.24, df = 6, P = 0.6444).

Conditioned mussel broodstock were spawned on 27 February 2019, and the larvae reared for 14 days until the pediveliger stage. On 13 March 2020, the prepared racks were

moved into two clean 3000 L tanks with filtered seawater. One tank received the racks holding New Zealand rope and the other received the racks holding used lobster pot warp with an airstone between each rack (Figure 1). 9,000,000 competent pediveliger larvae were added to each tank. Larvae were fed a mixture of cultured phytoplankton once a day and the contents of both tanks drained every 72 hours until all larvae had metamorphosed. Three weeks after settlement, each tank received ambient, flow-through seawater, and the racks remained in the hatchery until spring when they were deployed at the nursery field site (Blue Hill Salt Pond) of Mr. Evan Young, owner of the mussel aquaculture company, Blue Hill Bay Mussel.

The ropes were deployed at the nursery field site on 17 April 2019, the same day they were removed from the flow-through tanks at the Downeast Institute. Sampling for initial density and size was carried out in the same manner described above. At that time, it was discovered that 100% mortality had occurred of mussels in the tank housing the New Zealand fuzzy rope. All used lobster pot warps were deployed at the farmer's lease site in the Salt Pond in Blue Hill, Maine.

To estimate mean density per 1 m of rope, mean number of mussels per sample of rope was multiplied by one of the two lengths (10 cm or 45 cm). For example, suppose that the two replicate mussel counts from the top 10 cm of a section of rope were 37 (sample size = 37.1 mm) and 26 (sample size = 38.87 mm). The average number of mussels for this 10 cm section of rope would be (37/37.1 mm + 26/38.87 mm)/2 x 100 (converting mm to 10 cm) = 83.3 individuals. Similarly, suppose that the two replicate mussel counts from the first 45 cm section of rope were 22 (sample size = 34.63 mm) and 16 (sample size = 40.42 mm). The average number of mussels for this 45 cm section of rope would be (22/34.63 mm + 16/40.42 mm)/2 x 450 (converting to 45 cm) = 232.0 individuals. For the third 45 cm section, suppose the two replicate mussel counts were 13 (sample size = 41.99 mm) and 16 (sample size = 38.62 mm). The average for this section would be 162.9 individuals. The estimate for number of mussels for the 1 m section of rope would be 83.3 + 232.0 + 162.9 = 478.2 individuals. Since 50 m of rope was wrapped around a single PVC frame (i.e., rack), then the estimate for the number of mussels to begin with on that 50 m would be 478.2 x 50 = 23,910 individuals. The cohort produced a mean (± 95% CI) number of cultured blue mussel juveniles per rack of 38,411.6 ± 22,104.9 (n = 5).

Ten racks containing cultured mussels that had been settled on the used lobster pot warp and deployed at the Salt Pond in South Blue Hill Bay on 17 April 2019 were sampled on 24 October 2019. The combined mass of seed was divided into small (8.6-42.6 mm; mean SL = 25.8 ± 6.0 mm, n = 100; 227 kg) and large (18.8-55.2 mm; 44.7 ± 4.6 mm, n = 99; 401kg). The latter were taken to a growout lease site in Blue Hill Bay, and will produce an estimated 2,739 kg (6,026 lbs, or 3.01 tons) of saleable product when removed from the water early in 2021.

C. ***Field trials at commercial field sites to test the efficacy of hatchery production methods, the effectiveness of settlement substrates to retain juvenile mussels, and the release of cultured ropes to farms at multiple times throughout the year.***

Objective #3: To examine the efficacy of hatchery production methods by testing the long-term viability of mussel spat from two origins: a) broodstock that are naturally-conditioned and reared directly and, b) broodstock that are naturally-conditioned, but larvae are cryopreserved.

Question #3: Which hatchery production method results in the lowest drop-off rate and/or fastest growth rate of blue mussel spat at the farm sites?

Since we were unable to produce viable larvae that had been cryopreserved, our tests were conducted on mussel spat from naturally-conditioned broodstock.

Objective #4: To determine if differences exist in growth and retention rates of juveniles among settlement substrates, and whether drop-off rates for a given settlement substrate vary among the three farm sites.

***Question #4*:** Which settlement substrate results in the lowest drop-off rate and/or fastest growth rate of blue mussel spat at the farm sites?

Methods

In 2018, hatchery seeded rope was deployed at three farm sites in Maine (Fig. 8), all with very different characteristics and farming strategies. The easternmost farm, owned and operated by Moosabec Mussel, was located in Trenton at the mouth of the Jordan River. This is a shallow water site (< 1 meter of seawater at low tide) that is exposed to southwest winds. This site employed horizontal longlines with very shallow (<0.75 meters) loops of seeded rope.

The next farm, owned and operated by Blue Hill Bay Mussels, was approximately 30 km west of Trenton in South Blue Hill, Maine. This site had water depths from 4-7 meters at low tide and was very sheltered. Horizontal loglines were used to suspend the hatchery rope with two meter loops placed every 0.75 meters.

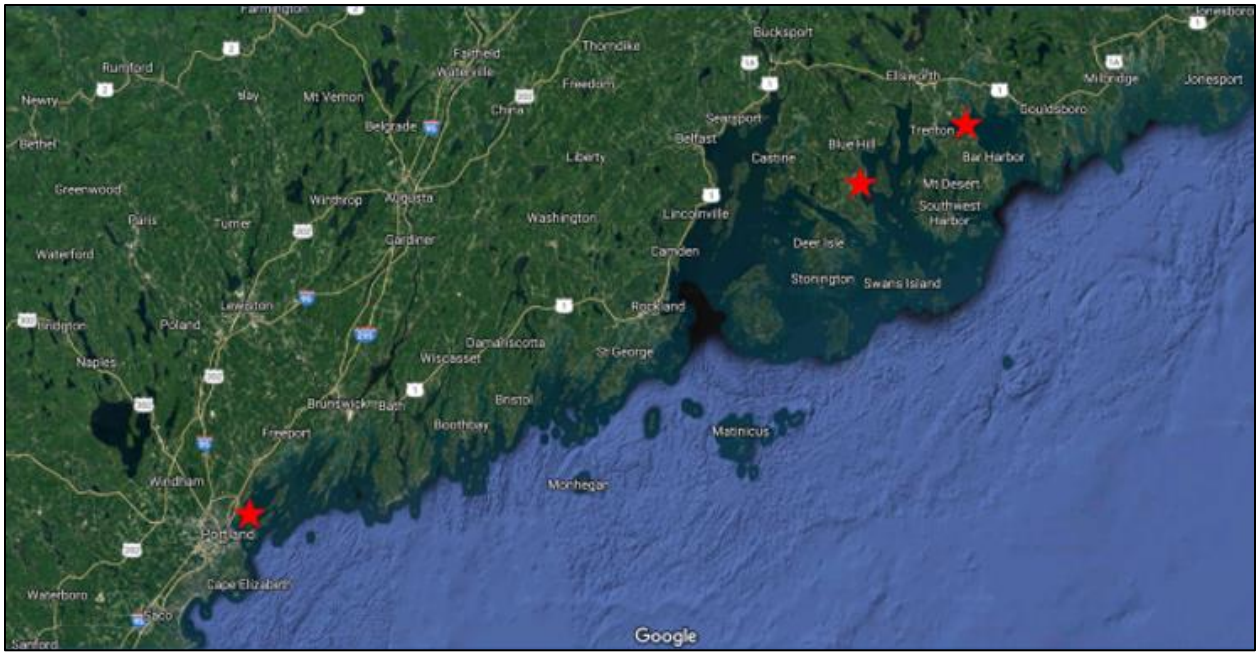

Figure 8. Farm sites in Trenton (Jordan River), South Blue Hill (Salt Pond), and in Portland (Casco Bay) where hatchery-seeded ropes were deployed on each of three occasions between March and May 2018.

The western most site, owned and operated by Calendar Island Mussels, was located in Casco Bay, Portland, Maine. This site had water depths of 10 meters at low tide and was very exposed from the southwest. 40-ft x40-ft steel mussel rafts were used to suspend the hatchery ropes with 10 meter loops attached to horizontal timbers.

Hatchery seeded ropes were deployed at each site on three different occasions March through May in 2018. Seed on ropes deployed at the easternmost (Trenton – Jordan River) and western-most (Portland – Casco Bay) sites suffered nearly 100% drop off likely due to interaction of seeded ropes with the bottom (Trenton) and exposure to high seas (Portland). Ropes deployed at the site in South Blue Hill had excellent retention of hatchery seed likely due to how sheltered the site was from weather conditions.

Seed produced for deployment was set on used lobster pot warp. Deployment dates and amount of rope were as follows: 31 March – 100 meters; 15 April – 150 meters; 15 May – 200 meters. Samples to determine initial density and size frequency were taken prior to deployment, as pre-viously described. No significant difference in initial density occurred between deployment dates (Fig. 9) between deployments. There was a significant difference ($P < 0.025$) in the initial mean shell length at deployment (Fig. 10) with the April 15th deployment having a smaller mean shell length compared to the other two deployments, although the difference was less than 0.5 mm.

Ropes were sampled for mussel growth periodically during the 2018 growing season (Fig. 11) by carefully lifting a section of rope to the water's surface and removing a small

amount (100-200) mussels at random. One hundred mussels were measured to the nearest 0.01 mm using digital calipers from each deployment on each sampling date. For example, mussels deployed on 31 March grew an average of 10 mm per month from July-August 2018 (Fig. 12).

Seed was removed from the ropes on August 29th and September 6th. The seed harvested was run through processing equipment operated by Blue Hill Bay Mussel to de-clump and clean seed prior to being socked into grow-out ropes. Total weight of seed harvested from each deployment was recorded and 10 sub-samples taken randomly from each deployment by removing approxi-mately 100 grams of mussel seed, weighing the sample, counting number of live mussels and measuring 20 individuals from a representative sample. The data enabled us to determine total number of mussels produced from each deployment of hatchery seeded ropes.

Results

Data presented below are from three sets of ropes that were deployed in the Salt Pond in South Blue Hill during the 2018 growing season, which was the only farm site that produced quanti-fiable data during and at the end of the growing season.

We observed a significant difference in final mussel density from the used lobster warp that was inversely related to deployment date (Fig. 13). Highest final density of mussels per linear meter of rope was from the final deployment (15 May), with lowest density from the first deployment (31 March). This trend was very similar to the mean initial density, which is a likely factor in the mean final density. Percent retention rates were calculated by comparing the initial density to the final density. This effort showed that the March 31st deployment had the lowest retention rate with 19.8% of mussels retained followed by the April 15th deployment with 24.2% retention and the best field retention observed in the May 15th deployment with 28.8% retention. Time of deployment is a likely factor in the retention rate of mussel seed on ropes with the earlier deployments having more time in the field and more time for mussels to fall off. Interestingly, only a slight difference was observed in mean final shell length between deployment dates. The first deployment (March 31st) yielded the largest mean shell length (32.8 ± 0.8 mm, n = 200). The mean shell length of mussels deployed on 15 April (30.1 ± 0.5 mm, n = 400) was not significantly different from the mean of mussels deployed on 15 May (30.8 ± 0.6 mm, n = 240; Fig. 14). The small difference in growth between deployments was likely due to cold weather early in spring that resulted in lower water temperatures at the time of deployment.

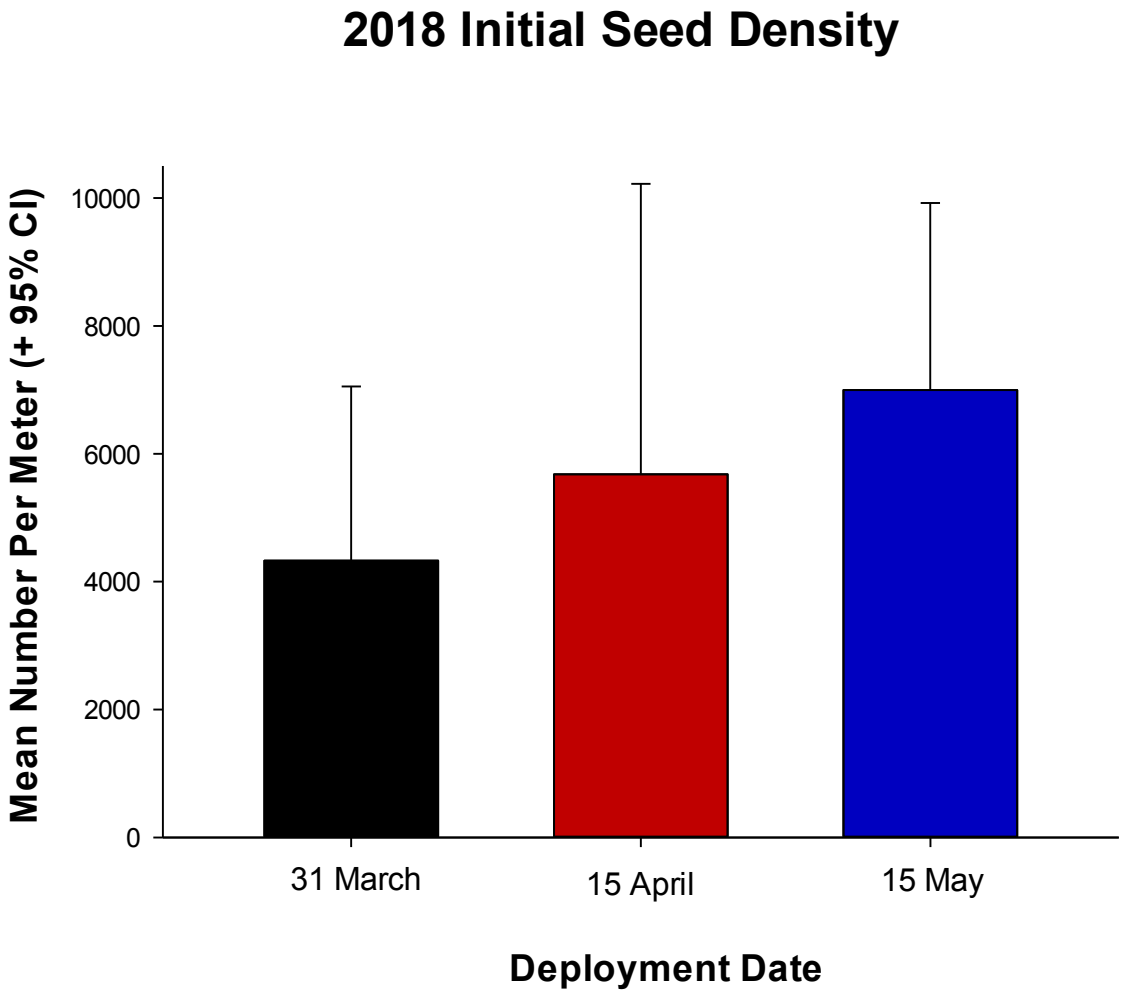

Figure 9. Mean (± 95% CI) initial cultured seed density on used lobster pot warp deployed in 2018 at the nursery field site at the Salt Pond in South Blue Hill, Maine. No significant difference in seed density occurred between deployment dates ($P > 0.15$).

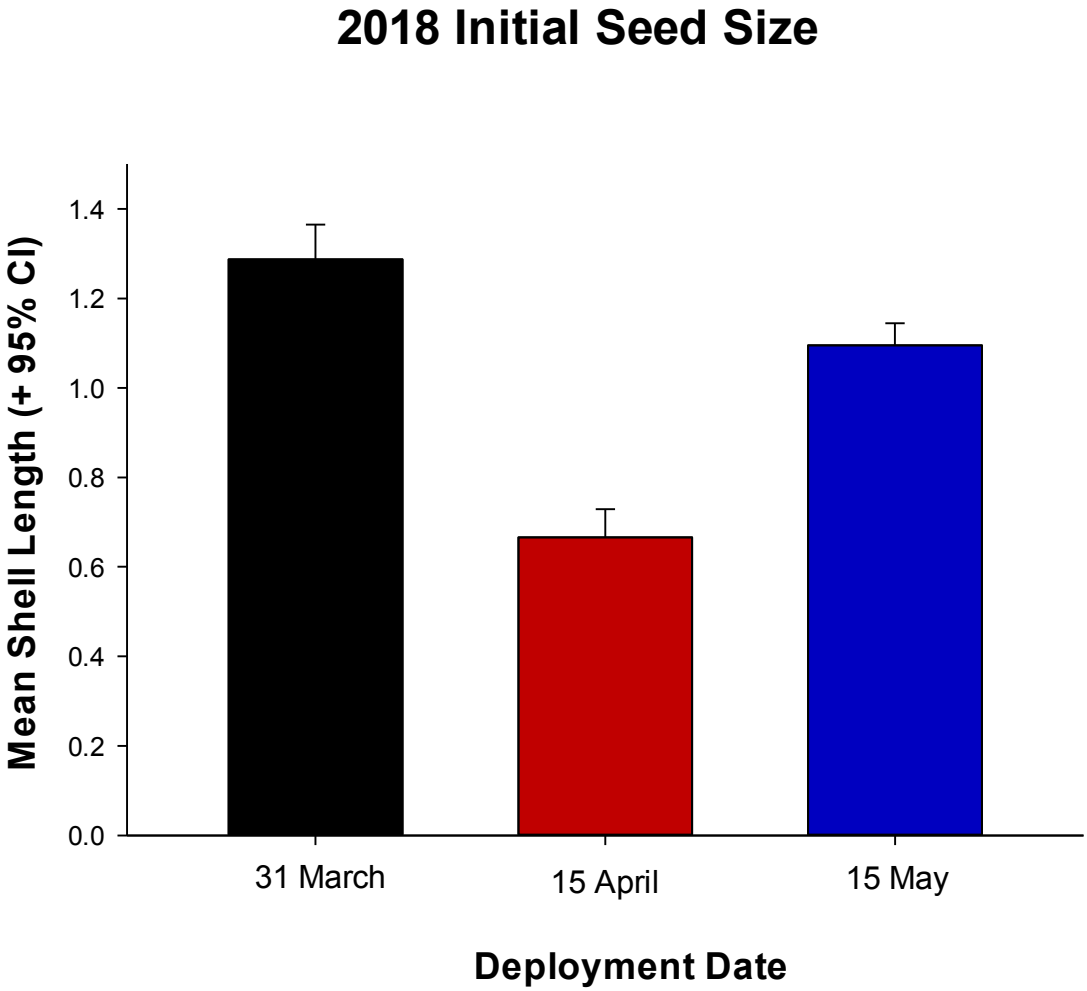

Figure 10. Initial mean shell length of cultured mussels on hatchery seeded ropes deployed in the Salt Pond at South Blue Hill in 2018.

Figure 11. Examples of hatchery-seeded ropes in the Salt Pond in South Blue Hill, Maine at the time of deployment in 2018, and on two sampling dates.

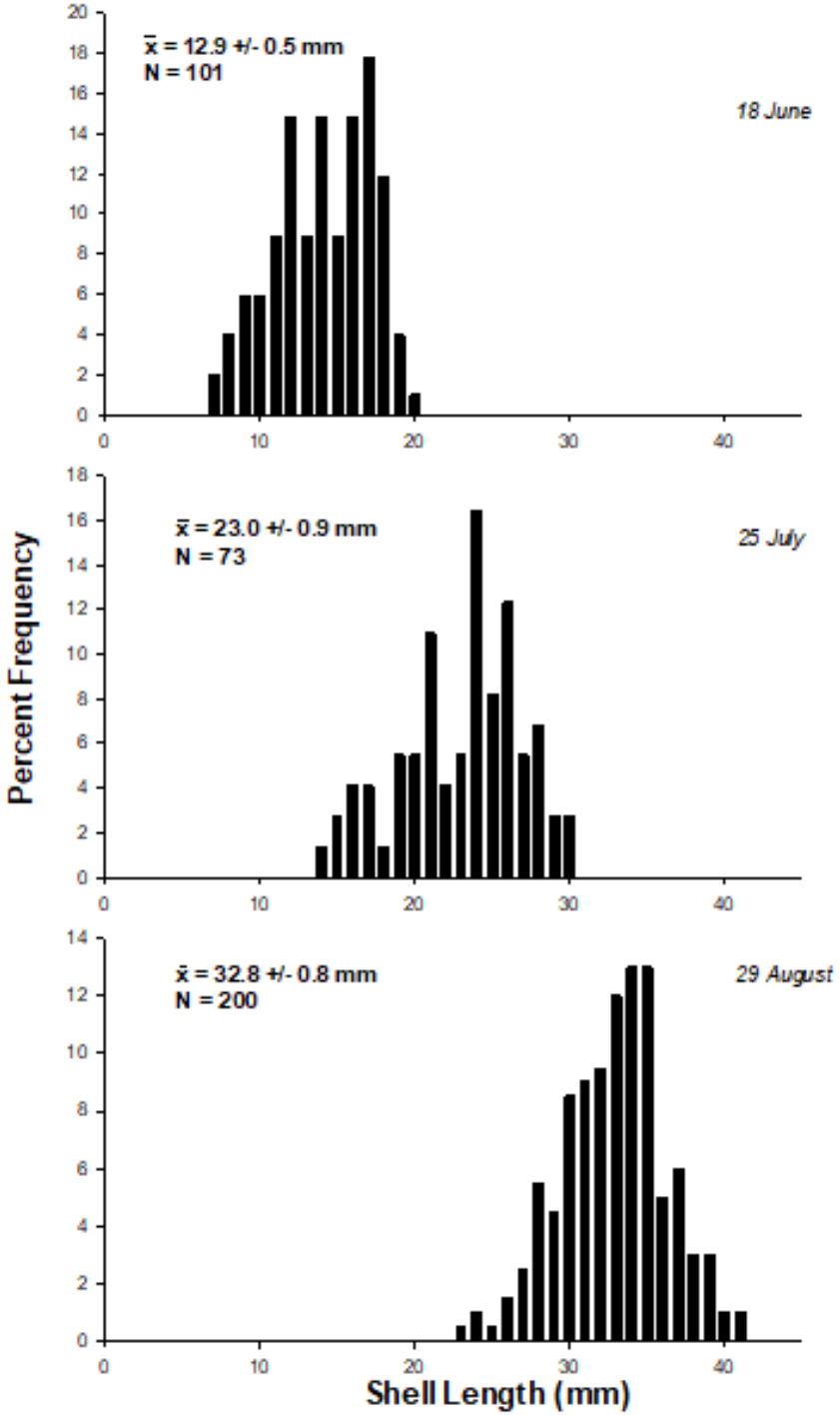

Figure 12. Size frequency distribution of cultured mussels on seeded ropes deployed at the Salt Pond in South Blue Hill on 31 March 2018. Ropes were sampled three times during the 2018 growing season.

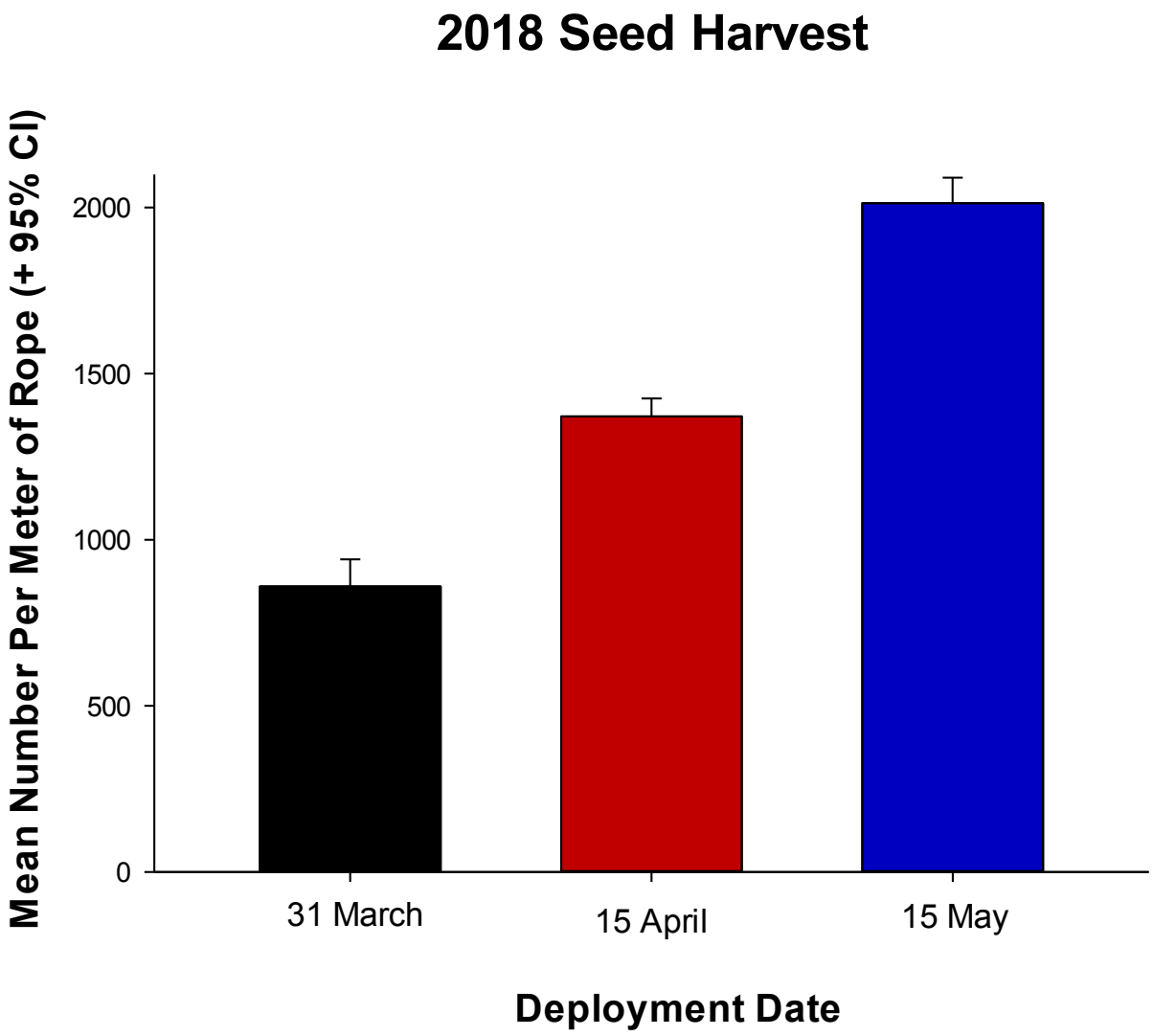

Figure 13. Mean number (± 95% CI) of mussel seed per meter of hatchery ropes when seed was harvested for socking (29 August and 6 September 2018).

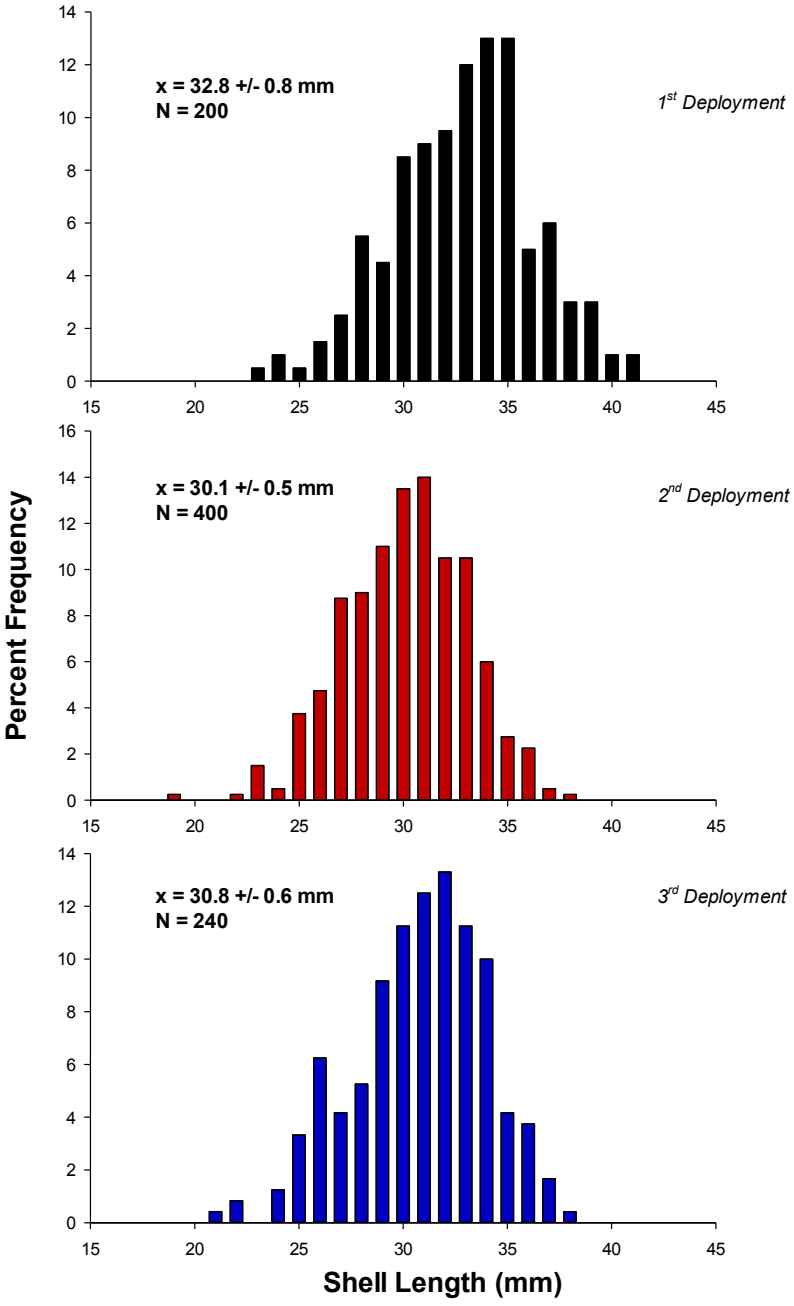

Figure 14. Size frequency distribution, at time of seed harvest, of three sets of ropes deployed at the Salt Pond in South Blue Hill, Maine during the 2018 growing season. Samples were taken in August and September 2018.

Objective #5: To determine whether working mussel farms would benefit from multiple cohorts of cultured seed in a single year.

Question #5: What is the fate and growth rate of multiple cohorts of mussel spat over a 9-month period at each farm site, and will the use of these cohorts increase annual farm production?

Two cohorts were produced for deployment at the nursery site in the Salt Pond in South Blue Hill during 2019. The fate of the first cohort (deployed on 17 April 2019) was reported (see above). A second cohort was produced from spawning wild conditioned animals on 10 June, and the larvae reared until 24 June, at which time they were competent to settle. Approximately 5,000,000 pediveligers were added to a 3,000 L tank housing 5 PVC racks, each holding 50 meters of used lobster pot warp. Using sampling methods described above, we estimated that each rack contained a mean number of individuals (± 95% CI) of 371,688.7 ± 130,823.8 (Fig. 15). This cohort was deployed at the nursery site in South Blue Hill on 8 July. Mussels were stripped from the ropes on 24 October 2019, and yielded 222 kg of small mussels ranging in shell length from 16.4-39.5 mm with a mean SL = 27.7 ± 4.5 mm (n = 100), and 90 kg of large mussels that ranged in SL from 18.2-43.9mm with a mean SL = 36.4 ± 4.5 mm (n = 40), that will ultimately yield and estimated 1,460 kg (3,212 lbs, or 1.61 tons) of saleable product.

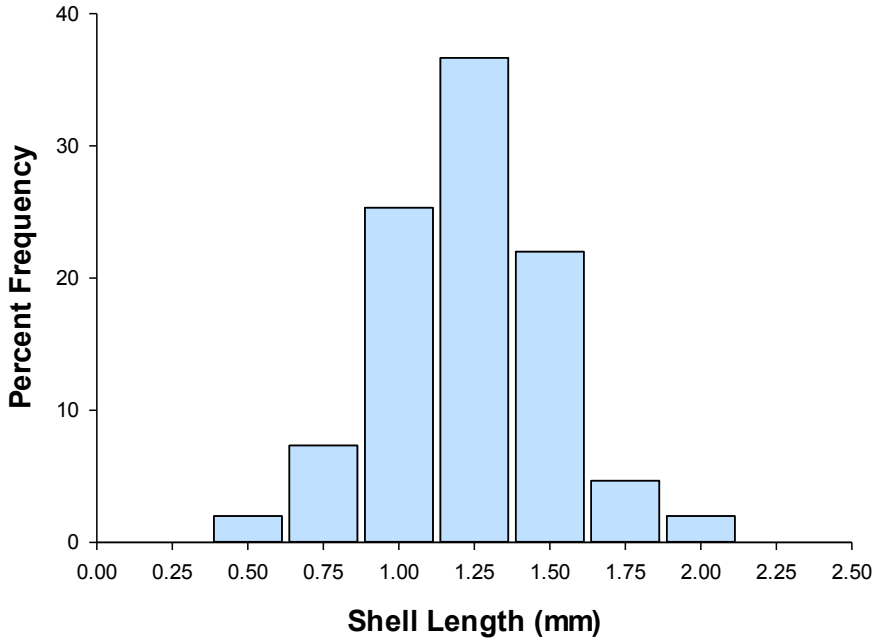

Figure 15. Initial size-frequency distribution of cultured blue mussels that were spawned on 10 June 2019 at the Downeast Institute, and deployed on racks containing used lobster pot warp at the Salt Pond in South Blue Hill on 8 July 2019. Mean shell length ± 95% CI = 1.2 ± 0.05 mm (n = 150).

During 2020, we produced 10 mussel cohorts from 10 separate spawnings each using conditioned broodstock. Each cohort comprised five PVC racks (Fig. 3) with 50 m of used lobster pot warp per rack (5 racks x 50 meters/rack x 10 cohorts = 2,500 m of rope, or 1.55 miles of rope). Estimated number of mussels per rack ranged from 50,000 to 800,000 (Fig. 16), and averaged 266,496.6 ± 50,010.8 individuals/ rack, which yields an estimated 13,324,830 mussel spat deployed at the Salt Pond in South Blue Hill of. Sizes of mussels at the time of deployment ranged from 0.25-3.9 mm (Fig. 17) with a mean shell length of 1.1 ± 0.02 mm (n = 3,016). Mussels from ropes randomly selected at the nursery site were sampled on 23 July 2020, and measured (as described above). This included Cohort 1 (deployed on 24 April 2020), Cohort 4 & 7 (deployed on 21 May 2020), and Cohort 11 (deployed on 18 June 2020). Means were significantly different from each other (ANOVA, F = 781.81, df = 2, 133) with those deployed the earliest nearly 100% larger than those deployed last (Fig. 18).

Beginning 29 September 2020, mussel seed that was deployed in the spring of 2020 was large enough to be stripped from the nursery ropes (Figs. 19-22) and socked (Fig. 23) to produce commercial grow-out ropes. DEI staff and Blue Hill Bay Mussel (BHBM) crew stripped 40 fish totes full of seed in South Blue Hill that were subsequently transferred to BHBM's grow-out raft adjacent to Hardwood Island in Frenchman's Bay. This process took several weeks to accomplish due to the massive volume of mussel seed and finding the appropriate weather conditions to work on the water. As the seed was stripped, totes were labeled to identify the specific cohort. The seed was then processed in preparation for socking using equipment owned by BHBM that included a brush de-clumper, de-bysser and grader. Seed from the cohorts deployed early in the year yielded two size classes ("small" and "large"), and the later cohorts having only small seed. Prior to socking onto grow-out ropes, the seed was weighted and ten subsamples (50-120 grams) were taken at random from each size class within a given cohort. The subsamples were weighed and the number of live mussels was recorded. Twenty mussels were selected at random from each sample and measured to create a size frequency distribution. The harvest of seed during Fall 2020 resulted in the harvest of 8,671 kg of mussel seed (19,076 pounds, or 9.54 tons) which is expected to produce 80,000 to 100,000 pounds of market mussels in 2021. Approximately 12% of the seed was categorized as large (SL range = 13.8-54.9 mm; mean = 41.8 ± 0.2 mm, n = 1,462), and the remaining as small (SL range = 10.5-51.2 mm; mean = 31.5 ± 0.3 mm, n = 2,527; Fig. 24). This additional volume of mussels, which are much larger than wild mussels of the same year class, will help farmers stagger their harvest, adding security to their business.

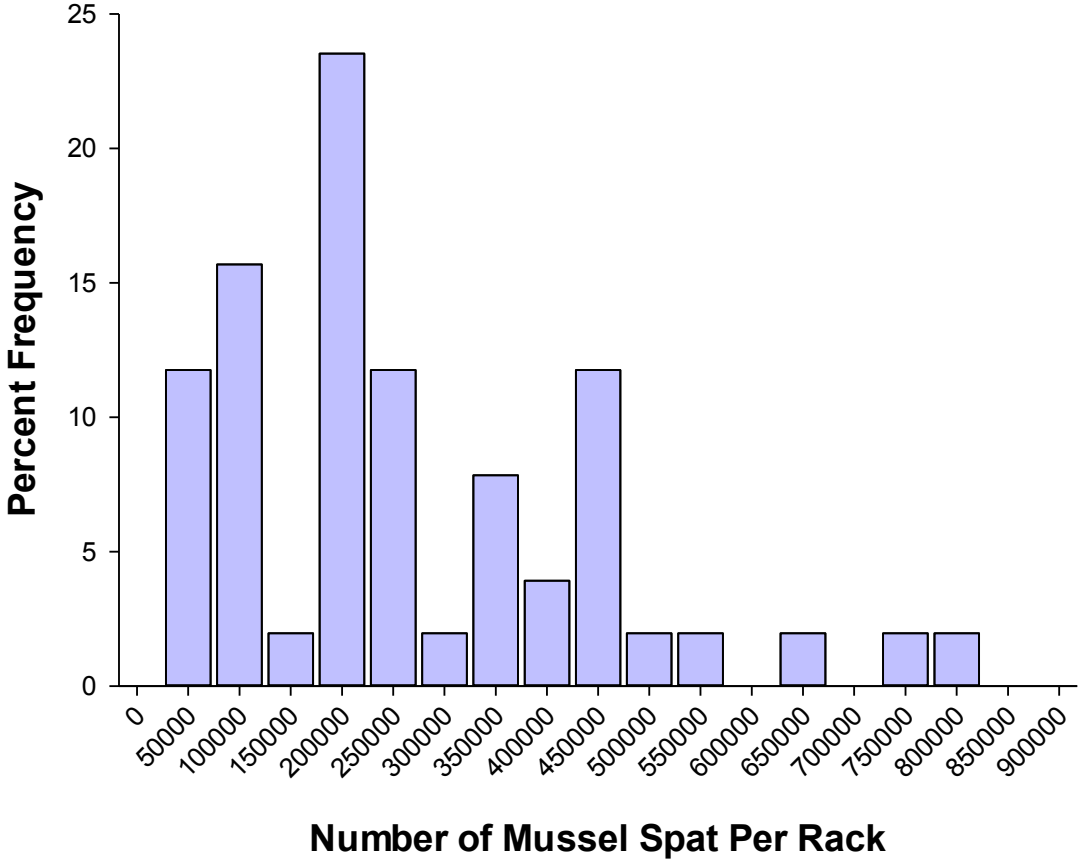

Figure 16. Estimated number of cultured mussel seed on 50 meters of rope (= 1 rack) for each of 51 racks produced during 2020 and deployed to the Salt Pond in South Blue Hill, Maine.

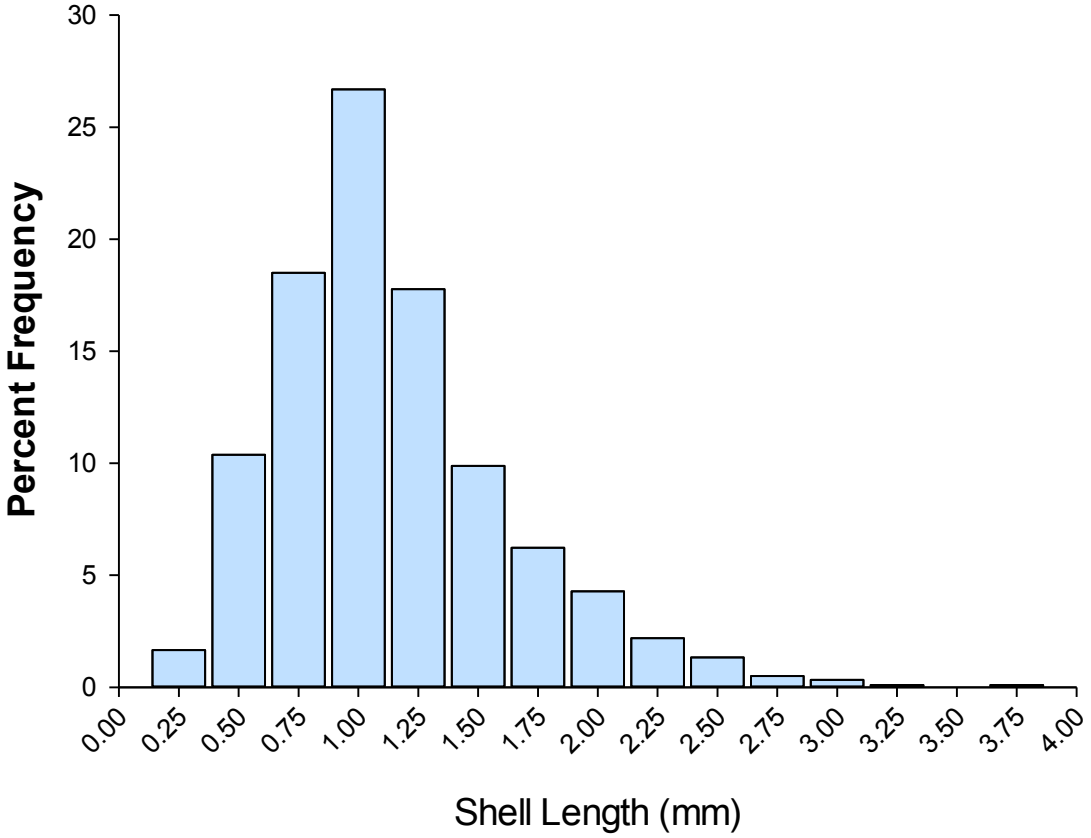

Figure 17. Size-frequency distribution of mussel spat on used lobster pot warp that was produced at the Downeast Institute during 2020, and subsequently deployed at a mussel lease site in the Salt Pond in South Blue Hill, Maine.

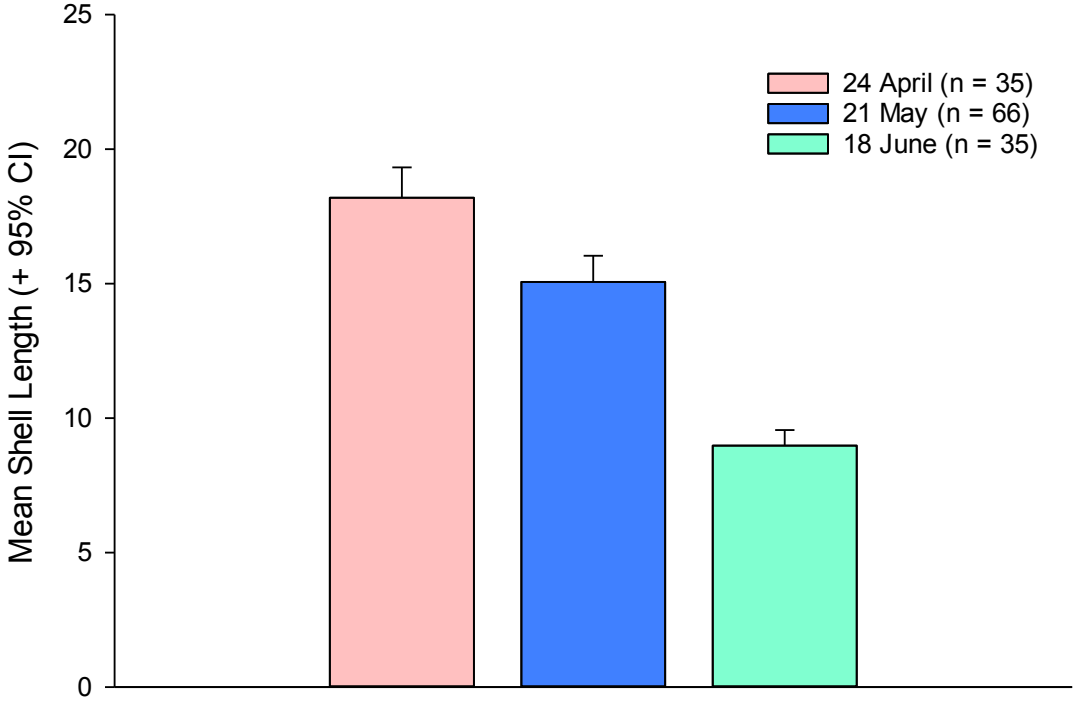

Figure 18. Mean shell length of cultured blue mussel juveniles settled on used lobster, and then deployed on three dates at the Salt Pond in South Blue Hill, Maine. Sampling occurred on 23 July 2020. Each mean is significantly different ($P < 0.05$) using ANOVA and a posteriori testing (Scheffe) that controls the Type I experimentwise error rate.

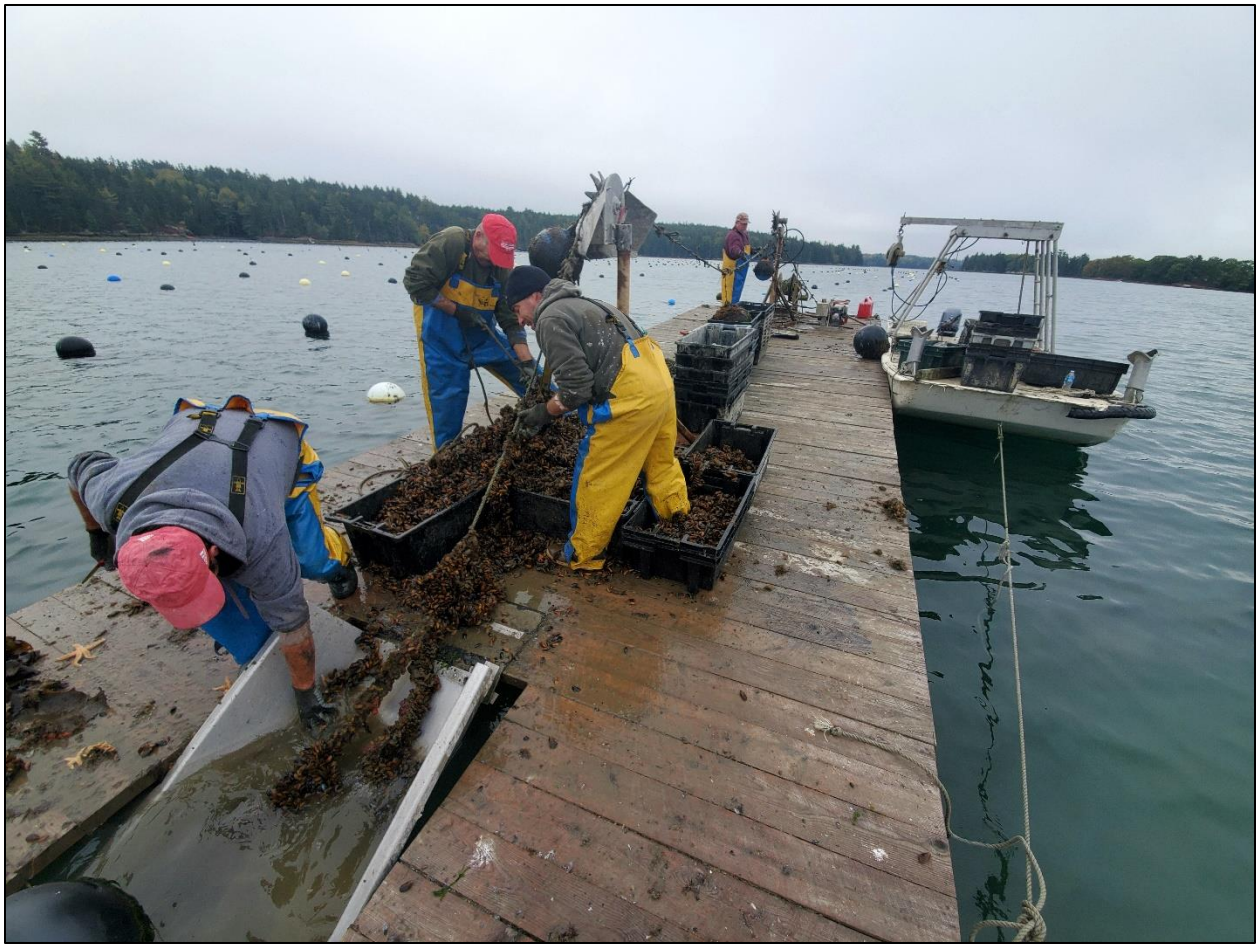

Figure 19. Stripping hatchery-seeded ropes at the Salt Pond in South Blue Hill during Fall 2020.

Figure 20. Example of a seeded rope that was deployed in the Salt Pond in South Blue Hill in April 2020, and is being stripped on 29 September 2020 in preparation for socking and re-deployment to a growout lease site in Frenchman's Bay.

Figure 21. Fish totes filled with seed mussels that are being held prior to socking at a mussel lease site in Frenchman's Bay.

Figure 22. Mussel de-clumper and size-sorting of seed mussels in preparation for socking and deployment at a commercial lease site in Frenchman's Bay (Fall 2020).

Figure 23. Cultured mussel seed that has been sorted by size being prepared for socking at a mussel lease site in Frenchman's Bay during Fall 2020.

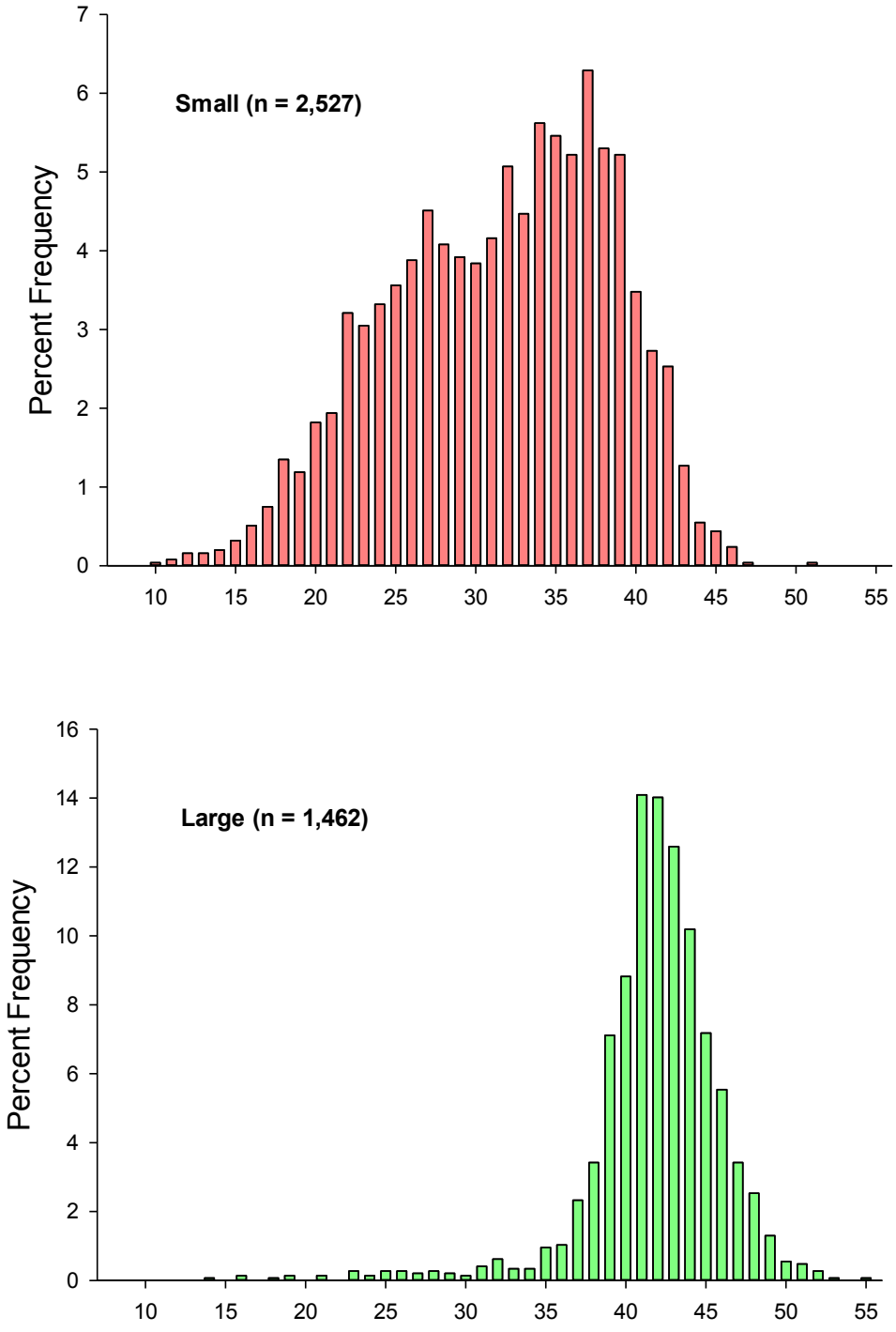

Figure 24. Size-frequency distribution of mussel juveniles that were deployed on used lobster pot warp at the Salt Pond in South Blue Hill (24 April - 20 July 2020), and sampled (29 September - 9 November 2020). All mussels were large enough to be socked and

transferred to a commercial growout site in Frenchman's Bay where it is anticipated that between 80,000 to 100,000 pounds of market mussels in 2021.

D. Examining the efficacy of remote setting platforms for mussel larvae

Objective #6: To determine the effectiveness of remote vs. traditional setting methods to decrease costs associated with production of cultured mussel seed.

Methods and Materials

Two trials were initiated in 2018 at the Salt Pond nursery site of Blue Hill Bay Mussel in South Blue Hill. A 1,500 L tank was set up adjacent to the BHBM pier with flowing seawater and aeration. The tank was filled with ambient seawater and lightly aerated. 10 liters of cultured algae was added to the tank along with 50 meters of used lobster rope. One million competent pediveliger larvae (10 days old) were transferred from DEI's facility in Beals to Blue Hill in a 4-liter container of filtered seawater. Larvae were introduced to the tank and allowed to settle. After 72 hours, during which time most larvae in the tank had settled onto the rope substrate, the tank was switched from a static system to flow-through for one week. The section of rope was then removed from the tank and deployed in shallow loops attached to a horizontal long line on the BHBM lease. Ropes were inspected two months later and very few mussels were observed at that time. We hypothesized that the larvae either were harmed in transit, perished in the remote settlement tank, or the flow-through was initiated too soon and the larvae were flushed out of the tank.

A second remote settlement trial was initiated in June 2020 using the same system described above. For this trial, the settlement tank was only half-filled with ambient seawater and 150 meters of rope was used (Figure 25). Three million competent pediveliger larvae were transported out of water to Blue Hill in a small pouch made of 150 micron Nitex mesh (Figure 26) that was placed in a cooler during transport from Beals. Larvae were added to the tank and started swimming immediately upon going into the water and allowed to settle. After 72 hours, the tank was filled with ambient seawater. After another 72 hours had elapsed (6 days after larvae were added to the tank), the tank was placed on flow-through seawater. After a week, the ropes were transported to the BHBM lease and suspended on a horizontal long line. The rope was inspected in October of 2020 and a large number of mussels were observed (Figure 27). It is evident that the mussels on the rope were not from wild recruitment because of the high incidence of gold mussels present on the rope, a unique phenotype that has been selected for by DEI shellfish hatchery staff and technicians. It is anticipated that mussels will be large enough to be stripped from the rope and socked in December 2020.

Figure 25. Remote settlement tank (June 2020) with lobster rope and approximately 3 million mussel larvae.

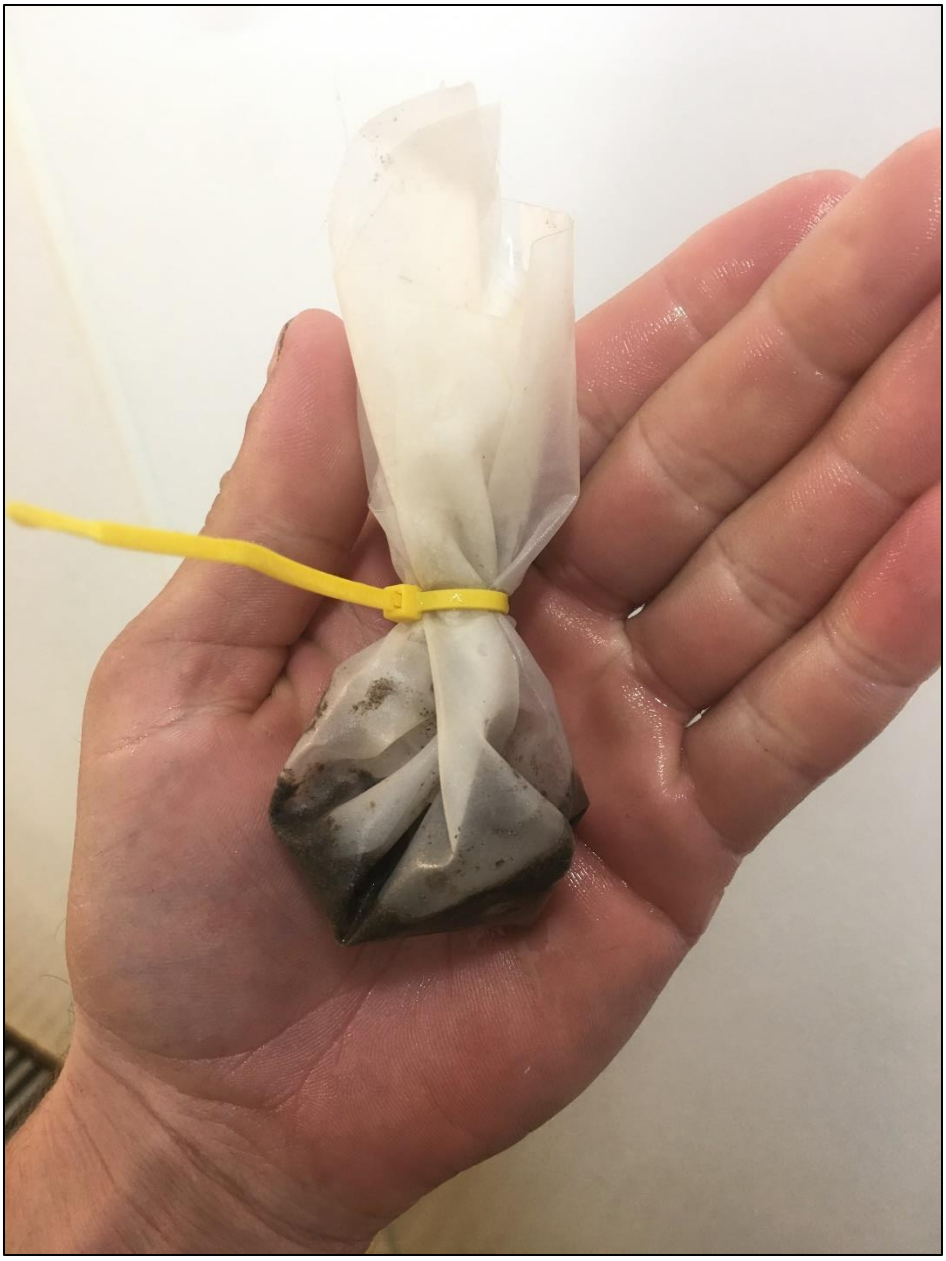

Figure 26. Packet of mussel larvae ready to be transported to Blue Hill for remote settlement trial held in a 150-micron Nitex screen.

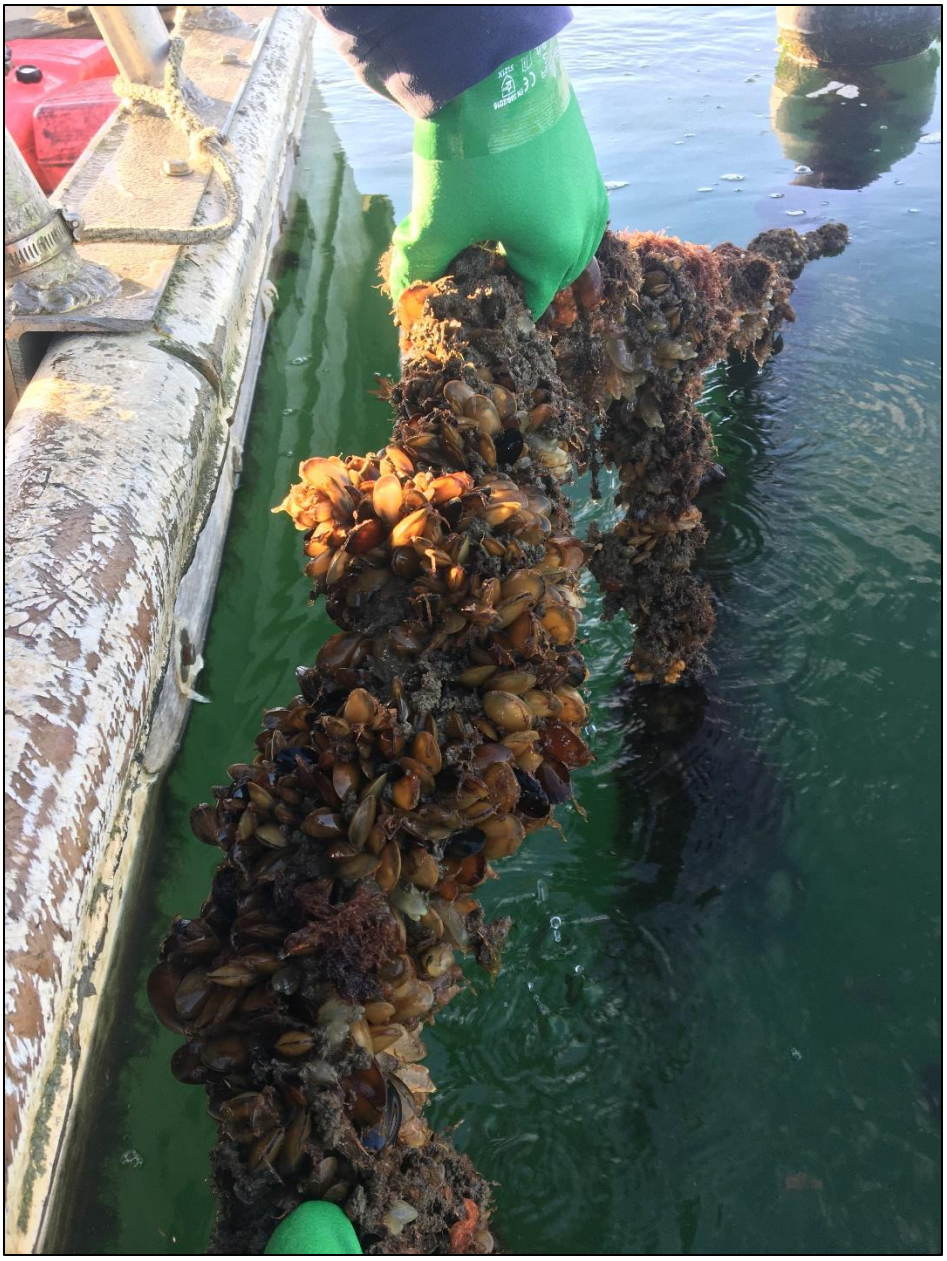

Figure 27. Remotely settled mussels on used lobster warp in October 2020. Note the prevalence of gold mussel seed.

D. Technology transfer plans

Objective #7: To develop a formal educational curriculum for mussel farmers to use to increase commercial production of farmed mussels.

Due to the COVID-19 pandemic, our plans to create an educational curriculum that was to accompany a 2-day live meeting held at the Downeast Institute were postponed, and we will recommence working on this objective during 2021-2022.

Figure 28. F3-generation "gold-striped" blue mussels.

References

Alfaro, A.C., Jeffs, A.G. 2003. Variability in mussel settlement on suspended ropes placed at Ahipara Bay, Northland, New Zealand Aquaculture, 216, 115– 126.

Parades, E., Adams, S.L., Tervit, H.R., Smith, J.F., McGowan, L.T., Gale, S.L., Morrish, J.R., Watts, E. 2012. Cryopreservation of Greenshell™ mussel (*Perna canaliculus*) trocophore larvae. Cryobiology 65, 256-262.

Protopopescu, G.C., Beal, B.F. 2015. Settlement response to various rope substrates in blue mussels (*Mytilus edulis* Linnaeus) in a hatchery setting. J. Shellfish Res. 34, 383-391.

Wang, H., Li, X., Wang, M., Clarke, S., Gluis, M., Zhang, Z. 2011. Effects of larval cryopreservation on subsequent development of the blue mussel, *Mytilus galloprovincialis* Lamarck. Aquaculture Res. 42, 1816-1823.