NRAC FINAL PROGRESS REPORT

Project Title	**Hatchery and Nursery Technologies for the Production of Blue Mussels**
Reporting Period	August 1, 2016 to October 30, 2017
Author (Project Coordinator)	Paul Rawson
Key Words	Mussel Aquaculture, Seed Production, Diversification
Funding Level	$46,419.
Participants	Paul Rawson, University of Maine; PI Dana Morse, Maine Sea Grant
Project Objectives	**Objective 1:** Demonstrate that mussels can be conditioned and spawned *contra-seasonally* in the fall. **Objective 2:** Determine the cost-effectiveness of standard live microalgal diets vs. new alternative diets (freeze-dried algae and algal paste) for conditioning blue mussel broodstock. **Objective 3:** Evaluate the suitability and cost effectiveness of alternative diets for setting mussel spat using new alternative diets (freeze-dried and algal paste) and comparing them to live microalgae. **Objective 4:** Evaluate the effect of nursery sites for spat deployment (at 1.5 mm average length) on the growth, survival and yield of seed for growout. **Objective 5.** Determine the relative performance of hatchery-produced mussel seed and wild caught seed in field trials. **Objective 6:** Determine the cost-benefit of hatchery-based seed production (accounting for alternative diets, seed yield and performance) compared to wild seed, including savings afforded by more efficient production cycles and potential market opportunity associated with favorable traits.
Anticipated Benefits	The direct beneficiaries of this research has been the fishermen/farmers in New England who have taken steps to established mussel farms and need a reliable source of mussel seed. The seafood-eating public, seafood processors, restaurants and retail outlets benefit from locally produced seafood. The measurable benefits are sustainable new enterprises conducting best management practices for locally-produced mussels.
Project Progress	**Objective 1:** Demonstrate that mussels can be conditioned and spawned *contra-seasonally* in the fall. During the reporting period we attempted 3 conditioning trials of blue mussels. On October 25th, 2016, we initiated conditioning of a group of 100 broods after mussels in natural populations had spawned out. Half of the broods were conditioned using a diatom-rich diet (LIVE) based on those used at the DownEast Institute. The second set of broods was

	conditioned using the macro-algal based Ori-One alternative feed from Skretting, Inc. Mussels in both treatments were fed at a daily ration of 4% (dry weight of food equivalent to 4% of estimated dry weight of broodstock) and gonad index (GI; relative wet weight of gonad compared to total mussel wet weight) was monitored weekly in a subset of 4-5 mussels from each feed type. After one week of conditioning GI for LIVE feed mussels had improved to from ~12 to 20%, but GI for the Ori-one conditioned mussels remained low, ~12-15%. After one month of conditioning, the GI of all conditioned broods was still <20%. On December 15th, after nearly seven weeks of conditioning, we tried spawning both sets of conditioned broods and a set of control mussels using a standard temperature-shock protocol. When mussels are naturally very ripe, this protocol usually results in a high proportion of broods (70-80%) spawning within two-three cycles of the water being warmed >5˚C above ambient for a short period followed by a return of the mussels to ambient temperature water. We applied 6 cycles of temperature shock to the broods, but no mussels spawned. These results are consistent with the low proportion of spawning mussels we obtained when conditioning broods in the fall of 2015 (10-40%), and mirror the experience at the DownEast Institute. Given that mussels require a period of quiescence to begin rebuilding the gonad after summer spawning, it is perhaps not surprising that fall conditioning has not been successful. Our expectation is that we should be able to condition mussel broods for spawning within 3-4 weeks; otherwise conditioning becomes time and cost prohibitive. ***Thus, the need for protracted conditioning and the low success rate for fall conditioning precludes conducting hatchery spawns for mussels in the late fall.*** **Objective 2:** Determine the cost-effectiveness of standard live microalgal diets vs. new alternative diets (freeze-dried algae and algal paste) for conditioning blue mussel broodstock. We conducted two winter conditioning trials to test effectiveness of broodstock diet. In each, we compared the efficacy of feeding mussel broodstock a diet of a commercially feed, Ori-One, fed at a ration of 4% of mussel dry weight per day to a diet of 100% LIVE algae consisting of a mix of *Isochrysis* (T-iso) or *Monochrysis*, *Chaetocerus calcitrans, C. muelleri, Rhodomonas*, and *Tetraselmis.*

The first trial was initiated January 31st using wild collected mussels. GI increased steadily over the first two weeks in the LIVE feed broods until it was almost double the GI of the initial control mussels (Fig. 1). Despite an initial increase in the Ori-One bro ods, the average GI in this group never exceeded 20%. During an unplanned change in personnel in the hatchery, there was an apparent spawning event in the live feed tanks that led to a steady decline in GI. Direct observations indicated that the gonads in the live feed broods contained mostly refractory material and few viable gametes. Given this event, the conditioning trial was abandoned on March 8th.

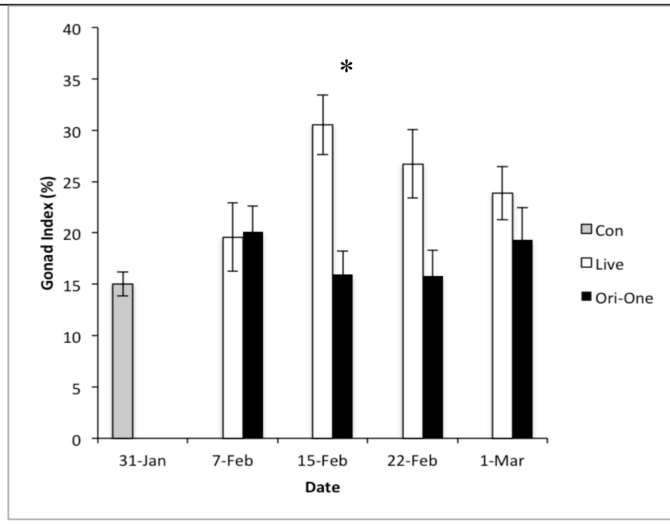


Fig. 1. Gonad index in live (white bars) and Ori-one broods fed at a 4% ration from January 31 to March 1. Asterisk marks the approximate data of spawning event in the live brood tanks.

A second trial with 200 mussel broods was initiated on March 13, 2016. A set of 50 control mussels was held on flow-through water at ambient conditions (6-8°C, 30 ppt) while the other 4 groups were gradual acclimated to 15°C, 30 ppt over a seven day period. Two of the groups were fed the same 4% ration of Live Food and 2 were fed the same 4% ration of Ori-One as in our previous conditioning attempts. GI in the Control broods at the start of the second conditioning experiment were slightly higher than in the first experiment of 2017 (Fig. 2). After one week of conditioning both the

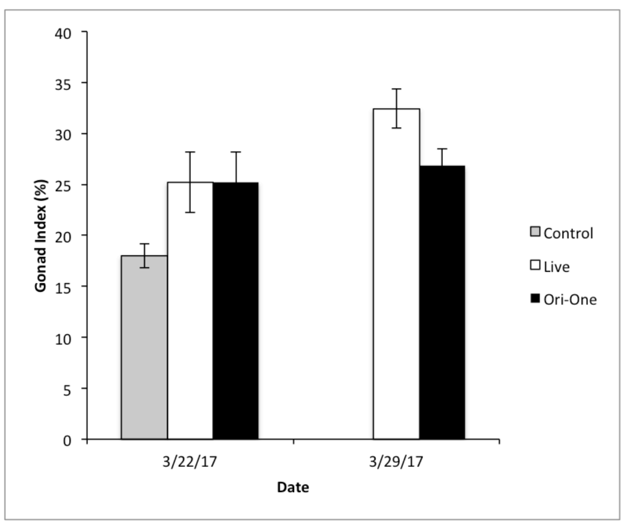


Fig. 2. Changes in gonad index for blue mussel broods fed a 4% ration of Live algae or Ori-One. Bars represent means ± one standard error.

LIVE feed and Ori-One broods had gained ~ 8% GI. By the end of the second week of full conditioning the mean GI in LIVE Feed broods was >30% with some individuals approaching 40%. Although some of the Ori-One broods had GI in excess of 30%, on average mussels in this treatment had gained little from the previous week. Part of the problem with Ori-one is that while it is accessible nutrition to bivalves it is hard to keep in suspension without vigorous aeration. This creates protein foam on the surface of the conditioning tank and the excess aeration can cause premature spawning. ***Thus, hatchery personnel dislike working with the Ori-one even in comparison to having to grow lots of live algae for the diatom-rich diets.***

Conditioning was terminated on April 1, 2016. After several cycles of "thermal stimulus", 23 female and 12 male mussels spawned. Eggs and sperm were obtained separately so that we could sample unfertilized eggs from each female for individual-based analyses (see below). Overall, spawning success was low (Fig. 3). Pronger et al. (2007) obtained over 60% spawning success in *M. edulis* conditioned on flagellates or a mix of flagellates and diatoms. We saw a maximum of 40% among control animals with a lower percentage of broods on LIVE feed spawning (~35%). The lowest spawning success was obtained in the Ori-One broods (20%). Among the LIVE feed broods, the proportion of female spawners was only slightly higher than in the Controls. These results suggest that, despite improvement in GI, our attempts to condition mussels actually brought about a decline in the proportion of mussels spawning activity. This is certainly true for the Ori-One broods where we also saw little improvement in GI over the two-week conditioning period.

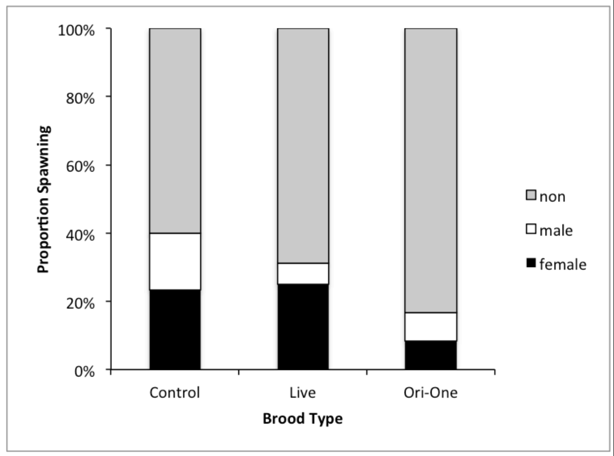
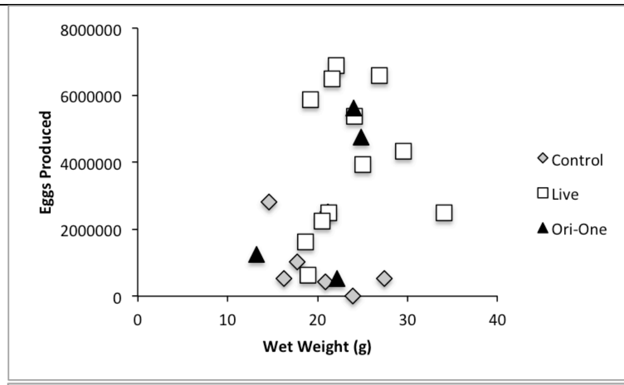


Fig. 3. Proportion of individuals that were spawning females (dark section of each bar), spawning males (white) or non-spawners (grey) in each of three treatments.

On the other hand, conditioning improved fecundity or egg production among the females that spawned. Overall, fecundity among females in all treatment groups was associated with wet weight (Fig. 4, top); the regression for these two variables was highly significant (P<0.001) although the R^2 for the model was only 0.08. A comparison of size-standardized egg production indicates that LIVE feed broods produced the highest number of eggs per female (Fig. 4, bottom). The roughly 4 million eggs per female among this treatment group rivals the results presented by Pronger et al. (2007) and is over 3-fold higher than the egg production obtained from the Control broods. Although few Ori-One broods s pawned, the female spawners in this treatment produced almost as many eggs as the LIVE feed broods. Our interpretation of these results is that conditioning is important to improving egg productivity or fecundity. We also feel that a longer conditioning period would likely have improved spawning success, as well, although our fear of another uncontrolled spawn led us to terminate conditioning early.



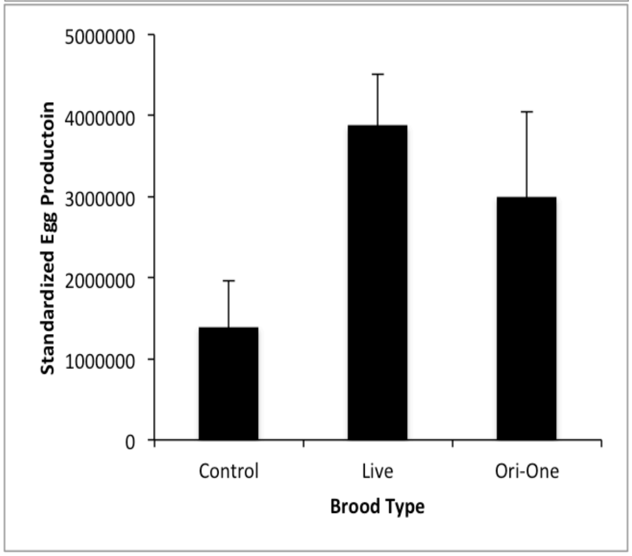


Fig. 4. Top - Total number of eggs produced by individual females as a function of wet weight and treatment. Regression analysis for fecundity of all females regardless of treatment indicated a significant but weak relationship between total eggs produced and wet weight. Bottom - The average number of eggs produced, standardized to a common wet weight of 22g for females in the three treatments. Error bars indicate the mean ± one s.e.

As part of our analysis we also tracked egg quality focusing primarily on differences in larval size among conditioning treatments. Generally, differences in larval size were subtle, but statistically significant. Approximately one week after fertilization, early veliger larvae from the Control broods were 8% smaller than the larvae from the LIVE feed and Ori-One broods (Fig. 5). The smaller size of the control larvae persisted throughout development. These results suggest that Control broods produced fewer eggs of lower quality.

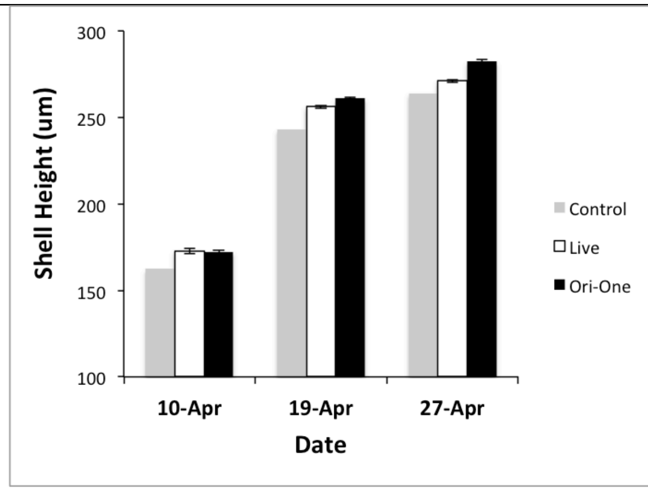


Fig. 5. Mean shell length (± s.e.) of veliger and pediveliger larvae belonging to each of our conditioning treatment groups.

Objective 3: Evaluate the suitability and cost effectiveness of alternative diets for setting mussel spat using new alternative diets (freeze-dried and algal paste) and comparing them to live micr oalgae.

Given our experience with Ori-one during the conditioning phase, above, we decided not to pursue this objective as original written. However, we conducted an alternative project in which we estimated the setting density and retention of mussel spat on two types of nursery rope.

We set mussel spat beginning on May 2, 2017, approximately 1 month post-sapwn. During setting, > 200,000 eyed larvae from the spawns of dark blue mussels were placed in each of six nursery tanks. Each tank was supplied with a 0.25 m^2 PVC frame; in three tanks the frame was covered with ~ 10 m of Portuguese rope and in the other three the frame was covered with ~8 m of "fuzzy" New Zealand rope. Each tank was supplied with vigorous aeration to discourage spat from setting on tanks sides and bottoms. On May 17th and June 28th we sampled ~3 cm segments of rope from near the top and bottom of the PVC frame as well as from a point midway between the top and bottom. Rope segments were stored in tubes containing 95% ethanol and spat counted on each using methods of Protopopescu (2014).

On May 17th, average spat density on the Portuguese rope (245/3cm) was higher than the estimated density on the New Zealand rope despite the greater surface area per unit length provided by the latter type of rope (Fig. 6). The heaviest settlement on the Portuguese rope appeared to have been on the middle of each rope while the greatest density of settlers on the New Zealand rope was on the bottom segments. These results need to be treated with caution for two reasons. First, although we took replicate samples from each height in each tank, early settlement appears to be very clumped and uneven. This is reflected in the high standard errors associated with the triplicate means from each tank by height combination. In addition, the New Zealand rope was very difficult to sample so that segment lengths were not uniform and once cut fibers and attached mussels came loose and were potentially lost (Fig. 7).

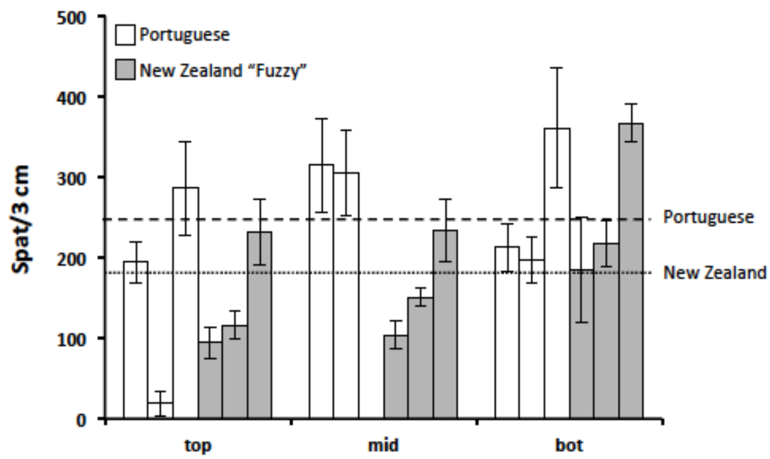


Figure 6. Mean (± one standard error) spat density for segments of rope taken at three different heights from the settlement ropes placed in three tanks with Portuguese rope (open bars) or New Zealand rope (grey bars) on May 17, 2017. The overall average densities on Portuguese and New Zealand rope are indicated by the wide dashed and dotted lines, respectively.

The unevenness of spat density persisted at the second sampling on June 28th. The highest number of spat per segment of rope was observed on a middle segment of New Zealand rope. At the second sampling, the mean density of spat was highest on the New Zealand rope, although estimated density on the Portuguese rope was >80% of that on the New Zealand rope. Our estimates suggest there was a shift in the distribution of mussels in each tank with higher densities in the top and middle segments and decreasing densities on the bottom segments, relative to the May sampling.

We used the estimated density of mussel set on each of two rope types at each sampling to estimate the total set per tank and setting success (see table, below). The former was calculated assuming even settlement across all 10m or 8m of rope for the Portuguese and New Zealand ropes, respectively while the latter indicates the proportion of the original 200,000 eyed larvae placed in each tank that set on each rope type and were subsequently field-deployed. These estimates suggest that setting density was higher on New Zealand rope at the June sampling. However, given the smaller length of New Zealand rope that can be deployed on the frames, the overall setting success was similar between the two rope types. ***Given how difficult the New Zealand rope can be to handle in the nursery tanks, our results suggest that the Portuguese style rope is the preferred rope in the nursery.***

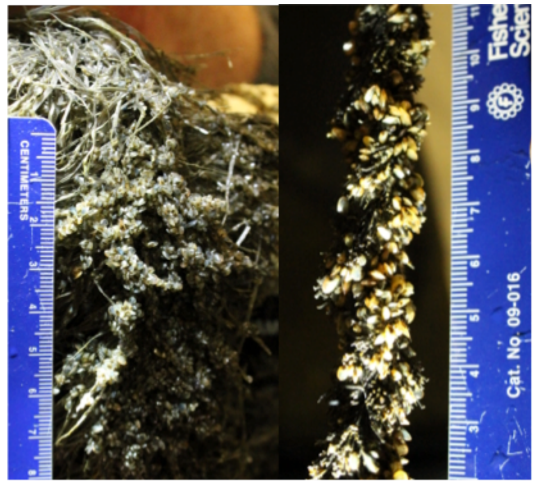
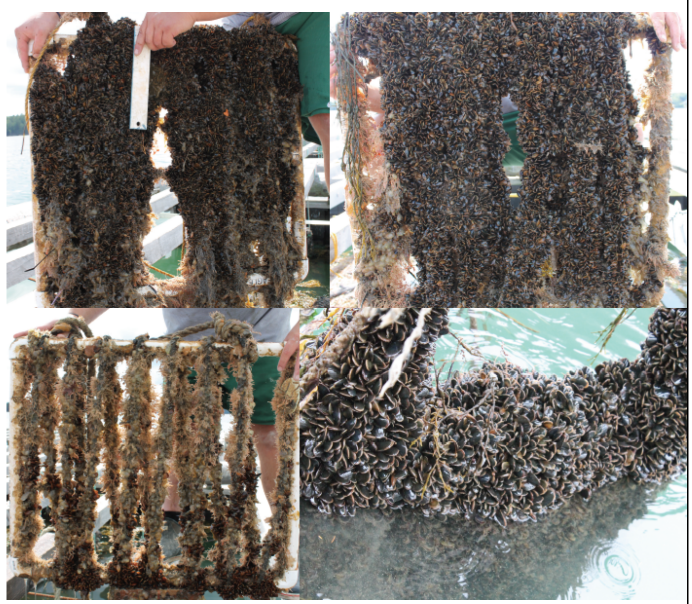


Figure 7. Mussel spat on New Zealand rope (left) initially were highly clumped toward the end of the long fibers while spat on the Portuguese rope (right) were more evenly spaced along the rope length.

Rope	Date	Mean Density (spat/m)	Total Set (per tank)	Setting Success
Portuguese	May 17	8167	81670	41%
	June 28	9307	93070	46%
New Zealand	May 17	6266	51036	25%
	June 28	11075	88600	44%

Our conclusion is predicated on the mussel spat remaining attached to Portuguese rope during field deployment. Spat frames were deployed at Dewey's Shellfish Lease Site at Clark's Cove, Damariscotta River. During two subsequent visits to the lease site, both types of rope were still attached to the PVC frames used in the settling tanks, anchored at two points to the mussel raft using "pot-warp" (lobster rope). The frames were weighted at the bottom using a standard clay brick that has kept the frames vertical except during strong tidal flows. At the first visit on August 25, 2017 we observed evidence of high spat retention on both types of rope (figure 8). The image at the top left of figure 8 is of a PVC frame with New Zealand rope while the image at top right is

	of a PVC frame with Portuguese rope. Preliminary estimates from these photos suggest approximately 60-65,000 mussels per frame with the Portuguese rope and upwards of 70,000 per from with the New Zealand rope. These are very rough approximations given the 3-dimensional nature of the ropes and our calculations assume that coverage is even on both faces of the rope. It is clear from the photos that, regardless of rope type, mussel spat were covering over 90% of rope surface and spat had moved onto guide ropes and PVC frame, itself. ***While there was some evidence at the August sampling of overset from wild spat, the majority of the rope surface was covered with hatchery-produced spat that precluded fouling by tunicates and other encrusting species***. For comparison, ropes from a separate project with low spat density were also deployed at the Dewey's Shellfish site; the light set resulted in a high rate of fouling (figure 8, lower left). We revisited Dewey's Shellfish lease site on October 9th to track mussel growth and retention. Blue mussels on both types of rope ranged in size from 14 to 32 mm in shell length while shell length for gold mussels ranged from 28 to 42 mm. Retention on the ropes continued to be excellent with 90% of rope surface still covered by mussels and the upward movement of mussels along the guide ropes in some cases had exceeded 1 m (figure 4, lower right). Unfortunately, given rough weather and the weight of the ropes we did not remove them completely from water for photos and further analysis.  **Objective 4:** Evaluate the effect of nursery sites for spat deployment (at 1.5 mm average length) on the growth, survival and yield of seed for growout. Although our intention was to test the two rope types under objective 3 at multiple nursery sites, pathology testing on the seed prior to deployment (by qPCR; Kennebec River Biosciences) found trace evidence of the agent causing the oyster disease MSX. Due to biosecurity concerns, we were not allowed to move the seed off the Damariscotta River and thus only deployed the seed at Dewey's

	Shellfish lease outside of Clarks Cove. **Objective 5.** Determine the relative performance of hatchery-produced mussel seed and wild caught seed in field trials. We were unable to address this objective, as natural set seed were not available until several months after we deployed hatchery seed at Dewey's Shellfish lease. **Objective 6:** Determine the cost-benefit of hatchery-based seed production (accounting for alternative diets, seed yield and performance) compared to wild seed, including savings afforded by more efficient production cycles and potential market opportunity associated with favorable traits. Summary: An economic assessment was presented at the NACE 2017 mussel workshop in Providence RI. **Our economic assessment suggests that if mussel farming were to depend on hatchery seed, the associated cost of that seed would need to be covered by 10 to 15% increase in sales price of market product (assuming a farm sale price of < $1 per pound).** There are several assumptions built into our economic assessment. First we assume there is demand for enough seed to entice a commercial hatchery to produce it. For planning purposes, we assume that half of the current 4 million pounds of farmed production will be met by hatchery seed (2 million market pounds). Thus hatcheries need to produce at least 40 million seed to meet the typical 20 mussels per pound at harvest. Assuming a 50% loss through the nursery phases, a target of 80 million 2mm seed ought to fulfill half the current commercial market need. Note that our best measurements for seed retention to market have only been a little over 25% thus far. Our other assumptions are: (i) that there is negligible cost to collecting and conditioning enough broodstock that spawn readily, (ii) no additional capital is needed since we will use existing hatchery capacity ("out of season"), (iii) raising mussel larvae is similar in cost to raising eyed oysters (market price = $400/million, or $0.40/K, (iv) the cost of remote-setting and raising field plantable mussels (> 2 mm) should be less than or no more than similar sized oyster seed (market price = $8/K). Therefore, if 2 mm mussel seed can be produced at $4/K that is less than half a penny per seed. At 20 mussels per pound, that would push price up almost $0.10/lb. which might be a 10 to 15% wholesale price increase. It seems feasible that the industry and market could absorb this, particularly if one could consistently deliver traits that the market desires (e.g shell color, higher meat yields).

Accomplishments:	
Outreach Overview	Describe in general how your results have been extended to the intended users. OR, if they haven't yet, explain when & how this will occur. Our outreach during this project included: 1) Distribution of mussel hatchery seed to participating growers (Dillon Shaw; Dewey's Shellfish Co.) along with communicating with other participants (Matt Moretti, Bang's Island Mussels) even though we could not send them seed due to biosecurity concerns. 2) Five talks on mussel seed production, nursery phase experiments and field deployment stemming from this project were coordinated and presented at a "Mussel Farming" session/workshop at the 2017 Northeast Aquaculture Conference and Expo in Providence, RI. We were joined in this effort by collaborators from the Woods Hole Oceanographic Institution, the DownEast Institute and Martha's Vineyard Shellfish Group.
Targeted Audiences	Provide information on the **target audience** for efforts designed to **cause a change in knowledge, actions, or conditions**. The target audience is our participating mussel farmers, and prospective entrepreneurs and fishermen who may be interested in starting a mussel farm. A secondary audience will be commercial shellfish hatcheries who might be engaged by mussel farmers to produce seed.
Outputs:	Outputs are tangible, measurable products (website, events, workshops, products [AV, curricula, models, software, technology, methods, websites, patents, etc.], trainees, etc.). Do NOT include publications as they're listed separately. We have drafted parts of a mussel hatchery and remote-set seed manual that constitutes one of our major products; much of what is in this manual was covered in the talks delivered at the NACE workshop. After completion, the manual will be made available through the Maine Sea Grant website (Morse) and the East Coast Shellfish Growers Association listserve.
Outcomes/Impacts:	Describe how findings, results, techniques, or other products that were developed or extended from the project generated or contributed to an outcome/impact. **Outcomes/impacts are defined as changes in Knowledge, Action, or Condition.** We have a better understanding of how hard it is to ripen mussels for spawning, and that one needs to start with hundreds of broodstock in order to assuredly have enough fertilized eggs. Once we have fertile eggs, the hatchery process is similar for other bivalves. Mussel seed

	are relatively easy to culture but retention on the settling ropes is uncertain prior to reaching a size of at least 2mm – thus remote set tanks should be operated for 2 or more months prior to stocking ropes in the open ocean. Remote setting frees up hatchery space sooner and is one way to obfuscate the biosecurity issues experienced during nursery phase culture of mussel seed in this project.
Impacts Summary	Provide short statements (2-3 sentences) about each of the following: (pre-established fields for Researchers to complete short statement answers) 1. **Relevance:** Issue – what was the problem? Lack of reliable wild mussel seed available and at times mussel farmers want it 2. **Response:** What was done? Develop hatchery techniques for reliable supply 3. **Results:** How did your work make a difference **(change in knowledge, actions, or conditions)** to the target audiences? Growers in our region now have an alternative that can help them hedge their bets that they will have sufficient seed if they contract with a hatchery for mussel seed. This was the message we broadcast at our NACE workshop. 4. **Recap:** One- sentence summary We have closed the life-cycle on one of the last aquacultured species that depends on wild progeny, and can provide a cost-effective means to secure the industries year round seed supply needs.
Publications	**Rawson, P., S. Lindell, K. Pepperman, M. Devin and D. Bailey.** *Conditioning of blue mussels (Mytilus edulis) using microalgal and alternative diets.* Oral presentation during the Blue Mussel Hatchery and Nursery Workshop at the Northeast Aquaculture Conference and Exposition, Providence, RI. January 2017.
Students/Participants:	Provide the following information for **every** student that worked with you during the reporting period: No students were involved in this project, primarily because employee turnover in our hatchery at the Darling Marine Center required extensive time commitment to working with new technicians and a lack of stable training environment for student project. However, Charlie Walsh and Tyler Hild, who both had extensive experience with microalgal culture, but only limited experience with shellfish hatchery culture, joined the project on an emergency basis in the fall of 2016. They quickly learned the necessary techniques and their dedication to the project ensured the success we had with the January to March 2017 conditioning and spawning of mussels.
	List any partners that you worked with on your project. Provide the

<table>
<tr><td rowspan="4">Partnerships</td><td colspan="4">following information for each Partner:</td></tr>
<tr><td>Partner</td><td>Specific Type</td><td>Level</td><td>Nature of Partnership</td></tr>
<tr><td>Bangs Island Mussels – Matt Moretti</td><td>Mussel Farmer</td><td>Provided Broodstock</td><td></td></tr>
<tr><td>Dewey’s Shellfish - Dylan Shaw</td><td>Mussel Farmer</td><td>Deployed Mussel Seed on Farm Rafts</td><td></td></tr>
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