Project Completion Report
Project Title: Optimization of Hatchery and Culture Technology for Razor Clams

Subaward # Z540402
Grant # 2010-38500-21074

SIGNATURE PAGE

PROJECT CODE: TRA-11-1 **SUBCONTRACT NO: Z540402**

PROJECT TITLE: Optimization of Hatchery and Culture Technology for Razor Clams

PREPARED BY:
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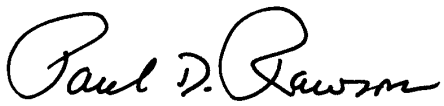

---------- March 1, 2015 ----------

Project Coordinator Date

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DATES OF WORK: November 1, 2011 to June 30, 2013

PARTICIPANTS:

Paul Rawson, University of Maine
Dale Leavitt, Roger Williams University
Dana Morse, Maine Sea Grant & UMaine Cooperative Extension University of Maine
Diane Murphy, Cape Cod Cooperative Extension & Woods Hole Sea Grant

REASON FOR TERMINATION: All project objectives completed.

PROJECT OBJECTIVES:

1) Develop improved hatchery methods for the production of razor clam seed in order to provide commercial shellfish hatcheries with the means to produce a steady, reliable source of seed.
2) Identify improvements in grow-out technology for the culture of razor clams and increase the industry's interest in and acceptance of this alternative species.
3) Track the marketability of razor clams in regional and broader markets.
4) Communicate the progress and results of proposed work directly to industry partners and the industry at-large.

ANTICIPATED BENEFITS:

This project sought to develop improved technologies for seed production of the razor clam, an alternative shellfish species, and make that technology available to shellfish hatcheries. Through our Razor Clam Roundtables, we worked closely with industry participants to design improved grow-out technologies. Over 20 growers representing shellfish operations throughout the NRAC region attended our two Roundtables and received an introduction to the biology, culture, and market potential of razor clams. By developing and demonstrating hatchery and grow-out culture systems for razor clams, our work will eventually lead to diversification of the shellfish culture industry of the northeastern U.S. Diversification will reduce the risks associated with an overreliance on just two species by the industry and allow shellfish growers to choose the most appropriate crops based on environmental and market conditions.

PRINCIPAL ACCOMPLISHMENTS:

A major goal of our project was to conduct multiple spawnings of razor clams using both field-collected, ripe broods and broods conditioned in the lab. We were successful at obtaining spawn from field and hatchery-ripe broods. We also compared the efficacy of using standard downwellers and upwellers versus trays filled with sand and mud substrates for nursery phase culture of razor clam offspring. The former methods represent the traditional methodologies for the early nursery phase

culture of shellfish (experimental controls), but have generally not supported the early growth and survival of razor clam spat. Sediment-filled trays represent a potential solution to problems often encountered with nursery phase culture of razor clams. Complete mortality of clams in downwellers indicated they are unsuitable for early nursery post-settlement for razor clams, as has been found in other hatchery experiments with razor clams. On the other hand, we found that the use of sediment filled trays provides a substantial increase in the growth and survival of immediate post-set razor clams in the hatchery. In our trials, we included both autoclaved and cleaned but non-autoclaved sediments. We recovered few clams from the tanks with autoclaved sediment, which may have been a result of changes in sediment chemistry and/or lack of preference for autoclaved sediments. In contrast, when provided with cleaned, washed sediments, razor clams exhibited a clear preference for coarse sand. The volume, biomass and growth for post-set razor clams in the coarse sand was higher in comparison to the clams that settled in the fine sand or natural sediment treatments in the tank which had non-autoclaved sediments. It is important to note that our experiment cannot directly determine differences in survival for clams in each of these treatments, as survival is confounded by differences in initial settlement density. Even so, the clear differences between total volume and biomass in the natural sediments and sand sediment treatments indicate there is greater settlement and survival in the latter. Based on our observations, the increased shell size and volume in the coarse sand treatment suggests that coarse sand should be used in aerated tank systems for successful survival and growth of *E. directus* post-settlement. These results, however, need to be viewed with caution as the handling and maintenance of sediments in a hatchery setting were identified as potential obstacles to the production and distribution of razor clam seed and the adoption of this species by the industry.

We also conducted experiments investigating sediment preference for razor clams at 6 months post-settlement. We recorded the burrowing behavior of individual clams placed in burrowing chambers containing polished-washed sand, natural mud or a sand-mud mix. Juvenile razor clams took over five times as long to explore the sediments surface and initiate burrowing when presented with mud sediments when compared to clams presented with sand or mixed sediments. The speed with which clams burrowed, once they started burrowing, was slightly faster in mud than in the other treatments. However, the large difference in the delay in initiating burrowing for clams exposed to mud overwhelms the small difference between treatments in burrowing speed. Thus, based on the differences in burrowing behavior juvenile razor clams expressed a clear preference for sand or sandy-mud substrates and clams exposed to mud remain exposed on the surface where they are prone to predation, disturbance, and are unlikely to feed which will affect their growth and survival. Future experiments will expand the analysis of sediment preference to other sediment types and ontogenetic stages.

We produced and convened two Razor Clam Roundtables. The first was held at the Darling Marine Center in Maine on May 2, 2012. The workshop covered the goals of the project, history of razor clam culture and research, and a discussion of life history characteristics of razor clams. Attendees joined in a broader discussion on the particulars of leasing, licensing and permitting, equipment and husbandry, site requirements, and razor clam biology. The second Razor Clam Roundtable was presented in Barnstable, MA on August 21, 2012. This second workshop complemented the one held at the University of Maine and focused on refining ideas for field deployment of razor clam seed as well as including more discussion on potential use of upwellers in hatchery systems, aspects of seed transfer and grow-out site characteristics.

IMPACTS:

We have no direct impacts to report, as yet. Our work focused on developing methods for the hatchery production of razor clam seed and there has not been time for a pipeline of production to develop. Follow-up work will test methods for seed transfer and field culture and we anticipate that this additional work will support the commercial production of razor clams.

RECOMMENDED FOLLOW-UP ACTIVITIES:

Our hatchery experiments sought to develop improved hatchery methods for the production of razor clam seed. Our Razor Clam Roundtables brought industry partners together to identify improvements in grow-out technology for the culture of razor clams and increase the industry's interest in and acceptance of this alternative species. Along the way, we found that hatchery operators and growers were concerned with the development of protocols for harvesting and distributing seed without damaging the fragile shells of razor clam seed. Thus, future work will not only continue to develop the optimal methods for hatchery production of seed, but will also focus on identifying optimal methods for processing seed and testing planting density effects on production of cultured razor clams. We also recommend revisiting the use of upweller technologies for the nursery-phase culture of razor clam seed.

SUPPORT:

YEAR	NRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1	93,616						93,616
TOTAL	93,616						93,616

PUBLICATIONS

Flanagan, Molly P., "Investigation of Early Development and Importance of Sediment Choice in the Hatchery Production of Razor Clams, Ensis Directus" (2013). *Honors College.* Paper 111. http://digitalcommons.library.umaine.edu/honors/111

ORAL REPORTS

Devin, M., M. Flanagan, P. Rawson, C. Devin and D. Morse. Variation in Settlement and Early Growth of Juvenile Razor Clams (*Ensis directus*)**.** Northeastern Aquaculture Conference and Exposition, Groton CT, December 2012.

Murphy, D., J. Reitsma. Investigating potential for razor clams, *Ensis directus*, to augment farm profitability: a case study in Massachusetts. World Aquaculture Society Annual Meetings, Nashville TN, February 2013.

Morse, D., P. Rawson and M. Devin. Steady steps toward pilot production of razor clams (*Ensis directus*) and sea scallops (*Placopecten magellanicus*) in Maine and Massachusetts. National Shellfisheries Association annual meeting, Jacksonville, FL. March 31, 2014.

Project Final Technical Report

Optimization of Hatchery and Culture Technology for Razor Clams

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Subcontract: Z540402

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4) Communicate the progress and results of proposed work directly to industry partners and the industry at-large.

METHODS AND PROCEDURES

Objective 1 – Develop improved hatchery methods for the production of razor clam seed in order to provide commercial shellfish hatcheries with the means to produce a steady, reliable source of seed.

Work toward this objective was conducted at both the Roger Williams University (RWU) and University of Maine Darling Marine Center (DMC) shellfish hatcheries. Our project focused on four distinct sub-objectives under objective 1.

> *Objective 1a:* Work closely with commercial hatcheries to design protocols for the production of razor clam seed that are consistent with the physical capacity and typical production schedule of most hatcheries.

We consulted with Dick Kraus of the Aquacultural Research Corporation (Dennis, MA), a commercial hatchery that has experience with the spawning and larval rearing of razor clams. He provided important advice to our research hatcheries, particularly with respect to the types of larval settlement treatments that are likely to be successful. Some of these suggestions were implemented in the work completed under this award, others are being implemented in a follow-up project, currently in progress.

> *Objective 1b:* Develop and test modified nursery phase culture systems within our experimental shellfish hatcheries at the University of Maine's Darling Marine Center (DMC) and at Roger Williams University (RWU).

> *Objective 1c:* We will produce a minimum of 1 million razor clam seed between our two hatcheries during this one year project to demonstrate the feasibility of large-scale seed razor clam seed production.

Conditioning and Spawning of Razor Clam Broods and Larval Rearing

A set of adult *E. directus* were collected in late May 2012 near the Darling Marine Center and brought to the hatchery. In the hatchery they were held in flow-through tanks using ambient Damariscotta River water. The water temperature in the Damariscotta River was approximately 15°C in late June. On June 28, 2012, after approximately a month of conditioning and feeding a mixed diet of *Isochrysis galbana*, *Tetraselmis* sp., *Pavlova lutheri*, and *Chaetoceros muelleri*, provided through a pulse-feeding system, nine of the broods were placed in a 12mm layer of filtered (1 um) and UV sterilized seawater (UVFSW, 15°C) in a 0.8m x 1.2m spawning table. To induce spawning the temperature of the water was gradually increased by adding heated UVFSW, raised a total of 7°C over three hours. Females were observed to release strings of eggs while males released a milky suspension of sperm. Six males and three females released gametes at water temperatures between 20°C and 22°C over a 2 hr 18 min time period (Table 1). Upon the initiation of spawning, individual razor clams were quickly placed into a 1 L beaker filled with UVFSW (15°C) to facilitate the collection of gametes at high concentrations. After spawning was complete, each brood individual was tagged via super gluing small slips of plastic, each numbered, to the exterior shell.

Fertilization of the eggs followed typical hatchery protocols for marine bivalves (Helm 2004). Marine bivalve eggs are prone to polyspermy, which occurs when multiple sperm fertilize a single egg leading to developmental abnormalities and eventually egg mortality. In an effort to minimize polyspermy, the egg-sperm suspensions were gently poured over a 50 µm sieve, capturing the fertilized eggs while allowing the water used for fertilization and excess sperm to pass through. The proportion of fertilized eggs was estimated by counting fertilized eggs in triplicate 1 ml samples loaded into a sedgewick rafter cell and observed at 100X magnification. The sieved fertilized eggs were held in a 20 L bucket for 48 h, during which time the embryos had progressed through the trochophore stage and entered the D-stage of larval development.

Sex	Time	Temperature °C	Tag #
Male	07:05	20	980
Male	07:55	20	891
Female	08:00	20	975
Male	08:04	20	976
Female	08:07	20	977
Male	08:28	20	982
Male	08:33	22	983
Female	09:23	22	984
Male	Unknown	22	985

Table 1: Temperature controlled spawn at the Darling Marine Center on June 28, 2012. Sex of individual broods was determined by gamete type. Time and temperature at which each brood spawned was recorded.

After 48 h, larvae were placed into four 350 L tanks of UVFSW at a density of 10 per ml and kept at ambient water temperatures (19°C) for the remainder of their larval development. The larval culture tanks were drained every other day; at each water change, the larvae were captured on an 80 um sieve and the tank water replaced with fresh UVFSW seawater. As the larvae developed, the density in the larval tanks was gradually reduced to 2 per ml and excess larvae were moved into three additional 350 L tanks containing UVFSW. In total, 7 tanks were used to culture approximately 700,000 razor clam larvae to metamorphosis. At each draining, a sample of larvae was placed on a deep-well slide and the larvae photographed using a Lumenera Infinity 2-1C 1.4 megapixel digital camera attached to an Olympus BX41 Compound Scope.

On July 10, 2012, larvae appeared to be reaching metamorphosis. The fastest growing larvae exhibited a foot and had reduced motility, suggesting that they were likely to settle. These larger larvae were isolated from slower growing larvae by grading larvae using a series of sieves (180 µm, 150 µm, and 80 µm). Those retained on the largest sieve were used in subsequent experiments investigating the importance of sediment type on settlement and early juvenile development.

Additional larval-stage culture was conducted at the Roger Williams University Shellfish Hatchery starting in mid-April, 2013 with the collection of broodstock from Point Judith Pond (Narragansett, RI). The broodstock were held in sediment and conditioned for a short interval (7 days) using standard hatchery conditioning strategies employed for other species of bivalves (e.g., oysters). The razor clams were spawned in the hatchery the last week in April and the larvae were cultured under standard larval rearing conditions (see Helm 2004).

Razor Clam Settlement and Nursery Phase Culture

Approximately 10,000 razor clam seed were provided to Leavitt (RWU) from Cape Cod Cooperative Extension (Murphy) on 12 July 2012; these seed were produced from MA razor clam broodstock spawned on 9 May 2012 at ARC in Dennis, MA. At RWU, the seed were maintained using two nursery culture strategies, upwelling and a sand-filled raceway, with 5,000 small razor clams (average size $\pm$SD = 6.15 mm $\pm$1.46) placed in each treatment. Due to the small number of individuals

available, only one replicate of each treatment was applied in this preliminary experiment. Growth was monitored monthly; due to the fragility of the shells handling was minimized to monthly.

The remainder of seed produced at the MA hatchery (ARC) was distributed among 10 growers on Cape Cod mid-July, 2012 and ~25,000 were held over at the Massachusetts Maritime Academy facility in Bourne to examine the effects of sediment type on clam survival and growth. Initially, these were placed into two 350µm sieves inside a larger tray with raw seawater flowing through the system on 19 July 2012.

The following day, a sand experiment was set up with 3 replicates of 4 different sand types. Small plastic trays (2-2.5" deep) were filled with sand and ~45ml (2,075 clams) of *E. directus* seed dispersed to each sand tray. Water inflow to the system was restricted for about 30 minutes to lessen further disturbance to clams; most dug in immediately in all sand types. The four sand types used were 1) silty/muddy marsh sand from Eastham near area of wild *Ensis* populations, collected wet; 2) very clean dry beach sand from Brewster above high tide line, sieved on 2mm screen at collection site to remove larger debris; 3) play sand (Quikrete) from Lowes, sieved on 2mm screen to remove gravel and rinsed with seawater over smaller 63µm screen; 4) silty sand from Barnstable Harbor area near wild *Ensis* populations, collected wet (Fig. 1).

Figure 1. Razor clam setting experiment at the ARC hatchery.

Clams were assessed weekly or bi-weekly for growth and survival. Subsamples were taken from each replicate tray for this purpose and clams were measured for length (mm) and width (mm). Tank and trays were routinely siphoned gently to clean away any accumulated algae or debris.

At the Darling Marine Center Hatchery, early nursery phase settlement preference by *E. directus* was examined using three different sediment types and two tank arrangements. Natural sediment was collected from Lowes Cove and sifted to remove large masses such as rocks and wood, leaving mainly fine-sized sediment. Fine sand (play sand) and coarse sand (construction grade) were purchased from a local supplier (Damariscotta Hardware). Sediments were prepared by rinsing several times with UV sterilized filtered seawater (UVFSW). Twelve 30cm x 15cm bins were filled with approximately 7-8cm of sediment and positioned within1.2m x 1.2 cm square tanks filled to a depth of 45cm with UVFSW. One received only autoclaved sediments while the other received non-autoclaved sediment. The three sediment types were randomly placed into each of ten different bins two of which were larger and split to hold two sediments replicates. Each sediment type had four assigned bins (Fig. 2). Approximately 200,000 competent razor clam larvae retained on a 180 µm sieve were introduced to each tank on July 10, 2012. Water in the tank was aerated throughout the experiment and water was

Figure 2. Setting experimental set-up at the Darling Marine Center.

changed every two days after the larvae settled. A second settling tank was set up in a manner identical to the first, except the sediments in the second tank were autoclaved (sterilized) prior to the experiment.

Settlement in "downweller" systems, commonly used for rearing recently settled bivalves in hatchery settings such as oysters, was also examined to test whether these systems can support razor clam settlement and early nursery phase culture (Fig. 2). Four downwellers were prepared by fitting 150 µm mesh screen on the bottom of a waxed wooden frame. The downwellers include PVC tubing that provides for water motion via an airlift so that water is circulated from the surrounding tank through the PVC pipe and downwells through the mesh screen. This method allows for continuous water flow through the screen. The screen is intended to hold competent larvae and promote settlement. Approximately 25,000 razor clam larvae were introduced into each downweller. Two downwellers contained sieved natural sediments and the remaining two had bare screen. Like the sediment treatment tanks, water in the downweller tanks was exchanged every other day.

Sediments in each treatment were regularly inspected to monitor presence and size of clams and shell durability. The size of individual clams and volume of clams in each treatment were measured to estimate growth and mortality in each treatment. Individuals were removed from the sediment via gentle sieving and counts of total volume and average of shell length were determined. Because recently settled razor clams have relatively fragile shells, the first measurement of sediment preference was conducted eight weeks post-settlement. The second count of individual's preference for settlement in different sediments was conducted twelve weeks post settlement. This count also included estimated individuals and biomass.

Sediment Preference Experiment

The burrowing rates of juvenile *E. directus* in three different sediment types were compared. Chambers for documenting variation in burrowing behavior of juvenile razor clams were constructed from two 12 x 12 x ¾ Lexan plates. In between the plates, a piece of 1" hollow tygon tubing was sandwiched and the plates were held together by a series of 10 stainless steel bolts with wing nuts. The tubing acted as both a spacer between the plates and as a seal for holding sediments between the plates and allow the behavior of clams placed on the sediments to be videotaped. Seawater flowed across the top of the sediment surface.

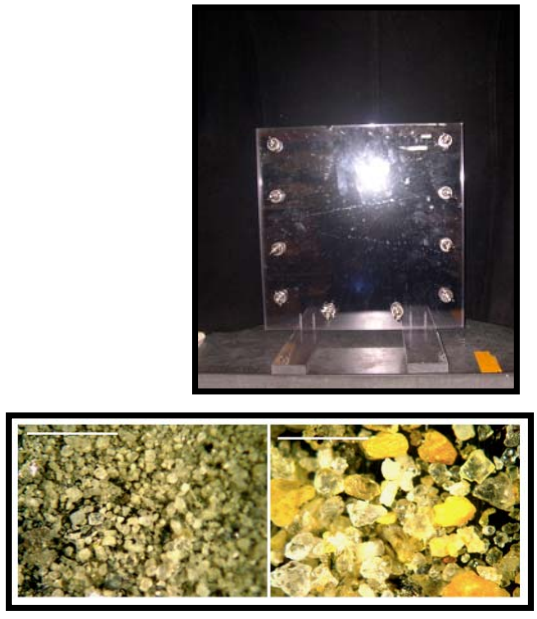


Figure 3. Burrowing chamber used to test sediment preferences for juvenile razor clams (top). Photomicrograph of natural mud (bottom, left) and sand (bottom right) substrates used in burrowing experiment.

Burrowing chambers (Fig. 3) were filled with one of three different sediment types leaving approximately 1" of space at the top for water flow and placement of individual clams. Three different sediment types were used, mud collected from Lowes Cove on the Damariscotta River, Maine, commercial play sand and a 50:50 mix (by volume of play sand and Lowes Cove mud). Juvenile razor clams (1.20 cm-1.85 cm in length) from the June 28 2012

spawning were transported from the Darling Marine Center hatchery and placed in a holding tank in Murray Hall at the University of Maine. The clams were fed live microalgae (*Isochrysis galbana,* strain *T-iso*), *ad libitum*, prior to the burrowing experiments.

The experimental trials were conducted between December 5 and December 14, 2012. The burrowing behavior of 12 juvenile clams was videotaped for each sediment type. Individual clams were retrieved from the holding tank using floppy forceps and their shell length recorded prior to deployment in the burrowing chamber. The burrowing activity of each clam was videotaped using a SONY Handycam; observations continued until burrowing was completed as evidence by either the valve being completely buried or lack of visual movement in the burrowing chamber. If a clam had not completed burrowing by the end of 15 min, the videotaping was suspended and the clam was recorded as having "not burrowed". Eighty three percent of clams burrowed within the 15 min period and most had completed burrowing by 8 min. Clams were removed from the burrowing chamber before the next recording began to limit any potential interference due to the presence of other razor clams in the test chamber. The videos were reviewed using iMovie to determine the time required for the exploration of sediment by siphon extension, the stages of burrowing (start of cycles, upright position, completion) and any other burrowing activity. Each variable was square root transformed prior to conducting single factor analysis of variance (ANOVA). For ANOVA where treatment factors were statistically significant a post-hoc test of means (Bonferroni correction) was used to test for differences among the different sediment types. ANOVA was conducted using SYSTAT (ver. 12) and the appropriateness of each model was determined via examination of model residuals. SYSTAT was used to identify potential outliers prior to running the models.

Objective 1d: We will report to commercial hatchery representatives semi-annually on our progress and discuss additional potential refinements to our hatchery protocols.

We had several opportunities to communicate with commercial hatcheries during the execution of this project. These included presentations and conversations during the second Razor Clam Roundtable in Barnstable, MA in August 2012 as well as at local and regional growers meetings. These conversations provided us with additional refinements and directions that have been incorporated into a follow-up project on razor clam hatchery and grows-out technology.

Objective 2: In conjunction with industry partners from throughout the NRAC region, we will identify potential improvements to the existing grow-out technology for the culture of razor clams. Previous NRAC-supported research determined that razor clam growth in the Northeast was highest in intertidal bottom trays or boarded raceways which provided for effective containment of this highly mobile species. This work also found that summer temperatures associated with intertidal culture of clams can lead to increased mortality. We will convene a series of Razor Clam Roundtable meetings with shellfish growers at which we will introduce as many growers to razor clam culture, as possible, review results from the previous razor clam project, discuss potential modifications to the grow-out protocols and technologies, and establish linkages between industry participants and extension partners. We anticipate that these roundtables will increase the industry's interest in and acceptance of this alternative species.

We produced and convened two Razor Clam Roundtables. The first was held at the Darling Marine Center on May 2, 2012. Dana Morse organized the Roundtable and PIs Leavitt and Rawson provided much of the information content (Diane Murphy could not attend due to an emergency). A diverse

array of participants was in attendance, including several growers from as far away as New Jersey. We were joined by special guest, Chantal Gionet from the Coastal Zone Research Institute in Shippagan New Brunswick, Canada, who shared her Institute's experience with razor clam culture with the workshop attendees. The workshop covered the goals of the project, history of razor clam culture and research, and a discussion of life history characteristics of razor clams. Attendees joined in a broader discussion on the particulars of leasing, licensing and permitting, equipment and husbandry, site requirements, and razor clam biology. The second Razor Clam Roundtable was presented in Barnstable, MA on August 21, 2012. This second workshop complemented the one held at the University of Maine and focused on refining ideas for field deployment of razor clam seed as well as including more discussion on potential use of upwellers in hatchery systems, aspects of seed transfer and grow-out site characteristics. Minutes of the Razor Clam Roundtables are included as an appendix to this technical report.

Objective 3 - Tracking the marketability of razor clams in regional and broader markets; We did not conduct any formal analysis of the market potential for razor clams. We collected some information on demand for, and pricing and acceptance of razor clams through periodic calls to local and regional shellfish dealers and by tracking inquiries on sourcing of razor clams. In a recent SEMAC-funded survey of wholesale shellfish dealers, Murphy and colleagues have sought to determine the market potential for alternative species such as razor clams. Their project is designed to collect information to better refine the market and 'marketability' for razor clams. Given the overlap with this complementary and more comprehensive effort to survey shellfish dealers we reduced our effort in addressing this objective.

Objective 4 - Communication of Results; In addition to the Razor Clam Roundtables conducted under objective 2, we will develop an NRAC Technical Bulletin as a follow-up to the recent NRAC bulletin on razor clam biology (Leavitt, NRAC 217-2010) and based on the advances and successful refinements in the hatchery protocols developed during this project. The technical bulletin will allow us to reach a broader group of shellfish growers in addition to those directly involved in the project. We propose to present the information at a number of additional venues, including local and regional shellfish culture workshops, an NRAC Regional Extension Program meeting, the Northeastern Aquaculture Conference and Exposition, and the Annual Meeting of the National Shellfisheries Association. The key strength of our proposal, however, is the direct involvement of industry partners, which provides for rapid technology transfer.

Production of the technical bulletin is in-progress. We are still working on refinements to the hatchery protocols in the second, follow-up project. Information from the follow-up work will be essential for producing an informative and useful technical bulletin. Information on the venues where we presented updates on project progress can be found at the end of this report.

RESULTS AND DISCUSSION

Conditioning, Spawning and Larviculture - A major goal of our project was to conduct multiple spawnings of razor clams using both field-collected, ripe broods and broods conditioned in the lab. Adult razor clams were spawned at the RWU hatchery the last week in April, 2013 and cultured under standard larval rearing conditions. Unfortunately, the larvae were contaminated with ciliates and bacteria and all were lost within 1 week. This pointedly demonstrated that, while razor clam larval development is similar to that of oysters and other clams, razor clams are more sensitive to their larval culture environment and need to be handled with more attention and care.

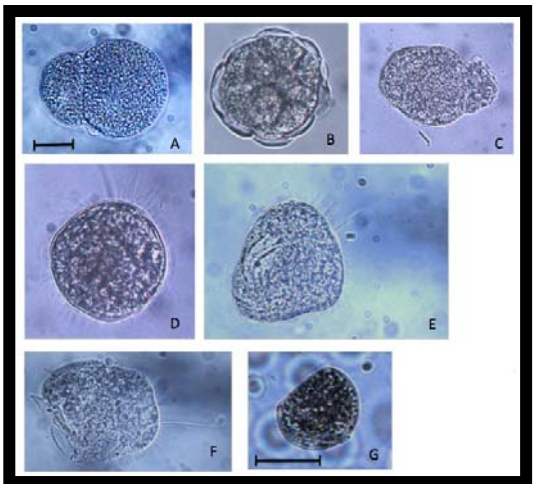


Figure 4: Embryonic development of *E. directus* spawned at the Darling Marine Center on June 28, 2012: A (1 hour post spawn (HPS)), B (2 HPS), C (8 HPS), D (12 HPS), E (20 HPS), F (21 HPS), G (24 HPS). Images A-F were taken at 40X magnification and the scale bar in A represents 10µm. Image G was taken at 10X magnification and the scale bar represents 50µm. A (36µm) shows two cells, B (36µm) shows several cell divisions, C (36µm) shows blastulation, D (36µm) shows cilia surrounding the group of cells, E (36µm) shows cilia surrounding and one larger cilia, F (37µm) shows the beginning of the veliger stage, and G (66µm) shows shell shape development and the D-Larva stage.

We were also able to obtain spawns from two groups of broodstock at the DMC hatchery. Several million fertilized eggs were obtained from the small set of broods spawned on June 28, 2012 and were used to track larval development and settlement. We closely tracked and photographically documented the early stages of embryonic and larval development for *E. directus*. During the first 36 h of development the water was kept at 16°C (+/- 0.5°C). Subsequent stages were kept at 19°C until settlement. The first cell division occurred within 1h of fertilization at 16°C (Fig. 4A). Cilia were apparent about 12 h post-fertilization (Fig. 4D) and one large cilia was visible 15 h post-fertilization and clearly evident by 20 h post-fertilization (Fig. 4E). Larvae reached the trochophore stage within 24 h post-fertilization in 16°C water. Initial shell formation was seen by 24 h indicating the beginning of the larval D-stage (Fig. 4G).

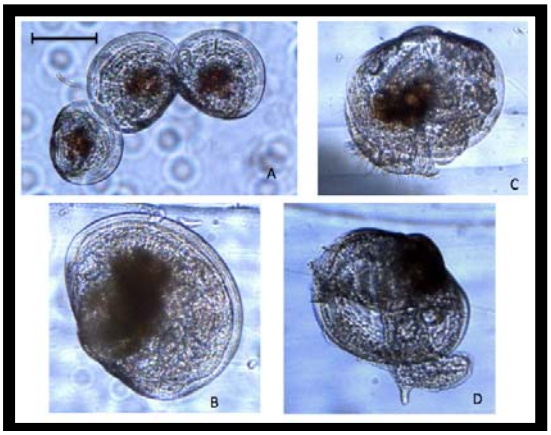


Figure 5: Larval development of *E. directus* spawned at the Darling Marine Center on June 28, 2012: A (5 day post spawn (DPS)), B (7DPS), C (13DPS), D (13DPS). All images were taken at 10X magnification and the scale bar in A represents 50µm. Image A (79 µm) shows three larva, B shows a single larva digital close up, C (117µm) shows a larvae with velum, and D (122µm) shows an early juvenile.

The larvae remained D-shaped until day 5 (Figs. 5A and B). As seen figure 5C, the velum, a prominent feeding structure surrounded by cilia, had formed by day 7. The appearance of the velum marks the transition to the veliger stage. A foot was evident in larvae as small as 120 µm in size; the development of the foot indicated the transition from the veliger stage to the pediveliger stage. The larvae demonstrated a remarkable shift in behavior upon the formation of a foot, they were no longer suspended in the water column and spent more time crawling along the bottom. In contrast, the individuals with just a velum and no foot remained suspended in the water column and were actively swimming. Generally, larvae over 150 µm in size had a developed foot; upon appearance of a foot ('competency') larvae were removed from the larval tanks and placement in the settlement tanks (July 10, 2012). Other than the development of the foot, no external

landmarks were observed (i.e., there were no obvious eye-spots as in eastern oysters).

Samples of sediment were taken over the first six days after "competent larvae" were placed in the settlement tanks; small, recently settled *E. directus* were observed indicating successful settlement. Figure 6 shows a razor clam approximately six days post-settlement. Gills can be seen through the transparent shell and the shell itself has begun to elongate. Based on clam densities, we estimated that one spawn at the DMC produced approximately 700,000 spat.

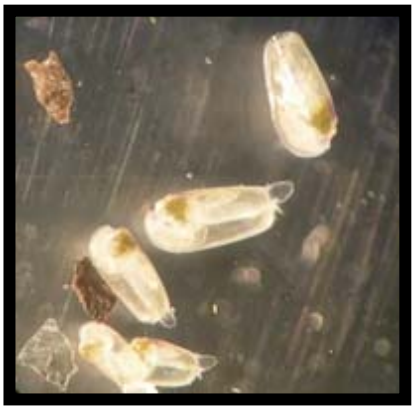


Figure 6. *E. directus* post settlement, courtesy of Dana Morse. Post metamorphic animals are generally less then 2-3mm in shell length. Post metamorphic animals are generally less

Post-set Nursery Culture - Approximately 10,000 razor clam seed were maintained at the RWU hatchery using two nursery culture strategies, upwelling and a sand-filled raceway, with 5,000 small razor clams (average size ±SD = 6.15 mm ±1.46) placed in each treatment. The upweller treatment was lost in mid-July due to an electrical system failure on the dock at RWU after a summer thunderstorm (problem subsequently repaired). The sand tray system was initially held in the flowing seawater wet lab at RWU and fed cultured microalgae (a mix of Tahitian *Isochrysis* and *Tetraselmis* sp.). On 15 August, the sand-filled raceway was moved to the RWU dock (intact) where it was maintained in a flow-through system on 50 µm bag-filtered seawater. Due to a pump failure, the razor clam seed in the sediment raceway was lost during the first week of September. However, growth of the seed in the sand raceway was monitored for the three months that the seed were held in culture and the growth is depicted in figure 7. The average daily gain of the razor clam seed was 0.21 mm/day which is consistent with the growth rates for razor clam spat that we and others have noted in prior work.

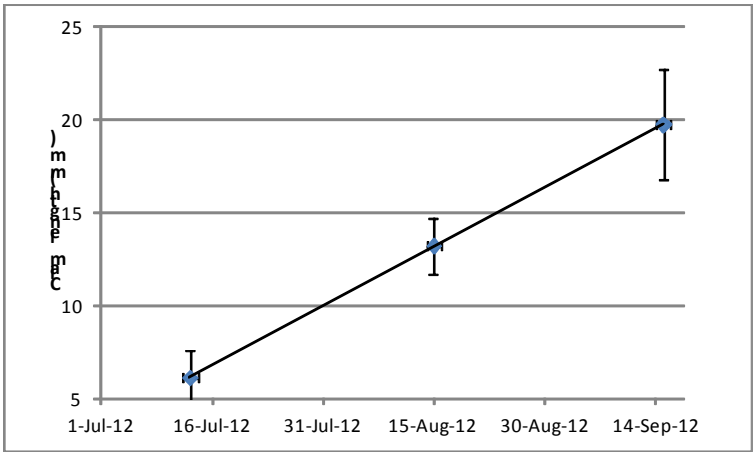


Figure 7 (top). Razor clam seed deployed in sand trays at the RWU hatchery as a nursery culture system. Successive images of seed in July, August and September (post-mortem) are presented from left to right. **(bottom)** Change in shell length for razor clam seed deployed in sand tray nursery culture system in July 2012.

In related work conducted under SEMAC funding, approximately 25,000 *E. directus* seed were received from ARC on 19 July 2012 at the MMA hatchery facility. Technically, the facility had been unused as a hatchery for several years, so only a rudimentary nursery system was available. Conditions were not ideal but sufficient to carry out post-set nursery culture experiments. Seed

averaged 45 clams/L and their average length was 6.62mm ±1.41 upon receipt from the ARC hatchery. Approximately 2,000 razor clams were deployed into sand trays representing 3 replicates of 4 different sand types and monitored for 6 weeks. As shown in figure 8, sand treatments had similar results but there was a significant difference in daily growth rate and average lengths between the dry beach sand trays (average size ±SD = 13.79 mm ±0.17) and marsh sand trays (average size +SD = 12.63 mm ±0.14).

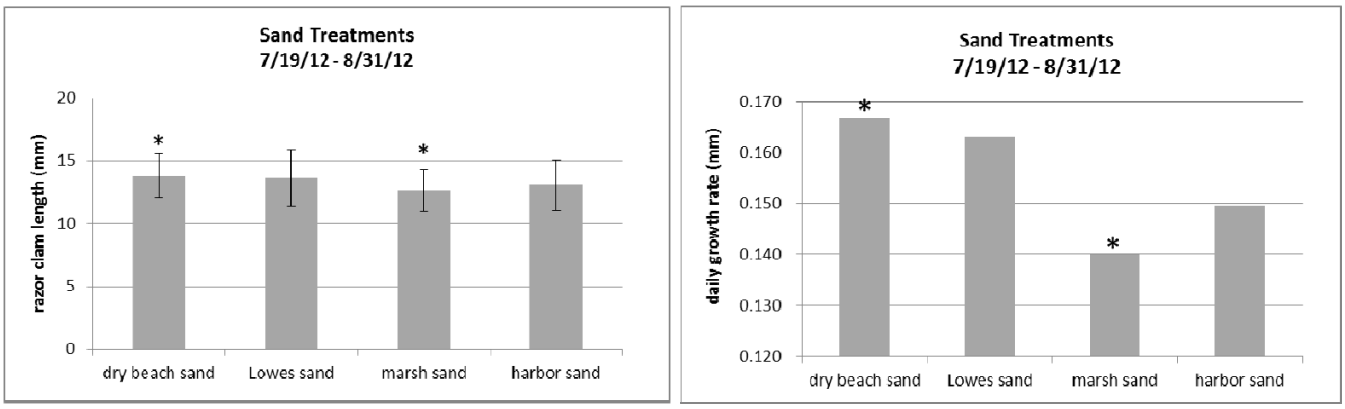


Figure 8: Differences in shell length and daily growth rates between sand types in the MMA culture system over 6 week period. Significant differences between dry beach sand and marsh sand treatments.

At the end of August, 2012 all 12 sand trays were removed, razor clams measured and survival assessed (50% survival) prior to redeployment into four larger sand trays for a density experiment. Densities per tray were 2160, 1893, 3530, and 5340, respectively (Fig. 9). The sand type, collected from nearby MMA beach, was uniform for all trays and approximately 12cm in depth. Percent survival after two months at these densities (ranged 12.3 to 20.2%) was very low and likely due to the presence of predatory worms and inadequate food since this was a flow through system.

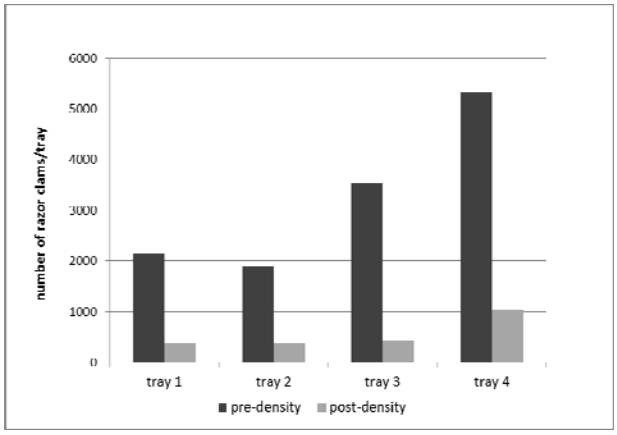


Figure 9: Two month (8/29/12 – 11/6/12) survival of razor clams at different densities.

Shellfish growers participating in this study utilized a variety of techniques to field culture post-set *E. directus*. Follow up with several growers revealed improved growth in razor clams that were deployed to field conditions earlier. Conversely, some problems arose with handling of seed and gear used, particularly netting. Small mesh clogged easily, becoming an attractive substrate for fouling organisms which contributed to reductions in water flow to the clams. Additionally, the typical use of water from the hose to wash down upweller bins proved too harsh for the seed.

Figure 10: Examples of mortality from intense water spray and fouling of mesh.

Most growers used a method typical for hard clams which consists of laying out a sand runway and covering it with mesh (Fig. 11, left). One grower attached small buoys to the underside of the netting to help lift it under water and relieve some of the accumulated siltation. Another grower experimented with the use of mesh-covered plastic pots buried into the sediment.

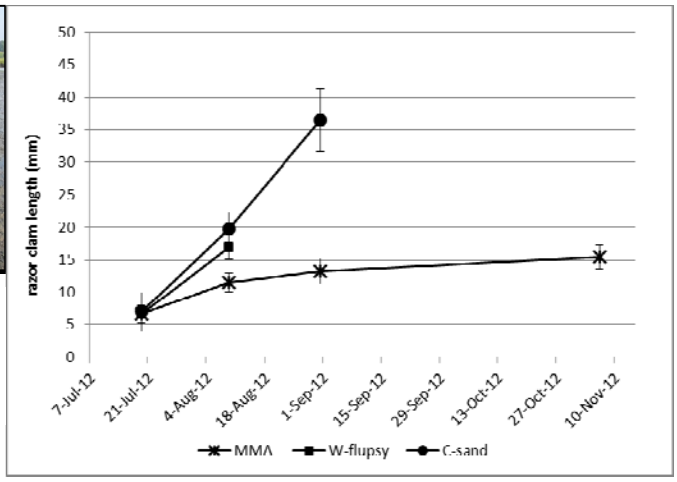
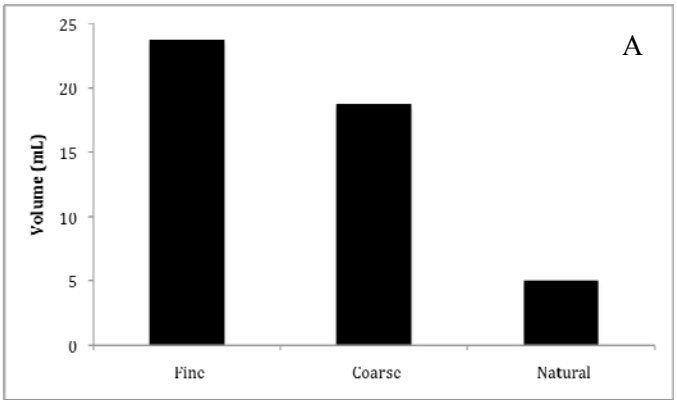


Figure 11. (right) Over the short term, comparisons of growth between MMA, grower flupsy (upweller), and grower field planting to sand reveals an advantage of moving *E. directus* seed earlier from a hatchery setting.

In post-set nursery research conducted at the Darling Marine Center, we found that the settlement, survival, and growth of *E. directus* varied substantially among tanks and treatments at the DMC hatchery. Mortality among the clams introduced to the tank with autoclaved sediment was 100% due to an unidentified contaminant that resulted in white film covering the sediment. Mortality was also 100% in the downweller treatments within the first week post-settlement, whether the downwellers contained sediment or not. In contrast, the volume of *E. directus* juveniles in the non-autoclaved sediments was quite high. At 8 wks post-settlement, the highest volume of juvenile *E. directus* was found in fine sediment while the lowest volume was observed in natural sediment (Fig. 12A). Seed volume provides an indication of both settlement density and survival to 8 wks. Given the fragile shells of small clams it was difficult to estimate these two variables independently. Growth also varied among the different treatments in the non-autoclaved sediments. Razor clams in the coarse sediments were found to have the greatest mean length at the 8 wks post-settlement sampling while the lowest mean length was observed for clams that settled in natural sediment (Fig. 12B). These observations indicate higher settlement and survival in both types of sand sediments and perhaps greater feeding



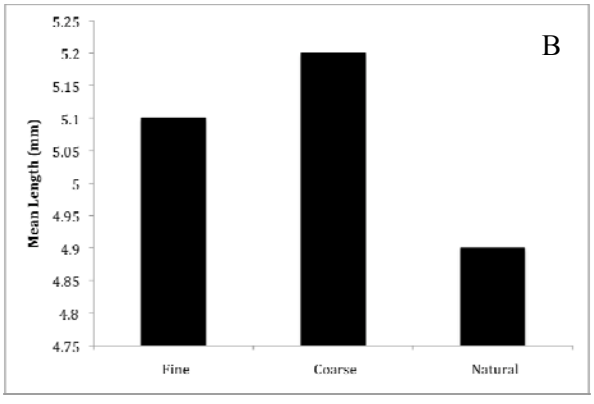


Figure 12: (A) Volume (mL) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank eight weeks post settlement. (B) Mean length (mm) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank eight weeks post settlement.

activity and health among the clams in the coarse sand treatment. By 12 wks post-settlement, the highest volume, largest mean length, greatest estimated number of individuals, and largest biomass was observed in the coarse sediment containers (Figs. 13 & 14). There were fewer, smaller clams observed in the fine sand compared to the coarse sand treatment and the lowest number and the smallest clams were observed in natural sediment treatment. The estimated growth rates for razor clam spat in the DMC hatchery ranged from 0.087 (natural sediment) to 0.092 mm•d^{-1} (coarse sand) over the first 8 wks post-settlement. Growth over the subsequent 4 wks increased (0.264 to 0.271 mm•d^{-1}) so that average growth after 12 wks post-settlement was 0.146 and 0.152 mm•d^{-1} in natural sediments and coarse sand, respectively.

The results of our settlement experiment provide a clear indication of the need for properly prepared sediments for the successful settlement and early growth and survival of razor clam seed. The high mortality seen in the tank with autoclaved sediment are likely the result of contamination; autoclaving sediments changes the organic content of the sediments and initiated the growth of what appeared to be a fungal contaminant the presence of which was harmful to young razor clams. The complete mortality of clams in downwellers indicates that they are unsuitable for early nursery post-settlement for razor

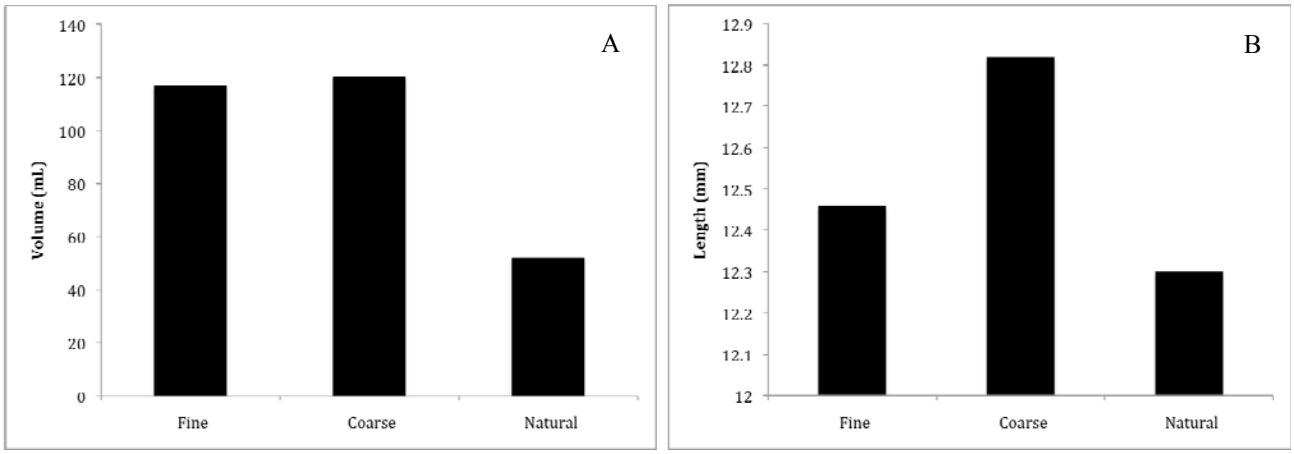


Figure 13. (A) Volume (mL) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank twelve weeks post settlement. (B) Mean length (mm) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank twelve weeks post settlement.

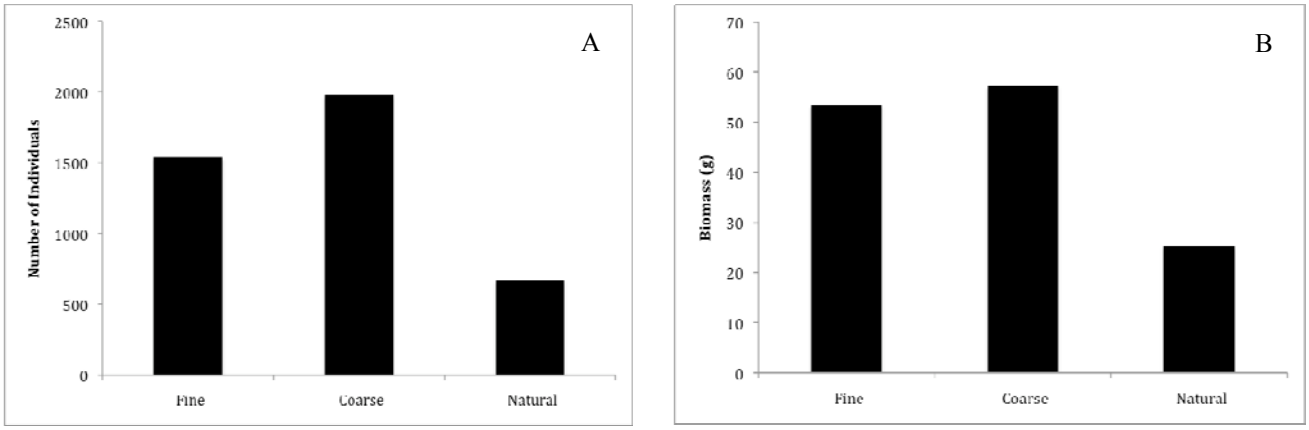


Figure 14. (A) The number of individuals estimated from counts of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank twelve weeks post settlement. (B) Estimated biomass (g) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank twelve weeks post settlement.

clams. Subsequent discussions with growers and hatchery operators indicated that this observation is not unique. The Aquaculture Research Corp. also observed very high mortality for razor clams held in downwellers. In contrast, the ARC had success with the use of upwellers and these will be used in our subsequent projects. With the fragile shells of newly settled juveniles and the fact that *E. directus* is a benthic bivalve that lives buried in the sediment (Christian 2010), it is not surprising that downwellers did not support its survival and growth.

On the other hand, when provided with cleaned, washed sediments, razor clams exhibited a clear preference for coarse sand. The volume, biomass and growth for post-set razor clams in the coarse sand treatment was higher in comparison to the clams that settled in the fine sand or natural sediment treatments. It is important to note that these experiments cannot directly determine differences in survival for clams in each of these treatments, as survival is confounded by differences in initial settlement density. However, the clear differences between total volume and biomass in the natural sediments and sand sediment treatments indicate there is greater settlement and survival in the latter. Based on our observations, the increased shell size and volume in the coarse sand treatment suggests that coarse sand should be used in aerated tank systems for successful survival and growth of *E. directus* post-settlement. Unfortunately, due to psuedoreplication of sediment treatments in the DMC hatchery experiment, no formal statistical tests of the difference in mean volume and shell size have been conducted and our findings should be considered preliminary observations.

Sediment Preferences and Burrowing Behavior for Juvenile Clams - Successful nursery culture and eventual outplanting of razor clams requires knowledge of the sediment characteristics preferred by various ontogenetic stages of this species. While adults can be found in sandy to muddy-sand habitats, these habitats may simply represent areas where predation or competition are low and not necessarily the types of habitats where growth and yield will be maximized. To develop a better understanding of sediment preferences for *E. directus*, we initiated sediment preference experiments. Adult razor clams are prolific burrowers and can quickly burrow up to 70 cm deep into the sediment (Winter *et al.* 2012). The burrowing behavior of razor clams, however, is likely to vary as a function of sediment characteristics. For example, Alexander (1993) has shown that sediment size influences the ability of adult *Ensis* to penetrate the sediment. Winter *et al.* (2012) have proposed that burrowing in *Ensis* proceeds through six steps (start of burrowing, downward extension of foot, upstroke of valve, valve contraction, foot contraction, and expansion of valve; Fig. 15). Before burrowing has initiated, the clam will use its foot to explore the sediment by extending it to the sediment. When conditions are suitable, the foot extends down into the sediment. As the valve of the shell contracts, blood fills the foot and allows it to act as an anchor for the animal to pull itself down into the sediments. The valve relaxes to start the next cycle. While the general burrowing mechanism outlined by Winter *et al.* (2012) likely holds for recently metamorphosed, juvenile and adult clams, it is not known whether the sediment preference and thus burrowing behavior differs among razor clams of different ontogenetic stages.

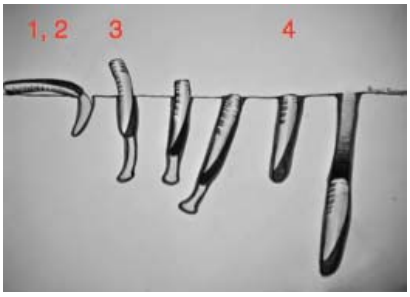


Figure 15. The burrowing cycle of *E. directus* (drawing courtesy of Abigail Flanagan). Initiation of burrowing (left=1), upright positioning (2), valve contractions (3-4), and completion of burrowing (right).

There were clear and statistically significant differences in the burrowing behavior of early juvenile razor clams (< 20 mm shell length) among the three sediment treatments included in this experiment.

Although all individuals used in this experiment were spawned on the same date (June 28, 2012), they were not all the same length. We used an analysis of variance to test whether the mean length of clams differed among the three sediment types. This analysis indicated there was no significant difference of lengths between treatment types ($F_{2,33}$ = 0.2; p = 0.82) and thus the effect of size on burrowing behavior was not considered further.

The burrowing cycles of 36 juvenile razor clams were visually recorded and analyzed for time to visual siphon extension, first evidence of foot extension, initiation of burrowing cycle, upright position of individual, and completion of burrowing cycle (Fig. 15). Some individuals in the mud treatment did not complete the burrowing cycle within 15 min and were not included in the analysis of burrowing behavior. In addition, siphon exposure was difficult to observe, particularly in the mud and mud/sand mix treatments due to suspended sediments so this variable was not analyzed. However, substantial and statistically significant differences among sediment types were detected for the three aspects of burrowing behavior that we quantified. The mean time from the start of exploration to the start of burrowing was found to be statistically different between the three sediment types ($F_{2,29}$ = 4.3 and p = 0.023). Clams in the mud treatment had the longest lag time between initiating exploration of the sediments and initiating burrowing (Fig. 16A). In contrast, the lag time for clams in the sand and mixed sediments was less than 25% of that observed for clams in the mud sediments. Pair-wise comparisons indicated that the means for the mud and mixed sediments were significantly different from one another (p = 0.025), although there was no difference between the sand and either of the other two treatments. In addition, while > 90% of the clams in the sand and mixed sediments completed burrowing within 15 min, only 67% of those in the mud treatment had completed burrowing in the same time frame (Fig. 16B); a difference that was statistically significant (R x C contingency test; χ^2_2 = 6.04; p < 0.05). Combined, these observations provide clear evidence that juvenile razor clams prefer and more readily burrow into coarser sediments.

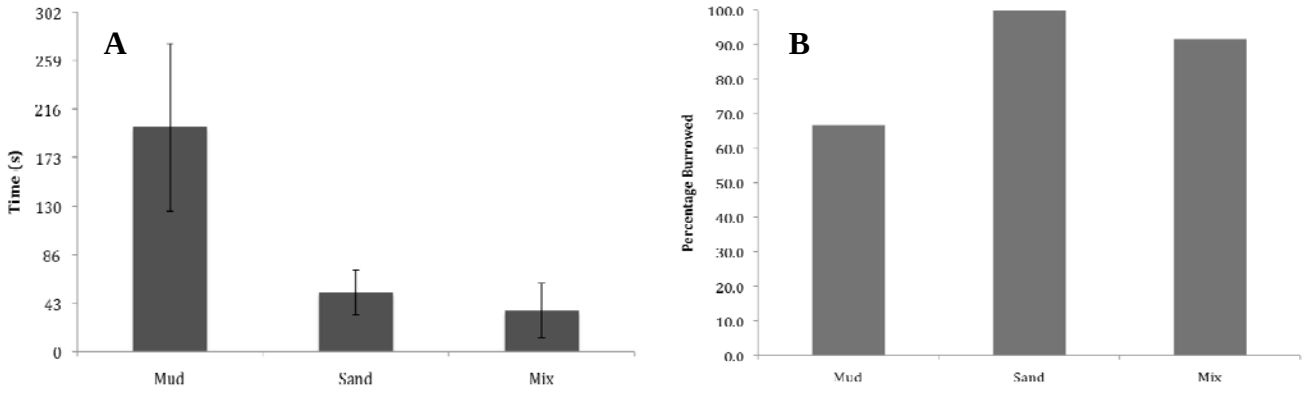


Figure 16. (A) Mean time in seconds (s) from start of exploration to initiation of burrowing cycle for juvenile *E. directus* in mud (n=9), sand (n=12) and mix (n=11) sediment treatments. The start of exploration was defined when foot of the individual was first visible while initiation of burrowing was defined as when the foot pushing into the sediment. Error bars represent the mean +/- one standard error for the untransformed values. (B) The proportion of juvenile *E. directus* that completed burrowing in 15 min in each of three sediment types (mud, sand, and mixed sediments).

The treatment-level effects that we observed for the early stages of burrowing were reversed for the other later aspects of burrowing behavior that we quantified. Razor clams in the mud treatment spent significantly less time completing the burrowing cycle once they were in the upright position (Fig.

17A; $F_{2,29} = 4.104$; $p = 0.028$). Similarly, after first initiating burrowing clams in the mud treatment completed burrowing twice as fast as the clams in the sand treatment and nearly 50% faster than the clams in the mixed sediments (Fig. 17B; $F_{2,29} = 14.47$; $p < 0.001$). These observations suggest that once clams make the commitment to burrow into the sediments, they have an easier time burrowing into mud than into mixed sediments and are much faster at completing burrowing in mud when compared to sand.

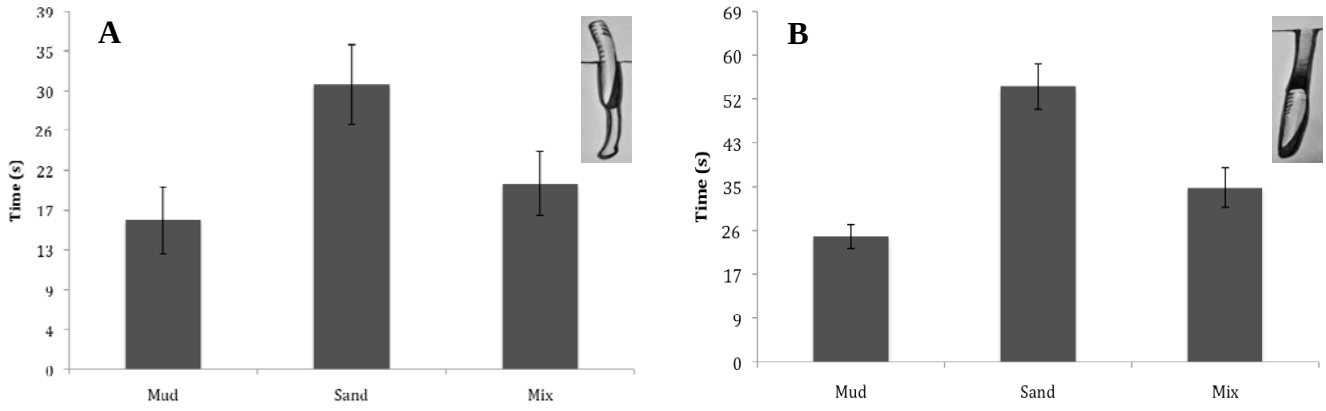


Figure 17. (A) Mean time in seconds (s) between when juvenile razor clams were in the upright position during burrowing cycle and when they completed the burrowing cycle. Error bars are the mean +/- one standard error for the untransformed values. (B) Mean time in seconds (s) between the start of burrowing cycle to completion of the burrowing cycle for juvenile *E. directus* in three sediment types (mud, sand, and mixed). The start of burrowing cycle was defined as when the steps of burrowing are first visible while the completion of the burrowing cycle is defined as when the burrowing steps are no longer visible. The error bars are the mean +/- the standard error for the untransformed values. The sketch in the upper right-hand corner of each panel depicts a visual representation of the portion of the cycle represented in the figure.

The whole burrowing cycle in razor clams includes a period of exploration prior to extension of the foot into the sediment at the start of burrowing (Fig. 15). In this experiment, the bulk of the total burrowing cycle time occurs during the exploration phase (Fig. 18). For the clams in the sand and mixed sediments, the exploration phase accounted for 48-58% of the total burrow cycle while it accounted for 78% of the total cycle among clams in the mud treatment. From an ecological perspective, juvenile razor clams are most vulnerable to predators and potentially exposed to adverse conditions or prone to being swept away if they spend a protracted period on the surface of the sediment (McDermott 1976). Thus, under field conditions, the differences in sediment preference are likely to translate into large differences in sediment-specific abundance for juvenile razor clams. In terms of the importance to aquaculture, the clear preference that clams display for coarser sediments as observed in this experiment, along with the

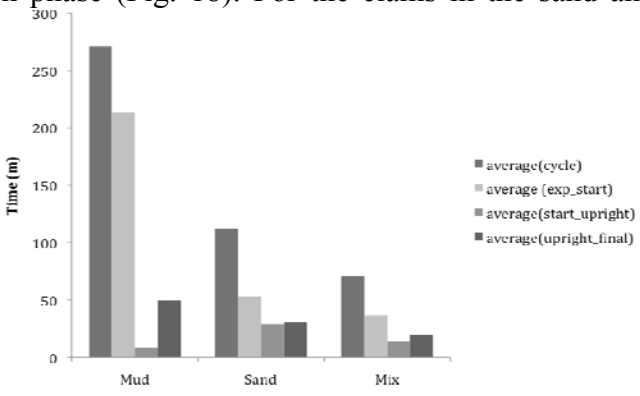


Figure 18. Average time for total burrowing cycle and diffferent phases of the burrowing cycle for juvenile *E. directus* in mud, sand, and sediment treatments. Each average total time was broken into the average times of explore to start of burrowing (exp_start), start of burrowing to upright (start_upright), and upright position to completion of burrowing (upright_final).

increased growth described earlier, indicates that hatcheries should use sand or mixed sediments and avoid fine grained mud for the nursery phase production of razor clam seed. Further, growers should identify grow-out sites with coarse-grained sediments to increase the likelihood of burrowing and early growth when razor clam seed are first deployed in the field.

Additional experiments with an increased array of sediment types would be beneficial for determining the optimal sediments that can be used in the nursery culture of razor clams. When extended to older age classes, such sediment preference experiments will also help to further define which sediment types will be the best for the post-nursery field grow-out culture of juvenile and adult *E. directus.* Future work should also strive to characterize sediments based on more than just grain size; including analyzing such variable as organic and sulfur content. Woodin *et al.* (1995) discussed different sediment variables and their influence on the burrowing behavior of the hard clam (*Mercenaria mercenaria*) and lugworm (*Arenicola cristata*). Differences in sediment grain size, which influences the redox state of the sediments, resulted in differing burrowing behaviors. More complete characterization of sediment characterization and the role of sediment-borne cues on burrowing behavior in razor clams will be critical in the identification of suitable culture sites and in developing appropriate grow-out protocols for this species. In addition, similar experiments as to the ones discussed above with juveniles of different ages will better show at what age juveniles have the best chance of burrowing under the sediment before predation or currents remove them from the culture lease site.

Market Analysis - Dana Morse received two inquiries from overseas about sourcing razor clams: one came from Singapore and one from Hong Kong. Regionally, it was clear that production from MA and ME was not enough to supply these inquiries, and both were interested to hear that aquaculture production was in development. One of these contacts specified 20 kg boxes made up of 1kg packs, and prices in the region of 9.0 euro/kg (approx. $5.10/lb) were possible for large, live clams, with large being 6" in length.

Calls to local dealers indicate that demand is sporadic, but that this is largely because of the sporadic supply off the flats - razors living in the lower intertidal and therefore only accessible at very low tides. Several shellfish dealers indicated that they could develop markets, if supply were improved and made more regular.

SUMMARY

Razor clams are readily spawned in the lab using individuals ripe from the field. We have also found that this species is amenable to conditioning to spawn using an approach similar to that used for other commercially cultured bivalves for which seed is produced in the lab (e.g., oysters, hard clams; Helm 2004). As efforts to increase the hatchery production of razor clam seed progress, we recommend being careful to keep number of broods sufficient to avoid potential inbreeding in hatchery stocks. In our experience, roughly 40-50% of a set of broods spawn at any given time. Thus, hatcheries will need to include upwards of 100 broods in a spawn in order to ensure at least 50 spawners with an approximate 1:1 gender ratio to minimize accumulation of inbreeding (1% per generation).

Larviculture with *E. directus* follows typical procedures used for other species. Past experience suggests that razor clam larvae are prone to "infection" from ciliates and bacteria and thus, hatcheries need to employ control measures to minimize risk of infection. These measures include thorough

sieving and washing of eggs to reduce transfer of infections from adult broods to larval cultures and use of fine filtered, UV sterilized seawater to limit the opportunity for contaminations to develop. The larval development of razor clams is much more rapid than with eastern oysters, despite colder water temperatures. For example, we typically find that eastern oysters take upwards of 21 d or more at water temperatures > 20ºC to complete larval development whereas some razor clam larvae developed a foot and began actively exploring benthic surfaces within 13 d post-fertilization at 19ºC. Razor clams do not develop any discernable eye spots or other external markers of competency, so hatchery operators will have to keep careful watch for the development of the foot. Future hatchery work with this species should examine the relationship between water temperature and development time and methods for determining when larvae reach competency.

The results of our settlement experiment provide a clear indication of the need for properly prepared sediments for the successful settlement and early growth and survival of razor clam seed. However, in independent experiments the ARC hatchery has had success at using upweller systems during early post-set nursery culture of razor clams; the use of upweller systems would greatly relieve the problems associated with sediments in hatchery settings. For example, the high mortality seen in the tank with autoclaved sediment was likely the result of contamination; autoclaving sediments changes the organic content of the sediments and initiated the growth of what appeared to be a fungal contaminant the presence of which was harmful to young razor clams. The complete mortality of clams in downwellers indicates that they are unsuitable for early nursery post-settlement for razor clams. Subsequent discussions with growers and hatchery operators indicated that this observation is not unique.

The overarching goal of our research collaborative is to develop hatchery and grow-out technology that leads to the culture of razor clams, *E. directus,* by the Northeastern shellfish aquaculture industry. The market potential for razor clams seems evident from our conversations with and a limited survey of shellfish dealers. The work presented in this report focused on developing improved protocols for the spawning, larviculture and early nursery culture of razor clams to facilitate the supply of cultured razor clams to the market. We were highly successful and spawning razor clams and carrying them through larviculture. Using sediment-filled trays, we successfully cultured razor clam seed to a size (10-20 mm) that would be appropriate for field-deployment. Through Razor Clam Roundtable discussions with growers we found that enthusiasm for culturing this species is high as is the market potential for aquacultured clams. Thus, there is ample potential for the culture of this species to provide an avenue for industry diversification.

References (cited in this report)

Alexander, Richard R., Robert J. Stanton, Jr., and J. R. Dodd. 1993. Influence of Sediment Grain Size on the Burrowing of Bivalves: Correlation with Distribution and Stratigraphic Persistence of Selected Neogene Clams." *PALAIOS* 8: 289-303.

Christian, J. R. 2010. Habitat Requirements and Life History Characteristics of Selected Marine Invertebrate Species Occurring in the Newfoundland and Labrador Region. *Canadian Manuscript Report of Fisheries and Aquatic Science* 2925: 28-29.

Helm, Michael M. 2004. The Hatchery Culture of Bivalves: A Practical Manual. *FAO Corporate Document Repository*. Fisheries and Aquaculture Department.

Winter, A.G., R.L.H. Deits and A.E. Hosoi. 2012. Localized fluidization burrowing mechanics of *Ensis directus*. *J. Exp Biol* 215: 2072-2080.

Woodin, S. A., S. M. Lindsay, and D. S. Wethey. 1995. Process-specific recruitment cues in marine sedimentary systems. *Biological Bulletin* 189: 49-58.

IMPACTS:

We have no direct impacts to report, as yet. Our work focused on developing methods for the hatchery production of razor clam seed and there has not been time for a pipeline of production to develop. Follow-up work will test methods for seed transfer and field culture and we anticipate that this additional work will support the commercial production of razor clams.

SUPPORT

YEAR	NRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1	93,616						93,616
TOTAL	93,616						93,616

PUBLICATIONS

Flanagan, Molly P., "Investigation of Early Development and Importance of Sediment Choice in the Hatchery Production of Razor Clams, Ensis Directus" (2013). *Honors College.* Paper 111. http://digitalcommons.library.umaine.edu/honors/111

ORAL REPORTS

Devin, M., M. Flanagan, P. Rawson, C. Devin and D. Morse. Variation in Settlement and Early Growth of Juvenile Razor Clams (*Ensis directus*)**.** Northeastern Aquaculture Conference and Exposition, Groton CT, December 2012.

Murphy, D., J. Reitsma. Investigating potential for razor clams, *Ensis directus*, to augment farm profitability: a case study in Massachusetts. World Aquaculture Society Annual Meetings, Nashville TN, February 2013.

Morse, D., P. Rawson and M. Devin. Steady steps toward pilot production of razor clams (*Ensis directus*) and sea scallops (*Placopecten magellanicus*) in Maine and Massachusetts. National Shellfisheries Association annual meeting, Jacksonville, FL. March 31, 2014.

OTHER

The information generated by this research has been incorporated into a business start-up course (Applied Shellfish Farming) taught annually by Co-PI Leavitt at Roger Williams University. He presents an evening of information on alternative shellfish species for culture and has inserted a section on razor clams based on information generated with NRAC support.