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INVESTIGATION OF EARLY DEVELOPMENT AND IMPORTANCE OF SEDIMENT CHOICE IN THE HATCHERY PRODUCTION OF RAZOR CLAMS, *ENSIS DIRECTUS*

by

Molly P. Flanagan

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Marine Science)

The Honors College

University of Maine

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Advisory Committee:

Paul D. Rawson, Associate Professor of Marine Sciences
Sara M. Lindsay, Associate Professor of Marine Sciences
Mark Haggerty, Rezendes Preceptor for Civic Engagement
Emmanuel Boss, Associate Professor of Marine Sciences
William G. Ellis, Associate Director of the School of Marine Sciences

ABSTRACT

Ensis directus, commonly known as the razor clam, is a bivalve species that lives in temperate sub-polar regions of the Atlantic Ocean. It is an infaunal species found in shallow, subtidal, sedimentary habitats. A recent increase in the market value for razor clams has resulted in heightened interest in the culture of this species. The experimental hatchery at the University of Maine's Darling Marine Center began work in 2012 to develop improved hatchery and grow-out techniques for this species. For my thesis, razor clam embryos from both spontaneous and controlled spawns were observed via video and still imagery to document the timing of early development. I obtained additional footage and images of clams during the larval phase through metamorphosis to determine morphological features that are associated with the onset of settlement in this species. I conducted experiments investigating the sediment preference of razor clam larvae and tested methods for improving the settlement rate and early post-settlement survival. Lastly, I determined the burrowing rates of juvenile razor clams to help identify appropriate sediments for nursery phase culture. The results of my research will aid in the development of razor clam aquaculture techniques that can be used by Maine's shellfish culture industry.

This thesis is dedicated to Charlie Slavin, a man who still inspires his students.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to my advisor, Paul Rawson, for his understanding, edits, and time in supporting the development of this thesis. This project would not have been possible without Mick and Chauncey Devin and staff at the Darling Marine Center, who played an important role in the beginnings of this process and data collection. Thanks to my thesis committee for their continual support. Sara Lindsay, thank you for your help in data analysis and suggestions on my burrowing experiments. Thank you to Mark Haggerty for support with my reading list. Thank you to my sister, Abigail Flanagan, who has supported me indirectly and directly via her amazing sketches of razor clams! As always, thank you to my family and friends for their support.

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INTRODUCTION

Ecology

Ensis directus, commonly known as the razor clam or the Atlantic jackknife, is a bivalve species that lives in temperate sub-polar regions of the Atlantic Ocean (Couñago and Tajes 2011, Christian 2010, Kenchington and Duggan 1998). The native range of the Atlantic razor clam extends from Georgia, USA to Labrador, Canada. This species was recently introduced along the northwest coast of Europe where it has been considered an invasive species (Palmer 2004). They are typically found in habitats with sand as the primary sediment (Couñago and Gómez 2011, Kenchington and Duggan 1998) but may also be found in habitats with mud to gravel sediments (Christian 2010). Although razor clams may inhabit the lower intertidal they are quite common in the subtidal zone, and may be found at depths up to 35m (Christian 2010).

Anatomy

Ensis, like other bivalves, has a shell that is made of three layers, the periostracum layer, the prismatic layer, and the nacreous layer (Couñago and Gómez 2011). The shells of clams in the Solenidae family, of which *Ensis* is a member, is elongated and laterally compressed when compared to other bivalves, such as the gaper clam (*Mya arenaria*). The diameter of the shell maintains an even diameter for the whole length of the animal except at the ends; the anterior end is slightly open at all times to allow for the foot to extend out for movement even with a closed shell (Drew 1907). Similarly, the posterior end of the shell has an opening to allow for the inhalant and exhalant siphons to be extended when the shell is closed. The internal anatomy of a razor clam is similar to that of other clam species with the exception of the foot being larger then in other species

(Figure 1C and Drew 1907). Razor clams are highly mobile relative to other clam species. This motility stems from the flexible, muscular foot. A simple diagram of the internal anatomy is presented in (Figures 1A and C) while Figure 1B shows an adult in with developed gonads.

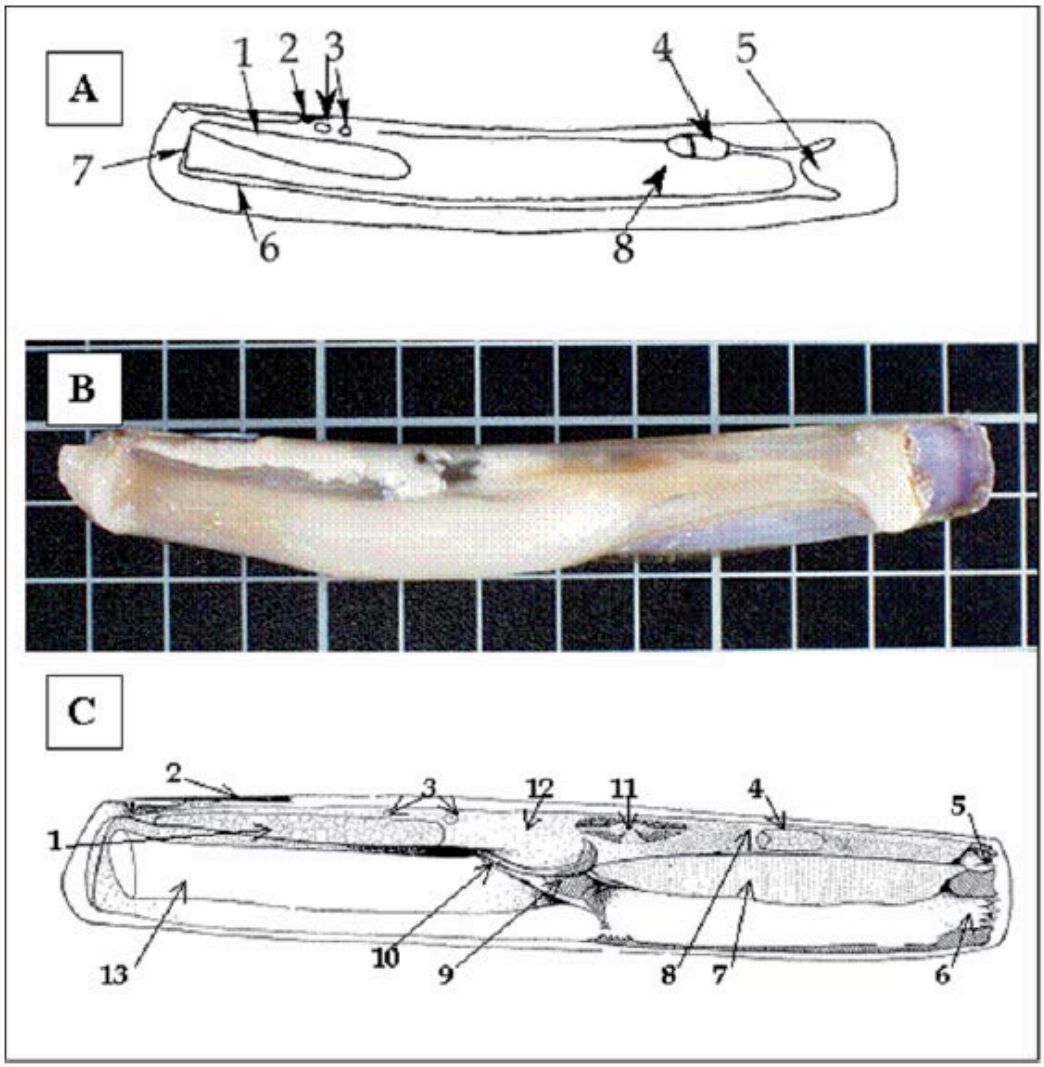

Figure 1: Internal Anatomy of *Ensis directus* as presented by Couñago and Gómez 2011. **(A)** Muscle scars on the internal surface of E. arcuatus bivalves.1: Scar of the anterior adductor muscle; 2: Distal area of the ligament insertion; 3: Scar of anterior retractor muscle of the foot; 4: Scar of posterior adductor muscle; 5: Pallial sinus; 6: Ventral pallial scar; 7: Anterior pallial image and 8: Scar of posterior retractor muscle of the foot. **(B)** Illustration of E. arcuatusesh, in mature stage, where gonad is emphasized. **(C)** Diagram of organ distribution in a specimen of E.siliqua. 1: Anterior adductor muscle; 2: Ligament; 3: Anterior retractor muscle of the foot; 4: Posterior adductor muscle; 5: Exhalant siphon; 6: Inhalant siphon; 7: Gills; 8: Posterior retractor muscle of the foot; 9: Labial palps; 10: Mouth; 11: Heart; 12:Digestive gland; 13: Foot. Taken from Darriba (2001).

The circulatory system of razor clams is open, as is common in all bivalves (Couñago and Gómez 2011). The heart pumps the blood into a series of spaces (sinuses) that act as “blood lakes” from which blood moves its way to the tissue and organs. Members of the Solenidae family have inner and outer gill lamellae in the gill chamber. The gills act not only as the respiratory organ, but cilia on the gills create water currents through the inhaleant siphon by which food enters the animal. The gills are also responsible for selecting particles that are “inhaled” by these currents. The food is then brought into the mouth, down the esophagus, into the stomach, through the digestive gland with wastes continuing to the intestine and out the anus. The mouth creates a ciliary current that sends the food from the gills down the digestive system (Couñago and Gómez 2011). A pair of ganglia with nerves that extend to the tissues makes up the simple nervous system of razor clams. Although bivalves have a pair of kidneys, most excretion takes place through the gills and body surface (Couñago and Gómez 2011).

Reproduction

Ensis directus, like most bivalves, is dioecious, with individuals having either male or female organs (i.e., the testes and ovary). External sexual dimorphism is minimal in these bivalves. (Christain 2010, Couñago and Gomez 2011). Sexual maturity in *Ensis directus* is reached at the end of the first year of life (Cardoso 2009). The yearly reproductive cycle of *Ensis directus* has been classified into five stages (Darriba 2004). Sexual rest (stage O) is defined by the presence of few follicles, or undeveloped gametes, in the gonads. At the start of gametogenesis (stage I), more follicles appear, are larger, and undeveloped sperm and egg can also be seen. The number of follicles continued to increase through stage I and whiter lamina, the visible part of the gonad, can be observed

near the digestive gland. In advanced gametogenesis gametes at all stages of development are present with mature gametes composing the smallest percentage (stage II). By stage IIIA, the gonads are ripe (all gametes are mature) and envelope the digestive gland and part of the foot. Both oocytes and spermatozoids are mature (stage IIIA). Upon spawning (stage IIIB), the gametes leave the enlarged gonads and empty spaces show where mature gametes were before spawning occurred. The onset of restoration (stage IIIC) is characterized by a decrease in the size of the gonad compared to the previous two stages as well as smaller follicles. The loss of gonad biomass continues through exhaustion (stage IV) until the cycle begins again at rest.

In general, the reproductive cycle of bivalve species is dependent on several interacting variables, such as temperature, food supply, and gonad development (Darriba *et al*. 2005). Typically, gonadal mass and gamete development begin as water temperatures increase (gonadal mass) and gametes are released in late April or early May in the Northern Hemisphere. Bivalves spawn as water temperatures increase when gametes are mature (Darriba *et al.* 2005). Reproduction is metabolically expensive and it is critical to an individual's survival that energy is diverted to reproduction only when conditions indicate the greatest success. Body growth (somatic mass) begins again after gamete release and often coincides with the spring bloom of algae, which supports the recovery of the energy lost in reproduction and the beginning of body growth (Cardoso 2009).

Aquaculture

There have been some attempts to culture razor clams, including *E. directus* and other members of the Solenidae. For example, people in China have been seafarming

Sinonovacula constricta, a close relative of *Ensis directus*, for nearly 500 years (Jaxin 1990). Aquaculture techniques are currently being developed for other species of razor clams in China, northern Europe, and Oregon (Jaxin 1990, Breese and Robinson 1981, Costa 2009). Research supporting these efforts has focused on a variety of aspects of the life cycle of razor clams including spawning, larval rearing, settlement, and grow-out techniques.

Objectives of this Thesis

Although the development of grow-out methods for razor clam aquaculture has progressed in Europe and China, there is little information available on larval rearing and early post-settlement culture of razor clams in hatchery settings. High mortality during these stages has precluded the consistent and reliable production of razor clam seed to support the culture of *E. directus* in the northeastern United States. The first objective of my thesis was to identify the external features of razor clam larvae that indicate their readiness to settle from the water column to the sediment. While at the Darling Marine Center in the summer of 2012, I documented the early development of *E. directus* from moment of fertilization through metamorphosis and settlement. For my second objective, I also conducted a sediment preference experiments for settling and juvenile razor clams. Species of infaunal (living within the sediments) bivalves are often found associated with specific sediment types (Compton *et al*. 2009). Several ecological factors can impact suitability of particular sedimentary habitats and clam growth and survival, such as the presence of predators, adequate delivery of suspended food, and the degree to which sediments get resuspended. The depth to which individuals burrow also has important ramifications on growth and productivity (deGoeij *et al.* 1998, Zacklan and Ydengerg

1997). Although the habitat for razor clams is often described as sandy to muddy gravel, these observations pertain primarily to the distribution of large adults while the distribution of juvenile razor clams has not been well described. The abundance of individuals in different sediments has been observed to change with age in several infaunal bivalve species. For example, in the Baltic clam (*Macoma balthica*,) juvenile clams settle in high intertidal mud habitats while adults are more often associated with sandier lower intertidal flats (Compton *et al.* 2009). It is presently unclear whether these patterns are due to age-specific changes in the sediment preference of clams or the result of other ecological processes.

What habitat characteristics are vital to razor clam growth and survival and are these conditions the same throughout the life cycle of *Ensis directus* are key questions; the answer to which will influence the choice of culture sites. There has been virtually no research on the abiotic and biotic processes that structure sediment-abundance associations in this species. The third objective of my research was to examine the burrowing rates of juvenile razor clams when they are presented with sand, mud, and a mixture of sand and mud. A set of burrowing chambers, like those employed designed by Sally Woodin colleagues at the University of South Carolina for monitoring burrowing activity in infaunal polychaetes, was used to observe burrowing (e.g., Volkenborn *et al.* 2010). Similar types of chambers were used by Winter *et al.* (2012) for examining the mechanics of razor clam burrowing. Time-lapse video analysis of burrowing when clams were placed on different sediments in these chambers and was used to estimate the time it takes for clams to initiate burrowing, the burrowing speed and the burrowing depth. An analysis of variance test determined whether the variance in burrowing behavior is

statistically associated with sediment type. The results of this work will aid farmers in the selection of suitable culture sites and whether one sediment type is suitable throughout nursery and grow-out phases of culture.

CHAPTER 1: LARVAL DEVELOPMENT OF *ENSIS DIRECTUS*

INTRODUCTION

Shellfish aquaculture is a thriving industry in the northeastern U.S. The production of cultured shellfish has increased steadily throughout the region over the past several decades, and now includes more than 350 culture operations generating products that value in excess of $50 million (Rhodes *et al.* 2005). The vast majority of farms in the region culture either Eastern oysters (*Crassostrea virginica*) or hard clams (*Mercenaria mercenaria*). Recent work at the Darling Marine Center has focused on developing culture techniques for alternative species, including the razor clam (*Ensis directus*). The razor clam is a bivalve species that lives in temperate sub-polar regions of the North Atlantic. It is commonly found in sandy and muddy-gravel habitats in shallow, subtidal regions. Although not currently cultured, this species is popular in the market and an increase in market value for the razor clam has resulted in greater interest in the farming of this species.

Recent research on *E. directus* and related species on the western coast of the United States, China, Galicia, and Chile have reported on the process of fertilization and developmental stages in razor clams (Costa 2007, Breese and Robinson 1980, Feudendahl 2005, Jaxin 1990). Proper conditioning and spawning of brood stock animals is a critical step for hatchery production of seed for any bivalve. For conditioning, adult animals or brood stock are kept in a controlled environment and feed a lipid-rich diet in order to maximize the number, quality and maturation of gametes. Conditioning can also provide for better management of species with different natural spawning cycles (González *et al.* 2011). For example, Darriba *et al.* (2004 and 2005) found that in Spain,

Ensis arcuatus spawns from January to April while *Ensis siliqua* has its spawning season from May to June. Controlling the maturation of brood stock of these two species allows hatcheries to spawn these two species together. To begin conditioning, adult razor clams are held in tanks with sediment and fed a high density of food at a constant temperature. Feudendahl (2005) conditioned *Ensis americanus* putting them into tanks with 15cm of sediment and holding the tank at a constant temperature.

Conditioning can also provide for better control of spawning. When wild broodstock are brought into the lab it is difficult to detect the level of maturity of their gametes and there is often variable success in inducing spawns from field collected stock. Conditioning followed by the induction of spawning through a variety of means allows for controlled collection of viable gametes (Utting 1997). The method is not foolproof; conditioning of *Ensis* by Feudendahl (2005) supported the maturation of gonads, but the brood stock released gametes before being induced to do so (spontaneous spawn).

Research in a variety of bivalves has investigated a variety of methods inducing controlled spawning (Helm 2004), several of which have been tried with razor clams. These methods include temperature shock, strip spawning, tidal stimulation, and the addition of potassium chloride (Breese and Robinson 1981, Kenchington *et a.l* 1998, González *et al.* 2011). The success of these spawning methods has been variable, and the degree to which any method is successful is likely to be species specific. Kenchington *et al.* (1998) found that in Nova Scotia a temperature shock protocol, which involves slowly increasing from ambient temperatures the water holding the brood animals by several degrees until gametes are released, will induce spawning. Feudendahl (2005) reported that *Ensis* had to be strip spawned in their work in Spain. The West coast species of razor

clams, *Siliqua,* responded to increased food density without need of temperature shock (Breese and Robinson 1981). Currently there is no clearly defined protocol for conditioning and spawning razor clams as there is for other species of bivalves (Helm 2004). Thus, there is a clear need of further work to establish optimal conditioning and spawning protocols for razor clams that allow for the consistent production of high quality gametes.

Early development in razor clams is similar, yet unique to that of other bivalve species. Costa (2007) described the early development of fertilized razor clams in hatchery settings. Like many other bivalves, the sperm and eggs are mixed together in UV-treated water and, post-fertilization, the eggs are sieved to get rid of surplus sperm. To minimize stress during larval development, the temperature in larval tanks is kept at stable (+/- 1-2°C) and Breese and Robinson (1980) suggest that the culture water be changed as often as possible (every other day or daily). Water temperature is a major determinant of the rate of larval development (Helm 2004). Faster development is fostered in warmer water temperatures up the point where it exceeds specie-specific temperature tolerance. González *et al.* (2011) theorized that there is an inverse relationship between egg size and time of embryonic development to the D-shaped veliger stage for razor clam species; the largest egg often has the shortest development time.

Larval development progresses through several stages up through competency, which is where an individual is morphologically and physiologically ready to settle and metamorphose. Helm (2004) describes the general developmental stages of embryonic and larval development for bivalves. By 24 to 36 h post-fertilization; the fertilized egg

has passed through the multicelled blastula and gastrula stages and becomes a motile trochophore, which has cilia surrounding an oval shaped body. As development proceeds, the larvae enter the straight-hinged D stage in which a complete digestive system, two valves, and the velum (a bivalve specific larval feeding organ) have formed. Given the time of the development of the velum, feeding of the larvae typically is initiated about 48 hours post-fertilization (González *el al.* 2011). During the veliger stage, reached between one to two weeks post-fertilization, the shell morphology of individual bivalve larvae generally takes on a species-specific shape. The gills and the foot develop in the beginning of the postveliger stage and at this point the larvae have attained competency and are ready to settle out of the water column. Oyster larvae develop a dark circle on their shell, known as the "eye" at this stage, which indicates readiness to settle. Not all species of bivalve demonstrate such easily discernable "landmarks" of competency and no landmarks have yet been noted for razor clams. At this point, individual larvae settle out of the water column and metamorphose into the benthic, juvenile form specific to each species.

Larval mortality, particularly during the latter stages of development and during settlement can represent a significant bottleneck to the successful production of high quality bivalve seed for culture operations. Recognizing when larvae reach competency and providing suitable substrates for settlement and early post-metamorphic growth and survival are crucial to the seed production process. The first objective of my thesis was to describe embryonic and larval development through settlement for *Ensis directus*. Although work to define developmental stages and "landmarks" of development associated with readiness to settle has been conducted with other razor clam species, it is

not presently clear whether these findings from that work will pertain to *Ensis directus* seed production in Maine. The findings of this research will provide for a better understand embryonic and larval development in *E. directus* and define developmental milestones that will aid in the establishment of hatchery protocols.

MATERIALS AND METHODS

A controlled spawn of *Enisis directus* brood stock was conducted on June 28, 2012. 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, 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 gradual increased by adding heated UVFSW, raised a total of 7°C over three hours. Females were observed to release a string of white pearls at spawning while males released a milky suspension. Six males and three females released gametes at water temperatures between 20°C and 22°C over a 2hr 18min 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.

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 of each spawn was recorded. Tag number was given post spawn.

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 over a period of several minutes, capturing the fertilized eggs while allowing the water used for fertilization and excess sperm to pass through the sieve. 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 progressed through the trochophore stage and entered the D-stage of larval development. During the first 36 hours of development the water was kept at 16°C (+/- 0.5°C). Subsequent stages were kept at 19°C until settlement.

At the end of 48 h, larvae were placed into four tanks containing 350 L of UVFSW at a density of 10 larvae•ml^{-1} 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 µm sieved and the tank water replaced with fresh UVFSW seawater. As the larvae developed, the density in the tanks was gradually reduced to 2 larvae•ml^{-1} 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 tank draining, a small sample of larvae was photographed using an Olympus BX41 Compound Scope and Lumenera Infinity 2-1C 1.4 megapixel digital camera. Ethanol was used to slow the movement of some clams in later stages of development as they were swimming too quickly to be photographed.

On July 10, 2012, the larvae appeared to be reaching competency and nearing settlement as evidenced the fastest growing larvae exhibiting a foot and had reduced motility. The faster growing 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 (see Chapter 2).

RESULTS AND DISCUSSION

Razor clam embryos and larvae exhibited all stages of larval development typical of marine bivalves (see Helm 2004). Embryonic development is shown in figure 2. The first cell division occurred within 1 h of fertilization at 16°C (Figure 2A). Cilia were apparent about twelve h post-fertilization (Figure 2D) and one large cilia was visible 15 h post-fertilization, although a clear image of the cilia was not taken until 20 h post-fertilization (Figure 2E). This observation shows that the larvae reached the trochophore

stage within 24 h post fertilization at a water temperature of 16°C water. Perhaps the most astonishing development began 21 h post-fertilization; at this point there was the continued development of the larger cilia and the initial signs of shell deposition at 24 h post-fertilization (Figure 2F and 2G).

Figure 3 shows major larval stages in razor clam development. Food can be seen in the intestines of all of the clams in these images which is an indication of the general health and activity of the larvae. The larvae remained in the shelled, D-shaped stage until day 5 (Figure 3A and B) and by day 7 there was clear development of the external feeding structure known as the velum (Figure 3C). The appearance of the velum marks the transition to the veliger stage. The development of the larval foot is clearly seen in Figure 3D. As the foot develops, the larvae transitions from the veliger stage to the pediveliger stage. The behavior of the larvae changed with the development of the foot; they spent less time in the water column and a substantial amount of time was spent crawling at the bottom. Larvae which had not developed a foot, remained suspended in the water column and were actively swimming. Generally larvae over 150 μm in size had a well developed foot, which resulted in their removal from the larval tanks and placement in the settlement tanks (see chapter 2) on July 10, 2012. Other than the development of the foot, no external landmarks were observed. Samples of sediment were taken over a period of six days post settlement from the settlement tanks and settled *E. directus* were observed, providing a clear indication of successful settlement. Figure 4 shows a razor clam at approximately 6 d post-settlement. Gills can be seen through the transparent shell and the shell itself has begun to elongate.

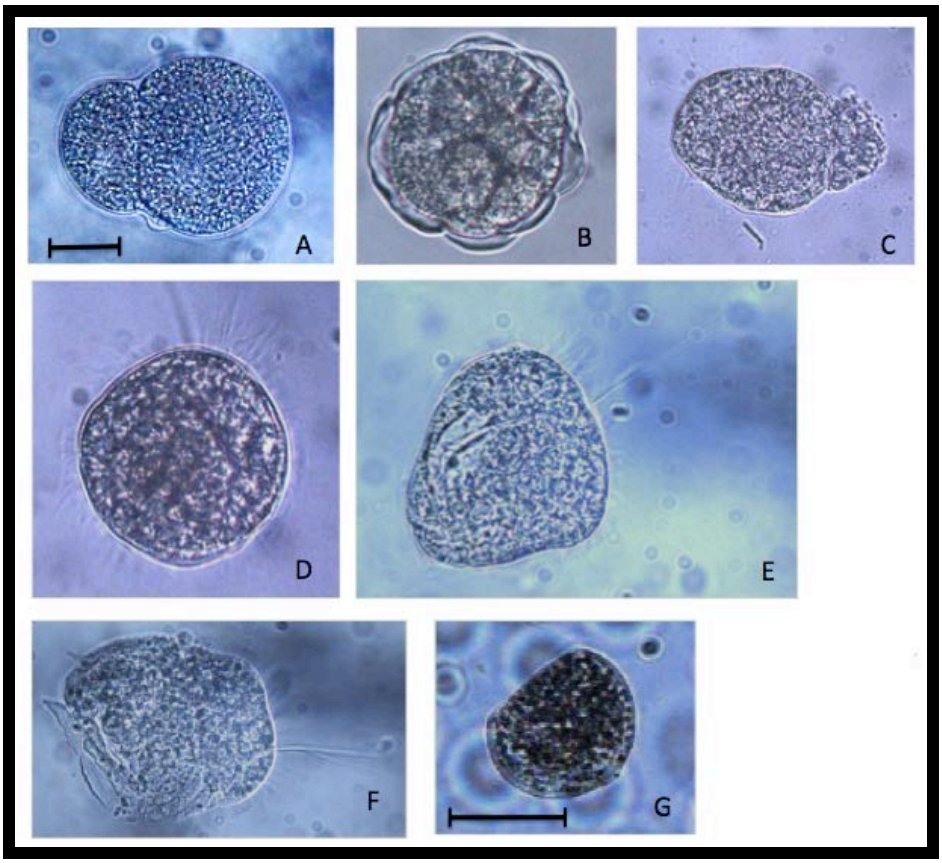

Figure 2: Embryonic development of *E. directus* spawned at the Darling Marine Center on June 28, 2012: A (1 hour post spawn (HPS)), B (2 HPS), C (8 HPS), D (12 HPS), E (20 HPS), F (21 HPS), G (24 HPS). Images A-F were taken at 40X magnification and the scale bar in A represents 10µm. Image G was taken 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.

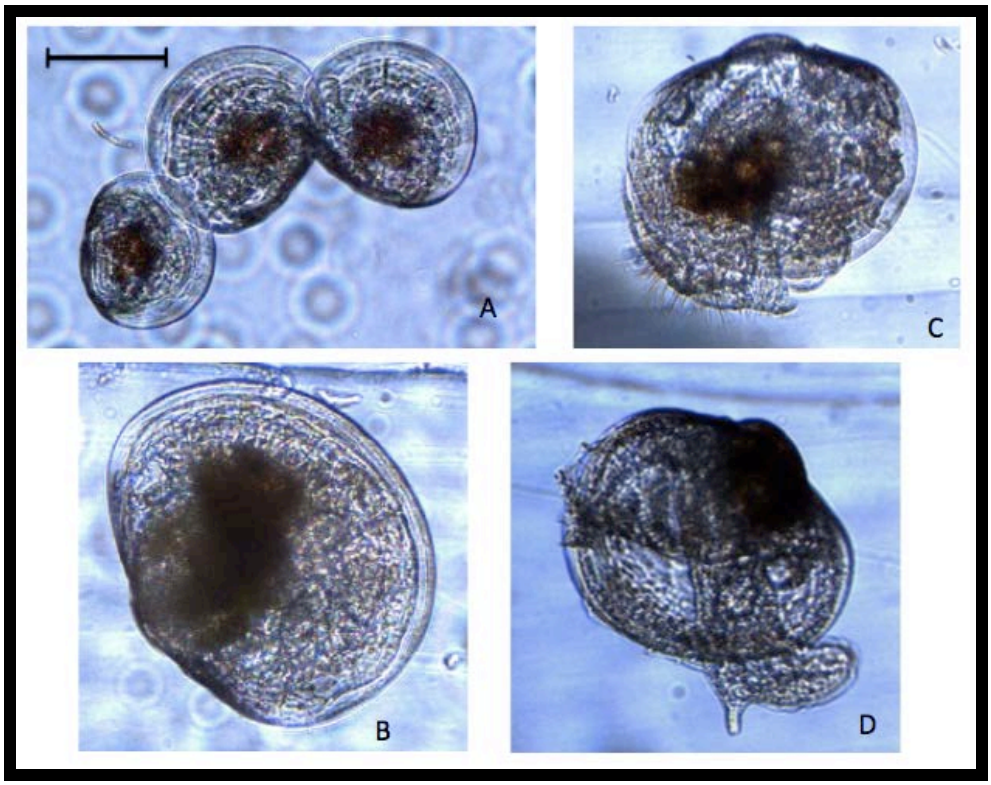

Figure 3: Larval development of *E. directus* spawned at the Darling Marine Center on June 28, 2012: A (5 day post spawn (DPS)), B (7DPS), C (13DPS), D (13DPS). All images were taken at 10X magnification and the scale bar in A represents 50µm. Image A (79 µm) shows three larva, B shows a single larva digital close up, C (117µm) shows a larvae with velum, and D (122µm) shows an early juvenile.

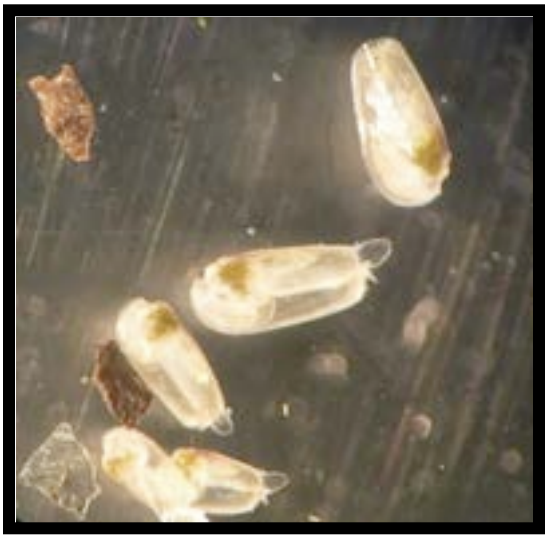

Figure 4: *E. directus* post settlement, courtesy of Dana Morse. Post metamorphic animals are generally less then 2-3mm in shell length.

CONCLUSION

The most prominent and discernible indication that razor clams are ready to settle is the presence of a well developed foot (see Figure 4). In addition, I observed notable differences in behavior as larvae neared competency they stopped swimming and spent more time crawling along the bottom. It is thus my recommendation that the development of the foot be used as a visual marker indicating the readiness of *E. directus* to settle. As described by Helm (2004), other bivalves that are cultured typically have features that indicate readiness to settle, an example being the dark eyespot seen on larval oysters. I found that the foot developed in a substantial portion of larvae by 13 d post-fertilization at 19°C. However, due to logistical constraints I only observed larval clams from one spawn and only at a narrow range of culture temperatures. The rate of larval development is highly dependent on temperature (Helm 2004). Increasing or decreasing the water temperature in which larvae are reared may result in shorter or longer times of development. *Mytilus edulis* (blue mussel) larvae will not grow at 5°C and show slowed development when reared in temperatures ranging 19°C to 22°C (Strathmann, 1987). On the other hand, mussel larvae have rapid development and grow well at 9°C, a temperature similar to the ambient temperature the adult habitat. In order to better understand the timing of development for *E. directus*, future research must examine larval development, survival and settlement success in different temperatures. The ultimate goal of such work will be to identify conditions that will maximize the production of reliable razor clam seed.

CHAPTER TWO: SETTLEMENT

INTRODUCTION

Successful settlement is critical to the production of bivalve seed in hatchery settings. Settlement and metamorphosis occur at the completion of larval development when larvae have reached competency. The duration of larval development varies considerable among species within a genus and even among populations and individuals within species (Strathmann 1987). Key environmental determinants of the rate of larval development include water temperature and food availability. At the completion of the larval stage larvae settle out of the water column, contact the substrate and, if the substrate is suitable, initiate metamorphosis. Competency and readiness to settle and metamorphose in different bivalve species are associated with a variety of physical characteristics. For example, American and European Oysters are classified as being ready to settle by their size and the appearance of an “eye spot”, or a small round darkening on their translucent shells (Helm 2004).

In hatchery settings, it is necessary to provide suitable conditions to induce controlled settlement. This includes identifying critical points in development. For example, once reaching metamorphosis special attention must be paid to larvae as some species have high mortality during or directly after this phase if not presented with suitable settlement conditions. In addition, competent larvae may settle indiscriminately on tank bottoms and sides of culture tanks if not provided with suitable substrates in a timely fashion. Some species, such as blue mussels, can delay metamorphosis if conditions for settlement are not present (Bayne 1976), but such delays can come at an energetic cost and may result in reduced survival and the substantial loss of settled larvae

(spat) and a decline in seed production. As shown by Breese and Robinson (1981) high mortality of larvae in the hatchery is associated with metamorphosis. Settlement also varies considerable between species

Technologies have been developed for some species, such as oysters and clams, to improve the recovery and survival of spat. Downwellers have been found to promote settlement in oysters and are used widely in oyster hatcheries. Downwellers distribute food evenly and relatively constantly to the seed that is held on sieves suspended in the tank, allowing for easy cleaning and access to the spat (see Figure 5). Improving the rate of successful settlement in oysters can be achieved by the use microcultch composed of finely ground cleaned oyster shell as a settlement substrate. This allows the spat to settle individually on the shell pieces rather then on each other; formation of “doubles” and larger clumps of oysters reduces the value relative to individual oysters sold to half shell markets. Settling methods that are used for oysters and other cultured species of bivalves, including other species of clams, have not been used successfully and ideal settlement protocols have not yet been identified for razor class (Breese 1981).

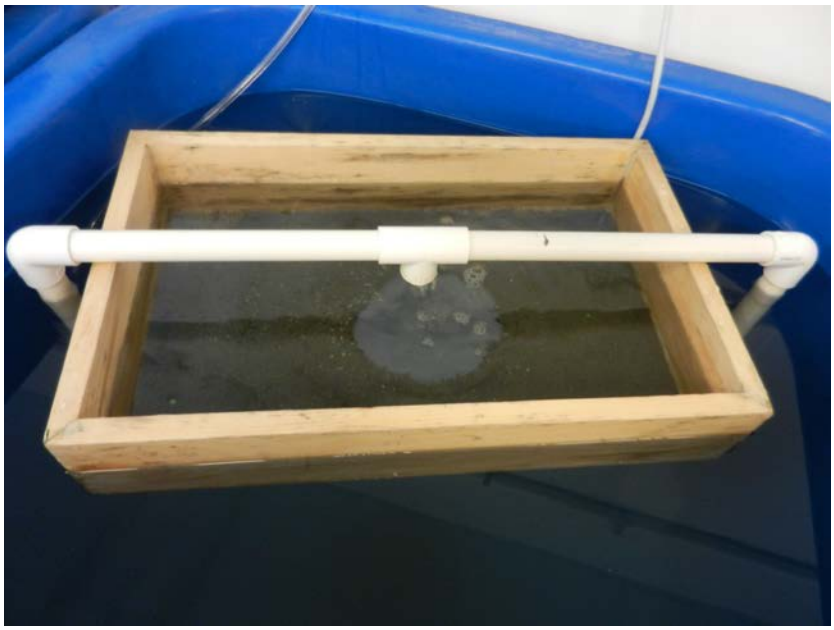

Figure 5: Downweller system containing natural sediment.

Larval development in *Ensis directus* proceeds as for many other bivalves. As described in chapter 1, larvae remain in the water column and after several days, depending upon environmental conditions, develop into veliger and pediveliger stages (González *et al.* 2011). During the pediveliger stage, the foot and gills form and the clams become competent. As described by González *et al.* (2011), *E. arcuatus* reached the postlarval stage 20 d post-fertilization while *E. siliqua* settled after only 14 d post-fertilization.

Several hatcheries have had success in spawning adult razor clams and obtaining large numbers of viable larvae. A key bottleneck in the production of seed has been carrying larvae through settlement and metamorphosis. The downweller system, typical in the production of oyster, mussel, and other bivalves has not been used successfully in the production of razor clam seed. Razor clams are benthic invertebrates and live in sandy and muddy gravel sediments from settlement through adulthood (Christian 2010). Dick Kraus (pers. comm.) of the Aquaculture Research Corporation has suggested that

razor clam spat are prone to bacterial and protozoan infections if not provided with sediment which is not provided by downweller systems.

As adults, razor clams are deep burrowers (Christian 2010). In natural environments, razor clam abundance appears to be correlated with grain size which may be a function of grain-size associated burrowing rates and predator avoidance. Razor clams are filter feeders and extend their siphon to the sediment surface for feeding and excretion. Thus, while clams may burrow to avoid exposure and predators they must also maintain a connection with the surface. The dynamics of razor clam burrowing has been studied by Winter *et al.* (2012), but whether sediment preference or sediment-specific burrowing capacity varies ontogenetically has not been thoroughly investigated in razor clams.

It is unclear whether sediment is an absolute requirement for settlement and post-metamorphic survival for razor clams. Costa (2009) documented that *E. directus* seed can survive without sediment until a shell length of 1 mm and until three months post-settlement. Other research suggests that razor clam larvae must be provided with appropriately sized sediments at settlement in order to improve survival through metamorphosis and production of seed. The second objective of my thesis is to better determine the preference for different sediment types in early post-larval *E. directus*. In developing culture methods for new species of bivalves, ease of culturing must be considered. It is important to maximize the use of equipment commonly employed for other cultured species to minimize expense and increase likelihood that the industry will adopt a new species. Key questions that need to be addressed include, how early sediment must be brought into the culture process, what sediment grain size is best suited for the

razor clams, and what conditions are necessary for minimal loss and greatest net production of seed.

MATERIALS AND METHODS

Settlement preference for three different sediment types and two methods of sediment preparation were determined for competent *Ensis directus*. Natural sediment was collected from Lowes Cove and sifted to remove large masses such as rocks and wood, leaving mainly fine-grained "mud" and silty sediment. Fine sand (play sand) and course 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 30 cm x 15 cm bins were filled with approximately 7-8 cm of one type of sediment were positioned in each 1.2 m x 1.2 cm square tank filled to a depth of 45 cm of UVFSW. These bins were placed into two tanks; the sediments placed in one tank had been further processed by autoclaving while the sediments in the second tank were not autoclaved. The three sediment types were randomly assembled into the ten different bins each containing a randomly assigned sediment type, two of which were larger and split to hold two sediments replicates. Thus, in each tank there were four assigned "bins" for each sediment type (Figure 6). 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 tanks was aerated throughout the experiment and water was changed every two days after the larvae settled.

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. Four downwellers were prepared by fitting 150 µm mesh screen on the bottom of a waxed wooden frame (Figure 5). 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 sieves and counts of total volume and average of shell length were determined. The first measurement of sediment preference was taken 8 week post-settlement. Juvenile *E. directus* have weaker shells when young and the shell gradual hardens with age. A count of individuals in different sediments did not happen prior to 8 weeks because the shells were not strong enough to withstand being sieved out of sediment. The second count of individual's preference for settlement in different sediments was conducted 12 weeks post-settlement. This count also included estimated individuals and biomass.

Figure 6: Autoclaved tank used in sediment preference experiments. Autoclaved tank used in sediment preference experiments. There were 10 bins containing sediments. The larger bins were split into two sections so that there were a total of 4 "bins" for each sediment type (natural, fine sand, and coarse sand).

RESULTS AND DISCUSSION

The settlement, survival, and growth of *E. directus* varied substantially among tanks and treatments. All clams introduced to the tank with autoclaved sediment perished due to an unidentified contaminant that resulted in white film covering the sediment. Mortality was also high in the downweller treatments. All individuals in both the bare downwellers and downwellers with fine sediment died within the first week of post-settlement. In contrast, the volume of *E. directus* juveniles in the non-autoclaved sediments was quite high. When counted 8 weeks post-settlement, the highest volume of juvenile *E. directus* was found in fine sediment while the lowest volume was observed in

natural sediment (Figure 7). 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 weeks post-settlement sampling while the lowest mean length was found in natural sediment (Figure 8). These observations indicate higher settlement and survival in both types of sand sediments and perhaps greater feeding activity and health among the clams in the coarse sand treatment. By 12 weeks post-settlement, the highest volume, largest mean length, greatest estimated number of individuals, and largest biomass was observed in the coarse sediment containers (Figures 9-12). 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.

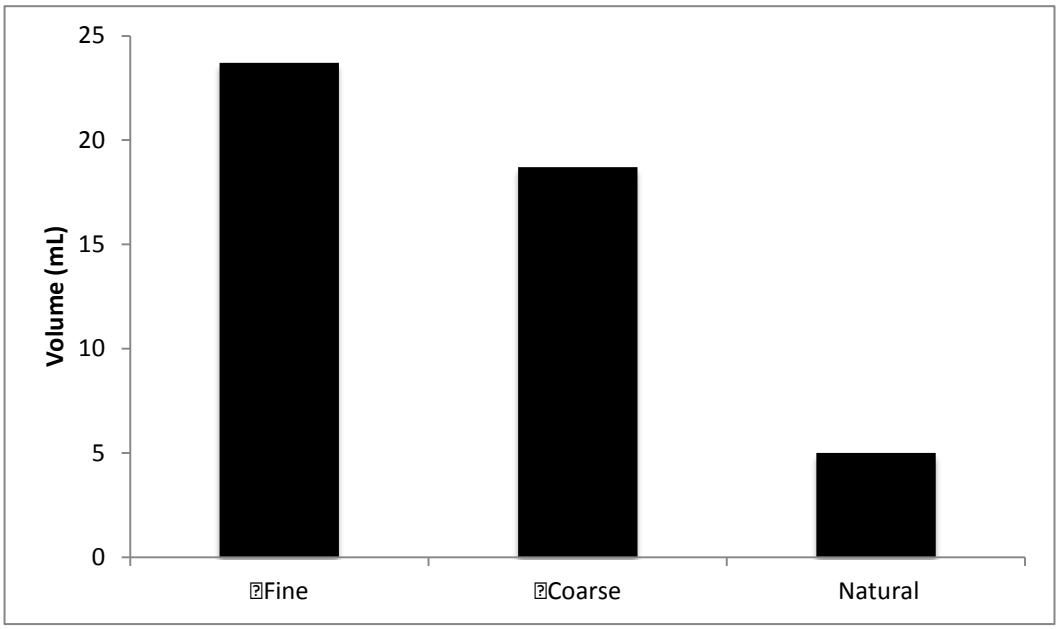

Figure 7: Volume (mL) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank eight weeks post settlement.

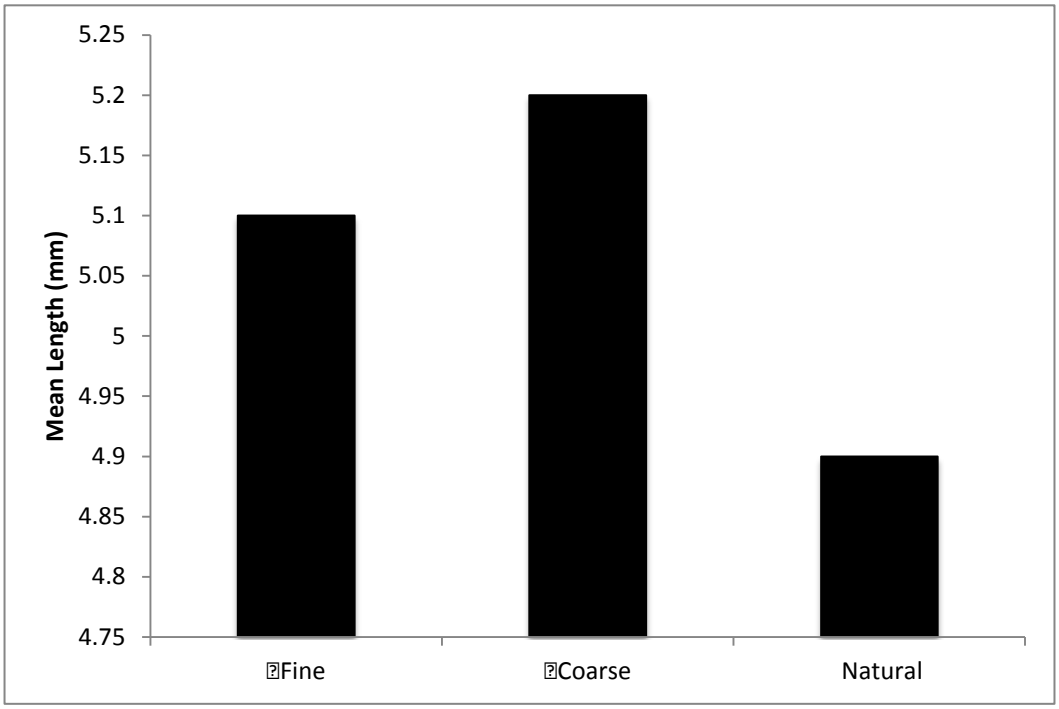

Figure 8: Mean length (mm) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank eight weeks post settlement.

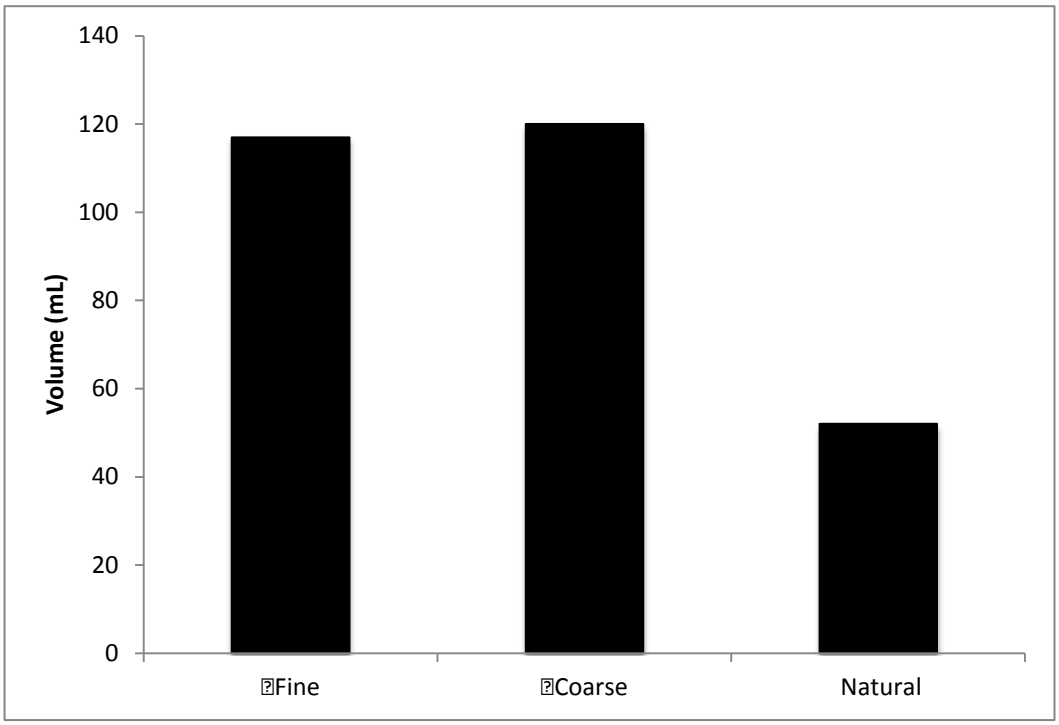

Figure 9: Volume (mL) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank twelve weeks post settlement.

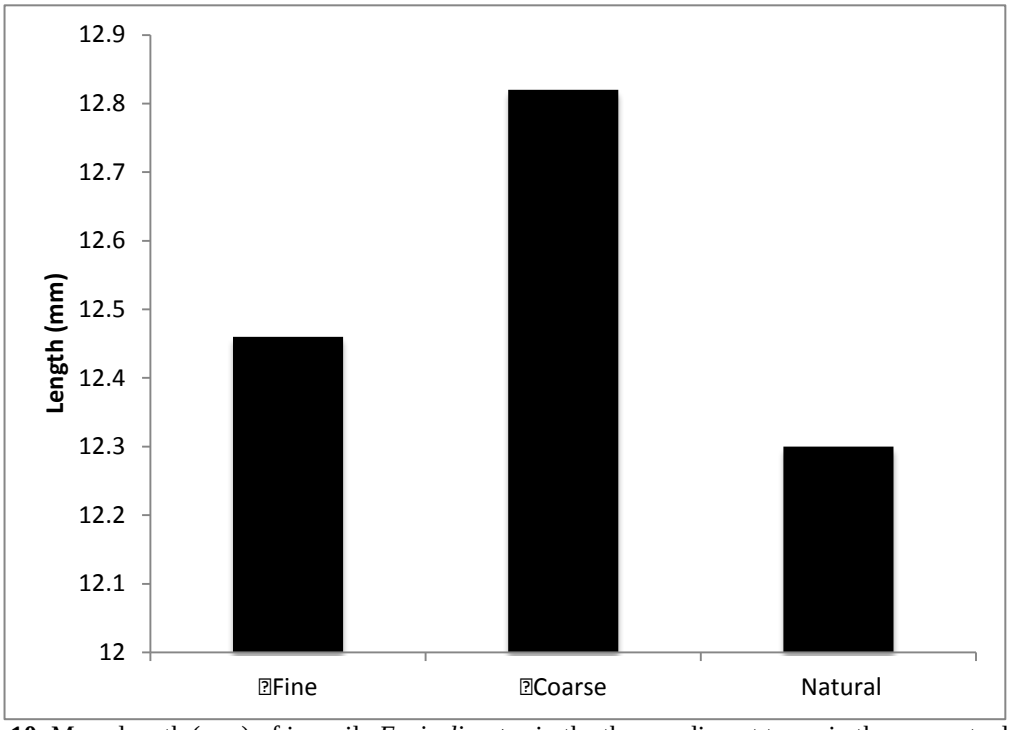

Figure 10: Mean length (mm) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank twelve weeks post settlement.

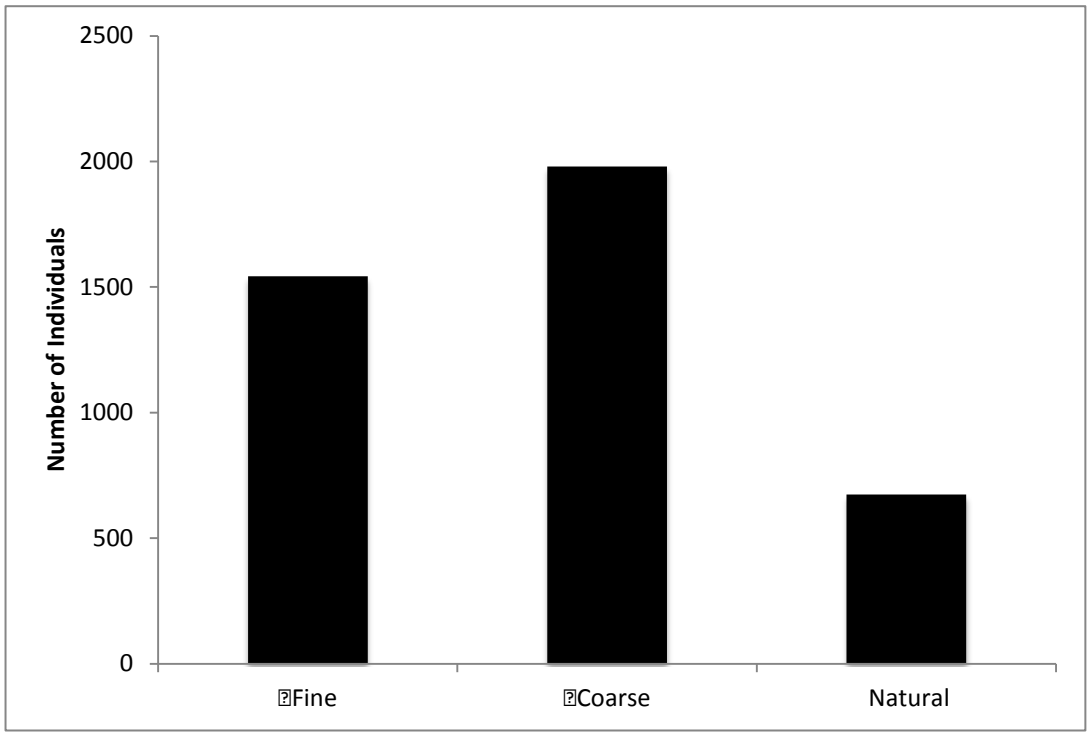

Figure 11: The number of individuals estimated from counts of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank twelve weeks post settlement.

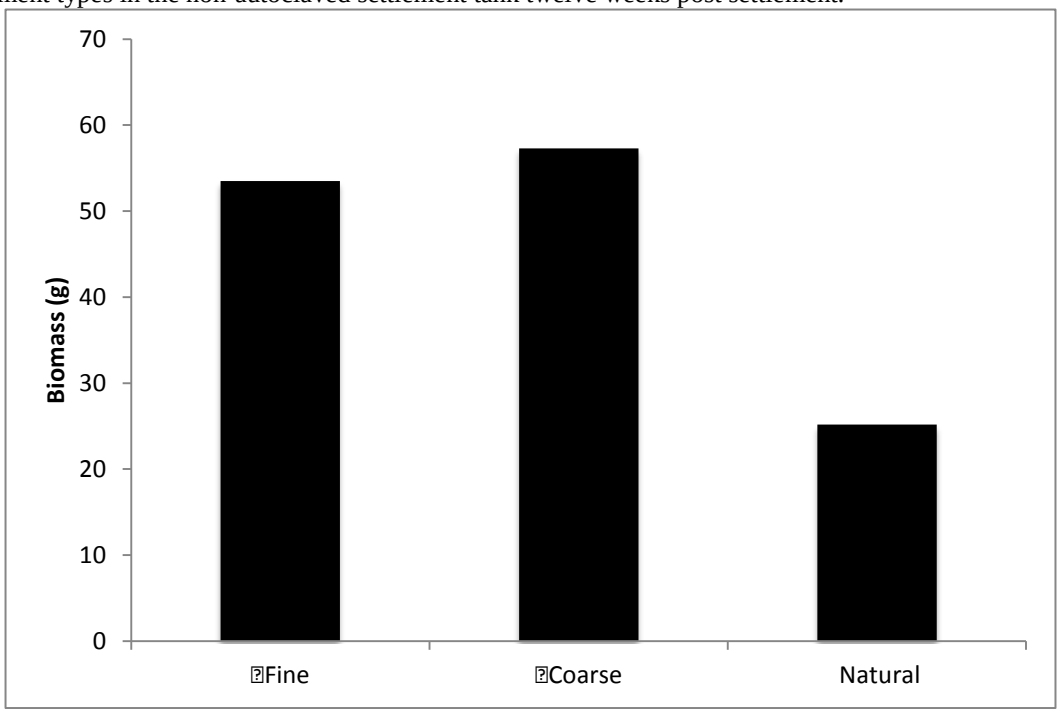

Figure 12: Estimated biomass (g) of juvenile *Ensis directus* in the three sediment types in the non-autoclaved settlement tank twelve weeks post settlement.

CONCLUSION

The results of my 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 may have been a result of contamination or lack of preference for autoclaved sediments. Complete mortality of clams in downwellers indicates that they are unsuitable for early nursery post-settlement for razor clams. 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 in the tank which had non-autoclaved sediments. It is important to note that my experiment 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 my 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 no formal statistical tests of the difference in mean volume and shell size have been

conducted. Thus, my findings should be considered preliminary observations and should be confirmed through additional research.

CHAPTER THREE: GROW-OUT

INTRODUCTION

Razor clams are benthic marine bivalves that burrow into sediments to reduce the risk of exposure and for protection from predators. When burrowed, they extend their siphon to the sediment surface to access the water column for feeding, excretion, defecation, and other activities. Razor clams have a strong muscular foot, which gives them the ability to move quickly through sediments. They can even "outrun" the spade used to harvest them which is a major factor that limits wild harvests of this species (Drew 1907). Sediment grain size influences the ability of *Ensis* to penetrate the sediment (Alexander 1993) and the need for sediments and ontogenetic variation in sediment preference complicates the culture of this species.

Several methods of culture systems have been proposed for *E. directus*. Garcia *et al.* (2011) used long-line, "in pond", and on-bottom culturing methods. Long-line culturing involves using cylinder like pens filled with sediment with small openings in the pen material to allow for water exchange. These pens are hung in the water column at different depths so that clams can feed on a natural supply of food. However, the cost of the material and the amount of sand needed to fill the pens makes this practice logistically difficult and highly expensive. "Pond culture" involves using large tubs filled with sediment held within a bivalve hatchery or seawater lab. While this approach allows for close monitoring of the clams and thus reduces the threat from predators, it requires hatchery space and extra expense to ensure adequate flow and delivery of food to the clams. On-bottom culture involves leasing bottom space in an estuary or coastal region and is the most common method used for clam culture. In this approach juveniles are

simply scattered on the seabed (Jaxin 1990). While this approach eliminates the logistical problems associated with transporting large amounts of sediment and does not require hatchery space, some predators of razor clams, such as the nemertean worm *Cerebratulus lacteus*can, also burrow into the sediment (McDermott 1976). Thus, the grow-out area needs to be protected from fish and bird predators by mesh and from benthic predators by board barriers. At present, however, there are no well established guidelines for site selection due to a lack of knowledge regarding the types of sediments that maximize retention, growth and survival or razor clams at various ontogenetic stages.

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; Figure 13). 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. The objective of the following experiment was to determine whether

the burrowing behavior (i.e., rate) of juvenile *Ensis* varies when the clams are presented with different sediment types

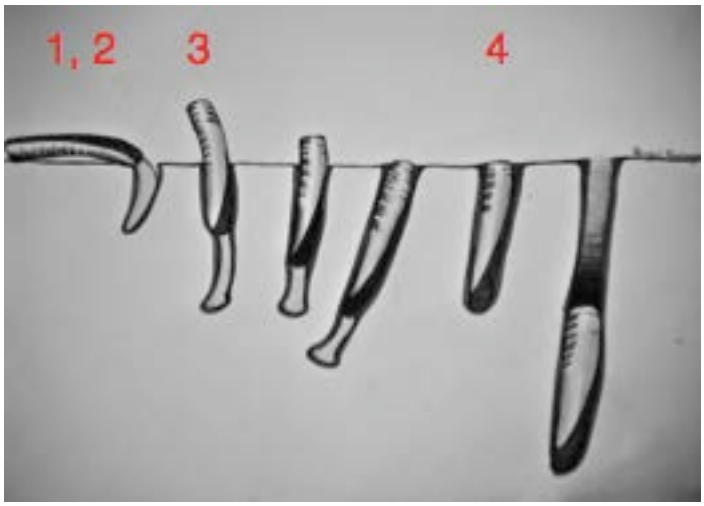

Figure 13: The burrowing cycle of *E. directus* (Abigail Flanagan 2013). Initiation of burrowing (left=1), upright positioning (2), valve contractions (3-5), and completion of burrowing (right=6).

MATERIALS AND METHODS

The burrowing rates of juvenile *E. directus* were compared between three sediment types. Chambers for documenting variation in burrowing behavior of juvenile razor clams were constructed from two 30.5 mm x 30.5 mm x 1.9 mm lexan plates. In between the plates, a piece of 2.5 cm 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 (see Figure 14) and allowed the behavior of clams placed on the sediments to be videotaped. A gentle stream of flowing seawater was passed across the top of the sediment surface during all burrowing trials.

Figure 14: Picture of the burrowing chamber without sediment but otherwise constructed

The burrowing chamber were filled with one of three different sediment types (Table 2), leaving approximately 2 cm of space at the top for water flow and placement of individual clams. Three different sediment types were used; mud collected from Lowes Cove commercial play sand (Figure 15) and a 50:50 mix (by volume of play sand and Lowes Cove mud). The mud was kindly provided by Dr. Sara Lindsay.

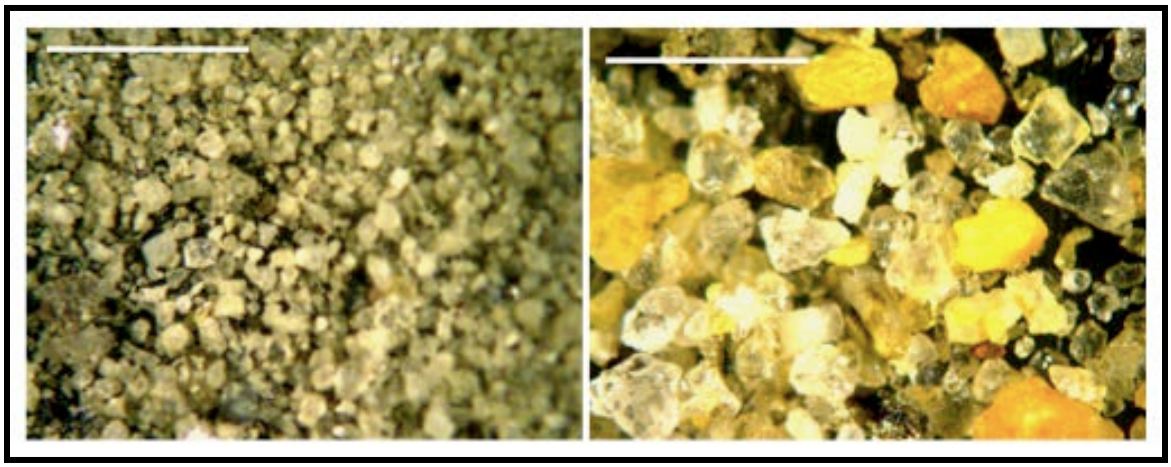

Figure 15: Mud (left) and Sand (right) sediment used in burrowing experiments. The picture was taken with 3X magnification. The scale bars represent 0.55mm. Based on an average of the diameters, found using ImageJ, of ten sediments, the sand grains are estimated to have ten times more area then the mud grains.

Juvenile razor clams (1.20cm-1.85cm 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 (table 2). Although I attempted to complete all of the trials for a single sediment type on the same day, the trials in mud took longer than expected and thus were completed over a three-day period. I used a recirculating seawater system to hold the clams and supply water for the burrowing chambers with the intent of providing constant conditions for all three

sediment trials. However, water temperature varied among the trials as indicated in Table 2.

Sediment Type	Number of *E. directus* counted	Testing Dates	Temperature of Water
Mud	1 9 2	12/5/12 12/10/12 12/11/12	18.6°C 13.2°C 14.1°C
Sand	12	12/3/2012	13°C
Mix	12	12/14/2012	16.5°C

Table 2: Sediment testing dates with observed water temperatures. Burrowing testing was completed in Murray Hall at The University of Maine.

The burrowing behavior of 12 juvenile clams was videotaped in 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. To video tape each clam, I used a SONY Handycam. Each clam was videotaped 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 across all treatments burrowed within the 15-min period and most had completed burrowing by 8 min. At the end of a trial, the individual was removed from the burrowing chamber before the next trial began to limit any potential interference due to the presence of other razor clams in the test chamber. The videos were analyzed using iMovie to determine the time clams spent exploring the sediment, the time required for clams to complete stages of burrowing described above (e.g., start of cycle, upright position, completion), and the timing of any other foot activity.

Each variable was square root transformed prior to conducting single factor analysis of variance (ANOVA). For ANOVA where the treatment effect was statistically significant, a post-hoc test of means (Bonferroni correction) was used to test for differences among the three sediment types. ANOVA was conducted using SYSTAT (version 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.

RESULTS AND DISCUSSION

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 my experiment. Although all individuals used in this experiment were spawned on the same date (June 28, 2012), they were not all the same length. I 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 (Figure 13). 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 I 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 (Figure 16). 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 (Figure 17); a difference that was statistically significant (R x C contingency test; $\chi^2_2 = 6.04$; $p < 0.05$). Combined, these observations provide clear evidence that juvenile razor clams prefer and more readily burrow into coarser sediments.

The treatment-level effects that I observed were reversed for the other two aspects of burrowing behavior that I quantified. Razor clams in the mud treatment spent significantly less time completing the burrowing cycle once they were in the upright position (Figure 18; $F_{2,29} = 4.104$; $p = 0.028$). Similarly, after first initiating burrowing clams in the mud treatment completed burrowing the nearly twice as fast as the clams in the sand treatment and nearly 50% faster than the clams in the mixed sediments (Figure 19; $F_{2,29} = 14.47$; $p < 0.001$). 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.

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 (Figure 15). In this experiment, the bulk of the total burrowing cycle time occurs during the exploration phase (Figure 20). 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 increased growth described in Chapter 2 of this thesis, indicates that hatcheries should use sand or mixed sediments and avoid fine grained mud for the nursery phase production of razor clam seed.

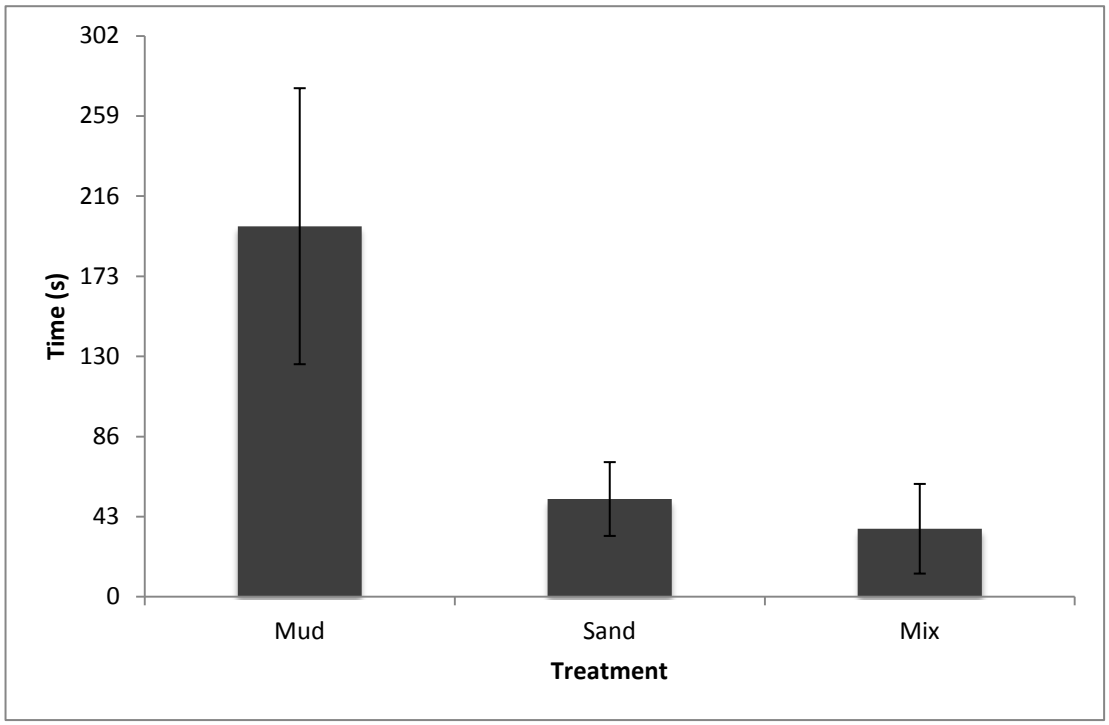

Figure 16: Mean time in seconds (s) from start of exploration to initiation of burrowing cycle for juvenile *E. directus* in mud (n=9), sand (n=12) and mix (n=11) sediment treatments. The start of exploration was defined when foot of the individual was first visible while initiation of burrowing was defined as when the foot pushing into the sediment. Error bars represent the mean +/- one standard error for the untransformed values. The sketch in the upper right-hand corner depicts a visual representation of the portion of the cycle represented in the figure.

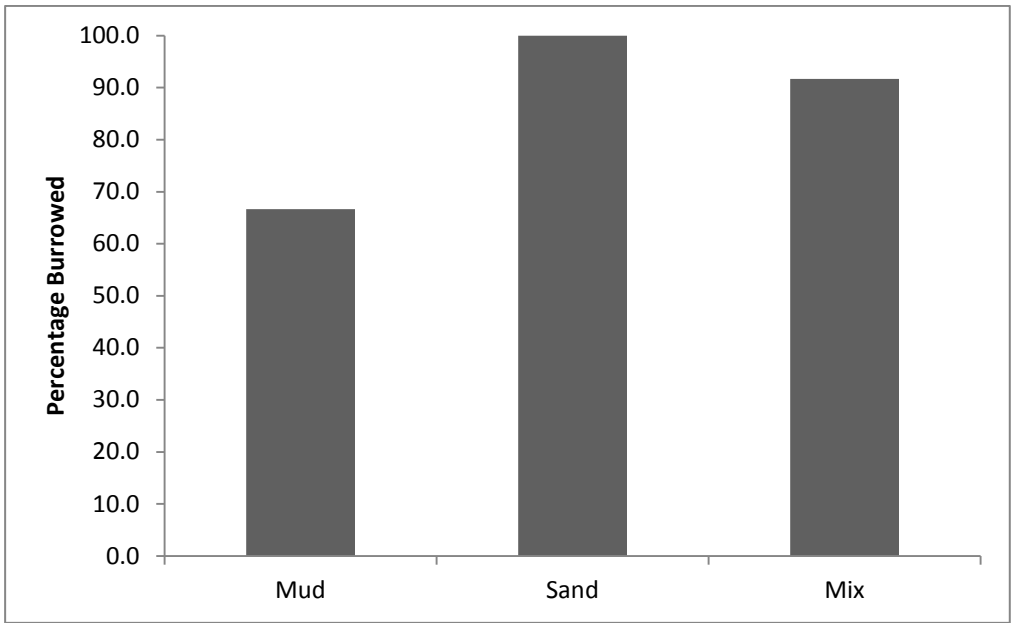

Figure 17: The proportion of juvenile *E. directus* that completed burrowing in 15 min in each of three sediment types (mud, sand, and mixed sediments).

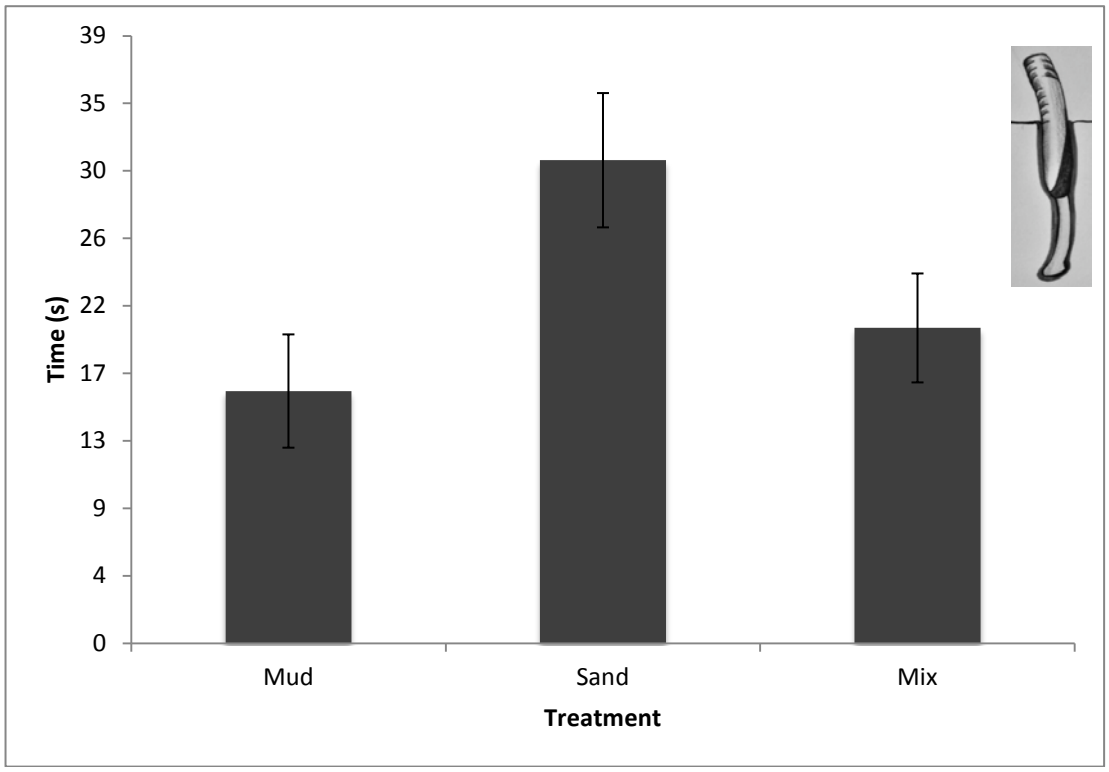

Figure 18: Mean time in seconds (s) between when juvenile razor clams were in the upright position during burrowing cycle and when they completed the burrowing cycle. Error bars are the mean +/- one standard error for the untransformed values. The sketch in the upper right-hand corner depicts a visual representation of the portion of the cycle represented in the figure.

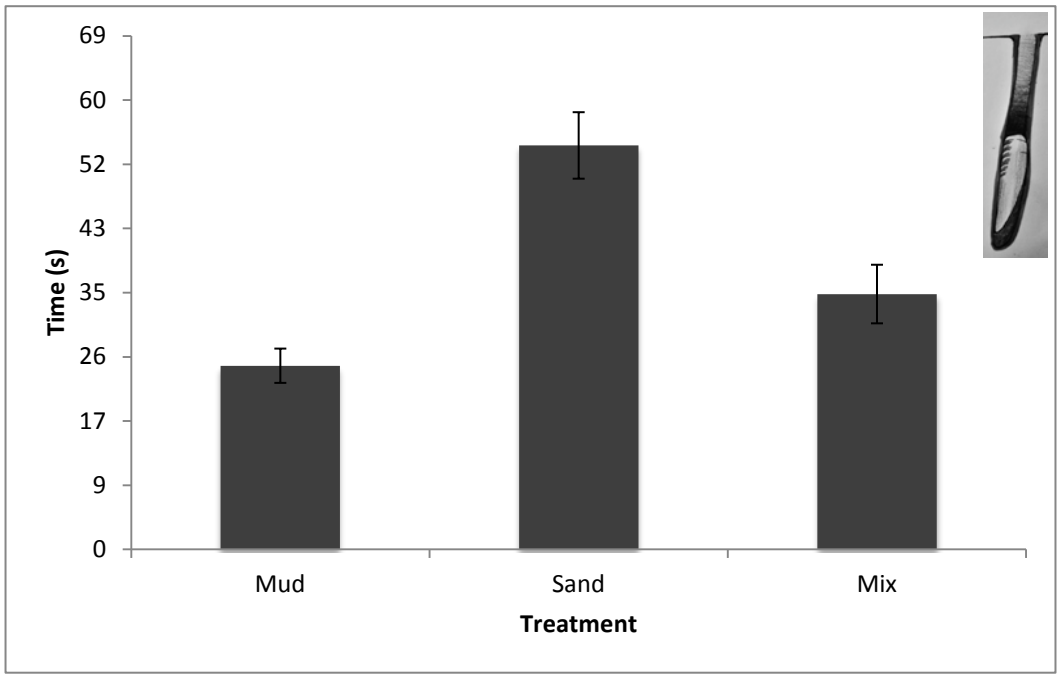

Figure 19: Mean time in seconds (s) between the start of burrowing cycle to completion of the burrowing cycle for juvenile *E. directus* in three sediment types (mud, sand, and mixed). The start of burrowing cycle was defined as when the steps of burrowing are first visible while the completion of the 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 depicts a visual representation of the portion of the cycle represented in the figure.

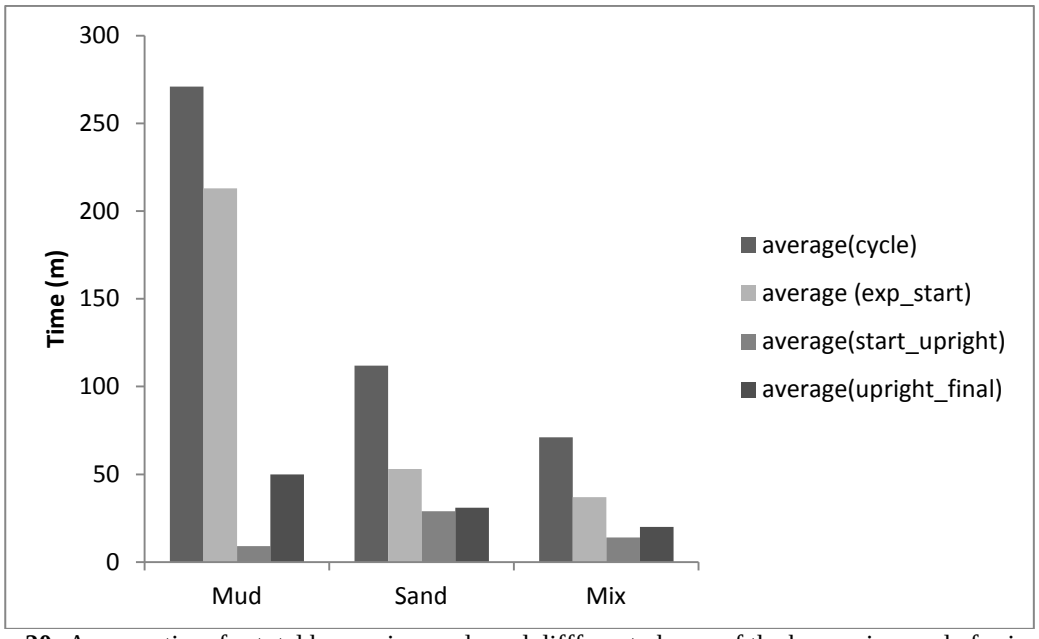

Figure 20: Average time for total burrowing cycle and diffferent phases of the burrowing cycle for juvenile *E. directus* in mud, sand, and sediment treatments. Each average total time was broken into the average times of explore to start of burrowing (exp_start), start of burrowing to upright (start_upright), and upright position to completion of burrowing (upright_final).

CONCLUSION

Based on the differences between razor clam burrowing behavior in the mud, sand, and mixed sediment treatments in the burrowing rate experiment, a mixed or mud sediment composition provides for the fastest completion of burrowing by 6 month-old juvenile *E. directus*. 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 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 type of sediment and original depth of sediment, which influences the redox state of the sediments, resulted in differing burrowing behaviors. Taking sediments from depth and the surface introduces the idea of sediment-borne cues and their influences on the burrowing rates of different species. 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 in this chapter 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.

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AUTHOR'S BIOGRAPHY

Molly Patricia Flanagan was born in Portland, Maine on October 28, 1990. She graduated from Mt. Blue High School in 2009. At the University of Maine, she majored in Marine Sciences, minored in premedical studies, and was a member of the Honor's College. While at UMaine, she was a member of the All Maine Women class of 2013 and a Sophomore Eagle. She also participated in other extracurricular activities and worked as a teaching assistant in the Organic Chemistry Department and the Honor's College. During her undergraduate career, she presented at two National Conference for Honors College events, worked at the Hatchery and the Darling Marine Center, and spent a summer doing nutritional and GI research at the Children's Hospital in Boston and Harvard Medical School. In addition, she played her cello in the University of Maine Orchestra and with her fiddle band Six Days Dry. Molly plans on attending Medical School.