ABSTRACT

Title of Thesis: FERTILIZATION SUCCESS IN THE EASTERN OYSTER *Crassostrea virginica* AND HYDRODYNAMIC INFLUENCES OF OYSTER SHELL ON LARVAL RETENTION

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Natural populations of the eastern oyster, *Crassostrea virginica* typically form dense, vertically-oriented shell assemblages comprised of rough, irregular surfaces which likely influence local water flow, affecting the transit of particles, including gametes and larvae, over them. Since oysters reproduce externally, dense assemblages of simultaneously spawning oysters may maximize gamete interactions before dilution occurs. In the water column, developing larvae may be transported both passively (with large-scale water flow) and/or actively (due to vertical swimming). Once near the bed, larvae may become entrained in interstitial shell spaces among oysters or oyster shells, further increasing the likelihood of settling within an oyster community.

Experiments conducted in this thesis showed fertilization success sharply decreased with increasing distance between introduced gametes in tanks without flow. In addition, more larvae were retained on flume beds covered with shell clumps than those without. Additional flume experiments suggested shell density and shell orientation significantly influenced larval retention.

FERTILIZATION SUCCESS IN THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* AND HYDRODYNAMIC INFLUENCES OF OYSTER SHELL ON LARVAL RETENTION

by

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DEDICATION

In loving memory of my mother, Peggy H. Pavlos
1941-2002

"Reach for the stars Baby, reach for the stars."

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Literature Review

The Eastern Oyster

The eastern oyster, *Crassostrea virginica* (Gmelin, 1791), is found along the Western Atlantic from the Gulf of St. Lawrence in Canada to the Gulf of Mexico, Caribbean, and the coasts of Brazil and Argentina. It is common in coastal areas and estuaries of reduced salinity. Natural *C. virginica* populations occur on hard substrates, both intertidally and subtidally (Carriker and Gaffney 1996). The eastern oyster is of great commercial importance (MacKenzie 1996), and is extensively exploited and cultivated (Andrews 1991; Menzel 1991) around the globe.

However, *C. virginica* is also of great ecological importance (Lenihan 1999). Eastern oysters naturally form dense aggregations and historically were able to maintain very high densities throughout the Chesapeake Bay (Brooks 1891; Alford 1973; Bahr 1976; Kennedy 1989; Hargis 1999). Numerous species of fish and invertebrate organisms depend on oyster reefs for habitat and protection (Breitburg and Miller 1995; Coen and Luckenbach 2000; Rodney and Paynter 2003). Oyster reefs provide three-dimensional hard substrate for benthic organism attachment (Wells 1961; Newell 1988; McCormick-Ray 1988; Kennedy 1996a). Experiments have shown complexity and species diversity of reef communities to increase with increasing oyster reef size (Lenihan and Peterson 1998; Coen and Luckenbach 2000; Breitburg et al. 2000; Harding and Mann 2001). Additionally, the spatial extent of *C. virginica* reefs has been shown to alter flow in the Chesapeake estuary (McCormick-Ray 1998; Kennedy and Sanford 1999). The presence of aggregate shell structure on the bottom produces a turbulent

benthic boundary layer, which leads to increased mixing within the current permitting increased delivery of food (phytoplankton) to the reef (Fréchette et al. 1989; Dame 1996; Lenihan 1999). This enhanced turbulent transport may also be a mechanism through which particles such as eggs and larvae get transported to the bed.

Estuarine Circulation

Another mechanism by which particle transport in the water column can occur is estuarine circulation. The Chesapeake Bay is classified as a partially mixed estuary. An estuary is a semi-enclosed coastal body of water with a free connection to the sea, and within which seawater is measurably diluted by freshwater from land drainage (Pritchard 1967). A partially mixed estuary has a distinct circulation pattern in which a vertical density gradient exists, but moderate tidal flow provides energy for turbulent mixing of fresh and salt water (Kennedy 1996b; Colling 2001). Friction between the estuary bottom and the overlying water column generates more turbulence than simple entrainment at the freshwater/saltwater interface. This can lead to the development of a steep vertical density gradient (Colling 2001).

Steep vertical density gradients observed in the estuary usually indicate salinity differences occurring between overlying water layers. Salinity gradients may be important to larval transport. Oyster larvae are sensitive to changes in salinity and larvae have the ability to actively change vertical position based on detected salinity gradients (Hidu and Haskin 1978; Mann et al. 1991; Dekshenieks et al. 1996). A further explanation of vertical positioning by oyster larvae can be found below in the section on larval transport.

Numerous physical processes such as molecular and turbulent diffusion, tides, storm mixing events, wind-driven currents, Langmuir circulation, internal waves, meso-scale eddies, and large-scale general circulation may also influence estuarine movement (Boicourt 1982, 1988; Yamazaki and Osborn 1988; Okubo 1994). These physical processes cover a broad range of temporal and spatial scales. Some or all of these processes may influence horizontal and vertical transport associated with dispersal and advection of oyster eggs and larvae.

Laminar flow is seldom found in nature (Denny 1988). Over oyster reefs, flow is generally chaotic and turbulent. Turbulent mixing can be either advantageous or disadvantageous for eggs and larvae in the water column. Turbulent mixing is important to organisms reproducing through external fertilization. Turbulence may serve to increase fertilization success by bringing egg and sperm together. However, it can also cause gametes expelled in low concentrations to be dispersed so far apart that fertilization success may be limited (Denny 1988). Likewise, increased water motion may have positive or negative effects on larval transport and retention.

External Fertilization and Spawning Cues

The dynamic nature of the estuarine environment poses many challenges for its inhabitants. One physiological process that can be challenging is reproduction. Many invertebrate organisms are free-spawners, reproducing by shedding their gametes into the water column. Before fertilization can occur, gametes from one individual sometimes must travel centimeters to meters before encountering same-species gametes (Denny 1988). During this time, gametes become quickly diluted in the water column, often to concentrations where fertilization success becomes unlikely (Pennington 1985; Denny

and Shibata 1989; Levitan 1991). It is ecologically disadvantageous for free-spawning organisms to expend energy producing gametes that have a very small chance of being successfully fertilized.

Therefore, many free-spawning organisms use synchronous spawning behavior to maximize fertilization success in the water column (Giese and Kanatani 1987; Levitan 1991; Levitan and Petersen 1995; Hay 1997). This process concentrates gametes in the water column, helping to enhance fertilization success and increase the likelihood of zygote production (Thompson et al. 1996; Hay 1997). The perpetuation of free-spawning marine invertebrates critically depends on successful fertilization (Pennington 1985; Levitan 1988; Denny and Shibata 1989).

Fecund estuarine organisms can detect exogenous factors from the surrounding water column enabling them to synchronize spawning (Thompson et al. 1996). The eastern oyster can use a variety of exogenous factors to induce spawning, including cues from temperature, algal blooms, salinity, and/or the presence of conspecific oyster gametes in the water column. Oysters sense conditions as they siphon surrounding seawater through their gills. It is very important that spawning cues trigger gamete release when surrounding environmental conditions are favorable (Starr et al. 1990).

One spawning cue used by oysters is change in water temperature. Research has been conducted in hatcheries regarding the effect of increasing water temperature on *C. virginica* (Nelson 1928a, b; Galtsoff 1964). This species of oyster can be induced to spawn between 15˚ and 34 ˚C. However, mass spawns in oyster populations most often occur in warm water above 22˚ to 23 ˚C (Galtsoff 1964). Experiments conducted on *C. virginica* showed a sudden rise in temperature of 20 - 30 ˚C instigated spawning in 51%

of males and 32% of females (Galtsoff 1940). There is debate as to if rate of temperature change has a greater effect in inducing spawning than attainment of a critical threshold temperature (Thompson et al. 1996). Orton (1920), Medcof (1939), and Galtsoff (1940) concluded that spawning was preceded by sudden rises in water temperature, yet Butler (1956) demonstrated that rate of temperature change was more important than a critical temperature level being attained.

At the Horn Point Laboratory hatchery in Cambridge, MD, oysters to be spawned are generally left under running, filtered ambient river water approximately 23˚ - 25 ˚C for 1 hour. Subsequently, the temperature is raised to between 27˚ - 30 ˚C, with oysters left to acclimate for another hour while being carefully monitored for the release of gametes. Generally, oysters are induced to spawn as temperature is gradually increased. Surrounding water temperatures can be increased to about 34 ˚C before temperature begins having deleterious effects on egg condition (Galtsoff 1964). Although temperature shock may induce spawning in some individuals of some species, it is unlikely that it is the sole cue for gamete release in free-spawning organisms. Erratic temperature fluctuations in the surrounding environment, especially within the intertidal zone, could lead to unpredictable timing of gamete release (Himmelman 1975). Thus the presence of another cue, suitable phytoplankton blooms, may also help synchronize spawning.

Coupling between phytoplankton blooms and gamete release occurs in numerous species of different taxonomic groups of marine invertebrates (Himmelman 1975, 1981; Falk-Petersen 1982; Starr et al. 1992). Oysters receive signals to spawn via algal ectocrine cues that indicate abundant food availability (Nelson 1955, 1957; Himmelman

1981; Giese and Kanatani 1987). This cue comes from a heat-stable metabolite released by various species of phytoplankton into the water column (Starr et al. 1990). Spawning during food abundance may also aid in reducing predation mortality (Starr et al. 1990). Some evidence suggests that chemical stimuli in the water column influences oyster fertilization at a greater rate than temperature (Galtsoff 1938). Since moderate phytoplankton blooms are the most favorable conditions for survival and growth of planktotrophic larvae, these blooms may be a more reliable spawning cue than water temperature since water temperature can vary unpredictably (Starr et al. 1990).

Salinity is one of the least important factors influencing spawning. The eastern oyster can spawn in various salinities. In estuaries on the Atlantic Coast, salinities are consistently below 15 (Butler 1949). In late spring and early summer, precipitation and river flooding lowers the salinity of the estuary. During this period of decreased salinity, gametogenesis and spawning still occur (Loosanoff 1953). Experiments conducted on *C. virginica* indicated successful gamete production was possible in water of 7.5 salinity, but was impaired at salinity 5 (Loosanoff 1953). Butler (1949) showed that gametogenesis in the eastern oyster was inhibited at salinities close to 0 in 90 % of the surviving population until salinity levels rose above 6. The threshold for deleterious effects due to salinity is most likely found below 5 or 6 (Butler 1949; Loosanoff 1953). Additionally, the rate of mortality of oysters subjected to lower salinities increases with increasing temperature (Loosanoff 1953).

In contrast to salinity, one of the most important and perhaps overriding exogenous factors that induce oysters to spawn is the presence of conspecific oyster gametes in the water column. Eastern oysters reproduce by shedding their gametes into

the water column. Natural populations of oysters tend to form in aggregations. This close proximity allows oysters to detect when neighbors spawn (Galtsoff 1964). Male oysters are generally first to spawn, discharging sperm into the water column (Galtsoff 1964). Gametes expelled by even just one oyster can induce surrounding oysters to spawn (Galtsoff 1964). Female oysters spawn after sensing the presence of male conspecific gametes (Galtsoff 1964). Female sensitivity to this cue may help prevent eggs from being discharged into water where no sperm is present (Galtsoff 1964). It should be noted however, that at the Horn Point Laboratory hatchery eggs have successfully been used to induce spawning in female as well as male oysters.

Mass-spawning occurs when simultaneous gamete release begins and then spreads over the entire oyster community (Galtsoff 1964). Gametes are expelled when the possibility of fertilization is high (Galtsoff 1964; Thompson et. al 1996). Synchronous spawning in the water column therefore may help maximize egg-sperm interactions.

Another advantage of dense formations is that they may help overcome dilution effects in the estuarine environment. As egg and sperm are expelled into the water column, their concentrations per unit volume get diluted, lessening the chance of successful fertilization. Additionally, spawned *C. virginica* eggs begin sinking to the bottom of the water column only a short time after they are spawned (Galtsoff 1964). Thus, male and female oysters spaced close together may also help maximize egg-sperm interactions early on.

Fertilization success has been extensively studied in many free-spawning invertebrates such as sea urchins, sea cucumbers, sea stars, sponges, anthozoans, and

polychaetes (see review by Levitan and Sewell 1998). Yet, few experiments have studied fertilization success in the eastern oyster (see Mann and Evans 1998). Factors influencing fertilization success include spawning synchrony (Pennington 1985; Levitan 1988; Sewell and Levitan 1992; Marshall 2002), dilution of expelled gametes (modeled by Denny 1988; Denny and Shibata 1989; empirical evidence by Pennington 1985; Levitan et al. 1991, 1992), distance between spawned gametes (modeled by Denny and Shibata 1989; empirical evidence by Pennington 1985; Yund 1990; Levitan 1991; Marshall 2002; Metaxas 2002), gamete longevity (Levitan et al. 1991; Williams and Bentley 2002), contact-time of egg-sperm interactions (Levitan et al. 1991), current velocity (Pennington 1985; Yund 1990; Levitan 1991; Levitan et al. 1992) and/or population density (Pennington 1985; Levitan 1991; Levitan et al. 1992; Mann and Evans 1998). Many of the experiments leading to these conclusions have been conducted both in the laboratory and *in situ.*

Laboratory experiments conducted by Pennington (1985) and Levitan et al. (1991) suggested that sperm dilution led to decreased fertilization success. Pennington (1985) found that high percentages of egg fertilization in the sea urchin *Strongylocentrotus droebachiensis* (*S. droebachiensis*) were only achieved using dense sperm suspensions. In suspensions containing greater than 10^6 sperm/l over 80% of the eggs were successfully fertilized, but percent fertilization declined as sperm suspensions were diluted. Essentially no fertilization success was observed in suspensions containing less than 10^4 sperm/l. The threshold where percent fertilization rapidly declined occurred when the sperm was diluted by 6-8 orders of magnitude (Pennington 1985).

A decrease in percent fertilization success was also observed upon diluting sperm from the sea urchin *Strongylocentrotus franciscanus* (*S. franciscanus*). Fertilization success remained high at the 10^2 dilution ($4.7x10^{10}$ sperm/l), but began to decline until the 10^6 dilution ($4.7x10^6$ sperm/l) when only 18% fertilization success was observed. Similar to Pennington's (1985) experiments, no fertilization success was observed past the 10^8 dilution ($4.7x10^4$ sperm/l) (Levitan et al. 1991).

Additionally, field experiments studying the effects of distance between gametes on fertilization success showed fertilization to rapidly decrease with increasing distance from spawning sea urchin males of the species *S. droebachiensis*. These experiments were conducted in San Juan Channel, Washington, where tidal current generally flows in the same direction (≈ 0.2 m/s). Over 90% fertilization success was observed in eggs fertilized directly over a spawning male. At distances greater than 10 cm downstream from the male, less than 25% fertilization success was observed. Fertilization success dwindled to 10% or less when eggs were located over 1 m from the spawning male. Eggs upstream from the spawning male (control) did not demonstrate any fertilization success (Pennington 1985).

Research has also been conducted on the effects of increased and decreased current velocity on fertilization success. Experiments conducted in Lameshur Bay, St. John, U.S. Virgin Islands, under relatively low flow conditions (0.009, SE = 0.003 m/s) also demonstrated decreased fertilization success with increased distance between sea urchin gametes of the species *Diadema antillarum* (*D. antillarum*). Carefully dispensed and monitored free drifting eggs demonstrated approximately 80% fertilization success 0.1 m the sperm source. Egg fertilization decreased to approximately 40% 1 m from the

sperm source and to approximately 10% at 3 m. In separate experiments where eggs were held in Nitex bags 1 m, 3 m, or 5 m away from a spawning male urchin, 18%, 5%, and 4% fertilization success was observed respectively. Fertilization estimates from the collection of free-drifting eggs were thought to be overestimates while data from the Nitex bags were thought to be underestimates. Actual fertilization success values are likely to be within the estimates of these two methods (Levitan 1991).

Yund (1990) made observations of sperm dispersal in the mouth of the Naragansett Bay, RI, at slack tide, regarding the colonial marine hydroid *Hydractinia echinata*. Fertilization success did not decrease significantly over the first 3 m from the sperm source, but decreased significantly beyond 3 m. Negligible fertilization success was found 5 m from the sperm source (Yund 1990; Levitan and Sewell 1998). *In situ* experiments conducted by both Levitan (1991) and Yund (1990) in either low flow conditions or at slack tide found no significant differences between up-current and down-current fertilization success.

Experiments conducted in fast current (> 0.2 m/s) showed fertilization success to be less at all points downstream than in slower currents (< 0.2 m/s). A decline in fertilization success was also observed with increasing distance from the spawning male at both high and low current velocities. Percent fertilization success was generally less than 20% at distances over 1 m, even in the slowest currents encountered (Pennington 1985). From his experiments, Pennington (1985) suggested that higher percentages of fertilization success might be achieved in the field if free-spawning organisms spawned into quiet but not stagnant environments rather than swift-moving water.

Levitan et al. (1992) also observed percent fertilization success to decrease with increasing current velocity in the sea urchin *S. franciscanus*. Fertilization success decreased from approximately 30% at zero current velocity to approximately 10% at a current velocity of 0.05 m/s.

In addition to current velocity and distance between spawned gametes various experiments have also demonstrated fertilization success to be a function of population density (Pennington 1985; Levitan 1991; Levitan et al. 1992). During a simulated mass-spawning, percent fertilization was higher at all distances (10 cm, 20 cm, 40 cm, 60 cm, 80 cm, 1 m, 2 m, 3 m, 4 m, 5 m) downstream from three spawning male urchins compared to experiments conducted on one spawning male urchin. Fertilization decreased with increasing distance from the three spawning males. Less than 50% of eggs were fertilized at distances greater than 1 m from the three spawning males (Pennington 1985).

Levitan (1991) manipulated population density in the sea urchin *D. antillarum* and also found that increased density led to increased fertilization success. Fertilization success increased from 7% to 40% as population density was changed from 1 through 16 males/m^2.

Levitan et al. (1992) examined the effects of levels of aggregation and population size on fertilization success in the sea urchin *S. franciscanus*. Simulated male and female urchins were placed in a grid at densities of 2 or 8 each, located either 0.5 m or 2 m apart. Experiments showed fertilization success to be sensitive to both nearest-neighbor distances and population size. Decreasing the distance between individuals and increasing population size led to an increase in fertilization success by 15% and 12%

respectively.

There are substantial challenges associated with achieving fertilization success as a free-spawning invertebrate. Exogenous factors such as spawning cues may help these organisms maximize their spawning efforts. Additionally, spawning synchronously, in close proximity to others, or in areas with slow current may also help increase the chance of successful fertilization.

Larval Transport

As end products of the zygotes produced by successful fertilization, developing larvae are also influenced by the hydrodynamics of the surrounding water column. Yet there is debate among scientists as to whether larval transport occurs mostly through active or passive processes (Mann 1986; Stancyk and Feller 1986; Mann et al. 1991). Some perceive larval transport to be mainly a passive process, based on physical processes such as tides, wind-driven currents, storm mixing events, Langumir circulation, internal waves, meso-scale eddies, and large-scale general circulation (Korringa 1941, 1952; Pritchard 1953; deWolf 1973, 1974; Andrews 1979, 1983; Boicourt 1982, 1988; Eckman 1990; Okubo 1994; Shanks 1995). Others believe larvae are able to actively exhibit some choice over their location in the water column by swimming vertically (Nelson 1912; Nelson 1926, 1927; Nelson and Perkins 1931; Carriker 1947, 1951; Hidu and Haskin 1978; Scheltema 1986; Mann et al. 1991; Dekshenieks et al. 1996). Yet it may be that both of these processes apply, occurring on different spatial or temporal scales, or under differing flow regimes (Butman 1986; Eckman 1990; Mann et al. 1991; Dekshenieks et al. 1996). Detailed explanations and supporting evidence for each view are mentioned below.

Before settling, oyster larvae develop in a vulnerable, short pelagic larval stage where they are influenced by both macroscale and microscale physical forces from the surrounding water column (Mann et al. 1991; Kennedy 1996b). On a macroscale level, circulation, mixing, and fronts may cause water to move in different horizontal and vertical directions. Passive transport of larvae occurs when macroscale processes overwhelm larval swimming abilities (Kennedy 1996b). Maximum distribution and larval survival are based on length of pelagic stage, and the direction and rate of surrounding transport currents (Scheltema 1986).

On a microscale level, oyster larvae are relatively small and live at very low Reynolds numbers (Vogel 1994; Young 1995; Kennedy 1996b). The Reynolds number (Re), is a dimensionless number represented by the equation: Re = (fluid density x velocity x object's linear dimension)/(fluid viscosity) (Vogel 1994; Kennedy 1996b). At low Reynolds numbers, small sizes and traveling velocities occur, and viscous forces are the predominant forces influencing motion (Vogel 1994; Kennedy 1996b; Crimaldi et al. 2002). Vogel (1994) estimated that a 0.3 mm-long invertebrate larvae would experience a Reynolds number of $3x10^{-1}$, which is noticeably minute compared to the Reynolds number that a large whale swimming at 10 m s^{-1} would experience, on the order of $3x10^{8}$. For larvae facing low Reynolds numbers, surrounding water acts as a viscous medium influencing swimming and food-capturing abilities (Vogel 1994).

Larval transport and retention is also influenced by the benthic boundary layer, a region near a surface (e.g., estuary bottom) where viscous forces result in diminished flow and where flow speed diminishes to zero upon contact with the surface. Boundary layers are thicker at low Reynolds numbers (Vogel 1994; Kennedy 1996b). Vogel (1994)

defined boundary layer thickness as the distance from a surface to the point at which the local velocity equals 99% of the "free stream" or laminar, non-affected velocity. Larvae within the viscous benthic boundary layer tend to be trapped very near the bottom, which may increase the likelihood of attachment to benthic substrate (Kennedy 1996b).

Many researchers have suggested invertebrate larvae are transported passively. For example, Korringa (1952) did not support the idea that oyster larvae could use vertical migration to help them travel up an estuary. He believed that transport occurred through large-scale horizontal transport. Pritchard (1953) stated that within a typical two-layered estuarine circulation system, oyster larvae near the bottom would tend to be carried up-estuary by physical processes. Experiments conducted by deWolf (1973, 1974) on barnacle larval dispersal demonstrated concurring results. He concluded that mechanical processes alone could explain retention of barnacle or bivalve larvae and that swimming behavior was not needed as an additional transport mechanism (Kennedy 1996b).

Andrews (1979, 1983) also argued that oyster larvae dispersal was predominately passive. He conducted experiments on a large number of bivalve species demonstrating similar abundance fluctuations due to changes in tidal cycle. Andrews (1983) proposed that although eastern oyster larvae showed evidence of transport up estuary, there was no need to correlate this transport with active larval participation (Kennedy 1996b). Okubo (1994) suggested that vertical mixing with vertical shear contributed to both horizontal tidal and non-tidal currents, which promoted the spread of larvae in the horizontal direction. A summary of passive transport mechanisms influencing larval transport can be found in a review by Shanks (1995).

In contrast to the idea of passive larval transport, other researchers have proposed that larvae may be able to swim vertically, giving them some choice over their location in the water column (Nelson 1926, 1927; Nelson and Perkins 1931; Carriker 1947, 1951; Hidu and Haskin 1978; Scheltema 1986; Mann et al. 1991; Dekshenieks et al. 1996). Planktotrophic oyster larvae use cilia to swim. These ciliated organisms may be able to exhibit some control over their horizontal movement by positioning themselves vertically so as to control their depth in the water column.

Due to stratification from temperature, salinity and density differences, different layers in the water column may experience different horizontal velocities (Shanks 1995). The net rate of vertical movement for *C. virginica* oyster larvae ranges from 0.8 – 3.1 mm s^{-1} depending on the surrounding water temperature (Hidu and Haskin 1978; Mann and Ranier 1990; Shanks 1995). Swimming speeds of this order of magnitude are sufficient to overcome most vertical movement in the estuarine water column. Thus, larvae that are able to position themselves vertically may then have some control over the layer they are being transported in horizontally.

Active larval transport has been observed in multiple field experiments. For example, J. Nelson (1912) observed more oyster larvae in the water during flood than ebb currents. He suggested that increased larval activity at increased salinities would lead to greater larval rising during flood currents causing larvae to be carried up estuary. Similar observations have been confirmed by other researchers including T.C. Nelson (1926,1955), Carriker (1951), Kunkle (1957), Haskin (1964), and Wood and Hargis (1971).

Additionally, T.C. Nelson (1927) found larvae of eastern oysters, other bivalves, and gastropods to be concentrated above the halocline (area where salinity changes rapidly with depth). Nelson and Perkins (1931) suggested that this phenomenon occurred as a result of larvae detecting and responding to changes in the vertical salinity gradient. Experimentation showed that as larvae descended and encountered higher salinity, they were stimulated to swim upward. Upon contacting lower salinity water, larvae sank back down (Nelson and Perkins 1931; Kennedy 1996b).

Similar to the observations by J. Nelson (1912), both Carriker (1951) and T.C. Nelson (1955) found an increased number of later-stage larvae to be present upstream, while younger larvae were found downstream near the adult beds. The presence of greater numbers of older-stage larvae upstream was thought to be associated with larval sensitivity to salinity and perhaps current velocity caused by flooding (Carriker 1947). Carriker (1947) also found early-stage larvae tended to be distributed homogeneously throughout the water column, while older larvae tended to congregate near the bottom (Kennedy 1996b).

Results from laboratory experiments and a model coincide with results of previously mentioned field experiments (Hidu and Haskin 1978; Mann et al. 1991; Dekshenieks et al. 1996). Hidu and Haskin (1978) found oyster larval swimming speeds to increase with increases in salinity. They also found larval swimming speeds increased with larval size (eyed veliger stage larvae swimming faster than early veliger stage larvae). Mann et al. (1991), however, found umbo stage larvae had greater swimming velocities than straight-hinge and pediveliger larvae. They suggested that these differences were due ontogenic (developmental) changes in larval weight and that at the

pediveliger stage, velum contractions were unable to effectively counter larval mass. These changes may help congregate older larvae near the bottom where they can then begin to search and test the bottom for settlement (Mann et al. 1991).

Additionally, larvae from three coastal mactrid bivalves responded to changes in salinity stratification by changing their vertical position in the water column (Mann et al. 1991). Increased salinity led to increased swimming velocity. Larvae tended to concentrate in areas with high salinity discontinuity regardless of species or developmental stage (Mann et al. 1991). Preferences for salinity discontinuities or higher salinity water may be an advantage in partially mixed estuaries and may help prevent larvae from being transported seaward in lower salinity surface water flow (Mann et al. 1991).

A model designed to study the effects of water column structure on *C. virginica* larvae led to the following results under conditions of well-mixed, partially stratified, and strongly stratified water columns. Under well-mixed conditions, smaller larvae were dispersed throughout the water column. Under strongly stratified conditions, smaller larvae clustered within the halocline. Intermediate size larvae clustered within or just below the halocline. Older larvae were found near the bottom under all salinity conditions (Dekshenieks et al. 1996). Thus, oyster larvae may be able to use depth regulation to position themselves in specific salinity ranges where chances of settlement and adult survival are greatest (Mann et al. 1991).

Despite contrasting views, it is very likely that oysters use both active and passive forms of transport during their larval stage (Butman 1986; Eckman 1990; Mann et al. 1991; Dekshenieks et al. 1996). Several researchers have found that larvae can be

delivered to the bed through a combination of physical processes and larval behavior (e.g., swimming, crawling, burrowing) (Mullineaux and Butman 1990; Butman and Grassle 1992; Grassle et al. 1992; Snelgrove et al. 1993; Crimaldi et al. 2002). Larvae may travel passively along following large-scale horizontal circulation patterns until they are advected close to the bottom. Close to the bottom, larvae are influenced by boundary-layer flow dynamics (Kennedy 1996b). Millimeters from the bottom a "no slip" condition exists where flow speed goes to zero upon reaching a boundary. Within this zone, flow speed is usually low enough to allow larvae to swim effectively (Butman 1986). Vertical positioning can be used by larvae to explore substrates separated by distances of millimeters or even meters. When an organism swims vertically up into the water column or is lifted off the bottom, it is advected into the overlying flow. Eventually it sinks onto a new site downstream. The distance over which larvae can travel is governed by the height at which they start above the bed (Butman 1986).

Settlement

Before settlement, older umbo larvae develop a pair of darkened "eye spots" as well as a foot containing a byssal or pedal gland (Galtsoff 1964; Kennedy 1996b). Oyster larvae demonstrate a "swim-crawl" behavior in which the foot is extended beyond the shell to aid in swimming (Kennedy 1996b). Settlement is defined as a behavioral activity in which larvae explore substrates before attachment occurs (Burke 1983). During this time, attachment is reversible. Larvae can reverse or repeat settling activity until they are satisfied with substrate conditions (Scheltema 1974). There are many physical factors in the environment that may signal *C. virginica* larvae to settle (Kennedy 1996b).

Young larvae are particularly attracted to bacterial surface films (biofilms) that form on oyster shell surfaces. Upon sensing these bacterial films, larvae are stimulated to search the substrate and begin to settle (Tamburri et al. 1992; Kennedy 1996b). As early as 1908, scientists began placing settlement cultch (shell for larvae to attach to) on the bottom for a few days to allow a "slime" or surface film to develop, a procedure thought to attract larval settlement (Nelson 1908; Kennedy 1996b).

One bacterium, *Shewanella colwelliana* was isolated from hatchery tanks containing eastern oyster spat. This bacterium produces L-3,4-dihydroxyphenylalanine (L-DOPA), a melanin precursor known to enhance eastern oyster settlement (Weiner et al. 1985, 1989; Kennedy 1996b).

In experiments conducted by Tamburri et al. (1992), eyed larvae rapidly responded to the presence of biofilm metabolites in still water. Larval responses included: larvae rapidly swimming downward in the water column, slowed swimming speed as the rate of turning increased (focusing activity near the bottom), and attachment to the bottom with their foot indicating settlement (Tamburri et. al 1992). The tri-peptide glycyl-glycyl-L-arginine (GGR) has also been found to induce larval settlement (Turner et al. 1994; Tamburri et al. 1996).

Another physical factor signaling oyster larvae to settle, is the presence of conspecifics (organisms of the same species). Typical of many sessile invertebrates, oyster larvae tend to settle gregariously producing large aggregations of conspecifics. Many believe that gregarious settlement occurs in response to chemical cues released by adult and juvenile conspecifics (Walne 1966; Bayne 1969; Hidu 1969, 1978; Keck et al. 1971; Veitch and Hidu 1971; see review by Crisp 1974; Burke 1986; Pawlik and

Hadfield 1990; Tamburri et al. 1992).

Crisp (1967) showed that the immersion of cultch in tissue extracts of eastern oyster bodies enhanced settlement of larvae on cultch. He also suggested that oyster larvae respond to the proteinaceous component of the surface of oyster shells (Crisp 1967; Kennedy 1996b). Hidu (1969) demonstrated that cultch with 1-day or 2-month old spat attracted significantly more spat than cultch containing no spat. In one experiment, he found that more larvae settled on clean shells held outside a bag containing 2-month old spat than on shells held around a control bag containing no spat. This led him to believe that the spat was producing a water-borne pheromone and that this pheromone was inducing other larvae to settle gregariously (Hidu 1969; Kennedy 1996b). Additionally, Tamburri et al. (1992) found eyed larvae to settle in response to the presence of chemicals released by adult conspecifics in still water. Larval responses were similar to those seen in the presence of biofilms (Tamburri et al. 1992).

Chemicals released by biofilms as well as by adult and juvenile oysters travel as waterborne cues (Tamburri et al. 1992). These cues have a molecular weight between 500 and 1000 Daltons (Zimmer-Faust and Tamburri 1994). If eyed larvae move rapidly, they can change their vertical position near the bottom in response to waterborne cues. Vertical swimming speeds average at 1.58 mm s^{-1} and peak at 3.13 mm s^{-1} in response to waterborne chemical cues (Zimmer-Faust and Tamburri 1994). Zimmer-Faust and Tamburri (1994) found these cues to be peptide inducers of low molecular weight, with arginine (an amino acid) at the C-terminal end. Peptide cues are introduced into the water via excreted, digested, and secreted materials from animals and bacteria (Jumars et al. 1989; Zimmer-Faust and Tamburri 1994). Turbulent mixing can cause cues released

by oysters to become diffusely distributed (Zimmer-Faust and Tamburri 1994).

Experiments conducted under conditions typical of estuarine flow showed oyster larvae to effectively settle in response to waterborne settlement cues (Turner et al. 1994; Tamburri et al. 1996). Oyster larvae settled more often in wells containing chemical cues than in those that did not contain the cue ($p < 0.025$) (Turner et al. 1994). Additionally, even in the absence of a vertical chemical gradient, a condition that may occur in turbulent benthic boundary layers, larvae were still able to respond to waterborne chemical cues (Tamburri et al. 1992).

Physical Nature of the Shell Bed and Shell Orientation

The dominant shell orientation for oysters living on natural reefs is vertical (Bahr and Lanier 1981). This orientation may be beneficial *in situ* allowing oysters to access improved water quality, increased food concentrations and decreased sedimentation. Oysters are autogenic (self-generating) ecosystem engineers. As one generation cements on top of the next, oysters form complex reef structures that can vary drastically from the surrounding soft sediment substrate (Jones et al. 1994, 1997).

There are many advantages associated with a gregarious lifestyle including, increased reproductive success, protection from predation, competitive ability, and filter-feeding efficiency (Knight-Jones and Stevenson 1950; Crisp 1979; Tamburri et al. 1992). Gregarious settlement may also help decrease juvenile and adult mortality (Tamburri et al. 1992).

Oyster reefs represent rough-turbulent boundary layers (Dame 1996). Increased boundary layer velocities can be found over bodies that protrude up into the water column, such as oyster shells, due to the presence of increased roughness elements

(Denny 1993; Abelson and Denny 1997). Increased roughness generated by these shell forms causes the overlying water to increase in velocity, generating random, chaotic patterns instead of laminar, linear ones. Individual fluid parcels move in small-scale eddying paths that may differ in direction from the main large-scale flow (Vogel 1994; Colling 2001). Bottom roughness created by protruding shells may significantly increase the transport of particles such as phytoplankton (Fréchette et al. 1989; Dame 1996; Lenihan 1999) and larvae (Eckman 1990) to the bed, due to enhanced turbulent transport.

Larvae advected near the bed may sense the presence of waterborne chemical cues, further inducing the likelihood of settlement to the bottom (Tamburri et al. 1992). Additionally, competent oyster larvae are negatively phototactic (travel away from light) (Zimmer-Faust and Tamburri 1994) and tend to accumulate near the bottom (Seliger et al. 1982). At the very bottom of the benthic boundary layer, velocity flows slow to near zero producing conditions where larvae are able swim vertically to explore the substrate (Butman 1986).

Additionally, oyster reefs are composed of many interstitial spaces. Pockets of space tend to form in between shells. Once larvae are already at the bottom exploring the substrate they may become entrained within these shell bed interstices. Hydrodynamics within the interstices may further aid in retaining larvae near the shell bed. Oyster larvae are also known to exhibit rugotrophism, settling in the small pits and crevices on the surfaces of oyster shells (Nelson 1953; Galtsoff 1964; Kennedy 1996b).

Some experiments have been conducted on larval retention. Experiments conducted by Breitburg et al. (1995), showed larvae from a benthic reef fish (naked goby *Gobiosoma bosc*) to accumulate in lower current flow wakes found down-current from

large rocks on an oyster reef. Retention was possibly due to either active behavioral preferences for these habitats or because larvae were being passively suspended and retained in these areas. Results from experimentation strongly suggested that aggregation of naked goby larvae in these low current areas was most likely due to an active behavioral response (Breitburg et al. 1995). Breitburg et al. (1995) commented that passive processes might be of greater significance to oyster larval retention since oyster larvae are smaller in size than fish larvae.

Large aggregations of oyster shell may be able to influence flow and retention in ways similar to large rocks. When flow confronts large obstacles such as aggregations of oyster shell on the bottom, areas of both accelerated and decelerated flow develop (Kennedy 1996b). Pyrtherch (1929) found more spat (young oysters) settled on the lee side of settlement substrates placed in the field. He concluded that eddies produced by the various tested settlement substrates reduced current speeds (Pyrtherch 1929). Similar to observations made by Breitburg et al. (1995), areas of reduced current speed may be important to larval retention and settlement.

Mann and Evans (1998) created a model, which estimated *C. virginica* larval retention within the James River, VA. The model estimated loss due to advection only and the cumulative number of competent to metamorphose larvae that were present in each of the cells of a grid overlaying the area of origin and an adjacent region. Run for 25 tidal cycles, the model suggested that larvae were being retained near their region of origin. Results from the model agreed with monitoring data from field surveys. This model did not however incorporate larval behavior into its estimates, which were thought to be minimal due to the lack of density stratification at their location. As mentioned

prior, larval behavior may play a larger role at other locations with steeper salinity gradients (Mann and Evans 1998).

Cementation and Metamorphosis

When an appropriate location is finally found, oyster larvae cement to hard substrate called cultch. During cementation, larvae cement their left valve to the cultch. During this process the pedal gland, which is located in the foot of the larva, produces and deposits a cement-like substance called a tanned mucopolysaccharide (Cranfield 1973a, b, c, 1974, 1975; Kennedy 1996b). The oyster's mantle (folds of the body wall that form and line the shell) and periostracum (thin, unmineralized sheet on shell protecting mineralized valves from corrosion) are also involved with attachment and adhesion to the substrate (Cranfield 1973a, b, c, 1974, 1975; Tomaszewski 1981; Carriker 1996; Kennedy 1996b). Harper (1991) found that the cement used in adhesion was derived from extrapallial fluid (secretion of mixed organic and inorganic substances by the outer mantle epithelium), continuously leaking to form periostracum, which filled in the spaces in between the cultch and the new spat shell. The extrapallial fluid also aided in ensuring that cultch and shell surfaces where close enough to ensure adhesion through electromagnetic interactions (Harper 1991; Kennedy 1996b).

Metamorphosis is an irreversible developmental process that begins with cementation of an oyster larva to the substrate. During metamorphosis, physiological and metabolic changes occur allowing larvae to switch from characteristics suitable for planktonic existence to those more suitable for adult life in the benthos. Some other changes that occur during metamorphosis are loss of the velum (ciliated swimming organ), resorption of the foot, and the development of gills (Kennedy 1996b). As adults,

oysters are sessile (non-moving) invertebrate organisms. Therefore, it is important to adult fitness that larvae settle on appropriate substrate (Grosberg 1987; Levitan 1991; Tamburri et al. 1996).

Summary

There are many factors that influence successful fertilization, transport, settlement, and larval retention in the eastern oyster *C. virginica*. The Chesapeake Bay estuary is a dynamic environment and these free-spawning invertebrates can use a variety of cues such as temperature, algal blooms, salinity, and/or the presence of conspecific oyster gametes in the water column to help increase the likelihood of successful fertilization and settlement. Additionally, larval transport and settlement may occur through a combination of both hydrodynamic processes and larval behavior. Once near the bed, eggs and larvae may further become retained in shell interstices.

While great strides have been made by many scientists to understand these complex processes further experimentation is still necessary to help fill in the gaps of our scientific knowledge. The eastern oyster is a valuable species both commercially and ecologically. Continuing to build a greater understanding of the processes that influence each life stage may have important ramifications including the development of new restorative techniques to help preserve this species.

Chapter 2

Introduction

The eastern oyster *Crassostrea virginica* (*C. virginica*) is a keystone species having profound ecological influence in the Chesapeake Bay estuary. This species is also very important commercially, but faces significant pressure from over-harvesting, habitat loss, and two major diseases *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) (Horton and Eichbaum 1991; MacKenzie 1996; Paynter 1996). Oyster reefs serve as filters in the Chesapeake Bay removing large amounts of phytoplankton from the water column (Lenihan et al. 1996; Jackson et al. 2001). Oyster reef shell structure provides habitat and predation refuge for numerous species of fish and invertebrates (Breitburg and Miller 1995; Coen and Luckenbach 2000; Rodney and Paynter 2003), yet the structure of oyster reefs *per se* has not been thoroughly examined.

Oysters are free-spawners; they cast their eggs and sperm into the water column. Fertilization success has been extensively studied in many free-spawning invertebrates such as sea urchins, sea cucumbers, sea stars, sponges, anthozoans, and polychaetes (see review by Levitan and Sewell 1998). Factors influencing fertilization success include spawning synchrony (Pennington 1985; Levitan 1988; Sewell and Levitan 1992; Marshall 2002), dilution of expelled gametes (modeled by Denny 1988; Denny and Shibata 1989; empirical evidence by Pennington 1985; Levitan et al. 1991, 1992), distance between spawned gametes (modeled by Denny and Shibata 1989; empirical evidence by Pennington 1985; Yund 1990; Levitan 1991; Marshall 2002; Metaxas 2002), gamete longevity (Levitan et al. 1991; Williams and Bentley 2002), contact-time of egg-sperm interactions (Levitan et al. 1991), current velocity (Pennington 1985; Yund 1990;

Levitan 1991; Levitan et al. 1992) and/or population density (Pennington 1985; Levitan 1991; Levitan et al. 1992; Mann and Evans 1998). Many of the experiments leading to these conclusions have been conducted both in the laboratory and *in situ*.

A dearth of information exists regarding fertilization success in the eastern oyster, *C. virginica* (see Mann and Evans 1998). Few experiments have examined the role that oyster density and/or shell bed structure might play in the retention of oyster larvae near the bed (see Tamburri et al. 1992, 1996; Turner et al. 1994 about attraction to the bed due to waterborne chemical cues). The oyster density at which maximum retention of larvae in a shell bed occurs is unknown.

Understanding factors influencing fertilization success and larval retention may be of great importance to perpetuating the oyster and many other free-spawning species. Thus, to elucidate some factors surrounding fertilization success and larval retention in the eastern oyster I designed and conducted a series of laboratory experiments to study the effects of distance between gametes on fertilization success and the effects of shell density and shell orientation on larval retention.

Materials and Methods

Gamete Separation and Fertilization Success Under No Flow

Experiments on eggs and sperm were conducted June - August 2000 at Horn Point Laboratory Oyster Hatchery in Cambridge, Maryland. Oysters were spawned in nine salinity water, 23˚ to 26 ˚C. Three, 200 L tanks (120 cm x 32 cm x 52 cm) were used to test the effect of gamete separation on fertilization success. Each tank was filled with 18 L filtered Choptank River water at 24 ˚C. Male and female gametes mixed together in small beakers served as controls representing fertilization success uninhibited by distance.

Oyster spawning was initiated on a spawning table. On average, each day, 100 oysters were placed on the spawning table to be spawned. Oyster shells were first gently cleaned, then scraped with an oyster knife to remove barnacle fouling. Fouling was removed to prevent barnacle predation on oyster larvae.

Cleaned oysters were held in ambient Choptank River water for 1 h, then temperature was raised to 30 ˚C and oysters were left at this elevated temperature for another hour. The gradual rise in temperature sometimes induced oysters to spawn spontaneously. If oysters did not respond, an oyster from the set was shucked and its gametes collected. Gametes were transferred to a small beaker and slightly diluted with 30 ˚C river water to make a solution. One ml of the gametic solution was squirted into the vicinity of the oysters. Some of the gametic material from the shucked oyster was placed under the compound microscope to determine if eggs or sperm were being used to induce the spawn. After sensing the presence of gametes in the water, some oysters spawned.

Oysters were sexed based on their spawning behavior. Female oysters expelled eggs by clapping their upper and lower shell valves together. Each clap or pulse would send a strong stream of eggs into the water column. Male oysters on the other hand constantly ejected a thin, milky-white stream of sperm (Galtsoff 1964). As soon as their sex could be determined from their spawning behavior, oysters were removed from the spawning table and male and female oysters were put into separate bins. To minimize contamination from the spawning table, male and female oysters were each allowed to spawn in three separate bins filled with 30 ˚C river water before the gametes for the experiment were collected. In each bin, female oysters were allowed three pulses and males were allowed to spawn continuously for ten seconds. Experimental gametes were selected from a fourth bin, with minimal opposite sex gamete contamination. For all experiments, fertilization success present in the fourth bin was quantified.

To minimize issues of incompatibility between egg and sperm, spawned gametes from about three male and three female oysters were used in each trial. A control beaker containing 10 ml of egg solution and 10 ml of sperm suspension with estimated concentration of half a million eggs to one million sperm was prepared. This ratio of gametes was thought to increase the probability of fertilization success and minimize polyspermy (more than one sperm fertilizing a single egg) (Stephano and Gould 1988). For each trial, gametes introduced directly into a control beaker without separation and mixed represented maximum fertilization success.

Ten ml of egg solution containing approximately half a million eggs and 10 ml of sperm suspension containing approximately one million sperm were simultaneously introduced 10 cm, 50 cm, or 116 cm apart in 200 L tanks without flow (Fig. 1). Each

experimental distance was run in a separate tank. Six replicate experiments were conducted.

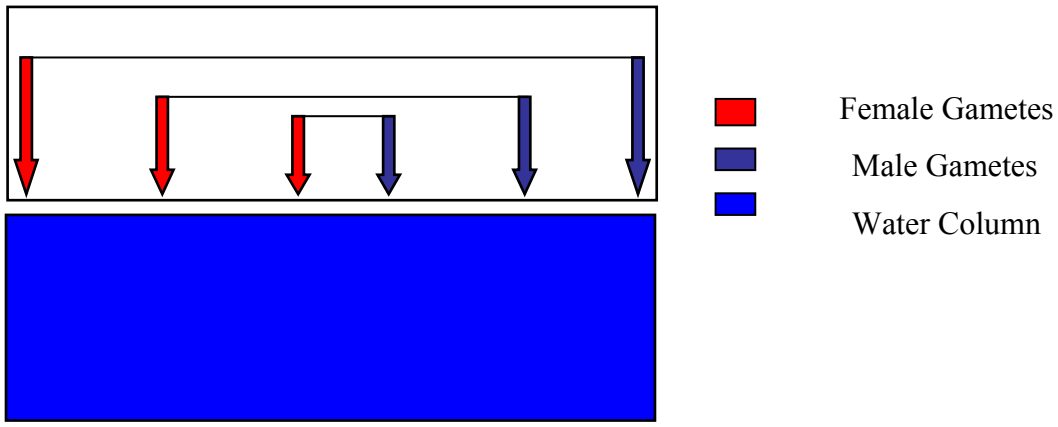


Figure 1. Experimental distances at which male and female gametes were introduced into the water column (10 cm, 50 cm, 116 cm). Each experimental distance was conducted in a separate tank.

After the spawn, every thirty minutes for 90 minutes, a beaker containing unfertilized eggs taken from the fourth bin was sampled to detect if any fertilization had occurred. After 70 minutes, the control beaker was surveyed for fertilization success. This time period was chosen because the first stage of cleavage was usually clearly visible by this time (Galtsoff 1964). Tanks were drained after 2 h, since eggs were predicted to remain viable for only 1 h after the spawn (Nelson 1891). However, it is thought that fertilization may still occur slightly past the estimated hour (Galtsoff 1964; Stephano and Gould 1988). Experimental tank water was drained through a 10 µ plankton net and eggs were placed in a container before bringing the final volume to 100 ml. Six ml of this sample was preserved in 1 ml of 10% formalin.

Two, 1 ml samples of preserved eggs from each experimental distance were surveyed for fertilization success in three columns of a Sedgewick rafter slide (two columns near the edges and one in the middle, each column consisting of 20 cells). Approximately 70 eggs were surveyed via compound microscope from each slide. Eggs were counted as successfully fertilized if they reached trefoil configuration or beyond by the end of the experiment. Trefoil is the first visible stage of cleavage, where eggs appear to consist of three cells (Galtsoff 1964).

Percent fertilization success at each distance was calculated by dividing the number of successfully fertilized eggs by the total number of eggs (fertilized and unfertilized) observed in six columns (3 columns from each sample, each column consisting of 20 cells) and multiplying by 100. Corrected values for fertilization success were calculated by subtracting the percentage of fertilization success in the fourth bin (residual contamination from spawning table) from total percent fertilization success at each of the varying distances. Three trials were eliminated from analysis because eggs from the fourth bin did not meet the criterion of demonstrating less than 45% fertilization success.

A one-way ANOVA was used to test the effect of distance on mean percent fertilization success (SAS version 8e, SAS Institute Inc., 2000).

Egg Experiments Under Flow

As an extension to the fertilization success experiments mentioned prior, flume experiments were designed to study the effects of unidirectional flow on fertilization success. Additionally, studies were proposed to determine the effects of unidirectional flow on the transport of *C. virginica* eggs and the retention of eggs by oyster shell under

flow conditions. These experiments were unsuccessful, as *C. virginica* eggs were unable to withstand shear stress associated with exiting the flume.

Larval Retention by Shell Clumps Under Flow

Umbo Larvae

Unidirectional flow was set-up in a re-circulating raceway flume (550 cm x 35 cm x 25 cm) at Beltsville Agricultural Research Station, Beltsville, MD. The flume consisted of Plexiglas panels making it easy to observe particle transport over the flume bed. Re-circulation was carried out by two jacuzzi pumps that transported water from a collection bin (≈ 106 L) at the weir end to two head tanks (≈ 95 L each) located in the ceiling. The flume itself held approximately 352 L of water. This flume has also been used in the following experiments: Palmer (1992), Palmer et al. (1992), and Turner et al. (1994) (Fig.2).

Oyster clumps, retrieved by divers from restored *C. virginica* reefs in the Chesapeake Bay were cleaned, shucked, bleached and dried to remove possible chemical cues (Fig. 3). A Sontek® 10 MHz Acoustic Doppler Velocimeter (ADV) probe was used to measure horizontal flow velocity. Flow velocity was then adjusted manually to approximately 1 cm/s for each experimental trial. A horizontal flow velocity of 1 cm/s was chosen as an estimate of slow tidal flow in the Chesapeake Bay. Two differing bed types, beds of clumped shell or bed with no shell were examined. Figure 4 shows an example of a typical shell clump. Horizontal flow velocity was manually adjusted to 1 cm/s for both beds. Umbo stage (≈ 100 μ) *C. virginica* larvae were tested in the flume to determine their retention as they passed over beds of clumped shell or no shell. All larvae were produced by the Horn Point Laboratory Oyster Hatchery. Flume water was

adjusted to the same salinity in which the larvae were reared (8 to 10) using Instant Ocean synthetic sea salt. Flume temperature ranged from 19 - 22 °C.

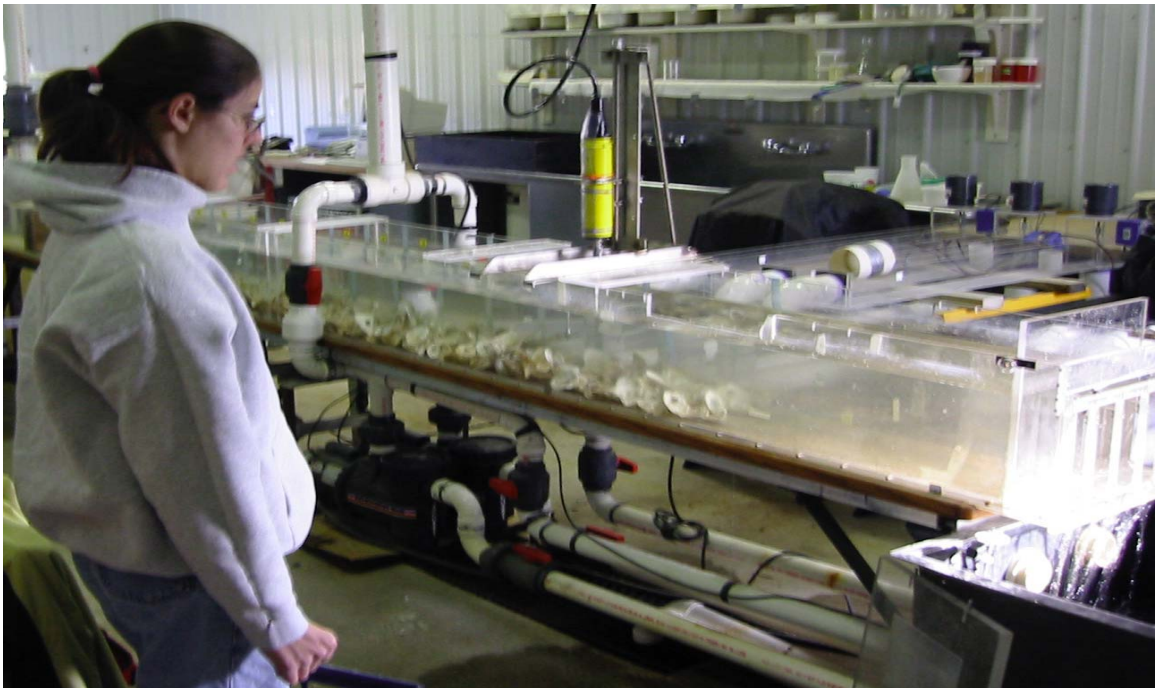


Figure 2. Flume setup at the Agricultural Research Station in Beltsville, MD. A Sontek® 10 MHz ADV probe is suspended above the flume working section. This probe was used to measure horizontal flow velocity so that it could be adjusted manually to approximately 1 cm/s for both bed types. Here, the working area of the flume is covered with shell clumps.

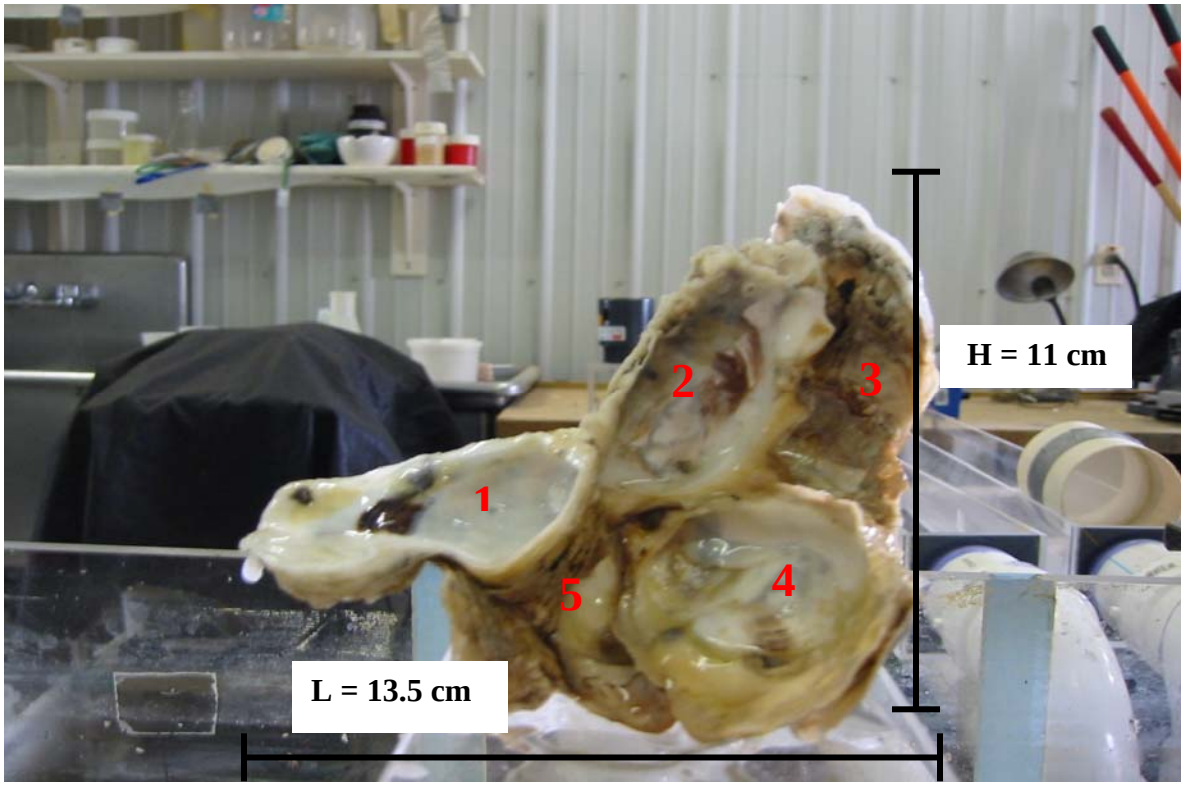


Figure 3. A natural oyster shell clump retrieved by divers from the Chesapeake Bay. Outer valves are removed. This shell clump represents the approximate size (H = 11 cm, L = 13.5 cm) and shape of shell clumps used to line the flume bottom in preliminary retention experiments. Notice how natural oysters settle gregariously. Numbers (1-5) represent where live oysters were present.

Figure 4. Flume bed covered with the clumped oyster shell. Approximate height of shell-covered bed is 11 cm. Look at the numerous interstitial spaces in between and underneath the shell clumps might be potential areas for larval and gamete retention.

For each trial, an 800 ml suspension of 250, 000 umbo-stage larvae was introduced into the flume via a beaker. The beaker was then quickly rinsed with small amount of seawater and poured into the flume. Thirty-micrometer mesh sieves were used (63 cm x 63 cm x 14 cm) to collect oyster larvae exiting the flume. Larvae passing through the flume were captured on mesh sieves at 3-minute intervals for 36 minutes. Between intervals, mesh sieves were rinsed so that larvae were washed into plastic cups labeled with the appropriate time interval. A sieve was also placed at the end of the flume after 36 minutes. This sieve collected larvae exiting the flume for the next 30 minutes (min 36 to min 66 after introduction). Four pairs of experiments over no shell and shelled bottom experiments were conducted. The experiment was ended after 66 min and larvae from the cups were transferred to appropriately labeled vials and stored at 4ºC until counted.

Vials containing oyster larvae were brought back to the laboratory where they were warmed to room temperature. Warming samples helped minimize clumping of larvae, which improved sampling accuracy. For each time interval sample, two separate counts were made of the total number of larvae present on a 1 ml Sedgewick Rafter slide. The average of the two slide counts was then multiplied by the total volume of the water in the vial to estimate the number of larvae collected during a given time interval.

Larval Retention by Differing Shell Densities and Orientations Under Flow

Vertically and horizontally oriented shell bottoms were created with densities of 0, 50, 100, 250, and 500 shells/m^2. The control was clay bed panels with no shell. These experimental densities were chosen to mirror the results of dredge and patent tong surveys of natural oyster bars in the Maryland portion of the Chesapeake Bay, which showed densities ranging from 0 to 250 oysters/m^2 (Vanisko et al. 2002). The base of the

shell bottoms was created from a 1.5 cm thick layer of non-toxic, sulfur-free plasteline spread on top of two Lexan® polycarbonate panels, each 112 cm x 37 cm x 0.64 cm. Loose oyster shell were cleaned, bleached and dried. Shells used were between 6.5 - 7.5 cm from umbo to tip. For the vertical shell orientation, the umbo of each shell was depressed into the clay bed panel at locations generated by a random number generator. Vertically oriented shells were inserted into the clay in staggered rows (modeled after Crimaldi et al. 2002) (Figs. 5, 6). For the horizontal shell orientation, depressions from the vertical shells were filled in with clay and shells were laid down horizontally and slightly depressed into the clay at the same locations. Horizontal shells were placed concave up for randomly generated numbers that were even and concave down for odd numbers. The salinity and temperature of the water in the flume was adjusted to the same conditions as the tanks in which the larvae were reared. The range of salinity varied by experiment between 9 and 10. Flume water temperature varied between 24 °C and 27 °C.

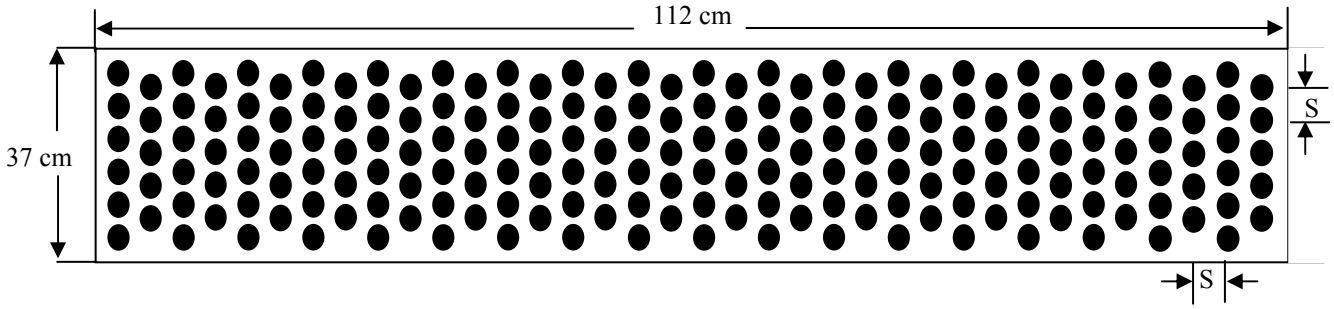


Figure 5. Top view schematic of the placement of oyster shells for shell density retention experiments. Flume working section consisted of two of these panels. Diagram represents the highest bed density, (250 shells/m^2 per panel x 2 = 500 shells/m^2). Spacing between shells, S = 3.1 cm. Flow is from left to right.

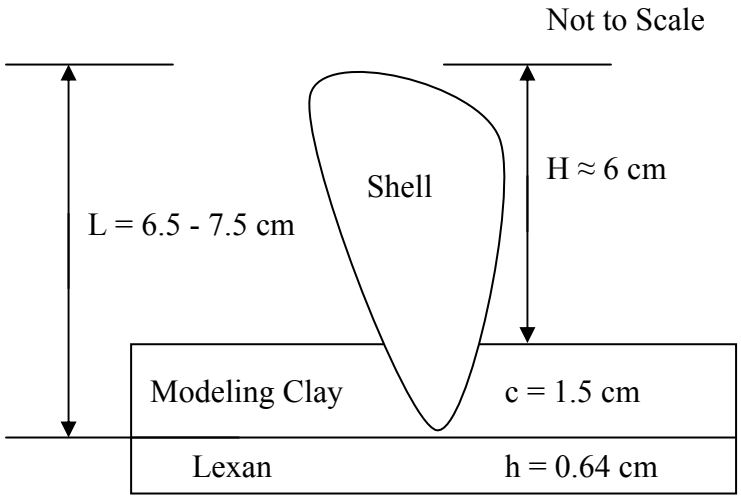


Figure 6. Positioning of vertically oriented oyster shells. Shell height L = 6.5 – 7.5 cm, height of the shell embedded in clay H ≈ 6 cm, height of lexan panel h = 0.64 cm. Bottom substrate is smooth modeling clay (c = 1.5 cm). All shells positioned with concave side of shell facing oncoming flow. Flow is from left to right.

Dye Observations and Pre-Eyed Larvae

Prior to each larval retention trial, dye was introduced into the flume. The passage of dye over a given distance per unit time was used to determine horizontal flow velocity. Horizontal flow velocity was adjusted manually to approximately 1 cm/s. Additionally, observations and photographs were taken concerning the influences of oyster shell density and orientation on surrounding hydrodynamics.

After the dye had cleared from the flume, approximately 200, 000 pre-eyed larvae (>202 μ) were introduced and collected as previously described, over the course of 36 minutes. This time period was chosen because preliminary experiments suggested that few larvae exited the flume past 36 minutes. After 36 minutes, water flow in the flume was stopped and the flume was then allowed to drain completely through a sieve. The flume, including the sides and bottom, was rinsed three times with seawater into a sieve. Sieve contents, containing larvae retained in the flume, were then rinsed down into cup and then placed in a vial. All vials were stored, transported and warmed as previously described.

Two, 1 ml samples from each time period vial were counted using three columns of a 1 ml Sedgewick Rafter slide (two columns near the edges and one in the middle, each column consisting of 20 cells). For each slide, the mean number of larvae counted in three columns was multiplied by 50 (the number of columns on a slide) to estimate the number of larvae present in 1 ml, then further multiplied by the total volume of water in the original vial to estimate the number of larvae collected during a given time interval. To ensure accuracy, larval counts using the three-column estimation method were compared to counts of the entire 1 ml Sedgewick Rafter slide. Relationship between the

two methods was approximately 1:1. Final larval counts for each time interval were obtained by taking the mean of the two previously determined larval counts for each vial.

Larval retention values were obtained by counting all larvae found in three 1 ml samples taken from the vial containing the flume bottom contents. Counts were then multiplied by the volume of water in the vial and the mean of the three estimates was used to determine the total number of larvae retained in the flume.

Out of the 27 trials conducted, three trials were eliminated prior to statistical analysis because they did not demonstrate the set criteria of having their total percentage of larvae accounted for being between 60 and 140 percent of the total larvae introduced. The trials that were removed were two trials of 250 shells/m^2 oriented horizontally, and one trial of 500 shells/m^2 oriented vertically.

For statistical analysis, a log transformation was performed on all percent retention data. A one-way analysis of covariance was conducted to determine the effects of shell density, shell orientation, and the interaction of shell density and shell orientation and on the percentage of larvae retained in the flume.

Results

Gamete Separation and Fertilization Success Under No Flow

Approximately half a million eggs and one million sperm were introduced at varying experimental distances in 200 L tanks without flow. After two hours, eggs showed remarkable differences in fertilization success. Distance between egg and sperm significantly influenced fertilization success (ANOVA, $p < 0.0005$). Fertilization success sharply decreased with increasing distance between introduced gametes (Fig. 7). Mean fertilization success for all trials decreased logarithmically with separation distance (Fig. 7).

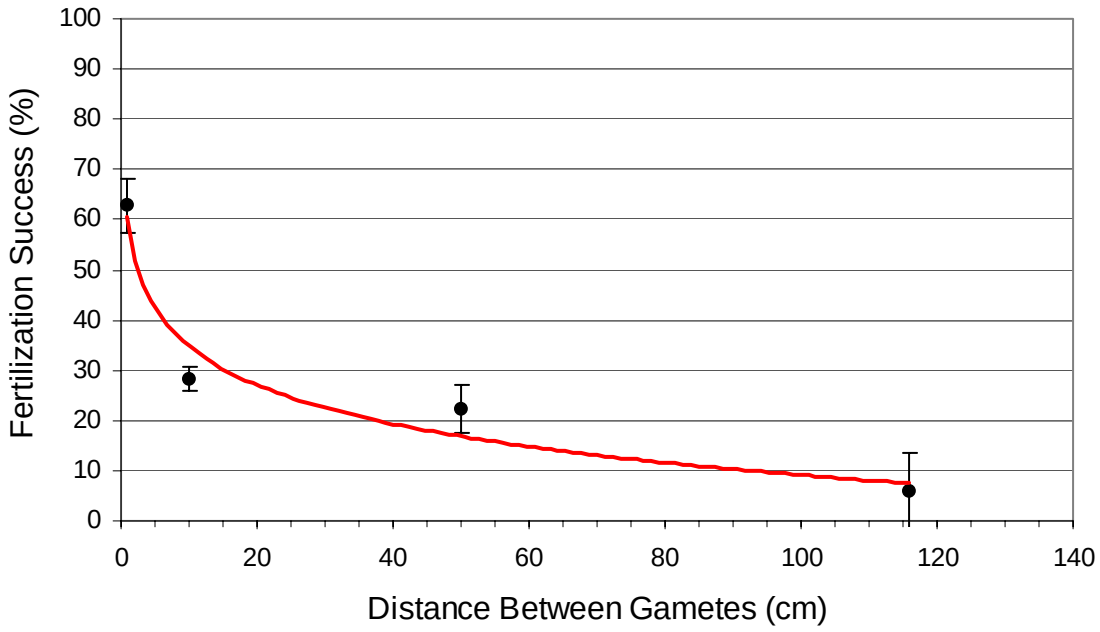


Figure 7. Logarithmic regression of mean percent fertilization success as a function of distance, $y = -11 \ln(x) + 60$, $R^2 = 0.96$. Note how fertilization success decreased rapidly to approximately 28% when gametes were separated by just 10 cm, and to almost zero at the far ends of the tank (116 cm).

Larval Retention by Shell Clumps Under Flow

Clumps vs. No Shell

Preliminary shell-effect experiments compared larval retention by beds of shell clumps versus beds without shell. Approximately 250, 000 umbo stage larvae were introduced over both bed types. Larvae exiting the flume were collected every three minutes for 36 minutes. Figure 8 shows trends of larval passage through the flume for each bed type.

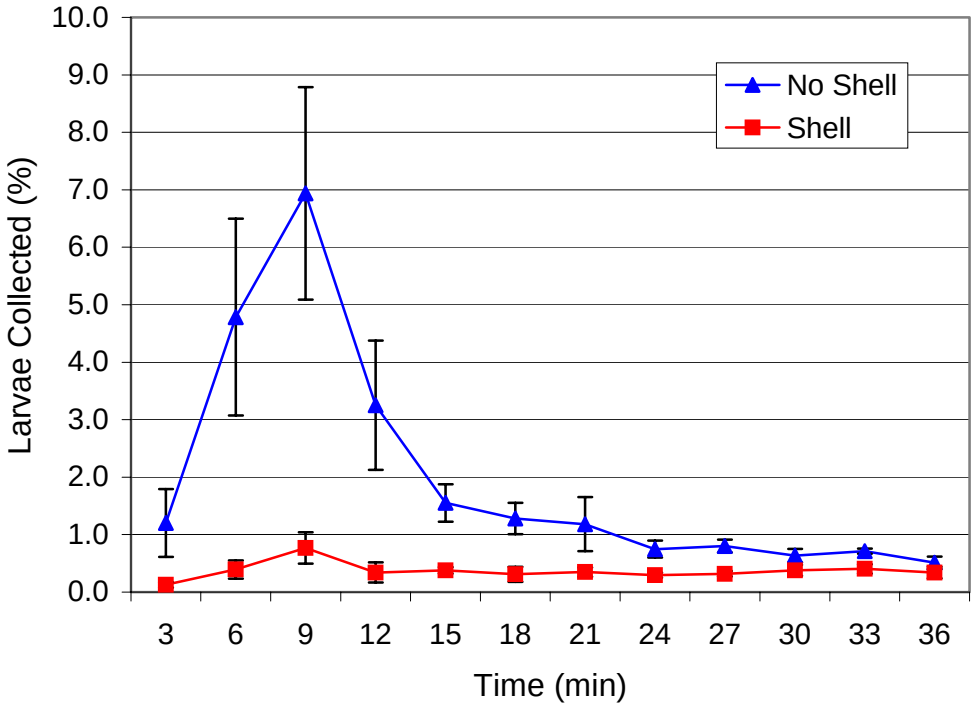


Figure 8. Mean percentage of umbo stage larvae collected on 30 µ sieves at 3-minute intervals for 36 minutes (N=4). Mean percentage is of the total number of larvae introduced into the flume. The greatest number of larvae exited the flume between 6-9 minutes. Larvae passing through/over the shelled bottom were retained relative to those passing over a bottom with no shell.

Retention was determined by subtracting the summation of larvae that passed through the flume in 36 minutes from the total number of larvae introduced. More umbo stage larvae were retained by beds containing clumped shell bottoms than those with no shell. 96% ± 0.79 SEM of umbo stage larvae introduced were retained on beds (11 cm high), whereas 77% ± 3.82 SEM were retained on beds with no shell (N=4). Although these experiments were relatively crude, they revealed that shell clumps had a substantial influence on the passage of oyster larvae through the water column.

Dye Observations

Qualitative observations of dye transport over shell beds of differing densities and orientations were made prior to experiments conducted with larvae. Shell densities and orientations used for the dye experiments were the same as those used for the pre-eyed larval retention experiments. Dye was introduced into the flume at the same location where larvae were to be introduced. Dye inserted into the flume over the greatest bed density, 500 shells/m^2, for both vertically and horizontally oriented beds, traveled over the tops of the shells with little dye visibly retained in the interstitial spaces between shells (Fig. 9). A similar pattern of dye transport was observed over horizontally and vertically oriented beds of 250 shells/m^2 density, with the exception that more dye was visibly retained within the interstitial spaces of some shells.

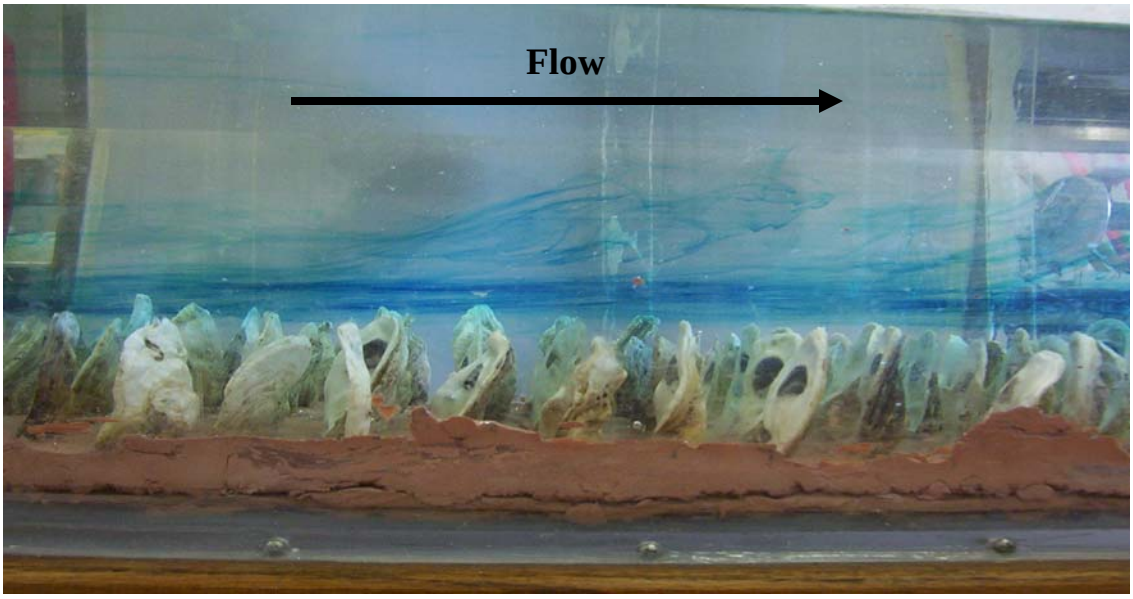


Figure 9. At a horizontal flow velocity of 1 cm/s, the presence of shell at the greatest bed density, 500 shells/m^2 oriented either vertically or horizontally, influenced hydrodynamics in such a way that dye passed directly over the tops of the shells with little dye visibly being retained in the interstitial spaces between shells. Vertical orientation is shown here.

In contrast, dye accumulated on both the concave and the convex side of shells, when passing through shell bed densities of 50 and 100 shells/m^2 oriented in the vertical (Fig. 10). At these same densities, for horizontally oriented beds, dye generally was retained on the bottom around the perimeter of shells (Fig. 11).

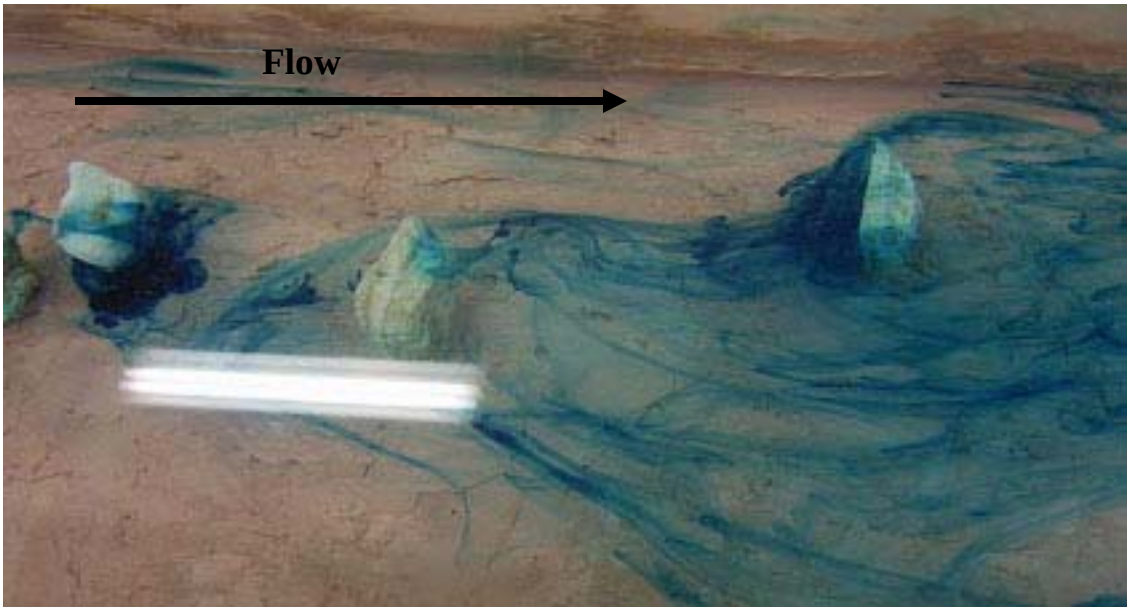


Figure 10. At a horizontal flow velocity of 1 cm/s, shell densities of 50 shells/m^2 and 100 shells/m^2 oriented vertically, influenced hydrodynamics in such a way that dye accumulated on both the concave and convex side of shells. 50 shells/m^2 oriented vertically shown here.

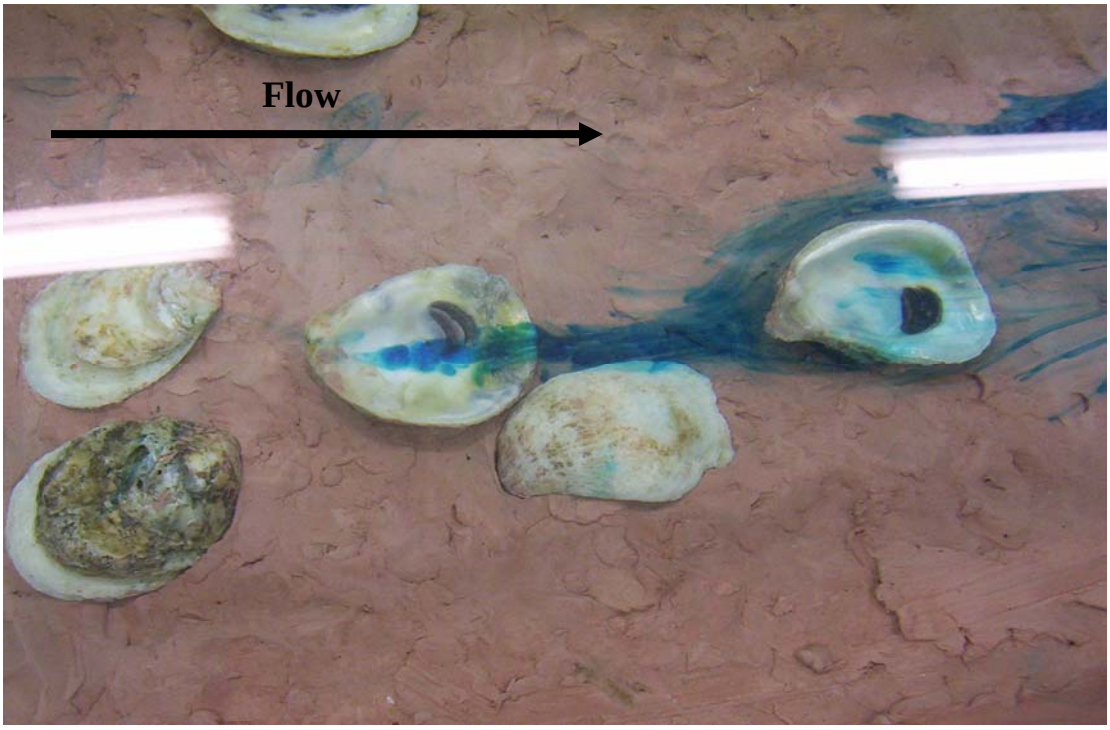


Figure 11. At a horizontal flow velocity of 1 cm/s, shell densities of 50 shells/m^2 and 100 shells/m^2 oriented horizontally, influenced hydrodynamics in such a way that dye was generally retained on the bottom around the perimeter of shells. 100 shells/m^2 oriented horizontally shown here.

Larval Retention by Differing Shell Bed Densities and Orientations Under Flow

Furthering preliminary experiments, more refined experiments were conducted to determine the effects of shell density and shell orientation on larval retention. Approximately 200, 000 pre-eyed larvae were introduced and allowed to pass over vertically and horizontally oriented shell bottoms with densities of 0, 50, 100, 250, and 500 shells/m^2. Similar to preliminary experiments, larvae exiting the flume were collected every three minutes for 36 minutes. Figure 12 shows trends of larval passage through the flume for each shell bed density.

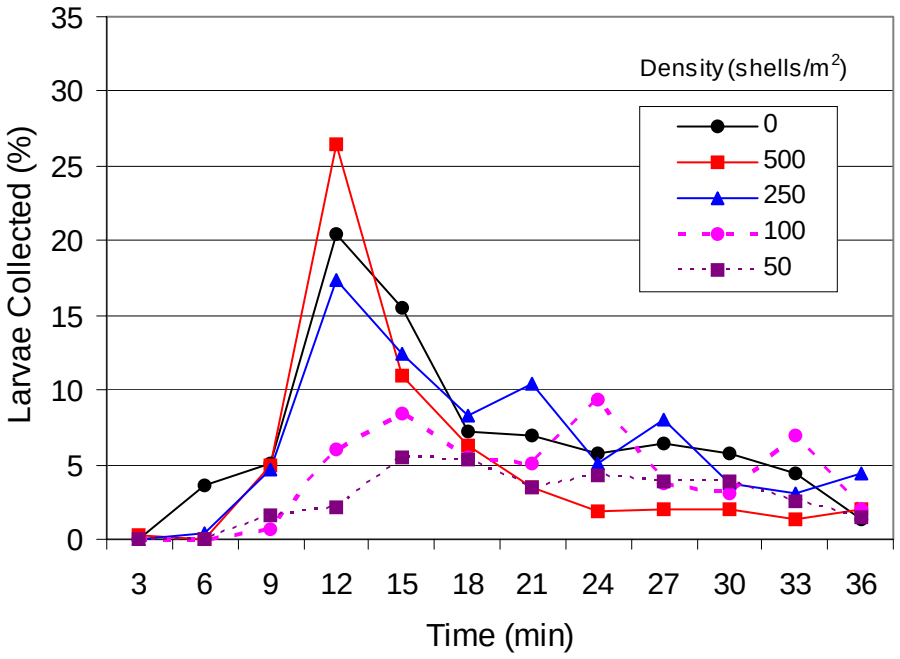


Figure 12. Mean percentage of pre-eyed larvae collected on 30 μ sieves at 3-minute intervals for 36 minutes over 5 different shell bed densities. Mean percentage is of the total number of larvae introduced into the flume. Error bars omitted for clarity (SEM approximately 3% for most data points). The greatest number of larvae exited the flume between 9-12 minutes at densities of 0, 250, and 500 shells/m^2 and 12-15 minutes at densities of 50 shells/m^2 and 100 shells/m^2. Larval retention was greatest at densities of 50 and 100 shells/m^2, relative to the other densities.

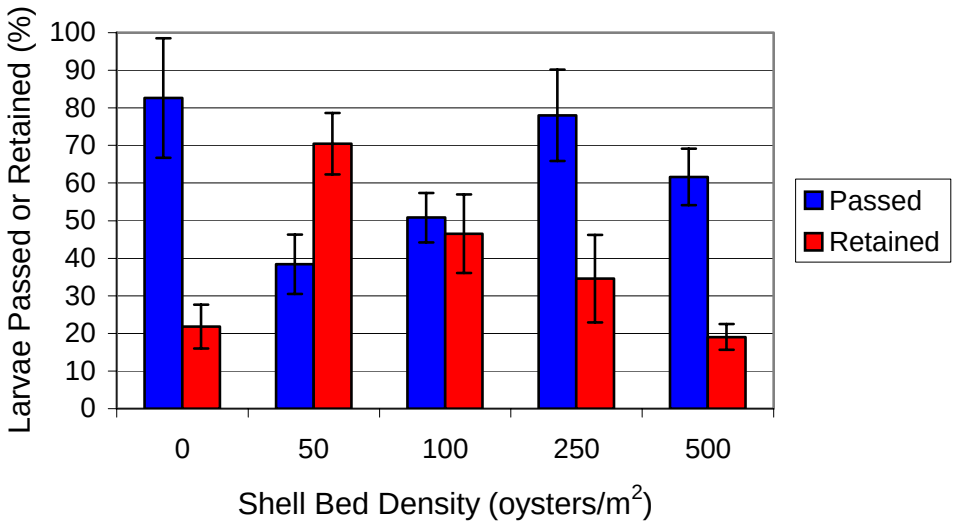
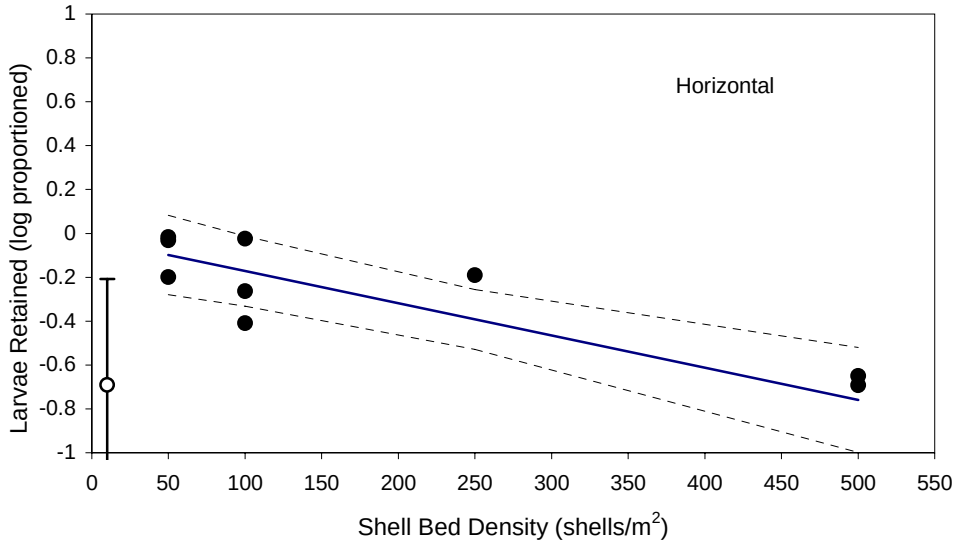


Figure 13. Mean percentage of pre-eyed larvae passed (in 36 min) or retained (after 36 min) in the flume over 5 different shell bed densities. Larval retention was greatest at densities of 50 and 100 shells/m^2 and decreased at densities of 250 and 500 shells/m^2. Low retention was also observed for the controls, 0 shells/m^2, suggesting that a threshold density may exist where further reduction of shell bed density will not lead to an increase in larval retention.

Larval retention was determined by rinsing down the flume bed and counting the number of larvae that did not pass through the flume in 36 minutes. Shell bed density had a significant effect on the percentage of larvae retained in the flume (ANCOVA, $p < 0.0002$). Larval retention was inversely related to shell bed density (Figs. 13, 14).

To determine effects of shell bed orientation on larval retention, trials were run with shells oriented both vertically and horizontally for each experimental shell bed density. Shell orientation significantly influenced larval retention (ANCOVA, $p < 0.0323$). More larvae were retained on horizontally oriented shell beds than vertically oriented shell beds (Fig. 14). No significant interaction was found between density and orientation (ANCOVA, $p > 0.2758$), regarding the percentage of larvae retained in the flume.



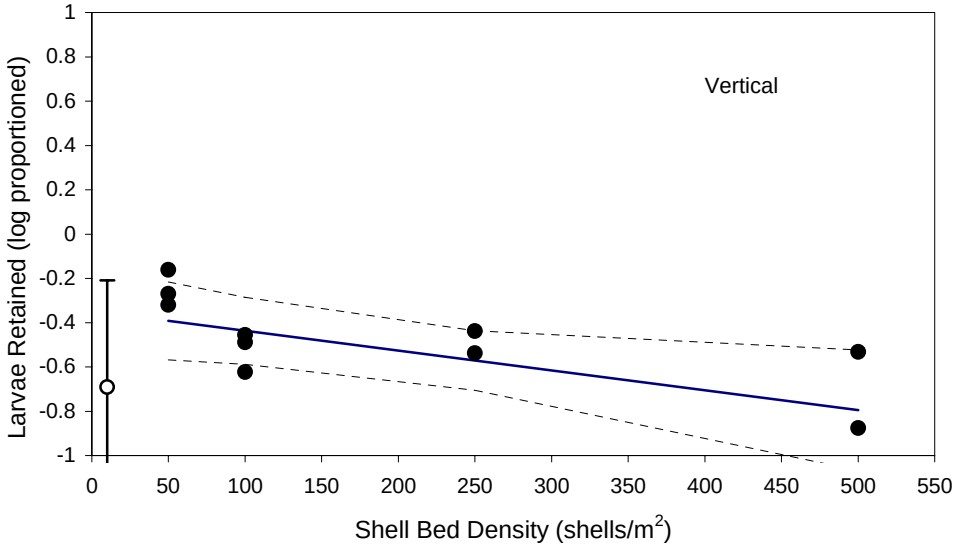


Figure 14. Larval retention was inversely related to shell bed density. Horizontally oriented shell beds retained more larvae than vertically oriented shell beds. Regression calculated from log proportioned percentage data (log (percent retained/100)), $y_H = -0.00147(\text{Density}) - 0.02543$, $R^2 = 0.77$, $y_V = -0.00090(\text{Density}) - 0.3467$, $R^2 = 0.32$. Data values and 95% CI (dotted lines) are log transformed values. Open circles with 95% CI represent control density, 0 shells/m^2.

Discussion

To shed light on the effects that natural, dense assemblages of oysters have on the perpetuation of the *C. virginica* reefs, a series of experiments were designed and carried out to study both fertilization success and the retention of larvae by oyster shell. Experiments conducted on egg-sperm interactions supported the hypothesis that fertilization success decreases with increasing distance between opposite sex parents. Larval retention experiments suggested that natural shell clumps retained more oyster larvae than beds with no shell. Finally, experiments studying the effects of shell bed density and shell bed orientation on the retention of oyster larvae refuted the hypothesis that shell beds of greater density, oriented in the vertical, would retain more larvae than lesser density beds oriented in the horizontal.

Gamete Separation and Fertilization Success

The trend of exponentially decreasing fertilization success with increased distance between opposite sex parents has been shown by many *in situ* experiments conducted on other free-spawning invertebrates (modeled by Denny and Shibata 1989; empirical evidence by Pennington 1985; Yund 1990; Levitan 1991; Marshall 2002; Metaxas 2002). Results suggest that the distance between spawned gametes may be an important factor contributing to overall fertilization success. Both in my experiments and in experiments conducted by Pennington (1985) at ≈ 0.2 m/s current velocity, the most dramatic decrease in fertilization success occurred within the first 10 cm of separation, a reduction of over 50%. In all of the previously mentioned experiments, eggs from females separated by only a few meters from their male counterparts suffered substantially reduced fertilization success compared to animals spawning at close proximities.

In addition to the distance between spawned gametes, there are many other factors that may influence fertilization success in the water column including spawning synchrony (Pennington 1985; Levitan 1988; Sewell and Levitan 1992; Marshall 2002), dilution of expelled gametes (modeled by Denny 1988; Denny and Shibata 1989; empirical evidence by Pennington 1985; Levitan et al. 1991, 1992), gamete longevity (Levitan et al. 1991; Williams and Bentley 2002), contact-time of egg-sperm interactions (Levitan et al. 1991), current velocity (Pennington 1985; Yund 1990; Levitan 1991; Levitan et al. 1992) and/or population density (Pennington 1985; Levitan 1991; Levitan et al. 1992; Mann and Evans 1998).

Gamete dilution also has a negative effect on fertilization success. Laboratory experiments conducted on sea urchin gametes by Pennington (1985) and Levitan et al. (1991) showed high levels of fertilization success at sperm concentrations $>10^6$ sperm/l, but limited fertilization success when sperm concentrations reached $\leq 10^4$ sperm/l. Pennington (1985) suggested that in order for successful fertilization to occur *in situ*, sperm concentrations needed to be dense and spawned minutes prior to encounters with eggs.

In addition to decreases observed due to distance between spawned gametes, my results also reflect reduced fertilization success due to increased gamete dilution. Gamete concentrations were highest for the controls, $5x10^7$ sperm/l and $5x10^4$ eggs/l, with the greatest amount of fertilization success observed at these concentrations. Upon introducing gametes to the aquaria marked dilution occurred, with viable gamete concentrations dropping to $5.5x10^4$ sperm/l and $5.5x10^1$ eggs/l. Observed low percentages of fertilization success in my experimental treatments (10 cm, 50 cm, and

116 cm distances) follow the results of both Pennington (1985) and Levitan et al. (1991) in which minimal fertilization success was observed when sperm concentrations neared 10^4 sperm/l. Additionally, laboratory experiments conducted in extremely still water have been shown to demonstrate very low levels of fertilization success because gametes tend not to mix (Pennington 1985).

Another factor that may substantially influence fertilization success is current velocity. While it is possible that currents may bring gametes together in the water column, various experiments have shown the presence of increased current (> 0.2 m/s) to decrease fertilization success (Pennington 1985; Levitan et al. 1992). Decreases in fertilization success due to increased current velocity have an additive effect on decreases observed due to increased distance between spawned gametes (Penninton 1985). This additional decrease in fertilization success typically occurs because the presence of a current facilitates dilution, spreading gametes even further apart from one another. In contrast, experiments conducted under low flow conditions (< 0.01 m/s) or at slack tide showed increased fertilization success both overall and at increasing distances from the sperm source compared to experiments conducted under increased flow conditions (Pennington 1985; Yund 1990; Levitan 1991; Levitan et al. 1992).

Decreased fertilization success due to increased current velocity may be one reason why Pennington's (1985) experiments conducted at ≈ 0.2 m/s demonstrated similar results to my experiments conducted under no flow conditions (with decreases due to increased distance between gametes, dilution and reduced mixing). Based on

the results of his experiments, Pennington (1985) suggested that higher percentages of fertilization success *in situ* may occur if free-spawning organisms spawn into quiet, but not stagnant environments rather than swift-moving water.

Fertilization success may also be a function of population density. Separate experiments conducted *in situ* on three species of sea urchins demonstrated decreased fertilization success in response to decreased population density (Pennington 1985; Levitan 1991; Levitan et al. 1992). Based on the results from these experiments, Levitan et al. (1992) suggested that low abundances or dispersed distribution of individuals may lead to decreased reproductive success. At low population densities, decreased reproductive success may occur due to the Allee effect.

The Allee effect occurs in populations with low abundances of individuals, where individual contributions to subsequent recruitment become dependent on population size or density (Allee 1931; Courchamp et al. 1999). Decreases in population size for free-spawning sessile invertebrates may lead to severe decreases in fertilization success and potentially to extinction (Levitan et al. 1992). At low population densities, there may be inadequate numbers of opposite sex gametes present in the water column at the same time to promote successful fertilization. Limited quantities of gametes in the water column during a mass-spawning event are likely to compound pre-existing reproductive limitations due to dilution effects (Levitan et al. 1992). Continuing the downward spiral, poor fertilization success leads to the production of few viable oyster larvae, further exacerbating problems of low population density.

In short, fertilization success in free-spawning invertebrates is likely influenced by a variety of factors such as spawning synchrony, distance between gametes, dilution

of expelled gametes, gamete longevity, contact time of egg-sperm interactions, current velocity and/or population density (Pennington 1985; Levitan 1991; Levitan et al. 1991, 1992; Marshall 2002; Williams and Bentley 2002). Different factors may be more important in influencing fertilization success in some species than others.

There is a need for on-going research studying the effects of previously mentioned factors on fertilization success in free-spawning invertebrates. Experimentation to determine additional factors that may influence fertilization success would also help to expand this science. Many free-spawning invertebrates are important both commercially and ecologically. Thus, it is important to gain a better understanding of the factors influencing their fertilization success. With regard to the eastern oyster *C. virginica*, *in situ* experiments studying the effects of increased distance between gametes, increased or decreased current velocity, or population density on fertilization success is still greatly needed.

Larval Retention

Results from preliminary experiments showed the presence of oyster shells to substantially influence the retention of larvae over a flume bed under uni-directional flow conditions. In these experiments, a greater number of umbo stage larvae were retained by beds containing clumped shell bottoms, than bottoms containing no shell. These early results suggested that shell clumps might be a location where larvae could be retained close to the bed.

Bed retention values for both shell and no shell treatments in the preliminary experiments are likely to be overestimates since larval retention was calculated by subtracting the summation of larvae captured on sieves during three-minute intervals

totaling 36 minutes from the total number of larvae introduced into the flume. Also, retention values may be overestimates because flume water temperature in the preliminary experiments was not continuously regulated with heaters. Larvae experiencing temperatures colder than they were accustomed to (19 - 22 °C vs. 24 - 27 °C), may have sunk to the bottom prematurely. The decreasing water temperature may also explain the fairly high larval retention observed on flume beds without shells.

In later experiments, a better estimate of larval retention was obtained by counting the number of larvae that were rinsed off of the bed onto a sieve. Larvae that exited the flume in 36 minutes plus the larvae retained on the bottom summed to approximately 100%. In order to minimize potential temperature shock to larvae, flume water temperature was maintained between 24 - 27 °C for these experiments.

In my second set of larval retention experiments, both shell bed density and shell orientation had significant effects on the percentage of pre-eyed larvae retained in the flume. Larval retention was inversely related to shell bed density and more larvae were retained on horizontally oriented shell beds than vertically oriented shell beds. Results from the qualitative dye observations may provide some insight about the observed patterns of retention.

I had hypothesized that the densest shell bed, 500 shells/m^2 oriented in the vertical would retain the greatest number of larvae. I thought that increasing shell density would increase the number of interstitial spaces available for retention. But what I discovered from the dye experiments was that at very high densities flow over the shells was sheet-like (Fig. 9). Shells used in these experiments were all of the same approximate height and dye parcels passed over the shells as though they were one solid boundary. One

reason that lower levels of larval retention might have been observed at the 500 shells/m^2 density is that a large number of larvae may have traveled in a pattern similar to the dye, moving up and over the tops of the shells and out of the flume in the sheet-like flow.

Interestingly, I found more larvae to be retained at bed densities of 50 and 100 shells/m^2. Increased distance between shells allowed time and space for distinct retention patterns to be set up. Dye observations at these densities showed dye parcels to be slowed and diverted by the presence of shell, with dye subsequently being retained. Dye retention occurred in-between shells, particularly at the base of concave and convex sides of shells oriented in the vertical. Dye retention was also seen around the perimeter of shells oriented in the horizontal. These observations are consistent with flow diagrams drawn by Abelson and Denny (1997) depicting both flow over protruding bodies in the water column and flow occurring in pits or depressions.

Though the greatest larval retention was observed at bed densities of 50 shells/m^2 and 100 shells/m^2, control experiments conducted at 0 shells/m^2 suggest that there is a limit to how low shell bed densities can become before adverse effects occur on larval retention capabilities. Mean larval retention at a density of 0 shells/m^2 was 69% less than retention seen by a density of 50 shells/m^2. These data suggest that the threshold density influencing the greatest amount of larval retention likely lies between 0 shells/m^2 and 50 shells/m^2. Additional experimentation is necessary to further explore this hypothesis.

Results from these experiments also indicated that more larvae were retained by horizontally oriented beds than vertically oriented beds. Some possible reasons for greater retention on horizontal beds are that larvae may have been retained in the cup-shape formed by those shells oriented convex side up, or that the area underneath convex

side up shells may have created conditions suitable for greater retention. Most likely, horizontally oriented beds retained more larvae than vertically oriented beds because shells were positioned closer to the bottom, trapping larvae already near the bottom or traveling as part of the bedload.

To better determine the effects of vertically oriented shell beds on larval retention additional experimentation is necessary. One limitation of this study was the height of the flume. The height of flume being only 25.4 cm, limited the height of the shells that could be used in the retention experiments. In the clumped shell experiments, shells protruded almost half the height of the flume (11 cm) and problems frequently occurred with the signaling capabilities of the ADV. For the density experiments, a shell height of 6 cm above the clay bed was chosen to minimize these problems. Natural shell heights can be much greater than this and it is likely that due to flume height restrictions, flow over the vertical shells didn't get a chance to fully develop. Different retention values might have been observed on vertical beds if the flume height had been higher.

Further replication of trials at each of the experimental densities and orientations may improve the precision of values for both larvae exiting the flume at each time period and larval retention on the flume bed. Variability was high between trials. One explanation for this variability is sampling error. Larvae in each of the experimental vials were very dense and did not remain in suspension very long after being mixed. This made it extremely difficult to get accurate 1 ml larval sub-samples. Subsequent estimates of the total number of larvae in a vial were determined by multiplying the 1 ml sub-sample estimates by the volume of liquid in the vial. This multiplicative step may have added to the original sampling error variability. Total counts were based on means of

vial estimates.

Additionally, larval retention values from vertically oriented shell beds displayed greater variability than retention values from horizontally oriented shell beds. This is demonstrated by the low R^2 value of 0.32 seen for vertically oriented beds compared to an R^2 value of 0.77 for horizontally oriented beds (Fig. 13). This increase in variability was most likely due to the increased turbulence generated by the vertically oriented shell beds.

My experiments were designed to specifically study the effects of shell bed density and shell orientation on larval retention. Thus, I did not characterize the mechanism(s) by which larvae were transported to the bed. Larvae could have been transported to the bed passively (hydrodynamically), actively (by vertical swimming), or through a combination of both of these processes. Additional experimentation is necessary to determine which mechanism(s) influenced larval transport to the bed.

In short, shell bed density was shown to have a significant effect on the percentage of larvae retained in the flume, with more larvae being retained at densities of 50 and 100 shells/m^2. Shell orientation was also shown to have a significant effect on larval retention, with greater retention observed on horizontally oriented beds than vertically oriented beds.

While my flume experiments begin to elucidate some of the effects of shell bed density and shell orientation on larval retention in the eastern oyster *C. virginica*, there is still a great need for experiments to be conducted *in situ*. The effects of tidal flow, changes to the height of the benthic boundary layer, and the amount of turbulence both in and above the bed may all influence larval retention. *In situ* experiments conducted on

larval retention may also have significant ecological implications. One particularly interesting experiment that could be conducted is to test if oyster densities of 50 and 100 oysters/m^2 lead to enhanced spat set. Results from these kinds of experiments may be of interest to those planning oyster restoration efforts.

In conclusion, dense oysters, and the structures they create, may have significant influences on both fertilization success and larval retention. My results showed that egg and sperm separated by only a few centimeters suffered substantially reduced fertilization success compared to mixed gametes. Since oysters reproduce externally, dense assemblages of simultaneously spawning oysters may maximize gamete interactions before dilution occurs. In the water column, developing larvae are likely transported both passively (with large-scale water flow) and actively (due to vertical swimming). Once near the bed, larvae may become entrained in interstitial spaces among oysters or oyster shells further increasing the likelihood of settling in an oyster community. My flume experiments showed increased larval retention at shell bed densities of 50 and 100 shells/m^2, and more larvae to be retained on horizontally oriented shell beds than vertically oriented beds. Further experimentation is necessary to determine the influence of oyster density on fertilization success and larval retention *in situ*.

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