Investigating the early life history of bay scallop, *Argopecten irradians*, using mesocosm experiments

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ABSTRACT OF THE DISSERTATION

Investigating the early life history of bay scallop, *Argopecten irradians*, using mesocosm experiments

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Bay scallops, *Argopecten irradians*, were once an abundant estuarine species, associated with seagrass beds ranging from the Gulf coast and along the Eastern coast of the United States. However, due to the presence of harmful algal blooms (HABs), loss of seagrass habitat, and increased predation, bay scallop populations have significantly declined across their entire range. Restoration efforts are underway in many coastal water bodies with varying success. Important data gaps regarding habitat requirements for bay scallops at all life history stages still remain. It was the overall goal of this dissertation project to identify and further examine those data gaps. Bay scallop life history can be divided into three segments: (1) pelagic larva, (2) juvenile, and (3) adult. Each stage has its own unique habitat requirements and specific selection pressures. This body of work was comprised of four main objectives, (1) determine if ctenophores prey upon bay scallop larvae, (2) quantify bay scallop larvae swimming behavior under different environmental conditions, (3) determine if juvenile bay scallops selectively prefer seagrass blades coated
with epiphytes, and (4) examine the effects of seagrass shoot density on adult bay scallop survival, growth, and physiological health. The main research findings were, (1) ctenophores are important predators of bay scallop larvae, (2) bay scallop larvae mostly hover when exposed to salinities in the range of 20 to 30 ppt, but when exposed to warm conditions (30°C), they swim upwards at a faster rate than those in 23°C conditions, (3) juvenile scallops do not demonstrate a preference for seagrass blades coated in epiphytes and were instead observed to attach to any available vertical substrate, and (4) increasing seagrass shoot density adversely impacted the concentration of bioavailable phytoplankton but did not have a negative effect on bay scallop health. Taken together, these results suggest that predation is an important driving factor inhibiting bay scallop population recovery. Restoration efforts need to account for predation at the larval stage. Suitable habitat for larval settlement needs to be identified and maintained as juveniles and adults will continue to require it as a spatial refuge from predation.
Dedication

I am deeply grateful to my parents who always believed in me, to Patrick who always supported me, to Patrick Joseph who inspired me, and to Nathaniel who motivated me.
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**Introduction**

Bay scallops, *Argopecten irradians*, (Figure 1) were once an important component of estuaries found along the Gulf Coast and the Eastern coast of the United States (Kirby-Smith 1972). They are commonly associated with seagrass beds and utilize this habitat throughout multiple life history stages. During their relatively short lifespan of 18-22 months, they will settle in seagrass as larvae, attach themselves to blades of seagrass as juveniles, and reside on the sandy bottom as adults (Belding 1910). Throughout these different stages, the vertical structure of seagrass provides a refuge from predation (Thayer and Stuart 1974). However, due to multiple factors, bay scallop populations have significantly declined along their entire extensive range.
Coastal ecosystems are experiencing adverse effects as surrounding land use is rapidly changing (Lathrop and Conway 2001, Lathrop and Bognar 2001, Zampella et al. 2007). As adjacent impervious surfaces increase, run-off contaminated with heavy metals, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), etc. is entering bays and estuaries (Niyogi et al 2001, Turja et al 2013, Danion et al 2014). Increased nutrient loads (Baker et al. 2014) are fueling harmful algal blooms (HABs)
(Hallegraeff 2003, Glibert and Burkholder 2006, Anderson 2009). For example, multiple variables assessed in Barnegat Bay-Little Egg Harbor, New Jersey (BB-LEH), including low dissolved oxygen concentrations, algal blooms (Gastrich et al. 2004, Kennish and de Jonge 2011), heavy epiphytic loading, and declines in seagrass biomass (Kennish et al. 2010, Fertig et al. 2013) all indicate the estuary is undergoing high levels of eutrophication (Bricker et al. 2007, Fertig et al. 2014).

Eutrophication of coastal water bodies has resulted in blooms of brown tide (Aureococcus anophagefferens) (Celestino 2007) and red tide (Ptychodiscus brevis) (Summerson and Peterson 1990) that have resulted in mass mortalities of adult bay scallops. Additionally, declines in seagrass biomass have effectively reduced available habitat for bay scallops (Kennish et al 2010, Celestino 2007). The overabundance of cow nose rays (Rhinoptera bonasus) due to the over-fishing of apex predator sharks has also contributed to the loss of bay scallop populations as these rays are voracious predators for scallops (Myers et al 2007, Peterson et al 2001). The rapid declines observed in bay scallop populations depleted the effective spawning stocks. An adequate supply of larvae was unable to be produced and recruitment limitation likely prevents populations from recovering (Peterson and Summerson 1992; Peterson et al. 1996; Tettelbach and Smith 2009).

There is a renewed interest in the restoration of bivalves. Efforts have been primarily focused on oyster restoration, although other filter-feeding mollusks, such as clams and scallops, have received attention. Motives behind bivalve restoration include the establishment of sustainable populations, enhancement of species diversity, and increased trophic complexity (Coen and Luckenbach 2000, Luckenbach et al. 2005, Coen
et al. 2007a, Coen et al 2007b, Grabowski and Peterson 2007). Additionally, as they graze on phytoplankton, bivalves have a localized influence on water quality (Grizzle et al. 2006). Extensive bay scallop restoration efforts have been initiated along the coast of North Carolina and in New York (Peterson et al. 1996, Tettelbach and Smith 2009).

However, data gaps remain that if filled, would provide useful information for the conservative and restoration of bay scallop populations. Therefore, it was the goal of this dissertation to identify and further investigate these data gaps. Bay scallops have three main life history stages: larva, juvenile, and adult. The role the surrounding habitat and its inhabitants plays during each of these stages was closely examined.

During the larval period, bay scallops are likely prey for planktonic predators. Ctenophores (*Mnemiopsis leidyi*) have been found in high densities in BB-LEH for decades (Nelson 1925) and known to consume oyster (*Crassostrea virginica*), mussel (*Mytilus edulis*), and clam (*Mulinia lateralis*) larvae (Purcell and Cowan 1995). However, it was currently unknown if ctenophores prey on bay scallop larvae. The identification of an important larval predator would help inform the understanding of recruitment and settlement patterns. Furthermore, very little is known regarding the swimming behavior of bay scallop larvae. Swimming behavior can be impacted by environmental conditions, such as water temperature, salinity, and light. This behavior can also have significant implications for larval dispersal and recruitment patterns.

After settlement occurs, the juvenile bay scallops then attach themselves via byssal threads to vertical blades of eelgrass, which provide protection against predation (Thayer and Stuart 1974, Pohle et al. 1991). As previously mentioned, algal blooms are occurring at an increased rate in coastal water bodies. Phytoplankton blooms and
epiphytes reduce light transmission to seagrass and can result in diebacks (Kennish et al. 2011, Fertig et al. 2014). However, while the effects of epiphytes directly on seagrass are well studied, their effects on bay scallop attachment are largely unknown. There is some indication that the presence of epiphytes on seagrass blades and macroalgae may enhance bivalve recruitment by increasing habitat complexity (Bologna and Heck 2000; Howarth et al. 2011). Another aim of this work is to assess the effects of these epiphytic blooms on the relationship between bay scallops and seagrasses in order to better understand the basic ecology of bay scallops and how eutrophication might influence their behavior.

As seagrass beds experience diebacks and reduced biomass due to the effects of eutrophication, it is important to understand how these habitat changes may impact bay scallops. The shoot density of seagrass beds can impact both predation rates (Orth 1992) and water flow and food supply (Eckman et al. 1989, Fonseca et al. 1982) and therefore may have an effect on the growth and survivorship of bay scallops (Irlandi et al. 1999).

It is the overall goal of this dissertation to provide decision-makers with information necessary to facilitate sustaining populations of bay scallops. The objectives of this study are fourfold: to better understand (1) bay scallop larval predation pressure by ctenophores (2) bay scallop larval swimming behavior under different environmental conditions (3) the effects of seagrass blade epiphytes on juvenile scallop attachment, and (4) the effects of seagrass patch density on scallop growth and survivorship.
Chapter 1

The predation of bay scallop (Argopecten irradians) larvae by ctenophores

(Mnemiopsis leidyi)

Abstract

Bay scallops were once an important component of New Jersey’s Barnegat Bay-Little Egg Harbor (BB-LEH) ecosystem and cultural heritage. Beginning in the 1970s, the New Jersey bay scallop population declined and has remained low. Bay scallops rely on seagrass habitats, and both habitat loss and recruitment limitation may play a role in preventing the population from recovering. It is not known if planktonic predators such as ctenophores (Mnemiopsis leidyi), which have been found in high densities in BB-LEH, act as important predators on bay scallop larvae and exert top-down control of population abundance. In order to assess potential predation pressure by ctenophores on bay scallops, the predation rate of individual ctenophores on high densities of hatchery-reared bay scallop veliger larvae was observed in the laboratory. After being allowed to feed on larvae, each ctenophore was then transferred to filtered seawater at hourly intervals to measure egestion rate. Egested living larvae were placed in beakers with food to determine larval survival after 24 hours. Ctenophores ingested 23% of bay scallop larvae. A small proportion of those larvae were egested live (<1%) and survived for 24 hours post-egestion. The results of this study suggest that ctenophores can contribute to predation of bay scallop larvae and may negatively impact bay scallop populations.

Key words:

Bay scallop, seagrass, predation, ctenophore
Introduction

The bay scallop, *Argopecten irradians*, is an ecologically and economically important bivalve found along the Atlantic and Gulf coasts of the United States. Historically, bay scallops have served as an important commercial and recreational fishery. Bay scallops were once an important component of the Barnegat Bay-Little Egg Harbor, New Jersey (BB-LEH) ecosystem and closely associated with the bay’s seagrass meadows. As reported by the National Marine Fisheries Service, the first landing records for BB-LEH were collected in 1956 and show 52,300 bushels of scallops were harvested. The New Jersey bay scallop fishery was successful over the next 12 years, yielding a total of 317,000 bushels. However, beginning in the 1970s, the New Jersey bay scallop population began to decline, and landings were only recorded in 1973 and 1974 (Celestino 2007). The low abundance of bay scallops could no longer support the fishery (Ford 1997). Successful restoration of the bay scallop population in BB-LEH would provide the potential for renewed recreational harvest as well as provide an ecosystem service benefit of increased water filtration.

Natural recovery of depauperate bay scallop populations is often limited by their relatively short life span of 18-22 months and their propensity to spawn only once in a lifetime (Belding 1910, Tettelbach and Smith 2009). However, bay scallop fecundity is high, with each hermaphroditic individual producing over 5 million eggs. Major spawning events in Barnegat Bay likely occur during mid-summer to early fall (Bologna et al. 2001). The planktonic free-swimming veliger larval period ranges from 1-2 weeks (Sastry 1965; Tettelbach and Wenczel 1993).
During the larval period, bay scallops are likely prey for planktonic predators. The two most abundant carnivorous gelatinous zooplankton species in BB-LEH are the scyphomedusan sea nettle jellyfish (*Chrysaora quinquecirrha*), and the local species of ctenophore (*Mnemiopsis leidyi*). Ctenophores have been found in high densities in BB-LEH over the past century (Nelson 1925) and are known consumers of oyster (*Crassostrea virginica*), mussel (*Mytilus edulis*), and clam (*Mulinia lateralis*) larvae (Purcell and Cowan 1995). However, it is currently unknown if ctenophores prey on bay scallop larvae. *M. leidyi* migrate to BB-LEH during late spring and early summer and leave the estuary during late fall (Nelson 1925). Their presence in the estuary coincides with the spawning period(s) of bay scallops (Bologna et al 2001), further supporting the likelihood that they are an important bay scallop larval predator.

Young 2016 estimated ctenophore abundances in the northern and southern sections of Barnegat Bay from 2012-2014. After sampling 6 locations in the northern part of the bay using a seine, an average of 200 individuals/tow was observed. Upwards to 400 individuals were observed in 2013. An increase in ctenophore density was also observed in the southern portion of the bay during 2013. Ctenophores were sampled using a plankton net at one location. An average of 40 mL jellies/L water were found during the months of June-October 2012 and increased to 60 mL jellies/L water in 2013.

The lobate ctenophore *M. leidyi*, like all other ctenophores, is exclusively carnivorous. This particular species is characterized by broad lobes at its oral end and a row of tentacles running along the sides of its mouth. They use another oral structure, auricles, to generate feeding currents (Haddock 2007). Prey encounter a ctenophore’s tentacles and become entangled on the lobes by sticky colloblast cells (Franc 1978,
Wagget and Costello 1999). Ctenophores do not have any stinging cells, so the larvae are not killed on contact. The tentacles then deliver the prey to the mouth and ingestion occurs as the prey enters the stomadaeum. Prey are then usually funneled to the center of the stomadaeum where digestion primarily occurs. Any undigested prey is passed back out through the mouth through a process termed egestion (Main 1928). Bay scallop veliger larvae are encased in a larval shell that likely offers some protection from digestion and makes them candidates for being egested from the ctenophore gut.

If predation pressure is in fact high, this pressure could have significant impacts on bay scallop settlement, recruitment, and ultimately population recovery. Assessing the potential predation pressure on bay scallop larvae by ctenophores was the aim of this project. The potential predation pressure on bay scallop larvae by ctenophores was evaluated using the following tests. I predicted that ctenophores would ingest bay scallop larvae at a rate greater than zero. Additionally, I hypothesized that the rate of egested empty bay scallop shells will be greater than the rate of egested live bay scallop larvae. Finally, I hypothesized that egested live bay scallop larvae could survive this egestion process.

**Methods**

During July 2016, bay scallop larvae were spawned from wild captured parents at the Rutgers Aquaculture Innovations Center in Cape May, N.J. After evaluating for gonadal maturity, seven fecund adults were selected and injected with a 2 mM serotonin solution. Self-fertilization was possible as gametes were pooled together for fertilization. Resulting larvae were maintained in the nursery until they reached the veliger stage (~115 µm; Figure S1). Ctenophores were collected from BB-LEH using a 1-m diameter
plankton net over Little Sheepshead Bridge in Tuckerton, N.J. during 5-minute tows (Figure S1). All organisms were kept in 1µm filtered natural seawater collected from Great Bay at ambient salinity and temperature. Individual ctenophores were held singly in plastic containers containing 2 L of filtered natural seawater for 12 hours to allow for gut clearance. Length and width measurements were obtained for each ctenophore used in the study, and all individuals used in feeding trials were of similar size (Table 1).

**Feeding:** Individual ctenophores were placed into 4-L containers containing 2 L filtered seawater and bay scallop larvae at a larval density of 2 ml⁻¹ (total of 4000 larvae). A total of 20 ctenophores were evaluated. A container of bay scallop larvae alone (no ctenophores) was used as a control to assess the number of larvae recaptured during each step of the experiment. Ctenophores were allowed to feed for 10 minutes under artificial light conditions and no aeration. Each ctenophore was then transferred to a separate container with filtered seawater and no larvae twice in 5-minute intervals to serve as a rinsing step to remove any larvae adhered to the outside of the ctenophores. Larvae remaining in the bucket after feeding as well those adhered to the sides of the ctenophores were counted to estimate feeding rate (Figure 1). An aquarium net was modified by wrapping a rubber band around the net portion to create a shallow scoop in order to transfer ctenophores. This net design was tested prior to the start of the experiment to ensure ctenophores would not be damaged in the transfer process.

**Hourly egestion rate.** Egestion rate was measured after rinsing by transferring each ctenophore to a new container filled with filtered seawater and no larvae at hourly intervals for six hours (Figure S2). Following removal of each ctenophore from the
container, the water was filtered over a 55 µm screen and all live larvae and empty larval shells were counted using a dissecting microscope (Figure 1).

At the conclusion of the experiment, ctenophores were gently blotted with a lint-free wipe and weighed to obtain wet weight. Ctenophores were then dried to a constant weight at 60 °C to obtain dry weight.

Figure 1. Illustration demonstrating the steps used to determine the ingestion and egestion of bay scallop larvae by ctenophores. Created with Biorender.com.

Larval survival. Egested living veligers from each single hour group were put in beakers according to their hour of egestion with food (*Isochrysis galbana*) and survival was monitored after 24 hours. A known number of larvae never exposed to a predator
were kept in a container with phytoplankton to serve as a control. At the end of the 24-hour period, larvae were filtered over a 55 µm screen and all live larvae were counted.

*Statistics.* Results from the larvae-only treatment used during the ctenophore feeding portion of the experiment were used to determine the percentage of larvae lost during the processing steps. The larvae-only treatment demonstrated that 62% of added larvae were accounted for at the end of the experiment and therefore, the prey values were falsely inflated by 38%. Despite our best efforts to carefully rinse the sides of the buckets and net, almost 40% of larvae were not able to be recaptured and included in the counts. The prey values were adjusted to reflect this recapture rate and then converted to percentages. Binomial tests were used to evaluate if predation on bay scallop larvae by ctenophores occurred. Comparisons between the percentage of surviving egested larvae and control larvae not exposed to ctenophores were completed by one-way analysis of variance.

**Results**

_Capture of bay scallop larvae by ctenophores_

*Mnemiopsis leidyi* evaluated in this study were of similar sizes (Table 1.) Ctenophores ingested an average of 979 (standard deviation = 545) bay scallop larvae during the 10-minute feeding time and digested 98.5% of these captured larvae. Ctenophore predation on bay scallop larvae was observed in all but one of the 20 replicates, and on average each ctenophore captured 22.3% (range = 0-46.3) of the available veligers (Figure 2).
Table 1. Ctenophore weights and sizes, mean ± SD

<table>
<thead>
<tr>
<th>Wet Weight (g)</th>
<th>Dry Weight (g)</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.41 ± 2.83</td>
<td>0.35± .11</td>
<td>44.8± 8.03</td>
<td>35.9 ± 4.49</td>
</tr>
</tbody>
</table>

Figure 2. Box plot diagram showing the percent of larvae ingested by ctenophores (n=20, median=22.3, range=0–46.3; binomial test, P < 2.2 x 10^{-16}). Dots represent individual data points.

*Egestion of bay scallop larvae by ctenophores*

A small percentage (median=0.57, range=0-11.6, n=20) of the captured larvae were egested live. Egestion of live larvae by ctenophores occurred primarily in the three hours following ingestion (Figure 3). Egestion of dead larvae, as evidenced by empty
shells, occurred less frequently than that of live larvae, and there was no egestion of live or dead larvae observed beyond the sixth hour (Figure 3).

Figure 3. Number of egested bay scallop larvae at each hourly interval following ingestion. Results shown as mean ± standard deviation.

Survival of live egested bay scallop larvae

There were no observed adverse effects on survival of bay scallop larvae by the capture and subsequent egestion processes of ctenophores. The survival of larvae that were egested live was not different from the survival of the non-prey veligers (control treatment) (1-way ANOVA, n ranges from 2-12, p=0.578). Dead larvae as evidenced by empty shells were also counted. Less than 10% of egested larvae were found dead. Additionally, the time spent in the digestive tract of ctenophores as represented by the hour of egestion did not impact larval survival (Figure 4).
Figure 4. Survival of bay scallop larvae 24 hours after live egestion from ctenophores (mean ± SD). Values along the x-axis refer to the total number of larvae still alive 24 hours post egestion/total number of larvae alive at time of egestion. The control consisted of larvae never exposed to ctenophores.

Discussion

This study documented *M. leidyi* predation upon bay scallop larvae, at relatively rapid and high rates (22% of available larvae). Previous studies have demonstrated the propensity for ctenophores to prey on crustacean zooplankton (Cronin et al 1962, Cargo and Shultz 1967, Bishop 1967, Herman et al 1968, Kremer 1979, Deason and Smayda 1982, Feigenbaum and Kelly 1984), ichthyoplankton (Cowan and Houde 1992, 1993), as well as other bivalve veliger larvae (Nelson 1925, Truitt and Mook 1925, Burrell and Van Engel 1976). Nelson (1925) concluded that 75% of evaluated *M. leidyi* had consumed bivalve larvae in BB-LEH and reported high larval settlement of bivalves in this estuary when ctenophore densities were low. Therefore, ctenophores can be important predators...
and likely impact the distribution and abundance of bay scallops in estuaries like BB-LEH.

A small percentage (<1%) of bay scallop veligers captured by *M. leidyi* were egested live. The larval shell likely serves as protection from digestion. Experiments conducted with the scyphozoan jellyfish *C. quinquecirrha* and oyster (*C. virginica*), mussel (*M. edulis*), and clam (*M. lateralis*) veligers demonstrated a larger percentage of dead veligers with open shells were ingested compared to live, closed-shell veligers. Open veligers were also retained longer than closed veligers (Purcell et al. 1991). In this study with *A. irradians* and *M. leidyi*, the highest rates of egestion of live veliger larvae occurred within the first hour after ingestion. There were no adverse effects observed of this predation process on the surviving larvae. These larvae could spend 1-4 hours in a ctenophore, being transported in the estuary differently than that of a small particle. If enough larvae are ingested and egested live, settlement patterns could be impacted. However, it is only a very small fraction of ingested larvae that was egested live. These results are similar to predation rates observed with ctenophores and oyster veligers, where 96% of captured oyster larvae were digested (Purcell et al. 1991).

It is important to identify predators that can have a major impact on larval settlement when considering restoration programs. Establishment of spawner sanctuaries as a bay scallop restoration strategy has been employed in North Carolina (Peterson et al. 1996) and Florida (Arnold et al. 2005). This procedure entails introducing natural or hatchery stock to specific sites. Occasionally, aquaculture gear is used to enclose and protect the stock from predators. The animals then spawn naturally and release their larvae into the water column. For this strategy to work, it is necessary that larvae remain
in the system and survive the planktonic phase (Goldberg et al. 2007). Under these conditions, the restoration effort has no control over the timing of spawning and when larvae will be present in the estuary. Alternatively, adults can be manipulated to spawn in a hatchery year-round under the proper conditions. By breeding these animals, larvae could be introduced to the estuarine system during times of low ctenophore abundance, thereby escaping this predation pressure. However, other environmental controls on bay scallop larvae must also be accounted for. The normal development of bay scallop embryos requires a narrow salinity and temperature range (20-25 °C and 25 °/oo S) (Tettelbach and Rhodes 1981). These conditions would be met artificially in the hatchery during spawning. Older bay scallop larvae (2-5 days post fertilization) can survive under a broader range of temperatures from 10-30°C. Larval salinity requirements are less flexible (Tettelbach and Rhodes 1981) but seasonal salinity changes are less significant unless extreme weather patterns are observed, or the system is heavily managed like the Caloosahatchee River Estuary in southwest Florida. Finally, the presence of suitable habitat to promote larval settlement and juvenile survival must be addressed. Ideally, this habitat would consist of seagrass which is ephemeral and seasonally regulated according to temperature requirements. However, bay scallops have been shown to interact positively with artificial seagrass composed of polypropylene “wrapping” ribbons (Bologna and Heck 1999). Artificial seagrass beds, or other submerged vertical structures, could be constructed in designated areas where settlement is predicted to occur based on larval dispersal patterns. Controlled larval releases could be viable restoration strategy for many Atlantic and Gulf estuaries if the right conditions are met.
The predator-prey interactions between gelatinous zooplankton limit the impacts
*M. leidy* have on top-down community structure (Hosia and Titelman 2011, Purcell and Cowan 1995). Sea nettle jellyfish (*C. quinquecirrh*ua) and another species of comb jelly (*Beroe ovata*) are two important predators of *M. leidy* and help control population abundances. Bay scallops are present primarily in the southern portion of BB-LEH estuary. This portion of the estuary maintains a higher salinity which is more conducive for bay scallops, as compared with the northern region of the bay. While densities of sea nettle jellyfish (*C. quinquecirrh*ua) are increasing in New Jersey waters, they are primarily found in the northern part of the bay (Bologna 2011). Predation on bay scallop veligers by *M. leidy* in estuarine systems may be limited when *C. quinquecirrh*ua are present. Sea nettle medusae are known to prey on ctenophores and subsequently reduce ctenophore densities (Feigenbaum and Kelly 1984). The presence of sea nettles also relieves predation pressure on bivalve larvae as *C. quinquecirrh*ua medusae do not consume bivalve larvae (Purcell et al 1991). *M. leidy* are ubiquitously distributed throughout the estuary and therefore may interact with both bay scallop larvae and sea nettles. The comb jelly, *Beroe ovata*, may also help to control the population abundance of ctenophores and thereby reduce the predation pressure on bay scallop larvae. *B. ovata* is a known predator of *M. leidy* and in fact, is thought to feed exclusively on ctenophores (Greeve 1970, Nelson 1925). It was proposed to introduce *B. ovata* to the Black Sea as a biological control for *M. leidy* populations, however the species invaded the system on its own (Konsulov and Kamburska 1998). *B. ovata* are present in mid-Atlantic estuaries (Burrell and Van Engle 1976, Kremer and Nixon 1976), however their population abundance and distribution may vary temporally. Howson *et al* 2017 rarely observed them during their
assessment in BB-LEH. They primarily found comb jellies in the northern portion of the bay and this temporally coincided with the periods of the largest *M. leidyi* abundances (spring and summer). While *M. leidyi* may be a voracious consumer of bivalve larvae, including *A. irradians*, they have their own predation pressures to contend with, which can limit their impacts on bay scallop populations.

The results presented herein suggest ctenophores are an important estuarine predator that should be considered when monitoring and restoring bay scallop populations. Restoration efforts rely on successful larval survival and settlement in order to produce sustainable adult populations. Therefore, bay scallop restoration efforts should consider the role of ctenophore populations in potentially reducing the larval pool. Identifying key predators is an important step in understanding larval predation and recruitment rates.

**Conclusion**

Ctenophores can be significant predators on bay scallop larvae. In these studies, ctenophores consumed between 5 and 50% of available scallop larvae. While ctenophore predation may adversely affect bay scallop populations, it is interesting that a small percentage of ingested larvae are egested live. Egestion from ctenophores, regardless of the time spent within the ctenophore gut, did not have a negative effect on 24-hour survival rates of larvae. Bay scallop restoration efforts, especially seeding programs, should avoid periods when ctenophore populations are abundant.
Supplementary Figures

Figure S1. Bay scallop veliger larvae (left) used in this predation experiment were hatchery-reared and approximately 115 µm in size. Ctenophores (right) were captured from Barnegat Bay, N.J.

Figure S2. Series of buckets used in the experiment to monitor ctenophore egestion of bay scallop larvae over time.
Chapter 2

Bay scallop larval swimming behavior in response to environmental stimuli

Abstract

Bay scallops (*Argopecten irradians*) are a relatively short-lived bivalve species historically found along the North American eastern seaboard. A mesocosm study was conducted to better understand how the swimming behavior of bay scallop larvae is influenced by environmental stimuli. The vertical swimming behavior of bay scallop veliger larvae was observed in response to light, temperature, and salinity. Late-stage scallop veliger larvae (10 days old) were exposed to three temperature conditions: cold (15°C), temperate (23°C), and warm (30°C). To evaluate the effect of salinity on swimming behavior, temperature was held constant at 23°C and veligers were exposed to either 20 ppt, 25 ppt, or 30 ppt conditions. The effect of light on bay scallop swimming response was evaluated under temperate conditions for a MidAtlantic estuary (23°C, 30 ppt) by exposing larvae to either white or red light. High definition video cameras were used to record the swimming behaviors of individual larvae. The swimming trajectories of individual larvae were mapped and average vertical velocities for 10 helical rotations were determined per larvae. There was no effect of light or salinity observed on bay scallop swimming behavior. Most larvae hovered in place with vertical velocities of 0.01 to 0.027 mm s\(^{-1}\). The cold conditions resulted in no swimming larvae as they all settled at the bottom of the aquaria. Increased temperature (30°C) elicited a significantly different response as compared to temperate conditions (23°C). A greater proportion of bay scallop larvae swam upwards at higher speeds of 0.054 mm s\(^{-1}\). Characterizing the swimming behavior of bay scallop
veliger larvae is a fundamental step to inform future larval dispersal models and ultimately to better understand population connectivity.

Key Words:
Bay scallop, larvae, swimming behavior, temperature, salinity, light

Introduction
The bay scallop, *Argopecten irradians*, is a bivalve mollusk commonly associated with seagrass beds along the Atlantic and Gulf Coasts of the United States (Kirby-Smith 1972). From the 1870s to the mid 1980s, bay scallop populations were stable enough to support commercial and recreational fisheries (Ford 1997, Mackenzie 2008). However, over the past six decades or more, bay scallop populations have significantly declined along their entire extensive range. This decline is likely due to the presence of brown tide (*Aureococcus anophagefferens*) as well as to the decline of seagrass meadows (*Zostera marina*), and over-harvesting (Celestino 2007). The rapid declines observed in bay scallop populations depleted the effective spawning stocks. An adequate supply of larvae was unable to be produced and recruitment limitation likely prevents populations from recovering (Peterson and Summerson 1993; Peterson et al. 1996; Tettelbach and Smith 2009).

Bay scallops have a relatively short life span of 18-22 months and usually only spawn once in a given lifetime (Belding 1910, Tettelbach and Smith 2009). Individuals are hermaphroditic and produce large amounts of gametes. Major spawning events in temperate estuaries like Barnegat Bay-Little Egg Harbor (BB-LEH), New Jersey (Bologna et al. 2001) and Bogue Sound, North Carolina (Peterson et al 1996) likely occur during mid-summer to early fall. The larval period ranges from 1-2 weeks and is
characterized by free-swimming veliger larvae (Sastry 1965; Tettelbach and Wenczel 1993). The planktotrophic larvae then settle as pediveligers in beds of eelgrass (*Zostera marina*) (Sastry 1965).

Larval dispersal patterns of estuarine bivalves are of considerable interest. Conservation and restoration strategies rely heavily on an understanding of population connectivity and recruitment dynamics (O’Connor et al. 2006, Krueck et al. 2017, D’Aloia et al. 2015, Kininmonth et al. 2011). Most estuarine bivalves, like bay scallops, are characterized by a short pelagic larval stage subject to relatively large-scale dispersal processes. Passive (advection) and active (swimming) processes impact larval dispersal and thus adult population distribution in estuarine ecosystems (Mann et al. 1991). However, the relative role of these processes is highly debated (Mann 1986a, Stancyk and Feller 1986), with evidence for some combination of passive transport via physical water properties and the ability of larvae to actively orient themselves in the water column (Carriker 1961, Nelson 1955, Kunkle 1957, Wood and Hargis 1971, Armsworth 2000, Paris and Cowen 2004, Largier 2004, Leis and Carson-Ewart 2003, Kough et al. 2013, Goodwin et al. 2019). It is likely that both types of processes are involved in the dispersal of larvae (Mann 1985, Mann 1986b, Cowan et al. 2007).

Bay scallops are an ideal candidate species for using the Regional Ocean Modeling System (ROMS) coupled with the Larval TRANSPORT Lagrangian (LTRANS) particle-tracking model to estimate metapopulation connectivity (Narvaez et al. 2012, Goodwin et al. 2019). However, in order to use such powerful models, attributes such as egg diameter, growth rate, size at settlement, and swimming behavior must be known for the species of interest. Bay scallop connectivity patterns were modeled in Buzzards Bay,
Massachusetts using the FVCOM I-State Configuration model (FISCM) to include physiological factors controlling biogeographical boundaries. The authors were unable to include swimming behavior in the simulations due to a dearth of data for this species (Liu et al 2015). Little is known regarding the swimming behavior of bay scallop larvae. The locomotory behavior of other planktonic bivalve larvae have been studied and while their swimming abilities are often characterized as weak, <2.3 mms\(^{-1}\) (Chia et al 1984), it has been shown to impact the direction and extent of larval dispersal (North et al 2008, Drake et al 2013, Goodwin et al 2019). Successful transport of larvae and the degree of the connectivity between subpopulations, therefore, can be dependent upon larval swimming patterns, largely in the vertical direction due to vertical shear in the currents (North et al 2008, Gilbert et al 2010).

Results from experiments with the king scallop (*Pecten maximus*) demonstrate that early stages of larval development, gastrula and trochophore stages, are either unable to propel themselves or exhibit erratic swimming behavior (Cragg 1980). It can be inferred then that they likely act as neutrally buoyant particles during this early developmental period (Nicolle et al 2013). Later in development, the veliger larvae demonstrate the ability to swim up vertically and intermittently stop swimming by retracting the velum and closing the shell valves (Cragg 1980). Bivalve larvae alternate between swimming upwards, some species swim in straight lines and others in vertical helices (Cragg and Gruffydd 1975, Cragg 1980), with periods of passive sinking (Mann and Wolf 1983). Any movement in the horizontal direction is considered to be passive (Mann and Wolf 1983). The larvae of various bivalve species have demonstrated the ability to respond to different environmental stimuli such as light (Bayne 1964, Wheeler

Bay scallops inhabit coastal estuaries that experience fluctuations in salinity and temperature. For example, BB-LEH is a shallow lagoonal estuary (average depth < 2m, range 1-6 m) and depending on the proximity to the inlets, salinity ranges from 18-25 ppt. Seasonal water temperatures range from -1.4 to 30°C (Kennish 2001). Bay scallops are found in the southern portion of BB-LEH, likely due to a higher salinity in this area. Natural bay scallop populations in eastern Connecticut are likely to experience a salinity of 25 ± 2 ppt, while Martha’s Vineyard populations inhabit waters with saline conditions (average salinity 30 ppt) (Tettelbach and Rhodes 1981). Normal development to straight hinge veliger larvae requires a narrow range of salinity and temperature conditions. Tettelbach and Rhodes (1981) observed greater than 70% normal development only at 20°C and 25‰ and 25°C and 25‰ for the northern subspecies *Argopecten irradians irradians* (as used in the present study). There were significantly reduced rates of normal development observed at salinities higher or lower than 25‰ and no normal development at 10 or 15‰ and 10°C or 35°C. Tolerable salinity and temperature ranges become wider as larvae age and grow. The optimal set of conditions for survival up to setting size was estimated at 17.7°C and 30‰ (Tettelbach and Rhodes 1981). The
salinities and temperatures evaluated in this study are all within a reasonable range of conditions typical for mid-Atlantic and Northeastern coastal estuaries.

To better understand the swimming behavior of bay scallop larvae and how that might affect dispersal patterns, I investigated the vertical locomotory behavior of bay scallop larvae at the veliger stage under a range of environmentally relevant salinity and temperature conditions likely to coincide with summer spawning events. A cold temperature was included (15°C) to investigate potential impacts to a late fall spawn in the Northeast region. I predict that the vertical velocity of bay scallop larvae will be (1) decreased under low saline conditions compared to high salinity conditions, (2) decreased under low temperature conditions compared to high temperature conditions, and (3) increased in the presence of light compared to dark conditions.

Methods

A series of mesocosm experiments were conducted at the Rutgers Aquaculture Innovations Center in Cape May, New Jersey to better understand the swimming response of bay scallop veliger larvae to temperature, salinity, and light. Bay scallop larvae used in this study were provided by the Martha’s Vineyard Shellfish Group. The veliger larvae were 8 days old and 150 μm in size at the time of the experiment. Scallop larvae were added to plastic, rectangular 2L aquariums (24.8 cm L x 9.7 cm W x 15.9 cm H) at a larval density of 2000L⁻¹ (Figure S1). Natural seawater from the Delaware Bay was passed through a 1 μm filter to minimize other particulates in the solution. Larvae were not fed during the experiment.

A series of salinity, temperature, and light regimes was evaluated. To test the effects of temperature, larvae were exposed to cold (15°C), temperate (23°C), and warm
(30°C) conditions, all held at a salinity of 30 ppt. To evaluate the impacts of salinity on swimming behavior, bay scallop larvae were exposed to 20 ppt, 25 ppt, and 30 ppt, all at a constant temperature of 23°C. The effect of light was also determined under 30 ppt and 23°C conditions (Table 1). The 2L experimental aquariums were placed into three different water baths to control temperature. A chiller was used to maintain 15±1°C in one water bath and heaters were used to maintain 23±1°C and 30±1°C in the other two. All treatments, except the light effect treatments, were observed under dark conditions with a waterproof, red LED flashlight (250 lumens, 1000K) (Cragg 1980) fully submerged and directed at the right side of the aquaria. This red light provided enough light to see the shelled larvae on the video while still limiting any effect light may have on swimming behavior. The effect of light was evaluated under similar conditions but with a white LED flashlight (250 lumens, 5000K) submerged and directed at the right side of the aquaria (Figure S1). Triplicate treatment groups were run on Day 1. The entire experiment was repeated on Day 2 with new larvae from the same cohort for a total of 6 replicate aquaria and 30 individual larvae tracked for each treatment group.

Veliger larvae were acclimated to conditions within the aquariums for 60 minutes prior to the initiation of filming. Plastic rulers were inserted through the tops of the aquariums into the center of the aquarium and into the center of the video frame to provide scale and calibrate the video tracking software. Go-pro Hero4 cameras (5 mm focal length) housed in waterproof casings were used to film swimming larvae. Cameras and aquaria were completely submerged underwater. Cameras were placed 2.5 cm from the outside surface of the aquarium and 7.35 cm from the scale in the center. Go-pro cameras have a fish-eye lens. This may cause some distortion in the captured images.
an effort to account for this, cameras were placed in the same position for each treatment and the outer regions of the images were not included in the analysis as these areas were not in focus. Larvae in each aquarium were filmed for a total of 10 minutes (1080p resolution, 60 fps). This time frame was chosen based on preliminary work at this larval density to ensure an adequate number of larvae were recorded in each video.

These videos were imported into Tracker software and the swimming trajectories of 5 individual larvae/aquarium were measured for 10 helical rotations. The first 2 minutes of each video were excluded to minimize any effects associated with starting the recording. Larvae were randomly selected amongst moving larvae that were in focus in the field of view. If a larva moved out of the field of view while being tracked, it was removed from the data set and another moving larva was randomly selected as a replacement. The vertical velocity (mm s\(^{-1}\)) for each measured larva was calculated using the equation \(V=K/T\), where \(K\) is the vertical distance traveled over one rotation and \(T\) is the time it took to complete one rotation (Mann and Wolf 1983; Figure 1). The vertical velocities for 10 consecutive individual rotations were determined for each larva. An average vertical velocity was then generated for each individual bay scallop larva. The video resolution was not high enough to determine if descending larvae were actively swimming or sinking with retracted velum. They will simply be identified as descending larvae. Mean ascending and descending speeds were determined for a given treatment group by calculating the average positive vertical velocity and negative vertical velocity, respectively.

The data were statistically analyzed using R to identify treatment effects. A p-value of <0.05 was \textit{a priori} defined as significant. Data from both days of experiments
were pooled together. The data were not normally distributed and therefore were analyzed via the Aligned Ranks Transformation (ART) ANOVA with aquarium included as a random effect (Mangiafico 2016, Wobbrock et al 2011). All results are presented as box plots demonstrating the median, interquartile range (IQR), minimum, and maximum.

Table 1. Bay scallop larvae swimming velocity was determined under a series of temperature and salinity combinations.

<table>
<thead>
<tr>
<th>Temperature (ºC)</th>
<th>Salinity (ppt)</th>
<th>Light</th>
<th>Replicate</th>
<th>Larvae/replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>20</td>
<td>Dark</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>23</td>
<td>25</td>
<td>Dark</td>
<td>6</td>
<td>5</td>
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<td>23</td>
<td>30</td>
<td>Dark</td>
<td>6</td>
<td>5</td>
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<tr>
<td>23</td>
<td>30</td>
<td>Light</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>Dark</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>Dark</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 1. Illustration demonstrating how the vertical velocity of one rotation was calculated. The Tracker software calculates the time and distance traveled in the y direction from points 1 to 2 and from points 2 to 3. The sum of these values provides the total vertical distance traveled and the time it took to complete one rotation. The total
distance divided by the time provides the vertical velocity for one rotation. Individuals were tracked for 10 consecutive rotations and a mean vertical velocity (mm s\(^{-1}\)) was calculated for each larva. Velocity is a vector quantity, so those individuals with a negative velocity on average were moving downwards and those with a positive velocity were moving upwards.

**Results**

Regardless of environmental stimuli or whether they were ascending or descending, bay scallops were observed to swim in a clockwise, helical pattern. Ascending larvae have positive values for vertical velocity and descending larvae have negative values. Those with vertical velocities at or near zero are hovering in place.

Bay scallop larvae were exposed to the three different salinity regimes, ranging from 20-30 ppt, all at a constant temperature of 23 °C. There was no observed effect of salinity on the vertical velocity of bay scallops (ART ANOVA, \(p=0.327, n=30\); Figure 2). Larvae under this range of salinities all appeared to hover, with little to no change in their vertical placement within the water column. Larvae with positive or negative velocities were grouped together respectively to determine the mean ascension speed (positive vertical velocity) and the mean descension speed (negative vertical velocity) (Table 2). As salinity increases, the ascending speed increases, but the descending speeds stays relatively the same (Table 2). The impact of light on vertical velocity was evaluated under 30 ppt and 23 °C conditions. Even in the presence of light, larvae hover in the water column (ART ANOVA, \(p=0.91865, n=30\); Figure 3).

A range of temperatures, all held at a constant salinity, were also evaluated. No larvae were found swimming in the coldest temperature, 15°C. They all settled on the
bottom of the aquarium. Their survival status was not determined and the 15°C treatment was not included in data analysis. This could be a shock response and larvae may require a longer acclimation period for these cold conditions. Active swimmers were readily observed in the moderate and warm temperatures. There was a significant difference in the vertical velocities measured under 23°C compared to those in the 30°C conditions (ART ANOVA, p=0.034988, n=30; Figure 4). The warmer temperature resulted in a shift in the direction of movement, with more larvae observed swimming upwards (Table 2).

Overall, as evidenced by the increased range of data, larvae were more active in the warmer conditions. More larvae were observed swimming upwards and downwards in the water column. The swimming speed also appears to have increased under warm conditions, with average observed speeds of 0.05 mm s⁻¹ and few larvae reaching speeds of 0.2 mm s⁻¹ (Table 2).

Table 2. Upward and downward swimming speeds of bay scallop veliger larvae under various environmental conditions. Vertical velocities of larvae were grouped according to positive and negative values. Positive values represent ascending larvae and negative values represent descending larvae. Speeds shown are mean ± standard deviation.
Figure 2. The vertical velocity of bay scallop veliger larvae measured under different salinity regimes. All salinity treatments were held under dark and 23 °C conditions. Dots represent individual data points (n=30).
Figure 3. The vertical velocity of bay scallop veliger larvae measured under light and dark conditions. All treatment groups were held under 23 °C and 30 ppt conditions. Dots represent individual data points (n=30).
Figure 4. The vertical velocity of bay scallop veliger larvae measured under different temperature regimes. All temperature treatments were held under 30 ppt conditions. Dots represent individual data points. Different letters represent significant difference between groups (ART ANOVA, p=0.034988; n=30). A third temperature, 15 °C, was included in the experiment but excluded from analysis as no larvae were found swimming in this treatment.

Discussion

The swimming behavior of *Argopecten irradians* larvae is poorly understood and understudied. To our knowledge, this is the first study to calculate vertical velocity and characterize bay scallop swimming behavior under different environmental conditions. Bay scallop veliger larvae were observed to swim in a clockwise, helical pattern along a straight line, consistent with observations made by Costello et al (1957), at speeds ranging from 0.01-0.05 mm s\(^{-1}\).
Most bay scallop larvae observed in this study, regardless of environmental stimuli presented, exhibited a tendency to hover, with little to no vertical movement. Increased temperature resulted in a shift in this pattern and greater swimming activity both upwards and downwards in the water column was observed, with a trend towards more larvae swimming upwards. Mann and Wolf (1983) observed the opposite effect of temperature on the swimming behavior of ocean quahog larvae (*Arctica islandica*). When presented with a thermal gradient, these larvae reduced their movement to avoid higher temperatures (stage dependent, ranging from 17°C-25°C). If a thermal gradient is present in an estuary, the warmer water will be located close to the surface. By swimming upwards under warm water conditions, bay scallop larvae will continue to encounter these high temperatures. They will also be located higher in the water column and possibly more susceptible to wind-driven dispersal. It has been hypothesized that by sinking in response to waterborne cues, such as turbulence, planktonic larvae may settle more successfully (Fuchs et al 2004, Wheeler et al 2017). The high temperature evaluated in the present study, 30°C, is well within the range of temperatures bay scallop larvae may encounter during late summer spawns (Chizmadia et al 1984 in Kennish and Lutz, Kennish 2001, Kennish, personal communication, 2021). It is possible that bay scallop veliger larvae may actively swim upwards in an effort to avoid settlement in that particular environment due to the higher water temperatures. When exposed to cold temperatures, bay scallop larvae reacted in a similar manner as that of ocean quahog larvae (Mann and Wolf 1983). Both species were observed to sink to the base of the aquarium (present study) or cylinder and remain closed for the duration of the experiment.
(Mann and Wolf 1983). When exposed to more temperate conditions (23°C), bay scallop larvae mainly hovered in place, with close to zero vertical velocities.

Bay scallop larvae did not exhibit any type of phototaxis in this study. Light did not have an effect on the vertical velocity of bay scallop larvae, the proportions of ascending and descending larvae, or the speed of movement. However, due to experimental design limitations, the light source came from the side and not from above as it would under natural conditions. Therefore, it is possible the larvae did not react to the light the same as they would to natural sunlight filtering through the water from above. Mann and Wolf (1983) concluded that ocean quahog larvae showed no evidence of phototaxis. Wheeler and colleagues (2017) also found that light did not affect the proportion of upward swimming eastern oyster (*Crassostrea virginica*) larvae. However, oyster larvae did respond to the presence of light in other ways, such as by changing their vertical and horizontal velocities by altering the helix geometry (Wheeler et al 2017).

The salinities evaluated in this study overall did not have an impact on bay scallop larvae swimming behavior. *Crassostrea virginica* larvae will change their vertical position in the water column in relation to salinity changes (Wood and Hargis 1971, Hidu and Haskin 1978). Active depth regulation can contribute to larval retention in the system as density differences in surface and bottom water contribute to net waterflow directions (Mann et al 1991). It is possible that when confronted with a salinity gradient, as opposed to a variety of intensities of environmental stimuli as used in the present study, differences in bay scallop swimming behavior and evidence of active depth regulation may be observed. Stimuli gradients more closely mimic natural discontinuities associated
with the length and depth of estuaries and tidal flow and may elicit a different response from swimming larvae (Mann et al. 1991).

Overall, observed swimming speeds for bay scallop larvae were lower than speeds reported for other bivalve larvae. Most pelagic larvae of marine benthic invertebrates have vertical speeds ranging from 0.67 to 2.00 mm s$^{-1}$ (as reviewed by Mileikovsky 1973). Published values for bivalve larvae fall within this range (Table 3). While lower than these other bivalve species, the values for *A. irradians* were closer to the other scallop on this list, *Pecten maximus*. Additionally, there is a wide range of variation in the measured speeds for bay scallop larvae (Table 2). Vertical displacement may be stage specific, as Cragg (1980) reported more early stage larvae swimming upwards. As development progressed, more larvae were observed lower in the water column. The rate of displacement may also be related to larval shell length, with larger larvae swimming upwards faster until a plateau at a particular shell length is reached, as described for *Crassostrea gigas* and *Mytilus edulis* (Troost 2008).
Table 3. Swimming speeds for bivalves as reported in the literature.

<table>
<thead>
<tr>
<th>Species</th>
<th>Swimming Speed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercenaria mercenaria</td>
<td>1.17 to 1.33 mm s⁻¹</td>
<td>Turner and George 1955</td>
</tr>
<tr>
<td>Teredo bartschi</td>
<td>7.7 mm s⁻¹</td>
<td>Isham and Tierney 1953</td>
</tr>
<tr>
<td>Mytilus edulis</td>
<td>1.1 mm s⁻¹</td>
<td>Konstantinova 1966</td>
</tr>
<tr>
<td>Crassostrea virginica</td>
<td>0.75 to 10 mm s⁻¹</td>
<td>Wood and Hargis 1971</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hidu and Haskin 1978</td>
</tr>
<tr>
<td>Ostrea edulis</td>
<td>1.23 mm s⁻¹</td>
<td>Cragg and Gruffydd 1975</td>
</tr>
<tr>
<td>Arctica islandica</td>
<td>0.2 to 0.52 mm s⁻¹</td>
<td>Mann and Wolf 1983</td>
</tr>
<tr>
<td>Spisula solidissima, Mulinia lateralis, and Rangia cuneata</td>
<td>0.18 to 0.53</td>
<td>Mann et al 1991</td>
</tr>
<tr>
<td>Pecten maximus</td>
<td>0.17 to 0.46 mm s⁻¹</td>
<td>Cragg 1980</td>
</tr>
</tbody>
</table>

Increasing our understanding of bay scallop swimming behavior allows for the use of powerful modeling tools to better predict population connectivity and identify habitats to protect. There is evidence to support the retention of bay scallop larvae within a given estuary. Different patterns of recruitment and settlement were observed between locations in separate but connected water bodies (Peterson and Summerson 1993). There is also evidence of more wide-scale larval transport occurring. Genetic data suggest the current population of bay scallops in Barnegat Bay-Little Egg Harbor were recruited from Long Island, New York. The prior, now extinct, population were more similar to North Carolina populations. A subspecies shift has occurred in this particular water body (Campanella et al 2007). To fully understand these population dynamics, larval transport mechanisms must be investigated. Physical circulation models used to estimate larval connectivity are increasingly utilizing a larval behavioral sub-component to better predict larval transport pathways and inter-population connectivity patterns with varying success.
As observed in the present study, bay scallops swam upwards at a faster rate when exposed to 30°C water. This temperature is typical of shallow estuaries during late summer when scallops are expected to spawn. Additionally, with climate change water temperatures are expected to rise. It is likely then that bay scallop larvae will encounter similar water temperatures and change their location within the water column, possibly making them less like passive particles. It is important for larval transport models to account for these changes to most accurately predict recruitment patterns.

Additional research is required to further understand the swimming behavior of bay scallop larvae and better predict how it will change under different environmental conditions. The present study determined the vertical velocity, ascension and descension speeds, and relative proportion of veliger larvae swimming upwards under distinct intensities of environmental stimuli (temperature, salinity, and light). It is recommended to expand on this body of data by including other larval stages, such as early trochophores, as environmental conditions required for normal development can be stage specific (Tettelbach and Rhodes 1981). Other swimming metrics, like the helix geometry and horizontal velocity, should also be evaluated. It has been hypothesized that changes in the helical geometry can serve an anti-predatory function (Wheeler et al 2017). The impact of thermal and salinity gradients on bay scallop larvae swimming behavior should be investigated as these discontinuities more closely mimic natural estuarine conditions (Mann et al 1991). Finally, the swimming data generated from this study and future work should be incorporated into larval dispersal models to further investigate the role of swimming behavior in dispersal patterns.
Conclusions

This is first study to closely examine bay scallop larvae swimming behavior. In general, larvae were observed to hover, with close to zero vertical velocity, under a variety of environmental conditions. When exposed to warm water conditions (30°C), more bay scallop larvae were observed to increase their swimming activity both upwards and downwards at faster rates than those exposed to 23°C. No swimming behavior was observed at 15°C as all larvae had settled on the bottom of the aquariums. It is critical to determine swimming speeds and vertical movement patterns for bay scallops so that powerful modeling tools can be utilized to further investigate larval dispersal. The selection of marine protected areas (MAPs) and environmentally sensitive areas (ESAs) can be better informed with a clear understanding of larval dispersal patterns for species of interest. The conservation and restoration of bay scallops promotes a healthy and diverse ecosystem and therefore lends itself as an ideal candidate for further research on dispersal and population connectivity.
Supplementary Figures

Figure S1. Photographs demonstrating the experimental set-up in one of the water baths (23°C). Aquariums are fully submerged in the water bath and rulers are positioned in the center of each aquarium. Overhead lights were on when the photograph on the left was taken to clearly show the set-up. However, during the experiment, the lights were off and only the submerged red light was on as seem in the picture on the right. During the light trials, the red light was replaced with a white light.
Chapter 3
The effects of seagrass epiphytes on juvenile bay scallop (*Argopecten irradians*) attachment under mesocosm conditions

Abstract

Juvenile bay scallops attach themselves via byssal threads to vertical blades of eelgrass, which provide protection against predation. Juvenile attachment can impact recruitment success. The eutrophication of coastal estuaries like Barnegat Bay-Little Egg Harbor, New Jersey is well documented within the scientific literature and microalgal blooms and epiphytes are occurring with increased frequency and intensity within this lagoonal estuary. While the effects of seagrass-associated epiphytes on bay scallop attachment are largely unknown, there is some indication that the presence of epiphytes on seagrass blades may enhance bivalve recruitment by increasing habitat complexity. In order to evaluate the relationship between epiphytic presence and scallop attachment, three experimental treatments were used: non-manipulated Artificial Seagrass Units (ASUs) (controls), ASUs with rasped ribbon to artificially mimic secondary structure, and ASUs primed with naturally occurring epiphytes (Bologna and Heck 1999). Primed ASUs were deployed in a nearby seagrass bed one week prior to the start of the experiment to allow for a natural epiphytic community to be established on the ribbons. Replicate treatments were arranged in a random design throughout the tank. Sand was added to the tank to serve as a bottom substrate. Bay scallops (5-10 mm in size) were randomly dispersed throughout the tank. Scallop attachment was monitored bi-hourly for the first 12 hours then once every 12 hours for an additional 72 hours. This monitoring
schedule allowed for the assessment of initial scallop dispersal patterns and prolonged distributions (Kamenos et al. 2004). Bay scallops did not demonstrate a preference among the experimental units; however, a tendency to attach to the aquarium walls was observed which could have implication for future field restoration strategies.

**Key Words**
Bay scallop, seagrass, attachment, epiphytes, eutrophication

**Introduction**

Increased development along the Atlantic coast has resulted in wide-spread changes to the adjacent estuaries and bays (Lathrop and Conway 2001, Lathrop and Bognar 2001, Zampella et al 2007). Low dissolved oxygen concentrations, algal blooms (Gastrich et al. 2004, Kennish and de Jonge 2011), heavy epiphytic loading, and declines in seagrass biomass (Kennish et al. 2010, Fertig et al. 2013) are all indicators of increased nutrient loads and have been observed in mid-Atlantic estuarine ecosystems, like Barnegat Bay-Little Egg Harbor (BB-LEH) (Baker et al 2014). The eutrophication of BB-LEH is well documented within the scientific literature (Bricker et al. 2007, Kennish 2009, Fertig et al. 2013, Fertig et al 2014, Kennish et al. 2007) and seagrass beds are declining in response to these elevated nutrient levels (Kennish et al. 2008, Kennish and Fertig 2012, Fertig et al. 2013). Additionally, macroalgal blooms are occurring with increased frequency and intensity within this lagoonal estuary. Ephemeral algal species like *Ulva lactuca* and *Enteromorpha intestinalis* are prevalent in this system and form dense mats that can block light transmission to underlying sea grass beds (Kennish et al. 2011, Fertig et al. 2014). Phytoplankton blooms and epiphytes also reduce light transmission to the seagrass. The seagrass beds, then, experience diebacks, which can
have deleterious effects on the estuarine ecosystem as these beds are critical primary producers (Lathrop et al. 2006) and provide an important benthic habitat for organisms such as bay scallops (Kennish and de Jonge 2011). While negatively affected by eutrophication, recent work suggests that seagrass meadows remain a significant component of the benthic habitat within BB-LEH but show high year-to-year spatial and temporal dynamics (Lathrop et al. 2014). While the effects of seagrass-associated epiphytes on bay scallop attachment are largely unknown, there is some indication that the presence of epiphytes on seagrass blades and macroalgae may enhance bivalve recruitment by increasing habitat complexity (Bologna and Heck 1999; Howarth et al. 2011).

The bay scallop larval period ranges from 1-2 weeks and consists of free-swimming veliger larvae (Sastry 1965; Tettelbach and Wenczel 1993). The planktotrophic larvae then settle as pediveligers in beds of eelgrass (Zostera marina) (Sastry 1965). After settlement occurs, the juvenile bay scallops attach themselves via byssal threads to vertical blades of eelgrass, which provide protection against predation and the effects of siltation (Thayer and Stuart 1974, Castagna 1975, Pohle et al 1991). Over time, the juveniles grow too heavy for the attachment and fall to the bottom of the bed (Thayer and Stuart 1974, Pohle et al. 1991).

The aim of this work was to assess the effects of these epiphytic blooms on the relationship between bay scallops and seagrasses in order to better understand the basic ecology of bay scallops and how eutrophication might influence their behavior. Attachment refers to the process by which newly metamorphosed juveniles attach themselves to eelgrass blades via byssal threads. Juvenile attachment can impact
recruitment success. Artificial seagrass units (ASUs) were used to address this question. The use of ASUs allows for the study to be carried out in tanks where the scallops can be closely monitored. Three experimental treatments were used: non-manipulated Artificial Seagrass Units (ASUs) (controls), ASUs with rasped ribbon to artificially mimic secondary structure, and ASUs primed with naturally occurring epiphytes (Bologna and Heck 1999). The experiment will test the following hypotheses:

1. A greater number of juvenile bay scallops will attach to the primed ribbons with an established epiphyte community compared to the non-manipulated control and rasped ribbons during the first 12 hours.

2. The initial dispersal pattern will persist over the duration of the experiment.

Methods

Artificial seagrass units were constructed of white egg crate styrene lighting panels cut into 8.89 cm x 21.59 cm rectangles (area = 191.9 cm²). Green polypropylene “wrapping” ribbons were tied on the panels to function as eel grass shoots, each with a width of 0.5 cm and a length of 18 cm. The surface area of ribbons in each treatment was 432 cm². Each ASU had 24 ribbon blades per unit at a density of 0.13 ribbons/cm². The control ASUs had clean, smooth ribbon. A rasp was used on the ribbon blades on the artificial control ASUs to mimic secondary structure without adding any biological material (Figures S3 and S4). The primed ASUs were deployed in a natural seagrass bed near Mordecai Island, New Jersey in Barnegat Bay for a week prior to the start of the experiment.

A series of eight glass aquaria (60.96 x 30.48 x 50.8 cm, L x W x H) were set up inside of the Rutgers Aquaculture Innovations Center as experimental replicates. Each
aquarium had 3L of clean sand, 38 L of filtered natural seawater, and each of the three ASUs (control, artificial control, and primed). Sand was collected from a nearby beach and washed twice. The surface area of available wall space submerged underwater was 4,645 cm$^2$. An air stone to provide aeration over the duration of the experiment was also installed in each of the aquaria. The arrangement of the ASUs in the aquarium was determined using a random number generator so that each type of ASU had an equal chance of being located on the edge or in the middle of the aquarium. The aquariums were set-up as described above approximately 12 hours before the addition of bay scallop seed and the initiation of the experiment.

Bay scallop seed were obtained from the Virginia Institute of Marine Science, Eastern Shore Laboratory for use in this experiment. Each replicate aquarium was stocked with 20 bay scallops. A random subsample (30%) of the scallops were photographed and measured using Image J (Schneider et al 2012) to obtain shell lengths (parallel to shell hinge). The average shell length of scallops used in this experiment was 9.24 mm ± 1.55 (standard deviation).

This experiment was conducted in two trials. Juvenile bay scallop attachment was monitored bi-hourly for the first 12 hours then once every 12 hours for an additional 48 hours during the first experimental run. The second iteration of the experiment repeated the monitoring of the initial attachment phase by observing attachment behavior bi-hourly for three hours. New bay scallops from the same cohort of seed were used for the second trial. Both experimental trials were conducted over a three-day period overall.

At the end of the experiment, 3 ribbon blades from each of the primed ASUs were randomly selected (n=24). These ribbons were weighed, wiped clean with ethanol, and
weighed again to estimate the epiphytic biomass associated with the primed ASUs (Table 1).

Table 1. Mean ± standard deviation (n = 3) of biomass on naturally primed ribbon blades.

<table>
<thead>
<tr>
<th>Replicate Aquaria</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiphytic Biomass (mg/cm)</td>
<td>4.78 ± 2.41</td>
<td>5.45 ± 0.918</td>
<td>2.75 ± 0.599</td>
<td>4.60 ± 2.25</td>
<td>8.64 ± 5.73</td>
<td>3.81 ± 0.580</td>
<td>2.60 ± 0.243</td>
<td>8.65 ± 3.63</td>
</tr>
</tbody>
</table>

The aquaria provided other surfaces for attachment. A large proportion of scallops attached to the aquarium walls and airlines. Scallops also remained on the sand or relocated back to the sand by the end of the experiment (Figures 1 and 2). Only the ASUs were included in the statistical analysis (Pohle et al 1991). Attachment site preference was evaluated at specific time points along the course of the trials. Hour 3 was chosen to represent initial dispersal patterns, and this was included in both trials. I also chose hours 7 and 12 to determine if there was any pattern in attachment preference. Finally, hour 48 was included to observe if any patterns persisted over time. Data were analyzed for normality via Shapiro test. In all cases, the data were not normally distributed and Kruskal-Wallis tests were used to determine any differences in attachment substrate. All results are presented as median ± interquartile range (IQR).

Results

During both trials the bay scallops attached to other available surfaces in the aquarium habitats such as walls and airlines (Figure 1). Many also failed to attach to a vertical substrate and remained on the sandy bottom or moved back to the sand (Figures 1 and 2).
Figure 1. Median ± IQR (n = 8) of bay scallops attached to each available substrate type over the course of trial 1. Dots represent outliers. The top grey bars represent the time points sampled in hours.
Figure 2. Median ± IQR (n = 8) of bay scallops attached to each available substrate type over the course of Trial 2. Dots represent outliers. The top grey bars represent the time points sampled in hours.

There was no significant difference in attachment site preference by juvenile bay scallops over the duration of the experiment. Similar dispersal patterns were observed in both trials by the third hour (Figure 3). A small number of scallops were attached to all
three ribbon types over the initial time period of the experiment. However, by 48 hours all bay scallops had settled back to the sandy bottom (Figures 3, 4, 5, and 6).

Figure 3. Median ± IQR (n = 8) of bay scallops attached to the ASUs 3 hours post introduction. Dots represent outliers. Note the different y axis scales between the two trials.
Figure 4. Median ± IQR (n = 8) of bay scallops attached to the ASUs 7 hours post introduction. Dots represent outliers.
Figure 5. Median ± IQR (n = 8) of bay scallops attached to the ASUs 12 hours post introduction. Dots represent outliers.
Figure 6. Median ± IQR (n = 8) of bay scallops attached to available substrates 48 hours post introduction.

Discussion

Seagrass blades provide a vertical spatial refuge when bay scallops are particularly vulnerable to predation. Predation is a primary driver controlling the abundance of bivalve populations, especially prior to growing large enough to a size refuge (Eggleston 1990, Peterson 1990). Bay scallops are a particularly vulnerable bivalve species due to their thin shells, inability to maintain prolonged valve closure, and epifaunal life history (Morgan et al 1980). By positioning themselves on vertical seagrass blades, bay scallops are awarded some protection from benthic predators such as crabs, starfish, oyster drills, and whelks (Morgan et al 1980, Tettelbach 1986, Pohle et al 1991).

Juvenile bay scallops as small as 150-190 μm in length have been shown to attach to seagrass blades (Eckman 1987). Once attached, they experience rapid growth and
eventually become too heavy and relocate to the bottom (Thayer and Stuart 1974). During the small juvenile stage, this attachment process is dynamic. Scallops can detach and reattach as necessary, which is useful in a seagrass system, where eelgrass can shed blades every 6-8 days (Eckman 1987).

Bay scallops are an interesting candidate for bivalve behavioral studies. Unlike other estuarine bivalve species, such as oysters, they have the ability to move throughout their environment over the course of their entire life cycle. It is logical then to predict they have habitat preferences. Kamenos et al 2003 demonstrated that juvenile queen scallops (*Aequipecten opercularis*) have a predetermined habitat attachment preference to pristine live maerl and their habitat usage changes in the presence of predators.

Our study did not demonstrate a bay scallop preference for eelgrass blade complexity or the presence of a biotic epiphytic community. Juveniles attached equally to clean and smooth eelgrass mimics, clean and rasped mimics, as well as eelgrass mimics that were covered in a natural epiphytic community. These results are different from what previous studies have observed regarding larval settlement. Primed substrates (allowed to accumulate a natural biofilm) have been shown to increase pelagic larval settlement of bryozoans and ascidians (Weiczorek and Todd 1997). Bologna and Heck 1999 observed a significant increase in the larval settlement of Mytilidae and *A. irradians* on naturally primed ASUs. They also found enhanced bivalve settlement on the rasped ASUs. It is possible that these settlement patterns may be species specific and *A. irradians* larvae would not show the same preference to primed ASUs. A more likely possibility is that since larval settlement and juvenile attachment are different life history events, different factors are influencing habitat selection. Sometimes the primary settlement site may be
suitable for juveniles of various species but not ideal for adults (Ambrose and Irlandi 1992). Often in these cases, the juveniles will at some point move to a new location by various species-specific means (Thayer and Stuart 1974, Sigurdsson et al 1976, Olivier et al 1996, and Commito et al 1995).

A strong tendency for bay scallops to attach other vertical structures present in the aquariums, such as the walls and airlines, was observed. This is consistent with the results from Pohle et al 1991. They observed an increased tendency to attach to aquarium walls with increasing scallop size and decreased eelgrass shoot density. The bay scallops in their study ranged from 6-20 mm. The scallops in the present experiment were ~9 mm and the shoot density was in the lower range. Relative surface area could be driving this propensity for scallops to attach to the aquarium walls which provided a significantly greater area compared to the ribbons. Alternatively, it is possible that these surfaces provided a more stable attachment point.

Bay scallop larvae are carried by marine currents and transported from spawning sites to juvenile habitats. They act largely as passive particles and rely on flow and water quality to survive and reach an appropriate settlement site (Liu et al 2015). Landscape characteristics play a critical role in recruitment success (Carroll et al 2012). As seagrass beds decline in temperate estuaries, like BB-LEH, a fragmented habitat emerges. This mosaic of seagrass patches is likely a driver of larval settlement patterns (Bologna and Heck 1999). Following settlement, juvenile success is dependent on suitable habitat to provide protection from predation (Irlandi et al 1995, 1999).

Bay scallop restoration efforts are underway in many different estuaries. Understanding the role habitat plays in juvenile bay scallop survival is critical to
informing successful restoration strategies. Establishment of spawner sanctuaries is a restoration strategy that has been used for bay scallops with moderate success (Arnold et al. 2005). Healthy bay scallops are introduced to specific areas and allowed to spawn naturally. Success of this approach is dependent on larval settlement in suitable sites that allow for the survival and growth of the bay scallops (Goldberg et al. 2000). However, as eutrophication increases in estuarine ecosystems, the seagrass beds that native and sanctuary scallop populations rely on for juvenile survivorship are declining. While it is still critical to protect seagrass beds, the results of this study suggest that alternative vertical structures could be used in suitable larval settlement sites to promote juvenile attachment. The design and implementation of these structures would require additional experimentation to determine the ideal material, size, and shape to optimize juvenile bay scallop success as well as to minimize any negative impacts on the natural fauna.

Additional consideration should be given to building large ASU’s in the field adjacent to natural seagrass beds to enhance conditions and improve larval settlement. The goal would be to increase the surface area of vertical substrates to create an alternate habitat where larvae settlement is predicted.

Numerous studies and anecdotal information suggest that alternative habitats for bay scallops exist naturally. One of the most important bay scallop fisheries in New York during the 1970s and early 1980s occurred in Flanders Bay, Long Island, NY (NYSDEC 2008). However, during this same time, there was no eelgrass present in the bay (Cashin Associates 1996). Some other submerged aquatic structure was providing settlement sites and refuge for young bay scallops. Fishermen in the Niantic River, Connecticut called a branching alga of unknown species “scallop weed” as it was covered with a dense set of
scallops (Marshal 1960). Bay scallops have been observed attached to macroalgae, like *Ulva, Chondrus, and Codium* (Kelly 1981, Tettelbach 1986, 1991). How these macroalgae habitats compare to eelgrass is still poorly understood. Carroll et al 2010 found that *Codium fragile* provided a refuge from predation comparable to eelgrass. However, they acknowledge other trade-offs may be occurring. *C. fragile* is associated with low dissolved oxygen (Tyler 2007). Water flow may be different between the two habitats and that would impact food availability, sedimentation, epiphyte composition, and larval settlement (Ferner et al 2009).

**Conclusions**

Juvenile bay scallops did not attach preferentially to artificial seagrass blades with enhanced 3-D structure, either composed of organic epiphytes or inorganic rasped edges. The results of this study add to a growing body of data which suggest alternative bay scallop habitats exist and bay scallops may not be as completely dependent on seagrasses for survival. Further research in the field is required to assess if these alternative habitats provide a spatial refuge comparable to seagrass beds. As populations of both species experience declines, there is some hope that the demise of one does not necessarily condemn the other to extinction as well.
Supplemental Figures

Figure S1. Experimental set-up at the Rutgers AIC.

Figure S2. Example aquarium with each of the ASU treatments: naturally primed, clean control, and rasped artificial control.
Figure S3. An example of the rasped artificial control with bay scallops on the sand.

Figure S4. Example of a clean control with a bay scallop attached (circled in red).
Chapter 4

The effects of seagrass shoot density on growth and survivorship of bay scallops, *Argopecten irradians*

Abstract

Bay scallops (*Argopecten irradians*) are a relatively short-lived bivalve species historically found along East Atlantic coast and Gulf regions of North America. Their populations have experienced significant declines attributed to loss of seagrass habitat. Estuarine systems are experiencing varying levels of eutrophication as a result of coastal development and seagrass beds are declining in response to these elevated nutrient levels. An important aim of this project was to target the impacts of eelgrass shoot density on bay scallop growth and survivorship. Experiments were carried out in annular flumes at Rutgers Aquaculture Innovations Center in Cape May, New Jersey. Artificial seagrass units (ASUs) were used in flume experiments to create a high shoot density environment (90% cover; 570 shoots/m$^2$) and a low shoot density environment (25% cover; 200 shoots/m$^2$). The absence of seagrass was also tested as a control treatment. Individually marked and measured bay scallops were added to each of the flumes. Filtered seawater was spiked with an environmentally relevant concentration of lab-cultured phytoplankton and this concentration was maintained throughout the duration of the experiment for a total of five weeks. Temperature was also maintained at 21°C to mimic summer conditions in an estuary. There was no impact of seagrass shoot density on bay scallop survivorship or shell growth. There was a trend toward reduced soft tissue weight in seagrass environments and no adverse impact on glutathione concentrations indicating energy reallocation could be occurring, prioritizing glutathione. The results of this study
suggest bay scallop restoration efforts would be best applied in seagrass beds with low shoot density patches.

**Key Words**

Bay scallop, seagrass, eutrophication, growth, survival, glutathione

**Introduction**

Increased coastal development has left many Atlantic estuaries and bays in a state of eutrophication. Community structures are changing as a result of these increased nutrient loads. Species that are more tolerant of various pollutants and poor water quality are finding greater success and outcompeting others (Rosenberg et al 2004), resulting in lowered species richness and diversity (Ruiz et al 1997) and loss of natural habitats (Lathrop and Bognar 2001). For example, the eutrophication of Barnegat Bay- Little Egg Harbor (BB-LEH) is well documented within the scientific literature (Bricker et al. 2007, Kennish 2009, Fertig et al. 2013, Kennish et al. 2007) and seagrass beds are declining in both patch size and density in response to these elevated nutrient levels, hazardous algal blooms (HABs), and shading effects (Kennish et al. 2008, Kennish and Fertig 2012, Fertig et al. 2013).

Juvenile and adult bay scallops (*Argopecten irradians*) use seagrass beds as their primary habitat. There is evidence that adult scallops will actively select against bare-bottom sandy habitats and are capable of traveling relatively long distances (> 0.5 km over three months) to locate a more suitable environment (Bologna and Heck 1999). The habitat they reside in may impact their survival, growth, and reproduction. Studies have demonstrated that bay scallops experience higher rates of predation at seagrass bed edges compared with the interior of the beds. However, those individuals residing at the
seagrass bed edges also exhibit higher growth rates and this is likely attributed to increased food availability (Bologna and Heck 1999; Carroll and Peterson 2013). Previous studies have focused primarily on evaluating the effects of seagrass patch size on bay scallop growth and mortality with varying results. However, the shoot density of seagrass beds can impact both predation rates (Orth 1992), water flow and food supply (Eckman et al. 1989, Cahalan et al. 1989, Fonseca et al. 1982). Generally, water flow speeds are highest outside of an eelgrass bed and slow within the interior of the canopy as a result of a loss of momentum through friction along leaf surfaces (Koch 2001). Eelgrass beds reduce water flow and likely the flux of phytoplankton concentrations as well. Thus, the shoot density of seagrass beds may then have an effect on the growth and survivorship of bay scallops (Irlandi et al. 1999). An important aim of this project is to strategically investigate the impacts of shoot density in the absence of predation.

Individual growth of bivalve species can be limited by food availability (Peterson 1982, Bertness and Grosholz 1985, Peterson and Black 1987, Olafsson et al 1994). While the evaluation of shell growth through the measurement of length and height is informative, bivalves have also demonstrated the propensity to lay down thinner, less dense shells at the expense of maintaining their growth rate when under environmentally stressful conditions (Ringwood 1992, Ruiz et al. 1995, Hoare et al. 1995). Prior studies have primarily used shell length and height as an indicator of growth. Shell size, density, and soft tissue weight were used as indicators of growth in these sets of experiments.

Glutathione (GSH), a tripeptide involved in oxidative stress response, is the most abundant antioxidant in all organisms. It functions to reduce reactive oxygen species, such as superoxide and hydroxyl radicals, as well as to serve as a substrate for glutathione
peroxidase, another antioxidant enzyme (Kelly et al. 1998). GSH is sensitive to food limitation (Jaegar et al. 1974, Gismondi et al. 2012) and reduced levels can increase the potential for incurring adverse effects due to other environmental stressors, such as algal toxins (Meister and Anderson 1983, Ringwood et al. 1998, Ringwood et al. 1999). GSH concentrations were evaluated as a physiological indicator of bay scallop health.

The objectives of this study were to determine the effects of seagrass patch density on scallop growth and survivorship. This study was used to evaluate the following hypotheses:

1. Greater seagrass shoot density will result in decreased concentrations of available phytoplankton compared to less dense seagrass beds and bare bottom sediments.
2. Exposure to greater seagrass shoot density will not have an effect on bay scallop mortality compared to those in less dense seagrass beds and bare bottom sediments.
3. Exposure to greater seagrass shoot density will not have an effect on bay scallop shell growth compared to those in less dense seagrass beds and bare bottom sediments.
4. Exposure to greater seagrass shoot density will result in reduced bay scallop shell weights compared to those in less dense seagrass beds and bare bottom sediments.
5. Exposure to greater seagrass shoot density will result in reduced bay scallop soft tissue weight compared to those in less dense seagrass beds and bare bottom sediments.
6. Exposure to greater seagrass shoot density will result in reduced glutathione concentrations compared to those in less dense seagrass beds and bare bottom sediments.

**Methods**

A series of mesocosm experiments were conducted using the annular flumes at the Rutgers Aquaculture Innovations Center (AIC) in Cape May, New Jersey to test these predictions. Artificial seagrass units (ASUs) were used to mimic eelgrass beds (*Zostera marina*) and were constructed of white egg crate styrene lighting panels. Green polypropylene “wrapping” ribbons were tied on the panels to function as eelgrass shoots, each with a length of 15 cm and a width of 0.5 cm (Bologna and Heck 1999) (Figures S1 and S2). The use of ASUs allowed these experiments to be carried out year-round without concern of eelgrass care and maintenance. Additionally, it ensured that shoot density in each treatment was uniform throughout the experiment and replicable.

Three counter-rotating annular (ring-shaped) flumes provided a consistent laminar water flow and temperature. These flumes are modeled on one from the Delft University of Technology in the Netherlands (Visser et al 1992). The flumes are constructed of a ring-shaped channel and a top plate. The top plate is in contact with the water surface and by rotating in one direction, it drives the water flow. The top plate is also capable of water temperature regulation. The flume channel rotates in the opposite direction to reduce secondary cross channel flow. A rotation ratio of 1.4:1 was selected to most effectively reduce this secondary flow (C.M. Fuller unpubl. data). The outside diameter of each flume is 400 cm (circumference 12.7 m) and the flume channel is 30 cm wide.
The volume of each flume with 20-cm water depth (as used in this study) is 700 l (Figure S2).

Three categories of seagrass densities were evaluated: (1) low density, (2) high density, and (3) sand (control treatment) following the differentiation utilized by Lathrop et al. (2006). The low-density treatment was designed to be ~25% coverage with 200 shoots/m². The high-density treatment had ~90% coverage with 570 shoots/m² (Worcester 1995; Figure S1). Sand was collected from a nearby beach. Artificial seagrass beds were constructed by placing ASUs along the bottom of each flume, in its entirety, then covering the egg crate with a 5 cm layer of sand. While the sand treatment did not have any ribbon shoots, egg crate was still placed under the sand as a control (Figure S3).

Filtered natural seawater (1µm) was added to the flumes to a depth of 20 cm and a moderate flow rate of ~10 cm/s was generated. Temperature was maintained at 20°C to mimic summer conditions in a shallow lagoonal estuary.

Water spiked with a known concentration of cultured phytoplankton, a mix of *Nannochloropsis* and *Isochrysis galbana* T-ISO (*Tisochrysis lutea*), was added to the flumes on a tri-weekly schedule at 100,000 cells/mL. This density was chosen to allow concentrations that the Coulter Counter could accurately measure and also be representative of an environmentally relevant concentration of algae. To provide an estimate of scallop food consumption, the remaining concentration of available phytoplankton, i.e. algal cells not consumed by scallops after 24 hours and still in the water column, was determined bi-weekly. For example, phytoplankton were added on Monday, Wednesday, and Friday. Samples were taken on Tuesday and Thursday. In an effort to determine if phytoplankton were evenly mixing throughout the water column or
if vertical stratification was occurring as a result of shoot density treatments, water samples were taken at the bottom, middle, and top of the water column. Water samples were obtained by inserting a 3 mL syringe attached to a piece of stainless-steel floral wire between a gap along the outer edge of the flume channel and the top plate. Water samples were collected immediately upon insertion into the water column at the desired depth, but some turbulent mixing may have occurred. These water samples were taken at three equidistant locations at the exterior edge of the ASU beds throughout each flume, 133 cm apart from each other. Real time phytoplankton concentrations were measured using the Coulter Counter.

This study was conducted through three separate trials (Table 1). The effects of seagrass blade density were first evaluated in separate individual flumes. Ribbon blades were uniformly distributed throughout each of the flumes as either low or high percent cover. The third flume consisted of the sand treatment. Due to the limitations imposed by the total number of flumes (three), this design was repeated a second time for replication purposes. However, due to time limitations, it is not a true replicate. The age, size and number of scallops are not equal across both trials. For this reason, these trials will be statistically analyzed separately but comparisons will be made. The third trial utilized a mixed design to mimic the flow of water through a natural seagrass bed with varying shoot densities from the edge to the interior of the bed (Table 1). Each of the three flumes were equally divided into three segments, each with an area of 1.17 m². Water flowed in a clockwise direction, passing through the sand treatment into the low shoot density treatment and followed into the high shoot density treatment (Figure S1). Each of the
three trials was conducted for a duration of five weeks to allow enough time for bay scallop growth to be observed but to also minimize water quality degradation.

Table 1. Seagrass shoot density experiments were conducted over three trials. Trials 1 and 2 were similar in design. Bay scallops used in each trial differed based on size, age, and number. An effort was made to keep the bio density consistent across Trials 1 and 2 by using smaller animals in the second trial.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Design</th>
<th>Date</th>
<th>Duration (Weeks)</th>
<th>Scallop Number (Flume)</th>
<th>Scallop Age (Months)</th>
<th>Scallop Size (Tb)(mm)</th>
<th>Scallop Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uniform</td>
<td>May 2017</td>
<td>5</td>
<td>12</td>
<td>10</td>
<td>~38</td>
<td>CCE 2016</td>
</tr>
<tr>
<td>2</td>
<td>Uniform</td>
<td>July 2017</td>
<td>5</td>
<td>20</td>
<td>3</td>
<td>~14</td>
<td>YIMS 2017</td>
</tr>
<tr>
<td>3</td>
<td>Mixed</td>
<td>September 2017</td>
<td>5</td>
<td>30 (10/treatment)</td>
<td>5</td>
<td>~30</td>
<td>YIMS 2017</td>
</tr>
</tbody>
</table>

Hatchery-reared bay scallops were used for these trials. Cornell Cooperative Extension of Suffolk County provided scallops that were used in the first trial. The Eastern Shore Laboratory at Virginia Institute of Marine Science provided the animals used in the second and third trials. At the start of each experiment, bay scallops were measured according to their shell length and height and individually tagged using marine-grade epoxy and color-coded, numbered honeybee tags. Scallops were randomly dispersed throughout each of the flumes in Trials 1 and 2 and allowed to move about freely. Scallops were tethered to their respective treatment environments in Trial 3. The same polypropylene ribbons used to construct the ASUs were used as tethers. One end of the ribbon was glued onto the shell using marine-grade epoxy and the other end was tied to the base of the ASU (egg crate composed of square cells) (Figure S5). The locations for tethering within each treatment were randomly selected.
At the conclusion of each trial, the growth and mortality of scallops exposed to each treatment were measured (Table 2). Mortality was determined by counting all dead scallops in each flume or treatment. Live scallops were sacrificed, and growth was estimated by measuring the shell height and length of each scallop (Figure S9). Additionally, shells were oven-dried to a constant weight and weighed for shell density. A sample of hepatopancreas tissue (approximately 0.05-0.1 g wet weight) was collected from each scallop and saved at -80°C for later GSH analysis. Soft tissue dry weight (hepatopancreas tissue removed) was measured for each animal (Figure S9).

A kinetic, spectrophotometric DTNB-GSSG reductase recycling assay was used to determine the total glutathione content in each sample (Ringwood et al 2003). Samples were homogenized in 5% sulfosalicylic acid (SSA) and centrifuged (13,000g, 5 minutes). Glutathione standards were prepared using a primary 1 mM reduced GSH stock (Sigma-Aldrich, St. Louis, MO) and diluted in 5% SSA. Standards and samples were added to the wells of a 96 well plate in triplicates, followed by the addition of βNADPH and 10mM 5,5’ dithiobis(2-nitrobenzoic acid) (DTNB). Glutathione reductase was added to each of these standards and samples, and absorbances were read immediately using a microplate reader at 412 nm at 30- second intervals for 2 minutes (μQuant, Bio-Tek Instruments). Sample glutathione concentrations were derived from the GSH standard curve and reported as nmol GSH/g wet weight.
Table 2. Various metrics used for evaluating the effects of seagrass density on multiple variables critical to bay scallop population dynamics.

<table>
<thead>
<tr>
<th>Variable Type</th>
<th>Metric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphological</td>
<td>Shell height and length</td>
</tr>
<tr>
<td></td>
<td>Shell dry weight</td>
</tr>
<tr>
<td></td>
<td>Soft tissue dry weight</td>
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<tr>
<td>Physiological</td>
<td>[GSH]</td>
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<td>Environmental</td>
<td>Phytoplankton concentration and distribution</td>
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All results are shown as mean values ± standard deviation. The data were statistically analyzed using R to identify treatment effects. A p-value of <0.05 was a priori defined as significant. Prior to analysis, the data were assessed for normality and homogeneity of variances. Data that did not pass either of these requirements were analyzed via the Aligned Ranks Transformation (ART) ANOVA followed by Tukey pairwise comparisons for significant results (Mangiafico 2016, Wobbrock et al 2011). All other data sets that met the requirements for parametric tests were analyzed via Analysis of variance (ANOVA) tests and Tukey pairwise comparisons. One-way ANOVAs were used to determine the effects of shoot density on scallop survivorship, growth and GSH concentration in Trials 1 and 2. Scallop metrics for Trial 3 were evaluated using one-way ANOVAs with a mixed effect model. ART Repeated Measures ANOVAs were used to evaluate the impacts of shoot density on phytoplankton concentration and spatial distribution.
Results

Each of three annular flumes were designated as either sand, low shoot density, or high shoot density in Trials 1 and 2. During a given trial, each flume received the same volume of the same algae-spiked water on each feeding day. Seagrass shoot density affected the measurable concentration of phytoplankton in both trials (Figures 1 and 2). Increased shoot density resulted in higher concentrations of remaining algae in Trial 1 (ART ANOVA, n=3, p=2.5500e-15, Figure 1). However, in Trial 2, the opposite effect was observed. The sand-only treatment had higher algae concentrations than the seagrass environments (ART ANOVA, n=3, p= 2.22e-16, Figure 2). The lowest concentrations of algae were consistently measured in the low shoot density treatment (Figures 1 and 2). The high shoot density treatment resulted in the highest concentrations of algae during Trial 1(Figure 1) whereas in Trial 2 (Figure 2), the sand treatment had the highest algae concentrations. Spikes in algae concentration were observed in Trial 1 at days 19 and 26. It is unclear why these high phytoplankton concentrations were suddenly observed, particularly in the high- and low-density treatments. Something may have occurred in the surrounding room that resulted in a temporary change to the feeding behavior of the scallops. It is important to note that when no bay scallops were present, the highest algae concentrations were measured in the low shoot density treatment and the lowest algae concentrations were found in the high-density treatment (Figures S6 and S7). This is the opposite of what was observed when scallops were present. It should also be noted that there was no observed vertical stratification of algae in any of the treatments across both trials. Algae appeared to be evenly mixed in the water column.
During Trial 3, each of the annular flumes contained an equal section of sand, low shoot density ASU, and high shoot density ASU. There was no observable difference in algae concentration across the three environment types (Figure 3).

Figure 1. Phytoplankton concentration (cells/mL) measured in each of the treatments (separate flume/treatment) during Trial 1. Filtered seawater spiked with 100,000 algal cells/mL was added to each flume according to the feeding schedule. Phytoplankton concentrations were measured on non-feeding days at three equidistant locations within each flume. At each location, water samples were collected from the bottom, middle, and
top of the water column. Lines represent means ± standard deviation (n = 3). Different letters represent significant statistical difference between treatments in the same water column location.

Figure 2. Phytoplankton concentration (cells/mL) measured in each of the treatments (separate flume/treatment) during Trial 2. Filtered seawater spiked with 100,000 algal cells/mL was added to each flume according to the feeding schedule. Phytoplankton concentrations were measured on non-feeding days at three equidistant locations within each flume. At each location, water samples were collected from the bottom, middle, and
top of the water column. Lines represent means ± standard deviation (n = 3). Different letters represent significant statistical difference between treatments in the same water column location.

Figure 3. Phytoplankton concentration (cells/mL) measured in each of the shoot densities (n= 3) during Trial 3. Filtered seawater spiked with 100,000 algal cells/mL was added to each flume according to the feeding schedule. Location in water column was not included in the statistical analysis of Trial 3. Based on the previous trials, it was not shown to have a significant effect. Lines represent means ± standard deviation.

A suite of metrics was used to evaluate the impacts of eelgrass shoot density on bay scallop biology. There was no effect of shoot density on scallop survivorship across all three trials (1-way ANOVA, Figure 4). Additionally, there was no observed impact of shoot density on bay scallop shell growth in either dimension (1-way ANOVA, Figure 5) or in regard to shell density (1-way ANOVA, Figure 6). Growth rates varied amongst the different trials but that likely is due to differences in initial scallop age and size. Seagrass shoot density had a negative effect on soft tissue mass in the second trial (1-way ANOVA, n=20, p = 0.04774). There is a trend towards lower tissue weights in seagrass environments (Figure 7).
Figure 4. Survivorship of bay scallops exposed to different seagrass shoot densities. Dead scallops were characterized by gaping shells that would not remain closed or empty shell boxes. Bars represent total percentage of live scallops in each treatment.
Figure 5. Shell growth (mm/5 weeks) characterized by height and length (mm) of bay scallops exposed to varying shoot densities of artificial seagrass for 5 weeks. Bars represent means + standard deviations (n ranges from 9-30).
Figure 6. Dried shell weights (g) of bay scallops exposed to different seagrass shoot densities for 5 weeks. Bars represent means + standard deviations (n ranges from 9-30).
Figure 7. Dry tissue weight (g) (except hepatopancreas tissue which was removed for GSH analysis) of bay scallops exposed to a range of seagrass shoot densities for 5 weeks. Bars represent means + standard deviations (n ranges from 9-30). There was a significant difference in soft tissue weight in Trial 2 (1-way ANOVA, p = 0.04774), with a trend toward lower tissue weights in seagrass environments (Tukey pairwise comparison between Sand and High shoot density, p = 0.0587).

There is an effect of seagrass shoot density on glutathione concentrations in hepatopancreas tissue. Significantly depleted GSH concentrations were observed in bay scallops exposed to the sand and low- shoot density treatments in Trial 1 (1-way ANOVA, n=12, p=4.069e-09). Trial 3 (ART ANOVA, n=30, p=1.923e-05) experienced elevated GSH levels compared to scallops from the low shoot density and sand conditions (Figure
8). There is no observable effect on GSH concentrations in Trial 2 (1-way ANOVA, Figure 8).

![Figure 8](image)

Figure 8. Glutathione concentrations (nmol/g wet wt.) of hepatopancreas tissue from bay scallops exposed to different artificial eelgrass shoot densities. Bars represent means + standard deviations (n ranges from 9-30). Different letters represent significant statistical difference between treatments in the same trial. Note the different y-axis scale used in Trial 3.

**Discussion**

Bay scallops rely on seagrass beds throughout their life history. Juvenile scallops settle on seagrass blades and the vertical structure of the blades provides refuge from predation. As they grow, bay scallops eventually become too heavy for the blades and fall to the bottom (Thayer and Stuart 1974, Pohle et al. 1991). As adults, they continue to live
in seagrass beds for the remainder of their lives, consuming phytoplankton, growing, and eventually reproducing. Seagrass beds provide the habitat in which every critical process of bay scallop ecology takes place (Belding 1910). It is therefore important to understand how the structure of this habitat may impact bay scallop ecology. This study closely examines the effects of seagrass shoot density on various aspects of bay scallop growth, physiological health, and survivorship.

The high shoot density treatment (Trial 1) and the sand treatment (Trial 2) resulted in the highest concentrations of measurable algae 24 hours after a feeding event. Since the flumes are closed systems, these are algae that the scallops did not consume; thereby suggesting bay scallops in the high shoot density and sand treatments were not consuming as much phytoplankton as those scallops in the low-density treatment. It is not clear if the bay scallops did not consume as much phytoplankton in these treatments due to behavioral changes or if the algae were in some way not as available. The complex interactions between eelgrass and the mussel Musculista senhousia have been closely studied in San Diego Bay. It has been demonstrated that as shoot density increases within a patch of eelgrass, the growth and survivorship of *M. senhousia* decreases. This decline is largely attributed to decreased food availability as a result of dampened water flow (Allen and Williams 2003). The idea that eelgrass beds diminish the concentration of phytoplankton for benthic filter-feeders is further supported by lower concentrations of chlorophyll *a* in the guts of mussels inside eelgrass patches compared to mussels in unvegetated plots (Reusch and Williams 1999). The results presented herein suggest that phytoplankton are being distributed throughout the seagrass bed, but the bay scallops in the high shoot density environment are not consuming as much of it. It is unlikely that the
presence of eelgrass in this scenario caused the phytoplankton to fall out of suspension, thus making it unavailable to filter-feeding bay scallops, as there was no evidence of vertical stratification observed in any treatment or trial. Microcurrents associated with eelgrass blades could also result in algal settlement on the blades themselves (Stevenson 1983, Medlin et al. 1985), rendering the phytoplankton unavailable to bay scallops.

However, phytoplankton abundance was similar for both the high shoot density and the sand environments, which suggests the presence of eelgrass was not directly responsible for the remaining phytoplankton concentrations. Rather, both environments influenced bay scallop feeding behavior to a similar effect.

The concentration of phytoplankton has been demonstrated to impact the feeding behavior of bay scallops. When comparing bay scallop feeding behavior and particle retention under varying concentrations of phytoplankton, low concentrations resulted in significant alterations of their behavior. Bay scallops were observed to adopt an extremely wide shell gape and the guard tentacles on the two inner mantle folds did not overlap. Additionally, the free margin of the gill protruded beyond shell margin. Little to no pseudofeces were formed under the low algae conditions. This behavior contrasts with that of scallops exposed to higher phytoplankton concentrations. These scallops exhibited less shell gape, guard tentacles formed a screen, and the free margin of the gill did not extend beyond the shell edge. Retention efficiency was also shown to be impacted by the concentration of suspending particles. Bay scallops in low algal density environments (0.88 mg l\(^{-1}\)) were less efficient at filtering small particles (1.73- 3.45 \(\mu\)m) compared to those in medium algal density conditions (6.08 mg l\(^{-1}\)) (Palmer and Williams 1980). Perhaps, there are more remaining phytoplankton in the flume system under high shoot...
density and sand environments because initially, the scallops in these habitats were less efficient at feeding.

The third trial consisted of both shoot densities and sand-only present in each flume in distinct, equal-sized sections. There was no observed difference in phytoplankton concentration across any of these habitats. It is possible that the patch sizes were not large enough to exert an effect on algae distribution as each treatment only took up 1/3 of the flume area. The use of annular flumes also means that the same water moving through the respective environment-types comes back around to repeat the cycle. The water would be moving through, carrying with it any phytoplankton that may have been swept up in the current from a previous treatment area, thus evenly distributing the phytoplankton throughout the flume regardless of shoot density.

Overall the remaining phytoplankton concentrations suggest that bay scallops in the high shoot density and sand environments experienced some degree of food limitation. The scallops may not have been as efficient in filtering the water column or the algae may not have been as available as compared to the low shoot density environment. However, the reduced feeding did not impact their shell growth, shell density, or survival. Likely, there was still sufficient algae present and consumed to support survivorship and shell growth of the scallops. Overall shell growth occurred at a higher rate during Trial 2. These animals were younger and smaller at the onset of the experiment compared to the other trials (Figures S4 and S5). The scallops used in Trials 1 and 3 were of a similar size. Comparable growth rates were observed in both the uniform flume design (Trial 1) and the mixed flume design (Trial 2). There was a stepwise decline of soft tissue weight with increasing seagrass shoot density observed in Trial 2 (Figure 7).
This trial was conducted with younger, smaller bay scallops that might be more sensitive to phytoplankton concentrations. Overall, tissue weight tends to be a more sensitive indicator of adverse effects (Ringwood 1991, Ringwood 1992, Ruiz et al 1995, Hoare et al 1995) compared to shell metrics. Due to predation selection pressures, smaller scallops allocate more energy to shell growth.

Interestingly, significant impacts to glutathione concentrations were observed in Trials 1 and 3. Few studies have measured GSH concentrations in bay scallops, so it is difficult to make a claim on a healthy concentration range, though it is likely in the 1200-1500 nmol g⁻¹ range (Ringwood unpublished data). Regoli et al 1998 measured total GSH concentrations in digestive gland tissue in a different scallop species, Adamussium colbecki, and found levels comparable to the lower range measured in this study (1430 nmol/g). Therefore, two different GSH scenarios are possible: a depletion scenario and an elevation scenario. In the depletion scenario, the GSH concentration remained at healthy levels during Trial 2, while soft tissue weight declined. This dichotomy suggests a trade-off was occurring. Young, small scallops in the high shoot density environment had less energy to allocate. Under these same baseline GSH conditions, sand and low shoot density treatments resulted in depleted GSH concentrations compared to the high shoot density treatment. The phytoplankton concentrations suggest the scallops in the high shoot density would be food-limited, but these animals appear to be unaffected. The bay scallops in the sand-only treatment are food-limited and expressing lower GSH concentrations. The low-shoot density provided the optimal food source in this study, but these animals are also experiencing low levels of GSH. All scallops have elevated GSH levels in Trial 3. These animals are from the same cohort as Trial 2 but were held in an
upweller for a couple months until the next trial. It is possible that these elevated GSH concentrations could be due to conditions in the upweller, although the bay scallops used in Trial 1 were held under similar conditions and do not seem to be affected. Bay scallops in Trial 3 were also tethered to their respective environments. The elevated GSH concentrations could be an artifact of tethering. While not a component of this study, bay scallops were not observed to move around the flumes frequently or for far distances during Trials 1 and 2. However, there was some movement that would have been restricted in Trial 3. Perhaps this restricted movement resulted in some form of stress that is being expressed in elevated GSH concentrations. Despite elevated GSH observations in all treatments, bay scallops exposed to the high shoot density environment had the highest concentrations. There were no differences in phytoplankton concentration observed in Trial 3, so it is likely that this is again due to a change in bay scallop behavior. The alternative scenario, though not likely, is one where the baseline GSH concentration is lower than previously discussed. In this case, GSH levels are uniformly elevated in Trial 2. This is still associated with a stepwise decline in soft tissue weight. The elevated GSH concentrations observed in scallops exposed to the high shoot density treatment in Trial 1 again suggest that energy reallocation is occurring, though from some metric not measured in this study. Under either GSH scenario (depletion or elevation), these results suggest maintaining glutathione concentrations is an important cellular process.

Ample GSH concentrations can help to mitigate impacts from toxicant exposure (Meister and Anderson 1983, Ringwood et al 1998, Ringwood et al 1999). Maintaining a robust antioxidant response system is important for estuarine organisms, especially those
living under stressed conditions. As nearby coastal development increases, run-off containing heavy metals, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) etc. is entering bays and estuaries at an increasing rate (Niyogi et al 2001, Turja et al 2013, Danion et al 2014). Anthropogenic inputs of nutrients are also fueling an increased occurrence of harmful algal blooms (HABs) (Hallegraeff 2003, Glibert and Burkholder 2006, Anderson 2009). Red tides in North Carolina devasted adult scallop populations and were associated with long-term reproductive failure (Peterson and Summerson 1993). When exposed to these environmental toxicants, organisms can experience varying degrees of oxidative stress which ultimately may lead to death (Regoli et al 1997). However, organisms are equipped with intricate defense systems that can sometimes detoxify harmful chemicals and scavenge dangerous reactive oxygen species (ROS). Glutathione is an integral part of these systems but can be depleted as it performs its detoxification and antioxidant functions. Cells must have enough energy to repair damage and maintain GSH and other antioxidant levels to avoid being inundated with ROS and eventually death (Di Giulio et al 1989, Winston et al 1990, Winston and Di Giulio 1991, Kelly et al. 1998).

Sand-only environments likely result in an increased predation potential which is a main driver of bay scallop population densities. For example, the loss of apex predatory sharks has resulted in a significant increase in cownose ray (*Rhinoptera bonasus*) population size and range. These rays can now be found from Southeast Florida to Long Island, NY and are known to consume bay scallops, among other bivalve species (Myers et al 2007). Predation by rays typically occurs prior to bay scallop spawning periods, further inhibiting scallop populations (Peterson et al 1996, Myers et al 2007, Peterson et
Adult bay scallops are extremely vulnerable to predation as they remain exposed on the sediment surface. Rays have been observed to target areas with high numbers of scallops and consume them until few to none are left, rendering the local population functionally extinct (Peterson et al 2001). The presence of seagrass or other vertical substrate may serve some level of predation refuge.

The preservation of native bay scallop populations and successful restoration efforts will promote a healthy, diverse ecosystem. Therefore, it is critical to better understand the impacts habitat may have on bay scallop ecology. This information is useful in informing regulatory decisions regarding the designation of protected habitats as well identifying key locations for restoration efforts. The results from this study suggest seagrass beds composed of patches with low shoot density would be optimal for bay scallop health. Food availability would be sufficient for growth and survivorship, and predation would also be minimized (Orth 1992). A food-risk trade-off has previously been identified for bay scallops across the seagrass edge (Bologna and Heck 1999, Carroll and Peterson 2013). Bay scallop growth was found to be the greatest in unvegetated areas even though chlorophyll concentration was similar or lower than in nearby seagrass (Carroll and Peterson 2013). This difference in growth is attributed to greater water flow rates outside of seagrass which provides a greater flux of food (Calahan 1989). Scallop growth rates were lowest in the interior of the bed and intermediate at the edge (Carroll and Peterson 2013). Therefore, what may be considered optimal for seagrass, may not apply to its associated fauna (Allen and Williams 2004). Patchy seagrass habitats may provide an environment where bay scallop growth and
survival are balanced (Irlandi et al 1995, Carroll and Peterson 2013) and scallop settlement is enhanced (Bolona and Heck 1999, Carroll et al 2012).

I suggest that the results from this series of mesocosm trials should be corroborated by field experiments conducted in natural eelgrass beds in situ. Adult bay scallops would be planted in enclosures in patches of eelgrass of varying densities. Enclosures with ASUs in adjacent sandy areas would be secured as well. Enclosures are necessary to contain the scallops as well as to exclude predators. Enclosures require routine maintenance to prevent fouling which would impact water flow. Additionally, further analysis of glutathione concentrations in bay scallop tissues is necessary. It is important to identify a baseline range for healthy Argopecten irradians that can be used to better understand the results of this study as well as any future oxidative stress response research. Finally, more research is needed in the field of bay scallop behavior. Through a combination of laboratory and field mesocosm studies we can better understand how bay scallop behavior in influenced by changes in habitat structure.

Conclusions

Overall, the results from this study suggest that high seagrass shoot density environments result in a decreased concentration of bioavailable algae, but bay scallops appeared to remain healthy. The low shoot density environment results in more optimal concentrations of bioavailable algae and had no impacts on soft tissue mass or GSH concentrations. Finally, the sand environment results in a reduction of the algal food supply and no impacts on soft tissue weight or GSH but likely results in the highest predation rates.
Figure S1. Illustration demonstrating the mesocosm design in each flume for the three trials. Each of the three flumes was a single density treatment in Trials 1 and 2. During Trial 3, each flume contained an equally sized segment of the density treatments. The arrow demonstrates the direction of water flow during Trial 3.
Figure S2. Annular flumes set-up for Trials 1 and 2. Each flume has a uniform distribution of artificial eelgrass per its respective treatment. The flume in the background is marked by a “X”, designating one of the phytoplankton sampling locations.
Figure S3. Tagged bay scallops in each of the different ASU treatments: Top left = High density, Top right = Low density, and Bottom center = Sand.
Figure S4. A subsample of the bay scallops used in Trial 1 (top) and Trial 2 (bottom).

Trial 2 consisted of younger, smaller scallops as compared to the other trials (see Figure S4).
Figure S5. A subsample of bay scallops being tagged and tethered for Trial 3. The same polypropylene ribbons used to construct the ASUs were used as tethers for Trial 3. One end of the ribbon was glued onto the shell and the other end was tied to the base of the ASU (egg crate).
Figure S6. Phytoplankton concentration (cells/mL) measured in each of the treatments (separate flume/treatment) during Trial 1- Phytoplankton Only. No bay scallops were added to the flumes. Filtered seawater spiked with 80,000 algal cells/mL was added to each flume on Day 1. Phytoplankton concentrations were measured over the next 7 days at three equidistant locations within each flume. At each location, water samples were collected from the bottom, middle, and top of the water column. Lines represent means ± standard deviation (n = 3).
Figure S7. Phytoplankton concentration (cells/mL) measured in each of the treatments (separate flume/treatment) during Trial 2- Phytoplankton Only. No bay scallops were added to the flumes. Filtered seawater spiked with 50,000 algal cells/mL was added to each flume on Days 1 and 3. Phytoplankton concentrations were measured over 4 days at three equidistant locations within each flume. At each location, water samples were collected from the bottom, middle, and top of the water column. Lines represent means ± standard deviation (n = 3).
Figure S8. Phytoplankton concentration (cells/mL) measured in each of the shoot densities (n= 3) during Trial 3- Phytoplankton Only. No bay scallops were added to the flumes. Filtered seawater spiked with 100,000 algal cells/mL was added to each flume on Days 1 and 3. Lines represent means ± standard deviation (n= 3).
Figure S9. Bay scallop shells were measured according to height and length (top). Bay scallop soft tissue (bottom) was dried and weighed. The hepatopancreas was isolated and analyzed for glutathione concentration.
Conclusion

The goal of this dissertation was to closely examine the effects of habitat quality on various stages of bay scallop life history. As water quality deteriorates and nutrient and pollution loads increase, coastal water body habitat undergoes changes. It is important to understand how these changes could impact bay scallop populations. Conservation and restoration efforts can be costly and labor intensive so efforts should be well informed in order to achieve the desired results.

Gelatinous zooplankton populations are increasing due to poor water quality associated with eutrophication. Ctenophores are likely to come in to contact with bay scallop larvae as they have similar salinity requirements, but it was previously unknown if they would prey on scallop larvae. I found that ctenophores can be significant predators on bay scallops, consuming between 5 and 50% of available larvae over a 10-minute period. A small percentage of ingested larvae are egested by the ctenophores alive and this process had no adverse impact on larval survival.

Restoration strategies heavily rely on an understanding of larval dispersal patterns to identify habitat necessary for settlement and recruitment. Recent modeling efforts for other species with pelagic larvae have incorporated larval swimming behavior to more accurately refine their results. The study presented herein was the first to quantify bay scallop larvae swimming behavior. The mean vertical velocity of scallop larvae was measured under various environmental conditions. In general, larvae were observed to hover, maintaining their vertical position in the water column with vertical speeds of ranging from 0.01 to 0.027 mm s\(^{-1}\) under salinity conditions ranging from 20, 25, and 30 ppt. Cold conditions (15ºC) resulted in no swimming larvae as they had all settled to the
bottom. However, when exposed to warm conditions (30°C), larvae swam upwards at faster rate of 0.054 mm s\(^{-1}\) and increased their activity overall compared to those exposed to 23 °C. Bay scallop larvae are likely to experience these warm water conditions as spawns typically occur in late summer and early fall. Water temperatures are expected to rise as climate change progresses as well. Light did not have an impact on vertical velocities of bay scallop larvae.

Eutrophication of coastal water bodies often results in phytoplankton blooms, which can coat the blades of seagrass, creating increased habitat complexity. Juvenile bay scallops attach to these seagrass blades via byssal threads. This vertical space likely provides protection from predation. It was previously unknown if juvenile scallops would preferentially seek out and attach to seagrass blades coated with this biotic film. The results of our study suggest that they do not attach preferentially to seagrass blades with or without increased complexity. They may not depend on seagrass to provide this spatial refuge at all, rather utilizing algal species and other vertical structures when necessary.

Seagrass beds are ephemeral and show increasing spatial variability with increased eutrophication. A goal of this study was to determine how seagrass shoot density affected adult bay scallop survival, growth, and physiological health in the absence of predation. The results suggest that high seagrass shoot density results in less available phytoplankton, however no adverse effects on overall bay scallop health were observed as evaluated in this study (growth metrics, GSH, and survivorship). Bay scallops in the low shoot density environment experienced more optimal concentrations of bioavailable phytoplankton. The sand-only environment resulted in a reduction of available phytoplankton but again, scallops appeared to remain healthy.
In summary, successful recovery of bay scallop populations will require a multi-scale effort aimed at promoting larval survival, identifying and protecting source population habitats and if necessary, creating alternative habitats for recruitment, and protecting adults from predation before they spawn. This body of work examined the impacts of habitat quality, quantity and neighboring species on all life history stages of bay scallops. Overall, it can be inferred that predation is a major driving factor inhibiting bay scallop population growth. Larvae experience predation from ctenophores in the water column, juveniles will be vulnerable if they cannot settle in appropriate habitat, and adults are highly susceptible when exposed on sandy substrates. As eutrophication and pollution continue to negatively impact coastal water bodies, these potential predation pressures are likely to increase. It is predicted gelatinous zooplankton will outcompete other native species and diversity will decline while their population densities increase. Seagrass beds will continue to experience diebacks and scallops will not have the habitat refugia they require. Adults are capable of producing substantial quantities of gametes during a spawning event which suggests a large adult population may not be necessary to bolster the population size of the next generation. However, refugia habitat is critical to ensuring successful larval settlement and growth. Therefore, restoration efforts divide their focus between both generating bay scallop larvae, whether through the establishment of spawner sanctuaries or the use of hatcheries, and the conservation of submerged aquatic vegetation. The data generated from this body of work should be used to inform efforts aimed at restoring bay scallop populations to promote healthy and diverse coastal ecosystems.
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