JUVENILE BLUE CRAB (*CALLINECTES SAPIDUS*) SURVIVAL IN SIMULATED SEAGRASS HABITATS (*ZOSTERA MARINA* AND *RUPPIA MARITIMA*)

By

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1. Abstract

Anthropogenic increases in global temperatures and nutrient loads are expected to reduce juvenile blue crab (*Callinectes sapidus*) survival in the Chesapeake Bay. These factors change habitat composition which can affect juvenile invertebrates and fishes that are dependent on these habitats. Eelgrass (*Zostera marina*) is declining due to rising water temperatures and increased nutrient loading, while widgeon grass (*Ruppia maritima*) can tolerate higher temperatures. An indoor mesocosm experiment was designed to test the suitability of *Zostera* and *Ruppia* as protective nursery habitats compared to sand. Artificial seagrass plots were placed in flow-through tanks. Juvenile blue crabs were tethered, and adult blue crabs and striped burrfish were introduced as predators in order to estimate juvenile crab survival in different substrates. Survival analysis revealed that *Zostera* provides more protection for juvenile crabs than sand. There was no significant difference between *Ruppia* and sand, and between *Zostera* and *Ruppia* in providing juvenile protection. This suggests juvenile survival may decrease in the future with *Zostera* loss and that stricter restrictions on the blue crab fishery in the Chesapeake Bay and mid-Atlantic region would be required to maintain healthy crab populations.

Key Words: Blue crab, *Callinectes sapidus*, survival, habitat, seagrass, *Zostera marina*, *Ruppia maritima,* hypoxia, *Hematodinium perezi*

2. Introduction

The blue crab (*Callinectes sapidus*) fishery is of great economic importance in the mid-Atlantic region, particularly in the Chesapeake Bay of Virginia and Maryland, USA (NOAA 2016). Blue crab populations are managed by state jurisdictions: the Virginia Marine Resource Commission (VMRC), Maryland Department of Natural Resources (MD DNR), and the Potomac River Fisheries Commission (CBSAC 2016, NOAA 2016). Management decisions are based on the reviews of annual surveys and harvest data by the Chesapeake Bay Stock Assessment Committee (CBSAC). This committee utilizes the bay-wide winter dredge survey because it provides a robust annual estimate of over-wintering blue crabs in Chesapeake Bay (CBSAC 2016).

In 2011, the VMRC, MD DNR, and NOAA Chesapeake Bay Office helped construct a stock assessment that recommends 215 million adult female crabs of spawning age (1+ years) persist in the Chesapeake Bay (Fig. 1) (CBSAC 2016, NOAA 2016). However, only 194 million were present at the start of the 2016 crabbing season. Although this estimate of abundance has increased since 2015 by 92% and the population is currently not overfished, it was overfished during the 1999, 2001, and 2002 seasons (CBSAC 2016, NOAA 2016). A dredge fishery was in operation every year from before the 1990's through 2008 near the mouth of Chesapeake Bay where gravid females burrow in the sediment to over-winter (Seitz, Personal Communication, 2016). This dredge fishery and other fisheries in operation (mainly commercial) may have been responsible for low numbers of female crabs in and prior to 2008. The continued low abundances from 2006-2008 (Fig. 1) motivated management action by CBSAC, and the dredge fishery has been closed since 2008 (MD DNR 2013, NOAA 2016). Additionally, a drop in 2014 to the

threshold level of 70 million female crabs may be due to juvenile blue crab predation by fish (Fig. 1) (Seitz, Personal Communication, 2016).

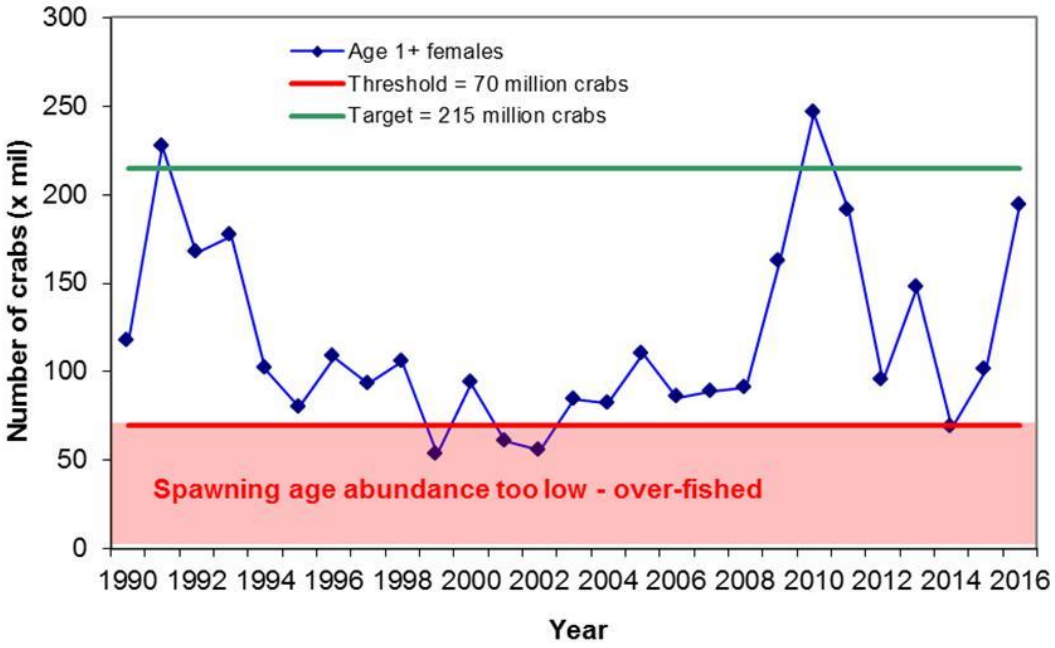

Figure 1. Winter dredge survey abundance estimates of female adult blue crabs of spawning age (1+ years) in Chesapeake Bay from 1990-2016. A dredge fishery in operation every year from before the 1990's to 2008 may be partly responsible for low abundances in and prior to 2008, including seasons of overfishing in 1999, 2001, and 2002 (Seitz, Personal Communication, 2016). Source: https://chesapeakebay.noaa.gov/fish-facts/blue-crab

In addition to their economic importance, blue crabs are opportunistic omnivores, scavengers, and prey in Chesapeake Bay. Juvenile blue crabs with 30-60 carapace width (CW), defined as the length in millimeters between the two longest lateral spines of the carapace, primarily feed on bivalves such as clams and oysters (39% of diet) (Lipcius et al. 2007). Plant matter and detritus (decaying organic matter) comprise 22% of their diet, while polychaetes, crustaceans, gastropods, and fish make up the rest. Blue crabs only comprise 3% of the 30-60 CW juvenile blue crab diet (Lipcius et al. 2007). Juvenile blue crabs <40 CW focus on smaller organisms like amphipods, mysids, polychaetes, plant matter, and detritus, while little evidence suggests they eat other blue crabs. In contrast, bivalves and blue crabs comprise most of the diets of larger juvenile and adult blue crabs >60 CW (46% and 16%) (Lipcius et al. 2007). Adult blue crabs have been responsible for 75-97% of juvenile blue crab mortality in the Rhode River, an unvegetated subestuary of the Chesapeake Bay (Hines & Ruiz 1995). Blue crabs are also prey for fish such as Atlantic croaker, striped bass, and red drum (NOAA 2016). Striped burrfish

(*Chilomycterus schoepfi*) visit seagrass beds in the Chesapeake Bay from late spring to autumn and eat blue crabs. Striped burrfish use strong beak-like jaws to consume invertebrates ("Chesapeake Bay Program: Striped Burrfish" 2012a).

2.1 Background

As part of their life cycle, blue crabs undergo ontogenetic (developmental stages) habitat shifts (Pardieck et al. 1999, Lipcius et al. 2007). In lower-salinity waters of Chesapeake Bay, mating begins in May after adult females molt for the final time (NOAA 2016). Many females remain in these waters for up to several months to build muscle and store energy for ovarian development and their migration to the mouth of the Chesapeake Bay up to 200 kilometers south (Turner et al. 2003). Signals such as changing water temperatures prompt female adults to appear at the mouth around mid- to late October. Some females that mate further north in Chesapeake Bay may not spawn until the following season (Turner et al. 2003). After arriving at the mouth, they finish producing a sponge of 750,000 to 3,200,000 eggs and release zoeae (first larval stage) that develop on the continental shelf (Fig. 2) (Lipcius et al. 2007, NOAA 2016). After zoeae develop into megalopae (the late larval stage), they migrate into brackish waters of Chesapeake Bay and its tributaries (e.g. York River) and settle in complex habitats such as seagrass beds (Lipcius et al. 2007). Here, they develop into the 1st benthic juvenile instar (phase between

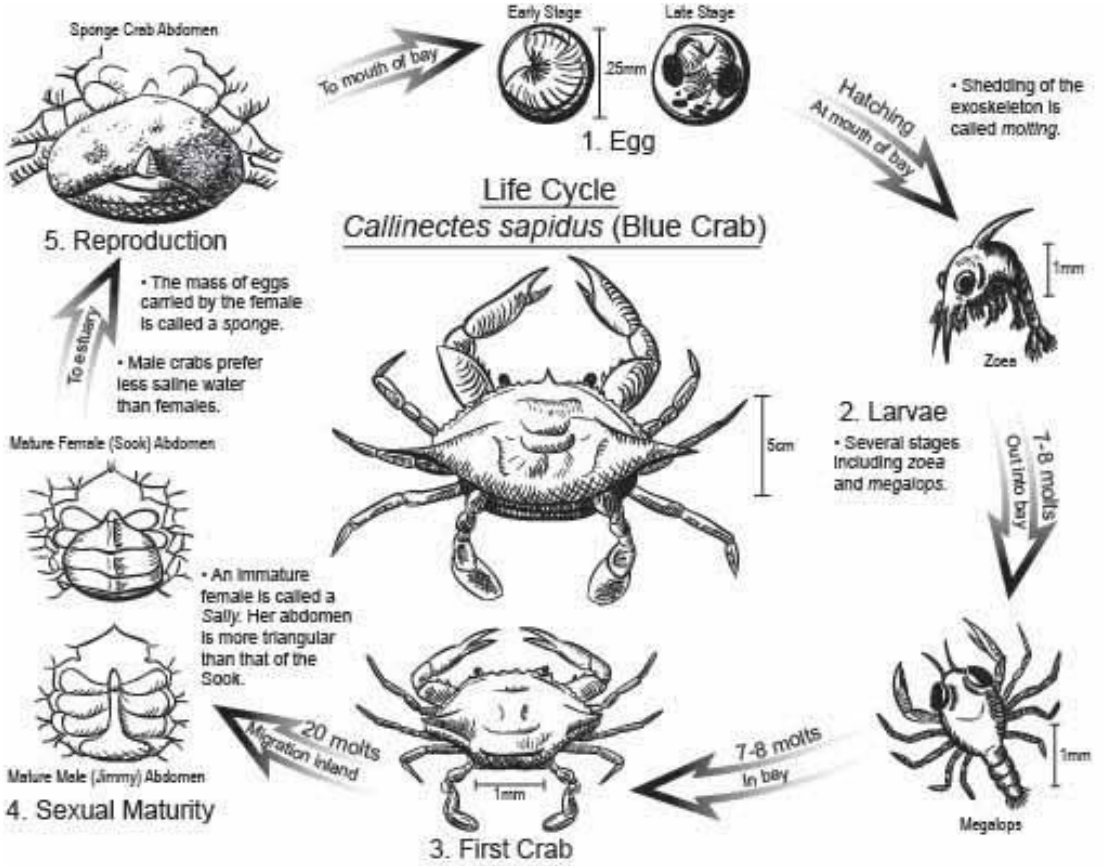

Figure 2. Complete life cycle of blue crabs (*Callinectes sapidus*). Source: https://www.behance.net/gallery/31876991/Blue-Crab-Life-Cycle

molting) at about 2.2-3.0 CW. Juveniles can remain in seagrass habitats until they grow to the 9th instar at about 20-25 CW (Lipcius et al. 2007). Seagrass habitats are primary nurseries for juvenile blue crabs as they provide protection from predation and high food abundance (Heck & Thoman 1984, Perkins-Visser et al. 1996).

Submerged aquatic vegetation, defined as grasses that grow just to the surface of shallow waters, enhances juvenile blue crab growth and survival more than unvegetated sediment habitats (Perkins-Visser et al. 1996). Eelgrass (*Zostera marina*) is a temperate subtidal seagrass species (Fig. 3) whose Atlantic range is from Nova Scotia to North Carolina. *Zostera* occupies deeper waters of the Chesapeake Bay and York River largely due to its low tolerance to warmer temperatures (Orth & Moore 1988). Other seagrass species in the mid-Atlantic region are shoalgrass (*Halodule wrightii*) and widgeon grass (*Ruppia maritima*) (Fig. 3). *Halodule* is a tropical intertidal seagrass that ranges from North Carolina to the Gulf of Mexico and Caribbean in the Atlantic (Micheli et al. 2008). *Ruppia* is a tropical and temperate species that ranges from Newfoundland to Texas, including Chesapeake Bay ("Smithsonian Marine Station at Fort Pierce: *Ruppia maritima*" 2001). Both *Halodule* and *Ruppia* tend to live in shallower waters due to their higher tolerance to warmer temperatures (Evans et al. 1986, Micheli et al. 2008). In Chesapeake Bay, *Ruppia* occupies shallower waters and co-occurs with *Zostera* at intermediate depths. *Zostera* typically occupies deeper waters (Orth & Moore 1988). This depth distribution is similar between *Zostera* and *Halodule* when they co-occur at intermediate depths (Micheli et al. 2008).

Zostera has declined by 29% in Chesapeake Bay since 1991 (Lefcheck et al. 2017). *Zostera* biomass and shoot density in June decreased from 1985-2004 in North Carolina (Micheli et al. 2008). This reduction in *Zostera* was due to high water temperatures and increased nutrient loading of nitrogen and phosphorous (Micheli et al. 2008, Lefcheck et al. 2017). Agricultural runoff and sewage disposal introduce large amounts of nitrogen and phosphorous that increase phytoplankton abundance and decrease light penetration to seagrasses (NOAA 2014). A reduction of 523-1403 million juvenile blue crabs (roughly $28.6-76.7 million) is expected to occur with the loss of *Zostera* (Lefcheck et al. 2017).

Halodule and *Ruppia* are more tolerant to environmental stressors and could potentially replace *Zostera*. *Halodule* biomass and shoot density in June remained consistent from 1985-2004 in North Carolina under conditions of high temperatures and nutrient loading (Micheli et al. 2008). *Halodule* can survive in deeper waters occupied by *Zostera*, but it may occupy shallower waters due to competitive interactions with *Zostera* (Micheli et al. 2008). *Ruppia* has a competitive advantage over *Zostera* at higher temperatures with regards to photosynthetic efficiency (Evans et al. 1986). *Ruppia* mainly remains in shallow waters because it is adapted for habitats with both high temperatures and high light intensity (Orth & Moore 1988).

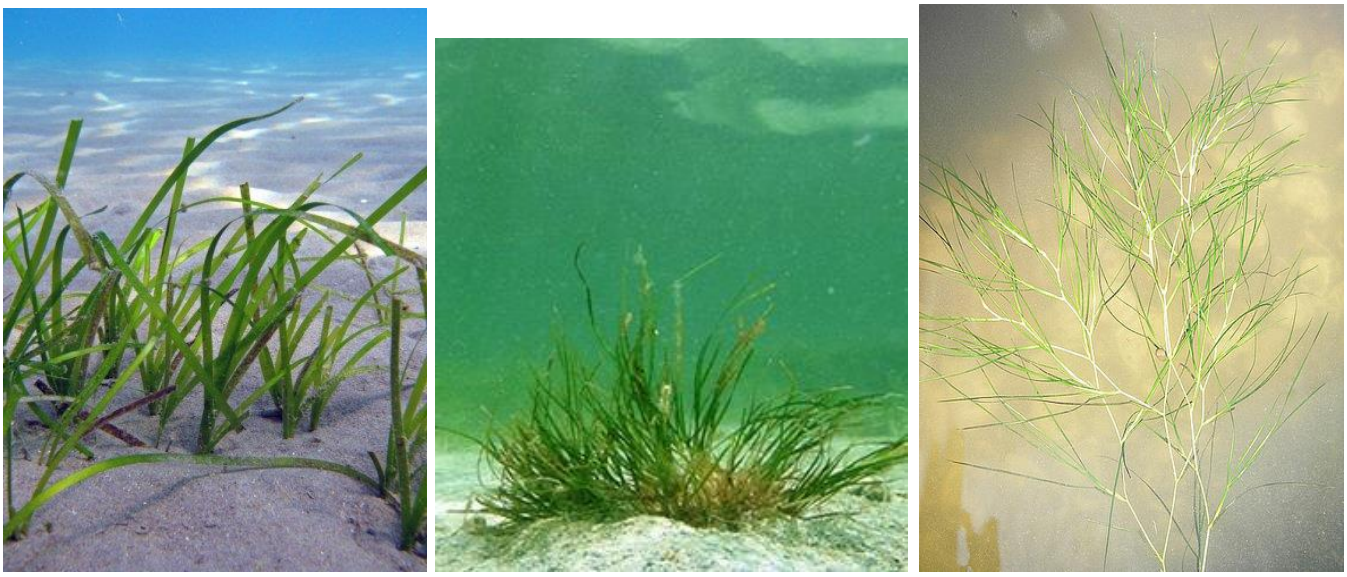

Figure 3. Left to right: eelgrass (*Zostera marina*), shoalgrass (*Halodule wrightii*), widgeon grass (*Ruppia maritima*). Sources in listed order: http://www.uicnmed.org/medras/en/galeria.htm; http://www.chesapeakebay.net/fieldguide/critter/shoal_grass; http://www.chesapeakebay.net/fieldguide/critter/widgeon_grass

2.2 Hypotheses

Anthropogenic changes such as a warming climate and increased nutrient loading will almost certainly impact the habitats that support blue crabs. To shed light on the impact of habitat change, the goal of this study was to determine if juvenile blue crab survival differs in sand, *Zostera*, and *Ruppia* habitats. I hypothesized *Zostera* and *Ruppia* habitats would enhance juvenile blue crab survival more than sand because of their protective structures, and that *Zostera* and *Ruppia* would provide equal protection for juvenile blue crabs. Therefore, *Ruppia* could replace *Zostera* as a protective nursery habitat for the blue crab as the Chesapeake Bay continues to warm.

3. Materials and Methods

An indoor mesocosm experiment investigating predation on juvenile blue crabs in three different habitats by two types of predators was performed in July 2016 at the Virginia Institute of Marine Science (VIMS). Artificial plots of *Zostera* have been used successfully to mimic natural seagrass patches and quantify survival of juvenile blue crabs (Hovel & Lipcius 2001). Artificial plots also control for seagrass density. Therefore, artificial *Zostera* and *Ruppia* meshes were constructed for this experiment. Each artificial seagrass plot was created by tying 20-cm strands of green polypropylene ribbon to circles of Vexar mesh (0.283 m^2) at natural shoot densities for the York River (*Zostera*: 500 shoots/m^2 ; *Ruppia*: 1000 shoots/m^2) (Fig. 4). A shoot was defined as an individual blade of seagrass. Ribbon widths were consistent with the natural blade widths of *Zostera* and *Ruppia*, 4- and 2- mm, respectively.

Figure 4. Artificial seagrass meshes of *Zostera marina* (left) and *Ruppia maritima* (right). Green polypropylene ribbons 20-cm in length were tied to black circular Vexar meshes (0.283 m^2).

Animal specimens for this experiment were collected from the lower York River using crab scrapes, dipnets, and seine nets: 43 juvenile blue crabs 12.9 – 43.6 CW (prey), 13 adult blue crabs 84.2 – 140.6 CW (predators), and five striped burrfish 160-175 mm (predators). Forty-three trials testing juvenile blue crab predation were conducted in six flow-through circular tanks (70.5 cm diameter) (Fig. 5). Two tanks held sand (control) from the York River (Fig. 6), two held *Zostera* mesh (Fig. 7), and two held *Ruppia* mesh (Fig. 8). The meshes were weighted to the tank bottom with rebar and covered with sand (~1-5 cm). Mesh-covered PVC pipes in the center of each tank allowed water to flow while preventing crabs from escaping. Juvenile crabs, adult crabs, and striped burrfish occupied individual holding tanks (Fig. 5).

Figure 5. Flow-through seawater system in the Seawater Research Lab at VIMS. Water was pumped from the York River, filtered, and distributed to each tank through pipes. Six circular tanks were used for trials. One held juvenile blue crabs, and one held striped burrfish. One long rectangular tank held adult blue crabs.

Figure 6. Two circular tanks were filled with several centimeters of sand from the York River. Sand was used as a control to compare juvenile blue crab survival between unvegetated and vegetated habitats.

Figure 7. Two circular tanks contained simulated *Zostera marina* meshes weighted with rebar and covered with 1-5 cm of sand. The green polypropylene ribbons attached to the meshes were pulled up from underneath the sand.

Figure 8. Two circular tanks contained simulated *Ruppia maritima* meshes weighted with rebar and covered with 1-5 cm of sand. The green polypropylene ribbons attached to the meshes were pulled up from underneath the sand.

Juvenile blue crab predation trials were performed with both untethered and tethered prey specimens. Untethered trials were limited and not statistically analyzed (see Appendix). However, tethering is a common method used in the field and laboratory to examine relative juvenile crab survival. It limits their range of movement while still allowing them to perform natural behaviors (i.e., walking, swimming, resting, burrowing) (Zimmer-Faust et al. 1994). Forty-three trials with tethered juvenile blue crabs were conducted with adult crab predators (39 trials, one adult per tank) and striped burrfish predators (four trials, two burrfish per tank). Two burrfish were used because solitary burrfish (starved 21 hours prior to trials) did not eat juvenile crabs after 24 hours. Note, a very large juvenile blue crab was used as a predator in some trials because not enough adults were available at the time of collection.

Each juvenile crab used in tethered trials had a 20-cm tether of monofilament fishing line attached to its carapace by cyanoacrylate and duct tape to ensure the crab remained on the seagrass mesh (Fig. 9). The opposite end of the tether was tied to a metal swivel clip which was tied loosely around the PVC center pipe with zip ties to allow crab movement around the entire pipe. Carapace width (CW) in millimeters of juvenile crabs (≤43.6 CW) and adult and large juvenile crabs (≥84.2 CW) was measured to the nearest tenth of a millimeter using calipers, and missing limbs were recorded (Figs. 10 & 11). Striped burrfish length was measured in centimeters from the tip of their mouth to the tip of their tail using a ruler (Fig. 12). Lengths were converted to millimeters so burrfish and juvenile crab sizes could be compared.

Figure 9. Tethered juvenile blue crab. Fishing line was tied to a metal swivel clip and the other end of the line was attached to the carapace by cyanoacrylate and duct tape. The tether was 20 cm in length (not including the swivel clip).

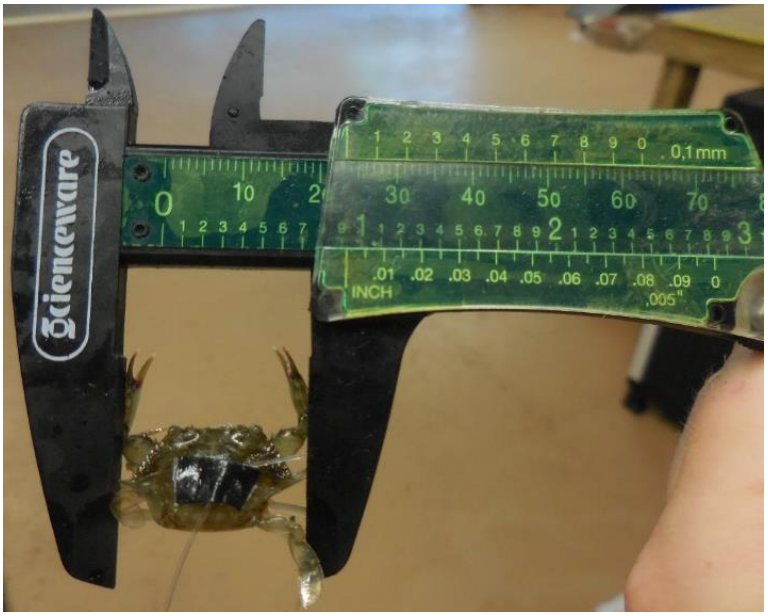

Figure 10. Calipers measured the distance between the longest lateral spines of a juvenile blue crab's carapace (≤43.6 CW) to the nearest tenth of a millimeter. This measurement of crab size is also referred to as carapace width (CW).

Figure 11. Calipers measured the distance between the longest lateral spines of the carapace of adult or large juveniles (≥84.2 CW) to the nearest tenth of a millimeter. This measurement of crab size is also referred to as carapace width (CW). This adult crab was held down with a brush and given tools such as a screwdriver to occupy its claws while its CW was measured.

Figure 12. Striped burrfish were measured from the tip of the mouth to the tip of the tail.

Figure 13. Predators (either one crab or two burrfish) were acclimated to the tank prior to the start of a trial. A mesh barricade (black) with PVC on either end isolated predators from tethered juvenile crabs already in tanks. A trial began once the mesh and PVC were removed and predators were no longer isolated from prey.

All predators were initially starved for about 24 hours prior to trials. Due to the limited time available to run trials, many predators were not starved after eating juveniles in trials and were used immediately in subsequent trials. After tethered juveniles were secured in tanks, either a predator crab or two striped burrfish were placed behind a barricade within the experimental tank for acclimation (Fig. 13). A trial began when the barricade was removed.

Trials lasted until the juvenile crab prey was captured. If juveniles were not consumed within a reasonable amount of time, a trial was terminated. The shortest trial lasted two hours and the longest lasted 48 hours. When a juvenile was found dead, it was immediately replaced with another and a new trial started. When the tanks were checked during trials, live juvenile and predator positions were recorded, as well as any missing limbs on the juvenile. When a piece of carapace and duct tape or only duct tape

remained on the tether, the juvenile was considered killed. If the tether was cut and no tape was found, the juvenile was not considered killed until the tank was checked. After each trial, the predators were removed and starved if possible prior to the start of the next trial. Predators that did not feed on tethered juveniles after a reasonable amount of time were removed and fed crab pieces to make sure they were able to feed. Habitats were assigned randomly to tanks at the onset of the study but not continually for each set of trials. Predators were introduced into randomly chosen tanks for new trials. Prior to this indoor mesocosm experiment, field tethering was attempted to estimate juvenile blue crab survival. These methods are included in the Appendix.

Sand trials (16 total) consisted of 14 predator crab trials and two burrfish trials. For crab predator trials, the average prey size was 26.2 mm and the average predator size was 119.9 mm. For burrfish predator trials, the average prey size was 22.4 mm and the average predator size was 160 mm. *Zostera* trials (14 total) consisted of 12 predator crab trials and two burrfish trials. For crab predator trials, the average prey size was 25 mm and the average predator size was 110.7 mm. For burrfish predator trials, the average prey size was 32.25 mm and the average predator size was 160 mm. *Ruppia* trials (13 total) only consisted of crab predators. The average prey size was 32.4 mm and average crab predator size was 122.5 mm.

3.1 Statistics

Survival analysis was used to examine the effects of predator type, habitat type, and crab predator-prey size ratios on juvenile crab survival. Time to event (death in this experiment) and event status comprise the outcome variable. This analysis correctly incorporates both uncensored and censored data. Data is uncensored if the event of interest occurs during the study, whereas data is censored if the event of interest does not occur during the study (information is incomplete). Ten trials were considered to be censored, meaning juvenile death did not occur during the trial length. R Project statistical software (version 0.98.1091) was employed for the survival analysis, using the Cox proportional hazards regression model (function coxph in the package survival) (Therneau 2015, R Core Team 2016). The function coxph allows for the inclusion of predictor variables. It estimates the effect of one variable while controlling for confounding effects of other variables. R was also used to create a boxplot comparing crab predator-prey size ratios in each habitat.

Excel was used to compare mean capture times for crab and burrfish predators, juvenile survival in each habitat over time, mean capture times in each habitat for predator crab trials, and the relationship between crab predator-prey size ratios and time to capture. This program cannot correctly account for censored data, so only dead juveniles were included.

Only tethered trials were statistically analyzed. An attempt was made to test the effect of predator type (adult crab vs striped burrfish) on juvenile survival while accounting for differences in habitat type and prey size. Due to limited burrfish trials, only predator crab trials were used to test the effect of habitat type while accounting for differences in the predator-prey size ratios. The effect of crab predator-prey size ratios on survival was tested while controlling for differences in habitat type.

4. Results

Predator type was not analyzed statistically because of the difference in sample sizes between adult crab predator trials and striped burrfish predator trials. Thirty-nine crab predator

trials were run, and juvenile crabs were eaten in 32 of them (82%). On the other hand, four striped burrfish predator trials were run and a juvenile crab was eaten in one of them (25%). The mean time to capture in crab predator trials was 16.56 ± 1 SE = 1.579 hours whereas striped burrfish ate one juvenile after 69.57 hours (Fig. 14).

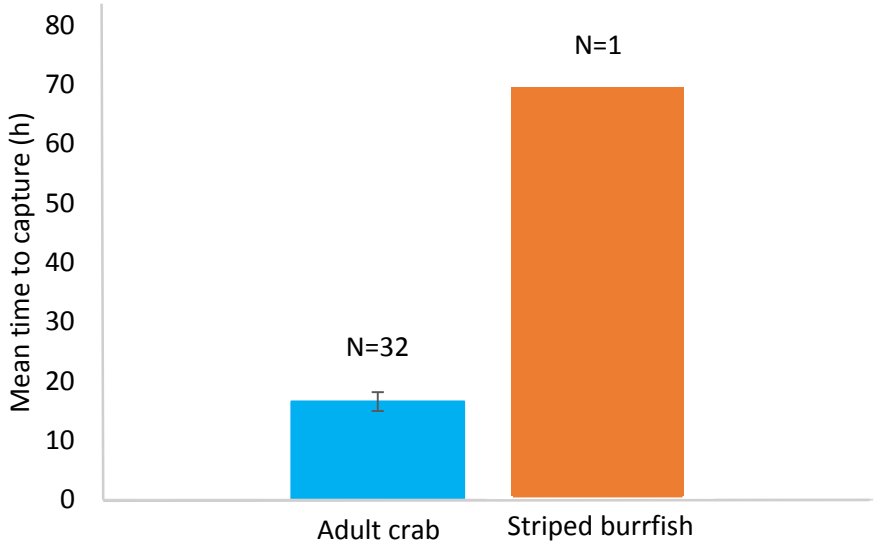

Figure 14. Mean time to capture (h) of juvenile crabs with adult crab and striped burrfish predators for all habitats. Only dead juveniles are included. Adult crabs have a SE because there was more than one trial.

Habitat type affected juvenile blue crab survival (Figs. 15 & 16). *Zostera* enhanced juvenile survival more than sand (p=0.0448). The percentage of live juveniles in *Zostera* after 48 hours was 33.33% compared to 7.14% in sand (Fig. 15). More juveniles (26.19%) were alive after 48 hours in *Zostera* than in sand. The mean capture time in sand was 14.96 ± 1 SE = 2.267 hours whereas that in *Zostera* was 18.82 ± 1 SE = 4.442 hours (Fig. 16). The difference between mean capture times in sand and *Zostera* was 3.86 hours. There was no significant difference between *Ruppia* and sand in providing juvenile protection (p=0.3742). After 48 hours, 15.38% of juveniles in *Ruppia* were still alive (Fig. 15). More juveniles (8.24%) were alive after 48 hours in *Ruppia* than in sand. The mean capture time in *Ruppia* was 16.82 ± 1 SE = 2.116 hours (Fig. 16). The difference between mean capture times in sand and *Ruppia* was 1.86 hours. From 16-22 hours, juvenile survival in sand and *Ruppia* followed a similar trend (Fig. 15). Juvenile survival dropped markedly from 16-22 hours and did not change from 22-24 hours. While juvenile survival in *Ruppia* remained constant from 22-48 hours, survival in sand dropped at 26 hours and then remained constant to 48 hours. In contrast, juvenile survival in *Zostera* gradually declined from 16-22 hours. Juvenile survival then decreased from 26-42 hours and remained constant until 48 hours.

There was no significant difference between *Zostera* and *Ruppia* in providing protection to juveniles (p=0.2305). More juveniles (17.95%) were alive in *Zostera* than *Ruppia* after 48

hours (Fig. 15). The difference between mean capture times in *Zostera* and *Ruppia* was two hours (Fig. 16). Juvenile survival dropped more gradually for *Zostera* and *Ruppia* from 0-4 hours than in sand (Fig. 15). Juvenile survival followed a similar decline in *Zostera* and *Ruppia* from 4-16 hours, while survival in sand remained constant. After 16 hours, survival trends diverged through the rapid decline in *Ruppia* and sand as compared to *Zostera*. Overall, juvenile survival appears to be lowest in sand, intermediate in *Ruppia*, and highest in *Zostera*.

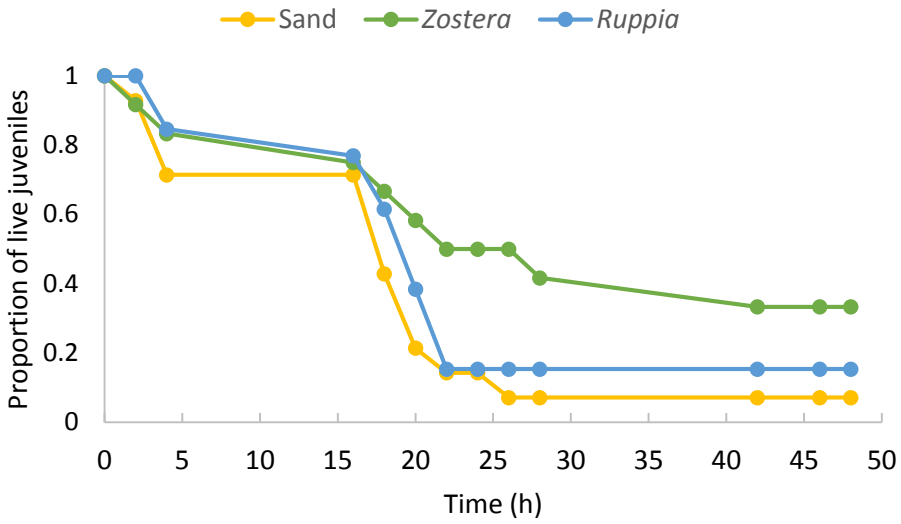

Figure 15. Juvenile crab survival in each habitat type from 0-50 hours. Proportion of live juveniles ranges from 0-100%. Only predator crab trials are included.

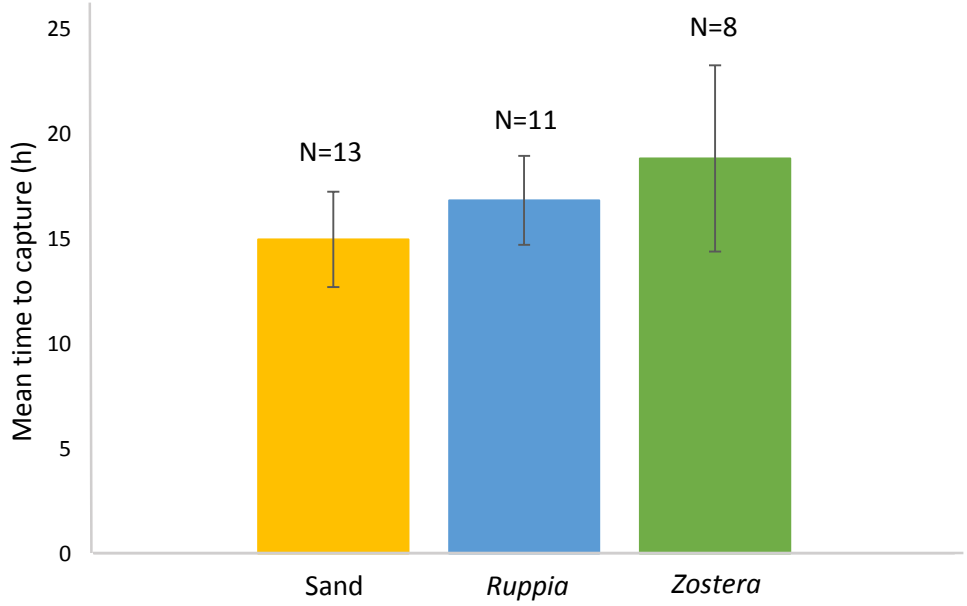

Figure 16. Mean time to capture (h) of juveniles in each habitat type ± 1 standard error (SE) for predator crab trials. Only dead juvenile crabs are included.

Crab predator-prey size ratios affected juvenile blue crab survival (p=0.0385). Ratios were analyzed because they did not differ between habitat types (ANOVA, $F_{2,36} = 1.97$, p=0.1918) (Fig. 17). Sand contained the largest range of size ratios from 2.71-9.85. *Zostera*'s range was from 2.73-6.68. *Ruppia*'s range was from 2.50-6.94. The medians for sand, *Zostera*, and *Ruppia* were 4.36, 4.65, and 3.92. Due to the small R^2 value of 0.1019 (Fig. 18), there only appears to be a negative relationship between crab predator-prey size ratio and time to capture for ratios 2.5-4.5. In this range, it seems that prey are caught faster when they are much smaller than their predators (higher ratio). Prey are not caught as quickly when closer in size to their predators (smaller ratio). For ratios higher than 4.5, there does not appear to be a relationship between crab predator-prey size ratio and time to capture.

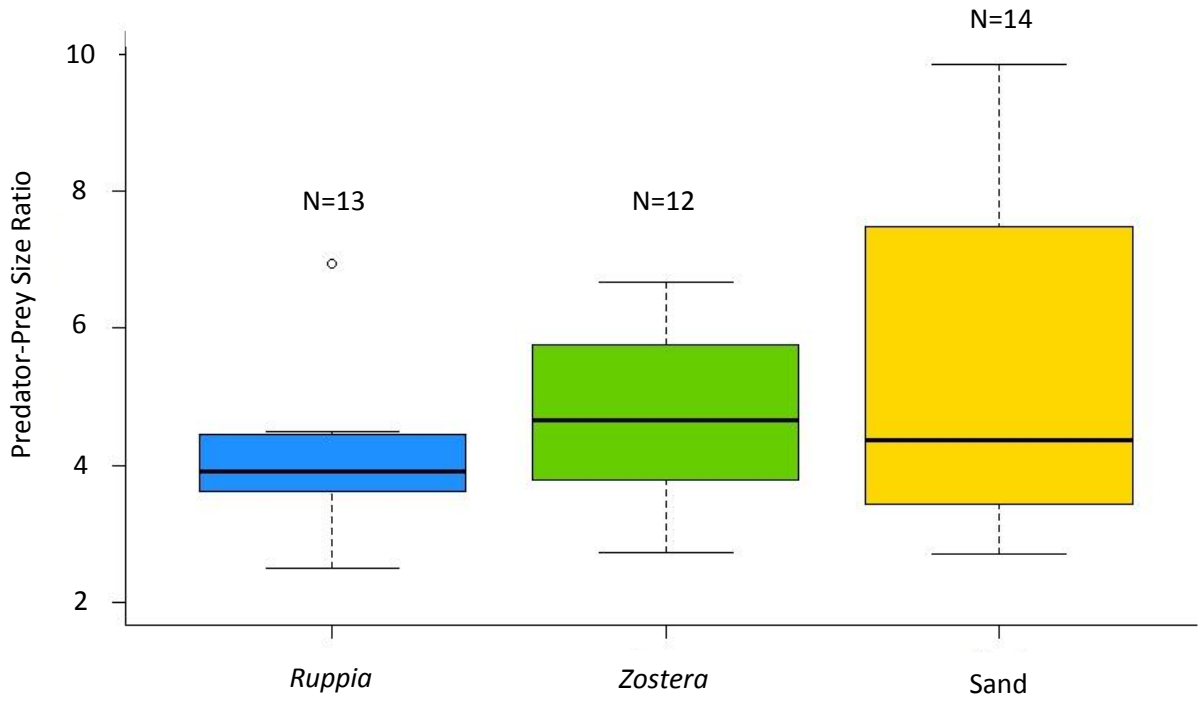

Figure 17. Boxplot of crab predator-prey size ratios in each habitat. The outlier in *Ruppia* is 6.94. Trials during which juveniles were eaten and trials during which they were not eaten are included. Ratios did not differ between habitats (ANOVA, $F_{2,36} = 1.97$, p=0.1918).

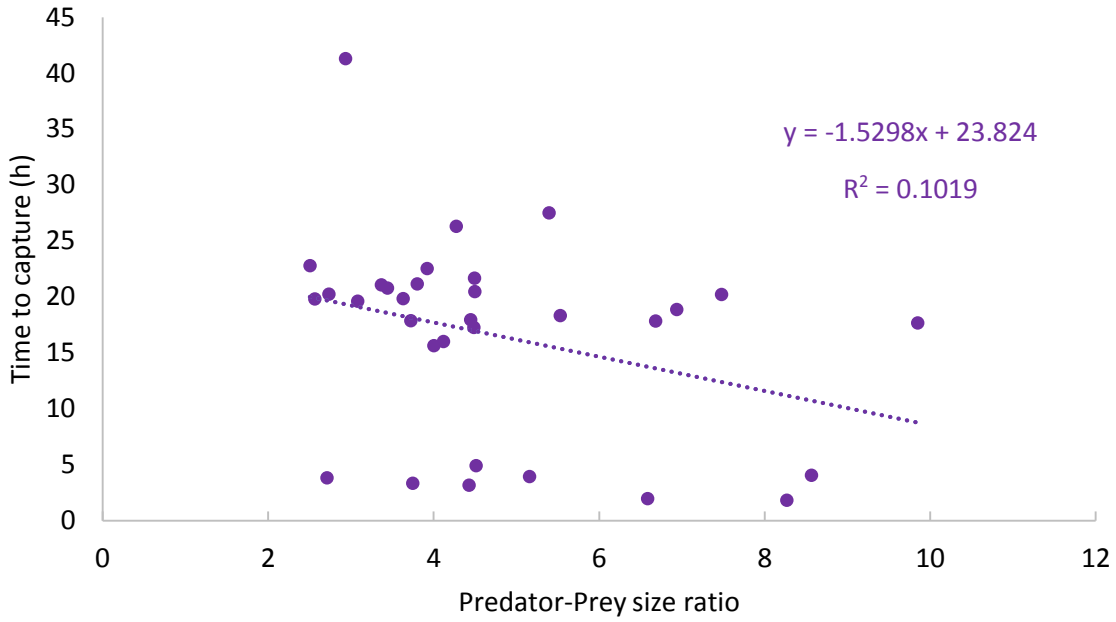

Figure 18. Linear regression of crab predator-prey size ratio vs time to capture (h) of juvenile crabs in predator crab trials. Only dead crabs are included.

5. Discussion

Predator type was not analyzed for an effect on juvenile survival in the current experiment. The small number of striped burrfish predator trials compared to crab predator trials hindered the survival analysis from running properly. Even though the mean time to capture of juveniles in crab predator trials could not be compared to the lone striped burrfish predator trial, it appears that adult crabs are more voracious predators. The average capture time by crabs was 53.01 hours shorter than that of the single burrfish trial. In future studies, equal and large numbers of crab and burrfish predator trials would be necessary to test for an effect of predator type on juvenile crab survival. Limited time and resources prevented performing more trials.

Habitat type affected juvenile crab survival. As predicted, *Zostera* enhanced juvenile survival more than sand. In contrast with the hypothesis, there was no significant difference between *Ruppia* and sand in providing juvenile protection. As hypothesized, there was no significant difference between *Zostera* and *Ruppia* in providing juvenile protection. Heck and Thoman (1984) found that *Zostera* supports more crabs than sand or *Ruppia* in terms of crab density. However, crab density is a different indicator of crab success than survival. Further investigation using large and equal sample sizes for both *Zostera* and *Ruppia* would be necessary to confirm the results that juvenile crab survival does not differ between *Zostera* and *Ruppia* and between *Ruppia* and sand.

Crab predator-prey size ratios affected juvenile survival. Even though the data do not fit the trend line well ($R^2 = 0.1019$), a relationship between predator-prey size ratio and time to capture is apparent for ratios ranging from 2.5-4.5 as the data cluster closer to the trend line. This trend is not apparent outside of this range. It seems that juveniles much smaller than their

predators are eaten faster. When juveniles are closer in size to their predators, they are not eaten as quickly. Larger predators may have a higher advantage over smaller prey, whereas larger prey may be less vulnerable to predation by smaller predators. This relationship could be strengthened in future work by increasing sample sizes and the length of the experiment.

5.1 Limitations

The current experiment was limited both by time and resources. Increased statistical power would be achieved through larger sample sizes and longer duration of the experiment. Seven trials were discarded for various reasons. Some include the addition of an extra adult crab in a tank mid-trial by an unknown person, a molting or dead predator crab, or intelligent juveniles that managed to climb out of predator reach. One trial was discarded because an additional live untethered juvenile crab was visible on top of the sand – this crab likely remained from an untethered trial. Another trial was discarded because the predator might have been sick since it did not eat after 74 hours and died soon afterwards.

Some trials included in the analysis had smaller complications. Tethered crabs were expected to exhibit limited movement, but some juveniles became tangled in the ribbon or had difficulty walking. Some tanks had murkier water than others at times, and this was due to backups in the pipe system. Both of these situations could have affected juvenile vulnerability. Several juveniles and some adults had missing claws or parts of claws and this could have increased prey vulnerability and affected the predator's ability to feed. Due to lack of time and resources, a predator crab was used in a trial a few days after molting, which could have affected its motivation to eat.

5.2 Future studies

In future research, more trials should be run in each habitat (50-100 each) with equal numbers comprising crab and burrfish predators in each habitat. Each predator would occupy an individual tank and be starved 24 hours prior to each trial to ensure it was hungry enough to hunt. Predators should not be used in more than one trial because they could learn how to maneuver in tanks (Saluta, Personal Communication, 2016). Juveniles and predators should also have intact claws. Although claws are lost naturally, missing claws could make prey more vulnerable to predation and affect the outcome. In future research, the habitats would be randomized prior to each trial. GoPro cameras would also be installed into each tank to record the exact time of predation events. This would allow experiments to run until prey are eaten, even if it occurs during the night. Lastly, it would be beneficial to put artificial meshes in the field with GoPro cameras attached to nearby PVC poles in order to determine the most common predators of juvenile crabs. This experiment could then be continued in a mesocosm using these predators to test juvenile survival in different substrates.

Incorporating all these factors into future studies may help refine the determination of whether *Ruppia* can fulfill the ecological role of *Zostera* as essential nursery habitat for juvenile blue crabs as the global climate continues warming. If future research shows *Ruppia* can replace *Zostera*, blue crab populations could continue to be ecologically and economically important in the Chesapeake Bay and the mid-Atlantic region. If future research shows *Ruppia* is unable to

replace *Zostera*, stricter restrictions on the blue crab fishery may be required to sustain healthy populations. Further research would be needed to determine if other habitats could replace *Ruppia.*

5.3 Other impacts on blue crab survival: hypoxia

Areas of hypoxia (low oxygen) at depth in coastal regions are becoming more prevalent worldwide due to increased fertilizer and sewage runoff (NOAA 2014). Increased stratification (layers of differing density) due to warming temperatures also allows hypoxia to persist in deeper waters (Williams 2012). The number of United States estuaries, including Chesapeake Bay, experiencing hypoxia has largely increased over the last few decades. Over 50% of these estuaries endure hypoxia in any given year (NOAA 2014). The effect of hypoxia on behavior and physiology of blue crabs, their prey, and their habitats may impact juvenile blue crab survival. Blue crabs exhibit the strongest avoidance responses to levels of low dissolved oxygen (DO) compared to other organisms including Atlantic croaker, pinfish, spot, and anchovies (Bell & Eggleston 2005). The relative abundance of blue crabs is significantly higher in mid-depths with higher DO than in deeper waters during chronic hypoxia (Bell & Eggleston 2005).

Occurrence of hypoxia can influence the feeding rates of blue crabs. Free-ranging blue crabs exposed to mild (DO = 2-4 mg/l) and severe (DO <2mg/l) hypoxia from upwelling generally decrease their proportion of time spent feeding compared to those exposed to normoxic (DO >4 mg/l) conditions (Bell et al. 2003). Blue crabs in severe hypoxia prior to relaxation events (when DO increases to mild hypoxia) require time to recover prior to traveling to deeper waters where benthic infauna reside (Bell et al. 2003). Benthic infauna are typically less buried in sediments during prolonged hypoxic events and are more vulnerable to predation. Even crabs exposed to mild hypoxia prior to relaxation events failed to take advantage of benthic infauna (Bell et al. 2003). This is fairly inconsistent with Taylor and Eggleston's (2000) study in which crabs did eat benthic infauna. Blue crabs in Bell et al. (2003)'s study could have utilized alternative food sources, or the hypoxic events were not prolonged enough for infauna to become vulnerable.

Juvenile blue crab feeding and growth significantly declines in hypoxic habitats (Das & Stickle 1993). Juveniles exposed to different hypoxic levels also take longer to molt and have longer intermolt intervals (Das & Stickle 1993). While these changes show that blue crab metabolic rates decline with reduced oxygen levels, blue crabs have also been found to maintain constant aerobic metabolic rates until a critical oxygen level is reached (Brill et al. 2015). This level represents the minimum amount of oxygen required to maintain aerobic metabolism, and it is about 20% air saturation for decapod crustaceans during resting metabolism (Brill et al. 2015).

As previously mentioned, hypoxia affects the prey of blue crabs. The soft-shelled infaunal clam *Mya arenaria* is prey for blue crabs. Taylor and Eggleston (2000) found that its sediment burial depths are significantly shallower and their siphon lengths are longer in low DO concentrations than at moderate or high DO concentrations (Taylor & Eggleston 2000). Blue crabs have significantly higher clam consumption rates in normoxic (DO ≥6 mg/l in this study) and moderate hypoxic (DO = 3-4 mg/l) conditions. In these cases, the clams were acclimated to severe hypoxia (DO ≤1.5 mg/l in this study) and were vulnerable to predation. When the clams

were acclimated to normoxia, they were less vulnerable and consumption rates by crabs were lower when conditions turned to moderate hypoxia (Taylor & Eggleston 2000).

Hypoxia affects the physiology of *Zostera* and *Ruppia*. Low oxygen (LO) conditions negatively impact the survival and growth of *Zostera*, especially in the presence of sulfides in sediment (Holmer & Bondgaard 2001). Sulfate reducing bacteria produce hydrogen sulfide during anaerobic decomposition of organic matter (carbon) in marine sediments (Canfield 1993). Sulfate reduction typically occurs deeper in sediments than oxic respiration, or aerobic decomposition. The relatively lower amount of oxygen in seawater compared to sulfate allows sulfate reduction to occur longer than oxic respiration. Ultimately, the amount of decomposed carbon on continental margins is about equal for sulfate reduction and oxic respiration (Canfield 1993). Sulfides and LO reduce *Zostera*'s photosynthetic rates, and sulfides rot young meristematic cells (undifferentiated cells analogous to stem cells in humans) (Holmer & Bondgaard 2001). LO reduces *Zostera*'s shoot densities and root sucrose (sugar) reserves. More sucrose accumulates in *Zostera* leaves under hypoxic conditions because sucrose transport to roots is blocked (Holmer & Bondgaard 2001).

Sulfides are phytotoxins at high concentrations. Young *Zostera* and *Ruppia* leak oxygen from their roots into the surrounding rhizosphere (sediments directly surrounding roots) to reduce exposure to sulfides (Jovanovic et al. 2015). Young *Ruppia* always maintains higher amounts of oxygen in its rhizosphere than *Zostera* because it leaks oxygen from both root tips and upper root sections. *Zostera* only loses oxygen from its root tips. Aside from more permeable root area, *Ruppia* also has larger biomass aboveground than *Zostera* that allows it to produce more oxygen via photosynthesis (Jovanovic et al. 2015). Oxygen leakage results in a less toxic rhizosphere because hydrogen sulfide is oxidized. Both *Zostera* and *Ruppia* are unable to maintain these protective oxic zones during nighttime when oxygen in the water column is 0-25% air saturation (Jovanovic et al. 2015). However, young *Ruppia* is better able to reestablish oxic zones than *Zostera* as oxygen concentrations increase. *Ruppia* is more protected against sulfide intrusion than *Zostera*. *Ruppia*'s ability to protect itself from sulfides partly explains why it is successful in recolonizing coastal shallow sediments that are low in oxygen and high in sulfides. As long as oxygen levels are not below a critical value, *Ruppia* has a competitive advantage over *Zostera* (Jovanovic et al. 2015). While *Ruppia* is more advantageous in these conditions, it is more vulnerable to damage by sulfide intrusion during times of hypoxia or anoxia due to its higher root permeability (Pedersen & Kristensen 2015). Another advantage *Ruppia* has over *Zostera* is its tolerance to higher water-column nitrate concentrations from agricultural runoff and sewage effluent (Burkholder et al. 1994). *Halodule* also has high tolerances to large nitrate concentrations (Burkholder et al. 1994).

As nutrient loading continues in Chesapeake Bay and hypoxia remains prevalent, blue crabs may avoid these regions and migrate to shallower waters. Juveniles may continue to have slower feeding and molting rates. It remains uncertain why blue crab aerobic metabolism has been observed to both slow down and remain unchanged in hypoxic conditions, and why blue crabs do not always migrate during relaxation events to seek exposed benthic infauna. *Ruppia*

has a competitive advantage over *Zostera* in hypoxic conditions and may serve as nursery habitat into the future.

5.4 Other impacts on blue crab survival: *Hematodinium perezi*

In addition to the effect of habitat and hypoxia on blue crab survival, *Hematodinium perezi*, a parasitic dinoflagellate, causes blue crab mortality. In coastal bays of Virginia and Maryland, analysis of blue crab fishery landings show a decline that corresponds to high mortality and *Hematodinium* infections (Messick & Shields 2000). *Hematodinium* is found in blue crabs from New Jersey to Florida, and along Texas' Gulf coast. Hemolymph (fluid similar to blood) has been examined for infections in over 13,000 blue crabs including 4,830 from coastal bays in Maryland, 1,542 from coastal bays in Virginia, and 5,076 from the lower Chesapeake Bay. Of naturally infected crabs kept in captivity, 100% died over 35 days at 20-24 ºC (Messick & Shields 2000). *Hematodinium* infections are more prevalent in higher salinity waters in regions of the lower Chesapeake Bay, and they are not found in crabs in northeastern parts of the bay where salinities are lower than 18‰ (Messick & Shields 2000). However, infections can occur in waters above 11‰ salinity (Newman & Johnson 1975).

Prevalence of *Hematodinium* is significantly highest in crabs from 3-9 ºC, while the infection intensity (activity) increases with temperature. Infections are more prevalent from August to November for crabs in coastal bays of Maryland and Virginia (Messick 1994, Messick & Shields 2000). In Maryland coastal bays, prevalence is significantly higher in crabs that measure 3-30 mm CW (Messick & Shields 2000). Smaller crabs (5-89 CW) have higher infection prevalence than crabs 90-180 CW in salinities of 19-32‰ and temperatures of 4-26 ºC in Virginia and Maryland coastal bays (Messick 1994). Juvenile blue crabs are more susceptible to *Hematodinium* infections than larger ones, but the explanations remain unknown.

Cannibalism can comprise 15-25% of adult blue crab diets (Lipcius et al. 2007, Li et al. 2011), and there is not a clear consensus on its effectiveness at transmitting *Hematodinium* to blue crabs. *Hematodinium* can infect blue crabs 16 hours after the consumption of infected blue crab tissues, and 63% of crabs were infected 24-48 hours after eating infected tissues (Walker et al. 2009). In contrast, Li et al. (2011) found no juvenile blue crabs infected with *Hematodinium* after their consumption of infected blue crab tissues. Only those crabs injected with infected hemolymph (positive control) showed infection and mortality (Li et al. 2011). Waterborne transmission of *Hematodinium* may be likely, as it has been detected in water samples in which blue crab infections were detected. In waters with uninfected crabs, *Hematodinium* was not detected (Frischer et al. 2006).

It remains uncertain why juvenile blue crabs may be more vulnerable to *Hematodinium* infections than adult crabs. It may be due to their presence in lower Chesapeake Bay where *Hematodinium* prefers the higher salinities. Even though Walker et al. (2009) did not specify if the experimental crabs were adults or juveniles, it seems that cannibalism may not be a likely transmission path for juveniles. Juveniles may become infected from surrounding waters, but it is unknown if it is transmitted from other prey sources. As the global climate continues warming, *Hematodinium* infections may become more severe as their activity increases with temperature.

5.5 Conclusions

Zostera provides more protection for juvenile blue crabs than sand, whereas there was no significant difference between *Ruppia* and sand in providing juvenile protection. There was not a significant difference between *Zostera* and *Ruppia* in providing protection for juvenile crabs. The continued decline of *Zostera* might not negatively affect juvenile blue crab survival if other habitats fill its ecological niche. *Ruppia* may be able to replace *Zostera* in Chesapeake Bay due to its higher tolerances to warm temperatures, hypoxia, and high nitrate concentrations. *Halodule* may be able to replace *Zostera* in North Carolina due to its higher tolerances to warm temperatures, low light intensity, and high nitrate concentrations. Other habitat types also provide nurseries for juvenile blue crabs. *Gracilaria vermiculophylla* (Fig. 19), an exotic species of red macroalga in Chesapeake Bay likely introduced along with invasive Asian oysters, provides favorable habitat for juvenile blue crabs (Johnston & Lipcius 2012). *Gracilaria* is not known to have negative effects on native species in Chesapeake Bay, and juveniles survive as well or better in *Gracilaria* than in mud or *Zostera* (Johnston & Lipcius 2012). Alternatively, *Gracilaria* is an invasive species in the Baltic Sea and it reduced the survival of *Zostera* in a mesocosm because it created anoxic conditions. Destruction to natural seagrass beds caused by *Gracilaria* has not been documented (Martínez-Lüscher & Holmer 2010). Salt marshes such as smooth cordgrass (*Spartina alterniflora*) (Fig. 19) also provide nursery habitat for juvenile blue crabs (Johnson & Eggleston 2010). Both survival and abundances of juvenile blue crabs are high in salt marshes (Johnson & Eggleston 2010). *Spartina* supports dense populations of post-larvae fishes and shellfish (Weinstein 1979). Management of *Gracilaria* and *Spartina* might become more important as they could potentially replace *Zostera* as essential juvenile crab nursery habitats.

As global temperatures continue rising and nutrient loads persist in coastal ecosystems, *Ruppia* and *Halodule* may be able to fulfill *Zostera*'s role as nursery habitat for juvenile blue crabs. Hypoxia will likely continue to affect blue crab feeding and molting rates, while *Hematodinium* infections will likely become more severe for juvenile crabs. Overall, the viability of future blue crab populations is uncertain because habitat type, hypoxia, and *Hematodinium* infections all have varying impacts on juvenile survival. These factors, among others, play critical roles in ensuring blue crab populations can remain ecologically and economically viable into the future.

Figure 19. Left to right: species of red macroalga (*Gracilaria vermiculophylla*), smooth cordgrass (*Spartina alterniflora*). Sources in listed order: http://cfb.unh.edu/phycokey/Choices/Rhodophyceae/Macroreds/GRACILARIA/Gracilaria_image_page.htm; https://plants.usda.gov/core/profile?symbol=spal#

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8. Appendix

8.1 Untethered trials

Juvenile blue crab predation trials were initially performed with untethered prey specimens. Nine trials, each with one untethered juvenile crab, were conducted with adult blue crab predators (six trials, one adult per tank) and striped burrfish predators (three trials, one burrfish per tank). Untethered trials were limited because juvenile crabs were difficult to locate once buried in sand and their status (alive/dead) was not easily confirmed. These trials were not statistically analyzed.

8.2 Field experiment

Juvenile blue crabs to be used as prey (8+) were collected from the lower York River using crab scrapes, dipnets, and seine nets. Field tethering experiments were attempted to estimate juvenile blue crab survival. Artificial seagrass plots were placed at the Goodwin Islands (37.2188°N, 76.4028°W) near the mouth of the York River, a tributary of Chesapeake Bay. Each plot had one tethered crab with a 15-cm tether. One end was tied to a metal swivel clip, which was attached to a metal stake, then placed in the center of the plot.

Four *Zostera* plots and four *Ruppia* plots were placed in unvegetated sand patches within a mixed bed of natural *Zostera* and *Ruppia*. Metal stakes, four on the outer rim and one in the center, secured each seagrass plot in the sediment. PVC poles marked plot locations and were placed about 0.3 m away from each plot. Ten tethering trials were to be conducted for each seagrass species (10 per plot).

The plots were placed in the field on 12 July 2016. Unfortunately, wind and choppy water disrupted the plots. In addition, cownose rays (*Rhinoptera bonasus*) were in the area when the plots were checked after approximately four hours, and they are known to reveal buried shellfish by flapping their fins on the bottom ("Chesapeake Bay Program: Cownose Ray" 2012b). One *Ruppia* and two *Zostera* meshes were lost and the field experiment was discontinued.