Understanding the Habitat Preferences of Juvenile Blue Crabs in Louisiana Estuaries When Presented with Food Cues

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Abstract:

Blue crabs (*Callinectes sapidus*), in addition to being ecologically critical in coastal systems, support a thriving fishery across the Southeastern United States, with the state of Louisiana leading the nation in harvests (Posadas 2020; LDWF 2022). Along with other decapods known to use a wide variety of habitats across their life cycle (Pittman and McAlpine, 2001; Moksnes & Heck Jr., 2006), the juvenile phase of a Blue crab's life cycle is characterized by intense association with estuarine structured habitat. It has been shown in previous research that submerged aquatic vegetation and oyster reefs potentially provide critical habitat to young crabs (Heck Jr. et. al., 2001; Longmire et al., 2021). However, studies investigating how strong food cues are in the process of how juvenile Blue crabs select habitats have been lacking. In the midst of changing environmental conditions in coastal Louisiana, understanding how young Blue crabs choose and associate with structured habitat in coastal estuaries will be critical in managing the fishery and ecosystem as a whole. The goal of this experiment was to identify key habitats chosen by juvenile Blue crabs when presented with food cues in order to provide habitat usage information to agencies involved in coastal restoration and fisheries management.

Introduction

Adult abundances of decapods, among many other invertebrates characteristic of marine and estuarine benthos, are seemingly determined by many different environmental variables, such as high predation among

juveniles, reproductive output of stocks, and subsequent development and migration of young (Moksnes & Heck Jr. 2006; Heck Jr. et. al., 2001). Common life-history strategies for these organisms include tri-phasic lifestyles, with complex spatial movements carried out by both juvenile and mature organisms throughout a variety of habitats (Pittman and McAlpine, 2001). In order to counter strong vulnerability to predation, juveniles of species whose life history strategies align with this tri-phasic lifestyle frequently are seemingly associated with strongly heterogeneous microhabitats (Moksnes & Heck Jr., 2006). Since these microhabitats are being perceived as an increasingly important link in decapod ontogeny, their emphasis in fishery and ecosystem management has gained significant traction.

The Atlantic Blue Crab (*Callinectes sapidus)* supports both massive commercial and recreational fisheries throughout the Atlantic and Gulf seaboards of the United States. The Louisiana fishery for the species is the largest in the nation (Louisiana Department of Wildlife & Fisheries [LDWF] 2022), with the state ultimately responsible for 30.6% of all national commercial landings in 2018 (Posadas 2020). Between 2000 and 2018, Louisiana commercial crabbers landed approximately 44.7 million pounds of Blue crabs, which fetched an annual real dockside value of approximately 43.3 million dollars (LDWF, 2022). In addition to their economic and cultural importance, Blue crabs are an important component of ecosystem function (LDWF 2022).

Blue crabs have been found to be associated with a wide range of microhabitats while in their juvenile phases of life, including submerged aquatic vegetation (SAV), seagrass beds, and coastal marshes (Heck Jr. et. al., 2001). These habitats, composed of a wide variety of submerged and emergent macrophytes, have been shown to support massive abundances of juvenile Blue crabs (Heck Jr. et. al., 2001). Salinity is perhaps a factor as well in distinguishing which types of SAV habitats juvenile Blue crabs use. Heck Jr. et. al. (2001) suggests that important nursery habitats may vary between juvenile Blue crabs based on size, with larger crabs perhaps being more likely to use oligotrophic zones of SAV and marsh while smaller crabs may more likely be found in higher salinity zones of SAV and marsh. In coastal Louisiana, native *Ruppia maritima* and introduced *Myriophyllum spicatum* are two SAV species frequently found in freshwater, oligohaline, and mesohaline zones, with differing tolerances to salinity and other abiotic water quality factors (Hillmann & La Peyre, 2019). Besides SAV, the Eastern Oyster (*Crassostrea virginica*) is an important, highly abundant ecosystem engineer along the Gulf of Mexico that frequently builds ecologically valuable reefs (La Peyre et al., 2019). Oyster reefs have been shown to contain and support biodiverse communities, including Blue crabs, due to their heterogenous structure and geometry (La Peyre et al., 2019). Past experiments have shown that oyster shell may be important in providing refugia from predators for juvenile Blue crabs, although the relative importance of oyster reefs compared to other habitats is not quite certain (Longmire et al., 2021). Amid reef loss caused by a wide variety of coastal challenges, oyster reef restoration has emerged as a new priority for estuarine restoration, with different types of reef restoration projects underway by organizations such as The Nature Conservancy and the Louisiana Department of Wildlife and Fisheries (The Nature Conservancy [TNC], n.d.; LeBreton 2022). Routine monitoring of these reef restorations will inform which styles of design work most efficiently in providing ecosystem services, which will in turn inform further restoration efforts (La Peyre et al., 2014).

Some past experiments have set out to analyze the dietary preferences of juvenile Blue crabs among nursery habitats, with juveniles found in the sub-estuaries of Chesapeake Bay frequently feeding on both bivalves and polychaetas (Seitz et al., 2011). However, research studying the direct relationship between habitat selection and availability of food have been, for the most part, lacking. While a study by Macreadie et al. (2012) did investigate how foraging habits of juvenile Blue crabs change around oyster reefs in response to predator cues and habitat complexity/continuity, this study minimally investigated which habitats juveniles select in conjunction with differing availabilities of food or foraging opportunities.

The coastline of Louisiana is currently highly susceptible to a wide variety of changing environmental conditions, with roughly 2000 square miles of land being lost since the 1930s (Coastal Protection & Restoration Authority [CPRA], 2013a). This alarming loss of coastal wetlands has been caused by both natural and anthropogenic factors, such as hydrological changes to the Mississippi River, oil & gas exploration, increased wave erosion, etc. (Roberts and Mendelssohn, 2022). To counter this crisis, dozens of coastal restoration projects have been completed or planned to reduce the severity of Louisiana's land loss (CPRA, 2013b). These local changes to coastal Louisiana, in combination with global climate change and sea level rise, will likely cause a change in environmental conditions that will be largely important in determining ecosystem function and services.

In order to provide comprehensive and effective management strategies for the Blue crab fishery in Louisiana and beyond, attention must be given to the microhabitats juvenile Blue crabs associate with, and specifically, how juveniles select habitats based on different foraging opportunities presented. From a management perspective, this is likely critical to ensuring successful recruitment of juvenile crabs to the stock, as well ensuring proper ecosystem function in estuaries. In this study, juvenile Blue crabs were exposed to different habitat types characteristic of Louisiana estuaries. These habitats included two species of submerged aquatic vegetation (SAV): *Ruppia maritima* and *Myriophyllum spicatum,* and two different geometries of oyster reefs designed to imitate both oyster culch reefs and live oyster reefs. Select habitats were supplemented with food to compare the relationship between habitat selection and foraging opportunities. The goal of this experiment was to successfully isolate the structural preferences of blue crabs to advance habitat restoration and conservation under changing environmental conditions.

Methods

Organism Collection

Juvenile Blue crabs, *Callinectes sapidus,* with carapace widths of 30-80 mm were captured on multiple sampling trips in coastal Louisiana using a variety of sampling techniques. The first sampling trip was conducted on Thursday, June 1st, 2023 from approximately 11:30 AM – 2:30 PM at Lake Pontchartrain near Lacombe, Louisiana. Discarded Spotted Sea Trout, *Cynoscion nebulosus*, carcasses were placed in a small, shallow, branch coming off Lake Pontchartrain (Figure 1). Researchers would wade out to the carcasses where they would attempt to use a long-handled dip net to scoop up juvenile crabs feeding on the carcasses. One crab was caught with this method. The crab was put in a mesh bag and stored in a cooler upon transport to LSU. The second sampling trip was conducted on June 13th, 2023, from approximately 9:00 AM – 3:30 PM. Oyster research mariculture cages from the Louisiana Universities Marine Consortium in Cocodrie, Louisiana and the Michael C. Voisin Oyster Hatchery in Grand Isle, Louisiana (Figure 1) were retrieved and brought to shore where all juvenile crabs within the range of accepted carapace widths were collected and placed into mesh bags. They were then placed in a cooler upon transport to LSU. The third sampling trip took place on June 15th, 2023, from approximately 7:00 PM – 11:00 PM at the Louisiana Universities Marine Consortium in Cocodrie, Louisiana. Juvenile crabs were found being swept out of the marsh by a strong outgoing tide, and long-handled dip nets were used to scoop up crabs as they ran under a bright dock light (Figure 1). Crabs were sorted by size before being stored in mesh bags upon transfer to LSU. The final sampling trip took place on July 3rd, 2023 from approximately 9:30 AM – 11:30 AM at Lake Pontchartrain in Lacombe, Louisiana. Small crab pots containing bread and sausages were set out along the shoreline of Lake Pontchartrain, next to stems of *Spartina alterniflora* and *Juncus roemerianus* (Figure 1). In addition, crabs were also caught in long-handled dip nets while wading across shallow flats in Lake Pontchartain, containing *Ruppia maritima* & *S. alterniflora* (Figure 1). Crabs were put in mesh bags and placed in a cooler upon transport to an old wet lab at LSU. Upon arrival to LSU, all crabs were stored in the experimental wet lab in flow-through tanks at a constant salinity of 10 PSU. Crabs were put in a series of 4 plastic trays, with each tray being divided by vexar mesh into 8-9 zones. One crab was assigned and placed in each zone. Every day, crabs were checked for mortality and molting, dead crabs and molts were removed, and salinity/temperature were measured by using a YSI Water Quality Probe. Once a week, ammonia, nitrate/nitrite, and pH were tested by using test strips. Crabs were fed every day with 0.5-1 cubic centimeter chunks of chicken gizzards, presented with a pair of forceps. Crabs were allowed to eat for 1-2 hours before food was removed.

Figure 1, Going clockwise: Locations of crab captures from June 1, 2023, and July 3, 2023 near Lacombe, Louisiana; location of crab captures from June 13, 2023 at the Michael C. Voisin Oyster Hatchery in Grand Isle, Louisiana; locations of crab captures from June 15, 2023, and June 13, 2023 at the Louisiana Universities Marine Consortium in Cocodrie, Louisiana

Eurasian Water Milfoil, *Myriophyllum spicatum,* was collected from an interior marsh pond at Big Branch Marsh National Wildlife Refuge on July 3, 2023 (Figure 2). The interior pond was reached via kayak, and milfoil was collected by wading in the water and extracting stems, rhizomes and roots with shovels. Approximately 100 stems were collected, rinsed with ambient water, and stored in mesh bags in a cooler during transport to LSU. Wideongrass, *Ruppia maritima,* was collected from the northern shore of Lake Pontchartrain at Big Branch Marsh National Wildlife Refuge on July 3rd, 2023 (Figure 2). Approximately 100 stems with attached rhizomes and roots were collected with shovels and trowels, rinsed with ambient water, and stored in mesh bags in a cooler for transport to LSU. All SAV was stored in small plastic containers in 5 PSU filtered, dechlorinated water. SAV was left outside on a patio of the experimental wet lab to help maximize exposure of SAV to sunlight.

Figure 2: Collection sites of *R. maritima* and *M. spicatum* from July 3, 2023 at Big Branch Marsh NWR in Lacombe, Louisiana

Experimental Habitats

Five habitats were included in each experimental replicate, including *R. maritima,* a species of SAV found in an ideal salinity of 4.7-22.6 PSU (SAV-Hi) (Tyler-Walters & d'Avack 2015), *M. spicatum,* an SAV found in an ideal salinity range of 0 – 10 PSU (SAV-Lo) (Jacobs & Mangold, 2009) vertical Eastern oyster (*Crassostrea virginica*) shell habitat (OR-V), horizontal Eastern oyster shell habitat (OR-H), and an unstructured habitat (UNS; Figure 3). To make SAV-Hi habitats, 9 similarly-sized clusters of *R. maritima* composed of 5 stems each were threaded through a 12.7 x 12.7 cm piece of vexar mesh. The clusters were evenly distributed and zip-tied to the vexar mesh, and the mesh was zip-tied to a 12.7 x 12.7 cm tile. Stem height was standardized by trimming to approximately 18 cm . To make SAV-Lo habitats, 9 similarly-sized clusters of *M. spicatum* composed of 3 stems each were threaded through a 12.7 x 12.7 cm piece of vexar mesh. The clusters were evenly distributed and zip-tied to the vexar mesh, and the mesh was zip-tied to a 12.7 x 12.7 cm tile. Stem height was standardized by trimming to approximately 18 cm. To make OR-V habitats, weathered and dried oyster shells of similar size from the experimental wet lab at LSU were cemented together with quick-dry concrete, and then cemented to a 12.7 x 12.7 cm tile. Shells were cemented together in a vertical orientation to mimic the geometry of an oyster reef with both cultch and live oysters growing on the surface. Calculated volume found via water displacement was used to standardize reef size, and completed reefs measured between 0.25-0.5 L in volume. To make OR-H habitats, piles of weathered and dried oyster shells of similar size from the experimental wet lab at LSU were spread and stacked loosely on a 12.7 x 12.7 cm tile. The shells were stacked in a horizontal orientation to mimic a cultch reef without live oysters. Calculated volume found via water displacement was used to standardize reef size, and all reef volumes measured between 0.25-0.5 Liters. For an unstructured control habitat (UNS), a 12.7 x 12.7 cm tile was used. Some habitats (1 in each habitat pair) were supplemented with food to compare habitat selection preferences among habitats with and without food. For habitats containing food, a galvanized metal screw was glued on to each tile of each habitat to act as a stake on which food could be attached, if the habitat was selected to contain food. If the screw fell off during the trial, it was attached to an oyster shell using rubber bands, or the tile swapped so that the tile with food had a screw.

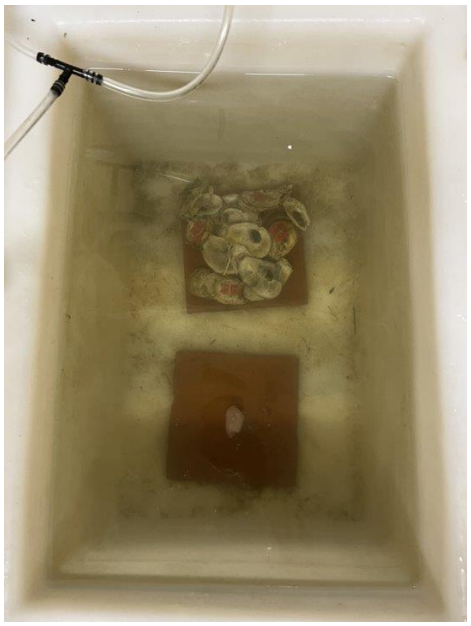

Figure 3: Constructed habitats utilized in each experimental replicate. The top picture displays an example of the SAV-Hi habitats. The bottom left picture displays an example of an OR-H habitat (top) and an Uns. habitat (bottom). The bottom right picture displays an example of an OR-V habitat (top) and a Sav-Lo habitat (bottom).

Experimental Process

To run the experiment, 10 translucent tubs with volumes of approximately 76 L each were set up in two rows along the wall of a wet lab at LSU in which the windows in the room were covered. All tanks were filled with filtered, dechlorinated artificial seawater at 10 PSU. Tanks were aerated with one air stone running off a main line above the tubs and strategically placed in the center of each tank near the surface of the water to minimize disturbing the crabs. As the tanks were used in a prior experiment, a single water change was performed prior to the experiment by draining and replacing ~1/3 of the water in each tank and replacing it with fresh 10 PSU carbon filtered, dechlorinated artificial seawater. Water was not changed again over the 3 day course of the experiment.

Habitat units were paired factorially (i.e. each habitat was paired once with all others) within individual tanks to form 10 possible habitat combinations (Figure 4). Given logistical limitations (availability of space, tanks, and crabs), a replicate is represented by a set of all ten tanks (all ten habitat combinations), run twice on a single day (replicate part A and part B). In replicate part 1, the food was randomly assigned to one habitat per pair using a coin toss, and in part 2, the food was placed in the opposite habitat of each pair.

At the start of replicate part A, temperature and salinity of the tanks were collected with a YSI water quality probe. Habitats were then placed into each tank according to their random assignments, at opposite ends of the tanks approximately 4.0 cm away from the tank edges, corners, and other habitats (Figure 4). Assigned crabs were placed into each tank at approximately 8:00 AM and left to acclimate for 30 minutes. If the assigned crab had been found to have recently molted, the new carapace width was measured and recorded. If the assigned crab died, or displayed a soft/paper shell, the crab was traded out for a replacement. After crabs were introduced, starting photos for each tank were taken with a cell phone camera. Food was introduced to the chosen habitats at approximately 8:25 AM. At 8:30 AM, after the 30 minute acclimation period, the starting positions of the crabs (edges of either habitat, insides of either habitat, or the middle of tank) were recorded. Position measurements were then recorded at 15-minute intervals (from approximately 8:30 AM – 10:00 AM) until seven total observations were taken. If crabs ate all food in their tank, this observation was noted. After the final observation was taken, final photographs of each tank were obtained, and crabs were removed and transferred back to the flow-through storage tanks. All food and habitats were removed, and the water in each tank was skimmed with a dip net. Habitats were then rearranged for part B. In the afternoon after replicate part A, the same protocol was then repeated for part B of each replicate, wherein the food was placed in the opposite habitat of each pair. Three full replicates (including both part A and part B) were conducted on July 12, 14, and 16, 2023,, every other day to ensure crabs were hungry during the trials. After replicate part B on July 12 and 14, the SAV units were placed back in their storage tubs to allow access to sunlight.

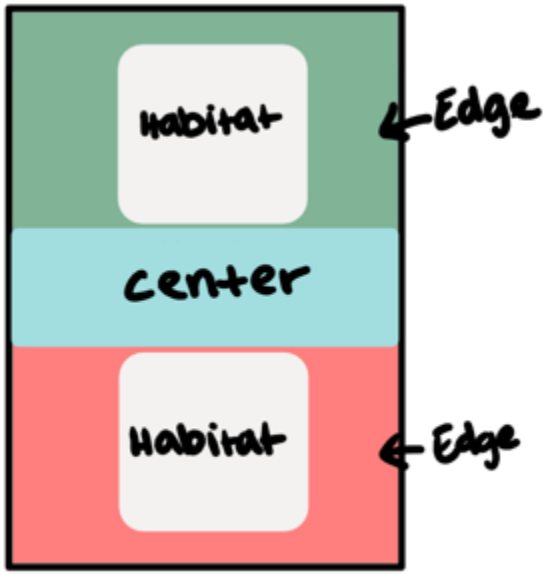

Figure 4: Example of how habitats were set up in a single experimental tank.

Tank 5						
Crab #		OR-V		Middle	SAV-LD	
Date	Time	Edge	Inside		Inside	Edge
7/12	12:30 PM				O	
Temp	12:45 PM				O	
26.9	1:00 PM				O	
	1:15 PM				O	
Saline	1:30 PM				O	
10.1	1:45 PM	X				
	2:00 PM				O	

Figure 5: Example of recorded data from one experimental trial. The circle markers indicate active eating behaviors. The X markers indicate eating behaviors are absent.

Data Analysis

During each experimental replicate, we recorded the tank's habitat combinations, crab ID (including sex and carapace width), habitat choice, whether or not habitat change occurred, position within habitat (edge/inside/center) (Figure 5), location of food, whether or not the crab was in food, and whether or not the crab was eating. The number of times a crab's position was documented in each of the habitats within each pair, within a replicate part (A or B) was summarized as the response variable, hereafter habitat count.

Mean habitat count were first compared across tanks using a one-way ANOVA. If there was no significant tank effect, the means of habitat count were compared across experimental replicate parts (a combination of day and part A and B) using a second one-way ANOVA. If there were no tank or replicate part effects, then differences in mean habitat count (response variable) were examined with a 2-way ANOVA that included habitats and whether or not the crabs were in the habitat with food (hereafter "food") as the main effects, and their interaction. Where assumptions of ANOVA (homogeneity of variance, independent observations, and normally distributed residuals) were not met, non-parametric Kruskal-Wallis Tests were conducted. If this occurred for the 2-way ANOVA (comparing mean habitat count by habitat, food, and their interaction), two separate Kruskal Wallis tests were run, one to examine the effect of being in the habitat with food on the mean habitat count, and the other to examine the effect of habitat on the mean habitat count.

Results

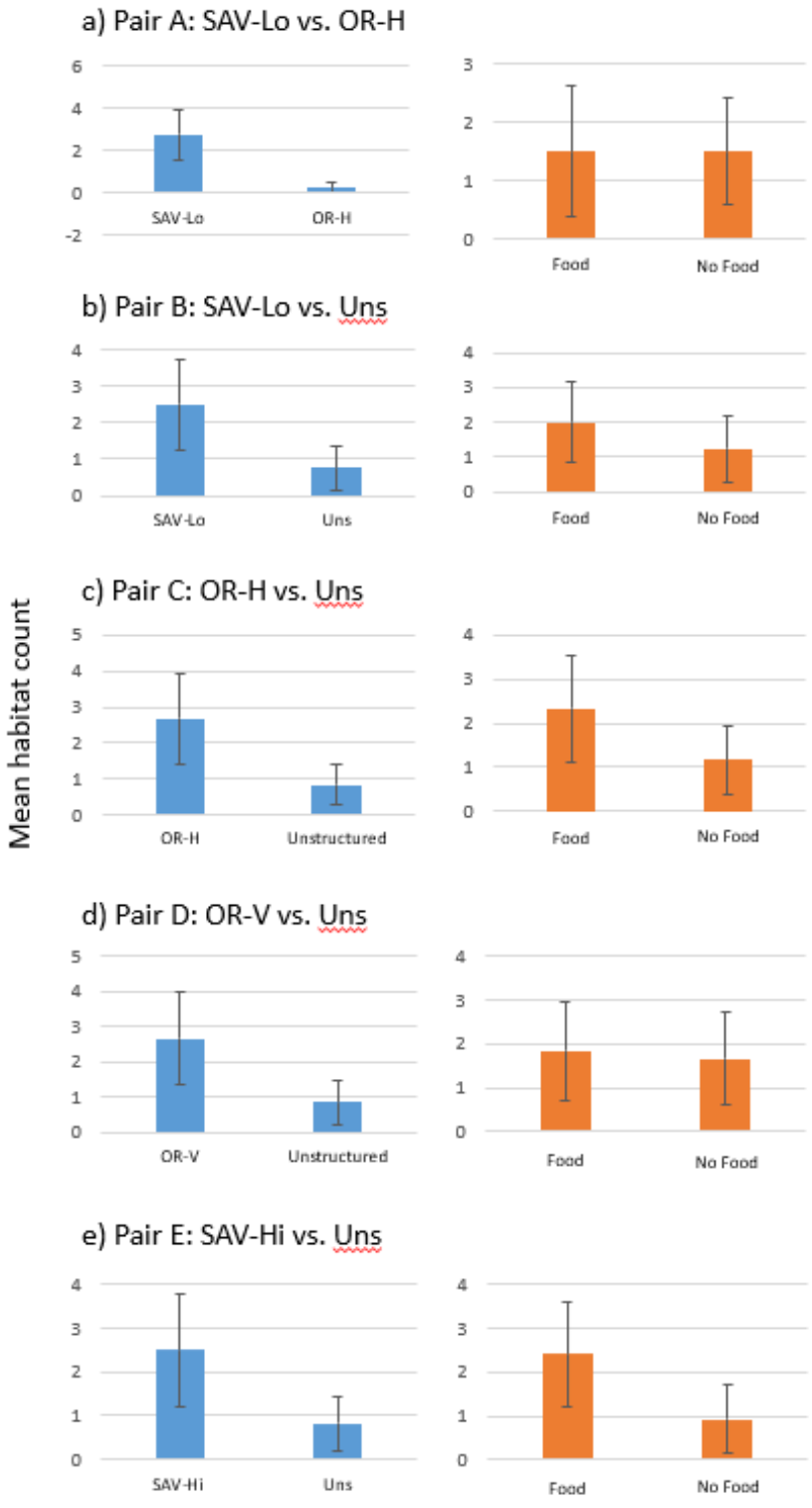

Figure 6: Number of times juvenile Blue crabs chose habitats among pairs A) SAV-Lo vs. OR-H, B) SAV-Lo vs. Unstructured, etc. among 6 replicates per pair. Also shown for each habitat pair is the mean occurrence of juvenile Blue crabs found in habitats containing or not containing food.

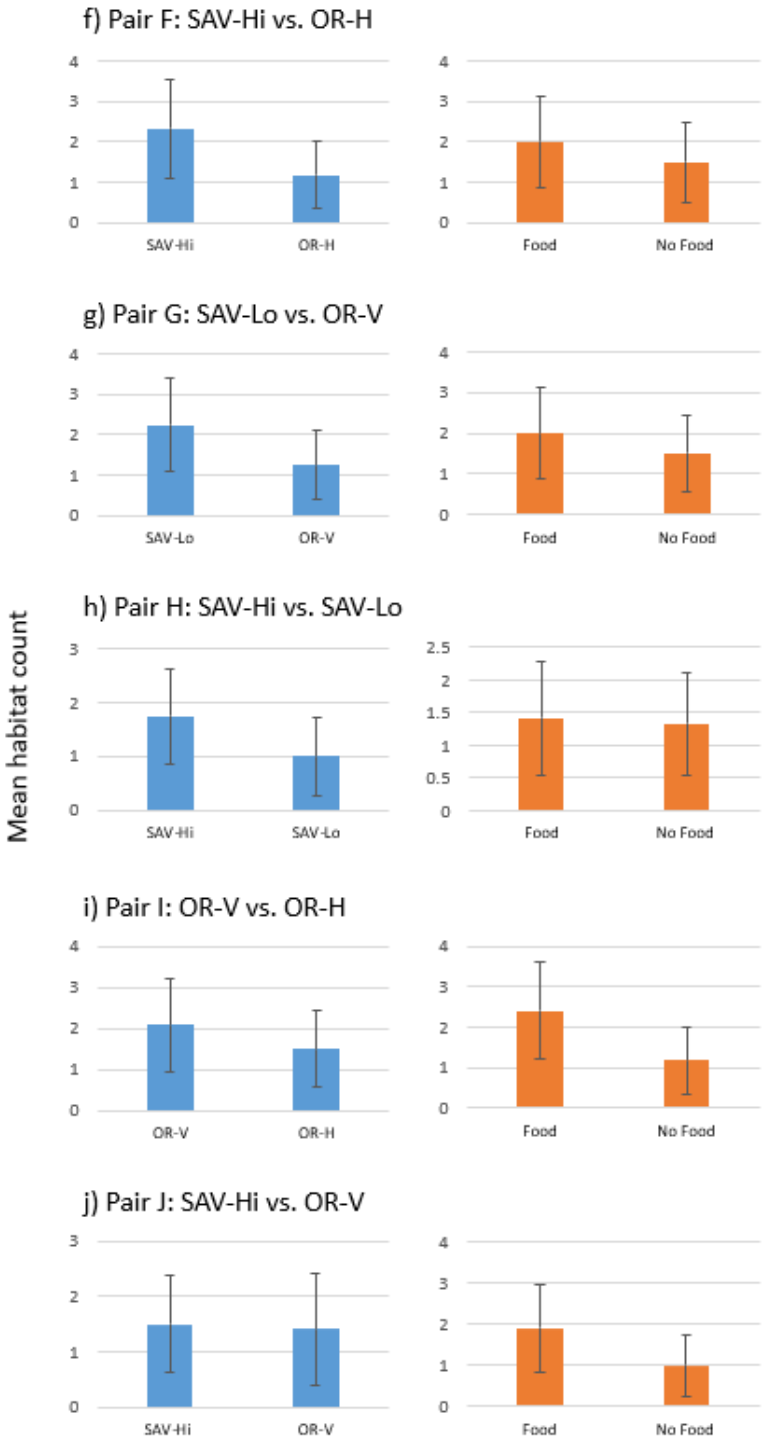

Figure 7: Number of times juvenile Blue crabs chose habitats among pairs F) SAV-Hi vs. OR-H, G) SAV-Lo vs. OR-V, etc. among 6 replicates per pair. Also shown for each habitat pair is the mean occurrence of juvenile Blue crabs found in habitats containing or not containing food.

Table 1: Tables displaying results of statistical tests carried out on each habitat pair. Parameters measured for each pair include differences measured among habitat preference, occurrence in habitats containing food or not, as well as potential habitat + food interactions. No statistically significant interactions were found between habitat and food variables.

OR-H vs. SAV-Hi			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	2.2	0.176
Food	1	0.40	0.543
Habitat x Food	1	0.045	0.837

OR-H vs. SAV-Lo			
	Habitat	Food	
Chi-Squared	8.67	0.00669	
DF	1	1	
P-Value	0.00323	0.9348	

SAV-Hi vs. Uns			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	11	0.0103
Food	1	9.0	0.0171
Habitat x Food	1	0.11	0.748

OR-V vs. SAV-Hi			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	0.013	0.913
Food	1	1.5	0.251
Habitat x Food	1	0.62	0.454

OR-V vs. SAV-Lo			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	1.7	0.227
Food	1	0.43	0.531
Habitat x Food	1	0.00	1.00

SAV-Hi vs. SAV-Lo			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	1.7	0.235
Food	1	0.020	0.890
Habitat x Food	1	1.7	0.235

SAV-Lo vs. Uns			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	6.9	0.0304
Food	1	1.3	0.293
Habitat x Food	1	0.016	0.904

OR-H vs. Uns			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	27	0.000837
Food	1	11	0.0109
Habitat x Food	1	0.22	0.650

OR-H vs. OR-V			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	0.68	0.433
Food	1	3.1	0.115
Habitat x Food	1	0.014	0.909

OR-V vs. Uns			
Factor	Degrees of Freedom	F-Value	P-Value
Habitat	1	6.7	0.0320
Food	1	0.056	0.820
Habitat x Food	1	0.056	0.820

In Habitat Pair A (OR-H vs. SAV-Lo), crabs were found in the SAV-Lo habitat 11 times as often as in the OR-H habitat (Figure 6). This difference was determined to be statistically significant (Table 1). Crabs were found an equal amount of times in both the habitat containing food and the habitat not containing food (Figure 6).

In Habitat Pair B (SAV-Lo vs. Uns.), crabs were found in the SAV-Lo habitat 3.33 times as often as in the unstructured habitat (Figure 6). This difference was determined to be statistically significant (Table 1). The mean occurrence of crabs in the habitat with food was determined to be 60% larger than the mean occurrence of crabs in the habitat without food (Figure 6). However, this difference was determined to not be statistically significant (Table 1).

In Habitat Pair C (OR-H vs. Uns.), crabs were found in the OR-H habitat 3.2 times as often as in the unstructured habitat (Figure 6). This difference was determined to be statistically significant (Table 1). Crabs were found in the habitat containing food 2 times more often than in the habitat not containing food (Figure 6). This difference was determined to be statistically significant (Table 1).

In Habitat Pair D (OR-V vs. Uns.), crabs were found in the OR-V habitat 3.2 times as often as in the unstructured habitat (Figure 6). This difference was determined to be statistically significant (Table 1). The

mean occurrence of crabs in the habitat containing the food was determined to be 10% larger than the mean occurrence of crabs in the habitat not containing the food (Figure 6). However, this difference was determined to not be statistically significant (Table 1).

In Habitat Pair E (SAV-Hi vs. Uns.), crabs were found in the SAV-Hi habitat 3 times as often as in the unstructured habitat (Figure 6). This difference was determined to be statistically significant (Table 1).Crabs were found in the habitat containing food 2.6 times as often as in the habitat not containing food (Figure 6). This difference was determined to be statistically significant (Table 1).

In Habitat Pair F (OR-H vs. SAV-Hi), crabs were found in the SAV-Hi habitat 2 times as often as in the OR-H habitat (Figure 7). However, this difference was determined to not be statistically significant (Table 1). The mean occurrence of crabs in the habitat containing food was 33% larger than the mean occurrence of crabs in the habitat not containing food (Figure 7). However, this difference was determined to not be statistically significant (Table 1).

In Habitat Pair G (OR-V vs. SAV-Lo), the mean occurrence of crabs in the SAV-Lo habitat was 80% larger than the mean occurrence of crabs in the OR-V habitat (Figure 7). However, this difference was determined to not be statistically significant (Table 1). The mean occurrence of crabs in the habitat containing food was 33% larger than the mean occurrence of crabs in the habitat not containing food (Figure 7). However, this difference was determined to not be statistically significant (Table 1).

In Habitat Pair H (SAV-Hi vs. SAV-Lo), the mean occurrence of crabs in the SAV-Hi habitat was 75% larger than the mean occurrence of crabs in the SAV-Lo habitat (Figure 7). However, this difference was determined to not be statistically significant (Table 1). The mean occurrence of crabs found in the habitat containing food was found to be 6.25% larger than the mean occurrence of crabs found in the habitat not containing food (Figure 7). However, this difference was determined to not be statistically significant (Table 1).

In Habitat Pair I (OR-H vs. OR-V), the mean occurrence of crabs in the OR-V habitat was 39% larger than the mean occurrence of crabs in the OR-H habitat (Figure 7). However, this difference was determined to not be statistically significant (Table 1). Crabs were found in the habitat containing food 2.1 times as often as the habitat not containing food (Figure 7). However, this difference was determined to not be statistically significant (Table 1).

In Habitat Pair J (OR-V vs. SAV-Hi), the mean occurrence of crabs in the SAV-Hi habitat was 6% larger than the mean occurrence of crabs in the OR-V habitat (Figure 7). However, this difference was determined to not be statistically significant (Table 1). The mean occurrence of crabs found in the habitat containing food was determined to be 91.7% larger than the mean occurrence of crabs found in the habitat not containing food (Figure 7). However, this difference was determined to not be statistically significant (Table 1).

Discussion

Unstructured habitats were least preferred by juvenile Blue crabs over the course of the experiment. This was expected, as oyster reefs and submerged aquatic vegetation provide more heterogenous habitat than unstructured habitat. This parallels previous research, such as that done by Moksnes & Heck (2006) who found that young Blue crabs actively avoid bare mud habitats in favor of more three-dimensional, structured varieties. Heck Jr. et. al. (2001) suggests predation as a key limiting factor in juvenile Blue crab recruitment, especially in

low latitudes. Therefore, the life history strategy of the Blue crab likely evolved to take advantage of these structured habitats in order to increase odds of survival, brought on by better cover from predators and increased opportunities for forage.

In general, juvenile Blue crabs associated with submerged aquatic vegetation habitats more frequently than unstructured habitats or oyster reef habitats, both vertical and horizontal in nature. One potential piece of reasoning for this trend lies in preexisting communities developed in collected submerged aquatic vegetation. Since both species of submerged aquatic vegetation were collected relatively recently before experiments were ran (approximately 1-2 weeks), organisms such as epiphytic plants, biofilms, and subsequently micro and macroinvertebrates may have been located on the stems of plants. The oyster reefs, on the other hand, were weathered and dried outside for long periods of time before the experiment, likely removing any existing aquatic communities already found on them. This potential addition of a new, unaccounted for food variable may have influenced crab behavior towards submerged aquatic vegetation.

Despite more scarce data than that used to induct the conclusions above, when the two species of SAV were compared head-to-head, it was found that a slight trend in habitat usage was evident towards *Ruppia maritima.* This was an unexpected find, as *Myriophyllum spicatum* is generally a more robust plant than *Ruppia maritima,* with an overall stockier build and robust, feathery, whorled leaves. It was thought that this thicker, more heterogenous structure may provide more ideal cover for the crabs. However, maybe due to its native status, perhaps *Ruppia maritima* offers more diverse biotic benefits to juvenile Blue crabs (Valinoti et. al., 2011). In fact, Valinoti et. al. 2011 suggested that species of *Palaemonetes* prefer *Ruppia maritima* for grazing purposes when compared to *Myriopyllum spicatum.* This perhaps acts as a starting place for our theory of more diverse food options for juvenile Blue crabs brought on by more diverse invertebrate and epiphytic plant assemblages. However, this comparison between species should be studied more in future experiments due to the relative scarcity of data in this experiment. Despite similar data scarcity, it was also hinted that juvenile Blue crabs preferred vertically oriented oyster reefs more than those that were horizontally oriented. This preference could have been brought on by the benefits that arise from more complex geometry (La Peyre et. al., 2019). However, this is a comparison that should also be studied more in future experiments due to the relative scarcity of data in this experiment.

During analysis of the experimental data, only 2 statistically significant cases of food effects were identified. This observation ran very contrary to our expectations. It was expected that crabs would have a strong preference for habitats with food present, as the crabs were starved for 1 day before each experimental replicate. However, this was not the case. Two potential explanations have arisen from this observation. The first potential explanation is that the crabs were simply not starved enough before the experiment and therefore did not have an adequate appetite to show strong preference between habitats with food vs. without food. During the initial storage phase before the experimental replicates were carried out, crabs were fed every 3 days, however, mortality became evident during that time period, so crabs were then fed every day before the 1 day starvation period prior to each experimental replicate. For future studies, careful consideration must be taken to effectively figure out how long crabs should be starved before experiments. Factors such as water temperature and nutritional value of food are likely to be important in this determination. Further studies could also be carried out with different types of food. For instance, in this particular study, chicken gizzards were used as the chosen diet for crabs. A more natural substitute that crabs are more likely to experience in the natural environment may be a better option for future diet and habitat studies. The other potential explanation proposes that crabs had a very strong habitat preference, so strong that it masked any possible food effects present. This is also possible, perhaps exacerbated by the relatively few experimental replicates conducted.

Some logistical challenges were encountered over the course of the experiment. Crab mortality was gradually seen over the course of the study, and out of the crabs that did not die, some experienced molting resulting in soft shells and paper shells not conducive for handling and transport to experimental tanks. Because of this slow decline in the number of available experimental crabs, only 3 replicates were able to be run. For the sake of statistical testing and accuracy/integrity of statistical analysis, more replicates should be run in future experiments. While SAV and oyster habitat units were standardized via water displacement, stem length, number of stem clusters per unit, etc., more refined methods that exhibit a higher level of both accuracy and precision will likely result in more reliable data. Use of laboratory-grade analytical equipment could be used to enact high-precision standardization in habitat volume.

In order to fully understand the habitat selection behaviors of juvenile Blue crabs in future laboratory studies, further water quality manipulations could be made in order to more fully encapsulate conditions juvenile Blue crabs are likely to encounter in their estuarine nursery grounds. For instance, in this study, water kept in experimental tanks consisted of dechlorinated municipal water with a marine mix of salt added until salinity reached approximately 10 PSU. No significant amount of fine substrate or suspended solids were intentionally added to the systems. Due to this lack of suspended solids, water was kept very clear, and therefore, it likely was not completely representative of coastal estuaries in Louisiana and beyond. Suspended solids, both biogenic and abiogenic, could be added to municipally-sourced water in future studies, or water sourced directly from coastal estuaries could be used to further explore the effects of physical water characteristics on juvenile crab behavior. A second water quality parameter that could be manipulated in future studies is water temperature. Manipulating water temperature throughout different experimental runs may provide valuable insight into how juvenile Blue crabs select habitat in varying seasons characteristic of temperate climates found in the Southeastern and Mid-Atlantic regions of the US.

While scientific literature suggests that juvenile Blue crabs may be more likely to be associated with submerged aquatic vegetation than emergent vegetation characteristic of coastal marshes, research suggests that coastal marshes are still likely important nursery habitats for juvenile Blue crabs (Heck Jr. et. al., 2001). Further habitat studies could incorporate emergent plant species characteristic of coastal wetlands, such as *Avicennia germinans*, *Sporobolus alterniflorus*, and *Juncus roemerianus*, into their experiments. While perhaps not especially useful to management in microtidal environments, data from these experiments would likely be especially valuable for management of Blue crab habitat in areas susceptible to flood tide events.

Overall, while fisheries management and ecosystem management are complex fields with lots of management angles and requirements, management for juvenile Blue crabs should take into account estuarine habitats, and specifically, structured habitat and their respective forage availability within estuaries. In Louisiana, climate change & sea level rise as well as unprecedented coastal restoration efforts will likely dramatically change both biotic and abiotic estuarine conditions (CPRA, 2023). Therefore, managing habitats and forage together into the survival and recruitment of juvenile Blue crabs will be a critical step in managing both the fishery and ecosystem function. Restoring the diversity of oyster reefs and submerged aquatic vegetation will likely be very important for the future of *Callinectes sapidus* and the locals and industries reliant upon them.

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