

HABITAT PREFERENCE OF THE DAGGERBLADE GRASS SHRIMP
PALAEMONETES PUGIO AND WHETHER FIELD PREFERENCE IS
CORRELATED WITH THE TREMATODE PARASITE *MICROPHALLUS TURGIDUS*

by

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DEDICATION

This thesis is dedicated to all those that believe in the power of science. To my mother, Samantha Stout, thank you for encouraging me to keep going even when I thought I couldn't. Thank you to the rest of my family, especially Auntie Al and Uncle Jer, for listening to me talk about the "sea monkeys" and their parasites. To my thesis advisors Dr. Carla Curran and Dr. Michele Guidone, thank you for your continual patience and guidance, I know I was difficult at times but you kept pushing me to do my best. To my undergraduate advisor Dr. Matt Draud, you were the person that inspired me to study marine biology and the person that encouraged me to apply for graduate school. Thank you to those that always believed that I could do this, it wasn't easy, but if it were then everyone would do it.

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ABSTRACT

Daggerblade grass shrimp *Palaemonetes pugio* reside in brackish estuarine systems and are thus exposed to the parasitic trematode *Microphallus turgidus*. The purpose of this study was to determine if the infection of *P. pugio* by *M. turgidus* was different across months and between two sites near Savannah, GA, and to determine habitat preference of infected *P. pugio* between the invasive red alga *Gracilaria vermiculophylla* and substrate-free open space in the laboratory. Trematode density was significantly higher in October-December 2016 and February 2017 (11.0-13.3) at one site and was significantly higher December 2016-January 2017 (10.1-10.3) and August-October 2017 (10.8-12.6) at the other site. In the laboratory, 76% of shrimp preferred tank edge locations whereas only 20% preferred *G. vermiculophylla*. Only 4% consistently selected the substrate-free open space. A wide range of factors such as food availability, presence of predators, interspecies competition, and abiotic conditions perhaps influence changes in parasite density and habitat preference.

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Chapter 1

Introduction and Overview

Crustaceans that select for habitat perhaps do so because of the physical features of that habitat. Alternatively, selection may not be because of the habitat itself but the influence of abiotic factors, alteration of native habitats, predation, interspecies interactions, food availability, or changes in host behavior by parasites. Abiotic factors such as salinity and temperature could be a driving force for habitat selection. Interspecies competition might also be a driving factor, as one species forces the other out of a common habitat into a new area. Crustaceans may use habitat to escape from predators or as foraging grounds. Invasive species that provide substrate could perhaps alter the utilization of a native habitat merely because of the physical presence of the new substrate. It is not well documented if invasive substrates alter the habitat selection behavior of grass shrimp, but it is known that parasites do change the behavior of grass shrimp hosts by altering activity levels, specifically in the presence of predators. It is possible that both parasitic infection of a host and the addition of an invasive substrate to new habitat could influence overall community dynamics.

Benthic crustaceans within the western Atlantic are capable of preferentially selecting for habitat (Herrnkind and Butler, 1986; Ramach et al., 2009). Juvenile and peuruli larvae of the spiny lobster *Panulirus argus* preferred the red algae *Laurencia* spp. compared to the seagrass *Thalassia testudinum* during habitat selection and settling experiments in the laboratory (Herrnkind and Butler, 1986). Peuruli settlement within *Laurencia* spp. was not because of food availability but because of the dark structural complexity of *Laurencia* spp. (Herrnkind and Butler, 1986). In contrast, juvenile *P. argus* only preferred *Laurencia* spp. because of the prey within it (Herrnkind and Butler, 1986). Adult male and female blue crabs *Callinectes sapidus* utilize different habitat depending

on tidal stage during nocturnal recapture studies in an embayment near Beaufort, NC (Ramach et al., 2009). Oviparous female crabs with late-egg stages were found at significantly deeper depths than females with early-egg stages (Ramach et al., 2009). Crabs with late-egg stages were transported closer to the entrance of the embayment during ebb tide just before larvae were released (Ramach et al., 2009). Molting crabs near the marsh edges also utilize shallow habitats where the smooth cordgrass *Spartina alterniflora* is found (Wolcott and Hines, 1990). Other crustaceans such as the slender sargassum shrimp *Latreutes fucorum* and the cerulean sargassum shrimp *Hippolyte curacaoensis* select structure such as eelgrass *Thalassia testudinum* during the night likely to avoid predation (Bauer, 1985). The daggerblade grass shrimp *Palaemonetes pugio* has a preference for marsh edge where *S. alterniflora* is abundant compared to an open water habitat near Galveston, Texas (Minello et al., 1994).

Palaemonetes pugio is a crustacean that inhabits submerged vegetation and hard bottom substrates such as oyster reefs near and around shallow marsh habitats (Heard, 1982) and is located along the southeastern coast of the United States (Kneib, 1987) and the Gulf of Mexico (Anderson, 1985). The most dominant vegetation along the marshes of the Atlantic and Gulf coasts is the smooth cordgrass *Spartina alterniflora* (Heard, 1982). Modeste (2009) observed a significant increase in the density of *P. pugio* near the edge of two tidal creeks (29.1 ± 6.32 shrimp/m³) and (10.8 ± 2.55 shrimp/m³) compared to the respective values farther from the edge (8.4 ± 2.26 shrimp/m³) and (4.5 ± 1.34 shrimp/m³), respectively. Female shrimp density was also higher near the edge (18.2 ± 3.79 shrimp) and (6.8 ± 1.76 shrimp) than males (10.9 ± 2.97 shrimp) and (3.9 ± 1.06 shrimp) at the two tidal creeks, respectively (Modeste, 2009). The higher number of *P.*

pugio near the marsh edge is perhaps due to the availability of refuge such as vegetation, oysters, and man-made structures near Savannah, GA (Modeste, 2009). Near New Jersey, when *P. pugio* was given a choice between *S. alterniflora* and the non-native reed *Phragmites australis* within the estuary, *P. pugio* preferred *S. alterniflora* as habitat (Robertson and Weis, 2007). This preference may be associated with the number of algal epiphytes and other food items on the stems (Robertson and Weis, 2007). Nematodes consisted of 82% of the epifauna on the stems; of those, 20 animals/stem over an 18 h time period were consumed by *P. pugio* compared to the 6 animals/stems on *P. australis* (Robertson and Weis, 2007).

The common grass shrimp *Palaemonetes vulgaris* and *Palaemonetes pugio* co-occur in dynamic environments; however, *P. pugio* is physiologically able to inhabit harsh abiotic areas (Rowe, 2002). *Palaemonetes pugio* tolerates a large range in salinity from less than 1 to greater than 30 ppt whereas *P. vulgaris* is more sensitive to salinity when it decreases below 10 ppt (Heard, 1982) but still tolerates salinities between 1-55 ppt (Anderson, 1985). Energy expenditure of *P. pugio* and *P. vulgaris* was lower at a salinity of 10 ppt than at 0.5 and 20 ppt in the laboratory (Rowe, 2002). *Palaemonetes pugio* was able to survive at the rate of 75% in a stressful salinity concentration at 0.5 ppt, which was higher than the survival rate of *P. vulgaris* at 25%. The ability of *P. pugio* to survive in this condition may be beneficial during habitat competition with *P. vulgaris* because *P. pugio* is able to move to different habitats where *P. vulgaris* is limited (Rowe, 2002). The survival of *P. pugio* and *P. vulgaris* is different as oxygen concentrations drop below 1.5 mg/l in the laboratory (Welsh, 1975). *Palaemonetes pugio* reduces its oxygen uptake when oxygen becomes depleted (Welsh, 1975). Also, a greater number of *P.*

pugio survive in low oxygen levels compared to *P. vulgaris* in caging experiments within an embayment (Welsh, 1975). Both species may be able to acclimate using alternative metabolic pathways in response to low oxygen concentrations (Welsh, 1975).

In the Gulf of Mexico, *Palaemonetes pugio* begins spawning in March and continues until October while *Palaemonetes vulgaris* begins spawning a month later in April and continues to October (Anderson, 1985). Those *P. pugio* that overwinter spawn at the beginning of the following year and die later the same year (Anderson, 1985). The reproductive season of *P. pugio* in South Carolina began later in April and ended earlier in August at 1 site and September at another site (Chaplin-Ebanks and Curran, 2007). During another study in South Carolina, it was estimated that the life span of *P. pugio* ranged between 6 and 13 months (Alon and Stancyk, 1982).

Palaemonetes pugio is an important crustacean in brackish estuarine systems (Kneib, 1987) as it facilitates the transfer of energy and nutrients along the food web from the producer and decomposer levels to higher trophic levels through their role as prey to the higher trophic levels within the estuary (Anderson, 1985). The predators of *P. pugio* include many fish, other crustaceans, and birds. Some finfish predators include the mummichog *Fundulus heteroclitus*, white perch *Morone americana*, striped bass *Morone saxatilis*, and the commercially important Atlantic croaker *Micropogonias undulatus* (Clark et al., 2003). The summer flounder *Paralichthys dentatus* preys upon *Palaemonetes* spp. in deep water habitats eliminating the need for *P. dentatus* to forage in shallow waters with high temperatures and intense light levels (Manderson et al., 2004). In addition, the blue crab *Callinectes sapidus* consumes species within the genus *Palaemonetes* in Florida estuaries (Laughin, 1982). *Palaemonetes pugio* is also prey for

other types of shrimp, such as the commercially important white shrimp *Litopenaeus setiferus* (Kneib and Knowlton, 1995). The red-breasted merganser *Mergus serrator* and the clapper rail *Rallus crepitans* are types of birds that consume *P. pugio* in Mississippi (Heard and Overstreet, 1983).

Palaemonetes pugio preys on benthic organisms living on and within vegetation. *Palaemonetes pugio* consumes animals such as rotifers (Morgan, 1980), nematodes (Morgan, 1980; Robertson and Weis, 2007), harpacticoid copepods (Robertson and Weis, 2007), protozoans (Morgan, 1980) and microscopic algae (Morgan, 1980; Fleeger et al., 1999). Predation rates of meiofauna by *P. pugio* on the stems of *Spartina alterniflora* were not similar between laboratory and field experiments in a Louisiana salt marsh (Gregg and Fleeger, 1998). *Palaemonetes pugio* did not significantly reduce meiofauna such as copepods, nematodes, and polychaetes on the stems of *S. alterniflora* or infauna during caging experiments but significantly reduced meiofauna (35 meiofauna/h) on the stems of *S. alterniflora* in the laboratory (Gregg and Fleeger, 1998). Lower predation rates of meiofauna on stems in the field could be due to the consumption of other food items such as detritus, small macrofauna, and algae by *P. pugio*, which were also present in the cages (Gregg and Fleeger, 1998). In addition, meiofaunal settlement may have increased within intertidal cages due to changes in water flow (Gregg and Fleeger, 1998). Shrimp within cages were perhaps exposed to environmental factors that could have caused a decrease in predation success (Gregg and Fleeger, 1998).

The red alga *Gracilaria vermiculophylla* is native to the northwest Pacific (Ohmi, 1956; Rueness, 2005) and has spread to the northeast Pacific including British Columbia (Saunders, 2009), and the Atlantic coast of North America including: New England

(Nettleton et al., 2013), Virginia (Thomsen and McGlathery, 2007), North Carolina (Freshwater et al., 2006), South Carolina (Byers et al., 2012) and Georgia (Byers et al., 2012; Wright et al., 2014). *Palaemonetes pugio* inhabits the areas where invasion of *G. vermiculophylla* is observed. The initial invasion date of *G. vermiculophylla* is unknown (Thomsen et al., 2009), but the existence of *G. vermiculophylla* in shallow estuarine systems is important as it provides a large coverage of macrophytic structure in areas that previously lacked vegetation (Byers et al., 2012). *Gracilaria vermiculophylla* attaches to hard substrates such as oysters and man-made objects such as clay bricks (Thomsen et al., 2007) and also the soft tubes of the polychaete tubeworm *Diopatra cuprea* (Byers et al., 2012; Zi-Min and Lopez-Bautista, 2014). The accumulation of this alga on mudflats appeared to alter the distribution and abundance of native amphipods near Savannah, GA (Wright et al., 2014), Virginia, and Denmark and also altered gastropod abundance in Sweden (Nyberg et al., 2009). Rapid reproduction of *G. vermiculophylla* is observed in Georgia and South Carolina, despite the inability of *G. vermiculophylla* to attach to soft substrates (Byers et al., 2012). Density and tube height of *D. cuprea* had a significant positive effect on the percent coverage of *G. vermiculophylla* (Byers et al., 2012). The attachment of *G. vermiculophylla* to the tubes of *D. cuprea* increased the biomass of *G. vermiculophylla* because the tubes acted as an anchor that attached the stipe of *G. vermiculophylla* to the soft muddy substrates in Georgia (Zi-Min and Lopez-Bautista, 2014). The addition of *G. vermiculophylla* may increase primary productivity and could be an important contributor to detrital foodwebs because of rapid decomposition (Byers et al., 2012). The biomass of *G. vermiculophylla* had a significant positive effect on the abundance of epifauna, which consisted of 71% amphipods and 10% gastropods living in

two sites near Savannah, GA and one site near Hilton Head, SC (Byers et al., 2012). *Gracilaria vermiculophylla* was effective at reducing the ability of the common grass shrimp *Palaemonetes vulgaris* to forage on the amphipods as it increased the survival of amphipods from 0% without *G. vermiculophylla* to $53.8 \pm 10.2\%$ with algae present in Savannah, GA (Wright et al., 2014).

Palaemonetes pugio uses habitat for refuge as a type of predator avoidance behavior depending on the presence or activities displayed by their predators (Sogard and Able, 1994; Clark et al., 2003; Canion and Heck, 2009). *Palaemonetes pugio* commonly inhabits seagrass beds and is an important link as it is a prey source for larger predators and is itself a detritivore that recycles the nutrients within the beds (Canion and Heck, 2009). It was once thought that habitat complexity, such as increased density of seagrass beds, increased the survival of prey (Mattila et al., 2008). However, this current paradigm was challenged by Mattila et al. (2008). They found new evidence about the role of different densities of artificial turtlegrass *Thalassia testudinum* as shelter in the laboratory on the survival of *P. pugio* in the presence of the pinfish *Lagodon rhomboides*. Higher densities of *T. testudinum* did not increase prey survival as previously documented. The predator-prey ratio was kept constant in relation to the surface area of the seagrass and tanks (Mattila et al., 2008). There was no difference in the survival of *P. pugio* as *T. testudinum* increased in density. The mean for the shrimp survival was $56.3 \pm 18.3\%$ (Mattila et al., 2008). The intermediate and high seagrass density treatments varied significantly from the sand treatment but not the low density treatment (Mattila et al., 2008). Although predation rates did not decrease as seagrass complexity increased, more prey survived in seagrass treatments than sand treatments (Mattila et al., 2008). In

previous experiments, the predator-prey ratio failed to increase as density and surface area of the seagrass beds increased, which indicated that predator-prey interactions would have been less than observed in natural settings (Mattila et al., 2008). Canion and Heck (2009) performed a field mesocosm experiment in which they increased the predator-prey ratio with density of *T. testudinum*. The survival of *P. pugio* was the same throughout all densities of *T. testudinum* even when prey and seagrass density were increased (Canion and Heck, 2009). Additionally, they used sand as a substrate and found that *L. rhomboides* consumed 83% of *P. pugio*, which was expected because there were no physical barriers to impair the vision of the predator (Canion and Heck, 2009). The sand treatment was significantly different from all three densities of *T. testudinum*. Only 27% of the shrimp were consumed in the low density treatment. This indicated that all densities of seagrass provided some refuge for prey (Canion and Heck, 2009). Heck et al. (2003) found that increased survival of prey in seagrass versus no vegetation is simply due to the effect of structure itself rather than the composition of the seagrass. Other aquatic habitats such as salt marshes and oyster reefs also offer protection (Minello et al., 1994; Heck et al., 2003). Furthermore, it is known that *Gracilaria vermiculophylla* increases the survival of organisms such as amphipods (Wright et al., 2014). The addition of *G. vermiculophylla* as a habitat may be beneficial for the common prey items of *P. pugio* and could prevent *P. pugio* from foraging successfully.

The interaction between organisms and different habitats is complex (Heck and Wetstone, 1977). Habitat heterogeneity was measured by comparing aboveground plant biomass and the number of species associated with the biomass (Heck and Wetstone, 1977). Plant biomass was strongly correlated and significantly related to the abundance

and number of species of invertebrates. However, the number of plant species is not an accurate representation of habitat complexity as these invertebrate species do not need one specific plant species but many types of vegetation containing a mixed composition of other invertebrates (Heck and Wetstone, 1977). If invertebrates specialized on one plant species over the other, the number of plant species would be an indicator of habitat complexity (Heck and Wetstone, 1977). Plant biomass might accurately reflect structural complexity because their generally compact leaves could provide higher food levels attracting more individuals as well as a larger living space for more species to inhabit (Heck and Wetstone, 1977). The increased biomass could also provide additional habitats for invertebrates such as the banded snapping shrimp *Alpheus armillatus* and *Alpheus viridiri* as the calcareous green algae *Halimeda optunia* and *Thalassia testudinum* provide increased shelter because of branches and dense leaves (Heck and Wetstone, 1977). Plant surface area has a positive effect on epifaunal density (Parker et al., 2001). The amphipod *Cymadusa compta* and the isopod *Erichsonella attenuata* were extremely abundant on the foliose green alga *Ulva* sp. and the branched red alga *Gracilaria verrucosa* during field studies near the subestuary of Chesapeake Bay, Virginia (Parker et al., 2001). *Gracilaria verrucosa* significantly enhanced the abundance of these epifauna, perhaps because of its structural complexity (Parker et al., 2001).

Palaemonetes pugio inhabits marsh areas and is thus exposed to parasites (Heard and Overstreet, 1983) including the bopyrid isopod *Probopyrus pandalicola* (Anderson, 1977; Heard, 1982), the microphallid trematode *Microphallus turgidus* (Heard and Overstreet, 1983; Pung et al., 2002), and the haplosporidian *Urosporidium crescens* (Sprague, 1970; Pung et al., 2002). The life cycle of *P. pandalicola* involves two hosts:

the planktonic copepod *Arcatia tonsa* and *P. pugio* (Heard, 1982). The epicaridean larvae of the bopyrid first attach to the copepod, which then mature to larger crytoniscus larvae (Heard, 1982). *Palaemonetes pugio* becomes infected by *Probopyrus pandalicola* when the crytoniscus larvae of the bopyrid attaches to the branchial chamber of *P. pugio* (Anderson, 1977; Heard, 1982). The bopyrid matures into an adult female when it migrates into the branchial chamber shortly followed by the attachment of a dwarf male (Heard, 1982). In general, no more than 1% of the adult population of *P. pugio* actually becomes infected by the isopod throughout the eastern coast of the United States and the Gulf of Mexico (Heard, 1982). However, infection rates were higher than 1% at two sites in both Georgia (3.1% and 2.0%) and South Carolina (1.8% and 1.3%) (Chaplin-Ebanks and Curran, 2007). The relationship between bopyrid prevalence and the gravidity of *P. pugio* was also examined (Chaplin-Ebanks and Curran, 2007). One South Carolina site had a higher prevalence of bopyrid infection during the reproductive season of *P. pugio* than in winter (Chaplin-Ebanks and Curran, 2007), but in Georgia, all sites had peak prevalence during the reproductive season (Chaplin-Ebanks and Curran, 2007). Peak gravidity during April and May corresponded with a 5.3% and 6.3% infection rate of the bopyrid, respectively (Chaplin-Ebanks and Curran, 2007). Sherman and Curran (2015) found that *P. pandalicola* sterilizes female grass shrimp. Sterilization occurs when the bopyrid derives energy from its grass shrimp host, which then prevents the shrimp from reproducing (Baudoin, 1975).

Trematode flatworms are an infective digenean parasite that can use up to four hosts to complete their life cycle, which generally begins with a mollusk as the first intermediate host and ends with a mammal as the definitive host (Shields and Overstreet,

2003). The digenean trematode *Microphallus basodactylophallus* is located in parts of Mississippi and Alabama and the southeastern coast of Florida spanning up to North Carolina (Heard and Overstreet, 1983). *Callinectes sapidus* is a common second intermediate host of *M. basodactylophallus* and becomes infected by free swimming cercariae larvae that attach to the branchial chamber after the gills are penetrated by the larvae (Shields and Overstreet, 2003). Death of *C. sapidus* is possible if infection of *M. basodactylophallus* is too high (Heard and Overstreet, 1983). The microphallid trematode *Microphallus turgidus* has a similar geographic range to that of *M. basodactylophallus* and is observed in marshes along the coast (Heard and Overstreet, 1983). *Microphallus turgidus* also transitions through multiple hosts such as a hydrobiid snail *Littoridinops monroensis* as the first intermediate host, *Palaemonetes pugio* as the second intermediate hosts, and *Mergus serrator* as the definitive host (Heard and Overstreet, 1983). The abdominal musculature of *P. pugio* commonly becomes infected by the larval stage of *M. turgidus* (Heard, 1982). The trematode penetrates through the gills and subsequently leaves cysts along the abdominal muscles (Heard and Overstreet, 1983). Larger *P. pugio* contain greater densities of the metacercarial cysts of *M. turgidus* than smaller shrimp (Pung et al., 2002). This indicates that density continues to increase as the length of *P. pugio* increases (Pung et al., 2002). These results suggest *M. turgidus* does not affect mortality rate as cysts accumulate within *P. pugio* (Pung et al., 2002). In the laboratory, *in vitro* excystation of the trematode cyst was greatest at 38°C and 7 ppt NaCl concentration and rapidly declined below 34°C (Khan et al., 2003). Maximum excystation rate in the laboratory occurred under similar conditions to that of the natural internal body conditions of the definitive prey that consumes *P. pugio* (Khan et al., 2003).

It is not understood if or how the reproduction of *P. pugio* is affected by the microphallid trematode (Pung et al., 2002).

The infection of *Microphallus turgidus* in *P. pugio* can affect host behavior by increasing activity levels (Kunz and Pung, 2004). The activities of *P. pugio* in the water column include normal movements such as swimming, walking, and hovering (Kunz and Pung, 2004). The frequency of these movements were altered in the presence of a predator when *P. pugio* was infected by *M. turgidus* (Kunz and Pung, 2004).

Palaemonetes pugio with a heavy trematode load of 30 or more cysts was consumed by *Fundulus heteroclitus* more than *P. pugio* without trematodes (Kunz and Pung, 2004). Infected *P. pugio* were more visible to *F. heteroclitus* through increased movements (Kunz and Pung, 2004). Movements of the shrimp may not be the only cause of increased visibility to its predators. There are other parasites that can increase the conspicuousness of its host (Holmes and Bethel, 1972). It is known that the ectoparasite *Probopyrus pandalicola* increases conspicuousness of the transparent shrimp body thus exposing the shrimp to its predators (Bass and Weis, 1999). It does so by removing the natural camouflage of *P. pugio* while significantly affecting behavior (Brinton and Curran, 2015). Parasitized shrimp do not exhibit a high percentage of backward thrusting, a normal anti-predation movement, but rather use a swimming movement that is more noticeable (Brinton and Curran, 2015). However, 100% of the parasitized shrimp that did backward thrust were consumed by predators, whereas only 85.3% of unparasitized shrimp were consumed (Brinton and Curran, 2015). Shrimp behavior was not affected during a predation event but immediately prior (Brinton and Curran, 2015). Shrimp significantly swam less when a predator was present (Brinton and Curran, 2015).

The trematodes of *Palaemonetes pugio* can become infected by a member of the phylum Haplosporidia *Urosporidium crescens* (Sprague, 1970). Haplosporidia contain protozoans that are spore forming (Shields and Overstreet, 2003) and are found along the Atlantic coast of North America and other places throughout the world (Perkins, 2000). The life cycle of *U. crescens* is not understood (Perkins, 2000). Some species of the genus *Urosporidium* are hyperparasites to digenean trematodes (Shields and Overstreet, 2003). For example, *U. crescens* parasitizes the metacercariae of the trematode *Microphallus basodactylophallus* in the blue crab *Callinectes sapidus* (Shields and Overstreet, 2003), and directly parasitizes the microphallid trematode *Microphallus turgidus* in *P. pugio* (Sprague, 1970; Pung et al., 2002). The haplosporidian does not harm the blue crab host, but is notably pathogenic to the trematode (Shields and Overstreet, 2003). When spores of the haplosporidian are produced, the trematode becomes encysted by the spores and become black, swollen (Shields and Overstreet, 2003; Sprague, 1970), and more visible to the naked eye (Shields and Overstreet, 2003). When spores of the haplosporidian are formed, energy is removed from the trematode, leaving it without reproductive abilities and causing paralysis once spores are excysted in the definitive host (Shields and Overstreet, 2003).

The purpose of the current study was to determine the monthly changes in parasite infection of *Palaemonetes pugio* and whether the location of *P. pugio* was correlated with the trematode parasite through field and laboratory studies. In chapter 2, prevalence, abundance, intensity, and density of *Microphallus turgidus* and *Urosporidium crescens* in *P. pugio* at two different study sites were documented. Monthly trends were examined as well as where shrimp were located. Shrimp could have

either been in sandy substrates or near the invasive red alga *Gracilaria vermiculophylla*.

The purpose of chapter 3 was to determine habitat preference of shrimp when given a choice between *G. vermiculophylla* and open space in the laboratory.

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Chapter 2

Monthly changes in the abundance, intensity, density, and prevalence of the microphallid trematode *Microphallus turgidus* and the haplosporidian hyperparasite *Urosporidium crescens* in the daggerblade grass shrimp *Palaemonetes pugio* and whether these parasites are correlated to where *P. pugio* is located in the environment

ABSTRACT

Shrimp that are near a habitat are perhaps influenced by behavioral changes as a result of parasitic infection. The daggerblade grass shrimp *Palaemonetes pugio* can be a host for many types of parasites including the microphallid trematode *Microphallus turgidus* and the haplosporidian hyperparasite *Urosporidium crescens*. The purpose of this study was to determine if prevalence (% of shrimp parasitized), abundance (# of trematodes in both uninfected and infected shrimp), intensity (# of trematodes per infected shrimp), and density (#/cm total length) of the microphallid trematode *Microphallus turgidus* changed monthly and if changes impacted whether *P. pugio* was located over as a sandy substrate or near rocks with the invasive red alga *Gracilaria vermiculophylla*. *Palaemonetes pugio* were sampled monthly at two sites near Savannah, GA using a drop trap on the sand and on the rocks with *G. vermiculophylla* (if present). Prevalence ranged from 84% to 100% at the Rodney J. Hall Boat Ramp and ranged from 69% to 100% at the Frank Downing Fishing Pier. There was no significant difference in prevalence between sites. Similarly, abundance and density were never significantly different between sites; however, intensity was significantly different between sites. Trematode density was significantly higher in October through December 2016 and February 2017 (11.0 ± 4.95 - 13.3 ± 7.05) than all other months at the Rodney J. Hall Boat Ramp and was significantly higher in December 2016 through January 2017 (10.1 ± 4.11 - 10.3 ± 3.89) and August through October 2017 (10.8 ± 5.49 - 12.6 ± 10.3) than all other months at the Frank Downing Fishing Pier. There was also a difference in parasite infection across months and with salinity. In addition, there was a significant difference in trematode density with shrimp length. Shrimp in the intermediate length category

(>2.9-3.2 cm) had a significantly higher trematode density (9.8 ± 8.14) than densities in the smaller (5.5 ± 5.85 to 7.7 ± 6.32) and larger (6.4 ± 4.80 to 6.7 ± 6.14) length categories, which was different than the previously reported finding of a positive relationship between trematode density and shrimp length. Fewer individuals were observed in the larger length categories perhaps because of altered growth rates from parasitic infection. As a result, this factor could affect predator-prey dynamics as smaller shrimp might be more susceptible to predation, which could impact overall shrimp population.

INTRODUCTION

Habitat preference is perhaps influenced by the complexities of parasite-host interactions. Parasites can alter the behavior of their host, which could affect habitat selection. Invasive substrates may also influence habitat preference just by their physical presence alone. Organisms may be redirected from their native habitat to the invasive habitat, thus altering density within those substrates, which could also alter predator-prey dynamics. Parasites may not only have an effect on the habitat preference of their host but could also alter host growth rate. Decreased growth could increase the likelihood of predation, perhaps altering the survival of an individual and ultimately decreasing population biomass.

Parasites in general are known to have various impacts on their hosts including altered survival (Scott, 1988) and reproduction (Baudoin, 1975; Scott, 1988). Some parasites may have pathological effects on their hosts, and others can indirectly increase the susceptibility of hosts to predators (Scott, 1988). The host population could be impacted if host survival and reproduction decreases because of infection (Scott, 1988).

For example, the ectoparasitic sea lice *Lepeophtheirus salmonis* can affect the survival of wild juvenile pink salmon *Oncorhynchus gorbuscha* after they migrate upstream (Krkošek et al., 2006). Farm-raised pink salmon that are upstream are already infected with the parasite, and as the wild salmon migrate through the farms, *L. salmonis* is transmitted to the wild juveniles resulting in host mortality (Krkošek et al., 2006) and can even eliminate entire populations of wild *O. gorbuscha* (Krkošek et al., 2007). Often parasites impact host reproduction and do so by castration (Baudoin, 1975). Trematode parasites that commonly infect the intertidal snail *Zeacumantus subcarinatus* completely replace host gonads, thereby reducing reproductive output in heavily infected individuals (Fredensborg et al., 2005).

Parasite-host interactions can result in altered host behavior such as disorientation, increased conspicuousness, and different activity levels (Holmes and Bethel, 1972). Parasitized killifish *Fundulus parvipinnis* infected with the trematode parasite *Euhaplorchis californiensis* exhibited conspicuous behaviors such as flashing, shimmying, contorting, and jerking more often than uninfected killifish when monitored in the laboratory (Lafferty and Morris, 1996). In field studies, more infected killifish were consumed than killifish without parasites, which supports the hypothesis that parasites modify host behavior to increase susceptibility to predators, thereby increasing transmission to definitive hosts (Lafferty and Morris, 1996). The bopyrid isopod *Probopyrus pandalicola* can infect the daggerblade grass shrimp *Palaemonetes pugio* (Anderson, 1977; Heard, 1982) and is known to increase conspicuousness of the transparent shrimp body, thus exposing the shrimp to its predators (Bass and Weis, 1999). It does so by decreasing the natural camouflage of *P. pugio* while significantly affecting

behavior (Brinton and Curran, 2015). Parasitized shrimp were observed to swim more (37.6% of the time) than unparasitized shrimp (21.2% of the time) immediately prior to consumption by the mummichog *Fundulus heteroclitus* (Brinton and Curran, 2015). Animals with parasites may also have altered movement patterns (Scott, 1988). The mud snail *Ilyanassa obsoleta* is commonly infected with the trematode parasite *Gynaecotyla adunca*, which causes the snails to move longer distances than those uninfected to higher sandbars and beaches, thus completely exposing the snails during nighttime low tides (Curtis, 1990). The parasite induces the snail to move farther inland so that the probability of transmission of the trematode cercariae increases to the semi-terrestrial crustacean second intermediate host, which is the beach hopper amphipods *Talorchestia longicoris* and *Talorchestia megalophthalmia*, and the fiddler crab *Uca pugilator* (Curtis, 1987; Curtis, 1990).

Palaemonetes pugio can be a host for other parasites such as the haplosporidian hyperparasite *Urosporidium crescens* (Sprague, 1970; Pung et al., 2002) and the microphallid trematode *Microphallus turgidus* (Heard and Overstreet, 1983; Pung et al., 2002). The abdominal musculature of *P. pugio* commonly becomes infected by the larval stage of *M. turgidus* (Heard, 1982). Larger *P. pugio* contained greater densities of the metacercarial cysts of *M. turgidus* than smaller shrimp during surveys from May 2000-March 2001 in Georgia (Pung et al., 2002). Density of the cysts continued to increase as the length of *P. pugio* increased (Pung et al., 2002; Sheehan et al., 2011). These results suggest *M. turgidus* does not affect mortality rate as cysts continue to accumulate (Pung et al., 2002). It is not understood if or how the reproduction of *P. pugio* is affected by the trematode (Pung et. al., 2002), but *M. turgidus* can affect the behavior of *P. pugio* (Pung

et al., 2002; Kunz and Pung, 2004). The frequency of normal movements of *P. pugio* such as swimming, walking, and hovering are increased in the presence of a predator when infected by *M. turgidus* (Kunz and Pung, 2004). Individuals with a heavy infection of 30 or more cysts were consumed by *Fundulus heteroclitus* more so than *P. pugio* without trematodes in the laboratory (Kunz and Pung, 2004). However, *P. pugio* parasitized by the bopyrid isopod *Probopyrus pandalicola* were captured less often by *F. heteroclitus* than uninfected shrimp (Bass and Weis, 1999), perhaps because infected *P. pugio* had reduced activity levels, which helped facilitate predator avoidance (Bass and Weis, 1999).

Although it is known that parasites alter activity levels of *Palaemonetes pugio* (Bass and Weis, 1999; Kunz and Pung, 2004), it is unknown if parasites affect habitat preference of *P. pugio*. *Palaemonetes pugio* inhabits submerged vegetation and hard bottom substrates such as oyster reefs near and around shallow marsh habitats (Heard, 1982). *Palaemonetes pugio* is located along southeastern coasts of the United States (Kneib, 1987) and the Gulf of Mexico (Anderson, 1985). The vegetation that is most dominant along the marshes of the Atlantic and Gulf coasts is the smooth cordgrass *Spartina alterniflora* (Heard, 1982). *Palaemonetes pugio* had a high density near the marsh edge where *S. alterniflora* is most abundant compared to an open water habitat in Halls Bay near Galveston, Texas (Minello et al., 1994). Modeste (2009) noted higher density of *P. pugio* near the edge of two tidal creeks (29.1 ± 6.32 shrimp/m³) and (10.8 ± 2.55 shrimp/m³) compared to away from the edge (8.4 ± 2.26 shrimp/m³) and (4.5 ± 1.34 shrimp/m³) near Savannah, GA. The higher number of *P. pugio* near the marsh edge is

perhaps due to the availability of refuge such as vegetation, oysters, and man-made structures (Modeste, 2009).

The red alga *Gracilaria vermiculophylla* has invaded the habitat of *Palaemonetes pugio*. *Gracilaria vermiculophylla* is native to the northwest Pacific (Ohmi, 1956; Rueness, 2005) and has spread to the northeast Pacific including British Columbia (Saunders, 2009), and the Atlantic coast of North America including New England (Nettleton et al., 2013), Virginia (Thomsen and McGlathery, 2007), North Carolina (Freshwater et al., 2006), South Carolina (Byers et al., 2012), and Georgia (Byers et al., 2012; Wright et al., 2014). The existence of *G. vermiculophylla* in shallow estuarine systems is important as it provides a large coverage of macrophytic structure in mudflats that previously lacked vegetation (Byers et al., 2012). *Gracilaria vermiculophylla* may increase primary productivity and could be an important contributor to detrital foodwebs because of rapid decomposition (Byers et al., 2012). There were over 10 native phyla within *G. vermiculophylla* perhaps because it was utilized for shelter, feeding or attachment in Virginia (Nyberg et al., 2009). The biomass of *G. vermiculophylla* had a significant positive effect on the abundance of epifauna that consisted of 71% amphipods and 10% gastropods in one site near Savannah, GA and multiple sites near Hilton Head, SC (Byers et al., 2012). *Gracilaria vermiculophylla* was effective at reducing the ability of the common grass shrimp *Palaemonetes vulgaris* to forage on the amphipods as it increased the survival rates of its prey from 0% without *G. vermiculophylla* to $53.8 \pm 10.2\%$ with algae present in Savannah, GA (Wright et al., 2014).

Now that an invasive substrate *Gracilaria vermiculophylla* has accumulated over mudflats once devoid of structure, *Palaemonetes pugio* is exposed to a structural change

within its habitat. *Palaemonetes pugio* may be redirected from native substrates to *G. vermiculophylla*, perhaps influenced by the number of parasites within *P. pugio*. In the current study, infected *P. pugio* were investigated to determine whether there is a relationship between parasite load and location of shrimp when a new substrate providing alga has invaded. The purpose of the present study was to determine if there were differences in trematode and haplosporidian prevalence (% of shrimp infected), abundance (number of parasites per infected and uninfected shrimp), intensity (number of parasites per infected shrimp), density (number of parasites per shrimp/cm total length), and prevalence (% of shrimp infected), across months and between sites and if they were correlated to where *P. pugio* was located in the environment such as a sandy substrate or near rocks with *G. vermiculophylla* if present.

HYPOTHESES

H_{A1}: There is a difference in *Microphallus turgidus* cyst abundance, intensity, density, and prevalence, and haplosporidian *Urosporidium crescens* cyst abundance, intensity, density, and prevalence in *Palaemonetes pugio* across months and/or between sites.

H_{A2}: *Microphallus turgidus* cyst abundance, intensity, density, and prevalence in *Palaemonetes pugio* correlates with where *P. pugio* is located in the natural environment such as near or in *Gracilaria vermiculophylla* or sandy substrates.

MATERIALS AND METHODS

Site Description

Two sites were sampled for grass shrimp. One site was located near the Rodney J. Hall Boat Ramp (31°56'51.07"N 81°04'05.65"W), which lies parallel to the Diamond Causeway Bridge and connects to the Skidaway River (Figure 2.1). The edges of this river are surrounded by large beds of the smooth cordgrass *Spartina alterniflora* that are habitat to many invertebrates and birds including the daggerblade grass shrimp *Palaemonetes pugio*, the common grass shrimp *Palaemonetes vulgaris*, the mud snail *Ilyanassa obsoleta*, the marsh periwinkle *Littoraria irrorata*, the fiddler crab *Uca pugilator*, the white shrimp *Litopenaeus setiferus*, and seagull and pelican species (pers. observ.). The other site was located next to the Frank Downing Fishing Pier (31°57'02.84"N 81°04'52.71"W) less than 1600 m east of the Rodney J. Hall Boat Ramp and lies perpendicular to Shipyard Creek, a tributary of the Skidaway River, which was used for sampling near the pier. This creek is diverse in its hydrology and substrate composition. It ranges from large rocks along the creek edge to sand in the center of the stream. The smooth cordgrass *Spartina alterniflora*, the red alga *Gracilaria vermiculophylla*, the common grass shrimp *Palaemonetes vulgaris*, and the blue crab *Callinectes sapidus* were also found near the edges of the creek. However, *G. vermiculophylla* was seasonal and appeared near the pier during winter beginning in January (pers. observ.) and remained until spring in May.

Field Surveys

Monthly samples of the daggerblade grass shrimp *Palaemonetes pugio* were collected beginning in October 2016 from the two sites near Savannah, GA to document trematode and hyperparasite abundance, intensity, density, and prevalence. Once shrimp were collected, measurements from the tip of the rostrum to the tip of the telson and ovigerity of the shrimp were also recorded. Sampling occurred during low tide when the edges of the marsh were accessible. Surface temperature and salinity were monitored using a thermometer and a refractometer, respectively. Shrimp were collected using a combination of drop trap and dip net methods near the marsh edge. The drop trap dimensions were 0.5 m x 0.5 m x 0.5 m; it was constructed using plastic mesh with a pore size of 1 mm x 1 mm, sewn to four edges of PVC pipe with the top and bottom of the box kept open. A small oval weight was placed along each bottom corner to keep the trap from rising when set in place. Once the drop trap was deployed, shrimp remained captured until a 0.20 m X 0.15 m aquarium dip net was used to remove them. The drop trap was used 10 replicate times on the sand substrate at the Rodney J. Hall Boat Ramp. At the Frank Downing Fishing Pier, the drop trap was also used 10 replicate times on the sand substrate as well as 10 replicate times on the rock substrate that had attached *G. vermiculophylla* present beginning in January 2017. If 100 shrimp were not collected using the drop trap, a 37.5 cm X 37.5 cm dip net was then dragged across a 10 m interval of sand, multiple times if necessary, to collect the remaining number needed. A sample size of 100 shrimp was needed to provide a large enough data set to properly represent the parasite abundance, intensity, density, and prevalence.

Shrimp at each site were identified to species using their rostrum and eyestalk coloration. The rostrum of each shrimp was examined by pulling down the antennae to expose its entire length. *Palaemonetes pugio* lacks serration at the tip of its rostrum unlike *Palaemonetes vulgaris* for which serration extends on both the top and the bottom of the rostrum (Anderson, 1985). Another common feature of *P. pugio* is a yellow pigmentation of the eyestalks (Anderson, 1985) while *P. vulgaris* has red pigmented eyestalks (pers. observ.). Total length of each shrimp was obtained by measuring from the tip of the rostrum to the end of the uropod using calipers to the nearest 0.01 mm. Identification of sex was not obtained in the field because it was difficult to differentiate. However, ovigerity was recorded if eggs were obvious on the ventral side of the abdomen.

Grass shrimp with parasites were also examined in the field. If *Palaemonetes pugio* was detected with the metacercarial stage of the microphallid trematode *Microphallus turgidus* or the haplosporidian hyperparasite *Urosporidium crescens*, the parasite number was then counted and recorded in the field. Metacercarial cysts appeared small with off-white pigmentation and were visible to the naked eye through the transparent integument of the live shrimp body. *Microphallus turgidus* cysts infected with the hyperparasite were much larger in size and contained black pigmentation. Following examination, shrimp were returned to the water.

Statistical Analyses

Summary statistics were calculated in Microsoft Excel to compare the average number of grass shrimp in each drop trap per site and month, average grass shrimp length, percent ovigerity, abundance (# parasites within infected and uninfected shrimp),

intensity (# of parasites within infected shrimp), density (# parasites/shrimp cm total length), and prevalence (presence or absence of parasite) of trematodes and hyperparasites across months, across months within each site, between sites, and between each drop trap collection per month. Regression analyses were also used in Excel to determine if salinity and temperature at each site were correlated with trematode and hyperparasite prevalence. Grass shrimp length, percent ovigerity, and the trematode and hyperparasite abundance, intensity, density, and prevalence were then analyzed using fully factorial two-way ANOVA for differences between the sampled sites, among months, and month-site interaction. Months sampled in both survey years (October 2016 through December 2017) were treated as unique sampling events. If there were significant differences among months, a Tukey's post hoc analysis was then used to determine which months were significantly different from one another. Trematode and hyperparasite prevalence were analyzed using the nominal logistic regression model to determine differences among months, sites, and month-site interaction. For shrimp collected by drop trap, trematode abundance, intensity, density, and prevalence. Shrimp density were further analyzed via a second two-way ANOVA for differences between the substrates and site, among months, and the interaction between months and site-substrate. Prior to all analyses, data were tested for normality via the Shapiro-Wilk test and homogeneity of variance using Levene's test. In all instances, the data were unable to be normalized via transformation. However, the analysis of variance test is robust to violations of these assumptions when the dataset is sufficiently large (Underwood, 1997), as the datasets were in this study. All analyses were conducted in JMP (Version 13).

RESULTS

A two-way ANOVA ($P < 0.0001$, $F = 44.0686$, $df = 29$, 2974) determined that there were significant differences in shrimp length from October 2016 through December 2017. There was a significant difference in length across all months ($P < 0.0001$, $F = 72.9815$, $df = 14$) and a significant month*site interaction ($P < 0.0001$, $F = 18.5152$, $df = 14$), but not between the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier ($P = 0.4580$, $F = 0.5510$, $df = 1$). The average grass shrimp length (cm) at the Rodney J. Hall Boat Ramp was smallest in October 2016 (2.3 ± 0.26), but was not significantly smaller than September 2017 (2.4 ± 0.42), October 2017 (2.4 ± 0.36), November 2017 (2.3 ± 0.33), and December 2017 (2.3 ± 0.51), and was largest in March 2017 (3.2 ± 0.44), although not significantly larger than May 2017 (3.2 ± 0.53) (Figure 2.2A). At the Frank Downing Fishing Pier, shrimp were smallest in December 2016 (2.3 ± 0.29) but not significantly smaller than January (2.5 ± 0.36), February (2.5 ± 0.30), June (2.3 ± 0.36), November 2017 (2.3 ± 0.34), and December 2017 (2.2 ± 0.66), and were largest in April 2017 (3.1 ± 0.34) but not significantly larger than May 2017 (3.0 ± 0.50) (Figure 2.2B). Shrimp length was generally higher in the spring and summer months (March 2017 through September 2017) and lower in the fall and winter (October 2016 through February 2017) at both sites. There was a significant relationship between shrimp length and trematode intensity (# of trematodes within infected shrimp), ($R^2 = 0.0587$, $P < 0.0001$) (Figure 2.3) and density (# trematodes/cm total length) ($R^2 = 0.0018$, $P = 0.0242$) (Figure 2.4).

The nominal logistic regression model ($P < 0.0001$, $\chi^2 = 624.6491$, $df = 29$, 3007) indicated significant differences in female ovigerity across months ($P < 0.0001$,

$\chi^2=549.0862$, $df=14$), but not between the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier ($P=0.9924$, $\chi^2=0.00009$, $df=1$) with a significant month*site interaction ($P<0.0001$, $\chi^2=41.75314$, $df=14$). The lowest percentage of ovigerous grass shrimp at the Rodney J. Hall Boat Ramp was in November 2016 (0%), January 2017 (0%), February 2017 (0%), and December 2017 (0%), with a maximum of 42% observed in May 2017 (Figure 2.5A). Shrimp were ovigerous in more months at the Rodney J. Hall Boat Ramp than at the Frank Downing Fishing Pier, although there were ovigerous shrimp in March through October 2017 at both sites (Figure 2.5). Ovigerity was 0% at the Frank Downing Fishing Pier from October 2016 through February 2017, which was different from the Rodney J. Hall Boat Ramp as there were ovigerous shrimp in October 2016, December 2016, and November 2017 (2%) (Figure 2.5). The maximum ovigerity at the Frank Downing Fishing Pier (50%) was observed a month earlier (April) than at the Rodney J. Hall Boat Ramp, after which values decreased to 0% in November and December 2017 (Figure 2.5).

A two-way ANOVA ($P<0.0001$, $F=33.6604$, $df=29$, 2975) determined significant differences for trematode abundance. However, abundance was not significantly different between the Rodney J. Hall Boat Ramp and Frank Downing Fishing Pier ($P=0.2342$, $F=1.4157$, $df=1$), but was significant across all months ($P<0.0001$, $F=56.6519$, $df=14$), with a significant month*site interaction ($P<0.0001$, $F=12.9525$, $df=14$). Abundance was higher in December 2016 (36.8 ± 20.99), but was not significantly different than November 2016 (30.3 ± 16.65) at the Rodney J. Hall Boat Ramp. Lowest abundance was in December 2017 (8.70 ± 9.81), but was not significantly lower than March 2017 (11.9 ± 13.58), April-July, and November 2017 (11.6 ± 13.58). At the Frank Downing Fishing

Pier, abundance was higher in September 2017 (33.9 ± 26.20), but was not significantly higher than August 2017 (28.2 ± 16.31) and October 2017 (34.3 ± 30.95). Abundance was lower in March 2017 (5.6 ± 6.93), but was not significantly different than April, June, and December 2017 (10.7 ± 15.22).

A two-way ANOVA ($P < 0.0001$, $F = 27.2727$, $df = 29, 2758$) indicated that there was a significant difference for trematode intensity between the Frank Downing Fishing Pier and the Rodney J. Hall Boat Ramp ($P = 0.0347$, $F = 4.4629$, $df = 1$) from October 2016 through December 2017, and a significant difference across all months ($P < 0.0001$, $F = 42.7072$, $df = 14$) with a significant month*site interaction ($P < 0.0001$, $F = 13.7333$, $df = 14$). The maximum number of trematodes (156) to infect a shrimp occurred in December 2016 at the Rodney J. Hall Boat Ramp.

There were significant differences in trematode density (two-way ANOVA; $P < 0.0001$, $F = 44.2945$, $df = 29, 2973$) across all months ($P < 0.0001$, $F = 79.8548$, $df = 14$) and the month*site interaction ($P < 0.0001$, $F = 11.9564$, $df = 14$), but not significantly different between the Rodney J. Hall Boat Ramp and Frank Downing Fishing Pier ($P = 0.3254$, $F = 0.9676$, $df = 1$). Average trematode density (# trematodes/ cm total length) was higher in December 2016 (13.3 ± 7.05) but was not significantly different than October 2016 (11.3 ± 3.50), November 2016 (11.8 ± 5.68), and February 2017 (11.0 ± 4.95) at the Rodney J. Hall Boat Ramp (Figure 2.6A). At the Frank Downing Fishing Pier, density was significantly higher in September 2017 (12.6 ± 9.07) and October 2017 (12.6 ± 10.25), but was not significantly different than December 2016 (10.3 ± 3.89), January 2017 (10.1 ± 4.11), August 2017 (10.8 ± 5.49), and October 2017 (12.6 ± 10.25) (Figure 2.6B). Density was lower in December 2017 (2.8 ± 3.79) at the Rodney J. Hall Boat Ramp but was not

significantly different than March through July and November 2017 (Figure 2.6A).

Density was lower in March 2017 (2.1 ± 1.86) at the Frank Downing Fishing Pier but was not significantly different than April through June or December 2017 (Figure 2.6B).

Trematode density tended to be higher in the fall and winter seasons and tended to be lower during the spring and summer at both the Rodney J. Hall Boat Ramp and Frank Downing Fishing Pier (Figure 2.6). Density tended to always be higher at the Rodney J. Hall Boat Ramp and was always greater than 2.8 ± 3.79 (Figure 2.6A).

There was a significant difference in trematode density among shrimp length categories (categories determined by Pung et al., 2002) (one-way ANOVA; $P < 0.0001$, $F = 18.7009$, $df = 6$, 2996) across months from October 2016 through December 2017. Average density was significantly lower in the < 2.1 cm, > 3.2 - 3.5 cm, and > 3.5 cm length categories (Figure 2.7). However, average density was significantly highest (9.9 ± 8.14) in the > 2.9 - 3.2 cm length category although it had fewer (366) individuals than the smallest length category (437). The higher number of individuals was in the > 2.3 - 2.6 cm length category with 709 shrimp with an average density of 7.5 ± 5.71 (Figure 2.7).

The nominal logistic regression model ($P < 0.0001$, $\chi^2 = 433.7737$, $df = 58$, 3030) determined significant differences in prevalence of shrimp parasitized by the trematode. There was no significant difference between the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier ($P = 1.0000$, $\chi^2 = 0.000002$, $df = 2$) from October 2016 through December 2017, and no significant month*site interaction ($P = 0.0750$, $\chi^2 = 39.38036$, $df = 28$), but there was a significant difference across all months ($P < 0.0001$, $\chi^2 = 344.1986$, $df = 28$). Prevalence of shrimp parasitized by the trematode remained constant at 100% from October 2016 through January 2017 at the Rodney J. Hall Boat Ramp (Figure 2.8A)

and from October 2016 through February 2017 at the Frank Downing Fishing Pier (Figure 2.8B). Prevalence was lowest in June and July with 84% and then increased to 99% in September at the Rodney J. Hall Boat Ramp before gradually decreasing to 87% in December 2017 (Figure 2.8A). At the Frank Downing Fishing Pier, prevalence increased from 76% in March 2017 to 98% in August, decreased again to its lowest in November 2017 at 69% and then increased to 77% in December (Figure 2.8B). Prevalence tended to be higher in the fall and winter months and lower in the spring and summer months at both the Rodney J. Hall Boat Ramp and Frank Downing Fishing Pier (Figure 2.8). Prevalence tended to be higher at the Rodney J. Hall Boat Ramp and was always higher than 84% (Figure 2.8).

A regression analysis indicated a significant relationship between prevalence and salinity (ppt) ($R^2=0.5686$, $P=0.0012$) at the Rodney J. Hall Boat Ramp (Figure 2.9A) and at the Frank Downing Fishing Pier ($R^2=0.4160$, $P=0.0094$) (Figure 2.9B) and no significant relationship between prevalence and temperature ($^{\circ}\text{C}$) ($R^2=0.1095$, $P=0.2282$) and ($R^2=0.0012$, $P=0.9033$), respectively (Figure 2.10). There was also no significant relationship between temperature collected from the Ft. Pulaski NOAA Data Buoy Center (averaging the minimum and maximum temperatures measured on the same day as sampling in the current study) and prevalence at the Rodney J. Hall Boat Ramp ($R^2=0.1069$, $P=0.2341$) (Figure 2.11A) and at the Frank Downing Fishing Pier ($R^2=0.0079$, $P=0.7530$) (Figure 2.11B). The relationship between prevalence and temperature when temperature was analyzed for 7 days (6 days prior to the sampling day and on the sampling day) from the Ft. Pulaski NOAA Data Buoy Center (averaging the minimum and maximum temperatures for all 7 days) was worse for the Rodney J. Hall

Boat Ramp ($R^2=0.0541$, $P=0.4042$) and at the Frank Downing Fishing Pier ($R^2=0.0026$, $P=0.8559$) (Figure 2.12) than the relationship between prevalence and temperature just on the sampling day (Figure 2.11).

A two-way ANOVA was used to determine if there were significant differences in abundance and intensity of shrimp infected by the hyperparasite. The two-way ANOVA for abundance indicated significant differences ($P=0.0133$, $F=1.6754$, $df=29$, 2976). However, there was no significant difference between the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier ($P=0.1483$, $F=2.0904$, $df=1$) from October 2016 through December 2017, and there was no significant difference across all months ($P=0.0674$, $F=1.6159$, $df=14$), but there was a significant month*site interaction ($P=0.0475$, $F=1.7087$, $df=14$). The maximum number of hyperparasites to infect a single shrimp was 6. There were 3 shrimp that had this maximum, each in a different month (November 2016, March 2017, and August 2017), but all were observed at the Frank Downing Fishing Pier. There were no significant differences determined for hyperparasite intensity (two-way ANOVA; $P=0.4909$, $F=0.9885$, $df=29$, 137) between the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier ($P=0.9121$, $F=0.0122$, $df=1$) from October 2016 through December 2017, and no significant difference across all months ($P=0.6135$, $F=0.8506$, $df=14$) and no significant month*site interaction ($P=0.3321$, $F=1.1364$, $df=14$).

There were significant differences determined for hyperparasite density (two-way ANOVA; $P=0.0164$, $F=1.6452$, $df=29$, 2973) between the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier ($P=0.1907$, $F=1.7129$, $df=1$), and no significant difference across all months ($P=0.0756$, $F=1.5848$, $df=14$), but there was a significant

month*site interaction ($P=0.0472$, $F=1.7100$, $df=14$). Average density was lower in October 2016 (0.00003 ± 0.000352) at the Rodney J. Hall Boat Ramp (Figure 2.13A) and in June 2017 (0.00002 ± 0.000287) at the Frank Downing Fishing Pier (Figure 2.13B). In contrast, density was higher at the Rodney J. Hall Boat Ramp in September 2017 (0.00043 ± 0.001495) and higher at the Frank Downing Fishing Pier in July 2017 (0.00075 ± 0.002021). No trend was observed because density was low in March (0.00012 ± 0.000705) and August (0.00077 ± 0.005490) and high in June 2017 (0.00037 ± 0.001422) at the Rodney J. Hall Boat Ramp (Figure 2.13A) and low in January (0.00009 ± 0.000556) and June (0.00002 ± 0.00028) and high in November 2016 (0.00051 ± 0.002386) at the Frank Downing fishing Pier (Figure 2.13B). Density then decreased again at the Rodney J. Hall Boat Ramp in October 2017 (0.00017 ± 0.000962) (Figure 2.13A) and September 2017 (0.00023 ± 0.001045) at the Frank Downing Fishing Pier (Figure 2.13B). Although there was no obvious seasonal trend in hyperparasite density at the Rodney J. Hall Boat Ramp and Frank Downing Fishing Pier, density at the Frank Downing Fishing Pier was always higher and was never lower than (0.00002 ± 0.000287) (Figure 2.13B).

The nominal logistic regression model ($P=0.0112$, $\chi^2=49.1308$, $df=29$) indicated significant differences in hyperparasite prevalence across months ($P=0.0218$, $\chi^2=26.58585$, $df=14$) from October 2016 through December 2017, and a significant month*site interaction ($P=0.0458$, $\chi^2=24.00562$, $df=14$), but not between the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier ($P=0.9216$, $\chi^2=0.009687$, $df=1$). Prevalence was highest (9%) at the Rodney J. Hall Boat Ramp in September 2017 (Figure 2.14A) and in July 2017 (15%) at the Frank Downing Fishing Pier (Figure

2.14B). Prevalence was lowest in October 2016 (2%) and August 2017 (2%) at the Rodney J. Hall Boat Ramp, and in June (1%) at the Frank Downing Fishing Pier. There was no consistent pattern in prevalence across seasons at the Rodney J. Hall Boat Ramp or at the Frank Downing Fishing Pier, but the Rodney J. Hall Boat Ramp tended to have higher prevalence in more months and was always higher than 2% (Figure 2.14A).

A regression analysis indicated no significant relationship between hyperparasite prevalence and salinity (ppt) at the Rodney J. Hall Boat Ramp ($R^2=0.0010$, $P=0.9124$) (Figure 2.15A) or at the Frank Downing Fishing Pier ($R^2=0.0033$, $P=0.8386$) (Figure 2.15B) between October 2016 through December 2017. There was no positive or significant relationship between prevalence and temperature ($^{\circ}\text{C}$) at the Rodney J. Hall Boat Ramp ($R^2=0.0423$, $P=0.4617$) (Figure 2.16A); however, there was a positive, although not significant, relationship between prevalence and temperature ($^{\circ}\text{C}$) at the Frank Downing Fishing Pier ($R^2=0.2126$, $P=0.0836$) (Figure 2.16B). The prevalence of shrimp parasitized by the hyperparasite was higher during the summer (22%) when the mean temperature was 33.0 ± 1.41 and the mean salinity was 23.0 ± 2.83 at the Frank Downing Fishing Pier (Table 2.1). However, the higher prevalence at the Rodney J. Hall Boat Ramp was during the spring (22%) when the mean temperature was 27.5 ± 2.38 and the mean salinity was 26.3 ± 1.50 . The lowest prevalence at both sites was during the winter with 8% at the Frank Downing Fishing Pier and 9% at the Rodney J. Hall Boat Ramp (Table 2.1).

A two-way ANOVA was used to determine if there were significant differences in trematode abundance and intensity, and a nominal logistic regression model was used to determine if there were significant differences in prevalence in the drop trap samples

(sand and rocks with *Gracilaria vermiculophylla*). For trematode abundance, a two-way ANOVA indicated significant differences ($P < 0.0001$, $F = 9.0728$, $df = 38, 1177$) across months ($P < 0.0001$, $F = 15.6473$, $df = 12$), with a significant interaction between months and site-substrates ($P < 0.0001$, $F = 3.5916$, $df = 24$). There was no significant difference among the site-substrates ($P = 0.0640$, $F = 2.7547$, $df = 2$). There was no abundance and intensity data recorded in November 2016 and June 2017 because no shrimp were collected in the rock with *G. vermiculophylla* drop trap samples at the Frank Downing Fishing Pier; therefore, these months were eliminated from the trematode abundance analyses. Similarly, there were significant differences in trematode intensity (two-way ANOVA; $P < 0.0001$, $F = 7.7527$, $df = 38, 1087$) in the drop trap samples across months ($P < 0.0001$, $F = 12.0778$, $df = 12$), with a significant interaction between months and site-substrates ($P < 0.0001$, $F = 3.8099$, $df = 24$). There was also a significant difference among site-substrates ($P = 0.0129$, $F = 4.3684$, $df = 2$). The nominal logistic regression model ($P < 0.0001$, $\chi^2 = 478.001$, $df = 76$) also showed significant differences in trematode prevalence in the drop trap samples across months ($P < 0.0001$, $\chi^2 = 164.3076$, $df = 24$), with a significant interaction between months and site-substrates ($P < 0.0001$, $\chi^2 = 142.4937$, $df = 48$), but there was no significant difference among the site-substrates ($P = 1.0000$, $\chi^2 < 0.0001$, $df = 4$).

Another two-way ANOVA ($P < 0.0001$, $F = 12.1240$, $df = 38, 1175$) determined significant differences in trematode density in the drop trap samples across months ($P < 0.0001$, $F = 20.4966$, $df = 12$), with a significant interaction between months and site-substrates ($P < 0.0001$, $F = 3.9480$, $df = 24$), and a significant difference among the site-substrates ($P = 0.0030$, $F = 5.8344$, $df = 2$). There was no trematode density data recorded in

November 2016 and June 2017 because no shrimp were collected in the rock with *G. vermiculophylla* samples at the Frank Downing Fishing Pier; therefore, these months were eliminated from the analyses. Density within the rock with *G. vermiculophylla* substrate at the Frank Downing Fishing Pier was not significantly different from the sand substrate at the Frank Downing Fishing Pier or from the sand substrate at the Rodney J. Hall Boat Ramp (Figure 2.17). However, density within the sand substrate at the Frank Downing Fishing Pier was significantly different from the sand substrate at the Rodney J. Hall Boat Ramp (Figure 2.17).

A two-way ANOVA ($P < 0.0001$, $F = 2.9267$, $df = 44$) indicated significant differences in shrimp density (# shrimp per drop trap sample) across months ($P = 0.0009$, $F = 2.6807$, $df = 14$), with a significant interaction between months and site-substrates ($P < 0.0001$, $F = 2.9854$, $df = 28$), and a significant difference among the site-substrates ($P < 0.0001$, $F = 10.5765$, $df = 2$) from October 2016 through December 2017. All months were included in the analyses even the months (November 2016 and June 2017) that no shrimp were collected in the drop trap samples with rocks with *Gracilaria vermiculophylla*. Total number of shrimp in the sand drop trap samples (1282) (including the dip net samples) at the Frank Downing Fishing Pier was significantly different from the rock with *G. vermiculophylla* samples (218) at the Frank Downing Fishing Pier, but was not significantly different from the density in the sand drop trap samples (1500) (including the dipnet samples) at the Rodney J. Hall Boat Ramp (Figure 2.18). The average number of shrimp near the rocks (regardless of whether or not *Gracilaria vermiculophylla* was present) was lower (14.5 ± 13.04) than the number near the sand substrate (35.1 ± 31.66) at the Frank Downing Fishing Pier from October 2016

through December 2017, which was 29% and 71% of the shrimp, respectively (Figure 2.19). However, the sand substrate at the Frank Downing Fishing Pier had a lower average number of shrimp than the sand substrate at the Rodney J. Hall Boat Ramp (40.9 ± 31.66) (Figure 2.19A). During the months when *G. vermiculophylla* was not present (October 2016 through December 2016 and June 2017 through December 2017), the average number of shrimp near the rocks was also lower (16.0 ± 14.91) (26%) than the number of shrimp near the sand substrate (45.5 ± 34.26) (74%) at the Frank Downing Fishing Pier (Figure 2.20), but the average number of shrimp near the sand substrate was higher than the average number of shrimp near the sand substrate at the Rodney J. Hall Boat Ramp (41.8 ± 37.71) (Figure 2.20A). The average number of shrimp near the rocks when *G. vermiculophylla* was present (January 2017-May 2017) was 11.6 ± 8.88 and was also lower than the average number of shrimp near the sand substrate (14.2 ± 7.16) at the Frank Downing Fishing Pier, and the average of number of shrimp near the sand substrate was lower than the sand substrate (39.2 ± 13.95) at the Rodney J. Hall Boat Ramp (Figure 2.21A). Although there were fewer shrimp collected when *G. vermiculophylla* was present, the percentage of shrimp near the rocks (45%) was nearly the same as the percentage near the sand substrate (55%) (Figure 2.21B). Overall, the average number and percentage of shrimp near the rocks (regardless of whether or not *G. vermiculophylla* was present) was always lower than the number near the sand (Figure 2.21) with the exception of January 2017 when 65% of shrimp were near the rocks and only 35% were near sand (Figure 2.22).

DISCUSSION

The major finding of this study was that significantly more *Palaemonetes pugio* were near sand substrates (526) than rocks (218) regardless of the presence of *Gracilaria vermiculophylla* at the Frank Downing Fishing Pier. There was no correlation between trematode density and location of shrimp, the null hypothesis was not rejected. Trematode density near the rocks was not significantly different than the density near the sand at the Frank Downing Fishing Pier or near sand at the Rodney J. Hall Boat Ramp. *Gracilaria vermiculophylla* was present from January 2017 through May 2017 at the Frank Downing Fishing Pier. The only month for which there was more *Palaemonetes pugio* near rocks with *G. vermiculophylla* was in January 2017 when the surface temperature was lowest (16°C). The Ft. Pulaski NOAA Data Buoy Center also recorded a low average temperature on the day that sampling occurred (15°C) and the mean value for the 7 days prior to sampling that year was 14.9°C in January 2017. It is possible that the number of grass shrimp shifted from one substrate to another because of temperature changes. *Palaemonetes pugio* may have selected sandy substrates instead of rocks with *G. vermiculophylla* when temperature increased, thus potentially increasing rate of transmission of the trematode parasite *Microphallus turgidus* to *P. pugio* as several hydrobiid snail species can be the first intermediate hosts of *M. turgidus* and are typically located within muddy substrates (Heard and Overstreet, 1983) that were perhaps in close proximity to grass shrimp. However, there was no significant effect of temperature on trematode prevalence. It is also possible that shrimp only preferred rocks with *G. vermiculophylla* one month out of all months because low temperatures prevented predators from utilizing that habitat. The blue crab *Callinectes sapidus* inhabited the

rocks (Guidone, pers. comm.). The cold water may have caused *C. sapidus* to utilize a different substrate or to be less active. During studies in Mobjack Bay, Virginia, the abundance of *C. sapidus* varied seasonally between a marsh creek tributary with *Spartina alterniflora* and a seagrass bed just downstream of the tributary (Orth and Montfrans, 1987). Mean density of *C. sapidus* was always highest in the seagrass beds through the fall and winter but gradually decreased through spring with its lowest density during summer (Orth and Montfrans, 1987). The highest density in the marsh creek was always highest in the fall and was lowest in the winter and summer (Orth and Montfrans, 1987). Although there were fewer grass shrimp collected from January 2017 through May 2017 (when *G. vermiculophylla* was present) the percentage of shrimp near the rocks (45%) was nearly the same as the percentage of shrimp near the sand (55%). Shrimp were near the rocks perhaps because cooler temperatures decreased the activity levels of predators or because of the presence of *G. vermiculophylla*.

Another finding of this study was that there was a significant effect of salinity but not temperature on the prevalence of *Palaemonetes pugio* infected by *Microphallus turgidus*, although similar trends within the literature were inconsistent (Pung et al., 2006; Sheehan et al., 2011). There was a significant decrease in prevalence as salinity increased at the Rodney J. Hall Boat Ramp and at the Frank Downing Fishing Pier. Salinity ranged from 17 ppt to 24 ppt at the Rodney J. Hall Boat Ramp when prevalence was 100%, while prevalence was lowest in June and July 2017 at 84% when salinity was 28 ppt and 26 ppt, respectively. Prevalence was lowest in November 2017 at the Frank Downing Fishing Pier when salinity was 29.5 ppt while salinity was lower and ranged from 17 ppt to 25 ppt during the months when prevalence was 100%. Perhaps prevalence

drastically decreased from 100% in February to 76% in March at the Frank Downing Fishing Pier because of precipitation levels. The National Weather Service recorded March 2017 as one of the driest months on record with only 0.50 in of rainfall. At the Rodney J. Hall Boat Ramp, prevalence also decreased but from 99% in February to 87% in March, while salinity increased from 18 ppt to 25 ppt. Pung et al. (2006) observed differences in both prevalence and abundance perhaps due to differences between collection locations, but only abundance was significantly correlated with salinity. The abundance of *M. turgidus* in both the Altamaha River and Ogeechee River was positively correlated with salinity, which ranged from 0.3 to 14 ppt and 5 to 25 ppt, respectively (Pung et al., 2006). In addition, 92% of *P. pugio* were infected with the trematode in locations where salinity was high and only 59% of *P. pugio* were infected in locations with lower salinity (Pung et al., 2002). However, Sheehan et al. (2011) observed trematode prevalence to decrease as salinity increased, but only at locations with wetlands as average salinity ranged from 5.4 ± 3.8 ppt to 27.7 ± 19.6 ppt. The results of the present study were similar to Sheehan et al. (2011) because there was a significant negative relationship between prevalence and salinity at the Rodney J. Hall Boat Ramp and at the Frank Downing Fishing Pier. In the current study, salinity ranged from 17 ppt to 32.5 ppt, which exceed the minimum and maximum salinity threshold for Sheehan et al. (2011). For comparison, if salinity was narrowed in the current study to the upper salinity in Sheehan et al. (2011), there was no longer a relationship with trematode prevalence. There was no significant relationship between water temperature (measured from the current study and from Ft. Pulaski) and prevalence at the Rodney J. Hall Boat Ramp or at the Frank Downing Fishing Pier from October 2016 through December 2017,

prevalence was generally higher when the water was cooler. At the Frank Downing Fishing Pier, prevalence was lowest in July 2017 (84%) when the temperature was 34°C and 30°C at Ft. Pulaski, but then increased to 97% in August 2017 when temperature decreased to 32°C, although temperature remained consistent at 30°C at Ft. Pulaski. However, Pung et al. (2006) found that there was a slight but significant correlation between abundance, but not prevalence, and temperature in the Altamaha and Ogeechee rivers. The National Weather Service also recorded high air temperature measurements in July and August, but found no “monthly extreme” (defined as average temperature of the warmest all time months) measurements throughout 2017 near Savannah, GA. However, in previous years (1875-2016), July (29.2°C-30.4°C) and August (29.2°C-29.3°C) were the only 2 months for which “monthly extremes” were recorded.

Prevalence varied seasonally at 1 of 3 other Georgia sites surveyed from summer 2001 to summer 2003 (Pung et al., 2006). The prevalence of *P. pugio* infected with the trematode at the Altamaha River, GA location was highest in fall and winter during one year, but summer and fall of the second year, which was different from the Ogeechee River, GA location. There was no seasonal trend at the Ogeechee River because prevalence was never lower than 90% (Pung et al., 2006). Overall, prevalence ranged from 63.7% to 93.3% from 2001 to 2003 at the 2 locations where *P. pugio* was obtained (Pung et al., 2006), and ranged from 69% to 100% during the current study. During the present study, prevalence of shrimp infected by *M. tigidus* was 100% in October 2016 through January 2017 at the Rodney J. Hall Boat Ramp and 100% in October-February at the Frank Downing Fishing Pier. Prevalence dropped in February 2017 and remained lower through July 2017 at the Rodney J. Hall Boat Ramp and dropped in March 2017 at

the Frank Downing Fishing Pier. Prevalence increased back to 98% and 96% in October 2017 at the Rodney J. Hall Boat Ramp and Frank Downing Fishing Pier, respectively. There was no significant difference in prevalence between the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier or among months within each site, but there was a significant difference across months. Trematode prevalence was lowest during the months when shrimp were ovigerous (March 2017-August 2017). There was also no significant difference in ovigerity between sites. However, at the Rodney J. Hall Boat Ramp, shrimp were ovigerous in more months than at the Frank Downing Fishing Pier. During another study near Savannah, Georgia, ovigerity ranged from March through September at one sample site, which was a longer period than another site (Chaplin-Ebanks and Curran, 2007). Similarly, when shrimp parasitized by the bopyrid isopod *Probopyrus pandalicola* was lowest there was a peak in ovigerity (Chaplin-Ebanks and Curran, 2007). Bopyrid prevalence in shrimp was opposite than trematode prevalence during the current study. *Probopyrus pandalicola* causes sexual sterilization in female *Palaemonetes pugio* (Sherman and Curran, 2015). There could be a significant impact on grass shrimp populations because those individuals parasitized before reaching sexual maturity are removed from the reproductive population (Chaplin-Ebanks and Curran, 2007). It is possible that shrimp reproduction is impacted by high densities of the trematode parasite, which could affect overall shrimp population (Pung et al., 2002). During the current study, the population of shrimp at the Frank Downing Fishing Pier may have been affected, as there were fewer months when shrimp were ovigerous.

Prevalence of *Palaemonetes pugio* infected with the hyperparasite *Urosporidium crescens* may also be correlated with salinity (Pung et al., 2002). When shrimp were

collected from coastal barrier islands in Georgia, only 2.9% of shrimp were parasitized by *U. crescens* at salinities greater than 22 ppt (Pung et al., 2002). An average of 3% of *Callinectes sapidus* were parasitized by *U. crescens* during the fall and winter of 1990-1992 within the Maryland portion of Chesapeake Bay (Messick, 1998). The increase of salinity by 1 ppt significantly increased the likelihood of parasitization in *C. sapidus* by 14.3% across locations in Louisiana (Rogers et al., 2015), whereas in this study the average prevalence of *P. pugio* parasitized during the fall and winter was 13% and when salinity increased by 1 ppt from May to June 2017, prevalence decreased from 10% to 1%. Prevalence increased from 1% in June to 15% in July when salinity decreased by 1 ppt at the Frank Downing Fishing Pier. Perhaps the reason *C. sapidus* was uninfected by *U. crescens* when examined at one site was because of the intolerance of *U. crescens* to low salinity (Rogers et al., 2015). The range in salinity in the current study at the Rodney J Hall Boat Ramp was between 17 ppt and 30 ppt and this occurred between December 2016 and November 2017. Even with this broad range in salinity, hyperparasite prevalence stayed about the same. Perhaps *Microphallus turgidus* was more susceptible to *U. crescens* at lower salinities than the trematode *Microphallus basodactylphallus* that infects the blue crab. In addition, *U. crescens* creates an unappetizing appearance of the blue crab and is referred to as black specks and pepper crabs (Sprague, 1970). The hyperparasite is unharmed to humans if consumed, but the black encysted metacercariae may keep seafood consumers away from eating blue crabs with the otherwise harmful trematode (Heard and Overstreet, 1983). Since the trematode becomes unproductive when infected by *U. crescens*, it is possible that future invertebrate hosts may benefit from infection. Fewer trematodes would be unable to reproduce, thereby, reducing the

number of larvae transmitted to the intermediate hosts. However, it may not be beneficial for commercial species such as blue crabs, as consumers preferentially select for crabs without pepper spots. The density of hyperparasite infection in grass shrimp may be correlated with site. Sheehan et al. (2011) observed that in areas where trematode abundance was high, so were hyperparasite infections. In the current study, hyperparasite infection was much higher at the Frank Downing Fishing Pier than at the Rodney J. Hall Boat Ramp even though the abundance of trematodes was not significantly different between sites. It is possible that the Frank Downing Fishing Pier had a higher abundance of other hyperparasite hosts.

There was also a significant relationship between the length of *Palaemonetes pugio* and density of the trematode *Mircophallus turgidus* among all months from October 2016 through December 2017. Density of trematode infection (#/cm TL) was significantly higher in October through December 2016 (11.3 ± 3.50 - 13.3 ± 7.05) and February 2017 (11.0 ± 4.95) at the Rodney J. Hall Boat Ramp and higher in December 2016 through January 2017 (10.1 ± 4.11 - 10.3 ± 3.89), and August through October 2017 (10.8 ± 5.49 - 12.6 ± 10.25) at the Frank Downing Fishing Pier, but there was no significant difference between sites. Trematode density was generally lowest during the months shrimp length was largest (March 2017 through July 2017). Sheehan et al. (2011) documented trematode intensity to increase with shrimp length. Pung et al. (2002) also found that larger shrimp had a higher density of trematodes than smaller shrimp, which could indicate that the accumulation of trematodes does not affect shrimp mortality. However, those studies were different than the present study because grass shrimp in the two largest length categories had significantly lower densities than shrimp in the >2.9-3.2 cm length

category. There was a smaller number of shrimp (265) collected in the >3.2-3.5 cm length range compared to the other length ranges except for the largest length (>3.5 cm) category (188). It is possible that mortality of shrimp was affected, because the average density of trematodes peaked in the >2.9-3.2 cm category, which could have caused the decline in shrimp numbers in the two largest size categories. It is possible that Pung et al. (2002) supported the idea that the accumulation of trematodes does not affect shrimp mortality because the average density of trematodes was not high enough. The maximum average density in Pung et al (2006) was approximately 4, whereas in the current study the maximum average density was 9.9. The decline of shrimp in the current study could be due to the sublethal effects that the trematode has on its host such as reduced growth rates that could make shrimp more susceptible to predation and could also lower reproduction because of decreased growth. Shrimp population size could be affected by trematode density; even though there is little evidence to support induced mortality by the trematode, it is possible that shrimp fecundity may be altered (Pung et al., 2002).

Parasites that reduce the growth of their hosts might increase transmission to the final definitive host. Further, parasites might function as background stressors and not be immediately detrimental to the host but could have stronger effects on the host over time (Thieltges, 2006). Decreased growth could increase the likelihood of predation partly because smaller shrimp could be displaced from refuge by larger shrimp. Decreased growth could also alter the survival of an individual, ultimately decreasing population biomass, which could affect the food web.

The combination of trematode and hyperparasite analyses could be used as a proxy to determine if environmental factors have an impact on parasite prevalence (Sheehan et

al., 2011). In the current study, it is known that prevalence was significantly correlated with salinity and not temperature. The continuation of field work is important as it is still unknown whether or not trematodes or hyperparasites can cause mortality in grass shrimp. Environmental conditions within areas with high parasite infections should be analyzed further to determine if the combination of environmental changes and parasites could affect grass shrimp populations. *Gracilaria vermiculophylla* should also be surveyed in areas where present year round, as it could impact the distribution of organisms near it, thus altering interactions within that community.

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Table 2.1. Prevalence of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the haplosporidian hyperparasite *Urosporidium crescens* De Turk at the Rodney J. Hall Boat Ramp (RJHBR) and the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from Fall 2016 through Fall 2017. Salinity and water temperatures were calculated as mean \pm 1 SD.

Site	Season	Hyperparasite prevalence (%)	Average Salinity (ppt)	Average Temperature (°C)
FDFP				
	Fall 2016	17	23.7 \pm 2.31	19.3 \pm 0.58
	Winter 2016-2017	8	18.5 \pm 1.73	18.0 \pm 2.83
	Spring 2017	19	25.7 \pm 0.50	27.9 \pm 2.39
	Summer 2017	22	23.0 \pm 2.83	33.0 \pm 1.41
	Fall 2017	21	27.5 \pm 5.34	25.3 \pm 6.22
RJHBR				
	Fall 2016	17	20.0 \pm 3.61	20.0 \pm 1.73
	Winter 2016-2017	9	20.5 \pm 3.54	19.5 \pm 2.12
	Spring 2017	22	26.3 \pm 1.50	27.5 \pm 2.38
	Summer 2017	10	24.0 \pm 2.83	33.0 \pm 1.41
	Fall 2017	34	26.0 \pm 6.66	23.9 \pm 5.66

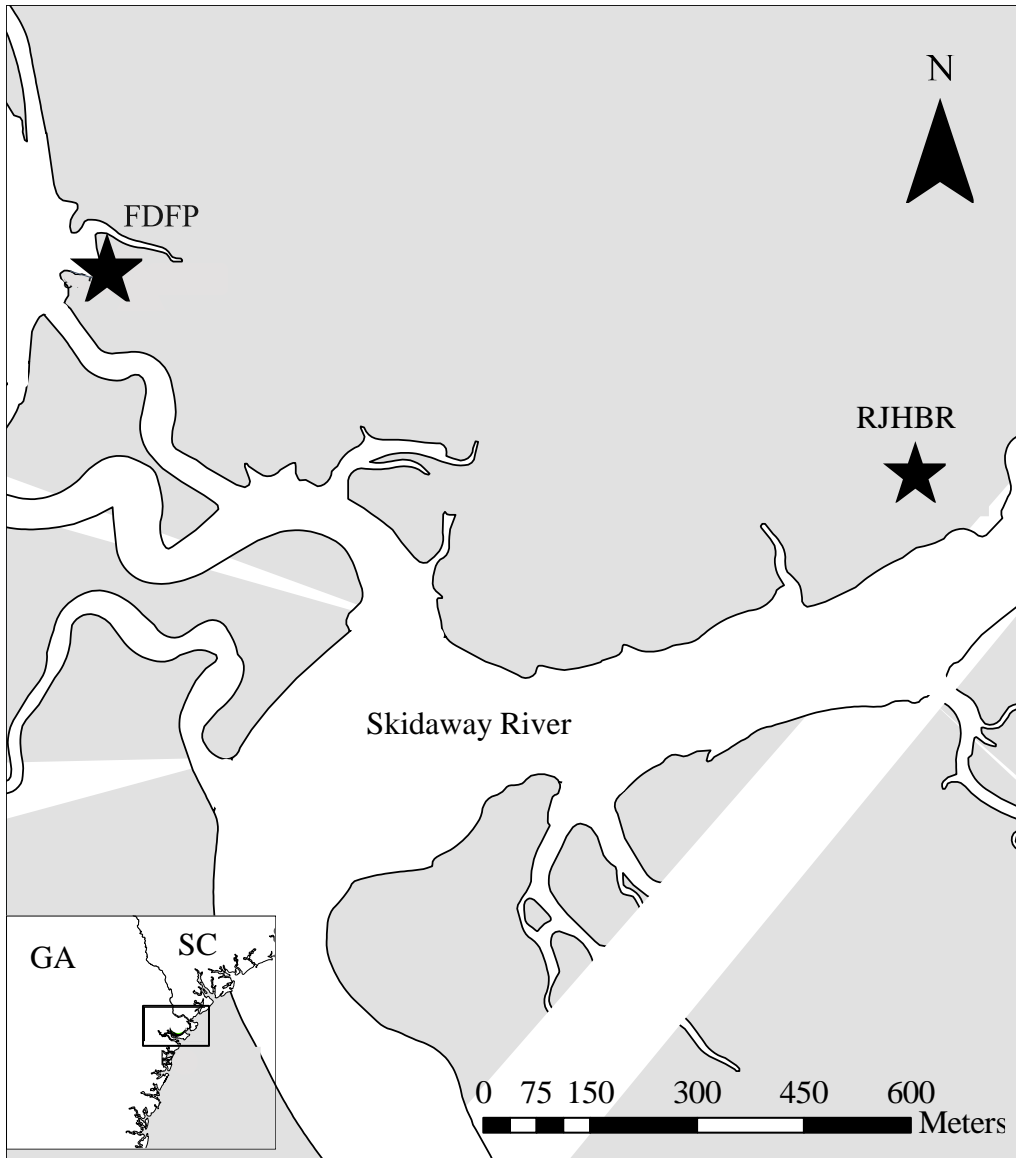


Figure 2.1. Map and location of the Rodney J. Hall Boat Ramp (RJHBR) and Frank Downing Fishing Pier (FDFP) sampling sites near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

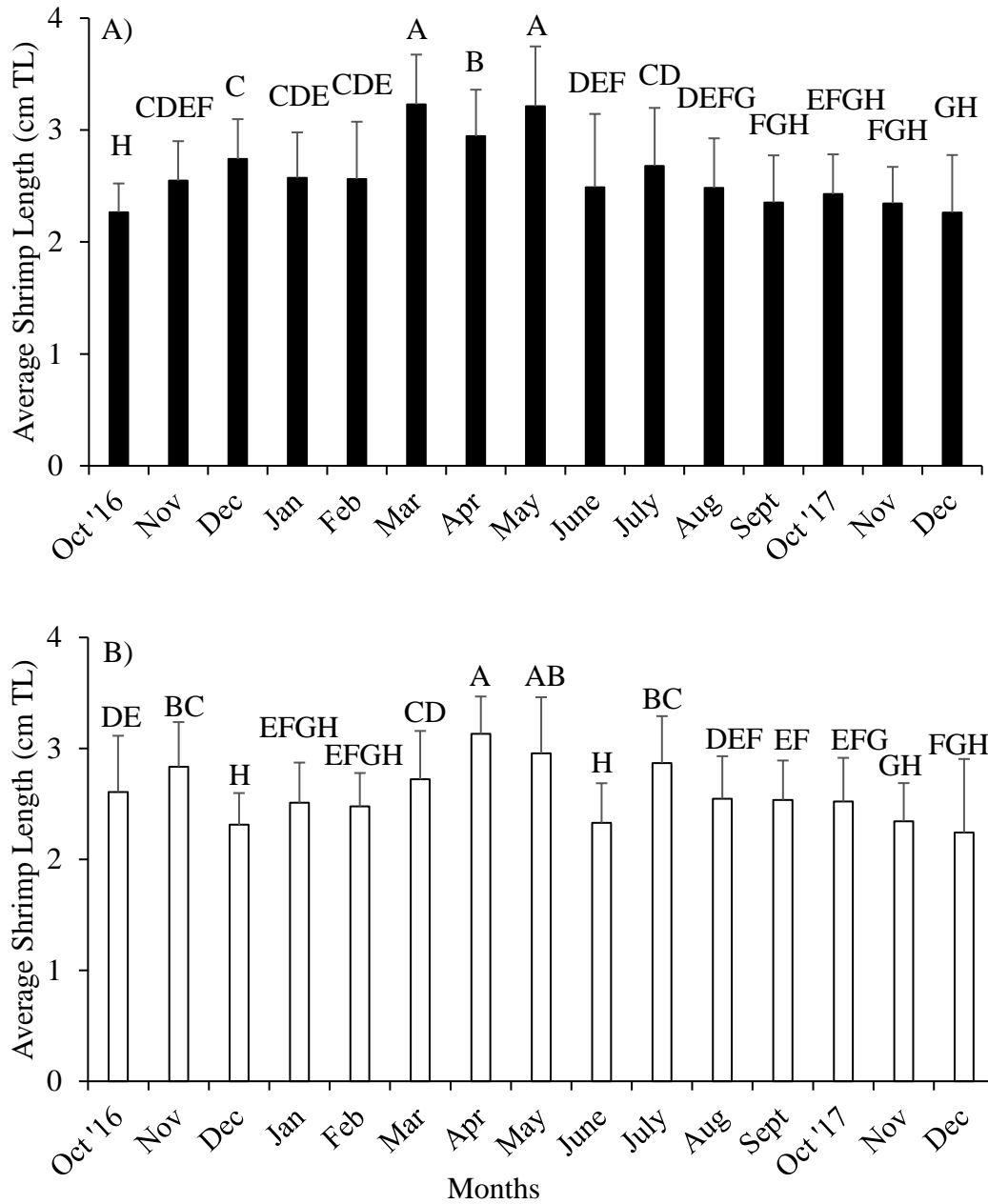


Figure 2.2. Average length and + 1 SD of the daggerblade grass shrimp *Palaemonetes pugio* at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017. Letters represent significant differences across months.

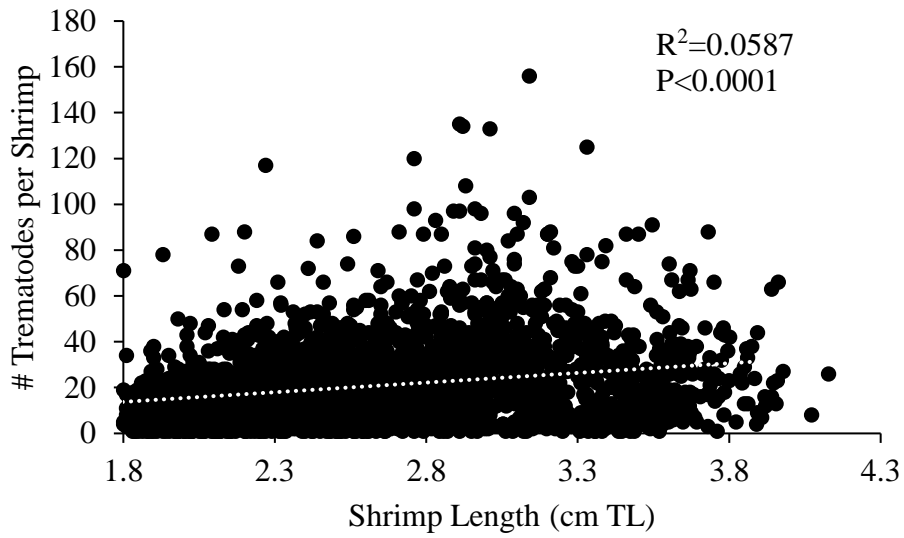


Figure 2.3. Intensity (# of trematodes within infected shrimp) of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the microphallid trematode *Microphallus turgidus* per grass shrimp length (cm TL) at the Rodney J. Hall Boat Ramp (RJHBR) and the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

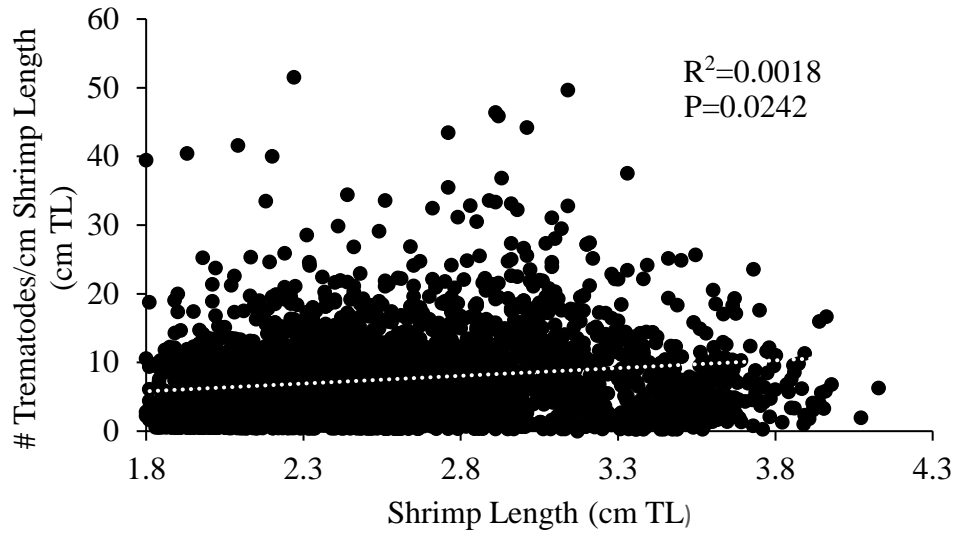


Figure 2.4. Density (# of trematodes/cm TL) of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the microphallid trematode *Microphallus turgidus* per grass shrimp length (cm TL) at the Rodney J. Hall Boat Ramp (RJHBR) and the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

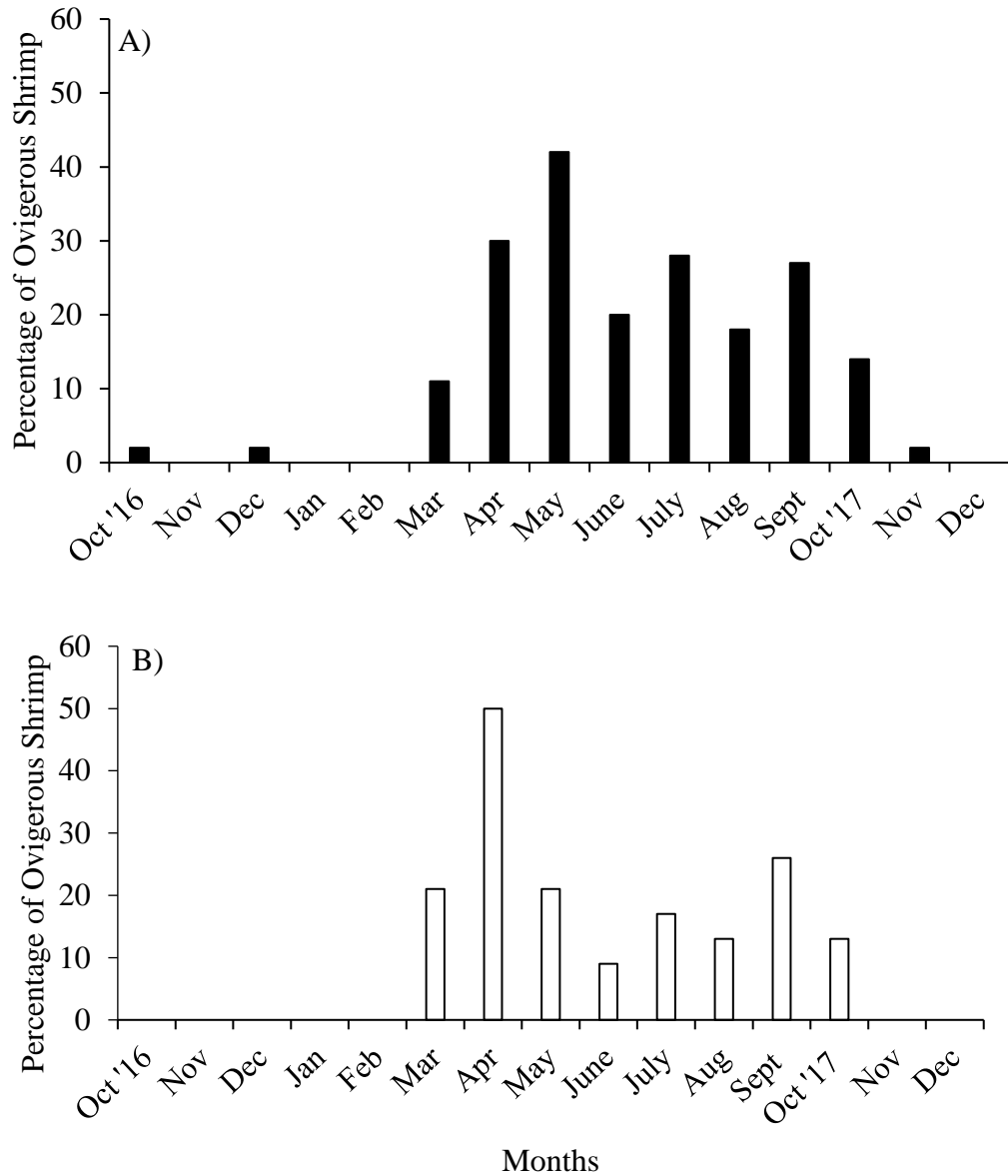


Figure 2.5. Percentage of ovigerous female daggerblade grass shrimp *Palaemonetes pugio* at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

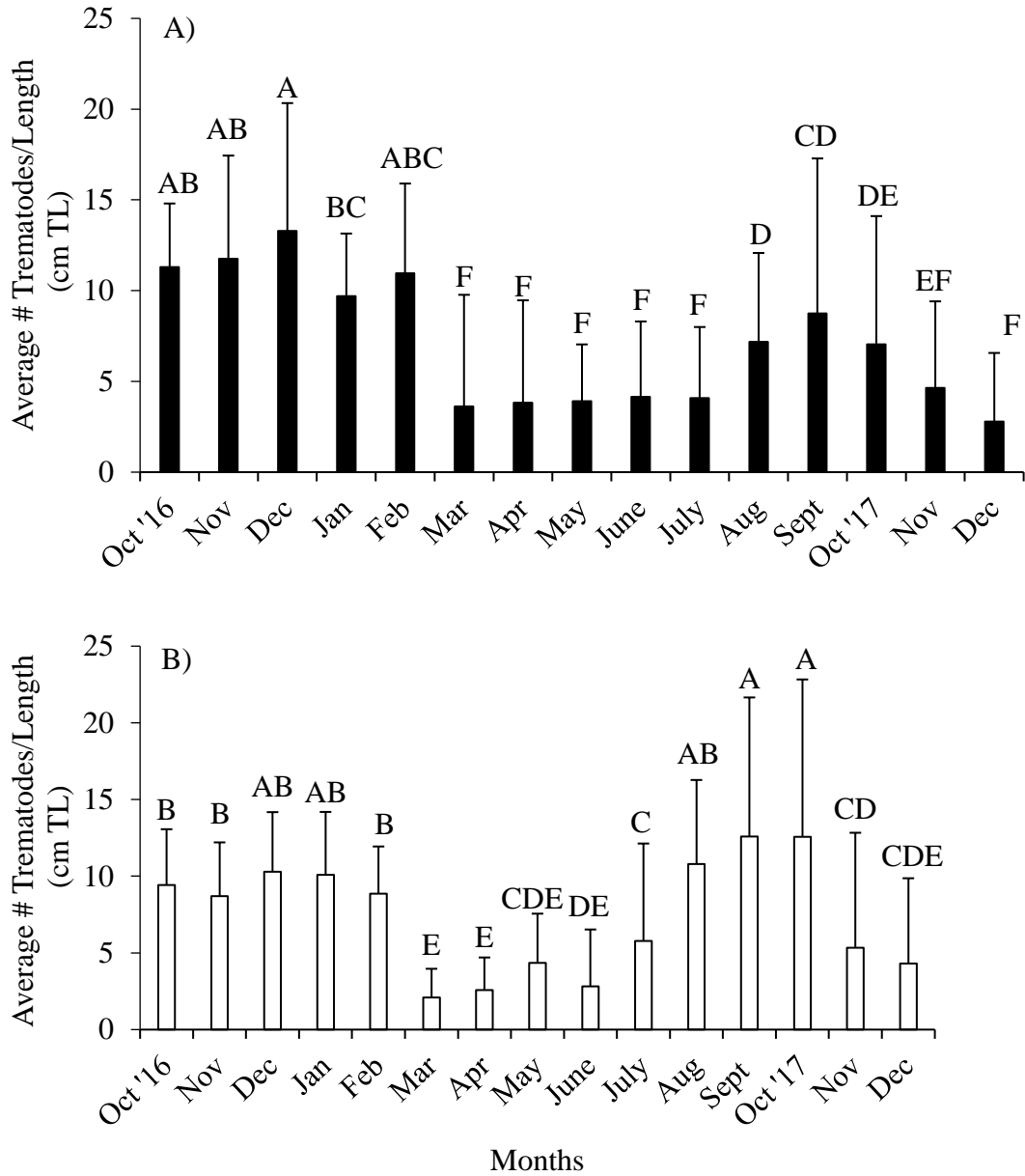


Figure 2.6. Density of trematode infection (#/cm TL) + 1 SD in the daggerblade grass shrimp *Palaemonetes pugio* at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017. Letters represent significant differences across months.

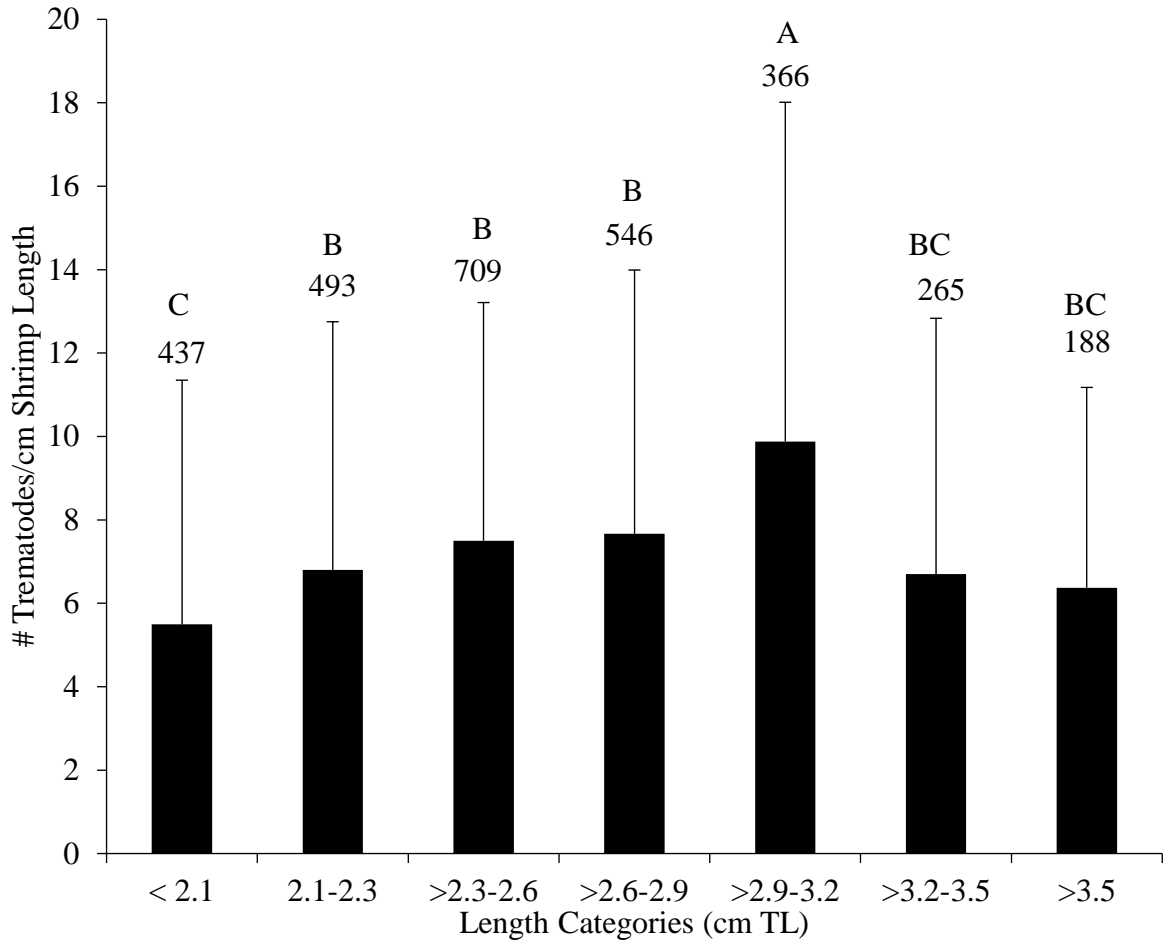


Figure 2.7. Mean density (# trematodes/cm shrimp length) of the daggerblade grass shrimp *Palaemonetes pugio* infected by the trematode *Microphallus turgidus* in different shrimp length categories at the Rodney J. Hall Boat Ramp and the Frank Downing Fishing Pier near the Skidaway River in Savannah, Georgia from October 2016 through December 2017. The mean salinity (ppt) combined for each site was 23.7 + 3.61 and the number above the error bars represents the number of shrimp in each category. Letters represent significance among categories.

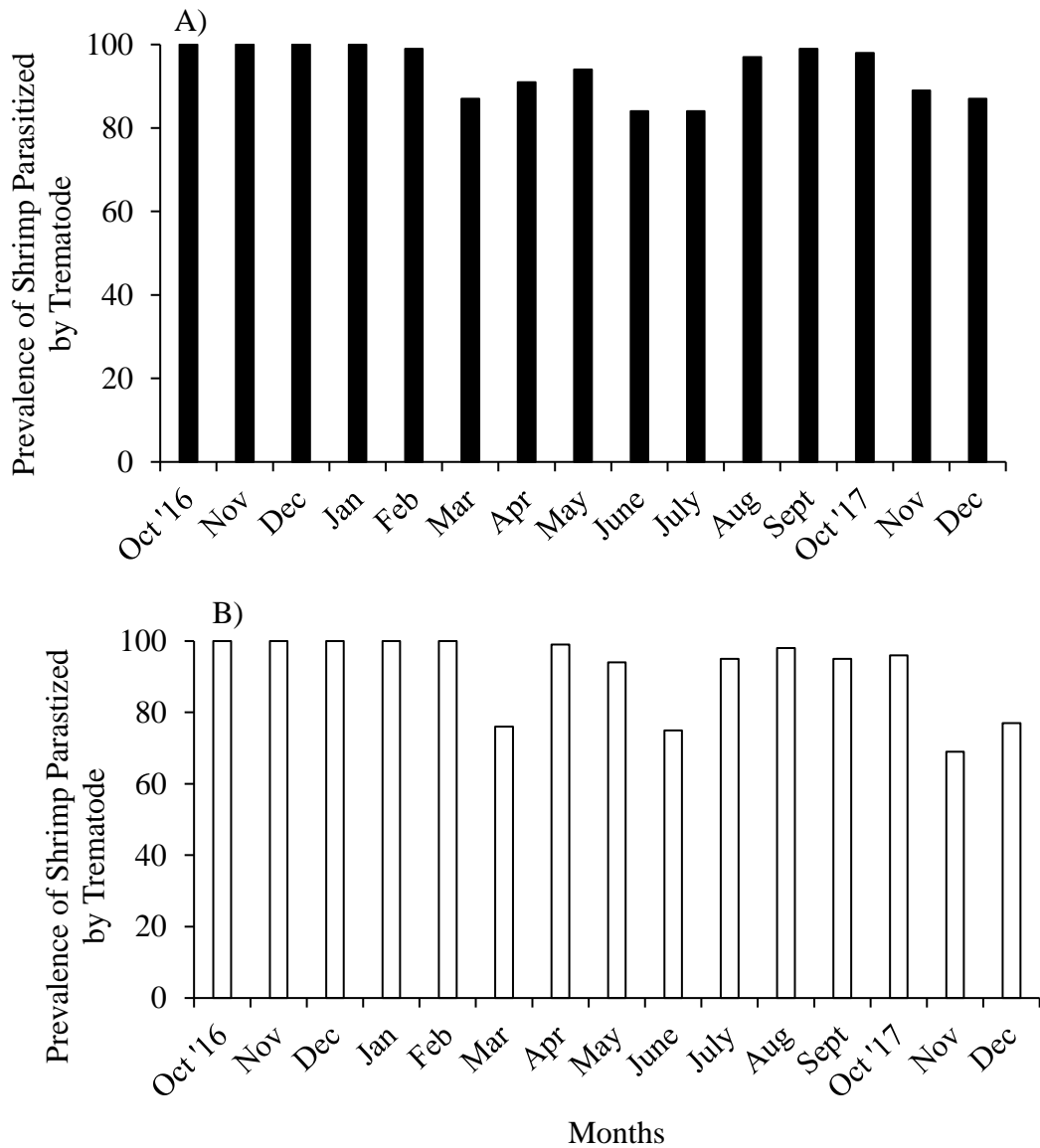


Figure 2.8. Prevalence (# individuals parasitized by the trematode) of parasitized daggerblade grass shrimp *Palaemonetes pugio* by a trematode at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

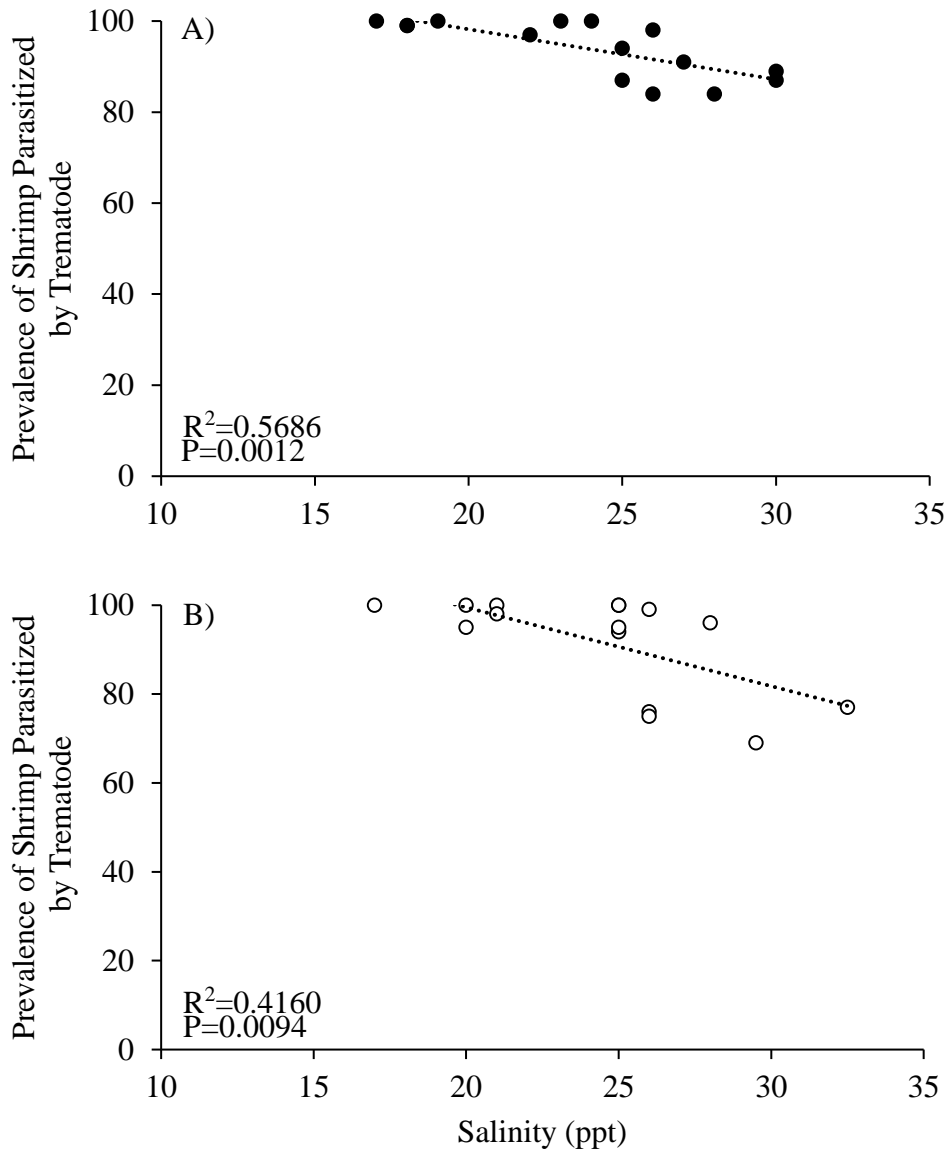


Figure 2.9. Prevalence of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the trematode parasite *Microphallus turgidus* with respect to salinity at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

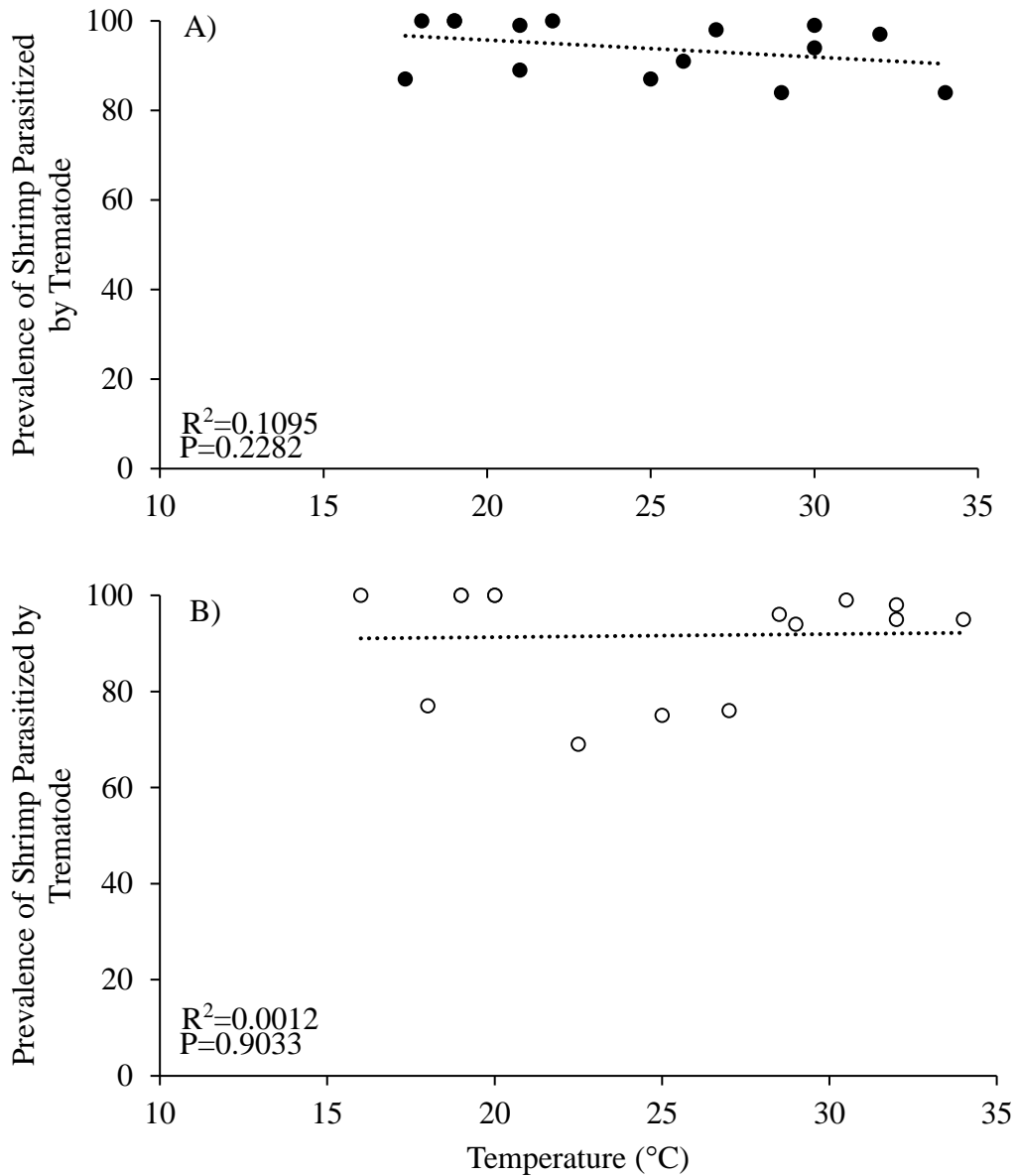


Figure 2.10. Prevalence of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the trematode parasite *Microphallus turgidus* with respect to temperature at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

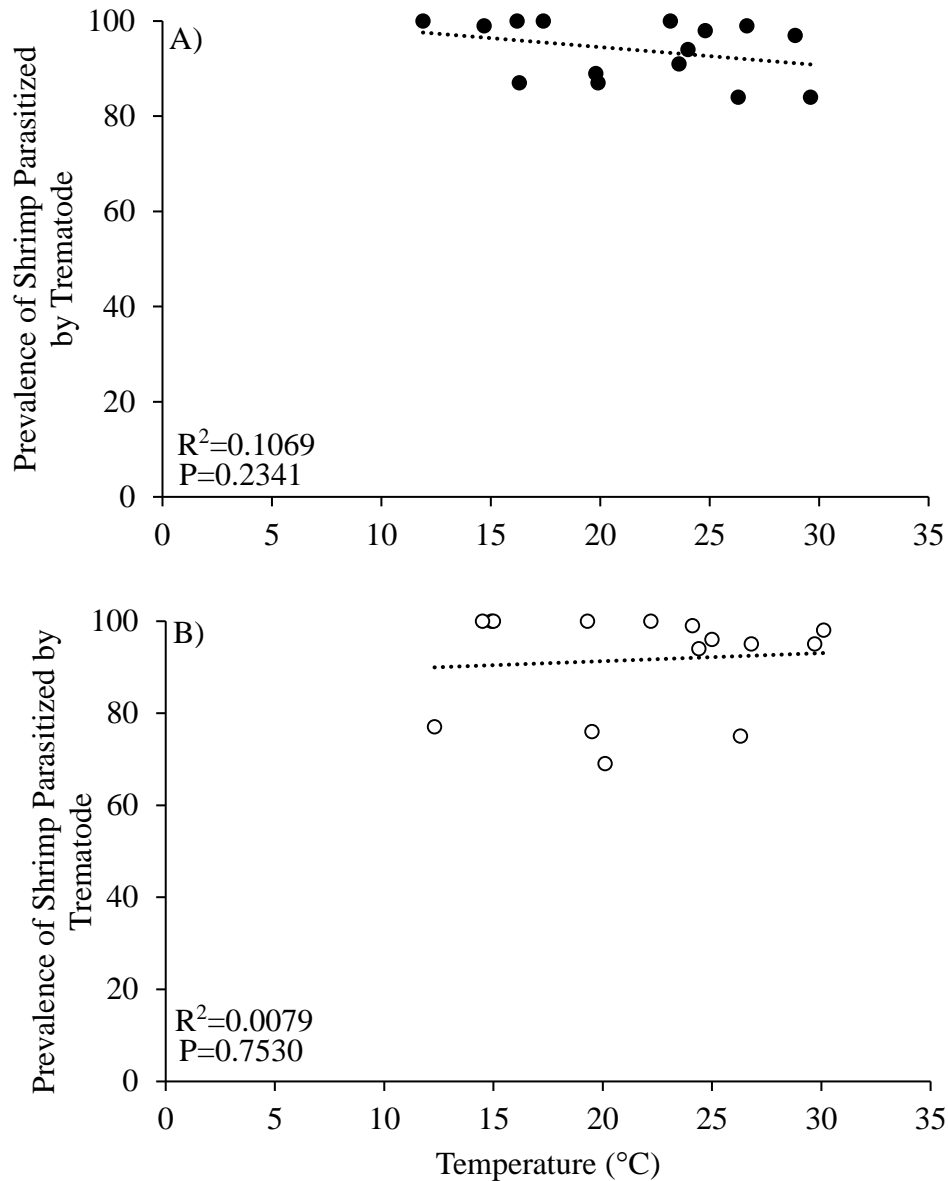


Figure 2.11. Prevalence of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the trematode parasite *Microphallus turgidus* with respect to temperature at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017. Temperature (averaging the minimum and maximum temperatures measured on the same day as sampling in the current study) was recorded from the Ft. Pulaski NOAA Data Buoy Center near Savannah, Georgia.

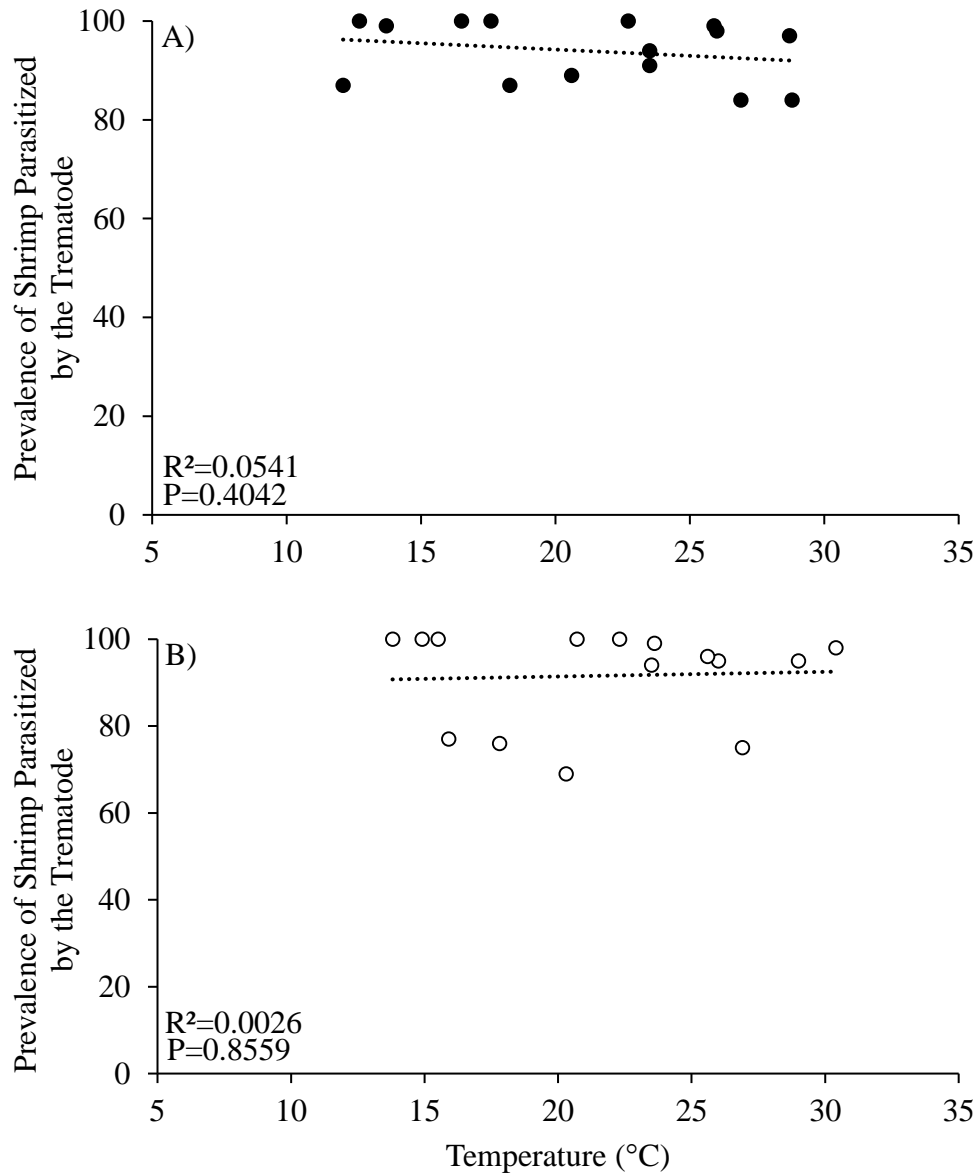


Figure 2.12. Prevalence of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the trematode parasite *Microphallus turgidus* with respect to temperature at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017. Temperature (averaging the minimum and maximum temperatures 6 days prior to the sampling day and the sampling day in the current study) was recorded from the Ft. Pulaski NOAA Data Buoy Center near Savannah, Georgia.

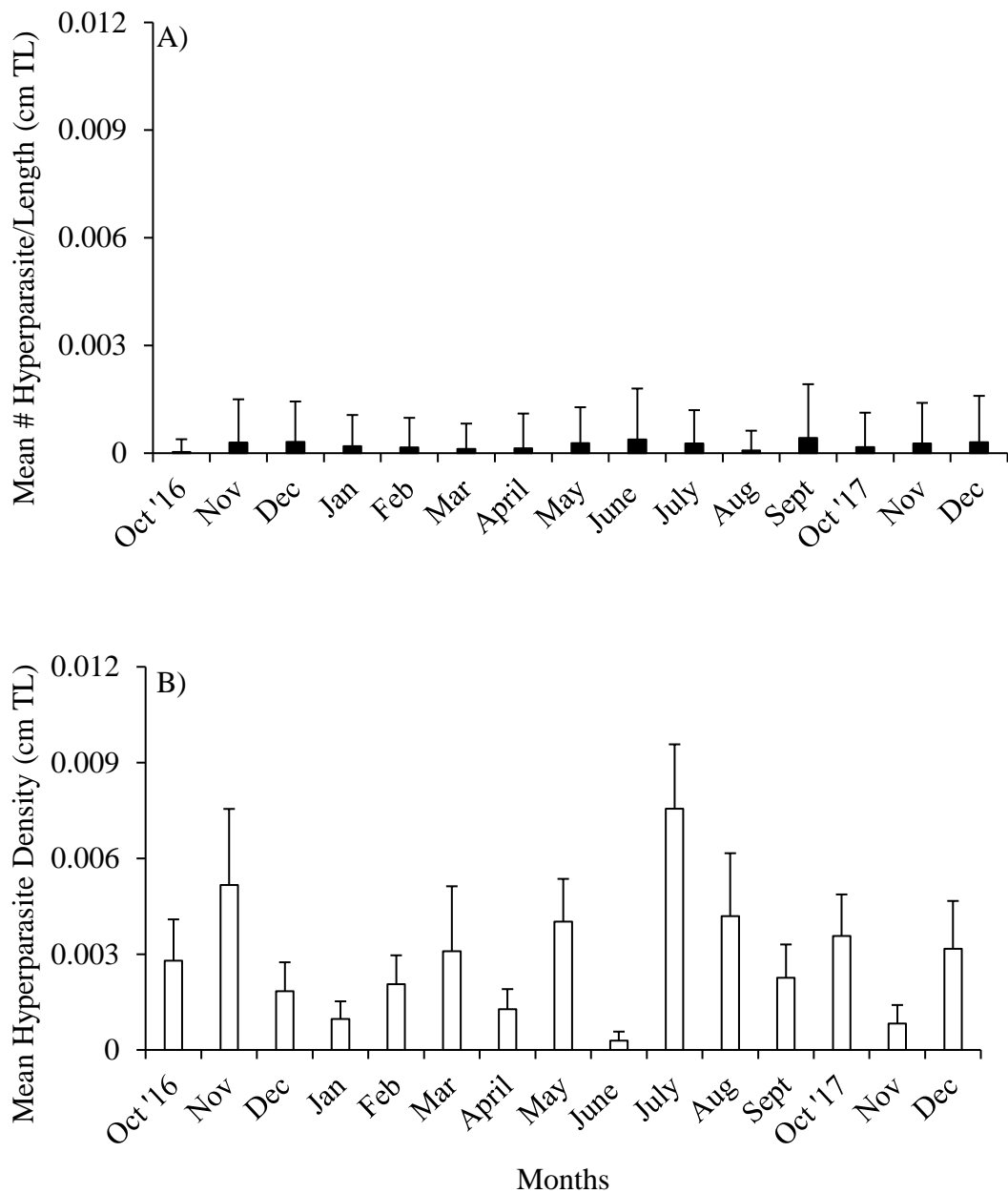


Figure 2.13. Density of a hyperparasite infection (#/cm TL) + 1 SD in the daggerblade grass shrimp *Palaemonetes pugio* at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

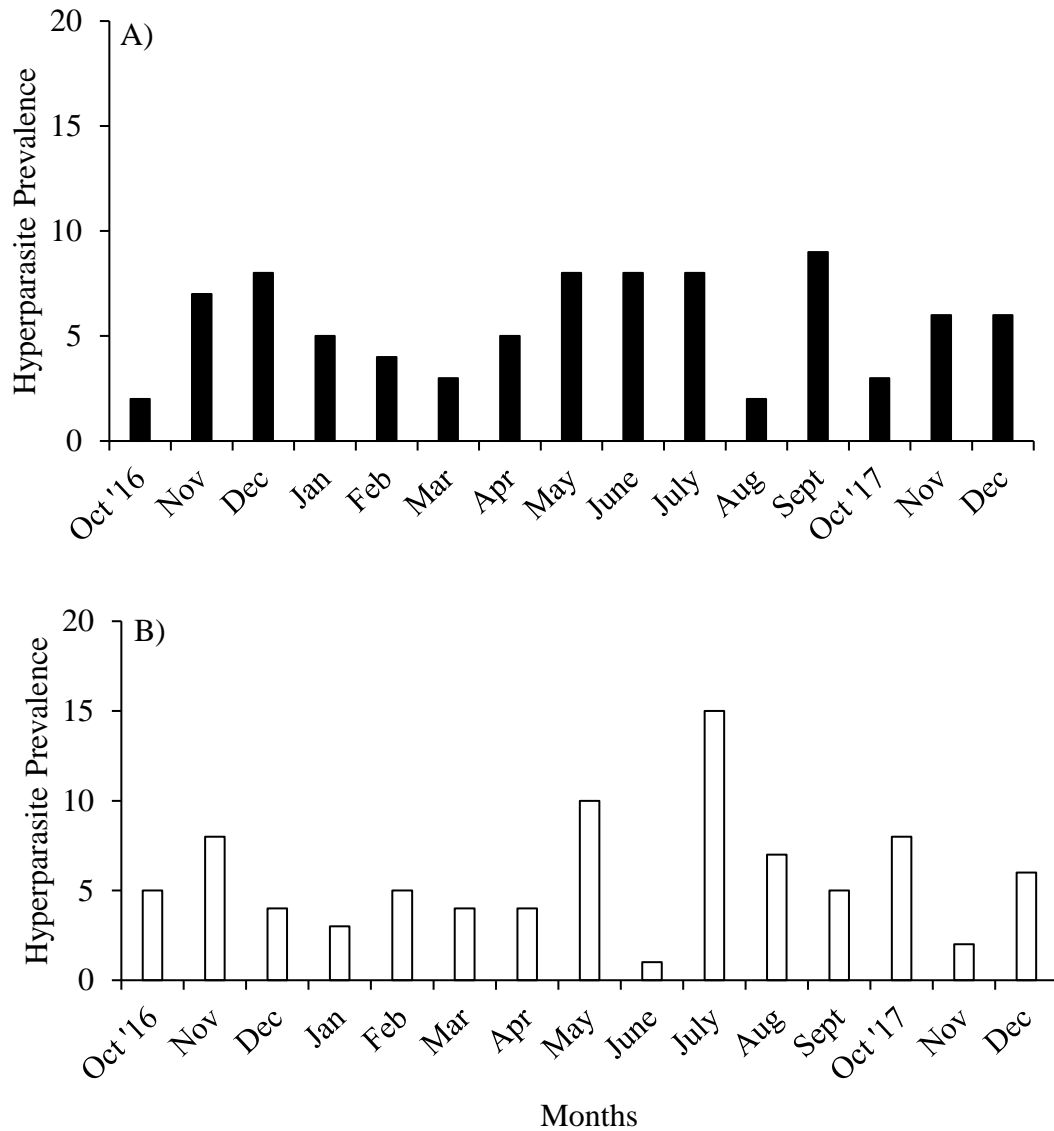


Figure 2.14. Prevalence (presence or absence) of parasitized daggerblade grass shrimp *Palaemonetes pugio* by a hyperparasite at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

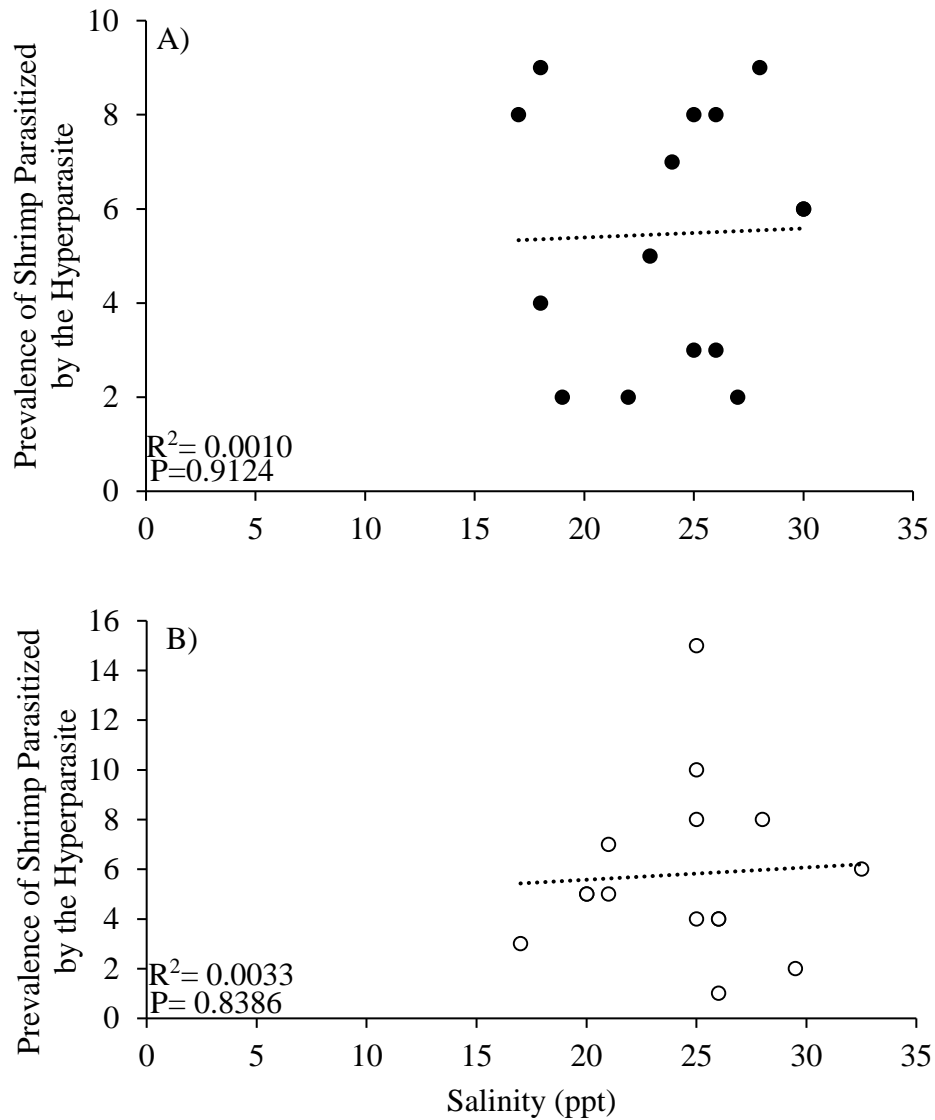


Figure 2.15. Prevalence of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the haplosporidian hyperparasite *Urosporidium crescens* De Turk with respect to salinity at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

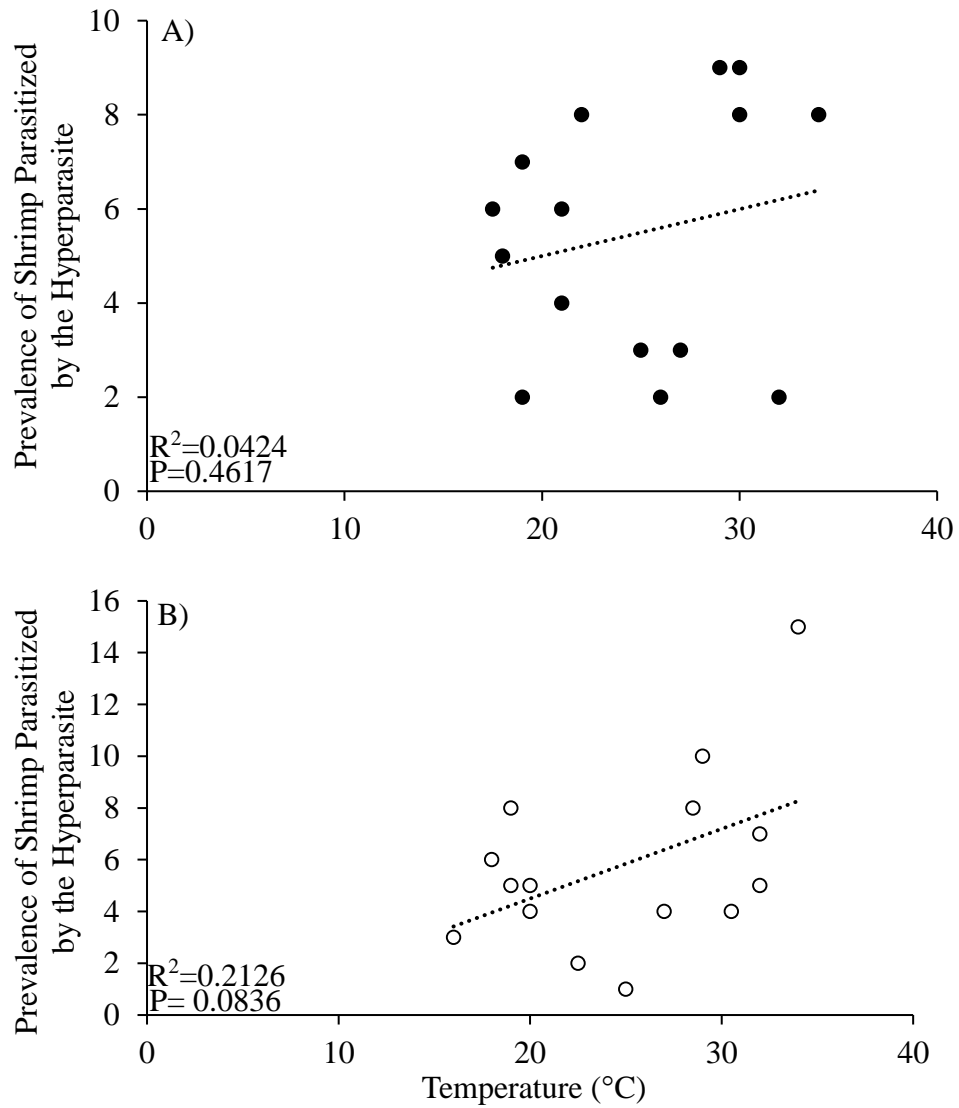


Figure 2.16. Prevalence of the daggerblade grass shrimp *Palaemonetes pugio* parasitized by the haplosporidian hyperparasite *Urosporidium crescens* De Turk with respect to temperature at A) the Rodney J. Hall Boat Ramp (RJHBR) and B) the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

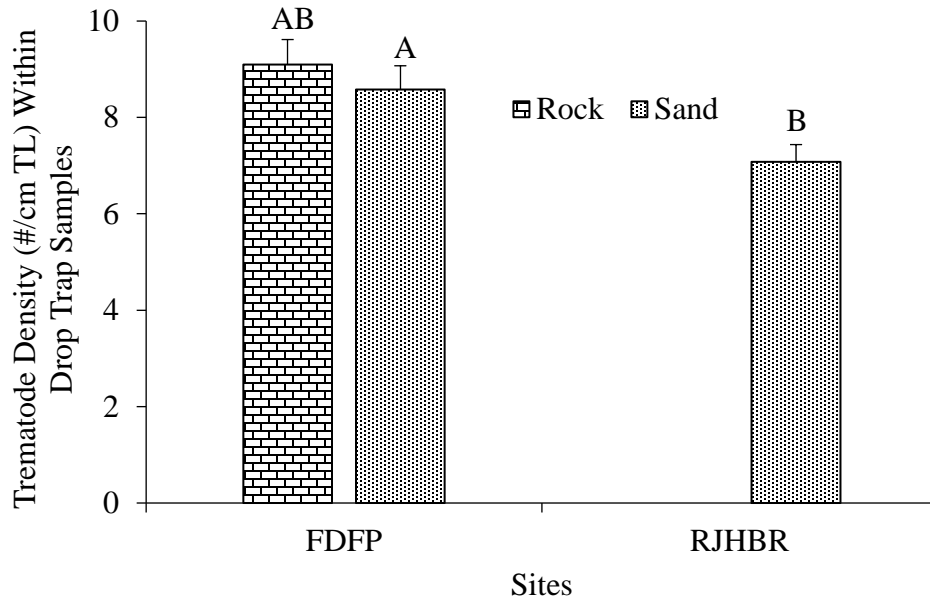


Figure 2.17. Density of the trematode *Microphallus turgidus* (#/cm TL) in the daggerblade grass shrimp *Palaemonetes pugio* in the rock with the red algae *Gracilaria vermiculophylla* and sand substrate at the Frank Downing Fishing Pier (FDFP) and the sand substrate at the Rodney J. Hall Boat Ramp (RJHBR) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017. November 2016 and June 2017 were excluded from the analysis because no shrimp were collected within the drop trap during these months at the FDFP.

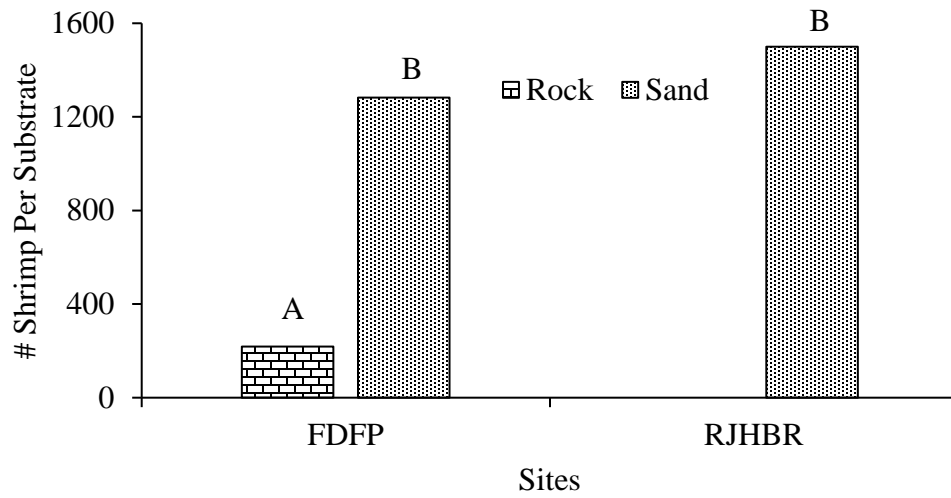


Figure 2.18. Number of daggerblade grass shrimp *Palaemonetes pugio* for all sampling events counted in the rock with the red algae *Gracilaria vermiculophylla* and sand substrate drop trap at the Frank Downing Fishing Pier (FDFP) and the sand substrate at the Rodney J. Hall Boat Ramp (RJHBR) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017. (Shrimp collected in the dip net were included for sand substrates).

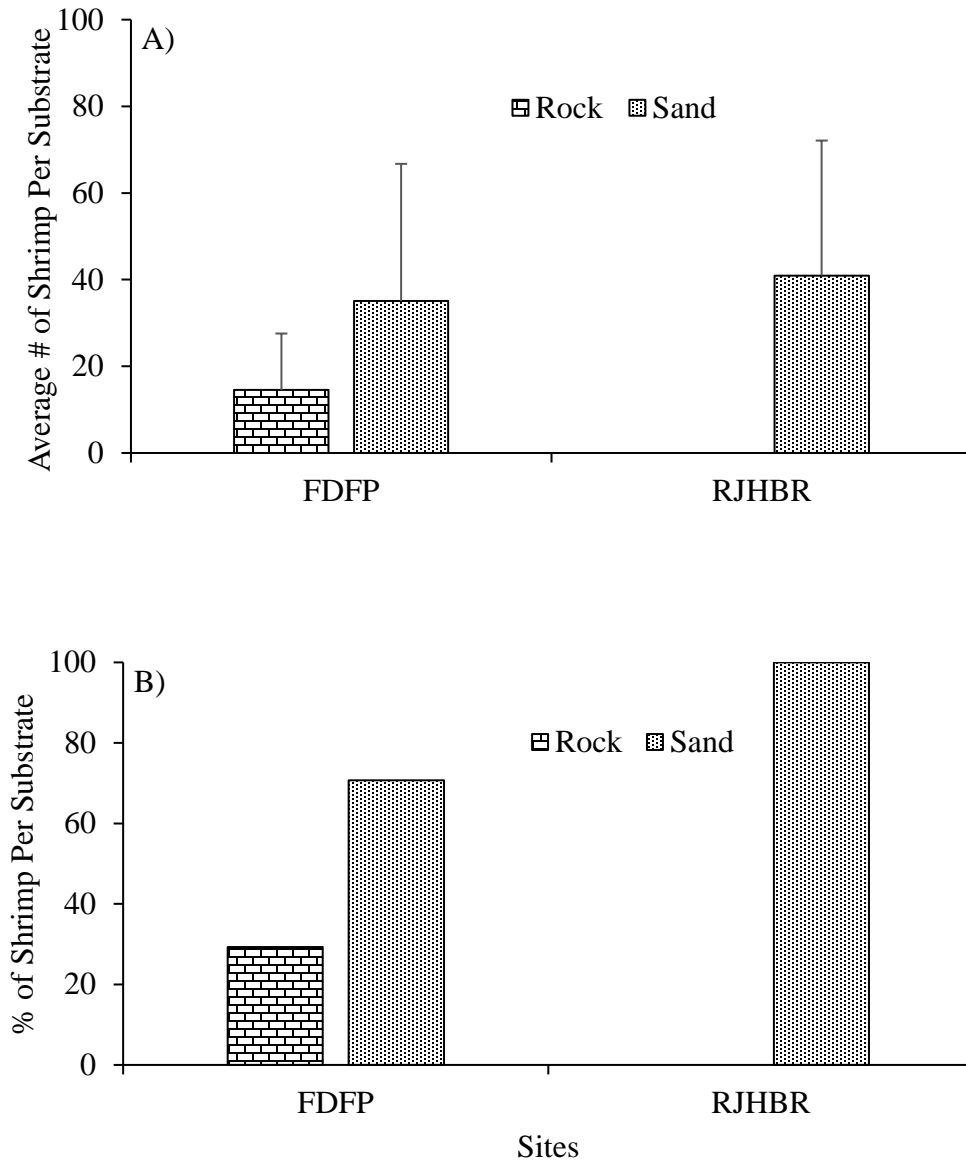


Figure 2.19. (A) Average number and (B) Percentage of daggerblade grass shrimp *Palaemonetes pugio* for all sampling events near the rock (regardless of whether or not the red algae *Gracilaria vermiculophylla* was present) and sand substrate at the Frank Downing Fishing Pier (FDFP) and the sand substrate at the Rodney J. Hall Boat Ramp (RJHBR) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017.

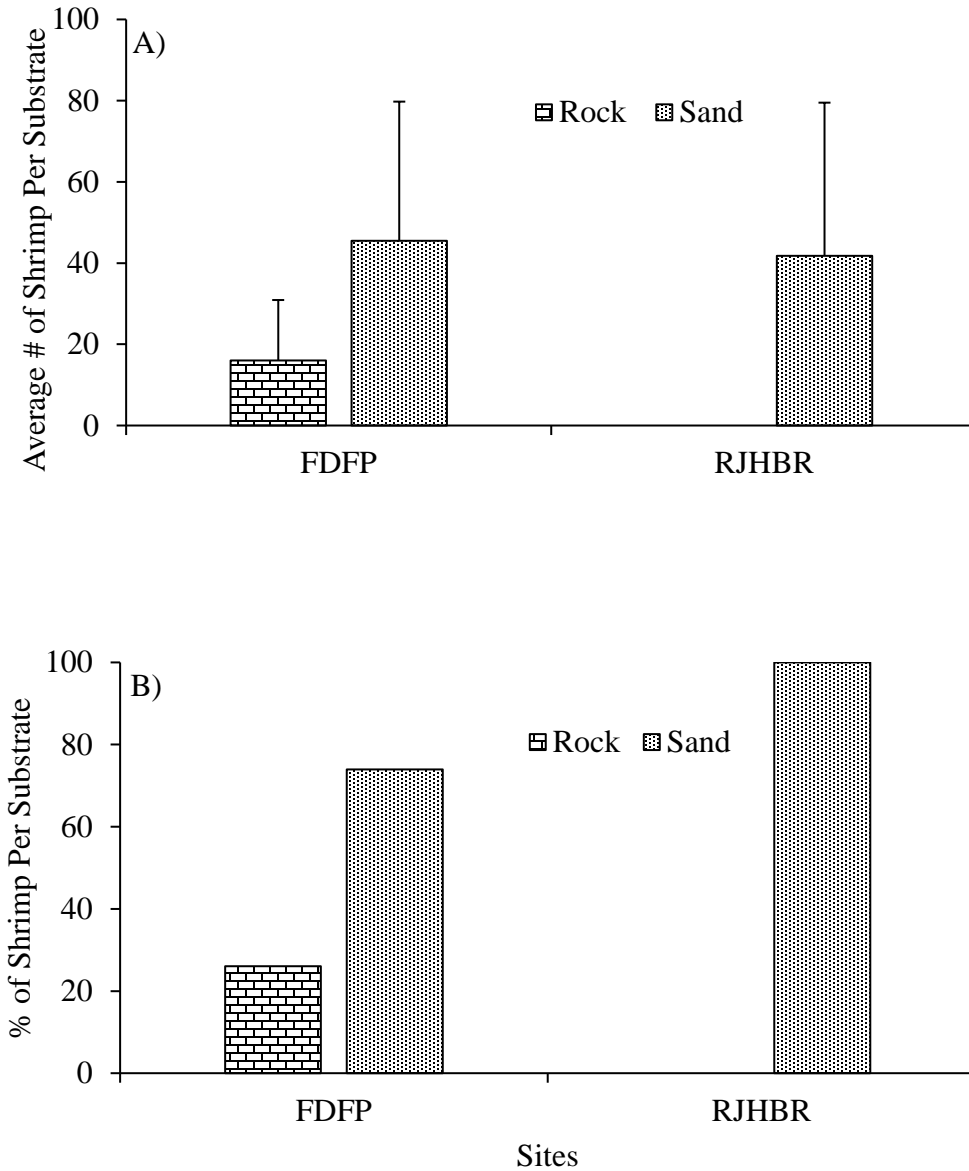


Figure 2.20. (A) Average number and (B) Percentage of daggerblade grass shrimp *Palaemonetes pugio* for all sampling events near the rock (without the red algae *Gracilaria vermiculophylla*) and sand substrate at the Frank Downing Fishing Pier (FDFP) and the sand substrate at the Rodney J. Hall Boat Ramp (RJHBR) near the Skidaway River in Savannah, Georgia from October 2016 through December 2016 and June 2017 through December 2017. (These were the months when *G. vermiculophylla* was not present).

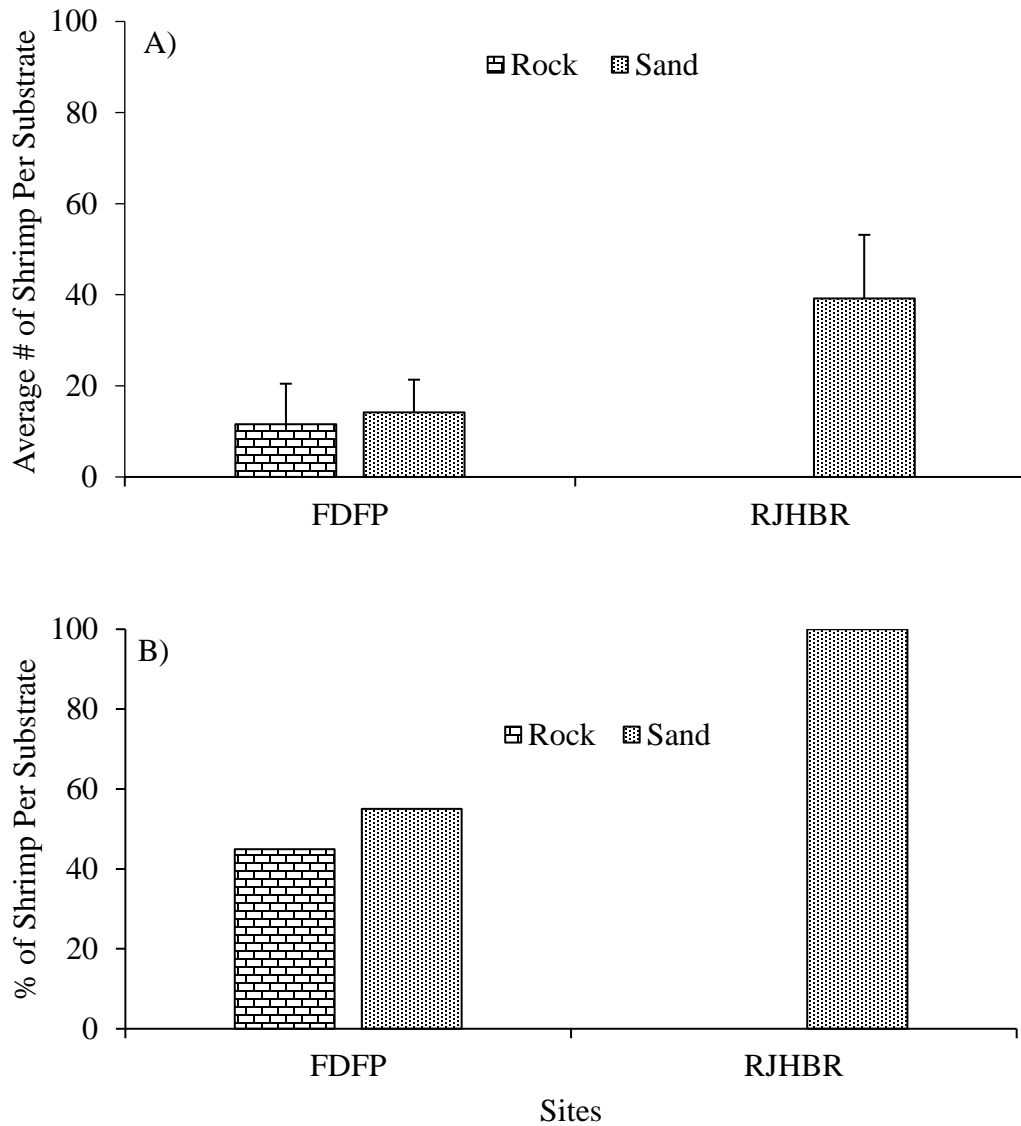


Figure 2.21. (A) Average number and (B) Percentage of daggerblade grass shrimp *Palaemonetes pugio* for all sampling events near the rock with the red algae *Gracilaria vermiculophylla* and sand substrate at the Frank Downing Fishing Pier (FDFP) and the sand substrate at the Rodney J. Hall Boat Ramp (RJHBR) near the Skidaway River in Savannah, Georgia from January 2017 through May 2017.

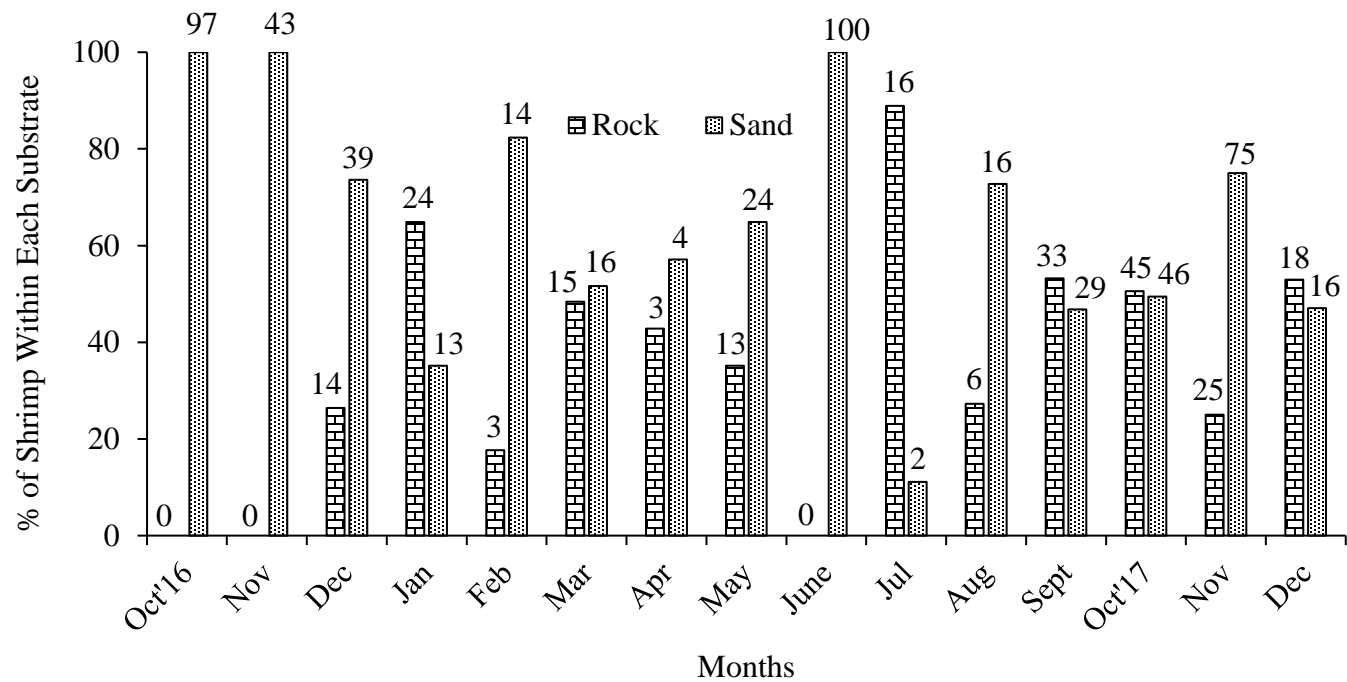


Figure 2.22. Percentage of daggerblade grass shrimp *Palaemonetes pugio* counted in the rock with the red algae *Gracilaria vermiculophylla* and sand substrate at the Frank Downing Fishing Pier (FDFP) near the Skidaway River in Savannah, Georgia from October 2016 through December 2017. The number above the bars represents the number of shrimp within each substrate. Some months have fewer than 100 shrimp because those that were caught with the dip net were excluded.

Chapter 3

Habitat preference of the daggerblade grass shrimp *Palaemonetes pugio* between the invasive red algae *Gracilaria vermiculophylla* and substrate-free open space in the laboratory

ABSTRACT

There are several factors that can influence the habitat selection of animals including food, shelter, predation, and perhaps parasites. The daggerblade grass shrimp *Palaemonetes pugio* inhabits vegetative and hard bottom substrates near areas where the infective stage of the trematode parasite *Microphallus turgidus* resides and where the red alga *Gracilaria vermiculophylla* has invaded. The purpose of this study was to determine substrate preference of infected *P. pugio* between *G. vermiculophylla* and substrate-free open space in the laboratory. Two trials were conducted on 6 separate days. During each trial, 1 shrimp was placed in each of 4 separate treatment tanks with *G. vermiculophylla* and 4 substrate-free control tanks, and shrimp were monitored every minute for 30 min. *Palaemonetes pugio* selected structure over open space in treatment tanks; however, they preferred edges of the tanks and not *G. vermiculophylla*. Only 9 out of 46 shrimp (20%) were observed the most often in *G. vermiculophylla* with a mean of $74.1 \pm 18.54\%$ of time. However, that was a stronger preference for those few shrimp than the 50% that preferred the right section of the treatment tank touching the edges (non-*G. vermiculophylla* section) with a mean of $60.6 \pm 16.81\%$ of time. Overall, 76% of treatment shrimp preferred tank edges. There were no obvious negative or positive impacts that the invasive *G. vermiculophylla* had on *P. pugio* in the laboratory, which could be ecologically relevant for *P. pugio* in the field. Habitat preference may be far more complicated than just substrate complexities itself, but could be influenced by a wide range of factors such as food availability, presence of predators, interspecies competition between *P. pugio* and *P. vulgaris*, and abiotic conditions.

INTRODUCTION

The driving force behind an organism selecting a specific habitat is perhaps because of its physical structure. However, there are many more variables to consider. Invasive species could introduce hard or soft structures to the benthic environment merely by its presence, which could alter a community by shifting members to this new habitat. Predation pressures or competition between species could also influence the selection of a habitat. Analyzing habitat preference is an effective way to determine species distributions within a certain area.

Habitat preference of animals can be affected by several factors (Boström and Mattila, 1999). Some include food, shelter (Boström and Mattila, 1999), and predation (Mittelbach, 1981; Gilliam and Fraser, 1987). Female yellow-headed blackbirds *Xanthocephalus xanthocephalus* establish territory where the emergence rates of odonates are high, which are an important food source for nestlings (Orians and Wittenberger, 1991). Female *X. xanthocephalus* also prefer dense vegetative habitat because it provides enough physical support for nests as well as refuge from predators (Orians and Wittenberger, 1991). The bluegill sunfish *Lepomis macrochirus* is capable of switching habitats from vegetation to open water as the availability of prey shifts throughout the summer at a mesotrophic marl lake in Barry County, Michigan (Mittelbach, 1981). In experimental studies, juvenile minnows *Semotilus atromaculatus* spent less time in their refuge when more food was provided, even when exposed to predators indicating that the reward of increased food outweighed the risk of predation (Gilliam and Fraser, 1987). Similarly, the isopod *Idotea baltica* preferred artificial seagrass with epiphytes over an area with shelter when exposed to a predator (Boström

and Mattila, 1999). However, habitat selection of the isopod *Erichsonella attenuata* was not because of food availability but because of predation, as *E. attenuata* preferred shelter over food (Boström and Mattila, 1999). In another laboratory experiment, the caridean shrimp *Tozeuma carolinense* shifted microhabitats when a predator was present (Main, 1987). The survival of the shrimp increased when *T. carolinense* migrated from the bottom of a seagrass blade to the top as the canopy of the upper blade appeared to offer a better refuge from the pinfish *Lagodon rhomboides* (Main, 1987).

The daggerblade grass shrimp *Palaemonetes pugio* is a crustacean that inhabits submerged vegetation and hard bottom substrates such as oyster reefs near and around shallow marsh habitats (Heard, 1982) and is located along southeastern coasts of the United States (Kneib, 1987) and the Gulf of Mexico (Anderson, 1985) and is predominantly located near the smooth cordgrass *Spartina alterniflora* (Heard, 1982). *Palaemonetes pugio* had a high density near the marsh edge where *S. alterniflora* was most abundant compared to an open water habitat near Galveston, Texas (Minello et al., 1994) and Savannah, GA (Modeste, 2009). When *P. pugio* was given a choice between *S. alterniflora* and the non-native reed *Phragmites australis* in a New Jersey estuary, *P. pugio* preferred *S. alterniflora* as habitat, perhaps because of the number of algal epiphytes and other food items attached to the stems of *S. alterniflora* (Robertson and Weis, 2007). Nematodes consisted of 82% of the epifauna on the stems; of those, 20 animals/stem were consumed by *P. pugio* over an 18 h time period compared to only 6 animals/stems on *P. australis* (Robertson and Weis, 2007). The surface area of plants and seaweeds could result in a greater density of epifauna, perhaps because of structural complexity or food and habitat availability (Parker et al., 2001). Epifaunal abundance and

biomass was significantly influenced by the abundance of the red alga *Gracilaria verrucosa*, but was not significantly influenced by the abundance of the seagrass *Zostera marina* during field studies near Chesapeake Bay, Virginia (Parker et al., 2001). The high surface area of *G. verrucosa* may have provided more food or habitat than *Z. marina* and might be the reason why higher epifaunal abundance was supported (Parker et al., 2001).

Palaemonetes pugio inhabits the marsh areas where invasion by the red alga *Gracilaria vermiculophylla* is observed. *Gracilaria vermiculophylla* is native to the northwest Pacific (Ohmi, 1956; Rueness, 2005) and has spread to the northeast Pacific including British Columbia (Saunders, 2009), and the Atlantic coast of North America including New England (Nettleton et al., 2013), Virginia (Thomsen and McGlathery, 2007), North Carolina (Freshwater et al., 2006), South Carolina (Byers et al., 2012) and Georgia (Byers et al., 2012; Wright et al., 2014). The existence of *G. vermiculophylla* in shallow estuarine systems is important as it provides a large coverage of macrophytic structure in areas that previously lacked vegetation (Byers et al., 2012). The accumulation of this alga on a mudflat appeared to alter the distribution and abundance of native amphipods as it decreased predation from the common grass shrimp *Palaemonetes vulgaris* near Savannah, GA (Wright et al., 2014). The addition of *G. vermiculophylla* may increase primary productivity and could be an important contributor to detrital foodwebs because of rapid decomposition (Byers et al., 2012). The addition increased the number of phyla that perhaps used *G. vermiculophylla* for shelter, feeding, or attachment (Nyberg et al., 2009). It is unknown if *G. vermiculophylla* impacts the habitat distribution of shrimp, but surveys have shown few shrimp in *G. vermiculophylla* (pers. observ.).

Palaemonetes pugio inhabits marsh areas and is thus exposed to parasites such as cercarian larvae of the trematode *Microphallus turgidus* (Heard and Overstreet, 1983; Pung et al., 2002; O’Leary and Pung, 2017). It is unknown whether this parasite impacts the habitat preference of *P. pugio*, but *M. turgidus* is known to affect activity. For example, *Microphallus turgidus* can affect the behavior of *P. pugio* by increasing shrimp activity levels (Kunz and Pung, 2004). The normal activities of *P. pugio* in the water column include movements such as swimming, walking, and hovering (Kunz and Pung, 2004). The frequency of these normal movements was altered in the presence of a predator when *P. pugio* was infected by *M. turgidus* (Kunz and Pung, 2004).

Palaemonetes pugio with a heavily infected trematode load of 30 or more cysts were consumed by the mummichog *Fundulus heteroclitus* more than those without trematodes in the laboratory (Kunz and Pung, 2004). *Palaemonetes pugio* that were infected had increased swimming, which caused *P. pugio* to move from its refuge and become more visible to *F. heteroclitus* (Kunz and Pung, 2004). The mechanism by which *M. turgidus* affects the behavior of *P. pugio* when exposed to a predator is not known (Kunz and Pung, 2004); even when trematode load was not considered, *P. pugio* exhibited predator avoidance depending on the activities of predators, which was documented during field experiments (Sogard and Able, 1994; Clark et al., 2003; Canion and Heck, 2009). During the day, the predation risk of *P. pugio* was different than at night as a larger number of *P. pugio* relocated to intermediate depths of the water column (30 cm) compared to more shallow depths (15 cm) (Clark et al., 2003). Migration to intermediate depths is perhaps due to both aquatic and terrestrial predatory pressures (Clark et al., 2003). There was also

a decrease in the use of shallow-water habitat and a shift in habitat utilization where grass shrimp densities were lower in refugia at night (Clark et al., 2003).

There are complexities of the parasite-host interaction related to habitat selection and predator-prey dynamics. It is known that *M. turgidus* alters the activities of *P. pugio* thus increasing the predation on *P. pugio* and that the presence of habitat structures does change the predation success of *P. pugio*. The interaction between an invasive substrate *Gracilaria vermiculophylla* and *P. pugio* has never been studied. *Palaemonetes pugio* is now exposed to a structural change within its habitat and the presence of this structure may affect the density of *P. pugio* and its prey both within *G. vermiculophylla* and away from *G. vermiculophylla*. Therefore, the purpose of this study was to determine the substrate preference of *P. pugio* between *G. vermiculophylla* and substrate-free open space in the laboratory.

HYPOTHESIS

H_{A1}: *Palaemonetes pugio* prefers *Gracilaria vermiculophylla* over substrate-free open space in the laboratory.

MATERIALS AND METHODS

A habitat preference study was conducted to determine the habitat preference of *Palaemonetes pugio* between *Gracilaria vermiculophylla* and substrate-free space. The experiment was conducted over 6 days; each day consisted of 2 trials, each with 4 treatment tanks and 4 control tanks (Table 3.1) that included:

1. Treatment: *Gracilaria vermiculophylla* + substrate-free space + one grass shrimp with a high cyst load (29+ cysts): 4 tanks

2. Substrate-free control: One grass shrimp with a high cyst load (29+ cysts): 4 tanks

Shrimp were collected from Country Club Creek (32°01'18"N 81°03'24"W) near Savannah State University in Savannah, Georgia using dip nets. *Palaemonetes pugio* was identified by examining the rostrum. The tip of the anterio-dorsal rostral margin lacks serration, as described by Anderson (1985). Only male shrimp were used for experiments and the sex was determined by the presence of the appendix masculina on the endopods of the second set of pleopods, as females lack this morphological feature (Anderson, 1985), using a dissecting microscope at 10X magnification. Shrimp were measured from the tip of the rostrum to the end of the telson and only shrimp larger than 18 mm were used. Metacercarial cysts of the trematode *Microphallus turgidus*, visible through the transparent integument of the body, were counted under the dissecting microscope and only shrimp with 29+ cysts were used for experiments. Shrimp were starved for 24 h prior to the trials because Bass and Weis (1999) found that shrimp activity decreased after 48 h of starvation. Shrimp were not fed during the experiment. Tanks used were 33 X 20 X 23 cm plastic tanks and were filled with 6 L of filtered estuarine water from Country Club Creek. Trials were conducted during the time that daytime ebb would occur at the collection site to control for the tidal rhythmicity shrimp exhibit during different tides, as Chaplin-Ebanks and Curran (2005) found that shrimp were most active during ebb tide. The first trial occurred 45 min before ebb tide and the second ended 45 min after ebb tide. All 8 shrimp were observed every min for 30 min during each trial similar to Brinton and Curran (2015) who assessed the behavior of *P. pugio* in the presence of *Fundulus heteroclitus* every 30 s for 30 min per replicate.

Trials (treatments and controls) were conducted with tanks sectioned into thirds. *Gracilaria vermiculophylla* was rinsed only placed on the left side 1/3 of the tank leaving the center 1/3 section and the right section 1/3 of the tank open (Figure 3.1). Shrimp were identified in different sections of the tanks either in the open space of each section or along the edges. Shrimp were considered touching the edges if they were observed swimming into or resting on any 4 sides of the tank including the corners. However, if shrimp were on the bottom of the tank (not swimming into or resting on the sides of the tank) they were counted as in the open space. Shrimp were considered in *G. vermiculophylla* if they were: resting on the branches, anywhere within the clump of algae, or anywhere in the sectioned 1/3 only if they were not touching the edges.

Treatment sections were labeled as:

GG=left section of the tank within *G. vermiculophylla*;

GS=left section of the tank touching the edges;

OO=center section in the open space;

OS=center section touching the edges;

EE=right section of the tank in the open space;

ES=right section touching the edges (Figure 3.1).

The sections in the substrate-free control tanks were labeled as:

EEL=left section of the tank in the open space;

ESL=left section touching the edges;

OO=center section in the open space;

OS=center section touching the edges;

EER=right section of the tank in the open space;

ESR=right section touching the edges (Figure 3.2).

This design was used to determine if the tank structure, the edges specifically, were preferentially selected by *P. pugio*. Summary statistics were calculated in Microsoft Excel to determine frequency (# of times shrimp were observed in any location), percentage (% of shrimp observed in preferred location), and mean percent (highest # of shrimp within preferred location/total # of shrimp (48) X 100). To resolve the repeated measures of observing each shrimp for 30 min, 1 value was measured for each shrimp and that value was the preferred location of each shrimp. A one-way ANOVA was conducted to test for the difference in shrimp preference between *G. vermiculophylla* and substrate-free open space. Prior to analysis, the data were analyzed via the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. All analyses were performed in SAS (Version 9.4).

RESULTS

In general, more shrimp preferred structure than open space in the treatment tanks. Grass shrimp preferred the right section of the tank touching the edges (ES) (502 times out of 1,440 observations) (Figure 3.3A). Grass shrimp were observed least in the center of the tank touching the edges (OS) with 53 times out of 1,440 observations (Figure 3.3A). To avoid having multiple observations for a shrimp, its preferred observation was also recorded. The highest percentage of grass shrimp that preferred any 1 location more than all others was 50% (Figure 3.3B) and those 23 grass shrimp chose

ES the most often with a mean of $60.6 \pm 16.81\%$ of time (Table 3.2). Conversely, no shrimp ever preferred open space with the lowest percentage of 0% in the center of the tank (OO), the center of the tank touching the edges (OS), and on the right section of the tank not touching the edges (EE) (Figure 3.3B).

In the control tanks, grass shrimp were counted more on the left section of the tank touching the edges (ESL) (417 times out of 1,440 observations) (Figure 3.4A). Shrimp preferred the center of the tank touching the edges (OS) the least with 48 times out of 1,440 observations (Figure 3.4A). To avoid having multiple observations for a shrimp, its preferred observation was also recorded. The highest percentage of grass shrimp that preferred any 1 location more than all others was 38% (Figure 3.4B) and those 17 shrimp preferred ESL to all other locations with a mean of $52.4 \pm 10.33\%$ of time (Table 3.2). No shrimp preferred OS (0%) (Figure 3.4B).

The number of times that grass shrimp was in *Gracilaria vermiculophylla* (GG) was compared to the number of times that grass shrimp was in the combined edge locations and in open space locations (Figure 3.5A). Shrimp were in *G. vermiculophylla* 300 times, which was higher than grass shrimp in the center of the tank and the right section of the tank not touching edges (OO+EE) with 262 times out of 1,440 observations. However, the number of grass shrimp in the combined edge locations (GS+ES+OS) was almost 3 times higher than the number of grass shrimp observed in the other locations with 878 times (Figure 3.5A). The percentage of shrimp within GG was 20%, which was also greater than the 0% of grass shrimp in OO+EE in the treatment tanks (Figure 3.5B). Only 9 out of 46 shrimp were observed the most often in *G. vermiculophylla* with a mean of $74.1 \pm 18.54\%$ of time. However, that was a stronger

preference for those few shrimp than the 76% that preferred the combined edge locations on the left and right section (GS+ES) ($53.3 \pm 17.64\%$ of time) (Table 3.2).

The location that paralleled GG in the control tanks was the left section of the tank not touching the edges (EEL). All edge and open space locations were also combined and compared to the number of times out of 1,440 observations shrimp were in the edge or open space locations. Grass shrimp were found more in the combined edge locations (ESL+ESR+OS) (836) than the center and right section locations (OO+EER) (357) and EEL (247) (Figure 3.6A). Twenty percent of shrimp were observed more in EEL than OO+EER (13%), but not ESL+ESR+OS (64%) (Figure 3.6B). There were 9 shrimp that preferred EEL with a mean of $39.3 \pm 10.24\%$ of time (Table 3.2); however, that was not greater than the 29 shrimp that preferred the edge locations the most often than any other location with a mean of $51.6 \pm 11.94\%$ of time.

The preference of grass shrimp to choose a specific structure was observed when all of the open space locations were combined (GG+OO+EE) (Figure 3.7A) and edge locations were combined (GS+OS+ES) (Figure 3.7B) in the treatment tanks; similarly, the open space locations (EEL+OO+EER) (Figure 3.7A) and edge locations (ESL+OS+ESR) (Figure 3.7B) in the control tanks were combined. Grass shrimp chose open space locations and the *Gracilaria vermiculophylla* location in the treatment tanks 562 times in GG+OO+EE, compared to 15 times in EEL+OO+EER in the control tanks (Figure 3.7A). However, grass shrimp preferred edge locations to open space locations GS+ES+OS (878) in the treatment tanks and in ESL+ESR+OS (836) in the control tanks, respectively (Figure 3.7B). Twenty percent of shrimp were observed in the open space locations in the treatment tanks while 33% of shrimp were observed in the open space

locations in the control tanks (Figure 3.8A). The percentage of shrimp in the edge location in the treatment tanks was 76% and 64% in the control tanks (Figure 3.8B).

There was a 1 out of 6 (17%) chance that shrimp would be in any of the 6 locations in the tank if there were no preference. The results were that a similar percent (19) of shrimp (9 out of 48 shrimp) were in the same location at min 1 as min 30 in the treatment tanks and there were only 23% of shrimp (11 out of 48 shrimp) that were in the same location at min 1 as min 30 in the control tanks. Therefore, the location of shrimp at min 1 did not seem to affect where shrimp were at at min 30, given that the percentage of shrimp at these same locations only slightly exceeded the likelihood they would be there by chance.

DISCUSSION

The major finding of this study was that structure in general was preferred over open space with 76% of shrimp preferring the tank edges, 20% of shrimp preferring *Gracilaria vermiculophylla* and only 4% of shrimp selecting open space. The hypothesis was supported in that shrimp preferred *G. vermiculophylla* to open space. The edge structure preference was not expected and could have been an artifact of the rectangular tanks used for habitat preference of *P. pugio* in the laboratory. However, it was expected that there would not be a high number of or percentage of *P. pugio* in the open space, because during field studies by Minello et al. (1994) the mean density of *P. pugio* was 97.5 ± 28.4 near the marsh edge where *Spartina alterniflora* was most abundant compared to open water 1.0 ± 0.70 . In the laboratory, the volume of water in contact with the edges of the tanks was much less than the volume of water in contact with the open space and *G. vermiculophylla*, yet the edges were still preferred. In other studies, the

abundance of some species such as the sand shrimp *Crangon septemspinosa* and *P. pugio* near marsh edges in less than 35 cm in depth was significantly greater than mid-zone and deep zone depths along subestuaries of the Chesapeake Bay River, Maryland (Ruiz et al., 1993). The preference of shrimp to choose edge locations was expected as per studies by Minello et al. (1994) and Ruiz et al. (1993). Ruiz et al. (1993) also observed greater abundance of *P. pugio* in locations where submerged aquatic vegetation (SAV) was present compared to areas without SAV. In addition, oyster reefs are another submerged structure that are an important hard bottom substrate that provides a complex 3D structure that results in many microhabitats (Tolley and Volety, 2005). The density of the bigclaw snapping shrimp *Alpheus heterochaelis*, the flatback mud crab *Eurypanopeus depressus*, and the green porcelain crab *Pertrolisthes armatus* and species richness were greater in oyster reefs than in sand substrates during field surveys near Tarpon Bay, Florida (Tolley and Volety, 2005). *Alpheus heterochaelis* had a mean density of 2.8 ± 0.5 in live oyster clusters and only a mean density of 0.3 ± 0.6 in sand bottom substrates (Tolley and Volety, 2005). Organisms are not dependent upon the oyster reefs themselves but the complexity of its 3D shape (Tolley and Volety, 2005). Oyster reefs and seagrass beds of *Thalassia testudinum* support a greater number of decapod crustaceans than non-vegetative marsh edges (Glancy et al., 2003). Oyster reefs supported 15 times more abundance of decapods than the marsh edge and 2 times more than seagrass near the St. Martins Aquatic Preserve within the Gulf Coast of Florida (Glancy et al., 2003). Even when density of *T. testudinum* was at its highest in the summer, the biomass of decapods was two times greater in the 3D-structured oyster reefs (27.98 g m^{-2}) than those observed in the seagrass (13.44 g m^{-2}) (Glancy et al., 2003). The mud crabs *Eurypanopeus*

depressus, *Panopeus herbstii*, and *Petrolisthes armatus* and *Alpheus heterochaelis* accounted for 95% and 5% of the decapods within the oysters during the summer and spring sampling periods, respectively (Glancy et al., 2003). In other studies, density of benthic infauna within vegetation near the marsh edge was greatest during the spring and early summer months near Galveston, Texas (Whaley and Minello, 2002).

Another 3D complex structure, the branched red macroalgae *Gracilaria verrucosa* increased the abundance of epifauna in manipulated field studies (Parker et al., 2001). The increased abundance of the isopod *Erichsonella attenuata* and the amphipods *Gammarus mucronatus* and *Cymadusa compta* was positively related to the abundance of *G. verrucosa* (Parker et al., 2001). *Gracilaria verrucosa* perhaps supports a greater abundance of epifauna than seagrass because of its morphological complexity or its surface area (Parker et al., 2001). Although seagrasses like *Zostera marina* have flat blades and are not considered more structurally complex than the branched alga *G. verrucosa* (Parker et al., 2001), the pistol shrimp *Alpheus pacificus* preferred to build burrows in areas with low abundances of the seagrass *Zostera capricornia* rather than areas where *Z. capricornia* was disturbed (Bell and Westoby, 1986). When given a choice between structure and no structure, all organisms preferred structure (Bell and Westoby, 1986). When given a choice between a flat structure such as *Z. marina* over a more relatively 3D complex structure such as *G. verrucosa*, the epifauna *E. attenuata*, *G. macronatus*, and *C. compta* preferred *G. verrucosa* (Parker et al., 2001). Although not directly comparable, in the present study when *P. pugio* was given the choice between a flat structure like the edges of the tank, and a 3D complex structure such as *G. vermiculophylla*, *P. pugio* preferred the flat edges. Knowlton et al. (1994) also conducted

habitat preference experiments in the laboratory, but with four different substrates: sand, mud, wood, and shell hash. The substrates were arranged so that a different substrate was in each corner of the tanks to alleviate any corner effect (Knowlton et al., 1994). Wood was held down in the tank by a zinc weight to eliminate floating, and to reduce turbidity, mud was placed over a 1 cm layer of sand (Knowlton et al., 1994). The mud substrate was selected most by *P. pugio* with about 48% of individuals and the least preferred substrate was shell hash with only about 12% of individuals (Knowlton et al., 1994). However, when *Palaemonetes vulgaris* was present there was a significant increase of *P. pugio* in the wood structure because of interspecies competition (Knowlton et al., 1994). In the present study, perhaps the reason why there was never a high percentage of *P. pugio* in different locations other than in the edge locations was because there was never any competition between species, nor was there any predatory pressures affecting *P. pugio* to seek refuge. A species interactions study should be conducted to determine whether there is an effect of interspecies interaction on the habitat preference of *P. pugio* and *P. vulgaris* and/or if *P. vulgaris* is able to displace *P. pugio* from the edges.

Edges of the tanks were always preferred, even when a more structurally complex substrate was present in the laboratory. However, *Gracilaria vermiculophylla* was always preferred over open space. Although it was surprising that shrimp preferred edges, it was not surprising that they selected a location consistently. During field studies, grass shrimp had high fidelity for sites in which they were captured and released (Allen et al., 2015). Tank size may not have been the issue for edge selection because in the field Allen et al. (2015) found that 13-23 days after *Palaemonetes pugio* was released, an average of 98.1% of individuals were recaptured at the same location of release (0 m). Since it was

unknown if shrimp ventured out of the site before recapture (Allen et al., 2015), it is possible that shrimp prefer to stay in small areas. Not much information is available on crustaceans and repeated movement patterns (Allen et al., 2015), but there could be an advantage to being familiar with an area. In fact, as observed in the current study, all but 2 shrimp had a strong location preference. Seventy six percent of the shrimp preferred edges. No shrimp preferred open space. Despite these preferences, there was little relationship between where a shrimp was in min 1 and where it was in min 30.

Palaemonetes pugio may not have left *G. vermiculophylla* once they were there. However, the structural complexity of *Gracilaria vermiculophylla* or the flat tank edges may not have been the reasons why *P. pugio* selected for these structures, but because there was no need to leave the structures once they were there. There were no predators present or interspecies conflict to displace them and even though there was not a food source, the risk of leaving to find food might have been more than the risk of starving in case predators were encountered. It might be advantageous for organisms to be familiar with a specific location because of benefits related to predator avoidance as well as energetic advantages from enhanced foraging success (Allen et al., 2015). There were not any obvious negative or positive impacts that the invasive *G. vermiculophylla* had on *P. pugio*, but perhaps if *G. vermiculophylla* had not been cleaned prior to experimentation and instead was a habitat for an epifaunal food source, or if predators were introduced, *P. pugio* may have preferred *G. vermiculophylla* to the edges of the experimental tanks. It is unclear whether the structure of *G. vermiculophylla* affected the preference of *P. pugio*, but the results in the current laboratory study parallel the results from the current field study in which significantly fewer shrimp were found near *G. vermiculophylla* than sandy

substrates. However, it is unknown if fewer *P. pugio* were near *G. vermiculophylla* because of *G. vermiculophylla* itself or if it was because of the rocks or potential predators near the rocks *G. vermiculophylla* was attached to. Habitat preference is far more complicated than just substrate complexity itself, and is also influenced by a wide range of factors such as food availability, presence of predators, and abiotic conditions. It is possible that *G. vermiculophylla* replaced a more desirable habitat of *P. pugio*, thus shifting overall community dynamics.

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Table 3.1. Experiments were conducted to determine the habitat preference of the daggerblade grass shrimp *Palaemonetes pugio* between the invasive red algae *Gracilaria vermiculophylla* and substrate-free open space in the laboratory. Shrimp were placed individually in 4 separate 33 cm X 20 cm X 23 cm substrate tanks and 4 separate substrate-free control tanks. All 8 shrimp were observed every min for 30 min during each trial to determine the preferred habitat of the shrimp.

	# Days	# Trials	# Reps	# Shrimp	# Min	# Observations
Treatment	6	2	4	48	30	1440
Control	6	2	4	48	30	1440

Table 3.2. The frequency and mean percentage (out of 1,440 observations) of the daggerblade grass shrimp *Palaemonetes pugio* was calculated to determine where *P. pugio* was located in treatment tanks. If shrimp were observed in multiple preferred locations, then those locations were excluded. Mean is calculated as percent of time. (GG=within *Gracilaria vermiculophylla*, GS=left section of the tank touching the edges, ES=right section of the tank touching the edges, GS+ES=combination of the edges).

Location	# Shrimp observed most often in location	Frequency	Mean % \pm 1 SD
GG	9	300	74.1 \pm 18.54
GS	12	323	39.4 \pm 8.74
ES	23	502	60.6 \pm 16.81
GS + ES	35	825	53.3 \pm 17.64
EEL	9	247	39.3 \pm 10.24
ESR	12	371	50.6 \pm 11.94
ESL	17	417	52.4 \pm 10.33
ESR+ESL	29	788	51.6 \pm 11.94

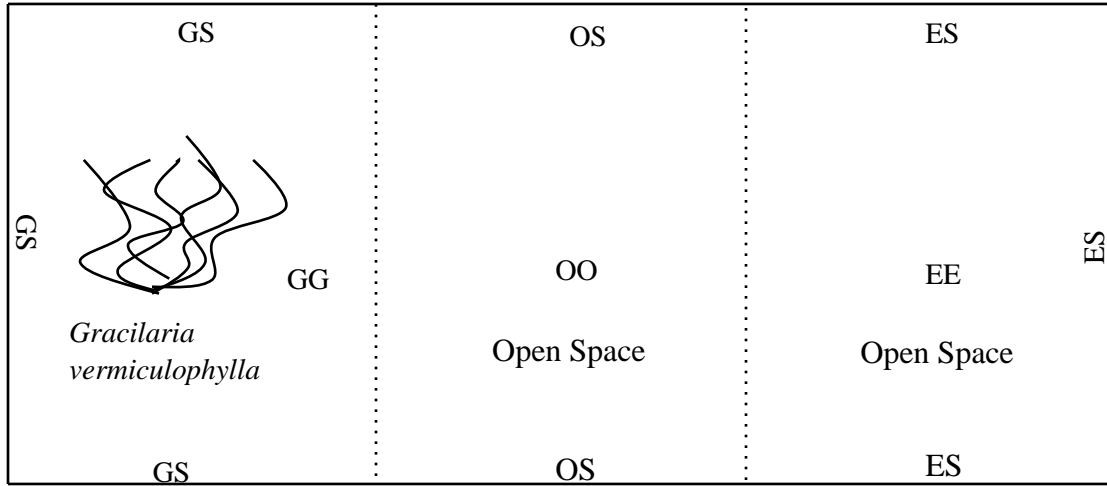


Figure 3.1. One *Palaemonetes pugio* was added to 4 replicate 33 cm X 20 cm X 23 treatment tanks with 2 habitats sectioned into thirds such as: 2/3 open space, and 1/3 *Gracilaria vermiculophylla*. Experiments were run for 30 min and shrimp were documented every 1 min. (GG= within the red alga *Gracilaria vermiculophylla*, EE= right section of the tank not touching the edges, OO= center of the tank not touching edges, OS= center of the tank touching edges, ES= right section of the tank touching edges, GS= left section of the tank touching edges).

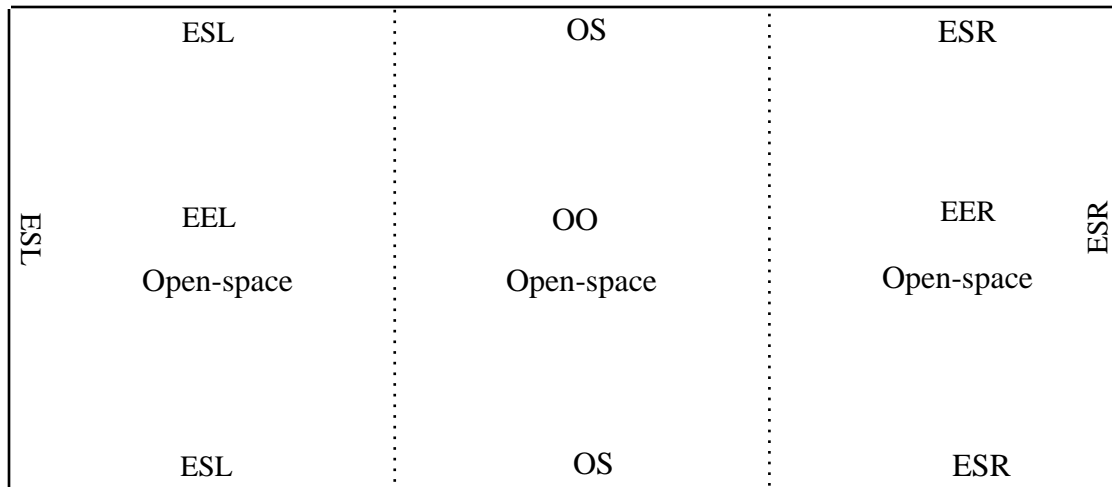


Figure 3.2. One *Palaemonetes pugio* was added to 4 replicate 33 cm X 20 cm X 23 control tanks sectioned into thirds such as: 1/3 open on the left side, 1/3 open in the center, 1/3 open on the right side. Experiments were run for 30 min and shrimp were documented every 1 min. (EEL= left section not touching edges, EER= right section of the tank not touching edges, OO= center of the tank not touching edges, OS= center of the tank touching edges, ESR= right section of the tank touching edges, ESL= left section of the tank touching edges).

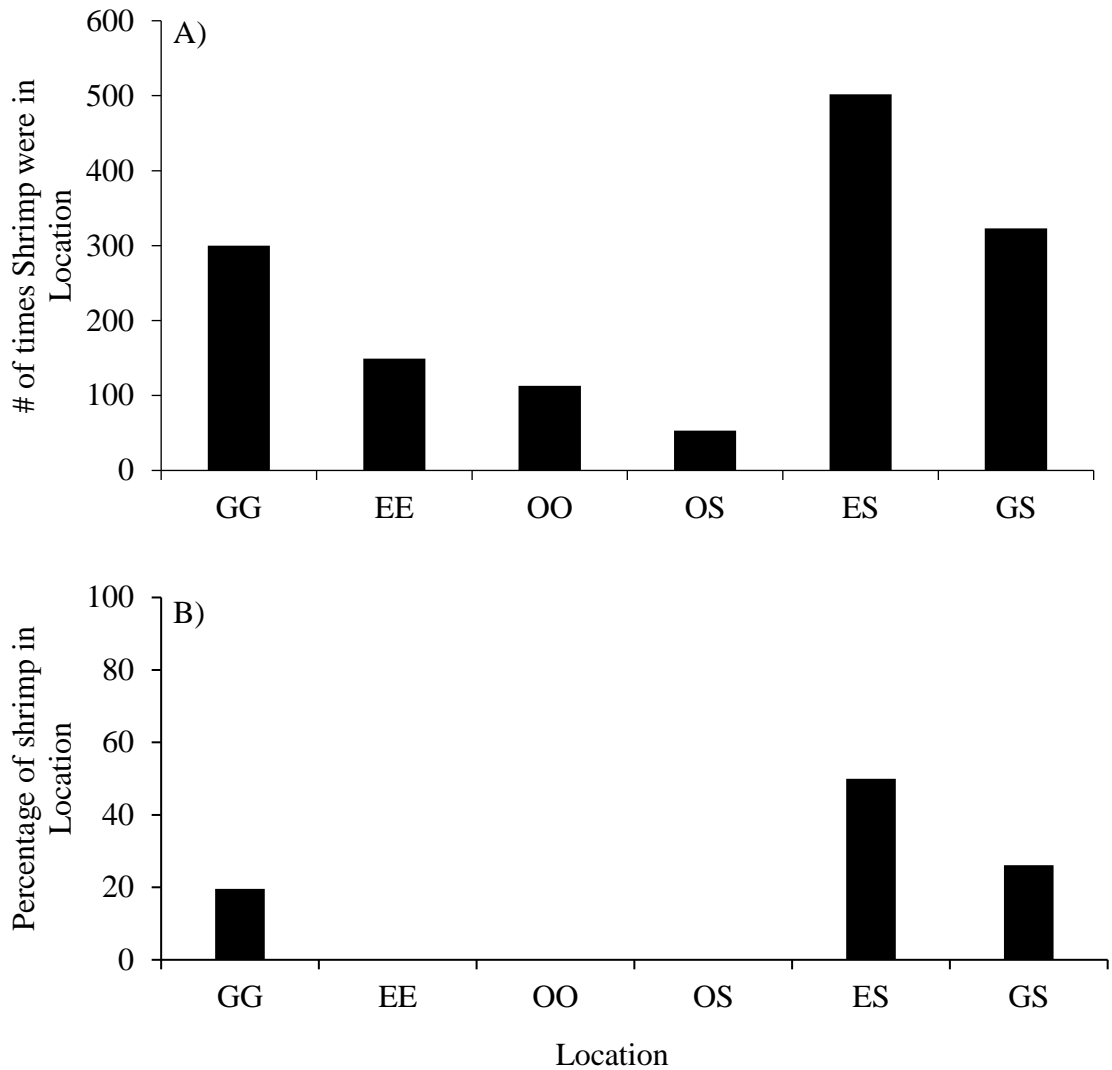


Figure 3.3. A) Frequency of daggerblade grass shrimp *Palaemonetes pugio* in a given location (# of times observed out of 1,440) and B) Percentage of shrimp that preferred a given location in the treatment tanks in the laboratory. For B), two out of 48 shrimp were excluded because those shrimp were observed in multiple preferred locations (found in more than one location the same percent of time). No shrimp preferred EE, OO, and OS. Location definitions: GG=within the red alga *Gracilaria vermiculophylla*, EE=right section of the tank not touching the edges, OO=center of the tank not touching the edges, OS=center of the tank touching the edges, ES=right section of the tank touching the edges, GS=left section of the tank touching the edges.

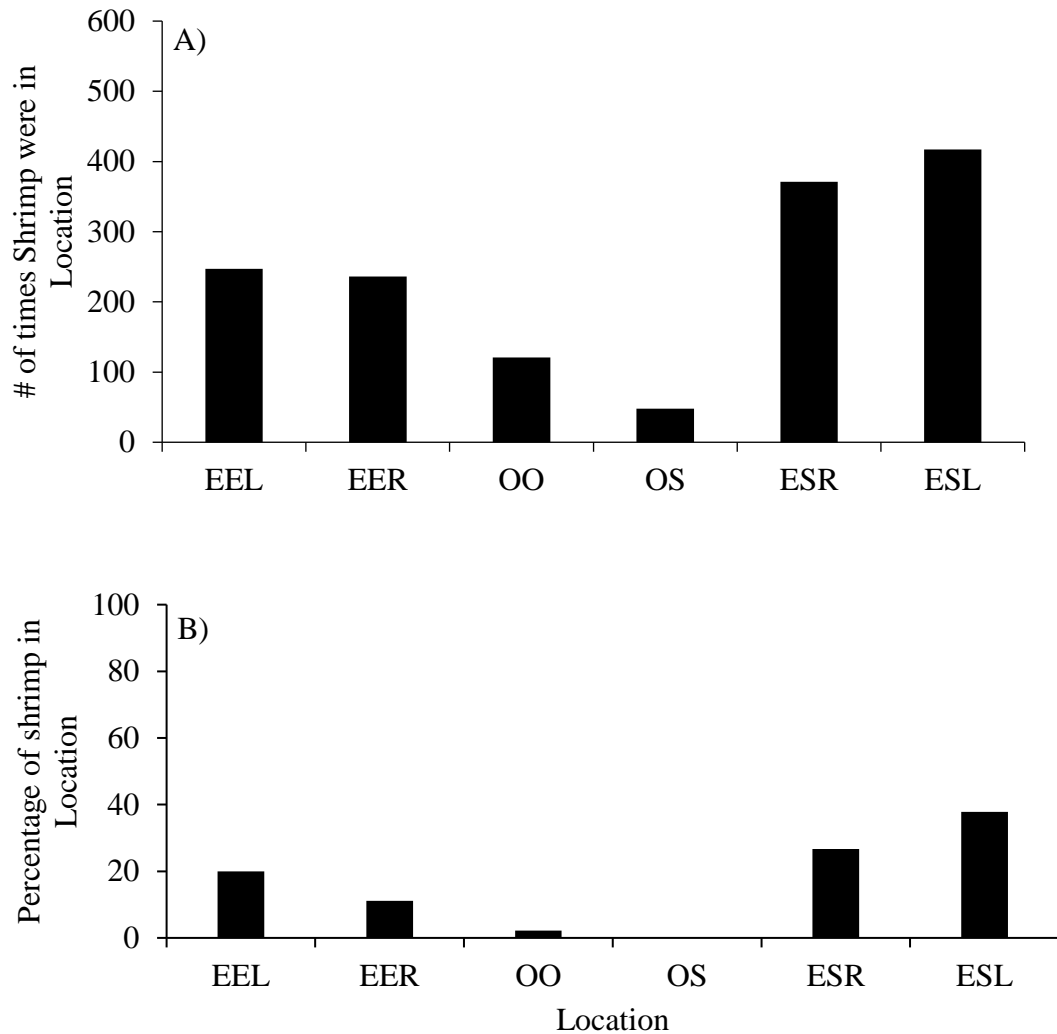


Figure 3.4. A) Frequency of daggerblade grass shrimp *Palaemonetes pugio* in a given location (# of times observed out of 1,440) and B) Percentage of shrimp that preferred a given location in the control tanks in the laboratory. For B), three out of 48 shrimp were excluded because those shrimp were observed in multiple preferred locations (found in more than one location the same percent of time). No shrimp preferred OS. Location definitions: EEL=left section of the tank not touching the edges, EER=right section of the tank not touching the edges, OO=center of the tank not touching the edges, OS=center of the tank touching the edges, ESR=right section of the tank touching the edges, ESL=left section of the tank touching the edges.

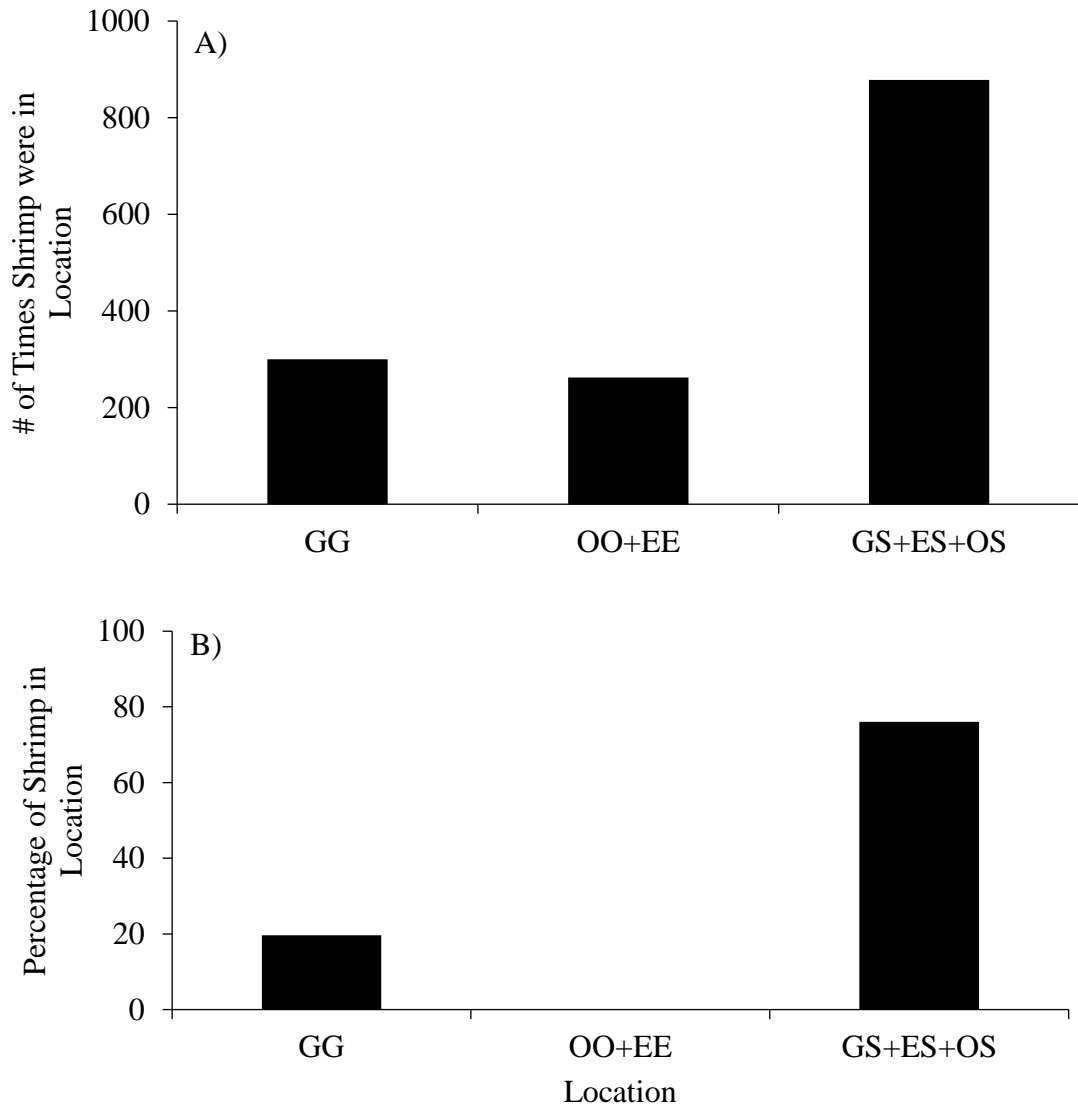


Figure 3.5. A) Frequency of daggerblade grass shrimp *Palaemonetes pugio* in a given location (# of times observed out of 1,440) and B) Percentage of shrimp that preferred a given location in the treatment tanks in the laboratory. For B), two out of 48 shrimp were excluded because those shrimp were observed in multiple preferred locations (found in more than one location the same percent of time). No shrimp preferred OO+EE. Location definitions: GG=within the red alga *Gracilaria vermiculophylla*, OO+EE=center and right section of the tank not touching the edges, GS+ES+OS=left section of the tank, center, and right section of the tank touching the edges.

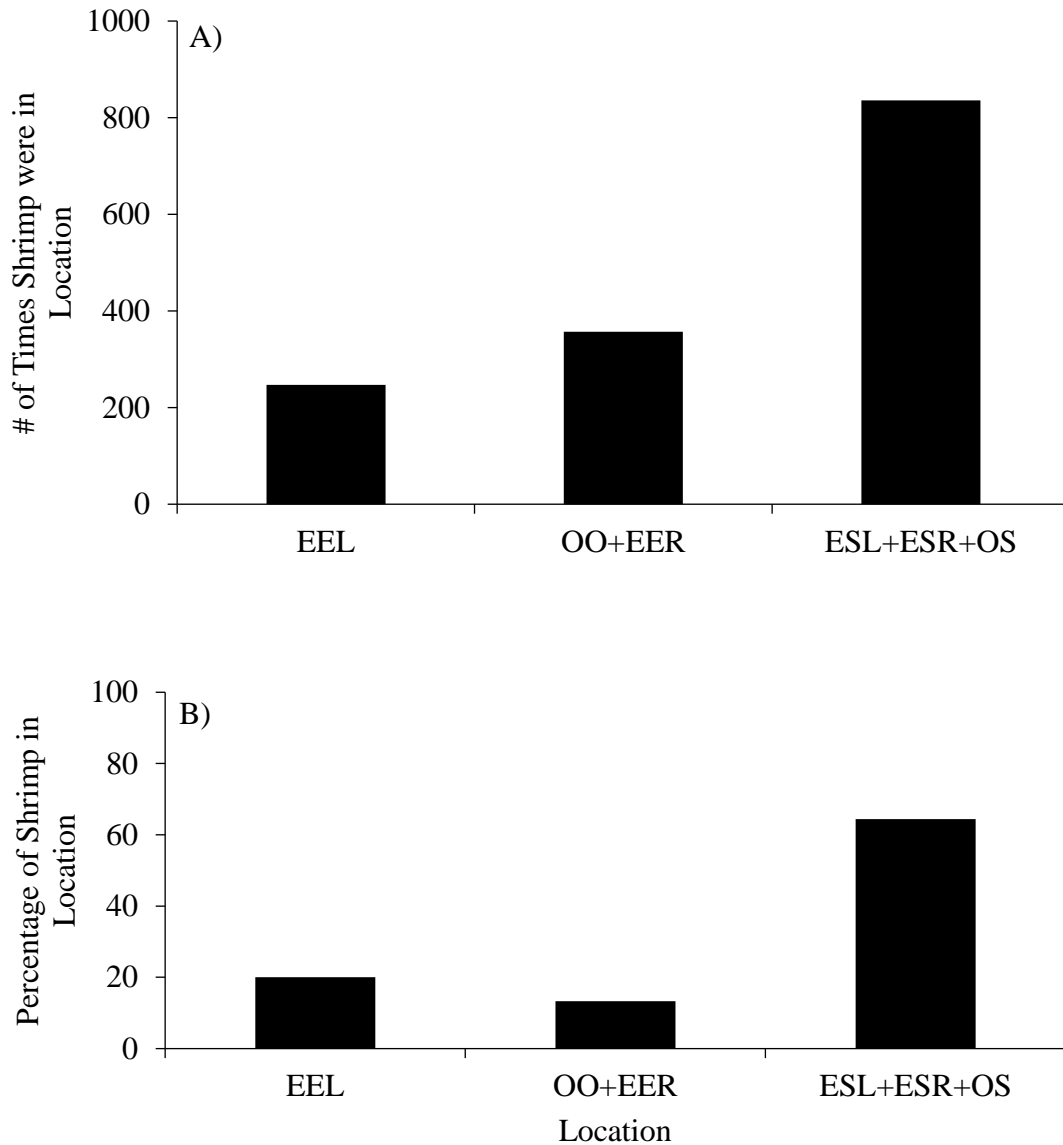


Figure 3.6. A) Frequency of daggerblade grass shrimp *Palaemonetes pugio* in a given location (# of times observed out of 1,440) and B) Percentage of shrimp that preferred a given location in the control tanks in the laboratory. For B), three out of 48 shrimp were excluded because those shrimp were observed in multiple preferred locations (found in more than one location the same percent of time). Location definitions: EEL=left section of the tank not touching the edges, OO+EER=center and right section of the tank not touching the edges, ESL+ESR+OS=left section, right section, and center of the tank touching the edges.

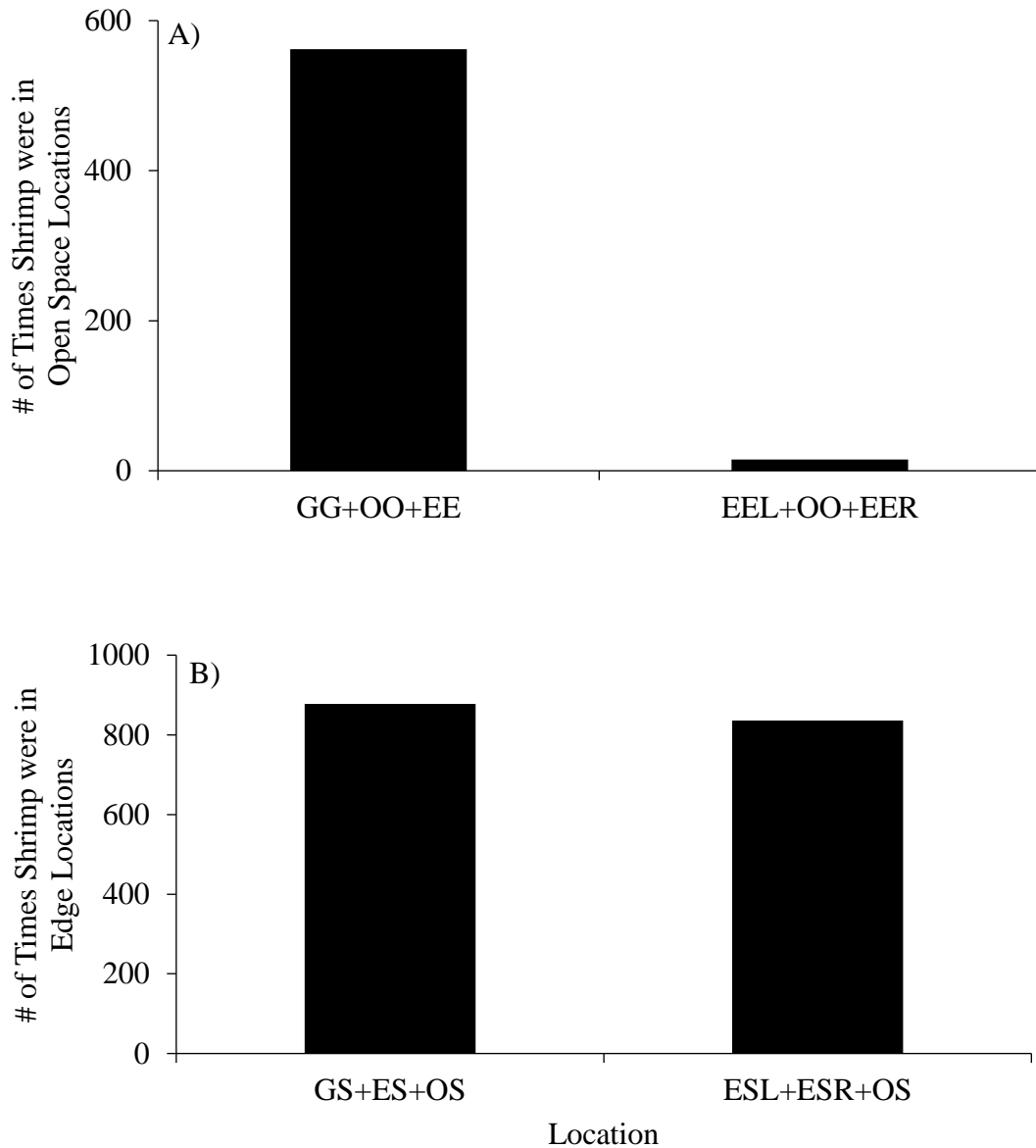


Figure 3.7. A) Frequency (# of times observed out of 1,440) of the daggerblade grass shrimp *Palaemonetes pugio* in *Gracilaria vermiculophylla*+open space locations in treatment tanks and open space in control tanks (Treatment: GG+OO+EE=left section in *G. vermiculophylla*, center of the tank, and right section of the tank not touching the edges, Control: EEL+OO+EER=left section, center, and right section of the tank not touching the edges). B) Frequency of *P. pugio* in edge locations in the treatment tanks and all edge locations in the control tanks (Treatment: GS+ES+OS=left section, right section, and center of the tank touching the edges, Control: ESL+ESR+OS=left section, right section, and center of the tank touching the edges).

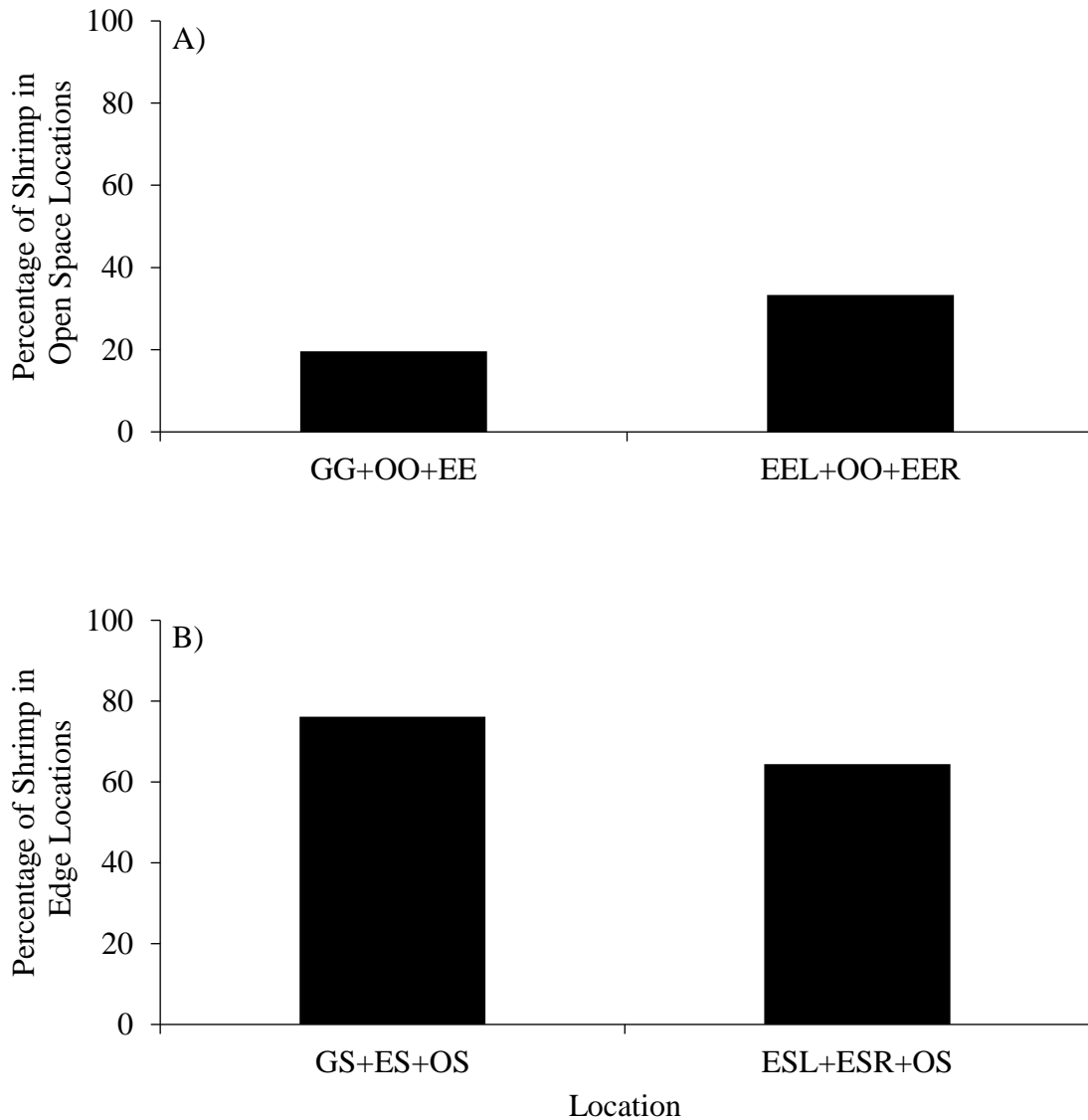


Figure 3.8. A) Percentage of the daggerblade grass shrimp *Palaemonetes pugio* in *Gracilaria vermiculophylla*+open space in the treatment tanks and open space in the control tanks (Treatment: GG+OO+EE=left section in *G. vermiculophylla*, center of the tank, and right section of the tank not touching the edges, Control: EEL+OO+EER=left section, center, and right section of the tank not touching the edges) in the laboratory. B) Percentage of *P. pugio* in edge locations in the treatment tanks and all edge locations in the control tanks (Treatment: GS+ES+OS=left section, right section, and center of the tank touching the edges, Control: ESL+ESR+OS=left section, right section, and center of the tank touching the edges).

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APPENDICES

APPENDIX A: Chapter 3 Daily Feeding

INTRODUCTION

Grass shrimp in the genus *Palaemonetes* consume a wide range of primary producers such as seagrasses, and algae but also plant detritus as well. For example, the detritus of mangroves and the needlerush *Juncus* in Florida estuaries were found in the gut content of a several species of *Palaemonetes* (Odum and Heald, 1972). In addition, plant and detrital material of *Zostera marina* were also a food source of *P. pugio* in the laboratory (Adams and Angelovic, 1970). Grazing of algal epiphytes by *P. pugio* enhanced the shoot growth of the widgeon grass *Ruppia maritima* by exposing the shoot to more available sunlight and by the deposition of fecal material (McCall and Rakocinski, 2007). This in turn prevents dieback of *R. maritima* in the estuary (McCall and Rakocinski, 2007). However, there is no evidence that *P. pugio* consumed the eelgrass *Halodule wrightii* based on fecal pellet production in the laboratory (Morgan, 1980). Similarly, *P. pugio* did not consume the sea lettuce *Ulva* in the laboratory even when starved, but the presence of *P. pugio* actually increased the growth of *U. rigida* approximately 6 mg d⁻¹ during a mesocosm experiment (Guidone et al., 2015). However, *Palaemonetes vulgaris* consumed a small amount of *Ulva compressa* and *Ulva rigida* (Guidone et al., 2015).

Palaemonetes pugio consumes a variety of organisms within the water column and within muddy and sandy substrates (Smith and Coull, 1987). Microalgae may be a substantial component to the diet of *P. pugio* as it ingests many types of algae such as the planktonic diatom *Thalassiosira weissflogii* and the planktonic dinoflagellate *Isochrysis galbana* although in smaller quantities than epiphytic algae (Fleeger et al., 1999). *Palaemonetes pugio* is also an opportunistic predator that relies on random encounters

with its mobile prey, such as the mysids *Mysidopsis bigelowi* and *Mysidopsis bahia* (Morgan, 1980). *Palaemonetes pugio* also preys on larvae of many crustacean species such as the fiddler crab *Uca minax* and the mud crab *Rhithropanopeus harrisi* in the laboratory (Morgan, 1992) as well as the postlarvae of *Callinectes sapidus* when abundance is high in the beds of the eelgrass *Zostera marina* (Olmi and Lipcius, 1991). Some meiofauna that were significantly reduced by *P. pugio* in the muddy substrates were bivalve larvae and tubellarians (Smith and Coull, 1987). One study indicated that *P. pugio* reduced the number of benthic invertebrates by either disturbing the sediments with their pleopods or by direct predation of the invertebrates (Kneib, 1985). Hydrobiid snails were affected by the presence of *P. pugio* when disturbance to the sediments altered feeding patterns that initiated migration to a new area (Kneib, 1985).

The mud snail *Ilyanassa obsoleta* is one gastropod associated with the invasive *G. vermiculophylla*, which deposits its egg capsules on the algae (Guidone et al., 2014). Some predators of the egg capsules such as the long-armed hermit crab *Pagurus longicarpus*, and the green crab *Carcinus maenas* consume intact egg capsules, while the periwinkle *Littorina littorea* punctures the wall of the egg capsules before consumption (Brenchley, 1982). Egg capsule presence may attract other potential predators such as *P. pugio* as the egg capsules contain snail larvae. *Palaemonetes pugio* is known to consume the larvae of other organisms in laboratory experiments such as the megalopae of *C. sapidus* (Olmi and Lipcius, 1991) and the larvae of the sheepshead minnow *Cyprindodon variegatus* (Bass and Weis, 1999).

MATERIALS AND METHODS

A proof of concept experiment was conducted beginning spring 2017 to determine if *Palaemonetes pugio* consumed snail veligers. Two treatments each with five replicates were designed as follows:

1. 15 veligers + one grass shrimp: 5 tanks
2. 15 veligers alone (non-predator control): 5 tanks

Prior to the experiment, grass shrimp were collected from the Frank Downing Fishing Pier (31°57'02.84"N 81°04'52.71"W) in Savannah, GA and placed in 1 L plastic aquaria. The shrimp were only collected from Frank Downing Fishing Pier because that is where snail egg deposition was prevalent on *Gracilaria vermiculophylla* and this species was not present at the Rodney J. Hall Boat Ramp. Male shrimp were chosen for this experiment to alleviate any hormonal factors exhibited by ovigerous, or soon to be ovigerous females. Only one shrimp was used in each tank to reduce competition for food, which eliminates the difficulty of determining which shrimp consumed the most food. Shrimp were collected 1 day before they were separated into tanks. They were then starved for 24 h in the tanks prior to each experiment. This timing was used because Bass and Weiss (1999) observed that grass shrimp significantly increased their activity level after a day of starvation, but decreased activity after 48 h. Experiments were conducted during the day when ebb tide occurred to control for known behavioral effects, as Chaplin-Ebanks and Curran (2005) detected shrimp activity to be highest at ebb tide and lowest at flood tide. Estuarine water from Country Club Creek, adjacent to Savannah State University, was used because the water was passed through a filtration system removing unwanted material from the water.

Veligers that were added to the tanks were reared in the laboratory. After veligers hatched from the egg capsules, they were collected with a plastic pipette and 15 were transferred to each replicate tank. A non-predator treatment was utilized to control for the natural mortality of the veligers. Shrimp used in this experiment had 25 or fewer trematodes, which was standardized to the number of trematodes/mm TL (0.14 ± 0.188) to control for behavioral factors as per Kunz and Pung (2004) who noticed an increase in shrimp swimming activity when parasite load exceeded 29 cysts. Shrimp in each replicate were given a maximum of 30 min to consume veligers based on a previous predation experiment by Bass and Weis (1999) as they permitted 30 min for the mummichog *Fundulus heteroclitus* to consume *P. pugio*. The remaining veligers were filtered through a 63 μm sieve and placed on a Petri dish and each was counted under the dissecting microscope at 10X total magnification. Veligers were considered dead if shells were empty. The number of live and dead veligers remaining were compared to the control. A t-test was performed to compare the number of veligers remaining in the treatment following the Shapiro-Wilks test for normality and homogeneity of variance using Levene's test.

An additional experiment was conducted to determine if the intensity of trematode cysts had an effect on the number of veligers consumed. A zero trematode cyst load was defined as having no cysts and a low trematode cyst load was defined as between one and nine cysts as per Kunz and Pung (2004). Grass shrimp were separated into two different treatments with ten replicates. There were five non-predator replicate controls and 2 replicate dead grass shrimp controls. The 2 shrimp died of natural causes before the trial. The treatments were as follows:

1. One grass shrimp with zero cyst load + 15 veligers: 10 tanks
2. One grass shrimp with low cyst load + 15 veligers: 10 tanks
3. One grass shrimp with high cyst load + 15 veligers: 10 tanks
4. 15 veligers alone (non-predator control): 5 tanks
5. One dead grass shrimp + 15 veligers (control): 2 tanks

All other aspects of the experiment were the same as the initial experiment except that shrimp were given 2 h to consume the veligers because during the proof of concept experiment the number veligers remaining after the experiment was too close to the number of veligers remaining within the controls. This indicated that 30 min was not enough time for the shrimp to consume the veligers. The dead grass shrimp control was used to assess if veligers were sticking to the shrimp body when shrimp were removed from the treatment tanks. An Independent t-test was conducted to determine the differences between trematode cyst intensity and the number veligers consumed, following adjustments for natural veliger mortality, if any. Prior to analysis, data were analyzed via the Shapiro-Wilks test for normality and Levene's test for homogeneity of variance.

RESULTS

The range and average number of veligers missing were calculated for feeding experiments that were conducted between 10 March to 21 May 2017. The lowest number of veligers missing was zero and the highest number of veligers missing was 15 (Table 1). The range of veligers missing on 11 March were similar for each trematode load. The highest missing for zero was 13, low was 15, and medium trematode load was 14 (Table

1). The range of veligers missing on 01 April for zero trematode load was 2-13 and was similar to low trematode load, which was between 0-15 (Table 1). The range of veligers missing for the high replicates on 21 May were comparable to the zero and low ranges on 01 April and 19 March. The lowest number of veligers missing on 21 May was zero and zero was also the lowest for the low trematode load on 01 April and zero trematode load on 19 March (Table 1). Twelve veligers missing was the highest number on 21 May for the high trematode load, which was only one more than the zero and low trematode loads on 19 March (Table 1).

There was a significant difference between the number of veligers missing and trematode load ($P=0.05$). There was no significant difference between a high trematode load and low trematode load ($P=0.302$) and there was also no difference between low and zero trematode load ($P=0.371$). However, there was a significant difference for the number of veligers missing between a high and zero trematode load ($P=0.037$). There seemed to be a relationship between the average number of veligers eaten and trematode load. The total average number of veligers missing for zero trematode load was 8.5 ± 4.29 and was greater than the high trematode load 5.8 ± 3.91 (Table 2). In addition, the total average number of veligers missing for low trematode load was 6.9 ± 4.24 , which was also higher than the high trematode load even though the replicates for the low trematode load ($n=52$) was more than the high trematode load (Table 2).

The range and average number of veligers missing was also calculated for control treatments from 10 March to 21 May 2017. The lowest number of veligers missing was 2 and the highest number of veligers missing was 4 (Table 3). The -2 value indicates that there were 2 more veligers counted than were added in at the beginning of the experiment.

The total number of veligers missing across all replicates was 37 and was statistically significant ($P=0.000$), which was smaller than our alpha level ($\alpha=0.05$). The average number of veligers missing was 1.1 ± 1.31 . There were no data for the number of veligers missing on 10 May and 14 May because no control replicates were used subsequent to the feeding experiment on these days (Table 3). The range of veligers missing seemed to stay consistent throughout experimentation. The range on 11 March and 01 April was between 0-2 and the range was also between 0-2 on 21 May (Table 3).

On 10 March, the mean for the low trematode load replicates and high load replicate was 4.4 ± 2.88 (Table 1). Only males were used for experiments except on 11 March females were used because not enough males were collected. The average number of veligers missing on March 11 for zero trematode load was 12.0 ± 1.73 , for low was 11.1 ± 3.02 , and for medium was 12.7 ± 1.53 (Table 2), respectively. The average number of veligers missing for the zero trematode load replicates was 5.8 ± 3.22 and 6.8 ± 3.12 for the low trematode load replicates on 19 March (Table 3). On 24 March, the average number of veligers missing for the low trematode load replicates was 4.3 ± 3.74 and for the medium trematode load replicates was 7.1 ± 3.73 (Table 4). The average number of veligers missing on 01 April for the zero trematode load replicates and the low trematode load replicates was 8.0 ± 4.78 and 9.0 ± 4.47 (Table 5), respectively. In addition, the average number of veligers missing on 07 April for the zero trematode load replicates and the low trematode low replicates was 11.1 ± 3.14 and 8.8 ± 3.65 (Table 6), respectively. Four high replicate trematode loads including one female were conducted on 10 May and the average number of veligers missing was 5.3 ± 6.08 (Table 7). Five more high replicate trematode loads with 2 females and 3 males were used on 14 May and the

average number of veligers missing was 9.6 ± 2.70 (Table 8). On 21 May, 20 high replicates with all males were used and the average number of veligers missing was 4.9 ± 3.33 (Table 9).

The average number of veligers missing on 10 March was 2.4 ± 1.14 (Table 10) and the average number of veligers missing on 11 March was 0.6 ± 0.89 (Table 11). On 19 March, there was a -1 total number of veligers missing was observed, meaning that one more veliger was found than was originally put in (Table 12). The average number of veligers missing on 19 March was 0.8 ± 1.30 (Table 12) and was 0.8 ± 1.48 on 24 March (Table 13). Two control replicates were used on 01 April to determine if veligers attached to the body of the shrimp. The average number of veligers missing on 01 April and 07 April was 0.6 ± 0.82 (Table 14) and 0.8 ± 1.79 (Table 15), respectively, indicating that they did not attach to the shrimp body. On 21 May, the average number of veligers missing was 1.4 ± 1.52 (Table 16).

DISCUSSION

The major finding of this study was that there was a significant difference between the number of veligers missing between high and zero trematode loads ($P=0.037$) and no significant difference between zero and low ($P=0.371$) and low and high ($P=0.302$) trematode loads. Uninfected *P. pugio* swim more often than infected shrimp in the absence a predator ($P=0.02$) (Kunz and Pung, 2004). Shrimp rely on random encounters with their prey (Morgan, 1980), so shrimp capable of swimming for longer may come in contact more often with their prey. There were more veligers consumed by *P. pugio* with zero trematode ($n=285$), while *P. pugio* with a high trematode load consumed fewer veligers ($n=157$). The swimming behavior of *P. pugio*

was similar to the results of Bass and Weis (1999). *Palaemonetes pugio* infected with *P. pandalicola* spent 93% swimming compared to 74% that were not infected.

Palaemonetes pugio were able to capture significantly more *Daphnia magna* unparasitized than parasitized ($P < 0.02$) (Bass and Weis, 1999). The larvae of *Cyprinodon* were also consumed more by unparasitized shrimp by *P. pandalicola* (0.39 ± 0.09) than parasitized shrimp (0.27 ± 0.08) (Bass and Weis, 1999).

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Table 1. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 10 replicate tanks and length (mm), sex, number of trematodes, number of alive, dead, and missing veligers were recorded and mean \pm 1 SD were calculated. The experiment was conducted on Friday 10 March 2017 using the Isle of Hope tide chart for 2 h starting 1 h before ebb tide 0830 and ending 1 h after ebb tide 1030. (H=29+ trematodes, L=1-28 trematodes).

Tank Replicates	Length (mm) TL	Sex	#Trematodes	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	#Veligers Missing (end)
1	30.11	M	34	H	7	0	8
2	24.92	M	1	L	6	1	8
3	27.35	M	8	L	9	2	4
4	25.66	M	9	L	7	3	5
5	26.57	M	3	L	10	2	3
6	26.48	M	3	L	12	3	0
7	24.05	M	4	L	10	1	4
8	27.00	M	3	L	6	1	8
9	23.96	M	9	L	13	1	1
10	27.15	M	7	L	11	1	3
Mean \pm SD	26.3 \pm 1.82		8.1 \pm 9.54				4.4 \pm 2.88

Table 2. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 13 replicate tanks and length (mm), sex, number of trematodes, and number of alive, dead, and missing veligers were recorded and mean \pm 1SD were calculated. The experiment was conducted on Saturday 11 March 2017 using the Isle of Hope tide chart for 2 h starting from 1 h before ebb tide 0956 and ending 1 h after ebb tide 1156. (None= no trematodes, L=1-9 trematodes, M=10-28 trematodes).

Tank Replicates	Length (mm) TL	Sex	Ovige (Y/N)	#Trematodes	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	# Veligers Missing (end)
1	33.6	F	Y	15	M	0	1	14
2	29.4	F	Y	7	L	3	0	12
3	23.8	M	N	9	L	2	0	13
4	31.1	F	N	15	M	3	1	11
5	31.0	F	N	3	L	2	0	13
6	24.1	M	N	4	L	4	1	10
7	26.4	M	N	1	L	2	0	13
8	25.6	M	N	0	None	1	1	13
9	30.4	F	N	11	M	5	4	6
10	29.6	F	N	8	L	0	0	15
11	25.9	F	N	3	L	5	1	9
12	28.3	F	N	0	None	1	1	13
13	26.6	F	N	0	None	5	0	10
Mean \pm SD	28.1 \pm 3.00			5.9 \pm 5.44	None 12.0 \pm 1.73 Low 11.1 \pm 3.02 Med 12.7 \pm 1.53			11.7 \pm 2.42

Table 3. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 20 replicate tanks and length (mm), sex, number of trematodes, number of alive, dead, and missing veligers were recorded and mean \pm 1SD were calculated. The replicates were conducted on Sunday 19 March 2017 using the Isle of Hope tide chart for 2 h starting from 1 h before ebb tide 1617 and ending 1 h after ebb tide 1817. (None=no trematodes, L=1-9 trematodes)

Tank Replicates	Length (mm) TL	Sex	#Trematodes	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	#Veligers Missing (end)
1	27.1	M	0	None	8	0	7
2	24.7	M	2	L	10	1	4
3	24.9	M	0	None	14	1	0
4	25.8	M	7	L	11	1	3
5	24.7	M	0	None	8	0	7
6	23.8	M	6	L	6	1	8
7	24.9	M	0	None	13	0	2
2	24.2	M	6	L	3	1	11
9	24.0	M	0	None	4	0	11
10	25.7	M	1	L	10	1	4
11	19.9	M	0	None	12	0	3
12	26.9	M	1	L	10	0	5
13	24.5	M	0	None	8	0	7
14	23.4	M	1	L	6	0	9
15	21.9	M	0	None	7	0	8
16	22.5	M	3	L	6	0	9
17	23.8	M	0	None	9	0	6
18	24.3	M	1	L	10	1	4
19	25.9	M	0	None	6	2	7
20	23.1	M	5	L	4	0	11
Mean \pm SD	24.3 \pm 1.68		1.7 \pm 2.39	None 5.8 \pm 3.22 Low 6.8 \pm 3.12			6.3 \pm 3.13

Table 4. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 20 replicate tanks and length (mm), sex, number of trematodes, number of alive, dead, and missing veligers were recorded and mean \pm 1 SD were calculated. The replicates were conducted on Friday 24 March 2017 using the Isle of Hope tide chart for 2 h starting from 1 h before ebb tide 0900 and ending 1 h after ebb tide 1100. (L=1-9 trematodes, M=10-28 trematodes).

Tank Replicates	Length (mm) TL	Sex	#Trematodes	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	#Veligers Missing (end)
1	22.5	M	6	L	5	0	10
2	26.3	M	18	M	2	0	13
3	23.4	M	9	L	11	1	3
4	23.8	M	14	M	4	0	11
5	22.1	M	1	L	12	1	2
6	27.2	M	13	M	13	0	2
7	23.5	M	1	L	9	2	4
2	25.8	M	13	M	7	0	8
9	24.3	M	1	L	9	2	4
10	24.2	M	10	M	8	1	6
11	24.3	M	5	L	9	2	4
12	23.3	M	18	M	8	1	6
13	26.2	M	1	L	3	0	12
14	28.3	M	21	M	4	0	11
15	24.9	M	5	L	14	0	1
16	28.5	M	27	M	13	0	2
17	25.6	M	1	L	13	0	2
18	23.9	M	14	M	8	0	7
19	22.1	M	3	L	13	1	1
20	25.9	M	16	M	10	0	5
Mean \pm SD	24.8 \pm 1.89		9.9 \pm 7.76	Low 4.3 \pm 3.74 Med 7.1 \pm 3.73			5.7 \pm 3.91

Table 5. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 20 replicate tanks. Length (mm), sex, number of trematodes, number of alive, dead, and missing veligers were recorded and means \pm 1 SD were calculated. The replicates were conducted for 2 h on Saturday 01 April 2017 using the Isle of Hope tide chart starting from 1 h before ebb tide 1512 and ending 1 h after ebb tide 1712. (None=no trematodes, L=1-9 trematodes).

Tank Replicates	Length (mm) TL	Sex	#Trematodes	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	#Veligers Missing (end)
1	26.9	M	8	L	1	0	14
2	27.0	M	0	None	5	1	9
3	24.2	M	2	L	11	0	4
4	25.6	M	0	None	3	0	12
5	26.1	M	6	L	6	1	8
6	26.2	M	0	None	0	0	15
7	28.9	M	4	L	3	0	12
8	27.0	M	0	None	11	0	4
9	24.9	M	5	L	3	0	12
10	26.7	M	0	None	3	1	11
11	26.9	M	4	L	6	0	9
12	25.4	M	0	None	3	0	12
13	25.9	M	1	L	5	1	9
14	26.7	M	0	None	1	0	14
15	26.0	M	5	L	9	0	6
16	24.9	M	0	None	2	0	13
17	24.6	M	3	L	12	0	3
18	25.8	M	0	None	5	1	9
19	26.7	M	1	L	4	0	11
20	24.5	M	0	None	3	0	12
Mean \pm SD	26.0 \pm 1.12		2.0 \pm 2.52	None 11.1 \pm 3.14 Low 8.8 \pm 3.65			10.0 \pm 3.50

Table 6. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 20 replicate tanks and length (mm), sex, number of trematodes, number of alive, dead, and missing veligers were recorded and mean \pm 1SD were calculated. The replicates were conducted for 2 h on Friday 07 April 2017 using the Isle of Hope tide chart starting 1 h before ebb tide 0854 and ending 1 h after ebb tide 1054. (None=no trematodes, L=1-9 trematodes).

Tank Replicates	Length (mm) TL	Sex	#Trematodes	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	#Veligers Missing (end)
1	25.2	M	3	L	2	1	12
2	26.4	M	0	None	8	0	7
3	24.8	M	1	L	6	0	9
4	27.4	M	0	None	12	0	3
5	23.8	M	7	L	15	0	0
6	25.3	M	0	None	13	0	2
7	25.5	M	2	L	10	0	5
8	22.7	M	0	None	2	0	13
9	23.7	M	8	L	10	0	5
10	23.4	M	0	None	10	1	4
11	25.8	M	1	L	2	0	13
12	25.9	M	0	None	2	0	13
13	22.7	M	4	L	5	0	10
14	21.8	M	0	None	11	0	4
15	28.7	M	2	L	0	0	15
16	24.2	M	0	None	1	0	14
17	27.3	M	1	L	4	0	11
18	24.3	M	0	None	8	0	7
19	28.8	M	2	L	5	0	10
20	23.3	M	0	None	2	0	13
Mean \pm SD	25.0 \pm 1.97		1.6 \pm 2.35	None 9.0 \pm 4.47 Low 8.0 \pm 4.78			8.5 \pm 4.54

Table 7. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 4 replicate tanks and length (mm), sex, number of trematodes, number of alive, dead, and missing veligers, were recorded and mean and standard deviations were calculated on Wednesday 10 May 2017 using the Isle of Hope tide chart. The replicates were conducted for 2 hours and started at (1017) and ended at (1217). (H=29+ trematodes)

Tank Replicates	Length (mm) TL	Sex	#Trematodes	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	#Veligers Missing
1	31.4	M	35	H	3	2	10
2	28.4	M	39	H	13	2	0
3	31.0	M	35	H	2	2	11
4	35.4	F	30	H	10	5	0
Mean ± SD	31.5 ± 2.90		34.8 ± 3.69				5.3 ± 6.08

Table 8. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 5 replicate tanks and length (mm), sex, number of trematodes, number of alive, dead, and missing veligers, were recorded and mean and standard deviations were calculated on Sunday 14 May 2017 using the Isle of Hope tide chart. The replicates were conducted for 2 hours and started at (1344) and ended at (1544). (H=29+ trematodes)

Tank Replicates	Length (mm) TL	Sex	#Trematodes	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	#Veligers Missing (end)
1	35.7	F	34	H	5	2	8
2	26.3	M	33	H	4	1	10
3	29.6	M	45	H	5	3	7
4	35.7	F	30	H	5	1	9
5	28.7	M	53	H	0	1	14
Mean ± SD	31.2 ± 4.29		39.0 ± 9.67				9.6 ± 2.70

Table 9. One daggerblade grass shrimp *Palaemonetes pugio* and 15 veligers of the mud snail *Ilyanassa obsoleta* were added to 20 replicate tanks and length (mm), sex, number of trematodes, number of alive, dead, and missing veligers, were recorded and mean and standard deviations were calculated on Sunday 21 May 2017 using the Isle of Hope tide charts. The replicates were conducted for 2 hours and started at (0726) and ended at (0926). (H=29+ trematodes).

Tank Replicates	Length (mm) TL	Sex	#Trematod es	Parasite Load	#Veligers Alive (end)	#Veligers Dead (end)	#Veligers Missing (end)
1	27.0	M	51	H	9	0	6
2	27.9	M	30	H	12	1	2
3	28.9	M	36	H	2	1	12
4	26.8	M	30	H	9	0	6
5	24.2	M	33	H	9	0	6
6	26.4	M	34	H	9	1	5
7	32.0	M	52	H	13	1	1
8	29.5	M	31	H	11	0	4
9	27.4	M	34	H	15	0	0
10	29.1	M	43	H	11	0	4
11	27.8	M	36	H	14	0	1
12	28.7	M	47	H	13	0	2
13	27.2	M	33	H	7	0	8
14	31.0	M	40	H	7	0	8
15	27.9	M	31	H	5	0	10
16	27.8	M	36	H	6	6	3
17	28.2	M	32	H	9	1	5
18	28.9	M	31	H	7	0	8
19	28.9	M	56	H	9	0	6
20	29.7	M	37	H	13	2	0
Mean ± SD	28.3 ± 1.68		37.7 ± 7.96				4.9 ± 3.33

Table 10. Fifteen veligers of the mud snail *Ilyanassa obsoleta* were added to 5 control tanks and the number of alive, dead, and missing veligers were recorded and mean \pm 1SD were calculated. The experiment was conducted on Friday 10 March 2017 for 2 h starting at 1035 and ending at 1235.

Tank Replicates Control	# Veligers Alive (end)	# Veligers Dead (end)	Total # Veligers Missing
1	11	0	4
2	5	7	3
3	11	2	2
4	13	1	1
5	10	3	2
Mean \pm SD			2.4 \pm 1.14

Table 11. Fifteen veligers of the mud snail *Ilyanassa obsoleta* were added to 5 control tanks and number alive, dead, and missing veligers, were recorded and mean and \pm 1SD were calculated. The experiment was conducted on Saturday 11 March 2017 for 2 h starting at 1130 and ending at 1330.

Tank Replicates Control	# Veligers Alive (end)	# Veligers Dead (end)	Total # Veligers Missing
1	12	3	0
2	14	1	0
3	13	0	2
4	13	2	0
5	12	2	1
Mean \pm SD			0.6 \pm 0.89

Table 12. Fifteen veligers of the mud snail *Ilyanassa obsoleta* were added to 5 control tanks and the number of alive, dead, and missing veligers, were recorded and mean \pm 1SD were calculated. The experiment was conducted on Sunday 19 March 2017 for 2 h starting at 1803 and ending at 2022.

Tank Replicates Control	# Veligers Alive (end)	# Veligers Dead (end)	Total # Veligers Missing
1	12	3	0
2	13	1	1
3	10	1	2
4	12	3	2
5	13	3	-1
Mean \pm SD			0.8 \pm 1.30

Table 13. Fifteen veligers of the mud snail *Ilyanassa obsoleta* were added to 5 control tanks and the number of alive, dead, and missing veligers, were recorded and mean and standard deviations were calculated on Friday 24 March 2017. The experiment was conducted for 2 h starting at 1100 and ending at 1310.

Tank Replicates Control	# Veligers Alive (end)	# Veligers Dead (end)	Total # Veligers Missing
1	12	0	3
2	11	3	1
3	11	5	-1
4	11	4	0
5	14	0	1
Mean \pm SD			0.8 \pm 1.48

Table 14. Fifteen veligers of the mud snail *Ilyanassa obsoleta* were added to 7 control tanks (tank 6 and 7 contained one dead shrimp) and the number of alive, dead, and missing veligers were recorded and mean \pm 1SD were calculated. The dead shrimp controls were used to assess if veligers were sticking to the shrimp body when shrimp were removed from the treatment tanks. The experiment was conducted on 01 April 2017 for 2 h starting at 1635 and ending at 1910.

Tank Replicates Control	# Veligers Alive (end)	# Veligers Dead (end)	Total # Veligers Missing
1	14	0	1
2	13	0	2
3	15	0	0
4	14	0	1
5	15	0	0
6	14	0	1
7	11	2	2
Mean \pm SD			0.6 \pm 0.82

Table 15. Fifteen veligers of the mud snail *Ilyanassa obsoleta* were added to 5 control tanks and the number of alive, dead, and missing veligers were recorded and mean \pm 1SD were calculated. The experiment was conducted on Friday 07 April 2017 for 2 h starting at 1035 and ending at 1248.

Tank Replicates Control	# Veligers Alive (end)	# Veligers Dead (end)	Total # Veligers Missing
1	13	0	2
2	13	0	2
3	14	1	0
4	8	5	2
5	16	1	-2
Mean \pm SD			0.8 \pm 1.79

Table 16. Fifteen veligers of the mud snail *Ilyanassa obsoleta* were added to 5 control tanks and the number of alive, dead, and missing veligers, were recorded and mean and standard deviations were calculated on Sunday 21 May 2017. The experiment was conducted for 2 hours and started at (830) and concluded at (1125).

Tank Replicates	# Veligers Alive (end)	# Veligers Dead (end)	Total # Veligers Missing
Control			
1	13	1	1
2	8	4	3
3	15	0	0
4	11	1	3
5	15	0	0
Mean \pm SD			1.4 \pm 1.52

Table 17. The length (mm) TL, number of trematodes, and the number of veligers of the mud snail *Ilyanassa obsoleta* consumed by the daggerblade grass shrimp *Palaemonetes pugio* was combined in to a range summary table for all experiment days from 10 March to 21 May 2017. Trematode categories are: (0= no trematodes, Low=1-9 trematodes, Medium=10-28 trematodes, High=29+ trematodes).

Date	Tank Replicates	Length (mm) TL Range	Trematode Range	#Veligers Missing Range (end)
3/10/17	1	30.1	34	8
	9	23.9-27.4	1-9	0-8
3/11/17	3	25.6-28.3	0	10-13
	7	23.8-31.0	1-9	9-15
	3	30.4-33.6	11-15	6-14
3/19/17	10	19.9-27.1	0	0-11
	10	22.5-26.9	1-7	3-11
3/24/17	10	22.1-26.2	1-9	1-12
	10	23.3-28.5	10-27	2-13
4/01/17	10	21.8-27.4	0	2-13
	10	22.7-28.8	1-8	0-15
4/07/17	10	24.5-27.0	0	4-15
	10	24.2-28.9	1-8	3-14
5/10/17	4	28.4-35.4	30-39	0-11
5/14/17	5	26.3-35.7	30-53	7-14
5/21/17	20	24.2-32.0	30-56	0-12

Table 18. The mean \pm SD and (number of replicates) for the total number of veligers consumed by the daggerblade grass shrimp *Palaemonetes pugio* were calculated for all experiments between 10 March to 21 May 2017. Females used on 11 March, 10 May, and 14 May were excluded from this table.

Date	Zero	Low	Medium	High	Overall Total Mean \pm SD
03/10/17	N/D	4.0 \pm 2.74 (n=9)	N/D	8.0 \pm 0.00 (n=1)	
03/11/17	13.0 \pm 0.00 (n=1)	12.0 \pm 1.73 (n=3)	N/D	N/D	
03/19/17	5.8 \pm 3.22 (n=10)	6.8 \pm 3.12 (n=10)	N/D	N/D	
03/24/17	N/D	4.3 \pm 3.74 (n=10)	7.1 \pm 3.73 (n=10)	N/D	
04/01/17	8.0 \pm 4.78 (n=10)	9.0 \pm 4.47 (n=10)	N/D	N/D	
04/07/17	11.1 \pm 3.14 (n=10)	8.8 \pm 3.61 (n=10)	N/D	N/D	
5/10/17	N/D	N/D	N/D	5.3 \pm 6.08 (n=3)	
5/14/17	N/D	N/D	N/D	9.6 \pm 2.70 (n=3)	
5/21/17	N/D	N/D	N/D	4.9 \pm 3.33 (n=20)	
Total Mean \pm SD	8.5 \pm 4.29 (n=31)	6.9 \pm 4.24 (n=52)	7.1 \pm 3.73 (n=10)	5.8 \pm 3.91 (n=27)	7.1 \pm 4.16 (n=120)

Table 19. The number of veligers of the mud snail *Ilyanassa obsoleta* missing in the control tanks were combined in to a range summary table for all experiment days from 10 March to 21 May 2017. Negative values indicate that there were more veligers at the end of the experiment than were added at the beginning of the experiment.

Date	Tank Replicates	#Veligers Missing Range (end)
3/10/17	5	1-4
3/11/17	5	0-2
3/19/17	5	-1-2
3/24/17	5	-1-3
4/01/17	7	0-2
4/07/17	5	-2-2
5/10/17	0	N/D
5/14/17	0	N/D
5/21/17	5	0-3

APPENDIX B: Chapter 3 Habitat Preference Results

Pilot Experiments

Pilot experiments were conducted for habitat preference of *Palaemonetes pugio* between *Gracilaria vermiculophylla*, artificial *Spartina alterniflora*, and substrate free space (Figure 1). There was no significant difference between the preference of habitat and substrate-free space ($P=0.249$), but there was a significant difference between native *S. alterniflora* and invasive *G. vermiculophylla* ($P=0.041$) from all trial days combined. On 11 May, the average number of times shrimp were observed in *S. alterniflora* was 3.7 ± 1.15 and the average for the *G. vermiculophylla* was 3.2 ± 1.34 (Table 1). The average number of times observed in *S. alterniflora* on 12 May was 4.3 ± 1.61 and the average for *G. vermiculophylla* was 3.0 ± 1.48 (Table 1). The total average number of times shrimp were observed for combined substrate was 3.5 ± 1.50 and the total average for the substrate free space was 3.0 ± 1.52 (Table 1). There was also no significant difference between substrate free space and artificial *G. vermiculophylla* and *S. alterniflora* ($P=0.227$). In contrast to the significant difference between real *G. vermiculophylla* and *S. alterniflora* there was no significant difference between artificial *G. vermiculophylla* and *S. alterniflora* ($P=0.858$). The average number of times shrimp were observed within artificial *G. vermiculophylla* for the first trial on 7 July was 3.0 ± 2.16 and *S. alterniflora* was 2.8 ± 0.96 (Table 2). The average for *G. vermiculophylla* on the second trial was 2.5 ± 1.29 and *S. alterniflora* was 3.8 ± 2.99 (Table 2). The total average for the combined substrate was 3.8 ± 1.86 and the combined substrate-free space was 4.0 ± 1.85 (Table 2).

Palaemonetes pugio with a low trematode load preferred *Gracilaria vermiculophylla* to the substrate free space during the trials where *G. vermiculophylla* was on the edges of the tanks (Figure 2). The total mean (number of times shrimp were

observed in each area) \pm SD for *G. vermiculophylla* for the first two trials was 0.8 ± 0.42 and the substrate free space was 0.2 ± 0.42 (Table 3). The preference for *G. vermiculophylla* slightly decreased for shrimp with a high trematode load (0.7 ± 0.45) and slightly increased for substrate-free space (0.3 ± 0.45) (Table 4). Control tanks were used to determine if edges were preferentially selected by *P. pugio*. The mean for the edge was 0.8 ± 0.41 and the substrate free space was 0.2 ± 0.41 (Table 5). Since there still was a preference for the edge of the tank, in the next trials *G. vermiculophylla* was moved to the middle of the tank and the edges were substrate-free (Figure 3). There was a greater preference for the edges than *G. vermiculophylla*. The mean for the edge was 0.7 ± 0.46 , while the mean for *G. vermiculophylla* was 0.3 ± 0.46 (Table 6).

Table 1. Ten daggerblade grass shrimp *Palaemonetes pugio* were added to each of the 3 substrate 33 cm X 20 cm X 23 cm replicate tanks with 1/3 of the tank *Gracilaria vermiculophylla*, 1/3 artificial *Spartina alterniflora* and a 1/3 with a substrate-free open section on 11 May 2017 and 12 May 2017. The mean \pm 1 SD were recorded for substrate and open habitat.

Trial	Habitat	Total Mean \pm SD
1	<i>G. vermiculophylla</i>	3.2 \pm 1.43
	<i>S. alterniflora</i>	3.7 \pm 1.15
	Open	3.2 \pm 1.34
2	<i>G. vermiculophylla</i>	3.0 \pm 1.48
	<i>S. alterniflora</i>	4.3 \pm 1.61
	Open	2.9 \pm 1.73
Total Mean \pm SD	Substrate	3.5 \pm 1.50
	Open	3.0 \pm 1.52

Table 2. Ten daggerblade grass shrimp *Palaemonetes pugio* were added to 2 of the 51 cm X 25 cm X 33 cm substrate tanks with a ¼ of artificial *Gracilaria vermiculophylla*, ¼ with artificial *Spartina alterniflora* and ½ of the tank with a substrate free open section on 12 July 2017. The mean \pm 1 SD were recorded for substrate and open habitat.

Trial	Habitat	Total Mean \pm SD
1	<i>G. vermiculophylla</i>	3.0 \pm 2.16
	<i>S. alterniflora</i>	2.8 \pm 0.96
	Open	4.3 \pm 1.71
2	<i>G. vermiculophylla</i>	2.5 \pm 1.29
	<i>S. alterniflora</i>	3.8 \pm 2.99
	Open	3.8 \pm 2.22
Total Mean \pm SD	Substrate	3.0 \pm 1.86
	Open	4.0 \pm 1.85

Table 3. One daggerblade grass shrimp *Palaemonetes pugio* with a low trematode load (1-9 cysts) was added to each of the 4 separate 33 cm X 20 cm X 23 cm substrate tanks with 1/2 of the tank covered by live *Gracilaria vermiculophylla* and 1/2 of the tank with a substrate free open section. Trials ran for 30 min observing behavior of shrimp every minute on 2 August 2017. The mean \pm 1 SD were recorded for substrate and open habitat. The total mean \pm 1 SD was calculated for every min during the 30 min trial, not just every 5 min.

Trial	Habitat	5 min	10 min	15 min	20 min	25 min	30 min	Total Mean \pm SD
1	<i>G. vermiculophylla</i>	0.75	1.0	0.5	0.75	1.0	1.0	0.8 \pm 0.42
	Open	0.25	0.0	0.5	0.25	0.0	0.0	0.2 \pm 0.42
2	<i>G. vermiculophylla</i>	0.75	0.75	1.0	1.0	0.5	1.0	0.8 \pm 0.42
	Open	0.25	0.25	0.0	0.0	0.5	0.0	0.2 \pm 0.42
Total Mean	<i>G. vermiculophylla</i>	0.75	0.87	0.75	0.87	0.75	1.0	0.8 \pm 0.42
	Open	0.25	0.13	0.25	0.13	0.25	0.0	0.2 \pm 0.42

Table 4. One daggerblade grass shrimp *Palaemonetes pugio* with a high trematode load (29+ cysts) was added to each of the 4 separate 33 cm X 20 cm X 23 cm substrate tanks with 1/2 of the tank covered by live *Gracilaria vermiculophylla* and 1/2 of the tank with a substrate free open section. Trials ran for 30 min observing behavior of shrimp every minute on 3 August 2017. The mean \pm 1 SD were recorded for substrate and open habitat. The total mean \pm 1 SD was calculated for every min during the 30 min trial, not just every 5 min.

Trial	Habitat	5 min	10 min	15 min	20 min	25 min	30 min	Total Mean \pm SD
1	<i>G. vermiculophylla</i>	0.5	1.0	0.5	0.75	1.0	0.25	0.8 \pm 0.43
	Open	0.5	0.0	0.5	0.25	0.0	0.75	0.2 \pm 0.43
2	<i>G. vermiculophylla</i>	0.0	0.75	0.5	0.75	0.75	0.5	0.7 \pm 0.48
	Open	1.0	0.25	0.5	0.25	0.25	0.5	0.4 \pm 0.48
Total Mean	<i>G. vermiculophylla</i>	0.25	0.87	0.5	0.75	0.87	0.37	0.7 \pm 0.45
	Open	0.75	0.13	0.5	0.25	0.13	0.63	0.3 \pm 0.45

Table 5. One daggerblade grass shrimp *Palaemonetes pugio* with a low trematode load (1-9 cysts) was added to each of the 4 separate 33 cm X 20 cm X 23 cm separate control tanks with no substrate to determine if there was an edge effect. Trials ran for 30 min observing behavior of shrimp every minute on 3 August 2017. The mean \pm 1 SD were recorded for open habitat. The total mean \pm 1 SD was calculated for every min during the 30 min trial, not just every 5 min.

Trial	Habitat	5 min	10 min	15 min	20 min	25 min	30 min	Total Mean \pm SD
1	Edge	1.0	1.0	1.0	0.75	0.75	1.0	0.8 \pm 0.43
	Middle	0.0	0.0	0.0	0.25	0.25	0.0	0.2 \pm 0.43
2	Edge	0.75	0.75	1.0	0.75	0.75	0.75	0.8 \pm 0.39
	Middle	0.25	0.25	0.0	0.25	0.25	0.25	0.2 \pm 0.39
Total Mean	Edge	0.87	0.87	1.0	0.75	0.75	0.87	0.8 \pm 0.41
	Middle	0.13	0.13	0.0	0.25	0.25	0.13	0.2 \pm 0.41

Table 6. One daggerblade grass shrimp *Palaemonetes pugio* with a high trematode load (29+ cysts) was added to each of the 4 separate 33 cm X 20 cm X 23 cm substrate tanks with 1/2 of the tank with live *Gracilaria vermiculophylla* in the middle of the tank and 1/2 of the tank with a substrate free open section on the edges. Trials ran for 30 min observing behavior of shrimp every minute on 9 August 2017. The mean \pm 1 SD were recorded for substrate and open habitat. The total mean \pm 1 SD was calculated for every min during the 30 min trial, not just every 5 min.

Trial	Habitat	5 min	10 min	15 min	20 min	25 min	30 min	Total Mean \pm SD
1	<i>G. vermiculophylla</i>	0.0	0.25	0.0	0.25	0.75	0.25	0.3 \pm 0.45
	Edge	1.0	0.75	1.0	0.75	0.25	0.75	0.7 \pm 0.45
2	<i>G. vermiculophylla</i>	0.75	0.0	0.0	0.25	0.0	0.5	0.3 \pm 0.46
	Edge	0.25	1.0	1.0	0.75	1.0	0.5	0.7 \pm 0.46
Total Mean	<i>G. vermiculophylla</i>	0.37	0.13	0.0	0.25	0.37	0.37	0.3 \pm 0.46
	Edge	0.63	0.87	1.0	0.75	0.63	0.63	0.7 \pm 0.46

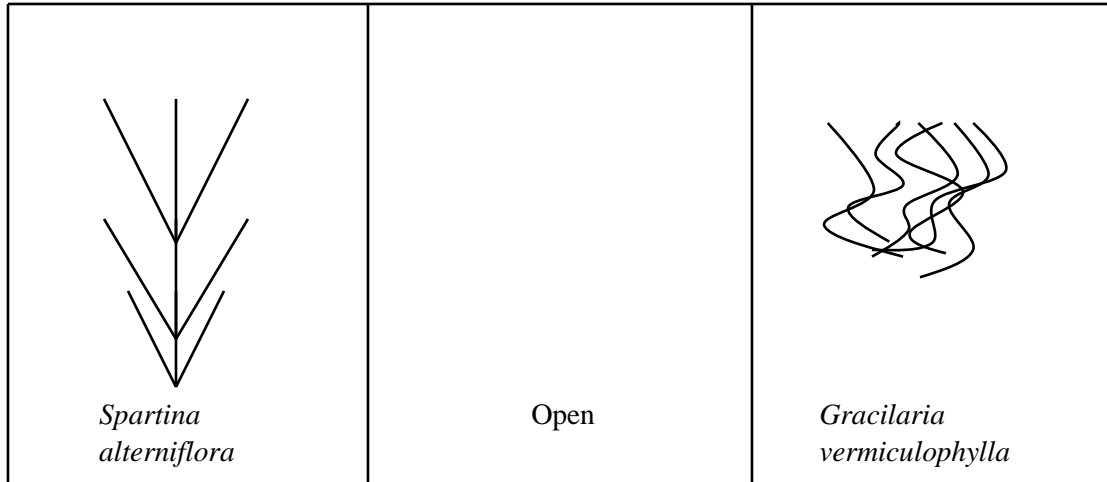


Figure 1. One *Palaemonetes pugio* was added to 4 replicate 33 cm X 20 cm X 23 tanks with 3 habitats sectioned into thirds. 1/3 *Spartina alterniflora*, 1/3 open space, and 1/3 *Gracilaria vermiculophylla*. Experiments were run for 30 min and shrimp were documented every 1 min.

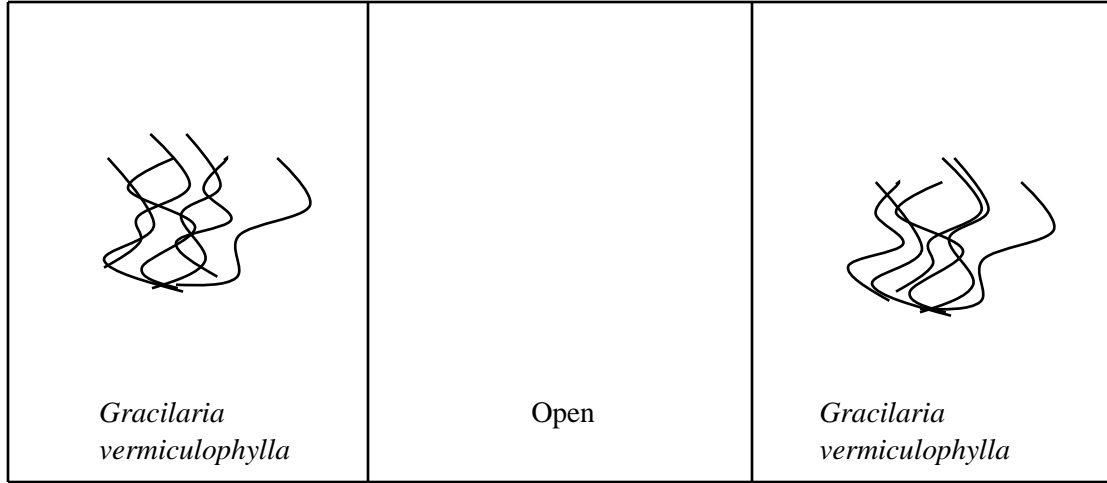


Figure 2. One *Palaemonetes pugio* was added to 4 replicate 33 cm X 20 cm X 23 tanks with 2 habitats sectioned into halves. 1/2 open space, and 1/2 *Gracilaria vermiculophylla*. Experiments were run for 30 min and shrimp were documented every 1 min.

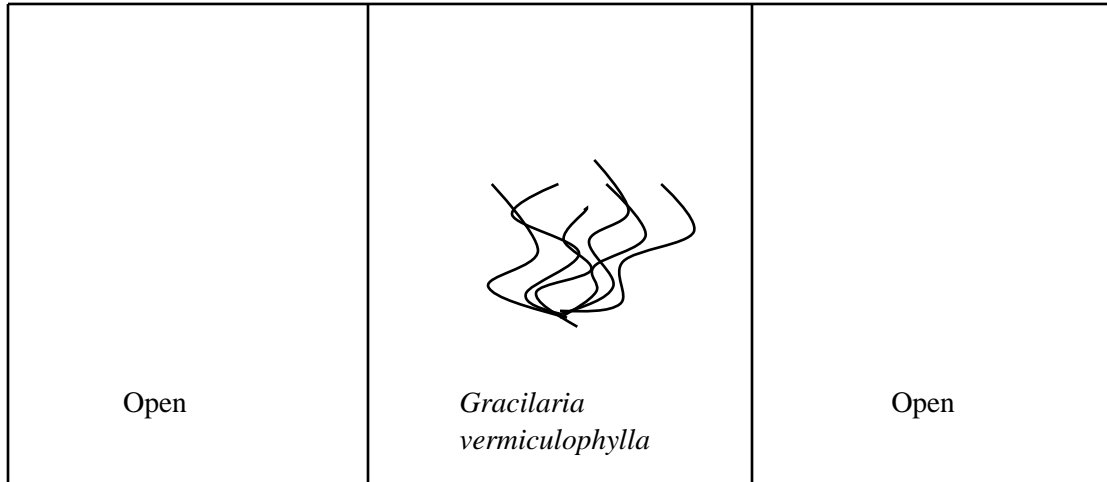


Figure 3. One *Palaemonetes pugio* was added to 4 replicate 33 cm X 20 cm X 23 tanks with 2 habitats sectioned into thirds. 2/3 open space, and 1/3 *Gracilaria vermiculophylla*. Experiments were run for 30 min and shrimp were documented every 1 min.

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EDUCATION

Savannah State University

Aug 2016-Present

Degree: Master of Science in Marine Sciences

Advisors: Dr. Carla Curran, Dr. Michele Guidone

Armstrong State University

May 2016

Degree: Bachelor of Science

Major: Biology Track: Marine

RESEARCH EXPERIENCE

Savannah State University

Oct 2016-Present

Thesis Research: Habitat Preference of the daggerblade grass shrimp *Palaemonetes pugio* and Whether Field Preference is Correlated with the Trematode Parasite *Microphallus turgidus*

Field Work

- Monthly surveys of the daggerblade grass shrimp *Palaemonetes pugio* using dip net and drop trap methods
- Identified different parasites within the shrimp including: the microphallid trematode *Microphallus turgidus*, the haplosporidian hyperparasite *Urosporidium crescens* De Turk, and the bopyrid isopod *Probopyrus pandalicola*
- Measured shrimp using calipers, shrimp species identification, trematode count and sexing shrimp using the dissecting microscope

Laboratory Work

- Feeding experiments using shrimp as predators and veligers of the mud snail *Ilyanassa obsoleta* as prey
- Habitat preference experiments using the smooth cordgrass *Spartina alterniflora* and the invasive red algae *Gracilaria vermiculophylla*

VOLUNTEER EXPERIENCE

Oct 2016-Present

- Boat experience collecting receiver data for the Atlantic stingray *Dasyatis sabina* and the cownose ray *Rhinoptera bonasus*
- Over 200+ logged boat hours
- Longline fishing to tag *R. bonasus* with a V13 internal transmitter
- Boat experience on a skiff collecting different species of flatfish using a beam trawl
- Identifying and measuring flatfishes

Jan 2017-Present

- Multi-day research cruises aboard the R/V Savannah: Water analysis: Fractionated DNA, Chlorophyll a (total and fractionated), POC, qPCR standards
- Handled a small plankton ½ m conical net, quantitative 1 m conical net, and 3 m tucker trawl

March 2017

- OCEARCH Research Expedition: Observed aboard the research vessel on techniques for catching Great White Sharks and learned about past and ongoing studies

November 2017-Present

- Measuring, sexing, and collecting otoliths from red snapper carcasses to document age/growth for the Georgia Department of Natural Resources Coastal Resources Division Carcass Recovery Project

Oct 2016

- Georgia Conservancy: Volunteered with the Georgia Conservancy to help with Hurricane Mathew relief. Cleared debris and restored nature trails.

CONFERENCES

February 2018

- Georgia Southern University, Armstrong Campus Science Seminar Series- Presented my thesis work to a seminar class. Thesis titled: Habitat preference of the daggerblade grass shrimp *Palaemonetes pugio* and whether field preference is correlated with the trematode parasite *Microphallus turgidus*

June 2017

- FACT Meeting- Hobe Sound, FL. Presented on the seasonal migration patterns of 2 cownose rays *Rhinoptera bonasus* along the South Atlantic Bight from 2014-2016

April 2017

- Savannah State University Research Day-Presented a GIS poster that displayed the seasonal migration patterns of *R. bonasus*

April 2016

- Armstrong State University Student Symposium-Presented findings on the reproductive advantages gained by the mud snail *Ilyanassa obsoleta* through the presence of an invasive red algal species *Gracilaria vermiculophylla*
- Armstrong State University Student Symposium-Presented findings on how form is correlated to function of different 3D replicate models of cephalofoils (hammerhead shark heads)

RELEVANT COURSEWORK

- MSCI 5401 Technical Writing and Communication
- MSCI 5402 Research/Marine Science Seminar
- MSCI 5202 Introduction to Coastal Oceanography
- MSCI 5560 Advanced Environmetrics
- MSCI 7564 Geographic Information Systems
- MSCI 6781 Benthic Ecology

UNDERGRADUATE MENTORING

Aug 2017-May 2018

- Helped undergraduates develop research projects based on outcomes of thesis research
- Trained undergraduates on how to identify shrimp to species, count parasites/sex shrimp under the dissecting microscope
- Trained undergraduates on how to collect, identify to species, identify and count parasites, and measure shrimp using calipers in the field
- Reviewing and editing undergraduate research papers and PowerPoint presentations

UNDERGRADUATE EXPERIENCE

Armstrong State University, Savannah, GA

Sept 2015-May 2016

Research/Lab Assistant

- Surveyed using quadrats and transects to collect the mud snail *Ilyanassa obsoleta*
- Measured *I. obsoleta* using calipers, and dissected the snails to determine sex ratios and trematode parasitic infection
- Examined reproductive advantages gained by the mud snail, *Ilyanassa obsoleta*, through the presence of an invasive algal species

Peer Mentor

Jan 2016-May 2016

- Sat in an introduction to cell and molecular biology class to help students formulate their own answers during class/group discussions and also held one study session per week.

Study Abroad Experience

Dec 2014

Field Studies in Marine Biology: Discovery Bay Marine Laboratory, Jamaica

- Explored and identified the varieties of hard and soft corals
- Discussed and observed the roles of anthropogenic and natural issues on coral reef degradation

SKILLS

- Experience using the statistical software SPSS and JMP **Jan 2017-Present**
- Experience using ArcGIS **Jan 2017-Present**
- Able to use Meshmixer software **Jan 2016**
- PADI Scuba Certified **Oct 2012**

EMPLOYMENT

GADNR-Coastal Resources Division

Mar 2018-Present

- Conduct angler surveys as a part of the APAIS, which is an intercept survey designed to assess the catch per unit effort of recreational fishermen
- Conduct Access-Point Angler Intercept Surveys (APAIS) at public marine fishing access points
- Collect individual catch data including species identification, total number of each species, length and weight measurements, as well as angler-specific information about the fishing trip and anglers' fishing behavior