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Vertical Distribution of Meroplankton and Bivalve Competition in a Well-Mixed Estuary

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UNIVERSITY *of* NORTH FLORIDA.

VERTICAL DISTRIBUTION OF MEROPLANKTON AND BIVALVE COMPETITION IN A
WELL-MIXED ESTUARY

by

Jennifer M. Raabe

A thesis submitted to the Department of Biology

In partial fulfillment of the requirements for the degree of

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College of Arts and Sciences

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CERTIFICATE OF APPROVAL

The thesis "Vertical distribution of meroplankton and bivalve settlement in a well-mixed estuary" submitted by Jennifer M. Raabe

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ABSTRACT

If we want to understand how meroplankton utilize the water column and how their vertical distribution may influence horizontal advection, it is important to study their behavior in the various environments where they exist. In a well-mixed system with physical cues dampened, and no vertical layering, these organisms will have to depend on environmental cues such as light, tidal current, and tide cycle, as well as their own swimming ability to migrate vertically. Plankton and water samples were collected at three depths (near surface, midwater, near bottom) during the summers of 2013 and 2014 from sites within the main channel of the Intracoastal Waterway. Six taxonomic groups were collected including polychaetes, bivalves, gastropods, barnacles, tunicates, and crabs, and fell into one of three categories of vertical distribution.

Certain preferences for vertical distribution, and habitat, of sessile invertebrates can increase, or provide refuge from, competition. To assess the potential competition for spatial resources between native and nonnative bivalves in the Guana Tolomato Matanzas estuary, settlement collectors with settlement plates at different depths were deployed for one month periods during the summers of 2013 and 2014 at two main channel sites and two feeder creek sites. Competition would likely be highest subtidally and within the main channel due to all species occurring in that habitat in higher numbers than the feeder creek.

CHAPTER 1

VERTICAL DISTRIBUTION OF MEROPLANKTON IN A WELL-MIXED ESTUARY IN

NORTHEAST FLORIDA

Introduction

Most benthic marine invertebrates have a free-swimming stage which allows larvae to repopulate and maintain the local population or increase the species' range by settling in new habitat away from the source population (Tapia et al., 2010). Once released, larvae can spend hours to months in the water column depending on the species, and may be transported great distances before transforming into the adult form (e.g. Pechenik, 1999; Pineda, 2000; Dobretsov & Miron, 2001). This life history trait may aid in limiting competition for resources with adults during early development, increasing genetic variation in the next generation, or connecting populations across spatial and temporal scales (e.g. Pechenik, 1999; Cowen et al., 2000). Invertebrate larvae of most species do not have the ability to actively swim horizontally against or at speeds greater than currents, meaning that dispersal of larvae is primarily at the whim of prevailing currents (e.g. Cowen et al., 2000; Bilton et al., 2002; Cowen & Sponaugle, 2009). Larvae of some species, however, have the ability to adjust their position vertically in ways that can allow them to control or modify dispersal (e.g. Carricker, 1951; Whalan et al., 2008; Lloyd et al., 2012a). The ability for larvae to overcome physical advection through their vertical migration behavior is dependent on size, and limiting environmental factors such as water density (Gallager et al., 1996) and turbulence (Garrison, 1999).

In some cases, vertical movement may allow larvae to take advantage of countercurrents in order to facilitate horizontal advection (Davis & Butler, 1989; McQuaid & Phillips, 2000; Dobretsov & Miron, 2001). This behavior may be triggered by biotic (e.g. food or predators) or abiotic (e.g. diurnal cycles, turbulence, gravity, density, salinity, and temperature) influences (e.g. Stancyk & Feller, 1986; Raby et al., 1994; Dobretsov & Miron, 2001; Hays, 2003; Knights et al., 2006; Breckenridge & Bollens, 2011). For example, zoea of most crab species swim near

the surface during flood tide, and descend during ebb tide using salinity and turbulence as cues in order to be transported out of the estuary or bay to continue development in coastal waters (e.g. Epifanio et al., 1984; Queiroga et al., 1997; Garrison, 1999; DiBacco et al., 2001). On the other hand, Banse (1986) determined that polychaete and echinoderm larvae behaved as passive, neutrally buoyant particles and their vertical distribution only changed due to hydrographic influences, with no changes due to the diurnal cycle.

Due to the various combinations of biological and hydrographic influences worldwide, different patterns of vertical larval distributions have been established for meroplankton. Much of this research has been conducted in natural stratified systems (e.g. Ouellet & Allard, 2006; Lloyd et al., 2012a; Walkusz et al., 2013), or in a laboratory setting under controlled conditions (Gallager et al., 1996). While laboratory experiments allow us to develop expectations of larval behaviors, they limit our understanding of the behaviors exhibited by larvae in the natural environment due the inability to recreate every condition found in the natural setting. Similarly, the utility of field studies is limited if they are only conducted in a subset of possible environments. Furthermore, most studies focus on the actions of a single species, so more general taxonomic patterns are harder to discern. Since many species differ markedly in their swimming mechanisms and abilities (e.g. Chia et al., 1984), findings in one taxonomic group are not always relatable to other groups. These shortcomings result in a substantial gap in our understanding of how larvae of many taxa behave in well-mixed systems.

The few studies conducted in a well-mixed water column primarily focus on different species of bivalves and the results of these studies suggest that patterns of vertical distribution of bivalve larvae are species-specific or even stage-specific. For example, the larvae of the sea scallop, *Placopecten magellanicus*, was evenly distributed throughout a mixed water column, yet

were concentrated above the pycnocline in stratified waters (Tramblay & Sinclair, 1990). A model was also created by utilizing information from laboratory studies to predict larval growth and behavior in the Eastern oyster, *Crassostrea virginica* (Dekshenieks et al., 1996). The model predicts that smaller (younger) larvae will be homogeneously distributed in well-mixed conditions, and the oldest larvae will primarily be found near the bottom (Dekshenieks et al., 1996). Similarly, late stage *C. virginica* larvae were observed to be most abundant near the benthos and least abundant near the surface (Baker & Mann, 2003). Baker and Mann (2003) also observed late stage larvae of other species and discovered that *Cyrtopleura costata* (clam) and *Bankia gouldi* (shipworm) displayed the same pattern as *C. virginica*, while *Geukensia demissa* (mussel) displayed the reverse pattern with larval abundance highest near the surface and least near the benthos. Since similar studies have only been conducted on bivalves and decapods, it leaves a gap in our current understanding.

Thus far, it is also rare to see studies which include patterns of vertical distribution of multiple taxonomic groups with different swimming mechanisms and life histories, simultaneously. In the few cases where these studies have been done, they have been carried out during upwelling and downwelling events in inner-shelf waters (Garland et al., 2002), through a deep, Antarctic straight with different hydrographic influences (Vazquz et al., 2007), and in a vertically stratified, shallow embayment (Lloyd et al., 2012a). To the Author's knowledge there have been no studies that have focused on the vertical distribution of meroplankton of multiple taxonomic groups in a shallow, well-mixed estuary. If we want to understand how meroplanktonic larvae of different species utilize the water column and how their vertical distribution may influence horizontal advection, it is important to study their behavior in the various environments where they exist.

In the current study, we examined the vertical distribution of 6 taxonomic groups with varying life histories, and swimming abilities. Different developmental types are utilized by marine invertebrates including planktotrophy, lecithotrophy, direct development, and mixed development, though about 70% of marine species utilize free-swimming planktotrophic development (e.g. Caswell, 1981; Jablonski & Lutz, 1983; Giangrande et al., 1994). Some taxonomic groups utilize different development types in different species (e.g. Jablonski & Lutz, 1983). For example, the gastropod *Littorina irrorata* has a planktotrophic development while the gastropod *Connus pennaceus* employs mixed development where the larvae are encapsulated in an egg, but then emerge as free-swimming, pre-metamorphic larvae (e.g. Caswell, 1981; Jablonski & Lutz, 1983). Many planktotrophic larvae use cilia for locomotion while others use muscular propulsion (e.g. Chia et al., 1984). We hypothesize that free-swimming planktotrophic larvae that utilize cilia for locomotion such as polychaetes, gastropods, and bivalves would find it more difficult to control their vertical position, and are more likely to show homogeneous vertical distribution. Conversely, we would expect that strong swimming larvae that utilize muscular propulsion such as crabs, shrimp, barnacles, and tunicates would more easily control their vertical distribution and, therefore, will be more likely to display stratification throughout the water column. In a well-mixed system with physical cues dampened, and no vertical layering, larvae will have to depend on environmental cues such as light, tidal current, and tide cycle, as well as their own swimming ability to migrate vertically. Therefore, the current study sought to determine whether larval abundances of various taxonomic groups varied with depths, tidal current, tide, and light levels.

Materials and Methods

Study sites

Sample collection was conducted from May to September 2013 and June to August 2014 within the Intracoastal Waterway (ICW) of Florida at 2 locations approximately 28.6 km apart (Fig. 1.1). The sampling location designated SS was located at the mouth of the San Sebastian River which empties into the ICW. Sampling took place at the green Daybeacon "1" (29° 52.131′N; 81° 18.446′W) which has a mean tidal range of 1.306 m and a mean depth of 3.9 m (NERRS, 2018). The second sampling location was located approximately 28.5 km south of site SS at the red Daybeacon "118" (29° 37.560'N; 81° 12.578'W) and designated BL since it is just north of the Bings Landing public boat ramp. Site BL has a mean tidal range of 0.449 m and a mean depth of 3.8 m (NERRS, 2018). Dix et al. (2013) found the site SS to be well-mixed, and it is assumed that BL is also well-mixed.

Collection cycles took place twice per month for three months (May – September 2103 and June – August 2014), and four collections were made at each site per collection cycle to include all tides within a 24-hour period (two flood and two ebb tides and both day and night). Collections were conducted at mid-incoming and mid-outgoing tides according to local tide tables, and within two days of either a spring or neap tide.

Water sample processing

To determine if the vertical distribution of larvae can be explained by temperature, salinity, or fluorescence, water samples were collected at each collection depth during each sampling event using a horizontal Alpha Water Sampler. Temperature and salinity were measured on-site using a YSI Pro 2030 water quality probe, while water samples were stored on ice and transported to the laboratory to test fluorescence using a Turner TD 700 Fluorometer.

Fluorescence was used as a proxy for food by measuring chlorophyll-*a* in each water sample; an estimate of phytoplankton abundance.

Plankton collection

The vertical distribution of meroplankton was assessed by using a modification of a technique described by Dobretsov & Miron (2001) in which plankton nets with 53-μm mesh were placed at 3 evenly distributed depths (near surface: 0.5 m from the surface; mid-water: ~ 1.5 – 3.0 m from surface; near bottom: ~ 0.5 – 1.0 m from bottom) on a mooring line (Fig. 1.2). Plankton nets were attached to swivels allowing them to rotate to face the water current at all times. Interchangeable lengths of line were used between plankton nets to adjust the nets to depth during different tides. A flow meter was attached to the cod-end of each plankton net to determine the volume of water moving through the net in order to determine larval concentrations during each collection. A single assembly was deployed for approximately 30 minutes during each collection period. Samples were fixed on site in a ~3:1 ratio of 99.5% ethanol and seawater to be identified and counted at the laboratory using a stereoscope.

Plankton sample processing

Samples were processed by decanting the ethanol and seawater solution using a 53-μm mesh sieve. Samples were then processed according to methods described by Britton & Greeson (1989). Once the solution was removed, samples were diluted with distilled water to a volume that could be managed under the microscope as determined by the individual conducting the sorting. Samples were stirred in a Z-shape to avoid creating a vortex, which could concentrate the plankton in the center of the container. Subsamples of 1 mL were then removed from the sample using a pipet, and placed in a Sedgewick-Rafter counting cell. At least three subsamples from each sample were counted and major taxonomic groups were identified: bivalvia,

gastropoda, polychaeta, crustaceans and urochordata (identification manuals: Todd et al., 1996; Johnson & Allen, 2012). Due to their morphology being more easily distinguished under the microscope than the other groups, crustaceans were further divided into the groups of barnacles and crabs for analysis. For data analysis, the mean larval abundance was calculated for each sample using subsample counts and abundances. First, each subsample count was multiplied by the stored sample volume to determine the total number of larvae of each group within the subsample. Second, the total number of larvae of each group in each subsample was then divided by the volume of water filtered during sampling as determined by the flow meter to determine the mean abundance of each group in each subsample. Then, the mean larval abundance (M), in the form of individuals per cubic meter (ind./m^{-3}), of group i in sample j was determined by using the following equation:

$$M_{ij} = \frac{1}{n_j} \sum_{i=1}^{n} D_{ij}$$

where n is the number of subsamples counted in sample j, and D is the mean abundance of group i found in each subsample.

Statistical analysis

Spearman's Rank-Order Correlation was performed to determine any relationships between depths and environmental variables (salinity, temperature, and fluorescence) in order to verify that the estuary was well-mixed during the time of sampling. To address the question: if larval abundances of various taxonomic groups vary with depths, tidal current, tide, and light levels, samples were pooled into two tidal current categories (ebb and flood), two tide categories (spring and neap), and two light level categories (day and night). Sampling events when the flowmeter was missing or displayed a negative distance were omitted from the dataset. Collections that took place during transitional times (dawn/dusk) were also removed from the

dataset. There were 134 daytime and 99 nighttimes samples; 117 neap tide and 116 spring tide samples; and 111 flood tide and 122 ebb tide samples. Since larval abundances failed to meet the assumptions of normality and heterogeneity of variances, they were $\log(x + 2)$ transformed (Lloyd et al., 2012a, 2012b). A series of 1-way and 2-way ANOVAs were utilized to test the main effects of larval depth with either tidal current, tide, or light level. Any significant differences among depths would signify that the larval abundance of each taxonomic groups differs with depth. Any significant interaction term would indicate larval densities are changing vertically in response to the environmental variables. For any significant interaction terms discovered, the simple main effects with Bonferonni adjustment was reported, and revealed the degree to which one factor (environmental parameter) effects each level of the second factor (depth). Due to tunicates continuing to fail the assumptions of normality and heterogeneity of variances after transformation, the group was analyzed using untransformed abundances and the non-parametric Kruskal-Wallis H test. The α-level for all statistical tests used was 0.05. All statistical tests were performed using SPSS 25.0.

Results

Using untransformed data, the most abundant taxa over all collections was polychaetes (37.65%), followed by bivalves (35.84%), gastropods (12.53%), barnacles (8.24%), tunicates (4.74%) and crabs (1%). Other taxonomic groups collected in fewer numbers were considered rare and were disregarded. Several groups showed different ontogenetic stages that could be reliably identified. For example, bivalve veligers and pediveligers, barnacle nauplii and cyprids, and crab zoea were all identified in some of the collections. In most cases, however, one of the life-stages was rare making statistical analysis of different age groups of the same taxa

unreliable. Therefore, all life stages were pooled for each taxonomic group. Although the system which was sampled had been determined as well-mixed in previous studies (e.g. Dix et al., 2013), temperature, salinity and fluorescence were tested at each depth during each sampling effort and no significant differences were found for any environmental variable across depths (Spearman's Rho tests: depth vs. salinity: $r_s = 0.078$, $p = 0.238$; depth vs. temperature: $r_s = -0.017$, $p = 0.799$; depth vs. fluorescence: $r_s = -0.002$, $p = 0.981$).

To determine if larval abundance for each taxonomic group differ across depths, larval abundance was pooled across collection dates and sites, and abundances among depths for each group was compared independently. Larval abundance did not differ significantly among depths in any of the taxonomic groups except gastropods. Gastropod larval abundance differed significantly among depths ($F_{(2,209)} = 3.471$, $MS_{Error} = 0.775$, $p = 0.033$) due primarily to greater abundance near bottom, although none of the pairwise comparisons were significantly different (Tukey's post hoc test: near surface vs. midwater: $p = 0.984$; near surface vs. near bottom: $p = 0.062$; midwater vs. near bottom: $p = 0.109$) (Fig. 1.3). There was a general (but not significant) trend towards greater abundance near bottom for all taxa, except barnacles and tunicates that both showed higher larval abundance near surface (Table 1.1., Fig. 1.3).

Crabs were the only taxonomic group to display a significant interaction term with an interaction between depth and tidal current (Fig. 1.5, Table 1.1). During flood tide, crab larvae were more abundant near bottom than near surface and midwater, while larval abundance did not differ among depths during ebb tide (pairwise comparisons: Flood tide: near surface (mean = 13.350) vs. midwater (mean = 10.605): $p = 0.791$; near surface vs. near bottom: (mean = 75.211) $p = 0.044$; midwater vs. near bottom: $p = 0.027$; Ebb tide: near surface (mean = 131.046) vs.

midwater (mean = 67.295) : p = 0.640; near surface vs. near bottom (mean = 18.388): p = 0.386;

midwater vs. near bottom: p = 0.139).

Homogeneity across depths with higher abundance in response to a single environmental variable were observed for gastropods and crabs. Gastropod and crab larvae were more abundant during spring tide and were more scarce during neap tide (Gastropods: $F_{(2,209)}$ = 5.006, MS_{Error} = 0.775, p = 0.026; Crabs: $F_{(2,209)}$ = 10.900, MS_{Error} = 0.457, p = 0.001) (Fig. 1.4). Tunicate larval abundance showed a significant difference between tidal currents with overall larval abundance greater during ebb tidal current than flood tidal current (Kruskall-Wallis H test: H = 4.636, df = 1, p = 0.031) (Table 1.1). Any interactions between depth and environmental factors were unable to be determined for tunicate larvae due to the use of a Kruskal-Wallis H test. Therefore, whether tunicate larvae utilize different depths in response to any of the environmental variables could not be tested.

Discussion

The current study sought to determine if larval abundance for each taxonomic group differs across depths, and if abundances change in response to environmental parameters (tidal currents, tides, and light levels) in a well-mixed, subtropical estuary. Meroplankton in the study system fall into one of three categories: (1) homogenous throughout the water column with no difference in abundance in response to environmental factors, (2) homogeneous across depth but with differences in abundance in response to a single environmental variable or (3) homogenous in some conditions and aggregated at a certain depth in others. Bivalve, polychaete, and barnacle larvae all fall into the first category with homogenous distributions throughout the water column regardless of light level, tide, or tidal current. The second category includes gastropod and tunicate larvae. Gastropods were homogeneous across depths but were more abundant during

spring tides, while tunicate larvae were homogeneous among depths but more abundant during ebbing tidal currents. Crab larvae were the lone taxonomic group in the third category with larvae homogenously distributed among depths during spring tides but aggregated near bottom during flood tide.

The homogeneous distribution observed for bivalve, polychaete, and barnacle larvae could be explained by one or more hypotheses. First, the pattern of homogeneity could be due to all species of these groups, and all age groups of these species, exhibiting no difference in larval abundance across depths due to their weak swimming abilities. Second, the majority of samples could be from a single species which displays patterns of homogeneity across depths and the other species are so rare that they do not disrupt the overall pattern. Third, the different species, or ontogenetic stages, within these groups show opposing patterns resulting in homogeneity when all species, or stages, are pooled together.

Previous research indicates bivalve larvae have a tendency towards aggregation at particular depths (e.g. Dekshenieks et al., 1996; Baker & Mann, 2003; Knights et al., 2006; Lloyd et al., 2012a). Therefore, it was unexpected that bivalves would display homogeneity in the current study. A higher abundance of oysters, *Crassostrea virginica*, than the other bivalve species, *Perna viridis, Geukensia demissa,* and *Mytella charruana* has been observed in the estuary (personal obs.). While the capture of multiple species is likely, it is more probable that oysters were dominating the bivalve pattern in most situations. *Crassostrea virginica* exhibits different vertical patterns and swimming behaviors at different ontogenetic stages (Chia et al., 1984; Dekshenieks et al., 1996; Baker & Mann, 2003). For example, a model based on oyster behaviors observed in the laboratory predicted smaller larvae to be distributed throughout the water column while the oldest were found near the benthos in a well-mixed water column

(Dekshenieks et al., 1996). A field study conducted in a well-mixed system also observed late stage oyster larvae more abundant near the benthos (Baker & Mann, 2003). The differences in vertical migrations at different ages may be the result of a change in larval behavior with competent larvae sinking towards the benthos (e.g. Chia et al., 1984; Dekshenieks et al., 1996; Baker & Mann, 2003). It has been suggested that the increase in mass may be responsible for the change in swimming abilities as younger, smaller bivalves are able to utilize cilia to support their mass, while the ciliated swimming of larger, more mature larvae is overpowered by sinking (Bayne, 1964; Chia et al., 1984; Baker & Mann, 2003). Thus, younger larvae would be higher in the water column while older larvae would be found nearer the benthos. Therefore, the most likely explanation for homogeneity found for bivalve larvae is that most of our samples were dominated by *C. virginica*, and opposing stage-specific distributions that have been pooled together were observed.

The same hypothesis can also be applied to the homogeneity observed for barnacle larvae. Ontogenetic stage-specific patterns have been described for some barnacles. For example, Tapia et al. (2012) observed that barnacle nauplii (early stage) were most abundant near the surface at all times, while cyprids (late stage) were in greater abundance in mid-depth and bottom layers. Since cyprid larvae are considered stronger swimmers than nauplii (e.g. Walker, 2004), it is unlikely that cyprids do not have the ability to swim near the surface. Therefore, the fact that they are typically found close to the bottom is better explained by more mature larvae sinking towards settlement substrate for further ontogenetic development. Since all ontogenetic stages of barnacles were pooled during our study, opposing depth patterns such as these could result in the observation of homogeneity across depths for this taxonomic group.

The homogeneous distribution of polychaete larvae observed in this study may be explained by their weak swimming speeds ($0.01 - 0.02$ cm s^{-1}; Chia et al., 1984). While various vertical distribution patterns have been described for polychaetes, these patterns may be tied to hydrographic processes in the study area (Banse, 1986). For example, early *Owenia fusiformis* larvae are mostly concentrated under the pycnocline when stratification is strong, yet are homogeneously distributed when stratification is weak (Thiébaut et al., 1992; Ataya et al., 2011). Thus, homogeneity would also be expected in a non-stratified system. Since polychaete larvae are considered one of the weakest swimmers, it is possible that larvae do not possess strong enough swimming abilities to penetrate through layers of different densities that are typically seen in stratified water columns. Therefore, the pattern observed in our study is likely due to vertical mixing overpowering larval swimming and controlling the distribution of polychaete larvae causing homogeneity.

Gastropods fall into the second category of larvae being uniformly distributed throughout the water column, but more abundant under certain environmental circumstances. Since gastropod larvae are able to regulate their vertical position within the water column with swimming speeds of 0.13 cm s^{-1} (Chia et al., 1984), the overall homogeneity of gastropod larvae among depths may be explained by a lack of stratification in phytoplankton, their food source. Lloyd et al. (2012b) found that the taxa *Littorinimorpha*, which includes *L. littorea,* had a strong, positive relationship with the fluorescence maximum. Since fluorescence was homogenous in the present study, there is no reason to expect *L. littorea* or other gastropods to congregate at a particular depth to feed.

Gastropod larvae were also significantly more abundant during spring tide than neap tide. The abundance of gastropod larvae during spring tide may be explained by gastropods spawning

in response to spring tides. *Littorina littorea*, the marsh periwinkle, is prominent within the estuary (Frazel, 2009) and is known to lay its eggs during spring tides. Larvae hatch after a few days spent in the egg capsule and spend between 11-30 days in the plankton (Fish, 1979). If gastropods regularly spawn during spring tides, mortality could lead to a pattern of greater abundance during spring tides than neap tides. Consequently, the pattern of gastropod larval abundance may be explained by larvae experiencing mortality after release from their egg capsules on the spring tide.

The overall homogeneity observed for tunicate larvae may be a result of different species utilizing distinct reproduction strategies. For instance, if brooded larvae and encapsulated larvae were released at different times, it could create a homogenous distribution. Several species of tunicates inhabit the estuary that undergo sexual reproduction in different ways. This includes two ovoviviparous species that release larvae after brooding, *Ecteinascidia turbinata* (mangrove tunicate) and *Amaroucium stellatum* (sea pork), and two species that utilize broadcast spawning which creates encapsulations, *Molgula manhattensis* (common sea grape) and *Styela plicata* (pleated sea squirt) (Berrill, 1931; Yamaguchi, 1975; Gotelli, 1987; Carballo et al., 2000; Frazel, 2009). Brooders release larvae approximately 7 to 9 days after fertilization (*E. turbinata*, Carballo et al., 2000), but larvae can be hatched from eggs after about 10 hours (*M. manhattensis*, Berrill, 1931). Therefore, the homogeneity throughout the water column was likely a combination of the lack of differentiation between species, and larvae being released at alternating intervals.

Broadcast spawning may be responsible for the influx of larvae during ebbing tides. Castilla et al. (2007) observed a broadcast spawning tunicate which spawned during flood tides, and subsequently observed tadpole larvae on the ebb tide a few hours later. The larval stage is

short-lived with larvae settling within minutes to hours after release (Yamaguchi, 1975; Davis & Butler, 1989), thus, quickly moving out of the water column and possibly beyond the depths sampled in this study soon after ebbing tides. Since some broadcast spawning tunicates can have multiple spawning events (*M. manhattensis,* Berrill, 1931; *Pyura stolonifera,* Marshall, 2002) it is likely this was also observed several times throughout the study causing the pattern of greater larval abundance during ebbing tides.

Crabs were the only group that showed significant differences in larval abundance among depths and changes in those depth patterns according to environmental variables. Crab larvae were more abundant near bottom during flood tide, homogenous during ebb tide, and showed greater overall abundance in spring tides than in neap tides. The pattern of greater abundance near bottom during a flood tide has been widely accepted as a transport mechanism (selective tidal-stream transport) for crab zoea to be exported offshore in taxa such as *Callinectes sapidus* (blue crab, Epifanio et al., 1984)*; Carcinus maenas* (green crab, Queiroga et al., 1997); Ocypodidae (ghost and fiddler crab). Pinnotheridae (pea crabs), and Panopeidae (mud crabs) (Garrison 1999); and *Pachygrapsus crassipes* (DiBacco et al., 2001). Crab larvae avoid being transported further into the estuary by sinking lower in the water column to evade the incoming tidal current. In contrast, Epifanio et al. (1988) observed fiddler crab (*Uca spp.*) zoea in the Delaware River estuary displaying the opposite pattern once they enter the primary estuary from the marsh creeks with larval abundance greater near the surface during flood tide, and more abundant near bottom during ebb tide. A pattern such as this would promote retention in the estuary, which would be beneficial to fiddler crabs as megalopae of this taxa are weak swimmers (Epifanio et al., 1988). Since our data show crab larvae displaying a different pattern than seen

for fiddler crabs, it is perhaps an indication that our samples included a majority of a species that utilize selective tidal-stream transport such as blue crabs or mud crabs.

The overall greater abundance of crab larvae during spring tides may be caused by larval release from encapsulation. Hatching during spring tide is a characteristic of many estuarine crabs including the most abundant species in our estuary, *Callinectes sapidus* (blue crab) and *Uca spp.* (fiddler crab) (Morgan, 1987; Morgan & Christy, 1995; Frazel, 2009). Timing the release of larvae during a spring tide can result in seaward transport (Christy, 1982) which is vital for metamorphosis. Therefore, the patterns observed for crabs are responses to either being exported, or retained within the estuary.

The current study has shown that major groups of meroplanktonic larvae in a shallow, well-mixed estuary in Northeast Florida display several different patterns throughout the water column: homogeneity across depth and consistent abundance across time, homogeneity across depth with higher abundance associated with environmental cues, homogenous across depth in some conditions and aggregated at a certain depth in others. The overall patterns observed here are not congruent with patterns observed in studies conducted on various meroplankton in stratified systems. In those studies, many taxa are associated with various layers (e.g. pycnocline, halocline, thermocline, fluorescence maximum) or display diel migration, while the same taxa in the current study mainly display homogeneity, with the exception of crabs. The contrast between this study and those conducted in stratified waters may suggest that larvae simply do not migrate or display stratification in well-mixed systems, but previous studies conducted in well-mixed waters certainly observe some aggregation or migration of larvae. Differences in larval patterns between the current study and previous field studies in mixed systems may lie in the taxonomic resolution utilized in each study (Tramblay & Sinclair, 1990; Dekshenieks et al., 1996; Baker &

Mann, 2003). Since many of these taxonomic groups show species-specific vertical distributions in previous studies, future studies in our estuary should focus on differentiating between species within each group. Sampling throughout the year, or having a longer sampling season, in order to capture different ontogenetic stages would also be beneficial as some groups display differences in vertical distribution as they mature.

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CHAPTER 2

NATIVE AND NON-NATIVE BIVALVE SETTLEMENT: POTENTIAL COMPETITION FOR SPATIAL RESOURCES IN A NORTHEAST FLORIDA ESTUARY

Introduction

Successful invasion of a species involves a number of stages, including initial introduction, survival and establishment in the new habitat, and range expansion (Andow et al., 1990). With the increase of trans-oceanic vessel traffic (Pimentel et al., 2005; Tan & Morton, 2006) and the warming of our oceans (e.g. Stachowicz et al., 2002), we see an increase in the introduction of marine invertebrates, especially tropical and sub-tropical species (Hilbish et al., 2010). Introduced species usually have a high tolerance for pollution and have the ability to reproduce quickly (Tan, 2006). Typically, these organisms include a larval stage capable of significant dispersal which provides the invading population the ability to increase its range rapidly, although rapid expansion may result in reduced reproductive output due to low population density (Allee effect) (Gascoigne & Lipcius, 2004; Leung et al., 2004; Tobin et al., 2011). Propagule pressure describes a measure of the number of individuals released into an area to which they are not native (Carlton, 1996) and is an important factor that may influence the success of an invasion (e.g. Johnston et al., 2009). Propagules, including larvae, may be released into an area but may not survive prior to settlement or may show low survival to reproductive age post-settlement (Johnston et al., 2009). Larval survival and subsequent settlement can be affected by both biotic (e.g. food or predators) and abiotic (e.g. diurnal cycles, turbulence, gravity, density, salinity, and temperature) influences (e.g. Stancyk & Feller, 1986; Raby et al., 1994; Dobretsov & Miron, 2001; Hays, 2003; Knights et al., 2006; Breckenridge & Bollens, 2011).

Bivalves are adept invaders with introductions documented worldwide that date back to the Middle Ages (e.g. Agard et al., 1992; Boudreaux & Walters, 2006; Tan & Morton, 2006; Karatayev et a., 2007; Spinuzzi et al., 2013; Herbert et al., 2016). Once a non-native species has

become established the potential of negative interactions with native species is likely to increase. Non-native bivalves have had detrimental effects on native communities in several instances such as the *Potamocorbula amurensis* in San Francisco Bay (e.g. Carlton et al., 1990; Nichols et al., 1990), *Dreissena polymorpha* in the Great Lakes (e.g. Burlakova et al., 2000), and *Crassostrea gigis* in the Oosterschelde estuary (e.g. Troost et al., 2009). Understanding the behavior of late stage larvae of native and non-native species of bivalves within the same ecosystem can allow us to better predict where non-native species might appear, and may also help us in understanding how these species might interact and potentially compete.

The Guana Tolomato Matanzas National Estuarine Research Reserve in Northeast Florida is home to several species of bivalves. The Eastern oyster, *Crassostrea virginica*, and the ribbed mussel, *Geukensia demissa*, are native bivalves while the Asian green mussel, *Perna viridis*, and the Charru mussel, *Mytella charruana*, are non-native species. The Asian green mussel, *Perna viridis,* is native to the coastal marine waters of the Indo-Pacific region, primarily distributed along the Indian and Southeast Asian coasts (Rajagopal et al., 2006) and was first discovered in Southeastern United States in 1999 (e.g. Benson et al., 2001; Buddo et al., 2003; Baker et al., 2007). *Mytella charruana* (charru mussel) is native to South America and Mexican Gulf Coast and was first discovered in Northeast Florida in 1989 (Boudreaux & Walters, 2006). Previous studies have revealed some of the negative effects that introduced bivalves in Northeast Florida may have on native oyster populations. Adults of both *P. viridis* and *M. charruana* have been shown to negatively influence *C. virginica* settlement and survival. *Perna viridis* adults can reduce *C. virginica* larval settlement; *M. charruana* can reduce spat growth; and both non-native species negatively affect the survival of juvenile oyster spat (Yuan et al., 2016b). Galimany et al. (2017) also discovered the non-native *M. charruana* is able to outcompete *C. virginica* due to its

ability to more rapidly intake and digest food sources. These studies, however, were conducted in the laboratory, so their relevance to natural systems is currently unknown. Much of their relevance depends on the degree to which these species overlap spatially and temporally in habitat use.

Competition among sessile invertebrates can take multiple forms, including preferences for similar habitat and substrate, as well as the relative timing at which settlement occurs. With each species in the current study occurring in subtropical to tropical climates, temperature and salinity tolerances are similar for each species (e.g. Bertness & Grosholz, 1985; Ortega & Sutherland, 1992; Bartol & Mann, 1997; Franz 2001; Spares & Dadswell, 2001; Wilson et al., 2005; Boudreaux & Walters, 2006; Rajagopal et al., 2006; Jost & Helmuth, 2007; Yuan et al., 2016a). Similarly, both non-native species have been found to preferentially settle on natural hard substrate including native oyster shells, instead of man-made hard substrates (Gilg et al., 2010). Since oyster spat were not investigated in Gilg et al. (2010), it is unknown to what degree the preferences of non-native species overlaps with those of the native species. Other studies have shown that *C. virginica* larvae preferentially settles on oyster shell (e.g. Nestlerode et al., 2007; George et al., 2014; Yuan et al., 2016b) though, settlement on other hard substrate such as concrete, porcelain, lime stone, and river rock has been observed (George et al., 2014). In fact, all of these species have been observed inhabiting the same intertidal oyster reef on the north end of the in the Indian River Lagoon in Florida (Yuan et al., 2016b, Walters pers. obs.). Peak settlement also occurs around the same time of year (summer to early fall) for each of these species, although some are known to have both major, and minor peaks of settlement (e.g. Báez, et al., 2005; Wilson et al., 2005; Gilg et al., 2014; Vallejo et al., 2017). Therefore, competent larvae of the bivalve species in the current study seem likely to be competing for similar

settlement substrate, in similar locations, at the same time. Another form of spatial competition that has not been tested, however, is preferential settlement depth, which has the potential to provide some spatial refuge.

Previous work at sites within their native range suggests that *P. viridis* preferentially settles at intermediate depths (4 m) as opposed to either shallow or deep depths (1m or 7m, respectively) (Rajagopal, 1998b). Late stage oyster larvae are found in higher densities near the benthos and late stage ribbed mussel larvae are found closer to the water surface (Baker & Mann, 2003). The depth preference of *M. charruana* settlement is undocumented.

A better understanding of the processes that govern settlement for these bivalve species will help us understand how introduced species might affect recruitment of native species. Can depth and habitat differences in settlement provide native bivalve species spatial refuge? Settlement plates were deployed at various depths within two different habitats during a time of peak settlement in a Northeast Florida estuary to determine (1) if spat abundance for each species differed among depths (top, mid, bottom of the water column), and (2) if spat abundance for each species differed among habitats (feeder creek, main channel).

Materials and Methods

Study sites

Settlement collection was conducted once a month from May to September 2013 and June to August 2014 at two locations within the main channel of the Atlantic Intracoastal Waterway (ICW) and within two creeks that feed into the ICW (Fig. 2.1). The timing of the collection periods coincided with what is typically peak settlement periods for all of these species (Báez, et al., 2005; Wilson et al., 2005; Gilg et al., 2014; Vallejo et al., 2017). Each feeder creek site was sampled in concurrence with a site located in the main channel of the ICW.

This sampling scheme provided two locations in close spatial proximity that differed markedly in habitat. Therefore, differences between the two sites are likely due to environmental differences as opposed to distance from source populations. The first feeder creek site (OC) was located near Oyster Creek, upstream the San Sebastian River. Collection at OC was located at green Daybeacon "35" (29° 53.267'N; 81° 19.210'W) where the mean depth is approximately 2.0 m (NERRS, 2018). The main channel site sampled along with OC was the sampling location designated SS, located where the San Sebastian River empties into the ICW. Sampling at SS took place at the green Daybeacon "1" (29° 52.131′N; 81° 18.446′W) which has a mean tidal range of 1.3 m and a mean depth of approximately 3.9 m (NERRS, 2018).

The second feeder creek site (PC) was located in Pellicer Creek off the end of a recreational boat dock within Faver-Dykes State Park (29° 40.024′N; 81° 15.444′W). This site had a mean depth of approximately 2.3 m with a mean tidal range of about 0.6 m (NERRS, 2018). The main channel site associated with PC was located at the red Daybeacon "118" (29° 37.560'N; 81° 12.578'W) and was designated as BL since it is just north of the Bings Landing public boat ramp. BL has a mean tidal range of 0.5 m and a mean depth of 3.8 m (NERRS, 2018). Dix et al. (2013) found the site SS to be well-mixed, and it is assumed that all other sites within this study are also well-mixed.

The vertical distribution of bivalve settlement was evaluated by placing spat collectors made of two 12 cm x 12 cm quarry tiles along a 3.1 m-long PVC pipe using plastic cable ties (Fig. 2.2). These settlement collectors were attached to the pilings of the day beacons or other posts at the study sites using steel hose clamps. Due to their shallower maximum depths, the feeder creek sites, OC and PC, had only two tile placements. The top tile was placed such that half of it would typically be exposed during spring low tides (spring low tide on the piling was

determined by the marking of fouling organisms) and the bottom tile was approximately 1.5 m below it. This positioned the bottom tile <1m from the bottom of the creek. The greater depths of main channel sites (SS and BL) allowed for three tile placements. The top collection plate was positioned ~0.6 m from the top of the PVC pipe and again placed so that half of it would be exposed during spring low tides. The bottom collection plate was placed just above the bottom of the PVC pipe and the middle plate was positioned at the mid-point between the top and bottom plates. This resulted in each plate being separated by ~1.2 m and the bottom plate rested approximately 1 m above the sediment.

Each of the 4 sites contained 1 spat collector which remained in the field for 1-month time periods after which they were retrieved, the quarry tiles removed and replaced with new tiles, and then the collector was returned to the water. Collected tiles were allowed to dry for approximately 2 weeks in a covered outdoor location with protection from rain. Bivalve spat were identified by using morphological characteristics and enumerated under a stereoscope. All the plates located at the Bing's Landing site during July 2013, and San Sebastian during September 2013 were lost when the equipment was torn from the piling, but data are available from all other locations and sampling periods.

Environmental variables were also collected at each site to verify the estuary was well-mixed during the time of sampling, and if not, to test whether settlement depth was associated with temperature, salinity, and fluorescence. We tested temperature, salinity, and fluorescence, by collecting water samples at three different depths using a horizontal Alpha Water Sampler. Temperature and salinity were measured on-site using a YSI Pro 2030 water quality probe, while water samples were stored on ice and transported to the laboratory to test fluorescence using a Turner TD 700 Fluorometer.

Statistical analysis

Since settlement densities were not normally distributed non-parametric Mann-Whitney U test was utilized to compare settlement abundance among depths (high, middle, and low) and habitats (feeder creek and main channel). Since environmental variables were found to not differ significantly among depths in this shallow, well-mixed system, analyses of environmental differences were restricted to comparisons among habitats utilizing a Kruskall-Wallis H test. Dates at which no settlement was detected for a given species were removed from analysis. The significance level for all statistical tests used was $\alpha = 0.05$ and all tests were performed using SPSS 25.0.

Results

All four of the species of interest settled on collectors over the course of the study, although *M. charruana* was only found in 2014. Oyster spat made up 75.35% of the total bivalves collected with a mean spat density 4.16X greater than that of the second most abundant species, *P. viridis*. *Mytella charruana* had the least amount of spat, making up 0.96% of the total bivalves collected throughout the entire study and only occurred in the main channel (Fig. 2.3). Most species showed fairly similar temporal settlement patterns with the greatest number of spat found in the later months of collection. Spat numbers during 2013 were lower by an order of magnitude compared to 2014 (121 total spat in 2013, 1,240 spat in 2014). In 2013, each species had low spat abundance in July, with peak abundance for all species in August and slightly decreased in September (Fig. 2.3). In 2014 *C. virginica* were abundant in all three months but peaked in July, while *P. viridis* were nearly absent in June then increased and remained steady in July and August (Fig. 2.3). Since settlement of the *M. charruana* was so rare, this species was removed from all analyses.

To determine differences among sites in each habitat (Main channel: BL and SS; Feeder creek: PC and OC), we pooled spat abundance across depths and collection dates, and compared spat density (mean number of spat per plate) at one site to spat density at the corresponding site within each habitat. Since *M. charruana* was never collected in the feeder creeks, and *G. demissa* was only collected at PC, these species were not included in the statistical analysis. Although *P. viridis* was found more in PC, and *C. virginica* spat was more abundant in OC, these differences were not significant (Kruskall-Wallis H Test results: U = 11.00, P =0.461; U = 21.00, P = 0.180, respectively) (Table 2.2). There were no significant differences between spat abundance for any species between the main channel sites, BL and SS (Table 2.2).

To test for potential differences in spat abundance between habitats, we pooled spat across depths and collection dates, and compared settlement abundance in the main channel with the settlement density in the feeder creek independently for each species. *Perna viridis* was the only species that showed a significant difference in spat abundance with the highest spat density found on the collectors placed in the main channel (U = 103.00, P = 0.042) (Table 2.3).

Spat abundance was compared across different depths for each species independently. Since the feeder creek sites only had settlement plates at two depths (top, bottom), compared to the main channel sites that had three (top, mid, bottom), depth analyses were conducted for each habitat separately. When each site was considered independently there was a trend toward lower spat density on the high plates than on the deeper plates (Top plate means: BL = 2.07, SS = 0.70, OC = 1.33, PC = 0.4; Middle plate means: BL = 13.19, SS = 19.31; Bottom plate means: BL = 21.69, SS = 21.85, OC = 16.50, PC = 2.20). That said, many of these comparisons suffer from low sample sizes making differences among depths difficult to verify. Therefore, the data for settlement depth were also analyzed by pooling data of sites within the same habitat (i.e: BL +

SS and OC + PC) which increased sample sizes substantially and made the comparisons among depths more powerful. In the feeder creeks, both *P. viridis* and *C. virginica* settled significantly more on the bottom plates than at the top, while *G. demissa* settlement did not differ among depths (Figure 2.4) (Mann-Whitney U tests: *P. viridis* top vs. bottom: U = 3.00, P = 0.015; *C. virginica* top vs. bottom: U = 17.50, P = 0.040; *G. demissa* top vs bottom: U = 0.00, P = 0.333). A similar pattern was observed in the main channel, with *P. viridis, C. virginica,* and *G. demissa* all having significantly lower settlement at the top collection plates than either the mid or bottom plates, while settlement at the mid and bottom plates did not differ from each other (Table 2.2) (Mann-Whitney U tests: top vs. mid: *P. viridis*: U = 19.50, P = 0.035, *C. virginica*: U = 14.50, P = 0.036, *G. demissa*: U = 7.50, P = 0.029; top vs bottom: *P. viridis*: U = 11.00, P = 0.004, *C. virginica*: U = 10.00, P = 0.011, *G. demissa*: U = 4.50, P = 0.008; mid vs bottom: *P. viridis*: U = 38.50, P = 0.393, *C. virginica*: U = 35.50, P = 0.666, *G. demissa*: U = 24.00, P = 0.442.) Although we did not perform statistical analysis on the depth distribution of *M. charruana,* it was most abundant at the mid depth plate, and was absent from the top plates.

To test if the differences in spat abundance between main channel and feeder creek sites could be explained by differences in environmental parameters, we compared temperature, salinity, and fluorescence among habitats. Feeder creeks showed lower than average salinity (H = 14.246, P < 0.001) and greater fluorescence (H = 13.311, P < 0.001) than the main channel sites while temperature did not differ significantly (H = 1.519, P = 0.218) (Fig. 2.5). Salinity and fluorescence in the feeder creeks fluctuated much more dramatically than in the main channel site, while temperature remained relatively steady in both habitats (Fig. 2.5).

Discussion

The current study investigated the potential for interspecific competition by determining: (1) if spat abundance for each species differ among depths (top, mid, bottom), and (2) if spat abundance for each species differ among habitats (feeder creek, main channel). All of the species showed similar settlement patterns, except for *M. charruana* for which the data are too limited to make any conclusive statements. *Perna viridis* and *C. virginica* preferentially settled at the mid to low depths rather than at the top settlement plates in both habitats, while *G. demissa* displayed this pattern in the main channel habitat but not in feeder creeks. All species were found in the main channel, but only *P. viridis* and *C. virginica* were collected in both feeder creek sites. Since *C. virginica* and *P. viridis* had the highest spat abundance throughout our study and both species exhibit the same settlement patterns associated with depth and habitat, spatial competition may be greatest between these two species. Competition would likely be highest in the main channel due to both nonnative species occurring in that habitat in higher numbers than the feeder creeks.

Our data show that *P. viridis, C. virginica,* and *G. demissa* preferentially settle subtidally since they all had a higher spat abundance on the mid and bottom collection plates which were always submerged, in contrast to the top collection plates which would be exposed on spring low tides. Subtidal settlement is an established pattern for both *P. viridis* and *C. virginica*. Rajagopal et al. (1998a) conducted a study within the native range of the Asian green mussel and found settlement most abundant at 4 m, and least abundant at the study's lowest depth of 7 m. The present study also showed the majority of settlement at ~3 m, but samples were not collected deeper than 3 m so it cannot be determined whether settlement would decrease at greater depths as in Rajagopal et al. (1998a). Subtidal settlement for *C. virginica* has also been a well-established pattern in the Eastern United States. Competent larvae are more abundant near the

benthos, and least abundant near the surface (Baker & Mann, 2003), and settlement generally follows the same pattern (Ortega & Sutherland, 1992; Bartol & Mann, 1997). Growth rates tend to be much higher in subtidal habitats than in the periodically submerged intertidal habitats (e.g. Sumner, 1981; Crosby et al., 1991), but higher predation rates have also been observed (e.g. Roegner & Mann, 1995). Therefore, preferential settlement in subtidal habitats such as was observed here, suggests that both *P. viridis* and *C. virginica* likely occupy areas that lack predators in their native ranges. If this is indeed the case, then the subtidal settlement behavior of *P. viridis* is unlikely to be deleterious in the Southeastern U.S. where it will tend to face the same predators as *C. virginica*.

Nielsen & Franz (1995) suggest a latitudinal difference in distribution patterns of *G. demissa* since with more settlement in the high intertidal zone for their Southern populations (e.g. Georgia and Alabama) (West & Williams, 1986; Lin, 1989). The conflicting results of vertical settlement of the ribbed mussel may be a result of post-settlement habitat selection where the mussel uses its byssal threads to continue searching for suitable habitat after initial settlement. Therefore, the differences in recruitment at certain depths is less important for the soft-bottom dwelling ribbed mussel because there is usually no limitation of space, and the aforementioned post-settlement movement allows them to be commitment free (Peterson, 1991). Since *G. demissa* has the ability to relocate, the spat abundance in our study may be an underestimate of the actual juvenile abundance at the study sites.

Perna viridis was the only species to display significantly greater settlement in the main channel of the ICW rather than its feeder creeks. This pattern is consistent with previous research on settlement in the same estuary (Gilg et al., 2014). In the feeder creeks, *C. virginica* had over 30x more settlers than *P. viridis*. The disparity between *P. viridis* and *C. virginica* suggests that

either oyster larvae are more adept in traveling into the feeder creeks, or oysters are better equipped to survive in the feeder creeks. Generally, it may also be more difficult for bivalve larvae to be transported into the feeder creeks from the parental source in the main channel due to being well flushed by tides through the nearby inlets and having flushing times of less than 2 days (Sheng et al., 2008). Since the feeder creeks become nearly fresh with salinities as low as 0.5 PSU (NERRS, 2018) at times, oyster salinity tolerance is the most likely explanation for exceptionally higher oyster spat abundance within the feeder creeks. Wilson et al. (2005) witnessed oysters surviving salinities <10 ppt while green mussels can survive salinities as low as 20 ppt (Rajagopal et al., 2006). Yuan et al. (2016b) also determined that *P. viridis* has a narrower range of temperature tolerances as salinity decreased, suggesting less survival in the low-salinity feeder creeks during the winter months. The disparity of settlement between these two species in the feeder creeks suggests this habitat could act as a refuge for oysters. Baker et al. (2011) also observed a potential refuge for oysters in the low-energy habitats such as mangrove prop roots. The low flow of the feeder creeks may also play a key role in providing refuge for *C. virginica*.

The lack of *M. charruana* in the collections could be due to lack of attraction to the settlement plates, low overall abundance of Charru mussels in the area, or because the settlement collectors were not placed in the right type of habitat. *Mytella charruana* may not have been attracted to the settlement plates since the mussel has been found to preferentially settle on natural substrate such as oyster and mussel shells rather than man-made substrate such as plexiglass, wood, and rock, including quarry tiles and brick (Gilg et al., 2010). Overall low abundance of *M. charruana* at the collection sites is a possible reason for the few spat found during this study since *M. charruana* was only found once at a location near the collection sites

during a 5-year survey (2006-2011) (Spinuzzi et al., 2013). The Charru mussel also has a low tolerance for cold weather events (Yuan et al., 2016a). For example, Spinuzzi et al. (2013) discovered a Northeast Florida population of *M. charruana* that experienced high mortality after an unusually cold winter in 2009 and by June 2010 when air temperatures fallen to 0°C or below and the population had not recovered. In the winter of 2012/2013, air temperatures in St. Augustine, Florida had dropped to or below 2°C for a few days throughout the winter (23 December, 17-18 February, 4 March; NOAA NWS). The winter of 2013/2014 was also fairly cold with air temperatures that dropped lower than 2012/2013 but only for two days (-3°C on 7 January; 0.56°C on 17 January; NOAA NWS). Therefore, in addition to using undesirable settlement substrate, the freezing temperatures in the winters leading up to spat collections could be responsible for the low abundance of *M. charruana* spat throughout our study. While *M. charruana* has been shown to occupy the same functional niche as *C. virginica* (Galimany et al., 2017), it appears less likely to be a significant competitor in Northeastern Florida due to its low numbers. If temperatures continue to drop to near freezing during the winters, it may be sufficient to keep *M. charruana* from becoming abundant enough to compete with *C. virginica*.

Since three of the species had higher subtidal settlement in the main channel, spatial overlap is likely to occur. In fact, *P. viridis* has been previously found to settle in greater numbers on disarticulated oyster and green mussel shell than man-made substrates (Gilg et al., 2010). Though oysters also preferentially settle on disarticulated oyster shell (e.g. Nestlerode et al., 2007; George et al., 2014), they have been observed settling less on live native and nonnative mussels (relative to settlement on oyster shell) (Yuan et al., 2016b). All species existing in such close proximity can significantly reduce survival and growth of oyster spat due to nonnative mussels decreasing the availability of settlement and food resources (Yuan et al., 2016b).

Nonnative bivalves can also negatively affect oyster larval settlement potentially due to predation of larvae (Yuan et al., 2016b).

Our data show a substantially higher number of *C. virginica* spat than *P. viridis* spat throughout the study which may be due to different settlement substrate preferences, peak spawning for green mussels took place before or after our collection dates, or higher abundance of oyster larvae than green mussel larvae. Gilg et al. (2010) discovered that *P. viridis* preferentially settles on natural hard substrate including native oyster shell and nonnative mussel shell. Previous studies have found that *C. virginica* larvae preferentially settles on oyster shell (e.g. Nestlerode et al., 2007; George et al., 2014; Yuan et al., 2016b), but settlement on man-made hard substrate has also been observed (George et al., 2014). Since both bivalves preferentially settle on calcium carbonate (e.g. oyster shells, travertine tiles, limestone; Nestlerode et al., 2007; George et al., 2014; Metz et al., 2015; Yuan et al., 2016b), the differences in spat abundance between oysters and green mussels in the current study are unlikely caused by settlement preferences. While settlement occurs between summer to early fall for both species (e.g. Dame, 1972; Wilson et al., 2005; Gilg et al., 2014), peak settlement for *P. viridis* and *C. virginica* may occur at different times. *Crassostrea virginica* in the Indian River Lagoon settle between March and September (Wilson et al., 2005), while *P. viridis* has been suggested to spawn twice per year in Tampa Bay, Florida with the first in April and the second in September (Barber et al., 2005) suggesting settlement would occur in around May and October since larvae have a 2-week life span (Rajagopal et al., 1998b). Since the current study took place between May – September in 2013 and June – August 2014, *P. viridis* spat were not collected during their potential second spawning event. Although, missing the Fall settlement peak may not be important in regards to the number of spat collected since Rajagopal et al. (2006)

discovered a substantially higher number of larvae during the summer months versus the fall

spawning event in their native range. Therefore, it is likely that the largest peak of settlement was

not missed during the collection period of the current study. Previous surveys conducted on

intertidal populations by researchers in the estuary found the greatest abundance of *P. viridis*

adults near two inlets in the estuary with few adults further from the inlets (see Gilg et al., 2014).

Due to the preference for subtidal settlement shown in the current study and others (e.g.

Rajagopal et al., 1998a), the current intertidal population may be an underestimate of the actual

population currently inhabiting the GTM Estuary. Although total population may be greater than

observed for intertidal communities, this study still collected fewer *P. viridis* spat than *C.*

virginica spat indicating that oysters are likely more abundant throughout the estuary than the

green mussel.

Currently, due to the low spat abundance of *M. charruana* and *P. viridis* compared to *C.*

virginica, it is unlikely the nonnative mussels will become a threat to the native oyster

populations unless ecological and environmental factors remain optimal for these species (i.e. no

unusually cold weather). Since climate scientists suggest that over the next few decades the

average air temperatures could increase to 1.1 to 5.4°C higher than it is today (US National

Centers for Environmental Information), conditions are likely to become more tolerable for these

nonnative bivalves. If conditions were to remain optimal long enough, these nonnative species

have the ability to devastate native oyster populations due to their tendency for fast growth and

high densities. For instance, *P. viridis* has a tendency for faster growth in areas with high flow

(Rajagopal et al., 1998a) and has been observed in densities as high as 12,000 individuals $/m^2$ in

Florida (Baker et al., 2002). *Mytella charruana* has been observed amassing in densities as high

as 11,000 individuals/m^2 (Pereira et al., 2003). These large aggregations could pose a threat by

displacing native oyster populations. In fact, *P. viridis* has already displayed its ability to dominate certain habitats in Tampa Bay (Baker et al., 2011).

While oysters are currently the dominant bivalve in the GTM Estuary, oyster reefs have been declining along the Atlantic and Gulf coasts (e.g. Coen et al., 2007). Oyster reefs provide many ecosystem services including increased native biodiversity (e.g. Grabowski et al., 2012). Invasion biology theory claims a positive relationship between native biodiversity and invasion resistance (Elton, 1958). If biodiversity were to decrease with the loss of oyster reefs, *P. viridis* and other non-native species could more easily invade estuaries with declining oyster populations. While cold temperatures currently limit *P. viridis* and *M. charruana* populations (Urian et al., 2010; Spinuzzi et al., 2013), global warming may allow these invasive mussels to not only persist and spread in the current study system, but to further expand their range throughout the Southeastern U.S. As increasing temperatures would only increase the potential for competition, spatial refuge could prevent the decline of *C. virginica*. Native species may not find refuge in their settlement depth preference since each species was found to settle subtidally. Though, with *M. charruana* spat absent and *P. viridis* spat abundance substantially lower than *C. virginica* in the feeder creek habitat, *C. virginica* may find spatial refuge from non-native competitors in the highly fluctuating, low salinity of feeder creeks.

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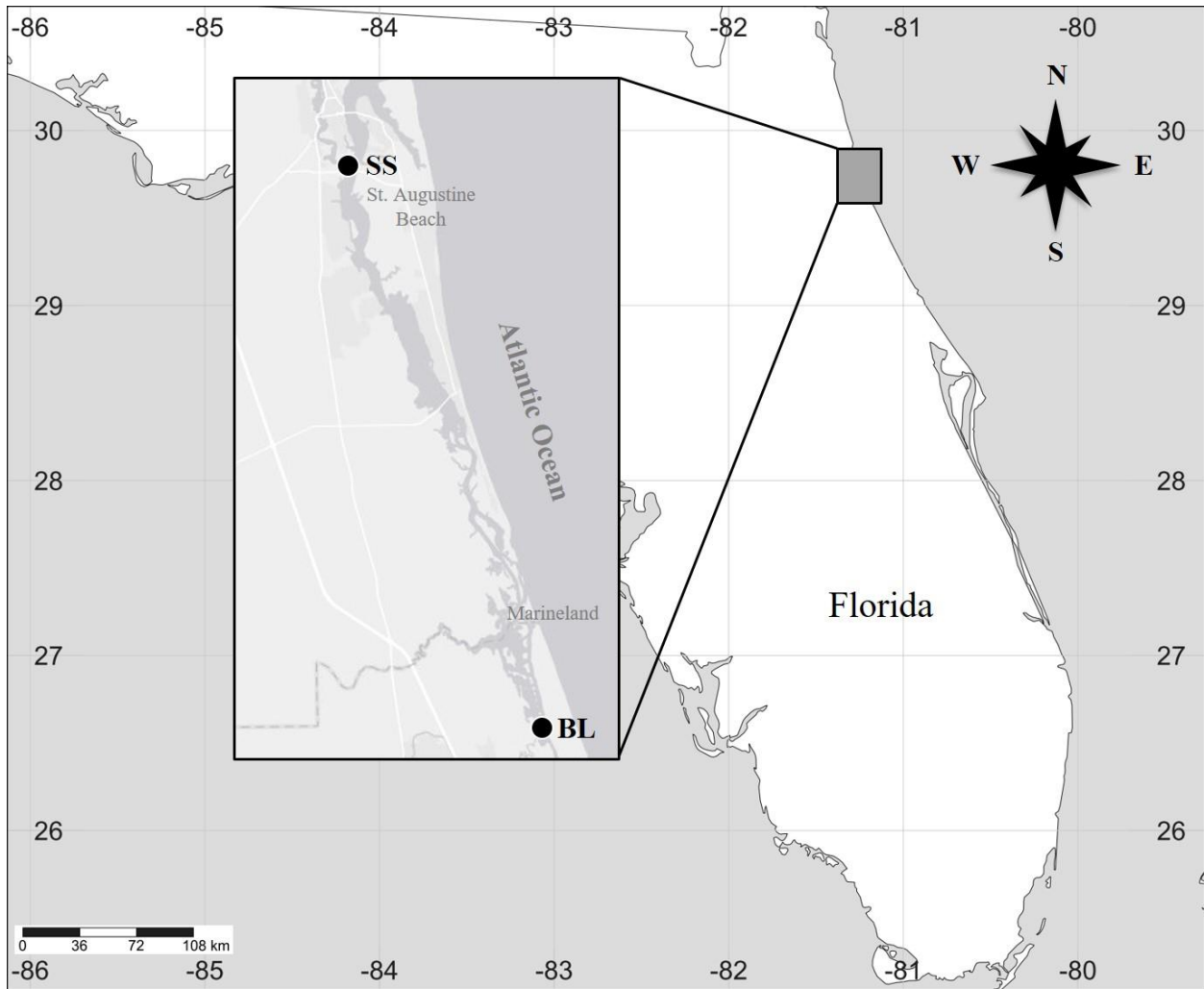
Figure 1.1. Site Map. The locations of the San Sebastian (SS) (29° 52.131′N; 81° 18.446′W) and Bing's Landing (BL) (29° 37.560'N; 81° 12.578'W) collection sites.
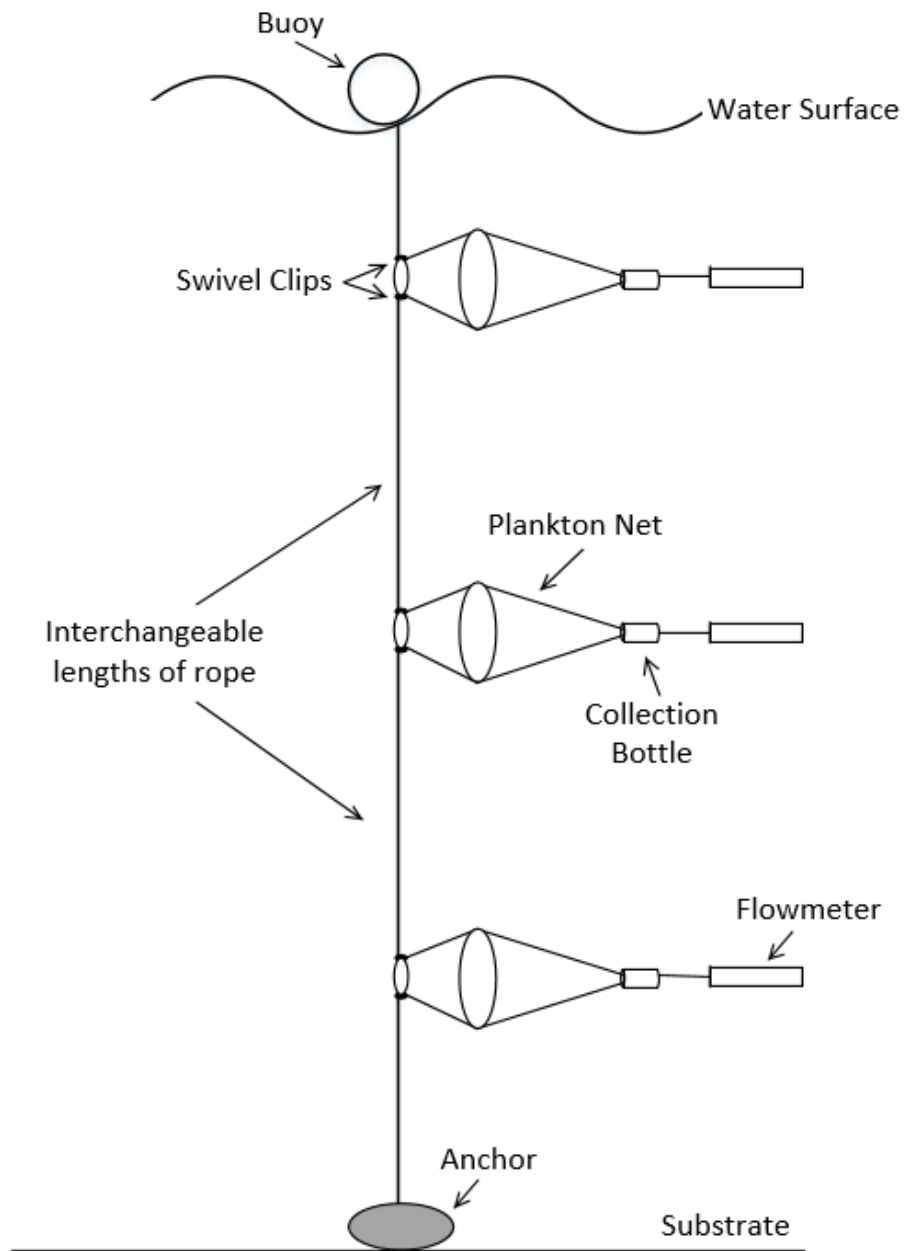
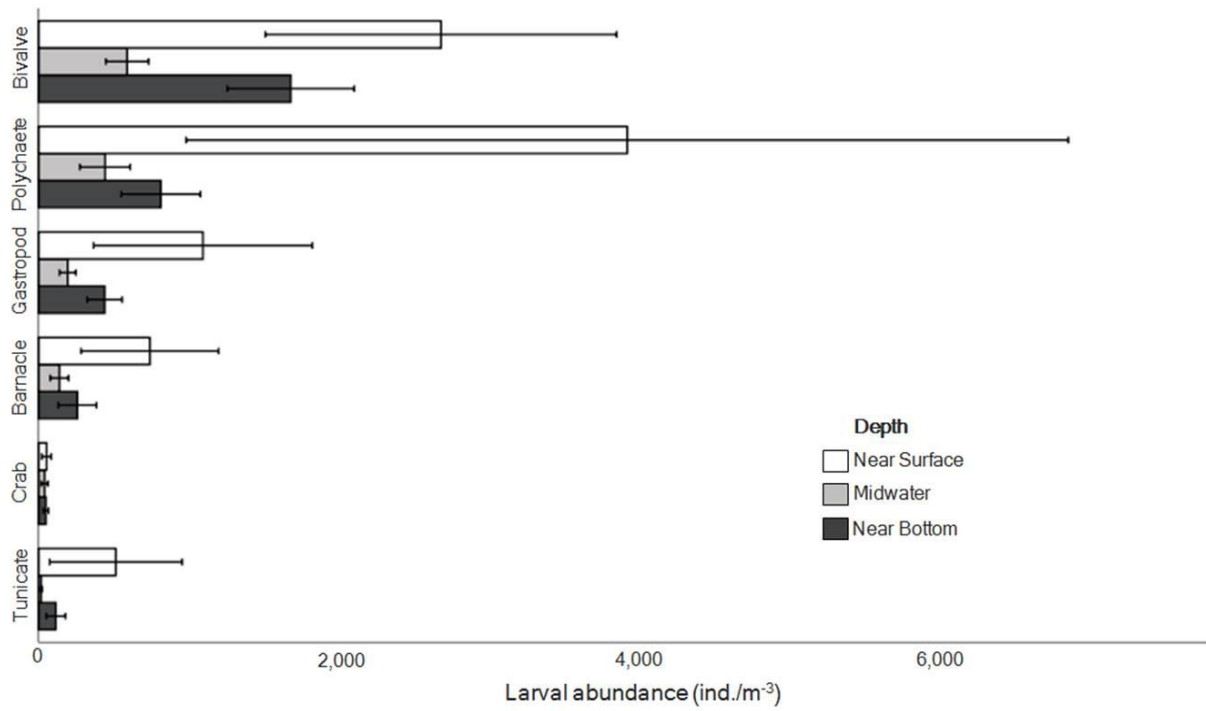**Figure 1.2.** Collection device modified from Dobretsov & Miron (2001).

Figure 1.3. Mean larval abundance (individual/m^3) (±SE) of each taxanomic group pooled for near surface depth (white bars), midwater depth (light grey bars), and near bottom depth (dark grey).
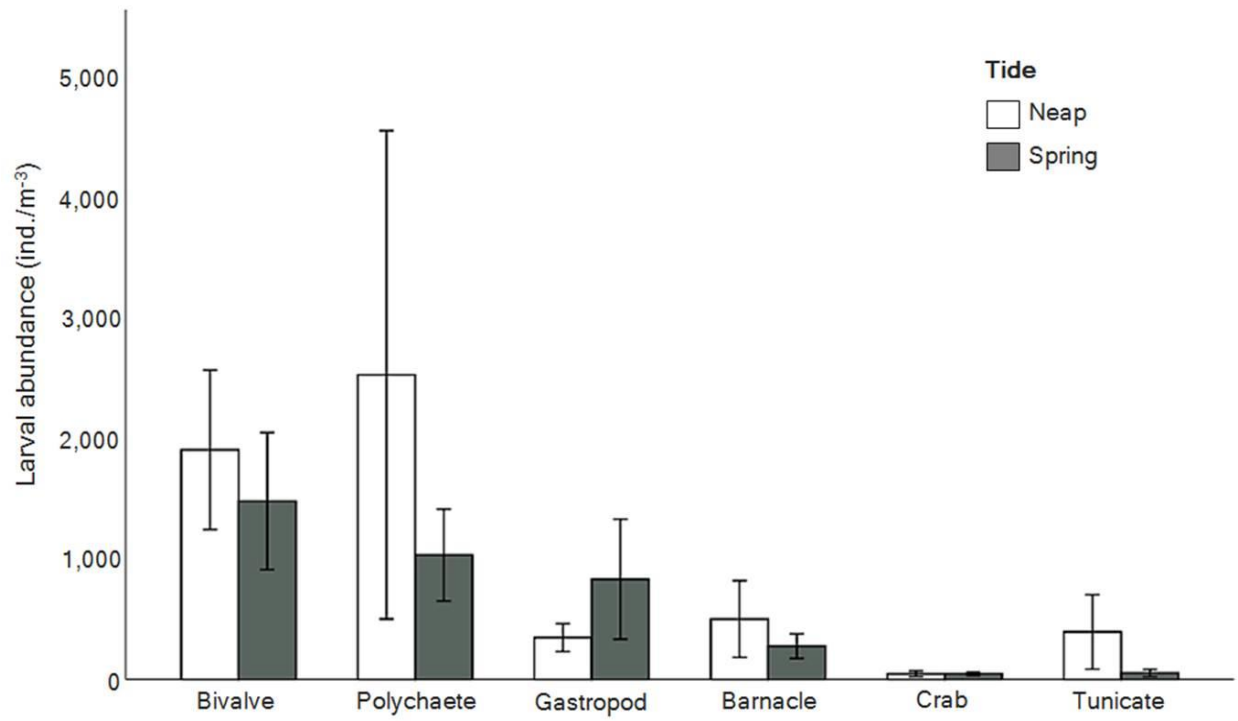
Figure 1.4. Mean larval abundance (individual/m^3) (±SE) of each taxonomic group pooled for spring tides (grey bars) and neap tides (grey bars).
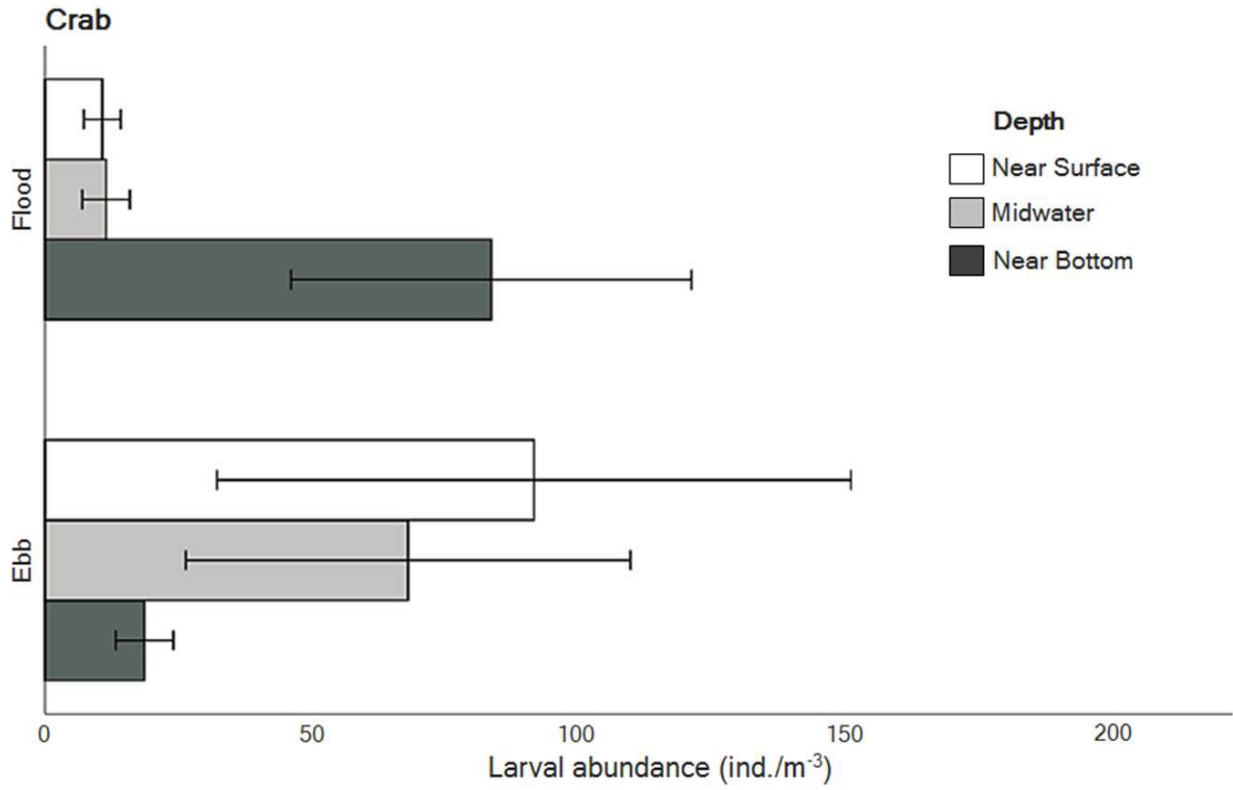
Figure 1.5. Mean larval abundance (±SE) of crab larvae with a significant interaction between depth (near surface, midwater, and near bottom) and tide (flood, ebb).

	Depth	Mean	SD	P-value		Depth	Mean	SD	P-value
Bivalve	Near Surface	2673.53	10503.29	*0.060*	**Polychaete**	Near Surface	3910.70	26374.96	*0.316*
	Midwater	588.87	1196.27			Midwater	441.78	1406.22	
	Near Bottom	1672.45	3794.60			Near Bottom	812.45	2363.99	
	Light					**Light**			
	Day	1310.50	1310.50	*0.816*		Day	879.44	879.44	*0.454*
	Night	2206.77	9443.92			Night	2990.87	23702.95	
	Tide					**Tide**			
	Spring	1479.38	6151.94	*0.230*		Spring	1032.31	4121.10	*0.162*
	Neap	1905.08	7127.02			Neap	2527.25	21810.85	
	Tidal Current					**Tidal Current**			
	Ebb	1144.89	2714.69	*0.448*		Ebb	743.15	3102.13	*0.154*
	Flood	2291.90	9181.95			Flood	2912.40	22435.64	
	Depth	**Mean**	**SD**	***P-value***		**Depth**	**Mean**	**SD**	***P-value***
Gastropod	Near Surface	1091.73	6539.58	**0.033**	**Barnacle**	Near Surface	739.45	4111.73	*0.359*
	Midwater	193.03	456.76			Midwater	138.38	511.31	
	Near Bottom	439.24	1043.21			Near Bottom	257.92	1140.57	
	Light					**Light**			
	Day	319.72	867.23	*0.488*		Day	214.94	839.80	*0.743*
	Night	958.30	5918.78			Night	624.34	3762.51	
	Tide					**Tide**			
	Spring	832.16	5384.27	**0.026**		Spring	276.95	1107.55	*0.913*
	Neap	347.85	1250.48			Neap	501.80	3420.45	
	Tidal Current					**Tidal Current**			
	Ebb	250.72	586.54	*0.051*		Ebb	290.58	1236.51	*0.138*
	Flood	965.09	5628.73			Flood	496.94	3441.99	
	Depth	**Mean**	**SD**	***P-value***		**Depth**	**Mean**	**SD**	***P-value***
Crab	Near Surface	52.60	278.50	*0.795*	**Tunicate**	Near Surface	513.33	3960.17	*0.235*
	Midwater	40.09	179.71			Midwater	17.20	44.98	
	Near Bottom	48.26	158.45			Near Bottom	114.67	567.40	
	Light					**Light**			
	Day	43.71	155.10	*0.706*		Day	58.35	316.87	*0.309*
	Night	52.12	272.49			Night	447.18	3598.97	
	Tide					**Tide**			
	Spring	48.31	160.01	**0.001**		Spring	53.75	317.71	*0.755*
	Neap	46.24	255.54			Neap	394.83	3326.88	
	Tidal Current					**Tidal Current**			
	Ebb	58.26	263.63	*0.199*		Ebb	112.57	560.44	**0.031**
	Flood	35.21	136.16			Flood	345.55	3371.21	

Table 1.1. Untransformed means and associated *p-values* for larval abundance. Values in bold represent significant differences ($\alpha = 0.05$) in one-way analysis of variance (bivalve, polychaete, gastropod, barnacles, crab) and Kruskall-Wallis H Test (tunicates).
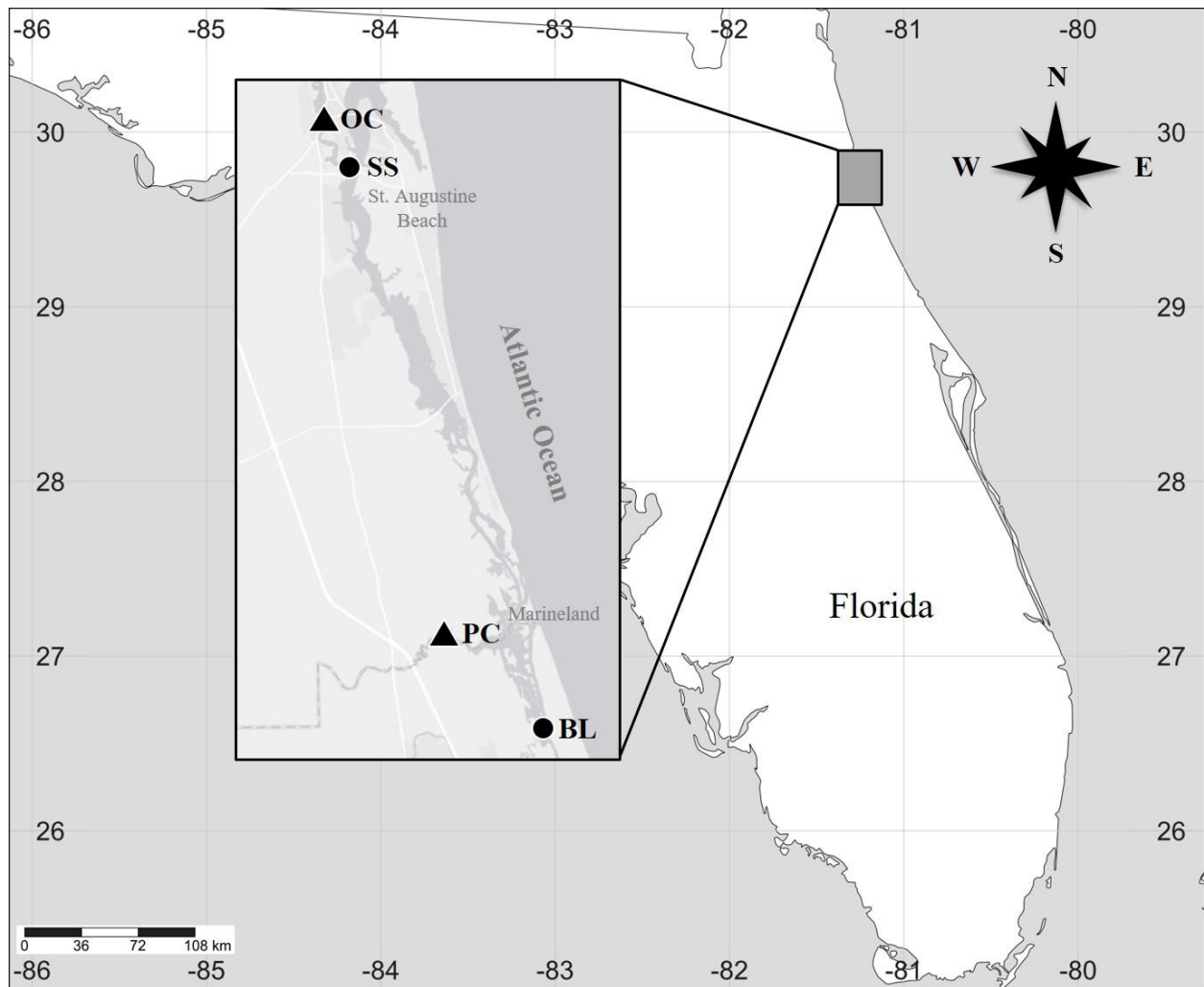
Figure 2.1. Site Map. The locations of the main channel collection sites (*circles)* San Sebastian (SS) (29° 52.131´N; 81° 18.446´W) and Bing's Landing (BL) (29° 37.560'N; 81° 12.578'W); and the feeder creek sites (*triangles*) Oyster Creek (OC) (29° 53.267'N; 81° 19.210'W) and Pellicer Creek (PC) (29° 40.024´N; 81° 15.444´W).
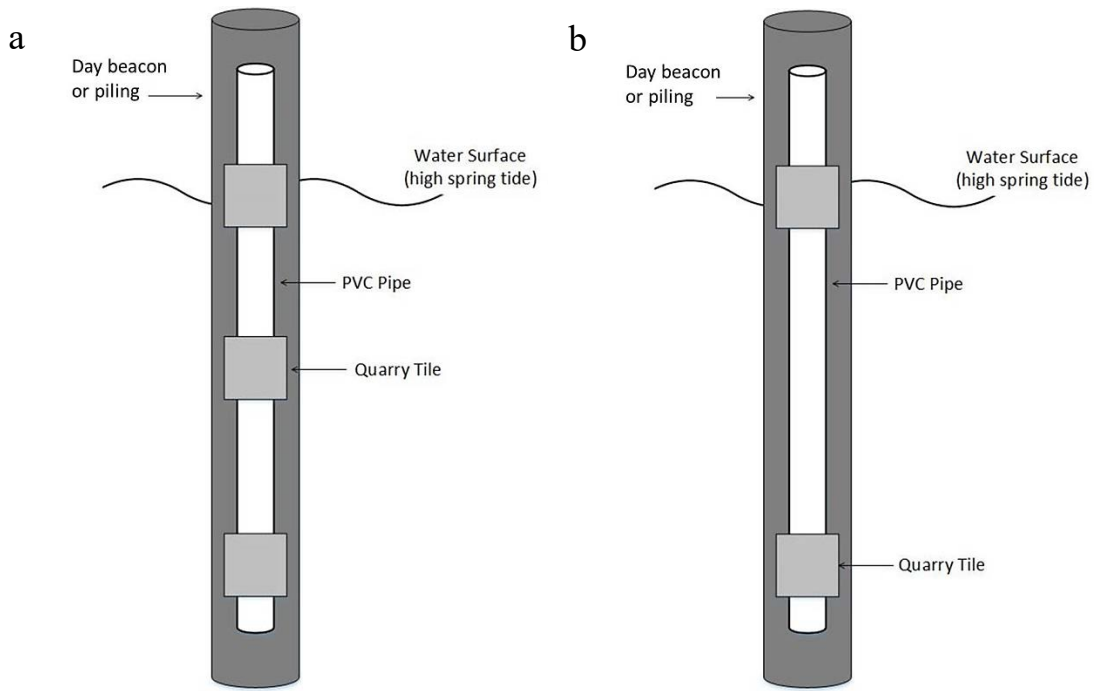
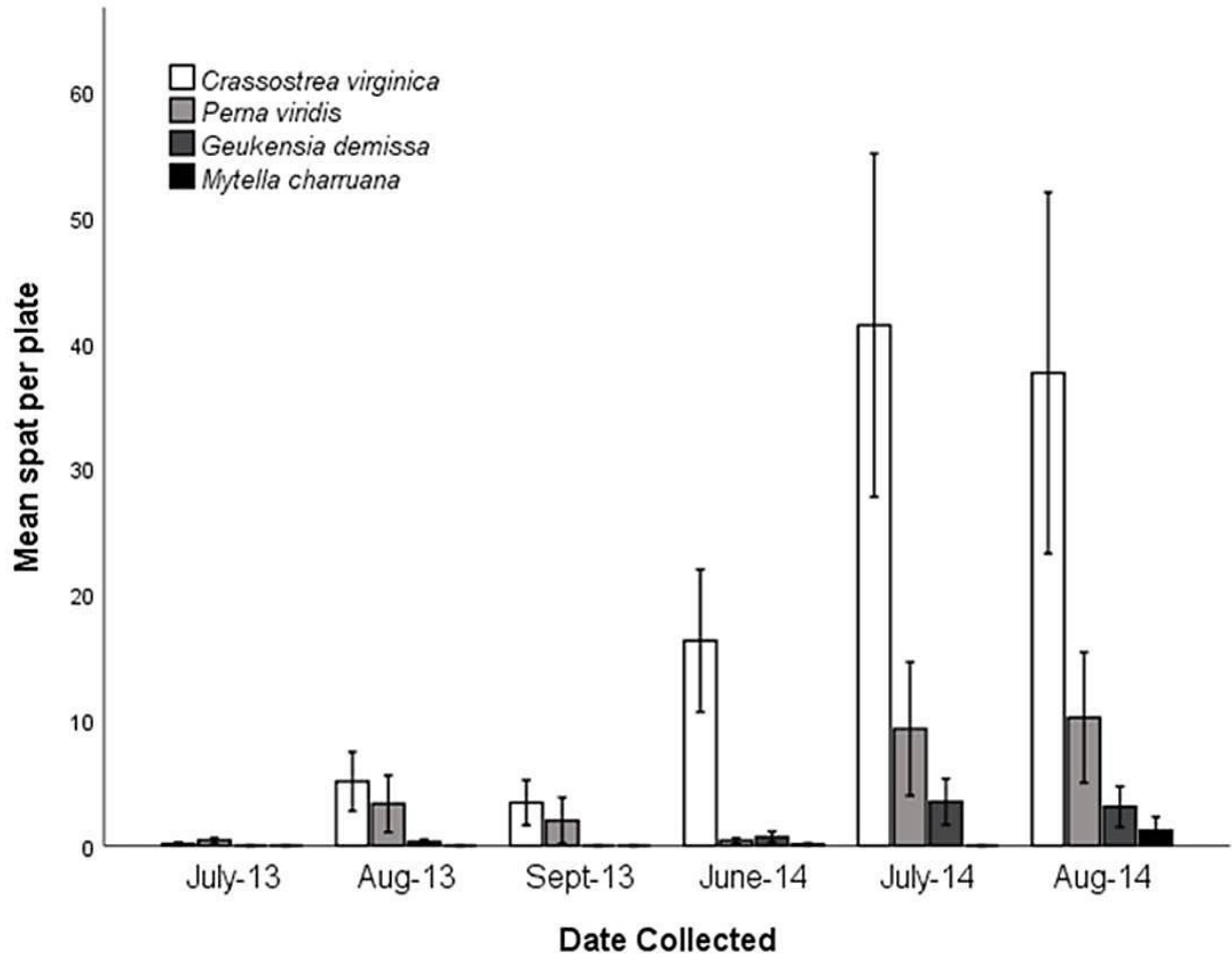**Figure 2.2**. Settlement collectors for (a) main channel sites and (b) feeder creek sites.

Figure 2.3. Mean spat (±SE) per plate (144 cm^2) of each species during each collection month.

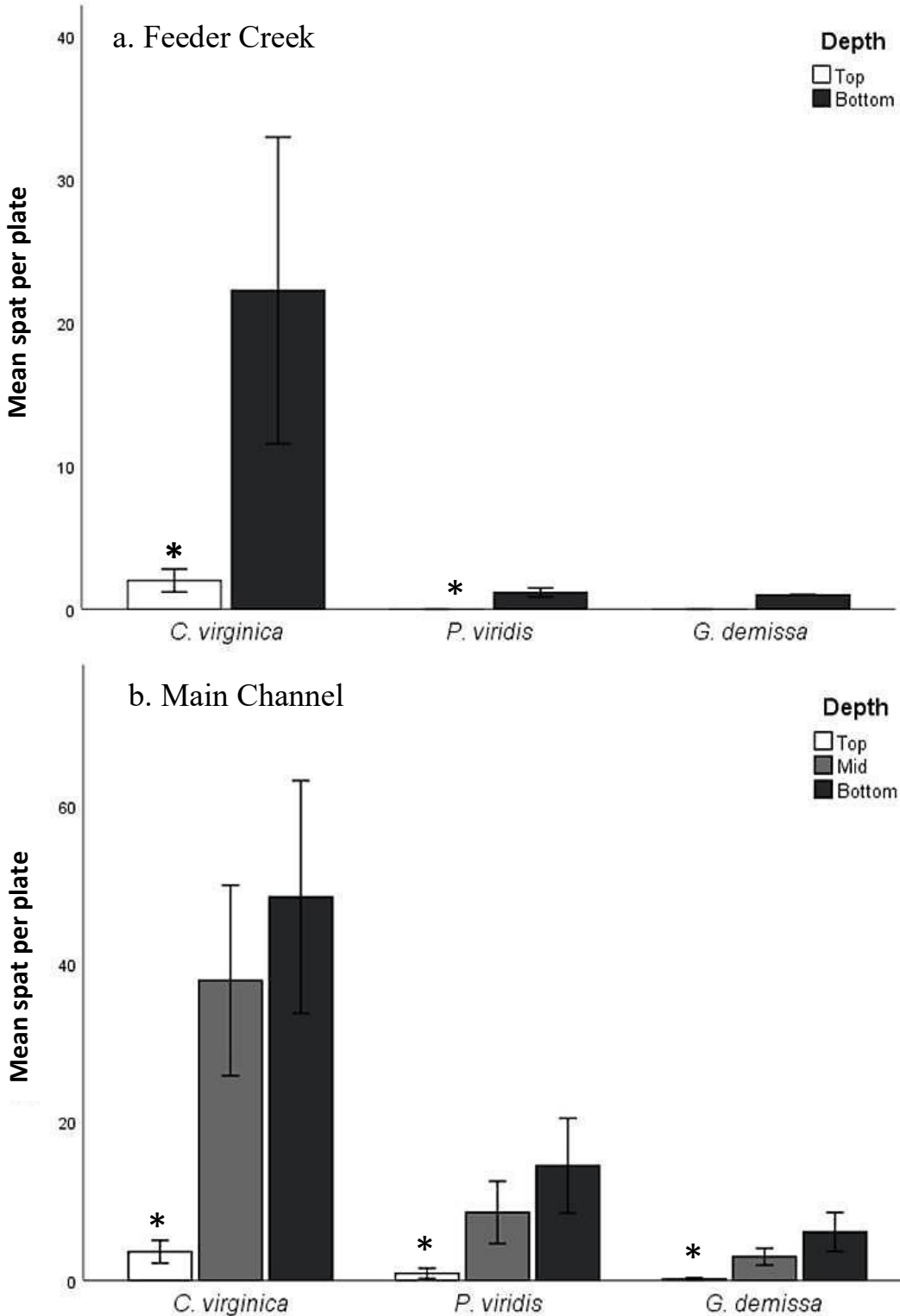**Figure 2.4.** Mean (±SE) spat per plate of bivalves between depths within (a) the feeder creeks, top (white) and bottom (dark grey) and (b) depths within the main channel, top (white), mid (light grey), and bottom (dark grey). Asterisks indicate a $p < 0.05$ obtained from Mann-Whitney U tests. *Mytella charruana* not included in either habitat depth comparison due to low sample size.

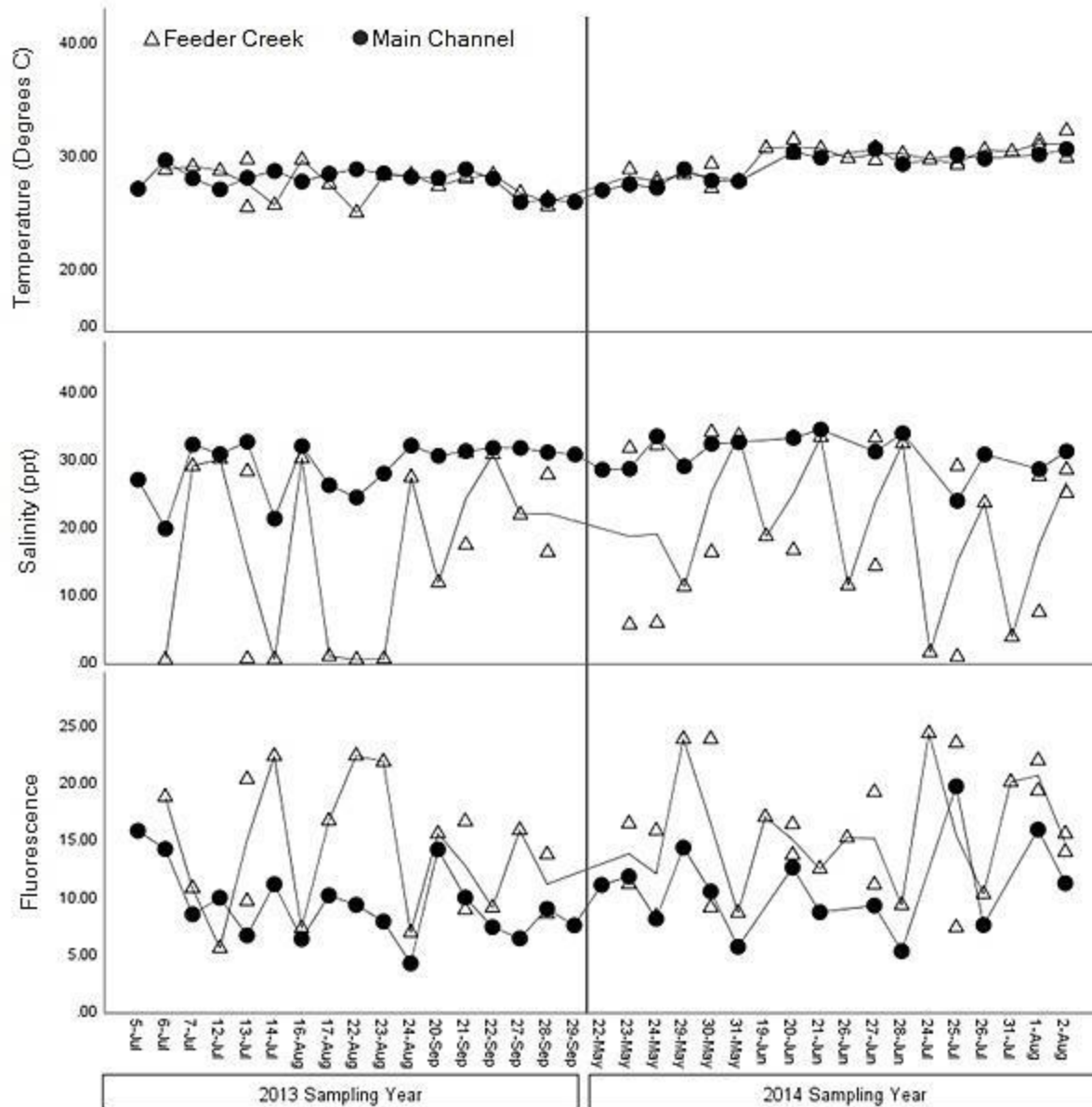**Figure 2.5.** Environmental variables (temperature (degrees celcius), salinity (ppt), and fluorescence) collected at different dates throughout the sampling periods at the main channel sites (closed circles) and feeder creek sites (open triangles).

Species	Mean Abundance	Mean Rank	Sum of Ranks	U	P
C. virginica vs.	19.32	62.72	3324.00	916.00	**0.001**
P. viridis	4.64	44.28	2347.00		
C. virginica vs.	19.32	66.34	3516.00	724.00	**0.000**
G. demissa	1.43	40.66	2155.00		
C. virginica vs.	19.32	71.08	3767.50	472.50	**0.000**
M. charruana	0.24	35.92	1903.50		
P. viridis vs.	4.64	58.28	3089.00	1151.00	0.067
G. demissa	1.43	48.72	2582.00		
P. viridis vs.	4.64	64.68	3428.00	812.00	**0.000**
M. charruana	0.24	42.32	2243.00		
G. demissa vs.	1.43	60.08	3184.00	1056.00	**0.001**
M. charruana	0.24	46.92	2487.00		

Table. 2.1. Results of Mann-Whitney U test using ranked spat per plate (N = 53) Mean abundance per spat plate. Bold indicates P ≤ 0.001.

Habitat	Species	Site	N	Mean Abundance	Mean Rank	Sum of Ranks	U	P
Feeder Creek	*C. virginica*	OC	12	17.42	10.75	129.00	21.00	0.155
		PC	6	1.50	7.00	42.00		
	P. viridis	OC	8	0.36	5.88	47.00	11.00	0.338
		PC	4	1.00	7.75	31.00		
Main Channel	*C. virginica*	SS	11	40.91	15.00	165.00	66.00	0.391
		BL	15	23.73	12.40	186.00		
	P. viridis	SS	14	2.57	12.86	180.00	75.00	0.181
		BL	15	13.53	17.00	255.00		
	G. demissa	SS	4	5.09	9.50	38.00	28.00	0.239
		BL	22	1.64	14.23	313.00		

Table 2.2 Results of Mann-Whitney U tests using ranked settlement of bivalves per plate between sites within the feeder creek (Oyster Creek, Pellicer Creek) and the main channel (San Sebastian, Bing's Landing). *Geukensia demissa* not included in feeder creek site comparisons.

Species	Habitat	N	Mean Abundance	Mean Rank	Sum of Ranks	U	P
C. virginica	Feeder Creek	18	12.11	18.06	325.00	154.00	0.055
	Main Channel	26	31.00	25.58	665.00		
P. viridis	Feeder Creek	12	0.58	15.08	181.00	103.00	**0.034**
	Main Channel	29	8.24	23.45	680.00		
G. demissa	Feeder Creek	4	0.50	9.50	38.00	28.00	0.239
	Main Channel	22	3.36	14.23	313.00		

Table 2.3 Results of Mann-Whitney U tests using ranked settlement of bivalves per plate between habitats (feeder creek, main channel). *M. charruana* not included. Bold indicates P < 0.05.

VITAE

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EDUCATION

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EXPERIENCE

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Florida Department of Health, Duval County, Florida

Marsh Bird Field Technician, April 2011 – July 2011
University of Georgia, Field sites in Pascagoula, Mississippi

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PRESENTATIONS

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Raabe J. and Gilg M. (2014, February) Determination of the vertical distribution of larvae of the invasive green mussel (*Perna viridis*) for use in an ecological niche model. Poster presented at the Guana Tolomato Matanzas National Estuarine Research Reserve State of the Reserve, Ponte Vedra, Florida.