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Hemichordates as a model system for investigating intertidal zonation in soft sediments

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by

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Abstract

Intertidal zonation of organisms is well studied on rocky shores but less so in soft sediment communities. On rocky shores, biotic factors such as predation often set the lower bound of a zone, while abiotic factors set the upper bound. Here I describe the zonation of hemichordate worms at two field sites in Maine and Virginia. In Virginia, *Saccoglossus kowalevskii* occurs in the mid-intertidal zone at densities up to 500 m^{-2}. In Maine, two hemichordate species, *Saccoglossus bromophenolosus* and *Protoglossus graveolens*, co-occur at densities approaching 100 m^{-2}. Hemichordates have chemical defenses that appear to deter fish, but not crustacean, predators. Six species of crustaceans and two species of predatory polychaetes were fed all three species of hemichordate. Crustaceans readily consumed hemichordates, while the polychaetes did not. In predator choice experiments, hermit crabs preferred hemichordates over the tissue of blue mussels, while green crabs preferred mussel tissue. These results suggest that, consistent with the rocky intertidal paradigm, the lower bound of the hemichordate zone could be set by crustacean predators, at least some of which appear to prefer hemichordates over palatable alternatives.

Introduction

As the tides rise and fall each day, organisms living at the edge of the sea experience dramatic fluctuations in their environment. The different responses of organisms to these fluctuating conditions results in a tendency for species, or groups of species, to occupy specific elevations within the intertidal, a pattern that is referred to as intertidal zonation (Stephenson & Stephenson, 1949). Intertidal zonation has been heavily studied in the rocky intertidal for decades (Baker, 1909, Stephenson & Stephenson, 1949, Connell, 1961a, Connell, 1961b, Lubchenco, 1980, Dayton, 1971, Wethey, 1983, Lindegarth et al., 2001, Tomanek and Helmuth, 2002, Fuentes and Brante, 2014), owing mostly to the fact that rocky intertidal prey species are sessile, predators are slow, and competition for space is two dimensional due to the hard substrate on which animals and algae occur.

Paradigms of zonation on rocky shores

Typically, the upper limits of zonation for rocky intertidal organisms are determined by abiotic factors, while the lower limits are determined by biotic factors (Connell, 1961a). There are numerous examples of this kind of interaction on rocky shores (Connell, 1961a, 1961b, Dayton, 1971, Lubchenco, 1980, Fuentes and Brante, 2014). For example, abiotic stresses associated with air exposure such as desiccation and time of feeding set upper limits of benthic survival based on species individual physiological tolerances (Davenport and MacAlister, 1996), allowing only select organisms to thrive (Dittman, 2000). Species diversity is thus highest at the low intertidal and decreases as air stresses increase higher in the intertidal (Read, 1984, Johnson, 1970). Dittman (2000) and Paavo (2011), however, have both suggested that species richness is highest at intermediate tide levels, mirroring the intermediate disturbance hypothesis (Connell, 1978). Numerous factors, including individual species tolerances, make certain zones within the vertical space of the intertidal suitable for some species and not others. While the possible habitat range of a species might be vertically broad, the other organisms sharing the space play a role in where they actually live. For example, in the lower intertidal, which remains submerged for longer periods, marine predators are able to forage longer, and often limit the populations of their prey to higher areas (Dayton, 1971, Dittman, 2000). Competition also can limit populations to the more stressful, air-dominated upper intertidal (Connell, 1961a,b).

In a classic set of studies, Connell (1961a,b) found that zonation of one barnacle species, *Chthamalus stellatus*, was due to competition with another barnacle species, *Balanus balanoides*. *B. balanoides* is faster growing and occupies the lower zone of the intertidal. In the absence of *B. balanoides, C. stellatus* was able to grow at lower elevations within the intertidal (Connell, 1961a,b). Individuals settling in areas inhabited by *B. balanoides* were outcompeted and only organisms living higher in the intertidal survived. *C. stellatus* has a higher tolerance to desiccation and heat stress and was therefore able to exist higher than *B. balanoides.* This classic example helped shape our understanding of ecological interactions in the rocky intertidal, but further studies identified additional factors impacting zonation.

A similar pattern was observed between a related set of barnacle species, but with external interactions from multiple species of the gastropod predator, *Thais* (*T. canaliculata, T. emarginata,* and *T. lamellosa*) (Dayton, 1971). *Thais* spp. selectively prey on *Balanus cariosus* and in the process free up space for *Chthamalus dalli,* the population of which increases (Dayton, 1971). Connell's (1961a, b) and Dayton's (1971) work showed that the lower limits of both species were dictated by biotic factors; the presence of the competitor *B. balanoides* for *C. stellatus*, and the presence of the predator *Thais* for *B. balanoides.* The upper limit for both species was determined by an abiotic factor, desiccation. Within this same environment, the asteroid *Pisaster ochraceus* preys on the mussel *Mytilus californianus,* which grows over other sessile species (Dayton, 1971, Paine, 1974). Both *M. californianus* and *B. cariosus* are inhibited from below by predators, but even in the absence of predators they are unable to form a monoculture in the space (Dayton, 1971). Abiotic factors such as log damage, desiccation, and ice scouring also keep dominant organisms from monopolizing space (Dayton, 1971, Lindegarth et al., 2001).

Ecological interactions and the resultant zonation are also found among algal species. For example, in New England the lower limit of the alga *Fucus vesiculosus* is set by competition for space with another alga, *Chondrus crispus*, and the upper limit is set by desiccation (Lubchenco, 1980). The upper limit of *C. crispus* is also set by desiccation, but its lower limit is set by sea urchin predation (Lubchenco, 1980), confirming the pattern of biotic stressors confining organisms from below, and abiotic stressors from above. These abiotic and biotic factors create biologically meaningful stress profiles that are confining species from both directions, a model that can be applied across a number of environments (Stafford et al., 2015). Other intertidal environments that experience similar stressors include mangrove forests, salt marshes, and sand and mud flats. Despite soft sediment marine environments being one of the most expansive habitats on earth (Wilson, 1991), there is still much to be discovered about the community structure, even in the accessible intertidal (Read, 1984).

Soft sediment factors for zonation

Unlike the sessile organisms of rocky shores, most animals on intertidal mud flats are mobile, small, and infaunal so even when zonation is present, it is difficult to detect (Peterson, 1991). Zonation is therefore hard to identify in the unstable, soft sediment ecosystems that dominate the east coast of the United States (Peterson, 1991, Ellis et al., 2000). The instability and variability of soft sediment habitats, in addition to the behavior and mobility of organisms, creates less obvious, and sometimes changing zonation patterns (Schlacher & Thompson, 2013). There is an inherent bias in marine ecology towards studying the more accessible intertidal and shallow subtidal environments (Wilson, 1991) but the general processes that structure near shore marine soft sediment communities are still poorly understood (Peterson, 1979). Soft sediment organisms exist in a unique three-dimensional ecosystem different from rocky shores (Peterson, 1979), but the environmental and biological drivers that impact rocky shores (desiccation, disturbance, recruitment, competition, and predation) may also contribute to species distribution in soft sediments.

Physiological stresses in soft sediments are less severe than those on rocky shores, as sediments create a buffer that can reduce desiccation, radiation, and temperature change by retaining water after the tide has departed (Peterson, 1991). The three dimensional space within the sediment remains moist longer than a rock face would when exposed for a low tide cycle but some organisms still reduce feeding and growth while exposed (Peterson, 1991). Species diversity is lowest in high intertidal regions, suggesting that soft sediment communities exist on a stress gradient based on species' physiological tolerances (Johnson, 1970). Areas high in the intertidal are submerged for only a short period of time so they provide a limited time for feeding and reproducing, which can lead to small individuals and populations (Peterson and Black, 1987). In addition, organisms living lower in the intertidal reduce food availability to those in the upper intertidal (Peterson and Black, 1987). While this is a biological limitation reducing the viability of species living higher in the intertidal, physical disturbances can have the same impact.

Disturbance can be more severe on soft sediments than on rocky shores, physically altering the habitat (Roberts et al., 2000). Waves, currents, tidal range, and shore morphology all influence the shape of mudflats (Roberts et al., 2000), and sediment can be deposited at a rate of multiple centimeters per day in some systems (Christie et al., 1999). In addition to shore morphology, disturbances such as storms burying parts of the flat, strong currents gouging out channels, and general wave action altering surface environments all influence the organisms living there (Woodin, 1978). Physical disturbance can also occur from biological processes like predator foraging. For example, blue crabs and horseshoe crabs can dig up to 9cm into the sand, having severe non-consumptive effects on the infauna (Woodin, 1978). Habitat features such as the pits and mounds created by these predators can impact species (Gerwing et al., 2016) through population limitation and creation of inhabitance patterns in soft sediment ecosystems. Like physical disturbances, predation keeps individual species from monopolizing resources (Paine, 1966, Dayton, 1971).

Predation is important in regulating macroinvertebrates in soft sediment intertidal communities (Riccardi and Bourget, 1999). There are two kinds of predators in soft sediments: epibenthic predators and infaunal predators (Ambrose, 1984). All are mobile, but epibenthic predators often retreat with the tide, while infaunal predators do not. Infaunal predators, such as the polychaetes *Nereis virens* and *Glycera dibranchiata,* are not limited by tidal fluctuations and have significant negative impacts on larval settlement and juvenile survivorship (Ambrose, 1984), which can strongly influence adult population distributions. The amount of time the upper intertidal is exposed is less than the low intertidal, which could reduce feeding by epibenthic predators in the upper-intertidal enough to see zonation. For example, the highly mobile subtidal crab *Cancer productus* preys on the snail *Littorina sitkana* at higher rates in the lower intertidal than in the mid and upper intertidal (Yamada & Boulding, 1996). The realized zone of *L. sitkana* is most likely due to this predation and could be a result of either escape behavior (non-consumptive effect) or high predation (consumptive effect) at sites accessible by the crabs. The retreat of epibenthic predators, like crabs, therefore creates a safer environment for prey higher in the intertidal (Yamada & Boulding, 1996, Byers, 2002). Some mobile predators, such as the horseshoe crab, *Limulus polyphemus*, are able to travel hundreds of meters up into the intertidal (at high tide) to forage (Lee, 2010), but would be able to spend the least time in the upper intertidal where the tide only accesses for a small part of the day. The mobility of soft sediment organisms not only influences predation, but can create a less severe competitive environment.

Competition for space, although less severe than on rocky shores, persists in soft sediments as well (Peterson, 1979). For example, competition among two species of burrowing amphipods severely reduces the survival and reproductive output of the higher-shore species when in combination with the lower-shore species (Crocker and Hatfield, 1980). Additionally, a study by Woodin (1974) found that the abundances of three species of tube building polychaetes negatively influenced the populations of burrowing species, a direct response to the lack of sediment available for the burrowers to use. So, if a population of tube building species is able to grow to a dense enough population, it can compete with burrowing organisms and keep them from surviving in their area. Certain polychaetes withdraw into their burrows upon interference by another polychaete, suggesting that although there may be space for both to burrow, they reduce each other's feeding time (Levin, 1982).

Competition for food resources can influence community structure (Levinton, 1972, Peterson, 1982). Some polychaetes fight with their palps (feeding appendage), stealing food and tube material (Levin, 1980). Organisms can avoid competition for feeding space by feeding on different things within the same space. Two species of snail, for example, coexist, but feed on different grain-sizes and therefore don't run out of food resources (Fenchel, 1975). The three-dimensional structure of soft sediment systems adds complexity to measuring competition in soft sediment systems because organisms can move both horizontally and vertically within the sediment (Levinton, 1977, Peterson, 1977). For some organisms, vertical stratification is pronounced, and multiple zones are created vertically within the sediment (Crocker & Hatfield, 1980). Suspension feeders, however, feed at the sediment surface and are limited in their feeding space, so moving apart horizontally is more optimal than moving vertically (Peterson & Andre, 1980). In addition to competition between adult organisms creating zonation either vertically or horizontally, competition can also exist between life stages.

Competitive interactions between adult and juvenile or larval populations can influence zonation. Some adult assemblages can reduce the settlement of other species' larvae and in doing so maintain their dominance (Woodin, 1976, Wilson, 1991). A first come, first served process is therefore reflected on mud flats based on larval recruitment (Gerwing et al., 2016). Recruitment can therefore impact the size and location of a species' zone. Once an adult population is established, it is then able to maintain its space, leading to dense patches of that species (Woodin, 1976). This can be done through competitive exclusion of space, or even ingestion of the larval forms of other species (Levin, 1980). Patchiness among organisms on small special scales on intertidal soft sediment flats has been observed in a number of organisms globally (Woodin, 1976, Levin, 1980, Ysebaert and Herman, 2002). On a mudflat on the York River in Virginia there is a dense patch of acorn worms (Phlyum Hemichordata) dominating the flat which could be used to describe zonation on a soft-sediment mud flat.

Hemichordates as a model system

The phylum Hemichordata is a sister phylum (closely related) to Echinoderms. Together, the two phyla form a superphylum, Ambulacraria and are sister to Chordates. Because hemichordates are abundant invertebrates with recent common ancestry with Chordates, they are heavily studied for their genetics and evolution (ex. Satoh et al., 2014, Fritzenwanker et al., 2014, and Lowe, 2008). Despite hemichordates' great abundance and evolutionary relevance, their ecology is poorly understood.

Hemichordates are split into two main classes, Enteropneusta (acorn worms), and Pterobranchia. Enteropneusts inhabit soft sediment estuarine and marine environments from the intertidal to the deep sea (Tassia et al., 2016). They are soft-bodied, tube-building worms that have a unique tripartite body plan: a proboscis, collar, and trunk, which makes them easily identifiable from segmented polychaete worms. The three species used in this study were all Enteropneusts: *Saccoglossus kowalevskii* (Agassiz, 1873)*, Saccoglossus bromophenolosus* (King et al., 1994)*,* and *Protoglossus graveolens* (Giray and King, 1996). The range of *S. kowalevskii* is from Georgia to southern Maine (Colwin & Colwin, 1953), while the range of *S. bromophenolosus* is thought to be from southern Maine to Nova Scotia (King et al., 1994). *P. graveolens* has only been collected at three locations in the Damariscotta River estuary, Maine (Giray & King, 1996), and at Stover's Point, Maine (this study), so its range is unknown.

Only a few studies have examined *S. bromophenolosus* or *P. graveolens*, but both are similar to *S. kowalevskii,* which is better studied. *S. kowalevskii*, resides in U-shaped burrows on mud flats (Ruppert & Fox, 1988) in high-density assemblages approaching 500 m^{-2} (*pers. obs.*). They are surface deposit feeders that probe the sediment surface near their burrows with their proboscis to feed (Knight-Jones, 1953). *S. kowalevskii* is able to regenerate its proboscis

(Tweedel, 1961, Luttrell et al., 2016), which is the only exposed part of the body during surface feeding, suggesting that they are preyed on by epibenthic predators.

Invertebrates living in soft sediment habitats are susceptible to heavy predation (Peterson, 1979, Quammen, 1984). One protection from this pressure is chemical defense. Brominated phenols for example, are present in a number of infaunal worm species (Yoon et al., 1994, Woodin et al., 1987, Kicklighter, 2003, Kicklighter et al., 2004). Yoon et al. (1994) found that in the polycahete *Notomastus lobatus*, the enzyme responsible for the production of bromophenols is primarily located in the tail. As this polychaete is a head-down deposit feeder, its tail is most vulnerable to predation, suggesting these compounds are used as chemical defenses (Yoon, 1994). Hemichordates are known to contain these compounds in high concentrations (Woodin et al., 1987, King et al., 1995, and Giray and King, 1997), and could potentially be used to deter predators.

To determine if hemichordates do in fact use these bromophenols as a predator defense, Kicklighter et al. (2004) conducted a prey choice experiment with *S. kowalevskii* and a palatable control in Georgia. The predators were two species of fish and one crab: the spot, *Leiostomus xantharus,* and the mummichog, *Fundulus heteroclitus,* and the lesser blue crab, *Callinectes similis*. Kicklighter et al. (2004) found that although *S. kowalevskii* was unpalatable to the two fish predators*,* it was readily consumed by the crab. Similarly, in Maine, Giray and King (1997) studied the palatability of the hemichordate *S. bromophenolosus*. Worms were offered as prey to two polychaete predators, the sand worm, *Nereis virens* and the blood worm *Glycera dibranchiata*, as well as to the hermit crab, *Pagurus longicarpus.* The hermit crab is a carnivore while the two polychaetes are omnivores (Ambrose, 1984, Volvenko, 1994). Giray and King (1997) found that *S. kowalevskii* were consumed by all three predators and the hermit crab preferred the *S. bromophenolosus* to a shrimp palatable control. Despite their chemical defense, hemichordates are still consumed by multiple species of crustacean and polychaete predators, thus it is possible that predation could influence their zonation.

In Virginia, there are abundant populations of blue crabs (*Callinectes sapidus*) and *P. longicarpus* that could potentially set the lower bounds of *S. kowalevskii* zonation. In Maine, the invasive green crab (*Carcinus maenas*) is prevalent (Williams et. al., 2015), along with numerous other crustacean predators. Because these crustacean predators are marine, it is assumed that they mainly feed while submerged, meaning that areas higher in the intertidal would be least affected by predation (Byers, 2002). However, these particular predators are highly mobile and able to retreat in time to avoid being exposed at low tide, meaning that inundation time, frequency, and predictability may play a role in whether or not predation influences zonation in infaunal communities (Kneib, 1984).

Significance and purpose

The coastal communities of the Chesapeake Bay and much of the United States Atlantic coast are dominated by unvegetated soft-sediment habitats (Seitz et al., 2006). By describing the distribution of *S. kowalevskii*, *S. bromophenolosus,* and *P. graveolens,* as well as any biotic and abiotic factors contributing to their distribution, I will begin to understand the mechanisms of zonation in select soft sediment communities at two locations in Maine and Virginia where these animals are abundant but understudied. Describing the mechanisms that structure soft sediment communities will allow me to compare my work with classic ecological paradigms developed for rocky shores.

The purpose of this study was to better understand hemichordate ecology while also using them as a model to determine what factors may be influencing intertidal zonation in two soft sediment intertidal ecosystems. I used three species of hemichordate; *S. kowalevskii, S. bromophenolosus, and P. graveolens* to investigate basic patterns of zonation on mud flats in locations in Maine and Virginia. I observed that at a site on the York River, Virginia*, S. kowalevskii* occupied a distinct zone in the intertidal. Based on this, as well as other studies of predation on hemichordates, I hypothesized that the lower limit of this zone is set in response to predation by crustacean predators.

Methods

Experiment sites

Field work in Virginia, including observational surveys and field manipulations, was conducted on the York River at a small sand flat (approximately 0.5 ha) just down river from Indian Field Creek (37°16'3.27"N, 76°33'12.02"W) (Figure 1). The flat is protected on either side by two small patches of *Spartina alterniflora*. All *Saccoglossus kowalevskii* used in predation experiments were collected from this site.

The study site in Maine was in Lowes Cove, a 5.9-ha tidal cove adjacent to University of Maine's Darling Marine Center in the Damariscotta River estuary (Figure 2). Within the cove, two inlets referred to as Cove 1 (43°56'10.60"N, 69°34'30.74"W) and Cove 2 (43°56'10.82"N, 69°34'26.59"W) were sampled. Coves were approximately 25 meters across and were shallow sloping. *Saccoglossus bromophenolosus* and *Protoglossus graveolens* used in predation assays were both collected from this site. Additional *P. graveolens* were collected from Stover's Point (43°45'28.1"N 69°59'52.1"W) in Casco Bay, Maine. Green crab survival experiments were conducted on the mud flat directly adjacent to the Bowden Coastal Studies Center (CSC) (43°47'24.1"N 69°57'33.2"W), also in Casco Bay. Most predators were collected from sites around Casco Bay. Blue crabs (*Callinectes sapidus)* were collected at the York River site, and some blood worms (used in predation trials with *S. kowalevskii*) were obtained from a bait supply store in Williamsburg, Virginia. Most *S. bromophenolosus* and *P. graveolens* predation trials were conducted in the Bowdoin CSC flow-through seawater lab, while all *S. kowalevskii* and *C. sapidus* predation trials were conducted in our lab at The College of William and Mary's main campus.

Casing to worm relationship

Hemichordates produce fecal casings that are visible at the sediment surface (Figure 3). In Virginia, the number of hemichordate casings present at the surface was quantified and correlated with the hemichordate counts. First, a shovelful of sediment was taken from a haphazardly selected location on the flat. Before the shovelful of sediment broke apart, the number of casings at the surface was recorded. Next, water was poured over the sediment to cause worms to fall out and worms were counted. This was repeated ten times on two occasions for a total of 20 replicates. In Maine, there was no quantitative account of the ratio, but in digging holes in Maine to collect hemichordates a similar pattern was detected.

Field observations

Observational field data was collected in both Maine and Virginia. In Virginia, transects were taken perpendicular to shore in the middle of the cove, and then parallel to each other. Between 4 and 6 transects were taken at a time, usually about 10m apart. Transects started at the high tide line (approximately in line with the edge of the fallen trees and clumps of vegetation at the site), and extended out to the water at low tide. Every 5 meters, a 0.25m^2 quadrat was placed on the north side of the transect tape with the back corner touching the meter marker. All hemichordate fecal casings were counted. This was repeated eight times at different times of year (between November and May) over two and a half years (Figure 4). In March of 2015, high populations of the tube building polychaete, *Spiochaetopterous oculatus* were observed and counted in transects along with the *S. kowalevskii* fecal casings. Because tides fluctuated, the length of transects were different each time I sampled, ranging from 25m to 50m. To account for this variation, only data from 0-25 meters was used when comparing average density of the flat across time.

In Maine, transects were conducted once, over the summer of 2016 in two coves within Lowes Cove. In each cove, transects were perpendicular to shore in the middle of the cove, then parallel to each other. Three transects were done (each approximately 7 meters from each other) in each cove. Transects started at the marsh line and extended out 50m. Every meter, a 1m^2 quadrat was laid and all hemichordate casings counted. Significantly lower densities of casings in Maine than in Virginia allowed for this more thorough transecting. Although Giray and King (1996) reported being able to easily distinguish between the two species based on casing, at my field sites the casings were indistinguishable, so I counted both species together. Hermit crabs were also recorded. Sand worms were counted in the immediate next meter square area because the movement of placing the quadrat caused them to retract. The quadrat was split by two observers, each counting only their half of the space.

Beach profiling

In Maine, beach profiles of both coves were taken. The high tide was determined by high water marks – sediment residue on *Spartina alterniflora* blades. The elevation change from there to the 0m mark was recorded. The 0m mark was at the bottom of the marsh ledge, where the mud flat began. Materials included a tape measure, two 1m PVC pipes (one marked with centimeters), a string attaching the two, and a string level. To determine the change in elevation from one point to the next, the PVC with cm markings (PVC 2) was placed at the lower elevation. The string attached to the other PVC (PVC 1) at the sediment surface. The string on PVC 2 was then adjusted until it was level (as determined by the string level) and the elevation was recorded. Then, PVC 1 was moved to the location of PVC 2 and PVC 2 was moved further down the flat. The distance between the two ranged from 1 meter to 8 meters based on the slope of the flat. In flat areas, 8 meters was used, in steep areas anywhere between 1 and 6 meters was used. The starting points were variable between coves 1 and 2, so elevation could not be directly compared across the two coves.

Green crab survival

To investigate green crab survival on a mudflat at low tide to better understand the crustacean distributions, a survival study was conducted. Green crabs were collected from the field and kept in flow-through tanks at the Coastal Studies Center. Crabs were chosen at random and assigned to either a "caged" or "tethered" condition. Caged crabs were put into small mesh baskets with a rock anchor. Tethered crabs had their carapaces glued to 0.5m of fishing line that was attached to a stake. During an evening low tide, 12 stakes and 12 cages were placed out in the field in two rows with alternating treatments (Figure 5). Crabs were placed at 2m intervals and the rows were 5m apart. After 24 hours, cages and stakes were collected and surviving crabs were recorded and released.

Predation assays

Predation assays were conducted across three species of hemichordate prey and eight species of predator. Predators included the American lobster (*Homarus americanus*), two species of rock crab (*Cancer borealis* and *Cancer irroratus*), green crabs (*Carcinus maenas*), hermit crabs (*Pagurus longicarpus*), blue crabs (*Callinectes sapidus*), sand worms (*Nereis virens*), and bloodworms (*Glycera dibranchiata*). Hemichordate prey species included *P. graveolens, S. bromophenolosus*, and *S. kowalevskii.* Crustacean predators were massed, measured, and randomly assigned to containers. They were individually placed into 20cm x 12cm plastic Sterilite containers containing seawater (approximately 1.5L) at flow-through temperatures; n = 10 per predator x prey assay besides *Nereis virens* (n = 2 for *S. kowalevskii* assays) and *Carcinus maenas* (n = 9 for *S. kowalevskii* assays). Live hemichordates were then weighed and randomly distributed among predators (Figure 6). Lids were added and flow-through seawater supplied via plastic tubing (Figure 7). Assays were left for 24 hours. At the end of 24 hours, remaining hemichordate tissue was weighed and predators released. Predators collected in Maine were given live *S. bromophenolosus* and *P. graveolens*. Ten of each species of predator were shipped to Virginia to use in predation assays with live *S. kowalevskii.* The Virginia predator, the blue crab, was fed a mix of live and dead *S. bromophenolosus* and *P. graveolens.* For predation trials involving blue crabs or *S. kowalevskii* (in Virginia), flow through tanks were not available. Instead, aerators were added to the containers, which sat at room temperature.

Crustacean choice

To determine if hemichordates were less palatable than a known palatable control, hermit crabs and green crabs were offered a choice between a dead *S. kowalevskii* and a piece of blue mussel tissue (a known palatable control). Predators were randomly placed into 10 plastic containers that measured 20cm x 12cm with approximately 1L seawater. Prey were thawed and massed. They were then ranked by weight and rank sets were added randomly to each container (Figure 8). The end of the container each prey item was added to was also randomly assigned. After observing a significant decrease in mass of prey in the container, the final mass of each prey item was recorded. This was repeated three times for a total of 30 assays for each predator. Hermit crabs were left for 4 hours (n = 10) or 16 hours (n = 20) depending on how much was consumed. Green crabs were left for a shorter period of time (1.5 hours) because they consumed prey at a faster rate overall. All data was recorded as “mass consumed per hour” to correct for differences in time within and between species of predator.

Hermit crab predation

To test for between species prey choice in hermit crabs, the same design as the crustacean choice experiment was used. The two prey species were *S. bromophenolosus* and *P. graveolens* (thawed), which are distributed on the same flat.

To determine if certain life stages were more or less defended than others, hermit crabs were fed *S. bromophenolosus* at three life stages; adult, juvenile, and egg. Adult assays were run for 24 hours. For predation on *S. bromophenolosus* eggs, ten eggs were pipetted into each of eleven bowls. Hermit crabs were then cleaned and placed randomly into ten of those bowls – one bowl had no crab as a control. After 30 minutes the crabs were removed and bowls observed under a microscope. Remaining eggs were counted in every bowl. For hermit crab predation on *S. bromophenolosus* juveniles, the same process was repeated except 10 juveniles were pipetted into each bowl and the remaining individuals were counted after 30 minutes.

Sand worm reactions

Sand worms (*N. virens*) were collected from the field and kept in flow-through seawater until used. They were placed individually into plastic containers that measured 20cm x 12cm with approximately 1L of seawater. After an adjustment period of 5 minutes, they were offered either a small piece of thawed shrimp, or a live hemichordate (*S. bromophenolosus*). They were monitored until they encountered the prey item. Their reaction upon initially encountering the prey item was recorded (n = 30). Categories included “recoil,” “turn around,” “ignore,” and “bite.” Each category was given a rank (1, 2, 3, and 4 respectively) and the reactions were averaged.

Transplant study

Field manipulations took place on the south bank of the York River (37°16'3.27"N, 76°33'12.02"W) on April 24th, 2017 on either side of a - 0.1' tide. Hemichordates were transplanted above and below their zone to test the hypothesis that lower bounds of the zone are set by biotic factors, and upper bounds are set by abiotic factors. There were three treatments; no cage, cage, and a cage control. There were five replicates of each treatment above the zone, below the zone, and within the zone (control).

Transplant cores were made from aluminum cans that measured 7in (17.7cm) tall by 6in(15.2cm) in diameter. The width of the aluminum was 2mm. The bottoms of cans were removed with can openers. Caged cans had ½ inch plastic hardware cloth mesh staked in above the can once transplanted. For cage control cans, mesh was cut to make bigger holes and allow access by predators.

Cores were taken from within the hemichordate zone and the number of hemichordate fecal casings was recorded. Cores were assigned random numbers to determine which position they were transplanted to (1-15 in each of the three zones). They were assigned in the repeating order of cage, no cage, half cage. Cores were pushed into the sediment until the top was even with the ground.

Cores were then removed from the sediment using a shovel and immediately placed in a hole at its designated location. The upper intertidal zone was 12 meters above the low tide line (0.1" tide) and 5 meters above the highest observed casing. The mid intertidal (control) was at the low tide line (approximate middle of zone). The lower intertidal was 23 meters below the mid intertidal where there were no casings present.

Cores were left out for 72 hours. Upon returning two cans had been removed by children, but all others remained. The casings in the remaining cores were counted. Cores were dug up and sieved through a 2000 micron mesh. Remaining hemichordates were recorded.

Statistical Analysis

To determine a relationship between casings and hemichordates, data were analyzed by testing multiple functions for best fit. For all relationships between species on the flat, an independent samples t-test was used. Where equal variance could not be determined, a Welches t-test was performed.

For the predation assays and the crustacean choice experiment, both parametric and non-parametric tests were performed. In both, the residuals of the data were not normally distributed (Kolmogorov-Smirnov and Shapiro-Wilk tests). Numerous studies have suggested that ANOVA is very robust to violations of assumption of normality (e.g. review by Glass et al., 1972). From this, I concluded that despite the non-normal distribution of residuals, a two-way ANOVA was still an appropriate test, but a non-parametric was performed as well. For both experiments, a Type III Test of Fixed Effects was performed using predator and prey as fixed factors as well as an interaction term of predator*prey. Then, a Bonferroni corrected post-hoc test of multiple comparisons was performed for the predation assays to evaluate specific relationships between factors. For the predation assays, an additional ANOVA was performed to compare the three prey items across the two categories of predators: crustaceans and polychaetes. In addition to performing the ANOVA for both the predation assays and the crustacean choice experiments despite non-normally distributed residuals, a non-parametric alternative was used. An ANOVA was performed on aligned rank transformed (ART) data in both R (Kay and Wobbrock, 2016) and SPSS for both data sets. The ART procedure removes all effects of additional independent variables and ranks dependent variables based on one independent variable at a time (Wobbrock et al., 2011). It does this for all independent variables, creating multiple ranks for the same dependent variable, which are then examined one at a time with an ANOVA (Wobbrock et al., 2011). Another Bonferroni corrected post hoc test of multiple comparisons was performed for the predation assays using the ART data. In using a non-parametric, the violations of normality were corrected for.

In the *P. graveolens* vs. *S. bromophenolosus* hermit crab choice experiment, the residuals were normal using both Kolmogorov-Smirnov and Shapiro-Wilk tests and a paired samples t-test was performed. To compare the reactions of *Nereis* to *S. bromophenolosus* and shrimp, a non-parametric Wilcoxon signed rank test was performed because the data was not normally distributed. As the green crab survival data were zeros and ones, and therefore not normally distributed, a nonparametric binomial test was used. An ANOVA was used to analyze the transplant study data.

Results

Casing to worm relationship

The relationship between fecal casings and worms was positive and significant ($p = 0.001$). The data were best fit by a cubic function ($F = 75.07$, adjusted $R^2 = 0.914$). A quadratic ($F = 85.61$, adjusted $R^2 = 0.882$) and linear ($F = 132.565$, adjusted $R^2 = 0.873$) function also fit well, and because there is no obvious biological explanation for either a cubic or quadratic function fitting the data more accurately, I have chosen to only report the linear function ($y = 1.4563x - 4.003$). This significant positive linear relationship indicates that counting fecal casings gives an accurate estimate of the number of worms present (Figure 9).

Field observations

Field observations consisted of counts of hemichordate fecal casings in relation to their distance from shore. The "shore" in Virginia was defined by an obvious visual change in the sediment as well as a rack line. In Maine, the "shore" was a discrete border between the edge of the mud flat and the high marsh. The additional organisms sampled were the polychaete *Spiochaetopterus oculatus* in Virginia*,* the polychaete *Nereis virens* (sand worm), and the hermit crab, *Pagurus longicarpus* in Maine. Overall, the distribution of hemichordates was similar in Maine and Virginia (Figure 10). The most obvious difference between the two populations was an order of magnitude difference in average hemichordate density on the flats. In Virginia, the mean density from 0-50m was 35.74 (±SE = 0.93) casings m^{-2} while the mean density from 0-50m in Maine was 2.89 (±SE = 0.45) casings m^{-2}. Both populations started around 5m from shore and were densest between 10 and 20 meters from shore. The York River, Virginia has a vertical tidal change of about 0.70 meters (NOAA tides and currents, Gloucester Point) and at our field site extended an average of 35 meters horizontally at low tide. At Lowes Cove, Maine, the vertical tidal drop is about 2.85 meters (NOAA tides and currents, Lowes Cove) and the whole cove drained on an average tide (hundreds of meters horizontally). Despite the average tides being so different, very few hemichordates were ever found extending beyond thirty meters in either system. The main population existed between five and thirty meters from shore, supporting the hypothesis that the "hemichordate zone" is in the mid-intertidal, meaning that they did not extend to high tide, but there was also a gap between the bottom of their distribution and the low tide line.

In Virginia, sampling took place from November 2014 to March 2017. When these values are averaged across time, the data are still bounded within a zone from approximately 5 to 30 meters (Figure 11). Average density (hemichordate casings m^{-2}) from 0-25 meters was highest in mid May (mean = 303 ±SE 111), and lowest in February (mean = 12.7, ± SE figure 16.8). From the fall of 2014 to the spring of 2015, the population decreased in the winter and then increased again in early spring, reaching its peak in mid May (Figure 12). Although only sampled twice in 2016, the pattern seemed to repeat itself, with increasing population densities in the spring. Sampling in March of 2017 yielded an identical average density for 0-25 meters (110.8 m^{-2}) as March of 2016, both lower than the densities found in March of 2015.

Zonation on the mudflat in Virginia was recorded in mid March of 2015, when the polychaete, *Spiochaetopterus oculatus* was found occupying a zone lower than *S. kowalevskii* (Figure 13). The main population of *S. kowalevskii* was found from 10m to 30m, while the population of *S. oculatus* was found from 25m to 40m. The mean *S. kowalevskii* position was 21.95 (±SE = 0.187) meters from shore, while the mean *S. oculatus* position was 35.07 (±SE = 0.174) meters from shore. These two populations had significantly different mean positions (Two sample t-test, t = -51.4, df = 2198, p < 0.001) and had an overlap of only about 5 meters.

The two coves in Maine were similar in their hemichordate distributions, although the zone of Cove 1 was slightly further from shore (Figure 14). In Cove 1, the mean distance from shore was 25 meters (±SE = 1.17) with a median of 22 meters from shore. In Cove 2, the mean distance from shore was 18.17 meters (±SE = 0.60) with a median of 15 meters from shore. Cove 2 was denser than Cove 1, with the highest average (n = 3 transects) density of 41 casings m^{-2} (±SE = 27.95), while the highest average density in Cove 1 was 10 casings m^{-2} (±SE = 8.19). A beach profile of Cove 2 revealed that from the top of the mud flat (not high tide) to the creek (35m), the elevation drop was 140cm (Figure 15). Within the first 10 meters there was a one meter drop. The remaining drop (40 cm) took place over the remaining 25 meters (averaging a 1.6 cm/m drop). The total change in elevation from the top to the bottom of the hemichordate zone was only 22 cm.

On both coves, there was a narrow zone of hemichordates, but some organisms are broadly distributed. For example, the sand worm, *Nereis virens* was distributed relatively uniformly starting around 5m and continuing all the way to 50m (Figure 16). Where present, the average density of *N. virens* was 5.46 m^{-2} (±SE = 0.38). They did not exhibit obvious zonation. The average sand worm densities within the hemichordate zone (5-30 m) and beyond it (30-50 m) were not significantly different (independent samples t-test, t = -.263, df = 35, p = 0.794). *N. virens* had a wider distribution and the mean distance from shore was 30.65 m (SE = 0.73), which is further than that of hemichordates, but there was still significant overlap (Figure 17). The hermit crab, *Pagurus longicarpus,* existed on the flat from 26 to 50 m. It occupied a unique zone from hemichordates (Figure 18). The mean distance from shore was 36.7 m (±SE = 0.51), while the mean distance from shore for hemichordates was 20.54m (±SE = 0.84). These means were significantly different (independent samples t-test, t = -16.452, df = 242.1, $p < 0.001$) and the overlapping area was only about 4 meters, suggesting the two species occupy two distinct zones. The spike in hermit crab density of 32.75 m^{-2} (±SE = 12.09**)** at 37m was the middle of the creek channel in Cove 2, suggesting the hermit crabs prefer to remain submerged.

Green crab survival

The green crab (*Carcinus maenas*) was unlikely to survive on a mudflat during low tide without the protection of a cage (Figure 19). All green crabs protected from predators by a cage (n = 12) survived for 24 hours (2 low tide cycles), while only 25% of the staked crabs (n = 12) survived. A non-parametric binomial test demonstrated that the proportion of surviving staked crabs differed significantly from the proportion of surviving caged crabs ($p < 0.001$).

Predation Assays

All six crustacean predators readily consumed all three prey species (mean proportion consumed = 0.892, ±SE = 0.018), while the average consumption by polychaetes was only 0.198 (±SE = 0.025) (Figure 20). Predation was observed directly for all six crustacean predators, but never for either of the polychaete predators, and there was no visible damage to the prey of polychaetes, only a minor loss of biomass. When combined, there was a significant difference in consumption by the crustacean versus polychaete predators, ($p < 0.001$) (Figure 21). A Bonferroni corrected post hoc test of multiple comparisons revealed that every crustacean predator was significantly different from both polychaete predators (n = 12, $p < 0.001$), and similar to each other (n = 15, $p > 0.05$). When averaged across all predators, prey species were also found to be significantly different from each other ($p = 0.007$). For the overall dataset, both a non-transformed ANOVA (Table 1) and an ART ANOVA (Table 2) were performed. When an ANOVA was run on the untransformed proportions consumed, predator ($p < 0.001$) and prey ($p = 0.001$) were both significant the interaction between predator and prey was not found to be significant ($p = 0.092$). When running the ANOVA on ART data, however, an interaction was detected ($p = 0.016$). This interaction was likely a result of the difference in predation on *S. kowalevskii* by the two Virginia crustacean predators in comparison to the pattern of predation by the Maine crustacean predators. The untransformed ANOVA revealed that *P. graveolens* and *S. kowalevskii* were consumed in similar proportions, 0.77 ± 0.041 and 0.79 ± 0.25, while on average, *S. bromophenolosus* was consumed less than either *S. kowalevskii* ($p < 0.001$) or *P. graveolens* ($p = 0.001$) (0.64 ± .046). *S. bromophenolosus* was the least consumed prey for all predators besides the two Virginia predators (*P. longicarpus* and *C. sapidus*). Instead, the Virginia predators consumed the least *S. kowalevskii,* suggesting *S. kowalevskii* is most chemically defended against the predators it naturally encounters.

Crustacean choice

In the choice experiments, hermit crabs and green crabs had opposite responses to the two prey options (Figure 22). The hermit crabs consumed an average of 11.48% (±SE = 1.53) of the hemichordate prey per hour and only 5.18% (±SE = 0.96**)** of the palatable blue mussel. The green crab on the other hand, consumed an average of 50.00% (±SE =5.10**)** of the hemichordate prey per hour and 62.96% (SE = 2.07**)** of the palatable blue mussel control. A two way ANOVA on the raw proportion consumed per hour data revealed that there was a significant effect of predator ($p < 0.001$), as well as an interaction effect of predator*prey ($p = 0.001$). There was no effect of prey ($p = 0.253$). The ART ANOVA revealed the same results. A significant effect of predator ($p < 0.001$), no effect of prey ($p = 0.483$), and an effect of the interaction ($p = 0.001$).

Hermit crab predation

Hermit crabs preferentially ate *P. graveolens* when given a choice between *S. bromophenolosus* and *P. graveolens* of approximately equal size (Figure 23). On average, they consumed 18.02% (SE = 4.91**)** of *S. bromophenolosus* and 45.54% (±SE = 6.21**)** of *P. graveolens* in a 24 hour period (n = 10). There was a significant difference in their consumption of the two hemichordate species (paired t-test, df = 9, t = 5.193, p = .001). When offered separately, however, they consumed them at almost equal rates, averaging 96.7% (±SE = 2.68) consumption of *P. longicarpus* and 98.7% (±SE = 0.91) consumption of *S. bromophenolosus*.

S. bromophenolosus was readily consumed during all three of its life stages by hermit crabs. Hermit crabs ate an average of 99% of an adult in 24 hours. In the egg assays, an average of 68% of the eggs were consumed after thirty minutes. Similarly, in the juvenile assays, an average of 82% of juveniles were consumed after thirty minutes. Controls with no crabs were also counted and all 10 eggs and all 10 juveniles were found in controls at the end of thirty minutes suggesting no observer error. The difference in size and detectability of prey items, as well as the time of exposure makes these assays difficult to compare.

Sand worm reactions

The sand worm, *Nereis virens*, had different reactions to the two prey types it was presented with (Figure 24*). N. virens* reacted to contact with hemichordates negatively. It ignored it, turned around, or recoiled. When encountering a piece of shrimp, however, it had either a neutral or sometimes aggressive reaction, ignoring, biting, and occasionally turning around. On a scale of 1- 4, 1 being recoil, and 4 being bite, hemichordates induced an average reaction of 1.65 (±SE = 0.13), which falls between recoil and turn. Shrimp induced an average reaction of 3.15 (±SE = 0.13), which falls between ignore and bite. The reaction to shrimp significantly higher on the scale than the reaction to hemichordates (n = 30, Z = -4.038, p < 0.001) when compared using a non-parametric Wilcoxon Signed Ranks Test.

Transplant Study

When hemichordates were transplanted higher into the intertidal only an average of 3.50 (SE = 0.74) hemichordates were remaining. In the mid intertidal there was an average of 7.86 (SE = 1.08), and in the lower intertidal, an average of 9.75 (SE = 1.39), but there was no effect of treatment (Figure 25a). The proportions remaining were 0.83 (SE = 0.18) in the lower intertidal, 0.44 (SE = 0.10) in the mid intertidal, and 0.009 (SE = 0.005) in the upper intertidal. There was a significant effect of zone on the proportion of casings remaining ($p < 0.001$), but no effect of treatment ($p = 0.708$) or an interaction ($p = 0.571$) (Figure 25b).

Discussion

Three hemichordate species, *Saccoglossus kowalevskii* (Virginia)*, Saccoglossus bromophenolosus* (Maine)*,* and *Protoglossus graveolens* (Maine), were the dominant infaunal macroinvertebrates on the mudflats in this study. In both locations (Virginia and Maine), hemichordates inhabit a specific mid-intertidal zone within the intertidal. Other species on the flats, specifically the polychaete *Spiochaetopterous oculatus* (Virginia)*,* and the hermit crab *Pagurus longicarpus* (Maine), also exhibited zonation, inhabiting the lower-intertidal. Lab experiments confirmed the palatability of hemichordates to multiple crustacean predators, suggesting that predation may play a role in setting the limits of their distribution. In further support of the role of predation in setting limits of zonation, all three hemichordate species had differential palatability to predators, and were actively avoided by some common infaunal predators.

Predation is just one of many possible forces structuring intertidal communities. For example, the rocky intertidal paradigm of intertidal zonation suggests that biotic forces such as competition (Connell, 1961a) and predation (Paine, 1974) limit a species' distribution from below, while abiotic forces such as desiccation and thermal stress can limit the distribution from above (Dayton, 1971), creating vertical zonation of organisms in the intertidal. Soft sediment systems may be fundamentally different from the rocky intertidal (Peterson, 1991) or could be governed by the same processes, just acting to different degrees.

The level to which factors such as predation, competition, disturbance, recruitment, and desiccation affect organisms in both soft sediment and rocky intertidal ecosystems can vary between the two systems (Peterson, 1991). Predators limiting the zone of prey organisms from lower in the intertidal has been observed on both rocky shores (Paine, 1974) and soft sediments

(Micheli, 1997). Competition plays a role in inhibiting the distribution of organisms from the lower intertidal in both systems (Connell, 1961, Woodin, 1974). Soft sediments are more dynamic and less stable than rocky shores because the fine particulate substrate of the soft sediment is easily altered by storms, currents, and daily tidal cycles (Roberts et. al., 2000). Although important on rocky shores, disturbances likely play a larger role in structuring the habitat of soft sediments than they do on rocky shores (Woodin, 1978, Peterson, 1991). Recruitment variation, however, can create patchy distributions and impact dominant species assemblage in both soft sediment and rocky systems (Woodin, 1976, Levin, 1980, Menge, 1991). Desiccation in soft sediments is less pronounced than on rocky shores due to the water holding capacity of the sediment at low tide (Peterson, 1991). Overall, the same pattern of biotic influences from the lower intertidal and abiotic influences from the upper intertidal arise in both systems, and in the case of my study, predation, competition, disturbance, recruitment, and desiccation could all be playing a role in hemichordate zonation.

Predation

Predation on infaunal macroinvertebrates by crab predators is generally higher than by fish or birds (Quammen, 1984). The potential for crab predation is more obvious in Maine because hermit crabs remain on the flat at low tide, and were therefore quantified. The hermit crabs in Maine existed at the highest abundances at the lowest tidal elevations, and many were seen in the subtidal right at the edge of the water, suggesting they retreat with the tide. The lower intertidal is submerged for more time than the upper intertidal, so hermit crabs can forage for longer periods of time in the lower intertidal than in the upper intertidal. More inundation time in the lower intertidal may also influence green crab predation. Green crabs were unable to survive on an intertdial mudflat for 24 hours without cage protection, suggesting they do not utilize the flat at low tide. They too likely feed more heavily in the lower intertidal and near the edges of the flat where they can retreat to the rocks more quickly. Two rock crabs were observed buried in the mud and not foraging on the flat in Maine, having no impact as predators at low tide. In Virginia, blue crabs and hermit crabs were never found on the flat at low tide, but in seine collection at both high and low tide on the York River flat in Virginia in Fall 2016, blue crabs were found in high abundances (*pers. obs.*). Hermit crabs were found in tide-pools and in the shallow subtidal. In addition to marine predators, shore birds have the potential to influence infaunal populations (e.g. Stempien, 2007), but shorebirds were not observed consuming hemichordates at either site, suggesting marine predators are the dominant predators on both flats.

Subtidal predators can be influenced by tidal inundation, which varied across sites. In Virginia, the tidal change is far less severe and the tide does not always extend past the hemichordate zone, whereas in Maine, even on high-low tides, low tide is far below the end of the hemichordate zone. This means that the hemichordates in Maine experience much more consistent tidal patterns; the tide fully retreats at every cycle. Predators in soft sediment communities are highly mobile and are able to venture high into the intertidal and retreat in time to avoid being exposed (Kneib, 1984). Inundation frequency may therefore be a factor in the magnitude of the effect of predators on infaunal communities. In areas with unpredictable (or more frequent) tidal coverage there may be less zonation due to predation, because the predators do not as predictably feed in particular areas or they have access to all areas far more often (Kneib, 1984). In consistent tidal areas, predators may access the intertidal in the same way each time and therefore influence zonation more strongly (Kneib, 1984). The retreat of the predators with each tidal cycle is what creates zonation, not predation on its own.

Infaunal predators do not retreat with the tide like epibenthic predators do, and may be able to feed for longer periods of time. In Maine, *N. virens* were found in high abundances with hemichordates. The effect of their predation can reduce other infaunal species populations by 30-96% (Ambrose 1984). Although *N. virens* population distribution was lower on the flat than that of hemichordates, they did not occupy a distinct zone, suggesting they are unlikely to strongly influence the zone of hemichordates. The other infaunal predator on the flat in Maine, *Glycera dibranchiata* actually increases some taxa by reducing *N. virens* populations (Ambrose, 1984), but, although present, no quantitative data on *G. dibranchiata* distribution was collected to know if they too impacted the distribution. Our lab results suggest that neither are major predators of *S. bromophenolosus, S. kowalevskii,* or *P. graveolens,* and would therefore not influence their zone, unless by competition for space. In addition to determining the range of the hemichordate zone, predators may also influence overall abundance patterns throughout the year.

There is higher risk of predation over the summer, when predators are abundant (Virnstein, 1977) creating a cyclical pattern in prey population. Although I did not sample in every month, I found that *S. kowalevskii* was most abundant on the flat in Virginia in the month of May, a similar finding to that of Kicklighter et al. (2004). They found that while the *S. kowalevskii* population was increasing in early summer, the populations of this palatable species declined. Increased densities of epibenthic predators have been recorded in late spring and summer in the inshore waters of the Southeastern U.S. (e.g. Nelson et al., 1991) and Virnstein (1977) has reported lower infaunal prey densities during summer months. Quammen (1984) found that the seasonality of prey abundance patterns correlate with the seasonality of major predators. Virnstein (1977) and Kicklighter et al.'s (2004) data suggest that high predation on infaunal organisms in the summer causes a decline in overall infaunal population during summer months. Hemichordates however, are not palatable to fish, and so do not experience this decline. Hemichordates maintain high populations throughout the summer, suggesting that they are unpalatable to some major predators on the flat. Other factors such as temperature, reproduction, and erosion influence variability throughout the year (Brazeiro & Defeo, 1996), which could confound effects of yearly predator cycles. Additionally, seasonal fluctuation adds evidence that abiotic factors alone are unable to structure intertidal environments.

Despite hemichordate's chemical defenses, they were preyed on by a number of predators in the lab. All crustacean predators readily consumed hemichordates, suggesting they are not completely deterred by the chemical defense, consistent with Kicklighter et al.'s (2004) finding that *S. kowalevskii*'s bromophenol chemical defense was ineffective against a crustacean predator, but effective against a fish predator. Based on the low predation by the two polychaete species, the bromophenols may also be effective against polychaete predators, a result inconsistent with that of Giray and King (1997). Not only were there overall trends based on groups of predators, but there were differences among both individual species of predator and prey, as well as interactions between predator and prey, suggesting different predators had different reactions to the different prey species. The overall consumption of *P. graveolens* and *S. kowalevskii* was very comparable, but the consumption of *S. bromophenolosus* was significantly less. All the Maine predators, besides the hermit crab maintained this pattern of high consumption of *S. kowalevskii* and *P. graveolens,* and lower consumption of *S. bromophenolosus,* suggesting maybe Maine predators have some aversion to *S. bromophenolosus,* the species they would encounter the most frequently. In the predators common in Virginia (*C. sapidus* and *P. longicarpus*), however, *S. bromophenolosus* was consumed in higher proportions, comparable to *P. graveolens*, while *S. kowalevskii* was consumed less. This suggests that *S. kowalevskii* is more chemically defended against its native Virginia predators more so than those in Maine. The Virginia predators are more averse to *S. kowalevskii's* defense than the defenses of *S. bromophenolosus* and *P. graveolens,* prey items *C. sapidus* would never have the opportunity to encounter.

When hermit crabs were given a choice, they preferred *S. bromophenolosus* to a palatable control, a result consistent with Giray and King's (1997) findings. This is evidence that hermit crabs have the potential to influence the lower bounds of the hemichordate zone in Maine where they were found in high abundances below the hemichordate zone. There are hermit crabs in Virginia as well that retreat with the tide and could impact the *S. kowalevskii* zone.

Neither of the polychaete predators consumed hemichordates, and although there was loss of hemichordate wet mass, there was no visible damage to the prey. This could have been error in wet-mass recordings, or an artifact of defecation and no food. This does not, however, align with Giray and King's (1997) findings. Although predation by the two polychaetes was not observed, there were fewer hemichordates in the container and telling concentrations of DBP (2,4-dibromophenol) measured in predator tissue (Giray and King, 1997). Polychaetes are difficult to manipulate in the lab (Woodin, *personal communication*) but further experiments that allow for direct observation of predation would help determine if *Nereis* and *Glycera* are in fact predators of hemichordates, and if they play a role in setting the bounds of the hemichordate zone. Additionally, competition could influence the hemichordate zone from below.

Competition

Organisms compete for space, food, and other resources and employ myriad methods to do so. Through competition organisms in the intertidal can create distinct zones (Lubchenco, 1980). In Virginia, the two dominant species on the York River study flat, *Saccoglossus* *kowalevskii,* and *Spiochaetopterus oculatus* inhabit two distinct zones. *S. kowalevskii* inhabited the mid intertidal, while *S. oculatus* occupied the lower intertidal. *S. oculatus* has previously been recorded as living in the lower intertidal in patches with densities of up to 200 m^{-2} (Barnes, 1964). While competition for space is less dominant on sand and mud flats than on rocky shores (Peterson, 1991), it can still be limiting for burrowing organisms, and the hard tubes of *S. oculatus* may create an infaunal habitat unsuitable for hemichordates. Even with enough space, they may competitively inhibit each other's feeding – both in time of feeding and food resources. *S. oculatus* exhibits both suspension and deposit-feeding behavior, and with palps reaching 4-6cm, may overlap with deposit feeders such as hemichordates (Turner and Miller, 1991). In Lowes Cove, phytoplankton depletion with each tide cycle over the summer months suggests that phytoplankton availability may be a limiting resource for benthic organisms (Carlson et al., 1984), so organisms lower in the intertidal have the potential to reduce food supply for those higher in the intertidal, creating a competitive interaction among or within species (Peterson & Black, 1987). Another possible competitive inhibition may be between the adults of one species and the juveniles of another (Woodin, 1976). Adult *S. kowalevskii* may be allelopathic, their chemical defense keeping *S. oculatus* larvae from settling in their zone, and settling deeper instead. The interactions between species, however, can be superseded by disturbance events which can impact an area indiscriminately.

Disturbance

Soft sediment communities experience disturbances that can completely alter habitat and therefore community structure. Shore morphology is influenced by waves, currents, and tidal range (Roberts et al., 2000), and daily removal and accumulation of sediment can be on the order of multiple centimeters per tide cycle (Christie et al., 1999). In addition to large-scale processes, individual organisms can influence the structure of a flat. For example, a ray can create a large pit in one feeding attempt, drastically influencing all the organisms in that space (Thrush et al., 1991). Other micro-topographical features like ridges and runnels occur on exposed flats because of wind waves (Bell et al., 1997). Not only do disturbances alter the habitat itself, but the infaunal community can be impacted as well. Storm events have been shown to alter community structure of soft sediment communities (e.g. Jaramillo et al., 2012, Noda et al., 2016). The flat on the York River has had major fluctuations in hemichordate population size, with populations declines after large disturbance events such as hurricanes; however, hemichordates have been observed recovering to pre-disturbance population sizes in a single season (Allen obs.). In addition to impacting the community structure, wave energy in intertidal regions frequently disrupts evidence of surface activity such as tracks, fecal casings, and feeding rosettes (Maurer & Aprill, 1979), which could account for some of the variation in densities recorded on the flat, and how they relate to the number of organisms present. Disruption of fecal casings could also account in part for the fluctuations in density on the York River through time. Therefore, the practical, non-invasive method of counting fecal casings when surveying for density may have underestimated worm abundance since casings are more likely to get washed away than the worms. In future studies, to mitigate this possible error, more frequent measures of the casing to hemichordate relationship should be recorded to account for disturbances. Small-scale disturbances, coupled with storms and strong currents can drastically change a flat and the community in a short period of time. Multiple stable states, a community existing and functioning with only some of the possible inhabitants, can arise through time as one population being wiped out by disturbance creates space for a new species (Sutherland, 1974).

The community on the York River flat has included years with dominant populations of

Diopatra (Allen obs.), a tube-building polychaete with a very large, hard tube. Despite historic densities, there were very few *Diopatra* tubes at the field site during this study. The population of *Diopatra* may have decreased due to a large-scale disturbance event, but they themselves can act as a refuge from smaller scale disturbances for other infaunal organisms (Woodin, 1978). Some macroinvertebrates distribute based on small-scale features within the flat ecosystem (Mills and Berkenbusch, 2009), and *Diopatra* creates such a feature. Infaunal organisms are densest around *Diopatra,* where they are shielded from disturbance by *Callinectes sapidus* and *Limulus polyphemus* (horseshoe crab), which can penetrate 3 to 9cm in depth (Woodin, 1978). Hemichordates burrow between 20 and 40cm and may be able to find refuge in depth, but have no additional physical protection from disturbance. No relationship between hemichordates and *Diopatra* was detected in field observations, but due to the hemichordate's lack of protection from disturbance, a relationship may exist. The other dense species on the York River flat was *S. oculatus,* which burrows at least 15cm deep, has a strong non-friable tube, and is unaffected by the presence of *Diopatra* tubes (Woodin, 1978) or predation by crabs and fish (Virnstein, 1977). The defenses of *S. oculatus* suggest that it does not require additional protection from small-scale disturbance, which may explain why it is able to occupy the lower portion of the intertidal. Alternatively, there may be supply-side (pre-settlement) components, to its distribution as well.

Recruitment

Soft sediment organisms are often patchily distributed (Levin 1980), resulting from selective recruitment in response to cues of adults or microorganism co-inhabitants (Woodin, 1976). These patches do not necessarily correspond to obvious changes in physical conditions and are perpetuated by differential recruitment (Woodin, 1976). Positive recruitment can lead to a sustained or new population. Alternatively, juveniles may fail to settle in an appropriate habitat because no adults are present, or they never arrive at a good settlement location. Recruitment of larvae or juveniles to an adult population is important in structuring that population and influence where a population is, its density, and distribution (Ysebaert and Herman, 2002).

Marine invertebrate species have different modes of development, which can lead to differences in recruitment patterns and success. Some marine invertebrates have planktonic larvae, which may travel hundreds of miles before settling in a new location. Hemichordate larvae, however, develop on the benthos, where they are far less mobile than planktonic larvae. Benthic larvae are more likely to settle back with their parent population than create a new population on a distant flat. Hemichordates spawn in the late summer and early fall, but based on the low densities of fecal casings found in the winter months, juveniles likely don't recruit to the population until the spring, when we found an increase in casing density. Differential recruitment patterns can lead to different species becoming dominant in habitats that are otherwise similar, creating alternate stable states through space (Sutherland, 1974).

Flats on both the York River, Virginia, and in Maine have similar physical characteristics but different species assemblages. In Maine, there were hemichordate, sand worm, peanut worm, bloodworm, and bamboo worm dominated flats. In Lowes Cove alone, there were patches of different infaunal macroinvertebrates that seemed to inhabit the same type of habitat. These flats represent multiple stable states – a thriving community without all its possible members (Sutherland, 1974). Environmental factors, migration, colonization, and biotic interactions can all influence actual distribution patterns of populations (Fenchel, 1975), which can vary across similar locations. In addition to supply side impacts on population distribution, post-settlement biotic interactions can influence patches of organisms in soft sediment intertidal regions.

Desiccation and lack of food resources

In soft sediment intertidal environments, desiccation is thought to be less substantial than on rocky shores because sediment can retain moisture and buffer organisms from air stress (Peterson, 1991). Although infaunal organisms may not desiccate, they can become food limited higher in the intertidal (Peterson and Black, 1987). The upper intertidal zone is submerged the least, and may not offer enough submergence time to allow for adequate feeding. Although the impact of air stress is diminished somewhat by sediment saturation at low tide, reduced feeding time may limit the hemichordate population in the upper intertidal. Food quality (as measured by Chlorophyll *a* levels, a measure of plankton present) is not an indication of feeding rates for *S. kowalevskii* (Karrh and Miller, 1994), but flow rates influence their feeding behavior (Miller et al., 1992). At high flows, *S. kowalevskii* withdraw their proboscis entirely (Miller et al.,1992), which means they are unable to feed. The response of hemichordates to flow rate and food quality is delayed by minutes to hours (Miller, 1992), which, especially in semi-diurnal intertidal habitats, can severely reduce feeding time. With less food, populations can shrink or disappear entirely, creating space for new populations. This could potentially be a bigger factor in Maine where the larger tidal fluctuations potentially create higher flows and therefore further limit those populations leading to smaller populations in Maine. My transplant study in Virginia confirmed that there was lower survival higher in the intertidal even 72 hours after transplantation, suggesting that adults are unable to survive in the upper intertidal.

Implications for general patterns of zonation

Intertidal zones are frequently divided into three areas: the upper, mid and lower intertidal. In addition to the processes structuring zonation that I consider above, there is interest in determining which zones may have the highest diversity and/or productivity (Paavo, 2011). Of over 72 studies on exposed sandy beaches, 62% found three distinct zones of benthic invertebrates (Schlacher & Thompson, 2013). In one such system of three distinct zones, species richness increased lower in the intertidal (Read, 1984). Other studies have found the highest species diversity within the mid-intertidal (Beukema, 1976, Beukema and Cadee, 1997, Paavo, 2011). In the present study in Virginia, there were two zones of obvious organisms, *S. kowalevskii* in the mid-intertidal, and *S. oculatus* in the low-intertidal, abutting the subtidal area. In Maine, although Lowes Cove drained hundreds of meters past Coves 1 and 2, a draining creek in the middle of the cove functioned as a subtidal refuge, and both *Nereis virens,* and *Pagurus longicarpus* inhabited the low-intertidal and subtidal creek bed. In both Virginia and Maine, there was a third zone of upper-intertidal which was relatively uninhabited. In both systems this upper zone was characterized by a change in substrate, in Virginia, coarser sediment and more shell material, and in Maine, large rocks and *Fucus*. These may create environmental boundaries that may make the habitat fundamentally unsuitable for certain infaunal organisms. Schlacher and Thompson (2013), however, found no correlation between environmental boundaries and biological zones, suggesting that biological factors such as predation, competition, and recruitment determine actual patches within larger environmental boundaries. In addition to the factors affecting distribution within zones, environmental differences between the flats may influence the overall distribution of the three hemichordate species.

The environmental factors impacting local zonation (e.g. temperature) can also contribute to geographic range limits of intertidal species (Wethey, 1983). For example, in the barnacles *Balanus balanoides* and *Chthamalus fragilis,* Wethey (1983) found that not only did temperature impact survival at the edge of zones, but competition by more tolerant species in those regions inhibited success and limited further distribution, effectively creating geographic zonation.

Although *S. kowalevskii* and *S. bromophenolosus* are closely related, *S. bromophenolosus*'s cold tolerance may allow it to outcompete *S. kowalevskii* in their overlapping zone of southern Maine (or *vice versa*). *S. bromophenolosus* and *P. graveolens* were found on the same flat in Maine, suggesting they have similar tolerances. Interestingly, however, *P. graveolens* were only found in the lower of the two coves (Cove 1), while the upper cove (Cove 2) was dominated by *Saccoglossus* spp. Although it was most likely *S. bromophenolosus* based on historical ranges, the morphological differences between the two *Saccoglossus* species are minor. There was a high abundance of smaller and lighter colored *Saccoglossus* spp. in the upper cove, which could have been *S. kowalevskii,* although they have not previously been reported north of York, Maine (King et al., 1995). There may be competitive interactions between all three species, leading to the differences in distribution across the East Coast as well as within the two coves in Maine. Additionally, although hemichordates only occupied a few meters of intertidal space, there is no obvious biological reason they couldn't survive submerged for longer periods. Most described species have been found in either the intertidal or shallow subtidal, but species inhabit the deep sea as well (Tassia et al., 2016). Hemichordates survive in a number of habitat types, each with their own set of biotic and abiotic factors influencing and structuring the community.

Ecosystems are dynamic, and individual factors do not act on their own. The idea that biological factors set lower limits and abiotic factors set upper limits is an over simplification (review by Tomanek and Helmuth, 2002). In reality, in addition to the basic paradigms of competition, predation, and desiccation, there are relationships between biotic and abiotic factors that influence the zone. For example, Wethey (1984) determined that sun exposure influenced competition between two barnacles and Menge and Sutherland (1987) concluded that while processes such as predation and disturbance may be distinct from each other, environmental conditions influence both. There are many individual factors structuring both the rocky intertidal and soft sediments; desiccation, disturbance, recruitment, competition, and predation, all of which may be interacting to influence the community in novel ways. The rocky and soft sediment intertidal habitats need not be studied differently – the same factors influence both, just at varying degrees. My study suggested that both predation and competition could be important in structuring two mudflats, adding to the growing literature on the structuring of soft sediment intertidal flats, which is not so different from that of rocky shores after all.

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Figures

Figure 1: York River, Virginia, field site in (A) 2014 and (B) 2017, showing both erosion and sedimentation over the past three years.

Figure 2: Lowes Cove on the Damariscotta River in Maine. The University of Maine, Darling Marine Center can be seen on the left. Embedded panel shows Cove 1 on the left and Cove 2 on the right, with a small cove in the middle (which was a higher elevation rocky area with no hemichordates).

Figure 3: (A) Dense patch of *S. kowalevskii* fecal casings on the York River. (B) Fecal casing with scale on a mudflat in Georgia (the southern range of the species).

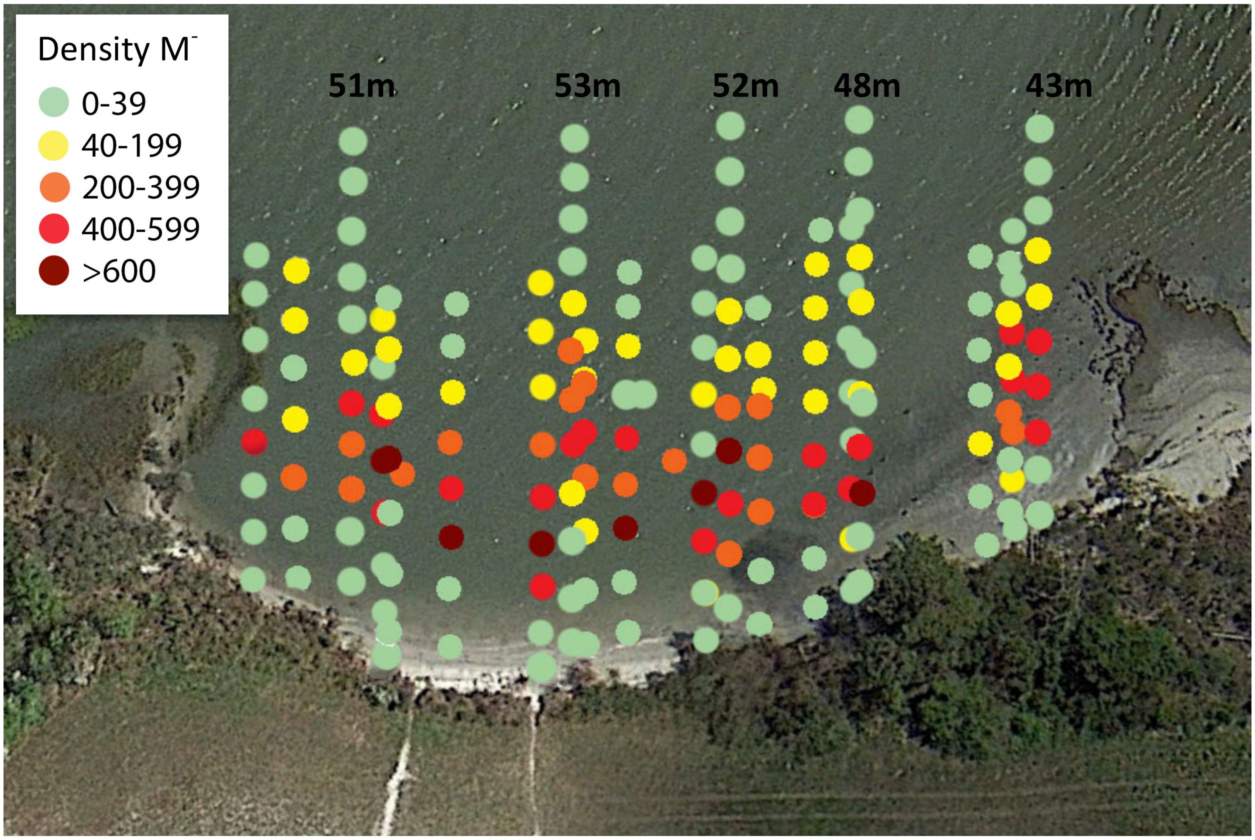

Figure 4: Arial view of the York River field site in Virginia with four sets of transects taken from November 2014 to February 2015. Transects originating from the high tide line (bottom of figure) and extending to low tide with higher densities of casings (M^{-2}) depicted as warmer colors. Numbers at the top are the distances from the high tide line for the longest set of transects.

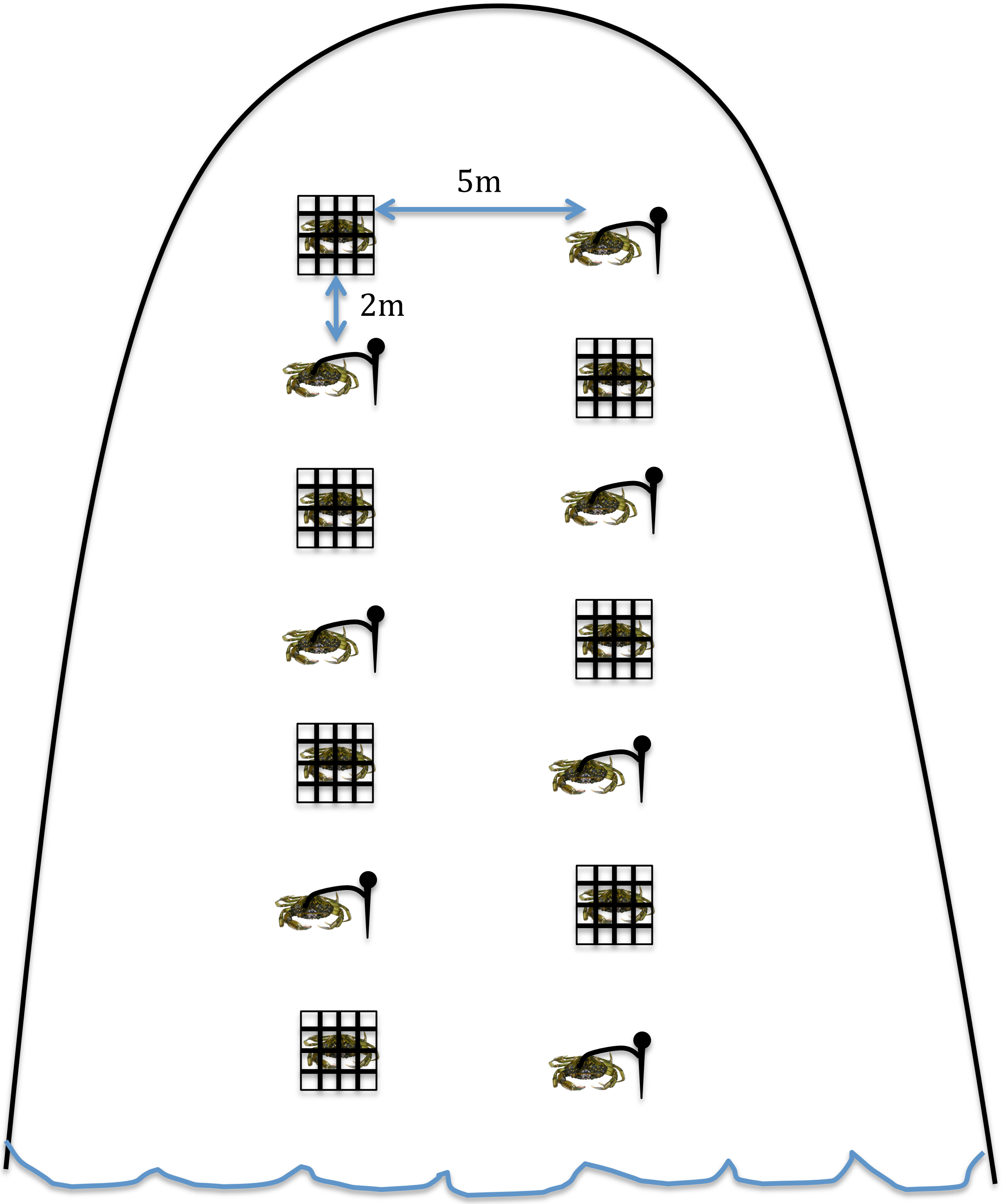

Figure 5: Partial diagram of green crab survival setup on the mudflat. 12 caged and 12 staked crabs total. Edge of flat in black and low tide line indicated by blue wave line.

Figure 6: Schematic of predation assay setup. One Sterilite container with a single predator and prey. N=10 per predator per prey item, with the exception of *C. maenas* with *S. kowalevskii* (n=9) and *N. virens* with *S. kowalevskii* (n=2).

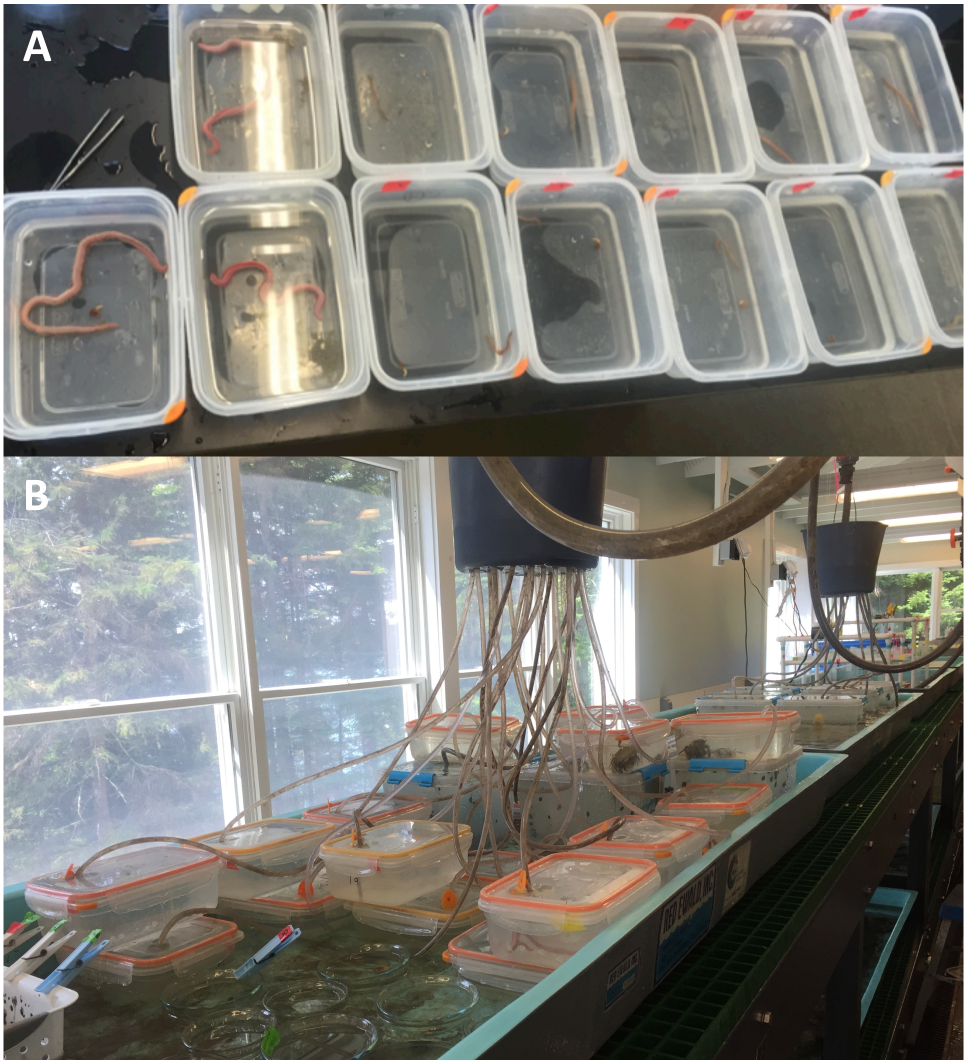

Figure 7: (A) Sterilite containers with predator and prey before being placed in the flow through system. (B) The lab flow through system at Bowdoin Coastal Studies Center. Water flowed into a bucket with tubes running out of holes in the bottom to Sterilite containers. This system was used for all predation assays.

Figure 8: Setup for crustacean choice experiments. Hermit crab (*P. longicarpus*) and green crab (*C. maenas*) were each given a choice between *S. bromophenolosus* and mussel tissue of approximately the same mass on either end of a Sterilite container.

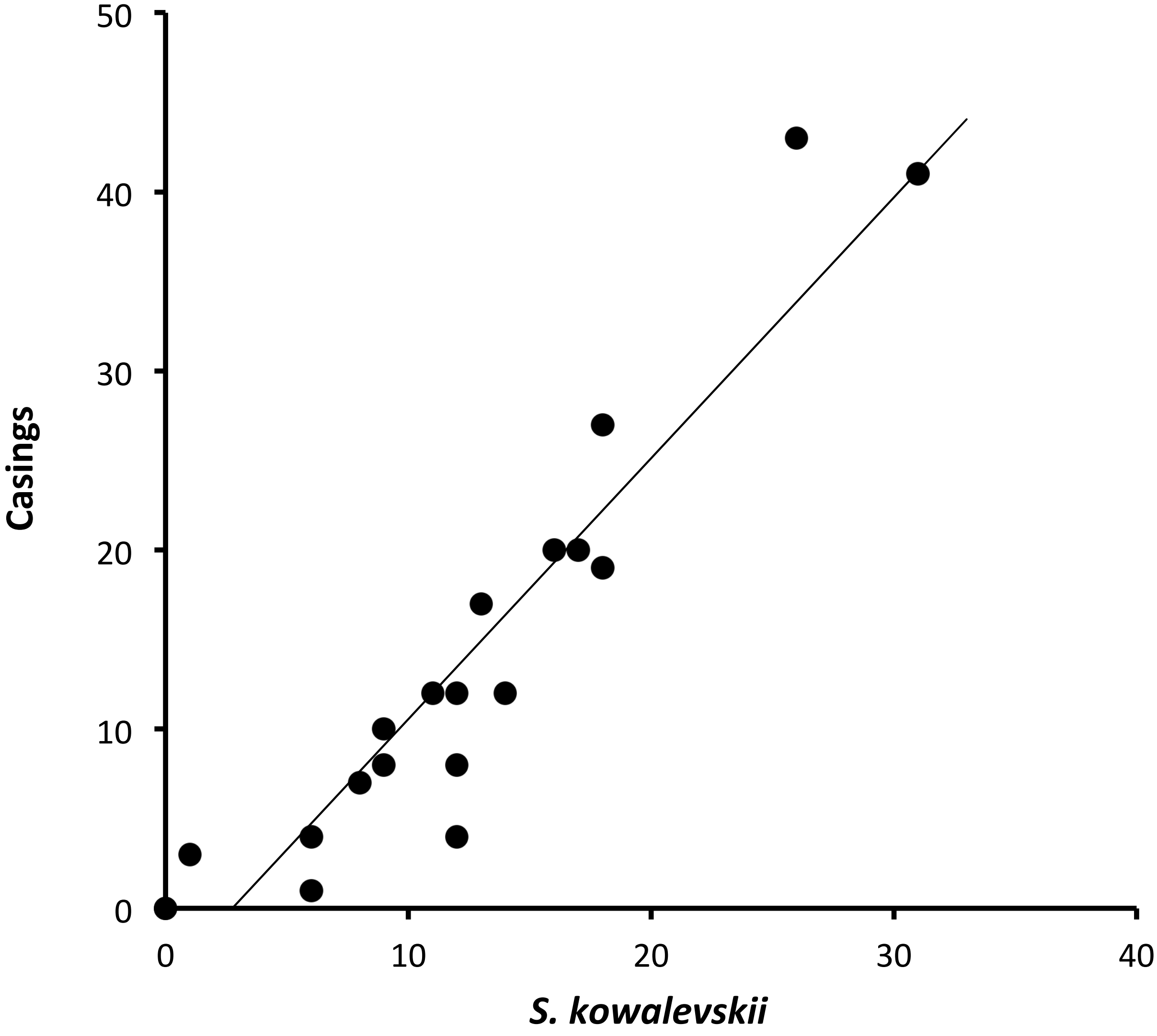

Figure 9: There is a positive correlation between the number of casings at the surface of the sediment, and the number of individual *S. kowalevskii* directly below it. (In a shovelfull). A linear function most appropriately represents the data showing a significant relationship (n=20, y = 1.4563x - 4.003, Adjusted R^2 = .873, p = 0.001). Each data point represents one shovelfull.

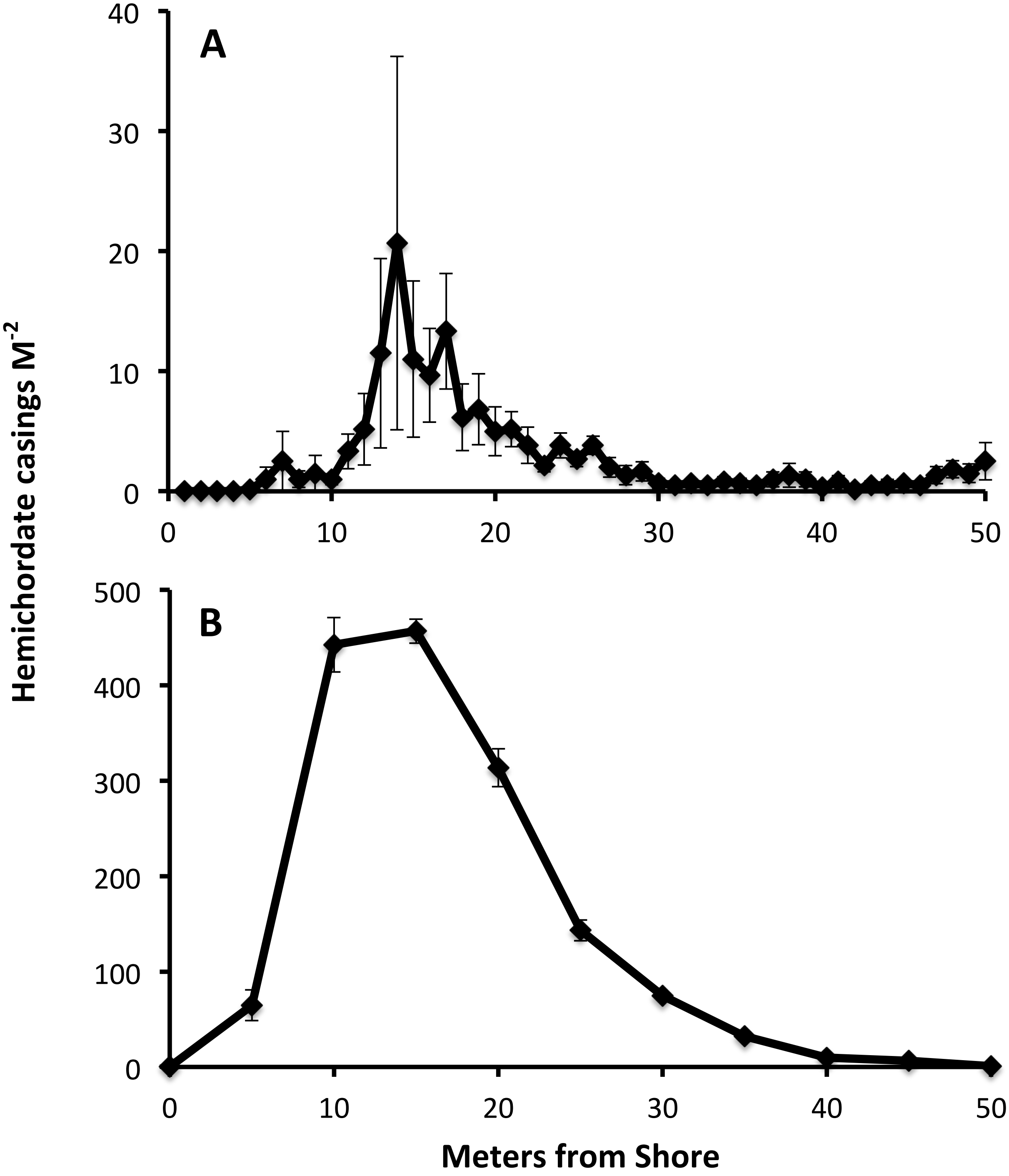

Figure 10: Average densities of hemichordate casings per meter across two flats in (A) Maine, and (B) Virginia. In Maine, data was taken from two adjacent flats measured on two days in July. In Virginia, all data was taken from one flat on one day in November. In both cases, the highest density of casings was found at approximately 15 meters from shore. Mean (n=6 transects) ± SE.

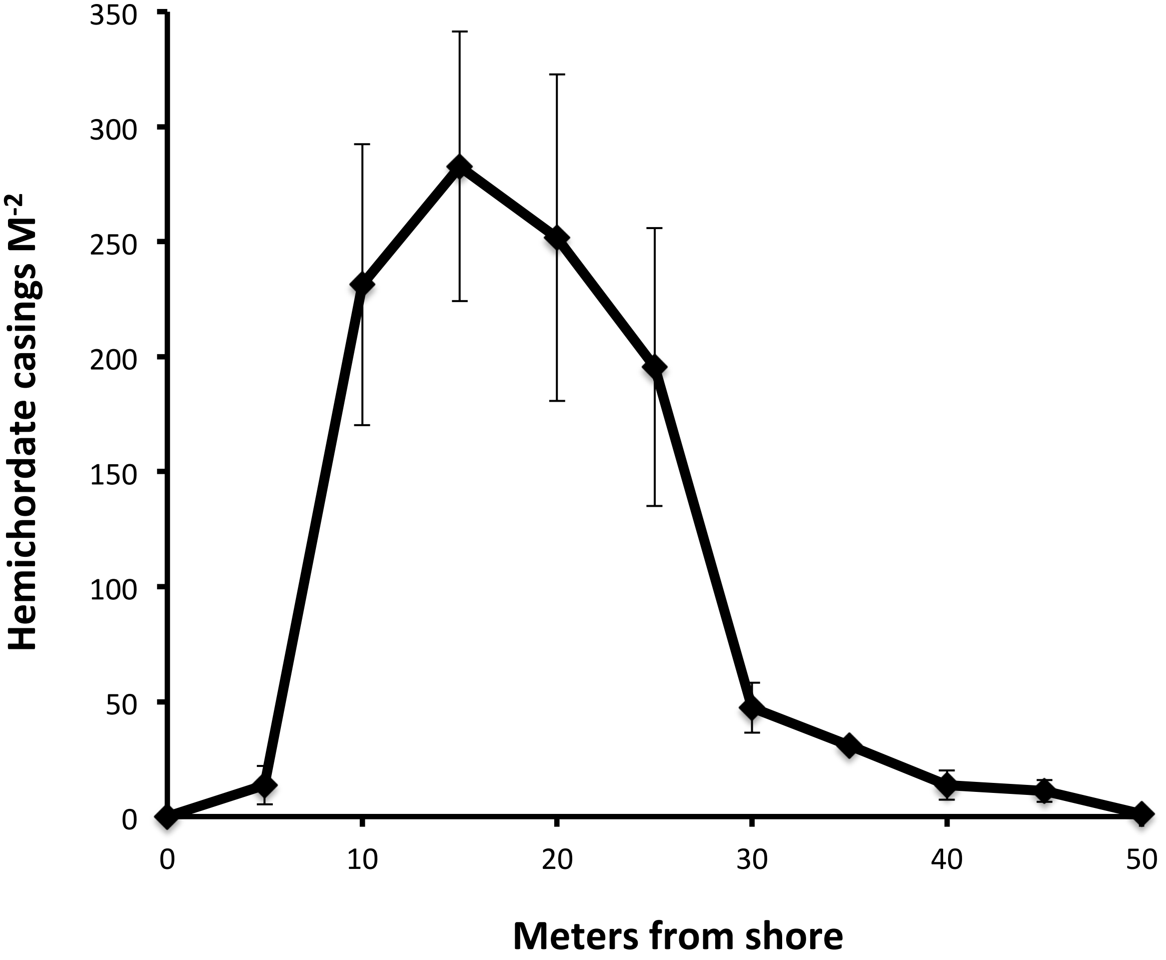

Figure 11: Hemichordate casing density in Virginia from November 2014 to March 2017. Averages ± SE, N=8.

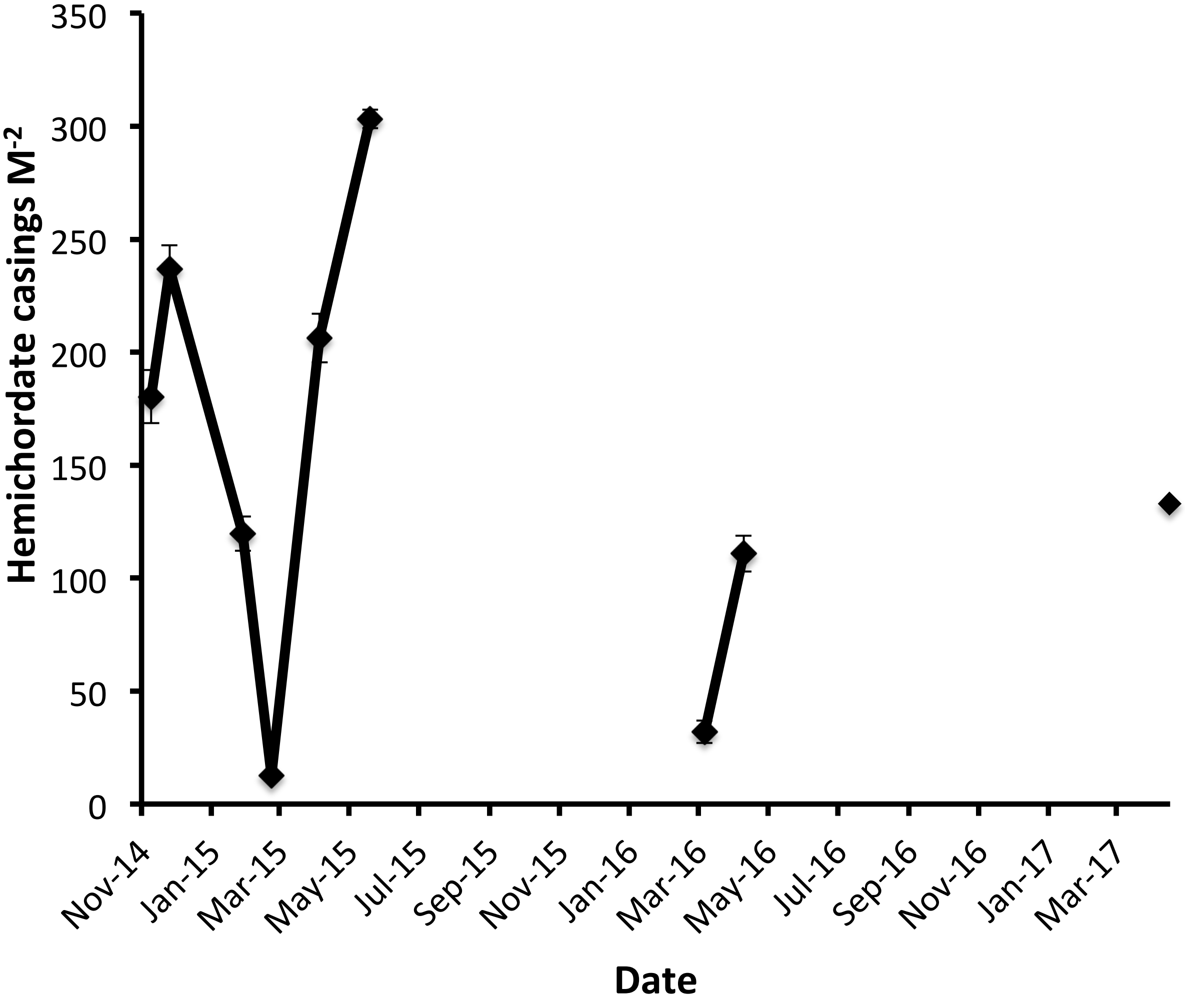

Figure 12: Density of casings on the York River form November, 2014 to March, 2017. A cyclical population pattern can be observed over the first year – high densities in the fall and summer and lower densities in the winter.

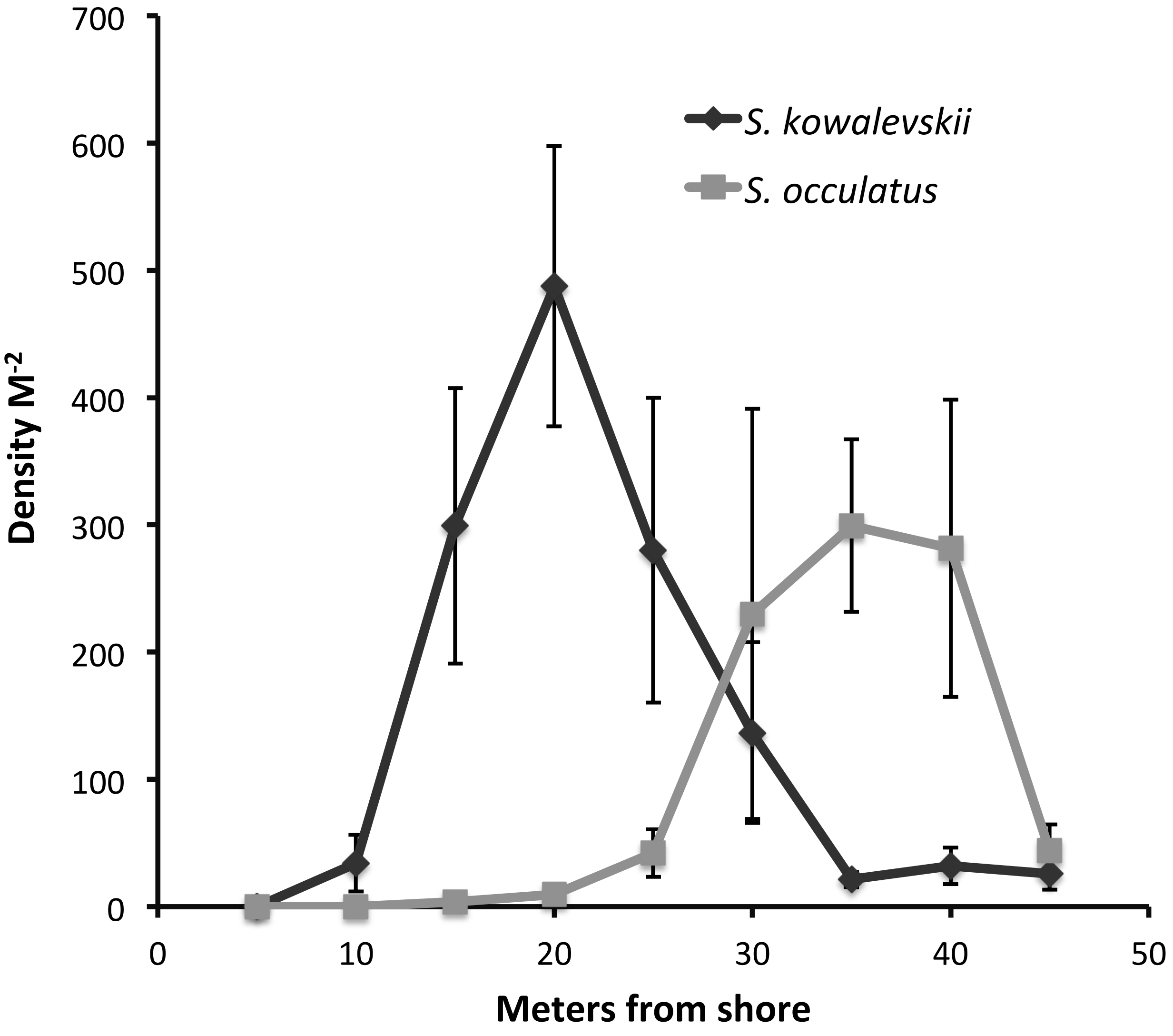

Figure 13: Population distributions of two species of infaunal worm on a mudflat in Virginia. The light grey represents the hemichordate, *Saccoglossus kowalevskii,* and the dark grey represents the polychaete *Spiochaetopterus oculatus*. Each data point represents the average density recorded across transects (n=6) ± SE. . The two species occupy distinct zones within the intertidal (p<0.001).

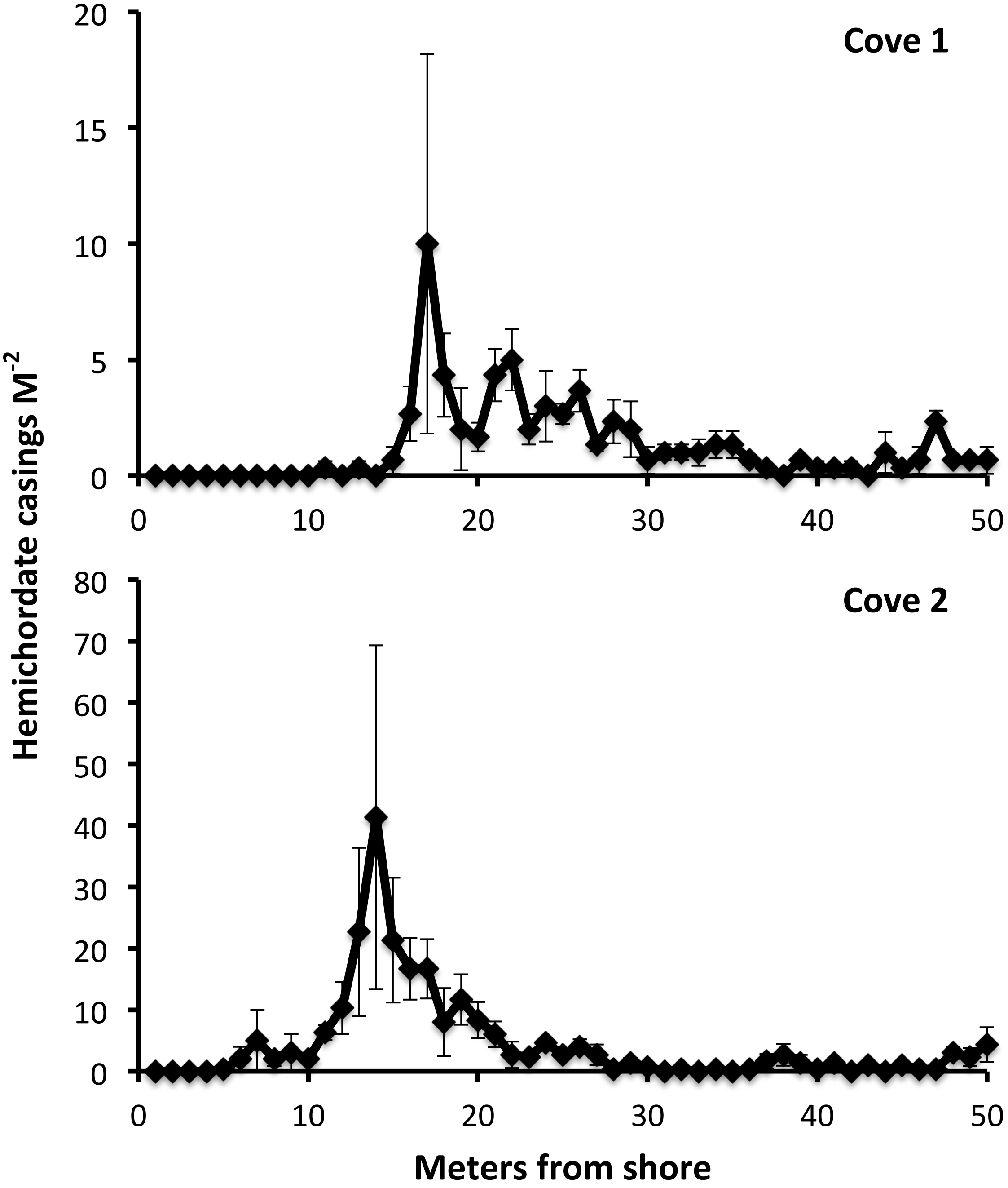

Figure 14: Intertidal distribution of hemichordates in each of the two coves in Maine showing slightly different distributions and densities. Each point represents average densisity across the flat (n = 3) ± SE.

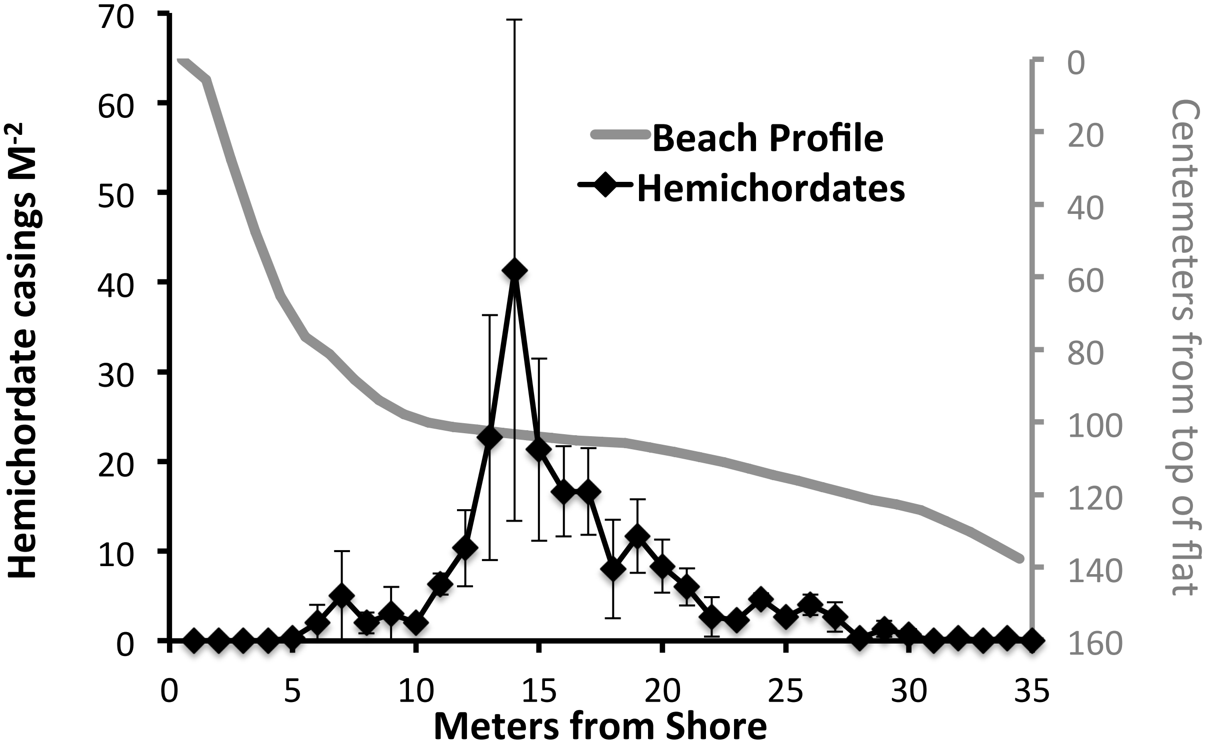

Figure 15: Beach profile overlaid with hemichordate densities from Cove 2 in Lowes Cove Maine. Most change in elevation took place in the first 10 meters of the flat, and the lowest elevation was at 35 meters. The hemichordate zone existed over an elevation change of only 22cm.

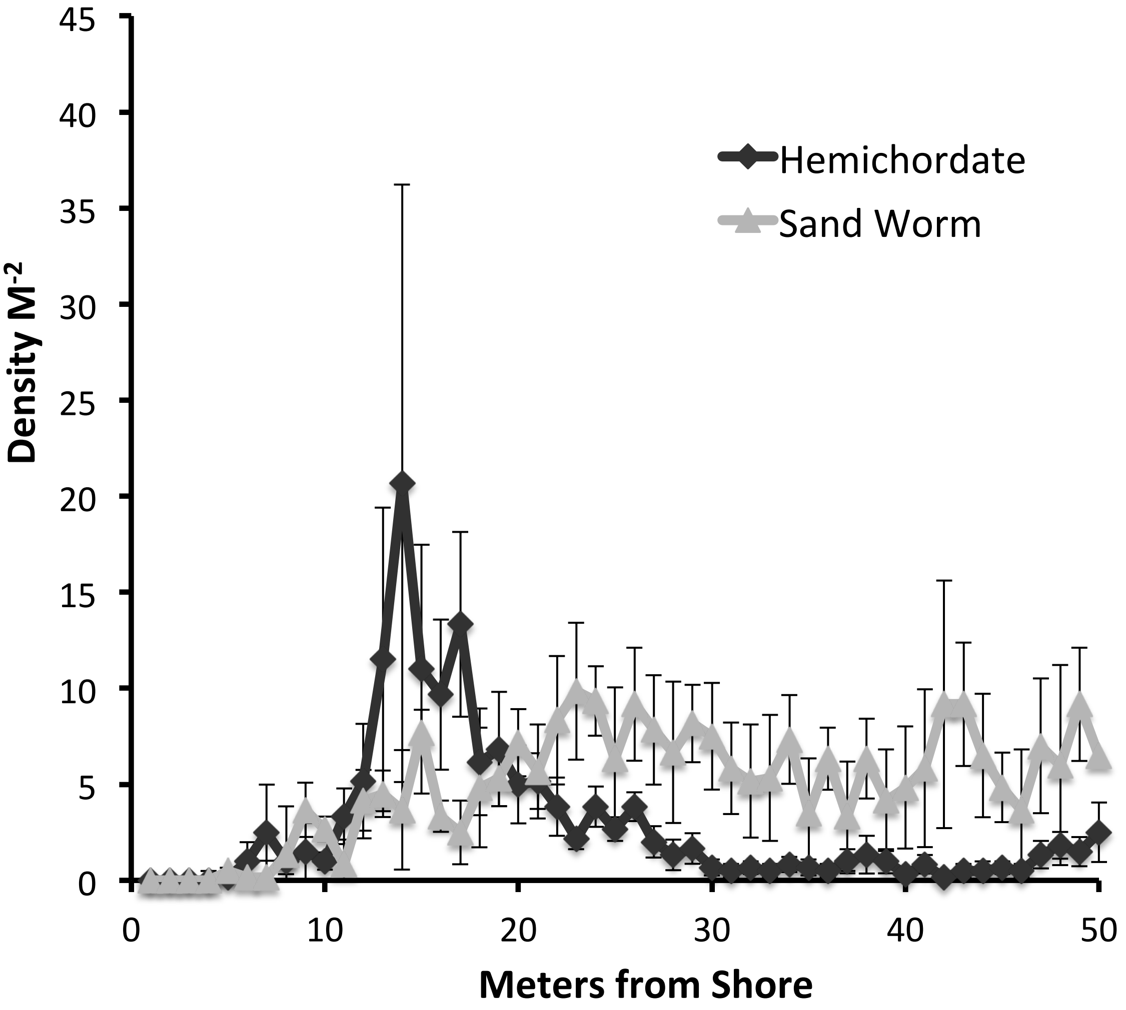

Figure 16: Hemichordate and sand worm distribution on two coves in Maine. The sandworms occupied a lower zone, but still completely overlapped with the hemichordates. There was no significant difference in the density of sand worms in and out of the hemichordate zone (p=0.794). Means (n = 6) ± SE.

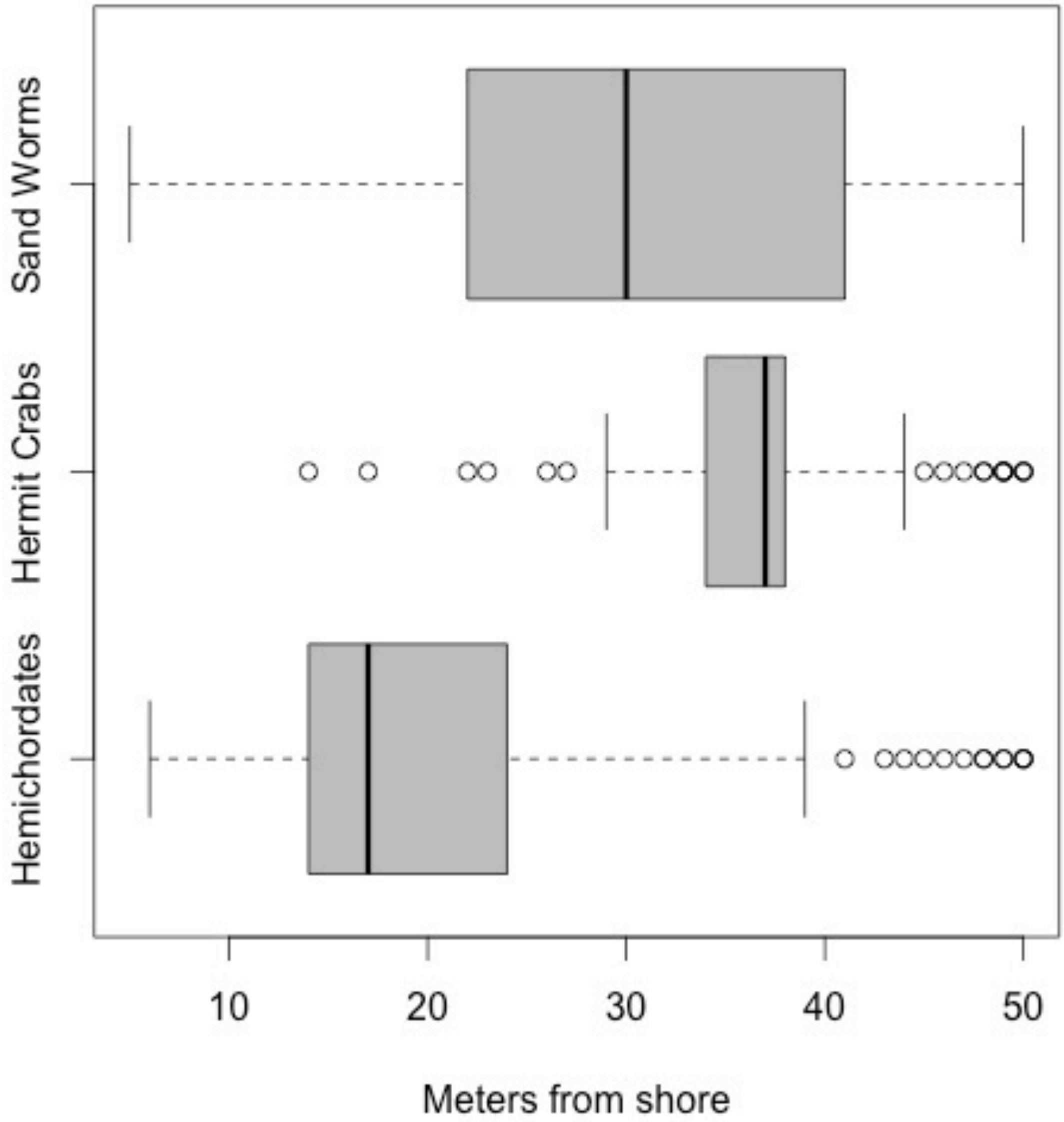

Figure 17: Sand worm, hermit crab, and hemichordate distribution on the flat in Maine. Boxes are interquartile range, circles are outliers. The large number of hemichordate outliers is likely due to the increasing slope on the far side of the creek.

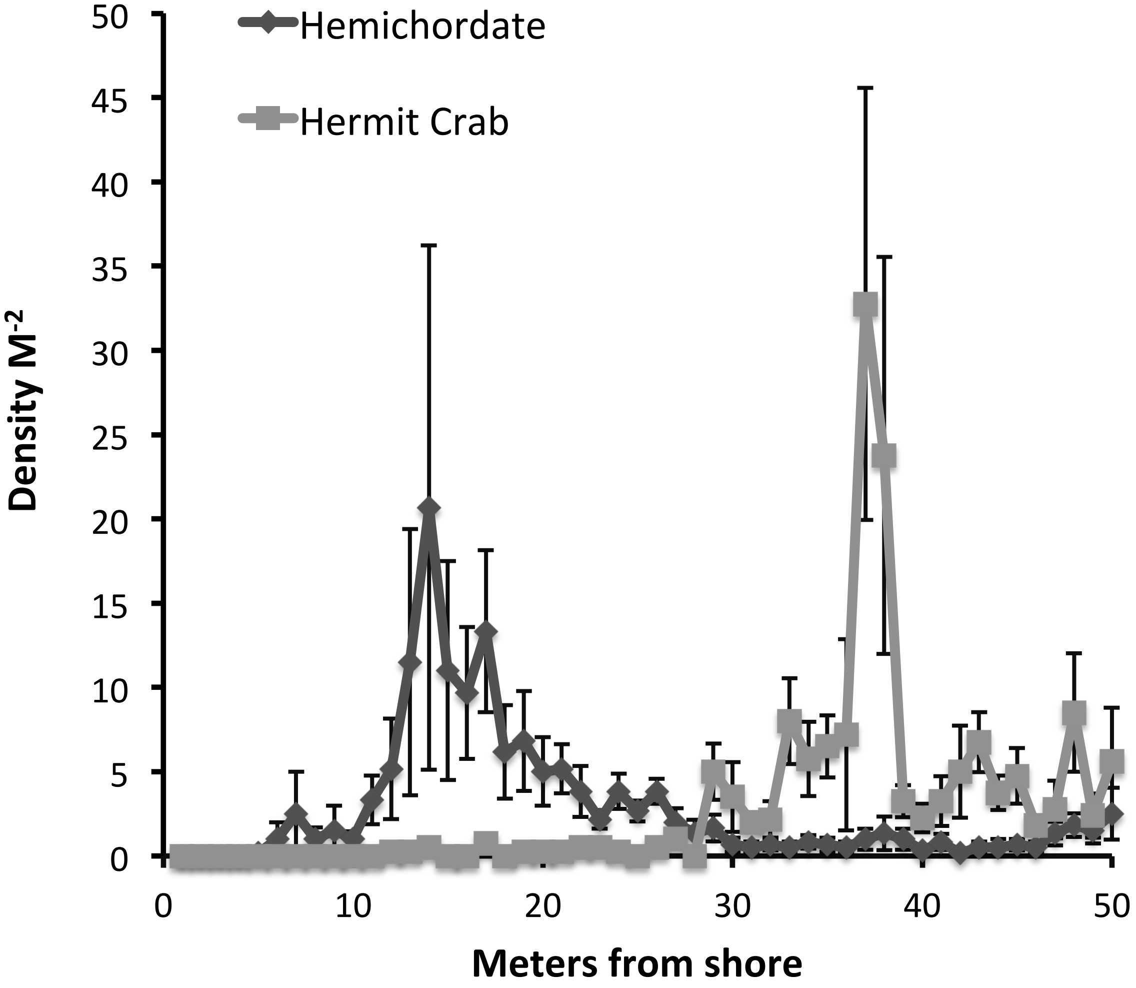

Figure 18: Average number of hemichordate casings per meter along transects in two coves in Maine. Dark points represent hemichordate casings, while light points represent hermit crabs. There was a significant difference in these two populations ($p<0.001$) and they only overlapped for 4 meters. Means (n=6) ± SE.

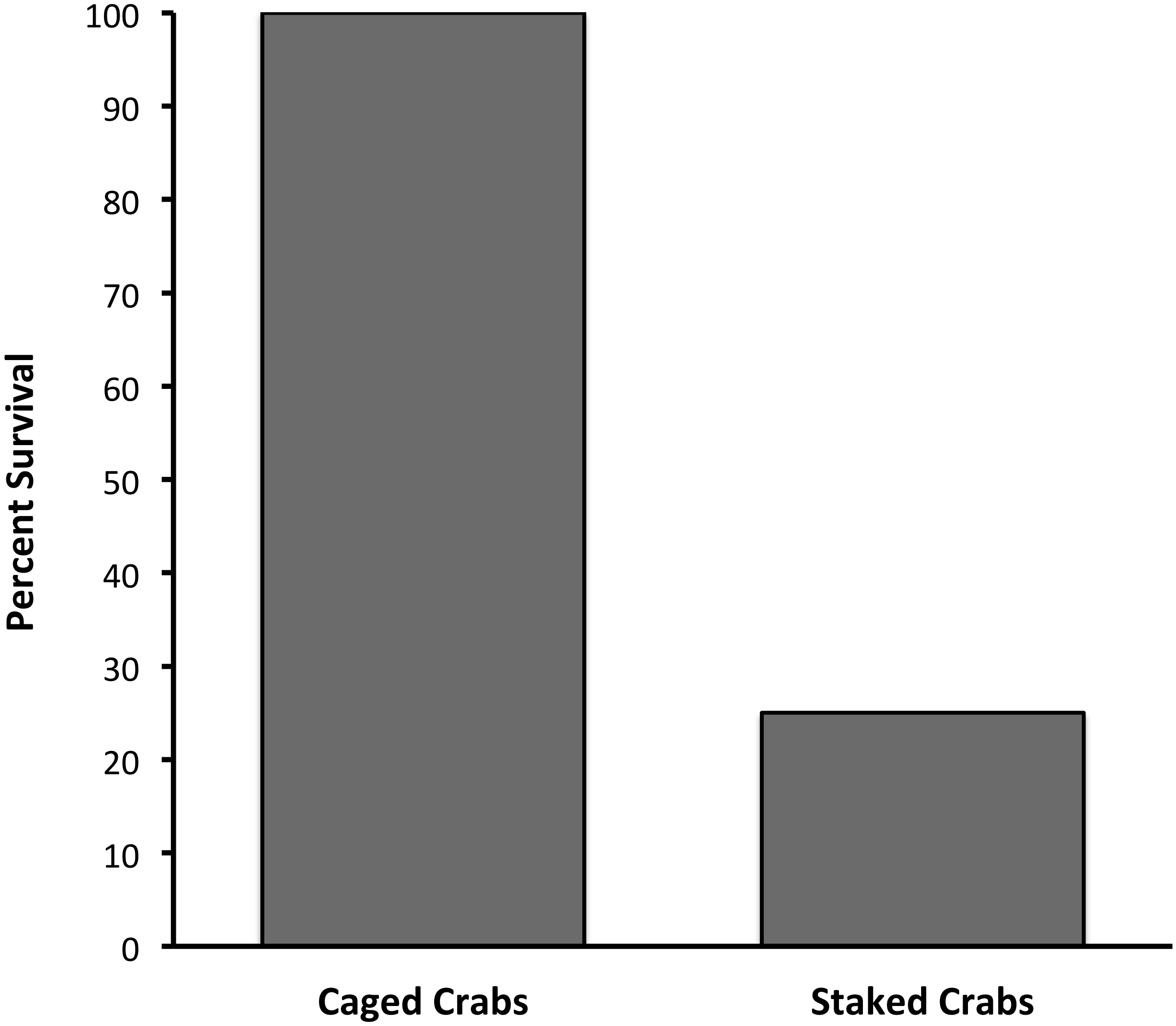

Figure 19 : Green crab survival on a mud flat in Maine at low tide. All caged crabs survived for two tide cycles (24 hours), while only 25% of staked crabs survived. **STATS.** Each bar represents the total percent of crabs that survived in each treatment (n=12).

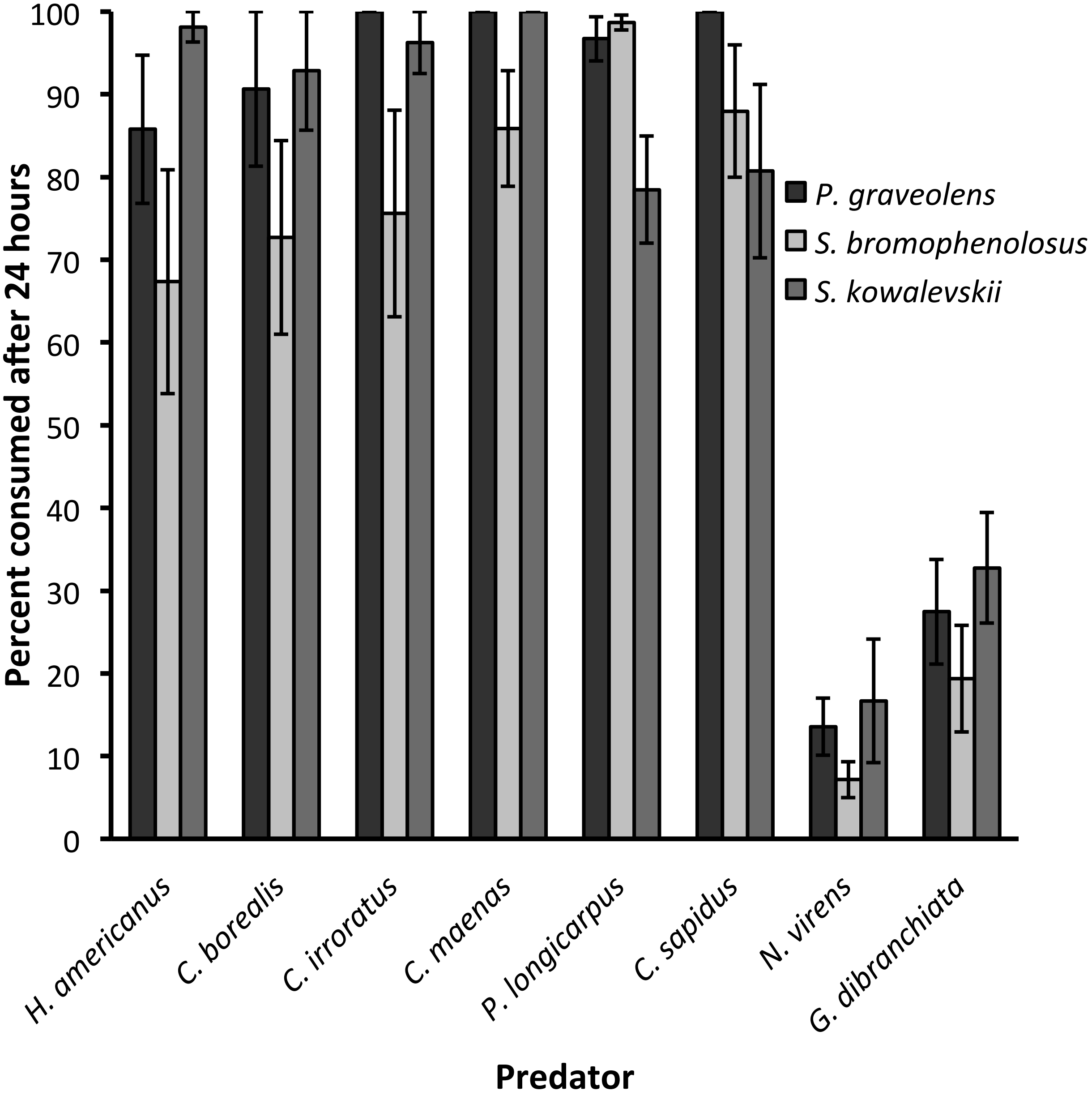

Figure 20: Predation assays of 8 predators on three species of hemichordate. Bars represent means ± SE. A Bonferonii corrected post-hoc comparison of means revealed no similarity between any of the crustacean predators with any of the polychaete predators. All crustacean predators were similar to each other ($p>0.05$) and the two polychaete predators were similar to each other ($p<0.05$). The prey species were all found to be different from one another when Aligned rank transformed.

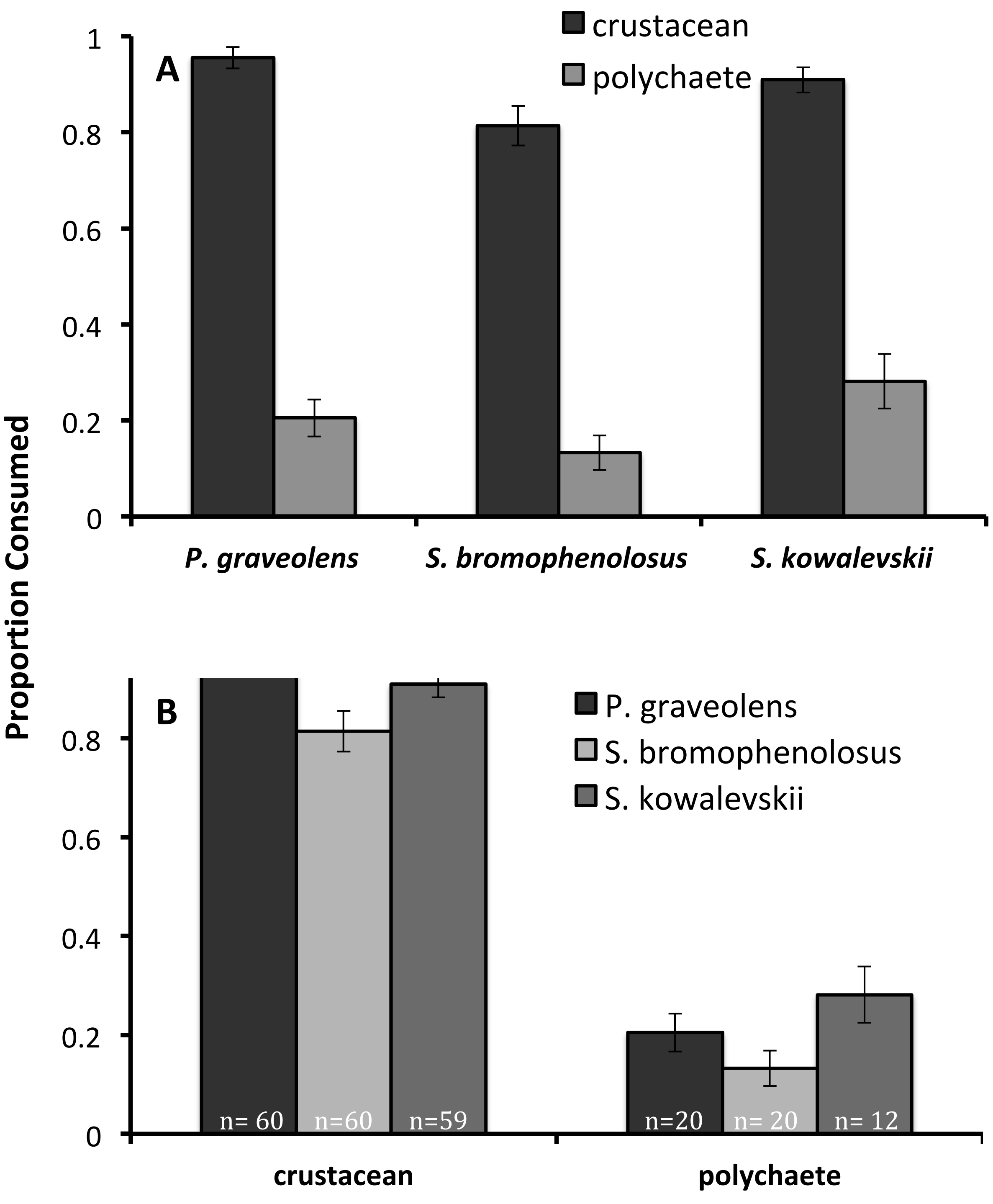

Figure 21: Predation assays by category. Bars represent average consumption of each prey item by predator category, grouped by prey item (A) and by predator type (B). When combined, crustaceans were significantly different from polychaete predators (p<0.001), and hemichordate prey was all different (p+0.007). Means ± SE.

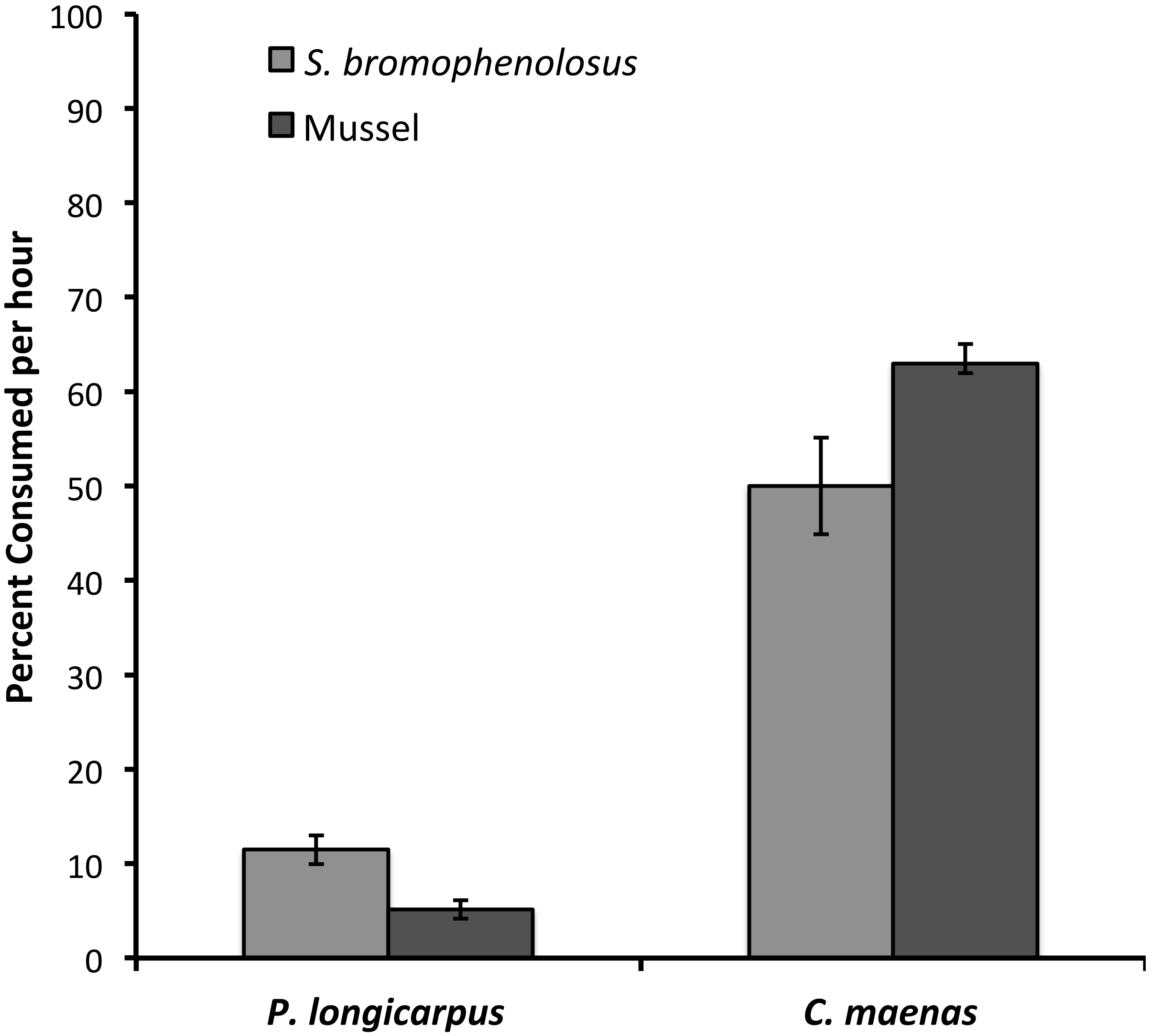

Figure 22: Crustacean prey choice. Means (n=30) ± SE. When given a choice between *S. bromophenolosus* and a palatable control of mussel tissue, the hermit crab, *P. longicarpus,* consumed more *S. bromophenolosus,* while the green crab, *C. maenas,* consumed more mussel. There was a significant effect of predator ($p<0.001$) and an interaction predator*prey ($p=0.001$), but prey was not found to be significant ($p=0.483$).

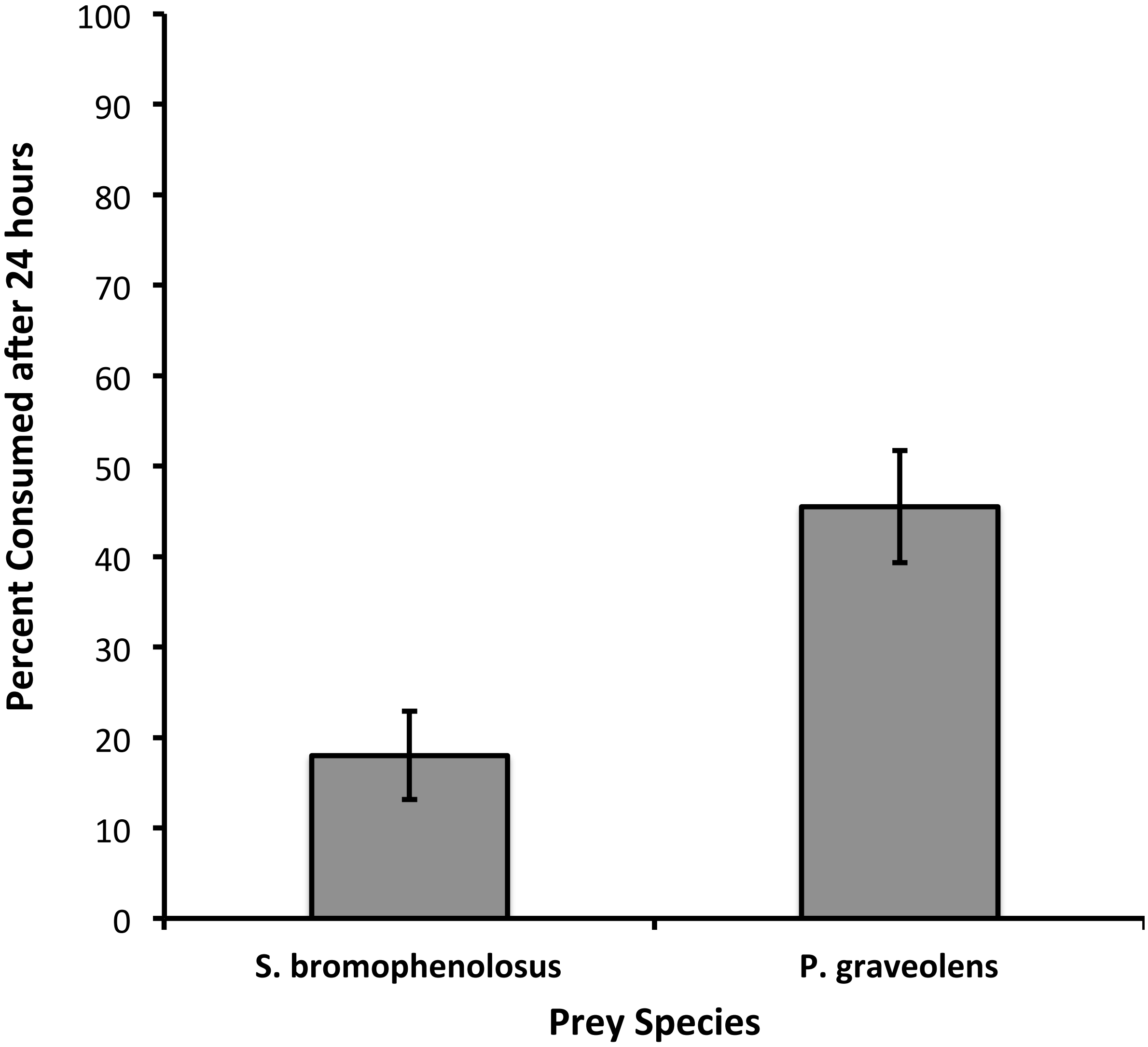

Figure 23: Hermit crab choice between two species of cohabiting species of hemichordate prey. Mean (n=10) ± SE. *S. bromophenolosus* was consumed less than the co-occurring, *P. graveolens* (p=0.001).

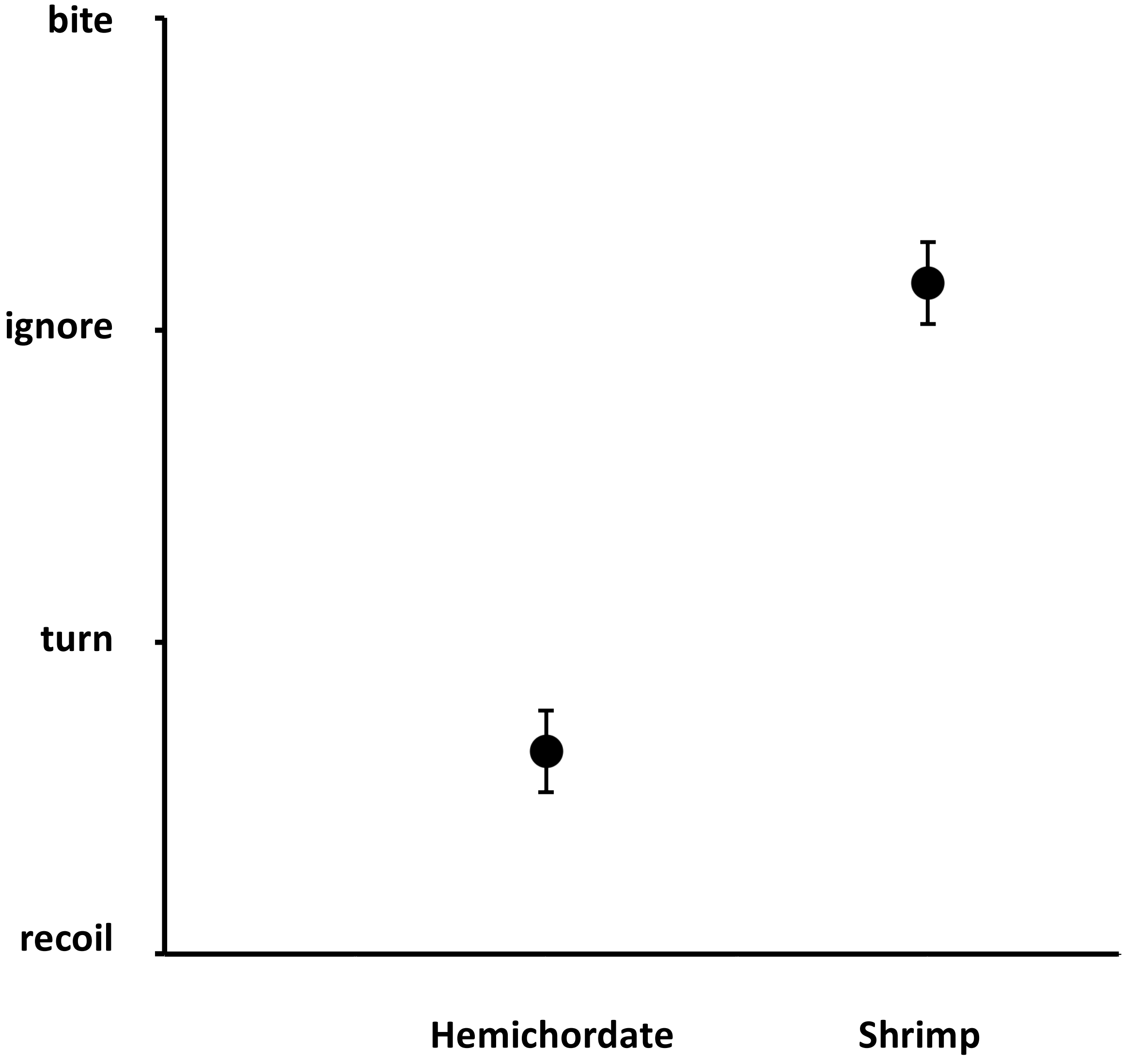

Figure 24: *Nereis* reaction to hemichordates and a palatable control. Mean (n=30) ± SE. The mean reaction of *Nereis* to hemichordate prey upon first encounter was significantly less positive than the reaction to a palatable control, shrimp ($p<0.001$).

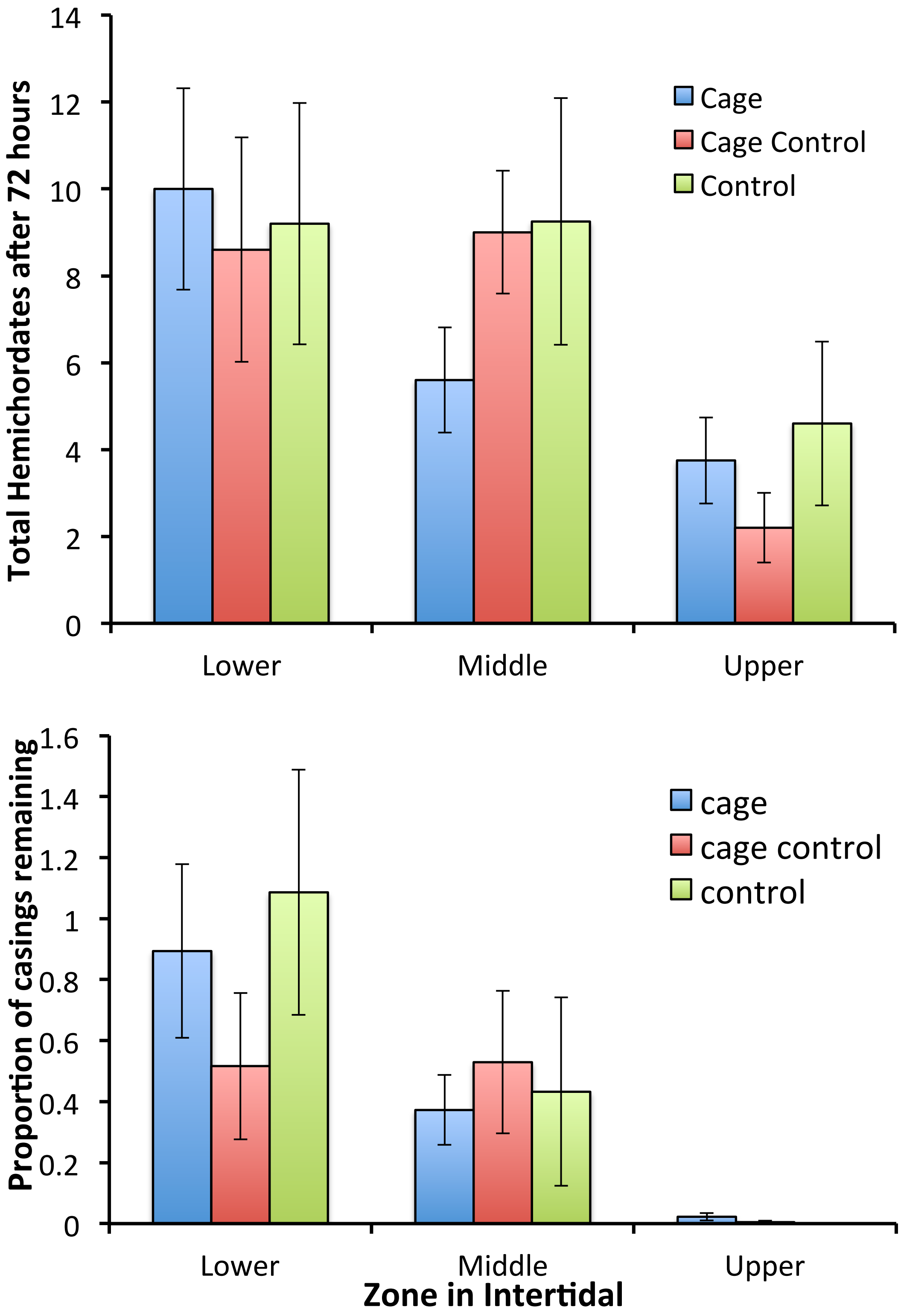

Figure 25: (A) Total hemichordates in transplants after 72 hours. Bars are means (n = 5) ± SE. There was a significant effect of zone (p = 0.003) but no effect of treatment (p = 0.720), or an interaction (p = 0.655). (B) Proportion of casings remaining after 72 hours. Singificant effect of zone (p < 0.001) but no effect of treatment (p = 0.708) or an interaction (p = 0.571).

Tables

Table 1: Univariate ANOVA for Overall Predation using raw proportion consumed. The residuals were not normally distributed, but ANOVA is robust to deviations in normality. These data suggest strong effects of both predator and prey, but no interaction. Predator includes six predators, while prey includes three species of hemichordate. Significant effects are bolded.

Factor	Type III Sum of Squares	df	Mean Square	F	P-value
Predator	15.884	7	2.269	46.009	**<.001**
Prey	.682	2	.341	6.917	**.001**
Predator * Prey	1.080	14	.077	1.564	.092

Table 2: Univariate ANOVA for Overall Predation using Align Ranked Transformed (ART) proportion consumed. This non-parametric alternative was used in concert with the standard ANOVA using raw data. Predator and prey were again considered significant, but the ART ANOVA revealed an interaction between predator and prey. The only deviation in response pattern was between the Virginia and Maine predators, suggesting the ART revealed an interaction based on predator origin. Significant effects are bolded.

Factor	Type III Sum of Squares	df	Mean Square	F	P-value
Predator	419562.753	7	59937.536	28.459	**<.001**
Prey	85293.291	2	42646.645	11.350	**<.001**
Predator * Prey	1026406.000	14	7452.415	2.043	**.016**

Table 3: Univariate ANOVA for Crustacean Choice experiments using raw proportion consumed per hour. The residuals were not normally distributed, but ANOVA is robust to deviations in normality. Predator includes only two predators, hermit crabs and green crabs, and prey includes two prey items, *S. bromophenolosus* hemichordates, and mussel tissue. These data suggest a significant effect of predator as well as an interaction but no significant effect of prey. Significant effects are bolded.

Factor	Type III Sum of Squares	df	Mean Square	F	P-value
Predator	6.954	1	6.954	276.065	**<.001**
Prey	.033	1	.033	1.320	.253
Predator * Prey	.278	1	.278	11.047	**.001**

Table 4: Univariate ANOVA for Crustacean Choice experiments using Aligned ranked transformed (ART) proportion consumed per hour. This non-parametric alternative was used in concert with a standard ANOVA using raw data. Although the F and P values were slightly different, they resulted in similar P-values, and did not alter any significance found with the raw dataset. Significant effects are bolded.

Factor	Type III Sum of Squares	df	Mean Square	F	P-value
Predator	80704.533	1	80704.533	156.688	**<.001**
Prey	512.533	1	512.533	.494	.483
Predator * Prey	9828.300	1	9828.300	10.662	**.001**