ABSTRACT

Title: INFLUENCES OF THE BIOTIC AND STRUCTURAL COMPONENTS OF *CRASSOSTREA VIRGINICA* ON THE OYSTER REEF COMMUNITY.

Karen E. Kesler, Doctor of Philosophy, 2015

Directed By: Professor Kennedy T. Paynter, Department of Biology

The hard, complex reef structure created by the eastern oyster, *Crassostrea virginica*, provides refuge and habitat that protects many organisms, allowing them to settle, survive, and spawn. In addition, oysters create copious amounts of biodeposits, which potentially serve as a basal nutrient resource for the reef ecosystem. I investigated the influence of oyster reef structure and oyster biodeposits on the reef community through a series of field experiments and mesocosm studies. Initially, the communities that colonized live oyster reefs were compared to communities that colonized empty oyster shell reefs, to evaluate the potential influence of live oysters that were actively feeding and creating biodeposits. Community assemblages on the two reef types were similar and no differences were seen with species level comparisons of abundance or biomass between the two treatments. The impact of oyster shell structure on energy

transfer up the food chain from the basal resource of oyster biodeposits to the predator, *Gobiosoma bosc*, through the amphipod, *Melita nitida* was then investigated. Oyster shell structure effectively provided protection to amphipods, with reduction of predation impacts in high complexity habitats when a predator was present. Next, stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of dominant reef species and basal resources, including oyster biodeposits, were measured seasonally to evaluate the major resource contributors to the reef. Overall, most carbon sources appeared to be pelagic in nature and an additional unidentified carbon source from outside of the oyster reef was incorporated into the food web. Finally, a $\delta^{15}N$ tracer study, utilizing biodeposits labeled with elevated $\delta^{15}N$ values, indicated that both *Melita nitida* and *Neanthes succinea* could incorporate *Crassostrea virginica* biodeposits and pass these nutrients to higher trophic levels. These studies suggested that oyster structure played a prominent role in defining the oyster reef community by providing habitat and protection for reef organisms. Mesocosm studies and isotopic analysis indicated that while some deposit feeders could consume oyster biodeposits, biodeposits were likely not a large component of their diet. Overall, these results suggest that structure was the dominant factor driving community organization on the reef, with minimal influence from oyster biodeposits.

INFLUENCES OF THE BIOTIC AND STRUCTURAL COMPONENTS OF *CRASSOSTREA VIRGINICA* ON THE OYSTER REEF COMMUNITY.

By

Karen Elizabeth Kesler

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Advisory Committee:
Associate Professor Kennedy T. Paynter, Chair
Associate Professor Jeffrey C. Cornwell
Adjunct Professor Anson H. Hines
Associate Professor Christopher M. Swan
Associate Professor William O. Lamp

Dedication

Dedicated to my parents, who always encouraged my love of science, and my siblings, who have always been there when I needed them. Your love and support has made this possible.

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Chapter 1: Introduction and Project Summary

The eastern oyster, *Crassostrea virginica* (Gmelin, 1791), is an essential species in the Chesapeake Bay ecosystem, both culturally and ecologically. Since the early 1800s, oyster populations in the Chesapeake Bay have declined an estimated 99.7% due to intense fishing pressures (Kennedy and Breisch 1983, Wilberg et al. 2011), habitat loss (Ford and Tripp 1996), water quality degradation (Kemp et al. 2005) and introduced diseases, MSX (*Haplosporidium nelson*) and Dermo (*Perkinsus marinus*) (Andrews and Hewatt 1957, Andrews and Wood 1967). Several restoration and management plans have been created over the past century in an effort to address the decrease in the oyster population in the Chesapeake Bay (Kennedy and Breisch 1983). Because of this dramatic decline in the *C. virginica* population, and the extensive investment in its restoration, it is important to understand this species' effects on the estuarine ecosystem.

In addition to its importance as a fishery, *C. virginica* also provides several ecological benefits to the Chesapeake Bay. One of the objectives of oyster restoration in the Chesapeake Bay is to restore the ecological functions of the oyster reef and help improve the health of the overall ecosystem. Oysters create habitat, including essential fish habitat (Wells 1961, Crabtree and Middaugh 1982, Coen et al. 1999, Grabowski and Powers 2004), enhance fish production (Peterson et al. 2003), filter water (Newell 1988), remove and cycle nutrients (Newell et al. 2005, Kellogg et al. 2013), and couple benthic and pelagic systems (Ulanowicz and Tuttle 1992). The ecological functions provided by the oyster stem from two important characteristics of the oyster: one, that it is a filter feeder, and two, that it creates biogenic reefs composed of hard, morphologically complex substrate.

As filter feeders, oysters are a key link between the pelagic and the benthic environments. Oysters filter seston composed of phytoplankton, flagellates, detritus, bacteria, and other suspended particles from the water column (Asmus and Asmus 1991, Bayne and Hawkins 1992). They preferentially consume certain food particles, particularly organic material, and reject the other particles as pseudofeces (Newell and Jordan 1983). Waste products from the ingested food particles are released as feces, which add organic material (Wotton and Malmqvist 2001) to the detritus pool and are available to detritus feeders as a nutrient resource (Odum and Cruz 1963). Collectively, feces and pseudofeces are known as biodeposits. As biodeposits are released, the oyster wraps them in mucus (Newell and Langdon 1996), which increases their density and allows them to settle out of the water column at a much faster rate than seston (Ostroumov 2005). They sink into the spaces between oysters and transfer energy and nutrients from the water column to the benthic community (Ulanowicz and Tuttle 1992). This process also reduces the turbidity surrounding oyster reefs, supporting seagrass and benthic algae growth (Ostroumov 2005).

Oyster biodeposits can follow a number of pathways once they are released onto the oyster reef. One possible end point for biodeposits is burial in the sediment (Haven and Morales-Alamo 1966a, Newell et al. 2002b). One study showed over the course of a month biodeposits had been incorporated into sediments to depths of 6-7 cm, however the largest quantities of biodeposits were generally found within the top 1.5 cm of sediment (Haven and Morales-Alamo 1966a). Another study showed that when large quantities of particulate organic matter (POM) overlaid sediment, simulating the presence of biodeposits, a large portion of that POM was not broken down microbially and

accumulated in the sediments (Newell et al. 2002b). This indicates the potential for biodeposit burial as well.

The nutrients from biodeposits can also be remineralized into the water column, where they can fuel additional phytoplankton growth. When POM simulating oyster biodeposits was added to sediment, nitrogen predominantly in the form of ammonium was released (Newell et al. 2002b). Ammonium release was enhanced when the sediment was anaerobic (Newell et al. 2002b), a typical environmental condition for Chesapeake Bay oyster reefs in the summer. Higher POM quantities generally led to increases in all nitrogen fluxes from aerobic and anaerobic sediment (Newell et al. 2002b). If the appropriate conditions were present, the nitrogen present in biodeposits could undergo denitrification and leave the system as nitrogen gas (Newell et al. 2002b, 2005, Kellogg et al. 2013). Clams have also been shown to increase benthic fluxes of nitrogen, specifically dissolved inorganic nitrogen in the form of NH_3, NO_2, and NO_3, leading to increased production levels (Doering et al. 1986).

Another end point for *C. virginica* biodeposits is incorporation into the food web of the oyster reef. Typically bivalves increase the amount of organic matter in sediments surrounding aggregations. This increase in organic matter is likely due to the deposition of biodeposits into the sediment (Stewart et al. 1998, Meyer and Townsend 2000, Norkko et al. 2001, Howard and Cuffey 2006). Increased levels of organic matter have been found in sediments surrounding several different kinds of bivalves including, *C. virginica*, *Margaritifera falcata*, *Dreissena* spp., and *Atrina zelandica* (Stewart et al. 1998; Meyer and Townsend 2000; Norkko et al. 2001; Howard and Cuffey 2006).

The increased nutrients in these sediments from biodeposits could potentially be incorporated into the food web through two pathways; absorption by plants and phytoplankton, or direct consumption by animals. One of the photosynthetic groups that has been shown to assimilate the nitrogen from biodeposits is microphytobenthos (MPB) (Newell et al. 2002b). MPB incorporated inorganic nitrogen from biodeposits when enough light was available for photosynthesis (Newell et al. 2002b). When MPB were not present and nitrates were added to aerobic sediments, nitrogen was lost from the system through denitrification (Newell et al. 2002b). NH_3 released from oysters through excretion and the decomposition of biodeposits can also be utilized by phytoplankton in the water column (Dame 1999). In some marine systems, bivalve biodeposits have increased the mass of benthic organic matter, which has led to seasonally increased macrophyte leaf area and increased abundance of macroinvertebrates (Reusch et al. 1994, Peterson and Heck 2001, Norkko et al. 2006). Carbon and nitrogen from biodeposits may have also enhanced densities of benthic organisms, particularly deposit feeders, located near the horse mussel *A. zelandica* (Norkko et al. 2001). In addition, zebra mussel biodeposits have been shown to serve as a food source for both native and non-native amphipods (Gergs and Rothhaupt 2008a, 2008b). Consumption of oyster biodeposits by deposit feeders has also been shown (Frankenberg and Smith 1967, Tenore and Gopalan 1974), however utilization of oyster biodeposits as a basal nutrient resource for the estuarine reef ecosystem has not been evaluated at the ecosystem level.

The potential nutritional value of bivalve biodeposits makes them a possible food source for meiofauna and macrofauna. The pseudofeces that are a component of biodeposits consist of microbes and particulate organic matter that has not been digested

(Bayne and Hawkins 1992), which can then be used by another organism. For example, organisms that live in the sediments can directly utilize the carbon present in oyster biodeposits (Dame 1999). Biodeposits also tend to have a low C:N ratio compared to seston from the water column, making them a higher quality food source (Prins and Smaal 1994). A lower C: N ratio in food resources helps reduce elemental imbalances, giving these foods greater nutritional value (Frost et al. 2002, Cross et al. 2005). Evrard et al. found that meiofauna preferred food that was rich in nitrogen, which led to the preferential consumption of food with low C: N ratios (2010).

Although few studies have evaluated the potential of *C. virginica* biodeposits as a food source, some studies with *Dreissena* spp. have documented biodeposit consumption by amphipods. *Gammarus fasciatus* and *Echinogammarus ischnus*, two amphipods species, consumed biodeposits in laboratory settings (Gonzalez and Burkart 2004). *G. fasciatus* had higher survival when it was fed biodeposits than when it was fed macrophytes and epiphytes, while *E. ischnus* displayed similar survival when it was fed either biodeposits or macrophytes and epiphytes (Gonzalez and Burkart 2004). *Gammarus roeselii*, another amphipod species, also consumed *Dreissena* spp. biodeposits (Gergs and Rothhaupt 2008a). However, the amphipod *Dikerogammarus villosus* only consumed small amounts of the biodeposit material (Gergs and Rothhaupt 2008a). These studies demonstrate the potential of bivalve biodeposits as a food source.

Field studies also observed the use of *Dreissena* spp. biodeposits as a food resource. Isotopic carbon analysis of *G. fasciatus* collected from Lake Erie supported the hypothesis that amphipods consume biodeposits (Limén et al. 2005). In addition, the stable isotope compositions of amphipods in Lake Constance, bordering Germany, were

positively related to zebra mussel production (Gergs et al. 2011). A similar correlation was seen after the invasion of *Dreissena* spp. in the Great Lakes region, where an increase in the zebra mussel population coincided with an increase in the amphipod population (Stewart and Haynes 1994). The increase in the amphipod population may have been due to the increased food supply as well as the increased habitat complexity associated with zebra mussel colonization (Gonzalez and Burkart 2004). Greenwood et al. also observed an increase in amphipod biomass when zebra mussels were present in streams, compared to gravel habitats that had similar structural complexity (2001). Stable isotope analysis of the amphipods and snails in this study indicated that zebra mussel biodeposits were one of a group of food resources utilized by these organisms (Greenwood et al. 2001).

One of the factors that may affect the utilization of biodeposits as a food resource is their availability to the community. Rates of biodeposition vary throughout the year due to the effect of environmental factors on oyster clearance rates. Oyster clearance rates are predominantly driven by temperature, but they can also be influenced by salinity, total suspended solids (TSS), and dissolved oxygen levels. For unimpaired filtration, dissolved oxygen levels need to be above 2 mg l^{-1} and salinity needs to be greater than three (Fulford et al. 2007, Cerco and Noel 2007). Clearance rates initially increase with greater TSS values up to a maximum rate at 25 mg l^{-1} of suspended solids, after which point clearance rates decrease (Fulford et al. 2007). Higher temperatures also increase clearance rates until a maximum rate is reached at 27°C (Newell and Langdon 1996), after which point higher temperatures begin to affect the physiology of the oyster reducing filtration rates (Fulford et al. 2007). These factors all vary seasonally, but

temperature seems to be the overall driving factor for oyster clearance rates (Fulford et al. 2007), making summer the peak season of biodeposit production.

With seasonal differences in biodeposit production, consumption of biodeposits may vary over the course of the year as well. In addition to seasonal differences in production, the ratio of feces to pseudofeces may vary over the course of the year as well, also causing differential consumption patterns. In horse mussels *Modiolus modiolus*, pseudofeces were only produced during the spring phytoplankton bloom (Navarro and Thompson 1997). The pseudofeces produced by the mussels had less inorganic material `than the feces and higher levels of organic carbon and nitrogen (Navarro and Thompson 1997). *C. virginica* held in flow through water from the York River, VA produced more pseudofeces than feces in August, September, and October (Haven and Morales-Alamo 1966b). Greater pseudofeces production was correlated with higher seston levels, while feces production remained relatively constant with increasing seston levels (Haven and Morales-Alamo 1966b). When *Geukensia demissa* were studied, the greatest amount of biodeposition occurred during the summer, corresponding with the highest water temperatures. There was negligible deposition over the winter (Smith and Frey 1985). This same pattern was also observed with *Mytilus edulis* (Tsuchiya 1980) and *C. virginica* (Haven and Morales-Alamo 1966b). Greater production of biodeposits in warmer months and negligible production in the winter may mean that the impacts of biodeposits as a food source may be observed more in summer months than in winter months.

Along with the production of large amounts of biodeposits, oysters also participate in the critical function of habitat creation in estuaries. As a species that has

the ability to modify the habitat of its surroundings, it have been characterized as an ecosystem engineer (Gutiérrez et al. 2003). When oyster larvae select settlement locations they prefer live oysters and oyster shells (Nestlerode et al. 2007), leading to the creation of oyster clumps and connected reef structures. An oyster clump is a structure that is formed when several larval oysters attach to the same oyster shell or live oyster, then grow and mature, resulting in a structure that contains several attached adult oysters. This clump formation provides a unique habitat with interstitial spaces available for settlement and colonization. When several of these clumps are found together, often attached to each other, this constitutes an oyster reef. This habitat has two critical characteristics that make it different from many of the other habitats available in the estuarine ecosystem: one, that it is a hard substrate that provides an appropriate surface for sessile organism attachment (Gutiérrez et al. 2003), and two, that it is a complex habitat that can provide refuge (Grabowski and Powers 2004) , spawning grounds (Crabtree and Middaugh 1982), and feeding grounds (Harding and Mann 2003) to numerous species.

Oyster reefs can facilitate an increase in diversity, abundance, and biomass of benthic fauna (Grabowski et al. 2005, Tolley and Volety 2005, Rodney and Paynter 2006). Reefs serve as essential fish habitat for crustaceans and finfish, providing a location for reproduction, feeding, and maturation of these taxa (Coen et al. 1999). In addition, reef structure baffles water movement, which encourages the settlement of food and larvae from the water column (Breitburg et al. 1995, Nestlerode et al. 2007). Benthic fauna often utilize oyster reef structure over bare mud or sand (Dauer et al. 1982, Posey et al. 1999) and the addition of oysters to mudflats increases the use of that area by other

bivalves, decapods and juvenile fish (Grabowski et al. 2005). Complex habitats such as oyster reefs potentially increase species diversity by reducing competitive interactions, allowing more species to coexist in one space (Crowder and Cooper 1982, Grabowski and Powers 2004) and by creating a variety of niches which can be occupied by many different species (Bruno et al. 2003). The expansion of an oyster reef into an area that was previously a mud flat creates new locations where sessile species can live, expanding their ranges and their realized niches (Bruno et al. 2003). This creation of habitat for sessile species is particularly important because hard substrate is often limited in estuarine environments (Meyer 1994), particularly in the Mid-Atlantic and Gulf of Mexico where oysters are the main source of hard substrate (Luckenbach et al. 2005).

Complex habitats can both help and hinder the organisms that live within these structures by providing protection, but also impeding foraging activities. Complexity can reduce both foraging efficiency and likelihood of prey ingestion by providing refuge for prey items and reducing predators' visual foraging capabilities (Bartholomew et al. 2000, Gergs and Rothhaupt 2008a). Success of prey ingestion is influenced by the likelihood of a predator-prey encounter, the ease with which a predator can capture prey (Greene 1986), and the probability that a predator will attack when prey are encountered. Structure can limit a predator's movement and increase the difficulty of prey capture (Bartholomew et al. 2000). This leads to complex structure reducing the likelihood of prey ingestion by both reducing the rate of encounters between a predator, and its prey, and by making it more difficult to capture that prey item once it is encountered.

The protection provided by structure can attract prey species to the habitat and provide them with protection that allows them to reproduce successfully, resulting in

greater prey densities than simple habitats (Diehl 1992, Gutiérrez et al. 2003). Greater prey density can be a benefit that outweighs the cost of reduced feeding efficiency due to structure (Longenecker 1993). In addition, at times structure can provide visual protection and decrease competitive interactions for foraging predators which can increase foraging success (Crowder and Cooper 1982, Winfield 1986, Grabowski and Powers 2004, Grabowski 2004). Given these advantages, even though feeding on reefs may be more challenging than feeding in simple environments, reefs are still highly utilized feeding grounds.

The ability of complex habitat to provide protection to prey species is dependent on a number of factors. The amount of cover provided by the complex habitat and the size/maneuverability of the predator both affect the survivorship of prey items, generally with greater cover resulting in greater survivorship and greater maneuverability of a predator resulting in greater mortality of the prey item (Bartholomew et al. 2000). Often the level of complexity needs to reach a specific threshold before any protection effect is experienced by the prey (Coull and Wells 1983, Gibbons 1988, Gotceitas and Colgan 1989, Bartholomew et al. 2000). In addition, the ability of a complex habitat to reduce predation pressures is not only dictated by the type of structure provided, but also by the species of the predator and prey items and how their behavior impacts likelihood of encounter and consumption. If a species that generally utilizes the pelagic environment is present in a complex benthic habitat, it can suffer higher mortality than a benthic- or demersal-oriented prey species in that habitat (Scharf et al. 2006). These factors can lead to instances where complex habitat does not provide protection to prey. A review of the protection provided by seagrasses to fish prey from fish predators found that 25% of the

time habitat did not affect the mortality of prey fishes (Horinouchi 2007). In addition Horinouchi (2007) found that the particular structure of the seagrass was more important than just the presence or absence of structure. However, generally complex structure does provide refuge for prey.

Several studies have quantified reduced consumption of prey items by predators when complex structure was present. Most of these studies have evaluated this phenomenon in submerged aquatic vegetation (SAV) habitats. In one study, the blenny *Helcogramma medium* consumed fewer meiofauna, copepods, and amphipods with increasing complexity (Coull and Wells 1983). Even at high complexities though, algae did not provide protection to polychaetes and species grouped into an 'other' taxa category (Coull and Wells 1983). *Perca fluviatilis* consumed fewer macroinvertebrates when submerged macrophytes were present than when they were absent (Diehl 1992). In another study, when three different plant structures were compared: macrophytes, emergent reeds, and water lilies, with three different fish species, it was found that there were specific plant structure-fish species responses in the protection provided to *Daphnia magna* prey. While reductions in consumption were present for all fish species, they did not all respond in the same manner to the various plant structures. These differences were likely linked to the body form of the fish and how that affected the fish's maneuverability and swimming speed. Fish with greater maneuverability were less affected by more dense plant structures (Winfield 1986).

While higher structural complexity often provided greater protection to prey items, intermediate complexities could lead to more constant growth for predators. When the protection provided by low, medium, and high densities of macrophytes to benthic

invertebrates from bluegills were compared, it was found that fish growth and survival were highest in intermediate densities (Crowder and Cooper 1982). At low densities, fish may deplete their food resources and at high densities fish may experience difficulty accessing prey, but at intermediate densities enough protection is provided to maintain the prey population and also allow access to those prey items. In this way, complexity can also benefit the predator, by maintaining a baseline prey population in the complex habitat refuge.

Bivalves, like submerged aquatic vegetation, can also reduce foraging efficiency (Longenecker 1993, Dittel et al. 1996, Posey et al. 1999, Grabowski et al. 2008), although the protection provided by this habitat has been less extensively evaluated. Zebra mussels provided protection to the amphipod species *E. ischnus* from predation by round gobies (Gonzalez and Burkart 2004), and grass shrimp preferred oyster reef habitats as refuges from mummichog predators over seagrass and shallow water refuges (Posey et al. 1999). Oyster shells have also been shown to provide protection to amphipods and mud crab megalopae from predators (Longenecker 1993, Dittel et al. 1996). In a study conducted with the simple food chain of oyster toadfish, mud crabs, and spat, predation by mud crabs on spat in a simple environment was significantly greater than in a complex reef environment both when an oyster toadfish predator was and was not present (Grabowski 2004). Oyster toadfish consumption of mud crabs was also greater on a simple reef than on a complex reef (Grabowski 2004). Oyster shell reefs have also been observed to provide protection to juvenile oysters from predatory crab species (Grabowski et al. 2008) and to hard clams and ribbed mussels from stone crabs and whelks (Hughes and Grabowski 2006).

Bivalve presence has been shown to increase abundance and, at times, biodiversity of associated communities. Higher densities and greater diversity of benthic and epibenthic communities have been associated with *A. zelandica* and mussel plots (*Dreissena* spp. and *M. edulis*) compared to bare plots (Botts et al. 1996, Ragnarsson and Raffaelli 1999, Norkko et al. 2001). However, it is still unclear if this increase in density and diversity is due to habitat structure alone, or if the presence of bivalve biodeposits enhances these bivalve habitats.

Several studies have found that the structure of a bivalve aggregation has more impact on the composition and abundance of the reef community than bivalve biodeposits (Botts et al. 1996, Stewart et al. 1998, Tolley and Volety 2005), but a few studies have found that biodeposits enhanced these habitats by attracting macroinvertebrates which use the biodeposits as a food source (Stewart and Haynes 1994, Ricciardi et al. 1997). Specifically, zebra mussel biodeposits have been shown to attract amphipods (Gergs and Rothhaupt 2008a) and increase colonization of zebra mussel druses (aggregations of attached zebra mussels) by chironomid larvae (Botts et al. 1996). Additionally, a study of *M. edulis*, found that species richness was driven by the structural properties of a mussel bed, but that the biotic activities of the mussels modified the species composition by driving changes in abundance and biomass (Norling and Kautsky 2007). Most studies evaluating the role of bivalve biodeposits have been conducted on zebra mussels in the Great Lakes region, while limited research has been performed on *C. virginica* and Chesapeake Bay systems, warranting additional investigation of these systems to determine the structuring mechanisms of these reef communities.

For my dissertation, I assessed the influences of both the structural components of the oyster as well as the impacts of their biodeposits on the reef community. I utilized several evaluation methods, including field studies, mesocosm experiments, and stable isotope analysis. Field studies and mesocosms evaluated the abundance and biomass of reef organisms in varying habitats and with differential access to biodeposits in order to assess the impact of both on the reef community. Stable isotope signatures of common reef species and basal resources were used to identify carbon sources for the reef and determine whether they included oyster biodeposits. I also used stable isotopes to determine relative trophic positions for common reef species, and as a tracer to evaluate the passage of nutrients through an oyster reef food chain.

Stable isotopes were used in these studies because they can help distinguish the origin of a food source as well as the trophic position of an organism, making them useful for food web analysis (Fry and Sherr 1984, Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999). In addition, stable isotopes reflect the incorporation of nutrients over time giving a more integrated picture of food consumption than gut content analysis (Cabana and Rasmussen 1994). The pairing of $\delta^{13}C$ and $\delta^{15}N$ stable isotope analyses allows for a thorough classification of the food web dynamics of the oyster reef (Fry and Sherr 1984). Little fractionation of $\delta^{13}C$ occurs between the source and consumer organisms (Fry and Sherr 1984), or during decomposition and consumption of plant material by microorganisms and invertebrate detritivores (Haines and Montague 1979). This makes $\delta^{13}C$ values useful in identifying carbon sources through trophic levels as values vary only ± 2‰ from food source to consumer, usually increasing 0.7-1.4‰ for each trophic level (Fry and Sherr 1984). In addition, organisms that were fed the same

diet had little variation (1-2‰) in their $\delta^{13}C$ signatures (Fry and Arnold 1982, Fry and Sherr 1984). Nitrogen isotopes effectively indicate trophic feeding position, with an average enrichment of $\delta^{15}N$ by 3.4 ± 1.1‰ for each trophic level (Minagawa and Wada 1984). This relationship tends to hold across habitats, age, and multiple forms of nitrogen excretion (Minagawa and Wada 1984). However, when using nitrogen as a determinant of isotopic position, it is important to identify the basal resource and quantify its isotopic composition, so that trophic position can be accurately assessed (Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999). Turnover time of organisms also needs to be taken into account, as smaller organisms have quicker turnover times which leads to more seasonal isotopic variation (Cabana and Rasmussen 1996).

Different carbon and nitrogen sources tend to have distinct isotopic signatures, which allows for food sources to be identified. In particular, stable isotopes can be used to distinguish between pelagic and benthic sources of organic matter since pelagic primary producers had a more depleted $\delta^{13}C$ signature than benthic primary producers (France 1995). In addition, benthic fish were found to have higher $\delta^{15}N$ values than pelagic fish (Estep and Vigg 1985).

A number of factors influence the isotopic signature of an organism. The carbon sources that are reflected in the isotope composition are typically biased toward food that was more recently consumed (Haines and Montague 1979). How quickly the isotopic composition of a consumer changes to reflect its diet is dependent on the species, the growth rate of the organism, and the tissue sampled (Fry and Arnold 1982, Estep and Vigg 1985). A change in isotopic composition of juvenile brown shrimp, for example, seemed to be linked to weight gain rather than the time elapsed (Fry and Arnold 1982).

Organism mobility and season also impact isotopic composition. Mobile species' food consumption may occur in a different location than where the organism was sampled, making food sources difficult to identify (Fry and Sherr 1984). Sessile species, on the other hand, can show localized isotopic patterns on a very small spatial scale (Fry and Sherr 1984). Changes in biogeochemical processes and diet over the year can also cause seasonal changes in the isotopic composition of organisms (Estep and Vigg 1985). Species that are generalists may have variation in their isotopic signal. Individuals of a generalist species may eat different proportions of a particular food type, resulting in variation in isotopic composition (Estep and Vigg 1985). Isotopic signatures can also be impacted by the habitat of the species. In estuaries a sharp change in composition occurs near the mouth of a river, reflecting a shift from the more depleted carbon signal of terrestrial carbon sources to the more enriched signal of marine carbon sources (Fry and Sherr 1984). When considering isotope data, all of these factors need to be considered to help with interpreting and understanding data acquired.

Questions and hypotheses

My dissertation evaluated the influence of *C. virginica* structure and biodeposits on the estuarine reef community. I conducted three studies to assess the impacts of these two characteristics of the oyster and their interactions.

My first study evaluated whether the *C. virginica* epifaunal reef community was enhanced by the presence of a live oyster. I compared species abundance, species biomass, and biodiversity of epibenthic macroinvertebrates and benthic resident reef fish between reefs created from live oyster clumps and structurally similar empty oyster shell clumps. This study asked, does the presence of a *live* oyster impact the abundance, biomass, or diversity of the oyster reef community? I hypothesized that some deposit

feeders, such as polychaetes and amphipods, may use oyster biodeposits as a food resource which may lead to an increase in their abundance or biomass on live oyster reefs. I also hypothesized that the additional nutrients may change the species composition of the community, by encouraging the growth of species' populations that utilize biodeposits as a food source. Finally, I expected that the species richness would be similar between the live oyster reefs and the empty oyster shell reefs since their structure and therefore the habitat that they were providing was similar.

My second study used mesocosms to look more closely at the influence of structure on the community. It assessed whether the complex structure of the oyster shell could impact trophic transfer. In addition, it addressed whether oyster biodeposits could support an amphipod population. I investigated the transfer of energy from biodeposits to higher trophic levels and how complexity impacted that transfer. Specifically, I evaluated the transfer of energy from oyster biodeposits to amphipods to naked gobies. Due to their prevalence on oyster reefs and their potential roles in a biodeposit based food chain, *Melita nitida* (amphipod) and *Gobiosoma bosc* (naked goby) were chosen for this study. A factorial study was conducted with two levels of complexity (high and low), two levels of predation (present and absent), and two levels of biodeposits (absent and present).

This study addressed the following questions: Can oyster biodeposits support abundance and biomass growth of an amphipod population? How does complex structure impact the transfer of energy up the food chain? What is the interaction between top-down and bottom-up effects on a small oyster reef food chain? I hypothesized that complex structure would provide protection for prey from predators, reducing the transfer of energy up the food chain, and that oyster biodeposits would

support a detritus based food chain. I also hypothesized that I would see the impacts of both bottom-up and top-down forces on the oyster reef chain.

My final study investigated whether oyster biodeposits could and were being used as a basal nutrient resource by the oyster reef community. In order to evaluate the use of biodeposits by reef species, I conducted a tracer study with oyster biodeposits that had an elevated $\delta^{15}N$ signature. I also seasonally sampled an oyster reef in the Severn River, MD to measure the communities' natural stable isotope signatures. The questions addressed by these studies were: Can oyster biodeposits be utilized as a food resource by deposits feeders and are reef communities in nature using this nutrient resource?

I hypothesized that the isotopic composition of the oyster reef organisms would demonstrate that some organisms were utilizing *C. virginica* biodeposits as a food resource. I believed that amphipods in particular would contain an isotopic signature that indicated their consumption of biodeposits. I also expected seasonal differences in the isotopic signatures of the reef organisms. Based on the mean environmental parameters of the Severn River (Table 1), low levels of biodeposit production would be expected from December to March while high levels of biodeposit production would be expected from June to September. Based on these differences in biodeposit production I expected a larger influence of biodeposits on the isotopic signature of organisms collected in early August and late September than the organisms collected in April. The nitrogen tracer study would further demonstrate that amphipods and polychaetes could consume biodeposits and that this energy could be transferred up the food chain to higher trophic organisms, such as naked gobies.

Table 1. Mean water quality seen in the Severn River from 1986 to 2012 from Maryland Department of Natural Resources's Eyes on the Bay monitoring program (Maryland Department of Natural Resources n.d.).

Month	Mean Bottom Water DO (mg l^{-1})	Mean Surface Water Salinity	Mean Surface Water Temperature (^{0}F)	Mean Secchi Depth (m)
January	10.49	9.92	38.37	1.46
February	10.57	9.90	38.38	1.56
March	9.90	8.20	44.51	1.35
April	7.75	6.98	53.91	1.04
May	5.07	6.70	64.71	0.93
June	2.41	7.23	77.28	0.84
July	2.00	8.25	81.23	0.84
August	2.77	9.58	81.21	0.90
September	3.92	10.54	75.25	1.02
October	6.41	11.94	64.95	1.19
November	8.42	11.77	53.06	1.52
December	9.34	10.39	44.00	1.24

Chapter 2: Evaluating the impact of biotic contributions of the eastern oyster, *Crassostrea virginica,* to the benthic reef community.

Introduction

Foundation species, species that modify their habitat by creating underlying structure that can be utilized by a larger community (Stachowicz 2001), are important for the creation of many marine and estuarine benthic habitats including oyster reefs, kelp forests, and seagrass beds (Bruno and Bertness 2001). The eastern oyster, *Crassostrea virginica,* is a foundation species that creates biogenic reefs that provide habitat and protection for many reef organisms. In addition, oysters are filter feeders that create copious amounts of biodeposits, which transfers nutrients from the water column to the benthic community (Ulanowicz and Tuttle 1992). While structure is known to create valuable habitat for reef species, it is unclear if the biological activities of a live oyster actively feeding and producing biodeposits enhances this habitat.

Oyster reefs provide large amounts of hard substrate and interstitial space that can facilitate an increase in diversity, abundance, and biomass in benthic fauna. Complex habitats, like *C. virginica* oyster reefs, can increase diversity by creating a variety of niches which can be occupied by many different species (Bruno et al. 2003). These habitats also reduce encounters between competing predators which reduces negative interactions, allowing the coexistence of competing species in one space (Crowder and Cooper 1982). *Crassostrea virginica* reefs provide a location for reproduction, feeding, and maturation of crustaceans and finfish, which has led to their classification as essential fish habitat (Coen et al. 1999). Oyster reefs are also critical to sessile species that need hard substrate as an attachment location. When an oyster reef expands into an area that was previously a mud flat, the range and realized niches of sessile species are extended

beyond their previous bounds (Bruno et al. 2003). The expansion of reefs also often leads to greater utilization of an area by juvenile fish, bivalves and decapods (Grabowski et al. 2005), as oyster reef structure is often utilized over bare mud or sand by benthic fauna (Dauer et al. 1982, Posey et al. 1999). In addition, water movement is baffled by reef structure, which encourages the settlement of food and larvae from the water column (Breitburg et al. 1995, Nestlerode et al. 2007). As one of the few providers of hard, complex substrate in the estuarine and coastal areas of the mid-Atlantic and Gulf of Mexico, oyster reefs are critically important to the continued existence of this habitat (Meyer 1994, Luckenbach et al. 2005).

In addition to their structural contributions, *C. virginica* are filter feeding bivalves that link the pelagic and benthic communities through the production and deposition of biodeposits. Biodeposits are comprised of phytoplankton, bacteria, and flagellates that were ingested by the oyster and released as feces and pseudofeces (Asmus and Asmus 1991, Bayne and Hawkins 1992). Feces are waste products produced after food has been assimilated while pseudofeces are material that has been passed through the oyster gill, wrapped in mucus, and then released from the oyster before any digestion has taken place (Newell and Langdon 1996). Biodeposition increases the amount of detritus, organic matter that was formerly living organisms or fecal material and its associated biota (Odum and Heald 1975), surrounding the reef. Increased levels of organic matter, likely due to biodeposition, have been observed around many species of bivalves, including, *C. virginica*, *Margaritifera falcata*, *Dreissena* spp., and *Atrina zelandica* (Stewart et al. 1998, Meyer and Townsend 2000, Norkko et al. 2001, Howard and Cuffey 2006). These

elevated levels of organic matter may contribute to changes in species abundance or biomass, which is sometimes observed around bivalves.

In particular, bivalve presence may cause changes in macroinvertebrate density. Plots associated with the horse mussel *A. zelandica* and the mussels, *Dreissena* spp. and *Mytilus edulis*, have displayed greater diversity and densities of species in the benthic and epibenthic communities than bare plots (Botts et al. 1996, Ragnarsson and Raffaelli 1999, Norkko et al. 2001). The bivalves may be providing the macroinvertebrates with a food source in the form of bivalve biodeposits, leading to these increases in abundance. The nutritional content of pseudofeces, in addition to the bacteria and digestive enzymes that are associated with the pseudofeces make them easier for consumers to assimilate, potentially making them a good food source (Izvekova and Ivova-Katchanova 1972). In addition, benthic animals have been shown to consume fecal pellets, including *C. virginica* fecal ribbons, exhibiting the importance of coprophagy in the benthic community's food web (Frankenberg and Smith 1967).

While oyster presence has been shown to increase abundance and, at times, biodiversity of species (Tolley and Volety 2005), it is still unclear if and how the presence of a live oyster enhances the habitat structure. One of the key contributions of live filter feeding bivalves to their communities is the production of biodeposits (Botts et al. 1996, Stewart et al. 1998, Norkko et al. 2001, Gonzalez and Burkart 2004, Norling and Kautsky 2007, Gergs and Rothhaupt 2008a). Biodeposits can potentially provide both a food resource to macroinvertebrates (Frankenberg and Smith 1967, Gergs and Rothhaupt 2008a) and enhance nutrient cycling (Newell et al. 2005). But in addition to this biodeposit production, the filtration process also improves the water clarity (Grizzle

et al. 2008). Greater water clarity allows for more benthic photosynthesis to occur, which can improve oxygen levels surrounding live bivalves (Ostroumov 2005). These bivalves themselves can also serve as a food resource. For oysters in particular, their spat are a target prey item for mud crabs and flatworms (Meyer 1994, Newell et al. 2007).

Colonization and recruitment of species to live oysters may be driven by the structure of the oyster, but in addition, may also be enhanced by biodeposit production, greater water clarity, or provision of oysters as a food resource. The structural component of a bivalve has been found to have the largest influence on the community's composition and abundance by many studies (Botts et al. 1996, Stewart et al. 1998, Tolley and Volety 2005), but some studies have seen enhancements of macroinvertebrate densities due to bivalve biodeposit presence (Stewart and Haynes 1994, Ricciardi et al. 1997). Amphipods and larval chironomids have been shown to be attracted to zebra mussel biodeposits (Botts et al. 1996, Gergs and Rothhaupt 2008a) and live *M. edulis* have been shown to alter species composition within a community (Norling and Kautsky 2007). The role of a live bivalve and its biodeposits in the community has predominantly been addressed in freshwater systems, and mainly in the Great Lakes region. Few studies have evaluated the contribution of live *C. virginica* to the structuring of the reef community (Tolley and Volety 2005), necessitating the need for greater investigation of this phenomenon on oyster reefs and within the Chesapeake Bay system.

This study evaluated how the oyster structure affected the estuarine reef community assemblage and if the biotic contributions and activities of a live oyster affected the response of the reef community. To assess this question, live oyster clumps and empty oyster shell clumps, with nearly identical structure to the live oyster clumps,

were used to create small reefs. These reefs were placed in the Patuxent River for 13 months, where they were colonized by reef animals. Species abundance, species biomass, and biodiversity of the reef communities of the two treatments were then determined and compared. I hypothesized that the biodeposits created by the live oysters would be utilized as a food source by deposit feeders, increasing either their biomass or abundance. I also anticipated that growth in these species would create differences in the species composition between the two treatments. While species composition may be altered, I hypothesized that I would find similar species richness between the two treatments as the habitat that was provided in the two treatments was similar.

Methods

Study Site

This study was conducted in a cove off the shore of the Patuxent River at the Solomons United States Navy Recreation Center in Solomons, Maryland (Figure 1). The cove was flanked on either side by stone breakwaters and the bottom was composed of a sandy substrate. Trays were initially placed in July 2009 on the shore side of the breakwaters, in water that ranged in depth from about 0.5 meter to 1.5 meters. Due to hydrodynamic disturbance at that depth, the trays were moved in November 2009 to deeper water on the river side of the breakwaters where water depth ranged from 1.5 to 2.5 meters.

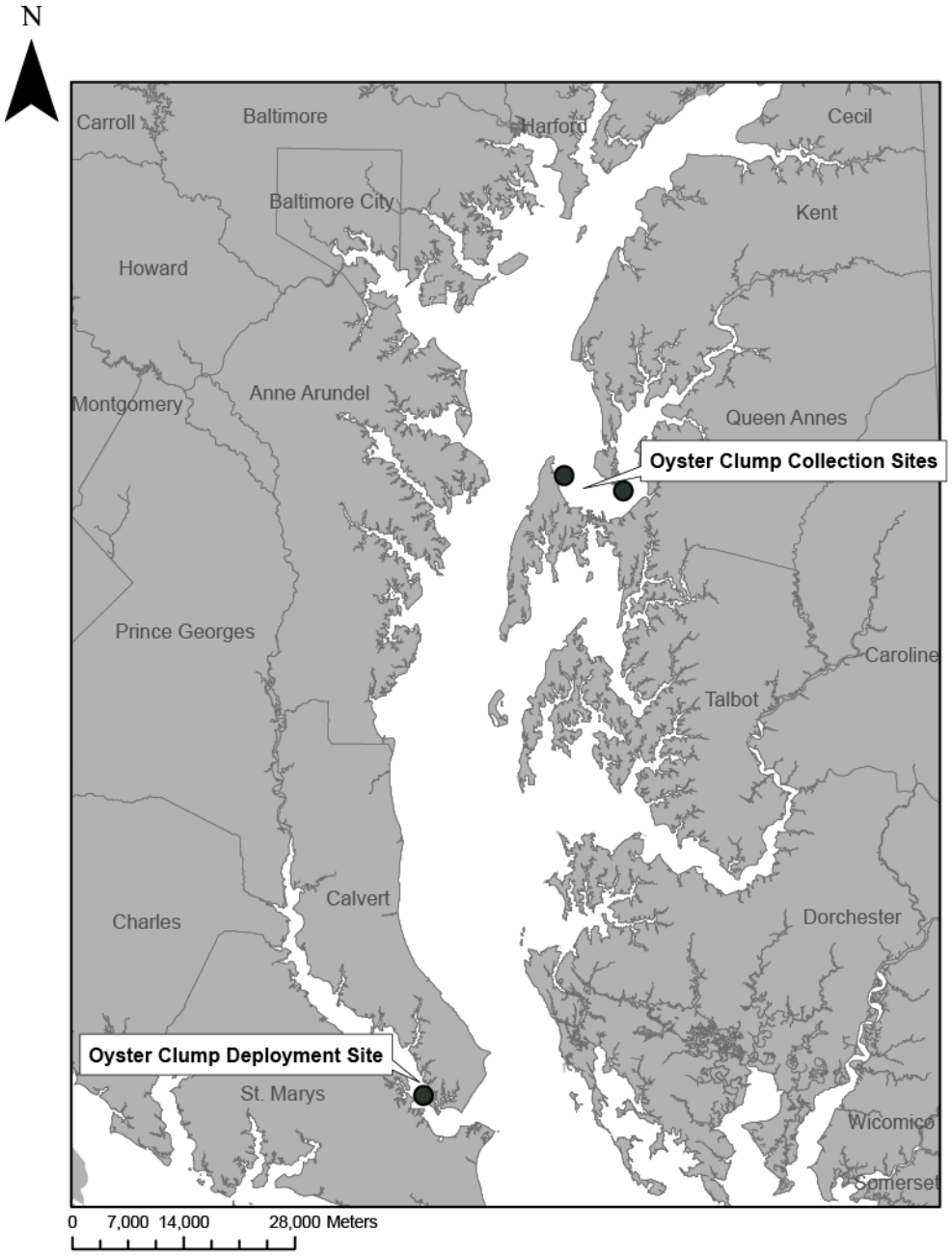

Figure 1. Location of oyster clump collection and oyster clump deployment in Chesapeake Bay tributaries.

Oyster Collection Sites

Crassostrea virginica clumps (groups of attached oysters) were collected from the lower Chester River, Maryland by divers. Specifically, 40 clumps were collected from the Hail Point managed reserve on June 25, 2009, and an additional 40 clumps were collected from the Strong Bay oyster sanctuary on July 1, 2009 (Figure 1). Clumps were

collected from the Chester River due to knowledge that older, larger oyster clumps were available from this location.

Experimental Unit Construction and Placement

Oysters located at these two sites were 4-5 years old and generally had a shell height of 100–140 mm. On average, the clumps consisted of 5-8 articulated oysters. Clumps from both locations were mixed and divided into a live oyster group and empty oyster shell group (40 clumps per treatment). Epiflora and epifauna were removed from each clump with shucking knives and brass, nylon, and stainless steel stiff bristled brushes after which clumps were rinsed with freshwater and placed into a 0.8 % domestic hypochlorite solution for two minutes to kill cryptic organisms. Clumps were then placed in freshwater for 6 minutes to rinse the oysters of hypochlorite and any remaining cryptic organisms (Newell and Jordan 1983). Oysters for the empty oyster shell treatment were carefully shucked and the oyster tissue was removed without breaking the articulated clump structure. Once the tissue was removed, the oysters' valves were glued back together with cyanoacrylate so that the live oyster and the empty oyster shell treatments had nearly identical structures and complexity. The final result was a live oyster treatment that consisted of articulated live oysters and an empty oyster shell treatment that consisted of articulated oyster shells that had their tissue removed and their valves glued together (Figure 2).

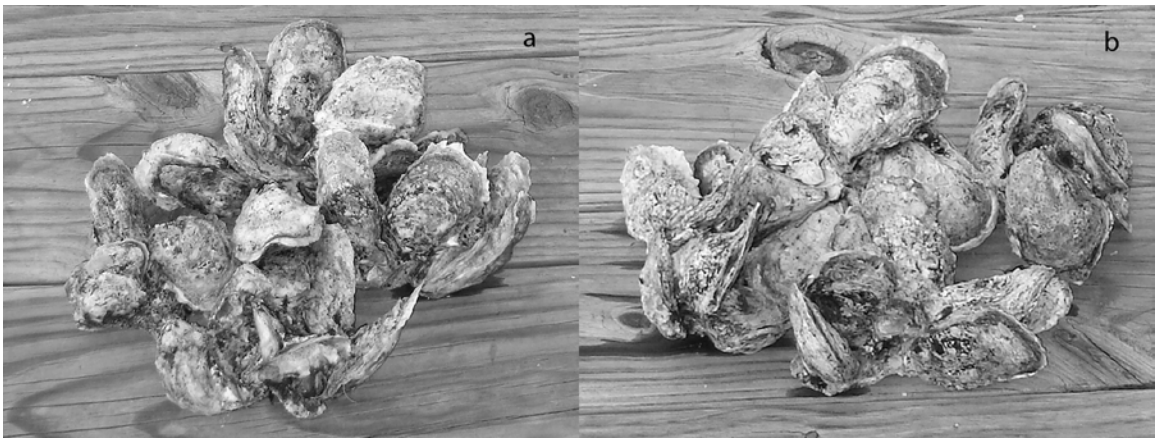

Figure 2. Examples of sets of oyster clumps used to create experimental units. a. Empty oyster shell treatment, which was created by removing the tissue from the oyster and gluing the two valves of the oyster shell together to mimic the structure of a live oyster. b. Live oyster treatment.

Experimental units consisted of a plastic bread tray (0.33 m^2) that contained holes in the sides and the bottom that allowed for water and sediments to move around and through the clumps. The trays were lined with a 1-mm fiberglass mesh that prevented motile fauna from escaping during the collection process. Four oyster clumps of the same treatment were secured to the center of a tray with a 63.5 cm by 0.86 cm plastic cable tie. Rugosity, a metric of complexity, was measured for each experimental unit and no significant difference was found between live (1.40 ± 0.04) and empty oyster shell treatments (1.38 ± 0.04) ($F=0.146$, $df=1, 18$, $P=0.707$). Rugosity was determined by running a chain across the width of the clumps, following the topography of the clumps along the way. The chain was then lifted from the clump and stretched to its full length and measured. Then the straight line distance across the width of the clump was measured. The initial value was divided by this straight line distance to give a rugosity value (this procedure was adapted from sinuosity index for freshwater stream ecology, Allan 1995).

The experimental site was a 15 m by 12 m plot that was divided into twenty 3 m^2 cells, to which treatments were randomly assigned. On June 30, 2009 and July 3, 2009 trays were deployed at the center of each cell and anchored with two horseshoe shaped pieces of reinforcing steel (0.61 m x 0.01 m). Water quality (dissolved oxygen, salinity, and temperature) was measured after the placement of the trays and each month during the course of the study using a 600 QS YSI probe.

Three trays from each treatment were randomly selected and removed on October 8, 2009 to evaluate the progress of the recruitment and colonization process and determine if any modifications were needed in the experimental design. This mid experiment assessment revealed a large accumulation of sand in the trays. In order to reduce sand accumulation, trays were relocated on February 2, 2010 to deeper water. The same grid arrangement used for the initial tray placement was replicated in the deeper waters when the trays were relocated. Trays were capped with a 1-mm mesh lid during transport to the new location in order to minimize fauna loss. On July 27, 2010 the remaining 14 trays were collected by capping the trays with 1 mm mesh lids and carrying the trays to shore.

The same procedure was used to collect organisms for the mid-experiment evaluation and for the final collection. Once trays were on shore, oyster clumps were removed from trays and surveyed for fishes. Fishes were removed and euthanized in a solution of 300 mg l^{-1} tricaine methanesulfonate buffered with sodium bicarbonate. Fishes were then placed on ice for transport to the lab where they were frozen at -20ºC. After fishes were removed, the clumps were placed into 7.57 l plastic containers filled with 70% ethanol. Any sediment or organisms in the tray were placed into the container

with the clumps. Sessile organisms that were attached to the tray were not included in the sample.

In the lab, organisms that colonized live oyster or empty oyster shells were washed from the shells onto a 63-µm mesh screen. Organisms were subsampled, sorted, identified and enumerated with the assistance of a dissecting microscope. Organisms were identified to class or order level for the mid experiment evaluation, and identified to the lowest possible taxon, which was typically species, for the final tray collection. Copepods were included in the mid experiment assessment but not included in the final assessment due to difficulty in identifying and sorting the taxa. Organisms were dried at 60°C for 72 hours to attain a dry biomass.

Statistical Analysis

Univariate Analysis

Mid experiment total abundance and total biomass data, collected in October 2009, were compared by one-way Analysis of Variance (ANOVA). In addition individual taxa abundance and biomass data were compared by one-way ANOVA with a Bonferroni adjusted alpha of .004 to account for multiple tests of significance. For all parametric tests conducted, normality of residuals was evaluated using a Shapiro-Wilk test and homogeneity of variances was evaluated with residuals graphically. For the mid experiment assessment, *actiniaria* biomass was log transformed and *gastropoda* biomass was square root transformed to meet normality assumptions.

For the final data set collected in July 2010, the total abundances and biomasses of the live oyster and empty oyster shell communities were compared using one-way ANOVA. In addition, based on the mid experiment evaluation and knowledge about feeding habits, species from the taxonomic groups of *amphipoda*, *turbellaria*, *polychaeta*,

cirripedia, and *ascidiacea*, were selected *a priori* to be compared using the final abundance and biomass data by one-way ANOVA (for normal data) or with a Kruskal-Wallis test (for non-normal data). These species were compared with a Bonferroni adjusted alpha of 0.008 for the 6 species compared for abundance and 0.007 for the seven species compared for biomass to account for multiple tests of significance. Abundances of *Apocorophium lacustre*, *Polydora* spp., *Balanus improvisus*, and *Stylochus elliptics*, were log transformed to meet assumptions of normality. Biomasses of *B. improvisus*, *Molgula manhattensis*, and *Neanthes succinea* did not meet normality assumptions and violations could not be corrected with transformations, therefore a Kruskal-Wallis test was used to make comparisons between the two treatments. SAS 9.1.3 (SAS Institute Inc. 2002) statistical program and JMP 9.0 (*JMP* 1989) statistical program were used for univariate analysis of live oyster and empty oyster shell treatment data.

Multivariate Analysis

In order to assess differences in community assemblage (by abundance and biomass) multivariate analyses were also conducted on mid experiment and final experiment data. This analysis utilized either abundance or biomass data for all of the species within a given replicate to assess differences between the two reef treatments. The community abundance and biomass data were both prepared the same way for analysis. First, abundance or biomass data of the whole community was square root transformed to minimize influences of dominant species on the community assemblage (Clarke and Warwick 2001). Bray Curtis similarity matrices were then created using this data set and the similarity matrices were then used to construct non-metric multidimensional scaling plots (MDS). These plots placed replicates in a non-dimensional space, with distances between replicates determined by their similarities to

each other. Points that were closer together were more similar than points that were farther apart. For this study, each point on this plot represented the community of one tray. MDS plots represented multidimensional data in two dimensions, so each plot was assigned a stress value that indicated how well the two dimensional plot represented the multidimensional data. A lower stress value indicated a better representation. Generally plots with a stress value below 0.2 gave a reliable representation of the data. One-way Analysis of Similarities (ANOSIM) was then conducted to determine if differences existed between the community assemblages of the two treatments at a significance level of 0.05. All multivariate analyses of community assemblage was conducted with Plymouth Routines in Multivariate Ecological Research (PRIMER) software (Clarke and Gorley 2006).

Diversity Data

Diversity of the final data set was assessed by calculating species richness and three diversity indices, the Margalef's diversity index, the Simpson index, and the Simpson evenness index. Three different indices were used because each of them gave us different information about the diversity of the community. The Margalef's diversity index represents a species richness measure that is less effected by sampling effort than a direct counting of species richness, the Simpson Index represents both the evenness and species richness of a community, and the Simpson evenness index only represents the evenness of the community, without being effected by species richness. The total number of species and the diversity indices were compared between the two treatments by one-way ANOVAs. The Margalef's diversity index (D_{Mg}) was calculated by the following equation(Clifford and Stephenson 1975, Magurran 2004):

Equation 1. $D_{Mg} = \frac{(S-1)}{lnN}$

Simpson index values were reported as D, where a greater D indicated a lower level of diversity and was calculated using the following equation (Simpson 1949, Magurran 2004):

Equation 2. $D = \sum \frac{(n_i[n_i-1])}{N[N-1]}$

The Simpson evenness index ($E_{1/D}$) was calculated with the inverse Simpson index value according to the following equation (Smith and Wilson 1996, Magurran 2004):

Equation 3. $E_{1/D} = \frac{(1/D)}{S}$

In equations 1, 2, and 3, S represents the number of species, N represents the total number of individuals, n_i represents the number of individuals in the *i*th species, and D represents the Simpson index value (Magurran 2004).

Results

Mid Experiment Evaluation

From the three trays of each treatment that were removed in October 2009 for a mid-experiment evaluation, no significant differences were observed between the community assemblages (calculated using biomass or abundance) on live oysters and empty oyster shells (ANOSIM, Biomass: Global R= -0.037, *P*=0.4, Abundance: Global R=0, *P*=0.5). Mean total abundance (± SE) on live oysters was 29570 ± 7516 and mean total abundance on empty oyster shells was 52133 ± 18394. There was no significant difference between the two treatments (ANOVA, F=1.289, df=1,4, *P*=0.320). Mean total dry biomass (± SE) of organisms collected on live oysters was 9.3070 ± 3.1033 g and 9.8465 ± 2.8527 g on empty oyster shells. The two treatments were not significantly different from one another (ANOVA, F=0.016, df=1,4, *P*=0.904). When abundances and

biomasses of individual taxa were compared, they were also similar between live oyster and empty oyster shell treatments, except *cirripedia* which had a significantly greater biomass on empty oyster shells than on live oysters (ANOVA, df=1,4, P>0.004 for all taxa except *cirripedia*, Figure 3, *cirripedia*: F=36.44, df=1,4, P=0.004, Figure 3b). In addition, similar taxa were found on both treatments.

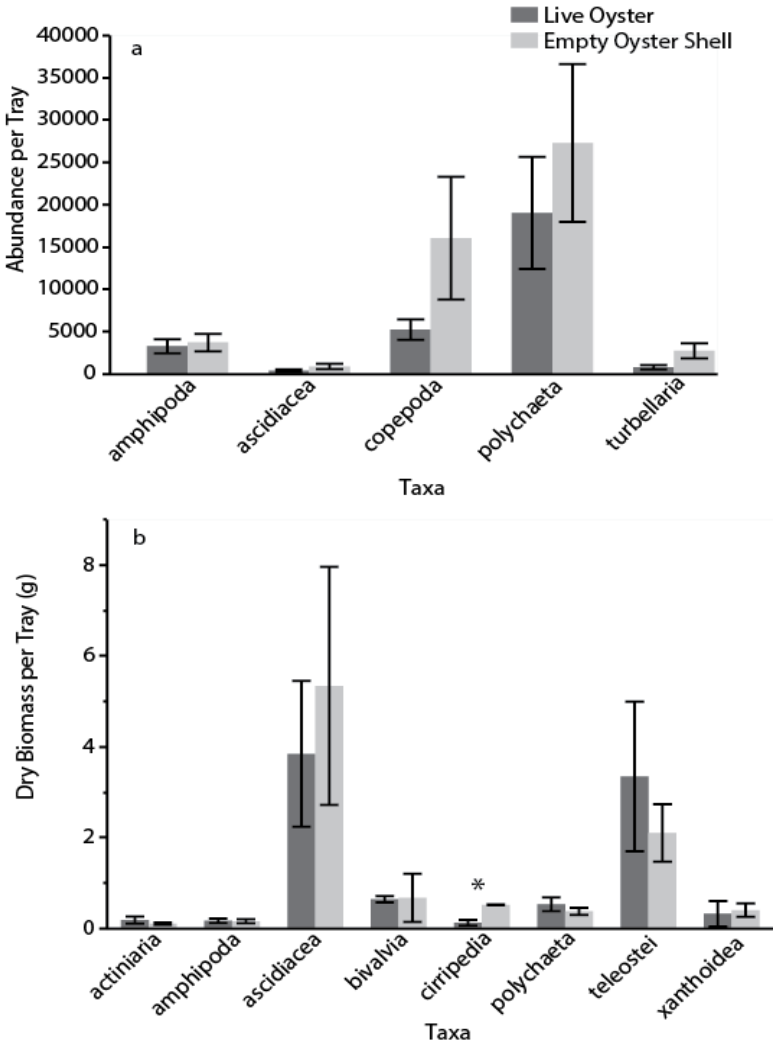

Figure 3. Mean (±SE) abundance and dry biomass (g) of dominant taxa on live oyster and empty oyster shell treatments collected in October 2009 after 3 months of recruitment and colonization. a. Mean abundance of dominant taxa (≥1% relative abundance). n_{live}=3 n_{shell}=3. b. Mean dry biomass for dominant taxa (≥1% relative biomass). n_{live}=3 n_{shell}=3. * indicates significance at P=0.004 (due to Bonferroni adjustments)

Final Collection Analysis - Abundance Data

The remaining seven trays of each treatment were collected in July 2010 and analyzed for the final data set. The benthic community assemblage (calculated with abundance) associated with empty oyster shell reefs and associated with live oyster reefs were not significantly different (ANOSIM, Global R= 0.123, *P*=0.104, Figure 4a). Mean total organism abundance (± SE) on live oysters, 25,572 ± 3,601 organisms per tray, was not significantly different from the mean total organism abundance on empty oyster shells, 18,779 ± 2,499 organisms per tray (ANOVA, F=2.4, df=1,12, *P*=0.147, Figure 5). The dominant species by abundance, defined as a species composing greater than or equal to 1% of the total abundance of all reef organisms, were *A. lacustre, B. improvisus, Melita nitida, N. succinea, Polydora* spp., and *Stylochus ellipticus* (Table 2). Abundances of *A. lacustre, M. nitida, N. succinea, Polydora* spp., *B. improvisus,* and *S. ellipticus,* were also not significantly different between live and empty oyster shell treatments when individual ANOVAs were conducted (ANOVA, Bonferroni adjusted alpha 0.008, df= 1,12, *P*>0.008, Figure 6). While not statistically significant, *A. lacustre* did display a marginal difference between the two treatments. Mean abundance (± SE) of *A. lacustre* for live oyster treatments was 12171 ± 2190 and the mean abundance for empty oyster shell treatments was 6488 ± 978 (ANOVA, F=6.16, df=1,12, *P*=0.029 (Bonferroni adjust alpha = 0.008)).

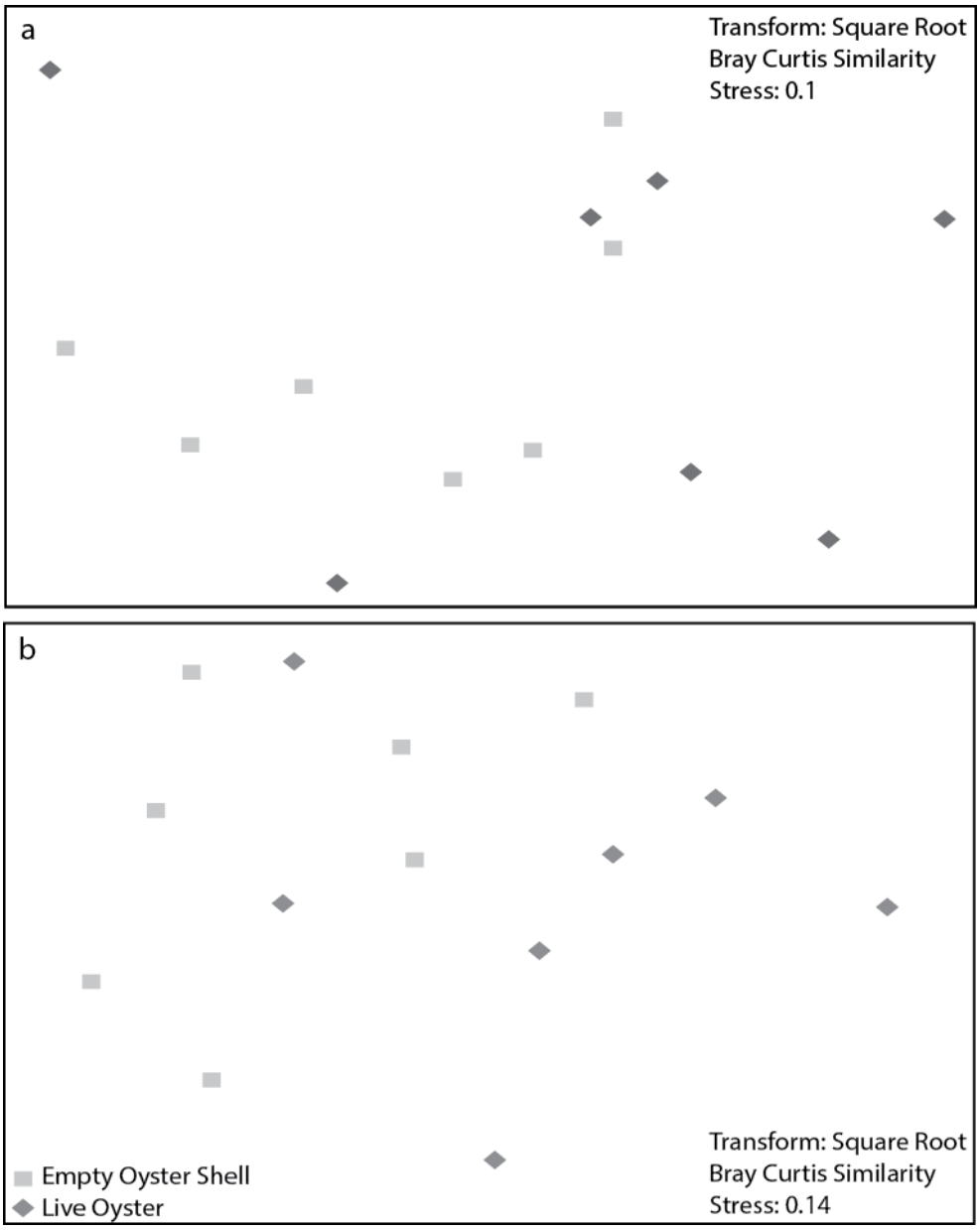

Figure 4. Non metric multidimensional scaling plots of square root transformed abundance or biomass data for live oyster and empty oyster shell treatments collected in July 2010 after 13 months of recruitment and colonization. a. Non metric multidimensional scaling plot of square root transformed abundance data. $n_{live}=7$ $n_{shell}=7$. b. Non metric multidimensional scaling plot of square root transformed biomass data. $n_{live}=7$ $n_{shell}=7$. Live oyster samples are represented by dark gray diamonds and empty oyster shell samples are represented by light gray squares. ANOSIM analysis indicated that treatments were not significantly different.

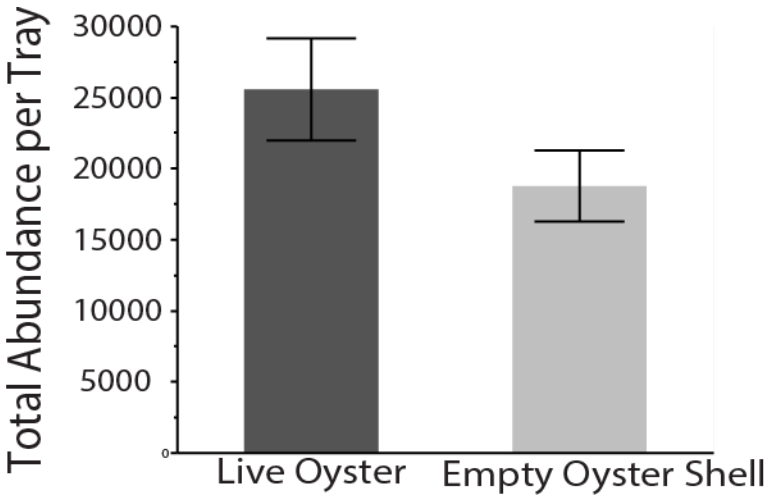

Figure 5. Mean total abundance ± SE of all organisms collected from live oyster and empty oyster shell treatments. Total abundance measured after 13 months of recruitment and colonization. Treatments were not significantly different. $n_{live}=7$ $n_{shell}=7$

Table 2. Mean abundance ± SE per tray and relative abundance per tray for dominant species on live oyster and empty oyster shell treatments ($n_{live}=7$, $n_{shell}=7$). Samples collected in July 2010 after 13 months of recruitment and colonization.

Species	Live Oyster		Empty Oyster Shell	
	Abundance	Relative Abundance	Abundance	Relative Abundance
Apocorophium lacustre	12,171 ± 2,190	0.48	6,488 ± 978	0.35
Polydora spp.	6,854 ± 1,761	0.27	5,475 ± 1,620	0.29
Neanthes succinea	4,288 ± 429	0.17	4,332 ± 442	0.23
Balanus improvisus	796 ± 106	0.03	841 ± 137	0.04
Melita nitida	759 ± 195	0.03	665 ± 56	0.04
Stylochus ellipticus	228 ± 65	0.01	303 ± 91	0.02

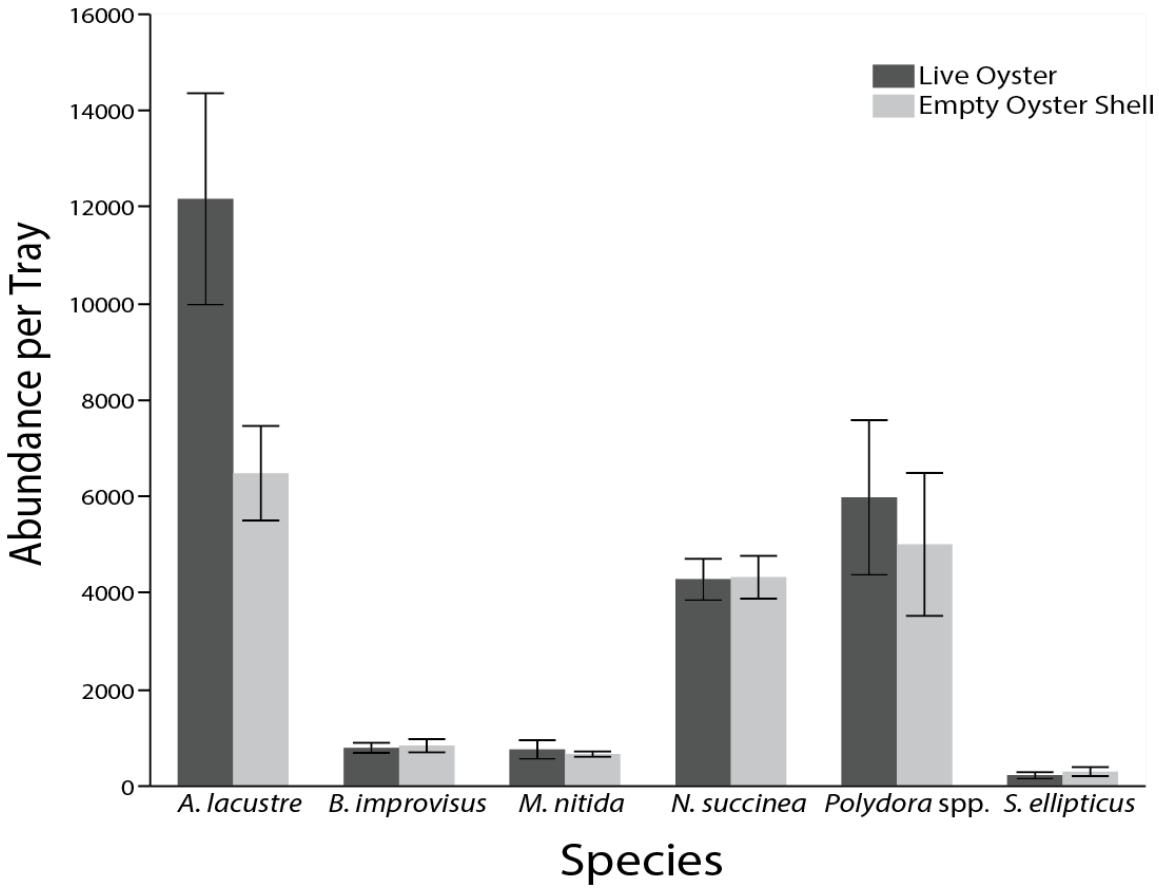

Figure 6. Mean abundance of dominant species on live oyster and empty oyster shell treatments. Abundance from trays removed in July 2010, after 13 months of recruitment and colonization. Values are abundance per tray ± standard error. Treatments were not significantly different. $n_{live}=7$ $n_{shell}=7$

Final Collection Analysis - Biomass Data

Biomass of organisms from the final seven trays collected in July 2010 were compared between the live oyster treatment and the empty oyster shell treatment. The benthic community assemblages (calculated with biomass) associated with empty oyster shell treatments and associated with live oyster treatments were not significantly different from each other (ANOSIM, Global R= 0.143, *P*=0.096, Figure 4b). The mean total dry biomass (±SE) for the live oyster treatment was 15.4505 ± 1.2672 g and the mean total dry biomass for the empty oyster shell treatment was 18.7172 ± 2.4291 g, which were not significantly different (F=1.41, df=1,12, *P*=0.258, Figure 7). The dominant species by

biomass, as defined as a species comprising greater than or equal to 1% of the total biomass, were *A. lacustre, B. eburneus, B. improvisus, Chasmodes bosquianus, C. virginica, Diadumene leucolena, Eurypanopeus depressus, Gobiesox strumosus, Gobiosoma bosc, Ischadium recurvum, M. nitida, M. manhattensis, Nassarius vibex, N. succinea, Opsanus tau, Polydora* spp., and *Rhithropanopeus harrisii* (Figure 8, Table 3). Biomasses of the individual species *A. lacustre, B. eburneus, B. improvisus, M. nitida, M. manhattensis, N. succinea,* and *Polydora* spp. were not significantly different between the live oyster and empty oyster shell treatments (ANOVA and Kruskal-Wallis, Bonferroni adjusted alpha 0.007, df=1,12, $P > 0.007$, Figure 8). However, while not statically significant, the biomass of *M. manhattensis* was marginally greater on the empty oyster shell treatment, with a mean biomass (±SE) of 0.3401 ± 0.1664 for the live oyster treatment and a mean biomass of 1.9008 ± 0.5630 for the empty oyster shell treatment (Kruskal-Wallis, H = 6.876, df=1, P=0.009, Figure 8 (Bonferonni adjusted alpha: 0.007)).

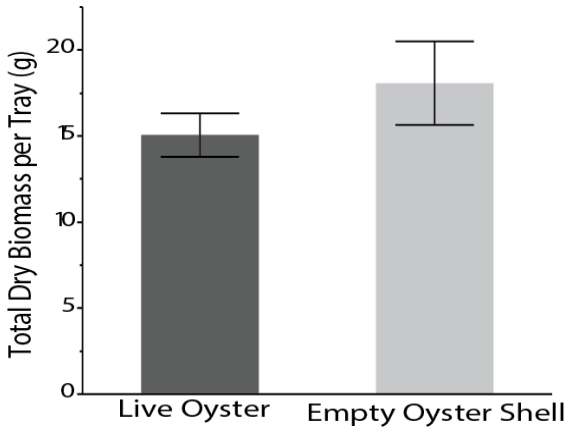

Figure 7. Mean total dry biomass (g) of all organisms collected from live oyster and empty oyster shell treatments. Total dry biomass measured after 13 months of recruitment and colonization. Values are mean total dry biomass per tray ± standard error. Treatments were not significantly different. $n_{live}=7$ $n_{shell}=7$

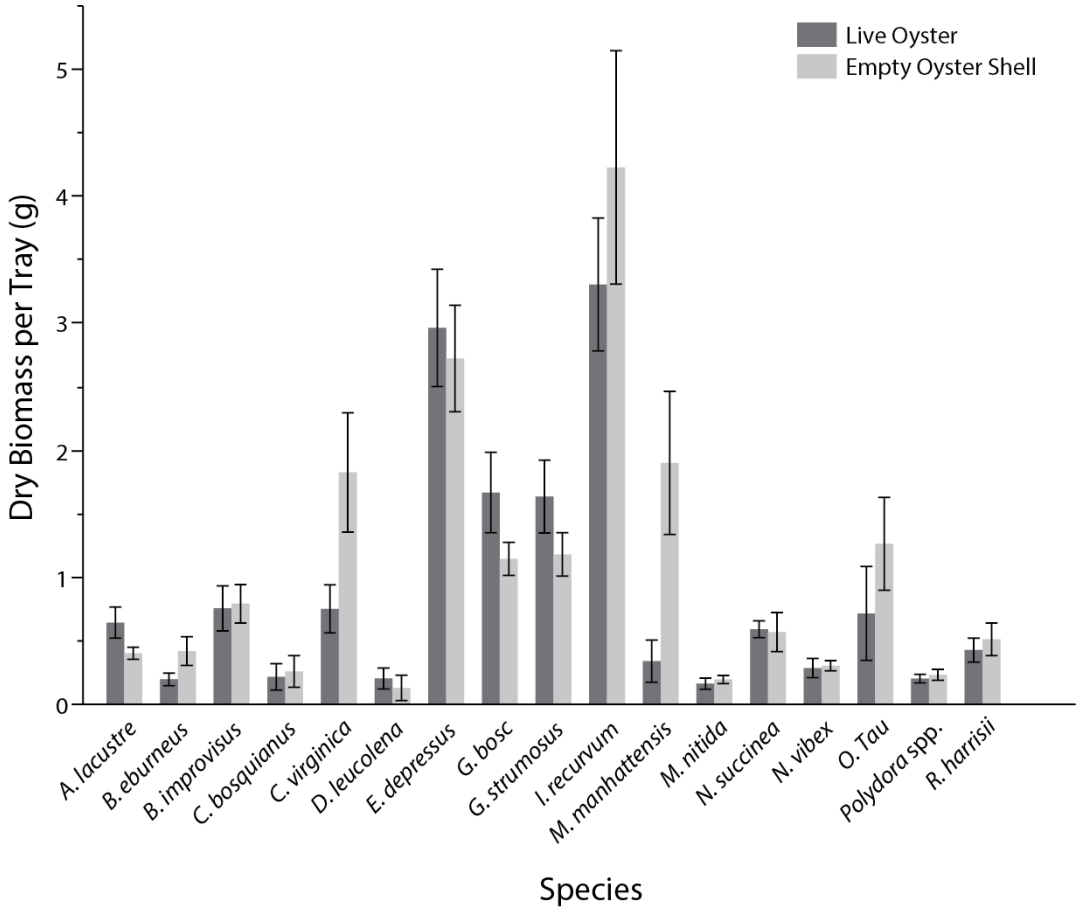

Figure 8. Mean dry biomass (g) of dominant species on live oyster and empty oyster shell treatments. Dry biomass from trays removed in July 2010, after 13 months of recruitment and colonization. Values are mean dry biomass per tray ± standard error. There were no significant differences for any of the species. $n_{live}=7$ $n_{shell}=7$

Table 3. Mean dry biomass (g) ± SE per tray and relative biomass per tray for dominant species on live oyster and empty oyster shell treatments (n_{live}=7, n_{shell}=7). Samples collected in July 2010 after 13 months of recruitment and colonization.

Species	Live Oyster		Empty Oyster Shell	
	Dry Biomass (g)	Relative Biomass	Dry Biomass (g)	Relative Biomass
Apocorophium lacustre	0.643 ± 0.122	0.04	0.401 ± 0.047	0.02
Balanus eburneus	0.196 ± 0.050	0.01	0.418 ± 0.113	0.02
Balanus improvisus	0.755 ± 0.177	0.05	0.791 ± 0.152	0.04
Chasmodes bosquianus	0.215 ± 0.104	0.01	0.259 ± 0.125	0.01
Crassostrea virginica	0.751 ± 0.189	0.05	1.826 ± 0.469	0.10
Diadumene leucolena	0.203 ± 0.082	0.01	0.129 ± 0.100	0.01
Eurypanopeus depressus	2.964 ± 0.463	0.19	2.723 ± 0.419	0.15
Gobiesox strumosus	1.635 ± 0.287	0.11	1.180 ± 0.170	0.06
Gobiosoma bosc	1.667 ± 0.838	0.11	1.145 ± 0.129	0.06
Ischadium recurvum	3.306 ± 0.523	0.21	4.228 ± 0.919	0.23
Melita nitida	0.162 ± 0.044	0.01	0.195 ± 0.032	0.01
Molgula manhattensis	0.340 ± 0.166	0.02	1.901 ± 0.563	0.10
Nassarius vibex	0.285 ± 0.076	0.02	0.303 ± 0.041	0.02
Neanthes succinea	0.591 ± 0.066	0.04	0.568 ± 0.154	0.03
Opsanus Tau	0.715 ± 0.371	0.05	1.264 ± 0.366	0.07
Polydora spp.	0.202 ± 0.034	0.01	0.231 ± 0.043	0.01
Rhithropanopeus harrisii	0.427 ± 0.095	0.03	0.512 ± 0.129	0.03

Final Collection Analysis - Diversity Data

Thirty-eight different species were identified on empty oyster shell reefs and 32 different species were identified on live oyster reefs (Table 4). The community found on the live oyster treatments had a mean species richness (±SE) of 24 ± 1 and empty oyster shell treatments had a mean species richness of 25 ± 1, demonstrating no significant difference in the number of species between the two treatments (ANOVA, F= 2.82, df= 1, 12, *P*=0.119). However, Margalef's diversity index, Simpson's Index, and the Simpson's Evenness Index each showed a significant difference between the two treatments. The live oyster treatment had a mean Margalef's diversity index (±SE) of 2.24 ± 0.07, a mean Simpson index (± SE) of 0.33 ± 0.02, and a mean Simpson evenness

index (±SE) of 0.12 ± 0.02. The empty oyster shell treatment had a mean Margalef's diversity index of 2.50 ± 0.08, a mean Simpson index of 0.27 ± 0.01, and a mean Simpson evenness index of 0.16 ± 0.01 (Figure 9). These values indicated higher diversity on empty oyster shell treatments (ANOVA, Margalef's diversity F=5.85, df=1,12, *P*=0.032, Simpson Index F=6.65, df= 1,12, *P*=0.024, Simpson evenness index F=8.05, df=1, 12, *P*=0.015).

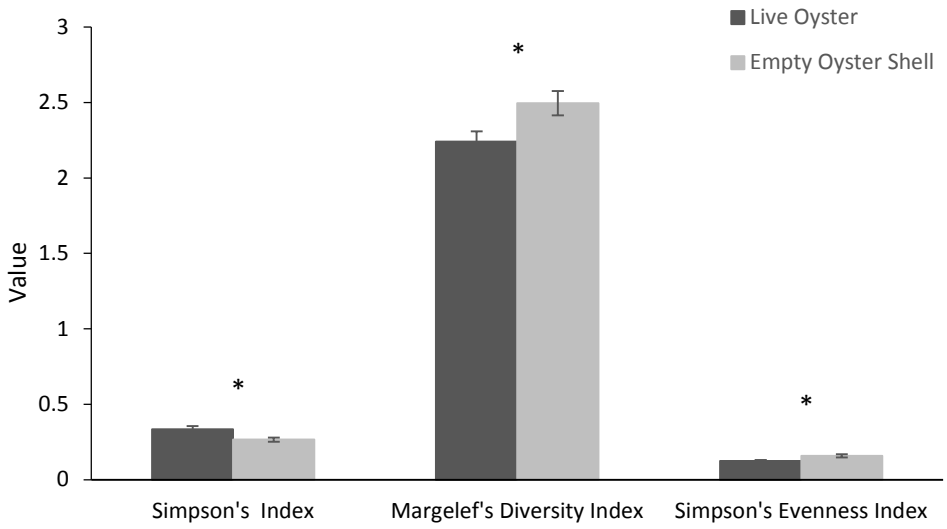

Figure 9. Mean Simpson Index, Margelef's Diversity Index, and Simpson's Evenness Index for the live oyster and empty oyster shell treatments. Error bars are standard error. n_{live}=7 n_{shell} =7. * indicates significance at P=.05.

Table 4. List of all taxa identified on live oyster and empty oyster shell treatments.

Order	Species	Common Name	Live Oyster	Empty Oyster Shell
Aciculata	*Neanthes succinea*	Clam worm	X	X
Actiniaria	*Diadumene leucolena*	Ghost anemone	X	X
Amphipoda	*Apocorophium lacustre*	Scud	X	X
	Melita nitida	Scud	X	X
	Mucrogammarus mucronatus	Spined-back scud		X
Batrachoidiformes	*Opsanus Tau*	Oyster toadfish	X	X

Table 4 (cont). List of all taxa identified on lived oyster and empty oyster shell treatments.

Decapoda	*Callinectes sapidus*	Blue crab		X
	Decapoda zoea	Larval decapod	X	
	Eurypanopeus depressus	Depressed mud crab	X	X
	Palaemonetes pugio	Daggerblade grass shrimp	X	X
	Rhithropanopeus harrisii	White-tipped mud crab	X	X
	Xanthidae	Xanthid crabs		X
Gobiesociformes	*Gobiesox strumosus*	Skilletfish	X	X
Haplotaxida	*Tubificidae imm. without capilliform chaetae*	Oligochaete		X
Isopoda	*Edotea triloba*	Isopod		X
Littorinimorpha	*Littoridinops tenuipes*	Henscomb hydrobe		X
Myoida	*Mya arenaria*	Soft shell clam	X	X
Mytiloida	*Geukensia demissa*	Ribbed mussel	X	X
	Ischadium recurvum	Hooked mussel	X	X
	Mytilidae	Salt water mussel	X	X
Neogastropoda	*Nassarius vibex*	Bruised nassa	X	X
Nudibranchia	*Doridella obscura*	Doridacean nudibranch	X	X
Ostreoida	*Crassostrea virginica*	Eastern oyster	X	X
Paleonemertea	*Carinoma tremaphoros*	Nemertean worm		X
Perciformes	*Chasmodes bosquianus*	Striped blenny	X	X
	Gobiosoma bosc	Naked goby	X	X
Phyllodocida	*Eteone heteropoda*	Polychaete	X	X
Pleurogona	*Molgula manhattensis*	Sea squirt	X	X
Polycladida	*Euplana gracilis*	Flatworm		X
	Stylochus ellipticus	Oyster flatworm	X	X
Sessilia	*Balanus ebruneus*	Ivory barnacle	X	X
	Balanus improvisus	Bay barnacle	X	X
Spionida	*Polydora cornuta*	Mud worm	X	X
	Polydora spp.	Mud worm	X	X
	Polydora websteri	Oyster mudworm	X	X
	Streblospio benedicti	Polychaete	X	X
Tanaidacea	*Hargeria rapax*	Tanaid	X	X

Table 4 (cont). List of all taxa identified on lived oyster and empty oyster shell treatments.

Veneroida	*Gemma gemma*	Amethyst gem clam	X	X
	Macoma balthica	Baltic clam	X	X
	Mytilopsis leucophaeata	Dark false mussel	X	

Discussion

The number of species, total abundance, and total biomass of the benthic macroinvertebrate and resident fish community found on live oyster reefs did not significantly vary from the community found on the empty oyster shell reefs (composed of articulated shells with oyster tissue removed) for either the mid experiment evaluation or the final data collection (Figure 5, Figure 7). In addition, multivariate comparisons of the community assemblage by abundance and biomass showed that the two treatments were similar (Figure 4). This indicated that structure was the defining characteristic of the community and that overall living oysters, including their biodeposit production and deposition, had little enriching effect on the oyster reef community. In addition, species level comparisons of reef organisms did not display a significantly enhanced response to the biotic presence of the oyster (Figure 6, Figure 8). However, a small subset of species displayed marginal changes in response to live oysters; *A. lacustre* increased in abundance and *M. manhattensis* decreased in biomass. In addition, *cirripedia* had significantly greater biomass on empty oyster shells when evaluated mid experiment (Figure 3b). The marginal change in abundance of *A. lacustre* effected the diversity of the communities, with the Margalef's diversity index, the Simpson's index, and the Simpson's Evenness Index all indicating higher diversity on the empty oyster shell treatment, even though there was no significant difference in species richness.

Several past studies that have compared the benthic communities on live bivalves to those on bivalve structure have also found little enhancement to the community diversity (Norling and Kautsky 2007), densities (Botts et al. 1996, Tolley and Volety 2005), and biomass (Tolley and Volety 2005) with the presence of a live bivalve. These studies were conducted with a variety of bivalve species, including *C. virginica, M. edulis,* and *Dreissena* spp., displaying how bivalve structure was important for community development across a number of systems. Oyster reef structure, in particular, provides many benefits to the oyster reef community which may be why structure was the dominating factor influencing community assemblage in this study. Motile species use oyster reefs for protection, foraging, and spawning while sessile species use the substrate as an attachment location and for protection (Wells 1961, Crabtree and Middaugh 1982, Meyer and Townsend 2000, Boudreaux et al. 2006, Hughes and Grabowski 2006). Spatial refuges created by the reef provide protection from predation to macrofauna and meiofauna, allowing for an increase in these organisms densities (Dauer et al. 1982, Stewart et al. 1998). These increases in prey populations can in turn enhance overall fish production (Peterson et al. 2003), which can enhance ecosystems outside of the reef community as transitory fish will feed on the reef and benefit from its provision of prey (Harding and Mann 2003).

One of the ways that a live oyster may have impacted the reef community would have been through biodeposit production, although this did not appear to largely influence the community. The limited impact of oyster biodeposits on the oyster reef community may be due to the eutrophic nature of the Chesapeake Bay. Posey et al. (2006) found nutrient addition to have little impact on estuaries that already had high

nutrient levels. Perhaps, since this system was nutrient saturated for part of the year (Karrh et al. 2007), additional nutrients from biodeposits did not have a large effect on the reef community. The biodeposits may have also been incorporated into the ecosystem in a manner that did not directly affect the abundance or biomass of the reef organisms. Biodeposits can be incorporated into the sediment, resulting in their subsequent burial and the nutrients from biodeposits can also be remineralized into the water column (Haven and Morales-Alamo 1966a, Newell et al. 2002a). If the biodeposits produced by these oysters went through one of these pathways and were not consumed or utilized by the reef organisms, than this may be why no difference was seen between the empty oyster shell and the live oyster communities.

In my treatments, while a similar number of species was found between the live oysters and empty oyster shells, the diversity of these two communities was impacted, most likely due to a shift in the evenness of the communities. In both the live and empty shell communities, *A. lacustre* was the dominant species, but it displayed greater dominance in the live oyster treatments than it did in the empty oyster shell treatments, composing 48% and 35% of the total number of organisms, respectively. The greater dominance of this species in the live oyster treatment affected the diversity of this community by lowering its evenness (Hixon and Menge 1991, Magurran 2004). This shift in evenness was displayed by a lower Simpson's evenness index, higher Simpson's index, and lower Margalef's diversity index. So even though a similar number of species was present in both treatments, the live oyster presence caused a shift in the composition of the community, with *A. lacustre* displaying greater dominance in the live oyster community.

Individual analysis of a few species selected *a priori* showed that a majority of the taxa were present in equal abundance and biomass for the two treatments. No species at the end of the yearlong experiment showed a significant difference between the two treatments, but this is likely due the conservative statistics that were conducted. A few species displayed trends that may indicate an ecological response to the presence of a live oyster. Norling and Kautsky (2007) found similar results, observing species specific responses to the presence of a live bivalve, some positive and some negative. In my study, after the yearlong experiment, *A. lacustre* responded positively to the biotic input of the Eastern oyster with almost twice the mean number of individuals on live oysters than on empty oyster shells (Figure 6). Biomass analysis showed a negative response of *M. manhattensis* which had a 5.6 times greater biomass on empty oyster shells than on live oysters, respectively (Figure 8). *Cirripedia,* over the initial three month colonization period, also had a negative response to live oysters (Figure 3b), however this difference was not seen with the final collection data.

Apocorophium lacustre, a tube dwelling epibenthic amphipod (Crawford 1937, Grigorovich et al. 2008), may have benefited from the presence of a live oyster because of the biodeposits produced by the live oyster. They may have used biodeposits as a food resource or as a building material for their protective tube structures. A similar species, *Apocorophium acutum*, gained significant protection from predation when it was allowed to form tubes from benthic material, such as detritus, and body secretions (Bousfield 1973), versus when it was not (Armsby and Tisch 2006). *Apocorophium lacustre* likely builds its tubes in a similar fashion, so the greater amount of benthic material present around live oysters may assist *A. lacustre* in its tube construction, protecting the species

from predation and elevating its abundance on live oyster reefs versus empty oyster shell reefs. This species may also feed on biodeposits since it consumes detritus (Walsh 1974), which may also lead to a greater abundance on live oysters.

The colonization or growth of *M. manhattensis* over the course of the year and *cirripedia* colonization or growth over the first three months of the study may have been affected by the presence of a live oyster as they both had lower biomasses on live oyster treatments than on empty oyster shell treatments. The biomass of *M. manhattensis* was not significantly different between the two treatments due to the conservative alpha needed for multiple tests of significance, but there was a trend towards lower biomass on live oysters. Biomass of *cirripedia* was significantly lower on live oysters than on empty oyster shells at the mid experiment evaluation. This lower biomass may be due to the ability of adult *C. virginica* to entrain pelagic larvae and competition between these filter feeding species. Adult oysters can filter pelagic larvae from the water column as they are feeding, and will often consume those larvae (Tamburri and Zimmer-Faust 1996). Even if the larvae are not consumed and released as pseudofeces, they generally cannot escape the mucus coating of the pseudofeces and will also die (Tamburri and Zimmer-Faust 1996). Predation has been shown to be an important influence on barnacle distribution in past studies (Gaines and Roughgarden 1987), and may be playing an influential role here. *M. manhattensis* and *cirripedia* both have pelagic larval stages (Jones and Crisp 1954, Costlow and Bookhout 1957, Costello and Henley 1971) that can potentially be consumed by adult *C. virginica*. Since empty oyster shells provide the settlement substrate for these species without the risk of consumption, more larvae may survive to settle on the empty oyster shell treatment than the live oyster treatment, leading to greater

biomasses on empty oyster shells than live oysters. *M. manhattensis* and *cirripedia* had a greater abundance on empty oyster shells than on live oysters, which suggests this process may have influenced the lower biomass on live oyster reefs. *M. manhattensis* (Randløv and Riisgård 1979), *cirripedia* and *C. virginica* are also all filter feeders that may compete with each other for food resources, leading to lower tunicate or barnacle biomass on the live oyster treatment.

Overall, my results have shown that the presence of a live oyster does not greatly effect species richness, abundance, and biomass. It also did not affect the overall community assemblage. This may indicate the importance of oyster habitat, particularly to reef dwelling species that are faced with a shortage of hard, complex substrate to serve as adequate habitat for refuge, feeding and spawning. In addition, oyster biodeposits were likely not being utilized by the majority of reef species. A few species displayed a marginal response to the presence of a live oyster, with greater *A. lacustre* abundances and lower *M. manhattensis* biomasses, but generally the presence of a live oyster did not affect species abundance and biomass differently than empty oyster shell structure in the *C. virginica* reef system.

Chapter 3: Evaluating the impact of complex structure on trophic transfer.

Introduction

The eastern oyster, *Crassostrea virginica*, creates biogenic reefs that are composed of interconnected oysters, creating a complex habitat that can impact the organisms that utilize the reef in both a positive and negative manner. The reef provides protection from predators and a safe location for spawning and growth, but it also reduces foraging efficiency making it more challenging to acquire food. Foraging efficiency is dependent upon the likelihood of a predator-prey encounter, the likelihood of a predator attack, and the ability of a predator to capture a prey item (Greene 1986). Complex habitats such as oyster reefs, can interfere with the foraging process by reducing the likelihood of a predator and prey encountering each other, as well as making it more challenging for a predator to capture prey once they meet (Bartholomew et al. 2000). However, complex habitats also typically have greater densities of prey items and may provide visual protection for predators, which can potentially improve foraging success (Crowder and Cooper 1982, Winfield 1986, Longenecker 1993).

A number of factors influence the level of protection provided by a complex habitat. Protection provided is dependent on predator maneuverability in the habitat and the level of coverage that is provided by the habitat (Bartholomew et al. 2000, Scharf et al. 2006). Predator maneuverability is often linked to its body form, which can impact its movement and swimming speed (Winfield 1986). If a predator has greater maneuverability then it will generally forage more successfully. Greater coverage provided by the habitat will generally lead to more protection for prey, increasing survivorship (Bartholomew et al. 2000).

Several studies have observed reduced prey consumption by predators in complex habitats. A majority of these studies have been conducted in submerged aquatic vegetation habitats, but this phenomenon has also been observed in bivalve habitats. These studies have displayed that the structure and species involved are important in determining the level of protection provided. The blenny, *Helcogramma medium*, had reduced foraging efficiency on meiofauna, copepods, and amphipods when feeding in more complex algae environments, but did not have reduced consumption of polychaetes or species grouped into an 'other' taxa category (Coull and Wells 1983). Protection for *Daphnia magna* from juvenile *Rutilus rutilus*, *Scardinius erythrophthalmus*, and *Perca fluviatilis* has been observed for macrophytes, emergent reeds, and water lilies; and fewer macroinvertebrates were consumed by perch amongst submerged macrophytes (Winfield 1986, Diehl 1992). Low, medium and high densities of macrophytes have also reduced predation on benthic invertebrates by bluegills, although medium macrophyte densities allowed for the greatest growth rates of predators (Crowder and Cooper 1982). Oyster shells have also provided protection to prey such as amphipods, grass shrimp, mud crabs, and mud crab megalopae, and other bivalves from a variety of fish and crab predators (Longenecker 1993, Dittel et al. 1996, Posey et al. 1999, Grabowski 2004, Hughes and Grabowski 2006, Grabowski et al. 2008). These studies demonstrate that complex habitats generally do provide protection for prey species. However, when reviewing "fish-on-fish" predation in seagrasses, Horinouchi found that in 25% of the studies seagrasses did not provide protection to prey species from predatory fish (2007).

In addition to providing protection with complex structure, bivalves can enhance their habitats through biodeposit production which can increase the mass of benthic

organic matter in marine systems (Reusch et al. 1994, Peterson and Heck 2001, Norkko et al. 2006). Carbon and nitrogen from biodeposits may have enhanced densities of the benthos, particularly deposit feeders, located near the bivalve filter feeder, *Atrina zelandica* (Norkko et al. 2001). In addition, zebra mussel biodeposits have been shown to serve as a food source for both native and non-native amphipods (Gergs and Rothhaupt 2008a, 2008b).

Several studies have addressed the use of zebra mussel biodeposits by macroinvertebrates in freshwater ecosystems (Stewart and Haynes 1994, Botts et al. 1996, Ricciardi et al. 1997, Stewart et al. 1998, Gergs and Rothhaupt 2008a, 2008b), but few studies have looked at the utilization of oyster biodeposits as a nutrient source (except see Frankenberg and Smith 1967, Tenore and Gopalan 1974, Tolley and Volety 2005). Past studies have documented the burial of biodeposits (Haven and Morales-Alamo 1966a), and the remineralization of the biodeposit nutrients into the water column (Newell et al. 2002b), but the direct consumption of *C. virginica* biodeposits has not been largely examined (except see Frankenberg and Smith 1967, Tenore and Gopalan 1974).

In order to assess the effect of complex structure on trophic transfer and to evaluate the direct consumption of *C. virginica* biodeposits, a small food chain was utilized for this study. The food chain consisted of *C. virginica* biodeposits, the deposit feeding amphipod *Melita nitida*, and a predatory fish, *Gobiosoma bosc*. The biodeposits served as a food resource for *M. nitida* and *G. bosc* was a teleost predator of *M. nitida*. Biodeposits are a combination of feces and pseudofeces that are produced by the oyster, which preferentially consume certain food particles, particularly organic material, and reject the other particles as pseudofeces (Newell and Jordan 1983). Waste products from

the ingested food particles are released as feces that enter the detritus pool, adding organic material (Wotton and Malmqvist 2001), and are thus made available to detritus feeders (Odum and Cruz 1963). *Melita nitida* is a highly mobile epibenthic amphipod species that is typically found in mesohaline waters, in the intertidal and subtidal zones (Borowsky 1980). The naked goby, *G. bosc,* is a permanent year round oyster reef resident (Nero 1976). Naked gobies display a preference for structured habitat, and for oyster shells in particular (Nero 1976, Able and Fahay 2010). The oyster reef habitat is essential for the naked goby lifecycle as they use the shells for laying and guarding eggs (Nero 1976, Coen et al. 1999). They are generalist predators that are opportunistic in their prey selection and are most likely visual daytime predators (D'Aguillo et al. 2014). Their diet consists of harpacticoids, ostracods, small eggs, amphipods, and polychaetes (Longenecker 1993, D'Aguillo et al. 2014). In the summer months naked gobies are one of the most abundant fish larvae in the mesohaline region of Chesapeake Bay tributaries, and they are also typically the most abundant resident reef fish (Breitburg 1999). Due to the pervasiveness of these species on the oyster reef, *G. bosc* and *M. nitida* were selected as model organisms for these studies.

Several past studies have investigated the impact of structural habitat complexity on trophic transfer in aquatic systems in seagrass habitats, while only a few have investigated this phenomenon on oyster reefs (Crowder and Cooper 1982, Coull and Wells 1983, Winfield 1986, Gibbons 1988, Diehl 1992, Bartholomew et al. 2000, Horinouchi 2007, Longenecker 1993, Grabowski et al. 2008, 2005, Posey et al. 1999, Dittel et al. 1996). In addition, many of these studies have measured only differences in abundances of species but have not evaluated changes in biomass. This study explored

how the complex habitat of the oyster reef impacted trophic transfer of nutrients from biodeposits up through the food web by quantifying both the biomass and abundance of organisms in the food chain to acquire a more complete picture of the process. Bivalves generate both biodeposits (biotic component of the reef) and complex habitat structure (physical component of the reef), so the provision of a nutrient resource and the creation of a complex habitat are intrinsically linked for these species. I evaluated the impact and interaction of these two components of oysters by assessing their effect on a primary consumer population (*M. nitida*) and on a secondary consumer (*G. bosc*) with a factorial study. A 2x2x2 factorial study was conducted with two levels of complexity (high and low), two levels of predation (present and absent), and two levels of biodeposits (present and absent). This study allowed me to observe bottom-up effects on the food chain through the provision of oyster biodeposits, top-down effects of predation by naked gobies on *M. nitida,* and how both of these interact and were influenced by shell structure. In addition, this study investigated whether a deposit feeder, *M. nitida,* could be supported by a biodeposits food source and what quantity of biodeposits was needed for this species' population to grow. I hypothesized that the shell structure would reduce the consumption of amphipods by the naked goby and that the biodeposits would increase the biomass of the amphipod population. I also hypothesized that oyster biodeposits would support an *M. nitida* population.

Methods

Biodeposit collection

Biodeposits were collected for two components of this study. First, they were collected for a study determining amphipod food requirements. Secondly, biodeposits were collected for the mesocosm study that tested impacts of complex habitat on trophic

transfer. Biodeposits were collected by the same methods for both components of this study. Three hundred and seventy-one oysters were placed in a 1.22 m by 2.44 m flow through tank at the Chesapeake Bay Laboratory in Solomons, MD. Raw, ambient seawater was pumped into the tank from the Patuxent River, MD. Oysters were placed in plastic trays (47 cm x 30.5 cm) on top of half inch PVC pipe. Holes in the base of the trays allowed biodeposits to drop below the trays and collect on the base of the tank. One to two times per week biodeposits were collected by siphoning them from the bottom of the flow through tank. The biodeposits were sieved through a 1-mm mesh screen to remove large macroinvertebrates and were collected on a 200-µm screen. The biodeposits were then inspected and any organisms that were seen were removed. The biodeposits were transferred into a plastic quart sized freezer bag and transported on ice back to the laboratory in College Park, MD. There the biodeposits were placed into an additional freezer bag, to protect them from freezer burn and placed in a -20^{0}C freezer until needed. Biodeposits were collected in fall 2011, spring 2012, fall 2012, and spring/summer 2013. A homogenate of biodeposits collected from fall 2011 was created by defrosting the samples and blending them together for 15 seconds. These biodeposits were used for the amphipod feeding studies. Three more homogenates of biodeposits were created from samples collected in spring 2012, fall 2012, and spring/summer 2013. These biodeposits were used for pilot studies and the complexity mesocosm study. In order to determine a conversion factor between the volume of biodeposit homogenate and dry biomass for biodeposits (biodeposit homogenate ml: dry biodeposits g), a 5-ml sample of a homogenate was dried in a 60ºC oven for three days, and weighed. This

conversion factor was used to determine the mass of biodeposits that was added during each experiment.

Amphipod Food Requirements

Melita nitida were collected from flow through tanks holding *C. virginica* and receiving unfiltered seawater from the Patuxent River in Solomons, MD. The amphipods were rinsed from oysters and their holding trays and collected on 200-µm mesh screens. They were then placed in containers of raw Patuxent River water and transported back to the laboratory in College Park, MD. Amphipods were identified under a dissecting scope and only *M. nitida* were kept for use in these studies.

Two studies were conducted to determine if amphipods could utilize biodeposits as a basal resource, and if so, determine what amount of biodeposits was needed for the species to grow and reproduce. For these studies, 9.46 l glass aquaria were filled with a one inch layer of sand and seawater created from Instant Ocean with a salinity matching the water from which the amphipods were collected (typically between 12 and 15). Water was held in tanks for one day before the amphipods were added to allow the water temperature to equilibrate with the room. For the first study, 28 individual amphipods were weighed and then added to each tank. Each amphipod population contained an equal number of mating pairs to allow for reproduction. Oyster biodeposits were added to amphipod populations in four quantities, 0.0 g dry weight, 0.2 g dry weight, 2.0 g dry weight, and 4.0 g dry weight. Quantities of biodeposits were randomly assigned to each tank and three replicates were conducted. For the second study, 55 amphipods were placed in each tank. Four quantities of biodeposits were tested, 4.0 g dry weight, 6.0 g dry weight, 8.0 g dry weight, and 9.0 g dry weight. Three replicates were also conducted for this study. To begin both studies, amphipods were enumerated and biomassed and

then added to experimental tanks. Amphipods were biomassed by placing them in a pretared sieve and then blotting the excess water from the sieve and from the amphipods with paper towels before being weighed. Once amphipods were added, biodeposits were then also added to the tanks. Each subsequent week, for four weeks, amphipod abundance and biomass were determined and fresh biodeposits were added to the tanks.

Statistical Analyses

Two-way repeated measures ANOVAs were conducted to determine if there were differences in amphipod biomass or abundance due to biodeposit treatments and week sampled. Normality of the residuals was tested using a Shapiro – Wilk test; homogeneity of variances of the residuals was evaluated with Bartlett and Levene Tests. Amphipod abundance data was natural log transformed in order to meet assumptions of normality. In addition, sphericity of the data was evaluated using a Mauchly's test. If the data did not meet assumptions of sphericity, a Huynh-Feldt corrected p-value was used to assess significance of the repeated measures ANOVA (Field et al. 2012). Pairwise t-tests with Bonferroni adjusted P-values were used to assess significance differences between factor levels for both biomass and abundance data. R statistical software was used to conduct all statistical analyses (R Core Team 2014). Repeated measures ANOVAs were conducted using the "ez" package in R statistical software (Lawrence 2013).

Complexity mesocosm

Organism and Oyster Shell Collection

Gobiosoma bosc were collected by deploying bags of oyster shell in the Choptank River, MD and the Patuxent River, MD. Starting one month after the initial deployment and afterwards when fish were needed, fish were retrieved from the bags as they colonized the shell habitat. *Gobiosoma bosc* between 33 and 45 mm in length were

collected from the shell bags and placed into plastic bags half filled with air and half filled with raw Chesapeake Bay water. Bags containing *G. bosc* were transported back to the College Park, MD in coolers to prevent overheating. *Melita nitida* were collected from flow through tanks that were holding *C. virginica* and receiving unfiltered seawater from the Patuxent River in Solomons, MD and from the Choptank River in Cambridge, MD. *Melita nitida* were also opportunistically collected from shell bags held in the Patuxent River, MD, the South River, MD, and the Choptank River, MD. *Melita nitida* were rinsed from the oysters or shell bags and collected on 200-µm sieves. They were then transferred to plastic containers filled with raw river water and transported to College Park, MD. Amphipods were identified and held in the laboratory until they were needed in experiments.

In order to create a complex habitat structure, loose disarticulated *C. virginica* shells were obtained from the Horn Point Oyster Hatchery in Cambridge, MD. Shells had been bleached and dried in the sun and were then scrubbed clean with wire brushes, soaked in the 10% bleach solution for two minutes, and then rinsed in a freshwater solution for six minutes (Newell and Jordan 1983).

Mesocosm Experiments

The effects of three factors on amphipod abundance and biomass and naked goby biomass were tested: predator presence (present and absent), biodeposit presence (present and absent), and complexity (high and low). All combinations of the three factors were tested, resulting in a full factorial design with eight treatments (Table 5). Treatment combinations were randomly assigned to mesocosms and replicated three times. This experiment was then repeated three times. Due to low amphipod numbers, the last round contained only two trials resulting in a total of eight replicates. Experimental tanks were

9.46 l glass aquaria filled with artificial seawater created from Instant Ocean and tap water at a salinity of approximately 13, which was similar to the salinity where the organisms were collected. The substrate in the low complexity treatments was a one-inch layer of sand. High complexity experimental units were filled with loose shell, in addition to the one-inch layer of sand on the bottom of the tank. Air stones were added to the tanks to keep dissolved oxygen levels near 8 mg l^{-1} or higher.

Table 5. Treatment combinations for the amphipod-naked goby-biodeposit study.

Treatment Number	Naked Goby	Complexity	Biodeposit
1	Present	Low	Present
2	Present	Low	Absent
3	Absent	Low	Present
4	Absent	Low	Absent
5	Present	High	Present
6	Present	High	Absent
7	Absent	High	Present
8	Absent	High	Absent

Newly assembled tanks sat for 24 hours to allow the temperature of the tanks to equilibrate with the room. After 24 hours, 35 individual amphipods were weighed and added to each tank. Amphipods were weighed by placing them in a pretared plastic container with the bottom replaced with 30-µm mesh screening. The container and amphipods were blotted with paper towels to remove excess water from the amphipods before they were weighed. Once weighed, amphipods were added to each mesocosm, with addition times staggered to allow for processing time of the tanks. A single naked goby, which had been starved for 24 hrs directly prior to addition, was also added to tanks that were assigned the predator present treatment. Before addition, naked gobies total length was measured and fish were blotted dry with a paper towel and weighed. The

equivalent of 5 g of dry biodeposits, 50.5 ml of fall 2012 homogenate biodeposits solution, was added to the appropriate mesocosms for round one and round two of the mesocosm experiment. Additional biodeposits were required for the final round of the mesocosm experiment, so biodeposits from the spring/summer 2013 homogenate were added. Forty-two ml of spring/summer 2013 homogenate biodeposits solution were added to biodeposit present treatments in the final round of mesocosm testing, which was the equivalent of 5 g dry weight of biodeposits. Amphipods and biodeposits were added in the same manner described above for three consecutive days until a total of 105 amphipods had been added to the tanks and the equivalent of 15 g (dry weight) of biodeposits had been added. Fish and amphipods fed for another 24 hours following the final additions of amphipods and biodeposits before final abundances and biomasses were determined. After this final 24 hours, naked gobies were removed and final weights and lengths were attained. Amphipods were removed from the low complexity treatments by sieving the tank water through stacked 1-mm and 200-µm sieves. The sand and sides of the tanks were carefully examined for any remaining amphipods. Amphipods were then removed from the sieves, counted, and collected on a pretared sieve in which they were weighed after excess water was blotted from the amphipods and sieve. Amphipods were removed from the high complexity tanks by rinsing each oyster shell from the mesocosm with fresh water over a 200-µm sieve. Shells were then placed in a freshwater bath, to force cryptic amphipods out of the ridges of the oyster shells. Once all the shells had been removed, the tank water was sieved and the sand and tank were observed for any remaining amphipods. Finally, the oyster shells in the freshwater bath were rinsed again with freshwater and the water from the bath was passed through

the 200-µm sieve. Amphipods were then removed from the sieve, enumerated, and collected on a pretared sieve in which they were weighed after excess water was blotted. The tanks were kept on a 12 hours on/12 hours off photoperiod each day.

Statistical Analysis

Generalized linear mixed models were used to analyze amphipod abundance. Abundance was analyzed with a three-way Analysis of Deviance with a quasibionomial distribution, to correct for overdispersion of the data (Crawley 2012). Complexity, biodeposits, and predator were included as fixed factors and round was included as a random block factor to account for conducting the experiment at three separate time points. Multiple mean comparisons were conducted using general linear hypotheses with a Tukey's adjustment. The Tukey's test was conducted on a single factor - treatment, a combined variable that represented the eight combinations of all the treatment factors.

Differences in initial amphipod biomass due to treatments were evaluated using a robust three-way ANOVA, since data did not meet the assumptions of normality. Final amphipod biomass data was analyzed with a linear mixed-effects model using a three-way ANCOVA with three fixed explanatory factors: complexity, biodeposits, and predation. In addition, round was incorporated as a random block factor to account for the three rounds of testing that were conducted. Due to violations in normality, initial and final amphipod biomasses were ranked and then a three-way ANCOVA was performed with the ranked values; ranked initial amphipod biomass serving as a covariate and ranked final amphipod biomass serving as the response variable. A variance covariate was included to account for increasing variance with increasing ranked initial amphipod biomass. The final model was reduced to remove the insignificant four and three-way interactions, and the insignificant two-way interactions between rank initial

amphipod biomass and complexity and rank initial amphipod biomass and biodeposits. Least squared means were used to make multiple mean comparisons after the ANCOVA was conducted, utilizing a Tukey's adjusted P-value.

Final naked goby biomass was analyzed using linear mixed-effects models. A two-way ANCOVA with a block factor for round and initial naked goby biomass as a covariate was conducted to test differences in final naked goby biomass. Round was treated as a random factor and complexity and biodeposits were treated as fixed factors. Initial and final naked goby biomass were log transformed to meet assumptions of normality. Normality of residuals was assessed using a Shapiro-Wilk test and homogeneity of variances was evaluated graphically and with a Bartlett's test.

R statistical software was used to conduct all statistical analyses (R Core Team 2014). Generalized linear mixed models were conducted using the "glmmPQL" function in the "MASS" package (Venables and Ripley 2002) and linear mixed-effects models were conducted using the "lme" function in the "nlme" package (Pinheiro et al. 2014). Robust ANOVA was conducted using the "t3way" function in the "WRS" package (Wilcox and Schönbrodt 2014). ANOVA tables were created using the "Anova" function in the "car" package (Fox and Weisberg 2011). Least squared means were generated using the "lsmeans" function from the "lsmeans" package (Lenth and Hervé 2014). General linear hypotheses testing was conducted using the "glht" function in the "multcomp" package (Hothorn et al. 2008).

Results

Amphipod Food Requirements

Pilot studies were conducted to determine the amount of biodeposits necessary for positive amphipod abundance and biomass growth. When 0.0 g (0.0 g/amphipod), 0.2 g

(0.007 g/amphipod), 2.0 g (0.071 g/amphipod), and 4.0 g (0.143 g/amphipod) of biodeposits were provided to 28 amphipods each week for four weeks, all treatments had lower final abundances and biomasses than initial values. When amphipod populations had a starting abundance of 55 and were provided with 4.0 g (0.073 g/amphipod) of biodeposits or more, all final amphipod abundances were greater than initial abundances, and when they were provided with 6.0 g (0.109 g/amphipod) of biodeposits or more, all final biomasses were greater than initial biomasses. Amphipods displayed the greatest amount of biomass growth after four weeks when given 8.0 g (0.145 g/amphipod) of oyster biodeposits per week. The greatest increase in amphipod abundance was seen with 9.0 g (0.167 g/amphipod) of oyster biodeposits, but this was just slightly higher than 8.0 g. The 4.0 g treatment experienced an 8.3% decrease in biomass, while the 6.0 g, 8.0 g, and 9.0 g, treatments increased in biomass by 13.0%, 36.7%, and 27.2%, respectively. When amphipod biomass was evaluated, week was found to have a significant effect (repeated measures ANOVA, F=4.387, df=4, P= 0.020, Huynh-Feldt corrected P-value). Multiple pairwise t-test with a Bonferroni correction were performed to compare weeks, and while none of the comparisons came out as significant when the first and fourth weeks were compared, there was a marginally significant P-value of 0.073. When abundance was evaluated, there was a significant biodeposit level by week interaction (repeated measures ANOVA, F=2.365, df=12, P=0.026). Due to the high number of multiple comparisons, the Bonferroni correction did not result in any significant comparisons to help understand which values differed from one another, but visual assessment of the data indicates differences between the biodeposit treatments at week 3 (Figure 9).

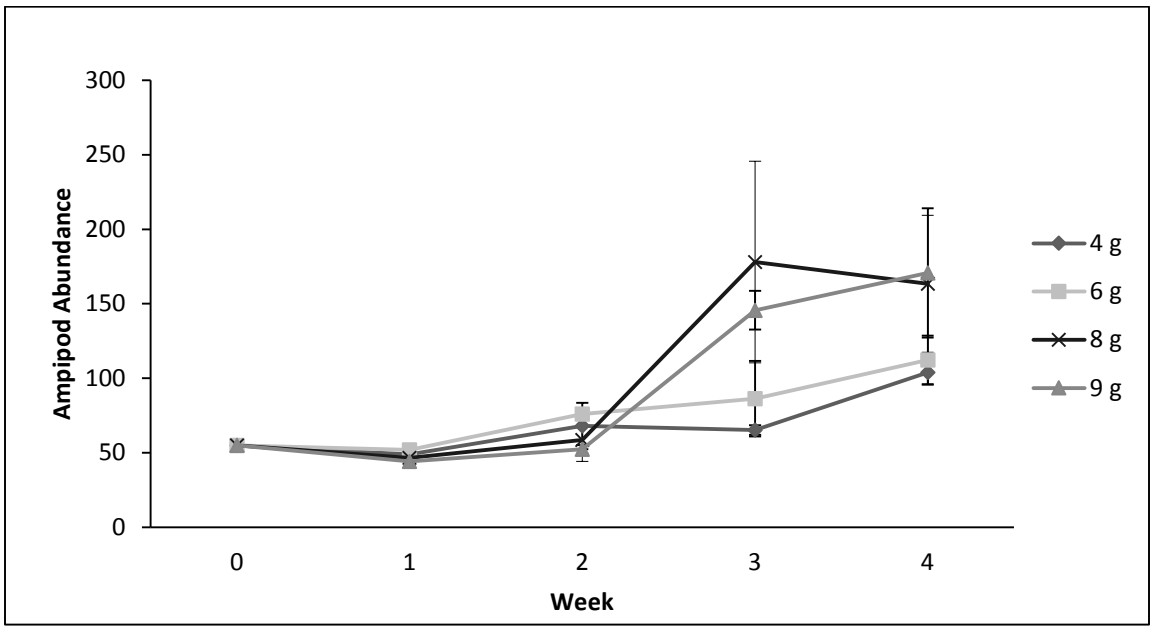

Figure 10. Mean total abundance (±SE) of *Melita nitida* populations after 4 weeks of growth with different amounts of oyster biodeposits available. Starting amphipod abundances were 55. n_4=3, n_6=3, n_8=3,n_9=3.

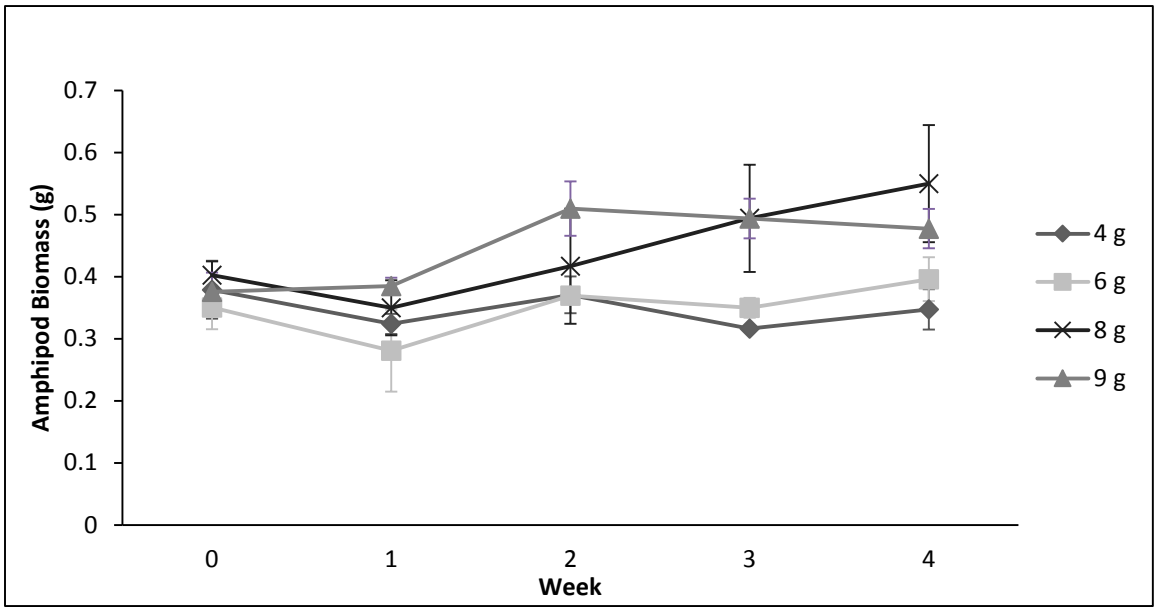

Figure 11. Mean total biomass (±SE) of *Melita nitida* populations after 4 weeks of growth with different amounts of oyster biodeposits. n_4=3, n_6=3, n_8=3,n_9=3.

Complexity Mesocosms – Melita nitida Abundance

There was no three-way interaction between the three factors tested: complexity, biodeposits, and predator. However, there was a significant interaction between complexity level and the presence of a predator (Analysis of Deviance, χ^2=7.179, df=1, *P*=0.007, Table 6), while biodeposit presence did not have a significant interaction with either predator presence or complexity level. Biodeposit presence alone also did not affect amphipod abundance. When a predator was present, high complexity treatments resulted in higher mean final amphipod abundances (±SE) (44.75 ± 4.41 biodeposits present, 35.38 ± 5.96 biodeposits absent) than low complexity treatments (9.75 ± 4.82 biodeposits present, 8.63 ± 5.33 biodeposits absent). However, when a predator was absent there was no difference in final amphipod abundance between high complexity (82. 13 ± 5.43 biodeposits present, 87.13 ± 4.14 biodeposits absent) and low complexity (80.25 ± 2.43 biodeposits present, 86.13 ± 1.77 biodeposits absent) treatments. Treatment eight (high complexity, biodeposits absent, predator absent) had the highest final mean amphipod abundance (± SE), 87.13 ± 4.14, while Treatment two (low complexity, biodeposits absent, and predator present) had the lowest final mean amphipod abundance, 8.63 ± 5.33. Treatments one and two (low complexity, predator present) were not significantly different from one another, but were significantly different from all other treatments. Treatments five and six were not significantly different from one another (high complexity, predator present), but were significantly different from all other treatments. Treatments 3, 4, 7, and 8 (predator absent) were not significantly different from one another, but were significantly different from 1, 2, 5, and 6 (predator present) (Tukey's HSD, *P*<.05, Figure 11).

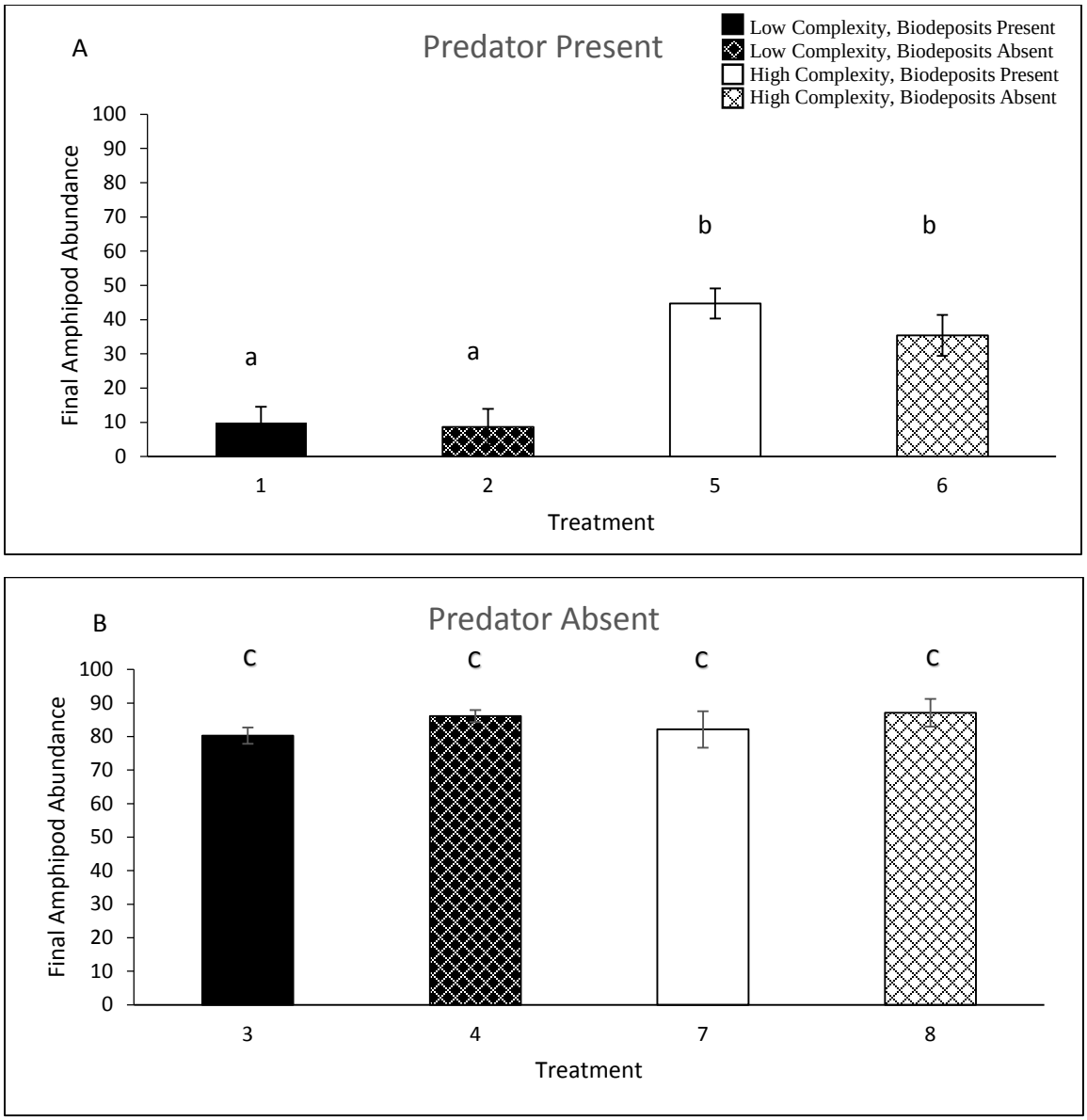

Figure 12. Final *Melita nitida* abundance (±SE) after three days of the experiment. Initial *M. nitida* abundance was 105. A. Final *M. nitida* abundance for treatments 1, 2, 5, and 6. All treatments had a predator present. B. Final *M. nitida* abundance for treatments 3, 4, 7, and 8. All treatments had no predator present. Black bars indicate low complexity, white bars indicate high complexity, solid bars indicate biodeposits present and hatched bars indicate biodeposits absent. Different letters indicate significant differences at α=0.05.

Table 6. Analysis of deviance table for *Melita nitida* abundance. Significant P values are bold.

Factor	DF	χ^2	P
(Intercept)	1	28.807	**<0.001**
Complexity	1	0.026	0.873
Biodeposits	1	0.587	0.444
Predator	1	35.962	**<0.001**
Complexity*Biodeposits	1	0.004	0.949
Complexity*Predator	1	7.179	**0.007**
Biodeposits*Predator	1	1.775	0.183
Complexity*Biodeposits*Predator	1	0.061	0.806

Complexity Mesocosms – Melita nitida Biomass

All initial *M. nitida* population biomasses were similar for all treatments (Robust ANOVA, $P>0.05$). The initial amphipod biomasses (± SE) for treatments 1-8 were 0.3626 ± 0.0280 g, 0.3931 ± 0.0338 g, 0.3974 ± 0.0331 g, 0.3827 ± 0.0325 g, 0.3387 ± 0.0333 g, 0.3795 ± 0.0296 g, 0.4016 ± 0.0324 g, 0.3801 ± 0.0350 g. There was a significant interaction between the complexity and predator factor (ANCOVA on ranked data, F= 14.943, df= 1,53, $P<0.001$) and biodeposits did not have an effect on amphipod biomass (Table 7). Treatments 3, 4, 7, and 8 (predator absent treatments) had the highest final amphipod biomasses, with final mean biomasses (± SE) of 0.3135 ± 0.0280 g, 0.3024 ± 0.0254 g, 0.2975 ± 0.0168 g, and 0.3360 ± 0.0360 g, respectively. The percent change for treatments 3, 4, 7, and 8, were -20.8%, -20.7%, -23.3%, and -11.7%, respectively. Treatments five and six both had a predator present in a high complexity habitat. The final mean amphipod biomass for treatments five and six were 0.1404 ± 0.0197 g and 0.1375 ± 0.0281 g, respectively, resulting in a 58.6% biomass loss for treatment five and a 64.6% biomass loss for treatment six. Treatments one and two both were low complexity treatments with a predator present and their mean final amphipod

biomasses were 0.0559 ± 0.0249 g and 0.0586 ± 0.0342 g, respectively (Figure 12, Figure 13). The biomass loss for treatment one was 86.4% and was 86.2% for treatment two.

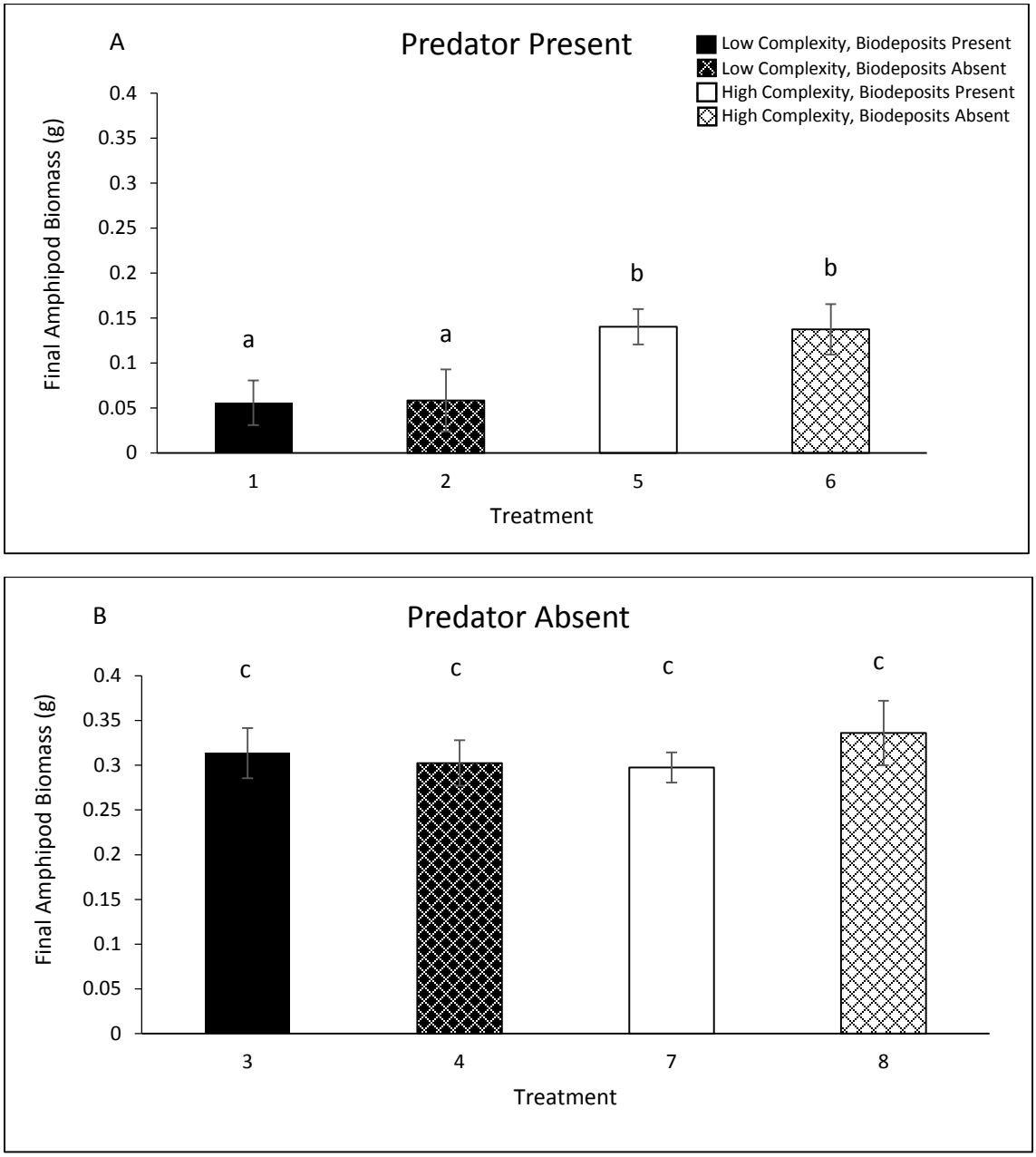

Figure 13. Final *Melita nitida* population biomass (±SE) after three days of the experiment. A. Final *M. nitida* biomass for treatments 1, 2, 5, and 6. All treatments had a predator present. B. Final *M. nitida* biomass for treatments 3, 4, 7, and 8. All treatments had no predator present. Black bars indicate low complexity, white bars indicate high complexity, solid bars indicate biodeposits present and hatched bars indicate biodeposits absent. Different letters indicate significant differences at α=0.05.

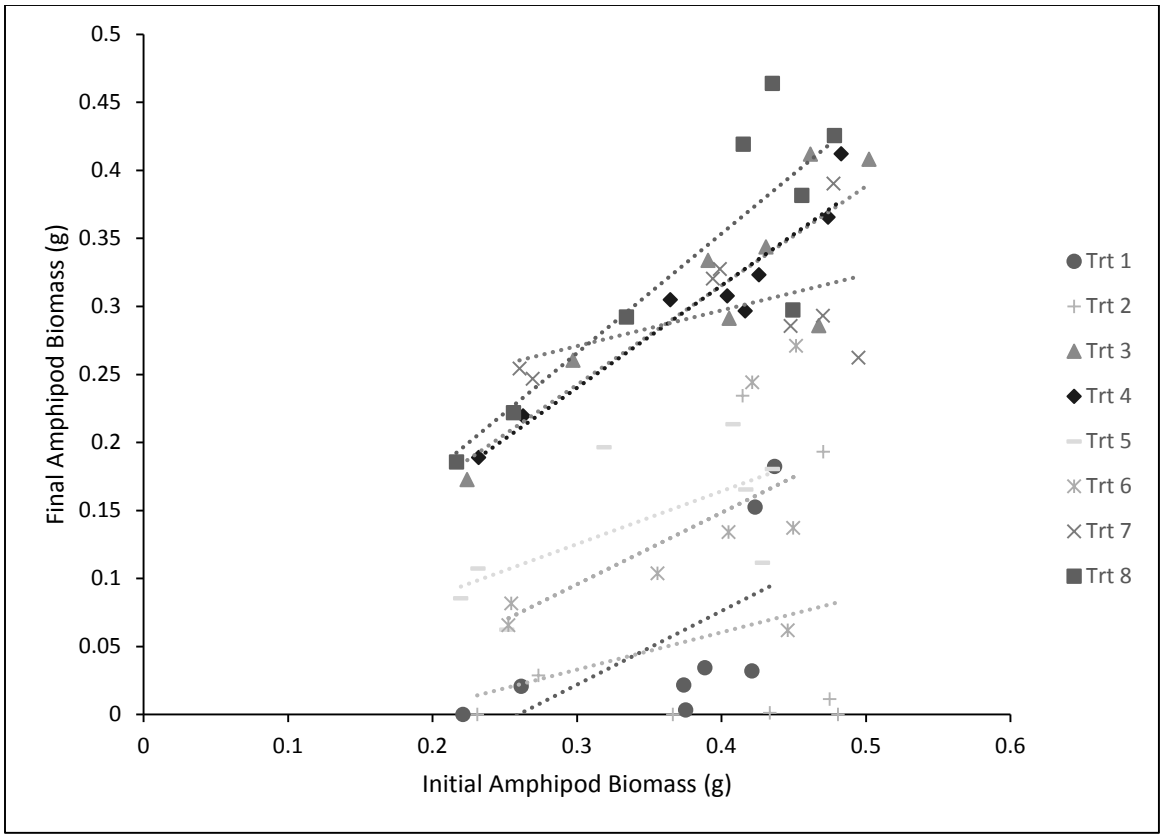

Figure 14. Analysis of Covariance for eight treatments. The covariate was initial *Melita nitida* biomass while final *M. nitida* biomass was the response variable. Each treatment is a combination of the three treatment factors: complexity, biodeposits, and predator (Table 5).

Table 7. ANCOVA table for final *Melita nitida* population biomass. ANCOVA was performed on ranked initial and ranked final amphipod biomass data. Significant P values are bold. Ranked initial amphipod biomass is abbreviated rankIABio.

Factor	numDF	denDF	F	P
Intercept	1	53	102.343	**<0.001**
rankIABio	1	53	28.057	**<0.001**
Predator	1	53	29.805	**<0.001**
Complexity	1	53	0.015	0.904
Biodeposits	1	53	0.018	0.894
rankIABio:Predator	1	53	11.187	**0.002**
Predator:Complexity	1	53	14.943	**<0.001**
Predator:Biodeposits	1	53	0.739	0.394
Complexity:Biodeposits	1	53	0.726	0.398

Complexity Mesocosms – Gobiosoma Bosc Biomass

Mean final *G. bosc* biomass increased for all treatments from mean initial *G. bosc* biomass over the course of the study. The largest percent fish biomass change was with treatment one (low complexity, biodeposits present) and two (low complexity, biodeposits absent) with an increase in fish biomass of 11.2% for both. The smallest change in fish biomass was seen with treatment five (high complexity, biodeposits present) which only increased 5.8%. Fish biomass in treatment six (high complexity, biodeposits absent) increased 9.0%. There was no significant difference in final fish biomass due to complexity level or biodeposit presence, although percent biomass increase was greater for the low complexity treatments than for the high complexity treatments.

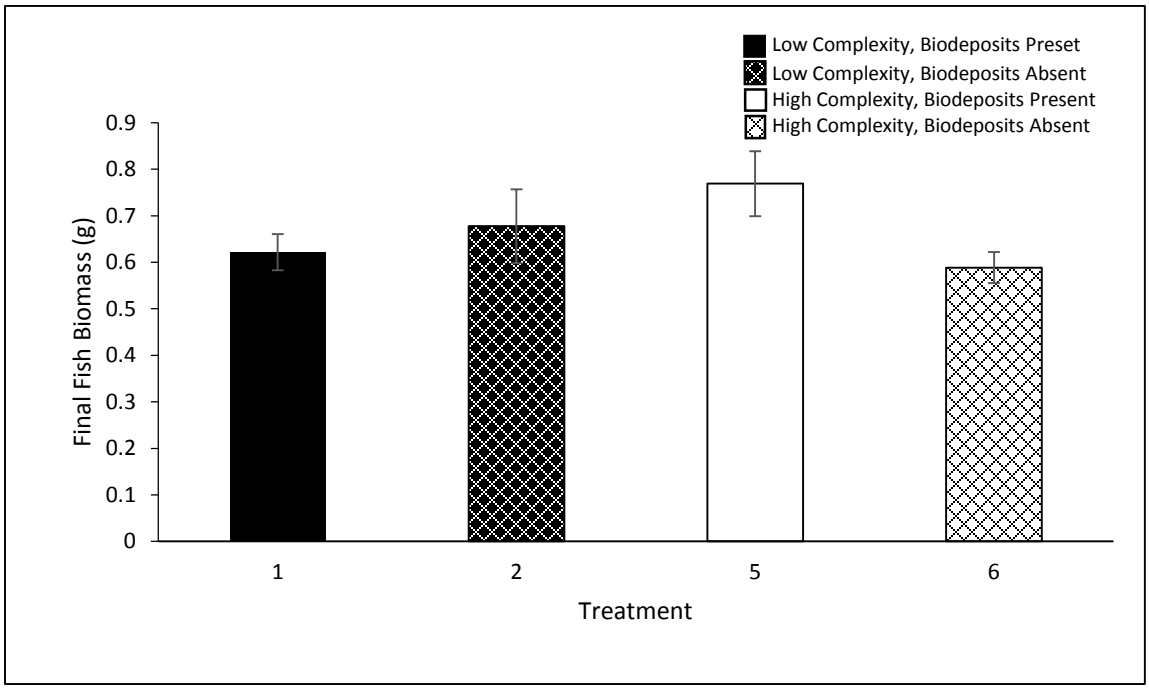

Figure 15. Final *Gobiosoma bosc* biomass (± SE) after three days of the experiment. Black bars indicate low complexity, white bars indicate high complexity, solid bars indicate biodeposits present and hatched bars indicate biodeposits absent. Treatments were not significantly different from one another.

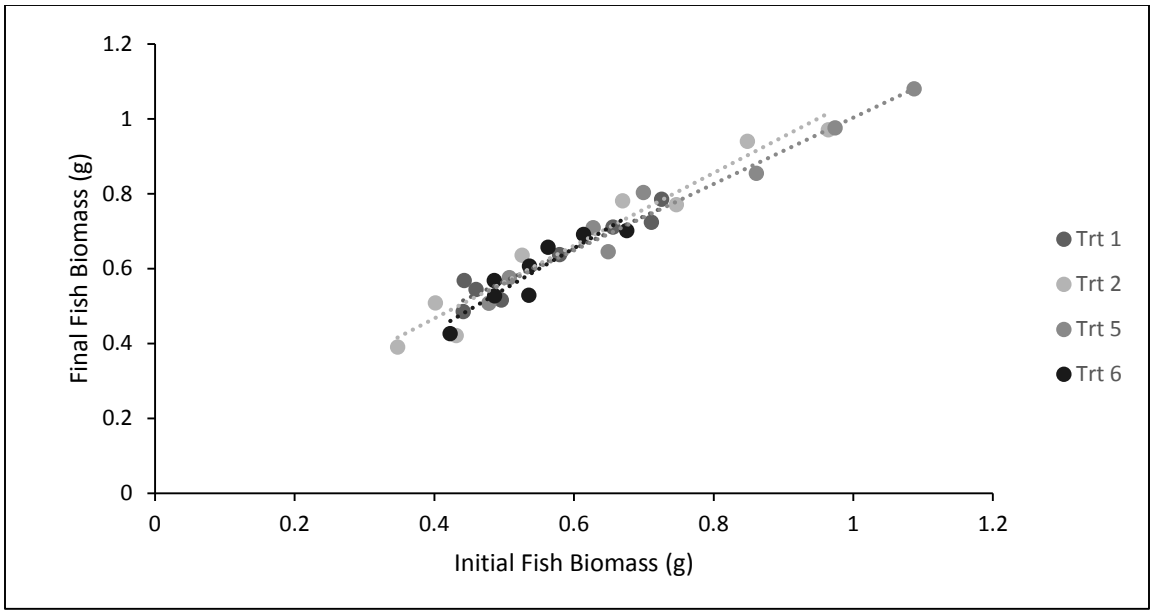

Figure 16. Analysis of covariance for predator biomass. The covariate was initial *Gobiosoma bosc* biomass plotted against the response variable final *G. bosc* biomass by treatments. Each treatment is a combination of the three treatment factors: complexity, biodeposits, and predator (Table 5). Treatments were not significantly different from one another.

Table 8. ANCOVA table for final *Gobiosoma bosc* biomass. Significant P values are bold.

Factor	numDF	denDF	F	P
Intercept	1	22	1.207	0.284
Complexity	1	22	0.301	0.589
LogInitialFishBiomass	1	22	37.293	**<0.001**
Biodeposits	1	22	1.091	0.308
Complexity*LogInitialFishBiomass	1	22	0.600	0.447
Complexity*Biodeposits	1	22	0.085	0.773
LogInitialFishBiomass*Biodeposits	1	22	1.224	0.281
Complexity*Biodeposits*LogInitialFishBiomass	1	22	0.124	0.728

Discussion

This study evaluated the effect of complexity on trophic transfer in a small oyster reef food chain. When amphipod abundance and biomass were evaluated, there was an interaction between the effect of predator presence and level of complexity. When a

predator was absent there was no difference in the mortality or final biomass of prey items between complexity levels; when a predator was present there was lower mortality and higher final biomasses of prey items in high complexity treatments compared to low complexity treatments (Figure 11, Figure 12). There was no effect of biodeposit presence on amphipod abundance or biomass. In addition, I did not see a difference in naked goby biomass with respect to biodeposit presence or complexity level.

This study found that oyster shells were able to provide protection from mortality to *M. nitida* from *G. bosc.* Both *M. nitida* and *G. bosc* are benthic-oriented species that typically use oyster reefs as their habitat. When the two species were placed together without any structure, naked gobies were able to easily capture the amphipods and the amphipod population's abundance and biomass were quickly reduced. However, when oyster shells were present, naked gobies' ability to detect and capture the amphipods was reduced, resulting in a greater final abundance and biomass in trials with structure present. Longnecker (1993) also found that naked gobies captured fewer amphipods in the presence of structure. While the feeding technique of naked gobies has not been extensively studied, evaluation of naked gobies' gut fullness at different times of day suggested that the goby was a visual predator that was mostly active during the daytime (D'Aguillo et al. 2014). Since the goby is most likely a visual predator, the presence of the oyster shells can block the goby from detecting the presence of the amphipods. In addition, *M. nitida* is an epibenthic species that will seek refuge in the ridges of the oyster shell, increasing the difficulty of detecting and capturing the amphipods (K.E. Kesler personal observation). The protection for the amphipods was observed not only with a greater number of amphipods surviving in the complex habitat, but also with a greater

biomass of amphipods remaining in the high complexity treatments after predation (Figure 11, Figure 12). In addition, there was no difference between high and low complexity treatments when a predator was absent, indicating that the mortality that was seen in the trials was due to the predator and not the presence of other amphipods or caused by the differences in the tank environment due to low complexity.

The reduction in prey consumption by a predator seen here was likely due to complex structure's ability to provide a habitat that allows for the coexistence of predator and prey species. Complex habitats contain microhabitats that allow multiple species to share a single space at once (Hixon and Menge 1991). They create refuges that provide protection to prey species from their predators, allow for separation between competing species, and protect species from disturbances (Diehl 1992, Norling and Kautsky 2007, Horinouchi 2007).

Two characteristics of a prey organism that can influence its probability of capture and consumption by a predator are its likelihood to encounter a predator and how easily that prey item can be captured by the predator (Greene 1986). Prey populations may be maintained in complex habitats because these structures can reduce the encounter rate between predator and prey individuals, reducing the likelihood of prey consumption (Nestlerode et al. 2007). This in turn reduces the efficiency of the predators in complex habitats (Grabowski 2004). Complex habitats can also provide refuge by limiting a predator's movement, reducing a predator's ability to detect a prey item, and by making the capture of prey items more difficult (Bartholomew et al. 2000). In this study, most likely the oyster shell is both reducing naked goby movement and limiting visual detection of prey items. In addition, *M. nitida* is an epibenthic species that can utilize the

structure of the oyster appropriately to gain protection from it. Benthically oriented species generally experience greater protection from complex benthic habitats than pelagically oriented species (Scharf et al. 2006).

The results of my study support the findings of past studies that structure provides protection to prey species from predators. Most studies evaluating the impacts of complexity have been conducted with submerged aquatic vegetation (SAV), with only a few studies evaluating the impact of oyster structure on foraging efficiency. Numerous submerged aquatic vegetation studies have demonstrated that the complex structure provides protection to macroinvertebrates from fish predators (Crowder and Cooper 1982, Coull and Wells 1983, Gilinsky 1984, Winfield 1986, Gibbons 1988, Diehl 1992, Bartholomew et al. 2000). A study evaluating juvenile naked goby growth rate found that the fish had higher growth rates in bare sand habitats than they did in eelgrass, indicating that the gobies had greater consumption, mostly of amphipods, in bare sand than eelgrass (Sogard 1992). This is similar to my results, where naked gobies consumed more amphipods in low complexity habitats compared to high complexity habitats. At times a threshold of complexity needs to be met, before the benefits of SAV structure can be attained by the prey items (Coull and Wells 1983, Gibbons 1988, Bartholomew et al. 2000). This seems to be the case with oyster structure as well, from pilot studies in which lower shell densities resulted in less protection. How different reef structures provide protection needs to be further investigated, to identify which structure and density provides the best refuge for prey.

While the literature on protection from seagrasses is more extensive, a few studies have evaluated the role of bivalves in providing refuge to prey items. Grabowksi et al.

(2008) found that complex habitat created by oysters can reduce foraging rates of blue crabs and mud crabs on juvenile oysters. In addition, Hughes and Grabowski (2006) found that stone crabs and knobbed whelks foraged less on hard clams and ribbed mussels in oyster reefs than on sand flats. But these results varied from the findings of Grabowski and Powers (2004), where at low and intermediate densities of mud crabs, there was no difference in mortality of juvenile hard clams between simple and complex habitats. My study supports the findings that greater habitat complexity results in greater survivorship of prey, with greater final abundances and biomasses of *M. nitida* in high complexity trials when a predator was present compared to low complexity treatments with a predator present. In addition naked gobies gained more weight in low complexity trials than in high complexity trials, although this difference was not significant.

There was no effect of biodeposits on the amphipod population's abundance or biomass in the mesocosm studies, but this may have been due to the length of time that the experiment ran. The mesocosms were only run for three days, while amphipod food requirement studies showed that growth in biomass and abundance was not seen until after four or three weeks, respectively (Figure 9, Figure 10). The response to nutrients from bivalve biodeposits in the field seems to be dependent on the availability of alternate resources in the ecosystem. If the alternate resources are limited, than the addition of biodeposits can impact the community (Howard and Cuffey 2006). In stream habitats, the benthic community will utilize invertebrate feces as a food source once other primary resources are depleted (Shepard and Minshall 1984). Also, over shorter time periods, there may be a weaker link between the abundance of consumers and the level of benthic production (Posey et al. 2002). The effect of increased benthic production may

only be seen over a longer time scale and greater spatial scale where consumers are less effected by predation pressures (Posey et al. 2002). However, some field enclosure experiments have seen an increase in abundance of invertebrates with the presence of a live bivalve, versus an empty bivalve shell, indicating that in some instances biodeposits can improve the abundance of invertebrates (Vaughn et al. 2008), although I did not observe this in my mesocosms. However, Vaughn et al. saw these results after one month, which may again indicate that a difference from biodeposits cannot be seen in the short time frame of this study.

While no differences in the amphipod population were seen in the mesocosm studies, the amphipod food requirement studies exhibited growth in the amphipod population when biodeposits were their only food resource available. This demonstrates that the amphipods can utilize biodeposits as a basal resource, but differences in growth due to biodeposits may not occur until several weeks have passed. The microbial content of feces and pseudofeces, as well as the undigested particulate organic matter present in biodeposits, is what allows the biodeposits to potentially be used as a food resource (Prins and Smaal 1994). The consumption of feces is directly correlated to the carbon content of the pellets (Frankenberg and Smith 1967), and oyster biodeposits have been shown to increase the carbon concentrations in surrounding sediments (Haven and Morales-Alamo 1966b), which may encourage the consumption of oyster biodeposits. In addition, several benthic species have been shown to consume feces, including *C. virginica* feces, demonstrating the potential for feces consumption and the potential importance of this food source in the benthic community (Frankenberg and Smith 1967). Other past studies have shown that amphipods will consume *Dreissena* spp. biodeposits

(Gonzalez and Burkart 2004, Gergs and Rothhaupt 2008b). This is a similar result to what I observed in my food requirement study, but again a longer time period appears to be necessary to see a growth in amphipod abundance and biomass due to biodeposits. These results may indicate that the amphipods exhibit a slow growth rate or that biodeposits are not readily consumed, so differences will not be seen until a greater time period has passed. In addition, survivorship on biodeposits appears to be species dependent for amphipods and potentially dependent on the species producing the biodeposits (Gonzalez and Burkart 2004, Limén et al. 2005, Gergs and Rothhaupt 2008b). These previous studies utilized biodeposits from *Dreissena* spp., while my study used biodeposits created by *C. virginica*, indicating that biodeposits from this species can also be used as a food resource, although perhaps not a primary one.

Even though I did not see an effect of biodeposits in my mesocosm studies, they have the potential to provide nutrition, which was exhibited by the pilot study where over the course of four weeks the amphipod population grew in abundance and biomass. Posey et al. (2006) also found an increase in biomass of crustaceans when nutrients were added to an estuarine ecosystem, but only when predators were excluded from the system. Along with exhibiting the potential of a basal resource of nutrients to encourage growth in the crustacean population, this study also exhibited the overwhelming effect that predation had on the population (Posey et al. 2006), which was also seen in my experiment. In my study, predator presence always resulted in lower amphipod biomass and abundance. This top-down control of the population appears to be operating on a faster time scale than the bottom-up effects of nutrient additions in the form of biodeposits.

This study indicates the ability of oyster shell structure to reduce the rate of trophic transfer from a primary consumer to its secondary consumer, as fewer amphipods were consumed in the presence of shell structure. The effects of the protection provided to macroinvertebrates by oyster structure can also be observed in the field. When amphipod abundances on non-restored oyster reefs were compared to abundances on restored oyster reefs, it was found that all amphipod species, except for the infaunal species *Leptocheirus plumulosus*, had much higher abundances on restored oyster reefs than on non-restored oyster reefs (Rodney and Paynter 2006). These amphipods may be found in higher numbers on reefs because of the protection that was provided to them from the reef structure. Rodney and Paynter (2006) also found greater numbers of naked gobies on restored reefs, and even with this predator present in greater numbers, amphipod numbers vastly exceeded those found on unrestored reefs. The naked goby was an extremely effective predator, causing a reduction in amphipod abundance and biomass in my study whenever it was present, even in complex habitats. I also saw that in addition to reducing amphipod abundance and biomass, naked gobies increased their biomass over the course of the study, indicating transfer of energy from the primary consumer to the secondary consumer. While I did not see a significant difference in fish biomass here with complexity, this again seems to be a difference that would increase with a longer time period.

Crowder and Cooper (1982) found that an intermediate seagrass density was important for long term stability of a fish's growth as it allowed for sufficient feeding by the predator but also enough protection of the prey population to prevent its depletion. My study showed that this relationship might potentially exist on the oyster reef as well,

where naked gobies still gain weight in high complexity trials but a baseline population of amphipods is maintained. This stability may allow for the persistence of the naked goby population, which could be important for the greater oyster reef and estuary food chain, as naked gobies are prey for the economically important predator striped bass (*Morone saxatilis*) (Harding and Mann 2003). Naked gobies were the dominant fish species consumed by striped bass at three different habitats sampled: bare sand, flat oyster bars, and vertical oyster reefs (Harding and Mann 2003). The provision of spatial refuges and prey items by reefs can enhance fish production as well as the overall survival and growth of a fish (Peterson et al. 2003). My study demonstrates how a reef can protect prey items, potentially allowing for the persistence of a constant prey source for resident and transitory fish predators on the reef. The next step would be to observe this relationship over a longer time scale to see if complexity allows for the reproduction and continued persistence of the prey population.

Chapter 4: Stable isotope analysis of the role of *Crassostrea virginica* biodeposits in an oyster reef food web.

Introduction

As a filter feeder, oysters link benthic and pelagic environments by transferring nutrients from the water column to the sediment surface. Oysters transfer these nutrients through biodeposition; the creation of feces and pseudofeces from seston that oysters filter from the water column (Ulanowicz and Tuttle 1992). Large amounts of biodeposits can potentially be formed by *Crassostrea virginica*, with one study estimating that in a 0.405 hectare area, 981 kg of biodeposits were produced by oysters in 1 week (Haven and Morales-Alamo 1966b). The amount of biodeposits produced at any given time is dependent upon water quality conditions. Temperature is the driving factor in biodeposit production, with higher temperatures leading to higher clearance rates for oysters, up to a maximum temperature of 27°C (Newell and Langdon 1996, Fulford et al. 2007). In addition, dissolved oxygen levels need to be above 2 mg l^{-1} (above hypoxic levels) and salinity needs to be greater than 3 for oysters to feed without impairment (Fulford et al. 2007, Cerco and Noel 2007). Total suspended solids (TSS) also impact filtration rates. As TSS levels increase, so do *C. virginica* filtration rates until TSS levels reach 25 mg l^{-1}, at which point filtration slows with greater increases in TSS levels (Fulford et al. 2007). As temperature is the main driver of filtration, peak biodeposit production rates are seen in summer months, when the water temperature is warmest and food is abundant.

Biodeposits can follow a few different pathways through the oyster reef ecosystem. They can be incorporated into the sediment, resulting in their subsequent burial, the nutrients from biodeposits can remineralize into the water column, and

biodeposits may be consumed by reef organisms (Haven and Morales-Alamo 1966a, Frankenberg and Smith 1967, Newell et al. 2002b). The first two processes have been demonstrated as possible pathways for *C. virginica* biodeposits; the third pathway has not been thoroughly evaluated, although there is some evidence of reef organisms using oyster biodeposits as a nutrient resource, as detailed below.

A few studies have observed the consumption of *C. virginica* biodeposits by macroinvertebrates in laboratory settings (Frankenberg and Smith 1967, Tenore and Gopalan 1974), but it has not been assessed in the larger estuarine ecosystem. However, several studies have assessed the role of bivalve biodeposits as a food resource using zebra mussel biodeposits. The amphipods *Gammarus fasciatus, Echinogammarus ischnus,* and *Gammarus roeselii* have all been observed consuming *Dreissena* spp. biodeposits (Gonzalez and Burkart 2004, Gergs and Rothhaupt 2008b). In addition, isotope analysis has indicated the use of zebra mussel biodeposits as a food source in the field by *G. fasciatus* and *G. roeselii* (Limén et al. 2005, Gergs et al. 2011).

In order to test the hypothesis that some oyster reef deposit feeders consume *C. virginica* biodeposits and that this energy is transferred into the food web, two stable isotope methods were utilized. First, oyster biodeposits were labeled with an elevated level of ^{15}N and were then fed to reef deposit feeders. These deposit feeders were then fed to higher trophic level organisms in order to determine if biodeposits' nutrients were being transferred up the food chain. Second, the natural abundances of $\delta^{13}C$ and $\delta^{15}N$ of reef organisms and basal resources from an oyster reef in a Chesapeake Bay tributary were measured to determine if oyster biodeposits were utilized as a food resource in the field. Reef organisms and basal resources were sampled in three different seasons for

stable isotope analysis. Biodeposit production varies over the course of the year due to changes in temperature and phytoplankton composition and concentrations, thus reef organisms were sampled in multiple seasons to see if the impact of biodeposits changed with production levels (Tsuchiya 1980, Smith and Frey 1985).

Stable isotopes were used for this analysis because $\delta^{13}C$ and $\delta^{15}N$ values provide information about the food web structure (Fry and Sherr 1984). Stable isotopes reflect the nutrients that were assimilated into an organism's tissue over time, providing an integrated picture of resource usage (Cabana and Rasmussen 1994). As a resource is consumed, its isotopes become incorporated into the consumer, so that the isotopic signature of the consumer now reflects its food sources. As the elements undergo biological processes like respiration, they experience fractionation, where light isotopes are more readily utilized in chemical reactions than heavy isotopes (Hecky and Hesslein 1995, Fry 2006). Fractionation leads to differences in the isotopic signature between a consumer and its prey (Fry 2006). ^{13}C undergoes only a small amount of fractionation between trophic levels, generally an enrichment of 0.7-1.4‰, which makes this value useful for determining carbon sources utilized by a consumer (Fry and Sherr 1984). A larger amount of fractionation is observed for ^{15}N, which increases 3.4 ± 1.1‰ for each trophic level, due to deamination processes in consumers (Minagawa and Wada 1984, Fry 2006). Because of this property, ^{15}N can be used to determine relative trophic position for organisms within a food web. Using both $\delta^{15}N$ and $\delta^{13}C$ values, inferences can be made about relative trophic position and carbon sources of reef organisms.

The main question assessed with these experiments was: Are oyster biodeposits being utilized as a nutrient resource by the oyster reef community? This question was

addressed using stable isotopes in two ways, by measuring the natural stable isotope signatures of an oyster reef community across multiple seasons and by utilizing a ^{15}N tracer. I hypothesized that the nitrogen tracer study would demonstrate that deposit feeders could utilize this resource, incorporating the nutrients into their tissues and then passing the nutrients to higher trophic levels, as indicated by elevated $\delta^{15}N$ values. I also hypothesized that the field study would show differential use of biodeposits as a basal resource dependent on the season, with a greater influence of the biodeposit resource in the summer when it was more available, and with a smaller influence in the early spring when biodeposit production was minimal.

Methods

δ^{15} Nitrogen Tracer Experiment

Two deposit feeders, *Melita nitida* (amphipod) and *Neanthes succinea* (polychaete) were fed isotopically labeled phytoplankton and isotopically labeled biodeposits. Labeling of phytoplankton and biodeposits was conducted in the same manner for both studies.

Isotopic Labeling of Algae

A dense culture of *Tetraselmis chuii* was grown. In order to determine the amount of algae that was present in a culture carboy, an algae culture volume: dry algae biomass (algae culture ml: dry algae g) ratio was determined. Algae cells from a 15-ml sample of the *T. chuii* culture were collected on a precombusted and preweighed glass fiber filter (25mm Whatman GF/F). The filter and algae were dried for 2 hours at 80°C and weighed. The initial weight of the filter was subtracted from the weight of the algae plus the filter in order to determine the biomass of the dried algae. The volume of algae culture: dry algae biomass (algae culture ml: dry algae g) ratio was used to estimate the

dry biomass of the algae in the entire culture carboy. Using the dry biomass, an estimate of the total mass of nitrogen in the tank was made. It was assumed that carbon composed 45% of the total weight of the algae sample (J. Cornwell, personal communication, 2013) and from this estimate of carbon weight, the Redfield ratio (106C:16N) was used to estimate the amount of nitrogen present in the algae in the carboy. Combining all these steps, grams of nitrogen in the carboy was equal to:

Equation 4. $N = A * 0.0679$

where N was the grams of nitrogen in the carboy and A was the grams of algae in the carboy. Based on this nitrogen value, it was calculated how much $Na^{15}NO_2$ (98 atom % ^{15}N) to add to the culture stock in order to make a 10% or 5.5% addition of the ^{15}N tracer. The amphipod study was conducted with a 10% ^{15}N enrichment of the algae stock and the polychaete study was conducted with a 5.5% ^{15}N enrichment of the algae stock. After the $Na^{15}NO_2$ was added to the culture it was given 48 hours to incorporate into the algae. Part of this algae culture was fed directly to either amphipods or polychaetes and part of the culture was fed to *C. virginica* in order to produce labeled oyster biodeposits.

Isotopic Labeling of Biodeposits

After the isotope had been incorporated into the algae, it was fed to cleaned *C. virginica*, held in an aerated, static tank. Approximately fifty oysters were used for labeled biodeposit generation. The volume of algae addition varied from day to day, in order to generate varying amounts of biodeposits for the tracer study, as there were fewer organisms to feed each day of the study. Oysters were allowed to produce biodeposits for two days before biodeposits were siphoned from the tank and collected on a 20-µm screen. A 5-ml sample of biodeposits was collected on a preweighed and precombusted glass fiber filter (25mm Whatman GF/F), which was dried for 2 hours at 80°C and

reweighed to determine a dry biomass of the biodeposits collected. This volume to dry biomass (biodeposits ml: dry biodeposits g) ratio was used to determine the amount of biodeposits added to each mesocosm.

Algae Condensing

In order to examine an alternate food source and its incorporation by potential deposit feeders of the oyster reef, labeled phytoplankton was also provided to deposit feeders as part of the tracer study. Algae cells, which had been labeled with an elevated level of ^{15}N, were consolidated by placing algae cultures in $-20^{0}C$ for approximately 8 hours and were then moved to 4°C for another 24 hours. As the algae chilled, the cells died and fell out of suspension, condensing at the base of the carboy. Water from the top of the carboy was then siphoned off, leaving the concentrated algae cells at the bottom of the carboy. Algae from a 5-ml sample of the condensed algae was collected on a preweighed and precombusted glass fiber filter (25 mm Whatman GF/F). The algae and filter were dried for 2 hours at 80°C and weighed to determine the dry biomass of the algae, establishing a ratio of condensed algae volume to dry algae biomass (condensed algae ml: dry algae g). This ratio was used to add specific masses of algae to mesocosms for the tracer experiments.

Tracer Experiment – Melita Nitida

Treatment tanks (9.46 l glass aquaria) were filled with 2-µm filtered sea water from the Choptank River, MD and each tank contained an air stone. Seventy-eight amphipods were added to each tank and were starved for 24 hours before the start of the study. Two samples of amphipods were collected after this 24 hours in order to get a baseline isotope value. Three tanks of amphipods received labeled algae and three tanks received labeled biodeposits as a food source. Biodeposit production was inconsistent

and at a lower rate than what was expected, so a consistent amount of biodeposits was not added to each tank each day. For Days 0 through 5, 0.4740 g, 0.0165 g, 0.0426 g, 0.0415 g, 0.1033 g, and 0.1748 g of biodeposits by dry weight were added to each of the appropriate treatment tanks, respectively. Average $\delta^{15}N$ (±SE) of the biodeposits was 2726.3 ± 420.6‰. On the first day 1.422 g ($\delta^{15}N$: 32760‰) of condensed algae by dry weight was added to each of the appropriate treatment tanks due to an error in calculation. On Day 5, an additional 0.1724 g ($\delta^{15}N$: 55978.2‰) of algae was added to each appropriate treatment tank.

Each day, for six days, samples of amphipods were collected from each tank, in order to observe the incorporation of the isotope label over time. Ten amphipods were removed from each tank each day, starting one day after food was added to the tanks. After amphipods were removed, they were placed into containers filled with 2-µm filtered sea water from the Choptank River, MD. Amphipods were held in these tanks for 24 hours to allow them to expel their guts before isotope analysis. Amphipods were then placed into scintillation vials and frozen at -20^{0}C until preparation for isotope analysis.

Tracer Experiment - Neanthes succinea and Gobiosoma bosc

Treatment tanks (9.46 l glass aquaria) were filled with 2-µm filtered sea water, air stones, and a layer of gravel. Ninety polychaetes were added to each tank and then starved for 24 hours before the start of the study. Three tanks received algae and three tanks received biodeposits as a food source. Biodeposit production rates were inconsistent and lower than expected, so inconsistent amounts of biodeposit additions were made. Biodeposits and algae were added to tanks every other day, to allow the oysters more time to produce biodeposits. The same biomass in dry weight was added to each tank, for both biodeposits and algae. On Day Zero 0.0065g of food by dry mass was

added, 0.1866g was added on Day 2, 0.1782g was added on Day 4, 0.271g was added on Day 6, and 0.3483g was added on Day 8. The mean $\delta^{15}N$ (±SE) of the labeled biodeposits and labeled phytoplankton was 5454.4 ± 899.8‰ and 19664.6 ± 2111.0‰, respectively. The study was run for a total of 10 days.

Each day, samples of polychaetes were collected from each tank, in order to observe how the isotope label was incorporated over time. Six polychaetes were sampled prior to the addition of either biodeposits or phytoplankton in order to get a baseline isotope signature for the polychaetes. Then one to two polychaetes, depending on size, were removed from each tank each day, starting one day after food was added to the tanks. After polychaetes were removed, they were placed into containers filled with 2-µm filtered sea water. Polychaetes were held in these tanks for 24 hours to allow them to expel their guts to prepare them for isotope analysis. Then polychaetes were placed in scintillation vials and frozen at -20^0C until preparation for isotope analysis. For one of the phytoplankton replicates polychaetes could not be sampled on day 9 or day 10 as not enough polychaetes were available for sampling on these days.

Individual *Gobiosoma bosc* were held in 9.46 l glass aquaria filled with 2-µm filtered Choptank River water and an airstone. Twelve naked gobies were initially held in tanks for 24 hrs without food. Six of these fish were then sacrificed by an overdose of buffered tricaine methanesulfonate (300 mg l^{-1}) in order to get a baseline $\delta^{15}N$ value for the fish. The remaining six fish were fed 1-2 polychaetes that had either been feeding on labeled biodeposits or labeled algae for the previous three days. Three replicates for both algae and biodeposits were conducted. Polychaetes were fed to fish for 7 days, after which, the fish were moved to new 9.46 l tanks with 2-µm filtered sea water and held for

24 hours to expel their guts in preparation for isotope analysis. Fish were then euthanized with an overdose of buffered tricaine methanesulfonate (300 mg l^{-1}) and frozen at -20^{0}C until preparation for isotope analysis.

Isotope Analysis Preparation

To prepare organisms for nitrogen isotope analysis, samples were defrosted and dried in aluminum weigh boats at 60°C for 72 hours. Dried samples were ground with a mortar and pestle, weighed, and packaged in tin capsules. Samples were sent to the University of California Davis Stable Isotope Facility for $\delta^{15}N$ analysis which was conducted with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Isotope values were reported using delta notation, $\delta^{15}N$, where $\delta^{15}N = ((R_{sample}-R_{standard})/R_{standard})*1000$ and the ratio of $^{15}N/^{14}N$ is R. Atmospheric nitrogen was used for the reference standard.

Statistical Analysis

Differences in $\delta^{15}N$ values for the treatment factor day for *N. succinea* and *M. nitida* were determined using repeated measures analysis followed by contrasts between day and baseline values for multiple mean comparisons. Analysis was conducted with a multilevel linear model. The model included a fixed factor, day, and a random factor, replicate within day, that accounted for the fact that organisms were sampled from the same experiment tank over the course of the study (Field et al. 2012). Differences in $\delta^{15}N$ values for *G. bosc* were determined using one-way ANOVAs with day as a fixed factor. $\delta^{15}N$ values for *N. succinea* that were fed phytoplankton were $\log_{10}$ transformed to reach assumptions of normality and homogeneity of variances. $\delta^{15}N$ values for *G. bosc* fed *N. succinea* that consumed phytoplankton were inverse transformed in order to

meet assumptions of normality and homogeneity of variances. $\delta^{15}N$ values for *G. bosc* fed *N. succinea* that consumed oyster biodeposits were $\log_{10}$ transformed in order to meet assumptions of normality and homogeneity of variances. All statistical test were conducted with R statistical program (R Core Team 2014). Analysis of Deviance and ANOVA tables were generated using the "Anova" function in the "car" package (Fox and Weisberg 2011) and multilevel linear models were tested using the "lme" function in the "nlme" package (Pinheiro et al. 2014). Multiple mean comparisons were conducted using the "glht" function in the "multcomp" package (Hothorn et al. 2008).

Seasonal Stable Isotope Sampling – Natural Abundances

Organisms from the oyster reef ecosystem that were potentially part of an oyster biodeposits based food web were collected on September 20, 2013, April 18, 2014 and August 8, 2014 from the oyster reef, Weems Upper, in the mainstem Severn River, MD. Samples of basal resource pools, including *C. virginica* biodeposits, were also collected at each sampling date in order to assess the contribution of each resource to the food web. This reef was constructed by the US Army Corps of Engineers and had a granite and shell veneer base substrate layer that was covered with oyster clumps. It was planted with oysters in 2001 and 2002. The reef depth was 2-3 m. This reef was located three miles from the mouth of the Severn River, and was flanked on either side by residential homes and docks and the waterway was heavily trafficked by personal and naval vessels. The main vegetation flanking the sides of the river in the stretch that contained the reef were grass lawns, with a few small patches of marsh grasses, predominantly *Phragmites australis*.

The basal resource pools that were sampled were surface sediment, benthic algae, seston, and *C. virginica* biodeposits. Surface sediment was collected two ways. First,

divers used an ice scoop to scoop off the top 1 cm of the sediment. The sediment was placed in a plastic resealable bag underwater and brought to the surface. Second, divers used a 10-ml syringe to siphon off the top 5 mm of sediment on the oyster reef. All sediment samples were frozen at -20°C until preparation for isotope analysis. Surface sediment was collected as a proxy for benthic algae, but benthic algae were also sampled by placing glass slides at the reef location. Glass slides were attached to bricks with silicon and placed at the study site by divers one month prior to sample collection. Benthic algae were allowed to colonize the glass slides and then they were retrieved on the sample collection day. Slides were transported back to the lab, where they were rinsed with distilled water; then material from the slides was removed with a razor blade and frozen at -20°C until analysis preparation. It was difficult to attain a sample of pure phytoplankton, but phytoplankton, zooplankton, bacteria and other suspended solids in the water column tend to have similar isotopic signatures (Fry and Sherr 1984). Given this knowledge, seston was sampled as a proxy for phytoplankton by collecting a 1-L sample of bottom water from the reef location. All water samples were frozen at -20ºC until prepared for analysis (Stribling and Cornwell 1997). In order to obtain pure biodeposit samples, oysters were collected from the field sample location. The oysters were rinsed with freshwater and epiflora and epifauna were removed with shucking knives and stiff bristled brushes. Freshwater was then used to rinse the oysters again before placing them into a 0.8% domestic hypochlorite solution for 2 minutes to remove and kill all cryptic organisms. Oysters were then placed in freshwater for six minutes to rinse the bleach solution and remove any remaining cryptic organisms. The oysters were then placed into a tank with Instant Ocean that was the same salinity as the site where the

oysters were collected for 48-72 hours to allow the oysters to evacuate their biodeposits. The biodeposits were collected on a 100-um screen, rinsed with distilled water and dried at 60^0C for 3 days or frozen at -20°C until isotope analysis, if samples could not be prepared for isotope analysis immediately.

Apocorophium lacustre, M. nitida, N. succinea, G. bosc, Ischadium recurvum and *Rhithropanopeus harrisii* were collected for stable isotope analysis on September 20, 2013, April 18, 2014, and August 8, 2014. In addition, *C. virginica* and *Palaemonetes pugio* were collected in April and August, and *Mucrogammarus mucronatus* was collected in April 2014. These species are common residents of oyster reefs and are potentially part of an oyster biodeposit based food chain. Organisms were collected by divers deploying six 0.58 m by 0.58 m plastic bread trays lined with 1-mm fiberglass mesh at the site, filling trays with oyster clumps from the reef, and collecting the trays one month after deployment. At the time of collection the trays were capped with a 1-mm mesh lid and brought up to the surface for processing. Five replicates of each species were collected, when possible, in order to attain an average isotopic signature for each species. Each tray collected served as a replicate. Fish were removed from the oyster clumps first and placed into plastic bags half filled with raw Severn River water and half filled with air. Fish were then placed into coolers for transport. Mud crabs were hand collected from the trays and placed into containers filled with raw water. Smaller macroinvertebrates were washed from the oyster shells into buckets of raw water. These macroinvertebrates were then transported back to the lab where they were sorted with the assistance of a dissecting microscope. Oysters from which the organisms were washed were collected and transported back to the lab to generate biodeposits. In September, the

trays and benthic algae slides could not be located at the reef; they were most likely removed by boaters or fishermen during the previous month. Therefore, in September organisms were collected directly from oyster clumps from the reef. Divers retrieved clumps and transported them to the boat, where fish were removed from between oysters and within boxes and other reef organisms were collected and rinsed off of the shells.

In the laboratory, organisms were divided into species and replicates and were individually held in tanks of Instant Ocean for 24 hours in order to allow the organisms to evacuate their guts prior to isotopic analysis. The salinity of the tanks matched the salinity of the collection location. After the organisms had evacuated their guts, they were rinsed with distilled water and frozen at -20°C, except for fish, which were euthanized using a buffered solution of 300 mg l^{-1} tricaine methanesulfonate (Yeager and Layman 2011) and then frozen at -20°C until analysis. Depending on the size of the organisms, either a single individual was used for isotope analysis, or multiple organisms of the same species were pooled together for a sample. Multiple organisms were sampled for *N. succinea, A. lacustre, M. nitida, M. mucronatus* and *R. harrisii* while individual organisms were sampled for *G. bosc, I. recurvum, C. virginica* and *P. pugio.*

To prepare the basal resources for isotope analysis, all samples were defrosted and sediment samples were rinsed with distilled water. Sediment samples and benthic algae samples were dried at 60°C for 72 hours. These dried samples, along with the dried biodeposits samples, were ground with a mortar and pestle or ball mill grinder to homogenize the samples. Access to a ball mill grinder was only available for April and August samples, so all September samples were ground by hand using a mortar and pestle. Samples were then fumed with HCl for 12-16 hours to remove any carbonates

present. Then 10 mg – 30 mg of ground material was packed into a tin capsule for isotope analysis. Defrosted water samples were passed through a precombusted (450°C for 4 hours) glass fiber filter (25 mm Whatman GF/F) (Gergs et al. 2011) until the filter clogged. The filter was then dried for 72 hours at 60°C. Filters were fumed with HCl for 12-16 hours, redried at 60°C for 48 hours, and were packed into tin capsules for isotope analysis.

To prepare organisms for nitrogen and carbon stable isotope analysis, they were defrosted and dried in aluminum weigh boats at 60 °C for 72 hours. Dried samples were ground with a mortar and pestle or ball mill grinder, fumed with HCl for 12-16 hours, weighed, and packaged in tin capsules.

September isotope samples were sent to the University of California Davis Stable Isotope Facility for $\delta^{13}C$ and $\delta^{15}N$ analysis which was conducted with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). April and August samples were sent to the Central Appalachians Stable Isotope Facility for $\delta^{13}C$ and $\delta^{15}N$ analysis with Thermo Fisher Delta V+ isotope ratio mass spectrometer interfaced with Carlo Erba NC 2500 Elemental Analyzer. Isotope values were reported using delta notation, where $\delta^{15}N$ or $\delta^{13}C = ((R_{sample}-R_{standard})/R_{standard})*1000$ and the ratio of heavy isotope to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) is R. Atmospheric N_2 gas and Pee Dee Belemnite were used as international reference standards for nitrogen and carbon, respectively.

Statistical Analysis

$\delta^{13}C$ and $\delta^{15}N$ values of organisms and basal resources were compared between sampling dates using one way ANOVAs or Kruskal-Wallis Tests for normal or non-

normal data, respectively. Two outliers were removed from the data set, one value for biodeposits sampled in April 2014 and one value of *A. lacustre* sampled in August 2014. Both of these values were much higher than what would have been expected biologically and were much higher than other replicates and so they were removed from the analyses. This created a sample size of one for *A. lacustre* for August, so no comparisons between this sample date and others could be made for this species. Tukey's HSD and Nemenyi test with Tukey's distribution (for normal and non-normal data, respectively) were used for multiple mean comparisons between sampling dates. For $\delta^{15}N$ values for biodeposits and $\delta^{13}C$ values for *M. nitida*, multiple mean comparisons were made by performing multiple Kruskal-Wallis test and then using a Bonferroni adjustment for the alpha (0.05/3=0.0167) due to unequal sample sizes for each sampling date which prevented the use of the Nemenyi test. All statistical test were conducted using R statistical program (R Core Team 2014). ANOVA tables were generated using the "Anova" function in the "car" package (Fox and Weisberg 2011). Multiple mean comparisons were conducted using the "glht" function in the "multcomp" package (Hothorn et al. 2008) and the "posthoc.kruskal.nemenyi.test" function in the "PMCMR" package (Thorsten 2014). In addition, trophic position was determined for organisms each season using the following equation:

Equation 5. $$TP = \lambda + \frac{\delta^{15}N_{secondary\ consumer} - \delta^{15}N_{base}}{3.4‰}$$

where λ is the trophic position of N_{base}, $\delta^{15}N_{secondary\ consumer}$ is the nitrogen isotopic value of the secondary consumer of interest, and $\delta^{15}N_{base}$ is the nitrogen isotopic value of the basal resource (Cabana and Rasmussen 1996). $\delta^{15}N_{base}$ was calculated by averaging the $\delta^{15}N$ values for all the basal resources in that season.

Results

δ^{15} Nitrogen Tracer Experiment

Melita nitida

Amphipods that were offered *T. chuii* labeled with an elevated $\delta^{15}N$ signature increased their average $\delta^{15}N$ signature over the course of six days of being fed the algae (Figure 16). Amphipod isotope signatures were significantly different from baseline levels starting after day 1 of sampling, and continued to be different from baseline levels for all remaining days of sampling (repeated measures χ^2=122.954, df=6, P<0.001, contrasts P<0.05, Figure 16). Baseline $\delta^{15}N$ values were 16.5 ± 0.2‰ and $\delta^{15}N$ values for days 1-6 were 1762.0 ± 304.6‰, 2674.3 ± 274.2‰ , 4050. 9 ± 122.1‰, 4218.4 ± 908.7‰, 5842.2 ± 470.6‰, and 5186.7 ± 784.8‰, respectively. In addition, amphipods that were offered oyster biodeposits that had been labeled with an elevated $\delta^{15}N$ signature also had an elevated $\delta^{15}N$ signature. A significant difference from the baseline amphipod $\delta^{15}N$ value, 16.5 ± 0.2‰, was seen starting on day 1, 462.9 ± 30.1‰, and the amphipod $\delta^{15}N$ values continued to be different from baseline values for days 2-6 with values of 743.4 ± 126.6‰ , 833.5 ± 139.5‰, 1340.1± 264.0‰, 1258.7 ± 182.4‰, 1633.4 ± 302.9‰, respectively (repeated measures χ^2=165.4, df=6, P<0.001, contrasts P<0.05, Figure 16).

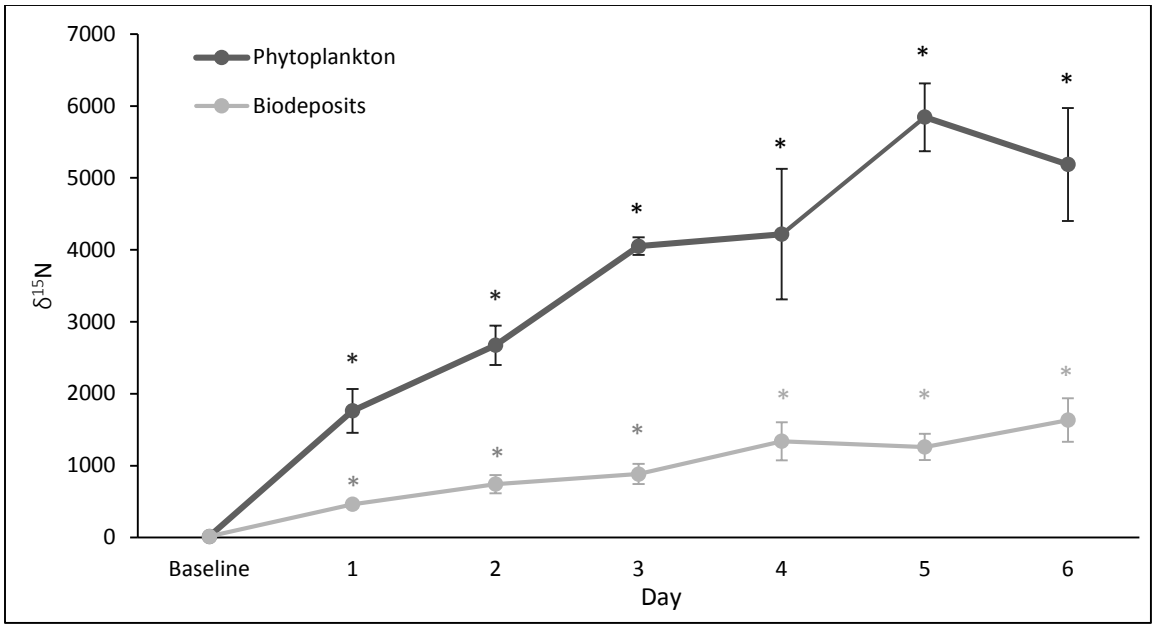

Figure 17. $\delta^{15}N$ values of *Melita nitida* collected on days 0-6 after being fed phytoplankton and biodeposits enriched with ^{15}N. Dark gray lines indicate isotope values for amphipods fed enriched phytoplankton while light gray lines indicate isotope values for amphipods fed enriched biodeposits. * indicates that value is significantly different from the baseline.

Neanthes succinea and Gobiosoma bosc

Polychaetes that were provided with phytoplankton labeled with elevated levels of $\delta^{15}N$ as a food source had significantly elevated levels of $\delta^{15}N$ after feeding on the phytoplankton for five days. Mean baseline $\delta^{15}N$ value of polychaetes was 96.0 ± 52.8‰ (±SE), while mean $\delta^{15}N$ values of polychaetes (±SE) on day 5-10 were 571.3 ± 100.1‰, 682.9 ± 121.1‰, 1829.7 ± 442.4‰, 1523.0 ± 526.3‰, 2244.6 ± 2118.3‰, and 2003.3 ± 1230.4‰, respectively (repeated measures χ^2=108.76, df=10 P<0.001, contrasts P<0.05, Figure 17). *G. bosc* that were fed polychaetes which had been feeding on labeled phytoplankton had a significantly enriched $\delta^{15}N$ signature after feeding on the polychaetes for seven days. Baseline *G. bosc* had a mean $\delta^{15}N$ value of 18.8 ± 0.2‰ and *G. bosc* sampled on day seven had a mean $\delta^{15}N$ value of 185.2 ± 19.1‰ (ANOVA F=3154.7, df=1, 4 P<0.001, Figure 18).

Polychaetes were also provided with biodeposits that had been labeled with an elevated $\delta^{15}N$ signature as a food source. These polychaetes had a $\delta^{15}N$ signature that was elevated compared to baseline levels on day 5, 7, 9 and 10. Mean baseline polychaete $\delta^{15}N$ value was 100.0 ± 39.1‰ and mean $\delta^{15}N$ values of polychaetes collected on day 5, 7, 9 and 10 of sampling were 795.0 ± 229.3‰, 833.5 ± 265.8‰, 2244.6 ± 402.8‰ and 1420.7 ± 174.9‰, respectively (repeated measures χ^2=149.4, df=10, P<0.001, contrasts P<0.05, Figure 17). *G. bosc* that consumed polychaetes that had consumed labeled biodeposits had significantly increased $\delta^{15}N$ signatures after seven days. Baseline gobies had a mean $\delta^{15}N$ value of 19.7 ± 1.3‰ while gobies sampled after consuming polychaetes for seven days had a mean $\delta^{15}N$ value of 245.6 ± 46.7‰ (ANOVA F=164.54, df=1, 4 P<0.001, Figure 18).

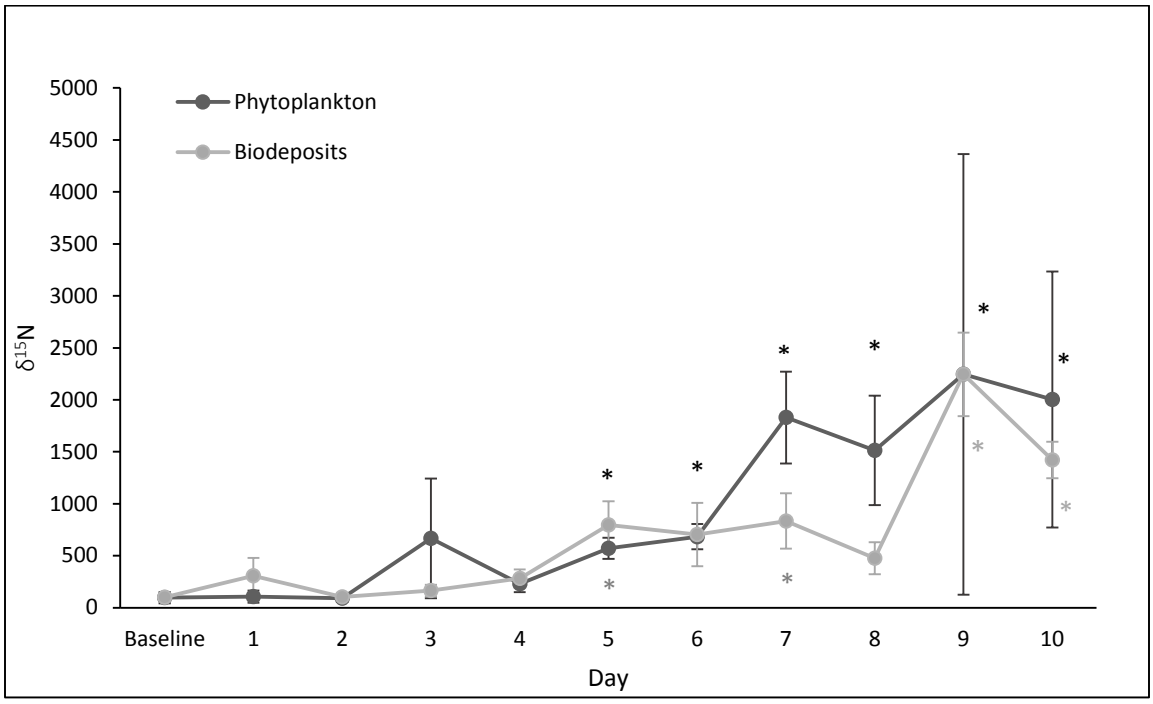

Figure 18. $\delta^{15}N$ values of *Neanthes succinea* collected on days 0-10 after being fed phytoplankton and biodeposits enriched with ^{15}N. Dark gray line indicates isotope values for polychaetes that were fed enriched phytoplankton and the light gray line indicates isotope values for polychaetes that were fed enriched biodeposits. * indicates that value is significantly different from the baseline

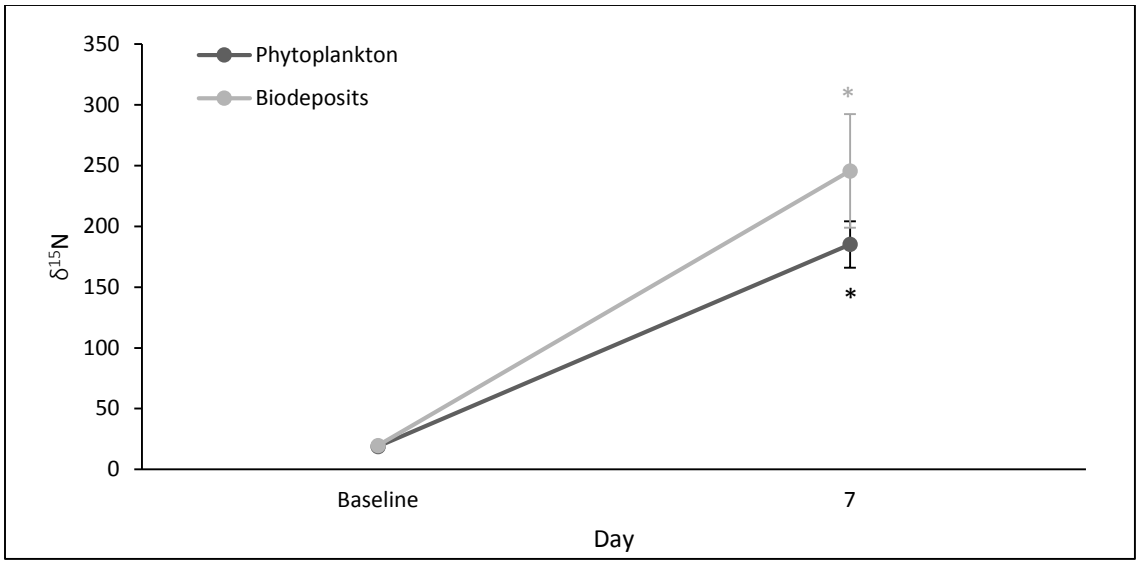

Figure 19. $\delta^{15}N$ values of *Gobiosoma bosc* collected on day 0 and 7 after consuming polychaetes which had consumed phytoplankton or biodeposits enriched with ^{15}N. Dark gray line indicates isotope values for naked gobies that consumed polychaetes fed enriched phytoplankton and the light gray line indicates isotope values for naked gobies that consumed polychaetes that were fed enriched biodeposits. * indicates that value is significantly different from the baseline.

Seasonal Stable Isotope Sampling – Natural Abundances

Seasonal sampling of oyster reefs showed most organisms and basal resources varied in their $\delta^{13}C$ values and $\delta^{15}N$ values over the course of the year (Figure 19, Figure 20). Basal resources and organisms were sampled in three seasons (fall, spring, and summer) on September 20, 2013 when water temperatures were 22.7°C, April 18, 2014 when water temperatures were 13.0°C and on August 6, 2014 when water temperatures were 26.6°C. Basal resources that were sampled included biodeposits, surface sediment sampled by a scoop, surface sediment sampled by a syringe, benthic algae collected on glass slides, and seston. Biodeposit $\delta^{13}C$ values were more enriched in September than April sampling, but all other seasons were similar (Kruskal–Wallis Test $H=10.82$, $df=2$, $P=0.004$). Biodeposits $\delta^{15}N$ values also differed between sampling dates, with greater enrichment of $\delta^{15}N$ in September compared to August and April (Kruskal-Wallis Test,

H=6.02, df=2, *P*=0.049). Surface sediment that was sampled by scooping the top centimeter of sediment from the reef location had significant differences in $\delta^{13}C$ but not $\delta^{15}N$ between sampling dates (Kruskal-Wallis Test H=9.62, df=2, *P*=0.008). Sediment sampled in September was more enriched in $\delta^{13}C$ than the sediment sampled in April, however, all other sampling dates did not differ from one another. Surface sediment that was sampled by a syringe placed into the top half centimeter of reef sediment also showed significant differences in $\delta^{13}C$ values between sampling dates, however it also showed differences in $\delta^{15}N$ values between sampling dates (Kruskal-Wallis Test $\delta^{13}C$: H=7.98, df=2, *P*=0.019; $\delta^{15}N$: H=11.18, df=2, *P*=0.004). Surface sediment was more enriched in $\delta^{13}C$ and $\delta^{15}N$ in September than April sampling (Nemenyi Test $\delta^{13}C$: *P*=0.029, $\delta^{15}N$: *P*=0.003). Benthic algae were collected on slides for two sampling dates, April and August, and they were significantly different in both $\delta^{13}C$ values and $\delta^{15}N$ values, with both values having greater enrichment in August (Kruskal-Wallis Test $\delta^{13}C$: H=6.82, df=1, *P*=0.009 $\delta^{15}N$: H=6.92, df=1, *P*=0.009). Significant differences in $\delta^{13}C$ and $\delta^{15}N$ were also found between sampling dates for seston samples, with more enriched $\delta^{13}C$ values in August compared to April and more enriched $\delta^{15}N$ values in September compared to April (Kruskal-Wallis Test $\delta^{13}C$: H=10.24, df=2, *P*=0.006 $\delta^{15}N$: H=9.80, df=2, *P*=0.007, Figure 21-23, Table 12).

All organisms, except for *R. harrisii* had different $\delta^{13}C$ values at different sampling dates. Of those organisms with significantly different values by sampling date, all except for *M. nitida* and *G. bosc*, had significantly depleted $\delta^{13}C$ values in April compared to August. $\delta^{13}C$ values were similar between April and August for *M. nitida* and *G. bosc*. When September $\delta^{13}C$ values were compared to April, September samples

were more depleted than April samples for *A. lacustre* and *I. recurvum,* but not between *N. succinea, M. nitida,* and *G. bosc,* which were similar between these two dates. $\delta^{13}C$ values from September differed for all organisms, except *R. harrisii*, when compared to August. All samples were more depleted in September than in August (Figure 19, 21-23, Table 12).

$\delta^{15}N$ values differ significantly between sampling dates for all organisms except for *P. pugio* and *G. bosc.* April and August samples were similar for all organisms except *C. virginica,* which was more enriched in August. $\delta^{15}N$ values differed for all species, except *P. pugio* and *G. bosc*, between September and April, with all species except for *N. succinea* showing greater enrichment in April. *Neanthes succinea* had greater enrichment in September. When $\delta^{15}N$ values were compared between September and August sampling, only *M. nitida* and *N. succinea* were significantly different. *Neanthes succinea* was more enriched in ^{15}N in September and *M. nitida* was more enriched in August (Figure 19, 21-23, Table 12).

Reef organisms were least enriched in September 2013 in $\delta^{13}C$ values, slightly more enriched in April 2014, and then enriched the most in August 2014 (Figure 19). Basal resources however, followed a different pattern, where they were the most depleted in April 2014, followed by more enriched basal resources in August 2014 and the most enriched basal resources in September 2013 (Figure 20). Trophically, as represented by $\delta^{15}N$ values, reef samples followed a similar trend at each sampling date. Basal resources had the lowest trophic position, followed by invertebrates that held a mid-range trophic position, and finally the vertebrate, *G. bosc*, held the highest trophic position in all seasons. Within this structure, the invertebrates seemed to break into two groups, one

with a lower trophic level than the other. *A. lacustre*, *I. recurvum*, *C. virginica*, and *M. mucronatus* (when it was sampled), held the lower trophic positions of the invertebrates and *M. nitida*, *R. harrisii*, *N. succinea*, and *P. pugio* held the higher trophic positions in the reef food web.

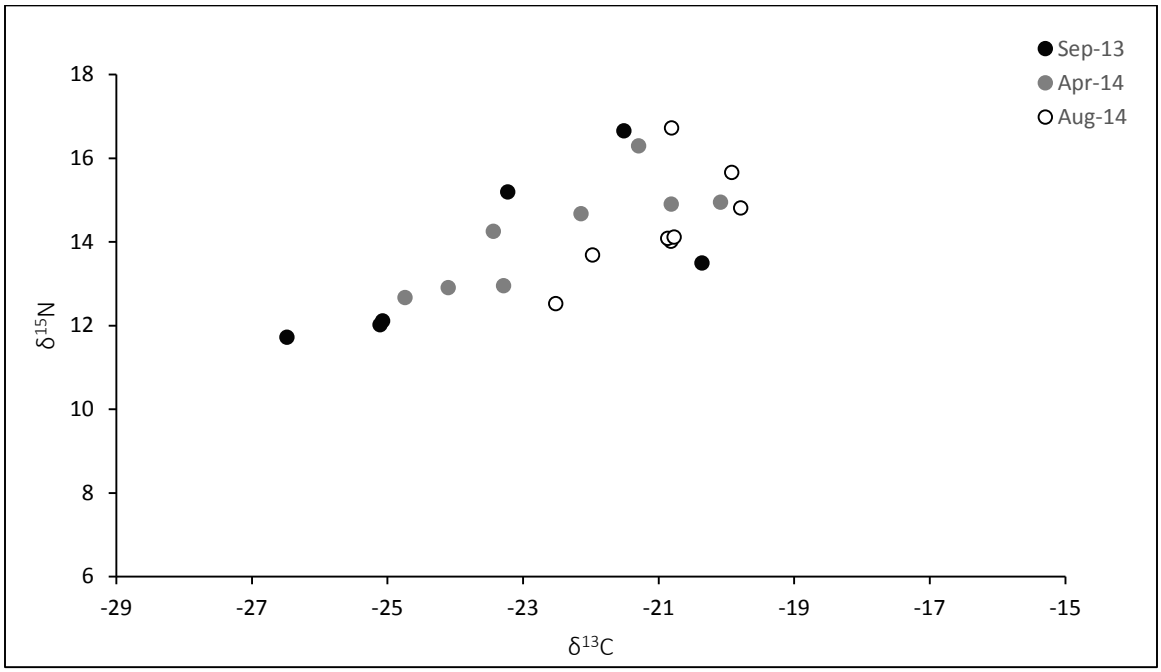

Figure 20. Mean stable isotope signature of reef organisms collected on September 20, 2013 (black), April 18, 2014 (gray), and August 8, 2014 (white) from Weems Upper oyster reef in Severn River, MD.

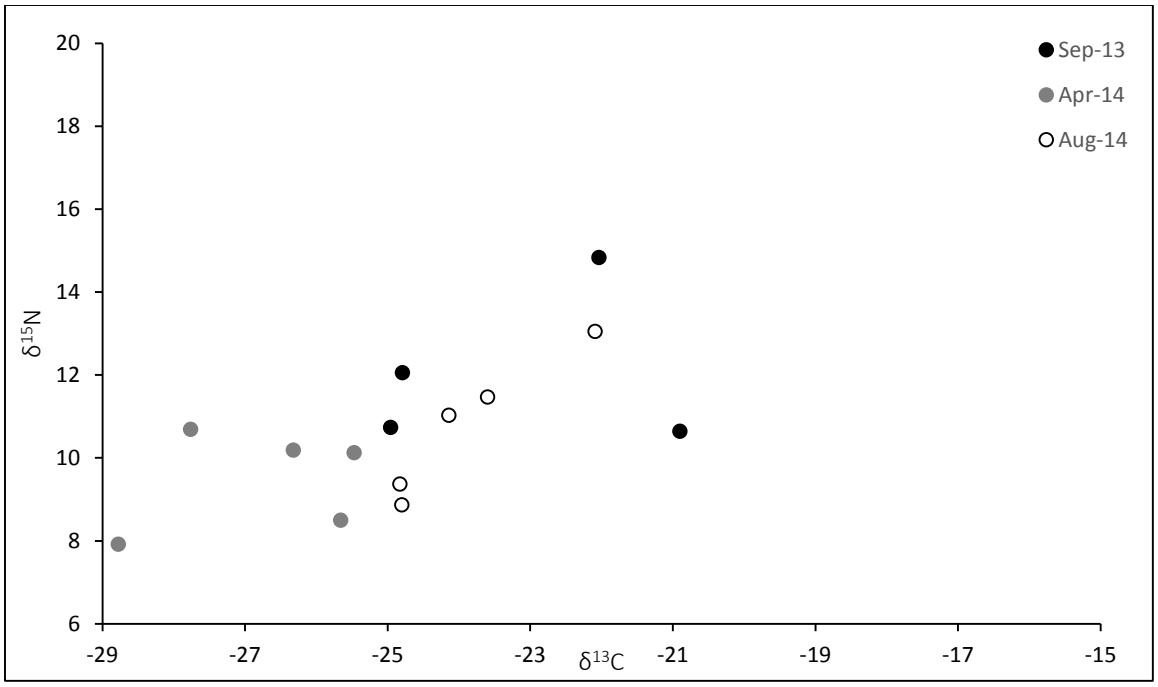

Figure 21. Mean stable isotope signature of reef basal resources collected on September 20, 2013 (black), April 18, 2014 (gray), and August 8, 2014 (white) from Weems Upper oyster reef in Severn River, MD.

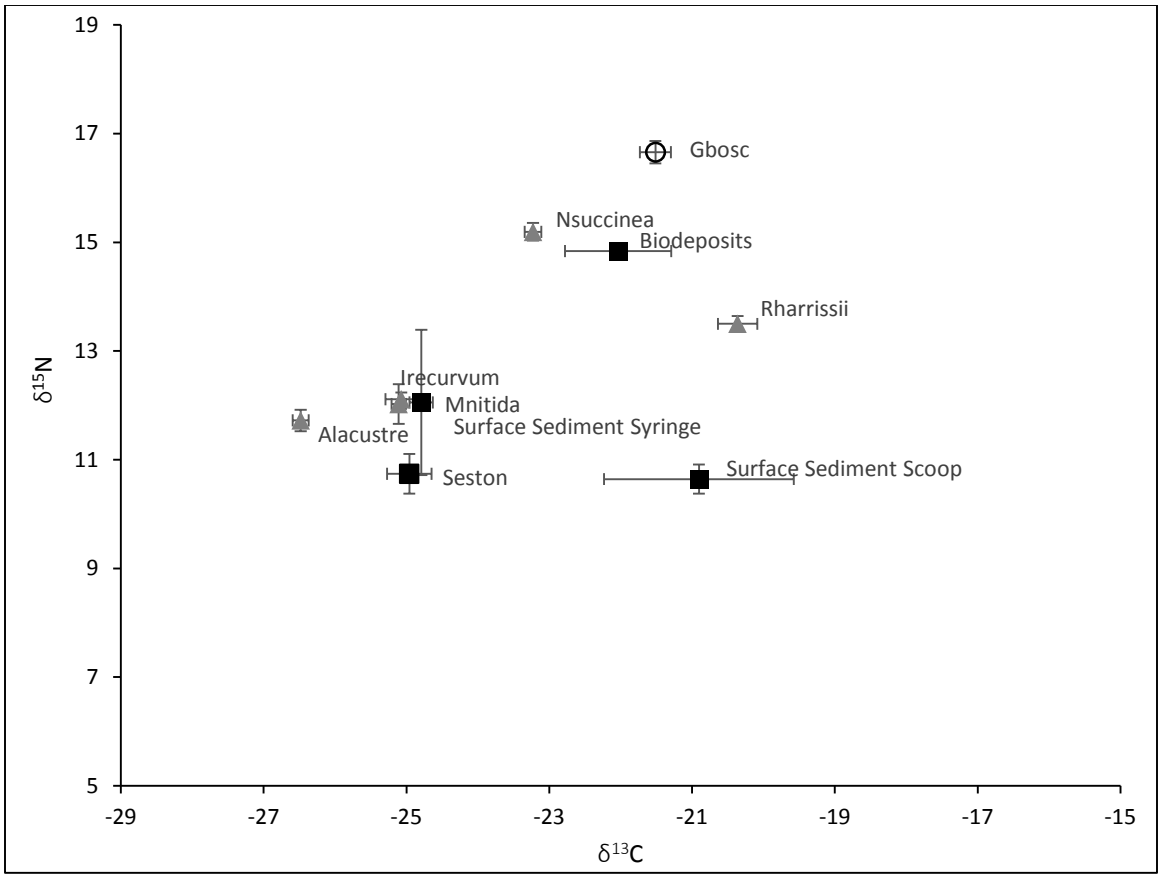

Figure 22. Mean stable isotope signature ± SE of reef organisms and basal resources collected from Weems Upper oyster reef in Severn River, MD on September 20, 2013. Basal resources are indicated by a black square, invertebrates are marked with a gray triangle, and vertebrates are represented with an open circle.

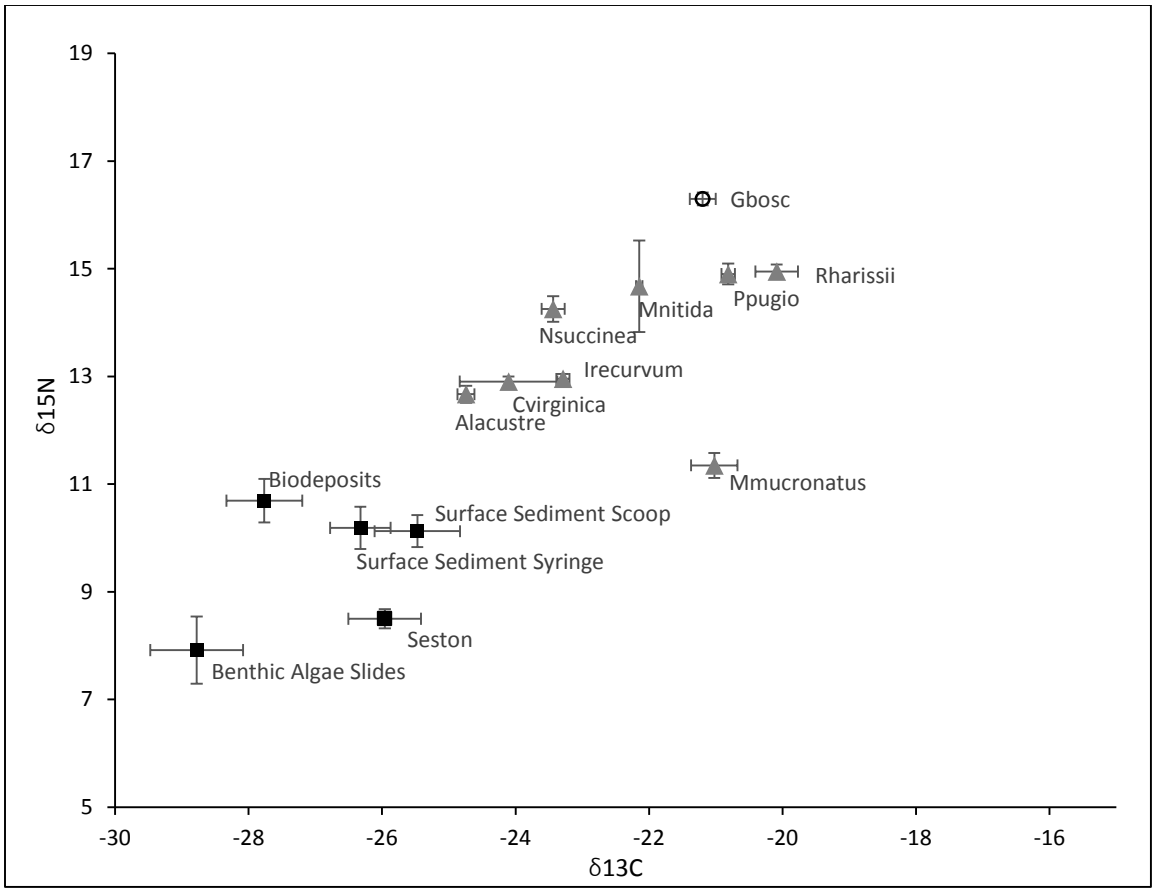

Figure 23. Mean stable isotope signature ± SE of reef organisms and basal resources collected from Weems Upper oyster reef in Severn River, MD on April 18, 2014. Black squares indicate basal resources, gray triangles indicate invertebrates, and open circles indicate vertebrates.

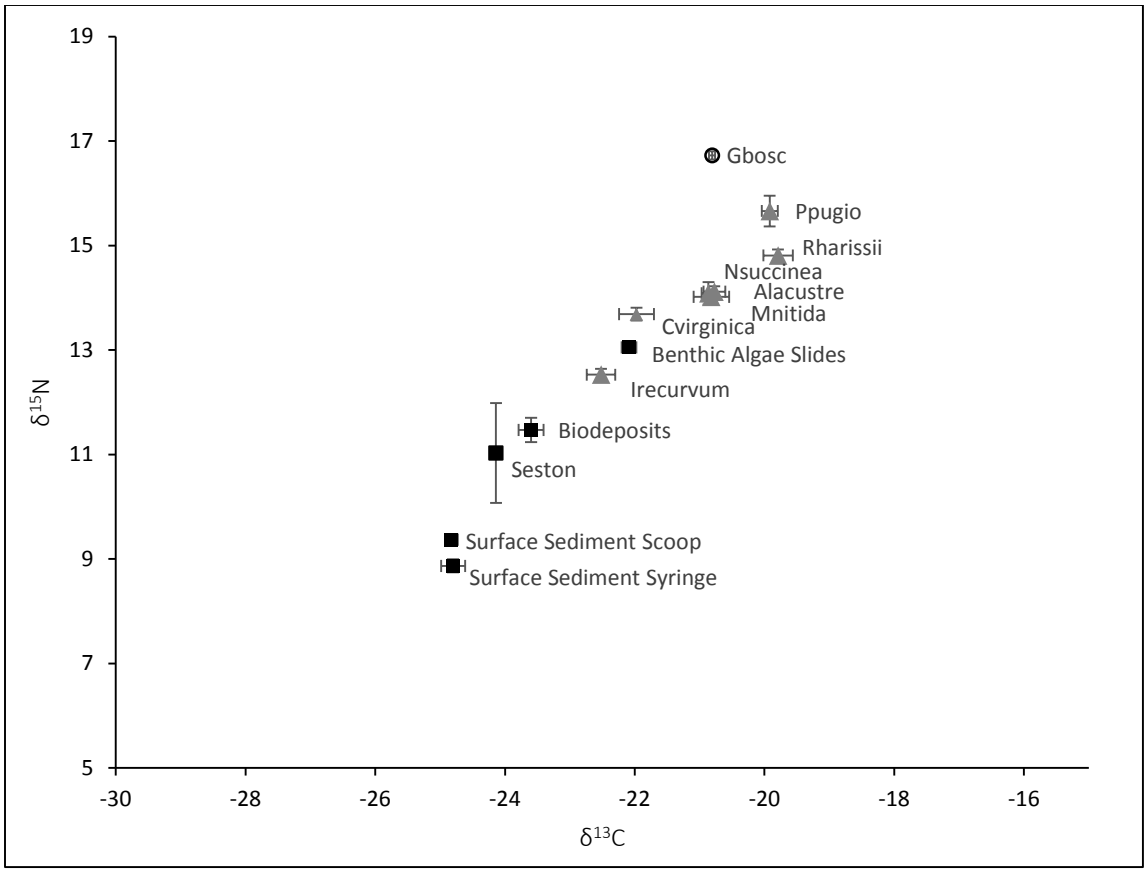

Figure 24. Mean stable isotope signature ± SE of reef organisms and basal resources collected from Weems Upper oyster reef in Severn River, MD on August 8, 2014. Basal resources are marked with a black square, invertebrates are marked with a gray triangle, and vertebrates are marked with an open circle.

Table 9. Trophic position of reef consumers from Weems Upper oyster reef in the Severn River, MD at three sampling dates, September 20, 2013, April 18, 2014, and August 8, 2014. Trophic position determined from the baseline of the average $\delta^{15}N$ of all basal resources on that sampling date. Blank spaces indicate that the species was not sampled on the date.

Species	September 2013	April 2014	August 2014
Mucrogammarus mucronatus		1.548	
Apocorophium lacustre	0.898	1.937	1.959
Crassostrea virginica		2.006	1.862
Ischadium recurvum	1.013	2.020	1.521
Neanthes succinea	1.919	2.403	1.988
Melita nitida	1.030	2.526	1.979
Palaemonetes pugio		2.593	2.443
Rhithropanopeus harrisii	1.421	2.607	2.192
Gobiosoma bosc	2.350	3.003	2.755

Table 10. Mean $\delta^{13}C$ values ± SE for organisms and basal resources sampled from the Weems Upper oyster reef in the Severn River, MD on September 20, 2013, April 18, 2014, and August 8, 2014. Blank spaces indicate that a species or resource was not sampled on that date.

	September 2013	April 2014	August 2014
Sample	$\delta^{13}C \pm SE$	$\delta^{13}C \pm SE$	$\delta^{13}C \pm SE$
Basal Resources			
Crassostrea virginica biodeposits	-22.04 ± 0.74	-27.77 ± 0.57	-23.60 ± 0.19
Surface sediment-scoop	-20.90 ± 1.33	-25.47 ± 0.64	-24.83 ± 0.10
Surface sediment-syringe	-24.79 ± 0.16	-26.33 ± 0.45	-24.80 ± 0.18
Seston	-24.96 ± 0.31	-25.66 ± 0.33	-24.14 ± 0.06
Benthic Algae		-28.78 ± 0.69	-22.09 ± 0.11
Consumers			
Mucrogammarus mucronatus		-21.02 ± 0.35	
Apocorophium lacustre	-26.48 ± 0.11	-24.74 ± 0.13	-20.86 ± 0.28
Crassostrea virginica		-24.11 ± 0.73	-21.97 ± 0.27
Ischadium recurvum	-25.07 ± 0.22	-23.29 ± 0.10	-22.52 ± 0.22
Neanthes succinea	-23.23 ± 0.12	-23.44 ± 0.17	-20.77 ± 0.17
Melita nitida	-25.11 ± 0.10	-22.15 ± 0.05	-20.86 ± 0.11
Palaemonetes pugio		-20.81 ± 0.10	-19.92 ± 0.12
Rhithropanopeus harrisii	-20.36 ± 0.28	-20.09 ± 0.32	-19.79 ± 0.23
Gobiosoma bosc	-21.51 ± 0.22	-21.20 ± 0.19	-20.81 ± 0.05

Table 11. Mean $\delta^{15}N$ values ± SE for organisms and basal resources sampled from the Weems Upper oyster reef in the Severn River, MD on September 20, 2013, April 18, 2014, and August 8, 2014. Blank spaces indicate that a species or resource was not sampled on that date.

	September 2013	April 2014	August 2014
Sample	$\delta^{15}N$ ± SE	$\delta^{15}N$ ± SE	$\delta^{15}N$ ± SE
Basal Resources			
Crassostrea virginica biodeposits	14.84 ± 0.11	10.69 ± 0.40	11.47 ± 0.23
Surface sediment-scoop	10.64 ± 0.27	10.13 ± 0.30	9.37 ± 0.08
Surface sediment-syringe	12.05 ± 1.34	10.19 ± 0.39	8.87 ± 0.13
Seston	10.74 ± 0.36	8.50 ± 0.18	11.03 ± 0.96
Benthic Algae		7.92 ± 0.63	13.05 ± 0.05
Consumers			
Mucrogammarus mucronatus		11.35 ± 0.24	
Apocorophium lacustre	11.72 ± 0.20	12.67 ± 0.16	14.02
Crassostrea virginica		12.91 ± 0.09	13.69 ± 0.12
Ischadium recurvum	12.11 ± 0.12	12.95 ± 0.12	12.53 ± 0.11
Neanthes succinea	15.19 ± 0.16	14.25 ± 0.24	14.12 ± 0.10
Melita nitida	12.03 ± 0.37	14.67 ± 0.85	14.09 ± 0.21
Palaemonetes pugio		14.90 ± 0.19	15.66 ± 0.29
Rhithropanopeus harrisii	13.50 ± 0.15	14.95 ± 0.13	14.81 ± 0.11
Gobiosoma bosc	16.66 ± 0.21	16.30 ± 0.12	16.73 ± 0.08

Table 12. P values for ANOVA and Kruskal-Wallis tests for differences in isotope signatures of reef basal resources and organisms between sampling dates: September 2013, April 2014, and August 2014. Multiple mean comparisons were conducted with a Tukey's HSD or Nemenyi for significant test. Comparisons of $\delta^{13}C$ for *Melita nitida* and $\delta^{15}N$ for *Crassostrea virginica* biodeposits were conducted with multiple pairwise Kruskal-Wallis test with a Bonferroni corrected alpha of 0.0167. Significant p-values were represented with bold type face. Blank spaces indicate that a species was not sampled on one of the dates being compared.

	----------$\delta^{13}C$----------					----------$\delta^{15}N$----------				
	P-values		Multiple Comparisons			P-values		Multiple Comparisons		
	ANOVA	Kruskal-Wallis	Aug:April	Sept:April	Sept:Aug	ANOVA	Kruskal-Wallis	Aug:April	Sept:April	Sept:Aug
Basal Resources										
Crassostrea virginica biodeposits		**0.004**	0.100	**0.003**	0.452		**0.049**	0.142	**0.014**	**0.009**
Surface sediment-scoop		**0.008**	0.583	**0.007**	0.100	**0.008**		0.099	0.303	**0.006**
Surface sediment-syringe		**0.019**	0.051	**0.029**	0.975		**0.004**	0.117	0.371	**0.003**
Seston		**0.006**	**0.005**	0.628	0.073		**0.007**	0.051	**0.008**	0.800
Benthic Algae		**0.009**	**0.009**				**0.009**	**0.009**		
Consumers										
Apocorophium lacustre	**<0.001**		**<0.001**	**<0.001**	**<0.001**		**0.020**		**0.020**	
Crassostrea virginica	**0.028**		**0.028**			**0.005**		**0.005**		
Ischadium recurvum	**<0.001**		**0.034**	**<0.001**	**<0.001**	**0.001**		0.066	**<0.001**	0.074
Neanthes succinea	**<0.001**		**<0.001**	0.654	**<0.001**	**0.002**		0.834	**0.006**	**0.002**
Melita nitida		**0.005**	0.025	0.025	**0.009**	**0.004**		0.628	**0.006**	**0.013**
Palaemonetes pugio	**0.001**		**0.001**			0.066				
Rhithropanopeus harrisii	0.411						**0.003**	0.298	**0.002**	0.136
Gobiosoma bosc	**0.027**		0.148	0.472	**0.024**	0.055				

Discussion

δ^{15} Nitrogen Tracer Experiment

The nitrogen tracer study indicated that both *M. nitida* and *N. succinea* incorporated nitrogen from the phytoplankton *T. chuii* and biodeposits produced by *C. virginica*. Over six days, the mean δ^{15}N value of *M. nitida* progressively increased when fed either labeled biodeposits or labeled phytoplankton. After one day, the isotope values of the amphipods that were fed phytoplankton were significantly different from baseline values (Figure 16). δ^{15}N values also increased for amphipods that were fed biodeposits and their nitrogen values were significantly different from baseline values after one day as well (Figure 16). This was similar to other invertebrates, including snails and freshwater crustaceans that rapidly accumulated a nitrogen tracer within two days (Aberle et al. 2005). This indicates that these amphipods were rapidly incorporating new nitrogen into their tissues, and that their isotopic signature reflects recent food sources. Amphipods fed labeled phytoplankton had a higher final enrichment values than those fed biodeposits, but the phytoplankton also had a higher initial δ^{15}N value. Amphipods that consumed phytoplankton only increased their nitrogen isotope level to about a sixth of the value of the enriched algae, while amphipods that incorporated biodeposits increased their nitrogen isotope level to about half that of the value of the labeled biodeposits. This may indicate that biodeposits are more easily or more quickly incorporated by these invertebrates than phytoplankton.

In addition to amphipods, polychaetes also showed an increase in δ^{15}N values over the course of the study. After five days, polychaetes that were fed phytoplankton had nitrogen signatures that were significantly different from baseline polychaete values (Figure 17). For polychaetes that were fed biodeposits, a significant difference in

nitrogen isotope signatures from baseline polychaete signatures was observed on day 5, 7, 9, and 10 (Figure 17). Polychaetes that were fed labeled phytoplankton and labeled biodeposits were then fed to a common predator on the oyster reef, the naked goby. After seven days of consuming polychaetes, both the naked gobies that consumed polychaetes fed phytoplankton and the naked gobies that consumed polychaetes fed biodeposits, had significantly elevated $\delta^{15}N$ values, indicating that they incorporated nitrogen from phytoplankton and biodeposit sources (Figure 18). Some contamination was present in these samples, artificially elevating the $\delta^{15}N$ values of some of the samples, however the trends that were observed were larger than the influences of the contamination, making the overall detection of the enrichment possible. However, this contamination did increase the amount of variation present in the samples, which may have masked differences between baseline levels and sampling on certain days.

The findings of this study support past findings that detritus can play a role as a basal nutrient source (Odum and Cruz 1963). Feces and pseudofeces, as components of the detrital pool in particular, have been demonstrated as viable food sources in a number of studies. Insect growth has been enhanced by feces compared to natural detritus in stream habitats (Ward and Cummins 1979). Invertebrates in stream ecosystems have also utilized feces as a nutrient source, after leaf litter and other terrestrial inputs of organic matter had been utilized (Shepard and Minshall 1984). In addition, incorporation of fish feces by crustaceans has been seen in kelp forest ecosystems (Rothans and Miller 1991). Also, in a study by Frankenberg and Smith, a large number of the animals from the benthic community including *Pagurus annulipes, Fundulus majalis, N. succinea, Panopeus herbstii, Urosalpinx cinerea, Penaues setiferus* and *Brachidontes exustus*

ingested more than 5% of their body weight in feces, demonstrating the importance of coprophagy in this ecosystem (1967). It has been hypothesized that pseudofeces can be easily assimilated due to a high nutritional value and its associated bacteria and digestive enzymes (Izvekova and Ivova-Katchanova 1972). When fed diets comprised of either zebra mussel biodeposits or macrophytes and epiphytes, the amphipod *G. fasciatus* had greater survivorship on the biodeposits diet and the amphipod *E. ischnus* had equal survivorship on each diet (Gonzalez and Burkart 2004). The amphipod *G. roeselii* has also been observed to consume zebra mussel biodeposits (Gergs and Rothhaupt 2008b). This wide breadth of ecosystems and species that can utilize feces or pseudofeces in their diet demonstrates the importance of this nutrient source in ecosystems.

Seasonal Stable Isotope Sampling – Natural Abundances

The oyster reef Weems Upper, which is located in the Severn River, MD, was sampled on September 20, 2013, April 18, 2014, and August 6, 2014. These represented a fall, spring, and summer sampling. Water temperatures at the time of collection were 23°C, 13°C, and 27°C, respectively. In each season, multiple basal resources were sampled. Surface sediment was sampled two ways, by scooping the surface of the sediment and by using a syringe to siphon off the top of the sediment. In addition, seston and oyster biodeposits were also sampled in each season. The last basal resource sampled was benthic algae, using glass slides, however these slides were only successfully retrieved at the April and August sampling dates. These basal resources were sampled in multiple seasons in order to identify the primary carbon sources being utilized by the oyster reef food web, and to determine if oyster biodeposits were contributing to the carbon sources that were being utilized. Based on the carbon values of the basal resources and the consumers, no one particular basal resource

overwhelmingly influenced the food web of the oyster reef, although the isotopic signatures of most of the basal resources were depleted in $\delta^{13}C$, which may indicate that the system is being influenced by pelagic carbon sources (Hecky and Hesslein 1995). Isotopic signatures of the consumers, with a few exceptions, did not change in relation to any specific seasonal changes in one of the basal resources, indicating that most likely the organisms were utilizing a combination of basal resources. In September, most of the carbon values of the consumers on the reef fell in between the carbon values of the basal resources sampled, indicating that the carbon sources utilized by the reef organisms were most likely originating from within the reef. In April, all of the basal resources were less enriched in carbon and in nitrogen than the consumers on the reef. Since they were all less enriched in carbon, this indicates that a carbon source that was not found directly on the reef must have been transported onto the reef and was being utilized by the reef organisms there. This outside carbon source would have a more enriched carbon value than the consumers on the reef, and was potentially a marsh grass, submerged aquatic vegetation, or benthic algae from shallower waters. It could also be a combination of these sources. In August, a similar pattern to what was seen in April was observed. All of the basal resources, except for benthic algae, had less enriched nitrogen and carbon values than the reef consumers. Again, there was no basal resource that was more enriched in ^{13}C than the primary consumers, indicating that some outside carbon source was influencing that reef food web and serving as one of the basal carbon sources.

Biodeposit production by oysters is predominantly driven by temperature, resulting in greater production in summer months, and minimal production in winter months (Fulford et al. 2007). Based on this knowledge, I hypothesized that if oyster

biodeposits were largely influencing the reef community food web, then I would see a large impact from them in the summer months and minimal influence in my spring sampling, as the water temperatures at the time of this sampling had just warmed enough for the oysters to start filtering again (Newell and Langdon 1996). My results do not indicate a large influence of biodeposits on the reef community. In September, while the carbon value of biodeposits indicated that they could potentially be a food source for *N. succinea* and *R. harrisii,* the nitrogen values of *N. succinea* and *R. harrisii* were not enriched enough to indicate that either of these species were utilizing them as a food resource. The nitrogen value of oyster biodeposits was similar to that of the omnivores on the reef, indicating that biodeposits were not largely being utilized as a carbon source by the reef organisms in this season, as the deposits feeders did not have an enriched nitrogen value relative to oyster biodeposits. If the deposit feeders were consuming biodeposits, I would expect the consumer to have a $\delta^{15}N$ value about 3.4‰ higher than that of the biodeposits. Even if the biodeposits were consumed in combination with other sources, I would still expect a nitrogen value enriched compared to biodeposits $\delta^{15}N$ values. In April sampling, as would be expected with the cold temperatures, there was little indication that any organisms were utilizing oyster biodeposits as a food source. The $\delta^{13}C$ value of the biodeposits was much more depleted than the rest of the reef organisms, so it was unlikely that a large amount of biodeposits was incorporated by any of the reef organisms. August was the only season where the reef organisms may have potentially utilized the oyster biodeposits as a nutrient source. Both the carbon and nitrogen signatures indicate that the reef organisms could potentially have consumed

biodeposits, although as they were more depleted in carbon than the organisms, it would only be in conjunction with another nutrient source.

When differences in $\delta^{13}C$ for basal resources for each month were compared to differences in $\delta^{13}C$ for consumers, similar trends were only seen between April and August sampling. In April, $\delta^{13}C$ of the basal resources seston and benthic algae were significantly more depleted than August. This coincided with significantly more depleted $\delta^{13}C$ values of the consumers, *A. lacustre, C. virginica, I. recurvum, N. succinea,* and *P. pugio.* This correlation may be an indication that some of these consumers were utilizing seston as a primary resource. All of these consumers except for *P. pugio* had $\delta^{13}C$ values that reflected the expected trophic fractionation from seston. *A. lacustre*, *C. virginica*, and *I. recurvum* are also known to be filter feeders (Gaston and Nasci 1988, Newell and Langdon 1996) which seems to be reflected in these isotope values. While this trend holds for August and April, it does not seem to hold when September values were considered, which may indicate that different basal resources were being utilized in September.

When significant differences between sampling dates for $\delta^{15}N$ of consumers and basal resources were evaluated only a few species' isotopic signatures shifted in a similar manner to the basal resources. When August values were compared to April, benthic algae and *C. virginica* were both significantly more depleted in April than August. However, the $\delta^{15}N$ values did not seem to indicate that *C. virginica* was consuming benthic algae, as the expected change in $\delta^{15}N$ due to fractionation between the consumer and resource was not seen. When September samples were compared to April samples, both *C. virginica* biodeposits and seston were significantly more enriched in $\delta^{15}N$ in

September. The only consumer that also followed this trend was *N. succinea*, which could potentially be feeding on biodeposits, as it is a deposit feeder (Pardo and Dauer 2003), however carbon values and September nitrogen values of *N. succinea* indicate that this would have to be in conjunction with another food source. When $\delta^{15}N$ values were compared between September and August, biodeposits and surface sediment were both significantly more enriched in September than August. Again, *N. succinea* was the only consumer to follow this trend, which again may reflect its role as a deposit feeder. Considering all the nitrogen and carbon values together indicated that while these resources were probably part *N. succinea*'s diet, they were not the only resources being utilized by this species. Overall, the lack of correlation in seasonal trends between the resources' isotope values and the consumers' isotope values seemed to indicate the consumers were utilizing a variety of resources in their diet and may be switching those resources depending on the season.

The organisms that reside on the reef were likely utilizing a carbon source that was not found directly on the oyster reef. Three potential sources of this outside carbon source were submerged aquatic vegetation, benthic algae, and marsh grasses. The three main species of submerged aquatic vegetation that were found in the Severn River when it was most recently surveyed in 2012 were widgeongrass (*Ruppia maritima)*, redhead grass (*Potamogeton perfoliatus*), and sago pondweed (*Stuckenia pectinata*) (Bergstorm 2012). While these plants were not sampled during this study, some past studies have conducted stable isotope analysis on these species. $\delta^{13}C$ values for *P. perfoliatus* have been observed between -18.5‰ and -11‰ and its $\delta^{15}N$ values were observed between 4.5‰ and 9.0‰ (Marcenko et al. 1989, Sensula et al. 2006, Lesutienė et al. 2008,

Cremona et al. 2009, Jaschinski et al. 2011). Widgeongrass had a similar isotopic signature to redhead grass, with $\delta^{13}C$ values observed between –11.1‰ and -19.1‰ and $\delta^{15}N$ values ranging from 2.8‰ to 12.21‰ (Harrigan et al. 1989, Chmura and Aharon 1995, Stribling and Cornwell 1997, Abreu et al. 2006, Winemiller et al. 2007). *S. pectinata* has also been observed to have isotopic signatures in a similar range, with carbon values ranging from -23‰ to 0‰, although most values were observed around -12‰, and nitrogen values observed between -11‰ and 13.8‰ although most values were observed around 5‰ (Herzschuh et al. 2010, Guinan et al. 2015). In addition to these three main SAVs, horned pondweed (*Zannichellia palustris)* has also been found in the Severn River, and has $\delta^{13}C$ values observed between -12.3‰ to -13.5‰ and $\delta^{15}N$ values between 7.1‰ and 11‰ (Haramis et al. 2001). These SAVs or detritus from these SAVs may have been utilized by species on the reef and may contribute to the more enriched $\delta^{13}C$ signature of some of the reef consumers.

In addition to these SAVs the outside carbon source may be benthic algae or microalgae which has washed onto the reef, which typically has a signature between -17.6‰ and -14. 9‰ $\delta^{13}C$ (Riera and Richard 1996, Herman et al. 2000). Benthic algae were sampled in this study in two ways, surface sediment was collected and glass slides were placed at the study location for a month before sampling in order to be colonized by benthic algae. While the goal with these methods was to sample benthic algae, the isotopic values of these samples seemed to indicate that in some seasons potentially little benthic algae was growing at this location, either due to temperature or light penetration. The typical benthic algae carbon signature is more enriched than phytoplankton, around -18‰ to -15‰ (Riera and Richard 1996, Herman et al. 2000), but my samples had values

of -29‰ and -22‰ for the benthic algae slides. The benthic algae slides in August were more enriched in $\delta^{13}C$ than the seston, which was expected, however they were more depleted in $\delta^{13}C$ in April than seston, which is atypical for benthic algae. In addition, surface sediment samples had $\delta^{13}C$ values around -25‰. These values were more depleted than what would be expected. Typically benthic material should be more enriched in $\delta^{13}C$ than pelagic material because of the microbial and meiofaunal processes that rework elements of planktonic carbon (Fry and Sherr 1984). In addition, benthic algae is typically more enriched than pelagic algae because of the boundary layer surrounding the algae. In faster moving water or more turbulent water, like in the water column, the boundary layer surrounding the algae where carbon fixation is occurring is thinner than in slow moving water, and will result in more depleted $\delta^{13}C$ values. With a thicker boundary layer, there is more resistance to diffusion, which allows for the incorporation of the heavier isotope into the carbon fixation process, resulting in more enriched $\delta^{13}C$ values in the benthic algae (France 1995, Peterson and Heck 2001). In addition, algae may be incorporating carbon from bicarbonate rather than carbon dioxide, which results in a more enriched signature of $\delta^{13}C$ (Hecky and Hesslein 1995). Benthic algae slide samples in August displayed this characteristic enhancement in $\delta^{13}C$, but April samples and sediment samples did not. Water temperatures in April were only around 13°C and were colder than that in the preceding month, so that, combined with low photosynthetically active radiation (PAR), may mean that there was limited benthic algae growth in that season. The study reef was at a depth of 2.5-3.5 m in a highly eutrophic and turbid river, so light penetration varied at this site over the course of the year. Typically the percent of light penetration from the surface in the Severn River to the

depth of about 2 meters varies from about 0.04% to 0.1% (Chesapeake Bay Program). This may be enough light to allow the benthic microalgae to still grow (Dennison et al. 1993), but when combined with cold temperatures, greater depths, or rain events, this may limit the amount of benthic algae that could have grown at this depth. If there was not a large amount of benthic algae growth, then this may be why the isotopic signature of these samples varied from previous samples of benthic algae. The surface sediment that was sampled generally had similar $\delta^{13}C$ values to the seston sampled from the water column in all seasons, except for scoop sampling in September (year round mean sediment:-25.24‰, year round mean seston: -24.92‰) so it may be influenced by the material settling out from the water column. Or, it's possible some of the sediment material was resuspended into the water column and was becoming part of the particulate organic matter (POM) of the water column (Riera et al. 2004). Past studies that have sampled benthic algae and particulate organic matter in a similar manner to this study found that POM and benthic microalgae had similar isotopic compositions, so the enrichment of benthic algae to POM may not always occur (Abeels et al. 2012).

A final outside carbon source that may have influenced the reef food web was marsh grasses, as *Spartina alterniflora* can have a $\delta^{13}C$ isotopic signature between -14.2‰ and -12.4‰ (Haines and Montague 1979). Estuarine wetlands have been identified along the banks and tributaries of the Severn river (U.S. Fish and Wildlife Service 2014), which can contain *S. alterniflora* (Baldwin et al. 2010). These marsh grasses may not be directly consumed by reef organisms, but their detritus may be utilized by species on the reef (Wrast 2008). In addition, *Phragmites australis* is commonly found along the banks of the Severn River. While this species would not have

been enriching consumer's $\delta^{13}C$, its detritus may be another food source for the reef with $\delta^{13}C$ values observed between -28‰ and -25‰ (Stribling and Cornwell 1997, Weinstein et al. 2000, Quan et al. 2012) and $\delta^{15}N$ values observed between 6.9‰ and 7.4‰ (Kang et al. 2003, Quan et al. 2012)

Basal resources had the highest enrichment in $\delta^{13}C$ values in September 2013, the least enrichment in April 2014, with values in August 2014 between these two. The enrichment patterns were correlated to rainfall amounts in the two months previous to sampling, with the least rain preceding the September sampling, the most rain preceding the April sampling, and rain totals in between these two preceding August sampling. The differences in observed enrichment may be due to these differences in rainfall. With greater rainfall there is greater terrestrial run off, which has a more depleted ^{13}C value than aquatic carbon sources typically. This contributed to brackish and freshwater particulate organic carbon (POC) having more depleted carbon values than oceanic POC (Fry and Sherr 1984). My sampling also took place near the mouth of the Severn River, where there may be an increased amount of terrestrial input compared to the main stem Chesapeake Bay. Many studies have observed that riverine samples were more depleted in their $\delta^{13}C$ values than estuarine or marine samples (Simenstad and Wissmar 1985, Riera and Richard 1996, McCallister et al. 2006). This is due in part to the influence of terrestrial plants and algal sources other than diatoms, which have a larger influence on the isotopic signatures of riverine communities (McCallister et al. 2006). Lower $\delta^{13}C$ values can also result from greater precipitation because the runoff can contain dissolved inorganic carbon that is more depleted in $\delta^{13}C$. In addition, rains can cause resuspension

of sediments which can result in the release of porewater, which can deplete carbon values (Fry 1999).

Seasonal sampling showed similar trophic structures on the oyster reef in all seasons. $\delta^{15}N$ values were utilized to identify trophic position, because they increase in a characteristic manner, generally by 3.4‰, for each trophic level (Minagawa and Wada 1984). Also, $\delta^{13}C$ does not usually increase substantially between trophic levels, with a fractionation of 0.7-1.4‰ between each trophic level (Fry and Sherr 1984). The average $\delta^{15}N$ of all the basal resources in September was enriched compared to the other two sampling dates while the organisms sampled in September were generally not more enriched in their $\delta^{15}N$ than the other sampling dates. The higher baseline level of $\delta^{15}N$ for the basal resources indicated that the organisms in September were all feeding at lower trophic positions than the other seasons (Table 9). The biggest differences were seen with *A. lacustre* and *I. recurvum*, which were both feeding at trophic levels of around 1 in September, but then were feeding at a trophic position closer to 2 in April and August, indicating that they were primary consumers. The omnivores, *P. pugio, R. harrisii, M. nitida,* and *N. succinea* were all feeding in between a trophic position of 2 and 2.5 in April and August, indicating a mix of consumption of basal resources and primary consumers. In September, *R. harrisii* and *M. nitida* were both feeding at about one trophic position lower than in the other seasons, with trophic positions of 1.4 and 1, respectively. *N. succinea* was still feeding around the same trophic position as the other seasons in September, around 2. *G. bosc* typically fed at a trophic position around 3 in April and August, and fed at a trophic position around 2.3 in September, indicating that it was primarily feeding on primary consumers (Table 9).

These trophic positions corresponded with expectations given knowledge of these species' diets. *C. virginica* and *I. recurvum* are both suspension feeders that primarily feed on phytoplankton (Newell and Langdon 1996). Both likely also feed on some detritus and bacteria while filtering seston from the water column (Riera and Richard 1996). *C. virginica's* diet may also consist of terrestrial detritus, benthic and planktonic diatoms, and macroalgae (Riera and Richard 1996). Their role as a primary consumers were reflected in their trophic positions of 2 or lower. *A. lacustre* is classified as both a suspension feeder and a surface deposit feeder (Gaston and Nasci 1988, Gaston et al. 1998), which was supported with the findings of my study with a trophic position ranging from .9 to 2, reflecting its role as a primary consumer. *Melita nitida* fed at a trophic level of 2 in August and 2.5 in April, which reflects the classification of this amphipod as a detritivore and algaevore (Odum and Heald 1975, Zimmerman et al. 1979, Hughes et al. 2000). *Melita nitida* can also be cannibalistic, which may be reflected in the higher trophic level of the April samples (K.E. Kesler, personal observation). *P. pugio* and *R. harrisii* are both classified as omnivores and detritivores (Adams and Angelovic 1970, Turoboyski 1973, Welsh 1975, Bell and Coull 1978, Deegan and Garritt 1997, Wallace et al. 1998, Fleeger et al. 1999), which was reflected in their trophic positions around 2.5, indicating a mix of consumption of basal resources and animal material. *N. succinea* is also an omnivore and detritivore, reflected in its trophic position of 1.9-2.4, consuming a mix of sediment, diatoms, protozoans, and metazoans (Pardo and Dauer 2003). *N. succinea* has also been observed consuming *C. virginica* biodeposits (Tenore and Gopalan 1974). Finally, *G. bosc* has been identified as a predator, consuming a large variety of macroinvertebrates, including amphipods, copepods, polychaetes, isopods and

oligochaetes (Douglass et al. 2011, D'Aguillo et al. 2014). This was reflected in this species feeding at the highest trophic position of all the species sampled, around 3, indicating that it predominately was consuming primary consumers (Table 9).

I. recurvum, C. virginica, M. nitida, and *A. lacustre,* all species that have been identified as using phytoplankton as part of their diet, had consistently more depleted carbon values than the other reef consumers (Table 10). This may be a reflection of their primary utilization of pelagic based carbon sources, which were more depleted than benthic carbon sources in $\delta^{13}C$ values (Chanton and Lewis 2002). This was similar to results seen by Quan et al. (2012) who found that in a *Crassostrea ariakensis* reef ecosystem, the bivalves had the most depleted carbon values, and that these values most resembled $\delta^{13}C$ values for particulate organic matter. The other primary consumers have been identified as omnivores/detritivores, and were likely consuming a larger fraction of benthic carbon sources. These consumers were likely incorporating more of the carbon source that was coming into the reef from an outside source than the suspension feeders, as their $\delta^{13}C$ values were more enriched. The naked goby, the top consumer sampled in this study, generally had a carbon signature that was in between these two groups of organisms, indicating that it most likely was consuming both organisms that primarily consume pelagic food sources and those that primarily consume benthic food sources.

The carbon sources sampled in this study tended to have isotopic values that reflected a pelagic origin, even though both seston and surface sediment were sampled. These sources seemed to make a partial contribution to reef organisms' diets, while an unidentified additional carbon source not located on the reef was the other carbon source being utilized. A study of oyster reefs in the Gulf of Mexico has demonstrated that the

oyster reef community was dominated more by pelagic food sources than by marsh plants, however this study did not sample benthic algae as a potential food source (Beck 2012). An assessment of a *C. ariakensis* oyster reef demonstrated that the major carbon sources to the food web were particulate organic matter and benthic microalgae (Quan et al. 2012). These studies indicate that potentially benthic algae or a marsh grass may have been playing a role in this oyster reef food web. In addition, SAVs that were in this river may also have been influencing this food web. This was the first study to investigate the oyster reef food web in the Chesapeake Bay, and the first one to classify reef organisms and basal resources with stable isotopes. In order to further classify the relationships on the oyster reef, additional research should potentially incorporate gut content analysis and sampling of basal resources from outside of the reef, to see what other carbon sources are important to this food web.

Chapter 5: Conclusions

Crassostrea virginica is an ecosystem engineer that alters its habitat by creating hard, complex three dimensional structure (Gutiérrez et al. 2003). *Crassostrea virginica* is also a filter feeder that produces a large amount of biodeposits (Haven and Morales-Alamo 1966b). Through three studies, I investigated the impacts of *C. virginica*'s reef structure and biodeposits on the oyster reef community, determining the importance of these two characteristics of the eastern oyster to structuring the reef community.

The first study that I conducted compared the communities that colonized small experimental oyster reefs that were composed of live oysters to small reefs composed of empty oyster shells. This study investigated whether the presence of a live oyster effected the oyster reef community. These two reef treatments had the same structural components, but reefs composed of live oysters contained oysters that were actively filtering the water column and producing biodeposits. The communities that colonized these two reefs were similar in abundance and biomass, both when individual species were evaluated and when the entire community assemblage was compared ($P>0.05$; Figure 4, Figure 6, Figure 8). Species richness was also similar between the two treatments ($P>0.05$), however the Margalef's diversity index, Simpson's Index, and Simpson's Evenness Index all indicated greater diversity on the empty oyster shell treatment ($P = 0.032$, $P=0.024$, $P=0.015$, respectively). This greater diversity was likely due to the dominance of *Apocorophium lacustre* in the live oyster reef communities, which lowered the evenness, and thus the diversity of this treatment. Overall, this study indicated the importance of oyster structure for the establishment of the oyster reef

community. The presence of a live oyster did not create differences in biomass, abundance, or community assemblage between the two treatments.

To further explore the role of oyster structure, particularly as a refuge, and to directly evaluate the impact of oyster biodeposit presence on a deposit feeder population, a study was conducted that manipulated three factors, habitat complexity, biodeposit presence, and predator presence. In this study, the habitat complexity factor and the predator factor had a significant interaction with their effect on the prey abundance and biomass (Figure 11, Figure 12). When a predator was present, high habitat complexity provided protection to amphipods and the final amphipod abundance and biomass was greater than in the low complexity treatments (abundance: P=0.007, biomass: P<0.001). When a predator was absent, a similar abundance and biomass of amphipods was present at the end of the study in the low and high complexity treatments. Neither amphipod abundance nor amphipod biomass was impacted by the presence or absence of oyster biodeposits (P>0.05). Fish biomass was also not impacted by the presence of oyster biodeposits, nor was it impacted by habitat complexity (Figure 14). This study demonstrated the importance of oyster structure as a refuge for prey items from reef predators. It demonstrated how protection was provided to these prey items and why species would seek refuge on the oyster reef. In addition, this study offered additional evidence that oyster biodeposits do not largely impact the reef community.

The final study that I conducted specifically addressed if oyster biodeposits could be utilized as a food resource, and if they were being utilized as a food resource on an oyster reef in the Severn River, MD. A ^{15}N tracer study was conducted to evaluate if oyster biodeposits could be incorporated by two deposit feeders, *Melita nitida* and

Neanthes succinea, and the natural abundances of stable isotopes of reef organisms from the field were determined to assess if oyster biodeposits were being incorporated as a food source on the reef. The tracer study demonstrated that oyster biodeposits could be utilized as a nutrient source by the deposit feeders *N. succinea* and *M. nitida*, as both species had elevated $\delta^{15}N$ values at the end of the study. In addition, when the deposit feeder *N. succinea* was fed to *G. bosc*, *G. bosc* had an elevated $\delta^{15}N$ signature, indicating that the nutrients were transferred to this next level of the food chain as well (Figure 16, Figure 17, Figure 18). The field evaluation of natural stable isotope signatures of reef organisms and basal resources indicated that *C. virginica* biodeposits were not a prominent nutrient resource utilized by the reef, but that they may be utilized in the summer. In addition, stable isotope analysis indicated that a carbon source originating from outside of the oyster reef was being utilized by the reef organisms.

These three studies together indicated that the structure created by *C. virginica* was extremely important to the community that resides on the oyster reef while the biodeposits that were produced by the oysters were only minimally utilized by reef organisms. These studies demonstrate the importance of oyster structure as a habitat, as indicated by the rapid colonization of reef material when placed in the Patuxent River, and the effectiveness of oyster structure as refuge for amphipods from a naked goby predator. In addition, my studies demonstrate that while oyster biodeposits can be utilized as a food source, as observed in studies with amphipod populations in Chapter 3 and with the ^{15}N tracer in Chapter 4, it is not a predominant food source utilized by the oyster reef community. No one basal resource was indicated as the primary food source for the reef from my data, but rather a combination of seston, surface sediment, and an

unknown carbon source from off the reef (potentially SAVs or marsh grass) likely served as the basis for the reef food web.

One of the key reasons that the structure of the oyster reef, like other structured habitats, is critical to the species that live there is that it can provide protection from predators, environmental stress and competitors (Lenihan 1999, Norling and Kautsky 2007, Horinouchi 2007, Grabowski et al. 2008). Protection from predators is provided by limiting a predator's movement, reducing prey detection, and also impairing a predator's ability to capture prey (Bartholomew et al. 2000). These same principles also reduce stress from competitors, where competing species encounter and detect each other less in complex habitats than they do in simple habitats (Grabowski et al. 2008). Protection from environmental stressors, like hypoxia, is provided by a reef elevating an oyster, and its associated taxa, from the sediment surface to a height that experiences fewer anoxic and hypoxic episodes (Lenihan 1999).

Complex habitats, like oyster reefs, also create spatial partitioning which results in a number of habitat niches which can be utilized by a variety of species, allowing for greater species diversity to develop (Hixon and Menge 1991). The oyster clump structure provides spaces in between shells where crabs and small fish can hide. In addition, when oysters die they create a box structure which consists of the two sides of the shell open but still attached at the hinge. This space is used both for protection and as a spawning location where eggs can be attached and defended (Runyan 1961, Crabtree and Middaugh 1982). The oyster shell itself also provides a number of habitat locations, as sessile species will use the shell as an attachment substrate, worms species will bore into and live within the shell, and other worm and crustacean species will hide amongst the ridges

of the oyster shell for protection (Haigler 1969, Faasse and van Moorsel 2003, Kennedy 2011). The variety of spaces available for habitation and protection on the oyster reef allows for the diversity of species that utilizes the reef and for the high abundances and biomasses of these species. In addition, this substrate is a limited resource in the Chesapeake Bay (Smith et al. 2005), making its presence even more desirable for reef species (Bahr 1974).

Overall the provision of complex habitat is beneficial to benthic and demersal marine species, as it can encourage greater juvenile survival and help avoid bottlenecks in recruitment due to habitat availability (Scharf et al. 2006). Since the reef supports greater survival and growth of prey species, it can enhance fish production both of resident reef fish and transitory fish which feed on those prey items (Peterson et al. 2003). This can be important economically to the Chesapeake region as some commercially important species feed on the oyster reef, such as striped bass (Harding and Mann 2003) and blue crabs (White and Wilson 1996).

Along with providing protection, the reef structure is important because of how it effects water movement. Reef structure can baffle water movement, which can encourage the settlement of food and larvae onto the reef structure (Nestlerode et al. 2007). This helps to provide food and encourage larval recruitment to the reef. Greater recruitment of sessile organisms can help with the spawning success of that species and greater food settlement can encourage growth on the reef.

The provision of unique hard substrate and protective complex structure by oyster reefs, and by bivalves in general, leads to greater densities and diversity on bivalve structures than on flat bottoms. This has been shown in multiple studies with *Musculista*

senhousia (Crooks and Khim 1999), *Dreissena* spp. (Botts et al. 1996), *Atrina zelandica* (Norkko et al. 2006), and *C. virginica* (Tolley and Volety 2005). Many of these studies used a bivalve mimic, either an empty shell or artificial bivalve, to create habitats and these structure-only habitats had greater organism densities than flat bottoms, demonstrating the importance of structure to establishing these communities (Botts et al. 1996, Crooks and Khim 1999, Tolley and Volety 2005). The results of my studies support the findings of these past studies; that the structure of the oyster reef is critical to the oyster reef community, driving the patterns of density and diversity seen on the reef.

While structure appears to be essential to the establishment of the reef community, biodeposits may only be minimally utilized by reef organisms. A few studies have shown that *C. virginica* biodeposits can be consumed (Frankenberg and Smith 1967, Tenore and Gopalan 1974), and this was also observed with the initial amphipod feeding study in Chapter 3 and isotope tracer study in Chapter 4, but biodeposits do not appear to be largely incorporated into the oyster reef food web in the field as seen with stable isotope analysis. The biodeposits may only be minimally used because they may be a lower quality food source than other available food sources. Newell and Jordan (1983) found that *C. virginica* pseudofeces, even though it was undigested, has less nitrogen and carbon than the original phytoplankton. *Crassostrea virginica* feces and pseudofeces also contained less energy than the original phytoplankton as well (Newell and Jordan 1983). Another possibility is that there is no food limitation on the reef, so the presence of biodeposits does not cause considerable differences in the reef community (Howard and Cuffey 2006). If consumers were not resource limited, then they may not have responded to the additional nutrient input of

oyster biodeposits. Nutrient additions may only make minimal changes to a community that already experiences high nutrient inputs (Posey et al. 2006). As the Chesapeake Bay is a highly eutrophic body of water (Kemp et al. 2005), the nutrients contributed by oyster biodeposits to the community may not be necessary for reef organisms. A third possibility is that top-down pressures were masking the effect of nutrient increases. The rate of predation may be higher than the rate of change in abundance or biomass due to additional nutrient resources, so that a change in these elements cannot be detected (Posey et al. 2002). This may be one of the reasons that an impact from biodeposits was not seen in the mesocosm studies. Naked gobies were extremely efficient predators that could quickly deplete an amphipod population, particularly when no refuge was available for the amphipods. Preliminary feeding studies with amphipods showed that differences in the population were not seen until after three weeks of being feed the biodeposit resource. It may be the predators were consuming prey items faster than I could detect changes in the prey population. However, stable isotope analysis of the oyster reef food web did not indicate biodeposits were a major resource either. These findings collectively indicate that biodeposits are not utilized as a primary food source by the reef organisms.

The results of these studies stress the importance of complex, hard substrate in establishing flourishing reef communities. Reestablishing oyster reefs can restore this unique habitat that is critical to so many species to the Chesapeake Bay. Artificial reefs may also be helpful in providing this habitat to the Chesapeake Bay, but these artificial structures must strive to mimic the diversity of spaces that are created and provided by the oyster reef in order to provide all of the ecological benefits to the reef community.

An actively growing and recruiting oyster reef may be beneficial over artificial substrate, as it can self-replenish the hard substrate and counter the impacts of sedimentation. However, currently most oyster reefs in Maryland are not self-sustaining and even after restoration efforts suffer from heavy sedimentation which buries shells and prevents attachment of new oyster larvae (Smith et al. 2005). In order to maintain these critical complex environments, changes in land use must also be incorporated into habitat restoration efforts. Reducing sedimentation input into the Chesapeake Bay can help improve oyster habitats. However, in the absence of an actively recruiting reef, the provision of artificial hard, complex substrate could still provide habitat benefits to organisms that would typically utilize the oyster reef. A combined effort at increasing the amount of hard substrate available in the Chesapeake Bay and also restoring reefs to a self-sustaining level is crucial to the organisms that utilize this ecosystem.

These studies assessed the influence of the physical oyster structure and the oyster biodeposits on the reef community. Overall the results indicated that oyster structure was the primary driver of species abundance and biomass, as well as community assemblage and species richness, while a live oyster reduced some metrics of diversity. In addition, oyster shell structure effectively provided protection from a common reef predator. Studies with oyster biodeposits showed that these biodeposits could be consumed and incorporated by deposit feeders, but that in the field they were not largely utilized as a food source and had little impact on the reef community. Overall, these studies showed that oyster structure was more importance to community organization than oyster biodeposits.

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