ABSTRACT

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Naked gobies (*Gobiosoma bosc*) are the most abundant resident fish on oyster reefs in the Chesapeake Bay and serve as a critical trophic link between the benthic and pelagic communities. Their response to oyster restoration may therefore be a useful indicator of ecosystem functioning. Experiments were conducted from 2005-2006 in the Severn and Patuxent Rivers to determine the response of naked gobies to increasing availability of oyster substrate. Naked gobies and other resident macrofauna were collected from experimental oyster reefs, constructed using adult oysters of various densities, loose oyster shell, and ambient sediment. Naked goby abundance, length, dry weight, and biomass increased with the availability of oyster substrate and were positively correlated with the abundance of other resident macrofauna. Individual dry weight and biomass were greater on reefs with a high density of oysters than on control plots, and may therefore serve as important indicators of oyster reef habitat quality.

NAKED GOBIES (*GOBIOSOMA BOSC*) AS INDICATORS OF OYSTER REEF RESTORATION SUCCESS

By

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Dedication

To Herb and Ted,
the great conservationists

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Chapter 1: Introduction

History of oysters in the Chesapeake Bay

Oyster reefs were once a dominant feature of the Chesapeake Bay. Dense assemblages of *Crassostrea virginica* historically posed navigational hazards to boats (Wharton, 1957), with densities estimated from the 100s to the 1000s m^{-2}. Oysters were also once culturally and economically important in the Chesapeake Bay region. Maryland once produced 39% of the total U.S. harvest of oysters, and the industry employed 20% of American fisheries workers (Kennedy, 1989), with peak oyster harvest reaching 15 million bushels in 1885 (Kennedy and Breisch, 1981). However, intense over-harvest of public stocks led to declining populations and greatly altered Maryland's economy. The low harvest numbers forced many to leave the industry, but continued to support a modest number of watermen until the 1960s when disease crippled the populations. Oyster landings averaged just over 100,000 bushels annually from the 2001/2002 season through the 2006/2007 season (Tarnowski, 2008). After many decades of decline, the mean density of oysters on historic oyster bars in the Chesapeake has been estimated to be less than 1 oyster m^{-2} (Jordan and Coakley, 2004).

Ecosystem services provided by oyster reefs

Oysters are believed to be a key species for the health of the Chesapeake Bay, providing many essential ecosystem services (Coen et al., 2007). Some of the most important services include water filtration, habitat formation, and nutrient cycling.

Water filtration

Oysters are filter-feeders, removing plankton and particles from the water column and depositing them as feces or pseudofeces onto the benthos. The decline in oyster populations has served to exacerbate the declining water quality of the Chesapeake Bay. Using harvest records, Newell (1988) estimated that the number of oysters in the Bay prior to the intensification of harvest in 1870 could filter the equivalent of the entire volume of the Bay within 3.3 days. He also estimated that 41.2% of the carbon produced daily in the Bay was removed by oysters. The population of oysters in the Bay in 1988 was estimated to take 325 days to filter the same volume, and was capable of removing just 0.4% of daily carbon production. Newell et al. (2004) later described the removal of nitrogen and phosphorus from the water by oysters, depositing the nutrients as feces and pseudofeces, preventing their regeneration into the water column. Oysters thus play an important role in the maintenance of water quality in Chesapeake Bay. The simultaneous decline of oysters and increase in nutrient inputs to the Bay has contributed to the degradation of its water quality.

Habitat formation

Oyster reefs are key habitat to many Bay organisms, providing both fuel for biogenesis and structural complexity that allows abundant and diverse benthic communities to thrive. Oyster larvae recruit to hard, clean surfaces on which they attach and grow into adults, producing reefs that extend vertically into the water column. The hard substrate also provides relief from the muddy bottom of the Bay, allowing oysters to avoid sedimentation (Lenihan and Peterson, 1998). Many studies

have highlighted the increased biodiversity and abundance of macrofauna on oyster reefs when compared to nearby non-structured habitats (Zimmerman et al., 1989; Harding and Mann, 1999; Harris, 2003; Soniat et al., 2004; Grabowski et al., 2005; Luckenbach et al., 2005; Tolley and Volety, 2005; Rodney and Paynter, 2006). Other filter and suspension feeders such as the hooked mussel (*Ischadium recurvum*), blue mussel (*Mytilus edulis*), and barnacles (*Balanus sp*.) also settle on oyster shells (Meyer and Townsend, 2000). Rodney and Paynter (2006) found mussels and barnacles in excess of 3,000 individuals per square meter on restored oyster reefs. The settlement of these suspension and filter feeders on oyster reefs further enhance the filtration capacity on the reef.

Many species of fish also utilize oyster reefs. Breitburg (1999) characterized three types of fish species that inhabit oyster reefs: resident fishes, which depend on the reef as their primary habitat, such as the naked goby (*Gobiosoma bosc*), skilletfish (*Gobiesox strumosus*), striped blenny (*Chasmodes bosquianus*), feather blenny (*Hypsoblennius hentz*), and oyster toadfish (*Opsanus tau*); facultative residents, which may utilize the structured habitat periodically, such as the black sea bass (*Centropristis striata*), northern pipefish (*Syngnathus fuscus*), and Atlantic spadefish (*Chaetodipterus faber*); and transients, which are wide ranging but will frequent oyster reefs to forage, such as striped bass (*Morone saxatilis*), juvenile summer flounder (*Paralichthys dentatus*), juvenile winter flounder (*Pseudopleuronectes americanus*), spot (*Leiostomus xanthurus*), pinfish (*Lagodon rhomboides*), inshore lizardfish (*Synodus foetens*), American eel (*Anguilla rostrata*), striped burrfish (*Chilomycterus schoepfii*), and Atlantic silverside (*Menidia menidia*).

The hard substrate and interstitial space created by dense oyster aggregations provide essential habitat to many fish species (Arve, 1960; Crabtree and Middaugh, 1982; Breitburg, 1999; Harding and Mann, 1999, 2000; Peterson et al., 2003; Tolley and Volety, 2005; Rodney and Paynter, 2006). Resident benthic fish, such as naked gobies and striped blennies, are reliant on this space and the insides of clean, articulated shells for nesting (Nero, 1976; Crabtree and Middaugh, 1982). Larvae produced by these resident fishes also aggregate near oyster reefs in the summer (Breitburg, 1999), and provide an important food source for commercially and recreationally important transient fish species such as striped bass, red drum, bluefish, and blue crabs (Nero, 1976; Breitburg, 1999; Harding and Mann, 2001; Stuntz et al., 2002). Thus, the interaction between oysters and resident benthic fishes is essential to the development of a healthy faunal community, which in turn supports upper trophic levels.

Nutrient cycling

The Chesapeake ecosystem is currently dominated by phytoplankton dependent on excess nutrients entering the Bay from the large surrounding watershed. Dame and Patten (1981) described the oyster reef as being made up of filter feeders, detritus, microbiota, meiofauna, deposit feeders, and predators. Oysters filter the phytoplankton from the water column and expel digested material as feces and excess food and refractory particles, such as sand and sediment, as pseudofeces, depositing them onto the bottom (Newell and Langdon, 1996). Oysters therefore convert the plankton into nutrient and energy-rich feces and pseudofeces which serve as a food source for deposit feeders such as amphipods and some polychaete worms. The

energy is then transferred to small predators, such as resident fish and crab species that feed on filter and deposit feeders. These small predators and their larvae are consumed by larger and often commercially or recreationally important transient species such as blue crabs and striped bass. (Lenihan, 1999). By filtering plankton from the water column and depositing material onto the Bay bottom, oysters control energy flow within the system by making planktonic energy sources available for non-filter feeding organisms, thereby coupling benthic and pelagic production (Dame and Patten, 1981; Dame et al., 1992; Dame et al., 2002).

Decline of oyster reefs

Populations of oysters in the Chesapeake Bay dropped dramatically in the late 1800s, and maintained a steady population from the 1930s until the 1950s before a rapid decline to today's current low levels. Multiple factors led to the decline of the stock, including over-harvest, loss of habitat, and disease. The recent dense human population within the watershed has led to dramatic increases in nutrient inputs to the Bay and its tributaries. Increasing runoff from agricultural operations, sewage treatment plants, storm drain overflows, and residential fertilizer use has led to the eutrophication of the Bay. With the loss of most of the historic oyster biomass in the Bay, excess phytoplankton remains in the water column, unavailable to many other species. Where oysters once controlled the flow of energy from the water column into the benthos, the energy entering the Bay through excess nutrient enrichment now remains in plankton form, changing the Chesapeake from a once benthic-dominated system to a pelagic-dominated system (Newell, 1988; Baird and Ulanowicz, 1989; Cooper, 1995; Dame et al., 2002).

Over-harvest and destructive harvest practices

Oysters in Chesapeake Bay are traditionally harvested using low-tech mechanical devices such as oyster dredges, oyster tongs, or by hand by SCUBA or hookah-divers. Dredging was outlawed in Maryland in the early 1800s due to the high efficiency at removing oysters from the reefs. Maryland again allowed the device to be used in 1865, leading to a frenzy of oyster harvest activity, peaking at 15 million bushels in 1885 (Kennedy and Breisch, 1981). Oyster dredging has since been implicated as the greatest contributor to the decline in oyster populations due to its destruction of oyster reefs in the 19th and 20th centuries (Brooks, 1891; Rothschild et al., 1994; Lenihan and Peterson, 2004). Dredging greatly reduced the height of many oyster reefs and led to high incidental oyster mortality (Lenihan and Peterson, 2004). Reduced structural height allowed the reef to become susceptible to sedimentation, leading to the loss of important habitat on which larvae could settle.

Today, hand tonging is widely used throughout the Bay. This simple, low-tech harvest method requires only a set of hand tongs and one waterman to operate them. However, this method is also non-selective in the size of oysters collected, and produces high incidental mortality and reef damage (Lenihan and Peterson, 2004).

Sedimentation and loss of habitat

Agricultural land use has increased in the Chesapeake region since the beginning of European settlement, leading to increased sedimentation rates and turbidity (Cooper, 1995). While agricultural land use has been steadily declining in the region, it still constitutes 25% of the Bay watershed (NRCS, 2006). Sediment runoff from farms during storm events carries heavy sediment loads into the Bay,

eventually settling out of the water column and covering oyster shells. Sediment loading into the Bay is also exacerbated by sea level rise that results in shoreline erosion. The decreased availability of clean hard substrate due to sedimentation prevents larval recruitment onto the reef as larval oysters cannot find suitable substrate on which to settle and metamorphose into juveniles (MacKenzie, 1996). Oyster habitat in the Maryland portion of the Chesapeake Bay has declined an estimated 70% in the last 25 years (OAC, 2007), with 90% of once productive habitat now classified as mud, sand, or heavily sedimented oyster shell and less than 1% of oyster bars mapped by Yates (Yates, 1913) still considered high quality oyster habitat (Smith et al., 2005).

Disease

While over-harvest and habitat loss caused a dramatic decrease in oyster stocks at the end of the 19th century, the harvest remained relatively steady from about 1930 to 1980 before experiencing a second dramatic decline. The appearance of two parasitic diseases decreased oyster stocks to less than 1% of historic levels and has since prevented the population from recovering despite massive restoration efforts (Andrews, 1988; Kennedy, 1989).

Many scientists speculate that the protozoan *Haplosporidium nelsoni,* commonly known as MSX (Multinucleated Sphere Unknown), was introduced in the Chesapeake following an attempted introduction of the Japanese oyster, *Crassostrea gigas* (Burreson, et al., 2000; Boesch and Greer, 2003). The disease was first discovered in the Chesapeake Bay in 1959, two years after it was discovered in Delaware Bay following the purported introduction of *C. gigas*. The disease has

since spread from Maine to Florida, but has not been found in the Gulf of Mexico. The parasite proliferates from 5 to 20°C and at salinities above 10. *Crassostrea virginica* generally succumb to the disease at salinities above 15 and temperatures greater than 20°C (Reece et al., 2001). The Virginia portion of the Chesapeake Bay has been more greatly affected than oysters in the waters of Maryland, where salinities rarely exceed 20. While transmission is not fully understood, the protozoan first infects the gills of the oyster, leading many scientists to believe that transmission occurs through an intermediate host in the water (Ewart, 1993). The infection eventually spreads to the digestive system of the oyster, producing severe metabolic stress, leading to a general degradation of tissue, reduced fecundity, and eventual death of the host (Barber et al., 1988). Most oysters do not survive past the first summer of infection (Ewart and Ford, 1993).

The second disease, known as Dermo, is caused by the protozoan *Perkinsus marinus*. Dermo is thought to be native to the Chesapeake Bay, but did not become a prominent threat to oysters in the Maryland portion of the Bay until the late 1980s following consecutive summers of drought and consistently high temperatures (Burreson and Andrews, 1988; Burreson and Ragone Calvo, 1996). Infection by the parasite is greatest at salinities above 15 and temperatures greater than 25°C. Though the protist is not virulent below a salinity of 12, it is able to persist and eventually spread when introduced to low salinity waters (Ragone and Burreson, 1993; Burreson et al., 1994). Infection causes sub-lethal effects on the physiology of the oyster, weakening its condition, thus decreasing growth and fecundity until finally the oyster succumbs (Andrews, 1965; Paynter and Burreson, 1991; Paynter, 1996). The disease

is rapidly transmitted from oyster to oyster, causing large scale mortality usually around the second or third summer of infection (Ewart and Ford, 1993).

The spread of Dermo to lower salinity areas of the Bay was probably exacerbated by Maryland's state-sponsored repletion program. In 1960, Maryland began a program that attempted to supplement the oyster industry while managing disease. Oyster shell, covered with either wild or hatchery-reared 'seed' oysters from high-salinity regions with high natural recruitment and growth rates, were transported to lower salinity regions where oyster disease was not prevalent. However, the transplanted oysters often had high levels of Dermo and MSX. The oysters were then allowed to grow until they reached market size, at which point they were harvested by watermen. This continual relocation of oysters and parasites from disease-ridden regions in the Bay to disease-free waters has been blamed for spreading the disease to populations with no natural defense to the parasite (Carlton and Mann, 1996). Oysters in the lower salinity regions of Maryland are more susceptible to Dermo-induced mortality due to their low natural recruitment and growth rate (Ford and Tripp, 1996).

Oyster restoration in the Chesapeake Bay

History of oyster restoration and management

To compensate for the loss of critical ecosystem services once provided by dense assemblages of oysters in the Chesapeake Bay, intensive oyster restoration efforts have been ongoing since the 1990s. In 1993, the Oyster Roundtable was established to develop an action plan for oyster restoration and management in Maryland. The roundtable was comprised of aquaculturists, watermen, managers,

scientists, environmentalists, and senior members of Maryland's Governor's office and Departments of Natural Resources, Agriculture, and Environment. The roundtable outlined an Oyster Action Plan, with final goals of restoring the ecosystem functions of oysters, providing a sustainable oyster fishery, and maximizing the government's ability to respond to the magnitude of the problem (MD ORT, 1993). These goals included reducing the impact of disease, increasing production of hatchery seed, and creating a disease-resistant native oyster strain (Paynter, 1999).

In 2007, the Governor of Maryland established the Oyster Advisory Commission (OAC), which was charged with developing strategies for restoring and managing oysters in the Maryland portion of the Bay. In its interim report, the OAC (2007) stressed a need to design a restoration plan, based on the best available science, which focuses on restoring the ecological function of oysters.

Goals of oyster restoration

The goal of oyster restoration in Maryland has traditionally focused on economic purposes (OAC, 2007). The Oyster Advisory Commission (2007) stated in its interim report that the goals of ecological and economic restoration are incompatible. The Commission therefore recommended an intensive restoration effort to create large oyster sanctuaries in the Maryland portion of the Chesapeake Bay that will eventually be self sustaining and ecologically useful, while the oyster industry shifts to private aquaculture.

With a renewed effort towards ecological restoration, many questions arise on how best to identify and achieve the necessary goals of the restoration program. Coen and Luckenbach (2000) reviewed the difficulties in defining success criteria and

goals for oyster reef restoration. They identified maintaining biodiversity, increasing finfish and shellfish production, and improving ecosystem services as three broad goals of all restoration efforts, but noted that quantifying the success of restoration efforts in relation to these goals is challenging.

The density at which oysters should be planted in order to restore ecological function is unknown (MacKenzie, 1996). Oyster fertilization success is dependent on a minimum density between individuals on the reef (Mann and Evans, 1998; Pavlos and Paynter, 2001). Jordan and Coakley (2004) also found that high density oyster reefs are more important to community development than a greater number of oysters spread more sparsely.

Metrics of restoration success

There are many possible metrics for monitoring the success of oyster restoration. In 2004, a group of oyster biologists and ecologists from around the United States formed the Oyster Restoration Workgroup with the goal of identifying metrics of oyster reef restoration success. In a workshop held by the group, the members identified reef density, size, architecture, and fragmentation, oyster size frequency, associated fauna, salinity, dissolved oxygen, and temperature as appropriate metrics for determining the success of habitat restoration (ORW, 2008). Resident fauna, according to the workgroup, is the most valuable metric of restored oyster reef ecosystem function. Peterson and Lipcius (2003) also argued that secondary production may be the most useful proxy for ecosystem restoration.

Many studies have attempted to quantify the development of macrofaunal communities and fish assemblages on oyster reefs compared to other habitats.

However, most of these studies have occurred on intertidal reefs in the Southeast United States or Gulf of Mexico region (Wells, 1961; Crabtree and Middaugh, 1982; Zimmerman et al., 1989; Meyer and Townsend, 2000; Lenihan et al., 2001; Stuntz et al. 2001, 2002; Peterson et al., 2003; Soniat et al., 2004; Grabowski et al., 2005; Luckenbach et al., 2005; Tolley and Volety, 2005; Walters and Coen, 2006; Shervette and Gelwick, 2008). Of those that were conducted in the Chesapeake Bay, most have focused on the Virginia polyhaline region (Harding and Mann, 1999, 2000; Luckenbach et al., 2005). Rodney and Paynter (2006) reviewed many of these studies and noted that none were conducted in subtidal regions of the mesohaline Maryland portion of the Chesapeake Bay using dense assemblages of adult oysters.

Many of the above cited studies also use natural reference reefs for comparison to restored oyster reefs. Unfortunately, few natural, dense assemblages of adult oysters exist in Maryland due to the depleted natural population and subsequent extensive restoration efforts. Hatchery raised seed oysters and natural seed from higher salinity regions have been planted on historically productive mesohaline bars since the 1960s. The future of healthy oyster populations in the Maryland portion of the Chesapeake Bay seems to be dependent on extensive hatchery production of oysters for use in restoration efforts. Therefore, at least in mesohaline waters of the Chesapeake Bay, studies on the development of macrofaunal communities for the purpose of measuring restoration success should be conducted on reefs representative of those produced by restoration.

Quantifying the macrofaunal community on oyster reefs is a very onerous and time consuming process. Performing statistical analyses on these types of data can

also be very complex. Walters and Coen (2006) evaluated the utility of various statistical approaches for comparing community convergence on natural and restored oyster reefs. The researchers had difficulty designing experiments to conform to the test requirements, as well as difficulties manipulating data into formats that would allow easy comparison.

The index of biotic integrity (IBI) was developed by Karr (1981) to monitor pollution effects on freshwater systems. It has been adapted many times for use in other habitats, including benthic estuarine habitats. Weisberg et al. (1997) adapted the IBI into a benthic index of biotic integrity (B-IBI) for the Chesapeake Bay. The B-IBI is based on the deviation of several metrics from the conditions found at an undisturbed reference site. The level of deviation is assigned a categorical value, producing a multi-metric benthic index of biotic condition. The range of metrics includes species diversity, abundance of taxonomic groups of varying sensitivities to disturbance, and depth distribution of species. This approach can be quite time consuming, requiring the collection and identification to species level of the benthic infauna and epifauna of the study site. It also requires the development of scoring criteria based on the values for each metric on the reference site. This scoring can be subjective and prone to error. Finally, as discussed above, very few undisturbed "reference" oyster reefs are available in the Chesapeake Bay which would hinder the development of reference scores in this approach.

Perhaps a more useful metric of restoration success, particularly for habitat function, can be the response of an indicator species. The use of indicator species for monitoring ecological condition has been well established (Soule and Kleppel, 1988).

The use of an indicator species can provide a simple and low tech method for monitoring restoration activities because it does not require the extensive sorting and identification of samples as macrofaunal community response indicators. Crosby and Reese (1996) developed a method for using butterfly fish as indicators of coral reef health. Their method can be used around the world and by developing countries that may not have the resources or expertise to conduct extensive sampling programs.

The response of an indicator species can be determined using several metrics. Monitoring programs often use the presence or absence of the species as the indicator of habitat quality. On oyster reefs, the abundance and total biomass of the indicator species is likely more indicative of the quality of the oyster reef habitat on which it is found since many of the reef residents are also found in low density on low quality reef habitat (for example, on control sites in Rodney and Paynter, 2006).

The health of an indicator species can also be used to determine habitat quality. Individual health of fish can be measured using metrics of individual condition. For example, total body lipid content may be a good indicator of individual health as it represents energy stored in the organism rather than lost through respiration. Lipids in prey species may also signify the availability of energy to higher trophic levels (Crosby and Reese, 1996). More lipid-dense individuals may therefore contribute to enhanced survival and productivity of predatory species frequenting the habitat. Another indicator of individual fish health is the Fulton condition factor (Ricker, 1975), which uses a length-weight relationship to describe the condition and health of a fish.

Naked gobies (Gobiosoma bosc)

Naked gobies (*Gobiosoma bosc*, Lacepède, 1800) are small resident fish of the Gobiidae family found in abundance on oyster reefs in the Chesapeake Bay. They reach sexual maturity during their second year, at an approximate total body length of 2.4 cm (Nero, 1976). Naked gobies are dependent on the structure created by oyster reefs. In laboratory mesocosm experiments, Nero (1976) found that *G. bosc* would always choose substrates that provided some type of physical cover. Breitburg (1999) called the fish "cryptic" in that it tends to shelter within the interstitial spaces of the reef matrix when approached by a predator. Gobies are not an aggressive species, and will share a space in articulated oyster shells with other fish of similar size until a larger fish intrudes (Nero, 1976).

Naked gobies also rely on oyster reefs for brooding. Females will lay their eggs on the underside of oyster shell and inside "boxes", or dead oysters whose shells are still attached at the hinge. Male naked gobies will then guard the eggs until they hatch (Nero, 1976; Crabtree and Middaugh, 1982). The structure created by high density oyster shell is therefore essential to the continued recruitment of resident naked goby populations.

Breitburg (1999) summarized several of her studies in which she found that naked goby recruitment rates are 1 to 2 orders of magnitude greater than the maximum recruitment rate of coral reef fish and other temperate reef fish. She also found that naked larval gobies are one of the two most abundant fish larvae in the mesohaline area of Chesapeake Bay tributaries, second only to menhaden in the mainstem of the Bay. Larval naked gobies dominate the icthyoplankton during the

summer months (Shenker et al., 1983), with peak recruitment in the York and Pamunkey Rivers in May (Massmann et al., 1963), June and July in the Patuxent River (Shenker et al., 1983; Breitburg, 1999), and August in the Piankatank River (Harding and Mann, 2000). The recruitment of such a large number of larval naked gobies into the benthos results in a transport of substantial energy and nutrients from the water column to the benthic community.

Naked gobies provide an essential link between the benthic and pelagic communities in the Chesapeake Bay. Oysters filter plankton from the water column and deposit nutrient-rich feces and pseudofeces into the benthos, which is consumed by macroinvertebrates in the oyster reef community such as amphipods and polychaete worms (Dame and Patten, 1981). Naked gobies feed on copepods, amphipods, barnacle cyprids, polychaetes, and fish eggs (Breitburg, 1999) found on the oyster reef, with a preference for amphipods (Longenecker, 1993) and polychaete worms (Nero, 1976). These important prey items are found in high quantities on restored oyster reefs in mesohaline Chesapeake Bay. Rodney and Paynter (2006) found mean amphipod densities of approximately 150 m^{-2} and polychaete densities greater than 1,200 m^{-2} on restored oyster reefs. Rodney and Paynter (2006) also classified naked gobies as "carnivore/omnivores", a functional group which they identified as being the highest trophic level of the reef resident community, representing the link between the benthic reef community and non-resident species and higher trophic levels.

Naked gobies and their larvae are important food sources for higher trophic levels. Since it is a permanent resident of the shallow near-shore area, the fish is

consistently available for piscivorous fish foraging on the reef (Nero, 1976). Larval naked gobies are also important food for juvenile striped bass, red drum, bluefish, and blue crabs (Nero, 1976; Breitburg, 1999; Harding and Mann, 2001; Stuntz et al., 2002).

The naked goby is thus an ideal candidate for use as an indicator of the value of oyster reefs as habitat for other Bay organisms as well as indicator of the success of oyster reef restoration. Oyster reefs provide an essential habitat for naked gobies, providing rich feeding grounds, protection from predation within the reef structure, and nesting sites during spawning periods. Naked gobies serve as an available source of energy, linking sources in the benthos and the pelagic community.

Objectives

Understanding the role of naked gobies in the Chesapeake Bay ecosystem, this study was designed to examine how naked gobies respond to the various substrate types currently available in the Maryland portion of the Chesapeake Bay, and whether their response can be used as a useful indicator of oyster habitat quality. The substrates chosen for this study represent the range of adult oyster densities that are available in the Bay either naturally or from restoration efforts, as well as loose oyster shell substrate and ambient sediment. The response of naked gobies to these substrate types was measured using abundance, length, individual dry weight, biomass, lipid content, and Fulton condition factor. The results were used to determine the value of the various habitat types to naked gobies. An additional study was designed to determine the effect that live oysters have on naked gobies when compared to the complex reef structure that oysters create.

Chapter 2: Methods

To determine the effect of substrate type and oyster density on naked goby abundance, length, dry weight, biomass, lipid content, and condition, as well as the utility of naked gobies as an indicator of restoration success, four experiments were conducted in the Maryland portion of the Chesapeake Bay (Figure 1A). Field portions of Experiment 1 were conducted during the fall of 2005 in the Severn River (Figure 1B). Experiments 2, 3, and 4 were conducted in summer and fall of 2006 in the Patuxent River (Figure 1C).

Experiment 1: Severn River, Fall 2005

Experimental trays were placed in the Severn River in September of 2005, on the western shore of the Chesapeake Bay at the United States Naval Academy. This site was chosen due to its easy access from shore, protection from public access, and proximity to the University of Maryland campus in College Park. The entire Severn River has also been designated as an oyster sanctuary and has been targeted for many oyster restoration projects. Experimental trays were placed within a shallow cove at the site to protect them from boaters and wave action.

Environmental data were collected from the site several times over the course of the two month experiment (Table 1). Mean salinity, temperature, and dissolved oxygen were 9.9, 19.2°C, and 8.6 mg/L, respectively, during the two month experiment. The bottom substrate at this site was characterized as muddy sand.

Oysters for experiment 1 were collected with patent tongs by watermen from a

restored oyster reef in the Chester River, under the supervision of University of Maryland scientists. Oysters were culled of all fouling organisms, soaked in freshwater for 5 minutes, dipped in dilute bleach, and soaked for another 5-10 minutes in freshwater to remove cryptic organisms prior to their use in the experiment.

Plastic bread trays (0.34 m^2) served as replicate containers for each treatment within the experiment. The trays were lined with 1 mm^2 fiberglass screening and weighted with two pieces of 15 cm long rebar, attached with plastic zip ties. The trays were spaced 1 meter apart in a grid pattern in sub-tidal, shallow depths (1 to 2 meters) of water. Each tray was anchored into the sediment with 1 or 2 pieces of 31 cm long hooked rebar. Each tray was also filled with ambient sediment before the treatment substrates were added.

After each tray was staked down and filled with ambient sediment, treatment substrates were randomly assigned to each tray. The five treatments included ambient sediment only, loose oyster shell, low density (8-10 $tray^{-1}$, ~27 m^{-2}) living oysters, high density (40 $tray^{-1}$, 118 m^{-2}) living oysters, and very high density (80 $tray^{-1}$, 235 m^{-2}) living oysters. Oysters were oriented vertically in the trays with the hinge pointing towards the bottom. Each treatment was replicated four times.

Treatment densities were determined according to the maximum number of oysters that could fit into a tray (very high density), a high density of one-half the maximum density, and a low density of one-tenth the maximum density. The current average density of oysters on historic oyster bars in Chesapeake Bay is approximately 1 oyster m^{-2} (Jordan and Coakley, 2004). This density could not be accurately

replicated within the confines of this experiment. Therefore, the low density treatments are actually much denser than current oyster bar conditions in the Chesapeake Bay.

During the experiment, the trays were left in the water for eight weeks and monitored several times for damage by waves or human interference. At the completion of the experiment, SCUBA divers latched lids (constructed of the same plastic bread trays and mesh screen) onto the trays to trap resident organisms prior to delivery to shore. Naked gobies are very inquisitive and not likely to flee when approached by a predator (Nero, 1976). Thus, the escape of this organism when approached by the diver and tray lid was not a concern as the fish were more likely to burrow into the available substrate if startled (Breitburg, 1999).

On shore, rugosity (surface complexity) of each treatment was measured using a thin plastic-coated wire. Rugosity is a measure of surface complexity adapted from the stream sinuosity index (Allan, 1995). It was estimated by molding the wire over the surface of the contents of each tray between two randomly assigned distances from the tray edge. The wire was then straightened and the length required to cover the surface was measured. The ratio of this length to the straight line length of the tray represents the unit-less rugosity. Two measurements were taken on each tray starting at randomly assigned points to determine the mean rugosity of the tray.

After rugosity was measured, the oysters were removed from each tray and soaked in freshwater to draw out cryptic organisms. Tray contents were rinsed and collected on an apparatus constructed of two sieves with mesh sizes of 1.6 cm^2 and 1 mm^2 (see Rodney and Paynter, 2006). Oysters remained in the freshwater for the

duration of each tray sieving process, which ranged from ~10 to 45 minutes, after which their lengths were recorded. Water from the soak was sieved and organisms collected and preserved in 70% ethanol for later identification.

All fish collected were placed in a solution of 0.6% tricaine methanesulfonate (MS-222) for approximately 10 minutes before being preserved on ice. Upon return to the lab, all naked gobies were placed in plastic bags in a freezer at -80°C.

Faunal samples collected and preserved in ethanol were later sorted in the lab. Using dissecting microscopes, the abundances of organisms within the following taxonomic groups were recorded: polychaetes (including *Neanthes succinea, Polydora websteri, Sabellaria vulgaris*), amphipods (*Gammarus sp.*), clams (*Gemma gemma*), fish (*Gobiosoma bosc, Gobiesox strumosus,* and *Chasmoides bosquianus*), xanthid crabs (*Eurypanopeus depressus,* and *Rhithropanopeus harrisii*), isopods (*Idotea sp.*), snails, nudibranchs (*Doridella obscura*), and shrimp (*Palaemonetes pugio*). The abundances of polychaetes, amphipods, clams, mud crabs, and fish were chosen for further analysis because they represented the most abundant taxonomic groups (excluding fouling organisms) found within the treatment trays. They were also the most abundant organisms found on restored oyster reefs by Rodney and Paynter (2006). Abundances of individual species within each group were not recorded, only the abundance of organisms within each group listed above (with the exception of *G. bosc*), because species diversity and richness were not being tested. Instead, the intent of this portion of the experiment was simply to be able to correlate the abundance of each faunal group with the abundance, length, dry weight, biomass, lipid content, and condition factor of *G. bosc.*

Frozen naked goby samples were later placed in a freeze-drier for 36 hours. Once removed from the freeze-drier, each individual naked goby was counted. All samples collected from an individual tray were pooled and homogenized in a coffee grinder (Braun KSM2). The entire sample homogenate was then weighed to determine biomass, and divided by the number of individuals within the sample to determine an average individual dry weight (g).

After biomass and dry weight measurements were recorded and all samples homogenized with the grinder, sub-samples were loaded into cellulose thimbles that had been previously weighed and stored in a humidity-controlled (≤10%) "dry box". After samples were loaded into the cellulose thimbles, they were allowed to equilibrate within the dry box for at least 36 hours. Each loaded thimble was then weighed to determine the sample mass inside the thimble.

Non-polar lipids were extracted from the thimbles with petroleum ether using the Soxhlet method (Rowe, 2003). Petroleum ether was used to extract the lipids because it primarily dissolves non-polar storage and other loosely held lipids (Geise, 1966). Extractions were allowed to run for 6 to 8 hours. Following the extraction, the thimbles were dried at 55°C for approximately 12 hours and then allowed to equilibrate in the humidity controlled dry box for at least 24 hours before their final weight was recorded. The non-polar lipid content of each sample was determined by the following equation:

$$[(I - P) / (I - E)] * 100 = \% \text{ lipid},$$

where I is the pre-extraction mass of loaded thimble, P is the post-extraction mass of loaded thimble, and E is the pre-extraction mass of the empty thimble.

Experiment 2: Patuxent River, Summer 2006

The second experiment was conducted from June to August 2006 in the Patuxent River, on the shore of the United States Naval Recreation Center in Solomons, MD. This site was chosen due to its proximity to the Chesapeake Biological Laboratory, where all lab work was conducted, and because the near-shore area is protected from boating traffic by the U.S. Navy. This site was much different from the Severn River site, being characterized by high wave action and sandy bottom. It was, however, located in a protected cove created by rock barriers.

Environmental data were collected from the site four times during the experiment. Mean salinity, temperature, and dissolved oxygen were 12.6, 28.4°C, and 8.0 mg/L, respectively, during the two month experiment from June to August (Table 1).

Oysters for the experiment were collected by dredge from Broadneck reserve in the Patuxent River with the help of scientists at Morgan State University's Estuarine Research Center. This bar had been planted in 2001 with 5.2 million hatchery produced spat on shell, and was open to harvest in the 2005/2006 season. The bar was then cleaned of remnant oysters with a dredge in 2006. Oysters collected by dredge with the ERC staff had been cleared of sediment and most fouling organisms by the previous bar cleaning and harvest activity. The dredged oysters were held for two days in flow-through tanks at the Chesapeake Biological Laboratory in Solomons, MD before being transported to the study site. The oysters were not put through the process of removing cryptic organisms in any of the Patuxent River experiments because the response of macrofauna was not being

determined and the prior handling of the oysters was deemed to have sufficiently removed most cryptic organisms.

Set-up for the experiment was the same as described for experiment 1 with a few exceptions. Since no differences were found between the high and very high density treatments in any measurement during experiment 1, the very high density oyster treatment was eliminated from experiments 2 and 3. Since an entire treatment was eliminated, replication was increased to include 5 trays of each of the remaining treatments.

Since reef-associated macrofaunal abundance was not tested in experiments 2-4, after surface complexity measurements were taken each tray was immediately placed on the sieving apparatus rather than into a freshwater soak. The trays were rinsed, and the length of each oyster was recorded as it was removed from the tray. The remaining tray contents were dumped onto the surface of the sieve and all naked gobies were collected and placed in 0.6% solution of MS-222 for 10 minutes before being preserved on ice. Upon return to the lab, naked gobies were immediately placed in plastic bags in a freezer at -80°C. Frozen naked goby samples were later freeze-dried for at least 36 hours.

The length of individual gobies was measured from the tip of the anterior end to the tip of the caudal fin. The individual dry weight of each goby was measured and the sum of the total dry weight of all individuals within a tray was used as the biomass measurement. Individual naked goby length and dry weight were used to determine the Fulton condition factor (K), an indicator of fish condition (Ricker, 1975), using the formula

$$K = (W*L^{-3})*1000,$$

where W is the dry weight of the fish in grams, L is the total body length of the fish in centimeters, and 1000 is used as a scaling constant to increase small decimals for the facilitation of statistical analyses.

Individual naked gobies were initially broken into pieces with mortar and pestle, and loaded into each cellulose thimble for the lipid extractions, rather than sub-samples of a pooled homogenate. However, high variability and low statistical power were associated with this method and it was therefore not used in the rest of the experiments.

Experiment 3: Patuxent River, Fall 2006

Experiment 3 was conducted in the Patuxent River at the same location as experiment 2. Environmental data were collected three times during the August to October experiment. The mean salinity, temperature, and dissolved oxygen were 12.7, 24.2°C, and 8.4 mg/L, respectively (see Table 1).

Set-up for the experiment, including the treatments and replication, was identical to that of experiment 2. Oysters from experiment 2 were re-used in experiment 3. The trays remained in the water from August 23, 2006 to October 19, 2006.

As in experiment 1, all naked gobies from each tray were freeze-dried, pooled, and homogenized in a grinder. Sub-samples (0.14 ± 0.02 g) of each pooled homogenate were loaded into the previously equilibrated and weighed cellulose thimbles. After loading into the cellulose thimbles, the non-polar lipids were extracted from the samples and the lipid content was determined via the soxhlet

method as described above. Lipids were extracted from multiple sub-samples of each pooled homogenate, and a mean for each tray was calculated.

Experiment 4: Live oysters vs. reef structure

A fourth experiment was conducted to differentiate between the effect of living oysters and the structure they create on naked goby abundance, length, dry weight, biomass, lipid content, and Fulton condition factor. Experiment 4 was conducted on the upriver side of the protected cove at the same location in the Patuxent River as both experiments 2 and 3, and during the same time period as experiment 3. Environmental data for this experiment are therefore the same as for experiment 3 (Table 1).

Oysters for this experiment were collected from Broadneck reserve along with those for experiments 2 and 3. Experimental treatments consisted of trays of high density (~ 35 oysters $tray^{-1}$) clumps of either living or dead oysters, replicated five times. “Dead” oyster clumps were created by placing clumped, living oysters in boiling water to loosen the adductor muscle, removing the meat, and gluing each oyster back shut using marine epoxy (Splash Zone Compound A-788).

Trays for this experiment were deployed in an identical manner to those for experiments 2 and 3, though they were arranged in a 3x3 grid with one additional tray in a 4^{th} row. The trays were left in the water for 8 weeks before being removed, sieved, and naked gobies collected and preserved in an identical fashion as experiments 2 and 3.

On September 1, 2006, Tropical Storm Ernesto hit the Maryland coast, with wind and rain causing tidal surges up to 6 feet in several Chesapeake tributaries

(Knabb and Mainelli, 2006) including the Patuxent River. The trays were checked on September 7, 2006, at which time it was discovered that some oysters from each treatment had washed out of the trays. Since it was impossible to determine which trays the oysters had come from, the oysters were removed from the area without disturbing the treatments. Therefore, the final oyster density in some trays for this experiment was slightly lower than the initial oyster density. There was no difference, however, in the final oyster density or rugosity between the live and dead oyster treatments.

At the completion of the experiment, the trays were retrieved and samples collected and stored in an identical fashion as experiments 2 and 3. Once removed from the freeze-drier, the abundance, length, and dry weight of each individual naked goby were determined. All individuals from the same tray were then pooled and homogenized with a grinder before a final tray biomass was determined. As in experiment 3, mean lipid content was determined from extractions of multiple sub-samples from each tray.

Statistical analyses

Data were analyzed using SigmaStat 3.1 (Systat, 2004) and SAS 9.1 (SAS Institute, Inc., 2006). Unless otherwise noted, analyses of variance (ANOVAs) and all pair-wise comparisons were made using the Student-Newman-Keuls Method (SNK) with Bonferroni adjusted alpha values of 0.01 for experiment 1 and 0.017 for experiments 2 through 4. Individual fish data were pooled within treatments when the power of the performed test was much lower than desired, as indicated in the results section and in figure legends. Data were transformed when necessary to

achieve normality and/or equality of variance. In cases where normality could not be obtained through transformation, pair-wise comparisons were made using the non-parametric Kruskal-Wallis method. In cases where equality of variance could not be obtained through transformation, the non-parametric Welch ANOVA was used because it does not assume homogeneity of variance (Welch, 1951). Pair-wise comparisons were then performed with the Kruskal-Wallis test. Correlations were conducted using Pearson product moment calculations.

Chapter 3: Results

Experiments 1-3:

Structural complexity

Surface complexity, or rugosity, increased with increasing availability of oysters and oyster shell for each experiment (Figure 2). Analyses of variance (ANOVAs) conducted on the rugosity of treatments in each experiment indicated that the rugosity in the shell, high, and very high density treatments was significantly greater than the rugosity of the mud and low density treatments in each experiment (expt. 1, $F = 20.0$; expt. 2, $F = 12.6$; expt. 3, $F = 12.2$; $p < 0.01$). Mean rugosity in experiment 2 was inverse transformed, and one outlier value in the low density treatment of experiment 3 was removed to achieve normality.

Macrofaunal community

Polychaetes, amphipods, clams, mud crabs, and fish were the most abundant resident macrofaunal categories collected from trays in experiment 1. The mean abundance of organisms within each of these categories, with the exception of amphipods, increased with oyster and shell density (Table 2, Figure 3). The abundances of polychaetes, amphipods, clams, mud crabs, and fish in the very high density treatment were significantly greater than in the mud and low density treatments. Mud crabs and fish were also significantly more abundant in the high density treatments than in the mud treatments. The low density oyster and mud treatments had no differences in abundance of any of the organisms counted.

Goby responses

Abundance

The total number of naked gobies residing in the treatment trays increased with increasing oyster and shell density (Figure 4). In the Patuxent River (experiments 2 and 3), significantly more gobies were found in the high density oyster and shell treatments than in the mud or low density treatments ($F = 25.8$ (expt. 2), 14.9 (expt. 3); $p < 0.01$). In experiment 1, gobies were more abundant in the shell treatment than in the mud or low density treatments ($F = 13.8$, $p < 0.01$).

In each experiment, the mean number of gobies found in the shell, high, or very high density treatments was more than twice those found in the mud and low density treatments (Figure 4). In experiment 2, which took place during the summer in the Patuxent River, the mean number of gobies found in the high density and shell treatments were more than twice those found in the same treatments of both fall experiments (1 and 3).

Length

The lengths of individual gobies from each replicate were pooled within treatments in experiments 2 and 3 due to the low power (0.377 and 0.591, respectively) of tests on the replicate means. Data in experiment 2 were natural log transformed to achieve normality, and pair-wise comparisons made using the Kruskal-Wallis test. Data in experiment 3 could not be normalized with transformation and pair-wise comparisons were therefore conducted with the Kruskal-Wallis test. The mean goby length was significantly greater in the high density treatment than in the low density treatment for both experiments 2 ($X^2 = 10.03$, $p = 0.0015$) and 3 ($X^2 = 7.98$, $p = 0.0047$). Mean goby length was also greater in the shell treatment than the mud treatment in experiment 2 ($X^2 = 10.75$, $p = 0.001$;

Figure 5). Naked goby length was measured for the Patuxent River experiments only.

Individual dry weight

The mean individual dry weight of naked gobies within each treatment increased with increasing oyster and shell density (Figure 6). The goby dry weights in the high density and shell treatments were significantly greater than the mud treatment in each experiment, ($F = 12.483$ (expt. 1); $X^2 = 14.08$ (expt. 2, High-Mud comparison); $X^2 = 9.33$ (expt. 2, Shell-Mud comparison); $F = 9.205$ (expt. 3); $p < 0.01$). The mean individual dry weight was also greater in the high density and shell treatments than in the low density treatment in experiments 1 and 3 ($F = 12.483$ (expt. 1); $F = 9.205$ (expt. 3); $p < 0.01$).

Goby individual dry weight in the low density oyster treatment of experiment 3 was also greater than the mud treatment ($F = 9.205$, $p < 0.01$).

The result of the SNK ANOVA in experiment 2 showed no differences between treatments ($F = 4.501$, $p = 0.018$), but because the p value was close to being significant the power of the test (0.688) was lower than desired, the dry weight of individual gobies from each replicate in experiment 2 were pooled within treatments, log transformed to achieve normality, and pair-wise comparisons conducted using the Kruskal-Wallis test. The result of this analysis showed significant differences between treatments, as reported above.

Biomass

The total goby biomass in the high density, very high density, and shell treatments were 4 to 12 times greater than the mean biomass in the mud and low treatments in all experiments ($F = 42.4$ (expt. 1), $F = 38.0$ (expt. 2), $F = 21.7$ (expt. 3); $p < 0.01$; Figure 7). Data were square root transformed in each experiment to achieve

equality in variance.

Fulton condition factor

Individual goby condition values were pooled in each treatment for both experiments due to the very low power of analyses conducted on replicate means (0.05 for experiment 2, 0.09 for experiment 3). Experiment 2 data were inverse transformed to achieve normality before pair-wise comparison with the Kruskal-Wallis test due to the inequality of variance. The high density treatment in experiment 2 was significantly greater than the shell treatment ($X^2 = 10.53$, $p = 0.0012$). No other trend was seen in the condition data for experiment 2 (Figure 8).

Goby conditions in the high density and shell treatments of experiment 3 were significantly greater than that of gobies in the mud treatment (ANOVA, $F = 3.712$, $p = 0.013$).

Lipid content

Lipid content of naked gobies increased with oyster and shell density in experiment 1 (see Figure 9). In this experiment, mean goby lipid content for shell, high, and very high density reef trays were 150-200% greater than those from mud and low density treatments ($F = 13.5$, $p < 0.01$). Two replicates within the mud treatment contained only 1 fish in the tray, and thus the resulting mass in the samples used for lipid analyses was very small (two orders of magnitude lower than any other sample). The lipid analyses on these samples showed a lipid content 2 to 4 times higher than any other replicate in all treatments. Therefore, these two samples were excluded from statistical analyses. In addition, one replicate within the low density oyster treatment was eliminated due to error in the extraction process.

There was no difference in naked goby lipid content between treatments in experiments 2 or 3.

Correlations between goby metrics, reef structure, and other macrofauna

When data were combined across experiments 1, 2, and 3, goby individual dry weight, and total goby biomass per tray were positively correlated with oyster density and rugosity ($p < 0.05$, Table 3). Rugosity was also positively correlated with goby abundance, length, individual dry weight, biomass, and lipid content.

Goby dry weight, biomass, and lipid content were positively correlated with each of the most abundant macrofaunal categories collected in experiment 1 (Table 4). Goby abundance was also positively correlated with all faunal categories besides amphipods.

Experiment 4

Though the mean surface complexity of the live oyster treatment appeared slightly higher than the dead oyster treatment (Table 5), the difference was not significant ($t = 2.0$, $df = 8$, $p = 0.084$). There were no differences in naked goby abundance, length, individual dry weight, total biomass, lipid content or condition between treatments (Table 5).

Chapter 4: Discussion

The goals of these experiments were twofold: to determine how a resident fish species, *G. bosc*, responds to differences in oyster abundance and surface complexity of bottom types common in the mesohaline region of the Chesapeake Bay, and to determine the utility of using *G. bosc* as an indicator of oyster reef restoration success. The results of this study indicate that increasing oyster density and surface complexity have a positive effect on the development of macrofaunal communities, including naked gobies, as measured by polychaete, amphipod, clam, mud crab, and fish abundance, and naked goby length, dry weight, biomass, lipid content, and Fulton condition factor. These metrics might be useful indicators of restoration success.

In experiments 1 through 3, the structural complexity of the experimental reef increased with oyster density, creating interstitial space for resident macrofauna to inhabit. The loose shell treatment attracted significant numbers of macrofauna despite not having any living oysters within the trays. The shell treatments may have had a greater amount of interstitial space than the treatments that contained living oysters. Less surface area is available on the two shells of an individual live oyster than is available on the two shells when they are not clamped shut (Tolley and Volety, 2005). Therefore, the loose shell treatments likely had more available hard surface than the live oyster treatments, and, despite providing little relief above the sediment surface, may have been able to provide significantly more substrate than the rugosity measurements alone would suggest.

There was no difference in rugosity or any goby metric (with the exception of

goby dry weight in experiment 3) between mud and low density treatments in any of the experiments. The minimal substrate provided by such a low density of oysters (approximately 27.5 m^{-2}) apparently provided no additional benefit to naked gobies than the soft mud and sand substrates available in the Severn and Patuxent Rivers. This supports findings by Rodney and Paynter (2006) that the density of oysters is more important to the health of the reef community than the number of oysters alone. The current density of oysters in the Chesapeake has been estimated to be < 1 m^{-2} (Jordan and Coakley, 2004), which is < 4% of the oyster density in the low density treatments of this experiment. These results suggest that the current average density of oysters in the Chesapeake Bay provides little substrate relief or benefit to naked goby populations, and suggests there may have been a large decline in Bay-wide goby populations with the loss of oyster reef habitat.

While these studies showed that the presence of shell had a significant positive effect on the attraction of naked gobies, similar results might not have been obtained if the reef trays were allowed to remain in the water for a longer period of time. For this experiment, oyster shells were left in the trays for two months before being retrieved. During this time, sedimentation was not great enough to cover or bury the shell. Vertical structure is important for the survival of juvenile oysters because it alleviates the effects of sedimentation which may smother the organisms (Lenihan, 1999; Soniat et al., 2004). If shell treatments in this experiment had been left for a longer period of time, especially in the Severn River which is subject to high sedimentation rates, shell substrate would likely be covered and would therefore become unavailable for resident macrofauna and fish. Reefs consisting of live

oysters, however, would continue to grow upwards and potentially outpace sedimentation rates.

The results of analyses of lipid content are interesting but somewhat confounding. While lipid content increased with oyster density and rugosity in experiment 1, there were no differences in lipid content for experiments 2 or 3. This trend may be a result of goby spawning activity. Naked gobies are known to spawn in the Mid-Atlantic region between April and August (Nero, 1976). While spawning, fish will use a significant portion of their gonadal lipid reserves (Meffe and Snelson, 1993). Naked gobies mature during their second year (Nero, 1976) and likely allocate more energy for reproduction than for growth (Anthony et al., 2000). The sexually mature gobies collected during experiment 2, which took place from June to August, had likely allocated more of their lipid reserves towards spawning activities than into storage lipids. Nero (1976) found that gobies generally become sexually mature at body lengths of 2.4 cm. The mean length of naked gobies found on the mud treatment in experiment 2 was 2.4 ± 0.1 cm while the mean length for the high density and shell treatments were 2.9 ± 0.1 cm and 2.8 ± 0.1 cm, respectively. Naked gobies found on the high density and shell treatments were capable of participating in spawning activities, while some of those on the mud treatment may not have reached maturity yet. Since the few smaller gobies found on the mud treatment had likely not allocated as much of their lipid reserves towards reproduction, their condition during this season may have been better than in those fish actually spawning. This is supported by the relatively high lipid content and condition of naked gobies found on the mud treatment despite having a significantly smaller length and dry weight than

those on the high density and shell treatments (see Figures 8 and 9).

Naked gobies are also more attracted to hard substrate during spawning season since male gobies are known to establish a nest within the interstitial spaces provided by oyster shells (Nero, 1976; Crabtree and Middaugh, 1982; Breitburg, 1999). Egg-laden oyster shells were observed during the retrieval of experimental trays over the course of all three experiments but were not quantified or recorded. This nesting behavior probably explains the extremely high abundance and biomass of naked gobies found on the high density and shell treatments during experiment 2 compared to those found on the same treatments in the fall experiments, when spawning activity had ended for the season (Figure 4).

Naked gobies feed on amphipods and polychaete worms found within the reef matrix (Nero, 1976; Longenecker, 1993; Breitburg, 1999). The increased abundance of these organisms with increasing substrate availability and reef complexity (Rodney and Paynter, 2006; this study) likely improved the quality of the reef habitat for naked gobies, and thereby the health of the individuals, as evidenced by almost every metric of goby health used in this study.

There were surprising trends in some of the naked goby length and individual dry weight measurements. Given the expected allometric relationship between length and weight, one would expect the differences in these values between treatments to be similar. However, in some cases there were differences in length but not weight, and vice versa. These results suggest habitat specific length-weight relationships, possibly related to available interstitial space, territorial behavior, or other cryptic ecological traits meriting further investigation.

While each goby metric analyzed in experiments 1 through 3 was highly correlated with oyster reef complexity (Table 3), further analysis shows that it may be the oysters themselves that provide the most significant benefit to naked gobies. There were no differences in any of the goby metrics between live and dead oyster treatments in experiment 4 (Table 5). Tolley and Volety (2005) found similar results in comparisons of live and "dead" oyster clumps, though the "dead" clumps in their study were left gaping and provided more surface complexity than the living clumps. Their results and the results of individual experiments in this study would suggest that the structure created by oysters is more important to the health of naked gobies than the oysters themselves. However, when data were combined from experiments 1-3, each goby metric identified in this study was highly correlated with oyster density, even when the lack of living oysters in the mud and shell treatments were accounted for by being assigned a value of zero oysters (Table 3). Therefore, while the availability of hard substrate was beneficial to naked gobies, the correlation data suggests that the actual living oyster provided an additional benefit.

Though abundance and biomass of naked gobies may indicate that the fish are attracted to specific habitat types, in some cases the individual health of the naked gobies (as indicated by lipid content and Fulton condition factor) did not follow the same trend. For example, there were no differences between naked goby abundance, length, individual dry weight, and biomass in the high density and shell treatments of experiment two, but the condition of the gobies in the shell treatment was actually significantly lower than the gobies found in the high density treatment (Figure 8). However, in the same location during the fall (experiment 3), the condition of naked

gobies was greater on the high density and shell treatments than on the mud treatment. These results suggest that, at least in the Patuxent River during the summer months when naked gobies are spawning, high density living oysters had a significant, positive effect on the health of individual naked gobies and the goby population on the reef than did areas covered with loose oyster shell but absent of living oysters.

Naked goby abundance, dry weight, biomass, and lipid content were positively correlated with the abundance of each macrofaunal category found on the various habitat treatments, with the exception of clams (Table 4). Individual dry weight and total biomass of naked gobies were also strongly correlated with oyster density and rugosity (Table 3). Total biomass was greater on the high density and shell treatments than on the mud or low density treatments in each experiment (Figure 7). Individual dry weight was greater on the shell and high density treatments than the mud treatment in each experiment, as well as the low density treatment in experiments 1 and 3 (Figure 6). These results suggest that dense assemblages of living oysters may provide significant ecological benefits compared to areas devoid of oysters.

Dense assemblages of living oysters provide increased nutrient material for amphipods and polychaetes, both of which are important goby prey items and were highly correlated with oyster density (Table 4). Increased availability of these prey items, as well as the structure created by the reef, were also strongly correlated with naked goby dry weight, biomass, and lipid content (Table 4), indicating the ecological benefit that the oyster reef presented to naked gobies. The enhanced health of

individual gobies and the goby population on the reef will likely lead to increased energy availability for non-resident species, such as striped bass and red drum, in the form of either larval gobies or the adult gobies themselves. Thus, mean naked goby individual dry weight and total biomass may be appropriate indicators of habitat quality, energy transfer, community development, and ecosystem function on restored oyster reefs.

Setting target levels of restoration metrics is important for determining whether ecosystem function is improving. In experiments 1 through 3, a mean individual dry weight of 0.134 g and a biomass of 7.568 g m^{-2} were associated with the high density oyster treatment, or a mean density of approximately 100 large (94.2 ± 14.7 mm in this study) oysters m^{-2} (Figure 10). Setting target naked goby dry weight and total biomass consistent with these findings may be a simpler and more efficient method of monitoring the ecosystem functioning of restored oyster reefs than the collection of the entire macrofaunal community.

Because we are unsure of the density of oysters on restored reefs necessary to achieve sufficient ecosystem functioning, many metrics of restored ecosystem services provided by oyster reefs will be necessary. Naked goby dry weight and biomass may be just two of many possible metrics used to monitor the success of the restoration of the suite of ecosystem services oysters provide.

These conclusions are based on three small-scale experiments and a limited range of oyster densities. However, based on the observations of macrofaunal abundance and goby dry weight and biomass, it is clear that oyster restoration does enhance the faunal abundance and diversity on the bottom of mesohaline tributaries

of the Chesapeake Bay, which is beneficial to the larger Bay ecosystem beyond the reef. Further studies on the response of gobies to large scale restoration projects over a wide range of oyster densities will be necessary to determine whether this simple monitoring technique is a viable alternative to sampling and identifying the macrofaunal community response to oyster restoration. Additional metrics of oyster restoration success must also be developed to determine whether the ecosystem functioning of restored oyster reefs has been achieved.

APPENDIX I: Tables

Table 1: Environmental data for each experiment.

	Dates	River	Salinity	Temperature	DO (mg/L)
Fall 2005*	09/09/05 – 11/04/05	Severn	9.9 ± 3.3	19.2 ± 10.8ºC	8.6 ± 1.8
Summer 2006†	06/23/06 – 08/23/06	Patuxent	12.6 ± 1.8	28.4 ± 1.2ºC	8.0 ± 0.1
Fall 2006+	08/23/06 – 10/19/06	Patuxent	12.7 ± 1.2	24.2 ± 5.3ºC	8.4 ± 1.0

*Mean of data collected August 17, October 18, and October 23, 2005; †Mean of data collected June 23, July 20, August 17, and August 23, 2006. +Mean of data collected August 23, September 18, and October 19, 2006.

Table 2: Mean abundance of each category of organisms collected within each experimental replicate (tray) in the Severn River, ± standard error. Within each row, values that share the same letter are not significantly different from each other.

	Substrate type				
Organisms	Mud	Low density oysters	High density oysters	Very high density oysters	Shell
Amphipods[1]	56.5 ± 16.13[a]	31.75 ± 11.09[a]	98.25 ± 15.91[a,b]	166 ± 31.84[b]	82.33 ± 29.54[a,b]
Isopods[2]	4.75 ± 1.89	2.50 ± 1.66	0	0	0
Clams	5.75 ± 2.29[a]	10.75 ± 2.66[a]	16.50 ± 6.23[a,b]	43.75 ± 11.30[b]	92.33 ± 13.25
Snails	0.75 ± 0.25	0.25 ± 0.25	0	1.25 ± 1.25	0
Nudibranchs[3]	0.50 ± 0.29	2.75 ± 1.55	4.00 ± 1.08	3.75 ± 1.25	5.00 ± 2.52
Shrimp[4]	2.00 ± 2.00	2.00 ± 1.15	0.50 ± 0.50	4.75 ± 2.63	3.33 ± 1.76
Polychaetes[5]	19.00 ± 5.21[a]	53.25 ± 13.24[a]	74.25 ± 10.42[a,b]	267.25 ±71.62[c]	231.33 ±34.17[b,c]
Mud crabs[6]	1.75 ± 1.44[a]	7.50 ± 2.75[a]	37.25 ± 3.17[b]	61.25 ± 7.08[c]	55.00 ± 6.67[b,c]
Fish[7]	3.75 ± 1.03[a]	7.50 ± 1.44[a,b]	14.50 ± 1.26[b,c]	18.50 ± 3.01[c]	24.25 ± 2.96[c]

[1]species of amphipods: *Gammarus sp.;* [2]species of isopods: *Idotea sp.*; [3]species of nudibranchs: *Doridella obscura;* [4]species of shrimp: *Palaemonetes pugio;* [5]species of polychaetes: *Nereis succinea, Polydora websteri, Sabellaria vulgaris*; [6]species of mud crabs: *Eurypanopeus depressus, Rithropanopeus harrisii;* [7]species of fish: *Gobiosoma bosc, Chasmodes bosquianus, Gobiesox strumosus.*

Table 3: Pearson product moment correlation between data from each treatment combined from experiments 1, 2, and 3. Upper value in each cell represents r and lower value represents p.

	Rugosity	**Goby abundance**	**Goby length**	**Goby dry weight**	**Goby biomass**	**% Lipid**	**K value**
Oyster Density	0.546 0.00000764	0.238 0.0669	0.250 0.120	0.353 0.00567	0.351 0.00598	0.0822 0.543	0.158 0.336
Rugosity		0.400 0.00168	0.547 0.000314	0.596 0.000000631	0.592 0.000000793	0.361 0.00621	-0.0528 0.749
Goby abundance			0.0499 0.760	0.110 0.401	0.907 2.02×10^{-23}	-0.268 0.0435	0.284 0.0793
Goby length				0.925 1.329×10^{-17}	0.297 0.0630	0.473 0.00207	-0.314 0.0512
Goby dry weight					0.377 0.00303	0.593 0.00000117	-0.241 0.140
Goby biomass						-0.0786 0.561	0.221 0.176
% Lipid							-0.372 0.0197

Table 4: Pearson product moment correlation between organism abundances and naked goby abundance, dry weight, biomass, and lipid content from all treatments in experiment 1. Upper value in each cell represents r and lower value represents p.

	Polychaetes	**Amphipods**	**Clams**	**Mud crabs**
Oyster density	0.539 0.0173	0.706 0.000723	0.0566 0.818	0.565 0.00944
Goby abundance	0.656 0.00228	0.298 0.215	0.737 0.000315	0.745 0.000164
Goby dry weight	0.765 0.000136	0.833 0.00000952	0.469 0.0428	0.880 0.000000308
Goby biomass	0.777 0.0000912	0.618 0.00484	0.520 0.0226	0.792 0.0000315
Goby % lipid	0.631 0.00662	0.534 0.0271	0.705 0.00159	0.779 0.000138

Table 5: Mean (± SEM) rugosity, goby abundance, length, individual dry weight, biomass, lipid content, and condition per treatment in experiment 4. There were no differences in any measurements between treatments.

	Treatment			
	Live oysters	**Dead oysters**	**F**	**p**
Rugosity	1.5 ± 0.4	1.4 ± 0.06	3.896	0.084
Goby abundance	30.8 ± 2.7	34.8 ± 3.0	0.989	0.349
Goby length (cm)	3.3 ± 0.1	3.3 ± 0.3	0.040	0.846
Goby individual dry weight (g)	0.15 ± 0.02	0.14 ± 0.03	0.195	0.671
Goby biomass (g)	1.2 ± 0.2	0.8 ± 0.1	3.246	0.109
Goby lipid content (%)	9.4 ± 0.7	9.5 ± 0.4	0.002	0.967
Goby condition (K value)	3.3 ± 0.1	3.1 ± 0.1	4.027	0.080

APPENDIX II: Figures

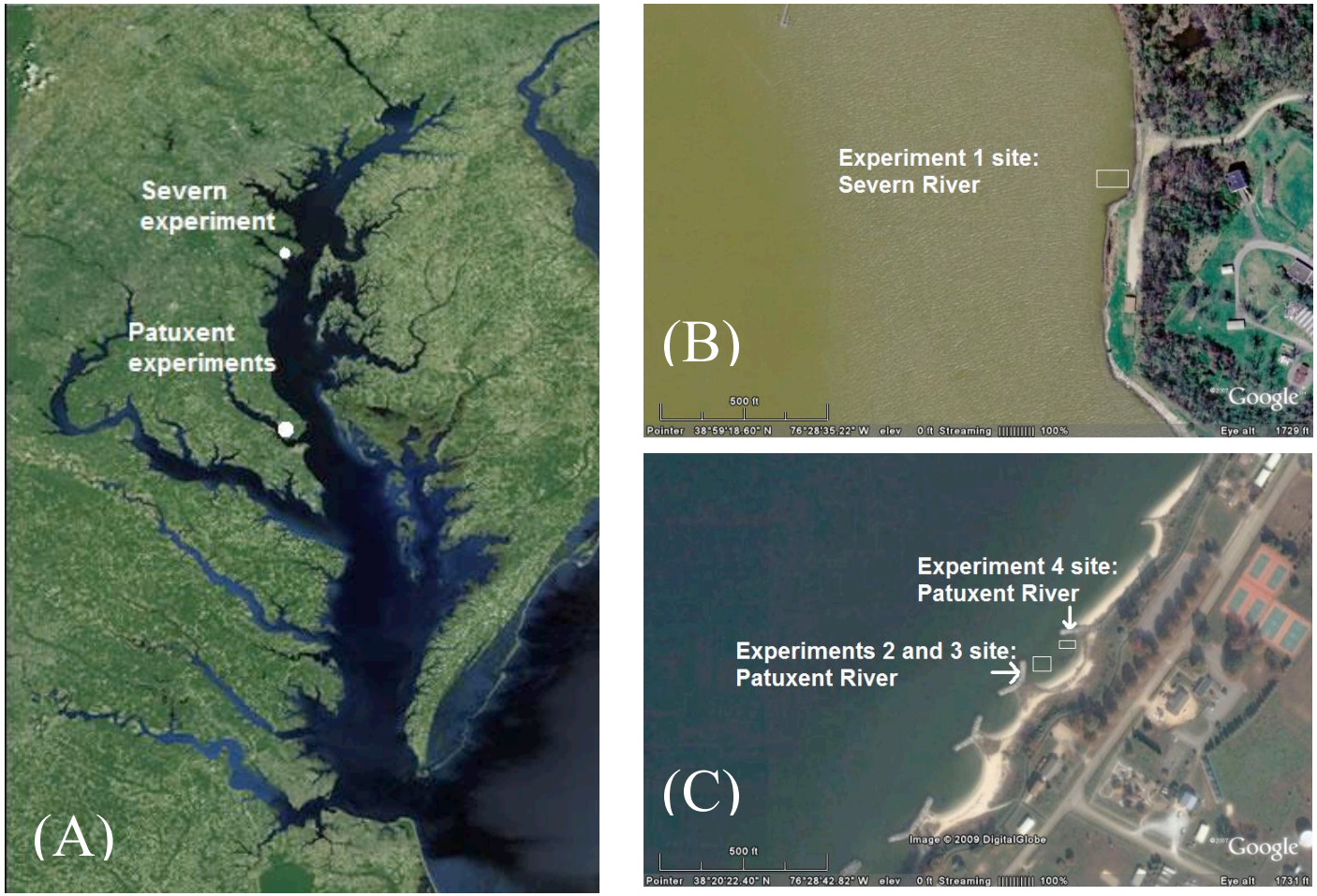


Figure 1: Location of field experiments. Image (A) shows the location of the Severn and Patuxent Rivers in the Maryland portion of the Chesapeake Bay while (B) and (C) indicate the placement of experimental trays in protective coves at each field site. Image (A): NASA/Goddard Space Flight Center Scientific Visualization Studio. Images (B) and (C): Google Earth.

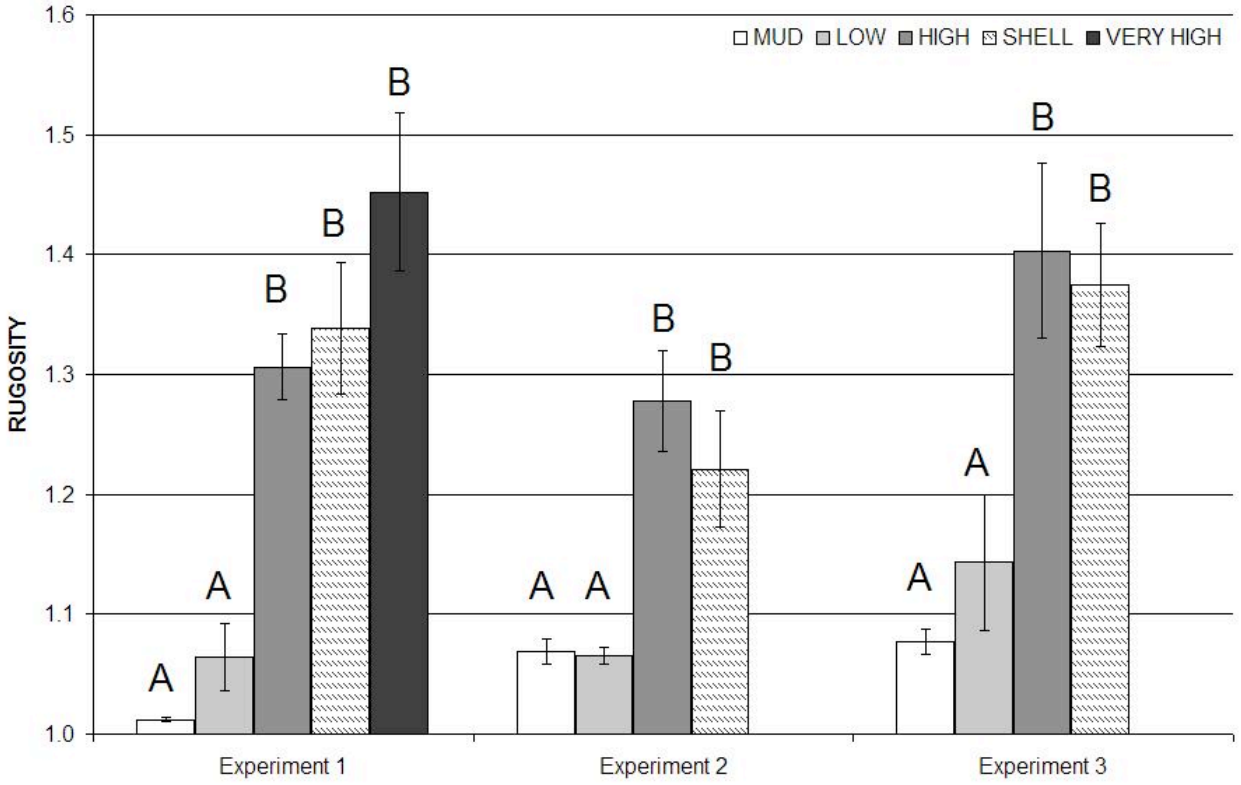


Figure 2: Surface complexity of each substrate type, ± standard error. In each experiment, shell, high, and very high density oyster treatments were significantly more complex than the mud and low density treatments (SNK ANOVA). Within each experiment, treatments sharing a letter are not significantly different from each other.

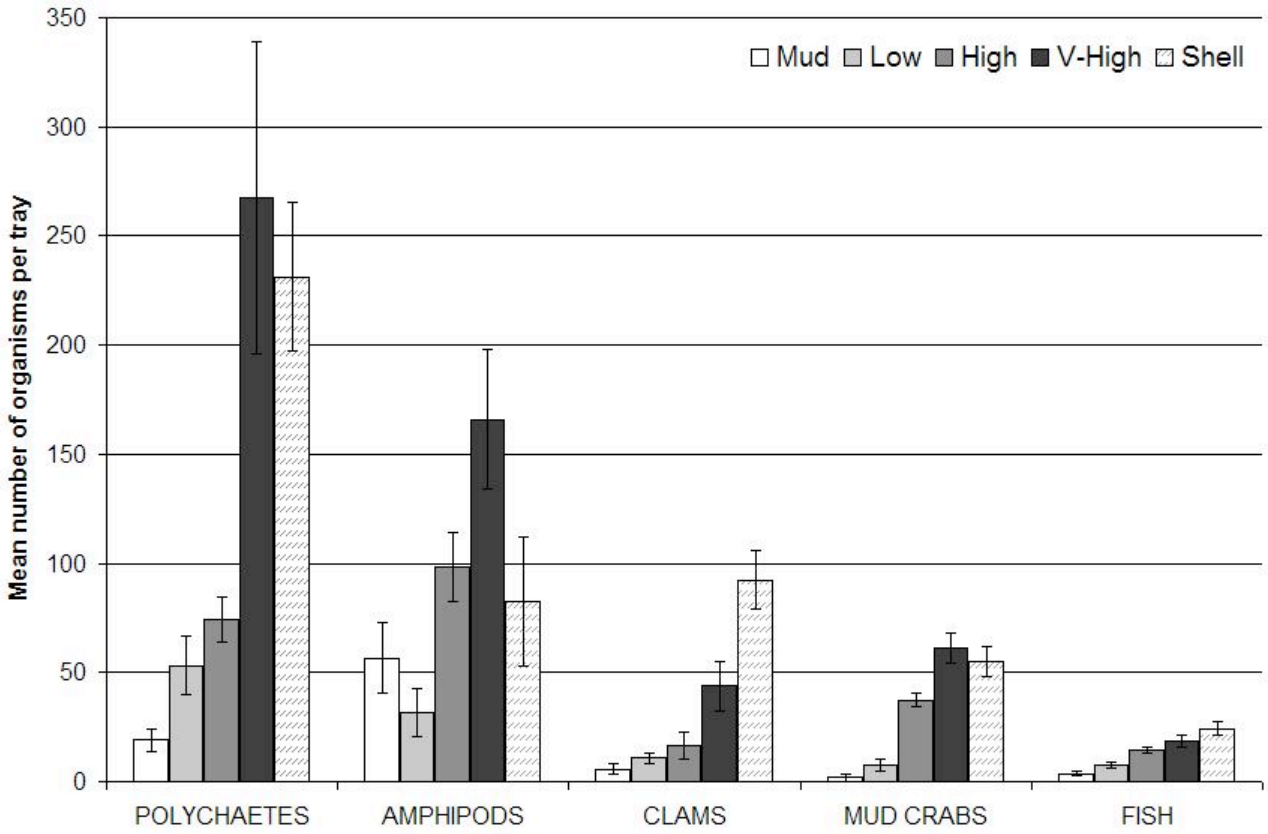


Figure 3: Mean abundance per tray of the most abundant macrofaunal categories found in experiment 1, ± standard error. See Table 2 for details on species contained within each category.

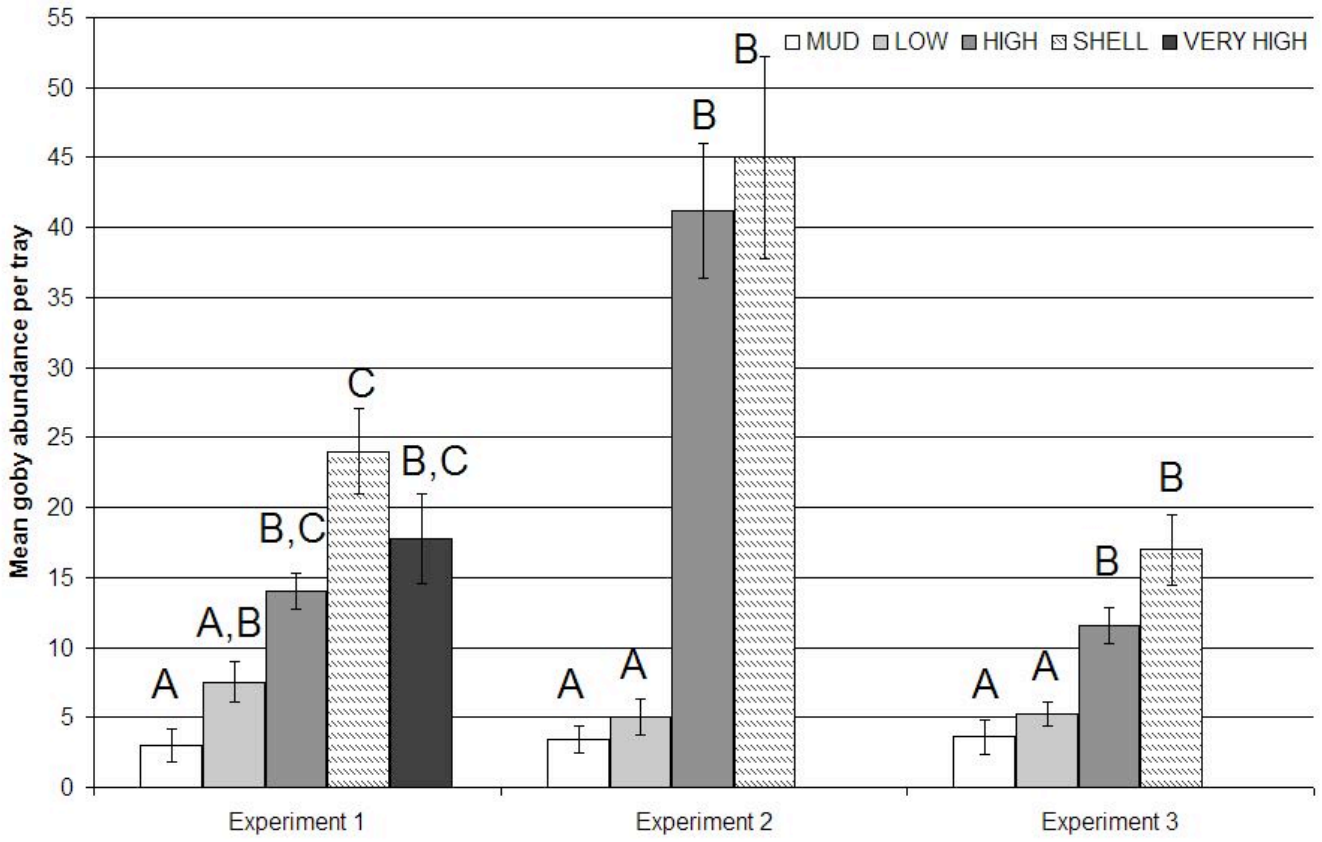


Figure 4: Mean total abundance of naked gobies per tray, ± standard error. Within each experiment, treatments sharing a letter are not significantly different from each other).

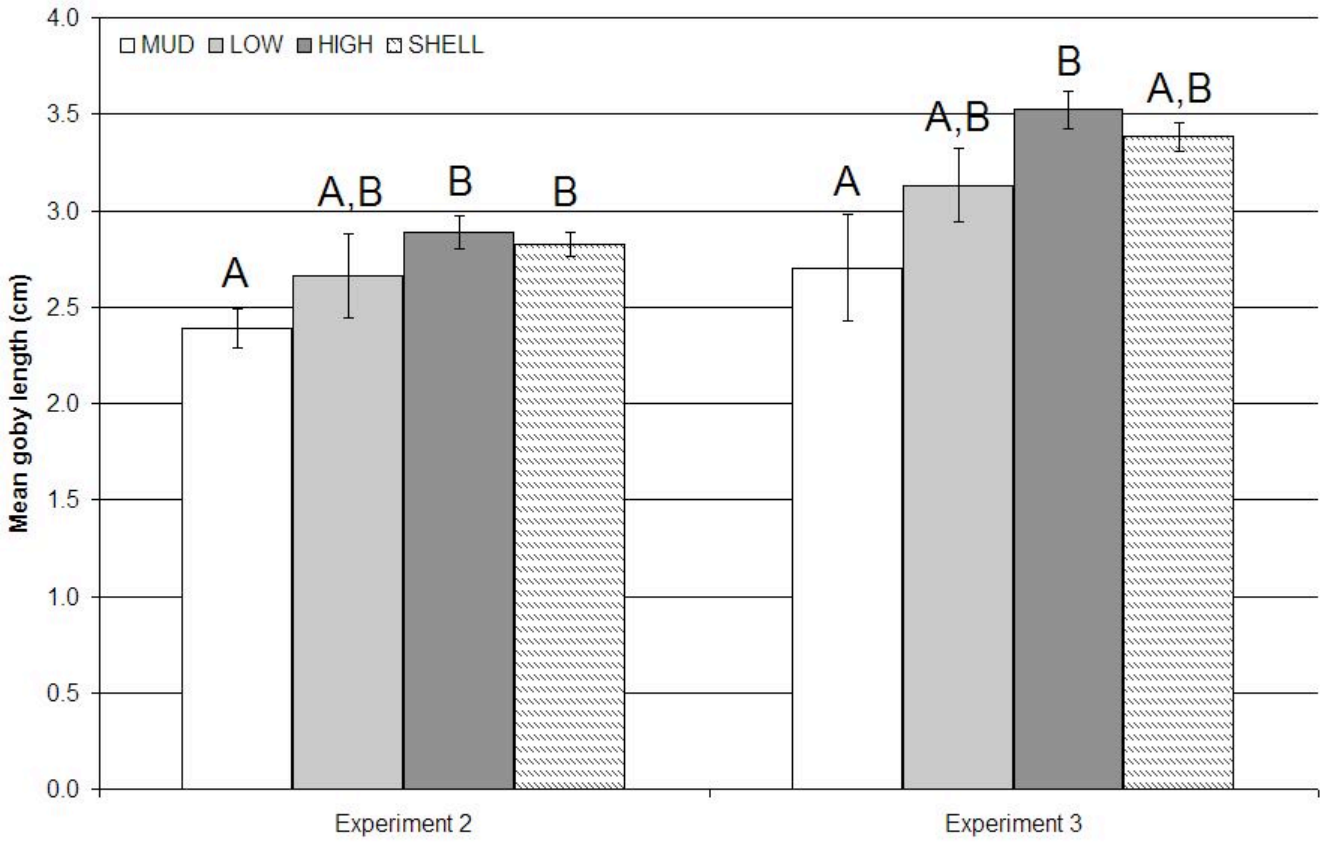


Figure 5: Mean total body length of naked gobies in each treatment, ± standard error. Within each experiment, treatments sharing a letter are not significantly different from each other).

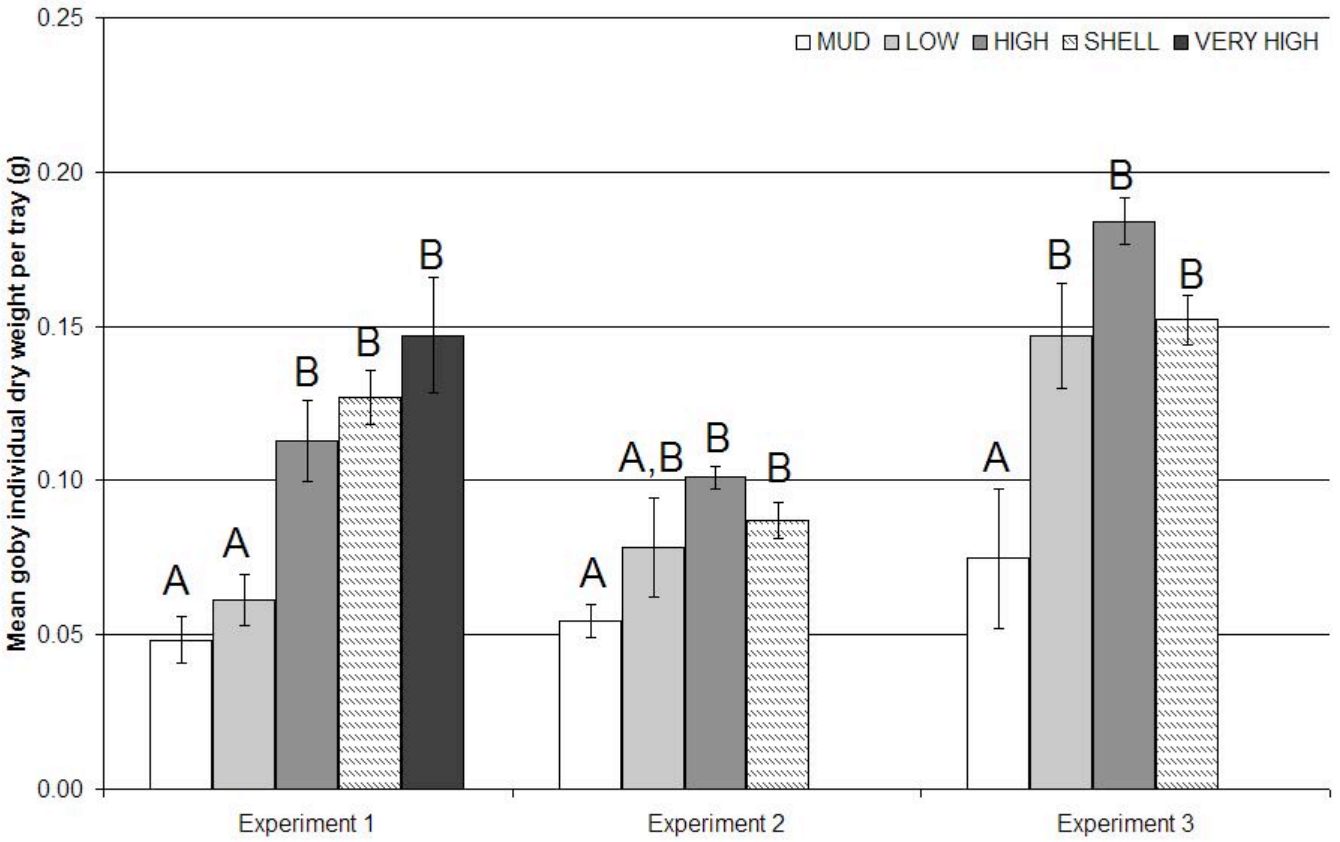


Figure 6: Mean individual dry weight of naked gobies in each treatment, ± standard error. Within each experiment, treatments sharing a letter are not significantly different from each other.

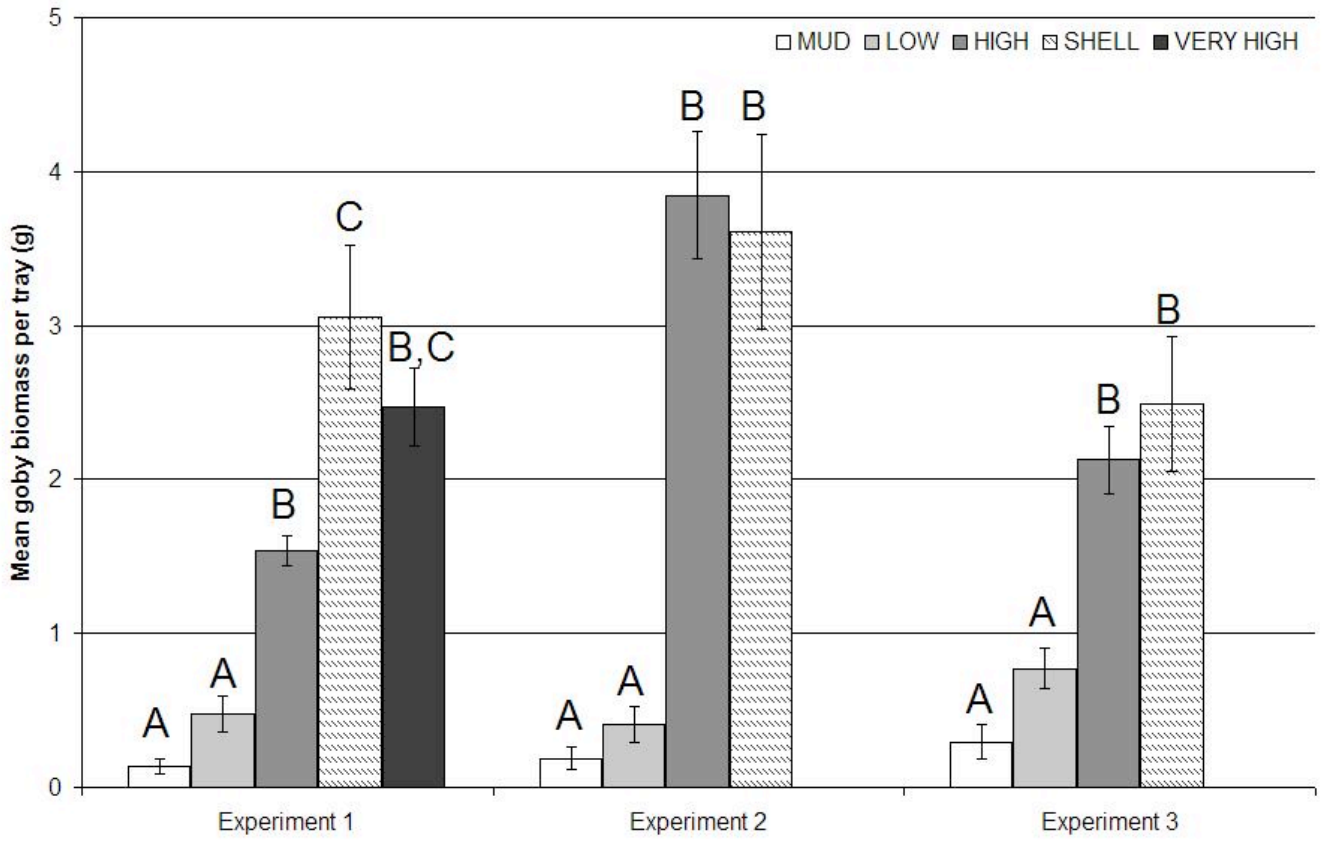


Figure 7: Mean total biomass of naked gobies in each tray, ± standard error. Within each experiment, treatments sharing a letter are not significantly different from each other.

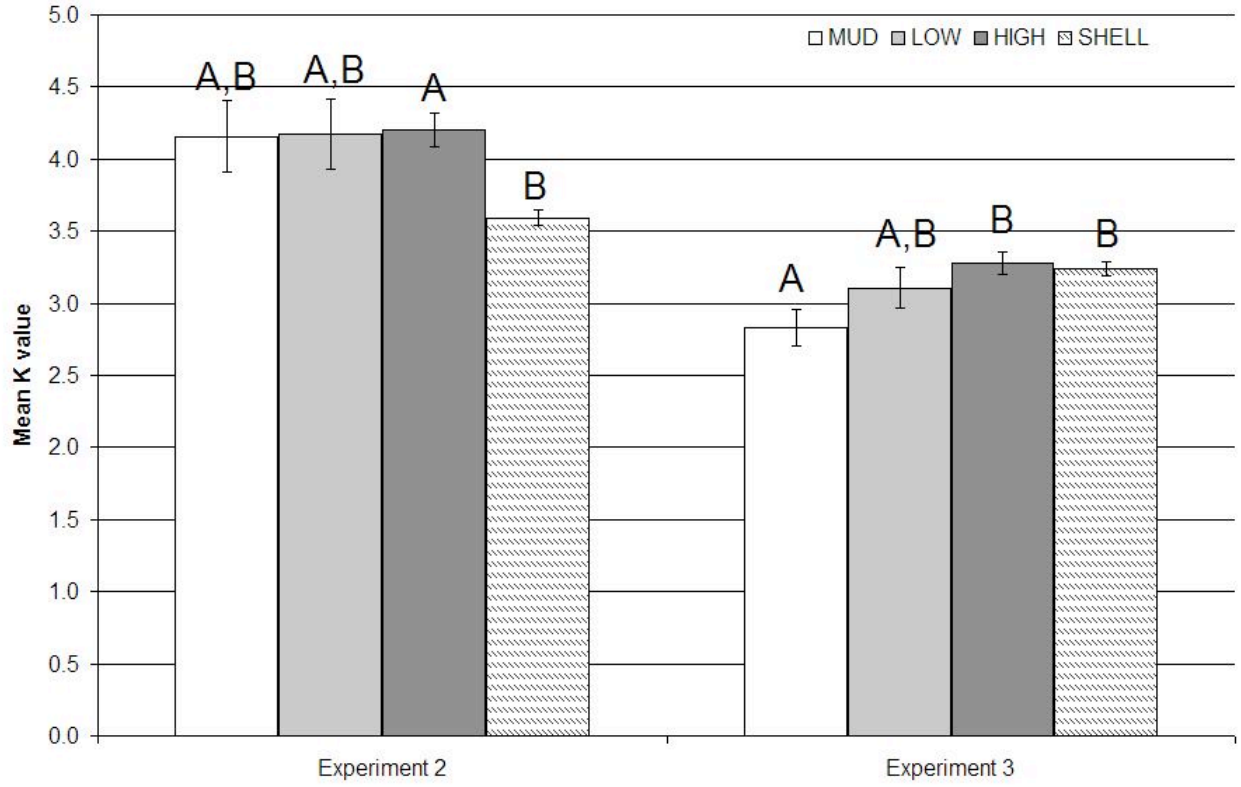


Figure 8: Mean Fulton Condition Index (K value) of naked gobies in experiments 2 and 3, ± standard error. Within each experiment, treatments sharing a letter are not significantly different from each other.

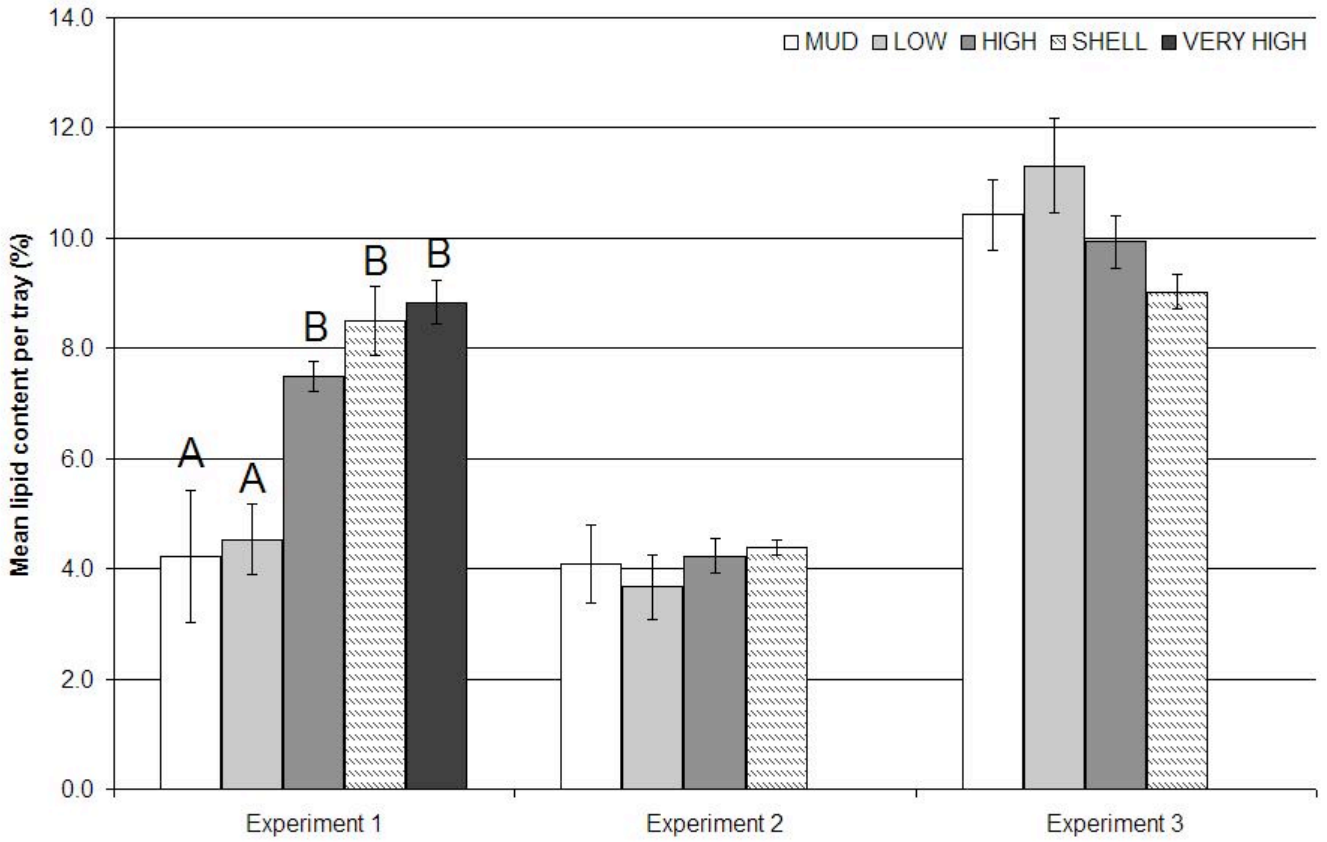
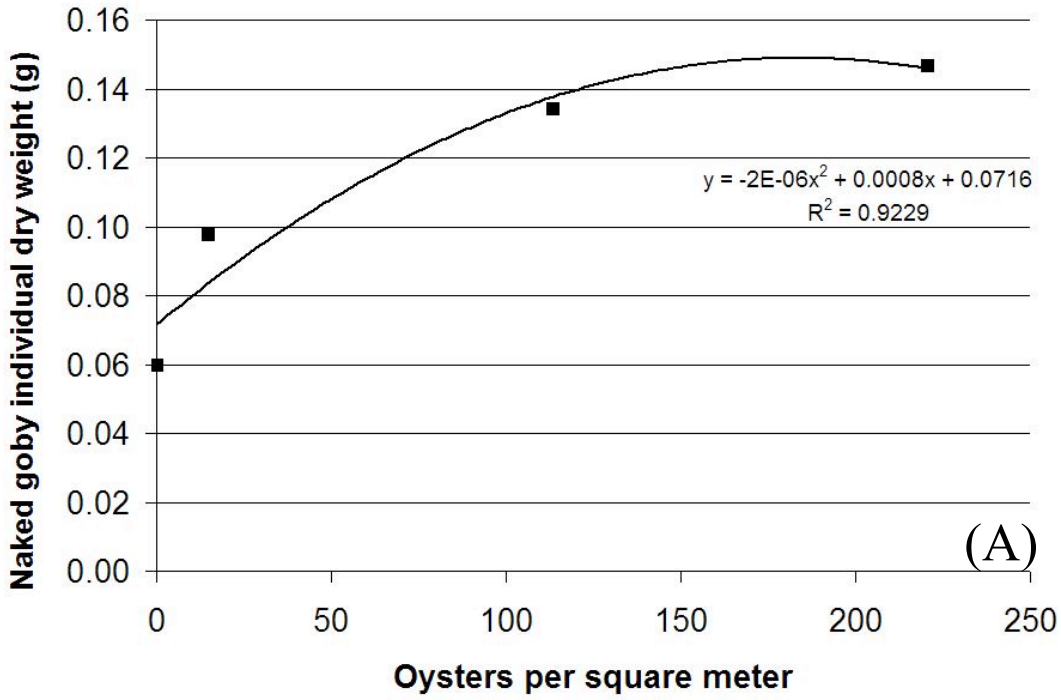


Figure 9: Mean lipid content of naked gobies in each tray, ± standard error. Within experiment 1, treatments sharing a letter are not significantly different from each other.



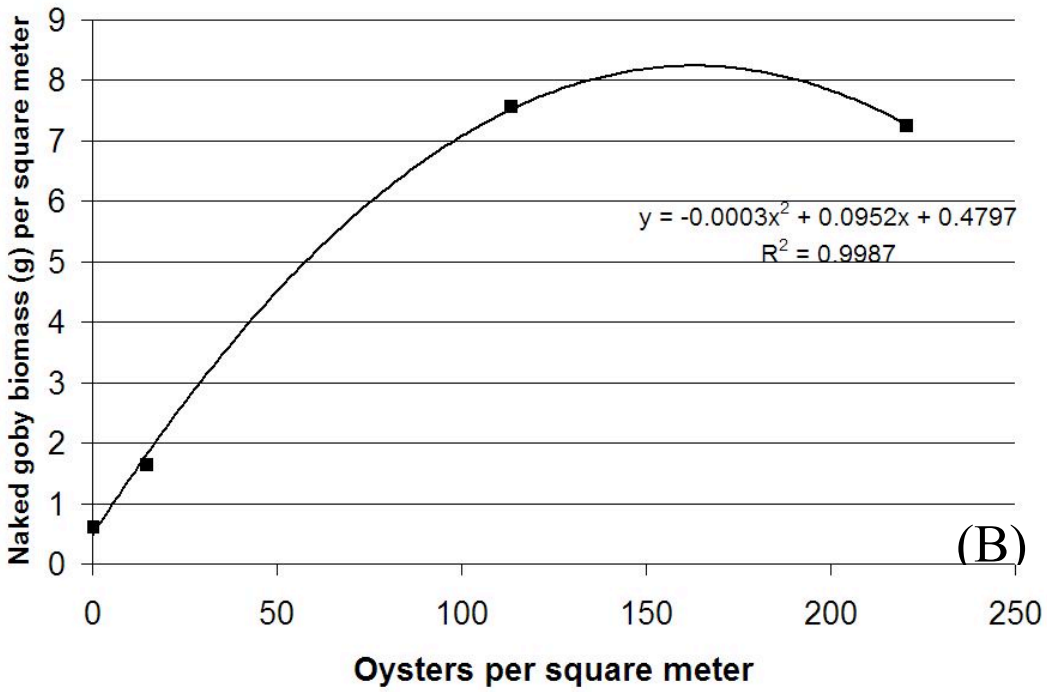


Figure 10: Increasing trend between adult oyster density m^{-2} and (A) naked goby individual dry weight and (B) naked goby biomass m^{-2}. Polynomial curves were fitted using Microsoft Excel (2003).

References

Allan, JD, 1995. Stream Ecology: Structure and Function of Running Waters. Kluwer Academic Publishers. Boston MA.

Andrews, JD. 1965. Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. *Chesapeake Science* 6: 60-67.

Andrews, JD. 1988. Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. Pages 47-63 in WS Fisher (ed). Disease Processes in Marine Bivalve Molluscs. American Fisheries Society Special Publication 18. Bethesda, Maryland.

Anthony, JA, DD Roby, and KRTurco. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. *Journal of Experimental Marine Biology and Ecology* 248: 53–78.

Arve, J. 1960. Preliminary report on attracting fish by oyster-shell plantings in Chincoteague Bay, Maryland. *Chesapeake Science* 1: 58-65.

Baird, D and RE Ulanowicz. 1989. The seasonal dynamics of the Chesapeake Bay ecosystem. *Ecological Monographs* 59:329-364

Barber, BJ, SE Ford, and HH Haskin. 1988. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. I. Condition Index and Relative Fecundity. *Journal of Shellfish Research* 7(1): 25-31.

Boesch, DF and J Greer (ed.). 2003. Chesapeake Futures: Choices for the 21st Century. An independent report by the Scientific and Technical Advisory Committee. Chesapeake Bay Program, STAC Publication Number: 03-001.

Breitburg, D.L. 1999. Are three-dimensional structure and healthy oyster populations the keys to an ecologically interesting and important fish community? Pages 239-250 in M.W. Luckenbach, R. Mann and J.A. Wesson (eds.) Oyster Reef Habitat Restoration. A Synopsis and Synthesis of Approaches. Virginia Institute of Marine Science, Gloucester Point, VA.

Brooks, WK. 1891. The Oyster. The Johns Hopkins University Press, Baltimore MD.

Burreson, EM and JD Andrews. 1988. Unusual intensification of Chesapeake Bay oyster diseases during recent drought conditions. Pages 799-802 in Oceans '88 Proceedings. The IEEE Service Center, Piscataway, New Jersey.

Burreson, EM, LMR Calvo, JF LA Peyre, F Counts, and KT Paynter. 1994. Acute osmotic tolerance of cultured cells of the oyster pathogen *Perkinsus marinus* (Apicomplexa: Perkinsida). *Comparative Biochemistry and Physiology* 109A: 575-582.

Burreson, EM and LM Ragone Calvo. 1996. Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. *Journal of Shellfish Research* 15: 17-34.

Burreson, EM, NA Stokes, and CS Friedman. 2000. Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. *Journal of Aquatic Animal Health* 12: 1-8.

Carlton, JT and R Mann. 1996. Transfers and world-wide introductions. Pages 691-706 in VS Kennedy, RIE Newell, and AF Able (eds.). The Eastern Oyster, *Crassostrea virginica*. Maryland Sea Grant Publications, College Park, MD.

Coen, LD, RD Brumbaugh, D Bushek, R Grizzle, MW Luckenbach, MH Posey, SP Powers, and SG Tolley. 2007. Ecosystem services related to oyster restoration. *Marine Ecology Progress Series* 341: 303-307.

Coen, LD and MW Luckenbach. 2000. Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? *Ecological Engineering* 15: 323-343.

Cooper, SR. 1995. Chesapeake Bay watershed historical land use: Impact on water quality and diatom communities. *Ecological Applications* 5: 703-723.

Crabtree, RE and DP Middaugh. 1982. Oyster shell size and the selection of spawning sites by *Chasmodes bosquianus, Hypleurochilus geminatus, Hypsoblennius ionthas* (Pisces, Blenniidae) and *Gobiosoma bosci* (Pisces, Gobiidae) in two South Carolina estuaries. *Estuaries* 5: 150-155.

Crosby, MP and ES Reese. 1996. A Manual for Monitoring Coral Reefs With Indicator Species: Butterflyfishes as Indicators of Change on Indo Pacific Reefs. Office of Ocean and Coastal Resource Management, National Oceanic and Atmospheric Administration, Silver Spring, MD. 45 pp.

Dame, RF, D Bushek, D Allen, A Lewitus, D Edwards, E Koepfler, and L Gregory. 2002. Ecosystem response to bivalve density reduction: management implications. *Aquatic Ecology* 36: 51-65.

Dame, RF and BC Patten. 1981. Analysis of energy flows in an intertidal oyster reef. *Marine Ecology Progress Series* 5: 115-124.

Dame, RF, JD Spurrier, and RG Zingmark. 1992. In situ metabolism of an oyster reef. *Journal of Experimental Marine Biology and Ecology* 164: 147-159.

Ernst, HR. 2003. Chesapeake Bay Blues: Science, Politics, and the Struggle to Save the Bay. Rowman and Littlefield Publishers. Inc. Lanham, Maryland.

Ewart, JW, and SE Ford. 1993. History and Impact of MSX and Dermo Diseases on Oyster Stocks in the Northeast Region. Northeastern Regional Aquaculture Center, University of Massachusetts -Dartmouth. NRAC Fact Sheet No. 200 – 1993.

Ford, SE and MR Tripp. 1996. Disease and defense mechanisms. Pages 581-660 in VS Kennedy, RIE Newell, and AF Able (eds.). The Eastern Oyster, *Crassostrea virginica*. Maryland Sea Grant Publications, College Park, MD.

Geise, AC. 1966. Lipids in the economy of marine invertebrates. *Physiological Reviews* 46: 244-298.

Grabowski, JH, AR Hughes, DL Kimbro, and MA Dolan. 2005. How habitat setting influences restored oyster reef communities. *Ecology* 86(7): 1926-1935.

Harding, JM and R Mann. 1999. Fish species richness in relation to restored oyster reefs, Piankatank River, Virginia. *Bulletin of Marine Science* 65: 289-300.

Harding, JM and R Mann. 2000. Estimates of naked goby (*Gobiosoma bosc*), striped blenny (*Chasmodes bosquianus*) and eastern oyster (*Crassostrea virginica*) larval production around a restored Chesapeake Bay oyster reef. *Bulletin of Marine Science* 66: 29-45.

Harding, JM and R Mann. 2001. Diet and habitat use by bluefish, *Pomatomus saltatrix*, in a Chesapeake Bay estuary. *Environmental Biology of Fishes* 60: 401-409.

Harris, CS. 2003. Eastern oyster (*Crassostrea virginica*) growth and epifaunal community development on bars of varying oyster density in Chesapeake Bay. Master's thesis, University of Maryland, College Park, Maryland, USA.

Jordan, SJ and JM Coakley. 2004. Long-term projections of eastern oyster populations under various management scenarios. *Journal of Shellfish Research* 23: 63-72.

Karr, JR. 1981. Assessment of biotic integrity using fish communities. *Fisheries* 6:21–27.

Kennedy, VS. 1989. The Chesapeake Bay oyster gishery: Traditional management practices. Pages 455-477 in JF Caddy (ed.). Offprints from marine invertebrate fisheries: Their assessment and management. John Wiley & Sons, Inc.

Kennedy, VS and LL Breisch. 1981. Sixteen decades of political management of the oyster fishery in Maryland's Chesapeake Bay. *Journal of Environmental Management* 16: 153-171.

Knabb, RD and M Mainelli. 2006. Tropical Cyclone Report – Hurricane Ernesto (AL052006). National Hurricane Center, National Oceanic and Atmospheric Administration (DOC/NOAA/NWS/NCEP/NHC/TPC). http://www.nhc.noaa.gov/pdf/TCR-AL052006_Ernesto.pdf.

Lenihan, HS, and CH Peterson. 1998. How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. *Ecological Applications* 8:128-140.

Lenihan, HS. 1999. Physical-biological coupling on oyster reefs: how habitat structure influences individual performances. *Ecological Monographs* 69: 251-275.

Lenihan, HS and CH Peterson. 2004. Conserving oyster reef habitat by switching from dredging and tonging to diver-harvesting. *Fish Bulletin* 102: 298-305.

Lenihan, HS, CH Peterson, JE Byers, JH Grabowski, GW Thayer, and DR Colby. 2001. Cascading of habitat degradation: Oyster reefs invaded by refugee fishes escaping stress. *Ecological Applications* 11: 764-782.

Longenecker, KR. 1993. Prey capture rates of *Gobiosoma bosc* on oyster reefs: a comparison with a soft-bottom habitat. Master's thesis, Department of Biology, University of South Florida, Tampa, Florida, USA.

Luckenback, MW, LD Coen, PG Ross Jr., and JA Stephen. 2005. Oyster reef habitat restoration: Relationships between oyster abundance and community development based on two studies in Virginia and South Carolina. *Journal of Coastal Research* 40: 64-78.

MacKenzie, CL. 1996. Management of natural populations. Pages 707-721 in VS Kennedy, RIE Newell, and AF Able (eds.). The Eastern Oyster, *Crassostrea virginica*. Maryland Sea Grant Publications, College Park, MD.

Mann, R and DA Evans. 1998. Estimation of oyster, *Crassostrea virginica*, standing stock, larval production and advection loss in relation to observed recruitment in the James River, Virginia. *Journal of Shellfish Research* 17: 239-253.
Massmann, WH, JJ Norcross, and EB Joseph. 1963. Distribution of larvae of the naked goby, *Gobiosoma bosci*, in the York River. *Chesapeake Science* 4: 120-125.

MD ORT. 1993. Maryland Oyster Roundtable Action Plan. Maryland Department of Natural Resources Fisheries Services, 30 pp.

Meffe, GK and FF Snelson. 1993. Annual Lipid Cycle in Eastern Mosquitofish (*Gambusia holbrooki: Poeciliidae*) from South Carolina. *Copeia* 1993(3): 596-604.

Meyer, DL, and EC Townsend. 2000. Faunal utilization of created intertidal eastern oyster (*Crassostrea virginica*) reefs in the southeastern United States. *Estuaries* 23: 34-45.

Microsoft, 2003. Microsoft ® Office Excel 2003, © 1985-2003 Microsoft Corporation.

Nero, LL. 1976. The natural history of the naked goby *Gobiosoma bosci* (Perciformes: Gobiidae). Master's thesis, Institute of Oceanography, Old Dominion University, Norfolk, Virginia, USA.

Newell, RIE. 1988. Ecological changes in the Chesapeake Bay: Are they the result of overharvesting the American oyster, *Crassostrea virginica*? Pages 536-546 in MP Lynch and EC Krome (eds.), Understanding the estuary: advances in Chesapeake Bay research. Chesapeake Bay Research Consortium, Publ 129 CBP/TRS 24/88, Gloucester Point, VA.

Newell, RIE and CJ Langdon. 1996. Mechanisms and physiology of larval and adult feeding. Pages 185-229 in VS Kennedy, RIE Newell, and AF Able (eds.). The Eastern Oyster, *Crassostrea virginica*. Maryland Sea Grant Publications, College Park, MD.

Newell, RIE, TR Fisher, RR Holyoke and JC Cornwell, 2004. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. Pages 93-120 in R Dame and S Olenin (eds.). The Comparative Roles of Suspension Feeders in Ecosystems, NATO Science Series: IV - Earth and Environmental Sciences. Kluwer Academic Publishers, Dordrecht, The Netherlands.

NRCS. 2006. "Chesapeake Bay and Agriculture". Conservation Resource Brief No. 0609. U.S. Department of Agriculture, Natural Resource Conservation Service. October 2006.

OAC, 2007. Maryland Oyster Advisory Commission 2007 Interim Report. Implementation of House Bill 133 Natural Resources - Chesapeake Bay – Oyster Restoration. Submitted to the Governor and General Assembly, January 4, 2008.

ORW, 2008. How to Monitor Sites using Oyster Restoration Metrics: Habitat. Oyster Restoration Workgroup. December 20, 2008. http://www.oyster-restoration.org/.

Pavlos, NV and KT Paynter. 2001. The effect of gamete proximity on fertilization success in the eastern oyster *Crassostrea virginica*. Page 518 in Aquaculture 2001:

Book of Abstracts, World Aquaculture Society, 143 J.M Parker Coliseum Louisiana State University Baton Rouge LA.

Paynter, KT. 1996. The effects of *Perkinsus marinus* infection on physiological processes in the eastern oyster, *Crassostrea virginica*. *Journal of Shellfish Research* 15: 119-125.

Paynter, KT. 1999. Ch 22: Managing Around Oyster Diseases in Maryland and Maryland Oyster Roundtable Strategies. Pages 317-327 in MW Luckenbach, R Mann, and JA Wesson (eds.), Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches Virginia Institute of Marine Science, Gloucester Point, VA.

Paynter, KT and EM Burreson. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: II. Disease development and impact on growth rate at different salinities. *Journal of Shellfish Research* 10: 425-431.

Peterson, CH, JH Grabowski, and SP Powers. 2003. Estimated enhancement of fish production resulting from restoring oyster reef habitat: quantitative valuation. *Marine Ecology Progress Series* 264: 249-264.

Peterson, CH and RN Lipcius. 2003. Conceptual progress towards predicting quantitative ecosystem benefits of ecological restorations. *Marine Ecology Progress Series* 264: 297-307.

Ragone, LM, and EM Burreson. 1993. Effect of salinity on infection progression and pathogenicity of *Perkinsus marinus* in the easter oyster, *Crassostrea virginica* (Gmelin). *Journal of Shellfish Research* 12: 1-7.

Reece, KS, D Bushek, KL Hudson and JE Graves. 2001. Geographic distribution of *Perkinsus marinus* genetic strains along the Atlantic and Gulf coasts of the USA. *Marine Biology* 139: 1047-1055.

Ricker, WE. 1975. Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* 191: 1-382.

Rodney, WS and KT Paynter. 2006. Comparisons of macrofaunal assemblages on restored and non-restored oyster reefs in mesohaline regions of Chesapeake Bay in Maryland. *Journal of Experimental Marine Biology and Ecology* 335:39–51.

Rothschild, BJ, JS Ault, P Goulletquer, and M Heral. 1994. Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. *Marine Ecology Progress Series* 111: 29-39.

Rowe, CL. 2003. Growth responses of an estuarine fish exposed to mixed trace elements in sediments over a full life cycle. *Ecotoxicology and Environmental Safety* 54: 229-239.

SAS Institute Inc. 2006. *Base SAS® 9.1.3 Procedures Guide, Second Edition, Volumes 1, 2, 3, and 4*. Cary, NC: SAS Institute Inc.

Shenker, JM, DJ Hepner, PE Frere, LE Currence, and WW Wakefield. 1983. Upriver migration and abundance of naked goby (*Gobiosoma bosc*) larvae in the Patuxent River estuary, Maryland. *Estuaries* 6: 36-42.

Shervette, VR and F Gelwick. 2008. Season and spatial variations in fish and macroinvertebrate communities of oyster and adjacent habitats in a Mississippi estuary. *Estuaries and Coasts* 31: 584-596.

Smith, GF, DG Bruce, EB Roach, A Hansen, RIE Newell, and AM McManus. 2005. Assessment of recent habitat conditions of eastern oyster (*Crassostrea virginica*) bars in mesohaline Chesapeake Bay. *North American Journal of Fisheries Management* 25: 1569-1590.

Soniat, TM, CM Finelli, and JT Ruiz. 2004. Vertical structure and predator refuge mediate oyster reef development and community dynamics. *Journal of Experimental Marine Biology and Ecology* 310: 163-182.

Soule, DF and GS Kleppel (eds.). 1988. Marine Organisms as Indicators. Springer-Verlag, New York, 342 pp.

Stuntz, GW, PS Levin, and TJ Minello. 2001. Selection of estuarine nursery habitats by wild-caught and hatchery-reared juvenile red drum in laboratory mesocosms *Environmental Biology of Fishes* 61: 305-313.

Stuntz, GW, TJ Minello, and PS Levin. 2002. A comparison of early juvenile red drum densities among various habitat types in Galveston Bay, Texas. *Estuaries* 25: 76-86.

Systat, 2004. SigmaStat for Windows version 3.11. Systat Software Inc.

Tarnowski, M. 2008. Maryland Oyster Population Status Report – 2007 Fall Survey. Maryland Department of Natural Resources, Shellfish Program and Cooperative Oxford Laboratory. MDNR Publ. No. 17-7302008-328.

Tolley, SG and AK Volety. 2005. The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. *Journal of Shellfish Research* 24: 1007-1012.

Walters, K and LD Coen. 2006. A comparison of statistical approaches to analyzing community convergence between natural and constructed oyster reefs. *Journal of Experimental Marine Biology and Ecology* 330: 31-95.

Weisberg, SB, JA Ranasinghe, LC Shaffner, RJ Diaz, DM Dauer, and JB Frithsen. 1997. An estuarine benthic index of biotic integrity (B-IBI) for Chesapeake Bay. *Estuaries* 20: 149-158.

Welch, BL. 1951. On the comparison of several mean values: an alternative approach. *Biometrika* 38: 330-336.

Wells, HW. 1961. The fauna of oyster beds, with special reference to the salinity factor. *Ecological Monographs* 31: 239-266.

Wharton, J. 1957. The bounty of the Chesapeake: Fishing in colonial Virginia. Charlottesville, Virginia: University Press of Virginia.

Yates, C. 1913. Survey of oyster bars of Maryland, 1906-1912. Maryland Shellfish Commission. United States Bureau of Fisheries. United States Coast and Geodetic Survey.

Zimmerman, R, T Minello, T Baumer, and M Castiglione. 1989. Oyster reef as habitat for estuarine macrofauna. NOAA Technical Memorandum NMFS-SEFC-249. October 1989.