Oyster reef restoration in the northern Gulf of Mexico: effect of substrate and age on ecosystem services

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OYSTER REEF RESTORATION IN THE NORTHERN GULF OF MEXICO: EFFECT OF SUBSTRATE AND AGE ON ECOSYSTEM SERVICES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Biological Sciences

by
Laura Alicia Brown
B.A. University of North Carolina at Chapel Hill, 2010
August 2012
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ABSTRACT

Oyster reefs provide estuaries with ecosystem services including carbon sequestration, shoreline stabilization, refugia for invertebrate biodiversity, and fisheries enhancement, but have been disappearing over the past century due to increasing overharvesting, disease, and eutrophication. The northern Gulf of Mexico has over 400 artificial oyster reefs constructed of various materials created through efforts to restore ecosystem services lost with declining oyster populations. The purpose of this study was to determine how two common artificial reef construction materials (rock vs. oyster shell) at various ages affect ecosystem services, specifically blue crab abundance, the commensal community, and oyster recruitment, as well as to determine the refuge value of oyster reefs. To determine whether the provision of ecosystem services varied over time, rock (n = 7) and oyster shell (n = 6) reefs of varying ages (new < 5 years since construction; old > 5 years since construction) were compared to natural oyster reefs (n = 7) and were sampled twice in the summer of 2011. Sampling devices included baited crab traps, spat settlement plates and commensal collectors. Results indicate no difference between reef category (reef by age) and blue crab abundances, most likely due to their mobility. Diversity, abundance and richness of the commensal community are lowest on old shell reefs. Old rock reefs were most similar to natural reefs in regards to commensal community assemblages, even surpassing them in commensal abundance seasonally. Spat densities and size varied across reef categories, and were more closely correlated with salinity than reef category. To determine refuge value of oyster structure, a mesocosm with a depth gradient and different oyster reef complexities in the deepest end was used. When high complexity shell areas were provided, juvenile crabs were more likely to use these deep water reef refugia when a predator was introduced to the tank; when no shell areas were available, juvenile crabs selected shallow
water refuge. The refuge value of oyster reefs, based both on the field survey and laboratory experiment, seems to be the driver of higher commensal abundances, richness, and diversity on older rock reefs, which last longer than old shell reefs which seem to disperse over time in sediments.
INTRODUCTION

Reefs of the Eastern Oyster (Crassostrea virginica) found along the Gulf of Mexico offer numerous ecosystem services that benefit surrounding environments as well as humans. Oyster industries help support the northern Gulf of Mexico’s economy, bringing in millions of dollars a year (Louisiana Department of Wildlife and Fisheries, LDWF 2004). The states along the Gulf of Mexico produce nearly two-thirds of the country’s oyster harvest by volume, and over one-half by value, and have been the dominant oyster production area in the U.S. since the mid-1980’s (LDWF 2004). Oysters filter water, assimilating organic matter, and at their peak filtered the volume of the Chesapeake Bay in 3.3 days (Dame et al., 1984, Newell 1988). Oysters remove nutrients that have been anthropogenically concentrated in bays and estuaries by agriculture and storm water run-off, lessening eutrophication (Cerco and Noel 2007). Oysters also filter sediments out of the water, increasing water clarity and helping sunlight reach submerged aquatic vegetation (SAV), aiding the role of SAV as nurseries for the juvenile blue crab, Callinectes sapidus (Perkins-Visser et al., 1996). Oysters are also capable of reducing phytoplankton biomass that has increased due to anthropogenic nutrient input (Newell et al., 2007). Carbon sequestration in oyster biomass is another ecosystem service of oyster reefs (Hargis et al., 1999). Since oysters secrete a calcium carbonate shell, they act as a carbon sink (Peterson and Lipcius 2003) and aid in reducing carbon in the atmosphere, as current levels of carbon dioxide are increase at a rate of 1.9 parts per million a year (Easterling and Karl 2008). Oysters have also been shown to aid in de-nitrification, removing nitrogen from coastal systems by transforming it into either N2 or N2O2 and releasing it into the atmosphere (Piehler and Smyth 2011).
Oysters aggregate together forming reefs, which in turn provide more ecosystem services. The reefs, when intertidal, act as a “living shoreline” for marshes. With the global mean sea level rising at a rate of 1.7 mm per year (Easterling and Karl 2008), as well as a 50% reduction of wetlands in America (Dahl 2006), oyster reefs parallel to the shore can reduce erosion on marshes from waves and boat wakes (Piazza et al., 2005, Scyphers et al., 2011). Protecting marshes is crucial to maintaining production of important fisheries such as shrimps and blue crabs (Zimmerman et al., 2002), as the marsh offers refuge as well its own ecosystem services. The vertical structure created by oysters allows smaller commensal organisms, such as the xanthid or mud crab family, to find refuge from predation (McDonald 1982). Oyster reefs are comparable to coral reefs in structural complexity (Harding and Mann 1999), and as oyster reef complexity increases, predator-prey interactions are altered (Grabowski 2004, Grabowski et al., 2005). Oyster reefs thus create vertical relief on muddy bottoms as well as a basal food resource for larger predators, such as black drum, blue crabs, stone crabs, and sheepshead (Lenihan et al., 2001, George et al., 2008 and O’Connor et al., 2008). By providing food as well as habitat, oyster reefs thus increase total numbers of sand seatrout, atlantic croaker, catfish, flounder, red drum, black drum, and blue crabs (Scyphers et al., 2011). Oyster reefs also support more biodiversity than mud flats surrounding them (Summerson and Peterson 1984, Posey et al., 1999, Coen and Luckenbach 2000, Plunket and La Peyre 2005, Tolley and Volety 2005, Grabowski et al., 2005, Scyphers et al., 2011).

Oyster populations have declined 85% from historical abundance worldwide (Beck et al., 2011), with more than 90% of oyster population declines resulting from anthropogenic factors (Lotze et al., 2006), indicating oysters and the ecosystem services they provide are at risk. Oyster reefs have deteriorated in many areas over the past century due to overharvesting and increased
eutrophication (Jackson et al., 2001, Kemp et al., 2005). More development along the coast leads to increased sediment loads, nutrient loads, primary productivity and hypoxic/anoxic conditions, which together have led to a sharp decline in oysters over time (Jackson et al., 2001, Lotze et al., 2006). In the Chesapeake Bay area alone populations have declined over 50 fold due to destructive harvesting procedures such as trawling and dredging (Rothschild et al., 1994). Trawling not only affects oysters by overharvesting, but also destroys the vertical complexity of oyster reefs, reducing refuge and habitat, as well as reducing recruitment (Baker and Mann 1991, Lenihan and Peterson 2004). Diseases such as Dermo and multinucleated sphere unknown (MSX) can also cause significant mortality at higher salinities (Rothschild et al., 1994, Melancon et al., 1998). The combination of disease, eutrophication, and overharvesting has thus, over a short period of time, greatly depleted oyster populations along the Atlantic coast of the United States.

Oyster reefs along the northern Gulf of Mexico are in fair condition, with only 50% to 89% of oyster reefs lost, compared to the Atlantic coast of North America, where reefs are considered in poor condition or functionally extinct (e.g. 90% to 100% loss, Beck et al., 2011). This is in part due to oyster fisheries along the Gulf of Mexico not reaching peak landings until the 1950’s, while peaks in the New York area were in the 1830’s, and in southern states were in the early 1900’s (Kirby 2004). This delay in overexploitation along the Gulf coast benefitted from lessons learned in Chesapeake and Long Island Sound (Rothschild et al., 1994, Kirby 2004). The fair condition of Gulf oyster populations can also partly be attributed to the Gulf’s management of the fishery. For example, in Louisiana, Louisiana Department of Wildlife and Fisheries (LDWF) requires that oystermen harvest off an oyster lease, instead of buying a permit. This reduces the “tragedy of the commons”, where everyone ultimately overharvests. The LDWF
works to license harvesters, oversee leases, seed public grounds, and continuously monitor water quality and environmental conditions (LDWF 2004), even controlling salinities in order to yield optimal production (La Peyre et al., 2009). The bays and bayous that contribute to the coastline of the northern Gulf of Mexico provide more area at optimal salinities (5-15 PSU) for oysters to grow with reduced rates of predation and disease (Melancon et al., 1998).

Although northern Gulf of Mexico oyster populations are in fair condition, lessons learned from the Atlantic coast, as well as threats from the 2010 Deepwater Horizon oil spill, disease, and hurricanes (Rothschild et al., 1994, Livingston et al., 1999, McCrea-Strub et al., 2011) all suggest that oyster populations along the Gulf of Mexico are in a delicate situation, warranting restoration efforts. Shellfish restoration, which includes creating artificial oyster reefs, has become a popular method to increase reef habitat (Lipcius and Burke, 2006) as well as restore ecological services (Grabowski and Peterson 2007). The restoration of oyster reefs has become a best management practice (BMP) for states along the Gulf of Mexico, as well as the eastern Atlantic coast. However, methods used to restore reefs can contribute to how well they succeed. Since the reef is not only habitat, but also a prey source for numerous predators (Brown and Richardson 1987, Eggleston 1990, Brown and Haight 1992, George et al., 2008), the health of a reef can impact the nekton and commensal community around it, although an easily determined index of reef health is difficult to develop (Coen and Luckenbach 2000). One good indicator of reef health is the number of oyster spat that settle on the reef, which affects the commensal community, as many organisms also consume juvenile oysters (Eggleston 1990, Grabowski 2004). Restoration efforts began as an effort to restore oyster populations, but recently the goal has shifted to restoring lost ecosystem services (Coen and Luckenbach 2000, Peterson et al., 2003, Luckenbach et al., 2005, Grabowski and Peterson 2007). In the northern
Gulf of Mexico alone, over 400 artificial oyster reefs have been constructed (Furlong 2012). State, federal, and private organizations, including The Nature Conservancy, the LDWF and the U.S. Army Corps of Engineers, have created reefs to promote habitat restoration, shoreline protection, and fisheries enhancement.

Reefs can be placed intertidally and parallel to shoreline, or subtidally and offshore, and a number of variables can affect the success of restored or artificial reefs. Factors such as salinity, height, and dissolved oxygen can all have significant effects on the survival of oyster reefs along the coast (Lenihan 1999), and could also indirectly affect the commensal community. Other factors are less studied, such as construction methods and materials used to restore the reef. Two common methods for artificial reef creation along the coast of the Gulf of Mexico are rock and recycled oyster shell (Coen and Luckenbach 2000, Luckenbach et al., 2005, Piazza et al., 2005, Powers et al., 2009). Little is known about how construction methods affect commensal and crab communities surrounding reefs. Rock reefs can be created out of limestone, marl, or concrete, and constructed in mounds, or hollow spheres, and range in size from cobble to large slabs. Shell reefs are often whole oyster shells returned to the water through recovery programs (LDWF 2004). Shells can be distributed across existing oyster reefs, or placed on bare substrate. Regardless of the material used, oyster spat are expected to recruit to the reef and help stabilize the restoration effort. While oysters are gregarious organisms preferring to settle with other oysters (Hidu 1969), results are mixed regarding whether shell or rock provide the best surface for recruitment. Crisp (1967) found that organic bio-films on an oyster shell promoted recruitment, compared to oyster shells with the bio-films removed, while the LDWF (2004) instead found greater recruitment to rock reefs than shell reefs. Few studies have been explicitly designed to determine the differences between shell versus rock as materials for artificial reefs.
(LDWF 2004), or how differing materials affect ecosystem services. Although some researchers have looked specifically at how their constructed reefs have fared (Lenihan 1999, Meyer and Townsend 2000, Grabowski et al., 2005, Luckenbach et al., 2005, Piazza et al., 2005, Gregalis et al., 2009), a detailed study examining how artificial reefs develop their ecosystem services over time is still much needed.

The purpose of my study is therefore to determine how artificial oyster reef construction material affects ecosystem services, and how those services change over time. In particular, I am interested in the effect that reef construction material and age have on commensal and crab communities surrounding reefs. I am particularly interested in habitat for commercially important blue crabs (*Callinectes sapidus*), and refuge value for commensal invertebrates and juvenile fish on the reef, which provide food for higher trophic levels (Grabowski 2004, Tolley and Volety 2005, Grabowski and Peterson 2007). My null hypothesis is that construction material and age of reefs will have no effect on surrounding commensal and crab communities. I am also interested in the effect of age and construction material on recruitment to the reef of oyster spat and barnacles, since higher juvenile recruitment sustains reefs (O’Beirn et al., 2000). My null hypothesis is that there will be no difference between age and construction material in promoting oyster spat colonization. To determine these effects of age and construction material on commensals, blue crabs, and oyster spat densities, a field study was conducted along the northern Gulf of Mexico. Finally, to determine the refuge value of oyster reefs for commensals like juvenile blue crabs, I also performed a mesocosm experiment in the laboratory.
METHODS

Field Site Selection

To determine how artificial oyster reef construction materials affect ecosystem services over time, a list of reefs created along the northern Gulf of Mexico for conservation and restoration purposes was compiled, along with the material, date, and location of their creation (Figure 1). Of over 400 artificial oyster reefs constructed, twenty reefs were chosen, spanning the northern Gulf of Mexico, from Copano Bay, Texas, to Apalachicola Bay, Florida (Figure 2). The independent variables were reef construction material (rock, shell, natural) and date of construction, from 1991-2009. Reefs included those constructed from rock materials (n = 7), oyster shell (n = 6), and natural oyster reefs as controls (n = 7). Oyster shell reefs were constructed by spreading oyster shells over an area, to provide a substrate for spat recruitment, and the second method involved rock substrates from small cobbles to larger concrete fragments spread over an area. Reefs were also divided into two age categories, reefs built before 2006, and after 2006. The final reef categories (reef material by age group) were thus old rock reefs (n = 6), new rock reefs (n = 2), old shell reefs (n = 2), new shell reefs (n = 3), and natural, control reefs (n = 7). I selected reefs that were not fringing reefs parallel to shorelines, but subtidal, with an average depth of 2.11 ± 0.60 meters. Reefs were also chosen with the understanding that they were not harvested for oysters, but created for conservation purposes only.

To compare commensal organisms on reefs to organisms on mudflats adjacent to the reef, I used two paired sampling sites: on-reef and adjacent to the reef. The first was on a reef, and the adjacent site was selected by using a PVC pole to determine where the reef ended and muddy substrata began, then after five consistent soundings with non-reef substrate, the boat was driven
at 1000 rpm for 10 seconds in the same direction as the five soundings. After those ten seconds, the substrate below was again checked for muddy bottom substrate, and if it was muddy bottom, this site was selected as the adjacent to reef site. All reefs were sampled in May and July 2011. At each site and date, salinity (PSU), dissolved oxygen (mg/L), and temperature (°C) were measured with an YSI 85 meter, as well as secchi depth (cm) (Table 1).

Figure 1. A map created by The Nature Conservancy of the oyster reef restoration efforts along the northern Gulf of Mexico, as well as the material reefs were created from.

**Adult Blue Crab Abundance**

To determine adult blue crab abundances at each site, two baited commercial crab traps were set per site (one on the reef and one adjacent to the reef). Traps were baited with chicken
legs and set out overnight. I recorded catch per unit effort (CPUE), which is total count of blue crabs per trap, and measured carapace widths (cm) and sex of the blue crabs.

**Commensal Community Assemblages**

To sample the commensal community, 0.3 m x 0.3 m bags constructed from 2 cm Vexar mesh were filled with clean, unbleached oyster shell, and attached to a cinderblock anchor. Commensal organisms recruit to the oyster shell within the bags (Stuck and Perry 1992). At each site, three bags were attached to a cinderblock with a rope and buoy. One cinderblock with three bags was placed on and adjacent to each reef and the bags were retrieved after one month. Bags were carefully lifted out of the water and collected in a tub so that loose invertebrates would also be collected. Organisms were kept on ice until they were returned to the LSU laboratory, where bags were opened, oyster shells washed over a 1mm mesh sieve, and all organisms placed in 80% ethanol. Organisms were identified to species in most cases (Hopkins and Valentine 1989). For each site, species richness, total commensal abundance, and Shannon’s Index of diversity (Spellerburg and Fedor 2003) were calculated. Commensal organisms were also divided into several functional groups, with total number of individual filter feeders, grazers, meso-predators and omnivores noted. Filter feeders were classified as any organism filtering water, grazers as consuming algae and detritus, meso-predators as oyster predators, and omnivores as consuming detrital matter and other organisms.

**Settlement**

Settlement of fouling organisms was quantified on a 15 cm x 15 cm clay tile caulked to the cinder block anchors, and enumerated taxa included oyster spat and barnacles (Banks and Brown 2002). The tile was attached with the rougher, unglazed side facing outwards. Settlement
plates were deployed for two one-month long sampling periods on and adjacent to the reef, and kept on ice or in a cold room until counted. Densities of oyster spat and barnacles per rough side of the plate were quantified, and the sizes of individual spat were measured.

**Oyster Density**

Oyster densities were sampled at each of the sites by SCUBA divers in October of 2011. Five 0.25 m\(^2\) quadrats were haphazardly thrown out at each site. Two divers collected samples by removing the top 10 cm of the reef using hands, hand tongs, and chisels. All loose material was placed in labeled buckets, kept on ice, and then brought to LSU laboratories. Material was sorted by live oyster, dead oyster, crushed shell, and rock, determining the total volume of each category at each of the sites. Oyster densities were used as a covariate in initial analyses, but were not significant in all cases.

**Statistical tests**

Dependent variables for adult blue crabs were CPUE, sex ratios, and size. Dependent variables for the commensal community were total commensal abundance, commensal species richness, commensal Shannon’s diversity, and functional group abundances. Finally, for settlement plates, spat densities, spat size, and barnacle densities were dependent variables.

In order to determining whether or not to pool samples from on and adjacent to the reef, a t-test was used with dependent variables of sex ratios, crab size, commensal abundances, species richness, Shannon’s diversity, and barnacle densities. For CPUE and spat densities, a Mann-Whitney test was used due to a lack of normality. Over all dependent variables, there was no significant difference between on the reef and adjacent to the reef, therefore all samples were pooled.
Figure 2. A map of 20 oyster reef sites along the northern Gulf of Mexico, with 7 natural reefs, 7 rock reefs, and 6 shell reefs evenly spread along the coast and sampled twice in the summer of 2011.

A two-way ANCOVA was used with reef category (each substrate/age group and season) as the categorical variables and salinity (if significant) as the covariate with all the above dependent variables. Other variables (dissolved oxygen, secchi depth, and oyster density) were initially used as covariates but did not have significant effects on dependent variables. If season was not a significant treatment, seasons were pooled, and only the reef category variable was looked at, though if seasons were a significant treatment, they were separated and two one-way ANCOVA was used. Significant differences among categories were analyzed in PROC GLIMMIX in SAS 9.2, with a binomial, negative binomial, Poisson or normal distribution
determined by the lowest AIC score. Differences between reef categories and seasons (when significant) were tested using Tukey’s *a posteriori* tests. Finally, a discriminant analysis was used as a holistic way to determine if the composition of the commensal community (e.g. functional groups, including filter feeders, omnivores, meso-predators, and grazers) differed among reef substrate and age categories. All of the statistical tests were completed with SAS 9.2.

Table 1. Water quality data, including means (+ SE), and ranges for salinity (PSU), dissolved oxygen (%), temperature (°C), and Secchi depth (cm) for all treatments during sampling trips April 2011 through November 2011.

<table>
<thead>
<tr>
<th>Category</th>
<th>Salinity Mean (PSU)</th>
<th>Salinity Range (PSU)</th>
<th>DO Mean (mg/L)</th>
<th>DO Range (mg/L)</th>
<th>Temperature Mean (°C)</th>
<th>Secchi Depth Mean (cm)</th>
<th>Secchi Depth Range (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.7±1.0</td>
<td>0.6-31.2</td>
<td>6.5±0.1</td>
<td>4.6-9.0</td>
<td>28.9±0.4</td>
<td>50±3</td>
<td>1-117</td>
</tr>
<tr>
<td>Old Rock</td>
<td>17.2±2.7</td>
<td>2.7-31.7</td>
<td>6.9±0.2</td>
<td>4.5-8.8</td>
<td>29.3±0.4</td>
<td>63±3</td>
<td>36-109</td>
</tr>
<tr>
<td>New Rock</td>
<td>13.9±2.3</td>
<td>2.1-29.5</td>
<td>7.4±0.2</td>
<td>5.4-8.9</td>
<td>29.7±0.9</td>
<td>36±5</td>
<td>12-61</td>
</tr>
<tr>
<td>Old Shell</td>
<td>8.2±1.6</td>
<td>0.2-16.0</td>
<td>6.4±0.3</td>
<td>4.8-7.7</td>
<td>30.7±0.4</td>
<td>33±4</td>
<td>9-57</td>
</tr>
<tr>
<td>New Shell</td>
<td>18.6±1.7</td>
<td>9.5-29.7</td>
<td>6.8±0.2</td>
<td>5.5-9.5</td>
<td>28.5±0.7</td>
<td>70±5</td>
<td>30-113</td>
</tr>
</tbody>
</table>

**Crab Refuge Experiment**

To determine the refuge value of oyster reefs to juvenile blue crabs in the presence of a cannibalistic adult blue crab, juvenile blue crabs were collected from oyster mariculture bags in Grand Isle, Louisiana, and brought back to LSU laboratories along with adult blue crabs collected from baited crab traps in Grand Isle, Louisiana. This experiment determined how
juvenile distribution changed with water depth and oyster refuge relief when juveniles were exposed to a cannibalistic adult blue crab. All crabs were maintained in aquaria at 15 PSU and 29°C and fed every 3 days. Two indoor tanks (1.25 m x 0.5 m x 0.45 m) held at a salinity of 15 PSU had a depth gradient, ranging from shallow (7 cm), medium (20 cm), and deep (42 cm), as in Dittel et al., 1995 (Figure 3). Three oyster shell treatments were constructed: 1) no complexity (no oyster shell), 2) low complexity (oyster shell = 10 cm relief), and 3) high complexity (oyster clumps = 40 cm relief) in the deep end of each tank. Each tank had only one oyster shell treatment. Treatment 1 tested if juvenile crabs prefer deep water or shallow water as a refuge, as in Dittel et al., (1995). Treatment 2 mimicked a degraded oyster reef in nature (shallow water versus low complexity deep water reef refuge). Treatment 3 mimicked a high complexity, healthy oyster reef (shallow water versus high complexity deep water reef refuge). By having a depth gradient, as well as a vertical complexity treatment, the interactive role of depth and structure in oyster reef refugia was assessed.

Figure 3. Diagram of the tank used in the experiment for testing refuge preference of juvenile blue crabs. Differing oyster complexities were placed in the deepest section of the tank to determine whether juvenile crabs prefer the shallow water refuge or the oyster reef refuge. A = 42 cm, B = 20 cm, C = 7 cm, D = 125 cm.

A juvenile blue crab (mean carapace width = 2.74 ± 0.27 cm) was placed in the medium depth of each treatment, and then allowed to acclimate for 24 hours (Dittel et al., 1995). At the
end of 24 hours, the final position of the crab was marked, and the adult blue crab (mean carapace width = 10.71 ± 0.83 cm) was placed in the deep end of the tank. After another 24 hours, juvenile crab positions were noted. Each treatment was replicated four times, with new crabs for each trial. A $\chi^2$ test in SAS 9.2 was used to determine the probability that the juvenile blue crab moved from the deep end of the tank to a shallower depth once the adult blue crab was added, with amount of oyster relief as the independent variable and juvenile crab position as the dependent variable.
RESULTS

Adult Blue Crab Abundance

Reef categories had no effect on blue crab CPUE, sex ratios, or size. Blue crab abundance on and adjacent to the reef were pooled (Mann-Whitney test, p = 0.65), as were seasons (two way ANCOVA, F = 3.47, p = 0.07). There was no significant difference in log-transformed CPUE’s among reef categories (two way ANCOVA, F = 1.70, p = 0.16), nor was salinity a significant covariate (F = 0.46, p = 0.50). In addition, there was no reef category by sampling date interaction (F = 2.01, p = 0.11).

Again, on and adjacent to the reefs (t-test, p = 0.54), as well as seasons (two way ANCOVA, F = 0.00, p = 0.95), were pooled in regards to sex ratios. Salinity had no effect on sex ratios (two way ANCOVA, Binomial Distribution, F = 0.34, p = 0.56), nor was there a difference among reef categories in sex ratios (F = 0.45, p = 0.77). In addition, there was no reef type by sampling date interaction (F = 0.19, p = 0.94).

On and adjacent to the reef samples were pooled (t-test, p = 0.75), as well as sampling dates (two way ANCOVA, F = 0.55, p = 0.46) in regards to adult blue crab size. Reef categories had no effect on the size of the adult blue crabs (two way ANCOVA, F = 2.03, p = 0.09), nor did salinity (F = 0.87, p = 0.35). Reef category and sampling dates did not interact (F = 0.77, p = 0.52).

Commensal Community Assemblages

Commensal abundances were pooled from on versus adjacent to the reefs (t-test, p = 0.09), but there were seasonal differences (two way ANCOVA, F = 6.55, p = 0.01). Even with
salinity as a significant covariate (two way ANCOVA, $F = 23.41, p = 0.0001$), there was still a significant difference in the log of total commensal abundance among reef categories ($F = 9.33, p = 0.0001$). However, there was no reef type by sampling date interaction ($F = 1.00, p = 0.42$). In May, commensal abundances on old rock reefs were significantly higher than old shell reefs (Tukey a posteriori test, $p = 0.01$), and control reefs (Tukey a posteriori test, $p = 0.04$). In July, the commensal abundances were however significantly higher on new shell reefs than new rock reefs (Tukey a posteriori test, $p = 0.03$) and old shell reefs (Tukey a posteriori test, $p = 0.007$). Old rock reef commensal abundances were higher than on new rock reefs (Tukey a posteriori test, $p = 0.04$) and old shell reefs (Tukey a posteriori test, $p = 0.008$) (Figure 4).

![Figure 4](image_url)

Figure 4. Log of mean total abundance of commensal organisms ± standard errors on four different types of artificial oyster reefs, old rock reefs, old shell reefs, new rock reefs and new shell reefs, compared to control reefs during May and July. Letters above histograms indicates significant differences in total abundance from Tukey’s tests.
Samples were pooled on and adjacent to the reef (t-test, p = 0.07), as well as seasonally (two way ANCOVA, F = 0.00, p = 0.56) for species richness. There was no significant difference in species richness among reef categories (two way ANCOVA, Poisson Distribution, F = 0.93, p = 0.46), although salinity was significant as a covariate (F = 27.59, p < 0.0001). There was no significant difference in species richness among reef categories (two way ANCOVA, Poisson Distribution, F = 0.93, p = 0.46), although salinity was significant as a covariate (F = 27.59, p < 0.0001). There was no significant difference in species richness among reef categories (two way ANCOVA, Poisson Distribution, F = 0.93, p = 0.46), although salinity was significant as a covariate (F = 27.59, p < 0.0001). There was no significant difference in species richness among reef categories (two way ANCOVA, Poisson Distribution, F = 0.93, p = 0.46), although salinity was significant as a covariate (F = 27.59, p < 0.0001). There was no reef category by sampling date interaction (F = 0.75, p = 0.56).

Shannon’s diversity was pooled from on and adjacent to the reef (t-test, p = 0.19), but was significantly different between seasons (two way ANCOVA, F = 8.57, p = 0.005). Shannon’s was significantly different among reef category (two way ANCOVA, F = 3.47, p = 0.01), and there was no category by sampling date interaction (F = 0.21, p = 0.93). Salinity was not a significant covariate (F = 0.44, p = 0.51). In May, old shell reefs had the lowest diversity of all reef types, differing from all others but new shell reefs (Figure 5). During July, there was no significant difference among reef categories (one way ANCOVA, F = 1.66, p = 0.18), with no salinity effect (F = 0.03, p = 0.86). All species found on reef types are indicated in Table 2, along with presence or absence on reef types, average salinities, and salinity ranges of organisms.

When the commensal community was divided into total numbers of individual omnivores, grazers, meso-predators, and filter feeders, the discriminant analysis was successful in separating reef categories (Wilks-λ = 0.64, F-value = 1.69, p = 0.05). Only the first discriminant function was significant, with the canonical correlation explaining 79% of the variation. All class means on canonical variables for each reef category were negative, except the old rock reefs (0.82) and new shell reefs (0.15), and old shell had the lowest discriminant score (-1.04) (Figure 6).
Figure 5. Average values for Shannon’s diversity Index of commensal organisms ± standard errors during May on four types of artificial oyster reefs, old rock reefs, old shell reefs, new rock reefs and new shells reefs, compared to control reefs. Letters above histograms indicates significant differences in diversity. July was not shown since there were no significant differences.

**Settlement**

Settlement was similar on and adjacent to the reef, and densities were pooled (Mann-Whitney test, p = 0.06). Densities were also pooled between sampling dates (two way ANCOVA, F = 0.60, p = 0.44). Oyster spat densities were higher on control reefs, as shown by the LSMeans. Reef type had a significant effect on oyster spat densities (two way ANCOVA, Negative Binomial Distribution, F = 3.90, p = 0.006), with control reefs having significantly higher spat densities than old rock reefs (Tukey *a posteriori* test, p = 0.0032) (Figure 7). Salinity had a significant effect on oyster spat densities (F = 111.54, p = 0.0001), with higher spat
densities at higher salinities (Figure 8). There was a significant reef type by sampling date interaction ($F = 2.58$, $p = 0.04$).

![Discriminant Function 1](image)

Figure 6. The class means on the first discriminant function. Old rock reefs are separated from other reef types, including the control reefs ($\text{Wilks-}\lambda = 0.64$, $F$-value $= 1.69$, $p = 0.05$)

Oyster spat were larger in July ($8.96 \pm 0.38$ mm) than in May ($4.91 \pm 0.13$ mm) (two way ANCOVA, Poisson Distribution, $F = 13.56$, $p = 0.003$). There was also a reef type by sampling date interaction for spat size ($F = 3.16$, $p = 0.04$). In May, reef category had no effect on spat size (two way ANCOVA, Poisson Distribution, $F = 0.21$, $p = 0.94$), and salinity was not significant ($F = 1.44$, $p = 0.23$). In July, there was a significant effect of reef category ($F = 27.50$, $p = 0.0001$), with salinity as a significant covariate ($F = 57.44$, $p = 0.0001$). Larger spat were found around 10 to 15 PSU (Figure 9). The LSMeans show significant difference in reef categories.
came from new shell reefs having larger spat sizes than control reefs (Tukey *a posteriori* test, *p* = 0.0001) and new rock reefs (Tukey *a posteriori* test, *p* = 0.004) (Figure 10). Since old rock reefs and old shell reefs had no spat in July, they were eliminated from the analysis of oyster spat size in July.

Figure 7. Mean oyster spat density per square meter ± standard errors on four types of artificial oyster reefs, old rock reefs, old shell reefs, new rock reefs and new shells reefs, compared to control reefs. Letters above histograms indicates significant differences in densities.

Barnacle densities on and adjacent to reefs were pooled (t-test, *p* = 0.55), as well as seasons (two way ANCOVA, F = 2.80, *p* = 0.10). Reef categories differed in barnacle densities (two way ANCOVA, Negative Binomial Distribution, F = 54.92, *p* = 0.0001), and salinity was important as well (F = 4.24, *p* = 0.04). Old shell reefs had the lowest barnacle densities (Tukey *a posteriori* test, *p* = 0.0001), and new rock reefs had the second lowest barnacle densities (Tukey *a posteriori* test, *p* = 0.0001).
*a posteriori* test, *p* < 0.01) (Figure 11). There was no sampling date and reef category interaction (F = 2.43, *p* = 0.06).

Figure 8– Mean oyster spat densities per square meter ± and standard errors versus salinity (PSU)s.

**Crab Refuge Experiment**

There was a significant difference between the level of structure and the probability that a juvenile blue crab would move from deeper water to shallower water when an adult blue crab was added to the tank ($\chi^2 = 8.91$, *p* = 0.01). There was no difference in the position of the juvenile in high structure reefs, but crab positions in low structure reefs were variable (Figure 12).
Table 2. Species list with Latin names, common names, average species abundance ± standard error, and average salinity where species were found ± standard error. An “X” indicates which reefs each species occurred in.

<table>
<thead>
<tr>
<th>Functional Guild</th>
<th>Common Name</th>
<th>Latin Names</th>
<th>Control</th>
<th>Old Rock</th>
<th>New Rock</th>
<th>Old Shell</th>
<th>New Shell</th>
<th>Average Number</th>
<th>Salinity Mean</th>
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<td>Blood Ark</td>
<td><em>Anadara ovalis</em></td>
<td>X</td>
<td>X</td>
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Figure 9. Mean spat sizes ± standard errors versus salinity (PSU) s. Larger spat sizes were found between 10 to 15 PSU.

Figure 10. Mean spat size in July ± standard errors on two types of artificial oyster reefs, new rock reefs and new shells reefs, compared to control reefs. Significant differences in densities are marked by asterisk.
Figure 11. Mean barnacle density per square meter ± and standard errors on four types of artificial oyster reefs, old rock reefs, old shell reefs, new rock reefs and new shells reefs, compared to control reefs. Letters indicates significant differences in densities.

To relate these data to the field, a linear regression was performed on the relationship between adult blue crab and juvenile blue crab CPUE from field collection, but it was not significant (simple linear regression, p = 0.64). Juvenile blue crab CPUE from field collection did not vary among reef categories (one way ANCOVA, F = 0.77, p = 0.59), nor was salinity significant (F = 0.38, p = 0.55).
Figure 12. Average juvenile blue crab position ± standard error with or without an adult blue crab. Zero = shallow water, 1 = intermediate water, and 2 = deep water. The treatments were no structure, low complexity, and high complexity. (N=4)
DISCUSSION

In this study, I investigated the effect of artificial oyster reef construction material on ecosystem services, and how those services change over time. Adult blue crab CPUE was not affected by reef category, most likely due to mobility of adults, while commensal communities had lowest diversity and abundance on old shell reefs. This result is most likely due to shell loss over long time periods, therefore decreasing habitat and refuge value. Spat densities and spat sizes were variable across reef categories, probably owing to variation in spawning times across bays and salinities. Ultimately, shell reefs seemed to lose ecosystem service function over time, while rock reefs were able to maintain structure and provide for commensal communities.

The lack of an effect of reef category on adult blue crab CPUE was most likely due to adult blue crab mobility. Females move long distances across estuaries to higher salinities to spawn (Van Engle 1958), and both males and females often move between habitat types in response to temperature, food availability, salinity, and tides (Meise and Stehlik 2003, Gregalis et al., 2009). Similarly, the mobile nature of these crabs may have resulted in the lack of difference in sex ratios between reef categories, and the not significant effect of salinity was most likely an artifact of the seasons sampled. Females prefer higher salinity waters during spawning, and lower salinities when they co-occur with males in pre- and post-spawn periods. Therefore a higher male to female ratio would be expected at lower salinities during spawning months (Meise and Stehlik 2003). However, sampling was not conducted during spawning, which occurs in early spring in the Gulf of Mexico (Darnell 1959). The lack of a difference in crab size among reef categories also suggested that no reef material or age offered greater resources for crab growth.
Perhaps also not surprising was the lack of a reef category effect on juvenile blue crab CPUE. That is, since there was no trend in adult CPUE, no trend in juvenile CPUE might be expected. The results of my laboratory experiment supported the findings of Dittel et al. (1995) who observed juvenile blue crabs taking refuge in shallow waters when structure was unavailable. In presence of degraded structure, juveniles were half as likely to take refuge in shallow water as they were to take refuge in oyster shells. When greater vertical complexity was provided, juveniles took refuge in oyster shell 100% of the time in the presence of cannibalistic adults, which are a principle source of mortality (Hines and Ruiz 1995). The greater vertical complexity of oyster reefs as superior refugia as determined in this laboratory study is corroborated by other studies of reefs as refugia (Grabowski 2004, Soniat et al., 2004, Tolley and Volety 2005). Refuge is quite important as it can enhance or inhibit the cascading effect of predators (Pace et al., 1999).

The different reef categories also appeared to offer similar ecosystem services compared to natural reefs in regards to commensal assemblages, with the exception of older shell reefs. Old shell reefs had lower diversity and abundance of commensals, and over time periods longer than 5 years, rock reefs had greater commensal species richness, diversity, and abundance than old shell reefs, and occasionally more than control reefs. This can likely be attributed to the presence of greater structure remaining at the old rock reefs compared to the old shell reefs. Furlong (2012) sampled the same reef treatments and found higher adult oyster density on rock reefs compared to shell reefs, as well as greater numbers of oyster clusters and hard substrates. Commensal organisms appear to utilize reef structure whether the oysters are alive or dead (Plunket and La Peyre 2005, Tolley and Volety 2005, Grabowski et al., 2008), with structure being the primary driving force and not food sources (Humphries 2010). Structure provides a
refuge, and oyster clusters are a nearly impenetrable refuge for decapod predators (Glancy et al., 2003). Even during periods of low spat recruitment, reefs anchored by heavy rock still provide structure. Vertical complexity from adult oysters, clusters, and hard substrate thus increase refugia from predation (McDonald 1982, Grabowski 2004, Gregalis et al., 2009) and provide increased chances for resource partitioning (Hughes and Grabowski 2006).

Shell scattering over time thus could explain the lack of commensal diversity and abundance on old shell reefs. In years of low spat recruitment, often at lower salinities, no live oysters are present on shell reefs to hold the reef together. In Delaware Bay, half of the oyster shell added to reefs was lost over a period of 2-10 years, with lowest shell half-lives at intermediate salinities (Powell et al., 2006). Oyster shell is less dense than rock and when shell reefs are created, loose shell is placed on top of substrate, making them more vulnerable to high flow velocities, resulting in shell movement (LDWF 2004, Lenihan 1999, Thompson and Amos 2002). With shifting, shell reefs either accrue sediment, or are spread too thinly and sink in the mud. Disturbances from fisheries trawling also increase shell spreading across sediment by dragging trawls across the shells, and shells spread thinly on a mud substrate do not add any vertical refuge, and thus protection from predation (Soniat et al., 2004). Once the shells are spread thinly, they can also be exposed to hypoxic conditions by being closer to the muddy hypoxic zone, further reducing oyster growth and settlement (Baker and Mann 1991, Lenihan 1999).

The finding of a lack of difference between nearby mud flats and reef treatments may be a sampling artifact. Scyphers et al., (2011) found higher blue crab densities on breakwater reefs compared to control areas based on a seine net sampling methods, which showed differences on and off the reef. For a subtidal oyster reef, a seine net would have been impractical, if not
impossible, to use as a sampling method. The use of crab pots introduced an experimental bias, since seine nets provide for an instantaneous grab sample whereas crab pots set overnight permitted crabs that were residing on the reef to swim to the crab pot adjacent to the reef. In regards to commensal samples, the commensal bags in this study created structure in mud flats, and may actually have attracted invertebrate recruits competing for space on nearby reefs. Other sampling methods besides bags include Grabowski et al. (2005)’s finger combing method, as well as lift net methods (Tolley and Volety 2005). Both of these studies, as well as others (Summerson and Peterson 1984, Posey et al., 1999, Coen and Luckenbach 2000, Plunket and La Peyre 2005) found greater numbers of commensals compared to the adjacent mudflats. Only Plunket and La Peyre (2005) used a method that was not instantaneous, but their method of using trays was done in a way that reduced structure on and off the reefs. While the commensal bags provide a good estimate of organisms in the area of the reef, regardless of structure, they may not provide an accurate representation of differences between adjacent mud flats and reefs.

All reef types yielded the same spat densities; with the exception that old rock had lower recruitment than natural control reefs, which could be related to differences in larval supply and flow velocities (Bushek 1998). Most of the natural reefs were located at the mouth of the bays sampled, which tend to be areas with higher flows, higher salinities, and potentially higher food sources, allowing oysters populations to thrive. Another factor that could leave to natural reefs having higher rates of recruitment is that if a natural reef was not successful at recruiting spat, it would not last over time and be present at the time of my sampling. By picking natural reefs that have lasted over time, I may bias my sampling by picking only successful natural reefs. Bushek (1988) found greater numbers of barnacles on offshore sites with higher flows, compared to oysters, which were found at lower flow, intertidal sites (Soniat et al., 2004). Barnacles recruit
year round, with highest peaks in the spring, whereas oysters have a settlement peak in early summer and again in late summer (Supan 1983, Banks and Brown 2002). Oyster spawning and settlement rates are a function of salinity and temperature (Kennedy et al., 1996). Since this survey spanned the entire northern Gulf of Mexico, and salinity was a significant covariate, it is possible that certain bays had passed their settlement peak before the settlement plates were deployed.

Oyster spawning is affected by salinity, with greater settlement at higher salinities (Ortega and Sutherland 1992), and my results agree. While the risk of predation and disease increases at higher salinities (Melancon et al., 1998), growth also increases (Eberline 2012). Lower salinities have less growth and recruitment, and extremely low salinities can actually halt spawning (Pollack et al., 2011, Eberline 2012). My results indicate the largest spat occur at intermediate salinities, but the higher number of predators at greater salinities may have also consumed the larger spat (Melancon et al., 1998). There are numerous predators of oyster spat, including xanthid mud crabs (Grabowski 2004), the flatworm *Stylochus ellipticus* (Newell et al., 2000), and blue crabs (personal observation). Oysters were larger in July compared to May, likely due to higher growth rates. Increases in oyster growth are correlated with increasing water temperature (Ingle and Dawson 1952, Dame 1972), which could have led to larger spat sizes in July compared to May. I attempted to standardize salinities across sites, but the variability in the Gulf of Mexico’s salinity range was too great. A flood in Louisiana brought salinities in Vermilion Bay to 0.1 PSU, while a drought in Texas brought salinities that average around 19.3 PSU to 32 PSU in August of 2011 (Pollack et al., 2011).

The temporal uncertainty of oyster spawning in different bays could also lead to error in interpreting spat data. Unless spat monitoring is ongoing through the whole spawning season, a
bay’s spawning season may be missed. Likewise, the seasonal effect on spat size was most likely determined by when oysters spawned and settled. Oysters spawning in July, as settlement plates were being deployed, had the opportunity to settle earlier and start growing earlier than oysters that spawned in early June, and settled just in time for May sampling settlement plate retrieval (Losee 1979). Since spat densities are variable and dependent upon salinities and temperature for spawning (Kenny et al., 1990, Ortega and Sutherland 1992), in retrospect, my protocol should have included monthly sampling of spat densities.

New rock and new shell reefs were thus generally similar to natural reefs with regard to ecosystem services provided. Oyster reefs are colonized rapidly by invertebrates (Meyer and Townsend 2000, Peterson et al., 2000, Peterson et al., 2003). Grabowski et al. (2005) found no significant seasonal effect on number of invertebrates; and concluded that most change in invertebrate densities is due to rapid colonization. When monitoring oyster reef restoration projects, the similarities between natural reefs and new rock and shell reefs, which are less than five years old, imply that there is no difference between construction material and commensal abundances. Though, if monitoring stops after a five-year period, the drastic change in commensal communities between construction materials is missed. This study, along with others, point out that long term monitoring of restored reefs is important to see if ecosystem services remain constant over time (Coen and Luckenbach 2000, Grabowski et al., 2005, Lukenbach et al., 2005, Grabowski and Peterson 2007, Kennedy et al., 2011).

In fact, this study suggested that over time, shell reefs lose their functional ecosystem services, while rock reefs retain them. Many of the shell reefs had degraded over time to the extent that only a few shells were found buried in the mud, with one reef being nonexistent. These reefs were still included in this study since the main question was to determine how
ecosystem service functions over time; clearly older shell reefs are more likely to lose their function. When creating an artificial oyster reef, my results thus suggest rock is a more resilient and effective material, although placement in regard to salinity variation is important as well.

From a management perspective, the first step in building artificial reefs would be to determine what ecosystem services to restore (Kennedy et al., 2011). If the goal is oyster settlement, and in turn ecosystem services like water filtration, de-nitrification, and other economic benefits, paying attention to the salinity ranges of potential areas is important, as salinity seems to have a greater effect on size and densities of oyster spat than reef material. If the goal is fisheries enhancement, and live oysters are not a priority (Tolley and Volety 2005), salinity is still critical, but reefs created from rock last longer.

Ideally, successfully restored reefs will provide all the ecosystem services lost by declining oyster populations (Luckenbach et al., 2005), although determining success on these reefs is complicated. While setting broad restoration goals like maintaining biodiversity, fish and shellfish production, and ecosystem services are important, the lack of methods to monitor success complicates designing projects, as well as determining if restoration goals have been met (Coen and Luckenbach 2000, Oyster Metrics Workgroup 2011, Furlong 2012). Metrics like habitat complexity, recruitment and species richness are good determinants of functional ecosystem services, although there is no universal standard to compare to. Referencing such metrics to a natural reef is a reasonable option, but often these reefs have been previously harvested, making them patchy and less effective as benchmarks for restoration success (Eggleston et al., 1999, Glancy 2003, Macreadie et al., 2011). The Oyster Metrics Workgroup, based in the Chesapeake Bay, noted that complete failures are much more easily noted than successful reefs, and that success must be measured in metrics that can apply to all reefs. They
suggested exploring relationships between different metrics, such as reef size, oyster biomass, total suspended solids and temperature in a controlled experiment, and using a few of these characteristics to determine the health of the reef, although such an approach is not politically popular nor cheap. By defining first what a reef is, success can be more easily measured. If a reef is simply structure (live or dead) that attracts fishes and offers refuge for invertebrates, success criteria could be high species richness, commensal abundance, and vertical complexity; whereas if a reef is a living community, oyster biomass and production may be determinants of success.

State and private restoration efforts ultimately will do what is most practical in an economic sense. One of the major considerations comes from shell being a limiting component. Oyster shell recycling programs, such as those in North Carolina, South Carolina, and Virginia benefit those states, but along the Gulf coast, recycled shell is difficult to find. Part of this results from most of the Gulf of Mexico’s oyster shell being shipped out to states like North and South Carolina, and Virginia, which end up in their shell recycling programs. Oyster shells that are not exported are used in road construction and poultry feed, ultimately creating a shell deficit along the Gulf coast (LDWF 2004). Economically, oyster shell is not the cheapest route, and rock or concrete is more cost effective (LWDF 2004).

This study provides support for a best management practice of using rock materials to create artificial oyster reefs along the Northern Gulf of Mexico. Rock material will not only result in more live oysters and make more sense economically (LDWF 2004), but it will continue to provide refuge and habitat for invertebrates at the bottom of the food web, regardless of variation in oyster recruitment. Determining the correct placement in regard to salinity regimes is still quite important to having successful recruitment and growth on the reef. However, if rock is used, if nothing recruits to the reef, all money spent and ecosystem services are not lost. In the
Gulf of Mexico, where sediment is mostly mud and salinity is highly variable, rock reefs may actually provide more ecosystem services than shell reefs over time, and in a few cases more services than natural reefs, therefore providing an optional if not optimal material to restore ecosystem services. As restoration efforts increase due to loss of ecosystem services from threats to Gulf of Mexico habitats, finding best management practices that efficiently and effectively restore lost ecosystem services of oyster reefs is crucial.


VITA

Laura Brown was born in November of 1987 in the beautiful mountains of Asheville, North Carolina. Although raised around mountain streams, after a summer program at the coast during her undergraduate days at the University of North Carolina-Chapel Hill (UNC), Laura found her niche in marine ecology. She worked for three summers as an intern in Dr. Charles Peterson’s laboratory, doing salt marsh and oyster reef ecology, and during the school year worked in Dr. Justin Ries’s laboratory working in marine biogeochemistry. Throughout her time at UNC, Laura helped answer questions about how increasing ocean acidification effects growth of organisms that calcify carbon, including oysters and corals. Other projects looked at trophic cascades across oyster reefs, and how overfishing can alter these interactions. She won the Mary Watts-Hill award from UNC to continue her research over the summer of 2009.

After graduating from UNC in 2010, Laura spent a summer working as a snorkel and SCUBA tour guide in Ketchikan, Alaska, taking cruise passengers through the kelp forests and rocky intertidal waters of the Pacific while teaching them fun facts about the ecosystem. This allowed Laura to expand her experience to cold water diving and Pacific ecosystems, and to facilitate her love of marine systems.

Once her summer stint was over, Laura traveled to Baton Rouge, Louisiana, to pursue her masters of science in biology at Louisiana State University (LSU) in Dr. Kenneth Brown’s laboratory. During her time at LSU, Laura has been deeply involved in active research on the Deepwater Horizon oil spill’s effect on oyster populations in Louisiana, including presenting findings at national conferences. Her thesis work has allowed her to collaborate with groups such as Louisiana Department of Wildlife and Fisheries, USGS, and The Nature Conservancy to help
determine how differing oyster reef restoration efforts affect ecosystem services and their functions over time. Her research will suggest best management practices for restoration projects across the northern Gulf of Mexico. This research was also presented at national conferences. Laura’s current interests are directed at maintaining sustainable marine ecosystems and lessening anthropogenic effects on these already fragile systems.