RELATIVE HABITAT VALUE OF ALTERNATIVE SUBSTRATES USED IN OYSTER REEF RESTORATION

A Thesis

by

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ABSTRACT

Oyster reef habitats have declined from historic levels due to a variety of reasons, including overharvest, disease, and degraded water quality. The harvesting of oysters has led to a loss of reef habitat for both oysters and reef-associated fauna. When oysters spawn, the larval oysters, or spat, depend on hard substrate for settlement and growth. Oyster shell is the preferred substrate for use in restoration because it most closely matches natural reef habitat, but it is often expensive and in limited supply. This study incorporated field and laboratory experiments to assess the relative habitat value of alternative substrates (crushed concrete, porcelain, crushed limestone, and river rock, as well as oyster shell) for larval oyster recruitment as well as reef resident fishes and macro-invertebrates. Replicate trays of each substrate type were deployed in St. Charles Bay, TX for four months during spring and summer 2012 and assessed for oyster recruitment and faunal diversity and density. Concrete, river rock, limestone and porcelain had similar spat recruitment densities compared to oyster shell (1300-2300 spat). Spat shell heights were also larger on these substrates (13-16 mm), while spat on porcelain substrates were slightly smaller (10-13 mm). All substrates except bare sediment had similar fauna species densities (200-500 individuals m^{-2}). Limestone had lower fauna diversity (H'; 0-1) than concrete and shell (1-2). Laboratory experiments compared the effectiveness of these substrates in providing prey refuge from pinfish and blue crab predators. All substrates performed similarly resulting in very low (<20 %) prey mortality rates for either predator. Results may enable future restoration plans to be implemented at a lower cost while providing similar habitat functions.

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LITERATURE REVIEW

Eastern oysters (*Crassostrea virginica*) are found in the Caribbean, Gulf of Mexico and Atlantic Ocean (Powell and Klinck 2007). These reef-building mollusks have great cultural, economic and ecological importance (Powell and Klinck 2007). Oysters provide a wide range of benefits to ecosystems and humans, including water filtration (Dame et al. 1981), nitrogen regulation (Piehler and Smyth 2011), habitat and refuge for fish and invertebrates (Peterson et al. 2003), shoreline protection (Piazza et al. 2005), food for higher trophic levels (Coen et al. 1999), and resources for human consumption. Oysters are also indicator species that can be used to gather information on the overall health of an estuary (Beseres Pollack et al. 2011).

Oysters act as biofilters, removing particulate matter through suspension feeding (Dame et al. 1984). Early studies in Chesapeake Area found that oysters can remove particulate matter between the sizes of 1 and 12 µm (Haven and Morales-Alamo 1972; Dame et al. 1981). In areas that have experienced drastic reductions in historical oyster abundance, loss of this filtration function has been linked to large-scale ecosystem changes like eutrophication and algal blooms (Newell 1988, Ulanowicz and Tuttle 1992, Lotze et al. 2006). The role of oysters in nitrogen regulation is also an important contributor to estuarine ecosystem function, particularly due to increasing nutrient influxes into area systems (Paerl et al. 1998, Piehler and Smyth 2011).

Oyster reefs are essential habitat for fish and invertebrates (Stunz et al. 2010, Harding and Mann 2001). Their 3-dimensional structure offers refuge for larval and juvenile fish species as well as smaller invertebrate species, such as mud crabs, porcelain crabs, brown shrimp and snapping shrimp (Stunz et al. 2010, Harding and Mann 2001, Gain et al. 2009). Of the organic particulate matter filtered by oysters, 70% is assimilated and the remaining 30% is used as food by reef resident species (Newell 1988, Tolley and Volety 2005). Juvenile fish and transient fish and crab species also feed on polychaetes, bivalves, decapods and smaller residents that inhabit these reefs (Grabowski et al. 2005, Tolley and Volety 2005). Faunal diversity on reefs is dependent on many factors including connectivity to natural reef populations, vertical relief of the reefs, distance from adjacent salt grass and sea grass habitats, and abiotic conditions (Pulliam 1988, French-McCay et al. 2003, Grabowski and Peterson 2007, Lipcius et al. 2008 Wilberg et al. 2013). In a North Carolina estuary, each $10m^2$ plot of restored reef was estimated to produce an additional 2.6 Kg yr^{-1} of oyster shell cover (Peterson et al. 2003). Restored reefs can therefore play a role in ameliorating habitat loss due to human consumption, disease and predation.

As reef builders, oysters are considered ecosystem engineers (Jones et al. 1994). Oyster reefs can contribute to shoreline stabilization, with their 3-dimensional reef structure serving to reduce wave energies and shoreline erosion (Meyer et al. 1997, Piazza et al. 2005). Shoreline protection efforts typically involve the construction of bulkheads, seawalls or other hard structures (Pilkey and Wright 1988, NRC 2007, Scyphers et al. 2012). These artificial barriers may increase erosion of adjacent shorelines through the reflection of wave energy off of the barriers (Scyphers et al. 2012) Current shoreline stabilization has focused more on the use of "living shorelines" whose 3-dimensional structure allow for decreased wave energy and increased sedimentation (NRC 2007, Scyphers et al. 2012). Living shorelines like oyster reef structures may serve to protect valuable adjacent habitats such as salt marsh and sea grass habitats while offering increased substrate for further oyster recruitment (Campbell and Hall 2002, Scyphers et al. 2012).

One of the most obvious ecosystem services of oyster reefs is as a fishery commodity for human consumption. National Marine Fisheries Service (NMFS) harvest data from 2009 reported that 35.6 million pounds of oysters were harvested in the United States, with a value of

$136.5 million (Lutz et al. 2011). Texas was the second largest producer in the Gulf in 2009, harvesting 2.7 million pounds of oysters and generating $28 million in revenue (NOAA 2009, Lutz et al. 2011).

Overharvesting of oysters and loss of reef habitat have caused a reduction in the benefits reefs provide. As such, oyster reefs are among the most degraded coastal habitats on earth, with estimates of only 15% currently existing throughout the world (Lotze et al. 2006, Beck et al. 2011). Besides historical losses in reef size, in some Gulf of Mexico estuaries, loss of oyster biomass has also been significant (zu Ermgassen et al. 2012). When reefs are degraded through dredging or oysters are overharvested, future habitat is lost (Powell and Klinck 2007). Loss of oyster habitat is especially critical because the free-swimming larvae of oysters depend on the structural foundation of oyster reefs for recruitment and growth (Rothschild et al. 1994, Lenihan and Peterson 1998) The loss of adult oysters through *P. marinus* and natural predators could also disrupt the natural balance of reef formation (Ray 1996, 2008).

Salinity tolerant predators and parasites are also damaging to oyster populations (Andrews and Ray 1988, Ray 1996, Ray 2008). Dermo (*Perkinsus marinus*), a parasitic protozoan, causes severe oyster mortalities from Chesapeake Area to the Gulf of Mexico (Mackin et al. 1950, Andrews and Ray 1988, Ray 2008), particularly during periods of warm temperatures and high salinities (Ray 1996, Ray 2008). In the 1980's, an extended period of drought allowed *Perkinsus marinus* to expand throughout Chesapeake Area (Andrews and Ray 1988). Similarly, *P. marinus* is prevalent throughout Texas areas due to drought-induced high salinities (Ray 2008). Oysters affected by *P. marinus* are unable to survive to market-size. Some states have had to reduce their market-size limits because of this threat (Ray 2008). With increasing salinities and temperatures throughout the Gulf of Mexico and western Atlantic, oysters continue to be threatened by these parasites (Andrews and Ray 1988, Ray 1996, Ray

2008). Oyster reef shell formation is dependent on the natural death of larger adult oysters (Powell et al. 2006, Carver et al. 2010). These shells are then buried by accumulating live oysters thereby creating a deep anoxic layer that excludes boring predators and parasites (Davies et al. 1989, Waldbusser et al. 2013). This allows preservation of shell calcium carbonate and further reef growth (Powell and Klinck 2007, Waldbusser et al. 2013).

Oyster reef restoration efforts are ongoing across the United States by a variety of federal, state, private, and NGO groups to ameliorate oyster population declines. However, efforts to restore oyster populations in Chesapeake Area have seen little success despite massive public support and investment. Some argue that the failure to restore oysters in this area is due to poorly defined goals and expectations (Mann and Powell 2007). The balance between fishing mortality and natural mortality typically leading to sustainable harvesting practices is further complicated by the need for restored reefs to grow through accumulation (Powell et al. 2006, Mann and Powell 2007).

Oyster reef restoration efforts generally involve placing oyster shells or other hard substrates back into estuaries to provide attachment points for larval oysters and reef development (Powers et al. 2009, Schulte et al. 2009). Shell is the natural substrate for restoring degraded oyster reefs—however, harvested oyster shells are often lost to landfills or competing uses such as road construction or as poultry feed additives (LDWF 2004). The limited availability and great expense of oyster shells is one of the major obstacles to oyster reef restoration on the large scale (LDWF 2004). In response, several states have come up with mechanisms to conserve existing oyster reefs and reclaim oyster shells for use in reef maintenance and restoration. In Mississippi, Senate Bill 2679 (1998) requires that all shell taken from oyster reefs be deposited back onto the reefs from which they are harvested. North Carolina Senate Bill 1272 (2010) extends tax credits for donation of oyster shells to the state for use in restoration. In Texas, Senate Bill 932 (2011) established a program to recover oyster shell and other cultch material for maintaining or enhancing natural reefs.

Because demand for oyster shells often exceeds supply, many alternative substrates are being used to restore reefs, including crushed concrete, gravel, limestone, and river rock, as well as other mollusk shells. Despite studies that have examined specific substrate types in relation to oyster recruitment and growth (e.g. Brumbaugh 2000, Soniat and Burton 2005, Nestlerode et al. 2007, White et al. 2009), substrates for restoration are still often selected based on price and availability rather than their ability to mimic important ecological functions (French-McCay et al. 2003, Brumbaugh and Coen 2009). In addition, although numerous oyster reef restoration efforts have been conducted in the western Atlantic and Gulf Coast, information is lacking on the relative habitat value for macrofauna of alternative substrates (French-McCay et al. 2003). As restoration efforts continue to increase, there is a critical need to understand the effectiveness of alternative substrates as replacements for natural oyster shell in reef building, not only for economic reasons but for both oyster recruitment and habitat creation for fish and macroinvertebrates.

INTRODUCTION

Eastern oysters (*Crassostrea virginica*) are reef-building bivalve mollusks found in the Caribbean, Gulf of Mexico and Atlantic Ocean. Oysters provide many cultural, economic and ecological benefits, including water filtration (Dame et al. 1981), nitrogen regulation (Piehler and Smyth 2011), habitat, refuge and foraging grounds for fish and invertebrates (Peterson et al. 2003), shoreline protection (Piazza et al. 2005), food for higher trophic levels (Coen et al. 1999), and resources for human consumption. Oysters are also indicator species that can be used to gather information on overall estuarine health (Pollack et al. 2011).

Oyster reefs, and the benefits they provide, have been steadily declining throughout much of the U.S. (zu Ermgassen et al. 2012). As such, oyster reefs are among the most degraded coastal habitats on earth, with about 15% currently existing worldwide compared to historic levels (Lotze et al. 2006, Beck et al. 2011). In some Gulf of Mexico estuaries, existing oyster reefs remain in a degraded state, with biomass, reef height, and broodstock declining while areal extent remains (zu Ermgassen et al. 2012). When reefs are degraded, future habitat is lost as is the ecological functions reefs provide (Powell and Klinck 2007). This loss of oyster habitat is especially critical because free-swimming oyster larvae depend on the structural foundation of existing oyster reefs for recruitment and growth (Rothschild et al. 1994, Lenihan and Peterson 1998).

Oyster reef restoration efforts are ongoing across the United States by a variety of federal, state, private, and NGO groups to ameliorate oyster population declines (Mann and Powell 2007). Oyster reef restoration efforts generally involve placing oyster shells or other hard substrates back into estuaries to provide attachment points for oyster colonization and reef development (Powers et al. 2009, Schulte et al. 2009). Shell is the natural substrate for restoring degraded oyster reefs—however, harvested oyster shells are often lost to landfills or competing uses (LDWF 2004). The limited availability and great expense of oyster shells is one of the major obstacles to oyster reef restoration on the large scale (LDWF 2004).

Because demand for oyster shells often exceeds supply, many alternative substrates are being used to restore reefs, including crushed concrete, gravel, limestone, and river rock, as well as other mollusk shells (LDWF 2004, TPWD 2011). Previous studies have examined specific substrate types solely for oyster recruitment and growth (e.g. Brumbaugh 2000, Soniat and Burton 2005, Nestlerode et al. 2007, White et al. 2009). However, it is also important to understand the ability of alternative substrates to mimic important ecological functions provided by natural reefs (French-McCay et al. 2003, Brumbaugh and Coen 2009). The goal of this study was to examine the habitat value of alternative substrates for use in oyster reef restoration projects. Field and laboratory experiments were conducted to compare oyster shell, crushed concrete, porcelain, crushed limestone and river rock in their ability to: 1) attract oyster larvae, 2) promote oyster growth to juvenile size and 3) provide habitat for fish and crustaceans. This study is unique in that five different substrates were examined simultaneously for their habitat value, not only for oysters, but also for the resident and transient species that depend on oyster reefs. Results from this study will help guide coastal resource managers in selecting appropriate alternative substrates that maximize restoration potential.

MATERIALS AND METHODS

Field Experiments

Field experiments were conducted in St. Charles Bay, Texas, part of the Mission-Aransas Estuary, USA (Fig. 1). The St. Charles Bay system (including the adjacent watershed) is approximately 530 km^2 (Asquith et al. 1997). The bay is relatively shallow with an average depth of 0.9 m, and maximum depth of 1.5 m (Scates and Shook 1999). The area comprises different habitat types including intertidal marsh, seagrass and oyster reef (Scates and Shook 1999). Commercial oyster harvest is uncommon and limited to the deeper central waters. No commercial oyster harvesting occurs in St. Charles Bay. Salinities and temperatures during the study period ranged from 22-25 and 25-28°C, respectively. Dissolved oxygen was consistent ranging from 6.97-7.12 mg/L.

Oyster shell that had been sun-bleached for 6 months was obtained from the Sink Your Shucks recycling program at Texas A&M University - Corpus Christi. Concrete was obtained from the chutes and hoppers of concrete trucks after construction projects. Porcelain was obtained from the City of Corpus Christi's municipal waste stream. Both the concrete and porcelain were crushed and graded to approximately 8 cm size. River rock and limestone were purchased in ~8 cm pieces from a local nursery. Individual sampling trays (0.75 m^2) were used to hold these substrates. The bottom of each tray was lined with 1 cm^2 mesh to prevent macroinvertebrates from falling through the tray during sampling. The trays were filled with 38 L of substrate (1 substrate per unit) for a total of 50 trays (10 trays per substrate). Ten shallow subtidal sites adjacent to natural oyster reef were selected throughout St. Charles Bay (Fig. 1). The natural reef that we chose in St. Charles is separated into two main areas, upper area and lower area, by a dredged boat channel. Because restoration success can vary on a site-by-site basis, 5 experimental sites were chosen on the reef in the lower area near the mouth of St. Charles and 5 were chosen at the reef in the upper area further back into St. Charles. In May 2012, 5 individual sampling trays were placed in a random grid pattern at each site, one filled with each substrate type: concrete, limestone, porcelain, river rock, or oyster shell. Placement areas were cleared of any oyster clusters to allow the trays to lay flat on the bay bottom. Trays were anchored with rebar to prevent movement or loss. Abiotic conditions (e.g., pH, DO, salinity and temperature) were measured at each site by using a Hydrolab sonde.

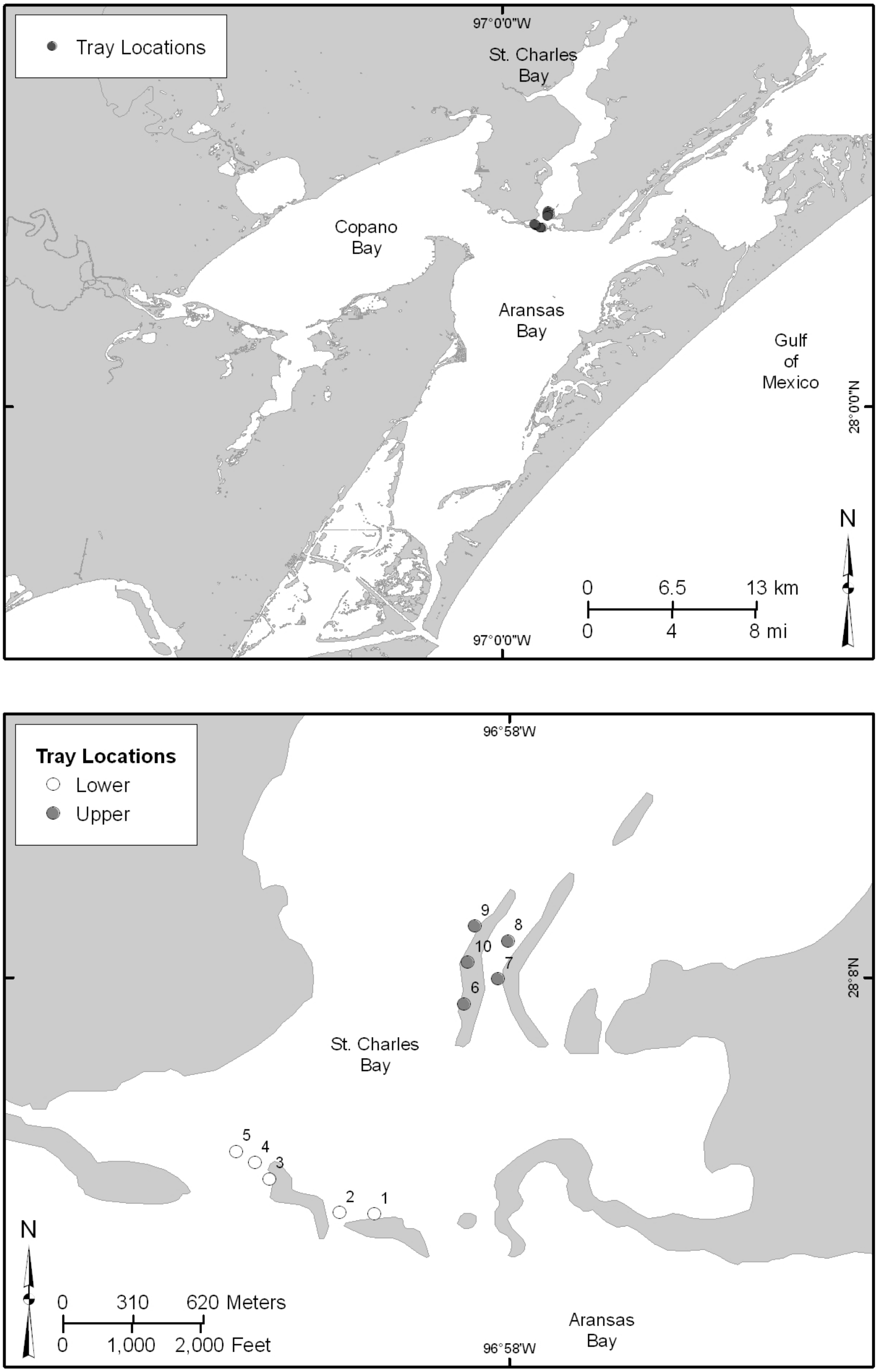


Figure 1. Sample site locations in St. Charles Bay, TX, part of the Mission-Aransas Estuary. Five individual sampling trays, 1 of each substrate type, were placed along natural reefs at 5 lower area and 5 upper area locations.

After 4 months, in September 2012, the trays were sampled using a modified throw trap. In addition to the sampling trays, bare sediment was also sampled at each site to provide a baseline comparison with each substrate. At each site, the 1 m^2 throw traps were deployed by rapidly enclosing the area around each of the 5 sampling trays, following the methods of Rozas and Minello (1997). The throw traps were pushed firmly into the sediment surrounding each tray to prevent loss of animals. The tray containing the experimental substrates was lifted from inside each throw trap and retained, while snug-fitting sweep nets (1.6 mm mesh) were passed through the enclosed area until all organisms were collected. All organisms were placed in 10% formalin and brought to the lab for processing. A 0.09 m^2 quadrat was placed randomly within each sampling unit for quantification of encrusting organisms such as oyster spat, barnacles, mussels, slipper shells, serpulid worms and algae. All substrates were retained and placed into bags for enumeration, spat height measurement and identification of reef-associated organisms in the lab. Any large (> 100 mm) macrofauna were identified to species in the field, measured for standard length using calipers and released. Hydrological parameters were again measured at each site. In the laboratory, organisms were sorted, identified to the lowest practical level, enumerated, and measured for standard length (mm) using calipers.

Laboratory Experiments

Laboratory experiments were conducted from June 2012 to August 2012 utilizing a flow-through tank system at the Texas A&M AgriLife Mariculture Research Laboratory in Port Aransas, TX. A total of 28-110 L rectangular (76 x 30 x 46 cm) fiberglass tanks were filled with one type of each substrate (oyster shell, river rock, limestone, concrete, porcelain) substrate each (Fig. 8). Two additional substrates, sand and bare bottom (no substrate), were added to the laboratory experiments as controls. Each experiment comprised 4 replicates of each substrate.

Predator organisms used for these experiments were blue crabs (*Callinectes sapidus*; 12.5-14 mm carapace length) and pinfish (*Lagodon rhomboides*; 12-14 mm standard length). Both species have been successfully used in similar experimental trials (Gain 2009). Mud crabs from the family Xanthidae were used as prey organisms in all of the trials. All organisms used in the laboratory experiments were collected from the Mission-Aransas Estuary, TX. Prior to the experiments, either two blue crabs or pinfish were placed into substrate tanks for a 24 hour starvation period. After 24 hours, predator organisms were cornered off to one side of the tank by plastic mesh to allow an acclimation area for the prey. Natural densities of Xanthid mud crabs (10 individuals per 1 m^2) were placed randomly throughout each tank the same day they were collected from the field and allowed 30 minutes to acclimate. For the size of tanks in these experiments, 10 mud crabs were used for each trial. Trials began when the divider mesh was removed. Trials lasted until 50% prey mortality was observed. For the blue crabs this was 48 hours and for pinfish, 72 hours. At the conclusion of each trial, all predators and surviving mud crabs were released back into the field at the collection site. A total of 12 trials per predator treatment were completed for an overall total of 24 experimental trials. Water quality was monitored each day to assure consistency between tanks.

Statistical Analysis

Data from the field experiments (spat recruitment, spat height, reef-associated faunal abundance and diversity) were examined using a one-way blocked partially hierarchical Analysis of Variance (SAS 9.3) with substrate as a fixed main effect and site and area as random effects with the equation:

$$y = a\ b\ c(b)$$

where a = substrate (5), b = area (2), c = site (5); $\alpha = 0.05$.

Adapting methods used by Gain (2009), all species counts were extrapolated to density of organisms (m^{-2}) prior to analysis. The mean and standard error (SE) for the total number of spat, fish, crustaceans, and other species was computed for each substrate type sampled. Normality of the residuals and homogeneity of the variance of all data were examined using R. Data transformation (log10[x+1]) was used to ensure homogeneity and normality (Gain 2009).

Data from the laboratory experiments were examined using a two-way ANOVA with both substrate and predator type as a fixed factor with $\alpha = 0.05$. A power analysis was also conducted. All ANOVAs and Tukey's HSD *post hoc* analyses were performed in SAS (9.3). Similarities between reef-associated faunal communities were analyzed using non-metric multidimensional scaling (MDS) using a Bray-Curtis similarity matrix in PRIMER-E v.6. Significant groupings of communities were determined using the SIMPER routine as part of cluster analysis (Clarke 1993). Significance between communities was tested using similarity percentages (SIMPER) procedures (Clarke 1993). The DIVERSE function in PRIMER-E was used to calculate the Shannon–Wiener diversity index (H'). Normality and homoscedasticity of the spat recruitment, spat height and fauna data were examined using R.

RESULTS

Field Experiments

Spat recruitment

Spat recruitment densities ranged from 322 m^2 to 2412 m^2 (Table 1). No spat were observed on the bare sediment substrates. There was a significant difference in spat recruitment between substrates ($p = <0.0001$). Using a Tukey's HSD test, there were no significant differences between any of the substrates except for bare sediment. Bare sediment contained significantly lower spat recruitment than all other substrates (Fig. 2). There was also a significant difference in spat recruitment between area (upper versus lower, $p = 0.0002$). Tukey's HSD test showed that the upper area had significantly higher spat recruitment than lower area for all substrates except concrete (Fig. 2).

Table 1. Spat m^{-2} recruitment data (mean spat densities and mean shell heights (± SE)) per area placement and substrate type in St. Charles Bay, TX

AREA	SUBSTRATE	MEAN SPAT RECRUITMENT (±SE)		MEAN SHELL HEIGHT (±SE)	
Lower	Concrete	2208.9	(680.4)	16.44	(3.4)
	Limestone	1032.3	(292.5)	13.87	(2.6)
	Porcelain	710.4	(222.2)	13.1	(4.8)
	River rock	1043.4	(267.3)	6.47	(2.8)
	Shell	1176.6	(283.5)	16.65	(4.0)
Upper	Concrete	2541.9	(696.0)	15.48	(3.4)
	Limestone	3019.2	(446.6)	12.75	(4.5)
	Porcelain	2031.3	(475.9)	10.89	(2.0)
	River rock	3141.3	(711.3)	15.25	(4.7)
	Shell	2697.3	(638.6)	14.88	(3.7)
Combined	Concrete	2375.4	(462.1)	15.96	(3.9)
	Limestone	2020.2	(415.9)	13.31	(3.5)
	Porcelain	1376.4	(739.2)	11.97	(2.9)
	River rock	2086.8	(1585.4)	15.86	(3.8)
	Shell	2020.2	(446.1)	15.67	(3.9)

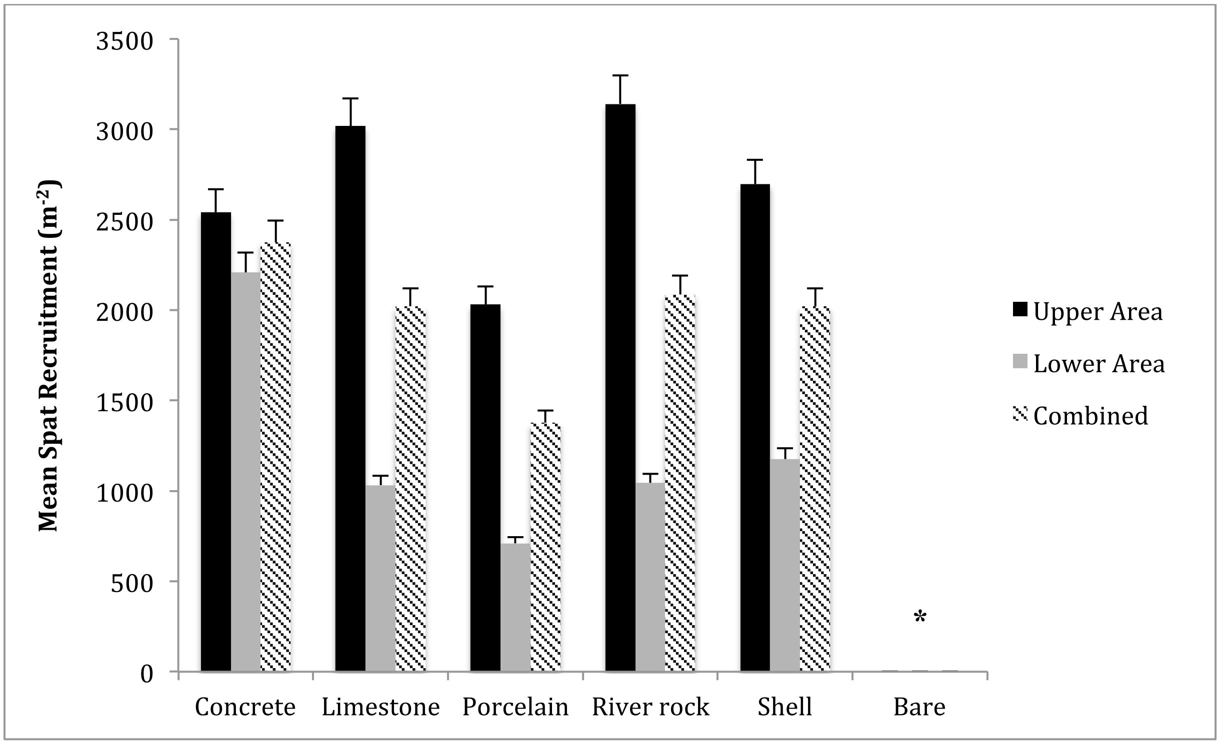


Figure 2. Mean spat recruitment (m^{-2}) for each substrate type for upper, lower, and combined tray placement areas in St. Charles Bay, TX.

Spat height

Spat shell heights (from hinge to lip) ranged from 1.4-41.5 mm. There was a significant difference in mean spat shell height between substrates ($p = <0.0001$) and between upper and lower areas ($p = 0.0002$) (Fig. 3). A Tukey's HSD test indicated that there were no significant differences in spat height between all substrates except bare sediment. Lower area had significantly higher mean spat lengths than upper area for both shell and porcelain substrates (Fig. 3).

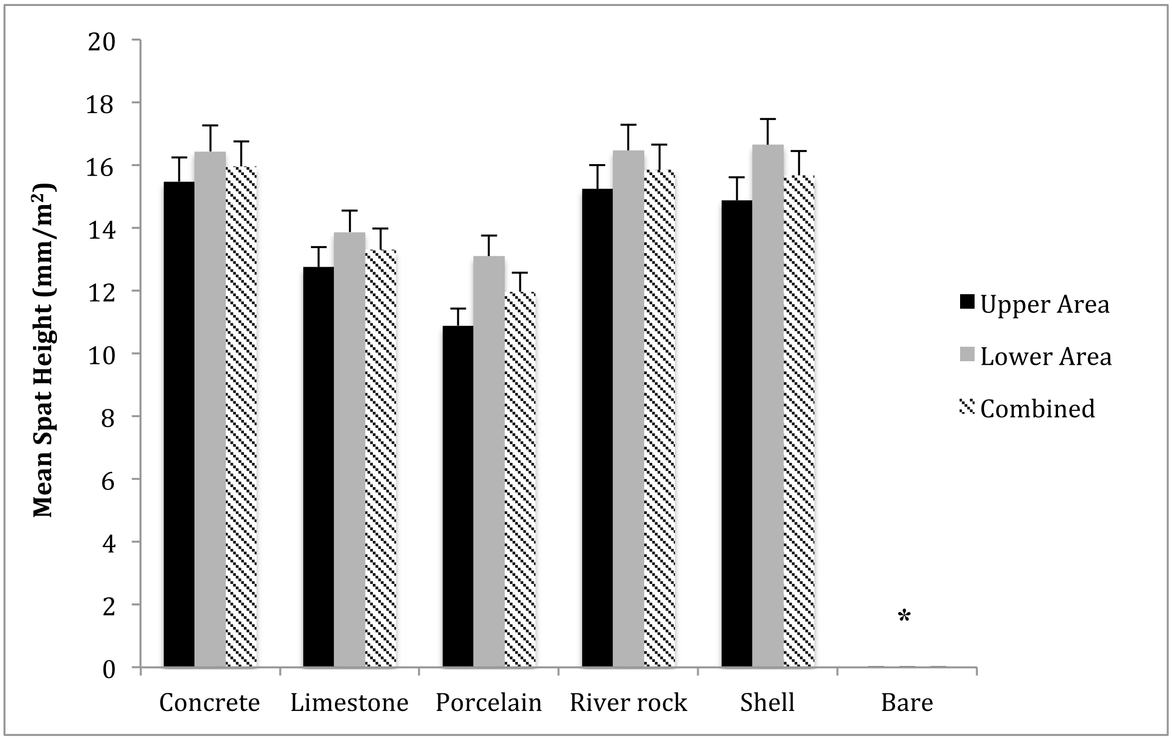


Figure 3. Mean spat shell height (mm/m^2) on each substrate type for upper, lower, and combined tray placement areas in St. Charles Bay, TX.

Reef-associated faunal density

Mean faunal species densities associated with all substrates except bare sediment ranged from 175-500 individuals m^{-2}. Mean densities on bare sediment ranged from 9 – 58 m^{-2}. Species densities were similar between all substrate types except bare sediment (p = <0.0001) (Table 2). There was a significant difference in species density between sites in upper and lower area areas (p = 0.0106), with lower area sites containing higher species densities (415.50 mean individuals per m^2; Fig. 4).

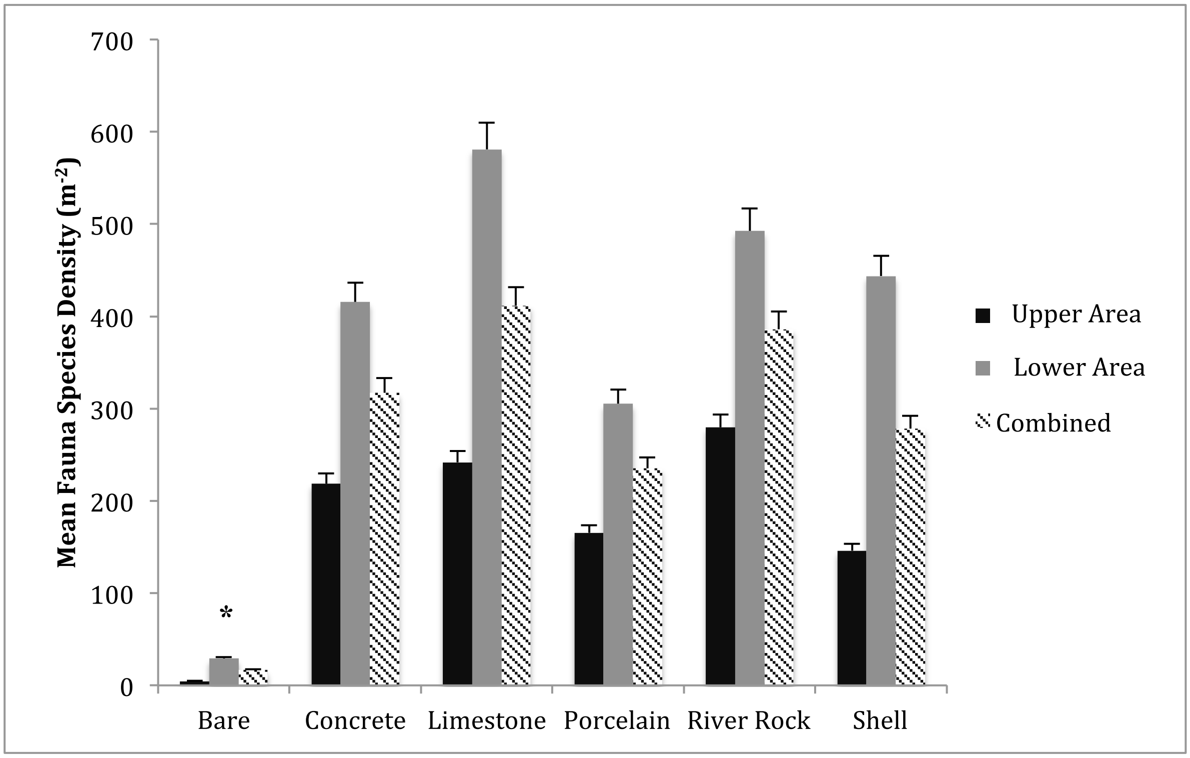


Figure 4. Faunal density (m^{-2}) associated with each substrate type for upper, lower, and combined tray placement areas in St. Charles Bay, TX.

Table 2. Reef associated fauna (total abundance and mean densities (± SE)) collected in association with each substrate type in St. Charles Bay, TX.

			Bare		Concrete		Limestone		Porcelain		River Rock		Shell	
COMMON NAME	SCIENTIFIC NAME	TOTAL NUMBER	MEAN	(±SE)	MEAN	(±SE)	MEAN	(±SE)	MEAN	(±SE)	MEAN	(±SE)	MEAN	(±SE)
Mud crab	Xanthidae	1,132	3.5	(1.5)	15.3	(2.8)	34.3	(7.2)	17.0	(2.6)	26.7	(2.1)	21.3	(3.6)
Porcellain crab	Porcellanidae	12,956	0.0	(0.0)	254.0	(65.9)	346.3	(119.8)	178.7	(51.7)	312.9	(62.4)	216.3	(92.2)
Grass shrimp	*Palaemontes spp.*	465	0.0	(0.0)	18.4	(5.2)	9.8	(3.4)	14.1	(3.0)	6.1	(1.2)	13.9	(4.9)
Stone crab	*Menippe adina*	129	0.0	(0.0)	4.1	(0.8)	2.4	(0.7)	2.6	(0.7)	3.1	(0.7)	2.7	(0.5)
Snapping shrimp	*Alpheus heterochaelis*	583	0.0	(0.0)	10.0	(2.0)	12.6	(4.2)	13.3	(2.8)	14.1	(4.2)	10.7	(3.1)
Code goby	*Gobiosoma robustum*	19	0.0	(0.0)	4.5	(0.5)	0.0	(0.0)	1.3	(0.3)	0.0	(0.0)	5.0	(0.0)
Naked goby	*Gobiosoma bosc*	350	2.0	(0.0)	5.9	(0.9)	8.9	(3.1)	7.6	(0.8)	8.9	(1.5)	6.3	(0.9)
Area anchovy	*Anchoa mitchilli*	137	27.5	(24.5)	12.5	(4.9)	4.0	(0.0)	1.0	(0.0)	5.7	(4.2)	5.0	(4.0)
Skillet fish	*Gobiesox strumosus*	86	0.0	(0.0)	2.3	(0.5)	1.8	(0.5)	3.4	(0.9)	2.3	(0.6)	4.2	(1.2)
Gulf toadfish	*Opsanus beta*	116	0.0	(0.0)	3.4	(0.8)	2.8	(0.8)	2.9	(0.5)	3.2	(0.9)	2.9	(0.6)
Silver perch	*Bairediella chrysoura*	10	0.0	(0.0)	1.0	(0.0)	0.0	(0.0)	3.5	(1.5)	0.0	(0.0)	2.0	(0.0)
Spotfin mojarra	*Eucinostomus argenteus*	4	0.0	(0.0)	1.5	(0.5)	0.0	(0.0)	1.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Striped blenny	*Chasmodes bosquianus*	1	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	1.0	(0.0)
Brown / Pink shrimp	*Farfantapenaeus spp.*	17	0.0	(0.0)	1.5	(0.0)	1.5	(0.0)	1.0	(0.0)	1.5	(0.0)	2.0	(0.0)
Blue crab	*Calinectes sapidus*	3	0.0	(0.0)	0.0	(0.0)	1.0	(0.0)	1.0	(0.0)	0.0	(0.0)	1.0	(0.0)
Pinfish	*Langodon rhomboides*	15	0.0	(0.0)	1.0	(0.0)	1.0	(0.0)	1.5	(0.5)	1.0	(0.0)	1.5	(0.5)
Sheepshead	*Archosargus probatocephalus*	35	0.0	(0.0)	2.3	(0.3)	1.0	(0.0)	1.3	(0.2)	1.4	(0.4)	1.3	(0.3)
Post-larval Penaids		1	0.0	(0.0)	1.0	(0.5)	0.0	(0.5)	0.0	(0.0)	0.0	(0.5)	0.0	(0.0)
Swimming crabs	Portunidae	2	1.0	(0.0)	1.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Feathered blenny	*Hypsoblennius hentz*	3	0.0	(0.0)	1.0	(0.0)	0.0	(0.0)	1.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Gafftopsail catfish	*Bagre marinus*	4	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	1.0	(0.0)	2.0	(0.0)	1.0	(0.0)
Grey snapper	*Lutjanus griseus*	1	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	1.0	(0.0)

Reef-associated faunal diversity

Species diversity (H') of reef-associated fauna ranged from 1.36 - 2.32 (Fig. 5). Species diversity was similar between upper and lower areas ($p = 0.1879$). There was a significant difference in mean species diversity between substrate types ($p = <0.0001$), with bare sediment having significantly lower mean species diversity (H' = 0.4) than the other substrates.

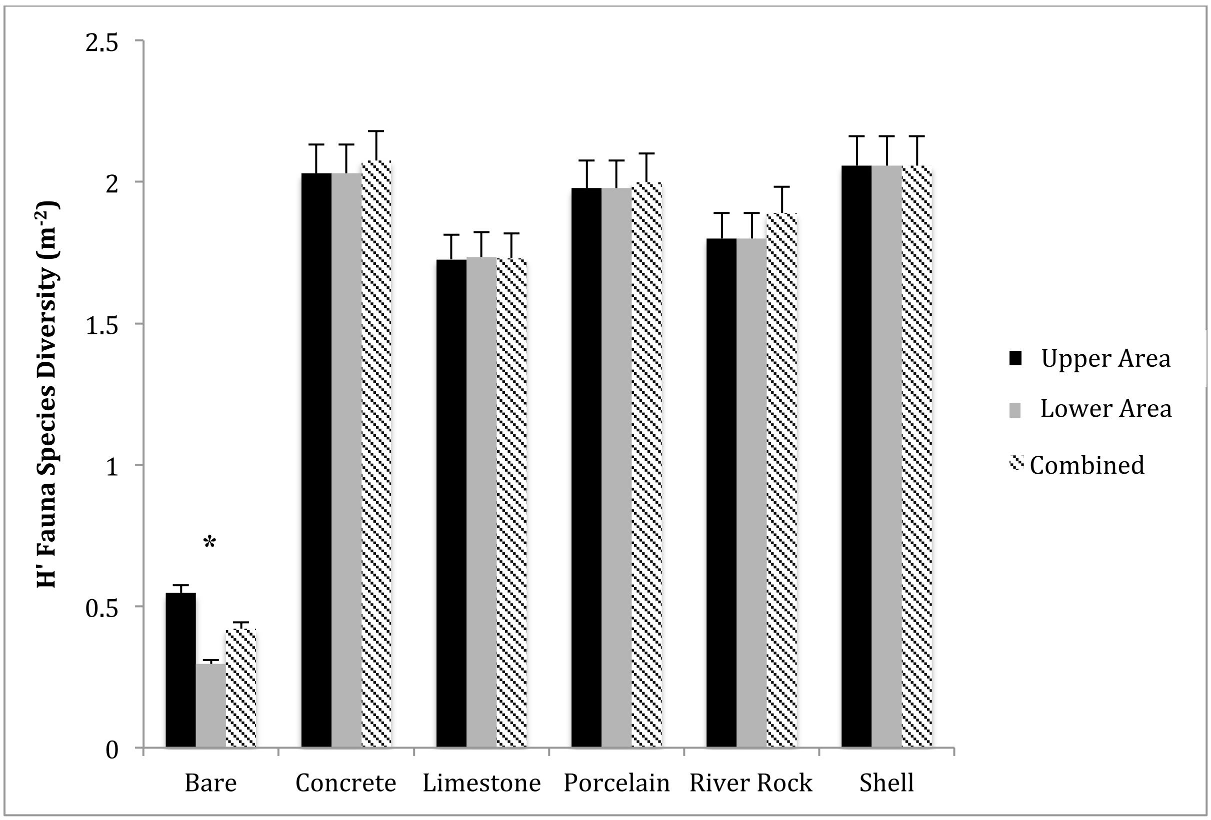
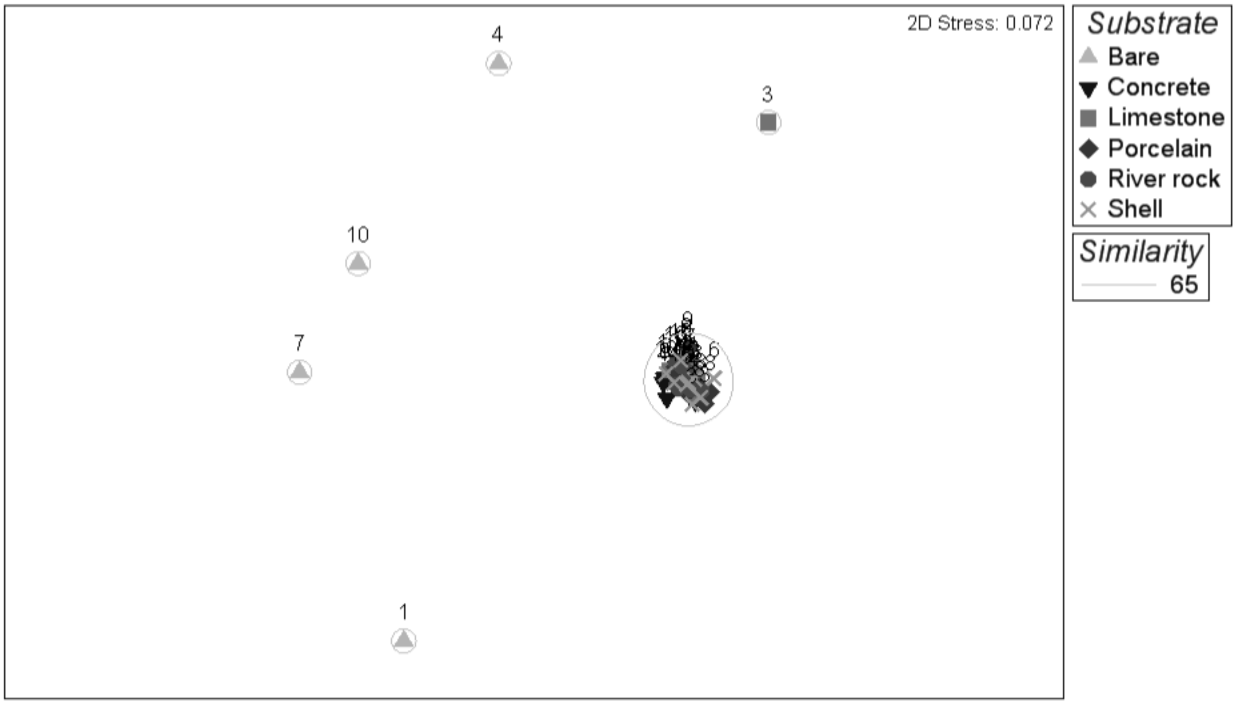


Figure 5. Species diversity (H') of reef associated fauna for each substrate type in lower and upper areas and combined in St. Charles Bay, TX.

Reef-associated faunal communities were generally similar to one another across substrate types, except for bare bottom (Fig. 6a). This was illustrated in the MDS analysis with almost all communities clustering into one large group with at least 67% similarity between substrate types (Fig. 6b).

A)



B)

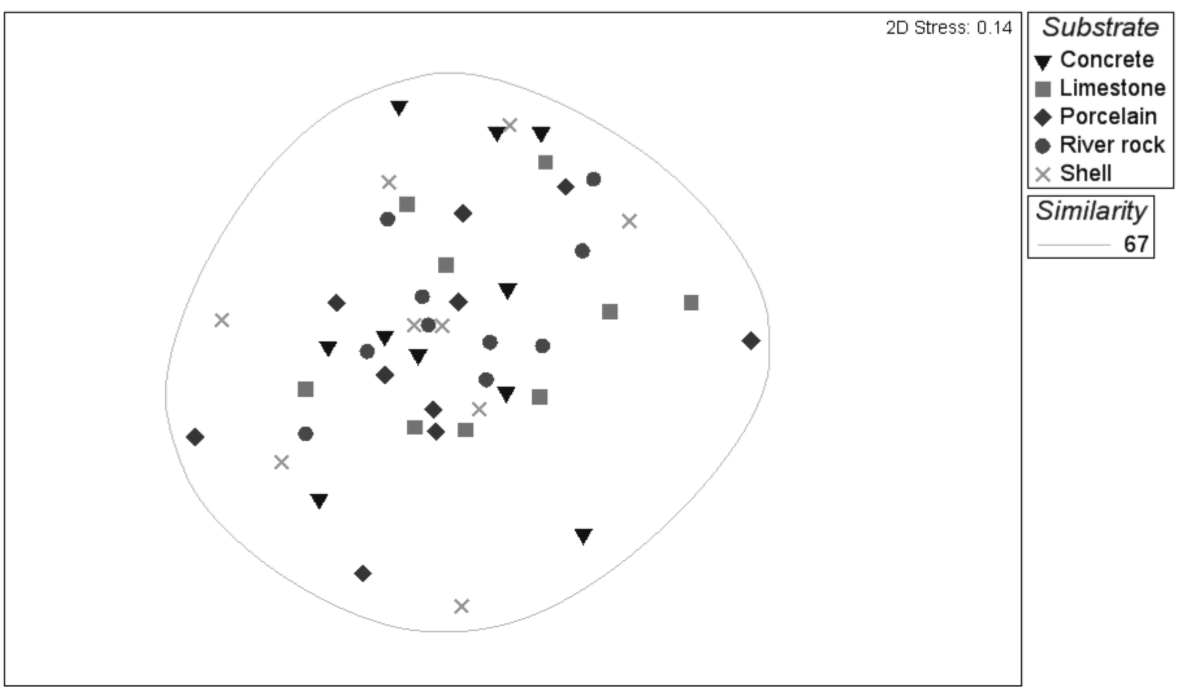


Figure 6. A) Multidimensional scaling plot of mean species-level community structure for each substrate type in St. Charles Bay, TX. **B)** Second-stage MDS plot, excluding bare sediment plots. Lines show similarity grouping result; 65, 67 refer to the percent similarity of samples within the cluster.

Reef-associated communities were also generally similar to one another from sites in upper and lower areas. The MDS analysis showed almost all communities clustering into one large group with at least 67% similarity between area areas (Fig. 7).

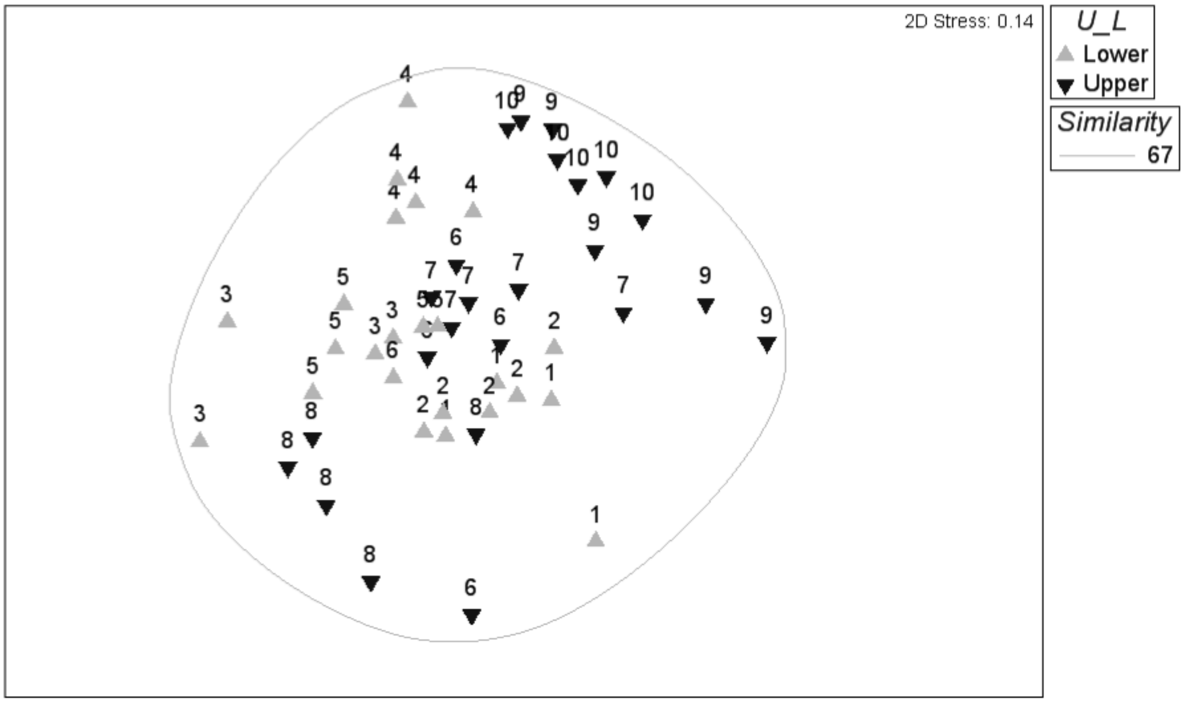


Figure 7. Multidimensional scaling plot of mean species-level community structure for lower and upper areas in St. Charles Bay, TX. Line shows similarity grouping; 67 refers to the percent similarity of the samples within the cluster; point labels refer to site numbers.

Analysis of Similarity results (excluding bare sediment) showed no significant differences in faunal communities between substrates (p = 0.111); however, there was a significant difference between faunal communities in sites 9 and 10 (p = 0.0340) when compared to all other sites. Porcellanid and Xanthid crabs were top contributors to each substrate, with naked gobies (*Gobiosoma bosc*) and snapping shrimp (*Alpheus heterochaelis*) switching back and forth between 3rd and 4th place in each grouping. Other frequently-occurring species were Palaemonetes shrimp, Gulf toadfish (*Opsanus beta*), stone crabs (*Menippe adina*) and skilletfish (*Gobiesox strumosis*). Blue crabs (*Callinectes sapidus*), spotfin mojarra (*Eucinostomus* *argenteus*) and sheepshead (*Archosargus probatocephalus*) were also found on some substrates, but not in large numbers.

Laboratory Experiments

Prey mortality in the laboratory experiments ranged from 0-100% (Fig. 8). There was a significant interaction between substrate and predator type ($p < 0.0001$), and therefore a simple main effects analysis was conducted. A new variable was created by converting treatments into a one-way ANOVA for each predator*substrate combination, to examine the effect of substrate type on mud crab mortality. The results from the one-way ANOVA were significant ($p = <0.001$). A Tukey's HSD test indicated that prey mortality due to blue crab predation was significantly higher on bare bottom or sand substrates than other substrates. Prey mortality due to pinfish predation on bare bottom and sand substrates were the next highest, however, pinfish predation on sand substrate was not significantly different than the shell, river rock, concrete, limestone and porcelain treatments. A power analysis showed that adequate sample sizes were used in these experiments.

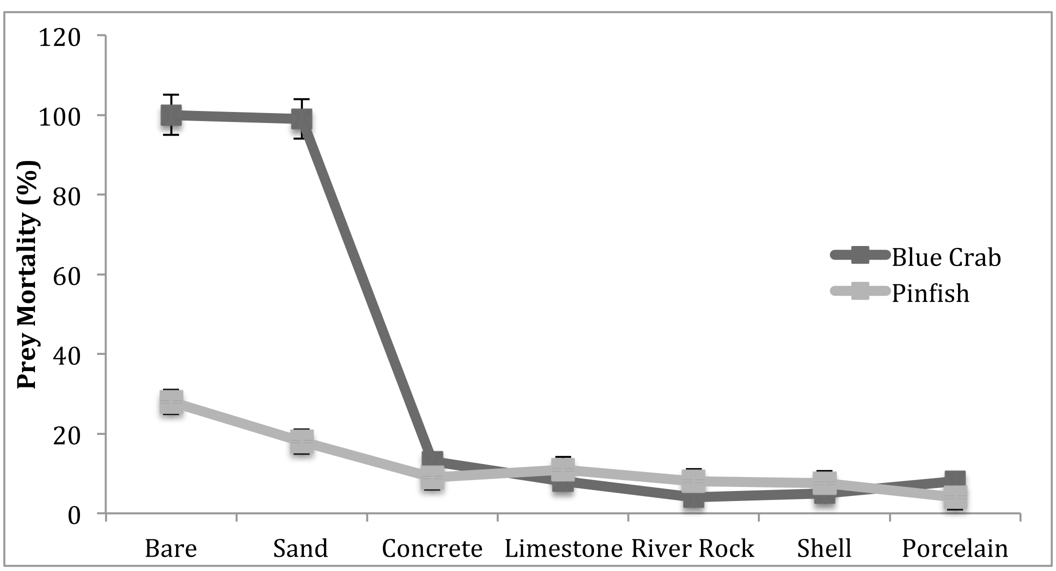


Figure 8. Mean (±SE) percent prey mortality from blue crab or pinfish predators across each substrate type from tank experiments conducted at the Texas A&M AgriLife Mariculture Research Laboratory in Port Aransas, TX.

DISCUSSION

Our results suggest that all of the alternative substrates examined are suitable for oyster spat recruitment and growth with all substrates containing spat that had grown to near juvenile size (>25 mm). Previous studies have found similar success using concrete and limestone as alternatives to shell for spat recruitment (LDWF 2004, Burton et al. 2005, Brumbaugh 2000, Coen et al. 2008). The Texas Parks and Wildlife Department has incorporated river rock and limestone into recent restoration efforts with recruitment success comparable to restored reefs using only oyster shell (TPWD 2011).

In our study, spat were observed to attach to the outer surfaces of the substrates as well as within the crevices of the physical structure of each sampling tray. Porcelain tended to have slightly lower spat recruitment when compared to the other substrates. Because porcelain substrates were obtained from the municipal waste stream (i.e. post-consumer use toilets, tubs, sinks), some surfaces were enameled and observations of oyster spat scarring on these surfaces suggest they were not as suitable for permanent attachment by oyster spat. Using porcelain substrates with a greater proportion of rougher surfaces may have increased spat settlement rates. The crushed concrete contained a mixture of materials (e.g. gravel, concrete, fibers). This provided a variety of different types of surfaces on which spat could settle, and may partially explain the somewhat higher spat densities observed. Because the oyster shell substrates comprised loose shells rather than vertically complex reef habitat, they tended to form a flat-layer within the trays. This diminished 3-dimensional structure may not have provided as suitable a recruitment space for spat (Coen et al. 2008, Manley et al. 2010), and may have contributed to the low recruitment densities, along with the high salinities we found. All of the substrates used in this study are suitable alternatives to oyster shell. Concrete and river rock even outperformed shell with higher spat densities and spat shell heights. While porcelain did not perform as well, it was still able to recruit spat and allow some to grow to juvenile size. If no other substrates are available, porcelain can still be used as a restoration aide.

Species densities of reef-associated fauna were surprisingly similar across substrate types, regardless of potential differences in habitat complexity due to differences in shapes of material (e.g. flat shells versus more rounded river rock). A study by Rodney and Paynter (2006) comparing macrofaunal assemblages on natural and restored reefs in Chesapeake Bay showed that restored reefs had an order of magnitude higher macrofauna density than natural reefs. The restored reef in their study consisted of loose oyster shell seeded by juvenile oysters (Rodney & Paynter 2006). Other studies have reported that restored reefs using seeded oysters on concrete structures display faunal communities similar to adjacent natural reefs after 1-5 years (Quan et al. 2009, Manley et al. 2010, Quan et al. 2012). Our results suggest that all substrates examined would be viable alternatives to shell for the purpose of supporting faunal communities.

Species diversity (H') was not significantly different across all substrates (mean values of 1-2) except for the bare sediment, which had a mean of 0.05. Excluding bare substrate samples, reef associated faunal community structure was generally similar across substrate types. The primary contributing species were consistent across substrates, including: mud crabs (Xanthidae), porcelain crabs (Porcellanidae), Gulf toadfish (*Opsanus beta*), naked gobies (*Gobiosoma bosc*) and grass shrimp (*Palaemonetes spp.*). These oyster reef residents are typical species found in this area (Gain 2009). Our results are similar to the findings that Kingsley-Smith et al. (2012) obtained on their study of natural reefs in South Carolina. Their fauna diversity (H') data matched our values with mean diversities ranging from 1-2 (Kingsley-Smith et al. 2012). All of the substrates used in this study displayed similar habitat function to oyster shell for reef-associated fauna.

Oyster reefs support a wide variety of molluscs, crustaceans, fish and polychaetes (Wells 1961, Grabowski et al. 2005, Boudreaux et al. 2006). The faunal communities observed in association with the various substrate types in this study are similar to those found on natural reefs with gobies (*Gobiosoma spp.*), blennies (*Hypsoblennius spp*.), Xanthid crabs and stone crabs (*Menippe adina*) recurring in many similar studies (Bahr & Lanier 1981, Newell 1988, Coen et al. 1999, Breitburg 1999, Tolley & Volety 2005, Kingsley-Smith et al. 2012). The observation of crab densities far exceeding other taxa, regardless of substrate type, supports results from previous studies on natural reefs (Zimmerman et al. 1989, Micheli & Peterson 1999, Minello 1999, Meyer & Townsend 2005, Tolley & Volety 2005). Gobies, Gulf toadfish and skilletfish, the most abundant fishes found associated with the substrates, have been shown to use shell crevices as spawning and foraging areas. The interstitial spaces formed by the substrates (Grabowski 2004, Grawbowski & Kimbro 2005, Gain 2009). This suggests that these alternative substrates may provide enhanced reproductive value for these species as well given the many interstitial spaces our substrates provide for shelter during spawning.

The results from the lab experiments indicate that increased habitat complexity yields lower prey mortality. This is quite intuitive given that greater species abundances are typically found in structurally complex habitats that allow refuge and concealment from predators (Laegdsgaard & Johnson 2001, Grabowski & Powers 2004). The lack of significant difference in prey mortality due to pinfish predation between sand substrates and the structurally complex substrates was unexpected. However, observations showed pinfish foraging behaviors were modified in the sand treatments, as they would bury themselves beneath the sand, perhaps as their own avoidance behavior. These fish species are commonly found on oyster reefs in this area and may utilize the 3-dimensional structures for refuge themselves due to their small size (Gain 2009). Studies on habitat complexity have found that predators have restricted mobility as complexity increases; furthermore, juvenile fish mobility may also be inhibited by highly complex habitats through the lack of visual detection of predators (Adams et al. 2004, Horinouchi 2007). Our results mirrored these findings in that the more structurally complex the habitat, the less predation by either predator was allowed to occur. Therefore, the reduced prey mortality rates in these experiments may have been due to the available complex habitat. Similar prey mortalities across substrate types and predators suggest that all of the materials examined (exclusing bare or sand bottom) provide viable prey refuge and suitable habitat for reef resident species.

Site selection is another important consideration when restoring oyster reef habitats, particularly as it relates to availability of larval oysters to colonize new substrates (Beseres Pollack et al. 2012). In this study, spat recruitment varied strongly between upper and lower areas with upper areas having higher recruitment. A variety of factors could contribute to this significant result including location of larval source reefs, water circulation patterns, and hydrological fluctuation. Similarly, in a South Carolina estuary, Kingsley-Smith et al. (2012) found little difference in spat recruitment between natural and enhanced (concrete blocks) reef treatments but significant differences between sites. Future restoration strongly depends on the choice of site, as well as, substrate used. Spat recruitment differences observed in the current study will help inform future oyster restoration projects in St. Charles Bay.

Spatial scale is also important to consider. Eggleston et al. (1998) examined differences in species density and diversity between small and large patches of oyster reef, and found that larger reefs supported higher numbers of faunal species (Eggleston et al. 1998). Although our study has shown that there are no difference in faunal densities between substrate types, larger scale, longer term studies should be conducted for more accurate pictures of each substrate's success as viable habitat for oysters and reef-associated fauna. Because each substrate has a different source, some of which were new and others of which were reclaimed or diverted from the waste stream, research should also focus on assessing environmental impacts associated with all the stages of a substrate's life. Studies pertaining to shell life cycle analysis found that shell spat accumulation and growth is important in stabilizing reefs (Powell & Klinck 2007, Waldbusser et al. 2013). Studies have yet to be done on the other substrates used in this study. In addition, each substrate also has a different chemical composition and information is also needed on the long-term sustainability of each material in estuarine and marine environments. One particular study by Miller et al. (2009) showed that oyster shell area decreased 16% and shell calcium content was reduced by 42% when exposed to high CO_2 levels. (Caldeira & Wickett, Orr et al. 2005, Miller 2009)

The main goals of this study were to determine if four different substrates were viable reef restoration alternatives to shell in their ability to recruit oyster spat, allow spat growth to juvenile size and provide refuge for reef-associated macroinvertebrates and fish. An understanding of the relative habitat value of alternative substrates for oyster reef restoration is important for sustainable management of this important coastal natural resource. Our findings suggest that concrete, limestone, porcelain and river rock are suitable alternatives to oyster shell in their ability to recruit spat and allow them to mature to juvenile size. Furthermore, all substrates used in this study are suitable alternatives to shell in providing valuable habitat for macrofauna. This study provides important information for future restoration planning, supporting the use of more readily available, less expensive, and/or environmentally friendly, recycled substrates.

REFERENCES CITED

Adams AJ, Locascio JV, Robbins BD (2004) Microhabitat use by a post-settlement stage estuarine fish: evidence from relative abundance and predation among habitats. *J Exp Mar Biol Ecol* 299:17-33.

Andrews JD, Ray SM (1988) Management strategies to control the disease caused by *Perkinsus marinus. American Fisheries Society Special Publication* 18: 257-264.

Asquith WH, Mosier JG , Bush PW (1997) *Corpus Christi Bay National Estuary Program*. 17: 1-38.

Bahr LM and Lanier WP(1981) The ecology of intertidal oyster reefs of the South Atlantic coast: a community profile. *U.S. Fish and Wildlife Service Program* FWS/OBS/-81/15. 105 pp.

Beseres Pollack J, Cleveland A, Palmer TA, Reisinger AS, Montagna PA (2012) A Restoration Suitability Index Model for the Eastern Oyster (*Crassostrea virginica*) in the Mission-Aransas Estuary, TX, USA. PLoS ONE 7(7): e40839.

Beseres Pollack J, Kim H-C, Morgan EK, Montagna PA(2011) Role of flood disturbance in natural oyster (*Crassostrea virginica*) population maintenance in an estuary in south Texas, USA. *Estuaries and Coasts* 34(1): 187-197.

Bittler K (2011) Salinity gradients in the Mission-Aransas National Estuarine Research Reserve. *GIS and Water Resources* pp. 1-19.

Breitburg D.C (1999). Are three-dimensional structure and healthy oyster populations the keys to an ecologically interesting and important fish community? In: M. W. Luckenbach, R. Mann & J. A. Wesson, editors. Oyster reef habitat restoration: a synopsis and synthesis of approaches. Gloucester Point, VA: *Virginia Institute of Marine Science Press*. pp. 239–250.

Brumbaugh RD (2000) An Analysis of Limestone Marl As A Settlement Substrate For Oysters In Chesapeake Bay. *Virginia Marine Resources Commission Report* pp. 1-15.

Brumbaugh RD, Cohen LD (2009) Contemporary approaches for small-scale oyster reef restoration to address substrate *versus* recruitment limitation: A review and comments relevant for the Olympia oyster, *Ostrea lurida* (Carpenter 1864). *Journal of Shellfish Research* 28(1): 147-161.

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. *Nature* 425: 365

Campbell, M., S.G. Hall (2002) Oyster Break: Engineering biologically dominated artificial reef systems for erosion reduction. Master's Thesis. Louisiana State University, Baton Rouge, L.A.

Coen LD, Knott DM, Wenner EL, Hadley NH, Ringwood AH , Bobo MY (1999) South Carolina intertidal oyster reef studies in South Carolina: design, sampling and focus for evaluating habitat value and function. In: M. W. Luckenbach, R. Mann & J. A. Wesson, editors. Oyster reef habitat restoration: a synopsis and synthesis of approaches. Gloucester Point, VA: *Virginia Institute of Marine Science Press*. pp. 133–158.

Coen LD, Schulte K, Powers A, Taylor LM (2008) Evaluating Oyster Shell Alternatives For Enhancing/Restoring Shellfish Beds and Associated Impacts. Marine Laboratory, Sanibel-Captiva Conservation Foundation.

Dame R, Zingmark R, Stevenson H, Nelson D (1981) Filter feeder coupling between the estuarine water column and the benthic subsystem. *Estuarine Perspectives* pp. 521-525.

Dame R, Bushek D, Prins T (2001) The Role of Suspension Feeders As Ecosystem Transformers in Shallow Coastal Environments. *Ecol. Comp. of Sed. Shores* pp. 11-37.

Eggleston DB, Elis WE, Etherington LL, Dahlgren CP, Posey MH (1999) Organism responses to habitat fragmentation and diversity: Habitat colonization by estuarine macrofauna *Journal of Experimental Marine Biology and Ecology* 236:107–132.

French-McCay DP, Peterson CH, DeAlteris JT, Catena J (2003) Restoration that targets function as opposed to structure: replacing lost bivalve production and filtration. *Marine Ecology Progress Series* 264: 197-212.

Gain I. (2009) Oyster reef as nekton habitat in estuarine ecosystems. Master's Degree Thesis. Texas A&M University – Corpus Christi.

Glausiusz J. (2010) Artificial Reefs to Buffer New York. *Nature* 464: 15.

Grabowski JH, Powers SP(2004) Habitat complexity mitigates trophic transfer on oyster reefs. *Mar. Ecol. Prog. Ser.* 277: 291-295.

Grabowski J H, Peterson CH(2007) Restoring oyster reefs to recover ecosystem services. *Socio-Eco. Iss. and Mgmt. Sols.* 4: 281-298.

Harding JM, Mann R (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. *Bull. Mar. Sci.* 66(1): 29-45.

Haven D and Morales-Alamo R (1966) Aspects of biodeposition in oysters and other invertebrate filer feeders. *Limnol. Oceanogr.* 11: 487-498.

Haven D and Morales-Alamo R (1970) Filtration of particles from suspension by the American oyster *Crassostrea virginica*. *Biol. Bull.* 139: 121-130.

Haven D and Morales-Alamo R (1972) Biodeposition as a factor in sedimentation of fine suspended solids in estuaries. *Geol. Soc. Mem.* 133:121-130.

Hewes W (1998) Senate Bill 2679. Ports and Marine Resources. Mississippi Legislature <http://billstatus.ls.state.ms.us/documents/1998/SB/2600-2699/SB2679IN.htm>.

Horinouchi M (2007) Review of the effects of within-patch scale structural complexity on seagrass fishes. *J Exp Mar Biol Ecol* 350:111-129.

Jackson JBC (2008) Ecological extinction and evolution in the brave new ocean. *Proc Natl Acad Sci USA* v.105 (Supplement_1): 11458–11465.

Kingsley-Smith PR, Joyce RE, Arnott SA, Roumillat WA, McDonough CJ, Reichert MJM(2012) *Journal of Shellfish Research* 31(4):1009-1021. 2012.

Leatherwood A. "St. Charles Bay," *Handbook of Texas Online*. Published by the Texas State Historical Association. <http://www.tshaonline.org/handbook/online/articles/rrs08> accessed April 16, 2012.

Laegdsgaard P, Johnson C (2001) Why do juvenile fishes utilize mangrove habitats? *J Exp Mar Biol Ecol* 257:229-253.

Lipcius RN, Eggleston DB, Schreiber SJ, Seitz RD, Shen J, Sisson M, Stockhausen WT, Wang HV (2008): Importance of Metapopulation Connectivity to Restocking and Restoration of Marine Species, *Reviews in Fisheries Science*, 16:1-3, 101-110.

Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC(2006) Depletion, Degradation, and Recovery Potential of Estuaries and Coastal Seas. *Science.* Vol. 312 (5781): 1806-1809.

Louisiana Department of Wildlife and Fisheries (2004) Final report for Louisiana's Oyster Shell Recovery Pilot Project. *Socioeconomics Research and Development Section and Marine Fisheries Division* NOAA Award No. NA96FK0188.

Louisiana Summary (2010) Agricultural and Natural Resources, Louisiana State University Agricultural Center.

Lutz. CG, Sambidi P, Harrison RW(2011) Oyster Profile. (AgMRC) Agricultural Marketing

Resource Center, Iowa State University. <http://www.agmrc.org/commodities__products/aquaculture/oyster_profile.cfm>.

Mackin JG, Owen HM, Collier A (1950) Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). *Science* 111: 328-329.

Manley J, Power A, Walker R, Hurley D, Belcher C, Richardson J (2010) Ecological Succession on Restored Intertidal Oyster Habitat in the Tidal Creeks of Coastal Georgia. *Journal of Shellfish Research*, 29(4): 917-926.

McAlister WH (1995) A Naturalists' Guide : Aransas. University of Texas Press, Austin, Texas. <http://www.tpwd.state.tx.us/landwater/water/conservation/txgems/anwr/index.phtml>.

Miller AW, Reynolds AC, Sobrino C, Riedel GF (2009) Shellfish Face Uncertain Future in High CO2 World: Influence of Acidification on Oyster Larvae Calcification and Growth in Estuaries. *PLoS ONE* 4(5): e5661.

Newell RIE (1988) Ecological changes in Chesapeake Bay: are they the result of over-harvesting the American oyster, *Crassostrea virginica*? In: M. P. Lynch & E. C. Chrome, editors. Understanding the estuary: advances in Chesapeake Bay research. Proceedings of a Conference. *Chesapeake Bay Research Consortium* publication 129. Baltimore,MD: Chesapeake Bay Research Consortium. pp. 536–546.

North Carolina Division of Marine Fisheries (2012) Oyster Shell Recycling. North Carolina Department of Natural Resources < http://portal.ncdenr.org/web/mf/oyster-shell-recycling-program>.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437(7059): 681–686.

Paerl HW, Pinckney JL, Fear JM, Peieris BL (1998) Ecosystem responses to internal and watershed organic matter loading: Consequences for hypoxia in the eutrophyling Neuse River Estuary, North Carolina, USA. *Marine Eco. Progress Series* 166:17-25.

Piazza BP, Banks PD, La Peyre MK (2005) The potential for created oyster reefs as a sustainable shoreline protection strategy in Louisiana. *Restoration Ecology* 13(3): 499-506.

Piehler MF, Smyth AR (2011) Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services. *Ecosphere* 2(12).

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

Powell EN, Klinck JM(2007) Is oyster shell a sustainable estuarine resource? *Journal of Shellfish Research* 26(1): 181-194.

Quan M, Zhu J, Ni Y, Shi L, Chen Y (2009) Faunal utilization of constructed intertidal oyster (*Crassostrea rivularis*) reef in the Yangtze River estuary, China *Ecological Engineering* 35: 1466–1475.

Quan W, Humphries AT, Shen X, Chen Y (2012) Oyster and Associated Benthic Macrofaunal Development on a Created Intertidal Oyster (Crassostrea Ariakensis) Reef in the Yangtze River Estuary, China. Journal of Shellfish Research, 31(3): 599-610.

Ray SM (1954) Biological studies of *Dermocystidium marinum*. *Rice Institute Pamphlet 41 (Special Issue)*, The Rice Institute, Houston, Texas.

Ray SM (1996) Historical perspective on *Perkinsus marinus* disease of oysters in the Gulf of Mexico Journal of Shellfish Research 15(10): 9-11.

Ray SM (2008) Historical perspective on Perkinsus marinus disease of oysters in the Gulf of

Mexico. *Journal of Shellfish Research* 24(5): 5-14.

Rodney WS, Paynter KT(2006) Comparisons of macrofaunal assemblages on restored and non-restored oyster reefs in mesohaline regions of Chesapeake Bay Maryland. *Journal of Experimental Marine Biology and Ecology* 355: 29-51.

Scyphers SB, Powers SP, Heck KL Jr, Byron D (2011) Oyster Reefs as Natural Breakwaters Mitigate Shoreline Loss and Facilitate Fisheries. PLoS ONE 6(8): e22396.

Skates C, Shook PH (1999) *Fly fishing the Texas Coast*. Pruett Publishing Company.

Texas Parks and Wildlife (2011) Galveston Bay Oyster Restoration Expanding. <http://www.tpwd.state.tx.us/newsmedia/releases/?req=20110815a>.

Tolley SG, Volety AK (2005) The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. *J Shellfish Res* 24(4): 1007-1012.

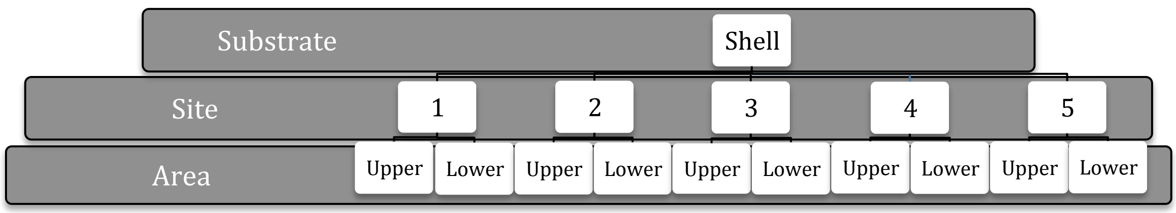
Ulanowicz RE, Tuttle J (1992) The trophic consequences of oyster stock rehabilitation in Chesapeake Bay.*Estuaries* 15:298-306.

Waldbusser GG, Powell EN, Mann R (2013) Ecosystem effects of shell aggregations and cycling in coastal waters: an example of Chesapeake Bay oyster reefs *Ecology* 94(4): 895–903.

Wilberg MJ, Wiedenmann JR, Robinson JM (2013) Sustainable exploitation and management of autogenic ecosystem engineers: application to oysters in Chesapeake Bay. *Ecological Applications*, 23(4), 2013, pp. 766–776.

Zu Ermgassen PS, Spalding MD, Blake B, Coen LD, Dumbauld B, Geiger S, Grabowski JH, Grizzle R, Luckenbach M, McGraw K, Rodney W, Ruesink JL, Powers SP, Brumbaugh R (2012) Historical ecology with real numbers: past and present extent and biomass of an imperilled estuarine habitat *Proc. R. Soc. B* doi:10.1098/rspb.2012.031.

APPENDIX A: Experimental Design



APPENDIX B. ANOVA and Tukey's HSD *post-hoc* Tables

Table 1. Two-way blocked partially hierarchical ANOVA on spat recruitment

	Source	DF	Type III SS	Mean Square	F Value	Pr > F
	Area	1	9.719860	9.719860	6.61	<.0001
*	Substrate	5	87.530490	17.506098	61.46	0.0445
	Site(Area)	8	11.692016	1.461502	5.13	0.0002
	Error: MS(Error)	38	10.823445	0.284828		
	Error	7.97	11.732373	1.471260		
	Error: 1.0083*MS(site(area)) - 0.0083*MS(Error)					

Table 2. Tukey's HSD *post-hoc* analysis on spat recruitment per substrate

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	substrate
A	5.1454	10	Concrete
A			
A	4.9939	9	Shell
A			
A	4.9529	10	Limestone
A			
A	4.9457	10	River rock
A			
A	4.4468	10	Porcelain
B	0.0000	4	Bare

Table 3. Two-way blocked partially hierarchical ANOVA on spat height

	Source	DF	Type III SS	Mean Square	F Value	Pr > F
	Area	1	0.112426	0.112426	0.52	0.4907
*	Substrate	5	16.113822	3.222764	58.07	<.0001
	Site(Area)	8	1.731646	0.216456	3.90	0.0002
	Error: MS(Error)	455	25.249605	0.055494		

Error	8.02	1.729526	0.215598

Error: 0.9947*MS(Site(Area)) + 0.0053*MS(Error)

Table 4. Tukey's HSD *post-hoc* analysis on spat height per substrate

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Substrate
A	1.17673	98	Concrete
A			
A	1.16795	95	Porcelain
A			
A	1.11293	85	River rock
A			
A	1.09663	93	Limestone
A			
A	1.07201	87	Shell
B	0.00000	12	Bare

Table 5. Two-way blocked partially hierarchical ANOVA on faunal density

	Source	DF	Type III SS	Mean Square	F Value	Pr > F
	Area	1	5.728121	5.728121	4.74	0.0613
*	Substrate	5	45.806219	9.161244	22.80	<.0001
	Site(Area)	8	9.609121	1.201140	2.99	0.0106
	Error: MS(Error)	38	15.271755	0.401888		

Error	7.956	9.609025	1.207768

Error: 1.0083*MS(Site(Area)) - 0.0083*MS(Error)

Table 6. Tukey's HSD *post-hoc* analysis on faunal density per area placement

Means with the same letter
are not significantly different.

Tukey Grouping	Mean	N	Area
A	415.50	26	Lower
B	195.04	27	Upper

Table 7. Two-way blocked partially hierarchical ANOVA on faunal diversity

	Source	DF	Type III SS	Mean Square	F Value	Pr > F
	Area	1	0.000184	0.000184	0.00	0.9631
*	Substrate	5	7.792707	1.558541	29.04	<.0001
	Site(Area)	8	0.646334	0.080792	1.51	0.1879
	Error: MS(Error)	38	2.039523	0.053672		

Error	7.91	0.641066	0.081017

Error: 1.0083*MS(Site(Area)) - 0.0083*MS(Error)

Table 8. Tukey's HSD *post-hoc* analysis on faunal diversity per substrate

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Substrate
A	2.0747	10	concrete
A			
A	2.0389	9	shell
A			
A	1.9993	10	porcelain
A			
A	1.8892	10	river rock
A			
A	1.7304	10	limestone
B	0.4211	4	bare

Table 9. Two-way AVOVA on prey mortality with regards to predator and substrate.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
predator	1	18021.4286	18021.4286	134.51	<.0001
substrate	6	100375.0000	16729.1667	124.86	<.0001
predator*substrate	6	53920.2381	8986.7063	67.07	<.0001

Table 10. One-way ANOVA on prey mortality with regards to predator and substrate.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Predator_Substrate	13	172316.6667	13255.1282	98.93	<.0001

Table 11. Tukey's HSD *post-hoc* analysis on simple main effects model for prey mortality.

Means with the same letter are not significantly different.				
Tukey Grouping		Mean	N	Predator_Substrate
	A	100.000	12	Blue crab_Bare
	A	99.167	12	Blue crab_Sand
	B	27.500	12	Pinfish_Bare
C	B	17.500	12	Pinfish_Sand
C		10.833	12	Pinfish_Limestone
C		9.167	12	Pinfish_Concrete
C		8.333	12	Blue crab_Limestone
C		8.333	12	Blue_crab_Porcelain
C		8.333	12	Pinfish_River rock
C		7.500	12	Pinfish_Shell
C		5.000	12	Blue crab_Shell
C		5.000	12	Blue crab_Concrete
C		4.167	12	Pinfish_Porcelain
C		4.167	12	Blue crab_River rock

APPENDIX C: ANOSIM Output

ANOSIM
Analysis of Similarities

Two-Way Crossed Analysis

Resemblance worksheet
Name: Resem1
Data type: Similarity
Selection: All

Parameters
Rank correlation method: Spearman

Factor Groups

Sample	Site	Substrate
Concrete1	1	Concrete
Limestone1	1	Limestone
Porcelain1	1	Porcelain
River rock1	1	River rock
Concrete4	4	Concrete
Limestone4	4	Limestone
Porcelain4	4	Porcelain
River rock4	4	River rock
Shell4	4	Shell
Concrete7	7	Concrete
Limestone7	7	Limestone
Porcelain7	7	Porcelain
River rock7	7	River rock
Shell7	7	Shell
Concrete10	10	Concrete
Limestone10	10	Limestone
Porcelain10	10	Porcelain
River rock10	10	River rock
Shell10	10	Shell
Concrete2	2	Concrete
Limestone2	2	Limestone
Porcelain2	2	Porcelain
River rock2	2	River rock
Shell2	2	Shell
Concrete3	3	Concrete
Limestone3	3	Limestone
Porcelain3	3	Porcelain
River rock3	3	River rock
Shell3	3	Shell
Concrete5	5	Concrete
Limestone5	5	Limestone
Porcelain5	5	Porcelain
River rock5	5	River rock
Shell5	5	Shell
Concrete6	6	Concrete
Limestone6	6	Limestone
Porcelain6	6	Porcelain
River rock6	6	River rock
Shell6	6	Shell

```
Concrete8     8    Concrete
Limestone8    8    Limestone
Porcelain8    8    Porcelain
River rock8   8    River rock
Shell8        8    Shell
Concrete9     9    Concrete
Limestone9    9    Limestone
Porcelain9    9    Porcelain
River rock9   9    River rock
Shell9        9    Shell
```

TESTS FOR DIFFERENCES BETWEEN Site GROUPS
(across all Substrate groups)
Global Test
Sample statistic (Rho): 0.109
Significance level of sample statistic: 3.4%
Number of permutations: 999 (Random sample)
Number of permuted statistics greater than or equal to Rho: 110

TESTS FOR DIFFERENCES BETWEEN Substrate GROUPS
(across all Site groups)
Global Test
Sample statistic (Rho): 0.159
Significance level of sample statistic: 11.1%
Number of permutations: 999 (Random sample)
Number of permuted statistics greater than or equal to Rho: 18

Outputs
Plot: Graph14
Plot: Graph15

APPENDIX D: Raw Data

Substrate Fouling (m^2)

Sample Date	site	substrate	spat (m^2)	barnacles (m^2)	mussels (m^2)	slipper shells (m^2)
9-Sep-12	1	Concrete	4484.4	765.9	299.7	22.2
9-Sep-12	1	Limestone	1676.1	66.6	344.1	510.6
9-Sep-12	1	Porcelain	1420.8	33.3	510.6	566.1
9-Sep-12	1	River rock	1753.8	33.3	122.1	66.6
9-Sep-12	2	Concrete	2120.1	44.4	410.7	155.4
9-Sep-12	2	Limestone	1776	44.4	288.6	0
9-Sep-12	2	Porcelain	888	44.4	410.7	210.9
9-Sep-12	2	River rock	943.5	0	499.5	277.5
9-Sep-12	2	Shell	1354.2	210.9	321.9	22.2
9-Sep-12	3	Concrete	333	55.5	166.5	0
9-Sep-12	3	Limestone	377.4	33.3	555	199.8
9-Sep-12	3	Porcelain	88.8	11.1	188.7	22.2
9-Sep-12	3	River rock	710.4	11.1	543.9	255.3
9-Sep-12	3	Shell	410.7	333	255.3	22.2
9-Sep-12	4	Concrete	2564.1	55.5	710.4	44.4
9-Sep-12	4	Limestone	532.8	22.2	954.6	355.2
9-Sep-12	4	Porcelain	455.1	55.5	299.7	33.3
9-Sep-12	4	River rock	277.5	55.5	55.5	77.7
9-Sep-12	4	Shell	1154.4	688.2	943.5	233.1
9-Sep-12	5	Concrete	1554	55.5	188.7	199.8
9-Sep-12	5	Limestone	777	55.5	210.9	133.2
9-Sep-12	5	Porcelain	721.5	44.4	355.2	210.9
9-Sep-12	5	River rock	1509.6	44.4	444	377.4
9-Sep-12	5	Shell	1764.9	66.6	310.8	99.9
8-Sep-12	6	Concrete	4872.9	777	266.4	233.1
8-Sep-12	6	Limestone	2453.1	133.2	355.2	122.1
8-Sep-12	6	Porcelain	2841.6	510.6	133.2	166.5
8-Sep-12	6	River rock	4573.2	233.1	410.7	355.2
8-Sep-12	6	Shell	2664	1087.8	477.3	133.2
8-Sep-12	7	Concrete	2020.2	532.8	521.7	66.6
8-Sep-12	7	Limestone	3907.2	488.4	555	288.6
8-Sep-12	7	Porcelain	2852.7	1143.3	155.4	210.9
8-Sep-12	7	River rock	2974.8	321.9	321.9	177.6
8-Sep-12	7	Shell	3796.2	1254.3	1653.9	210.9
8-Sep-12	8	Concrete	1454.1	954.6	421.8	88.8
8-Sep-12	8	Limestone	1809.3	188.7	399.6	310.8

8-Sep-12	8	Porcelain	1087.8	532.8	144.3	210.9
8-Sep-12	8	River rock	1598.4	111	555	222
8-Sep-12	8	Shell	1531.8	455.1	344.1	155.4
8-Sep-12	9	Concrete	3307.8	754.8	321.9	22.2
8-Sep-12	9	Limestone	4173.6	377.4	222	244.2
8-Sep-12	9	Porcelain	2697.3	321.9	0	33.3
8-Sep-12	9	River Rock	1609.5	266.4	466.2	310.8
8-Sep-12	9	Shell	1087.8	954.6	66.6	33.3
9-Sep-12	10	Concrete	1054.5	77.7	177.6	88.8
9-Sep-12	10	Limestone	2730.6	1098.9	122.1	11.1
9-Sep-12	10	Porcelain	666	33.3	199.8	177.6
8-Sep-12	10	River rock	4961.7	577.2	122.1	66.6
9-Sep-12	10	Shell	4428.9	3019.2	732.6	155.4

Spat Height (mm)

Sample Date	site	substrate	spat lengths
9-Sep-12	1	Concrete	29.0
9-Sep-12	1	Concrete	22.7
9-Sep-12	1	Concrete	8.7
9-Sep-12	1	Concrete	19.5
9-Sep-12	1	Concrete	12.7
9-Sep-12	1	Concrete	30.4
9-Sep-12	1	Concrete	12.4
9-Sep-12	1	Concrete	8.7
9-Sep-12	1	Concrete	10.2
9-Sep-12	1	Concrete	24.5
9-Sep-12	2	Concrete	24.8
9-Sep-12	2	Concrete	17.7
9-Sep-12	2	Concrete	6.6
9-Sep-12	2	Concrete	16.4
9-Sep-12	2	Concrete	31.7
9-Sep-12	2	Concrete	3.5
9-Sep-12	2	Concrete	22.0
9-Sep-12	2	Concrete	16.6
9-Sep-12	2	Concrete	6.0
9-Sep-12	2	Concrete	18.8
9-Sep-12	3	Concrete	6.5
9-Sep-12	3	Concrete	15.6
9-Sep-12	3	Concrete	7.0
9-Sep-12	3	Concrete	17.9
9-Sep-12	3	Concrete	4.9

9-Sep-12	3	Concrete	12.7
9-Sep-12	3	Concrete	9.5
9-Sep-12	3	Concrete	13.5
9-Sep-12	3	Concrete	9.6
9-Sep-12	3	Concrete	24.6
9-Sep-12	4	Concrete	28.0
9-Sep-12	4	Concrete	11.9
9-Sep-12	4	Concrete	8.4
9-Sep-12	4	Concrete	9.4
9-Sep-12	4	Concrete	5.8
9-Sep-12	4	Concrete	8.1
9-Sep-12	4	Concrete	12.9
9-Sep-12	4	Concrete	25.2
9-Sep-12	4	Concrete	28.6
9-Sep-12	4	Concrete	10.1
9-Sep-12	5	Concrete	19.9
9-Sep-12	5	Concrete	30.2
9-Sep-12	5	Concrete	22.2
9-Sep-12	5	Concrete	12.6
9-Sep-12	5	Concrete	20.0
9-Sep-12	5	Concrete	27.7
9-Sep-12	5	Concrete	14.7
9-Sep-12	5	Concrete	23.3
9-Sep-12	5	Concrete	19.1
9-Sep-12	5	Concrete	19.2
8-Sep-12	6	Concrete	17.88
8-Sep-12	6	Concrete	16.41
8-Sep-12	6	Concrete	12.18
8-Sep-12	6	Concrete	14.84
8-Sep-12	6	Concrete	20.89
8-Sep-12	6	Concrete	12.45
8-Sep-12	6	Concrete	8.02
8-Sep-12	6	Concrete	20.67
8-Sep-12	6	Concrete	21.29
8-Sep-12	6	Concrete	5.2
8-Sep-12	7	Concrete	10.5
8-Sep-12	7	Concrete	18.8
8-Sep-12	7	Concrete	9.6
8-Sep-12	7	Concrete	10.2
8-Sep-12	7	Concrete	13.9
8-Sep-12	7	Concrete	14.6
8-Sep-12	7	Concrete	5.0
8-Sep-12	7	Concrete	15.1

8-Sep-12	7	Concrete	5.5
8-Sep-12	7	Concrete	21.3
9-Sep-12	8	Concrete	4.5
9-Sep-12	8	Concrete	4.0
9-Sep-12	8	Concrete	8.4
9-Sep-12	8	Concrete	18.9
9-Sep-12	8	Concrete	4.7
9-Sep-12	8	Concrete	9.8
9-Sep-12	8	Concrete	3.9
9-Sep-12	8	Concrete	3.4
9-Sep-12	8	Concrete	4.5
9-Sep-12	8	Concrete	18.1
9-Sep-12	9	Concrete	28.8
9-Sep-12	9	Concrete	20.2
9-Sep-12	9	Concrete	16.7
9-Sep-12	9	Concrete	15.1
9-Sep-12	9	Concrete	32.6
9-Sep-12	9	Concrete	27.7
9-Sep-12	9	Concrete	10.1
9-Sep-12	9	Concrete	15.0
9-Sep-12	9	Concrete	17.5
9-Sep-12	9	Concrete	23.0
9-Sep-12	10	Concrete	29.0
9-Sep-12	10	Concrete	8.9
9-Sep-12	10	Concrete	17.9
9-Sep-12	10	Concrete	23.9
9-Sep-12	10	Concrete	20.3
9-Sep-12	10	Concrete	19.1
9-Sep-12	10	Concrete	22.0
9-Sep-12	10	Concrete	25.8
9-Sep-12	10	Concrete	27.2
9-Sep-12	10	Concrete	18.8
9-Sep-12	1	Limestone	23.0
9-Sep-12	1	Limestone	8.0
9-Sep-12	1	Limestone	5.1
9-Sep-12	1	Limestone	4.2
9-Sep-12	1	Limestone	3.7
9-Sep-12	1	Limestone	4.9
9-Sep-12	1	Limestone	17.3
9-Sep-12	1	Limestone	8.4
9-Sep-12	1	Limestone	15.2
9-Sep-12	1	Limestone	17.9
9-Sep-12	2	Limestone	3.6

9-Sep-12	2	Limestone	10.1
9-Sep-12	2	Limestone	8.9
9-Sep-12	2	Limestone	6.8
9-Sep-12	2	Limestone	16.8
9-Sep-12	2	Limestone	5.9
9-Sep-12	2	Limestone	1.8
9-Sep-12	2	Limestone	6.6
9-Sep-12	2	Limestone	8.7
9-Sep-12	2	Limestone	3.9
9-Sep-12	3	Limestone	20.6
9-Sep-12	3	Limestone	22.6
9-Sep-12	3	Limestone	25.7
9-Sep-12	3	Limestone	20.1
9-Sep-12	3	Limestone	10.2
9-Sep-12	3	Limestone	15.5
9-Sep-12	3	Limestone	8.7
9-Sep-12	3	Limestone	15.0
9-Sep-12	3	Limestone	6.8
9-Sep-12	3	Limestone	23.8
9-Sep-12	4	Limestone	20.8
9-Sep-12	4	Limestone	22.0
9-Sep-12	4	Limestone	15.4
9-Sep-12	4	Limestone	14.4
9-Sep-12	4	Limestone	17.5
9-Sep-12	4	Limestone	15.0
9-Sep-12	4	Limestone	19.4
9-Sep-12	4	Limestone	27.6
9-Sep-12	4	Limestone	15.5
9-Sep-12	4	Limestone	16.7
9-Sep-12	5	Limestone	5.8
9-Sep-12	5	Limestone	24.2
9-Sep-12	5	Limestone	21.6
9-Sep-12	5	Limestone	6.6
9-Sep-12	5	Limestone	21.2
9-Sep-12	5	Limestone	7.8
9-Sep-12	5	Limestone	13.1
9-Sep-12	5	Limestone	18.6
9-Sep-12	5	Limestone	24.2
9-Sep-12	5	Limestone	16.3
8-Sep-12	6	Limestone	10.77
8-Sep-12	6	Limestone	7.31
8-Sep-12	6	Limestone	16.9
8-Sep-12	6	Limestone	18.43

8-Sep-12	6	Limestone	15.94
8-Sep-12	6	Limestone	12.43
8-Sep-12	6	Limestone	12.82
8-Sep-12	6	Limestone	13.51
8-Sep-12	6	Limestone	10.84
8-Sep-12	6	Limestone	22.3
8-Sep-12	7	Limestone	24.7
8-Sep-12	7	Limestone	5.8
8-Sep-12	7	Limestone	6.2
8-Sep-12	7	Limestone	20.4
8-Sep-12	7	Limestone	8.4
8-Sep-12	7	Limestone	10.5
8-Sep-12	7	Limestone	23.6
8-Sep-12	7	Limestone	7.8
8-Sep-12	7	Limestone	5.7
8-Sep-12	7	Limestone	11.2
9-Sep-12	8	Limestone	25.5
9-Sep-12	8	Limestone	29.7
9-Sep-12	8	Limestone	18.0
9-Sep-12	8	Limestone	10.7
9-Sep-12	8	Limestone	15.9
9-Sep-12	8	Limestone	6.0
9-Sep-12	8	Limestone	9.8
9-Sep-12	8	Limestone	3.9
9-Sep-12	8	Limestone	3.1
9-Sep-12	8	Limestone	5.6
9-Sep-12	9	Limestone	27.4
9-Sep-12	9	Limestone	7.2
9-Sep-12	9	Limestone	6.3
9-Sep-12	9	Limestone	4.9
9-Sep-12	9	Limestone	9.3
9-Sep-12	9	Limestone	19.5
9-Sep-12	9	Limestone	18.9
9-Sep-12	9	Limestone	17.0
9-Sep-12	9	Limestone	5.5
9-Sep-12	9	Limestone	19.1
9-Sep-12	10	Limestone	6.9
9-Sep-12	10	Limestone	7.7
9-Sep-12	10	Limestone	5.4
9-Sep-12	10	Limestone	6.6
9-Sep-12	10	Limestone	7.5
9-Sep-12	10	Limestone	7.1
9-Sep-12	10	Limestone	10.1

9-Sep-12	10	Limestone	24.5
9-Sep-12	10	Limestone	27.5
9-Sep-12	10	Limestone	5.1
9-Sep-12	1	Porcelain	6.1
9-Sep-12	1	Porcelain	10.1
9-Sep-12	1	Porcelain	9.0
9-Sep-12	1	Porcelain	3.3
9-Sep-12	1	Porcelain	6.7
9-Sep-12	1	Porcelain	8.8
9-Sep-12	1	Porcelain	7.5
9-Sep-12	1	Porcelain	39.9
9-Sep-12	1	Porcelain	10.7
9-Sep-12	1	Porcelain	6.5
9-Sep-12	2	Porcelain	33.2
9-Sep-12	2	Porcelain	9.7
9-Sep-12	2	Porcelain	8.5
9-Sep-12	2	Porcelain	34.4
9-Sep-12	2	Porcelain	9.6
9-Sep-12	2	Porcelain	6.4
9-Sep-12	2	Porcelain	4.8
9-Sep-12	2	Porcelain	15.6
9-Sep-12	2	Porcelain	8.0
9-Sep-12	2	Porcelain	7.3
9-Sep-12	3	Porcelain	4.7
9-Sep-12	3	Porcelain	4.9
9-Sep-12	3	Porcelain	28.0
9-Sep-12	3	Porcelain	3.8
9-Sep-12	3	Porcelain	5.7
9-Sep-12	3	Porcelain	1.4
9-Sep-12	3	Porcelain	15.5
9-Sep-12	3	Porcelain	7.4
9-Sep-12	4	Porcelain	20.4
9-Sep-12	4	Porcelain	10.1
9-Sep-12	4	Porcelain	27.3
9-Sep-12	4	Porcelain	30.0
9-Sep-12	4	Porcelain	26.6
9-Sep-12	4	Porcelain	13.3
9-Sep-12	4	Porcelain	25.0
9-Sep-12	4	Porcelain	12.6
9-Sep-12	4	Porcelain	12.5
9-Sep-12	4	Porcelain	21.1
9-Sep-12	5	Porcelain	4.2
9-Sep-12	5	Porcelain	14.5

9-Sep-12	5	Porcelain	32.0
9-Sep-12	5	Porcelain	7.6
9-Sep-12	5	Porcelain	2.9
9-Sep-12	5	Porcelain	5.5
9-Sep-12	5	Porcelain	13.4
9-Sep-12	5	Porcelain	8.9
9-Sep-12	5	Porcelain	8.2
9-Sep-12	5	Porcelain	15.1
8-Sep-12	6	Porcelain	5.2
8-Sep-12	6	Porcelain	3.4
8-Sep-12	6	Porcelain	6.5
8-Sep-12	6	Porcelain	10.8
8-Sep-12	6	Porcelain	34.7
8-Sep-12	6	Porcelain	23.3
8-Sep-12	6	Porcelain	13.9
8-Sep-12	6	Porcelain	9.1
8-Sep-12	6	Porcelain	2.1
8-Sep-12	6	Porcelain	2.8
8-Sep-12	7	Porcelain	24.3
8-Sep-12	7	Porcelain	10.3
8-Sep-12	7	Porcelain	8.2
8-Sep-12	7	Porcelain	10.4
8-Sep-12	7	Porcelain	11.6
8-Sep-12	7	Porcelain	13.1
8-Sep-12	7	Porcelain	6.8
8-Sep-12	7	Porcelain	4.8
8-Sep-12	7	Porcelain	20.0
8-Sep-12	7	Porcelain	5.6
9-Sep-12	8	Porcelain	7.6
9-Sep-12	8	Porcelain	4.7
9-Sep-12	8	Porcelain	4.3
9-Sep-12	8	Porcelain	4.5
9-Sep-12	8	Porcelain	4.1
9-Sep-12	8	Porcelain	3.3
9-Sep-12	8	Porcelain	6.4
9-Sep-12	8	Porcelain	6.3
9-Sep-12	8	Porcelain	6.1
9-Sep-12	8	Porcelain	4.4
9-Sep-12	9	Porcelain	35.6
9-Sep-12	9	Porcelain	14.5
9-Sep-12	9	Porcelain	10.5
9-Sep-12	9	Porcelain	8.3
9-Sep-12	9	Porcelain	8.7

9-Sep-12	9	Porcelain	6.3
9-Sep-12	9	Porcelain	9.1
9-Sep-12	9	Porcelain	10.5
9-Sep-12	9	Porcelain	18.7
9-Sep-12	9	Porcelain	7.2
9-Sep-12	10	Porcelain	35.0
9-Sep-12	10	Porcelain	25.3
9-Sep-12	10	Porcelain	10.0
9-Sep-12	10	Porcelain	9.5
9-Sep-12	10	Porcelain	18.1
9-Sep-12	10	Porcelain	12.7
9-Sep-12	10	Porcelain	8.7
9-Sep-12	10	Porcelain	7.7
9-Sep-12	10	Porcelain	4.6
9-Sep-12	10	Porcelain	5.0
9-Sep-12	1	River rock	14.9
9-Sep-12	1	River rock	17.7
9-Sep-12	1	River rock	19.3
9-Sep-12	1	River rock	21.0
9-Sep-12	1	River rock	22.2
9-Sep-12	1	River rock	23.8
9-Sep-12	1	River rock	24.0
9-Sep-12	1	River rock	25.2
9-Sep-12	1	River rock	27.6
9-Sep-12	1	River rock	30.9
9-Sep-12	2	River rock	4.0
9-Sep-12	2	River rock	3.4
9-Sep-12	2	River rock	5.5
9-Sep-12	2	River rock	26.7
9-Sep-12	2	River rock	9.9
9-Sep-12	2	River rock	19.2
9-Sep-12	2	River rock	8.5
9-Sep-12	2	River rock	15.7
9-Sep-12	2	River rock	20.9
9-Sep-12	2	River rock	5.9
9-Sep-12	3	River rock	20.7
9-Sep-12	3	River rock	15.5
9-Sep-12	3	River rock	20.7
9-Sep-12	3	River rock	7.9
9-Sep-12	3	River rock	6.2
9-Sep-12	3	River rock	6.9
9-Sep-12	3	River rock	16.7
9-Sep-12	3	River rock	17.3

9-Sep-12	3	River rock	8.3
9-Sep-12	3	River rock	19.4
9-Sep-12	4	River rock	3.0
9-Sep-12	4	River rock	14.5
9-Sep-12	4	River rock	13.8
9-Sep-12	4	River rock	10.0
9-Sep-12	4	River rock	19.8
9-Sep-12	4	River rock	8.5
9-Sep-12	4	River rock	25.0
9-Sep-12	4	River rock	22.0
9-Sep-12	4	River rock	18.4
9-Sep-12	4	River rock	12.3
9-Sep-12	5	River rock	41.5
9-Sep-12	5	River rock	20.1
9-Sep-12	5	River rock	25.3
9-Sep-12	5	River rock	4.2
9-Sep-12	5	River rock	16.4
9-Sep-12	5	River rock	11.8
9-Sep-12	5	River rock	27.2
9-Sep-12	5	River rock	10.2
9-Sep-12	5	River rock	18.0
9-Sep-12	5	River rock	15.5
8-Sep-12	6	River rock	22.66
8-Sep-12	6	River rock	11.97
8-Sep-12	6	River rock	8.3
8-Sep-12	6	River rock	14.73
8-Sep-12	6	River rock	19.02
8-Sep-12	6	River rock	5.7
8-Sep-12	6	River rock	16.21
8-Sep-12	6	River rock	13.9
8-Sep-12	6	River rock	17.42
8-Sep-12	6	River rock	12.2
8-Sep-12	7	River rock	27.9
8-Sep-12	7	River rock	8.1
8-Sep-12	7	River rock	6.2
8-Sep-12	7	River rock	21.4
8-Sep-12	7	River rock	20.5
8-Sep-12	7	River rock	8.4
8-Sep-12	7	River rock	5.8
8-Sep-12	7	River rock	8.6
8-Sep-12	7	River rock	19.3
8-Sep-12	7	River rock	18.7
9-Sep-12	8	River rock	22.8

9-Sep-12	8	River rock	5.8
9-Sep-12	8	River rock	5.4
9-Sep-12	8	River rock	24.6
9-Sep-12	8	River rock	26.5
9-Sep-12	8	River rock	27.4
9-Sep-12	8	River rock	15.2
9-Sep-12	8	River rock	9.9
9-Sep-12	8	River rock	16.4
9-Sep-12	8	River rock	8.1
9-Sep-12	9	River Rock	20.2
9-Sep-12	9	River Rock	18.5
9-Sep-12	9	River Rock	20.1
9-Sep-12	9	River Rock	15.4
9-Sep-12	9	River Rock	5.4
9-Sep-12	9	River Rock	12.4
9-Sep-12	9	River Rock	2.6
9-Sep-12	9	River Rock	12.8
9-Sep-12	9	River Rock	22.7
9-Sep-12	9	River Rock	8.9
9-Sep-12	10	River rock	23.5
9-Sep-12	10	River rock	11.0
9-Sep-12	10	River rock	7.3
9-Sep-12	10	River rock	19.8
9-Sep-12	10	River rock	10.8
9-Sep-12	10	River rock	27.3
9-Sep-12	10	River rock	27.0
9-Sep-12	10	River rock	22.6
9-Sep-12	10	River rock	15.9
9-Sep-12	10	River rock	9.0
9-Sep-12	2	Shell	24.0
9-Sep-12	2	Shell	28.5
9-Sep-12	2	Shell	30.4
9-Sep-12	2	Shell	27.0
9-Sep-12	2	Shell	22.1
9-Sep-12	2	Shell	11.0
9-Sep-12	2	Shell	7.0
9-Sep-12	2	Shell	24.9
9-Sep-12	2	Shell	12.5
9-Sep-12	2	Shell	4.9
9-Sep-12	3	Shell	18.8
9-Sep-12	3	Shell	16.5
9-Sep-12	3	Shell	22.5
9-Sep-12	3	Shell	38.5

9-Sep-12	3	Shell	7.0
9-Sep-12	3	Shell	27.6
9-Sep-12	3	Shell	8.7
9-Sep-12	3	Shell	22.7
9-Sep-12	3	Shell	15.7
9-Sep-12	3	Shell	28.8
9-Sep-12	4	Shell	5.8
9-Sep-12	4	Shell	3.9
9-Sep-12	4	Shell	5.5
9-Sep-12	4	Shell	9.4
9-Sep-12	4	Shell	12.2
9-Sep-12	4	Shell	8.8
9-Sep-12	4	Shell	29.9
9-Sep-12	4	Shell	13.2
9-Sep-12	4	Shell	7.8
9-Sep-12	4	Shell	24.4
9-Sep-12	5	Shell	24.1
9-Sep-12	5	Shell	24.4
9-Sep-12	5	Shell	14.8
9-Sep-12	5	Shell	7.8
9-Sep-12	5	Shell	5.0
9-Sep-12	5	Shell	22.1
9-Sep-12	5	Shell	23.2
9-Sep-12	5	Shell	6.9
9-Sep-12	5	Shell	8.1
9-Sep-12	5	Shell	10.1
8-Sep-12	6	Shell	29.0
8-Sep-12	6	Shell	24.1
8-Sep-12	6	Shell	7.3
8-Sep-12	6	Shell	23.1
8-Sep-12	6	Shell	8.0
8-Sep-12	6	Shell	8.9
8-Sep-12	6	Shell	5.4
8-Sep-12	6	Shell	4.9
8-Sep-12	6	Shell	17.7
8-Sep-12	6	Shell	8.8
8-Sep-12	7	Shell	13.6
8-Sep-12	7	Shell	11.3
8-Sep-12	7	Shell	9.0
8-Sep-12	7	Shell	4.3
8-Sep-12	7	Shell	18.8
8-Sep-12	7	Shell	33.5
8-Sep-12	7	Shell	7.4

8-Sep-12	7	Shell	25.0
8-Sep-12	7	Shell	12.9
8-Sep-12	7	Shell	9.2
9-Sep-12	8	Shell	17.1
9-Sep-12	8	Shell	28.1
9-Sep-12	8	Shell	7.0
9-Sep-12	8	Shell	31.6
9-Sep-12	8	Shell	18.2
9-Sep-12	8	Shell	3.9
9-Sep-12	8	Shell	5.0
9-Sep-12	8	Shell	4.8
9-Sep-12	8	Shell	11.1
9-Sep-12	8	Shell	4.0
9-Sep-12	9	Shell	11.9
9-Sep-12	9	Shell	28.5
9-Sep-12	9	Shell	9.8
9-Sep-12	9	Shell	9.6
9-Sep-12	9	Shell	6.3
9-Sep-12	9	Shell	9.3
9-Sep-12	9	Shell	30.0
9-Sep-12	9	Shell	15.3
9-Sep-12	9	Shell	8.2
9-Sep-12	9	Shell	5.2
9-Sep-12	10	Shell	20.2
9-Sep-12	10	Shell	15.2
9-Sep-12	10	Shell	19.5
9-Sep-12	10	Shell	11.5
9-Sep-12	10	Shell	24.6
9-Sep-12	10	Shell	25.0
9-Sep-12	10	Shell	23.5
9-Sep-12	10	Shell	14.3
9-Sep-12	10	Shell	21.3
9-Sep-12	10	Shell	22.0

Faunal Species Diversity

substrate	placement	site	S	N	d	J'	Brillouin	H'	1-Lambda'	N1
concrete	lower	1	7	17.73104738	2.086726298	0.944717401	1.479896676	1.838335179	0.87350873	6.286064373
concrete	lower	2	7	15.10391862	2.209982014	0.865166647	1.328915054	1.683536559	0.833217933	5.384565168
concrete	lower	3	10	20.96500884	2.957748754	0.942956112	1.748640293	2.171236688	0.911931578	8.769122003
concrete	lower	4	12	23.85908868	3.46766301	0.905559902	1.708419694	2.250231821	0.904212404	9.489935551
concrete	lower	5	11	25.10760065	3.10253511	0.920271948	1.758785362	2.206715753	0.906141307	9.085827238
concrete	upper	6	9	19.6349939	2.686986214	0.904276387	1.539327597	1.986898302	0.883200804	7.292878342
concrete	upper	7	10	20.20775727	2.993945689	0.893642915	1.559600853	2.057688853	0.891649543	7.827857562
concrete	upper	8	12	23.49604254	3.484505981	0.937530333	1.721200323	2.329675359	0.929380373	10.27460544
concrete	upper	9	9	18.88751937	2.722476201	0.94072855	1.578318119	2.06699189	0.903785563	7.901020197
concrete	upper	10	10	19.28493483	3.041234875	0.936225836	1.636139818	2.155739654	0.91525923	8.634274197
limestone	lower	1	7	14.27748202	2.256755927	0.890417457	1.230405401	1.732672367	0.848904689	5.655747967
limestone	lower	2	7	20.72765427	1.979238603	0.936911475	1.407352775	1.823145547	0.865591085	6.191302887
limestone	lower	3	5	6.473890696	2.141582971	0.846484077	0.79791529	1.362363566	0.801425502	3.905413104
limestone	lower	4	7	18.48834238	2.05680879	0.87595323	1.233740187	1.704526281	0.824311963	5.498780171
limestone	lower	5	10	26.60336765	2.743034527	0.890578993	1.732811031	2.050633914	0.87997983	7.772826852
limestone	upper	6	8	17.84063365	2.429308304	0.894367453	1.441388499	1.859784836	0.858780704	6.42235476
limestone	upper	7	6	13.06133868	1.94578527	0.883000463	1.068728546	1.58212444	0.821991936	4.865280838
limestone	upper	8	7	17.39694733	2.100623544	0.906838768	1.328506918	1.764626763	0.859508824	5.839392474
limestone	upper	9	7	12.69182658	2.361313923	0.853108419	1.175366574	1.66007233	0.8286033	5.259691264
limestone	upper	10	7	16.2183391	2.153515008	0.906347992	1.37582296	1.763671756	0.851379902	5.833818474
porcelain	lower	1	9	18.08292602	2.763415521	0.91003977	1.540930692	1.999561749	0.891913187	7.385818547
porcelain	lower	2	7	17.06919121	2.114705016	0.939709251	1.291529938	1.828589769	0.875848042	6.225101633
porcelain	lower	3	11	20.08527571	3.333347784	0.921141695	1.709387467	2.208801316	0.917242204	9.104796076
porcelain	lower	4	7	17.26645764	2.106175276	0.907519221	1.35615419	1.765950862	0.848061293	5.84712953
porcelain	lower	5	13	24.22639001	3.764773754	0.898586266	1.789499284	2.304828266	0.911308865	10.02245691
porcelain	upper	6	10	22.49285638	2.890918175	0.917502111	1.678278038	2.112626684	0.901075625	8.269935287
porcelain	upper	7	9	19.4694202	2.694650577	0.933602606	1.55697785	2.051334592	0.901550815	7.778275008
porcelain	upper	8	9	17.89680392	2.773326834	0.931129407	1.585292231	2.045900418	0.907893882	7.736121145
porcelain	upper	9	8	12.08336249	2.809180959	0.910713791	1.285238859	1.893776089	0.896897412	6.644411259
porcelain	upper	10	7	14.82227068	2.225411298	0.915690151	1.402155873	1.781850758	0.858015461	5.940841311
river rock	lower	1	8	20.16036261	2.330444802	0.92721152	1.449739624	1.928082152	0.879722526	6.876309871

river rock	lower	2	8	16.41659896	2.501525077	0.868477098	1.307159691	1.805947356	0.84773118	6.085734072
river rock	lower	3	11	22.94349558	3.191793792	0.905405461	1.681361595	2.171067475	0.903860732	8.767638283
river rock	lower	4	9	20.98263843	2.628383944	0.904700627	1.577996534	1.987830453	0.8754974	7.299679572
river rock	lower	5	9	20.66445664	2.641645743	0.910045873	1.663318413	1.99957516	0.876179868	7.385917597
river rock	upper	6	7	17.386554	2.101063134	0.914640835	1.461551806	1.779808883	0.857827349	5.928723233
river rock	upper	7	10	22.48518915	2.8912348	0.905167525	1.596834426	2.084225249	0.892449132	8.038361337
river rock	upper	8	10	21.20173751	2.946874593	0.928370482	1.621629426	2.137652033	0.911211449	8.479504641
river rock	upper	9	5	13.6609598	1.529904597	0.931355911	1.081873862	1.498959513	0.812440517	4.477028358
river rock	upper	10	6	12.83819512	1.958921631	0.836373892	1.090857936	1.49858084	0.782957784	4.475333348
shell	lower	2	7	15.36149736	2.196302431	0.924334679	1.436210915	1.798672232	0.871818484	6.041620268
shell	lower	3	12	22.48796715	3.533591183	0.92227357	1.69670827	2.291763727	0.922889581	9.892369742
shell	lower	4	9	20.00799574	2.670109345	0.884082405	1.462305345	1.942527589	0.861723865	6.976362077
shell	lower	5	9	22.01980114	2.587370572	0.92422765	1.646771348	2.030735708	0.885947937	7.619690164
shell	upper	6	10	15.22789363	3.305021718	0.951616756	1.628902365	2.191178557	0.940393933	8.945749982
shell	upper	7	10	21.17310268	2.94817923	0.909199687	1.630311405	2.093509645	0.897016741	8.113340198
shell	upper	8	12	23.75389639	3.472500005	0.929055237	1.810783685	2.308615537	0.926715211	10.06048664
shell	upper	9	9	16.80578798	2.835146826	0.934246698	1.620848876	2.052749805	0.908228168	7.789290718
shell	upper	10	6	13.17685156	1.93914075	0.915582075	1.206556198	1.640502853	0.84335478	5.157762459

Overall Fauna Data

Substrate	Xanthidae	Porcella nidae	Palaemo ntes	Menippe adina	Alpheus heteroch aelis	Gobios oma robust um	Gobioso ma bosc	Anchoa mitchilli	Gobiesox strumosus	Opsanus beta	Bairedi ella chryso ura	Eucinost omus argente us	Chasm odes bosqui anus	Farfant apenae us sp.	Caline ctes sapid us	Langod on rhombo ides	Archosar gus probato cephalus	Brown shrim p	Post- larval Penaid	Portunid ae	Hypsoble nnius hentz	Bagre marinus
Bare-1								52														
Bare-10	2						2	3														
Bare-4	5																			1		
Bare-7							2															
Concrete -1	3	139	19		7		4	27		3												
Concrete -10	8	108		8	6		7	9	2	1						1	3					
Concrete -2	22	223	10	1	7		1			1												
Concrete -3	9	187	13	3	11		6		3	3							2	2				
Concrete -4	19	742	1	7	2		5	6	5	7						1	3			1		
Concrete -5	14	485	30	3	15	5	10		2	6							1	1				
Concrete -6	14	269	9	2	20		10		2	1						1						
Concrete -7	35	199	17	7	3		8		1			1							1		1	
Concrete -8	17	47	48	3	20	4	4		1	4	1	2					2					
Concrete -9	12	141		3	9		4	8		5							3				1	
Limesto ne-1	15	154	4		7		3			1						1						
Limesto ne-10	27	234		6	3		5	4	1													
Limesto ne-2	33	313	19		15		20			6						1						
Limesto ne-3	1	26		1						2					1							

Limestone-4	48	852		1	15		4		3	3												
Limestone-5	80	1186	22	2	30		28		1	4						1		2				
Limestone-6	26	383	5	4	2		9		2									1				
Limestone-7	34	111	4	3	2		1															
Limestone-8	59	76	5	1	35		8			1												
Limestone-9	20	128		1	4		2									1	1					
Porcelain-1	8	138	8	1	17	1	10	1		3												
Porcelain-10	11	180		6	3		4		2								2					
Porcelain-2	15	106	13		17		5		1	4												
Porcelain-3	20	83	4		25	2			3	3	5				1		1	1				
Porcelain-4	19	390		3	6		7		8	1												
Porcelain-5	9	533	8	2	19	1	11		5	4						1	1	1				1
Porcelain-6	24	206	20	1	24	1	9		2	5							2					
Porcelain-7	25	98	20	3	4		10		3							2					1	
Porcelain-8	31	15	26		16		6			2	2						1	1				
Porcelain-9	8	38		2	2		6			1		1					1					
River rock-1	23	256	5	1	18		8	14		2												
River rock-10	20	279		3	1		3		1													
River rock-2	23	324	1	1	5		8			3							1					
River rock-3	21	291	9	1	27		7		4	7						1	1	1				
River rock-4	25	627	1	8	13		6	2	2	4												
River rock-5	22	658	8	3	7		7		2	2												2
River rock-6	32	211	10	2	6		10										1					

River rock-7	27	402	8	6	15		15	1	1							1	3					
River rock-8	41	28	7	2	45		19		4	1							1	2				
River rock-9	33	143		4	4		6															
Shell-10	9	109		5	3		9			1												
Shell-2	24	78	8	1	10		3			2												
Shell-3	18	120	6	4	26	5			3	6				1	1	1	1					
Shell-4	25	854	1	4	4		3	1	10	4												
Shell-5	25	476	25	2	8		6		3	4						2						
Shell-6	8	11	7	1	10		5			2							1	2				1
Shell-7	37	198	23	3	5		9		2	1			1				2					
Shell-8	35	28	40	1	27		9		3	5	2						1	2				
Shell-9	11	73	1	3	3		6	9	4	1												