OYSTER REEF RESTORATION: SUBSTRATE SUITABILITY MAY DEPEND ON SPECIFIC RESTORATION GOALS

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Abstract

Oyster reef restoration is an increasingly used tool to combat habitat loss and restore ecosystem services that reefs provide. A limited supply of oyster shell for restoration practices has prompted research focused on understanding the value of alternative substrates for reef construction. We restored 6 acres of subtidal oyster reef complex in the Mission-Aransas Estuary, TX, in July 2013 using replicated sections of concrete, limestone, river rock, and oyster shell substrates. Oyster and reef-associated faunal development were assessed for 18 months post-construction. Oyster populations varied seasonally and by substrate; the highest oyster abundance across all substrates was observed during July 2014 ($\overline{X}$= 1288 m^{-2}). Concrete ($\overline{X}$ = 1022 m^{-2}) and limestone ($\overline{X}$ = 939 m^{-2}) supported the greatest number of oysters over all dates. Motile macrofauna also varied with season and substrate type; abundance and was highest during July 2014 ($\overline{X}$ = 2766 m^{-2}) and October 2014 ($\overline{X}$ = 1748 m^{-2}). Oyster shell ($\overline{X}$ =1533 m^{-2}) and concrete ($\overline{X}$ = 1047 m^{-2}) substrates supported the highest abundances of motile fauna. Faunal diversity (Hill's N1) peaked in April 2014 ($\overline{X}$ = 4.1) and did not vary by substrate material. All substrates were successful at providing habitat for oyster and faunal communities—but were effective to varying degrees for different metrics—suggesting that substrate choice should be dependent on restoration goals. We developed a simple benefit-cost ratio to determine which substrates had the best return on investment for our restoration goals. The metric is flexible so practitioners can adapt it to suit their own project goals and substrate costs. As oyster reef restoration activities continue at small and large scales, substrate selection criteria are critical for assisting stakeholders in ensuring restoration investments are maximizing environmental benefits per dollar spent.

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General Introduction

Eastern Oysters, *Crassostrea virginica*, are bivalve mollusks that form subtidal and intertidal reefs in estuaries along the Caribbean, Atlantic Ocean, and Gulf of Mexico. Reefs, formed from the generational settlement of oysters, provide refuge habitat and spawning substrate for numerous fish and motile crustaceans (Breitburg 1999; Peterson et al. 2003) along with important biogenic habitat for a number of benthic invertebrates (Zimmerman et al. 1989). Other environmental benefits provided by oyster reef habitat include water filtration (Dame et al. 1980) and shoreline stabilization (Piazza et al. 2005). Due to the three-dimensional habitat structure they create, oysters are recognized as important ecosystem engineers in otherwise soft-bottom systems (Jones et al. 1996; Lenihan & Peterson 1998). Oysters are also key indicator species, providing information on the overall health of an estuary (Pollack et al. 2011).

Oyster reefs form extensive three-dimensional habitat with varying levels of complexity. Oyster shell is the preferable habitat for larval oysters and colonizing fauna (Gutierrez et al. 2003), thus sustaining the longevity of the reef and its associated benefits. In North Carolina, each 10 m^2 of restored reef was estimated to produce an additional 2.6 kg yr^{-1} of oyster shell cover (Peterson et al. 2003). Reefs create numerous microhabitats for use by macrofauna (Tolley & Volety 2005) which may include larval bivalves (Harding & Mann 1999), gastropods, polychaetes, mud and portunid crabs, and reef-associated fishes including gobies and skilletfish (Breitburg 1999). The presence of lower trophic levels attracts predators including fish species such as pinfish (*Lagodon rhomboides*), spot (*Leiostomus xanthurus*), and gulf toadfish (*Opsanus beta*) (Stunz et al.

2010). The increase in fish and invertebrate production created by oyster reef habitat is economically favorable as well, providing prey for the sport fishes that drive recreational fishing within coastal environments. A study in Louisiana reported that 23% of annual marine fishing days were spent over oyster reef generating $2 million in revenue (Henderson & O'Neil 2003).

In addition to creating large scale habitats for a variety of marine species, oysters help improve the water quality of estuarine environments through their feeding activities. Large populations of suspension-feeding bivalves can have significant impacts on water quality and phytoplankton dynamics within a system (Cloern 1982; Cohen et al. 1984; Dame 1996; Coen & Luckenbach 2000). Oysters ingest edible phytoplankton from the water column while binding rejected material with mucous, ultimately depositing this as pseudofeces onto the sea surface (Haven & Morales-Alamo 1972; zu Ermgassen et al. 2012). This process brings suspended material to the benthos, reducing turbidity within the water column. Research in the Chesapeake Bay area has shown that oysters can extract particulate matter between 1 and 12 µm from the water column (Haven & Morales-Alamo 1972; Dame et al. 1980). The filtration of phytoplankton increases light penetration in shallow water which benefits nearby seagrass habitat (Newell & Koch 2004) and aids in preventing large-scale algal blooms and eutrophication (Ulanowicz & Tuttle 1992; Coen & Luckenbach 2000). Oysters also bioaccumulate environmental toxicants and bacteria (Newell 2004), which otherwise negatively affect water quality and the overall health of a system (Mott 2008).

Estuarine ecosystems comprise multiple habitats that are vulnerable to degradation due to pollution and overharvesting, and environmental factors such as erosion due to heavy storms. Although vegetation has proven effective in reducing erosion of estuarine habitats (Gleason et al. 1979), its effectiveness is reduced in high energy areas prone to heavy storms, strong wave action, and anthropogenic disturbances such as boat wakes (Williams 1993). The three-dimensional structure of oyster reefs can serve as a breakwater, dissipating harmful wave energy and protecting nearby habitats such as salt marsh (Meyer et al. 1996). The natural protection of oyster reefs may be advantageous over artificial methods, like bulkheads, which can reflect wave energies to adjacent habitats and cause further erosion (Scyphers et. al 2011; George et al. 2014). For Texas bays, which are prone to strong storms and heavily used by recreational boaters, the use of oyster reefs may be a viable alternative to traditional coastline protection.

Oyster production generates large revenues for the Texas and US Gulf of Mexico seafood industries. The oystermen in the United States harvested 29.3 million pounds of oysters in 2012, with a net worth of $104.4 million (NMFS 2014). Texas oystermen generated an estimated $21.3 million from oyster harvests during 2012, the second most profitable state in the US. Although commercial harvest produces substantial economic benefits, the downside is its contribution to the degradation of oyster reef habitat. Along with harvest for human consumption, historical dredging for industry and road construction has altered the physical landscape of oyster reefs in Texas bays (Doran 1965), and has contributed to the decline of oyster reef habitat throughout the Gulf of Mexico.

Oyster populations have declined drastically worldwide in response to poor resource management, disease, increased sedimentation, and environmental degradation

(Hargis & Haven, 1988; 1999; Nestlerode et al. 2007). Shellfish reefs are the most degraded marine habitats on earth, with estimated losses of up to 85% relative to historic abundances (Beck et al. 2011). The Texas coastline, with its warm temperatures, strong storms and increasing industrialization is no exception to the threat of habitat loss due to disease or habitat degradation. Oyster shells are crucial habitat for oyster larvae that require their solid foundation for recruitment and growth (Rothschild et al. 1994; Lenihan & Peterson 1998). However, when oyster reefs are overharvested or dredged for shell, channels or other coastal developments, this important substrate is lost (Powell & Klinck 2007).

Oyster populations are also at risk due to natural causes, including molluscan predators and protozoan parasites. Dermo disease, caused by the protozoan parasite *Perkinsus marinus*, causes massive mortalities in oyster populations in estuaries across the US Atlantic and Gulf of Mexico (Andrews & Ray 1988; Ray 2008). *Perkinsus marinus* is prevalent in Texas waters due to the year-round warm temperatures and high salinities associated with its persistence (Ray 2008). Dermo reduces growth and inhibits gonadal development of oysters, preventing them from reaching market size (White et al. 1984) while reducing the surface area available for recruitment of future generations. In response to the declining oyster populations, oyster reef restoration activities have increased to combat further losses of the biogenic habitat.

Reef restoration efforts are occurring across much of the range of *C. virginica* to recover some of the ecosystem functions and services provided by oysters (Dunn et al. 2014). In areas where oyster reef habitat has declined, but where there are still reproductively viable populations of oysters, typically the first step for restoration is laying down hard substrate, which serves as a base for oyster recruitment and faunal community development (Powers et al. 2009; Schulte et al. 2009). The habitat provided by restored reefs, if successful, mimics that of natural oyster reef and over time provides similar ecosystem services. The preferred substrate used in oyster reef restoration is *C. virginica* shell, collected from local shucking operations or from dredging historic reef deposits (Nesterlode et al. 2007). However, harvested oyster shell is often disposed of in landfills or lost to competing uses such as road construction or as poultry feed additives (LDWF 2004; George et al. 2014). Shortages of oyster shell have led to investigations of alternative substrates for reef construction (Mann et al. 1990; Soniat & Burton 2005; Dunn et al. 2014; George et al. 2014).

A variety of substrates have been tested for the ability to replace natural oyster shell for reef restoration including mollusk shells, rubber material, porcelain, and gravel. Unfortunately, in many restoration studies, the choice of substrate material is based solely on price and availability rather than the ecological potential (Brumbaugh & Coen 2009; George et al. 2014). A number of studies have examined recruitment and survival of oyster larvae (Mann et al. 1990; Soniat & Burton 2005; Nesterlode et al. 2007), but fewer focus on the relative habitat value for macrofauna (French-McCray et al. 2003; George et al. 2014). As reef restoration efforts expand throughout the United States with the desire to mimic ecosystem services provided by natural reefs, it is important to better understand the effect of substrate type on relative habitat value.

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Introduction

Estuarine ecosystems have undergone profound ecological and physical changes due to natural and anthropogenic causes, and are widely considered the most degraded of marine ecosystems (Officer et al. 1984; Nixon et al. 1995; Jackson et al. 2001). A number of human activities have been linked to the decline of estuarine habitats including, increased sedimentation and turbidity due to land use change (Thrush et al. 2004), increased hypoxia and anoxia from excess nutrient inputs (Cooper and Brush 1993; Paerl 2006), and overexploitation of estuarine resources (Lotze et al. 2006; Rick and Erlandson 2009).

Eastern oysters, *Crassostrea virginica*, are bivalve mollusks that form extensive reefs in estuaries along the Caribbean, Atlantic Ocean and Gulf of Mexico. Oyster reefs, formed from the generational settlement of oysters, are key components of estuarine ecosystems. These biogenic reefs provide complex habitat used as refuge and spawning substrate for numerous fish and motile crustaceans (Breitburg 1999; Peterson et al. 2003) along with important biogenic habitat for many benthic invertebrates (Zimmerman et al. 1989). Oysters are recognized as important ecosystem engineers in otherwise soft-bottom systems due to the three-dimensional habitat they create (Jones et al. 1996; Lenihan & Peterson 1998). Other environmental benefits provided by oyster reef habitat include water filtration (Dame et al. 1980), shoreline stabilization (Piazza et al. 2005), and nitrogen regulation (Beseres Pollack et al. 2013).

Oysters are one of the few reef building species that are actively harvested for human consumption. When oysters are harvested or reefs are purposely dredged, the resulting decline in shell resources can reduce bathymetric complexity and may ultimately lead to the disappearance of reef habitat (Bergquist et al. 2006; Powell & Klinck 2007). Oyster populations have declined drastically worldwide (Hargis & Haven, 1998; 1999; Nestlerode et al. 2007), with estimated losses of up to 85% relative to historic abundances (Beck et al. 2011). Because oyster larvae depend on the solid foundation provided by older generations for recruitment and growth, when reef substrates are removed essential habitat is lost (Rothschild et al. 1994; Lenihan & Peterson 1998; Powell & Klinck 2007). Oyster reefs add complexity to otherwise soft-bottom estuaries in the Gulf of Mexico, increasing species diversity of associated organisms (Harding & Mann 2000, Gutierrez et al. 2003). Therefore, reductions of reef habitat may also lead to losses of biodiversity (Lotze et al. 2006; Airoldi et al. 2008; Brown et al. 2014).

In response to losses of oyster habitat, ecological restoration efforts are being implemented worldwide (Clewell & Aronson 2013) with goals of assisting the recovery of ecosystems that have been degraded or destroyed (Jordan et al. 1990; Benayas et al. 2009). Since 1990, over 400 artificial reefs have been constructed in the northern Gulf of Mexico alone (Furlong 2012). Because a main bottleneck to oyster population recovery is lack of hard substrate, restoration activities focus on providing shells or other materials as a base for oyster recruitment and faunal community development (Powers et al. 2009; Schulte et al. 2009). If successful, the habitat provided by restored reefs mimics that of natural oyster reef and over time provides similar ecosystem services (Grabowski et al. 2012). The preferred substrate used in reef restoration is *C. virginica* shell, collected from shucking operations or from dredged deposits of oyster reef (Nesterlode et al. 2007). However, harvested oyster shell is often disposed of in landfills or lost to competing uses such as road construction or poultry feed additives (LDWF 2004; George et al. 2014), making its availability limited and often times expensive. Decreased availability of oyster shell has led to investigations of alternative substrates for reef construction (Mann et al. 1990; Soniat & Burton 2005; Dunn et al. 2014; George et al. 2014). Studies have evaluated a variety of substrates for oyster reef restoration, but once options of suitable materials have been identified, further evaluations should examine how to maximize the ecological benefits in relation to project costs.

Although ecological benefits are the main focus of restoration projects, the funds available often influence the decision-making process. Restoration planning involves many steps including site selection, pre-construction monitoring and assessments, and reef design and construction. With a limited budget to work with, accurate decision making and appropriate allocation of funds is crucial to the success of the project. To account for this, restoration practitioners can apply benefit-cost analysis, incorporating the ecological values provided by the substrate (oyster abundance, biodiversity) with the economic logistics required to maximize return on investments in the project (Goldstein et al. 2008; Daily et al. 2009).

The goal of this study was to evaluate the efficacy of four substrates in oyster reef restoration: concrete, limestone, river rock, and oyster shell. Specific objectives were to: 1) characterize and compare effects of substrate type on oyster populations, 2) describe and compare resident fauna community structure and species-substrate relationships, and 3) calculate and compare benefit-cost ratios for each substrate type to guide restoration planning.

Materials and Methods

Study Area

The Mission-Aransas Estuary is a shallow, bar-built estuary on the south Texas coast (Fig. 1). The estuary is approximately 540 km^2, has an average depth of 2 m, and is one of the southernmost regions of commercial oyster harvest in Texas (Beseres Pollack et al. 2013). The estuary comprises several bays, the largest of which are Aransas Bay and Copano Bay. There is a natural salinity gradient within the estuary, with higher salinities near the Gulf inlets and lower salinities near the river sources.

In July 2013, six acres (0.24 km^2) of subtidal oyster reef were restored in Aransas Bay, Texas. Twelve rectangular reef mounds (each 27.4 m x 18.5 m x 0.3 m) were constructed using barges for hauling, with three replicate mounds of four substrate types: concrete, river rock, limestone, and oyster shell (Fig. 1c) For this project, oyster shell was provided at no cost to the project by the “Sink Your Shucks” recycling program at Texas A&M University-Corpus Christi. Shells were subject to a six-month quarantine prior to use. Concrete, river rock, and limestone substrates of similar sizes were purchased from a materials yard in Corpus Christi, Texas. Dimensions of the concrete and limestone were approximately 12.7 X 15.2 cm and for the river rock and oyster shell were approximately 7.6 x 10.2 cm. Three months post-construction, 72 sampling trays (dimensions 0.5 x 0.3 m) were filled with ~19 L of the different substrates, separately. Six sampling trays (containing substrate matching the reef mound of interest) were anchored to each of the 12 reef mounds using rebar stakes. The margins of each mound and the location of all sampling trays were marked with PVC poles.

Field Sampling

Sampling of the reef occurred quarterly (seasonally) from January 2014 – April 2015 (six sampling periods). During each sampling period, one tray per mound was randomly selected and removed. Substrate and organisms from each sampling tray were transferred to individual 19 L containers and transported to the laboratory for processing. Hydrological parameters (salinity (psu), dissolved oxygen (mg l^{-1}), temperature (°C)) were measured at 3 locations around the reef using a Hydrolab MS5 datasonde during each sampling event. In the laboratory each of the tray samples (N=12) was washed thoroughly across a 0.5 mm sieve to separate reef-associated motile organisms from sessile organisms permanently attached to the substrate.

Laboratory Analyses

Macrofaunal organisms were separated from tray substrates, sieved on a 4 mm mesh and transferred to jars with 10% buffered formalin. Fauna were sorted on a dissecting tray using forceps and examined using a stereo microscope. All organisms were quantified and identified to the lowest practical taxonomic level. Dry-weight biomass was obtained after placing organisms in a drying oven at 60 °C for 48 hours.

A 10% (1L) subsample of substrate from each sampling tray was used for counting oyster spat and other sessile organisms. Shell height of oyster spat was measured (nearest 0.1 mm) from the hinge to the ventral shell margin using mechanical calipers. Other sessile species were also measured and counted. The coverage of substrate by each species was standardized by converting to percent (%) area cover using the abundance, mean size and total surface area of each representative substrate (% area cover = n*size *100 / total area). The total surface area of the substrate was determined using a flexible plastic mesh with a known grid size (2.0 x 2.5 cm). Presence/absence of encrusting algae and bryozoans was noted throughout the study.

Data Analysis and Statistics

Abundance (n m^{-2}), diversity (N1) of motile and sessile fauna, size (shell height) of oysters, and percent area cover of sessile fauna, were analyzed using a two-way analysis of variance (ANOVA) with sampling date and substrate type as fixed factors using the equation:

$$Y = d\ s\ d*s$$

Where d = sampling date and s = substrate type. Motile fauna were aggregated into major taxonomic categories (crab, fish, shrimp) for analysis. Dry weight biomass (g m^{-2}) was analyzed using the two-way model for motile fauna but was not possible for sessile organisms, due to attachment to substrates. Tukey's HSD post hoc tests were used to determine differences among substrate types and dates if significant differences occurred. Data were transformed (log, square root, arcsine) where necessary to improve normality prior to analysis. ANOVA tests and data transformations were performed using SAS 9.4 (SAS Institute Inc. 2015) and results were considered statistically significant at $\alpha \leq 0.05$.

Similarities between motile fauna communities were analyzed using non-metric multidimensional scaling (MDS) using a Bray-Curtis similarity matrix. Significant similarity groupings were determined using the SIMPROF routine as part of cluster analysis (Clarke 1993; George et al. 2014). To test for differences among substrate types and sampling dates, an analysis of similarities (ANOSIM) test was used. In addition to the ANOSIM, a similarity percentages (SIMPER) test was used to examine pairwise differences in individual species among dates and substrates. Data were log transformed where necessary prior to analysis. All multivariate statistics were calculated using PRIMER v.7 software (Clarke et al. 2014) following the methods of Clarke (1993).

A metric incorporating the mean abundance of oysters and motile fauna over the ~18 month monitoring period, coupled with the substrate and transportation costs (dollars m^{-3}) was used to rank our substrates from an ecological and economic standpoint. The Benefit Cost Ratio (BCR) equation (separate for oysters and fauna) was applied simply:

$$BCR = \frac{B_t}{\sum C_t}$$

Where (B_t) is benefits over time, which in this case was mean abundance of oysters (n m^{-2}) and motile fauna (n m^{-2}) and (C_t) is the combined costs, which includes material and transportation costs ($US m^{-3}). Our (t) was the duration of our monitoring period, which was ~18 months.

Results

Oysters and other sessile fauna

Hydrological parameters showed expected seasonal variation. Water temperatures ranged from 6.8-29.7 °C, averaging 21.9 °C. The average salinity was 28.3, and was lowest in the spring (16.3) and highest in the summer (34.0). Dissolved oxygen ranged from 5.2-10.8 mg l^{-1}, averaging 7.5 mg l^{-1}.

A total of 3,892 oysters were collected and measured throughout the study. Oyster abundance (n m^{-2}) varied seasonally (Fig 2a), abundances in January ($\overline{X}$= 260 m^{-2}) and April 2014 ($\overline{X}$= 231 m^{-2}) were significantly less than the other four sampling dates ($p < 0.001$) (Table 2). The largest oyster abundance was observed during July 2014 ($\overline{X}$= 1288 m^{-2}). Oyster abundance also varied significantly between substrate materials, with concrete ($\overline{X}$= 1022 m^{-2}) and limestone ($\overline{X}$= 939 m^{-2}) supporting the greatest number of oysters ($p < 0.0001$) over all dates.

Oyster shell height ranged from 3.2 to 76.1 mm across all substrates and dates. A significant interaction was detected using the two-way ANOVA, and thus a simple main effects analysis was used for analysis of shell heights. There was a significant difference in oyster size between sampling dates and substrate types ($p < 0.0001$). The smallest oysters were collected in January 2014, the first sampling period of the study, on oyster shell ($\overline{X}$= 7.7 mm; Fig.3). The largest oysters were collected in April 2015, the last sampling period of the study, on concrete material ($\overline{X}$= 21.8 mm; Fig. 3). Juvenile oysters (>25 mm) were most abundant in January ($\overline{X}$= 137 m^{-2}) and April 2015 ($\overline{X}$= 283 m^{-2}), concrete ($\overline{X}$= 128 m^{-2}) and limestone ($\overline{X}$= 98 m^{-2}) substrates supported the highest numbers of oysters in that size class (Fig 2c). One market-sized oyster (≥ 76 mm) was collected during the final sampling period, but multiple juveniles were approaching market size by the final sampling event.

Oysters dominated percent coverage across all substrate types (Fig. 4). Percent coverage of oysters was significantly less in January and April 2014. *Balanus* sp. (acorn barnacles) and serpulid polychaete worms were the next most common species across substrates and sampling dates. Other sessile organisms observed on the substrates included *Anomia simplex* (jingle shell), *Crepidula* sp. (slipper shell), mussels from the family Mytillidae and various Tunicata (sea squirt) species. River rock supported the lowest % area cover by all sessile organisms with the exception of *Crepidula* sp., which showed no substrate preference.

Motile fauna

A total of 11,362 motile fauna were collected, representing 8 fish species and 10 decapod crustacean taxa (Tables 1, 2). There was a significant difference in motile fauna abundance between sampling dates ($p < 0.0001$) and substrate types ($p=0.0086$). Abundance was greatest during July 2014 ($\overline{X}$= 2766 m^{-2}) and October 2014 ($\overline{X}$= 1748 m^{-2}) and least in January 2014 ($\overline{X}$= 77 m^{-2}; Fig. 5A). Oyster shell ($\overline{X}$= 1533 m^{-2}) and concrete ($\overline{X}$= 1047 m^{-2}) supported the largest overall abundances of motile fauna. Motile fauna biomass was also significantly different between sampling dates ($p < 0.0001$) and substrate types ($p < 0.0001$). Mirroring the abundance patterns, motile fauna biomass was greatest in July 2014 ($\overline{X}$= 80.9 g m^{-2}) and October 2014 ($\overline{X}$= 81.2 g m^{-2}; Fig. 5B). Oyster shell ($\overline{X}$= 80.9 g m^{-2}) and limestone ($\overline{X}$= 57.8 g m^{-2}) supported the greatest overall biomass of motile fauna. There were significant differences in motile fauna diversity between sampling dates ($p < .0001$) but not substrate types. Diversity (N1) peaked in April 2014 ($\overline{X}$= 4.1), prior to peaks in abundance and biomass, declined to a low in July 2014 ($\overline{X}$= 1.9), and then slowly increased again through time (Fig. 5C).

Crabs (all species) were the most abundant motile fauna on the restored reef (Fig. 6A). Crab abundances varied significantly between sampling dates ($p <0.0001$) but not substrate types. Crab abundance was greater in July 2014 ($\overline{X}$= 2674 m^{-2}), October 2014 ($\overline{X}$= 1618 m^{-2}) and January 2015 ($\overline{X}$= 878 m^{-2}) than any other sampling dates. Crab biomass was greatest in July 2014 ($\overline{X}$= 64.2 g m^{-2}) and on oyster shell substrates ($\overline{X}$= 52.7 g m^{-2}; Fig. 7A). Fish were most abundant on oyster shell substrate ($\overline{X}$= 37 m^{-2}) and followed similar abundance patterns as crabs (Fig. 6B). Fish biomass varied both seasonally ($p<.0001$) and by substrate type ($p<.0001$; Fig. 7B). Fish biomass generally increased over time, with the greatest values occurring in the collections made during the final two sampling dates (January 2015, April 2015). Oyster shell ($\overline{X}$= 21.8 g m^{-2}) and limestone ($\overline{X}$= 19.4 g m^{-2}) supported the greatest amount of fish biomass. Oyster shell also supported the largest abundances ($\overline{X}$= 129 m^{-2}) and greatest biomass ($\overline{X}$= 6.4 g m^{-2}) of shrimp (Fig. 6C, 7C). Shrimp abundance was significantly different among sampling dates ($p <0.0001$) but did not follow the same general patterns as crabs and fish; instead abundances increased gradually over time, January 2015 ($\overline{X}$= 169 m^{-2}) and April 2015 ($\overline{X}$= 136 m^{-2}) supporting the largest abundances. Shrimp biomass was greatest in October 2014 ($\overline{X}$= 6.9 g m^{-2}) and April 2015 ($\overline{X}$= 4.9 g m^{-2}).

Non-metric multidimensional scaling analysis on species abundance demonstrated that motile fauna communities separated into two main groups driven by sampling dates (Fig. 8). The group on the left comprises all samples from January 2014 and April 2014 sampling dates, during early reef development. The group on the right includes all samples from July 2014-April 2015. The tighter spacing between points in this second group indicates greater similarity in community structure among samples compared to those collected earlier in reef development. Motile fauna communities were significantly different between substrate type and sampling date (ANOSIM: substrates p=0.003, dates p= 0.0001). Differences in substrate-related community structure were driven by oyster shell, which supported a community of motile fauna that were significantly different than river rock (p= 0.0005) and concrete (p= 0.011) substrates. The main fauna affecting the substrate groupings were *Palaemonetes vulgaris* (marsh shrimp), *Gobiosoma bosc* (naked goby), *Eurypanopeus turgidus* (ridgeback mud crab) and *Opsanus beta* (gulf toadfish). The most abundant species across all substrates were *Petrolisthes* sp. (porcelain crabs), *Alpheus heterochaelis* (snapping shrimp), and *Dyspanopeus texanus* (gulf grassflat crab).

MDS analysis on species biomass mirrored that of abundance with community biomass varying significantly between substrate types and sampling dates (ANOSIM: substrates p= 0.001, dates p= 0.001). Oyster shell and limestone supported the greatest faunal biomass with gulf toadfish, porcelain crabs and *Menippe adina* (gulf stone crab) having the largest influence in the differences between substrates. Porcelain crabs were the largest contributor to faunal biomass across all substrates.

Benefit-cost ratios

Benefit-cost ratios (BCRs) were calculated for oyster and motile fauna production separately (Table 3). The higher ratios correlate to higher ecological benefits in relation to the cost of material. Market prices for each substrate type varied as follows: concrete rubble (\$27.14 m^{-3}), limestone (\$38.25 m^{-3}), river rock (\$42.18 m^{-3}) and oyster shell (\$32.70 m^{-3}). Similar to our tests for oyster abundance, concrete (34.4) and limestone (23.3) scored higher than oyster shell (19.6) and river rock (12.9) when evaluating BCRs for oyster production. When applying the BCR equation to motile fauna production, oyster shell (36.1) scored slightly higher than concrete (35.3); both substrates scored well above limestone (20.8) and river rock (17.1). The BCR's for our tested substrates ranged from 17.1- 36.1 but were all considered successful based on our project goals. Any scores lower than 5 may be cause for consideration of other materials.

Discussion

This study focused on the effect substrate material has on oyster and motile fauna community development during reef restoration. The ability of a substrate to support oyster populations and provide habitat for other organisms is the basic component in reef restoration. Once substrates are deemed suitable for habitat creation, restoration practitioners should choose a substrate that's both economically and biologically favorable based on their restoration goals and budget. We tested four substrate types and tracked the community development on the reef for ~18 months. All examined substrates were suitable for attracting oysters and establishing oyster reef communities. Variability in the performance of these substrates, regarding different aspects of the reef development, indicates substrate choice should be considered carefully and be dependent on the restoration goals.

Oyster Communities

Oysters were present at low abundances on all substrates by the first sampling period, 3 months post tray deployment. Nine months after tray deployment, in July 2014, there were large increases in oyster abundance across all substrate types, corresponding with the summer recruitment period. Oyster abundance after nine months ($\overline{X}$= 1288 m^{-2}) was similar to other restoration studies conducted in the area (George et al. 2014; 617-1556 m^{-2}) and elsewhere in the Gulf of Mexico (Soniat & Burton 2005; 338- 2156 m^{-2}).

Oyster abundance (in relation to substrate type) fluctuated somewhat after the first summer but generally stayed similar across time, indicating no other seasonal influences once oysters were established.

Concrete and limestone supported the greatest abundances of oysters ($\overline{X}$= 1022 and 939 m^{-2}, respectively) during the study. Concrete also proved to be a successful material in a study conducted by Dunn et al. 2014 in North Carolina, while limestone attracted more oysters than sandstone in a study done in Louisiana (Soniat & Burton 2005). The size and texture of reef material influences the attractiveness of substrates to oysters (Fuchs & Reidenbach 2013) and may have played a role in our study. Rough textured materials (i.e. concrete, limestone) create more turbulence near the reef surface and may increase oyster settlement compared to smooth river rock (Fuchs & Reidenbach 2013). Oyster shells are relatively flat compared to the other substrates and tended to settle into even layers, reducing the three-dimensional complexity that normally promotes prey survivorship and oyster recruitment success (Grabowski & Powers 2004; Humphries et al. 2011). The relatively smooth texture of river rock may have also decreased the ability of oysters to attach permanently; another smooth-textured material, polished marble, is used for hatchery-setting of oysters because its surface aids in the removal of juvenile oysters (Hidu et al. 1975; George et al. 2014). Concrete and limestone rocks were slightly larger (12.7 X 15.2 cm) than the oyster shells and river rocks (7.6 X 10.2 cm), which may have provided more surface area for recruitment, and complexity and interstitial space for predator refuge (Bartol et al. 1999). The smaller substrates may also have been less structurally stabile and organisms may have been crushed or detached by substrate movement, which underscores the importance of substrate size in areas of turbulent water (Shelly 1979). Qualitatively we noticed more oyster scars, remnants of a settled oyster after mortality, on oyster shell and river rock than the other substrates.

Oyster shell heights increased gradually on all substrates between January 2014, during early reef development, and April 2014. Shell heights declined in the summer, most likely due to the large numbers of small recruits colonizing the reef during spawning season. From July 2014 until the end of the monitoring period oyster sizes increased, with a few approaching market size (76 mm) by the last sampling event and one reaching that benchmark. Mean shell heights on the experimental reefs were all within the classification of spat (< 25 mm) which is consistent with other restoration studies of similar duration (Luckenbach et al. 2005; Nestlerode et al. 2007). Trends in mean oyster size were similar across substrate type, but the largest oysters were observed on concrete material. It is important to note that concrete and limestone not only supported the greatest overall abundances of oysters (all sizes combined), but that of juveniles as well. Post-settlement survival and growth is key to the ultimate success of the oyster communities, and substrates able to promote this should be utilized. The large numbers of small oysters on the reef could be due to the warm climate in south Texas, where temperatures usually stay warm enough to support oyster spawning year round (Quast et al. 1988). The constant supply of recruits could delay the emergence of larger size classes during the early stages of the life of a reef. Despite the smaller size classes, oyster size increased on all substrate types indicating survival and growth on all examined materials.

Sessile Fauna

The most abundant sessile organisms observed on the substrates (besides oysters) were *Balanus* spp. and serpulid worms. Both were abundant throughout the monitoring period but occupied minimal area due to their small size. *Balanus* spp. were most abundant during the spring and summer months, one of their expected recruitment periods (Brown & Swearingen 1998). Barnacles did not demonstrate preference for a particular substrate, nor did their abundance appear to be negatively correlated with oyster populations. This coexistence could be due to settlement preferences of the two sessile organisms. A study conducted in Galveston Bay, Texas, found that oysters were the dominant species on pilings within 10 m of shore, and barnacles dominated the pilings 10 m out and further (Bushek 1988). The other sessile organisms (i.e. slipper shells, jingle shells, mytillid mussels and tunicates) were most abundant during the winter and spring, similar to previous studies (Dean & Hurd 1980). These organisms showed no clear substrate preference, but were all the least abundant on river rock material, indicating a relative inability of this substrate to promote long-term organism attachment.

Qualitatively, I observed a consistent presence of the red alga *Gracilaria tikvahiae* algae on some reef substrates. *Gracilaria tikvahiae* is a eurythermal, euryhaline red alga (Bird & McLachlan 1988) that is ubiquitous in bays, inlets, and estuaries on the Atlantic coast, but ranges from Canada to southern Mexico (McLachlan 1979; Gurgel et al 2004). Algae were most frequently observed on limestone (6/6 sampling dates) and concrete (5/6 sampling dates) substrates. Under eutrophic conditions, *G. tikvahiae* can form dense algal mats and create hypoxic or anoxic conditions (Peckol & Rivers 1995). On a few occasions *G. tikvahiae* completely covered the substrate material within the sampling trays and parts of the reef complex, but was not observed to form mats. Its presence did not seem to hinder oyster recruitment since concrete and limestone supported similarly high abundances of oysters during its presence. Practitioners should monitor algal populations in future restoration efforts to ensure that its presence does not influence water quality or faunal population dynamics.

Motile Fauna Communities

The structure of natural and restored oyster reefs provides crucial habitat for a multitude of organisms including fish (with both ecological and commercial importance) and decapod crustaceans (Zimmerman et al. 1989; Breitburg 1999; Lenihan et al. 2001; Peterson et al. 2003; Toley & Volety 2005; Stunz et al. 2010; Quan et al. 2012). MDS analysis indicated a reef age influence on species abundance and biomass, community structure in January and April 2014 was different than all other sampling months, indicating a shift from early to later stage community development. This is consistent with other studies, indicating that the age of a reef affects oyster development and associated faunal community characteristics (Burt et al. 2011; Quan et al. 2012; Brown et al. 2014). Diversity of motile fauna was greatest in early reef development, and least in the summer (driven by fewer organisms at high abundances), indicating dominant organisms colonizing the reef. Oyster shell outperformed all other substrates for attracting motile fauna as evidenced by the overall greater abundances and biomasses. Concrete supported the next greatest faunal abundances, while limestone was second overall in supporting faunal biomass. Along with species-specific substrate relationships, seasonal variability in utilization of the reef may also play a role in the observed trends (Nevins et al. 2014).

Crabs were the most abundant motile fauna across all substrate types, ultimately driving the observed patterns in faunal abundance and biomass. The most common crabs collected were porcelain crabs, Gulf stone crabs and the mud crabs *Dyspanopeus texanus* and *Eurypanopeus turgidus*. The abundance and biomass of crabs was greatest in the summer and on oyster shell substrate, as has been seen in previous studies (Tolley & Volety 2005). Porcelain crabs were found in large numbers during the summer. Several species are commonly associated with oyster reefs, including the green porcelain crab *Petrolisthes armatus*, which dominates crab communities on oyster reefs in Georgia and South Carolina (Knott et al. 1999; Hollebone 2006; Tillburg et al. 2010). Porcelain crabs are usually filter feeders (Caine 1975) that use oyster matrices for access to the water column (Tolley & Volety 2005). These crabs play an important role in the trophic structure of oyster reefs, serving as a prey item for multiple reef residents including commercially important species like grey snapper (Yeager & Layman 2011). Unlike porcelain crabs, mud crabs are a common intermediate predator on oyster reefs, consuming juvenile oysters (McDermott & Flower 1952; Meyer 1994; Grabowski 2004) and ribbed mussels (Seed 1980), both of which were present on the reef. It is not uncommon for a few crab species to dominate shallow reef communities. In a study comparing fauna communities in estuarine habitats, Glancey et al. (2003) found that two species of mud crabs and the green porcelain crab made up 95% of the faunal abundance on oyster reef habitat. Gulf stone crabs, a commercially important decapod predator (Rinedone & Eggleston 2011), were not observed in tray samples until 6 months post-deployment, but were consistently found thereafter. Stone crabs consume small oysters and other invertebrates (Brown & Haight 1992) and the delayed presence of these crabs could be because there were few oyster present during early reef development. Although the crab community on the reef was not diverse, they were very abundant and contributed a great deal of biomass which is promising due to their important role in reef ecosystems, both as prey items and predators.

The other decapod group of interest, shrimp, were most abundant with large amounts of biomass on oyster shell substrate. Snapping shrimp were the dominant shrimp on the reef, consistent with previous studies (Lehnert & Allen 2002; Bourdreaux et al. 2006). Snapping shrimp are symbionts of the mud crab *Panopeous herbstii*, living in its burrows in salt marshes (Silliman et al. 2003). Although *P. herbstii* was not present in our samples, other mud crab species, *E. turgidus* and *D. texanus* were commonly collected. *Palaemonetes* spp. (grass shrimp) were the next most abundant shrimp found during the study. Although usually associated with marsh ecosystems, grass shrimp have been documented in high abundances on oyster reefs (Wells 1961; Lenihan et al. 2001; Stunz et al. 2010), and preferred oyster reef habitat as a refuge in the presence of predators in (Humphries et al. 2011). These shrimp are an important part of estuarine ecosystems, serving as prey items for commercially important species like *Sciaenops occelatus* (red drum) (Scharf et al. 2000).

The largest numbers of fish were observed during the summer and on oyster shell substrates. Fish biomass gradually increased over time on oyster shell but remained fairly constant on river rock and concrete. The abundance of fish was greatest in July 2014 on all substrates, but much greater increases were seen on oyster shell than any other substrate during that time, almost doubling from April 2014. The large increase on oyster shell alone may suggest that fish communities prefer the oyster shell over other substrates, specifically for spawning and refuge habitat. The three dominant fish species observed were naked goby, Gulf toadfish and *Gobiesox strumosus* (skilletfish). Naked gobies use the inside of the empty shells as an egg attachment site (Nero 1976) and live inside the cracks and crevices provided by rocks, oyster shells, and other hard substrates for refuge (Breitburg 1991; Harding & Mann 2000; Tolley & Volety 2005). Goby larvae often selectively feed on oyster larvae (Harding & Mann 1999). Like naked gobies, skilletfish utilize the reef for reproduction and have shown preferences for the unfouled inner surfaces of shells as spawning substrate (Kuhlmann 1998). Gulf toadfish are a common reef predator, feeding on abundant crab populations (Grabowski 2004; Grabowski et al. 2008; Heithaus et al. 2008). Crab abundance increased greatly during the summer on oyster shell which may be attributed to the activities for which these fish species use reef habitats, attesting to the importance of substrate choice in restoration projects.

Benefit-costs of substrates

The benefits of successful reef restoration have been well documented which solidifies the need for continued restoration practice. Successfully restored reefs can provide ecosystem services worth between $5,500 and $99,000 per hectare per year and regain the restoration costs in 2-14 years (Grabowski et al. 2012). Restoration efforts are not always successful based on the original goals which wastes time, effort, and project funds. A study in Alabama restored oyster reefs adjacent to salt marsh habitat but found no significant increase in overall fish or motile crustacean abundance, possibly due to the redundancy of the site selection since habitat was already available (Geraldi et al. 2009). To avoid restoration failure, and maximize the benefits provided by the reef it is important plan carefully. Once a site location and a reef design/size are selected, the costs of construction will be dependent on the choice of the material used.

All substrates used in this study were capable of supporting populations of oysters and motile fauna. Although all substrates were successful according to my restoration goals, there were differences in their performances that should be considered when choosing materials for future projects. When I applied BCR analysis to the substrate performances, concrete was better for sustaining oyster populations while oyster shell was better for creating habitat for motile fauna communities (Table 3.). These results were similar to the original analysis, prior to incorporating the substrate economics and BCR equations. This does not take away from the importance of applying BCR analysis in future projects, since all situations are different and certain aspects can alter the BCR ratings.

This project used materials that were obtained from the same region, thus transportation costs did not significantly alter the benefit-cost analysis. However, in many cases, transportation costs, and associated environmental footprints should be incorporated when assessing the cost of materials. Gonzales et al. (in press) found that when looking at risk of various environmental impacts (global warming potential, acidification potential, eutrophication potential), limestone and river rock ranked highest among the tested substrates, mainly due to the extraction and transportation processes of these mined materials. These findings demonstrate the complex decision making involved when choosing reef material for restoration. Circumstances and material costs will vary among restoration projects, but using the necessary attributes and applying them to a metric will be beneficial when planning future restorations.

Conclusions

The purpose of this study was to evaluate four substrates for reef restoration, based on their capacity to support oyster populations and motile fauna communities. The future success of habitat restoration relies upon the continued improvements of the entire process. Reef construction is one of the first steps in the process, thus substrate choice could determine the success or failure of the project. Our results show that concrete, oyster shell, limestone and river rock are suitable substrates for reef restoration but vary in their ability to attract oyster and faunal communities. The larger, rougher textured substrates (concrete, limestone) outperformed the others for attracting oysters while oyster shell was preferable in creating habitat for motile fauna. The choice of materials cannot always be based solely on the ecological benefits, but I have suggested a simple metric to use when there are options between suitable substrates, based on ecological performance and economic logistics. These findings can guide restoration practitioners towards the right substrate based on the specific restoration goals.

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Table 4. Mean abundances (±SE) of motile fauna species across substrate types.

		Substrate Material							
Common name	Scientific name	Concrete	(SE±)	Limestone	(SE±)	Oyster Shell	(SE±)	River rock	(SE±)
Porcelain crabs	*Petrolisthes* sp.	778.9	(44.1)	620.0	(105.1)	1188.5	(305.9)	569.3	(46.8)
Texas mud crab	*Dyspanopeus texana*	148.5	(49.1)	91.1	(23.2)	123.3	(27.0)	101.9	(27.8)
Snapping shrimp	*Alpheus heterochaelis*	63.3	(14.5)	64.1	(16.9)	120.7	(7.2)	70.0	(14.2)
Gulf Stone crab	*Menippe adina*	25.9	(7.7)	21.9	(10.4)	35.9	(8.0)	25.2	(7.1)
Naked goby	*Gobiosoma bosc*	5.6	(1.3)	14.8	(3.5)	20.7	(3.0)	10.0	(1.3)
Ridgeback mud crab	*Eurypanopeus turgidus*	7.0	(2.1)	10.0	(5.1)	14.8	(10.4)	11.9	(5.7)
Grass shrimp	*Palaemonetes vulgaris*	7.0	(3.5)	6.3	(3.3)	7.4	(3.0)	1.1	(1.1)
Skilletfish	*Gobiesox strumosus*	3.3	(1.7)	1.5	(0.7)	9.3	(2.4)	1.1	(0.6)
Gulf Toadfish	*Opsanus beta*	1.1	(1.1)	5.6	(0.0)	6.7	(0.6)	0.0	(0.0)
Blue crab	*Callinectes sapidus*	4.4	(1.7)	1.5	(1.0)	4.1	(2.1)	1.5	(1.0)
Grass shrimp	*Palaemonetes pugio*	1.5	(1.0)	0.0	(0.0)	0.4	(0.4)	0.0	(0.0)
Larval fish	Unidentified larval fish	0.0	(0.0)	1.5	(1.5)	0.0	(0.0)	0.0	(0.0)
Speckled snapping shrimp	*Synalpheus fritzmuelleri*	0.0	(0.0)	0.4	(0.4)	0.4	(0.4)	0.0	(0.0)
Blennies	Blenniidae sp.	0.0	(0.0)	0.0	(0.0)	0.4	(0.4)	0.0	(0.0)
Stretchjaw blenny	*Chasmodes longimaxilla*	0.0	(0.0)	0.0	(0.0)	0.4	(0.4)	0.0	(0.0)
Brown grass shrimp	*Leander tenucornis*	0.4	(0.4)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Larval Sciaenids	Sciaenidae sp.	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.4	(0.4)
Pipefish	Syngnathidae sp.	0.0	(0.0)	0.4	(0.4)	0.0	(0.0)	0.0	(0.0)

Table 5. Mean abundances (±SE) of motile fauna across sampling dates.

		Sampling dates											
Common name	Scientific name	Jan-14	(SE±)	April-14	(SE±)	July-14	(SE±)	Oct-14	(SE±)	January-15	(SE±)	April-14	(SE±)
Porcelain crabs	*Petrolisthes* sp.	27.8	(5.0)	66.1	(11.3)	2346.7	(618.8)	1318.3	(204.0)	690.0	(57.0)	286.1	(23.2)
Texas mud crab	*Dyspanopeus texana*	3.3	(1.1)	2.8	(2.1)	213.3	(45.1)	248.9	(37.7)	162.8	(10.1)	66.1	(4.3)
Snapping shrimp	*Alpheus heterochaelis*	5.6	(1.4)	10.0	(2.1)	53.3	(9.6)	108.9	(29.0)	168.3	(34.1)	131.1	(17.7)
Gulf Stone crab	*Menippe adina*	0.0	(0.0)	0.0	(0.0)	76.7	(9.3)	51.1	(9.1)	20.6	(5.2)	15.0	(1.7)
Naked goby	*Gobiosoma bosc*	15.6	(4.7)	25.6	(5.6)	13.9	(7.3)	11.7	(4.2)	7.2	(2.8)	2.8	(1.7)
Ridgeback mud crab	*Eurypanopeus turgidus*	1.7	(0.6)	15.6	(3.1)	37.2	(11.0)	0.0	(0.0)	5.6	(3.7)	5.6	(0.6)
Grass shrimp	*Palaemonetes vulgaris*	19.4	(8.2)	2.8	(1.7)	5.0	(5.0)	0.6	(0.6)	1.1	(0.6)	3.9	(1.4)
Skilletfish	*Gobiesox strumosus*	0.0	(0.0)	0.6	(0.6)	11.1	(7.5)	5.6	(3.7)	5.6	(1.9)	0.0	(0.0)
Gulf Toadfish	*Opsanus beta*	0.0	(0.0)	0.6	(0.6)	8.9	(4.0)	3.3	(2.1)	3.9	(1.9)	3.3	(2.1)
Blue crab	*Callinectes sapidus*	2.8	(2.1)	14.4	(3.7)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Grass shrimp	*Palaemonetes pugio*	0.0	(0.0)	1.7	(1.1)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	1.1	(1.1)

		Sampling dates											
Common name	Scientific name	Jan-14	(SE±)	April-14	(SE±)	July-14	(SE±)	Oct-14	(SE±)	January-15	(SE±)	April-14	(SE±)
Larval fish	Unidentified larval fish	0.0	0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	2.2	(2.2)
Speckled snapping shrimp	*Synalpheus fritzmuelleri*	0.6	(0.6)	0.6	(0.6)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Blennies	Blenniidae sp.	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.6	(0.6)	0.0	(0.0)	0.0	(0.0)
Stretchjaw blenny	*Chasmodes longimaxilla*	0.0	(0.0)	0.0	(0.0)	0.6	(0.6)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Brown grass shrimp	*Leander tenucornis*	0.0	(0.0)	0.6	(0.6)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Larval Sciaenids	Sciaenidae sp.	0.6	(0.6)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Pipefish	Syngnathidae sp.	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.6	(0.6)	0.0	(0.0)

Table 6. Substrate characteristics including price, mean abundances of oysters and motile fauna and associated Benefit Cost ratios.

	Cost (Dollars m^{-3})		*Benefit*			
Substrate	*Price*	*Transportation*	*Oysters (m^{-2})*	*Fauna (m^{-2})*	*BCR (Oysters)*	*BCR (Fauna)*
Shell	32.7	9.8	832	1533	19.6	36.1
Concrete	27.1	2.6	1022	1047	34.4	35.3
Limestone	38.3	2.0	939	838.9	23.3	20.8
River rock	42.2	4.1	597	792.2	12.9	17.1

Figure captions:

Figure 1. Location of the restored oyster reef in Aransas Bay, TX, part of the Mission-Aransas Estuary, shown in state (a) estuary (b) and local (c) scales. Repeated colors indicate reef mounds of the same substrate type.

Figure 2. Abundance (n m^{-2}) of A) Total oysters B) Spat (< 25 mm) and C) juvenile oysters (≥ 25 mm) : quarterly averages ± standard error across four substrate types and six sampling dates in the Mission-Aransas Estuary, TX.

Figure 3. Oyster size (shell height (mm)): quarterly averages ± standard error across four substrate types and six sampling dates in the Mission-Aransas Estuary, TX.

Figure 4. Percent (%) area cover: quarterly averages ± standard error for most abundant sessile organisms across four substrate types and six sampling dates in the Mission-Aransas Estuary, TX.

Figure 5. Motile fauna: quarterly averages ± standard error for A) abundance (n m^{-2}), B) Biomass (g m^{-2}), and C) N1 diversity across four substrate types and six sampling dates in the Mission-Aransas Estuary, TX.

Figure 6. Individual motile fauna taxa: quarterly averages ± standard error of abundance (n m^{-2}) for A) Crab B) Fish C) Shrimp across four substrate types and six sampling dates in the Mission-Aransas Estuary, TX.

Figure 7. Individual motile fauna taxa: quarterly averages ± standard error of biomass (g m^{-2}) for A) Crab B) Fish C) Shrimp across four substrate types and six sampling dates in the Mission-Aransas Estuary, TX.

Figure 8. Multidimensional scaling plot of community structure by sampling date and substrate type. Letters refer to substrate type, colors to sampling date.

Figure 1.

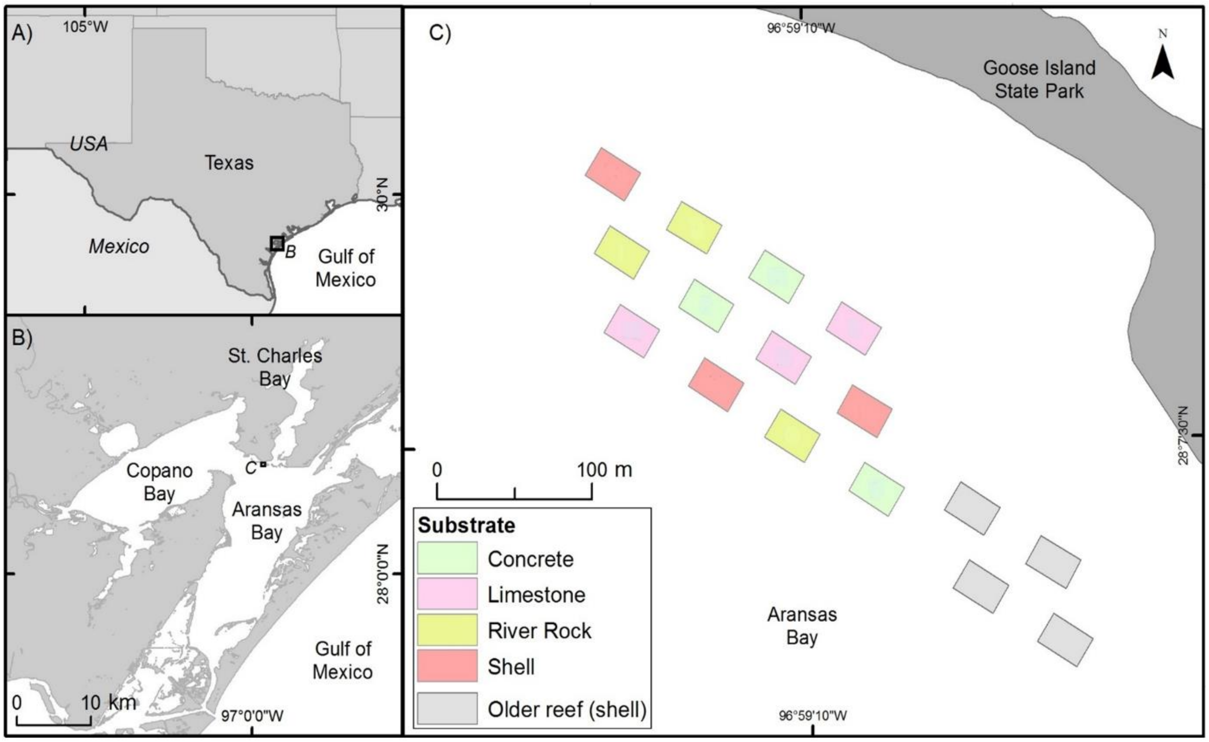

A)
105°W
USA
Texas
30°N
Mexico
B
Gulf of
Mexico
B)
St. Charles
Bay
Copano
Bay
C
Aransas
Bay
28°0'0"N
Gulf of
Mexico
0
10 km
97°0'0"W
C)
96°59'10"W
Goose Island
State Park
N
28°7'30"N
0
100 m
Substrate
Concrete
Limestone
River Rock
Shell
Older reef (shell)
Aransas
Bay
96°59'10"W

Figure 2.

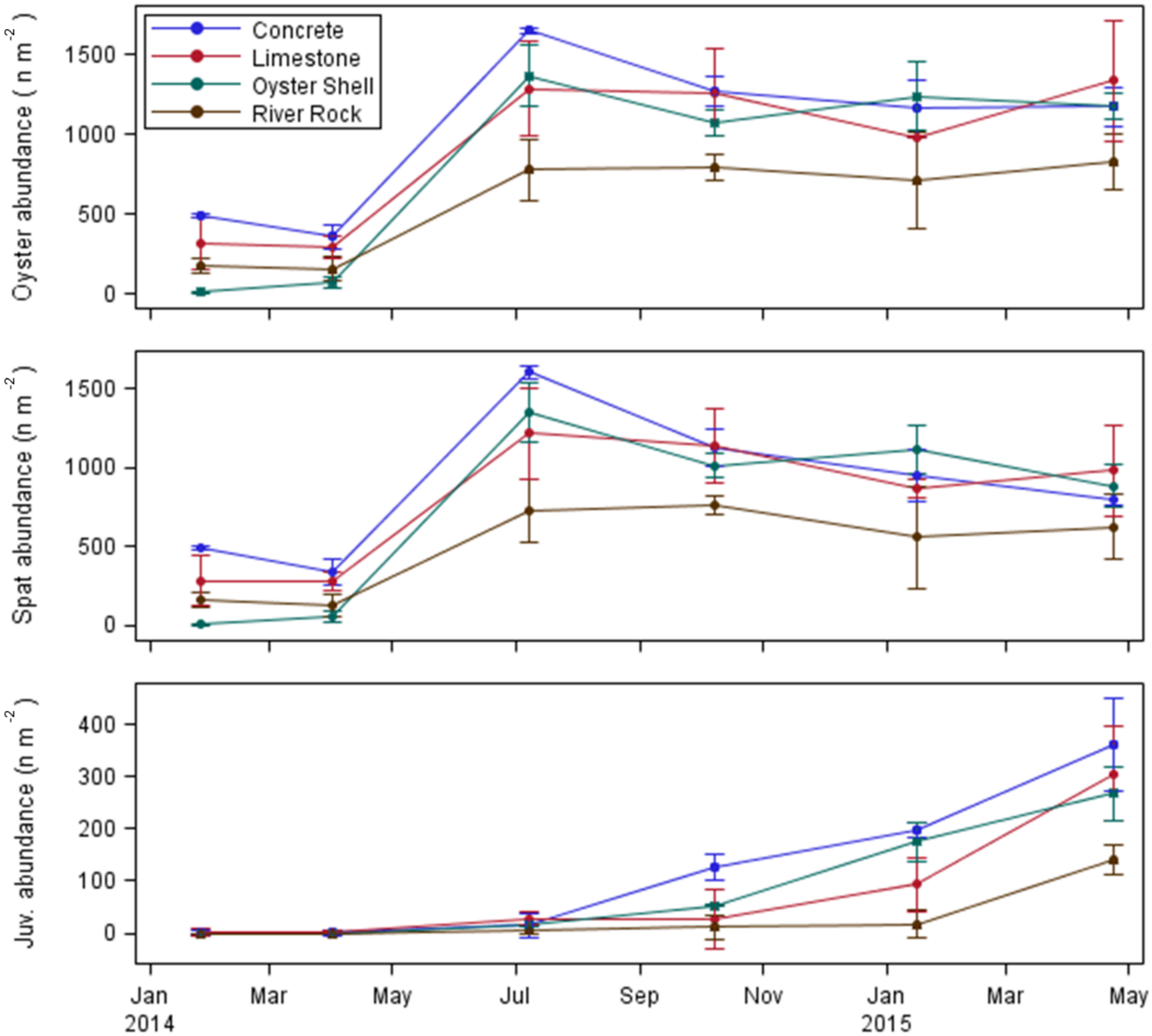

Concrete
Limestone
Oyster Shell
River Rock
Oyster abundance (n m -2)
Spat abundance (n m -2)
Juv. abundance (n m -2)
1500
1000
500
0
400
300
200
100
Jan 2014
Mar
May
Jul
Sep
Nov
Jan 2015
Mar
May

Figure 3.

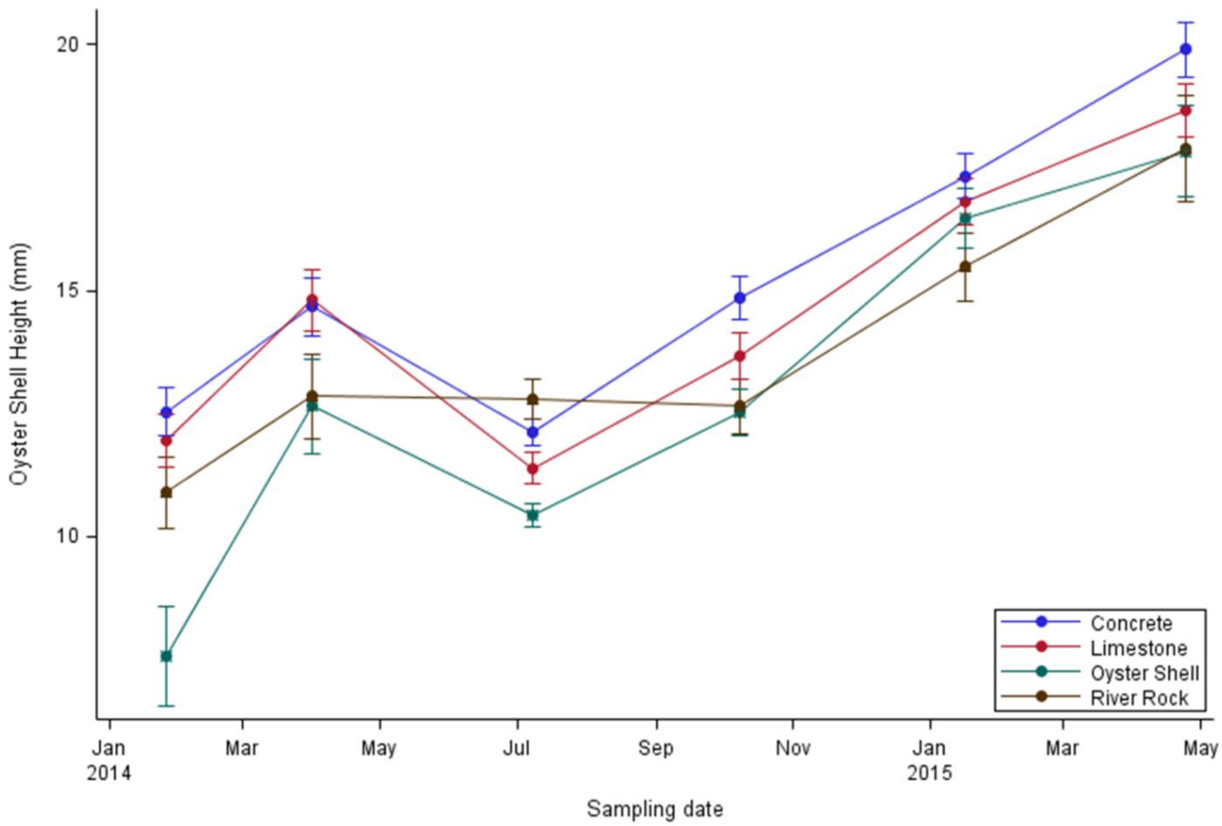

Oyster Shell Height (mm)
20
15
10
Jan 2014
Mar
May
Jul
Sep
Nov
Jan 2015
Mar
May
Sampling date
Concrete
Limestone
Oyster Shell
River Rock

Figure 4.

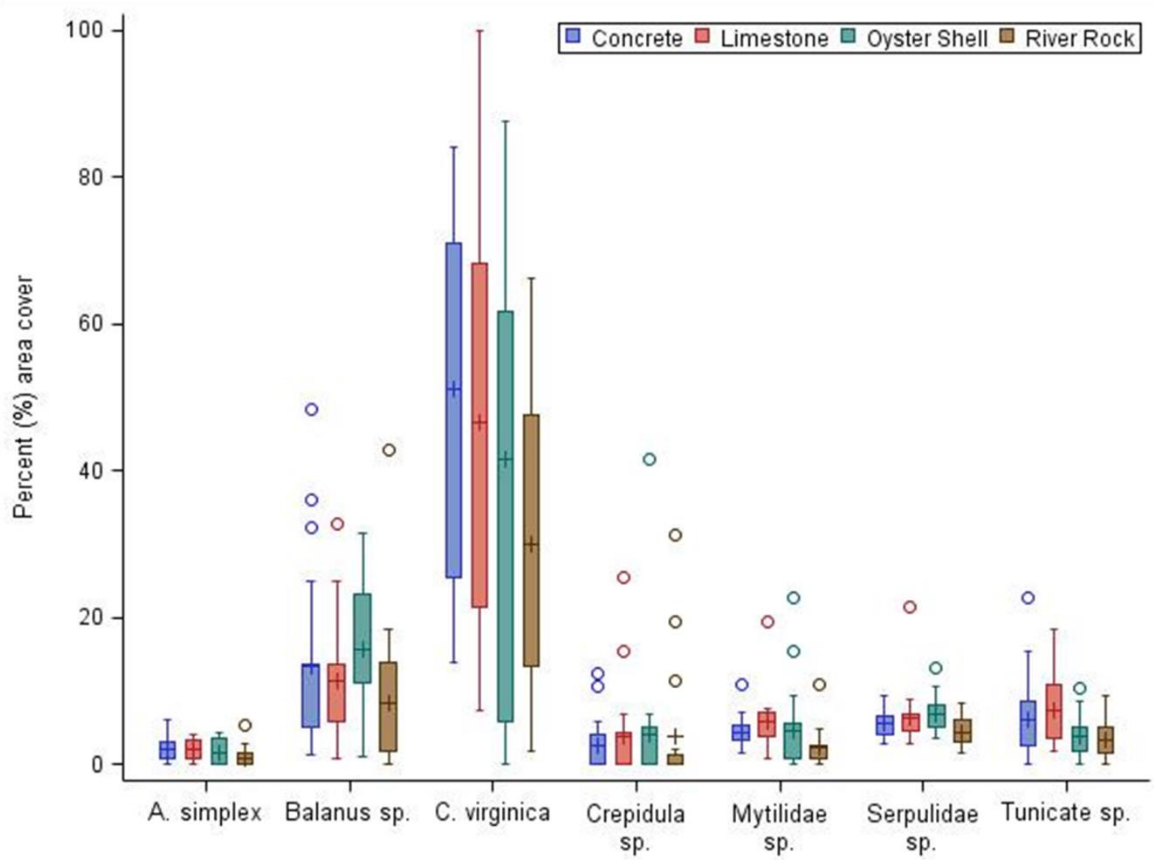

Concrete
Limestone
Oyster Shell
River Rock
Percent (%) area cover
100
80
60
40
20
0
A. simplex
Balanus sp.
C. virginica
Crepidula sp.
Mytilidae sp.
Serpulidae sp.
Tunicate sp.

Figure 5.

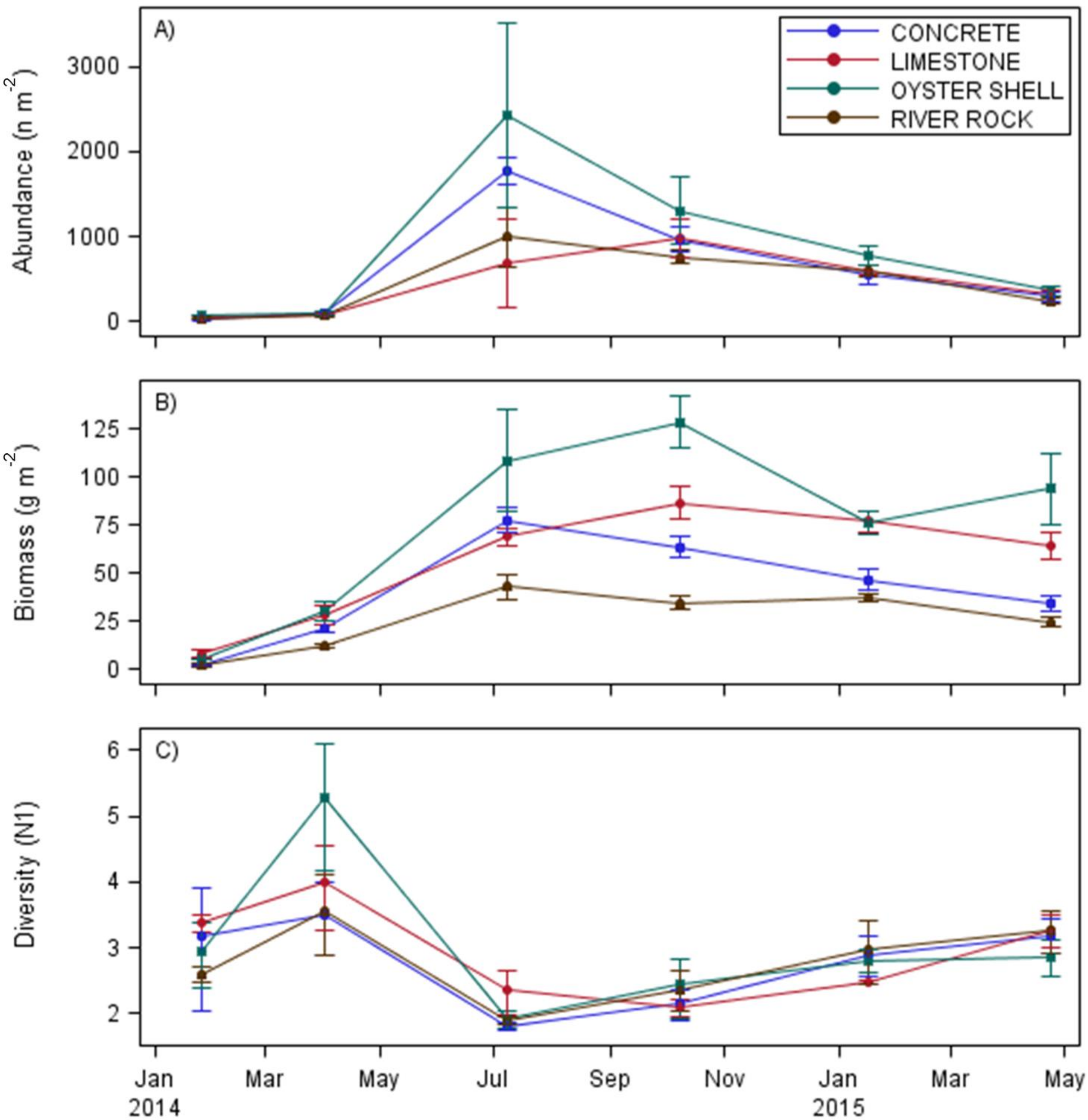

A)
CONCRETE
LIMESTONE
OYSTER SHELL
RIVER ROCK
Abundance (n m-2)
0
1000
2000
3000
B)
Biomass (g m-2)
0
25
50
75
100
125
C)
Diversity (N1)
2
3
4
5
6
Jan 2014
Mar
May
Jul
Sep
Nov
Jan 2015
Mar
May

Figure 6.

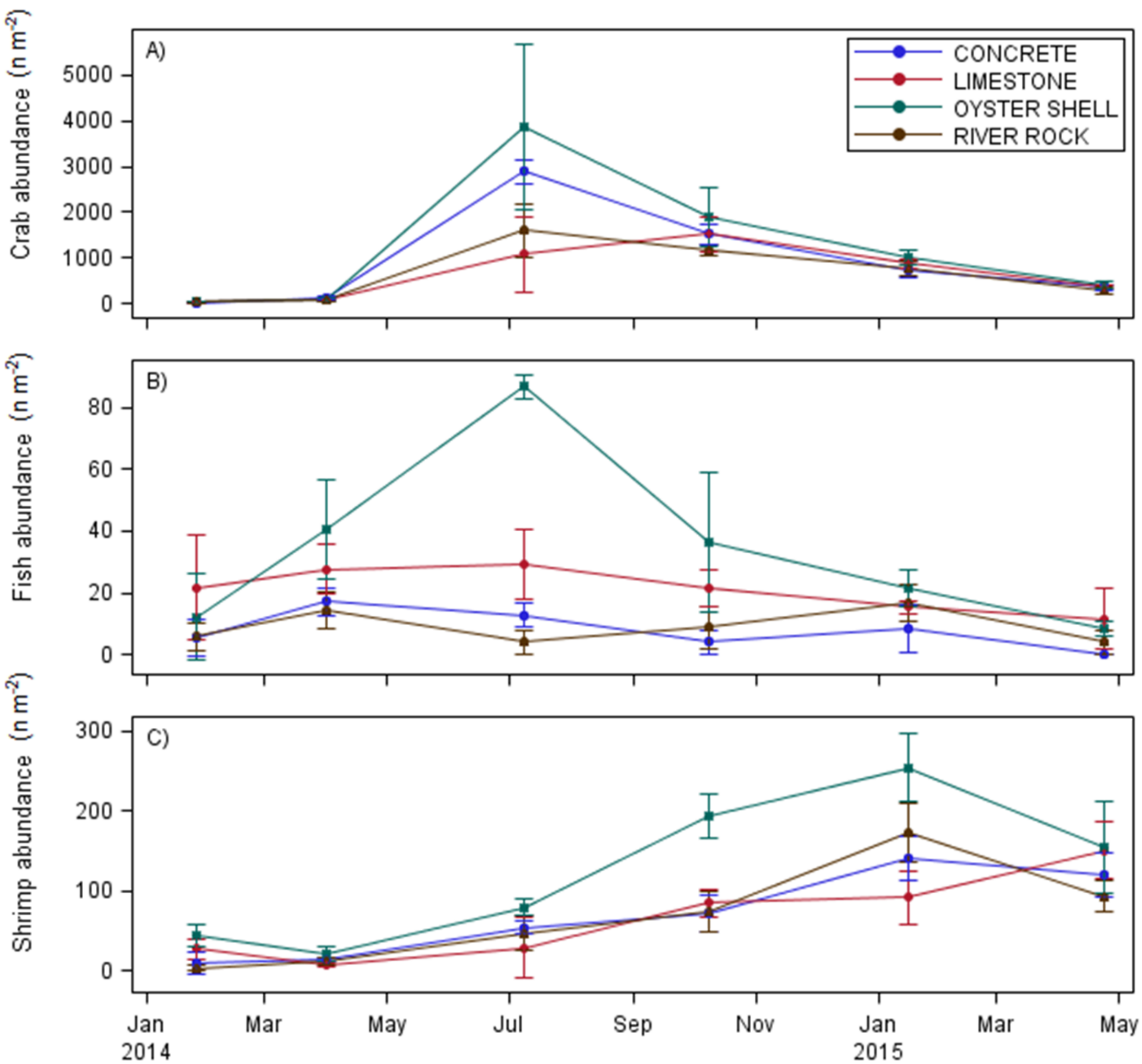
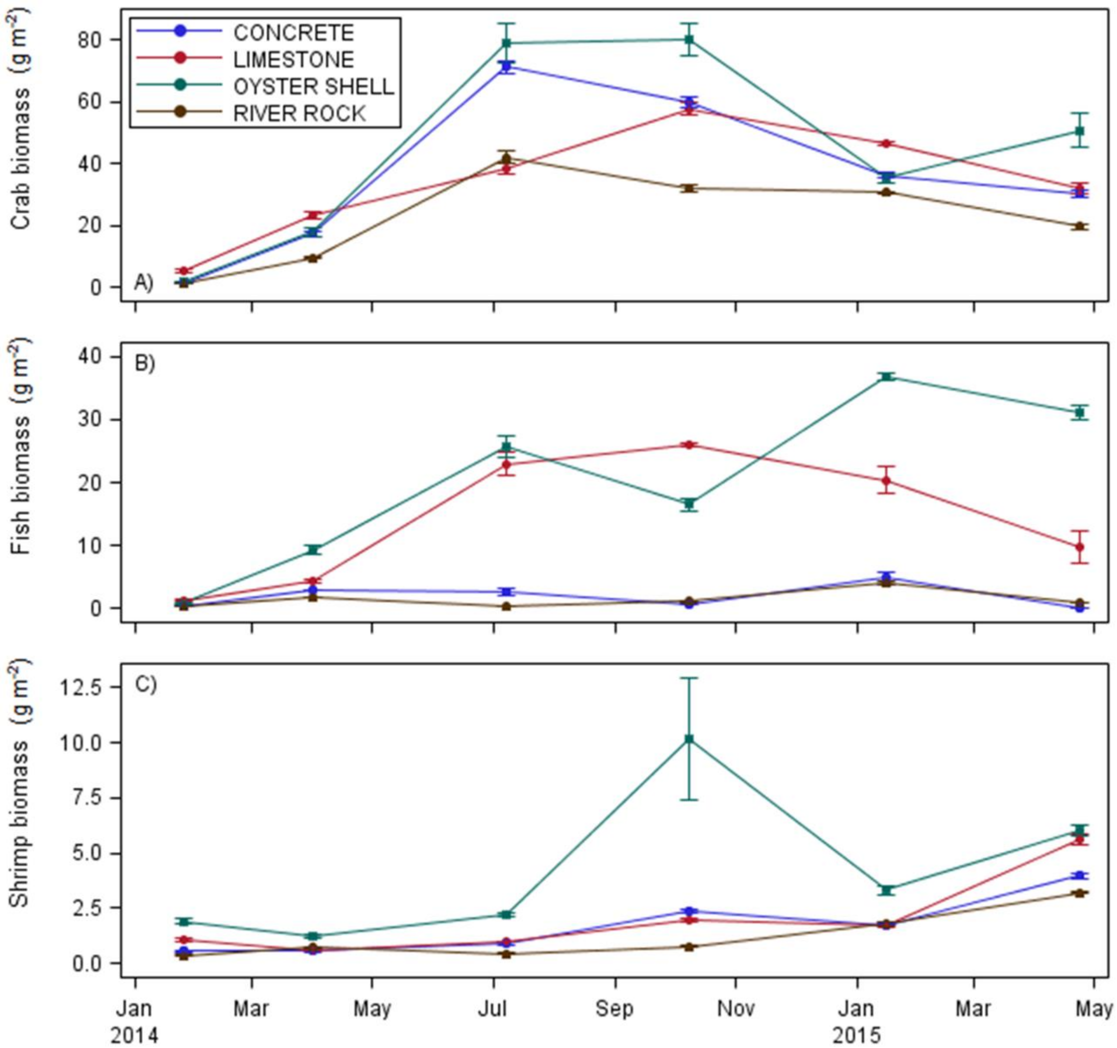
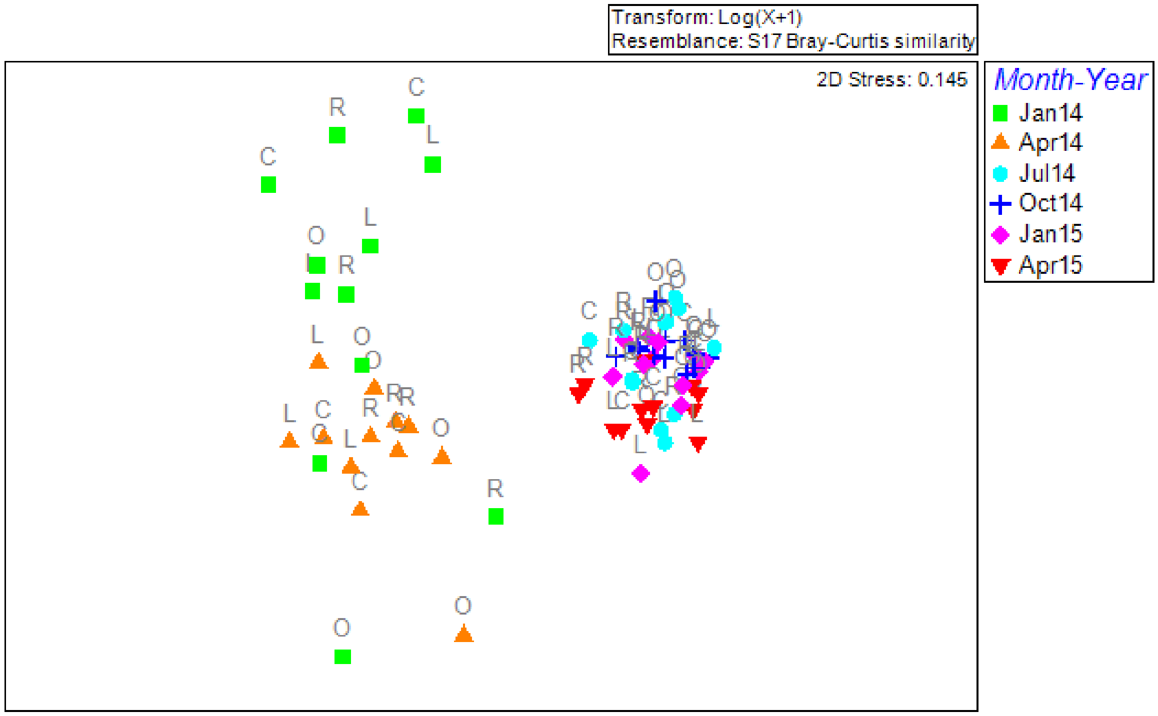

A)
B)
C)
CONCRETE
LIMESTONE
OYSTER SHELL
RIVER ROCK
Crab abundance (n m^{-2})
Fish abundance (n m^{-2})
Shrimp abundance (n m^{-2})
0
1000
2000
3000
4000
5000
0
20
40
60
80
0
100
200
300
Jan 2014
Mar
May
Jul
Sep
Nov
Jan 2015
Mar
May

Figure 7.



CONCRETE
LIMESTONE
OYSTER SHELL
RIVER ROCK
Crab biomass (g m-2)
A)
Fish biomass (g m-2)
B)
Shrimp biomass (g m-2)
C)
Jan 2014
Mar
May
Jul
Sep
Nov
Jan 2015
Mar
May

Figure 8.


Transform: Log(X+1)
Resemblance: S17 Bray-Curtis similarity
2D Stress: 0.145
Month-Year
Jan14
Apr14
Jul14
Oct14
Jan15
Apr15

Appendix A: ANOVA and Tukey's *post hoc* outputs

Table 7. ANOVA results for oyster abundance (square root transformed) by sampling date and substrate type.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Date	5	6004.710054	1200.942011	49.59	<.0001
Substrate	3	724.021754	241.340585	9.97	<.0001
Date*Substrate	15	672.051545	44.803436	1.85	0.0545

Table 8. Tukey's post hoc analysis for oyster abundance by sampling date.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**Date**
A	35.325	12	08JUL2014
A			
A	33.464	12	24APR2015
A			
A	33.047	12	08OCT2014
A			
A	31.844	12	16JAN2015
B	14.259	12	01APR2014
B			
B	14.080	12	26JAN2014

Table 9. Tukey's post hoc analysis on oyster abundance by substrate type.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Substrate**
	A	30.944	18	Concrete
	A			
B	A	29.044	18	Limestone
B				
B	C	25.161	18	Oyster Shell
	C			
	C	22.864	18	River Rock

Table 10. ANOVA results for fauna abundance (log-transformed) by substrate type and sampling date.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Date	5	118.7265934	23.7453187	127.23	<.0001
SUBSTRATE	3	2.8227199	0.9409066	5.04	0.0041
Date*SUBSTRATE	15	3.3001224	0.2200082	1.18	0.3197

Table 11. Tukey's post hoc analysis of faunal abundance by substrate type.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**SUBSTRATE**
	A	6.0367	18	OYSTER SHELL
	A			
B	A	5.6839	18	CONCRETE
B				
B		5.6296	18	LIMESTONE
B				
B		5.5027	18	RIVER ROCK

Table 12. Tukey's post hoc analysis of faunal abundance by sampling date.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Date**
	A	7.1739	12	08JUL2014
	A			
B	A	6.8910	12	08OCT2014
B				
B		6.4299	12	16JAN2015
	C	5.7105	12	24APR2015
	D	4.3832	12	01APR2014
	E	3.6907	12	26JAN2014

Table 13. ANOVA results of mean Hill's N1 diversity of motile fauna by substrate type and sampling date.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Date	5	32.79712299	6.55942460	12.10	<.0001
SUBSTRATE	3	0.82855765	0.27618588	0.51	0.6778
Date*SUBSTRATE	15	7.80130639	0.52008709	0.96	0.5101

Table 14. Tukey's post hoc analysis of mean N1 diversity by substrate type.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**SUBSTRATE**
A	1.35599	18	OYSTER SHELL
A			
A	1.34642	18	LIMESTONE
A			
A	1.31069	18	RIVER ROCK
A			
A	1.30314	18	CONCRETE

Table 15. Tukey's post hoc analysis of Hill's N1 diversity of motile fauna by sampling date.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Date**
	A	1.59968	12	01APR2014
	A			
B	A	1.41513	12	24APR2015
B				
B	C	1.37236	12	26JAN2014
B	C			
B	C	1.32415	12	16JAN2015
	C			
D	C	1.17373	12	08OCT2014
D				
D		1.08930	12	08JUL2014

Table 16. ANOVA results showing of crab abundance by sampling date and substrate type.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Date	5	165.1296841	33.0259368	134.60	<.0001
SUBSTRATE	3	1.7630372	0.5876791	2.40	0.0798
Date*SUBSTRATE	15	3.4491155	0.2299410	0.94	0.5317

Table 17. Tukey's post hoc analysis on crab abundance by substrate (log-transformed)

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**SUBSTRATE**
A	6.1672	18	OYSTER SHELL
A			
A	5.9413	18	CONCRETE
A			
A	5.8022	18	LIMESTONE
A			
A	5.7699	18	RIVER ROCK

Table 18. Tukey's post hoc analysis on crab abundance by sampling date

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Date**
	A	7.6471	12	08JUL2014
	A			
B	A	7.3190	12	08OCT2014
B				
B		6.7388	12	16JAN2015
	C	5.8773	12	24APR2015
	D	4.4882	12	01APR2014
	E	3.4505	12	26JAN2014

Table 19. ANOVA results of fish abundance by substrate type and sampling date (square root transformation).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Date	5	72.6517065	14.5303413	3.91	0.0047
SUBSTRATE	3	103.7680319	34.5893440	9.31	<.0001
Date*SUBSTRATE	15	59.5936555	3.9729104	1.07	0.4081

Table 20. Tukey's post hoc analysis of fish abundance by substrate type.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**SUBSTRATE**
	A	5.4641	18	OYSTER SHELL
	A			
B	A	4.5497	18	LIMESTONE
B				
B	C	2.9072	18	RIVER ROCK
	C			
	C	2.5153	18	CONCRETE

Table 21. Tukey's post hoc analysis of fish abundance by sampling date.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Date**
	A	5.0918	12	08JUL2014
	A			
	A	4.8944	12	01APR2014
	A			
B	A	3.9333	12	08OCT2014
B	A			
B	A	3.8921	12	16JAN2015
B	A			
B	A	3.2349	12	26JAN2014
B				
B		2.1079	12	24APR2015

Table 22. ANOVA results of shrimp abundance by substrate type and sampling date.

Source	DF	Type III SS	Mean Square	F Value	Pr > F

Date	5	832.4153280	166.4830656	28.62	<.0001
SUBSTRATE	3	118.6112923	39.5370974	6.80	0.0007
Date*SUBSTRATE	15	81.7250466	5.4483364	0.94	0.5321

Table 23. Tukey's post hoc analysis of shrimp abundance by substrate.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**SUBSTRATE**
A	10.4326	18	OYSTER SHELL
B	7.6417	18	CONCRETE
B			
B	7.4442	18	LIMESTONE
B			
B	7.3496	18	RIVER ROCK

Table 24. Tukey's post hoc results of shrimp abundance by sampling date.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Date**
	A	12.6602	12	16JAN2015
	A			
	A	11.3617	12	24APR2015
	A			
B	A	10.0696	12	08OCT2014
B				
B	C	7.1481	12	08JUL2014
	C			
D	C	4.2771	12	26JAN2014
D				
D		3.7854	12	01APR2014

Sessile organism coverage:

C. virginica: used arc sin transformation (arsinpct)

Table 25. ANOVA results for percent cover of *C.virginica* by sampling date and substrate type.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Date	5	4.40633460	0.88126692	21.13	<.0001
Substrate	3	0.68832077	0.22944026	5.50	0.0025
Date*Substrate	15	0.54372587	0.03624839	0.87	0.6005

Table 26. Tukey's post hoc analysis of C.virginica percent coverage by sampling date.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**Date**
A	0.73367	12	08JUL2014
A			
A	0.64714	12	24APR2015
A			
A	0.60217	12	08OCT2014
A			
A	0.56610	12	16JAN2015
B	0.13136	12	26JAN2014
B			
B	0.11644	12	01APR2014

Table 27. Tukey's post hoc analysis of C. virginica percent coverage by substrate type.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Substrate**
	A	0.56135	18	Concrete
	A			
	A	0.53624	18	Limestone
	A			
B	A	0.45630	18	Oyster Shell
B				
B		0.31069	18	River Rock

Balanus sp.

Table 28. ANOVA results of *Balanus sp*. percent coverage by substrate type and sampling date.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Date	5	0.43117225	0.08623445	18.97	<.0001
Substrate	3	0.05298601	0.01766200	3.89	0.0145
Date*Substrate	15	0.11649710	0.00776647	1.71	0.0810

Table 29. Tukey's post hoc analysis of *Balanus sp*. percent covereage by substrate type.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Substrate**
	A	15.679	18	Oyster Shell
	A			
B	A	13.426	18	Concrete
B	A			
B	A	11.365	18	Limestone
B				
B		8.379	18	River Rock

Table 30. Tukey's post hoc analysis of *Balanus sp*. percent coverage by sampling date.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Date**
	A	27.345	12	08JUL2014
	B	14.166	12	24APR2015
	B			
C	B	11.343	12	16JAN2015
C	B			
C	B	10.323	12	08OCT2014
C	B			
C	B	6.183	12	01APR2014
C				
C		3.915	12	26JAN2014

Serpulidae sp.

Table 31. ANOVA results of *Serpulidae sp*. percent coverage by substrate type and sampling date.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	5	71.28956753	14.25791351	2.03	0.0904
Substrate	3	62.22026178	20.74008726	2.96	0.0415
Date*Substrate	15	88.43316266	5.89554418	0.84	0.6291

Table 32. Tukey's post hoc analysis of *Serpulidae sp*. percent coverage by substrate type.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Substrate**
	A	6.8209	18	Oyster Shell
	A			
B	A	6.3343	18	Limestone
B	A			
B	A	5.4676	18	Concrete
B				
B		4.3757	18	River Rock

BIOMASS TABLES:

COMBINED TAXA (square root GM2)

Table 33. ANOVA results of combined motile fauna biomass by date and substrate type.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	5	404.2490025	80.8498005	33.45	<.0001
SUBSTRATE	3	115.0465426	38.3488475	15.86	<.0001
Date*SUBSTRATE	15	34.6513097	2.3100873	0.96	0.5134

Table 34. Tukey's post hoc analysis of combined fauna biomass by substrate type.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**SUBSTRATE**
	A	8.1756	18	OYSTER SHELL
	A			
B	A	7.1897	18	LIMESTONE

B				
B	C	5.9738	18	CONCRETE
	C			
	C	4.8156	18	RIVER ROCK

Table 35. Tukey's post hoc analysis of combined fauna biomass by sampling date.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**Date**
A	8.6961	12	08OCT2014
A			
A	8.6819	12	08JUL2014
A			
A	7.6572	12	16JAN2015
A			
A	7.2642	12	24APR2015
B	4.8414	12	01APR2014
C	2.0911	12	26JAN2014

CRABs: used log (g/m²):

Table 36. ANOVA results for crab biomass by substrate type and sampling date.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	5	72.74366613	14.54873323	45.76	<.0001
SUBSTRATE	3	3.64491268	1.21497089	3.82	0.0156
Date*SUBSTRATE	15	3.51042998	0.23402867	0.74	0.7365

Table 37. Tukey's post hoc analysis of crab biomass by substrate type.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**SUBSTRATE**
	A	3.3862	18	OYSTER SHELL
	A			
	A	3.3519	18	LIMESTONE
	A			

B	A	3.1908	18	CONCRETE
B				
B		2.8188	18	RIVER ROCK

Table 38. Tukey's post hoc analysis of crab biomass by sampling date.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Date**
	A	4.0249	12	08JUL2014
	A			
	A	4.0196	12	08OCT2014
	A			
	A	3.6302	12	16JAN2015
	A			
B	A	3.4800	12	24APR2015
B				
B		2.8459	12	01APR2014
	C	1.1211	12	26JAN2014

Fish biomass: used log (g/m^2):

Table 39. ANOVA results for fish biomass by substrate type and sampling date.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	5	26.73295455	5.34659091	8.42	<.0001
SUBSTRATE	3	54.74307579	18.24769193	28.74	<.0001
Date*SUBSTRATE	15	18.30041361	1.22002757	1.92	0.0444

Table 40. Tukey's post hoc analysis of fish biomass by substrate type.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**SUBSTRATE**
A	2.6935	18	OYSTER SHELL
A			
A	2.3978	18	LIMESTONE

B	0.8538	18	CONCRETE
B			
B	0.7766	18	RIVER ROCK

Table 41. Tukey's post hoc analysis of fish abundance by sampling date.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**Date**
A	2.5208	12	16JAN2015
A			
A	1.9986	12	08JUL2014
A			
A	1.8476	12	08OCT2014
A			
A	1.6193	12	24APR2015
A			
A	1.5907	12	01APR2014
B	0.5055	12	26JAN2014

Shrimp biomass: log (g/m²):

Table 42. ANOVA results of shrimp biomass by substrate type and sampling date.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Date	5	12.33465106	2.46693021	10.38	<.0001
SUBSTRATE	3	5.74528138	1.91509379	8.06	0.0002
Date*SUBSTRATE	15	2.88558788	0.19237253	0.81	0.6619

Table 43. Tukey's post hoc analysis of shrimp biomass by substrate type.

Means with the same letter are not significantly different.			
Tukey Grouping	**Mean**	**N**	**SUBSTRATE**
A	1.4777	18	OYSTER SHELL
B	0.9796	18	LIMESTONE

B			
B	0.9014	18	CONCRETE
B			
B	0.7135	18	RIVER ROCK

Table 44. Tukey's post hoc analysis of shrimp biomass by sampling date.

Means with the same letter are not significantly different.				
Tukey Grouping		**Mean**	**N**	**Date**
	A	1.7190	12	24APR2015
	A			
	A	1.3252	12	08OCT2014
	A			
B	A	1.1304	12	16JAN2015
B				
B		0.7218	12	08JUL2014
B				
B		0.6378	12	26JAN2014
B				
B		0.5740	12	01APR2014

Appendix B: ANOSIM outputs

ANOSIM- Abundance data
Analysis of Similarities

Two-Way Crossed - AxB

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

Factors

Place	Name	Type	Levels
A	SUBSTRATE	Unordered	4
B	monyy	Unordered	6

SUBSTRATE levels
OYSTER SHELL
RIVER ROCK
CONCRETE
LIMESTONE

monyy levels
Apr14
Apr15
Jan14
Jan15
Jul14
Oct14

Tests for differences between unordered SUBSTRATE groups
(across all monyy groups)
Global Test
Sample statistic (Average R): 0.185
Significance level of sample statistic: 0.3%
Number of permutations: 9999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Average R: 26

Pairwise Tests

R Significance Possible
Actual Number >=

Groups	Statistic	Level %	Permutations	Permutations	Observed
OYSTER SHELL, RIVER ROCK	0.389	0.05	1000000	9999	4
OYSTER SHELL, CONCRETE	0.265	1.1	1000000	9999	112
OYSTER SHELL, LIMESTONE	0.037	33.5	1000000	9999	3353
RIVER ROCK, CONCRETE	0.049	32.9	1000000	9999	3291
RIVER ROCK, LIMESTONE	0.179	7.2	1000000	9999	715
CONCRETE, LIMESTONE	0.198	6.6	1000000	9999	658

Tests for differences between unordered monyy groups
(across all SUBSTRATE groups)
Global Test
Sample statistic (Average R): 0.544
Significance level of sample statistic: 0.01%
Number of permutations: 9999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Average R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Apr14, Apr15	0.972	0.02	10000	9999	1
Apr14, Jan14	0.278	2.5	10000	9999	244
Apr14, Jan15	0.963	0.02	10000	9999	1
Apr14, Jul14	0.972	0.01	10000	9999	0
Apr14, Oct14	0.981	0.02	10000	9999	1
Apr15, Jan14	0.787	0.02	10000	9999	1
Apr15, Jan15	0.296	3.5	10000	9999	353
Apr15, Jul14	0.657	0.09	10000	9999	8
Apr15, Oct14	0.63	0.02	10000	9999	1
Jan14, Jan15	0.88	0.03	10000	9999	2
Jan14, Jul14	0.796	0.01	10000	9999	0
Jan14, Oct14	0.88	0.01	10000	9999	0
Jan15, Jul14	0.463	0.7	10000	9999	68
Jan15, Oct14	0.176	3	10000	9999	296
Jul14, Oct14	0.407	0.4	10000	9999	40

ANOSIM- Biomass data

Analysis of Similarities

Two-Way Crossed Analysis

Resemblance worksheet
Name: Resem2
Data type: Similarity
Selection: All

Factor Values
Factor: SUBSTRATE
OYSTER SHELL
RIVER ROCK
CONCRETE
LIMESTONE
Factor: Month- year
Apr14
Apr15
Jan14
Jan15
Jul14
Oct14

Factor Groups

Sample	SUBSTRATE	Month- year
E-OYSTERSHELL-Apr14	OYSTER SHELL	Apr14
L-OYSTERSHELL-Apr14	OYSTER SHELL	Apr14
N-OYSTERSHELL-Apr14	OYSTER SHELL	Apr14
E-OYSTERSHELL-Apr15	OYSTER SHELL	Apr15
L-OYSTERSHELL-Apr15	OYSTER SHELL	Apr15
N-OYSTERSHELL-Apr15	OYSTER SHELL	Apr15
E-OYSTERSHELL-Jan14	OYSTER SHELL	Jan14
L-OYSTERSHELL-Jan14	OYSTER SHELL	Jan14
N-OYSTERSHELL-Jan14	OYSTER SHELL	Jan14
E-OYSTERSHELL-Jan15	OYSTER SHELL	Jan15
L-OYSTERSHELL-Jan15	OYSTER SHELL	Jan15
N-OYSTERSHELL-Jan15	OYSTER SHELL	Jan15
E-OYSTERSHELL-Jul14	OYSTER SHELL	Jul14
L-OYSTERSHELL-Jul14	OYSTER SHELL	Jul14
N-OYSTERSHELL-Jul14	OYSTER SHELL	Jul14
E-OYSTERSHELL-Oct14	OYSTER SHELL	Oct14
L-OYSTERSHELL-Oct14	OYSTER SHELL	Oct14
N-OYSTERSHELL-Oct14	OYSTER SHELL	Oct14
F-RIVERROCK-Apr14	RIVER ROCK	Apr14
I-RIVERROCK-Apr14	RIVER ROCK	Apr14

O-RIVERROCK-Apr14		RIVER ROCK	Apr14
F-RIVERROCK-Apr15		RIVER ROCK	Apr15
I-RIVERROCK-Apr15		RIVER ROCK	Apr15
O-RIVERROCK-Apr15		RIVER ROCK	Apr15
F-RIVERROCK-Jan14		RIVER ROCK	Jan14
I-RIVERROCK-Jan14		RIVER ROCK	Jan14
O-RIVERROCK-Jan14	RIVER ROCK	Jan14	
F-RIVERROCK-Jan15	RIVER ROCK	Jan15	
I-RIVERROCK-Jan15	RIVER ROCK	Jan15	
O-RIVERROCK-Jan15	RIVER ROCK	Jan15	
F-RIVERROCK-Jul14	RIVER ROCK	Jul14	
I-RIVERROCK-Jul14	RIVER ROCK	Jul14	
O-RIVERROCK-Jul14	RIVER ROCK	Jul14	
F-RIVERROCK-Oct14	RIVER ROCK	Oct14	
I-RIVERROCK-Oct14	RIVER ROCK	Oct14	
O-RIVERROCK-Oct14	RIVER ROCK	Oct14	
G-CONCRETE-Apr14	CONCRETE	Apr14	
J-CONCRETE-Apr14	CONCRETE	Apr14	
P-CONCRETE-Apr14	CONCRETE	Apr14	
G-CONCRETE-Apr15	CONCRETE	Apr15	
J-CONCRETE-Apr15	CONCRETE	Apr15	
P-CONCRETE-Apr15	CONCRETE	Apr15	
G-CONCRETE-Jan14	CONCRETE	Jan14	
J-CONCRETE-Jan14	CONCRETE	Jan14	
P-CONCRETE-Jan14	CONCRETE	Jan14	
G-CONCRETE-Jan15	CONCRETE	Jan15	
J-CONCRETE-Jan15	CONCRETE	Jan15	
P-CONCRETE-Jan15	CONCRETE	Jan15	
G-CONCRETE-Jul14	CONCRETE	Jul14	
J-CONCRETE-Jul14	CONCRETE	Jul14	
P-CONCRETE-Jul14	CONCRETE	Jul14	
G-CONCRETE-Oct14	CONCRETE	Oct14	
J-CONCRETE-Oct14	CONCRETE	Oct14	
P-CONCRETE-Oct14	CONCRETE	Oct14	
H-LIMESTONE-Apr14	LIMESTONE	Apr14	
K-LIMESTONE-Apr14	LIMESTONE	Apr14	
M-LIMESTONE-Apr14	LIMESTONE	Apr14	
H-LIMESTONE-Apr15	LIMESTONE	Apr15	
K-LIMESTONE-Apr15	LIMESTONE	Apr15	
M-LIMESTONE-Apr15	LIMESTONE	Apr15	
H-LIMESTONE-Jan14	LIMESTONE	Jan14	
K-LIMESTONE-Jan14	LIMESTONE	Jan14	
M-LIMESTONE-Jan14	LIMESTONE	Jan14	
H-LIMESTONE-Jan15	LIMESTONE	Jan15	

K-LIMESTONE-Jan15	LIMESTONE	Jan15
M-LIMESTONE-Jan15	LIMESTONE	Jan15
H-LIMESTONE-Jul14	LIMESTONE	Jul14
K-LIMESTONE-Jul14	LIMESTONE	Jul14
M-LIMESTONE-Jul14	LIMESTONE	Jul14
H-LIMESTONE-Oct14	LIMESTONE	Oct14
K-LIMESTONE-Oct14	LIMESTONE	Oct14
M-LIMESTONE-Oct14	LIMESTONE	Oct14

TESTS FOR DIFFERENCES BETWEEN SUBSTRATE GROUPS
(across all Month- year groups)
Global Test
Sample statistic (Global R): 0.255
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

	R	Significance	Possible	Actual	Number
Groups	Statistic	Level %	Permutations		
	Permutations		Observed		
OYSTER SHELL, RIVER ROCK		0.543	0.1		
1000000	999	0			
OYSTER SHELL, CONCRETE		0.284	0.8		
1000000	999	7			
OYSTER SHELL, LIMESTONE		0.019	45.1		
1000000	999	450			
RIVER ROCK, CONCRETE	0.08	21.9	1000000	999	218
RIVER ROCK, LIMESTONE	0.42	0.3	1000000	999	2
CONCRETE, LIMESTONE	0.309	1	1000000	999	9

TESTS FOR DIFFERENCES BETWEEN Month- year GROUPS
(across all SUBSTRATE groups)
Global Test
Sample statistic (Global R): 0.524
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

	R	Significance	Possible	Actual	Number
>=					
Groups	Statistic	Level %	Permutations	Permutations	
Observed					

Apr14, Apr15	0.917	0.1	10000	999	0
Apr14, Jan14	0.565	0.1	10000	999	0
Apr14, Jan15	0.944	0.1	10000	999	0
Apr14, Jul14	0.935	0.1	10000	999	0
Apr14, Oct14	0.954	0.1	10000	999	0
Apr15, Jan14	0.824	0.1	10000	999	0
Apr15, Jan15	0.056	32.6	10000	999	325
Apr15, Jul14	0.556	0.1	10000	999	0
Apr15, Oct14	0.324	0.1	10000	999	0
Jan14, Jan15	0.926	0.1	10000	999	0
Jan14, Jul14	0.907	0.1	10000	999	0
Jan14, Oct14	0.926	0.2	10000	999	1
Jan15, Jul14	0.222	4.5	10000	999	44
Jan15, Oct14	0.167	8.2	10000	999	81
Jul14, Oct14	0.296	3.6	10000	999	35

Appendix C: Additional figures

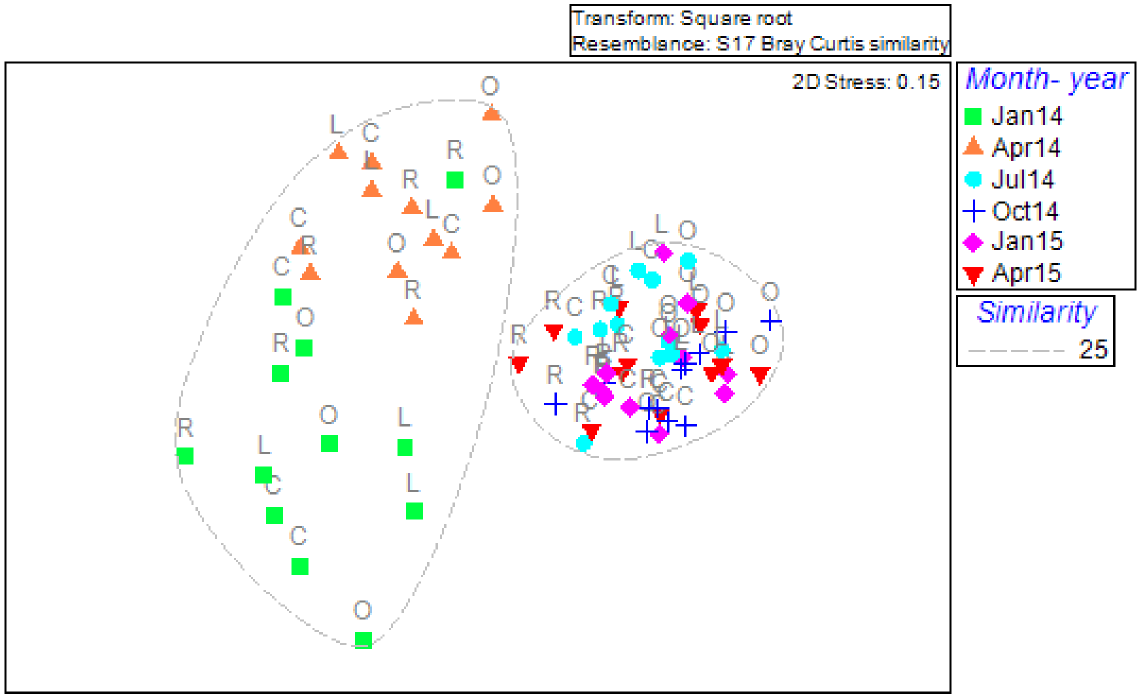


Figure 1. Multidimensional scaling plot of fauna biomass by sampling date and substrate type. Letters refer to substrate type, colors to sampling date; Lines show similarity grouping result, 25 refers to the percent similarity of samples within each c