

EVALUATION OF THE ECOLOGICAL VALUE OF CONSTRUCTED INTERTIDAL
OYSTER REEFS AND AQUACULTURE STRUCTURES IN DELAWARE BAY:
HABITAT UTILIZATION BY MOTILE MACROFAUNA

By

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ABSTRACT OF THE THESIS

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The Cape Shore on the New Jersey side of Delaware Bay USA is an extensive high-energy polyhaline intertidal zone that consistently receives the highest oyster (*Crassostrea virginica*) settlement in the Bay. Intertidal oyster reefs in lower Delaware Bay are ephemeral, and it is generally assumed that oyster mortality due to predation, disease and winter ice scouring inhibit their persistence. If protected, however, oysters survive and grow well; hence, oyster aquaculture is developing at the site. In June 2006, shell-bag oyster reefs of varying height were constructed on the intertidal sand flats in lower Delaware Bay to determine the potential for oyster reef restoration in this temperate estuary. Oysters survived a heavy formation of ice during winter 2007, but shifting sediments nearly buried the shortest reef by April 2007, a process that may be important in limiting the development of oyster reefs in this system.

In May 2007, six 2-layer shell-bag reefs were constructed. These constructed reefs as well as rack and bag oyster culture systems provided semi-permanent structures

that form potential habitats for motile fauna on the sand flats of the Cape Shore. To assess the habitat potential of these structures, constructed reefs, aquaculture racks and adjacent sand flats were monitored for utilization by motile macrofauna using wire mesh traps, crab pots and eel traps from May through October 2007. Species richness for aquaculture racks (25 species) and shell-bag reefs (22 species) were comparable and significantly greater than on the sand flats (17 species). Seven species were unique to aquaculture racks. Species abundance was five times greater around aquaculture racks and three times greater around shell-bag reefs compared to sand flats. Intertidal oyster reefs and aquaculture structures increased habitat complexity, attracted similar assemblages of motile macrofauna and supported an increased species abundance, biomass and species richness compared to sand flats. Based on these metrics, oyster aquaculture rack and bag structures are comparable habitat to intertidal oyster reef habitat in Delaware Bay.

Preface

Included in the thesis is the preliminary study on the survival and persistence of constructed shell-bag reefs in Delaware Bay conducted in summer 2006 which is now published.

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INTRODUCTION

Marine ecosystems have long been studied for their species diversity and ecological complexity. These inherent attributes also spawned a long-standing dependence on the world's fishery stocks. Humans have been using the resources of the oceans since they first learned how to fish (Jackson et al. 2001). At that time, the direct and indirect effects of these disturbances were a foreign concept. In the 1800's a commonly accepted idea was that the fisheries of the world were boundless (Botsford et al. 1997). Jackson et al. (2001) compare the drastic before and after fishing effects on three common marine ecosystems. Today, nearly half of the world's commercially important fisheries stocks are exploited (Botsford et al. 1997).

The Eastern oyster, *Crassostrea virginica*, is a filter-feeding bivalve found in estuarine habitats of varying salinity in North America. *C. virginica* form intricate reef systems and have been called "ecosystem engineers" (Jones et al. 1994, Lenihan & Peterson 1998). The formation of Eastern oyster reefs was summarized by Dame (1996). *C. virginica* larvae initially settle on a hard substrate, grow and then serve a new substrate for incoming recruits. The oysters are cemented together and extra substrate is also created through the settling and recruitment of other invertebrates such as tubeworms, bivalves and barnacles. This ongoing process creates a growing vertical reef structure.

C. virginica is a species that has high commercial and ecological value; however, the latter is often overlooked. The Eastern oyster can be considered a water quality monitor and processor. Nutrient loading has caused the eutrophication of many estuaries, which leads to an increase in primary production. The decreased oyster populations cannot process large amounts of phytoplankton caused by eutrophication. Newell (1988)

estimated that oyster densities in the Chesapeake Bay during the mid-1800's could filter a volume of water equal to that contained in the Bay in only 3.3 days. By comparison, would take present day oyster populations about 325 days to master this same feat. Temperate oyster reefs are analogous to tropical coral reefs in terms of their ecological diversity (Lenihan 1998). Oyster reefs grow via a positive feed-back loop by providing new substrate for the continual recruitment, settlement, and survival of *C. virginica* larvae. In addition, macro-zooplankton and nekton utilize oyster reefs for refuge and resource acquisition. Wells (1961) conducted a study on species diversity in oyster bed habitats of varying salinity and found 303 species associated with *C. virginica* beds in North Carolina. A follow up to Wells' study by Dame (1979) showed 37 benthic species living in the reef structure. In Maryland, 17 finfish species have been associated with oyster reefs, and in South Carolina there have been 87 resident species and 60 transient species documented utilizing oyster reefs (Breitburg 1999, Luckenbach et al. 2005).

Subtidal and intertidal oyster reefs form along the Eastern coast of the United States. Subtidal reefs exist throughout most of its range in North America; however, from North Carolina to Florida intertidal oyster reefs are pre-dominant (Bahr & Lanier 1981, Burrell 1986). Historically, ephemeral intertidal oyster reefs have been known to form in lower Delaware Bay, USA (Fig. 1). Why don't these intertidal reefs persist in this high recruitment zone? If these reefs did persist, or even as ephemeral structures, what type of fauna do they attract? In 2006, a preliminary study was designed to address these questions. Based on data from 2006, a study was conducted to compare how motile macrofauna utilize intertidal oyster reefs, nearby aquaculture structures and the adjacent sand flats.

Preliminary Study 2006 Introduction

As in many North American Atlantic Coast estuaries, the Eastern oyster, *Crassostrea virginica* Gmelin, is strongly linked to the history and socio-economic structure of communities surrounding Delaware Bay, USA. Post-World War II technological improvements facilitated a rapid expansion of the oyster fishery until overfishing and two virulent oyster diseases (Dermo and MSX) decimated the industry (Farley 1988, Ford 1996). A sustainable subtidal population remains and supports a small but viable fishery (Canzonier et al. 1998); however, recent recruitment failures nearly forced its closure (Powell et al. 2006, 2007). As a result, a major subtidal shell-planting program is in progress to increase oyster recruitment and production for the fishery (ACE 2006).

In addition to the socio-economic structure it supports, *Crassostrea virginica* is also ecologically valuable. Dame (1996) summarizes a variety of ecosystem processes influenced by marine bivalve molluscs, one of which is creation of habitat. Gregariously settling oysters form beds and reefs that create spatial heterogeneity, giving the beds an intrinsic habitat value (Breitburg 1992, Harding & Mann 2000). This habitat provides nursery grounds and refuge from predation, as well as reproduction and foraging sites for a variety of estuarine species (Coen & Luckenbach 2000, Harding & Mann 2001, Luckenbach et al. 2005, Rodney & Paynter 2006). Studies in Virginia, Maryland, Louisiana and the Carolinas have quantified (1) increases in species diversity and abundance (Coen et al. 1999, Breitburg et al. 2000, Coen & Luckenbach 2000, Luckenbach et al. 2005), (2) impacts of intertidal reefs on reducing shoreline erosion

(Meyer et al. 1997, Piazza et al. 2005), and (3) effects of reef height on recruitment and persistence (Lenihan 1999, Nestlerode et al. 2007) via the creation and restoration of oyster beds and reefs. In Delaware Bay, USA, Maurer and Watling (1971) surveyed the fauna associated with subtidal oyster beds. Intertidal oyster reefs occasionally form along the Cape Shore in lower Delaware Bay (Fig. 1), but few studies have examined their ecology. The Cape Shore consistently receives high oyster recruitment (Powell et al. 2007), but substrate is limiting and mortality is high, resulting in the formation of ephemeral oyster reef habitat. This study investigated the use of shell-bags to construct intertidal oyster reefs. Our objective was to gather information about the effects of reef height on (1) oyster recruitment and mortality, (2) habitat utilization by motile fauna, and (3) reef persistence within the intertidal zone.

Preliminary Study 2006 Materials and Methods

In June 2006, three oyster shell-bag reefs were constructed on the sand flats of the Cape Shore region of lower Delaware Bay (Fig. 1A). Mesh bags (14.3 mm opening) were filled with 19L of oyster shell and arranged side-by-side in a 1.5 m x 3 m rectangular footprint to form the base of each reef. The shell-bag reefs mimic the small oyster reefs that form periodically on the sand flats (Fig. 1B-E). Reefs were separated by 10 m, aligned parallel to the beach and contained one (Reef 1), two (Reef 2) or three (Reef 3) layers of shell-bags; each layer was about 16 cm high. Maximum tidal amplitude at the Cape Shore is ~2.75 m. During high tide, the mean water depth was ~2 m where the constructed reefs were located. Reefs were exposed for ~4 h during low tide.

On 5 October 2006 and 10 April 2007, live and dead oysters were counted on all three shell-bag reefs without disturbing reef structure by using a 10-cm² quadrat haphazardly placed along four transects (n=16 per reef). Transects were perpendicular to the beach and approximately evenly spaced across each reef. Settlement was estimated as the sum of live and dead oysters m⁻² in October 2006. Dead oysters included boxes (empty valves), gapers (boxes containing oyster tissue), and scars (marks left from recently detached oysters). Post-settlement mortality was estimated as the number of dead oysters divided by the number of dead plus live oysters in October 2006. Recruitment was defined as the number of live oysters m⁻² in October 2006. Over-winter survival was calculated as the number of live oysters m⁻² in April 2007 divided by October oyster recruitment. One-way analysis of variance (ANOVA) and Tukey's post-hoc comparisons ($\alpha = 0.05$) were used to test for differences among reefs in oyster settlement, recruitment, post-settlement mortality, and April 2007 oyster abundance. Reef persistence over time was documented photographically.

Motile fauna were sampled bi-weekly with unbaited, galvanized steel 6.35-mm mesh traps (length = 45 cm, diameter = 23 cm, 25.4 mm funnel entrances at each end) secured to the reef base or in a control sand area (10 m from the nearest reef) during low tide. At the following low, all individuals captured were identified to species and enumerated. Sixteen collections were conducted from 27 July to 16 October 2006 using one trap per treatment, while four collections completed from 17 October to 26 October 2006 used five traps per treatment.

Preliminary Study 2006 Results

Natural oyster settlement occurred shortly after reefs were constructed and extensive recruitment was evident in October (Fig. 1E). Estimated settlement and recruitment through October 2006 for Reefs 1 and 2 were significantly greater and nearly double that of Reef 3 (Fig. 2A and C). Post-settlement mortality through October 2006 increased from Reef 1 to Reef 3, reinforcing the pattern of differential settlement. At this time, mortality was 10% or less for all reefs and differences among reefs were not statistically significant (Fig. 2B, $p = 0.08$). In contrast, over-winter mortality was greatest on Reef 1 (64% vs. 34% and 16% on Reefs 2 and 3, respectively) and altered live oyster abundances among reefs by April 2007 (Fig. 2D). Highest oyster abundance in April 2007 occurred on Reef 2 and was significantly greater than abundances on Reef 1 and Reef 3.

The primary cause of over-winter mortality on Reef 1 was sedimentation (Fig. 3). Shortly after reef construction, sediments accumulated in front of each reef. Small, shallow (<10 cm depth) tidal pools often formed behind each reef, increasing in area with the height of the reef. By April 2007, sediments covered most of Reef 1, eliminating any remnants of a tidal pool. The heights of Reef 2 and Reef 3 protected them from the effects of sedimentation. Sediments accumulated in the first layer of each reef, but the accumulation decreased with reef height (Fig. 3).

Thirteen different motile species were collected on the reefs while only seven species were captured on the sand flat (Table 1). All species collected on the sand flat were present on the reefs, and faunal abundances were higher on reefs compared to the sand flat. Total abundance was inversely related to reef height (Table 1). The most

commonly observed reef species were *Palaemonetes pugio*, *Nassarius obsoletus* and *Pagurus longicarpus*. *Palaemonetes pugio* was the dominant species on all four sampling sites, while *N. obsoletus* was collected exclusively on the reefs. Yellow-phase American eels, *Anguilla rostrata*, were the most abundant of the five fish species and were associated solely with the reefs.

Preliminary Study 2006 Discussion

This study demonstrates the utility of shell-bag reefs for creating oyster reef habitat in temperate estuaries. The reefs consisted of wild oysters set on disarticulated shell, creating a habitat matrix similar to a natural oyster bed, which was quickly utilized by typical oyster reef fauna (Maurer & Watling 1971, Luckenbach et al. 2005). Oyster settlement, recruitment, survival and reef persistence varied according to reef height.

Lower Delaware Bay is the highest oyster settlement zone in the bay (Powell et al. 2006, 2007); yet persistent intertidal reefs do not form. The absence of intertidal reefs in this region of the bay is often attributed to predation, disease, and freezing or ice shear. Our data demonstrate that shifting sediments contribute to the ephemeral nature of intertidal reefs. During winter 2007, several feet of ice accumulated on the Cape Shore flats (G. DeBrosse, Rutgers Cape Shore Shellfish Hatchery Manager, pers. comm.), yet oysters survived and Reefs 2 and 3 persisted. Therefore, it seems more likely that shifting sediments inhibit the formation of intertidal reefs rather than predation or harsh winter conditions. Both MSX and dermo disease are highly prevalent at the Cape Shore (Haskin & Andrews 1988, Ford 1996) but generally cause mortality in older oysters and were not expected to significantly impact the oysters observed in this study.

Results indicated that oyster habitat supports a greater abundance and perhaps a greater species richness of motile species than the adjacent sand flats. Decapod crustacean abundance on reefs was twice that on the sand flat, and five fish species utilized the reefs while only three species were captured on the sand flats. The highest species richness and abundance observed corresponded with the reef that supported greatest oyster recruitment, Reef 1, although this reef did not persist. Interestingly, five American eels (*Anguilla rostrata*), of increasingly larger size were captured over the course of the summer and into the fall on reefs but not on the adjacent sand flat. The timing of these captures corresponds to eel migrations and may indicate that the oyster reef structure provides a useful refuge or foraging habitat for eels during their migrations.

Other studies have found similar responses. For example, in Chesapeake Bay, Rodney and Paynter (2006) observed a four-fold increase in xanthid crabs and demersal fish on restored subtidal oyster reefs compared to non-restored controls, and *Palaemonetes pugio* abundance was more than ten times greater on restored reefs. On the Eastern Shore of Virginia, Arve (1960) found that three times as many fish were captured over subtidal shell plantings versus unplanted controls. Results from the present study show a four-fold increase in xanthid crabs and almost twice the number of fish species on intertidal reefs versus sand flat. In South Carolina, Luckenbach et al. (2005) found that the decapod crabs *Eurypanopeus depressus* and *Panopeus herbstii*, which naturally inhabit intertidal reefs, were good indicators of community development on restored intertidal oyster reefs. Both crab species were captured in the present study, albeit at low frequencies with the gear used. These species are common on subtidal oyster beds elsewhere in Delaware Bay, and may likewise represent good indicator

species for intertidal oyster reefs in Delaware Bay with a more appropriate sampling method. Sampling gear is known to influence size and composition of fauna collected. For example, Able et al. (2005b) found that fish <20 mm TL were never captured in wire mesh traps. In the present study, small naked gobies (*Gobiosoma bosc*) were often observed in the reefs, but were never trapped. A more comprehensive sampling regime will likely reveal additional insights into the habitat value of intertidal oyster reefs in temperate estuaries like Delaware Bay.

Intertidal oyster reefs have been shown to reduce shoreline erosion along the southeastern Atlantic coast of the United States (Meyer et al. 1997) and in the northern Gulf of Mexico (Piazza et al. 2005). Rates of erosion are high in many areas of Delaware Bay including the Cape Shore (Phillips 1986). In addition to property loss, erosion has eliminated valuable beach, dune, and marsh habitats negatively impacting fisheries production, horseshoe crab spawning, and migratory shore bird foraging. The survival of the two taller intertidal reefs indicates that constructed intertidal oyster reefs represent a potentially viable strategy to reduce erosion in Delaware Bay and similar temperate estuaries, while also providing additional ecological services through the creation of a novel habitat and the ecological functions associated with oyster reefs. The results from this preliminary study were used to design a more in depth study of habitat utilization of constructed intertidal oyster reefs by motile macrofauna in Delaware Bay.

Habitat utilization of three habitat types

In Delaware Bay, several efforts are underway to increase oyster abundance. These efforts are focused on increasing oyster production for the oyster fishery and the

socio-economic structure it supports. Oyster aquaculture is one method that can alleviate the strain that is placed on the natural oyster population. Unfortunately, outdated regulations, a lack of information on ecological effects, and some public misconceptions inhibit the development of oyster culture in New Jersey. The state of New Jersey is currently deliberating on legislation to permit aquaculture development zones. A bone of contention is the perception that aquaculture activities will negatively impact intertidal flat habitats. Hence, there is a need to demonstrate the ecological outcomes of the aquaculture activities.

Oyster aquaculture research is being conducted by Rutgers University on the tidal flats of Cape Shore, and two commercial oyster aquaculture farms operate on either side of the research station. Oysters are grown off-bottom in a rack and bag system consisting of plastic ADPI[®] culture bags on rebar racks. These aquaculture racks provide structure and vertical relief on the broad sand flats while protecting the cultured oysters from predators. These structures could be considered artificial versions of oyster reefs and may provide similar ecological services.

Ulanowicz and Tuttle (1992) produced a quasi-equilibrium model which demonstrated that aquaculture activities would not only help the failing Chesapeake Bay oyster fishery but also ameliorate the worsening ecological conditions of the Chesapeake Bay. More recent studies on the East and West Coast as well as Europe have shown an increase in abundance of benthic invertebrates and fish species associated with the habitat created by oyster aquaculture operations (Dumbauld 1997, Dumbauld et al. 2000, Laffargue et al. 2006). Therefore, oyster aquaculture systems may have an intrinsic habitat value but do they attract fauna assemblages similar to intertidal oyster reefs?

The preliminary wire mesh trap sampling data from 2006 showed an increase in macrofauna abundance, biomass and species richness associated with the shell-bag reefs compared to adjacent control sand areas. While similar observations have been reported from other estuaries, it is noteworthy that the American eel (*A. rostrata*) was the most abundant species of the finfish captured on the shell-bags reefs, while no eels were caught on the adjacent sand areas. Furthermore, the total length of *A. rostrata* increased from 35 cm to 55 cm during the June through October sampling season, indicating that American eels may utilize oyster reefs as habitat for foraging or shelter. This initial data indicates the potential habitat value of constructed reefs and aquaculture for *A. rostrata* as well as other species in the lower Delaware Bay.

The American eel (*A. rostrata*) is an important commercial fishery species. This eel is a catadromous species with a complex life history undergoing multiple metamorphoses. While in the yellow phase, the American eel resides in estuarine habitats for two to twenty years (Able & Fahay 1998). The quality of estuarine habitats are vital to the growth and productivity of *A. rostrata* since the final life stage that triggers the spawning migration is thought to be linked more to length and weight rather than age (Oliveira 1999). *A. rostrata* begins its migration in fall to spawning grounds in the Sargasso Sea (Able & Fahay 1998). Because of *A. rostrata*'s semelparous reproduction, the species is highly susceptible to overfishing and its complex life cycle makes it a difficult commercial stock to manage (Vecchio 2001). Unfortunately, along much of the Atlantic Coast, almost all life stages of the American eel are subjected to fishing mortality. Therefore, *A. rostrata* was chosen as a species of interest to further investigate the habitat utilization by motile macrofauna.

This study was designed to compare the habitat utilization by motile fauna of three habitat types: rack and bag oyster culture systems, constructed shell-bag reefs and the surrounding sand flats. The objectives of the study were (1) to determine if constructed intertidal shell-bag reefs and oyster aquaculture structures support increased motile macrofauna species richness, abundance and biomass; (2) to determine if constructed shell-bag reefs and aquaculture racks maintain similar motile fauna species assemblages and (3) to determine if American eels *A. rostrata* utilize intertidal constructed reefs and oyster aquaculture racks as habitat.

MATERIALS AND METHODS

Study site description

The study was conducted on the intertidal sand flats at the Rutgers University Cape Shore Hatchery Facility near Green Creek, NJ in the lower Delaware Bay (Fig. 1A). The Cape Shore region consists of extensive tidal flats that extends about 1 km from shore during low tide. Maximum tidal amplitude at the Cape Shore is ~2.75 m. The time frame for this study was 9 May 2007 to 16 October 2007.

Water temperature was monitored by a HOBO Water Temp Pro [H20-001] temperature logger (logging frequency of 15 min, resolution of 0.2°C) from 8 June 2007 to 15 October 2007. The temperature logger was deployed inside a PVC tube and placed in the sediment in front of the Rutgers University Cape Shore Hatchery Building in an area that was rarely exposed to the air. Therefore, temperature data corresponds to the times the sand flats were not exposed. Weekly temperatures were averaged over 15 minute intervals for the duration of the temperature logger deployment. On 26

September 2007, the logger was brought onto the beach for about 3 hours; the corresponding logger readings were omitted from analyses.

Cape Shore salinity data were obtained from the NOAA Center for Operational Oceanographic Products and Services (CO-OPS), Physical Oceanographic Real-Time System (PORTS) data retrieval site (<http://tidesandcurrents.noaa.gov/ports.html>). The station closest to Cape Shore is Brandywine Shoal Light, Delaware Bay station buoy (station ID 8555889) located at 38° 59.2' N, 75° 6.8' W (Fig. 1A). Weekly mean salinity was obtained by averaging the 6 minute data from the Brandywine Shoal station. Station salinity data are missing from 21 June to 25 June 2007 and were not included in the weekly average.

Study Design

On 9 May 2007, six shell-bag reefs were constructed on the sand flats at Cape Shore. Mesh bags (14.3 mm opening) were filled with 19L of oyster shell and arranged to form 1.5 m x 3 m x 0.3 m reefs that were aligned parallel to the beach on a sand bar 160 m from shore (Fig. 4A). The bottom layer was about 16 cm high and consisted of 4 rows of 10 bags. The four corners were anchored with rebar poles and a second layer of 27 bags added to create a pyramid approximately 30 cm tall.

The corners of six sand plots (1.5 m x 3 m) were marked with rebar poles (Fig. 4B). Paired reef and sand treatments were at least 10 m apart and positioned in two groups across the sand bar (Fig. 5) to avoid interfering with the Rutgers University Cape Shore Hatchery aquaculture racks. Paired treatments were distributed equally across the

front, the middle and the back of the sandbar to avoid crowding treatments and to ensure at least 10 m of separation between any two replicates (Fig. 5).

The oyster aquaculture racks used for this study were part of Atlantic Capes Fisheries, Inc. aquaculture operations managed by James Tweed which is located immediately south of the Cape Shore Hatchery Facility (Fig. 4C). The specific aquaculture racks chosen for this study were located on the same sand bar as the shell-bag reef and sand treatments and the distance from the first aquaculture rack to the first sand treatment was 71 m (Fig. 5). Six oyster aquaculture rack and bag structures were marked with wooden stakes (Fig 4D). Three wooden stakes were placed next to the aquaculture racks to mark treatment locations and to provide anchorage for sampling gear. The aquaculture racks were 0.9 m wide and stakes were placed alternately on each side of the racks across a distance of about three meters for each replicate location.

Sediment elevations were taken for all sites using a Lasermark Wizard LM30 laser level to quantify and compare the relative depths of shell-bag reef, sand and aquaculture treatments. Elevations were taken from the benchmark of the USGS well number 090089 (Oyster Lab 4 Obs.) located in front of the Rutgers University Cape Shore Hatchery Building. The USGS well is 3.44 m above sea level according to the National Geodetic Vertical Datum of 1929(NGVD 1929). Treatment elevations from this benchmark were used to calculate height (m) above sea level for sand, shell-bag reef and aquaculture treatments.

Elevations of the paired reef and sand plots were matched in the initial design, but the aquaculture sites were added post-hoc and site selection limited by the physical location of the aquaculture operation. One-way analysis of variance (ANOVA) and

Tukey's post-hoc comparisons ($\alpha = 0.05$) indicated there was no significant difference among habitat type elevations (p-value = 0.785). All treatments were within a base sediment elevation of 22.9 cm. Ranges of elevations for sand plots were within 15.3 cm, shell-bag reefs were within 15.1 cm and aquaculture racks were within 22.9 cm of each other above sea level. Reef 6 was the deepest of the shell-bag reef treatments with an elevation of 0.824 m above sea level (Table 2). Its corresponding paired treatment, Sand 6 was also the deepest sand treatment with an elevation of 0.843 m above sea level (Table 2). These two treatments were the only shell-bag reef and sand treatments that were consistently surrounded by sloughs of water. Reef 1 (0.976 m) and Reef 3 (0.958 m) as well as their paired sand treatments had the shallowest depths of the shell-bag reef treatments (Table 2). As depicted in Fig. 5, Aquaculture treatments 1, 2 and 5 were positioned in sloughs and were associated with the deepest depths of 0.824 m, 0.777 m and 0.814 m above sea level respectively. The exposed aquaculture treatments (Aquaculture 3,4 and 6) had relative depths comparable to the more shallow shell-bag reef and sand treatments (Table 2).

Oyster settlement, recruitment and mortality

In September 2007, oyster settlement, recruitment and post-settlement mortality were determined for all six reefs as described in previous Material and Methods for the preliminary 2006 study.

Trap sampling

Motile macrofauna were sampled bi-weekly with 18 galvanized steel 6.35-mm mesh traps (length = 45 cm, diameter = 23 cm, 25.4 mm funnel entrances at each end), 18 galvanized steel 6.35-mm mesh eel traps (length = 79 cm, diameter = 23 cm, 25.4 mm funnel entrances at each end) and 6 double funnel 4-cm mesh crab traps (length = 62 cm, width = 25 cm, height = 30 cm). Using a randomized block design, unbaited traps were secured to the base of shell-bag reefs, aquaculture racks and control sand plots during low tide (Fig. 6). At the following low tide, all individuals captured were bagged and taken back to the laboratory for analysis. Species were identified, enumerated and measured for total length (TL) and biomass (g). Thirty-nine collections were conducted from 20 May to 16 October 2007.

***Anguilla rostrata* mark-recapture study**

An American eel, *Anguilla rostrata*, mark-recapture study was conducted during bi-weekly trap sampling from 20 May to 16 October 2007. Subcutaneous acrylic paint injections were used to mark eels (Lotrich & Meredith 1974, Naismith & Knight 1988). All *A. rostrata* captured were measured for TL (cm) and biomass (g), and then anaesthetized by placing them on ice for a short period of time prior to injection. Non-toxic Liquitex® soft body professional acrylic artist paints were injected ventrally behind the anus using a 1cc syringe with a 30 gauge needle. Paint marks were unique for each individual captured. Six paint colors were used in varying combinations and injected on the right side (Reef), left side (Aquaculture) and both sides (Sand) of the body in order to identify habitat residency when recaptured. To assess mark retention, three eels were marked in the lab and held in an aquarium for seven days (Fig. 7).

Seine sampling

Motile macrofauna were sampled monthly during mid ebb tide with a seine (3.5 m x 1.5 m seine with a 1.5 x 1.5 x 1.5 m bag, 3.2 mm mesh size) around two shell-bag reef treatments and two sand treatments from June to September 2007. Aquaculture racks were not sampled by seine due to the structural complexity of the aquaculture operations. In order to locate the shell-bag reefs and sand plots during high tide, PVC poles (3 m) were used to mark the corners of the replicates being sampled by seine. The same replicates were sampled every month. Three consecutive seine tows swept the perimeter of each replicate and seine catches were combined in a bucket for a total composite seine catch. Species were identified, enumerated and measured for TL and biomass (g). Large specimens were processed and released whenever possible.

Shell-bag sampling

To assess the shell-bag reef species utilizing the shell-bags as habitat that could have been missed by trap and seine sampling, resident shell-bag reef fauna were collected monthly from June to September 2007. Plastic 3 mm mesh vexar (length = 70 cm, width = 35 cm) was placed underneath three shell-bags on all six shell-bag reefs to prevent escape of resident fauna. Once a month at low tide, three shell-bags from three reefs (n=9) were washed in buckets to remove a sub-sample of motile fauna that were then collected using a 1 mm sieve. Rodrigues et al. (2007) found that sorting with a 1 mm sieve was sufficient for sampling benthic estuarine macrofauna. Samples were bagged and frozen for laboratory analysis and shell-bags were placed back on the reefs. In the

laboratory, samples were sieved a second time (1 mm sieve) and sorted to species when possible using a dissecting microscope. Abundance and biomass (g) was determined for each species. The same shell-bags were sampled for the duration of the study, but the set of three replicate reefs sampled was alternated each month.

Data analysis

Oyster settlement, recruitment and mortality

One-way analysis of variance (ANOVA) and Tukey's post-hoc comparisons ($\alpha = 0.05$) were used to test for differences among reefs in oyster settlement, recruitment and post-settlement mortality.

Trap sampling

A three-factor ANOVA and Tukey's post-hoc comparisons ($\alpha = 0.05$) were used to test for differences among habitat types in trap species abundance, biomass and species richness. The three factors used in the analysis were habitat treatment (shell-bag reef, aquaculture rack and sand), sampling time (day/night) and trap type (wire mesh, eel and crab). Species richness was measured as the number of different species present during a particular sampling event. Due to unforeseen circumstances (poor weather, trap loss, etc.), habitat treatment sampling varied from 18 to 21 collections which is less than the predicted sampling of the randomized block design ($n=22$). Since time is not an independent variable, abundance, biomass and richness were averaged over time for the analysis.

A two-factor (habitat treatment and sampling time) multivariate analysis of variance (MANOVA) and Bonferroni post-hoc comparisons ($\alpha = 0.05$) were conducted using the GLM function in SAS (SAS, Version 9.1) to test if species with a combined total abundance of 20 individuals or greater across the three habitat types differed in abundance and biomass among habitat types. The eight species used in the analysis were *P. pugio*, *N. obsoletus*, *P. longicarpus*, *Callinectes sapidus*, *Crangon septemspinosa*, *A. rostrata*, *Limulus polyphemus* and *Bairdiella chrysoura*. A three-factor ANOVA indicated there was no difference in species abundance, biomass and richness for wire mesh and eel traps (see results for previously described analysis); therefore, data for wire mesh and eel traps were combined and averaged over time for the MANOVA analysis. Crab trap data were excluded from the MANOVA analysis since only one of the eight species was captured in the traps during the sampling period. A discriminant function analysis was performed using the DISCRIM procedure (SAS, Version 9.1) to determine the species that were contributing to the differences among habitat treatments and sampling time.

Principal Component Analysis (PCA) was used to examine the variability in macrofauna composition for the three habitat types (shell-bag reef, aquaculture rack and sand). PCA is a multivariate ordination technique that reduces a large number of correlated variables through linear combinations to form uncorrelated principal components. These new components represent the variation in the data set with the first principal component accounting for the maximum variance. The eight most abundant species discussed above were used in the PCA. This excluded the rare species in the data set which are most likely not contributing to the overall variation in species composition.

Species abundances averaged over time were used in the analysis. Principal component analysis was conducted using the PRINCOMP function with the covariance matrix in SAS (SAS, Version 9.1). Principal component 1 (PC1) was used as a substitute for species composition and a linear regression was performed using relative treatment depth and PC1.

Seine sampling

A two-sample T-test was used to test for differences in seine species abundance, biomass and species richness between shell-bag reefs and sand treatments. In addition, a Bonferroni adjusted two-sample T-test for abundance and biomass was conducted on the seven most abundant species collected during the seine sampling period to test for differences between shell-bag reef and sand habitats. The most abundant species, *Neomysis americana*, was not included in the T-test analysis since it was only collected one time in June.

Shell-bag sampling

A non-parametric Spearman rank correlation was used to analyze the correlation between shell-bag reef species collected by resident fauna sampling and trap sampling. Species collected by trap sampling and resident fauna sampling were ranked according to abundance and only species overlapping in the two data sets were used in the Spearman Rank Correlation. The analysis was performed for total shell-bag reef abundance and separately for each replicate shell-bag reef.

RESULTS

Oyster settlement, recruitment and mortality

During the timeframe of this study, temperature ranged from 14°C to 30°C (Fig. 8A) and salinity ranged from 16 to 25 (Fig. 8B). Estimated settlement (sum of live and dead oysters m⁻²) for Reef 4 was significantly greater and nearly double that of Reef 1 and Reef 3 (p-values = 0.036 and 0.024 respectively) (Fig. 9). Estimated recruitment (number of live oysters m⁻²) for Reef 4 was also nearly double and significantly greater than Reef 1 and Reef 3 (p-values = 0.031 and 0.026 respectively). In September 2007, post-settlement mortality was consistently low (<5%) and was not significantly different among reefs (Fig. 9).

Motile Fauna

A total of 30 species were collected during the trap sampling period with species richness lowest on the sand (n=17) and highest on the aquaculture racks (n=25) (Table 3). Of the 30 species collected, seven were unique to the aquaculture habitat, while one species was unique to sand and shell-bag reef habitats, the Asian shore crab, *Hemigrapsus sanguineus*, and the Black sea bass, *Centropristis striata*, respectively. The most abundant trap species for all three habitats was *P. pugio*. Seventeen trap species (57%) were not collected using seine sampling (Table 3). In addition, eighteen trap species (60%) were not collected during monthly shell-bag sampling (Table 3). Thirteen of the trap species were only collected as juveniles: *L. polyphemus*, *B. chrysoura*, *Menidia menidia*, *Lagodon rhomboides*, *Etropus microstomus*, *Morone saxatilis*, *Micropogonias undulates*, *C. striata*, *Paralichthys dentatus*, *Alosa pseudoharengus*,

Lutjanus griseus, *Leiostomus xanthurus*, *Cynoscion regalis*. The eight most abundant species were associated with all three habitat types in varying abundances and of these eight species only two species, *C. septemspinosa* and *C. sapidus*, were more abundant on the sand habitat (Table 3).

Species abundance box plots indicate average species abundance was highest on the aquaculture racks and was more than five times greater than sand average species abundance (Fig. 10A). Also, average shell-bag reef species abundance is three times greater than the sand habitat. The greatest average species abundance was associated with the Aquaculture racks, median abundance values for shell-bag reef and aquaculture rack habitats were comparable (Fig. 10A). Box plots of traps species biomass reveal that the highest average biomass was also associated with the aquaculture racks and average biomass for both shell-bag reef and aquaculture racks was three times greater than biomass on the sand flats (Fig. 10B). Average species richness was comparable for all three habitat types ranging from 2.18 species on the sand and 3.29 species on the aquaculture racks (Fig. 10C).

Three-factor ANOVA and Tukey's post-hoc comparison results revealed that sand species abundance was significantly less than reef ($p = 0.008$) and aquaculture ($p < 0.0001$) treatments, while there was no difference detected between reef and aquaculture (Table 4 and Fig. 10A). Time significantly affected abundance with higher species abundances occurring during nighttime sampling. In addition, trap type significantly affected species abundances. There was no difference in abundances for wire mesh and eel traps, but crab traps collected significantly less individuals than both wire mesh and eel traps ($p < 0.0001$). There was a significant effect of the Treatment * Trap interaction,

largely because wire mesh and eel traps collected five times fewer animals on the sand flats than on the reef and aquaculture treatments.

Species biomass for sand treatments was significantly less than reef ($p = 0.001$) and aquaculture ($p < 0.0001$) treatments and there was no difference between reef and aquaculture biomass (Table 4 and Fig. 10B). There was no effect of time or trap time on species biomass. The only significant interaction effect on biomass was the trap * time interaction. Crab trap biomass was greater during daytime collections while wire mesh and eel traps had higher biomass yields during night sampling which contributed to the significant interaction.

Three-factor ANOVA results for trap species richness indicated sand species richness was significantly less than species richness on aquaculture racks ($p = 0.036$) (Table 4 and Fig. 10C). There was no difference in species richness between sand and shell-bag reef habitats as well as reef and aquaculture racks. Day and night sampling showed no differences in species richness. The type of trap used for sampling significantly influences the number of species that were collected. While there was no difference between eel and wire mesh traps, the number of species collected using crab traps was significantly less than those in wire mesh and eel traps ($p < 0.0001$). On the sand flats, the number of species collected in wire mesh and eel traps was lower than shell-bag reef and aquaculture racks which contributed to the significant interaction effect of treatment * time (Table 4).

A two-factor MANOVA was used to test for differences in abundance and biomass for the eight most abundant trap species (Table 3) across habitat types.

MANOVA results indicated that abundance differences for the eight trap species were significant for habitat type, time of sampling and the interaction effect (Table 5).

Discriminant function analysis indicated that abundances of *P. pugio*, *N. obsoletus*, *A. rostrata* and *B. chrysoura* were contributing to the significant effect of habitat treatment detected by the MANOVA (Table 6). *P. pugio*, *A. rostrata* and *B. chrysoura* abundances were significantly higher around the aquaculture racks while significantly lower numbers of *N. obsoletus* were collected on the sand flats. Abundances for the hermit crab, *P. longicarpus*, were highly correlated with habitat type and time which contributed to the significant effect of these two factors (Table 6). *P. longicarpus* was lower for the sand flats and it was the only species that was collected more often during daytime sampling. Abundance of the sand shrimp, *C. septemspinosa*, was only correlated with sampling time with higher numbers being collected during night sampling events (Table 6). Also, *P. pugio*, *N. obsoletus*, *A. rostrata* and *L. polyphemus* were the species that contributed to the significant interaction effect of treatment * time.

MANOVA biomass results also detected a significant effect for habitat type and sampling time but there was no interaction effect for species biomass (Table 7).

Discriminant function analysis indicated that biomass for *P. pugio* and *N. obsoletus* was contributing to the significant effect of habitat treatment (Table 8). *P. longicarpus* and *C. septemspinosa* were highly correlated with both habitat treatment and sampling time.

The biomass of *L. polyphemus* was only correlated with sampling time (Table 8).

Results from the PCA indicated two principal components characterized the species composition and explained 99% of the variation in the original data set. Principal component 1 accounted for most of the data set variation (95%) and is associated with

habitat depth during the sampling period. Habitat treatments that were sampled with traps at lower heights above sea level were associated with positive PC1 scores while habitats sampled at higher heights above sea level were associated with negative PC1 scores (Fig. 11). The distribution along PC2 is not as distinct and PC2 explained only 4% of the variation of the original data set. Since most the variance is explained by PC1, PC2 was not examined further.

Species composition associated with depth above sea level and the three habitat types was explained by plotting the species eigenvector loading scores (Fig. 11). Five of the eight species were characterized with small eigenvector loadings and for visual impact these species were omitted from the vector plot. The three species depicted, *P. pugio*, *N. obsoletus* and *P. longicarpus*, were the most abundant species collected during the sampling period (Table 3). Six of the eight species were correlated with PC1 (Table 9). *P. pugio* was highly correlated with PC1 and associated with deeper depths (Fig. 11). While *N. obsoletus* and *P. longicarpus* were also highly correlated with PC1, these two species were associated with more average depths. A linear regression of height above sea level and PC1 confirmed that species composition was associated with water depth with a significant R^2 value of 0.325 (Fig. 12). The linear regression explains only 33% of the variation between PC1 and depth above sea level; therefore, other factors contribute significantly to the pattern depicted by PC1. Linear regression results for habitat treatment height above sea level show no correlation for sand ($R^2 = 0.24$), a significant correlation for reef ($R^2 = 0.87$, p-value = 0.006) and a strong but not significant correlation for aquaculture ($R^2 = 0.56$, p-value = 0.077). Therefore, height above sea level and habitat type are contributing to variation in species composition.

***Anguilla rostrata* mark-recapture study**

Fifty-two American eels, *A. rostrata*, were marked during the May to October 2007 trap sampling period. During this sampling period, *A. rostrata* was the most abundant finfish species collected (Table 3). Two-factor MANOVA results confirmed that there were significantly more *A. rostrata* associated with the aquaculture racks (42 eels) than both reef (15 eels) and sand (2 eels) habitats (Table 5 and Fig.13A). Despite only two eels being captured on the sand flats, there was no statistical difference in *A. rostrata* abundance between reef and sand habitats. A similar pattern was evident for *A. rostrata* biomass. Aquaculture eel biomass was significantly greater than reef and sand and there was no difference in biomass between reef and sand habitats (Table 7 and Fig.13B). *A. rostrata* average biomass was highest on reefs ($72 \text{ g} \pm 8.85 \text{ SE}$), followed by aquaculture racks ($59 \text{ g} \pm 4.77 \text{ SE}$) and lowest on the sand ($55 \text{ g} \pm 4.5 \text{ SE}$). Also, *A. rostrata* average length was highest on the reefs ($32.7 \text{ cm} \pm 1.61 \text{ SE}$), followed by the aquaculture racks ($31.2 \text{ cm} \pm 0.97 \text{ SE}$) and lowest on the sand flats ($30.5 \text{ cm} \pm 1.5 \text{ SE}$). The largest eel was collected on the shell-bag reefs measuring 48 cm and 152 g.

Seven *A. rostrata* were recaptured during the mark-recapture study period resulting in a 13.5% recapture rate. No eels were recaptured on the sand flats. Recaptured eels exhibited no preference between reef and aquaculture habitats: four eels were recaptured around the aquaculture racks and 3 eels were recaptured on the shell-bag reefs (Fig.13A). One eel originally marked on an aquaculture rack was recaptured twice on Reef 6.

Seasonal abundance of *A. rostrata* increased from May to July on reef and aquaculture racks. *A. rostrata* abundances around aquaculture racks reached a peak in August with 17 eels captured (Fig. 14A). Following the peak in August, *A. rostrata* abundance leveled off through October for all three habitats.

Average seasonal biomass of *A. rostrata* for reefs was equal to or greater than aquaculture racks every month during the sampling period except for May (Fig. 14B). In May, two eels weighing 3 g and 140 g were captured which is causing the unusually large SE. From July to October, average reef biomass was greater than racks. Eel abundance peaked in August on the aquaculture racks, and average eel biomass also peaked in August but on the shell-bag reefs (Fig. 14B). In August, there was a greater abundance of smaller eels associated with the aquaculture racks.

Seine sampling

A total of 23 motile species were collected during monthly seine sampling with higher species richness on shell-bag reef treatments (21 species) compared to sand treatments (17 species) (Table 10). Twelve seine species (>50%) were not collected using the three trap sampling methods, and sixteen species (70%) were not collected during shell-bag sampling (Table 10). Six species were unique to the shell-bag reef habitats while there were only two species were found solely on the sand habitat, Ocellate lady crab (*Ovalipes ocellatus*) and Summer flounder (*Paralichthys dentatus*) (Table 10). Nine juvenile and two adult finfish species were associated with the sand habitat, while ten juvenile and 6 adult finfish species were collected around the shell-bag reef habitat (Table 10). Average species abundance and biomass for shell-bag reefs was nearly

double that of the adjacent sand flats; however, average species richness for the two habitat types was comparable (Fig. 15). The high variance for Reef species abundance is the result of a single occurrence of a large number of *N. americana*.

Results from two-sample tests showed no significant differences in species abundance ($p = 0.489$), biomass ($p = 0.362$) and species richness ($p = 0.374$) between shell-bag reefs and sand treatments. In addition, two-sample t-tests with *N. americana* removed from the analyses indicated no significant differences in species abundance ($p = 0.82$), biomass ($p = 0.373$) and species richness ($p = 0.297$) between shell-bag reefs and sand treatments. Bonferroni adjusted two-sample T-tests detected no significant differences in abundance ($p = 1.0$) and biomass ($p = 1.0$) for the two habitat types for the seven most abundant species: *L. polyphemus*, *Anchoa mitchilli*, *C. septemspinosa*, *P. pugio*, *M. menidia*, *C. sapidus* and *M. undulatus*.

Shell-bag sampling

During monthly shell-bag reef sampling from June to September 2007, a total of 28 resident species were associated with the constructed shell-bag reefs (Table 11). Fourteen shell-bag species (50%) were not collected via seine or trap sampling (Table 11). Many of the fourteen species would not be collected using traps or seine since they were juvenile infauna species. Eleven of the shell-bag species were only collected as juveniles: *Geukensia demissa*, *Tagelus plebeius*, *L. polyphemus*, *Macoma* sp., *Urosalpinx cinerea*, *Acteocina canaliculata*, *Mulinia lateralis*, *Petricolaria pholadiformis*, *Mya arenaria*, *C. sapidus* and *C. septemspinosa*. Species richness was highest on Reef 3 (23 species) and Reef 1 (21 species) while Reef 4 and Reef 6 consisted of the highest species

abundance (Table 11). The most abundant shell-bag reef species was the Lunar dovesnail, *Astiris lunata*. *A. lunata* also contributed the highest biomass of all the shell-bag species with 194.04 g collected during the sampling period.

Box plots indicate species abundance, biomass and richness varied for all six replicate shell-bag reefs (Fig. 16). Average species abundance ranged from 250 individuals on Reef 2 to 850 individuals on Reef 4 which is due to the high numbers of *A. lunata* and *Gammarus sp.* associated with Reef 4 (Fig. 16A and Table 11). Average biomass (g) varied from 21 g on Reef 1 to 12 g on Reef 2 and average species richness ranged from 9 species found on Reef 4 to 13 species on Reef 3 (Fig. 16B-C).

Average abundance for all species consistently increased from June to September (Fig. 17A). This striking trend is largely due to the increasing abundance of the *A. lunata*. The average abundance for three mud crab species commonly associated with oyster reefs, *Panopeus herbstii*, *Eurypanopeus depressus* and *Rhithropanopeus harrisii*, increased from June to September with the highest abundance in August (Fig. 17B).

Spearman rank correlations were used to analyze the correlation between shell-bag reef species collected by two different sampling methods: shell-bag fauna sampling and trap sampling. There was no significant correlation among overlapping species for all reefs combined as well as the six individual shell-bag reef replicates (Table 12). These results indicate that the two sampling methods were targeting different species and size classes of individuals.

DISCUSSION

Evaluation of sampling techniques

A well-designed sampling regime is necessary to identify habitat utilization by motile macrofauna. Sampling gear is known to target different fauna species as was evident in this study. Passive sampling was conducted using wire mesh, eel and crab traps, and 60% of the trap species caught were not present in the other two sampling methods. Wire mesh and eel traps consisted of the same mesh size and opening diameter but the eel traps were 34 cm longer. This variation in length did not affect the abundance, biomass or species richness of fauna captured in wire mesh and eel traps. However, there are capture efficiency limitations associated with wire mesh traps. The 6.35 mm mesh size limits the ability to capture juveniles and smaller species (Able et al. 2005b) and the 23 cm diameter also excludes larger species. In another study by Able et al. (2005a), fish species have varying behavioral responses to wire mesh traps including escape and intraspecific aggression which can alter trap species composition. Large mesh crab traps were chosen as the third trap sampling gear to target the larger species that were excluded by wire mesh and eel traps. Only four species were captured in the crab traps: *O. tau*, *P. dentatus*, *Morone americana* and *C. sapidus*. Not surprisingly, *C. sapidus* was the most common crab trap species. Species abundance and richness was lower in crab traps compared to wire mesh and eel traps. Even though crab traps captured larger species, there was no difference in biomass among the three trap types which is most likely due to the low abundances of the larger fauna collected using crab trap sampling.

The second sampling method used in this study was seining, which was designed to capture the larger motile species that were utilizing the three habitats during high tide. Over 50% of the species captured with this method were not collected with the other

sampling methods and seining yielded higher finfish abundances than trap sampling. However, a limitation of this seining is that seines have highly variable and low catch efficiency in estuaries as a result of net avoidance by fauna that can escape over or under the seine or swim away faster than the seine tow rate (Rozas & Minello 1997).

The third sampling method employed during this study consisted of washing motile fauna out of shell bags. Shell-bag samples mainly consisted of juvenile decapod crustaceans, mollusks and fish. This sampling method was designed to collect macrofauna larger than 1 mm that were not collected during the other sampling efforts.

A comparison of the methods used in this study reiterates that there is no perfect macrofauna sampling protocol. Life history characteristics and relative body size of the targeted faunal groups should be considered before choosing a sampling regime. Furthermore, multiple gear considerations should be made when trying to determine macrofauna habitat utilization.

Oyster Habitats in Delaware Bay

This study demonstrates the habitat utilization of constructed shell-bag reefs and oyster aquaculture systems in a temperate estuary. Natural oyster settlement occurred on the shell-bag reefs shortly after construction, and high recruitment rates typical for the Cape Shore region were evident in September 2007. Hidu (1978) observed two patterns of high oyster larvae settlement at Cape Shore. The highest oyster settlement occurred at the slope area in 1.8 m to 3.66 m of water and in the intertidal zone at MLW. In this study, highest settlement occurred on the shell-bag reefs with the lowest elevation above sea level. In contrast, the reefs with the highest elevation above sea level received the

lowest oyster settlement. Growth and survival of intertidal oysters was influenced by tidal zonation. Oysters in the high intertidal zone (> 25 cm above MLW) experience higher mortality and slower growth rates than oysters at or below MLW (Roegner & Mann 1995, Bartol et al. 1999). Relative to height above sea level, the constructed shell-bag reefs were within a 15 cm elevation range which is less than the elevation difference between MLW and the high intertidal zone. Therefore, it is unlikely that this change in elevation is contributing to the differences in oyster settlement.

Intertidal constructed shell-bag reefs and oyster aquaculture operations supported an increased species abundance and biomass of motile macrofauna compared to the adjacent sand flats. Abundances of species utilizing these two habitat types were more than triple that of the sand habitat. The habitat created by shell-bag reefs and oyster aquaculture structures also maintains high species richness and fauna communities similar to natural oyster reef habitats (Maurer & Watling 1971, Lenihan et al. 2001, Rodney & Paynter 2006).

Species composition with regards to the eight most abundant species was similar for the three habitat types, but the abundances of these species varied greatly among the habitat types. Differences in species abundances and diversity can be attributed to the habitat created through constructed oyster reefs and aquaculture structures in comparison to the barren sand flats. However, the differences in species composition of crustaceans and gastropods between constructed reefs and aquaculture racks are more difficult to discern because of the differences in habitat depth and structure. In this study, PCA analysis indicates macrofauna composition is associated with height above sea level. Therefore, the greater species abundances associated with the oyster aquaculture racks is

not necessarily due to an increase in habitat value when compared to constructed shell-bag reefs. Nevertheless, it can be concluded that oyster aquaculture structures and intertidal oyster reefs provide habitats that attract similar assemblages of motile fauna.

The linear regression indicates that height above sea level is not the only factor driving the separation along PC1. Macrofauna sampling was conducted during spring, summer and fall months and a seasonal gradient may be influencing the species composition. Another factor possibly influencing species composition of the three habitat types is habitat structure. The aquaculture racks provide vertical relief while allowing fauna to pass freely underneath the rack, hide from predators and stalk prey, whereas fauna cannot pass freely under the more solid structure of the reefs. Nonetheless, the PCA results confirm the intrinsic habitat value associated with both types of oyster structure.

The habitat matrix created by oyster aquaculture structures and shell-bag reefs serve as nursery grounds for juvenile species where they can forage while being protected from predators. Nine juvenile finfish species were associated with these oyster habitats and not the adjacent sand flats. In previous studies, juvenile species documented utilizing oyster habitat included: *L. rhomboides*, *O. tau*, *C. striata*, *P. dentatus*, *A. mitchilli*, *L. griseus*, *L. xanthurus* and *C. regalis* (Breitburg 1999, Harding & Mann 1999, Lehnert & Allen 2002). In this study, all of these juvenile species were associated with either shell-bag reefs or oyster aquaculture structures. Therefore, these constructed oyster structures create nursery grounds for juvenile species similar to oyster reef habitat.

Another ecological value of intertidal shell-bag reefs and aquaculture racks is that they act as foraging sites and refugia from predation for a variety of species in Delaware

Bay. Many studies have documented the utilization of oyster reefs for refuge and foraging by finfish and decapod crustaceans in other estuarine systems (Breitburg 1999, Coen & Luckenbach 2000, Luckenbach et al. 2005, Rodney & Paynter 2006). In Delaware Bay, grass shrimp, *Palaemonetes* sp., are widely abundant and important prey items for demersal fish and invertebrates (Leatham & Maurer 1980). The pattern of higher fish species richness associated with aquaculture racks (15 species) and shell-bag reefs (12 species) compared to the sand flats (7) may indicate that both types of oyster habitat serve as foraging grounds for finfish species. Increased prey availability could contribute to the higher biomass associated with constructed shell-bag reefs and oyster aquaculture structures. In this study, *P. pugio* was three times more abundant on shell-bag reefs than sand flats and six times more abundant on aquaculture racks. MANOVA results indicated that *P. pugio* abundance and biomass was significantly greater on oyster habitat and PCA showed *P. pugio* had a strong influence on species composition. Posey (1999) found that *P. pugio* would hide in oyster shell habitat to escape predators. The shell-bag reef habitat created predator refuge for *P. pugio*. The higher numbers of *P. pugio* associated with oyster aquaculture racks indicate these systems create a different oyster structure with predatory protection supplied by both the oysters and the plastic mesh bags.

Intertidal constructed oyster structures can simultaneously provide multiple ecological functions. Xanthid crabs utilize oyster reefs as foraging grounds while feeding on oyster spat and predator refuge from species such as the oyster toadfish, *O. tau* (McDonald 1982, Tolley & Volety 2005). In this study, two adult mud crab species *R. harrisi* and *E. depressus* were three times more abundant in traps on shell-bag reefs than

both aquaculture racks and sand flats. During this study, the shell-bag reefs consisted of disarticulated shell with newly settled spat, while the oyster aquaculture racks contained varying sizes of adult oysters. Oyster spat is the preferred diet over clams, mussels and barnacles (Leatham & Maurer 1980, McDonald 1982) for *R. harrisii*, *E. depressus* and *P. herbstii* which could attract them preferentially to the shell-bag reefs over the other two habitat types. Also, the niche-filled structure of the shell-bags reefs may serve as better refugia from predation than the aquaculture racks. With regards to Xanthid crabs, intertidal constructed reefs habitat value is greater than oyster aquaculture racks.

Intertidal rack and bag oyster aquaculture structures and constructed shell-bag reefs support similar macrofauna assemblages and abundances. Aquaculture racks did support an increased species abundance, biomass and richness compared to shell-bag reefs and sand flats. However, there are several caveats of this study that confound the conclusion that oyster aquaculture operations are superior habitat than oyster reefs. The sand bars along the tidal flats at Cape Shore are not uniform in sediment elevation. At low tide, the sand and shell-bag reef treatments were completely exposed while three of the aquaculture treatments remained in a shallow portion of a slough with about 2-6 cm of water. Traps that are in sloughs experience a prolonged fishing time which could artificially increase species abundance, biomass and richness. Sediment elevations taken at each treatment replicate indicated that all habitat treatments were within a sediment elevation range less than 22.9 cm, and this change in elevation is less than the height of the reefs (30 cm) and was not significant. A second caveat is that the aquaculture operation consisted of a dense cluster of rack and bag structures which was much more complex than the constructed shell-bag reefs. The aquaculture operation is spread over

three sand bars with rows containing 10-12 aquaculture racks. All six shell-bag reef treatments were at least 10 m apart while the aquaculture racks were an average of 2 m apart. Also, the total lengths of the racks ranged from 15 to 18 m which is over 5 times the length of the shell-bag reefs. The large structure matrix of the aquaculture operation is on a much larger scale than the constructed shell-bag reefs and could be creating an increased species abundance, biomass and richness artifact. Finally, only one sampling method was used in this study to identify habitat utilization of aquaculture racks. A more inclusive sampling regime should be used in order to better define the habitat value of these structures. Therefore, a conservative conclusion is that intertidal aquaculture rack and bag structures attract nearly equivalent assemblages of motile fauna.

During the mark-recapture study, American eels, *A. rostrata*, were most commonly associated with aquaculture racks. *A. rostrata* biomass was significantly greater on aquaculture racks. However, this may be a result of the high abundances captured since average monthly biomass was consistently higher on shell-bag reefs. *A. rostrata* has been previously associated with subtidal and intertidal oyster reefs (Coen et al. 1999, Harding & Mann 1999). Despite the lack of statistical significance, eels were seven times more abundant on shell-bag reefs than sand flats.

Eels were recaptured on both oyster habitats but not on the sand flats. While the recapture rate was 13%, the total number of eels marked and released (n=52) was low. Recaptured eels demonstrated no obvious preference between shell-bag reef and aquaculture habitats. Previous mark-recapture studies have shown that when estuarine environmental conditions are favorable, yellow phase American eels exhibit a limited home range (Bozeman et al. 1985, Ford & Mercer 1986, Morrison & Secor 2003).

Yellow phase eels prefer dark areas where they can be in constant contact with their surroundings which is why they often hide in hollow crevices or burrow in the mud (Tesch 1977). Off-bottom aquaculture operations provide dark areas and access to sediment under the racks while constructed shell-bag reefs provide a multitude of crevices for hiding. Anecdotally, during shell-bag sampling, eels were found hiding under shell-bags at low tide. While a more comprehensive mark-recapture study is necessary to determine home range and residency, data from the present study indicate that the American eel will readily use intertidal shell-bag reefs and oyster aquaculture racks as habitat.

Seining was conducted around shell-bag reef and sand habitats to sample the transient species that were excluded from trap sampling. Breitburg (1999) defined transient oyster reef species as species that inhabit a wide range of habitats but can often be abundant around oyster reefs. Seining was conducted along the perimeter of the habitats sampling fauna associated with the sand flats immediately adjacent to each habitat. Although not significant, trends followed patterns observed with trap sampling. Namely, shell-bag reefs had higher species abundance, biomass and richness than the sand flats. Fourteen finfish species were associated with the shell-bag reefs and there was increased prey availability for these species around the reef habitat. In Delaware Bay, mysids are a dominant zooplankton food for fish species and have been characterized as a transient species on Delaware Bay oyster beds (Maurer & Watling 1971, Leatham & Maurer 1980). In some samples, the mysid, *N. Americana*, was four times greater around the shell-bag reefs and *P. pugio* was six times greater compared to the sand flats. In this study, the only previously documented transient species that was

more abundant on shell-bag reefs was the bay anchovy, *A. mitchilli* and this association was not significant (Breitburg 1999, Harding & Mann 1999, 2001, Lehnert & Allen 2002). The lack of significance of seining data indicates that the shell-bag reefs did not alter the sand flat fauna immediately adjacent to the reef. Also, it is difficult to accurately quantitatively sample transient species using a seine. The utilization of shell-bag reef habitat by transient species cannot be determined from the results of this study.

Shell-bag fauna sampling was conducted to assess motile fauna that were residing in the shell-bags. Oyster reef resident species are reliant on oyster reefs as their principal habitat (Breitburg 1999). Nineteen juvenile species (68% of species collected) were utilizing the shell-bag habitat. The most abundant species was the gastropod, *A. lunata* which was noted as a common species in Delaware Bay by Maurer and Watling (1971). Average species abundances increased from June to September coinciding with the settlement and growth of *C. virginica* on the constructed reefs. Meyer and Townsend (2000) found that the mud crab, *E. depressus*, recruited to constructed oyster reefs after oyster settlement occurred. The greatest species abundances occurred on the reefs with the lowest height above sea level and the highest oyster settlement, Reef 4 and Reef 6. Adult and juvenile mud crabs were common species residing in the shell-bags with *P. herbstii* the most abundant followed by *E. depressus* and *R. harrisii*. *E. depressus* is the most abundant mud crab species on Delaware Bay oyster beds while *P. herbstii* is more common in the lower bay and *R. harrisii* in the upper bay (Maurer & Watling 1971, Leatham & Maurer 1980). Shell-bag sampling confirmed that constructed shell-bag reefs are habitat for juvenile, resident fauna and the increasing resident community indicates the shell-bag reefs were beginning to attract oyster reef fauna.

As *C. virginica* populations continue to decline, fishery managers turn towards oyster reef restoration and aquaculture to ameliorate the current deteriorating situation. A properly designed large or small-scale restoration project can improve the oyster populations and provide ecosystem services such as habitat and benthic-pelagic coupling (Coen & Luckenbach 2000, Brumbaugh et al. 2006). Constructed shell-structures aid in restoring the oyster populations. This habitat utilization study showed that intertidal constructed shell-bag reefs can (1) persist, (2) support increased species richness, abundance and biomass compared to sand flats, and (3) attract similar fauna as oyster reef habitat in Delaware Bay.

Similar to oyster reef restoration, oyster aquaculture can aid the failing oyster populations by alleviating demand on the wild populations. The public has growing concerns over the environmental impact of oyster aquaculture, including eutrophication and loss of habitat. While these concerns are valid, there are ecological benefits of oyster aquaculture that are being overlooked. This study demonstrated that intertidal oyster aquaculture rack and bag structures create valuable habitat that is similar to oyster reef habitat. The loss of sand flat habitat is mitigated by the increase in species richness associated with the aquaculture racks. There are few studies that document the ecological services of shellfish aquaculture operations. The ecological benefits and habitat value of aquaculture apparent in this study have also been associated with clam aquaculture (Powers et al. 2007) and oyster aquaculture operations (DeAlteris et al. 2004, Laffargue et al. 2006). Aquaculture is a viable method to help alleviate the declining oyster populations while creating sustainable habitat for many species.

Table 1. List of species sampled with wire mesh traps on the three intertidal shell-bag reefs and control sand plot. Number denotes total number of individuals collected. Total abundance and species richness is shown for all four sampling sites. Reef names are as follows: Reef 1 (1 shell-bag layer), Reef 2 (2 shell-bag layers), and Reef 3 (3 shell-bag layers). Fish species and common names referenced from (Nelson et al. 2004). Invertebrate species and common names referenced from (Turgeon et al. 1998, McLaughlin et al. 2005).

Species Name	Common Name	Sand	Reef 1	Reef 2	Reef 3
Teleosts					
<i>Anguilla rostrata</i>	American eel	0	1	2	2
<i>Bairdiella chrysoura</i>	Silver Perch	1	1	1	0
<i>Lagodon rhomboides</i>	Pinfish	1	1	0	1
<i>Menidia menidia</i>	Atlantic silverside	2	0	0	1
<i>Opsanus tau</i>	Oyster Toadfish	0	0	1	0
Crustaceans					
<i>Palaemonetes pugio</i>	Daggerblade grass shrimp	15	40	35	29
<i>Pagurus longicarpus</i>	Longwrist hermit crab	10	19	11	20
<i>Callinectes sapidus</i>	Blue crab	4	2	5	2
<i>Panopeus herbstii</i>	Atlantic mud crab	1	3	0	0
<i>Crangon septemspinosa</i>	Seven spine sand shrimp	0	2	1	0
<i>Eurypanopeus depressus</i>	Flatback mud crab	0	1	0	1
<i>Rhithropanopeus harrisii</i>	Harris mud crab	0	1	0	0
Gastropods					
<i>Nassarius obsoletus</i>	Eastern mud snail	0	15	11	6
Total Abundance		34	86	67	62
Species Richness		7	11	8	8

Table 2. Height (m) above sea level of replicate sampling locations. Reef and sand sites were paired as indicated in Fig. 5. Aquaculture sites were selected independently to represent a similar range of depths.

Replicate	Sand	Reef	Aquaculture
1	0.996	0.958	1.006
2	0.956	0.976	0.981
3	0.934	0.865	0.956
4	0.902	0.895	0.824
5	0.892	0.865	0.814
6	0.843	0.824	0.777

Table 3. Species captured with wire mesh, eel and crab traps on the three habitat types: sand (n=122), shell-bag reefs (n=122) and aquaculture racks (n=120) from May through October 2007. Values denote total number of individuals collected. Fish species and common names referenced from (Nelson et al. 2004). Invertebrate species and common names referenced from (Turgeon et al. 1998, McLaughlin et al. 2005). * indicates species not collected during seine sampling. + indicates species not collected during shell-bag sampling. Superscripts indicate if species are a = adult and juvenile or b = juvenile.

Common Name	Species Name	Sand	Reef	Aquaculture	Total
Daggerblade grass shrimp	<i>Palaemonetes pugio</i>	541	1890	3534	5965
Eastern mud snail	<i>Nassarius obsoletus</i>	117	869	840	1826
Longwrist hermit crab	<i>Pagurus longicarpus</i>	235	867	658	1760
Blue crab	<i>Callinectes sapidus</i>	60 ^a	53 ^a	58 ^a	171
Seven spine bay shrimp	<i>Crangon septemspinosa</i>	70 ^a	30	33 ^a	133
American eel	<i>Anguilla rostrata</i> *+	2	15	42	59
Atlantic horseshoe crab	<i>Limulus polyphemus</i>	13 ^b	16 ^b	1 ^b	30
Silver perch	<i>Bairdiella chrysoura</i> +	1 ^b	2 ^b	17 ^b	20
Estuarine mud crab	<i>Rhithropanopeus harrisi</i> *	3	12	4	19
Atlantic silverside	<i>Menidia menidia</i> +	3 ^b	6 ^b	7 ^b	16
Striped cusk-eel	<i>Ophidion marginatum</i> *+	5	3	2	10
Flatback mud crab	<i>Eurypanopeus depressus</i> *	3	10	1	14
Naked goby	<i>Gobiosoma bosc</i>	0	6	5	11
Atlantic mud crab	<i>Panopeus herbstii</i> *	2	2	2	6
Pinfish	<i>Lagodon rhomboides</i> *+	0	2 ^b	2 ^b	4
Smallmouth flounder	<i>Etropus microstomus</i> *+	3 ^b	1 ^b	0	4
Oyster toadfish	<i>Opsanus tau</i> *+	0	2	1 ^b	3
Green crab	<i>Carcinus maenas</i> *	0	1	1	2
Striped bass	<i>Morone saxatilis</i> +	1 ^b	1 ^b	0	2
Asian shore crab	<i>Hemigrapsus sanguineus</i> *	1	0	0	1
Atlantic croaker	<i>Micropogonias undulatus</i> +	1 ^b	3 ^b	0	4
Black sea bass	<i>Centropristis striata</i> *+	0	1 ^b	0	1
Summer flounder	<i>Paralichthys dentatus</i> +	0	1	1 ^b	2
Alewife	<i>Alosa pseudoharengus</i> *+	0	0	2 ^b	2
Bay anchovy	<i>Anchoa mitchilli</i> +	0	0	2 ^a	2
Gray snapper	<i>Lutjanus griseus</i> *+	0	0	1 ^b	1
Spot	<i>Leiostomus xanthurus</i> *+	0	0	1 ^b	1
Striped killifish	<i>Fundulus majalis</i> *+	0	0	1	1
Weakfish	<i>Cynoscion regalis</i> *+	0	0	1 ^b	1
White perch	<i>Morone americana</i> *+	0	0	1	1
Total		1061	3793	5218	10072
Species Richness		17	22	25	30

Table 4. Summary of three-factor ANOVA testing differences in trap species abundance, biomass and species richness among habitat types. Multiple comparisons of the means were analyzed using Tukey's post-hoc comparisons ($\alpha = 0.05$). Treatments are S, sand; R, reef and A, aquaculture. Time is D, day or N, night. Traps are E, eel; M, wire mesh and C, crab.

	Source	df	MS	F	P	Multiple Comparisons
Abundance	Treatment	2	5181.97	28.97	<0.0001	S < R = A
	Time	1	1662.47	9.29	0.003	D < N
	Trap	2	9110.77	50.94	<0.0001	C < E = M
	Treatment*Time	2	312.59	1.75	0.18	
	Treatment*Trap	4	1298.21	7.26	<0.0001	
	Time*Trap	2	441.44	2.47	0.0905	
	Treatment*Time*Trap	4	108.76	0.61	0.6579	
	Error	90	178.87			
Biomass	Treatment	2	10852.74	12.48	<0.0001	S < R = A
	Time	1	416.58	0.48	0.4906	D = N
	Trap	2	310.70	0.36	0.7005	E = C = M
	Treatment*Time	2	460.88	0.53	0.5903	
	Treatment*Trap	4	1905.35	2.19	0.0762	
	Time*Trap	2	4073.98	4.69	0.0116	
	Treatment*Time*Trap	4	301.67	0.35	0.8455	
	Error	90	869.27			
Richness	Treatment	2	5.66	21.79	<0.0001	S = R, S < A, R = A
	Time	1	0.60	2.32	0.1316	D = N
	Trap	2	69.88	269.01	<0.0001	C < E = M
	Treatment*Time	2	0.07	0.29	0.7515	
	Treatment*Trap	4	1.45	5.59	0.0005	
	Time*Trap	2	0.51	1.97	0.1453	
	Treatment*Time*Trap	4	0.21	0.80	0.5303	
	Error	90	0.26			

Table 5. Multivariate and univariate ANOVA results for effects on the eight most abundant trap species of habitat species abundances. Highlighted P-value for ANOVA's are significant at the Bonferroni-corrected significance level ($\alpha = .006$) for 8 tests, one for each species.

MANOVA					
	Source	df	Wilk's λ	F	P
	Treatment	16, 46	0.1004	6.20	<0.0001
	Time	8, 23	0.2892	7.06	0.0001
	Treatment*Time	16, 46	0.3198	2.21	0.0185
ANOVA					
	Source	df	SS	F	P
<i>Palaemonetes pugio</i>	Treatment	2	3983.32	16.85	<0.0001
	Time	1	670.81	5.67	0.0238
	Treatment*Time	2	292.69	1.24	0.3044
<i>Nassarius obsoletus</i>	Treatment	2	295.15	6.61	0.0042
	Time	1	171.52	7.68	0.0095
	Treatment*Time	2	54.16	1.21	0.3114
<i>Pagurus longicarpus</i>	Treatment	2	175.82	17.16	<0.0001
	Time	1	26.20	5.11	0.0311
	Treatment*Time	2	13.77	1.34	0.2761
<i>Callinectes sapidus</i>	Treatment	2	0.0450	0.44	0.6452
	Time	1	0.1190	2.35	0.1357
	Treatment*Time	2	0.1776	1.75	0.1903
<i>Crangon septemspinosus</i>	Treatment	2	0.6710	3.20	0.0550
	Time	1	1.8496	17.64	0.0002
	Treatment*Time	2	0.2348	1.12	0.3397
<i>Anguilla rostrata</i>	Treatment	2	0.7330	10.79	0.0003
	Time	1	0.1863	5.49	0.0260
	Treatment*Time	2	0.1321	1.95	0.1606
<i>Limulus polyphemus</i>	Treatment	2	0.0851	5.93	0.0068
	Time	1	0.0110	1.54	0.2249
	Treatment*Time	2	0.0613	4.27	0.0234
<i>Bairdiella chrysoura</i>	Treatment	2	0.1598	7.25	0.0027
	Time	1	0.0117	1.07	0.3102
	Treatment*Time	2	0.0118	0.54	0.5898

Table 6. Summary of Discriminant Function Analysis for differences in trap species abundances among habitat types. Significant P-values are highlighted.

Species	Statistic	Treatment	Time	Treatment*Time
<i>Palaemonetes pugio</i>	Coefficient	0.016	0.003	-0.013
	Correlation	0.996	0.231	-0.562
	P	<0.0001	0.1749	0.0004
<i>Nassarius obsoletus</i>	Coefficient	-0.013	0.014	0.007
	Correlation	0.707	0.295	-0.394
	P	<0.0001	0.0806	0.0174
<i>Pagurus longicarpus</i>	Coefficient	0.024	-0.051	0.049
	Correlation	0.350	-0.548	0.226
	P	0.0363	0.0005	0.1855
<i>Callinectes sapidus</i>	Coefficient	-0.581	0.354	0.534
	Correlation	0.263	0.279	0.170
	P	0.1209	0.0994	0.3215
<i>Crangon septemspinosa</i>	Coefficient	-0.171	0.451	0.078
	Correlation	-0.185	0.708	0.001
	P	0.2802	<0.0001	0.9953
<i>Anguilla rostrata</i>	Coefficient	0.250	0.439	-0.724
	Correlation	0.802	0.305	-0.626
	P	<0.0001	0.0702	<0.0001
<i>Limulus polyphemus</i>	Coefficient	-0.970	-0.176	1.383
	Correlation	-0.185	0.095	0.429
	P	0.2799	0.5809	0.009
<i>Bairdiella chrysoura</i>	Coefficient	0.222	-1.151	1.289
	Correlation	0.511	-0.206	-0.080
	P	0.0015	0.2281	0.6438

Table 7. Multivariate and univariate ANOVA results for effects on the eight most abundant trap species of habitat species biomass. Highlighted P-value for ANOVA's are significant at the Bonferroni-corrected significance level ($\alpha = .006$) for 8 tests, one for each species.

MANOVA					
	Source	df	Wilk's λ	F	P
	Treatment	16, 46	0.1006	6.19	<0.0001
	Time	8, 23	0.2892	7.07	0.0001
	Treatment*Time	16, 46	0.4639	1.35	0.2117
ANOVA					
	Source	df	SS	F	P
<i>Palaemonetes pugio</i>	Treatment	2	866.26	14.50	<0.0001
	Time	1	137.51	4.60	0.0401
	Treatment*Time	2	50.39	0.84	0.4401
<i>Nassarius obsoletus</i>	Treatment	2	618.11	6.92	0.0034
	Time	1	387.89	8.68	0.0062
	Treatment*Time	2	116.65	1.31	0.286
<i>Pagurus longicarpus</i>	Treatment	2	440.90	16.73	<0.0001
	Time	1	90.19	6.84	0.0138
	Treatment*Time	2	26.13	0.99	0.3828
<i>Callinectes sapidus</i>	Treatment	2	0.695	0.23	0.7938
	Time	1	0.102	0.07	0.7953
	Treatment*Time	2	5.003	1.67	0.2045
<i>Crangon septemspinosus</i>	Treatment	2	0.101	3.02	0.0637
	Time	1	0.209	12.5	0.0013
	Treatment*Time	2	0.078	2.34	0.1134
<i>Anguilla rostrata</i>	Treatment	2	2526.92	11.48	0.0002
	Time	1	694.06	6.3	0.0177
	Treatment*Time	2	255.82	1.16	0.3266
<i>Limulus polyphemus</i>	Treatment	2	0.005	5.11	0.0123
	Time	1	0.004	6.75	0.0144
	Treatment*Time	2	0.004	3.7	0.0365
<i>Bairdiella chrysoura</i>	Treatment	2	0.623	2.62	0.0892
	Time	1	0.021	0.17	0.6805
	Treatment*Time	2	0.016	0.07	0.9367

Table 8. Summary of Discriminant Function Analysis for differences in trap species biomass among habitat types. Significant P-values are highlighted.

Species	Statistic	Treatment	Time	Treatment*Time
<i>Palaemonetes pugio</i>	Coefficient	0.002	0.010	-0.013
	Correlation	0.360	0.211	-0.526
	P	0.0312	0.2177	0.001
<i>Nassarius obsoletus</i>	Coefficient	0.003	0.015	-0.001
	Correlation	0.427	0.303	-0.325
	P	0.0093	0.0726	0.053
<i>Pagurus longicarpus</i>	Coefficient	0.054	-0.038	0.025
	Correlation	0.841	-0.564	0.254
	P	<0.0001	0.0003	0.135
<i>Callinectes sapidus</i>	Coefficient	-0.112	0.080	0.052
	Correlation	-0.183	0.089	0.300
	P	0.2859	0.6043	0.0757
<i>Crangon septemspinosa</i>	Coefficient	-0.645	1.094	0.448
	Correlation	-0.541	0.643	0.344
	P	0.0007	<0.0001	0.0399
<i>Anguilla rostrata</i>	Coefficient	0.000	0.005	-0.007
	Correlation	0.298	0.285	-0.509
	P	0.0775	0.0925	0.0015
<i>Limulus polyphemus</i>	Coefficient	2.454	1.477	5.385
	Correlation	0.045	0.324	0.572
	P	0.7943	0.0542	0.0003
<i>Bairdiella chrysoura</i>	Coefficient	0.064	-0.334	0.221
	Correlation	0.195	-0.104	-0.131
	P	0.2551	0.5478	0.4477

Table 9. Eigenvector loadings and Pearson correlations of species abundances for principal components 1 and 2 for sand, shell-bag reef and aquaculture habitats. Product-moment correlation probability (P) significance: ^aP < 0.001, ^bP < 0.01, ^cP < 0.05.

	PC1		PC2	
	Eigenvector	Correlation	Eigenvector	Correlation
<i>Palaemonetes pugio</i>	0.958	0.998 ^a	-0.269	-0.055
<i>Nassarius obsoletus</i>	0.263	0.865 ^a	0.698	0.455
<i>Pagurus longicarpus</i>	0.112	0.600 ^b	0.663	0.703 ^b
<i>Callinectes sapidus</i>	0.005	0.416	-0.014	-0.244
<i>Crangon septemspinosa</i>	-0.008	-0.461 ^c	-0.035	-0.379
<i>Anguilla rostrata</i>	0.014	0.925 ^a	-0.003	-0.034
<i>Limulus polyphemus</i>	-0.002	-0.375	0.010	0.380
<i>Bairdiella chrysoura</i>	0.005	0.670 ^c	-0.010	-0.237

Table 10. Species captured by seine around shell-bag reefs (n=8) and sand habitats (n=8) from June to September 2007. Values denote total number of individuals collected.

Fish species and common names referenced from (Nelson et al. 2004). Invertebrate species and common names referenced from (Turgeon et al. 1998, McLaughlin et al. 2005). * indicates species not collected during trap sampling. + indicates species not collected during shell-bag sampling. Superscripts indicate if species are a = adult and juvenile or b = juvenile.

Common Name	Species Name	Sand	Reef	Total
Opossum shrimp	<i>Neomysis americana</i> *	820	3063	3883
Atlantic horseshoe crab	<i>Limulus polyphemus</i>	583 ^a	426 ^a	1009
Bay anchovy	<i>Anchoa mitchilli</i> +	303 ^a	330 ^a	633
Seven spine bay shrimp	<i>Crangon septemspinosa</i>	236 ^a	208 ^a	444
Daggerblade grass shrimp	<i>Palaemonetes pugio</i>	46	296	342
Blue crab	<i>Callinectes sapidus</i>	32 ^a	105 ^a	137
Atlantic silverside	<i>Menidia menidia</i> +	42 ^a	20 ^a	62
Atlantic Croaker	<i>Micropogonias undulates</i> +	23 ^b	16 ^b	39
Bluefish	<i>Pomatomus saltatrix</i> *+	7 ^b	3 ^b	10
Valviferan isopod	<i>Synidotea laticauda</i> *+	1	8	9
Northern pipefish	<i>Syngnathus fuscus</i> *+	4 ^b	1 ^b	5
Atlantic menhaden	<i>Brevoortia tyrannus</i> *+	2 ^b	1 ^b	3
Striped Bass	<i>Morone saxatilis</i> +	2 ^b	1 ^b	3
Black drum	<i>Pogonias cromis</i> *+	0	2 ^b	2
Edotia isopod	<i>Edotia tribola</i> *	1	1	2
Hogchoker	<i>Trinectes maculatus</i> *+	1 ^b	1	2
Ocellate lady crab	<i>Ovalipes ocellatus</i> *+	2	0	2
Summer flounder	<i>Paralichthys dentatus</i> +	2 ^b	0	2
Atlantic needlefish	<i>Strongylura marina</i> *+	0	1 ^b	1
Feather blenny	<i>Hypsoblennius hentz</i> *+	0	1	1
Naked goby	<i>Gobiosoma bosc</i>	0	1	1
Silver Perch	<i>Bairdiella chrysoura</i> +	0	1	1
Windowpane	<i>Scophthalmus aquosus</i> *+	0	1 ^b	1
Total		2107	4487	6594
Species Richness		17	21	23

Table 11. List of species collected from shell-bags (n=6) from June to September 2007. Values denote total number of individuals. Fish species and common names referenced from (Nelson et al. 2004). Invertebrate species and common names referenced from (Turgeon et al. 1998, McLaughlin et al. 2005). * indicates species not collected during trap or seine sampling. Superscripts indicate if species are a = adult and juvenile, b = juvenile or c = could not determine.

Common Name	Species Name	Reef 1	Reef 2	Reef 3	Reef 4	Reef 5	Reef 6	Total
Lunar dovesnail	<i>Astyris lunata</i> *	2595 ^a	1089 ^a	2171 ^a	3600 ^a	2400 ^a	4341 ^a	16196
Scud amphipod	<i>Gammarus sp.</i> *	150	127	130	1282	354	459	2502
Atlantic mud crab	<i>Panopeus herbstii</i>	118 ^a	93 ^a	99 ^a	79 ^a	119 ^a	127 ^a	635
Clam worm	<i>Nereis cuprea</i> *	57 ^a	35 ^a	32 ^a	42 ^a	32 ^a	23 ^a	221
Eastern mud snail	<i>Nassarius obsoletus</i>	44 ^a	21	27 ^a	28 ^a	20 ^a	38 ^a	178
Flatback mud crab	<i>Eurypanopeus depressus</i>	26 ^a	22 ^a	53 ^a	22 ^a	31 ^a	23 ^a	177
Daggerblade grass shrimp	<i>Palaemonetes pugio</i>	38 ^a	34 ^a	45 ^a	2 ^b	1 ^b	20 ^a	140
Edotia isopod	<i>Edotia tribola</i>	34	19	43	20	10	3	129
Estuarine mud crab	<i>Rhithropanopeus harrisii</i>	10 ^b	11 ^b	48 ^a	7 ^b	10 ^a	6 ^a	92
Longwrist hermit crab	<i>Pagurus longicarpus</i>	4	4	24	13	7	3	55
Plumed worm	<i>Diopatra sp.</i> *	12 ^c	1 ^c	21 ^c	1 ^c	1 ^c	10 ^c	46
Ribbed mussel	<i>Geukensia demissa</i> *	8 ^b	6 ^b	15 ^b	0	3 ^b	2 ^b	34
Stout tagelus	<i>Tagelus plebeius</i> *	6 ^b	1 ^b	12 ^b	1 ^b	0	0	20
Atlantic horseshoe crab	<i>Limulus polyphemus</i>	1 ^b	1 ^b	2 ^b	4 ^b	5 ^b	5 ^b	18
Asian shore crab	<i>Hemigrapsus sanguineus</i>	6 ^a	2 ^a	1	0	4 ^a	3 ^b	16
Amethyst gemclam	<i>Gemma gemma</i> *	1	1	2	3	1	1	9
Sabellaria	<i>Sabellaria vulgaris</i> *	0	0	2 ^c	2 ^c	1 ^c	4 ^c	9
Macoma clam	<i>Macoma sp.</i> *	2 ^b	2 ^b	1 ^b	0	1 ^b	1 ^b	7
Atlantic oyster drill	<i>Urosalpinx cinerea</i> *	2 ^b	0	0	0	0	2 ^b	4
Channeled barrel-bubble	<i>Acteocina canaliculata</i> *	0	0	2 ^b	0	0	2 ^b	4
Dwarf surfclam	<i>Mulinia lateralis</i> *	0	0	2 ^b	0	1 ^b	0	3
False angelwing	<i>Petricolaria pholadiformis</i> *	0	1 ^b	1 ^b	1 ^b	0	0	3
Softshell clam	<i>Mya arenaria</i> *	1 ^b	0	0	0	2 ^b	0	3
Blue crab	<i>Callinectes sapidus</i>	1 ^b	0	0	0	0	0	1
Opossum shrimp	<i>Neomysis americana</i>	1	0	0	0	0	0	1
Naked goby	<i>Gobiosoma bosc</i>	0	0	1	0	0	0	1
Seven spine bay shrimp	<i>Crangon septemspinosa</i>	0	0	1 ^b	0	0	0	1
Green crab	<i>Carcinus maenas</i>	0	0	0	1	0	0	1
Total		3117	1470	2735	5108	3003	5073	20506
Species Richness		21	18	23	17	19	19	28

Table 12. Spearman rank correlation coefficients for all shell-bag reefs combined and six individual shell-bag reefs. n=number of overlapping species. Spearman Rank Critical values for $\alpha = 0.05$.

Treatment	n	Correlation	Critical Value
All Reefs	11	0.115	0.523
Reef 1	8	-0.167	0.643
Reef 2	7	0.000	0.714
Reef 3	9	-0.283	0.600
Reef 4	8	-0.071	0.643
Reef 5	7	-0.679	0.714
Reef 6	7	-0.143	0.714

Fig. 1. Intertidal Cape Shore Study Site. A) Asterisk marks location of study site ($39^{\circ} 04' 25''$ N, $74^{\circ} 54' 46''$ W) at Rutgers' Cape Shore Hatchery Facility near Green Creek, New Jersey in lower Delaware Bay. Star marks location of Brandywine Shoal Light, NOAA PORTS Delaware Bay station buoy (station ID 8555889 at $38^{\circ} 59.2'$ N, $75^{\circ} 6.8'$ W) which was used to obtain salinity data B) Historical (ca 1940) intertidal oyster reef at study site. C) Recent (2004) intertidal oyster reef at study site. D) Two-layer shell-bag reef constructed on intertidal sand flats, 26 June 2006 before recruitment. E) Two-layer shell-bag reef on 5 October 2006 after natural oyster recruitment.

Fig. 2. 2006-07 oyster data A) estimated oyster settlement, B) post-settlement mortality through October 2006, C) oyster recruitment through October 2006, and D) oyster abundance in April 2007. Data are means \pm SE calculated from 10 cm² quadrats (n = 16 per reef). *denotes $p < 0.001$ for Tukey's post-hoc comparisons.

Fig. 3. Photographs comparing sedimentation around A) Reef 1, B) Reef 2, and C) Reef 3 in April 2007.

Fig. 4. Habitat Treatments. A) Two-layer shell-bag reef constructed on intertidal sand flats, 9 May 2007. B) Sand treatment marked with rebar poles on 9 May 2007. C) Atlantic Capes Fisheries, Inc oyster aquaculture operation on the intertidal sand flats. D) Example of an aquaculture rack treatment

Fig. 5. Study Design Diagram. Scale diagram of reef (R), sand (S) and aquaculture (A) treatment locations on the intertidal sand flats at Cape Shore (n = 6 replicates per treatment). All treatments were located on the same sand bar. Numbers denote replicate treatment. Blue lines approximate slough locations during low water. Axis break indicates 71 m distance between aquaculture racks and S1 treatment. The total lengths of the aquaculture racks are 15.2 m (racks with A1 and A2) and 18.3 m (racks with A3-6). Diagram is drawn to scale.

Fig. 6. Bi-weekly Trap Sampling. A) Shell-bag reef sampled with 3 wire mesh traps and 1 crab trap. B) Sand treatment sampled with 3 wire mesh traps and 1 crab trap. C) Aquaculture rack sample with 3 wire mesh eel traps.

Fig. 7. *A. rostrata* marking. *Anguilla rostrata* marked ventrally with a blue acrylic paint injection on the right side. * indicates anus. A) Acrylic paint mark at the time of injection. B) Same acrylic paint mark seven days after injection

Fig. 8. Temperature and Salinity. A) Cape Shore average weekly temperature. \pm SE of the mean. B) NOAA Brandywine Shoal Light, DE average weekly salinity. \pm SE of the mean. Gap indicates missing salinity data for a 5 day period (21 June to 25 June 2007).

Fig. 9. 2008 Oyster Settlement and Mortality. Estimated oyster settlement for shell-bag reefs (upper panel), letters indicate significant differences by Tukey's post-hoc comparison. Post-settlement mortality through September 2007 (lower panel). Data are means \pm SE calculated

from 10 cm² quadrats (n = 16 per reef). Inset picture of oyster settlement for Reef 4 in November 2007.

Fig. 10. Trap Species Box Plots. Seine species Box plots for A) species abundance, B) species biomass (g) and C) species richness collected via trap sampling from May to October 2007. Squares indicate mean values. * and letters indicate $p < 0.05$ for Tukey's post-hoc comparisons.

Fig. 11. PCA Ordination. (Top) Principal component ordination of macrofauna community for sand (■), shell-bag reef (●) and oyster aquaculture habitats (▲). 95% of the variation is explained by PC1 and 4% of the variation is explained by PC2. (Bottom) Vector plot of species eigenvector loadings.

Fig. 12. PCA Regression. Linear regression of height above sea level and PC1 for macrofauna community for sand (■), shell-bag reef (●) and oyster aquaculture habitats (▲). Linear regression ANOVA P-value = 0.013.

Fig. 13. *A. rostrata* Abundance and Biomass. A) Total number of *A. rostrata* marked and recaptured during trap sampling from May to October 2007. B) Total biomass (g) of marked and recaptured *A. rostrata*. * indicates $p < 0.05$ for Tukey's post-hoc comparisons.

Fig. 14. *A. rostrata* Seasonal Trends. A) Monthly total abundance of *A. rostrata* for sand (■), shell-bag reef (●) and oyster aquaculture habitats (▲) from May to October 2007. Totals

include marked and recapture eels. B) Average monthly biomass (g) \pm SE of *A. rostrata* for sand (■), shell-bag reef (●) and oyster aquaculture habitats (▲).

Fig. 15. Seine Species Box Plots. Seine species Box plots for A) species abundance, B) species biomass (g) and C) species richness collected via seine sampling from June to September 2007. Squares indicate mean values.

Fig. 16. Shell-bag Species Box Plots. Box plots of shell-bag species from six replicate shell-bag reefs for A) species abundance, B) species biomass (g) and C) species richness collected via shell-bag sampling from June to September 2007. Squares indicate mean values.

Fig. 17. Shell-bag Species Seasonal Trends. Shell-bag reef species average abundances from June-September 2007 via shell-bag sampling. A) Average abundances \pm SE for all resident species (■) and the most abundant resident reef species *Astyris lunata* (●). B) Average abundances \pm SE for three resident species of mud crabs: *P. herbstii* (■), *E. depressus* (●) and *R. harrisii* (▲).

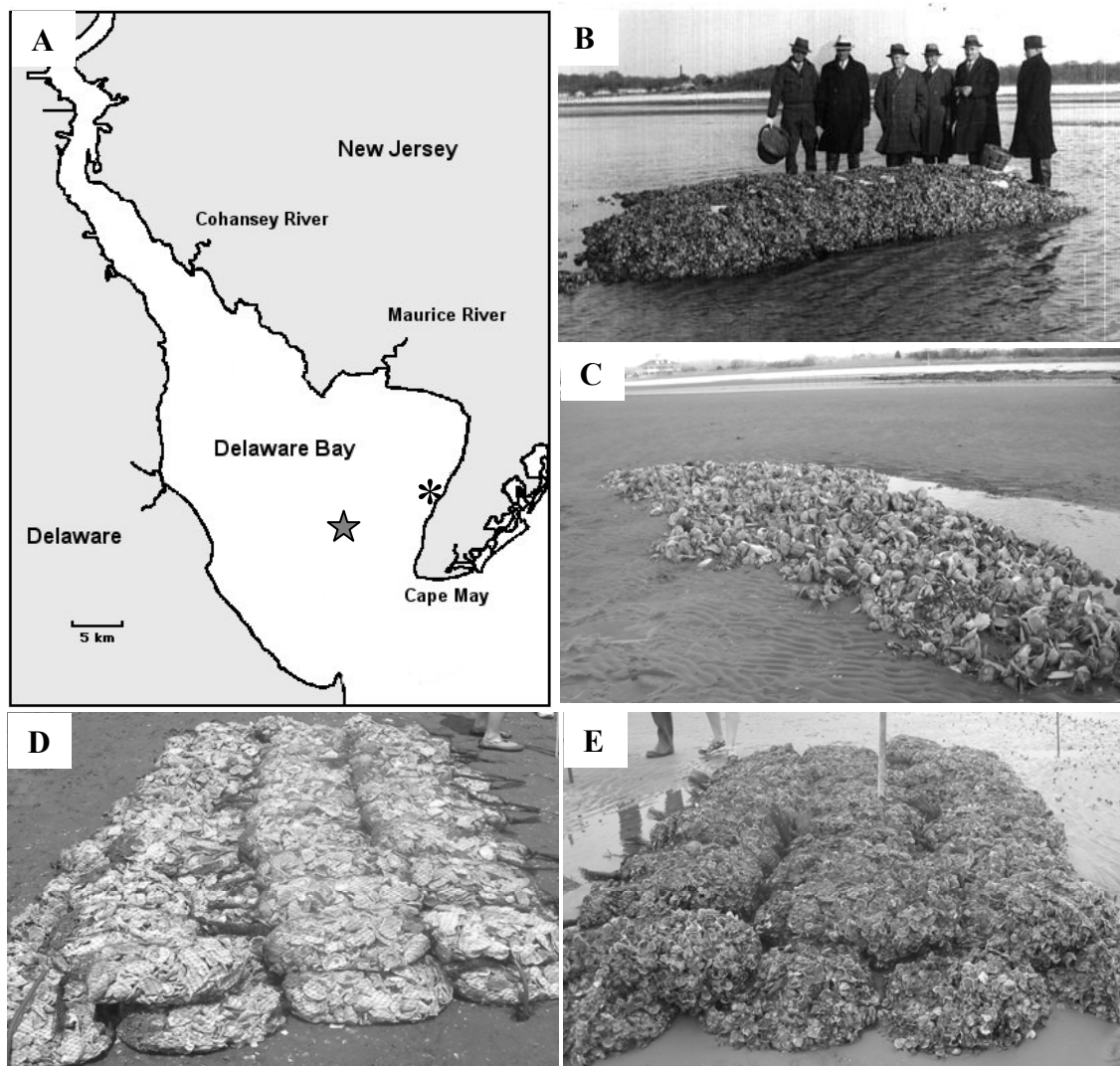


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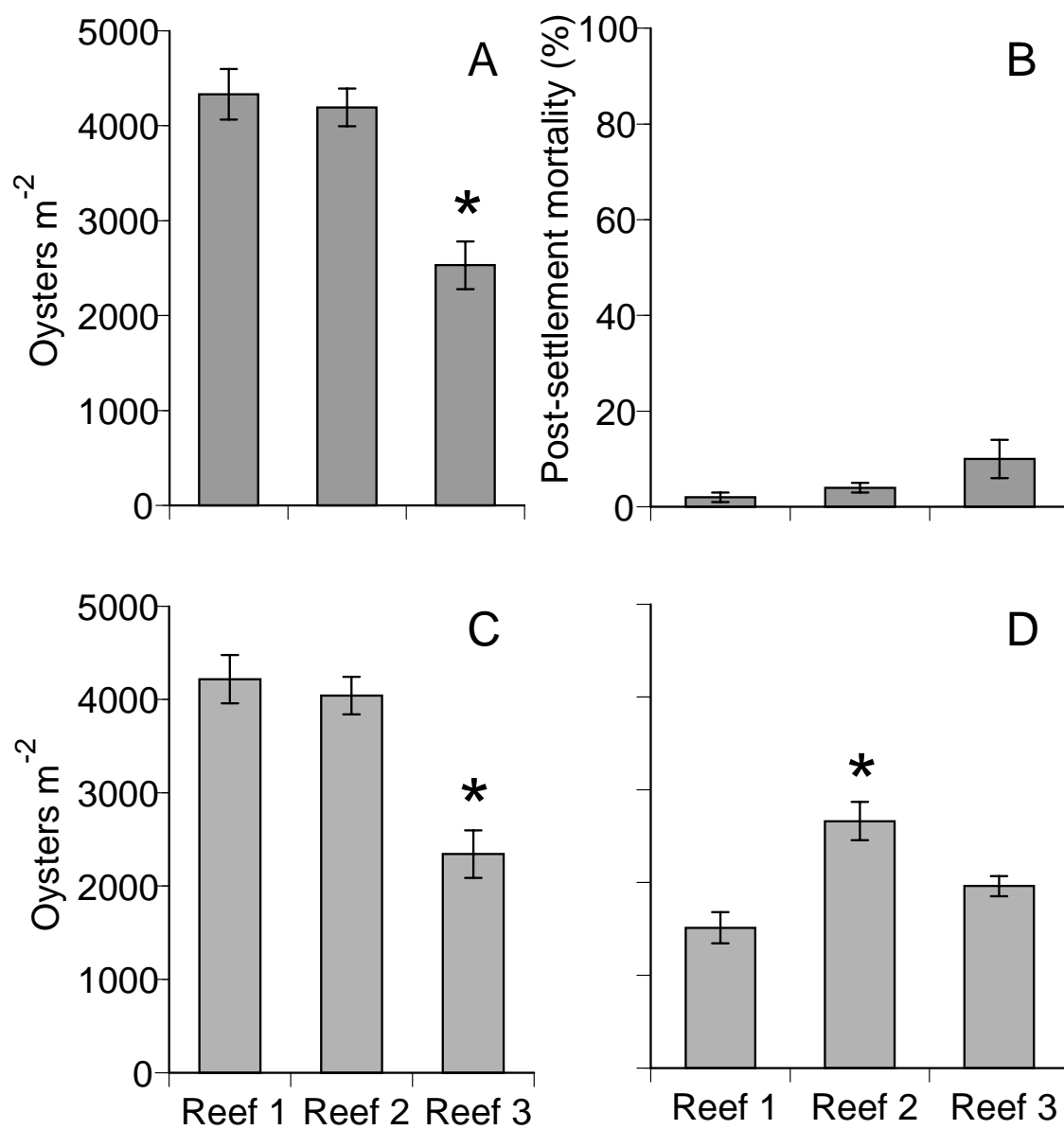


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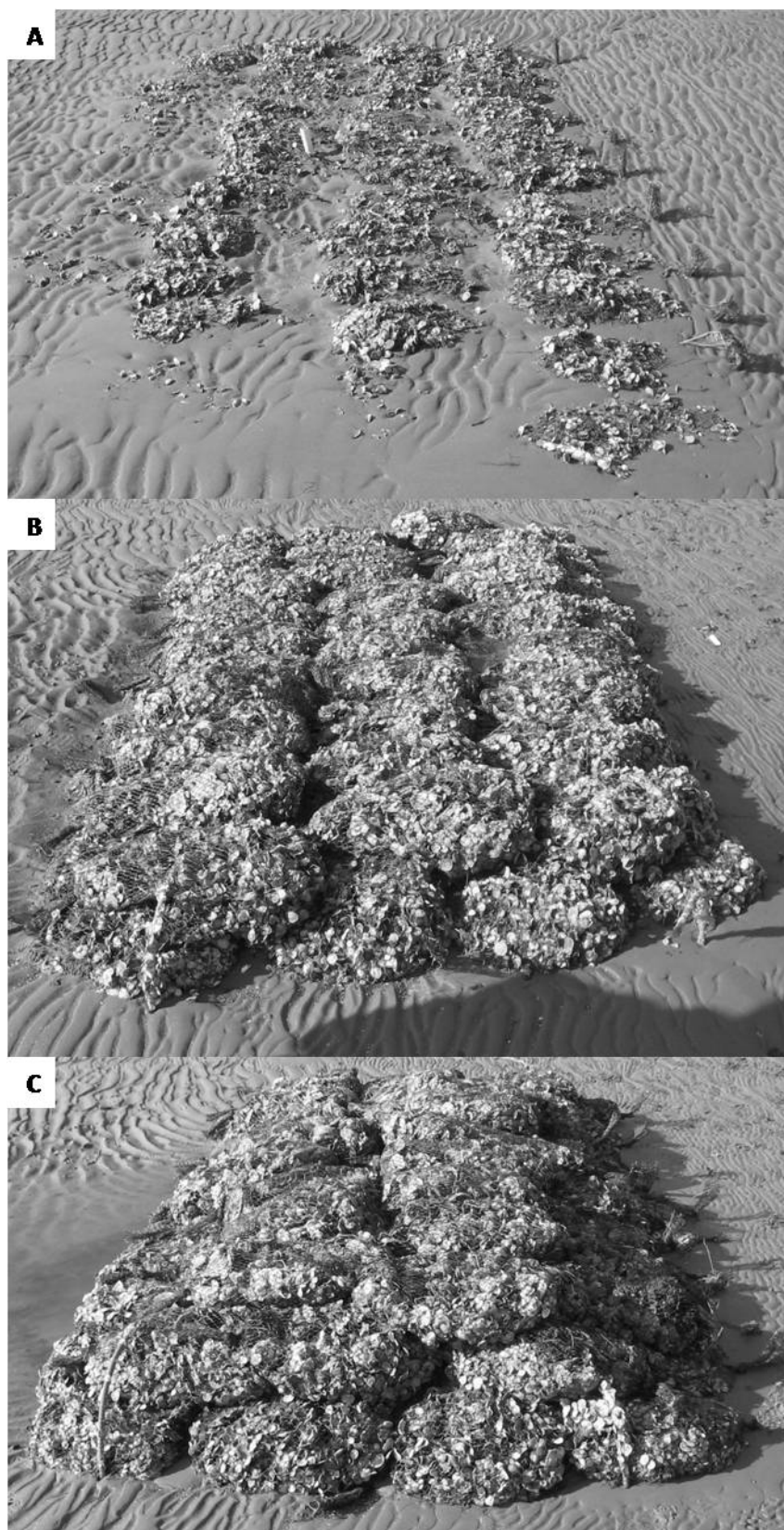


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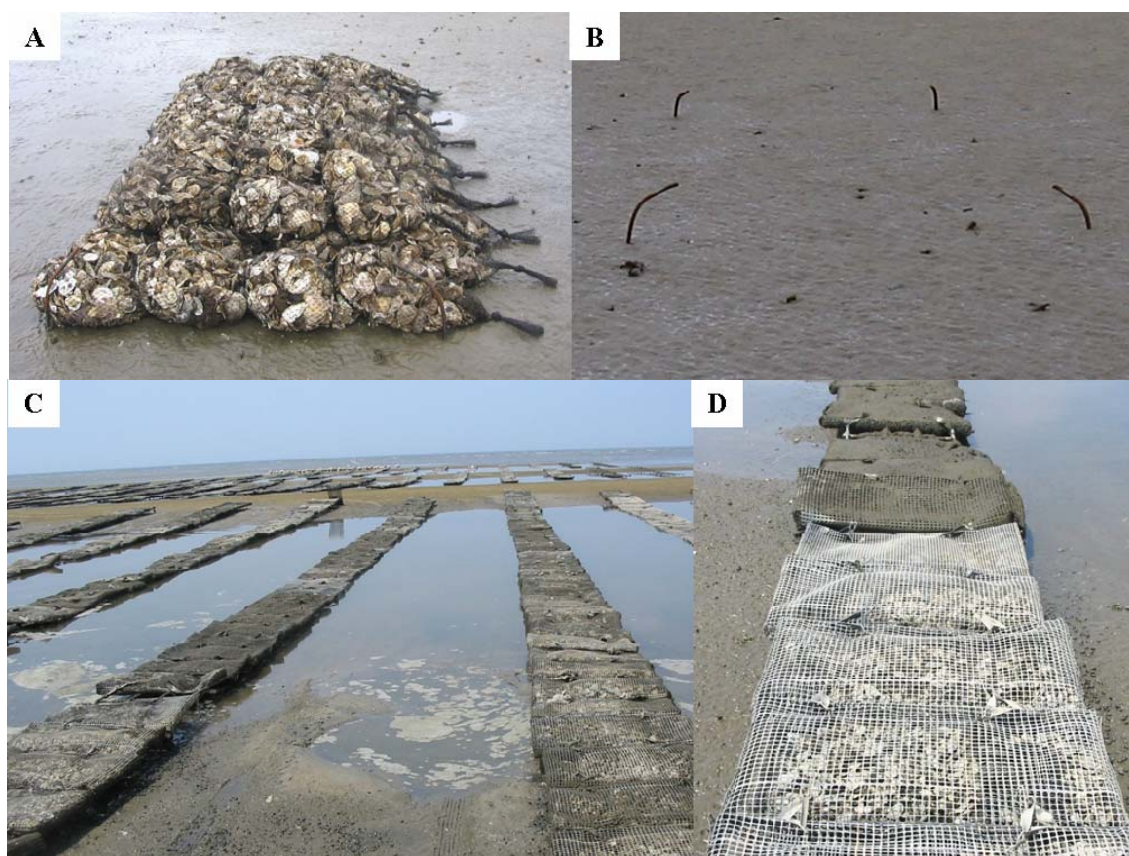


Fig. 4.

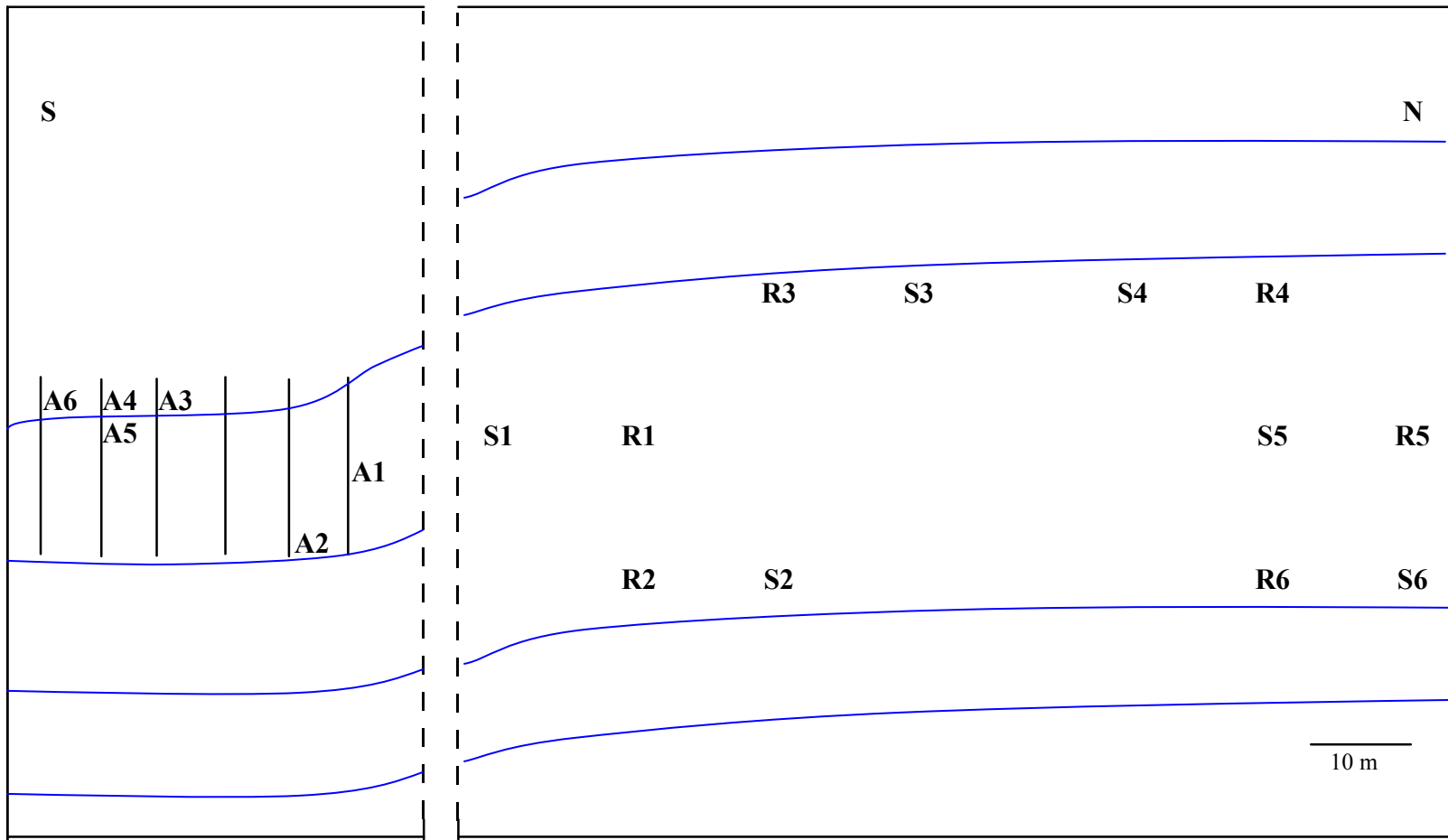


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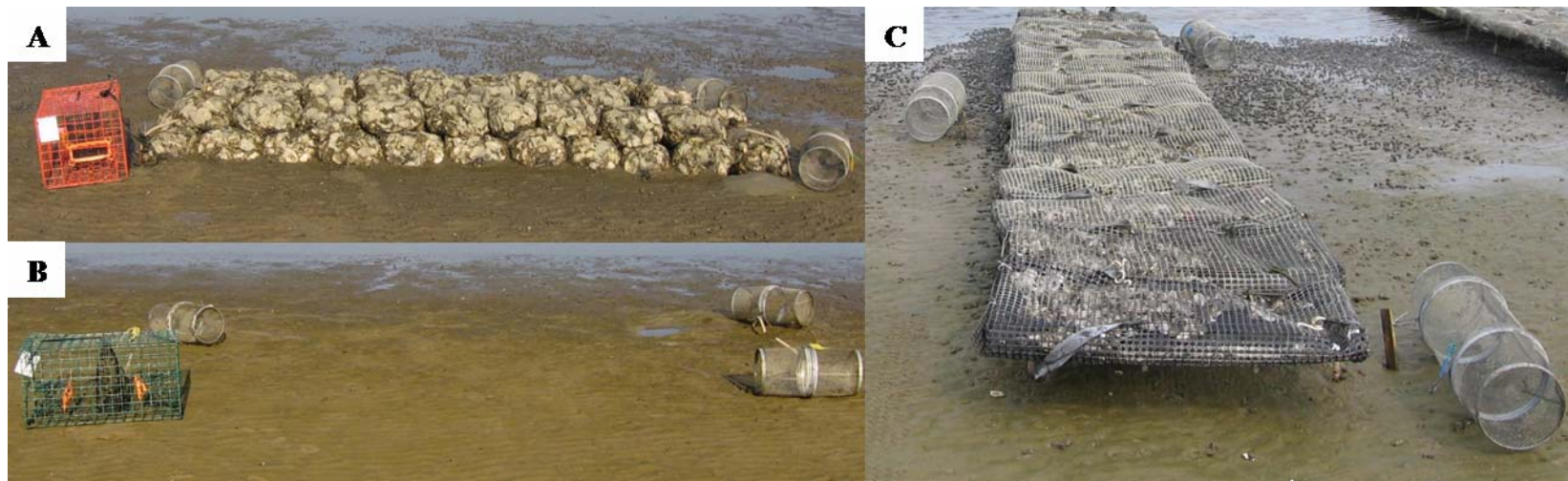


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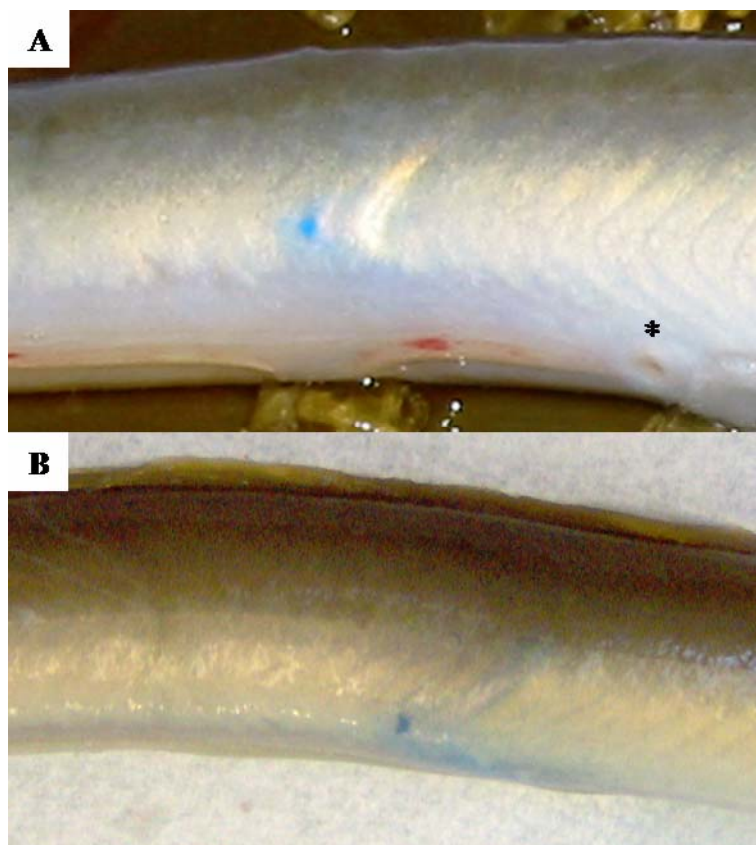


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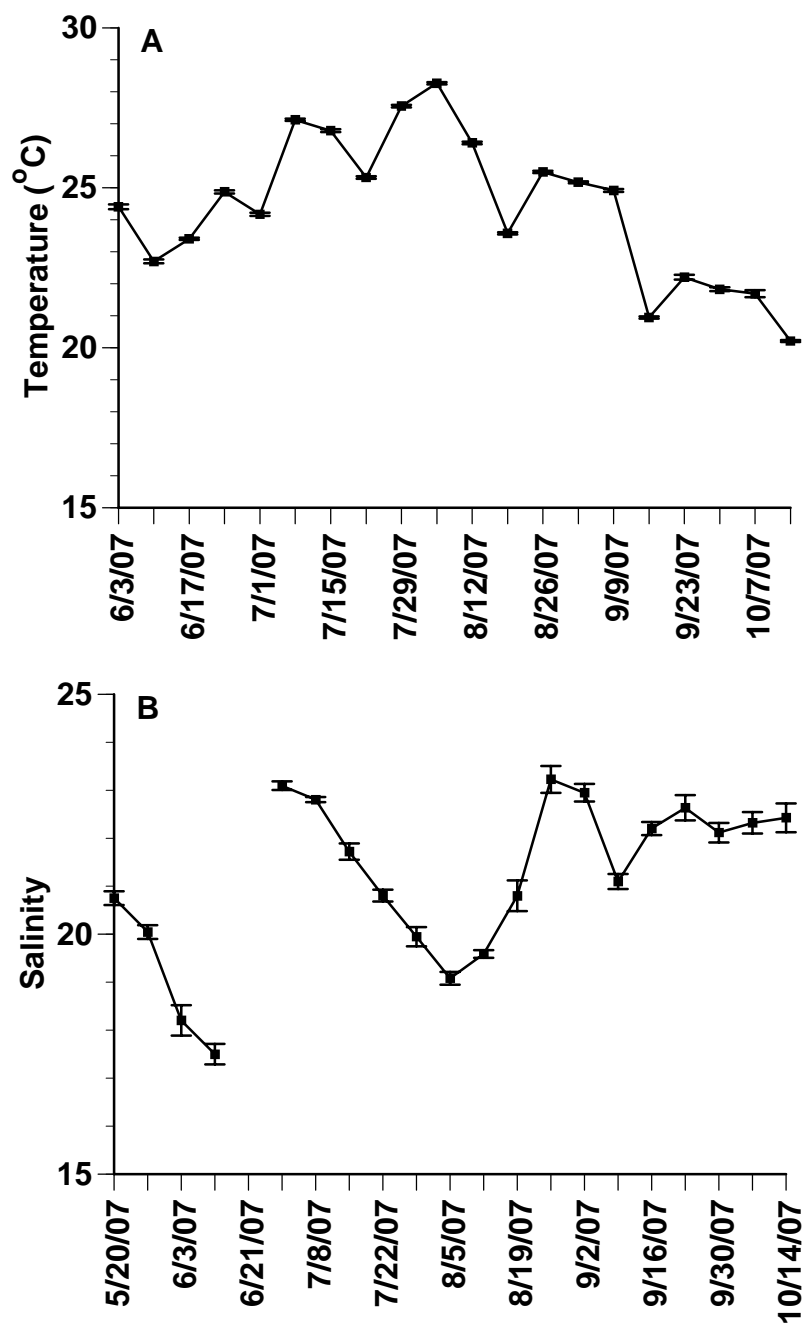


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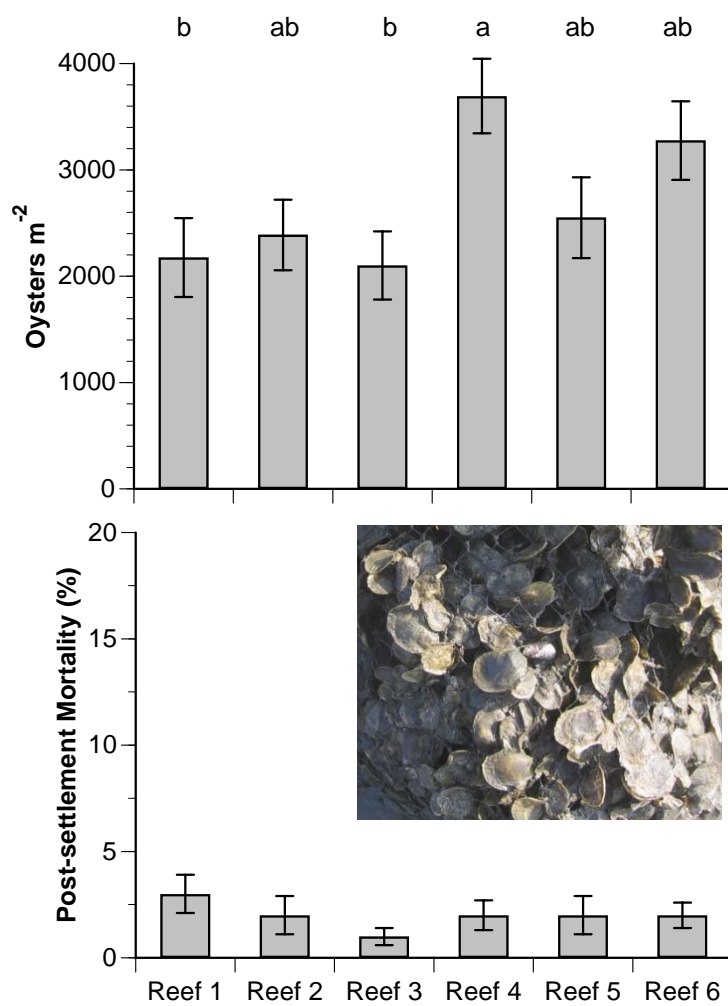


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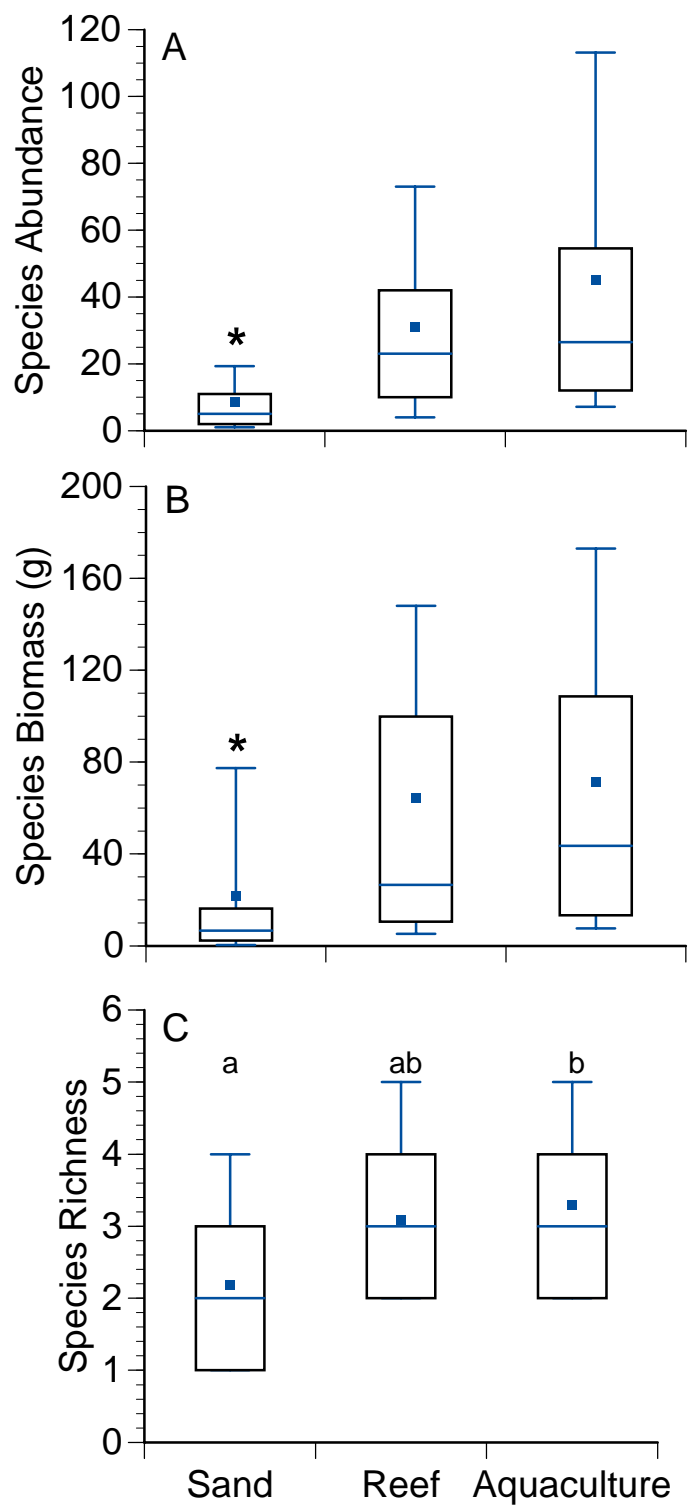


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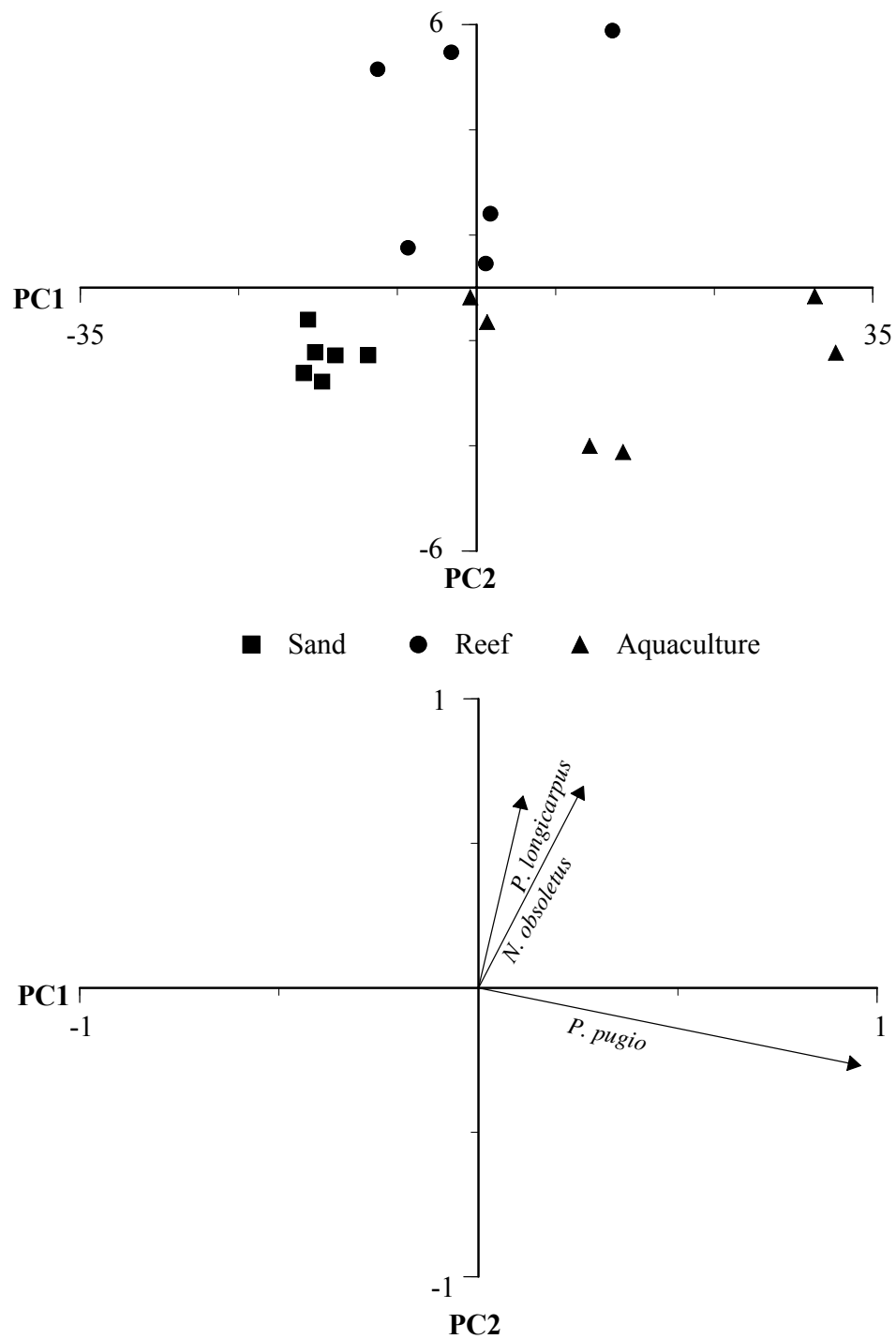


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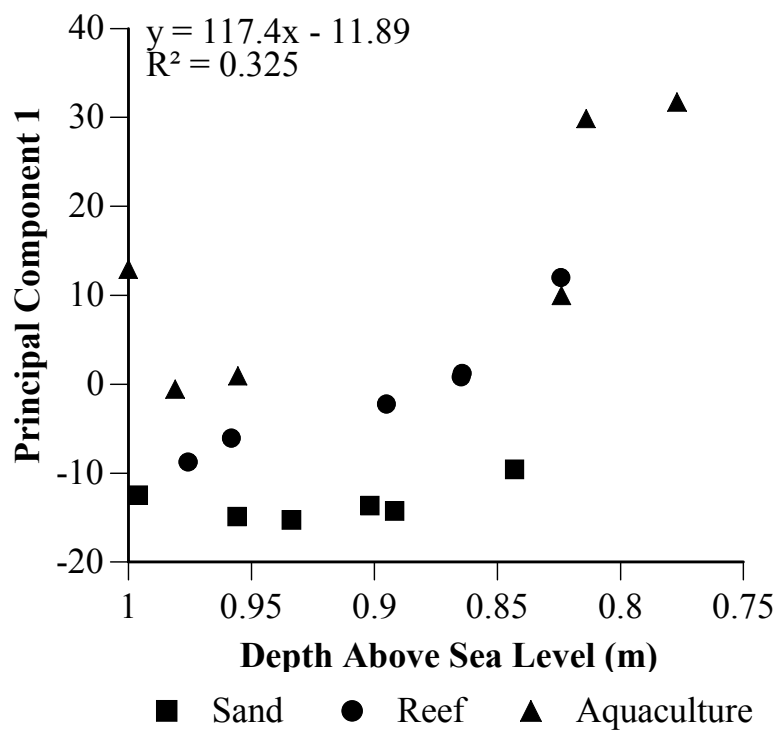


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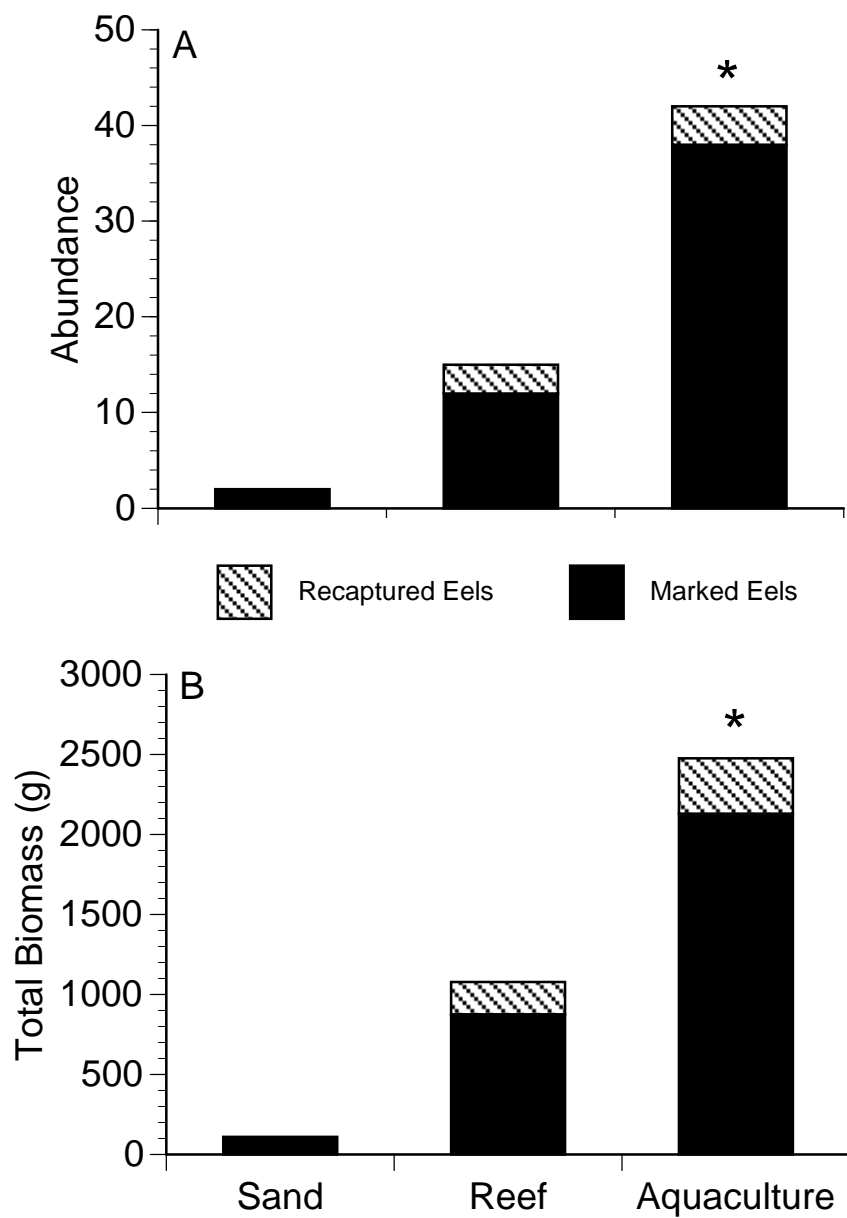


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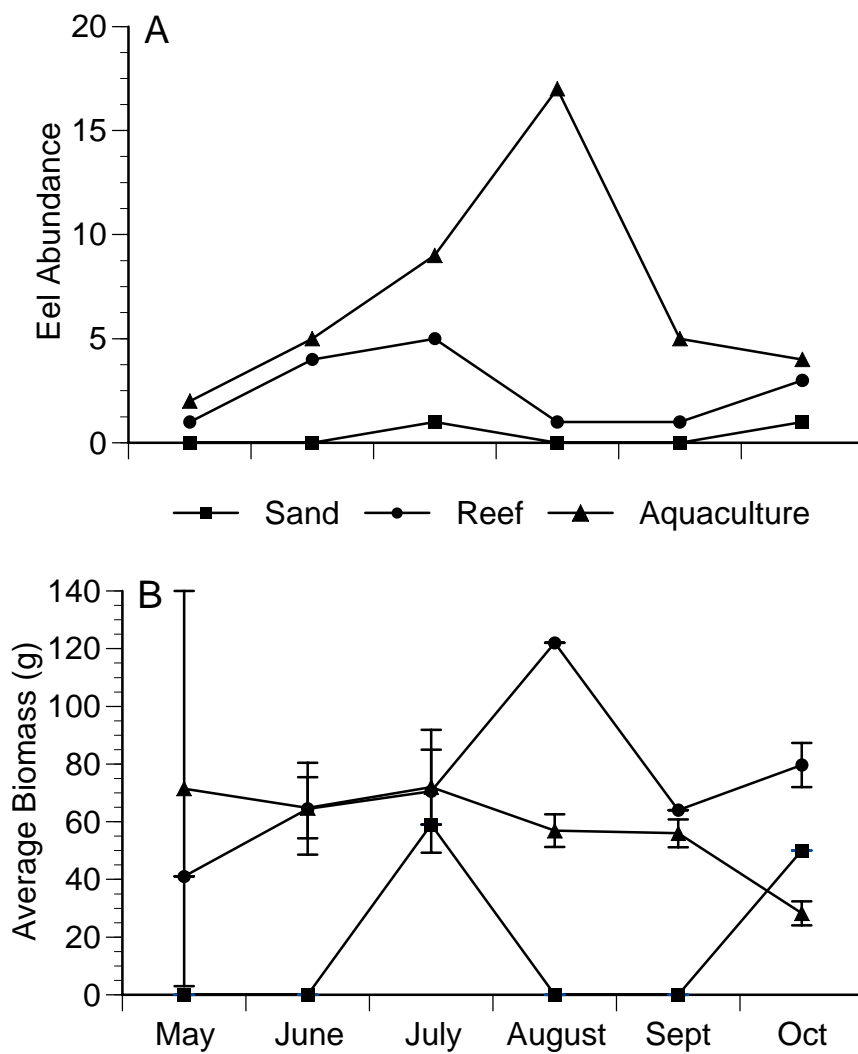


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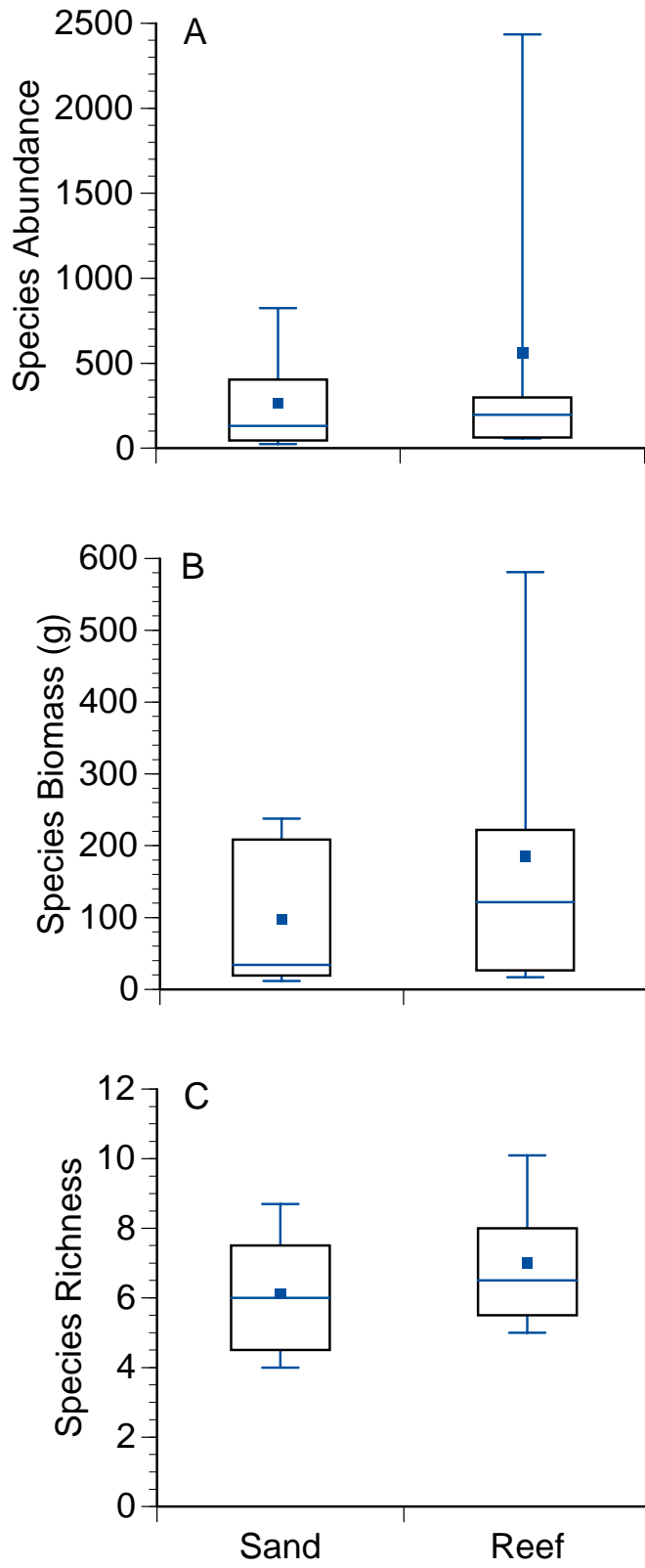


Fig. 15.

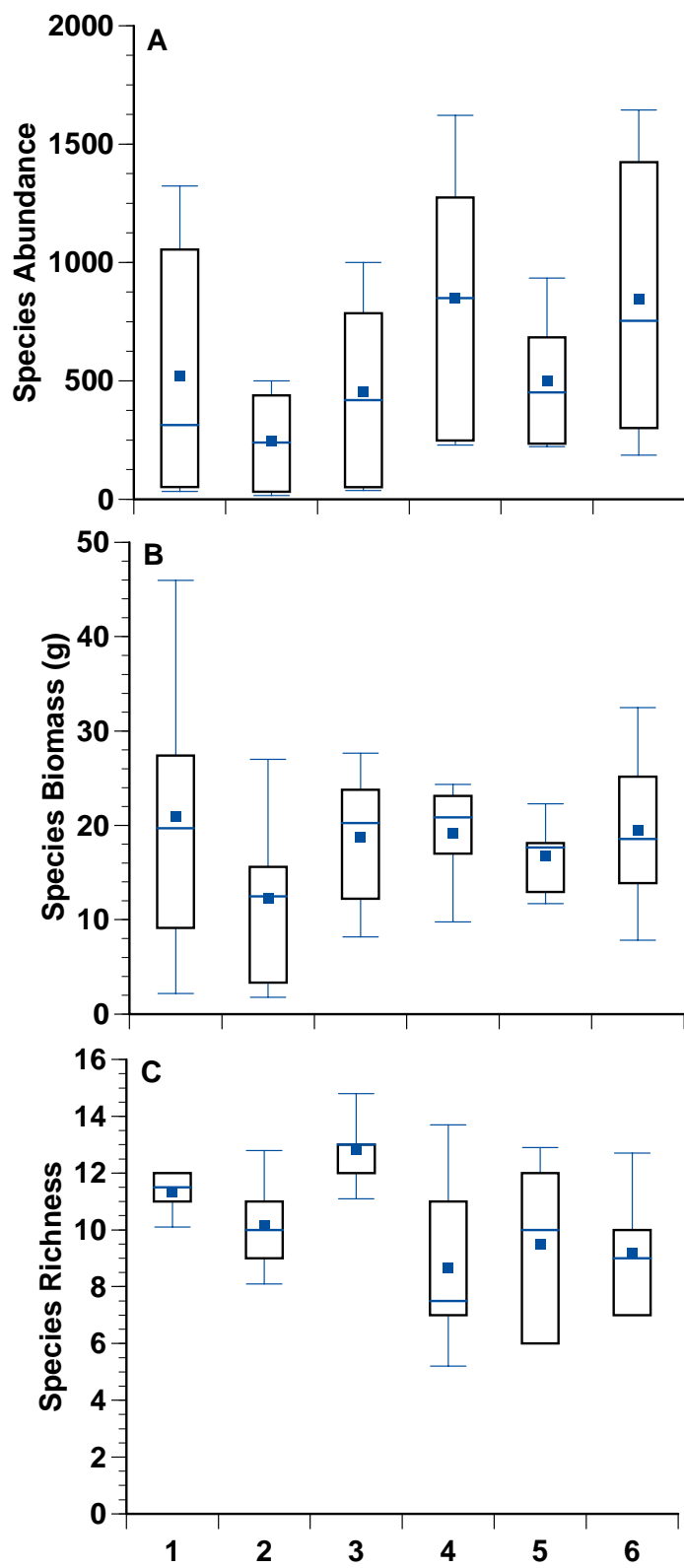


Fig. 16.

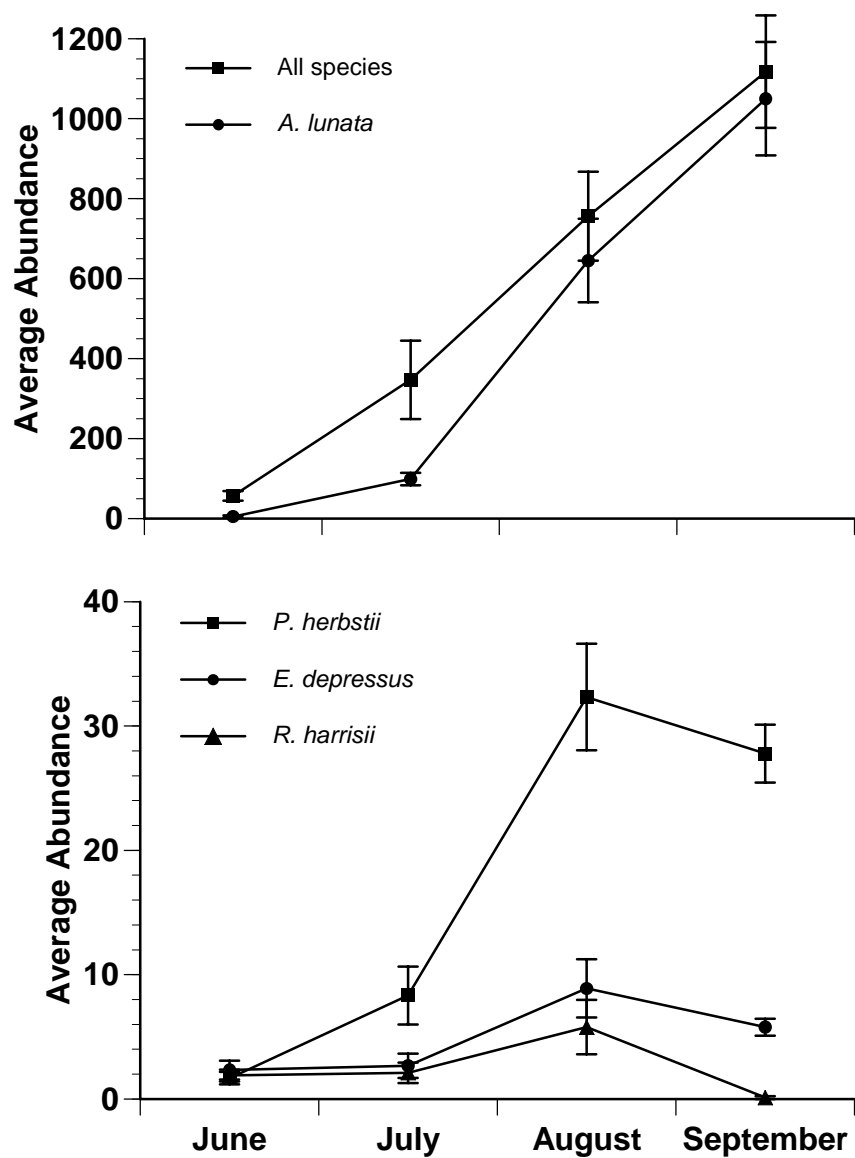


Fig. 17.

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