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Natural cultch type influences habitat preference and predation, but not survival, in reef-associated species

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Cultch type influences species and interactions

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Abstract

A shared origin with fresh and dredged cultch and availability via mining have made fossil cultch a commonly used reef restoration substrate. However, important differences in shape and size between whole-shell cultch and fossil cultch may impact the complexity of reefs constructed from these materials. To determine if these differences may impact the development of restored reefs, we quantified the interstitial space each cultch type provides and constructed reef mesocosms to measure (1) the immediate effects of exposure to each cultch type on mortality of blue crab (*Callinectes sapidus*) and pink shrimp (*Farfantepenaeus duorarum*); (2) the tendency of crab, shrimp, and Florida crown conch (*Melongena corona*) to be found on habitats composed of each substrate type and their position within each in split-substrate mesocosms; and (3) the influence of cultch type on predation of Eastern oysters (*Crassostrea virginica*) by crabs and conch. Aggregation of fossil cultch contain more shells and provide less interstitial space than an equivalent volume of whole-shell cultch. Although immediate mortality following deployment was low and did not differ among cultch types, we found that all species were more likely to be found on fresh cultch over fossil cultch in choice experiments and used each habitat type differently. Cultch type also impacted the size of oysters consumed by crabs in short-term feeding trials. The structure and traits of habitats created by various materials should be added to the growing list of issues considered when natural communities are to be restored in oyster reefs and other environments.

Key words: *Callinectes sapidus*; *Crassostrea virginica; Farfantepenaeus duorarum;* habitat complexity; interstitial space; *Melongena corona;* oyster reef; restoration

Implications:

- Differences in the number of shells and total interstitial space per unit volume exist between whole-shell fresh cultch and broken-shell fossil cultch, with fossil cultch aggregations packing more tightly and providing less interstitial space.
- Reef-associated organisms are more likely to be found on fresh cultch in mesocosm habitats containing both fresh and fossil cultch and use these two cultch habitats differently. Some predator-prey interactions also differ based on cultch type.
- In addition to chemical composition, the size and shape of materials used for oyster reef restoration may affect reef restoration progress.
- Restoration efforts, and selection and design of materials, should aim to replicate natural shell shape and variation.
- Considering reef-associated organisms when designing and monitoring restoration projects may be crucial to gauging progress and success.

Introduction

The materials present in an area and the way those materials are arranged determine the structure and complexity (e.g., variation in surface area and relief) of a site and can influence organisms, interactions, and communities (Byrne 2007). For example, organisms may require specific conditions to persist in the environment, and habitat structure may contribute to meeting those needs (e.g. refuge space) or to modifying other factors (e.g., hydrodynamic pressure) to be within a tolerable range. Habitat complexity can also influence species interaction; for example, predation rates may be altered due to the ability of predators to perceive and pursue prey (Crowder & Cooper 1982; Penczykowski et al. 2014; Boeye et al. 2014). Because of this, habitat considerations are critical in both ecological research design and restoration projects. This may be especially important when focal organisms are the ones that actually define communities via their contribution to structure, such as in mussel beds and kelp forests (Gosnell et al. 2012).

The eastern oyster (*Crassostrea virginica,* Gmelin 1791) forms important habitat and has received a great deal of attention in both research and restoration activities. By forming complex structures that provide spaces for feeding, nesting, and refuge from predators and other environmental stressors (Bartol et al. 1999 p. 199; Bartholomew et al. 2000; Gutiérrez et al. 2003; Coen et al. 2007), oysters play a crucial role as ecosystem engineers. The complex habitat created by oyster reefs supports a suite of species not found in nearby habitats (Coen & Luckenbach 2000; Tolley & Volety 2005; Scyphers et al. 2011; Brown et al. 2014) and variation in complexity impacts survival and interactions among species (Lenihan 1999; Grabowski 2004; Grabowski et al. 2008). Reefs also serve important roles in the protection of shorelines (Coen et al. 2007; Furlong 2012) and the stabilization of marsh areas (Meyer et al. 1997; Piazza et al. 2005; Scyphers et al. 2011). With the loss of up to 85% of worldwide oyster reefs in the last century through overharvesting, disease, and dredging (Cohen & Zabin 2009; Beck et al. 2011), major conservation and economic concerns have emerged. In response to these concerns, restoration efforts have increased in an attempt to preserve and enhance associated fisheries.

Adding fresh cultch (whole oyster shell obtained from shucking houses, restaurants, or other sources) to degraded reefs was once the primary method of reef restoration. However, the concurrent decline in oyster populations and increased demand for substrate for use in reef restoration, along with the loss of oyster shells to landfills, road construction, and the historic use of tabby concrete, has led to an increase in the use of alternative materials for reef restoration. Reefs have been constructed from alternative abiotic materials, such as rock, limestone, and concrete (Furlong 2012; La Peyre et al. 2014), and alternative biotic substrate options, including shells from the surf clam *Spisula solidissima* (Schulte et al. 2009b). A lack of fresh cultch has also led to extensive efforts focused on procuring additional oyster shell by dredging degraded reefs (hereafter, *dredged cultch*) or mining ancient oyster beds (hereafter, *fossil cultch*).

Although dredging and mining cultch can often have unwanted side effects (e.g., disturbance of substrate and organisms) and older cultch may not persist for as long as rock and concrete, the use of cultch in any form has been encouraged as it may promote reef development and is considered a more natural source that will not need to be eventually removed from sites (Schulte et al. 2009b). For example, oyster shell may attract more larvae than artificial substrates and support different community assemblages, though results from various experiments differ and outcomes may be location specific (Gibbons et al. 1989; Schulte et al. 2009b; La Peyre et al. 2014; George et al. 2015). Dredged cultch may also last longer than fresh cultch in low-pH environments (Waldbusser et al. 2011) and potentially prolong reef persistence (Luckenbach et al. 2005; Waldbusser et al. 2011), allowing more time for the development of a reef community. The higher availability of dredged relative to fresh cultch has led to its growing use and study over the past twenty years (Schulte et al. 2009a).

Although fossil cultch may share some traits with dredged cultch, it differs markedly in shape from whole-shell fresh and dredged cultch. Whereas dredged material often still resembles oyster shells with eroded or rounded edges (for images, see Waldbusser et al. 2011), fossil cultch more closely resembles gravel, likely due to prolonged time spent weathering over geologic time scales and the mining process itself (Appendix 1). Reefs are constructed by depositing several inches of oyster shell or alternative substrate on sites identified as suitable for reef development or restoration (Schulte et al. 2009b). The smaller, more uniform pieces of fossil cultch visually appear to form reefs that are more compact and dense compared to reefs constructed of whole-shell cultch due to the wide range in size and shape of fresh cultch pieces. For these reasons, reefs formed of fossil cultch may differ from reefs formed of whole-shell natural or dredged cultch in habitat structure, including interstitial space, which is an important habitat characteristic in determining reef outcomes (Schulte et al. 2009b; Brown et al. 2014). Interstitial spaces and the habitat heterogeneity they create across tidal gradients are crucial for recruitment and survival of oysters and benthic macroinvertebrates (Bartol et al. 1999; Soniat et al. 2004; Tolley & Volety 2005; Gregalis et al. 2009; Brown et al. 2014) and influence local sedimentation and hydrodynamics (Soniat et al. 2004; Schulte et al. 2009a). The size and arrangement of cultch pieces might also influence reef stability, movement potential, or vertical relief. These differences may be crucial to reef health and stability and important to consider in choosing cultch type for restoration projects. Yet, to our knowledge, such factors have not been considered, even as fossil cultch has become the primary source of shell material for major restoration projects (Schulte et al. 2009b; Blankenship 2013).

In addition to the noted variations in size and shape, there are additional perceived but untested differences of impacts to the reef environment between these cultch types. In fact, we were initially contacted about impacts of fossil cultch by management agencies who had been contacted with concerns from local fishermen (Apalachicola, FL, USA) about the sediment released during fossil cultch deployment and its potential impacts on reef-associated species. The mining and weathering of fossil cultch produces small particles of shell dust that are then released into the water column, creating a noticeable plume of dust clouding the water until the particles settle out. This dust was proposed to have negative effects on economically important reef-associated species. Although most assessments of restorations focus on the presence and density of oysters or on the number of harvestable oysters (Schulte et al. 2009a; Beck et al. 2011;

Furlong 2012; La Peyre et al. 2014), the biological diversity of restored reefs may also be an important factor in the selection of materials and techniques when restoring reefs and in engendering the support of local stakeholders.

The use of fossil cultch presents an opportunity to investigate how habitat complexity influences organisms, interactions, and communities. Understanding these differences would also inform our understanding of trade-offs associated with different substrate use in oyster reef restoration projects. To assess these concerns, we quantified differences in traits (available interstitial space per unit volume, number of shell components, average shell volume and weight) between fresh and fossil cultch and designed a suite of mesocosm experiments to determine differences between fresh and fossil cultch. These experiments addressed the following questions: (1) Do the particles released during deployment of each cultch type lead to a difference in immediate mortality in economically important species located on the site? (2) Are macroinvertebrates (e.g., blue crab (*Callinectes sapidus*, Rathbun 1896*)*, pink shrimp (*Farfantepenaeus duorarum*, Burkenroad 1939*)*, and Florida crown conch (*Melongena corona*, Gmelin 1791)) more likely to be found on reefs composed of either cultch type in mixed reef mesocosms, and does their habitat usage differ among reef habitats? and (3) Does cultch type affect predation rates of blue crab and crown conch on Eastern oysters (*C. virginica)*? If cultch type affects community membership or predation rates, it could influence the speed with which communities develop and the trajectory they follow, ultimately influencing the ecological and economic outcomes of restoration projects. If mortality, preference for certain habitats, or restriction to certain spaces results in species being absent from cultch deposit sites or present at lower densities, species diversity would be lower at sites using less suitable materials for restoration than at other restoration sites. The absence of reef-associated species from restoration sites could also reduce support from local stakeholders, including fishermen, for restoration projects. Together, this set of experiments aimed to better our understanding of how the use of fresh and fossil cultch might impact the life cycle of reef restoration efforts.

Methods

Overview

All mescosm experiments were conducted between June and November 2014 in outdoor mesocosms at the Florida State University Coastal and Marine Laboratory (St. Teresa, FL, USA). Sixteen tanks (1.2 m diameter, 0.3 m height) were set up on an outdoor platform. Seawater was pumped from the bay adjacent to the lab into the tanks to a depth of ~0.20 m, similar to the depth of nearby oyster reefs at low tide, at an average rate of 0.18 L/s. Sixteen 0.012 m (diameter) holes were drilled along the top edge of the pools to allow for drainage while preventing organisms from escaping. Aluminum flashing wrapped around each of the tanks prevented organisms from escaping or entering the tanks. Water temperature was recorded using Thermochron ibuttons (Maxim Integrated, model DS1921G-F5#) and compared against local conditions recorded daily by staff at the FSU Coastal and Marine Laboratory. Over the course of our study, water temperature ranged from 15.0 to 30.6°C, and salinity ranged from 27.7 to 34.6 ppt.

Fresh and fossil cultch from the Eastern oyster, *Crassostrea virginica*, was provided by the Florida Fish and Wildlife Conservation Commission. Fossil cultch was mined north of Carrabelle, FL (Gulf Coast Aggregates), where it had been rinsed, stored outside, and exposed to sun and rain before use, a common practice when using cultch (Cohen & Zabin 2009). Cultch was reused in multiple experiments. Between each set of experiments and trials, cultch was removed from the tanks, rinsed, and exposed to sun for at least 4 days. Tanks were rinsed, scrubbed, and dried fully to avoid confounding factors of prior cultch type and organism presence. Organisms used in experiments were either collected from waters directly adjacent to the lab or purchased from local suppliers. Analyses were carried out using R (R Core Team 2014) and installed packages including ggplot2 (for graphical analysis and figures, Wickham 2009), lme4 (for generalized linear mixed effect models, Bates 2010), boot (for calculating inverse logit of model coefficients, Canty & Ripley 2014), car (for testing nested models, 2011), multcomp (for post-hoc tests, Hothorn et al. 2008), plyr (for database manipulation, Wickham 2011), and optimx (to aid in fitting generalized linear mixed effect models, Nash & Varadhan 2014).

Quantifying differences between fresh and fossil cultch

To estimate differences in habitat traits between fresh and fossil cultch reefs, we measured cultch piece density, mass, and available interstitial space per unit volume. We measured these traits by filling a 3-liter beaker of known weight with fresh or fossil cultch and weighing it, then adding in water to fill the interstitial space among the cultch pieces and weighing the beaker a final time. We repeated these measures five times for each cultch type. To estimate how much settling and configuration may differ among trials using the same shells, we filled the beaker 4 more times with a single set of cultch we had previously used and measured variation among the fills. Knowing the volume of the container and weight of water the beaker held when empty and when filled with cultch allowed us to determine the available interstitial space per liter. Counting the number of shells added each time allowed us to determine average mass, volume, and density for each cultch type. Differences among cultch types were tested using linear models with cultch type as a fixed factor (for the shells that we measured five times, their average value was used in the final analysis).

Impacts of immediate exposure

The effect of immediate exposure to the particles released into the water by fresh and fossil shell during deposition and of direct contact for crabs was examined by measuring mortality of blue crabs and pink shrimp after cultch addition. Cultch treatments (fresh cultch, fossil cultch, and a no cultch control) were randomly assigned to 12 tanks, with 4 replicates per treatment. After the tanks were filled and water was continuously flowing, each treatment tank received 16.3 kg of cultch to create a layer several centimeters thick covering the bottom of the tank (Schulte et al. 2009b). Since fossil and fresh cultch are both composed of oyster shell, we used a common mass between experiments to standardize the amount of shell used for each cultch type. Within 60 s of cultch addition, each tank received 4 crabs with a mean carapace width of 115 mm and 10 shrimp. Crabs were placed directly in the tanks, and shrimp were placed together in a single 18.9 L container with 4 mesh-covered holes allowing for water flow and protection from predators. The separate container for shrimp in the tanks provided protection from the crabs while allowing both species to be exposed to any shell particles released. Crabs and shrimp were collected the previous day through typical commercial harvesting methods: crab traps in Apalachicola Bay, FL, and shrimp nets in Horseshoe Beach, FL. Crabs and shrimp were checked and dead individuals removed at 16:30 on the first day (4 hours after experimental setup), at 09:00 and 16:30 for the next 4 days, and at 09:00 on the last day.

The effect of cultch treatment on mortality of shrimp and crabs was analyzed using a generalized linear mixed-effects model since mortality, the number of organisms remaining alive (successes) contrasted with the number found dead (failures) during each sampling period, was a binomial variable, and tank was added as an observation-level random effect to account for potential similarities in mortality within experimental units (Harrison 2014). Correlated random effects were also fit for each tank to account for the repeated measures aspect of the experiment (Bates 2010). P-values were obtained by comparing nested models using chi-square tests (Zuur 2009).

Location among and within reef types

We used simple location survey experiments to determine whether organisms exhibited differential patterns of habitat use or access for the two cultch types. Differences may be due to preferences for one cultch type over another or to limiting factors (if one cultch type did not provide easily accessible interstitial space or was unable to be burrowed into); preferences or limitations would suggest that cultch type could affect an organism's presence on oyster reefs. Since differences in the size and shape of individual cultch pieces would lead to equal weights of fossil and fresh cultch resulting in different volumes of shell (and thus reefs of different areas), we used a consistent volume of cultch to create artificial reef mesocosms in each tank. Two cultch formations were constructed in each tank by adding 0.019 m^3 of fresh cultch to one half of the tank and 0.019 m^3 of fossil cultch to the other half with an empty corridor (15.2 cm wide) between them. Reefs were level and equal in height to avoid any bias due to water depth above the cultch, and reef orientation differed among tanks to reduce bias due to external environmental factors. Water flow was started at least 6 hours before organisms were added to the tanks.

We ran separate trials for pink shrimp, crown conch, and blue crab. We ran 5 replicates of 15 shrimp each; 4 replicates of 10 small (<60 mm shell length) crown conch; 4 replicates of 10 large (>78 mm) crown conch; 5 replicates of 4 small (<105 mm carapace width) blue crabs; and 5 replicates of 3 large (>115 mm carapace width) blue crabs.

Each trial was started by placing individuals in the center of the tank simultaneously and allowing them to acclimate for ~15 hours before starting observations. At 09:00 and 16:30 for 4 days, the number of individuals on the fresh, fossil, or center portions of the tank was counted during observations that lasted up to 60 seconds. Since the location of organisms on top of or within the interstitial space of a reef determines predation risk and resource access, we also recorded the number of individuals found on top of or within reef habitats of each cultch type. Individuals on top of cultch included those on the sides of the tank above or near the cultch and those on shell pieces with less than half of their bodies covered by shell. Individuals were considered to be within the interstitial space of reef habitats if they were tucked under or into oyster shells and if half or more of their bodies were covered by shell or below the exterior surface of the reef (i.e., in a depression within the reef surface). Since shrimp moved around more than crabs and conchs and could more easily get between individual pieces of shell, observations for shrimp followed additional guidelines. Tanks with shrimp were observed for 180 seconds at a time, and shrimp had to be located within depressions or tucked into shells for at least 5 seconds before being considered to be within interstitial reef space. Any shrimp not found within 180 seconds of observation were counted as within fresh cultch since any shrimp on top of either reef or within the fossil reef habitat could be found more easily. After the initial acclimation period, organism location was measured by determining the number of individuals on top of and within each reef during observation periods.

Location data were analyzed using a generalized linear mixed-effects model. Because both types of cultch occupied the same volume, a null hypothesis was developed that organisms would be found equally on both substrates. If this was true, the intercept for the model would not deviate significantly from zero. In addition to considering the intercept, we again included a random effect of sampling time within each tank to account for repeated measures and potential similarities within each tank. P-values were determined based on models fit by maximum likelihood. For crabs and conchs, we also considered the impact of organism size on location and retained this variable in the final model only if it was significant; however, including or excluding size did not change the overall results regarding relative number found on each cultch type. For the subset of individuals found on each type of cultch, we used a similar model structure to consider differences in habitat usage, focusing on the numbers of organisms found on top of reefs as compared to those within interstitial space. Finally, we used a similar model structure including cultch type as a factor to compare differences in habitat usage between the substrates for each organism.

Predation

Effects of cultch type on predation rate by crab and conch on oysters were also examined. Four cultch treatments (fresh, fossil, mixture of fresh and fossil, and no cultch as a control) were randomly applied to 16 tanks. Each tank with cultch had 0.04 m^3 of cultch added: either 0.04 m^3 fresh, 0.04 m^3 fossil, or 0.02 $m^3$of fresh mixed with 0.02 m^3 of fossil. For each trial, we randomly distributed six ceramic tiles (10.80 cm. x 1.90 cm x 0.64 cm) per tank on top of the cultch so that oysters were accessible to predators. Two tiles had five small (~20 mm length) oysters glued to each tile, two had five large (~40 mm length) oysters, and two contained a mix of large and small oysters. We added 2 tiles of control oysters in small cages to each tank (10 oysters: 5 large and 5 small) to ensure oyster death was due to predation rather than tile preparation or experiment effects. All were single oysters, set on microcultch by hatchery and raised in waters adjacent to the bay. For the initial conch predation trials lasting 4 days, we added 4 conchs with a mean length of 70 mm and mean weight of 49 g to each tank. For crab predation trials lasting 2 days, we added 3 crabs with a mean carapace width of 132 mm to each tank. After the trial period ended, tiles were removed and oyster consumption was noted by the presence of empty shells or absence of oysters altogether, indicating that the oyster shell was removed by a predator. In subsequent trials, we reduced the experimental period by half, with 2 day trials for conchs and 1 day trials for crabs, to consider potential short-term differences among cultch types. New conchs were collected for the 2 day trials (mean length of 69 mm and mean weight of 49 g). After a holding period during which crabs were not fed, the crabs from the 2 day trials were used for the 1 day trials. The crabs were mixed and haphazardly assigned to pools for the 1 day trials.

We analyzed oyster consumption using a generalized linear mixed-effects model. Oyster consumption was modeled as a binomial variable, and the effect of treatment and oyster size was considered a fixed effect; interactions among oyster size and cultch type were also considered but were removed from the model if not significant. Tank effects were again included as an observation-level random effect to account for overdispersion in the data. When significant differences existed based on treatment, multiple comparison tests were carried out using the multcomp package in R (Hothorn et al. 2008) to compare differences among fresh, fossil, and mixed cultch; family-wise error rates were corrected for using the Holm method (Holm 1979). Provided p-values were computed using Type III analysis of variance tables.

Results

Quantifying differences among fresh and fossil cultch

There was a significant difference in the number of pieces of fresh (mean: 14.13, range: 11.33 - 16) and fossil (mean: 146.67, range: 138.33 - 153.33) cultch per L (t = -46.230, p < 0.001). Fossil cultch also had a significantly lower average weight per piece than fresh cultch

(8.12 g vs 45.81 g, $t = 12.270$, $p < 0.001$). There was significantly more interstitial space per L (hereafter, *proportion of interstitial space*) for fresh cultch than fossil cultch (0.59 vs 0.41, $t = 24.03$, $p < 0.001$; Fig. 1), which corresponded to a significantly lower volume per shell for fossil vs fresh cultch (3.8 mL vs 26.0 mL, $t = 14.205$, $p < 0.001$). Fossil cultch also was denser than fresh cultch (1.99 kg/L vs 1.71 kg/L, $t = -8.822$, $p < 0.001$). Graphical analysis of repeated measurements indicated that changes that occurred among trials were minimal relative to the overall difference between fresh and fossil cultch (Fig. 2); for each cultch type the proportion of interstitial space varied by less than .02 per fill.

<u>*Immediate exposure*</u>

Low mortality was noted throughout the experiment, with only 7 crabs (14.6%) and 20 shrimp (16.7%) dying. Mortality occurred in all treatments regardless of cultch type, and there was no significant difference between the treatments in crab ($X^2_2 = 0.925$, $p = 0.6297$) or shrimp ($X^2_2 = 5.110$, $p = 0.078$) mortality.

<u>*Location among and within reef types*</u>

Overall, organisms displayed a significant tendency to be located on sides of tanks containing fresh cultch as opposed to fossil cultch (all $p \leq 0.018$; Table 1, Fig. 3. Pink shrimp had the largest discrepancy in likelihood to be observed on the fresh or fossil side of mescosms. Species also demonstrated different habitat usage within cultch types (Table 1). Shrimp were more likely to be within interstitial space of reef habitats composed of fresh cultch than on top of the same habitat type (Intercept Coefficient (IC) = 2.694, $p <$ 0,001; Fig. 4), but more likely to be on top of fossil cultch than within fossil cultch interstitial space (IC = -3.277, $p < 0.001$). The difference in habitat usage among cultch types by shrimp was significant ($X^2_1 = 180.17$, $p < 0.001$). Conchs were more likely to be found on top of each type of cultch than within them (fossil cultch: IC = -4.464, $p < 0.001$; fresh cultch: IC = -1.753, $p < 0.001$; Fig. 4). For fresh cultch reefs, the difference was even stronger for larger conchs, but both sizes had similar preferences while using fossil cultch. The difference in habitat usage among cultch types by conchs was also significant ($X^2_1 = 66.848$, $p < 0.001$). Crabs were more likely to be found in than on fresh cultch (IC = 0.890, $p < 0.018$), and in fresh cultch habitats small crabs were even more likely than large to be found in cultch. Although crabs showed no differences in habitat usage in fossil cultch reef habitats, there was a significant difference in habitat usage among the two cultch types ($X^2_1 = 12.002$, $p < 0.001$).

Predation

There was a significant interaction between cultch type and the size of oysters consumed during 1 day trials of crab predation (Table 2). Post-hoc tests indicated crabs consumed significantly more small oysters than large oysters on fossil cultch and in control tanks ($X^2_1 = 4.657$, $p = 0.031$; $X^2_1 = 13.697$, $p < 0.001$), while on fresh cultch they consumed marginally more large oysters ($X^2_1 = 3.205$, $p = 0.073$; Fig. 5). No difference in size of oysters consumed was observed in mixed cultch treatments. For the 2 day trials, more small oysters than large oysters were consumed overall, but no differences were observed between cultch treatments.

While neither cultch treatment nor oyster size affected predation by conchs after 2 days, both influenced conch predation after 4 days. Overall, conchs consumed more large oysters than small oysters, and significant differences in oyster consumption existed among cultch treatments (Table 2). However, comparison between cultch treatments did not reveal any significant differences among our planned comparisons (fresh vs. fossil, fresh vs. mixed, and mixed vs. fossil), indicating the difference likely existed between cultch and control treatments.

Discussion

Overall, we found clear differences between cultch types in the average size, weight, and density per shell and in the amount of interstitial space aggregations of each cultch type. In exposure experiments, the type of cultch used had no immediate impact on the species examined as a result of cultch deployment. Although the pool mesocosms are highly simplified versions of the field environment, the exposure to shell dust in the confined pool may be more concentrated than it would be in a natural reef setting, thus compounding the potential impacts of shell dust on mortality. The low mortality observed thus suggests that, even with the more concentrated shell dust, the cultch type did not impact species mortality. The mortality we observed in blue crabs and pink shrimp likely resulted from pre-experimental exposure.

While cultch type did not affect mortality, blue crabs, pink shrimp, and crown conch were all more likely to be found on the side of tanks containing fresh cultch as opposed to fossil cultch. Habitat use on reef habitats composed of each cultch type also differed for all organisms, with organisms being less likely to be found within reef structures formed of fossil cultch. Cultch type also influenced how predators consumed oysters, with crabs in short-term trials consuming more small oysters on fossil cultch but showing no size preference on reefs of mixed or fresh cultch. Together these results suggest cultch type may impact community structure and restoration success by influencing the complexity of the habitat and predation interactions between species.

Differences we observed between reefs constructed of these two cultch types may be due to the noted differences in habitat traits. Our data suggest that reefs composed of fossil cultch have less interstitial space, and the habitat usage studies indicate this may lead or allow organisms to use interior reef space less in fossil cultch reefs. Differences in interstitial space are likely due to the fact that the smaller size of fossil cultch pieces causes them to pack together more tightly, hence the greater number of shells of fossil cultch per liter. This nestled effect limits the space between fossil cultch pieces. Conversely, the lower density of fresh cultch pieces may reflect the tendency of these larger, more structurally varied cultch pieces to settle with more space between the pieces. The complexity of fresh cultch could limit predation on small organisms by providing more refuge, making prey more difficult to locate, and by being more difficult for predators to navigate. This may be particularly important for juveniles recruiting to the reef and may lead to a preference for fresh cultch reefs. As species get larger, their reliance on available interstitial space decreases, as may their ability to fit into small spaces. This may explain why large conchs and crabs were found more often on top of fresh cultch than within the cultch. For these reasons, fresh cultch may be better for building new reefs where many small organisms arrive via recruitment while fossil cultch may be better as a supplement to degrading reefs with an already established community.

The lack of integration of habitat complexity into studies focused on predator effects and community ecology in general is a previously cited concern (Baber & Babbitt 2004; Grabowski et al. 2008; Gosnell et al. 2012) that has often been echoed for management scenarios (Lepori et al. 2005; Byrne 2007). These results demonstrate that habitat complexity may play an essential role in determining the trajectory of restored communities by affecting the major species that rely on the reef and must be considered in addition to the origin of reef materials. They also indicate that including reef-associated species in the monitoring of restored sites or evaluation of restoration techniques is essential to determining how closely restored reefs resemble natural sites. The degree of habitat complexity associated with each cultch type could determine which drivers affect species more on restored reefs. Fresh cultch, with a greater habitat complexity, could support a more diverse community by providing more refuge and altering predator-prey interactions (Grabowski 2004; Grabowski & Kimbro 2005; Grabowski et al. 2008), allowing non-consumptive effects to have more influence. Fossil cultch, which creates less complex habitat, could limit species abundances and cause communities to be driven more by consumptive effects, possibly limiting species diversity to fewer large species. Habitat that promotes consumptive and non-consumptive effects, both of which have been shown to drive community dynamics on reefs (Grabowski & Kimbro 2005; Hill & Weissburg 2012) and elsewhere (Preisser et al. 2005), may be essential to rebuilding persistent reef communities that closely resemble natural sites. Although our results suggest these differences may occur, long-term monitoring of reefs constructed of varying percentages of fossil and fresh cultch are needed to fully address these issues. For example, does the use of fossil cultch lead to early changes in community membership, larval settlement, and interaction strength, such as excluding predators or changing competitive dynamics, which have long-term repercussions for reef development? Or does settlement of oysters eventually produce a mixed cultch reef that then progresses to more closely resemble a naturally occurring reef in species diversity and interactions? On what time scale do these changes occur, and how do they relate to goals for restoration projects? Studies of how cultch type influences other interactions (predator-predator for food or space) may also be important in determining how cultch impacts reef development. Similarly, on a landscape scale, does using fossil cultch lead to an overall decline in diversity or just cause the distribution of predators to favor natural cultch sites? Such studies could also offer insight into how impacts of complexity vary across an organism's life cycle and development. Within a species, smaller organisms will be able to use interstitial space differently and may be less able to manipulate cultch, so the effect of cultch type may also differ as organisms grow and change in size. The direct effect of the sediment released by fossil cultch on oysters should also be more closely considered, as species included here may be less impacted than filter feeders.

Our results also suggest that efforts should be made to manipulate abiotic materials to increase habitat complexity. This may inform the use of current materials such as concrete as well as new suggested substrates. For example, porcelain is a commonly available material that is being considered for use as a reef substrate, primarily in crushed form (George 2013). Varying the size and shape of the crushed material could yield reefs that exhibit complexity similar to that of natural reefs. These questions should be more directly considered to offer guidance to restoration efforts.

Besides these noted differences in cultch shape, size, and resulting habitat complexity, other differences may also exist among the cultch types that were not quantified here. Although all cultch was dried for more than 6 months prior to use, as is common practice for cultch used in restoration processes, it is possible that biofilms or other biological components may still remain on fresh cultch, leading to chemical signals that differ among the cultch types. Likewise, although we have considered macroscale differences in habitat complexity here, weathering and other processes (including the actual mining) may lead to differences in the texture of cultch that may impact the ability of larvae to settle or other organisms to move throughout reefs composed of various materials. These issues warrant further study, especially as they may inform the actual development of alternative, non-biological cultch replacements. Habitat complexity itself could also be better measured, as could aspects of habitat components. For example, although we noted a visible difference in the cloudiness of water following the release of fossil cultch, sedimentation rates or initial water clarity would offer a more quantitative measure of differences between cultch type.

The relative importance of substrate and complexity may depend on restoration goals and availability of restoration materials. For example, if the primary goal is to grow oysters or promote specific ecological services provided by oyster reefs, such as water filtration, without developing a community, oysters may be housed in floating aquaculture cages. While these are not traditional reefs, they may be significantly less expensive to produce and may be employed when other factors, such as substrate availability or the ability to deploy substrate, are limited. When ecological community health and diversity are the primary goals, using cultch to provide a more natural substrate as well as greater habitat complexity may be the best choice, and our results suggest fresh cultch or similarly-shaped materials should be used when possible.

However, fresh cultch is in limited supply, and, while most coastal states have shell recycling programs, oyster shells are not always recycled. Greater incentives, such as the tax credits of $1/bushel given in North Carolina (State of North Carolina 2016) and $5/bushel in Maryland (Comptroller of Maryland 2016), may help increase shell recycling. Extending these polices to other states could help to increase the amount of oyster shell recycled, but it still may not be enough to supply all of the reefs requiring restoration. To extend the use of available oyster shell, other materials such as clam shell or sand covered by oyster shell (Nestlerode et al. 2007; Schulte et al. 2009b) could be used to supplement fresh cultch and extend its use. Since fossil cultch is in greater supply than fresh cultch and is chemically more similar to fresh cultch than are human-made materials, mixing the cultch types together before depositing on reef sites or covering a base deposit of fossil cultch with fresh cultch could be used to extend the supply of fresh cultch. Although the latter configuration was not used in these experiments, it may provide a more effective use of materials by using less of the more limited resource while maintaining the preferred characteristics of fresh cultch.

While further long-term studies comparing fresh cultch, fossil cultch, and mixed fresh and fossil cultch to human-made materials could increase our understanding of their relative impacts on oysters reefs, this study has helped to show that a material's persistence in the environment and its origin should not be the only factors considered when restoring reefs. Habitat traits and complexity may also be important in promoting reef development. All three species we observed utilized habitat differently among reefs composed of fresh and fossil cultch and were more likely to be found on the structurally complex fresh cultch reefs. Predation rates on small oysters also differed among cultch types. The higher rates of predation on small oysters observed on fossil cultch reefs may lead to fewer oysters overall and fewer large oysters over time, further reducing the potential for habitat complexity to increase at a site and for sites to contain populations capable of reproducing. Fewer oysters and less refuge space could in turn cause abundance and diversity of species on less complex reefs to initially be lower than on more complex reefs and to continue to decline over time despite restoration efforts. If fossil cultch was used for reef restoration solely because it may persist longer in the environment, the community attracted to the reef may not be as stable or as healthy as the community associated with a fresh cultch reef. In order to reach a balance between reef persistence and healthy community structure, fresh cultch should be used and supplemented with other materials to extend the number of reefs that can be successfully restored. Overall, future restoration efforts in oyster reefs and elsewhere should more fully consider how best to create habitat complexity and promote diversity in order to restore natural communities and achieve other restoration objectives.

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Tables

Table 1. Summary of results from models testing whether organisms were more likely to be located on the fresh or fossil side of a mixed substrate mesocosm and whether they were more likely to be found within the reef for each substrate type. Bolded values indicate significance at the *p* = 0.05 level. IC = Intercept Coefficient. Size only retained in models used to determine intercept coefficients for the species when significant.

	Location: Fresh cultch			**Location: Within fresh**			**Location: Within fossil**		
Species	IC	Z value	*p*	IC	Z value	*p*	IC	Z value	*p*
Shrimp	2.43	9.321	<**0.001**	2.694	10.49	<**0.001**	-3.277	-4.55	<**0.001**
Conch	0.711	3.478	<**0.001**	-1.753	-6.509	<**0.001**	-4.464	-5.05	<**0.001**
Size	0.141	0.477	0.634	1.245	4.24	<**0.001**	20.70	0.001	0.999
Crab	0.673	2.36	**0.018**	-.543	-.818	0.414	-0.159	-0.629	0.529
Size	0.757	1.481	0.138	1.553	1.985	**0.047**	-0.227	-0.476	0.634

Table 2. Summary of results from generalized linear mixed-effects models testing the effect of cultch treatment and oyster size on conch and crab predation on oysters. For post-hoc tests see text. Missing values (**--**) were not included in the final model. Bolded values indicate significance at the p = 0.05 level.

		Impact of:		
Species	**Time Period**	**Cultch Type**	**Size**	**Cultch Type & Size**
		X_3^2 statistic (p value)	X_1^2 statistic (p value)	X_3^2 statistic (p value)
Crab	1 Day	4.981 (0.173)	**4.858 (0.028)**	**20.254 (< 0.001)**
	2 Days	3.261 (0.353)	**4.254 (0.039)**	--
Conch	2 Days	2.741 (0.433)	0.866 (0.352)	--
	4 Days	**8.09 (0.044)**	**14.630 (< 0.001)**	--

Figure Legends

Figure 1. Difference in proportion of interstitial space found in fresh and fossil cultch aggregations. Points are arranged to prevent overlay.

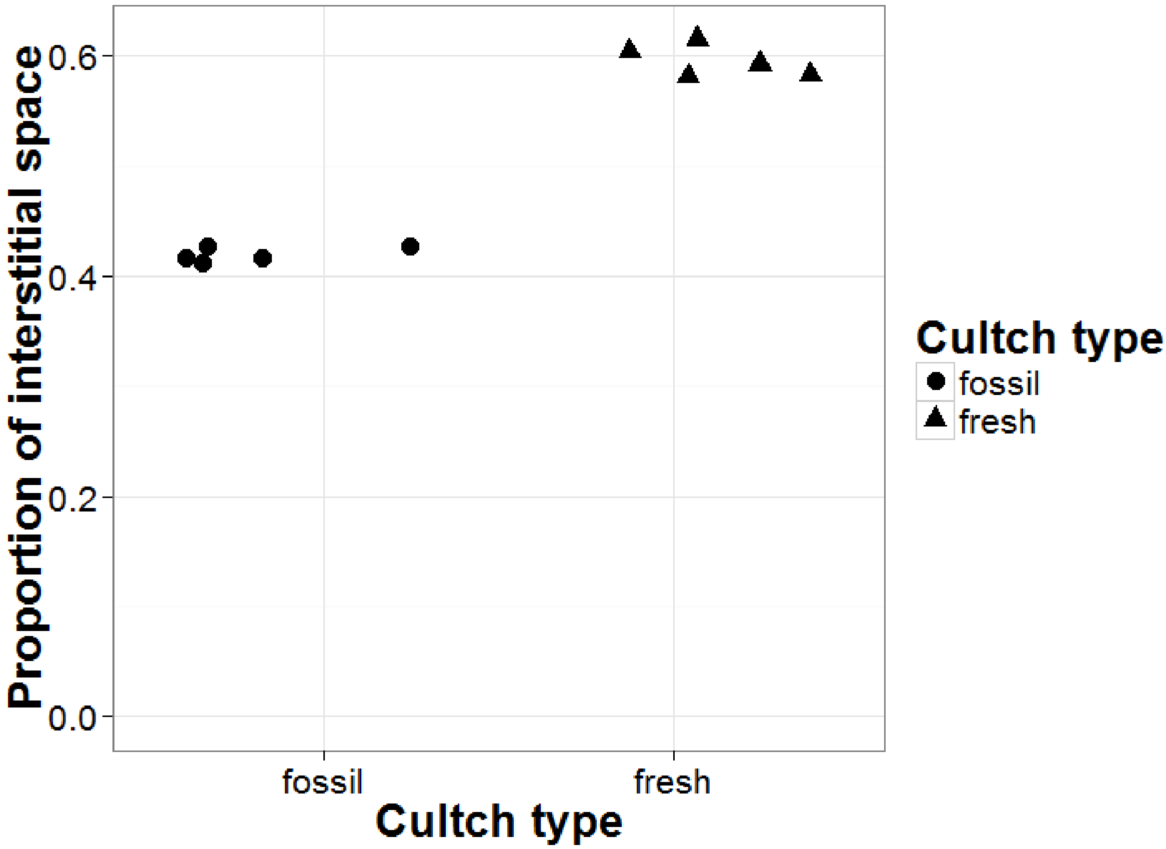
Figure 2. Variation in measurements of proportion of interstitial space found in fresh and fossil cultch aggregations when a single set of shells were placed in beaker five times for each type of cultch. Points are arranged to prevent overlay.

Figure 3. Location probability of (A) pink shrimp, (B) crown conch, and (C) blue crab determined by number of individuals observed on fossil or fresh cultch sides of a mixed substrate mesocosm. Black dashed lines at 0.50 represent the null hypothesis of no preference.

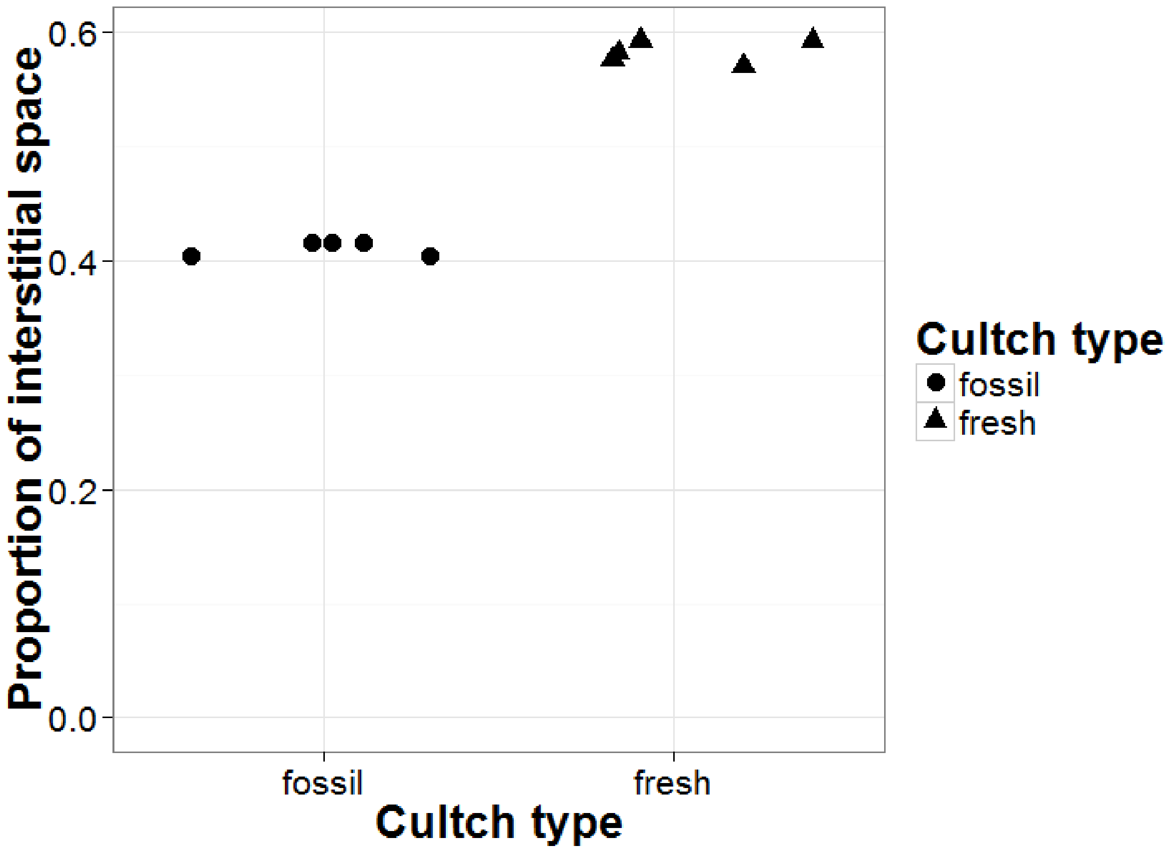
Figure 4. Location probability of pink shrimp, crown conch, and blue crab for being in or on fresh (A, C, E) and fossil (B, D, F) cultch determined by the number of individuals observed in each cultch type. Black dashed line at 0.50 represents the null hypothesis of no preference.

Figure 5. Predation of blue crab and crown conch upon small (~20mm length, light gray bars) and large (~40mm, dark gray bars) Eastern oysters on fossil, fresh, mixed, and no cultch treatments with +95% confidence intervals. A) 1-day crab trials, B) 2-day crab trials, C) 2-day conch trials, D) 4-day conch trials.

Figure 1


Proportion of interstitial space
0.6
0.4
0.2
0.0
fossil
fresh
Cultch type
Cultch type
fossil
fresh

657 **Figure 2**



0.6
0.4
0.2
0.0
Proportion of interstitial space
fossil
fresh
Cultch type
Cultch type
fossil
fresh

658 **Figure 3.**

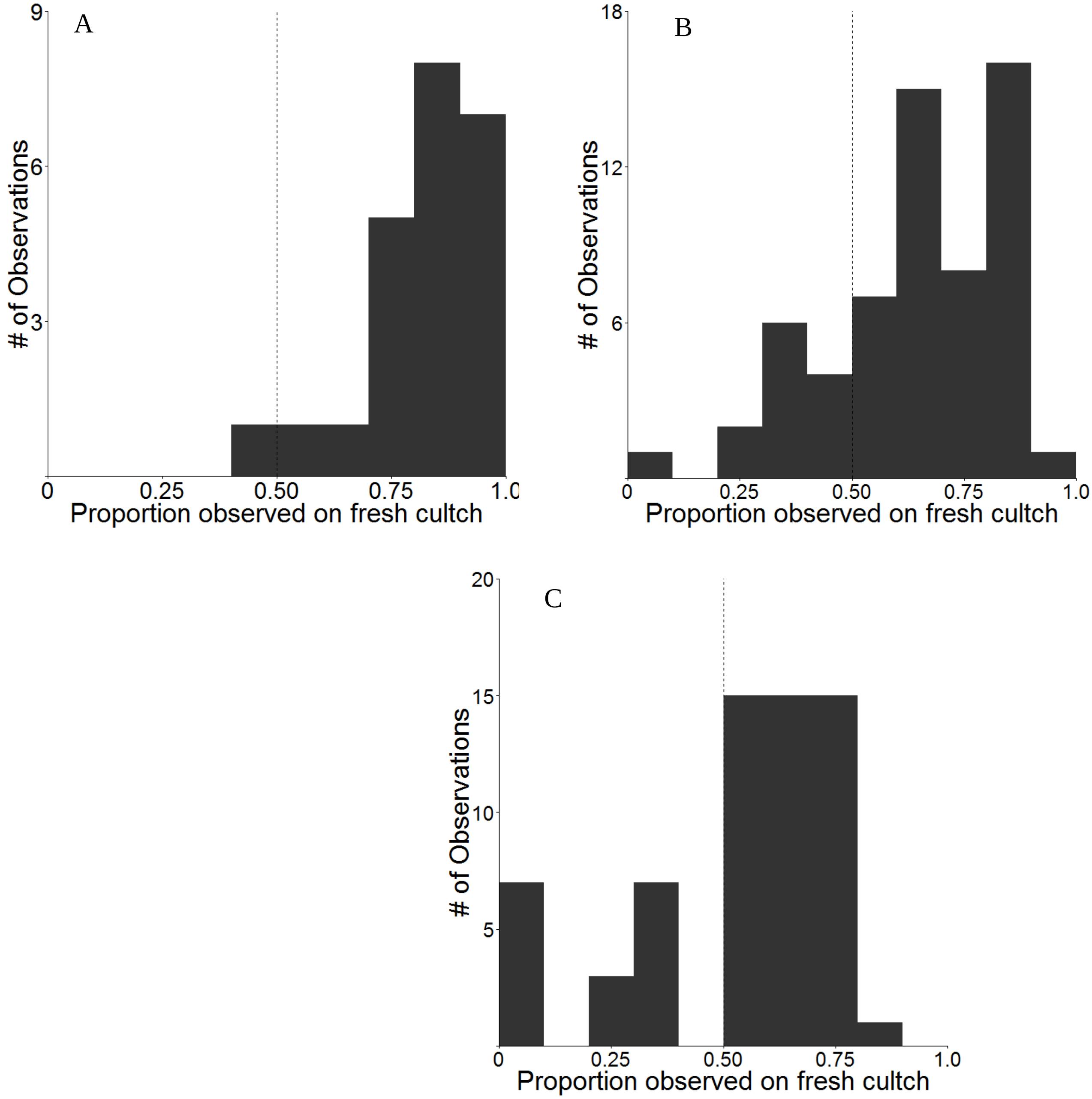

A
B
C
# of Observations
Proportion observed on fresh cultch
0
0.25
0.50
0.75
1.0
3
6
9
12
18
5
10
15
20

Figure 4.

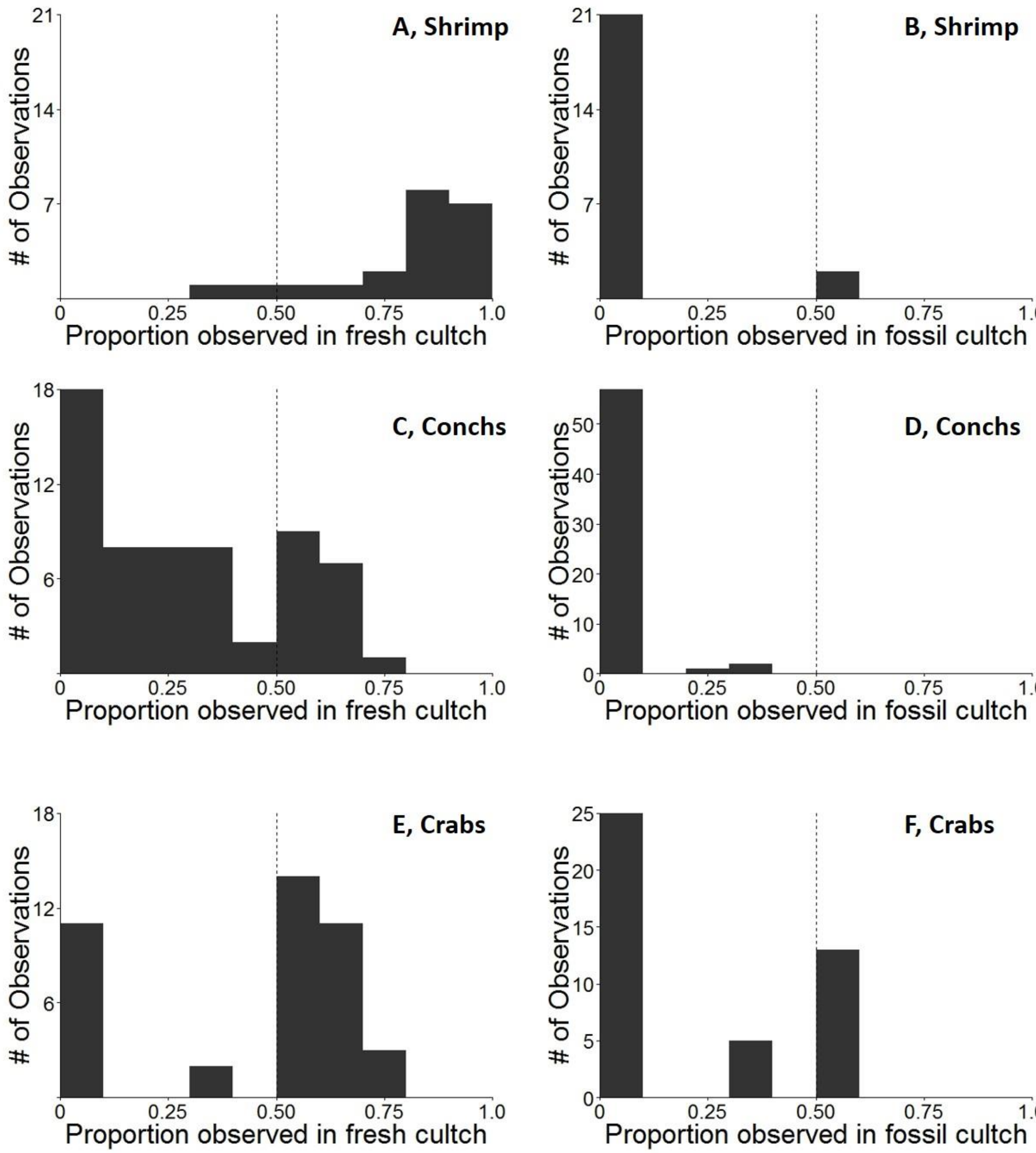
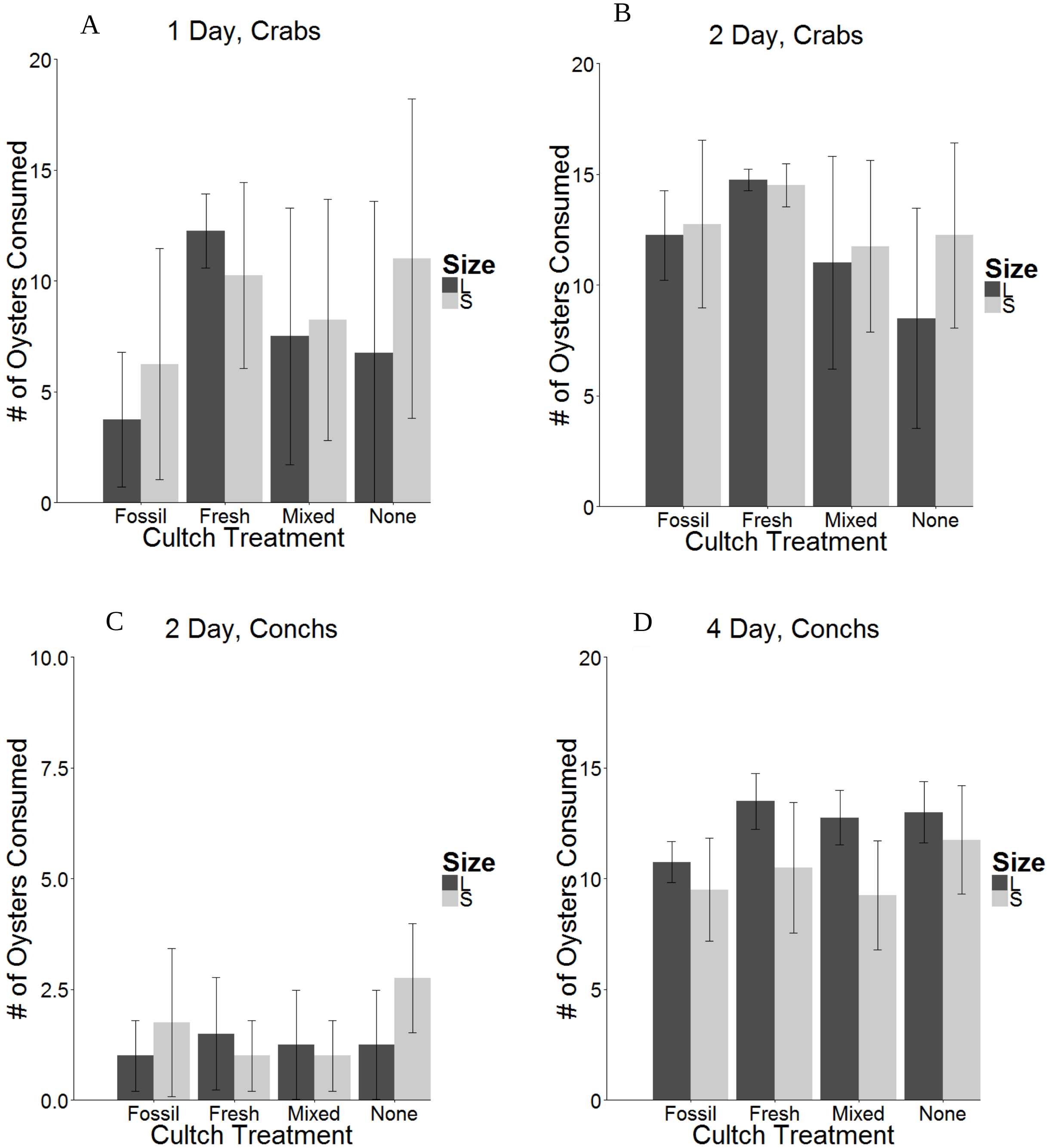

A, Shrimp
B, Shrimp
C, Conchs
D, Conchs
E, Crabs
F, Crabs
# of Observations
Proportion observed in fresh cultch
Proportion observed in fossil cultch
0
0.25
0.50
0.75
1.0
7
14
21
6
12
18
10
20
30
40
50
5
15
20
25

Figure 5.


A
1 Day, Crabs
B
2 Day, Crabs
C
2 Day, Conchs
D
4 Day, Conchs
# of Oysters Consumed
Cultch Treatment
Fossil
Fresh
Mixed
None
Size
L
S
0
5
10
15
20
0.0
2.5
5.0
7.5
10.0