BORROWING ECOLOGICAL PRINCIPLES: INFLUENCE OF REEF PLACEMENT AND HABITAT COMPLEXITY ON FREE-LIVING AND PARASITE DIVERSITY

By

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Abstract

Biodiversity is indicative of a healthy ecosystem. Fish and invertebrate species are key members of oyster reef food webs and are useful in evaluating biodiversity and restoration success. Eastern oyster populations have severely declined in estuaries along the US Atlantic Coast. They are known for their ecosystem services creating three-dimensional structures that provide habitat for organisms. Past studies explored how factors such as substrate composition, reef relief, and physical drivers influence oyster development and associated communities (both free-living and parasitic). Fewer studies explored how placement (i.e., landward vs. seaward) relative to the reef, along with a reef's relief (i.e., higher or lower), may affect reef associated species assemblages. These latter factors are imperative to evaluate as reef placement and reef relief alter hydrodynamics and sedimentation. Additionally, structural complexity in a reef may influence host-parasite interactions, with free-living and parasite diversity increasing in response to restoration. Still, little is known about the role of parasites in reef communities. These gaps in knowledge required a more in-depth study to determine the role of placement in the establishment and evolution of restored reefs. I conducted field surveys to evaluate how reef placement and reef-relief affected biodiversity.

This study took place in the RCR located in Beaufort, NC, United States. I had 12 study sites along the Taylor's Creek shoreline (4 low relief, four high-relief, and 4 control). At each site, two passive samplers were placed landward and seaward of a restored reef. Over a one-year sampling period, I collected fish and crab species, recorder their abundance, and dissected and checked them for parasites. Abiotic measurements including water temperature (°C), DO (mg/L), and salinity (ppt) were also collected. I predicted that landward sides of restored reefs would support higher species abundance and richness of mobile fauna (both free-living and parasite organisms) and that the seaward side of restored reefs would support higher sessile faunal abundance and richness. I also predicted that high-relief reefs would support the highest biodiversity for both mobile and sessile fauna. For sessile fauna, this prediction was upheld, with the exception that reef-relief didn't influence sessile species abundance. However, this prediction was not upheld for mobile free-living fauna. In fact, results from my analyses showed that mobile fauna abundance was greatest seaward of control plots. This result may suggest that structure in a habitat is a driver of species diversity, where control sites had two passive samplers placed landward and seaward, despite no physical reef being present. In addition, parasite prevalence in crab and fish host species was low, finding no differences between the placements and reef reliefs. Such low abundances may be a result of the young age of the one-year-old restored reefs. One-year post restoration may be insufficient to observe free-living and parasitic communities matching that of a natural reef. Altogether, this study provides important baseline understanding regarding how structure and time affect species assemblages in restored reefs. Future restoration projects should consider time as a predictor of restoration success.

Borrowing ecological principles: Influence of reef placement and habitat complexity on free-living and parasite diversity

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Borrowing ecological principles: Influence of reef placement and habitat complexity on free-living and parasite diversity

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Introduction

Biodiversity is an essential indicator of ecosystem health (Kremen, 2005). Biological diversity can aid in the provision of certain ecosystem services, such as enhancing water quality, resilience and resistance to invasive species, and fisheries productivity (Lamb et al., 2017; Stachowicz et al., 2007; Worm et al., 2006). Oyster reefs represent a biodiverse habitat in estuarine systems, providing numerous ecological services (Coen et al., 1999; Luckenbach et al., 2005; Coen et al., 2007). Through water filtration, 70% of the organic matter filtered by oysters assimilated, while the remainder serves as a food source for benthic organisms (Newell, 1988; Coen & Luckenbach, 2000). By creating three-dimensional reef structures, oyster reefs create complex biogenic habitat that attracts and provides substrate for invertebrate and fish species to settle on or take refuge within (Coen et al., 1999; Coen & Luckenbach, 2000; Tolley et al., 2005; Luckenbach et al., 2005; Moore et al., 2020). The structure of oyster reefs can increase biodiversity by providing interstitial space as well as shelter, as the oysters settle, recruit, and grow (Wells, 1961; Grabowski & Powers, 2004; Schulte et al., 2009). However, as oyster populations have declined, so has the biodiversity that is associated with this biogenic habitat (Rothschild et al., 1994; Costanza et al., 1997).

When restoring an oyster reef, ecologists hope to restore valuable ecosystem services and biodiversity (Coen et al., 2007). Key members of oyster reef communities, like fish and crustaceans, can be used as indicator taxa to evaluate biodiversity and restoration success. The three-dimensional structure of an oyster reef provides structural complexity that can positively affect species biodiversity (McCormick 1994; Nash et al., 2012; Darling et al., 2017, Denis et al., 2017). Both resident and transient fish species have been sampled as a measure of structural complexity (Tolley & Volety, 2005; Lenihan et al., 2001). For example, oyster shells have served as a suitable substrate for spawning benthic eggs by blenny and goby species, where the fish select for a specific length and gape of empty oyster shells (Peters, 1981; Breitburg, 1999; Crabtree & Middaugh, 1982). Additionally, the interstitial space within a reef structure provides refuge from predation for mud crabs, such as *Eurypanopeus depressus* and *Panopeus herbstii* (McDonald, 1982). Additionally, in the presence of predators, grass shrimp *Palaemonetus pugio* seek refuge in oyster shell (Posey et al., 1999). Structural complexity can also influence host-parasite interactions (Johnson et al., 2016). Recently, Moore et al. (2020) found that restoration of oyster reef habitats resulted in enhanced community biodiversity, with free-living and parasite diversity increasing in response to oyster restoration. Furthermore, several past studies have found trophically transmitted parasites can not only inform free-living diversity in a system (Huspeni and Lafferty; 2004; Bass & Weis, 2008; Anderson & Sukhedo, 2013) but they may also better forecast restoration trajectories through time compared with free-living species because parasite diversity captures multiple trophic links across a variety of invertebrate and vertebrate host species (Moore et al., *in revision*).

Past studies have found that factors such as reef relief have altered the availability of habitat. For example, Gregalis and colleagues (2014) found that high relief oyster reefs support higher abundances of crab species, where the increased height allowed them to escape hypoxic environments. In contrast, low relief oyster reefs support higher abundances of transient fish. However, determining if organismal assemblages differ as a function of both reef relief and position (landward/seaward) relative to the shoreline, a critical component of oyster reef restoration, has not been explored.

Restored oyster reefs are often designed in a manner such that they are constructed parallel to the shore to provide coastal protection services, often modifying the hydrodynamics of the environment (Kaplan et al., 2016; Martin et al., 2005). The hydrodynamics and physical stressors on the seaward side differ from the landward side, with the highest erosion rates occurring on the seaward side and higher sedimentation rates occurring on the landward side (Chowdhurry et al., 2019). Abiotic processes, such as water movement and sedimentation, may alter the structural availability and complexity of the reef (Martin et al., 2005). Low-crested defense structures provide habitat for mobile and sessile fauna, and the landward side of these structures decreases exposure and hydrodynamic regime, influencing the diversity of crustaceans and fish (Martin et al., 2005). The landward side of an oyster reef can also provide refuge from predation for mobile species (Connell 1972; Menge 1978). However, low-crested oyster reefs may be more susceptible to sedimentation on the landward side, leading to burial and ultimately adversely affecting epifaunal communities, including oysters (Colden and Lipcius, 2015). Additionally, low-crested reefs are submerged for a longer amount of time, which may lead to higher oyster predation (Fodrie et al., 2014).

In this study, I quantified mobile and sessile species abundance and richness both landward and seaward of high- and low-relief restored oyster reefs. I hypothesized that:

(1) the landward side of restored oyster reefs will support higher mobile fauna (both free-living and parasitic organisms) species abundance and richness than the seaward side of restored oyster reefs regardless of height;

(2) while low-relief oyster reefs will initially provide refuge from physical stressors for mobile fauna, the oysters on low-relief reefs will be more susceptible to sedimentation and predation over time than high-relief reefs;

(3) the landward side of high-relief oyster reefs will ultimately support the highest biodiversity of mobile fauna, with the difference in supported diversity between low-relief and high-relief reefs increasing over time;

(4) the seaward side of high-relief oyster reefs will support higher sessile fauna species richness than the landward side of restored oyster reefs, where the seaward side will promote more direct water movement and delivery of food items for sessile organisms.

Methods

Study Site

This study took place in the Rachel Carson Reserve (RCR), part of the North Carolina Coastal Reserve and the NOAA National Estuarine Research Reserve, located in Beaufort, NC, United States (Figure 1). Taylor's Creek, an estuarine tidal creek, directly abutting the RCR was the focus of this study. In July 2020, eight 15 m by 1.5 m oyster reefs (4 high relief [0.3 m tall] and 4 low relief [0.2 m tall]) were constructed using Oyster Catcher^{TM} material along the Taylor's Creek marsh shoreline (Figure 2). Additionally, four control sites (no reef construction) were established. Oyster breakwater site selection was conducted in coordination with the Rachel Carson Reserve Site Manager and the NC Coastal Reserve Coordinator. Across 12 sites, each site consisted of a single treatment in a randomized block design, with four replicates per

Figure 1. The Rachel Carson Reserve, Beaufort, NC and sample area, Taylor's Creek. Colored blocks are representative of 12 intertidal sites. Green block=high relief sites, orange block=low relief sites, yellow block=control sites. Red numbers depict the block design. Block 1 (sites 1-3), block 2 (sites 4-6), block 3(sites 7-9), and block 4 (sites 10-12). Photo credit: Google Earth

treatment (Figure 1). Breakwaters were modeled after the Oyster Catcher^{TM} design produced by Sandbar Oyster Co. and made with biodegradable material. The bases of the reefs were initially set at approximately -0.4 m North American Vertical Datum of 1988 (NAVD88), respectively, within the optimal growth zone (OGZ, -0.6 to -0.3 m NAVD88) for intertidal oysters (Fodrie et al., 2014; Ridge et al., 2015). High- and low-relief reef crests were located at approximately -0.1 and -0.2 NAVD88 respectively. Treatments were placed at least 25m apart.

Figure 2. Low-relief Oyster Catcher^{TM} reef 7 months post-construction (Site 4, February 2021). Photo credit: Nina Woodard

Reef fauna sampling

To fully understand the influence of placement on the structural complexity and habitat availability of an oyster reef, it is important to evaluate the associated community assemblages. It has been demonstrated that traditional survey methods are often limited for a variety of reasons, ranging from inadequate funding to habitat sensitivity (Yoccuz et al., 2001; Bates et al., 2007). As a result, more recent studies have begun to include surrogate species – organisms that can be reliably used to predict the presence of another species – for monitoring diversity (Rodriguez & Brooks, 2007; Moore et al., 2020). Trophically transmitted parasites have been identified as valuable surrogate species as their life cycle includes various host species, thus providing us with knowledge on the type of trophic connections supported by different ecosystems (Lafferty et al., 2006; Dunne et al., 2013). It has also been demonstrated that parasite diversity is positively correlated with host diversity as well as structural complexity (Kamiya et al., 2014; Wood & Johnson, 2016; Johnson et al., 2016). In estuarine systems, the hosts that parasites might often target as intermediate hosts include mollusks, mud crabs, and smaller resident fish species, such as blennies and gobies (Toscano et al., 2014; Hardin et al., 2019). Therefore, we sampled both resident free-living organisms and parasites associated with restored reefs and control sites to understand the influence of restoration more fully on community structure and assembly.

Figure 3. Landward passive sampler with a HOBO temperature logger connected to the top. Photo credit: Rachel Gittman

We sampled fauna monthly for a year, from October 2020 until October 2021. Over the course of this project, crustaceans, fish, and other mobile free-living organisms were collected using two passive samplers at each site. These samplers are plastic milk crates (19 x 22 x 16 cm) (Figure 3) filled with 3.5 lbs. of dried out oyster shells and zip-tied shut with mesh on top (Roche and Torchin, 2007). These crates allow the organisms to freely move inside and outside of the crates, while being attracted to the three-dimensional habitat that the oyster shell provides (Moore et al., 2020; Blakeslee et al., 2021). Each crate was zip-tied to wooden stakes approximately 5 meters from the middle of the oyster reef. One crate was oriented directly landward of the oyster reef and the second crate was oriented directly seaward of the oyster reef. Altogether, 24 crates were deployed: 2 per site (landward/seaward) with 12 total sites made up of 4 replicates per each of our 3 treatments (high-relief, low-relief, and control). Target resident (i.e., primarily site specific) crabs and fishes (see below) were collected approximately four weeks post deployment for later parasite analysis by sorting through the contents of each crate using a floating sieve (69 x 56 x 12 cm) with a 2 mm mesh. We also recorded the free-living diversity of other mobile taxa such as blue crabs, shrimps, and transient fishes.

The target resident fish species for parasite dissections included two goby species, the naked goby, *Gobiosoma bosc*, and the seaboard goby*, Gobiosoma ginsburgi*, two blenny species, the crested blenny, *Parablennius laticlavius*, and the feathered blenny, *Hypsoblennius hentz*, and the oyster toadfish, *Opsanus tau*. The target resident crab species for parasite dissections included the stone crab, *Menippe mercenaria*, and four mud crab species: the common mud crab, *Panopeus herbstii,* the white-fingered mud crab, *Rhithropanopeus harisii,* the black-fingered mud crab, *Dyspanopeus sayi* and the flatback mud crab, *Eurypanopeus depressus*. These target species served as models to study the impacts of orientation and reef-relief on free-living and parasite abundance as they represent resident taxa.

Once the target species were collected, they were transported back to the laboratory at East Carolina University alive in a bucket with aerated water (taken from the collection site). The fish were euthanized by decapitation and then placed in a -20°C freezer until dissection. The crabs were placed in a -20°C freezer until dissection. Before dissections, target resident crab species were identified, sexed, and the widest part of the carapace measured using calipers and recorded in millimeters. The crabs were identified to species level using a guide by Williams (1984). Before dissections, target resident fish species were identified using two guides and the total length (from head to end of the caudal fin) was measured with calipers and recorded in millimeters (Kals & Carpenter 2011; Murdy & Musick 2013).

The influence of orientation and reef relief on sessile invertebrates was quantified using 13.5 cm x 13.5 cm plastic fouling tiles attached to the milk crates. The plastic tiles were removed February 2021, July 2021, and September 2021, and then replaced with fresh tiles after each removal. To determine differences in biomass of recruiting individuals landward versus seaward, the plastic fouling tiles were taken back the laboratory at East Carolina University, and the fouling organisms on each tile were brushed and scraped off into an aluminum foil and placed into a drying oven at 75°C for 120 hours. Samples were then weighed on a fine-resolution digital scale (0.01 grams) and the dry weight of each sample was recorded. Before scraping the tiles, each tile was photographed to document the sessile organisms on each plastic tile, as well as the differences in percent cover of sessile taxa landward versus seaward (Figure 4). Percent cover and species composition were measured using an image analysis program Fiji/ImageJ (version 2.3.0/1.53f) (Figure 4).

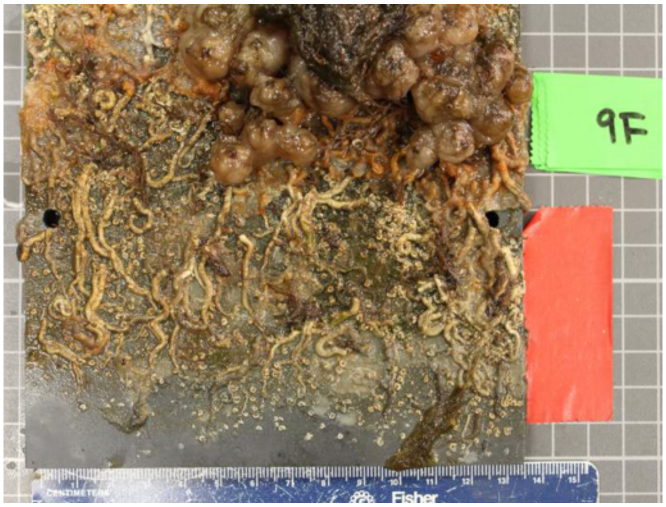

Figure 4. Plastic fouling tile (Landward, Site 9) depicting sessile organism settlement. Photo credit: Nina Woodard

We conducted post-restoration monitoring of oyster reef characteristics in October 2020, December 2020, March 2021, June 2021, September 2021, and March 2022 to assess the impacts of wave energy, reef relief, and exposure on oyster abundance. Oyster abundance was quantified by counting the number of live oysters within ¼ of a 0.0625m^2 quadrat, with the sample section (1-4) chosen randomly (Figure 5). Lengths (mm) of five oysters were also recorded in each quarter quadrat using calipers. Sampling was conducted for three breakwater ‘tables’ per site, with the quadrat placed on the landward side of the reef. Elevation points were taken on the reef crest in the middle of the sampled quadrat using a Trimble R10 Real-Time Kinematic (RTK) GNSS (GPS; 0.5-1.0cm horizontal and 1.0-4.0 cm vertical resolution) (Albright *et al.*, *in preparation).*

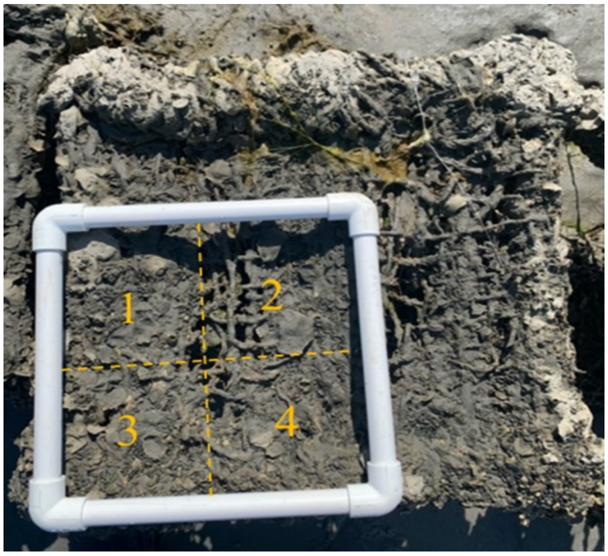

Figure 5. Oyster sampling using 0.0625m quadrat. Quadrat placed on the table surface (landward) and oyster abundance was enumerated within 1 of the 4 squares within the quadrat.

Abiotic data

Abiotic measurements included water temperature (°C), dissolved oxygen (mg/L), and salinity (ppt). These measurements were collected using a handled YSI (Pro30 Model) at the location of each reef. Attached to each plastic fouling tile was a HOBO pendant data logger (Figure 3) recording temperature. Starting in May 2021, HOBO salinity loggers were deployed. For all statistical analyses, only the handheld YSI abiotic data was included.

Host dissections and parasite identification

We quantified the relative abundance and taxa richness of endoparasites that have multi-host life cycles (e.g., nematodes, trematodes, entoniscid isopods, cestodes, acanthocephalans) and additionally a rhizocephalan which has a direct life cycle, infecting mud crab hosts. These endoparasites may reside in a variety of common host organisms, which were separated into two host groups including our target resident fish and crab species. Both the fish (> 20mm) and crabs (>4mm) were dissected using established protocols (Moore et al., 2020; Blakeslee et al., 2015). In the fish, the entire gastrointestinal tract was removed. The stomach, liver, gallbladder, spleen, and intestine were checked for parasites under a dissecting microscope at 4-6x (with 10x oculars). The head, body, and fins were checked for trematode cysts under a dissecting microscope at 4x. In the crabs, the gonad, ganglia, and hepatopancreas tissue were removed and examined under a compound microscope at low power (4x, with 10x oculars). After dissection, parasites that were large enough to be separated from the host tissue were placed in 1.5 ml tube and kept in -20°C freezer. All other host tissue was placed in a 1.5 mL tube and kept in a -20°C freezer. The rest of the body was discarded.

Statistical Analyses

We performed exploratory data visualization and analyses to determine the distribution of the data: crab abundance, fish abundance, biomass of recruiting sessile individuals, percent cover of recruiting sessile individuals, parasite prevalence in crab and fish species, and parasite intensity in fish species. To test for differences in total abundances of free-living fishes and crabs we fit generalized linear mixed models (GLMMs) with placement (landward/seaward), reef relief (high, low, control), time post reef restoration (3-15 months), and dissolved oxygen (DO) treated as fixed effects and site (1-12) nested within block (1-4) as random effects. For these analyses, we assumed negative binomial distribution (Bolker 2008). To test for differences in the total number of free-living fishes and crabs captured over the entire study period we fit GLMMs with a poisson distribution with the fixed effects of placement (landward/seaward), relief (high, low, control), DO, and height change. Site (1-12), nested within block (1-4), was included as a random effect.

To analyze percent cover of recruiting sessile individuals we used generalized linear mixed effect models with fixed effects of placement (landward/seaward), reef relief (high, low, control), date (February 2021, July 2021, October 2021), and salinity. Site (1-12), nested within block (1-4), was included as a random effect. Because percent cover is a continuous variable that is bound between zero and 1, we assumed a Beta family error distribution. We also used a GLMM with beta errors to analyze parasite prevalence in free-living crab and fish host species with the fixed effects of species identity (Crabs: *Menippe mercenaria*, *Panopeus herbstii, Rhithropanopeus harisii, Dyspanopeus sayi, Eurypanopeus depressus* and Fishes: *Gobiosoma bosc*, *Gobiosoma ginsburgi*, *Parablennius laticlavius*, *Hypsoblennius hentz*, *Opsanus tau*), average carapace width for the crabs and average length for fish, total abundance of dissected crabs and fish, and salinity, and with Site nested within block as random effects. We also used a GLMM with poisson distribution to analyze parasite intensity of free-living fish hosts with the fixed effects of fish species identity (*Gobiosoma bosc*, *Gobiosoma ginsburgi*, *Parablennius laticlavius*, *Hypsoblennius hentz*, *Opsanus tau*), average fish length, total abundance of dissected fishes, and DO, and with site nested within block as random effects. Lastly, we used a GLMM with a gaussian distribution to analyze biomass of recruiting sessile invertebrates with the fixed effects of placement (landward/seaward), reef relief (high, low, control), date (February 2021, July 2021, October 2021), and salinity, and site nested within block as random effects. Both DO and salinity measurements were found to be correlated fixed effects throughout the study period. Salinity was used in mixed models analyzing sessile invertebrate recruitment, in addition to parasite prevalence, as a response variable. Salinity plays a major role in species distribution throughout estuarine habitats, and as a result, changes to salinity can determine species assemblage throughout a habitat. DO was used in mixed models to analyze free-living crab and fish abundance, in addition to parasite intensity in fish host samples, to observe the seasonal effects and time post reef restoration on free-living and parasite abundance.

For each endpoint, we built alternative simplified models by sequentially dropping all terms from the full models outlined above, down to the null model of no fixed effects and compared them using sample size Corrected Akaike's Information Criterion (AICc). For most analyses we based our inferences on the most parsimonious models (i.e. the model with the lowest-AICc score). However, when models were largely indistinguishable (i.e. delta AICc <2), we were conservative and chose to interpret the most inclusive models. If interactions were significant, estimated marginal means were used to assess comparisons. All analyses were performed in the R statistical computing environment (version 4.1.3 (2022-03-10)) using the packages glmmTMB (Brooks et al., 2017), bblme (Bolker 2021), car (Fox & Weisberg, 2019), AICcmodavg (Mazerolle, 2020) and emmeans (Lenth, 2022).

Results

Impact of time, substrate placement, and reef-relief on free-living community structure

Free-living crustacean and fish abundance varied with time post reef restoration (3-15 months) (Figures 6 & 7, Tables 1 & 2). Over the course of the entire study period, average crab abundance was 5.97 ± 0.25 (mean ± SE) and average fish abundance was 0.69 ± 0.09. There were no detectable differences in average target crab abundance over the course of the experiment, but it is notable that the abundances were greatest 4 months (November 2021) post-

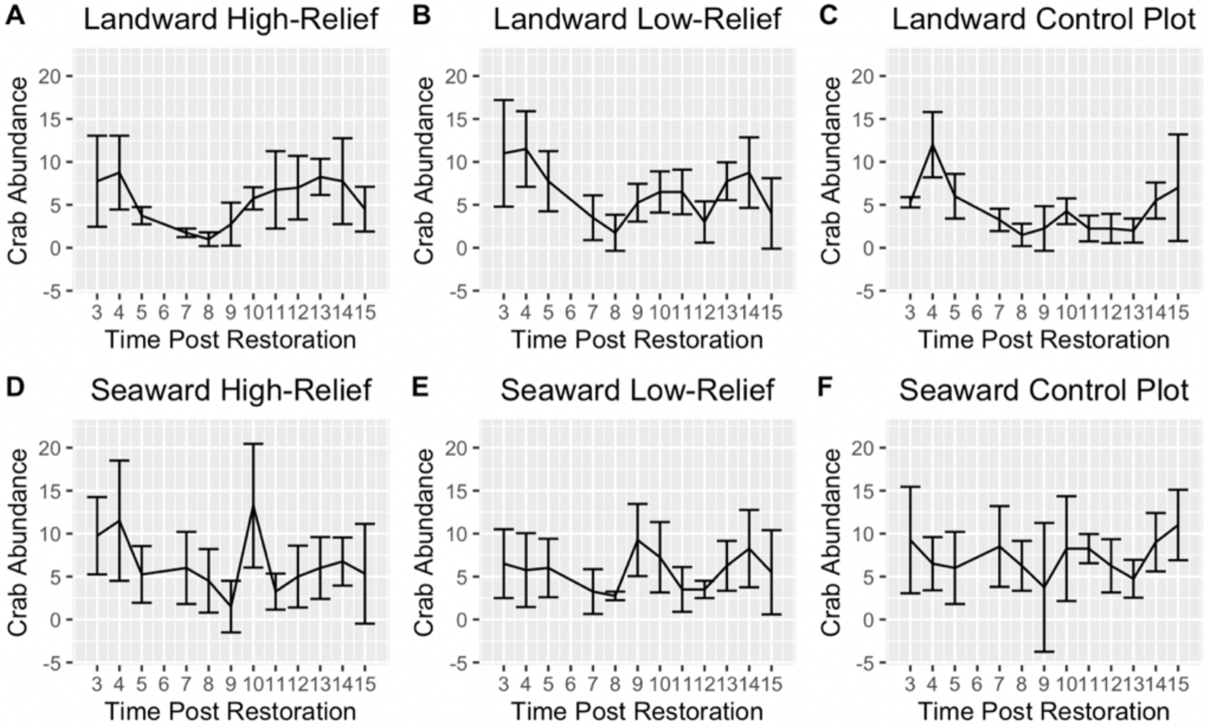

Figure 6. Abundance (mean ± SE) of the four mud crab species (*P. herbstii, R. harisii, D. sayi, E. depressus*) and the stone crab (*M. mercenaria*). Graphs A-F show crab abundance as a response to time post reef restoration across two placements (landward/seaward) and three reef relief treatments (high, low, control) from October 2020 to October 2021 (3-15 months).

reef restoration (9.3 ± 0.99). The seaward side of control plots promoted the greatest average target crab species abundance throughout the experiment (7.3 ± 0.63) (Figure 6F, Table 1). Average target fish species abundance was greatest at 15 months post-reef restoration (2.43 ± 0.55, $p=7.87e^{-5}$) across all sites, but these differences were not statistically distinguishable between high-relief, low-relief, and control sites (Figure 7, Table 2). Differences in free-living crab and fish abundance were associated with shifts in dissolved oxygen throughout the study period ($p<0.0001$: Table 1 & 2).

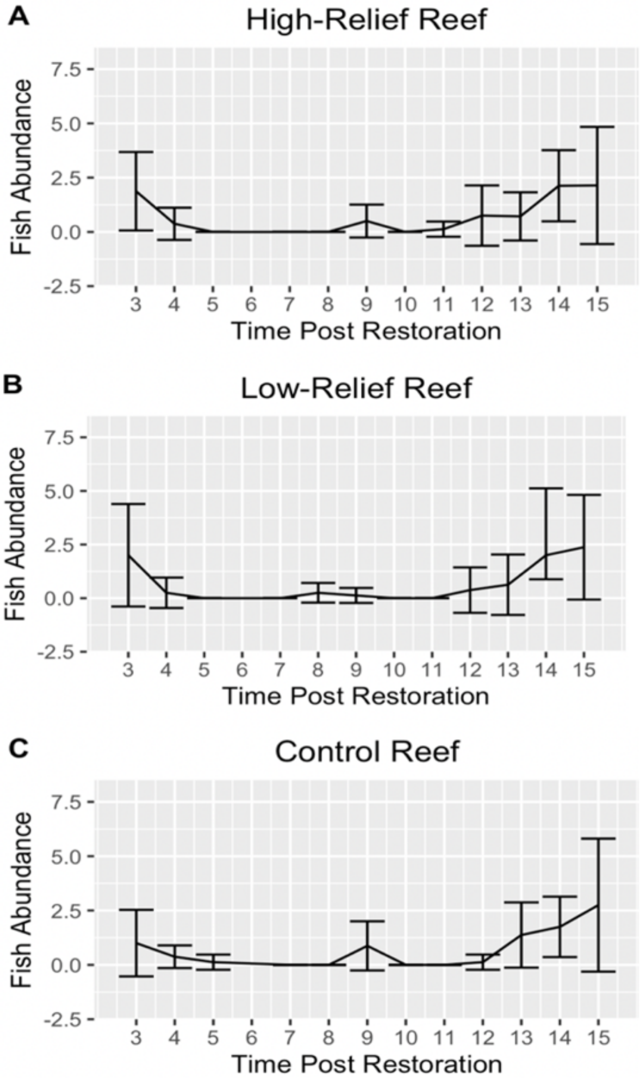

Figure 7. Abundance (mean ± SE) of two goby species, two blenny species, and the oyster toadfish (*Gobiosoma bosc, Gobiosoma ginsburgi, Parablennius lactivius, Hypsoblennius bentz, and Opsanus tau).* Graphs A-C show fish abundance as a response to time post reef restoration across three reef relief treatments (high, low, control) from October 2020 to October 2021 (3-15 months).

Changes in the total number of crabs captured over the entire study period varied according to placement (landward/seaward) and reef relief (high, low, control) (Table 1). At control sites the average target crab abundance was 52.5 ± 4.5 on the landward side and 87.5 ± 14.37 on the seaward side. At high-relief sites the average target crab abundance was 65.5 ± 7.53 on the landward side and 73.25 ± 12.82 on the seaward side. At low-relief sites the average target crab abundance was 77.25 ± 4.01 on the landward side and 67.75 ± 6.142 on the seaward side (Table 10). Total target crab abundance was highest on the seaward side of control sites (p=0.012, Tukey posthoc pairwise comparisons: Figure 8, Table 3). Fish abundance was similar across high-relief reefs (16.5 ± 3.3), low-relief reefs (16 ± 3.5) and control sites (16.5 ± 3.1) (Figure 8, Table 4 & 11).

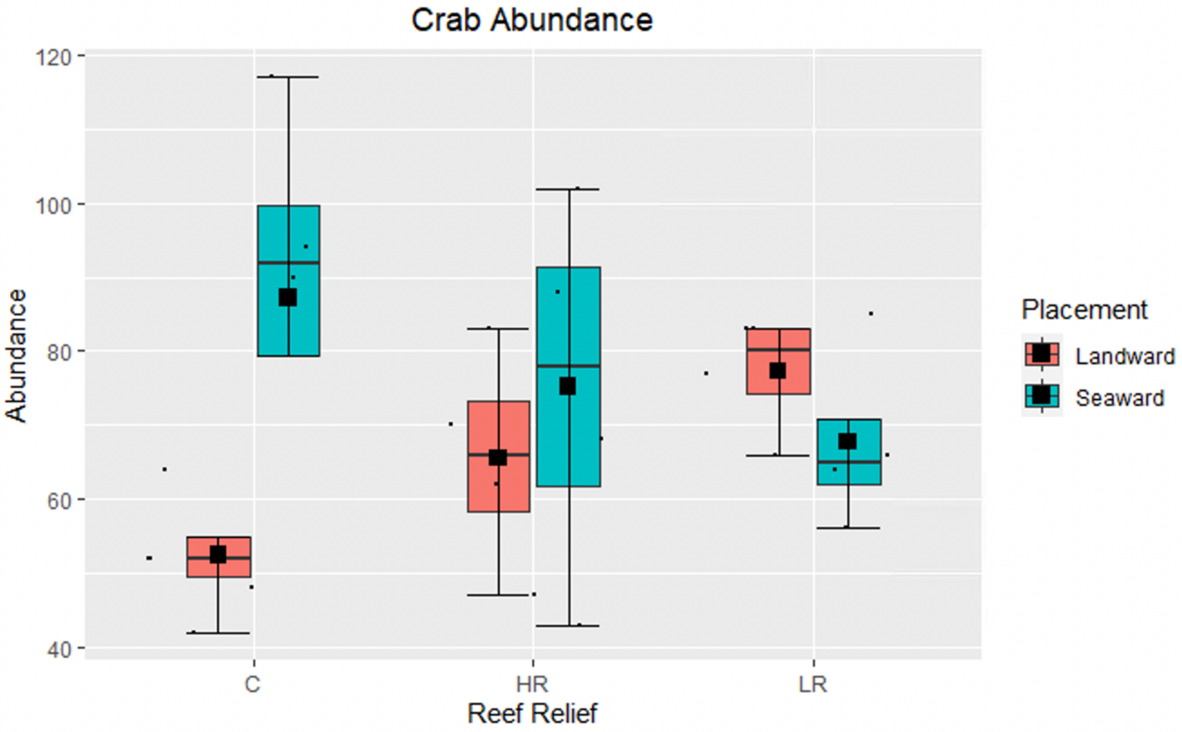

Figure 8. Crab abundance as a response to reef relief (control, high relief, low relief) comparing the two different placements (landward/seaward) from October 2020 to October 2021.

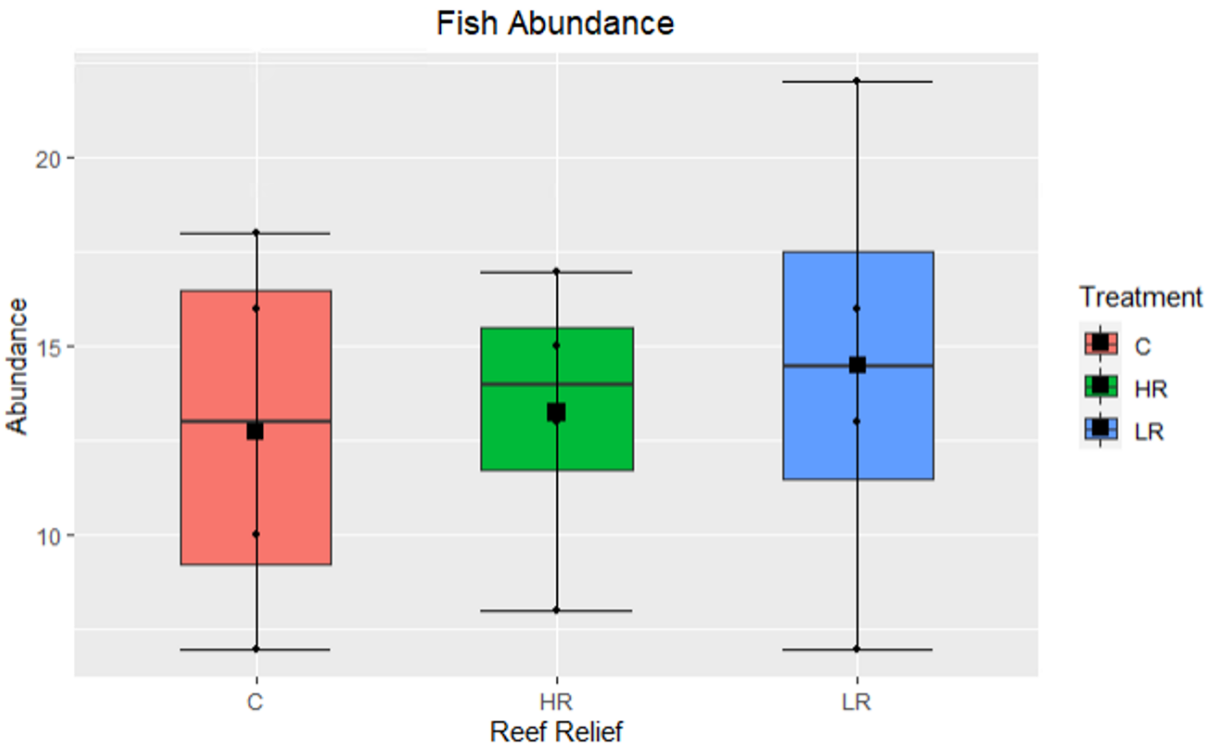

Figure 9. Fish abundance as a response to reef relief (control, high relief, low relief) from October 2020 to October 2021.

Target crab species richness over the course of the entire study period was relatively low (2.22 ± 0.06) and as a result no statistical models were constructed analyzing species richness as a response variable. However, in simple comparisons of placement (landward/seaward) and reef relief (high, low, control) across all sites and sampling events, target crab species richness was higher on the seaward side of restored reefs (2.5 ± 0.09) compared to the landward side of reefs (1.9 ± 0.07) (Figure 10), but was similar in average richness among high-relief reefs (2.3 ± 0.11), low-relief reefs (2.15 ± 0.10) and control sites (2.22 ± 0.11). The most abundant free-living crab species was *P. herbstii* (49.08 ± 12.84), followed by *D. sayi* (26.33 ± 11.83. The least abundant free-living crab species was *E. depressus* (0.25 ± 0.62: Table 12).

Similarly, overall target fish richness was low (0.42 ± 0.05) and as a result, no statistical models were constructed. For examination of reef relief across sites and sampling events, there were no clear trends or differences (Figure 11). The most abundant free-living fish species was *P. laticlavius* (4.67 ± 2.61), followed by *H. hentz* (3.75 ± 3.28). The least abundant free-living fish species was *G. bosc* (1.83 ± 1.64: Table 15).

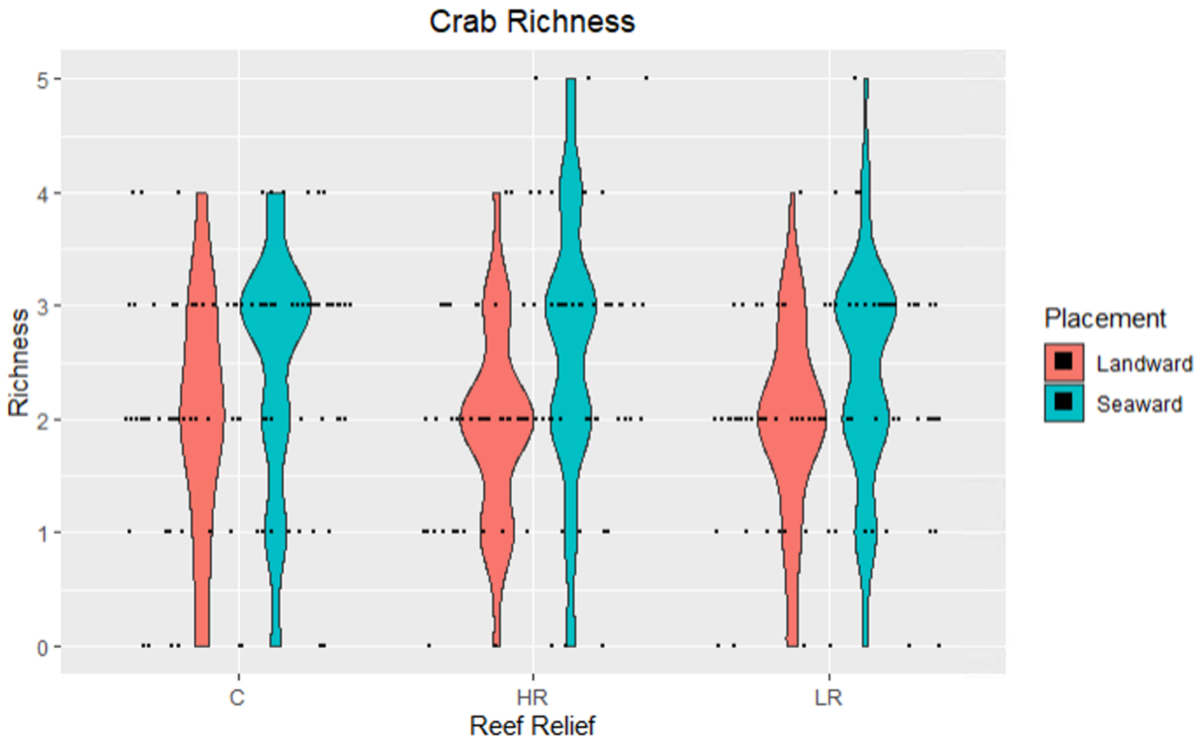

Figure 10. Crab richness as response of reef relief (control, high relief, low relief) and grouped by placement

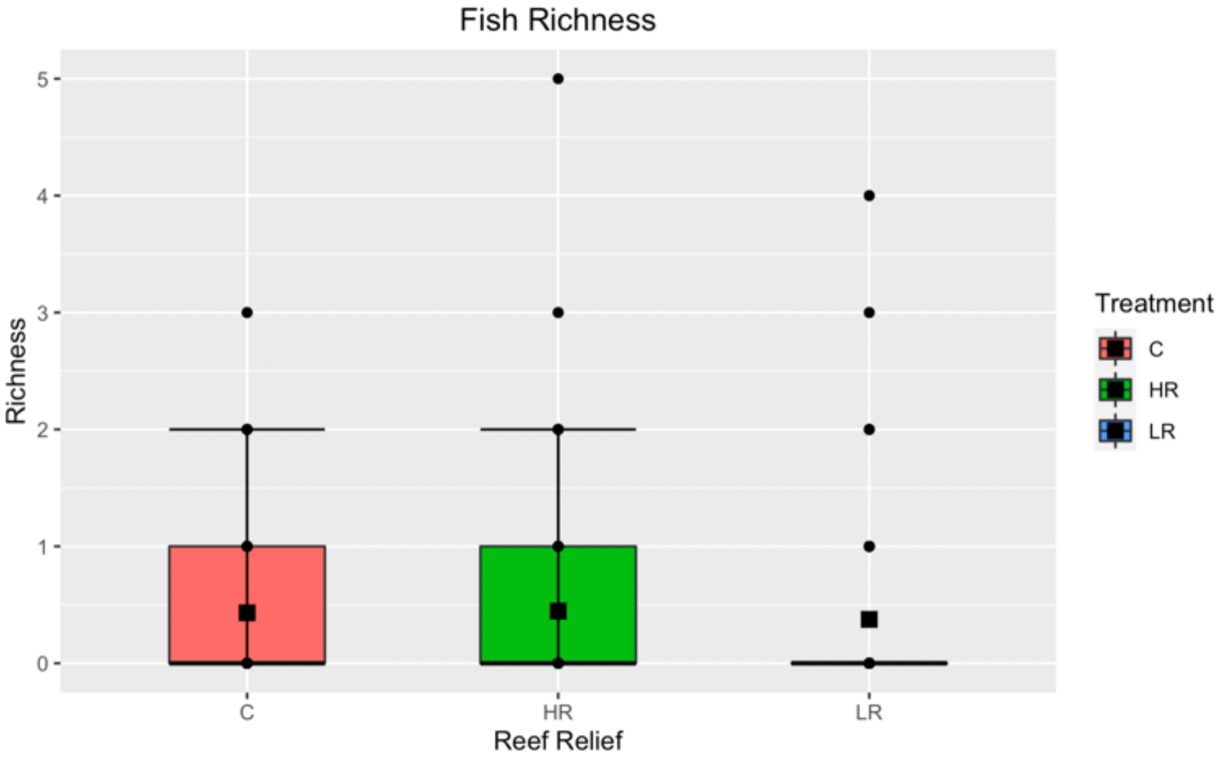

Figure 11. Fish richness as a response to reef relief (control, high relief, low relief) from October 2020 to October 2021.

Influence of substrate placement, reef-relief, and time on parasite diversity

About eight percent of the crab host samples were infected with parasites, with the most common parasite group being the entoniscid *Cancrion spp*, followed by nematodes. Forty-eight percent of the host samples that were infected by parasites were found on the landward side of the restored oyster reefs, while fifty-two percent of the host samples that were infected by parasites were found on the seaward side of restored oyster reefs (p=0.4330: Table 5, Figure 12). The most abundant free-living crab species infected by parasites was *P. herbstii* (55.58%), followed by *D. sayii* (29.17%). The least abundant free-living crab species infected by parasites was *E. depressus* (0.31%) (Figure 13). Parasite prevalence in target host crab species was the greatest in crabs that were at least 24 mm in carapace width (Figure 13). The largest crab

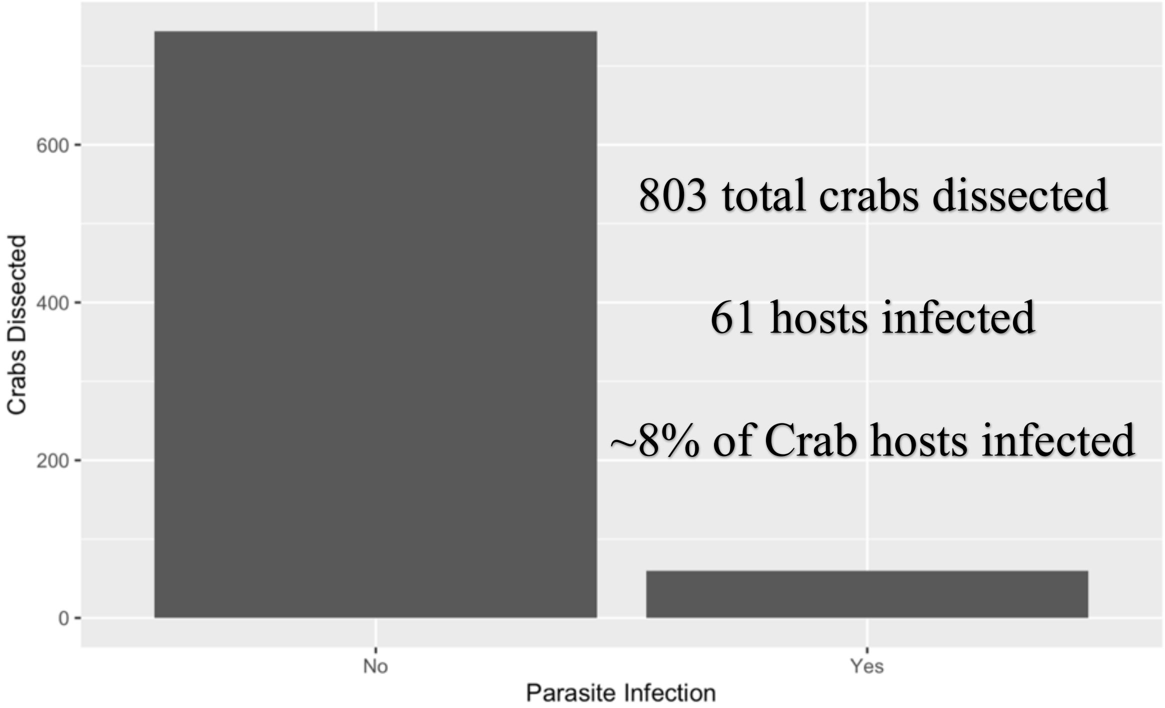

Figure 12. Total abundance of crabs dissected, and the amount of crab host samples infected by parasites across all reef-relief treatments (high, low, control) and placement (seaward/landward).

carapace width belonged to *M. mercenaria*, which also happened to have the lowest average parasite prevalence (1% ± 0.000). However, there was no interaction between crab species identity and crab width on parasite prevalence (Table 5).

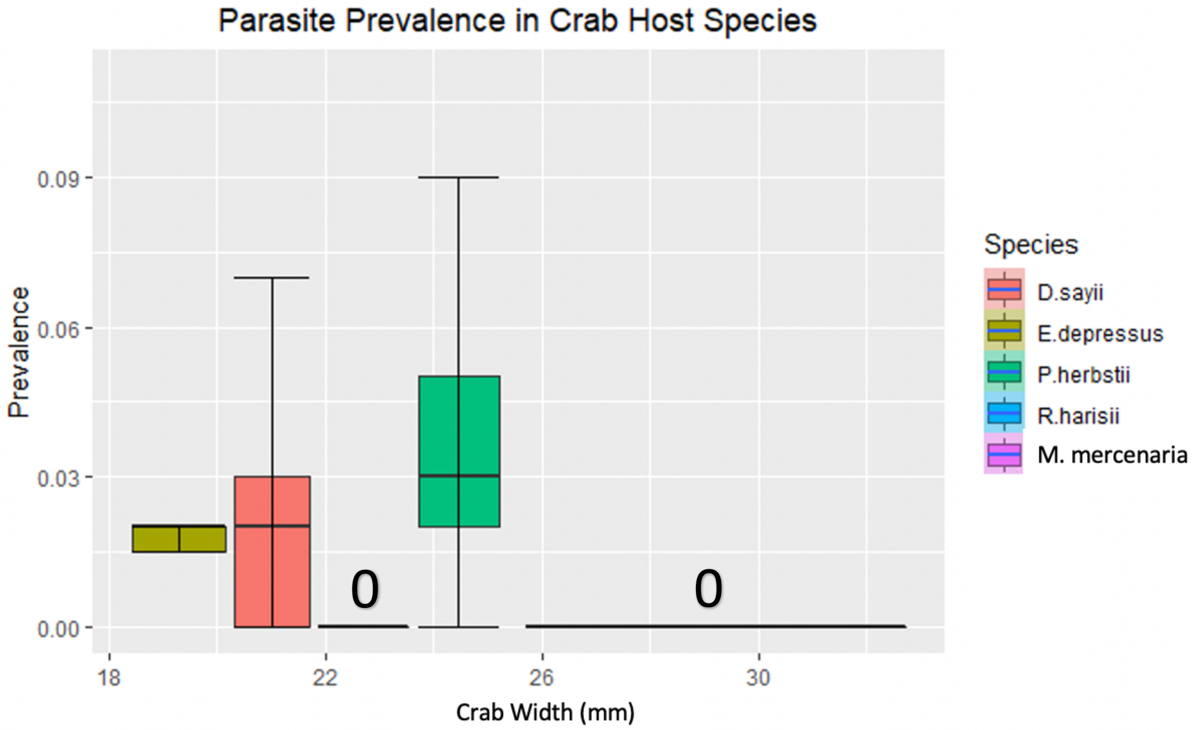

Figure 13. Parasite prevalence in target crab host species (*D. sayii, E. depressus, P. herbstii, R.harisii, and the M. mercenaria)* based on the width (mm) of the crab carapace.

Parasite prevalence in the five target fish host species captured during the study period was significantly impacted by the total number of host fish dissected (p=0.001929, Table 6). Forty-three of the fish host samples dissected were infected by parasites, with the most common parasite group being trematodes (larval metacercarial cysts). The most abundant free-living fish species infected by parasites was *P. laticlavius* (35%), followed by *H. hentz* (34%). The least abundant free-living fish species to be infected by parasites was *G. ginsburgi* (3%). *G. bosc* was the only fish species to not be infected by parasites.

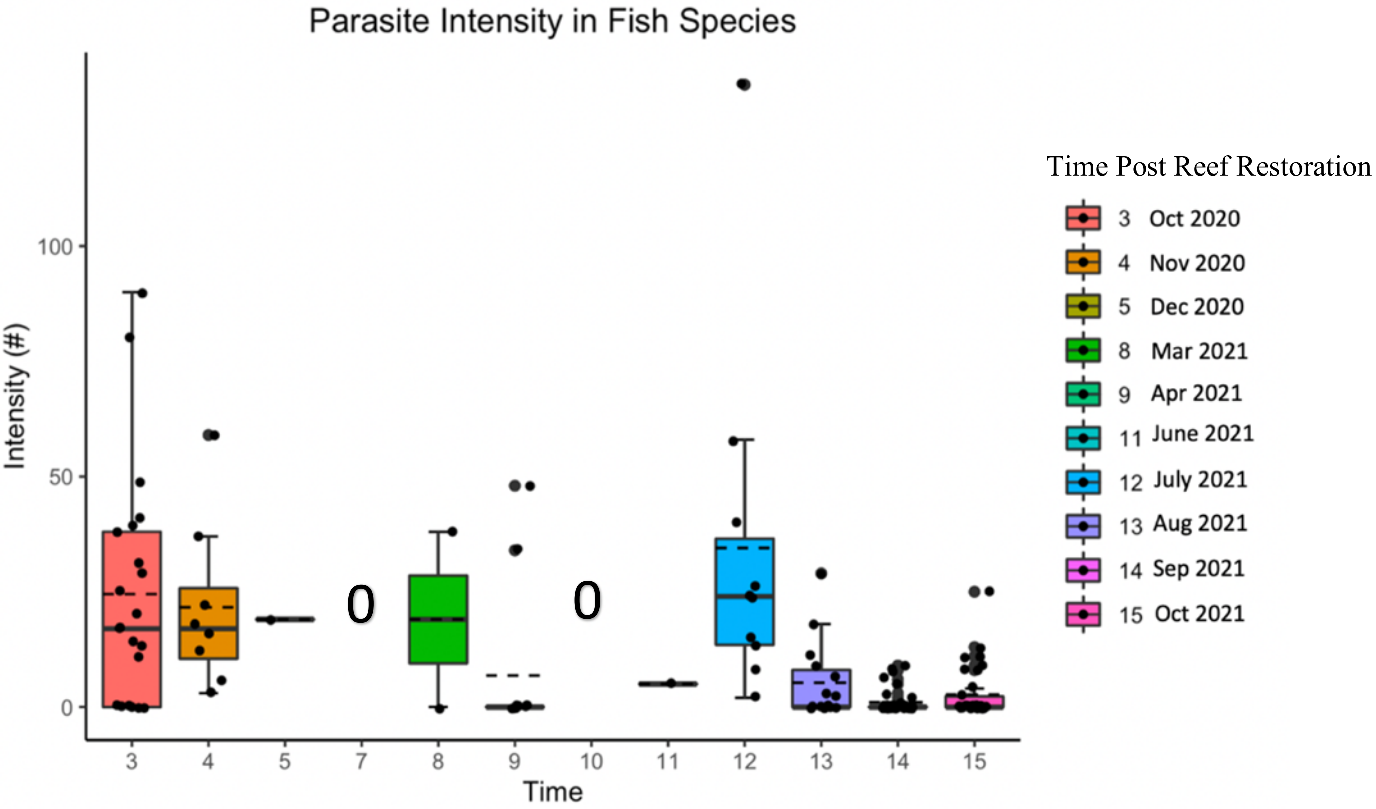

Figure 14. Parasite intensity in fish host samples as a response to time post reef restoration (3-15 months). Zeroes indicate missing box plots where those months (February [month 7]and May [month 10]) did not have any fish host samples infected with parasites.

Parasite intensity in fish host samples was higher at low-relief reefs (13.1 ± 3.85), compared to high-relief reefs (9.08 ± 2.52) and control sites (7.18 ± 1.83) but these differences were not statistically significant ($p > 0.05$: Tukey posthoc pairwise comparisons). In response to time post reef restoration (3-15 months), parasite intensity in fish host samples was greatest at 12 months (34.5 ± 12.3, p=7.97 $e^{-9:}$ Tukey posthoc pairwise comparisons) post restoration (July 2021) (Figure 14, Table 6). Across the entire post restoration period, free-living and parasite responses were greatest during the first (October-November) and fourth (June-August) season (Figure 15). Additionally, parasite intensity was associated with the length of fish host samples (p=0.002676: Table 7). When we considered the interaction between fish species identity and

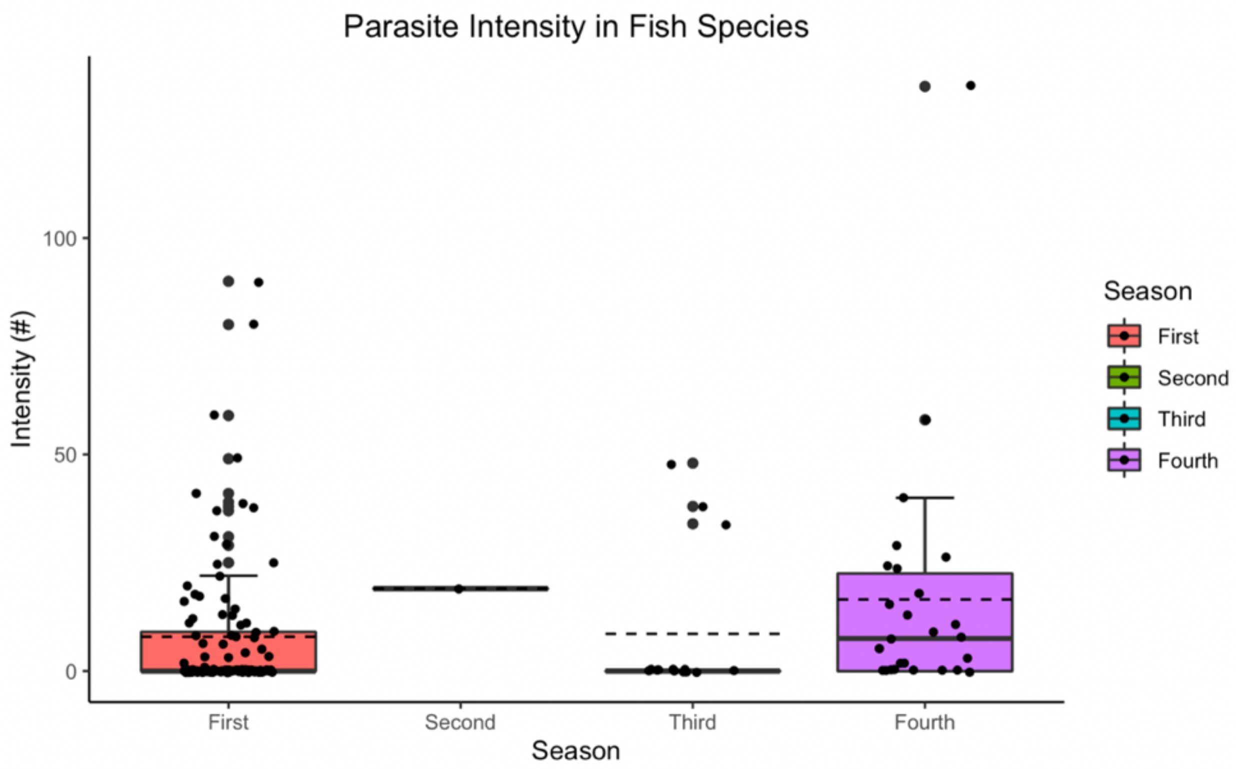

Figure 15. Parasite intensity in fish host samples as a response to season (1-4). Season 1 includes September-November, Season 2 includes December-February, Season 3 includes March-May, and Season 4 includes June-August. Parasite intensity in fish host samples was greatest during the first and fourth season.

total fish length (mm), oyster toadfish were found to have the greatest parasite intensity, with an average intensity of 40.74 ± 8.12.

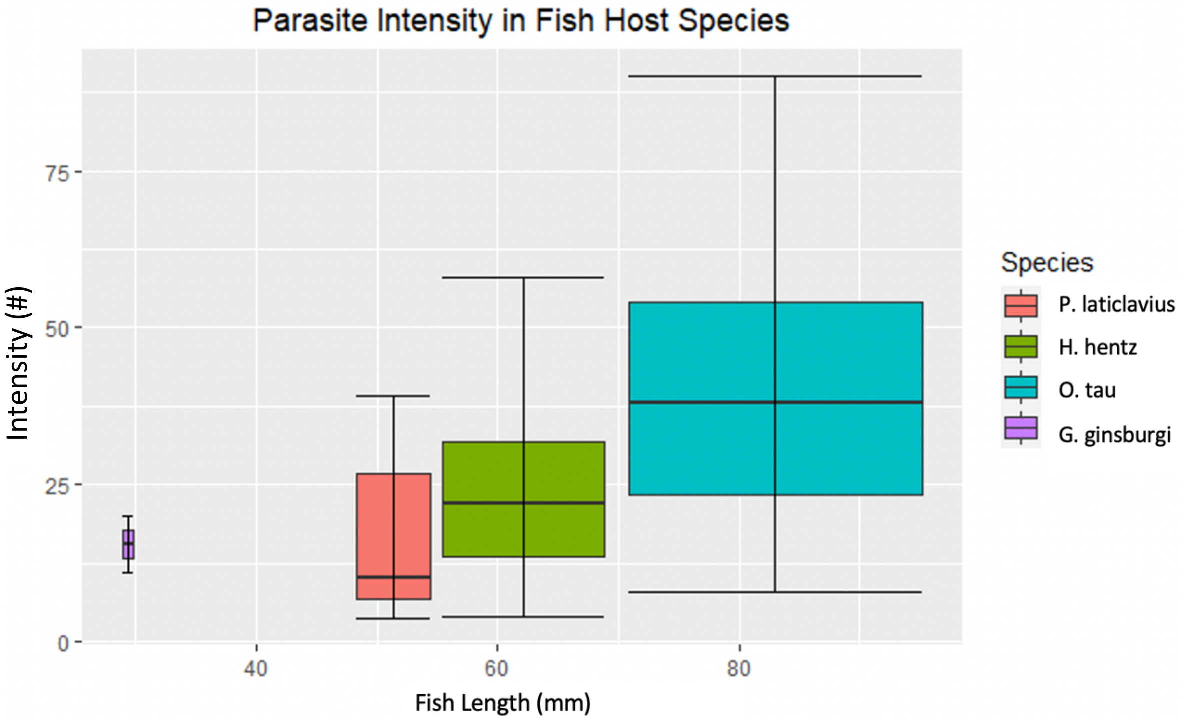

Figure 16. Parasite intensity in fish host samples as a response to total fish length (mm), grouped by fish species categories.

Impact of substrate placement and reef relief on percent cover and biomass of sessile invertebrate communities

To understand the influence of reef placement and reef relief on sessile invertebrate communities, I analyzed the differences in percent cover and biomass of recruiting individuals landward and seaward of high-relief and low-relief reefs and control sites. Changes to sessile communities between October 2020 and October 2021 varied according to placement relative to the shoreline (landward/seaward), reef-relief (high, low, control) and the collection month (February 2021, July 2021, October 2021) (Table 8 & 9).

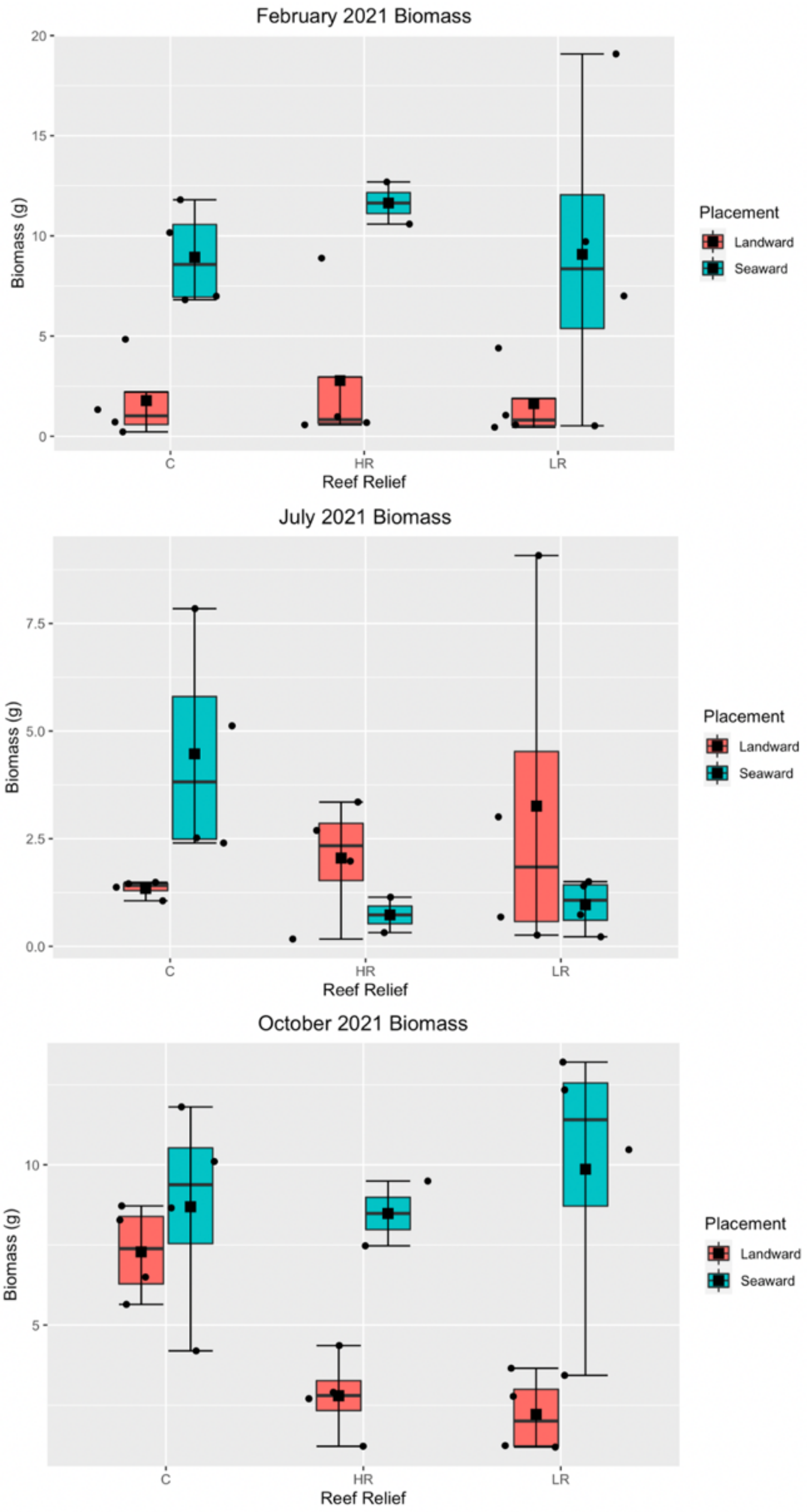

Figure 17. Epifaunal biomass on plastic fouling tiles between three reef-relief treatments (control, high, low), comparing two placements (landward/seaward) for the months of February, July, and October (2021).

Across the three collection months, percent cover of sessile organisms differed in response to placement, where individuals trended higher on the seaward side of restored reefs (89% ± 0.016) compared to the landward side (75% ± 0.03) (Table 9).

Across all three collection months, differences in biomass of recruiting individuals were associated with date ($p=1.245e^{-6}$, Table 8). In February 2021, biomass of recruiting individuals trended higher on the seaward side of restored reefs (9.5 ± 1.5 g) compared to the landward side (2.05+ 0.76 g, p=.00017: Tukey posthoc pairwise comparisons). In response to reef relief, biomass was higher at high-relief reefs (5.73 ± 2.3 g) compared to low-relief reefs (5.35 ± 2.31 g) and control sites (5.36 ± 1.55 g) , but this difference was not statistically significant ($p > 0.05$: Tukey posthoc pairwise comparisons) (Figure 17, Table 8).

In July 2021, biomass of recruiting individuals trended higher on the seaward side of the high-relief and low-relief restored reefs and control sites (2.3 ± 0.76 g) compared to the landward side (2.22 ± 0.69 g). Additionally, biomass trended higher at control sites (2.9 ± 0.85 g) compared to high-relief (1.61 ± 0.53 g) and low-relief reefs (2.11 ± 1.05 g). However, these differences were not statistically significant ($p > 0.05$: Tukey posthoc pairwise comparisons: Figure 17, Table 8).

In October 2021, biomass of recruiting individuals trended higher on the seaward side of the restored reefs (9.1 ± 1.03 g) compared to the landward side (4.1 ± 0.77 g, p=0.0007). In response to reef relief, biomass was higher at the control sites (7.9 ± 0.86 g) compared to high-relief (4.69 ± 1.29 g) and low-relief reefs (6.04 ± 1.79 g), but this difference was not statistically significant ($p > 0.05$: Tukey posthoc pairwise comparisons: Figure 17, Table 8).

Discussion

Restored oyster reefs are valued for their ecosystem services and shoreline protection (Coen et al., 2007; Fodrie et al., 2014). The three-dimensional reef structure attracts and provides valuable substrate for invertebrate and fish species (Coen et al., 1999; Coen & Luckenbach, 2000; Tolley et al., 2005; Luckenbach et al., 2005; Moore et al., 2020). Additionally, restoration of biogenic habitat has provided enhanced community biodiversity, where host-parasite interactions increase in response to oyster restoration (Moore et al., 2020). I quantified mobile and sessile species abundance and richness landward and seaward of high- and low-relief restored oyster reefs, as well as control sites, to examine how location relative to a biogenic reef habitat would influence free-living and parasite diversity. Predictions for mobile free-living fauna were not upheld, with low abundances throughout the entire study period. In contrast, predictions for sessile species abundance were upheld, where stark contrasts were seen between abundances on the seaward side of reefs compared to the landward side. In the sections that follow, we discuss in detail the changes in free-living and parasite community structure throughout the study period, post-restoration.

Changes in free-living mobile and parasite community structure

We found that free-living crab and fish species associated with the reefs were present at all sites and similar in composition to what would be found in natural or older restored reefs (Moore et al., in review). However, abundance across all sites was relatively low compared to abundances found at an older restored reef or natural reef. Additionally, parasite prevalence in crab species was low as compared to prevalence found in natural or older restored reefs, suggesting that habitat function is not yet fully restored in this system (Smith et al., 2022; Moore et al., in review). Previous studies have shown that free-living communities quickly recruit to oyster reef habitats within 1-2 years post reef restoration (Humphries et al., 2011; Moore et al., 2020). However, free-living abundances on restored reefs do not match populations on older or natural reefs until approximately 6 years post reef restoration (Smith et al., 2022, Moore et al., in review). Monitoring for oyster reef restoration projects often stop at 2 years (Bayraktarov et al., 2016); however long-term monitoring (6-10 years) is needed before seeing complete oyster restoration success (Baggett et al., 2015; Smith et al., 2022). Due to low abundances across all sites, parasite prevalence in restored reefs may also increase after several years post reef restoration (Moore et al., in review). Similar studies in restored and natural coral reefs found that parasite differences were more apparent as time post restoration increased (Burt et al., 2011; Hill et al. 2021). As a result, through long-term monitoring, free-living and parasite community structure could very well increase as time post reef restoration increases (Smith et al., 2022, Moore et al., in review).

Reef fauna sampling for mobile species only exhibited a clear difference in crab abundance between the landward and seaward substrate placement at control sites, where average abundance was greatest on the seaward side of these sites. Structure within a given community is a driver of species diversity, and while control sites were without a restored reef, they were not without structure in the utilization of samplers filled with dried oyster shell (Gittman et al., 2016; Moore et al., 2020). Indeed, the passive samplers were essentially the only available structure within control plots for recruiting organisms. In contrast, at restored reef plots, both the high- and low-relief restored reefs had much greater structure, including a restored oyster reef, a landward placed sampler, and a seaward placed sampler. Thus, we may have underestimated our crab abundances at reef sites and overestimated abundances at our control sites.

For free-living target crab species, the common mud crab (*Panopeus herbstii*) remained the most abundant species throughout the study period, while the flat-back mud crab (*Eurypanopeus depressus*) was the least abundant. The common mud crab is often found amongst loose oyster shell at the base of oyster reefs (McDonald, 1977, 1982; Meyer 1994), which may explain why it was the most abundant species collected using our passive samplers. The flatback mud crab (*E. depressus*) is often found at elevated sections of restored reefs (Gregalis et al., 2009). As a result, our passive sampling technique might have attracted certain crab species over others in this location. In 2016-2019 surveys of the Pamlico and Neuse estuaries using the same passive sampling design, *E. depressus* was the second-most abundant crab species detected from moderate salinity sites sampled during the study (Blakeslee et al., 2021). Thus, it may also be that different abiotic (salinity, temperature) and biotic factors (competitors, predators, and habitat) at the coastline within estuaries may play a role in the species' lower abundance in our study compared to Blakeslee et al. (2021).

For free-living fish species, the crested blenny (*Parablennius laticlavius*) remained the most abundant species throughout the study period, followed by the feathered blenny (*Hypsoblennius hentz*), while the naked goby (*Gobiosoma bosc*) remained the least abundant. *G. bosc* is an abundant fish species often found in benthic habitats. However, *G. bosc* is also a habitat generalist, which could be why it is the least abundant fish species found across all sites (Moore et al., 2018). Additionally, the abiotic and biotic conditions along our study area differ from those of estuarine studies, and as a result, this could have contributed to the species' lower abundance. In lower to moderate salinities, *G. bosc* is the most common fish species. However, there is more diversity and likely more competition in the higher salinity sites that we investigated (Moore et al., 2018). In contrast, the *P. laticlavius* and the *H. hentz* exhibit strong site fidelity throughout their lives, and as a result, they remained the most abundant fish species (Harding et al., 2019; Moore et al., 2020).

For the free-living target crab host species, parasite prevalence did not differ in response to the landward/seaward reef placement. Free-living crab abundance was found to only be greater on the seaward side of the control plots. This suggests that habitat function is not yet fully restored in this system, where multiple years may be needed before abundances can match that of an older reef (Smith et al., 2022; Moore et al., in revision). Due to the low free-living crab abundances, parasite prevalence in restored reefs may be lagging even further behind the free-living diversity and may need more time to eventually catch up. *P. herbstii* had the highest infection prevalence, followed by *D. sayii*. By the end of the post-restoration period (15 months, October 2021), only 8% of target crab hosts were infected. This could be due to habitat disturbance due to restoration, where the young age of the habitat could play a vital role in the low parasite prevalence and host-parasite community structure (Anderson and Sukhdeo, 2013, Song and Proctor, 2020; Moore et al., 2020). As this habitat continues to age, hosts may continue to colonize the restored reefs and parasite prevalence and diversity are expected to increase (e.g., Moore et al., in review). For the free-living target fish host species, sixty-nine percent of hosts were infected with trematode cysts, where intensity was the greatest 12 months post restoration, July 2021. In response to season, trematode parasite intensity in fish host samples were greatest during the warmer months from September to November and June to August. This could be partially due to the small collection count of free-living fish during the winter and spring months, where the total number of fish to dissect was much smaller, and thus, infections decrease. Abiotic factors, such as warmer temperatures or increased salinities, can cause parasitic infections to peak during the warmer seasons in aquatic ecosystems (Upatham et al., 1984; Sawabe and Makiya, 1995, Poulin 2020). For example, in response to increasing water temperature, *H. trivolvis* snails were found to have higher trematode parasite infection rates (Paull & Johnson, 2014). However, our mixed model results for fish-host parasite intensity showed that temperature did not have a meaningful impact on intensity. This could be due to limited fish abundance data. While 42% of fish species collected were infected with trematode parasites, we only collected 145 fish across the entire study period. Lack of data could be due to our passive sampling technique and the fact that collection occurred during low tide. Future studies could include using minnow traps so that we are not limited in our sampling.

Moreover, parasite intensity increased as a response to the interaction between fish species and total fish length, where the oyster toadfish had the greatest average parasite intensities with the greatest total fish lengths. Larger juvenile or adult fish can eat larger amounts and ranges of prey items (Wilson et al., 1982; Bisker et al., 1989; Moore et al., in review). As a result, these larger oyster toadfish may be consuming a more diverse and increased amount of prey items infected with parasitic organisms (Moore et al., in review).

Community changes in recruiting sessile individuals

Overall biomass of sessile species was greatest on the seaward side of our restored oyster reefs. Longshore flow and depth current velocity is higher on the seaward side of reefs and reduced on the landward side of reefs (Chowdhurry et al., 2019). As a result, sessile species biomass may have been influenced by increased exposure to flow on the seaward side of the oyster reef, where species rely on transport of food by the water currents. Natural oyster reefs are oriented shore perpendicular whereas restored oyster reefs run shore parallel. Perpendicular reefs experience much greater flow compared to parallel reefs, where parallel reefs are built to provide coastal shoreline protection (Lenihan, 1999; Colden et al., 2016). As a result, there may be tradeoffs between shore stabilization and oyster reef, as well as sessile fauna, productivity.

During the month of July of 2021, sessile species biomass was the greatest on the landward side of both high-relief and low-relief restored oyster reefs (Figure 15). One possible reason could be due to the increased disturbance on the seaward side of the reefs during the summer season due to boat wakes. As a result, this increased disturbance may have led to dislodgement or death (Kimbro and Grosholz 2006). Another explanation could be a shift to an algal dominated environment during the summer months. For example, algae may reduce abundance of other sessile organisms by smothering them or interfering with feeding (Day 1983, Roth et al., 2020). As a result, the landward side may have experienced less disturbances and may have been the more desirable residence for sessile species during this time.

While no statistical analyses were constructed on taxa identification and composition, I did observe general trends between the landward and seaward side of the restored reefs. The landward side of restored reefs and control sites consisted of algae and tube worms, whereas the seaward side of restored reefs generally consisted of tunicates, bryozoan, mussels, barnacles, and oysters. Additionally, smaller sessile organisms were observed to fill smaller niches on the fouling plate while others mounted on top of other sessile organisms, creating three-dimensional structures on the plate. This three-dimensional structure provides greater structural complexity (Darling et al., 2017; Denis et al., 2017).

Conclusion

This field experiment provided new insight into the importance of long-term monitoring and time post-restoration as a predictor of free-living reef associated species abundance, as well as the relationship between substrate placement and reef relief. Our results indicate that 1 year post restoration may be insufficient to see free-living and parasite communities matching that of a natural reef. Compared to natural or older reefs, abundance was too low at our 1 year restored reefs to see if there was a clear relationship between substrate placement and reef relief across all sites. Additionally, free-living crab abundance only differed between the landward and seaward placement at control plots, indicating that structure may be the most important factor for recruitment of mobile individuals. In contrast to the free-living mobile fauna abundances, I did see greater sessile fauna abundances in response to placement, where recruiting individuals were greatest on the seaward side of our restored reefs. Future restoration projects should continue to consider time as a predictor of restoration success. Failure to consider long-term monitoring may lead to nonoptimal reef development and associated community assembly.

Mixed Model Table Results

Table 1: Mixed model results for the change in target crab abundance from October 2020 to October 2021

Fixed Effect	χ^2	DF	Prob > χ^2
Placement (landward, seaward)	12.8926	1	**0.0003412**
Reef-Relief (high, low, control)	6.0224	2	**0.0492337**
DO	26.1769	1	**< 0.0001**
Time Post Restoration (3-15 months)	20.7851	1	**< 0.0001**
Oyster Abundance	0.8490	1	0.3568316
Placement*Reef-Relief	11.3180	2	**0.0034860**
Time Post Restoration*Oyster Abundance	0.5431	1	0.4611477

Table 2: Mixed model results for the change in target fish abundance from October 2020 to October 2021

Fixed Effect	χ^2	DF	Prob > χ^2
Reef-Relief (high, low, control)	0.0937	2	0.954223
DO	35.0099	1	**< 0.0001**
Time Post Restoration (3-15 months)	8.4532	1	**0.003644**
Oyster Abundance	0.0181	1	0.892996
Time Post Restoration*Oyster Abundance	0.0589	1	0.808310

Table 3: Mixed model results for change in the total target crab abundance across all time post reef restoration

Fixed Effect	χ^2	DF	Prob > χ^2
Placement (landward, seaward)	11.0647	1	**0.0008799**
Reef-Relief (high, low, control)	5.2420	2	0.691823
DO	0.6150	1	0.4328937
Reef Height Change	0.5966	1	0.4398774
Placement*Reef-Relief	9.161	2	**0.0102085**

Table 4. Mixed model results for change in the total target fish abundance across all time post reef restoration

Fixed Effect	χ^2	DF	Prob > χ^2
Treatment	0.0045	1	0.9168
DO	2.4773	1	0.1155
Reef Height Change	0.0018	1	0.9662

Table 5: Mixed model results for analysis of parasite prevalence in target host crab species across all time post reef restoration

Fixed Effect	χ^2	DF	Prob > χ^2
Placement (landward, seaward)	0.6146	1	**0.4330**
Crab Species Type	28.9430	4	**< 0.0001**
Total Host Crabs Dissected	24.7488	1	**< 0.0001**
Crab Carapace Width	1.2620	1	**0.2613**
DO	0.9504	1	0.3296
Total Prevalence Across All Crab Species	47.4701	1	**< 0.0001**
Crab Species Type*Crab Carapace Width	6.4711	4	0.1666

Table 6: Mixed model results for analysis of parasite prevalence in target host fish species across all time post reef restoration

Fixed Effect	χ^2	DF	Prob > χ^2
Fish Species Type	9.2013	4	0.056261
Total Fish Length	0.1039	1	0.747251
Total Host Fish Dissected	9.6162	1	**0.001929**
DO	1.5628	1	0.211255
Total Prevalence Across All Fish Species	26.1494	1	**< 0.0001**
Fish Species Type*Total Fish Length	0.5233	4	0.970991

Table 7: Mixed model results for analysis of parasite intensity in infected target host fish species across all time post reef restoration

Fixed Effect	χ^2	DF	Prob > χ^2
Fish Species Type	14.1751	3	**0.002676**
Total Fish Length	0.1450	1	0.703387
Total Host Fish Dissected	0.8668	1	0.351846
DO	0.0190	1	0.890337
Fish Species Type*Total Fish Length	8.8729	3	**0.031029**

Table 8: Mixed model results for analysis of biomass of recruiting sessile individuals for February 2021, July 2021, and October 2021.

Fixed Effect	χ^2	DF	Prob > χ^2
Placement (landward, seaward)	10.1392	1	**0.001452**
Relief (high, low, control)	5.4458	2	0.065685
Salinity	1.5249	1	0.216875
Oyster Abundance	2.7163	1	0.099326
Date	27.1929	1	**< 0.0001**
Placement*Relief	5.0408	2	0.080439

Table 9: Mixed model results for analysis of percent cover of recruiting sessile individuals for February 2021, July 2021, and October 2021.

Fixed Effect	χ^2	DF	Prob > χ^2
Placement (landward, seaward)	20.299	1	**< 0.0001**
Relief (high, low, control)	0.8488	2	0.654172
Salinity	1.7388	1	0.187285
Date	9.2379	2	**0.009863**
Placement*Relief	5.2343	2	0.073012

Species Abundance Descriptive Table Results

Table 10: Total average target crab abundance as a response to placement (landward/seaward) and reef relief treatment over the entire study period

Placement	Mean ± SE
Landward/Control	52.5 ± 4.5
Seaward/Control	87.5 ± 14.37
Landward/High-Relief	65.5 ± 7.53
Seaward/High-Relief	73.25 ± 12.82
Landward/Low-Relief	77.25 ± 4.01
Seaward/Low-Relief	67.75 ± 6.14

Table 11: Total average target fish abundance as a response to reef-relief (high, low, control) over the entire study period

Reef Relief Treatment	Mean ± SE
High-Relief	16.5 ± 3.3
Low-Relief	16 ± 3.5
Control	16.5 ± 3.1

Table 12: Average target crab species abundance from October 2020 to October 2021

Crab Species	Mean ± SE
Common Mud Crab (*P. herbstii*)	49.08 ± 12.84
Black-fingered Mud Crab (*D. sayi*)	26.33 ± 11.83
White-fingered Mud Crab (*R. harisii)*	8.33 ± 4.46
Flatback Mud Crab (*E. depressus*)	0.25 ± 0.62
Stone Crab (*M. mercenaria*)	9.50 ± 4.56
Unknown Mud Crab	48.33 ± 11.74

Table 13: Average target crab species as a response to placement (landward/seaward) from October 2020 to October 2021

Crab Species	Landward [Mean ± SE]	Seaward [Mean ± SE]
Common Mud Crab (*P. herbstii*)	33.75 ± 3.96	15.33 ± 1.42
Black-fingered Mud Crab (*D. sayi*)	6.83 ± 1.15	20.17 ± 2.72
White-fingered Mud Crab (*R. harisii)*	3.08 ± 0.78	5.83 ± 0.89
Flatback Mud Crab (*E. depressus*)	0.08 ± 0.08	0.17 ± 0.11
Stone Crab (*M. mercenaria*)	2.25 ± 0.59	6.50 ± 1.08
Unknown Mud Crab	19.83 ± 1.37	28 ± 3.46

Target 14: Average target crab species as a response to reef-relief (high-relief, low-relief, control) from October 2020 to October 2021

Crab Species	High-Relief [Mean ± SE]	Low-Relief [Mean ± SE]	Control [Mean ± SE]
Crab Species	51.75 ± 6.70	56.50 ± 6.45	39 ± 3.19
Common Mud Crab (*P. herbstii*)	23.5 ± 5.24	26 ± 5.67	29.5 ± 7.92
Black-fingered Mud Crab (*D. sayi*)	9.5 ± 2.90	8.25 ± 0.85	7.25 ± 2.87
White-fingered Mud Crab (*R. harisii)*	0.25 ± 0.25	0.50 ± 0.50	0.00 ± 0.00
Flatback Mud Crab (*E. depressus*)	8.75 ± 2.25	6.25 ± 1.49	13.5 ± 1.66

Stone Crab (*M. mercenaria*)	47 ± 7.63	47.50 ± 3.30	50.5 ± 7.41

Table 15: Average target fish species abundance from October 2020 to October 2021

Fish Species	Mean ± SE
Seaboard Goby (*G. ginsburgi*)	3.50 ± 2.15
Naked Goby (*G. bosc*)	1.83 ± 1.64
Crested Blenny (*P. laticlavius*)	4.67 ± 2.61
Feathered Blenny (*H. hentz*)	3.75 ± 3.28
Oyster Toadfish (*O. tau*)	2.58 ± 1.56

Target 16: Average target fish species as a response to reef-relief (high-relief, low-relief, control) from October 2020 to October 2021

Fish Species	High-Relief [Mean ± SE]	Low-Relief [Mean ± SE]	Control [Mean ± SE]
Seaboard Goby (*G. ginsburgi*)	4.25 ± 1.03	3.75 ± 1.75	2.5 ± 1.19
Naked Goby (*G. bosc*)	1.5 ± 0.96	2.75 ± 0.48	1.25 ± 1.25
Crested Blenny (*P. laticlavius*)	3.5 ± 2.84	4.75 ± 3.20	5.75 ± 2.46
Feathered Blenny (*H. hentz*)	2.5 ± 1.66	5 ± 2.48	3.75 ± 2.78
Oyster Toadfish (*O. tau*)	2 ± 1.22	3.50 ± 1.26	2.25 ± 1.31

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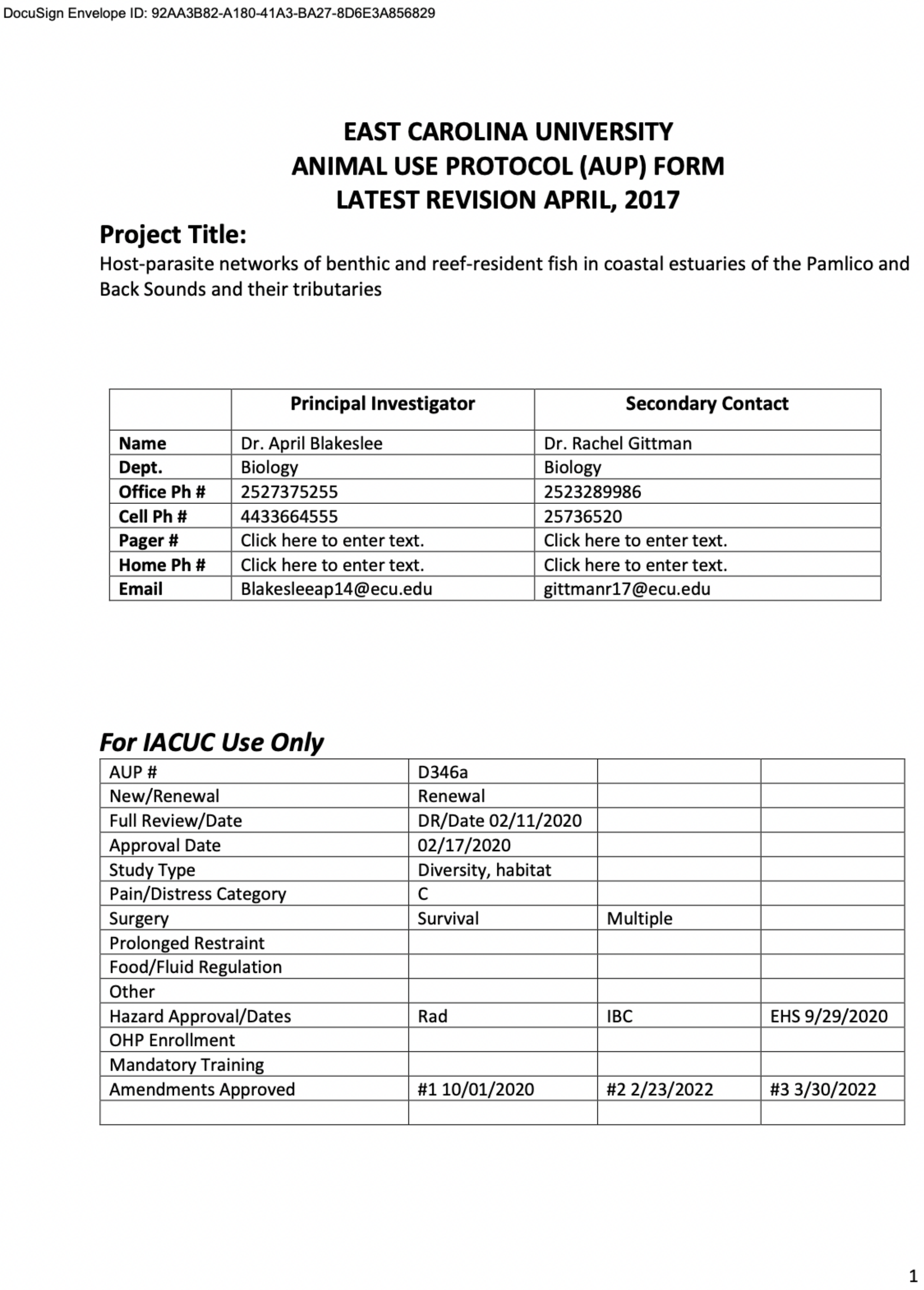
APPENDIX A: IACUC Approval Memo



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EAST CAROLINA UNIVERSITY
ANIMAL USE PROTOCOL (AUP) FORM
LATEST REVISION APRIL, 2017

Project Title:

Host-parasite networks of benthic and reef-resident fish in coastal estuaries of the Pamlico and Back Sounds and their tributaries

	Principal Investigator	**Secondary Contact**
Name	Dr. April Blakeslee	Dr. Rachel Gittman
Dept.	Biology	Biology
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Cell Ph #	4433664555	25736520
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AUP #	D346a		
New/Renewal	Renewal		
Full Review/Date	DR/Date 02/11/2020		
Approval Date	02/17/2020		
Study Type	Diversity, habitat		
Pain/Distress Category	C		
Surgery	Survival	Multiple	
Prolonged Restraint			
Food/Fluid Regulation			
Other			
Hazard Approval/Dates	Rad	IBC	EHS 9/29/2020
OHP Enrollment			
Mandatory Training			
Amendments Approved	#1 10/01/2020	#2 2/23/2022	#3 3/30/2022

I. Personnel

A. Principal Investigator(s):

Dr. April MH Blakeslee

B. Department(s):

Biology

C. List all personnel (PI's, co-investigators, technicians, students) that will be working with live animals and describe their qualifications and experience with these specific procedures. If people are to be trained, indicate by whom:

Name/Degree/Certification	Position/Role(s)/ Responsibilities in this Project	Required Online IACUC Training (Yes/No)	Relevant Animal Experience/Training (include species, procedures, number of years, etc.)
Dr. April MH Blakeslee, PhD	PI/field collections/laboratory care	Yes	20 years of experience sampling, handling, and experimenting with live marine organisms, including multiple invertebrate species and in the last 6 years nearshore fish.
Dr. Rachel Gittman, PhD	coPI/field collections/laboratory care	Yes	12 years of experience sampling, handling, and experimenting with live marine organisms, including multiple invertebrate species and nearshore fish
Christopher Moore, BS	Graduate student/co-investigator/field collections/laboratory care/dissections	Yes	Extensive personal and professional experience collecting and maintaining fishes; IACUC approved project over the past 3 years.
Megan Geesin, BS	Graduate student/co-investigator/field collections/laboratory care/dissections	Yes	Megan has field experience in estuarine ecosystems, including sampling marsh plants and multiple invertebrate species, such as oysters and crabs .
Stacy Trackenberg, BS	Graduate student/ field collections	Yes	5 years of experience sampling, handling, and experimenting with live marine organisms, including multiple invertebrate species and with nearshore fish
Georgette Tso, MS	Graduate student field collections	Yes	Georgette has field experience in estuarine ecosystems, including sampling marsh plants

			and multiple invertebrate species, such as oysters and crabs
Nina Woodard, BS	Graduate student / field collections/laboratory care/dissections	Yes	Nina has 2 years of field and lab experience in estuarine systems asa masters student .
Anna Albright, BS	Graduate student/ field collections	Yes	Anna has 2 years of experience of field and lab work in estuarine systems as a masters student.
Haley Hagemeier, BS	Graduate student/ field collections	Yes	Haley has been working in the Blakeslee lab since fall 2021. She has received field and lab training with Dr. Blakeslee over the past 4 months.
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