EFFECTS OF LANDSCAPE-SCALE OYSTER-REEF RESTORATION ON NEKTON COMMUNITIES IN A TEMPERATE ESTUARY

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ABSTRACT

Owen Mulvey-McFerron: Effects of landscape-scale oyster-reef restoration on nekton communities in a temperate estuary
(Under the direction of F. Joel Fodrie)

Restoration of degraded biogenic habitats is a common practice to recover lost biodiversity and ecosystem services. Oyster reefs are globally imperiled essential fish habitat, motivating interest in novel restoration approaches. Therefore, I examined fish community response to construction of six 180-oyster-patch-reef complexes in a section of a North Carolina estuary with no extant oyster reefs. I assessed nekton communities at reef and control sites with nets, traps and dual frequency identification sonar (DIDSON). Occurrences of nekton were higher at reef sites than controls across gear types. DIDSON footage revealed >300% more nekton, while catch data showed only ~5% increase at reefs over controls. Several species including pinfish, silver perch, blue crab and mullet showed greatest difference at reefs highlighting that restoration benefits vary across species. Restoring oyster reefs with a landscape-scale patch reef approach is an effective way to maximize the ecosystem services available to a wide variety of species.

To the oysters and fish of the New River. Godspeed, friends.

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LIST OF SYMBOLS AND ABBREVIATIONS

α	alpha level significance
CPUE	catch per unit effort
DIDSON	Dual Ferequency Identification Sonar
ie	for example
MHz	megahertz
m	meter
mm	millimeter
n	sample size
nMDS	non-metric multidimensional scaling
%	percent
“	inch
>	greater than
<	less than
+/-	plus or minus

Introduction

All animals share similar basic necessities for survival and reproduction, and among these is the unavoidable need for sufficient habitat (Tilman et al 1994). Habitat includes not only the physical space and structure of an environment, but the resources provided to the animal (Hayes et al 1996). As development and anthropogenic climate change, a hallmark of the Anthropocene era, removes and alters available habitats, population declines and extinctions have been observed across myriad taxa in terrestrial, freshwater, and marine environments (Pimm et al 2014). Habitat loss in coastal marine systems has been particularly apparent as this suite of threats cause declines in habitat quality and area (Sunday et al 2016). Biogenic habitats, including marshes, coral reefs, mangroves, and oyster reefs are important to a wide variety of species, due in part to their structural complexity and ability to provide a wide variety of ecosystem services (Sunday et al 2016). These biogenic habitats in particular have proven particularly vulnerable, with considerable global losses surpassing 85% (Beck et al 2011).

One increasingly common tool for mitigating habitat loss is habitat restoration. Early restoration efforts focused primarily on restoring lost structures, however as our understanding of the role of habitat in biodiversity maintenance has progressed, restoration has become more focused on restoring lost ecosystem functions. Habitat extends beyond the physical space an organism inhabits to the resources that space provides for survival (Hayes et al 1996), so this refocusing of restoration goals has largely proven beneficial by shifting the focus to restoring lost ecosystem services. Restoration can span reconstructing lost structural habitat components

(termed “Type A ecoengineering”), to reintroducing living components of the habitat, such as live oysters or mangrove seedlings (termed “Type B ecoengineering”) (Gilby et al 2017). Frequently, a combination of the two (initial construction followed by natural recruitment) is utilized in marine ecosystems. Once new habitat is created, colonization through the arrival of mobile species (across a variety of life stages), as well as larval dispersal of sessile organisms typically occurs. After initial colonization, there is some debate over whether or not biomass on reefs represents truly new production as a result of the new habitat, or aggregation of existing biomass from surrounding areas; in either case, both processes have significant ecological implications (Bohnsack 1989).

Nekton communities have shown mixed responses to habitat restoration, particularly in the case of estuarine oyster reefs. In systems where oyster reefs currently exist and bare substrate is converted into new oyster reefs, substantial increase of nekton at reef sites over bare substrate has been noted (George et al 2015, Humphries et al 2015). Additionally, the location of an oyster reef within the seascape can have impacts upon that reef’s utility to species. Connectivity to other habitats (ie: seagrass, marsh edge) can increase utilization, as these habitats can provide a wider variety of resources useful to nekton throughout time and space (Grabowski et al 2005; Ziegler et al. 2018). Substantial spatial overlap or very high connectivity, however, can lead to functional redundancy, where multiple habitats provide the same services to organisms thus reducing the utilization of the restored habitat (Grabowski et al 2005, Geraldi et al 2009). While habitat position and size are important, substrate type seems to be less so (George et al 2015). Simply replacing the lost structural complexity in an ecosystem is often enough of a stimulus to promote a positive response from species impacted by prior habitat loss.

We constructed a series of six subtidal oyster reefs in the New River Estuary, North Carolina as our study region for this project. The upper portion of the New River Estuary is currently devoid of oysters, and historical studies as early as 1886 did not record oysters outside of the strongly-tidal lower estuary and Intercoastal Waterway (Winslow 1886) (Figure 1). Additionally, the New River has a long history of water quality concerns, including the distinction of having some of the highest levels of eutrophication in the region in the late 1900's (Peierls et al 2012). Toxic, bloom inducing algae still persists in the estuary today, although significant improvements with water treatment has significantly reduced phytoplankton biomass and nutrient loads (Tomas et al 2007, Mallin et al 2005). Improvements in water quality in the New River have positively impacted fishing, recreation, and military training exercises over the recent two decades. The construction of new wastewater treatment plants and updated pollution regulations have restored habitat conditions to a suitable level for oysters, with the primary factor limiting oyster resurgence being the lack of larval supply and hard settling substrate. Restoring oysters to suitable habitat in the middle portion of the estuary can accomplish many of the goals associated with the ecosystem services provided by oysters including further improving water quality (Peterson et al 2003), improved larval connectivity due to higher larvae load, and increased available fish habitat. Many recreationally and commercially important species have a well-documented affinity with oyster reefs, thus constructing oyster reefs in suitable environments could have profound impacts on fish assemblages such as pinfish, red drum, flounder, and others (Coen 1999, Lenihan et al 2001, Peterson et al 2003, Ziegler et al 2018).

Given the mobile nature of many estuarine nekton species, single site restoration may be too small of a scale to effectively provide broader habitat benefits (ie: all of the foraging needs of a predatory fish). Larger species (red drum, black drum, and southern flounder) have

demonstrated no particular fidelity to restored cultch reefs in the lower New River, with some species preferring a large field of patch reefs (Kenworthy 2019). In fact, most oyster reef restoration studies looking at fish enhancement have used a single, relatively small reef (ie: Ziegler et al 2018, Rutledge et al 2018, Geraldi et al 2009, Grabowski et al 2005). Considering that many oyster reef restoration efforts are done in this manner, evaluating restoration benefits on a reef-by-reef basis is appropriate. With the recognized link between fisheries enhancement and oyster reefs (Hernandez et al 2018), it may be necessary to adjust the scale and design of oyster reef restoration, moving toward a field of patch reefs to better address the habitat needs of mobile nekton. This approach was used in the New River, and while the project had a variety of restoration goals, the potential for unique nekton responses warranted examination.

I had three primary goals of this study centered around the nekton community I expected to establish itself on the restored oyster-reef complexes. First, I wanted to describe a New River oyster reef nekton community, as this is a novel community type in this portion of the estuary. Second, I wanted to quantify how the modification of the existing estuary habitat changed the nekton community. Finally I qualitatively compared the use of traditional sampling gears and active acoustic technology to explore the utility of each method in structurally complex habitats.

Methods

Reef site selection and construction

Six 70m by 30m reef-complex sites were selected along the east and west banks of Farnell Bay (Figure 1). At each site, 180 patch reefs were constructed, 90 patch reefs were made using Allied Concrete Company Oyster Castles, and the other 90 were constructed with Sandbar Oyster Company Oyster Catchers (Figure 2). Reef complexes were constructed over the course of two weeks in April, 2019. Each reef had four quadrants (inshore Oyster Catchers, offshore Oyster Catchers, inshore Oyster Castles, offshore Oyster Castles) separated by two 8m corridors, one oriented perpendicular to shore and one oriented parallel to shore. Within each quadrant, patch reefs were organized into three rows parallel to shore (Figure 2). The 15 patch reefs in each row were 2m apart, and each row was 4m apart (Figure 2). Oyster Castle patch reefs were arranged in a 2x4 block rectangle, with two blocks on top to secure each patch reef for a total dimension of 36”x24”x16” (Figure 2). Oyster Catchers were set out on racks in nearby estuaries (Lower New River, Newport River, and Back Sound, NC) for several months to collect natural oyster spat prior to relocation to the New River. Once relocated, eight Oyster Catchers were arranged in a 2x2x2 Oyster Catcher rectangle and wired together, with a bamboo pole sunk into the sediment in the middle of the rectangle. Two Oyster Catchers were placed over the bamboo pole and wired in place (patch reef dimensions: 24”x24”x16”).

Each reef complex was constructed at subtidal locations with mean depth between 0.3-1.2m. Prior to construction, bottom composition was bare sandy-mud substrate, with no submerged aquatic vegetation or substantial depth contours in the study sites. Control sites were selected 300-400m away from reef sites to reduce crossover of fish during the study period. All

sites had similar depths, bottom types, distances from shore, and fetch to make them reasonably comparable.

DIDSON sampling

Once per month from May to October 2019, a Dual Frequency Identification Sonar (DIDSON) was used to non-invasively sample the reef sites. The DIDSON was bolted to an aluminum stand which was placed in the water at eight positions (two per quadrant) at each reef and control site, and one video was taken at each position (Figure 3). The DIDSON frequency was set to 1.8 MHz, 10 frames per second with a viewing range of 2.08 m to 7.08 m (5m depth of field). Four minute recordings were made for four videos per reef site. The depth of field was reduced to 2.5m to increase fine-scale resolution of the patch reefs in the other four recordings (one minute each), repeated at all reef and control sites (Figure 3). At reef sites, at least two patch reefs were positioned in frame for each four minute video, and one patch reef was in frame for each one minute video. Temperature and salinity were collected at each site prior to conducting sampling.

During analysis, the first minute of the four minute recordings was dropped to account for fish disturbance caused by setting the DIDSON stand near the patch reefs. No time was removed from the one minute videos prior to analysis since the DIDSON was already in place and required only minor adjustment. The software environment R was used to randomly select ten frames per minute of all videos for analysis. The selected frames were spaced throughout the video in an effort to reduce double counting fish. For the month of August, only five frames/minute of video were sampled. I conducted a maximum average abundance test and determined that when the CPUE (fish/ten frames) was compared across a variety of sampling rates (3, 5, 7, 10 frames/ minute), there was no increase in CPUE after 5 frames/minute meaning

that all fish had been sampled in the video. This was done by re-analyzing a representative set of DIDSON videos with the various frame sampling rates and comparing the mean CPUEs and standard errors.

The fish measurement tool in the DIDSON software package (Didson Version V5.26.06) was used to count measure each fish in the selected frames. In each selected frame, fish were identified and measured by hand using the 'fish measurement' tool. I would toggle between the selected frame and up to ten frames before and after to ensure that all fish in the selected frame were detected and measured accurately. Once all videos were analyzed, the average number of fish/ten frames (or fish/second) was calculated and used as the CPUE for a specific reef site. Standard error was calculated between the CPUEs of each video within each reef site. Similar methods (MeanCount) have been found in video studies to effectively estimate true abundance at reef sites, and due to the similarities between traditional video and DIDSON technology, mean CPUE was the best way to approach abundance estimations (Schobernd et al 2013) Since the sampling effort and subsequent analysis was maintained across all videos, the one and four minute videos were pooled at all sites.

Net and trap sampling

At each site, two 15m gill nets, two commercial grade crab pots (24"x24"x18", 1-1/2" hexagonal mesh, three 2-5/16"cull rings), and four Gee-style minnow traps (1/4" mesh, 1-1/2" opening) were set two hours before sundown and collected two hours after sundown. I followed the gillnet protocol that the North Carolina Division of Marine Fisheries utilizes on the New River. A four hour, evening soak time reduced marine mammal and sea turtle encounters and minimized blue crab predation on fish caught in the gill nets. One gill net at each site had 2.5-inch bar mesh/5-inch stretch mesh (5-inch net), and the other gill net (experimental net) was

constructed of three five-meter panels of 0.5,0.75, and 1-inch bar mesh. These different mesh sizes each selected for a different size class of fish (Hickford and Schiel 2008), so by varying our mesh sizes we were able to sample across many of the size classes of fish in the New River. The 5-inch net was deployed perpendicular to shore between the Oyster Catchers and Castles, while the experimental net was run parallel to shore between the inshore and offshore quadrant, alternating between the Oyster Catcher and Oyster Castle sides of the reefs each sampling period (Figure 3). This arrangement allowed us to sample as close to the structure as possible, where the target nekton are present, without fouling the nets in the patch reefs. The Gee minnow traps were baited with 5-6 pieces of dog food and deployed next to a patch reef in each quadrant for a total of four traps per site, and crab pots were baited with 1-2 dead mullet, Atlantic menhaden, or pinfish; one trap was placed next to an Oyster Castle patch reef, and the other was placed next to an Oyster Catcher reef. The bait differentiated the traps and crab pots from reef structures by providing an additional attractant for nekton, making them susceptible to capture and sampling. Gear were deployed at control sites in the same arrangement as at the respectively paired reef site.

Each fish captured was identified to species level, standard length was measured in mm, and the total weight in grams of each species (in aggregate) in each gear type was measured. Carapace width was used in place of standard length on crabs, and shrimp were measured from the tip of the tail to tip of the rostrum.

Statistical analysis

All analysis was conducted using a pairwise approach. Individual reef sites were compared directly to their associated control, or all reefs were pooled and compared to all pooled control sites.

Catch per unit effort (CPUE) was calculated as mean number of nekton per site when comparing all reefs to all controls, or a mean number of nekton per sampling event when comparing individual reefs to their associated control sites. Paired T-tests were used to determine the statistical clarity of any differences in these comparisons (α=0.05). I calculated standard error within each tested group to quantify the variance in CPUE across sites or sampling events. I calculated mean number of nekton per site for each sampling event at reef and control sites and constructed a seasonal catch curve. Standard error was calculated for each reef and control value, and a paired T-test (α=0.05) was used to determine the statistical difference between reef and control values across the sampling period (July-October). The ten most abundant species, based on gear captures, were compared between reefs and controls, with standard error calculated for each treatment type, and a paired T-test (α=0.05) run between each reef/control for each species. Size distributions for both DIDSON data and gear sampling data were calculated using 100mm bins at all reef and control sites. I calculated mean standard error for each bin-treatment permutation, and a paired T-test (α=0.05) between reef and control values for each bin. Species richness and Shannon diversity between all reef and control sites were calculated with standard error in R, and pairwise T-tests were used to compare the richness and diversity values at each (α=0.05).

I compared the nekton communities at reef and control sites using permanova analysis with the vegan package in R to quantify the treatments effects of reefs at all sites. Once the treatment and residual R^2 had been calculated, I used a Bray-Curtis dissimilarity matrix and plotted the data using an nMDS plot with 95% confidence intervals. I tested the stress of the ordination to ensure that it was optimized for the analysis.

Results

DIDSON Sampling

A total of 6,103 individuals were sampled, with 4,858 coming from reef sites and 1,245 coming from control sites. We saw an extremely statistically clear ($p<<0.001$) 490% increase in the overall CPUE across all reef sites as compared to control sites (Figure 4).

There were variable increases in CPUE across sites ranging from 235% (Site 4, $p=0.003$) to 933% (Site 6, $p=0.004$). The remaining four reefs (1, 2, 3 and 5) fell between reefs 4 and 6, with increases in catch of 244% ($p=0.002$), 655% ($p<0.001$), 310% ($p<0.001$), and 453% ($p<0.001$), respectively.

Regarding the size structure of the fish sampled with the DIDSON, the mean length of fish at control sites was 10.7% larger than at reef sites (control mean=122mm, reef mean=110mm, $p=0.035$). The mean length was higher at all control sites than reef sites

The preponderance (>98%) of all observed fish were <300mm, and of those fish, 79.6% occurred on reefs (Figure 5). Of fish >300mm, about half (43%) were observed at reefs, while a slightly larger proportion (57%) were observed at the controls (Figure 5).

Net and Trap Sampling

A total of 3,751 individuals were collected across 42 taxa. 1823 individuals across 32 taxa came from control sites, and 1,928 individuals across 36 taxa were collected at reef sites.

Site 2 and Site 5 had statistically higher catches at the reefs of 31.9% ($p=0.012$) and 19.1% ($p=0.044$), respectively. The remaining four sites were not clearly different between reefs and controls, however the means at Reefs 4 and 6 were higher than at controls (Reef 4 +5.5%,

p=0.700, Reef 6 +14.7%, p=0.106). Mean catches at controls were higher than reefs at Sites 1 (-4.1%, p=0.660) and 3 (-235%, p=0.133).

Overall abundance over time between reef and control sites showed a seasonal curve (minimums early and late in the study period, with elevated abundances from late July to late September), but there were no clear differences between reef and control sites (18 July p=0.273, 30 July p=0.189, 14 August p=0.606, 27 August p=0.304, 10 September p=0.177, 26 September p=0.779, 16 October p=0.225) (Figure 7). When the ten most abundant species (threadfin herring *Opisthonema oglinum*, Atlantic menhaden *Brevoortia tyrannus*, pinfish *Lagodon rhombiodes*, blue crab *Callinectes sapidus*, silver perch *Baridella chrysura*, spot *Leiostomus xanthurus*, mullet *Mugil spp.*, penaeid shrimp *Farfantepenaeus spp./Litopenaeus spp.*, pigfish *Orthopristus chrysoptera*, Atlantic croaker *Micropogon undulates*) were individually examined, species-specific responses to oyster reefs did emerge from the overlying seasonality (Figure 8). Mean catches were higher at reef sites across the study period for pinfish (+82%, p=0.003), silver perch (+427%, p=0.012), blue crabs (+42%, p= 0.044), mullet (+137%, p=0.122) and pigfish (+61%, p=0.052). Atlantic menhaden (-45%, p= 0.069), penaeid shrimp (-54%, p= 0.007), and Atlantic croaker (-63%, p= 0.027) occurred in higher abundance at the control sites.

The remaining species showed some oscillation during the sampling period, with threadfin herring displaying an early season affinity for the control sites, but later occurring in higher abundances on reefs resulting on only an overall increase of 7.2% (p=0.197). Mullet abundances on reef sites started nearly 90% higher than at controls, however a decrease on reefs and an increase at control sites over time resulted in a convergence of the two in late August that remained until the end of the sampling period, although overall abundance was higher at reef sites (+137%, p=0.012) (Figure 8). Atlantic croaker displayed the opposite trend, with initial

control abundances being higher than at reef sites, with a slightly later convergence (September through October). Spot appeared to display no temporal discrepancies between reef and control sites, however there was a clear seasonal peak in late August (Figure 8).

Recreationally important species (recreationally important for the purposes of this study is defined as a fish that has a set size or bag limit in the state of North Carolina: red drum *Sciaenops ocellata*, black drum *Pogonias cromis,* bluefish *Pomatomus saltatrix,* cobia *Rachycentron canadum,* Florida pompano *Trachinotus carolinus,* sheepshead *Archosargus probatocephalus*, southern flounder *Paralichthys lethostigma*, spadefish *Chaetodipterus faber*, Spanish mackerel *Scomberomorus maculatus*, speckled trout *Cynoscion nebulosus*) were seen in an overall low abundance. Of the total number of individuals sampled, 35.6% (n=32) were sampled at reef sites, and the remaining 64.4% (n=58) came from controls.

The size distribution of nekton sampled using gill nets, crab pots and minnow traps was predominantly composed of individuals <200 mm standard length (95.6%), with an even split between reef (48.8%) and control (51.2%) (Figure 9). Of the 4.4% of sampled fish > 200mm, 44.5% (73 fish) occurred on control sites and 55.4% (91 fish) occurred on reefs (Figure 9). There were no statistical differences between reef and control sites.

There was no clear difference in the species richness index (p=0.591) or Shannon diversity index (p=0.940) between reef and control sites (Figure 10). The aggregate fish communities at the reefs and control sites were not impacted by treatment (reef vs. control) effects (Figure 11). The residuals alone explain 96.7% of the variance, while the treatment effects only accounted for 3.3% (at p=0.01). There was also no clear difference between the individual reef communities and their respective controls, with no treatment effect accounting for

more than 10% of the community variance (Site 1 R^2=0.063, Site 2 R^2=0.080, Site 3 R^2=0.099, Site 4 R^2=0.076, Site 5 R^2=0.067, Site 6 R^2=0.046).

Discussion

We noted higher catch rates across all reef sites with all gear types, although the magnitude by which catch rates were enhanced varied by gear type. Overall catch rates were 5.4% higher at reef sites based on gill nets, minnow traps and crab pot catch data, but based on the DIDSON footage, reefs had 3.9 times as many fish as controls (Figures 4 and 6). There were no substantial species community differences between reef and control sites over the study period with any of the gears (Figure 10).

The stark disparity between gear and DIDSON catch rates is driven by differences in the manner in which each gear type samples. We conducted net and trap sampling during the day prior to the study period and had negligible catches, while DIDSON sampling during this time period returned high CPUE. This suggests diel homogeneity in fish abundance, demonstrating that while present at reefs and controls, the fish were not interacting with the nets and traps. Since these gear require fish to strike or enter them to be sampled, periods of high mobility and feeding are required to collect nekton, justifying the evening crepuscular deployment (when fish were moving between reef patches or across the control sites). Additionally, increased visibility during the day likely lead to gear avoidance behavior, particularly with the unbaited gill nets. As catchability of reef affiliated species in nets and traps is inversely related to distance from reef patches, the DIDSON has a distinct advantage in its ability to sample in and on highly structured environments without physically disturbing the reef substrate.

Additionally, soak times of nets and traps play an important role in catchability (Olin et al 2004). It was not uncommon to find the gill nets saturated (typically with Atlantic menhaden and threadfin herring) to the point of being unable to collect additional fish, or having a substantially reduced catch rate. The four hour soak times minimized the period during which the gill nets

were fishing with sub-maximal catch rates, thus informing a more accurate CPUE measure. The DIDSON does not reach saturation, nor does the acoustic beam cause avoidance behaviors in fish, making it an ideal sampling methodology in areas with high fish density or studies when long soak times are necessary.

Gear selectivity and bias are very well documented in ecological studies (Hamley 1975, Hansson and Rudstam 1995, etc.), and this study brings this issue into focus. One challenge that arises from sampling in a complex habitat is the restriction of appropriate sampling methods. Often this occurs because particular methods simply do not work (ie: trawling, seine nets), while in other cases certain practices do not return data in either the quantity or the quality necessary for this study. For instance, in highly complex habitats, hook and line surveys may only be utilized in a small portion of the study area or experience low catch rates due to gear fouling, while in environments where water clarity is very low, visual/video surveys will not observe and record large portions of the present nekton community. For these reasons, the combination of DIDSON and nets and traps allowed us to most effectively sample in and around the oyster reefs.

Aside from differences in catch rates, the qualitative similarities between the size structure data from the net/trap and DIDSON footage indicate that acoustic sampling may provide an alternative to nets and traps for sampling structurally complex habitats. Both gears have inherent size selectivity (DIDSON has a lower limit of detection contingent on the image resolution, nets and traps have upper and lower limit mesh size selection) but it appears that the combination of gears used in this study select for the same sizes of fish. Quantitative comparison in a controlled system would be necessary to determine the precise degree of similarity, but the catalogue of environments in which application of acoustic video imaging augments or replaces nets and traps is growing, with promising opportunities in highly structured shallow water

habitats. Although the overall community did not appear changed by the presence of oyster reefs, there were several species with enhanced catch rates at reef sites. Among these were pinfish, silver perch, blue crabs, and pigfish- all species that flourish in structurally complex habitats (Shervette and Gelwick 2007, Harding and Mann 2001). Habitat value is ultimately derived from the context of that habitat in the larger seascape (Nagelkerken et al 2015), so due to the lack of any other structurally complex habitats in the portion of the estuary where the reefs were constructed, these species found these reefs to be more ecologically useful in some form. These species have similar diets, small crustaceans, infaunal invertebrates, and epiphytic organisms, which are known to flourish on oyster reefs suggesting that food availability was a one reason for their abundance on these reefs (Peterson et al 2003). While found across many estuarine habitats, studies have shown that these fish favor complexity over simplicity with regards to habitat types, which can result in higher growth rates and abundances on reefs (Harding and Mann 2001).

Threadfin herring, Atlantic menhaden, penaeid shrimp and Atlantic croaker appeared to show no clear affinity towards oyster reefs. These species are habitat generalists that utilize a wide variety of habitats, often including oyster reefs during certain portions of their life history (Kingsley-Smith et al 2012). Penaeid shrimp in particular are known to utilize vegetated habitats preferentially in estuaries so their absence from the reef sites is not unexpected (Minello 1991). Threadfin herring, Atlantic menhaden and Atlantic croaker are not reef obligate species, so restoring a variety structurally complex estuarine habitats might better meet the resource and habitat needs of these species.

Given the known affinity of a number of recreationally important species for oyster reef habitat, it was somewhat unexpected that our overall sampling collected such a low number of

these species. Studies in similar systems employing different sampling protocols have noted low abundances of these larger predators and have cited the social behaviors of these species as a reason for their low capture rates (Kingsley-Smith et al 2012,). While the solitary or small-schooling behaviors of these species explain the low abundance, their feeding behaviors may also influence their occurrence on or off reef. Bluefish, speckled trout and Spanish mackerel all occurred in higher abundances at control sites- these species are pelagic predators and predominantly piscivorous. Structurally complex habitats provide refugia for juveniles and low trophic level prey species, which may make them less productive feeding areas for mobile predators, resulting in lower abundance at reef sites during peak feeding periods (typically crepuscular periods, during which our sampling took place).

While still uncommon in this study, all other recreationally important species were more abundant at reef sites (red drum, black drum, cobia, Florida pompano, sheepshead, southern flounder, and spadefish). These species span a variety of life histories and feeding strategies, but one commonality is a large diet component associated with benthic or demersal prey types (often invertebrates) which are frequently abundant on oyster reefs (Llanso et al 1998, Hayes 1990, Armitage and Alevizon 1980, Miller and Dunn 1980). This abundance of forage may explain the presence of these species Over time, recreational fisheries catch enhancement may develop as one of the many services provided by these reefs. As oysters grow, habitat complexity, and subsequently the scale of the services provided to reef fauna increase (Gilby et al 2017).

Colonization through mobile individuals relocating from off-reef habitats to the reefs by several species was nearly instant. While under construction, pinfish and pigfish were observed in and around new reef patches on multiple occasions. Due to the almost complete lack of structurally complex habitats in Farnell Bay, this rapid colonization by reef affiliates suggests that there may

have been habitat limitation occurring. Studies have shown that pinfish growth rates can be five times higher in oyster reefs than on bare substrate, so there is a strong likelihood that after this initial aggregation, pinfish production was enhanced through increased growth at reef sites (Shervette and Gelwick 2006).

Future study determining how these reefs are impacting fish in the New River would require an understanding of individual or species specific residency patterns on the reefs. Conducting acoustic telemetry studies of dominant community species using on and off reef receiver arrays would enhance understanding of temporal use patterns across a variety of scales. This would also help inform understanding of how these reefs are impacting species specific survival rates. Gut content and stable isotope analysis of reef predators would explore how much biomass is actually being consumed and whether or not significant predation is occurring on reefs. This is essential for quantifying the production vs. aggregation of these reefs, which in turn would inform future restoration efforts in similar systems.

The community and species impacts of oyster restoration observed in this study may prove useful in targeting restoration work, while the comparison between net and trap sampling and DIDSON sampling has implications for how restoration projects are studied. Low impact sampling that is effective in quantifying nekton abundance in structured habitats or environments with sensitive species may permit research in areas historically precluded from such study. As restoration ecology continues its expansion as an essential science, advances in sampling methods and technology will provide more opportunities to understand the environments being restored.

Figures

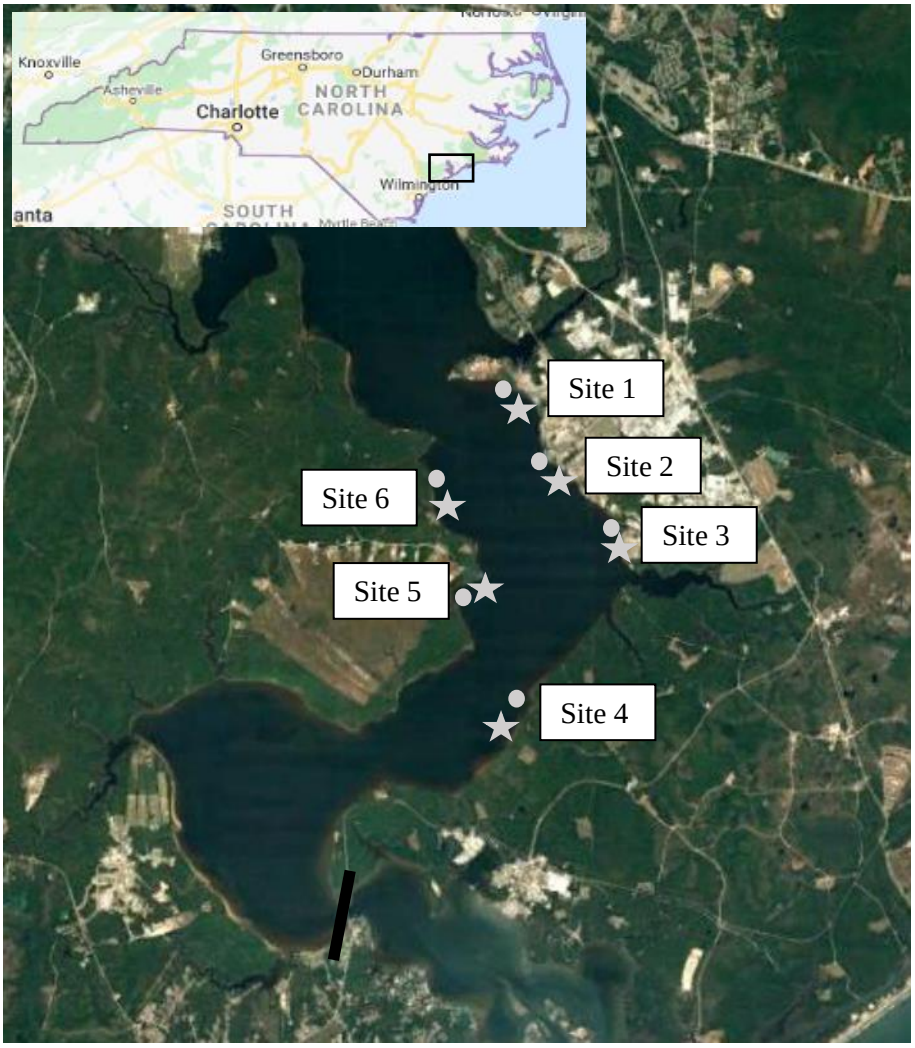

Figure 1: New River Estuary, North Carolina. Reefs and control sites are located in the Farnell Bay portion of the estuary. Reefs are indicated by stars, and controls are indicated with circles. Black line indicates upriver extent of natural oyster occurrence noted by Winslow, 1886.

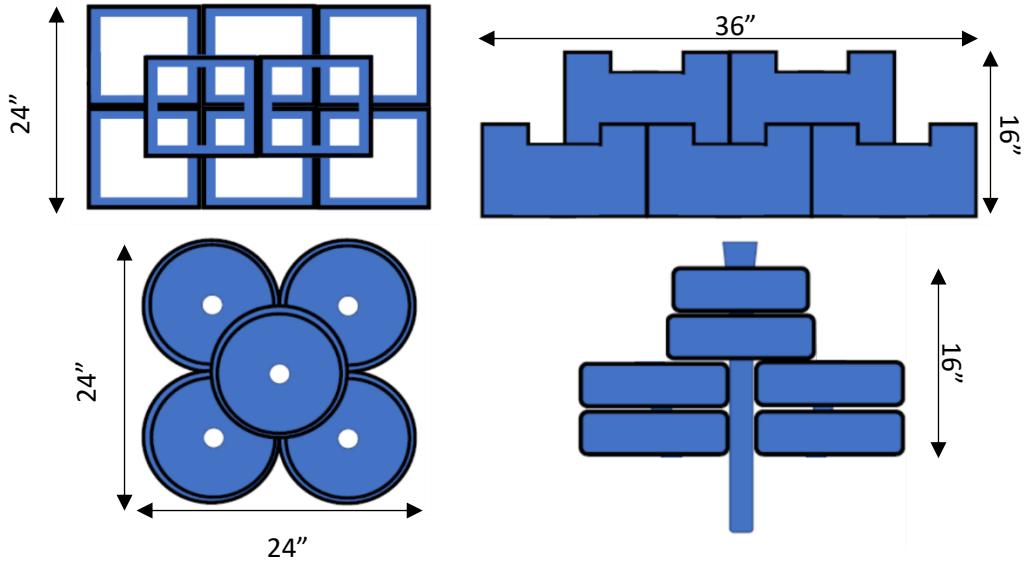

Figure 2: Oyster Catcher and Oyster Castle patch reef schematics. 90 bare Oyster Castle patch reefs and 90 seeded Oyster Catcher patch reefs were deployed at each site.

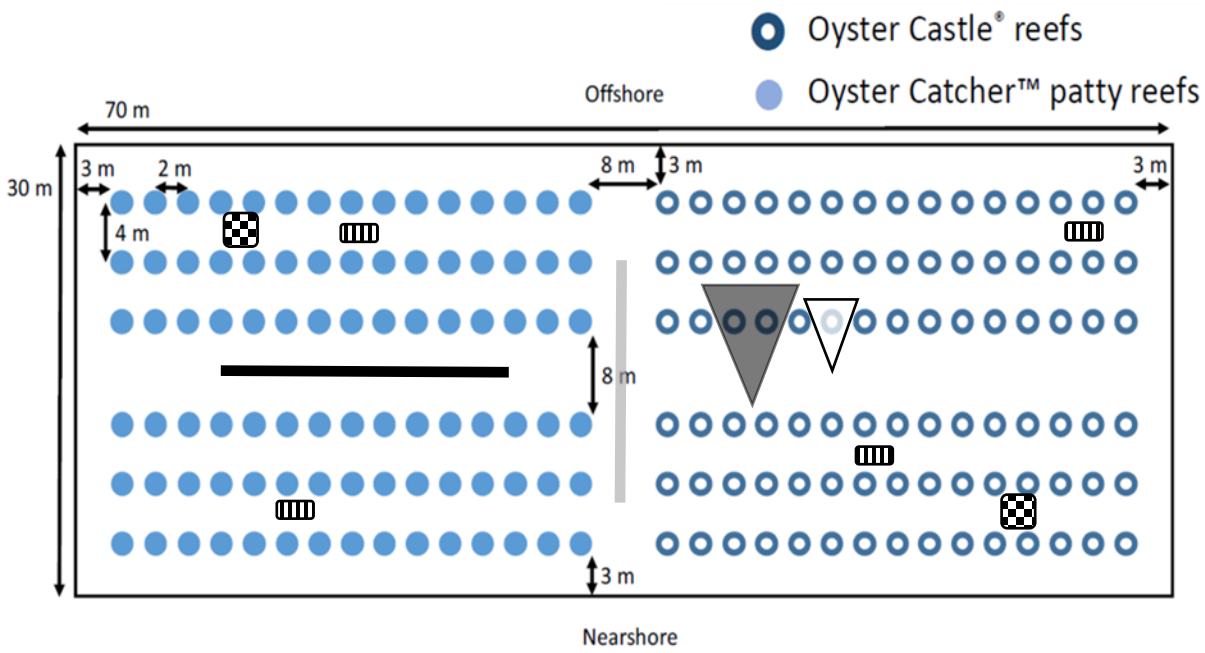

Figure 3: Reef schematic with sampling gear. One 15m experimental net (black line, alternated between Oyster Castle and Oyster Catcher side of reef each sampling event), one 15m 5” mesh gill net (gray line), four minnow traps (vertical bars), and two crab pots (checkered squares) were deployed twice monthly. Two DIDSON videos were recorded in each reef quadrant, one with a 5m field depth (gray cone), and one with a 2.5m field depth (white cone). This sampling design was replicated at control sites.

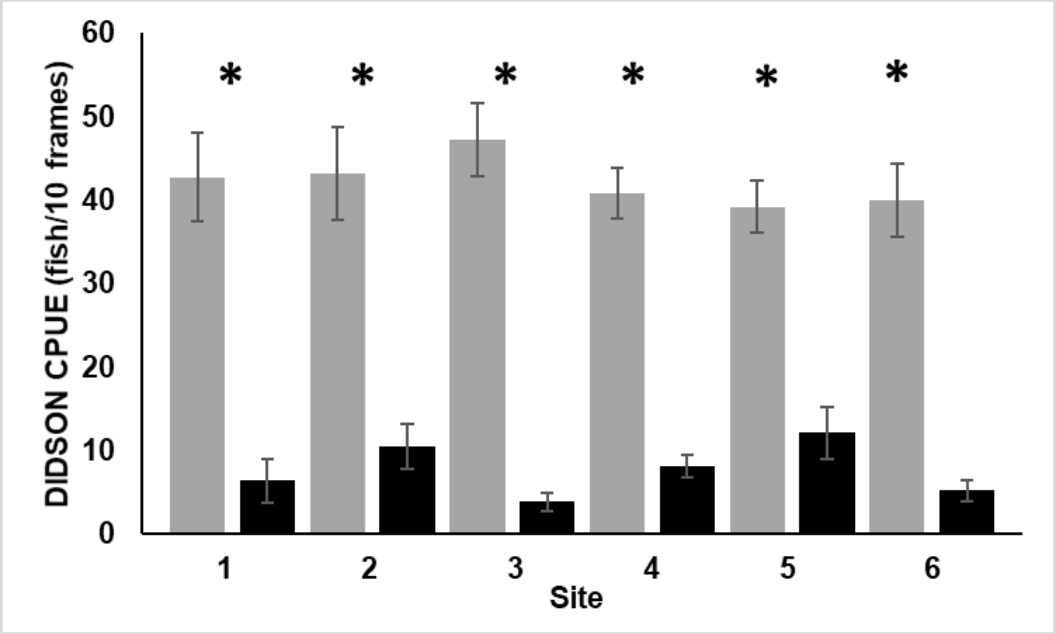

Figure 4: CPUE of fish at reef and control sites observed during July and August DIDSON sampling events. Gray indicates reefs and black indicates control sites. Error bars display +/- standard error within each site. An asterisk represents a statistical difference between reef and control CPUEs ($p<0.05$) between paired reefs and controls.

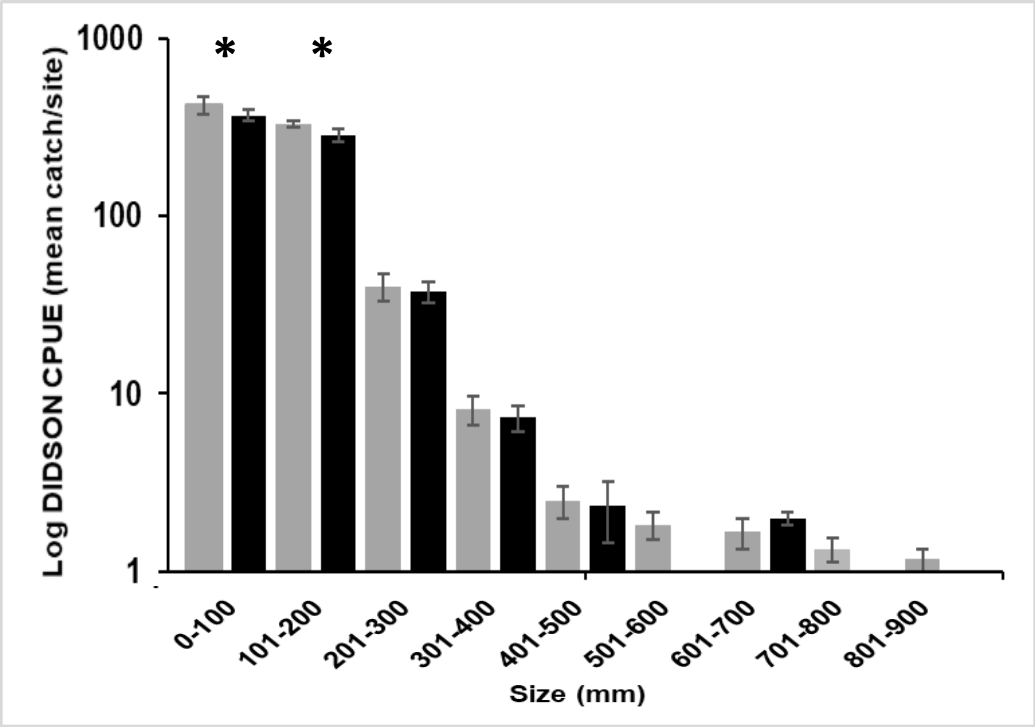

Figure 5: Size distribution of fish observed during July and August DIDSON sampling events. Reef sites are notated with gray bars, while control sites correspond to the black bars. Error bars display +/- standard error within each site. An asterisk represents a statistical difference between reef and control CPUEs ($p<0.05$) between paired reefs and controls.

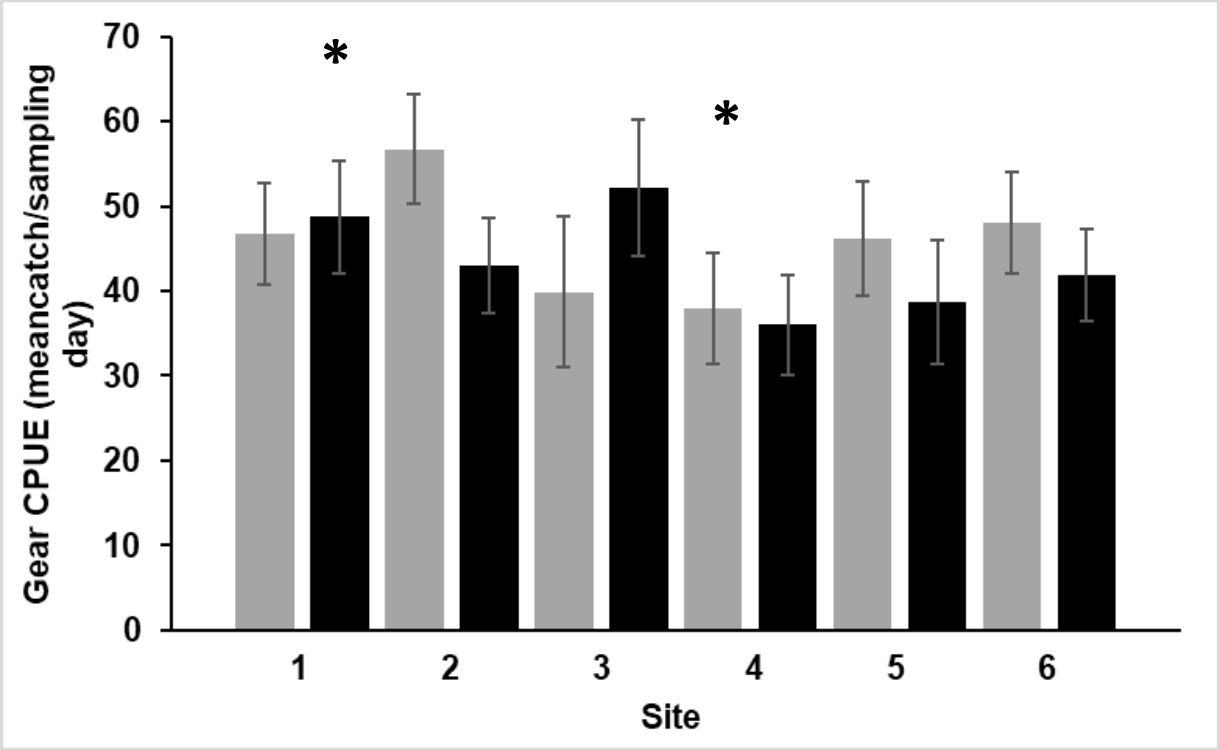

Figure 6: Catch at reef and control sites based on net and trap captures. All net and trap types are pooled. Reef sites are notated with gray bars, while control sites correspond to the black bars. Error bars display +/- standard error within each site. An asterisk represents a statistical difference between reef and control CPUEs ($p<0.05$) between paired reefs and controls.

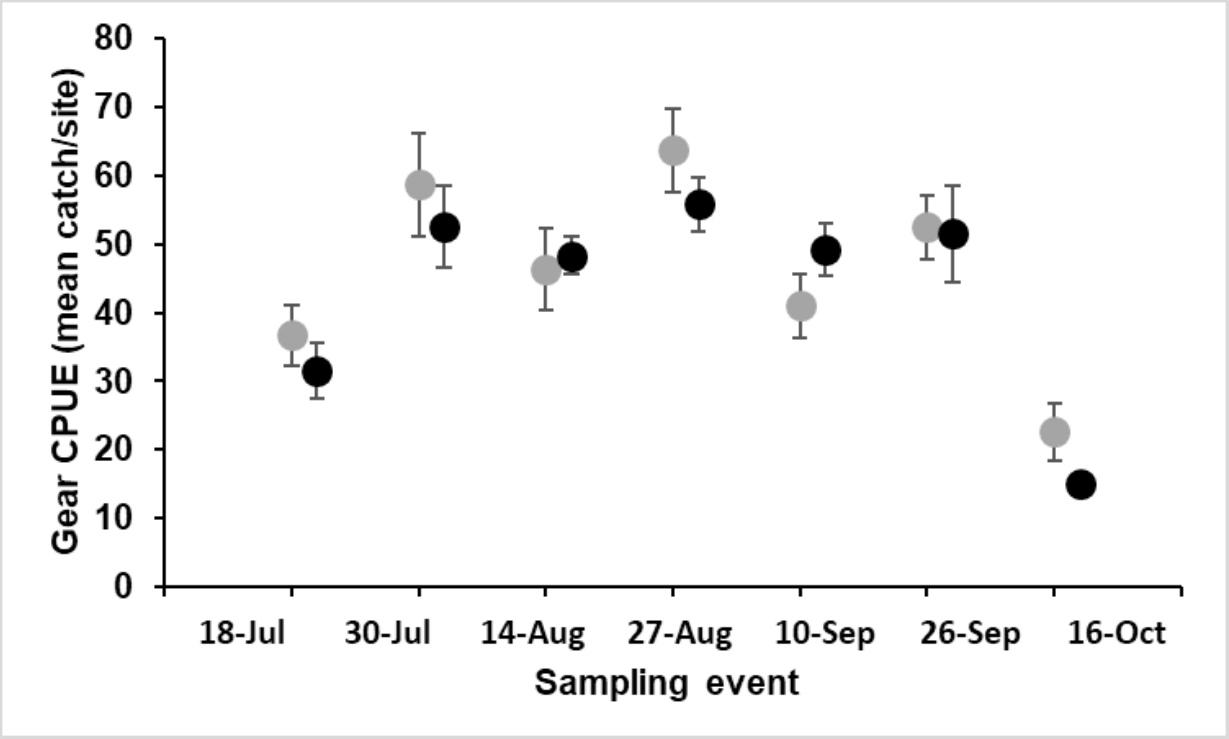

Figure 7: Catch over time from July to October at reef and controls based on gear captures. Black points indicate control sites, gray points indicate reef sites. Error bars display +/- standard error within each site. An asterisk represents a statistical difference between reef and control CPUEs ($p<0.05$) between paired reefs and controls.

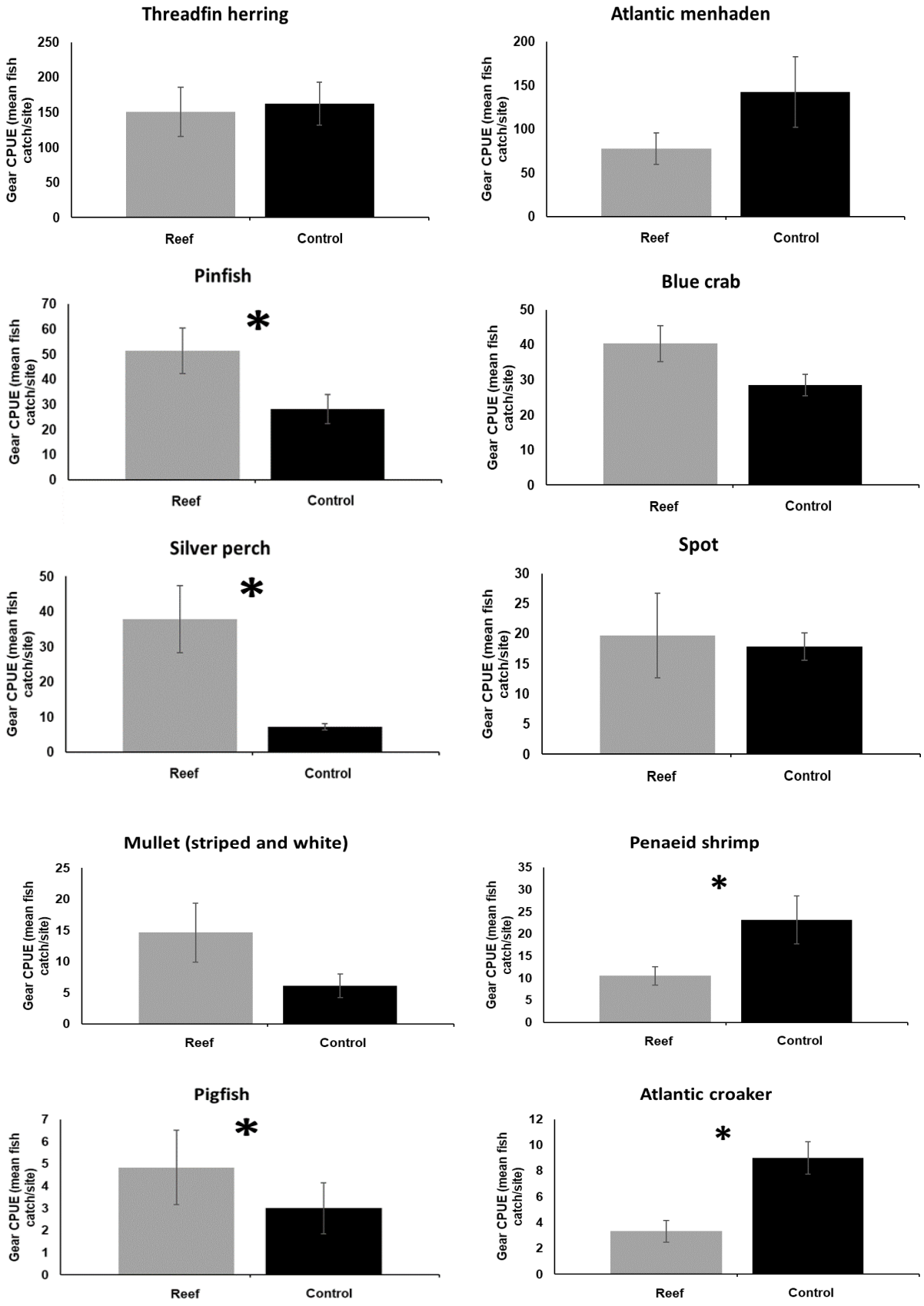
Threadfin herring
Gear CPUE (mean fish catch/site)
250
200
150
100
50
0
Reef
Control
Atlantic menhaden
Gear CPUE (mean fish catch/site)
200
150
100
50
0
Reef
Control
Pinfish
*
Gear CPUE (mean fish catch/site)
70
60
50
40
30
20
10
0
Reef
Control
Blue crab
Gear CPUE (mean fish catch/site)
50
40
30
20
10
0
Reef
Control
Silver perch
*
Gear CPUE (mean fish catch/site)
50
40
30
20
10
0
Reef
Control
Spot
Gear CPUE (mean fish catch/site)
30
25
20
15
10
5
0
Reef
Control
Mullet (striped and white)
Gear CPUE (mean fish catch/site)
25
20
15
10
5
0
Reef
Control
Penaeid shrimp
*
Gear CPUE (mean fish catch/site)
35
30
25
20
15
10
5
0
Reef
Control
Pigfish
*
Gear CPUE (mean fish catch/site)
7
6
5
4
3
2
1
0
Reef
Control
Atlantic croaker
*
Gear CPUE (mean fish catch/site)
12
10
8
6
4
2
0
Reef
Control

Figure 8: CPUE for the ten most abundant species sampled at all reef and control sites. Reef sites are notated with gray bars, while control sites correspond to the black bars. Error bars display +/- standard error within reef or control. An asterisk represents a statistical difference between reef and control CPUEs ($p<0.05$).

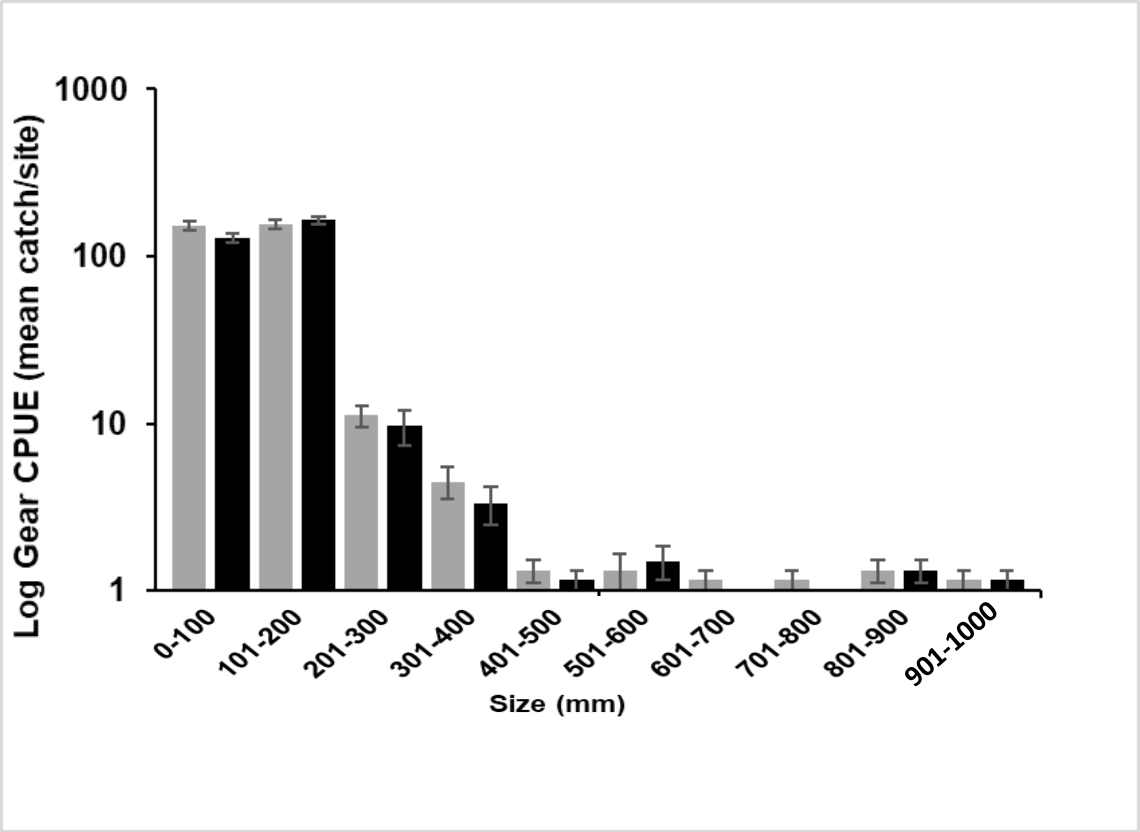

Figure 9: Size distribution of fish based on net and trap sampling. All net and trap catches are pooled. Reef sites are notated with gray bars, while control sites correspond to the black bars. Error bars display +/- standard error within each site. An asterisk represents a statistical difference between reef and control CPUEs ($p<0.05$) between paired reef and controls.

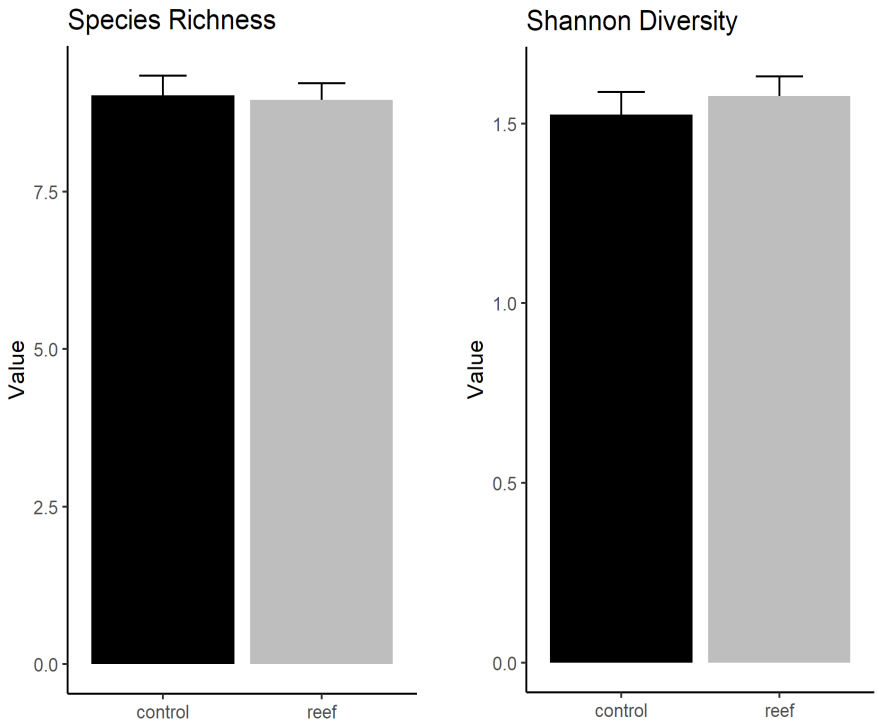

Figure 10: Species richness and Shannon diversity based on gear sampling data between July and October. Black indicates reef, gray indicates control. Error bars indicate mean standard error. Neither species richness nor Shannon diversity differed statistically ($p<0.05$) between reef and control.

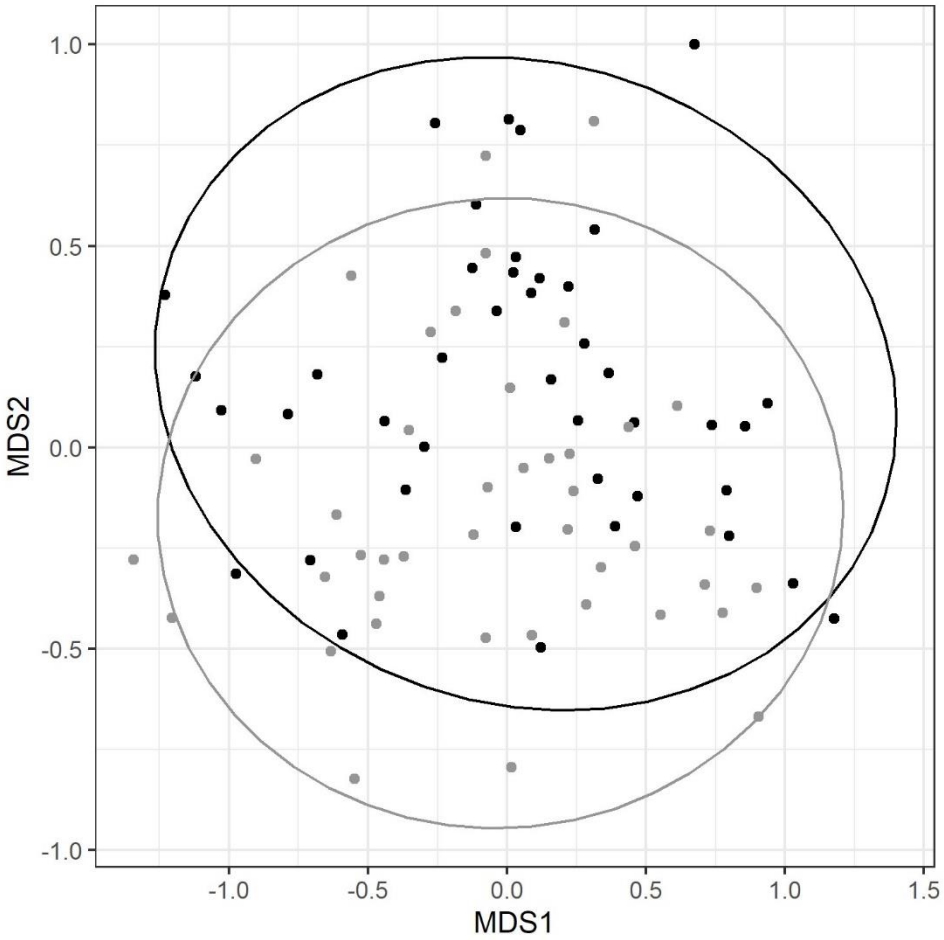

Figure 11: NMDS comparison of the nekton communities at all reef and control sites between July to October. Black indicates control sites, gray indicates reef sites. Ellipses represent 95% confidence intervals. Data were not transformed or standardized. Stress=0.164. R^2=0.033, F=2.843. There are no clear nekton community differences between reef and control treatments.

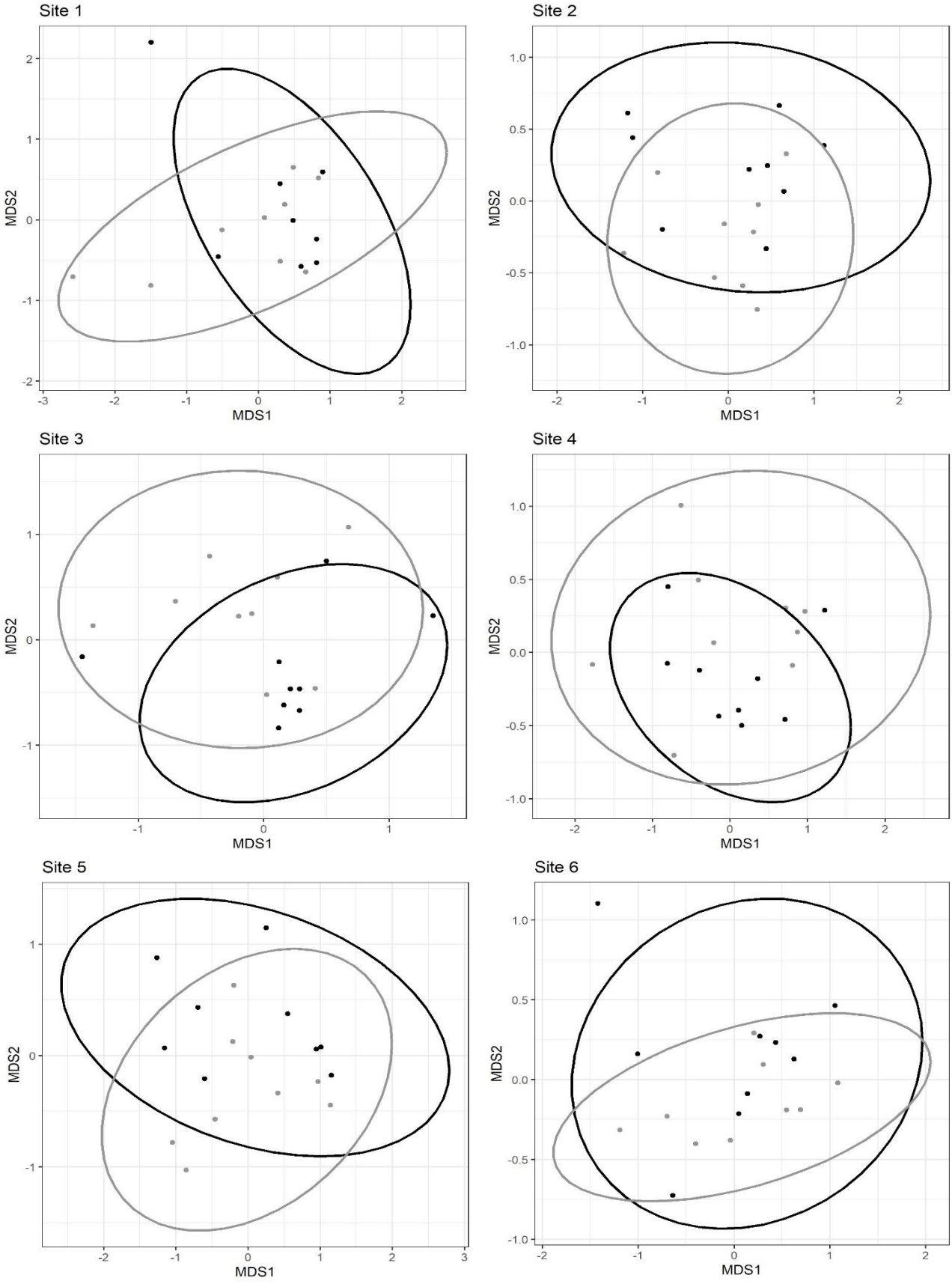
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
MDS1
MDS2

Figure 12: NMDS comparison at each reef and control site. Black indicates control, red indicates reef sites. Ellipses are 95% confidence intervals. Data were not transformed or standardized. Site 1 Stress=0.010, R^2=0.063, F=1.011. Site 2 Stress =0.120, R^2=0.080, F=1.392. Site 3 Stress=0.133, R^2=0.099, F=1.765. Site 4 Stress=0.123, R^2=0.076, F=1.318. Site 5 Stress=0.116, R^2=0.067, F=1.140. Site 6 Stress= 0.081, R^2=0.046, F=0.780. There are no clear differences between the nekton communities at any of the reef/control pair sites.

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