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PELAGIC FISH DIVERSITY AND DENSITY ON AND OFF RESTORED OYSTER REEF HABITAT

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PELAGIC FISH DIVERSITY AND DENSITY ON AND OFF RESTORED OYSTER REEF HABITAT

A thesis submitted in partial fulfillment of the requirements of the degree of Master of Science at Virginia Commonwealth University

by

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Abstract

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The heterogeneity provided by structured habitats is important in supporting diverse and
dense fish communities. The biogenic reefs created by the native Eastern Oyster, *Crassostrea
virginica*, were once the dominant structural habitat in Chesapeake Bay, and have since declined
to less than 1% of historic estimates. Conflicting results on the effects of oyster reef restoration
on pelagic fish assemblages make further investigation necessary. Incorporating multiple
sampling strategies may help elucidate oyster reef habitat influence on fish assemblages. This
study used multi-panel gillnets, hydroacoustic technology, and day-night sampling to describe
pelagic fish assemblages on and off oyster reef habitat in the lower Piankatank River, VA. Data
from oyster reef habitat, adjacent sandy-mud bottom habitat, and unstructured sandy habitat
outside of a reef restoration area compared fish diversity, species composition, and density

among habitat types. A multivariate analysis using day of the year, day or night, and habitat type as model terms found temporal factors explained variation in fish distribution more than habitat. Fish diversity varied significantly with day or night and habitat type. Diversity and density were significantly higher at night, demonstrating the necessity of nocturnal sampling in fish assemblage research. Results from this study conclude that fish assemblages were not significantly more diverse or denser on reef than non-reef habitat. We suggest that future work should concentrate on studying areas where oyster reef habitat comprises a larger proportion of the study area.

Introduction

The heterogeneity provided by structured habitats is important in supporting diverse and dense fish communities. Structurally complex environments have the ability to sustain more diverse and dense communities by altering the effect of predation and competition. The increased refuge provided by structured habitat decreases predation efficiency and moderates predator-prey interactions, thereby sustaining prey populations (Menge et al. 1976, Hixon 1991, Diehl 1992, Humphries et al. 2011). Structurally complex environments also limit interaction among competing species, increasing diversity by allowing functionally similar species to coexist (Luckhurst and Luckhurst 1978, Menge et al. 1985, Caley & St John 1996, Almany et al. 2004). In otherwise homogenous environments, the addition of structure and habitat complexity has the ability to increase abundance and species diversity in communities.

Common structured habitats that provide physical obstacles and refuge for fish in aquatic environments include rocky intertidal zones, aquatic vegetation and coral reefs. Many studies have described the increased diversity and density of fish assemblages associated with these physically complex habitats (Menge et al. 1985, Sale 1991, Hixon and Beets 1993, Jenkins et al. 2015), demonstrating the importance of structured habitat to fish. Eutrophication, climate change, pollution, and anthropogenic development have degraded these habitats, which has implications for associated fish assemblages (Turner et al. 1999, Lotze et al. 2006, Komyakova et al. 2013). Research on the influence of structured habitat on fish assemblages is necessary for properly managing habitat and fish resources.

In Chesapeake Bay, submerged aquatic vegetation, coastal marshes, and oyster reefs provide natural structural complexity. The biogenic reefs created by the native Eastern Oyster, *Crassostrea virginica*, were once the dominant structural habitat, but have declined to less than 1% of historic estimates (Rothschild et al. 1994, Wilberg et al. 2011). The rugosity, vertical relief and cohesion of oyster reefs provide the structure necessary to support a diverse and dense resident community. Oyster reefs may be essential to certain fish stocks since they provide structure and habitat heterogeneity for prey. Loss of reef habitat has direct implications for reef-specific epifaunal community (Zimmerman et al. 1989), but impacts to pelagic fish assemblages are relatively unknown.

Restoration of oyster reefs is expected to increase diversity and density of pelagic fish assemblages, similar to aquatic vegetation, coral reefs, and other structured habitat. Though some research indicates oyster reefs increase fish diversity and density (Harding and Mann 1999, Stunz 2010), other research does not support that conclusion (Geraldi et al. 2009, Pierson & Eggleston 2014). Harding and Mann (1999) used multi-panel gillnets on oyster reef and unstructured bottom and concluded that species richness is significantly higher on oyster reefs than unstructured bottom. Stunz (2010) used 2.6 m drop net enclosures to sample reef habitat, marsh edge and non-vegetated bottom, and found reef habitat had significantly more diverse and dense fish assemblages than the other habitats. Alternatively, Geraldi et al. (2009) used a before/ after/ impact/ control (BACI) design to sample fish with seines and block nets, and determined that the addition of oyster reef did not significantly impact fish assemblages. Pierson and Eggleston (2014) used gill nets and fish traps and found that fish density on unstructured bottom was higher than or similar to oyster reef fish density, but diversity was higher on reef habitat.

Differences in study locations, methods, sampling regime, and other confounding variables may be contributing to the conflicting results associated with these studies. Incorporating novel techniques to pelagic fish sampling may elucidate oyster reef habitat influence on fish assemblages. Fish sampling traditionally employs selective passive gear types (e.g. traps and nets), which are dependent on fish behavior and size for capture. In contrast to traditional passive sampling methods, a hydroacoustic survey uses the reflection of sound to attain precise abundance and size assessments (Simmonds and MacLennan 2005; Boswell et al. 2007). A key advantage of hydroacoustic technology is that gear avoidance, fish size, and fish behavior do not restrict which individuals reflect echoes. Though hydroacoustics can collect accurate density and size data, this technology cannot differentiate species, so passive sampling is necessary to assess species composition. Multi-panel gillnets incorporate a variety of mesh sizes, widening the size and species range of captured fish. Additionally, fish sampling typically occurs during daytime hours. Typical daytime sampling neglects data collection on nocturnal species composition and ecological processes (Hammerschlag and Serafy 2010). Combining hydroacoustic and gillnet data collection, both day and night, may improve our understanding of fish utilization of oyster reef habitat.

The objective of this study was to use a combination of traditional (gillnet) and novel (lateral-aspect hydroacoustics) sampling techniques to describe pelagic fish assemblages on and off restored oyster reef habitat. Multi-panel gillnets and hydroacoustics were employed to test the hypothesis that oyster reef habitat supports more diverse and dense fish assemblages than non-reef habitat. Data from structured oyster reef habitat, adjacent sandy-mud bottom habitat, and unstructured sandy habitat outside of a reef restoration area were analyzed to compare fish species composition, diversity, and density among habitat types.

Study Sites

This research studied the oyster reef restoration area in the lower Piankatank River, Virginia (Figure 1). The Piankatank River, a western shore tributary of lower Chesapeake Bay, has a tidal fluctuation of 0.4 m and a salinity range of 10 to 18 ppt. This estuary is ideal for oyster restoration because its eddying hydrodynamics successfully retain oyster larvae. The National Oceanic and Atmospheric Administration (NOAA) facilitates oyster reef restoration in the Piankatank River. To help guide state, federal and non-governmental partners, NOAA delineated a restoration priority area ideal for oyster reef restoration efforts within the river (Figure 2) based on hydrodynamics and salinity data. This oyster restoration complex area is 5,022 acres and contains an estimated 240 acres of natural oyster reef habitat and 130 acres of restored habitat, to date. Restored reefs Palace Bar Reef, Burton Point Reef, and Fishing Bay Reef were selected for study because they had the most area of all natural or restored reefs within the oyster reef restoration complex. These reefs are also ideal for fish assemblage research because they are state-protected from commercial disturbance and have retained their emergent structures. Palace Bar reef is fifty-three acres of shell mounds and reef balls, and has an average oyster density of 175.7 per m^2. Palace Bar reef is located furthest upriver. Fishing Bay reef is twenty acres of small stone rubble, located on the north shore between Palace Bar and the mouth of the river. Though oyster density data has not yet been collected on this reef, underwater observation in spring of 2016 indicated that the stone rubble foundation successfully grew into a cohesive reef. Burton Point Reef is a sixty acres reef located near the mouth of the river, with an

average oyster density of 77.8 per m^2. Scattered shell habitat is prevalent throughout the oyster restoration complex. Habitat outside of the reef is homogenous sand flats. Sampling outside of the reef complex, we can compare assemblages on unstructured habitat within a network of oyster reefs to unstructured habitat outside of the reef network. Comparing the pelagic fish assemblages on unstructured habitat within the restoration complex to the assemblages on unstructured habitat outside of the complex area will allow us to assess the effect of oyster reef habitat proximity on fish.

Data were collected at eight sampling locations: three restored oyster reef sites (Palace, Burton, Fishing Bay), three adjacent, unstructured habitat off-reef sites and two unstructured habitat sites outside of the NOAA-designated reef-restoration complex (Figure 2). All sampling sites had a depth range of 1.4 m to 2.4 m mean low water. Esri's ArcGIS® software was used to select sampling sites from NOAA-compiled geospatial data on reefs, habitat and bathymetry. On-reef sites were selected at the center of Palace Bar Reef, Fishing Bay Reef and Burton Point Reef. Off-reef sites were selected on unstructured sandy-mud bottom of comparable depth, within one nautical mile of an associated reef site. Sampling sites outside the reef complex were on unstructured, homogenous sand flats. The two sites established outside of the reef complex were selected due to their close proximity to the complex, comparable depths, homogenous sandy bottom, and physical partition from the reef complex. Stove Point, a narrow land peninsula, provides a physical land barrier between the outside sites and the restored oyster reef complex (Figure 2). On reef sites, unstructured reef-adjacent sites, and sites outside of the reef complex will be referred to as "Reef", "Off" (or "Off-reef") and "Outside", respectively.

Methods

Data Collection

Fishes were collected from experimental gillnets during the day and night, from May through September of 2011, 2012, and 2015-2017. Fish were collected on each site using 53 m x 2.5 m multi-panel gillnets composed of 2.5 cm, 3.8 cm, 6.4 cm and 7.6 cm monofilament mesh panels. Nets were set on georeferenced sampling sites and soaked for approximately three hours. There were thirty-eight net sets deployed on Reef and Off habitat prior to 2016. In 2016, Outside sites were established. In 2016 and 2017, each sampling event deployed gillnets on a reef site, an adjacent off-reef site and two outside sites. Confounding variables such as tide, time of day, and season were comparable for the three habitat types during a single sampling event. Upon capture, fish were pulled out of nets and placed on ice for processing in the laboratory where they were identified to species and measured for total length (TL) to nearest millimeter. Large specimens (Cownose Ray *Rhinoptera bonasus* and Sandbar Shark *Carcharhinus plumbeus*) were processed and released live on site.

Fish density data were collected day and night, from May through September of 2016 and 2017, using a BioSonics™ 430 kHz split-beam DT-X Digital Scientific Echosounder connected to a transducer affixed to an aluminum 1.23 m x 1.23 m x 1.23 m frame lowered onto the bottom (Figure 3).

A receiver timed, amplified and filtered the "echoes", which were measured and recorded by the echosounder and displayed by a computer. Echoes were processed with specialized software to create high-resolution fish density, size frequency, and distribution estimates (Fernandes et al. 2002; John Simmonds 2005; Boswell, Wilson & Wilson 2007). Equipment was

calibrated and tested by BioSonics professionals at the beginning of each sampling season. Settings were adjusted for depth and environmental conditions prior to each survey. Data collection started approximately ten minutes after equipment deployment to decrease effects on nearby individuals. Stationary, lateral-aspect hydroacoustic data collected feedback from 2,500 pings (approximately ten minutes of surveying) during a single sample. The 6.7° acoustic beam transmitted horizontally through the water column across reef habitats (or parallel to channels for unstructured habitat sites), at 5 pings/sec. Fish swimming within a 2-8 m conical pinging range of the transducer were counted using Biosonics ™ tracking software, Visual Acquisition® (version 6.2). Echo decibel strength was recorded and a movement-based algorithm was used to identify and track fish. This tracking technology discerns fish from emergent structures to prevent double counts. Echo outputs from each survey were scanned for disparities before being converted into raw target strength data. Visual Acquisition® (version 6.2) was used to convert echoes into spreadsheets of target strength decibels. Hydroacoustic density data and the gillnet species composition data were analyzed to assess species composition, diversity and density on and off restored oyster reefs.

Data Analysis

Gillnet data were used to compare fish species composition and diversity among habitat types and sampling sites. Since the Piankatank River system is dominated by clupeid species, data were analyzed with and without clupeid data. A Shannon-Weiner Index was calculated to rank the diversity of fish assemblages caught on Reef, Off and Outside habitat. Jaccard's coefficient was calculated on species presence data to quantify the similarity of species composition among habitat types. A cluster dendrogram was created from a Bray-Curtis dissimilarity matrix on species catch per unit effort to visually assess similarities in samples and

among sampling sites. To assess differences in community among habitat types, gillnet data were

first transformed into catch per unit effort (CPUE) by species. For gillnet data, catch per unit

effort was calculated as

$$CPUE = \# \text{ of fish /soak time} * \text{net length unit}$$

where 30.5 m of net was equal to one net length unit (since a few sampling days employed the

use of longer nets). Raw abundance data were converted into catch per unit effort by species,

then used to calculate species diversity (H') using the Shannon-Weiner Diversity Index

$$H' = -\sum_{i=1}^{S} (p_i)(log_e p_i)$$

where p_i is the proportion of individuals belonging to the ith species. Habitat and sampling site

assemblages were ranked by diversity based on H'. To assess community similarity among

habitat types, gillnet species presence data were analyzed using Jaccard's index of dissimilarity

$$J(A, B) = \frac{A \cup B}{A \cap B} = \frac{|A \cup B|}{|A| + |B| - |A \cap B|}$$

where A is a matrix of species presence and absence for one habitat type, and B is a matrix of

species presence for another habitat type.

Hydroacoustic density data were collected using Biosonics [TM] equipment and technology.

Visual Acquisition® software (version 6.2) was used to process and analyze raw survey data. A

target's echo integration and backscattering were calculated into a target strength decibel (dB),

which was used to estimate target size. Updates to lateral-aspect hydroacoustic technology and

target-strength-to-size-equations, have reduced the bias associated with shallow-water

assessments. Love's lateral-aspect equation, Love_Lat was used to calculate total fish length (L, in

cm) from acoustic target strength (TS) since it minimizes bias associated with shallow-water

estuarine environments (Love 1969; Boswell et al. 2008). Love's lateral-aspect equation, Love$_{Lat}$ calculated fish total length as

$$TS_{Lateral} = 24.1 * log_{10}(TL_{cm}) - 61$$

where TS$_{lateral}$ was an acoustic target strength from a lateral aspect hydroacoustic echo in decibels, and L$_{cm}$ was total fish length in centimeters. Based on Love's lateral-aspect equation, fish with a target strength of less than -45 dB are smaller than 46 mm. Since all fish caught in the gillnet had measured total lengths greater than 46 mm (Table 1), tracks with a decibel less than -45 dB were analyzed separately. Track data were transformed into catch per unit effort (CPUE) calculated as

$$CPUE = \text{\# of tracks per } 3260.911 \text{ m}^3$$

where 3260 m^3 is the volume of water that was surveyed by the hydroacoustic beam after five intervals of 500 pings (approximately ten minutes).

All statistical analyses were performed using R v3.3.2 statistical software. Data were tested for normality using a Shapiro-Wilk test. An analysis of variance test (ANOVA) assessed gillnet and hydroacoustic data for differences in fish density among reef, off-reef and outside reef complex habitat types for gillnet-sized fish and forage fishes. A multifactorial ANOVA was run on habitat type, day of year, and time of day, along with their interaction terms, to determine which factors most affect total caught-fish CPUE, clupeid CPUE and non-clupeid CPUE from gillnets. A multifactorial ANOVA was also run on habitat type, day of year, and time of day to determine which factors most effect gillnet-sized fish (fish with TS > 45 dB) and smaller than gillnet-sized fish (fish with TS < -45 dB) from hydroacoustic tracks. Significance levels for all statistical tests were established at alpha = 0.05 a priori.

Results

Species Composition and Diversity

A total of 2513 individuals representing 31 species and 24 families were captured from 84 gillnet sets (50 during the day and 34 at night; Table 1). Pelagic fish species richness was highest on reef habitat (23 species), followed by off reef habitat (20 species) and then habitat outside the reef complex (16 species; Figure 4). Reef habitat had 7 unique species associated with it, off reef habitat had 4, and outside the reef complex habitat had 1 (Figure 4). Reef and off reef assemblages shared 17 species. The three habitat types shared 14 species, demonstrating a similarity in overall pelagic fish community in the lower Piankatank River.

Diversity varied significantly with day or night ($p < 0.000$) and habitat type ($p = 0.048$; Table 2). Diversity varied most significantly with day or night sampling time, with total pelagic fish diversity greater at night (mean H' = 0.99 ± 0.03) than day (mean H' = 0.59 ± 0.03). Though reef habitat had the highest species richness, pelagic fish assemblages outside of the reef complex had the highest diversity (mean H' = 0.95 ± 0.05), followed by reef habitat (mean H' = 0.74 ± 0.05) and off reef habitat (mean H' = 0.63 ± 0.03; Figure 5). Reef habitat had the highest diversity at night (mean H' = 1.16 ± 0.07), but the lowest diversity during the day (mean H' = 0.48 ± 0.06; Figure 6).

The most abundant species caught in the study was Atlantic Menhaden *Brevoortia tyrannus* (59% of total catch), followed by Atlantic Thread Herring *Opisthonema oglinum* (10% of total catch). Atlantic Menhaden comprised 58% of reef catch, 67% of off reef catch, and 55% of catch outside reef complex (Figure 7). Total Clupeid catch comprised 67% of reef catch, 75%

of off reef catch, and 68% of outside reef complex catch. Excluding Clupeids, Striped Bass *Morone saxatilis* (24%) was the most abundant species caught in the study, followed by Atlantic Croaker *Micropogonias undulatus* (22%), Bluefish *Pomatomus salatrix* (15%), Spot *Leiostomus xanthurus* (12%), Southern Kingfish *Menticirrhus americanus* (8%), Cobia *Rachycentron canadum* (3%), Cownose Ray *Rhinoptera bonasus* (3%), Weakfish (3%) *Cynoscion regalis*, Silver Perch *Bairdiella chrysoura* (2%), Harvestfish *Peprilus paru* (2%), Spadefish *Chaetodipterus faber* (2%) with all other species comprising 1% or less of total catch.

Jaccard's Index of Dissimilarity was calculated to quantitatively assess the dissimilarity of pelagic fish community among habitat types. Off-reef sites and sites outside the reef complex were 68.2% dissimilar. Reef and off-reef sites were 55.2% dissimilar, and reef and outside reef complex sites were 50.0% dissimilar. A Bray-Curtis dissimilarity matrix was calculated from species composition data of each gillnet set then structured as a cluster dendrogram, illustrating clustering with day or night (Figure 8). A cluster dendrogram was created from species composition data by sampling site, and showed clustering among sites with similar river location, although this was not statistically analyzed (Figure 9).

Density

Fish density was analyzed from 32 reef gillnet sets, 32 off reef gillnet sets and 20 outside reef complex gillnet sets. Gillnets caught fish between 46 mm and 1280 mm total length (TL). Clupeid and non-Clupeid fish density varied significantly with temporal factors, but not significantly by habitat type (Table 3; Figure 10). Total gillnet catch was significantly higher at night, with 81% of total catch being caught at night (Figure 11). Clupeid density varied significantly with day or night and day of year (Table 3). Clupeid fish density was greater at night (mean CPUE = 3.63 ± 0.26) than day (mean CPUE = 0.63 ± 0.05; Figure 12). Non-Clupeid

catch varied significantly with day or night (Table 3). Non-Clupeid fish density was greater at night (mean CPUE = 8.72 ± 0.51) than day (mean CPUE = 1.32 ± 0.23, Figure 12).

Out of the 6 most abundant non-Clupeid species caught in the study, Southern Kingfish was the only species that had density vary significantly with habitat type (p = 0.001), being found in higher abundances on unstructured habitat outside of the reef complex.

Fifty-four hydroacoustic surveys were analyzed for differences in fish density among habitat types: 17 on reef, 17 off reef and 20 outside the reef complex. Twenty-seven of the surveys were conducted during the day, and 27 at night. The size range for fish tracked by hydroacoustics was calculated from target strength and estimated to be between 1.5mm to 1076 mm TL. Fish large enough to be caught in gill nets (total length greater than 46 mm or target strength greater than -45dB) and small forage fish were analyzed separately since forage fish less than 46 mm were not caught in gillnets, but were tracked by hydroacoustic equipment. Hydroacoustic gillnet-sized fish (fish with TS > -45 dB) density differed significantly by day of year (p = 0.00; Table 3). Small forage fish (fish with TS < -45 dB) densities did not vary significantly with habitat type, day of year or sampling time.

Discussion

Fish diversity varied significantly with day or night and habitat type. Diversity was greater at night, which may be a function of an overall increase in fish activity at night. Though relatively more species were caught on reef habitat, the reef complex (reef sites and off reef sites) did not harbor a more unique, reef-dependent community compared to outside the reef

complex as hypothesized. The outside complex habitat was more diverse than unstructured habitat within the reef complex. This could be because the outside complex sites were frequented by both the reef complex community and the open water community, as evident from the presence of species such as Spanish Mackerel *Scomberomorus maculatus* and Sandbar Shark *Carcharhinus plumbeus*. Though outside habitat had the highest diversity, all three habitat types shared 14 species, showing a general similarity of community for the lower Piankatank River. Reef and off reef habitat shared more species than any other habitat pair, suggesting the pelagic fish community within the reef complex is similar and perhaps different from the outside reef complex pelagic fish community.

The presence of oyster reefs and other biogeographical features unique to this estuarine environment may be attracting juveniles from the larger Chesapeake Bay pelagic fish species pool. Species-specific size frequency data from gillnetted fish suggests that most Striped Bass, Silver Perch and Atlantic Croaker caught in the lower Piankatank are juveniles or sub-adults (McCulloch and McIninch, unpublished data). The lower Piankatank reefs may be providing refuge habitat, like oyster reefs, important to the survival and growth of these species.

Atlantic Menhaden dominated the catch at all sites, regardless of habitat type, position in the river, or time of sampling. Attempts to combine gillnet and hydroacoustic length frequency data to infer species abundance on reefs was not possible because Atlantic Menhaden were so abundant and had such a large size range. Previous studies suggest the decline of oysters and their nutrient sequestering capabilities contributes to increased nutrient concentrations in the water, leading to increased phytoplankton production (Tuttle 1987). Phytoplankton increases in the bay could contribute to this domination of phytoplankton-feeding Clupeids (Friedland et al. 1989, Ulanowicz and Tuttle 1992) in our samples.

Species-specific analyses were performed on the five most abundant non-Clupeid species, including Striped Bass, Atlantic Croaker, Spot and Southern Kingfish. Southern Kingfish was the only species that had densities vary with habitat type, found in higher densities outside of the reef complex. Peterson and Grabowski (2003) conducted a meta-analysis on oyster reef to fish relationship studies and suggest that Striped Bass, Atlantic Croaker, and Bluefish are not enhanced by oyster reefs (though they note the conflicting reports on Striped Bass). Our results conclude that Striped Bass, Atlantic Croaker and Bluefish varied significantly with factors other than habitat. This could be due to the fact that these species are common and were caught on all habitat types. Variation in overall fish density was also explained more by temporal factors than habitat. Specifically, day or night contributed to more variation in diversity and density for all gillnet-sized and gillnet caught fish.

Time of sampling (day versus night) explained most of the variability in density for both Clupeid and non-Clupeid catch. This variation between day and night could be due to daytime fish avoidance of nets. Fish avoidance is higher during daytime hours or in clearer waters, suggesting that fish avoidance is a function of visibility and not space limitation within the net. Previous studies suggest that catchability rates of gillnets decrease as fish accumulate in nets due to fish avoidance, even for small catches (Olin et al. 2004). Species composition was slightly different at night, with Weakfish and Silver Perch caught only at night. A species visual acuity and present environmental conditions may affect catchability more than diel shifts in activity. The capture of some species, like Weakfish and Silver Perch, may only occur at night when visibility is lowest. Gillnet CPUE can be used as an index of fish abundance during periods of low visibility (Olin et al. 2004), making nocturnal sampling key to fish assemblage research.

For hydroacoustic data, day of the year was the independent variables that most explained variation in the density of fish tracks with a target strength greater than -45 dB. Though habitat explained 8% of variation in density of gillnet-sized fish ($p = 0.073$; Table 2), day of year is a more influential factor, explaining 23% of variation ($p = 0.000$; Table 2). Though hydroacoustic technology allowed us to sample the small pelagic forage fishes that gillnets could not, the factors considered did not significantly influence forage fish distribution. This could be because forage fishes are difficult to quantify due to their small size and formation of dense schools (Campbell 2008). Results from this study suggest that forage fish distribute randomly or according to independent variables not considered in this study.

The cluster analysis reveals relationships with assemblages sampled at the same time (day versus night) and within close proximity to each other. Fish assemblages up river are more similar than fish assemblages down river, suggesting that location is yet another factor influencing variation in diversity and species composition. Pelagic fish move and congregate according to many factors. Tide cycle, availability of food resources, salinity, and temperature are a few examples of potential factors that also influence fish. Many of these factors have a temporal component or correlation, making diel shifts in distribution, or seasonal shifts in distribution appropriate factors to include in our model. Habitat seems to be explaining some of the variation in pelagic fish diversity and densities, but temporal factors explain more. This could be because oyster reef habitat made up less than 8% of the reef complex area. Perhaps reef restoration has not yet reached a scale large enough to significantly influence the pelagic fish community in the Piankatank reef complex more than other factors.

Conclusion

The main objective of this research was to compare diversity and density of pelagic fish assemblages on structured reef habitat, on unstructured off reef habitat and unstructured habitat outside of the reef network. Pelagic fish were studied using multi-mesh gillnets, lateral-aspect hydroacoustic technology, and nocturnal sampling regimes. Gillnets provided information on species composition utilizing each habitat type, while hydroacoustic equipment provided density estimates of pelagic fish, including smaller, forage fish not caught in gillnets. Our hypothesis that fish assemblages are more diverse on reef habitat in comparison to unstructured habitat was not supported. Variation in fish diversity was influenced by day or night and habitat factors, with night assemblages being more diverse than day assemblages, and outside reef complex assemblages being more diverse than other habitat types. Our hypothesis that fish assemblages are more abundant on reef habitat in comparison to unstructured habitat was not supported. This study found that temporal factors explain most of the variation in fish density. Specifically, fish are denser at night than day. Overall, temporal factors, like day or night or day of the year influence fish density and diversity more than structured habitat presence, but it is important to consider scale in this assessment. Total oyster reef habitat comprised less than 8% of the reef complex area. Understanding how pelagic fish are influenced by oyster reef habitat may require studying reef restoration at a larger scale.

Future Work

Though abundance and species composition data is a good start to determining which fish species to focus on, future research should concentrate on establishing solid reef-to-fish relationships. Understanding fish to reef connections using stable isotopes or diet analysis can further explain how oyster reefs benefit fisheries. Future research should consider fish density and community among all structured habitat types (e.g. marsh, submerged aquatic vegetation, anthropogenic structures, etc). The structure of oyster reefs is certainly attracting fish, but the community composition of reef residents, or species that stay on reef year-round, may be more influential than this study was able to determine. Also, exploring the feasibility of using the hydroacoustic technology to create a Piankatank River-specific target strength library would be beneficial to future fish research in this restoration area. Hydroacoustic survey using two transducers (one facing towards reef, the other away from reef) would be an interesting method to compare fish assemblages closest to reef and fish assemblages away from reefs. Overall, additional research should consider the importance of nocturnal sampling and use of different data collection methods. The Piankatank reef restoration effort is considered large-scale by Chesapeake Bay standards, but to fully understand the effect of reef habitat on fish, future work should consider studying reef habitat that comprises more than 8% of the study area.

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Appendix

Table 1: Species Captured in Gillnets

Species collected in gillnets in the Piankatank River from 2015 to 2017 as part of a study on oyster reef restoration.

Common Name	Species	Family	Total Length (cm)		Target Strength (dB)		Economically relevant?
			Min	Max	Min	Max	
Sandbar Shark	*Carcharhinus plumbeus*	Carcharhinida	128.0	128.0	-10.4	-10.4	
Cobia	*Rachycentron canadum*	Rachycentrida	27.8	99.0	-26.3	-13.1	yes
Cownose Ray	*Rhinoptera bonasus*	Myliobatidae	30.0	80.0	-25.5	-15.3	
Houndfish	*Tylosurus crocodilus*	Belonidae	77.5	77.5	-15.7	-15.7	
Spanish mackerel	*Scomberomorus macula*	Scombridae	45.0	56.0	-21.3	-19.0	yes
Bluefish	*Pomatomus saltatrix*	Pomatomida	16.0	42.0	-32.1	-22.0	yes
Atlantic Menhaden	*Brevoortia tyrannus*	Clupidae	8.6	39.7	-38.6	-22.6	yes
Striped Bass	*Morone saxatilis*	Percichthyidae	12.8	39.0	-34.4	-22.8	yes
Whitefin sharksucker	*Echeneis neucratoides*	Echeneidae	28.0	36.3	-26.3	-23.6	
Gizzard shad	*Dorosoma cepedianum*	Clupidae	36.1	36.1	-23.6	-23.6	
Red drum	*Sciaenops ocellatus*	Sciaendidae	22.0	36.1	-28.8	-23.6	yes
Speckled trout	*Cynoscion nebulosus*	Sciaendidae	24.3	35.1	-27.7	-23.9	yes
Northern Pufferfish	*Sphoeroides maculatus*	Tetradontidae	35.0	35.0	-23.9	-23.9	
Atlantic Croaker	*Micropogonias undulat*	Sciaendidae	12.0	32.7	-35.1	-24.7	yes
Southern Kingfish	*Menticerrhus american*	Sciaendidae	21.1	31.4	-29.2	-25.1	yes
Oyster Toadfish	*Opsanus tau*	Batrachoidida	15.0	30.0	-32.8	-25.5	
Atlantic Threadfin Herring	*Opisthonema oglinum*	Clupidae	11.9	29.6	-35.2	-25.7	
Summer flounder	*Paralichthys dentatus*	Bothidae	15.5	28.1	-32.4	-26.2	yes
Weakfish	*Cynoscion regalis*	Sciaendidae	12.7	28.0	-34.5	-26.3	yes
Spot	*Leiostomus xanthurus*	Sciaendidae	14.0	23.5	-33.5	-28.1	yes
Atlantic stingray	*Dasyatis sabina*	Dasyatidae	22.7	22.7	-28.5	-28.5	
Harvestfish	*Peprilus alepidotus*	Stromateidae	17.0	22.0	-31.5	-28.8	
Black seabass	*Centropristis striata*	Serranidae	18.5	18.5	-30.6	-30.6	yes
Silver perch	*Bairdiella chrysoura*	Sciaendidae	15.0	18.2	-32.8	-30.8	
Spadefish	*Chaetodipterus faber*	Ephippidae	10.0	16.0	-37.0	-32.1	yes
Hogchoker	*Trinectes maculatus*	Soleidae	4.6	11.7	-45.1	-35.4	
Pinfish	*Lagodon rhomboides*	Sparidae	4.6	9	-45.1	-38.1	

Table 2: ANOVA Results for Assemblage Diversity

Multifactorial ANOVA results assessing diversity of fish caught in gillnets. Habitat, day of the year and day or night sampling time were factors tested for sources of variation in the data.

Source of Variation	df	SS	Term SS / Total SS	F	*p*
Diversity (H')					
Habitat	2	1.42	0.06	3.18	0.048
Day of Year	1	0.06	0.00	0.00	0.958
Day or Night	1	2.88	0.13	12.86	0.001
Habitat * Day of Year	2	0.69	0.03	1.54	0.221
Habitat * Day or Night	2	1.07	0.05	2.39	0.099
Day of Year * Day or Night	1	0.00	0.00	0.00	0.973
Habitat * Day of Year * Day or Night	2	0.17	0.01	0.38	0.685
Residuals	72	16.12	0.72		

Residual SE = 0.47 on 72 degrees of freedom

Model R^2= 0.29

Table 3: ANOVA Results for Caught Fish Density

Multifactorial ANOVA results assessing density of fish caught in gillnets for Clupeid catch and non-Clupeid catch. Habitat, day of the year and day or night sampling time were factors tested for sources of variation in the data.

Source of Variation	df	SS	$\dfrac{\text{Term SS}}{\text{Total SS}}$	F	p
Gillnet Density (CPUE)					
Clupeid Catch					
Habitat	2	16.08	0.01	0.38	0.686
Day of Year	1	144.06	0.05	6.79	0.011
Day or Night	1	1029.20	0.36	48.54	0.000
Habitat * Day of Year	2	32.43	0.01	0.76	0.469
Habitat * Day or Night	2	0.07	0.00	0.00	0.998
Day of Year * Day or Night	1	9.19	0.00	0.43	0.512
Habitat * Day of Year * Day or Night	2	76.64	0.03	1.81	0.171
Residuals	72	1526.54	0.54		

Residual SE = 4.60 on 72 degrees of freedom
Model R^2 = 0.46

Source of Variation	df	SS	$\dfrac{\text{Term SS}}{\text{Total SS}}$	F	p
Non-Clupeid Catch					
Habitat	2	7.42	0.01	0.89	0.416
Day of Year	1	0.07	0.00	0.02	0.895
Day or Night	1	181.21	0.35	43.44	0.000
Habitat * Day of Year	2	0.45	0.00	0.05	0.948
Habitat * Day or Night	2	8.52	0.02	1.02	0.365
Day of Year * Day or Night	1	10.08	0.02	2.42	0.124
Habitat * Day of Year * Day or Night	2	8.99	0.02	1.08	0.346
Residuals	72	300.34	0.58		

Residual SE = 2.04 on 72 degrees of freedom
Model R^2 = 0.42

Table 4: ANOVA Results for Track Density

Multifactorial ANOVA results assessing density of fish surveyed with hydroacoustics for tracks with a target strength greater than -45 dB and for tracks with a target strength less than -45 dB. Habitat, day of the year and day or night sampling time were factors tested for sources of variation in the data.

Source of Variation	df	SS	Term SS / Total SS	F	p
Hydroacoustic Density (CPUE)					
Tracks with TS > -45 dB					
Habitat	2	112843.00	0.08	2.80	0.073
Day of Year	1	309249.00	0.23	15.34	0.000
Day or Night	1	54855.00	0.04	2.72	0.106
Habitat * Day of Year	2	25115.00	0.02	0.62	0.541
Habitat * Day or Night	2	28797.00	0.02	0.71	0.495
Day of Year * Day or Night	1	1460.00	0.00	0.07	0.789
Habitat * Day of Year * Day or Night	2	7750.00	0.01	0.19	0.826
Residuals	41	826396.00	0.60		

Residual SE = 142 on 41 degrees of freedom
Model R^2 = 0.40

Source of Variation	df	SS	Term SS / Total SS	F	p
Tracks with TS < -45 dB					
Habitat	2	419001.00	0.08	2.08	0.138
Day of Year	1	237340.00	0.05	2.36	0.132
Day or Night	1	18900.00	0.00	0.19	0.667
Habitat * Day of Year	2	114952.00	0.02	0.57	0.569
Habitat * Day or Night	2	35845.00	0.01	0.18	0.838
Day of Year * Day or Night	1	279300.00	0.05	2.77	0.103
Habitat * Day of Year * Day or Night	2	8418.00	0.00	0.04	0.959
Residuals	41	4127704.00	0.79		

Residual SE = 317.3 on 41 degrees of freedom
Model R^2 = 0.21

Figure 1: Map of Study Area in Piankatank River, VA.
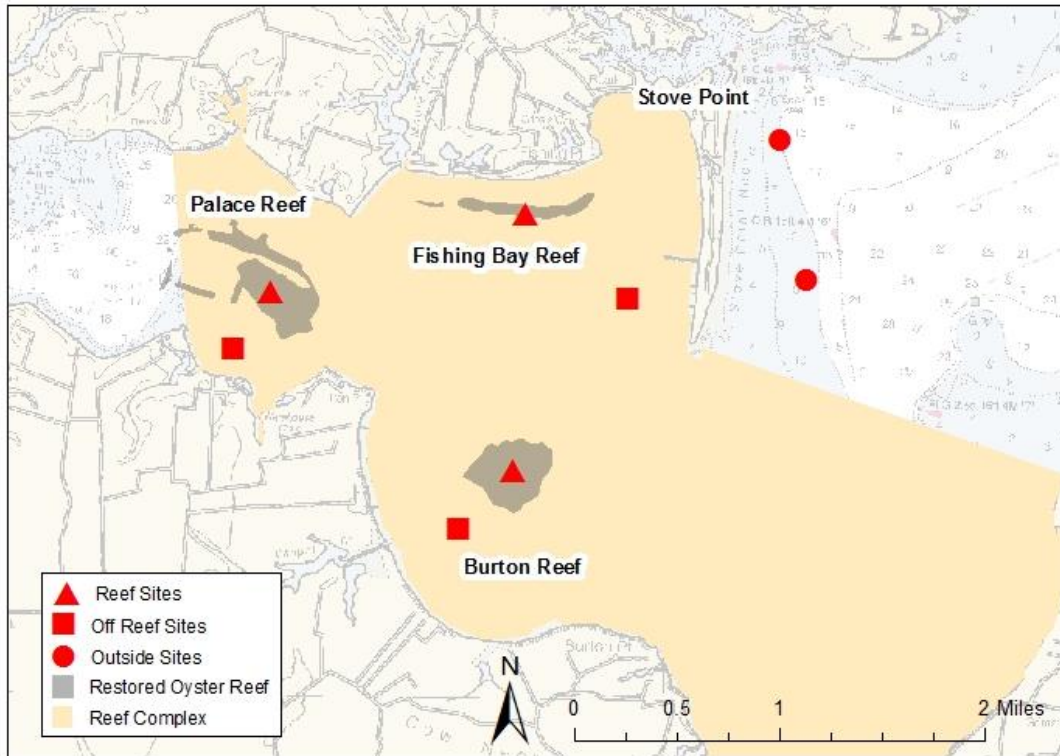Located on western shore of lower Chesapeake Bay.

Figure 2: Map of Sampling Locations

Project sampling sites, restored oyster reefs, and delineated reef complex polygon in the Piankatank River, VA. Reef complex area, represented by tan polygon, delineated by Piankatank River Oyster Restoration workgroup, and based on oyster larvae retention and flow regime. Outside sites are adjacent to, but outside of reef complex area. Off-reef sites are adjacent to and within 1 nautical mile of reef sites.
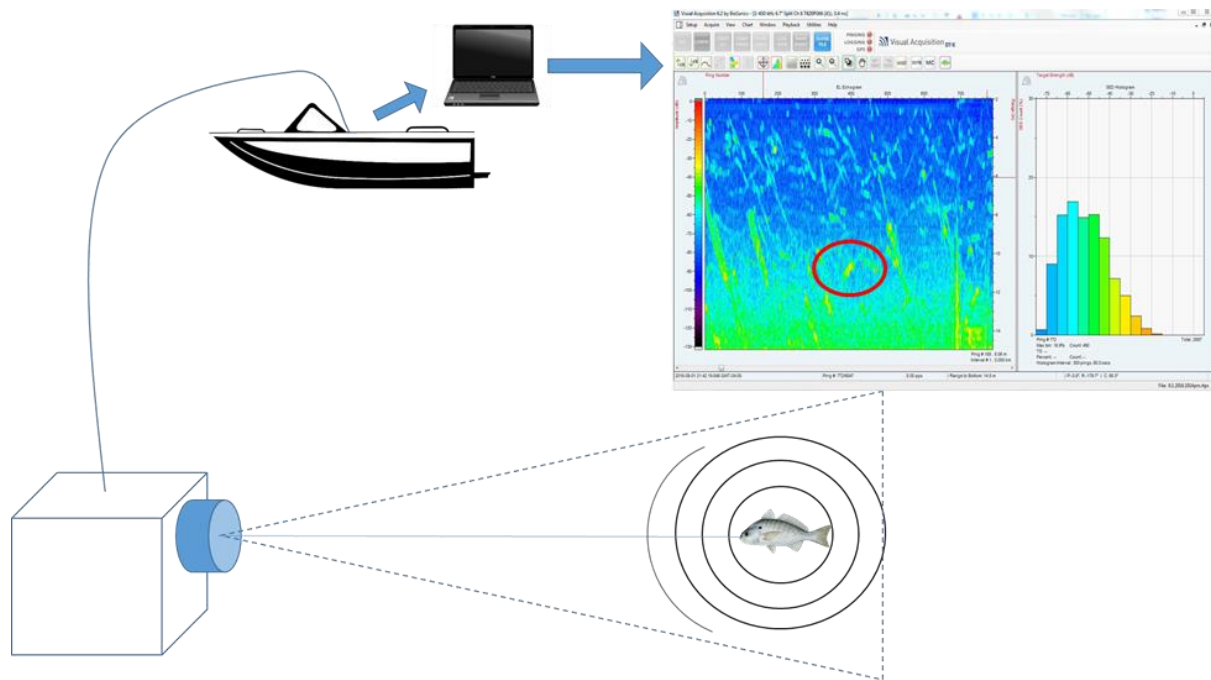
Figure 3: Hydroacoustic Equipment Set Up

Diagram of hydroacoustic equipment field sampling set-up. Figure depicts a stationary frame set on the habitat bottom, with side-scanning transducer attached. A beam angle scans across habitat area, and detects targets. Signals are sent through a receiver, where they are interpreted and displayed on the laptop, for real-time analysis. All data is recorded and saved for later processing.
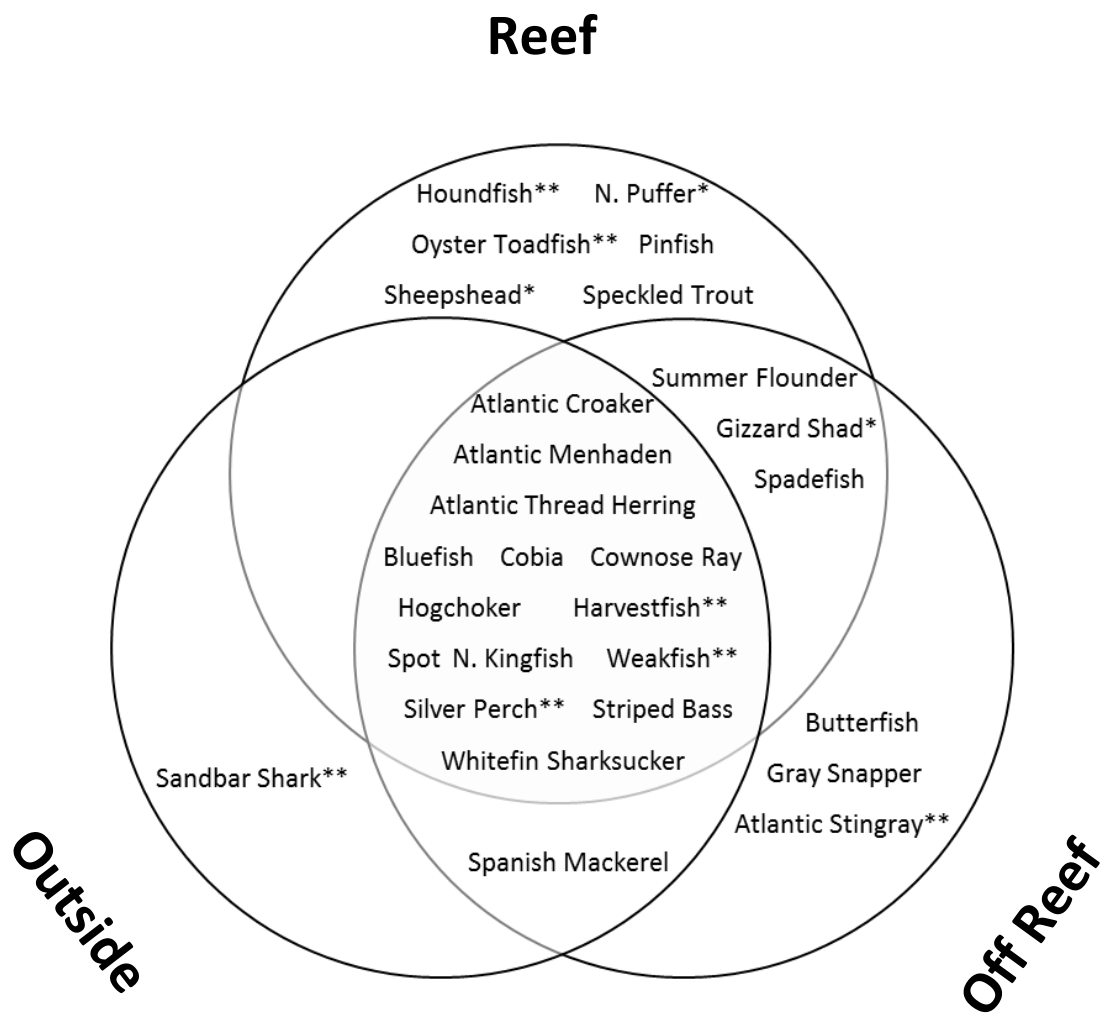
Reef

Houndfish**　　N. Puffer*

Oyster Toadfish**　Pinfish

Sheepshead*　　Speckled Trout

Summer Flounder

Atlantic Croaker

Gizzard Shad*

Atlantic Menhaden

Spadefish

Atlantic Thread Herring

Bluefish　Cobia　Cownose Ray

Hogchoker　　Harvestfish**

Spot N. Kingfish　　Weakfish**

Silver Perch**　Striped Bass

Butterfish

Whitefin Sharksucker

Gray Snapper

Sandbar Shark**

Atlantic Stingray**

Spanish Mackerel

Outside

Off Reef

Figure 4: Venn Diagram of Species Composition for Each Habitat Type

*Denotes species only caught during the day
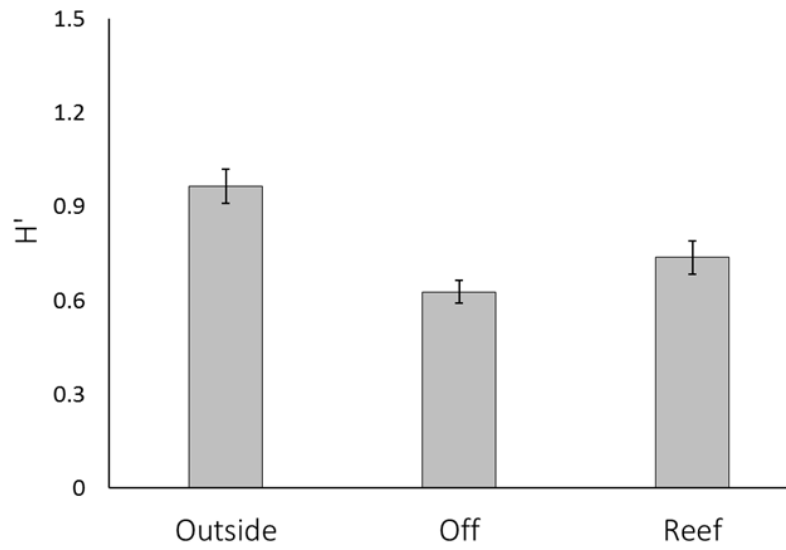**Denotes species only caught at night

Figure 5: Fish Diversity by Habitat Type

Fish diversity by habitat type, calculated using Shannon-Weiner Index (H').
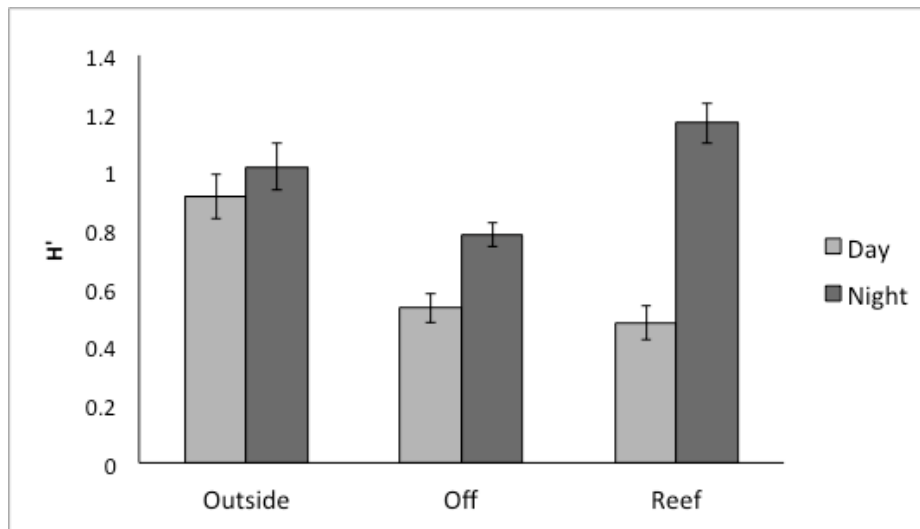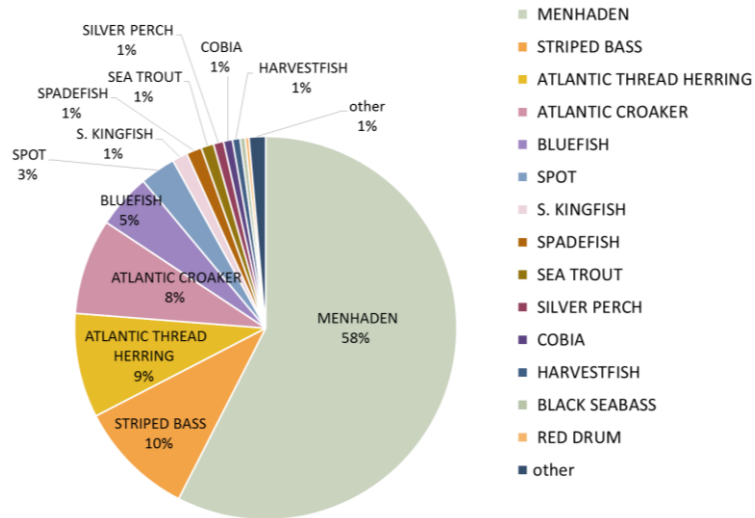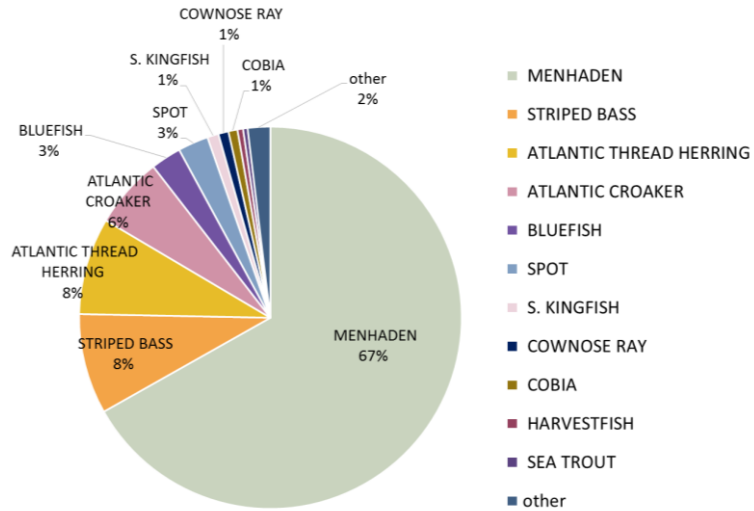
Figure 6: Fish Diversity by Habitat Type for Day and Night

Fish diversity by habitat type for Day and Night, calculated using Shannon-Weiner Index (H').

On Reef

SILVER PERCH
1%   COBIA
                     1%   HARVESTFISH
SEA TROUT   1%
1%
SPADEFISH   other
1%   1%
S. KINGFISH
1%
SPOT
3%

BLUEFISH
5%
                                              MENHADEN
ATLANTIC CROAKER   58%
8%

ATLANTIC THREAD
HERRING
9%

STRIPED BASS
10%

- MENHADEN
- STRIPED BASS
- ATLANTIC THREAD HERRING
- ATLANTIC CROAKER
- BLUEFISH
- SPOT
- S. KINGFISH
- SPADEFISH
- SEA TROUT
- SILVER PERCH
- COBIA
- HARVESTFISH
- BLACK SEABASS
- RED DRUM
- other

Off Reef

                     COWNOSE RAY
                     1%
        S. KINGFISH   COBIA
        1%   1%
              SPOT   other
              3%   2%
BLUEFISH
3%
ATLANTIC
CROAKER
6%
ATLANTIC THREAD
HERRING   MENHADEN
8%   67%

STRIPED BASS
8%

- MENHADEN
- STRIPED BASS
- ATLANTIC THREAD HERRING
- ATLANTIC CROAKER
- BLUEFISH
- SPOT
- S. KINGFISH
- COWNOSE RAY
- COBIA
- HARVESTFISH
- SEA TROUT
- other

Outside

                         HARVESTFISH
          SANDBAR SHARK   1%   STRIPED BASS
COBIA   1%   1%
1%   SEA
COWNOSE RAY   TROUT
2%   1%
ATLANTIC CROAKER   other
5%   1%
BLUEFISH
5%

SPOT
6%
                                              MENHADEN
S. KINGFISH   55%
8%

ATLANTIC
THREAD
HERRING
13%

- MENHADEN
- ATLANTIC THREAD HERRING
- S. KINGFISH
- SPOT
- BLUEFISH
- ATLANTIC CROAKER
- COWNOSE RAY
- COBIA
- SEA TROUT
- SANDBAR SHARK
- HARVESTFISH
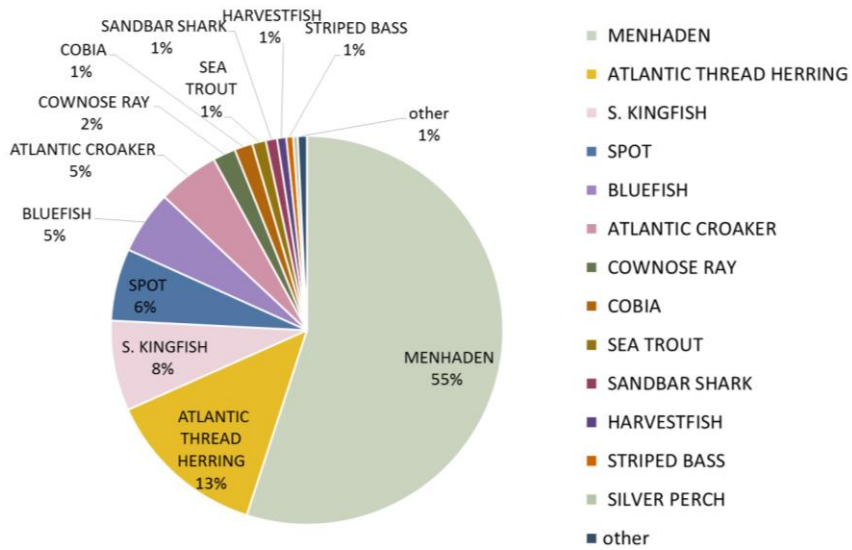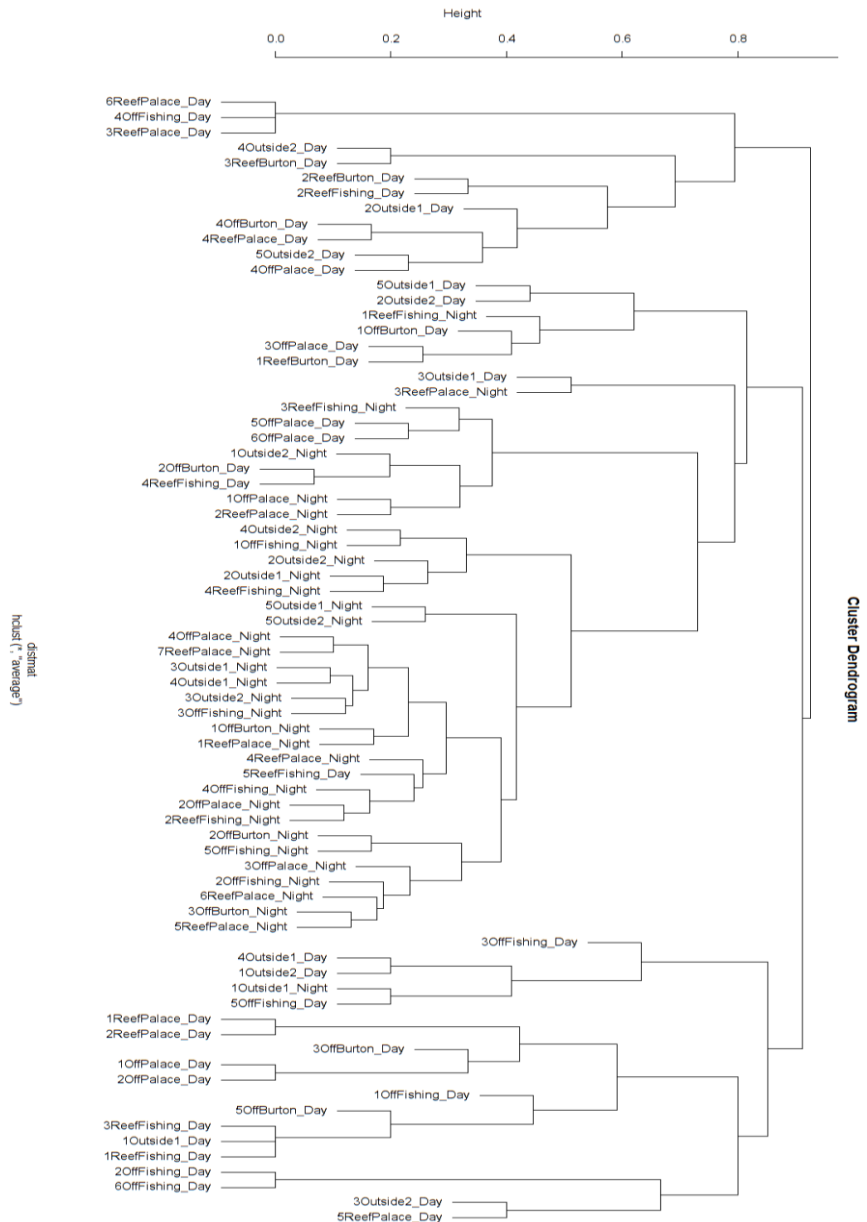- STRIPED BASS
- SILVER PERCH
- other

Figure 7: Species Composition by Habitat Type

Figure 8: Cluster Dendrogram by Gill Net Set

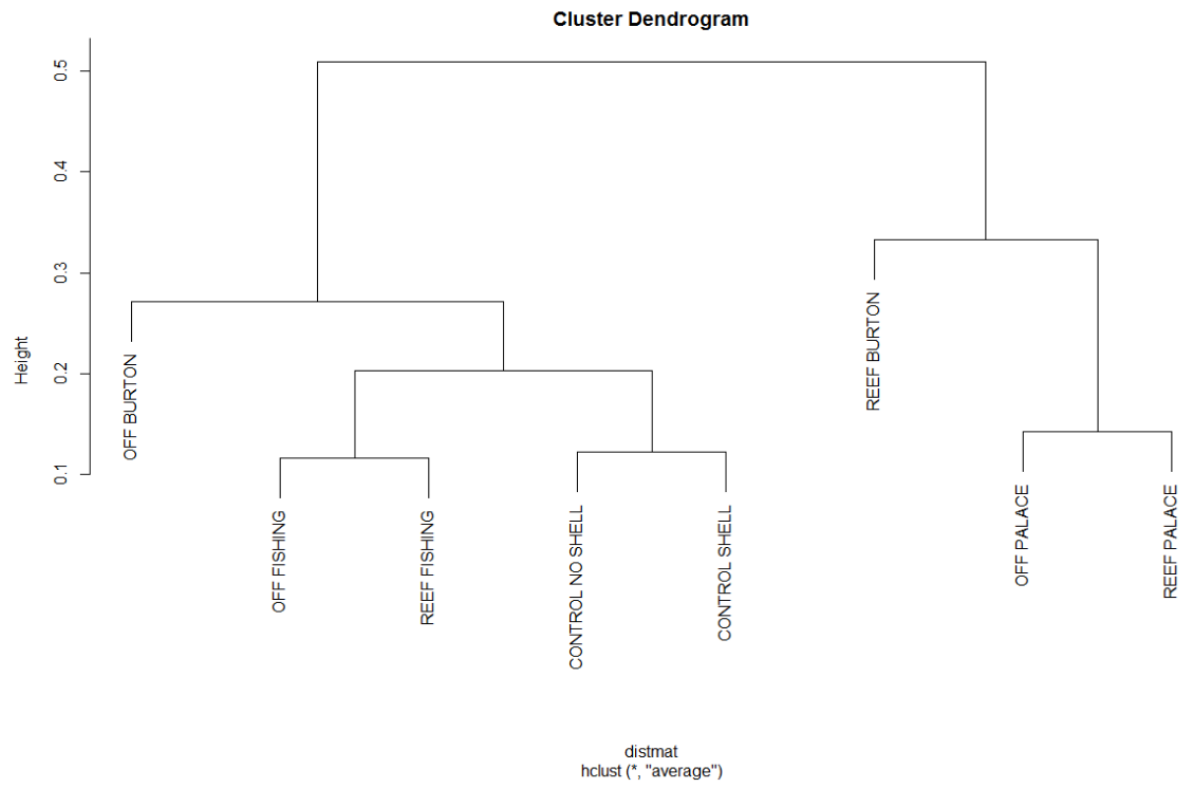Clustering based on a Bray-Curtis dissimilarity matrix of Species CPUE by each gill net set.

Figure 9: Cluster Dendrogram by Sampling Site.

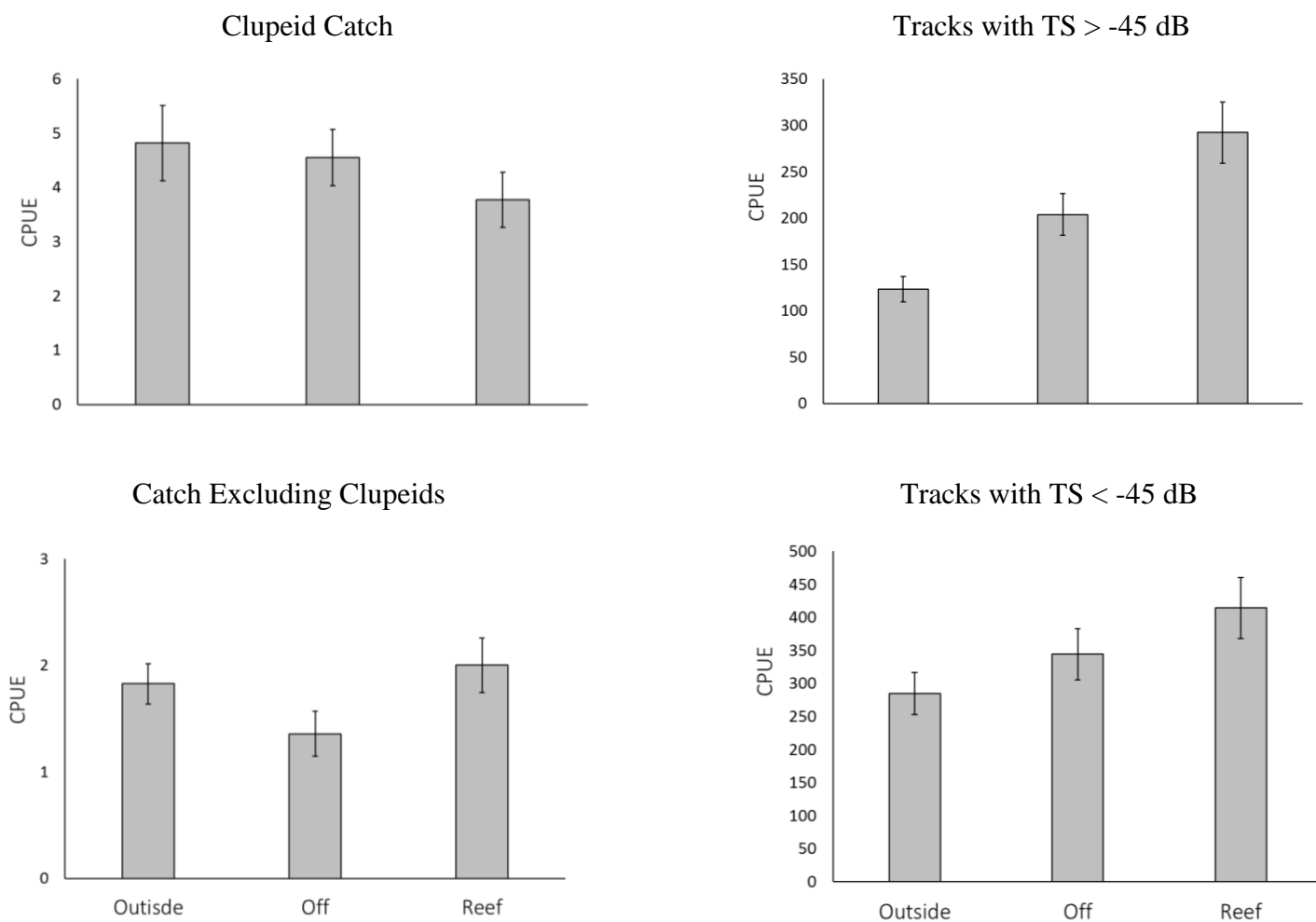Clustering based on Bray-Curtis dissimilarity matrix on species CPUE of each sampling site

Clupeid Catch

Tracks with TS > -45 dB

Catch Excluding Clupeids

Tracks with TS < -45 dB

Figure 10: Fish Density by Habitat Type.

Density by habitat type, in mean catch per unit effort ± standard error from gillnet data and hydroacoustic tracks.
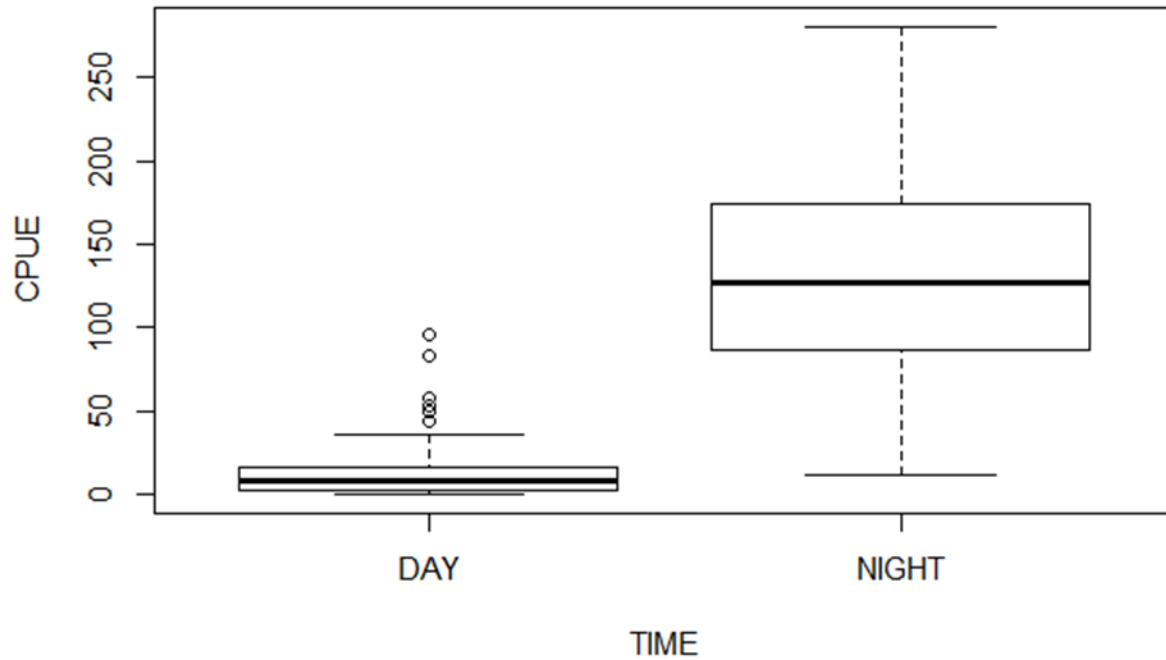
Figure 11: Total Fish Density for Day vs Night Sampling Events from Gillnet Catch CPUE in mean catch per unit effort ± standard error. The difference was significant ($p = 0.001$).
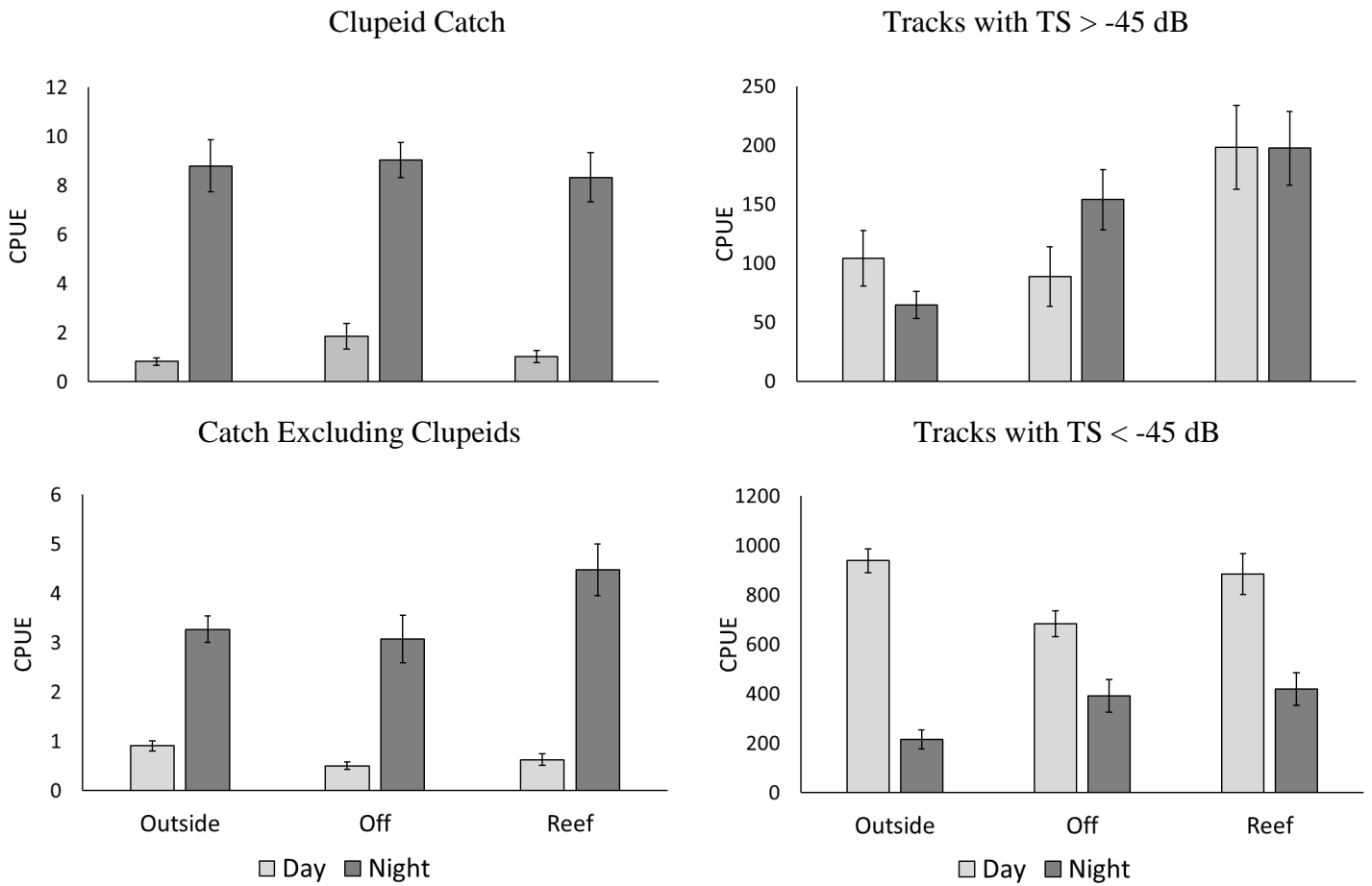
Figure 12: Day and Night Fish Density for Each Habitat Type.

Density by habitat type, in mean catch per unit effort ± standard error from gillnet data and hydroacoustic tracks.