COMMUNITY STRUCTURE AND FEEDING ECOLOGY OF FISHES ON

ARTIFICIAL REEFS IN THE NORTHWEST GULF OF MEXICO

A Thesis

by

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ABSTRACT

Artificial reefs serve as important habitat for several marine fish species in the northwest Gulf of Mexico (NW GoM). Structure type, relief, and depth of artificial reefs have been shown to affect the community composition and trophic relationships of reef associated fishes. The purpose of this study is to investigate these relationships using a variety of metrics examining both fish assemblage and trophic ecology on several nearshore artificial reefs in the northwest Gulf of Mexico. Chapter I uses a suite of traditional fisheries methods to observe the effects of individual structure on the assemblage of marine fish. We investigated three individual reef types (concrete, rig, and ship) using three survey methods (fish trap, vertical longline, and active acoustics) over four years of sampling (2014 -2017). Two reef types, rig and ship, were found to have a more diverse assemblage of fish than concrete reefs using traditional fishing methods (vertical longline and fish trap); however, concrete reefs were found to have higher concentrations of fish using active acoustics. These results indicate that increased reef relief and complexity offer habitat for a wider range of species, while low relief habitats attract less diverse assemblages of fish in higher concentrations. The differences in trophic structure were also investigated on high and low relief structure types in Chapter II. Using both stable isotope and fatty acid analyses we examined the feeding ecology of three reef associated fishes, tomtate (*Haemulon aurolineatum*), pigfish (*Orthopristis chrysoptera*), and red snapper (*Lutjanus campechanus*). The three species were compared on high relief habitats, while one species, red snapper, was also investigated

on low relief habitats. The three species exhibited different feeding strategies using stable isotope values and fatty acid ratios that reflected known diets from other regions. Red snapper feeding ecology was different between the two structure types. Red snapper that were collected on low relief habitats fed on a higher trophic level than those collected on high relief habitat types. This difference among the structure types may be due to the lack of intraguild competition that may occur on more diverse high relief reefs relative to less diverse low relief reefs. Overall results suggest that artificial reef structure type and design may support unique assemblages and provide different functions to reef associated species.

DEDICATION

For my family, who has encouraged and supported me throughout my young career. As well as for Sarah, who gave me unconditional love and support.

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CONTRIBUTORS AND FUNDING SOURCES

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CHAPTER I: INTRODUCTION

The goals of using artificial substrates range, from building barriers against erosion, creating vertical structure, enhancing biodiversity, and increasing fisheries yield (Baine, 2001). For fish and other marine fauna, artificial reefs serve as important habitat in coastal ecosystems (Baine, 2001; Bohnsack and Sutherland, 1985; Pickering and Whitmarsh, 1997). Artificial reefs have been shown to increase habitat complexity which promotes recruitment of sessile benthic organisms (Dupont, 2008; Grossman et al., 1997), and can reduce the risk of predation (Shulman, 1984; Svane and Petersen, 2001). However, the role of artificial reefs as a source of production attracting and concentrating reef associated organisms is uncertain (Brickhill et al., 2005; Powers et al., 2003).

Artificial reefs serve as important habitat for reef associated species in the northern Gulf of Mexico (Rooker et al., 1997; Streich et al., 2017b). Fish assemblage on artificial reefs are affected by both increased reef relief (Rilov and Benayahu, 2000) complexity (Gorham and Alevizon, 1989; McClanahan, 1994). Artificial reefs have been shown to mimic the assemblage of fishes that utilize natural reefs, which make artificial reefs usable analogs for studying these communities in a controlled setting (Granneman and Steele, 2015). Elements that affect the fish assemblage on artificial reefs also affect the trophic structure as well, promoting foraging across multiple trophic groups (Rooker et al., 1997), and can lead to competition over limited adjacent resources due to high densities of fish (Shipley and Cowan Jr, 2011).

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The specific goals of this research are to examine the influence of artificial structure type on the assemblage and trophic structure of reef fishes. The northwest Gulf of Mexico (NW GoM) contains both natural and artificial reef structures that support a diversity of fishes (Ajemian et al., 2015a; Rooker et al., 1997; Streich et al., 2017a). However, there are data deficiencies for the nearshore assemblages that utilize nearshore artificial reefs on the northern Texas coast, which require further study (Chapter 1). In addition to a lack of information regarding the assemblage of fishes, there is little information regarding the trophic structure of fish that utilize these habitats in this region, and if structure type plays an important role in the feeding ecology of reef associated fishes (Chapter 2). We hypothesize that, both assemblage and trophic structure are altered depending on reef types, and that these relationships are species specific in the NW GoM.

CHAPTER II: COMMUNITY STRUCTURE

Synopsis

Quantitative surveys of fishes associated with artificial reefs in the northwest Gulf of Mexico (NW GoM) were conducted over a four-year period (2014 - 2017). Artificial reefs were comprised of three types including concrete structures, toppled rig jacket, and decommissioned ships. Reefs were surveyed using vertical long line (VLL), fish traps, and dual frequency identification sonar (ARIS 1800). VLL fishing was accomplished with replicated five-minute soaks, while traps consisted of replicated one-hour soaks. Catch per unit effort (CPUE) and diversity were calculated for each gear type. The relative abundance of fishes (CPUE), across all species, significantly differed among reef type and year. CPUE estimates of fishes were highest at toppled rig jackets for both VLL (1.7 ± 2.2 SD, CPUE) and traps (6.2 ± 3.8), while results from sonar surveys indicated the highest relative abundance of fishes on concrete reefs (15.3 ± 26.8 fish*frame^{-1}). Red snapper (n = 792), followed by gray triggerfish (n = 130), pigfish (n = 70), tomtate (n = 69), and hardhead catfish (n = 57) were the most numerically abundant species using VLL and trap, where red snapper comprised 90.7% of total catch using VLL and 43.9% using traps. Mean diversity (H') was highest on toppled rig jackets using both fishing methods (VLL 0.1 ± 0.2 and traps 0.7 ± 0.5). Findings from this study highlight the utility of using multiple gear types to survey reef fish assemblages associated with artificial reefs.

INTRODUCTION

Size and species-selectivity gear bias can hinder the ability of single gear types to be used to estimate the relative abundance of fishes (Diaz et al., 2003; Willis et al., 2000). Fisheries independent surveys using trawls, longlines, entanglement nets, and traps, can result in varied catch composition with each gear having size and species-specific biases (Wells et al., 2008a). Historically, alternative methods (i.e. active acoustics) to traditional fishing gear (e.g. netting, trapping, and hook and line) were expensive, had poor resolution, and were not powerful enough to count individual fish aside from creating biomass estimates (Jolly and Hampton, 1990). However, increased resolution with the innovation of multi-beam transducers has enhanced the ability to enumerate and identify fishes taxonomically (Holmes et al., 2006). Dual identification sonar (DIDSON) is one tool that allows users to obtain near video-quality imaging to enumerate, identify, and observe fish in habitats that are turbid or inaccessible (Able et al., 2013; Baumann et al., 2016; Boswell et al., 2008; Doehring et al., 2011; Mueller et al., 2006). The northwestern Gulf of Mexico (NW GoM) can be a challenging location to conduct visual surveys due to a strong nepheloid layer (Rezak and Bright, 1985; Shideler, 1981) and high levels of turbidity in coastal waters (Kennicutt, 2017). DIDSON uses a high operating frequency, 1.1 MHz at distances greater than 15m and 1.8 MHz at distances less than 15m, to track moving objects, such as fish, with higher resolution than traditional single transducer sonar techniques (Belcher et al., 2002). Advantages of this technique over others is the ability to observe fish throughout the

water column allowing them to remain undisturbed to observe relationships that would normally be affected by removal from their environment (i.e. fishing, netting) (Boswell et al., 2010). Relative abundance estimates using active acoustics, in conjunction more traditional sampling techniques can help to evaluate fish community structure, especially when visual surveys are not an option.

Fisheries independent surveys are often used in attempt to remove the bias of catch data normally associated with fisheries dependent sampling (Rotherham et al., 2007). Fisheries independent surveys using vertical longlines (VLLs) is commonly used to sample fishes associated with artificial reefs in the northern GoM (Gregalis et al., 2012), but has been shown to be strongly biased towards red snapper (*Lutjanus campechanus*) (Scott-Denton et al., 2011). Fish traps are also effective tools for sampling fishes associated with complex structures (Newman and Williams, 1995) and can sample a broader range of size classes and species than VLLs (Streich et al., 2017a), but are still size selective based upon the size of the trap and mesh (Wells et al., 2008a). Acoustic surveys are effective at estimating relative fish abundance and biomass (Boswell et al., 2010) and are highly effective in turbid environments (Mueller et al., 2006) but have limited taxonomic resolution (Mueller et al., 2010) and may fail to accurately represent abundance of more sedentary benthic fishes (Able et al., 2014). Thus, a combination of these techniques can balance the shortcomings and caveats of their counterparts, and be used to better estimate fish assemblage structure.

Fish assemblages associated with artificial reefs are affected by differences in reef structure, depth, and size of habitats (Perkol-Finkel et al., 2006; Rilov and

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Benayahu, 2000; Rooker et al., 1997; Strelcheck et al., 2005). The factors affecting the assemblage of fishes on artificial reefs has been widely studied in the northern GoM and found to have similar drivers to other reef systems with vertical relief, rugosity, and available surface for colonizing organisms being among the most important (Ajemian et al., 2015a; Boswell et al., 2010; Dance et al., 2011a; Gregalis et al., 2012; Rooker et al., 1997). Large marine structures used to create artificial reefs (i.e. free standing rig jackets, decommissioned ships) with high vertical relief and complex structure, harbor diverse fish assemblages off the Texas coast (Ajemian et al., 2015a), and throughout the GoM (Boswell et al., 2010; Reynolds et al., 2018). While smaller, low lying concrete structures (quarry rocks, pyramids, reef balls) have been widely implemented to supplement benthic structure across the region (Dance et al., 2011a; Strelcheck et al., 2005) including the Texas coast (Arney et al., 2017; Streich et al., 2017a). Low lying reefs have also been shown to attract similar assemblages of fish to high relief habitats; however, these assemblages differ in demography, composition, and reduced diversity (Rilov and Benayahu, 2000).

Artificial reef programs have been widely implemented in coastal areas to supplement or replace benthic habitat (Baine, 2001). In 1984, the United States implemented the "National Fishing Enhancement Act" (NEFA) calling for the responsible and effective use of artificial reefs to enhance recreational and commercial fisheries. Within the northern GoM there are numerous artificial structures that serve as potential reef habitat including nearly 3,000 individual active oil platforms, 515 of which have been decommissioned to be exclusively used as artificial reefs (as of 2012,

www.bsee.gov). Individual states have taken responsibility for many reefing programs

often permitting reefing sites in addition to decommissioning freestanding marine

structures. In Texas, the Texas Parks and Wildlife Department (TPWD) has deployed

artificial reefs on the inner continental shelf off the Texas coast since 1990

(www.tpwd.texas.gov) in response to NEFA. According to TPWD, three programs

operating within the artificial reef program have been developed to help with the design

and implementation of these artificial reefs within the scope of the greater Artificial Reef

Program: Rigs-to-Reefs, Ships-to-Reefs, and the Nearshore Reefing program. TPWD's

goal of creating these artificial reefs was to supplement the NW GoM's low density of

natural reef structures. Artificial reefs monitored by TPWD consist of structures

including ships, rocks and prefabricated concrete, and toppled rig jackets. These

artificial reef structures, differ with respect to vertical relief, water depth, proximity to

shore, and are used to accomplish different goals of the Artificial Reef Program, such as

increasing local fishery production and tourism. This network of diverse reef structures

along the Texas coast provides an ideal location for examining the effects of reef

structure on associated reef fish assemblages.

Surveys of fishes on nearshore artificial reefs along the northern Texas coast is

currently limiting and been focused along the mid to south Texas coastline (Ajemian et

al., 2015a; Arney et al., 2017; Rooker et al., 1997; Streich et al., 2017a; Streich et al.,

2017b). Only two other studies have addressed the questions of gear selectivity on fishes

associated with artificial reefs of Texas (Ajemian et al., 2015b; Streich et al., 2018) and

another on the validity of acoustic techniques to assess fish biomass (Bollinger and

Kline, 2017). Objectives of this study were to use traditional fisheries methods and active acoustics to observe temporal and spatial differences in assemblage structure across three different artificial reef structures. We hypothesize that due to the increased rugosity and depth, toppled rig jackets and ships will have higher diversity and biomass than low lying concrete reefs in the NW GoM.

MATERIALS AND METHODS

Surveys

 Quantitative fisheries independent surveys were conducted at eight artificial reef

sites, representing three reef types including toppled concrete structures, rig jackets, and

ships (Figure A.1). Reef sites varied between both depth (m) and distance from shore

(km). Concrete (A, B, and C) were sites with low relief (< 2 m) and consisted of hard

structure, including quarry rock, buoy pieces, experimental reef pyramids, and concrete

anchors and were located in the northern region of the sampling area. Rig (A, B, and C)

were artificial reefs primarily constructed from toppled rig jackets, cut-off rig jackets,

and concrete blocks located in the central region of the sampling area. Ship (A and B)

were reef structures comprised of decommissioned sunken ships, toppled rig jackets, and

concrete pyramids located further south than the other reef types (Figure A.2). Abiotic

parameters including both salinity (psu) and temperature (C°) were first measured at

maximum depth each site. Each site was then sampled using VLL and fish trap

replicated in triplicate. VLL fishing was accomplished using bandit reels spooled with

136 kg test mainline and outfitted with a 7.3 m backbone of 181 kg test monofilament

containing ten, 45 kg test, 45.7 cm gangions. The terminal end of the backbone was

weighted with a 3.6 kg mushroom weight and each gangion was baited with cut Atlantic

Mackerel (*Scomber scombrus*). VLL sampling of each replicate consisted of four

separate drops of a backbone outfitted with gangions of one hook size (2/0, 8/0, 11/0,

and 15/0) which was fished haphazardly on one side of the boat for five minutes. In

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addition to VLL, two small fish traps (63.5 cm width x 96.5 cm length x 50.8cm height, 1.6 cm mesh with two 15.2 cm x 13.7 cm openings) also baited with cut Atlantic Mackerel, were concurrently deployed at each replicate, and soaked for one hour. All fish, after being caught, were identified, enumerated, and measured to the nearest mm fork length (FL).

On non-concurrent sampling events, a five-minute drift transect survey using the ARIS (ARIS 1800, SoundMetrics) sonar was conducted at each site with three replicates to estimate the relative abundance of fish (fish*frame^{-1}). The ARIS unit was attached to a rotating arm (AR2, SoundMetrics) for axis control, and to a hydrofoil prior to being submerged for each drift transect. Once submerged, the ARIS unit was pointed downwards (angle varied from 0 - 20°) towards the target structure and set to record (Figure A.1.). Each site was visited at least once during the first two years (2014 and 2015) with additional surveys of select reefs (Rig B and Rig C) in the following two years (2016 and 2017).

Catch from the VLL and trap surveys were converted into catch per unit effort (CPUE) to estimate species abundance by gear type at each site (Table A.1). Because effort differed between the two gear types, (5 minute VLL soak vs. 1 hour trap soak), they were not comparable statistically. To identify trends in less abundant species, CPUE was 4th root transformed and used to create a Bray-Curtis similarity matrix. Two-factor permutational MANOVA (PERMANOVA) was then used on the matrix in PRIMER v.7 (Clarke and Gorley, 2015) to examine relative abundance using reef type and year. Paired with two-factor PERMANOVAs were SIMPER analysis, which were

analyses used to identify individual species driving the differences in assemblage structure among reef type and year. Two-factor nested PERMANOVAs were also conducted, nesting site within reef type, and year within reef type, to determine differences in assemblage structure among sites and years within reef type. In addition to identifying assemblage differences using relative abundance, biodiversity indices and size selectivity analyses were used to differentiate the effectiveness of each gear type. Differences in diversity were identified using the Brillouin's diversity index (H_B). The Brillouin index is calculated as:

$$H_B = \frac{\ln N! - \sum_{i-1}^{s} \ln n_i!}{N}$$

where N is the total number of individuals in the sample, n_i is the number of individuals belonging to the *ith* species, and *s* is the number of species (Brillouin, 1962). Size selectivity between fishing gear types were investigated using a one-way ANOVA between species FL and gear type (VLL or Trap) for the three species that were caught at least two times by each gear type. The species used for size selectivity analyses were red snapper, gray triggerfish (*Balistes capriscus*), and hardhead catfish (*Ariopsis felis*), and significance for all statistical tests was determined at 0.05.

Transect Analysis

Acoustic transect data were imported using ARIScope v2.0 (SoundMetrics). Each ARIS transect was preprocessed using 1) platform motion, 2) beam pattern correction, and 3) crosstalk reduction functions to maximize the clarity of each individual frame. Transects were then reviewed to determine if the structure and fish could be qualitatively separated from background noise. Individual frames

containing structure were then isolated using video software VLC (VideoLAN). One frame in fifty (2%) were analyzed sequentially from the original transect video and used for analysis, which is comparable to other non-automated surveys using DIDSON (Grote et al., 2014; Makabe et al., 2012). All frames to be analyzed were then imported into Image-J for analysis. Frames were first set to scale as it was recorded, and then the color threshold was adjusted to maximum contrast to separate background from structure and fish. The analyze particle function was then set to identify particles between 0.005 m^2 and 0.5 m^2 to avoid identifying objects that were too large (structure) or too small. Particles isolated via the identify particles function were qualitatively reviewed, to omit background noise or structure, and counted. Analysis was conducted on the total fish counted per frame (fish*frame^{-1}). Fish*frame^{-1} was compared across reef types, years, and sites using Kruskal-Wallis models due to lack of homogeneity of variance of fish counts and zero inflation. Individual tests were followed by a Mann-Whitney pairwise analysis and used to elucidate individual differences in relative abundance among reef type.

Further investigation of species abundance, gear type, and structure type was conducted using redundancy analysis (RDA) (Lewis et al.), which is a method of direct gradient analysis using CANOCO (ter Braak and Smilauer, 2012). Correlations between variables (gear type, reef type, salinity, temperature, and distance from shore) and canonical axes were used to explain each variable's contribution to the total abundance of fish species, and for those species that were caught more than once during sampling (Table A.2). Each axis used in the analysis was derived from an iterative reciprocal weighted

averaging of species abundance among samples in addition to the ordination scores for

the linear combinations of categorical variables.

RESULTS

Species comparison

A total of 72 fishing survey replicates across three varied reef types (Concrete n=24; Rig n=33; Ship n=15) were analyzed for this study along with 39 acoustic surveys replicates (Concrete n=9; Rig n=21; Ship n=9). Relative abundance among all fish species (total CPUE) was highest on toppled rig jackets for both VLL (1.7 ± 2.2 SD, CPUE) and traps (6.2 ± 3.8) (Figure A.3). Abiotic variables, salinity (concrete, 34.35 ± 1.85 ppt; rig, 35.72 ± 0.83 ppt; ship, 35.42 ± 2.21 ppt), temperature (concrete, 28.94 ± 2.33 C°; rig, 28.42 ± 1.54 C°; ship, 28.27 ± 1.53 C°), and distance from shore (concrete, 37.05 ± 3.69 km; rig, 38.89 ± 8.60 km; ship, 33.64 ± 23.55 km) were not found to be significantly different. However, depth ($F\text{-value}_{2,24} = 15.62$, p-value < 0.001) significantly differed with concrete reefs (12.88 ± 1.25 m) being significantly shallower than both rig (20.61 ± 1.42 m) and ship reefs (23.52 ± 8.21 m). A total of 1,185 fishes were collected in fishing surveys representing 66.8% red snapper (n = 792), 11.0% gray triggerfish (n=130), 5.9% pigfish (*Orthopristis chrysoptera*) (n=70), 5.7% tomtate (*Haemulon aurolineatum*) (n=69), 4.8% hardhead catfish (n=57), 1.3% lane snapper (*Lutjanus synagris*) (n=15), 1.0% Atlantic croaker (*Micropogonias undulatus*) (n=12), 1.0% sand seatrout (*Cynoscion arenarius*) (n=12), 0.74% pinfish (*Lagodon rhomboides*) (n=9), 0.6% spadefish (*Chaetodipterus faber*) (n=7), 0.3% oyster toadfish (*Opsanus tau*) (n=4). Remaining species (black drum (*Pogonias cromis*), sharksucker (*Echeneis naucrates*), blue runner (*Caranx crysos*), Atlantic sharpnose shark (*Rhizoprionodon*

terraenovae), gray snapper (*Lutjanus griseus*), southern kingfish (*Menticirrhus americanus*), gag grouper (*Mycteroperca microlepis*), Atlantic bumper (*Chloroscombrus chrysurus*), and spinner shark (*Carcharhinus brevipinna*)) were all caught once (Table A.2). Of the three species analyzed for size selectivity between gear types, both red snapper ($F_{1,724}$ = 490.64, p-value < 0.001) and gray triggerfish ($F_{1,127}$ = 35.38, p-value < 0.001) had larger fish caught using VLL (373 ± 76 and 358 ± 49) than caught in fish traps (251 ± 54 and 270 ± 74), respectively (Table A.2). No significant difference in size was found for hardhead catfish collected between gear types ($F_{1,53}$ = 0.235, p-value = 0.630).

Fishing Surveys

Fish assemblage assessed from VLL surveys significantly differed across reef types (pseudo-$F_{2,193}$ = 6.736, p = 0.001) and years (pseudo-$F_{3,192}$ = 2.275, p = 0.036). Significant differences existed in fish assemblages on rig reefs (t = 2.718, p = 0.001) and ships (t = 3.730, p = 0.001) compared to concrete reefs, with no significant difference between rig reefs and ships. Fish assemblages differed between 2014 and 2015 (t = 2.093, p = 0.012) as well as 2014 and 2017 (t = 2.016, p = 0.005), but not other years. Sites also significantly differed when nested within reef type (pseudo-$F_{7,186}$ = 2.411, p = 0.008), where toppled rig reefs Rig - B (t = 3.135, p = 0.003) and Rig - C (t = 2.457, p = 0.007) had significantly different assemblage of fish than Rig - A; all other pairwise relationships were non-significant. Year was also significantly different when nested within reef type (pseudo-$F_{7,186}$ = 2.006, p = 0.016), where fish assemblages on toppled

rig reefs in 2014 differed from rig reefs in 2017 (t = 1.961, p = 0.016), all other pairwise relationships were non-significant.

Species driving the differences among reef types and years were primarily red snapper and gray triggerfish. Rig reefs had higher abundances of red snapper (1.52 ± 2.12; CPUE ± SD) than ships (0.88 ± 1.45 CPUE; mean dissimilarity 21.13%), but lower abundance than on concrete reefs (1.64 ± 2.00 CPUE; mean dissimilarity 13.34%). Similarly, ship artificial reefs had lower relative abundances of red snapper than concrete reefs (0.88 ± 1.5; mean dissimilarity 20.28%), but higher abundances of gray triggerfish (0.17 ± 0.50 CPUE) than both concrete (0.13 ± 0.51 CPUE; mean dissimilarity 10.04%) and rig reefs (0.01 ± 0.11 CPUE; mean dissimilarity 13.04%) (Figure A.5). The average relative abundance of red snapper collected with VLL doubled from 2014 (0.92 ± 1.39; mean dissimilarity 18.06%) to 2017 (1.96 ± 2.60; mean dissimilarity 13.75%). Relative abundance of red snapper was also higher in 2015 (1.67 ± 1.92; mean dissimilarity 14.21%) than 2016 (1.22 ± 1.92; mean dissimilarity 13.84%). Species diversity (H_B) using VLL among years was highest in 2014 (0.47 ± 0.33) followed by 2015 (0.27 ± 0.25) and 2016 (0.23 ± 0.34), with the lowest diversity in 2017 (0.09 ± 0.18). Species diversity was highest on ships (0.57 ± 0.16 SD), followed by toppled rigs (0.37 ± 0.31 SD) and concrete reefs (0.09 ± 0.12) (Figure A.4).

When examining fish abundance from trap surveys, fish assemblage structure significantly differed by reef type (pseudo-$F_{2,101}$ = 5.501, p = 0.001) and year (pseudo-$F_{3,100}$ = 2.403, p = 0.014). Similar to results from VLL surveys, fish abundance was higher at rig reefs (t = 2.921, p = 0.001) and ships (t = 2.357, p = 0.004) than at concrete

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reefs, while there was no difference between rig and ship reefs. Concrete reef sites significantly differed (pseudo-$F_{6,95}$ = 4.136, p = 0.001) when site was nested within reef type. Fish assemblage on Concrete - A differed from Concrete – B (t = 3.695, p = 0.001), and Concrete - B fish assemblage differed from Concrete - C (t = 3.667, p = 0.001). Similar to results using VLL, rig reef sites also significantly differed when nested within reef type, where fish assemblage differed at Rig - B (t = 2.141, p = 0.004) and Rig - C (t = 2.003, p = 0.002) compared to Rig - A. Year (pseudo-$F_{7,94}$ = 3.022, p = 0.001) was a significant factor when nested within reef type using trap surveys. For concrete reefs, 2015 differed in fish assemblage from 2017 (t = 1.829, p = 0.045), with no other differences occurring among years. For rig reefs, only 2014 and 2015 had similar fish assemblages, 2014 differed from 2016 (t = 2.286, p = 0.002) and 2017 (t = 2.563, p = 0.001), 2015 differed from 2016 (t = 1.703, p = 0.024) and 2017 (t = 2.631, p = 0.001), and 2016 differed from 2017 (t = 2.731, p = 0.009). In contrast to results using VLL, fish assemblages in traps for concrete reefs (1.0 ± 2.3 CPUE) had lower abundances of red snapper than both rig reefs (2.5 ± 3.8 SD CPUE; mean dissimilarity 27.78%) and ship reefs (1.1 ± 1.8 CPUE; mean dissimilarity 37.67%).

Concrete reefs had higher abundance of hardhead catfish (0.42 ± 0.78 CPUE) compared to rigs (0.32 ± 0.79 CPUE; mean dissimilarity 16.13%) and ships (0.03 ± 0.18 CPUE; mean dissimilarity 20.61%) (Figure A.6). Differences in relative abundances of red snapper and gray triggerfish varied between rigs and ship habitat types. Gray triggerfish abundance was higher on rig reefs (0.95 ± 1.97 CPUE; mean dissimilarity 12.40%) than on ships (0.47 ± 1.12 CPUE). red snapper, Gray triggerfish, and pigfish

abundance differed among years. Red snapper abundance was higher in 2014 (2.48 ±
3.99 CPUE; mean dissimilarity 11.24 %) vs. 2015 (1.44 ± 2.52 CPUE) on all reefs. gray
triggerfish were not collected in traps in 2016 or 2017, and pigfish were not collected in
traps in 2017, however both were collected in 2014 and 2015 (Figure A.6). Species
diversity using traps among years was highest in 2014 (0.96 ± 0.60) followed by 2015
(0.81 ± 0.50) and 2016 (0.19 ± 0.30), with the lowest diversity in 2017 (0.20 ± 0.35).
Species diversity among reef types, was highest on rig reefs (1.01 ± 0.61 SD), followed
by ship reefs (0.83 ± 0.45), and concrete reefs (0.41 ± 0.37) (Figure A.4).

Acoustic Surveys

Relative abundance of fish (fish*frame^{-1}) estimated using ARIS transects
significantly differed among reef type ($x^2 = 55.53$, $p < 0.001$). Acoustic surveys
conducted on concrete structures (n = 9), rigs (n = 21), and ships (n = 9), contained
56,796 frames, of which 90.1% (n = 51,173) met the criteria for analyses, leading to
1033 (2%) frames being analyzed. Pairwise analyses show significant differences exist
among all reef types (p < 0.001), Fish relative abundance was highest on concrete
structures (15.31 ± 26.83 SD), followed by ships (5.84 ± 13.55 SD), and then rigs (4.52
± 10.59) (Figure A.3). Individual differences exist among sites within reef type ($x^2 =$
97.1, $p < 0.001$), with pairwise differences between Concrete - B and the other two sites.
Mean relative abundance (fish*frame^{-1}) was highest on Concrete - B (30.64 ± 33.31)
when compared to the other concrete reefs (Concrete - A; 1.73 ± 2.28, and Concrete – C;
2.63 ± 3.39) so analyses of among reef type differences were rerun excluding Concrete -
B, and were still determined to be significantly different ($x^2 = 12.2$, $p = 0.001$).

Gear type, reef type, salinity, temperature, depth, and distance from shore were significantly correlated with canonical axes (pseudo-F = 15.9, p-value = 0.002). Eigenvalues for the first three multivariate axes were 0.090 (Axis 1), 0.021 (Axis 2), 0.013 (Axis 3), and 0.004 (Axis 4). Pseudo-canonical correlation coefficients for the three axes were 0.538, 0.298, 0.336, and 0.161 respectively. The total cumulative percentage of variance explained by the first four axes was 12.39%. Axes 1 and 2 accounted for 9.04% and 2.10% of the variation, respectively, and 99.12% of the cumulative variance modeled by the RDA (Figure A.7). Fish were ordinated in multivariate space proportionally along the gradients that most affected their abundance. Gray triggerfish, tomtates, lane snapper, and pinfish had positive relationships with rig and ship reefs, increasing depth and salinity, as well as being primarily collected using fish traps. Several other species, oyster toadfish, pigfish, Atlantic croaker, hardhead catfish, and spadefish were also positively associated with trap collections with similar numbers between rig and concrete reefs. Red snapper was positively associated with concrete reefs and VLLs. Sand seatrout also have fewer associations with specific reef type but were primarily collected using VLL.

DISCUSSION

Results of this study highlight fish assemblage structure on artificial reefs in the NW GoM and how they differ according to structure type. Toppled rig jackets and ships were shown to have higher CPUE for multiple species and higher diversity than low relief concrete structures. Increased relief on artificial structures have been hypothesized to change natural reef assemblages in addition to pelagic fish assemblages that are not normally associated with natural reefs (Rilov and Benayahu, 2000). This study concluded that these trends also exist within NW GoM artificial reefs and can be attributed to the sampling done using multiple gear types. Gear types with known bias used in conjunction account for the sampling gaps that occur when gears are used individually (Wells et al., 2008a). Diversity and relative abundance of fish differed among VLL and trap sampling which were shown to target different communities of fish. In addition to more diverse assemblages being found on larger, higher relief structures, Bohnsack et al. (1994) observed that larger higher relief habitats have lower densities of fish than low relief smaller habitats. This was also confirmed through our sampling using ARIS surveys which showed that the highest densities of fish exist on smaller, low relief concrete habitats, vs. larger more rugose structures. This unique description of the fish assemblage on nearshore artificial reefs in the NW GoM illustrates the utility of using three comprehensive techniques to describe the differences in assemblage driven by the structure of individual reefs.

Several estuarine-associated fish species were collected over artifical reefs during the course of this study. Fish species included four sciaenids (Atlantic croaker, sand seatrout, black drum, and southern kingfish), oyster toadfish, pigfish, and pinfish. Benthic substrate on the inner continental shelf in the NW GoM has limited hard structure (i.e. natural reef), and is comprised primarily of mud and sand with interspersed natural shell rubble banks (Rezak et al., 1990). A study conducted by Wells et al. (2009) over a natural drowned barrier island in the NW GoM indicated that the most abundant species collected were predominantly estuarine associated (i.e. sciaenids). Fish assemblages on naturally occurring unconsolidated sand or mud bottom in close proximity to artificial reefs indicate that there is substantial connectivity with surrounding habitat acting as a source of biodiversity to artificial reefs (Bohnsack, 1989). Bohnsack et al. (1994) found that artificial reefs had fish assemblages comprised of species from both surrounding natural reef and surrounding unconsolidated sand habitat. Fish assemblages on artificial reefs have also been described as having high levels of transience with "settled" fish accounting for very little biomass (5.7%) (Bohnsack et al., 1994). This connectivity serves as an important role for the implementation of artificial reefs and is often the goal of managers when weighing potential benefits to regional fisheries (Pickering and Whitmarsh, 1997). Continued supplementation of nearshore artificial reefs in the NW GoM can potentially increase connectivity among fish assemblages that utilize these natural low relief sand and mud features with reef fishes commonly found throughout other parts of the GoM.

Fish assemblages on artificial reefs observed in this study were similar to findings in other regions of the GoM using similar gear. Species richness on artificial reefs has been reported to be high using visual gear type (i.e. ROV surveys) in the NE and NW GoM (Ajemian et al., 2015a; Dance et al., 2011a). Sampling with entrapment or entanglement gear reduces diversity estimates due to the effect of gear bias when independent unique gears are used (Connell et al., 1998). VLLs are an effective tool for collecting predatory marine fish such as red snapper (Gregalis et al., 2012; Streich et al., 2018). Gregalis et al. (2012) found using VLLs, the assemblages of artificial reefs off the coast of Alabama were dominated by red snapper comprising 87% of total catch with 19 other species comprising the remaining 13%. Similarly, in this study, red snapper comprised 90.7% of all fish caught on VLLs, while 11 other species comprised the remainder of the total catch. Fish traps were effective at collecting a more diverse assemblage of fishes than VLLs. Likewise, small fish traps had a species richness of 14 species over the course of post-reefing surveys on prefabricated concrete pyramids off the Texas coast, while VLL only collected 7 species (Streich et al., 2017a). While small fish traps have been shown to collect a diverse fish assemblage, albeit smaller fish than VLL, red snapper was still by far the most dominant species in fish traps comprising 43.9% of all fish caught. High proportions of red snapper on artificial reef assemblages in the northern GoM has been well documented using alternative methods aside from fishing gear (Dance et al., 2011a; Redman and Szedlmayer, 2009; Wells and Cowan, 2007). Red snapper have also been shown higher densities on artificial structures relative to other structures (e.g. natural reefs and unconsolidated sand and mud bottom)

(Karnauskas et al., 2017; Streich et al., 2017b) and their high densities may play a role

in shaping the assemblage structures on artificial reefs.

Reef type is often a product of opportunity, utilizing available structure with the

lowest cost of implementation that will produce the greatest desired effect (Baine, 2001).

Large artificial structures are costly to implement and this cost can be offset by using

existing decommissioned marine structures (Dafforn et al., 2015). However, to

maximize the desired effects of artificial reefs (i.e. fisheries production) specific design

features need to be incorporated such as vertical relief, reef footprint, and rugosity. In

this study, reefs with the highest diversity were large and had higher vertical relief which

is a trend that has been described in numerous studies examining reef fish assemblages

(Bohnsack et al., 1994; Rooker et al., 1997). A study by Rilov and Benayahu (2000)

observed that an increase in vertical relief resulted in a higher proportion of pelagic

fishes that utilized the artificial reef vs. low relief habitats which had smaller proportions

of pelagic fishes. In Ajemian et al. (2015a), the authors observed differences in fish

assemblage structure among reef types when looking at differences among ships and

toppled, cutoff, and freestanding rig platforms. These differences were a function of the

combination of water depth and vertical relief with water depth being the primary factor

in determining fish assemblage (Ajemian et al., 2015a). Water depth in this study varied

from 13 to 32m, with the mean depth of concrete reef sites (13.82 ± 1.23 m SD) being

significantly shallower than both rig (20.93 ± 0.70 m) and ship (23.93 ± 10.13 m) reef

sites which may have affected the assemblages of fish that were observed. Water depth

is also highly correlated with the distance from shore on the Texas coast and may affect

fish assemblage structure (Hyndes et al., 1999). While not one individual factor can be isolated among water depth, distance from shore, and vertical relief, the reef structure likely affects reef fish assemblage.

The relative abundance of fish from ARIS surveys was almost three times higher on low-lying artificial reefs relative to ship or rig reefs which may be due to differences in habitat rugosity and complexity. Habitat complexity has been linked to the ability to observe fish on natural reefs, as reef habitats with a more complex topography were strongly correlated with lower species richness estimates during visual diver surveys (Wilson et al., 2007). Furthermore, ARIS surveys produce mixed results when used in environments with potential obstructions to the field of view, evidenced by the high abundance estimates on less rugose low lying concrete reefs relative to highly rugose toppled rig jackets and ships. Demersal and sedentary fishes, or species that closely associate with structure were unlikely to be counted using acoustic surveys in estuaries (Able et al., 2014), which may also be true for highly complex benthic structures that are in offshore marine environments. Certain species of fish can be attracted to artificial reefs that lack complex structure almost immediately after the structures are deployed, such as red snapper, gray triggerfish, and tomtates (Arney et al., 2017; Mills et al., 2017; Streich et al., 2017a). Red snapper, for example, utilize unstructured habitats, (i.e. shell rubble and sand) during their first year of life (Wells et al., 2008c), and have been shown to recruit to higher relief habitats thereafter, by age 2 (Gallaway et al., 2009). Red snapper have higher site fidelity on smaller low relief artificial reefs that are isolated from other reef structures (Strelcheck et al., 2007). High site fidelity in addition to sparse

isolated structure can concentrate fish into close proximity (Boswell et al., 2010). The ARIS sonar has a narrow fixed beam angle which can cover an entire, granite block, or reef pyramid at a time while, the high relief habitats surveyed (toppled rig jackets and decommissioned ships) have a much larger footprint, which do not fit within a single frame. In this study, low-lying concrete reefs had higher relative abundance of red snapper compared to both Rig and Ship reefs. Which may be due to high concentrations of fish on smaller less complex concrete reefs, or sampling bias due to the narrow fixed with of the ARIS unit. In future studies, caveats such as structure size and complexity need to be taken into consideration when interpreting the relative abundance of fish on artificial reefs.

The varied results in artificial reef assemblage structure among different gear types provide additional evidence for the need to use multiple gears when estimating fish abundances. Gear bias is a common issue throughout fisheries surveys when estimating the relative abundance and size of multiple species (Jackson and Harvey, 1997). Wells et al. (2008a) found that across four gear types, there was distinct size selectivity and bias involved in each, with demersal trawls being the most effective tool for collecting high numbers of juvenile red snapper, but missing larger individuals that were collected in higher abundance using chevron traps and observed using underwater camera arrays. With exclusively passive gear types, the effectiveness of the gear in our study was based on our ability to be proximate to structure, baiting fish. However, the act of baiting, can specifically attract carnivorous or omnivorous fish (Løkkeborg, 1990). Baiting is often species or guild specific, and does less to attract planktivorous or herbivorous fish,

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which have been shown to comprise a large portion of fish assemblage structure in studies that utilize visual surveys on artificial reefs (Dance et al., 2011a; Rooker et al., 1997). In our study, aside from a few limited instances, planktivores and herbivores were excluded from our sampling which is a caveat to exploring the assemblage structure of fish using baited gear. Combining baited gear types, that may be size and species-specific, with acoustic surveys that can provide taxonomic resolution is necessary to fully describe the assemblage structure of fish on artificial reef habitats.

Temporal and regional differences in fish assemblage are common and hypothesized to be driven by a combination of both biotic and abiotic factors (Attrill and Power, 2002). These changes in fish assemblage can be much stronger in coastal areas that have increased seasonal variability in salinity and temperature (Feyrer et al., 2015). Sites representing reef types were spatially distinct with concrete sites being located in the northern portion, ships in the southern portion, and rigs in the central portion of the sampling area. A study by Neves dos Santos et al. (2005) conducted fish assemblage surveys on low relief artificial reefs on the southern coast of Portugal, examining the differences between two reef locations. Each location was spatially distinct with varied differences in abiotic (salinity and temperature) and biotic (distance to estuaries or natural reef) factors and was shown to alter fish assemblage, with higher biomass and species richness on reefs closer to the estuary (Neves dos Santos et al., 2005). For the reefs used in this study, neither salinity nor temperature significantly differed among sites. The sites sampled in this work were spatially distinct varying in distance to the nearest estuary as well as being surveyed exclusively in the summer which may have

ignored seasonal assemblage changes common in coastal waters. Estimating reef fish assemblage is challenging when both temporal and spatial factors vary within the sampling design. Future studies should attempt to include these factors in investigations into artificial reef fish assemblages in coastal ecosystems.

This study highlights the importance of using multiple gear types to sample fish assemblages associated with artificial reefs due to size selectivity and caveats involved with traditional fisheries methods. Reef fish assemblages in shallow coastal environments of the NW GoM are similar to adjacent regions and are comprised of both reef associated fishes and transient fishes that utilize surrounding unconsolidated sand and mud bottom. Artificial structures that have both high relief and rugosity promote increased diversity in fish assemblages, while low relief habitats supported higher relative abundance of fishes based on acoustic surveys. However, low relief artificial reefs had lower assemblage diversity than higher relief habitats. Future work examining fish assemblages associated with artificial reefs should focus on developing methods to balance shortcomings of individual techniques to better estimate the relative abundance of reef fishes.

CHAPTER III: BIOMARKERS

Synopsis

Artificial reefs harbor rich biodiversity and support complex trophic relationships and food webs. The predator-prey dynamics within localized food webs can vary over time, and shift among habitats for many reef associated species. How these trophic relationships and food webs affect the functionality of artificial reefs as habitat for fishes is widely debated and requires further study using multiple techniques. The feeding ecology of three reef fishes; tomtate (*Haemulon aurolineatum*), pigfish (*Orthopristis chrysoptera*), and red snapper (*Lutjanus campechanus*) was examined in the northwestern Gulf of Mexico (NW GoM) in 2016 and 2017. For this study, we had two objectives, 1) examine feeding ecology of three reef associated fishes, and 2) compare the feeding ecology of red snapper, over high and low relief artificial reefs. The feeding ecology of individual fish were assessed using stable isotope ratios, carbon (^{13}C), nitrogen (^{15}N), and sulfur (^{34}S), as well as poly-unsaturated fatty acid ratios, linoleic acid (LA; 18:2n-6), -linoleic acid (ALA; 18:3n-3), stearidonic acid (SDA; 18:4n-3), arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n3) and docosahexaenoic acid (DHA; 22:6n-3). All individuals had both epaxial muscle and liver tissue analyzed to reveal long-term (months to years) and short-term (days to weeks) feeding strategies, respectively. Pigfish exhibited lower ^{15}N and ^{34}S than the other two species, suggesting this species fed on a lower trophic level and more benthic prey, respectively. Pigfish also exhibited significantly higher muscle ^{13}C and lower ^{34}S than both red snapper and tomtate, that

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was more reflective of an estuarine signature. However, the difference in ^{13}C for pigfish was not reflected in faster turnover liver tissue, suggesting that the movement from estuary to offshore reef likely occurred within a shorter timeframe. Tomtate feeding ecology was shown to be similar to red snapper although reflected a greater proportion of benthic prey in their diet with lower ^{34}S and higher ^{13}C and EPA. ^{15}N values were higher in red snapper collected from low relief artificial reefs than high relief reefs, which indicated habitat specific differences that may be due to increased abundance of conspecifics. Differences between reef relief were more pronounced in 2017 than in 2016 highlighting the seasonal changes that occur in source primary production. The use of both tissue types and biomarkers provide useful feeding information on different timescales of several common reef associated fishes and the effect of different structure types on the trophic ecology of red snapper in the NW GoM.

INTRODUCTION

Artificial reefs serve a variety of functions; such as barriers against erosion, providing vertical structure, enhancing biodiversity, and increasing fisheries yield (Baine, 2001; Bohnsack, 1989). Artificial reefs have been shown to attract a diverse community of fishes, and serve as important reef habitat for some species (Arena et al., 2007; Folpp et al., 2013; Granneman and Steele, 2015; Rooker et al., 1997). The factors that have been identified as important in diversifying community structure include vertical relief (Rilov and Benayahu, 2000), rugosity (Jennings et al., 1996), and productive adjacent habitat (Bohnsack and Sutherland, 1985). As diversity increases on artificial reefs, so does the diversity within trophic guilds (Dance et al., 2011b). Some species utilize artificial reefs as foraging grounds (Fabi et al., 2006) while other species take refuge on artificial reefs and forage in the surrounding substrate proximate to the structure (Lindquist et al., 1994). With these varied modes of feeding and interaction of trophic guilds, it is expected that as the community structure changes so do trophic interactions (McCann et al., 1998).

Quantifying trophic interactions among reef-associated species is important for estimating the productivity of a reef. This can be accomplished via dietary biomarkers (i.e., stable isotope ratios, fatty acids) using the paradigm "you are what you eat" (Peterson and Fry, 1987). Stable isotope ratios are based upon the principles that differing pathways of carbon fixation occur during photosynthesis. Stable isotope values can indicate trophic position, dietary shifts, and movement when paired with baseline

isotopic ratios generated by primary producers (Bird et al., 2018; Post, 2002; Trueman et al., 2012). Carbon (^{13}C) isotope ratios are widely used to identify potential carbon sources (DeNiro and Epstein, 1978), and nitrogen (^{15}N) can determine trophic level (Deniro and Epstein, 1981). Both ^{13}C and ^{15}N ratios have been shown to undergo fractionation increasing through each trophic level, 0.5 to 1.5‰ and 2 to 5‰ respectively (Post, 2002). Sulfur (^{34}S) is useful to contrast benthic versus pelagic foraging strategies in fishes, and undergoes levels of fractionation that are less than ^{13}C and ^{15}N increasing only slightly with increasing trophic levels (Peterson and Fry, 1987; Wells et al., 2008b). ^{34}S values tend to be lower in benthic zones due to the increased percentage of sulfides in the sediment, while ^{34}S values are higher in the water column where an increase in sulfates occur (Fry et al., 2008). Sulfur (^{34}S) ratios have also been shown to parallel ^{13}C isoscapes along coastal estuarine gradients, due to the effects that freshwater input has on sulfates which are more depleted than marine sulfates (Peterson and Fry, 1987). When used together all three stable isotope ratios can provide valuable information on the feeding ecology of marine fishes and identify interspecific and intraspecific trophic relationships.

Fatty acids (FAs) are another dietary tracer that can be useful to provide information on trophic structure. FAs are typically conserved when passing from producer to consumer, making them a useful time-integrated biomarker, similar to stable isotope ratios (Dalsgaard et al., 2003; Iverson, 2009). Unlike stable isotope ratios, FAs, specifically poly-unsaturated fatty acids (PUFAs), reflect individual specific primary producers more accurately than stable isotope ratios (Rooker et al., 2006). Essential fatty

acids (EFAs) are PUFAs that cannot be synthesized efficiently by organisms at a rate

that is sufficient to meet their biochemical requirements and must be obtained through

their diet (Kainz et al., 2004). PUFAs remain in their original state within tissues

(Iverson, 2009) and are stored, accumulating over the lifespan of the organism, or are

metabolized as needed (Dalsgaard et al., 2003). As defined, the primary EFAs in marine

fishes are arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and

docosahexaenoic acid (DHA; 22:6n-3) (Sargent et al., 1999; Tocher, 2003). Each of

these EFA's have been identified as nutritionally significant biological compounds to

marine fishes derived predominately from marine source primary production (Kainz et

al., 2004; Parrish, 2009). Ratios of PUFAs including EFAs have been used in

conjunction with other indicators including biomagnifying pollutants and stable isotope

ratios and have shown to correlate with piscivory, and increasing trophic level (Litz et

al., 2017; Rooker et al., 2006; Sardenne et al., 2017). PUFAs used in conjunction with

other FAs can be a powerful tool in identifying species specific and spatially relevant

feeding trends.

The use of stable isotope and FAs ratios can encompass the feeding ecology of

organisms over varied timeframes using multiple tissue types. However, it is important

to understand the specific rates in which the tissue "turns over", or begins to reflect food

resources (Hobson et al., 2010) . Tissue turnover can be affected by several potential

factors including, temperature, growth rate, and metabolism for both stable isotope and

FA ratios (Boecklen et al., 2011). Tissues with higher metabolic activity can quickly

reflect changes in dietary nutrient sources, while tissues that have lower metabolic

activity take longer to reflect changes in diet (Davis et al., 2015; Matich et al., 2011).

Few in-situ studies have been performed on marine fishes to look at tissue specific

turnover of both FAs and stable isotopes, however, companion studies Mohan et al.

(2016a) and Mohan et al. (2016b) found that liver had a faster turnover for both stable

isotope and FA ratios (1-2 months) relative to muscle tissue (3-4 months) in a marine

omnivore, Atlantic croaker (*Micropogonias undulatus*). Multiple tissues, when used in

conjunction, can identify temporal changes in feeding ecology and movement, for fishes

that utilize artificial reefs, and may help identify seasonal and ontogenetic habitat shifts.

The feeding ecology of red snapper has been widely investigated in the northern

Gulf of Mexico (GoM) (McCawley, 2007; Ouzts and Szedlmayer, 2003; Simonsen et al.,

2015; Szedlmayer and Lee, 2004; Wells et al., 2008b; Zapp et al., 2013), however rarely

in conjunction with co-occurring species. The use of both stable isotope and FA

biomarkers, have also not been used in conjunction to investigate the feeding ecology of

marine fish on artificial reefs. The objective of this study were to 1) examine feeding

ecology of three reef associated fishes, and 2) compare the feeding ecology of red

snapper, over high and low relief artificial reefs. Bulk stable isotope ratios of ^{13}C, ^{15}N,

and ^{34}S along with seven PUFAs, LA (18:2n-6), ALA (18:3n-3), SDA (18:4n-3), ARA

(20:4n-6), EPA (20:5n-3), DPA (22:5n3) and DHA (22:6n-3) were compared, from both

epaxial white muscle tissue and liver tissue in order to utilize slow and fast turnover

times, respectively.

MATERIALS AND METHODS

Sample Collection and Processing

Commonly occurring reef fish (Chapter 1), tomtate (*Haemulon aurolineatum*), pigfish (*Orthopristis chrysoptera*), and red snapper (*Lutjanus campechanus*) were collected during quantitative surveys from June through August over a two-year period (2016 - 2017) at two artificial reef complexes. Sites included a high relief complex (>2 meters vertical relief) comprised predominantly of toppled freestanding rig jackets, while the low relief complex (<2 meters) consisted of quarry rock, buoy pieces, and concrete anchors. Each site consisted of three replicate locations for quantitative sampling using either vertical longline (VLL) or fish traps (see methods; Chapter 1). Upon collection, samples were measured to the nearest mm fork length (FL) and sacrificed by immersion in a bath containing a lethal dose of MS-222 (250 – 625 mg/L) for 10 minutes under the guidelines of IACUC AUP 2017-0057. Each fish collected had epaxial white muscle tissue removed at a location anterior to the dorsal fin, along with a sample of liver tissue, for both stable isotope and FA analysis. Muscle and liver tissue collected for stable isotope and FA analysis was frozen, with tissue for FA analyses additionally being placed in a 15 mL conical tube with 2 mL of chloroform prior to being frozen.

Stable Isotope Analysis

Samples for stable isotope analysis were lyophilized 48 hours in a FreeZone (Labconco) freeze dryer and lipids were then extracted via an Accelerated Solvent

Extractor 35 (Dionex). Varied lipid content has been shown to alter estimates of carbon

(^{13}C) so lipid correction, or extraction, is a necessary process when comparing ^{13}C

values among organisms (Post et al., 2007). The extraction process used 34 mL cells

packed with layered tissue samples separated by 30 mm filter papers (Whatman), and

was run in cycles of 5 min saturations with petroleum ether at 100°C and 105.5 k/cm^2 in

order to reach thermal equilibrium, followed by a flush with fresh solvent. This

procedure was repeated three times per cell to ensure the removal of lipids. Following

lipid extraction, tissue was homogenized via a Wig-L-Bug grinding mill and

encapsulated using 5×9 mm tin capsules, placed in a 96 plate well, and shipped to the

Stable Isotope Facility at the University of California at Davis for analysis. Samples for

^{13}C and ^{15}N analyses were weighed to the nearest 1 mg, while samples for ^{34}S

analyses were weighed to the nearest 4 mg. Analysis of the stable isotopes ^{13}C and ^{15}N

was performed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ

Europa 20-20 isotope ratio mass spectrometer (IRMS) (Sercon), and ^{34}S analysis was

done using an Elementar vario ISOTOPE cube interfaced to a 20-22 IRMS (Sercon).

Heavy isotopes were compared to laboratory standards; carbon was compared via

Vienna PeeDee Belemnite, nitrogen was compared via atmospheric N_2, and sulfur was

compared via Vienna Canon Diablo Troilite. Stable isotope data was presented in delta

notation, $X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is the heavy isotope, R_{sample} is the

ratio of heavy to light isotope in the sample, and $R_{standard}$ is the ratio of heavy to light

isotope in the reference standard.

Fatty Acid Analysis

Lipids were extracted from muscle tissue samples using a 2:1:0.5 ratio of chloroform:methanol:water to optimize extraction from aquatic samples. Tissue was ground and then sonicated in the chloroform mixture to ensure full saturation and then centrifuged to separate lipids from tissue. The extraction process was repeated three times per sample and the lipid rich solution was then dried via nitrogen (N_2) evaporator to remove remaining solvent from the solution. The extracted lipids were derivatized into fatty acid methyl esters (FAMEs) using BF3-methanol as described in Parrish (1999). FAMEs were quantified using a HP 6890 Series Gas Chromatography system paired with an Agilent 5973 inert Mass Selective Detector outfitted with a 30m Agilent DB-Wax UI column. The column temperature began at 50°C for 1 min, then was increased (25 °C min^{-1}) to 200 °C, held for 2 min, then increased (3 °C min^{-1}) to 240 °C and held for 20 minutes. The carrier gas was helium, flowing at a rate of 1 ml min^{-1}. Injector temperature was set at 220 °C and the detector temperature was constant at 250 °C. We identified peaks using retention time and individual m/z ion ratios using a single ion scan. FAME peaks were initially identified in Supelco standards, 37 component FAME mix and marine source poly-unsaturated FAMEs (PUFA no. 1), and all samples included an internal standard (methyl tricosanoate, 23:0). FAMEs were analyzed using Enhanced ChemStation (Agilent) analysis software to identify FAME peaks within samples. Seventeen (17) individual FAMEs were quantified using this method including; myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1n9), vaccenic acid (18:1n7), linoleic acid (LA;

18:2n6), -linoleic acid (ALA; 18:3n3), steradonic acid (SDA; 18: 4n3), cis-11-

eicosenoic acid (20:1n9), heneicosanoic acid (21:0), arachidonic acid (ARA; 20:4n-6),

eicosapentaenoic acid (EPA; 20:5n-3), behinic acid (22:0), and lignoceric acid (24:0),

docosapentaenoic acid (DPA; 225n-3), and docosahexaenoic acid (DHA; 22:6n-3).

These FAs have been shown to comprise a majority of fatty acids found within a similar

marine fish omnivore in the NW GoM (Mohan et al., 2016b). After identification all

PUFAs (LA, ALA, SDA, ARA, EPA, DPA, and DHA) were expressed as a proportion

(%) to all measured FAs within the sample.

Data Analysis

Each objective was investigated independently using varied statistical analyses.

For objective one, examination of trophic structure among the three reef fish species

(tomtate, pigfish, red snapper) was accomplished using samples collected in one year

(2016) from the high relief reef site. For objective two, habitat specific differences were

examined using red snapper collected from high and low relief sites during both years

(2016 and 2017). Among the three species (tomtate, pigfish, and red snapper)

MANOVA models were applied to incorporate ^{13}C, ^{15}N, ^{34}S and seven PUFAs (LA,

ALA, SDA, ARA, EPA, DPA, and DHA) to assess differences for those samples for

which all biomarkers were analyzed. For all samples, both muscle tissue and liver tissue

were analyzed separately. Individual ANOVA models were then used to compare

differences among species using stable isotope and PUFA ratios for all samples

collected. To investigate habitat type as a factor for red snapper, MANOVA models

were used to evaluate intraspecific comparisons that occur between habitat type and

year. Two-way ANOVAs were then used to investigate the relationships of habitat type and year for each tracer by tissue type (muscle and liver). Pairwise post-hoc testing was performed using Shaffer's multiple comparison procedure using the multcomp package in R (Hothorn et al., 2008), and significance was determined at p 0.05. Ontogenetic shifts were investigated for each species using linear regression to determine significant linear relationships among stable isotope and PUFA ratios when compared to fork length (FL). Linear regression was analyzed separately for each tracer among species and tissue types.

Nonmetric multidimensional scaling (nMDS) was used to visualize differences among species and between reefs for red snapper by tissue type (McCune et al., 2002). Before ordination, data for each tracer was modified into an untransformed resemblance matrix using a Euclidian distance measure, and run using PRIMER (Clarke and Gorley, 2015). All biomarkers used in ordination were also used in quadratic discriminate function analysis (QDFA). QDFA was used to test the ability of stable isotope and PUFA ratios to distinguish the uniqueness of feeding strategies among species and between reefs for red snapper, by tissue type. Jackknife cross-validated classifications were used to quantify the classification success to respective species and habitat types based upon both SI and PUFA biomarkers. Classification success was based upon the residuals of individual biomarkers. Stepwise variable selection was used to optimize QDFA models using Uschi's classification performance measures, correctness rate (CR), as an estimator for the correctness of a classification rule (1-error rate). Variables will be added and removed through both stepwise removal and addition to improve the CR of

reclassification estimates to a minimum of 0.1%, which is the cutoff of model

optimization. Classification success has an inverse relationship to levels of dietary

overlap: high levels of overlap are reflected by low percent classification success, while

low levels of overlap will reflect high percent classification success among species and

between habitat type. QDFA and stepwise variable selection were performed using the

KlaR package in R (Roever et al., 2018).

RESULTS

A total of 75 (n = 28 tomtate, n = 18 pigfish, and n = 29 red snapper) fish were collected in 2016 and used in species specific analyses. For analyses between relief types (low and high) 84 red snapper were collected over the two-year sampling period, 48 from high relief (2016, n = 29; 2017, n = 19), 36 from low relief (2016, n =18; 2017, n = 18). All samples collected had both muscle and liver tissue analyzed for stable isotope ratios (^{13}C, ^{15}N, ^{34}S) and a subset of samples for species specific analysis (n = 12 tomtate, n= 10 pigfish, and n = 11 red snapper), and red snapper for habitat specific analyses for samples collected in 2016 (high relief, n = 11; low relief, n = 11) and 2017 (high relief, n = 9; low relief, n = 8) were also analyzed for FA ratios. Mean size range varied by species with tomtates ranging from 178 – 245mm FL (212.3 ± 17.1mm; Mean ± SD), pigfish ranged from 158 – 241mm FL (202.4 ± 20.3 mm), and red snapper ranged from 118 - 540mm FL (2016 high relief, 301.1 ± 78.7 mm; 2016 low relief, 347.3 ± 51.8 mm; 2017 high relief, 355.1 ± 121.6; 2017 low relief, 369.1 ± 64.1) (Figure A.8).

Ontogeny

Species specific relationships in ontogeny varied between tissue types. Pigfish were found to have no linear relationship between fork length and any tracer using liver tissue. Using muscle tissue, pigfish had significantly negative linear relationships between length and EPA (slope = -0.031, R^2 = 0.41, p-value = 0.02). Tomtates had varied ontogenetic relationships between tissue types. For tomtates, biomarkers within muscle tissue, ^{13}C (slope = 0.007, R^2 = 0.21, p-value = 0.009) and ^{15}N (slope = 0.018,

$R^2 = 0.51$, p-value < 0.001) had significant positive linear relationships with FL. While DHA (slope = -0.081, $R^2 = 0.48$, p-value = 0.011) had a significantly negative linear relationship with FL within tomtate muscle tissue. In tomtate liver tissue, LA (slope = 0.006, $R^2 = 0.37$, p-value = 0.02) had a significant positive linear relationship with FL, while ARA (slope = -0.024, $R^2 = 0.36$, p-value = 0.02) and DHA (slope = -0.173, $R^2 = 0.57$, p-value = 0.002) had negative linear relationships with FL. In red snapper muscle, ^{15}N (slope = 0.002, $R^2 = 0.17$, p-value < 0.001), ALA (slope = 0.002, $R^2 = 0.08$, p-value = 0.04) had positive linear relationships, while ARA (slope = - 0.004, $R^2 = 0.14$, p-value = 0.012), and DPA (slope = -0.002, $R^2 = 0.15$, p-value = 0.008) had negative linear relationships with FL. In red snapper liver tissue, several biomarkers had significant relationships with FL, ^{15}N (slope = 0.004, $R^2 = 0.11$, p-value = 0.001), ALA (slope = 0.001, $R^2 = 0.10$, p-value = 0.03), SDA (slope = 0.001, $R^2 = 0.13$, p-value = 0.02) all had positive linear relationships while ARA (slope = -0.004, $R^2 = 0.09$, p-value = 0.04) had a negative linear relationship with FL.

Objective 1: Examine feeding ecology of three reef associated fishes.

Relationships among the three species using stable isotope values (^{13}C, ^{15}N, and ^{34}S) and PUFA ratios (LA, ALA, SDA, ARA, EPA, DPA, and DHA) were significant for both muscle ($F_{2,31} = 6.288$, p < 0.001) and liver tissue ($F_{2,29} = 4.789$, p < 0.001). Comparing biomarkers in muscle tissue among the three species, all except for ALA and SDA were found to be significantly different (Table A.3). Regarding specific tracer differences in muscle tissue, ^{13}C was significantly different in muscle tissue among the three species with the highest values found in pigfish samples (-16.11 ± 0.82)

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and lowest in tomtate samples (-16.90 ± 0.24). Pigfish samples had significantly lower

^{15}N values (14.88 ± 0.93) than the other two species (tomtate, 15.86 ± 0.41; red

snapper, 15.66 ± 0.24) as well as ^{34}S (pigfish, 15.20 ± 2.38, tomtate, 18.47 ± 0.57; red

snapper, 18.63 ± 0.34). Muscle samples collected from pigfish also had significantly

lower LA ratios (0.48 ± 0.09) than tomtate (0.53 ± 0.07) or red snapper (0.58 ± 0.09).

The remaining differences among biomarkers were in red snapper muscle tissue, which

had significantly lower ARA ratios (3.18 ± 0.97), EPA (3.87 ± 1.07), and DPA (1.70 ±

0.54) than tomtates (ARA, 4.21 ± 1.49; EPA, 5.71 ± 0.61; DPA, 2.99 ± 1.05) and pigfish

(ARA, 4.85 ± 1.15; EPA, 5.61 ± 1.13; DPA, 2.88 ± 0.54). DHA was the only PUFA that

was higher in red snapper muscle tissue (20.35 ± 2.54) than the other species, tomtates

(14.35 ± 2.48) and pigfish (16.43 ± 2.93) (Figure A.9).

Using liver tissue, ^{13}C, ^{15}N, ^{34}S, ALA, and EPA were found to be

significantly different among species, similarly to muscle tissue, while LA, ARA, DPA,

and DHA were not (Table A.4). Pairwise differences in stable isotope ratios using liver

tissue indicated species specific trends with red snapper samples having significantly

lower ^{13}C values than tomtates (-17.09 ± 0.65) but did not significantly differ in values

from pigfish ^{13}C samples. Pigfish had significantly lower ^{15}N values (13.35 ± 0.55)

than the other two species, tomtate (13.96 ± 0.56) and red snapper (13.78 ± 0.28). All

three species had significantly different ^{34}S values using liver tissue with pigfish

samples having the lowest (17.05 ± 1.06) and red snapper having the highest (19.14 ±

0.46). PUFA differences varied among species. ALA ratios were significantly different

between tomtate (0.19 ± 0.08) and pigfish (0.08 ± 0.02), while no differences existed for

red snapper and the other two species. EPA ratios were lowest for pigfish samples in liver tissue (3.19 ± 0.97) than tomtate (5.07 ± 0.83) and red snapper (4.48 ± 1.32) (Figure A.10).

QDFA classification success

The variables that were most important for species-specific classification success using QDFA varied between tissue types. Classification success among species using muscle was highest with ^{34}S, ARA, and DHA yielding 97.1% (tomtate, 100.0%; pigfish, 100.0%; red snapper, 91.7%) classification success rate. For classification using liver tissue among the three species, the combination of ^{34}S, SDA, and ARA, resulted in a 90.6% success among species (tomtate, 92.3%; pigfish, 100.0%; red snapper, 80.0%) (Figure A.11).

Objective 2: Compare the feeding ecology of red snapper, over high and low relief artificial reefs.

Relationships between high and low relief reefs and years using all biomarkers were significantly different for both muscle tissue and liver tissue. Using muscle tissue, both factors were significant (relief: $F_{1,37} = 6.731$, $p < 0.001$; year: $F_{1,37} = 5.662$, $p < 0.001$). In liver tissue, both factors were again significant (relief: $F_{1,35} = 28.099$, $p < 0.001$; year: $F_{1,34} = 5.671$, $p < 0.001$). Differences in muscle tissue for samples collected on high and low relief reefs varied between years. SDA, DPA, and DHA were not found to differ among red snapper samples collected on high and low relief reefs for either year in muscle tissue. Only ^{15}N and ^{34}S were significantly different in muscle tissue for samples collected in 2016, and were significantly different for both years (2016-2017),

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with low relief reef samples having higher ^{15}N (2016 high relief, 15.66 ± 0.24; 2016 low relief, 16.09 ± 0.16; 2017 high relief, 16.04 ± 0.26; 2017 low relief, 16.45 ± 0.29) and lower ^{34}S (2016 high relief, 18.63 ± 0.34; 2016 low relief, 18.11 ± 0.44; 2017 high relief, 18.68 ± 0.35; 2017 low relief, 18.21 ± 0.48). Several biomarkers significantly differed in muscle tissue of fish collected on the two reef relief types in 2017. ^{13}C was higher on low relief reefs vs. high relief reefs (high relief, -16.85 ± 0.16; low relief, -16.71 ± 0.18) in 2017, as were ARA ratios (high relief, 2.80 ± 0.49; low relief, 3.71 ± 0.4.72). Two biomarkers were higher on high relief reefs vs. low relief in 2017 ALA (high relief, 0.19 ± 0.05; low relief, 0.15 ± 0.04) and EPA ratios (high relief, 4.36 ± 0.42; low relief, 3.09 ± 0.53) (Figure A.12) (Table A.5).

Individual differences in liver tissue also varied between years, and between red snapper collected at high and low relief reefs. Liver tissue differed for ^{13}C, ^{15}N, and ^{34}S values between years. ^{13}C was lower in red snapper liver tissue for both years on low relief reefs vs. high relief reefs (2016 high relief, -17.09 ± 0.65; 2016 low relief, -17.78 ± 0.68; 2017 high relief, -17.39 ± 0.46; 2017 low relief, -17.91 ± 0.73), while ^{15}N was higher in red snapper liver tissue on low relief reefs vs. high relief reefs (2016 high relief, 13.78 ± 0.28; 2016 low relief, 15.27 ± 0.36; 2017 high relief, 14.63 ± 0.46; 2017 low relief, 15.39 ± 0.35). The relationship with ^{34}S values and relief types varied between 2016 and 2017 in liver tissue. In 2016, ^{34}S was higher in red snapper liver tissue samples on high relief (19.14 ± 0.45) relative to low relief reefs (18.51 ± 0.49). However, in 2017 red snapper samples collected on high relief (18.70 ± 0.29) had lower ^{34}S values than on low relief reefs (19.15 ± 0.57). Samples collected in 2017 had more

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differences among the biomarkers than samples collected in 2016, with ALA, SDA, EPA and DPA having significant differences between habitat types. ALA ratios in the liver tissue of red snapper samples collected on low relief reefs were higher (0.37 ± 0.16) vs. high relief reefs (0.23 ± 0.05) as were SDA ratios (low relief, 0.28 ± 0.17; high relief, 0.10 ± 0.04). EPA and DPA ratios were higher for red snapper liver samples collected on high relief reefs (EPA, 5.96 ± 0.56; DPA, 3.36 ± 1.02) vs. low relief reefs (EPA, 3.67 ± 0.55; DPA, 1.80 ± 0.52) in 2017 (Figure A.13) (Table A.5).

QDFA classification success

Variables used in classification using QDFA between high and low relief caught red snapper also differed between tissue types and years. Classification success of muscle tissue samples collected in 2016 between reef types was 85.7% using ^{13}C, ^{15}N, and ARA (high relief, 81.8%; low relief, 90.0%). Samples collected in 2017 were best classified using ^{15}N, ARA, and EPA at a 100% success rate. Classification success of liver tissue samples in 2016, was 100% between low and high relief reefs exclusively using ^{15}N. For liver tissue from red snapper collected in 2017, classification resulted in a 100% success rate using ^{15}N and SDA and between high and low relief reefs (Figure A.14).

DISCUSSION

Results of this study illustrate the species specific and habitat related differences in trophic ecology that occur on artificial reefs in the northwest Gulf of Mexico. Species-specific results suggest that over a longer temporal scale differences relate to movement (muscle tissue), while short term relationships may be a function of trophic structure (liver tissue). Matich and Heithaus (2014) used stable isotopes to determine that during the dry season juvenile bull sharks in the everglades moved into upstream channels to capitalize on the abundance of prey moving into the system from the marsh, and confirmed this movement with acoustic telemetry. Using a short term turnover tissue (blood plasma) and long term turnover tissue (whole blood), the researchers were able to identify the trophic response increased marsh prey consumption (Matich and Heithaus, 2014). Our study also used multiple tissue types to elucidate long term trophic relationships (i.e. movement), as well as short term trends (i.e. habitat specific feeding). Pigfish illustrated similar trends with long term tissue reflecting a move from inshore estuaries to offshore rigs while short term tissues were more likely a reflection of habitat related resource partitioning. Habitat related differences were also identified for red snapper as an important factor in determining trophic structure. Habitat for fish in marine environments are strongly associated with prey availability and prey type which can alter specific feeding trends of generalist predators like red snapper (Schwartzkopf et al., 2017). However, feeding changes over ontogeny occur over a longer period of time are more pronounced than changes in habitat associated feeding, which occur quickly

(Wells et al., 2008b). Varied tissue types that reflect trophic ecology over different timescales and multiple biomarkers (i.e. stable isotope values and FA ratios) are important to understanding the full trophic breadth of individual species.

Species Differences

All three of the species investigated in this study are considered epibenthic predators, yet each exhibit differences among feeding strategies, that are site specific and vary over ontogeny. Dietary preferences among the three species have been examined throughout the GoM and the southeastern US. Adult pigfish forage on benthic prey including crabs, shrimp and annelids (Darcy, 1983) while red snapper and tomtate feed on benthic crustaceans and other invertebrates; however, both species have more fish in their diets relative to pigfish (Norberg, 2015; Szedlmayer and Lee, 2004). Diets of red snapper have been shown to have a high proportion of fish, for example, age 1+ red snapper had diets comprised of 70% fish (Wells et al., 2008b). For small and medium (151-250 mm) sized tomtates collected on artificial reefs, 70% of individual diets were comprised of fish, while large (> 250mm) tomtates collected on artificial reefs consumed a higher proportion of decapod crustaceans (Norberg, 2015). Multiple biomarkers confirmed these feeding differences among species. Pigfish, who have been shown to feed primarily on benthic invertebrates (Darcy, 1983), had lower ^{34}S and ^{15}N values than red snapper or tomtate. ^{34}S is useful for determining pelagic vs. benthic feeding and ^{15}N is used for trophic level. Comparatively low ^{34}S values indicate a preference for benthic prey (relative to pelagic prey), and comparatively lower ^{15}N values indicate feeding on lower trophic level prey when comparing among species (Post, 2002). In

combination, these two biomarkers are useful biomarkers in identifying preference for benthic invertebrates among multiple species that have varied trophic strategies, including piscivory (Plumlee and Wells, 2016). DHA is another tracer that can be used to identify higher trophic level feeding and piscivory (Rooker et al., 2006). Litz et al. (2017) found that for piscivorous fishes, high DHA and low EPA comparatively indicate individual diets that consist of higher amounts of fish. Red snapper had significantly higher DHA and lower EPA ratios in muscle tissue relative to the other two species, which may be due to a high fish based diet by red snapper. These results highlight the species specific dietary differences among commonly occurring marine fishes at artificial reefs.

Biomarkers in varied tissue types provide different information regarding the feeding ecology of marine organisms. ^{13}C values are typically higher in estuaries than coastal environments due to changes in salinity affecting phytoplankton communities (Fry, 2002). ^{34}S values are also affected by salinity due to higher proportions of sulfates that occur in fresh water which make them good indicators of movement from low salinity environments (Fry and Chumchal, 2011). Davis et al. (2015) used both muscle and liver tissue and found a significant proportion of bream (*Acanthopargus australis*) showed a seasonal migration from inshore marsh areas to nearshore reef sites. They infer this movement due to the high proportion of bream liver tissue having significantly lower ^{13}C (-15 to 20‰) which is reflective of a marine signature compared to the values found in muscle tissue (-11 to -18‰) which were more reflective of an estuarine signature. The differences between the two tissues reflected an inshore to offshore

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movement. The migration habits of pigfish not well known but are estimated to be seasonal movements leaving the estuary and towards offshore habitats (Darcy, 1983). The use of long term tissues vs. short term tissues in this study helped to identify potential differences in habitat use by pigfish when compared to red snapper and tomtates who are found relatively ubiquitously throughout shelf waters in the northern GoM. ^{13}C and ^{34}S, which are both useful tracers when observing movements of marine organisms across isoscapes (Tucker et al., 2014), was significantly higher and significantly lower, respectively, for pigfish muscle tissue compared to the other two species. However, in liver tissue, it was ^{34}S and not ^{13}C that differed among the three species with pigfish having the lowest values. Thomas and Cahoon (1993) found that ^{34}S was useful identifying benthic opportunist from other piscivorous or planktivorous fishes on rocky reef structures off of the coast of California. Due to the lower ^{34}S values in pigfish liver tissue when compared to the other two species, it appears that they feed almost exclusively on benthic prey. Identifying these specific habitat shifts and dietary preferences on artificial reefs help us to better understand the role these reefs play on the ecology of fish communities.

Habitat Differences

Red snapper have been shown to have different feeding strategies based upon habitat (Szedlmayer and Lee, 2004; Wells et al., 2008b). Simonsen et al. (2015) found that red snapper collected on toppled platforms and natural reefs had higher ^{15}N values and had diets that were more diverse in prey than those collected on freestanding platforms. Our study also found that on low relief artificial reefs, ^{15}N was higher

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relative to high relief artificial reefs. A survey of these sites indicated that the diversity

of the fish assemblage was found to be higher on toppled rig jackets, when compared to

low lying concrete reefs (Chapter 1). Less diverse reefs may allow opportunistic

generalists like red snapper, to take advantage of unoccupied trophic roles. Red snapper

have been hypothesized to feed off the reef on surrounding unconsolidated sand and mud

bottom targeting benthic invertebrates and fish (McCawley, 2007). Tomtates have also

shown this foraging strategy, feeding primarily off the reef (Arena et al., 2007). Reef

relief may play an important role in allowing fish who feed similarly to partition their

habitat. Red snapper and tomtate feeding were significantly different using both stable

isotope and PUFA ratios. Significantly lower ^{34}S values in tomtate liver tissue indicate

that they feed more on benthic prey than red snapper. Higher ^{13}C values and EPA ratios

in liver tissue also indicated further resource partitioning, and indicate differences in

primary productivity. In the NW GoM ^{13}C values vary from carbon sources in the

benthos, vs those throughout the water column (Wells et al., 2008b) with benthic ^{13}C

values being slightly higher (-18.80 ‰) than pelagic particular organic matter (-22.50

‰) (Dance et al. *in review*). The benthic microalgae communities in the northern Gulf of

Mexico are comprised primarily of diatoms (Wells et al., 2008b) which are

proportionally reflected in diets with higher EPA ratios within tissues (Goedkoop et al.,

2000). Diatoms are enriched in EPA which is an essential fatty acid that is conserved in

marine fishes, making them a reliable indicator for diet (Kainz et al., 2004). Both

species, red snapper and tomtate are considered epibenthic reef associated predators,

however, it appears that they may partition their prey resources. Tomtates were shown to

feed more on benthic associated prey using varied bioindicators. While red snapper, when compared to tomtates, were shown to feed higher on more pelagic prey. On low relief habitats, it appears that red snapper feed similarly to tomtates on high relief habitats with lower ^{34}S values and higher EPA ratios. Fisheries surveys in this area show that tomtates were not present on these low relief habitats (Chapter 1) so it may be that red snapper are taking advantage of the potential niche vacancy. These findings increase the general body of knowledge on the feeding ecology of these two relatively ubiquitous predators when they occur on artificial reefs in the NW GOM.

Annual differences in primary production were evident between the two habitat types. In 2016, muscle tissue only differed in ^{15}N and ^{34}S, while liver differed similarly in addition to ^{13}C. However, in 2017 the differences were widespread among biomarkers with seven of ten biomarkers that occurred in either tissue type exhibiting significant relationships. Seasonal changes in abiotic factors in environments along the Texas coast are common and are driven primarily by pulses of freshwater (Tolan, 2007). These freshwater pulses affect community composition of both fish and primary producers that occur within coastal environments (Litz et al., 2014; Wawrik and Paul, 2004). Budge et al. (2002) noted strong geographic differences in FA composition of fish collected within the Gulf of Lawrence across multiple trophic levels. These differences were attributed to the geographic differences in phytoplankton community driven by varied abiotic and geographically relevant variables (Budge et al., 2002). We noted a similar trend in annual differences that may have been driven by changes in phytoplankton communities determined by seasonal changes in precipitation and

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salinity. In addition to differences among the three stable isotopes, FA ratios including long chain (C_{18}, C_{22}, and C_{20}) PUFAs differed in red snapper tissue between the two reefs in 2017. A study of Sargassum communities in the Gulf of Mexico indicated that long chain FA's were important in isolating specific carbon sources among several potential sources (Rooker et al., 2006). As these phytoplankton communities shift with salinity and varied freshwater sources so do the bioindicators that vary with their assemblage. While this study did not sample primary producers due to the low assumed variability that occurred during the summer sampling period, the results highlight the value of sampling a variety of regionally specific primary production sources across multiple years and seasons to help identify specific source contribution to marine taxa.

Conclusions

This study illustrates that the feeding ecology of fishes on artificial reefs is complex and habitat specific. On high relief reefs, the three species were shown to have differences in both the long term vs. the short term. We hypothesize that long term differences are due to seasonal movement, while short term differences observed among species indicate resource partitioning. Habitat related differences for red snapper show they feed on higher trophic levels on low relief reefs vs. high relief reefs, which may be due to the increased relative abundance trophically similar species on high relief reefs. This study highlights the usefulness of multiple tracers reflected over short and long term timescales in the interpretation of the complex trophic relationships occurring on artificial reefs in the NW GoM.

CHAPTER IV: CONCLUSIONS

Throughout this study artificial reefs have been identified as important habitat for a wide range of fishes. Chapter 1 illustrated that fish assemblages on artificial reefs in the coastal waters of the NW GoM vary according to reef type. Higher relief, more rugose structure, supported increased diversity of fish. While low relief structure appears to have higher relative abundance of fish using active acoustics (ARIS 1800). Findings here illustrate the importance of utilizing multiple techniques when estimating fish assemblage. Vertical longlines were shown to be especially effective in sampling predatory reef fish, however were highly biased in their catches of red snapper. Fish traps collected a wider range of fishes but were biased in size based upon trap size and mesh. Active acoustics were most effective in estimating fish densities but provided no taxonomic resolution. The use of these multiple gear types in conjunction created a more accurate estimate of fish assemblage, highlighting the use of multiple techniques when collecting community data.

Chapter 2 examined the trophic ecology of fishes associated with different artificial reef types utilizing multiple tracers (i.e. stable isotopes and fatty acids) across multiple tissues (i.e. muscle and liver). Species specific differences were examined on high relief habitats for pigfish, tomtate, and red snapper while habitat related differences were examined between high and low relief habitats for red snapper. Both stable isotope and fatty acid analyses revealed trophic partitioning among species. Long term differences among species may be linked to seasonal migrations, while short term

differences may be linked to trophic partitioning. Habitat related differences showed that red snapper, on lower relief habitats, exhibited higher trophic level feeding than high relief habitats. Trophic differences among habitat types may be due to lower intra-guild diversity. The use of multiple tracers that reflected short-term and long-term timescales was found to be useful in identifying trends across food webs on artificial reefs.

Results show that artificial reefs have complex relationships with their associated fish assemblages. Reef structure and reef relief are important factors to consider for both fish assemblage and trophic structure. These findings add to the growing body of literature on the benefits of artificial reefs and how their design influences the ecology of surrounding organisms. Reef design and implementation is a mandate for many state agencies for both recreational and commercial marine activities. Fisheries managers and coastal planners can use this information to make future decisions on the creation of near shore reefs throughout the NW GoM.

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Table A.1: Table of surveyed replicates for each structure type over the 4 year (2014 – 2017) sampling period.

VLL and Trap Surveys					
Structure	Years Sampled	2014	2015	2016	2017
Concrete	3	0	12	6	6
Rig	4	9	9	9	6
Ship	3	6	6	3	0
ARIS Surveys					
Concrete	1	0	9	0	0
Rig	3	9	9	3	0
Ship	1	0	9	0	0

Table A.2: Total abundance and mean size (± SD) for fish caught during both VLL and trap surveys on artificial reef sites.

Species	Concrete	Rig	Ship	Trap Mean Size (mm)	VLL Mean Size (mm)
Red Snapper (*Lutjanus campechanus*)	357	350	85	251 ± 54	373 ± 76
Gray Triggerfish (*Balistes capriscus*)	32	74	24	270 ± 74	358 ± 49
Pigfish (*Orthopristis chrysoptera*)	1	58	11	203 ± 20	N/A
Tomtate (*Haemulon aurolineatum*)	0	54	14	228 ± 22	N/A
Hardhead Catfish (*Ariopsis felis*)	37	19	1	333 ± 24	347 ± 33
Lane Snapper (*Lutjanus synagris*)	0	12	3	233 ± 33	N/A
Atlantic Croaker (*Micropogonias undulatus*)	3	9	0	228 ± 24	264 ± 24
Sand Seatrout (*Cynoscion arenarius*)	2	10	0	N/A	336 ± 36
Pinfish (*Lagodon rhomboides*)	0	8	1	185 ± 35	153 ± 0
Spadefish (*Chaetodipterus faber*)	5	1	1	117 ± 25	224 ± 0
Oyster Toadfish (*Opsanus tau*)	0	4	0	311 ± 43	N/A
Black Drum (*Pogonias cromis*)	0	0	1	N/A	538 ± 0
Sharksucker (*Echeneis naucrates*)	0	1	0	N/A	672 ± 0
Blue Runner (*Caranx crysos*)	0	0	1	N/A	210 ± 0
Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*)	0	0	1	N/A	690 ± 0
Gray Snapper (*Lutjanus griseus*)	0	1	0	331 ± 0	N/A
Southern Kingfish (*Menticirrhus americanus)*	0	1	0	298 ± 0	N/A
Gag Grouper (*Mycteroperca microlepis*)	0	1	0	283 ± 0	N/A
Atlantic Bumper (*Chloroscombrus chrysurus*)	0	1	0	182 ± 0	N/A
Spinner Shark (*Carcharhinus brevipinna*)	1	0	0	N/A	N/A

Table A.3: Test results for one-way ANOVA among all species tomtate (TT), pigfish (PF), and red snapper (RS) for all biomarkers found in muscle tissue. Significant relationships are in **bold** (p 0.05).

	df	F-Ratio	p-value	Pairwise	p-value
^{13}C	71	16.441	**< 0.001**	TT – RS	**0.003**
				PF – TT	**< 0.001**
				PF – RS	**0.003**
^{15}N	71	19.036	**< 0.001**	TT – RS	0.169
				PF – TT	**< 0.001**
				PF – RS	**< 0.001**
^{34}S	71	50.271	**< 0.001**	TT – RS	0.638
				PF – TT	**< 0.001**
				PF – RS	**< 0.001**
% LA	31	3.991	**0.029**	TT – RS	0.185
				PF – TT	0.160
				PF – RS	**0.025**
% ALA	31	1.775	0.186	TT – RS	0.930
				PF – TT	0.300
				PF – RS	0.300
% SDA	31	0.910	0.413	TT – RS	0.566
				PF – TT	0.566
				PF – RS	0.597
% ARA	31	5.568	**0.009**	TT – RS	**0.050**
				PF – TT	0.230
				PF – RS	**0.007**
% EPA	31	13.073	**< 0.001**	TT – RS	**< 0.001**
				PF – TT	0.830
				PF – RS	**< 0.001**
% DPA	31	10.637	**< 0.001**	TT – RS	**< 0.001**
				PF – TT	0.739
				PF – RS	**< 0.001**
% DHA	31	15.260	**< 0.001**	TT – RS	**< 0.001**
				PF – TT	0.076
				PF – RS	**< 0.001**

Table A.4: Test results for one-way ANOVA among all species tomtate (TT), pigfish (PF), and red snapper (RS) for all biomarkers found in liver tissue. Significant relationships are in **bold** (p 0.05).

	df	F-Ratio	p-value	Pairwise	p-value
^{13}C	71	3.808	**0.027**	TT – RS	**0.031**
				PF – TT	0.668
				PF – RS	0.068
^{15}N	70	9.007	**< 0.001**	TT – RS	0.160
				PF – TT	**< 0.001**
				PF – RS	**0.004**
^{34}S	72	51.596	**< 0.001**	TT – RS	**< 0.001**
				PF – TT	**< 0.001**
				PF – RS	**< 0.001**
% LA	29	1.584	0.222	TT – RS	0.312
				PF – TT	0.272
				PF – RS	0.483
% ALA	29	4.126	**0.027**	TT – RS	0.208
				PF – TT	**0.023**
				PF – RS	0.137
% SDA	29	2.037	0.149	TT – RS	0.243
				PF – TT	0.172
				PF – RS	0.443
% ARA	29	1.544	0.231	TT – RS	0.356
				PF – TT	0.972
				PF – RS	0.356
% EPA	29	8.721	**0.001**	TT – RS	0.189
				PF – TT	**< 0.001**
				PF – RS	**0.012**
% DPA	29	1.377	0.268	TT – RS	0.339
				PF – TT	0.674
				PF – RS	0.339
% DHA	29	2.713	0.083	TT – RS	0.082
				PF – TT	0.418
				PF – RS	0.187

Table A.5: Test results of two-factor ANOVA for red snapper among habitat type using relief (high/low) and year (2016/2017) as factors for all biomarkers. Significant relationships are in **bold** (p 0.05).

|		Muscle			Liver		
		df	F-Ratio	p-value	df	F-Ratio	p-value
$\delta^{13}C$	Relief (2016)	45	0.178	0.675	47	12.648	**< 0.001**
	Relief (2017)	34	6.413	**0.161**	33	6.606	**0.015**
	Year	79	35.827	**< 0.001**	80	2.561	0.114
	Relief x Year	79	1.541	0.218	80	0.311	0.579
$\delta^{15}N$	Relief (2016)	45	45.063	**< 0.001**	46	265.740	**< 0.001**
	Relief (2017)	34	20.232	**< 0.001**	33	44.307	**< 0.001**
	Year	79	46.854	**< 0.001**	79	50.937	**< 0.001**
	Relief x Year	79	0.0511	0.813	79	20.525	**< 0.001**
$\delta^{34}S$	Relief (2016)	45	20.571	**< 0.001**	48	21.601	**< 0.001**
	Relief (2017)	34	11.231	**0.002**	33	8.951	**0.005**
	Year	79	0.711	0.402	81	0.197	0.658
	Relief x Year	79	0.070	0.793	81	27.940	**< 0.001**
% LA	Relief (2016)	21	2.949	0.101	19	0.787	0.386
	Relief (2017)	15	8.155	**0.012**	14	0.780	0.392
	Year	36	0.495	0.486	33	7.742	**0.009**
	Relief x Year	36	0.820	0.371	33	0.083	0.775
% ALA	Relief (2016)	21	0.001	0.992	19	2.223	0.152
	Relief (2017)	15	2.496	**0.135**	14	6.424	**0.024**
	Year	36	8.361	**0.007**	33	12.728	**0.001**
	Relief x Year	36	1.770	0.192	33	1.439	0.239
% SDA	Relief (2016)	21	0.770	0.390	19	0.306	0.587
	Relief (2017)	15	0.576	0.460	14	9.412	**0.008**
	Year	36	0.234	0.631	33	2.361	0.134
	Relief x Year	36	0.761	0.389	33	2.671	0.112
% ARA	Relief (2016)	21	0.860	0.364	19	0.209	0.653
	Relief (2017)	15	15.132	**0.001**	14	2.985	0.106
	Year	36	0.836	0.367	33	2.721	0.109
	Relief x Year	36	8.744	**0.006**	33	0.152	0.699
% EPA	Relief (2016)	21	0.425	0.522	19	0.177	0.679
	Relief (2017)	15	30.359	**< 0.001**	14	66.633	**< 0.001**
	Year	36	0.929	0.342	33	2.777	0.105
	Relief x Year	36	10.235	**0.003**	33	12.486	**0.001**
% DPA	Relief (2016)	21	1.642	0.214	19	1.700	0.106
	Relief (2017)	15	4.451	0.052	14	13.615	**0.002**
	Year	36	2.318	0.137	33	10.797	**0.002**
	Relief x Year	36	0.007	0.979	33	18.969	**< 0.001**
% DHA	Relief (2016)	21	0.939	0.344	19	0.255	0.620
	Relief (2017)	15	0.150	0.704	14	1.212	0.289
	Year	36	1.000	0.324	33	0.429	0.517
	Relief x Year	36	0.757	0.390	33	1.487	0.231
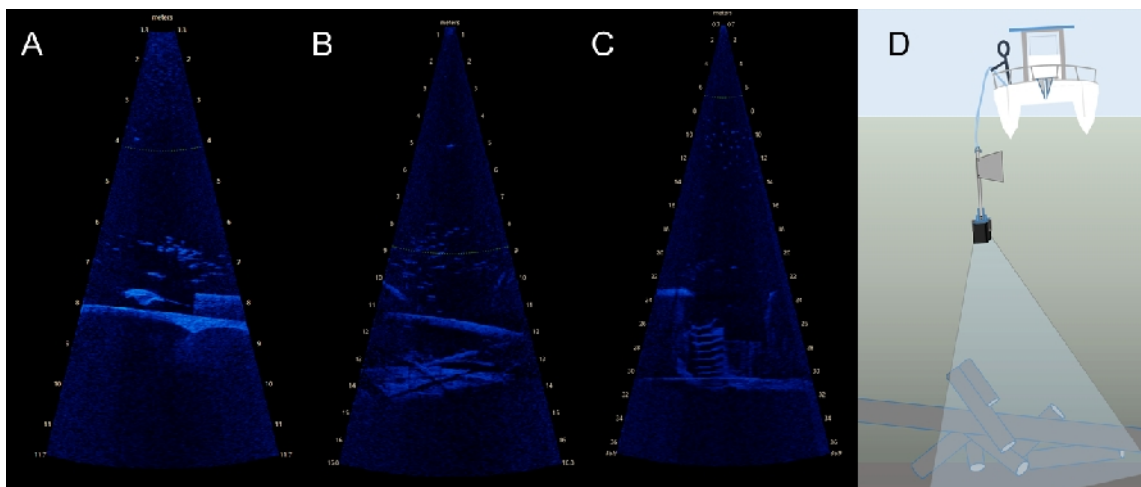
Figure A.1: ARIS images representing each reef type, A) concrete, B) toppled rig jackets, C) ship, and D) an "Artist" rendition of ARIS surveys.
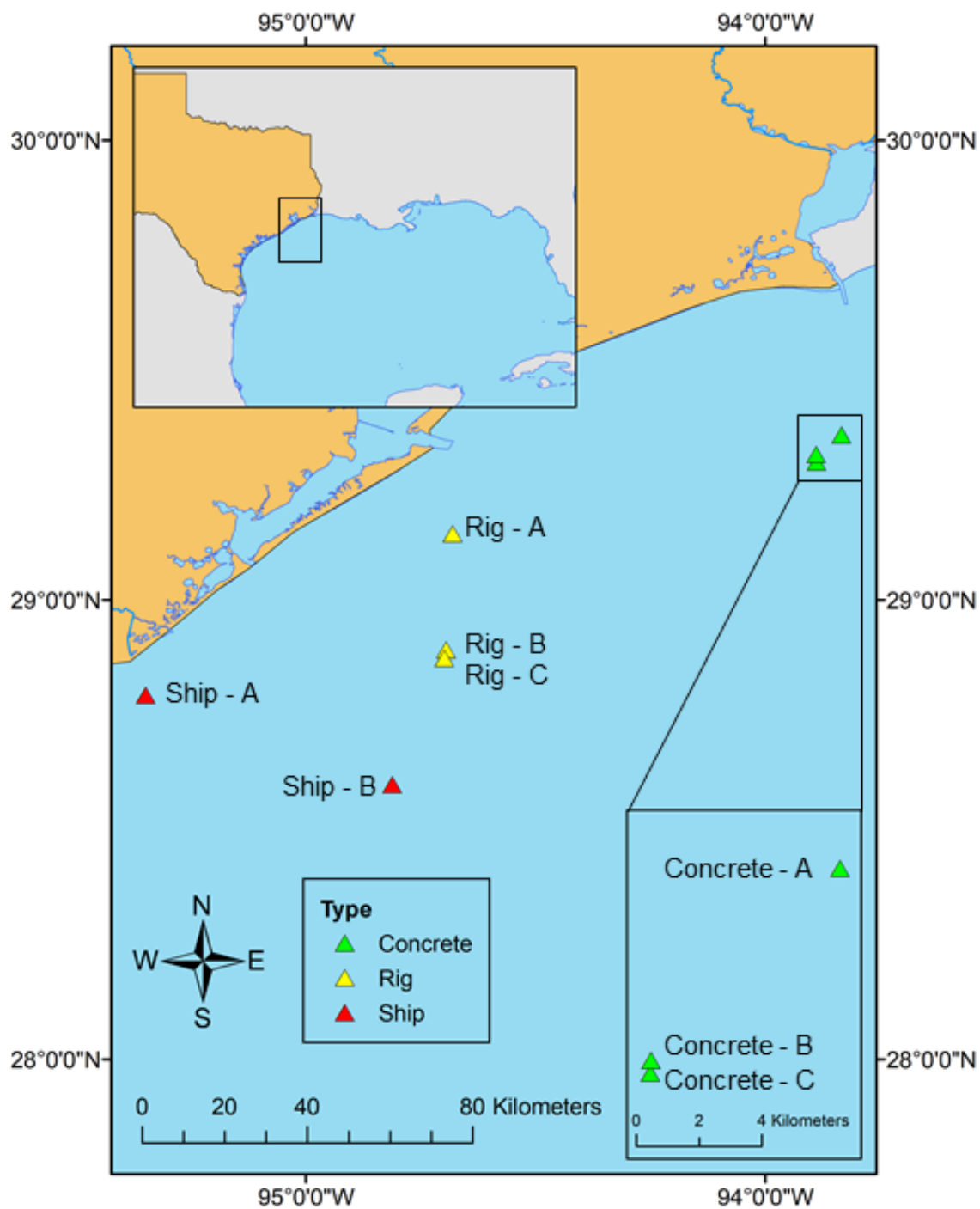
Figure A.2: Map of the artificial reef sites and associated replicate locations along the northern Texas coast.
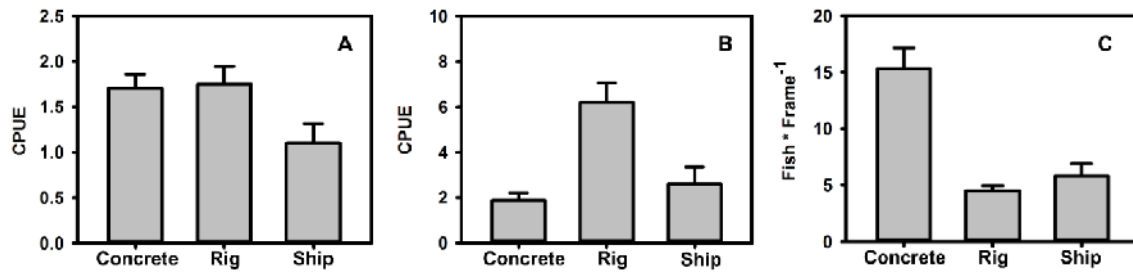
Figure A.3: Mean (± SE) A) total fish CPUE using VLL, B) total fish CPUE using fish traps, and C) Fish*frame^{-1} using ARIS for concrete, rig, and ship artificial reefs in the NW GoM.
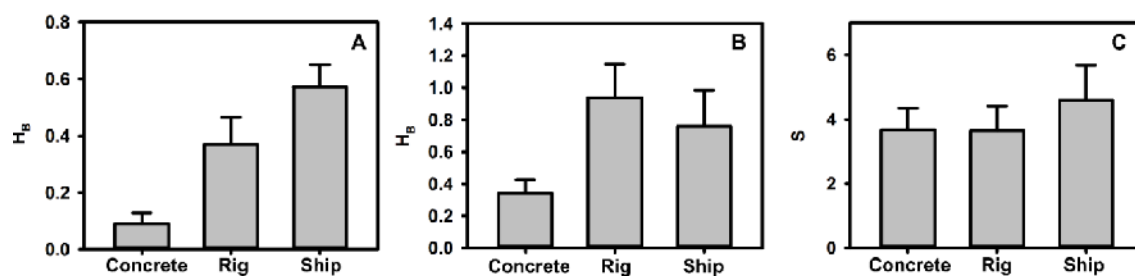
Figure A.4: Mean (± SE) A) diversity (H_B) using VLL, B) diversity (H_B) using traps, and C) total species richness (S) for all fish collected, on concrete, rig, and ship artificial reefs in the NW GoM.
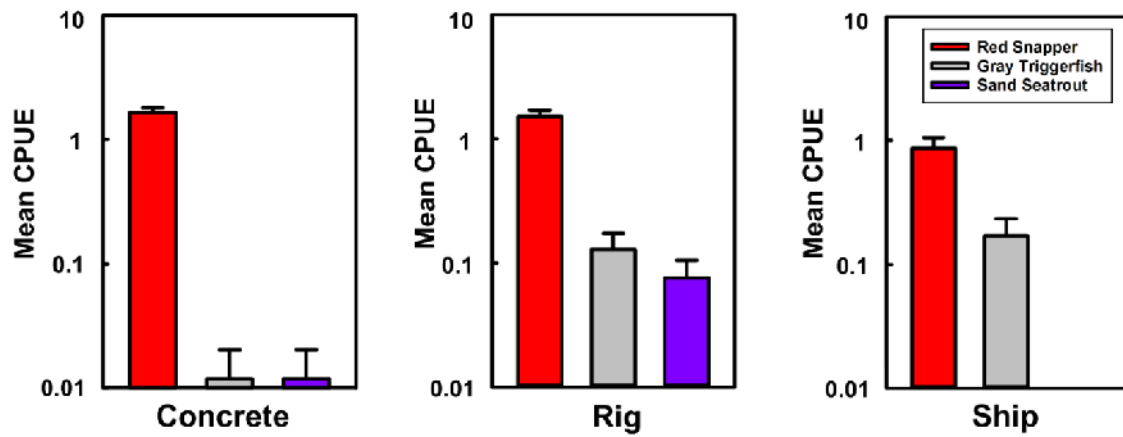
Figure A.5: Mean (±SE) CPUE using VLL for three species collected from multiple reef types on in the NW GoM.
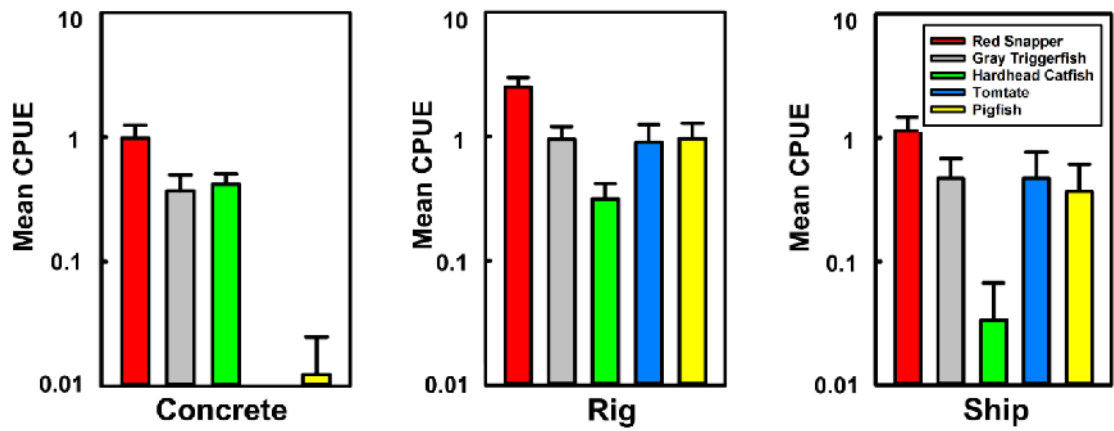
Figure A.6: Mean (±SE) CPUE using fish traps for five species collected from multiple artificial reef types in the NW GoM.
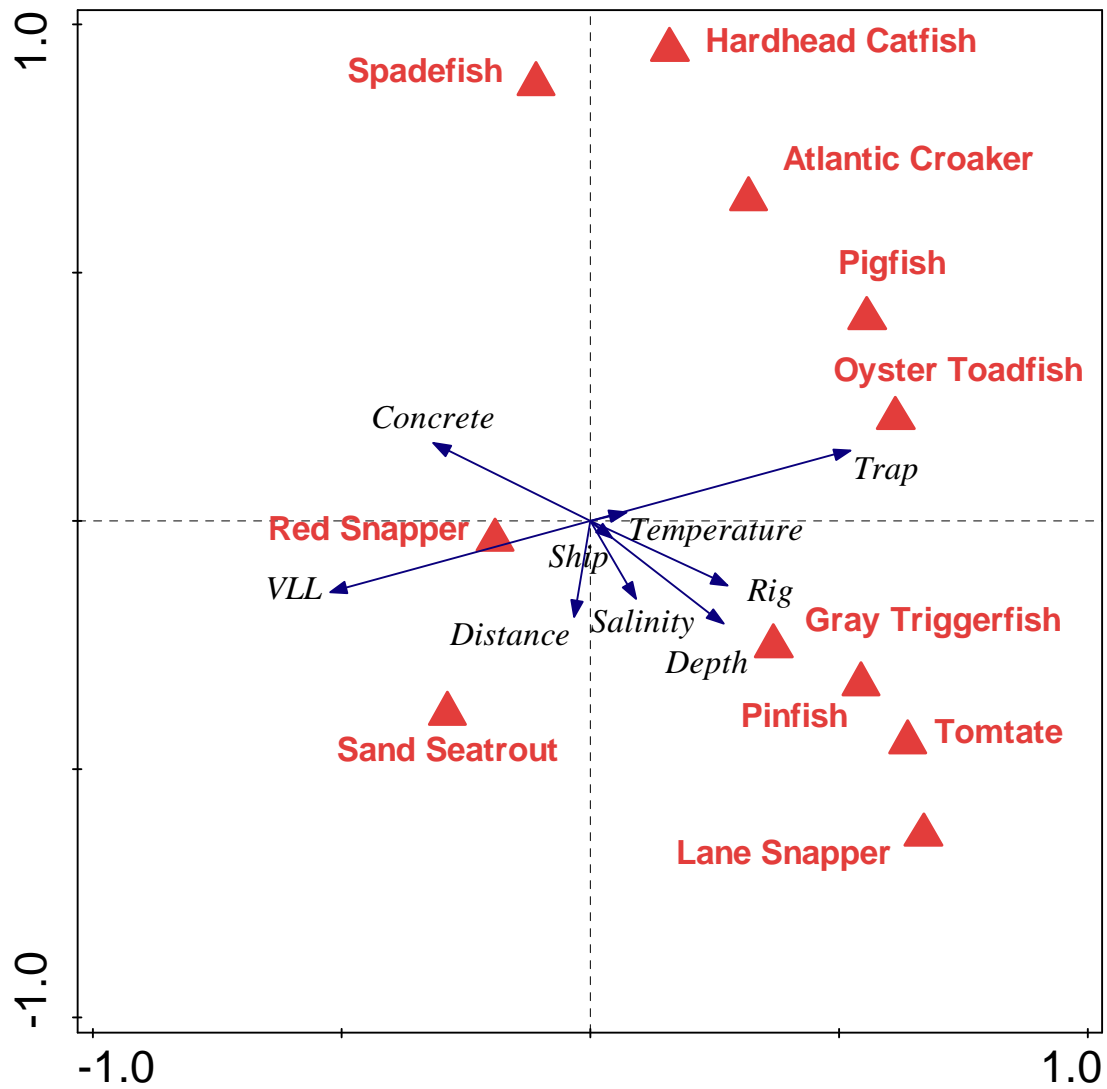
Figure A.7: Plot of fish the species and RDA scores on the first two axes, with gear type (VLL or Trap), reef type (Concrete, Rig, and Ship), salinity, temperature, depth, and distance from shore as factors relative to species abundance.
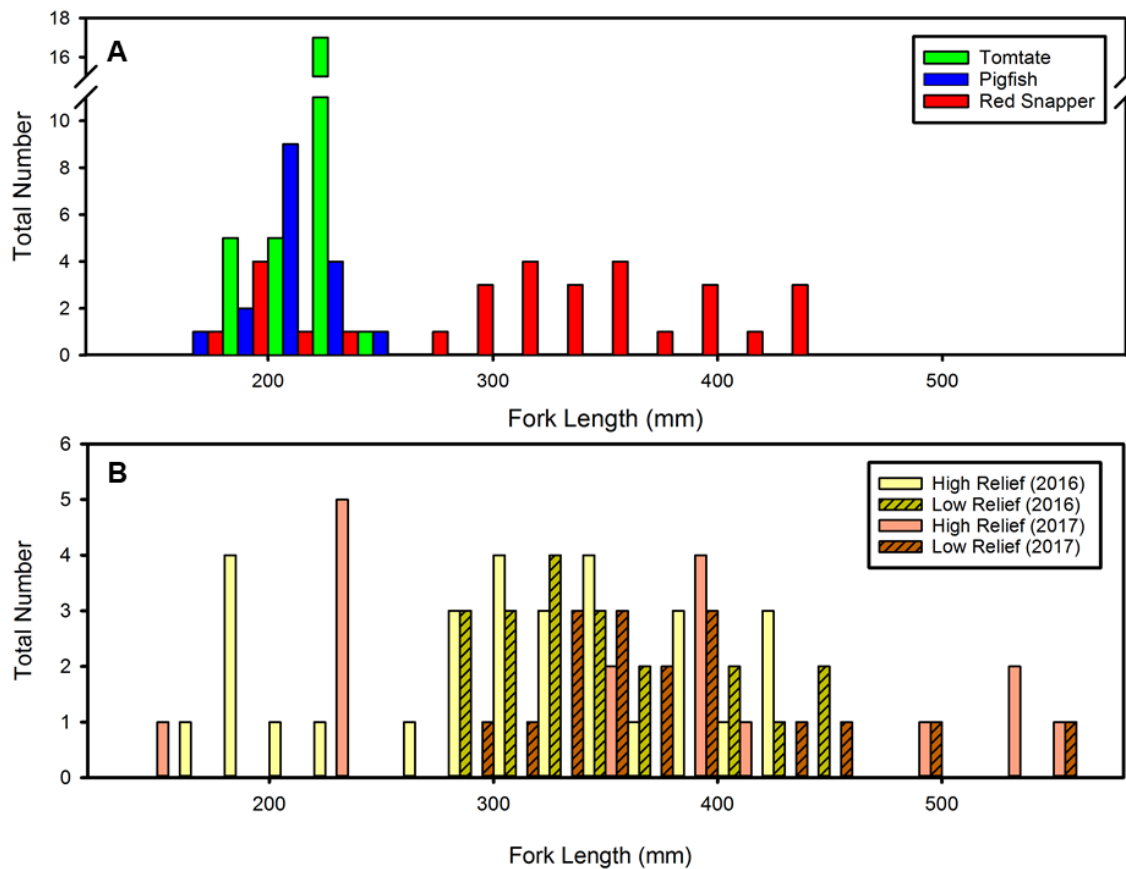
Figure A.8: Fork length (FL) histograms for A) tomtate (n= 28), pigfish (n=18), red snapper (n = 29) collected on high relief reefs in 2016, and B) red snapper collected on high (2016, n = 29; 2017, n = 19) and low (2016, n =18; 2017, n = 18) relief artificial reefs.
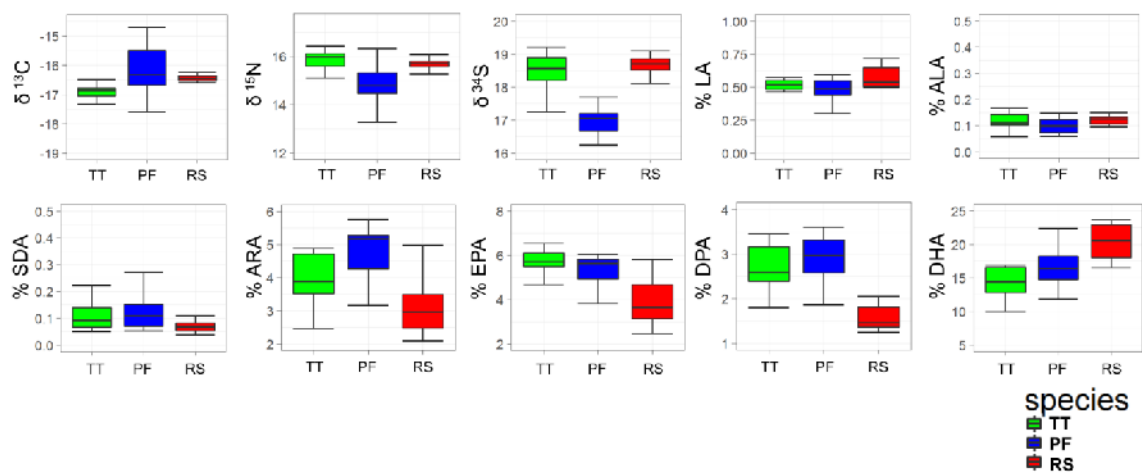
Figure A.9: Boxplots of the mean values for all biomarkers among the three species tomtate (TT), pigfish (PF), and red snapper (RS) in muscle tissue.
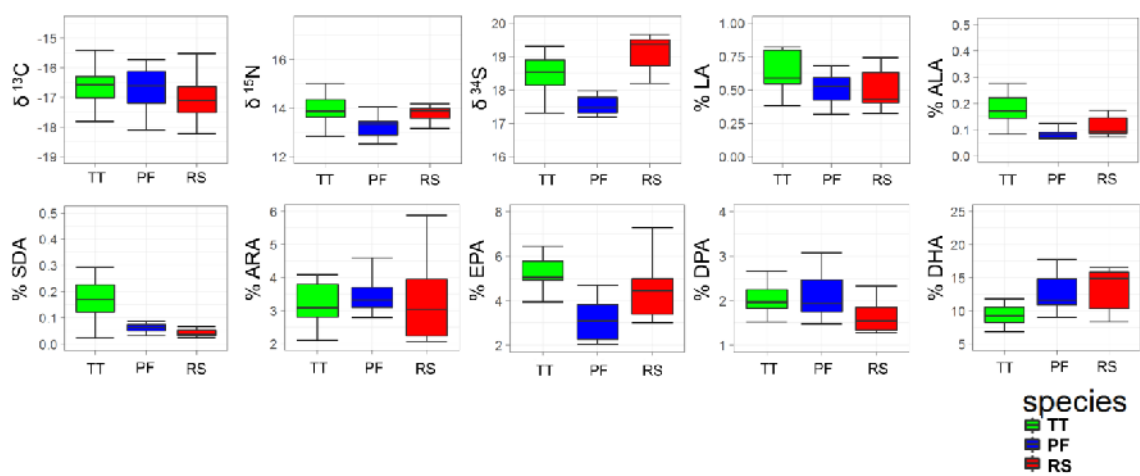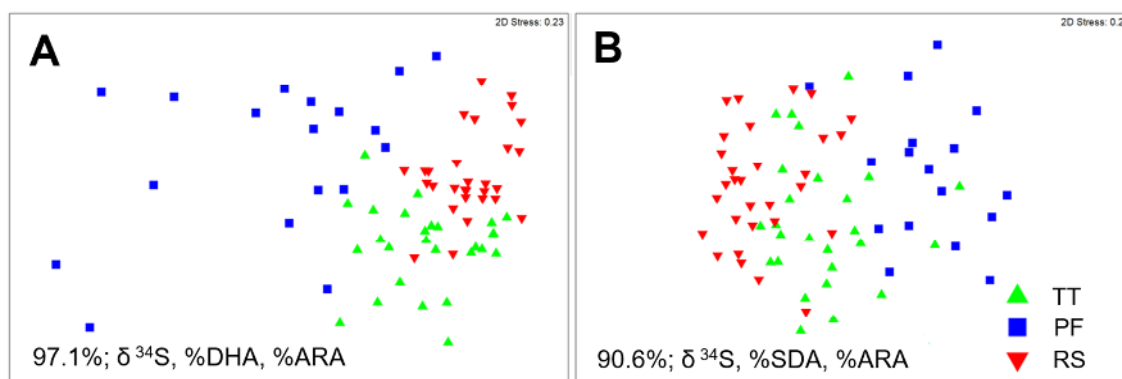
Figure A.10: Boxplots of the mean values for all biomarkers among the three species tomtate (TT), pigfish (PF), and red snapper (RS) in liver tissue.

Figure A.11: nMDS plots using ordination among all biomarkers to separate points, illustrating species specific differences between A) muscle and B) liver. Percentages on the bottom of the plots indicate the classification success and the tracers used to discriminate between species using QDFA.
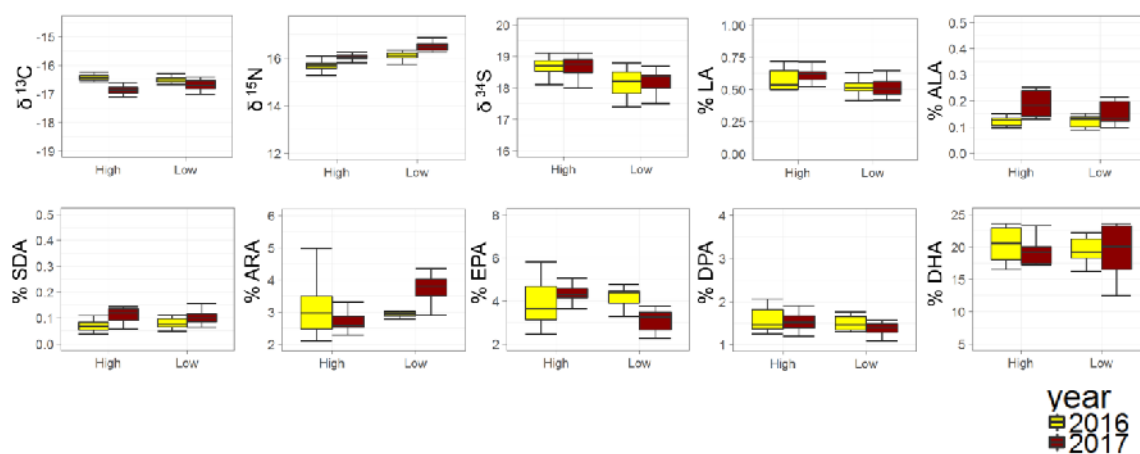
Figure A.12: Boxplots of the mean values for all biomarkers in muscle tissue for red snapper collected on high relief and low relief reefs from years 2016 and 2017.
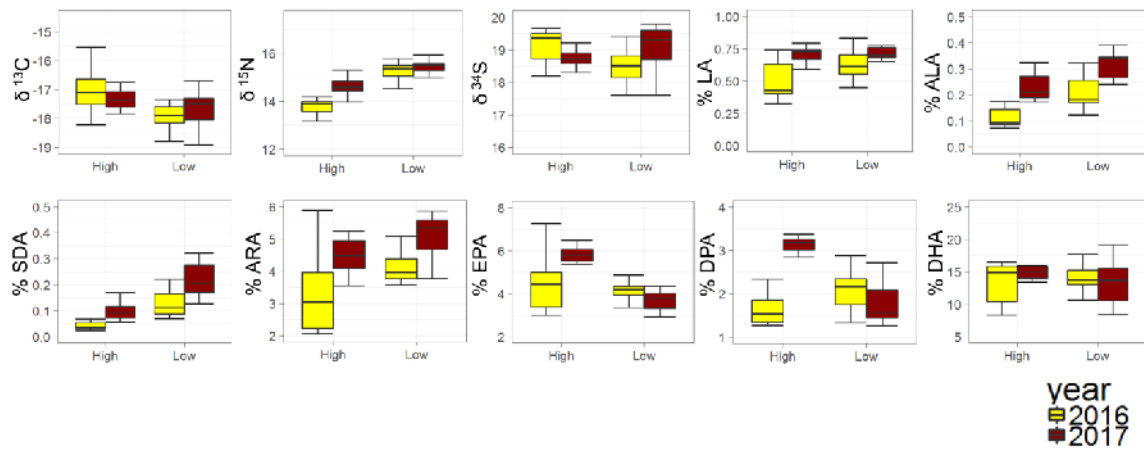
Figure A.13: Boxplots of the mean values all biomarkers in liver tissue for red snapper collected on high relief and low relief reefs from years 2016 and 2017.
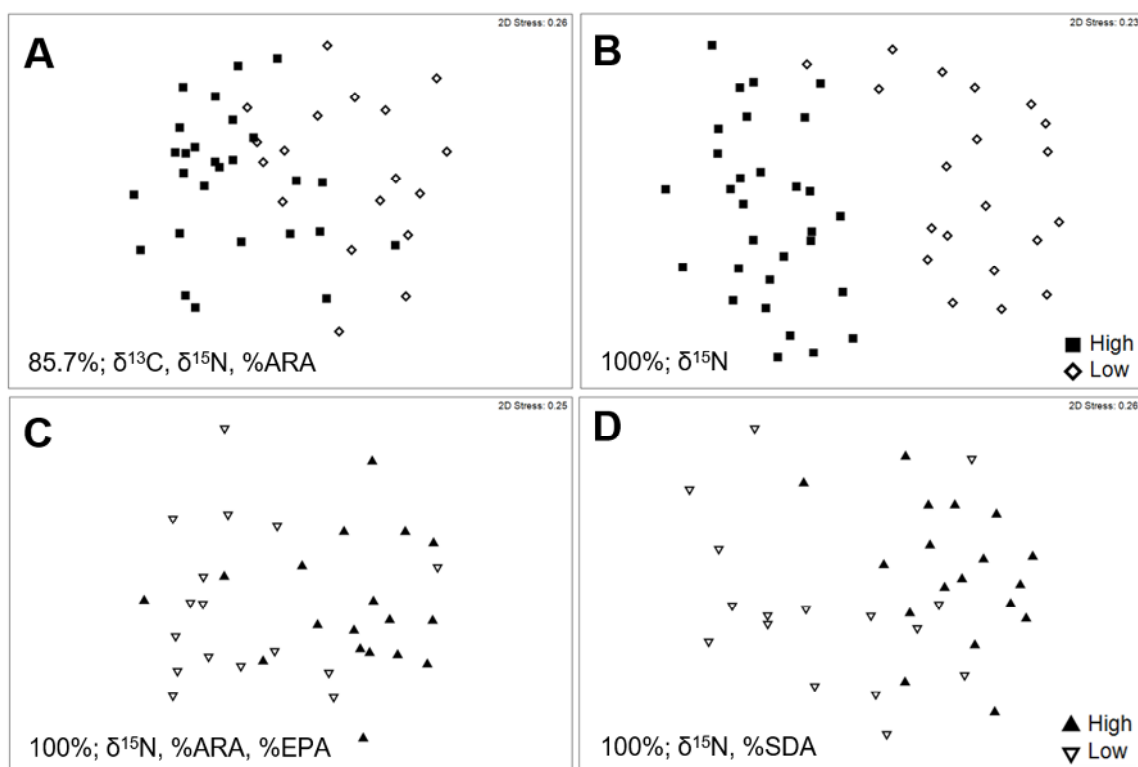
Figure A.14: nMDS plots using ordination among all biomarkers to separate points, illustrating habitat relief related differences for red snapper in 2016 between A) muscle and B) liver, and in 2017 between C) muscle and D) liver. Percentages on the bottom of the plots indicate the classification success and the tracers used to discriminate between high and low relief reefs using QDFA.