Hydrobiologia
https://doi.org/10.1007/s10750-021-04647-1

PRIMARY RESEARCH PAPER

Trophic ecology of fishes associated with artificial reefs assessed using multiple biomarkers

Jeffrey D. Plumlee · David N. Hala · Jay R. Rooker · J. Brooke Shipley · R. J. David Wells

Received: 4 February 2021 / Revised: 11 June 2021 / Accepted: 17 June 2021

Abstract Understanding trophic relationships within artificial reef communities, especially those of the most numerically abundant fish, provides value to ecologists and managers looking to prioritize healthy food webs. Here we elucidate the trophic interactions of three common fish species on high relief (> 5 m) and low relief (< 5 m) artificial reefs in the northwestern Gulf of Mexico. Biomarkers including stable isotopes, ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$), and essential fatty acids (18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6, 20:5n-3, 22:5n3, and 22:6n-3) were analyzed within muscle and liver tissue. Species-specific comparisons among tomtate (*Haemulon aurolineatum*), pigfish (*Orthopristis chrysoptera*), and red snapper (*Lutjanus campechanus*), revealed differences in biomarkers within muscle tissue (long-term) namely $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, EPA (20:5n-3), and DHA (22:6n-3). However, using liver tissue (short-term) significant differences existed among a fewer number of biomarkers ($\delta^{15}N$, $\delta^{34}S$, and EPA) among the three species, indicating increasing trophic similarity. Red snapper collected from low relief reefs had higher $\delta^{15}N$ values than those on high relief reefs which may be due to higher forage trophic level due to the lack of co-occurring congeners. This study highlights the importance of interspecific food web observations that aid in the interpretation of the complex trophic relationships occurring on artificial reefs.

Keywords Stable isotopes · Essential fatty acids · Red snapper · Artificial reefs · Food webs

Handling editor: Ivan Nagelkerken

J. D. Plumlee (✉)
Institute of Marine Sciences, University of North Carolina At Chapel Hill, Morehead City, NC 28557, USA
e-mail: jplumlee@live.unc.edu

D. N. Hala · J. R. Rooker · R. J. D. Wells
Department of Marine Biology, Texas A&M University at Galveston, 1001 Texas Clipper Rd., Galveston, TX 77553, USA

D. N. Hala · J. R. Rooker · R. J. D. Wells
Department of Ecology & Conservation Biology, Texas A&M University, College Station, TX 77843, USA

J. B. Shipley
Texas Parks and Wildlife Department, Artificial Reef Program, Austin, TX 78744, USA

Introduction

Artificial reefs serve a variety of functions; such as erosion control, enhancing biodiversity, and increasing fisheries yield (Bohnsack, 1989; Baine, 2001). As faunal habitat, artificial reefs are designed to mimic natural reefs to attract a diverse community of structure-associated fauna and serve as important foraging and refuge habitat (Rooker et al., 1997; Arena et al., 2007; Folpp et al., 2013; Granneman & Steele, 2015). To accomplish the goal of establishing

Published online: 05 July 2021

quality habitat to structure-associated fauna, reef design should include factors such as vertical relief (Rilov & Benayahu, 2000), rugosity (Jennings et al., 1996), and productive adjacent habitat (Bohnsack & Sutherland, 1985). As artificial habitat increases the localized diversity of structure-associated fauna, it is expected there are similar localized changes within the food web (McCann et al., 1998). Artificial habitat adds complexity, therein resilience, to localized food webs by increasing the diversity within trophic guilds (Dance et al., 2011; Cresson et al., 2019). The trophic benefits of the artificial structure itself to localized food webs are species-specific, while some species utilize structure as foraging grounds (Fabi et al., 2006), other species use the structure as refuge and forage in the surrounding substrate proximate to the structure (Lindquist et al., 1994).

Quantifying trophic interactions among reef-associated species is important for estimating the productivity of a reef. The feeding ecology of red snapper has been widely investigated throughout the Gulf of Mexico, however the studies that have investigated the diets of both pigfish and tomtate are rare. All three species are observed consumers of benthic crustaceans including crabs and shrimp, while red snapper and tomtate have been shown to feed on higher proportions of fish (up to 70%) although this changes throughout their ontogeny (Darcy, 1983; Szedlmayer & Lee, 2004; Wells et al., 2008; Norberg, 2015). However in lieu of the presence of dietary information we can use biomarkers (i.e., stable isotope ratios, fatty acids) using the paradigm "you are what you eat" to infer trophic relationships (Peterson & Fry, 1987). Stable isotope values can indicate trophic position, dietary shifts, and movement when paired with baseline isotopic ratios generated by primary producers (Post, 2002; Trueman et al., 2012). Carbon ($\delta^{13}C$) isotope ratios are widely used to identify potential carbon sources based upon the principles guiding the differing pathways of carbon fixation during photosynthesis (DeNiro & Epstein, 1978). Nitrogen ($\delta^{15}N$) can be used to determine trophic level as well as food web complexity (Deniro & Epstein, 1981). Both $\delta^{13}C$ and $\delta^{15}N$ 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 ($\delta^{34}S$) is useful to contrast benthic versus pelagic foraging strategies in fishes, and undergoes levels of fractionation that are less than $\delta^{13}C$ and $\delta^{15}N$ increasing only slightly with increasing trophic levels (Peterson & Fry, 1987; Wells et al., 2008). In marine systems, $\delta^{34}S$ values tend to be lower in benthic zones due to the increased percentage of sulfides in the sediment, while $\delta^{34}S$ values are higher in the water column where inorganic sulfur occurs mostly as sulfates (Fry et al., 2008). Sulfur ($\delta^{34}S$) ratios have also been shown to parallel $\delta^{13}C$ isoscapes along coastal estuarine gradients, due to the effects that freshwater input has on sulfates which are more depleted than marine sulfates (Peterson & 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) can also be useful in providing information on trophic structure. FAs are typically conserved when passing from producer to consumer, making them a useful trophic biomarker, similar to stable isotope ratios (Dalsgaard et al., 2003; Iverson, 2009). Unlike stable isotope ratios, FAs, specifically polyunsaturated fatty acids (PUFAs), reflect specific primary producers better than stable isotope ratios (Rooker et al., 2006). 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). 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). However, in some extreme cases EFAs can be metabolized in periods of starvation or nutrient limitation (Gourtay et al., 2018; Galloway & Budge, 2020). 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 been shown to correlate with piscivory and trophic level (Rooker et al., 2006; Litz et al., 2017; Sardenne et al., 2017).

The use of stable isotope and FA ratios can encompass the feeding ecology of organisms over varied timeframes using multiple tissue types. However, it is important to understand the various tissue turnover rates in order to relate to the food resources (Hobson et al., 2010). Tissue turnover can be affected by several potential confounding factors including, temperature, growth rate, and metabolism for both stable isotope and FA ratios (Boecklen et al., 2011). However, it is generally agreed upon that tissues with higher metabolic activity can quickly reflect changes in dietary nutrient sources than tissues with lower metabolic activity (Matich et al., 2011; Davis et al., 2015). Few in situ studies have been performed on marine fishes to look at tissue-specific turnover of both stable isotope and FA ratios; however, companion studies by 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* (Linnaeus 1766). For larger adult predatory fish, these estimates were longer, as noted in a species of grouper, *Plectropomus leopardus* (Lacepède 1802), with a 50% turnover in liver tissue at three weeks (21 days) but a 95% turnover in liver tissue at nearly three months (91 days), which had similar trends in muscle tissue (50%, 126 days; 95%, 543 days) (Matley et al., 2016). While tissue-specific estimates appear to differ by species, utilizing multiple tissue types can be useful to identify temporal changes in feeding ecology and movement of fishes. Biomarkers in animal tissues provide time-relevant information regarding the feeding ecology of marine organisms across ontogeny, but they can also reflect the different sources of primary production that support an animal across space (Matich et al., 2011).

Here a combination of both fatty acid and stable isotope biomarkers across multiple tissue types were used to investigate the feeding ecology of three common reef fishes (tomtate, pigfish, and red snapper) that co-occur on artificial reefs in the northwestern Gulf of Mexico (NW GoM). Each of these species are numerically abundant, structure-associated, mesopredators that serve as important trophic links in NW GoM artificial reef food webs. Fish communities that utilize nearshore artificial reefs in the NW GoM have a high proportion of residents that are estuarine-dependent (Plumlee et al., 2020) including Atlantic croaker; sand seatrout, *Cynoscion arenarius* (Ginsburg 1930); black drum, *Pogonias cromis* (Linnaeus 1766); southern kingfish, *Menticirrhus americanus* (Linnaeus 1758); oyster toadfish, *Opsanus tau* (Linnaeus 1766); and pinfish, *Lagodon rhomboides* (Linnaeus 1766). Understanding a few of the trophic relationships within this community, especially those of the most numerically abundant fish, provides value to ecologists and managers looking to prioritize healthy food webs. The primary objective of this study was to characterize dietary biomarkers of three different reef taxa to identify trophic similarities and dissimilarities. Additionally, for red snapper, ubiquitous occupancy on artificial reefs in the northwest Gulf of Mexico and the lack of co-occurring congeners on low relief reefs may indicate a change in food web structure compared to high relief reefs. This being the case, we also aim to contrast the feeding trends of fish on high and low relief artificial reefs, respectively. Species-specific comparisons of dietary biomarkers were accomplished using samples collected in a single year with all fish from high relief artificial reefs. Habitat specific trends examined using red snapper collected from both high and low relief complexes during two years. Biomarkers comprised three bulk stable isotope ratios along with seven PUFAs and compared from both epaxial muscle and liver tissue in order to utilize slow and fast turnover times, respectively.

Materials and methods

Sample collection and processing

Three reef fish species including tomtate, *Haemulon aurolineatum* (Cuvier 1830); pigfish, *Orthopristis chrysoptera* (Linnaeus 1766); and red snapper, *Lutjanus campechanus* (Poey 1860) were collected during quantitative surveys from June through August over a two-year period (2016–2017) at two artificial reef complexes (high relief W 94° 41′ 48″, N 28° 52′ 56″; low relief W 94° 54′ 20″, N 29° 18′ 12″). Collection locations within the high relief complex (> 5 m) were primarily constructed from toppled and cutoff rig jackets, as well as concrete blocks, while collection locations from the low relief complex (< 5 m) primarily consisted of quarry rock, Marine Administration (MARAD) buoy pieces, experimental reef pyramids, and large concrete anchors. The high relief

reef complex was approximately 38.9 km from shore and at a depth of 20.9 m. While the low relief reef complex was approximately 37.1 km from shore and at a slightly shallower depth of 13 m. Both were surrounded by unconsolidated sand and mud bottom. Sample collection and determination of relative abundance was assessed using quantitative sampling using both vertical longline or fish traps (see methods; Plumlee et al., 2020). Upon collection, samples were measured to the nearest mm fork length. 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 fatty acid (FA) analyses. Muscle and liver tissue collected for stable isotope and FA analyses were frozen at − 80° C. Tissue for FA analysis was initially placed in a 15 ml conical tube with 2 ml of chloroform prior to being frozen.

A total of 75 (n = 28 tomtate, n = 18 pigfish, and n = 29 red snapper) fish was collected in 2016 and used in species-specific analyses. All samples collected had both muscle and liver tissue analyzed for stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) and a subset of samples (n = 12 tomtate, n = 10 pigfish, and n = 11 red snapper) for species-specific analysis of FA ratios. For analyses between habitat types (high and low relief) 84 red snapper were collected over the two-year sampling period, 48 from high relief reefs (2016, n = 29; 2017, n = 19), and 36 from low relief reefs (2016, n = 18; 2017, n = 18). Subsamples of red snapper were also used for habitat specific FA in 2016 (high relief, n = 11; low relief, n = 11) and 2017 (high relief, n = 9; low relief, n = 8). Mean size range varied by species with tomtates ranging from 178 to 245 mm fork length (FL) (212.3 ± 17.1 mm; mean ± standard deviation), pigfish ranged from 158 to 241 mm FL (202.4 ± 20.3 mm), and red snapper ranged from 118 to 540 mm FL (2016 high relief, 301.1 ± 78.7 mm; 2016 low relief, 347.3 ± 51.8 mm; 2017 high relief, 355.1 ± 121.6 mm; 2017 low relief, 369.1 ± 64.1 mm) (Fig. 1).

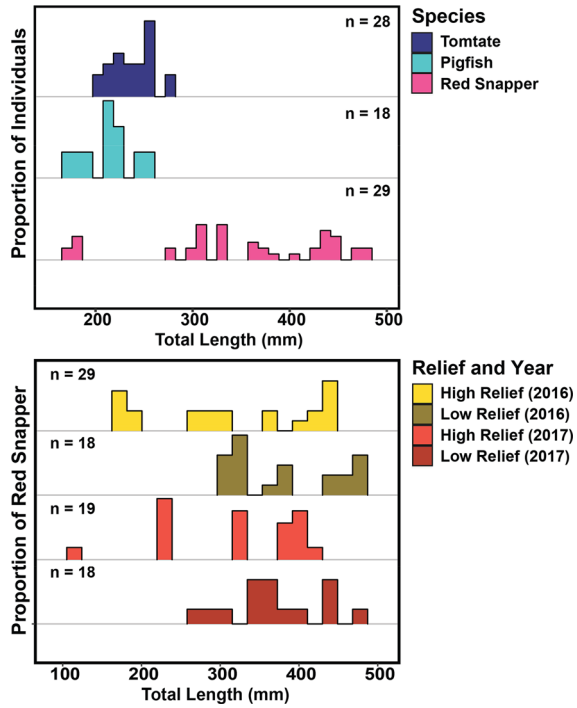

Fig. 1 Fork length (FL) histograms for **A** tomtate (n = 28), pigfish (n = 18), red snapper (n = 29) collected on high relief reefs in 2016, and **B** red snapper collected on high (2016, n = 29; 2017, n = 19) and low (2016, n = 18; 2017, n = 18) relief artificial reefs

Stable isotope analysis

Sample of both muscle and liver tissue for stable isotope analysis were lyophilized 48 h 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 measurement of stable isotopic composition of carbon ($\delta^{13}C$) so lipid correction, or extraction, is a necessary process when comparing $\delta^{13}C$ values among organisms (Post et al., 2007). The extraction process used 34 ml cells packed with layered tissue samples separated by 30 mm glass fiber filter papers (GF/934-AH, 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 $\delta^{13}C$ and $\delta^{15}N$ analyses were weighed to the nearest 1 mg, while samples for $\delta^{34}S$ analysis were weighed to the nearest 4 mg. Analysis of the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ was performed using

a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (IRMS) (Sercon), and $\delta^{34}S$ analysis was done using an Elementar vario ISOTOPE cube interfaced to a 20–22 IRMS (Sercon). Isotopic ratios were presented as delta values, using Vienna PeeDee belemnite, atmospheric N_2 and Vienna Canon Diablo troilite as international standard according to the following equation: $\delta X = [(R_{sample}/R_{standard})- - 1] \times 1000$, 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. Samples were run at UC Davis with interspersed laboratory standards using the provisional values to correct the finalized values with an accuracy of ± 0.2‰ ^{13}C, ± 0.3‰ ^{15}N, and ± 0.4‰ ^{34}S respectively.

Fatty acid analysis

Lipids were extracted from muscle and liver tissue 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 30 m Agilent DB-Wax UI column. The column temperature began at 50°C for 1 min, then was increased (25°C/min) to 200°C, held for 2 min, then increased (3°C/min) to 240°C and held for 20 min. The carrier gas was helium, flowing at a rate of 1 ml/min. 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 polyunsaturated 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), α-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; 22:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). These FAs have been shown to comprise a majority of fatty acids (> 80%) found within a similar omnivorous fish species in the northwestern GoM (Mohan et al., 2016b). After identification all PUFAs (LA, ALA, SDA, ARA, EPA, DPA, and DHA) were expressed as a proportion (%) to all explicitly measured FAs within the sample.

Data analysis

Each objective was investigated independently using varied statistical analyses. MANCOVA models were applied to examine for differences among species (tomtate, pigfish, and red snapper) using the three stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) and seven PUFAs (LA, ALA, SDA, ARA, EPA, DPA, and DHA), with fork length included as a covariate. Muscle and liver tissue were analyzed separately for each of the fish samples. Subsequent ANCOVA models were then used to compare each individual biomarker among the three reef fishes. MANCOVA models were also used to examine two habitat types (high and low relief) and year of collection (2016 and 2017). Two-way ANCOVAs were then used to investigate the relationships of habitat type and year for red snapper by tracer and tissue type (muscle and liver), with fork length added as a covariate. To further examine the effect of species, habitat type, and year on the feeding ecology of individual fish, differences between the values of measured biomarkers in liver and muscle tissue were analyzed similarly to the biomarkers themselves using MANOVA and subsequent individual ANOVAs. 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 $P \leq 0.05$. Due to the number of multiple comparisons among all biomarkers using ANCOVA, a Bonferroni

correction was applied to all *P*-values within each main test, and post hoc comparison.

In addition to determining statistical differences among biomarkers, random forest models were also used to determine the relative importance of each biomarkers species and habitat type comparisons. Year was not examined as a factor due to the low sample size creating a lack of meaningful subset of data in addition to the best practices of including variation to elucidate meaningful relationships. Tree creation within the random forest models was created using subsetted data, 75% of the dataset was used to test the random forest reclassification and the remaining 25% to predict. Each random forest model was created using 500 trees and model accuracy was determined using an initial out of the bag (OOB) error rate, combined with the prediction percentages generated from the remaining 25% of the dataset (Liaw & Wiener, 2002). Rank biomarker importance was determined using mean variable importance, which is generated through the measured decrease in node impurities averaged across all trees within the random forest model. Nonmetric multidimensional scaling (nMDS) was used to visualize differences among species and habitat type factors using all biomarkers (McCune et al., 2002). Before ordination, data for each tracer were modified into an untransformed resemblance matrix using a Bray–Curtis distance measure in the vegan package in R (Clarke & Gorley, 2015).

Results

Species-specific comparison of biomarkers

The differences among these three species using stable isotope values (δ^{13}C, δ^{15}N, and δ^{34}S) and PUFA ratios (LA, ALA, SDA, ARA, EPA, DPA, and DHA) were statistically significant for both muscle ($F_{2,31} = 6.29$, $P < 0.001$) and liver tissue ($F_{2,29} = 4.79$, $P < 0.001$). More specifically, several biomarkers in muscle tissue among the three species were significantly different including δ^{13}C, δ^{15}N, δ^{34}S, EPA, DPA, and DHA (Tables 1,2). Regarding specific tracer differences in muscle tissue, δ^{13}C was significantly different in muscle tissue among the three species with the highest values found in pigfish samples ($-$ 16.11 $\pm$ 0.82‰) and lowest in tomtate samples ($-$ 16.89 $\pm$ 0.24‰). Pigfish samples had significantly lower δ^{15}N values (14.88 $\pm$ 0.92‰) than the other two species (tomtate, 15.86 $\pm$ 0.41‰; red snapper, 15.82 $\pm$ 0.3‰) as well as δ^{34}S (pigfish, 15.21 $\pm$ 2.37‰, tomtate, 18.47 $\pm$ 0.57‰; red snapper, 18.43 $\pm$ 0.45‰). The remaining differences among biomarkers were in red snapper muscle tissue, which had significantly lower EPA (3.87 $\pm$ 1.07%) than tomtates (5.71 $\pm$ 0.61%) and pigfish (EPA 5.61 $\pm$ 1.13%). DHA was the only PUFA that was higher in red snapper muscle tissue (20.35 $\pm$ 2.54%) than the other species, tomtates (14.35 $\pm$ 2.48%) and pigfish (16.43 $\pm$ 2.93%) (Table 1).

Using liver tissue, δ^{15}N, δ^{34}S, and EPA were found to be significantly different among species (Tables 1, 2). Pigfish had significantly lower δ^{15}N values (13.35 $\pm$ 0.55‰) than the other two species, tomtate (13.96 $\pm$ 0.56‰) and red snapper (14.4 $\pm$ 0.8‰). All three species had significantly different δ^{34}S values using liver tissue with pigfish samples having the lowest (17.05 $\pm$ 1.05‰) and red snapper having the highest (18.89 $\pm$ 0.56‰). EPA was lowest for pigfish liver tissue (3.19 $\pm$ 0.97%) relative to tomtate (5.07 $\pm$ 0.83%) and red snapper (4.37 $\pm$ 1.04%) (Table 1).

The mean difference between biomarkers in muscle and liver tissue for individual fish was also found to be significantly different among species ($F_{2,\ 29} = 4.60$, $P < 0.01$) using MANCOVA. However, with further investigation using ANCOVA, this was due to one biomarker, δ^{34}S which was highest for pigfish (2.06 $\pm$ 1.46) followed by red snapper (0.52 $\pm$ 0.49) and tomtate ($-$ 0.03 $\pm$ 0.60) ($F_{2,\ 29} = 31.90$, $P < 0.01$).

The variables that were most important for species-specific classification success using random forest models varied between the two tissue types. Random forest reclassification for species using biomarkers in muscle tissue had an OOB error rate of 18.2% and a prediction success rate of 100% (tomtate, 100%; pigfish, 100%; red snapper, 100%). The highest mean importance for reclassification of random samples to species using biomarkers in muscle tissue belonged to δ^{34}S (mean, 11.19; tomtate, 4.10; pigfish, 23.90; red snapper, 5.58) and DPA (mean, 7.66; tomtate, 7.31; pigfish, 6.37; red snapper, 9.31). The mean importance of DHA (mean, 4.85; tomtate, 8.21; pigfish, $-$ 0.11; red snapper, 6.44) and δ^{13}C (mean, 4.83; tomtate, 8.01; pigfish, 4.49; red snapper, 1.98) were next highest and only differed by 0.02 mean importance

Table 1 Mean values (± standard deviation) for all biomarkers within both muscle and liver tissue for species-specific comparisons

Biomarkers	Muscle			Liver		
	Tomtate	Pigfish	Red Snapper	Tomtate	Pigfish	Red Snapper
$\delta^{13}C$	− 16.89 ± 0.24	**− 16.11 ± 0.82**	− 16.51 ± 0.23	− 16.62 ± 0.7	− 16.71 ± 0.67	− 17.37 ± 0.74
$\delta^{15}N$	15.86 ± 0.41	**14.88 ± 0.92**	15.82 ± 0.3	13.96 ± 0.56	**13.35 ± 0.55**	14.4 ± 0.8
$\delta^{34}S$	18.47 ± 0.57	**15.21 ± 2.37**	18.43 ± 0.45	18.46 ± 0.6	**17.05 ± 1.05**	18.89 ± 0.56
LA	0.53 ± 0.07	0.48 ± 0.08	0.55 ± 0.08	0.63 ± 0.15	0.51 ± 0.12	0.6 ± 0.17
ALA	0.13 ± 0.05	0.1 ± 0.03	0.13 ± 0.04	0.19 ± 0.08	0.08 ± 0.02	0.18 ± 0.1
SDA	0.17 ± 0.2	0.13 ± 0.08	0.13 ± 0.22	0.16 ± 0.08	0.06 ± 0.02	0.12 ± 0.14
ARA	4.21 ± 1.49	4.85 ± 1.15	3.05 ± 0.71	3.19 ± 0.65	3.21 ± 0.89	4.15 ± 1.37
EPA	5.7 ± 0.61	5.61 ± 1.13	**3.99 ± 0.86**	5.07 ± 0.83	**3.19 ± 0.97**	4.37 ± 1.04
DPA	2.99 ± 1.05	2.88 ± 0.55	1.6 ± 0.41	2.09 ± 0.39	1.99 ± 0.72	1.91 ± 0.52
DHA	14.35 ± 2.48	16.43 ± 2.93	**19.9 ± 2.33**	9.55 ± 3.81	10.93 ± 4.61	13.63 ± 2.55

Significant relationships are in bold ($P \leq 0.05$)

(Figs. 2, 4). Reclassification of species using biomarkers in liver tissue had an OOB error rate of 20.0% and a prediction rate of 85.7% (tomtate, 66.6%; pigfish, 100%; red snapper, 100%). The highest mean importance belonged to $\delta^{34}S$ (mean, 10.67; tomtate, 3.48; pigfish, 17.84; red snapper, 10.68), followed by ALA (mean, 5.71; tomtate, 7.84; pigfish, 7.22; red snapper, 2.06), and SDA (mean, 5.56; tomtate, 9.22; pigfish, 0.32; red snapper, 7.15) respectively (Figs. 2, 4).

Red snapper over high and low relief artificial reefs

Trophic biomarkers significantly differed in red snapper tissue collected between high and low relief reefs and years (2016 and 2017) regardless of tissue type (muscle and liver). Biomarkers in red snapper muscle tissue differed significantly between habitat types and between years (relief: $F_{1,37} = 6.73$, $P < 0.001$; year: $F_{1,37} = 5.66$, $P < 0.001$). For red snapper collected in 2016 low relief reef samples had higher $\delta^{15}N$ (high relief, 15.66 ± 0.24‰; low relief, 16.09 ± 0.16‰) and lower $\delta^{34}S$ (high relief, 18.63 ± 0.34‰; low relief, 18.11 ± 0.44‰) in muscle tissue. In 2017, red snapper collected on low relief reefs also had higher $\delta^{15}N$ (high relief, 16.04 ± 0.26‰; low relief, 16.45 ± 0.29‰) in addition to higher ARA (high relief, 2.80 ± 0.49%; low relief, 3.71 ± 0.4.72%). However, EPA was higher for red snapper muscle samples collected on high relief reefs (4.36 ± 0.42%) than low relief reefs (3.09 ± 0.53%) in 2017 (Tables 3, 4, 5).

Trophic biomarkers in red snapper liver tissue were also different between habitat types and years (habitat type: $F_{1,35} = 28.10$, $P < 0.001$; year: $F_{1,34} = 5.67$, $P < 0.001$). For red snapper collected in 2016, both $\delta^{13}C$ (high relief, − 17.09 ± 0.65‰; low relief, − 17.77 ± 0.68‰) and $\delta^{34}S$ (high relief, 19.14 ± 0.45‰; low relief, 18.51 ± 0.49‰) were lower in red snapper liver tissue at low relief reefs relative to high relief reefs. Only $\delta^{15}N$ showed consistent differences in red snapper liver tissue across years, with red snapper liver samples from both years having higher $\delta^{15}N$ in samples collected on low relief reef relative to 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‰). Additionally, in 2017 EPA was significantly different in red snapper liver tissue for fish collected on high relief reefs (5.96 ± 0.56%) relative to. low relief reefs (3.67 ± 0.55%) (Tables 3, 4, 5).

The difference between biomarkers found in muscle and liver tissue was also found to be significantly different between red snapper collected on low and high relief reefs using MANCOVA (relief: $F_{1,37} = 4.30$, $P < 0.001$; year: $F_{1,37} = 2.01$, $P = 0.045$). No statistical differences existed between measured muscle and liver biomarkers in 2016, but in 2017 the differences between $\delta^{34}S$ values measured in muscle and liver tissues were higher ($F_{1,37} = 56.91$, $P = 0.04$) for red snapper collected on low relief reefs

Table 2 Test results for one-way ANCOVA among all species tomtate (TT), pigfish (PF), and red snapper (RS) for all biomarkers found in muscle and liver tissue

Biomarkers	Muscle					Liver				
	df	*F*-Ratio	*P*-value*	Pairwise	*P*-value*	df	*F*-Ratio	*P*-value*	Pairwise	*P*-value*
$\delta^{13}C$	71	16.91	**0.01**	TT–RS	1.00	71	3.76	0.28	TT–RS	1.00
				PF–TT	**0.03**				PF–TT	1.00
				PF–RS	**0.03**				PF–RS	1.00
$\delta^{15}N$	71	19.97	**0.01**	TT–RS	0.54	70	9.11	**0.01**	TT–RS	1.00
				PF–TT	**0.03**				PF–TT	**0.03**
				PF–RS	0.27				PF–RS	1.00
$\delta^{34}S$	71	49.57	**0.01**	TT–RS	1.00	72	53.53	**0.01**	TT–RS	1.00
				PF–TT	**0.03**				PF–TT	**0.03**
				PF–RS	**0.03**				PF–RS	**0.03**
LA	31	4.09	0.27	TT–RS	1.00	29	1.55	1.00	TT–RS	1.00
				PF–TT	1.00				PF–TT	1.00
				PF–RS	0.39				PF–RS	1.00
ALA	31	1.76	1.00	TT–RS	1.00	29	4.18	1.00	TT–RS	1.00
				PF–TT	1.00				PF–TT	0.84
				PF–RS	1.00				PF–RS	1.00
SDA	31	0.88	1.00	TT–RS	1.00	29	2.26	0.50	TT–RS	1.00
				PF–TT	1.00				PF–TT	1.00
				PF–RS	1.00				PF–RS	1.00
ARA	31	5.57	0.06	TT–RS	1.00	29	2.30	1.00	TT–RS	0.09
				PF–TT	1.00				PF–TT	1.00
				PF–RS	1.00				PF–RS	0.09
EPA	31	12.75	**0.01**	TT–RS	**0.03**	29	8.42	**0.01**	TT–RS	1.00
				PF–TT	1.00				PF–TT	**0.03**
				PF–RS	**0.03**				PF–RS	0.87
DPA	31	11.20	**0.01**	TT–RS	1.00	29	1.38	1.00	TT–RS	1.00
				PF–TT	1.00				PF–TT	1.00
				PF–RS	1.00				PF–RS	1.00
DHA	31	15.58	**0.01**	TT–RS	**0.03**	29	2.88	0.72	TT–RS	0.63
				PF–TT	1.00				PF–TT	1.00
				PF–RS	**0.03**				PF–RS	1.00

Significant relationships are in bold ($P \leq 0.05$)

*All *P*-values were adjusted using the Bonferroni correction for multiple comparisons

(0.72 ± 0.50) compared to red snapper collected on high relief reefs (0.33 ± 0.50).

The variables that were most important for habitat specific classification success using random forest models varied between tissue types. Random forest reclassification of red snapper muscle tissue using biomarkers between low relief and high relief habitat had an OOB error rate of 16.67% and a prediction success rate of 80% (high relief 80% and low relief 80%). The highest mean importance for reclassification of random samples to species using biomarkers in muscle tissue belonged to $\delta^{34}S$ (high relief, 13.30; low relief, 14.89) followed by $\delta^{15}N$ (high relief, 7.25; low relief, 4.62). All other biomarkers in muscle tissue had a mean importance of less than three (Figs. 3, 5). Reclassification of species using biomarkers in liver

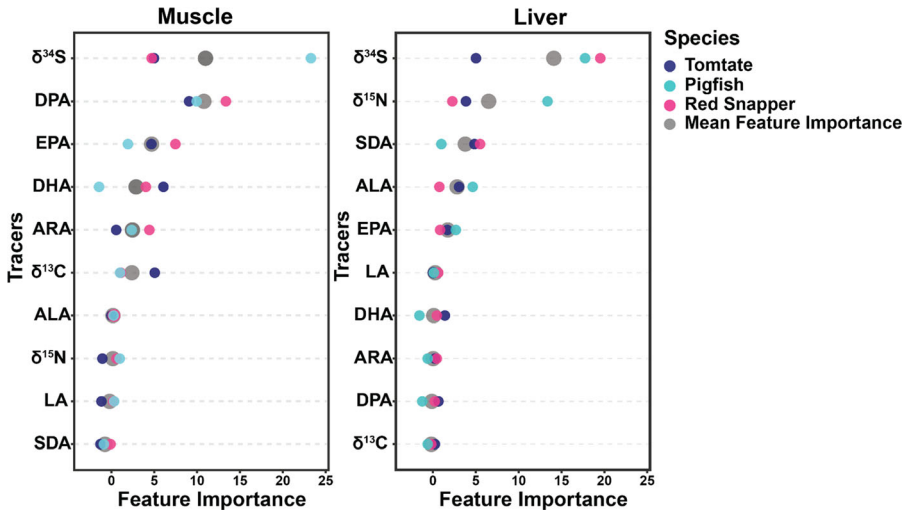

Fig. 2 Plots denoting model importance for each tracer within random forest model ranked from highest to lowest importance in determining correct classification within nodes. Color for tomtate is blue, red snapper is pink, and pigfish is cyan

Table 3 Mean values (± standard deviation) for all biomarkers within both muscle and liver tissue for habitat type comparisons for samples collected in 2016

Biomarkers	Muscle		Liver	
	High-relief	Low-relief	High-relief	Low-relief
δ^{13}C	− 16.52 ± 0.26	− 16.49 ± 0.19	**− 17.09 ± 0.65**	**− 17.77 ± 0.68**
δ^{15}N	**15.66 ± 0.24**	**16.09 ± 0.16**	**13.78 ± 0.28**	**15.27 ± 0.35**
δ^{34}S	**18.63 ± 0.34**	**18.12 ± 0.43**	**19.15 ± 0.46**	**18.51 ± 0.49**
LA	0.58 ± 0.09	0.52 ± 0.06	0.56 ± 0.21	0.63 ± 0.12
ALA	0.13 ± 0.04	0.13 ± 0.04	0.14 ± 0.12	0.21 ± 0.06
SDA	0.1 ± 0.09	0.18 ± 0.3	0.1 ± 0.19	0.14 ± 0.07
ARA	3.18 ± 0.97	2.9 ± 0.2	4.01 ± 1.85	4.28 ± 0.77
EPA	3.87 ± 1.07	4.11 ± 0.59	4.48 ± 1.32	4.28 ± 0.77
DPA	1.7 ± 0.54	1.49 ± 0.18	1.72 ± 0.54	2.09 ± 0.45
DHA	20.35 ± 2.54	19.4 ± 2.09	13.33 ± 3.13	13.9 ± 2.02

Significant relationships are in bold ($P \leq 0.05$)

Table 4 Mean values (± standard deviation) for all biomarkers within both muscle and liver tissue for habitat type comparisons for samples collected in 2017

Biomarkers	Muscle		Liver	
	High-relief	Low-relief	High-relief	Low-relief
δ^{13}C	− 16.85 ± 0.16	− 16.71 ± 0.18	− 17.39 ± 0.46	− 17.91 ± 0.73
δ^{15}N	**16.04 ± 0.26**	**16.45 ± 0.29**	**14.63 ± 0.32**	**15.39 ± 0.35**
δ^{34}S	18.68 ± 0.35	18.22 ± 0.48	18.71 ± 0.28	19.17 ± 0.57
LA	0.61 ± 0.06	0.52 ± 0.08	0.71 ± 0.06	0.75 ± 0.11
ALA	0.19 ± 0.05	0.15 ± 0.05	0.23 ± 0.05	0.37 ± 0.16
SDA	0.12 ± 0.04	0.1 ± 0.03	0.1 ± 0.04	0.28 ± 0.17
ARA	**2.8 ± 0.49**	**3.71 ± 0.47**	4.49 ± 0.55	5.07 ± 0.78
EPA	**4.36 ± 0.42**	**3.09 ± 0.53**	**5.96 ± 0.56**	**3.67 ± 0.55**
DPA	1.54 ± 0.23	1.32 ± 0.21	**3.36 ± 1.02**	**1.8 ± 0.52**
DHA	18.67 ± 2.75	19.33 ± 4.21	15.04 ± 2.4	13.31 ± 3.88

Significant relationships are in bold ($P \leq 0.05$)

Table 5 Test results of two-factor ANCOVA for red snapper among habitat type using habitat type (high and low relief) and year (2016/2017) as factors for all biomarkers

Biomarkers	Comparisons	Muscle			Liver		
		df	*F*-Ratio	*P*-value*	df	*F*-Ratio	*P*-value*
$\delta^{13}C$	Habitat (2016)	45	0.18	1.00	47	12.65	**0.04**
	Habitat (2017)	34	6.41	0.6	33	6.61	0.92
	Year	79	42.77	**0.04**	80	2.43	1.00
	Habitat × Year	79	3.43	1.00	80	0.29	1.00
$\delta^{15}N$	Habitat (2016)	45	45.06	**0.04**	46	265.74	**0.04**
	Habitat (2017)	34	20.23	**0.04**	33	44.31	**0.04**
	Year	79	50.56	**0.04**	79	51.06	**0.04**
	Habitat × Year	79	0.01	1.00	79	24.84	**0.04**
$\delta^{34}S$	Habitat (2016)	45	20.57	**0.04**	48	21.60	**0.04**
	Habitat (2017)	34	11.23	0.08	33	8.95	0.24
	Year	79	0.71	1.00	81	0.28	1.00
	Habitat x Year	79	0.07	1.00	81	27.71	**0.04**
LA	Habitat (2016)	21	2.95	1.00	19	0.79	1.00
	Habitat (2017)	15	8.16	0.68	14	0.78	1.00
	Year	36	0.50	1.00	33	7.52	0.36
	Habitat × Year	36	0.96	1.00	33	0.09	1.00
ALA	Habitat (2016)	21	0.01	1.00	19	2.22	1.00
	Habitat (2017)	15	2.50	1.00	14	6.42	1.00
	Year	36	9.32	0.16	33	13.25	**0.04**
	Habitat × Year	36	1.53	1.00	33	1.31	1.00
SDA	Habitat (2016)	21	0.77	1.00	19	0.31	1.00
	Habitat (2017)	15	0.58	1.00	14	9.41	1.00
	Year	36	0.23	1.00	33	2.51	1.00
	Habitat × Year	36	0.85	1.00	33	2.54	1.00
ARA	Habitat (2016)	21	0.86	1.00	19	0.21	1.00
	Habitat (2017)	15	15.13	**0.04**	14	2.99	0.96
	Year	36	1.12	1.00	33	3.44	1.00
	Habitat × Year	36	9.89	0.12	33	0.35	1.00
EPA	Habitat (2016)	21	0.43	1.00	19	0.18	1.00
	Habitat (2017)	15	30.36	**0.04**	14	66.63	**0.04**
	Year	36	0.96	1.00	33	2.74	1.00
	Habitat × Year	36	9.85	0.12	33	12.54	**0.04**
DPA	Habitat (2016)	21	1.64	1.00	19	1.70	1.00
	Habitat (2017)	15	4.45	1.00	14	13.62	**0.04**
	Year	36	2.53	1.00	33	12.69	**0.04**
	Habitat × Year	36	0.03	1.00	33	23.49	**0.04**
DHA	Habitat (2016)	21	0.94	1.00	19	0.26	1.00
	Habitat (2017)	15	0.15	1.00	14	1.21	1.00
	Year	36	1.02	1.00	33	0.43	1.00
	Habitat × Year	36	0.61	1.00	33	1.36	1.00

Significant relationships are in bold ($P \leq 0.05$)

*All *P*-values were adjusted using the Bonferroni correction for multiple comparisons

tissue had an OOB error rate of 7.14% and a prediction rate of 100.0% (high relief 100% and low relief 100%). The highest mean importance belonged to $\delta^{15}N$ (high relief, 17.32; low relief, 18.10), EPA (high relief, 9.81; low relief, 9.78), and SDA (high relief, 6.27; low relief, 8.96) (Figs. 3, 5).

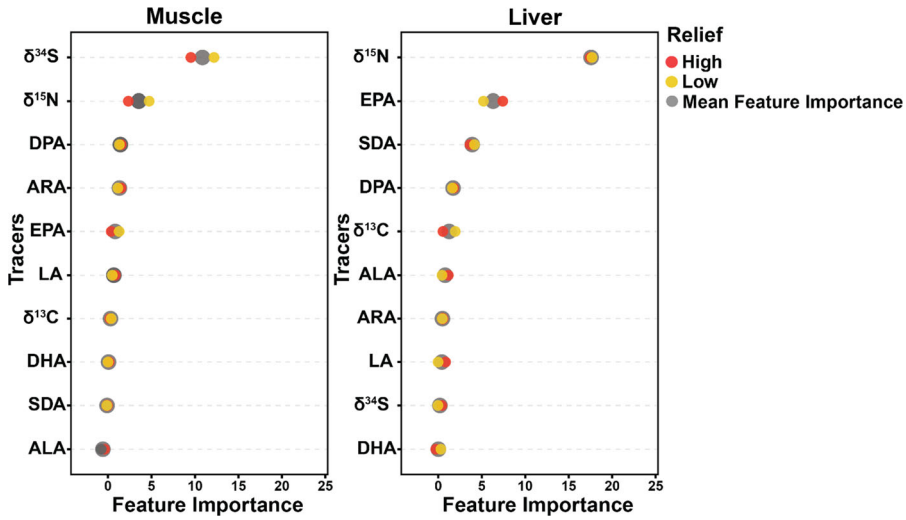

Fig. 3 Plots denoting model importance for each tracer within a random forest model ranked from highest to lowest importance in determining correct classification within nodes. High relief reefs are in red and low relief reefs are in orange

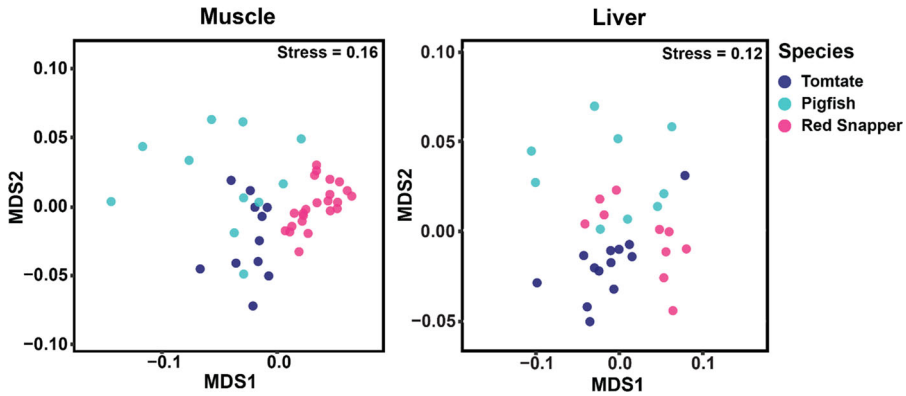

Fig. 4 nMDS plots for all biomarkers plotted in multivariate space for both muscle and liver tissue across all three species. Color for tomtate is blue, red snapper is pink, and pigfish is cyan

Discussion

We found that tomtate, pigfish, and red snapper had numerous significant differences in their measured stable isotope and FA trophic biomarkers. These differences occurred in a higher rate for biomarkers measured in muscle tissue (100% prediction success rate) when compared to liver tissue (86% prediction success rate), indicating an increasing similarity in these three species diets over the short-term. Previous work has shown that habitat configuration and structure type for artificial reefs are closely tied to both species' composition (Rilov & Benayahu, 2000; Ajemian et al., 2015; Paxton et al., 2017; Plumlee et al., 2020) and food web structure (Dance et al., 2011; Cresson et al., 2019; Paxton et al., 2019). On these reefs, tomtate, pigfish and red snapper appear to feed within similar resource pools over the short-term through high trophic overlap. In our investigation of the effect of reef relief on red snapper trophic ecology. Red snapper collected on high and low relief habitats had substantially more differences in the liver tissue (100% prediction success rate) than muscle tissue (80% prediction success rate), indicating the feeding preferences of red snapper may differ with habitat. For these reefs in particular, previous fisheries independent sampling noted that diversity was greater on high relief reefs while low relief reefs maintained high

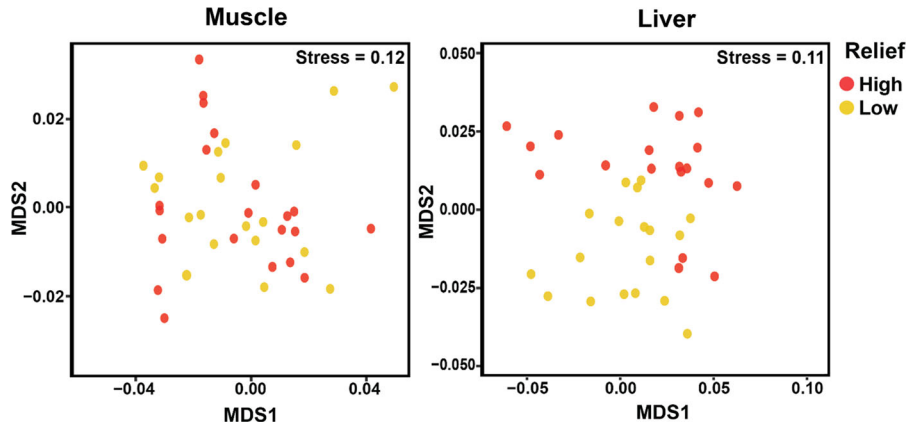

Fig. 5 nMDS plots for all biomarkers plotted in multivariate space for both muscle and liver tissue between the two habitat types. High relief reefs are in red and low relief reefs are in orange

abundances of single-species dominated fish assemblages (Plumlee et al., 2020). It is important for ecosystem managers to prioritize food web relationships in restored habitats, especially those undergoing chronic anthropogenic disturbance such as fishing, as a path to rehabilitating and enhancing ecosystem function.

All three of the species investigated in this study forage primarily in benthic habitats, yet primary literature estimations of their diets vary. Literature estimations of the diets for these three species correlate with our results using stable isotopes and FA biomarkers. Pigfish, which feed primarily on benthic invertebrates (Darcy, 1983), had lower δ^{34}S and δ^{15}N values than tomtate or red snapper. Conversely, piscivory is associated with comparatively higher δ^{34}S and δ^{15} N when measured together (Plumlee & Wells, 2016), which were observed in both red snapper and tomtate tissues when compared to pigfish. DHA and EPA are also important bioindicators that, respectively, reflect piscivory and higher relative trophic level feeding (Rooker et al., 2006; Litz et al., 2017). Mechanistically, DHA comprises higher proportions of the fatty acid profile of marine dinoflagellates which are found throughout the water column but in higher concentrations towards the surface in marine systems. While EPA makes up a larger proportion of the fatty acid profile of marine diatoms, which are the most numerically abundant phytoplankton in northern GOM benthic substrates (Qian et al., 2003; Jónasdóttir, 2019). Red snapper had higher DHA and lower EPA ratios in muscle tissue relative to the other two species, which reflect potentially moderate proportions of fish in their diet. However, DHA and EPA in red snapper liver did not differ compared to the fatty acid proportions in tomtate and pigfish liver tissue. Dietary analyses using stomach contents for red snapper collected on the same reefs indicate their primary forage were benthic crustaceans including portunid crabs and stomatapods but not fishes, as the stable isotope and FA biomarkers in the muscle indicated (Dance et al., 2018). These results indicate that for the red snapper on high relief artificial reefs their diets reflect the available prey items surrounding the reef, and utilize an apparent shared resource pool with co-occurring congeners such as tomtate and pigfish.

There were comparatively large differences in the biomarkers within pigfish muscle tissues compared to the biomarkers found in muscle tissue of red snapper and tomtate, but less so in those found in liver tissues. As nearshore reefs are important nodes of connectivity for inshore/nearshore fish communities, they may also serve a role to facilitate estuarine food subsidies to neritic marine ecosystems through movement of allochthonous resources. For example, δ^{13}C values are variable in coastal environments due to changes in biogeochemistry, turbidity, and salinity that alter phytoplankton communities (Fry, 2002). δ^{34}S values in marine systems are also indicators of freshwater input due to higher proportions of sulfates that occur in fresh-water which make them reliable indicators of movement from low salinity environments (Fry & Chumchal, 2011). Davis et al. (2015), using

biomarkers in both muscle and liver tissue, found a significant proportion of yellowfin bream, *Acanthopargus australis* (Günther 1859), underwent seasonal migration from inshore marsh areas to nearshore reef sites. Using stable isotope biomarkers, the authors inferred this movement due to the high proportion of bream liver tissue having lower $\delta^{13}C$ (− 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. In the pigfish collected for this study, we noticed a very similar trend in the tissues from individuals collected on nearshore artificial reefs with minor differences in $\delta^{13}C$ values in muscle tissue (− 16.71 ± 0.67‰) compared to liver tissue (− 16.11 ± 0.82‰) but significantly higher $\delta^{34}S$ values in muscle tissue (17.05 ± 1.05‰) than was found in liver tissue (15.21 ± 2.37‰). The migratory patterns of pigfish are not well established, but there is evidence to support seasonal movements leaving the estuary to offshore marine habitats (Darcy, 1983). There are several examples of estuarine-dependent fishes contributing to offshore food webs. Pinfish an estuarine-dependent seagrass consumer, contributed to the diets of gag grouper, *Mycteroperca microlepis* (Goode & Bean 1879), found up to 90 km from the source seagrass beds where pinfish are seasonally resident resulting in a 18.5–25% grouper seagrass derived biomass (Nelson et al., 2012). Similarly, pigfish may play a role in the connectivity of coastal marine habitats by carrying allochthonous resources to nearshore artificial reefs.

Red snapper collected on toppled platforms and natural reefs had diets that were more diverse in prey than those collected on freestanding platforms which reflected the overall benthic prey fields (Simonsen et al., 2015). Additionally, Simonsen et al. (2015) found that $\delta^{15}N$ values in the tissue of red snapper were higher on natural and low relief cut-off platforms, similarly to what we observed in red snapper in this study. The less diverse reefs may allow opportunistic generalists like red snapper, to take advantage of unoccupied trophic roles, including feeding on higher trophic level prey. Red snapper feed off the reef on surrounding unconsolidated sand and mud bottom targeting benthic invertebrates and fishes (McCawley, 2007; Dance et al., 2018) and co-occurring reef fishes, like tomtate, also feed primarily off the reef (Arena et al., 2007). Biomarkers in red snapper and tomtate liver tissues significantly differed but reflected very slight differences in feeding preferences, namely only in $\delta^{34}S$ (by ≈ 0.5‰). The lack of difference in the feeding among red snapper and other heterospecifics is not surprising given how red snapper behave when in the presence of other reef fishes. Studies of red snapper observed that when in the presence of other congener (gray snapper, *Lutjanus griseus* [Linnaeus 1758]; and lane snapper, *Lutjanus synagris* [Linnaeus 1758]) individuals appeared to reduce their swimming activity and movement, but still consumed an equal amount of prey to the other commonly co-occurring snapper species (gray snapper), while outcompeting others (lane snapper) (Marshak & Heck, 2017). However, red snapper collected on low relief habitats (2016, 368.4 ± 54.80 cm TL; 2017, 380.76 ± 66.53 cm TL) were slightly larger (< 5 cm mean TL) than those collected on high relief habitat (2016, 322.47 ± 85.56 cm TL; 2017, 364.24 ± 141.30 cm TL) over both years which may be another reason that we observed higher $\delta^{15}N$ on low relief habitats. Albeit a slight difference in size, red snapper do undergo a well-documented increase in trophic level as they grow, along with changes in habitat use which are relatively difficult to uncouple (Wells et al., 2008; Dance et al., 2018). Future work should focus on how the reduction in habitat space (reduced reef relief), decreased faunal diversity, and increased fish size each contribute to allowing red snapper to feed on higher trophic level prey on low relief habitats in the northwest Gulf of Mexico.

This study illustrates that the feeding ecology of fishes on artificial reefs is complex and habitat specific. Short-term species-specific relationships on high relief reefs reflected high levels of trophic overlap, while long-term relationships revealed differences in food web position for each of the three species. We hypothesize that these long-term (muscle tissue) differences are due to seasonal movement and migration, while short-term (liver tissue) differences observed among species indicate immediate resource use. For habitat related differences in the trophic ecology of red snapper collected on low relief reefs and high relief reefs, there was a notable increase in trophic level on low relief reefs that may be due to the decreased relative abundance of congeners. This study highlights the importance of observations made in the context of habitat and inter-specific food web interactions to aid in the interpretation of the complex trophic

relationships occurring on artificial reefs in the NW GoM.

Acknowledgements This project was funded by Texas Parks and Wildlife Artificial Reef Program (Award #443017) to R. J. David Wells and Jay R. Rooker (http://tpwd.texas.gov/landwater/water/habitats/artificial_reef/). We thank the members of the Shark Biology and Fisheries Science and Fisheries Ecology Labs at Texas A&M University at Galveston for their assistance in the field and in the lab.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest in publishing this research.

References

Ajemian, M.J.; Wetz, J.J.; Shipley-Lozano, B.; Shively, J.D.; Stunz, G.W. An analysis of artificial reef fish community structure along the northwestern Gulf of Mexico shelf: potential impacts of "Rigs-to-Reefs" programs. PLoS ONE. 10:e0126354; 2015

Arena, P.T.; Jordan, L.K.B.; Spieler, R.E. Fish assemblages on sunken vessels and natural reefs in southeast Florida, USA. Hydrobiologia. 580:157-171; 2007

Baine, M. Artificial reefs: a review of their design, application, management and performance. Ocean & Coastal Management. 44:241-259; 2001

Boecklen, W.J.; Yarnes, C.T.; Cook, B.A.; James, A.C. On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics. 42:411-440; 2011

Bohnsack, J.A. Are high-densities of fishes at artificial reefs the result of habitat limitation or behavioral preference. Bulletin of Marine Science. 44:631-645; 1989

Bohnsack, J.A.; Sutherland, D.L. Artificial reef research: a review with recommendations for future priorities. Bulletin of Marine Science. 37:11-39; 1985

Clarke, K. R. & R. N. Gorley, 2015. PRIMER v.7: User Manual and Tutorial Plymoth, UK: PRIMER-E

Cresson, P.; Le Direach, L.; Rouanet, E.; Goberville, E.; Astruch, P.; Ourgaud, M.; Harmelin-Vivien, M. Functional traits unravel temporal changes in fish biomass production on artificial reefs. Marine Environmental Research. 145:137-146; 2019

Dalsgaard, J.; St. John, M.; Kattner, G.; Müller-Navarra, D.; Hagen, W. Fatty acid trophic markers in the pelagic marine environment. In: Advances in Marine Biology: Academic Press; Cambridge, 2003

Dance, K.M.; Rooker, J.R.; Shipley, J.B.; Dance, M.A.; Wells, R.D. Feeding ecology of fishes associated with artificial reefs in the northwest Gulf of Mexico. PLoS ONE. 13; 2018

Dance, M.A.; Patterson, W.F.; Addis, D.T. Fish community and trophic structure at artificial reef sites in the northeastern Gulf of Mexico. Bulletin of Marine Science. 87:301-324; 2011

Darcy, G. H. 1983. Synopsis of biological data on the pigfish, *Orthopristis chrysoptera* (Pisces: Haemulidae). NOAA Technical Report

Davis, J.P.; Pitt, K.A.; Fry, B.; Connolly, R.M. Stable isotopes as tracers of residency for fish on inshore coral reefs. Estuarine Coastal and Shelf Science. 167:368-376; 2015

DeNiro, M.J.; Epstein, S. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta. 42:495-506; 1978

Deniro, M.J.; Epstein, S. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica Et Cosmochimica Acta. 45:341-351; 1981

Fabi, G.; Manoukian, S.; Spagnolo, A. Feeding behavior of three common fishes at an artificial reef in the northern Adriatic Sea. Bulletin of Marine Science. 78:39-56; 2006

Folpp, H.; Lowry, M.; Gregson, M.; Suthers, I.M. Fish assemblages on estuarine artificial reefs: natural rocky-reef mimics or discrete assemblages? PLoS ONE. 8:e63505; 2013

Fry, B. Conservative mixing of stable isotopes across estuarine salinity gradients: a conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries. 25:264-271; 2002

Fry, B.; Chumchal, M.M. Sulfur stable isotope indicators of residency in estuarine fish. Limnology and Oceanography. 56:1563-1576; 2011

Fry, B.; Cieri, M.; Hughes, J.; Tobias, C.; Deegan, L.A.; Peterson, B. Stable isotope monitoring of benthic–planktonic coupling using salt marsh fish. Marine Ecology Progress Series. 369:193-204; 2008

Galloway, A.W.; Budge, S.M. The critical importance of experimentation in biomarker-based trophic ecology. The Royal Society; 2020; https://doi.org/10.1098/rstb.2019.0638

Gourtay, C.; Chabot, D.; Audet, C.; Le Delliou, H.; Quazuguel, P.; Claireaux, G.; Zambonino-Infante, J.-L. Will global warming affect the functional need for essential fatty acids in juvenile sea bass (Dicentrarchus labrax)? A first overview of the consequences of lower availability of nutritional fatty acids on growth performance. Marine Biology. 165:1-15; 2018

Granneman, J.E.; Steele, M.A. Effects of reef attributes on fish assemblage similarity between artificial and natural reefs. ICES Journal of Marine Science. 72:2385-2397; 2015

Hobson, K.A.; Barnett-Johnson, R.; Cerling, T. Using Isoscapes to track animal migration. in: West J.B., Bowen G.J., Dawson T.E., Tu K.P., eds Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping. Dordrecht: Springer Netherlands; 2010

Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric models. Biometrical Journal. 50:346-363; 2008

Iverson, S.J. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. in: Kainz M., Brett T.M., Arts T.M., eds Lipids in Aquatic Ecosystems. New York: Springer New York; 2009

Jennings, S.; Boulle, D.P.; Polunin, N.V.C. Habitat correlates of the distribution and biomass of Seychelles' reef fishes. Environmental Biology of Fishes. 46:15-25; 1996

Jónasdóttir, S.H. Fatty acid profiles and production in marine phytoplankton. Marine Drugs. 17:151; 2019

Kainz, M.; Arts, M.T.; Mazumder, A. Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnology and Oceanography. 49:1784-1793; 2004

Liaw, A.; Wiener, M. Classification and regression by randomForest. R news. 2:18-22; 2002

Lindquist, D.G.; Cahoon, L.B.; Clavijo, I.E.; Posey, M.H.; Bolden, S.K.; Pike, L.A.; Burk, S.W.; Cardullo, P.A. Reef fish stomach contents and prey abundance on reef and sand substrata associated with adjacent artificial and natural reefs in Onslow Bay, North Carolina. Bulletin of Marine Science. 55:308-318; 1994

Litz, M.N.C.; Miller, J.A.; Copeman, L.A.; Teel, D.J.; Weitkamp, L.A.; Daly, E.A.; Claiborne, A.M. Ontogenetic shifts in the diets of juvenile Chinook Salmon: new insight from stable isotopes and fatty acids. Environmental Biology of Fishes. 100:337-360; 2017

Marshak, A.R.; Heck, K.L. Interactions between range-expanding tropical fishes and the northern Gulf of Mexico red snapper Lutjanus campechanus. Journal of Fish Biology. 91:1139-1165; 2017

Matich, P.; Heithaus, M.R.; Layman, C.A. Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. Journal of Animal Ecology. 80:294-305; 2011

Matley, J.; Fisk, A.T.; Tobin, A.J.; Heupel, M.R.; Simpfendorfer, C.A. Diet-tissue discrimination factors and turnover of carbon and nitrogen stable isotopes in tissues of an adult predatory coral reef fish, *Plectropomus leopardus*. Rapid Communications in Mass Spectrometry. 30:29-44; 2016

McCann, K.; Hastings, A.; Huxel, G.R. Weak trophic interactions and the balance of nature. Nature. 395:794-798; 1998

McCawley, J. R. Seasonal and size specific diet and prey demand of red snapper on Alabama artificial reefs; 2007

McCune, B.; Grace, J.B.; Urban, D.L. Analysis of Ecological Communities. MjM software design, Gleneden Beach, OR; 2002

Mohan, J.A.; Smith, S.D.; Connelly, T.L.; Attwood, E.T.; McClelland, J.W.; Herzka, S.Z.; Walther, B.D. Tissue-specific isotope turnover and discrimination factors are affected by diet quality and lipid content in an omnivorous consumer. Journal of Experimental Marine Biology and Ecology. 479:35-45; 2016a

Mohan, S.D.; Mohan, J.A.; Connelly, T.L.; Walther, B.D.; McClelland, J.W. Fatty-acid biomarkers and tissue-specific turnover: validation from a controlled feeding study in juvenile Atlantic croaker *Micropogonias undulatus*. Journal of Fish Biology. 89:2004-2023; 2016b

Nelson, J.; Wilson, R.; Coleman, F.; Koenig, C.; DeVries, D.; Gardner, C.; Chanton, J. Flux by fin: fish-mediated carbon and nutrient flux in the northeastern Gulf of Mexico. Marine Biology. 159:365-372; 2012

Norberg, M. J. The ecology of tomtate, *Haemulon aurolineatum*, in the northern Gulf of Mexico and effects of the Deepwater Horizon oil spill: University of South Alabama; 2015

Parrish, C.C. Essential fatty acids in aquatic food webs. Lipids in aquatic ecosystems. Springer, New York; 2009

Paxton, A.B.; Pickering, E.A.; Adler, A.M.; Taylor, J.C.; Peterson, C.H. Flat and complex temperate reefs provide similar support for fish: evidence for a unimodal species-habitat relationship. PLoS ONE. 12:e0183906; 2017

Paxton, A.B.; Taylor, J.C.; Peterson, C.H.; Fegley, S.R.; Rosman, J.H. Consistent spatial patterns in multiple trophic levels occur around artificial habitats. Marine Ecology Progress Series. 611:189-202; 2019

Peterson, B.J.; Fry, B. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics. 18:293-320; 1987

Plumlee, J.D.; Dance, K.M.; Dance, M.A.; Rooker, J.R.; Tin-Han, T.C.; Shipley, J.B.; Wells, R. Fish assemblages associated with artificial reefs assessed using multiple gear types in the northwest Gulf of Mexico. Bulletin of Marine Science; 2020; 96: 655

Plumlee, J.D.; Wells, R.J.D. Feeding ecology of three coastal shark species in the northwest Gulf of Mexico. Marine Ecology Progress Series. 550:163-174; 2016

Post, D.M. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology. 83:703-718; 2002

Post, D.M.; Layman, C.A.; Arrington, D.A.; Takimoto, G.; Quattrochi, J.; Montana, C.G. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia. 152:179-189; 2007

Qian, Y.; Jochens, A.E.; Kennicutt Ii, M.C.; Biggs, D.C. Spatial and temporal variability of phytoplankton biomass and community structure over the continental margin of the northeast Gulf of Mexico based on pigment analysis. Continental Shelf Research. 23:1-17; 2003

Rilov, G.; Benayahu, Y. Fish assemblage on natural versus vertical artificial reefs: the rehabilitation perspective. Marine Biology. 136:931-942; 2000

Rooker, J.R.; Dokken, Q.R.; Pattengill, C.V.; Holt, G.J. Fish assemblages on artificial and natural reefs in the Flower Garden Banks National Marine Sanctuary, USA. Coral Reefs. 16:83-92; 1997

Rooker, J.R.; Turner, J.P.; Holt, S.A. Trophic ecology of Sargassum-associated fishes in the Gulf of Mexico determined from stable isotopes and fatty acids. Marine Ecology Progress Series. 313:249-259; 2006

Sardenne, F.; Hollanda, S.; Lawrence, S.; Albert-Arrisol, R.; Degroote, M.; Bodin, N. Trophic structures in tropical marine ecosystems: a comparative investigation using three different ecological tracers. Ecological Indicators. 81:315-324; 2017

Sargent, J.; Bell, G.; McEvoy, L.; Tocher, D.; Estevez, A. Recent developments in the essential fatty acid nutrition of fish. Aquaculture. 177:191-199; 1999

Simonsen, K.A.; Cowan, J.H.; Boswell, K.M. Habitat differences in the feeding ecology of red snapper (Lutjanus campechanus, Poey 1860): a comparison between artificial and natural reefs in the northern Gulf of Mexico. Environmental Biology of Fishes. 98:811-824; 2015

Szedlmayer, S.T.; Lee, J.D. Diet shifts of juvenile red snapper (*Lutjanus campechanus*) with changes in habitat and fish size. Fishery Bulletin. 102:366-375; 2004

Tocher, D.R. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science. 11:107-184; 2003

Trueman, C.N.; MacKenzie, K.; Palmer, M. Identifying migrations in marine fishes through stable-isotope analysis. Journal of Fish Biology. 81:826-847; 2012

Wells, R.J.D.; Cowan, J.H.; Fry, B. Feeding ecology of red snapper *Lutjanus campechanus* in the northern Gulf of Mexico. Marine Ecology Progress Series. 361:213-225; 2008