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Spatial Variation in Basal Resources and Trophic Position of Selected Fishes of the North-Central Gulf of Mexico

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Spatial Variation in Basal Resources and Trophic Position of Selected Fishes of the North-Central Gulf of Mexico

by

Christopher R. Fleming

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ABSTRACT

The North-central Gulf of Mexico is a complex hydrologic environment with freshwater influx that varies on spatial and temporal scales. Freshwater input exerts influence on the isotope values of organisms living in coastal ecosystems. The objectives of this study were to determine relationships between total length and isotope value, estimate basal resource usage and trophic position of target species from Mississippi Sound, reef, and pelagic environments, and identify differences and similarities in spatial patterns of collection. Muscle tissue samples were collected from October 2014 through September 2015. Stable isotope analysis identified a trophic gradient extending from nearshore to offshore, with $\delta^{13}C$ values becoming enriched as distance from shore increased, while $\delta^{15}N$ values decreased. Species from the Mississippi Sound exhibited varying degrees of habitat usage, with Red Drum being the most diverse, while Gafftopsail Catfish and Atlantic Sharpnose shark had more habitat specificity. This study presented evidence that freshwater inputs influenced the isotope values of reef fish species. $\delta^{15}N$ values of Vermilion Snapper, Red Porgy, Red Snapper, and Tomtate were statistically higher near sources of freshwater input. Stable isotope data identified variable habitat usage in Cobia. Application of this knowledge when developing statistical models may help increase efficacy of management decisions.

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DEDICATION

I would like to dedicate this work to Jonathan Daniel Becker. He was a son, a grandson, a brother, a nephew, and a friend. He worked as a deckhand, and never shied away from a conversation about fishing or video games. Every time I saw him, he would ask me how this project was going. I only wish he could be here to see it finished. He will be forever missed by all that knew and loved him.

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CHAPTER I - INTRODUCTION

A common approach to understanding of trophic dynamics is based on stomach content analysis. Although they provide a direct representation of predator/prey relationships, these studies are limited in their efficacy because they provide a snapshot of diet that fails to consider source contribution dynamics over time (Stoner & Zimmerman 1988; Polis & Strong 1996; Pinnegar & Polunin 1999). Because various food items are digested at different rates, more readily digestible materials may be easily overlooked (Michener & Schell 1994; Melville & Connolly 2002). Feeding activity and morphological characteristics such as pharyngeal teeth further complicate gut analysis, as prey items can be rendered unidentifiable by grinding (Khoury 1987).

A common approach to understanding of trophic dynamics is based on stomach content analysis. Although they provide a direct representation of predator/prey relationships, these studies are limited in their efficacy because they provide a snapshot of diet that fails to consider source contribution dynamics over time (Stoner & Zimmerman 1988; Polis & Strong 1996; Pinnegar & Polunin 1999). Because various food items are digested at different rates, more readily digestible materials may be easily overlooked (Michener & Schell 1994; Melville & Connolly 2002). Feeding activity and morphological characteristics such as pharyngeal teeth further complicate gut analysis, as prey items can be rendered unidentifiable by grinding (Khoury 1987).

Nutrient and particulate organic matter (POM) input to marine systems via freshwater inflow exerts strong influence on food webs and the stable isotopic compositions of the animals living in coastal ecosystems (Fry 2002). For example, bivalves from the Great Sippewissett marsh in Massachusetts, USA (Peterson et al.

1985), and the Marennes–Oleron basin, an oyster cultivation region near the estuary of the Charente River in France (Riera & Richard 1996) show distinct isotopic gradients along transects from open ocean to upper estuary, with stable isotopic enrichment occurring closer to higher salinity waters. In a comparative study of two adjacent estuaries in Maine, USA, bivalves from the estuary with more freshwater inflow showed differing δ^{13}C values than those from the estuary with less freshwater input (Incze et al. 1982). In the north-central Gulf of Mexico (NGOM), similar gradients have been evident in Spotted Seatrout (*Cynoscion nebulosus*) (Fulford & Dillon 2013), Atlantic Sharpnose Shark (*Rhizoprionodon terranovae*, Drymon et al. 2012) and Eastern oysters (*Crassostrea virginica*, Dillon et al. 2015).

The NGOM is a region with potential freshwater biogeographic barriers due to riverine discharges across the region that vary spatially and temporally. The Mississippi River on the western side of this region acts as the primary drainage for the midwestern United States. The western side of the NGOM is characterized by low salinities, muddy bottoms and extensive salt marshes (Beck & Odaya 2001). East of Mobile Bay, riverine input is much lower, resulting in clear water of higher salinity, with an abundance of seagrass beds (Beck & Odaya 2001).

The Mississippi River accounts for about 90% of the freshwater inflow to the GOM (Milliman & Meade 1983; Rabalais et al. 1996) with a combined mean annual inflow to the GOM for the Mississippi and Atchafalaya Rivers since 1980 is nearly 22,000 m^3s^{-1} (Dunn 1996). This discharge also delivers about 1.3×10^{11} moles y^{-1} of nitrogen to waters of the GOM (Howarth et al. 1996). Much of this nitrogen is anthropogenic, with primary sources being fertilizers, nitrogen fixation from crop associated legumes, and atmospheric deposition of nitrogen oxides from fossil fuel combustion (Dagg & Breed 2003) but nitrogen loading to the GOM fluctuates with seasonal patterns of freshwater discharge (Bratkovich et al. 1994). In comparison, net riverine transport of total organic carbon to the GOM averages 4.8×10^{11} mol y^{-1}, with 66% delivered in particulate form. Concentrations of particulate organic carbon can be as high as 600 µmol l^{-1} in the river and < 0.8 µmol l^{-1} in offshore waters (Trefry et al. 1994). Finally, the Mobile Bay is the easternmost source of significant freshwater input in the NGOM. Mean freshwater discharge from Mobile Bay is about 1,512 m^3s^{-1}, but during winter through spring rainfalls it can exceed 9,000 $m^3s^{-1}$and be as low as 80 m^3s^{-1} during the summer (Mobile Bay Modeling Report 2012).

In contrast, East of this area, riverine input is minimal and waters become increasingly oligotrophic. These areas include Perdido Bay, which is fed by the 105 km long Perdido River and the Escambia and Blackwater Rivers feed that he Pensacola Bay complex, which is composed of Pensacola Bay, Escambia Bay, East Bay, and Blackwater Bay. At the easternmost area of the study area is the Choctawhatchee River, which feeds the Choctawhatchee Bay near Destin Florida. The barrier islands Perdido Key, Santa Rosa Island, and Okaloosa Island mitigate freshwater inflow into this region of the GOM. These hydrologic characteristics provide an opportunity to examine how this variable freshwater input affects the trophic ecology of marine fishes across the NGOM. This study will have three objectives: to determine if total length influences isotope values, to estimate trophic position and energy source use of reef, pelagic, and Mississippi Sound fish in the NGOM, and determine differences and similarities associated with spatial patterns of collection.

CHAPTER II - METHODS

Muscle tissue samples for the study were taken from fish landed in the NGOM between Port Fourchon, Louisiana, and Destin, Florida (-90.50 to -85 degrees longitude, 30 to 28 degrees latitude, Fig. B.1). Samples were taken from the catch of recreational, charter, and “headboat” fishing vessels in the NGOM in the Fall of 2014, as well as the Spring and Summer of 2015. Total length (TL, mm) was recorded. Catch location for each fish was obtained via angler interview or by trip reports filed with the NOAA Southeast Region Headboat Survey eLog system. Sampling locations were Port Fourchon and Venice Louisiana; Biloxi, Mississippi; Dauphin Island and Orange Beach, Alabama and Destin, Florida.

To establish an isotopic baseline for reef and pelagic species, plankton and POM samples were collected on SEAMAP research cruises in May and September 2015 (Fig. B.4). Plankton samples were collected using 947 µm Neuston and 333 µm Bongo nets, as well as a 53 µm hand held surface net deployed behind the vessel. Water was collected for POM samples with a Niskin bottle. A known volume of water was then filtered onto 25mm glass fiber filters in the field using a syringe and an acid washed stainless steel inline filter holder. POM samples were collected at all SEAMAP stations during the September 2015 cruise. Zooplankton was collected on the Spring 2015 cruise whereas both zooplankton/phytoplankton were collected on the September 2015 cruise.

Muscle samples collected in the field were put n plastic bags, placed on ice, and transported to the lab where they were stored in an ultralow freezer (-70^{o}C) before being freeze dried in a Labconco FreeZone 6 lyophilizer for at least 48 hours. Freeze-dried tissue samples were hand ground to a fine powder using a ceramic mortar and pestle.

Ground tissue was stored in labeled 20 ml scintillation vials kept in a desiccator. Samples were weighed using a Mettler Toledo XP26 microbalance and packed into tin capsules and stored in well plates until isotopic analysis. Glass fiber filters were folded, packed into tin capsules, and then analyzed whole. Plankton samples collected in the field were stored in freezers on board the ship and were kept frozen until being oven dried (70°C). Once dried, plankton samples and POM filters were placed into an acid fume bath for 24 hours to remove inorganic carbonates and then stored in a desiccator until analysis.

842 individuals from 49 taxa were sampled during the study. To eliminate rare species and those with a small number of samples, target species from each ecotype were identified. Target species from the Mississippi Sound and pelagic ecotypes were those with a minimum of n = 5, while reef target species were those with a minimum of n = 5 from at least two of the sampling locations (Fig. B.3). Target species from the Mississippi Sound were collected in Biloxi, Mississippi and included Spanish Mackerel (*Scomberomorus maculatus,* n = 51), King Mackerel (*Scomberomorus cavalla,* n = 12), Red Drum (*Sciaenops ocellatus,* n = 23), Southern Kingfish (*Menticirrhus americanus,* n = 11), Sand Seatrout (*Cynoscion arenarius,* n = 18), Blacktip Shark (*Carcharhinus limbatus,* n = 8), Atlantic Sharpnose Shark (*Rhizoprionodon terranovae,* n = 18) and Gafftopsail Catfish (*Bagre marinus,* n = 5). Pelagic target species, which were sampled in Port Fourchon and Venice, La., Biloxi, Ms., and Dauphin Island Al., were Blackfin Tuna (*Thunnus atlanticus*, n = 48), Yellowfin Tuna (*Thunnus albacares*, n = 29), Cobia (*Rachycentron canadum*, n = 53), Dolphinfish (*Coryphaena hippurus,* n = 19), Wahoo (*Acanthocybium solandri,* n = 5), and Blue Marlin (*Makaira nigricans,* n = 7). Reef target species (Table A.1) were Red Snapper (*Lutjanus campechanus*) and Vermilion

Snapper (*Rhomboplites aurorubens*), Red Porgy (*Pagrus pagrus*), Tomtate (*Haemulon aurolineatum*), and Greater Amberjack (*Seriola dumerili*) Prior to any quantitative or descriptive analysis, reef target species were given a species and location code. Red Snapper is RS, Red Porgy is RP, Tomtate is TT, Greater Amberjack is GAJ, and Vermilion Snapper is VS. Locations sampled were Louisiana (LA), Dauphin Island (DI), Orange Beach (OB) and Destin, Florida (FL). For example, Vermilion Snapper sampled in Orange Beach are coded as VSOB, Greater Amberjack sampled in Louisiana are GAJLA, Red Porgy sampled on Dauphin Island are RPDI, etc.

Carbon and nitrogen stable isotope biplots were created to evaluate potential source contributions for each species and region. Primary producer (source) data points are plotted in isotope space with lines connecting the source points creating a source mixing polygon. This polygon is then shifted up two trophic levels, and overlaid on a plot containing data points for potential consumers in isotope space. By following the pattern of ~1‰ enrichment for $\delta^{13}C$, and a~3‰ for $\delta^{15}N$, one can make inferences regarding the use different basal resources by different consumers.

Correlation analyses were conducted using Spearman rank test to determine if a relationship existed between isotope value and total length (mm). Target species from the Mississippi Sound and Pelagic ecotypes were tested by species, while reef species were tested by region sampled, and as a single species group without consideration to sampling area. Performing this test determined if age related feeding behavior or habitat utilization is contributing factor in the variation among species. If no correlation exists, it will allow total length to be eliminated as an influencing factor on isotope values.

Using R (version 3.4.1, www.r-project.org) stable isotope values of each species were tested for normal distribution and homogeneity of variance. Parametric tests make the assumption that data will have a normal distribution and homogenous variance. If either of these assumptions were violated, the non-parametric equivalent tests were used. A Shapiro-Wilk test was used to determine if the $\delta^{13}C$ and $\delta^{15}N$ values for each species collected follow a normal distribution. Based upon the results of this test, a Bartlett's test or Levene's test was used to assess homogeneity of variance among species. The Levene's test is more robust to deviations from the normal distribution, so this was used if the majority of the isotope data for each species is non-normal.

The non-parametric Kruskal-Wallis test was used to determine if differences existed in ranked means of $\delta^{13}C$ and $\delta^{15}N$ isotope values of species sampled in the Mississippi Sound and pelagic environments. Multiple pairwise comparisons using a Bonferroni corrected Mann-Whitney U-test were used to determine differences among ranked mean isotope values. Conducting multiple pairwise comparisons increases the probability of a rare event. The Bonferroni correction takes the desired p-value and divides it by the number of comparisons to be made, resulting in a more conservative test. This correction was used for species comparisons from all ecotypes.

PERMANOVA is a resampling approach that permutates the data, eliminating the requirement of parametric assumptions since no distribution is assumed. Significance is determined using a pseudo-F statistic, as distributions will be created from the data collected. PERMANOVA partitions variability, allowing for multiple factors and interaction effects (Anderson 2001).. $\delta^{13}C$ and $\delta^{15}N$ would each be tested separately using species and location as factors. PRIMER software (version 7.0.13, www.primer-e.com) was used to perform the PERMANOVA test. Before creating the PERMANOVA design, a Euclidean distance dissimilarity matrix to be used as the basis for the PERMANOVA was calculated for each of the two isotope values. With the resemblance matrix calculated, a 2-way design was used, with species (n = 5) and location (n = 4) as factors. The number of unique permutations for each factor, as well as the interaction factor, was set at 999.If a significant interaction existed, the square root of the estimated components of variance (ECV) was used to determine the primary source of the variation driving the interaction term. The ECV is a numeric value, the highest of which is the factor that contributes most to the variation. The factor with the highest ECV was further analyzed to visualize the variation within that factor.

Once a potential factor with the highest ECV was identified, the variation was visually represented using non-metric multi-dimensional scaling (nmMDS) based on a Euclidean Distance dissimilarity matrix. The nmMDS plot is an iterative technique that produces a visual representation of this dissimilarity, with those points furthest away from one another being the most dissimilar. For the purpose of this study, the factor (species or location) that had the highest ECV for each isotope would be used with the raw isotope data to make the nmMDS plots. For example, if the species factor had the highest ECV for $\delta^{13}C$, the plots were constructed using the raw $\delta^{13}C$ values by species. Because the plots are non-metric, the data will be presented in an ordination space, without labeled axes.

CHAPTER III – RESULTS

Size range (TL, mm), and mean values with standard errors for δ^{13}C and δ^{15}N were calculated for all 842 individuals sampled. (Table A.2). This included all rare species as well as target species (Fig. B.5). Species collected from the Mississippi Sound were the most depleted in δ^{13}C, followed by reef species, then pelagic species. Conversely, species from the Mississippi Sound were the most enriched in δ^{15}N, followed again by reef species, and pelagic species, which were the most depleted in δ^{15}N.

Primary producer or source isotope data for isotopic baselines in the Mississippi Sound were collected from the literature (Table A.3). Primary sources for this data were Sullivan and Moncrieff 2001, which was conducted in Graveline Bayou, and Dillon et al. 2015, which were from Grand Bay National Estuarine Research Reserve. These included *Halodule* seagrass and associated epiphytic algae, plankton and POM, benthic microalgae (microphytobenthos), and macroalgae. These sources were used to construct the mixing space bi-plots for the Mississippi Sound (Fig. B.6).

All fish sampled in the Mississippi Sound and barrier islands (n = 157) had δ^{13}C values that ranged from -9.9 to -23.7‰ (Fig. B.7), which reflects a wide range of basal carbon sources in this system. Mean values for most individual species sampled from the Mississippi Sound had narrow ranges, with mean δ^{13}C values that ranged from -19.9 ± 1.6‰ for the Gafftopsail Catfish which was the most depleted, to -17.2 ± 0.3‰ for the King Mackerel, which was the most enriched. (Fig. B.9B). Range in individual δ^{15}N values spanned 7.8‰, about two and a half trophic levels (Fig. B.8). Range in mean δ^{15}N values was considerably less, with only 1.4‰ separating the most depleted (14.3 ± 0.2‰, King Mackerel) from the most enriched (Gafftopsail Catfish, 15.6 ± 0.3‰, Fig.B. 8).

Only the Sand Seatrout exhibited a positive correlation in either isotope value, with $\delta^{15}N$ increasing with total length.

Red Drum showed the highest variation in isotopic values for the Mississippi Sound fish group (Fig. B.7), with $\delta^{13}C$ values that ranged from -9.9 to -23.6ç (n=35, mean = -19.1 ± 0.6‰). Although most of the specimens sampled fell between -20.3‰ and -23.6‰, three had values that were considerably more enriched, with $\delta^{13}C$ values of -13.6‰, -11.7‰, and -9.9‰. These three specimens were also appreciably more depleted in $\delta^{15}N$, which were more than one trophic level (~4‰) less than other Red Drum. Isotope values for Red Drum were not correlated to length (Table A.4).

Spanish Mackerel was the most common species captured in the Mississippi Sound (n = 51), and had $\delta^{13}C$ values that ranged from -16.3to -21.3‰ (mean =-18.1 ± 0.2‰). $\delta^{15}N$ values were as variable as those of Red Drum, ranging from 9.5 to 16.8‰ (mean = 14.4 ± 0.3‰). King Mackerel exhibited less variation in isotopic values when compared to Spanish Mackerel. Carbon stable isotope values ranged from -16.4 to -19.1‰ (mean =-17.2 ± 0.3‰) while $\delta^{15}N$ values ranged from 13.4 to 16.3‰ (mean = 14.3 ± 0.2‰). Isotope values and length were not correlated for both species.

The range of carbon stable isotope values for the Atlantic Sharpnose Shark (n = 18) were fairly narrow, from -16.6 to -18.0‰ (mean = -17.4 ± 0.1‰). The range in $\delta^{15}N$ values was also very narrow, spanning about 1.5‰, from 13.6 to 15.2 ‰ (mean = 14.2 ± 0.1‰). Blacktip Shark had mean $\delta^{13}C$ values that were similar to those of the Sharpnose at 17.4 ± 0.2‰, with individuals that ranged from -16.3 to -18.14‰. Individual $\delta^{15}N$values ranged from 14.3 to 16.2‰. The mean $\delta^{15}N$ for Blacktips was slightly more enriched than Sharpnose at 15.0 ± 0.2‰ and there was no correlation in isotope values with total length.

Southern Kingfish and Sand Seatrout had identical mean $\delta^{13}C$ values at -18.6‰. Sand Seatrout ($n = 18$), had a 6.1‰ range in $\delta^{13}C$, (-16.3 to -22.3‰) while Southern Kingfish ($n = 11$) had a much narrower range of 3.2 ‰ (-16.3 to -19.5‰). Range in $\delta^{15}N$ for Southern Kingfish was 13.9 to 15.5‰. Sand Seatrout had a total $\delta^{15}N$ range of 5.0‰, (14.4 to 16.7‰) with one outlier at 11.7‰. Mean $\delta^{15}N$ values for these two species were also very similar, with Southern Kingfish at 15.0 ± 0.1‰, and Sand Seatrout at 15.3 ± 0 .2‰. Sand Seatrout was the only species to show a positive correlation to length and ^{15}N values.

The Gafftopsail Catfish had the most depleted mean $\delta^{13}C$ value (-19.9 ± 1.6‰) and most enriched $\delta^{15}N$ value (15.6 ± 0.3‰) of all species sampled in the Mississippi Sound, but was one of the least common captures ($n = 5$). Carbon isotope values ranged from -18.6 to -23.7‰. $\delta^{15}N$ values were more tightly clustered, with 1.5‰ separating the most depleted (15.1‰) from the most enriched (16.6‰).

Results of Shapiro-Wilk tests (significance at $p < 0.05$) on species from the Mississippi Sound resulted in two species that were non-normal in $\delta^{13}C$, and three non-normal species in $\delta^{15}N$ (Table A.5.) Variance among Mississippi Sound species was not homogenous (Bartlett's test p-values $\delta^{13}C = 2.2\text{x}10^{-16}$,$\delta^{15}N = 4.347\text{x}10^{-11}$). This result violates the assumption of homogenous variance, so the non-parametric Kruskal-Wallis test was used to determine if differences exist in mean isotope values.

Results of the Kruskal-Wallis test indicate that differences exist in means of ranked values of $\delta^{13}C$ ($H = 34.211$, $df = 7$, $p = 1.573\text{x}10^{-5}$) and $\delta^{15}N$ ($H = 20.744$, $df = 7$,

$p = 4x10^{-3}$). As a post-hoc test, multiple pairwise comparisons were conducted using the non-parametric Mann-Whitney U test with a Bonferroni correction, resulting in a significance level of $p \leq 7x10^{-3}$.

$\delta^{13}C$ values for the Atlantic Sharpnose Shark were different from most species in the Mississippi Sound, with the only similarities being with the King Mackerel and Blacktip Shark. (Table A.6). King Mackerel had $\delta^{13}C$ values that were more enriched, differing statistically from those of Gafftopsail Catfish, Red Drum, and Sand Seatrout. Blacktip Sharks (mean $\delta^{13}C = 17.5 \pm 0.2$ ‰) were dissimilar to Gafftopsail Catfish, which had $\delta^{13}C$ values that were 2.5‰ more depleted. Sharpnose Sharks, which had the most depleted mean $\delta^{15}N$ value (14.2 ± 0.1‰) of all the species from the Mississippi Sound and Barrier Island were also statistically dissimilar the most species for$\delta^{15}N$ values. After the Bonferroni correction, only one species, the Spanish Mackerel, was statistically similar in $\delta^{15}N$ (Table A.7). All other species were statistically similar.

Mean POM $\delta^{13}C$ values showed little variation among stations, with a range of 1.5‰ between the most depleted and enriched (-25.5 ± 0.2‰ to -24.0 ± 0.1‰, Table A. 7.). A Kruskal-Wallis test on $\delta^{13}C$ values from each station showed no significant differences among the means of all stations ($H = 10.375$, $df = 7$, $p = 0.16$). Significant differences did exist in $\delta^{15}N$ among stations ($H = 14.978$, $df = 7$, $p = 0.04$), however they were negated when a Bonferroni correction was applied to the pairwise comparisons.

Mean values of $\delta^{13}C$ for all net sizes were within 1.3‰, with the 53 μm net being the most enriched at -19.7‰ (Table A.8). Variation between Bongo and Neuston nets was minimal, with 0.2‰ separating the means. Mean $\delta^{15}N$ values for all plankton samples were between 5.1‰ and 6.9‰, with the 53 μm net being the most depleted.

0.7‰ separated the mean $\delta^{15}N$ values of the Bongo and Neuston nets. When values for all stations were pooled by net size, no significant differences in $\delta^{13}C$ or $\delta^{15}N$ were detected (H = 4.29, df = 2, p = 0.12, H = 0.16, df = 2, p = 0.92, respectively).

A mixing polygon of POM and planktonic basal resources was plotted in isotope space for reef fish (Fig. B.10). Plankton samples collected on the 2015 SEAMAP cruises are presented by net type. In addition to the data obtained on SEAMAP cruises, values for other potential basal sources of carbon and nitrogen were collected from the literature.

$\delta^{13}C$ values for all reef fish (n = 332) were primarily between -18‰ and -16‰, while $\delta^{15}N$ values ranged from 9.2‰ to 16.7‰, encompassing slightly more than two trophic levels. (Fig. B.11). Mean $\delta^{13}C$ values had a range of about 2‰, with Vermillion Snapper from Florida (VSFL) being the most depleted (-18.3 ± 0.03), and Greater Amberjack from Florida (GAJFL) being the most enriched (-16.2 ± 0.2‰, Fig.13B). The range in mean $\delta^{15}N$ values was 3.3‰, with RSLA having the highest at 15.2 ± 0.15‰, and RPOB with the lowest at 11.9 ± 0.1‰. (Fig. B.13A).

Most species showed no discernable trends in $\delta^{13}C$values with location, however there were noticeable trends in $\delta^{15}N$ values. Samples taken from Red Porgy, and Tomtate showed a slight depletion in $\delta^{15}N$ from Mobile Bay to waters of Orange Beach and Destin, Florida (Fig. B.13B). Mean $\delta^{15}N$ for both species were more enriched near Mobile Bay, with values 1.1‰ higher in Tomtate, and 1.7‰ higher in Red Porgy. Red Snapper showed also showed a consistent depletion in $\delta^{15}N$, with those from Louisiana being nearly 1 trophic level (2.3‰) higher than those in Orange Beach. Vermilion Snapper sampled in Orange Beach and Florida were isotopically more depleted in $\delta^{13}C$ than those from Louisiana and Dauphin Island.

Three reef species had isotope values that were correlated to total length. Vermilion Snapper (n = 149) showed enrichment in δ^{13}C, and depletion in δ^{15}N values as length increased. The correlation in δ^{15}N was weak, with an R-value of -0.16. This pattern of δ^{15}N depletion with increasing size was also evident in Vermilion Snapper from Orange Beach (R = -0.48). Greater Amberjack (n = 28) showed a pattern of enrichment in δ^{13}C (R = 0.46) as size increased. Red Porgy (n = 38) exhibited a positive correlation in δ^{15}N with increasing length (R = 0.49). This correlation was also evident in Red Porgy from Florida, having an R-value of 0.51.

Vermilion Snapper were sampled in each of the four ecoregions. δ^{13}C values for all locations had a range of about ~3‰, with VSDI (n = 33) having the largest spread (Fig. B.15A). VSDI and VSOB (n = 36) had δ^{13}C values that overlapped the other groups, but VSLA (n = 33) and VSFL (n = 47) were each distinct, with no overlapping values between the two locations. δ^{15}N values had a range of about 4‰ with the exception of VSDI, where δ^{15}N were as high as 16.7‰. Mean δ^{13}C values showed a very slight depletion (~1‰) from Louisiana to Destin Florida, with each region significantly different from the others (Table A.11) but this trend was not evident in δ^{15}N values. Vermilion Snapper from Louisiana, Orange Beach, and Destin were statistically similar to one another, with only 0.1‰ separating mean δ^{15}N values of the three groups. Those sampled on Dauphin Island had a slightly higher mean δ^{15}N value that was about 1‰ higher than the other sampling areas, and were significantly different from all other sampling areas (Table A.12).

Greater Amberjack were collected in Louisiana, Orange Beach, and Destin, Florida. The difference in mean δ^{13}C values from the most depleted (Orange Beach) to the most enriched (Florida) was < 1%. Greater Amberjack from Orange Beach (n = 6) had the highest mean δ^{15}N, followed by Louisiana (n = 15), then Florida (n = 6). No statistical differences in δ^{13}C or δ^{15}N values across all sampling locations were detected after the Bonferroni correction was applied, (Table A.11, A.12) but the range of values for both isotopes of GAJ sampled in Louisiana encompassed the values of GAJ from the other two sampling areas (Fig. B.15E). Those from Orange Beach and Florida were each distinct from one another, with a range of < 1% for δ^{13}C and δ^{15}N. The range in isotope values for GAJ from Louisiana were more pronounced, with 1.6‰ in δ^{13}C and 6.1‰ (approximately two trophic levels) for δ^{15}N.

Red Snapper were sampled in Louisiana (n = 47), Dauphin Island (n = 10), and Orange Beach (n = 33). RSLA had the widest range of δ^{13}C and δ^{15}N values, with the most depleted and enriched individuals exhibiting a range of 3‰ and 4‰, respectively (Fig. B.15D). Range in δ^{15}N for RSDI and RSOB was about 1‰, with RSOB exhibiting a slightly larger range in δ^{13}C values. Mean δ^{13}C values for all 3 locations were separated by < 1‰, and were not statistically different from one another (Table A.11). δ^{15}N values were all statistically different (Table A.12), and showed a noticeable pattern of depletion from west to east (Fig. B.15D).

Tomtate were only collected from Dauphin Island (n = 15) and Orange Beach (n=14). Tomtate from both regions showed a similar spread of about 2‰ in δ^{13}C, and a range of δ^{15}N values of about 1‰ (Fig. B.15B). Mean δ^{13}C values were statistically similar between locations, (Table A.11) while mean δ^{15}N values were statistically different, with 1.1‰ separating the means (Table A.12).

Red Porgy were sampled in Orange Beach (n = 11), Florida (n = 22), and on Dauphin Island (n = 5, Fig. B.15C). RPOB and RPFL were nearly identical in both isotope values, and had no significant differences. RPDI had δ^{13}C values that were slightly enriched with a mean that was approximately 0.6‰ higher, but were statistically similar to the other regions after correction (Table A.11). Although exhibiting a higher value for δ^{15}N compared to the other 2 regions, RPDI were only statistically different from RPOB after correction (Table A.12). δ^{15}N values for Red Porgy sampled in Florida had a positive correlation to total length ($p=0.01$, $R^2=0.27$), which is indicative of increasing prey size as gape size increased.

Shaprio-Wilk tests revealed that three groups (RSLA, VSDI, VSLA) had non-normal distributions of δ^{13}C, and four (RSLA, GAJLA, VSDI, VSOB) had non-normal distributions of δ^{15}N (Table A.10). Variance for both isotopes among the groups was not homogenous (Bartlett's test δ^{13}C p-value = 2.2×10^{-16}, δ^{15}N p-value = 4.347×10^{-11}). Both assumptions for the use of parametric tests were violated, so a PERMANOVA was used to determine if significant differences in isotope values exist among the groups.

Results of the PERMANOVA test on δ^{13}C values returned significant values for species (pseudo-F = 37.95; p [perm] = 0.001, Fig. B.12A), location (pseudo-F = 12.33; p [perm] = 0.001), and the interaction term (pseudo-F = 7.32; p [perm] = 0.001). The significant value for the interaction term precludes making any inferences about each of the two factors. The square root of ECV showed that species was accountable for most of the variation (0.56), followed by the interaction term (0.28), and location (0.24).

The PERMANOVA test for δ^{15}N was also significant for species (pseudo-F = 21.3; p [perm] = 0.001), location (pseudo-F = 44.4; p [perm] = 0.001, Fig B.12B), and interaction factor (pseudo-F = 22.3; p [perm] = 0.001). As with $\delta^{13}C$, the significant interaction factor for $\delta^{15}N$prevents drawing conclusions regarding location and species. The square root of the ECV had the interaction factor contributing most of the variation (0.84), but was followed closely by location (0.76). Unlike the ECV for $\delta^{13}C$, species contributed the least to the variation with 0.68.

Non-Metric Multi-dimensional scaling (nmMDS) was used to illustrate the dissimilarities in isotopic values by species and location. The PERMANOVA revealed that the species factor had the highest square root of the ECV for $\delta^{13}C$ values. To illustrate this variation, the plots were constructed using raw isotope $\delta^{13}C$ data for all five reef target species. The ordination by species showed the most separation between Vermilion and Red Snappers (Fig. B.16). Moderate separation was also evident with Greater Amberjack and Vermilion Snapper. Tomtates and Red Porgies showed the least amount of spread, but both had high degrees of dissimilarity to Red Snapper. The PERMANOVA on the $\delta^{15}N$ values showed that the location factor contributed to more of the variation than the species factor. Again, raw $\delta^{15}N$ data was used, with all four sampling locations set as factors. Orange Beach and Destin Florida had a low degree of dissimilarity between the two locations (Fig. B.17). Samples taken from Louisiana had a wide spread on the ordination, when compared to the other three sampling locations. Differences become especially evident when the location factor was narrowed to two locations, with Mobile Bay being used to divide the entire sampling area into western and eastern regions (Fig. B.18).

Large offshore pelagic fish (n = 112) had mean values of -16.8 ± 0.05‰ for $\delta^{13}C$ (Fig. B.23B). Mean $\delta^{15}N$ value was 12.0 ± 0.19‰ for $\delta^{15}N$, which was about 1‰ less than the mean value for reef species. $\delta^{13}C$ values were similar to reef fish, with most falling between -18‰ and -16‰. $\delta^{15}N$ values ranged from 7.6‰, to 15.7‰, spanning about three trophic levels. Mean $\delta^{13}C$ values for each of the six species sampled had a range of about 1‰ from the most depleted to most enriched, while $\delta^{15}N$ had a range of about 2‰. Yellowfin Tuna and Dolphin fish were the most depleted in $\delta^{15}N$, with both species having mean values of 10.8‰. Cobia was the most enriched in $\delta^{15}N$ (12.7 ± 0.2‰).

Three species from the pelagic ecotype had isotope values that were correlated to total length. Blackfin (n = 48) and Yellowfin Tuna (n = 29) had $\delta^{15}N$ values that increased with total length. Cobia (n = 53) exhibited a positive correlation in $\delta^{13}C$ and a negative correlation in $\delta^{15}N$ as length increased. Blue Marlin, Dolphinfish, and Wahoo had no correlations for either isotope.

The Shapiro-Wilk test for normal distribution revealed that 4 of the five pelagic species had non-normal $\delta^{13}C$ value distributions, and one species had a non-normal nitrogen distribution (Table A.14). Variance among pelagic species was not homogenous (Bartlett's test, $\delta^{13}C$ $p = 1.8 \times 10^{-3}$, nitrogen $p = 2.4 \times 10^{-3}$). This violates the assumptions for ANOVA, so the non-parametric test was used. The Kruskal-Wallis test of the ranked isotope data revealed significant differences in $\delta^{13}C$ ($H = 24.4$, $p = 1.8 \times 10^{-4}$) and $\delta^{15}N$ ($H = 37.44$, $p = 5.9 \times 10^{-7}$) isotope values. Differences among individual species were elucidated using the non-parametric Mann-Whitney U test with a Bonferroni correction, which reduced the significance level to $p = 0.01$. Blue Marlin had more enriched $\delta^{13}C$ values than most species, similar only to Cobia ($p = 0.13$) and Wahoo after correction ($p = 0.03$, Table A.15). Blackfin Tuna, which were 0.33‰ more depleted than Cobia, were also statistically dissimilar ($p = 9.6 \times 10^{-4}$). No other differences were detected in values of $\delta^{13}C$. Blackfin and Yellowfin Tuna exhibited statistical dissimilarity ($p = 0.004$), with the Blackfin having mean $\delta^{15}N$ value that was 1.7‰ more enriched than Yellowfin Tuna. The Blackfin was also dissimilar to Dolphinfish,($p = 3.5 \times 10^{-3}$), which was the more depleted of the two species. Cobia, which was the most enriched in $\delta^{15}N$ of all the pelagic species, differed from the more depleted Yellowfin Tuna ($p = 1 \times 10^{-4}$), and Dolphinfish (1×10^{-5}). (Table A.16).

CHAPTER IV – DISCUSSION

The Mississippi Sound is a complex estuarine system with numerous possible basal carbon and nitrogen sources. Sampling location, and varying hydrologic conditions such as seasonal freshwater influx may affect values of potential baseline sources of carbon and nitrogen. Isotopically heavy carbon (i.e. less negative) sources in the Sound are *Spartina alterniflora* nearshore and *Halodule wrightii* seagrass beds along the north side of some barrier islands while the isotopically depleted (lighter) sources consisted of phytoplankton and *Juncus romarianus*.

Red Drum from the Mississippi Sound and barrier islands had a high degree of variability in both $\delta^{13}C$ and $\delta^{15}N$ values suggesting that Red Drum can exploit a wide range of habitats in Mississippi Sound that have different source isotope values. Is also possible that Red Drum in different habitats occupy different trophic positions due to differences in prey availability between habitats. Spanish Mackerel $\delta^{13}C$ values (-16.3 to -21.3‰) suggest they are also utilizing various carbon sources but occupy a narrower isotope niche space than Red Drum. $\delta^{15}N$ values for Spanish Mackerel also showed a high degree of variation (9.6 to 16.6‰), but like Red Drum, determining if this variation is indicative of differences in individual trophic position, utilization of different habitats or a combination of both.

Isotope data suggest that Blacktip Shark, Sharpnose Shark, and King Mackerel rely on similar basal resources in Mississippi Sound, likely utilizing prey items supported by planktonic and benthic basal carbon sources. On the mixing polygon, the data points for all three species'$\delta^{13}C$ values fell between the isotopically lighter phytoplankton and the more enriched diatom substrate, which was used as a proxy for benthic microfauna. It is also possible that *S. alterniflora* or *H. wrightii* are important carbon sources however it seems unlikely given the narrow habitat extent of both of these species in Mississippi Sound: *S. alterinfora* is a fringing marsh species in Mississippi Sound's *Juncus* dominated marshes and *H. wrightii* is only found in shallow waters north of the barrier islands. $\delta^{13}C$ values had a lesser degree of variation for these three species, which indicates a narrow use of habitats and/or prey resources. Although dietary overlap does occur in the diet of the two shark species (Hoffmayer and Parsons 2003), Blacktip Sharks rely less on benthic crustaceans than the Sharpnose Shark so the statistically higher $\delta^{15}N$ values in Blacktips likely reflect a higher trophic level due to a higher degree of piscivory, with less dependency on the prey items such as portunids and stomatopods that are commonly found in the Sharpnose diet. The King Mackerel, which has a diet consisting primarily of teleost fishes (Devane 1978),was more similar to Blacktip Sharks, but showed a higher degree of variability in $\delta^{15}N$ values (13.4 – 16.6‰), which may indicate varying usage of nearshore and offshore habitats.

The Sand Seatrout and Southern Kingfish occupy similar isotopic niches in the Mississippi Sound. Although statistically similar, the Sand Seatrout exhibited a higher degree of variability in both $\delta^{13}C$ (-16.4 to -22.3‰) and $\delta^{15}N$ values (11.7 to 16.7‰), which suggests that this species is utilizing a wider range of habitats than the Southern Kingfish. Stomach content analysis for these fish is limited, but the wider range of values in the Sand Seatrout could reflect the differences in feeding morphology, which allow the trout to consume larger prey items.

The Gafftopsail catfish had the most depleted mean $\delta^{13}C$ values of any species sampled (-19.9 ± 1.6‰), but this depleted mean value was due to one individual with an exceptionally low value of -23.7‰. Variation in the remaining four specimens was very low, with a $\delta^{13}C$ range of about 0.6‰. Mean value without this low outlier was 19.0‰, which was closer to the means of Sand Seatrout (-18.6 ± 0.5), Southern Kingfish (-18.6 ± 0.3), and Red Drum (-19.1 ± 0.6). With only five individuals sampled, a larger sample size, along with detailed catch location data would be needed to determine if this more depleted value resulted from utilization of habitats supported by *Juncus* or terrestrial carbon. Mean $\delta^{15}N$ value for this species was the highest of the Missisippi Sound target species at 15.65 ± 0.3‰. This could indicate that these fish are feeding at a high trophic level similar to most other target species in this study, or utilizing a habitat with a higher degree of freshwater input with higher $\delta^{15}N$ values.

Proximity to freshwater sources appeared to influence plankton isotope values for all three net sizes. At each of the six near-shore stations, < 2‰ separated the $\delta^{13}C$ values of all three net types. The two offshore stations, B180 and B322, had a greater range of $\delta^{13}C$ among net types (range = 3.8‰ and 5.6‰, respectively). Decreasing $\delta^{15}N$ values from inshore to offshore was only measured in the 53 µm sized plankton. Plankton from Bongo (333 µm) and Neuston (947 µm) nets at offshore stations had $\delta^{15}N$ values similar to stations closer to shore. This difference may have been due to reduced freshwater discharge from the Mississippi River from Spring to Fall.

Reef fish in the NGOM had isotopic values reflective of a plankton-based food web, with mean values clustered around the values for plankton collected on the 2015 SEAMAP cruises (Fig. B.11, B.12). Red Snapper from Louisiana (-21.6 to -16.3‰) and Vermilion Snapper from Dauphin Island (-18.6 to -15.7‰) exhibited the widest range in $\delta^{13}C$ values when compared all other reef species and reef locations, which may reflect these species inhabiting the nexus between nearshore and offshore habitats. Reef habitats in the waters of Louisiana experience a high degree of terrestrial carbon loading via variable freshwater influx from the Mississippi River, which contributes to the wider spread in $\delta^{13}C$ values of these species. The range of $\delta^{13}C$ for all other species ranged from 0.5 to 2‰, which could be expected with species found in a more isotopically stable environment that results from minimal freshwater input, or increasing depth, with little variability in prey resources. Ranges in $\delta^{15}N$ values among reef species were variable, with those sampled near freshwater sources generally exhibiting the widest ranges. The exception were Vermilion Snapper landed in Florida, with a range of 4.2 per mil, however it should be noted that this range was due to one outlying individual that was more than 1‰ enriched than the individual with the next highest $\delta^{15}N$ value.

The data presented suggests that freshwater input influenced the isotopic compositions of several reef species in the NGOM. Vermilion Snapper and Red Porgy from Orange Beach and Florida had mean values that were slightly depleted in $\delta^{13}C$ and $\delta^{15}N$ versus those sampled from Louisiana and Dauphin Island, which lie closer to riverine inputs from the Mississippi River and Mobile Bay. This gradient of isotopic enrichment from estuary to open ocean follows those noted by Riera and Richard (1996) as well as Dillon et al (2015). Although Tomtate and Red Snapper did not exhibit the same pattern of $\delta^{13}C$ depletion from West to East, $\delta^{15}N$ values of both species were isotopically lighter in fish collected at Orange Beach and Florida. The decrease of nearly 3‰ in Red Snapper, and ~1‰ in Tomtate would be consistent with the difference between $\delta^{15}N$ enriched riverine waters and $\delta^{15}N$ depleted offshore oligotrophic waters. Though not definitive, examination of the ECV from the PERMANOVA offers further evidence of freshwater influence on isotope values, with the “location” (second only to the interaction) factor contributing to the variation in the δ^{15}Nvalues of reef fish. This suggests that isotopically enriched nitrogen from freshwater sources (Mississippi and Mobile Rivers) do influence the δ^{15}N values of these species. The ECV shows the “species” factor contributed most of the variation in δ^{13}C values, likely reflecting differences in feeding strategy between species. Although this data does provide support for freshwater influence on isotopic values of these species, it does have limitations, as the test design was unbalanced due to lack of availability of all species from all sampling locations.

Large offshore pelagic species also appear to be supported by planktonic sources, with most δ^{13}C values falling in the same range as reef fish, between -16 and -18‰. Mean δ^{13}C values for all pelagic fish (-16.8 ± 0.05‰) were more enriched than reef fish (-17.4 ± 0.04‰) and Mississippi Sound fish (-18.3 ± 0.1‰), while mean δ^{15}N (12.0 ± 0.1‰) was more depleted when compared to mean values of those from the Mississippi Sound (14.9 ± 0.1‰) and reef environments (13.0‰) This pattern reflects a difference in baseline C and N sources as freshwater input diminishes further offshore. Although the total span in δ^{15}N values for all species in both groups was about 8‰, the lower mean value of the pelagic group shows the existence of an isotopic gradient from nearshore to offshore environments. The largest variation in δ^{15}N values for pelagic fish was measured for Blackfin Tuna, which showed a pattern of carbon and nitrogen enrichment as total length increased, likely due to consumption of higher trophic level prey items with increasing gape size. Isotope values indicate Yellowfin Tuna utilize similar prey items however isotope values were not correlated with length. Cobia had the widest range in pelagic $\delta^{13}C$ values (-15.8 to -17.6‰), and $\delta^{15}N$ values did not go below 10‰, which suggest this species consistently occupies a higher trophic position than other pelagic species or rely more on nearshore nitrogen sources than the other large offshore pelagic species. The Blue Marlin, an offshore pelagic feeder, occupied the most narrow isotopic niche, showing minimal variation in the range of $\delta^{13}C$ (-16.0 to -16.3‰), and $\delta^{15}N$ (9.7 to 12.8‰), which reflects the utilization of the isotopically stable habitat that results from a lack of freshwater influence. Wahoo and Dolphinfish, two species that are frequently associated with *Sargassum* mats, also occupied a similar isotopic space in the NGOM. Although not statistically different, the Wahoo exhibited a higher degree of variability in $\delta^{15}N$ values. Range in $\delta^{15}N$ values for Wahoo were 6.6‰, compared to 4.3‰ for Dolphinfish. Both exhibited a similarly narrow range in $\delta^{13}C$ value (< 2‰), suggesting that despite similar habitat usage, the Wahoo may be using a larger variety of prey resources.

Pelagic target species are primarily offshore predators that reside in an oligotrophic environment providing contrast to reef target species, which exhibited higher degree of spatial variability in $\delta^{15}N$ values. The offshore environment is less isotopically dynamic due to minimal freshwater influx, but the large offshore pelagic species presented in this study appear to occupy different niches in isotopic space.

This study shows that carbon and nitrogen stable isotopes are an effective method for identifying variable trophic niche space among fish assemblages of inshore, reef, and offshore fish. Although data from this study provides evidence of variable trophic niche space among fish guilds and shows that an isotopic gradient exists with distance from shore, further studies of this nature would benefit from the incorporation of other data parameters. Catch locations were reported by charter vessel captains or obtained via angler interview but could not be verified. The reporting system used by many of the charter vessel captains only allowed one catch location to be reported per trip, even if multiple sites had been fished or if fish were caught while trolling. Additionally, no information was collected regarding salinity, depth, reef type, etc., all of which may affect isotopic values. The inclusion of these easily obtained data parameters would benefit future studies tremendously.

Using stable isotopes in conjunction with detailed stomach content analysis on spatial and temporal scales would help determine if variations in isotopic values are the result of freshwater influence, or are indeed indicative of differences in food webs among locations. Many of the species examined in this study such as Vermilion Snapper, Tomtate, Southern Kingfish, and Red Porgy have limited isotope or stomach content data for the GOM region, and what little data does exist is constrained to a small temporal and spatial scales.

The efficacy of future studies may be increased by incorporating sulfur stable isotope (^{34}S) analysis, polyunsaturated fatty acids (PUFAs) profiles, or compound specific stable isotope analysis of amino acids and/or fatty acids in addition to the more traditional $\delta^{13}C$ and $\delta^{15}N$ measurements. Similar to $\delta^{13}C$, $\delta^{34}S$ exhibits minimal trophic enrichment (~0.2‰) in animal tissues, and may be an informative tracer for determining which basal resources are important to each predator type (Fry 1987). A study using ^{34}S as a tracer on similar reef species strengthened feeding classifications of 5 reef predators, allowing inferences to be drawn about their trophic pathways that were not possible with carbon and nitrogen stable isotopes alone (Thomas and Cahoon 1993). Many primary producers also have distinct PUFA profiles, and have become increasingly useful as additional tracers to compliment the results of stable isotope analyses. Rooker et al. (2006) compared fatty acid profiles of producers and consumers of *Sargassum* communities to more effectively delineate the importance of different basal resources for *Sargassum* associated predators.

The development of isoscapes has been useful in determining spatial differences in basal resources in marine environments. These isotopic gradients have been documented in plankton (Graham et al 2010) and POM worldwide (Hoffman et al 2000). This distinction is also evident in sediments (Nerot et al. 1998), primary producers (Fry 1988), and consumers (Jennings and Warr 2003) on continental shelves. In the GOM, Radabaugh et al. (2013) documented an isotopic gradation of fishes on the West Florida shelf.T his study may contribute to the body of knowledge needed for the development of a Gulf-wide isoscape, which would increase the understanding of the isotopic dynamics in the NGOM.

APPENDIX A – TABLES

Table A.1

Sample sizes of each reef target species by location

Species by Location	**Louisiana (LA)**	**Dauphin Island (DI)**	**Orange Beach (OB)**	**Destin (FL)**
Greater Amberjack (GAJ)	15	ND	6	6
Red Snapper (RS)	47	10	33	ND
Red Porgy (RP)	ND	5	31	22
Tomtate (TT)	ND	15	14	ND
Vermilion Snapper (VS)	33	33	36	47

Table A.2

Mean isotope values for all species by ecotype. Asterisks identify target species

Species	**Common Name**	**Size Range, TL (mm)**	**n**	**Mean, SE δ^{13}C (‰)**	**Mean, SE δ^{15}N (‰)**
Inshore Fishes					
*Cynoscion arenarius**	Sand Seatrout	244 - 355	18	-19.2 ± 0.5	15.3 ± 0.3
Cynoscion nebulosis	Spotted Seatrout	282 - 575	21	-20.0 ± 0.2	14.4 ± 0.3
*Menticirrhus americanus**	Southern Kingfish	212 - 331	11	-18.6 ± 0.3	14.0 ± 0.3
*Sciaenops ocellatus**	Red Drum	480 - 985	35	-18.9 ± 0.5	15.0 ± 0.1
Pagonias cromis	Black Drum	480	1	-20.9	17.2
*Bagre marinus**	Gafftopsail Catfish	525 - 650	5	-19.9 ± 0.9	15.6 ± 0.3
Archosargus probatocephalus	Sheepshead	440 - 480	2	-19.2 ± 2.4	15.3 ± 2.7
Ariopsis felis	Hardhead Catfish		3	-19.0 ± 0.2	14.1 ± 0.4
Caranx crysos	Blue Runner	510	1	-16.6	13.9
Atractosteus spatula	Alligator Gar	1531	1	-17.3	16.9
Paralichthys lethostigma	Southern Flounder	330	1	-20.7	13.8
*Scomberomorus maculatus**	Spanish Mackerel	321 - 554	59	-18.1 ±.14	14.4 ± 0.2
*Scomberomorus cavala**	King Mackerel	750 - 1225	37	-17.2 ± 0.1	14.4 ± 0.3
Euthynnus alletteratus	Little Tunny	630 - 752	4	-16.8 ± 0.2	13.4 ± 0.6

*Carcharhinus limbatus******	Blacktip Shark	1115 - 1595	8	-17.4 ± 0.2	15.0 ± 0.3
Carcharhinus acronotus	Blacknose Shark	1180	1	-17.1	14.4
*Rhizoprionodon terraenovae******	Sharpnose Shark	702 - 960	19	-17.4 ± 0.1	14.2 ± 0.1
Reef Fishes					
*Lutjanus campechanus******	Red Snapper	410 - 742	98	-17.3 ± 0.1	14.0 ± 0.1
Lutjanus synagris	Lane Snapper	359 - 435	11	-16.3 ± 0.1	13.7 ± 0.3
Lutjanus griseus	Gray Snapper	342 - 565	30	-16.6 ± 0.1	14.1 ± 0.0
*Rhomboplites aurorubens******	Vermilion Snapper	208 - 565	151	-17.9 ± 0.04	12.4 ± 0.1
*Pagrus pagrus******	Red Porgy	275 - 465	38	-17.3 ± 0.1	12.2 ± 0.1
Calamus leucosteus	Whitebone Porgy	249 - 442	5	-16.8 ± 0.2	12.6 ± 0.2
Calamus proridens	Littlehead Porgy	335 - 365	2	-17.3 ± 0.3	12.1 ± 0.7
Mycteroperca interstitialis	Yellowmouth Grouper	575	1	-17.8	11.9
Mycteroperca phenax	Scamp	485 - 950	7	-17.3 ± 0.2	14.2 ± 0.8
Mycteroperca microlepis	Gag	722 - 815	3	-17.5 ± 0.2	14.0 ± 1.5
Epinephelus niveatus	Snowy Grouper	557 - 635	3	-16.8 ± 0.3	13.6 ± 0.8
Epinephelus morio	Red Grouper	575 - 680	5	-17.0 ± 0.2	13.5 ± 0.8
*Haemulon aurolineatum******	Tomtate	212 - 251	29	-16.8 ± 0.1	12.6 ± 0.1
Chaetodipterus faber	Atlantic Spadefish	280 - 310	2	-18.4 ± 0.2	13.1 ± 0.8
Cephalopholis cruentata	Graysby	312 - 350	2	-17.6 ± 0.4	13.4 ± 0.4
Pristigenys alta	Short Bigeye	287 - 295	1	-17.9	13.8
Sphyraena barracuda	Great Barracuda	1165 - 1305	2	-17.2 ± 0.0	13.3 ± 2.2
Seriola rivoliana	Almaco Jack	400 - 1030	11	-17.0 ± 0.2	11.3 ± 0.6
*Seriola dumerili******	Greater Amberjack	600 - 1400	28	-16.5 ± 0.1	13.7 ± 0.2
Seriola zonata	Banded Rudderfish	590	1	-16.2	13.6
Alectis cilialis	African Pompano	900	1	-16.1	14
Caranx hippos	Crevalle Jack	980	1	-21.1	13.9
Centropristis ocyurus	Bank Seabass	219 - 282	3	-17.2 ± 0.1	11.8 ± 0.3
Pterois sp.	Lionfish	225 - 340	11	-16.9 ± 0.2	12.4 ± 0.1
Epinephelus nigritus	Warsaw Grouper	NA	1	-16.2	14.4

Pelagic Fishes					
*Thunnus atlanticus**	Blackfin Tuna	395 - 930	48	-17.0 ± 0.1	12.5 ± 0.3
*Thunnus albacares**	Yellowfin Tuna	1200 -1600	31	-16.9 ± 0.1	10.8 ± 0.3
*Acanthocybium solandri**	Wahoo	1560 - 1760	6	-16.7 ± 0.2	11.8 ± 1.1
Elegatus bipinnulata	Rainbow Runner	520 -695	3	-17.2 ± 0.1	11.2 ± 2.3
*Rachycentron canadum**	Cobia	960 - 1545	55	-16.7 ± 0.1	12.7 ± 0.2
*Coryphaena hippurus**	Dolphin	520 - 1285	19	-16.9 ± 0.2	10.9 ± 0.3
*Makaira nigricans**	Blue Marlin	2570 -2870	7	-16.0 ± 0.1	11.2 ± 0.4

Table A.3

Producer data for the Mississippi Sound. Obtained from the literature. Dillon et al. collected from Grand Bay National Estuarine Research Reserve. Moncrieff & Sullivan collected from Graveline Bayou.

Producer	Mean δ^{13}C	Mean δ^{15}N	Source
BMA	-21.8	4	Dillon et al. 2015
POM	-21.8	2.5	Dillon et al. 2015
Plankton	-23	5.5	Dillon et al. 2015
Halodule.wrightii	-12.2	6	Moncreiff & Sullivan 2001
Epiphytic Algae	-17.5	5.9	Moncreiff & Sullivan 2001
Diatom rich substrate	-14.7	7.8	Moncreiff & Sullivan 2001
Phytoplankton	-21.8	9.9	Moncreiff & Sullivan 2001
Macroalgae	-16.8	7	Moncreiff & Sullivan 2001

Table A.4

Correlation data of $\delta^{13}C$ and $\delta^{15}N$ as a function of total length (mm) for species from the Mississippi Sound and barrier islands Values are significant when $p < 0.05$

	p-value		**R**	
Species	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**
Red Drum	0.17	0.37	0.29	-0.19
Gafftopsail Catfish	0.62	0.87	0.30	0.10
Sand Seatrout	0.63	0.02	0.11	0.52
Southern Kingfish	0.65	0.79	-0.15	0.09
Sharpnose Shark	0.69	0.61	-0.10	-0.13
Blacktip Shark	0.23	0.91	0.47	0.04
King Mackerel	0.67	0.38	-0.11	0.22
Spanish Mackerel	0.36	0.19	-0.13	-0.18

Table A.5

Shapiro-wilk tests for normal distribution of isotopic data for Mississippi Sound species. Significance ($p < 0.05$) indicates non-normal distribution.

Species	**$\delta^{13}C$**	**$\delta^{15}N$**
King Mackerel	0.56	0.22
Spanish Mackerel	0.08	$3x10^{-5}$
Southern Kingfish	0.34	0.30
Sand Seatrout	0.40	$2x10^{-3}$
Red Drum	0.02	$6x10^{-4}$
Gafftopsail Catfish	$4x10^{-3}$	0.43
Sharpnose Shark	0.34	0.09
Blacktip Shark	0.28	0.12

Table A.6

Matrix of Bonferroni corrected p-values resulting from multiple pairwise Mann-Whitney U comparisons of δ^{13}C values of Mississippi Sound species. Values are significant when $p < 0.007$

Species δ^{13}C	**Spanish Mackerel**	**Gafftopsail Catfish**	**Red Drum**	**Sand Seatrout**	**Southern Kingfish**	**Sharpnose Shark**	**Blacktip Shark**
King Mackerel	0.02	2×10^{-3}	2×10^{-3}	9×10^{-3}	**0.01**	0.69	0.69
Spanish Mackerel		0.31	0.01	0.11	0.23	5×10^{-3}	0.06
Gafftopsail Catfish			0.70	0.22	0.43	9×10^{-4}	4×10^{-3}
Red Drum				0.71	0.25	1×10^{-3}	0.02
Sand Seatrout					0.78	9×10^{-3}	0.01
Southern Kingfish						3×10^{-3}	0.02
Sharpnose Shark							0.97

Table A.7

Matrix of Bonferroni corrected p-values resulting from multiple pairwise Mann-Whitney U comparisons of δ^{15}N values of Mississippi species. Values are significant when $p < 0.007$.

Species δ^{15}N	Spanish Mackerel	Gafftopsail Catfish	Red Drum	Sand Seatrout	Southern Kingfish	Sharpnose Shark	Blacktip Shark
King Mackerel	0.25	0.83	0.79	0.98	0.11	8x10^{-4}	0.31
Spanish Mackerel		0.16	0.26	0.22	0.86	**0.02**	0.94
Gafftopsail Catfish			0.50	0.73	0.09	2x10^{-3}	0.17
Red Drum				0.78	0.45	2x10^{-3}	0.39
Sand Seatrout					0.11	9x10^{-3}	0.17
Southern Kingfish						1x10^{-2}	0.71
Sharpnose Shark							2x10^{-3}

Table A.8

Mean (±SE) POM isotope values (‰) by SEAMAP station. Plankton net values are based on 1 sample from each station.

			POM (‰)			**53 µm (‰)**			**333 µm (‰)**			**947 µm (‰)**		
Station #	**Latitude**	**Longitude**	**$\delta^{13}C$**	**$\delta^{15}N$**	**C:N Ratio**	**$\delta^{13}C$**	**$\delta^{15}N$**	**C:N Ratio**	**$\delta^{13}C$**	**$\delta^{15}N$**	**C:N Ratio**	**$\delta^{13}C$**	**$\delta^{15}N$**	**C:N Ratio**
B176	29.50	-88.04	-25.5 ± 0.2	1.2 ± 1.1	3.2 ± 0.2	-19.7	7.1	4.97	-20.2	7.2	4.8	-20.2	6.8	4.7
B177	30.00	-87.95	-24.9 ± 0.2	4.5 ± 0.4	3.0 ± 0.1	-19.8	8.4	4.98	-20.0	6.9	4.7	-21.4	6.5	6.9
B178	30.00	-88.47	-24.3 ± 0.9	1.4 ± 0.8	4.2 ± 1.4	-18.5	5.3	5.8	-19.6	6.9	4.5	-16.5	5.4	6.0
B179	29.50	-88.50	-24.7 ± 0.1	2.6 ± 0.2	2.9 ± 0.0	-20.2	8.8	4.42	-20.2	5.9	4.5	-19.1	7.8	4.6
B180	29.00	-88.50	-24.7 ± 0.2	3.2 ± 0.7	3.8 ± 0.4	-18.0	1.8	7.59	-20.3	5.0	4.6	No data	No data	No data
B183	29.00	-89.00	-241 ± 0.1	4.8 ± 0.7	2.5 ± 0.2	-19.8	9.5	4.52	-21.0	7.6	4.4	-21.4	6.9	8.2
B322	29.25	-88.00	-25.4± 0.4	-1.4 ± 0.4	4.3 ± 0.4	-18.0	1.8	6.07	-19.7	7.3	4.4	-23.6	6.7	6.6
B323	29.22	-88.50	-25.5 ± 1.0	3.4 ± 0.8	4.0 ± 0.4	-20.0	7.1	4.94	-19.8	7.9	4.6	-19.9	8.5	4.0

Table A.9

Correlation data of $\delta^{13}C$ and $\delta^{15}N$ as a function of total length (mm) for reef target by species and location. Values are significant when $p < 0.05$.

	p-value		**R**	
Species by location	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**
All VS	2.5×10^{-3}	0.04	0.29	-0.10
All RS	0.61	0.36	0.05	0.49
All GAJ	0.01	0.13	0.46	0.29
All TT	0.53	0.87	0.12	0.03
All RP	0.19	1×10^{-3}	0.21	0.49
VSLA	0.23	0.09	-0.20	-0.34
RSLA	0.09	0.37	0.05	-0.13
GAJLA	0.13	0.05	0.40	0.51
VSDI	0.09	0.60	0.24	0.09
RSDI	0.45	0.10	0.27	0.55
TTDI	0.30	0.45	0.30	0.29
VSOB	0.09	4×10^{-3}	-0.31	-0.48
RSOB	0.99	0.68	0.01	-0.07
GAJOB	0.16	0.95	-0.66	0.03
TTOB	0.91	0.10	-0.03	0.40
RPOB	0.39	0.96	-0.29	-0.07
VSFL	0.43	0.20	-0.11	-0.18
GAJFL	0.78	0.26	0.14	-0.54
RPFL	0.35	0.01	0.20	0.51

Table A.10

Shapiro-Wilk tetsts for normal distribution of isotopic values of reef species. Significance ($p < 0.05$) indicates non-normal distribution

Species by location	**$\delta^{13}C$**	**$\delta^{15}N$**
VSLA	0.02	0.60
RSLA	3.9×10^{-07}	2×10^{-3}
GAJLA	0.244	2×10^{-3}
VSDI	6.1×10^{-05}	1×10^{-3}
RSDI	0.08	0.56
TTDI	0.38	0.15
VSOB	0.18	4×10^{-4}
RSOB	0.10	0.62
GAJOB	0.42	0.52
TTOB	0.81	0.71

Table A.11

. Matrix of Bonferroni corrected p-values resulting from multiple pairwise Mann-Whitney U comparisons of δ^{13}C values of reef species by location. Values are significant when $p < 0.0038$.

Species δ^{13}C	**RSLA**	**GAJLA**	**VSDI**	**RSDI**	**TTDI**	**VSOB**	**RSOB**	**TTOB**	**RPOB**	**GAJOB**	**VSFL**	**RPFL**	**GAJFL**
VSLA	0.61	$1.1\text{x}10^{-7}$	$8\text{x}10^{-4}$	$4\text{x}10^{-3}$	$6.2\text{x}10^{-4}$	$2.6\text{x}10^{-11}$	0.86	$7\text{x}10^{-4}$	0.74	0.06	$7.9\text{x}10^{-15}$	0.90	$1.2\text{x}10^{-4}$
RSLA		$7.2\text{x}10^{-6}$	$1.8\text{x}10^{-3}$	0.02	$3\text{x}10^{-3}$	$1.6\text{x}10^{-7}$	0.40	0.01	0.87	0.16	$3.1\text{x}10^{-10}$	0.45	$3\text{x}10^{-4}$
GAJLA			$7.\text{x}10^{-6}$	$4\text{x}10^{-3}$	0.02	$4.4\text{x}10^{-8}$	$5.891\text{x}10^{-6}$	$4\text{x}10^{-3}$	$5\text{x}10^{-4}$	0.01	$7.2\text{x}10^{-9}$	$1.923\text{x}10^{-6}$	0.46
VSDI				$9\text{x}10^{-4}$	$2\text{x}10^{-4}$	$8\text{x}10^{-4}$	$1.2\text{x}10^{-3}$	$2\text{x}10^{-4}$	0.04	$9.6\text{x}10^{-3}$	$2.6\text{x}10^{-9}$	$4.2\text{x}10^{-3}$	$8.6\text{x}10^{-4}$
RSDI					0.23	$2.2\text{x}10^{-6}$	$1.7\text{x}10^{-3}$	0.46	0.06	0.87	$8.6\text{x}10^{-7}$	$8\text{x}10^{-3}$	$4\text{x}10^{-3}$
TTDI						$2\text{x}10^{-4}$	$9.6\text{x}10^{-4}$	0.35	$9.6\text{x}10^{-3}$	0.17	$7.9\text{x}10^{-9}$	$1\text{x}10^{-3}$	0.02
VSOB							$2.0\text{x}10^{-10}$	$1\text{x}10^{-7}$	$1.4\text{x}10^{-4}$	$1.4\text{x}10^{-4}$	$2\text{x}10^{-4}$	$8.5\text{x}10^{-7}$	$1.2\text{x}10^{-4}$
RSOB								$1.3\text{x}10^{-3}$	0.72	0.05	$7.1\text{x}10^{-14}$	0.80	$2.6\text{x}10^{-4}$
TTOB									0.05	0.43	$1.7\text{x}10^{-8}$	$4\text{x}10^{-3}$	$6\text{x}10^{-3}$
RPOB										0.29	$8.1\text{x}10^{-6}$	0.55	$1.5\text{x}10^{-3}$
GAJOB											$8\text{x}10^{-5}$	$3.8\text{x}10^{-4}$	$8\text{x}10^{-3}$
VSFL												$2.7\text{x}10^{-10}$	0.11
RPFL													$3.8\text{x}10^{-4}$

Table A.12

Matrix of Bonferroni corrected p-values resulting from multiple pairwise Mann-Whitney U comparisons of $\delta^{15}N$ values of reef species by location.

Values are significant when $p < 0.0038$.

Species $\delta^{15}N$	**RSLA**	**GAJLA**	**VSDI**	**RSDI**	**TTDI**	**VSOB**	**RSOB**	**TTOB**	**RPOB**	**GAJOB**	**VSFL**	**RPFL**	**GAJFL**
VSLA	$4.9x10^{-14}$	$1.2x10^{-5}$	$3.2x10^{-3}$	$2.9x10^{-6}$	$7.1x10^{-7}$	0.30	0.24	0.19	0.06	1.2x10-4	0.48	0.18	0.11
RSLA		$2.6x10^{-4}$	$1.9x10^{-9}$	$1.2x10^{-4}$	$3.5x10^{-7}$	$3.6x10^{-14}$	$1.5x10^{-13}$	$1.8x10^{-8}$	$3.1x10^{-7}$	0.02	$1.7x10^{-15}$	$6.9x10^{-11}$	$2.2x10^{-4}$
GAJLA			0.02	$4x10^{-3}$	0.01	$5.1x10^{-6}$	$5.1x10^{-5}$	$1.6x10^{-4}$	$2.8x10^{-4}$	0.67	$5.8x10^{-6}$	7.10^{-6}	0.02
VSDI				0.07	0.24	$1x10^{-4}$	0.08	$3.4x10^{-4}$	$1x10^{-4}$	$6.7x10^{-3}$	3.6x10-4	$2.2x10^{-4}$	0.80
RSDI					0.02	$2.2x10^{-6}$	$8.9x10^{-6}$	$1.0x10^{-6}$	$1.2x10^{-4}$	$4.1x10^{-3}$	$5.1x10^{-5}$	$6x10^{-6}$	0.01
TTDI						$3.9x10^{-8}$	$4.3x10^{-7}$	$2.6x10^{-8}$	$2.6x10^{-7}$	$5x10^{-4}$	$8x10^{-5}$	$4.6x10^{-6}$	0.02
VSOB							$6.2x10^{-4}$	0.29	0.03	$1.2x10^{-4}$	0.8	0.31	$5x10^{-3}$
RSOB								$1x10^{-3}$	$2x10^{-4}$	$1.4x10^{-4}$	0.07	2x10-3	0.18
TTOB									0.28	$6.2x10^{-4}$	0.76	0.98	$7.4x10^{-3}$
RPOB										$1x10^{-3}$	0.40	0.41	$3.1x10^{-3}$
GAJOB											$1.6x10^{-4}$	$2.4x10^{-4}$	$5.1x10^{-3}$
VSFL												0.79	0.10
RPFL													0.02

Table A.13

Correlation data of $\delta^{13}C$ and $\delta^{15}N$ as a function of total length (mm) for pelagic Values are significant when $p < 0.05$. P-values are significant at $p < 0.05$.

	p-value		**R**	
Species	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**
Blackfin Tuna	$2.4x10^{-4}$	$2x10^{-3}$	0.62	0.42
Blue Marlin	0.09	0.44	-0.67	0.30
Cobia	0.01	$3.7x10^{-3}$	0.33	-0.39
Dolphinfish	0.21	045	0.21	-0.18
Wahoo	0.20	0.46	-0.12	0.37
Yellowfin Tuna	0.68	$6x10^{-3}$	-0.06	0.49

Table A.14

Shapiro-wilk tests for normal distribution of isotopic data for pelagic species.

Significance ($p < 0.05$) indicates non-normal distribution

Species	**$\delta^{13}C$**	**$\delta^{15}N$**
Blackfin Tuna	0.03	0.12
Yellowfin Tuna	0.06	0.22
Blue Marlin	0.04	0.85
Wahoo	0.6	0.33
Cobia	8.8×10^{-6}	1.6×10^{-4}
Dolphinfish	0.01	0.25

Table A.15

Matrix of Bonferroni corrected p-values resulting from multiple pairwise Mann-Whitney U comparisons of δ^{13}C values of pelagic species. Values are significant at $p < 0.01$.

Species δ^{13}C	**Yellowfin Tuna**	**Blue Marlin**	**Wahoo**	**Dolphinfish**	**Cobia**
Blackfin Tuna	0.86	$1x10^{-4}$	0.21	0.32	$1x10^{-3}$
Yellowfin Tuna		$4x10^{-3}$	0.41	0.70	0.02
Blue Marlin			0.03	$4x10^{-3}$	0.13
Wahoo				0.68	0.62
Dolphinfish					0.22

Table A.16

Matrix of Bonferroni corrected p-values resulting from multiple pairwise Mann-Whitney U comparisons of $\delta^{15}N$ values of pelagic species. Values are significant at $p < 0.01$.

Species $\delta^{15}N$	**Yellowfin Tuna**	**Blue Marlin**	**Wahoo**	**Dolphinfish**	**Cobia**
Blackfin Tuna	4×10^{-3}	0.07	0.43	3.5×10^{-3}	0.80
Yellowfin Tuna		0.34	0.57	0.47	1×10^{-4}
Blue Marlin			0.94	0.64	0.13
Wahoo				0.63	0.52
Dolphinfish					1×10^{-5}

APPENDIX B ILLUSTRATIONS

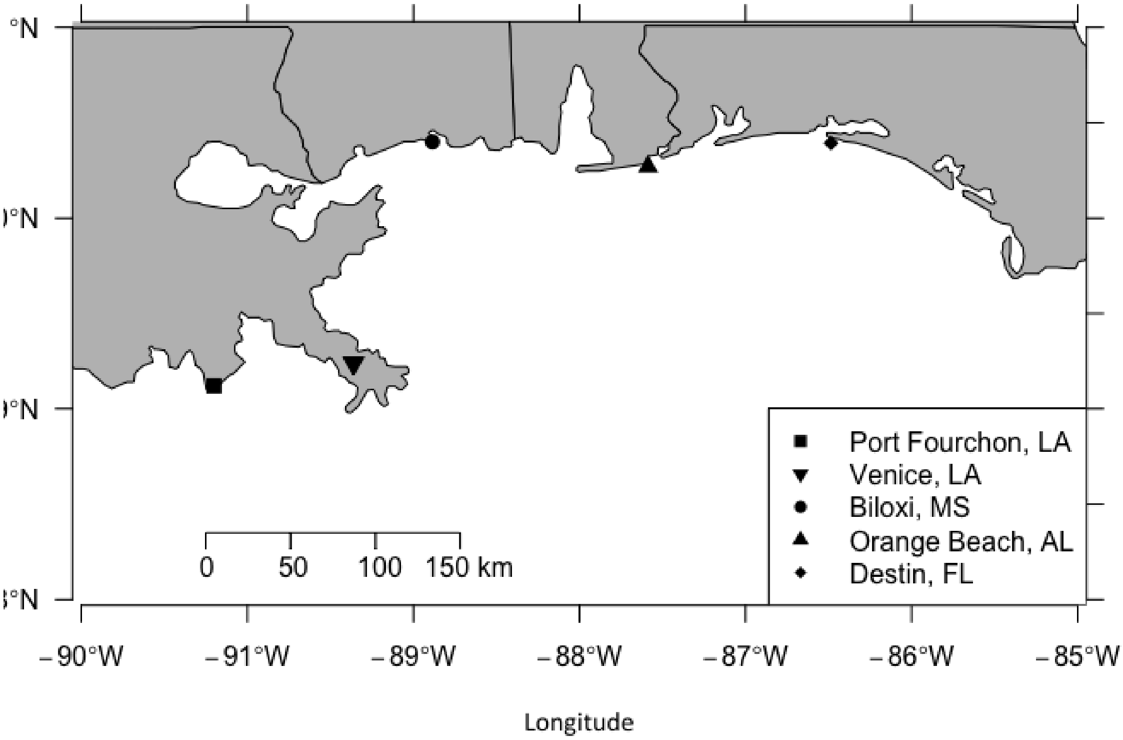


Figure B.1 Study Area including ports of sampling activity

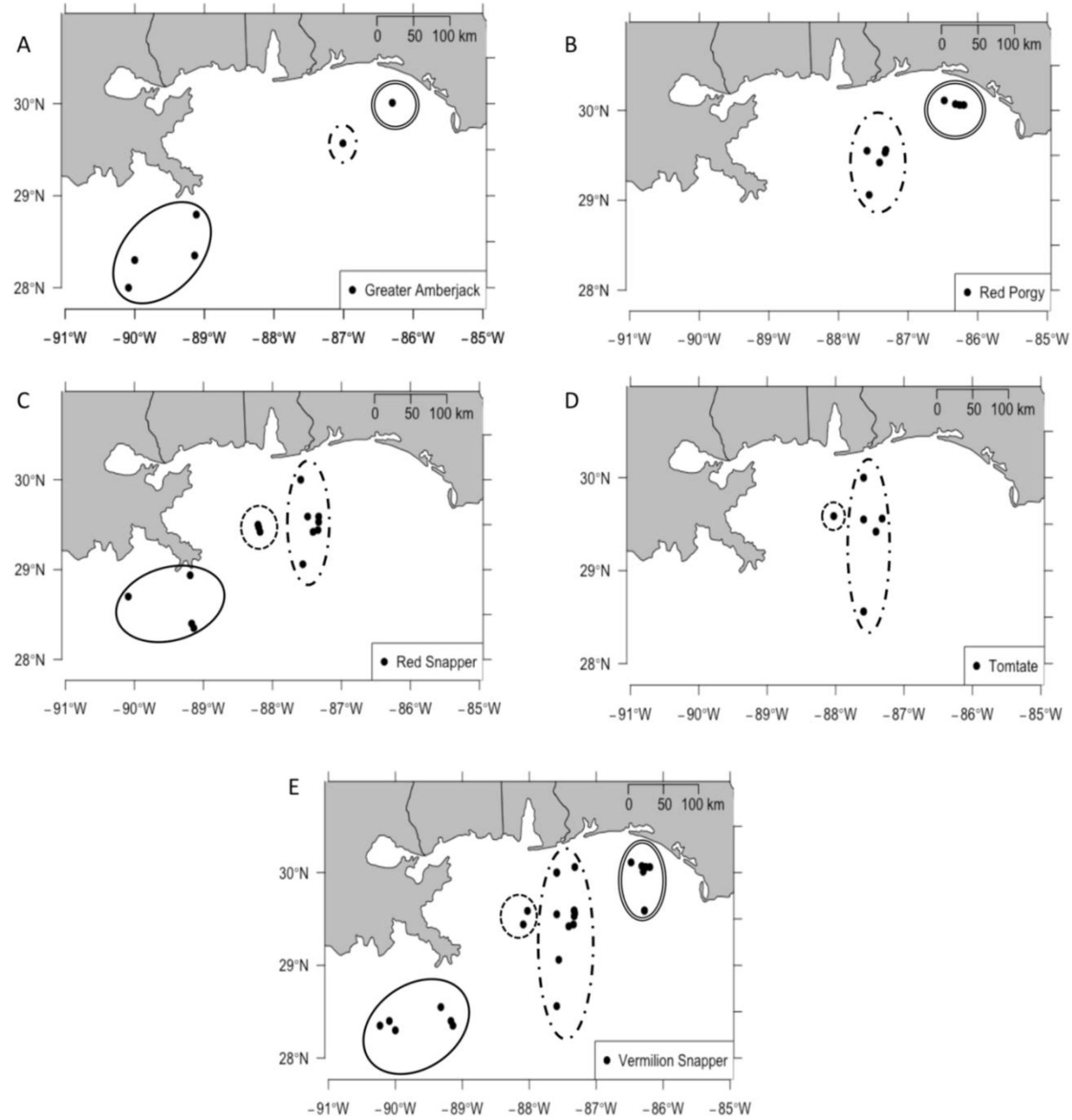


Figure B.2 Catch locations for Greater Amberjack (A), Red Porgy (B), Red Snapper (C), Tomtate (D) and Vermilion Snapper (E). Ovals represent locations reported for each sampling area: Louisiana (solid), Dauphin Island (dashed), Orange Beach (dash/dot) and Destin, Florida (double line)

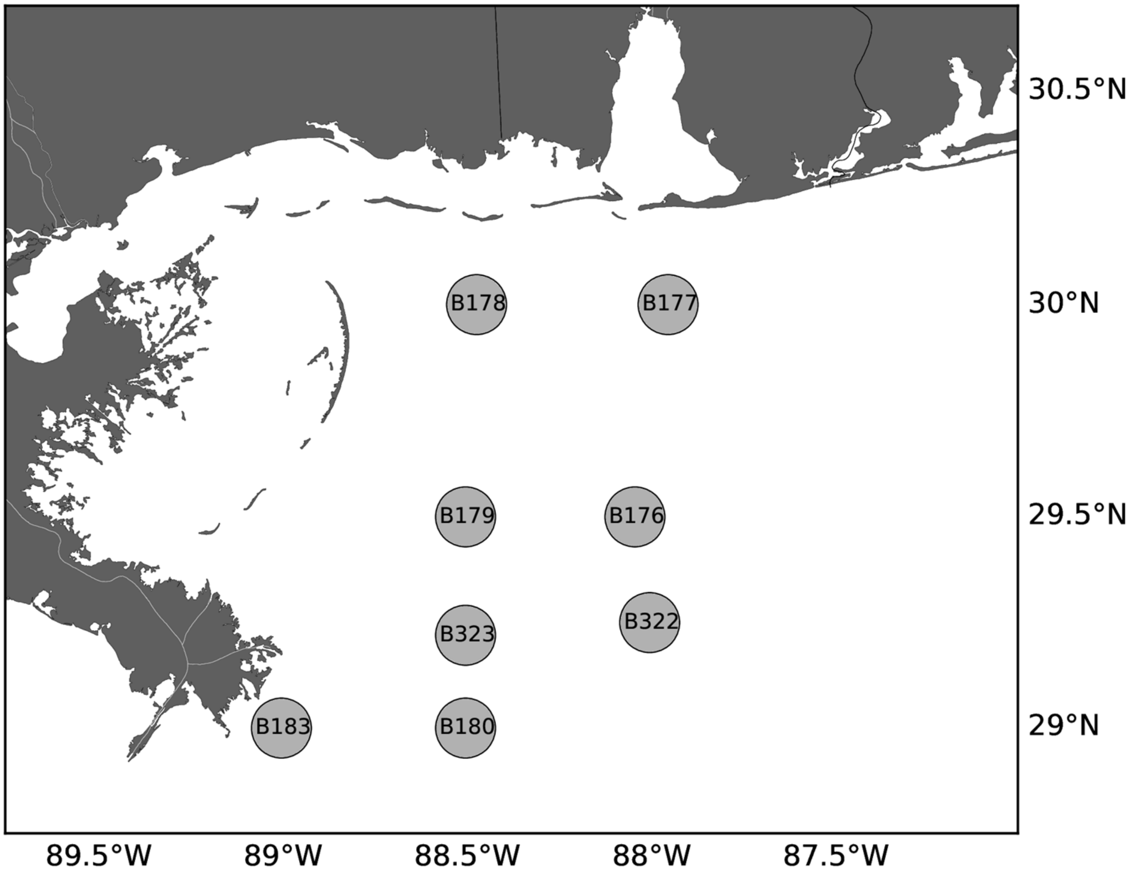


Figure B.3 SEAMAP station locations where POM and plankton samples were collected. (Image credit: SEAMAP) Numbered circles represent SEAMAP sampling locations.

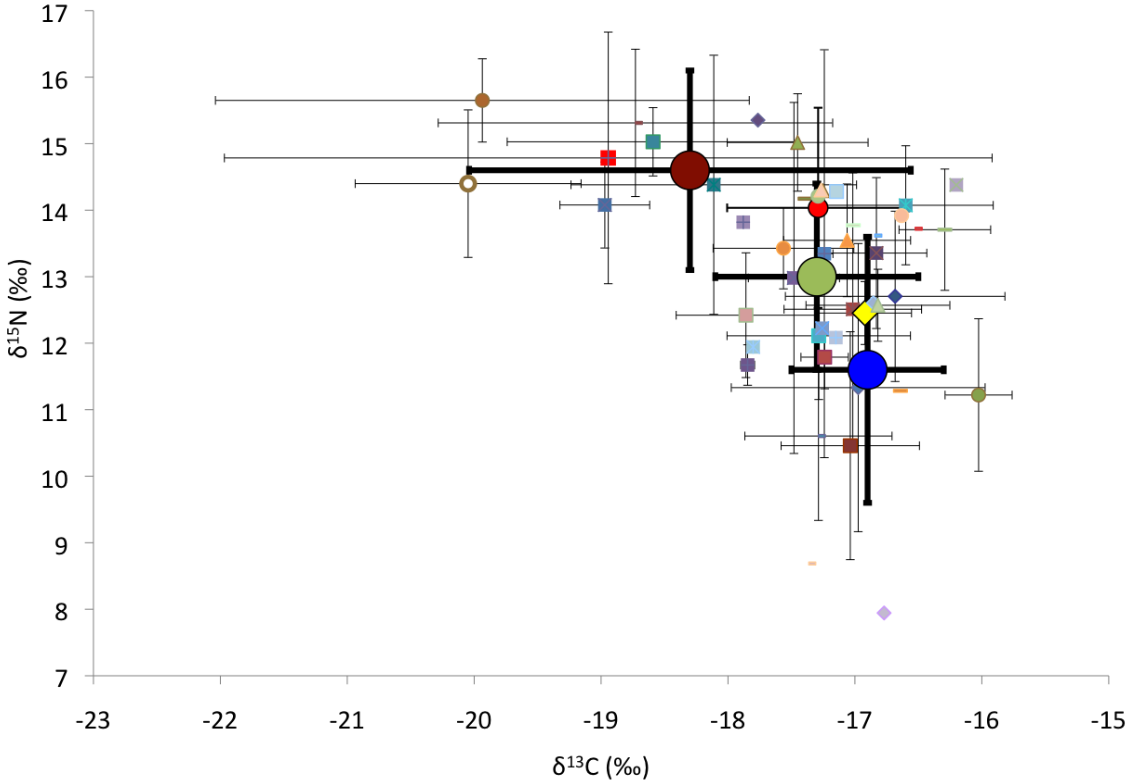


Figure B.4 Biplot of all species sampled in the north-central Gulf of Mexico, including those from the Mississippi Sound, Reef, and Pelagic habitats. Data points are mean values, error bars represent standard deviation. Large data points indicate mean isotopic values of all species from each ecotype. Large red point represents species from the Mississippi Sound and barrier Islands, large green point represents all reef species, and large blue point represents all pelagic species. This pattern of enrichment in δ^{13}C and depletion in δ^{15}N illustrates a trophic gradient from near shore to offshore. Sample sizes by species listed in Table 2.

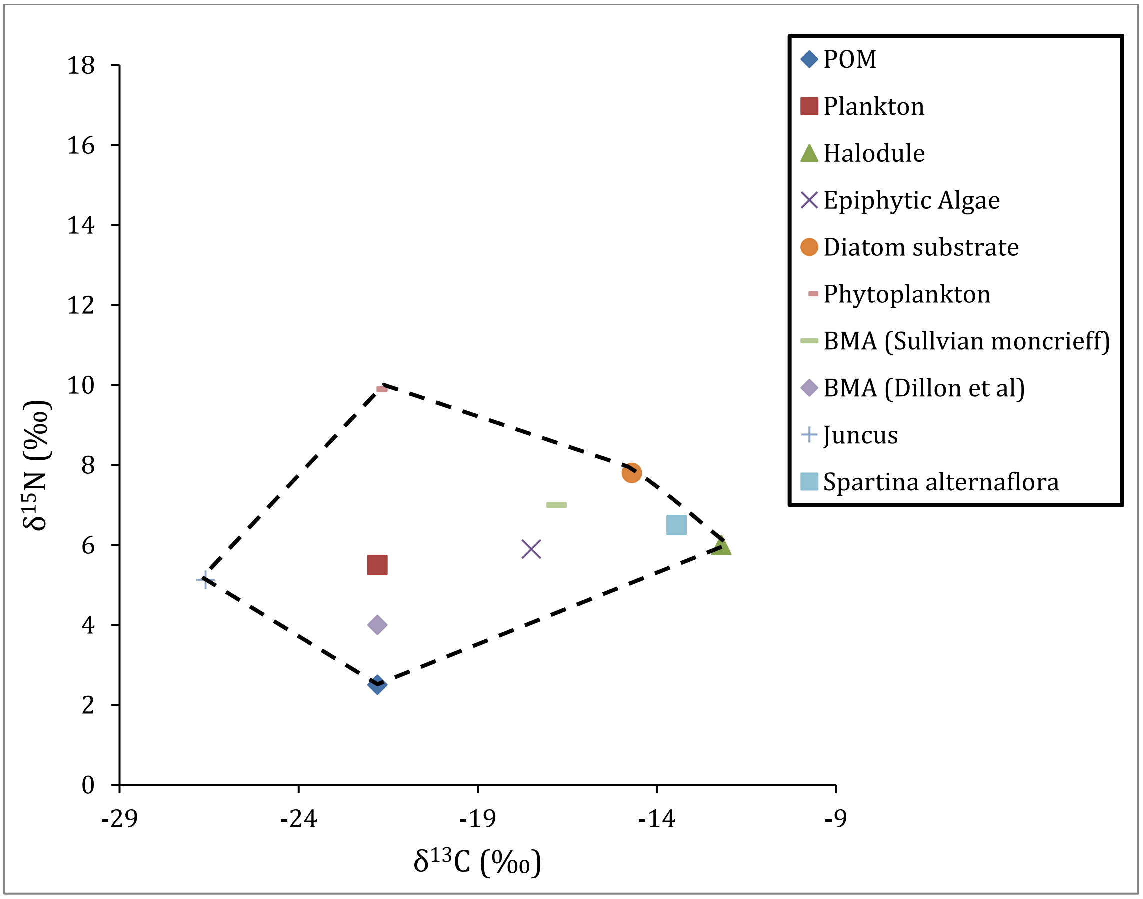


Figure B.5 Biplot of isotope source data for the Mississippi Sound. Total isotopic mixing space for the Mississippi Sound is created by drawing a polygon around outermost potential basal food web sources.

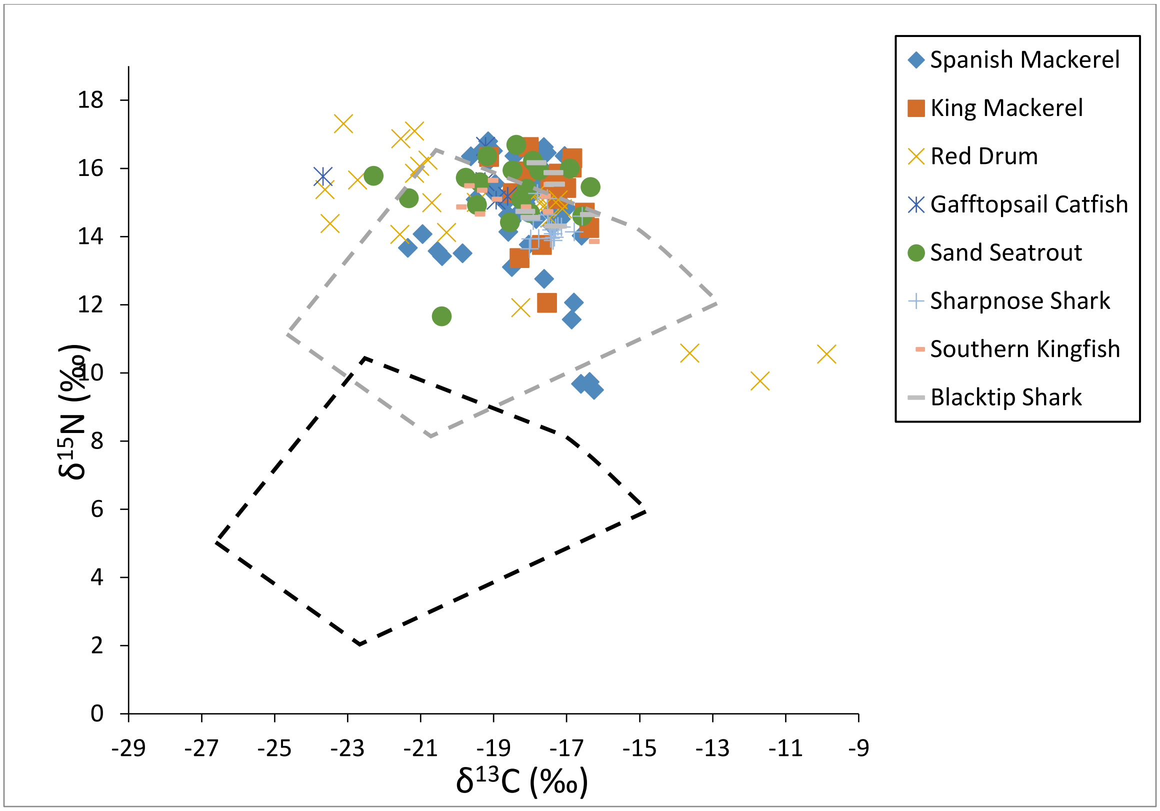


Figure B.6 Biplot of all individual species from the Mississippi Sound. Dashed polygons represent mixing space. Lower polygon represents basal resources at trophic level one, upper polygon represents trophic level 3. This allows estimates to be made about sources supporting species from the Mississippi Sound.

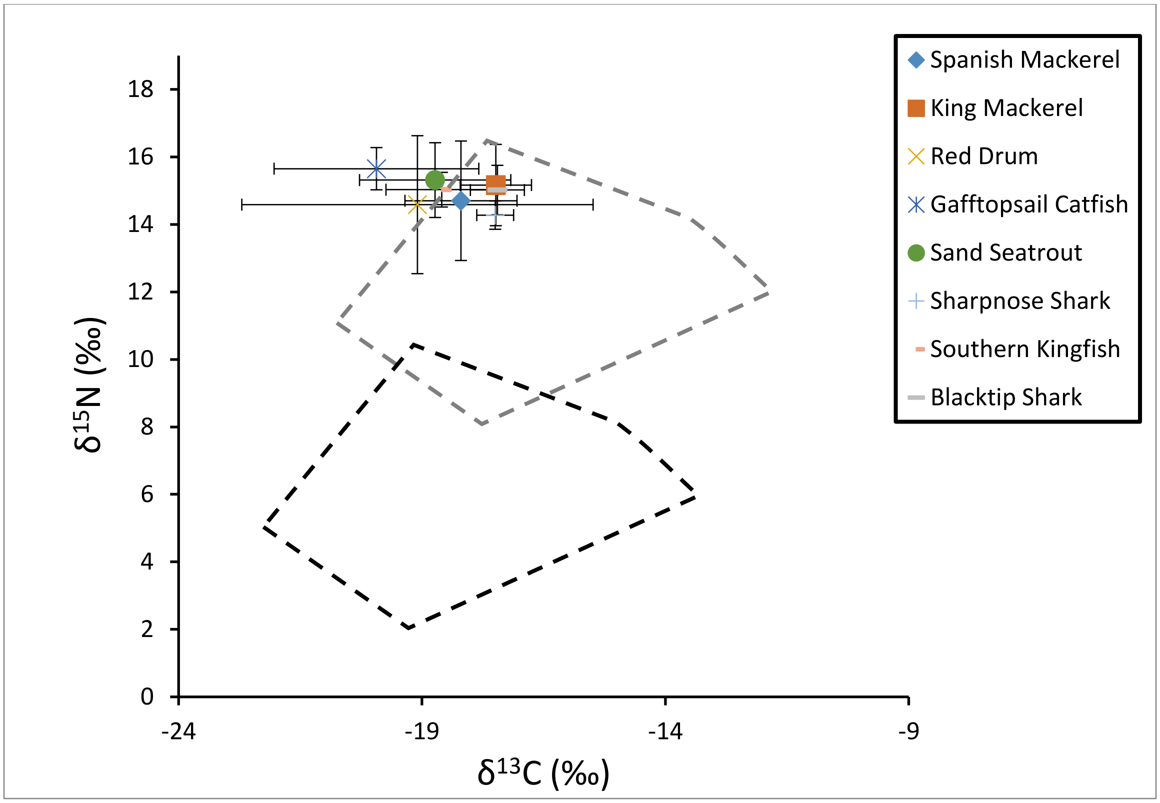
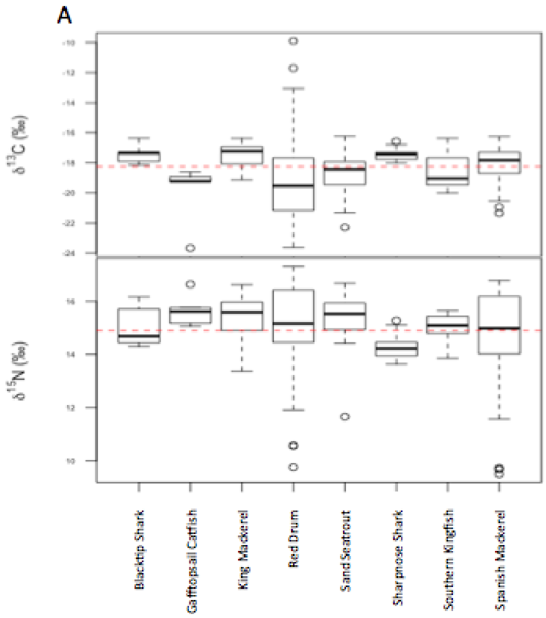
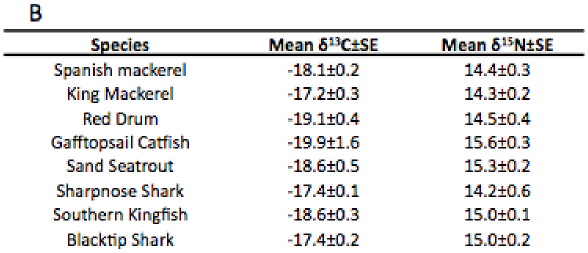


Figure B.7 Biplot with mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ values for target species of the Mississippi Sound. Lower polygon represents basal resources at trophic level one, upper polygon represents trophic level 3. This allows estimates to be made about sources supporting species from the Mississippi Sound.




B

Species	Mean δ13C±SE	Mean δ15N±SE
Spanish mackerel	-18.1±0.2	14.4±0.3
King Mackerel	-17.2±0.3	14.3±0.2
Red Drum	-19.1±0.4	14.5±0.4
Gafftopsail Catfish	-19.9±1.6	15.6±0.3
Sand Seatrout	-18.6±0.5	15.3±0.2
Sharpnose Shark	-17.4±0.1	14.2±0.6
Southern Kingfish	-18.6±0.3	15.0±0.1
Blacktip Shark	-17.4±0.2	15.0±0.2

Figure B.8 Boxplots of δ^{13}C and δ^{15}N (6A) and values (6B) for target species of the Mississippi Sound. Dashed line on box plots represent grand mean for all species.

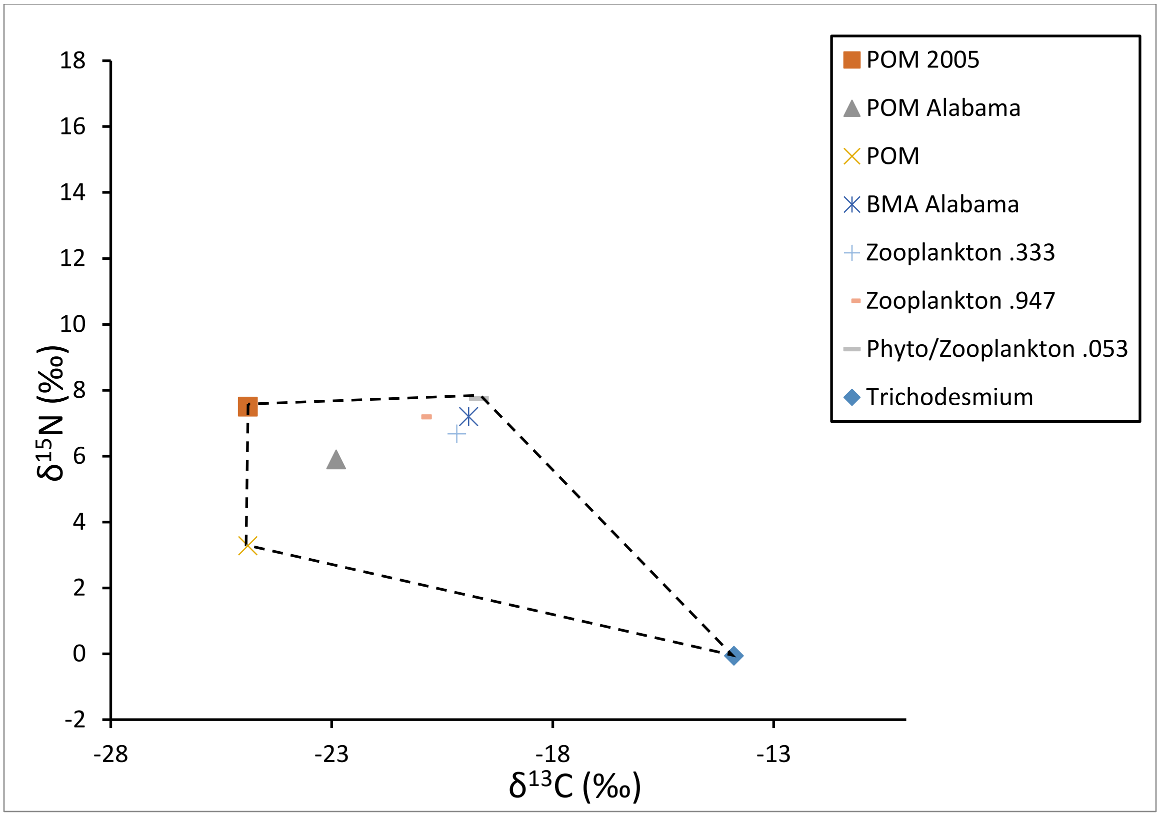


Figure B.9 Biplot of possible $\delta^{13}C$ and $\delta^{15}N$ sources for reef fish. Dashed polygon represents total isotopic mixing space for the reef ecotype. Total mixing space is created by drawing the polygon around outermost potential baseline food web sources

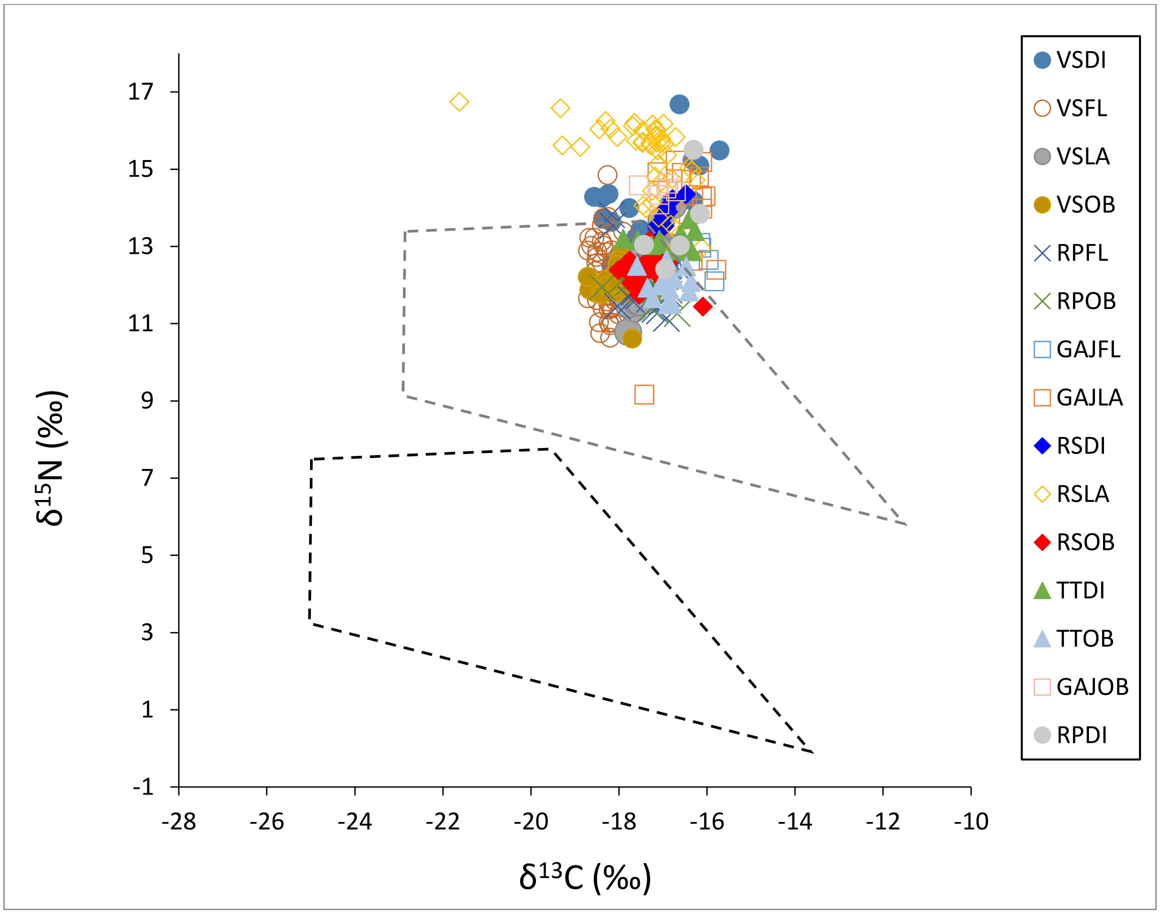


Figure B.10 Biplot with δ^{13}C andδ^{15}N values for individual target reef species. Lower polygon represents basal resources at trophic level one, upper polygon represents trophic level 3. This allows estimates to be made about sources supporting reef species in the north-central Gulf of Mexico.

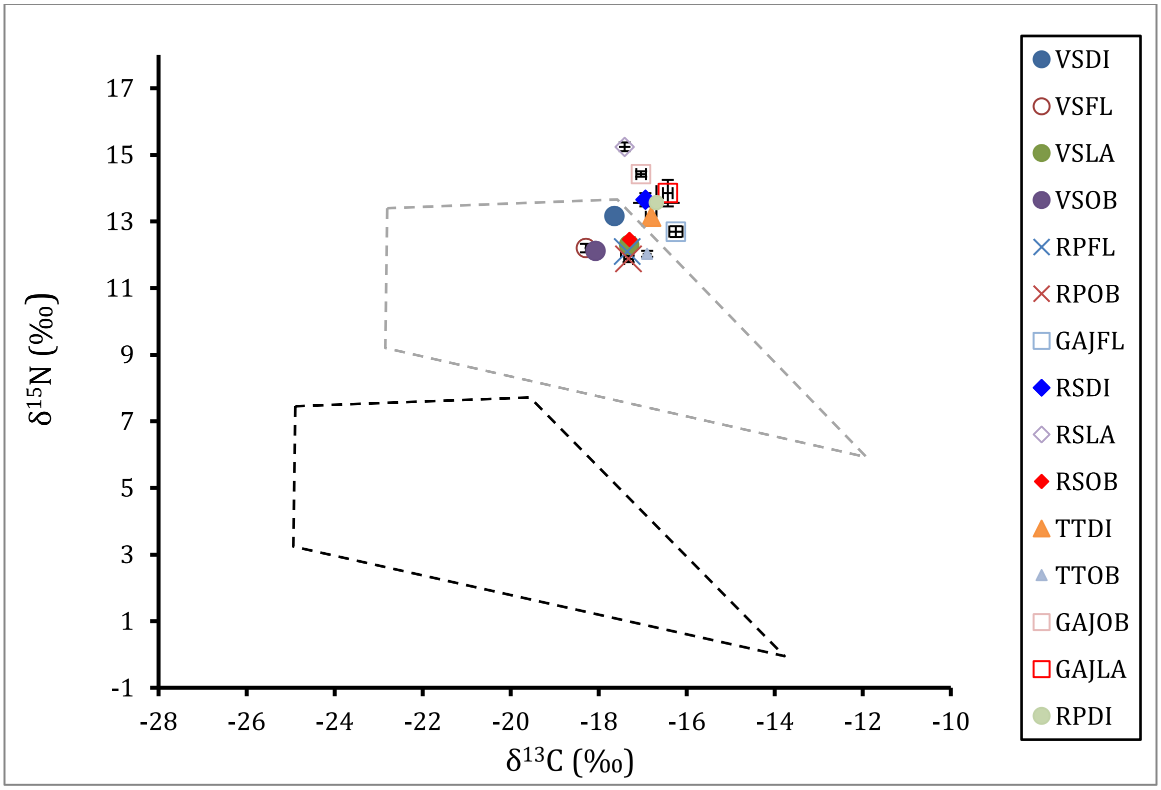
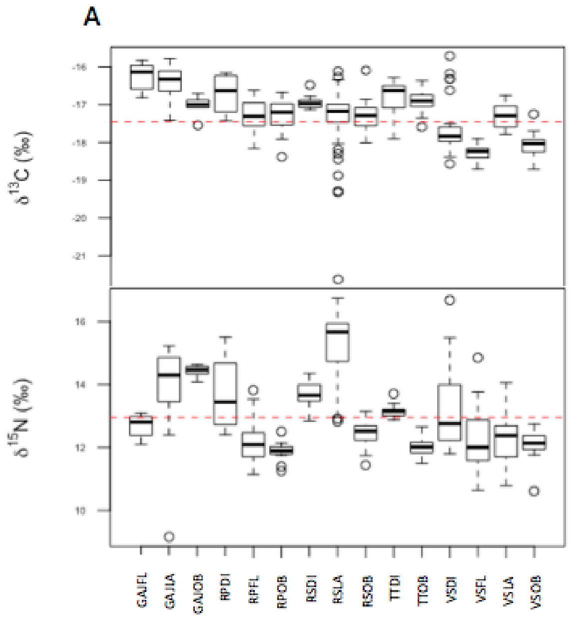


Figure B.11 Biplot with mean δ^{13}C and δ^{15}N for reef species. Bars represent standard errors. Lower polygon represents basal resources at trophic level one, upper polygon represents trophic level 3. This allows estimates to be made about sources supporting reef species in the north-central Gulf of Mexico.

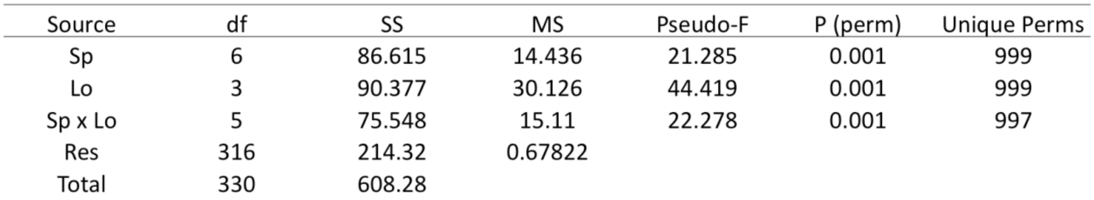


B

Reef Fishes	Mean $\delta^{13}C$±SE	Mean $\delta^{15}N$±SE
GAJFL	-16.2±0.1	12.7±0.2
GAJLA	-16.4±0.1	13.5±0.4
GAJOB	-17.0±0.1	14.2±0.1
RPDI	-16.7±0.2	13.6±0.5
RPFL	-17.3±0.0	12.1±0.1
RPOB	-17.3±0.0	11.9±0.1
RSDI	-16.9±0.1	13.5±0.2
RSLA	-17.4±0.1	15.4±0.1
RSOB	-17.3±0.1	12.5±0.1
TTDI	-16.8±0.1	13.4±0.1
TTOB	-16.9±0.1	12.0±0.1
VSDI	-17.6±0.1	13.2±0.2
VSFL	-18.3±0.0	12.2±0.1
VSLA	-17.3±0.1	12.3±0.1
VSOB	-18.1±0.1	12.1±0.1

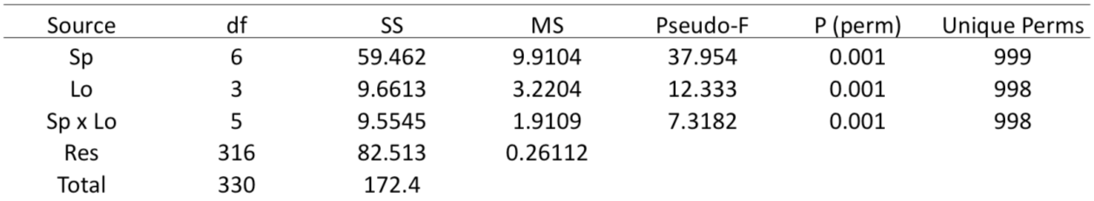
Figure B.12 Boxplots of $\delta^{13}C$ and $\delta^{15}N$ (A) and mean values(±SE)(B) for Greater Amberjack (GAJ), Red Porgy (RP), Red Snapper (RS), Tomtate (TT) and Vermilion Snapper (VS) by location. Locations are Dauphin Island (DI), Florida (FL), Louisiana (LA), and Orange Beach (OB). Dashed line on boxplots represents grand mean.

A



Source	df	SS	MS	Pseudo-F	P (perm)	Unique Perms
Sp	6	86.615	14.436	21.285	0.001	999
Lo	3	90.377	30.126	44.419	0.001	999
Sp x Lo	5	75.548	15.11	22.278	0.001	997
Res	316	214.32	0.67822			
Total	330	608.28				

B



Source	df	SS	MS	Pseudo-F	P (perm)	Unique Perms
Sp	6	59.462	9.9104	37.954	0.001	999
Lo	3	9.6613	3.2204	12.333	0.001	998
Sp x Lo	5	9.5545	1.9109	7.3182	0.001	998
Res	316	82.513	0.26112			
Total	330	172.4				

Figure B.13 PERMANOVA tables for δ^{15}N (A) and δ^{13}C (B).

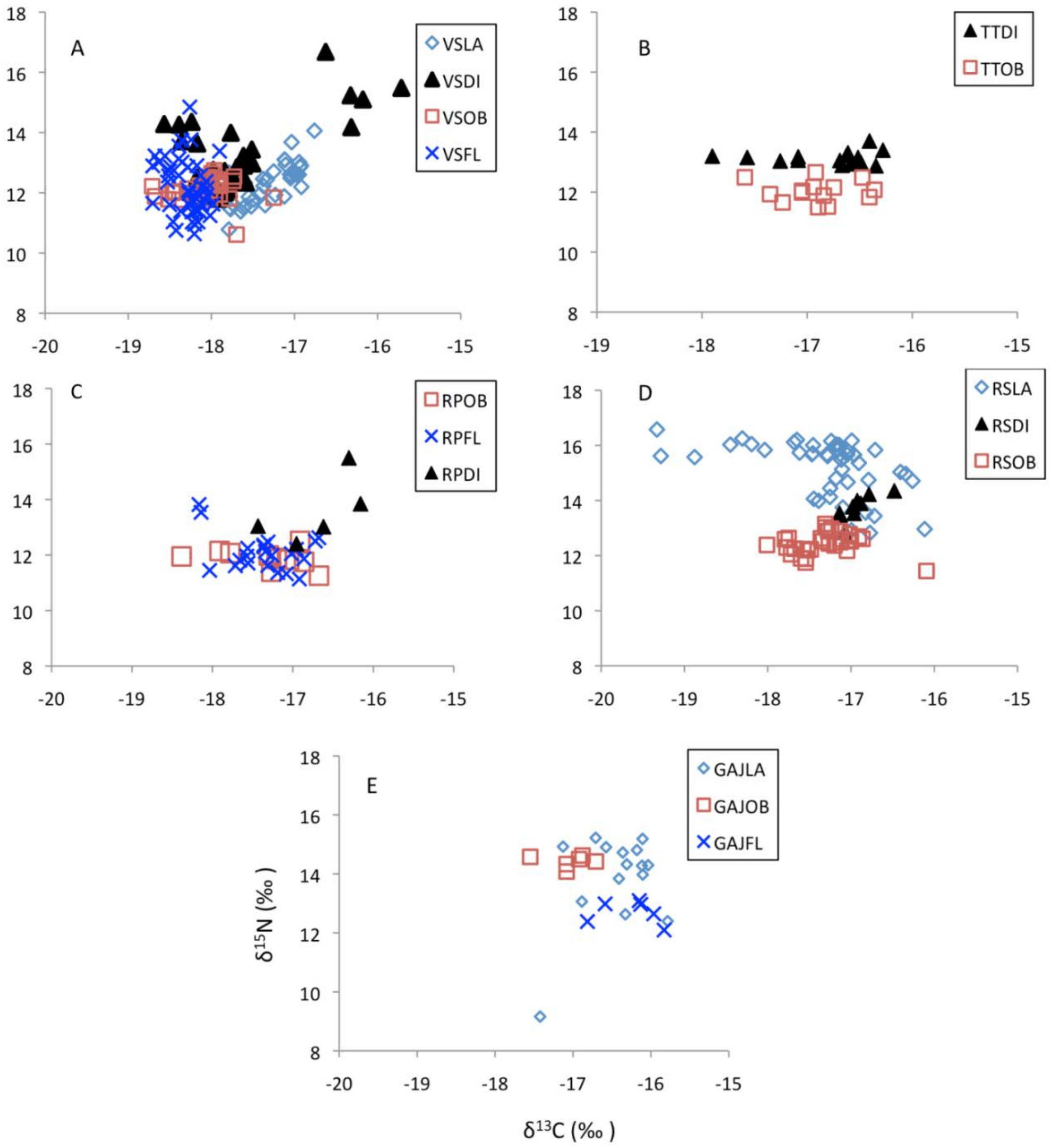


Figure B.14 Carbon/Nitrogen biplots for all individual Vermilion Snapper (A), Tomtate (B), Red Porgy (C), Red Snapper (D), and Greater Amberjack (E).

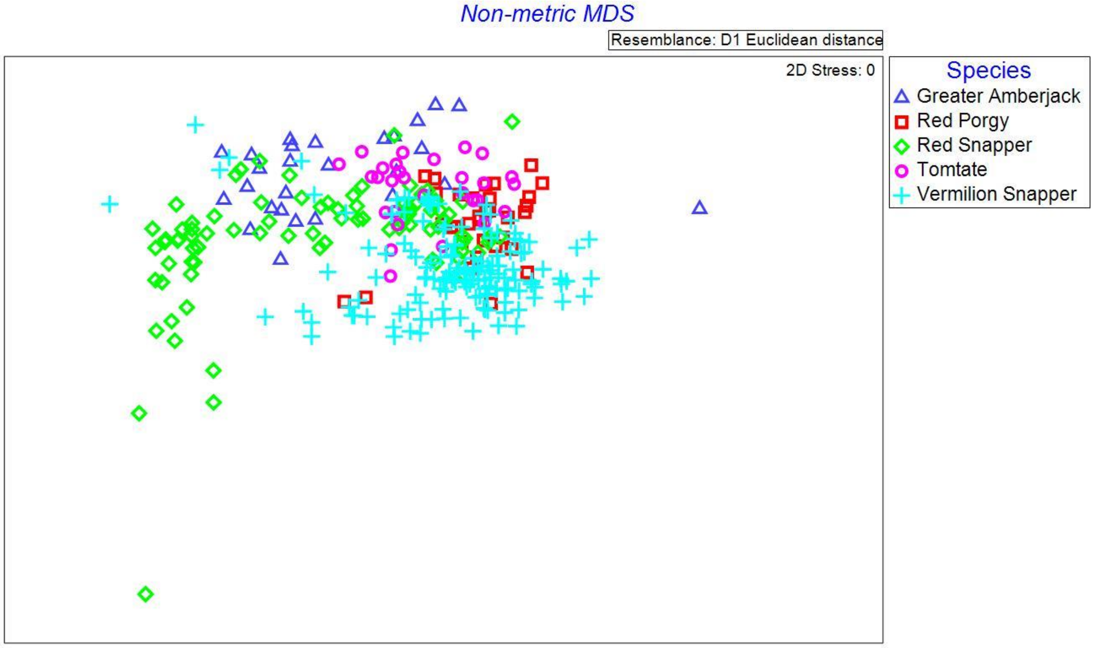


Figure B.15 MDS plot of raw $\delta^{13}C$ values by species based on Euclidean distance dissimilarity for all species.

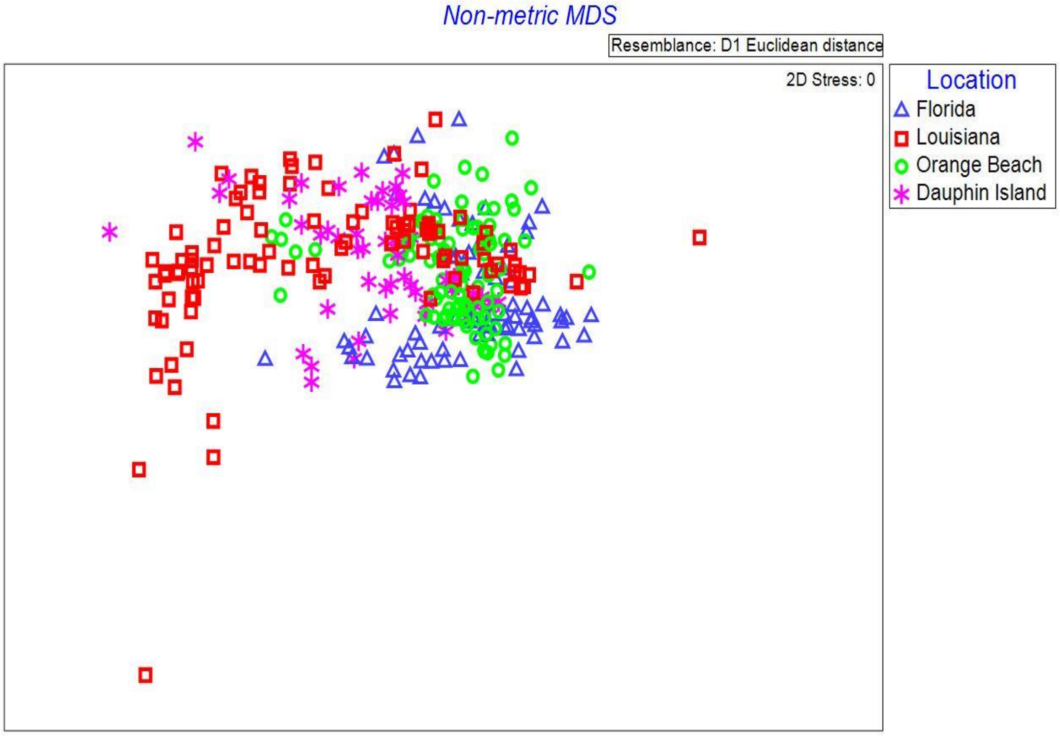


Figure B.16 MDS plot raw $\delta^{15}N$ values by sampling location based on Euclidean distance dissimilarity for all species by location.

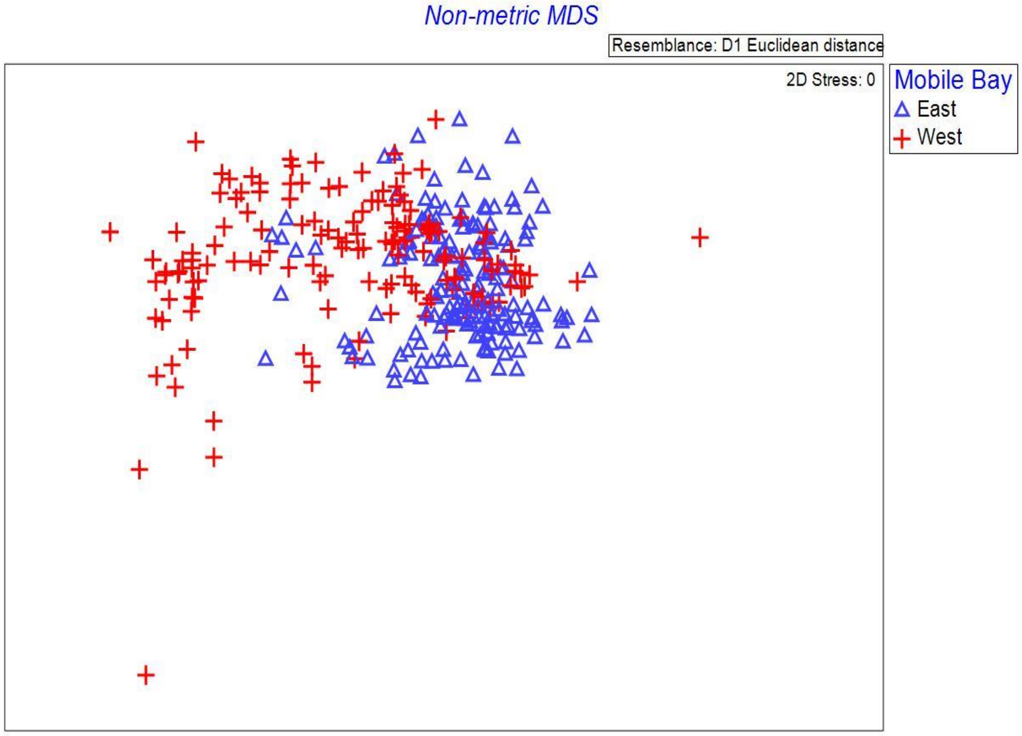


Figure B.17 MDS plot of raw δ^{15}N values by region based on Euclidean distance dissimilarity. Louisiana and Dauphin Island are labeled west of Mobile Bay, while Orange Beach and Destin Florida are labeled as east of Mobile Bay.

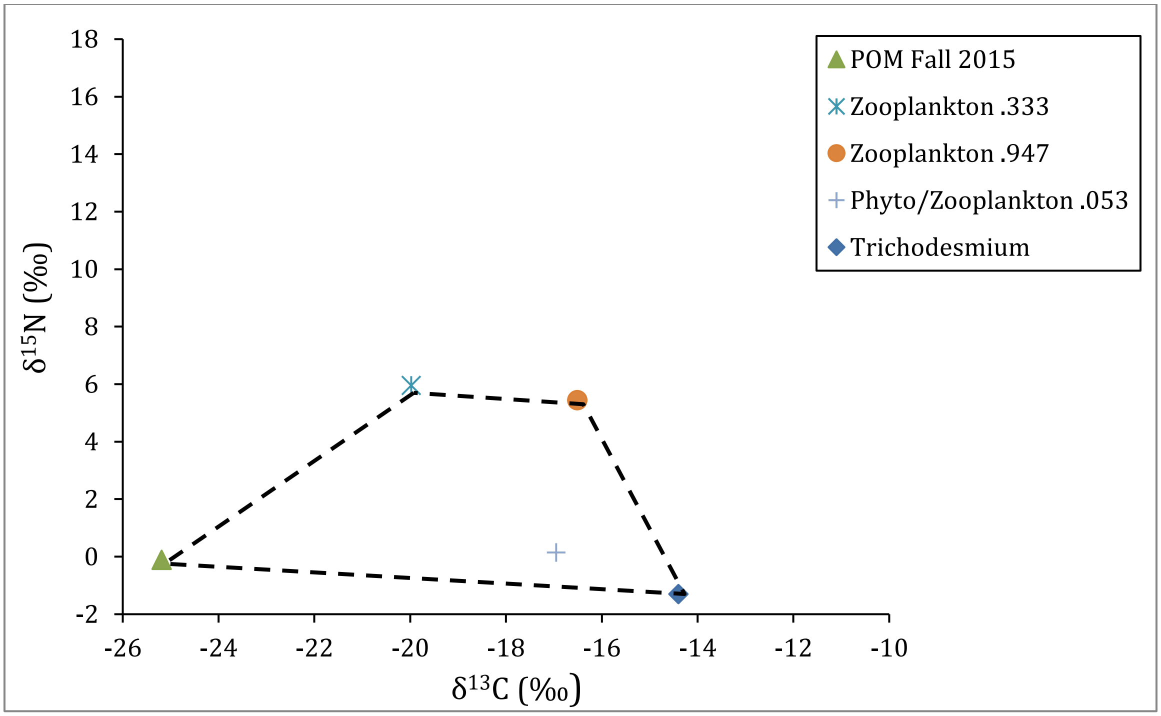


Figure B.18 Mixing polygon created from δ^{13}C and δ^{15}N of offshore producers. Plankton samples were collected on 2015 SEAMAP surveys. Value for offshore Trichodesmium found in Dorado et al. 2012

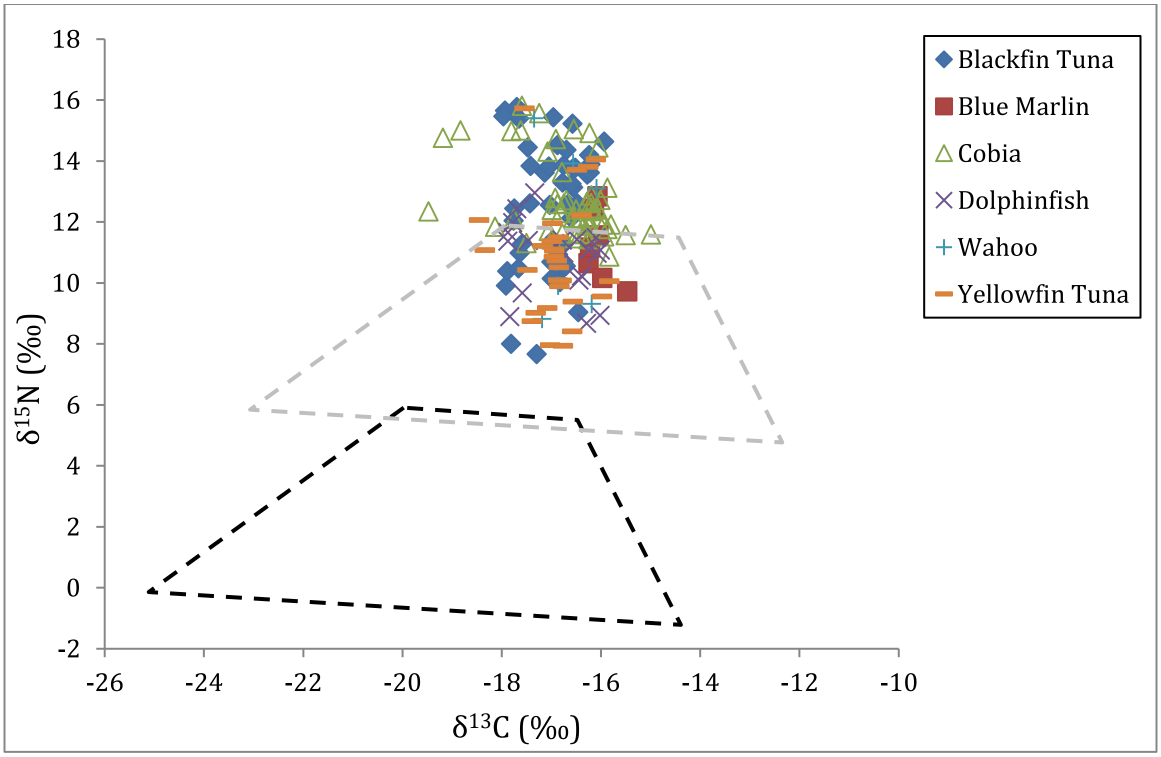


Figure B.19 Mixing polygon of δ^{13}C and δ^{15}N values including individual pelagic species. Gray polygon represents a tropic increase of two levels.

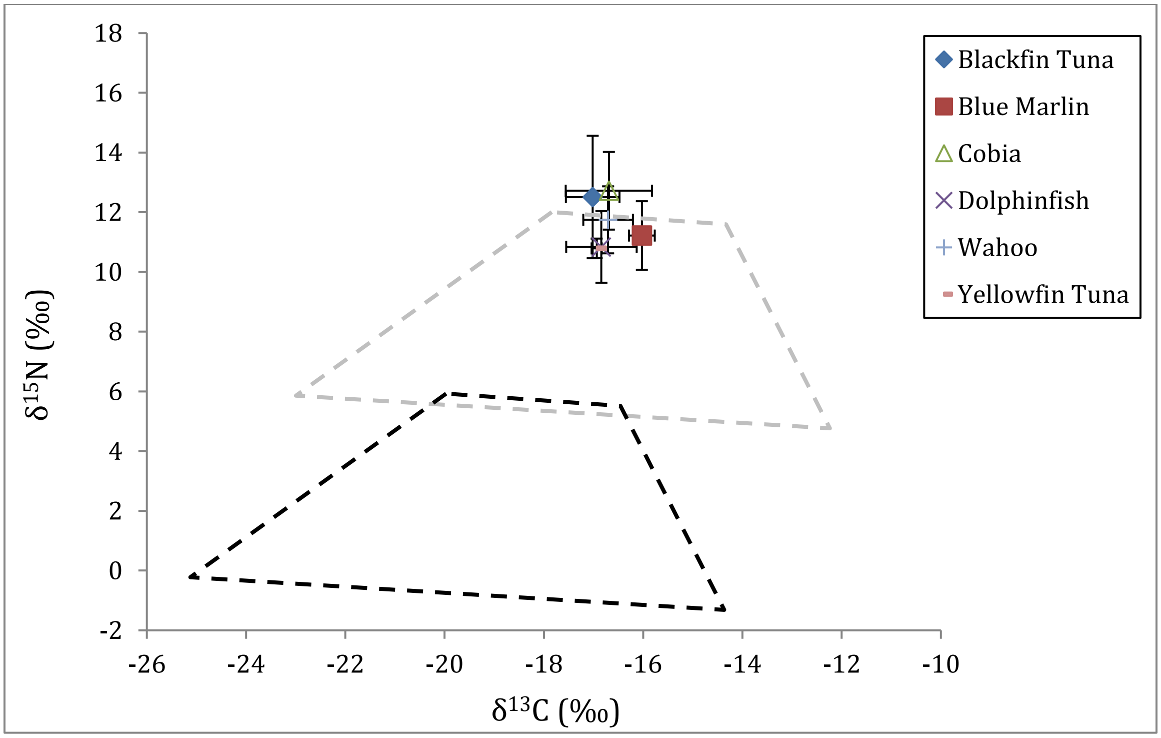
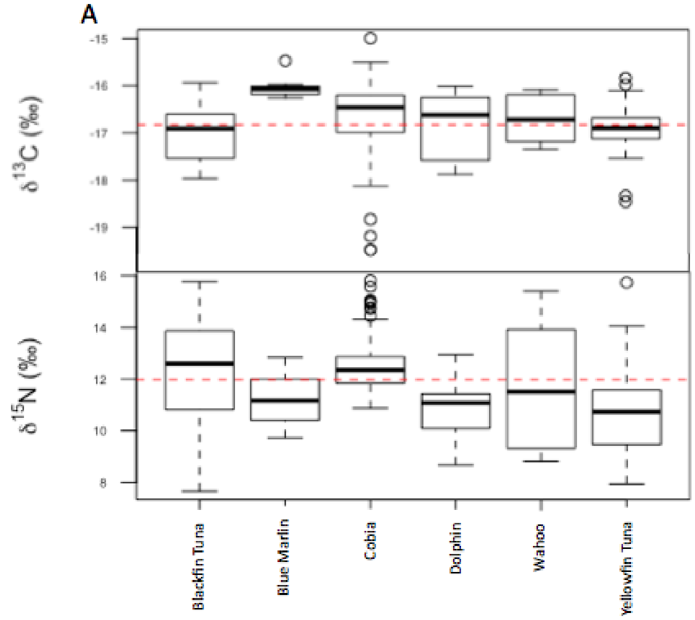


Figure B.20 Bipolot of mean δ^{13}C and δ^{15}Nvalues for each pelagic species. Bars represent standard error.



B

Species	Mean $\delta^{13}C$±SE	Mean $\delta^{15}N$±SE
Blackfin Tuna	-17.0±0.1	12.5±0.3
Blue Marlin	-16.0±0.1	11.2±0.4
Cobia	-16.7±0.1	12.7±0.2
Dolphin	-16.8±0.2	10.8±0.3
Wahoo	-16.7±0.2	11.7±1.1
Yellowfin Tuna	-16.9±0.1	10.8±0.3

Figure B.21 Figure B.22. Boxplot of $\delta^{13}C$ and $\delta^{15}N$ (B.21A) and table of mean values with standard error (B.21B).

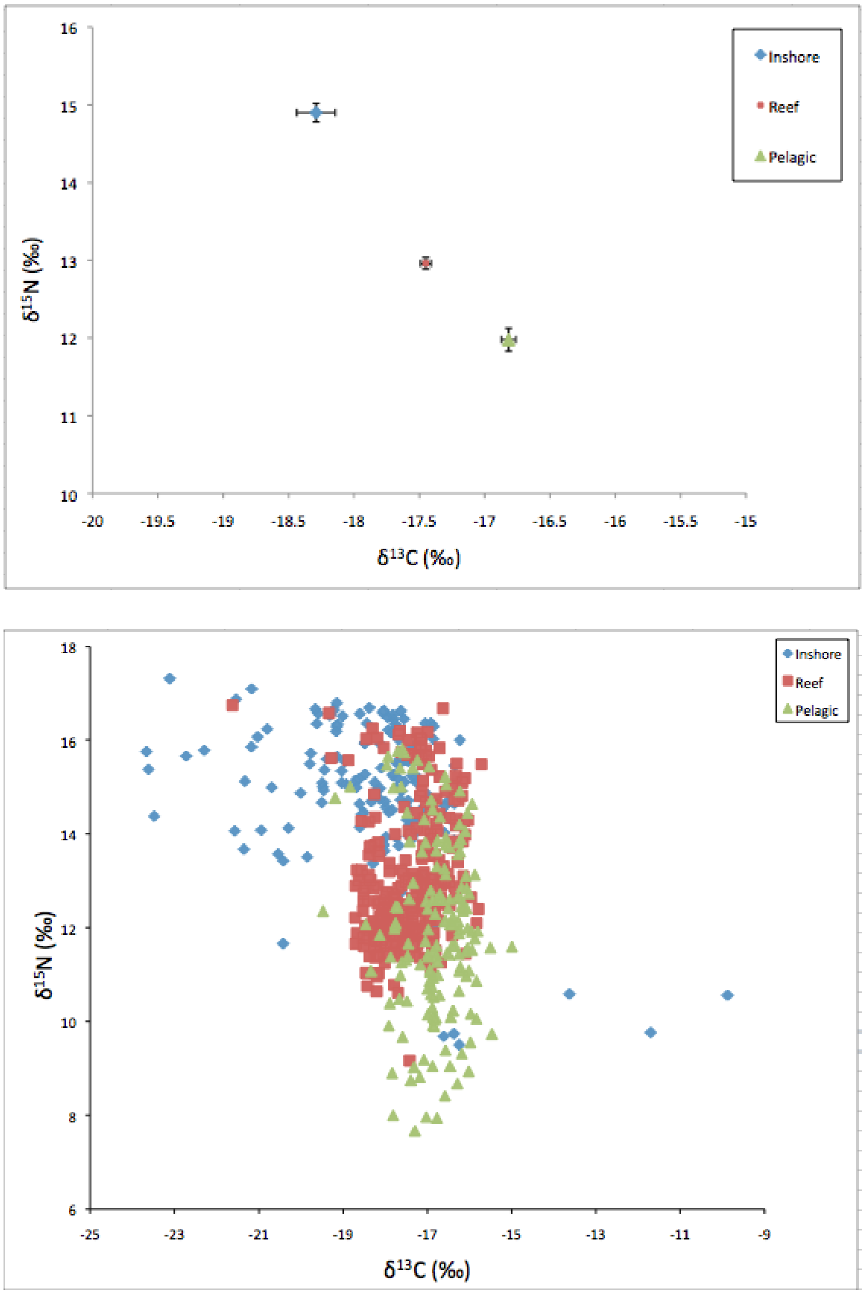


Figure B.22 Biplots of all target species, and meanδ^{13}C andδ^{15}N values by guild with standard error. The depletion in nitrogen and enrichment in carbon illustrates a shift in basal carbon and nitrogen resources from inshore to offshore environments

APPENDIX C – SUPPLEMENTAL BIPLOTS OF ALL SPECIES

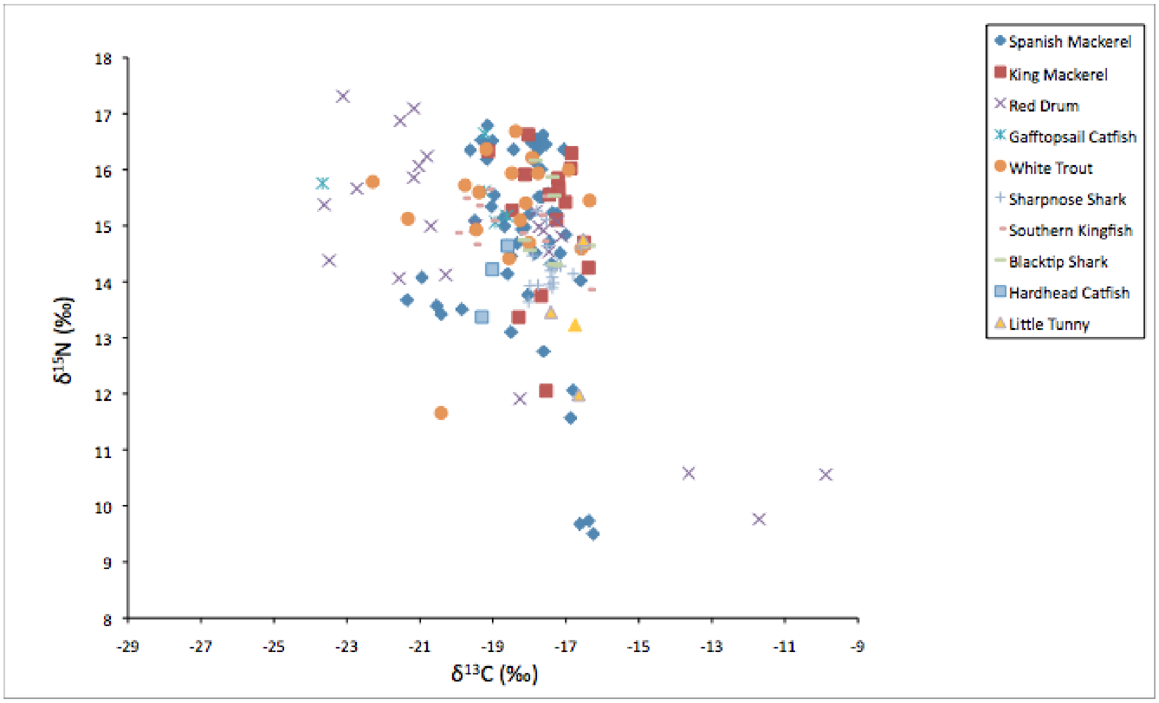


Figure C.1 Non-target species were those with less than the minimum sampling volume to conduct meaningful statistical operations. For the Mississippi Sound and Barrier Islands, this was any species with less than n = 5. Data for all species, including non-target species, is in Table 2

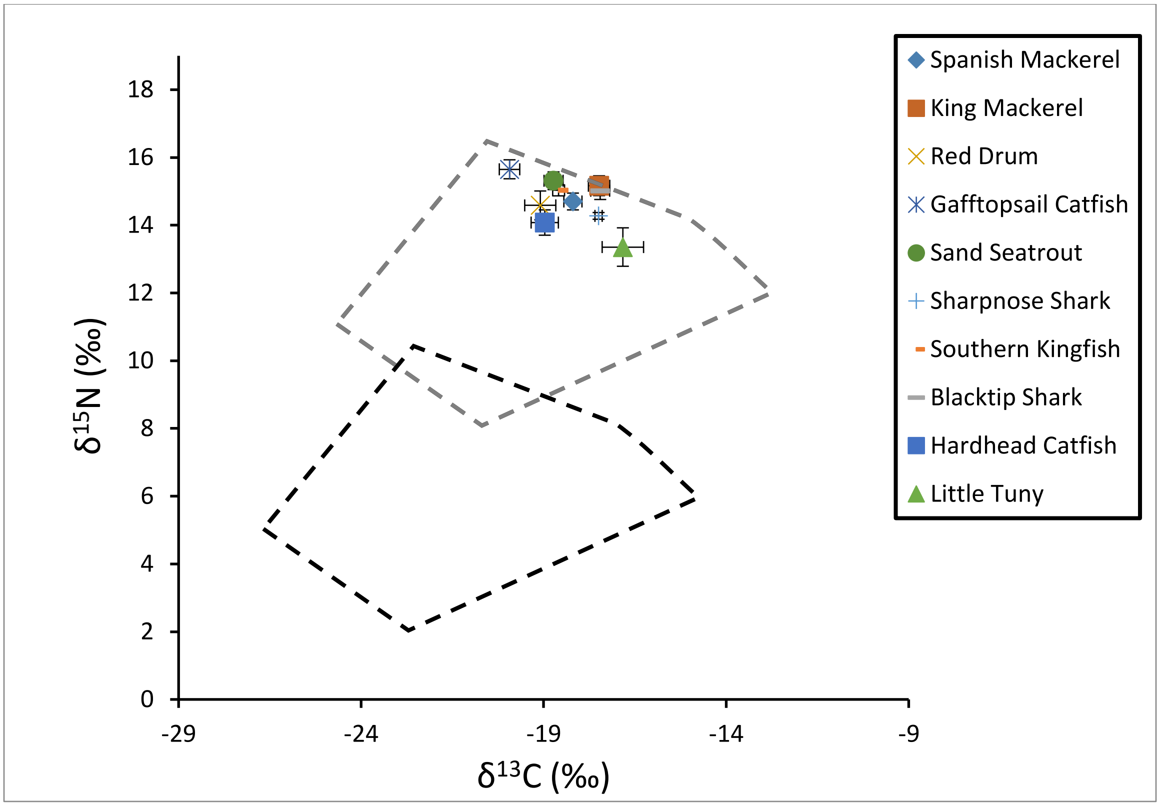


Figure C.2 Biplot of mean δ^{13}C and δ^{15}N values with standard error for all Mississippi Sound species, including non-target species. Dashed polygons represent potential producers, and producers shifted 2 trophic levels.

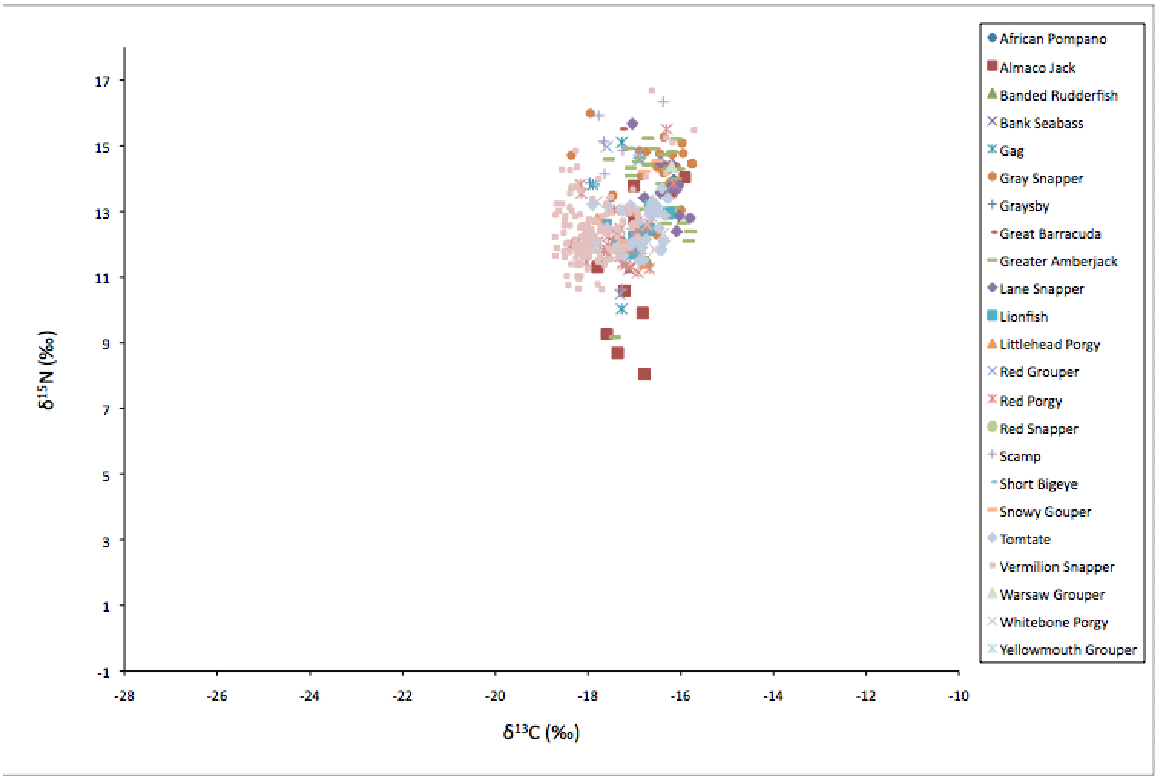


Figure C.3 Biplot of δ^{13}C and δ^{15}N values for all reef species, including non-target species

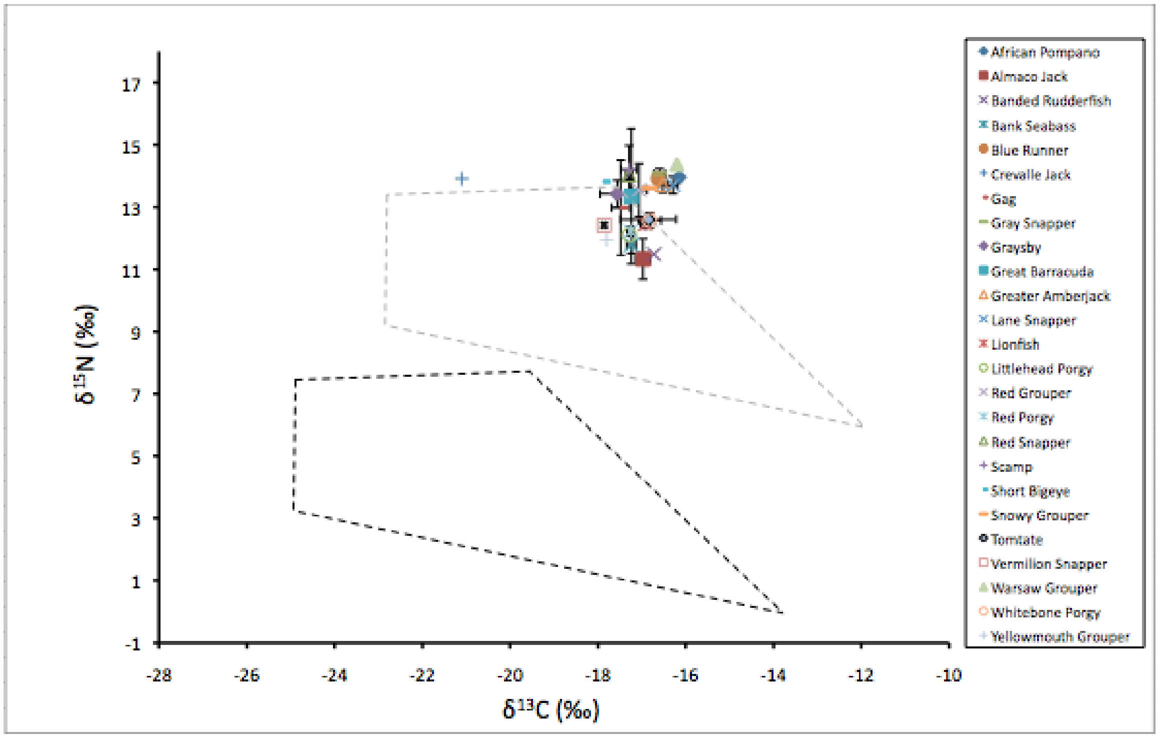


Figure C.4 Biplot of mean δ^{13}C and δ^{15}N values with standard error for all Reef species, including non-target species. Dashed polygons represent potential producers, and producers shifted 2 trophic levels.

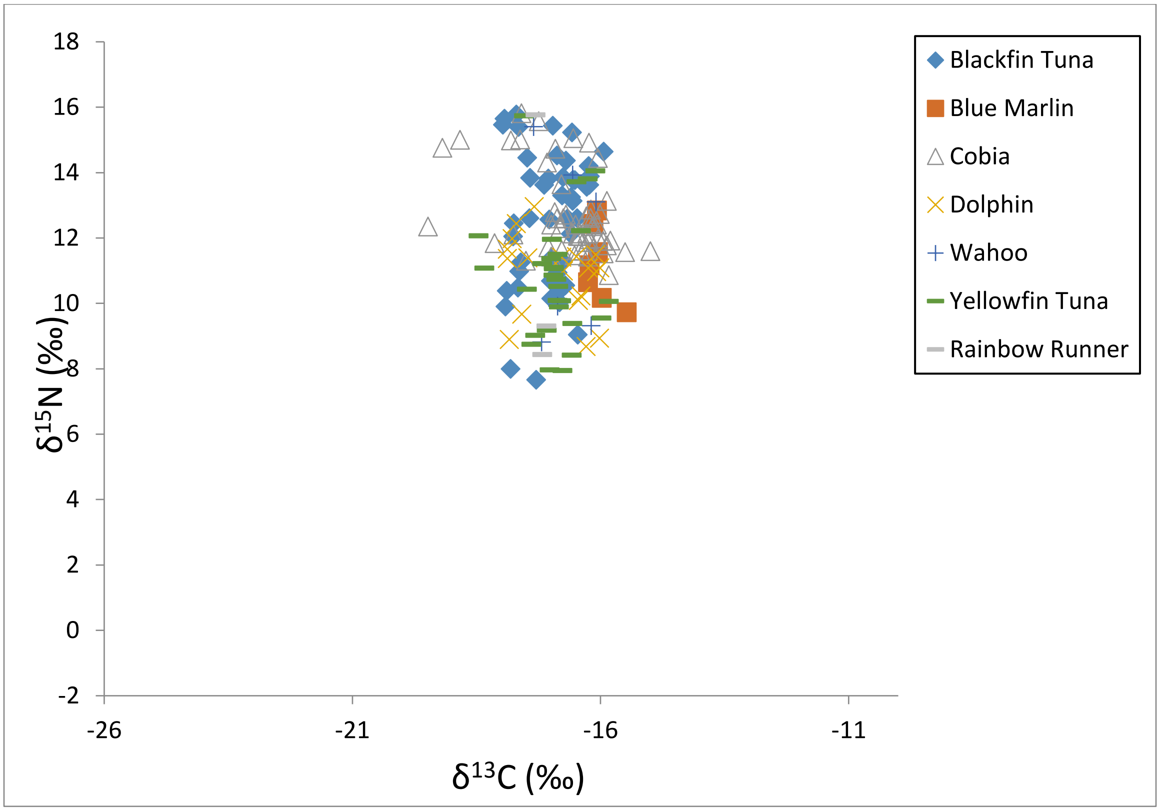


Figure C.5 Biplot of $\delta^{13}C$ and $\delta^{15}N$ values for all pelagic species, including non-target species.

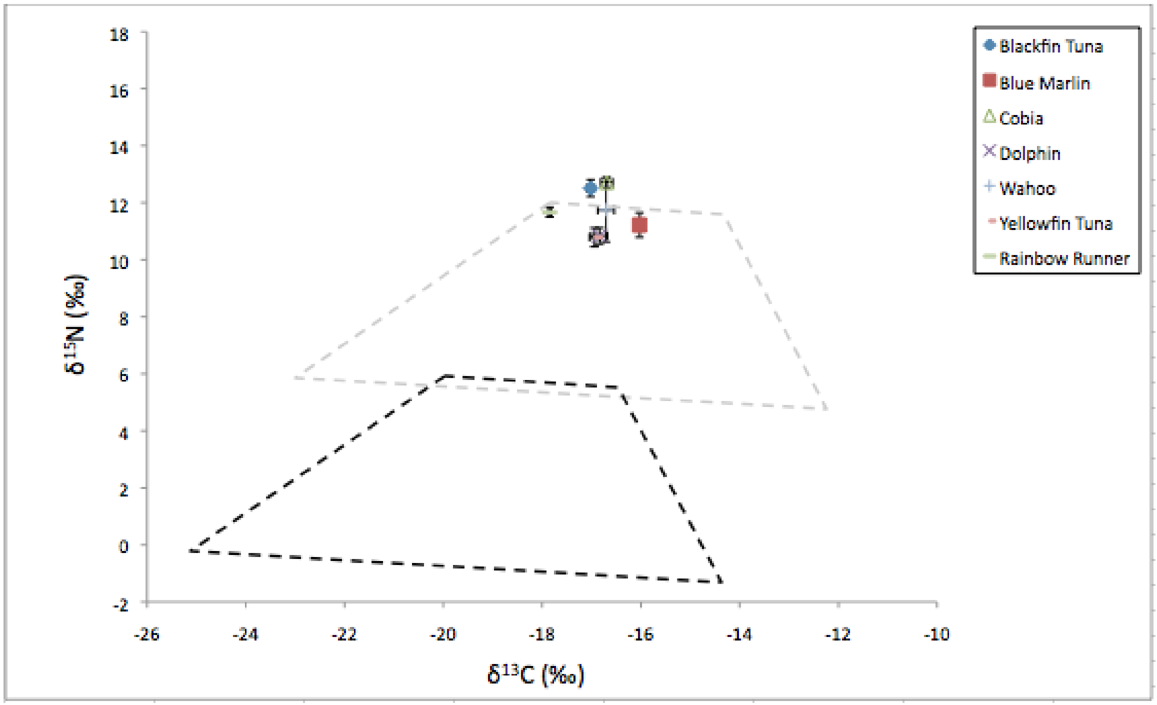


Figure C.6 Biplot of mean δ^{13}C and δ^{15}Nvalues with standard error for all pelagic species, including non-target species. Dashed polygons represent potential producers, and producers shifted 2 trophic levels.

Table C.2

Prey items for each target species with references. Isotope values are in ‰ and include standard errors

Trophic Guild	Prey Items	Reference	δ^{13}C (Mean + SD)	δ^{15}N (Mean + SD)
Pelagic Fishes				
Yellowfin Tuna	*Auxissp, Brevoortiapetronus, Caranxcrysos, Mugil cephalus, Micropogoniasundulatus,* exocoetids. Cephalopods, Malacostracan decapods.	Franks et al. 2016	-16.9 ± 0.57	10.8 ± 1.83
Blue Marlin	Scombrid, Carangid, and Coryphaenid fishes.	Davies and Bortone 1976	-16.0 ± 0.26	11.2 ± 1.15
Wahoo	Scombrid, exoceoetid, elupeid, earangid, balistid fishes.	Manooch and Hogarth 1983, Franks et al. 2007	-16.7 ± 0.52	11.7 ± 2.75
Dolphin	Balistids, crustacea, carangids, exocoetids, syngnathids, diodontids	Manooch et al. 1984	-16.9 ± 0.72	10.9 ± 1.18
Blackfin Tuna (Caribbean)	Engraulid, clupeid, carangid fishes, mysid, stenopodid, portunid, euphausid crustaceans, loliginid cephalopods.	Headley et al. 2009	-17.0 ± 0.59	12.5± 2.05
Cobia	Portunid, ovalipid, decapodid crustacea, hardhead catfish, sea robins, round scad, dwarf sand perch, loliginid and octopodid cephalopods.	Meyer and Franks 1996	-16.7 ± 0.86	12.7 ±1 .28
Reef Fishes				
Red Snapper	Fish, crab, squid.	Wells et al. 2008	-17.3 ± 0.72	14.0 ± 1.51
Vermilion Snapper (Atlantic)	Pelagic crustacea, squid, pelagic gastropods, fish, misc. invertebrates.	Grimes 1979 Sedbury & Cuellar 1993	-17.9 ± 0.55	12.4 ± 0.94
Red Porgy (Atlantic)	Majid, portunid, calappid crabs, fish higher in winter, echinoderms.	Manooch 1977	-17.3 ± 0.50	12.2 ± 0.85

Tomtate (Atlantic)	Polychaetes, benthic crustacea including copepods, stomatopods, cumaceans. mollusks, algae	Sedbury 1985	-16.9 ± 0.41	12.6 ± 0.63
MS Sound Fishes				
Red Drum	Decapodid shrimp, callinectid crabs, fish, penaeid shrimps, stomatopods – Fish and penaeid shrimps higher in winter.	Overstreet and Heard 1978	-18.9 ± 3.02	14.6 ± 0.43
Sand Seatrout	Penaeid, sergestid, caridean shrimps, callinectid crabs, fish and fish parts.	Overstreet and Heard 1982	-19.2 ±2 .34	15.0 ± 0.51
Sharpnose Shark	Teleost fishes including clupeids, ariids, engraulids, scombrids. penaeid, stomatopod and portunid crustaceans.	Hoffmayer and Parsons 2003	-17.4 ± 0.43	14.2 ± 0.60
Blacktip Shark	Teleost fishes including clupeids, stromateids, soleids, sciaenids, triglids. Penaeid crustaceans, and unidentified chondricthyes.	Hoffmayer and Parsons 2003	-17.4 ± 0.55	15.0 ± 0.73
Gafftopsail Catfish	Organic debris, crabs, fish including menhaden and worm eel. Small *Callinectes sapidus*, six crab species, 11 fish species (Port Aransas). Amphipod *Ampelisca abdita*, unidentifiable fish, *Farfantepenaeu sduorarum*, unidentified crabs, *Callinectes sapidus* (Florida).	Miles 1949 Reid et al. 1956 Odum and Heald 1972 Rudershausen and Locascio 2001	-19.9 ± 2.10	15.6 ± 3.77
King Mackerel	Teleost fishes, predominantly carangids, clupeids. Small amounts of penaeid shrimp.	Devane 1978 Moore 2014	-17.2 ± 0.65	14.4 ± 1.94
Spanish Mackerel	Engraulid, clupeid, and carangid fishes. Penaeid shrimps and loliginid squid.	Saloman and Naughton 1983	-18.1 ± 1.12	14.4 ± 1.94

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