Nueces Delta Wetland Functionality Study

by

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TABLE OF CONTENTS

Introduction

The Nueces estuary has endured significant physical damage including the mining/removal of the entire hard-bottom that formerly consisted of oyster shell, as well as pipeline construction throughout the Nueces Bay. These modifications have changed the Bay from a system having a hard bottom, to one having wind-driven sediment resuspension of flocculent sediments.

The Nueces Estuary has also undergone tremendous changes in water delivery during the past 100 yrs (Hill et al. 2011), including the Calallen Diversion Dam in 1989, La Fruta Dam (1929 rebuilt in 1935) that was replaced by Lake Corpus Christi (1958), and Choke Canyon Reservoir in 1982. Collectively these impoundments have reduced flows to the estuary by more than 50% since the 1980s. Mitigation efforts have included pass through agreed orders from the Texas Commission on Environmental Quality to deliver up to 3,000 acre-feet of water daily to the Rincon Bayou.

A major concern with this legislation is that sufficient flow reaches the marsh community to foster both numerical and healthy populations of recreationally and commercially important fish and shellfish. Vascular plant communities provide substrate to stabilize the marsh soil, and produce significant carbon for detrital foodweb pathways. Based on recent work by Montagna et al. (2017) the current water quantity is not sufficient to maintain marsh plant health.

Primary productivity enters aquatic food webs either directly via grazing on phytoplankton or other aquatic producers, or indirectly through the detrital food web which relies on secondary producers (usually invertebrates) to convert detrital material to usable animal biomass that can be transferred to higher trophic levels. Functionality of estuaries depend on stability of food resources to support higher trophic levels. The movement of naturally occurring isotopes such as carbon, nitrogen, and sulfur through the food web provides a mechanism to identify source material for food webs. The weight of the more common isotope form is less than the more rare heavier isotopes, causing selective enzyme/chemical reactions to favor the lighter isotope being incorporated into tissue. This approach has been widely used to assess food web coupling. Previously, Riera et al. (2000) tracked brown shrimp growth in the Nueces Estuary from 11 mm to 90 mm size during December- July and identified a detrital/benthic diatom pathway as providing carbon and nitrogen needed for shrimp maturation using isotopic ratios. Typically nitrogen isotopes are used to evaluate trophic relationships, as there is an isotopic enrichment of ~3-5 for each trophic transfer.

The one problem with isotopic analyses is that the food resources are collected typically on the same scale as that of the consumers. If the food web is dependent on microalgae, their successional pattern and doubling rate is much faster than the food webs supported by them. Consequently, changes in microalgal composition may occur during previous weeks, resulting in a confounding interpretation of the food web. This was recently circumvented in the analysis of pigments, gut contents in porcelain crabs (Zimba et al. 2016). Jackson et al. (2011) developed a Bayesian modelling approach to statistically compare higher trophic levels

independent of the basal food sources. This approach (SIBER) was used to help deconvolute our interpretation of the food web in Nueces Bay. An alternative method SISUS (statistical model for stable isotope sourcing) was used to assess what sources of of food proportionally contribute to a consumer's diet (Plotting was done using R-studio software.

METHODS

Four sites were selected for sampling in the Nueces Estuary to identify variation in food resources in the estuary (Figure 1). These included Rincon Bayou (the former Nueces River main channel-RB), the Delta Access Channel (DAC), and a site in Nueces Bay (NB).

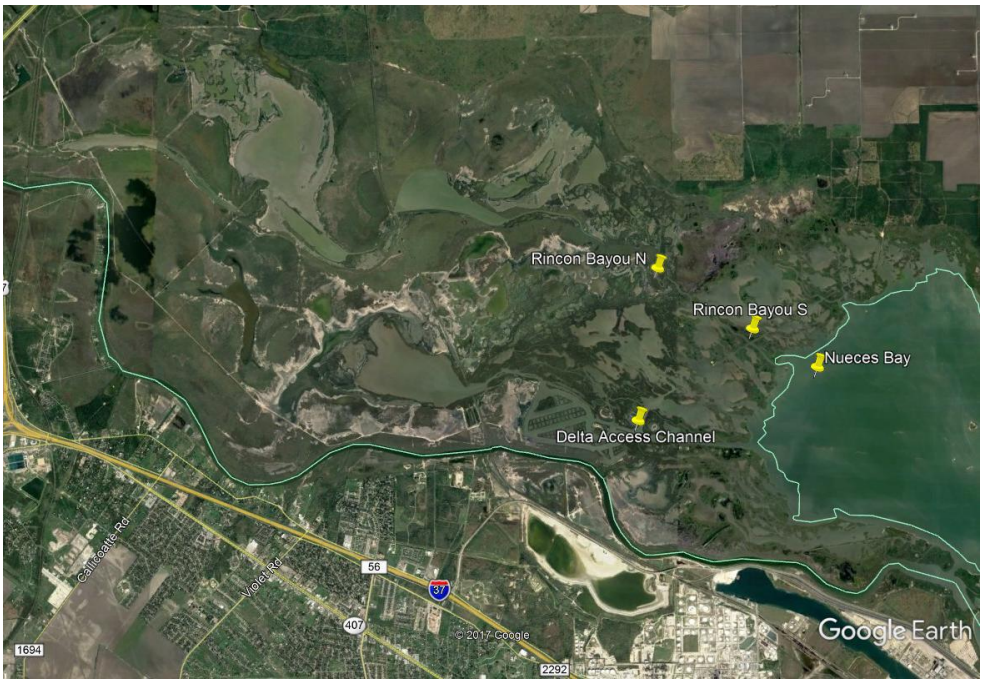

Figure 1. Location of Nueces Estuary sampling sites.

Sampling dates were chosen to capture breeding seasons during the year. Emphasis was on Spring (February), late Spring (May), late Fall (October). Sampling dates were 2/15-2/19/2016, 5/23-5/27/2016 and 10/27-11/3/2016).

Sampling Design

Samples were collected for primary producers, benthic invertebrates, and fish. The methodology for each is described below.

Primary producers were collected from each site. In the DAC and RB aboveground vascular plant samples were collected from the dominant vegetation (*Batis maritima* and *Salicornia virginica*). Samples were dried at 60C, then ground. Benthic algal mats consisting largely of cyanobacteria and benthic diatoms were collected, rinsed to remove sediment inorganics, then dried at 60C. Phytoplankton samples were collected by pumping water through a 7 micrometer mesh net. At each site we filtered 3,584 L of water. Net retained material was concentrated by centrifugation (1500g x 10 minutes). Samples were examined visually and inorganic (sand/silt) was removed by a timed settling procedure and evaluation microscopically. Clean phytoplankton samples were dried in a freeze dryer, and homogenized using a mortar and pestle.

Zooplankton was concentrated in the field using essentially the same procedure as used for phytoplankton. Water for phytoplankton samples were prefiltered with a75 micrometer net. Zooplankton samples were observed under a dissecting microscope (10-65x) and contaminants were removed. Zooplankton samples were dried in a freeze dryer, then homogenized with a mortar and pestle.

Benthic algal mats were sampled from each site by collecting multiple trays if sediment (each 3 cm deep x 25 cm x 55 cm). Nitex cloth was overlaid the sediment surface (75 micrometer), with a surface second layer of 7 micrometer used to harvest motile algae. Sediment samples were incubated overnight on a !2:12 light:dark cycle at room temperature to enhance algal diurnal migration. The Nitex layers were removed from the sediment between 10:00 a.m.-2:00 pm. The netting was place in 0 ppt deionized water and the netting was stomached (kneaded) to release attached algae. The algal slurry was centrifuged to remove water, freeze dried, then ground with a mortar and pestle to homogeneity.

Benthic polychaetes and amphipods were collected using 10 cm cores. Forty-fifty cores were collected per site. Cores were hand-seived through 500 mm mesh screening to remove sediments, then each organism was identified using 10x magnification on a dissecting microscope and placed in labelled sample vials. Sufficient biomass of the dominant large polychaete species *Leonis culveri* and amphipod *Edotia trioba* were identified and analyzed for isotopic composition.

Other invertebrates such as comb jellyfish (*Beroe ovata*), water boatmen (Corixidae), fly larvae (Chironimidae) were separated to lowest taxonomic unit using a stereo microscope, then freeze-dried, and homogenized using a mortar and pestle. For the dwarf surf clam (*Mulinia lateralis*) muscle tissue was removed from each clam, freeze-dried, and homogenized.

Pelagic invertebrates (mysids-*Americamysis almyra*, brown shrimp (*Farfantepenaeus aztecus*), and white shrimp (*Litopenaeus setiferus*) were identified, divided by size (shrimp) in three sizes, freeze-dried, then homogenized with a bead-beater. Blue crabs (*Callinects sapidus*) had their carapaces removed, and muscle tissue was freeze dried, then homogenized.

Fish greater than 4 cm were identified and fillets were removed from muscle tissue and each fish was individually prossed for isotope analyses. For smaller fish, fish were filleted (>1 cm) and the muscle tissue combined to obtain sufficient biomass for analyses. For ≤1 cm fish, the entire fish was freeze dried and ground to homogenize samples.

Each sample was homogenized then subsampled for isotopic analyses. Sample weights were adjusted to optimize the elemental content being injected, meaning that the mass of each element had to fall within the optimal concentration within the regression curve for each element. Typically, this required ~5, 4, and 3 mg sample sizes to be accurately measured, the weight recorded, then the aluminum cups to be sealed (folded). Samples were then placed in 96 well plates. All samples were analyzed in duplicate to identify spurious sample results.

Solid materials were analyzed for ^{13}C and ^{15}N isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at University of California, Davis. Samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flowed through a water trap (magnesium perchlorate) and an optional CO_2 trap (for N-only analyses). N_2 and CO_2 were separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS.

Stable isotope ratios of ^{34}S in solid samples are measured using an Elementar vario ISOTOPE cube interfaced to a SerCon 20-22 IRMS (Sercon Ltd., Cheshire, UK). Samples are combusted at 1150°C in a reactor packed with tungsten oxide. Immediately following combustion, sample gases are reduced with elemental copper at 880°C and subsequently pass through a buffering reactor filled with quartz chips held at 900°C. SO_2 and CO_2 were then separated by purge and trap. Following separation, the SO_2 adsorption trap was heated and the sample SO_2 passes directly to the IRMS for measurement. Standards for each isotope were used to quantify isotopic composition, including the international standards VPDB (Vienna PeeDee Belemnite) three reference sulfur standards, and air for carbon, sulfur, and nitrogen, respectively.

Data were analyzed using R, a public statistical package. Data was first visualized using 1,2, and 3 factor plots, then analyzed using SIBER to assess foodweb relationships (Jackson et al. 2012). Finally, food sources accounting for the observed carbon, nitrogen, and sulfur values were compared using procedures defined in the program SISUS (Erhardt et al. 2014).

RESULTS AND DISCUSSION

Two trays were damaged in shipment, and required an additional set of preparation and mailing. A total of 158 samples were analyzed from this project. This include primary producers (16 discrete samples), particulate organic matter (2 samples), invertebrates (92), and the remainder fish (50 samples). The particulate organic matter samples were actually

phytoplankton-size material, but microscopically was determined to contain large detrital material.

Primary Producers

Primary producer C,N,S triplots (Figure 2) displayed several immediate features including a highly variable N ratio (+2 to +8). Producers included both C3 and C4 plants as evidenced by the values near -25 (C3) and -13 (C4).

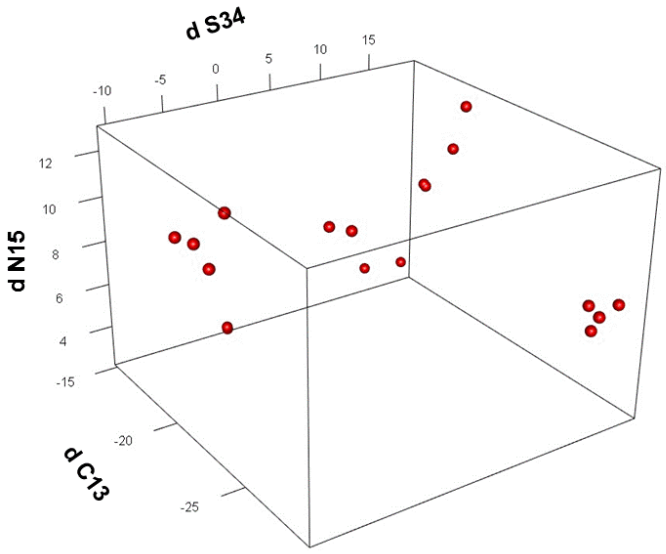

Figure 2. Isotopic tri-plot of carbon, nitrogen, and sulfur isotopic ratios for Nueces functionality study.

To make this more clear, Figure 3 shows a biplot of C,N ratios for the primary producers. The values near -27 are rooted salt marsh plants (*Batis, Salicornia)*, whereas phytoplankton and benthic algae fall between -15 to -22. The N values suggest that the phytoplankton are receiving new N, whereas the salt marsh plants and benthic algae had had nitrogen that has 1-2 levels through the food chain prior to their use. Average del N values for primary producers is 7.7.

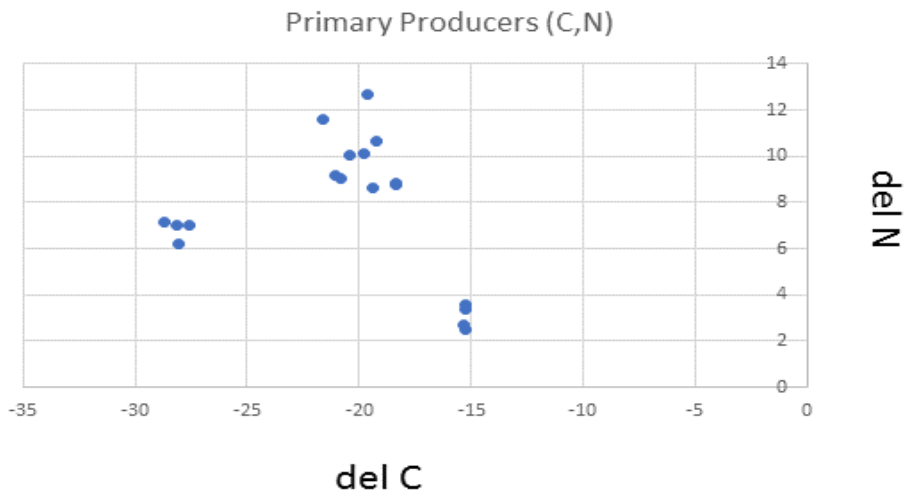

Figure 3. Isotopic biplot of del C and N ratios for the primary producers of Nueces Bay. Rooted macrophytes are C3 like with values above -25, whereas phytoplankton and algae are about -15 to -22.

The isotopic biplot of del C/S is presented in Figure 4. Low sulfur ratios were generally found in the aquatic samples- phytoplankton, benthic diatoms, and the particulate organic matter samples, with cyanobacteria/diatoms from the salt marsh having the next lowest values. One set of phytoplankton samples from the Nueces Bay had extremely high sulfur values, suggesting a different sulfur source.

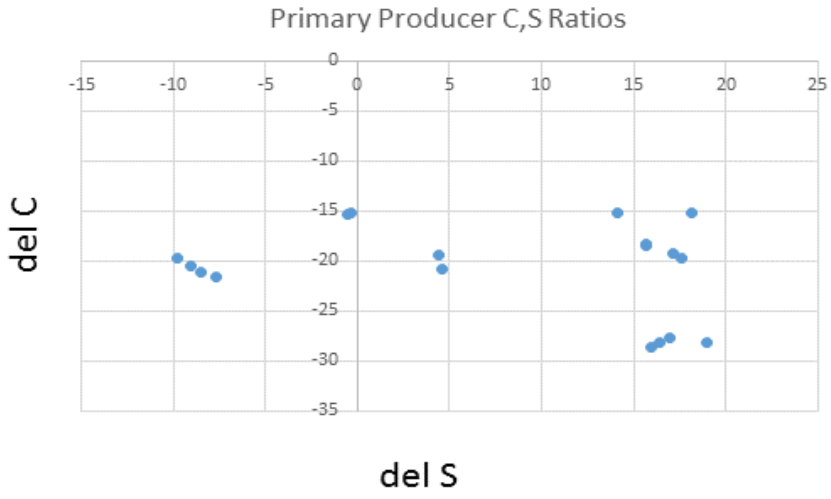

Figure 4. Isotopic biplot of del carbon and nitrogen ratios.

Invertebrate and Benthic Fish Isotopic Ratios

Isotopic triplots of invertebrates and small fish (sailfin mollys, silverside, goby) showed a range of del N ratios exceeding 2 foodweb levels (4-8-12), whereas most organisms are consuming carbon sources having del C ratios of -18 to -22 (Figure 5). Isotopic sulfur content ranges from 5-19, with most species less than 15 del S ratio.

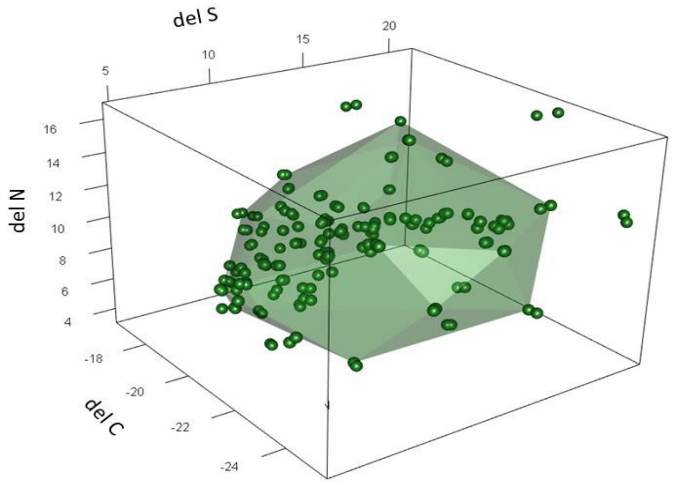

Figure 5. Isotopic triplot of del C,N, and S for invertebrate and small fishes from Nueces Bay

Closer analysis of the isotopic data using biplots provides clearer evidence of the range of food webs being consumed by this class. The large majority of organisms are close to a del N isotopic ratio of 10 (9.9 mean).

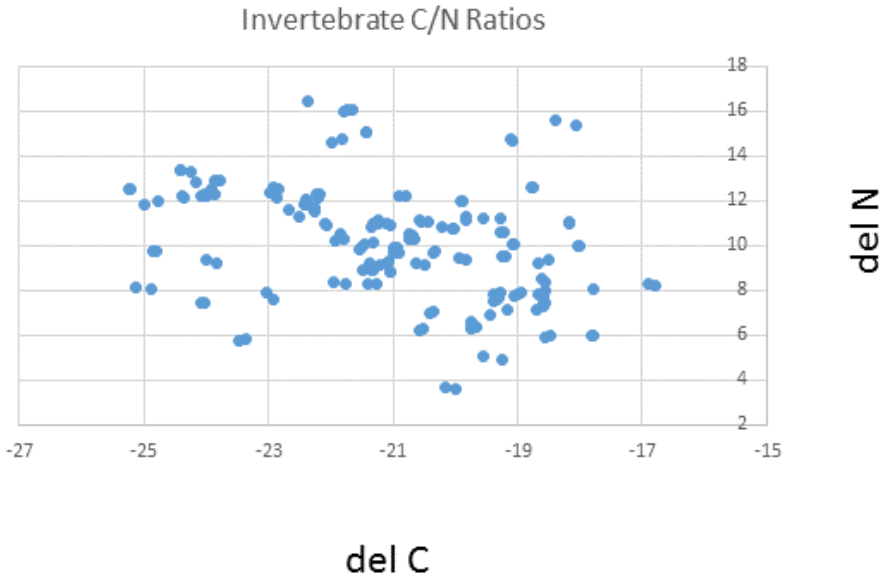

Figure 6. Isotopic biplot of del C and N ratios in invertebrates and small herbivorous fish.

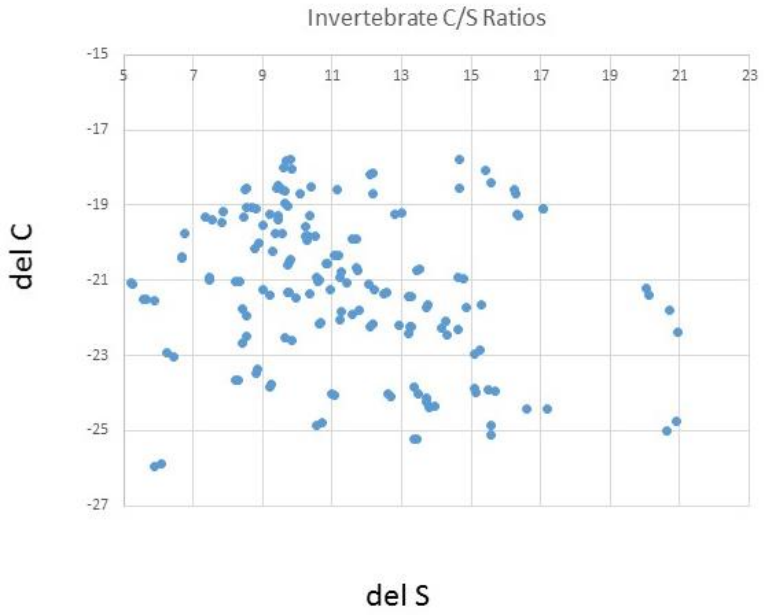

Figure 6. Isotopic ratios of del C and S for invertebrates.

Invertebrates would be expected to feed primarily on the primary producers and the highest del S ratios were in zooplankton (~20) which compares with phytoplankton at 19. The benthic shellfish *Tellina alternata* had a similar trophic level as zooplankton, suggesting that the zooplankton species were largely benthic copepods. Mysids would graze largely on planktonic zooplankton, so values for residual zooplankton would be more like benthic taxa such as the detritivore-feeders like *Tellina* (Rothschild 2004). This is most obvious in the isotopic plot of N/S (Figure 7).

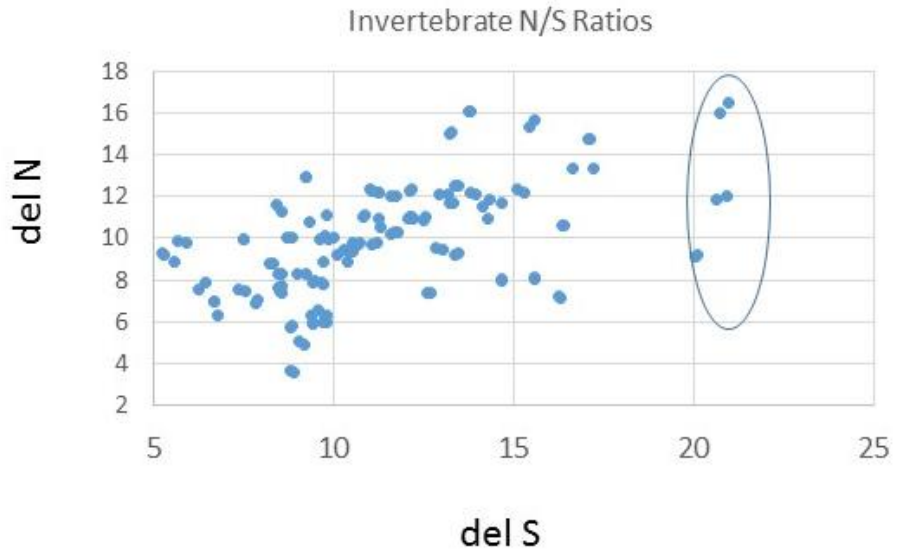

Figure 7. Isotopic biplot of del N/S ratios for invertebrates and small fish. Note the circle on the right side containing zooplankton and the benthic feeding shellfish *Tellina alternata.* These clearly are a unique feeding consortia relative to most animals in Nueces Bay.

Fish Isotopic Ratios

Triplot isotopic relationships are shown in Figure 8. It is immediately apparent that fish do not have any signal from the vascular plants, whereas values for phytoplankton, and benthic algae are in the middle of fish values (-18 to -22). N values ranged from the same as primary producers to 2 trophic levels above those values.

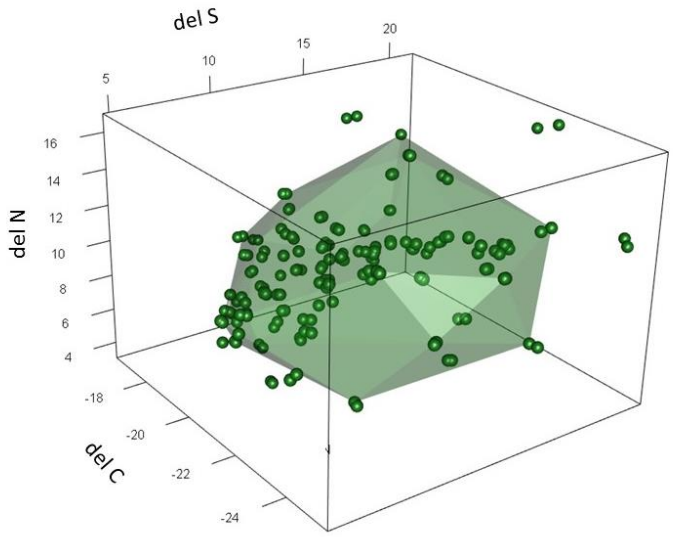

Figure 8. Isotopic triplot of fish collected from Nueces Bay.

These relationships can be better seen in biplots of carbon and nitrogen isotopes (Figure 9. Highest nitrogen values were found in gaftop catfish, red and black drum, and alligator gar, followed by spotted seatrout, hardhead catfish, croaker, and layfish. This order does not make sense at first glance, given published food preferences of these fish. It is likely this reflects the food being consumed (1st order) is affecting the second/third trophic ratios.

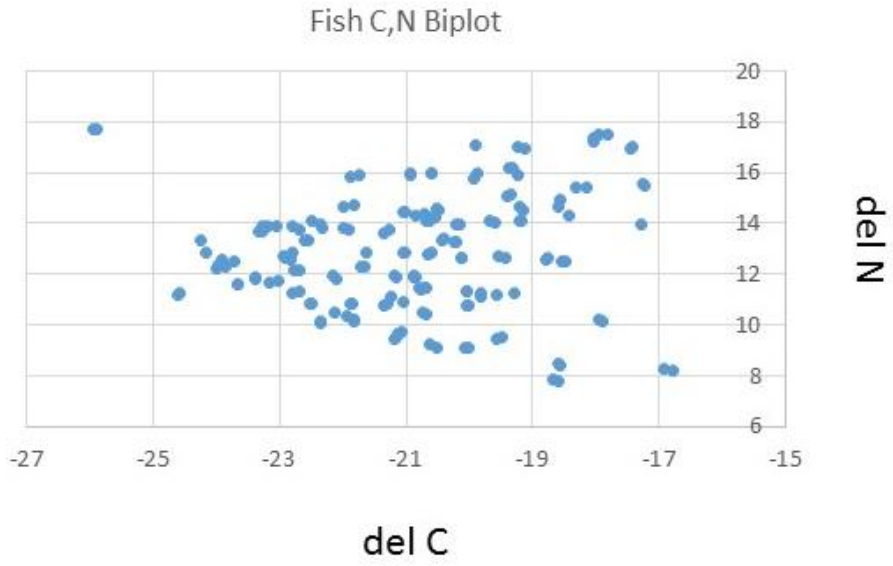

Figure 9. Isotopic biplot of fish carbon to nitrogen ratios in Nueces Bay.

An evaluation of carbon to nitrogen isoplots reveals some details (Figure 10). It is clear that most carbon is sourced from non-vascular plant sources. There are at least two trophic levels within the fishes, based on N increases from 8-17.

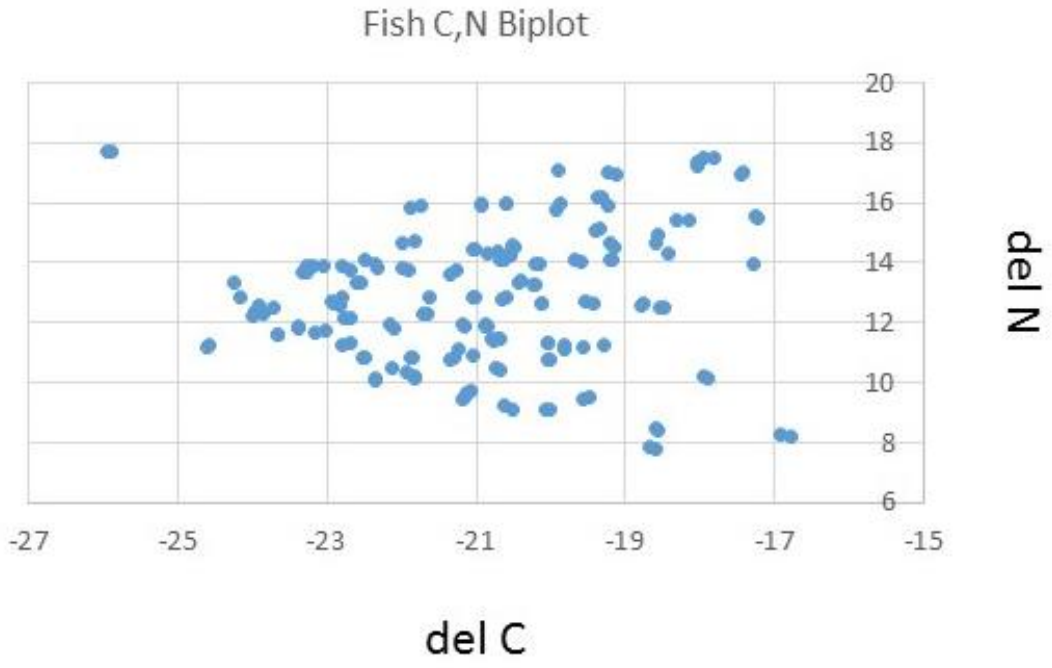

Figure 10. Fish del N to N isotopic biplot for Nueces Bay.

An assessment of fish C to S ratios (Figures 11) provides some better detail of the S sources to discriminate food sources. S values for fish fall into two classes centered at 10 and 14. This is useful in comparing the invertebrate sources that would serve as food for fishes at these levels (*Penaeus* at 14 and benthic invertebrates like *Mulinia*).

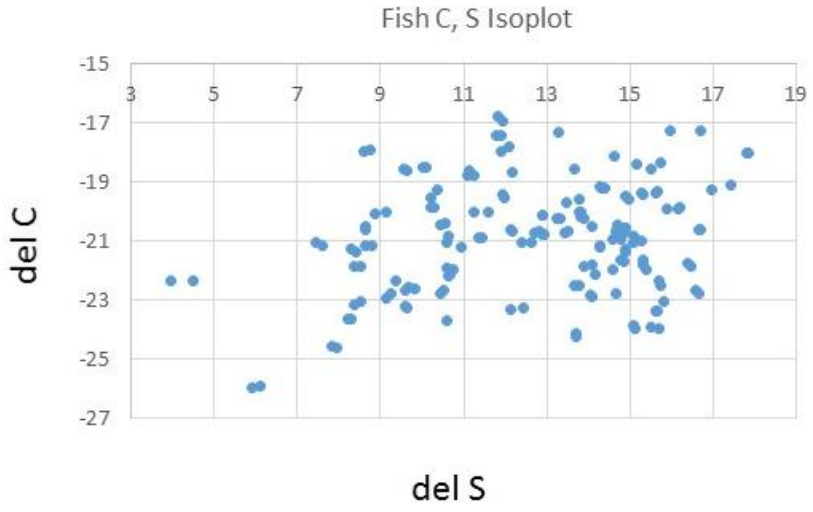

Figure 11. Fish carbon to sulfur isotopic biplot.

Analysis of the fish nitrogen to sulfur biplot is informative concerning the sources of food. There are clearly two groupings based on S values, with a use of lower food resources by half of the fish, and a higher trophic level of food resources by the other portion.

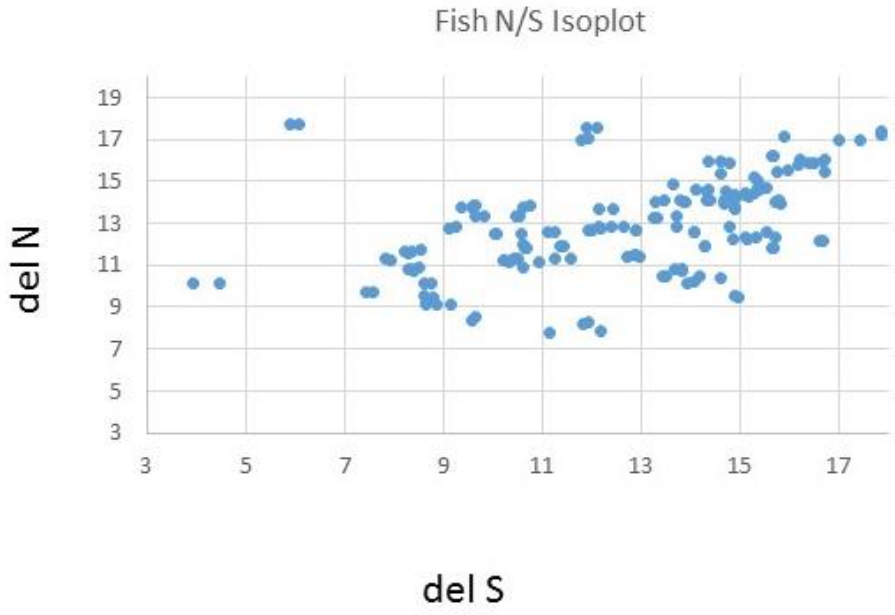

Figure 12. Fish isotopic plot of del N and S ratio for Nueces Bay.

Food Preference Based on Bayesian Analyses for Important Fish Species

An analysis of food sources accounting for observed isotopic signatures was calculated using Bayesian methods. For *P. cromis* (black drum), less than 20% of food is derived from the vascular vegetation (*Batis, Salicornia*), whereas POM and benthic algae account for over 60% of the food consumed (Figure 12).

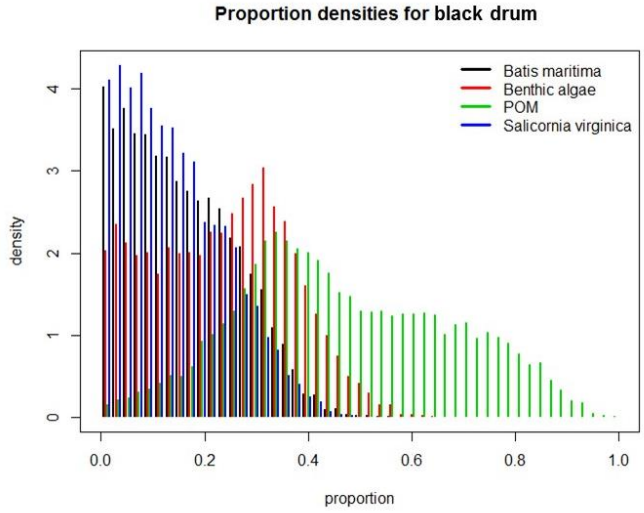

Figure 12. Food preferences for black drum (*P. cromis*) in Nueces Bay.

A similar plot was made for brown shrimp (*Farfantepenaeus aztecus)* and it clearly points to particulate organic matter as contributing 80% to the diet of these shrimp (Figure 13). *Salicornia* account for the second largest contribution to diet. POM can include both phytoplankton and benthic algae in Nueces Bay given the tychoplanktonic nature of the sediments. Mixing is strongly controlled by wind mixing/resuspension of sediments.

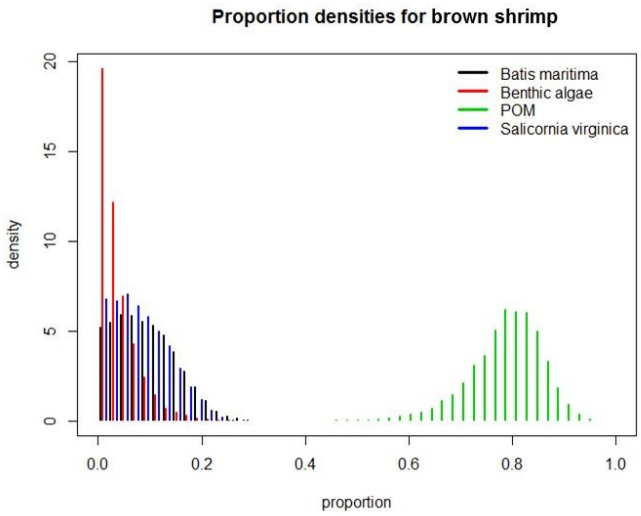

Figure 13. Brown shrimp diet elucidated by Bayesian isotopic analyses.

While small, there is a catfish fishery and in Nueces Bay this fish depends on particulate organic matter and benthic microalgae as the ultimate food resource (Figure 14).

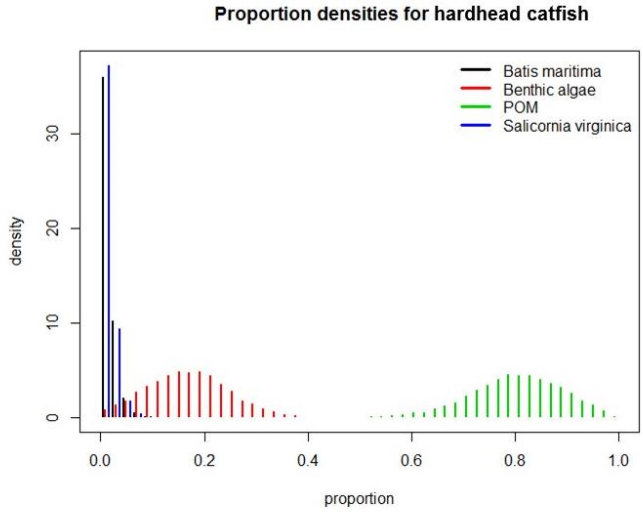

Figure 14. Dietary sources for hardhead catfish in Nueces Bay inferred from Bayesian analyses.

Mullet serve as a recreationally important fish, and are harvested for use in crabbing. Isotopic analysis of diets suggest that the benthic algae and particulate organic matter (POM) contribute over 70% to the diet.

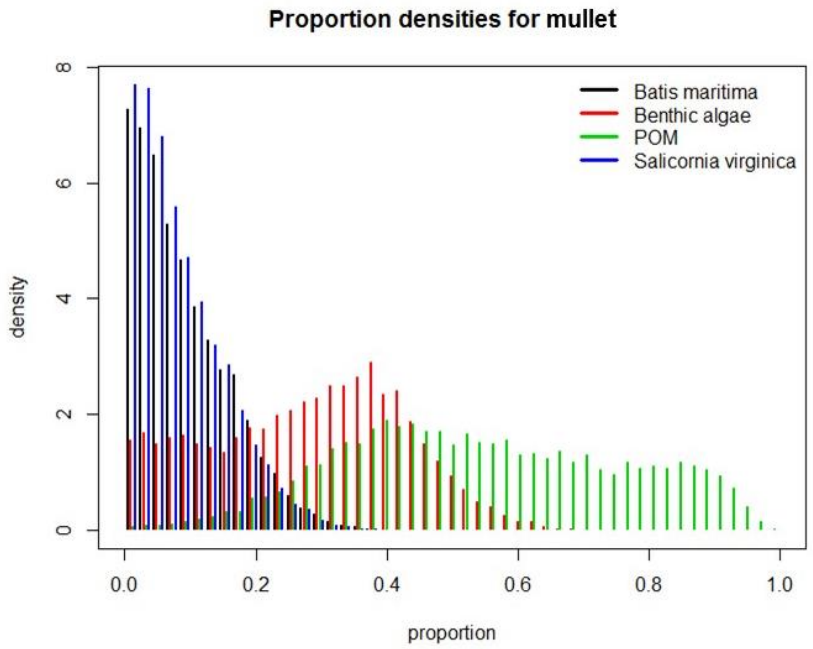

Figure 15. Dietary preferences of mullet in Nueces Bay as inferred from isotopic composition.

Red drum depend on POM and benthic algae for over 50% of the dietary needs based on isotopic mixing models (Figure 16). This is somewhat surprising compared to literature descriptions having their preferred diet as shrimp and other fish.

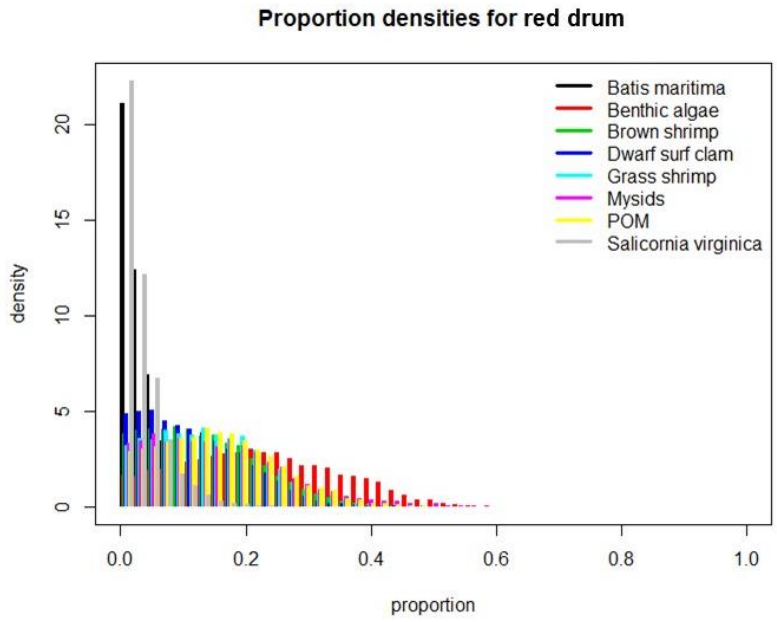

Figure 16. Red drum feeding preferences estimated using isotope dilution models for Nueces Bay.

Black drum invertebrate preferences included dwarf surf clam, grass shrimp, and brown shrimp. This strongly agrees with literature on this species (Figure 17).

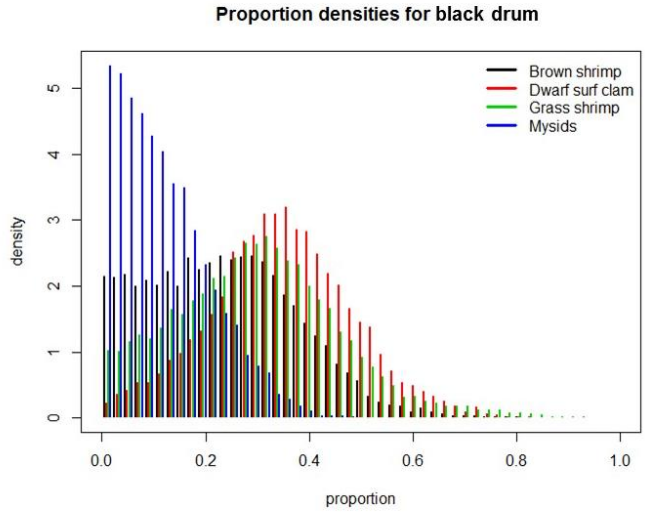

Figure 17. Dietary preference of hardhead catfish in Nueces Bay.

Hardhead catfish (Figure 18) showed marked preference for mysids (Figure 19). Grass shrimp were also a minor component of their diet.

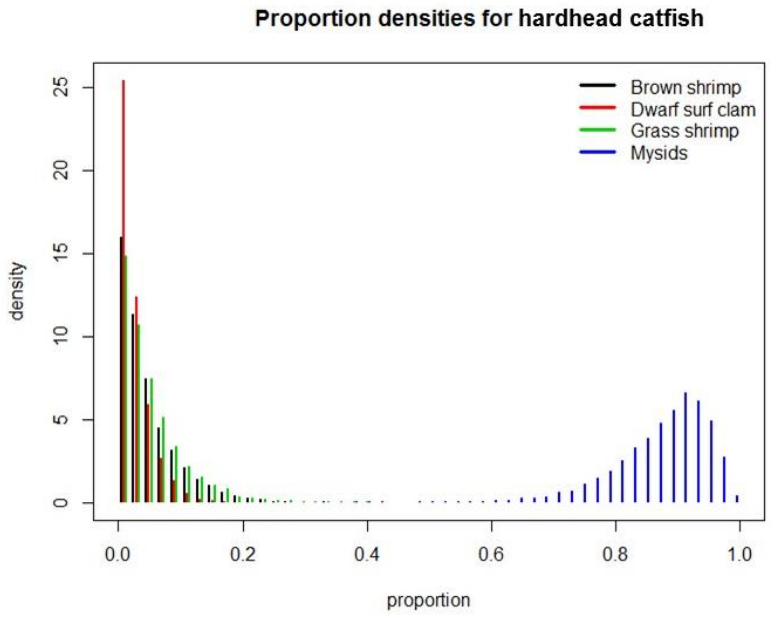

Figure 18. Hardhead catfish food preferences in Nueces Bay.

Convex hull data analysis using ACCUMEN/SUSIS approaches

The isotopic data has also independently analyzed using convex hull analyses. The results are shown in Figures 19a,b,c. In the plots for C, and N there is a strong indication of POM driving the dietary composition of the fish and shrimp. Food resources for the organisms are derived from particulate organic matter and benthic microalgae, rather than rooted salt marsh vascular plants.

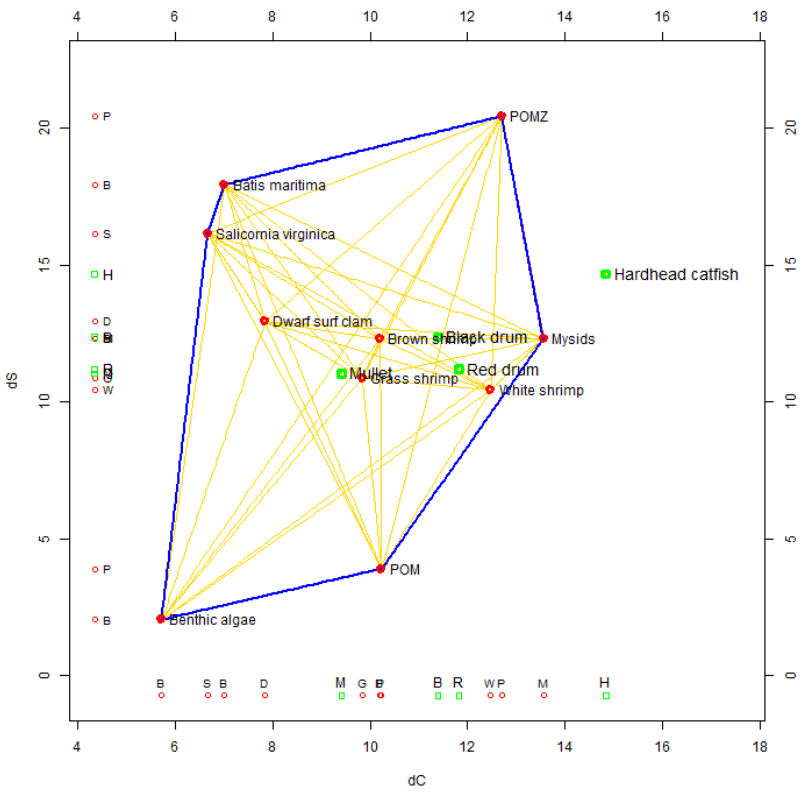

Figure 19A. C/N isoplot for major producers and consumers in Nueces Bay.

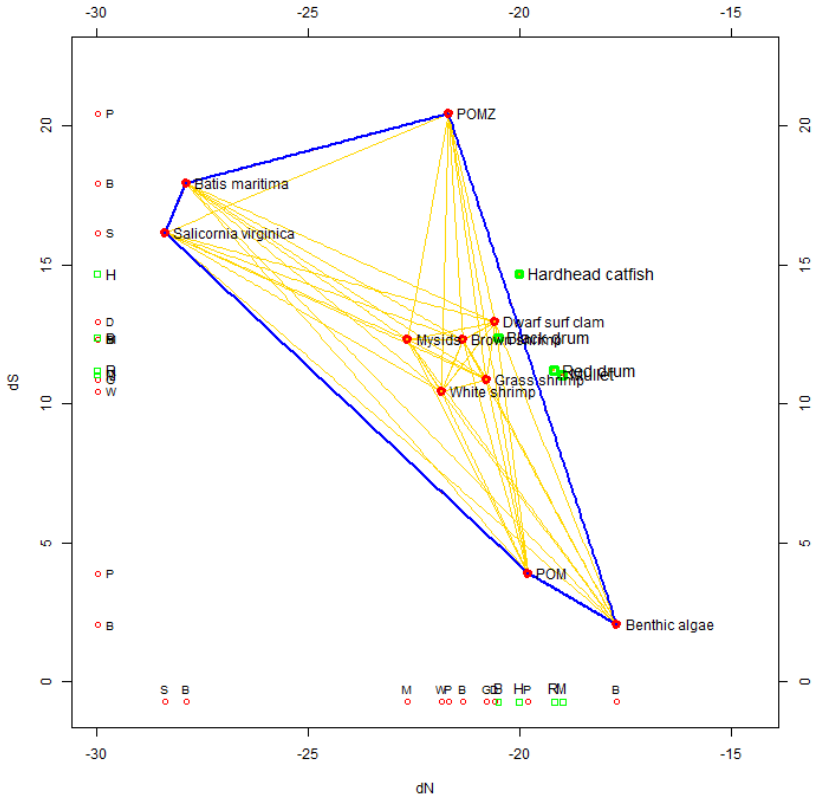

Figure 19b. N/S isoplot for major producers and consumers in the Nueces Bay.

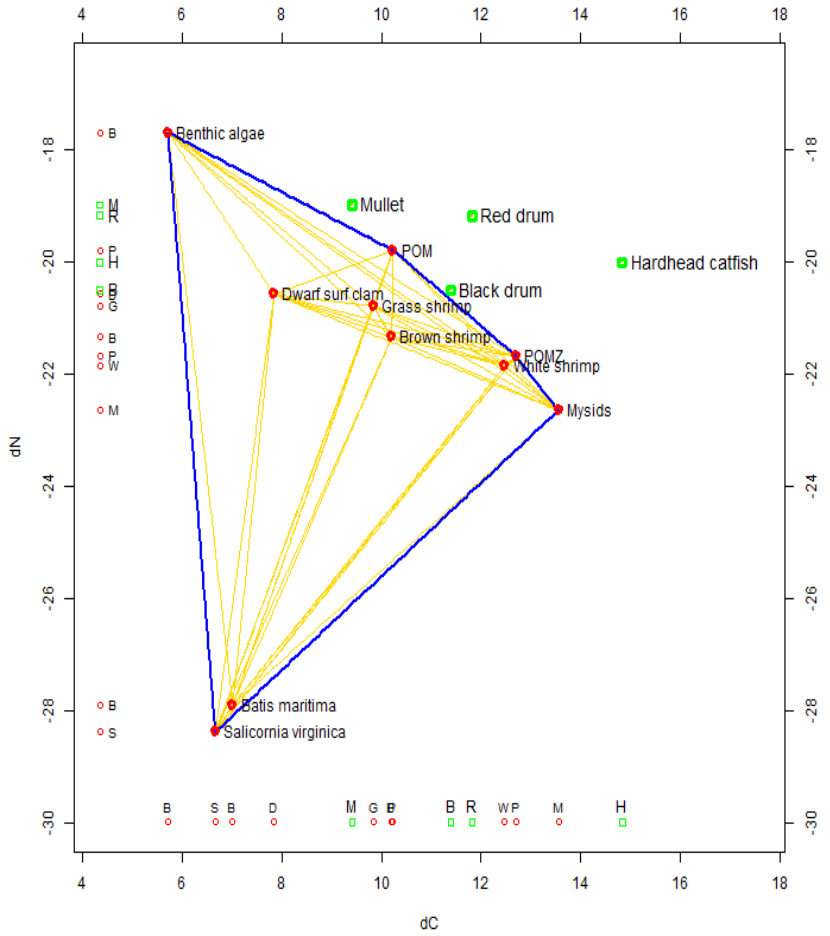

Figure 19c. C/N isotopic ratios for major producers and consumers.

Conclusions

The Nueces Bay has been impacted by removal of hard-bottom and pipeline construction. Water diversions have reduced freshwater flow into the Rincon Bayou, affecting salt marsh function. Stable isotopic analyses demonstrate that both the marsh and the Bay act as a detrital pathway for higher trophic level nutrition, based on particulate organic material and secondarily on benthic algae. This determination was evident from direct plotting of data (biplot and triplots) as well as Bayesian analysis procedures. Use of sulfur provide to be an important isotopic element for separation of food resources.

Acknowledgements

This project would not have been possible with the dedicated efforts of Erin Hill, Aaron Baxter, and Bobby Duke. Lab processing efforts by Beth Almaraz, I-Shuo Huang, and Ben Long facilitated timely sample processing. I-Shuo Huang provided R expertise to develop isotopic diagrams. Support for this work was provided by the TGLO and Center for Coastal Studies, TAMUCC.

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