Investigating isotopic niche width overlap between submerged aquatic vegetation, fringing marsh grasses and nekton in an oligohaline ecosystem

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Abstract

Fringing saltmarshes and submerged aquatic vegetation are two critical components of estuarine ecosystems that provide numerous benefits to resident and transient nekton. Back Bay in Biloxi, Mississippi is an oligohaline estuary that is dominated by the salt-tolerant submerged aquatic vegetation, Vallisneria americana and the fringing salt marsh vegetation, Juncus roemerianus. Given that submerged aquatic vegetation often grows adjacent to fringing saltmarsh in estuarine systems, nekton may have the opportunity to use both habitats daily. This study investigated the isotopic niche width of each of these species and the overlap with lower trophic level consumers. Carbon (13C/12C) and sulfur (34S/32S) stable isotope ratio analyses were used to identify isotopic niche width of Menidia beryllina, Fundulus grandis, and Lepomis macrochirus. Overlap was analyzed using Stable Isotope Bayesian Ellipses in R to compare the overlapping space between fishes and basal carbon sources bimonthly from May 2021 through May 2022. Fishes had greater than 50% isotopic niche overlap with submerged aquatic vegetation compared to fringing saltmarshes. Overlap was less than 23% for Juncus roemerianus and negligible for other saltmarsh vegetation. These results suggest that the isotopic niche space of Vallisneria americana primarily overlaps with the niche space of these fishes and should be considered a high priority for habitat conservation and restoration efforts in this area.

Introduction

Fringing saltmarshes and submerged aquatic vegetation (SAV) are two critical components of estuarine ecosystems that provide numerous benefits to resident and transient nekton as well as humankind (Rozas and Odum, 1987; Castellanos and Rozas, 2001; Jerabek et al., 2017). A subset of these benefits for nekton include refuge from predation, a source of food, and nursery habitat (Peterson and Turner, 1994; Castellanos and Rozas, 2001; Harrison-Day et al., 2020). Given that SAV often grows adjacent to fringing saltmarsh in estuarine systems, nekton may have the opportunity to use both habitats daily. Studies in meso- and polyhaline environments where seagrasses are the dominant SAV indicate that fringing saltmarshes and seagrasses may serve as redundant habitats (Minello et al., 2012; Rozas et al., 2012; McDonald et al., 2016); however, the roles of SAV and fringing saltmarshes at providing habitat benefits such as nutrition are poorly understood in oligohaline environments, particularly along the northern US Gulf Coast (Harrison-Day et al., 2020).

Oligohaline ecosystems are often characterized as highly productive and diverse, particularly for euryhaline species (Rozas and Odum, 1987; Castellanos and Rozas, 2001; Peyre and Gordon, 2012). Fish, decapod crustaceans, and other free-swimming organisms readily depend on the ecosystem services provided by oligohaline environments (Peterson and Turner, 1994; Castellanos and Rozas, 2001). Along the northern US Gulf Coast, fringing saltmarshes in Mississippi are primarily dominated by Black Needlerush (*Juncus roemerianus*), Smooth Cordgrass (*Spartina alterniflora*), Big Cordgrass (*Spartina cynosuroides*), and Saltmeadow Cordgrass (*Spartina patens*; Cho et al., 2012; Mendelssohn et al., 2017). American Eelgrass (*Vallisneria americana*) is one of the dominant SAV species along the northern US Gulf Coast (Cho et al., 2012), where it grows in waters less than 12 but can withstand salinities up to 15 for short periods of time (Doering, P. et al. 1999; French and Moore, 2003; Boustany, Michot and Moss, 2010). Studies have reported that faunal communities in brackish and mesohaline waters are similar in terms of abundance and biodiversity between SAV and fringing saltmarsh habitat (Rozas and Odum, 1987; Castellanos and Rozas, 2001). Particularly along the northern US Gulf Coast, the abundance of estuarine fish and invertebrates has a strong positive relationship with the presence of both fringing saltmarsh and SAV (Turner, 1977; Rozas and Minello, 2012; Shakeri et al., 2020); however, few studies have directly compared isotopic niche overlap between SAV, fringing saltmarsh and nekton in a *Vallisneria americana*- and *Juncus roemerianus*-dominated ecosystem.

Detrital export from saltmarsh grasses is a major source contribution to estuarine and marine communities (Boesch and Turner, 1984; Haines and Montague, 1987) and is influenced primarily by tidal intensity, amplitude, and wave action (Odum et al., 1973; Boesch and Turner, 1984). Detritivores depend upon detritus as a main source of nutrition (Haines and Montague, 1979) and detrital export from saltmarshes is considered one of the leading nutrient influxes of organic carbon into the base of estuarine food webs (Haines, 1977) with contributions to net primary production ranging from 40% to 75% (Borey, Harcombe, and Fisher, 1983; Boesch and Turner, 1984; Garcia et al., 2007). The correlation between fishery biomass and saltmarsh varies regionally, but studies along the northern US Gulf Coast region indicate a positive correlation (Turner 1977); for example, Turner (1977) reported that annual yields of penaeid shrimp caught inshore were highly correlated with the area of vegetated wetlands including aquatic habitats featuring seagrasses (Turner, 1977; Boesch and Turner, 1984). This suggests coastal faunal communities in the region are highly dependent upon detrital export as a major nutritional contributor to the base of the food web.

Numerous studies have evaluated food webs to delineate ecosystem structure (Peterson and Fry, 1987; Connally et al., 2003; Garcia et al., 2007). Naturally occurring stable isotope ratios can be used in aquatic ecosystems to delineate trophic levels, understand community dynamics, and assess the isotopic niche space organisms of interest occupy within the community (Peterson and Fry, 1987; Bearhop et al., 2002; Hayden et al., 2017). Carbon ($^{13}C/^{12}C$), sulfur ($^{35}S/^{34}S$), and nitrogen ($^{15}N/^{14}N$) stable isotope ratios have been successfully used to identify habitat use in estuarine environments (Peterson and Fry, 1987; Fry, 2002; Cullen et al., 2019). Carbon and nitrogen isotope ratios both increase predictably with trophic level, about 1‰ and 3‰ per level, respectively (DeNiro and Epstein, 1978; Peterson and Fry, 1987; Logan and Lutcavage, 2010), which can be useful for determining trophic structure. Additionally, carbon isotope ratios may be used to identify primary producers based on differences in glucose production and photosynthetic pathways (Peterson and Fry, 1987, Fry, 2006). By analyzing the carbon ratios of basal sources, comparisons of isotopic niche overlap can be assessed using the sources' unique isotope signature. When combined with carbon, sulfur isotopes can refine source tracing and further help identify the isotopic niche space between signatures. (Peterson and Fry, 1987; Fry, 2006; Cullen et al., 2019). Unlike carbon and nitrogen isotopes, fractionation of sulfur isotope ratios is negligible with increasing trophic levels, making it an ideal indicator when basal sources have large differences in sulfur values (Peterson and Fry, 1987; Bouillion, Conolly, and Lee, 2008).

Few studies have compared the isotopic niche space shared between submerged aquatic vegetation and fringing marsh in a *Vallisneria americana* and *Juncus roemerianus* dominated oligohaline system as ultimate carbon sources using stable isotope ratio analysis. Therefore, we assessed the overlap of submerged aquatic vegetation and fringing saltmarsh and compared that to nekton using Back Bay, Mississippi as the study system. The objective of this study was to evaluate isotopic niche overlap in this system to a subset or consumers at the base of the food web.

Methods

a) Study sites

Back Bay of Biloxi, Mississippi (hereafter Back Bay) is a low salinity estuary within the Mississippi Sound. Back Bay is approximately 20 kilometers (km) long and its width ranges from 1.5-km at the bay mouth to 0.80-km in the east (Eleuterius, 1978; Hashim, 1995). The average depth of Back Bay is 1.3 meters (m) at mean low water and depth ranges from 1.3 to 3-m (Eleuterius, 1978; Hashim, 1995). Excluding its tributaries, Back Bay covers approximately 42-km^2 (Eleuterius, 1978; Hashim, 1995). Two rivers discharge freshwater into Back Bay among several smaller bayous and the Industrial Waterway (Fig. 1).

The Biloxi and the Tchouticabouffa Rivers discharge approximately 13.97-m^3/s and 12.36-m^3/s, respectively (Eleuterius, 1978; Hashim, 1995), making the far western portion of Back Bay oligohaline (Eleuterius, 1978; Hashim, 1995). However, salinity at the mouth of the Biloxi River in the western portion of the bay can range from oligohaline to mesohaline depending on the time of year and river discharge rate (Eleuterius, 1978; Hashim, 1995). Back Bay traditionally becomes more stratified in the summer when river discharge increases, then it transitions to well-mixed during the winter when discharge decreases (Eleuterius, 1978; Hashim, 1995). Despite broad changes in water quality across salinity gradients, the vegetative community within Back Bay remains diverse with a recent survey reporting over 40 plant species across the bay's nearshore, water bottom, and upper shoreline habitats (Cho, 2011, Cho and Biber, 2017).

In October 2019, a high-resolution drone survey of the shoreline and nearshore area of Back Bay was performed. Imagery from this survey was then used to classify shoreline and nearshore environments, including fringing saltmarsh and submerged aquatic vegetation habitat. This classification was used to select five replicate study sites on the western edge of Back Bay (Fig. 1). Each site was dominated by *Vallisneria americana* and *Juncus roemerianus*. Study sites consisted of 120-m tracts of fringing saltmarsh and SAV habitat. Each of the five study sites had at least 60-m tracts of SAV and saltmarsh. These were specific criteria for selection of each of the five sites so sites would have similar habitats. These criteria were quantified using aerial imagery imported into ArcGIS Pro.

b) Field sampling and processing

The primary sampling gears used in this study were fyke and seine nets. Nekton in this study are defined as free swimming organisms and include estuarine fishes and macroinvertebrates. All nekton were analyzed as composite samples of at least five individuals from each of the seven sampling events. Sampling was conducted bimonthly (every other month) for 12-months.

Samples pooled into composite samples were from the same site. The minimum high tide needed for sampling was determined to be 0.41-m above mean low water, which was the average high tide during winter months (December through March) over the last five years as measured from the USGS monitoring station (8743735) at Cadet Point in Back Bay. Within each site, sampling locations were chosen haphazardly on each sampling date. To sample the fringing saltmarsh, fyke nets six meters in length with 0.635-centimeter mesh webbing were placed against the marsh edge. Fyke nets were deployed haphazardly along each site within a two-hour window of low tide when the saltmarsh platform was exposed and no water remained. Nets were deployed for less than 24-hours and were retrieved in the next tidal cycle at low tide. Submerged aquatic vegetation habitat was sampled with a seine net (10-m wide x1-m high, with 0.635-centimeter mesh) at or above the defined high tide, within a 24-hour window of fyke net sampling. Sampling occurred over 200-m^2 parallel to the saltmarsh edge. The area seined started 1-m away from the saltmarsh edge (Fig. 2).

All fishes and macroinvertebrates collected via seine and fyke net were subject to cold shock treatment immediately following collection as recommended by the American Fisheries Society (Uses of Fishes in Research Committee, 2014). All samples collected were frozen prior to analysis.

Vegetation surveys of the fringing saltmarsh and SAV were conducted to estimate the percent cover of species present (ESM_1, ESM_2). Three percent cover estimates were measured 0.5-m landward of the marsh edge using a 0.25-m^2 quadrat at 2-m intervals along the length of the fyke net (Fig. 2). Percent cover of each vegetation species was determined by having two researchers visually identify vegetation and independently determine the percentage of ground cover for each species within each quadrat. These estimations were averaged for analysis. Vegetation surveys of SAV were conducted using a presence/absence method (Offner, 2023). A 1-m^2 quadrat was subdivided into 16 subunits, each representing 6.25% of the total area (Offner, 2023). The presence of SAV was determined by laying the quadrat over the water bottom and feeling within each unit for vegetation (Offner, 2023). After field scouting, initial sampling events, and analysis of aerial imagery, it was determined that *Vallisneria americana* was considerably dense and ubiquitous at all sites; therefore, all vegetation identified was assumed to be *V. americana*. To capture the full extent of SAV within sites and the area sampled by the seine nets, surveys were performed 6-m seaward from the marsh edge in seven-meter increments parallel to the marsh platform (Fig. 2). Vegetation for stable isotope analysis was collected from within the sampling quadrats and placed on ice following collection. All samples were frozen prior to analysis.

To capture the detrital contribution to nekton in this area, particulate organic matter (POM) was collected by filtering approximately 250-milliliters of water through a 0.25-nanometer glass microfiber filter. To avoid sediment resuspended from the benthos due to sampling, particulate organic matter was collected 10-m seaward from the marsh edge from 0.3-m under the surface. All other vegetation including benthic macroalgae and epiphytes were collected haphazardly while sampling and transported on ice for isotopic analysis.

c) Stable isotope analysis

A subset of fish and prominent vegetation species were used for carbon ($^{13}C/^{12}C$), sulfur ($^{35}S/^{34}S$), and nitrogen ($^{15}N/^{14}N$) stable isotopic analysis (SIA). Basal carbon sources were selected because of their prevalence at each site based on the 2019 drone imagery as well as field measurements during the initial months of this project in 2021. Cho (2011 & 2016) have also reported these species throughout Back Bay. All vegetation was thoroughly rinsed with deionized (DI) water, wiped with Kimtech™ Kimwipes®, rinsed, then dried for at least 72 hours at 60°C. To avoid contamination, the drying oven was cleaned before and after each use with 99.9% methanol and Kimwipes®. Macroinvertebrates with a carapace width and length less than 2 centimeters were used for analysis and were included as composite samples. Macroinvertebrates were thoroughly rinsed with DI water and dried as whole samples. Samples of *Callinectes sapidus* were acid washed with 10% hydrochloric acid. Samples of Palaemonidae were not acid washed. Composite samples were used to achieve sufficient weight of at least five times the mass per sample for SIA of carbon, sulfur, and nitrogen. This technique also accounts for the dietary variability among individuals (Peterson and Fry, 1987; Fry, 2006).

All fish and vegetation samples (except for POM) were soaked in DI water for 15 minutes after preparation to dissolve any remaining salts prior to being dried. Particulate organic matter was filtered through glass microfiber filters that were combusted at 450°C for 4 hours to burn off organic carbon. Samples (including POM filters) were placed into beakers that were cleaned with 99.9% methanol and covered with sterile aluminum foil to reduce contaminants from the oven (Levin and Currin, 2012). After drying samples were stored in airtight containers until they were ground with a Micro-Mill® Grinder II, also cleaned with 99.9% methanol before and after each sample. Powdered samples were then stored in sterile airtight scintillation vials until a subset of samples were packed into tin

boats as part of the isotopic analysis preparation process. For carbon and sulfur SIA, fish were analyzed as composite samples of at least five individuals within ± 15 millimeters standard length of each other to reduce the variability among individual diets as well as ontogenetic shifts. Fishes smaller than 10 centimeters in total length were used in analysis. Macroinvertebrates were combined into composite samples, when necessary, as described above. All samples were packed into 5 x 9 millimeter tin boats in 96 well sample trays then stored with Dry-Rite™ until analysis. Dry-Rite™ was poured into plastic bags and then poked with holes so it would not come into direct contact with the sample trays. Samples that needed acid washing were packed into silver boats designed to withstand hydrochloric acid. It is important to note, we retained mass of all samples throughout the project. A subset of samples were shipped to the University of Connecticut where they were analyzed for carbon and nitrogen ratios using a Costech 4010 Elemental Analyzer. During the project, we were awarded a United States Fish and Wildlife Service grant to pay for analysis of sulfur isotope rations. A subset of samples were shipped to Louisiana State University where they were analyzed, also in a continuous flow isotope mass spectrometer.

d) Statistical analysis

Stable Isotope Bayesian Ellipses in R (SIBER, Jackson 2011) was used to estimate the standard ellipse area of SAV and fringing marsh grasses and the overlap in niches between those two vegetation species and a subset of fishes and macroinvertebrates (Jackson and Parnell, 2023). Using Bayesian metrics, SIBER plots standard ellipses use bivariate data to group individual elements in a biplot using the mean as the center of the hull. This method of using a standard ellipse is analogous to using a standard deviation with univariate data (Batschelet, 1981; Jackson et al. 2011). By comparing niche areas and calculating the percentage of overlap, we can compare the overlap of nekton to the two dominate vegetation types investigated in this study (Solomon et al., 2011; Ponce et al., 2021; Cybulski et al., 2022).

Data were pooled into average values across the duration of the study prior to analysis. Using the SIBER package (Jackson and Parnell, 2023) in RStudio 4.3.1, ellipses of carbon and sulfur isotopes were created. Carbon was plotted along the x-axis and sulfur was plotted on the y-axis. Isotopic fractionation corrections of + 1‰ were made to consumer values of carbon. An additional plot with nitrogen isotopes was created with corrections of +3‰ of consumer values for visualization only in the early stages on the project (Peterson and Fry, 1987). Standard ellipse area values were not calculated with biplot data using Nitrogen values. Sulfur isotope fractionation is considered negligible and thus consumer values did not have corrections (Peterson and Fry, 1987). Standard ellipses represented 40% of all data values of each species represented in the biplot as recommended by Jackson (2011). Proportions of ellipse overlap were calculated using the Max Likely Overlap function in SIBER. This function used the estimated means of posterior distributions to calculate the percentage of overlap as per mill squared units $(‰)^2$. The higher percentages of overlap between two sources are, the greater the similarity and alignment between isotopic niches. Next, the Bayesian Overlap function was called with Just Another Gibbs Sampler (JAGS) to fit a Bayesian normal distribution over two isotopic niches of comparison (Jackson, 2011). The credible interval was set at 0.95, the number of draws (model simulations) was 100, and all other parameters were accepted default (Jackson, 2011). Carbon and sulfur and carbon and nitrogen data of all fauna and flora collected over the sampling period are represented as mean values in biplots. Error bars are shown with respect to the X and Y axes with a credible interval of 0.95.

Results

In total, 226 samples were analyzed for carbon ($^{13}C/^{12}C$), sulfur ($^{35}S/^{34}S$), and nitrogen ($^{15}N/^{14}N$) stable isotope ratios. Mean values for consumers ranged from –19.05‰ to –27.06‰, 9.9‰ to 13.31‰, and 15.22‰ to 18.62‰ for carbon, sulfur, and nitrogen respectively (Table 1).

Table 1 Mean and standard error (SE) of all fauna and vegetation displayed. The number (n) represents the number of composite samples analyzed over the sampling period. Samples are listed by decreasing number of samples analyzed. Fauna includes all fish and crustaceans. Flora includes all saltmarsh and submerged aquatic vegetation.

Species	Type	Number (n)	$\delta^{13}C$		$\delta^{34}S$		$\delta^{15}N$	
			Mean	SE	Mean	SE	Mean	SE
Menidia beryllina	Fauna	21	-23.99	0.43	12.27	0.51	17.38	0.13
Micropterus salmoides	Fauna	15	-26.82	0.54	10.56	0.46	15.64	0.45
Fundulus grandis	Fauna	15	-22.19	0.55	10.95	0.54	15.76	0.32
Vallisneria americana	Flora	15	-25.20	1.34	13.80	0.96	9.64	0.37
Sagittaria lancifolia	Flora	15	-28.00	0.48	5.60	0.94	7.60	0.37
Spartina cynosuroides	Flora	15	-13.36	0.16	6.35	0.62	6.31	0.37
Juncus roemerianus	Flora	13	-28.63	0.23	3.85	0.92	7.80	0.28
Myriophyllum spicatum	Flora	12	-22.37	0.98	14.28	1.44	8.39	0.94
Spartina alterniflora	Flora	12	-14.38	0.20	5.26	1.37	6.62	0.28
Lucania parva	Fauna	12	-22.48	0.45	10.47	0.49	15.22	0.24
Callinectes sapidus	Fauna	12	-22.54	0.60	15.29	0.30	18.62	1.79
Lepomis macrochirus	Fauna	9	-26.41	0.51	10.1	0.84	15.97	0.56
Palaemonidae	Fauna	9	-23.92	0.26	13.31	0.07	15.53	0.20
Lepomis microlophus	Fauna	8	-24.50	0.84	11.58	0.67	18.00	0.05
Benthic macroalgae	Flora	8	-31.16	1.39	12.91	0.72	12.11	0.86
Ruppia maritima	Flora	6	-21.93	0.10	15.72	0.02	10.66	1.55
Lagodon rhomboides	Fauna	6	-22.32	0.31	11.2	1.01	16.55	0.12
Micropogonias undulatus	Fauna	3	-21.93	0.03	12.33	0.13	16.92	0.29
Brevoortia patronus	Fauna	3	-22.60	0.09	12.57	0.03	15.31	0.02
Fundulus diaphanus	Fauna	3	-22.59	0.09	12.57	0.03	16.13	0.21
Mugil cephalis	Fauna	3	-21.99	0.03	9.9	0.00	16.69	0.05
Anchoa mitchilli	Fauna	3	-27.06	0.03	13.23	0.03	17.2	0.03
Cyprinodon variegatus	Fauna	3	-19.05	0.22	11.53	0.09	18.31	0.54
Spartina patens	Flora	3	-14.20	0.02	11.17	0.03	7.95	0.22
Ceratophyllum demersum	Flora	3	-27.21	0.12	13.07	0.03	15.06	0.25

Mean values of primary producers ranged from −13.36‰ to −31.16‰, 3.85‰ to 15.72‰, and 6.31‰ to 15.06‰, for carbon, sulfur, and nitrogen respectively (Table 1). Fifteen fish species, ten vegetation species, and two crustacean species or families (e.g. Palaemonidae) were analyzed for SIA of carbon, sulfur and nitrogen isotope ratios (Table 1, Figures 3-4).

The Inland Silverside (*Menidia beryllina*), Gulf Killifish (*Fundulus grandis*), and Bluegill (*Lepomis macrochirus*) were the most abundant and consistent species caught during the sampling period. Therefore, this subset of fish was selected for carbon and sulfur SIA niche overlap comparisons (Fig. 5, ESM_3, ESM_4).

Other fishes were analyzed for carbon and nitrogen SIA (Table 1, Fig. 4) but were not included in niche overlap comparisons given infrequent encounters and small sample sizes. There were 10 basal carbon sources included for carbon and nitrogen SIA (Table 1, Fig. 4), but only a subset of these primary producers were included in carbon and sulfur SIA given infrequent encounters and small

sample sizes (Fig. 5). V*allisneria americana* and *Juncus roemerianus* were overwhelmingly prevalent at all sites during the study. Other basal sources analyzed for carbon and sulfur SIA that occurred in the study region were benthic macroalgae, *Spartina patens*, *S. alterniflora*, *S. cynosuroides, Sagittaria lancifolia*, *Ruppia maritima*, *Myriophyllum spicatum*, and *Ceratophyllum demersum* (Table 1).

Benthic macroalgae was the most depleted basal carbon source with mean isotope ratio values of −31.16 ± 1.39‰, 12.91 ± 0.72‰, and 12.11 ± 0.86‰ for carbon, sulfur and nitrogen, respectively (Table 1). The next most depleted carbon source was *J. roemerianus* with mean isotope values of −28.63 ± 0.23‰, 3.85 ± 0.92‰, and 7.8 ± 0.28‰ for carbon, sulfur and nitrogen, respectively (Table 1). *Sagittaria lancifolia* was similarly depleted with carbon values falling within the same range as *J. roemerianus*. *S. lancifolia* had mean isotope values of −28.2 ± 0.48‰, 5.6 ± 0.94‰, and 7.6 ± 0.37‰ for carbon, sulfur and nitrogen, respectively (Table 1). *Vallisneria americana* had isotope values that were more enriched than that of *J. roemerianus* with mean isotope values of −25.2 ± 1.34‰, 13.8 ± 0.96‰, and 9.64 ± 0.37‰ for carbon, sulfur and nitrogen, respectively (Table 1). *Myriophyllum spicatum* was more enriched in carbon values than *J. roemerianus* with mean isotope values of −22.37 ± 0.98‰, 14.28 ± 1.44‰, and 8.39 ± 0.94‰ for carbon, sulfur and nitrogen, respectively (Table 1). *Ruppia maritima* fell within the same carbon range as *M. spicatum* with mean isotope values of −21.93 ± 0.1‰, 15.72 ± 0.02‰, and 10.66 ± 1.55‰ for carbon, sulfur and nitrogen, respectively (Table 1).

Fundulus grandis, *M. beryllina*, and *L. macrochirus* were all depleted in carbon values ranging from −22.19 ± 0.55‰ to −26.41 ± 0.51‰ but remained in the center spread of all basal carbon sources (Table 1, Fig. 5). *Lepomis macrochirus* was the most depleted in carbon with mean isotope values of −26.41 ± 0.51‰, 10.1 ± 0.84‰, and 15.97 ± 0.56‰ for carbon, sulfur and nitrogen, respectively (Table 1). The carbon values fell within the range of *V. americana* (Table 1). *Fundulus grandis* was less depleted with mean isotope values of −22.19 ± 0.55‰, 10.95 ± 054‰, and 15.76 ± 0.32‰ for carbon, sulfur and nitrogen, respectively (Table 1). The carbon values fell within the ranges of the SAV species *R. maritima*, *M. spicatum*, and *V. americana* (Table 1). *Menidia beryllina* was the most enriched in carbon values of the 3 fishes. Mean isotope values were −23.99 ± 0.43‰, 12.27 ± 0.51‰, and 17.38 ± 0.13‰ for carbon, sulfur and nitrogen, respectively (Table 1). The carbon values fell within the ranges of *V. americana* and *M. spicatum* (Table 1).

Standard ellipses were created using SIBER to display isotopic niches of carbon and sulfur isotopes to compare overlap between isotopic niches (Fig. 5, Table 2).

Table 2 Standard Ellipse Overlap (SEA) was calculated using the Max Likely Overlap function; p was set at 0.95 and the units are per mil squared $(‰)^2$. Upper and lower quartiles of Bayesian Overlap were calculated along with mean overlap performed in a pairwise manner; P.Interval was set at 0.95, Draw was set to 100. Mean = $\bar{x}$. Samples are listed in decreasing order of Standard Ellipse Area.

Species	*Menidia beryllina*				*Lepomis macrochirus*				*Fundulus grandis*			
	SEA	2.5%	97.5%	$\bar{x}$	SEA	2.5%	97.5%	$\bar{x}$	SEA	2.5%	97.5%	$\bar{x}$
Vallisneria americana	68.8	50.66	99.13	70.2	59.12	34.77	115.23	60.97	65.5	38.2	103.49	64.21
Myriophyllum spicatum	58.23	44.53	88.89	60.09	47.74	32.1	98.12	51.41	39.69	28.85	85.76	53.58
Juncus roemerianus	9.07	1.51	23.87	8.48	25.57	9.44	43.55	23.23	2.8		17.55	4.1
Benthic macroalgae	7.07	2.55	41.21	14.57	6.68	1.44	41.58	15.01	0	0	19.2	2.44
Ruppia maritima	0.21	0.01	0.41	0.21	0	0	0.24	0.04	0	0	0.3	0.07
Spartina alterniflora	0	0	0	0	0	0	0	0	0	0	5.71	0.77
Spartina cynosuroides	0	0	0	0	0	0	0	0	0	0	0	0

Menidia beryllina's isotopic niche had greater than 50% overlap with the SAV *V. americana* and *M. spicatum.* Results were calculated as proportions of overlap by per mill units squared $(‰)^2$. Displayed in Table 2 are the lower 2.5% and upper 97.5% quartiles along with the mean. *Vallisneria americana* and *M. spicatum* have means of overlap greater than 50% with *M. beryllina*, *F. grandis*, and *L. macrochirus*. *Juncus roemerianus* and other *Spartina* species. have means of overlap less than 23%.

Niche overlap was detected between these fishes and primary producers due to similar isotopic ranges. When analyzing 40% of the data to determine niche overlap with respect to carbon, there are several pairwise comparisons that result in greater than 50% isotopic niche overlap. When compared to the primary producers, the isotopic niche for *Menidia beryllina* overlaps greater than 60% with that of *V. americana* and *M. spicatum*, overlaps less than 15% with that of *R. maritima*, benthic macroalgae, and *J. roemerianus*, and has no overlap with *S. patens*, *S. cynosuroides*, or *S. alterniflora* (Table 2, Fig. 5). *Lepomis macrochirus* overlaps greater than 50% with that of *V. americana* and *M. spicatum*, overlaps less than 24% with that of *R. maritima*, benthic macroalgae, and *J. roemerianus*, and has no overlap with *S. patens*, *S. cynosuroides*, or *S. alterniflora* (Table 2, Fig. 5). *Fundulus grandis* overlaps greater than 53% with that of *V. americana* and *M. spicatum*, overlaps less than 4% with that of *R. maritima*, benthic macroalgae, and *J. roemerianus*, and *S. alterniflora*, but there was no overlap with *S. cynosuroides* (Table 2, Fig. 5).

Discussion

This study aimed tocompare isotopic niche overlap of nekton in an oligohaline system dominated by the SAV *Vallisneria americana* and the marsh plant *Juncus romerianus*. Our results indicate that submerged aquatic vegetation, specifically *V. americana*, played a large role in the base of the food web for lower trophic level consumers during this study. Within the project's study area in west Back Bay, *Vallisneria americana* and *Juncus roemerianus* were the dominant shoreline and nearshore vegetation, which is corroborated by other studies in the area (Cho et al., 2012; 2016). We performed stable isotope analyses of three common resident fishes (*F. grandis*, *M. beryllina*, and *L. macrochirus)* to identify the primary source contributions to the diet of these fishes. For each of the three fishes, isotopic niche overlap was greatest for *V. americana* than any other measured basal carbon source. These findings highlight the importance of *V. americana* as a critical habitat and is supported by other research in other estuaries along the northern US Gulf Coast. In Mobile Bay and coastal Louisiana, *V. americana* beds have been highlighted as important nursery habitats for invertebrates (Heck et al., 2021) that also support a higher nekton density and species richness than nearby saltmarsh edge (Rozas and Minello 2006). However, ours is the first known study to directly assess niche overlap of *V. americana* and saltmarsh vegetation against fishes using both carbon and sulfur isotopes.

Each of these fishes have different feeding habits and trophic ecologies and their physiological salinity tolerance allow them to occupy *V. americana* habitats within oligohaline estuaries. As our results suggest, submerged aquatic vegetation as a food source is the driver behind the the high niche overlap between the the studied fishes and SAV, in addition to refuge from predation. Studies conducted within *V. americana* have indicated this habitat is a resource for prey production. Within the Mobile-Tensaw Delta, Chaplin and Valentine (2009) concluded that 77-95% of total macroinvertebrate production within submerged aquatic vegetation (i.e., *V. americana)* were amphipods. These macroinvertebrates are known to be a primary food source for *F. grandis* in Mississippi waters (Rozas and LaSalle 1990). It is likely *F. grandis* is feeding on amphipods within the *V. americana* habitat and thus getting the majority of its source nutrition from *V. americana*, driving the strong isotopic niche overlap with *V. americana* observed in our study.

Menidia beryllina is commonly found in fresher (oligohaline) portions of estuaries and is primarily planktivorous (Bengtson (1984) and Peck et al. (2003)). Based on Chaplin and Valentine (2009), it is assumed that high prevalences of zooplankton are associated with *V. americana* beds (Bolduc, Bertolo, Hudon, and Pinel-Alloul, 2020). Results of the niche overlap analyses indicate that *M. beryllina* is obtaining most of its source nutrition from the two SAV, *V. americana* and M. spicatum. *Menidia beryllina* is drawn to vertically complex habitats such as submerged aquatic vegetation canopy cover (Peck et al. 2003). In our study, *V. americana* was dense across all study sites and grew among other structurally complex SAV, such as *M. spicatum*.

A study by Peterson (1991) demonstrated that *L. macrochirus* and other fishes in Centrarchidae historically occupy oligohaline estuaries to exploit reduced competition for food resources. Feeding habits of *L. macrochirus* in Davis Bayou of east Back Bay, where it is more mesohaline, were evaluated by Vanderkooy et al. (2000) who reported that *Lepomis* spp. fed on macroinvertebrates. The top three prey items in Vanderkooy et al. (2000) were Oligochaeta, Gammaridae, and Copepoda. Referencing Chaplin and Valentine (2009), it is expected to see most macroinvertebrate production in submerged aquatic vegetation. These results help explain why *L. macrochirus* has greater overlap with *V. americana* than any of the saltmarsh grasses evaluated. These three fishes, *F.*

grandis, *M. beryllina*, and *L. macrochirus*, are assumed to be feeding on macroinvertebrates within *V. americana* beds of west Back Bay and as a result *V. americana* is the primary source of basal carbon for these fishes (Peterson 1991; Chaplin and Valentine ,2009; Vanderkooy et al., 2009).

This study reinforces the important role of SAV for supporting nearshore ecosystems and has implications for management of these critical habitats. First, these results highlight the importance of preserving native submerged aquatic vegetation. Niche overlap showed that *V. americana* based food web was primarily used by these three fishes as a resource for nutrition. Other studies in oligohaline ecosystems have reported similar findings that demonstrate the importance of *V. americana* habitat to secondary production (Chaplin and Valentine, 2009). Second, the significant reliance of these species on this habitat type indicates an importance to other commercially important fish that may use these fishes as prey. As mentioned by Peterson (1991), other centrarchids (i.e., *Micropterus salmoides*) occupy oligohaline portions of estuaries to reduce competition for food. We expect that saltmarshes had lower source contributions in this study due to the location and timing of sampling. Within the mouth of the Biloxi River, there was substantial submerged aquatic vegetation cover compared to saltmarsh grasses. Submerged aquatic vegetation was also found to be available year-round despite lower percent cover during the winter months. Combined these factors help explain why there was a strong niche overlap with submerged aquatic vegetation. However, there were still some traces of source contributions from saltmarsh grasses such as *J. roemerianus*. As reported in this study, *J. roemerianus* and other saltmarsh grasses contribute some nutritional value to the fishes analyzed thus the conservation and restoration of saltmarsh grasses should be a priority.

This study focused on a subset of nekton present in the study system. We recommend that future studies use SIA to examine a broader range of fishes and macroinvertebrates in this system that occupy different tropic levels. Additionally, we recommend future studies explore mixing models that may enable diet estimations and analyses across trophic levels such as MixSIAR or Niche Rover. The SIBER model we employed is restricted to two isotopes, while other models such as Niche Rover allow for the use of three isotopes. Additionally, SIBER cannot be used as a quantitative tool to estimate resource partitioning. In this study, trophic levels were not being evaluated, so using SIBER to assess isotopic niche overlap was an appropriate approach.

We also recommend that future studies examine changes in niche overlap across seasons. This was an original objective of our study, but a lack of sufficient number of nekton during winter and fall months restricted our ability to make this comparison. Future studies should sample monthly for more than one year to retain enough samples to make comparisons for niche overlap. Additionally, SIA on tissue with faster assimilation rates such as blood may also be useful if enough samples are acquired of an appropriate size to enable blood collection (>10 cm fish total length). Future studies that aim to evaluate seasonal niche overlap should: 1) use bigger fishes that would contain sufficient blood tissue and/or 2) conduct aggressive monthly sampling to ensure enough samples to perform robust comparisons.

Declarations

Authors acknowledge that they have no competing interests related to this work.

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Author Contributions

Keith Chenier Jr: Study design, Data analysis, Interpretation of results, Writing & Review.

Eric Sparks: Study design, Interpretation of results, Review.

Kelly M. Darnell: Study design, Interpretation of results, Review.

J. Marcus Drymon: Study design, Interpretation of results, Review.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and the supplementary information files. The datasets and analysis code used in this study can be requested from the corresponding author upon a reasonable request.

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Figures

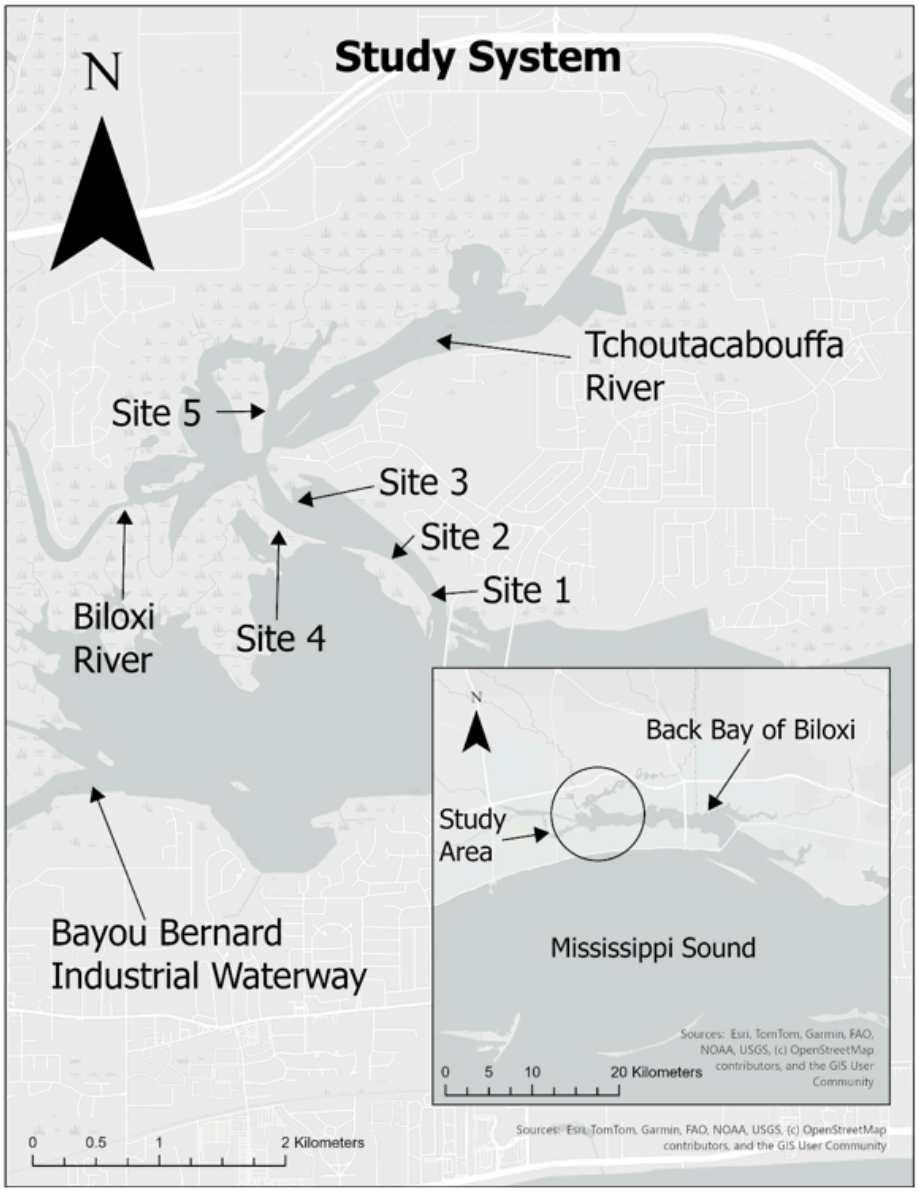

Figure 1

Location of study sites within Back Bay, Mississippi (ArcGIS Pro 3.5, 2025).

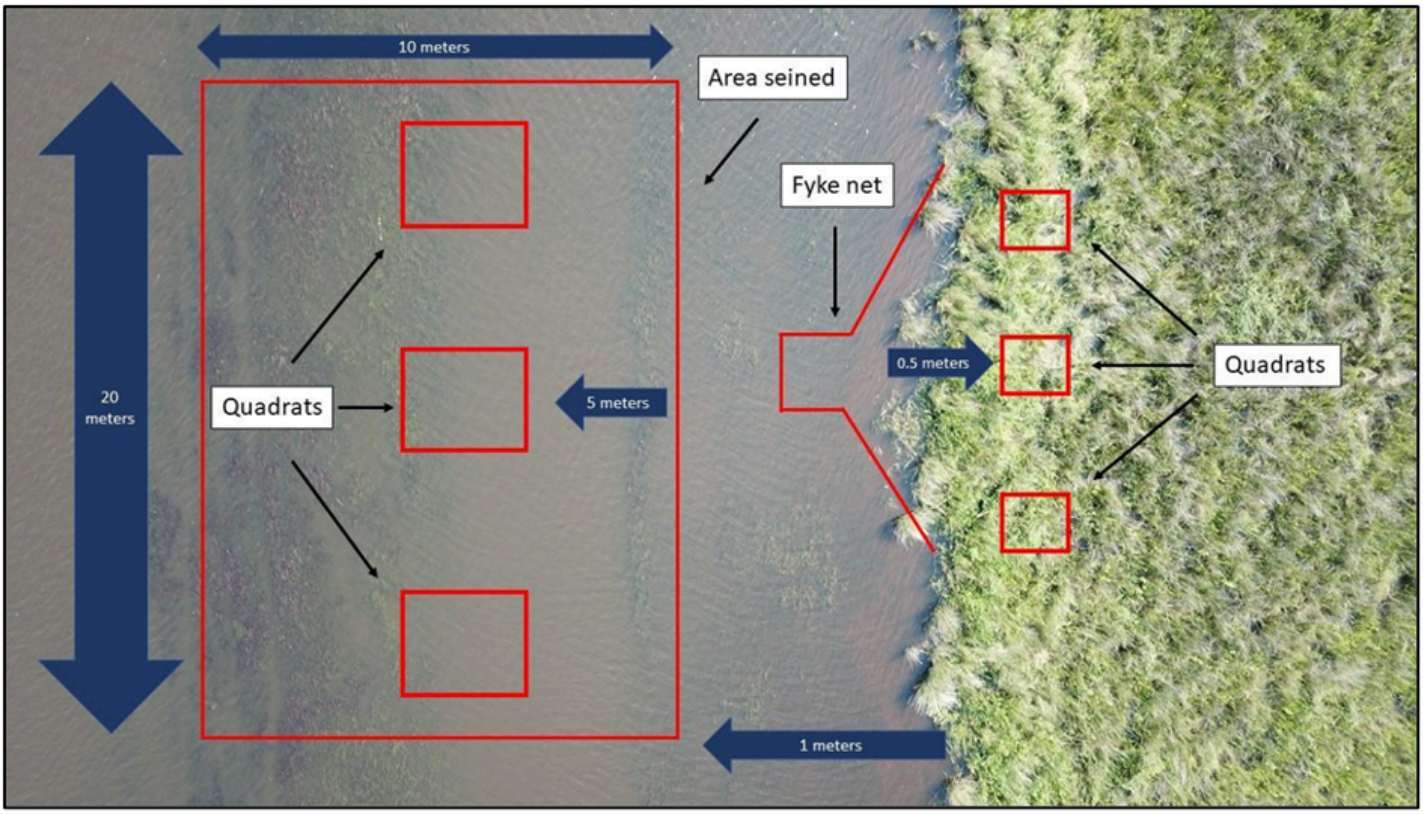

Figure 2

Aerial photograph and diagram of sampling methods taken via quadcopter UAV at 60-m altitude with a resolution of 2 centimeter/pixel; diagram not drawn to scale.

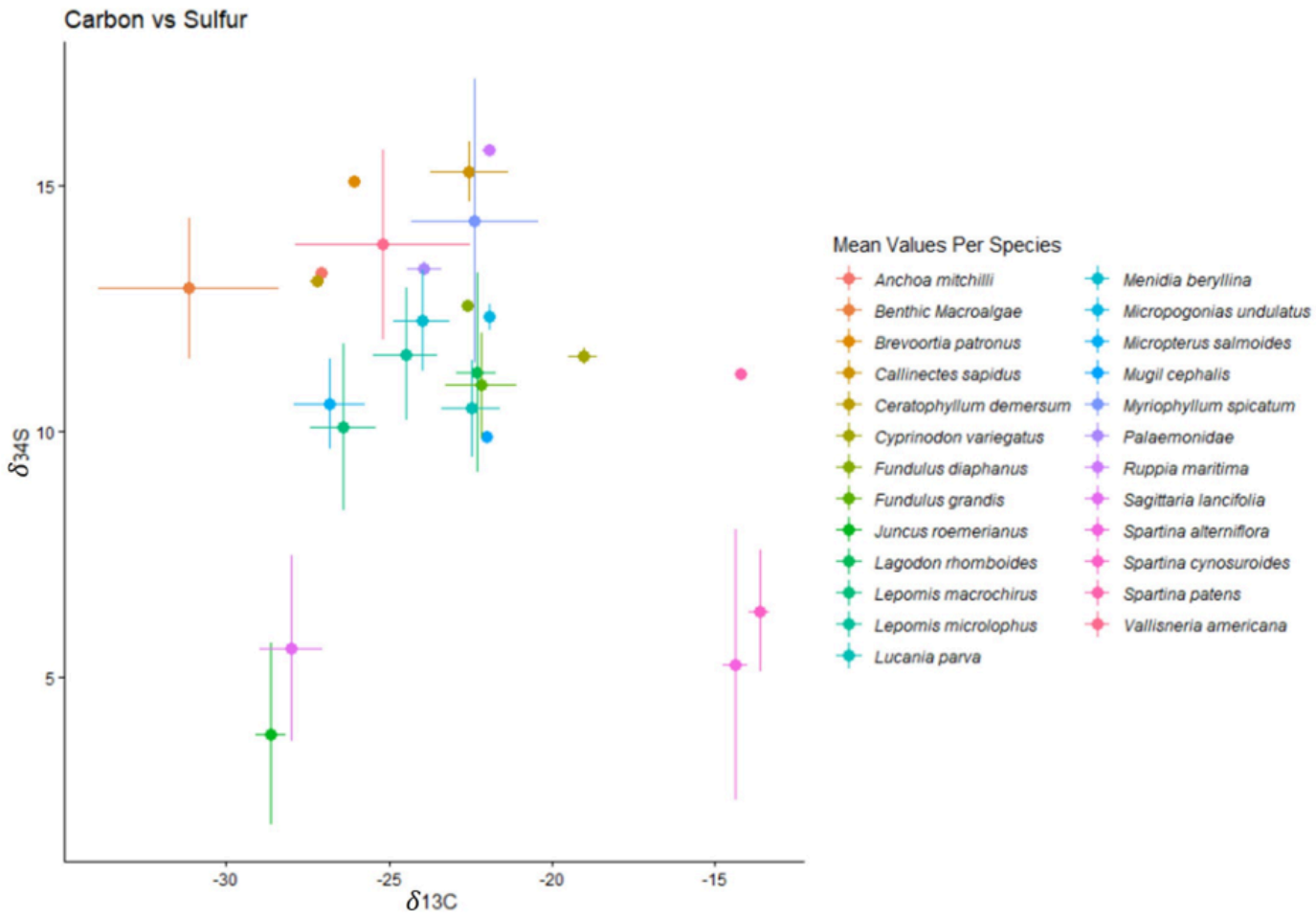

Figure 3

Biplot produced in SIBER of carbon and sulfur isotopes for fauna and flora analyzed. Mean and standard error are shown with the credible interval set at 0.95.

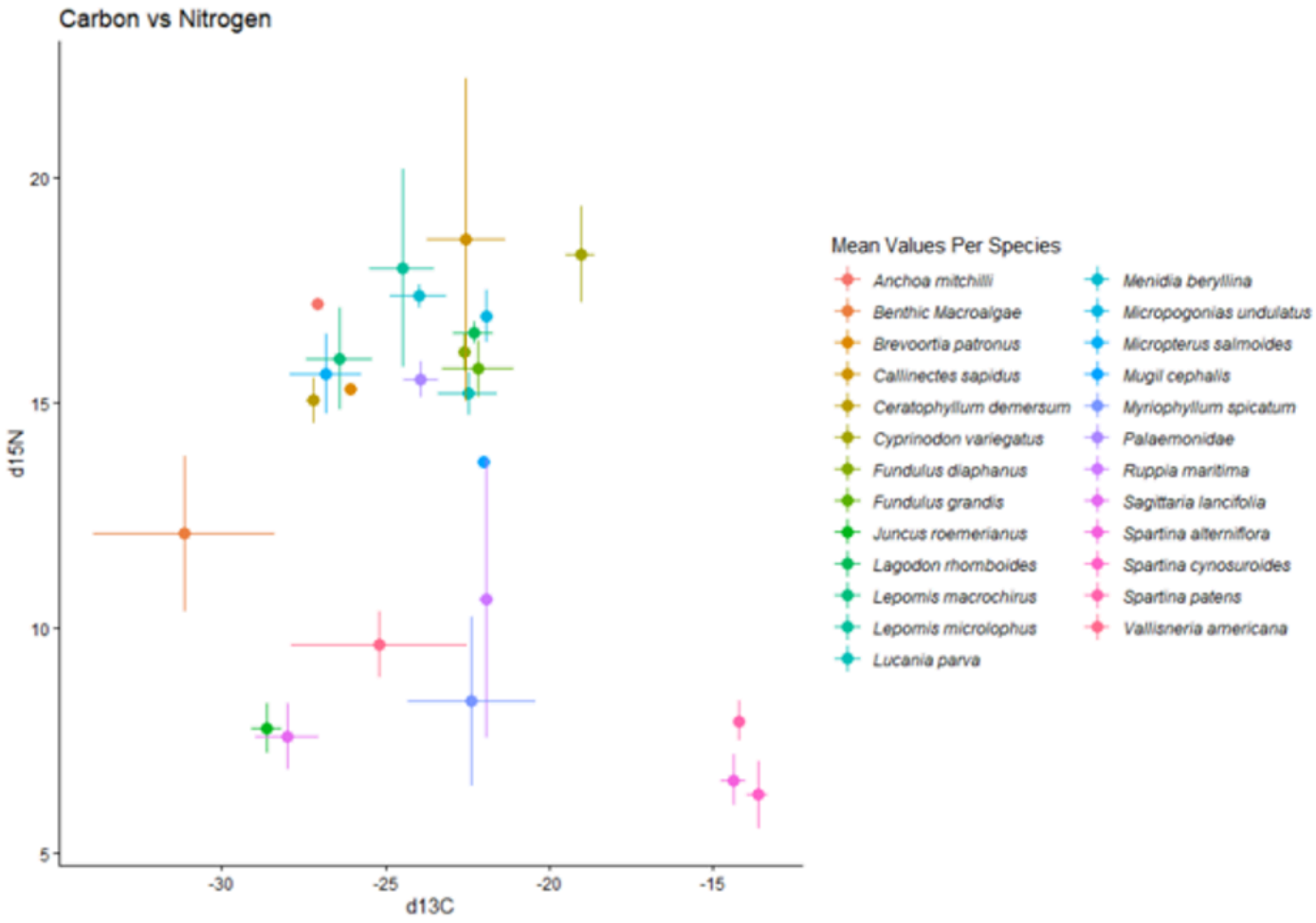

Figure 4

Biplot produced in SIBER of carbon and nitrogen isotopes for fauna and flora analyzed. Mean and standard error are shown with the credible interval set at 0.95.

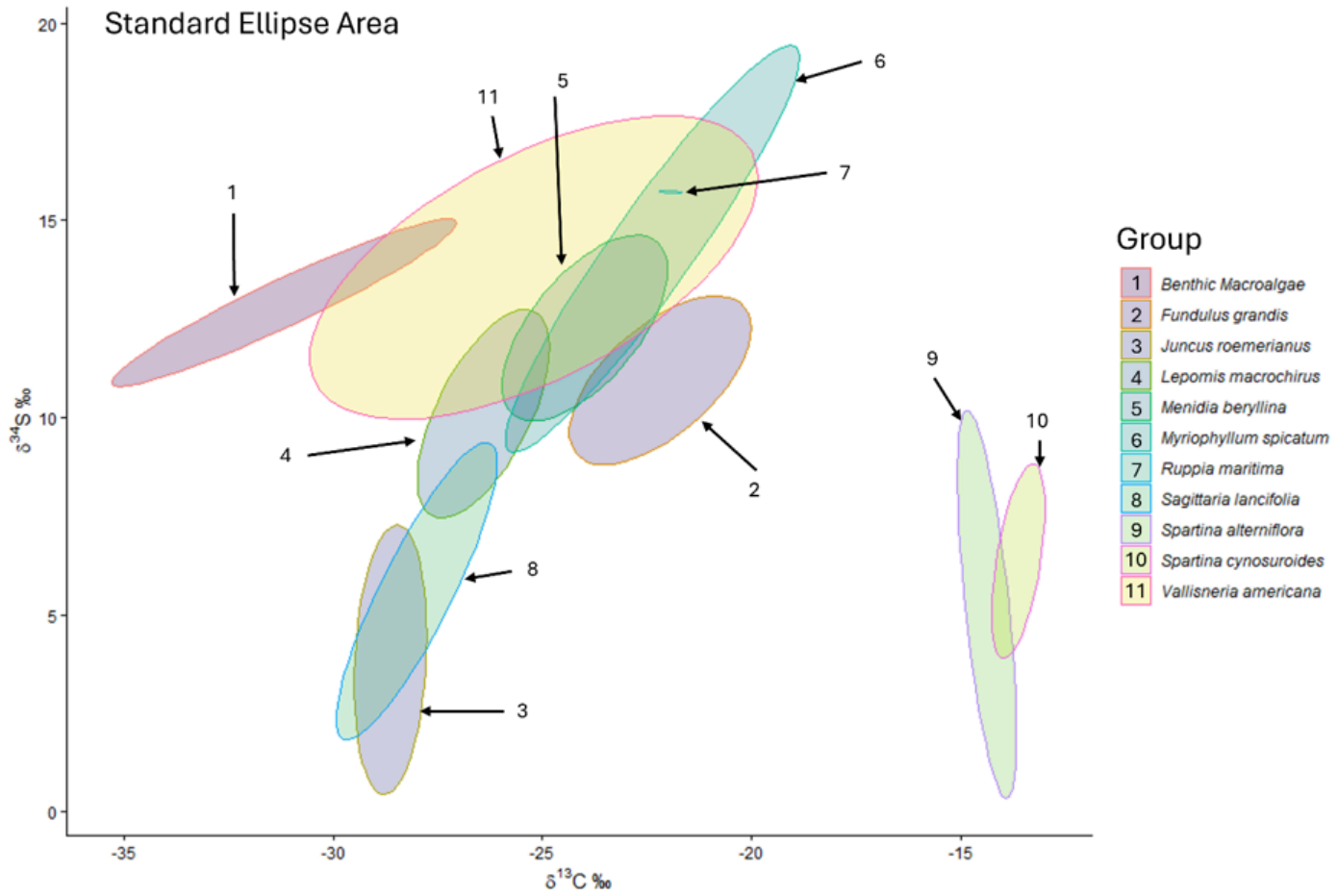

Figure 5

Standard ellipses for species of interest within Back Bay. The center of each ellipse represents mean values with ellipses encapsulating 40% of groups' values (percent of ellipse was set to 0.4).

Supplementary Files

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