# Assessing trophic flexibility of a predator assemblage across a large estuarine seascape using blood plasma stable isotope analysis

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## ARTICLE INFO

## ABSTRACT

The northcentral Gulf of Mexico (nGoM) encompasses a range of environments that support a speciose predatory assemblage. Large predatory species are often viewed as trophically-analogous, sharing similar isotopic niches. To investigate the regional and seasonal isotopic variability of a predator assemblage across the coastal waters of the nGoM (Louisiana, Mississippi, and Alabama), we analyzed the stable isotopes of carbon, nitrogen, and sulfur using a fast turnover tissue, blood plasma, for seven dominant predator species. Carbon, nitrogen, and sulfur isotope values varied regionally, with a significant interaction between region and season for nitrogen and sulfur. Species' isotopic niches varied regionally, as well as seasonally, leading to varying levels of isotopic niche overlap among species. Blacktip sharks *Carcharhinus limbatus* and Atlantic sharpnose sharks *Rhizoprionodon terraenovae* in particular demonstrated flexibility in their isotopic relationships, whereas blacknose sharks *C. acronotus* maintained a more consistent isotopic niche regardless of region or season. No biologically significant overlap was observed for blacknose sharks, bull sharks *C. leucas*, or spinner sharks *C. brevipinna*, suggesting that these species occupy distinct isotopic space. Overlap calculations using two isotopes (carbon and nitrogen) produced markedly higher overlap versus three isotopes (carbon, nitrogen, and sulfur), which demonstrates that predatory roles may be oversimplified when using only carbon and nitrogen. These findings highlight the value of examining seasonal variation in trophic roles using fast turnover tissues and provide the first triple-isotope characterization of a common predatory assemblage in the northcentral Gulf of Mexico.

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## 1. Introduction

Trophic relationships can vary across ecosystems, particularly in the marine environment where limited physical barriers allow for the movement of organisms among regions. Highly mobile predators, such as elasmobranchs (sharks, skates, and rays), occupy vital roles across marine ecosystems by eliciting strong top-down regulation of prey communities (Heithaus et al., 2008; Heupel et al., 2014; Shaw et al., 2016). Historically, predators have been characterized into the same trophic role or functional group, but a growing body of literature is demonstrating that trophic roles of predatory assemblages may be more complex than previously assumed (Hussey et al., 2015; Bizzarro et al., 2017; Shipley et al., 2019). The accepted notion that large predators, particularly sharks, are universally "top consumers" has become antiquated; recent research has demonstrated the trophic plasticity of predators both regionally and seasonally (Hussey et al., 2015; Bizzarro

et al., 2017; Shipley et al., 2019). Consequently, assessing trophic relationships among predators in an ecosystem, including seasonal shifts in shared isotopic space, provides a more holistic view of the roles of marine predators, with implications for elasmobranch management and conservation (Bizzarro et al., 2017; Pool et al., 2017; Espinoza et al., 2019).

A species' trophic role within an ecosystem can be estimated through stable isotope ratios measured in tissues collected non-lethally from many individuals in a population (Layman et al., 2007; Newsome et al., 2007). The stable isotope ratios of carbon, nitrogen, and sulfur provide information about an individual's dietary habits (nitrogen) as well as the location where the individual is feeding (carbon and sulfur). Specifically, the stable isotope of carbon, $^{13}C$, shows conservative isotopic fractionation associated with the incorporation of a resource into a consumer, but $\delta^{13}C$ values can vary markedly between primary producers (e.g., seagrass versus mangrove, or terrestrial versus aquatic) (DeNiro and Epstein, 1978; Thayer et al., 1983; Chasar et al., 2005). The limited fractionation and source distinction of $\delta^{13}C$ thereby can be used to indicate basal carbon sources within a food web

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(DeNiro and Epstein, 1978; Peterson and Fry, 1987). Values of the stable isotope of nitrogen, $^{15}$N, are enriched in an individual's tissues relative to its food, designating the isotopic signature of nitrogen as a predictable indicator of trophic position or the length of a food web (Peterson and Fry, 1987; Fry, 1988). More recently, sulfur has been increasingly used as an additional indicator of basal resource to assess trophic relationships within aquatic systems (Pool et al., 2017; Curnick et al., 2019; Heuvel et al., 2019). Sulfides in seawater (~20‰) are distinctly different from sulfides in sediments (~−24‰), making the stable isotope of sulfur, $^{34}$S, and the resulting isotope ratio a useful delineator between pelagic and benthic baselines of an individual's diet (Connolly et al., 2004; Chasar et al., 2005). In regions such as large estuaries, where $\delta^{13}$C values overlap across primary producers, sulfur stable isotopes can provide increased resolution into foraging patterns of an individual (Connolly et al., 2004; Chasar et al., 2005; Hussey et al., 2012a).

Stable isotope signatures from an individual's tissues can also be used to estimate isotopic niche (Bearhop et al., 2004; Jackson et al., 2011; Swanson et al., 2015). The isotopic niche of an organism serves as a proxy for its trophic niche and provides a useful metric for assessing the degree of interspecific and intraspecific competition, as well as resource partitioning among populations (Bearhop et al., 2004; Jackson et al., 2011; Swanson et al., 2015). Isotopic niches can change seasonally, making fast turnover tissues ideal to identify any potentially rapid trophic shifts (Matich et al., 2011; Bizzarro et al., 2017; Espinoza et al., 2019). While muscle tissue turns over slowly (e.g., annually), blood plasma tissue has an isotopic half-life of approximately 30 days in elasmobranchs; therefore, it can provide dietary information on shorter (i.e., seasonal) timescales (Kim et al., 2012; Matich et al., 2011). The use of a faster turnover tissue could prove critical when investigating trophic relationships across highly variable spatial and temporal seascapes, such as the northcentral Gulf of Mexico (nGoM).

The coastline of the nGoM is often referred to as the "fertile fishery crescent" (Grimes, 2001). It includes many bays and inlets, which create a mosaic of environments ranging from shallow, brackish regions with strong terrestrial influences to deeper, marine-like areas, even across a relatively narrow longitudinal gradient. These coastal regions are home to a wide array of marine predators (Froeschke et al., 2010; Drymon et al., 2010; Plumlee et al., 2018). The trophic ecology of predator populations is well described off the coasts of Texas (Scharf and Schlicht, 2000; Froeschke et al., 2010; Plumlee and Wells, 2016) and Florida (Snelson et al., 1984; Bethea et al., 2004; Matich et al., 2011), but fewer studies have investigated the trophic diversity of predators across the nGoM (i.e., the coastlines of Louisiana, Mississippi, and Alabama) (Drymon et al., 2010, 2012). As such, the objectives of this study were to: 1) use stable isotope ratios of carbon, nitrogen, and sulfur to examine the isotopic niches and isotopic overlap of predatory species across the nGoM; 2) use a fast turnover tissue to detect potential seasonal variations in isotopic niches of predators across a large estuarine seascape.

## 2. Materials and methods

### 2.1. Study region

The nGoM encompasses coastal waters of Louisiana, Mississippi, and Alabama, extending from the Chandeleur Islands, LA in the west to Mobile Bay, AL in the east (Sanial et al., 2019). This region spans three sub-regions which effectively illustrate the diversity of ecosystems within the nGoM. First, the Chandeleur Islands off of Louisiana, which can only be accessed via boat or seaplane, represent relatively pristine habitat, influenced by the Mississippi River Delta and Chandeleur Sound (Poirrier and Handley, 2007; Sanial et al., 2019). This curvilineal chain of islands acts as a barrier against wind and wave energy from offshore, creating habitat for the seagrass meadows that spread along the western shelf of the islands (Pham et al., 2014; Kenworthy et al., 2017). Moving eastward, the Mississippi Sound is a highly productive estuarine

region off the coast of Mississippi that connects Lake Borgne, LA to Mobile Bay, AL (Pham et al., 2014; Mickle et al., 2018). The southern extent of Mississippi Sound is marked by a chain of barrier islands bordering both the Mississippi and Alabama coasts (Pham et al., 2014). Seagrass beds dominated by shoal grass (*Halodule wrightii*) are common along the northern coasts of these barrier islands and provide habitat for ecologically and economically important species like blue crabs (*Callinectes sapidus*) and red drum (*Sciaenops ocellatus*) (Moncreiff, 2007; Pham et al., 2014). Lastly, the Mobile Bay estuary is a large, shallow system broken into two smaller bays: Mobile Bay (the main basin) and Bon Secour Bay (the southeast basin). The Mobile River System represents the eighth largest average discharge in North America (Osterman and Smith, 2012); this freshwater influx results in large fluctuations in salinity in the Mobile and Bon Secour Bays, particularly in early spring when discharge is highest. Saltwater intrusion into these bays is limited by the barrier island of Dauphin Island and the Fort Morgan Peninsula located at the southern end of the Bay (Osterman and Smith, 2012). These three sub-regions (Chandeleur Sound, Mississippi Sound, and Mobile Bay/Bon Secour Bay) present a unique opportunity to investigate the trophic role of predators across highly variable regions within a larger estuarine seascape (nGoM). These three sub-regions roughly align with state boundaries; therefore, for simplicity's sake, we will refer to them as Louisiana or LA (Chandeleur Sound), Mississippi or MS (Mississippi Sound), and Alabama or AL (Bon Secour Bay).

### 2.2. Sample collection

Sampling occurred seasonally (April/May and September/October) from fall 2015 to spring 2017 across the study region (Fig. 1) using bottom longline protocols standardized by the National Marine Fisheries Service and previously used in the region (Drymon et al., 2010). The gear consisted of 1.85 km (1 nmi) of 4 mm monofilament (545 kg test) set with 100 gangions baited with Atlantic mackerel (*Scomber scombrus*). Each gangion was comprised of a longline snap, a 15/0 circle hook, and 3.66 m of 3 mm (320 kg test) monofilament. Soak time per bottom longline set was standardized at 1 h. All individuals that could be safely boarded were removed from the main line, unhooked, enumerated by species, measured (to the nearest mm total length), weighed (to the nearest kg), and released. Prior to release, a 5 mL blood sample was collected from individuals via caudal venipuncture. Blood samples were placed in additive-free vacutainers and centrifuged onboard to extract blood plasma. Plasma samples were stored in a −18 °C freezer onboard, then transferred to a −80 °C freezer once on land until later processing. To eliminate the potential of maternal transfer influencing isotopic values from mother to offspring, individuals with open umbilical scars (an indication of recent parturition) were not sampled for blood (Olin et al., 2011).

### 2.3. Stable isotope analysis

The seven most abundant predator species were selected for subsequent analyses: Atlantic sharpnose shark (*Rhizoprionodon terraenovae*, n = 82), red drum (n = 77), blacktip shark (*Carcharhinus limbatus*, n = 65), southern stingray (*Hypanus americanus*, n = 38), blacknose shark (*C. acronotus*, n = 21), spinner shark (*C. brevipinna*, n = 19), and bull shark (*C. leucas*, n = 13). Blood plasma samples were dried at 60 °C and then homogenized using a mortar and pestle. Lipids in animal tissues are depleted in $\delta^{13}$C when compared with whole organisms or proteins, which may compromise interspecies comparisons (Post et al., 2007; Kim and Koch, 2012; Carlisle et al., 2017). Therefore, all tissues underwent lipid extraction using a 2:1 chloroform:methanol rinse following modified protocols from Bligh and Dyer (1959) and Hussey et al. (2012b). Additionally, urea is a metabolically-formed waste product prevalent in elasmobranch tissues that is depleted in $^{15}$N (Post et al., 2007; Kim and Koch, 2012; Carlisle et al., 2017). Thus, urea was also extracted from all elasmobranch tissues using three
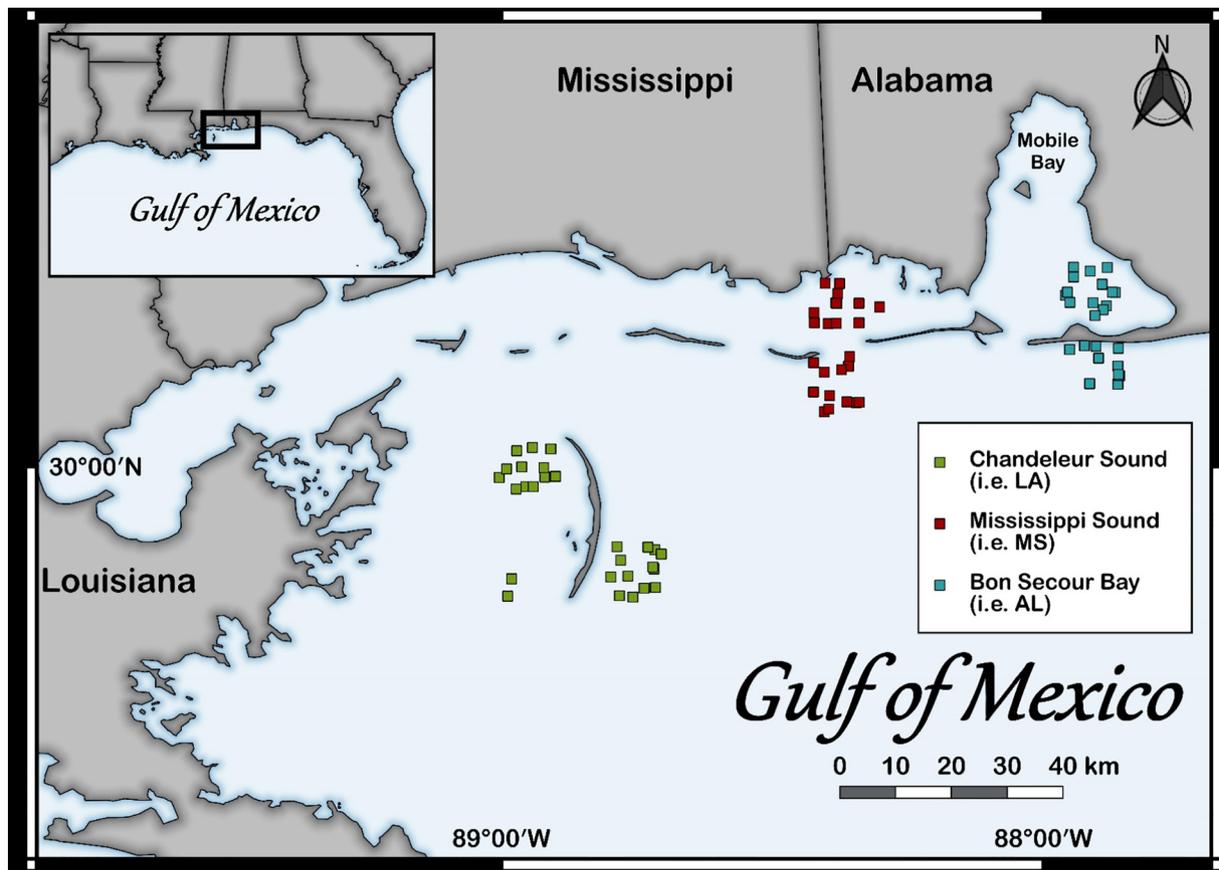
**Fig. 1.** Bottom longline sampling effort ($n = 77$) in the coastal waters of the northcentral Gulf of Mexico from fall 2015 through spring 2017. For simplicity's sake, these three regions will be described by state; specifically, Louisiana (i.e., Chandeleur Sound), Mississippi (i.e., Mississippi Sound), and Alabama (i.e., Bon Secour Bay).

rounds of milli-Q water sonication, according to a modified protocol from Kim and Koch (2012). Teleost samples were not urea-extracted due to the low amount of urea in their tissues compared to elasmobranchs (Kim and Koch, 2012). Following extractions, the samples were then re-dried, re-homogenized, weighed using a microbalance, packed in tin capsules, and placed in 96-well plates until analysis.

Stable isotope ratios of $^{13}$C:$^{12}$C and $^{15}$N:$^{14}$N were measured using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California, Davis (UCD) Stable Isotope Facility. Stable isotope ratios of $^{34}$S:$^{32}$S were measured using an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA) and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen) at the Washington State University (WSU) Stable Isotope Core Laboratory. Stable isotope ratios for carbon, nitrogen, and sulfur are expressed in delta notation following the formula:

$$\delta X = \left[ (R_{sample} / R_{standard}) - 1 \right] \times 1000$$

where X is the heavy isotope, $R_{sample}$ is the ratio of heavy to light isotope in the sample, and $R_{standard}$ is the ratio of heavy to light isotope in the reference standard. The reference standard materials were Pee Dee Belemnite for carbon, atmospheric $N_2$ for nitrogen, and Vienna Canyon Diablo Troilite for sulfur. Values for carbon, nitrogen, and sulfur isotope ratios are expressed as per mil (‰). The long-term standard deviations were 0.2‰ for $\delta^{13}$C, 0.3‰ for $\delta^{15}$N, and 0.5‰ for $\delta^{34}$S.

### 2.4. Statistical analysis

All statistical analyses were performed in PRIMER (Plymouth Routines in Multivariate Ecological Research, version 7.0.13) statistical package and the PERMANOVA+ add-on package (Anderson et al., 2008; Clarke and Gorley, 2015) or Rstudio (version 1.2.1335, R Core Team, 2019) with significant values set at $p < 0.05$ where appropriate.

We used a PERMANOVA on normalized isotope data (Anderson et al., 2008) to investigate differences in each stable isotope ($\delta^{13}$C, $\delta^{15}$N, or $\delta^{34}$S) across region, season, and their interaction. Linear regressions were used to examine relationships between individual isotopes and fork length (cm) or disk width (cm) for each species. Standard ellipse areas corrected for small sample sizes (SEAc) were then calculated for each species using the SIAR package (Parnell et al., 2008). The SEAc of a species represents its core isotopic niche for each bivariate combination of isotope ($\delta^{13}$C and $\delta^{15}$N, $\delta^{34}$S and $\delta^{15}$N, $\delta^{13}$C and $\delta^{34}$S) based on approximately 40% of the data (Jackson et al., 2011; Every et al., 2017; Shipley et al., 2019). Bivariate ellipses were calculated for each species by region and season, then averaged to produce the mean SEAc values for each species regionally and seasonally.

Total trophic niche overlap was then calculated using the nicheROVER package, which uses a Bayesian framework with 95% of the data to calculate the probability of species A sharing isotopic space with species B and vice versa (Swanson et al., 2015). The degree of isotopic niche overlap was calculated as follows: 1) by examining seasonal differences in isotopic niche overlap of the predatory species in Louisiana, Mississippi, and Alabama using only $\delta^{13}$C and $\delta^{15}$N, the most common isotopes used in trophic ecology studies, and 2) by incorporating all three tracers, $\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S, in seasonal isotopic niche overlap calculations of the same predator assemblage. A minimum of five individuals of each species in each region and season was required to calculate isotopic overlap, yet a sample size of 10 was preferred (Jackson et al., 2011; Swanson et al., 2015). Biologically significant overlap was defined as overlap >60%, in accordance with criteria defined in recent
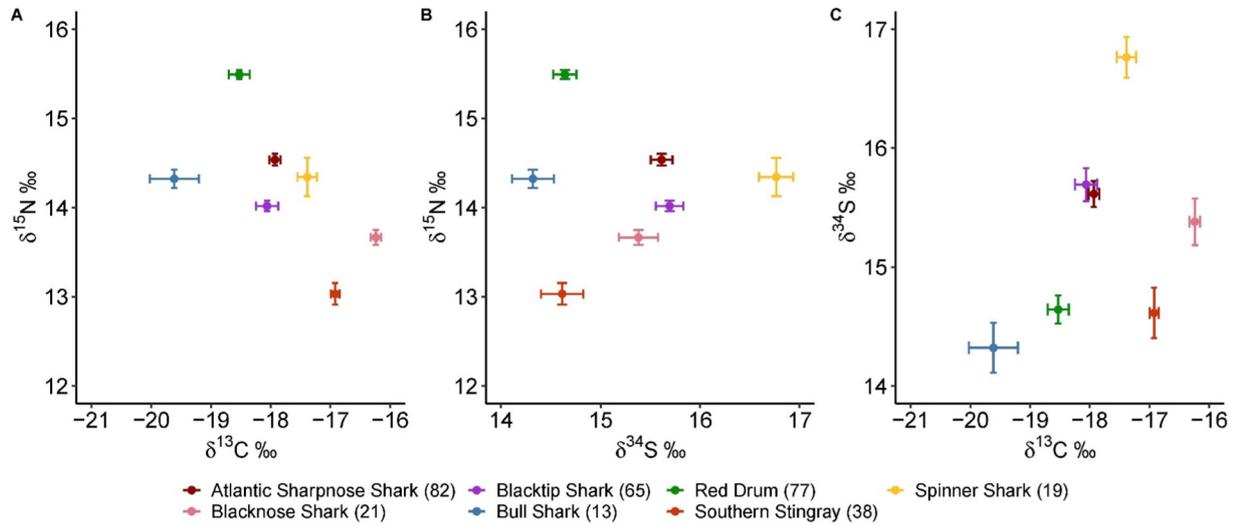
**Fig. 2.** Isotope biplots of the mean ($\pm$SE) values for A) $\delta^{13}$C vs. $\delta^{15}$N, B) $\delta^{34}$S vs. $\delta^{15}$N, and C) $\delta^{13}$C vs. $\delta^{34}$S for the seven species in this study. Sample sizes are noted in parentheses.

stable isotope comparisons (Guzzo et al., 2013; Dance et al., 2018; Heuvel et al., 2019).

## 3. Results

Stable isotope values varied across regions and seasons. Carbon stable isotope values were significantly different across regions (PERMANOVA: Pseudo-$F$ = 35.8, $p$ < 0.01). Nitrogen and sulfur stable isotope values were significantly different across regions (PERMANOVA ($^{15}$N): Pseudo-$F$ = 9.1, p < 0.01; PERMANOVA ($^{34}$S): Pseudo-$F$ = 7.5, p < 0.01) and a significant interaction was identified between region and season (PERMANOVA ($^{15}$N): Pseudo-$F$ = 11.1, p < 0.01; PERMANOVA ($^{34}$S): Pseudo-$F$ = 9.2, p < 0.01). Blacknose sharks had the highest average $\delta^{13}$C values ($-16.2 \pm 0.1$‰) and bull sharks had the lowest ($-19.6 \pm 0.4$‰). Red drum exhibited the highest average $\delta^{15}$N values ($15.5 \pm 0.1$‰) while southern stingray had the lowest ($13.0 \pm 0.1$‰). Spinner sharks had the highest average $\delta^{34}$S values ($16.8 \pm 0.8$‰) and bull sharks had the lowest ($14.3 \pm 0.8$‰) (Fig. 2). Length and $\delta^{13}$C were significantly related for Atlantic sharpnose sharks, blacktip sharks, bull sharks, and red drum. Length and $\delta^{15}$N were significantly related for Atlantic sharpnose sharks and spinner sharks. Length and $\delta^{34}$S were significantly related for Atlantic sharpnose sharks, red drum, and spinner sharks (Table 1).

### 3.1. Louisiana

In Louisiana, Atlantic sharpnose sharks, blacktip sharks, red drum, and spinner sharks were encountered in both seasons ($n \geq 5$), while

southern stingrays were only captured in spring (Table 2). The largest mean SEAc ($2.5 \pm 0.6$‰$^2$) was occupied by red drum in the fall and the smallest mean SEAc values ($0.7 \pm 0.1$‰$^2$ and $0.7 \pm 0.2$‰$^2$) were occupied by spinner sharks in fall and red drum in spring, respectively (Table 2). In spring, the only biologically significant isotopic overlap was observed for blacktip sharks within the isotopic space of southern stingrays (73%) (Table 3; Fig. 3). No biologically significant overlap was observed in fall (Table 3).

### 3.2. Mississippi

In Mississippi, Atlantic sharpnose sharks, blacktip sharks, and southern stingrays were encountered in both seasons ($n \geq 5$), while blacknose sharks, bull sharks, and red drum were only captured in the spring (Table 2). The largest mean SEAc ($1.9 \pm 0.4$‰$^2$) was occupied by blacktip sharks in the fall and the smallest mean SEAc ($0.8 \pm 0.1$‰$^2$) was occupied by blacktip sharks in the spring (Table 2). In spring, biologically significant overlap was observed for blacktip sharks and red drum within the isotopic space of Atlantic sharpnose sharks (87% and 71%, respectively) (Table 4; Fig. 4). In fall, biologically significant overlap was observed for Atlantic sharpnose sharks in the isotopic space of blacktip sharks (79%) (Table 4).

### 3.3. Alabama

In Alabama, blacknose sharks and red drum were encountered in both seasons (n ≥ 5), while blacktip sharks and bull sharks were only captured in the fall (Table 2). The largest mean SEAc ($2.1 \pm 0.5$‰$^2$)

**Table 1**

Results from linear regressions testing the relationships between fork length (or disk width for stingrays) and each individual isotope ($\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S). Species data were combined across regions and seasons. Significant values ($p < 0.05$) are written in **bold**.

| | $\delta^{13}$C | | | $\delta^{15}$N | | | $\delta^{34}$S | | |
|---|---|---|---|---|---|---|---|---|---|
| Species | Slope | Adjusted R$^2$ | p-Value | Slope | Adjusted R$^2$ | p-Value | Slope | Adjusted R$^2$ | p-Value |
| Atlantic sharpnose shark | 0.053 | 0.285 | **<0.001** | 0.033 | 0.212 | **<0.001** | 0.053 | 0.200 | **<0.001** |
| Blacknose shark | 0.008 | −0.015 | 0.414 | 0.009 | 0.002 | 0.319 | 0.027 | 0.033 | 0.210 |
| Blacktip shark | 0.047 | 0.423 | **<0.001** | 0.002 | −0.006 | 0.438 | −0.002 | −0.015 | 0.818 |
| Bull shark | 0.062 | 0.682 | **<0.001** | −0.007 | 0.073 | 0.190 | −0.008 | −0.046 | 0.508 |
| Red drum | −0.146 | 0.272 | **<0.001** | 0.006 | −0.006 | 0.478 | −0.041 | 0.038 | **0.048** |
| Southern stingray | 0.005 | 0.013 | 0.230 | 0.000 | −0.023 | 0.956 | 0.013 | 0.003 | 0.297 |
| Spinner shark | 0.000 | −0.059 | 0.970 | −0.031 | 0.595 | **<0.001** | 0.023 | 0.530 | **<0.001** |

**Table 2**

Average (mean ± SE) fork length (FL) or disk width (DW), δ¹³C, δ¹⁵N, δ³⁴S, and average SEAc values for each species by region and season. n = number of samples analyzed for all three isotopes. Dashes indicate where species were not present in sufficient numbers for overlap analyses in that community. Overall community averages are listed under all species, indicated by the row names of "*Average.*" Some species had low sample size (n < 10) and therefore results should be cautiously interpreted.

| | | Spring | | | | | | Fall | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| State | Species | n | FL/DW (cm) | δ¹³C (‰) | δ¹⁵N (‰) | δ³⁴S (‰) | Avg SEAc (‰²) | n | FL/DW (cm) | δ¹³C (‰) | δ¹⁵N (‰) | δ³⁴S (‰) | Avg SEAc (‰²) |
| LA | Atlantic sharpnose shark | 21 | 72.2 ± 1.3 | −17.7 ± 0.2 | 14.6 ± 0.2 | 15.9 ± 1.1 | 1.6 ± 0.8 | 6 | 65.6 ± 2.8 | −17.4 ± 0.2 | 14.5 ± 0.1 | 15.8 ± 0.6 | 0.9 ± 0.2 |
| | Blacktip shark | 14 | 103.9 ± 2.4 | −17.2 ± 0.1 | 14.0 ± 0.2 | 16.6 ± 0.6 | 0.8 ± 0.2 | 8 | 96.4 ± 3.9 | −17.0 ± 0.2 | 14.2 ± 0.2 | 15.1 ± 0.5 | 0.8 ± 0.1 |
| | Red drum | 23 | 84.8 ± 0.7 | −17.3 ± 0.1 | 15.3 ± 0.1 | 14.7 ± 0.9 | 0.7 ± 0.2 | 10 | 84.5 ± 1.0 | −18.1 ± 0.3 | 15.5 ± 0.3 | 14.0 ± 1.3 | 2.5 ± 0.6 |
| | Southern stingray | 15 | 84.4 ± 3.3 | −16.7 ± 0.1 | 13.2 ± 0.2 | 15.3 ± 1.3 | 2.2 ± 0.6 | 0 | – | – | – | – | – |
| | Spinner shark | 8 | 106.8 ± 5.2 | −17.7 ± 0.3 | 13.4 ± 0.1 | 17.2 ± 0.9 | 1.1 ± 0.1 | 11 | 70.0 ± 2.2 | −17.2 ± 0.2 | 15.0 ± 0.1 | 16.4 ± 0.4 | 0.7 ± 0.1 |
| | *Average* | 16.2 | 90.4 ± 2.6 | −17.3 ± 0.1 | 14.3 ± 0.1 | 15.7 ± 1.3 | 1.3 ± 0.3 | 8.8 | 79.1 ± 2.5 | −17.4 ± 0.1 | 14.9 ± 0.1 | 15.3 ± 1.3 | 1.2 ± 0.2 |
| MS | Atlantic sharpnose shark | 34 | 70.4 ± 1.2 | −18.2 ± 0.1 | 14.5 ± 0.1 | 15.8 ± 0.9 | 1.8 ± 0.1 | 21 | 74.4 ± 0.5 | −18.0 ± 0.1 | 14.6 ± 0.1 | 14.9 ± 0.9 | 1.1 ± 0.3 |
| | Blacknose shark | 6 | 93.6 ± 2.2 | −16.1 ± 0.1 | 13.7 ± 0.2 | 15.9 ± 0.9 | 0.9 ± 0.2 | 0 | – | – | – | – | – |
| | Blacktip shark | 14 | 105.8 ± 4.8 | −17.3 ± 0.1 | 14.1 ± 0.1 | 15.9 ± 0.6 | 0.8 ± 0.1 | 19 | 86.4 ± 2.4 | −18.2 ± 0.2 | 14.0 ± 0.1 | 14.7 ± 1.3 | 1.9 ± 0.4 |
| | Bull shark | 8 | 102.1 ± 4.0 | −18.9 ± 0.5 | 14.1 ± 0.1 | 14.2 ± 0.6 | 1.7 ± 0.9 | 0 | – | – | – | – | – |
| | Red drum | 27 | 89.4 ± 0.5 | −18.5 ± 0.1 | 15.4 ± 0.1 | 14.8 ± 0.8 | 0.9 ± 0.3 | 0 | – | – | – | – | – |
| | Southern stingray | 14 | 98.4 ± 3.0 | −16.9 ± 0.1 | 13.3 ± 0.2 | 14.6 ± 1.1 | 1.3 ± 0.5 | 9 | 82.4 ± 3.0 | −17.3 ± 0.1 | 12.3 ± 0.1 | 13.6 ± 1.0 | 0.9 ± 0.3 |
| | *Average* | 17.2 | 93.3 ± 2.6 | −17.9 ± 0.1 | 14.4 ± 0.1 | 15.3 ± 1.0 | 1.2 ± 0.4 | 16.3 | 81.1 ± 1.9 | −17.9 ± 0.1 | 13.9 ± 0.1 | 14.6 ± 1.2 | 1.3 ± 0.4 |
| AL | Blacknose shark | 8 | 89.6 ± 1.9 | −16.2 ± 0.1 | 13.8 ± 0.2 | 15.4 ± 1.0 | 0.9 ± 0.2 | 7 | 85.7 ± 3.3 | −16.4 ± 0.2 | 13.5 ± 0.1 | 14.9 ± 0.7 | 0.9 ± 0.2 |
| | Blacktip shark | 0 | – | – | – | – | – | 10 | 68.9 ± 3.0 | −21.0 ± 0.3 | 14.0 ± 0.1 | 16.5 ± 0.5 | 0.8 ± 0.3 |
| | Bull shark | 0 | – | – | – | – | – | 5 | 71.4 ± 0.9 | −20.7 ± 0.2 | 14.6 ± 0.2 | 14.6 ± 1.0 | 1.0 ± 0.3 |
| | Red drum | 9 | 92.2 ± 1.5 | −21.8 ± 0.5 | 16.1 ± 0.1 | 13.8 ± 0.9 | 1.6 ± 0.8 | 8 | 86.0 ± 2.1 | −19.0 ± 0.3 | 15.6 ± 0.3 | 15.7 ± 0.9 | 2.1 ± 0.5 |
| | *Average* | 8.5 | 90.9 ± 1.7 | −19.2 ± 0.7 | 15.0 ± 0.3 | 14.53 ± 1.21 | 1.3 ± 0.5 | 7.5 | 78.0 ± 2.4 | −19.4 ± 0.4 | 14.4 ± 0.2 | 15.6 ± 1.0 | 1.2 ± 0.3 |

was occupied by red drum in the fall and the smallest mean SEAc (0.8 ± 0.3‰²) was occupied by blacktip sharks, also in the fall (Table 2). No biologically significant overlap was observed in spring or fall for Alabama (Table 5; Fig. 5).

### 3.4. Two versus three isotopes

Substantially higher overlap was shown when calculating overlap using two (carbon and nitrogen) versus three (carbon, nitrogen, and sulfur) isotopes. In Louisiana, the average total isotopic overlap in spring for two isotopes was 30.6 ± 5.3%, compared to 15.1 ± 3.7% for three isotopes. For fall in Louisiana, the average total isotopic overlap for two isotopes was 49.5 ± 6.8%, compared to 14.3 ± 5.0% for three isotopes

(Fig. 6). In Mississippi, the average total isotopic overlap in spring for two isotopes was 21.3 ± 4.6%, compared to 13.6% ± 3.8% for three isotopes. For fall in Mississippi, the average total isotopic overlap for two isotopes was 23.8 ± 14.8%, compared to 20.0 ± 13.1% for three isotopes (Fig. 6). In Alabama, the average total isotopic overlap in spring for both two and three isotopes was 0.0 ± 0.0%. For fall in Alabama, the average total isotopic overlap for two isotopes was 7.7 ± 3.5%, compared to 2.4 ± 1.1% for three isotopes (Fig. 6).

## 4. Discussion

The nGoM is a dynamic, complex region, characterized by a wide range of baseline resources differing in carbon, nitrogen, and sulfur

**Table 3**

Total isotopic niche overlap probability (%) for the species present in Louisiana according to all three isotopes (carbon, nitrogen, and sulfur), divided by season (spring on the left, fall on the right). Results are presented as two different overlap estimates for each species comparison, based on whether species A is being compared to B and vice versa. For example, in spring, 23% of Atlantic sharpnose sharks' isotopic niche overlapped with the isotopic niche of blacktip sharks, but 31% of blacktip sharks' isotopic niche overlapped with the isotopic niche of Atlantic sharpnose sharks. Not all species were represented in each season; species absence is indicated by dashes. A color gradient was applied such that darker colors coordinate with higher overlap; a scale is present underneath the matrix. Values in white indicate biologically meaningful overlap, defined as overlap >60%.



**Species B**

| | | SPRING | | | | | FALL | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species A | | Atlantic Sharpnose | Blacktip | Red Drum | Southern Stingray | Spinner | Atlantic Sharpnose | Blacktip | Red Drum | Southern Stingray | Spinner |
| | Atlantic Sharpnose | | 23 | 8 | 24 | 5 | | 37 | 3 | --- | 44 |
| | Blacktip | 31 | | 2 | 73 | 13 | 34 | | 3 | --- | 5 |
| | Red Drum | 19 | 4 | | 20 | 0 | 2 | 2 | | --- | 0 |
| | Southern Stingray | 5 | 15 | 2 | | 2 | --- | --- | --- | | --- |
| | Spinner | 9 | 26 | 0 | 21 | | 35 | 5 | 1 | --- | |

| Color | % overlap | Color | % overlap |
|---|---|---|---|
| | 0 - 10 | | 51 - 60 |
| | 11 - 20 | | 61 - 70 |
| | 21 - 30 | | 71 - 80 |
| | 31 - 40 | | 81 - 90 |
| | 41 - 50 | | 91 - 100 |
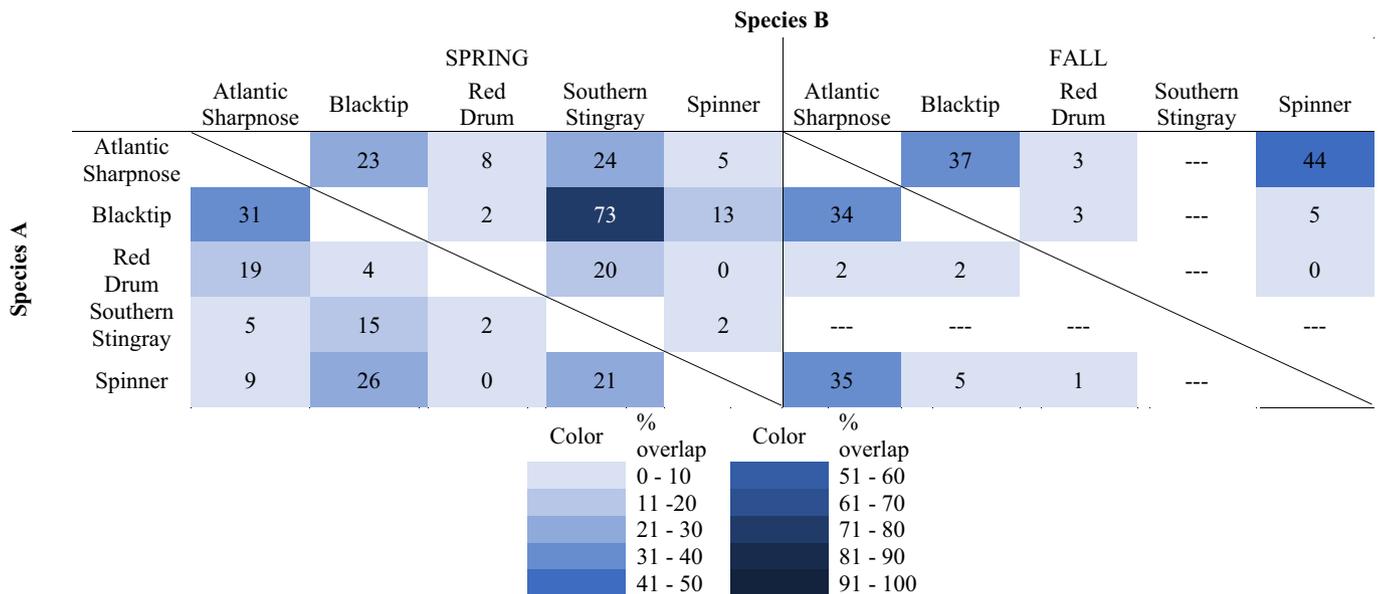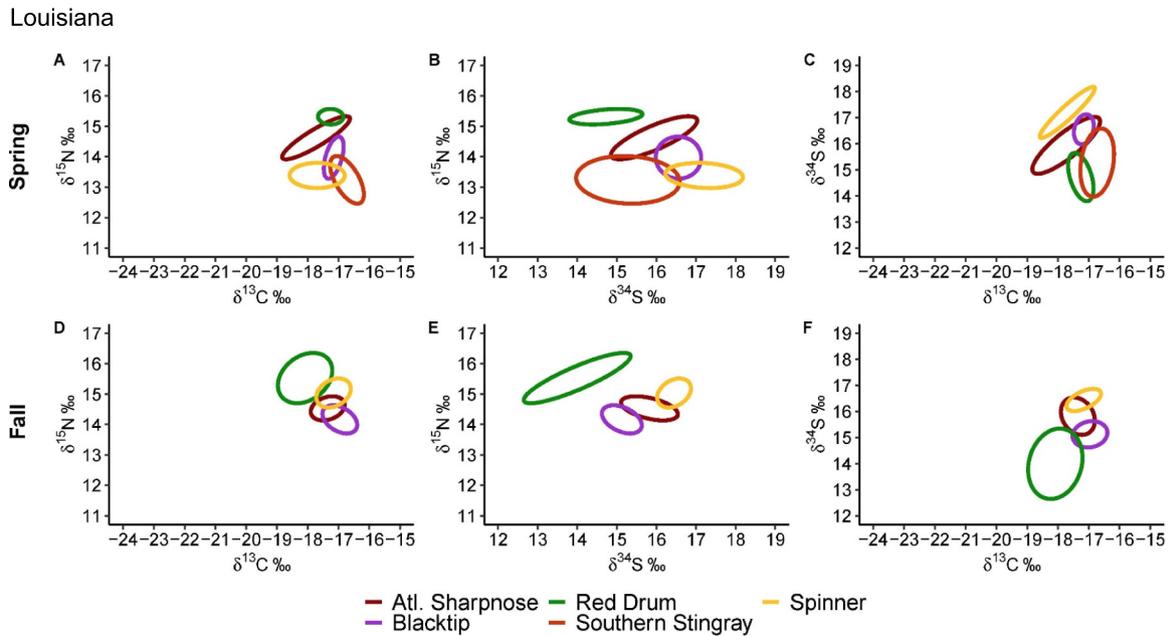
**Fig. 3.** Standard ellipse areas corrected for small sample size (SEAc) incorporating 40% of the data for $\delta^{13}C$ vs. $\delta^{15}N$ (A, D), $\delta^{34}S$ vs. $\delta^{15}N$ (B, E), and $\delta^{13}C$ vs. $\delta^{34}S$ (C, F) for Louisiana in spring (A–C) and fall (D–F). Colors coordinate with species involved in overlap calculations for Louisiana.

isotope values. Within this region, multiple inlets and bays create several estuarine seascapes that vary in environmental parameters and prey assemblages. These regions are subject to large fluctuations in salinity, temperature, and dissolved oxygen, as well as seasonal shifts in weather patterns. For the seven species examined in this study, both regional and seasonal isotopic niche shifts were identified, implying that the predatory species in this region exhibit greater trophic flexibility than previously assumed.

The most enriched ^{15}N values were observed in red drum, yet it is unlikely that these fish feed at a higher trophic level than the other predatory species examined. Instead, this discrepancy may be attributed to the resources in regions where these fish spend the majority of their time. Red drum occupy estuaries and coastal waters <20 m, feeding on crabs and teleosts in nearshore, shallow water habitats (Scharf and Schlicht, 2000; Powers et al., 2012). The estuarine environments of the nGoM, notably the Mobile Bay Estuary, are susceptible to

**Table 4**

Total isotopic niche overlap probability (%) for the species present in Mississippi according to all three isotopes (carbon, nitrogen, and sulfur), divided by season (spring on the left, fall on the right). Results are presented as two different overlap estimates for each species comparison, based on whether species A is being compared to B and vice versa. For example, in spring, 27% of Atlantic sharpnose sharks' isotopic niche overlapped with the isotopic niche of blacktip sharks, but 87% of blacktip sharks' isotopic niche overlapped with the isotopic niche of Atlantic sharpnose sharks. Not all species were represented in each season; species absence is indicated by dashes. A color gradient was applied such that darker colors coordinate with higher overlap; a scale is present underneath the matrix. Values in white indicate biologically meaningful overlap, defined as overlap >60%.

| | **Species B** | | | | | | | | | | | |
| | | SPRING | | | | | | FALL | | | | |
| Species A | Atlantic Sharpnose | Blacknose | Blacktip | Bull | Red Drum | Southern Stingray | Atlantic Sharpnose | Blacknose | Blacktip | Bull | Red Drum | Southern Stingray |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Atlantic Sharpnose | | 1 | 27 | 10 | 17 | 1 | | --- | 79 | --- | --- | 0 |
| Blacknose | 8 | | 15 | 6 | 0 | 31 | --- | | --- | --- | --- | --- |
| Blacktip | 87 | 6 | | 13 | 1 | 9 | 37 | --- | | --- | --- | 0 |
| Bull | 50 | 1 | 13 | | 0 | 5 | --- | --- | --- | | --- | --- |
| Red Drum | 71 | 0 | 1 | 0 | | 0 | --- | --- | --- | --- | | --- |
| Southern Stingray | 4 | 15 | 9 | 7 | 0 | | 0 | --- | 4 | --- | --- | |

| Color | % overlap | Color | % overlap |
|---|---|---|---|
| | 0 - 10 | | 51 - 60 |
| | 11 - 20 | | 61 - 70 |
| | 21 - 30 | | 71 - 80 |
| | 31 - 40 | | 81 - 90 |
| | 41 - 50 | | 91 - 100 |
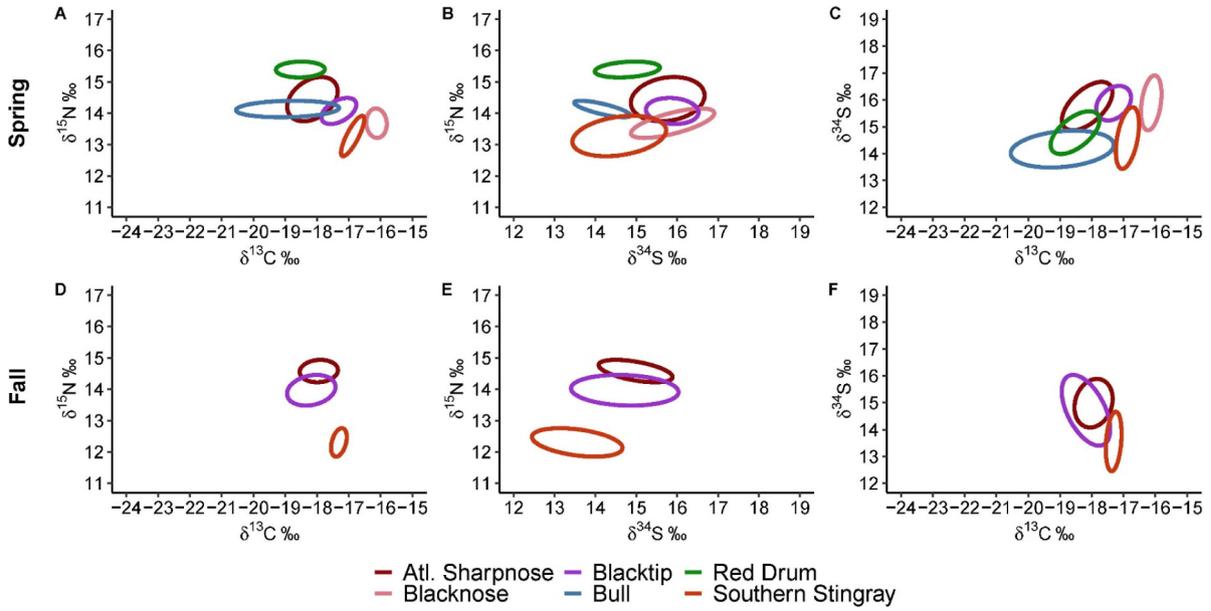
Mississippi



**Fig. 4.** Standard ellipse areas corrected for small sample size (SEAc) incorporating 40% of the data for δ¹³C vs. δ¹⁵N (A, D), δ³⁴S vs. δ¹⁵N (B, E), and δ¹³C vs. δ³⁴S (C, F) for Mississippi in spring (A–C) and fall (D–F). Colors coordinate with species involved in overlap calculations for Mississippi.

nitrogenous waste runoff and other terrestrial influences, especially during heavy rains in spring (Osterman and Smith, 2012). Sewage, farm waste, and other nitrogenous waste from terrestrial sources may contribute to higher δ¹⁵N values in predators in coastal ecosystems (Montoya, 2007; Mancinelli and Vizzini, 2015). Consequently, the high average δ¹⁵N value of red drum in Alabama during the spring suggests that the basal nitrogen sources for red drum may be heavily influenced by an influx of freshwater and terrestrial sources, rather than indicative of higher trophic level feeding.

Size was a significant factor for all predatory species examined for at least one isotope, with the exception of blacknose sharks and southern stingrays. The positive relationship between size and δ¹³C observed in both blacktip and bull sharks suggests an expansion in foraging grounds inshore and offshore (Plumlee and Wells, 2016), while the negative relationship for red drum suggests an increase in estuarine-based sources into the diet of larger individuals (Thayer et al., 1983; Koch, 2007; Matich and Heithaus, 2014). Larger red drum also had significantly lower δ³⁴S values, suggesting increased consumption of benthic prey

**Table 5**
Total isotopic niche overlap probability (%) for the species present in Alabama according to all three isotopes (carbon, nitrogen, and sulfur), divided by season (spring on the left, fall on the right). Results are presented as two different overlap estimates for each species comparison, based on whether species A is being compared to B and vice versa. For example, in fall, 14% of bull sharks' isotopic niche overlapped with the isotopic niche of red drum, but 3% of red drum's isotopic niche overlapped with the isotopic niche of bull sharks. Not all species were represented in each season; species absence is indicated by dashes. A color gradient was applied such that darker colors coordinate with higher overlap; a scale is present underneath the matrix. Values in white indicate biologically meaningful overlap, defined as overlap >60%.

|  |  | Species B | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
|  |  | SPRING | | | | FALL | | | |
|  |  | Blacknose | Blacktip | Bull | Red Drum | Blacknose | Blacktip | Bull | Red Drum |
| Species A | Blacknose |  | --- | --- | 0 |  | 0 | 0 | 1 |
|  | Blacktip | --- |  | --- | --- | 0 |  | 2 | 5 |
|  | Bull | --- | --- |  | --- | 0 | 2 |  | 14 |
|  | Red Drum | 0 | --- | --- |  | 0 | 2 | 3 |  |

| Color | % overlap | Color | % overlap |
|---|---|---|---|
|  | 0 - 10 |  | 51 - 60 |
|  | 11 - 20 |  | 61 - 70 |
|  | 21 - 30 |  | 71 - 80 |
|  | 31 - 40 |  | 81 - 90 |
|  | 41 - 50 |  | 91 - 100 |
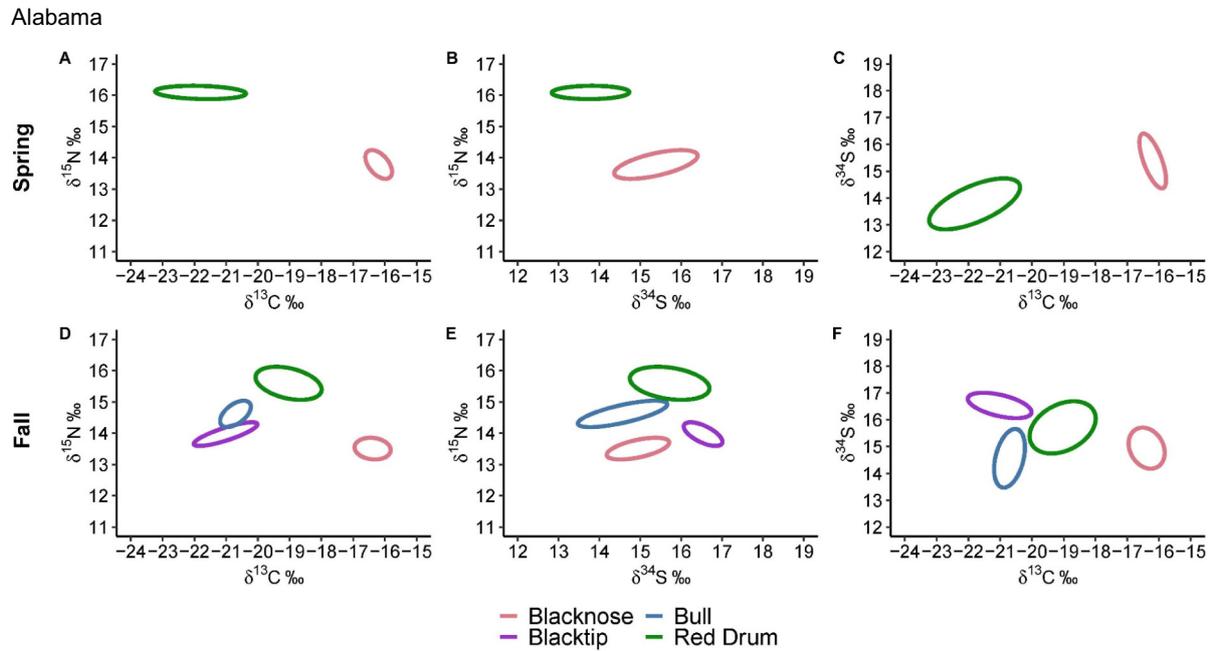
**Fig. 5.** Standard ellipse areas corrected for small sample size (SEAc) incorporating 40% of the data for δ¹³C vs. δ¹⁵N (A, D), δ³⁴S vs. δ¹⁵N (B, E), and δ¹³C vs. δ³⁴S (C, F) for Alabama in spring (A–C) and fall (D–F). Colors coordinate with species involved in overlap calculations for Alabama.

(Chasar et al., 2005; Heuvel et al., 2019). Spinner sharks were the only species to have a significant negative relationship between length and δ¹⁵N. This was likely a result of a strong seasonal influence on isotope values rather than size itself; nitrogen values were lower in spring in Louisiana, possibly related to a shift in the prey base and/or anthropogenic inputs. A positive relationship was observed between length and δ³⁴S for spinner sharks, suggesting larger individuals are incorporating more pelagic prey into their diet, corroborating previous work with stomach contents of spinner sharks (Bethea et al., 2004; Connolly et al., 2004). The positive correlations between length and all three

isotopes observed for Atlantic sharpnose sharks suggest that this species undergoes ontogenetic dietary shifts. Previous studies corroborate these results, describing smaller individuals as dietary specialists, preying mainly on invertebrates, while larger individuals consume a wider range of prey items, including teleosts and other elasmobranchs, which can lead to increased carbon, nitrogen, and sulfur values (Hoffmayer and Parsons, 2003; Drymon et al., 2012).

A fast turnover tissue was imperative for identifying seasonal changes in isotopic niche patterns among predatory species across the nGoM. The seasonal fluctuations that occur across this large estuarine
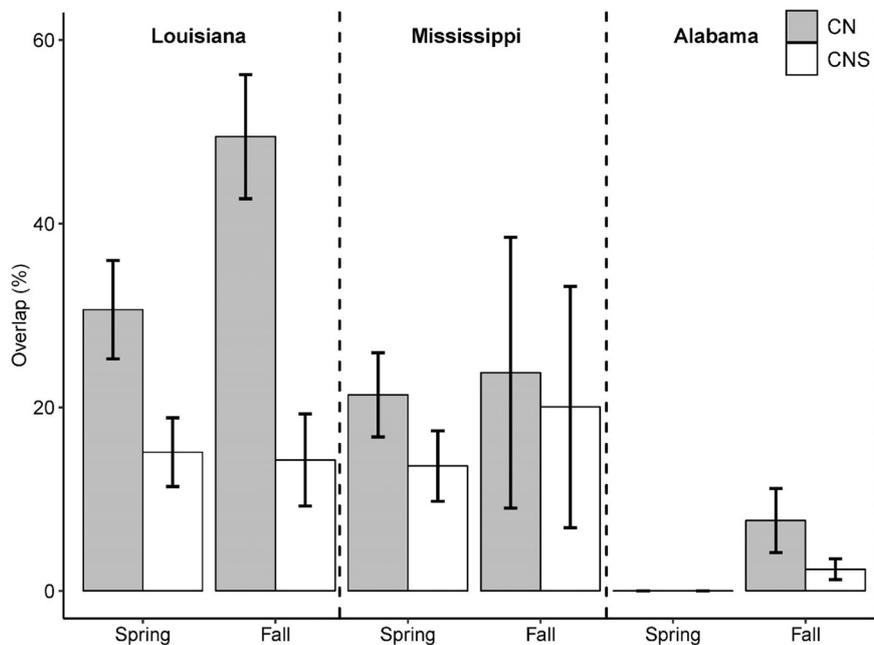


**Fig. 6.** Bar plot of means plus standard errors of the total isotopic niche overlap calculations using two isotopes (δ¹³C and δ¹⁵N, indicated by the gray bars) or all three isotopes (δ¹³C, δ¹⁵N, and δ³⁴S, indicated by the white bars) for each region by season.

seascape affect habitat suitability and prey availability, especially for large, mobile predators (Bizzaro et al., 2017; Pool et al., 2017). In Louisiana, for example, seasonal shifts in the isotopic relationships of spinner sharks versus Atlantic sharpnose sharks or blacktip sharks suggest that spinner sharks demonstrate flexibility in their isotopic space according to seasonal pulses of available prey items. Both spinner and blacktip sharks prefer to feed on the planktivorous Gulf menhaden *Brevoortia patronus*, which are common in large schools in nearshore waters in the spring but move offshore to spawn in the fall (Roithmayr and Waller, 1963; Hoffmayer and Parsons, 2003; Bethea et al., 2004). With fewer Gulf menhaden available, spinner sharks may expand their diet to include other small teleost species in the fall, thereby increasing their isotopic niche and sharing isotopic space with another generalist, the Atlantic sharpnose shark. Seasonal shifts in Gulf menhaden may have also resulted in seasonal shifts of isotopic niche overlap between Atlantic sharpnose sharks and blacktip sharks in Mississippi. Fall blacktip sharks likely broadened their diet to include the benthic-feeding Atlantic croaker (*Micropogonias undulatus*), which expanded the total isotopic space occupied by blacktip sharks (Moncreiff and Sullivan, 2001). Seasonal fluctuations in the isotopic relationships of these species may have been oversimplified with the use of a longer turnover tissue, such as muscle.

Other instances of inter- and intraspecific fluctuations in isotopic niche patterns were observed among predatory species across regions and seasons. Given the partitioning of species by region and season, it should be noted that some isotopic patterns of species with low sample size ($n < 10$) should be interpreted with caution, particularly in Alabama where overall sample size was low. In Louisiana in spring, blacktip sharks showed significant isotopic overlap with southern stingrays. Blacktip sharks occupied a small isotopic niche, while southern stingrays occupied a larger isotopic space because of the wide variety of prey items that they consume (Funicelli, 1975; Gilliam and Sullivan, 1993). The biologically significant overlap suggests that these species may forage in the same area and potentially compete for prey items. Limited isotopic niche flexibility of other predators was identified. For example, the SEAc values of blacknose sharks in Mississippi and Alabama were virtually identical, suggesting that this species may have more limited variability in its diet and foraging location. Lastly, blacknose sharks, along with bull sharks and spinner sharks, showed no biologically significant isotopic niche overlap with any other species in this study, regardless of region or season. This indicates that, although these species are taxonomically similar (i.e., all in the genus Carcharhinus), they occupy unique isotopic, and therefore presumably trophic, space across the nGoM.

Using carbon, nitrogen, and sulfur in concert allowed for a comprehensive examination of the wide variability in resources incorporated by the different predatory species across a large estuarine gradient. Unlike the stable isotopes of nitrogen or carbon, the stable isotope of sulfur does not fractionate metabolically or trophically, rendering it a conservative natural tracer (Chasar et al., 2005). Further, $\delta^{34}S$ signatures distinctly differ from benthic to pelagic resources by as much as 30‰, which makes sulfur effective in resolving predator diet choices and foraging areas, particularly in estuarine areas where $\delta^{13}C$ signatures of dominant primary producers may overlap (Connolly et al., 2004; Chasar et al., 2005). For example, the $\delta^{13}C$ values in this study were significantly different regionally, but not seasonally, which suggests that the predatory species showed no seasonal shift in diet. However, significant region and season interactions with the stable isotope values for sulfur and nitrogen indicate otherwise, implying that seasonal carbon stable isotope signatures of primary producers may be indistinguishable in large estuarine areas (Chasar et al., 2005). Further, sulfur was useful for detecting different diet habits of the predatory species in this study, such as red drum and spinner sharks in Louisiana. The $\delta^{13}C$ values of both species suggest they feed in or near seagrass regions, according to previously published values of seagrass beds in the region by Moncreiff and Sullivan (2001). However, the $\delta^{34}S$ values of both species

show distinct differences in diet, with spinner sharks demonstrating a pelagic-based feeding strategy (higher $\delta^{34}S$) and red drum consuming more benthic prey items (lower $\delta^{34}S$) (Connolly et al., 2004; Chasar et al., 2005). In estuarine environments, $\delta^{34}S$ signatures provide finer resolution of an animal's foraging habits than $\delta^{13}C$ and $\delta^{15}N$ alone (Chasar et al., 2005; Fry and Chumchal, 2011).

While the consistently higher overlap values calculated using two isotopes (carbon and nitrogen) suggest that two-isotope characterizations may oversimplify the trophic role of predators, an alternate possibility exists. Some of the reduced overlap we observed may be mathematically induced, and not necessarily biologically relevant. Specifically, the addition of a third dimension (i.e., sulfur) inherently increases variability in n-dimensional isotopic space, which would thus reduce overlap between species. Consequently, lower overlap would be expected, as was seen in this study and others (Curnick et al., 2019; Heuvel et al., 2019). Acknowledging this potential, we still advocate that future studies consider using the combination of carbon, nitrogen, and sulfur isotopes for characterizing isotopic overlap (Pool et al., 2017; Curnick et al., 2019; Heuvel et al., 2019).

The multidimensionality of this study, which examined seasonal shifts across seven mobile predators, each of which likely consume numerous potential prey species that may incorporate different basal resources, presents a challenge when interpreting data patterns. For example, the use of blood plasma, though advantageous for detecting seasonal fluctuations in an individual's diet and foraging location, may also increase the isotopic variation of an individual within a population due to the rapid turnover (Bearhop et al., 2004). The majority of the predatory species in this study exhibit generalist feeding tendencies and a variable diet, which can complicate conclusions drawn from the stable isotope values of a fast turnover tissue (Bearhop et al., 2004; Layman et al., 2007; Newsome et al., 2007). In addition, it is important to note that without seasonally correlated stable isotope values characterizing the base of the food web, it is difficult to make direct comparisons among individual species across regions. Regional or even seasonal differences in predator stable isotope values could be an artifact of regional differences in the base of the food web (Layman et al., 2007). However, the isotopic niche relationships observed among species are likely a fair representation of the isotopic patterns in these ecosystems and provide a strong indication of variable and flexible feeding and habitat use patterns. Previous studies across these regions suggest that the potential prey bases are isotopically similar among the study sites (Moncreiff and Sullivan, 2001; Drymon et al., 2012); however, continued work to characterize carbon and sulfur isoscapes in this region will provide additional insight into future trophic investigations using stable isotope ratios.

## 5. Conclusion

The variable predatory isotopic relationships witnessed in the regions examined in this study are indicative of the variable food web dynamics that permeate the estuarine seascapes of the nGoM. The use of three isotopes (i.e., $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) provided finer resolution when describing the predatory assemblage across Louisiana, Mississippi, and Alabama, consistent with other recent studies that highlight the benefits of using three stable isotope tracers in dietary studies, particularly in regions with estuarine and fresh-water influence (Curnick et al., 2019; Heuvel et al., 2019). Limited biologically significant isotopic overlap among predatory species across these regions and seasons suggests that predatory species are occupying more nuanced roles than previously assumed. Moreover, these niches appear to be fluid, shifting in response to potential changes in prey availability and environmental parameters. Finally, this study demonstrates the importance of seasonal sampling schemes and the use of fast turnover tissues in stable isotope studies to accurately describe the trophic structure of species in a dynamic environment.

## Declaration of competing interest

None.

## Acknowledgments

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