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## Interspecific and intraspecific variability in the trophic ecology of three co-habiting apex predators in coastal Texas

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*Les analyses et les conclusions de ce travail d'étudiant n'engagent que la responsabilité de son auteur et non celle d'AGROCAMPUS OUEST*



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## Résumé

Les estuaires sont des milieux caractérisés par de fortes variations en termes de conditions environnementales et par de fortes pressions anthropiques. Pour autant, les effets de ces variations sur les relations trophiques des prédateurs apicaux restent méconnus. Plus particulièrement les prédateurs apicaux sont connus pour avoir des rôles importants dans les réseaux trophiques, conditionnant leur bon fonctionnement. Pour obtenir davantage d'information concernant ces rôles dans les estuaires du Texas, nous proposons ici de combiner analyses de contenus stomacaux et analyses des isotopes stables pour étudier l'écologie trophique de trois prédateurs apicaux : le garpique alligator (*Atractosteus spatula*), le requin bouledogue (*Carcharhinus leucas*) et le requin bordé (*Carcharhinus limbatus*). Les trois espèces se nourrissent tous de mullets en grande quantité mais se différencient par le reste de leur régime. Les garpiques se nourrissent aussi de Clupeidae alors que le requin bouledogue se nourrit de poissons-chats ou d'ombrine tachetée. Les requins bouledogues se nourrissent à un plus haut niveau trophique que les garpiques alligators. Même s'ils ciblent en partie les mêmes proies, il semblerait que les requins bouledogues limitent la compétition interspécifique en se nourrissant davantage dans les eaux à forte salinité, auxquelles les garpiques ont un accès limité. Ces résultats démontrent l'intérêt de l'utilisation conjointe des analyses de contenus stomacaux et des isotopes stables qui permettent d'apprécier plus finement ces problématiques.

## Abstract

Estuaries show high environmental condition variability and face many anthropogenic disturbances. Effects of these variations on top predators trophic relationships remain poorly understood. More specifically, top predators have key roles in food webs health and integrity. To get a better understanding of top predators roles in Texas estuaries, we proposed here to combine stomach contents analyses and stable isotopes analyses to study trophic ecology of three top predators: the alligator gar (*Atractosteus spatula*), the bull shark (*Carcharhinus leucas*) and the blacktip shark (*Carcharhinus limbatus*). The three species extensively feed on mullets but differ in the rest of their diet. Gars feed also on Clupeidae while bull sharks feed on catfish or red drums. Bull sharks have higher trophic position than alligator gars. Even if they partially target similar prey, it seems that bull shark limit interspecific competition by foraging more in high salinity waters, where gars access is restricted. These results support the joint use of stomach content analyses and stable isotopes analysis, which enable us to get a better understanding of trophic issues.



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# 1. Introduction

## 1.1. Estuaries: dynamic environments facing many threats

Estuaries constitute a significant interface between continental and marine aquatic environments and show unique spatiotemporal dynamics. The presence of permanent or periodical input of freshwater and output to the ocean generate environmental condition gradients, especially regarding salinity (Potter et al., 2010) but also in terms of temperature and dissolved oxygen. Moreover, differences in rainfall between seasons influence the river input water flow and environmental gradients throughout the year. Therefore, estuaries systems are characterized by high dynamism in terms of environmental conditions.

In addition to high natural dynamism, estuarine environments face many changes caused by anthropogenic disturbances (Kennish, 2002). Indeed, estuary areas represent hotspots for human development. This constant urbanization involves destruction and artificialization of wetlands, causing habitat loss, and substantial water flow changes. These last are also the result of upstream dam construction and intensive agriculture, which retain considerable quantities of water. The surrounding human activities are also responsible for massive pollutant inputs such as sewage and industrial chemicals, which impact water quality (van de Merwe et al., 2016). Population growth and climate change predicted for the future will likely exacerbate these disturbances on estuaries.

From an ecological perspective, estuaries are crucial areas for many species since they contain many habitats. Many species are partially or fully dependent on estuarine habitats. Indeed, estuaries provide physical shelters and high food availability due to high productivity for juveniles and thus have a nursery role (Worm et al., 2006). In addition to their ecological importance, estuaries also provide a wide range of ecosystem services (Barbier et al. 2011). These ecosystems provide coastal protection by dissipating waves and preventing erosion by retaining sediments. Also, many coastal fishery activities occur in estuary areas or rely on estuary-dependent species.

In this context, humans positively benefit from estuary services. Nevertheless, all the anthropogenic activities affect estuarine ecosystem integrity and health. Studies showed that these disturbances affect community structure and abundance in estuarine ecosystems (Baptista et al., 2010). However, the impacts of these changes on energy flow, trophic relationships, and food web structures remain poorly understood. Ecosystem management cannot be successful without identifying the trophic relationships and their variability according to ecological contexts. This knowledge would be highly valuable to face the challenges indicated by mid- and long-term estuaries integrity predictions.

## 1.2. Importance of top predators

Trophic interactions are a crucial aspect of community ecology structure because of its implications in various ecological mechanisms such as predation (when an organisms forage on another), competition (when organisms forage on the same prey), or resource partitioning (when organisms forage on different resources). Therefore, trophic interactions shape community structure and energy flow through food webs, influencing ecosystem health and functioning. Nevertheless, these relationships are not straightforward because they link a wide range of organisms, sometimes from several ecosystems, thus creating complex food webs instead of linear food chains as commonly thought (Levine, 1980). Qualitative and quantitative knowledge on the diet of each organism in its ecosystem is essential to understand their roles through different notions such as the trophic position and the trophic niche. The trophic position is the average length of the path over which an organism obtains

energy from the primary source and thus illustrate the position of an organism within the food web. Even if the ecological niche concept remains elusive (Newsome et al., 2007), it is commonly defined as a multidimensional object in a space where the axes are environmental variables (Hutchinson, 1957). When these variables are food resources, the multidimensional object is called the trophic niche.

In addition to understanding the specific roles and identifying ecological mechanisms involved, this knowledge is crucial to have a holistic vision of ecosystem functioning. In a climate change context, environmental conditions are subject to change in aquatic systems, potentially impacting food webs and trophic dynamics. Moreover, direct anthropogenic disturbances such as habitat alteration, pollution, and fishing also significantly impact trophic dynamics and represent major threats to marine ecosystem health. In this context, trophic models are useful for predicting changes and implementing efficient and sustainable management measures, especially regarding fisheries. For decades, fisheries management is mostly based on single-stock and monospecific approaches. Now, the scientific community promotes an ecosystem approach which would integrate biotic, abiotic, and human component (FAO, 2003; Garcia & Cochrane, 2005). Trophic interaction data are necessary to build and set trophic models, which are essential tools for ecosystem-based management (Schmitz., 2007; Ainsworth et al., 2014; Link & Browman, 2014). However, gaps remain for many species in many ecosystems and need to be filled to update management strategies (Braga et al., 2012).

Top predators, which are large and highly mobile species such as some elasmobranchs, large teleosts, or marine mammals, occupy important roles in aquatic food webs. Their trophic ecology recently became a major interest for ecologists. At the top of the food webs, they regulate lower trophic levels by top-down effects via both consumptive (predation) and non-consumptive (fear) effects, affecting community structures and ecosystem health (Preisser, 2007; Heithaus et al., 2008; Estes et al., 2011). It has long been considered that these predators all have similar trophic relationships and thus share similar roles in aquatic ecosystems (Cortés, 1999). However, recent studies suggest that it may be more complex than that. Indeed, some co-habiting marine predators compete for the same food resource while others have different trophic niches (Bethea et al., 2004; Bizzarro et al., 2017; Shiffman et al., 2019; Peterson et al., 2020) probably to partition prey and minimize competition (Papastamatiou et al., 2006; Heithaus et al., 2013). Besides, it appears that many species show ontogenetic shift in diet (Bethea et al., 2004, 2006, 2007; Lucifora et al., 2009; Matich et al., 2015a, 2015b; Butler et al., 2018; Shipley et al., 2019) or variability across region and season (Bethea et al., 2007; Matich et al., 2014; Varela et al., 2019), which suggest that environmental conditions may impact their diet. Studying top predators' trophic interactions and their flexibility is important to understand their roles in ecosystems and to predict environmental change consequences. These implications are crucial to improve ecosystem management strategies and for top predators conservation, especially for sharks whose populations have declined dramatically over the past decades (Myers & Worms, 2003; Fowler et al., 2005), although many species may be keystone species.

### 1.3. Stomach Contents Analyses and Stable Isotopes Analysis to study trophic ecology

Stomach Content Analysis (SCA) and Stable Isotope Analysis (SIA) are two methods commonly used to investigate trophic interactions and roles in marine environments.

SCA has been used for several decades to study different aspects of fish diet. This framework allows a wide range of qualitative and quantitative methods to identify prey and assess their relative importance, thus providing detailed insight about diet composition, prey diversity, and feeding habits (Hyslop, 1980; Cortés, 1997; Marshall & Elliott, 1997). The data obtained is beneficial for investigating species-specific trophic issues such as trophic position and trophic niche breadth but is also crucial to

compare species and highlight consumer-prey relationships within an ecosystem. Despite its precision, SCA is a snapshot of individuals diet, only providing information about recent feeding events. This approach also requires a large sample size to be representative of dietary habits (Hyslop, 1980), which can be problematic. A significant amount of individuals shows empty stomachs, especially in piscivorous species (Arrington et al., 2002; Pedreschi et al., 2015). In addition, this may be intensified in sharks, which sometimes evert their stomach under stress conditions generated during catch events (Brunnschweiler et al., 2011). This could explain that some studies reported most individuals with empty stomachs (Hoffmayer & Parsons, 2003; Lucifora et al., 2009; Estupiñán-Montaña et al., 2017). Extensive sampling is also not always achievable or appropriate because stomach content collection is invasive or lethal. Moreover, potential prey misidentification due to digestion and differences in digestion rates between prey types can lead to other biases. Therefore, SCA provides very valuable and unique insights about short term feeding behavior.

SIA recently became a popular method to study trophic structure, function, and dynamics in aquatic ecosystems (Post, 2002; Michener & Kaufman, 2007). This method relies on the natural presence of different isotopes for some elements and their variability in abundance in natural systems, making it possible to use them as natural tracers (Peterson & Fry, 1987). Nitrogen and carbon are the two most used elements to conduct SIA in marine and freshwater ecosystems (Layman et al., 2007), and their isotope composition, *i.e.* the ratio between the heavy isotope and the light isotope of the same element, provides different information. The nitrogen ratio, referred as  $\delta^{15}\text{N}$  value, is primarily used as an indicator of trophic position. Heavy elements are often conserved during chemical reactions, while light elements are consumed. This phenomenon, called isotopic fractionation, causes a high  $^{15}\text{N}$  enrichment of an individual's tissues relative to its diet, which lead to a difference in isotope composition between the consumer and its diet. The Diet-Tissue Discrimination Factor (DTDF), which quantifies this enrichment, is challenging to calculate since it requires controlled laboratory studies (Hussey et al., 2012). That is why many studies use the average value of 3.4 ‰ estimated by Post (2002). However, DTDF values are species-specific and vary across tissues, diet composition, and environmental conditions (Pinnegar & Polunin, 1999; Vanderklift & Ponsard, 2003; Barnes et al., 2007; Olin et al., 2013). Carbon ratio, referred as  $\delta^{13}\text{C}$  value, is highly variable between primary producers, and  $^{13}\text{C}$  does not show a high enrichment when incorporated as  $^{15}\text{N}$  does. Therefore,  $\delta^{13}\text{C}$  is used to predict basal carbon sources and provide information about the habitat in which individuals forage. Together,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values form a bi-dimensional area called the isotopic niche. Many metrics exist to estimate isotopic niches (Layman et al., 2007, 2012; Jackson et al., 2011), making SIA an excellent tool for ecologists to make interspecific comparisons and study niche variation (Newsome et al., 2007). However, SIA is not always straightforward, and many aspects must be considered before analysis. Because isotopes are not instantly incorporated in tissues after ingestion, isotopic values do not reflect the diet at the sampling date but provide a long-term vision. Thus, turnover values, which are species-specific and tissues-specific, need to be estimated before any interpretation. Similarly, DTDF for the species and the tissue studied needs to be acknowledged since trophic position estimation is highly sensitive to variability in DTDF. It is also possible that isotopic values need to be adjusted based on baseline, *i.e.* primary consumers, isotopic values before making any comparisons (Day et al., 2019). Interpretations should also be conducted with caution since the isotopic and trophic niche are two related but different concepts. Isotopic niche overlap between two species does not necessarily mean they feed on the same species and potentially compete.

Unlike SCA, which tells about the ingested diet, SIA provides information about the assimilated diet. SCA provides detailed information about prey taxonomy and recent feeding event while SIA informs about long term dietary habits. Even if the results obtained from these two methods are not always consistent (Petta et al., 2020), this complementarity is an opportunity to get a more robust understanding of diet variability (Clarke et al., 2005), especially for species which are not easy to study like top predators. So far, only a few studies combined SCA and SIA to investigate variations in dietary

habits within and across top predator populations (McMeans et al., 2010; Abrantes & Barnett, 2011; Trystram et al., 2016; Varela et al., 2019).

Studying trophic ecology of top predators in estuaries thus gather different central point of interest:

- Increasing our knowledge of top predators trophic ecology is essential to understand their roles and implement optimal measures to manage their populations or prevent their decline
- Benefiting from estuaries unique dynamism to study the impact of environmental conditions on predators diet.
- Understanding estuaries trophic food web functioning to be able to predict and mitigate anthropogenic disturbances impact

#### 1.4. Trophic ecology of three top predators in Texas estuaries

Texas estuarine systems are particularly concerned about natural and anthropogenic disturbances. Texas coastline is affected by frequent extreme meteorological events such as tropical storms and hurricanes. These events strongly erode the coastline, impacting shape and water flow in coastal bays and estuaries (Xu et al., 2018). Moreover, climate change is likely to increase the frequency of major hurricanes, especially in the Gulf of Mexico (Bender et al., 2010), which would exacerbate estuaries alteration. Besides, Texas estuaries face many anthropogenic disturbances such as oil and natural gas production, mining, massive agricultural production, and dam construction (TPWD, 2012). In addition to pollutant input, human activities substantially impact freshwater inflow and alter environmental conditions in estuarine ecosystems, especially regarding salinity (Palmer et al., 2011). Therefore, management need is crucial to maintain the integrity and health of Texas estuaries.

This study focuses on the two most abundant top predators within Texas estuaries and coastal bays: the alligator gar and the bull shark (*Carcharhinus leucas*). The blacktip shark (*Carcharhinus limbatus*) has also been sampled to a lesser extent. It was therefore included in the analyses when possible.

The **alligator gar** (*Atractosteus spatula*) is a freshwater fish from the Lepisosteidae family found in rivers and estuaries of the southern United States. With a maximum size of the more than 2.5 m, the alligator gar is the biggest gar species. *A. spatula* has a brown-greenish torpedo-shaped body, a large and flat head, and a long mouth full of serrated teeth (Figure 1).



Figure 1: Lateral view of the alligator gar, *Atractosteus spatula*

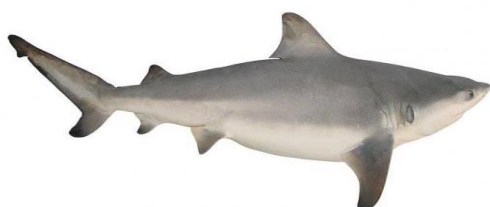


Figure 2: Lateral view of the bull shark, *Carcharhinus leucas*

The **bull shark** (*Carcharhinus leucas*) is a coastal shark from the Carcharhinidae family distributed in tropical and subtropical waters worldwide. *C. leucas* is recognizable by its massive body and small eyes (Figure 2). The bull shark is known for its osmoregulation ability allowing freshwater tolerance. Females give birth near or in estuaries, which serve as nursery grounds for juveniles.

The **blacktip shark** (*Carcharhinus limbatus*) is also a shark from the Carcharhinidae family. Common in tropical and subtropical waters, its maximal size is smaller than the bull sharks. As suggested by its name, this shark often exhibits black tips at on all its fins, excepted the anal fin (Figure 3).



Figure 3: Lateral view of the blacktip shark, *Carcharhinus limbatus*

Alligator gars, bull sharks, and blacktip sharks are abundant top predators and co-occur in the estuaries of the northwestern Gulf of Mexico (Froeschke et al., 2010; Dougherty et al., 2018). It is known that their distribution is conditioned by biotic factors such as body size or competition (Matich et al., 2017), and abiotic factors such as temperature or salinity (Froeschke et al., 2010). Previous studies also reported the influence of these factors on diverse top predators dietary habits (Espinoza et al., 2019; Grainger et al., 2020). To better understand the role of these animals in food webs, we propose to investigate the trophic ecology of these three species in two of the seven major Texas estuarine systems: Sabine Lake and San Antonio Bay. As Texas estuaries are undergoing changes, this knowledge will be valuable to predict consequences on food web structure and better understand top predators roles in estuaries.

### 1.5. Question & Hypotheses

This study aims to answer the two following questions:

- 1) Do alligator gars, bull sharks, and blacktip sharks exhibit interspecific variability in trophic interactions and niche breadth?
- 2) Do these predators exhibit intraspecific variability in trophic interactions and niche breadth between estuaries, across season, water temperature, salinities and throughout ontogeny?

Main hypotheses:

#### **Interspecific variability**

The alligator gar is a freshwater species that tolerates high salinities, and the blacktip shark is a marine species that tolerates brackish waters. Thus, we expect that alligator gars feed mostly on prey found in low salinity and blacktip shark mostly on marine prey. The bull shark is the only species that is adapted to low and high salinities, thanks to osmoregulation mechanisms (Pillans et al., 2006). As a euryhaline species, it may feed on both prey source and may show broader niche breadth. This hypothesis is supported by studies which reported higher isotopic niche in bull sharks in comparison to other predators (Espinoza et al., 2019; Shiffman et al., 2019).

#### **Variability between estuaries**

Even if San Antonio Bay and Sabine Lake are geographically relatively close, biotic and abiotic contexts are likely to differ, thus exhibiting different food web structures (Shiffman et al., 2019).

### **Variability across seasons**

We expect seasonal variability in trophic interactions because (1) prey can show seasonality in terms of presence or abundance (Matich & Heithaus, 2014); (2) environmental conditions show high variability in estuaries, especially in terms of salinity and temperature, which affect alligator gars, bull sharks and blacktip sharks distribution (Allen et al., 2017; Heupel & Simpfendorfer, 2008; Froeschke et al., 2010) and probably their diet; (3) energy requirements may vary across seasons.

### **Variability across environmental conditions**

Environmental condition impact may be complex to assess since the water temperature and salinity at sampling sites may be linked to season and ontogeny. Alligator gars show more efficient osmoregulation abilities with ontogeny and bull sharks juveniles often move to higher salinity waters (Matich et al., 2015). However, we can expect variation in diet due to prey availability changes or the impact of environmental conditions, especially temperature, on metabolism.

### **Variability throughout ontogeny**

Predators growth often involves morphological, physiological, and behavioral changes such as mouth size increasing and hunting skills improvement, which enable them to catch bigger prey. This has been observed in several shark species such as the lemon shark (*Negaprion brevirostris*, Wheterbee et al., 1990), the copper shark (*Carcharhinus brachyurus*, Lucifora et al., 2009), and also in alligator gar juveniles (Butler et al., 2018). We thus expect that *A. spatula*, *C. leucas*, and *C. limbatus* diversify their diet, feed at increasing trophic level, and expand their trophic niche throughout ontogeny. It is also possible that intermediate body size individuals show broader trophic niche, reflecting a transition state as part of a trophic shift, especially for bull sharks in which trophic ontogenetic shifts have been reported (Matich et al., 2015b).

## **2. Methods**

### **2.1. Study areas**

The State of Texas is located in the Southern USA and the North-Western Gulf of Mexico (Figure 4a). The Texas coastline is shaped by long sandy beaches and many barrier islands (Wilkinson, 1975) shelter its bays and estuaries (Powell et al., 2002; Dougherty et al., 2018; Xu, 2018). This study focuses on two majors bays of the Texas coast: San Antonio Bay (SAB) and Sabine Lake (SL) (Figures 4b and 4c). They are characterized by shallow depths (average less than 2 m) and massive but variable freshwater inflow from many rivers and saltwater influx, that create strong salinity gradients. San Antonio Bay is on the central Texas Coast. This estuarine system covers around 530 km<sup>2</sup>, including the bay itself and several extensions such as Espiritu Santo Bay and Hynes Bay. Matagorda Island separates the estuary system from the open water of the Gulf of Mexico. Sabine Lake is located in the Eastern part of the Texas coast, on the border with Louisiana. Sabine Lake area is only approximately 350 km<sup>2</sup>, and is separated from the Gulf of Mexico by a pass instead of a barrier island. In this geographic context, the freshwater influence is critical and leads to lower salinities than in San Antonio Bay.

Alligator gars, *A. spatula*, and bull sharks, *C. leucas*, are both abundant in Sabine Lake and San Antonio Bay but in different proportions. Indeed, their abundances are equivalent in San Antonio Bay whereas the alligator gar is much more present in Sabine Lake (more than 17 times more abundant, Dougherty et al., 2018). Blacktip sharks are only found in San Antonio Bay, to a lower extent than the two other species.



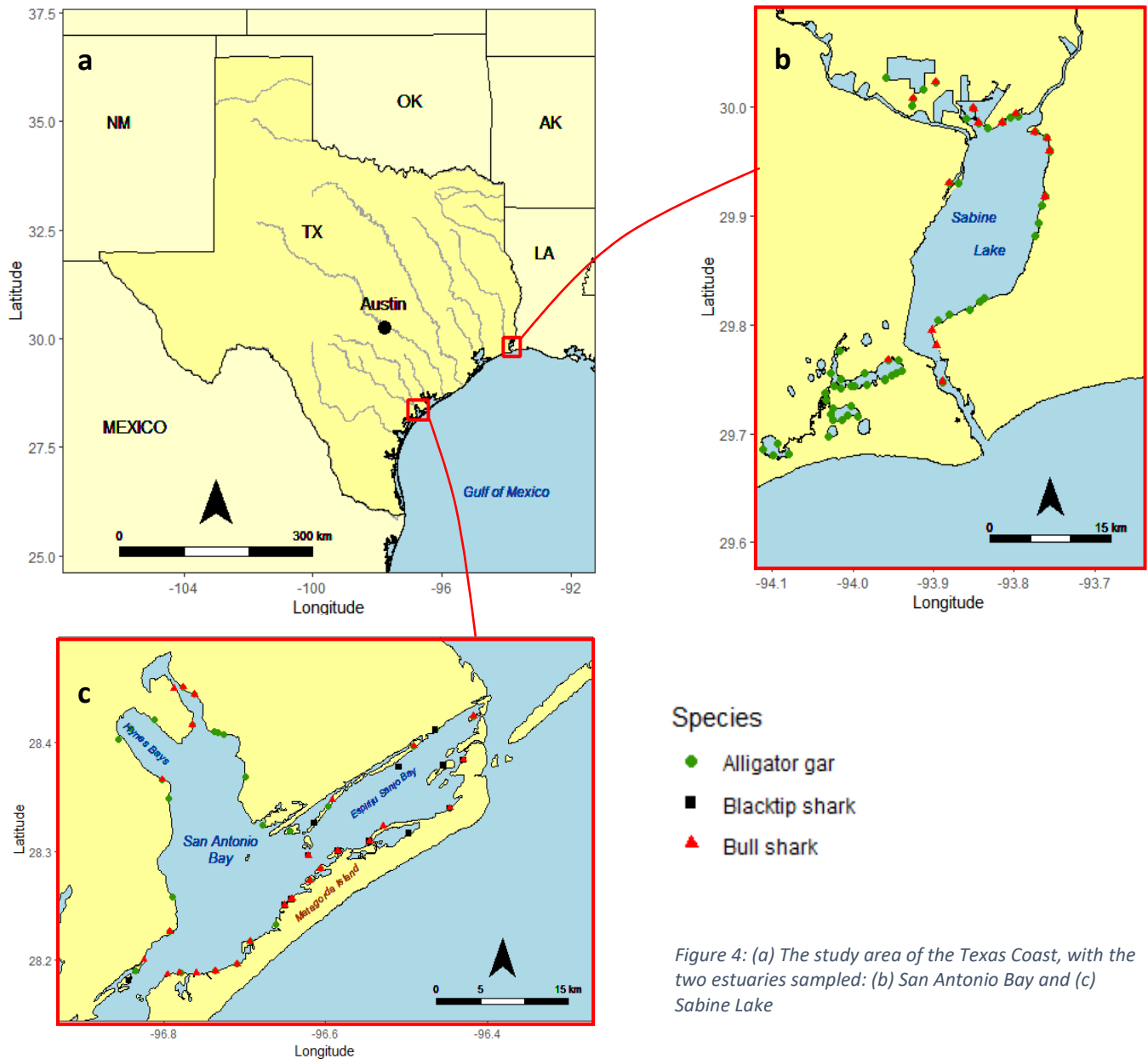


Figure 4: (a) The study area of the Texas Coast, with the two estuaries sampled: (b) San Antonio Bay and (c) Sabine Lake

## 2.2. Sampling collection

The samples used in this study were part of a larger project monitoring Texas estuaries led by the Texas Park and Wildlife Department (TPWD) Coastal Fisheries Division. Our species of interest were sampled in San Antonio Bay and Sabine Lake in Spring (April to June) and Fall (September and early October) 2018 by TPWD staff. Specimens were all collected with monofilament gill nets set perpendicularly to the shoreline. Only animals found dead were kept for analysis, while all live animals were released. Geospatial and hydrologic data such as salinity and temperature were systematically collected. The specimens collected were bagged and labeled on board and processed or frozen immediately upon return to shore.

### 2.3. Laboratory analyses

Each specimen was identified to species and measured to the nearest millimeter (Pre-caudal and total length). Stomachs were removed by dissecting the animals and their content extracted then stored in ethanol until identification. All contents (prey items) of each stomach were visually analyzed and identified at the lowest possible taxonomic level (Figure 5). Each of these prey items was individually counted and weighted.

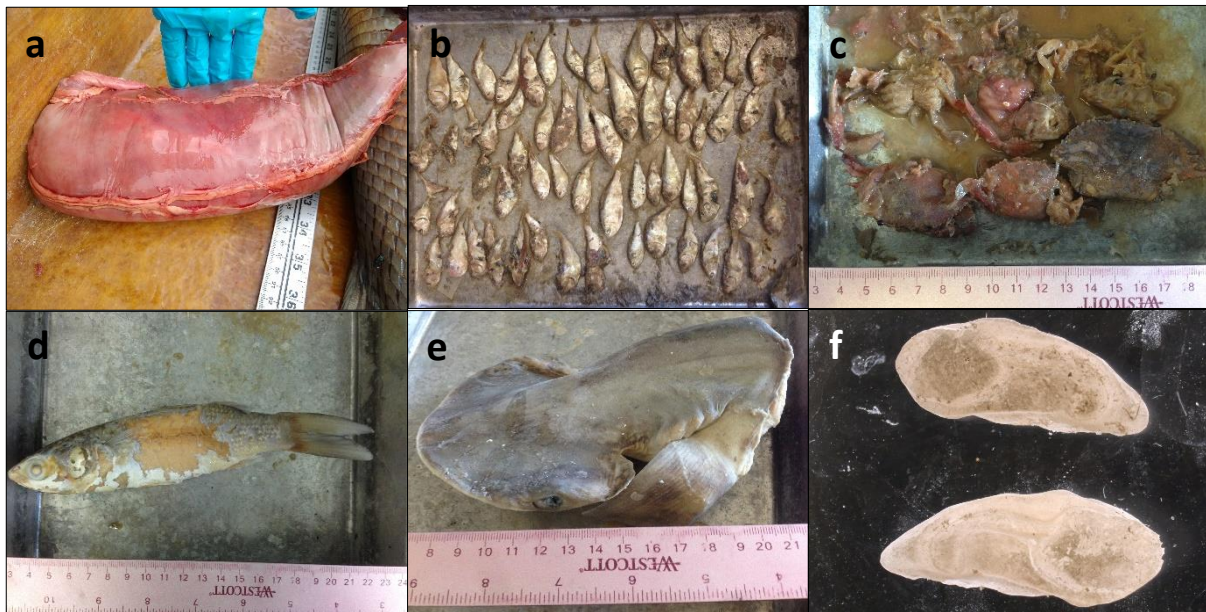


Figure 5:(a) Alligator gar stomach, and examples of prey items found in study species stomachs (b) gulf menhaden (*Brevoortia patronus*) (c) blue crabs (*Callinectes sapidus*) (d) mullet (*Mugil spp.*) (e) bonnethead shark (*Sphyrna tiburo*) head (f) seatrout (*Cynoscion nebulosus*) otoliths

Different tissue types were sampled to perform stable isotope analysis. Muscle samples were collected from most of the bull shark, blacktip shark, and alligator gar specimens, whereas the livers were only sampled on a random subset of them. All the samples were stored frozen (Sweeting et al., 2004) and dried, homogenized, and lipid extracted using a 2:1 chloroform:methanol solution (Churchill et al., 2015), considered to be the most efficient solvent in this case (Hussey et al., 2012). Indeed, lipids show significant depletion in  $^{13}\text{C}$  compared to proteins and carbohydrates (DeNiro & Epstein, 1977), and their abundance can vary across individuals, tissues, and species. Even if lipid extraction is not always required (Matich et al., 2010), it enables the data standardization and thus more robust comparisons (Post et al., 2007; Hussey et al., 2012). This protocol is especially important since we use high lipid content tissue, such as elasmobranch liver (Ballantyne, 1997). Samples were sent to the Florida International University Stable Isotope Laboratory where a mass spectrometer with industry standards were used to quantify the  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  stable isotope ratios, noted  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  respectively, as expressed here:

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

where X is the elements studied (either C or N) and R is the ratio of heavy to light isotopes ( $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ). The international standards are defined as the PeeDee Belemnite for carbon and the air for nitrogen.

## 2.4. Data analyses

All the statistical analyses were conducted using R 4.0.2 (R Core Team, 2020).

- Stomach Content Analysis

### Sample size and classes definition

The SCA was used to make interspecific comparisons of diet between the three predator species, and intraspecific comparisons across estuaries, seasons, body sizes, and environmental factors. Because the SCA approach only applies to groups of individuals, classes were defined for quantitative factors, based on ecological context (Table 1). Water temperature and salinity classes apply for all species and are based on the range of values observed in SAB and SL during sampling seasons. Body size classes are species-specific and related to their life-history traits and growth (Branstetter, 1987; Killam & Parsons, 1989; Neer et al., 2005; Heithaus et al., 2009; Natanson et al., 2014; Binion et al., 2015). The first class approximately matches the total length (TL) range of young of the year sharks, and alligator gars less than two years of age. The second class represents small juveniles. Finally, the largest class encompasses large juveniles and even small adult alligator gars, since they mature around 1100 cm TL.

Factor	Classes		
	<i>T1</i>	<i>T2</i>	<i>T3</i>
Water Temperature (°C)	≤ 23	]23 , 28.5]	> 28.5
	<i>Low</i>	<i>Brackish</i>	<i>High</i>
Water Salinity (ppu)	≤ 9	]9 , 18]	> 18
	<i>Small</i>	<i>Inter</i>	<i>Large</i>
Alligator gar Total Length (mm)	≤ 800	]800 , 1000]	>1000
Blacktip shark Total Length (mm)	≤ 850	]850 , 1100]	> 1100
Bull shark Total Length (mm)	≤ 900	]900 , 1200]	> 1200

Table 1: Classes boundaries used to define the groups for the stomach content analyses

Rarefaction curves (also known as cumulative curve) were extrapolated for each group to assess the sample sizes using to the 'iNEXT' package (Hsieh et al., 2016). This approach consists of plotting the cumulative number of stomachs against the cumulative species richness for each group (Appendix 1). The number of stomachs analyzed, *i.e.* the sample size, is a sufficient representation of the overall diet if the interpolated curve reaches an asymptotic phase (Cortés, 1997). The minimal threshold of 9 individuals with stomach contents, *i.e.* not empty stomachs, was chosen here based on curves, even though it was not always a large enough sample to reach an asymptote. Small sample sizes are common in large marine predator diet studies due to their relatively low abundance (Papastamatiou et al., 2006; Heithaus et al., 2013).

### Vacuity Index

The vacuity index represents the proportion of empty stomachs observed among the total number of stomachs sampled (Brown et al., 2012). The vacuity index was calculated for each population and for each group of individuals within population defined by Table 1. Differences in Vacuity index between groups were assessed by Chi square test if the sample size allowed it. To fulfil this requirement, contingency table, *i.e.* table of the number of empty and non-empty stomachs by group, must contain at least 80 % of cells with values superior or equal to 5 (McHugh, 2013). If this condition was not respected, the difference of Vacuity Index between group were tested with Fisher's exact test.

### **Diet Description and prey importance**

First, we considered all the diet items at the lowest possible taxonomic level to establish a broad dietary description. Stomach contents consisted of 44 different prey types, including identified and unidentified animals, plants, and nonorganic items. The percent frequency of occurrence %O (proportion of stomach containing a specific prey item), the relative weight %W (percentage by weight represented by a specific prey item), and the relative number %N (percentage by number represented by a specific prey item) were calculated for each prey-item. Each of these indices gives different information on the diet (Cortés, 1997). Thus, we calculated the Index of Relative Importance (IRI) (Pinkas et al., 1971), which integrates the three indices and reduces the biases related to the use of each individually. To be more meaningful and to make inter and intraspecific comparisons possible (Cortés, 1997), we expressed IRI on a percentage basis (%IRI) as follow:

$$IRI = \%O * (\%N + \%W) \qquad \%IRI_i = \frac{IRI_i}{\sum_{a=1}^n IRI_a}$$

where n is the total number of prey categories.

Second, we excluded plants and nonorganic items because they were highly infrequent and they are not representative of the diets of the study species since they are exclusively carnivorous (Castro, 1996; Trystram et al., 2016; Estupiñan-Montaño et al., 2017; Butler et al., 2018). Prey items were also gathered under higher taxonomic levels to include non-identified items at the species level. After grouping some of the 44 items, diet data included 23 prey items.

Then the frequency of occurrence, the relative weight and number, and Index of Relative Importance were re-calculated and gathered in tables to provide an overview of the importance of each prey group in alligator gars, bull sharks and blacktip sharks diet (Appendix 5, 6, 7). In addition to the Index of Relative Importance, we also built 3D Costello diagrams that provide graphical visualization of prey importance (Appendix 8-12, Cortés, 1997). Each prey item is represented by a point in which coordinates are given by the %O, %W, and %N associated.

### **Diet comparisons**

Permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was used to test the variation in dietary composition between species and between groups within species (Table 1 au dessus). Similar to traditional multivariate analysis of variance (MANOVA), the PERMANOVA compares geometric structure and position of multidimensional data clouds from dissimilarity matrix. However, this approach is permutation-based (set to 999 permutations here) and is thus more flexible in terms of assumptions. Differences in diet were then tested between species within each ecosystem, between ecosystems for each species and across seasons, water temperatures, salinities, and body size classes. Bray-Curtis similarity matrix of relative weight (%W) was used because this index quantifies the nutritional contribution of each prey-item, providing the most ecological relevance to trophic ecology. Homogeneity of dispersion was tested by PERMDISP (Anderson 2017), which compares the distance between observations and the centroid. PERMANOVA is robust to dispersion heterogeneity if the groups included in the analyses are balanced. We considered that it was the case for the groups presenting a significant difference in dispersion. Furthermore, we assessed the contribution of individual prey items to the Bray-Curtis dissimilarity matrix by performing similar percentage analysis (SIMPER) (Clarke, 1993). Such contributions allow us to identify prey responsible for the difference between diets. These analyses were conducted using the 'vegan' package (Oksanen et al., 2019).

### Trophic niche breadth

The Levin measure and the Shannon-Wiener Index are two common metrics used to estimate the dietary niche breadth (Marshall & Elliott, 1997). The Levin measure gives more weight to abundant prey items, while the Shannon-Wiener Index gives more weight to rare prey items (Krebs, 2013). We used the Levin measure in this study, because it was , considering the low sample sizes. The Levin measure (B) was thus calculated and standardized (BA) as followed:

$$B = \frac{1}{\sum p_j^2} \quad B_A = \frac{B-1}{n-1}$$

where  $p_j$  is the %W of prey item  $j$  and  $n$  the total number of prey species.

The prey weight was used instead of the %IRI because of its ecological relevance. Standardization led to values between 0 and 1 and thus allowed for comparisons between groups.

### Niche overlap

Dietary niche overlap indicates the similarity in trophic resources among species or groups. It is thus a crucial aspect to understand interspecific relationships within assemblages. The dietary niche overlap was estimated with the Renkonen index, also known as percentage overlap or Schoener overlap index and defined as follow:

$$P_{jk} = \left[ \sum \min (\%W_{ij}, \%W_{ik}) \right] * 100$$

where  $P_{jk}$  is the percentage overlap between the two groups  $j$  and  $k$ , and  $\%W_{ij}$  and  $\%W_{ik}$  are the weight proportions of prey  $i$  in the diet of  $j$  and  $k$ .

- Stable Isotope Analysis

### Tissue turnover

Isotopic incorporation is element-specific and varies depending on different factors such as species, tissues, or diet composition (Bosley et al., 2002; German & Miles, 2010; Mont'Alverne et al., 2016; Franssen et al., 2017). Ideally, interspecific comparisons of isotope values must be conducted between tissues presenting a similar turnover rate. Indeed, isotope values must relate information about dietary habits within the same time scale. However, although numerous studies have quantified turnover rates of different tissues across a wide range of species (Vander Zanden et al., 2015), values have never been estimated yet for any of the three species studied here to our knowledge. We assumed that bull shark and blacktip shark turnover rates are similar in the same tissue because they both belong to the *Carcharhinus* genus and probably share similar metabolic processes. However, as a Teleost, the alligator gar is not a close relative to the two other species, so there is no indication that alligator gars and the studied sharks share similar turnover rates for the same tissue type.

We reviewed existing studies that estimated the turnover rates of both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in both muscle and liver tissues in piscivorous teleosts and sharks. We then used the mean of results from these studies to estimate alligator gar and shark element-specific turnover rates for muscle and liver tissue. It appears that  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  turnover rates are faster in liver than in muscle for both sharks and teleosts. However, sharks isotope incorporation generally takes several hundred days in both tissues (Logan & Lutcavage, 2010; Kim et al., 2012; Malpica-Cruz et al., 2012; Galvan et al., 2016), while teleosts takes approximately one hundred days (Suzuki et al., 2005; Sweeting et al., 2007a, 2007b). To limit the potential bias related to this difference in turnover rates, we decided to use isotopic values from the liver of sharks and the muscle of alligator gars, because turnover was most comparable from the tissues collected. Unfortunately, the sample size for blacktip shark liver was too small ( $n = 8$ ) to conduct reliable inferences so this species was removed from any stable isotope analysis.

### DTDF correction

The DTDF - Diet-Tissue Discrimination Factor – represents the enrichment in heavy isotope of a consumer tissue relative to its diet. Similar to isotopic turnover rates, DTDF values are element-specific, and vary depending on different factors such as species, tissues, or diet composition (Pinnegar & Polunin, 1999; Vanderklift & Ponsard, 2003; Barnes et al., 2007; Olin et al., 2013). No estimation has been made for the species studied to our knowledge, so we used results from existing studies conducted on similar species, which include estimation for carbon and nitrogen on lipid-extracted tissues. For the alligator gar, we calculated the mean between Pinnegar & Polunin (1999) and Trueman et al. (2005). For the bull shark, we used the values from Hussey et al. (2010) and Malpica-Cruz et al. (2012) (Table 2).

Species	Tissue type	$\Delta^{15}\text{N}$ (‰)	$\Delta^{13}\text{C}$ (‰)
Alligator gar	Muscle	2.8	2.35
Bull shark	Liver	1.5	0.22

Table 2: DTDF values estimated from the literature values and used in this study

### Maternal effect

Investigating trophic relationships in newborns using stable isotope can be difficult for placental species. Indeed, embryos feed on maternal resource via the placenta, influencing their tissues isotopic compositions (Matich et al., 2010; Ollin et al., 2010). Because of the long turnover in tissues, the mother signature does not dissipate immediately after birth and tissues of young juveniles show enriched values in  $^{15}\text{N}$  and  $^{13}\text{C}$ . The bull shark *C. leucas* is a placental species. We therefore removed young of the year individuals, *i.e.* individuals inferior to 900 mm in total length (Branstetter, 1987; Neer et al., 2005), from the analyses to avoid any bias due to maternal effect on isotope values.

### Baseline adjustment

We used 10 species of primary consumers (1 mollusk, 3 crustaceans and 6 fish) to establish the isotopic baseline in this study: wedge clam (*Rangia cuneate*), brown shrimp (*Farfantepenaeus aztecus*), grass shrimp (*Palaemonetes pugio*), white shrimp (*Litopenaeus setiferus*), gulf menhaden (*Brevoortia patronus*), striped mullet (*Mugil cephalus*), white mullet (*Mugil curema*), gizzard shad (*Dorosoma cepedianum*) and sheepshead minnow (*Cyprinodon variegatus*).

Preliminary analyses revealed variation in isotopic composition within these primary consumers along a salinity gradient (Appendix 13), especially in San Antonio Bay, where primary consumers  $\delta^{15}\text{N}$  values significantly decrease with salinity while  $\delta^{13}\text{C}$  increases (Table 3). Variations of baseline isotope values along environmental gradients can occur in marine systems (Nerot et al., 2012; Schaal et al., 2016) and need to be considered to adjust the values of primary producers (Day et al., 2019). After checking the assumption of homoscedasticity and normality of the residuals graphically, we used the slope of the linear Gaussian regression model of the relationship between  $\delta^{13}\text{C}_{\text{baseline}}$  or  $\delta^{15}\text{N}_{\text{baseline}}$  and salinity in each ecosystem, expressed as  $\alpha$  (Table 3), to adjust alligator gars and bull sharks values according to the following equation:

$$\delta X_{\text{adjusted}} = \delta X_{\text{raw}} - \alpha * \text{Sal}$$

where X represents either  $^{15}\text{N}$  or  $^{13}\text{C}$  and  $\alpha$  the slope of the regression line whose equation is  $\delta X_{\text{baseline}} = \alpha * \text{Salinity} + \beta + \varepsilon \sim \mathcal{N}(0, \sigma^2)$ .

	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
San Antonio Bay	- 0.247	0.174
Sabine Lake	0.126	0.144

Table 3: Values of  $\alpha$  for both elements in both estuaries

This adjustment neutralizes the influence of salinity on the baseline isotope value and its propagation through the food web, which could lead to errors in interpretation.

### Trophic position

Trophic position of study species was inferred with the equation from Post (2002):

$$TP_i = \frac{\delta^{15}N_i - \delta^{15}N_{baseline}}{\Delta^{15}N} + \lambda$$

where  $TP_i$  is the trophic position of the individual  $i$ ,  $\delta^{15}N_{baseline}$  is the mean isotope ratio of baseline,  $\Delta^{15}N$  is the DTDF of the species in question, and  $\lambda$  is the trophic position of the baseline species, which is 2 here since they are primary consumers. The trophic position of a group of individuals is the mean trophic position of all the individuals from this group.

### Comparisons between species within ecosystems

The DTDF were subtracted from the isotope values to correct the difference due to the different fractionation between species. The effect of the categorical variable *Species* was tested within each ecosystem for each element using an analysis of variance (ANOVA). Assumptions were tested graphically and with a Levene test as well as a Shapiro-Wilk test.

$$\delta X_i = \mu + A_i + \varepsilon$$

where  $\mu$  is constant and  $A_i$  the effect of the modality  $i$  of the factor *Species* and  $\varepsilon$  a residual error which has normal distribution of parameters  $(0, \sigma^2)$ .

### Influence of season and body size on isotope values

Stable isotopes take a long time to be incorporated in tissues (weeks-months), which means that the ratios do not reflect the feeding behavior in the conditions of sampling, *i.e.* of catching. Thus, the influence of water temperature and salinity was not investigated in this study.

The effects of seasonality and body size were tested using analysis of covariance (ANCOVA). Season was included, because of broad-scale temporal variability in environmental conditions between Spring, *i.e.* colder, and Fall, *e.g.* warmer. Models were element, species, and ecosystem-specifics. The isotope value was the dependent variable, season the factor and total length the covariate, as expressed in the following equation:

$$\delta X_i = \beta_0 + \beta_1 * TL + Season_i + \varepsilon$$

where  $\beta_0$  is constant,  $TL$  is the Total Length,  $\beta_1$  is the slope in the linear relationship between  $\delta X$  and Total Length,  $S_i$  is the effect of the modality  $i$  of the factor Season, and  $\varepsilon$  a residual error which has normal distribution of parameters  $(0, \sigma^2)$

ANCOVA assumptions (linearity, homogeneity of regression slopes, normality and homoscedasticity of residuals) were checked graphically and with the Shapiro-Wilk test and the Levene test.=

### Isotopic niche breadth and overlap

Isotopic niche position and shape for a population can be assessed using their positions in the bi-dimensional isotopic space. We used the approach proposed by Jackson et al. (2011), which uses ellipses to represent the isotopic niche. The standard ellipse includes a 40% estimate of the isotopic niche similar to a kernel density, based on the variance of isotope values in bivariate space, and is robust to sample size and extreme values. Accordingly, the isotopic niche breadth is given by the Standard Ellipse Area (SEA).

We first used this approach to represent species isotopic niches within each ecosystem graphically. Then, we divided the individuals into groups based on their total length in the same fashion as in the stomach content analysis (Table 1), to evaluate change in isotopic niche with growth. However, due to the limited sample size in some groups, using the sample size-corrected standard ellipse area (SEA<sub>c</sub>) appears more appropriate. This correction inflates the standard ellipse to compensate for systematic underestimation when the sample size is low (Jackson et al., 2011).

SEA were also estimated in a Bayesian framework to consider uncertainties. By providing posterior distributions of the Standard Ellipse Area (SEA<sub>B</sub>), this method enables comparisons of isotopic niche breadth between groups statistically. We conducted Monte Carlo Markov Chain simulations with 20 000 iterations, 2 chains, and a burnin of 1000. We also used the uninformative priors recommended by Jackson et al. (2011).

The isotopic niche overlap was measured as the area of overlap between two SEA<sub>c</sub> and express as a percentage for each species.

These analyses were conducted with the 'SIBER' package (Jackson et al., 2011), and the Bayesian distributions were fitted with the JAGS software using the 'rjags' package (Plummer, 2019).

## 3. Results

A total of 308 alligator gars, 127 bull sharks and 52 blacktip sharks were sampled during the study (Table 4).

Species	Stomach contents		Stable isotope		Total Length (mm)	
	Number of stomachs sampled	Number of empty stomachs	Number of muscle sample	Number of liver sample	Range	Mean ± SD
<b>San Antonio Bay</b>						
Alligator gar	119	82	60		670 - 1306	990 ± 147
Bull shark	82	11		68	775 - 1526	1120 ± 166
Blacktip shark	52	21		8	640 - 1777	963 ± 274
<b>Sabine Lake</b>						
Alligator gar	189	115	51		517 - 1545	898 ± 188
Bull shark	45	9		37	745 - 1109	923 ± 108

Table 4: Data summary of the alligator gars, bull sharks and blacktip sharks used in SCA and SIA



### 3.1. Stomach content analyses

#### 3.1.1. Vacuity index

The proportion of individuals with empty stomach varied considerably between species in both estuaries (in San Antonio:  $\chi^2 = 60.967$ ,  $df = 2$ ,  $p < 0.001$ ; in Sabine Lake:  $\chi^2 = 24.344$ ,  $df = 1$ ,  $p < 0.001$ ; Figure 6). The alligator gars showed the highest vacuity index with 69 % and 61 % of empty stomachs in SAB and SL respectively. Comparatively, most of the bull sharks had prey in their stomachs with vacuity index of only 13 % (SAB) and 20 % (SL). The blacktip shark, which were only sampled in SAB, showed an intermediate vacuity index of 40 %.

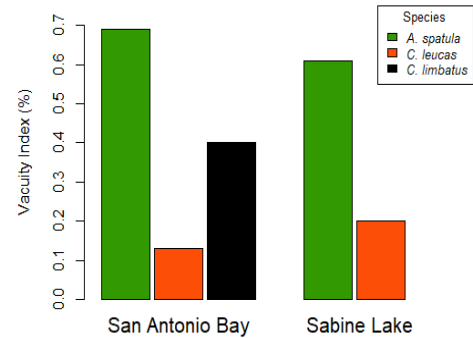


Figure 6: Vacuity index of alligator gars, bull sharks and blacktip sharks

The proportion of empty stomachs was consistent across estuary for alligator gars ( $\chi^2 = 2.0585$ ,  $df = 1$ ,  $p > 0.05$ ). However, alligator gar vacuity index varied according to other factors. In San Antonio Bay, index of vacuity rates significantly differed across temperature group only (Fisher's exact test,  $p < 0.005$ ). The frequency of empty stomach within individuals caught in water temperature (T) ranging from 23°C to 28.5°C were very low (36%) compared to the two other group (77% for  $T \leq 23.5^\circ\text{C}$  and 78% for  $T > 28^\circ\text{C}$ ) (Appendix 14). In this estuary, groups based on season, salinity and body size did not show any significant variation in vacuity index. However, we still observed a trend to decrease with salinity for the vacuity index in San Antonio Bay. In Sabine Lake, vacuity index did not vary across water temperature groups but across groups based on season ( $\chi^2 = 4.8467$ ,  $df = 1$ ,  $p < 0.05$ ) and salinity ( $\chi^2 = 4.1528$ ,  $df = 1$ ,  $p < 0.05$ ). Indeed, 67% of the stomach examined in Spring were empty while only 51% of the stomachs in Fall had prey. Similarly, the vacuity rate was higher among individuals caught in low salinity waters, *i.e.* salinity  $< 9$  ppu, than in intermediate salinity waters, *i.e.* between 9 and 18 ppu. Body size did not affect the proportion of empty stomachs among alligator gars in San Antonio Bay or Sabine Lake.

Bull sharks also showed a consistent vacuity index across estuaries ( $\chi^2 = 0.94968$ ,  $df = 1$ ,  $p > 0.05$ ). Vacuity index also did not vary among bull sharks based on season, water temperature, salinity or body size (Fisher's exact test,  $p > 0.05$ ).

Vacuity rates showed important variation across groups of blacktip sharks, but none were significant, potentially due to low sample sizes.

Species	Total	Season		Water temperature (°C)			Water salinity (ppu)			Body size		
		Spring	Fall	$T \leq 23^\circ\text{C}$	$23 < T \leq 28.5$	$T > 28.5$	$S \leq 9$	$9 < S \leq 18$	$S > 18$	Small	Inter	Large
<b>San Antonio Bay</b>												
Alligator gar	35	27	8	3	16	13	4	14	14	6	13	16
Bull shark	41	21	20	1	15	25	3	10	28	1	21	19
Blacktip shark	14	8	6	0	11	3	0	0	14	3	4	7
<b>Sabine Lake</b>												
Alligator gar	41	20	29	17	16	16	32	17	0	20	9	20
Bull shark	23	11	12	0	13	10	19	4	0	12	11	0

Table 5: numbers of individuals of each group included in stomach contents analyses. Red numbers indicate too low sample size ( $n < 9$ ) to consider the stomach contents representative of the diet

### 3.1.2. Diet description

A total of 617 prey items were found in the 249 stomachs examined with contents. 64 % of them were identified at the species level, 16 % at a higher taxonomic level and the rest were unidentified. 25 different species were represented, mostly teleosts fish but also elasmobranchs, crustaceans, and snakes (Appendix 2, 3, 4). Teleost fish were the most dominant prey type, representing at least 84 % of the diet in alligator gars, bull sharks and blacktip sharks. Other prey types such as crustaceans and elasmobranchs were marginal in their importance due to low occurrences. Additionally, a few stomachs contained inorganic matter such as fishing gear, and organic non animal matter such as algae. Unfortunately, non-animal and unidentified items did not provide informative data and were removed from the further calculations. %IRI was calculated after removal of these groups (Appendix 5, 6, 7). Table 5 presents the sample size in each group after the removal of unidentifiable and non-diet items. Because of the threshold of 9 individuals established, some groups of individuals were considered irrelevant for the following analyses.

Most of the groups compared to each other did not show significant differences in dispersion.

Alligator gars and bull sharks both exhibited statistically different diet between estuaries based on prey weight data, suggesting their diet is context-specific (PERMANOVA(*Alligator gar*): pseudo-F = 3.0182,  $p < 0.01$ ; PERMANOVA(*Bull shark*): pseudo-F = 1.9335,  $p < 0.05$ ).

#### ➤ San Antonio Bay

Stomach contents of San Antonio Bay alligator gar, bull shark and blacktip shark expressed as %W were significantly different from one another (PERMANOVA: pseudo-F = 2.48,  $p < 0.01$ ). The interspecific dissimilarities in stomach contents compositions were mostly based on the quantities of Mugilids found, and on the species that was second most important (SIMPER, Appendix 15).

#### **Alligator gar**

After removal of the non-desired prey groups, 35 alligator gars were included in the analyses. Stomach contents revealed the preponderance of two prey types in San Antonio Bay – Mugilids and Clupeids. Mugilids were the most important prey group (%IRI = 54%; Figure 7, Appendix 5), and were found in most of the stomachs examined, representing 75% of the total weight of the stomach contents for alligator gars in San Antonio Bay (Figure 7). Almost all the identified Mugilids were striped mullet, *Mugil cephalus*. Clupeids were the second most dominant prey type with a %IRI of 45%, and were nearly all gulf menhadens, *Brevoortia patronus* (Appendix 2). These small pelagic fish were frequently found in high numbers (Appendix 8) but were less important than Mugilids in terms of weight. Therefore, 99% of alligator gars diets were composed of mullets and small pelagic fish in San Antonio Bay.

Intraspecific variability in stomach content compositions based on IRI was detected among alligator gars in San Antonio Bay. Stomach contents of individuals caught in water temperatures from 23 to 28.5°C (group *T2*) was composed of 80% Clupeids and 20% Mugilids, while the stomach contents of the individuals caught in warmer waters (group *T3*) were almost exclusively characterized by Mugilids (%IRI = 95%). Similarly, salinity (*Brackish* and *High*) and body size (*Inter* and *Large*) had effects on alligator gars diets. Clupeids were found in high proportions in *Brackish* (%IRI = 74%) and *Inter* (%IRI = 70%) groups, but were almost absent in *High* (%IRI = 4%) and *Large* (%IRI = 8%), which were mostly comprised of mullets (%IRI ≥ 90%).

Permutation analysis revealed significant differences in stomach content gravimetric composition between groups based on environmental conditions (PERMANOVA (*T2-T3*): pseudo-F = 2.868,  $p < 0.05$ ;

PERMANOVA (*Brackish-High*): pseudo-F = 2.9853 ,  $p < 0.05$ ). The SIMPER analyses showed that these differences were mostly due to change in Mugilids and Clupeids weight. No significant change was detected between groups based on ontogeny (PERMANOVA (*Inter-Large*): pseudo-F = 1.8713 ,  $p > 0.05$ ). Differences between seasons were not investigated because of low sample size for *Fall* (n= 8).

### **Bull shark**

Forty-one bull sharks sampled in San Antonio Bay estuary had stomachs containing identifiable prey items. Similar to alligator gars, the stomach contents of bull sharks were mostly composed of 2 prey types (Appendix 9). The dominant prey were catfish from the Ariidae family, which represented 51% of relative importance in the overall diet (Figure 7). A large amount of these catfish were unidentifiable at the species level. But only two identifiable species were present in stomachs: the gafftopsail catfish, *Bagre marinus*, and the hardhead catfish, *Ariopsis felis*. The second major prey group was Mugilids (%IRI=31%), predominantly striped mullets (Appendix 3). Together, Ariids and Mugilids represented 80 % of San Antonio bull shark stomach contents. The remaining prey included Clupeids and Sciaenids with %IRI of 8% and 7%, respectively.

Mugilids and Ariids relative importance were consistent across seasons (respectively 34% and 52% in *Spring* and 26% and 47% in *Fall*). However, Clupeids were present in higher proportion in *Spring* and were replaced by Sciaenids in *Fall*. Stomach contents were similar between *T2* and *T3*, with no substantial change in prey importance ( $\Delta\%IRIMugilids = 15\%$   $\Delta\%IRIAriids = 8\%$ ) (Figure 7). Ariids %IRI did not show variation between salinity groups. *Brackish* stomach contents were characterized by a high proportion of seatrout, *Cynoscion spp.* (from the Sciaenidae family,  $\Sigma\%IRI = 26\%$ ), and the *High* group by a high value for Mugilids (%IRI = 41%). Finally, stomach compositions varied across body size groups (*Inter* and *Large*). Clupeids and Sciaenids %IRI were both superior to 10% in *Inter* but were trivial in larger individuals stomachs, which contained Ariids and Mugilids.

Percentage of weight %W of prey groups did not differ across groups based on seasons (PERMANOVA(*Spring-Fall*): pseudo-F = 0.63529 ,  $p > 0.05$ ), temperature (PERMANOVA(*T2-T3*): pseudo-F = 0.40376 ,  $p > 0.05$ ), salinity (PERMANOVA(*Brackish-High*): pseudo-F = 1.0779 ,  $p > 0.05$ ) or ontogeny (PERMANOVA(*Inter-Large*): pseudo-F = 1.0951 ,  $p > 0.05$ ).

### **Blacktip shark**

Only 14 of the 31 blacktip sharks which had non-empty stomachs had identifiable prey in their stomachs. Mugilids were the only major prey (Appendix 10), with a relative importance of 66%. Other prey types such as Sciaenids (red drum, *Scienops ocellatus*, and atlantic croaker, *Micropogonias undulates* essentially) or other shark species were also found in a lesser extent (Figure 7; Appendix 4, 7). Because of low sample sizes, intraspecific variations were not investigated in this species.

#### ➤ Sabine Lake

Only bull sharks and alligator gar were caught in Sabine Lake estuary. For both species, sampled individuals predominantly fed on mullets. However, alligator gars stomach contents were also composed of Clupeids in high quantities while bull sharks also fed on red drums (Sciaenids). Specific gravimetric compositions showed significant dissimilarities (PERMANOVA(*Alligator gar-bull shark*): pseudo-F = 2.1904 ,  $p < 0.01$ ). SIMPER analysis revealed that Mugilids mostly contributed to this dissimilarity with 33%, followed by red drums, which contributed to 24%.

## Alligator gar

Forty-one alligator gars had identifiable stomach contents. As observed in San Antonio Bay, alligator gars stomach contents were mostly composed of two prey (Figure 7). Clupeids were the most represented with %IRI = 69% (Appendix 5). Clupeids were almost exclusively represented by gulf menhaden and were found frequently in high numbers in alligator gars stomachs (Appendix 11). Mulletts were also abundant in stomach contents, with a relative importance of 20%. The rest of the prey identified were Sciaenids, and crustaceans.

Sabine Lake alligator gars stomach contents were essentially composed of gulf menhaden in *Spring* (%IRI Clupeids = 86%). However, Clupeids %IRI was lower in *Fall* (33%) while mulletts, which were almost absent in *Spring*, had an index of relative importance of 45%. In terms of water temperature, Clupeids, Mugilids, and Sciaenids relative importance varied greatly between groups. Clupeids %IRI was 34% in *T1*, 5% in *T2*, and 95% in *T3* and Mugilids %IRI from 25% to 71% to less than 1%. Indexes of relative importance were consistent across salinity groups. Finally, there was no major change in relative importance of Clupeids or Mugilids between body size groups. However, we denoted the unusual high importance of crustaceans in individuals  $\leq 800$  mm ( $\Sigma$ %IRI = 14%).

Difference in gravimetric stomach content compositions significantly different across season groups (PERMANOVA(*Spring-Fall*): pseudo-F = 3.0054,  $p < 0.001$ ), water temperature groups (PERMANOVA(*T1-T2-T3*): pseudo-F = 3.0054,  $p < 0.01$ ), but not across salinity groups (PERMANOVA(*Low-Brackish*): pseudo-F = 0.91597,  $p > 0.05$ ).

## Bull shark

Sabine Lake bull sharks stomach contents were also characterized by the preponderance of two types of prey (Figure 7, Appendix 3, 6, 12). Mugilids appeared as a dominant species with %IRI of 59%, followed by red drums with a %IRI of 32%.

Bull sharks exhibited variability in stomach contents, especially seasonally. *Spring* bull sharks stomachs predominantly contained red drums (%IRI = 76%) and almost no Mugilids (%IRI < 5%), while individuals sampled in *Fall* consumed nearly only Mugilids (%IRI > 97%). Relative Importance of mulletts and red drums were 38% and 44% respectively in *T2*, and 75% and 11% in *T3*. Differences between salinities could not be investigated because the *Brackish* group sample size was inadequate ( $n = 4$ ), and no individuals were caught in salinity > 18 ppt. The smallest individuals had stomach contents mostly composed of red drums (%IRI = 47%) and larger sharks, from the *Inter* group mainly consumed Mugilids (%IRI = 78%). PERMANOVA procedures only detected significant change between season groups (PERMANOVA(*Spring-Fall*): pseudo-F = 4.6167,  $p < 0.001$ ) and the SIMPER analysis showed that this difference was mainly driven by variation in red drums and Mugilids weight in the stomach contents.

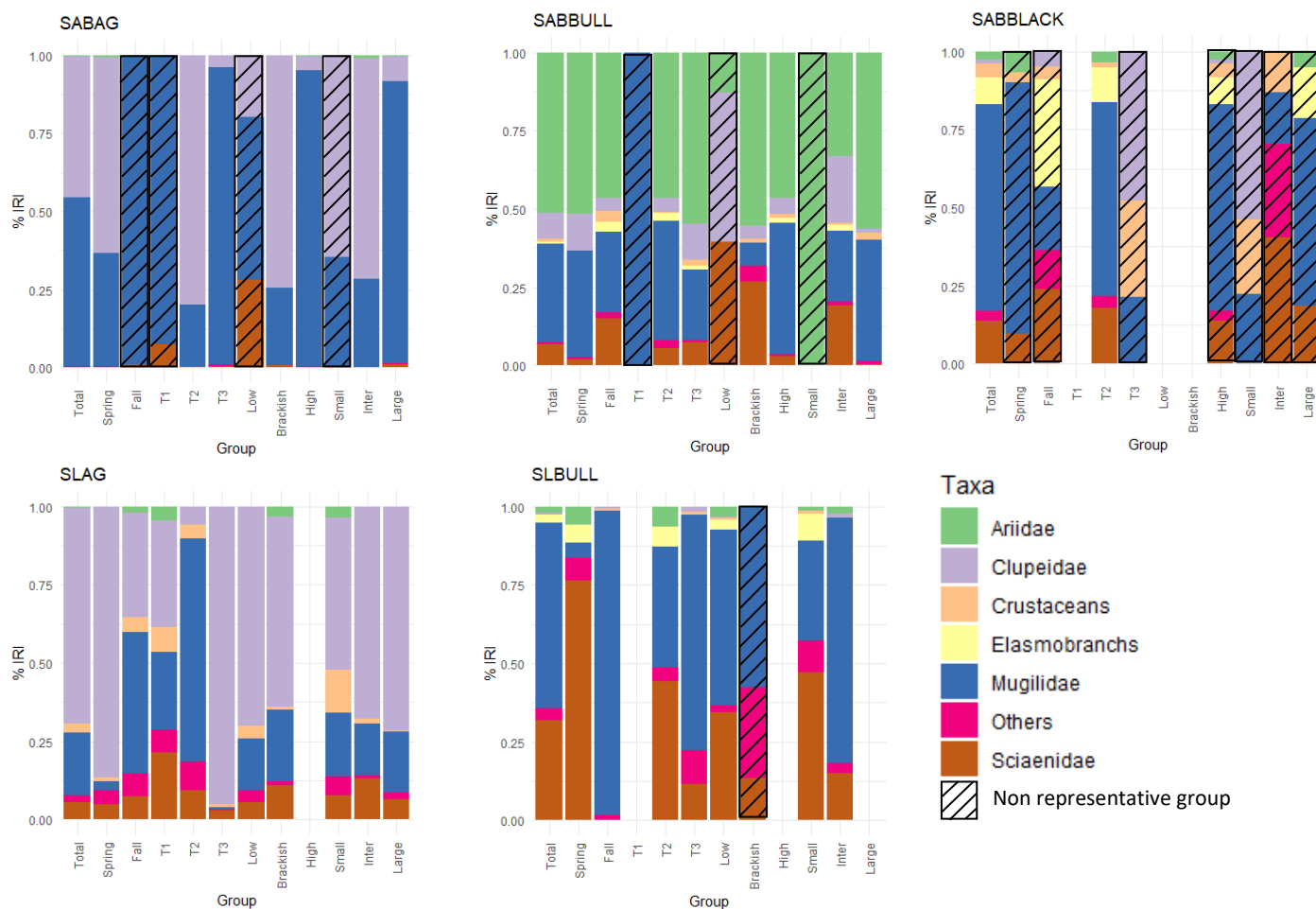


Figure 7: Stomach contents composition in taxonomic groups of prey measured with sum of %IRI. For San Antonio Bay (on top) and Sabine Lake SL (bottom) alligator gars (AG), bull sharks (BULL) and blacktip sharks (BLACK). Vacant bars indicate no individuals were sampled for particular statistical groups

### 3.1.3. Niche breadth

Species	Total	Season		Water temperature (°C)			Water salinity (ppu)			Body size*		
		Spring	Fall	T <sub>≤23°C</sub>	23<T <sub>≤28.5</sub>	T <sub>&gt;28.5</sub>	S <sub>≤9</sub>	9<S <sub>≤18</sub>	S <sub>&gt;18</sub>	Small	Inter	Large
<b>San Antonio Bay</b>												
Alligator gar	0.13	0.19	0.01	0.01	0.23	0.03	0.03	0.21	0.07	0.17	0.24	0.05
Bull shark	0.33	0.25	0.34	0	0.22	0.36	0.07	0.25	0.25	0	0.38	0.11
Blacktip shark	0.39	0.07	0.31	NA	0.37	0.14	NA	NA	0.39	0.09	0.16	0.20
<b>Sabine Lake</b>												
Alligator gar	0.28	0.50	0.27	0.38	0.22	0.27	0.44	0.28	NA	0.34	0.27	0.34
Bull shark	0.29	0.28	0.11	NA	0.40	0.54	0.30	0.70	NA	0.38	0.51	NA

Table 6: Standardized Levin measures of trophic niche breadth for alligator gars, bull sharks and blacktip sharks sampled. Red numbers indicate too low sample size (n<9) to consider the stomach contents representative of the diet

The standardized Levin measure provided variable niche breadth estimates across species and groups of individuals (Table 6). In San Antonio Bay, the two shark species showed wider trophic niche than alligator gars. The bull shark and the blacktip shark had higher Levin measures ( $B_A = 0.33$  and  $B_A = 0.39$  respectively) than the alligator gar ( $B_A = 0.13$ ) in San Antonio Bay. Comparatively, bull sharks and alligator gars had similar values in Sabine Lake ( $B_A = 0.29$  and  $B_A = 0.28$  respectively). Variation in niche breadth estimates across groups did not follow the same trends across estuaries or across species when comparisons were relevant. Niche breadth was higher in *Fall* than in *Spring* for San Antonio Bay

bull sharks while it was the opposite in Sabine Lake bull sharks. Values also decreased with water temperature for San Antonio Bay alligator gars, while it increased for Sabine Lake alligator gars and for San Antonio Bay and Sabine Lake bull sharks. San Antonio Bay alligator gars and bull sharks trophic niche breadth also decreased with body size while it increased for Sabine Lake alligator gars.

### 3.1.4. Trophic niche overlap

Most of the values of the Renkonen index are based on unreliable values of percentage weight due to slow sample sizes in many groups of individuals. Globally, trophic niche overlap estimates were similar between all pairs of predators and in both estuaries (Table 7).

Niche overlap estimates between body size groups ranged from 0 to 0.90 in San Antonio (Table 8). However, most values are irrelevant. Interspecific niche overlap was globally higher than intraspecific niche overlap. The niche overlap between alligator gars and bull sharks increased with bull shark total length and decreased with alligator gar total length. Moreover, the overlap between small and large bull sharks (P=0.36) was surprisingly higher than the overlap between small and intermediate (P=0.09).

Because of the higher sample size in Sabine Lake, all the Renkonen index calculated were considered relevant (Table 9). Interspecific overlap increased with alligator total length and with bull shark total length, reaching a maximum of P=0.56 for the overlap between large gars and large sharks. Intraspecific overlap was globally higher than interspecific overlap.

	Total	Season		Water temperature (°C)			Water salinity (ppu)		
		Spring	Fall	T≤23°C	23<T≤ 28.5	T>28.5	S≤9	9<S≤18	S>18
<b>San Antonio Bay</b>									
Alligator gar - Bull shark	0,49	0,58	0,32	0,98	0,50	0,29	0,03	0,24	0,49
Alligator gar - Blacktip shark	0,43	0,72	0,04	NA	0,43	0,13	NA	NA	0,41
Bull shark - Blacktip shark	0,48	0,46	0,19	NA	0,61	0,26	NA	NA	0,53
<b>Sabine Lake</b>									
Alligator gar – Bull shark	0.53	0.17	0.39	NA	0.23	0.16	0.30	0.54	NA

Table 7: Renkonen index for groups of individuals based on Season and environmental conditions

	AG Small	AG Inter	AG Large	Blacktip Small	Blacktip Inter	Blacktip Large	Bull Small	Bull Inter	Bull Large
AG Small	1								
AG Inter	0,90	1							
AG Large	0,73	0,69	1						
Blacktip Small	0,44	0,39	0,18	1					
Blacktip Inter	0,10	0,10	0,08	0,09	1				
Blacktip Large	0,52	0,55	0,53	0,11	0,08	1			
Bull Small	0,00	0,10	0,01	0	0	0,03	1		
Bull Inter	0,36	0,45	0,27	0,30	0,14	0,36	0,09	1	
Bull Large	0,56	0,66	0,59	0,17	0,09	0,54	0,36	0,32	1

Table 8: Renkonen percentage of overlap between groups based on body size in San Antonio Bay

	AG Small	AG Inter	AG Large	Bull Small	Bull Inter	Bull Large
AG Small	1					
AG Inter	0.58	1				
AG Large	0.50	0.39	1			
Bull Small	0.25	0.36	0.54	1		
Bull Inter	0.38	0.45	0.56	0.70	1	
Bull Large	NA	NA	NA	NA	NA	NA

Table 9: Renkonen percentage of overlap between groups based on body size in Sabine Lake

### 3.2. Stable Isotope Analyses

After removing individuals smaller than 900 mm in total length and 5 outliers, 105 alligator gars and 78 bull sharks were included in the analysis. Table 10 summarizes the isotope data collected in this study after being adjusted for salinity.

	n	$\delta^{15}\text{N}$ (‰)		$\delta^{13}\text{C}$ (‰)	
		Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
<b>San Antonio Bay</b>	114				
Alligator gar	54	15.75 , 25.31	19.99 $\pm$ 2.25	-23.75 , -13.71	-18.98 $\pm$ 2.43
Bull shark	60	16.68, 24.43	20.96 $\pm$ 1.78	-21.75, -15.32	-18.16 $\pm$ 1.61
<b>Sabine Lake</b>	69				
Alligator gar	51	8.20, 16.07	11.72 $\pm$ 1.70	-25.72, -16.38	-19.83 $\pm$ 2.17
Bull shark	18	11.37, 15.24	12.85 $\pm$ 1.17	-22.33, -17.61	-19.65 $\pm$ 1.48

Table 10: Summary of the isotope data on alligator gars and bull sharks sampled in San Antonio Bay and Sabine Lake

For  $\delta^{15}\text{N}$ , mean specific isotope values ranged from 11.72  $\pm$  1.70 ‰ (mean  $\pm$  SD) in Sabine Lake alligator gars to 20.96  $\pm$  1.78 ‰ in San Antonio Bay bull sharks. The same groups showed the minimum and maximum mean values for  $\delta^{13}\text{C}$ , with means of -19.83  $\pm$  2.17 ‰ and -18.16  $\pm$  1.61 ‰. Ranges of isotope values were higher in San Antonio Bay than in Sabine Lake for both species (Table 10).

#### 3.2.1. Interspecific comparisons

To prevent the bias due to the difference of DTFD values between species and between tissues, the DTFD were subtracted from the raw isotope values before being adjusted to enable relevant interspecific comparisons in terms of trophic relationships.

Alligator gars and bull sharks diets differed significantly in their isotope values in both estuaries (Table 11). ANOVA indicated a significant effect of *Species*, which explained 15%-32% of the variability in isotope values. ANOVA assumptions of homoscedasticity and normality of the residuals were checked graphically (Appendix 16-19) and with the Levene test and the Shapiro-Wilk test. Globally, after subtraction of the DTFD, bull sharks had more enriched  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in both ecosystems (Figure 8).

	$\delta^{15}\text{N}$				$\delta^{13}\text{C}$			
	Df	Sum sq	F value	p value	Df	Sum sq	F value	p value
<b>San Antonio Bay</b>								
Species	1	147.36	38.67	<b>2.20e-08</b>	1	102.25	27.44	<b>7.70e-07</b>
Residuals	112	454.66			112	417.41		
<b>Sabine Lake</b>								
Species	1	79.04	40.99	<b>3.95e-07</b>	1	48.67	12.31	<b>1.02e-03</b>
Residuals	63	167.45			63	276.33		

Table 11: Analysis of variance table of the effect of the Species factor on isotope values in each estuary

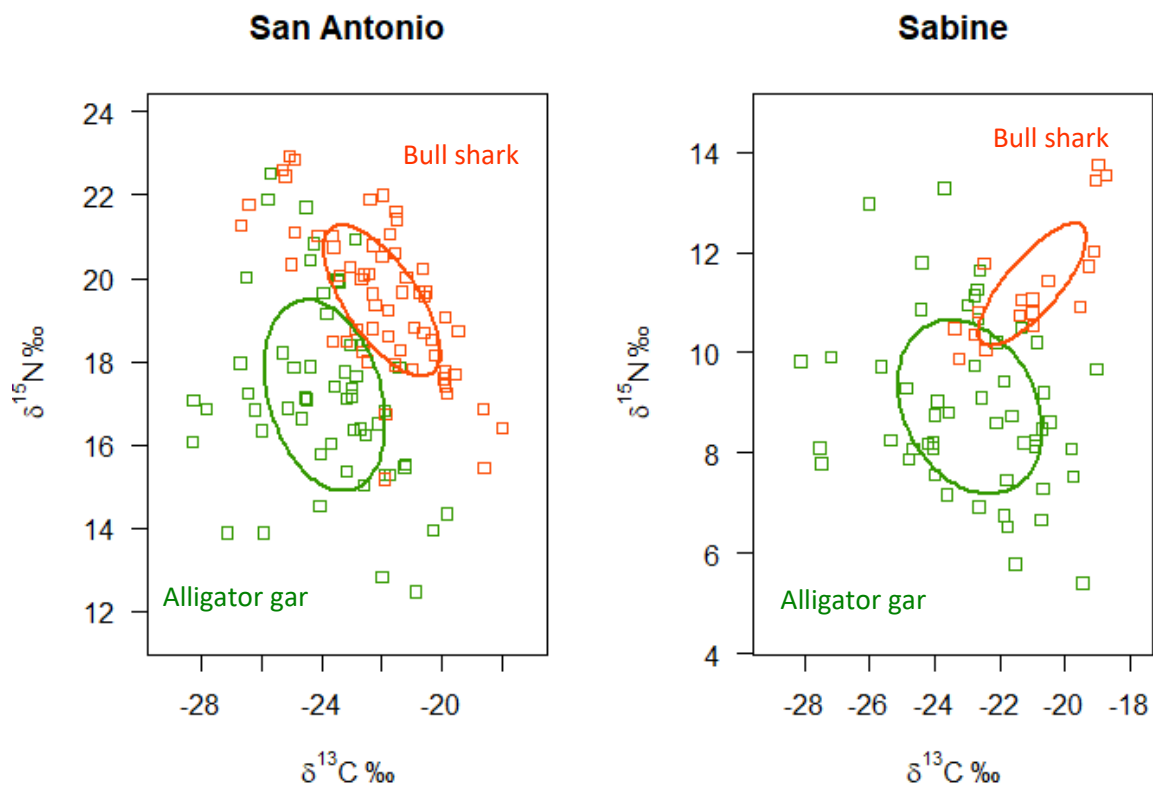


Figure 8: SIBER ellipses of alligator gars and bull sharks sampled in San Antonio Bay (left) and Sabine Lake (right)

### 3.2.2. Effects of season and body size for each predator in each estuary

ANCOVA procedures revealed that body size and season affected isotope values (Table 12). The Total length of individuals had a significant positive effect on alligator gars muscle  $\delta^{15}\text{N}$  in both estuaries and on bull sharks liver  $\delta^{15}\text{N}$  in Sabine Lake. With slopes of  $0.009\text{‰}\cdot\text{mm}^{-1}$  and  $0.003\text{‰}\cdot\text{mm}^{-1}$  in San Antonio Bay and Sabine lake, respectively, *A. spatula* showed a higher increase of  $\delta^{15}\text{N}$  with body size than Sabine Lake bull sharks (slope =  $0.001\text{‰}\cdot\text{mm}^{-1}$ ). Moreover, body size significantly affected  $\delta^{13}\text{C}$  only in San Antonio Bay bull sharks, where  $\delta^{13}\text{C}$  decreased with the individuals total length. Therefore, the relationship between body size and isotope values was different between alligator gars and bull sharks. The influence of body size on isotope values is also visible in Figure 9, where individuals represented in the isotopic space are grouped by size class.

Season impacted  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in bull sharks but not in alligator gar (Table 12, Figure 10). The interaction between season and total length had a significant effect only on Sabine Lake bull shark  $\delta^{15}\text{N}$  values.



	$\delta^{15}\text{N}$				$\delta^{13}\text{C}$			
	Df	Sum sq	F value	p value	Df	Sum sq	F value	p value
<b>San Antonio Bay</b>								
<b>Alligator gar</b>								
TL	1	14.54	3.13	<b>0.083</b>	1	7.47	1.99	0.165
Season	1	6.41	1.38	0.246	1	4.25	1.13	0.293
TL*Season	1	14.34	3.09	0.085	1	5.45	1.45	0.234
Residuals	51	246.68			51	188.08		
<b>Bull shark</b>								
TL	1	9.906	3.36	0.072	1	24.19	13.16	<b>6.19e-04</b>
Season	1	12.21	4.15	<b>0.047</b>	1	83.09	45.21	<b>9.88e-09</b>
TL*Season	1	0.01	0.01	0.984	1	1.95	1.06	0.307
Residuals	56	164.91			56	102.92		
<b>Sabine Lake</b>								
<b>Alligator gar</b>								
TL	1	29.25	12.02	<b>1.13e-03</b>	1	0.62	0.13	0.721
Season	1	0.20	0.08	0.776	1	0.06	0.01	0.909
TL*Season	1	0.47	0.19	0.664	1	7.86	1.64	0.206
Residuals	47	114.32			47	224.90		
<b>Bull shark</b>								
TL	1	1.67	6.17	<b>0.026</b>	1	3.20	2.76	0.119
Season	1	14.85	55.02	<b>3.263e-06</b>	1	23.47	20.27	<b>4.97e-04</b>
TL*Season	1	2.93	10.84	<b>5.334e-03</b>	1	0.01	0.01	0.914
Residuals	14	3.78			14	16.21		

Table 12: Analyses of variance table of the effect of the factor Season and the covariate TL (Total Length) on isotope values in each estuary

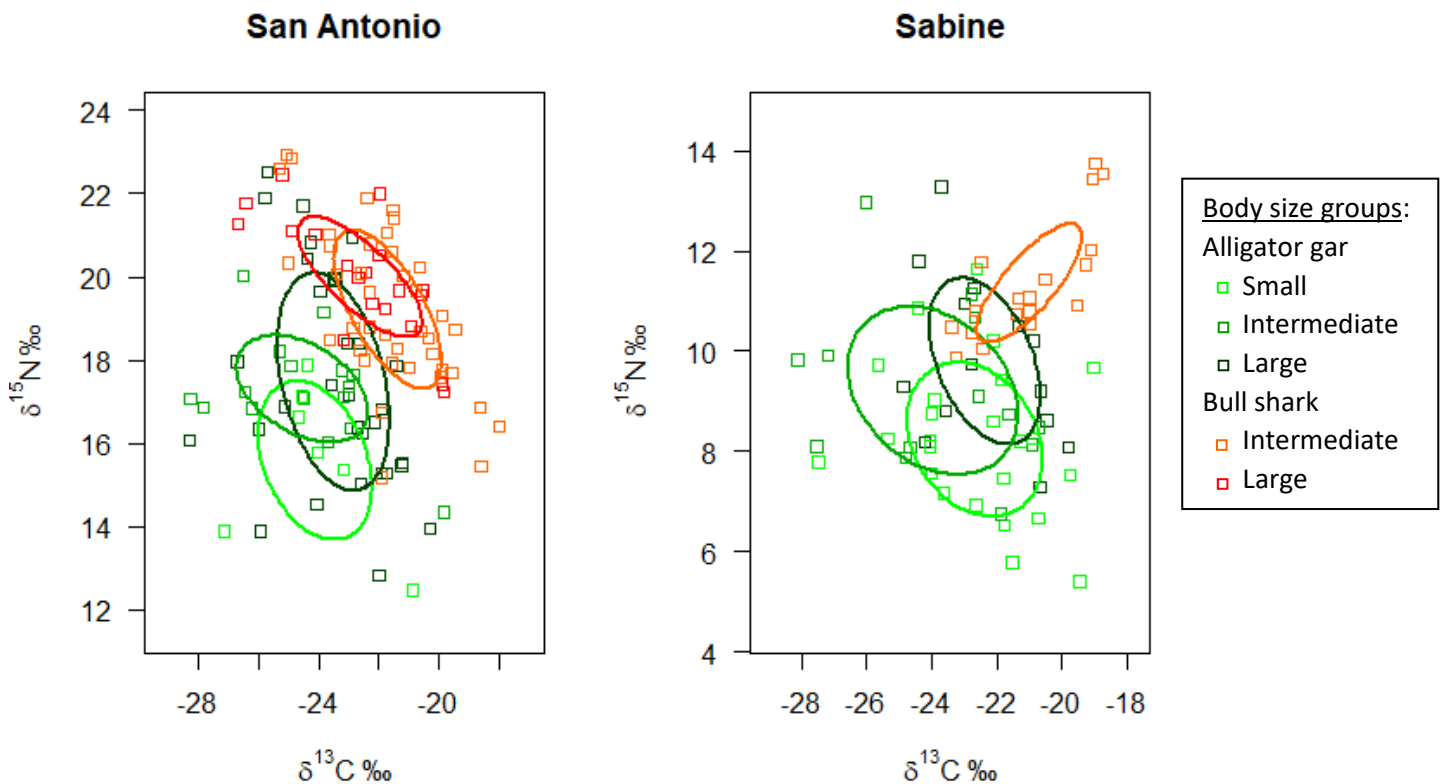


Figure 9: SIBER ellipses of alligator gars and bull sharks body size groups sampled in San Antonio Bay (left) and Sabine Lake (right)

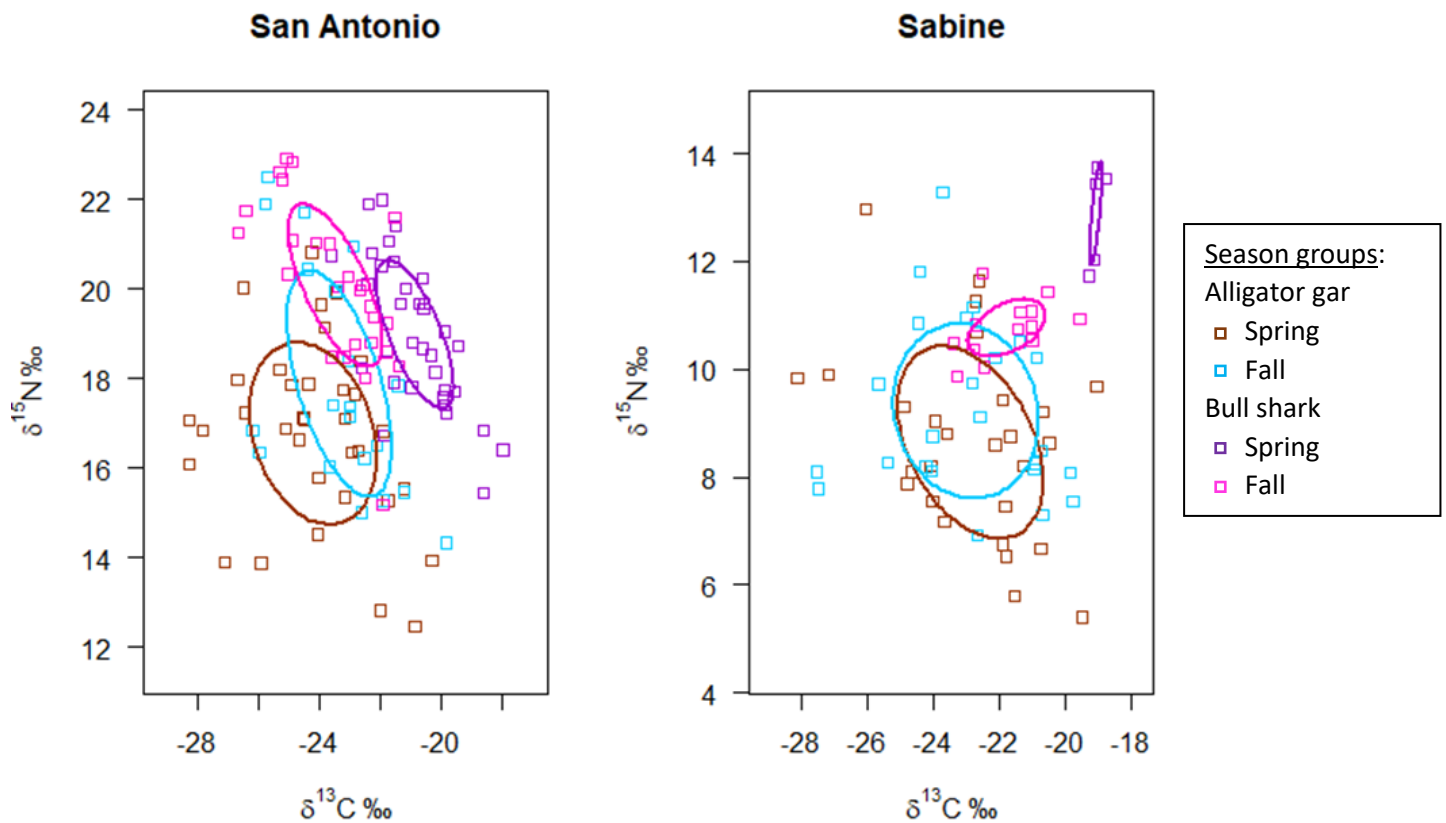


Figure 10: SIBER ellipses of alligator gars and bull sharks season groups sampled in San Antonio Bay (left) and Sabine Lake (right)

### 3.2.3. Trophic position

Globally, alligator gars had a lower trophic position in comparison to bull sharks in both estuaries. In San Antonio Bay, alligator gars and bull sharks had a mean trophic position of 3.2 and 4.9, respectively. Mean TP values were lower in Sabine Lake: 2.7 for *A. spatula* and 4.1 for *C. leucas* (Figure 11). It was also noticeable that alligator gars from both estuaries and bull sharks from Sabine Lake showed a similar range of individual trophic position while San Antonio Bay bull shark individuals showed a wider range of trophic position.

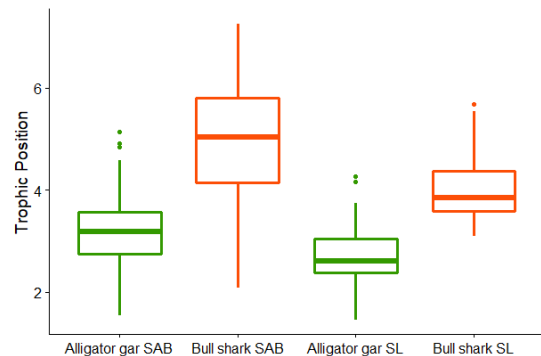


Figure 11: Trophic positions of *A. spatula* and *C. leucas* in San Antonio Bay (SAB) and Sabine Lake (SL)

### 3.2.4. Isotopic niche

The calculation of the isotopic niche breadth with  $SEA_C$  and with the mode of the  $SEA_B$  posterior distribution led to similar results (Figure 12, Table 13). Alligator gar's isotopic niche was approximately two times wider than bull sharks isotopic niche in San Antonio and approximately three times wider in Sabine Lake (Table 13). The uncertainties of the  $SEA_B$  estimation was higher for alligator gars than bull sharks.

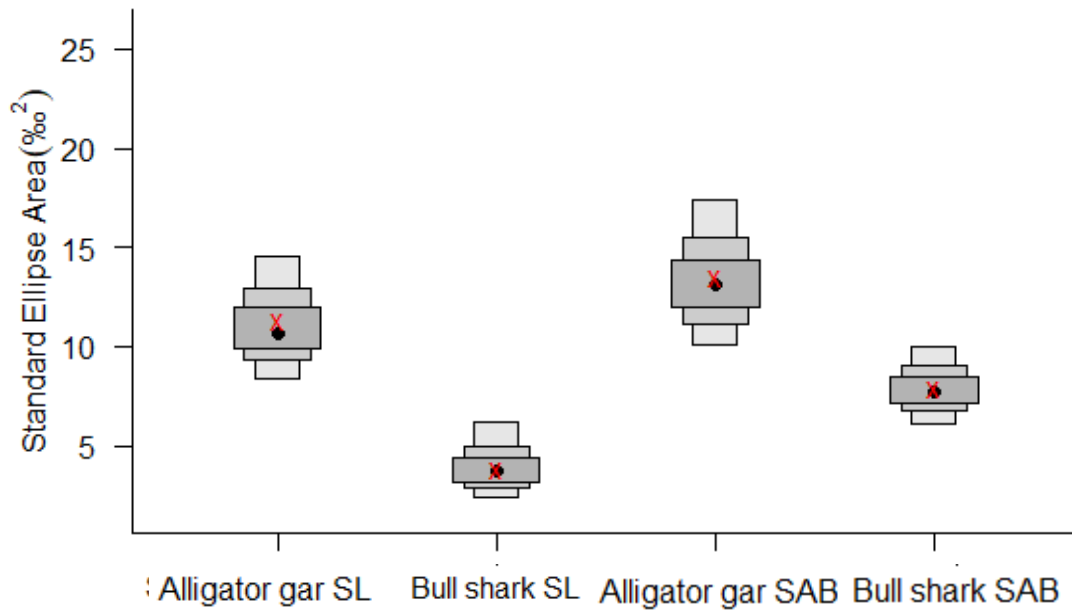


Figure 12: Posterior distributions of the SEAB estimated by with a Bayesian approach. The red crosses represent SEAC values estimated with a frequentist approach

Isotopic niche overlap between species was very low or even null. In Sabine Lake, the overlap area between species niche  $0.15\text{‰}^2$ , representing around 1% of alligator gar  $SEA_c$  and around 2% of bull shark  $SEA_c$ , which is negligible. In San Antonio Bay, there was no overlap between species  $SEA_c$ .

When individuals are separated by body size groups,  $SEA_c$  are slightly higher than  $SEA_b$  (Table 14). Alligator gars isotopic niche breadth varied differently across estuaries. Intermediate body size gars had a narrower niche in comparison to smaller and larger individuals in San Antonio Bay. However, they had the widest isotopic niche in Sabine Lake.

Bull shark isotopic niche slightly decreased with body size in San Antonio Bay. Only large gars isotopic niche overlap bull sharks niche (Table 15). Intraspecific niche overlap between body size groups were higher within bull sharks in SAB and within alligator gars within SL.

$SEA_c$  and  $SEA_b$  by season provided similar estimates of isotopic niche breadth in both estuaries. Alligator gars niche were smaller in Fall than in Spring in San Antonio Bay and were equivalent in Sabine Lake (Table 15). Bull sharks showed similar variation between season in both estuaries with higher isotopic niche breadth estimates in Fall than in Spring. Interspecific and intraspecific overlap were not calculated here because turnover rates were different between species and because overlap across season does not have ecological sense.

Species	San Antonio Bay		Sabine Lake	
	SEA <sub>c</sub>	Mode SEA <sub>B</sub>	SEA <sub>c</sub>	Mode SEA <sub>B</sub>
Alligator gar	13.5	13.2	11.3	10.7
Bull shark	7.9	7.8	3.9	3.8

Table 13: Estimation of the sample size-corrected Standard Ellipse Area (SEA<sub>c</sub>) and the Bayesian Standard Ellipse Area (SEA<sub>B</sub>) for the alligator gar and the bull shark in both estuaries. Values are expressed in %<sup>2</sup>

Species	San Antonio Bay		Sabine Lake	
	SEA <sub>c</sub>	Mode SEA <sub>B</sub>	SEA <sub>c</sub>	Mode SEA <sub>B</sub>
<b>Alligator gar</b>				
Small	12.6	9.9	9.9	9.1
Inter	8.4	7.5	14.1	12.3
Large	14.5	13.9	8.5	7.6
<b>Bull shark</b>				
Inter	8.1	7.8	3.9	3.7
Large	6.4	6.2		

Table 14: Estimation of the sample size-corrected Standard Ellipse Area (SEAC) and the Bayesian Standard Ellipse Area (SEAB) for the alligator gar and the bull shark body size

Species	San Antonio Bay		Sabine Lake	
	SEA <sub>c</sub>	Mode SEA <sub>B</sub>	SEA <sub>c</sub>	Mode SEA <sub>B</sub>
<b>Alligator gar</b>				
Spring	13.0	12.4	11.6	11.0
Fall	11.6	11.0	11.4	10.9
<b>Bull shark</b>				
Spring	4.8	4.6	0.5	0.4
Fall	6.6	6.3	1.9	1.6

Table 15: Estimation of the sample size-corrected Standard Ellipse Area (SEAC) and the Bayesian Standard Ellipse Area (SEAB) for the alligator gar and the bull shark seasonal groups in both estuaries. Values are expressed in %<sup>2</sup>

	AG Small	AG Inter	AG Large	Bull Inter	Bull Large
(a) San Antonio					
AG Small	1	0.48	0.37	0	0
AG Inter	0.32	1	0.41	0	0
AG Large	0.43	0.70	1	0.09	0.09
Bull Inter	0	0	0.05	1	0.73
Bull Large	0	0	0.04	0.58	1

	AG Small	AG Inter	AG Large	Bull Inter	Bull Large
(b) Sabine					
AG Small	1	0.48	0.39	0	
AG Inter	0.68	1	0.58	0.02	
AG Large	0.33	0.35	1	0.26	
Bull Inter	0	0	0.12	1	
Bull Large					

Table 16: Estimations of isotopic niche overlap between body size groups in (a) San Antonio Bay and (b) Sabine Lake. Values are expressed as percentage of the group in column SEAC

## 4. Discussion

This study aimed to investigate the trophic ecology of the three most abundant top predators found in Texas estuaries: the alligator gar *Atractosteus spatula*, the bull shark, *Carcharhinus leucas*, and the blacktip shark *Carcharhinus limbatus*. For that, we used two different approaches, stomach content analyses and stable isotope analyses, to get data about their trophic relationships within Texas estuaries food webs. Each method provided different information about interspecific and intraspecific variability in trophic ecology. Intraspecific variability was investigated across extrinsic factors, such as seasons and environmental conditions, and intrinsic factors such as ontogeny. This knowledge will improve our understanding of the trophic roles and flexibility of these three top predators. SCA provided precise basic information about diet composition while SIA provided a smoothed long-term dietary habits vision. In a context where Texas estuarine environments are facing changes and threats, this knowledge is essential to predict the impacts of these changes and implement efficient ecosystem management measures. This study was part of a wider project aimed at understanding the global trophic structures of the Texas estuaries food webs.

### 4.1. Overall diet habits: interspecific similarities and differences

Globally, the three top predators studied here share an important similarity: their diet mostly rely on one or two prey items. More specifically, alligator gars. In San Antonio Bay and Sabine Lake respectively, 99% and 89% of alligator gars diet, and 82% and 91% of bull sharks diet are represented by two prey items. Also, a single prey type represents 66% of San Antonio Bay bull sharks in San Antonio Bay. Moreover, they all share mullets species, and more precisely striped mullets, as a major component of their diet (Alligator gars:  $\Sigma\%IRI=54\%$  in SAB,  $\Sigma\%IRI=20\%$ ; Bull sharks:  $\Sigma\%IRI=31\%$  in SAB,  $\Sigma\%IRI=59\%$  in SL; Blacktip shark:  $\Sigma\%IRI=66\%$  in SAB). Mugilids are demersal and gregarious species (Bagarinao & Vetter, 1989), reaching more than 50 cm. Such relative importance of Mugilids is consistent with Wenner et al. (1990) who found that mullets have a role of forage fishes in estuaries for many high trophic level predators. Surprisingly, alligator gars feed more on Mugilids in San Antonio Bay than in Sabine Lake while it is the opposite in bull sharks. In addition to these similarities, alligator gars, bull sharks, and blacktip sharks also show some crucial differences in terms of diet composition.

**Alligator gars** feed extensively on gulf menhadens, *Brevoortia patronus*, in San Antonio Bay and Sabine Lake. Although there is a lack of study about alligator gars diet, our findings are consistent with those found in close relative species. Smylie et al. (2015) reported the predominance of Clupeids from the *Brevoortia* genus in the diet of the longnose gar, *Lepisosteus osseus*. The gulf menhaden is a small pelagic fish which have a gregarious behavior. Finding high numbers of this prey item in alligator gar stomachs (up to 62 gulf menhadens in a single stomach) (Appendix 8 and 11) suggests that this predator most likely feeds on gulf menhaden schools. In addition, high vacuity index values in alligator gars (69% in SAB and 61% in SL) are consistent with high values found by Bonham (1941) and showed that feeding events are time spaced. This suggests that alligator gars may be ambush foragers which sit and wait for gulf menhaden schools to then strike and eat high numbers of individuals. This predation strategy is consistent with the alligator gar morphology, which is designed to make speed bursts and ambush prey (Lemberg et al., 2018). Such a feeding strategy could also be related to a low metabolism or low activity level (Fu et al., 2009). Other prey items can also be found in their stomachs, but their importance can be considered negligible compared to Mugilids and Clupeids. Clupeids and Mugilids are primary consumers and have a low trophic position (Ollin et al., 2013; Matich, unpublished data). The fact they both represent the main diet of alligator gars explains the trophic position around 3 found with the stable isotope analysis approach. Therefore, the alligator gar appears as a relatively

specialist species that take advantage of mullet and gulf menhaden schools, depending on ecological conditions (discussed further), to feed massively at a low energetic cost.

**Bull sharks** major prey item in addition to Mugilids was different from alligator gars. In San Antonio Bay, catfish from the Ariidae family represent half of bull sharks diet, followed by Mugilids and Clupeids and Sciaenids, to a lesser extent. Two species were identified among these catfish: the hardhead catfish, *Ariopsis felis*, and the gafftopsail catfish, *Bagre marinus*. This is consistent with Snelson et al. (1984), who reported high quantities of *A. felis* and *B. marinus* in bull sharks diet on the Florida west coast. However, catfish are almost totally absent from Sabine Lake bull sharks diet ( $\Sigma\%IRI=2\%$ ), which is dominated by Mugilids ( $\Sigma\%IRI=59\%$ ) and completed by Sciaenids ( $\Sigma\%IRI=32\%$ ). These Sciaenids are mainly represented by red drums, *Sciaenops ocellatus*. Hardhead catfish, gafftopsail catfish, and red drums are demersal predators (Yanez-Arancibia & Lara-Dominguez, 1988; Scharf & Schlight, 2000; Matich et al., 2020), which suggest that bull sharks may feed close to the substratum. This is also suggested by the presence of sediments and vegetation in some of the stomach examined. Targeting demersal species may be related to their morphology since their mouth is located on the ventral face, which is adapted to feed close to the bottom. Trophic levels estimated using stable isotopes are  $>4$ , which is consistent with a diet mainly composed of predators and the trophic level of 4.2 estimated by Cortés (1999) for this species. Unlike alligator gars, most bull sharks stomachs contained prey, suggesting they feed very frequently. Bull shark diet studies reported different vacuity index values, ranging from 22% to 56% (Snelson et al., 1984; Trystram et al. 2016; Estupiñan-Montaño et al., 2017). Vacuity index values of 13% (SAB) and 20% (SL) were consistent with the value of 22% found by Snelson et al. (1984), who studied juvenile bull sharks caught with gillnets. The other studies focused on adult individuals caught with commercial fishing gear. Therefore, this may suggest that the sampled method may lead to different vacuity indexes or that the vacuity index increases with body size. The first hypothesis is credible since shark can evert their stomach during catching events, and the second also makes sense from an anatomic standpoint because juveniles have smaller stomachs and greater metabolic needs per unit body mass (faster growth) than adults. The high frequency of feeding events may be explained by high energy requirement due to the osmoregulation mechanisms used by the bull shark to support low salinity waters.

**Blacktip sharks mostly fed on Mugilids and prey on higher numbers of low important preys** than the previous species. Indeed, prey items such as Sciaenids ( $\Sigma\%IRI=14\%$ ), Elasmobranchs ( $\Sigma\%IRI=9\%$ ) or Crustaceans ( $\Sigma\%IRI=4\%$ ) are more abundant in *C. limbatus* diet than in alligator gars or bull sharks. Blacktip sharks showed intermediate vacuity rate value (60%), which show they forage less frequently than bull sharks. However, it is unlikely that blacktip sharks are ambush forager. Vacuity index value is relatively high (60%), and above all it is a continuous swimmer species. The blacktip shark is a marine species and does not benefit from osmoregulation abilities that could make it support low salinities like the bull shark. This physiological limitation may explain why blacktip sharks were only found in San Antonio Bay and in low numbers. It is possible that small individuals enter San Antonio Bay to take advantage of the physical protection against predator and high prey availability, such as shrimp species. However, Sabine Lake water salinity is probably too low for this species.

Isotope  $\delta^{13}C$  ratios were significantly lower for alligator gars than bull sharks in both estuaries (Table 11, Figure 8). This suggest that alligator gars feed more in freshwater habitat or from preys more related to freshwater food webs than the bull shark, which feed more on marine species or marine food webs related preys.

Therefore, diet composition and feeding strategies of alligator gars, bull sharks and blacktip sharks are likely related to their morphology and metabolism. Alligator gars are ambush foragers, waiting for big gulf menhaden schools to forage high quantities at low energetic cost. Bull sharks are continuous swimmers and use osmoregulation mechanisms to support low salinity waters (Pillans et al., 2006). This may explain frequent feeding events on prey adapted to their morphology. Blacktip sharks do not benefit from osmoregulation capabilities as efficient as the bull shark. This may lead to lower energetic

requirements and then low frequency of feeding events. Prey items identified using SCA are consistent with the trophic positions estimated using SIA. Bull sharks feed at higher trophic levels than alligator gars in both estuaries. Finally, isotope  $\delta^{13}\text{C}$  ratios were significantly lower for alligator gars than bull sharks in both estuaries (Figure 8). This shows that alligator gars feed more in freshwater habitat or from preys more related to freshwater food webs than the bull shark, which feed more on marine species or marine food webs related preys. This is consistent with life-history traits of study predators because the alligator gar is a freshwater species while the bull shark is a marine species, but also suggest the presence of mechanisms to minimize interspecific competition (See further Discussion 4.2).

#### 4.2. Trophic niche breadth and overlap

SCA and SIA provided different results in terms of niche breadth. The standardized Levin index values obtained from stomach contents analysis suggest that alligator gars have a narrower niche breadth than bull sharks and blacktip sharks in San Antonio Bay (Table 6). This is explained by the fact the alligator gars rely almost exclusively on its two major prey items, while both sharks also include numerous low importance preys in their diet. Such low importance preys have been found in Sabine Lake alligator gars, which have a similar niche breadth than bull sharks.

However, isotopic niche breadth estimated with stable isotope was inconsistent with the previous findings. Estimations of standard ellipse area revealed broader isotopic niches in alligator gars than bull sharks in both estuaries. Indeed, bull sharks isotopic niche were almost two times and three times narrower the alligator gars in San Antonio Bay and Sabine Lake, respectively (Figure 8, Table 13). The consistency of the isotopic niche breadth estimation between  $\text{SEA}_c$  and  $\text{SEA}_b$  shows that both methods lead to similar estimations for sample size  $> 37$  individuals. The differences between the two approaches in niche breadth estimation highlights the complexity of the stable isotope analysis. Indeed, this method provides information on the isotopic niche breadth, which is a different concept from the actual niche breadth. Thus, the two approaches are not necessarily contradictory here. Alligator broad isotopic niches can be explained by high ranges of isotope values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in both ecosystems. Alligator gar higher range of  $\delta^{13}\text{C}$  (-23.75, 13.71 ‰ in SAB; -25.72, -16.38 ‰ in SL) than bull shark (-21.75, -13.71 in SAB; -22.33, -17.61 in SL) suggests diversity in foraging locations across a wider geographic range. In other words, this shows a high intraspecific variability in terms of forage grounds among alligator gars individuals and probably in terms of habitat use in general. High range of  $\delta^{15}\text{N}$  also suggest variability among alligator gars individuals. Indeed, it shows a diversity of trophic levels at which individuals feed, which means that they feed on different species or that prey exhibited variability in trophic position. The first option seems more plausible since alligator gars prey are mostly primary consumers and thus should not exhibit such a variability in trophic position. Therefore, the Levin measure of trophic niche shows that bull sharks and blacktip sharks diet is more diverse than alligator gars in San Antonio Bay and that bull sharks and alligator gars diet are as diverse in Sabine Lake from a taxonomic standpoint. Isotopic niche inferences show that alligator gars exhibit more intraspecific variability in terms of feeding habitat and of prey trophic position.

In terms of niche overlap, the two methods also provided different results. The Renkonen percentage overlap indicated values comprised between 40% and 50% (Table 7), which suggests that alligator gars, bull sharks and blacktip sharks all share approximately half of their diet with each other. This interspecific overlap is consistent with the high presence of Mugilids in the three predators diets. Alligator gars, bull sharks, and blacktip sharks share and compete for this resource in San Antonio Bay and Sabine Lake. Mugilids are demersal species that can also form big schools and swim in open waters. Because of this ambivalence, they can meet both alligator gars and sharks preferences in terms of feeding strategy. The dissimilarity in the rest of the diet composition and the feeding strategy may be resource partitioning to reduce interspecific competition or related to prey preferences, morphological differences, or energetic requirements. Specific  $\text{SEA}_c$  overlaps were null in San Antonio Bay and small

in Sabine Lake (Figure 8, Table 15), suggesting the absence of interspecific competition. This may be due to more depleted  $\delta^{13}\text{C}$  values in alligator gars than bull sharks in both estuaries, which suggests that alligator gars globally feed in lower salinity waters than bull sharks, as mentioned earlier. Despite their good osmoregulation abilities, alligator gars cannot tolerate high salinity waters for extensive period (Allen et al., 2017). It is possible that bull sharks take advantage of the high productivity of higher salinity waters, thus minimizing the energetic cost of osmoregulation and minimizing competition with alligator gars at the same time. This hypothesis is supported by the higher isotopic overlap in Sabine Lake, where salinities are lower, and thus where alligator gars access is less restricted. In addition, this is also supported by the fact that niche interspecific isotopic niche overlap increase with alligator gar body size (Figure 9, Table 16), because larger gars benefit from more efficient osmoregulation abilities (Allen et al., 2017), and therefore more access to high salinity. Therefore, even if alligator gars and bull sharks partially feed on similar species, bull sharks decrease competition and partition the resources by taking advantage of alligator gars limited access to high salinities. However, it is possible that it leads to an increase of competition with blacktip sharks, whose access is not restricted, but this aspect could not be investigated here. Globally, our results highlight that SCA and SIA provide different information, and that results must be interpreted with caution.

### 4.3. Variability in diet

In this study, we also investigated how top predators diets vary between two estuaries, among season, environmental conditions, and with ontogeny. However, these issues were not examined in for blacktip sharks because of low sample sizes, which prevented us from getting reliable results.

#### 4.3.1. Alligator gar

##### **Regional variability**

Alligator gars exhibit similar dietary habits in San Antonio Bay and Sabine Lake from a statistical standpoint. Globally, *A. spatula* individuals feed on the same prey with a similar intensity. However, %IRI values revealed that Clupeids were more important in Sabine Lake than San Antonio Bay (%IRI=45% in SAB, %IRI=69% in SL). This may be related to difference in prey availability between estuaries. Except for the low value of Levin index for San Antonio Bay, niche breadths were also consistent in both estuaries. Our results thus suggest that alligator gars have similar trophic interactions and trophic roles in San Antonio Bay and Sabine Lake food webs.

##### **Seasonal variability**

The stomach contents approach did not allow us to investigate seasonal diet variability of gars in San Antonio Bay. Nevertheless, Sabine Lake individuals clearly show a diet shift between seasons. Spring diet is based on gulf menhaden, while Mugilids dominate Fall diet. Spring diets then coincide with Spring large aggregations of Clupeids, observed in Texas bays (Scharf & Schlicht, 2000). Our results then suggest that alligator gar take advantage of the high availability of Clupeids to feed at low energetic cost in Spring. We also found that the vacuity rate was higher in Spring than in Fall (Appendix 14). This supports the hypothesis that the high vacuity rate is related to a diet based on small pelagic fish, characterized by non-frequent feeding events in which preys are caught in high numbers.

We did not detect any change in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  isotope values neither major variation of isotopic niche breadth across seasons. We want to draw attention to the fact that SIA do not relate Spring and Fall feeding habits. The slow incorporation of elements in tissues suggests that SIA provides information about Winter and Summer diets, but the exact timescale remains too uncertain to draw conclusions. However, this approach is still valuable to provide information about eventual variability in the diet



throughout the year. Our results show that alligator gars trophic role does not vary through the year even if they take advantage of a seasonal Clupeids pulse in Spring.

### **Effect of environmental conditions**

Alligator gars diet varies across environmental conditions. First, *A. spatula* feeds more on Clupeids in low salinity waters. This may be related to the lower salinity of Texas estuaries in Spring rather than a direct impact of salinity on the trophic behavior. Diet variability across water temperature was inconsistent across estuaries. In San Antonio Bay, alligator gars feed more on Clupeids when water temperature  $\leq 28.5^{\circ}\text{C}$  while it was the opposite in Sabine Lake. Niche breadths were also hard to compare between groups based on salinity or temperature because of the low sample sizes. Changes in diet are likely related to variability in prey availability or variability in energy requirements across environmental conditions. The first option is plausible since the fact that Clupeids importance in diet was higher in Sabine Lake, which is characterized by low salinities, than in San Antonio Bay. Therefore, the variability of alligator gar trophic ecology across environmental conditions remains unclear.

### **Ontogenetic shift**

Dietary composition varied among body size groups in San Antonio Bay and Sabine Lake. Even if the lack of information on the diet of gars  $< 800$  mm TL makes comparison difficult, consumption of Clupeids seems to decrease with body size in San Antonio Bay while it seems to increase in Sabine Lake. Therefore, alligator gars total length does not explain this variation. This suggests that the variation of prey relative importance observed between body size group is not due to morphological change, but rather probably to environmental conditions. However, crustaceans were only found in small individuals stomachs, highlighting an ontogenetic shift to piscivory. Such transition has been reported in small juvenile alligator gars, which shifted from a diet based on small invertebrates to piscivory during their growth (Butler et al., 2018). The significant increase of  $\delta^{15}\text{N}$  values with total length also supports this (Table 12; Snow et al., 2020). Large alligator gars feed on bigger fish prey, which explained why large gars show more diet overlap with bull sharks (Table 15), which feed at higher trophic levels (See Discussion 4.1).

## **4.3.2. Bull shark**

### **Regional variability**

Bull sharks show partially different diet composition between estuaries. San Antonio Bay individuals feed on Ariids while Sabine Lake individuals feed on red drums. Even if they do not belong to the same taxonomic groups, these two preys belong to the same functional group of demersal predators. Catfish and red drums are present in both ecosystems but may show regional variability in abundance. It is conceivable that bull sharks adapt their diet to different prey availabilities. Also, bull sharks niche breadth estimated with SCA and SIA were consistent across estuaries. Therefore, bull sharks show different trophic interactions but have similar roles in San Antonio Bay and Sabine Lake food webs.

### **Seasonal variability**

Bull sharks diet shows minor seasonal changes in San Antonio Bay. Clupeids presence in Spring and absence in Fall is consistent with alligator gars stomach contents analysis results, suggesting predators take advantage of Clupeids seasonal high abundance. Major prey items proportions do not exhibit variation across seasons in San Antonio Bay. Sabine Lake bull sharks diet is essentially composed of red drums, which are then absent from the diet of individuals sampled in Fall and replaced by Mugilids prey. The Fall sampling period, *i.e.* September and October, is the time of the year when red drums abundance is the lowest in Sabine Lake (Scharf, 2000). This explains their scarcity in bull shark stomach at this period. Bull sharks then fall back on Mugilids, even if it increases competition with alligator gars for this resource. The fact that bull sharks feed on the same prey in Spring and Fall in San Antonio Bay

and adapt its diet in Sabine Lake to face trophic resource scarcity suggests two things: (1) bull sharks are flexible predators and (2) Mugilids likely have a role of forage fish for bull sharks but is not a preferred prey.

Individuals caught in Spring and Fall also show different  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios. First, even if the unknown timescale prevents us from linking these variations with the results of the SCA, it suggests that bull shark trophic interactions vary in terms of trophic level and habitat use throughout the year. Second, the opposite variation of isotope values between San Antonio Bay and Sabine Lake individuals suggests that bull sharks exhibit different change in diet, and that these changes may be driven by extrinsic factors, such as salinity.

### **Effect of environmental conditions**

SCA results did not show any changes in bull sharks dietary habits based on water temperature or salinity.

### **Ontogenetic shift**

We did not detect ontogenetic diet composition change in this study using PERMANOVA on relative prey weight, although Figure 7 exhibits some changes in San Antonio Bay and Sabine Lake. Intermediate body size bull sharks eat more Clupeids while Large individuals do not in San Antonio Bay. Besides, small bull sharks, *i.e.* TL < 900 mm, feed much more upon Sciaenids than Intermediate body size sharks. The discrepancy of these results may be attributed to the difference of metrics used (%W and %IRI, See discussion 4). Stable isotope supports the presence of ontogenetic shift. The increase of  $\delta^{15}\text{N}$  with body size in Sabine Lake bull sharks (Table 12), suggest that bigger individuals shark feed at higher trophic levels. This has been reported in many shark species.

Therefore, the bull shark appears as a flexible predator, which can adapt its diet depending on prey availability. This species diet is then probably driven by prey availability more than interspecific competition with other top predators such as the alligator gar.

## **4.4. Limitations of stomach contents analyses**

SCA provides a high-resolution vision of trophic relationships. It has the advantage of identifying the preys foraged by predators, which is necessary to understand relationships within food webs. However, examining stomach content only provides a snapshot of what an individual has been eating recently (Michener & Kaufman, 2007) and does not reflect the dietary habit of the individuals examined. Thus, a high number of individuals must be included in the analysis to provide a reliable representation of the diet of a group of individuals. In this study, we chose the threshold of 9 individuals to consider the stomach compositions representative of groups dietary habits. However, in most cases, 9 individuals are not enough to reach an asymptotic phase on species richness cumulative curves (Appendix 1). Even if we probably identified the major prey items in the three study predators diets, it is possible that we missed a part of the diet composition due to a too-small sample size. This implies that the trophic niche breadths and eventually, the niche overlaps obtained from SCA could be underestimated in this study. Moreover, some groups did not even reach the size of 9 individuals and could not be included in any interpretation, making interpretation of the shift across seasons, environmental conditions, or ontogeny difficult or even impossible. This limitation of SCA can be exacerbated by the regurgitation of prey upon capture, especially in shark species, which often evert their stomach under stress (Brunnschweiler et al., 2011). Therefore, this study highlights the difficulty of conducting stomach contents on species, associated to the difficulty to obtain a large sample size such as top predators (Cortés, 1997; Trystam et al., 2016).

The index of relative importance %IRI is a common metric widely used to study marine predators diet composition (Cortés, 1997; Bethea et al., 2004, 2006, 2007; Rosende-Pereiro et al., 2019). Nevertheless, this metric also shows some limits. This metric provides a synthetic vision of the diet composition by integrating the different components of prey importance (%W, %N and %O). However, this index is not additive, which means that %IRI is sensitive to the number of prey groups included in the analyses (Cortés, 1997). The sum of the %IRI of all preys from a family is not equivalent to the %IRI of this family, and the difference in between can be substantial (Appendix 2,3,4 vs. 5,6,7). Furthermore, %IRI is calculated for each prey type and cannot be used to conduct multivariate analysis such as permutation analysis of variance as we did here.

Finally, the stomach content analysis provides informative data about prey that alligator gars, bull sharks, and blacktip sharks catch and ingest but not about what they assimilate (Michener & Kaufman, 2007). Therefore, SCA is more related to a behavioral aspect of the diet than a physiological or nutritional aspect.

#### 4.5. Limitations of stable isotope analyses

The number of alligator gar muscle samples and bull shark liver samples were large enough to conduct reliable stable isotope analysis in San Antonio Bay and Sabine Lake. Unfortunately, the number of blacktip shark liver samples was too low to include them in the analyses. SIA provided valuable insight into the roles played by alligator gars and bull sharks, especially in terms of trophic position. Nonetheless, our inferences rely on strong assumptions that need to be considered when interpreting the results obtained.

Firstly, we determined the Diet-Trophic Discrimination Factor based on previous studies conducted on similar species. The common-used DTDF value of 3.4‰ recommended by Post (2002) is associated with high uncertainties and many studies showed the presence of high variability in DTDF values, which differ among species, diet composition and environmental conditions (Harvey et al., 2002; Vanderklift & Ponsard, 2003; Suzuki et al., 2006; Sweeting et al., 2006a, 2006b; Barnes et al., 2007; Voltaire et al., 2007; German & Miles, 2010; Heady & Moore, 2013; Mont'Alverne et al., 2016; Franssen et al., 2017; Taylor et al., 2017). The DTDF values corresponding to the alligator gar and the bull shark have never been estimated to our knowledge. For the alligator gar, we decided to use the mean values found in previous studies that estimated DTDF for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , conducted on similar species (piscivorous teleosts) and lipid-extracted tissues, with reasonable sample sizes. We found only two studies that met these criteria (Pinnegar & Polunin, 1999; Trueman et al., 2005). For the bull sharks, we used the mean of the values estimated by Hussey et al. (2010) and Malpica-Cruz et al. (2013). Therefore, the DTDF values used in this study are associated with high uncertainties. Trophic position estimates are highly sensitive to small errors on DTDF (McCutchan et al., 2003), and this uncertainty thus propagates through the comparisons of isotope values. DTDF improvement based on diet composition is not realistic, considering the difficulty to match the natural diet in laboratory conditions. However, a more accurate estimation of the discrimination factor associated with bull shark liver and alligator gar muscle would be very beneficial to increase the reliability of the methodology used here.

The second important limitation of the SIA conducted in this study is the difference in isotopic turnover rates between alligator gar muscle and bull shark liver. Previous studies suggest that turnover rates are faster in teleosts than chondrichthyans tissues (Hussey et al., 2012). This turnover rate difference must be considered when interpreting the data, especially for interspecific comparisons.

Finally, our approach includes an adjustment of the data made based on the baseline. We used isotope values of 10 species of primary consumers as an isotopic baseline.

Baseline isotope values varied in function of salinity, especially in San Antonio Bay. Thus, we adjusted alligator gars and bull sharks isotope values based on each estuary baseline variation to be consistent. This adjustment was necessary to prevent interference in isotope values due to salinity, which would lead to inaccurate interpretation. However, this approach assumes that the salinity at time and place of capture represents the environment in which the predator foraged more than 100 days before, *i.e.* the time of incorporation of stable isotopes. This assumption is likely erroneous because alligator gars and bull sharks can exhibit long-distance movement in estuaries (Buckmeier et al., 2013; Solomon et al., 2013; Matich & Heithaus, 2015).

Therefore, the methods used in this study show important limitations which need to be considered. The joint use of SCA and SIA, with their advantages and their weaknesses give more robustness to our findings.

## 5. Conclusion & Perspectives

Alligator gars, Bull sharks and blacktip sharks all share mullets as a relatively important part of their diet. To face such a competition for foraging resource, bull sharks may adapt to limit this competition by spatial partitioning. They feed in high salinity waters where the alligator gar has a restricted access due to physiological limitation. We also found that alligator gars show high intraspecific variability in habitat use and trophic position. This could also be a mechanism to decrease interspecific competition. These two hypotheses are plausible and suggest that alligator gars and bull shark have high flexibility abilities. Unfortunately, our study did not investigate trophic ecology of blacktip sharks as far as the two other species. It is possible that competition between bull sharks and blacktip sharks increase with salinity. Thus, it would be very valuable to increase our sample size to investigate this question and have a better understanding of the role of blacktip sharks.

The alligator gar is ambush forager which feed frequently on large schools of Clupeids while the bull shark feed frequently on bigger predator fish. These interspecific differences are related to morphological and behavioral, and probably metabolically differences. Data on these predators energy requirement would be valuable to confirm this.

Variability in diet have been observed in the three species. It is difficult to untangled the effect of the different variables when investigating intraspecific variability in dietary habits. Indeed, regions, seasons, water temperature and ontogeny are not independent variables. We encourage TPWD to keep sampling predators, when found dead, in San Antonio Bay and Sabine lake to get more data and keep investigating these questions. Our results suggest that intraspecific variability in diet may be related to variability in prey availability. It would thus be interesting to compare our results with abundance index (or CPUE from fisheries), of the major prey identified: Mugilids, Ariids and Sciaenids (red drums especially).

This study also shows the usefulness of combining stomach content analyses and stable isotope analyses. A single approach would have considerably limited our abilities to understand the roles of these predators. We highly encourage the combination of these two approaches to investigate trophic ecology in aquatic ecosystems.

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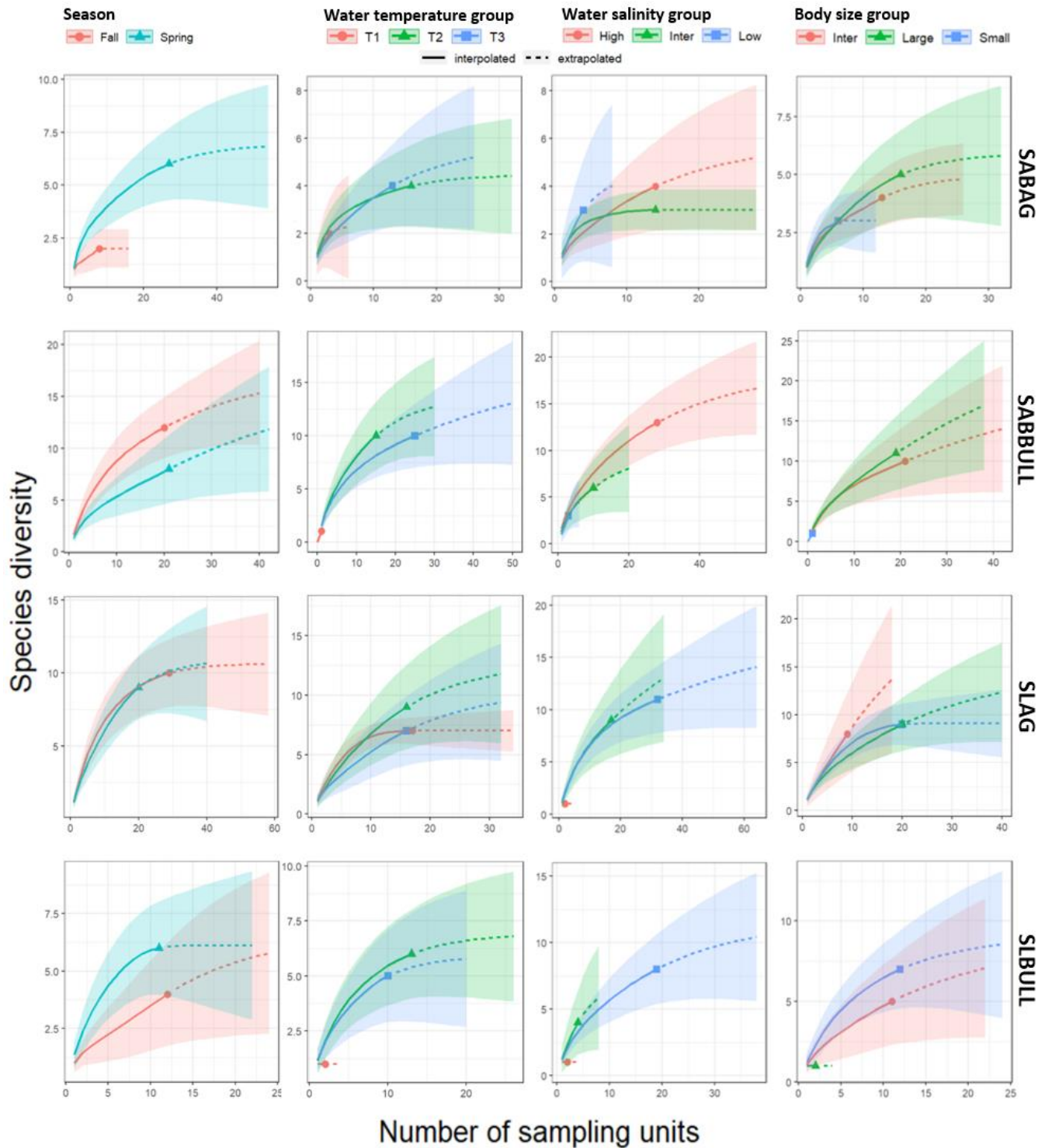
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## Appendix





Appendix 1: Cumulative curves of species diversity (number of species) for alligator gar, bull shark groups. From top to bottom: San Antonio Bay Alligator Gars (SABAG), San Antonio Bay Bull sharks (SABBULL), Sabine Lake Alligator gars (SLAG), Sabine Lake Bull sharks (SLBULL)



Tarpon	0,00	0,01	0,00	0	0,04	0	0	0	0,01	0	0,10	0	0	0	0,01	0,02	0	NA	0	0	0,01	0	0	0,01
<b>Mugiliformes</b>	<b>0,36</b>	<b>0,12</b>	<b>0,26</b>	<b>0,98</b>	<b>0,03</b>	<b>0,28</b>	<b>0,86</b>	<b>0,11</b>	<b>0,92</b>	<b>0,09</b>	<b>0,67</b>	<b>0,01</b>	<b>0,52</b>	<b>0,15</b>	<b>0,90</b>	<b>0,22</b>	<b>0,11</b>	<b>0,00</b>	<b>0,25</b>	<b>0,22</b>	<b>0,82</b>	<b>0,18</b>	<b>0,18</b>	<b>0,14</b>
unID Mugilidae	0,09	0,09	0,01	0,89	0,03	0,20	0,25	0,08	0,04	0,04	0,62	0,01	0	0,10	0,12	0,22	0,01	NA	0,02	0	0,53	0,12	0	0,14
Striped mullet	0,27	0,02	0,25	0,10	0	0,07	0,61	0,03	0,86	0,04	0,05	0	0,52	0,04	0,77	0	0,09	NA	0,23	0,21	0,29	0,07	0,18	0
White mullet	0,00	0,00	0,00	0	0	0,01	0	0	0,02	0,02	0	0	0	0	0,01	0	0,01	NA	0	0,01	0	0	0	0,01
<b>Perciformes</b>	<b>0,00</b>	<b>0,08</b>	<b>0,00</b>	<b>0,02</b>	<b>0,06</b>	<b>0,15</b>	<b>0,14</b>	<b>0,00</b>	<b>0,01</b>	<b>0,31</b>	<b>0,13</b>	<b>0,03</b>	<b>0,28</b>	<b>0,01</b>	<b>0,00</b>	<b>0,06</b>	<b>0,16</b>	<b>0,00</b>	<b>0,01</b>	<b>0,00</b>	<b>0,02</b>	<b>0,21</b>	<b>0,14</b>	<b>0,08</b>
Warmouth	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0,00	0	0
unID Sciaenidae	0	0,00	0	0	0,00	0,00	0	0	0	0,01	0,01	0	0	0	0	0,01	0	NA	0	0	0	0,05	0	0
Atlantic croaker	0,00	0,00	0,00	0	0	0,01	0	0,00	0	0	0,05	0	0	0,01	0	0,01	0	NA	0,01	0,00	0	0,01	0,02	0
unID Drum	0	0,01	0	0	0	0,02	0	0	0	0,04	0	0	0	0	0	0	0,03	NA	0	0	0	0,01	0	0,01
Red drum	0	0,05	0	0	0,01	0,10	0	0	0	0,27	0	0	0	0	0	0,01	0,13	NA	0	0	0	0	0,05	0,07
unID Seatrout	0,00	0,01	0,00	0,02	0,03	0	0,14	0	0,01	0	0,07	0,00	0,28	0	0	0,02	0	NA	0	0	0,02	0,13	0	0
Spotted seatrout	0	0,00	0	0,00	0	0,01	0	0	0	0	0	0,01	0	0	0	0,01	0	NA	0	0	0	0	0,04	0
Spot croaker	0	0,00	0	0	0,01	0	0	0	0	0	0	0,01	0	0	0	0,01	0	NA	0	0	0	0	0,05	0
Pinfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Sheepshead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
<b>Pleuronectiformes</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
Southern flounder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
<b>Siluriformes</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0</b>	<b>0</b>	<b>0,02</b>	<b>0</b>	<b>0,00</b>	<b>0</b>	<b>0,03</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0,01</b>	<b>0</b>	<b>0,02</b>	<b>0</b>	<b>0</b>	<b>0,01</b>	<b>0,01</b>	<b>0,07</b>	<b>0</b>	<b>0,00</b>
unID Ariidae	0	0,00	0	0	0	0,01	0	0	0	0,02	0	0	0	0	0	0	0,02	NA	0	0	0	0,07	0	0
Gafftopsail catfish	0,00	0	0,00	0	0	0	0	0,00	0	0	0	0	0	0	0,01	0	0	NA	0	0	0,01	0	0	0
Hardhead catfish	0,00	0,00	0,00	0	0	0,00	0	0	0	0,00	0	0	0	0	0	0	0,00	NA	0	0,01	0	0	0	0,00

Appendix 2: %IRI of the all the prey items identified at the lowest taxonomic level, after removal of non-animal prey, for the alligator gar, *Atractosteus spatula*, in San Antonio Bay and Sabine Lake. Taxonomic levels in bold are sums of %IRI.

	SAB	SL	SAB		SL		SAB			SL			SAB			SL			SAB			SL		
	Total	Total	Spring	Fall	Spring	Fall	T1	T2	T3	T1	T2	T3	Low	h	High	Low	h	High	Small	Inter	Large	Small	Inter	Large
<b>Crustaceans</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	NA	<b>0,00</b>	<b>0,01</b>	<b>0,00</b>	<b>0,01</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,01</b>	<b>0,00</b>	<b>NA</b>
unID Portunidae	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Blue crab	0,00	0	0,00	0	0	0	0	0	0,00	NA	0	0	0	0,01	0	0	0	NA	0	0,00	0	0	0	NA
Panopeidae	0,00	0	0	0,00	0	0	0	0,00	0	NA	0	0	0	0	0,00	0	0	NA	0	0	0,00	0	0	NA
unID Shrimp	0,00	0	0	0,00	0	0	0	0	0,00	NA	0	0	0	0	0,00	0	0	NA	0	0	0,00	0	0	NA
White shrimp	0,00	0,00	0	0,01	0	0,01	0	0	0,01	NA	0	0,01	0	0	0,01	0,00	0	NA	0	0	0,02	0,01	0	NA
<b>Snake</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	NA	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>
Western ribbon	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Nerodia	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
<b>Elasmobranchs</b>	<b>0,01</b>	<b>0,02</b>	<b>0,00</b>	<b>0,03</b>	<b>0,03</b>	<b>0,00</b>	<b>0,00</b>	<b>0,01</b>	<b>0,02</b>	NA	<b>0,04</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,02</b>	<b>0,00</b>	<b>NA</b>	<b>0,00</b>	<b>0,03</b>	<b>0,00</b>	<b>0,05</b>	<b>0,00</b>	<b>NA</b>
<b>Carcharhiniformes</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	NA	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>	<b>0,00</b>	<b>0,03</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>
Bonnethead shark	0,01	0	0	0,02	0	0	0	0	0,02	NA	0	0	0	0	0,01	0	0	NA	0	0,03	0	0	0	NA
Atlantic sharpnose	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
<b>Myliobatiformes</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	<b>0,01</b>	<b>0,03</b>	<b>0,00</b>	<b>0,00</b>	<b>0,01</b>	<b>0,00</b>	NA	<b>0,04</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	<b>NA</b>	<b>0,00</b>	<b>0,01</b>	<b>0,00</b>	<b>0,05</b>	<b>0,00</b>	<b>NA</b>
unID Myliobat.	0,00	0,01	0	0,00	0,02	0	0	0,01	0	NA	0,02	0	0	0	0,00	0,01	0	NA	0	0	0,00	0,03	0	NA
Bluntnose stingray	0,00	0,01	0	0,00	0,01	0	0	0,01	0	NA	0,02	0	0	0	0,00	0,01	0	NA	0	0,01	0	0,02	0	NA
<b>Teleost</b>	<b>0,98</b>	<b>0,98</b>	<b>1,00</b>	<b>0,95</b>	<b>0,97</b>	<b>0,99</b>	<b>1,00</b>	<b>0,98</b>	<b>0,96</b>	NA	<b>0,96</b>	<b>0,99</b>	<b>1,00</b>	<b>0,99</b>	<b>0,97</b>	<b>0,97</b>	<b>1,00</b>	<b>NA</b>	<b>1,00</b>	<b>0,97</b>	<b>0,97</b>	<b>0,94</b>	<b>1,00</b>	<b>NA</b>
<b>Anguilliformes</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	NA	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>
Anguillidae	0,00	0	0,00	0	0	0	0	0,00	0	NA	0	0	0	0	0,00	0	0	NA	0	0	0,00	0	0	NA
Shrimp eel	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
<b>Clupeiformes</b>	<b>0,06</b>	<b>0,00</b>	<b>0,16</b>	<b>0,02</b>	<b>0,00</b>	<b>0,01</b>	<b>0,00</b>	<b>0,03</b>	<b>0,10</b>	NA	<b>0,00</b>	<b>0,02</b>	<b>0,47</b>	<b>0,05</b>	<b>0,03</b>	<b>0,01</b>	<b>0,00</b>	<b>NA</b>	<b>0,00</b>	<b>0,14</b>	<b>0,01</b>	<b>0,00</b>	<b>0,02</b>	<b>NA</b>
unID Clupeidae	0,00	0	0,00	0,00	0	0	0	0	0,01	NA	0	0	0	0	0,01	0	0	NA	0	0,00	0,00	0	0	NA
Gizzard shad	0,06	0,00	0,15	0,01	0	0,01	0	0,02	0,09	NA	0	0,02	0,47	0,05	0,02	0,01	0	NA	0	0,13	0,01	0	0,02	NA
Gulf menhaden	0,00	0	0	0,01	0	0	0	0,01	0	NA	0	0	0	0	0,00	0	0	NA	0	0,01	0	0	0	NA
Bay anchovy	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
<b>Elopiformes</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	NA	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,06</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>	<b>0,00</b>	<b>0,01</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>
Ladyfish	0,00	0	0	0,01	0	0	0	0,02	0	NA	0	0	0	0,06	0	0	0	NA	0	0,01	0	0	0	NA

Tarpon	0,00	0	0	0,01	0	0	0	0	0,00	NA	0	0	0	0	0	0	0	NA	0	0	0,01	0	0	NA
<b>Mugiliformes</b>	<b>0,35</b>	<b>0,40</b>	<b>0,36</b>	<b>0,31</b>	<b>0,02</b>	<b>0,95</b>	<b>1,00</b>	<b>0,52</b>	<b>0,22</b>	NA	<b>0,26</b>	<b>0,58</b>	<b>0,00</b>	<b>0,07</b>	<b>0,49</b>	<b>0,37</b>	<b>0,42</b>	NA	<b>0,00</b>	<b>0,21</b>	<b>0,46</b>	<b>0,19</b>	<b>0,61</b>	NA
unID Mugilidae	0,34	0,23	0,30	0,31	0,01	0,54	0	0,52	0,21	NA	0,05	0,50	0	0,07	0,47	0,25	0,07	NA	0	0,21	0,44	0,05	0,46	NA
Striped mullet	0,01	0,17	0,06	0	0,02	0,40	1	0	0,02	NA	0,20	0,08	0	0	0,02	0,12	0,35	NA	0	0,00	0,02	0,14	0,15	NA
White mullet	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
<b>Perciformes</b>	<b>0,12</b>	<b>0,54</b>	<b>0,08</b>	<b>0,18</b>	<b>0,87</b>	<b>0,03</b>	<b>0,00</b>	<b>0,08</b>	<b>0,15</b>	NA	<b>0,60</b>	<b>0,38</b>	<b>0,43</b>	<b>0,26</b>	<b>0,08</b>	<b>0,53</b>	<b>0,58</b>	NA	<b>0,00</b>	<b>0,29</b>	<b>0,02</b>	<b>0,73</b>	<b>0,28</b>	NA
Warmouth	0	0,01	0	0	0	0,03	0	0	0	NA	0,02	0	0	0	0	0	0,22	NA	0	0	0	0,03	0	NA
unID Sciaenidae	0	0,01	0	0	0,02	0	0	0	0	NA	0	0,05	0	0	0	0,01	0	NA	0	0	0	0,03	0	NA
Atlantic croaker	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
unID Drum	0,01	0	0,03	0,00	0	0	0	0,01	0,02	NA	0	0	0	0	0,03	0	0	NA	0	0,03	0,00	0	0	NA
Red drum	0,04	0,49	0,02	0,05	0,81	0	0	0,04	0,04	NA	0,58	0,17	0,43	0	0,03	0,51	0,19	NA	0	0,09	0,00	0,58	0,28	NA
unID Seatrout	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Spotted seatrout	0,07	0	0,02	0,12	0	0	0	0,03	0,09	NA	0	0	0	0,26	0,02	0	0	NA	0	0,17	0,01	0	0	NA
Spot croaker	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Pinfish	0,00	0	0,01	0	0	0	0	0	0,00	NA	0	0	0	0	0,00	0	0	NA	0	0	0,01	0	0	NA
Sheepshead	0,00	0,03	0	0,00	0,05	0	0	0,00	0	NA	0	0,16	0	0	0,00	0,01	0,16	NA	0	0,00	0	0,09	0	NA
<b>Pleuronectiformes</b>	<b>0,00</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	NA	<b>0,03</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	NA	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,06</b>	NA
Southern flounder	0	0,01	0	0	0,02	0	0	0	0	NA	0,03	0	0	0	0	0,02	0	NA	0	0	0	0	0,06	NA
<b>Siluriformes</b>	<b>0,44</b>	<b>0,03</b>	<b>0,40</b>	<b>0,42</b>	<b>0,05</b>	<b>0,00</b>	<b>0,00</b>	<b>0,33</b>	<b>0,49</b>	NA	<b>0,07</b>	<b>0,00</b>	<b>0,10</b>	<b>0,55</b>	<b>0,36</b>	<b>0,04</b>	<b>0,00</b>	NA	<b>1,00</b>	<b>0,30</b>	<b>0,47</b>	<b>0,01</b>	<b>0,03</b>	NA
unID Ariidae	0,42	0,03	0,35	0,42	0,05	0	0	0,30	0,47	NA	0,07	0	0,10	0,55	0,33	0,04	0	NA	1	0,29	0,42	0,01	0,03	NA
Gafftopsail catfish	0,01	0	0,03	0	0	0	0	0	0,02	NA	0	0	0	0	0,01	0	0	NA	0	0	0,03	0	0	NA
Hardhead catfish	0,01	0	0,02	0,00	0	0	0	0,03	0,00	NA	0	0	0	0	0,02	0	0	NA	0	0,00	0,02	0	0	NA

Appendix 3: %IRI of the all the prey items identified at the lowest taxonomic level, after removal of non-animal prey, for the bull shark, *Carcharhinus leucas*, in San Antonio Bay and Sabine Lake. Taxonomic levels in bold are sums of %IRI.

SAB

	Total	Spring	Fall	T1	T2	T3	Low	Brackish	High	Small	Inter	Large
<b>Crustaceans</b>	<b>0,02</b>	<b>0,03</b>	<b>0,03</b>	<b>NA</b>	<b>0,01</b>	<b>0,16</b>	<b>0,00</b>	<b>NA</b>	<b>0,02</b>	<b>0,22</b>	<b>0,09</b>	<b>0,00</b>
unID Portunidae	0	0	0	NA	0	0	NA	NA	0	0	0	0
Blue crab	0	0	0	NA	0	0	NA	NA	0	0	0	0
Panopeidae	0	0	0	NA	0	0	NA	NA	0	0	0	0
unID Shrimp	0,009	0	0,030	NA	0,011	0	NA	NA	0,009	0,223	0	0
White shrimp	0,010	0,025	0	NA	0	0,158	NA	NA	0,010	0	0,094	0
<b>Snakes</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
Western ribbon	0	0	0	NA	0	0	NA	NA	0	0	0	0
Nerodia	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Elasmobranchs</b>	<b>0,10</b>	<b>0,00</b>	<b>0,36</b>	<b>NA</b>	<b>0,11</b>	<b>0,00</b>	<b>NA</b>	<b>NA</b>	<b>0,10</b>	<b>0,00</b>	<b>0,00</b>	<b>0,15</b>
<b>Carcharhiniformes</b>	<b>0,10</b>	<b>0,00</b>	<b>0,36</b>	<b>NA</b>	<b>0,11</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>	<b>0,10</b>	<b>0,00</b>	<b>0,00</b>	<b>0,15</b>
Bonnethead shark	0	0	0	NA	0	0	NA	NA	0	0	0	0
Atlantic sharpnose	0,099	0	0,363	NA	0,105	0	NA	NA	0,099	0	0	0,152
<b>Myliobatiformes</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
unID Myliobat.	0	0	0	NA	0	0	NA	NA	0	0	0	0
Bluntnose stingray	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Teleost</b>	<b>0,882</b>	<b>0,975</b>	<b>0,607</b>	<b>NA</b>	<b>0,884</b>	<b>0,842</b>	<b>0,000</b>	<b>NA</b>	<b>0,882</b>	<b>0,777</b>	<b>0,906</b>	<b>0,848</b>
<b>Anguiliformes</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
Anguillidae	0	0	0	NA	0	0	NA	NA	0	0	0	0
Shrimp eel	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Clupeiformes</b>	<b>0,011</b>	<b>0,000</b>	<b>0,038</b>	<b>NA</b>	<b>0,000</b>	<b>0,215</b>	<b>0,000</b>	<b>NA</b>	<b>0,011</b>	<b>0,574</b>	<b>0,000</b>	<b>0,000</b>
unID Clupeidae	0,011	0	0,038	NA	0	0,215	NA	NA	0,011	0,574	0	0
Gizzard shad	0	0	0	NA	0	0	NA	NA	0	0	0	0
Gulf menhaden	0	0	0	NA	0	0	NA	NA	0	0	0	0
Bay anchovy	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Elopiformes</b>	<b>0,038</b>	<b>0,000</b>	<b>0,142</b>	<b>NA</b>	<b>0,039</b>	<b>0,000</b>	<b>0,000</b>	<b>NA</b>	<b>0,038</b>	<b>0,000</b>	<b>0,315</b>	<b>0,000</b>
Ladyfish	0,038	0	0,142	NA	0,039	0	NA	NA	0,038	0	0,315	0
Tarpon	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Mugiliformes</b>	<b>0,61</b>	<b>0,80</b>	<b>0,08</b>	<b>NA</b>	<b>0,63</b>	<b>0,13</b>	<b>0,00</b>	<b>NA</b>	<b>0,61</b>	<b>0,20</b>	<b>0,14</b>	<b>0,58</b>

unID Mugilidae	0,01	0	0,03	NA	0	0,13	NA	NA	0,01	0,20	0	0
Striped mullet	0,60	0,80	0,05	NA	0,63	0	NA	NA	0,60	0	0,14	0,58
White mullet	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Perciformes</b>	<b>0,20</b>	<b>0,12</b>	<b>0,34</b>	<b>NA</b>	<b>0,19</b>	<b>0,50</b>	<b>0,00</b>	<b>NA</b>	<b>0,20</b>	<b>0,00</b>	<b>0,45</b>	<b>0,23</b>
Warmouth	0	0	0	NA	0	0	NA	NA	0	0	0	0
unID Sciaenidae	0,02	0	0,07	NA	0	0,50	NA	NA	0,02	0	0	0,03
Atlantic croaker	0,05	0	0,21	NA	0,06	0	NA	NA	0,05	0	0,45	0
unID Drum	0,01	0,03	0	NA	0,02	0	NA	NA	0,01	0	0	0,03
Red drum	0,10	0,06	0,07	NA	0,11	0	NA	NA	0,10	0	0	0,15
unID Seatrout	0	0	0	NA	0	0	NA	NA	0	0	0	0
Spotted seatrout	0,01	0,02	0	NA	0,01	0	NA	NA	0,01	0	0	0,02
Spot croaker	0	0	0	NA	0	0	NA	NA	0	0	0	0
Pinfish	0	0	0	NA	0	0	NA	NA	0	0	0	0
Sheepshead	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Pleuronectiformes</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
Southern flounder	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Siluriformes</b>	<b>0,02</b>	<b>0,06</b>	<b>0,00</b>	<b>NA</b>	<b>0,03</b>	<b>0,00</b>	<b>0,00</b>	<b>NA</b>	<b>0,02</b>	<b>0,00</b>	<b>0,00</b>	<b>0,04</b>
unID Ariidae	0	0	0	NA	0	0	NA	NA	0	0	0	0
Gafftopsail catfish	0,02	0,06	0	NA	0,03	0	NA	NA	0,02	0	0	0,04
Hardhead catfish	0	0	0	NA	0	0	NA	NA	0	0	0	0

Appendix 4: %IRI of the all the prey items identified at the lowest taxonomic level, after removal of non-animal prey, for the blacktip shark, *Carcharhinus limbatus*, in San Antonio Bay. Taxonomic levels in bold are sums of %IRI.

	SAB		SL		SAB		SL		SAB			SL			SAB			SL						
	Total	Total	Spring	Fall	Spring	Fall	T1	T2	T3	T1	T2	T3	Low	Brackish	High	Low	Brackish	High	Small	Inter	Large	Small	Inter	Large
<b>Crustaceans</b>	<b>0</b>	<b>0,03</b>	<b>0</b>	<b>0</b>	<b>0,01</b>	<b>0,05</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0,08</b>	<b>0,04</b>	<b>0,01</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0,04</b>	<b>0,01</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0,14</b>	<b>0,02</b>	<b>0,00</b>
Portunidae	0	0,01	0	0	0,00	0,02	0	0	0	0,02	0,04	0	0	0	0	0,01	0,00	NA	0	0	0	0,01	0,02	0,00
Panopeidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Shrimp	0	0,02	0	0	0,01	0,03	0	0	0	0,06	0	0,01	0	0	0	0,03	0,00	NA	0	0	0	0,12	0	0,00
<b>Elasmobranchs</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
Bonnethead shark	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Atlantic sharpnose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Myliobatiformes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
<b>Clupeidae</b>	<b>0,45</b>	<b>0,69</b>	<b>0,63</b>	<b>0</b>	<b>0,86</b>	<b>0,33</b>	<b>0</b>	<b>0,80</b>	<b>0,04</b>	<b>0,34</b>	<b>0,06</b>	<b>0,95</b>	<b>0,20</b>	<b>0,74</b>	<b>0,04</b>	<b>0,70</b>	<b>0,61</b>	<b>NA</b>	<b>0,65</b>	<b>0,71</b>	<b>0,08</b>	<b>0,49</b>	<b>0,68</b>	<b>0,71</b>
<b>Mugilidae</b>	<b>0,54</b>	<b>0,20</b>	<b>0,36</b>	<b>0,99</b>	<b>0,03</b>	<b>0,45</b>	<b>0,93</b>	<b>0,20</b>	<b>0,95</b>	<b>0,25</b>	<b>0,71</b>	<b>0,01</b>	<b>0,52</b>	<b>0,25</b>	<b>0,95</b>	<b>0,16</b>	<b>0,23</b>	<b>NA</b>	<b>0,35</b>	<b>0,28</b>	<b>0,90</b>	<b>0,20</b>	<b>0,16</b>	<b>0,19</b>
<b>Sciaenidae</b>	<b>0,00</b>	<b>0,06</b>	<b>0,00</b>	<b>0,01</b>	<b>0,05</b>	<b>0,07</b>	<b>0,07</b>	<b>0,00</b>	<b>0,00</b>	<b>0,21</b>	<b>0,09</b>	<b>0,03</b>	<b>0,28</b>	<b>0,01</b>	<b>0,00</b>	<b>0,05</b>	<b>0,11</b>	<b>NA</b>	<b>0,00</b>	<b>0,00</b>	<b>0,01</b>	<b>0,08</b>	<b>0,13</b>	<b>0,07</b>
Atlantic croaker	0,00	0,00	0,00	0	0	0,01	0	0,00	0	0	0,04	0	0	0,01	0	0,01	0	NA	0,00	0,00	0	0,01	0,01	0
Red drum	0	0,04	0	0	0,01	0,06	0	0	0	0,21	0	0	0	0	0	0,01	0,11	NA	0	0	0	0	0,04	0,07
Seatrout	0,00	0,01	0,00	0,01	0,03	0,00	0,07	0	0,00	0	0,05	0,02	0,28	0	0	0,04	0	NA	0	0	0,01	0,07	0,03	0
Spot croaker	0	0,00	0	0	0,01	0	0	0	0	0	0	0,01	0	0	0	0,01	0	NA	0	0	0	0	0,04	0
<b>Ariidae</b>	<b>0,00</b>	<b>0,00</b>	<b>0,01</b>	<b>0</b>	<b>0</b>	<b>0,02</b>	<b>0</b>	<b>0,00</b>	<b>0</b>	<b>0,04</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0,00</b>	<b>0</b>	<b>0,03</b>	<b>NA</b>	<b>0</b>	<b>0,01</b>	<b>0,00</b>	<b>0,04</b>	<b>0</b>	<b>0,00</b>
<b>Others</b>	<b>0,00</b>	<b>0,02</b>	<b>0,00</b>	<b>0,00</b>	<b>0,05</b>	<b>0,07</b>	<b>0,00</b>	<b>0,00</b>	<b>0,01</b>	<b>0,07</b>	<b>0,09</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,01</b>	<b>0,04</b>	<b>0,01</b>	<b>NA</b>	<b>0,00</b>	<b>0,00</b>	<b>0,00</b>	<b>0,06</b>	<b>0,01</b>	<b>0,02</b>
Snake	0	0,00	0	0	0,01	0	0	0	0	0	0,01	0,00	0	0	0	0,00	0,00	NA	0	0	0	0,04	0	0
Anguillidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Shrimp eel	0	0,01	0	0	0	0,07	0	0	0	0,07	0,01	0,00	0	0	0	0,02	0,01	NA	0	0	0	0,03	0,01	0,01
Bay anchovy	0	0,00	0	0	0	0,00	0	0	0	0	0,01	0	0	0	0	0	0,00	NA	0	0	0	0	0	0,00
Ladyfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Tarpon	0,00	0,00	0,00	0	0,03	0	0	0	0,01	0	0,07	0	0	0	0,01	0,02	0	NA	0	0	0,00	0	0	0,01
Warmouth	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Pinfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Sheepshead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
Southern flounder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0

Appendix 5: %IRI of the preys after removal of the unidentified items for the alligator gar, *Atractosteus spatula*, in San Antonio Bay and Sabine Lake. Crustaceans, Elasmobranchs, Sciaenidae and Other are sums of %IRI.

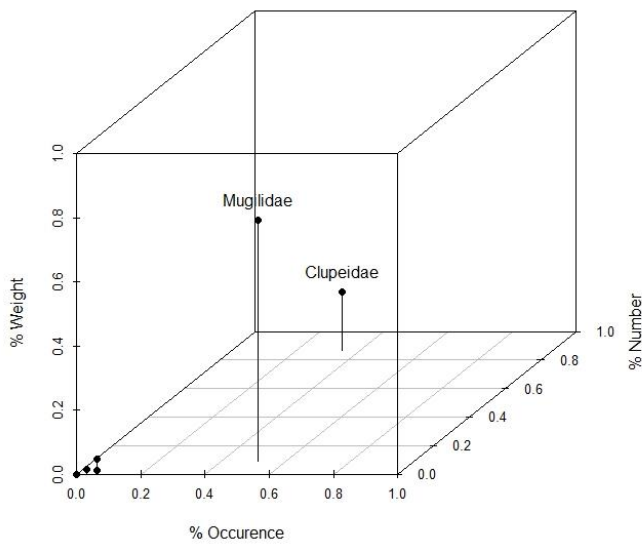


	SAB		SL		SAB		SL		SAB			SL			SAB			SL						
	Total	Total	Spring	Fall	Spring	Fall	T1	T2	T3	T1	T2	T3	Low	Brackish	High	Low	Brackish	High	Small	Inter	Large	Small	Inter	Large
<b>Crustaceans</b>	<b>0,01</b>	<b>0,00</b>	<b>0,00</b>	<b>0,03</b>	<b>0</b>	<b>0,01</b>	<b>0</b>	<b>0,00</b>	<b>0,02</b>	<b>NA</b>	<b>0</b>	<b>0,01</b>	<b>0</b>	<b>0,01</b>	<b>0,01</b>	<b>0,00</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0,00</b>	<b>0,02</b>	<b>0,01</b>	<b>0</b>	<b>NA</b>
Portunidae	0,00	0	0,00	0	0	0	0	0	0,00	NA	0	0	0	0,01	0	0	0	NA	0	0,00	0	0	0	NA
Panopeidae	0,00	0	0	0,00	0	0	0	0,00	0	NA	0	0	0	0	0,00	0	0	NA	0	0	0,00	0	0	NA
Shrimp	0,01	0,00	0	0,03	0	0,01	0	0	0,02	NA	0	0,01	0	0	0,01	0,00	0	NA	0	0	0,02	0,01	0	NA
<b>Elasmobranchs</b>	<b>0,01</b>	<b>0,02</b>	<b>0</b>	<b>0,03</b>	<b>0,06</b>	<b>0</b>	<b>0</b>	<b>0,02</b>	<b>0,01</b>	<b>NA</b>	<b>0,06</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0,01</b>	<b>0,03</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0,02</b>	<b>0,00</b>	<b>0,09</b>	<b>0</b>	<b>NA</b>
Bonnethead_shark	0,00	0	0	0,02	0	0	0	0	0,01	NA	0	0	0	0	0,01	0	0	NA	0	0,02	0	0	0	NA
Atlantic_sharpnose	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Myliobatiformes	0,00	0,02	0	0,02	0,06	0	0	0,02	0	NA	0,06	0	0	0	0,01	0,03	0	NA	0	0,00	0,00	0,09	0	NA
<b>Clupeidae</b>	<b>0,08</b>	<b>0,00</b>	<b>0,12</b>	<b>0,04</b>	<b>0</b>	<b>0,01</b>	<b>0</b>	<b>0,04</b>	<b>0,11</b>	<b>NA</b>	<b>0</b>	<b>0,02</b>	<b>0,48</b>	<b>0,04</b>	<b>0,05</b>	<b>0,01</b>	<b>0</b>	<b>NA</b>	<b>0</b>	<b>0,21</b>	<b>0,01</b>	<b>0</b>	<b>0,01</b>	<b>NA</b>
<b>Mugilidae</b>	<b>0,31</b>	<b>0,59</b>	<b>0,34</b>	<b>0,26</b>	<b>0,05</b>	<b>0,97</b>	<b>1</b>	<b>0,38</b>	<b>0,23</b>	<b>NA</b>	<b>0,39</b>	<b>0,75</b>	<b>0</b>	<b>0,07</b>	<b>0,42</b>	<b>0,56</b>	<b>0,58</b>	<b>NA</b>	<b>0</b>	<b>0,22</b>	<b>0,39</b>	<b>0,32</b>	<b>0,78</b>	<b>NA</b>
<b>Sciaenidae</b>	<b>0,58</b>	<b>0,34</b>	<b>0,54</b>	<b>0,62</b>	<b>0,82</b>	<b>0</b>	<b>0</b>	<b>0,52</b>	<b>0,62</b>	<b>NA</b>	<b>0,51</b>	<b>0,11</b>	<b>0,52</b>	<b>0,82</b>	<b>0,50</b>	<b>0,38</b>	<b>0,13</b>	<b>NA</b>	<b>1</b>	<b>0,52</b>	<b>0,57</b>	<b>0,48</b>	<b>0,18</b>	<b>NA</b>
Atlantic_croaker	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Red_drum	0,02	0,32	0,01	0,04	0,76	0	0	0,03	0,02	NA	0,44	0,11	0,40	0	0,02	0,35	0,13	NA	0	0,06	0,00	0,47	0,15	NA
Seatrout	0,04	0	0,01	0,11	0	0	0	0,03	0,05	NA	0	0	0	0,27	0,01	0	0	NA	0	0,13	0,00	0	0	NA
Spot_croaker	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
<b>Ariidae</b>	<b>0,51</b>	<b>0,02</b>	<b>0,52</b>	<b>0,47</b>	<b>0,06</b>	<b>0</b>	<b>0</b>	<b>0,47</b>	<b>0,55</b>	<b>NA</b>	<b>0,06</b>	<b>0</b>	<b>0,13</b>	<b>0,55</b>	<b>0,46</b>	<b>0,03</b>	<b>0</b>	<b>NA</b>	<b>1</b>	<b>0,33</b>	<b>0,56</b>	<b>0,01</b>	<b>0,02</b>	<b>NA</b>
<b>Others</b>	<b>0,01</b>	<b>0,04</b>	<b>0,01</b>	<b>0,02</b>	<b>0,07</b>	<b>0,02</b>	<b>0</b>	<b>0,03</b>	<b>0,01</b>	<b>NA</b>	<b>0,05</b>	<b>0,11</b>	<b>0</b>	<b>0,05</b>	<b>0,01</b>	<b>0,02</b>	<b>0,29</b>	<b>NA</b>	<b>0</b>	<b>0,01</b>	<b>0,01</b>	<b>0,10</b>	<b>0,03</b>	<b>NA</b>
Snake	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Anguillidae	0,00	0	0,00	0	0	0	0	0,00	0	NA	0	0	0	0	0,00	0	0	NA	0	0	0,00	0	0	NA
Shrimp_eel	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Bay_anchovy	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Ladyfish	0,00	0	0	0,01	0	0	0	0,02	0	NA	0	0	0	0,05	0	0	0	NA	0	0,01	0	0	0	NA
Tarpon	0,00	0	0	0,00	0	0	0	0	0,00	NA	0	0	0	0	0,00	0	0	NA	0	0	0	0	0	NA
Warmouth	0	0,01	0	0	0	0,02	0	0	0	NA	0,02	0	0	0	0	0,17	NA	0	0	0	0,03	0	NA	
Pinfish	0,00	0	0,00	0	0	0	0	0	0,00	NA	0	0	0	0	0	0	0	NA	0	0	0	0	0	NA
Sheepshead	0,00	0,02	0	0,00	0,05	0	0	0,00	0	NA	0	0,11	0	0	0	0,01	0,11	NA	0	0	0	0,08	0	NA
Southern_flounder	0	0,01	0	0	0,02	0	0	0	0	NA	0,02	0	0	0	0	0,01	0	NA	0	0	0	0	0,03	NA

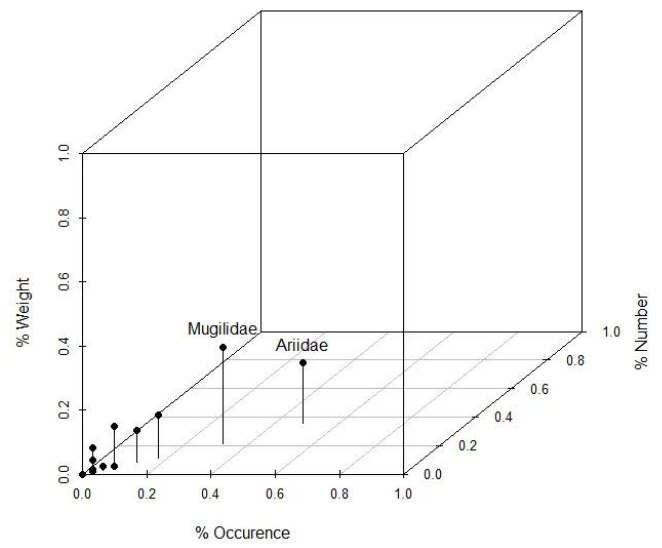
Appendix 6: %IRI of the preys after removal of the unidentified items for the bull shark, *Carcharhinus leucas*, in San Antonio Bay and Sabine Lake. Crustaceans, Elasmobranchs, Sciaenidae and Other are sums of %IRI.

	Total	Spring	Fall	T1	T2	T3	Low	Brackish	High	Small	Inter	Large
<b>Crustaceans</b>	<b>0,04</b>	<b>0,03</b>	<b>0,04</b>	<b>NA</b>	<b>0,02</b>	<b>0,31</b>	<b>NA</b>	<b>NA</b>	<b>0,04</b>	<b>0,24</b>	<b>0,13</b>	<b>0</b>
Portunidae	0	0	0	NA	0	0	NA	NA	0	0	0	0
Panopeidae	0	0	0	NA	0	0	NA	NA	0	0	0	0
Shrimp	0,04	0,03	0,04	NA	0,02	0,31	NA	NA	0,04	0,24	0,13	0
<b>Elasmobranchs</b>	<b>0,09</b>	<b>0</b>	<b>0,34</b>	<b>NA</b>	<b>0,11</b>	<b>0</b>	<b>NA</b>	<b>NA</b>	<b>0,09</b>	<b>0</b>	<b>0</b>	<b>0,16</b>
Bonnethead shark	0	0	0	NA	0	0	NA	NA	0	0	0	0
Atlantic sharpnose	0,09	0	0,34	NA	0,11	0	NA	NA	0,09	0	0	0,16
Myliobatiformes	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Clupeidae</b>	<b>0,01</b>	<b>0</b>	<b>0,05</b>	<b>NA</b>	<b>0</b>	<b>0,48</b>	<b>NA</b>	<b>NA</b>	<b>0,01</b>	<b>0,54</b>	<b>0</b>	<b>0</b>
<b>Mugilidae</b>	<b>0,66</b>	<b>0,81</b>	<b>0,20</b>	<b>NA</b>	<b>0,62</b>	<b>0,21</b>	<b>NA</b>	<b>NA</b>	<b>0,66</b>	<b>0,22</b>	<b>0,16</b>	<b>0,60</b>
<b>Sciaenidae</b>	<b>0,10</b>	<b>0,10</b>	<b>0,07</b>	<b>NA</b>	<b>0,13</b>	<b>0</b>	<b>NA</b>	<b>NA</b>	<b>0,10</b>	<b>0</b>	<b>0</b>	<b>0,19</b>
Red drum	0,09	0,07	0,07	NA	0,11	0	NA	NA	0,09	0	0	0,16
Seatrout	0,01	0,03	0	NA	0,02	0	NA	NA	0,01	0	0	0,02
Spot croaker	0	0	0	NA	0	0	NA	NA	0	0	0	0
<b>Ariidae</b>	<b>0,03</b>	<b>0,07</b>	<b>0</b>	<b>NA</b>	<b>0,03</b>	<b>0</b>	<b>NA</b>	<b>NA</b>	<b>0,03</b>	<b>0</b>	<b>0</b>	<b>0,05</b>
<b>Others</b>	<b>0,07</b>	<b>0</b>	<b>0,29</b>	<b>NA</b>	<b>0,09</b>	<b>0</b>	<b>NA</b>	<b>NA</b>	<b>0,07</b>	<b>0</b>	<b>0,70</b>	<b>0</b>
Snake	0	0	0	NA	0	0	NA	NA	0	0	0	0
Anguillidae	0	0	0	NA	0	0	NA	NA	0	0	0	0
Shrimp eel	0	0	0	NA	0	0	NA	NA	0	0	0	0
Bay anchovy	0	0	0	NA	0	0	NA	NA	0	0	0	0
Ladyfish	0,03	0	0,12	NA	0,04	0	NA	NA	0,03	0	0,30	0
Tarpon	0	0	0	NA	0	0	NA	NA	0	0	0	0
Warmouth	0	0	0	NA	0	0	NA	NA	0	0	0	0
Atlantic croaker	0,04	0	0,17	NA	0,05	0	NA	NA	0,04	0	0,40	0
Pinfish	0	0	0	NA	0	0	NA	NA	0	0	0	0
Sheepshead	0	0	0	NA	0	0	NA	NA	0	0	0	0
Southern flounder	0	0	0	NA	0	0	NA	NA	0	0	0	0

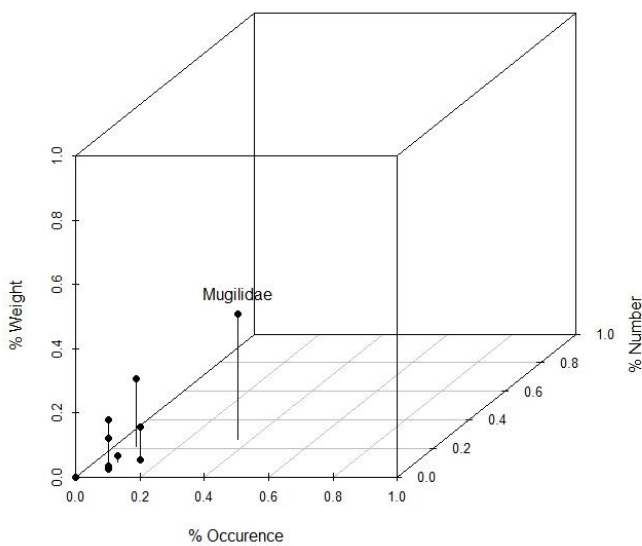
Appendix 7: %IRI of the preys after removal of the unidentified items for the blacktip shark, *Carcharhinus limbatus*, in San Antonio Bay. Crustaceans, Elasmobranchs, Sciaenidae and Other are sums of %IRI.



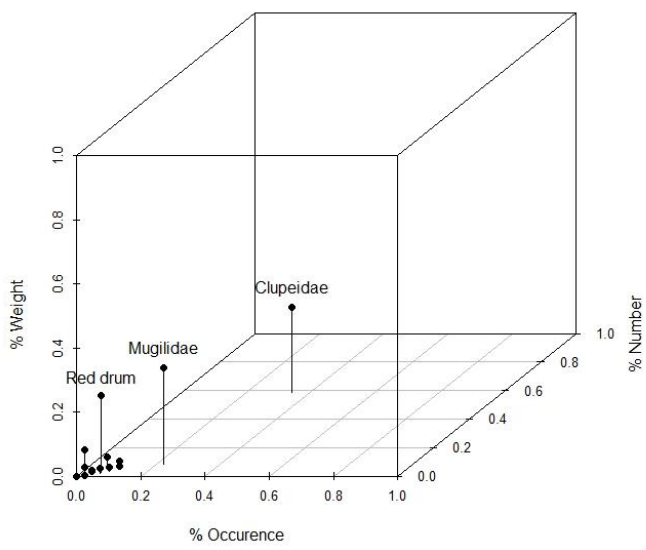
Appendix 8: 3D Costello representation of San Antonio Bay alligator gars diet. Major prey items are labelled



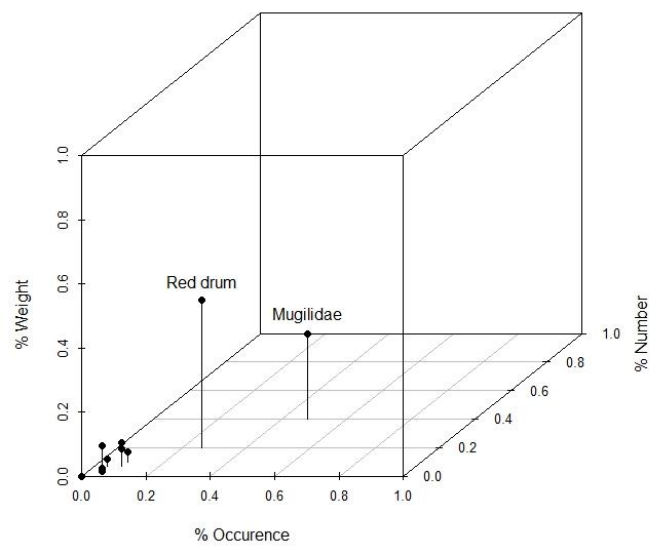
Appendix 9: 3D Costello representation of San Antonio Bay bull sharks diet. Major prey items are labelled



Appendix 10: 3D Costello representation of San Antonio Bay blacktip sharks diet. Major prey items are labelled

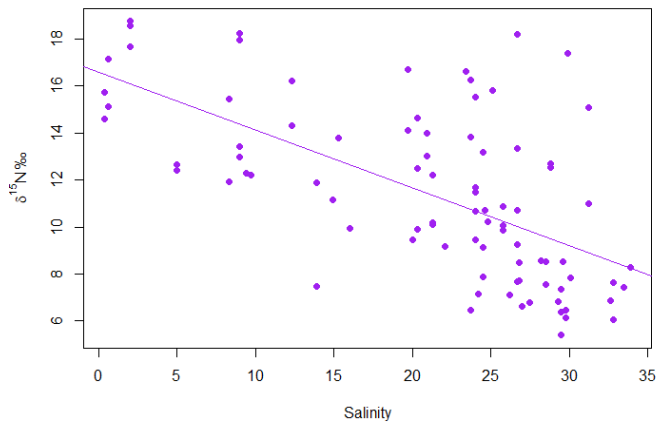


Appendix 11: 3D Costello representation of Sabine Lake alligator gars diet. Major prey items are labelled

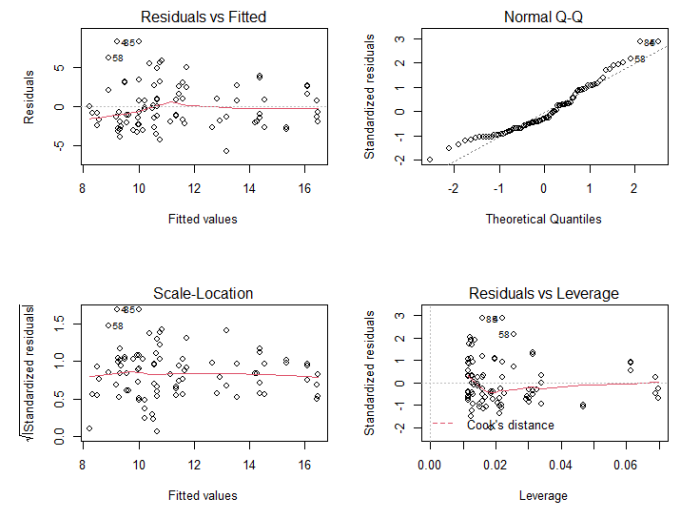
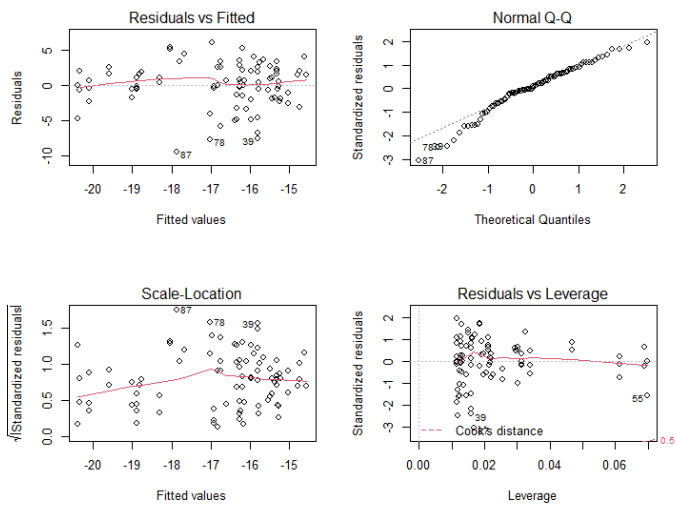
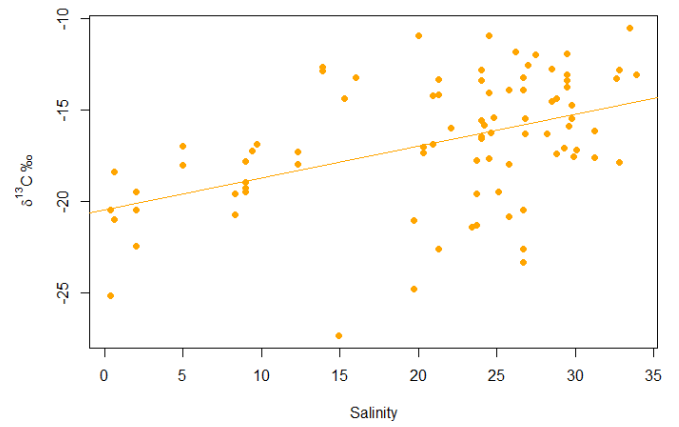


Appendix 12: 3D Costello representation of Sabine Lake bull sharks diet. Major prey items are labelled

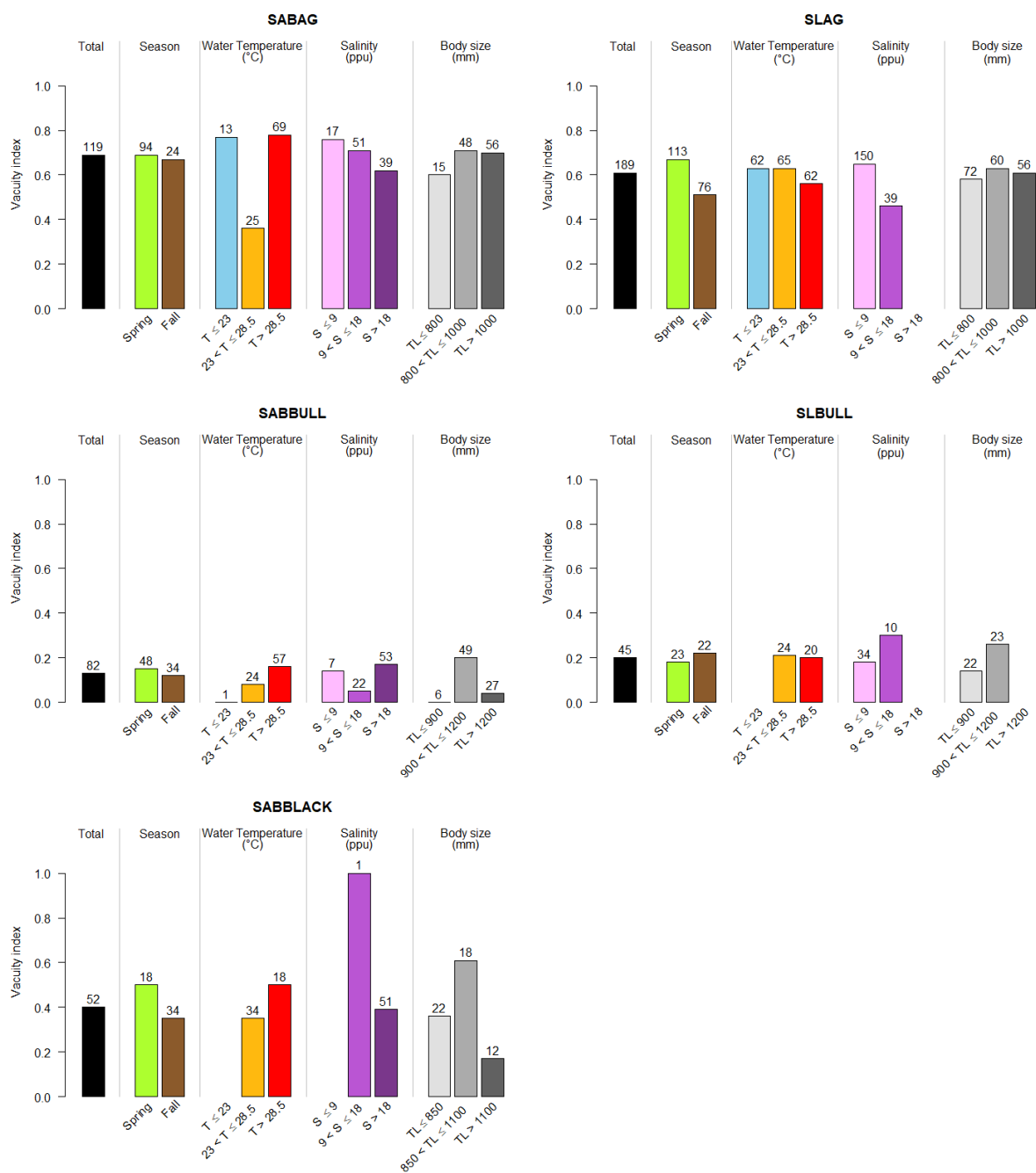
San Antonio Bay primary consumers - Nitrogen



San Antonio Bay primary consumers - Carbon



Appendix 13 : Linear regression of the relationship between salinity and nitrogen (left) or carbon (right) isotope values in primary consumers, and plot associated



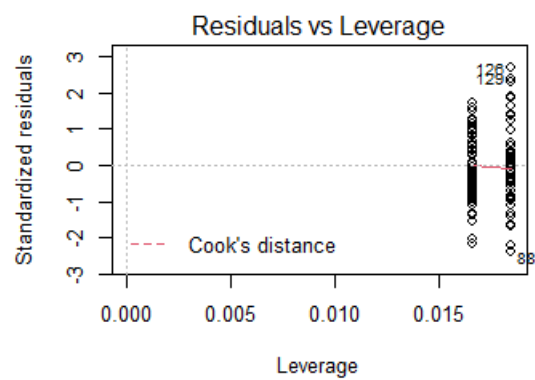
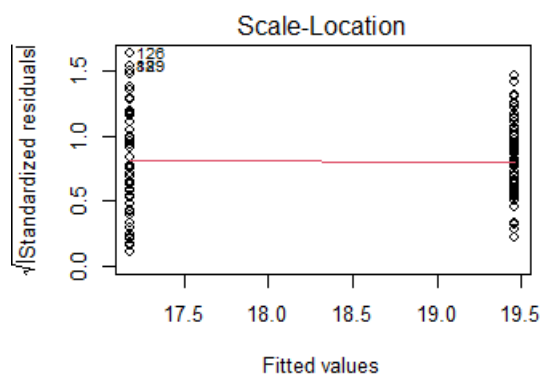
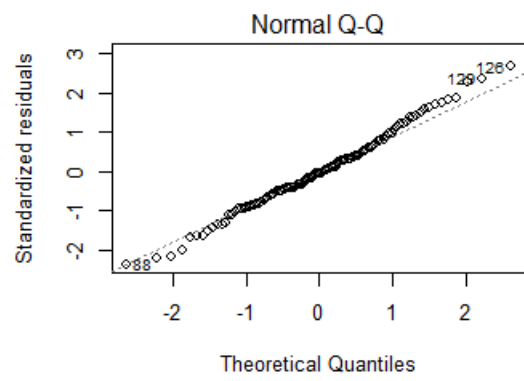
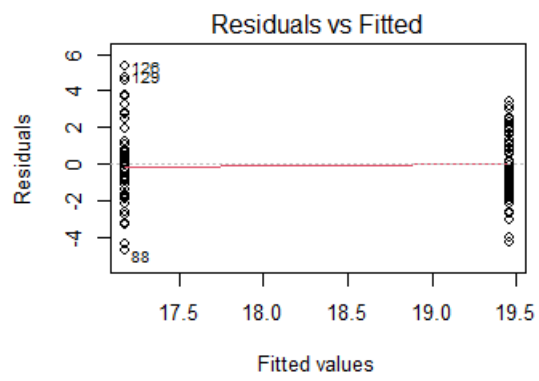
Appendix 14 : Percentages of stomachs containing prey for each population, by season, by water temperature, salinity, and body size group. From top to bottom alligator gar, bull shark and blacktip shark, San Antonio Bay is on the left and Sabine Lake on the right. The values at the top of bars are the total numbers of individuals in each group.

Résultats SIMPER

SIMPER SAB	Average a	Average b	Average dissimilarity $\pm$ SD	Contribution %	Cumulative %
Alligator gar x Bull shark					
Mugilidae	77.14	28.77	0.39 $\pm$ 0.37	43.79	43.79
Clupeidae	18.69	12.95	0.19 $\pm$ 0.30	21.79	65.58
Ariidae	4.05	18.04	0.13 $\pm$ 0.22	14.17	79.75
Alligator gar x Blacktip shark					
Mugilidae	77.14	46.84	0.40 $\pm$ 0.37	45.06	45.06
Clupeidae	18.69	1.05	0.15 $\pm$ 0.27	16.99	62.05
Red Drum	0	12.84	0.07 $\pm$ 0.21	8.64	70.69
Bull shark x blacktip shark					
Mugilidae	28.77	46.84	0.29 $\pm$ 0.36	31.53	31.53
Ariidae	18.04	2.42	0.14 $\pm$ 0.24	14.95	46.48
Red Drum	12.63	12.83	0.12 $\pm$ 0.27	12.50	58.98

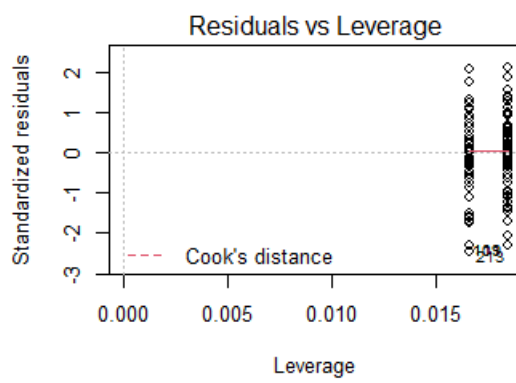
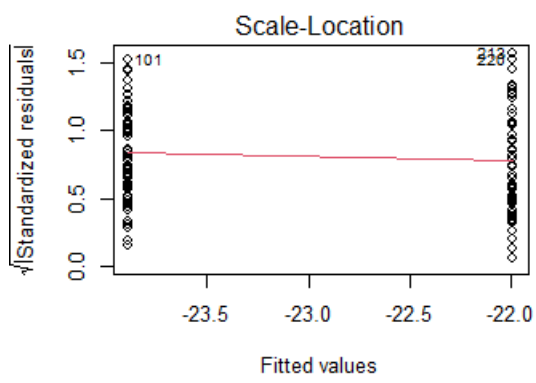
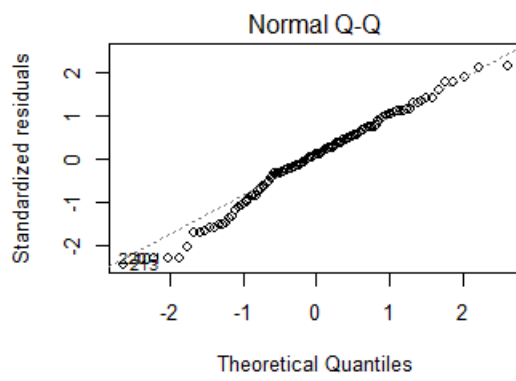
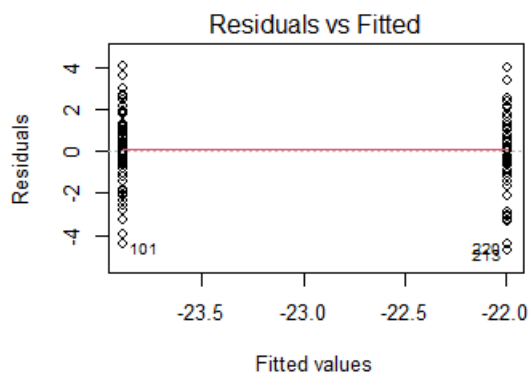
SIMPER SL	Average a	Average b	Average dissimilarity $\pm$ SD	Contribution %	Cumulative %
Alligator gar x Bull shark					
Mugilidae	20.95	46.06	0.31 $\pm$ 0.37	33.37	33.37
Red Drum	16.52	16.52	0.22 $\pm$ 0.36	23.36	56.73
Clupeidae	18.46	18.46	0.10 $\pm$ 0.22	10.54	67.27

Appendix 15: Result of the dissimilarity percentage analysis (SIMPER) for interspecific comparisons within each estuary

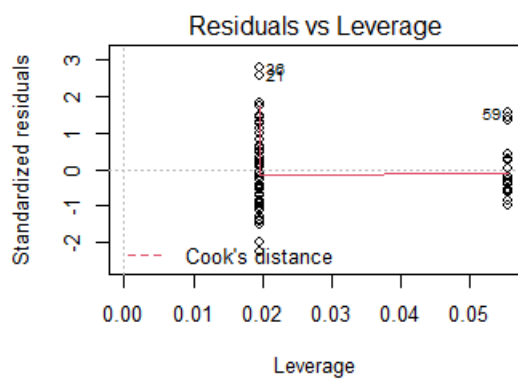
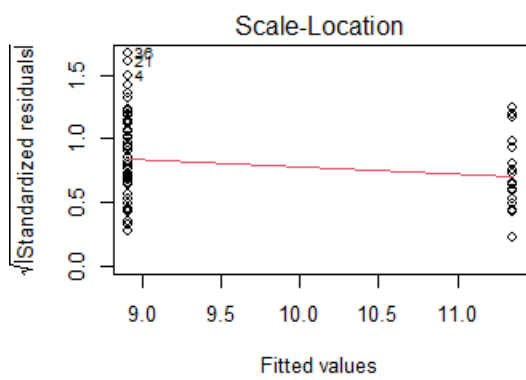
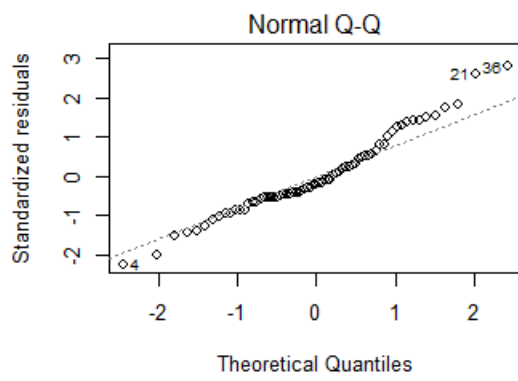
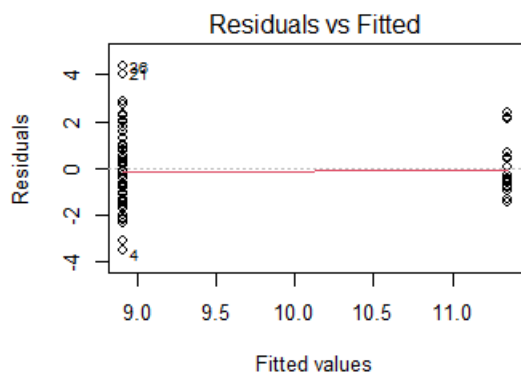


Appendix 16 : Interspecific difference of  $\delta^{15}\text{N}$  in San Antonio Bay: ANOVA plots

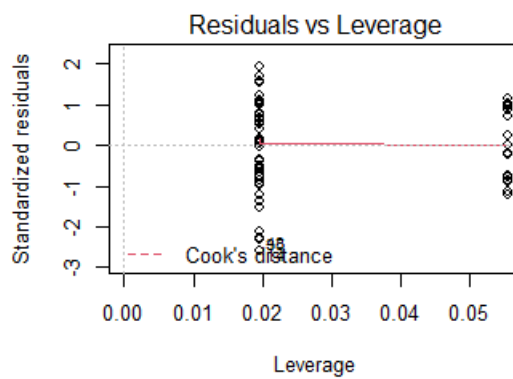
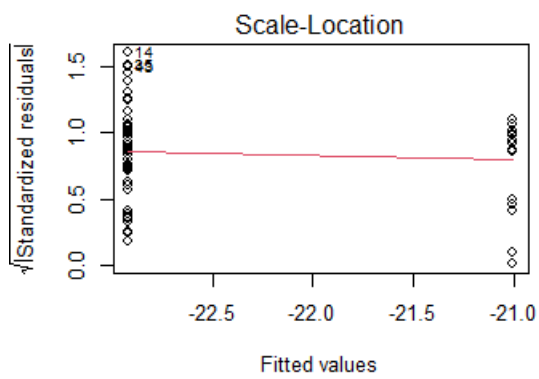
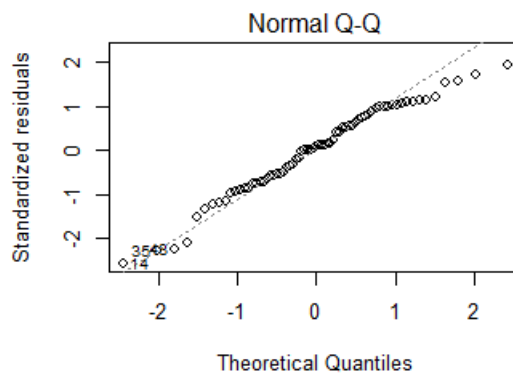
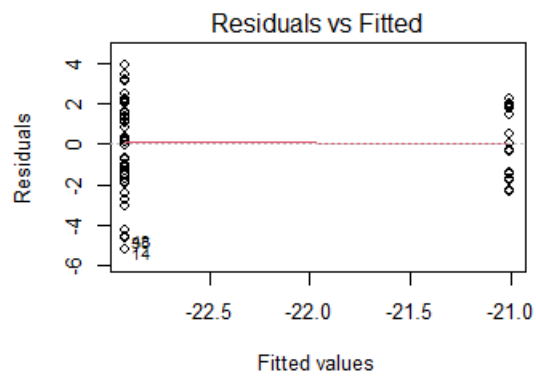




Appendix 17 : Interspecific difference of  $\delta^{13}\text{C}$  in San Antonio Bay: ANOVA plots



Appendix 18 : Interspecific difference of  $\delta^{15}\text{N}$  in Sabine Lake: ANOVA plots



Appendix 19 : Interspecific difference of  $\delta^{13}\text{C}$  in Sabine Lake: ANOVA plots