VARIATION IN HABITAT USE AND TROPHIC DYNAMICS OF A CATADROMOUS FISH (*ANGUILLA ROSTRATA*) IN SUB-TROPICAL TEXAS

A Thesis

by

MICHAEL CURTIS

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

Benjamin Walther, PhD
Chair

Dean Hendrickson, PhD
Committee Member

James Hogan, PhD
Committee Member

August 2021

ABSTRACT

American Eel is an economically, ecologically, and culturally valuable fish. Increased fishing pressure and other stressors have earned international threatened and endangered designations in an effort to protect the species. U.S. Fish and Wildlife Service (USFW) species status reviews resulted in no protection for the species in the U.S. due partly to data gaps about the dynamics of facultatively catadromous strategies and migratory plasticity of the species. The outcome of the reviews has led to questions about the species in lesser-known regions of its range resulting in the creation of a multi-tiered project to assess population structure, distribution, habitat use, and parasite affliction of Gulf of Mexico (GOM) eels, specifically in Texas.

For this study, American Eel were caught in different hydrological systems in Texas from 2012 – 2019. Muscle tissue stable isotope analyses (SIA) of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were used to elucidate habitat use and trophic ecology. SIA is widely used for studies focused on food web interactions and has seen increased interest in elucidation of migratory histories. Isotopic values differing across salinity gradients are reflected in primary producers and then assimilated in the tissues of consumers. Individuals were sampled from river and coastal basins in Texas from 2009 to 2019.

Univariate and multivariate analysis revealed that individuals displayed highly variable isotopic values both within and between major hydrologic system groups, perhaps suggesting differences in life history strategies that could fill in a gap in knowledge of the species in this lesser-known region of its range.

DEDICATION

My work is dedicated to my family and friends, who have always supported and motivated me to work toward and complete my goals. My wife Shannon has been a constant inspiration. She always pushes me, especially during difficult times, and I can always count on her love and support. I am excited to work toward our next big adventure.

I couldn't have made it this far without the motivation and help from my parents and brother or the support of Shannon's family. Words cannot express my gratitude for all of their help.

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Introduction to Habitat Use and Diadromy

Habitat use can be defined as the way a species, or individual, uses a habitat's biotic or abiotic resources (Krausman 1999). This includes, but is not limited to, using a habitat as a source for prey, for predator evasion, or as a location for spawning or rearing young. Species with complicated life histories, typically involving multiple ontogenetic shifts in distributions, will use resources from many different habitats throughout their lives (McDowall 1988; Secor 2015). In many cases, these species are marked by individuals that pursue different life history strategies or varied ways to use their habitats, often referred to as a 'bet-hedging' strategy, to maximize individual fitness (Sogard 1992; Tyler & Rose 1994). Studies focused on within-species variability, or individual variability, are crucial when quantifying or predicting population responses to environmental change and are key to implementing effective management and conservation strategies (Helfman 2007; Kerr et al. 2010; Lomnicki 1980; Levin 1976; Tyler & Rose 1994).

Fishes that undertake migrations between freshwater and marine habitats are collectively described as diadromous and make up less than 1% of total fish species (Limburg & Waldman 2009; McDowall 1988; McDowall 1997; Myers 1949). Diadromous fishes connect multiple habitats by transporting nutrients between freshwater and marine biomes and thus provide periodic pulses of prey availability to multiple consumers across environmental gradients (Engman et al. 2018; Deegan 1993; Greene et al 2009; Limburg & Waldman 2009). This migration is reciprocal, in many cases, and occurs at a particular and predictable life history phase of the individual or species (McCleave & Edeldine 2009; McDowall 1997). Many

diadromous species have historically been a significant dietary resource for humans, mostly due to consistent annual migration periods that facilitate large captures by humans with little effort. Atlantic Sturgeon, American Eel, and American Shad are examples of diadromous species that have supported major historic fisheries and that have seen drastic declines in populations due in part to overfishing (Greene et al. 2009).

Anadromy and catadromy are life history categories under the umbrella term diadromy that describe migrations from marine to freshwater habitats and freshwater to marine habitats, respectively (McDowall 1997; Myers 1949). Anadromous species, such as Salmons (*Salmonidae*) are thought to be more prevalent in temperate climates due partially to a higher abundance of food resources in temperate oceanic environments; conversely, catadromous species are more commonly found in tropical climates due to more available freshwater food resources in those environments (Gross 1988; McDowall 1988). This thinking is based off the 'Productivity Hypothesis' which postulates that the geographic distribution of anadromous and catadromous species depends on the relative productivity of adjacent fresh and marine systems, and that anadromy and catadromy are evolutionary responses to relative availability and distribution of prey in temperate and tropical environments respectively (Gross 1987; Gross et al. 1988; Roberts et al. 2019; Secor 2015). Individual variability regarding habitat use and intraspecific diversity of life history strategies is a lynch pin to the productivity hypothesis and can be referred to as migratory or habitat plasticity (Augspurger et al. 2017; Gross 1987; Gross et al. 1988). Further designations of obligate or facultative diadromy describe whether migration between fresh and marine environments is a necessary component of life histories for all individuals or whether some individuals do not undertake transhaline migrations, respectively (Crook et al. 2006; Potter et al. 2015). More specifically, facultative catadromy is a powerful

strategy in which some American Eel populations may reside in marine environments their entire life while other individuals make excursions between marine and freshwater environments (McCleave & Edeline 2009). Facultative catadromy may be driven by individualized responses to specific biotic or abiotic drivers such as searching for food, habitat fragmentation, or predator avoidance that do not result in uniform migratory movements throughout a population.

Anguillids

Fishes of the family Anguillidae and genus *Anguilla* have traditionally been the token embodiment of catadromous fishes and have played prominent social and economic roles in numerous human cultures across the world. Historically, Anguillid migrations into rivers has led to increased economic and societal values of many species. For instance, European Eel (*Anguilla anguilla*) was traditionally so abundant it was used as rent and tax payments to landlords, sometimes topping 100,000 eels per annum (Greenlee 2020). In addition, American Eel (*Anguilla rostrata*) was a valuable food source for Native Americans and early settlers from Europe, and many Indigenous peoples of Oceania fished Anguillids and deified them (Greene et al. 2009; Noble et al. 2016).

American Eel (*Anguilla rostrata*) inhabit a vast latitudinal habitat range from Venezuela to Greenland (~10°N - 70°N), including the Caribbean and Gulf of Mexico, and reside in multiple habitat types from coastal estuaries to inland rivers and lakes (Busch et al. 1998; Greene et al. 2009; Jacoby et al. 2017; Rypina et al. 2014; Shepard 2015). American Eel, like other Anguillids, have a complex and dynamic life history based around a panmictic and semelparous reproduction strategy, and are oviparous, depending on external fertilization of eggs (Busch et al. 1998; Greene et al. 2009; Haro et al. 2000; MacGregor et al. 2008; Oliveira 1999). Sexually

mature Silver Eels migrate from coastal boundaries to spawn in the Sargasso Sea, typically in the winter to early spring (Greene et al. 2009; Shepard 2015). The precise location of spawning is a mystery as no direct observation of American Eel spawning has been observed, though locations of leptocephali larval catches have indicated they spawn in the southwest Sargasso Sea (Busch et al. 1998; Greene et al. 2009; Shepard 2015). The leaf-like leptocephali larvae are transported from spawning areas by both active swimming and passive drift with currents, the latter being the primary source of motility until larvae metamorphose into glass eels upon crossing the continental slope and enter coastal margins (Greene et al. 2009; McCleave 1993; Rypina et al. 2014). The unpigmented glass eels move into brackish estuaries and lower freshwater margins whereupon they change into brown pigmented elvers and they are considered young of year (YOY) (Busch et al. 1998; Greene et al. 2009; Rypina et al. 2014; Shepard 2015). Elvers then transform into sexually immature yellow eels for anywhere from 3 – 60 years until the final transformation into sexually mature silver eels that begin their return to the Sargasso Sea to spawn (Busch et al. 1998; Greene et al. 2009; Helfman et al. 1984; Shepard 2015). Massive seasonal rainfall followed by freshwater discharge is an aiding force to outward migration (Busch et al. 1998; Greene et al. 2009; Shepard 2015).

The habitats of American Eel shift throughout the life history stages of the species. The mostly planktonic leptocephali larvae occupy the uppermost 300 meters of the water column in marine environments (Greene et al. 2009; Shepard 2015). Glass eels and elvers are mostly nocturnal and begin the glass eel stage in marine environments drifting on tides and currents to coastal margins. Elvers use flood tides to enter estuaries, and habitats with various soft substrate are valuable to burrow and avoid ebb tides as they rest to avoid swifter water flows (Greene et al. 2009; Pratt et al. 2014; Shepard 2015). Yellow stage American Eels are able to exploit a variety

of habitats upon entry to coastal zones, either remaining in brackish habitats or migrating further upstream to more freshwater environments (Greene et al. 2009; Pratt et al. 2014; Shepard 2015). Previous studies have used otolith microchemistry to quantify movements of American Eel across salinity gradients during this phase with results showing that some individuals never leave brackish estuaries, others enter freshwater initially and make multiple trans-haline migrations, and others enter rivers only once and remain there for the entirety of their lives before migrating to spawn in the ocean (Daverat et al. 2006; Greene et al. 2009; Jessop et al. 2006; Jessop et al. 2008; Jessop et al. 2012; Lamson et al. 2006; Pratt et al. 2014; Shepard 2015).

Mystery obscures the timing and cues employed by American Eel when migrating or shifting habitats. Some studies suggest that movement over the continental slope is directly related to the transition to the glass eel stage from the leptocephali larvae stage, after which the glass eel begins moving toward coastal margins (Miller 2009; Pratt et al. 2014; Shepard 2015). Work by Cresci (2020) has suggested that the entrance to coastal margins for glass eel and elver stages of European Eel (*Anguilla anguilla*) is facilitated by magnetic and lunar cues, as well as recognition of specific freshwater or salinity chemical compositions via enhanced olfactory senses. Many observations of American Eel glass eel and elver stages have noted that individuals stall prior to entry into brackish and freshwater zones, hypothetically awaiting physiological adjustments to their ionic balance physiology before switching between hyperosmotic and hypoosmotic environments (Greene et al. 2009). Yellow Eel individual life history strategies are not totally clear, but many suspect the ability to transition between habitats provides a greater opportunity to consume diverse prey taxa as well as avoid predators more easily. Notably, female eels tend to be longer and be more represented in larger rivers further upstream while males are smaller and are more abundant in narrow creeks, marshes, or estuarine

environments (Greene et al. 2009; Helfman et al. 1984; Jessop et al. 2006; Shepard 2015). It is unclear if there is a physiological cue or pre-determination of female eels to migrate further upstream. There is substantial evidence that some individuals remain in the same location for multiple seasons or years only moving, hypothetically, to attain food or avoid predation or abiotic stressors (Bozeman et al. 1985; Greene et al. 2009; Ford & Mercer 1986; Morrison et al. 2003; Shepard 2015). Environmental factors are thought to play a role in the timing of yellow eel metamorphosis into silver eel stage with temperature, food availability, and overall habitat condition possibly sparking the stage transition (Shepard 2015).

The feeding ecology of American Eels changes significantly across the varied metamorphic stages. Leptocephali larvae do not suffer a high metabolic demand and most have been caught without any food items in their stomachs, though some studies have shown the larvae feed on marine snow consisting of detritus and a range of small plankton (Busch et al. 1998; Greene et al. 2009; Shepard 2015). Glass eels and elvers have been documented to primarily feed on insects, insect larvae, and benthic macroinvertebrates (Busch et al. 1998; Greene et al. 2009; Jessop 2000; Pratt et al. 2014). Yellow eels are opportunistic feeders. They have been known to feed on benthic macroinvertebrates, mussels, insects, snails, small fishes (including other eels), detritus, and carrion (Greene et al. 2009; Shepard 2015). A study by Bouchereau et al. (2009) looked at the diet of European eel ranging in size from 15.6 to 72.0 centimeters in a lagoon over the period of 1 year with 4 sampling events, one during each season. Up to 13 different prey were found in the stomachs with most containing insects and polychaetes. Another study by Denoncourt and Stauffer Jr. (1993) analyzed stomach contents of American Eel in the Delaware River and identified 56 different prey taxa across all individuals sampled.

There are periods in which American Eel do not regularly consume prey, primarily driven by physiological influences. At the yellow stage, American Eels can regulate their metabolic needs depending on seasonal temperatures or access to food allowing individuals to enter a state of torpor until conditions return to normal (Shepard 2015; Walsh et al. 1983). Silver stage American Eels are also believed to fast during their migration to the Sargasso Sea, relying on sufficient fat stores prior to metamorphosing to sustain them on their journey (Busch et al. 1998; Greene et al. 2009; Shepard 2015).

Management and Stressors

The American Eel is considered panmictic with one single spawning stock (Brust et al. 2017). Glass stage American Eel is the primary target for commercial fishers with landings totaling 928,358 pounds in 2016, which is substantially lower than the peak landing of over 3,670,000 million pounds in 1979 (Brust et al. 2017; MacGregor et al. 2008; Shepard 2015). The landings are primarily exported to Europe and Asia as a substitute for imperiled regional species (*A. japonica and A. anguilla*). Domestic consumption of American Eel in the United States is minimal and most of the landings that stay in the U.S. are used as bait. However, the glass stage American Eel fishery has seen dramatic increase in demand since its inception in 1970. The Atlantic States Marine Fisheries Commission's (ASMFC) Eel management board first convened in 1995 and implemented an interstate management plan in 1999 consisting of size restrictions and possession limits (Brust et al. 2017; Shepard 2015). In 2001, Maine and South Carolina prohibited commercial fisheries for glass eels, and they remain the only states with harvest prohibitions (Brust et al. 2017; Shepard 2015). The ASMFC American Eel stock assessment update in 2017 stated the price of glass stage American Eel has increased to over

$1,000 and peaked over $2,000 per pound with highest reported value being $40.38 million for 21,611 pounds in 2012 (Brust et al. 2017). The driving force behind the increase in demand is growing desire for American Eel in Asian aquiculture markets. European Eel and Japanese Eel are preferred over American Eel in Asian markets, but populations of the preferred species have drastically declined over the past decade driving the demand and value of imported American Eel upwards (MacGregor et al. 2008; Shepard 2015). There is no recreational fishery for American eel in the U.S. and most fish that are recreationally landed are believed to be bycatch. Subsistence fishing for American Eel was traditionally recognized for Native Americans and early European settlers but declined to insignificant levels after 1900 (Brust et al. 2017). Use of American Eel as a replacement species for international markets has led to high fishing pressure and drastic declines in abundance of which in turn has led to more focus on conservation and understanding of the species across the entirety of its range.

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) listed the species as threatened in 2012 as a response to declining population (Jacoby et al. 2017; Shepard 2015). Following COSEWIC's threatened designation of American Eel the International Union for Conservation of Nature (IUCN) responded by red listing the species as endangered. Two separate status reviews were performed by the U.S. Fish and Wildlife Service (USFW), once in 2007 and again in 2015. Both reviews concluded that while the population was in decline, there was no evidence suggesting consideration for protection under the Endangered Species Act (ESA) (Jacoby et al. 2017; Shepard 2015). Shepard concluded in the 2015 USFW review that while there were many stressors affecting American Eel at isolated locations, the stressors were not consistent across the entire population. Shepard went on to state that to be considered a threat to the species the stressor would have to be a detriment across most of the population's

range or significantly alter mortality, reproduction, or recruitment (Shepard 2015). The abundance and distribution of American Eel is not as well documented in many parts of its range, particularly in the Gulf of Mexico (GOM), Caribbean, Central America, and South America. This absence of data for U.S. states along the GOM has also led to a gap of knowledge regarding stressors that may threaten American Eel in that portion of its range.

The main stressors affecting American Eel population and distribution in the U.S. are increased fishing pressure, habitat modification (both fragmentation and loss), and the unmitigated threat of climate change (Helfman 2007; Bonhommeau et al. 2008; Limburg & Waldman 2009). Helfman (2007) details major drivers of habitat loss such as decline of riparian vegetation, impoundments, degraded water quality, and the introduction of invasive species as stressors affecting numerous aquatic species. Impoundments are particularly damaging to aquatic systems and migratory species that require free movement into and within tributaries. Busch et al. (1998) used GIS databases to assess fragmentation of Atlantic coastal streams due to hydroelectric dams and discovered that American Eel, and other migratory diadromous species, lost access to ~84% of historic portions of their range. Flow control systems are constructed in a way that if no electricity is needed the water discharge can cease or be set at a low enough level that it creates a stagnant pool or reduce habitat livability for days (Helfman 2007). The discontinued gene flow and the genetic structure of the population due to the isolation and alteration of fish communities in man-made reservoirs is another detriment, as is the direct threat of mortality by turbines (Helfman 2007; Hoeinghaus 2018; Limburg & Waldman 2009). Helfman (2007) describes a surprising situation in which reservoirs exacerbate negative climate effects because the flooded landscape vegetation ceases to be a net sink of carbon dioxide (CO_2) and instead exude CO_2 and methane (CH_4). Increasing global temperatures due to climate

change has been predicted to affect larval development of fish, accessible habitat, and distribution boundaries of freshwater and marine fish and diadromous species in particular (Lassalle & Rochard 2009; Limburg & Waldman 2009). Large scale oscillations, such as the North Atlantic Oscillation (NAO) and El Niño Southern Oscillation (ENSO), account for a considerable amount of variation in sea surface temperature (SST), wind pattern alterations, regional cooling and warming, and varied precipitation (Bonhommeau et al. 2008; Drouineau et al. 2017; Knights 2003; Secor 2015). The NAO and ENSO events can also influence migration and distribution of migratory fishes and their progeny by altering oceanic currents thereby affecting larval transport, limit the quality of planktonic food for larval fishes, and alter salinity zones which can affect migration of adults to spawning grounds (Bonhommeau et al. 2008; Drouineau et al. 2017; Secor 2015). Bonhommeau et al. (2008) looked at decadal shifts in SST in eel spawning grounds as an indicator of primary production and found that there was lower recruitment of juvenile eels that coincided with the increased SST of the spawning grounds. This suggests that the increase in SST is related to lower food quality over the period of migration for leptocephali larvae (Bonhommeau et al. 2008; Drouineau et al. 2017).

Shepard's most recent American Eel status review (2015) concluded with a summary of reasons for not designating the species as endangered under the ESA, but it also provided the foundation for research on the species moving forward. One of the highlighted issues in the status review was the lack of research or documentation of American eel in the totality of its range including sub-tropical GOM, sub-tropical and tropical Atlantic, and tropical islands of the Caribbean (Shepard 2015). A number of studies have used otolith microchemistry to elucidate habitat use and shifts in the northeastern U.S. and eastern Canada (Benchetrit et al. 2017; Daverat et al. 2006; Jessop et al. 2002; Jessop et al. 2006; Jessop et al. 2008; Jessop et al. 2012;

Lamson et al. 2006; Morrison et al. 2003). These studies have shown that American Eel in the northwest Atlantic pursue individual life history strategies with some eels spending their entire life in brackish marine environments, others being long-term residents of freshwater systems, and some making multiple transhaline shifts throughout their lives. Given that latitudinal gradients in freshwater versus oceanic productivity has been hypothesized to influence migratory life history types, American Eel research in subtropical systems is needed to evaluate if unique combinations of migratory and habitat uses exist in latitudes where catadromy is theoretically more favorable (Gross 1987; Gross et al. 1988; McDowall 1988). There have been promising signs of research expanding to these underrepresented regions recently. A study by Kwak et al (2018) synthesized American Eel research in the Caribbean from the years 2005 – 2016 and discovered similarities to life history strategies that mirror that of those in the northwest Atlantic; specifically, that the eels occupied a dynamic range of habitats such as large freshwater streams and coastal plains.

Shepard's status review (2015) left many questions that required answering to gain better knowledge of American eel in the entirety of its range. A multi-tiered project has since been proposed to tackle many of these questions with the combined efforts of Texas A&M University Corpus Christi, the University of Texas at Austin, and Texas Parks and Wildlife (TPWD) River Studies program. Key aspects of the project are to elucidate the historical and current distribution of American Eel in Texas, introduce and implement a glass eel/elver monitoring program, assess the genetic relationship between eels caught in the GOM to eel in the Atlantic, describe the population structure of eel in Texas, characterize habitat use of eel in Texas, and assess the presence of the parasite *Aguillicola crassus* in the GOM population. The specific purpose and objective of this thesis is to discern if American Eel display varied individual life

history strategies regarding habitat use and trophic ecology in sub-tropical Texas using muscle tissue stable isotope analysis (SIA); and if so, are those strategies similar to habitat use and trophic ecology employed by conspecifics in the northwest Atlantic. The resulting work, and completion of the entire project, will be a major step in understanding American Eel in a severely underrepresented portion of its range.

Introduction to Stable Isotope Analysis

Numerous methods have been used to assess movement ecology of fishes: visual observations, various internal or external electronic tags (applied tags), and the analysis of natural structures and tissues (natural tags) (Elsdon et al. 2008; Secor 2015). Natural tags are recorded in metabolically active and time-integrated tissues (liver, blood, or muscle) or structures that are incrementally accreted and therefore time-separated (such as otoliths, scales, vertebrae, or fin spines). These samples are relatively easy to obtain and can be analyzed to deduce the life history of the subject, whereas many applied tags allow a brief window of scrutiny from the time of capture, or implantation, until the subject leaves a pre-designed telemetry array or the tag detaches (Trueman et al. 2012). Stable isotope analysis (SIA) is another powerful tool to elucidate ecological information. It is based on three principles and concepts: 1) the tissue stable isotope values of interest reflect dietary and ambient water sources, 2) tissue type represents a window of observation depending on the temporal change of metabolically active tissue (tissue turnover), and 3) the material being taken up by the consumer is reflected in the interaction of light and heavy isotopes of the element of interest (fractionation) (Hobson et al. 2019; Trueman et al. 2012). The primary use of tissue SIA has been to characterize food web structure but it can also be employed to track migration across isotopic gradients.

A major consideration for tissue SIA is the turnover rate of the analyzed tissue, which is defined as the length of time it takes for the tissue of an individual to reflect an isotopically distinct diet following a diet switch (Buchheister & Latour 2010; Tieszen et al. 1983; Zilversmit et al. 1943). Different tissue types have different turnover rates depending on their relative rates of metabolic activity. Mucus, blood, and liver typically have the shortest turnover rates while muscle has a longer turnover rate, typically months or even years (Church et al. 2009; Trueman et al. 2012). Turnover rates are species-specific, and they are also heavily influenced by metabolism, ontogenetic stages, feeding rate, and temperature (Buchheister & Latour 2010; Walsh et al. 1983). Turnover rates depend on both the metabolism and replacement of existing biomass as well as addition of new biomass that effectively dilutes the isotope signature of any existing biomass. The relative contributions of these two processes differ by ontogenetic stage. Juvenile fish typically have higher tissue turnover rates due to rapid growth and high rates of biomass addition compared to adult fish, whose turnover is dominated by the slower process of metabolic replacement (Trueman et al. 2012). The rate of isotopic turnover in tissues is ideally quantified with laboratory experiments but may also be estimated with field samples if experiments are not feasible (Buchheister & Latour 2010; Eberhardt et al. 2015; Tieszen et al. 1983; Zilversmit et al. 1943). In a study investigating ecological plasticity of European Eel, Harrod et al. (2005) estimated white muscle tissue turnover rate of up to 1 year for adult eels. A more recent project on American Eel estimated muscle tissue turnover for yellow eel at approximately 191 days (Eberhardt et al. 2015).

Fractionation of elements through a system can also heavily influence isotopic signatures of individuals. Fractionation occurs when biological, chemical, or physical processes cause a change in the isotopic ratio of a reactant or product and is driven by the mass of respective

isotopes, with heavier isotopes reacting slower than lighter isotopes due to differential masses and stronger elemental bonds. (Peterson & Fry 1987; Trueman et al. 2012). As lighter isotopes have faster reaction rates and are preferentially metabolized and respired or excreted relative to heavier isotopes, heavier isotopes accumulate and isotope ratios become more enriched (i.e. have higher values in the ‰ notation) as individuals move further up in the food web. The main assumption when applying this metric to trophic interactions is that the fractionation and isotope enrichment per trophic level of $\delta^{15}N$ is generally ~3.4‰, whereas the enrichment per trophic level of $\delta^{13}C$ is ~0-1‰ (Buchheister & Latour 2010; DeNiro & Epstein 1978; DeNiro & Epstein 1981; Peterson & Fry 1987; Post 2002). The enrichment value of 3.4‰ for $\delta^{15}N$ is a generalization and differs both across and within species. For example, Kaifu et al. (2013) and Eberhardt et al. (2015) performed dietary studies on Japanese Eel and American Eel respectively with results for $\delta^{15}N$ enrichment being 2.1‰ per trophic level for Japanese Eel and 1.2‰ for American eel. Enrichment of $\delta^{34}S$ is nearly negligible per trophic level but has a higher variance between primary producers across salinity gradients (Connolly et al. 2004; Fry 1988; Peterson & Fry 1987).

Isotope ratios of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ (stable isotopes of carbon, nitrogen, and sulfur) can thus provide insight into individual movement and migration across isotopic gradients, or ‘isoscapes’ (Cunjak et al. 2005; Fry 2002; Gilbert et al. 2019; Herzka 2005; Hobson 1999; Peterson & Fry 1987; Tieszen et al. 1983). Environmental stable isotope ratios can differ due to terrestrial or aquatic origins of organic material, salinity, decomposition, natural geological processes, and anthropogenic influences (Fry 2002; Herzka 2005; Hobson 1999; Peterson & Fry 1987; Tieszen et al. 1983). These processes contribute to the isotopic make-up of primary producers, thereby creating an isotopic baseline for the ecosystems and the foundation for developing isoscapes.

Isoscapes are a powerful way to track animal migrations as it models migration based upon organic source material moving through the system in the form of dietary intake (Gilbert et al. 2019; Hobson et al. 2010; Hobson et al. 2019; Trueman et al. 2017). The three main assumptions when using isoscape information are: the isotopic make-up of the location through which the animal moves must be known, trophic fractionation factors must be known, and tissue turnover rates must be known (Hobson et al. 2010; Hobson et al. 2019; Trueman et al. 2012; Trueman et al. 2017). If these parameters are well characterized, then recent migrants can be identified given that they retain isotopic signatures of their prior habitat if they immigrated before their tissues have turned over and equilibrated with local dietary signatures. Conversely, non-migratory individuals should isotopically resemble one another due to theoretical lack of variation in movement and diet. These assumptions are most useful when the baseline signatures are known for the systems being studied, but analyses can still provide valuable information when comparing individual variability across systems in which baseline signatures are not fully known.

Values of $\delta^{13}C$ vary greatly in plants depending on their photosynthetic pathways. For instance, C_3 plant values are approximately -28‰ and C_4 plant values are approximately -13‰ (DeNiro & Epstein 1978; Hobson 1999; Middelburg 2014; Peterson & Fry 1987). Enrichment of $\delta^{13}C$ values is minimal at ~0-1‰ going up the food chain, making this isotope tracer a good indicator of primary food sources (Buchheister & Latour 2010; DeNiro & Epstein 1978; Peterson & Fry 1987). Values of $\delta^{13}C$ also differ depending on the system being studied. For example, the $\delta^{13}C$ values for marine waters is the result of exchange interactions of CO_2 between the Earth's atmosphere and total bicarbonate in the ocean's surface water (Peterson & Fry 1987). Freshwater $\delta^{13}C$ values are more variable than marine values and can be affected by the

weathering of carbonate rocks, mineral springs, CO_2 levels in the atmosphere, and organic matter respiration (Peterson & Fry 1987). Typically, $\delta^{13}C$ values are higher in marine than in freshwater systems, and they mix linearly or curvilinearly across salinity gradients within estuaries (Fry 2002; Peterson & Fry 1987). Bishop et al. (2017) provide an example of shifts in $\delta^{13}C$ values across salinity gradients in a Texas bay. Values of $\delta^{13}C$ range from ~ -26‰ to -17‰ over a salinity range of 7 to 38 in adductor muscle tissue of oysters

Nitrogen fractionation does not directly influence plants as much as carbon fractionation due to nitrogen being the limiting agent in plant growth, meaning that all nitrogen can be utilized by primary producers without measurable fractionation (Peterson & Fry 1987). Despite this, $\delta^{15}N$ is extremely valuable in dietary studies due to the general ~3.4‰ enrichment up the food chain providing a powerful measure for trophic level studies (DeNiro & Epstein 1981; Middelburg 2014; Pinnegar & Polunin 1999). Values of $\delta^{15}N$ may also be used to identify migration patterns where significant gradients in baseline signatures exist, such as across many estuaries (Peterson & Fry 1987). In Texas estuarine systems ranging in salinity from 2 to 35, suspended particulate organic matter (SPOM) $\delta^{15}N$ values range from 12‰ to -2‰ (Bishop et al. 2017). Anthropogenic nitrogen inputs are increasingly affecting natural nitrogen processes, which complicates movement studies, but in turn can aid in the identification of nitrogen input due to human activity (Peterson & Fry 1987; Wada 2009).

Values of $\delta^{34}S$ in marine environments are consistent at 21‰ in the water column, while terrestrial sources are much lower, ranging from 2-6‰ (Fry & Chumchal 2011; Peterson & Fry 1987; Yamanaka et al. 2003). Values of $\delta^{34}S$ in the marine and freshwater benthos are typically lower than pelagic values due to bacterial sulfides in anoxic environments, which range from approximately -20‰ to - 24‰ (Connolly et al. 2004; Fry & Chumchal 2011; Yamanaka et al.

2003). Freshwater to marine transition zones are often marked by a sharp increase until approximately 5ppt salinity, when the $\delta^{34}S$ values level off at marine values for the remainder of the increasing salinity gradient (Fry 2002; Fry & Chumchal 2011). This makes muscle tissue sulfur stable isotope analysis a powerful tool to delineate recent movement between estuarine and freshwater habitats. Values $\delta^{34}S$ can be a powerful "tie-breaker" in source primary production studies when incorporated in studies where $\delta^{13}C$ and $\delta^{15}N$ values are ambiguous (Connelly et al. 2004). The combined use of these isotopes could thus reveal variation in recent migration histories within a capture location if some individuals still retain marine-type isotope ratios while others have equilibrated to local freshwater food webs. Regressions between pairwise combinations of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ can thus potentially reveal variable immigration patterns given known differences in baseline isotope values across salinity gradients.

A multi-proxy approach to diet and movement studies is a more powerful and effective way to analyze and elucidate natural history of individuals, whether using natural tags or a combination of natural and applied tags (Herzka 2005; Middelburg 2014; Trueman et al. 2012). The 'More is Better' approach is particularly beneficial in dynamic systems like the ones seen in Texas. The state of Texas provides a broad range of systems and environments from freshwater rivers, to streams, to hypersaline lagoons, many of which are punctuated by aperiodic drought and flooding events (Nielsen-Gammon et al. 2005; Walther & Nims 2014; Ward 2000; Wurbs & Zhang 2014). The state is defined by a strong longitudinal precipitation gradient and latitudinal temperature gradient (Hudson & Heitmuller 2008). Hydrological systems are dynamic across the state's 15 major river basins and 8 major coastal basins that span the gamut of environmental classification ranging from arid deserts to humid wetlands including rivers that are equally as diverse in size and water flow (Hudson & Heitmuller 2008; Wurbs & Zhang 2014). These rivers

are crucial suppliers of nutrients and sediment to Texas' coastal zones. Salinity is dependent on rainwater flow into bays and estuaries with the general trend of a gradual and steady increase in $\delta^{13}C$ values and a decline in $\delta^{15}N$ values from freshwater to marine habitats. Values of $\delta^{34}S$ drastically increase at salinities above 5. Therefore, if recent salinity is the dominant influence on tissues of diadromous fish captured in freshwater, marine immigrants should contain high values of $\delta^{13}C$ and $\delta^{34}S$ along with low values of $\delta^{15}N$ compared to longer-term freshwater residents.

The purpose and objectives for this study are to infer habitat use and trophic ecology of American Eel in Texas using SIA of muscle tissue for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. Isotopic values compared within and across systems will inform any variability within and across groups that could be attributed to differences in diet or habitat use. However, care must be taken when analyzing the results due to possible influences of isotopic values. For example, if an individual caught far upstream in freshwater displays high $\delta^{13}C$ values compared to others caught in that system it may be due to either recent immigration from a marine environment or alternatively by having fed within a system with a higher $\delta^{13}C$ baseline signal independent of salinity. If the turnover rate calculated by Eberhardt et al. (2015) was applied here, then the individual would have had approximately 6 months to shift habitats before the isotopic signal ceased to reflect that of the former environment. The outcome of this study, and the overall project on American eel in Texas, will provide a foundation to build upon and help fill a substantial knowledge gap for the species across the state.

CHAPTER II: American Eel in Texas: Employing Muscle Tissue Stable Isotope Analysis to Elucidate Habitat Use and Feeding Ecology

INTRODUCTION

American Eel (*Anguilla rostrata*) has a complex life history that makes use of a diverse range of habitats across its developmental stages. The totality of the species' range includes northern portions of South America (Venezuela), the Caribbean, Gulf of Mexico (GOM), western Atlantic along the east coasts of the United States and Canada, and the north Atlantic to Greenland; with environments including marine waters, brackish estuaries, and freshwater rivers and lakes (Busch et al. 1998; Greene et al. 2009; Jacoby et al. 2017; Shepard 2015). American Eel, like other Anguillids, are catadromous fish that make up one panmictic population characterized by a semelparous spawning event in the southwestern Sargasso Sea (Haro et al. 2000; Macgregor et al. 2008; Oliveira et al. 1999). The life history of the species can best be defined by a series of physiological and morphological metamorphosis events which are accompanied by individually varied strategies of habitat use and dispersion (Greene et al. 2009; Jessop et al. 2002; McCleave & Edeline 2009). Leptocephali larvae are transported from the Sargasso Sea mostly via passive drifting along currents before metamorphosing into glass eel upon crossing continental slopes, and again into elvers when entering coastal margins (Busch et al. 1998; Greene et al. 2009; Helfman 1984; McCleave 1993; Miller et al. 2009). The elvers then transform into sexually immature yellow eel and disperse into local systems. Most of the leptocephali larvae ride the Florida Current and Gulf Stream as they make their way to the coastal margins of the U.S. and Canada, fewer make their way into the Caribbean Sea and GOM with the determination of distribution being unknown (McCleave 1993). American Eel employ

an opportunistic generalist feeding strategy, specifically in the juvenile elver and yellow eel phases, when they may consume prey items including insects, macroinvertebrates, small fishes, and even other eels. Stomach contents of yellow American Eel can include up to 56 different taxa (Denoncourt & Stauffer Jr. 1993).

American Eel abundance has drastically declined compared to historical values due to stressors such as: increased fishing pressure, habitat loss or fragmentation, effects of rampant climate change, and parasitic infection (Helfman 2007; Bonhommeau et al. 2008; Limburg & Waldman 2009). There is little demand for American Eel as a food source in the U.S., so the main concern is the increased landings of glass eel and elver stage individuals for aquaculture in overseas markets (Haro et al. 2000; MacGregor et al. 2008; Shepard 2015). The Atlantic States Marine Fisheries Commission (ASMFC) noted that the price for glass eels increased to over $1000, with peaks over $2000, with the highest reported value being $40.38 million for 21,611 pounds in 2012 (Brust et al. 2017). In addition to harvest, population declines have also been attributed to habitat fragmentation due to dams, impaired freshwater habitat quality, and climate change impacts on marine systems (Bonhommeau et al. 2008; Busch et al. 1998; Drouineau et al. 2017; Helfman 2007; Hoeinghaus 2018; Knights 2003; Lasalle & Rochard 2009; Limburg & Waldman 2009; Secor 2015). As a result of the continued population declines, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and the International Union for Conservation of Nature (IUCN) designated the species as threatened and endangered, respectively (Jacoby et al. 1027; Shepard 2015). The U.S. Fish and Wildlife Service (USFW) responded with two species status reviews (2007 & 2015), with both concluding the impact of stressors did not extend to the entirety of the population (Shepard 2015). Shepard's status review (2015), while encompassing most of the available distribution and abundance information about

American Eel, led to more questions regarding the species in lesser-known regions of its range. A specific limitation of the report was a lack of data about historical and current abundance and distribution in the GOM compared to knowledge of the species along the temperate Atlantic coast. This data gap prompted the formation of a multi-tiered project with goals to elucidate historical and current distributions of American Eel in GOM, implement a standardized glass eel phase monitoring program comparable to that of Atlantic eels, quantify the population structure of GOM eels compared to Atlantic eels, assess the presence of the parasite *Anguillicola crassus* in GOM eels, and characterize habitat use of GOM eels, particularly in Texas. The specific task of this thesis is to characterize the habitat use and feeding ecology of American Eel in Texas using muscle tissue stable isotope analyses (SIA) of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values.

Bulk tissue SIA is a powerful tool used to elucidate ecological information and is based around specific assumptions and concepts: the tissue SIA values reflect dietary and ambient water sources, the species-specific turnover rate of metabolically active tissue is known, and the material being assimilated into the consumer is impacted by fractionation between light and heavy isotopes between the producer and the consumer (Hobson et al. 2019; Peterson & Fry 1987; Trueman et al. 2012). A species' turnover rate varies depending on factors including which tissue is analyzed, the ontogenetic stage and growth rate of the organism, the feeding rate, and the ambient temperature (Buchheister & Latour 2010; Walsh et al. 1983). The most widespread use of tissue SIA has been to quantify food web structure and consumer interactions, but it has also been employed to reconstruct movement between habitats or food webs with distinct isotope signatures. Knowing the source, or primary producer, SIA values is key to understanding baseline signatures within and across systems and quantify gradients of isotope variability across landscapes, or 'isoscapes', that provide a powerful tool to track migration

between systems (Hobson et al. 2010; Hobson et al. 2019; Trueman et al. 2012). When incorporating the use of isoscapes, one must consider the isotopic baselines of each system the individual has moved through, the trophic discrimination factors for the isotopes of interest, and tissue turnover rates to determine the time window over which movement may be resolved (Hobson et al. 2010; Hobson et al. 2019; Trueman et al. 2012; Trueman et al. 2017).

Values of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in consumer tissues reflect those of the individual's food web and are spatially heterogenous across salinity gradients (DeNiro & Epstein 1978; DeNiro & Epstein 1981; Peterson & Fry 1987; Trueman et al. 2017). A key assumption when working with SIA is the magnitude of enrichment per trophic level. Generally, isotope values increase by ~3.4‰ for $\delta^{15}N$ and ~1.0‰ for $\delta^{13}C$ per trophic level, while enrichment is negligible for $\delta^{34}S$ (Buchheister & Latour 2010; Connolly et al. 2004; DeNiro & Epstein 1978; DeNiro & Epstein 1981; Peterson & Fry 1987). Values of $\delta^{15}N$ are particularly useful when discerning trophic position given its higher enrichment value, but trophic enrichment values can be species specific. Studies performed by Kaifu et al. (2013) and Eberhardt et al. (2015) performed diet studies on Japanese Eel (*Anguilla japonica*) and American Eel, respectively, with the resulting enrichment values for $\delta^{15}N$ being 2.1‰ for Japanese Eel and 1.2‰ for American Eel. Values for $\delta^{13}C$ and $\delta^{34}S$, while only being minimally enriched between trophic levels, are useful to identify movement across a salinity gradient. Values of $\delta^{13}C$ vary greatly in plants depending on photosynthetic pathways and provenance, where primarily marine plants' carbon uptake is a third that of terrestrial plants (DeNiro & Epstein 1978; Hobson 1998; Middelburg 2014; Peterson & Fry 1987). Sulfur values are relatively stable in marine environments, with values of ~21‰ in the water column, while terrestrial values are generally much lower, with primary producers

having values typically between 2‰ and 6‰ (Fry & Chumchal 2011; Peterson & Fry 1987; Yamanaka et al. 2003).

Texas provides a unique location for this study due to the range of systems and environments from freshwater rivers and streams that are punctuated by monthly and annual aperiodic drought and flooding events to hypersaline lagoons (Nielsen-Gammon et al. 2005; Walther & Nims 2014; Ward 2000; Wurbs & Zhang 2014). The state's geology is limestone dominant making dissolution of carbonates a major source of riverine dissolved inorganic carbon (DIC) and provides powerful indication of geological based carbon transport versus anthropogenic nutrient loading (Zeng et al. 2011). There are few completely free flowing hydrologic systems in Texas as many are impounded at multiple sites. These modes of fragmentation greatly affect the natural flows of Texas' rivers and streams as well as inhibit movement of many vertebrate and invertebrate species (Hoeinghaus 2018; Wurbs & Zhang 2014). The state is defined by a strong longitudinal precipitation gradient and latitudinal temperature gradient (Hudson & Heitmuller 2008). Hydrological systems are dynamic across the 15 major river basins and 8 major coastal basins in a state that runs the gamut of environmental classification ranging from arid deserts to humid wetlands including rivers that are equally as diverse in size and water flow (Hudson & Heitmuller 2008; Wurbs & Zhang 2014). These rivers are crucial to Texas' coastal zones due to freshwater, nutrient and sediment transportation to estuaries.

Previous studies on isotope variability in Texas estuaries have characterized isotope mixing patterns across salinity gradients using DIC, dissolved inorganic nitrogen (DIN), particulate organic matter (POM), or bivalves as indicators of local stable isotope values (Aguilar, 2017; Bishop et al. 2017; Fry 2002; Kaldy et al. 2005; Kendall et al. 2001; Riera et al.

2000; Zeng et al. 2011). Research in Texas' systems correspond with general interpretations that $\delta^{13}C$ values increase linearly or nonlinearly as salinity increases, while $\delta^{15}N$ values show a gradual decrease salinity increases (Aguilar 2017; Bishop et al. 2017; Fry 2002). Bishop et al. (2017) reported the values of $\delta^{13}C$ and $\delta^{15}N$ in suspended particulate organic matter (SPOM) ranged -30‰ to -20‰ and 12‰ to -2‰, respectively, in Aransas and San Antonio Bays where salinities ranged from about 2 to 35. Values of $\delta^{34}S$ found in sulfates sharply increase until plateauing at salinities of approximately 5 (Fry 2002).

The purpose and objectives of this thesis is to determine habitat use and trophic ecology of American Eel in Texas using muscle tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values. This research aims to see if individuals across, and within, systems have varied isotopic values which would suggest they undertake different life history strategies. My specific goals were to 1.) compare differences in univariate and bivariate isotope combinations among eels collected from different drainage basins to determine if broad location-specific differences in isotope values were present; and 2.) compare individual pair-wise isotope ratios within each location to assess if recent immigration from high salinity waters could explain (in part) variation in isotope ratios that may reflect divergent migratory histories. These results could help confirm if American Eel employs a facultatively catadromous strategy in Texas. The outcome of this study, and the broader multi-tiered project, aims to fill a gap in knowledge of American Eel in an understudied portion of its geographic range.

METHODS

Study Area

American Eel were collected across a broad range of Texas aquatic systems. The sites were designated using the United States Geological Survey's (USGS) national Hydrologic Unit Code (HUC), with some sites being combined due to geographic proximity and/or low sample numbers (Figure 1). From North-Northeast to South-Southwest: Sabine River Basin (S), Brazos-San Jacinto River Basins (B-SJ), Colorado River Basin (C), Guadalupe-Lavaca River Basins (G-L), Nueces River Basin (N), Coastal Basin (COAST). A seventh group, Wastewater (WW), was created due to several individuals being captured in an isolated section of the Port Lavaca, TX wastewater treatment plant.

Sampling & Processing

American Eel collections occurred during 2012-2019 in Texas rivers and estuaries and individuals were deposited at the Ichthyology Collection at the University of Texas at Austin (Hendrickson et al. 2021). Methods of collection included electro-fishing, fyke net, and rod and reel. These collections were part of a collaborative effort with TPWD, university labs, and a citizen science outreach program targeting Eels using diverse sampling methods across diverse habitat types including barrier islands, freshwater rivers and streams, dam spillways, wastewater treatment plants, and estuaries.

Spatial, temporal, and environmental data were recorded at the time of collection for the 77 total American Eel used for the analyses reported here. Individual samples were either placed on ice or frozen and taken to the Ichthyology Collection at the University of Texas at Austin for

processing where the samples were given identification numbers. The Eels were placed in an ice bath during processing where standard length and weight were recorded. Eels were dissected and processed for other analyses not reported here (otoliths, gut contents, parasites). The individuals were initially fixed in buffered 10% formalin for 7-10 days before being placed in 35% ethanol. After another 7-10 days the eels were moved to permanent storage in 70% ethanol.

Sample Stable Isotope Analysis

Dorsal muscle tissue with skin removed was collected from 77 individuals for $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis and preserved in ethanol, and additional subset of tissue from 4 eels was preserved in formalin to evaluate if sample preservation method affected isotope values. Muscle tissue samples for all stable isotope analyses were placed in a drying oven for 72 hours at 60°C. Once dried, the tissues were ground into a fine homogenous powder using a mortar and pestle and then placed into glass vials. Approximately 1.0 mg (+/- 0.5mg) of powdered tissue from each sample was enclosed in individual tin capsules for analyses. Samples analyzed for $\delta^{13}C$ and $\delta^{15}N$ values included replicate powdered tissue subsamples from 10 randomly selected ethanol-preserved samples as well as the 4 formalin preserved samples.

Values of $\delta^{34}S$ were also quantified for a subset of 60 individuals that had sufficient remaining powdered muscle tissue to provide enough sample mass for additional isotope analyses. These analyses also included 2 of the individuals with muscle tissue samples having counterparts that were preserved in formalin. After being dried and ground, 3-5 mg were packed into tin capsules for analyses, including replicates from 8 randomly selected ethanol-preserved samples as well as the 2 formalin preserved samples.

All prepared samples were sent to the University of California Davis Stable Isotope Facility for analysis. Dual $\delta^{13}C$, $\delta^{15}N$ analyses of natural abundance in solid samples were performed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Analyses of $\delta^{34}S$ natural abundance in solid samples was performed using an Elementar vario ISOTOPE cube interfaced to a SerCon 20-22 IRMS (Sercon Ltd., Cheshire, UK).

Results were reported using standard δ-notation where

$$\delta^{13}C \text{ (or } \delta^{15}N, \delta^{34}S) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000 \textit{(represented as ‰)}$$

and R_{sample} and $R_{standard}$ equal the ratio $^{13}C/^{12}C$ (or $^{15}N/^{14}N$, $^{34}S/^{32}S$) of the sample and the certified reference material, respectively. All δ-values were reported in relation to VPDB (Vienna Pee Dee Belemnite), Air, and VCDT (Vienna-Canyon Diablo Troilite) for carbon, nitrogen, and sulfur, respectively. Carbon and nitrogen samples were compared against 8 reference materials with δ-values representing means of multiple analyses: Amaranth Flour (Quality Control) $\delta^{13}C$ = -12.82, $\delta^{15}N$ = 2.45, %C = 42.11, %N = 2.45; Bovine Liver (Quality Control) $\delta^{13}C$ = -21.58, $\delta^{15}N$ = 7.72, %C = N/A, %N = N/A; Caffeine (^{13}C Scale Normalization, ^{15}N Quality Control) $\delta^{13}C$ = -35.05, $\delta^{15}N$ = -2.89, %C = 47.64, %N = 28.35; Chitin (Linearity Correction, Elemental Totals) $\delta^{13}C$ = -18.86, $\delta^{15}N$ = 2.24, %C = 44.52, %N = 6.75; Enriched Alanine (^{15}N Scale Normalization) $\delta^{13}C$ = N/A, $\delta^{15}N$ = 41.13, %C = N/A, %N = 15.72; Glutamic Acid (GLAC)(^{13}C, ^{15}N Scale Normalization) $\delta^{13}C$ = -11.07, $\delta^{15}N$ = -8.53, %C = 40.82, %N = 9.52; Keratin (Quality Control) $\delta^{13}C$ = -24.35, $\delta^{15}N$ = 4.78, %C = 49.31, %N = 14.91; Nylon Powder (NYPOW)(Drift Correction) $\delta^{13}C$ = -24.67, $\delta^{15}N$ = -1.03, %C = N/A, %N = N/A. Sulfur

samples were compared against 5 reference materials with δ-values representing means of multiple analyses: Cysteine (Scale Normalization) $\delta^{34}S = 34.22$, %S = 26.40; Hair (Quality Control) $\delta^{34}S = 2.32$, %S = 2.19; Mahi-Mahi Muscle (Quality Control) $\delta^{34}S = 19.39$, %S = 1.00; Taurine (Scale Normalization) $\delta^{34}S = -2.46$, %S = 26.40; Whale Baleen (Linearity Correction, Elemental Totals) $\delta^{34}S = 17.48$, %S = 3.20.

The mean standard deviation for reference materials replicates was 0.06‰ for $\delta^{13}C$, 0.03‰ for $\delta^{15}N$, and 0.30‰ for $\delta^{34}S$. The absolute accuracy for calibrated reference material was 0.05‰ for $\delta^{13}C$, 0.04‰ for $\delta^{15}N$, and 0.11‰ for $\delta^{34}S$.

Lipid Correction

Lipid correction models were used to correct for any bias in $\delta^{13}C$ values due to lipid content and corrections were applied to all samples for consistency. The modified lipid correction model used for this study was a two-step equation given by Kiljunen et al. (2006) where

$$L = \frac{93}{1+(0.246 \times (C{:}N) - 0.775)^{-1}}$$

followed by

$$\delta^{13}C' = \delta^{13}C + D \times \left(I + \frac{3.90}{1+287/L}\right)$$

where L equals the proportional lipid content, $C{:}N$ is the ratio of total carbon to total nitrogen in μg, $\delta^{13}C'$ is the lipid normalized sample value, $\delta^{13}C$ is the original measured sample value, D is a constant representing the isotopic difference between protein and lipid (7.018 ±0.263), and I is a constant (0.048 ±0.013; Kiljunen et al. 2006). The constants D and I define the curvature of the slope and intersection on the x-axis, respectively for the modified model (Kiljunen et al. 2006).

Statistical Analysis

All statistical analyses were performed using R statistical software (R core team 2021). The impacts of preservation method, either formalin and ethanol, were compared in 4 samples for $\delta^{13}C$ and $\delta^{15}N$ and 2 samples for $\delta^{34}S$ using paired t-tests. The remaining statistical analyses were performed comparing samples that had been preserved in ethanol. Differences in isotope signatures among geographically defined groups were assessed with univariate and bivariate methods. Shapiro-Wilk tests were used to assess normality for univariate comparison of isotopic values between groups, with values of $\delta^{13}C$ and $\delta^{34}S$ not being normally distributed while the values of $\delta^{15}N$ were normally distributed. Kruskal-Wallis tests were used to compare $\delta^{13}C$ and $\delta^{34}S$ values between locations due to lack of normality then followed by Dunn's-Bonferroni post-hoc tests. Values of $\delta^{15}N$ between locations were compared using one-way Analysis of Variance (ANOVA), due to normally distributed data, and followed by Holm post-hoc tests. Within-group covariance of isotope combinations was assessed by calculating linear regression models between each isotope pair for each defined group.

Bivariate overlap of groups in isotope niche space were determined using Stable Isotope Bayesian Ellipses in R (SIBER) separately for the following combinations of isotopes: 1.) $\delta^{13}C$

and $\delta^{15}N$, 2.) $\delta^{13}C$ and $\delta^{34}S$, and 3.) $\delta^{15}N$ and $\delta^{34}S$ (Jackson et al 2011). The Bayesian standard ellipses (which encompass 40% of the data for each group) were used to calculate Standard Ellipse Area with a correction for small sample sizes (SEA_c) for each group and bivariate isotope comparison. Overlaps in bivariate ellipses were calculated using the maximum likelihood function (maxLikOverlap) in SIBER. This function uses the maximum likelihood estimated means and covariance of the group's ellipses to calculate overlap area, which were reported in $‰^2$.

Linear regressions were fit to comparisons of individual fish isotope values across all groups pooled and for each location separately with the purpose of comparing within group variance. These regression models were calculated for each location using all three bivariate isotope combinations (Model A: $\delta^{13}C$ against $\delta^{15}N$, Model B: $\delta^{13}C$ against $\delta^{34}S$, and Model C: $\delta^{15}N$ against $\delta^{34}S$).

RESULTS

Sample Collection and Analyses

Sample size for muscle tissue SIA are as follows: S (n = 11), B-SJ (n = 16), C (n = 5), G-L (n = 10), N (n = 12), COAST (n = 6), WW (n = 17). Standard length for all 77 individuals ranged from 151 mm minimum to 1047 mm maximum, while mean standard lengths (± 1 standard deviation) for each group ranged from 262.5 mm (±92.6) in the Nueces basin to 571.6 mm (±170.8) in the Wastewater group (Figure 2; Table 1). The standard length was significantly different across groups (Kruskal-Wallis: $p = 5.332e^{-05}$) and Dunn's post hoc comparison showed that there were two distinct subgroups showing length similarities (Figure 2).

Muscle tissue samples for stable isotope analysis (n = 77 for $\delta^{13}C$ & $\delta^{15}N$, n = 60 for $\delta^{34}S$) were compared against reference materials and ran with 10 duplicate samples for $\delta^{13}C$ and $\delta^{15}N$ and 8 duplicate samples for $\delta^{34}S$. The mean differences (± 1 standard deviation) in values between duplicates for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were 0.25‰ (±0.29‰), 0.06‰ (±0.05‰), and 0.28‰ (±0.21‰) respectively. Duplicate sample isotope values were averaged and assigned to the respective individual for the subsequent statistical analyses.

Preservation Method

A small subset of samples from each analysis, $\delta^{13}C$ and $\delta^{15}N$ (n = 4) and $\delta^{34}S$ (n = 2), was used to assess whether preservation in formalin resulted in significantly altered isotope ratios compared to ethanol preserved samples. Preservation method (ethanol vs formalin) had no significant effects on $\delta^{13}C$ ($t = 0.6839$, $p = 0.6839$), $\delta^{15}N$ ($t = -1.1115$, $p = 0.3473$), and $\delta^{34}S$ ($t = -1.0920$, $p = 0.4720$) values (Figure 3). For all subsequent statistical analyses, only the isotope values obtained from ethanol-preserved samples were used, for consistency.

Isotope Bias Assessments

The primary goal of the subsequent statistical analyses was to compare differences in isotope values among and within locations. In order to avoid any alternative sources of bias, isotope values were examined for global relationships between standard length and isotope ratios(which could bias isotopes values if eels consume a specialist diet or if isotopic values of prey varied greatly between locations), first we conducted a standard lipid correction using C:N ratios as a proxy for lipids, which are known to alter $\delta^{13}C$ values.

I found a significant positive correlation between C:N ratios and $\delta^{13}C$ ($p = 0.0164$; Figure 4), although the adjusted R^2 value was low ($AR^2 = 0.06$). There were 7 individuals with high values for C:N (>3.5), and thus a lipid correction model was used to avoid any potential bias of lipids on this isotope value (Post et al. 2007). After the lipid-correction was applied, a linear regression comparing the original lipid-uncorrected $\delta^{13}C$ values against lipid-corrected $\delta^{13}C$ values which showed these values were strongly positively correlated ($p = 2.200e^{-16}$ and $AR^2 = 0.97$) and the correction procedure only influenced the few high C:N value specimens, as expected (Figure 5).

There was no significant relationship between C:N and $\delta^{13}C$ ($p = 0.3988$; $AR^2 = 0.00$), but the relationship between C:N and $\delta^{15}N$ was significant ($p = 0.0252$, $AR^2 = 0.05$; Figure 4). C:N and $\delta^{34}S$ did not result in a significant relationship ($p = 0.1746$; $AR^2 = 0.01$), but C:N ratios did show statistically significant relationship with SL ($p = 0.0011$, $AR^2 = 0.12$; Figure 4).

There was a positive significant relationship between SL and $\delta^{13}C$ ($p = 0.0015$; AR^2 0.11) and there was no significant relationship between SL and $\delta^{15}N$ ($p = 0.133$; $AR^2 = 0.02$) or $\delta^{34}S$ ($p = 0.8357$; $AR^2 = -0.02$; Figure 6). The low explanatory value of SL and isotope values indicated it was not necessary to apply a length correction to isotope values.

Stable Isotope Values Between Groups

Ranges and mean tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of American Eel grouped by capture location are reported in Table 1. The total range for isotopic values for all individuals were: $\delta^{13}C$ = -31.6‰ - -18.5‰, $\delta^{15}N$ = 10.8‰ – 20.6‰, and $\delta^{34}S$ = -2.2‰ – 13.1‰ (Table 1). Values of $\delta^{13}C$ were significantly different between groups ($p = 4.959e^{-09}$), and post-hoc comparisons found that $\delta^{13}C$ values for WW were significantly different from values for every

other location except C and COAST (Figure 7). Values of $\delta^{15}N$ were significantly different among groups ($p = 3.869e^{-07}$) and post-hoc comparisons found that there were 3 groupings of similarity with S and B-SJ sharing the least amount of similarities with other groups, S having lower values and B-SJ having higher values (Figure 7). Finally, values of $\delta^{34}S$ were significantly different among groups ($p = 3.797e^{-09}$) and post-hoc comparisons found that there were two distinct clusters with similar values that were not significantly different from one another within clusters. The first cluster consisted of fish from the G-L, N, and COAST groups had higher $\delta^{34}S$ values and the second cluster, consisting of fish from S, B-SJ, and C groups, had lower $\delta^{34}S$ values (Figure 7). Fish from the WW had $\delta^{34}S$ values that were only significantly different from the B-SJ group but overlapped with all other groups (Figure 7).

Isotopic Niche Widths & Group Overlap

SEA_c values calculated for $\delta^{15}N$ against $\delta^{13}C$ values yielded the following niche width estimates: S = 5.7‰^{2}, B-SJ = 9.3‰^{2}, C = 3.9‰^{2}, G-L = 8.4‰^{2}, N = 2.9‰^{2}, COAST = 18.4‰^{2}, WW = 0.7‰^{2} (Figure 8). The COAST group had the highest occurrence of overlap in this bivariate space, with every group except WW, with the largest overlap area between the COAST and G-L groups (4.9‰^{2}) and the COAST and B-SJ groups (4.7‰^{2}). The WW group had no overlap with any other group (Table 2).

SEA_c values calculated for $\delta^{34}S$ against $\delta^{13}C$ yielded the following niche width estimates: S = 5.5‰^{2}, B-SJ = 8.5‰^{2}, C = 2.9‰^{2}, G-L = 18.8‰^{2}, N = 3.1‰^{2}, COAST = 31.6‰^{2}, WW = 0.4‰^{2} (Figure 9). There was minimal overlap between groups in this bivariate space. The B-SJ and G-L groups both had the highest amount of overlap with two other groups. The largest overlap was between the G-L and COAST groups at 5.5‰^{2} (Table 2).

SEA_c values calculated for $\delta^{34}S$ against $\delta^{15}N$ yielded the following niche width estimates: S = 3.2‰^{2}, B-SJ = 4.1‰^{2}, C = 2.7‰^{2}, G-L = 2.6‰^{2}, N = 1.5‰^{2}, COAST = 26.3‰^{2}, WW = 0.9‰^{2} (Figure 10). There was minimal overlap between groups in this bivariate space as well. Values of fish in the C, G-L, and COAST groups had the highest amount of overlap. The G-L and COAST groups had the highest overlap with each other at 2.0‰^{2}. The COAST group had the largest estimated niche widths as quantified by SEA_c values while the WW group had the smallest niche widths as measured by SEA_c values consistently across all three bivariate isotope combinations (Table 2).

Isotopic Variance Within Locations

Location N was the only location with significant relationships across all comparisons (Figure 16; Table 3). The only other group with significant relationships for Models A and B was WW (Figure 18; Table 3). The only other significant relationships were seen in Model C for S, B-SJ, and G-L (Figure 12, 13, 15; Table 3). The S, C, and COAST groups all showed positive relationships for Model A which is contrary to expectations if isotopic values primarily reflecting movements from high to low salinities (Figures 12, 14, 17; Table 3). S, G-L, and WW were the only groups to have positive relationships for Model B, the expected outcome for $\delta^{13}C$ and $\delta^{34}S$ if fish recently immigrated from high salinity environments (Figures 12, 15, 18; Table 3).

DISCUSSION

This study used SIA of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ to elucidate habitat use and trophic ecology of American Eel in Texas' hydrological systems. Muscle tissue SIA does not provide a lifelong

record, but it does provide a time-integrated record going back approximately 6 months for American Eel (Eberhardt et al. 2015). Thus, the results obtained in this study indicate recent feeding experience and may reflect migratory movements if eels had recently inhabited and fed in isotopically distinct locations, including estuarine or marine habitats. Overall, this study found that there were significant differences in univariate and multivariate isotope values between eels captured in different river basins across the Texas coast, with some isotopes showing substantial variability among individuals within a basin. While recent immigration from estuarine or marine waters could potentially be a driver of some of the individual variability observed, the observed patterns may also reflect diversity in prey specialization within freshwater systems as well as movement within river systems that may contain isotopically diverse baselines.

An important step before interpreting isotope values is to ensure that the method of collection and preservation does not bias results and confound ecological explanations for the observed patterns. Preservation effects are of particular concern where specimens may be sourced from archival and museum catalogs where either ethanol or formalin may have been used for long term storage. Although the sample sizes available for comparison were limited in the current study, I found there were no significant differences between preservation methods for any of the three isotope ratios assessed in this thesis, suggesting either ethanol or formalin may be suitable for muscle tissue preservation prior to SIA of $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$ for American Eels (Figure 3). Previous work comparing preservation methods resulted in the 'Goldilocks and the Three Bears' gamut of possibilities: ethanol depleted $\delta^{13}C$, ethanol enriched $\delta^{13}C$, or the $\delta^{13}C$ values were not significantly different from formalin-preserved specimens (Arrington & Winemiller 2002; Kaehler & Pakhomov 2001; Kelly et al. 2006). My results support the lack of

a difference due to preservation method, although continued assessments of the impact of preservation methods with expanded sample sizes should still be performed. Previous research has suggested that the length of time the sample was preserved before analysis could be an additional factor that determines whether preservation method results in altered isotope values. Specifically, Le Bourg et al. (2019) and Kaehler & Pakhomov (2001) noticed a greater effect of formalin over the analysis periods of 1, 4, and 12 weeks. Individuals in the current study were initially formalin-fixed for 7 – 10 days prior to permanent fixation in ethanol. The formalin samples for comparison were taken from ethanol-preserved fish and placed in formalin for approximately 6 months until preparation for SIA. Thus, the results presented here may not apply to samples preserved for years or decades prior to SIA quantification.

An important finding of this thesis is that isotope values varied significantly among certain river basins across much of Texas, while values from other locations overlapped. Univariate analyses of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ separately identified large-scale geographic patterns of variation that in some instances suggested underlying latitudinal gradients in isotope baselines (Figure 7). Most notably for $\delta^{15}N$ values were the distinct variation between eels captured in the Sabine and the Brazos-San Jacinto locations. Values of $\delta^{13}C$ appeared to have a mild, although not entirely linear, trend with values increasing at more southern locations such as the Nueces River. Values for $\delta^{34}S$ displayed a major shift, with a distinct geographic break in values between the Colorado River and the Guadalupe-Lavaca rivers. Values for the Coastal eels were also distinct, as they had the highest $\delta^{13}C$ and $\delta^{34}S$ values. In a study performed using European Eel, Harrod et al. (2005) compared isotopic means of individuals sampled from three distinct salinity zones: freshwater (FW) = <0.1 per mil, brackish water (BW) = ~15.0 per mil, and marine waters (MW) = >25.0 per mil. The $\delta^{13}C$ values were -25.2‰, -23.9‰, and 17.3‰ for FW, BW,

and MW respectively. While the values may differ in Texas' systems, similar trends in elevated $\delta^{13}C$ values in addition to $\delta^{34}S$ values for individuals caught in brackish or marine waters were observed, as expected for individuals recently inhabiting marine or brackish waters.

The eels in the Wastewater group showed highly clustered $\delta^{13}C$ and $\delta^{34}S$ values with $\delta^{13}C$ values elevated above all groups, including the Coastal eels. These eels were unique in that they were residing between two different phases of effluent sanitation at the city of Port Lavaca's Wastewater Treatment plant (Stephen Curtis, personal communication). The eels were isolated in a freshwater system within the treatment plant and stomach contents were comprised solely of snails (*Physa sp.*; Melissa Casarez, personal communication). The length of time these eels were isolated in the effluent system is unknown, but the monotypic diet could explain the lack of variation within the Wastewater group. Diet availability, diversity, and abundance in a system can alter the isotopic makeup of an individual, the Wastewater group is a prime example (DeNiro & Epstein 1978; DeNiro & Epstein 1981; Hobson et al. 2019; Trueman et al. 2012). Nutrient loading, whether industrial, agricultural, or urban, affects baseline values and can provide stark differences between systems. Hydrological systems in urban areas routinely receive outflows from wastewater treatment plants or runoff of other wastes. Morrissey et al. (2013) conducted a study monitoring isotope values in macroinvertebrates living in urban discharge and discovered $\delta^{15}N$ values were enriched downstream of a wastewater treatment plant, individuals upstream were unaffected by the treatment plant. The study included values of $\delta^{13}C$ and $\delta^{34}S$ as well, with neither showing a significant difference between upstream and downstream environments in relation to the treatment plant. Multiple studies have looked at the influences wastewater treatment plants have on isotopic values and have come to the conclusion that individuals downstream of treatment facilities have higher $\delta^{15}N$ values and lower $\delta^{13}C$ and $\delta^{34}S$ (Loomer et

al. 2015; Morrissey et al. 2013; Northington & Hershey 2006). The large quantity of *Physa sp.* found within the stomachs of WW eels may explain the lack of variation in values of $\delta^{13}C$ within the group, but it does not explain the values being significantly higher than the other groups. One possible source for increased $\delta^{13}C$ values is supplemental carbon needed to account for the chemical oxygen demand (COD) of wastewater treatment plants. The Environmental Protection Agency (EPA; 2013) details the necessity for supplemental carbon to be introduced for denitrifying organisms to use. This supplemental carbon can come in many forms (added corn syrup, ethanol, methanol, or glycerin) and is added after the initial aeration process. The supplemental carbon, now in the form of waste activated sludge, is removed from treatment at the secondary clarifiers. The WW eels were free to roam between the secondary clarifiers and UV sterilization, so it is possible this supplementary carbon source could influence the $\delta^{13}C$ values of the group. Sampling of the effluent between stages, and the activated waste, would be helpful in confirming this.

Values of $\delta^{34}S$ follow a steep incline in isotopic values between salinities of 0 and 5 salinity before plateauing at $\delta^{34}S$ values of 21 in marine environments. The reason for variation in $\delta^{34}S$ values seen across the groups is not easy to pinpoint, but one possible driver is the fossilized ancient reef systems of west Texas. Present et al. (2019) detail deposition and paleogeography of sulphate evaporites that incorporate into calcium carbonate lattice work of corals of the Delaware basin. These incorporations are higher than terrestrial $\delta^{34}S$ values and were representative of marine $\delta^{34}S$ values from the Precambrian to the early Mesozoic. These formations have resulted in a medley of terrestrial $\delta^{34}S$ values with the ancient marine sulfates being hotspots of higher isotopic values (Peterson & Fry 1987; Present et al. 2019). One study was able to deduce the $\delta^{34}S$ values in ancient marine habitats using evaporites from a west Texas

reef formation with the resulting values being ~10‰ (Present et al. 2019). Considering average terrestrial $\delta^{34}S$ values are 2‰ - 6‰, erosion and runoff from ancient reefs into freshwater systems may provide an explanation for elevated $\delta^{34}S$ values in individuals from southwestern locations. Another consideration is the flow variation of different systems, particularly in regard to where the eel were collected. Some of the isotopic values may be driven by massive freshwater inflow, fluctuation in isotopically distinct groundwater contributions or, in some instances, salt water encroachment as a result from drought. These hydrologic events could influence the isotopic make-up of systems and create inconsistencies in diet pathways. Extensive sampling of rivers throughout west Texas' drainage basins, including dietary items, needs to be performed to better pinpoint any influence these formations have on $\delta^{34}S$ values.

Overall, isotope values, particularly $\delta^{13}C$ and $\delta^{34}S$ values, followed a latitudinal gradient with values increasing from northeast to southwest, suggesting high variability both within and between groups. There are many possible drivers for the outcome of the univariate and multivariate analyses that may influence results. The patterns could be the result of recency of individual migration orhabitat shift. Previous studies have focused on the facultatively catadromous nature of American Eel along the Atlantic coast and the ability of individuals to make multiple transhaline migrations in their lifetime (Daverat et al. 2006; Greene et al. 2009; Jessop et al. 2006; Lamson et al. 2006; Pratt et al. 2014; Shepard 2015). Ontogeny and physiology could alter the turnover rate of individuals, speeding up assimilation of isotopic material in the case of younger fish (Buchheister & Latour 2010). The primary production isotopic values could vary for the different locations over the ten-year sampling period, driven by underlying geological make-up or hydrological influences, causing system baselines to shift.

The main takeaway is that variation in isotopic values could be seen both within and among groups and could be the result of multiple influences on individuals.

The bivariate niche widths also showed a mixture of patterns with some locations having high niche overlap and others having limited overlap (Figures 7, 8, 9; Table 3). The Coastal group had the highest estimated ellipse area over all three biplots suggesting it was the group with the largest niche and highest in group variation of isotopic values. The Coastal group also had the most overlap with other locations, including the complete overlap with the Colorado River group in $\delta^{15}N$ and $\delta^{13}C$ values. The Wastewater group consistently had the narrowest niche widths in bivariate isotope space across all biplots with no overlap with other groups. These results suggest that, for the most part, the groups occupy a fairly similar niche breadth across the locations, save the extreme high and low values of the Coastal and Wastewater groups. These could be driven by the dietary variation within specific systems. American Eel is a generalist feeder and will consume any prey item it is able to, which will then be reflected isotopically in the eel. Bouchereau et al. (2009) detailed prey numbers and frequency of occurrence in stomachs of European Eel, noticing that diets shifted seasonally and with the abundance of particular prey items; namely fish were present more in autumn and spring, and stomachs sampled in the summer months had the highest diversity of prey. Another study on feeding selectivity of American Eel reported that of the 325 eels sampled 171 had contents and the number of organisms in a stomach ranged from 1 - 145 (average of 17.6) while the number of taxa ranged from 1 – 12 (average of 4.4; Denoncourt & Stauffer Jr. 1993). Riera et al. (2000) provided $\delta^{13}C$ and $\delta^{15}N$ ranges of POM from the Nueces River to Nueces Bay and further to Aransas Pass. The river values of $\delta^{13}C$ and $\delta^{15}N$ were -28.8‰ - -26.3‰ and 8.8‰ – 10.8‰, marsh values of $\delta^{13}C$ and $\delta^{15}N$ were -26.3‰ - -17.1‰ and 2.6‰ – 9.3‰. Isotopic values of $\delta^{13}C$

and $\delta^{15}N$ in Nueces Bay were -27.2‰ - -20.8‰ and 9.2‰ – 9.4‰, while Aransas Pass values of $\delta^{13}C$ and $\delta^{15}N$ were -24.8‰ - -22.0‰ and 3.0‰ – 11.1‰. The $\delta^{13}C$ values for group N ranged from -27‰ - -22‰ while the $\delta^{15}N$ values ranged from 14‰ - 17‰. These isotopic values most resemble the POM values of Nueces River, especially considering the estimated trophic position for American Eel is 4.0 (with a slightly lower position (2.2) for individuals caught in restricted systems) and the discrimination factor for $\delta^{15}N$ was calculated as 1.2 (Eberhardt et al. 2017). Expanding upon the dietary variation within a system, baseline and primary producer values may be influenced by nutrient loading as a result of proximity to agricultural, industrial, or urban centers which has been shown to increase $\delta^{15}N$ values.

If recent salinity experience was the main driver of tissue stable isotope values, we would generally expect to see increased $\delta^{13}C$ and $\delta^{34}S$ values and decreased $\delta^{15}N$ values in individuals caught in brackish or marine waters, or in freshwater-caught individuals that recently immigrated from high salinity habitats (Fry 2002). When analyzing SIA values of suspended POM for Matagorda Bay, Aguilar (2017) reported an increasing linear trend in $\delta^{13}C$ values from -26.1‰ to -25.0‰ with a salinity range from 14 – 28. $\delta^{15}N$ had a negative relationship in Matagorda Bay with values ranging from 8.5‰ to 6.1‰ over the same salinity range. Similarly, when analyzing SIA values of suspended POM in Aransas and San Antonio Bays, Bishop et al. (2017) reported an increasing linear trend in $\delta^{13}C$ from -28.3‰ at salinity of 2.5 to -21.2‰ at 34 for samples collected in August 2010. Values for $\delta^{15}N$ ranged from 4.4‰ - 11.2‰ over the same salinity range. Bishop et al. (2017) further analyzed time-integrated oyster muscle tissue, turnover rate of approximately 1 year, and noticed similar results to the suspended POM with corresponding August 2010 $\delta^{13}C$ values ranging from ~ -25.0‰ to -17.5‰ over the same salinity gradient. While these values don't necessarily describe migratory plasticity of individuals, it

does show enrichment of isotopic values from a primary producer to primary consumer over a salinity gradient.

Linear regression models comparing stable isotopes were run to assess variation of individuals within locations. The expectation for relationships between isotope values, assuming habitat residence is the main driver, is that $\delta^{13}C$ and $\delta^{34}S$ will have a positive relationship, while $\delta^{15}N$ and $\delta^{13}C$, and $\delta^{13}C$, will have a negative relationship. The Nueces River was the only group that displayed significant differences when comparing $\delta^{13}C$ against $\delta^{15}N$, $\delta^{13}C$ against $\delta^{34}S$, and $\delta^{15}N$ against $\delta^{34}S$. Values of $\delta^{13}C$ and $\delta^{15}N$ had a negative relationship, which is expected if individuals are shifting to a marine environment. Groups varied in the ranges of isotopic values with G-L having ~10‰ and C having ~2.5‰ (second only to the WW group) variation in $\delta^{13}C$ values. Values of $\delta^{15}N$ ranged in the COAST and B-SJ groups by 5‰ and C and G-L groups by 2.5‰. The COAST group had $\delta^{34}S$ values consisting of a 9‰ range while the N group only varied by 1.5‰. The magnitude of differences between individuals mirrors observations from previous work in regard to isotopic values across a salinity gradient. Specifically, that Bishop et al. (2017) reported a ~7‰ range in $\delta^{13}C$ and $\delta^{15}N$ values of POM across a salinity gradient in Texas. Values of $\delta^{34}S$ drastically increase until salinity of 5 before leveling off at a constant 21‰. One individual, thought to have been caught in a marine environment, had the highest $\delta^{34}S$ values of the entire collection at over 14‰. This is under the 21‰ threshold seen in marine $\delta^{34}S$ values but may not discount the individual from spending a lot of time in saline environments. Much of American Eel diet is found in the benthos or lower bounds of the water column. Values of $\delta^{34}S$ are typically lower in benthic food webs compared to higher in the water column. Fry and Chumchal (2011) noted a ~1‰ - 2‰ variation in species specific $\delta^{34}S$ values based on consumption of benthic diets in estuarine and marine environments. These results

could suggest that some individuals may have recently migrated between marine and fresh environments, but more analyses need to be performed to confirm the results. Future work could include the application of otolith microchemistry as a tool to reconstruct migration history across salinity gradients. This method is widely used and works well in concert with SIA to delineate habitat shifts.

CONCLUSION

The goal of this project was to fill a knowledge gap of habitat use of GOM eels in Texas and contribute to the larger project focused on American Eel ecology and population dynamics in a lesser-known region of their range. Muscle tissue stable isotope analysis of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ have become more widely used in reconstructing migration history and habitat use in aquatic organisms. The results of the analyses provide strong evidence of high individual variability within groups and high variability between groups suggesting that American eel in Texas use different habitats.

Future directions for the project, and a passing of the torch for this study, could include muscle tissue compound specific stable isotope analysis (CSIA) to assess diet quality and better describe American Eel occupied trophic position (Whiteman et al. 2019). A study focused on eels in a specific river system, all collected during the same season/year, may provide a more detailed look into individual variability within the system. The inclusion of primary producers and other consumers would provide a more detailed look into the trophic dynamics of the community. Thirdly, Mueller et al. (2019) detail the use of a specialized acoustic transmitter

targeting juvenile American Eel. This would be crucial in detailing movements and shifts in specific habitats and how movements are affected by structures.

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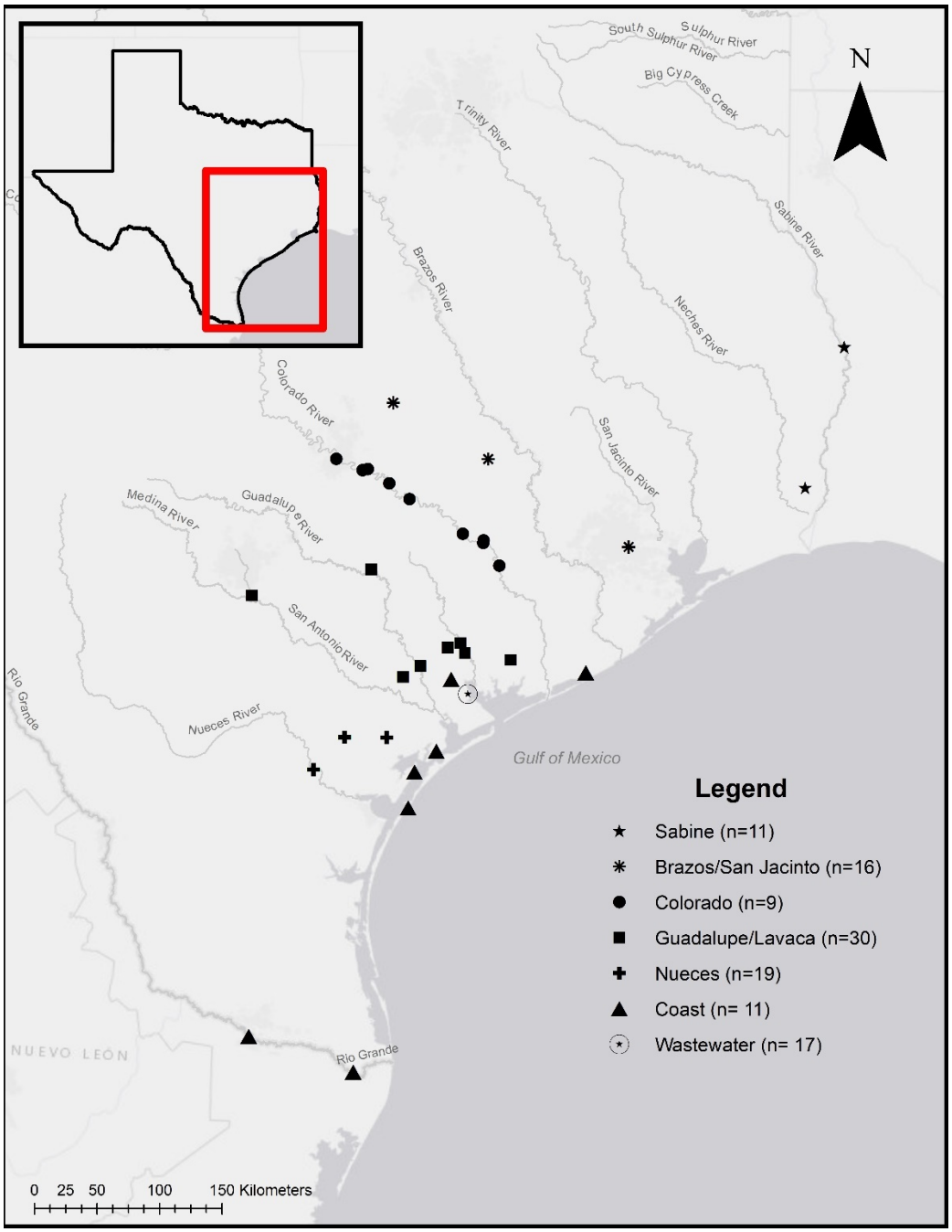
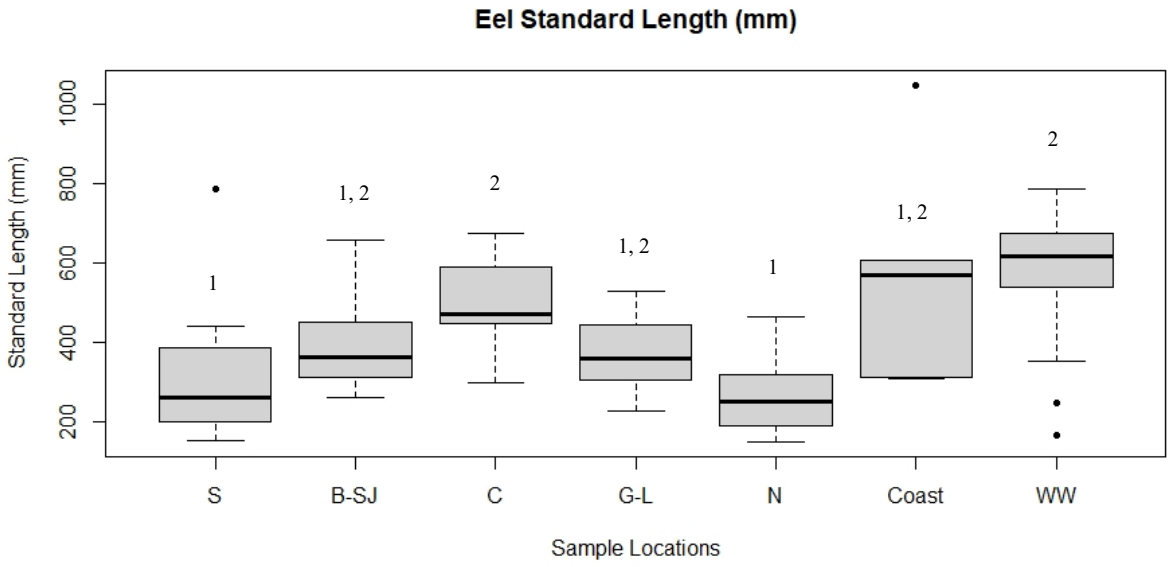


Figure 1: Sampling location of American Eel in Texas.



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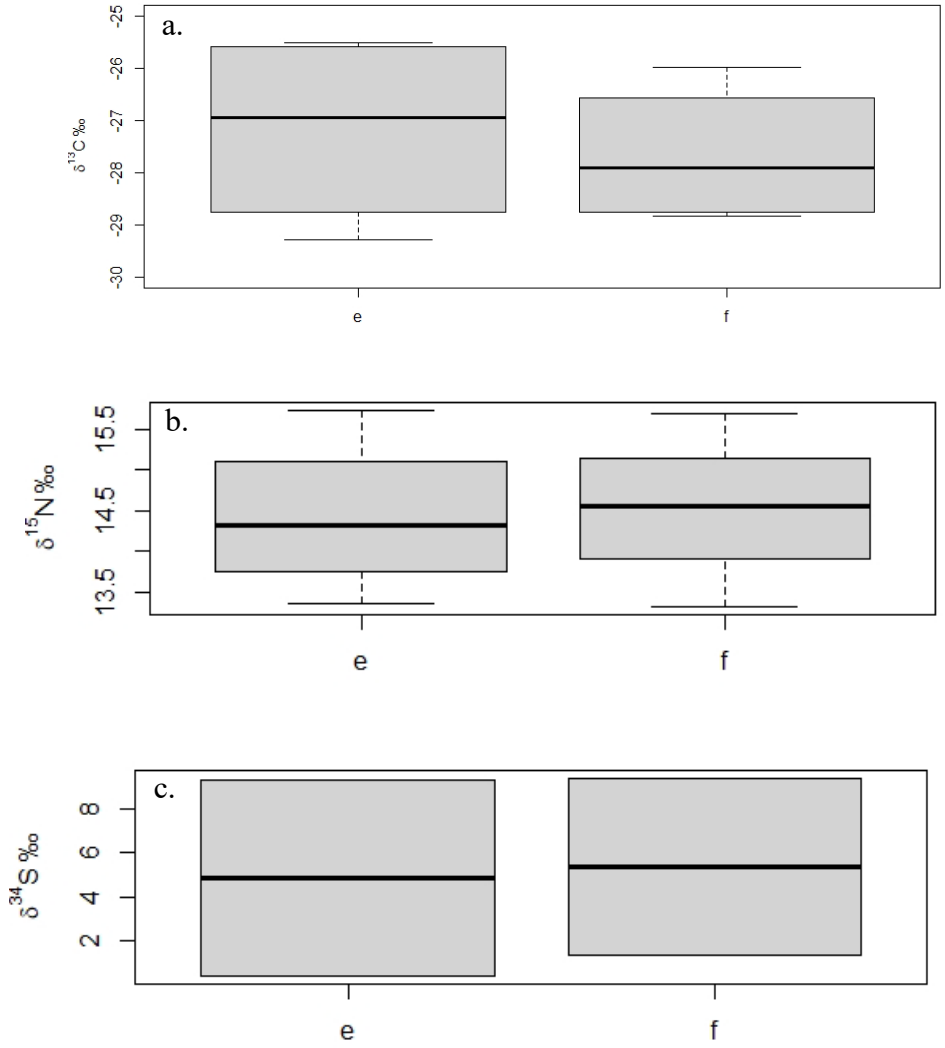

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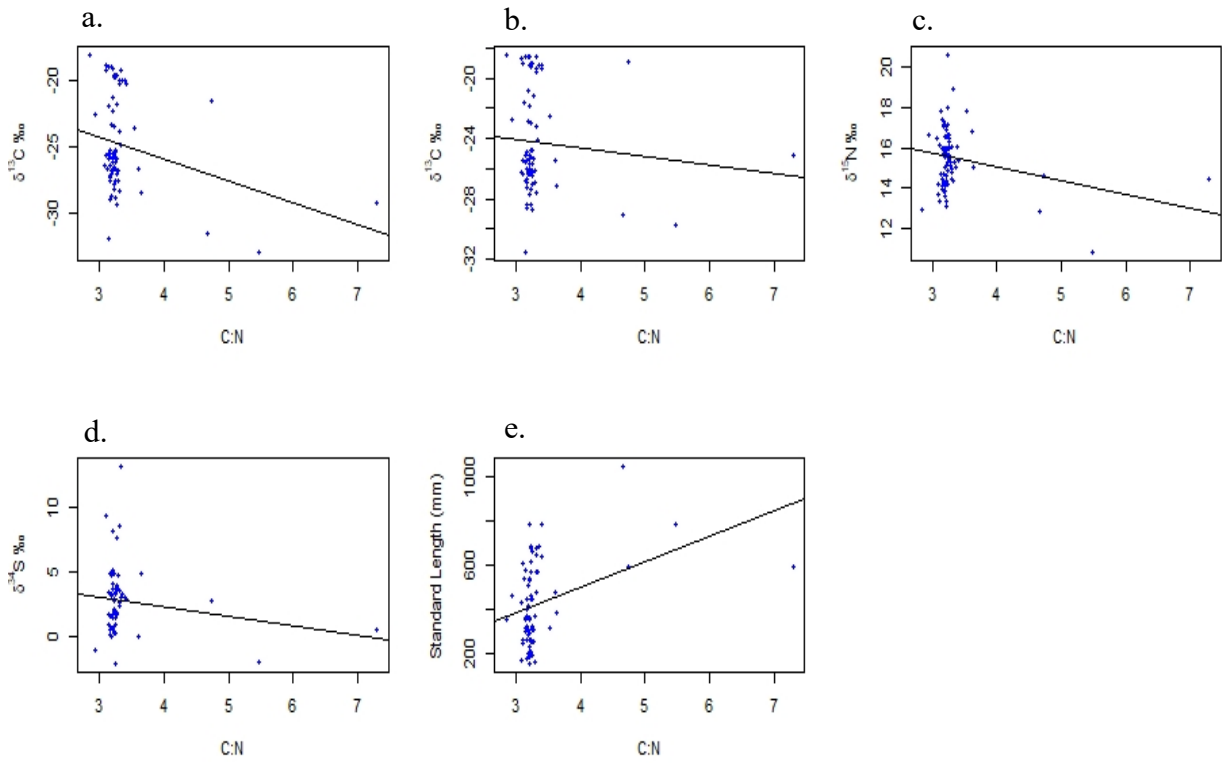

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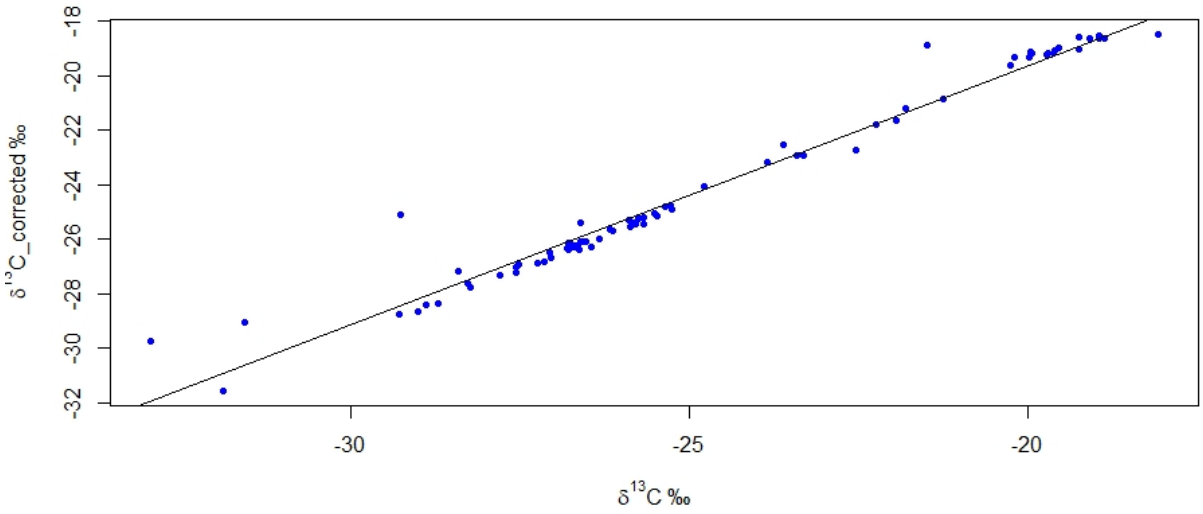

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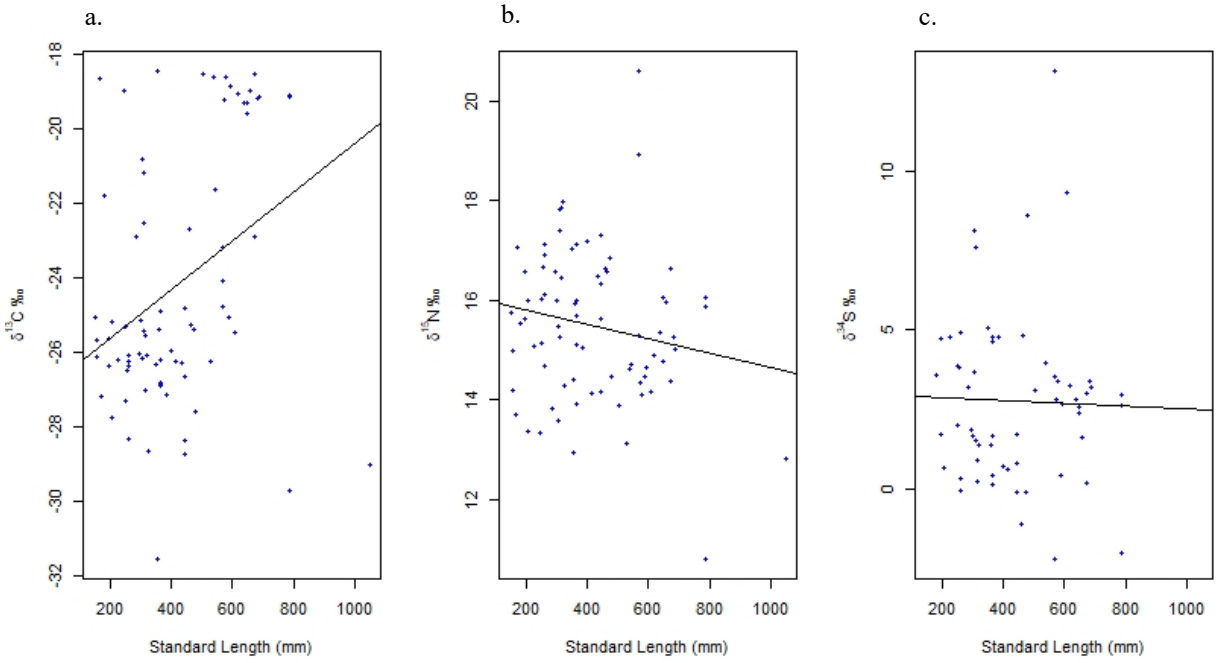

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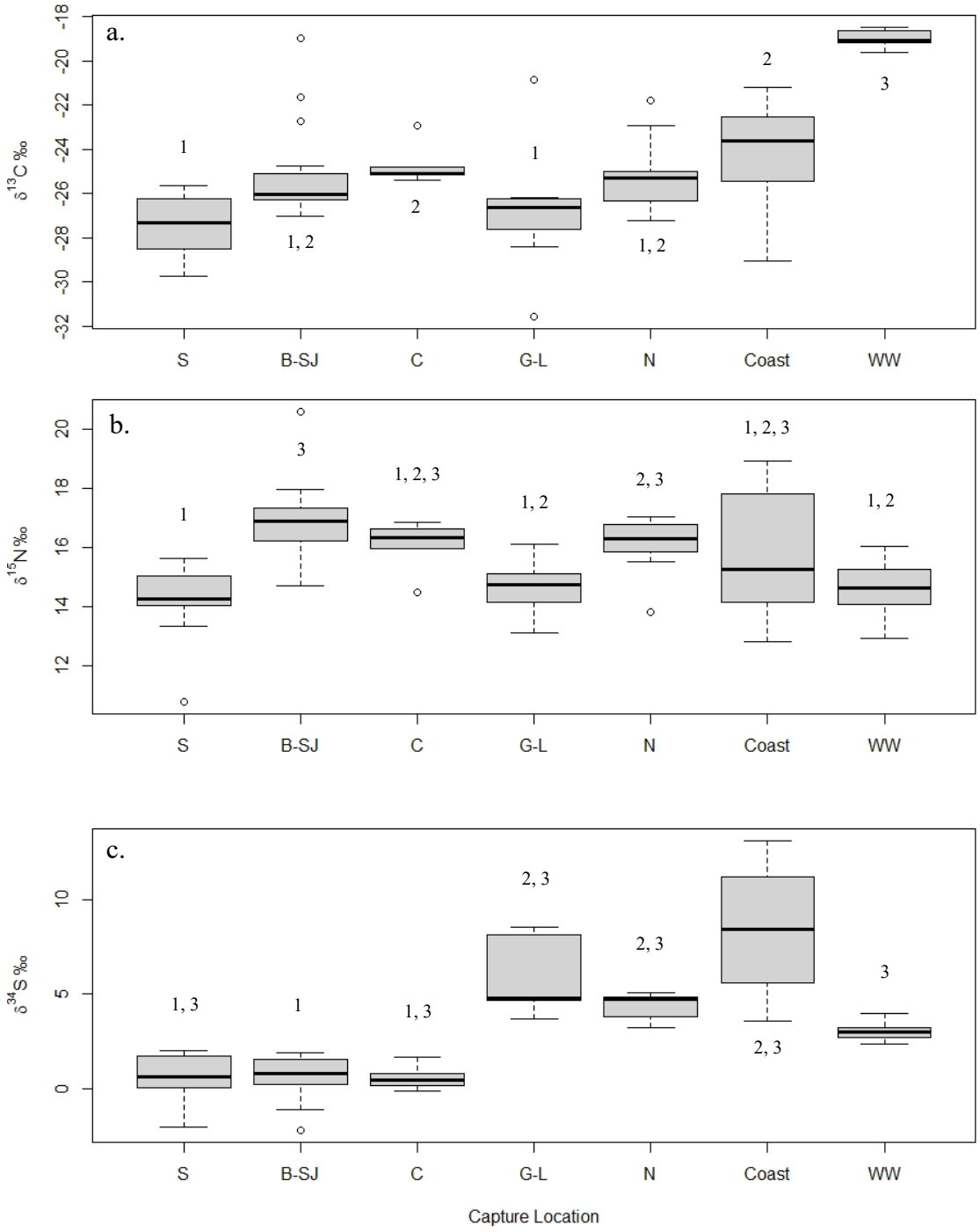

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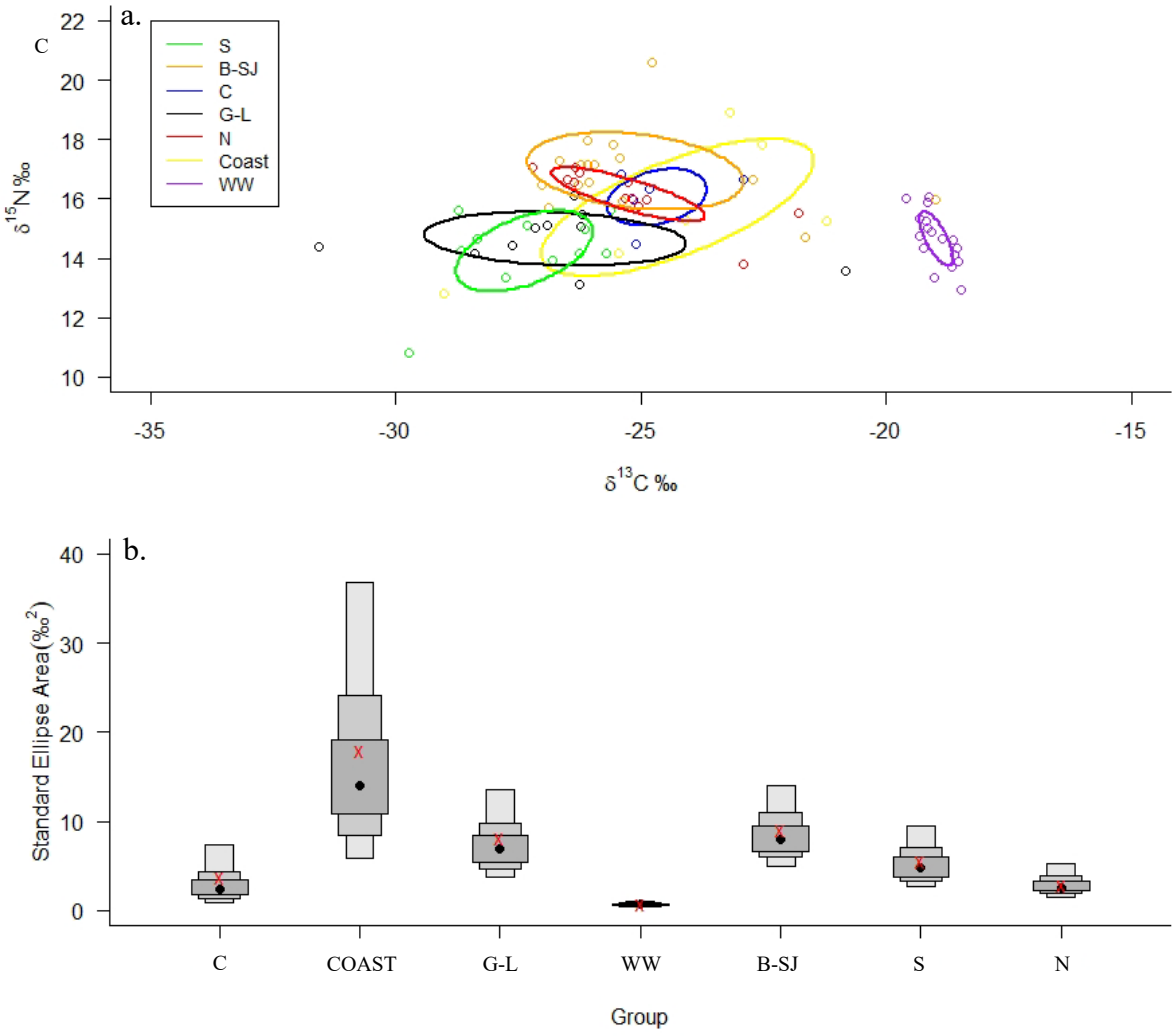

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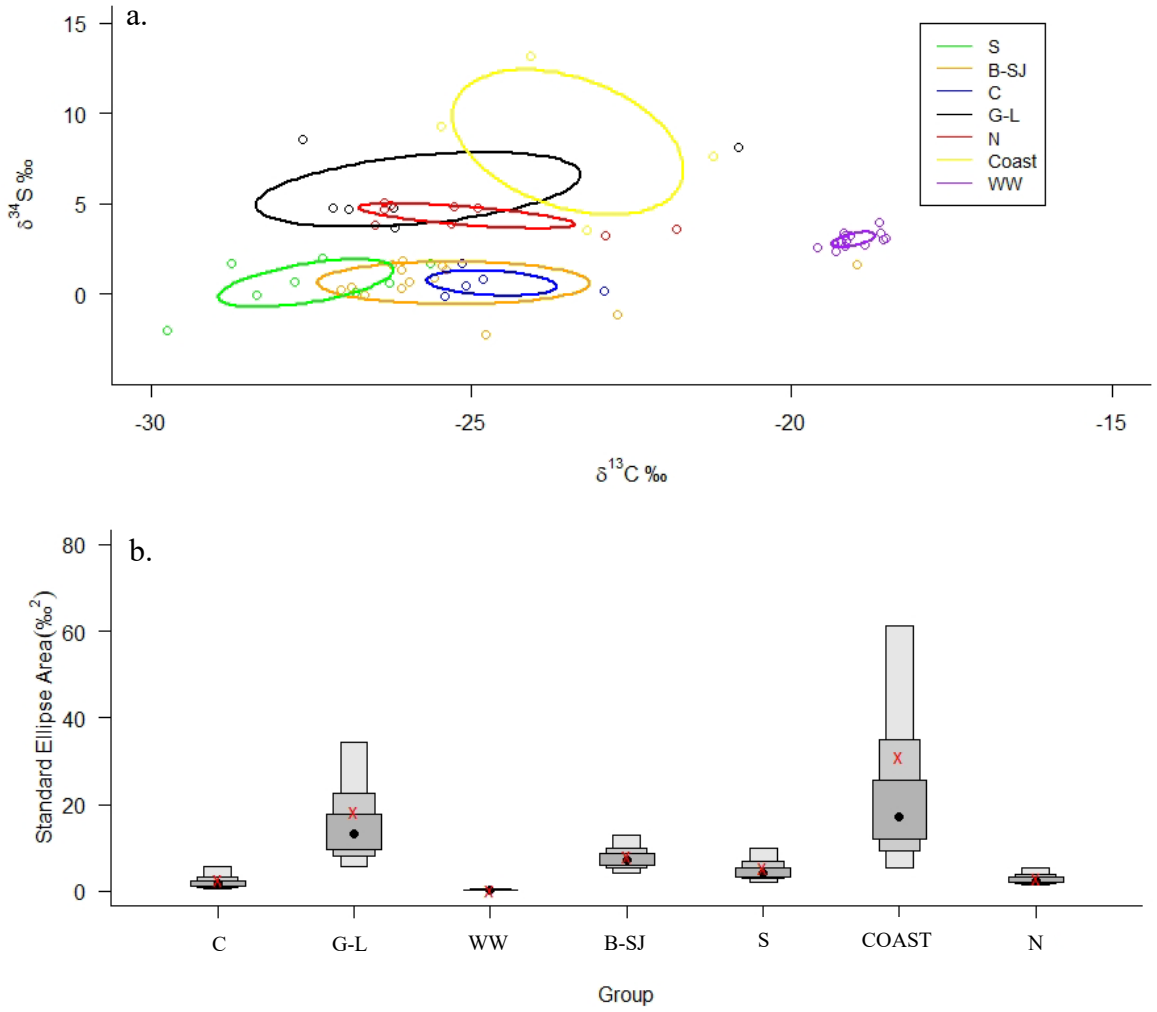

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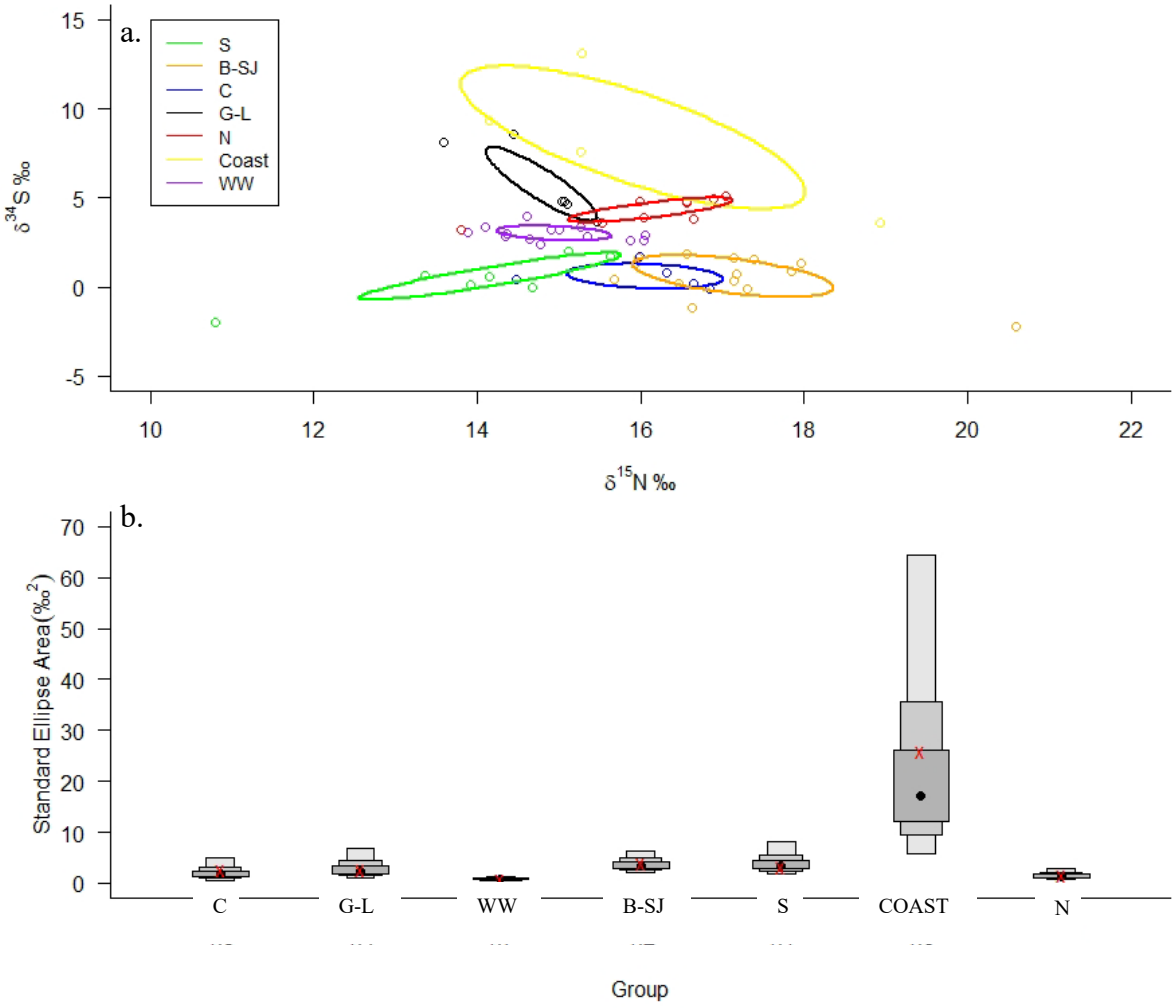

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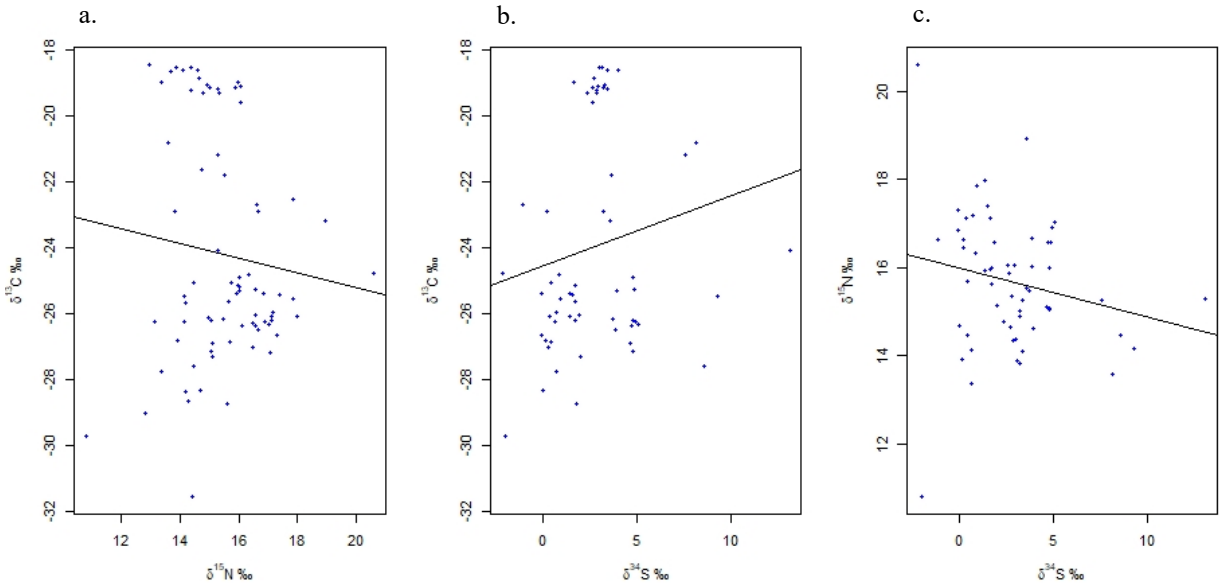

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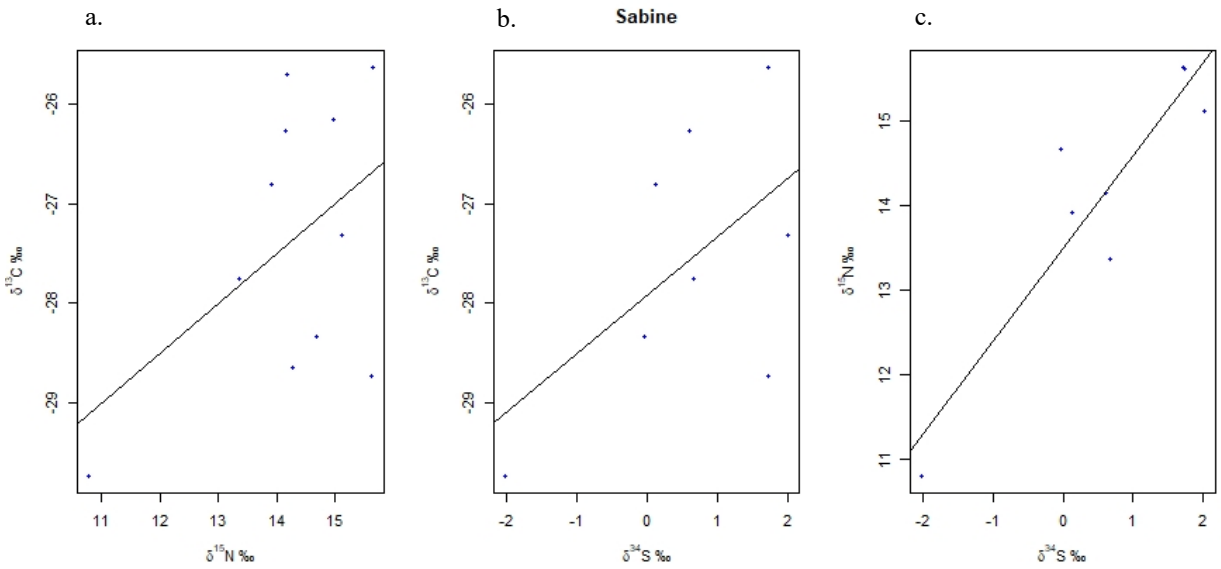

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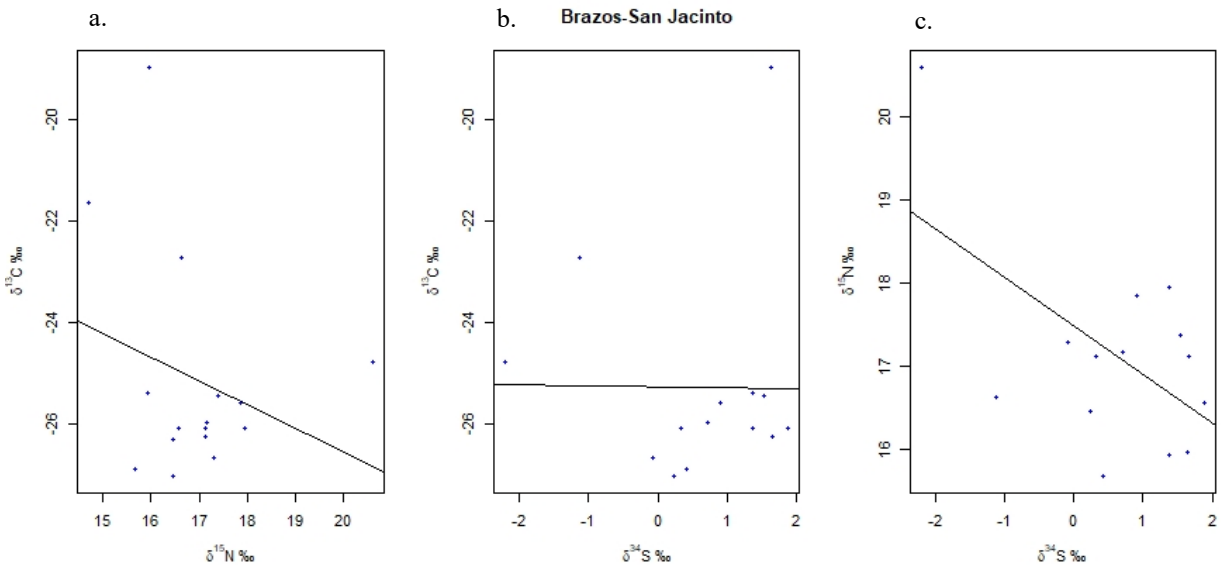

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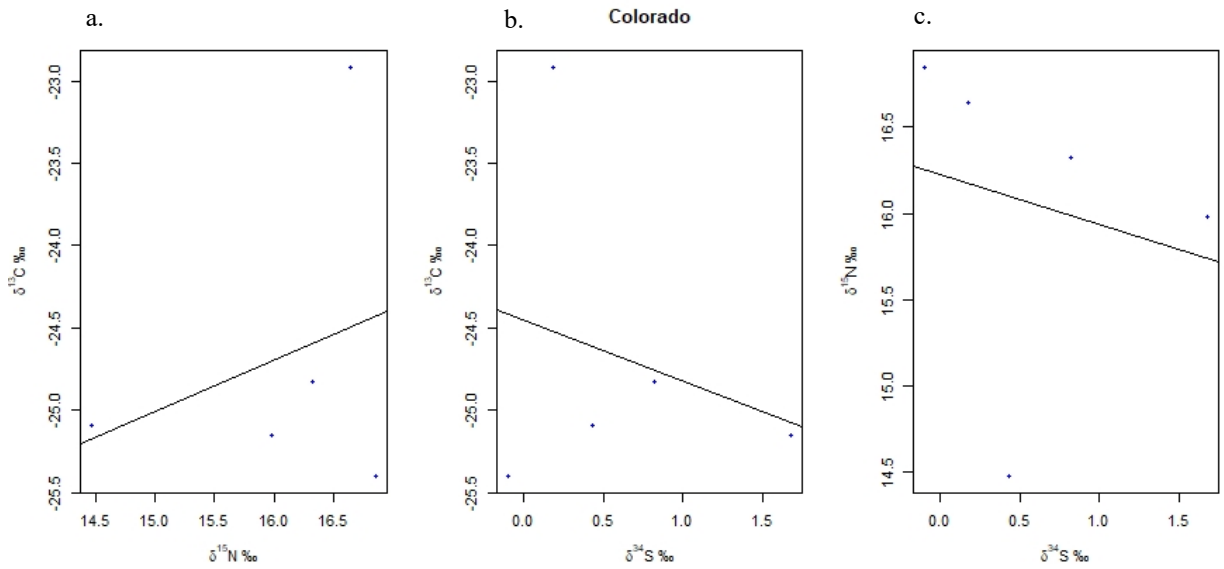

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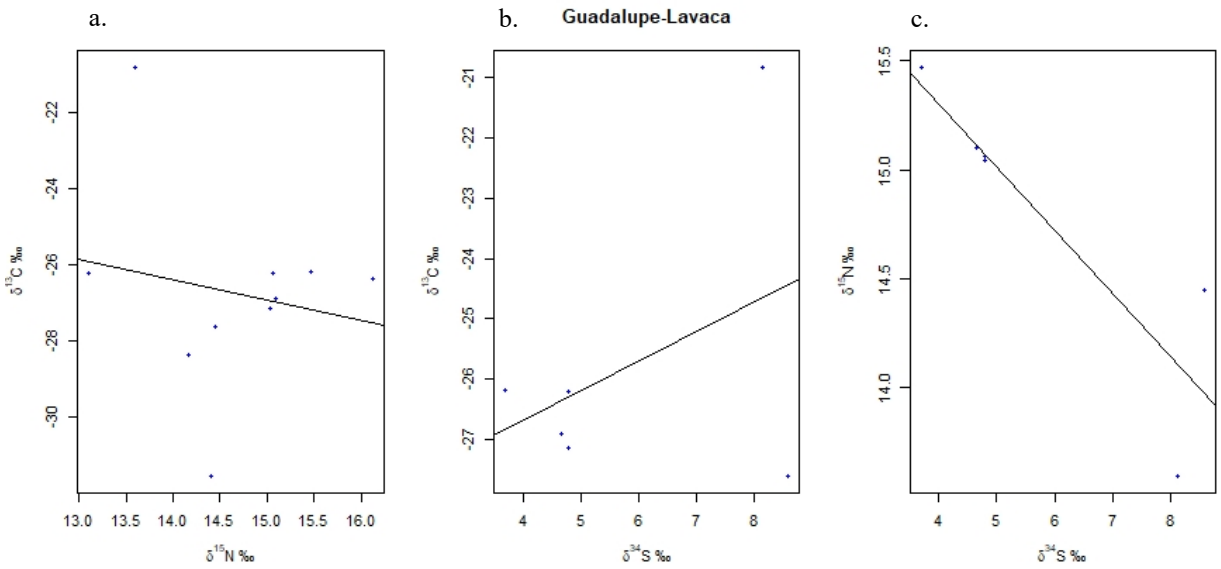

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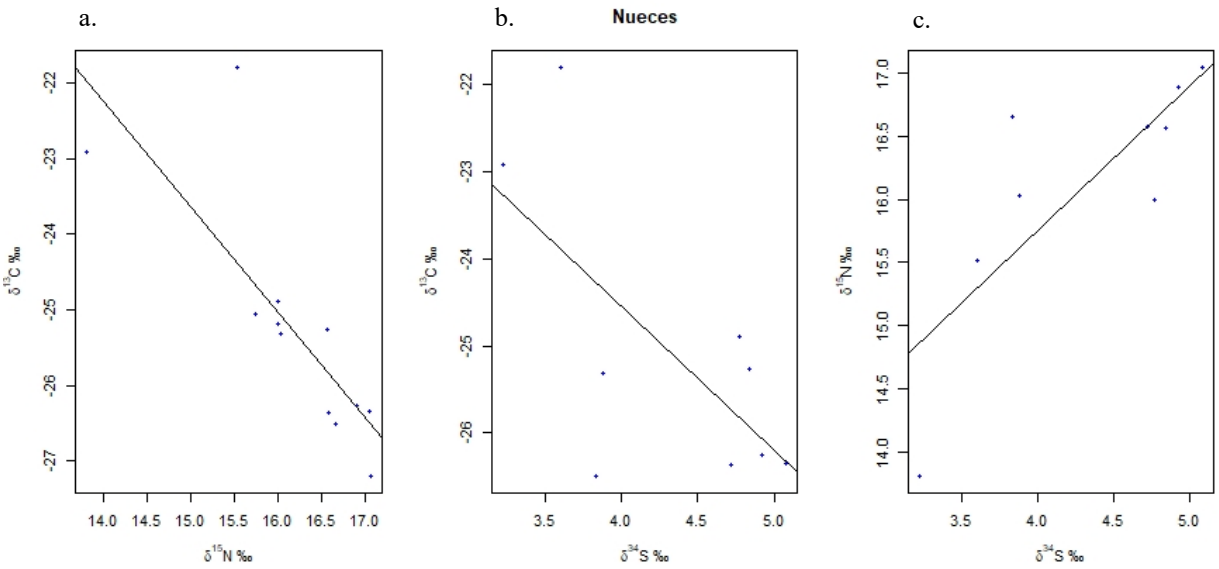

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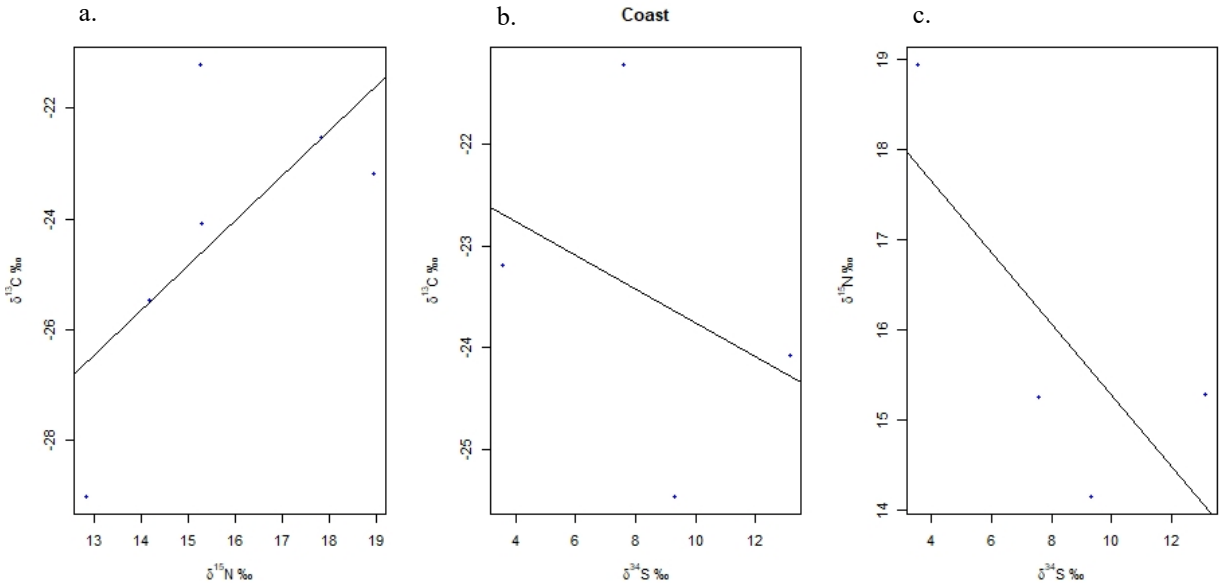

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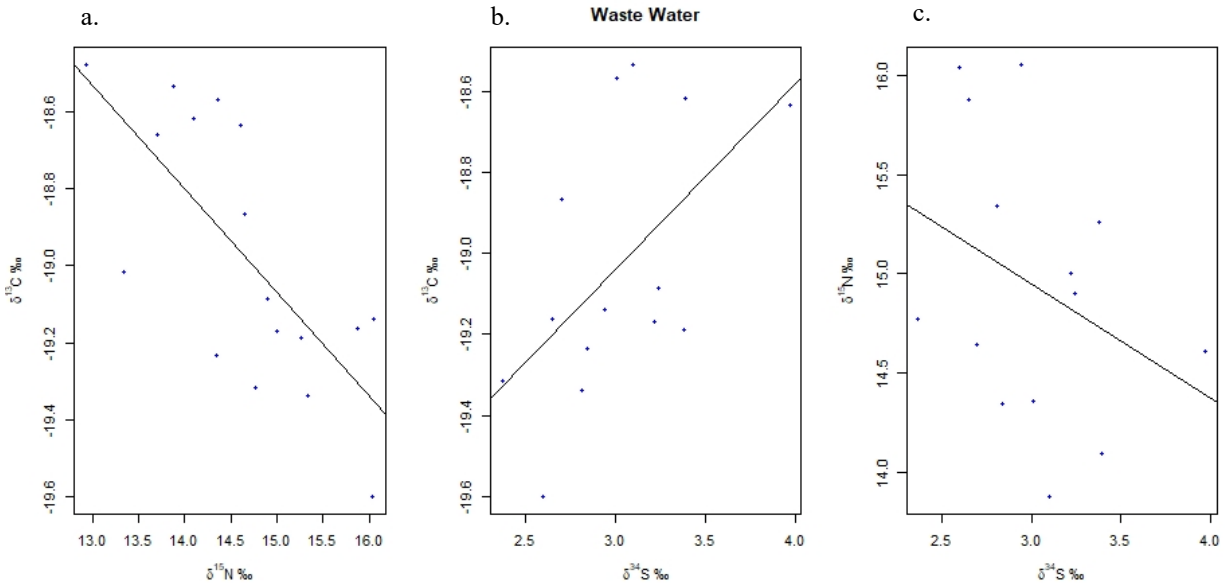

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Location	Total *n* (*n* for $\delta^{34}S$)	SL (mm)	$\delta^{13}C‰$	$\delta^{15}N‰$	$\delta^{34}S‰$
S	11 (8)	155 - 786 (324.0 ± 182.3)	-29.7 – -25.6 (-27.4 ± 1.4)	10.8 – 15.1 (14.2 ± 1.3)	-2.0 – 2.0 (0.6 ± 1.3)
B-SJ	16 (14)	262 - 659 (401.1 ± 111.0)	-27.0 – -19.0 (-25.1 ± 2.2)	14.7 – 20.6 (16.9 ± 1.3)	-2.2 – 1.9 (0.6 ± 1.2)
C	5 (5)	301 - 674 (497.0 ± 142.7)	-25.4 – -22.9 (-24.7 ± 1.0)	14.5 – 16.8 (16.1 ± 0.9)	-0.1 – 1.7 (0.6 ± 0.7)
G-L	10 (6)	229 - 530 (366.2 ± 95.9)	-31.6 – -20.8 (-26.7 ± 2.6)	13.1 – 16.1 (14.7 ± 0.9)	3.7 – 8.5 (5.8 ± 2.0)
N	12 (9)	151 - 164 (262.5 ± 92.6)	-27.2 – -21.8 (-25.3 ± 1.5)	13.8 – 17.1 (16.2 ± 0.9)	3.2 – 5.1 (4.3 ± 0.7)
COAST	6 (4)	309 - 1047 (569.0 ± 269.4)	-29.0 – -21.2 (-24.3 ± 2.7)	12.8 – 18.9 (15.7 ± 2.3)	3.6 – 13.1 (8.4 ± 4.0)
WW	17 (14)	167 - 787 (571.6 ± 170.8)	-19.6 – -18.5 (-19.0 ± 0.3)	12.9 – 16.1 (14.7 ± 0.9)	2.4 – 4.0 (3.0 ± 0.4)

Table 2: Bayesian niche overlap between groups in ‰^{2}

		S	B-SJ	C	G-L	N	COAST	WW
$\delta^{15}N \sim \delta^{13}C$	S	-	0.0	0.0	3.8	0.0	2.2	0.0
	B-SJ	0.0	-	2.8	0.0	2.4	4.7	0.0
	C	0.0	2.8	-	0.3	1.7	3.9	0.0
	G-L	3.8	0.0	0.3	-	0.0	4.9	0.0
	N	0.0	2.4	1.7	0.0	-	2.1	0.0
	COAST	2.2	4.7	3.9	4.9	2.1	-	0.0
	WW	0.0	0.0	0.0	0.0	0.0	0.0	-
$\delta^{34}S \sim \delta^{13}C$	S	-	1.6	0.0	0.0	0.0	0.0	0.0
	B-SJ	1.6	-	2.9	0.0	0.0	0.0	0.0
	C	0.0	2.9	-	0.0	0.0	0.0	0.0
	G-L	0.0	0.0	0.0	-	1.9	5.5	0.0
	N	0.0	0.0	0.0	1.9	-	0.2	0.0
	COAST	0.0	0.0	0.0	5.5	0.2	-	0.0
	WW	0.0	0.0	0.0	0.0	0.0	0.0	-
$\delta^{34}S \sim \delta^{15}N$	S	-	0.0	0.1	0.0	0.0	0.0	0.0
	B-SJ	0.0	-	0.3	0.0	0.0	0.0	0.0
	C	0.1	1.2	-	0.0	0.0	0.0	0.0
	G-L	0.0	0.0	0.0	-	0.2	2.0	0.0
	N	0.0	0.0	0.0	0.2	-	0.5	0.0
	COAST	0.0	0.0	0.0	2.0	0.5	-	0.0
	WW	0.0	0.0	0.0	0.0	0.0	0.0	-

Table 3: Results of linear regression models across all samples and by group locations.

Location	LM	Slope	y-intercept	*p*-value	AR^2
Global	$\delta^{13}C \sim \delta^{15}N$	-0.2238	-20.7572	0.3864	0.00
	$\delta^{13}C \sim \delta^{34}S$	0.2116	-24.5648	0.1837	0.01
	$\delta^{15}N \sim \delta^{34}S$	-0.11015	-15.96853	0.1265	0.02
S	$\delta^{13}C \sim \delta^{15}N$	0.5041	-34.5513	0.1242	0.16
	$\delta^{13}C \sim \delta^{34}S$	0.5844	-27.9251	0.1429	0.21
	$\delta^{15}N \sim \delta^{34}S$	1.0995	13.4944	0.0017	0.80
B-SJ	$\delta^{13}C \sim \delta^{15}N$	-0.4654	-17.2306	0.3033	0.01
	$\delta^{13}C \sim \delta^{34}S$	-0.01712	-25.26517	0.9744	-0.08
	$\delta^{15}N \sim \delta^{34}S$	-0.5820	17.4853	0.0390	0.25
C	$\delta^{13}C \sim \delta^{15}N$	0.3126	-29.6935	0.6325	-0.22
	$\delta^{13}C \sim \delta^{34}S$	-0.3677	-24.4547	0.6812	-0.25
	$\delta^{15}N \sim \delta^{34}S$	-0.2894	16.2262	0.7313	-0.27
G-L	$\delta^{13}C \sim \delta^{15}N$	-0.5387	-18.8555	0.6140	-0.09
	$\delta^{13}C \sim \delta^{34}S$	0.4964	-28.6861	0.4255	-0.04
	$\delta^{15}N \sim \delta^{34}S$	-0.28936	16.45604	0.0200	0.72
N	$\delta^{13}C \sim \delta^{15}N$	-1.396	-2.707	0.0014	0.62
	$\delta^{13}C \sim \delta^{34}S$	-1.6462	-17.9653	0.046	0.38
	$\delta^{15}N \sim \delta^{34}S$	1.1425	11.1848	0.0116	0.57
COAST	$\delta^{13}C \sim \delta^{15}N$	0.8192	-37.1246	0.1361	0.33
	$\delta^{13}C \sim \delta^{34}S$	-0.1664	-22.0914	0.6290	-0.29
	$\delta^{15}N \sim \delta^{34}S$	-0.3942	19.2154	0.2493	0.35
WW	$\delta^{13}C \sim \delta^{15}N$	-0.26976	-15.02333	0.0011	0.49
	$\delta^{13}C \sim \delta^{34}S$	0.4571	-20.4108	0.0354	0.26
	$\delta^{15}N \sim \delta^{34}S$	-0.5742	16.6730	0.2379	0.04

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Appendix 1: Supplemental Isotope Information

Table A: Individual ID, Capture Location, Standard Length, corrected $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of all American Eel individuals used for this study (Hendrickson et al. 2021). Values shown are for samples preserved in ethanol only.

TNHCi	Capture Location	Standard Length (mm)	$\delta^{13}C$‰	$\delta^{15}N$‰	$\delta^{34}S$‰
64851	C	447	-24.8	16.3	0.8
64852	COAST	313	-22.5	17.8	NA
64853	G-L	229	-26.2	15.1	4.8
64854	G-L	262	-26.4	16.1	NA
64855	G-L	530	-26.2	13.1	NA
64856	WW	646	-19.3	14.8	2.4
64857	WW	787	-19.2	15.9	2.7
64858	WW	683	-19.2	15.3	3.4
64859	WW	784	-19.1	16.1	2.9
64860	WW	638	-19.3	15.3	2.8
64861	WW	649	-19.6	16.0	2.6
64862	WW	674	-18.6	14.4	3.0
64863	WW	616	-19.1	14.9	3.2
64864	WW	576	-18.6	14.1	3.4
64865	WW	538	-18.6	14.6	4.0
64866	WW	685	-19.2	15.0	3.2
64867	WW	506	-18.5	13.9	3.1
64868	WW	572	-19.2	14.4	2.8
64869	WW	354	-18.5	12.9	NA
64870	WW	248	-19.0	13.3	NA
64871	WW	167	-18.7	13.7	NA
64872	B-SJ	567	-24.8	20.6	-2.2
64873	B-SJ	542	-21.6	14.7	NA
64874	B-SJ	461	-22.7	16.6	-1.1
64875	B-SJ	434	-26.3	16.5	NA
64876	B-SJ	659	-19.0	16.0	1.6
65229	COAST	1047	-29.0	12.8	NA
68436	S	443	-28.7	15.6	1.7
68437	S	364	-26.8	13.9	0.1
68438	S	414	-26.3	14.1	0.6
68439	S	263	-28.3	14.7	-0.0
68440	S	325	-28.6	14.3	NA
68441	S	253	-27.3	15.1	2.0
68442	S	195	-25.6	15.6	1.7

68443	S	207	-27.8	13.4	0.7
68444	S	155	-25.7	14.2	NA
68445	S	159	-26.2	15.0	NA
68446	G-L	385	-27.2	15.0	4.8
68447	G-L	367	-26.9	15.1	4.7
68495	C	301	-25.1	16.0	1.7
68496	COAST	606	-25.5	14.2	9.3
68497	G-L	354	-31.6	14.4	NA
68498	G-L	478	-27.6	14.5	8.6
68499	G-L	306	-20.8	13.6	8.1
68500	COAST	570	-23.2	18.9	3.6
68501	G-L	307	-26.2	15.5	3.7
68503	C	674	-22.9	16.6	0.2
68504	G-L	444	-28.4	14.2	NA
69316	N	151	-25.1	15.7	NA
69317	N	173	-27.2	17.1	NA
69318	N	184	-21.8	15.5	3.6
69319	N	197	-26.4	16.6	4.7
69320	N	208	-25.2	16.0	NA
69321	N	353	-26.3	17.0	5.1
69322	N	255	-26.5	16.7	3.8
69323	N	285	-22.9	13.8	3.2
69324	N	250	-25.3	16.0	3.9
69325	N	464	-25.3	16.6	4.8
69326	N	263	-26.3	16.9	4.9
69327	N	367	-24.9	16.0	4.8
69328	B-SJ	315	-27.0	16.5	0.2
69329	B-SJ	314	-25.6	17.9	0.9
69330	B-SJ	445	-26.7	17.3	-0.1
69331	B-SJ	364	-26.2	17.1	1.7
69332	B-SJ	295	-26.1	16.6	1.9
69333	B-SJ	366	-26.9	15.7	0.4
69334	B-SJ	321	-26.1	18.0	1.4
69335	B-SJ	311	-25.4	17.4	1.5
69336	B-SJ	262	-26.1	17.1	0.3
69337	B-SJ	400	-26.0	17.2	0.7
69338	B-SJ	362	-25.4	15.9	1.4
72219	COAST	309	-21.2	15.3	7.6
72226	C	473	-25.4	16.8	-0.1
72227	C	590	-25.1	14.5	0.4
72228	COAST	569	-24.1	15.3	13.1

72229	S	786	-29.7	10.8	-2.0
72230	WW	595	-18.9	14.6	2.7