Clemson University
TigerPrints

All Theses Theses

August 2020

Assessing Residency Patterns and Trophic Ecology of Southern Flounder in Alabama's Coastal Waters

Jared Chrisp
Clemson University, jared.chrisp@gmail.com

Follow this and additional works at: https://tigerprints.clemson.edu/all_theses

Recommended Citation

Chrisp, Jared, "Assessing Residency Patterns and Trophic Ecology of Southern Flounder in Alabama's Coastal Waters" (2020). *All Theses*. 3377.
https://tigerprints.clemson.edu/all_theses/3377

This Thesis is brought to you for free and open access by the Theses at TigerPrints. It has been accepted for inclusion in All Theses by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.

ASSESSING RESIDENCY PATTERNS AND TROPHIC ECOLOGY OF SOUTHERN FLOUNDER IN ALABAMA'S COASTAL WATERS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

---

by
Jared Keith Chrisp
August 2020

Accepted by:
Dr. Troy Farmer, Committee Chair
Dr. Brandon Peoples
Dr. Dana Sackett

ABSTRACT

Southern Flounder (*Paralichthys lethostigma*) is an economically important species along the northern Gulf of Mexico. Over the last several years, Southern Flounder populations have experienced drastic declines. Analysis of natural tags, such as otolith chemistry and stable isotopes, can be used to examine habitat-specific contributions to commercial and recreational fisheries. A better understanding of habitat-use patterns and food web dynamics of this species could provide insight into habitat conservation and harvest regulations to promote sustainability of this species.

Water and otolith chemistry were used to quantify the proportional contributions of various residency patterns to the commercial and recreational harvest of historic (2004 – 2007) and recent (2018 – 2019) Southern Flounder populations. Otolith strontium to calcium (Sr:Ca) values from laser ablation inductively coupled plasma mass spectrometry were used to quantify age-specific and lifetime residency patterns for Southern Flounder across Alabama's seasonal salinity gradient. Flounder were classified into one of three contingent types: freshwater, estuarine, or transient. Our results suggest that contributions to the commercial and recreational fisheries were predominately from estuarine habitats, and freshwater habitats were important during the settlement phase. Specifically, 3% of commercially and recreationally harvested flounder were lifetime freshwater contingents, but 57% utilized freshwater during the first year of life.

We used bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes, compound specific $\delta^{15}N$ isotopes (AA-CSIA), and stomach content analysis (SCA) to determine trophic ecology and food web dynamics of Southern Flounder. We assigned location of harvest for

commercially and recreationally harvested flounder using $\delta^{13}C$ and $\delta^{15}N$ values from fishery-independent samples. In agreeance with otolith chemistry, isotope analysis results indicated greater contributions to commercial and recreational fisheries from estuarine habitats than freshwater habitats. Additionally, flounder harvested in lower portions of Mobile Bay appear to be consuming prey at higher trophic levels than other areas along Alabama's coastal waters.

DEDICATION

I dedicate this thesis to my Mom and Dad, Lynette and Brett, for fostering my love for the outdoors and always encouraging me to fulfill my dreams. Also, to my brother, Neil, for being my best friend and supporting me through all of my endeavors.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Troy Farmer, for his tutelage and patience in teaching me how to conduct proper research and scientific writing of fisheries science. You have been an amazing example of a mentor and researcher and I am honored to be one of your first graduate students. Thank you to my committee, Brandon Peoples and Dana Sackett, for your assistance and mentorship in helping me understand project development and statistical analyses. Thank you to Meghan Angelina for supporting me through every aspect of this project by providing constant encouragement and inspiring me as both a scientist and friend. To our field technicians, Mason Collins, Jacob Moreland, and Hannah Mulligan, thank you for all of your hard work and enthusiasm. Lastly, thank you to my friends and the graduate student community who have helped me grow as a person and a scientist.

This projected was funded by the Marine Resources Division of Alabama Department of Conservation and Natural Resources and the Sportfish Restoration Program of the U.S. Fish & Wildlife Service. I would like to thank Kevin Anson, John Mareska, and Craig Newton from MRD for all of your support over these last two years. Also, thank you to Dave Armstrong and Tommy Purcell from Wildlife and Freshwater Fisheries District 5.

This project received extensive assistance in laboratory processing and sample collections. Thank you to Laura Linn and Reid Nelson from Dauphin Island Sea Lab for assisting with otolith chemistry laboratory processing and analyses. Thank you to Crystal Hightower and the Powers Lab for providing samples from fishing tournaments. Thank

you to Natalie Wallsgrove for processing stable isotope samples. Thank you to Matthew Catalano, Dennis DeVries, and Rusty Wright for providing assistance and historic otolith samples. Finally, thank you to the Clemson Creative Inquiry students that helped process hundreds of otoliths and isotope samples.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER 1: SOUTHERN FLOUNDER RESIDENCY PATTERNS ACROSS A SEASONAL SALINITY GRADIENT

Figure Page

LIST OF FIGURES (CONT.)

Figure Page

LIST OF FIGURES (CONT.)

Figure Page

CHAPTER ONE

SOUTHERN FLOUNDER RESIDENCY PATTERNS ACROSS A SEASONAL SALINITY GRADIENT

INTRODUCTION

Estuaries are essential to the ontogenetic development of many recreationally and commercially important fishes. As an interface between freshwater and marine ecosystems, estuaries are highly productive and extremely complex ecosystems that provide nursery habitat to numerous fish species (Beck et al. 2001; Able 2005). Understanding the benefits these habitats provide and the physical and bioenergetic movements of nutrients and organisms across estuarine habitats is essential to developing appropriate management and conservation actions (Nathan et al. 2008). Currently, anthropogenic impacts on estuaries are severely degrading their ecological and economic benefits (Creighton et al. 2015; Baker et al. 2017), by altering habitat and food web dynamics, affecting estuarine fish at various life stages (Courrat et al. 2009; Houde and Rutherford 2016). A better understanding of how fish utilize and benefit from a diverse suite of estuarine habitats is therefore essential to protect critical estuarine habitats and the fisheries that rely on them.

Being a euryhaline, estuarine-dependent species, Southern Flounder (*Paralichthys lethostigma*) rely on estuaries for growth and ontogenetic development. Adult flounder spawn offshore and eggs are carried by tidal currents into estuaries where larvae undergo sinistral, craniofacial metamorphosis and begin settlement (Jager 1999; Schreiber 2006). Numerous abiotic (e.g., salinity, dissolved oxygen, temperature, nutrient flow) and biotic (e.g., prey availability, species competition, predator abundance) factors impact their

survival and distribution (Polis et al. 1997). Previous studies have shown that post-larval juveniles settle in sandy or muddy habitats, generally near vegetation (Burke et al. 1991; Powell and Schwartz 2006; Nañez-James et al. 2009). Larval flounder are tolerant of several environmental parameters, therefore settlement can occur across large salinity (0 – 35 psu), temperature (12 – 39° C), and dissolved oxygen (2.8 – 6.5mg/L) levels (Taylor and Miller 2001; Nañez-James et al. 2009; Furey et al. 2013). As flounder require inshore habitats for survival through juvenile stages and growth to harvestable sizes, their accessibility to commercial and recreational harvest is also dependent on the suitability and quality of these critical inshore habitats.

Southern Flounder sustain economically important recreational and commercial fisheries across their geographic range (Froeschke et al. 2011; Flowers et al. 2019). Over the last several years, this species has seen drastic declines in adult abundance across their entire range (VanderKooy 2015; Flowers et al. 2019). For Alabama in particular, population declines, and the resulting diminished harvest, have resulted in recent landings that are less than a quarter of historic averages (VanderKooy 2015). As a result of the declining adult abundance, increased attention is being focused on ecological factors that may be contributing to these declines. A recently completed stock assessment indicated Alabama's Southern Flounder stock is experiencing a decline in overall abundance most likely due to low recruitment, although overfishing may also play an important role by reducing spawning stock biomass (Powers et al. 2018). As production of new recruits to the adult spawning population is vital for the sustainability of any fishery, quantifying

habitat-specific contributions to the adult population would assist with identifying key habitats that are likely essential in rebuilding Southern Flounder stocks.

While the use of oligohaline and mesohaline waters by flatfish (family Paralichthyidae) species is well documented (Rozas and Hackney 1984; Glass et al. 2008; Nañez-James et al. 2009; Smith and Scharf 2010), recent work indicates age-0 and juvenile flatfish species may use, or even prefer, tidal freshwater habitats (Zucchetta et al. 2010; Lowe et al. 2011). In addition, Farmer et al. (2013) discovered Southern Flounder may migrate to low-salinity estuaries during the first two years of life, which was previously believed to occur only during the first year. Much of this increased understanding of Southern Flounder habitat use across salinity gradients was accomplished through the use of natural tracers, such as otolith chemistry (Lowe et al. 2011; Farmer et al. 2013; Nims and Walther 2014).

Analysis of otolith chemistry is a useful tool in quantifying fish migratory and residency patterns across salinity. Otoliths are acellular and not primarily influenced by metabolic turnover experienced by other tissues (Elsdon et al. 2008). This guarantees permanent encapsulation of trace elements into the otoliths' chemical makeup, resulting in a unique chemical signature throughout the lifetime of each fish (Campana et al. 2000). Otoliths contain diel, geochemical accretions of trace elements which may be more representative of the ambient water chemistry than an individual's diet or physiological condition (Campana 1999; Walther and Limburg 2012). Therefore, a relationship between otoliths and the ambient water chemistry must be developed to fully understand otolith chemistry. Water chemistry is the concentration of trace metals elements known

as endmembers, which occur at variable rates based on geochemical weathering of upstream geological materials within the watershed (Elsdon et al. 2008; Macdonald and Crook 2010; Walther and Limburg 2012). Previous studies have shown potential positive (e.g., magnesium, calcium, strontium) and negative (e.g., barium) relationships between water chemistry endmembers and salinity (Surge and Lohmann 2002; Walther and Limburg 2012). Seasonal fluctuations in freshwater discharge shifts the locality of estuary salinity classifications (i.e., oligohaline (0.5 – 5 psu), mesohaline (5 – 18 psu), polyhaline (18 – 30 psu)), so spatiotemporal variations in water chemistry may need to be addressed (Teichert et al. 2017). Additionally, elemental concentrations in water do not scale exactly with otolith elemental concentrations. Partition coefficients, which describe the proportional incorporation of water chemistry elements into otoliths, can be developed by analyzing elemental signatures along the edge of the otolith concurrently with ambient water chemistry (Nelson and Powers 2019). Elemental partition coefficients can then be used to interpret otolith elemental chronologies into migratory and residency patterns that shed light onto life-history and movement patterns across salinity gradients within an estuary (Macdonald and Crook 2010).

While relationships between the environmental conditions and otolith chemistry are useful and hold considerable promise in interpreting migratory and residency patterns, many limitations exist. One potential limitation is that otolith signatures may be a result of fish migration across salinity gradients or salinity fluctuations over a relatively stationary fish. Estuaries have highly variable freshwater fluctuations in which salinity delineations may move several kilometers within a single year (Lowe and Peterson

2014). As these freshwater fluxes occur, estuaries may experience spatiotemporal variation in water endmember concentrations (Gillanders 2002; Tournois et al. 2013). Fortunately, select elemental endmembers within otolith chemistry scale with salinity (Nelson and Powers 2020). As a result, otolith chemistry can be used as an index of salinity residency and migratory patterns, but exact locality cannot be determined without high-resolution water quality (i.e., salinity) or telemetry for exact locations of interest. Another limitation is that otolith and water elemental concentrations cannot be used alone, but must be considered in a ratio with calcium (i.e., element:Ca). Ratios are necessary due to substitution rates of trace elements for calcium into the $CaCO_3$ matrix of otoliths (Sturrock et al. 2012; Loewen et al. 2016). Calcium can be used as an internal standard for otolith analyses and external standard for water chemistry analyses to offset any elemental concentration issues (Craig et al. 2000; Nelson and Powers 2020).

Our specific objectives to address the essential need for a better understanding of Southern Flounder estuarine habitat use were to 1) use otolith chemistry from fishery-independent and fishery-dependent collections from 2004 – 2007 and 2018 – 2019 to quantify large-scale patterns of habitat use, 2) determine any sex-specific or age-specific differences in habitat-use patterns, and 3) examine which habitat-use patterns contributing to the commercial and recreational Southern Flounder fisheries in Alabama's coastal waters. Ultimately, results from this study aim to inform management and conservation actions for a species currently experiencing population declines across its range.

METHODS

Study system

This study was conducted in the Mobile-Tensaw River Delta (hereafter referred to as the "Delta") and Mobile Bay in Alabama (Figure 1). With an average daily discharge of 1850 m^3/s, the Mobile-Tensaw River system is the fourth largest river system in the contiguous United States (Schroeder et al. 1990, Morisawa 1968). As the primary source of freshwater to Mobile Bay, this system influences the biochemical and hydrographical variations in this estuary (Dzwonkowski et al. 2011). There are three smaller freshwater sources including Dog River (watershed area 237 km^2), Fowl River (watershed area 184 km^2), and Week's Bay (watershed area 521 km^2), which have regional influences on salinity and nutrients in Mobile Bay (Lehrter 2008; Mortazavi et al. 2012). Mobile Bay averages 3 m depth across an area 15-35 km wide and 45-50 km long with a drainage basin of 115,467 km^2 (Dzwonkowski et al. 2011). Seasonal discharge fluctuations determine the spatiotemporal intrusion of salinity into the Delta, with northern reaches only experiencing high salinity during periods of low flow (Noble et al. 1996; Norris et al. 2010; Lee et al. 2019).

Sample collections

Fishery-independent collections

During 2004 – 2007, Southern Flounder were collected by Auburn University from six sites in tidal freshwater to oligohaline habitats located in the northeastern corner of Mobile Bay and up to 32 river km into the Delta (Figure 1). Sampling was conducted monthly using pulsed DC boat electrofishing (Smith-Root, Inc.). Two 15-minute boom

mounted electrofishing transects and three 10-minute prod-pole electrofishing transects were conducted at each site. Complete descriptions of sampling efforts during 2004 – 2007 can be found in Lowe et al. (2011) and Glover et al. (2013). While previous studies (Lowe et al. 2011; Farmer et al. 2013) reported Southern Flounder otolith chemistry results from these collections, we processed archived otolith samples that had not previously been analyzed for otolith chemistry. Results reported here for 2004 – 2007 represent new data, not previously reported in the literature.

During 2018 – 2019, juvenile and adult Southern Flounder were collected by Clemson University from ten sites located along a 60+ km seasonal salinity gradient of saltmarshes, bays, tidal creeks, and freshwater ecosystems. Sites at the lower end of the estuary were located on the landward side of barrier islands and within tributaries of Mobile Bay in meso- to polyhaline habitats (south of I-10, Figure 1). Sites at the upper end of the estuary were located 23 river km into the Delta in tidal freshwater to oligohaline habitats (Figure 1). Sites were sampled 1 – 2 times monthly during May – July of 2018 and March, May – July of 2019.

Four sampling methods were used to collect Southern Flounder during the 2018 – 2019 sampling period. These included beam trawls, gill nets, electrofishing, and hook-and-line. A one-meter wide beam trawl with 2 mm mesh was used at all sampling locations to target small juveniles (≤ 100 mm total length (TL)). Beam trawl transects (eight at meso- to polyhaline sites and three at oligohaline sites) were hauled by boat in 2-minute trawls during each site visit. Gillnets were used to target large juveniles and adults (≥100 mm TL). Four soaking hours (two 2-hour sets) of 30 m by 2.4 m gillnets with 127

mm stretch mesh were set at meso- to polyhaline sites. Nets were set parallel to shore with a hook towards shore at the downstream end. At freshwater sites, pulsed DC boat electrofishing (Midwest Lake Electrofishing Systems Infinity Box) was used along shorelines. Six 15-minute boom mounted electrofishing transects were conducted during each site visit. Hook-and-line sampling was conducted after all standardized sampling was completed or in areas not accessible by the previous methods. At each site we recorded date, time, GPS coordinates, and water depth at the beginning and end of each sampling transect or gillnet set. All Southern Flounder collections were conducted according to use guidelines outlined in IACUC protocol #AUP2018-001 at Clemson University.

Additional flounder were collected by Alabama Marine Resources Division (MRD) during their Fisheries Monitoring and Assessment Program (FAMP). This survey program used a 4.88 m otter trawl with 4.76 mm mesh pulled for 10 minutes at 2 – 2.5 knots. Surveys occur monthly at 24 locations across all of Alabama's coastal waters south of I-10. Trawl samples were placed on ice and returned to MRD's Dauphin Island laboratory for processing.

Fishery-dependent collections

Southern Flounder were collected from the commercial and recreational fisheries during both 2004 – 2007 and 2018 – 2019 sampling periods. MRD (2004 – 2007) collected from recreational anglers using protocols from NOAA Fisheries Marine Recreational Information Program (MRIP) and from commercial fish houses. MRD used Access Point Angler Intercept Survey (APAIS) to randomly select public access locations

across Mobile Bay and Alabama's coast at selected times to creel recreational anglers fishing from shore and vessel. Clemson University (2018 – 2019) collections included commercial fish houses, opportunistic collections from boat access points, and two large annual fishing tournaments.

Water quality and chemistry

At each site visit during 2004 – 2007 and 2018 – 2019, a water column profile was completed using a YSI ProPlus handheld unit to record temperature (°C), dissolved oxygen (mg/L), and salinity (psu). Measurements were taken at one-meter depth increments starting at the surface. Additionally, daily time-series salinity data were collected from three Dauphin Island Sea Lab stations (Meaher State Park, Middle Bay, Dauphin Island, https://arcos.disl.org/). Water chemistry samples for quantifying elemental concentrations were collected in conjunction with water column profiles during July 2018 and March, May – July 2019. Water chemistry samples were collected at 1-meter depth using a Van Dorn water sampler, filtered through 0.45 μm filters using a vacuum filtration system, fixed with 95% nitric acid (HNO_3) at 2%, and stored in 200 mL acid washed bottles.

Laboratory processing

Measurements from Southern Flounder included total length (mm), weight (g), and macroscopic inspection of gonads. Additionally, sagittal otoliths were removed, cleaned of tissue in research-grade ultrapure water, and air dried. Otoliths were embedded in individual wells with Buehler EpoKwick epoxy and hardener (2004 – 2007 samples) or Struers EpoFix epoxy and hardener (2018 – 2019 samples). Otolith wells were

sectioned with a Buehler IsoMet low speed saw making two cuts perpendicular to the sulcal groove, one on each side of the otolith's core, approximately 2 mm wide. Sectioned otoliths were polished using a Buehler circular polishing station with 600 and 1000 grit paper until the core and annuli were exposed. Otoliths were fixed to a glass slide with Crystalbond 509, imaged with digital imaging analysis system, and aged by two readers before being processed for otolith chemistry.

Otolith chemistry samples were processed at the Dauphin Island Sea Lab instrumentation lab using an Agilent 7700x quadrupole inductively coupled plasma mass spectrometer (ICPMS) coupled to a 213 nm Nd:YAG NWR laser. Mounted otoliths were rinsed with deionized water and cleaned using a low power cleaning pre-ablation (40 µm spot, 100 µm/sec, 20% laser power, 5 Hz) to remove contaminants on the otolith surface along the same transect as the chemistry analysis ablation (Gover et al. 2014). Prior to chemistry analysis, an argon gas carrier was analyzed for 60 seconds. Following methods from Lowe et al. (2011), otoliths were ablated (25 µm spot, 5 µm/sec, 30% laser power, 10 Hz, energy around 5 J/cm^2) from the core to the distal edge along a straight transect parallel to the sulcal groove. Otolith chemistry analysis targeted concentrations for a suite of elements (i.e., magnesium (^{24}Mg), calcium (^{43}Ca), manganese (^{55}Mn), zinc (^{65}Zn), strontium (^{88}Sr), and barium (^{137}Ba)) with each element being sampled every 0.6 seconds. Analytical precision was assessed using a reference standard (NIST-612) which was run at the beginning, end, and every hour between to assess instrumental drift. Trace Element IS data reduction scheme in Iolite v3 addressed limits of detection, background signals, and corrected for instrument drift. Raw elemental counts were converted to

concentrations (ppm) using Ca (37.69%) as an internal standard (Longerich et al. 1996), then elemental concentrations were converted to molar ratios with calcium (element:Ca). Individual values for each otolith were scaled to years by assuming the last reading along the otolith's edge was laid on date of harvest and the core was a hatch date of January 1st (Fitzhugh et al. 1996; Glass et al. 2008).

Water samples were processed for elemental concentrations of ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{65}Zn, ^{88}Sr, ^{137}Ba with the same ICPMS system in solution mode coupled with an Agilent autosampler. Samples were diluted based on salinity at 10x (0 – 5 psu), 20x (5 – 10 psu), 50x (10 – 20 psu), or 100-fold (≥ 20 psu) with 2% nitric acid. Internal standards (IS) beryllium (^{9}Be) and indium (^{115}In) were added to each sample at 10 and 1 ppb concentrations, respectively. Following methods from Nelson and Powers (2020), an external 5-point calibration curve of elemental concentrations and IS was processed before running the water samples. Lab calibrated reference standards and 2% nitric acid blanks were run every hour to assess instrumental drift and background signals. Within the Agilent Masshunter software, the calibration curve was used to correct for instrumental drift, mass bias, and convert count data into elemental concentrations (ppb). Concentrations of each element were converted to molar ratios with calcium to compare with otolith chemistry.

Statistical analysis

Water to otolith partition coefficient

Using best fitting nonlinear models from Nelson and Powers (2020), we quantified the relationships between water and otolith element:Ca with salinity. For Sr:Ca and Mg:Ca, the asymptotic equation

$$\text{element:Ca} = asy[1 - e^{-k(\text{Salinity} - s0)}] \qquad \textit{Equation 1}$$

quantified the asymptote (*asy*), increase coefficient (*k*), and intercept (*s0*). For Ba:Ca the exponential equation

$$\text{element:Ca} = s0 * e^{-k(\text{Salinity})} \qquad \textit{Equation 2}$$

quantified the increase coefficient and intercept. Paired water and otolith chemistry samples were used to quantify partition coefficients, or the fractional incorporation of ambient water elemental concentrations into the otolith. Using the equation

$$D_{\text{element:Ca}} = [(\text{element:Ca})_{\text{otolith}}] / [(\text{element:Ca})_{\text{water}}] \qquad \textit{Equation 3}$$

(Morse and Bender 1990), partition coefficients ($D_{\text{element:Ca}}$) for each flounder were calculated by using the element:Ca (mmol:mol) from the last thirty days of otolith growth (element:$Ca_{otolith}$; which ranged from 25 – 92 µm, depending on fish age) and water chemistry (element:Ca_{water}) from samples collected on the same day from March to July of 2019 (N = 43). Due to limited samples at higher salinities, individual flounder $D_{\text{element:Ca}}$ were averaged by water salinity classification (i.e., freshwater (<1 psu), mesohaline (5 – 18 psu), and polyhaline (>18 psu)) to test if partition coefficients were consistent across the range of salinities. The grand mean partition coefficient used to determine residency status was calculated by averaging the partition coefficient from each salinity classification. All analyses were completed in R version 3.6.1 (R Development Core Team 2019).

Residency classification

Our goal in analyzing otolith elemental data was to classify each Southern Flounder as a freshwater (salinity ≤ 1 psu) or estuarine (salinity > 1 psu) resident during each year of life. To accomplish this, we needed to quantify the relationship between otolith element:Ca ratios and salinity from our ambient water sampling. We fit non-linear models of water element:Ca (mmol:mol) versus ambient salinity at time of sample collection for all water samples collected during 2018 and 2019. From this relationship we quantified the expected water element:Ca value for 1 psu salinity (i.e., the threshold value for residency classification). Variance was estimated using bootstrapped 95% confidence intervals generated using 1000 iterations in the R package nlsBoot. The predicted water element:Ca value for 1 psu was then multiplied by the partition coefficient to develop the expected mean otolith element:Ca value at 1 psu salinity. To quantify the uncertainty in the otolith element:Ca threshold value at 1 psu salinity, we multiplied the 95% upper and lower confidence intervals of the predicted water element:Ca value by the partition coefficient.

To summarize time series of otolith element:Ca values, we used a regime shift detection algorithm to detect significant shifts in otolith element:Ca values along the laser ablation transects (Rodionov 2004). Following methods from Turner and Limburg (2015) and Seeley and Walther (2018), algorithm parameters were set at a significance level of 0.05, cut-off length of 10 cells (approximately 27 μm), and a Huber's weight parameter of 1 for omitting outliers. The algorithm used these parameters to identify regime shifts,

or discontinuity, in element:Ca values along otolith transects and create a smoothed average between shifts.

Using the otolith element:Ca freshwater threshold determined earlier, smoothed time series of otolith element:Ca values were classified as above or below this 1 psu salinity threshold value at each time step (i.e., fractional ages). An element:Ca value equal to or below the threshold value for 1 psu salinity was classified as a freshwater resident for a given time step, while an element:Ca value above the threshold value was classified as an estuarine resident for a given time step. The proportion of total values above or below this threshold value were then summarized in each year of life (i.e., between each annuli) and across the entire lifetime for each individual. If greater than 90% of the values across each age or lifetime fell into one classification (i.e., freshwater or estuarine), residency patterns were assigned to that classification. If neither classification consisted of 90% of the transect, then a 'transient' classification was assigned to indicate a fish that either moved between freshwater and estuarine habitats or a fish that resided in an area that experienced seasonal changes in salinity.

Fisher's exact tests used 3 x 2 contingency tables to test the null hypothesis that lifetime and age-specific residency classifications (rows) were independent of location of collection, sex, and fishery-dependent method of collection (columns). Specifically, these tests evaluated if lifetime residency patterns differed by location of collection (i.e., Delta versus Mobile Bay) or by fishery-dependent method of harvest (i.e., commercial versus recreational). These tests also evaluated if age-specific residency patterns within location of collection differed by sex or by fishery-dependent method of collection. A separate

contingency table was used to test for differences between the four analyses listed above. A Bonferroni correction was applied to age-specific analyses to account for multiple comparisons and control familywise error rates.

RESULTS

Temporal trends in water salinity values were consistent across Mobile Bay and the Delta. Annual salinity patterns across all sites were lowest in the spring, increased throughout the summer and then decreased during the fall (Figure 2). Salinities in Mobile Bay (Middle Bay and Dauphin Island loggers; Figure 1) ranged from 2 – 30 psu, while salinity in the lower Delta (Meaher State Park logger; Figure 1) ranged from 0 – 14 psu during this study. Lower Delta summer and fall salinity values were above the 1 psu salinity threshold, but remained below 1 psu during winter and spring for most years of this study (Figure 2). On average, 62% of lower Delta annual salinity values were below 1 psu.

Elemental concentrations of dissolved Ca, Sr, and Mg from 55 water samples showed positive, linear relationships with salinity ($R^2 > 0.99$, $p < 0.001$), while Ba showed no relationship (R^2=0.004, $p = 0.27$) (Figure 3). In ratios with water Ca (element:Ca), Mg:Ca (*asy* = 4.52, *k* = 0.95, *s0* = -0.02) and Sr:Ca (*asy* = 7.62, *k* = 0.99, *s0* = -0.31) showed positive, asymptotic relationships, while Ba:Ca (*k* = 0.29, *s0* = 580.55) showed a negative, exponential relationship (Figure 4). Otolith element:Ca ratios each showed unique relationships with ambient salinity. Otolith Mg:Ca had no relationship, while Sr:Ca had a positive, asymptotic relationship (*asy* = 2.42, *k* = 0.72, *s0* = -0.42), and

Ba:Ca had a negative, exponential relationship (k = 0.73, $s0$ = 25.19) with salinity (Figure 5). Of the elements analyzed in this study, only dissolved concentrations of Sr and Sr:Ca showed relationships with salinities ranging from 0 – 25 psu.

Mean partition coefficients ($D_{\text{element:Ca}}$) for each element showed unique trends across salinity regions (i.e., freshwater, mesohaline, polyhaline). $D_{\text{Mg:Ca}}$ and $D_{\text{Ba:Ca}}$ exhibited 3 – 5 times more variability between regions than $D_{\text{Sr:Ca}}$ (Table 1). $D_{\text{Mg:Ca}}$ decreased 0.135 mmol:mol from freshwater to polyhaline, while $D_{\text{Ba:Ca}}$ increased 0.075 mmol:mol from freshwater to polyhaline. $D_{\text{Sr:Ca}}$ experienced a mid-salinity peak, but remained relatively constant with a range of 0.024 mmol:mol across all salinity regions. By averaging the mean partition coefficient for each salinity region, the grand mean partition coefficients were $D_{\text{Mg:Ca}}$ = 0.06, $D_{\text{Ba:Ca}}$ = 0.07, and $D_{\text{Sr:Ca}}$ = 0.31. Due to inconsistencies in Mg and Ba water and otolith relationships with salinity, only Sr:Ca ratios were used as a marker for salinity exposure and residency classifications.

Using the nonlinear relationship determined for water Sr:Ca regressed against salinity (Figure 4), the predicted water Sr:Ca values for 1 psu was 5.53 mmol:mol (95% confidence interval = 5.25 – 5.89). When multiplied by the grand mean partition coefficient for Sr:Ca, the freshwater threshold for 1 psu salinity in Southern Flounder otoliths was 1.71 mmol:mol Sr:Ca (95% confidence interval = 1.62 – 1.82). Uncertainty surrounding the 1.71 mmol:mol Sr:Ca threshold was not incorporated into further analyses, as the bootstrapped 95% confidence interval was narrow relative to other studies that formally included the uncertainty of Sr:Ca thresholds into their analyses (Seeley and Walther 2018). The narrow 95% confidence interval presented here suggests

that including uncertainty in this threshold value would have little impact on final residency classifications.

Transect otolith chemistry data from 417 Southern Flounder (263 fishery-dependent, 154 fishery-independent) were used to examine proportional occurrence of residency classifications (i.e., freshwater, transient, estuarine). Across all years, more females were collected than males (342 females, 43 males, 32 unidentified) and females had larger mean lengths, weights, and older ages than males (Table 2). Of the three lifetime residency classifications, transient was the most common (n = 188, 45%), followed by estuarine residency (n = 139, 33%), then freshwater residency (n = 90, 22%). Lifetime transient flounder exhibited a wide diversity of patterns in their use of freshwater habitats, which ranged from 10% - 90% of their lifetime. The distribution of individuals across this gradient of lifetime transient habitat use was fairly uniform with 15%, 11%, 9%, and 8% of transients with 10 – 30%, 30 – 50%, 50 – 70%, and 70 – 90% of lifetime freshwater habitat use, respectively. There was a declining trend in freshwater habitat use (freshwater residents and transients) with age (65% age-0, 41% age-1, 36% age-2, 25% age-3, and 0% age-4; Figure 7).

Southern Flounder lifetime residency patterns differed significantly by area of collection (Fisher's exact test: $p < 0.001$). Southern Flounder collected in the Delta were predominately freshwater lifetime residents (69%) or transient, while those collected in Mobile Bay were predominately estuarine lifetime residents (46%) or transient (Figure 8). Only 3% of individuals collected in Mobile Bay were lifetime freshwater residents, while <1% of Delta collected individuals were lifetime estuarine residents.

Male and female Southern Flounder residency patterns were similar across younger ages. Within location of collection (i.e., Delta or Mobile Bay), no significant differences in the frequency of residency patterns occurred between age-0 males and age-0 females (Delta Bonferroni corrected $p = 1$, Mobile Bay Bonferroni corrected $p = 0.8$) or age-1 males and age-1 females (Delta Bonferroni corrected $p = 1$, Mobile Bay Bonferroni corrected $p = 0.8$) (Figure 9). Females utilized freshwater habitats (i.e., freshwater or transient residency) up to age-3, while males utilized freshwater habitats up to age-1. Older males were collected less frequently than females, with only a single age-3 male, but several age-3 and age-4 females ($n = 23$) (Figure 9).

Southern Flounder lifetime residency patterns between Alabama's commercial and recreational fisheries did not differ ($p = 0.3$; Figure 10). Although lifetime freshwater residencies combined across all cohorts occurred in only 3% of fishery-dependent samples, at least one individual harvested by the commercial and recreational fisheries exhibited lifetime freshwater residency in 45% of the cohorts (i.e., 2001 – 2006 and 2014 – 2018) analyzed in this study (Figure 10). On average, 62% of the individuals harvested from each cohort had a lifetime residency indicating at least some level of freshwater habitat utilization (i.e., freshwater residency or transient). Age-specific residencies for commercially and recreationally harvested Southern Flounder revealed no significant differences in the distributions of residency classifications for age-0 (Bonferroni corrected $p = 1$), age-1 (Bonferroni corrected $p = 0.5$), age-2 (Bonferroni corrected $p = 1$), and age-3 (Bonferroni corrected $p = 1$) (Figure 11). Combining across the recreational and commercial harvest, the percent of individuals utilizing freshwater habitats (annual

resident and transients) declined with age, with 57% of fish being classified as freshwater or transient during age-0 but 0% by age-4.

DISCUSSION

Trace metal relationships with water and otoliths

This study effectively tested the ability to use three trace metals as salinity proxies by assessing their relationship with water salinity and Southern Flounder otoliths in Alabama's coastal waters. Validating the use of a trace element as a salinity proxy requires addressing the assumption that elemental endmembers are distinguishable between freshwater and marine salinities in the ambient environment (Walther and Limburg 2012). From water samples collected in this study, Sr and Mg concentrations exhibited conservative, linear relationships with salinity, as well as positive, asymptotic relationships between water Sr:Ca and Mg:Ca with salinity. Sr:Ca and Mg:Ca ratios were similar to regional and global freshwater (< 0.5 psu) and polyhaline (> 18 psu) endmembers. Freshwater Sr:Ca ratios ranged from 2.08 – 3.66 mmol:mol (global median 2.39 mmol:mol, Brown and Severin 2009), while Mg:Ca freshwater ratios ranged from 0.35 – 1.39 mol:mol (global mean 0.45 mol:mol, Walther and Nims 2015). Polyhaline Sr:Ca ratios ranged from 7.68 – 7.96 (global mean 8.54 mmol:mol, de Villiers 1999), while Mg:Ca polyhaline ratios ranged from 4.6 – 4.9 (regional mean around 4.6, Mohan and Walther 2015). The distinct differences between freshwater and marine elemental endmembers indicated these elements could be used as a salinity proxy, if incorporated into otoliths in proportion to ambient environment concentrations. Ba concentrations

exhibited a slight decreasing trend with increasing salinity, however high variability at lower salinities and a mid salinity peak indicated a limited relationship with salinity. Although water Ba concentrations were not conservative with salinity, water Ba:Ca exhibited a strong, exponential decline with increasing salinity, indicating potential use as a salinity proxy for biogenic carbonates (Figure 4). Water chemistry results in this study were consistent with previous studies in this system (Nelson and Powers 2019, 2020).

Otolith Sr:Ca ratios exhibited positive, asymptotic relationships with salinity, indicating Sr:Ca as a proxy for salinity exposure and habitat use for Southern Flounder in this estuarine system. Additionally, Sr:Ca had the lowest range in differences between mean partition coefficients across salinity classifications (Table 1). Otolith Mg:Ca ratios showed no relationship with salinity and exhibited large ranges in mean partition coefficients, distinguishing Mg as a poor salinity proxy for this species in Alabama. Similar to water Ba:Ca, otolith Ba:Ca showed a negative, exponential relationship with salinity; however, large increases in partition coefficients (D_{Ba}) were observed with increasing salinity. This trend is not unique to Southern Flounder and has been observed by several other species (detailed list in Nelson and Powers 2020). Since water Ba concentrations across salinity gradients were not highly distinguishable, the increased partition coefficient limited the ability to differentiate freshwater versus marine residency (Nelson and Powers 2020). As a result of water and otolith relationships with salinity, only Sr:Ca ratios were validated as a proxy for salinity exposure and habitat-use for Southern Flounder in this study.

Water and otolith Sr:Ca values were used to develop a freshwater threshold (≤ 1 psu) for Southern Flounder in Alabama's coastal waters. The water Sr:Ca freshwater threshold of 5.53 ± 0.16 (mean ± standard deviation) was similar to the Sr:Ca oligohaline (< 5 psu) threshold of 5.23 ± 1.23 from Texas (Seeley and Walther 2018), with lower variability. The otolith freshwater (≤ 1 psu) threshold value calculated in this study (1.71 Sr:Ca) aligns with previous studies in this estuarine system, however the salinity threshold was empirically quantified to be 1 psu rather than assumed to approximate 2 psu (Lowe et al. 2011; Farmer et al. 2013). However, our results indicate that the previous threshold from these studies was generally indicative of residence in low salinity (tidal freshwater (≤ 5 psu) to oligohaline (0.5 – 5 psu)) versus high salinity (mesohaline (5 – 18 psu) and polyhaline (18 – 30 psu)) habitats.

Salinity exposure and residency patterns

Southern Flounder otolith signatures revealed high utilization of low salinity habitats, which is consistent with the putative life history of the species (Stokes 1977; Fischer and Thompson 2004). 65% of all individuals used freshwater habitats during their first year of life (i.e., freshwater residency or transient patterns during first year of otolith growth). Additionally, 41% of all individuals were classified as transient or freshwater residents during their second year of life, indicating freshwater habitats may be serving as more than postsettlement nursery habitats for a large portion of Alabama's Southern Flounder population. The results of this study demonstrated three important findings: 1) individuals collected in the Delta showed significantly higher lifetime freshwater residency percentages versus those collected in Mobile Bay, 2) the fishery-dependent

harvested flounder exhibited few lifetime freshwater residents, and 3) fishery-dependent harvested flounder exhibited a decreasing trend in freshwater utilization with increasing age.

Lifetime transient and estuarine residents comprised 97% of fishery-dependent samples, yet more than half of all harvested individuals utilized freshwater in some capacity (i.e., at least one age specific transient or freshwater residency). The majority (57%) of harvested flounder experienced freshwater during age-0, but only 21% by age-1. Additionally, age-specific analyses of fishery-dependent samples revealed significantly higher estuarine residency patterns for those collected in Mobile Bay versus the Delta. This suggests that Delta habitats are contributing less to the commercial and recreational fisheries than Mobile Bay habitats. It appears that the majority of Southern Flounder contributing to the commercial and recreational fisheries begin settlement in or near freshwater habitats, but reside in estuarine habitats after age-0.

Southern Flounder are marine migrants, requiring offshore habitats to spawn, but inshore estuarine habitats for juvenile settlement and development (Elliott et al. 2007). Locality of settlement and habitat-use appears to be highly variable across salinity gradients, suggesting the existence of distinct migratory contingents (i.e., divergent migratory tactics within a stock (Secor 1999)). From previous studies, both freshwater and estuarine habitats played an important role in providing suitable habitat for growth and development within estuaries (Lowe et al. 2011; Farmer et al. 2013; Nims and Walther 2014). Specifically, Farmer et al. (2013) Southern Flounder samples collected in freshwater and oligohaline habitats (< 5 psu) exhibited lifetime residency classifications

(95% flounder assayed: 16% freshwater, 37% transient, 42% estuarine residencies) proportionally similar to the results of this study (22% freshwater, 45% transient, 33% estuarine residencies). Southern Flounder collected in Texas estuarine habitats (> 5 psu) showed much lower utilization of freshwater habitats (Nims and Walther 2014). Analysis of Nims and Walther (2014) residency data using the methods from this study (i.e., proportional freshwater residencies of 0 – 10% = estuarine, 10 – 90% = transient, and 90 – 100% = freshwater residencies), revealed lifetime residency classifications of Texas Southern Flounder exhibiting 4% oligohaline (< 5 psu), 36% transient, 60% estuarine residencies (data acquired using GraphGrabber V2.0, Quintessa 2020). Additionally, Nims and Walther (2014) used a 5 psu oligohaline threshold rather than a 1 psu freshwater threshold, indicating potentially lower freshwater residency proportions than indicated above. Higher concentrations of Southern Flounder within Texas estuarine habitats versus freshwater habitats has also been recorded in other studies (Glass et al. 2008; Nañez-James et al. 2009). Previous studies have indicated estuarine contingency patterns may be linked to individual genetic (Darden et al. 2014) or behavioral (Nims and Walther 2014) adaptations. For example, Texas flounder displayed greater proportional use of estuarine habitats, but Texas estuaries also have disproportionately more estuarine habitat than Alabama estuaries due to lower annual discharge (Bianchi et al. 1998). Additionally, Blandon et al. (2001) found distinct genetic structuring in Southern Flounder west of Galveston Bay, suggesting evolutionary adaptation in Texas migratory contingents due to habitat availability. Since the Mobile-Tensaw River System has higher discharge and greater proportional Southern Flounder freshwater residencies than Texas,

similar mechanisms may be underlying the observed patterns in this study. Future research is required to understand if these patterns are genetically based and maintained across generations, or if they simply represent a wide degree of plasticity in habitat use.

To our knowledge, this is the first to study to use otolith chemistry from Southern Flounder collected across all available inshore habitats (i.e., freshwater and estuarine) within an estuary. Our findings suggest the importance of collecting individuals across the entire salinity gradient when evaluating residency to ensure all potential contingents are represented in the data. When quantifying residency characteristics of euryhaline species, future studies should consider collecting samples across all salinity regions within a study system to capture the full range of habitats used by the species.

Several assumptions were necessary when classifying otolith chemistry data into residency patterns (Elsdon et al. 2008; Walther 2018). First, when developing the 1 psu freshwater threshold, we assumed flounder movements were minimal and water Sr:Ca values were stable (i.e., identical to those measured at the time of collection) over the 30 days prior to collection. Conventional tagging of Southern Flounder in the mid-Atlantic showed limited movement (< 1 km) during summer estuarine residency (Craig et al. 2015). Additionally, salinity in the Delta remained below 1 psu and estuarine signatures in Mobile Bay remained above 5 psu (i.e., the asymptotic threshold for water Sr:Ca ratios) during the 2019 sampling period. Consequently, the 1 psu threshold for water and Southern Flounder otoliths developed in this study should be accurate, although controlled experiments are required to validate this. Secondly, residency classifications may be a result of fish movement or seasonal fluctuations in water chemistry over a

relatively stationary fish. Alabama's coastal waters have variable seasonal salinity regimes, with large proportions of Mobile Bay experiencing annual freshwater influxes (Dzwonkowski et al. 2017). If freshwater influxes were the primary influence on the occurrence of freshwater residency, there would be far fewer freshwater and transient residents during years of low freshwater discharge. Seasonal fluctuations in discharge could be impacting the proportion of transient individuals (sensu Farmer et al. 2013), so future analyses should evaluate the relative importance of freshwater habitats against metrics of freshwater habitat availability to evaluate if residency patterns change in proportion to fluctuations in habitat availability. Lastly, several factors may influence flounder residency patterns that are not recognizable with otolith chemistry alone. Internal factors, including diet and physiology, could impact the incorporation of trace elements into the otolith's calcium carbonate matrix (Campana and Thorrold 2001; Sturrock et al. 2014). This is evident with Mg in this study, which was not incorporated into otoliths in proportion to the ambient concentrations. Additionally, external factors including food web dynamics and abiotic environmental variables impact habitat utilization, potentially driving fish residency and movements (Burke 1995; Zucchetta et al. 2010; Furey and Rooker 2013). Future evaluation of prey availability, diets, and growth rates across estuarine salinity gradients may provide further insights into the relative importance of freshwater versus estuarine habitats.

Results of this study have several management implications as Southern Flounder are currently experiencing a population decline across their entire range. Following the nursery-role hypothesis from Beck et al. (2001) and Dahlgren et al. (2006), it appears that

freshwater nursery habitats are playing an important role in Southern Flounder settlement and contribution of recruits to the adult (age-1+) population. As Southern Flounder otoliths displayed variable distributions across freshwater and estuarine habitats at age-0, protecting low- and high-salinity habitats ensures connectively between all potential habitats exploited by flounder during ontogenetic growth and development. Protection of diverse habitats within estuaries would preserve the potentially distinct migratory contingents of this species (Schindler et al. 2010), potentially increasing resiliency against future environmental variables and harvest pressures.

LITERATURE CITED

Able, K. W. 2005. A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. Estuarine, Coastal and Shelf Science 64(1 SPEC. ISS.):5–17.

Baker, M. C., M. A. Steinhoff, and G. F. Fricano. 2017. Integrated effects of the Deepwater Horizon oil spill on nearshore ecosystems. Marine Ecology Progress Series 576:219–234.

Beck, M. W., K. L. J. Heck, K. W. Able, D. L. Childers, D. B. Eggleston, B. M. Gillanders, B. Halpern, C. G. Hays, K. Hoshino, T. J. Minello, R. J. Orth, P. F. Sheridan, and M. P. Weinstein. 2001. The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates. BioScience 51(8):633–641.

Bianchi, T. S., J. R. Pennock, and R. R. Twilley. 1998. Biogeochemistry of Gulf of Mexico Estuaries.

Blandon, I. R., T. L. King, W. Virginia, W. J. Karel, and J. P. M. Jr. 2001. Preliminary genetic population structure of southern flounder , Paralichthys lethostigma , along the Atlantic Coast and Gulf of Mexico 678(November 1975):671–678.

Brown, R. J., and K. P. Severin. 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. Canadian Journal of Fisheries and Aquatic Sciences 66(10):1790–1808.

Burke, J. S. 1995. Role of feeding and prey distribution of summer and southern flounder

in selection of estuarine nursery habitats. Journal of Fish 47:355–366.

Burke, J. S., J. M. Miller, and D. E. Hoss. 1991. Immigration and settlement pattern of Paralichthys dentatus and P. lethostigma in an estuarine nursery ground, North Carolina, U.S.A. Netherlands Journal of Sea Research 27(3–4):393–405.

Campana, S. 1999. Chemistry and composition of fish otoliths:pathways, mechanisms and applications. Marine Ecology Progress Series 188:263–297.

Campana, S. E., G. A. Chouinard, J. M. Hanson, A. Frechet, and J. Brattery. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fisheries Research 46(1):343–357.

Campana, S. E., and S. R. Thorrold. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences 58(1):30–38.

Courrat, A., J. Lobry, D. Nicolas, P. Laffargue, R. Amara, M. Lepage, M. Girardin, and O. Le Pape. 2009. Anthropogenic disturbance on nursery function of estuarine areas for marine species. Estuarine, Coastal and Shelf Science 81(2):179–190.

Craig, C. A., K. E. Jarvis, and L. J. Clarkej. 2000. An assessment of calibration strategies for the quantitative and semiquantitative analysis of calcium carbonate matrices by laser ablationinductively coupled plasma-mass spectrometry (LA-ICP-MS). Journal of Analytical Atomic Spectrometry 15(8):1001–1008.

Craig, J. K., W. E. Smith, F. S. Scharf, and J. P. Monaghan. 2015. Estuarine residency and migration of southern flounder inferred from conventional tag returns at multiple spatial scales. Marine and Coastal Fisheries 7:450–463.

Creighton, C., P. I. Boon, J. D. Brookes, and M. Sheaves. 2015. Repairing Australia's estuaries for improved fisheries production - What benefits, at what cost? Marine and Freshwater Research 66(6):493–507.

Dahlgren, C. P., G. T. Kellison, A. J. Adams, B. M. Gillanders, M. S. Kendall, C. A. Layman, J. A. Ley, I. Nagelkerken, and J. E. Serafy. 2006. Marine nurseries and effective juvenile habitats: Concepts and applications. Marine Ecology Progress Series 312(April):291–295.

Darden, T. L., M. J. Walker, K. Brenkert, J. R. Yost, and M. R. Denson. 2014. Population genetics of Cobia (Rachycentron canadum): Implications for fi shery management along the coast of the southeastern United States. Fishery Bulletin 112(1):24–35.

Dzwonkowski, B., A. T. Greer, C. Briseño-Avena, J. W. Krause, I. M. Soto, F. J. Hernandez, A. L. Deary, J. D. Wiggert, D. Joung, P. J. Fitzpatrick, S. J. O'Brien, S. L. Dykstra, Y. Lau, M. K. Cambazoglu, G. Lockridge, S. D. Howden, A. M. Shiller, and W. M. Graham. 2017. Estuarine influence on biogeochemical properties of the Alabama shelf during the fall season. Continental Shelf Research 140(January):96–109. Elsevier Ltd.

Dzwonkowski, B., K. Park, H. Kyung Ha, W. M. Graham, F. J. Hernandez, and S. P. Powers. 2011. Hydrographic variability on a coastal shelf directly influenced by estuarine outflow. Continental Shelf Research 31(9):939–950. Elsevier.

Elliott, M., A. K. Whitfield, I. C. Potter, S. J. M. Blaber, D. P. Cyrus, F. G. Nordlie, and T. D. Harrison. 2007. The guild approach to categorizing estuarine fish assemblages: A global review. Fish and Fisheries 8(3):241–268.

Elsdon, T., B. Wells, S. Campana, B. Gillanders, C. Jones, K. Limburg, D. Secor, S. Thorrold, and B. Walther. 2008. Otolith Chemistry To Describe Movements And Life-History Parameters Of Fishes (1):297–330.

Farmer, T. M., D. R. DeVries, and J. E. Gagnon. 2013. Using seasonal variationin otolith microchemical composition to indicate largemouth bass and southern flounder residency patterns across an estuarine salinity gradient. Transactions of the American Fisheries Society 142(5):1415–1429.

Fischer, A. J., and B. A. Thompson. 2004. The age and growth of southern flounder, *Paralichthys lethostigma,* from Louisiana estuarine and offshore waters. Bulletin of Marine Science 75(1):63–77.

Fitzhugh, G. R., L. B. Crowder, and J. P. Monaghan. 1996. Mechanisms contributing to variable growth in juvenile southern flounder (Paralichthys lethostigma). Canadian Journal of Fisheries and Aquatic Sciences (53):1964–1973.

Flowers, A. M., S. D. Allen, A. L. Markwith, and L. M. Lee. 2019. Stock Assessment of Southern Flounder (Paralichthys lethostigma) in the South Atlantic , 1989 – 2017:1–228.

Froeschke, B. F., B. Sterba-Boatwright, and G. W. Stunz. 2011. Assessing southern flounder (Paralichthys lethostigma) long-term population trends in the northern Gulf of Mexico using time series analyses. Fisheries Research 108(2–3):291–298. Elsevier B.V.

Furey, N. B., M. A. Dance, and J. R. Rooker. 2013. Fine-scale movements and habitat use of juvenile southern flounder Paralichthys lethostigma in an estuarine seascape.

Journal of Fish Biology 82(5):1469–1483.

Furey, N. B., and J. R. Rooker. 2013. Spatial and temporal shifts in suitable habitat of juvenile southern flounder (Paralichthys lethostigma). Journal of Sea Research 76:161–169. Elsevier B.V.

Gillanders, B. M. 2002. Temporal and spatial variability in elemental composition of otoliths: implications for determining stock identity and connectivity of populations. Canadian Journal of Fisheries and Aquatic Sciences 59(4):669–679.

Glass, L. A., J. R. Rooker, R. T. Kraus, and G. J. Holt. 2008. Distribution, condition, and growth of newly settled southern flounder (Paralichthys lethostigma) in the Galveston Bay Estuary , TX. Journal of Sea Research 59:259–268.

Glover, D. C., D. R. DeVries, and R. A. Wright. 2013. Growth of largemouth bass in a dynamic estuarine environment: An evaluation of the relative effects of salinity, diet, and temperature. Canadian Journal of Fisheries and Aquatic Sciences 70(3):485–501.

Gover, T. R., M. K. Nims, J. J. Van Tassell, P. D. Collingsworth, J. W. Olesik, S. A. Ludsin, and E. A. Marschall. 2014. How Much Cleaning is Needed When Processing Otoliths from Fish Larvae for Microchemical Analysis? Transactions of the American Fisheries Society 143(3):779–783.

Houde, E. D., and E. S. Rutherford. 2016. Coastal and Estuarine Research Federation Recent Trends in Estuarine Fisheries : Predictions of Fish Production and Yield 16(2):161–176.

Jager, Z. 1999. Selective Tidal Stream Transport of Flounder Larvae (Platichthys

flesusL.) in the Dollard (Ems Estuary). Estuarine, Coastal and Shelf Science 49(3):347–362.

Lee, J., B. M. Webb, B. Dzwonkowski, A. Valle-Levinson, and J. Lee. 2019. Characteristics of exchange flow in a multiple inlet diurnal estuary: Mobile Bay, Alabama. Journal of Marine Systems 191(July 2018):38–50. Elsevier.

Lehrter, J. C. 2008. Regulation of eutrophication susceptibility in oligohaline regions of a northern Gulf of Mexico estuary, Mobile Bay, Alabama. Marine Pollution Bulletin 56(8):1446–1460.

Loewen, T. N., B. Carriere, J. D. Reist, N. M. Halden, and W. G. Anderson. 2016. Linking physiology and biomineralization processes to ecological inferences on the life history of fishes. Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology 202:123–140. Elsevier Inc.

Longerich, H. P., S. E. Jackson, and D. Günther. 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. Journal of Analytical Atomic Spectrometry 11(9):899–904.

Lowe, M. R., S. A. Ludsin, B. J. Fryer, R. A. Wright, D. R. DeVries, and T. M. Farmer. 2011. Otolith Microchemistry Reveals Substantial Use of Freshwater by Southern Flounder in the Northern Gulf of Mexico. Estuaries and Coasts 35(3):907–910.

Lowe, M. R., and M. S. Peterson. 2014. Effects of Coastal Urbanization on Salt-Marsh Faunal Assemblages in the Northern Gulf of Mexico. Marine and Coastal Fisheries 6(1):89–107.

Macdonald, J. I., and D. A. Crook. 2010. Variability in Sr:Ca and Ba:Ca ratios in water and fish otoliths across an estuarine salinity gradient. Marine Ecology Progress Series 413(January):147–161.

Mohan, J. A., and B. D. Walther. 2015. Spatiotemporal Variation of Trace Elements and Stable Isotopes in Subtropical Estuaries: II. Regional, Local, and Seasonal Salinity-Element Relationships. Estuaries and Coasts 38(3):769–781.

Morse, J. W., and M. L. Bender. 1990. Partition coefficients in calcite: Examination of factors influencing the validity of experimental results and their application to natural systems. Chemical Geology 82:265–277.

Mortazavi, B., A. A. Riggs, J. M. Caffrey, H. Genet, and S. W. Phipps. 2012. The Contribution of Benthic Nutrient Regeneration to Primary Production in a Shallow Eutrophic Estuary ,. Estuaries and Coasts 35(3):862–877.

Nañez-James, S. E., G. W. Stunz, and S. A. Holt. 2009. Habitat use patterns of newly settled southern flounder, paralichthys lethostigma, in aransas-copano bay, Texas. Estuaries and Coasts 32(2):350–359.

Nathan, R., W. M. Getz, E. Revilla, M. Holyoak, R. Kadmon, D. Saltz, and P. E. Smouse. 2008. A movement eoclogy paradigm for unifying organismal movement research. Proceedings of the National Academy of Sciences 105(49):19052–19059.

Nelson, T. R., and S. P. Powers. 2019. Validation of species specific otolith chemistry and salinity relationships. Environmental Biology of Fishes 102:801–815. Environmental Biology of Fishes.

Nelson, T. R., and S. P. Powers. 2020. Elemental Concentrations of Water and Otoliths as

Salinity Proxies in a Northern Gulf of Mexico Estuary. Estuaries and Coasts. Estuaries and Coasts.

Nims, M. K., and B. D. Walther. 2014. Contingents of Southern Flounder from Subtropical Estuaries Revealed by Otolith Chemistry. Transactions of the American Fisheries Society 143(3):721–731.

Noble, M. A., W. W. Schroeder, W. J. Wiseman, H. F. Ryan, and G. Gelfenbaum. 1996. Subtidal circulation patterns in a shallow, highly stratified estuary: Mobile Bay, Alabama. Journal of Geophysical Research: Oceans 101(C11):25689–25703.

Norris, A. J., D. R. DeVries, and R. A. Wright. 2010. Coastal Estuaries as Habitat for a Freshwater Fish Species: Exploring Population-Level Effects of Salinity on Largemouth Bass. Transactions of the American Fisheries Society 139(2):610–625.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an Integration of Landscape and Food Web Ecology: The Dynamics of Spatially Subsidized Food Webs. Annual Review of Ecology and Systematics 28(1):289–316.

Powell, A. B., and F. J. Schwartz. 2006. Distribution of Paralichthid Flounders (Bothidae: Paralichthys) in North Carolina Estuaries. Chesapeake Science 18(4):334.

Powers, S. P., M. Albins, and J. Mareska. 2018. An Assessment of Southern Flounder in Alabama Coastal Waters.

Rodionov, S. N. 2004. A sequential algorithm for testing climate regime shifts. Geophysical Research Letters 31(L09204).

Rozas, L. P., and C. T. Hackney. 1984. Use of Oligohaline Marshes by Fishes and Macrofaunal Crustaceans in North Carolina. Coastal and Estuarine Research

Federation 7(3):213–224.

Schindler, D. E., R. Hilborn, B. Chasco, C. P. Boatright, T. P. Quinn, L. A. Rogers, and M. S. Webster. 2010. Population diversity and the portfolio effect in an exploited species. Nature 465(7298):609–612. Nature Publishing Group.

Schreiber, A. M. 2006. Asymmetric craniofacial remodeling and liberalized behavior in larval flatfish. Journal of Experimental Biology 209(4):610–621.

Schroeder, W. W., S. P. Dinnel, and W. J. Wiseman. 1990. Salinity Stratification in a River-Dominated Estuary. Estuaries 13(2):145–154.

Secor, D. H. 1999. Specifying divergent migrations in the concept of stock: The contingent hypothesis. Fisheries Research 43(1–3):13–34.

Seeley, M. E., and B. D. Walther. 2018. Facultative oligohaline habitat use in a mobile fish inferred from scale chemistry. Marine Ecology Progress Series 598:233–245.

Smith, W. E., and F. S. Scharf. 2010. Demographic characteristics of southern flounder, Paralichthys lethostigma, harvested by an estuarine gillnet fishery. Fisheries Management and Ecology 17(6):532–543.

Stokes, G. M. 1977. Life history studies of southern flounder (*Paralichthys lethostigma*) and gulf flounder (*P. albigutta*) in the Aransas Bay area of Texas. Texas Parks and Wildlife Department Technical Series 25:1–37.

Sturrock, A. M., C. N. Trueman, A. M. Darnaude, and E. Hunter. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? Journal of Fish Biology 81(2):766–795.

Sturrock, A. M., C. N. Trueman, J. A. Milton, C. P. Waring, M. J. Cooper, and E. Hunter.

2014. Physiological influences can outweigh environmental signals in otolith microchemistry research. Marine Ecology Progress Series 500(Campana 1999):245–264.

Surge, D. M., and K. C. Lohmann. 2002. Temporal and spatial differences in salinity and water chemistry in SW Florida estuaries: Effects of human-impacted watersheds. Estuaries 25(3):393–408.

Taylor, J. C., and J. M. Miller. 2001. Physiological performance of juvenile southern flounder, Paralichthys lethostigma (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. Journal of Experimental Marine Biology and Ecology 258(2):195–214.

Teichert, N., S. Pasquaud, A. Borja, G. Chust, A. Uriarte, and M. Lepage. 2017. Living under stressful conditions: Fish life history strategies across environmental gradients in estuaries. Estuarine, Coastal and Shelf Science 188:18–26.

Tournois, J., F. Ferraton, L. Velez, L. Mercier, A. M. Darnaude, C. Aliaume, and D. J. McKenzie. 2013. Temporal stability of otolith elemental fingerprints discriminates among lagoon nursery habitats. Estuarine, Coastal and Shelf Science 131:182–193. Elsevier Ltd.

Turner, S. M., and K. E. Limburg. 2015. Does Daily Growth Affect the Rate of Manganese Uptake in Juvenile River Herring Otoliths? Transactions of the American Fisheries Society 144:873–881.

VanderKooy, S. 2015. Management profile for the gulf and Southern flounder fishery in the Gulf of Mexico. Gulf States Marine Fisheries Commission (247).

de Villiers, S. 1999. Seawater strontium and Sr/Ca variability in the Atlantic and Pacific oceans. Earth and Planetary Science Letters 171(4):623–634.

Walther, B. 2018. The Art of Otolith Chemistry : interpreting patterns by integrating perspectives Is otolith Mg adding nuance to the hypoxia story ?

Walther, B. D., and K. E. Limburg. 2012. The use of otolith chemistry to characterize diadromous migrations. Journal of Fish Biology 81(2):796–825.

Walther, B. D., and M. K. Nims. 2015. Spatiotemporal Variation of Trace Elements and Stable Isotopes in Subtropical Estuaries: I. Freshwater Endmembers and Mixing Curves. Estuaries and Coasts 38(3):754–768.

Zucchetta, M., A. Franco, P. Torricelli, and P. Franzoi. 2010. Habitat distribution model for European flounder juveniles in the Venice lagoon. Journal of Sea Research 64(1–2):133–144. Elsevier B.V.

TABLES AND FIGURES

Table 1. Means and standard errors (in parentheses) for water element to calcium (Ca), otolith element to calcium, and partition coefficient (mmol:mol) for magnesium (^{24}Mg), strontium (^{88}Sr), and barium (^{137}Ba) for Southern Flounder from three salinity regions in the Mobile-Tensaw River Delta and Mobile Bay during May – July, 2019. Water and otoliths samples were collected on the same day.

Salinity (psu)	n	^{24}Mg			^{88}Sr			^{137}Ba		
		Water Mg:Ca	Otolith Mg:Ca	D_{Mg}	Water Sr:Ca	Otolith Sr:Ca	D_{Sr}	Water Ba:Ca	Otolith Ba:Ca	D_{Ba}
Freshwater (<1)	29	0.680 (0.066)	0.082 (0.004)	0.149 (0.013)	2.824 (0.084)	0.824 (0.029)	0.295 (0.010)	527.916 (6.146)	18.980 (1.364)	0.036 (0.003)
Mesohaline (5-17)	13	4.443 (0.022)	0.076 (0.004)	0.017 (0.001)	7.557 (0.051)	2.418 (0.079)	0.319 (0.009)	61.669 (2.464)	3.101 (0.208)	0.053 (0.007)
Polyhaline (24-25)	2	4.738 (0.060)	0.068 (0.008)	0.014 (0.001)	7.799 (0.104)	2.462 (0.249)	0.316 (0.036)	24.018 (3.567)	2.635 (0.243)	0.111 (0.009)

Table 2. Southern Flounder collected in the Mobile-Tensaw River Delta and Mobile Bay from 2004 – 2007 and 2018 – 2019 by different sources in descending order from largest to smallest sample sizes. Sample size (n) and ranges for total length, weight, age, and year of harvest or collection for all Southern Flounder otoliths used in this study.

Collector	Males					Females					Unidentified				
	n	Length (mm)	Weight (g)	Age	Year	n	Length (mm)	Weight (g)	Age	Year	n	Length (mm)	Weight (g)	Age	Year
Recreational Fishery	1	321		1	2005	124	242 - 586	250 - 2450	0 - 3	2004 - 2007, 2018 - 2019	14	351 - 529	800 - 1908	1 - 3	2007, 2018 - 2019
Commerical Fishery	10	300 - 391	313 - 618	1 - 3	2006, 2019	112	339 - 564	435 - 1880	1 - 4	2005 - 2007, 2019	3			1	2018
Clemson University	17	233 - 333	125 - 460	0 - 1	2018 - 2019	63	166 - 547	44 - 1913	0 - 2	2018 - 2019	16	53 - 191	1.29 - 69	0	2018 - 2019
Auburn University	13	174 - 339	48 - 390	0 - 1	2005, 2007	32	192 - 470	68 - 1312	0 - 3	2005 - 2007	0				
AL Marine Resources Division	2	289 - 306	300 - 380	1	2018	11	191 - 463	80 - 1320	0 - 1	2018 - 2019	0				

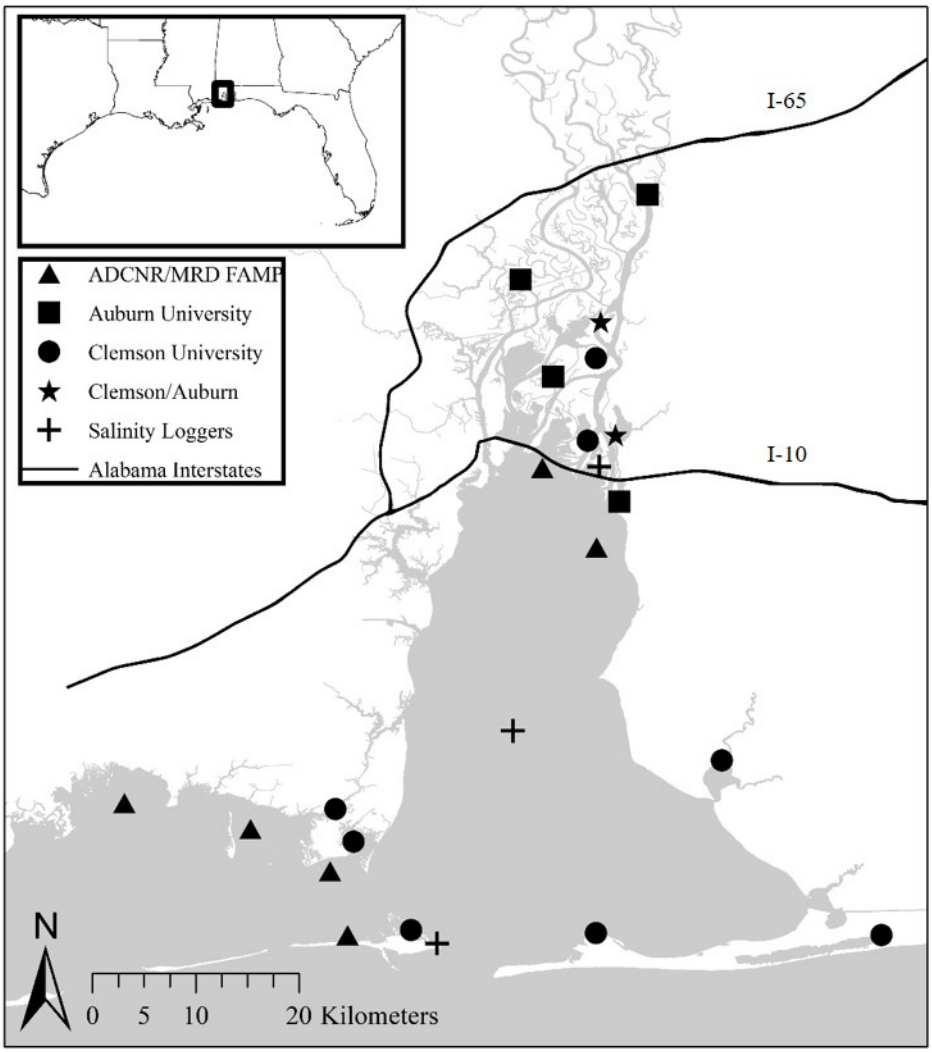

Figure 1. Map of the Mobile-Tensaw River Delta (north of I-10) and Mobile Bay (south of I-10) in Alabama showing the ten Clemson University (2018 – 2019) sampling locations for Southern Flounder and water chemistry collections (circles and stars), six Auburn University (2004 – 2007) sampling locations (squares and stars), Alabama Marine Resources Division's Fisheries Assessment and Monitoring Program (MRD FAMP) sampling locations (triangles), and three Dauphin Island Sea Lab salinity loggers (from north to south: Meaher State Park, Middle Bay, Dauphin Island; cross). Black stars

indicate sampling locations by both Clemson University and Auburn University. Sites adjacent to and north of I-10 have an average annual oligohaline salinity, while sites south of I-10 have an average annual mesohaline to polyhaline salinity.

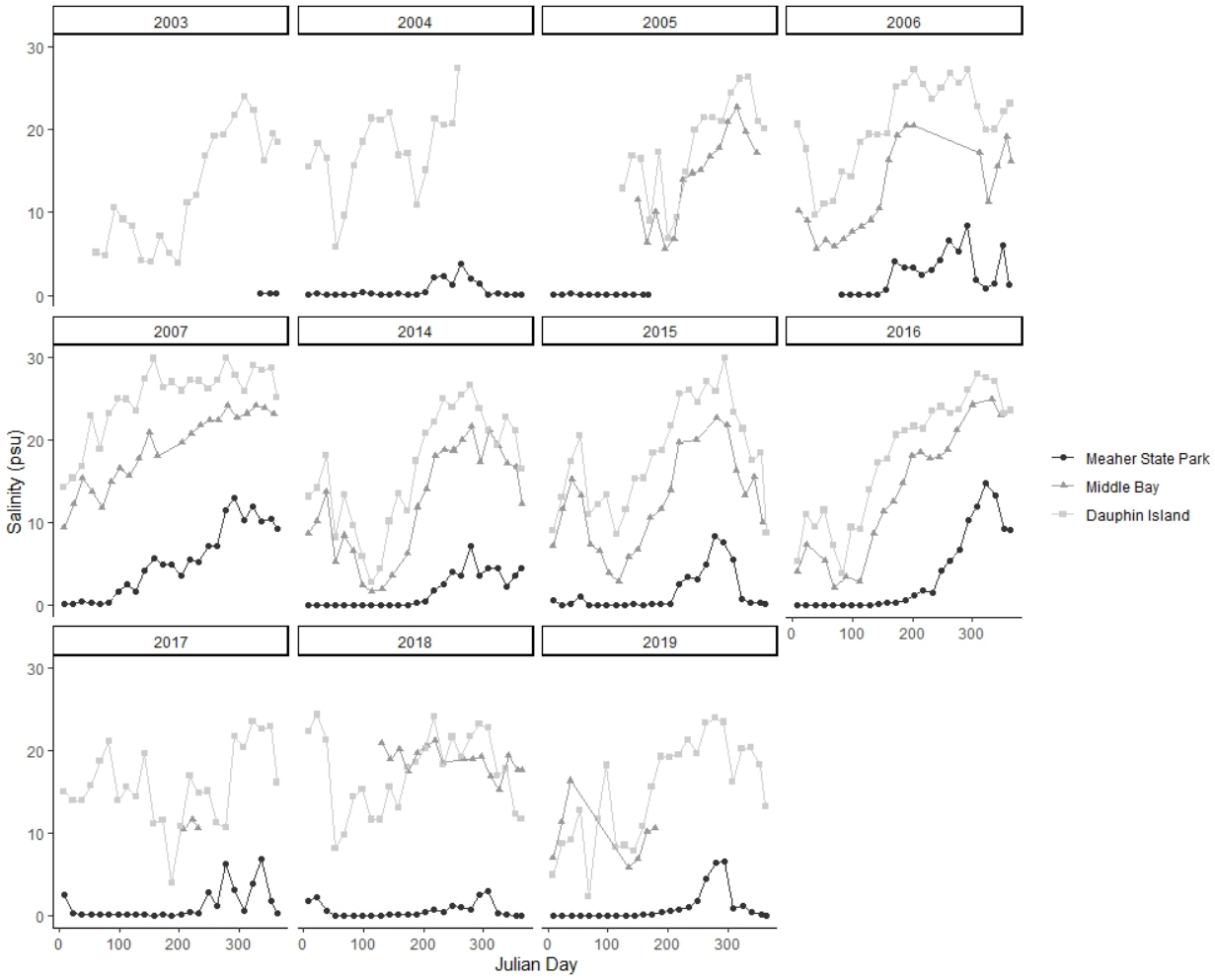

Figure 2. Fifteen-day mean values of salinity (psu) from three salinity loggers in Mobile Bay, Alabama. Logger locations span the entire bay from north to south (Figure 1). Located at the northern extent of Mobile Bay, Meaher State Park salinity values are representative of salinities in the lower Delta, while Middle Bay and Dauphin Island represent Mobile Bay salinities. Data were downloaded from Alabama's Real-Time Coastal Observation System (ARCOS) for these three loggers which were installed and operated by Dauphin Island Sea Lab.

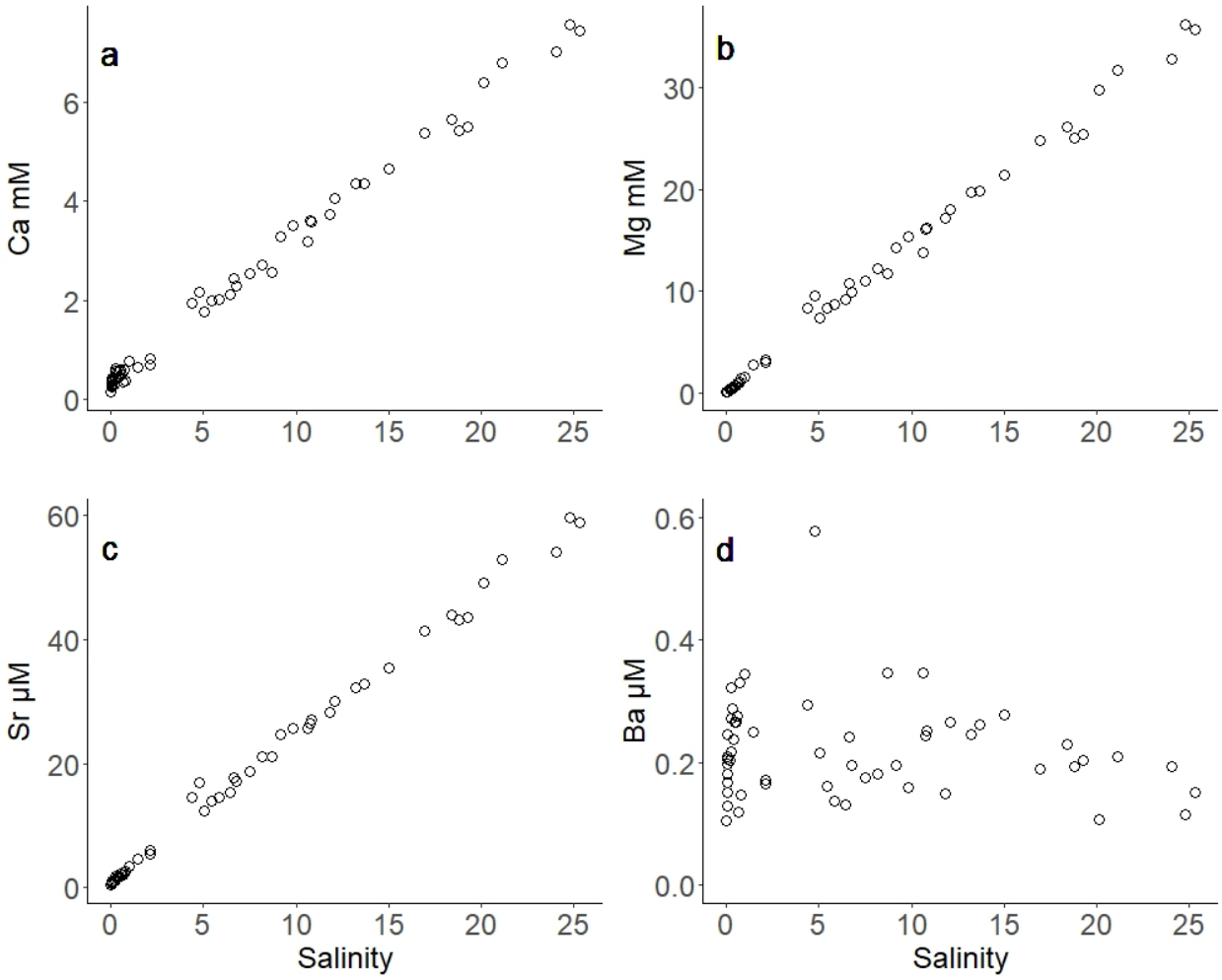

Figure 3. Water elemental concentrations of a) calcium (Ca), b) magnesium (Mg), c) strontium (Sr), and d) barium (Ba) with salinity for 55 water samples from the Mobile-Tensaw River Delta and Mobile Bay. Samples were collected during July 2018 and March through July of 2019 at 1 m depth by Clemson University.

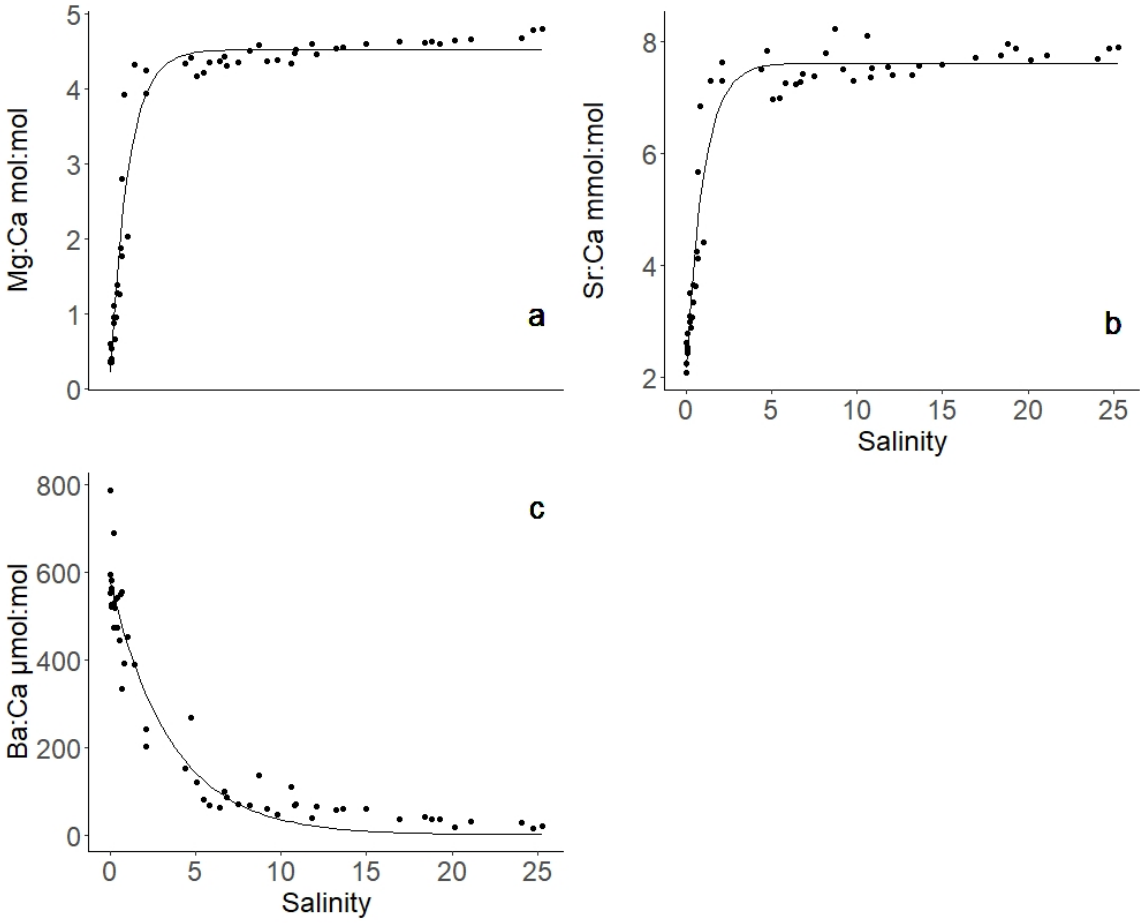

Figure 4. Water element to calcium ratios for a) magnesium (Mg), b) strontium (Sr), and c) barium (Ba) with salinity for 55 water samples from the Mobile-Tensaw River Delta and Mobile Bay. Samples were collected during July 2018 and March through July of 2019 at 1 m depth by Clemson University. Lines represent modelled relationship of element:Ca ratios with salinity.

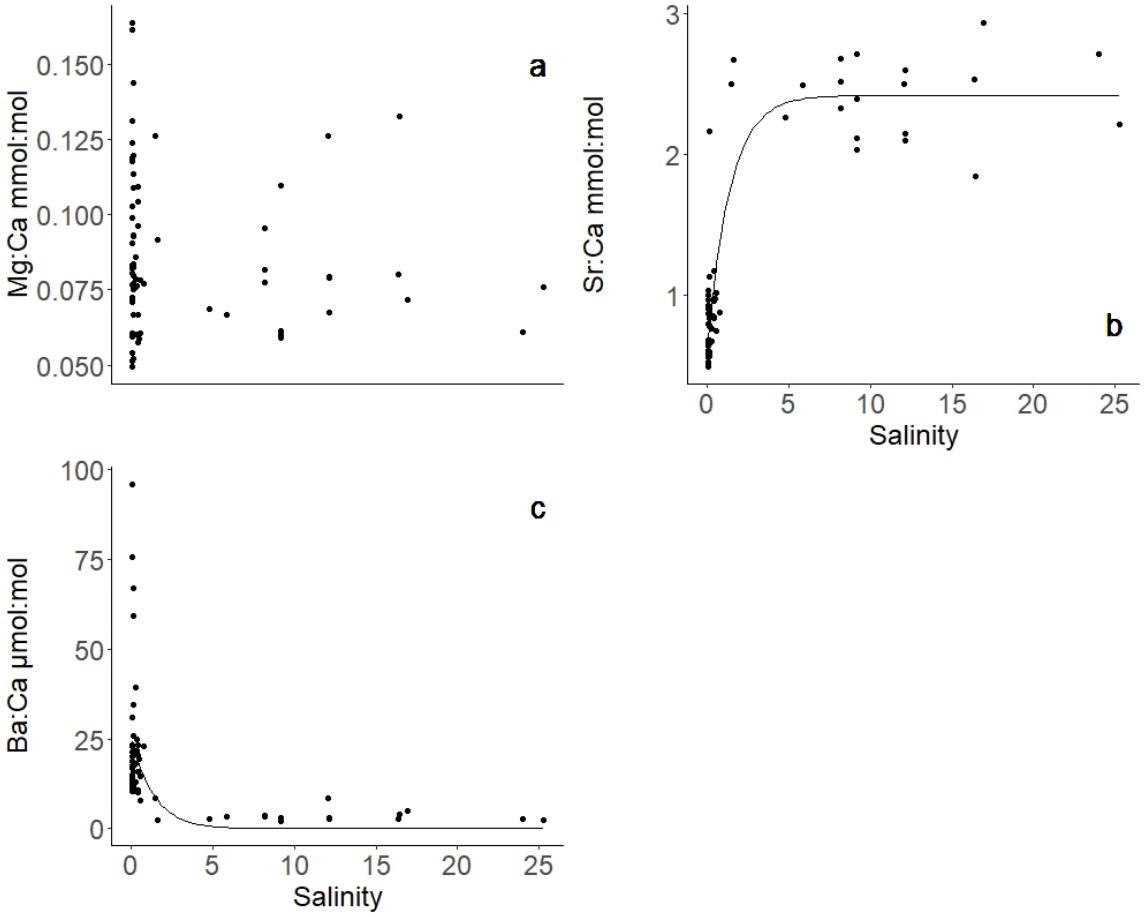

Figure 5. Otolith element to calcium ratios for a) magnesium (Mg), b) strontium (Sr), and c) barium (Ba) with salinity for mean otolith values from the last 30 days of otolith growth of 73 Southern Flounder from the Mobile-Tensaw River Delta and Mobile Bay. Samples were collected during 2018 and 2019 by Clemson University. Lines represent modelled relationship of element:Ca ratios with salinity.

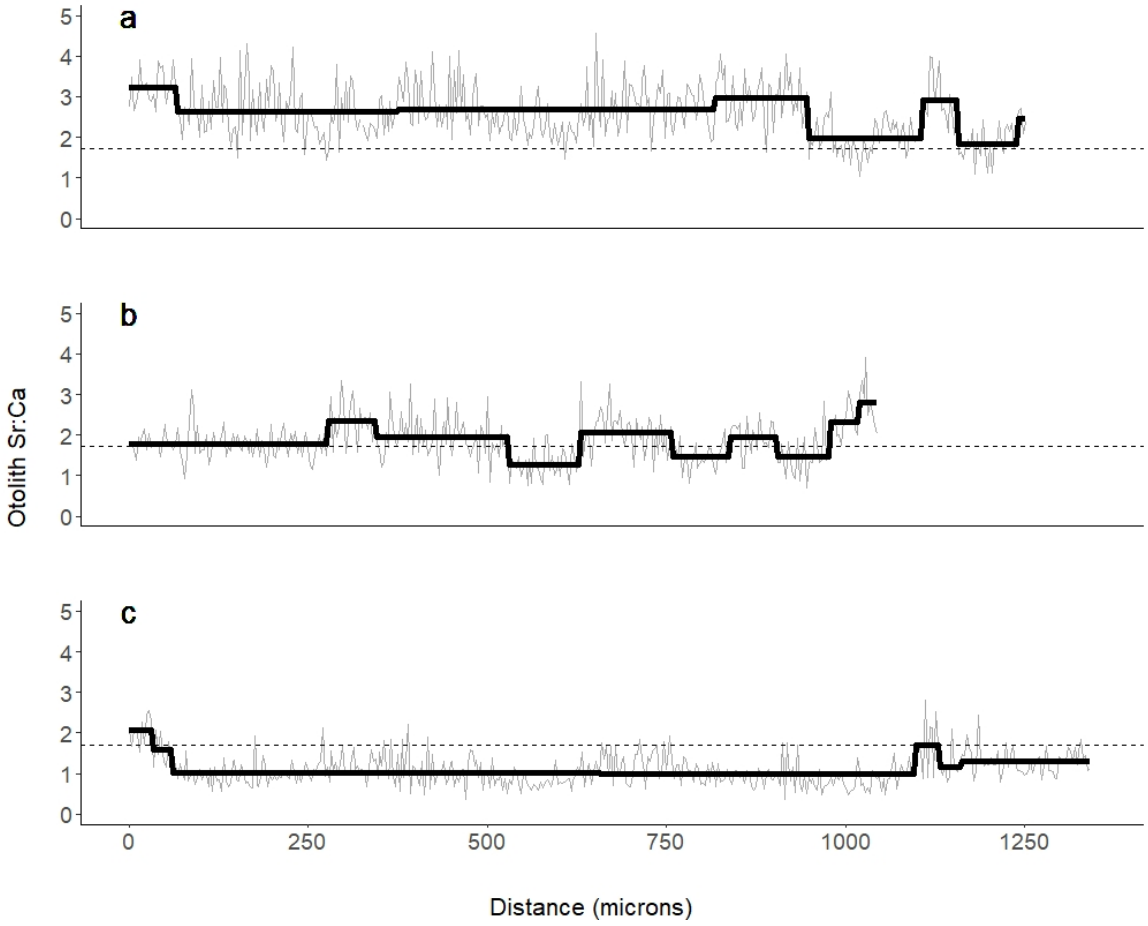

Figure 6. Otolith Sr:Ca values from Southern Flounder collected in Alabama's coastal waters. Raw values (grey lines) from laser ablation ICPMS output were smoothed using a regime shift detector (black lines) from Rodionov (2004). An otolith Sr:Ca value ≤ 1.71 mmol:mol (horizontal, dotted line) was used to indicate residence in freshwater (salinity ≤ 1 psu). Plots represent a) estuarine, b) transient, and c) freshwater classifications for Southern Flounder based on proportion of smoothed Sr:Ca values above or below the freshwater threshold (90% below = freshwater resident; 90% above = estuarine resident; all others = transient).

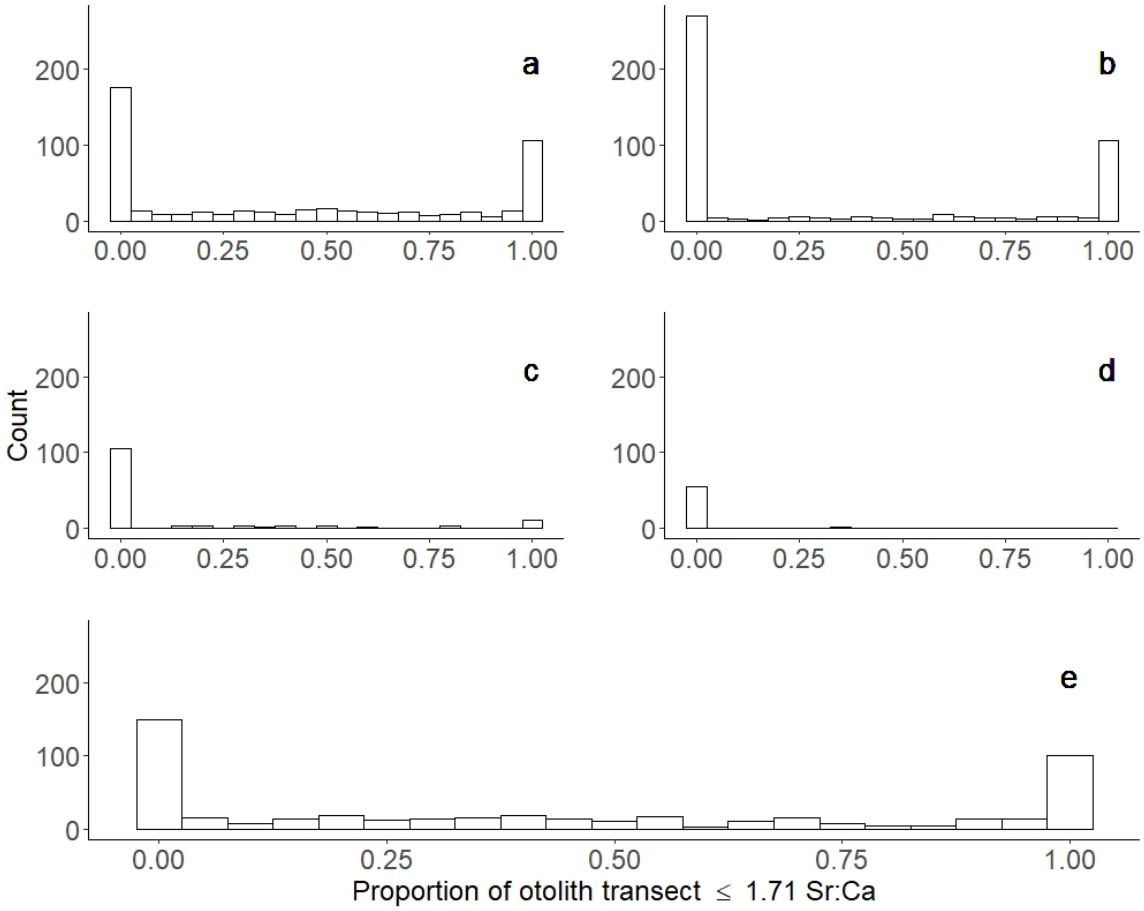

Figure 7. Frequency distributions of Southern Flounder grouped by proportion for a) age-0, b) age-1, c) age-2, d) age-3, and e) lifetime otolith transect ≤ 1.71 Sr:Ca (mmol:mol) which indicates the proportional lifetime or age-specific residence in freshwater (salinity ≤ 1 psu). Counts shown are for 417 Southern Flounder from the Mobile-Tensaw River Delta and Mobile Bay during 2004 – 2007 and 2018 – 2019 collected from both fishery-dependent and fishery-independent collections. Southern Flounder with ≥ 90% of their otolith transect below 1.71 Sr:Ca were classified as freshwater residents, those with ≤ 10% of their otolith transect below 1.71 Sr:Ca were classified as estuarine residents, and those with 11 – 89% of their otolith transect below

1.71 Sr:Ca were classified as transients. Two age-4 individuals, not imaged above, consisted of estuarine residents only.

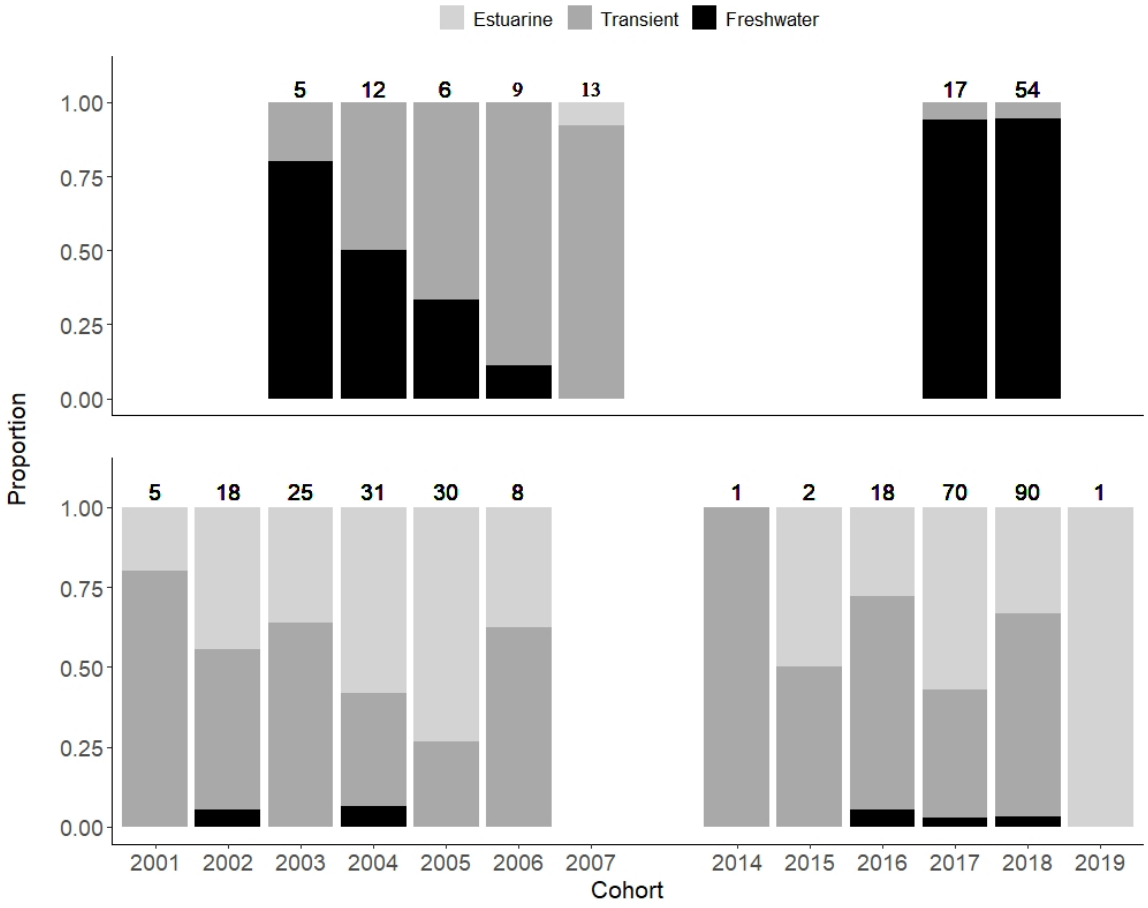

Figure 8. Proportions of lifetime residency classification for 417 Southern Flounder collected in the Mobile-Tensaw River Delta (top) and Mobile Bay (bottom) from 2004 – 2007 and 2018 – 2019 by cohort. Fishery-dependent and fishery-independent samples are combined in all of the plots above. Total sample sizes by cohort are located above each bar.

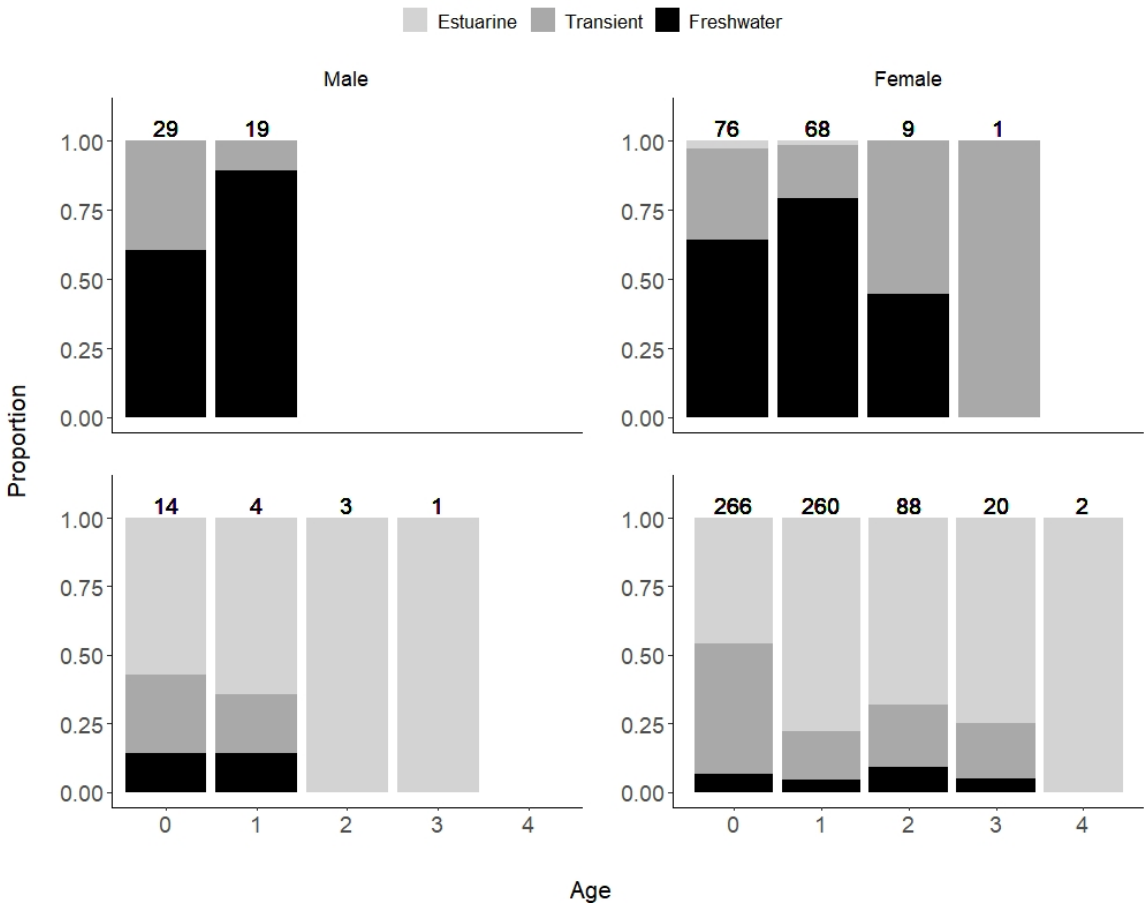

Figure 9. Proportions of age-specific and sex-specific residency classifications for Southern Flounder males (left) and females (right) collected in the Mobile-Tensaw River Delta (top) and Mobile Bay (bottom) during 2004 – 2007 and 2018 – 2019. Delta samples consisted of only fishery independent, while Mobile Bay samples consisted of fishery-dependent and fishery-independent samples. Total sample sizes by age-group are located above each bar.

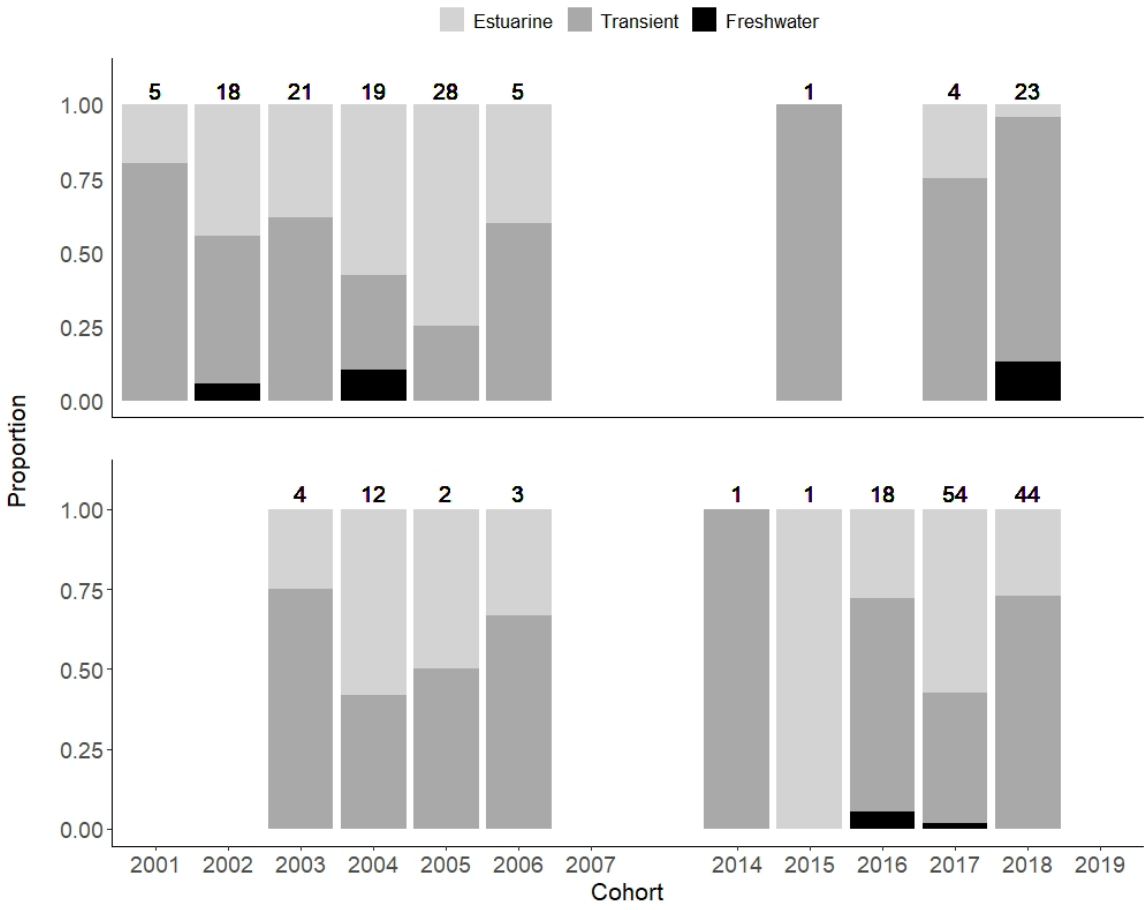

Figure 10. Proportion of lifetime residency classifications of Southern Flounder harvested by commercial (top) and recreational (bottom) fisheries in Mobile Bay during 2004 – 2007 and 2018 – 2019 by cohort. Total sample sizes by cohort are located above each bar.

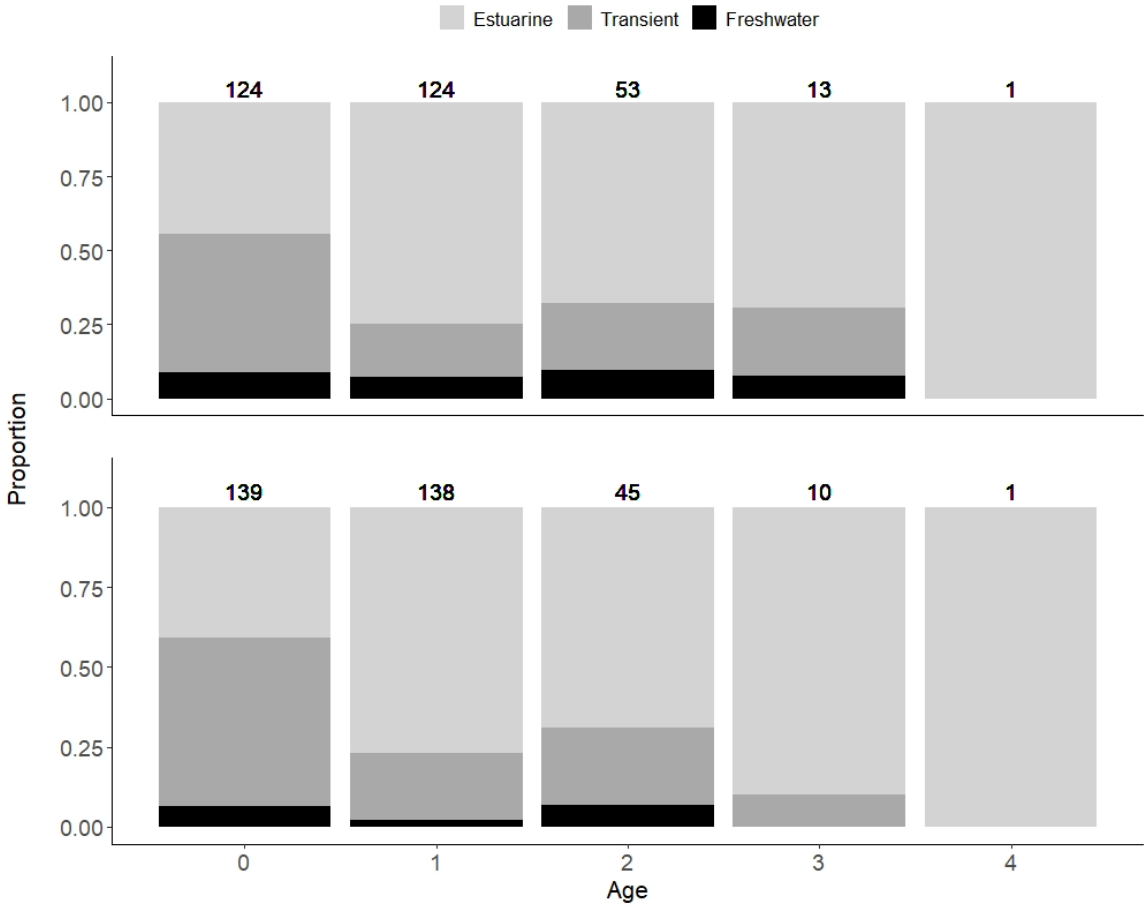

Figure 11. Age-specific residency classifications of Southern Flounder harvested by commercial and recreational fisheries in Mobile Bay during 2004 – 2007 and 2018 – 2019. Residencies of commercially harvested fish (top) and recreationally harvested fish (bottom) are labelled with total sample sizes by age-group above each bar.

CHAPTER TWO

TROPHIC ECOLOGY AND FOOD WEB DYNAMICS OF SOUTHERN FLOUNDER IN ALABAMA'S COASTAL WATERS

INTRODUCTION

As fish grow and develop, their nutritional needs, prey availability, and habitat-use have a direct impact on survival. Consequently, understanding foraging ecology and habitat use, particularly for economically important species, are essential to support sustainable fisheries. For fish experiencing population declines, understanding the role food sources and habitat, or lack thereof, may play in survival is vital. One such species is Southern Flounder (*Paralichthys lethostigma*), an estuarine dependent species which exhibit the putative life history of fall to winter offshore spawning followed by larvae ingress into estuarine habitats (Stokes 1977; Fischer and Thompson 2004; Glass et al. 2008). Southern Flounder are currently experiencing a population decline across the entire range of the species, which has increased the need for understanding life history characteristics (VanderKooy 2015; Powers et al. 2018; Flowers et al. 2019). For developing Southern Flounder, as with any species, to obtain the most benefit from a nursery, spatiotemporal alignment of ecosystem provisions must be met (Sheaves et al. 2014). These provisions may include dietary needs, refuge from predators, or suitable abiotic conditions (i.e. temperature, oxygen, salinity) (Polis et al. 1997; Kennish 2002; Nagelkerken et al. 2015). Overall, a thorough understanding of fish movements and habitat use within estuaries in lacking (Beck et al. 2001; Able 2005), but natural tags, such as otolith chemistry and stable isotopes, can be used to better understand fish life history characteristics.

One method of examining habitat use across various salinity concentrations in estuarine environments is otolith chemistry analysis. Otoliths are metabolically inert and incorporate a matrix of trace elements, which are often more representative of the ambient water chemistry than a fish's diet (Campana 1999; Walther and Limburg 2012; Sturrock et al. 2014). Elemental concentrations, also known as endmembers, within the ambient water chemistry are a result of upstream geochemical weathering and may exhibit conservative relationships with salinity (Elsdon et al. 2008; Nelson and Powers 2020). Incorporation rates of elemental endmembers into an otolith is highly variable between species and estuary, so water to otolith partition coefficients are needed to assess the utility of otoliths as a marker for salinity exposure (Macdonald and Crook 2010; Nelson and Powers 2020). Overall, this method is useful for reconstructing migratory and residency patterns of fishes across salinity gradients, but gives little insight into how individuals are utilizing these habitats. For Southern Flounder specifically, these techniques have revealed highly variable habitat-use patterns throughout ontogenetic growth and development within and between estuaries across the Gulf of Mexico (Lowe et al. 2011; Farmer et al. 2013; Nims and Walther 2014). These studies have demonstrated distinct migratory contingents within the species, however little is known about the relative importance of these habitats and the ecological consequences of Southern Flounder residency patterns.

Stable isotope analysis (SIA) is another useful tool to evaluate food web dynamics and trophic ecology. Similar to otolith chemistry, SIA works by testing biological material for different isotopic endmembers (e.g. nitrogen (δ^{15}N) and carbon (δ^{13}C))

(Peterson and Howarth 1987). Instead of physical habitat, endmembers indicate differences in trophic position and the food web sources of nutrients due to reaction kinetics of heavy and light isotopes (Trueman et al. 2012). Nitrogen isotopes, for example, can be used as a trophic level indicator due to the excretion of lighter isotopes (δ^{14}N and δ^{13}N) and the incorporation of heavier isotopes (δ^{15}N) retained in body tissues (Peterson 1999). Carbon isotopes represent terrestrial, benthic, and pelagic influences based on primary productivity at the base of the food web (McCutchan et al. 2003; Trueman et al. 2012). Ultimately, these isotopic endmember concentrations can be used to determine ontogenetic patterns and trophic position (Post 2002; Buchheister and Latour 2011), estuary connectivity and migratory characteristics (Herzka 2005; Trueman et al. 2012), and diet breadth (Scharf et al. 2000) of an individual fish.

While SIA provides a better understanding of estuarine fish habitat-use and trophic positions, some limitations apply. For instance, stable isotopes represent the accumulation of prey isotopic signatures over an extended period of time. Thus, understanding the temporal window that endmember values represent involves a knowledge of trophic fractionation and tissue turnover rates that are not always available for every species or life stage (Thomas and Crowther 2015; Vander Zanden et al. 2015). Trophic fractionation is the partitioning and mixing of heavy and light isotopes from prey to predator and can be variable for some endmembers (Peterson and Fry 1987; Post 2002). However, nitrogen isotopes have been particularly useful in food web studies as the averaged fractionation with each progression of trophic position has been well established in many species (3 – 4‰; Vander Zanden and Rasmussen 2001; Post 2002;

Fry 2008). Turnover rate is the amount of time it takes isotopic signatures within an organism to reflect that of their prey after undergoing an isotopically distinct dietary shift, likely due to ontogenetic development or migration to different habitats (Trueman et al. 2012; Busst and Britton 2018). The variability of turnover rates between species, biological material (i.e. liver, muscle, skeleton), and ontogenetic life stages can introduce greater error and uncertainty than fractionation because of greater variation from environmental variables and ontogenetic stages among organisms (Fry 2008; Vander Zanden et al. 2015). Buchheister and Latour (2010), for example, discovered half-life turnover of muscle tissue in adult summer flounder (*Paralichthys dentatus)* could take 69 days and 96 days for $\delta^{13}C$ and $\delta^{15}N$, respectively. Alternatively, Bosley et al. (2002) discovered half-life turnover of muscle tissue in juvenile winter flounder (*Pseudopleuronectes americanus*) took 4.1 days and 3.9 days for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Since turnover rates can be highly variable, especially between ontogenetic stages, stomach content analysis can be useful to offset unknown species-specific turnover rates (Wells et al. 2008). Furthermore, if sample collections consist of different size classes of the same species along salinity gradients, ontogenetic development and habitat-specific contributions can be examined (Powell and Schwartz 1979; Winemiller et al. 2007). As with other habitat and food web methods, limitations do exist. High occurrence of slowly digestible material could overestimate consumption rates, while unidentifiable, highly digested prey may decrease the diet breadth of the species within different habitats (Hyslop 1980; Buckland et al. 2017). The limitation of each of these methods

demonstrates the difficulty in broadly understanding foraging ecology and habitat-use for any species when only a single technique is used. We therefore incorporated several natural tags (i.e., otolith chemistry and stable isotopes) and SCA to increase the resolution of habitat-specific residencies and food web dynamics of Southern Flounder within Alabama's coastal waters.

To better understand habitat-use and foraging ecology, and the roles these factors play in the current decline of the Southern Flounder population, our specific goals were to 1) investigate the ability to use bulk carbon and nitrogen isotopic ratios as a marker for habitat-use across a large seasonal salinity gradient, 2) use compound specific $\delta^{15}N$ isotopes to determine trophic position, and 3) relate isotopic values to residency classifications determined from otolith chemistry analyses for Southern Flounder in Alabama's coastal waters. While previous studies have assessed otoliths, stable isotopes, or a combined approach on other flatfish species (order Pleuronectiformes), to our knowledge this is the first study to use a multiple natural tag approach on Southern Flounder. Ultimately, results from this study aim to inform management and conservation actions about the habitat-use characteristics of a species currently experiencing population declines.

METHODS

Study system

This study was conducted in the Mobile-Tensaw River Delta (hereafter referred to as the "Delta") and Mobile Bay in Alabama (Figure 1). With an average daily discharge

of 1850 m^3/s, the Mobile-Tensaw River system is the fourth largest river system in the contiguous United States (Schroeder et al. 1990, Morisawa 1968). As the primary source of freshwater to Mobile Bay, this system strongly influences the biochemical and hydrographical variations in the estuary (Dzwonkowski et al. 2011). There are multiple smaller freshwater sources including Dog River (watershed area 237 km^2), Fowl River (watershed area 184 km^2), and Week's Bay (watershed area 521 km^2), which have regional influences on salinity and nutrients in Mobile Bay (Lehrter 2008; Mortazavi et al. 2012). Additionally, Alabama has one small (surface area 9.3 km^2) tidally influenced lagoon, Little Lagoon, which is not connected to the Mobile-Tensaw River System, but instead receives nutrients directly from the Gulf of Mexico. Mobile Bay averages 3m depth across an area 15-35 km wide and 45-50 km long with a drainage basin of 115,467 km^2 (Dzwonkowski et al. 2011).

Sample collections

Fishery-independent collections

We collected juvenile and adult Southern Flounder from nine sites located along a 60+ km seasonal salinity gradient of saltmarshes, bays, tidal creeks, and freshwater ecosystems. Sites at the lower end of the estuary were located on the landward side of barrier islands and within tributaries of Mobile Bay in meso- to polyhaline habitats (south of I-10; Figure 1). Sites at the upper end of the estuary were located at the confluence of the Delta and Mobile Bay and up to 23 river km into the Delta in tidal freshwater to oligohaline habitats (north of I-10; Figure 1). Sites were sampled 1 – 2 times monthly during May – July of 2018 and March, May – July of 2019.

Four sampling methods were used to collect Southern Flounder. These included beam trawls, gill nets, electrofishing, and hook-and-line. A one-meter wide beam trawl with 2 mm mesh was used at all sampling locations to target small juveniles (≤ 100 mm total length (TL)). Beam trawl transects (minimum of eight at meso- to polyhaline sites and three at oligohaline to freshwater sites) were hauled by boat in 2-minute trawls during each site visit. Gillnets were used to target large juveniles and adults (≥100 mm TL). Four soaking hours (two 2-hour sets) of 30 m by 2.4 m gillnets with 127 mm stretch mesh were set at meso- to polyhaline sites. Nets were set parallel to shore with a hook towards shore at the downstream end. At freshwater sites, pulsed DC boat electrofishing (Midwest Lake Electrofishing Systems Infinity Box) was used along shorelines. Six boom mounted electrofishing transects were conducted for 15 minutes during each site visit. Hook-and-line sampling was conducted after all standardized sampling was completed or in areas within our sampling sites that were not accessible by the previous methods. At each site we recorded date, time, GPS coordinates, and water depth at the beginning and end of each sampling transect or gillnet set. All Southern Flounder collections were conducted according to use guidelines outlined in IACUC protocol #AUP2018-001 at Clemson University.

Additional flounder were provided from Alabama Marine Resources Division's (MRD) Fisheries Monitoring and Assessment Program (FAMP). This survey program used a 4.88 m otter trawl with 4.76 mm mesh pulled for ten minutes at 2 – 2.5 knots. Surveys occur monthly at 24 locations across all of Alabama's coastal waters below I-10.

Trawl samples were placed on ice and returned to MRD's Dauphin Island laboratory for processing.

Fishery-dependent collections

Southern Flounder were collected from the commercial and recreational fisheries throughout Alabama's coastal waters. Opportunistic collections of the recreational fishery came from boat access points at Little Lagoon and various locations in Mobile Bay (Figure 1). Commercial samples were purchased from two commercial fish houses along the eastern shore of Mobile Bay. Exact locality of harvest for commercially and recreationally harvested flounder were unknown.

Laboratory processing

Several measurements were taken from Southern Flounder including length (mm), weight (g), and macroscopic inspection of gonads. Additionally, we removed stomachs (preserved in 95% ethanol), sagittal otoliths, and a muscle tissue sample from the ocular (left) side of each individual. Tissue samples were freeze dried in a Labconco FreeZone 2.5 at -50°C for ≤ 5 days and ground to a homogenous powder using a stainless steel mortar and pestle. Samples were processed for bulk carbon and nitrogen isotopic composition (hereafter $\delta^{13}C$ and $\delta^{15}N$) and nitrogen compound specific amino acids (AA-CSIA) at the University of Hawai´i at Mānoa's Biogeochemical Stable Isotope Facility. Detailed descriptions of $\delta^{13}C$, $\delta^{15}N$, and AA-CSIA methodology and instrumentation used for this study can be found in Hannides et al. (2009), Dale et al. (2011), and Bradley et al. (2015). Briefly, $\delta^{13}C$ and $\delta^{15}N$ values of Southern Flounder muscle tissue were analyzed on a Costech ECS 4010 Elemental Combustion System coupled to an isotope

ratio mass spectrometer (Thermo Finnigan DELTAplus XP or DELTA V Advantage) via a Conflo IV interface. International glycine reference materials and in-house standards were analyzed in triplicate prior to, after, and between every 6-12 samples to assess instrumental drift. All bulk isotope data had an accuracy of ± 0.2‰. On a subset of samples (n = 16), AA-CSIA of derivatized samples was conducted with a Thermo Scientific DELTA V Plus or MAT 253 mass spectrometer interfaced to a Trace GC gas chromatograph via a GC-C III combustion furnace. Accuracy and precision were determined by co-injecting internal reference compounds (L-2 Aminoadipic acid (AAA) and L-(+)-Norleucine (Nor)) of known nitrogen isotopic composition with Southern Flounder tissue samples. The mean difference between known and measured values for AAA and Nor was 0.84‰ ± 0.77‰ standard deviation (SD). Samples were analyzed in triplicate and isotopic accuracy of amino acids analyzed in this study (glutamic acid, glycine, lysine, and phenylalanine) averaged 0.38‰ SD and ranged from 0.27‰ – 0.61‰ SD. Individuals selected for AA-CSIA encompassed the range of sizes across collections from the Delta and Mobile Bay by Clemson University. All isotope values were reported in δ-notation (as ‰) relative to Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 for carbon and nitrogen, respectively.

Residency patterns and contingent types of Southern Flounder were determined with otolith chemistry. Detailed descriptions of otolith chemistry methodology and instrumentation used in this study can be found in Chapter 1. Briefly, sectioned and polished otoliths were analyzed for strontium (^{88}Sr) and calcium (^{43}Ca) elemental signatures using an Agilent 7700z quadrupole inductively coupled plasma mass

spectrometer (ICPMS) coupled to a 213 nm Nd:YAG NWR laser at the Dauphin Island Sea Lab (DISL) instrumentation lab. Otoliths were ablated along a straight transect from the core to the distal edge parallel to the sulcal groove. Standard methods for otolith cleaning and instrumental precision analyses were conducted to assess limits of detection and correct for instrumental drift (Longerich et al. 1996; Gover et al. 2014). Significant shifts and smoothed means in time series otolith Sr:Ca ratios were analyzed using a regime shift detection algorithm across the entire laser ablation transect (i.e., lifetime of the individual flounder) (Rodionov 2004). Sr:Ca ratios were used as a marker for salinity exposure.

Analysis of ambient water chemistry, salinity, and otolith edge chemistry showed that a threshold of 1.71 mmol:mol Sr:Ca could be used to indicate habitat-use above or below 1 psu salinity (see Chapter 1). Any Sr:Ca value above this threshold indicated estuarine habitat-use and anything below, tidal freshwater habitat-use. The proportion of values above and below the Sr:Ca threshold were quantified across the entire laser ablation transect, and flounder were classified into one of three lifetime contingency types. Freshwater contingents had ≥ 90% of lifetime Sr:Ca values below the threshold, while estuarine contingents had ≥ 90% of lifetime Sr:Ca values above the threshold. Individuals with less than 90% of lifetime Sr:Ca values in either habitat-use category were classified as transient.

To assess the food web dynamics of Southern Flounder, stomachs were macroscopically inspected for prey. Prey items were identified to the lowest taxonomic level, counted, and measured to the nearest mm. Standard length, carapace width, and

rostrum and telson measurements were completed for fish, crabs, and shrimps, respectively.

Statistical analysis

Spatial patterns in isotopic signatures

To test if tissue bulk isotopic ratios (δ^{13}C and δ^{15}N) in Clemson University (n = 89) and MRD (n = 14) collected Southern Flounder differed across the seasonal salinity gradient in the Delta and Mobile Bay, samples were analyzed using permutational multivariate analysis of variance (PERMANOVA) with Euclidean distance dissimilarity matrices on two separate models. We compared the fit of two PERMANOVA models using Akaike information criterion (AICc) corrected for small sample size (Burnham and Anderson 2002), which varied in spatial resolution, to investigate the spatial scale at which tissue isotopic ratios differed. The broad scale PERMANOVA model included two groups, one for flounder collected in Mobile Bay and one for flounder collected in the Delta. The regional model included groups from four spatial regions (i.e., Delta, Upper Bay, Middle Bay, and Lower Bay; Figure 1). Additionally, principal coordinate analysis (PCoA) ordination plots were generated from a multivariate Levene's homoscedasticity test from the package 'vegan' in R version 3.6.1 (R Development Core Team 2019). Ordination plots were used to visually assess distributions and overlap in δ^{13}C and δ^{15}N values around centroid grouping variables for each model above. All analyses were completed in R version 3.6.1 (R Development Core Team 2019).

Bulk isotopic ratios from Southern Flounder with known collection locations were used to assign a location of harvest for commercially and recreationally harvested

flounder. Harvest locations were assigned to fishery-independent samples using quadratic discriminate analysis (QDA). Flounder were assigned into one of four regions (Delta, Upper Bay, Middle Bay, Lower Bay). QDA accuracy was assessed using leave-one-out cross validation with uninformative priors (0.25 for each region). Lastly, fishery-dependent samples were graphical inspected for similarities with assignment location.

Relating Isotopes Ratios to Lifetime Residency Patterns

Otolith chemistry habitat-use delineation is limited to interpretations above or below 1 psu salinity. Additionally, laser ablation techniques provide limited insight into habitat-use of older flounder due to daily otolith accretions becoming concentrated along the edge of otoliths. To determine recent habitat-use and dietary influences, Southern Flounder bulk isotopic ratios were regressed with otolith derived contingent types (freshwater, transient, estuarine). A PERMANOVA model assessed bulk isotopic ratios by each contingent type for Southern Flounder collected by Clemson and MRD.

Trophic position

Inferring trophic position from bulk isotopic values requires knowing baseline isotopic values in the ambient environment. This includes all levels of the food web from primary producers to recently consumed prey. For highly migratory species, such as Southern Flounder, this would entail prey collections across several different ecosystems. Alternatively, trophic position can be derived from AA-CSIA, in which select amino acids can be used to interpret primary production (source) and trophic interactions. To calculate amino acid derived trophic position (TP_{CSIA}), individuals analyzed for AA-CSIA (n = 16) were assessed using a modified equation from Chikaraishi et al. (2009):

$$TP_{CSIA} = ((\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta) / TEF) + 1 \qquad \textit{Equation 1}$$

where β is the difference in δ^{15}N values from trophic (glutamic acid; $\delta^{15}N_{Glu}$) and source (phenylalanine; $\delta^{15}N_{Phe}$) amino acids between primary producers, and the trophic enrichment factor (TEF) is the relative change in trophic and source amino acids with each trophic transfer. We used constant values for β (3.6 ± 0.5‰) and TEF (5.7 ± 0.3‰) developed from Bradley et al. (2015), as the constants developed in their study are the most relevant to ours and cover a wide range of trophic levels and species. To test differences in trophic position across Alabama's coastal waters, flounder were grouped to the smallest spatial resolution possible. Since replicate samples from Upper Bay were not represented in AA-CSIA samples, the smallest spatial scale consisted of regional groupings into Delta, Middle Bay, Lower Bay. To test for differences in TP_{CSIA} between regions, values were regressed with flounder grouped by region using analysis of variance (ANOVA).

Bulk δ^{15}N were used to assess trophic position for a larger set of individuals (n = 128). To calculate trophic position from bulk δ^{15}N, a spatial baseline correction factor was derived from the subset of AA-CSIA samples. Since β and TEF are unknown for bulk δ^{15}N, a weighted mean δ^{15}N value from three source amino acids (glycine, lysine, and phenylalanine) was calculated for each region using the following equation from Bradley et al. (2015):

$$\delta^{15}N_{source} = \frac{\sum \frac{\delta^{15}N_x}{\sigma_x^2}}{\sum \frac{1}{\sigma_x^2}} \qquad \textit{Equation 2}$$

where $\delta^{15}N_x$ is the $\delta^{15}N$ from each source amino acid and σ_x is the standard deviation of triplicate isotopic analysis for each source amino acid. A grand mean was calculated by averaging the weighted means from the three source amino acids. The grand mean was then subtracted from individual bulk $\delta^{15}N$ samples to remove baseline $\delta^{15}N$ values and create a proxy for trophic position ($\Delta\delta^{15}N$) for each individual Southern Flounder. $\Delta\delta^{15}N$ values were regressed with sex, total length, weight, temporal stability (month to month consistency in $\Delta\delta^{15}N$ values), and contingent types to assess physiological and ecological impacts on trophic position.

Stomach content analysis

Southern Flounder stomach content data were separated into individuals collected in Mobile Bay or the Delta. Frequency of occurrence was determined by summing the number of times a prey item occurred within non-empty stomachs divided by the total number of non-empty stomachs. Consumed fish prey were separated to the family level, while crustaceans were grouped into either shrimp or crab. To calculate differences in size of fish prey between locations, fish prey lengths were divided by flounder lengths to determine relative size of fish prey to flounder size

RESULTS

A total of 128 Southern Flounder were collected and processed for muscle tissue stable isotopes. Of those, 27 were collected by fishery-dependent sources and 101 by fishery-independent sources. Clemson University collections comprised the majority of flounder samples processed for bulk $\delta^{13}C$ and $\delta^{15}N$ (n = 89) and all samples processed for

AA-CSIA (n = 16). Within Clemson samples, the majority (n = 67) were collected in the Delta using boat electrofishing, while Mobile Bay sample sizes using gillnets (n = 19) and beams trawls (n = 3) were much smaller. MRD collections (n = 14) encompassed the majority of Alabama's coastal waters south of I-10 (Figure 1). Fishery-dependent samples consisted primarily of purchases from commercial fish houses (n = 23), but also included a few fish harvested by the recreational fishery (n = 4). Collected flounder were primarily females (80%) and ranged in age from 0 – 2, and spanned a range of lengths (113 – 547 mm) and weights (12 – 1913 g).

Bulk isotopes

Analysis of fishery-dependent collected flounder revealed significant differences ($p < 0.01$) in bulk $\delta^{13}C$ values between all collection regions (i.e., Delta, Upper Bay, Middle Bay, and Lower Bay). Bulk $\delta^{13}C$ values gradually increased from north to south (Figure 2). Bulk $\delta^{15}N$ values were significantly different ($p < 0.01$) between regions with no spatial trend (Figure 2). No significant differences were detected in $\delta^{13}C$ or $\delta^{15}N$ between years among regions with flounder collections in both years of this study ($p > 0.2$), indicating annual site-specific stability for the duration of this study.

Multivariate analysis of $\delta^{13}C$ and $\delta^{15}N$ ratios for each flounder allowed further spatial delineation. The best PERMANOVA model describing spatial variation in isotopic differences, determined by AICc, was the regional model (R^2 = 0.85, Table 1). An ordination plot of this model revealed connectivity between Lower Bay and Middle Bay regions, but differences in isotopic ratios between these regions and the Delta and Upper Bay (Figure 3).

QDA analysis of bulk $\delta^{13}C$ and $\delta^{15}N$ ratios had an accuracy of 88%. Fishery-dependent samples with unknown harvest locations were classified into one of three regions. Eleven (40%), four (14%), and 13 (46%) fishery-dependent samples were classified as Upper Bay, Middle Bay, and Lower Bay harvest location, respectively (Figure 4). No samples were classified as being harvested within the Delta. Visual inspection revealed a group of seven individuals (25%) exhibiting enriched $\delta^{13}C$ (> -20‰) and depleted $\delta^{15}N$ (< 11‰) consistent with one fishery-dependent flounder with a known harvest location in Little Lagoon (Figure 4). Although QDA assigned Middle Bay and Lower Bay harvest locations for these individuals, their isotopic signatures are visually different from other flounder in those two regions. These flounder were likely harvested outside of the Middle Bay and Lower Bay regions, but within Alabama's waters not surveyed by fishery-independent collections (such as Little Lagoon).

Graphical inspection of bulk $\delta^{13}C$ and $\delta^{15}N$ ratios displayed differences between freshwater, estuarine, and transient lifetime contingents (Figure 5). PERMANOVA of bulk $\delta^{13}C$ and $\delta^{15}N$ ratios by contingent types revealed significant differences between contingent types ($R^2 = 0.71$, $p = 0.001$). Similar to lifetime otolith residency patterns, transient contingents exhibited isotopic overlap with both estuarine and freshwater contingents. Additionally, transient flounder exhibited a much wider range of dissimilarity from one another and exhibited isotopic values outside the range of estuarine and freshwater contingents (Figure 5).

Trophic position

The trophic position of Southern Flounder determined through AA-CSIA was 2.80 ± 0.12 (mean ± standard error), 2.73 ± 0.10, and 3.36 ± 0.05 for the Delta, Middle Bay, and Lower Bay, respectively. Trophic position was similar between the Delta and Middle Bay sites ($p = 0.98$), but elevated at Lower Bay sites ($p = 0.02$). After combining lysine, glycine, and phenylalanine $\delta^{15}N_{source}$ values, the grand mean baseline correction factor by region was 8.64‰, 6.04‰, and 8.07‰ for the Delta, Middle Bay, and Lower Bay, respectively. Proxy trophic position values ($\Delta\delta^{15}N$), normalized with baseline correction factors, were highly correlated with trophic positions developed through AA-CSIA ($p = 0.003$, $R^2 = 0.44$; Figure 6).

In agreeance with AA-CSIA values, $\Delta\delta^{15}N$ showed higher trophic levels for flounder collected in Mobile Bay than those collected in the Delta (Figure 7). Estuarine contingents also exhibited higher trophic levels than freshwater contingents ($p = 0.04$), but statistically similar trophic levels with transient contingents ($p = 0.86$). Freshwater contingents also exhibited similar trophic levels with transient contingents ($p = 0.12$). As $\Delta\delta^{15}N$ did not differ ($p > 0.4$) between months of harvest at sites where Southern Flounder were collected over several months (i.e., March, May, June, and July), we assumed consistent trophic dynamics across these time periods. Additionally, no significant differences in $\Delta\delta^{15}N$ were exhibited between males and females ($p = 0.36$), fish length ($p = 0.19$), or fish weight ($p = 0.29$).

Stomach contents

Fish comprised the majority of Southern Flounder diets in this study. In Mobile Bay, flounder consumed mostly fish with some shrimp, while individuals collected in the

Delta consumed fish, shrimp, and crabs (Figure 8). Specifically, fish prey consumption by flounder in freshwater habitats consisted primarily of sunfish and largemouth bass (Centrarchidae), while fish prey in estuarine habitats consisted primarily of drum and seatrout (Sciaenidae) or anchovies (Engraulidae). On average, consumed fish prey in Mobile Bay were larger relative to flounder body size than fish prey in the Delta (Figure 9).

DISCUSSION

This study effectively used two natural tags and stomach content analysis to determine food web dynamics and trophic ecology of Southern Flounder in Alabama's coastal waters. Although samples consisted of proportionally more females than males, no difference was detected between male and female isotopic signatures, indicating results from this study are indicative of food web dynamics for both sexes. Demonstrated by both AA-CSIA and $\Delta\delta^{15}$N values, Southern Flounder collected in Lower Bay are consuming prey at higher trophic levels than those in Middle Bay and the Delta. It is important to note that fish in Mobile Bay were, on average, 90mm larger than those in the Delta. However, length and weight had no significant effect on trophic position. Additionally, stomach content analysis suggests flounder in Delta are consuming smaller prey relative to body size and a wider diversity of prey, including shrimp and crab which have lower trophic position signatures than fish prey (Akin and Winemiller 2008). Overall, region-specific analyses indicated variability in Southern Flounder food web dynamics across Alabama's coastal waters.

Bulk $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios allowed inferences of regional-scale differences across Alabama's coastal waters. The regional model revealed significant differences in flounder isotopic values from north to south across Alabama's coastal waters. As flounder isotopic values are indicative of the supply of $\delta^{13}C$ and $\delta^{15}N$ from prey field, these values indicate that isotopes are different within prey across regions (Ishikawa 2018). Bulk $\delta^{13}C$ and $\delta^{15}N$ spatial patterns demonstrated that a wide diversity of isotopically distinct environments across Alabama's salinity gradient are contributing to the commercial and recreational flounder fisheries. Visual inspection of these collections revealed locally distinct isotopic values, such as those experienced in Little Lagoon. Distinct isotopic values are likely caused by locally influenced nutrient inputs (Fry 2008). For Little Lagoon specifically, depleted $\delta^{13}C$ and enriched $\delta^{15}N$ values were likely a result of groundwater sources and seagrasses rather than upstream fluvial processes, like signatures exhibited in the Delta and Mobile Bay (Su et al. 2012). Little Lagoon is a small portion of Alabama's coastal waters that potentially contributes a disproportionate number of recruits to the commercial and recreational fisheries. To understand proportional contributions to fishery-dependent samples, additional samples would need to be collected across all of Alabama's distinctly different isotopic habitats.

Southern Flounder $\delta^{13}C$ values were influenced by local carbon sources. Sources exhibit a gradual enrichment in $\delta^{13}C$ from the Delta to Mobile Bay. These sources included C3 terrestrial plants (~ -30 to -20‰), marine algae and phytoplankton (~ -20 to -15‰), and seagrass (~ -15 to 10‰) (Fry 2008). Flounder collected by MRD in the Upper Bay displayed $\delta^{13}C$ values consistent with both Delta and Middle Bay signatures,

indicating Upper Bay as a likely transition point between depleted and enriched $\delta^{13}C$ values. Several fishery-dependent flounder were classified as harvested within Upper Bay. While these flounder may have been harvested in the Upper Bay, Mobile Bay has multiple freshwater tributaries with highly forested watersheds (Lehrter 2008; Mortazavi et al. 2012). The depleted $\delta^{13}C$ in flounder tissues may be a result of residency within Upper Bay, or $\delta^{13}C$ values could have been influenced by prey contributions from productive tributaries along the southern portion of Mobile Bay. Samples would need to be collected and compared within all of Mobile Bay's major tributaries to gain a better understanding of watershed-specific effects on Southern Flounder $\delta^{13}C$ values in Alabama.

One challenge in interpreting stable isotopes is understanding if fish isotopic values represent the local trophic ecology or if isotopes are representative of distinctly different food webs from a recently immigrated individual. As Southern Flounder in this study ranged in size from 113 to 547 mm, the time required to reach equilibrium to local isotopic conditions could range from days or months (Bosley et al. 2002; Buchheister and Latour 2010). Additionally, turnover and fractionation rates can be highly variable at different trophic levels (Bosley et al. 2002; Witting et al. 2005). Based on our distinct region-specific isotopic results, flounder appeared to be in equilibrium with site of collection and exhibit high site fidelity (did not migrate across isotopically distinct habitats) prior to collection. Quantifying isotopic breaths of region-specific prey and controlled experiments quantify turnover and fractionation rates of Southern Flounder

would allow for greater ability to infer short-term movement patterns from tissue isotopic signatures.

Southern Flounder were classified into one of three contingency types based on otolith chemistry signatures (see Chapter 1). Otolith chemistry displayed broad scale patterns in habitat use across salinity gradients, but lacked the resolution of short-term movements. By combining two natural tags, we gained a much greater understanding of seasonal movement dynamics of these contingents. Estuarine and freshwater contingents exhibited isotopic signatures consistent with Middle to Lower Bay and the Delta, respectively. For these two contingency types, isotopes confirmed short-term isotopic signatures were reflective of lifetime residency patterns. Transient contingents had isotopic signatures aligning with estuarine and freshwater residents, but also had values not observed by these two contingency types. This indicates transient flounder may have recently moved into collection locations or consumed recently immigrated prey from locations outside the area of this study (i.e., offshore or nearby estuaries).

This study builds on previous research supporting the concept of distinct migratory contingents in Southern Flounder (Farmer et al. 2013; Nims and Walther 2014). To fully understand the resilience of a population and implications of distinct migratory contingents on spawning stock biomass, future studies would need to quantify annual variability in contributions by each contingency type (Kraus and Secor 2004). Additionally, understanding harvest dynamics and Southern Flounder life history could lead to improvements in future stock assessments. Flounder spawning occurs in offshore habitats in fall to winter months, resulting in a rapid spawning migration out of estuarine

habitats in the fall (Stokes 1977; Fischer and Thompson 2004). Assuming larger flounder captured at the mouth of estuaries to Gulf of Mexico during fall are migrating to spawning grounds, otolith chemistry and isotopic analysis of these individuals could determine location of residency prior to the migration. This could provide insight into habitat-specific contributions to the spawning stock biomass by various contingency types. This greater understanding provided by natural tags could aid managers in selecting priority areas of ongoing habitat conservation efforts across Alabama's coastal regions (e.g., Forever Wild Land Trust, Alabama Coastal Management Program). Overall, maintaining the diversity of migratory patterns and the habitats in which they occupy, could lead to the sustainability and resilience of fish populations to natural and anthropogenic stressors (Schindler et al. 2010).

LITERATURE CITED

Able, K. W. 2005. A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. Estuarine, Coastal and Shelf Science 64(1 SPEC. ISS.):5–17.

Akin, S., and K. O. Winemiller. 2008. Body size and trophic position in a temperate estuarine food web. Acta Oecologica 33(2):144–153.

Beck, M. W., K. L. J. Heck, K. W. Able, D. L. Childers, D. B. Eggleston, B. M. Gillanders, B. Halpern, C. G. Hays, K. Hoshino, T. J. Minello, R. J. Orth, P. F. Sheridan, and M. P. Weinstein. 2001. The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates. BioScience 51(8):633–641.

Bosley, K. L., D. A. Witting, R. C. Chambers, and S. C. Wainright. 2002. Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder pseudopleuronectes americanus with stable isotopes. Marine Ecology Progress Series 236(July):233–240.

Bradley, C. J., N. J. Wallsgrove, C. A. Choy, J. C. Drazen, E. D. Hetherington, D. K. Hoen, and B. N. Popp. 2015. Trophic position estimates of marine teleosts using amino acid compound specific isotopic analysis. Limnology and Oceanography: Methods 13(9):476–493.

Buchheister, A., and R. J. Latour. 2010. Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (Paralichthys dentatus). Canadian Journal of Fisheries and Aquatic Sciences

67(3):445–461.

Buchheister, A., and R. J. Latour. 2011. Trophic ecology of summer flounder in lower Chesapeake Bay inferred from stomach content and stable isotope analyses. Transactions of the American Fisheries Society 140(5):1240–1254.

Buckland, A., R. Baker, N. Loneragan, and M. Sheaves. 2017. Standardising fish stomach content analysis: The importance of prey condition. Fisheries Research 196(August):126–140. Elsevier.

Burnham, K. P., and D. R. Anderson. 2002. Avoiding Pitfalls When Using Information-Theoretic Methods Author (s): David R . Anderson and Kenneth P . Burnham. The Journal of Wildlife Management 66(3):912–918.

Busst, G. M. A., and J. R. Britton. 2018. Tissue-specific turnover rates of the nitrogen stable isotope as functions of time and growth in a cyprinid fish. Hydrobiologia 805(1):49–60. Springer International Publishing.

Campana, S. 1999. Chemistry and composition of fish otoliths:pathways, mechanisms and applications. Marine Ecology Progress Series 188:263–297.

Chikaraishi, Y., N. O. Ogawa, Y. Kashiyama, Y. Takano, H. Suga, A. Tomitani, H. Miyashita, H. Kitazato, and N. Ohkouchi. 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnology and Oceanography: Methods 7(11):740–750.

Dale, J. J., N. J. Wallsgrove, B. N. Popp, and K. N. Holland. 2011. Nursery habitat use and foraging ecology of the brown stingray Dasyatis lata determined from stomach contents, bulk and amino acid stable isotopes. Marine Ecology Progress Series

433:221–236.

Dzwonkowski, B., K. Park, H. Kyung Ha, W. M. Graham, F. J. Hernandez, and S. P. Powers. 2011. Hydrographic variability on a coastal shelf directly influenced by estuarine outflow. Continental Shelf Research 31(9):939–950. Elsevier.

Elsdon, T., B. Wells, S. Campana, B. Gillanders, C. Jones, K. Limburg, D. Secor, S. Thorrold, and B. Walther. 2008. Otolith Chemistry To Describe Movements And Life-History Parameters Of Fishes (1):297–330.

Farmer, T. M., D. R. DeVries, and J. E. Gagnon. 2013. Using seasonal variationin otolith microchemical composition to indicate largemouth bass and southern flounder residency patterns across an estuarine salinity gradient. Transactions of the American Fisheries Society 142(5):1415–1429.

Fischer, A. J., and B. A. Thompson. 2004. The age and growth of southern flounder, *Paralichthys lethostigma,* from Louisiana estuarine and offshore waters. Bulletin of Marine Science 75(1):63–77.

Flowers, A. M., S. D. Allen, A. L. Markwith, and L. M. Lee. 2019. Stock Assessment of Southern Flounder (Paralichthys lethostigma) in the South Atlantic , 1989 – 2017:1–228.

Fry, B. 2008. Stable Isotope Ecology. Page Springer.

Glass, L. A., J. R. Rooker, R. T. Kraus, and G. J. Holt. 2008. Distribution, condition, and growth of newly settled southern flounder (Paralichthys lethostigma) in the Galveston Bay Estuary , TX. Journal of Sea Research 59:259–268.

Gover, T. R., M. K. Nims, J. J. Van Tassell, P. D. Collingsworth, J. W. Olesik, S. A.

Ludsin, and E. A. Marschall. 2014. How Much Cleaning is Needed When Processing Otoliths from Fish Larvae for Microchemical Analysis? Transactions of the American Fisheries Society 143(3):779–783.

Hannides, C. C. S., B. N. Popp, M. R. Landry, and B. S. Graham. 2009. Quantification of zooplankton trophic position in the North Pacific Subtropical Gyre using stable nitrogen isotopes. Limnology and Oceanography 54(1):50–61.

Herzka, S. Z. 2005. Assessing connectivity of estuarine fishes based on stable isotope ratio analysis. Estuarine, Coastal and Shelf Science 64(1 SPEC. ISS.):58–69.

Hyslop, E. J. 1980. Stomach contents analysis—a review of methods and their application. Journal of Fish Biology 17(4):411–429.

Ishikawa, N. F. 2018. Use of compound-specific nitrogen isotope analysis of amino acids in trophic ecology: assumptions, applications, and implications. Ecological Research 33(5):825–837. Springer Japan.

Kennish, M. J. 2002. Environmental threats and environmental future of estuaries. Environmental Conservation 29(1):78–107.

Kraus, R. T., and D. H. Secor. 2004. Dynamics of white perch Morone americana population contingents in the Patuxent River estuary, Maryland, USA. Marine Ecology Progress Series 279(September 2004):247–259.

Lehrter, J. C. 2008. Regulation of eutrophication susceptibility in oligohaline regions of a northern Gulf of Mexico estuary, Mobile Bay, Alabama. Marine Pollution Bulletin 56(8):1446–1460.

Longerich, H. P., S. E. Jackson, and D. Günther. 1996. Laser ablation inductively

coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. Journal of Analytical Atomic Spectrometry 11(9):899–904.

Lowe, M. R., S. A. Ludsin, B. J. Fryer, R. A. Wright, D. R. DeVries, and T. M. Farmer. 2011. Otolith Microchemistry Reveals Substantial Use of Freshwater by Southern Flounder in the Northern Gulf of Mexico. Estuaries and Coasts 35(3):907–910.

Macdonald, J. I., and D. A. Crook. 2010. Variability in Sr:Ca and Ba:Ca ratios in water and fish otoliths across an estuarine salinity gradient. Marine Ecology Progress Series 413(January):147–161.

McCutchan, J. H., W. M. Lewis, C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102(2):378–390.

Mortazavi, B., A. A. Riggs, J. M. Caffrey, H. Genet, and S. W. Phipps. 2012. The Contribution of Benthic Nutrient Regeneration to Primary Production in a Shallow Eutrophic Estuary ,. Estuaries and Coasts 35(3):862–877.

Nagelkerken, I., M. Sheaves, R. Baker, and R. M. Connolly. 2015. The seascape nursery: A novel spatial approach to identify and manage nurseries for coastal marine fauna. Fish and Fisheries 16(2):362–371.

Nelson, T. R., and S. P. Powers. 2020. Elemental Concentrations of Water and Otoliths as Salinity Proxies in a Northern Gulf of Mexico Estuary. Estuaries and Coasts. Estuaries and Coasts.

Nims, M. K., and B. D. Walther. 2014. Contingents of Southern Flounder from

Subtropical Estuaries Revealed by Otolith Chemistry. Transactions of the American Fisheries Society 143(3):721–731.

Peterson, B. J. 1999. Stable isotopes as tracers of organic matter input and transfer\rin benthic food webs: A review. Acta Oecologica 20(4):479–487.

Peterson, B. J., and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics 18(1):293–320.

Peterson, B. J., and R. W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnology and Oceanography 32(6):1195–1213.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an Integration of Landscape and Food Web Ecology: The Dynamics of Spatially Subsidized Food Webs. Annual Review of Ecology and Systematics 28(1):289–316.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83(3):703–718.

Powell, A. B., and F. J. Schwartz. 1979. Food of Paralichthys dentatus and P . lethostigma (Pisces : Bothidae) in North Carolina Estuaries. Coastal and Estuarine Research Federation 2(4):276–279.

Powers, S. P., M. Albins, and J. Mareska. 2018. An Assessment of Southern Flounder in Alabama Coastal Waters.

Rodionov, S. N. 2004. A sequential algorithm for testing climate regime shifts. Geophysical Research Letters 31(L09204).

Scharf, F. S., F. Juanes, and R. A. Rountree. 2000. Predator size - Prey size relationships

of marine fish predators: Interspecific variation and effects of ontogeny and body size on trophic-niche breadth. Marine Ecology Progress Series 208:229–248.

Schindler, D. E., R. Hilborn, B. Chasco, C. P. Boatright, T. P. Quinn, L. A. Rogers, and M. S. Webster. 2010. Population diversity and the portfolio effect in an exploited species. Nature 465(7298):609–612. Nature Publishing Group.

Schroeder, W. W., S. P. Dinnel, and W. J. Wiseman. 1990. Salinity Stratification in a River-Dominated Estuary. Estuaries 13(2):145–154.

Sheaves, M., R. Baker, I. Nagelkerken, and R. M. Connolly. 2014. True Value of Estuarine and Coastal Nurseries for Fish: Incorporating Complexity and Dynamics. Estuaries and Coasts 38(2):401–414.

Stokes, G. M. 1977. Life history studies of southern flounder (*Paralichthys lethostigma*) and gulf flounder (*P. albigutta*) in the Aransas Bay area of Texas. Texas Parks and Wildlife Department Technical Series 25:1–37.

Sturrock, A. M., C. N. Trueman, J. A. Milton, C. P. Waring, M. J. Cooper, and E. Hunter. 2014. Physiological influences can outweigh environmental signals in otolith microchemistry research. Marine Ecology Progress Series 500(Campana 1999):245–264.

Su, N., W. C. Burnett, K. T. Eller, H. L. MacIntyre, B. Mortazavi, J. Leifer, and L. Novoveska. 2012. Radon and radium isotopes, groundwater discharge and harmful algal blooms in Little Lagoon, Alabama. Interdisciplinary studies on environmental chemistry, Vol 6: Advanced environmental studies by young scientists (November 2015):329–338.

Thomas, S. M., and T. W. Crowther. 2015. Predicting rates of isotopic turnover across the animal kingdom: A synthesis of existing data. Journal of Animal Ecology 84(3):861–870.

Trueman, C. N., K. M. Mackenzie, and M. R. Palmer. 2012. Identifying migrations in marine fishes through stable-isotope analysis. Journal of Fish Biology 81(2):826–847.

VanderKooy, S. 2015. Management profile for the gulf and Southern flounder fishery in the Gulf of Mexico. Gulf States Marine Fisheries Commission (247).

Walther, B. D., and K. E. Limburg. 2012. The use of otolith chemistry to characterize diadromous migrations. Journal of Fish Biology 81(2):796–825.

Wells, R. J. D., J. H. Cowan, and B. Fry. 2008. Feeding ecology of red snapper Lutjanus campechanus in the northern Gulf of Mexico. Marine Ecology Progress Series 361(Bowen 1996):213–225.

Winemiller, K. O., S. Akin, and S. C. Zeug. 2007. Production sources and food web structure of a temperate tidal estuary: Integration of dietary and stable isotope data. Marine Ecology Progress Series 343:63–76.

Witting, D. A., R. C. Chambers, K. L. Bosley, and S. C. Wainright. 2005. Experimental evaluation of ontogenetic diet transitions in summer flounder (Paralichthys dentatus), using stable isotopes as diet tracers . Canadian Journal of Fisheries and Aquatic Sciences 61(11):2069–2084.

Vander Zanden, M. J., M. K. Clayton, E. K. Moody, C. T. Solomon, and B. C. Weidel. 2015. Stable isotope turnover and half-life in animal tissues: A literature synthesis.

PLoS ONE 10(1):1–16.

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in Delta 15N and Delta13C trophic fractionation: Implications for awuatic food web studies. Limnology and Oceanography 46(8):2061–2066.

TABLES AND FIGURES

Table 1. Model selection results from PERMANOVA and sum of squares AICc explaining dissimilarities in Southern Flounder (n = 103) bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values in Alabama's coastal waters. Models represent groupings by location of collection for fishery-independent flounder during the summers of 2018 and 2019 (Figure 1).

Model Name	K	RSS	AICc	Delta_AICc	AICcWt	R^2
Regional (Delta, Upper, Middle, Lower)	4	265.62	105.85	0	0.999	0.85
Broad-scale (Delta, Mobile Bay)	2	389.09	161.65	55.8	0.001	0.78

K = number of parameters; RSS = residual sum of squares; AICc, Akaike Information Criterion; AICcWt = model weights; R^2 = R^2 from PERMANOVA

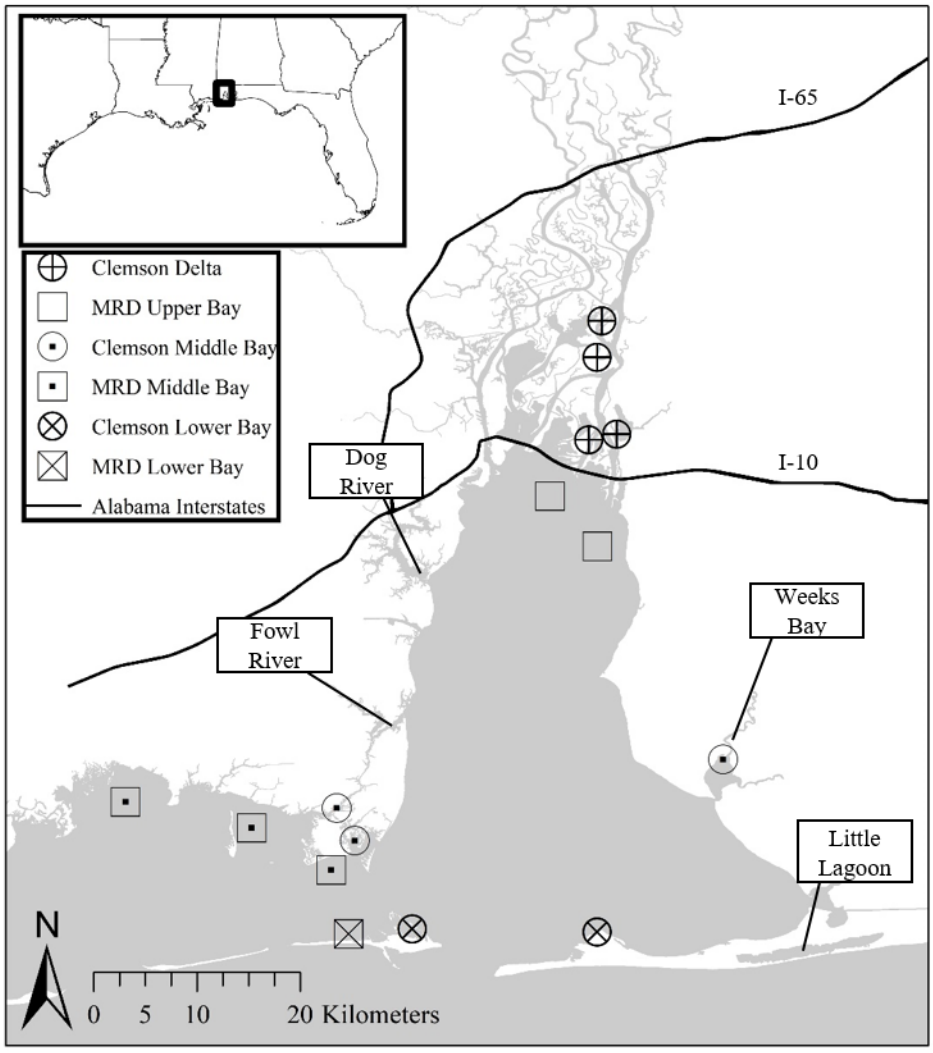

Figure 1. Map of the Mobile-Tensaw River Delta (north of I-10) and Mobile Bay (south of I-10) in Alabama showing the collection locations of Southern Flounder during 2018 and 2019. Nine Clemson University sampling locations (circles; two-letter site code) were classified into one of three regions (Delta, Middle Bay, Lower Bay) based on habitat and salinity similarities. Additional fishery-independent samples (squares) were provided by Alabama Marine Resource Division's Fishery Assessment and Monitoring

Program (MRD FAMP). MRD samples were classified into Upper Bay, Middle Bay, and Lower Bay.

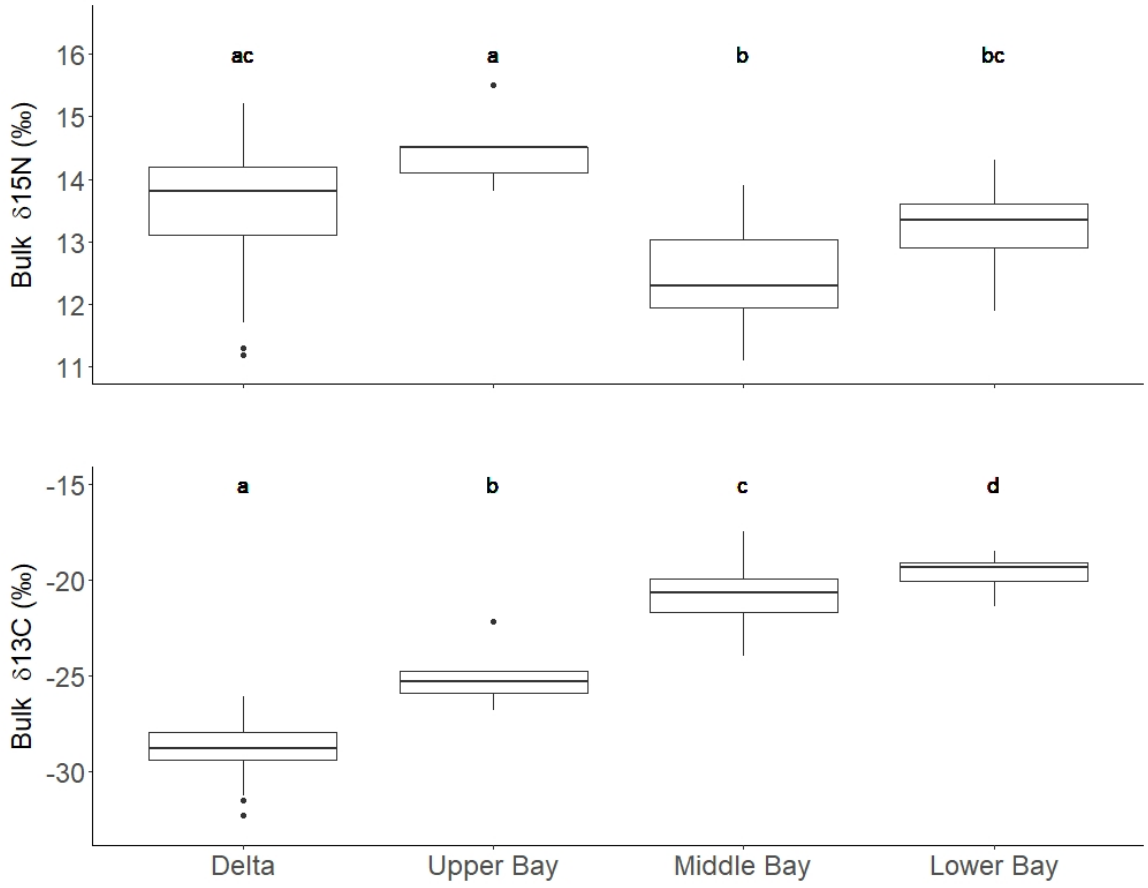

Figure 2. Bulk carbon (δ^{13}C) and nitrogen (δ^{15}N) by site of collection from Southern Flounder (n = 103) collected in Alabama's coastal waters by Clemson University and MRD from 2018 to 2019. δ^{13}C values were significantly different between all regions and gradually increased in a north to south direction. δ^{15}N values exhibited no trend across Alabama.

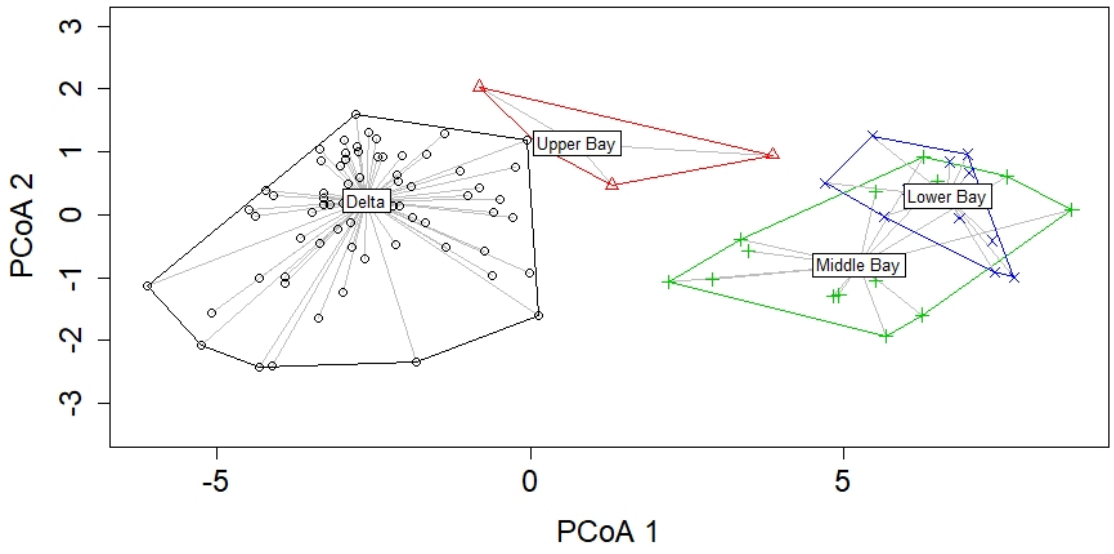

Figure 3. Principal coordinate analysis (PCoA) ordination plot of Southern Flounder bulk $\delta^{13}C$ and $\delta^{15}N$ ratios from a multivariate Levene's homoscedasticity test from the package 'vegan' in R version 3.6.1. Centroid points are flounder collection locations for fishery-dependent sample the Delta and Mobile Bay in 2018 and 2019 (Figure 1). Overlapping convex hulls indicated similarity in isotopic values.

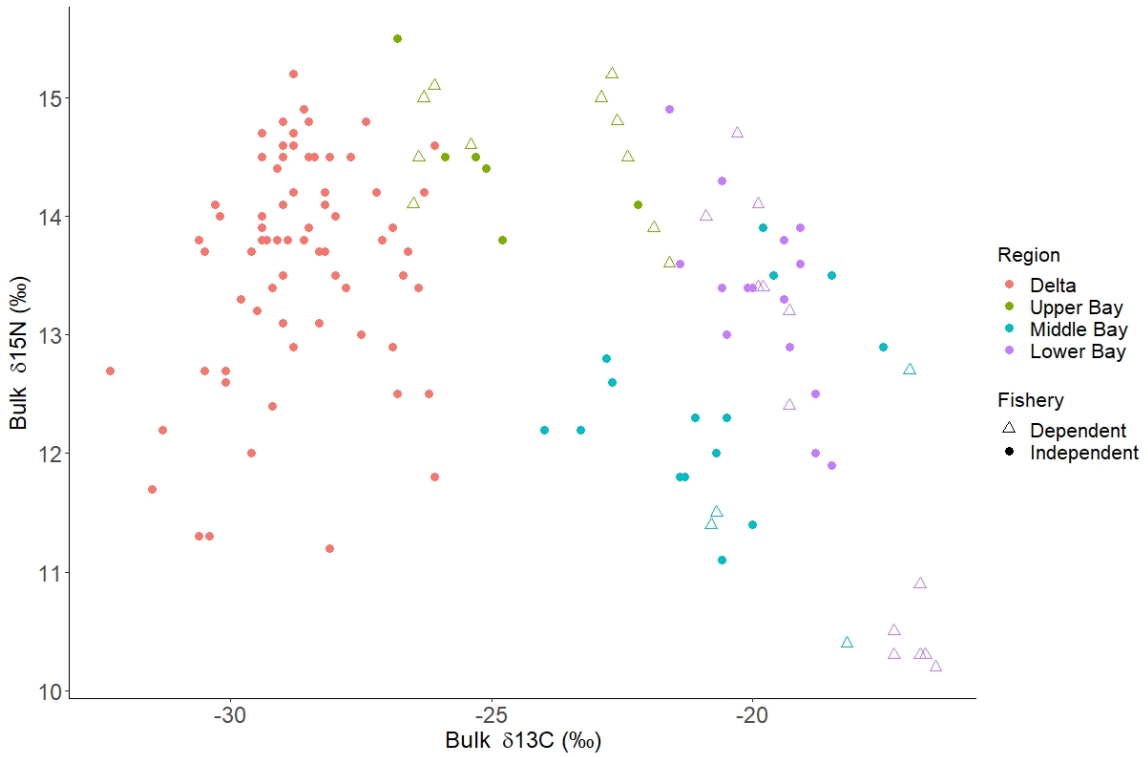

Figure 4. Southern Flounder (n = 128) bulk carbon (δ^{13}C) and nitrogen (δ^{15}N) ratios by location of collection for fishery-dependent (triangles) and fishery-independent (circles) collections during 2018 and 2019. Fishery-independent flounder were collected in four regions of Alabama's coastal waters including the Delta (red), Upper Bay (green), Middle Bay (blue), or Lower Bay (purple; Figure 1). Fishery-dependent collections were assigned location of collection using quadratic discriminate analysis. Flounder exhibiting enriched δ^{13}C (> -20‰) and depleted δ^{15}N (< 11‰) were outside the isotopic range of fishery-independent samples in Middle Bay and Lower Bay, but were consistent with one fishery-dependent flounder with a known harvest location in Little Lagoon (blue triangle with 10.4‰ δ^{15}N and 17.5‰ δ^{13}C).

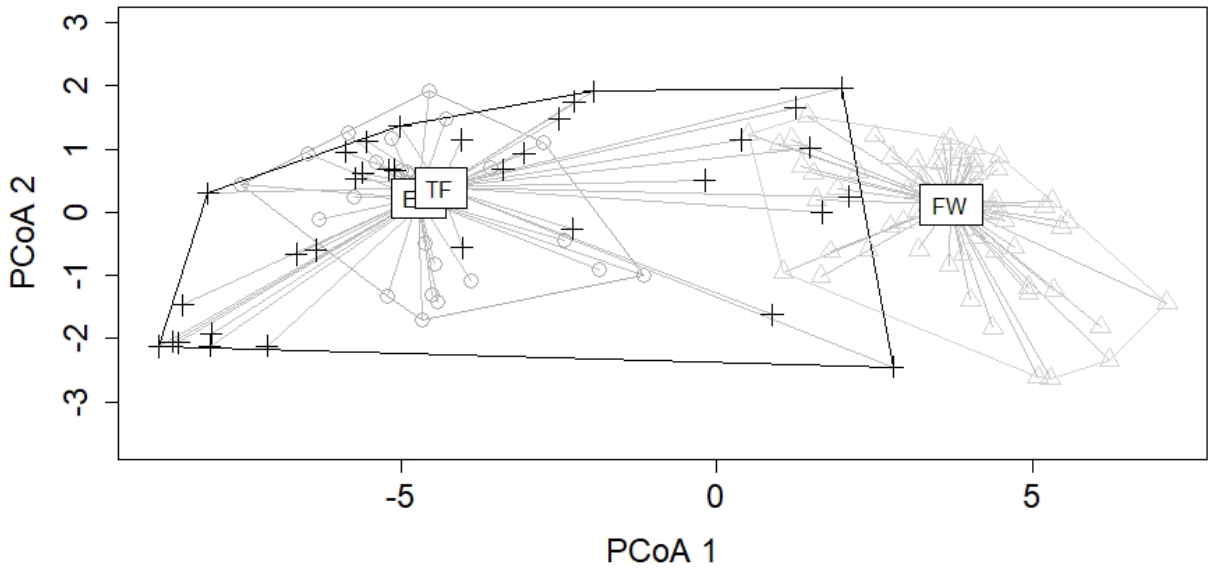

Figure 5. Ordination plot from the PERMANOVA output of Southern Flounder (N = 128) bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values by lifetime residency classification determined from otolith chemistry. Freshwater Southern Flounder contingents (FW) had significantly different isotope values from estuarine contingents (E). Convex hull of isotope values from transient contingents (TF) overlapped both estuarine and freshwater contingent, as well as consisted of unique values.

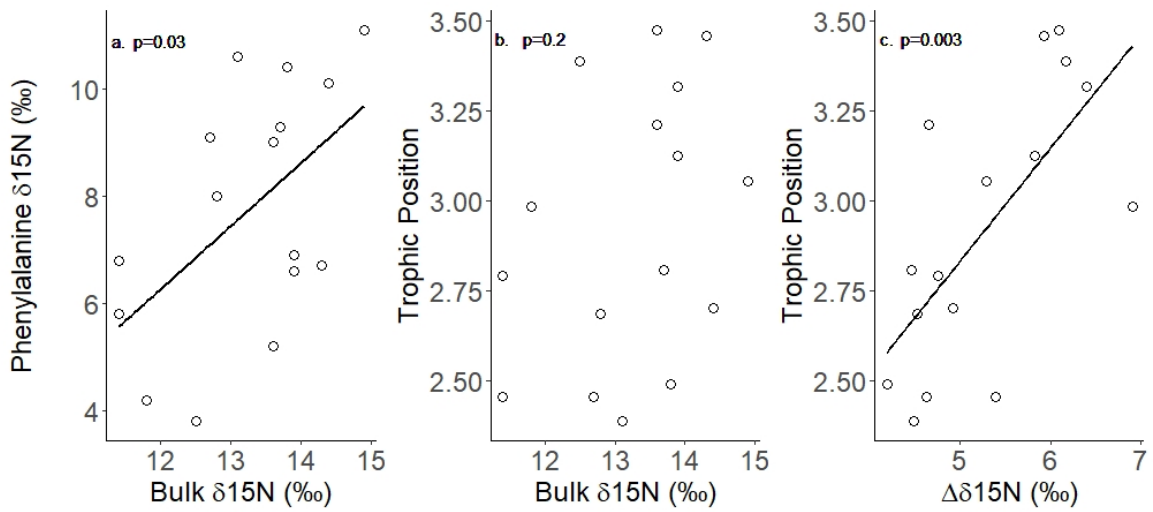

Figure 6. Bulk δ^{15}N values compared to a) the source amino acid phenylalanine and b) trophic position based on AA-CSIA values (using constants from Bradley et al. (2015)) for Southern Flounder (n = 16) from Alabama's coastal waters. Trophic position was also compared with c) a proxy for trophic position ($\Delta\delta^{15}$N) in which δ^{15}N values were corrected for baseline values by region of collection.

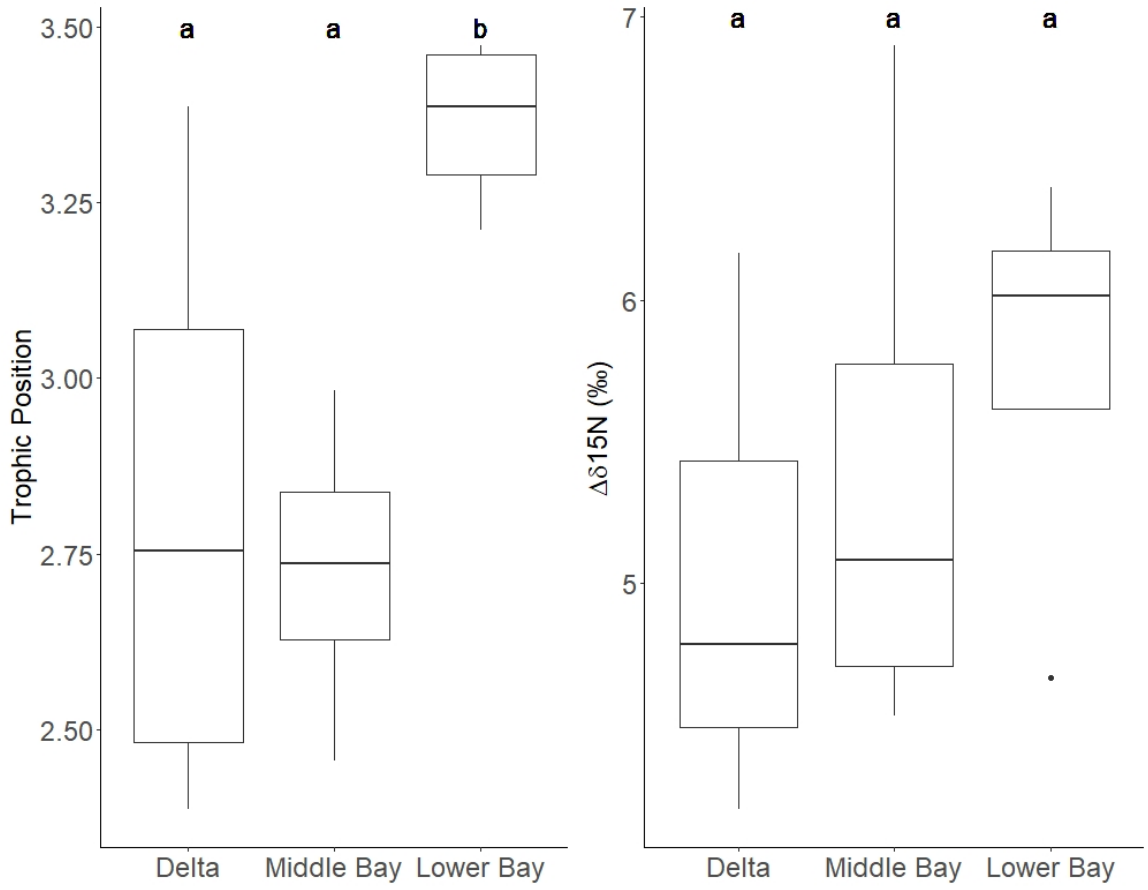

Figure 7. Trophic position of Southern Flounder (n = 16) calculated from AA-CSIA (left) and a proxy for trophic position ($\Delta\delta^{15}$) (right). Southern Flounder were collected in the Delta and Mobile Bay in Alabama by Clemson University during 2018 and 2019.

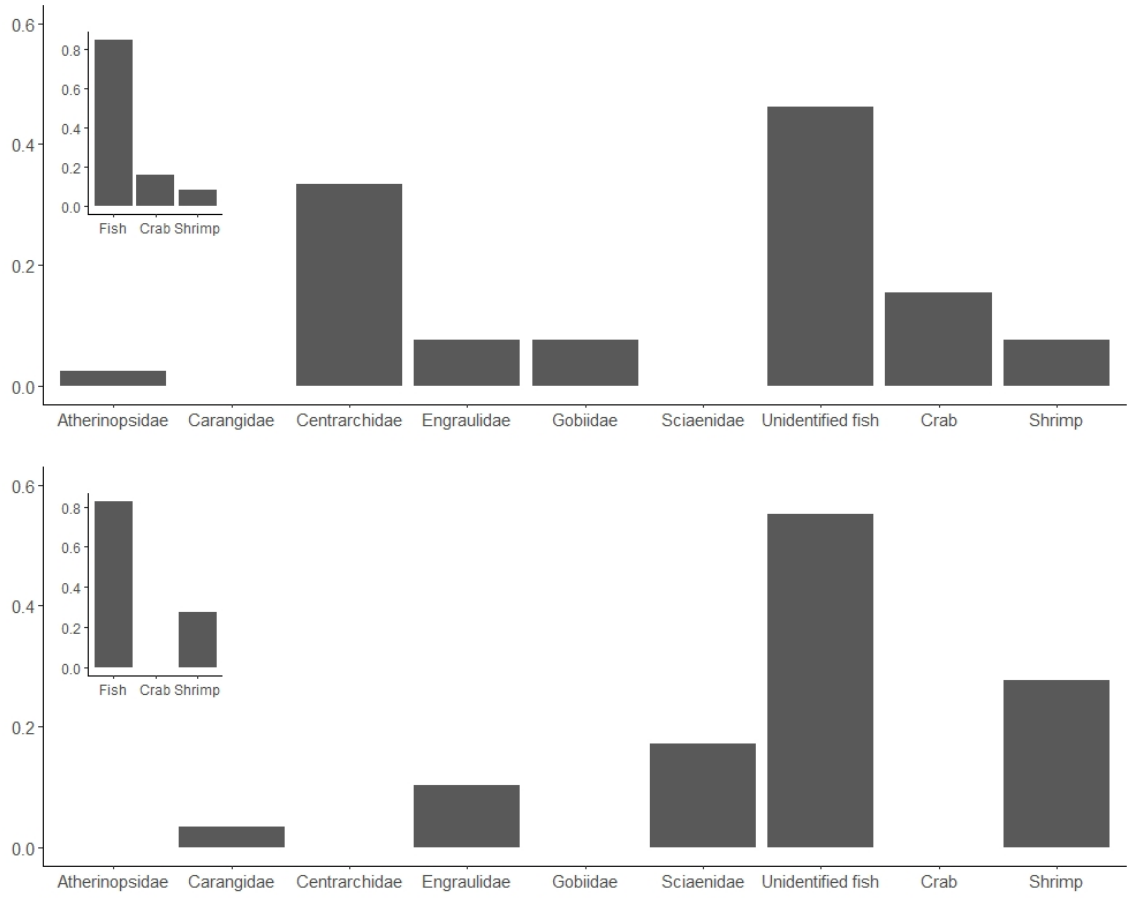

Figure 8. Prey item frequency of occurrence in Southern Flounder stomachs from Alabama's coastal waters during 2018 and 2019. Southern Flounder collected in the Delta (top, *n* = 39) consumed fish, shrimp, and crabs, while individuals collected in Mobile Bay (bottom, *n* = 28) consumed fish and shrimp. Plot inserts represent prey groupings, while plots separate fish prey by family.

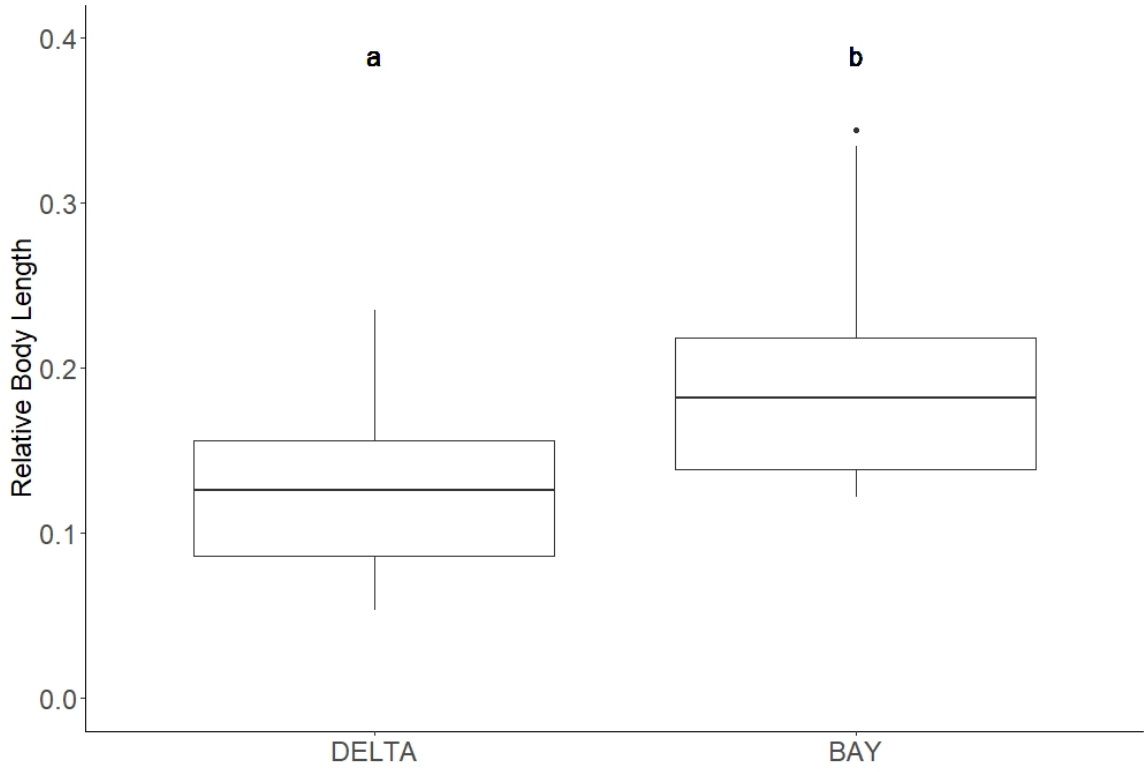

Figure 9. Stomach content analysis of Southern Flounder collected in the Delta and Mobile Bay during 2018 and 2019. Relative predator to prey length for Southern Flounder and fish prey items calculated by dividing flounder length by prey length. Flounder in Mobile Bay were consuming significantly larger prey, relative to body size, than fish in the Delta.