

**Assessing Southern Flounder *Paralichthys lethostigma* Populations
in the Northern Gulf of Mexico**

by

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Abstract

Despite Southern Flounder being a commercially and recreationally important flatfish species in the northern Gulf of Mexico (GOM), our understanding is poorly described of its population dynamics. I collected Southern Flounder from three sites along the northern GOM-- Barataria Bay, LA, Mobile Bay, AL, and Apalachicola Bay, FL-- and used sagittal otoliths to estimate age-and-growth, as well as mortality. The lifetime variation in the elemental composition of otoliths (also was assayed to infer patterns of past habitat use throughout life, particularly as related to salinity) among regions. Male Southern Flounder collected from Alabama coastal waters grew faster and had a greater estimated von Bertalanffy length asymptote than did male Southern Flounder from Louisiana or Florida coastal waters. Female Southern Flounder collected from Louisiana and Alabama coastal waters grew faster than fish from Florida coastal waters, but the von Bertalanffy length asymptote estimate for Southern Flounder collected from Florida was larger than those for Louisiana and Alabama coastal waters. Length-frequency distributions differed across all three populations, with fish being larger in Alabama versus both Louisiana and Florida. Weight-length relationships also differed across these populations, with Southern Flounder from Louisiana and Alabama coastal waters putting on more weight per length than fish from Florida coastal waters. Based on catch-curve analysis, total annual mortality for these populations was 82% in Louisiana, 66% in Alabama, and 54% in Florida. Results from age-and-growth analyses of Southern Flounder from these three populations indicate they are exhibiting different growth patterns.

Laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) was used to assay otolith chemical composition through time in an effort to explore lifetime habitat use and potential population differences in chemical constituents, as well as to assess whether otoliths of Southern Flounder along the northern GOM were exposed to oil as a result of the 2010 Deepwater Horizon (DWH) oil spill. None of the elements thought to be markers indicative of the DWH oil spill met the limit of detection criteria for any population, indicating that fish collected in my study did not accumulate elevated levels of elements previously identified from the oiling event. I also used changes in otolith chemical signatures, with particular emphasis on Sr^{88} , along the growth axis to examine past salinity exposure, and thus past environment experienced by fish collected along the northern GOM during their otolith formation. Based on otolith microchemistry, a group of Southern Flounder from each population had low Sr^{88} concentrations during the development of their otolith core, indicating a period of freshwater residency after ingress from the marine environment. Concentrations of Ba^{137} represent an effective marker for distinguishing Southern Flounder collected from Louisiana when compared to Alabama or Florida.

In order to test for possible effects of the DWH oil spill of April 2010 on the Southern Flounder population structure from areas that experienced heavier oiling (i.e., Louisiana, and to a much lesser extent Alabama), growth and condition estimates from my study were compared to historical studies conducted from the same areas. When compared to pre-oil spill data, I found that male Southern Flounder collected from Louisiana reached a larger theoretical maximum length (L_{∞}) at a slower rate, while females reached a smaller L_{∞} at a faster rate after the oiling

event. Pre-oil comparisons of condition suggest that Southern Flounder from Louisiana put on more weight per length than did fish collected in my study. Alabama comparisons suggest that males reached a larger L_{∞} at a faster rate, while females reached a smaller L_{∞} at a faster rate after the oiling event. No differences of condition between pre-oil and post-oil data were observed from this region.

Results from this study suggest that Southern Flounder populations across the northern GOM are growing differently from one another and further support the need for freshwater habitats to be included when identifying nursery habitat for this important flatfish species. More work is needed to evaluate the importance of such habitats for their migratory life history strategies and how these freshwater habitats affect the overall population dynamics of Southern Flounder.

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Introduction

Southern Flounder *Paralichthys lethostigma* populations have historically supported important commercial and recreational fisheries in the northern Gulf of Mexico (GOM), and are among the nearshore fishes most often targeted by recreational anglers (Stunz 2000; Fischer and Thompson 2004). Due to the importance of this nearshore fishery, declines in commercial and recreational landings of Southern Flounder in the northern GOM have prompted concern and more restrictive regulation by management agencies (Vanderkooy 2000; Fischer and Thompson, 2004; Froeschke et al. 2011). Although there is increased concern with their status, there is little empirical analysis of the population dynamics of Southern Flounder across the northern GOM. Fish population characteristics such as size structure, abundance, growth, recruitment, and mortality can vary both spatially and temporally. Spatially explicit data are needed to determine the best management approaches for this important northern GOM fishery. While the causes for variation within and among stocks are not well understood, individual fish growth and mortality are likely to be important influences. This understanding is important because changes in size structure and abundance of a fish stock can have implications for ecological processes such as recruitment (Ricker 1954; Beverton-Holt 1957).

Some stocks or populations consist of identifiable groups, or contingents (Secor 1999; Nims and Walther 2014), that have unique behaviors or habitat use. Certain groups within populations can exhibit different migratory and habitat use patterns. For example, one group can exhibit migratory behavior, moving in and out of different areas or habitats, while another group can remain resident in an area or habitat type. These differences in migratory behaviors and

habitat use can contribute to differences in spawning behavior and individual growth rates (Kerr and Secor 2010). Examining otolith chemical constituents can reveal such contingents that may exist in a population (Campana 1999; Elsdon et al. 2008). Otoliths provide a valuable structure for assessing many aspects of fish biology because they are metabolically inert, grow continuously, and may also contain environmental markers making them reliable indicators of a fish's broad scale location and migratory history throughout its life (Campana and Neilson 1985; Campana 1999; Campana and Thorrold 2001; Lowe et al. 2009, 2011; Sturrock et al. 2012; Farmer et al 2013; Nims and Walther 2014; Nelson et al 2015). On a smaller spatial scale, otoliths have been used to quantify fish movement patterns because they incorporate some elements in proportion to their dissolved abundances in the surrounding water (Bath et al. 2000; Walther & Thorrold 2006; Lowe et al. 2009, 2011; Farmer et al 2013; Nelson et al 2015). Southern Flounder stocks across the northern GOM provide an opportunity to investigate how growth, mortality, recruitment variation, and relative abundance may differ among contingent or local populations.

Southern Flounder is the largest member of the family Paralichthyidae occurring in the GOM (Fischer and Thompson, 2004). Its endemic range on the Atlantic coast extends from Albemarle Sound, North Carolina, to the Loxahatchee River on the lower eastern coast of Florida. Southern Flounder do not inhabit the southernmost tip of peninsular Florida, but occur from the Caloosahatchee River, FL northward along the western coast of Florida around the northern GOM to northern Mexico (Manooch 1984; Hoese and Moore 1998) and are found in the GOM coasts of Alabama, Mississippi, Louisiana, and Texas from the barrier islands to the

outer shelf and in Florida on the inner shelf from Apalachee Bay to north of Tampa Bay (Reagan and Wingo 1985), being more abundant in the northwest portion of the GOM (Nall 1979; as reviewed in GSMFC 2000, 2015).

Southern Flounder reach sexual maturity at age-2 (Powell 1974; Stokes 1977; Manooch 1984). Most Southern Flounder in the northern GOM spawn in late fall and early winter, but some have been observed to spawn as late as early spring (Stokes 1977; Reagan and Wingo 1985; Gilbert 1986). With the onset of cold weather in the fall, Southern Flounder move offshore into deeper and thermally more stable waters to spawn (Hildebrand and Cable 1930; Gunter 1945; Ginsburg 1952; Stokes 1977). Males begin their migration into deeper waters earlier than females and few remain in the estuaries beyond November in the northern GOM. Sampling during these spawning migrations has led to reports of Southern Flounder being collected in water depths up to 120 m (Hildebrand 1954; Stokes 1977; Darnell 1985; Gilbert 1986; Sanders et al. 1990).

Southern Flounder eggs and larvae are pelagic, buoyed by a single oil globule in the yolk, and drift inshore on the prevailing wind and tide-driven currents until they transform and settle in estuarine and nearshore habitats (Stokes 1977; Gilbert 1986; Glass et al. 2008; Nanez-James et al. 2009). Total transformation for Southern Flounder is temperature-dependent, but is usually complete within approximately 50 days after spawning (Arnold et al. 1977). After settling, juveniles remain in marsh habitats that experience varying salinities (Powell and Schwartz 1977; Lowe et al. 2011) for the first two to three years of life. The length of time spent in these habitats depends on individual growth rates.

The Deepwater Horizon (DWH) oil spill that occurred in April of 2010 was the largest oil spill in U.S. history (Abbriano et al. 2011) and was a dramatic event for the northern GOM. Despite the research that has been conducted since its occurrence more than 6 years ago, many questions still remain regarding the ecological effects of the estimated 4.9 million barrels of oil that spilled into the GOM throughout the summer of 2010. Potential impact of the DWH oil spill on the recruitment of Southern Flounder would have likely occurred during the juvenile post-settlement phase of life. The event occurred after the time when juvenile Southern Flounder would have been pelagic. Juvenile fish could have been particularly vulnerable to the oil as it came ashore given their small size, benthic nature, and relative inability to actively avoid it. Continued chronic exposure to contaminated sediments may reduce their growth and potentially increase mortality of juvenile and larger fish in the time since the event. A better understanding of the impact of the 2010 DWH oil spill on the population structure of Southern Flounder will be pivotal for being able to better predict the outcomes of future oil spills in the GOM.

Here I first quantify and compare age structure of Southern Flounder populations from collections across 3 regions: the northeast Florida Panhandle waters, Alabama waters, and the relatively heavily oiled regions of Louisiana in order to assess the strength of the 2010 year class across the northern GOM and investigate whether effects differ between areas that received greater oiling versus those that did not. Second, I quantify and compare growth rates and condition of both male and female Southern Flounder among regions and compare my findings to previous studies conducted in the northern GOM to evaluate any differences in size structure between and among regions. Finally, I quantify the otolith chemical constituents of Southern

Flounder collected from each region by using laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) to determine if otolith elemental composition differs across the northern GOM and to infer the salinity that fish had experienced through their lives.

Methods

Study Area

Southern Flounder were collected from (1) Barataria Bay and associated nearshore waters of Louisiana, (2) Mobile Bay and associated sounds and nearshore waters of Alabama, and (3) nearshore waters and sounds of Apalachicola Bay, Florida (Figure 1). Sites were chosen to span a large spatial area of the northern GOM as well to capture a gradient of potential impact from the 2010 DWH oil spill, with the greatest potential impacts most likely in Louisiana marshes, with progressively less oil exposure moving eastward (gomex.erma.noaa.gov; Nixon et al. 2016).

Field Collections

Juvenile and adult Southern Flounder were collected from June 2014 through June 2015. To fully characterize the age distribution and accurately quantify Southern Flounder growth, I collected between 203 – 229 individuals (Table 1) from each of the three regions with bottom trawling and gill nets.

Beginning in June 2014, an otter trawl (4.9-m head rope, 6.4-mm bar mesh wings and body, 3.2-mm bar mesh cod end) was towed for 1-hour during the day. All Southern Flounder were euthanized with MS-222 and immediately put on ice for transport to the laboratory. All by-catch captured during trawl sampling was released immediately. Due to the ineffectiveness for

collecting larger individuals, bottom trawling was discontinued in October 2014 and gill nets were used for the remainder of the study.

After discontinuation of bottom trawls, experimental gill nets were used at night in each region. Seven gill nets were set: six 38.1-m x 2.44-m experimental nets with 5 – 7.62-m panels with mesh stretch sizes ranging from 76.2-mm to 177.8-mm, and one 228.6-m x 2.44-m gill net with 127-mm mesh throughout the entire net. Gill nets were set parallel to the shore in shallow (0.5 – 2 meters depth) water in an effort to capture Southern Flounder as they moved into shallower waters to feed at night (Dugas 1975). Gill nets were allowed to soak for six hours, after which all collected Southern Flounder were removed, euthanized with MS-222, placed on ice, and returned to the laboratory. All by-catch was released immediately or disposed of in an Institutional Animal Care and Use Committee (IACUC) approved facility.

In addition to my collections, I obtained individuals from local fish houses in each region to assure that I had individuals representing all age classes for my age-and-growth analyses. I also obtained Southern Flounder samples collected by the National Oceanic and Atmospheric Administration (NOAA) fall 2014 Southeastern Area Monitoring and Assessment Program (SEAMAP) ground fish survey and I submitted sample request forms to the Louisiana Department of Wildlife and Fisheries (LDWF), the Alabama Marine Resources Division (MRD), and the Florida Fish and Wildlife Conservation Commission (FWC). I received specimens captured by LDWF from the Barataria Bay, LA area, Alabama MRD historic (2001 – 2010) Southern Flounder independent gill net fisheries data, specimens captured by Florida FWC from Apalachicola Bay, FL, and the Florida FWC historic (2004 – 2010) independent fisheries data.

Abiotic Measurements

Temperature, salinity, and conductivity were measured in each area where Southern Flounder were collected (Yellow Springs Instruments Model 30). Measures were taken 0.25-m below the surface.

Processing of Samples in the Laboratory Age-and-Growth Analysis

In the lab, Southern Flounder were measured (total length [TL] in mm), weighed (total wet weight [TW] in g), their gonads removed and weighed (g), sex determined, and sagittal otoliths removed. Extracted sagittal otoliths were cleaned in 30% H₂O₂ to remove any excess tissue, rinsed with ultra-filtered triple-distilled water (DIUF), and stored in vials for preparation for ageing and microchemical analysis. Due to asymmetry of Southern Flounder sagittae (Fischer and Thompson 2004), only the left otoliths were used as an ageing structure (Wenner et al. 1990; Stunz et al. 2000; Nims and Walther 2014). Sagittae were mounted in epoxy resin and a transverse core section removed with a low-speed diamond bladed double wheel saw (South Bay Technology INC. Model 650) and mounted to a slide for age determination under a compound microscope. Opaque zones in each otolith were counted by two independent readers to estimate fish age. If the readers agreed on the age of the fish, that age was accepted. If the readers did not agree, the otolith was re-examined by each reader until an age was agreed upon. If the discrepancy could not be resolved, a third independent reader examined the otolith. If the third reader agreed with one of the original readers, that age was accepted. If all readers did not agree, the otolith was discarded from further analysis. The distance from the core to each

annulus and to the outer edge was measured with an image analysis system to allow for back-calculations of annual growth history.

Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry Analysis

Due to the small number of older individuals collected, all three year-old and older fish from each region were considered for microchemical analysis. Samples were randomly selected from these older fish within each region until a sample size of 100 individuals from each region was achieved. Each sectioned otolith was ground and polished to expose the otolith core (with 30 micron lapping film and 3 micron lapping film; 3M, St. Paul, Minnesota, USA). Polished otolith sections were rinsed with triple distilled ultra-filtered water (DIUF), dried, removed from the microscope slide and remounted to new triple DIUF rinsed microscope slides (40 otoliths per slide) for elemental analysis. Slides containing the remounted otoliths were submerged in a sonicating water bath for five minutes (50/60 Hz), and then triple rinsed with triple DIUF water. Slides were then air dried and sealed in acid washed glass beakers for transport.

Elemental composition of Southern Flounder otoliths was assayed at the Dauphin Island Sea Lab, Dauphin Island, Alabama. Trace elements were measured with laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) on an Agilent Technologies 7700 series ICPMS coupled to a 213 nm New Wave NWR-213 internally homogenized, optically attenuated laser system. Otolith runs were bracketed by two certified reference materials (CRMs) - NIST 612 and MACS3. Analysis transects across otoliths were pre-ablated using a low-powered (5 Hz) laser cleaning pulse with a spot size of 50- μm to remove surface contamination. Otoliths were analyzed to quantify barium and strontium concentrations (in ppm)

to estimate the salinity experienced by each Southern Flounder from each region. In addition, Al, V, Cr, Ni, Cu, Zn, and Se, all of which have been identified in DWH crude oil (Grosser et al. 2012), were quantified. In order for an element to be included in the comparative analysis across regions, its concentration had to be above the limits of detection (LOD) in at least 50% of the otoliths. Each otolith was ablated from the focus of the core, across the core, and to the edge of the otolith along the sulcal groove with a 25- μm laser spot size and a scan speed of 5- $\mu\text{m/s}$, allowing me to recreate individual lifetime exposure to the measured elements. Southern Flounder otoliths have been successfully analyzed for elemental signatures from the Mobile-Tensaw Delta, AL (Lowe et al. 2011, 2012; Farmer et al 2013) as well as from Texas coastal waters (Nims and Walther 2014).

Statistical Analysis

Relative Abundance

Catch-per-unit-effort (CPUE) for experimental and single mesh gill nets was calculated for Southern Flounder collected from each region as the mean number of fish collected per hour of effort. Differences in CPUE across regions were tested with an ANOVA. CPUE calculations were used to evaluate the relative abundance of Southern Flounder from each region.

Age Structure

I used a catch curve analysis, or a linear regression of the natural log of numbers collected as a function of age, to determine the relative abundance of year classes up to the maximum age where multiple individuals were collected. Assumptions of this analysis are that cohorts have relatively constant recruitment, catchability, and mortality over the study period (Chapman and Robson 1960). Catch curve analysis allowed me to quantify instantaneous total mortality rate (Z) of Southern Flounder from each region as:

$$\ln(N_t) = \ln(N_o) - Z(t)$$

where $\ln(N_t)$ is the natural logarithm of the number of fish in a year class at time t , $\ln(N_o)$ is the natural logarithm of the original number of fish in a year class, and Z is the instantaneous rate of total annual mortality. The slope of the regression was interpreted as an estimate of Z . Average annual survival was calculated as:

$$S = e^{-Z},$$

where S is survival and Z is total instantaneous mortality. Total annual mortality (A), was calculated as $A = 1 - S$. Maceina (1997) suggested that positive and negative residuals associated with catch-curve regressions represented strong and weak year classes in Largemouth Bass *Micropterus salmoides* populations from two northern Alabama reservoirs. Following this approach, the deviations above or below the exponential decline curve were examined to assess relative year-class strengths of Southern Flounder collected from each region. Mortality and age structure of Southern Flounder were compared among regions to determine if the 2010 year class was unusually weak across the northern GOM, as well as whether there were stronger effects in

areas that received greater oiling. Slopes were compared among populations using a one-way analysis of variance (ANOVA).

Age-frequency distributions were also generated for Southern Flounder collections from each region. These distributions allowed for visual representation of the mortality estimates by following the decline in numbers across year-classes.

Growth and Condition

Due to sexual dimorphism in Southern Flounder (Stunz et al. 2000; Fischer and Thompson 2004), separate von Bertalanffy growth equations were estimated for males and females. Von Bertalanffy growth models of total length at age were fitted with nonlinear regression to all fish collected from each region. Maximum likelihood analysis was conducted to estimate equation parameters in R statistical software (v 3.2.2) with the following equation:

$$L_t = L_\infty \{ 1 - e^{-k(t-t_0)} \},$$

where L_t is the total length at time t (years), L_∞ is the length asymptote (mm), k is the growth coefficient per year, and t_0 is the time (years) when length theoretically would be zero (von Bertalanffy 1957). These models were developed using only length at capture and estimated age at capture.

To quantify growth differences between pairs of regions, as well as to gain an idea of which model best explained my Southern Flounder data, an age range was selected to assure the comparisons were consistent. Eight von Bertalanffy growth models with varying growth parameters were created in R statistical software (v 3.2.2). Pairwise comparisons of male and female Southern Flounder from two regions at a time were evaluated. The eight different von

Bertalanffy growth models chosen for this analysis resulted in 28 pairwise model comparisons. Under a normal distribution, and the assumption that sigma, the amount of variation in the set of values, did not differ between regions, the eight growth models ranged from the null model with no variation in the growth parameters to the full model where all growth parameters varied (Table 2). Von Bertalanffy parameter values were named in the model function and log transformed to constrain them to remain positive (except t_0). Because the t_0 parameter of the equation can have a negative value, it was not log transformed. An optimization algorithm was then used to find parameter values that minimized the negative log likelihood. Each growth model computed the corrected Akaike's information criterion (AICc) to be used as an index to select the growth model that displayed the best fit for these data. Corrected AIC differences were also computed to compare incremental differences among models and were used to compute weights (w_i) for each model. These weights were then used to obtain the probability that a given model provided the most parsimonious fit to the data, given the candidate set of models (Burnham and Anderson 2001).

Body condition was evaluated via weight-length relationships for each region, expressed as:

$$W = a L^b,$$

where W is the weight of the fish (g), L is the total length of the fish (mm), a is a constant (intercept), and b is a constant (slope of non-linear regression line). The weight-length relationship was then \log_{10} transformed into a linear form and reported as:

$$\log_{10}(W) = a' + b \cdot \log_{10}(L),$$

where a' is $\log_{10} a$ and is the y-axis intercept and b is the slope of the equation. Differences in the slopes of the transformed weight-length relationship were compared across regions with a one-way analysis of covariance (ANCOVA).

Length-frequency distributions of Southern Flounder collected in this study were examined in 100 mm TL increments or bins to examine among-region differences. A Komolgorov-Smirnov two-sample test was used to test for distribution differences between regions.

Pre vs. Post Oiling Event Comparison

To determine if evidence supported impacts of the 2010 DWH oil spill on Southern Flounder growth and condition, the von Bertalanffy growth parameters and slope estimates of the weight-length relationship from previous studies and long term monitoring conducted in the same regions prior to the oiling event were qualitatively compared to our current findings. The findings from each region in this study were compared to: (1) a previous study conducted in Barataria Bay, LA (Fischer and Thompson 2004), (2) pre oil spill samples and data from the Alabama MRD, and (3) pre oil spill data from the Florida FWC.

Otolith Microchemistry

Two runs ablating the NIST 612 glass reference standard with known concentrations of isotopes were analyzed both before and after approximately every 12 otolith samples. These standard runs allowed me to quantify and correct for instrumental drift, determine the precision (CV) of elemental concentrations measurements, and served as external calibration standards. Estimates of background levels of isotopes in the Ar carrier gas were analyzed for 30 seconds

before every otolith analysis, which allowed limits of detection (LODs) to be calculated based on the following formula from Ludsin et al. (2006):

$$\text{LOD} = \frac{3 \cdot \sigma_{\text{bgd}}}{S \cdot Y} \cdot \sqrt{\frac{1}{N_{\text{bgd}}} + \frac{1}{N_{\text{pk}}}}$$

where σ_{bgd} equals the standard deviation (SD) of the pre-ablation background determination of isotopes, N_{bgd} and N_{pk} are numbers of replicate determinations used in the integration of the background and ablation signal, respectively, S is mean sensitivity (counts/s per unit concentration) for the NIST reference standard, and Y is the ablation yield relative to the NIST reference standard (Longerich et al. 1996). Based on this formula an isotope's concentration had to be greater than 3 SDs above background levels to be considered greater than detection limits. I only included an isotope in the analysis if it was greater than the detection limit in at least 50% of otolith samples for each population.

In order to convert raw isotope concentrations from counts per second (cps) to parts per million (ppm), Ca^{43} was used as an internal standard held constant at 400,432 ppm for samples and the published 81,830 ppm for the NIST 612 standard (Longerich et al. 1996; Ludsin et al. 2006; Lowe et al. 2011; Nelson et al. 2015). Counts per second were transformed to ppm using iolite v3.x built under IGOR Pro 6.37 software from WaveMetrics. Conversions from cps to ppm were computed by using the formula from Ludden et al. (1995). A detailed description of this formula is given in Lowe (2007). The unit used to report otolith elemental concentrations varies across the literature, with concentrations either being reported in ppm (Ludsin et al. 2006; Ranaldi and Gagnon 2008), $\mu\text{g g}^{-1}$ (Albuquerque et al. 2012), mmol mol^{-1} (Lowe et al. 2009;

Nims and Walther 2014), or $\mu\text{mol mol}^{-1}$ (Sohn et al. 2005; Nims and Walther 2014). Given that $\mu\text{g g}^{-1}$ is equivalent to ppm and $\mu\text{mol mol}^{-1}$ can be easily converted to mmol mol^{-1} , otolith elemental concentrations were reported in ppm and comparative analysis was conducted using ppm for this study.

In order to facilitate comparison of transect elemental data between fish of differing ages, elemental data were scaled to years for each fish. Similar to techniques used by Farmer et al. (2013), this was accomplished by measuring the total distance of each ablation as well as the distance between the core and each succeeding annuli the laser ablation traversed. Distance increments were re-scaled by using the ablation speed recorded for each transect (every 1.531 s). Distance measurements were converted to estimated ages of fish under the assumption that the distance between the core and edge of first annuli, as well as distance between adjacent annuli, represented 365 days.

When conversions were made, the estimated age at capture corresponded to the last elemental measurement at the otolith edge. The same hatch date (January 1) assumed for age-and-growth analysis was used here. For example, an age-3 individual collected in March 2015 would have elemental data scaled from 2012.00 in the core to 2015.25 (90 days / 365 days per year ~ 0.25).

Nonlinear regression was used to quantify Sr and Ba concentrations as a function of proportional distance (proportion of overall transect length of each individual fish) from the otolith core. Locally-weighted scatterplot smoothing (LOWESS) was used to smooth untransformed Sr and Ba profiles (span=0.25; Trexler and Travis 1993; Rooker et al. 2004; Lowe

et al. 2011). Similar to Lowe et al. (2011), the core (i.e., otolith material deposited during and shortly after hatch) region of the otolith was defined to be the inner 20% of the ablated distance/time and the edge region of the otolith (i.e., otolith material laid down prior to collection) was defined as the outer 80% to 100% of the ablated distance/time. Individuals were then categorized into discrete groups based on their defined core region Sr concentrations. Individuals with a core Sr profile ≥ 1500 or < 1500 ppm were then categorized as having experienced elevated salinity or freshwater/oligohaline conditions, respectively, at some point throughout their core (natal) profile (Lowe 2007; Lowe et al. 2009; Farmer et al. 2013; Nims and Walther 2014).

To determine if elemental concentrations averaged across life profile regions along the otolith ablation differed among sites for each element that was above LODs at least 50% of the time, a one-way ANOVA was used. Mean core, edge, first year of life, and whole otolith elemental concentrations (ppm) were analyzed to test for any differences among the three northern GOM sites.

Results

Relative Abundance

Both gear types (experimental and single mesh gill nets) yielded variable CPUE values for Southern Flounder from each region. Overall, single mesh gill net CPUE was generally higher than for experimental gill nets. Single mesh gill net CPUEs did not differ among sites (LA vs. AL $F_{2,47} = 1.05$, $P = 0.45$; LA vs. FL $F_{2,47} = 1.05$, $P = 0.15$; AL vs. FL $F_{2,47} = 1.05$, $P = 0.73$), but experimental gill net CPUEs were significantly higher in Louisiana than in Florida ($F_{2,47}$).

$_{47} = 6.23, P < 0.001$; Figure 2) while not differing between Alabama and either Louisiana ($F_{2, 47} = 6.23, P = 0.06$) or Florida ($F_{2, 47} = 6.23, P = 0.47$; Figure 2).

Age Structure

In Louisiana coastal waters, strong Southern Flounder year classes were evident in 2011 and 2014 while weaker year class production seemed apparent in 2012 and 2013 (Figure 3). In Alabama coastal waters, slightly stronger Southern Flounder year classes were evident in 2010 and 2014 while year class production appeared weaker in 2012 (Figure 3). Finally, in Florida coastal waters, strong Southern Flounder year classes were evident in 2010, 2011, and 2014 while weaker year class production seemed apparent in 2012 and 2013 (Figure 3).

Total annual mortality (A) estimates for Southern Flounder collected from Louisiana, Alabama, and Florida coastal waters were $A = 0.82 (F_{1, 2} = 13.06)$, $A = 0.66 (F_{1, 3} = 155.1)$, and $A = 0.54 (F_{1, 3} = 1.68)$, respectively, indicating a gradient of mortality that decreased from west to east (Figure 3). Estimates of Z showed the same west-east pattern (Figure 3), although differences were not significant (LA vs. AL $F_{5, 8} = 3.97, P = 0.67$; LA vs. FL $F_{5, 8} = 3.97, P = 0.41$; AL vs. FL $F_{5, 8} = 3.97, P = 0.66$).

Age-frequency distributions generated for Southern Flounder collected from Louisiana, Alabama, and Florida (Figure 4) coastal waters provided additional evidence of the decline in numbers of each year-class from each region. Age-0 and age-1 Southern Flounder contributed the largest proportion at age for Louisiana and Alabama while age-1 contributed the largest proportion at age in Florida coastal waters.

Growth and Condition

The von Bertalanffy growth coefficients (k) for male Southern Flounder from Louisiana, Alabama, and Florida coastal waters were $k = 0.63$, $k = 0.81$, and $k = 0.63$, respectively (Figure 5). The maximum theoretical attainable total lengths (L_{∞}) for male Southern Flounder from Louisiana, Alabama, and Florida coastal waters were $L_{\infty} = 358.8$, $L_{\infty} = 377.8$, and $L_{\infty} = 349.7$, respectively (Figure 5), indicating that male Southern Flounder collected from Alabama coastal waters exhibited faster growth and greater maximum total length than did Southern Flounder collected from Louisiana or Florida coastal waters. The growth coefficients (k) for female Southern Flounder from Louisiana, Alabama, and Florida coastal waters were $k = 0.97$, $k = 0.84$, and $k = 0.55$, respectively (Figure 6). The length asymptote (L_{∞}) for female Southern Flounder from Louisiana, Alabama, and Florida coastal waters were $L_{\infty} = 483.2$, $L_{\infty} = 484.1$, and $L_{\infty} = 507.7$, respectively (Figure 6), indicating that female Southern Flounder collected from Louisiana and Alabama coastal waters exhibited faster growth than fish collected from Florida coastal waters, but the maximum total length estimate for Southern Flounder collected from Florida coastal waters was larger than the estimates generated from Louisiana and Alabama coastal waters.

Multi-model pairwise comparison of either male or female length at a particular age using AICc supported no differences in male growth trajectories among regions (Figure 7 and 8; Table 2 and 3). While differences in models could not be assessed as significant from AIC numbers, the differences in AIC numbers from each growth model to the next best growth model were less than four for both male and female Southern Flounder (Table 2 and 3) suggesting that these eight von Bertalanffy growth models were equivalent. The equivalency of the eight growth

models for male Southern Flounder suggested that even when growth parameters differed for each region in the pairwise comparison, the model still drew the same growth trajectory. Multi-model pairwise comparison between selected ages suggested equivalent L_{∞} for female Southern Flounder from Louisiana, Alabama, and Florida coastal waters, but greater k for Louisiana coastal waters relative to Alabama and Florida (Figure 8; Table 3).

The b exponent generated from the weight-length relationship for Louisiana and Alabama Southern Flounder was > 3 indicating positive allometric growth, while fish from Florida coastal waters showed negative allometric growth with a b exponent that was < 3 (Figure 9). Significant differences in the weight-length relationships among sites (all $F_{5, 819} = 5286$, $P \leq 0.007$) indicated that Southern Flounder collected from Louisiana and Alabama coastal waters put on more weight per length than did fish from Florida coastal waters and that Southern Flounder collected from Louisiana put on more weight per length than did fish from Alabama coastal waters (Figure 9).

A Komolgorov-Smirnov two-sample test indicated significant differences in total length-frequency distributions between Louisiana versus Alabama ($D = 0.19$, $P = 0.002$), Louisiana versus Florida ($D = 0.19$, $P = 0.001$), and Alabama versus Florida ($D = 0.3$, $P < 0.001$). Southern Flounder larger than 400 mm were most abundant in Louisiana and Alabama coastal waters compared to Florida (Figure 10).

Pre vs. Post Oiling Event Comparison

Pre-oil spill theoretical maximum attainable total length (L_{∞}) for males from a previous study in Barataria Bay, LA (Fischer and Thompson 2004) was smaller ($L_{\infty} = 332$) compared to post-oil spill ($L_{\infty} = 358.8$; Figure 11). Pre-oil spill growth coefficient (k) was higher ($k = 1.03$) than the post-oil spill growth rate observed in the present study ($k = 0.63$; Figure 11) suggesting that male Southern Flounder from this region reached a larger L_{∞} at a slower rate after the 2010 DWH oil spill. In contrast, pre-oil spill growth coefficient (k) for female was lower ($k = 0.51$) than the post-oil spill growth rate observed in the present study ($k = 0.97$; Figure 11). Pre-oil spill theoretical maximum attainable total length (L_{∞}) for female Southern Flounder was larger ($L_{\infty} = 556$) compared to post-oil spill ($L_{\infty} = 483.2$; Figure 11) indicating that female Southern Flounder from this region reached a smaller L_{∞} at a much faster rate after the 2010 DWH oil spill. Pre-oil spill condition of Southern Flounder was compared to post-oil spill condition via slope estimates (b) generated by the weight-length relationship. The pre-oil spill slope estimate was larger ($b = 3.47$) than the slope estimate from the present study ($b = 3.2752$; Figure 11).

Pre-oil spill growth coefficient (k) for males from the Alabama MRD was lower ($k = 0.54$) than the post-oil spill growth rate observed in the present study ($k = 1.17$; Figure 12). Pre-oil spill theoretical maximum attainable total length (L_{∞}) for male Southern Flounder was smaller ($L_{\infty} = 342.5$) compared to post-oil spill ($L_{\infty} = 373.9$; Figure 12) indicating that male Southern Flounder from this region reached a larger L_{∞} at a much faster rate after the 2010 DWH oil spill. Pre-oil spill growth coefficient (k) for females was lower ($k = 0.44$) than the post-oil spill growth rate observed in the present study ($k = 0.84$; Figure 12). Pre-oil spill theoretical

maximum attainable total length (L_{∞}) for female Southern Flounder was larger ($L_{\infty} = 542.7$) compared to post-oil spill ($L_{\infty} = 484.1$; Figure 12) indicating that female Southern Flounder from this region reached a smaller L_{∞} at a much faster rate after the 2010 DWH oil spill. The pre-oil spill condition was compared to the post-oil spill condition via slope estimates (b) generated by the weight length relationship. I found no significant difference ($F_{3, 564} = 7156, P = 0.52$) in the slope estimates between pre-oil spill data provided by the Alabama MRD ($b = 3.125$) and post-oil spill data from the present study ($b = 3.153$; Figure 12) indicating that post-oil spill Southern Flounder from Alabama waters are putting on the same weight per length as pre-oil spill individuals.

I found a significant difference ($F_{2, 513} = 2279, P < 0.001$) in the slope estimates between pre-oil spill data provided by the Florida FWC ($b = 3.258$) and post-oil spill data from the present study ($b = 2.878$; Figure 13) indicating that pre-oil spill Southern Flounder from Florida waters were putting on more weight per length than post-oil spill individuals.

Otolith Microchemistry

Out of the 11 elements examined with LA-ICPMS, four (Ca, Mn, Sr, and Ba) met the criteria (> 50% LOD) to be used in otolith comparisons across all regions in this study. Given that Ca was used as an internal standard, it was not used for otolith analysis. Therefore three elements (Table 4) were used for all profile (i.e., core, edge, year one, and whole otolith) comparisons across all regions. None of the elements chosen to test for potential markers from the DWH oil spill (Al, V, Cr, Ni, Cu, Zn, and Se) met the LOD criteria for analysis within each region separately, indicating that Southern Flounder collected in this study did not accumulate

elevated levels of elements identified from the DWH oil spill crude oil (Grosser et al. 2012) in their otoliths from their surrounding environment.

Otolith core mean Sr⁸⁸ concentrations differed significantly across all regions (LA vs. AL $F_{2, 276} = 28.35, P < 0.001$; LA vs. FL $F_{2, 276} = 28.35, P = 0.01$; AL vs. FL $F_{2, 276} = 28.35, P < 0.001$; Figure 14). However, otolith edge mean Sr⁸⁸ concentrations only differed significantly between Louisiana and Alabama ($F_{2, 276} = 4.96, P = 0.002$), while not differing between Louisiana versus Florida ($F_{2, 276} = 4.96, P = 0.19$) or Alabama versus Florida ($P = 0.49$; Figure 14). Otolith core mean Ba¹³⁷ concentrations differed significantly between Louisiana and Alabama ($F_{2, 276} = 7.58, P = 0.02$) and Louisiana and Florida ($F_{2, 276} = 7.58, P < 0.001$), but not between Alabama and Florida ($F_{2, 276} = 7.58, P = 0.16$; Figure 15). However, otolith edge mean Ba¹³⁷ concentrations differed significantly across all regions (LA vs. AL $F_{2, 276} = 66.83, P < 0.001$; LA vs. FL $F_{2, 276} = 66.83, P < 0.001$; AL vs. FL $F_{2, 276} = 66.83, P < 0.001$; Figure 15).

Otolith mean Sr⁸⁸ concentrations during the first year of life differed significantly between Louisiana and Alabama ($F_{2, 167} = 13.61, P = 0.001$) and Alabama and Florida ($F_{2, 167} = 13.61, P < 0.001$), but did not differ between Louisiana and Florida ($F_{2, 167} = 13.61, P = 0.14$; Figure 14). Otolith mean Ba¹³⁷ concentration from the first year of life differed significantly between Louisiana and Alabama ($F_{2, 167} = 10.51, P = 0.001$) and Louisiana and Florida ($F_{2, 167} = 10.51, P < 0.001$), but did not differ between Alabama and Florida ($F_{2, 167} = 10.51, P = 0.24$; Figure 15).

Mean whole otolith Sr⁸⁸ concentrations differed significantly between Louisiana and Alabama ($F_{2, 276} = 12.08, P < 0.001$) and between Florida and Alabama ($F_{2, 276} = 12.08, P <$

0.001), but did not differ between Louisiana and Florida ($F_{2, 276} = 12.08, P = 0.23$), being highest in Alabama samples (Figure 14). Whole otolith Ba^{137} concentrations differed across all regions (LA vs. AL $F_{2, 276} = 31.59, P < 0.001$; LA vs. FL $F_{2, 276} = 31.59, P < 0.001$; AL vs. FL $F_{2, 276} = 31.59, P = 0.005$) with mean Ba^{137} concentrations being highest in Louisiana and decreasing to the east (Figure 15). Elevated Ba^{137} concentrations, similar to the findings in this study, were documented in *F. grandis* (Nelson et al. 2015) collected from Louisiana estuaries.

Otolith mean core Mn^{55} concentration did not differ among regions (LA vs. AL $F_{2, 276} = 0.52, P = 0.54$; LA vs. FL $F_{2, 276} = 0.52, P = 0.71$; AL vs. FL $F_{2, 276} = 0.52, P = 0.31$; Figure 16). Otolith mean edge Mn^{55} concentration differed between Louisiana and Florida ($F_{2, 276} = 3.75, P = 0.03$) and Alabama and Florida ($F_{2, 276} = 3.75, P = 0.01$). No significant differences were detected between Louisiana and Alabama ($F_{2, 276} = 3.75, P = 0.83$; Figure 16). Otolith region (core vs edge) Mn^{55} chemical signatures differed significantly within regions (LA $F_{1, 184} = 154, P < 0.001$; AL $F_{1, 176} = 156.2, P < 0.001$; FL $F_{1, 192} = 229.4, P < 0.001$). The increased concentration of Mn^{55} in the core region compared to the edge region of Southern Flounder otoliths in this study were similar to other studies conducted across northern GOM coastal waters (Lowe 2007; Lowe et al. 2011; Farmer et al. 2013; Nims and Walther 2014; Nelson et al. 2015) and served as added evidence that the core region was in fact ablated, which is common in the vaterite formation in the otolith core (Melancon et al. 2005; Lowe et al. 2011).

Of the 95 Southern Flounder otoliths analyzed from Louisiana coastal waters, 65% demonstrated a Sr^{88} concentration ≥ 1500 ppm in otolith cores, suggesting that these individuals formed their core in areas with elevated salinity. Of the 65%, 42% demonstrated an elevated

(≥ 1500 ppm) Sr^{88} concentration throughout the entire core profile (Figure 17A). Of the 65%, 14% demonstrated an elevated (≥ 1500 ppm) Sr^{88} concentration at the beginning of the core formation and declined to freshwater/oligohaline conditions for the remainder of core profile (Figure 17B). An additional 9% of the 65% demonstrated elevated Sr^{88} concentrations at the beginning of the core formation with a decline in concentrations indicative of freshwater/oligohaline conditions for some portion in the middle before increasing to a marine concentration at the edge of the otolith core profile (Figure 17C). The remaining 35% analyzed from this study had a Sr^{88} concentration < 1500 ppm indicating otolith core formation in freshwater/oligohaline conditions. Of the 35%, 11% demonstrated freshwater/oligohaline conditions for the entire core profile (Figure 17D), and 24% demonstrated these same conditions at the beginning of core formation then had an increase to marine concentrations for the remainder of the otolith core profile (Figure 17E).

Of the 93 Southern Flounder otoliths analyzed from Alabama coastal waters, 93% demonstrated a Sr^{88} concentration ≥ 1500 ppm in otolith cores, suggesting that these individuals formed their core in areas with increased salinity. Of the 93%, 84% demonstrated an elevated (≥ 1500 ppm) Sr^{88} concentration throughout the entire core profile (Figure 18A). Of the 93%, 9% demonstrated marine conditions at the formation of the otolith core then concentrations declined to freshwater/oligohaline conditions for some portion before increasing back to marine concentrations at the edge of the otolith core profile (Figure 18B). I found 4% of the Southern Flounder analyzed from Alabama demonstrated a Sr^{88} concentration < 1500 ppm throughout the entire core profile (Figure 18C) and 3% that demonstrated a Sr^{88} concentration < 1500 ppm at the

beginning of core formation, then increasing to a marine signature (≥ 1500 ppm) for the remainder of the core profile (Figure 18D), indicating otolith core formation in freshwater/oligohaline conditions.

Of the 97 Southern Flounder otoliths analyzed from Florida coastal waters, 52% demonstrated a Sr^{88} concentration ≥ 1500 ppm in otolith core profiles, suggesting that these individuals formed their core in areas with increased salinity. Of the 52%, 32% demonstrated an elevated (≥ 1500 ppm) Sr^{88} concentration throughout the entire core profile (Figure 19A). Of the 52%, 17% demonstrated an elevated (≥ 1500 ppm) Sr^{88} concentration at the beginning of otolith core formation and declined to freshwater/oligohaline conditions for the remainder of core profile (Figure 19B). An additional 3% of the 52% demonstrated elevated Sr^{88} concentrations at the beginning of core formation with a decline in concentrations indicative of freshwater/oligohaline conditions for some portion in the middle before increasing back to marine concentrations at the edge of the otolith core profile (Figure 19C). The remaining 48% analyzed from this study had a Sr^{88} concentration < 1500 ppm indicating otolith core formation in freshwater/oligohaline conditions. Of the 48%, 20% demonstrated freshwater/oligohaline conditions for the entire core profile (Figure 19D), and 28% demonstrated these same conditions for the beginning of core formation then had a rapid increase to marine conditions for the remainder of the otolith core profile (Figure 19E).

Discussion

In this study I examined population vital rates and spatial trends exhibited by Southern Flounder from three regions along the northern GOM to both expand on the available empirical data and to test for possible regional population differences for this commercially and recreationally important flatfish species. Historic data from these areas were used to compare with current data from this study to test for any possible effects that these Southern Flounder populations may have experienced due to the April 2010 Deepwater Horizon oil spill. Otolith microchemical analysis was used to infer natal habitat use of individuals from each area as well as to test for population differences in habitat use across the broader northern GOM.

Relative Abundance and Age Structure

I detected a west-to-east Southern Flounder relative abundance gradient, with higher CPUE estimates in Louisiana coastal waters versus those in Alabama and Florida. I also found that the proportion of older individuals in the population increased from Louisiana to Florida. In general, younger individuals dominated my collections; these findings are similar to other studies conducted along the northern GOM (Stokes 1977; Music and Pafford 1984; Stunz et al. 2000; Fischer and Thompson 2004; Nimms and Walther 2014). Older individuals are usually collected with size selective gear (i.e., gigging) which is not traditionally used in age structure analyses and was not used here. Across my sample sites, habitat that was sampled (i.e., depth, substrate, salt marsh vegetation nearby, time of set, and placement of nets in regards to tides each night) was replicated as closely as possible. Replicating the conditions for these gill net sets across all regions supports the proportion-at-age results that I observed, i.e., that older individuals are more

abundant in Florida waters when compared to Louisiana and Alabama. Given that Southern Flounder may exceed 764 mm in Louisiana (Fischer and Thompson 2004), 675 mm in Alabama (GSMFC 2000), and the world record recreationally-caught Southern Flounder was caught in Florida and was documented at 838 mm (GSMFC 2000), it is clearly possible that larger and perhaps older fish are present in the population but are likely so uncommon that they did not appear in my catches.

Mortality

Results from my catch-curve analysis also confirmed a west-to-east gradient in total annual mortality (A) estimates in these Southern Flounder populations. I found the highest estimates of total annual mortality to be from Louisiana followed by Alabama and then Florida. These findings suggest that Southern Flounder experienced higher rates of mortality in areas that had also experienced heavier oiling (Louisiana) relative to moderately oiled Alabama and non-oiled Florida. However, similar estimates of mortality have been documented from Southern Flounder in South Carolina (75.8%) and Texas (75%) coastal waters (Matlock 1992), suggesting that the high mortality estimates that I obtained may not be attributable to the oiling event. Many possible causes for increased total annual mortality can exist in large estuarine systems and the estimates generated here can be used in future maximum yield modeling and management actions of northern GOM Southern Flounder.

Growth and Condition

When growth estimates from my study were compared with historic data from Louisiana (Fischer and Thompson 2004; Blanchet 2010) and Alabama (ALMRD independent gill net

fishery data, 2001-2010), male Southern Flounder were estimated to reach a larger theoretical maximum age in post versus pre-oil data. Among regions, Louisiana and Florida male Southern Flounder reached similar length asymptotes, but Alabama males reached a much larger size at a faster rate. In contrast, female Southern Flounder from my study exhibited a west-to-east length gradient with smaller length asymptote estimates observed in Louisiana and the largest length asymptote estimates in Florida. Pre-oiling event growth estimates from Fischer and Thompson (2004) and Blanchet (2010) of length asymptote parameters in Louisiana were greater than the findings here of Southern Flounder collected from the same region. Growth estimates for females in Alabama from my study differed from historic data provided by the Alabama MRD. Female Southern Flounder collected prior to the oiling event reached a larger length asymptote ($L_{\infty} = 542$) than my estimate ($L_{\infty} = 484$). Due to the questioned accuracy of the Florida growth data submitted in Nall (1979) by subsequent studies (GSMFC 2000; Stunz et al. 2000), I was unable to reliably compare length-at-age data to the historic data collected from Florida coastal waters for both males and females.

Southern Flounder condition differed significantly across the study area, exhibiting a decreasing west-to-east gradient with Southern Flounder from Louisiana putting on more weight per length than fish from Alabama, which had greater condition than did fish from Florida. Southern Flounder collected from Louisiana and Alabama exhibited allometric growth while growth exhibited by Florida Southern Flounder was isometric. My findings for Florida fish are similar to those from Nall (1979). These results suggest that Southern Flounder are growing differently across the region. Only the condition of Southern Flounder from Florida in my study

differed from pre-oil spill data used for comparison. However, given the lack of oiling in Florida, I did not expect to see a significant difference in this region, suggesting that some other factors from this system are affecting fish condition.

Otolith Microchemistry

I used changes in the elemental composition of otoliths from Southern Flounder collected in all three areas to infer patterns of past habitat use throughout life, particularly as related to salinity. Otolith Sr concentrations have been found to be a reliable indicator of salinity in a variety of species and systems (Milton et al. 2000; Krause and Secore 2004; Lowe 2007; Whitley et al. 2007; Elsdon et al. 2008; Lowe et al. 2011; Farmer et al. 2013). Strontium concentrations across fish otoliths have also been used to examine salinity exposure throughout life for numerous species (Milton et al. 2000; Rooker et al. 2004; McCulloch et al. 2005; Brenkman et al. 2007; Farmer et al. 2013) and they were used in a similar manner in this study. Due to the positive relationship previously described between otolith Sr and salinity (Bath et al. 2000; Lowe et al. 2009; Albuquerque et al. 2012; Nelson et al. 2015), coupled with the findings of elevated Ba¹³⁷ concentrations even in the face of increased salinity (around 15 ppt) in Barataria Bay, Louisiana (attributed to the high riverine suspended particulate matter the Mississippi River drainage contains; Nelson et al. 2015), I decided Sr⁸⁸ would be the best indicator of salinity exposure of Southern Flounder for my study areas. I considered otolith Sr⁸⁸ concentrations ≥ 1500 ppm to indicate habitat use where salinity was elevated; Sr⁸⁸ concentrations < 1500 ppm were considered representative of freshwater/oligohaline habitat conditions (as in Farmer et al. 2013).

Southern Flounder is an estuarine-dependent marine species thought to spawn in marine waters near tidal inlets and whose larvae are transported inshore where they settle at the head of the estuary (Powell and Schwartz 1977; Burke et al. 1991). However, adult Southern Flounder have also routinely been reported in coastal freshwater habitats (Keup and Bayless 1964; Rogers et al. 1984; Castellanos and Rozas 2001; Lowe et al. 2011; Farmer et al. 2013; Nims and Walther 2014). Both temporal trends and mean otolith Sr⁸⁸ concentrations in Southern Flounder from these three regions suggest variable life history strategies relative to use of freshwater versus salt water habitats. My study demonstrates that 35% of Southern Flounder examined from Louisiana, 7% from Alabama, and 48% from Florida used low salinity habitat at some time during the development of their otolith core, indicating a period of freshwater residency after ingress from the marine environment. Surprisingly, of these percentages, a subset of these individuals from each region even had mean Sr⁸⁸ concentrations that remained below 1500 ppm throughout the entire otolith core region. This indicates that these fish used a freshwater or low salinity environment at the formation of the otolith core and remained there throughout the entire core development. These findings are the first of their kind for Southern Flounder from Louisiana and Florida estuaries and further validate previous results from Alabama waters (Lowe et al. 2011; Farmer et al. 2013). Similar findings have been observed in Southern Flounder populations from Texas estuaries (46% freshwater/oligohaline residency found in Nims and Walther 2014). The findings from my study on natal habitat use, combined with previous studies along the United States Gulf of Mexico coast, support that a broader definition of critical nursery

habitat (i.e., to include freshwater/oligohaline areas) may be needed for juvenile Southern Flounder.

Elements identified in crude DWH oil spill oil (Al, V, Cr, Ni, Cu, Zn, and Se) (Grosser et al. 2012) were analyzed and found to be below detection limits for all Southern Flounder from all three sample areas. Given this, no indication of oil exposure from the DWH oil spill oil was deposited in the otoliths of Southern Flounder when compared across sites. A trend of minimal effect from the DWH oil spill on Southern Flounder from this study has also been observed for other nearshore fauna (Fodrie and Heck 2011; Carmichael et al. 2012; Moody et al. 2013; Nelson et al. 2015) in the northern GOM.

I expected to find a trace metal signature in otoliths of Southern Flounder analyzed from Barataria Bay, Louisiana that reflected oil exposure given that this region received heavy amounts of oiling that persisted in estuaries well into 2011 (gomex.erma.noaa.gov/erma.html). My fish collections did not start until November 2014 for this region. Given this, collection of older individuals (including the 2010 year class) was required to strengthen my ability to find evidence of an oil signature in my analysis. Not finding elements in otoliths that were associated with oil exposure in Alabama and Florida regions was not surprising, given that the amount of oiling was light and patchy or nonexistent.

Barium concentrations from Louisiana were highest across all otolith profiles that were analyzed (i.e., core, edge, first year, and whole otolith), decreasing to the east into Alabama and Florida estuarine waters. The decreasing west-to-east gradient of Ba¹³⁷ concentrations observed here are similar to Nelson et al. (2015) for Gulf Killifish *Fundulus grandis*, and given the high

concentrations of Ba¹³⁷ in Southern Flounder otoliths collected from Barataria Bay, LA it would be logical that Ba¹³⁷ would be a good otolith marker for identifying fish that use estuarine waters around the Mississippi River due to the high amounts of riverine suspended particulate matter this drainage contains. Florida samples had the highest percentage ([Sr⁸⁸] <1500 ppm = 48%) of Southern Flounder across all regions that experienced freshwater/oligohaline conditions during otolith development, but these fish also had the lowest Ba¹³⁷ concentrations across all otolith profiles from all regions, demonstrating that Ba¹³⁷ is not solely an indicator of freshwater and supports that Sr⁸⁸ may be the better salinity indicator of habitat use throughout the northern GOM.

Strontium concentrations from Alabama were highest across all otolith profiles analyzed (i.e., core, edge, first year, and whole otolith) with little difference between Louisiana and Florida estuarine waters. Alabama also exhibited the highest percentage ([Sr⁸⁸] ≥1500 ppm = 93%) of Southern Flounder across all regions that experienced elevated salinity conditions during otolith development. Southern Flounder used in this analysis were collected in waters with higher salinities (18.64 ppt average) than those from previous studies conducted in Alabama (e.g., <5 ppt primarily in Lowe et al. 2011; Farmer et al. 2013). Yet I still found that 7% of my samples from Alabama experienced freshwater/oligohaline conditions during otolith development. These findings further support the previous findings of freshwater/oligohaline use by some juvenile Southern Flounder from this region (Lowe et al. 2011; Farmer et al. 2013).

Manganese (Mn²⁵) concentrations from otolith core and edge profiles of samples did not differ across the three areas. However, otolith core concentrations of Mn²⁵ were significantly

higher than edge concentrations in all areas. Elevated core concentrations of Mn^{25} , as well as other elements, have been documented previously (Morales-Nin et al. 2005; Ruttenberg et al. 2005; Melancon et al. 2008; Farmer et al. 2013) and it has been suggested that this phenomenon is driven by lower calcium concentrations in the core (Dove et al. 1996) or maternal element contributions during the embryonic stage (Chittaro et al. 2006). Manganese appears to be a good indicator of the core region of an otolith for Southern Flounder, but not a good otolith marker to distinguish collection locations.

Management Implications

In order to develop comprehensive fishery management plans, a better understanding of migratory behaviors of Southern Flounder juveniles is critical. It is also important to determine the importance of low salinity habitat for sustaining Southern Flounder populations. Although more research will be needed to fully quantify the life history dynamics of Southern Flounder across the northern GOM, I have demonstrated that Southern Flounder are growing differently across my three study regions. Differences in growth, mortality, size structure, and habitat use of these important recreationally and commercially nearshore fisheries identify some issues relative to the management of these species. These populations grow differently and therefore require different management strategies. Further study of Southern Flounder habitat use during early life across broad spatial scales may be needed to more fully understand the complex life history strategy of male and female Southern Flounder across their northern GOM distribution.

I collected females in greater numbers than males from all three regions. This finding was similar to those found by other authors (Music and Pafford 1984; Stunz et al. 2000; Fischer

and Thompson 2004). Due to the migratory habit of the male Southern Flounder (Stokes 1977), it would be useful in the future to target males to gain a better understanding of their age-and-growth, condition, and migration patterns such that we more fully understand male Southern Flounder and the complex life history strategy of the species as a whole throughout the northern GOM.

My otolith microchemical results further strengthen the argument for these Southern Flounder populations to be managed differently. Barium (Ba^{137}) may be a reliable marker for Louisiana Southern Flounder stock discrimination or nursery area discrimination due to their use of estuarine waters close to the Mississippi River. Also, the differences in whole otolith mean concentrations of Sr^{88} found in my study could be used to discriminate between stocks collected from Mobile Bay, Alabama versus Apalachicola Bay, Florida. Differences in otolith Ba^{137} and Sr^{88} concentrations have also been used in many other studies (Thorrold et al. 1998; Campana et al. 2000; Gillanders and Kings 2003; Vasconcelos et al. 2007; Tanner et al. 2001; Nelson et al. 2015) to classify fish to collection areas. This study represents the first evidence suggesting Southern Flounder hatching in or near freshwater environments from Louisiana and Florida waters.

Results from this study, much like previous studies examining Southern Flounder along the northern GOM (Lowe et al. 2011; Farmer et al. 2013; Nims and Walther 2014), suggest that freshwater habitats located within estuaries need to be included when defining nursery habitat for this recreationally and commercially important flatfish species. It is important to identify, delineate, and protect critical freshwater habitats used by Southern Flounder throughout their

natural distribution to increase recruitment for the stocks studied here as well as their congeners located along the U.S. Atlantic coast estuaries. More work is needed to evaluate the importance of these habitats for the migratory life history strategies and how they affect the overall population dynamics of Southern Flounder.

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Table 1. Number (n) of Southern Flounder collected from three regions of the northern Gulf of Mexico using bottom trawling gear and gill nets.

Region	n
Louisiana	229
Alabama	221
Florida	203

Table 2. Multi-model pairwise comparison of von Bertalanffy growth parameter differences between two regions of the northern Gulf of Mexico for male Southern Flounder using lengths-at-age (mm). Full model allowed growth parameters (L_{∞} , k , and t_0) to differ between regions and Null model assumes growth parameters are constant between regions. Weight is the certainty that the model is the true “best” model of the models considered.

Comparison	Sex	Model Parameters	AICc	Δ AIC	Weight	
LA vs. AL	Male	t_0	5	391.83	0.00	0.37
		k	5	392.69	0.87	0.24
		k, t_0	6	394.44	2.62	0.10
		L_{∞}, t_0	6	394.53	2.70	0.10
		L_{∞}	5	394.69	2.86	0.09
		L_{∞}, k	6	395.16	3.33	0.07
		Full	7	397.09	5.27	0.03
		Null	4	401.17	9.35	0.00
LA vs. FL	Male	Null	4	352.19	0.00	0.40
		t_0	5	353.94	1.74	0.17
		k	5	354.07	1.88	0.16
		L_{∞}	5	354.26	2.06	0.14
		k, t_0	6	356.64	4.45	0.04
		L_{∞}, t_0	6	356.75	4.56	0.04
		L_{∞}, k	6	356.88	4.69	0.04
		Full	7	359.59	7.40	0.01
AL vs. FL	Male	t_0	5	264.52	0.00	0.38
		k	5	265.34	0.83	0.25
		L_{∞}	5	266.92	2.40	0.11
		k, t_0	6	267.76	3.24	0.08
		L_{∞}, t_0	6	267.85	3.34	0.07
		L_{∞}, k	6	268.37	3.85	0.06
		Null	4	269.10	4.58	0.04
		Full	7	271.12	6.61	0.01

Table 3. Multi-model pairwise comparison of von Bertalanffy growth parameter differences between two regions of the northern Gulf of Mexico for female Southern Flounder using lengths-at-age (mm). Full model allowed growth parameters (L_{∞} , k , and t_0) to differ between regions and Null model assumes growth parameters are constant between regions. Weight is the certainty that the model is the true “best” model of the models considered.

Comparison	Sex	Model	Parameters	AICc	Δ AIC	Weight
LA vs. AL	Female	k, t_0	6	4012.37	0.00	0.42
		Full	7	4013.53	1.16	0.24
		L_{∞} , t_0	6	4013.90	1.54	0.20
		t_0	5	4015.20	2.84	0.10
		L_{∞} , k	6	4018.03	5.66	0.02
		k	5	4019.92	7.55	0.01
		L_{∞}	5	4022.69	10.33	0.00
		Null	4	4025.15	12.78	0.00
LA vs. FL	Female	k, t_0	6	5497.63	0.00	0.51
		Full	7	5498.82	1.19	0.28
		L_{∞} , t_0	6	5500.47	2.84	0.12
		t_0	5	5502.84	5.21	0.04
		L_{∞} , k	6	5504.07	6.44	0.02
		k	5	5504.60	6.97	0.02
		Null	4	5505.78	8.15	0.01
		L_{∞}	5	5505.80	8.17	0.01
AL vs. FL	Female	L_{∞}	5	5414.648	0	0.26
		k	5	5414.998	0.35	0.22
		k, t_0	6	5416.072	1.42	0.13
		t_0	5	5416.116	1.46	0.12
		L_{∞} , k	6	5416.666	2.01	0.09
		L_{∞} , t_0	6	5416.69	2.04	0.09
		Full	7	5418.045	3.39	0.05
		Null	4	5420.699	6.05	0.01

Table 4. Elements that met our criteria for inclusion in profile comparisons across regions. Elements were quantified during trace element analysis of Southern Flounder otoliths. Element limit of detection (LOD) is the mean LOD averaged for all runs. Coefficient of variation (CV) was determined from NIST 612 data from each bracketed run and is presented here as overall average. The percent of otoliths above LOD (%>LOD) for Southern Flounder from all regions is given for each element used in analyses.

Element	Element LOD	CV (%)	(%>LOD) N=279
Mn ⁵⁵	0.12	8.49	94.6
Sr ⁸⁸	0.02	8.73	100
Ba ¹³⁷	0.02	10.39	100

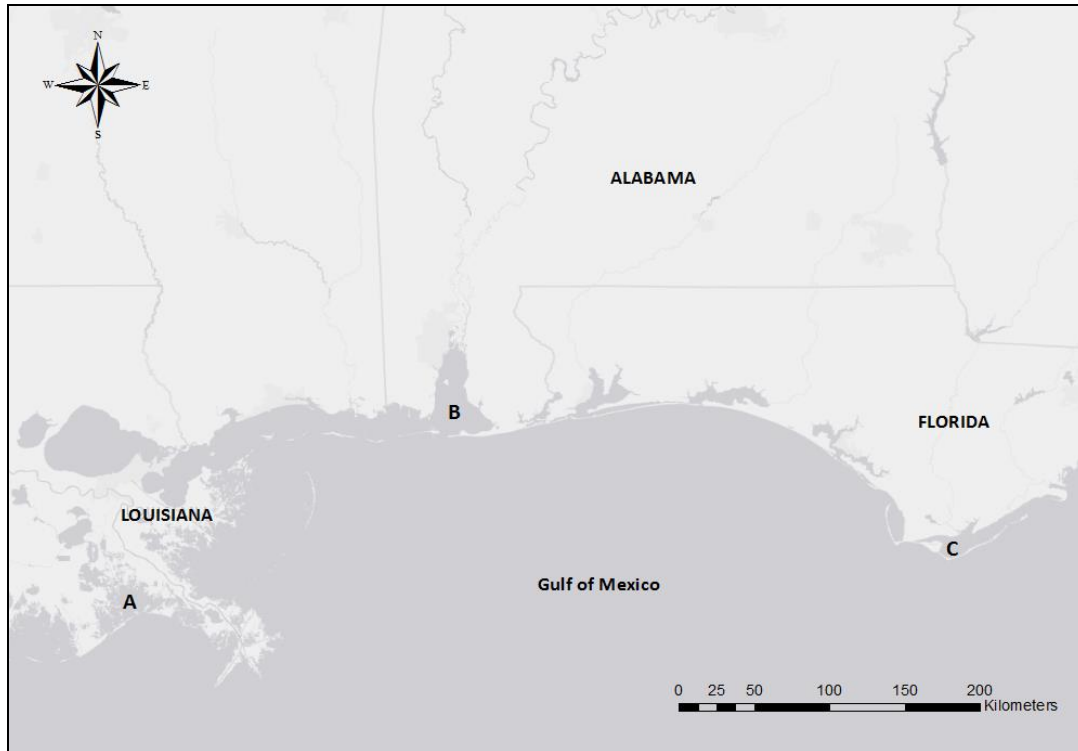


Figure 1. Study area site map indicating nearshore waters and associated sounds of Batataria Bay, LA (A), Mobile Bay, AL (B), and Apalachicola Bay, FL (C) where Southern Flounder were collected for this study.

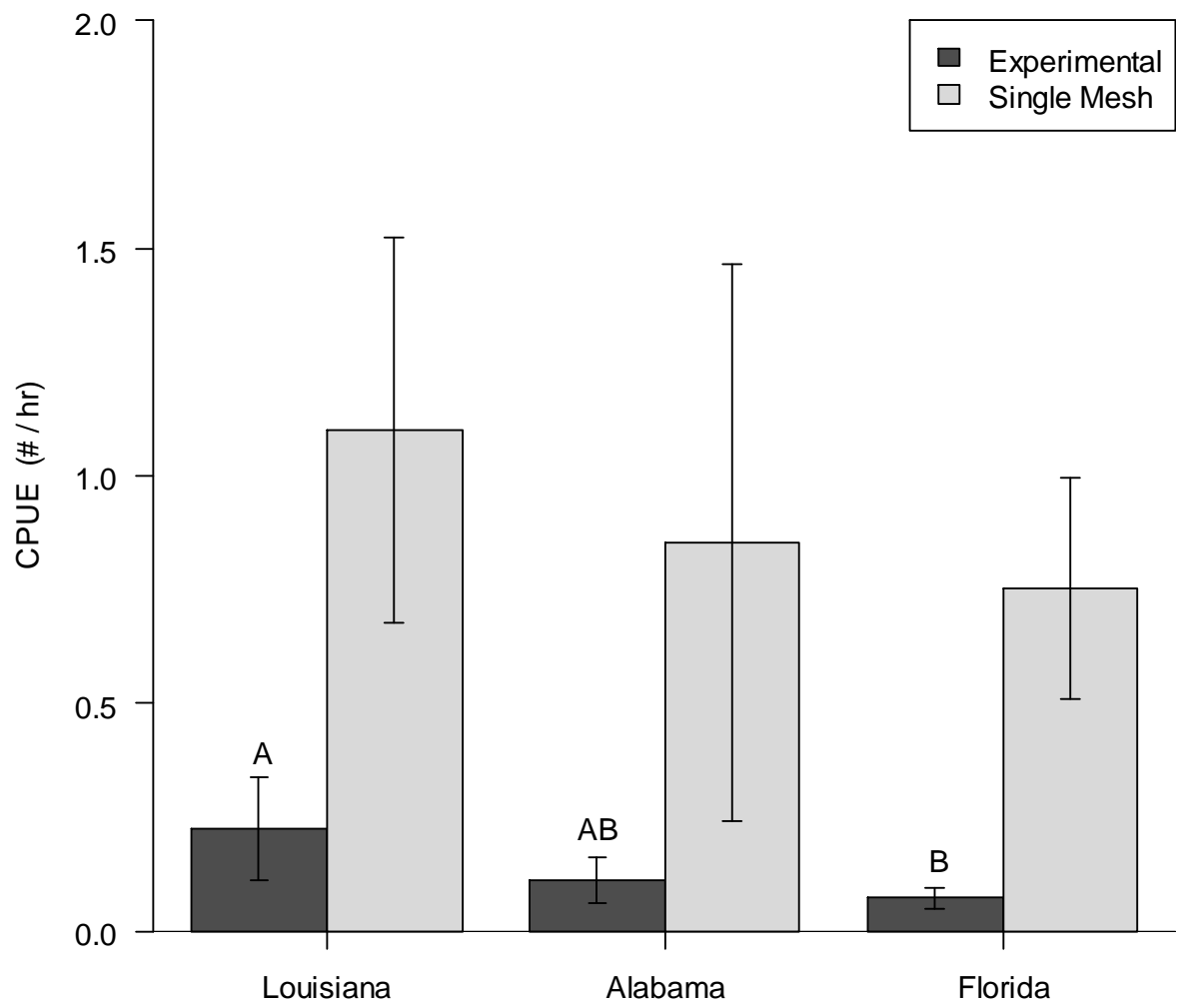


Figure 2. Gill net CPUE (#/net hr) by gear type (experimental and single mesh gill nets) and by region. Significant differences for experimental gill nets are indicated by letters. No significant differences were observed for single mesh gill net comparisons across regions.

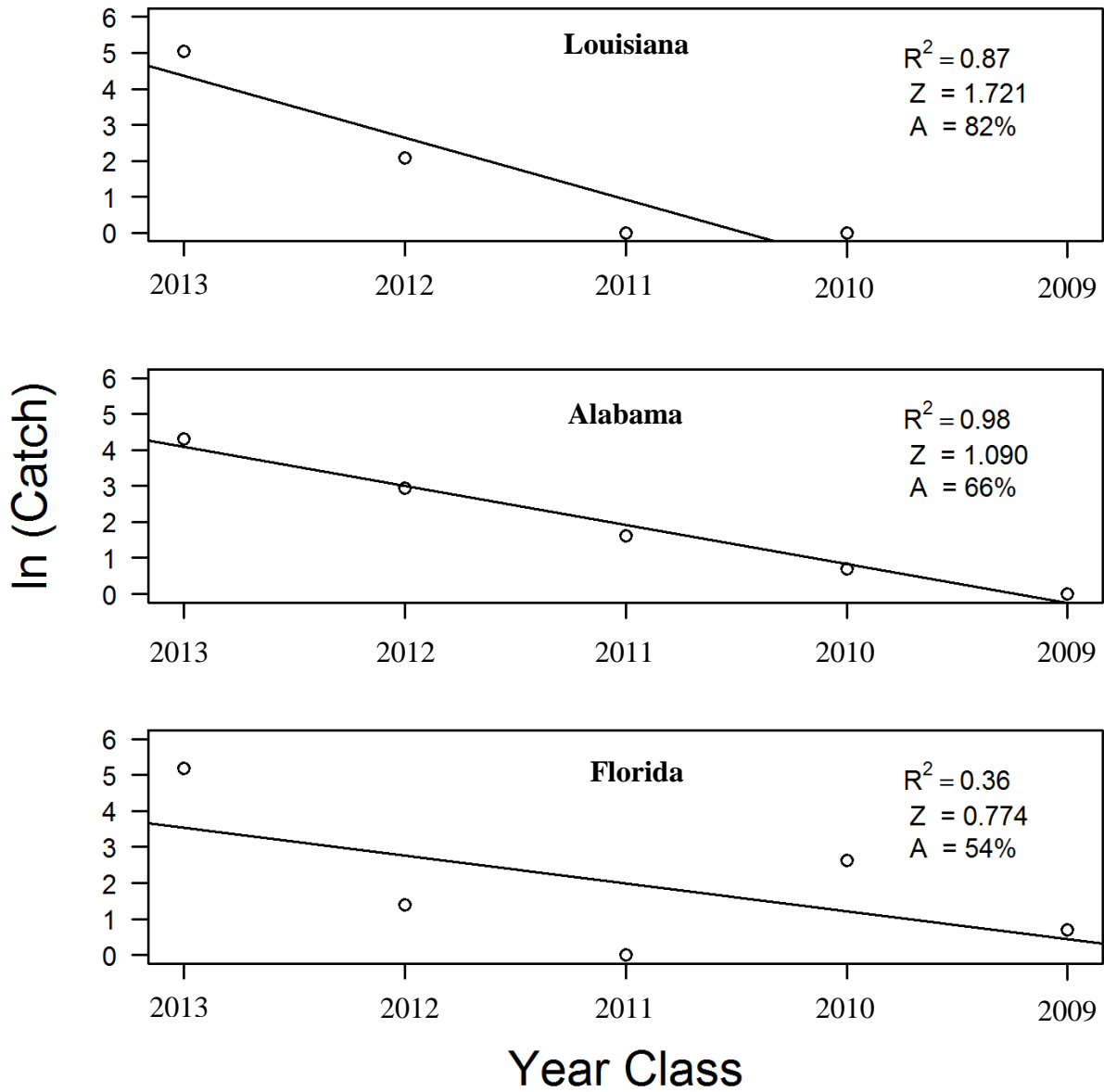


Figure 3. Log transformed catch-at-age regression (R^2), instantaneous total mortality rate (Z), and annual mortality rates (A) estimated from Southern Flounder collected from three regions of the northern Gulf of Mexico.

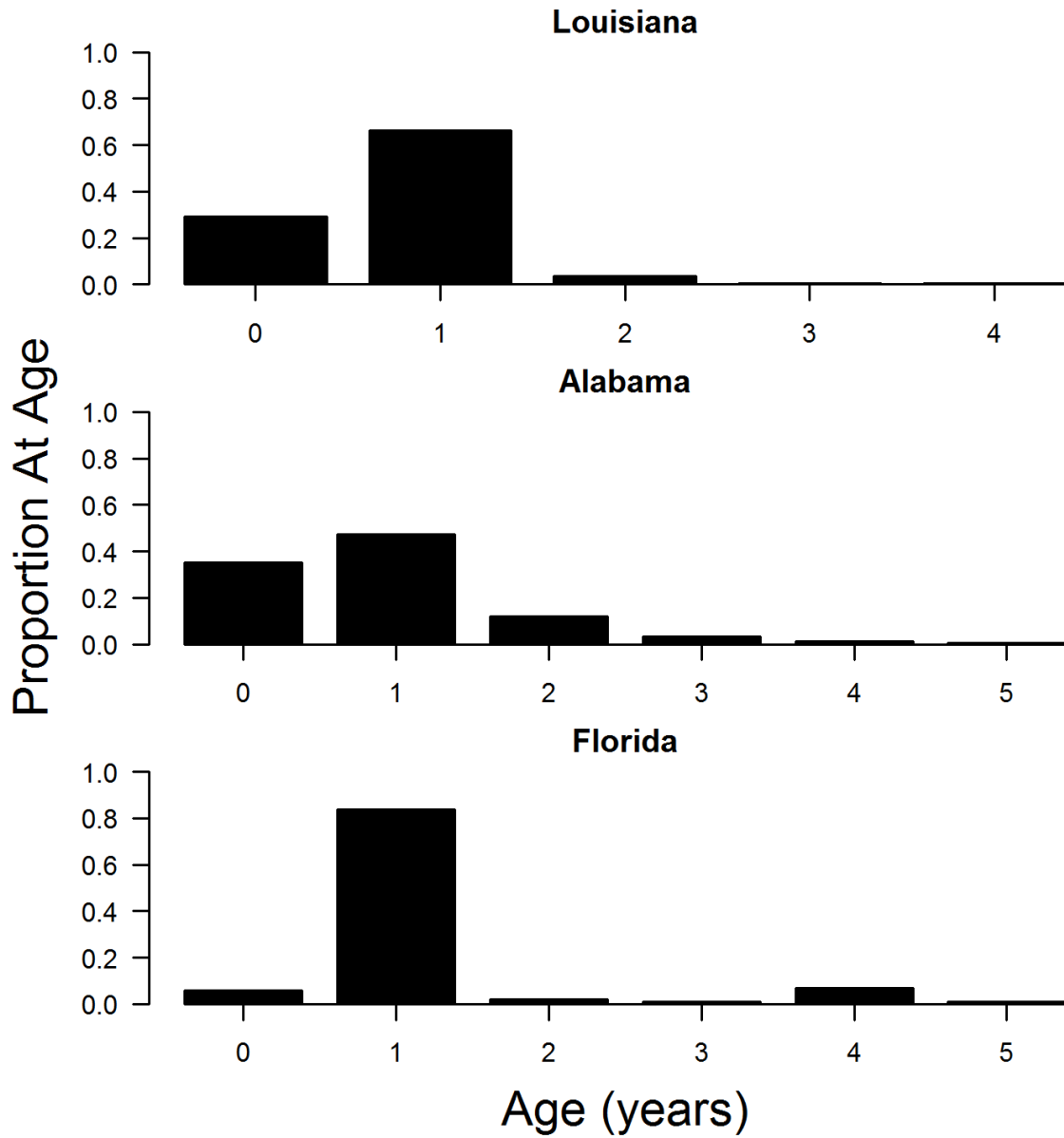


Figure 4. Age frequency distributions of Southern Flounder collected from three regions in the northern Gulf of Mexico.

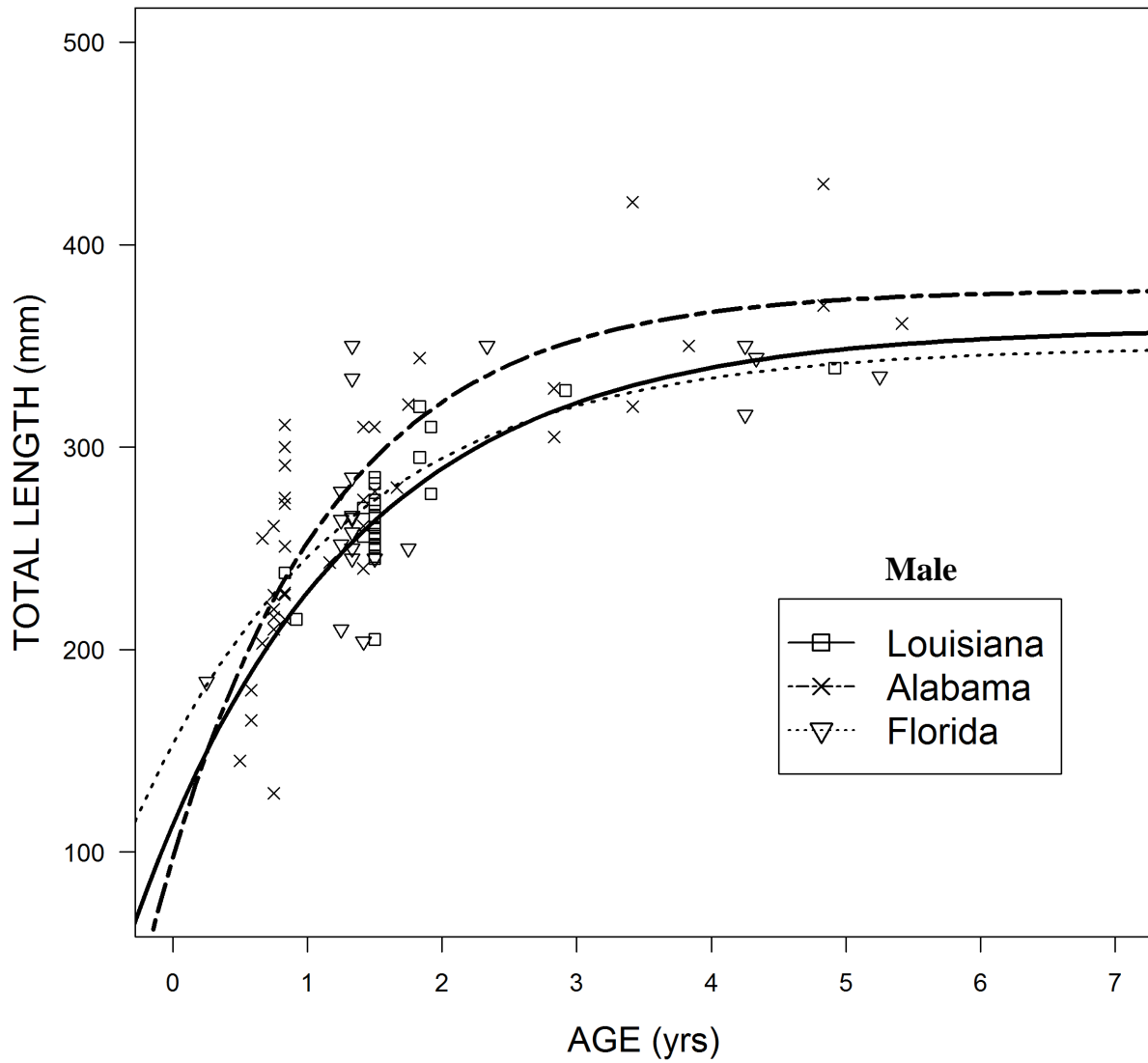


Figure 5. von Bertalanffy growth curves of male Southern Flounder collected from three regions in the northern Gulf of Mexico.

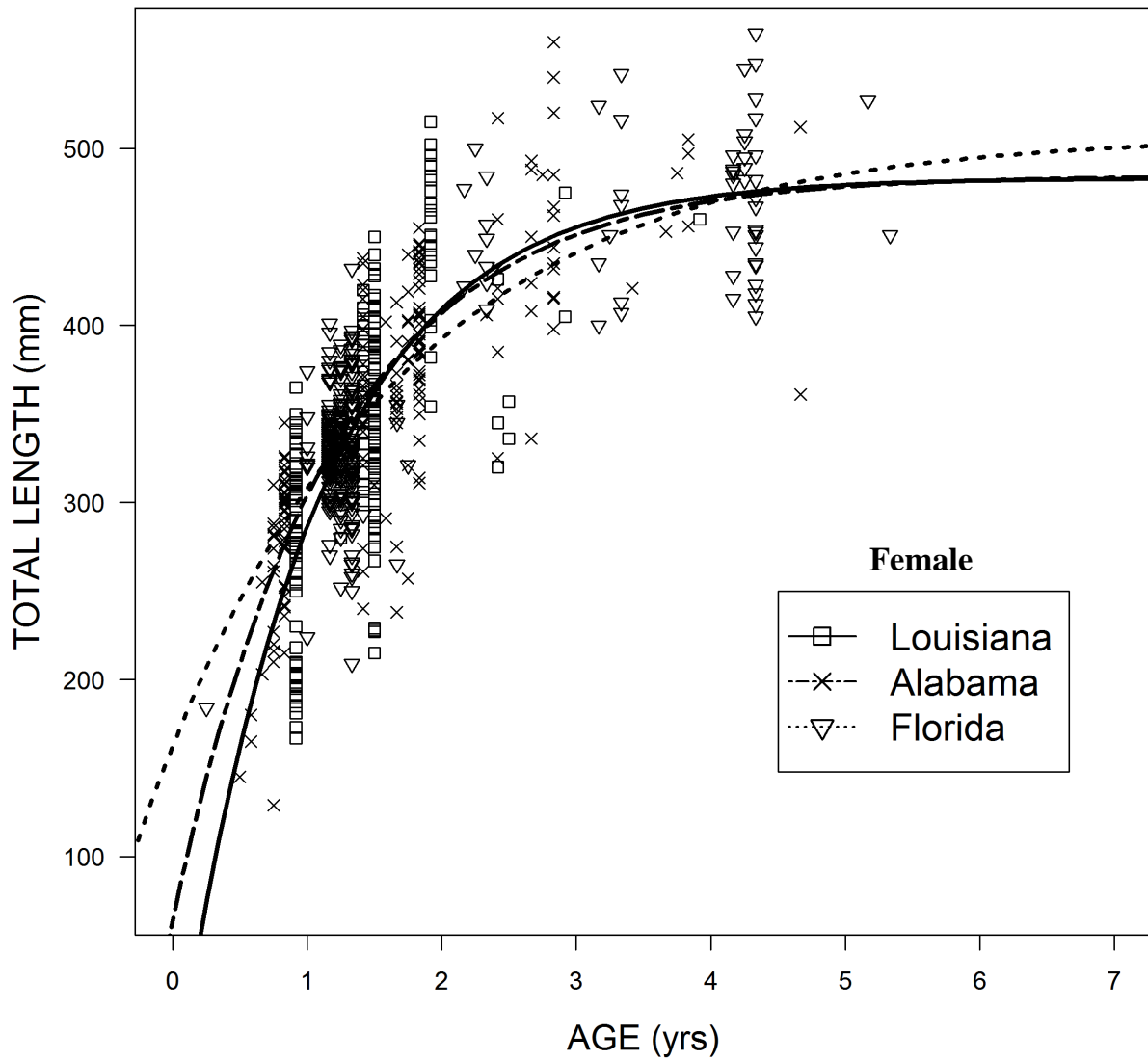


Figure 6. von Bertalanffy growth curves of female Southern Flounder collected from three regions in the northern Gulf of Mexico.

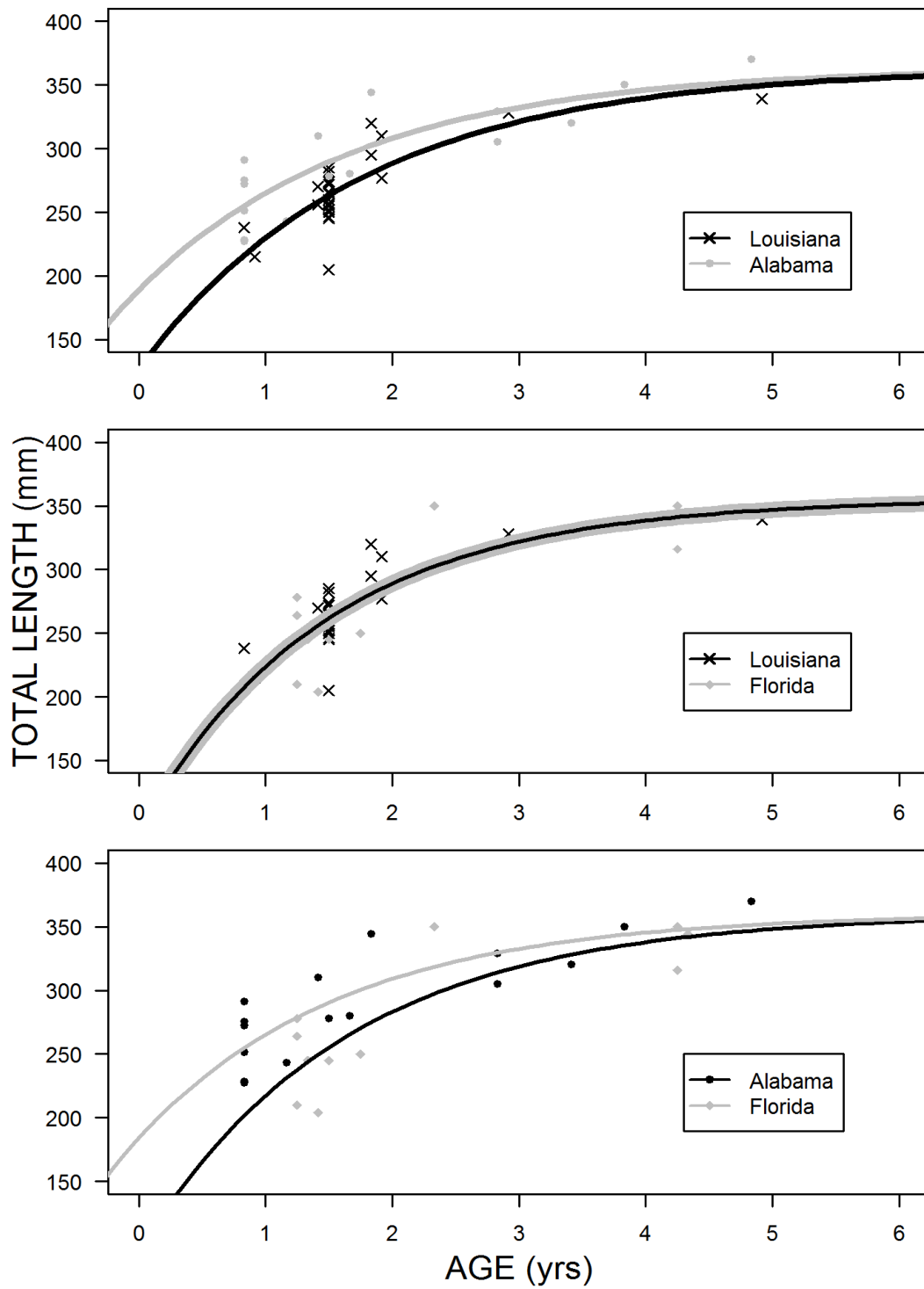


Figure 7. Multi-model pairwise comparison of male Southern Flounder length at a particular age (mm).

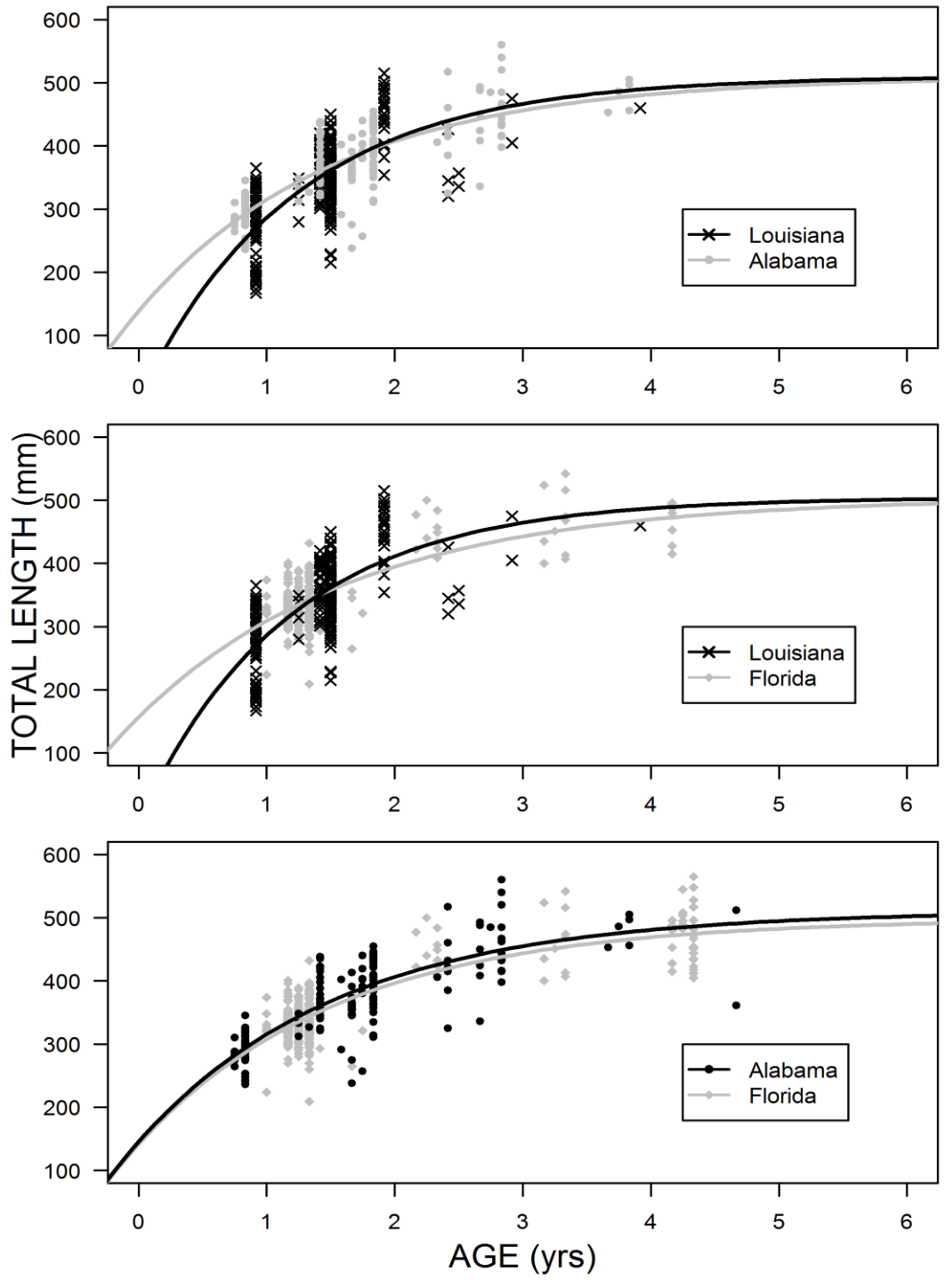


Figure 8. Multi-model pairwise comparison of female Southern Flounder length at a particular age (mm).

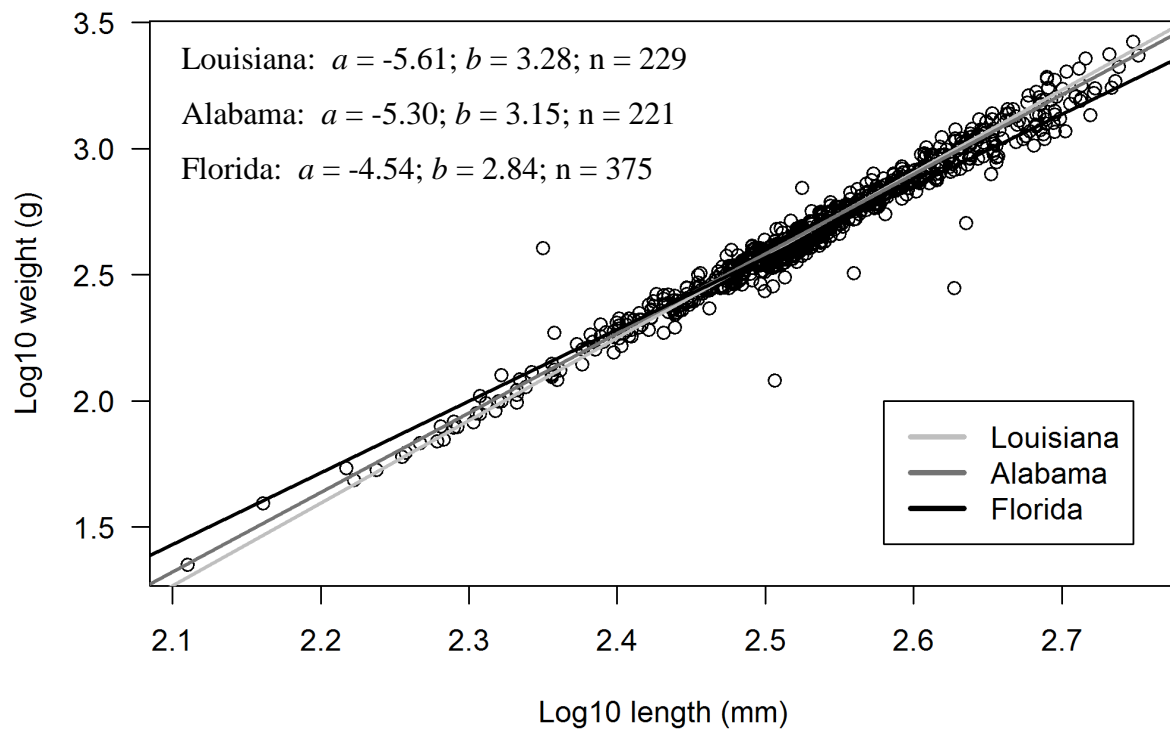


Figure 9. Weight-length relationship of Southern Flounder from three regions of the northern Gulf of Mexico. Shown are the a and b exponents from the \log_{10} transformed weight-length relationship equation and the sample size (n) used for each region. Significant differences across all regions (all $p \leq 0.007$) were observed.

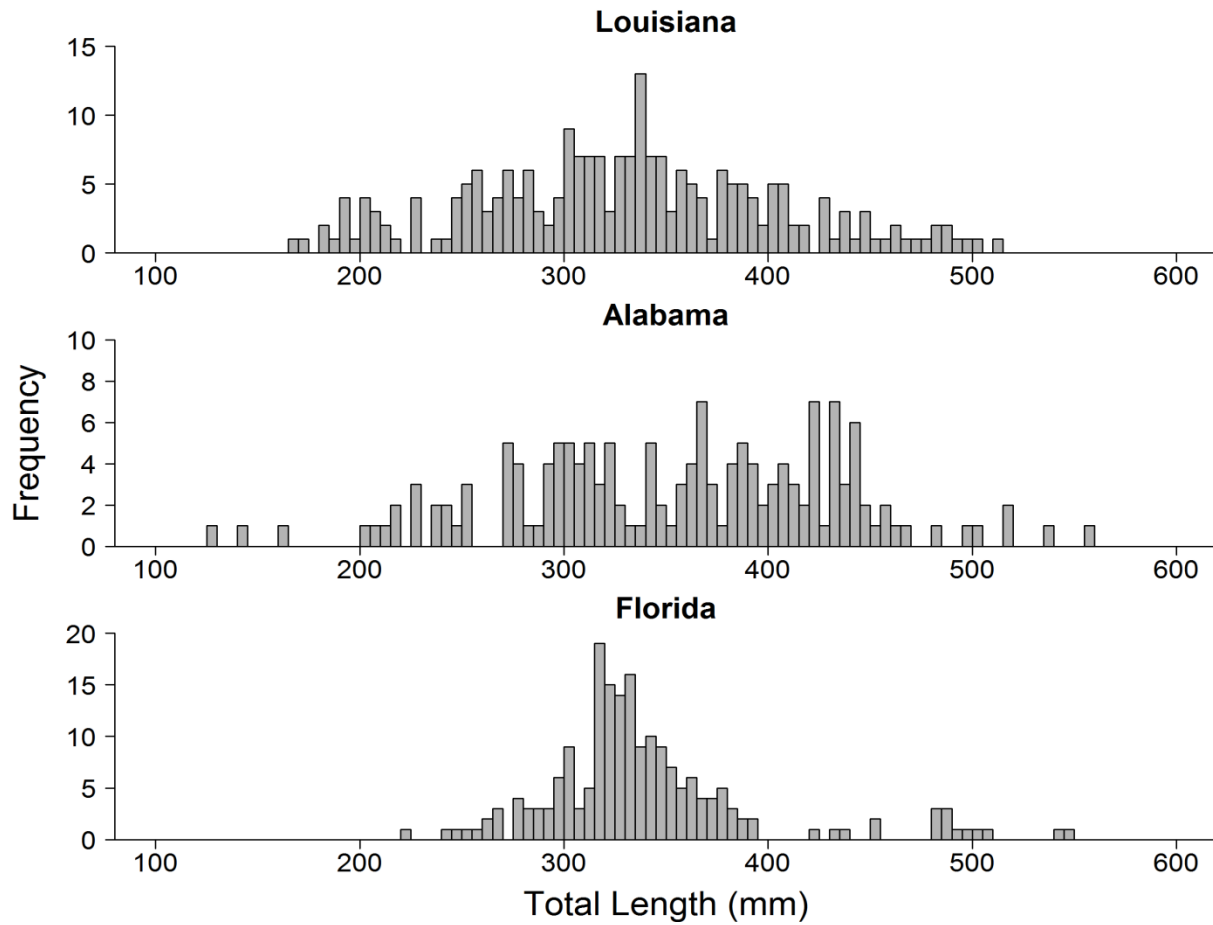


Figure 10. Length frequency distributions of Southern Flounder collected from three regions in the northern Gulf of Mexico.

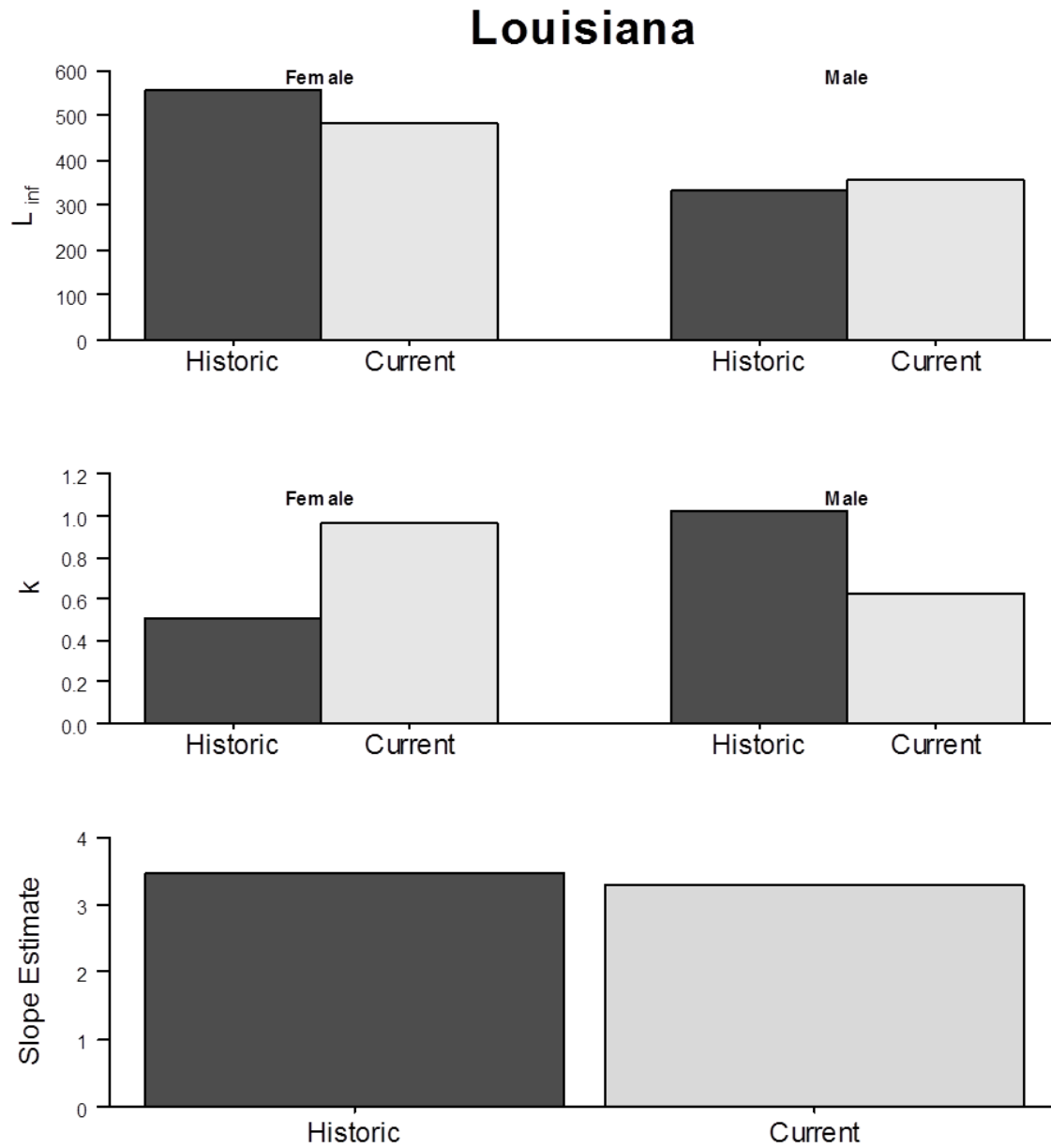


Figure 11. Historic and current von Bertalanffy growth parameter estimates and slope estimates from weight-length relationship for Southern Flounder collected from Barataria Bay, Louisiana.

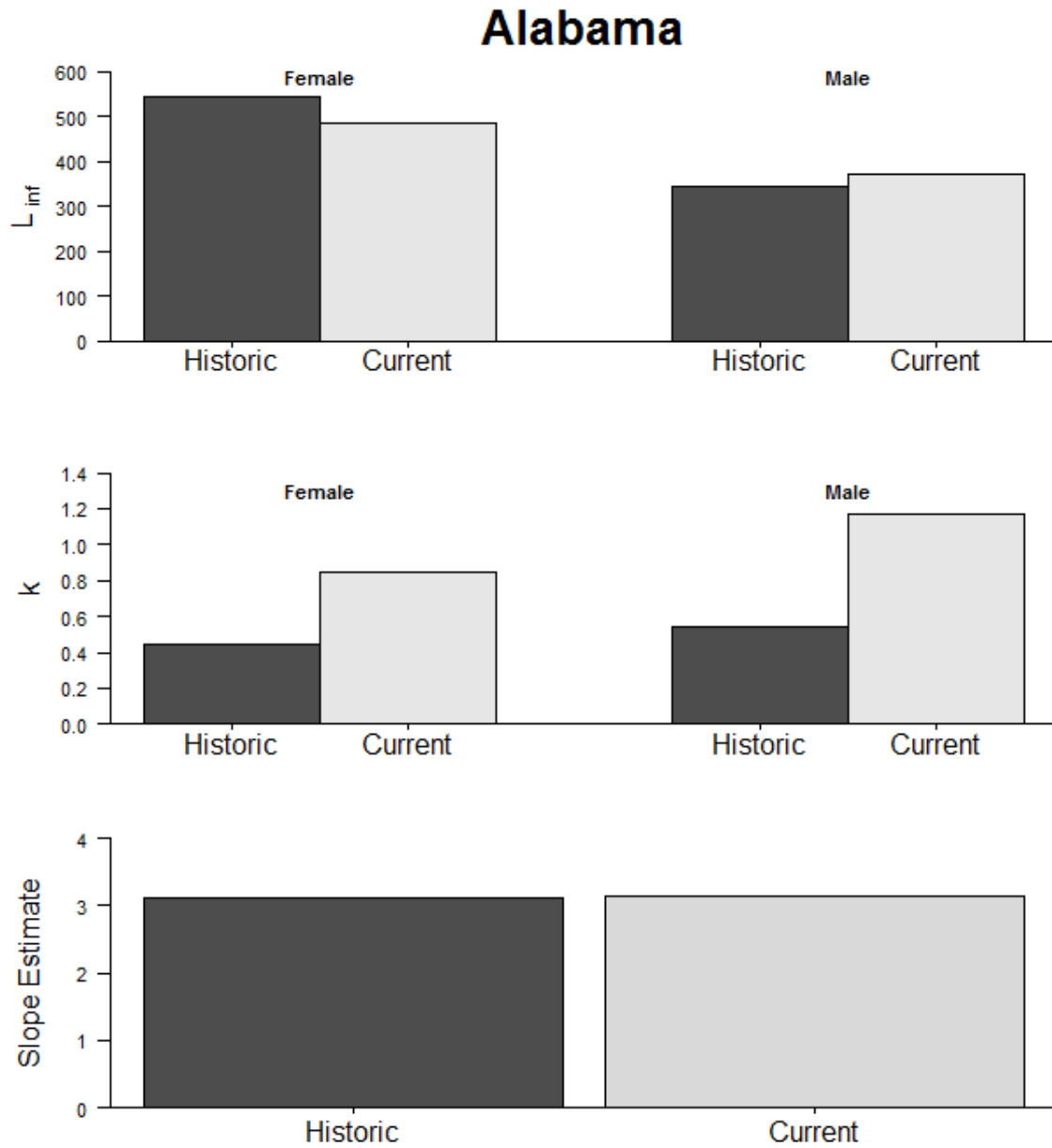


Figure 12. Historic and current von Bertalanffy growth parameter estimates and slope estimates from weight-length relationship for Southern Flounder collected from Alabama coastal waters.

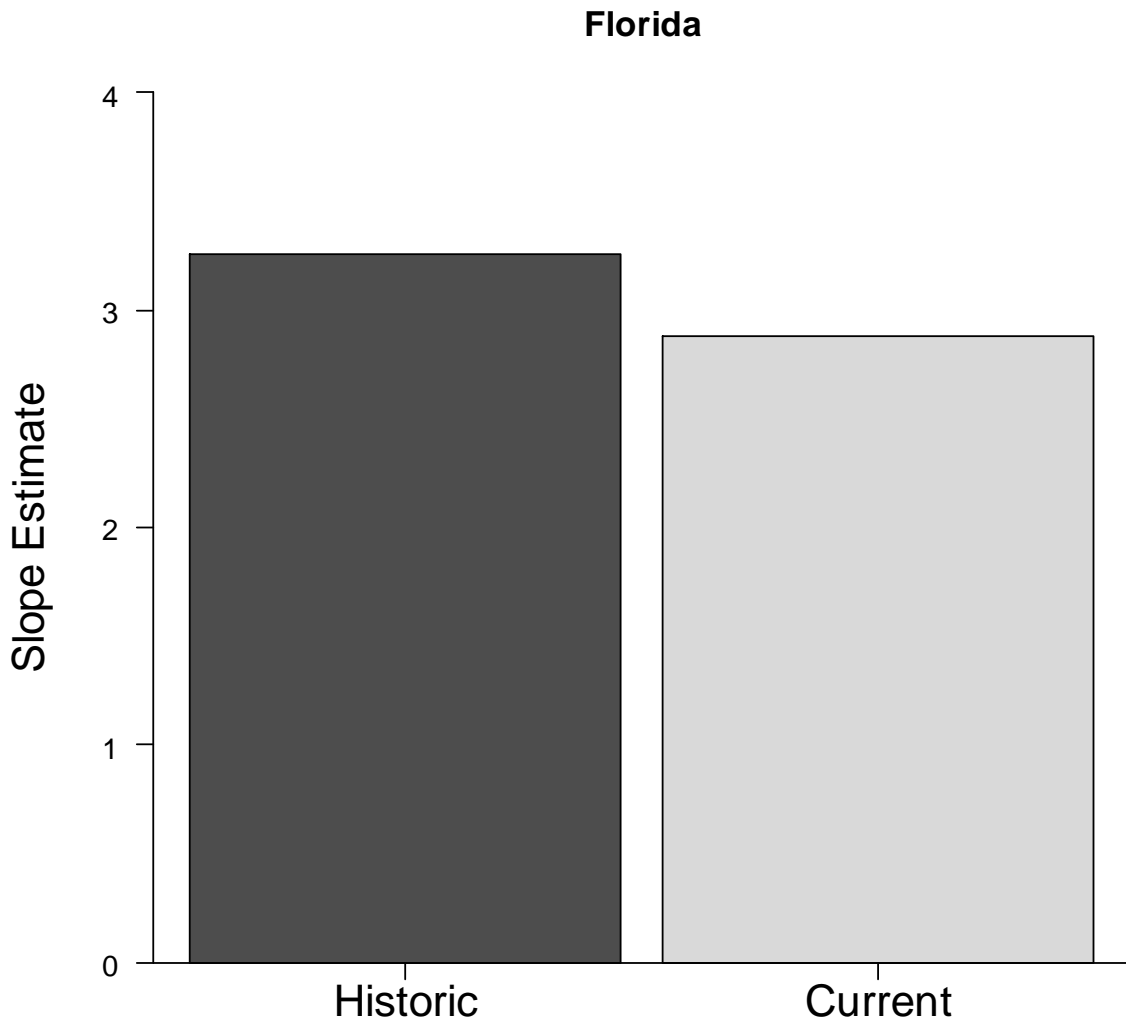


Figure 13. Historic and current slope estimates from weight-length relationship of Southern Flounder collected from Florida.

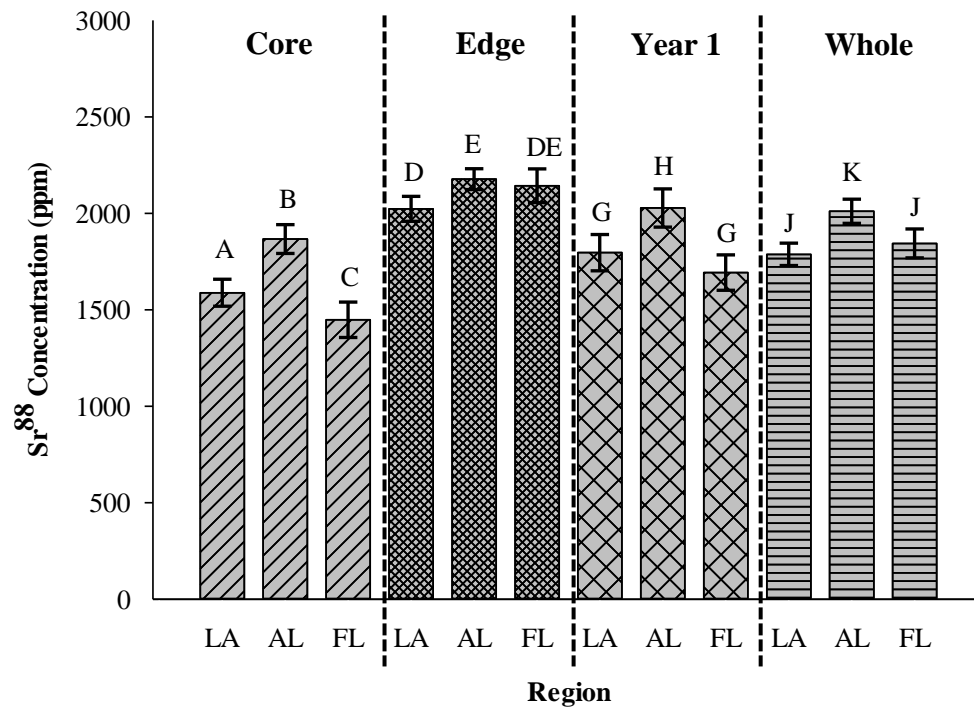


Figure 14. Mean Sr⁸⁸ concentrations for all otolith profile (displayed in legend) comparisons tested across all regions (error bars represent 95% confidence intervals). Letters above error bars correspond to significant differences detected only between profile comparisons across the three regions at a time; letters do not depict differences in comparisons between all otolith profiles across regions.

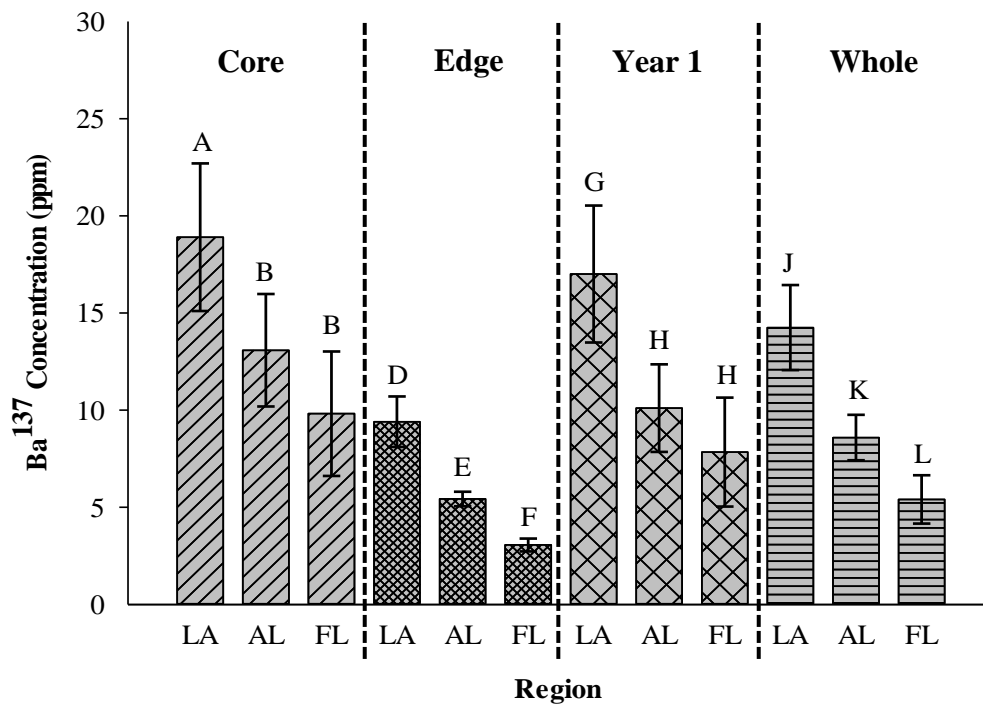


Figure 15. Mean Ba¹³⁷ concentrations for all otolith profile (displayed in legend) comparisons tested across all regions (error bars represent 95% confidence intervals). Letters above error bars correspond to significant differences detected only between profile comparisons across the three regions at a time; letters do not depict differences in comparisons between all otolith profiles across regions

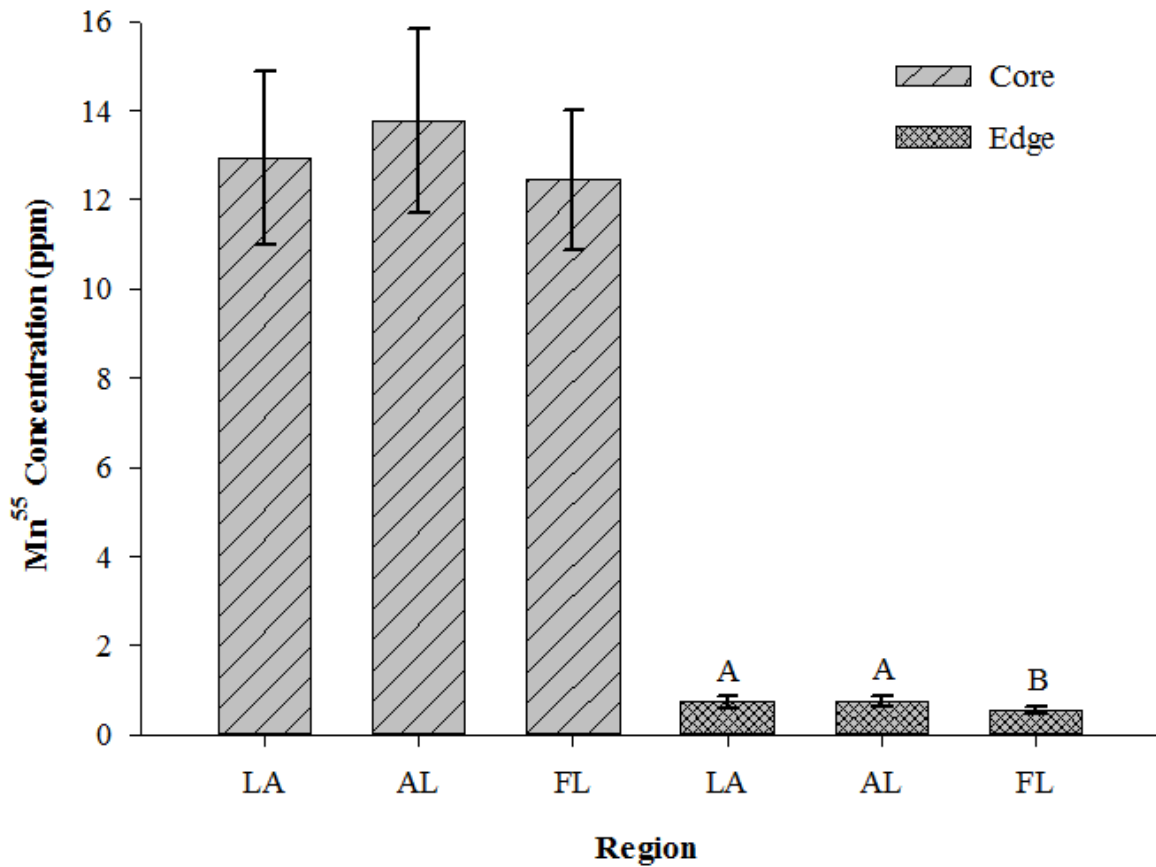


Figure 16. Mean Mn⁵⁵ concentrations for core and edge otolith profile (displayed in legend) comparisons tested across all regions (error bars represent 95% confidence intervals). Letters above error bars correspond to significant differences detected only between edge profile comparisons across the three regions at a time; letters do not depict differences in comparisons between core and edge otolith profiles across regions. No significant differences were detected in core profile comparisons across regions.

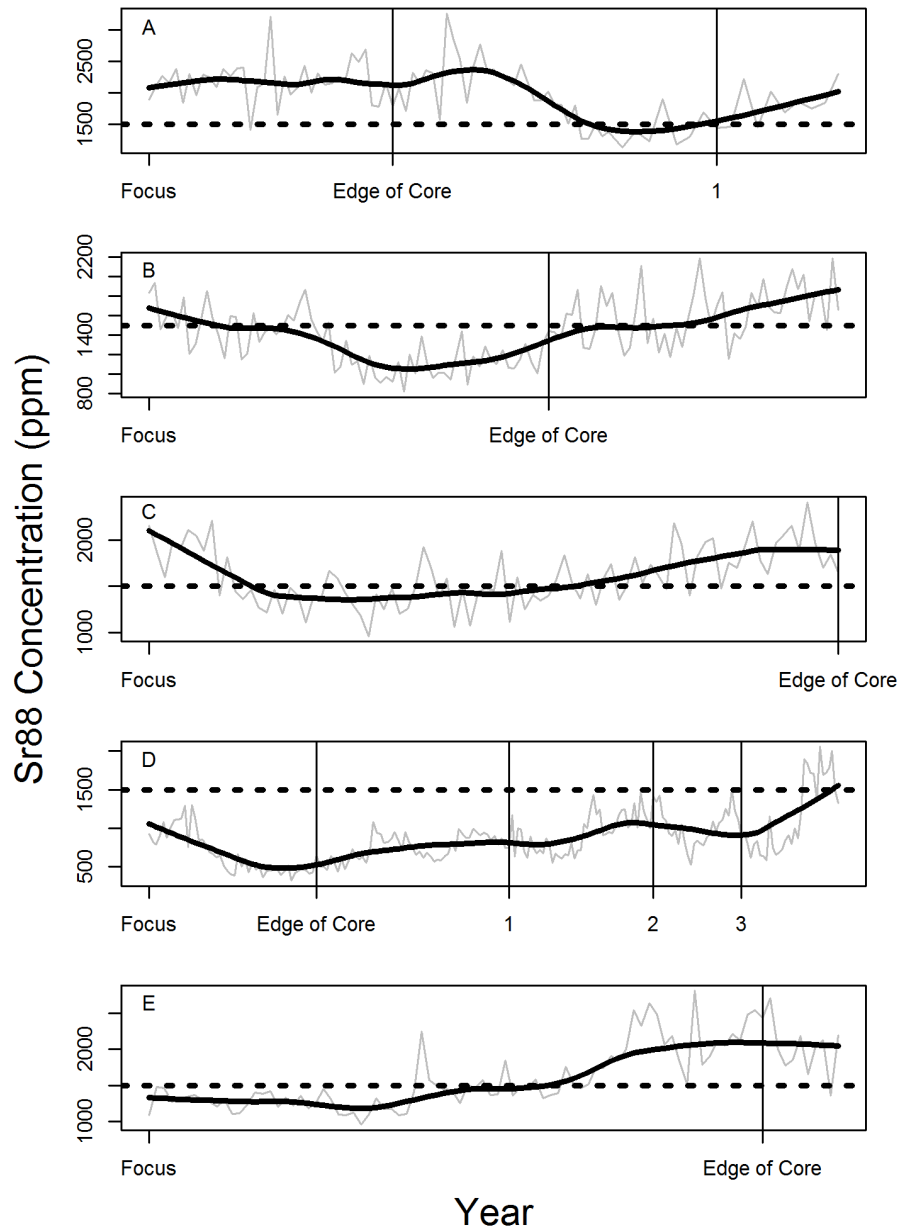


Figure 17. Otolith Sr⁸⁸ profiles for Southern Flounder collected from Louisiana coastal waters. Plots demonstrate: (A) individuals with a marine signature throughout the entire core region, (B) individuals with a marine signature at beginning of core region and declined to freshwater/oligohaline for remainder of core profile, (C) individuals with marine signature at beginning of core region and declined to freshwater/oligohaline conditions for a portion of the core profile before elevating back up to a marine signature, (D) individuals with a freshwater/oligohaline signature throughout the entire core region, and (E) individuals with a freshwater/oligohaline signature at beginning of core region and increased to marine conditions for remainder of core profile. Gray and black plots represent the raw and LOWESS (span=0.25) smoothed data, respectively. The dotted line indicates the threshold between marine and freshwater/oligohaline environments.

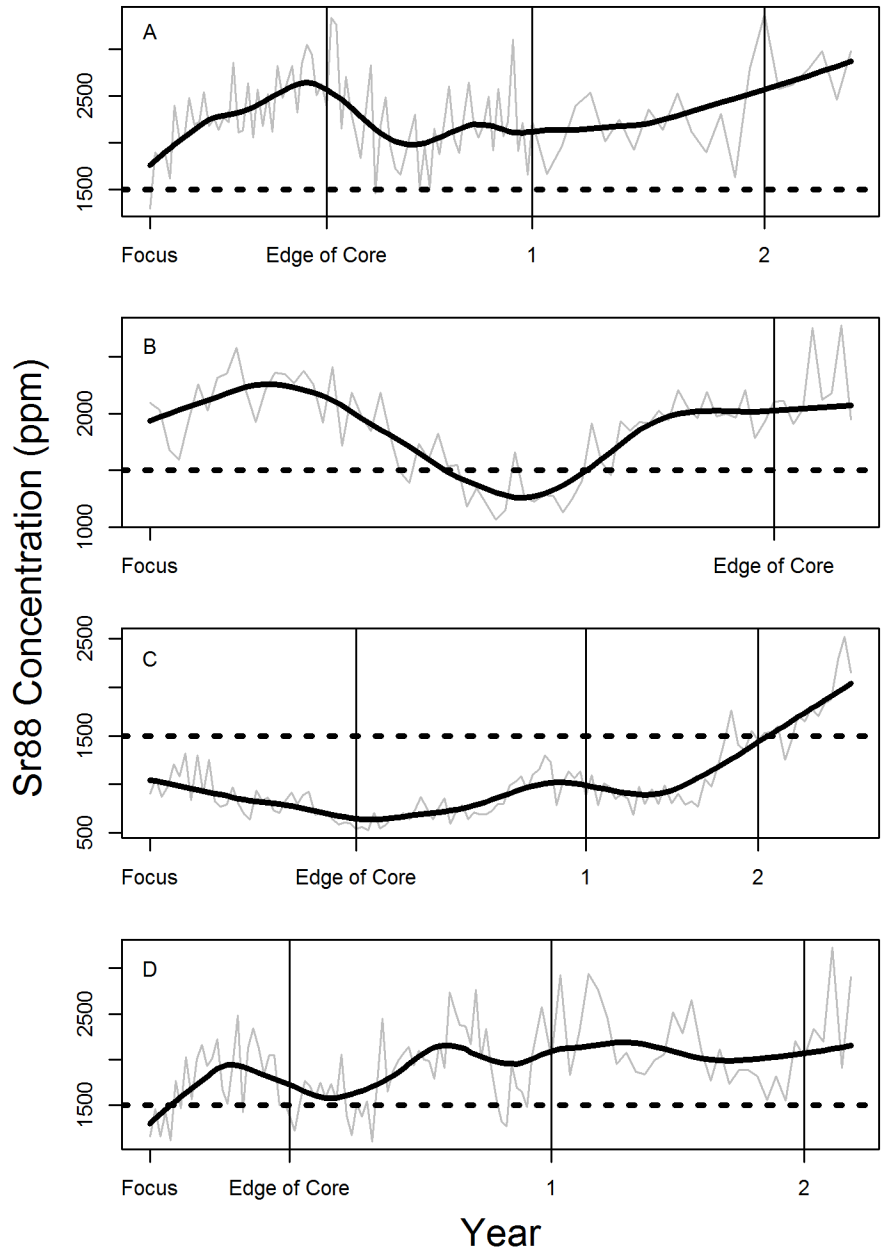


Figure 18. Otolith Sr⁸⁸ profiles for Southern Flounder collected from Alabama coastal waters. Plots demonstrate: (A) individuals with a marine signature throughout the entire core region, (B) individuals with marine signature at beginning of core region and declined to freshwater/oligohaline conditions for a portion of the core profile before elevating back up to a marine signature, (C) individuals with a freshwater/oligohaline signature throughout the entire core region, and (D) individuals with a freshwater/oligohaline signature at beginning of core region and increased to marine conditions for remainder of core profile. Gray and black plots represent the raw and LOWESS (span=0.25) smoothed data, respectively. The dotted line indicates the threshold between marine and freshwater/oligohaline environment.

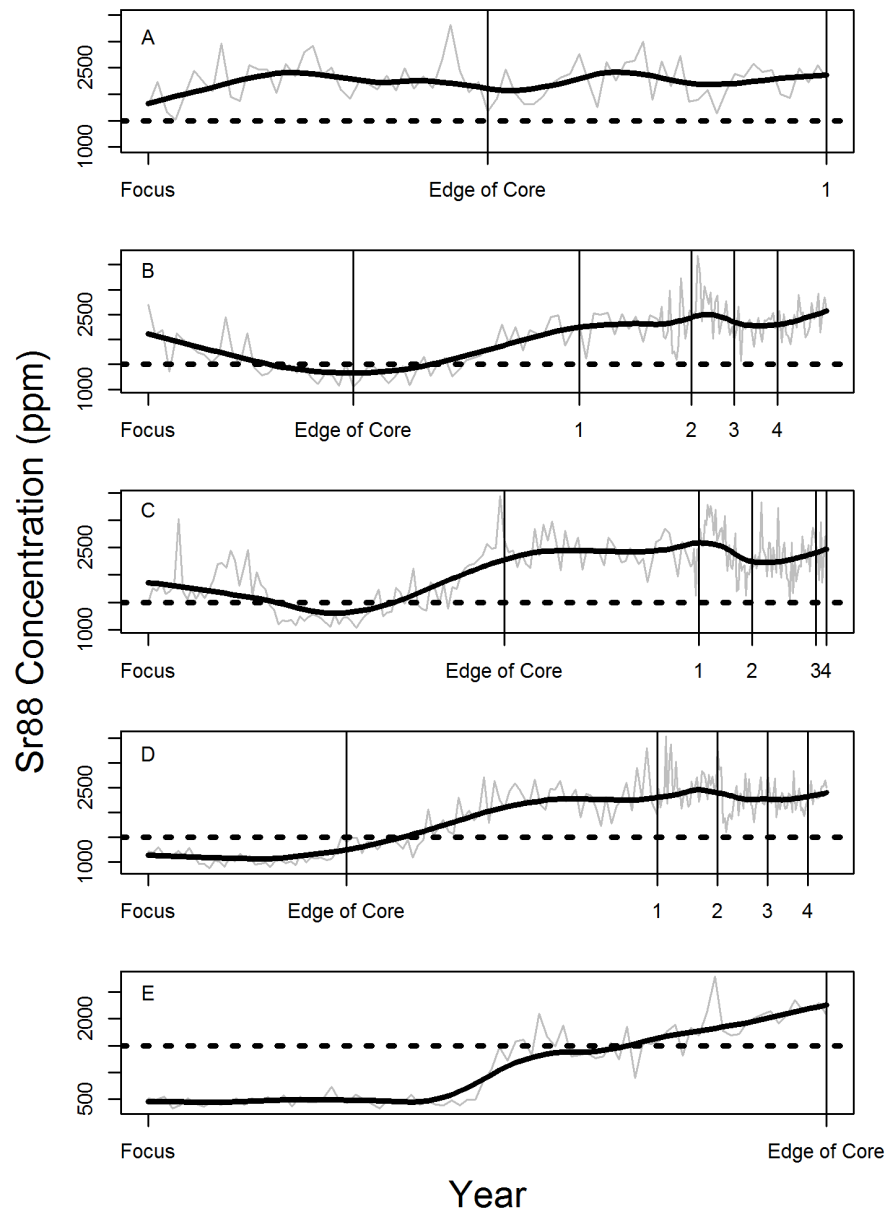


Figure 19. Otolith Sr⁸⁸ profiles for Southern Flounder collected from Florida coastal waters. Plots demonstrate: (A) individuals with a marine signature throughout the entire core region, (B) individuals with a marine signature at beginning of core region and declined to freshwater/oligohaline for remainder of core profile, (C) individuals with marine signature at beginning of core region and declined to freshwater/oligohaline conditions for a portion of the core profile before elevating back up to a marine signature, (D) individuals with a freshwater/oligohaline signature throughout the entire core region, and (E) individuals with a freshwater/oligohaline signature at beginning of core region and increased to marine conditions for remainder of core profile. Gray and black plots represent the raw and LOWESS (span=0.25) smoothed data, respectively. The dotted line indicates the threshold between marine and freshwater/oligohaline environments