

RECONSTRUCTING THE PAST SALINITIES EXPERIENCED BY A FRESHWATER  
AND MARINE PISCIVORE IN THE MOBILE-TENSAW RIVER DELTA  
USING OTOLITH MICROCHEMISTRY

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A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Masters of Science

Auburn, Alabama  
December 17, 2007

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## VITA

Michael Robert Lowe, son of Michael William and Sandra Kay Lowe, was born July 5, 1975 in Indianapolis, Indiana. He graduated from Ben Davis High School in 1993. He attended Indiana University Purdue University at Indianapolis for two years before deciding that college was not for him. He held various jobs from the Finish Line to Federal Express until he accepted in the Tool and Die Engineering Apprenticeship with WAND Enterprises in Wheeling, Illinois. During which time, he received a formal education in Mechanical Engineering from Oakton Community College in Des Plaines, Illinois. However, he decided he was in the wrong line of work and went back to school. In the summer of 2000, he enrolled in the Marine Biology/Fisheries program at Texas A&M University at Galveston, Texas. During his academic career, he worked for two years as a laboratory and field technician for the National Marine Fisheries Service and conducted independent research in Dr. Jay Rooker's laboratory. He received a B.S. degree in Marine Fisheries in May of 2004 and promptly entered graduate school in the Department of Fisheries and Allied Aquacultures, Auburn University, in June of 2004. He will work toward his Ph.D. in Coastal Sciences at the University of Southern Mississippi.

THESIS ABSTRACT

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Master of Science, December 12, 2007  
(B.S., Texas A&M University, 2004)

183 Typed Pages

Directed by Dennis R. DeVries and Russell A. Wright

In this study, we used changes in otolith microchemistry, with particular emphasis on otolith Sr:Ca, along the otolith growth axis to examine the past salinities, and thus the past environments, experienced by age-0 largemouth bass *Micropterus salmoides* and age-0 southern flounder *Paralichthys lethostigma* collected along an upstream to downstream gradient in the Mobile-Tensaw Delta (MTD). Results from a laboratory experiment indicated that there is a significant time lag (~ 21 days) between initial changes in salinity and maximum saturation levels in the otoliths of age-0 largemouth bass. For age-0 largemouth bass collected from the MTD, spatial and temporal variation in salinity, and also Sr:Ca<sub>water</sub>, resulted in otoliths from spring collected fish having markedly different Sr:Ca<sub>otolith</sub> profiles than those of fall-collected fish. Spring-collected fish had relatively stable profiles below 1500  $\mu\text{mol mol}^{-1}$ , which is indicative of a

freshwater environment. Strontium:Ca<sub>otolith</sub> profiles for fall-collected fish, particularly from sites that experienced increased salinity from late summer until their collection, were stable and below 1500  $\mu\text{mol mol}^{-1}$  for the first 70% of the profile (i.e., while ambient water was fresh), but showed an abrupt increase for the remaining 30% of the otolith.

Further, our ability to classify age-0 largemouth bass to their hatch and collection sites was also driven by spatial and temporal variation in salinity, and, as a result, water chemistry. In the early spring, when largemouth bass hatch, water chemistry in the MTD was relatively homogenous due to freshwater, and thus elementally homogenous, environments that existed from upstream to downstream. Thus, our ability to correctly classify spring-collected fish to their collection sites was poor. However, increased salinity during the fall at downstream sites resulted in a water chemistry that differed from upstream to downstream. As a result, our ability to correctly classify fall-collected fish to their collection sites improved.

Based on otolith microchemistry, 68% of age-0 southern flounder appeared to hatch in higher salinity waters before moving into the MTD. For these fish, Sr:Ca<sub>otolith</sub> was high in the otolith core and declined rapidly to  $\leq 1500 \mu\text{mol mol}^{-1}$  for the remainder of the otolith, indicating a prolonged period of freshwater residency after moving there from the marine environment. Surprisingly, not all southern flounder exhibited this pattern; 32% of fish from the MTD had Sr:Ca<sub>otolith</sub> concentrations that remained  $\leq 1500 \mu\text{mol mol}^{-1}$  throughout the entire otolith; suggesting that these fish hatched in a freshwater or low salinity environment and remained there for their entire first year of life.

## ACKNOWLEDGEMENTS

This work could not have been completed without the help and support of many people. I would like to thank my advisors, Dennis DeVries and Rusty Wright, for their guidance and support during my studies at Auburn University. Though we did not always see eye to eye on certain subjects, I learned a lot about myself as a student and a scientist as a result of their persistence, and I am better for it. I cannot thank Stuart Ludsin enough for his guidance and technical assistance throughout my M.S. studies. Further, the technical support provided by Brian Fryer, Joel Gagnon, Zhaoping Yang, and J.C. Barrette, of the University of Windsor's Great Lakes Institute of Environmental Research, was a valuable commodity.

There are many people that either were in or worked with the "Delta Force" during my stay at Auburn University. Specifically, I have to thank Dave Glover and Troy Farmer; this project would have not been nearly as interesting or fun without them. I would like to extend a heart felt thanks to Tammy DeVries, who always kept us on schedule and never, ever, let us forget a birthday in the laboratory. I also would like to thank Dave Armstrong, Mark Fritz, Adam Peer, Alicia Norris, Jennifer Barkman, Stephen DeVries, Zach DeVries, Cheryl Cortemeglia, and Mike Leonard for their assistance in the field.

Finally, I cannot express enough how extremely grateful I am to my parents for their love, support, and encouragement. Without whom, I would have never made it this far and certainly would not dare to go further.

Style manual or journal used - Ecological Applications

Computer software used - Microsoft Word, Microsoft Excel, Sigmaplot 10.0, SAS  
Version 8, and STATISTICA.



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## INTRODUCTION

Fish movement potentially influences and is influenced by a wide range of ecological processes (Deegan 1993; Winemiller and Jepsen 1998). At the local scale, fish may maximize fitness by moving among habitat patches. Further, fish may migrate through multiple environments at the regional-scale, linking ‘source’ and ‘sink’ populations (Schlosser 1998). Fish movements are often related to ontogeny (Werner and Gilliam 1984) or in response to changes in local conditions (Gross et al. 1988; Schlosser 1998). Despite the importance of fish movements to the ecology and management of aquatic systems as well as individual stocks, fish movement often is not fully characterized and remains difficult to quantify (Beck et al. 2001).

The inability to track individual fish movements can be attributed to the limitations associated with conventional tagging techniques. Traditional mark-recapture techniques, while providing important demographic information, can only indicate movement from release point to recapture site (Lucas and Baras 2000). Another limitation of mark-recapture studies is low recapture rate which may be further compounded in open systems, such as rivers and estuaries. Electronic tags, such as acoustic and archival tags, while overcoming some of these limitations, are limited to larger fish and therefore can not be used on small individuals (e.g., during early life stages) or small-bodied species. A potential solution to such limitations is the use of the elemental composition of calcified structures of fish, such as otoliths (Campana 1999; Milton and Chenery 2003; Brazner et al. 2004).

Otoliths are paired calcified structures used primarily for proprioception in teleost fishes (Popper and Lu 2000). These structures are composed of metabolically inert, aragonitic calcium carbonate that forms sequential, concentric rings on a proteinaceous matrix through continuous accretion (Panella 1971; Campana and Neilson 1985). The rate of accretion is governed physiologically and forms sequential daily, seasonal, and annual increments that indicate age for many species. Further, as sequential layers are deposited, elements from the surrounding water are incorporated into the carbonate matrix (Campana 1999). This process results in a natural marker that is independent of fish size and keeps a temporal record of the elemental composition of past environments experienced by an individual fish. The underlying premise of otolith microchemistry is that elemental concentrations in the otolith reflect the environments in which an individual fish has resided (Campana 1999). The most common application of otolith microchemistry has been identification of fish nursery areas using a suite of elements within the core region of the otolith (e.g., Secor and Zdanowicz 1998; Rooker et al. 2003). However, recent advances in analytical techniques have allowed for the analysis of the elemental composition along the otolith growth axis. Specifically, strontium (Sr) standardized to calcium (hereafter Sr:Ca) has been well studied and proven useful in reconstructing the past salinities (i.e., previous environments) experienced by an individual fish. The premise for salinity inferences based on otolith Sr:Ca are two-fold. First, the correlation between otolith Sr:Ca and the Sr:Ca concentration of the surrounding water has shown a consistently positive relationship (Farrell and Campana 1996; Elsdon and Gillanders 2004; Kraus and Secor 2004; Martin et al. 2004). Second, ambient Sr:Ca concentrations vary along freshwater-marine gradients and the relationship

between salinity and the Sr:Ca is a power-type function, with the majority of variation occurring below 8‰ salinity (Surge and Lohmann 2002). Given these relationships, several studies have reconstructed the past salinities experienced by estuarine (e.g., Chesney et al. 1998; Kafemann et al. 2000; Tzeng et al. 2002; Arai et al. 2003; Rooker et al. 2004).

Because estuaries are areas of mixing between fresh and marine waters, they are structured along a salinity gradient and, therefore, are ideal systems for using otolith microchemistry to reconstruct the past environments of individual fish as they relate to salinity. As a result of this gradient, salinity is considered the dominant abiotic factor influencing the spatial and temporal distribution of estuarine communities (Bulger et al. 1993; Jassby et al. 1995; Wagner 1999; Martino and Able 2003). However, within the freshwater portion of the estuary, the impact of salinity may be even more dramatic given the temporal variation that may occur (Odum 1988). For example, throughout much of the year this portion of the estuary is freshwater (0-0.5‰). However, during periods of reduced freshwater input, the combination of local hydrology, wind, and tides, allows saltwater to penetrate upstream into freshwater habitats creating an environment that grades from freshwater in the upstream reaches, to mesohaline (5-18‰) downstream (Odum 1988).

Such spatial and temporal variation in salinity results in a dynamic environment and potentially affects both the resident freshwater and estuarine fish communities in ways not predicted by studies conducted solely in freshwater or marine systems. First, resident freshwater fishes are exposed to increased salinity, which can be osmotically stressful (Moser and Gerry 1989; Lankford and Targett 1994; Altinok and Grizzle 2001).

For example, largemouth bass *Micropterus salmoides* is an important recreational fish species in many freshwater systems throughout North America, but also can occur in freshwater habitats of the Atlantic and Gulf coast estuaries (Meador and Kelso 1989, 1990a, 1990b; Peterson and Meador 1994). Though adult largemouth bass may respond to increased salinity by moving to freshwater (Swingle and Bland 1974; Meador and Kelso 1989), the response of age-0 largemouth bass to increased salinity has not been quantified. Previous work suggests that age-0 largemouth bass are tolerant of short periods of salinity up to 12‰ (Susanto and Peterson 1996). In natural settings, age-0 largemouth bass appear not to move during periods of increased salinity (Swingle and Bland 1974; Meador and Kelso 1989).

Second, many estuarine-dependent species are known to use the freshwater to oligohaline portions of estuaries during their first year of life (Gunter 1957; Rogers et al. 1984; Peterson and Ross 1991; Ross 2003; Posey et al. 2005). Southern flounder *Paralichthys lethostigma*, for example, is a commercially and recreationally valuable species that is thought to use freshwater habitats of estuaries during the first year (Keup and Bayless 1964; Rogers et al. 1984; Castellanos and Roza 2001). Laboratory studies suggest that salinities as low as 0‰ have little effect on the growth and survival of age-0 southern flounder (Daniels and Borski 1998; Smith et al. 1999). In Louisiana marshes, the absence of age-0 southern flounder from flatfish surveys may indicate that they are indeed using freshwater habitats (Allen and Baltz 1997). However, the biology and ecology of southern flounder in freshwater habitats has received relatively little attention.

In this study, we used changes in elemental concentrations along the growth axis of otoliths to examine the past environments experienced by age-0 fish. Specifically, we

used variation in Sr:Ca in the otolith as a proxy for the past salinities that age-0 fish may have encountered in the tidal river portion of an estuary. Our specific objectives were four-fold. First, we used otolith Sr:Ca concentrations to examine how coastal age-0 largemouth bass, collected before and after saltwater intrusion into a tidal river system, responded to increased salinities. Second, we used the same approach to examine when age-0 southern flounder potentially move into freshwater habitats. Third, we used a suite of elements in the otolith core and edge regions to retrospectively classify hatch and collections sites for age-0 largemouth bass. Fourth, we conducted a laboratory experiment to examine how changes in salinity (i.e. ambient Sr:Ca) affect Sr:Ca concentrations in the age-0 largemouth bass otoliths. Ultimately, this study will advance our understanding of how two economically important fishes, with contrasting life-history strategies, use freshwater and low salinity habitats of estuaries during the first year of life.

## METHODS

### *Study Area and Site Description*

The Mobile–Tensaw Delta (hereafter referred to as MTD) is ~55 km long, 10-15 km at its widest point, and is the terminus of the Mobile River Drainage Basin. The Mobile River basin, at 69,200 km<sup>2</sup>, has the sixth largest drainage area in North America (Tucker 1985), draining portions of four southeastern states (Alabama, Mississippi, Tennessee, and Georgia). The MTD includes portions of both the Mobile and Tensaw rivers and is characterized by a reticulated network of channels, creeks, lakes, marshes, and small embayments. Upstream, the MTD remains largely undeveloped; however, downstream areas, particularly those adjacent to Mobile Bay, are bordered by the city of Mobile and several developing communities.

The MTD is a river-dominated system and mean discharge is 1,750 m<sup>3</sup> s<sup>-1</sup>. Depending on the amount of rainfall in the basin, the MTD experiences considerable seasonal variation in salinity along its latitudinal axis. During high-flow periods, freshwater may extend into Mobile Bay (Schroeder 1978; MBNEP 1998). Conversely, during periods of low-flow (July through December) a salt wedge may intrude upstream into the Delta for several km (Schroeder 1978; Chadwick and Feminella 2001). The extent of the salt wedge also is influenced by wind direction and tides (diurnal, mean range of 0.4 m in Mobile Bay). In addition to variable salinity, habitats within the MTD are spatially complex, from seasonally-flooded bottomland hardwoods to dense stands of emergent marsh and submerged aquatic vegetation (SAV) downstream (Chaplin 2001).

We sampled six fixed sites located within the lower portion of the MTD (Figure 1). These sites, arranged along an upstream to downstream gradient, are Dennis Lake, McReynold's Lake, Gravine Island, Crab Creek, Bay Minette, and D'Olive Bay. Site selection was based upon the dominant habitat types along the main stem of the Tensaw River. The two sites within the upstream region of our study area represent small riverine channels that are either directly (i.e., Dennis Lake) or indirectly (i.e., McReynold's Lake) connected to the Tensaw River. Both of these sites are characterized by forested shorelines and stenohaline SAV. These two sites rarely experience increased salinity during low flow periods in the fall (Peer et al. 2006). The downstream sites (i.e., D'Olive Bay and Bay Minette) are shallow embayments dominated by emergent salt marsh vegetation and euryhaline SAV. During low flow in the fall, salinity can be elevated at these sites for a prolonged period of time (Peer et al. 2006). However, salinity in Bay Minette is much lower than in D'Olive Bay due to the influence of freshwater input from Bay Minette Creek. Within the middle region, Gravine Island and Crab Creek represent transitions-zone sites, thus containing characteristics of both upstream and downstream sites. During low flow, the salt wedge can extend upriver to these two sites. However, salinity at these two sites is vertically stratified and temporally variable (Peer et al. 2006).

#### *Fish Collection*

Age-0 largemouth bass and age-0 southern flounder were collected monthly from all six sites from May 2004 through October 2005. All fishes were collected via electrofishing and trawling. Boat-mounted pulsed-DC electrofishing (Smith-Root DC electrofisher, 7.5 GPP, 7500 watts) was used to collect both fish species in shallow, nearshore habitats. Electrofishing transects were 10 to 15-min in duration and total effort



was equal to at least one-hour of pedal time at each site during each month. In addition, fishes inhabiting deeper water were collected using 4.9-m head rope otter trawl (6.4-mm bar mesh wings and body and 3.2-mm bar mesh cod end). Two 5-minute trawls were conducted quarterly at each site, except McReynold's Lake due to the dominance of woody debris along the river bed. All fishes were placed on ice and returned to the laboratory for otolith extraction and preparation.

For the laboratory experiment, we used boat-mounted pulsed-DC electrofishing to collect 500 age-0 largemouth bass from Monroe County Lake, AL. Monroe County Lake is a state managed lake that was drained and restocked in 1999 with largemouth bass from the MTD. Fish were transported back to Auburn University in aerated tanks, where they were placed in holding tanks until the start of the experiment.

#### *Environmental and Water Samples*

Concurrent with fish collections, salinity, water temperature, and dissolved oxygen profiles were conducted at fixed stations at 1-m depth intervals. Data loggers (Solinst Levlogger 3001®) also were deployed at fixed depths (1 m below mean high tide) at Gravine Island and D'Olive Bay to monitor changes in water temperature, level, and salinity at half-hour intervals. Water samples for elemental analysis were collected before and during peak salinity in 2005 at each site using a Van Dorn water bottle (2200 ml) at a depth of 1 m. Each sample (~200 ml) was filtered through a 0.45- $\mu$ m filter, fixed with 125- $\mu$ l of high grade nitric acid, stored in acid-washed, 125-ml polypropylene bottles, and refrigerated until processing.

*Effect of Salinity and Ambient Water Chemistry on the Otolith Microchemistry of Age-0  
Largemouth Bass*

From 27 July to 26 August 2006 (30 days), we conducted a laboratory experiment to measure the effect of variation in salinity on the microchemistry of age-0 largemouth bass otoliths. Experimental fish were marked before the experiment with alizarin red (50 mg/L), then weighed, measured, and acclimated (increased salinity 5‰ every 24 hr) to salinities over a period of 48 h before being randomly assigned (n = 12 per tank) to one of 3 salinities (0‰, 5‰, 10 ‰; within the range of salinity that occurs in the MTD during the fall low-flow period). Six replicate 190 L propylene tanks were established for each salinity treatment (n = 18 tanks). Salinities were established by mixing bioassay grade Crystal Sea Marine Mix (Marine Enterprises International, Baltimore, MD) with local well water in a 1892 L holding tank. Each tank was independently supplied oxygen and temperature was maintained at the ambient temperature of the facility (31.6 °C). After 15 days, fish within a replicate were moved to another salinity level: 4 fish went from 0‰ to 5 ‰, 4 fish went from 0‰ to 10‰, 4 fish went from 5‰ to 0‰, and 4 fish went from 10‰ to 0‰ with the remaining fish staying in their original salinity. Prior to movement between treatments, fish received a treatment-specific fin clip. Fish remained at these new salinities for the remainder of the experiment (15 days). Salinity, temperature, and dissolved oxygen were monitored daily and experimental fish were fed live guppies *Poecilia reticulata* that were reared in 0‰ water. Seventy-five percent water changes occurred every other day to avoid increased ammonia levels and waste material on the bottom of each tank was removed at that time. At the end of the experiment, fish were

removed from each treatment tank, weighed, measured, and frozen until otolith extraction and preparation.

### *Otolith Preparation*

Sagittal otoliths were extracted from both field-collected and experimental fish, cleaned in 30% H<sub>2</sub>O<sub>2</sub> to remove any excess tissue, rinsed with ultra-filtered double-distilled water (DIUF), and stored dry in polyethylene vials for preparation for microelemental analysis. Otoliths were set in epoxy resin and a transverse section containing the core was removed using a low-speed diamond-blade saw (South Bay Technologies, Inc., San Clemente, California). Thin sections were mounted on petrographic slides with thermoplastic glue, ground on 320-, 600-, and 800-grit paper to expose the core, and polished to a smooth appearance (thickness ~ 50 µm). Prior to elemental analysis, all otoliths were examined for annuli. If annuli were absent in the otoliths of largemouth bass collected in the June or October, the fish was classified as an age-0. Southern flounder were considered age-0 if they were collected after March 1<sup>st</sup> and their otoliths lacked an annulus; therefore, we were able to include fish from February as age-0s from the previous year class. Polished otolith sections were rinsed with DIUF, dried, and transferred to round petrographic slides for elemental analysis.

### *Elemental Analysis of Otoliths*

In a class-100 clean room, prepared otoliths, 8 per round slide, were placed in acid-washed Petri dishes, covered with ultra-pure MilliQ water (MQW), and sonicated for 10 min in a MQW bath with in an ULTRASONIK cleaner (model 57X; Ney Dental, Inc., Bloomfield, Connecticut). Each slide was rinsed three times with MQW and then dried for 24 hr under a class-100 laminar flow fume hood. Elemental analysis was done

using laser-ablation inductively coupled plasma mass spectrometry (LA-ICPMS). LA-ICPMS consisted of a custom Continuum Surelite I solid state Nd:YAG laser (Field-collected fish: wavelength: 266 nm; maximum power: 20mJ; pulse rate: 20 Hz; pulse width: 4-6 ns; laser spot diameter: 16-30  $\mu\text{m}$ ; Experimental fish: wavelength: 266 nm; maximum power: 20mJ; pulse rate: 20 Hz; pulse width: 4-6 ns; laser spot diameter: 16-30  $\mu\text{m}$ ) coupled to a Thermo-Elemental X7 ICPMS. Otoliths from field-collected fish were ablated from the core to the distal edge (i.e., to provide Sr:Ca profile for an individual's entire life) while otoliths from the experimental fish were ablated only in the region corresponding to the time of the experiment (i.e., after the alizarin mark). All ablations were in a continuous straight-line transect parallel to the otolith sulcus at  $\sim 5 \mu\text{m s}^{-1}$ . For field-collected fish, we quantified the concentrations of 17 isotopes representing 13 elements;  $\text{Li}_7$ ,  $\text{Mg}_{25}$ ,  $\text{Ca}_{43}$ ,  $\text{Ca}_{44}$ ,  $\text{Mn}_{55}$ ,  $\text{Fe}_{57}$ ,  $\text{Cu}_{65}$ ,  $\text{Zn}_{66}$ ,  $\text{Rb}_{85}$ ,  $\text{Sr}_{86}$ ,  $\text{Sr}_{88}$ ,  $\text{Cd}_{111}$ ,  $\text{Sn}_{118}$ ,  $\text{Sn}_{120}$ ,  $\text{Ba}_{137}$ ,  $\text{Ba}_{138}$ , and  $\text{Pb}_{208}$ . For the experimental fish, we quantified the concentrations of 6 isotopes of 3 elements;  $\text{Ca}_{43}$ ,  $\text{Ca}_{44}$ ,  $\text{Sr}_{86}$ ,  $\text{Sr}_{88}$ ,  $\text{Ba}_{137}$ , and  $\text{Ba}_{138}$ .

To control for instrument drift, a glass reference standard (NIST 610) with known elemental concentrations was analyzed twice prior to and twice after every 16 samples. Doing so allowed us to determine precision in estimating the otolith elemental concentrations. Before each otolith was ablated, the argon (Ar) carrier gas was analyzed for 60 s (i.e., background levels) to calculate the limits of detection (*LOD*) for each element (X) in a sample using the following equation (Ludsin et al. 2006):

$$LOD_X = \frac{3 * \sigma_{\text{bgd}}}{S * Y} * \sqrt{\frac{1}{N_{\text{bgd}}} + \frac{1}{N_{\text{samp}}}}$$

where  $\sigma_{\text{bgd}}$  is the standard deviation of the background levels for a specific element;  $S$  is the mean sensitivity for a specific element (counts  $\text{s}^{-1}$  per unit concentration);  $Y$  is the ablation yield of an element standardized against the reference material;  $N_{\text{bgd}}$  and  $N_{\text{samp}}$  are replicate determinations used in the integration of the background and ablation signals, respectively. For an element to be measured reliably, it must be at least 3 SD above background levels after corrections for instrument drift, ablation yield, and sensitivity (Ludsin et al. 2006).

### *Data Preparation*

All analyses of field-collected age-0 largemouth bass were restricted to only those fish collected in June (i.e., pre-salinity; high-flow) and October (i.e., post-salinity; low-flow) of both 2004 and 2005. Due to lower relative abundances of age-0 southern flounder, we analyzed the elemental content of otoliths from throughout the year to maximize sample size. Further, we assumed a mean hatch date of April 1 for age-0 largemouth bass (Peer et al. 2006) and January 1 for age-0 southern flounder (Glass 2006). Additionally, we combined sites into upstream, middle, and downstream regions for all analyses.

Raw ablation data were converted from counts per second to parts per million content (ppm) using the following equation (Ludden et al. 1995):

$$[C_X]_{\text{samp}} = \frac{\left[ \frac{I_{m,X}}{I_{m,Is}} \right]_{\text{samp}} * (C_{Is})_{\text{samp}} * (C_{m,X})_{\text{std}}}{\left[ \frac{I_{m,X}}{I_{m,Is}} \right]_{\text{std}} * (C_{Is})_{\text{std}}}$$

where  $C$  is the concentration,  $X$  is the element being considered, and  $Is$  is the internal standard ( $\text{Ca}_{44}$  in this case). The samp and std subscripts distinguish between elements in

the sample and standard (NIST 610), respectively.  $I$  is the intensity of mass ( $m$ ) of the element and  $C$  is the concentration of mass of the element.

For an element to be used in subsequent analyses, it had to meet two criteria (Gillanders and Kingsford 1996; Rooker et al. 2001; Hedges et al. 2004; Ludsin et al. 2006). First, elements had to be above the average limits of detection (LODs) for at least 75% of fish from a given site. Second, the average coefficient of variation (CV) for element, as determined from the glass reference standard, had to be less than 10%. Based on our LA-ICMPS analyses of age-0 largemouth bass otoliths, Mg<sub>25</sub>, Ca<sub>43</sub>, Ca<sub>44</sub>, Mn<sub>55</sub>, Zn<sub>66</sub>, Sr<sub>86</sub>, Sr<sub>88</sub>, Ba<sub>137</sub>, Ba<sub>138</sub>, and Pb<sub>208</sub> fit these criteria for inclusion in the analyses (Table 1).

### *Statistical Analysis*

*Strontium:Calcium Profiles*: In order to examine the past environments experienced by field-collected age-0 largemouth bass, age-0 southern flounder, and experimental fish, we constructed plots of otolith Sr as a function of proportional distance (proportion of overall burn length of each individual fish). Raw Sr:Ca profiles were smoothed at a span of 0.25 using locally-weighted scatterplot smoothing (LOWESS) techniques (Trexler and Travis 1993; Rooker et al. 2004). The LOWESS technique put all profiles on the same x-axis scale (i.e., 0-100, by 1) and, therefore, simplified direct comparisons of individual fish.

For field-collected fish, a multivariate repeated-measures analysis of variance (MV-RM-ANOVA) that treated collection region as the main effect, proportional distance (i.e., distance from the core along the otolith growth axis) as the repeated measure, and mean smoothed Sr:Ca concentration of all individuals from a collection

region at a every 5% of the transect (N = 21 data points) as the response, was used to examine proportional distance, collection region, and proportional distance \* collection region interactions for both spring and fall-collected age-0 largemouth bass. Because age-0 southern flounder were pooled across all months of capture, we were unable to conduct MV-RM-ANOVA. For the experimental fish, we used a piecewise linear regression (PLR) to estimate slope and Knot values (i.e., the point along the otolith axis in which there was a change in Sr:Ca). MV-RM-ANOVA was used to examine the effect of salinity on Sr:Ca<sub>otolith</sub> profiles. Treatment group (i.e., original salinity to final salinity treatment), proportional distance (i.e., distance from the core along the otolith growth axis), and the mean smoothed Sr:Ca concentration at a given point along the growth axis (N = 101 data points) for all fish from a treatment group were treated as the main effect, the repeated measure, and the response, respectively. Wilks' criterion was used to assess deviations from flatness (within the main effect) and parallelism (between main effects). Mean Sr:Ca concentrations at each point along the proportional distance axis were compared among main effect levels using univariate ANOVA. Significant differences were examined further with Scheffe's test to control for experimentwise error rates.

*Classification of fish to collection sites:* To test for otolith elemental differences among the three regions, a nested multivariate analysis of variance (ANOVA) was used with site nested within collection region. Significant difference in elemental concentrations were further examined with Student-Nueman-Keuls (SNK) multiple comparison tests to test for significant mean effects. Linear discriminant function analysis (LDFA) was performed using a suite of elements that met criteria for inclusion (Table 1) to classify field-collected age-0 largemouth bass to hatch and collection regions

using the core and edge portions of the otolith, respectively. Otolith core and edge portions were defined as the first and last 10 s of each laser ablation across the otolith, respectively. Due to temporal differences in water chemistry, we conducted six separate LDFAs: 1 & 2) core microchemistry for fish collected in both 2004 and 2005; 3 & 4) edge microchemistry for fish collected in spring (i.e. pre-salinity) of both 2004 and 2005; and 5 & 6) edge microchemistry for fish collected in the fall (i.e. post-salinity) of both 2004 and 2005. Data were tested *a priori* for deviations from normality using Kolmogorov-Smirnov normality test (all  $p \leq 0.20$ ). As a result, all data were log transformed prior to analysis. Missing data (i.e., elements below LOD) were randomly distributed over predictor and grouping variables and therefore were deleted (Tabachnick and Fidell 2001). We removed outliers with elevated Mahalanobis  $D^2$  ( $\chi^2_{4, 0.05}=14.8602$ ). The removal of data points resulted in unequal sample sizes among the grouping variables. The probability of assignment to a collection region, however, was equal among groups in our LDFAs.

*Experimental Fish:* In order to quantify how salinity influenced Sr:Ca<sub>otolith</sub> in experimental age-0 largemouth bass, we plotted otolith Sr:Ca as a function of proportional distance from the otolith core. We used the same LOWESS technique as with the field-collected fish for smoothing the data. Individual fish within tanks were not independent, so tank means were treated as replicate units for the analysis. Three variables were measured from the Sr:Ca plots of experimental fish (Elsdon and Gillanders 2005b); height (defined as the smoothed asymptotic value of the plot, equates to the concentration at which saturation occurs), distance (defined as the distance, along the otolith, between the initial change and the asymptote equates to the time it takes for



saturation to occur), and slope (defined as the angle between the initial change and saturation equates to the rate at which saturation occurs). Three MANOVAs were performed to test for the effects of salinity and ambient Sr/Ca on height, distance, and slope. When a significant effect was detected, SNK multiple comparison tests were used to test for significant mean effects. ANOVA was used to test for differences in rearing conditions (i.e., salinity, temperature, and dissolved oxygen), ambient elemental concentrations (Sr/Ca), and mean fish and otolith growth among treatment groups (all  $p \leq 0.05$ ). SNK test were used to further investigate significant differences.

## RESULTS

### *Effort and Fish Collection*

Between May 2004 and January 2006, we electrofished for a total of 112 hr during 21 monthly sampling trips in the Mobile Tensaw MTD. We also bottom trawled for a total of 3.5 hr during five quarterly sampling trips. A total of 3715 age-0 largemouth bass (12 to 209 mm total length [TL]) were collected via electrofishing with no largemouth bass captured in the trawls. We collected 388 age-0 flounder (14 to 250 mm TL) via electrofishing. Bottom trawling collected an additional 18 age-0 flounder (12-260 mm TL).

### *Physicochemical Sampling and Field Water Chemistry*

Water temperature (Figure 2) and dissolved oxygen (Figure 3) were similar among collection sites and regions. Dissolved oxygen levels at the bottom tended to be lower than those at the surface during summer (Figure 3). Salinity varied both spatially and temporally, being higher during the fall in the middle and downstream sites (Figure 4). Also, salinity was generally higher in 2004 than 2005 due to differences in river inputs between years (Figure 5). Data from salinity loggers indicated considerable daily variation that was not apparent in the monthly sampling (Figure 6).

Elemental composition of water samples collected in March of 2005 was similar among all sites (Table 2). Later in the year, water chemistry differed among sites, particularly downstream (Table 2). With the exception of Pb, all elements used in the analysis were positively correlated with salinity (Figure 7). In addition, two of the

element:Ca concentrations (Mg:Ca and Sr:Ca) were correlated with salinity (Figure 8). Neither Mn:Ca or Ba:Ca were functionally correlated to salinity; however, the relationship between Ba:Ca was significantly non-random (Figure 8; 2DKS D = 0.13, p = 0.008).

#### *Sr:Ca<sub>otolith</sub> Profiles for Age-0 Largemouth Bass*

All Sr:Ca<sub>otolith</sub> profiles for spring-collected age-0 bass from Dennis Lake, for both the 2004 and 2005 year classes, were stable and consistently below 1500  $\mu\text{mol mol}^{-1}$  for the entire transect (Figure 9 and 10). With few exceptions, this pattern was repeated for spring-collected age-0 largemouth bass from all sites during both years (Figures 11-20). MV-RM-ANOVA of the smoothed profiles for spring-collected age-0 largemouth bass (Figure 21) indicated that there were clear changes in Sr:Ca across the otolith (Wilks' Lambda = 0.486;  $F_{0.05, 20, 84} = 4.43$ ;  $P \leq 0.0001$ ) and the changes did differ among collection regions (Wilks' Lambda = 0.498;  $F_{0.05, 40, 168} = 1.75$ ;  $P = 0.007$ ). Univariate contrasts showed that, with the exception of a few randomly distributed data points (Range  $F_{0.05, 1, 103} = 3.98 - 19.98$ ;  $P \leq 0.05$ ), mean Sr:Ca<sub>otolith</sub> for all collection regions at each point did not differ from the mean at the last point (Range  $F_{0.05, 1, 103} = 0.02 - 3.21$ ;  $P \geq 0.0005$ ). Further, mean Sr:Ca<sub>otolith</sub> differed significantly among collection regions at each data point (Range  $F_{0.05, 2, 103} = 5.41 - 95.96$ ;  $P \leq 0.0005$ ). Scheffe's multiple comparison test indicated that, from the otolith core to the edge, age-0 largemouth bass for the middle and downstream sites had greater Sr:Ca<sub>otolith</sub> concentrations than fish from the upstream site.

The same pattern, Sr:Ca<sub>otolith</sub> consistently less than 1500  $\mu\text{mol mol}^{-1}$  across the entire otolith, was evident in fall-collected (October) age-0 bass from Dennis Lake in

2004 (Figure 22) and 2005 (Figure 23) and McReynolds Lake in 2004 (Figure 24) and 2005 (Figure 25). Patterns for fall-collected age-0 largemouth bass from the middle and downstream sites were different from upstream fish. For example, Sr:Ca<sub>otolith</sub> for fish collected from Gravine Island in 2004 ranged in magnitude from below 1500  $\mu\text{mol mol}^{-1}$  for the first 75% of the transect to 3500  $\mu\text{mol mol}^{-1}$  near the otolith edge (Figure 26). This pattern was repeated in 2005, although the Sr:Ca<sub>otolith</sub> peaks were smaller (Figure 27). There were a couple of deviations from this general pattern in 2004 (i.e. fish numbers 6550 and 6664) where profiles lacked a distinct Sr:Ca<sub>otolith</sub> peak at the edge of the otolith (Figure 26). Strontium:Ca<sub>otolith</sub> profiles for Crab Creek fish also had a conspicuous peak at the otolith edge in both 2004 (Figure 28) and 2005 (Figure 29), though lower than those in Gravine Island. Likewise, Sr:Ca peaks were evident in 2004 (Figure 30) and 2005 (Figure 31) otolith edges from age-0 largemouth bass from Bay Minette; of all sites with a Sr:Ca peak at the edge of the otolith, this site had the smallest peaks. Both Crab Creek and Bay Minette produced individual fish that lacked a Sr:Ca<sub>otolith</sub> peak at the otolith edge. Strontium:Ca<sub>otolith</sub> profiles for D'Olive Bay age-0 largemouth bass showed a peak at the otolith edge that remained high rather than declining in both 2004 (Figure 32) and 2005 (Figure 33).

Repeated measures MANOVA of the smoothed profiles for fall-collected age-0 largemouth bass (Figure 34) indicated that there were marked changes in Sr:Ca across the otolith (Wilks' Lambda = 0.175;  $F_{0.05, 20, 86} = 20.25$ ;  $P \leq 0.0001$ ) and the changes differed significantly among collection regions (Wilks' Lambda = 0.216;  $F_{0.05, 40, 172} = 4.95$ ;  $P \leq 0.0001$ ). Univariate contrasts showed that for the first 70% of the transect, mean Sr:Ca<sub>otolith</sub> for all collection regions at each point did not differ from the mean at the last

point (Range  $F_{0.05, 1, 105} = 0.15 - 11.99$ ;  $P \geq 0.05$ ). However, between 70 and 95% of the transect, mean values differed significantly from the last data point at 100% (Range  $F_{0.0005, 1, 105} = 33.49 - 151.24$ ;  $P \leq 0.05$ ). Further, a similar trend in mean Sr:Ca<sub>otolith</sub> among groups was observed. Scheffe's multiple comparison test indicated that, from the otolith core to the edge, age-0 largemouth bass for the middle and downstream sites generally had greater Sr:Ca<sub>otolith</sub> concentrations than for fish from the upstream site and that these differences were more pronounced in toward the otolith edge (Figure 34).

#### *Sr:Ca<sub>otolith</sub> Profiles for Age-0 Southern Flounder*

Two patterns were apparent in the Sr:Ca<sub>otolith</sub> profiles for both the 2004 and 2005 year classes of flounder collected in the Mobile Tensaw Delta. Roughly two-thirds of fish had patterns where Sr:Ca<sub>otolith</sub> peaked near the core region (first 20-30% of the laser burn) and was consistently below 1500  $\mu\text{mol mol}^{-1}$  to the otolith edge. These fish were termed the 'higher-salinity origin' group. The other group, termed 'lower-salinity origin', had Sr:Ca<sub>otolith</sub> values that were below 1500  $\mu\text{mol mol}^{-1}$  from the core to the otolith edge. For flounder collected at Dennis Lake, 66 and 57% of individuals from the 2004 (6 of 9; Figure 35) and 2005 (4 of 7; Figure 36) year classes, respectively, were of the 'higher-salinity origin' group. Maximum Sr:Ca<sub>otolith</sub> values in the cores of these otoliths ranged from 1600 to 3900  $\mu\text{mol mol}^{-1}$ . For flounder collected at Graving Island, 78 and 62% of the individuals were of the 'higher-salinity origin' and had peak Sr:Ca<sub>otolith</sub> values ranging from 2000 to 3900  $\mu\text{mol mol}^{-1}$  for the 2004 year class (14 of 18; Figure 37) and 2100 to 3200  $\mu\text{mol mol}^{-1}$  for the 2005 year class (8 of 13; Figure 38), respectively. Seventy percent of the 2004 year class (7 of 10; Figure 39) and 63% of the 2005 year class (5 of 8; Figure 40) of the flounder from Crab Creek had a Sr:Ca peak

greater than 1500 in the core followed by levels below 1500  $\mu\text{mol mol}^{-1}$ . Peak Sr:Ca<sub>otolith</sub> values in the core regions ranged from 2000 to 3300  $\mu\text{mol mol}^{-1}$  for both year classes. For flounder collected at Bay Minette, 70 and 56% of individuals from the 2004 (7 of 10; Figure 41) and 2005 (5 of 9; Figure 42) year class, respectively, were of the ‘higher-salinity origin’ group. Maximum Sr:Ca values in the cores of Bay Minette otoliths ranged from 1600 to 3800  $\mu\text{mol mol}^{-1}$ . Sixty percent of the 2004 year class (3 of 5; Figure 43) and 71% of the 2005 year class (5 of 7; Figure 44) from D’Olive Bay were from the ‘high-salinity origin’ group; their otoliths had a Sr:Ca peak in the core followed by levels consistently below 1500  $\mu\text{mol mol}^{-1}$ . Peak Sr:Ca values in the core regions ranged from 1600 to 3000  $\mu\text{mol mol}^{-1}$  for both year classes. In all cases, the remaining flounder from each site were placed in the ‘low-salinity origin’ group.

#### *Classification of Hatch and Collection Regions for Age-0 Largemouth Bass*

In 2004 and 2005, otolith elemental concentrations of age-0 largemouth bass varied among collection regions (i.e. upstream, middle, or downstream) and otolith regions (i.e. core, edge [spring collection], edge [fall collection]) (Figures 45 and 46). In 2004, significant effects among the suite of elemental concentrations were detected for collection region (MANOVA; Wilks’ Lambda = 0.59; df = 10,306;  $F = 9.2$ ;  $P < 0.001$ ), otolith region (Wilks’ Lambda = 0.32; df = 10,306;  $F = 23.6$ ;  $P < 0.001$ ), as well as the interaction between collection region and otolith region (Wilks’ Lambda = 0.31; df = 20,508;  $F = 4.1$ ;  $P < 0.001$ ). Univariate analyses indicated that Mg, Ca, Sr, and Ba, differed significantly among otolith regions but not collection regions, nor was there any interaction. In contrast, Sr differed significantly among collection regions, otolith regions, and showed a significant interaction between collection region and otolith region

(Table 3). Results were similar for 2005; significant effects among the elemental concentrations were detected for collection region (MANOVA; Wilks' Lambda = 0.69;  $df = 10,264$ ;  $F = 5.5$ ;  $P < 0.001$ ), otolith region (Wilks' Lambda = 0.18;  $df = 10,264$ ;  $F = 36.5$ ;  $P < 0.001$ ), and their interaction (Wilks' Lambda = 0.68;  $df = 20,439$ ;  $F = 2.7$ ;  $P < 0.001$ ). Magnesium, Ca, Sr, and Ba differed significantly among otolith regions while Sr and Ba showed significant interactions between collection region and otolith region (ANOVA; Table 3). Further, there was a significant collection region effect for Sr (Table 3).

Based on the MANOVA results, three LDFAs were performed for each year: one comparing elemental concentrations in otolith cores; one comparing elemental concentrations of the otolith edge for spring-collected bass; and one comparing the concentrations of the otolith edge for fall-collected bass. Significant discrimination was observed (Wilks' Lambda = 0.76;  $df = 12,154$ ;  $F = 1.9$ ;  $P = 0.042$ ) for the core LDA comparing the elemental concentrations for the 2004 year-class among collection regions. One significant function ( $\chi^2 = 21.7$ ;  $df = 12$ ;  $P = 0.041$ ) was produced that explained 24% of the variation. Standardized coefficients indicated that Sr and Mn were important elements in the separating group along Root 1 and Root 2, respectively (Figure 47; Table 4). High Partial Wilks' Lambda values suggest that all of the elements contributed weakly to the discrimination. Overall, the LDA was able to correctly classify 58% of age-0 bass to collection regions (Table 5). Significant discrimination was observed (Wilks' Lambda = 0.72;  $df = 12,132$ ;  $F = 2.0$ ;  $P = 0.033$ ) for the core LDA comparing the elemental concentrations for the 2005 year class among collection regions. One significant function ( $\chi^2 = 22.4$ ;  $df = 12$ ;  $P = 0.033$ ) was produced that explained 20% of

the canonical variation. Standardized coefficients indicated that Sr and Ba were important elements in the separating group along Root 1 and Root 2, respectively (Table 4;). Like the core LDFA for 2004, elevated Partial Wilks' Lambda values suggest that all of the elements contributed weakly to the discrimination (Figure 48). Overall, the LDFA was able to correctly classify 53% of age-0 bass to collection regions (Table 5).

The LDFA comparing elemental concentrations of the otolith edge for spring-collected bass in 2004 was significant (Wilks' Lambda = 0.52;  $df = 10,100$ ;  $F = 3.9$ ;  $P < 0.002$ ) and produced one significant function ( $\chi^2 = 34.09$ ;  $df = 10$ ;  $P < 0.001$ ) that explained 41% of canonical variation. Barium was the most important element in Root 1 and Mg was important for Root 2 (Table 4). Overall, 67% of age-0 bass were correctly classified to collection regions (Figure 49, Table 5). The LDFA comparing elemental concentrations of the otolith edge for spring-collected bass in 2005 was significant (Wilks' Lambda = 0.66;  $df = 10,92$ ;  $F = 2.1$ ;  $P = 0.029$ ) and produced one significant function ( $\chi^2 = 20.09$ ;  $df = 10$ ;  $P = 0.028$ ) that explained 23% of canonical variation. Strontium was the most important element in the discrimination along Root 1 and Ba along Root 2. (Table 4). However, Partial Wilks' Lambda was high for all elements and suggests that the elements contributed weakly to the LDFA. Overall, 57% of age-0 bass were correctly classified to collection regions (Figure 50; Table 5).

The LDFA comparing elemental concentrations of the otolith edge for fall-collected bass in 2004 was significant (Wilks' Lambda = 0.29;  $df = 10,98$ ;  $F = 8.4$ ;  $P < 0.00001$ ) and produced one significant function ( $\chi^2 = 63.4$ ;  $df = 10$ ;  $P < 0.000001$ ) that explained 65% of canonical variation. Strontium was the most important element in the discrimination along Root 1 and Ca was important to Root 2 (Table 4). Overall, 71% of



age-0 bass were classified to their correct collection region (Figure 51; Table 5). The LDA comparing elemental concentrations of the otolith edge for fall-collected bass in 2005 was significant (Wilks' Lambda = 0.46;  $df = 10,92$ ;  $F = 4.34$ ;  $P < 0.0001$ ) and produced one significant function ( $\chi^2 = 63.4$ ;  $df = 10$ ;  $P < 0.000001$ ) that explained 51% of canonical variation. Strontium and Ba were the most important elements in the discrimination along Roots 1 and 2, respectively (Table 4). Overall, 70% of age-0 bass were classified to their correct collection region (Figure 52; Table 5).

*Effect of Salinity and Ambient Water Chemistry on the Otolith Microchemistry of age-0 Largemouth Bass*

Dissolved oxygen in the experiment did not differ among treatments (Figure 53, lower panel; ANOVA;  $F_{2, 531} = 0.260$ ;  $P = 0.165$ ) or time periods ( $F_{2, 531} = 0.034$ ;  $P = 0.790$ ). Temperature did not differ among treatments ( $F_{2, 531} = 0.0302$ ;  $P = 0.711$ ), although temperature did differ among time periods ( $F_{2, 531} = 81.7$ ;  $P = 0.00000$ ); water temperature was 0.5 °C warmer toward the end of the experiment (Figure 53, center panel). Salinity differed among treatment levels (Figure 53, upper panel;  $F_{2, 531} = 11.2 \times 10^4$ ;  $P = 0.00000$ ) but not time periods ( $F_{2, 531} = 0.020$ ;  $P = 0.431$ ). As a result, Sr:Ca<sub>water</sub> differed among treatments (Figure 54;  $F_{2, 16} = 20.029$ ;  $P = 0.00000$ ) and those differences were consistent for the duration of the experiment ( $F_{2, 16} = 0.076$ ;  $P = 0.673$ ).

Linear regression indicated that the slope of the “0 - 0” group ( $n = 5$ ) did not differ from zero ( $P = 0.913$ ); therefore, Sr:Ca<sub>otolith</sub> did not increase over time (Figure 55A). Piecewise linear regression (PLR) indicated that knot values for the “0 - 5” ( $n = 22$ ) and “0 - 10” ( $n = 24$ ) groups occurred at 50.6 % (SE = 1.45) and 52.6 (SE = 0.352), respectively. Assuming constant growth and that the change from 0 ppt to higher salinity

occurred at 50 % (15 d) and, therefore, Sr:Ca<sub>otolith</sub> in the “0 - 5” and “0 - 10” groups began to increase at 15.2 and 15.8 days, respectively (Figure 55B and C). For both groups, slopes after the knot were significantly different from 0 ( $P \leq 0.001$ ) but never reached an asymptote or saturation with ambient Sr:Ca<sub>water</sub>. In the “5 - 5” group ( $n = 34$ ; Figure 55D), the saturation was reached in about 22 days (knot = 73.519; SE = 0.825) and an asymptote was reached at 2479.11 (SE = 17.506)  $\mu\text{mol mol}^{-1}$ . Prior to knot, the slope was significantly greater than 0 ( $P \leq 0.0001$ ), while, after the knot, slopes did not differ from zero ( $P = 0.91$ ). Sr:Ca<sub>otolith</sub> in the “10 - 10” group ( $n = 32$ ; Figure 55E) reached saturation at 4200.28 (SE = 20.535)  $\mu\text{mol mol}^{-1}$  at approximately 21 days (knot = 71.28; SE = 0.450). Prior to the knot, the slope was significantly greater than 0 ( $P \leq 0.0001$ ), while after the knot the slope did not differ from zero ( $P = 0.74$ ). Slopes differed significantly among treatments (ANOVA;  $F_{4,112} = 8350.012$ ;  $P = 0.00$ ): slopes were greatest in the “10 - 10” and “0 - 10” treatments (Tukey’s HSD, Figure 56A). Further, in the treatments that reached saturation (i.e. “0 - 0”, “5 - 5”, and “10 - 10”), maximum levels differed significantly ( $F_{2,68} = 863.87$ ;  $P = 0.00$ ) and saturation was greatest in the “10 - 10” group (Figure 56B).

Smoothed profiles for each treatment group (Figure 57) showed significant changes in Sr:Ca<sub>otolith</sub> across the otolith (MV-RM-ANOVA; Wilks’ Lambda = 0.00000;  $F_{0.05,400,27} = 258.18$ ;  $P \leq 0.0001$ ) and the changes differed among treatment groups (Wilks’ Lambda = 0.000013;  $F_{0.05,100,6} = 4623.93$ ;  $P \leq 0.0001$ ). Univariate contrasts indicated that, except for the first 3% of the profiles for each treatment group, mean Sr:Ca<sub>otolith</sub> of all treatment groups at each point along the proportional distance axis differed significantly from the mean at the last point (Range  $F_{0.05,1,105} = 16.44 - 331.28$ ;

$P \leq 0.0005$ ). Further, there was significant mean Sr:Ca<sub>otolith</sub> by treatment group interaction (Range  $F_{0.05, 4, 105} = 5.41 - 95.96$ ;  $P \leq 0.0005$ ). The “5 - 5” and “10 - 10” treatments became significantly greater from the “0 - 5”, “0 - 10”, and “0 - 0” treatments after 3% of the transect, or about 1 day assuming constant growth (Figure 57). After 17% of the transect (i.e., 5 days), Sr:Ca<sub>otolith</sub> in the “10 - 10” became significantly greater than all treatments for the remainder of the experiment (Figure 57). After the change-over among salinity levels at 15 days, Sr:Ca<sub>otolith</sub> in the “0 - 5” and “0 - 10” differed significantly from the “0 - 0” group within 3 days (60%) after the change-over occurred. From day 18 of the experiment (i.e., 60%) to about day 27 (92%) profiles from all five treatments differed significantly. After day 27, the “5 - 5” and “0 - 10” groups converged and were statistically indistinguishable based on mean Sr:Ca<sub>otolith</sub>.

## DISCUSSION

We used changes in the elemental composition of otoliths to infer patterns of past habitat use, as they relate to salinity, for both age-0 largemouth bass and age-0 southern flounder in a tidal river estuary (i.e., the Mobile-Tensaw Delta). Specifically, we used the relationship between salinity and  $\text{Sr}:\text{Ca}_{\text{otolith}}$  observed in both our study and others (Bath et al. 2000; Milton and Chenery 2001; Kraus and Secor 2004) to infer how these species responded to changes in salinity. Largemouth bass did not appear to move away from habitats as salinity increased. Southern flounder may have moved into freshwater habitats earlier in life than previously expected and showed variable responses to increased fall salinity.

### *Age-0 Largemouth Bass*

From January to June (i.e., the collection month for spring fish), there was little spatial variation in both salinity and  $\text{Sr}:\text{Ca}_{\text{water}}$  among sites during both 2004 and 2005. As a result,  $\text{Sr}:\text{Ca}_{\text{otolith}}$  profiles for spring-collected age-0 largemouth bass expressed little variation and concentrations were generally below  $1500 \mu\text{mol mol}^{-1}$ , which has been shown in work by others to be a threshold value between freshwater and elevated salinity (Limburg 2001; Zimmerman 2005; Brenkman et al. 2007). Salinity at upstream sites remained within the freshwater range (0-0.5 ‰) during summer and fall, similar to spring-time conditions. However, salinity increased at both the middle and downstream sites during both years from August through November. Strontium: $\text{Ca}_{\text{otolith}}$  profiles for

fall-collected age-0 largemouth bass from upstream sites were similar to the profiles of spring-collected fish (i.e., low concentrations with little variation). Profiles for fall-collected fish from the middle and downstream sites were stable and below  $1500 \mu\text{mol mol}^{-1}$  for the first 70% of the profile (i.e., while ambient water was fresh), but then showed an abrupt increase for the remaining 30% of the otolith, consistent with the timing of increased salinity and ambient Sr:Ca concentrations that occurred prior to collection.

Experimental results provide additional support that increased Sr:Ca<sub>otolith</sub> at the otolith edge of fall-collected fish from downstream sites is due to a lack of movement in response to increased salinity. First, in treatment groups exposed to either 5 or 10‰ salinity for the duration of the experiment, Sr:Ca<sub>otolith</sub> reached saturation at about 21 days (assuming constant growth). A 21 d lag between environmental change and saturation in the otolith has also been suggested for several other species (Milton and Chenery 2001; Elsdon and Gillanders 2004). Second, though fall-collected age-0 largemouth bass from the middle and downstream sites were not exposed to constant salinity levels from late-July until their collection, peak Sr:Ca<sub>otolith</sub> concentrations were similar to those from experimental fish exposed to constant salinity levels, particularly the “5-5” and “0-10” treatments. This suggests that observed Sr:Ca<sub>otolith</sub> concentrations for field-collected age-0 largemouth bass, particularly at the otolith edge, were the summation of exposure to varying levels of salinity or ambient Sr:Ca. Given the time needed for to achieve saturation relative to ambient conditions, age-0 largemouth bass must be residing in areas of increased salinity rather than dispersing in search of freshwater.

Our ability to correctly classify age-0 largemouth bass to their collection regions was driven by both seasonal and regional variability in ambient water chemistry. Classification rates, based on mean element concentrations of the otolith core (both spring and fall-collected fish) and edge regions (only spring-collected fish) of age-0 largemouth bass, were poor. This likely resulted from the freshwater conditions that persisted throughout the MTD during the spring of both years such that water chemistry was relatively homogenous among sites. However, classification rates for otolith edge concentrations of fall-collected largemouth bass improved due to the middle and downstream collection regions being elementally distinct from the upstream region as a result of increased salinity. Again, these findings support that age-0 largemouth do not move into lower salinity areas in response to increased salinity. If age-0 largemouth bass were moving to remain in freshwater, we would expect edge concentrations from fall-collected fish to be similar among collection regions and our classification rates would remain poor.

Several studies have suggested that adult largemouth bass in coastal systems disperse to freshwater environments in response to increased salinity (Swingle and Bland 1974; Meador and Kelso 1989; but see Norris et al. 2005), but none have attempted to directly quantify the movements of age-0s due to the difficulties associated with tagging small fish. By not dispersing from areas in response to seasonal increases in salinity, and age-0 largemouth bass are exposed to additional factors that potentially alter vital rates and eventual recruitment dynamics (Houde 1987). Early life growth, in particular, is important in determining year-class strength and, subsequently, recruitment to later life stages (Gutreuter and Anderson 1985; Garvey et al. 2002; Ludsin and DeVries 1997). In

coastal systems, increased osmoregulatory costs at salinity above 10‰ result in increased routine metabolism (Susanto and Peterson 1996). As a result, less energy can be allocated to growth and coastal largemouth bass populations often express slower growth during their first year of life than populations from freshwater systems (Herring 1981; Meador and Kelso 1990a; but see Peer et al. 2006). Meador and Kelso (1990a) compared mean length at first annulus formation (i.e., age-1 fish) for largemouth bass from coastal and freshwater locations along the Gulf of Mexico and mid-Atlantic coasts. Coastal largemouth bass were always smaller at age-1 than freshwater conspecifics; however, the discrepancy was much larger for the Gulf of Mexico versus the mid-Atlantic region. However, in the MTD, age-0 largemouth bass grew faster at sites exposed to increased fall salinity when compared to fish from freshwater sites (Peer et al. 2006).

Factors other than salinity (or even correlates of salinity) can differ markedly within an estuary. For example, the upper reaches of estuaries provide critical habitat for many estuarine and marine fishes and invertebrates (Gunter 1957; Rogers et al. 1984; Peterson and Ross 1991; Ross 2003; Posey et al. 2005). Estuarine and marine species may integrate with the already present freshwater food web to provide mixed prey assemblages that may alter growth patterns of largemouth bass (Micucci et al. 2003). Diets rich in fish have profound effects on growth (Olson 1996) and may ameliorate the negative effects of salinity on the growth of coastal largemouth bass (Peer et al. 2006). However, diets composed primarily of invertebrates, such as blue crabs (*Callinectes sapidus*), may not be as energetically dense and may contribute to the negative effects of salinity on growth.

### *Age-0 Southern Flounder*

Unlike largemouth bass, southern flounder is an estuarine-dependent marine species; spawning is thought to occur in marine waters near tidal inlets and larvae are transported inshore where they settle at the head of the estuary (Powell and Schwartz 1977; Burke et al. 1991). Though most studies have indicated that low salinity areas are important age-0 habitats, sampling efforts have focused on areas where salinity is  $\geq 2\text{‰}$  (Burke et al. 1991; Reichert and Van der Veer 1991; Allen and Baltz 1997; Nanez-James 2006). However, southern flounder have routinely been reported in coastal freshwater habitats (Keup and Bayless 1964; Rogers et al. 1984; Castellanos and Rozas 2001). Further, the absence of age-0 southern flounder during extensive flatfish surveys in Georgia and Louisiana estuaries was attributed to age-0s using freshwater habitats (Reichert and Van der Veer 1991; Allen and Baltz 1997). In our study, 68% of age-0 southern flounder appeared to hatch in higher salinity waters before moving into the MTD. For these fish,  $\text{Sr}:\text{Ca}_{\text{otolith}}$  was high in the otolith core and declined rapidly to  $\leq 1500 \mu\text{mol mol}^{-1}$  for the remainder of the otolith, indicating a prolonged period of freshwater residency after ingress from the marine environment. Surprisingly, however, not all southern flounder exhibited this pattern; a subset of fish from the MTD had  $\text{Sr}:\text{Ca}_{\text{otolith}}$  profiles that were  $\leq 1500 \mu\text{mol mol}^{-1}$  throughout the entire otolith from the core to the edge. This indicates that these fish hatched in either a freshwater or low salinity environment and remained there for their entire first year of life.

Further, many of the flounder collected in the MTD during or after the low-flow, high salinity period, lacked an abrupt increase near the end of their  $\text{Sr}:\text{Ca}_{\text{otolith}}$  profiles, which would have been expected had these fish encountered increased salinity. The lack



of an abrupt increase in Sr:Ca may have resulted for several reasons. First, differential microhabitat use may have resulted in individuals constantly seeking lower salinity environments. In other estuaries, salinity appears to be an important driver of microhabitat use for age-0 southern flounder (Allen and Baltz 1997; Walsh et al. 1999). Second, individuals may have been in the process of migrating from far upstream down into the estuary. Both Allen and Baltz (1997) and Riechert and Van der Veer (1991) attributed the conspicuous absence of age-0 southern flounder in flatfish surveys to the species moving further upstream and out of their sampling area. However, in both cases the authors focused on low salinity habitats and did not target freshwater. Third, elemental uptake rates may not have been consistent throughout the life of southern flounder. Several species, including some cold-water flatfishes, have exhibited ontogenetic changes in element concentrations (Toole et al. 1993; de Pontual et al. 2003; Chittaro et al. 2006), however, the effects of ontogeny on otolith microchemistry has not been examined for southern flounder.

Prolonged use of freshwater habitats by age-0 southern flounder contradicts results of previous experimental work. Though salinity does not appear to have an effect on southern flounder growth in the laboratory (Daniels and Borski 1998; Smith et al. 1999), larval mortality, but not age-0 mortality, has been shown to be higher in freshwater compared to salinities  $\geq 10\text{‰}$  (Smith et al. 1999). Further, both maximum and minimum thermal tolerances during age-0 stages were positively related to salinity (Prentice 1989; Taylor et al. 2000). Prentice (1989) also observed feeding cessation in both age-0s and adults when temperatures fell below 9.3 °C in freshwater treatments but

not at 15‰. Clearly the nursery value of freshwater habitats for southern flounder needs further investigation.

Interestingly, studies from Louisiana (Allen and Baltz 1997), Alabama (this study), Georgia (Rogers et al. 1984; Richert and Van der Veer 1991), and North Carolina (Keup and Bayless 1964; Burke et al. 1991) have either observed or suggested freshwater residency for southern flounder, while studies in Texas have generally found highest densities in salinity  $\geq 5\text{‰}$  (Stokes 1977; Nañez-James 2006; Glass 2006). Recent genetic analysis identified two genetic clusters: individuals from the eastern Gulf of Mexico (i.e., LA, MS, and AL) grouped with fish from the Atlantic coast (i.e. northern FL and NC), while fish from sites within Texas clustered as a separate group (Blandon et al. 2001). Greater use of freshwater habitats by southern flounder in the MTD (and elsewhere) versus Texas populations may be related to genetic differences among regional stocks and such potential stock issues warrant further examination.

#### *Management Implications*

Both largemouth bass and southern flounder are economically important in coastal systems throughout the southeastern United States (Malvesuto et al. 1982; Krause 2002; GSMFC 2000). Despite having markedly different life-history strategies, both species are commonly found in freshwater habitats. Yet, little work has focused on the ecology of either species in freshwater habitats of estuarine systems.

Our study suggests that age-0 largemouth bass do not avoid seasonal increases in salinity by moving to fresher areas. However, previous research indicates that salinity tolerance changes with ontogeny and that adult largemouth bass respond to increased salinity by moving to freshwater (Swingle and Bland 1974; Meador and Kelso 1989).

The lack of movement by age-0 largemouth bass, particularly in response to increased salinity, could lead to specific areas in the MTD that may be more favorable for recruitment relative to others, such as those downstream areas that are exposed to salinity and its correlates that may affect local growth rates. As a result, management strategies should treat this system more like a mosaic of small connected lakes and streams, rather than a single large unit. For example, stocking of age-0 fish to compensate for localized fish kills in the MTD, as were observed after hurricane Ivan in 2004, (D. Armstrong, Alabama Department of Conservation and Natural Resources, Spanish Fort, Alabama, personal communication) might be an effective management tool. However, stocking should occur with age-0s because of their apparent tolerance of increased salinity during the fall low-flow period. Further, only genetic strains of largemouth bass specific to the MTD (Hallerman et al. 1986) should be stocked. Other strains, particularly those from completely freshwater systems or that may attain a larger body size, may not grow as well in low salinity habitats (Meador and Kelso 1990a,b) or may alter local stock genetics (Cooke et al. 2001). Clearly, an assessment of recruitment needs to be conducted across the MTD, with specific comparisons made between those sites that experience seasonal increases in salinity (i.e., downstream areas) and those that generally remain fresh (i.e., upstream areas). Combining this assessment with routine salinity monitoring will help identify what levels of salinity may compromise or enhance recruitment.

Our results counter conventional wisdom regarding the early-life stages of southern flounder and suggest that the freshwater habitats within an estuary may also be important to the management of southern flounder. Under the essential fish habitat provisions of the Magnuson-Stevens Fishery Conservation and Management Act and

Sustainable Fisheries Act of 1996, federal agencies are required to identify those waters and substrates that are necessary to the production and sustainability of federally managed species, such as southern flounder. The relative importance of certain habitats is commonly assessed with basic biological data (i.e., occurrence, density, survival, and growth) for a species. However, the relative contribution of freshwater habitats to the adult population still needs to be determined. Should a significant fraction of the adult stock originate from freshwater nurseries, which is indicated by our study, then it becomes even more important to delineate and protect the potentially critical habitats. More work is clearly needed to evaluate the importance of both freshwater and estuarine habitats in population dynamics of southern flounder.

Like most estuaries throughout the southeastern United States, the Mobile-Tensaw MTD faces a number of issues that may lead to the alteration of the salinity regime, habitat availability, and habitat quality; including reduced freshwater input (Naiman et al. 1995), deeper salt-wedge penetration as a result of sea-level rise (Day et al. 1995), and other anthropogenic changes to the estuarine landscape. Changes in salinity may lead to changes in not only the distribution of suitable nursery habitats for both largemouth bass and southern flounder in coastal systems, but also the overall quality of these habitats in general (Whitfield and Wooldridge 1994). Some habitats or conditions may facilitate survival of a species to later life-stages while others do not (Petrik et al. 1999; Rozas and Zimmerman 2000). In order to effectively manage both largemouth bass and southern flounder in coastal systems, it is important to identify and conserve those habitats and conditions that are necessary for the propagation of early-life stages.

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Table 1. Isotopes (including calcium) used in DFA for both age-0 largemouth bass (LMB) and southern flounder (FLDR) using LA-ICP-MS. Mean limits of detection (LOD) were calculated based on all sample runs. The coefficient of variation (CV), as determined from NIST 610 standards, is the average for all runs, and was calculated by dividing standard deviations of runs by their means. The percentage of samples greater than detection limits for an element (% > LOD) also is provided. Bolded values are for elements that met our criteria for inclusion in analysis.

Isotope	Mg	Ca	Mn	Fe	Cu	Zn	Rb	<sup>86</sup> Sr	<sup>88</sup> Sr	Cd	<sup>118</sup> Sn	<sup>120</sup> Sn	<sup>137</sup> Ba	<sup>138</sup> Ba	Pb
Element LOD (ppm)	15.0	663.3	0.9	165.9	5.2	1.1	0.8	6.7	0.8	0.6	1.1	0.8	1.4	0.2	0.1
CV (%)	6.4	1.1	2.0	162.4	4.7	4.1	6.0	1.9	2.0	5.6	5.9	8.6	3.0	3.6	5.2
<b>FLDR (Core) % &gt; LOD</b>															
<b>2004</b>	<b>94.6</b>	<b>100.0</b>	<b>100.0</b>	34.6	19.1	63.6	18.2	<b>100.0</b>	<b>100.0</b>	10.9	45.5	65.5	<b>100.0</b>	<b>100.0</b>	<b>84.6</b>
<b>2005</b>	<b>84.0</b>	<b>100.0</b>	<b>87.1</b>	82.6	35.4	65.3	30.6	<b>100.0</b>	<b>100.0</b>	31.3	65.3	47.7	<b>100.0</b>	<b>100.0</b>	<b>80.2</b>
<b>LMB (Core) % &gt; LOD</b>															
<b>2004</b>	<b>100.0</b>	<b>100.0</b>	<b>94.7</b>	71.7	61.1	<b>90.3</b>	54.9	<b>100.0</b>	<b>100.0</b>	54.9	61.0	67.3	<b>100.0</b>	<b>99.1</b>	<b>92.0</b>
<b>2005</b>	<b>100.0</b>	<b>100.0</b>	<b>86.8</b>	75.5	55.7	<b>88.3</b>	54.7	<b>100.0</b>	<b>100.0</b>	52.0	50.9	51.9	<b>99.1</b>	<b>100.0</b>	<b>83.0</b>
<b>LMB (Edge) % &gt; LOD</b>															
<b>Spring 2004</b>	<b>93.0</b>	<b>100.0</b>	49.0	40.4	21.1	61.4	26.3	<b>100.0</b>	<b>100.0</b>	17.5	66.7	52.5	<b>100.0</b>	<b>100.0</b>	<b>87.7</b>
<b>Spring 2005</b>	<b>98.1</b>	<b>100.0</b>	62.3	41.5	17.0	64.2	26.4	<b>100.0</b>	<b>100.0</b>	3.8	56.6	69.8	<b>100.0</b>	<b>100.0</b>	<b>83.0</b>
<b>Fall 2004</b>	<b>85.0</b>	<b>100.0</b>	10.2	66.7	18.0	66.1	14.3	<b>100.0</b>	<b>100.0</b>	18.2	35.8	50.0	<b>100.0</b>	<b>98.6</b>	<b>80.3</b>
<b>Fall 2005</b>	<b>82.3</b>	<b>100.0</b>	10.9	56.6	1.9	49.1	7.6	<b>100.0</b>	<b>100.0</b>	0.0	18.9	26.4	<b>98.1</b>	<b>100.0</b>	<b>89.8</b>

Table 2. Raw element concentrations of water samples collected at 1 m from all six sites in 2005. March samples represent approximate elemental concentrations of water at time of hatch for largemouth bass. Salinity and water samples were at a 1 meter depth.

	Site	Salinity	Mg	Ca	Mn	Sr	Ba	Pb	Mg:Ca	Mn:Ca	Sr:Ca	Ba:Ca	
	March-05	ppt				**All values in $\mu\text{mol mol}^{-1}$ **							
	Upstream	DL	0.1	1630.684	5759.193	5.608	29.383	12.768	6.658	0.283	0.001	0.005	0.002
		ML	0.2	2176.392	4302.749	2.738	31.618	10.490	7.494	0.506	0.001	0.007	0.002
	Middle	GI	0.1	2704.704	11107.118	14.493	64.231	23.826	2.532	0.244	0.001	0.006	0.002
		CC	0.1	3260.190	12381.823	15.281	73.115	45.359	7.230	0.263	0.001	0.006	0.004
	Downstream	BM	0.1	1443.281	5588.664	10.296	30.055	15.599	15.287	0.258	0.002	0.005	0.003
		DB	0.1	3135.157	10893.046	18.246	64.964	21.976	4.924	0.288	0.002	0.006	0.002
	June-05												
	Upstream	DL	0.1	459.780	922.363	3.039	9.161	11.427	-	0.498	0.003	0.010	0.012
		ML	0.1	3259.022	12131.816	19.253	75.311	25.369	8.266	0.269	0.002	0.006	0.002
	Middle	GI	0.1	1688.988	5928.332	5.890	35.958	13.868	4.930	0.285	0.001	0.006	0.002
		CC	-	-	-	-	-	-	-				
	Downstream	BM	0.1	1487.615	2436.829	40.025	22.571	25.794	6.061	0.610	0.016	0.009	0.011
		DB	1.1	36115.473	21152.423	245.353	255.690	40.025	2.844	1.707	0.012	0.012	0.002
	August-05												
	Upstream	DL	0.1	2028.086	8408.505	18.876	50.821	24.782	-	0.241	0.002	0.006	0.003
		ML	0.5	16873.501	20381.539	69.573	160.172	72.716	8.092	0.828	0.003	0.008	0.004
	Middle	GI	2.5	33700.057	14329.842	34.558	204.190	14.292	5.084	2.352	0.002	0.014	0.001
		CC	1.7	58264.967	30065.804	85.895	394.289	38.777	8.371	1.938	0.003	0.013	0.001
	Downstream	BM	2.4	85665.078	37231.509	234.531	518.696	75.701	17.883	2.301	0.006	0.014	0.002
		DB	14.3	315116.421	106941.575	507.387	1727.229	210.526	14.300	2.947	0.005	0.016	0.002

Table 3. Univariate results of the Nested ANOVA for otolith element concentrations of age-0 largemouth bass from the Mobile-Tensaw Delta. Significant effects are indicated by bolded values (Bonferroni-adjusted  $\alpha = 0.008$ ).

Effect	2004							2005						
	df	MS	F	P<	MS	F	P<	df	MS	F	P<	MS	F	P<
			<b>25Mg</b>			<b>43Ca</b>				<b>25Mg</b>			<b>43Ca</b>	
Region	2	0.033	0.647	NS	0.005	5.133	NS	2	0.069	1.528	NS	0.002	1.336	NS
Otolith	2	2.327	45.683	0.004	0.008	7.471	0.004	2	1.229	27.041	0.004	0.005	4.124	0.004
Site(Region)	3	0.016	0.317	NS	0.001	0.529	NS	3	0.051	1.132	NS	0.000	0.121	NS
Region*Otolith	4	0.168	3.299	NS	0.002	1.616	NS	4	0.008	0.179	NS	0.000	0.243	NS
Otolith*Site(Region)	6	0.059	1.164	NS	0.001	0.503	NS	6	0.133	2.922	NS	0.000	0.377	NS
Error	157	0.051			0.001			136	0.045			0.001		
			<b>88Sr</b>			<b>138Ba</b>				<b>88Sr</b>			<b>138Ba</b>	
Region	2	0.301	22.328	0.004	0.129	2.524	NS	2	0.170	18.814	0.004	0.017	0.537	NS
Otolith	2	0.229	17.013	0.004	0.640	12.555	0.004	2	0.519	57.523	0.004	2.248	70.354	0.004
Site(Region)	3	0.025	1.872	NS	0.105	2.062	NS	3	0.005	0.607	NS	0.143	4.477	NS
Region*Otolith	4	0.115	8.547	0.004	0.171	3.349	NS	4	0.061	6.710	0.004	0.142	4.459	0.004
Otolith*Site(Region)	6	0.014	1.047	NS	0.054	1.060	NS	6	0.016	1.738	NS	0.050	1.554	NS
Error	157	0.013			0.051			136	0.009			0.032		
			<b>208Pb</b>							<b>208Pb</b>				
Un	2	0.280	1.210	NS				2	0.116	0.738	NS			
Otolith	2	0.982	4.241	NS				2	0.121	0.771	NS			
Site(Region)	3	0.234	1.011	NS				3	0.172	1.095	NS			
Region*Otolith	4	0.051	0.222	NS				4	0.110	0.702	NS			
Otolith*Site(Region)	6	0.055	0.238	NS				6	0.089	0.569	NS			
Error	157	0.232						136	0.157					



Table 1 Standardized canonical scores for LDFA Root 1 and Root 2, from analysis of otolith cores, edges of spring-collected, and edges of fall-collected age-0 largemouth bass from both year classes. Bold numbers indicate the elements most strongly associated with that root. Cumulative proportion of variation explained by each root is provided. LOD = limit of detection.

	Core		Edge (Spring)		Edge (Spring)	
	Root 1	Root 2	Root 1	Root 2	Root 1	Root 2
<b>2004</b>						
Log(Mg)	0.026	0.260	-0.305	<b>0.916</b>	0.016	-0.216
Log(Ca)	-0.507	0.065	-0.043	0.017	0.422	<b>0.849</b>
Log(Mn)	-0.457	<b>-0.981</b>		<b>**Below LOD**</b>		
Log(Sr)	<b>0.813</b>	-0.795	-0.718	0.378	<b>1.039</b>	-0.176
Log(Ba)	-0.529	0.470	<b>1.248</b>	0.171	-0.320	0.226
Log(Pb)	-0.059	-0.280	-0.338	-0.287	0.032	-0.199
Cumm. Prop.	0.720	1.000	0.842	1.000	0.908	1.000
<b>2005</b>						
Log(Mg)	0.478	-0.103	0.210	0.403	0.244	-0.566
Log(Ca)	-0.154	-0.572	0.071	-0.032	-0.224	0.270
Log(Mn)	-0.434	0.030		<b>**Below LOD**</b>		
Log(Sr)	<b>0.822</b>	-0.463	<b>0.988</b>	0.517	<b>0.912</b>	0.145
Log(Ba)	0.216	<b>0.816</b>	-0.105	<b>-1.211</b>	-0.045	<b>0.720</b>
Log(Pb)	-0.151	-0.408	0.258	-0.304	-0.255	0.029
Cumm. Prop.	0.727	1.000	0.618	1.000	0.939	1.000

Table 2 Classification matrices for LDFAs of otolith cores, edges of spring-collected, and edges of fall-collected age-0 largemouth bass from both year classes. Bold numbers represent the number of fish correctly classified to their collection region. Total sample size for each region displayed in the bottom row.

	% Correct	Upstream	Middle	Downstream
<b>Core</b>				
<b>2004</b>				
Upstream	68	<b>19</b>	5	4
Middle	53	6	<b>16</b>	8
Downstream	52	5	8	<b>14</b>
Total	58	30	29	26
<b>2005</b>				
Upstream	62	<b>16</b>	5	5
Middle	46	8	<b>11</b>	5
Downstream	50	7	5	<b>12</b>
Total	53	31	21	22
<b>Edge (Spring)</b>				
<b>2004</b>				
Upstream	75	<b>15</b>	3	2
Middle	65	1	<b>11</b>	5
Downstream	60	3	5	<b>12</b>
Total	67	19	19	19
<b>2005</b>				
Upstream	47	<b>8</b>	4	5
Middle	56	3	<b>10</b>	5
Downstream	67	5	1	<b>12</b>
Total	57	16	15	22
<b>Edge (Fall)</b>				
<b>2004</b>				
Upstream	74	<b>14</b>	5	0
Middle	58	5	<b>11</b>	3
Downstream	83	2	1	<b>15</b>
Total	71	21	17	18
<b>2005</b>				
Upstream	59	<b>10</b>	5	2
Middle	53	4	<b>9</b>	4
Downstream	95	1	0	<b>18</b>
Total	70	15	14	24

Table 1 Univariate results of the Nested ANOVA for otolith element concentrations of age-0 southern flounder otolith cores from the Mobile-Tensaw Delta. Significant effects are indicated by bolded values (Bonferroni-adjusted  $\alpha = 0.008$ ).

Effect	df	2004						2005						
		MS	F	P<	MS	F	P<	MS	F	P<	MS	F	P<	
		<b>25Mg</b>			<b>43Ca</b>			<b>25Mg</b>			<b>43Ca</b>			
Region	2	0.020	0.454	NS	<b>0.020</b>	<b>11.464</b>	<b>0.000</b>	2	0.057	2.627	NS	0.002	1.771	NS
Origin	1	0.012	0.281	NS	0.003	1.834	NS	1	0.155	7.189	NS	0.001	1.025	NS
Site(Region)	2	0.027	0.619	NS	0.006	3.619	NS	2	0.016	0.734	NS	0.000	0.143	NS
Region*Origin	2	0.019	0.429	NS	0.004	2.089	NS	2	0.015	0.717	NS	0.001	0.555	NS
Origin*Site(Region)	2	0.053	1.196	NS	0.003	1.613	NS	2	0.009	0.409	NS	0.001	1.051	NS
Error	44	0.044			0.002			37	0.022			0.001		
		<b>55Mn</b>			<b>88Sr</b>			<b>55Mn</b>			<b>88Sr</b>			
Region	2	0.006	0.130	0.878	0.025	2.007	NS	2	0.101	2.068	NS	0.010	0.724	0.492
Origin	1	0.211	4.494	0.040	<b>1.861</b>	<b>150.3</b>	<b>0.000</b>	1	0.308	6.306	NS	<b>1.936</b>	<b>143.5</b>	<b>0.000</b>
Site(Region)	2	0.020	0.427	0.655	0.013	1.021	NS	2	0.010	0.213	NS	0.094	6.924	NS
Region*Origin	2	0.153	3.264	0.048	0.065	5.275	NS	2	0.096	1.969	NS	0.010	0.770	NS
Origin*Site(Region)	2	0.095	2.033	0.143	0.005	0.441	NS	2	0.028	0.574	NS	0.010	0.736	NS
Error	44	0.047			0.012			37	0.049			0.014		
		<b>138Ba</b>			<b>208Pb</b>			<b>138Ba</b>			<b>208Pb</b>			
Region	2	0.078	1.483	NS	0.336	2.121	NS	2	0.042	0.948	NS	0.085	0.738	NS
Origin	1	<b>3.375</b>	<b>64.583</b>	<b>0.000</b>	0.038	0.239	NS	1	<b>4.051</b>	<b>90.821</b>	<b>0.000</b>	0.054	0.467	NS
Site(Region)	2	0.030	0.583	NS	0.016	0.104	NS	2	0.014	0.320	NS	0.041	0.351	NS
Region*Origin	2	0.106	2.031	NS	0.325	2.051	NS	2	0.038	0.855	NS	0.455	3.946	NS
Origin*Site(Region)	2	0.018	0.348	NS	0.699	4.417	NS	2	0.019	0.426	NS	0.323	2.801	NS
Error	44	0.052			0.158			37	0.045			0.115		

Figure 1 Map of Mobile-Tensaw Delta, Alabama, USA, From upstream to downstream, samples sites are Dennis Lake, McReynolds Lake, Gravine Island, Crab Creek, Bay Minette, and D'Olive Bay.

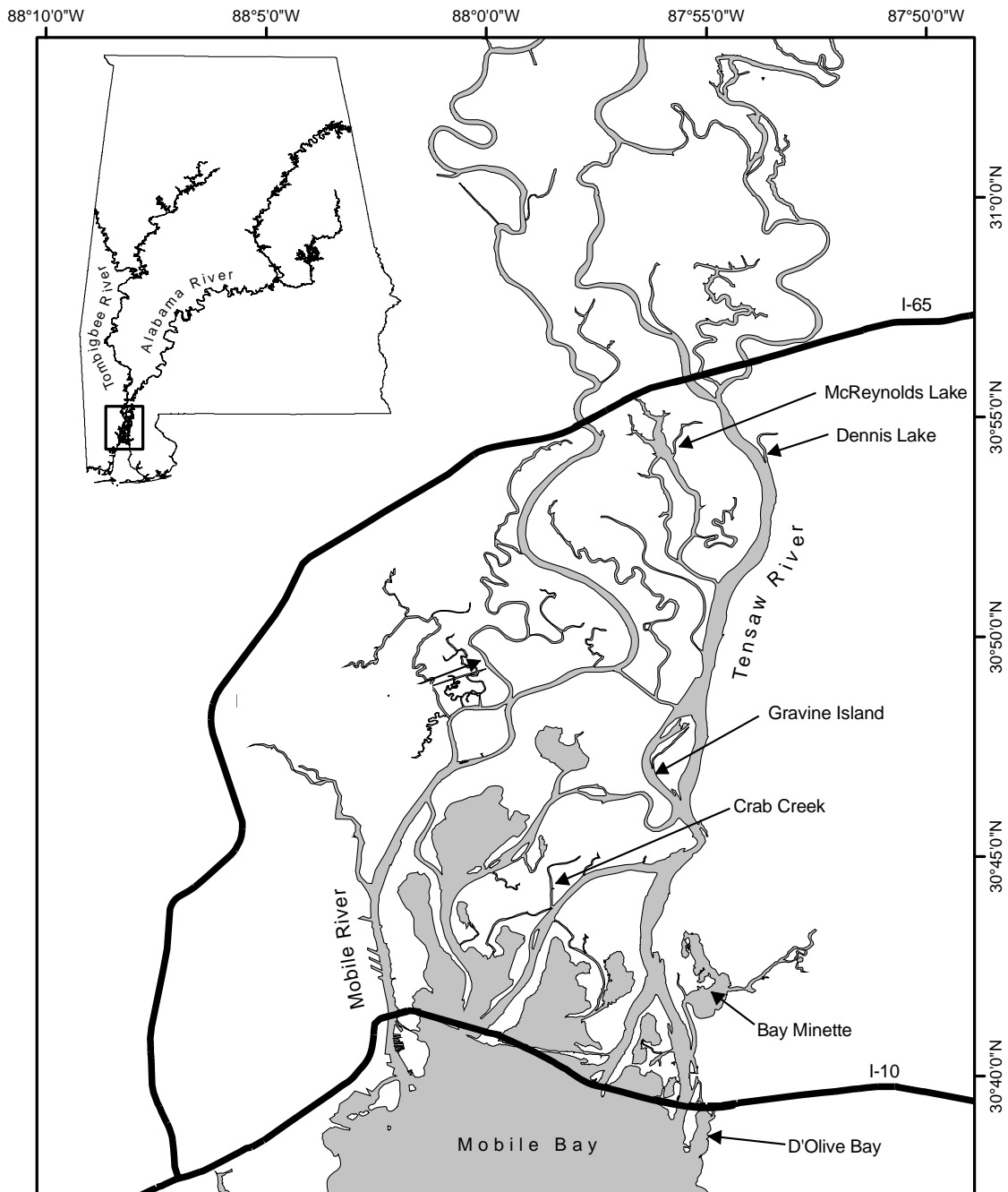


Figure 2. Monthly surface (●) and bottom (○) temperatures at six sites in the Mobile Tensaw Delta. From top to bottom, sites are arranged from upstream to downstream with 2004 and 2005 in the left and right side of each panel, respectively.

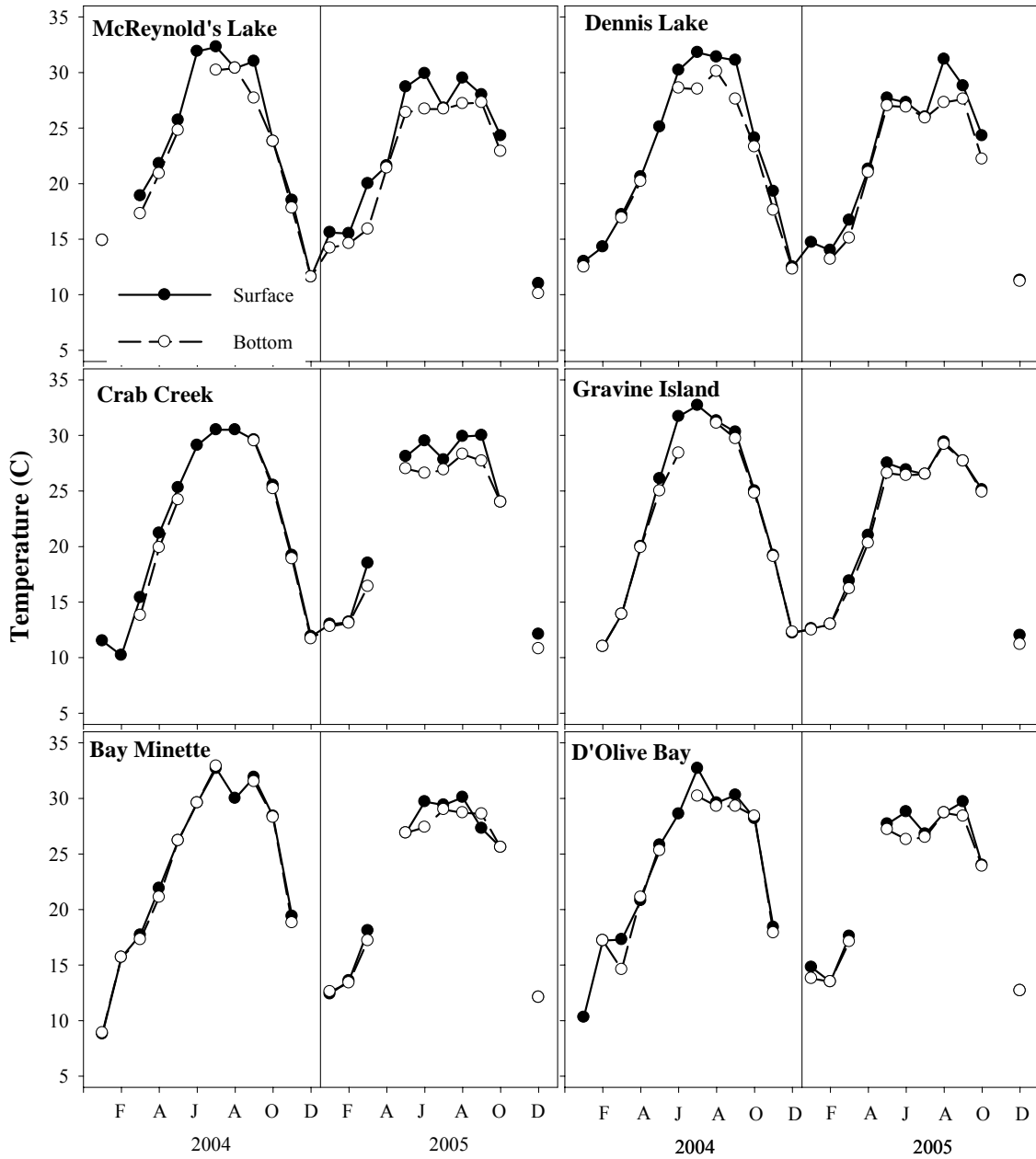


Figure 3. Monthly surface (●) and bottom (○) dissolved oxygen concentrations at six sites in the Mobile Tensaw Delta. From top to bottom, sites are arranged from upstream to downstream with 2004 and 2005 in the left and right side of each panel, respectively.



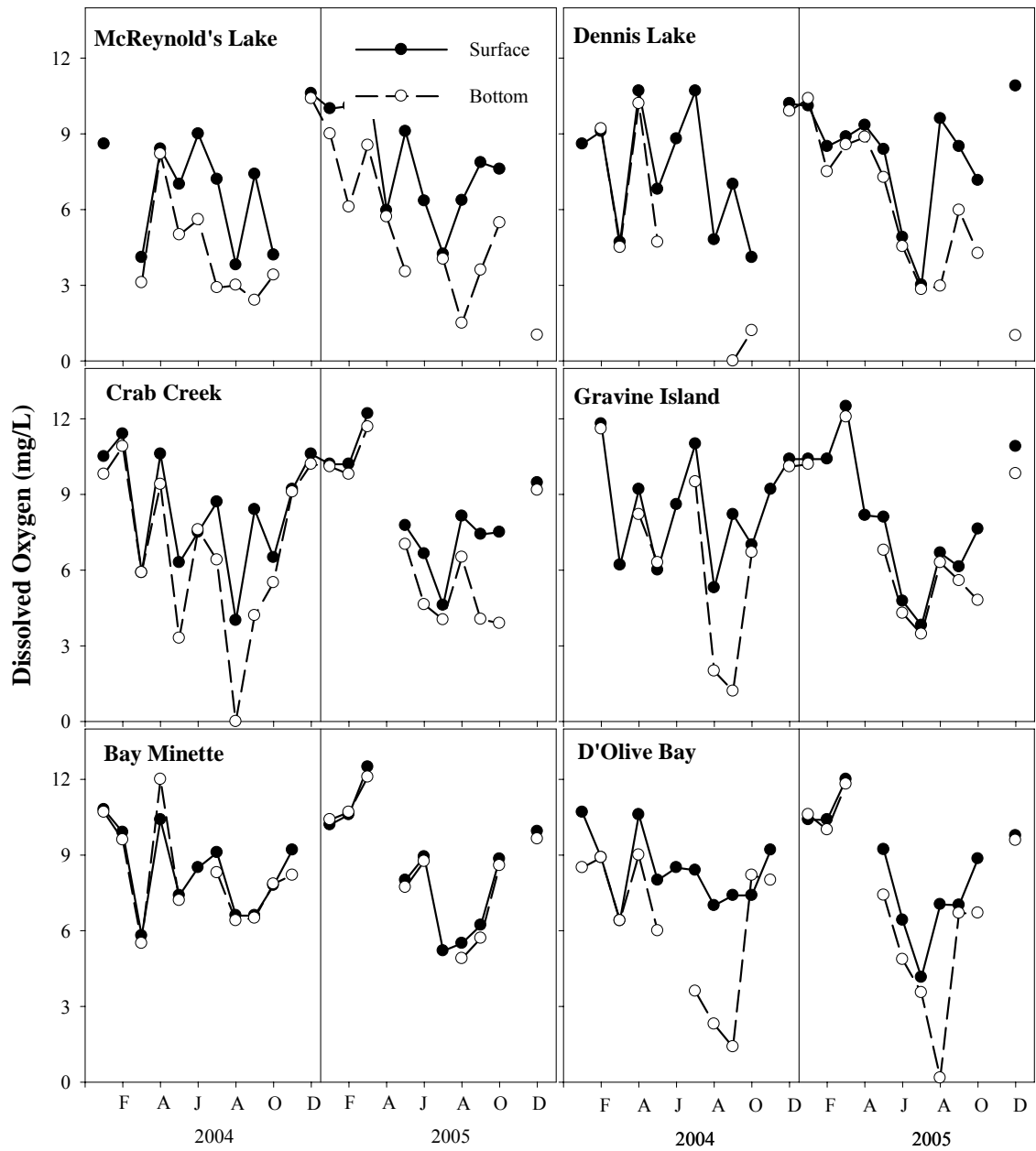


Figure 4. Monthly surface (●) and bottom (○) salinity at six sites in the Mobile Tensaw Delta. From top to bottom, sites are arranged from upstream to downstream with 2004 and 2005 in the left and right side of each panel, respectively.

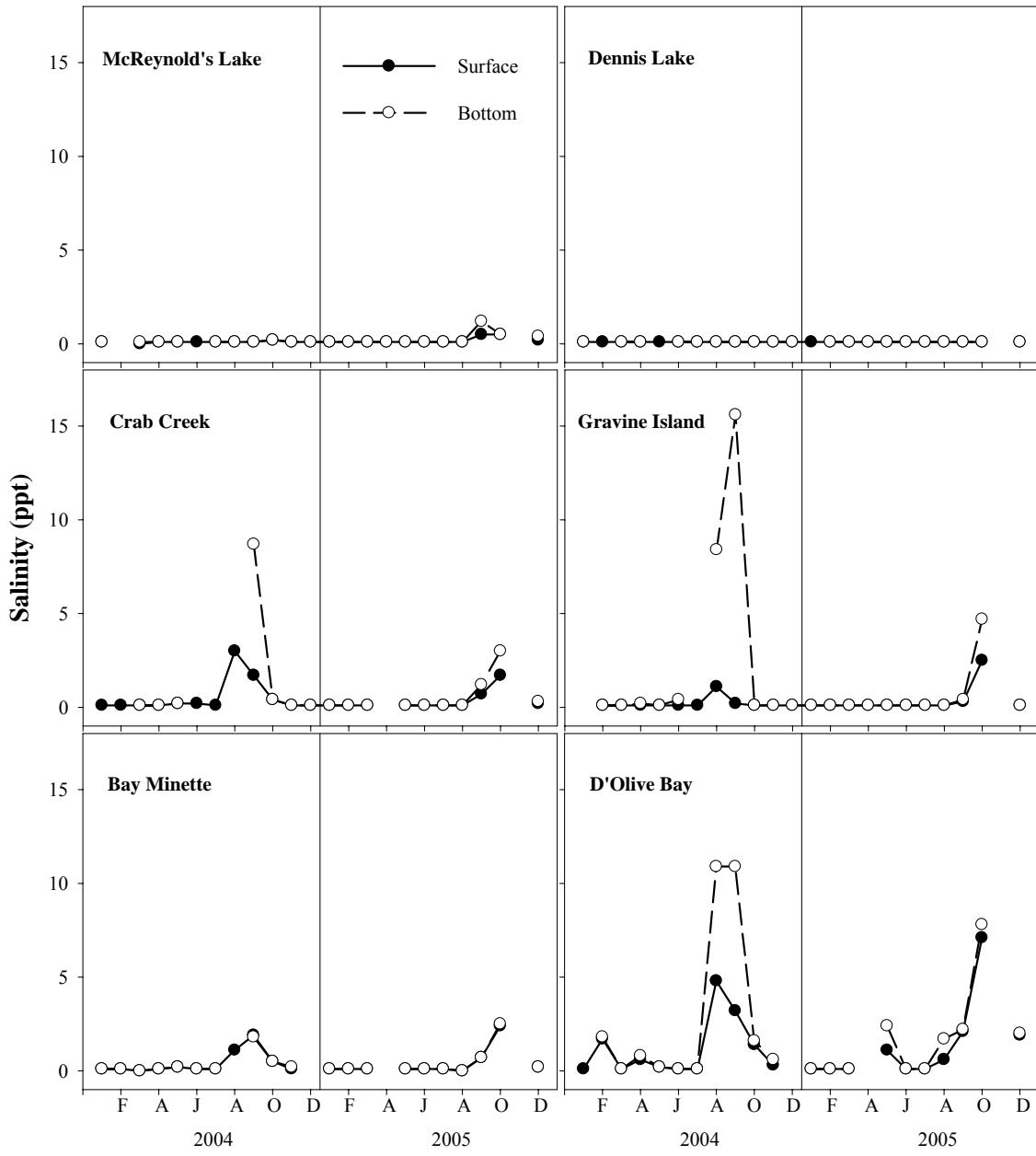


Figure 5 Mean discharge rates from Claiborne (Tombigbee River) and Coffeerville (Alabama River) Dams.

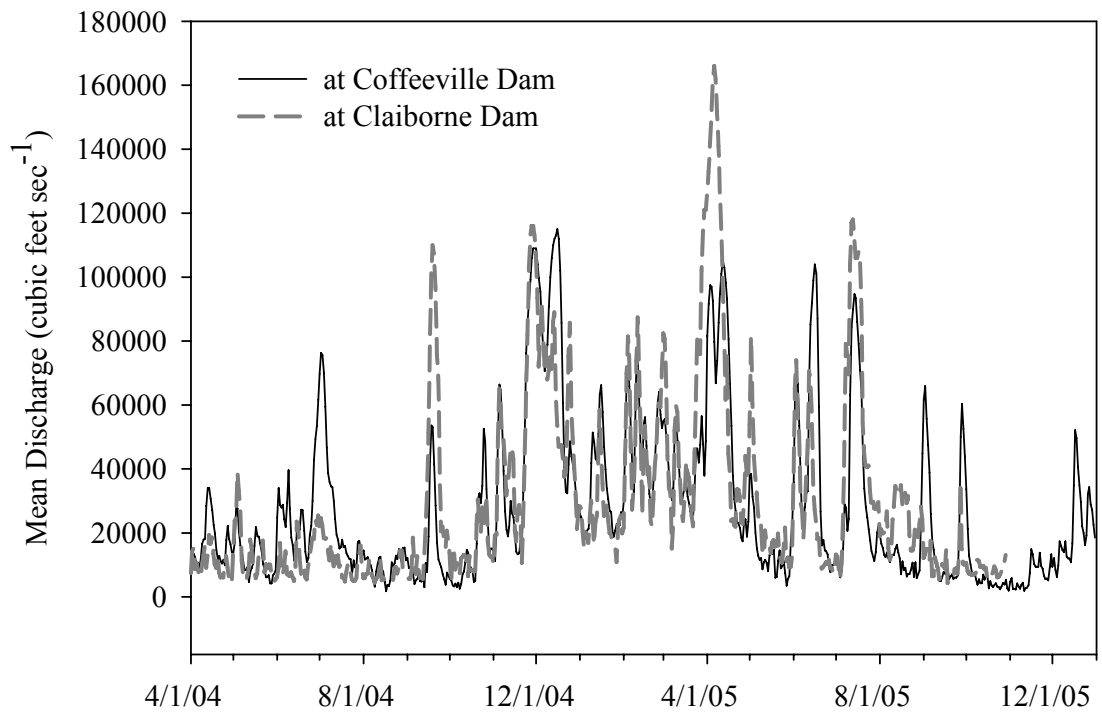


Figure 6. Mean salinity ( $\pm$  SE) measured at 30-min intervals at 1 m for Gravinge Island (upper panel) and D'Olive Bay (lower panel) in 2005. Triangles represent surface measurements from monthly Physicochemical sampling.

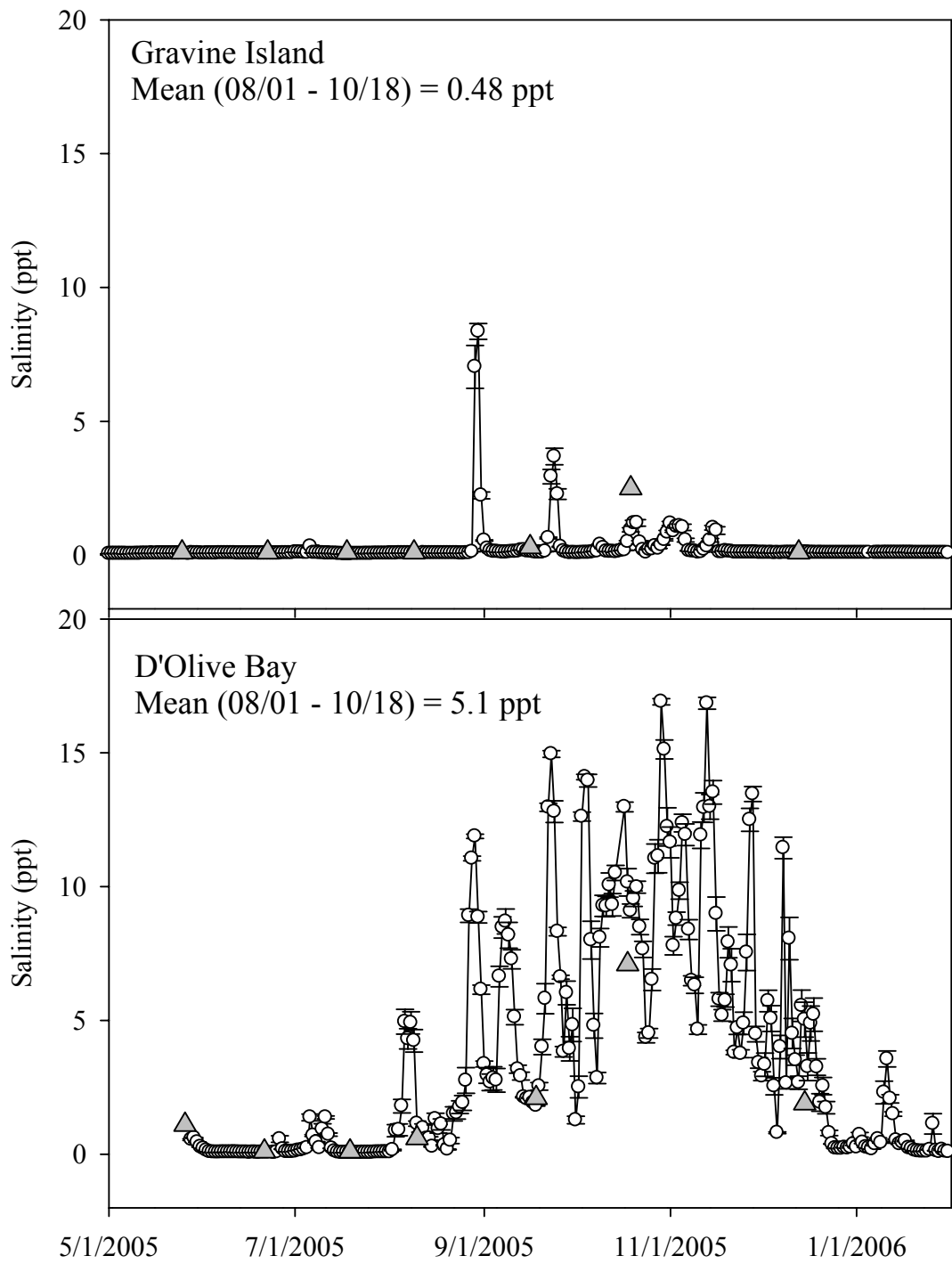


Figure 7. Relationships between aqueous element concentrations and salinity in the Mobile Tensaw Delta, collected across all dates. All water samples were collected at a depth of 1 m.



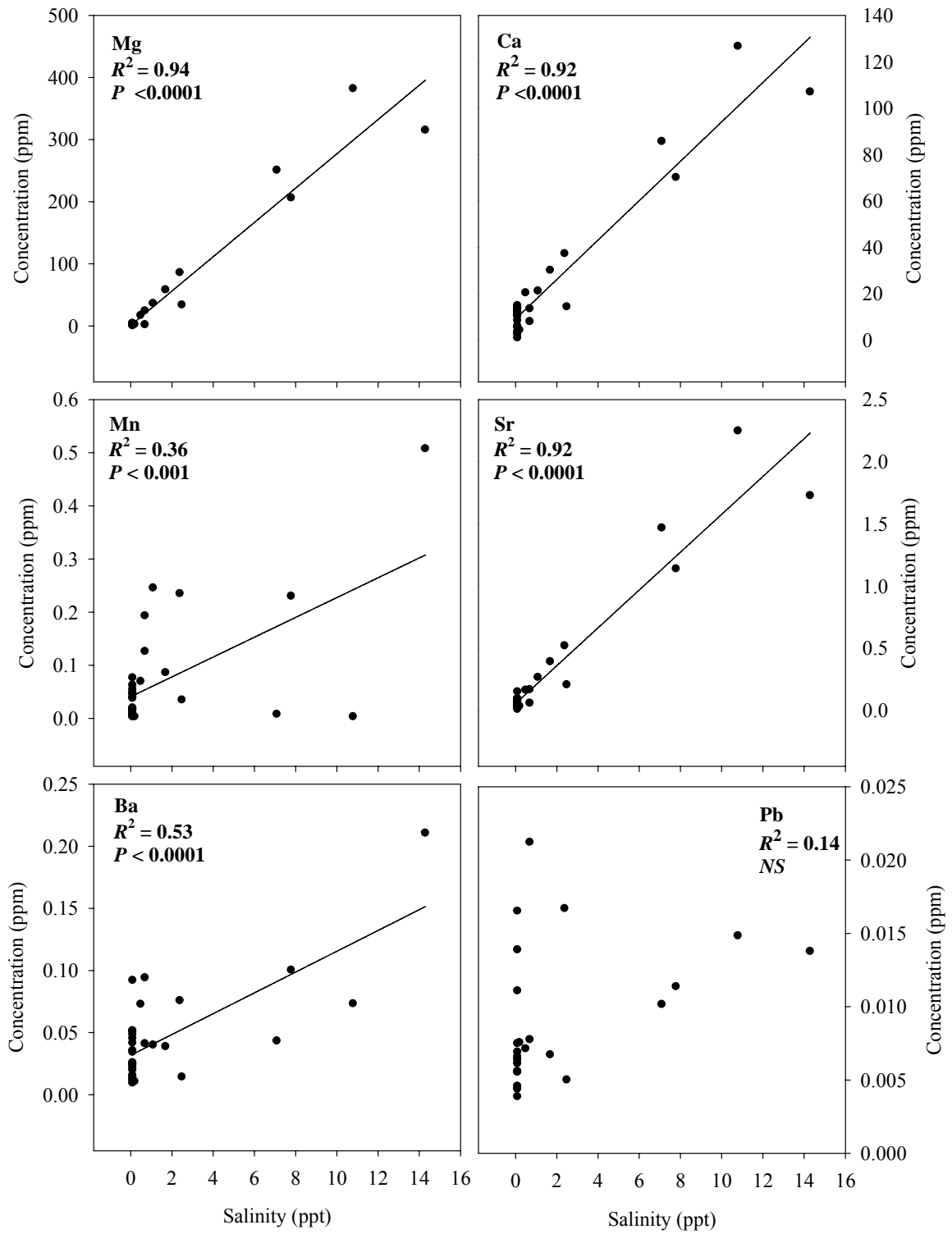


Figure 8. Relationships between aqueous Element:Ca concentrations and salinity from the Mobile Tensaw Delta. All water samples were collected at a depth of 1 m. Gray triangles indicate that maximum separation occurred at a salinity of at salinity of 0 ‰ for both Mn and Ba (2DKS).

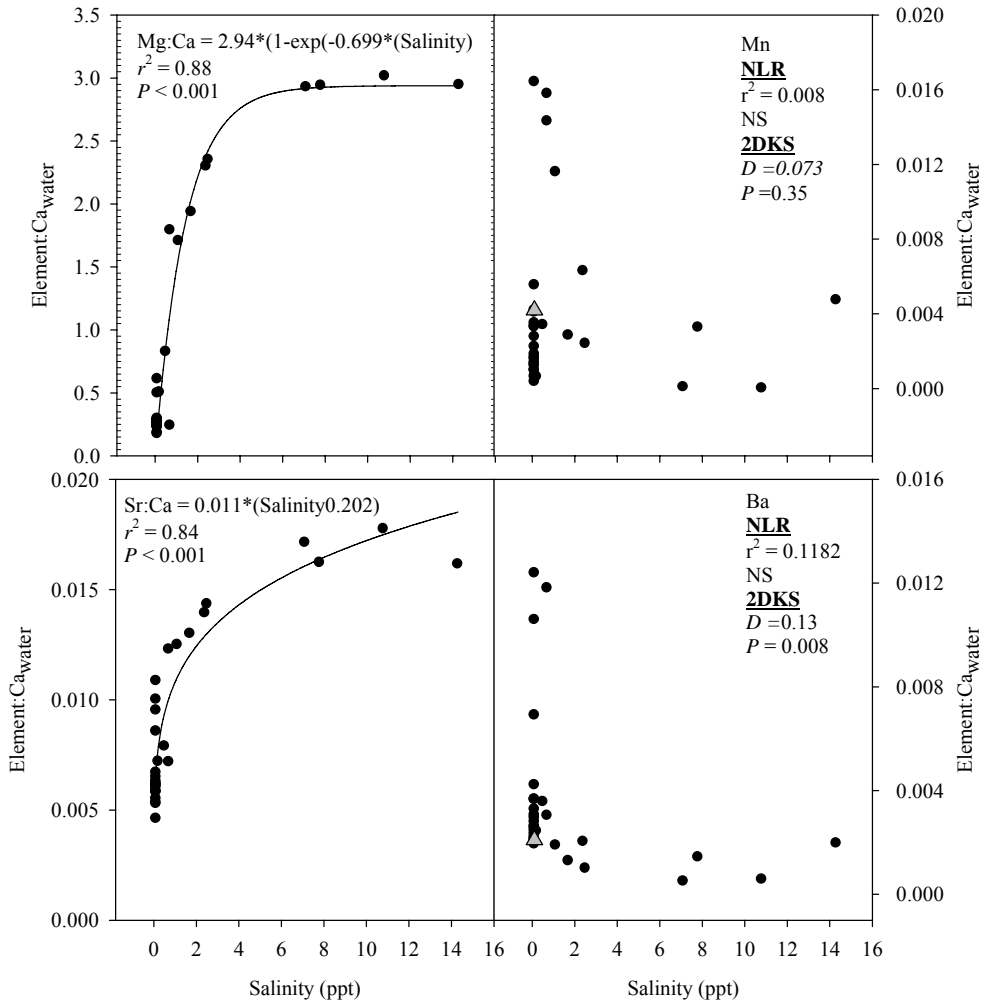


Figure 9 Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year class collected from Dennis Lake (Upstream Region) in Spring 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

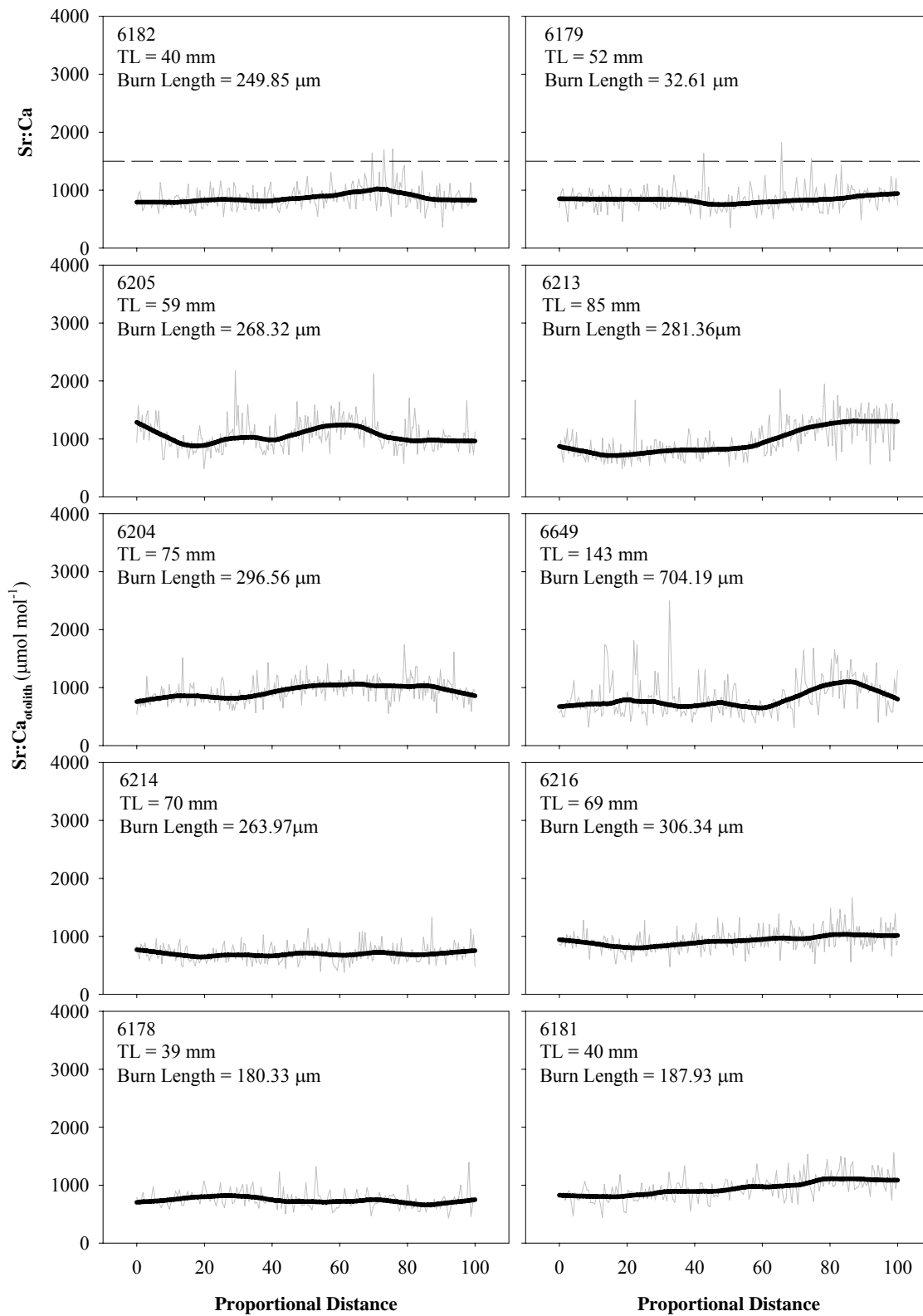


Figure 10. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year class collected from Dennis Lake (Upstream Region) in Spring 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

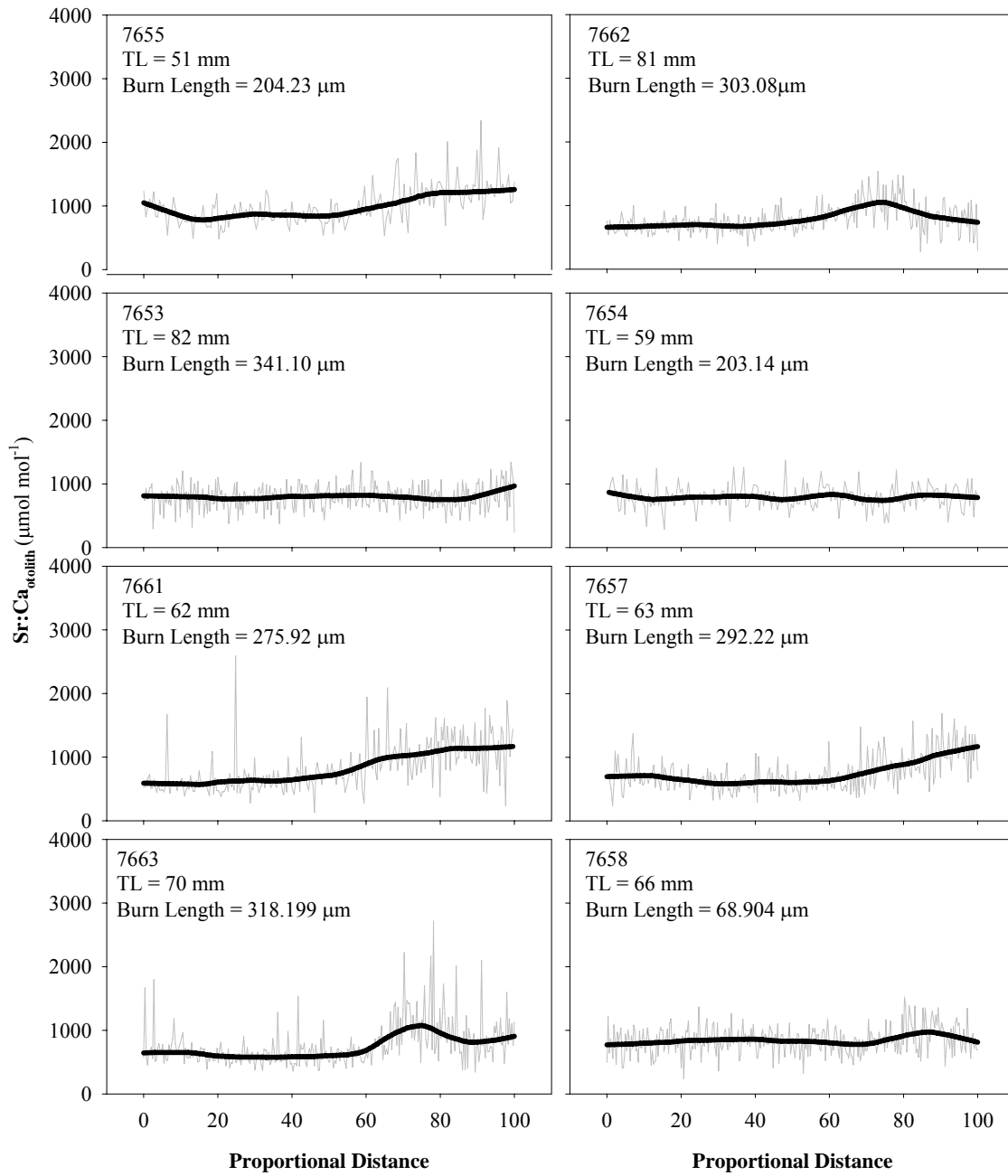


Figure 11. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year class collected from McReynolds Lake (Upstream Region) in Spring 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.



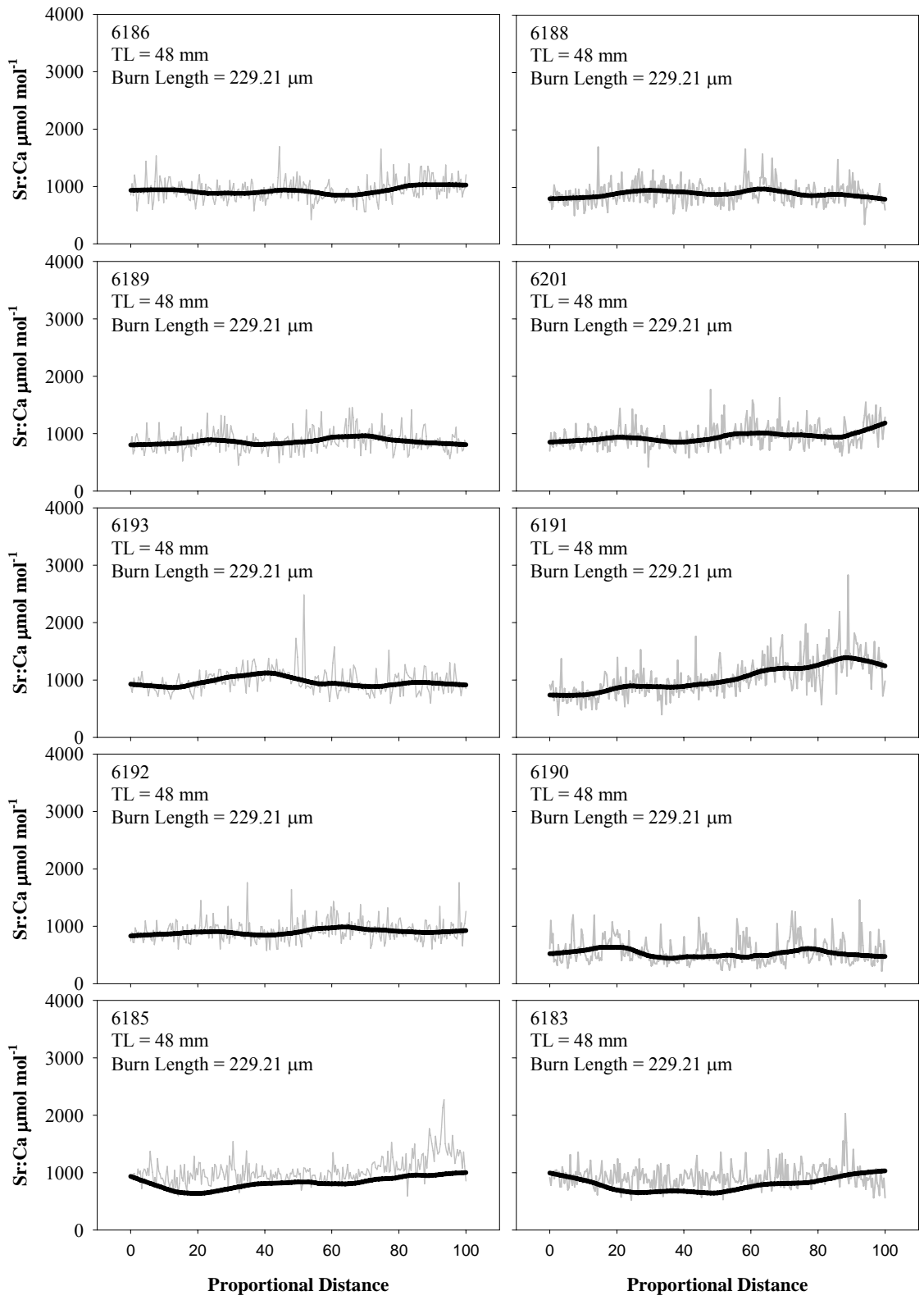


Figure 12. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year class collected from McReynolds Lake (Upstream Region) in Spring 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

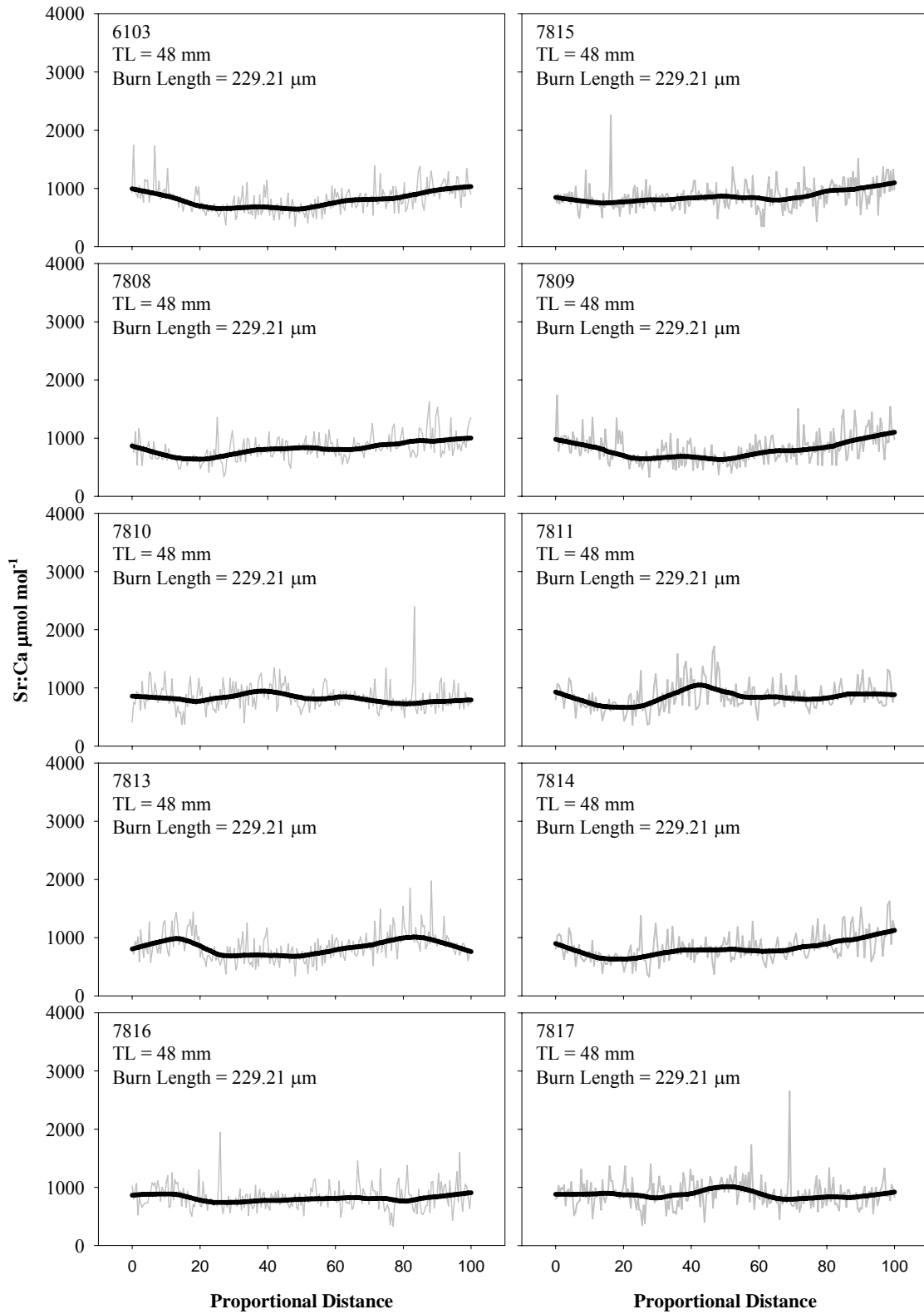


Figure 13. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year class collected from Gravine Island (Middle Region) in Spring 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

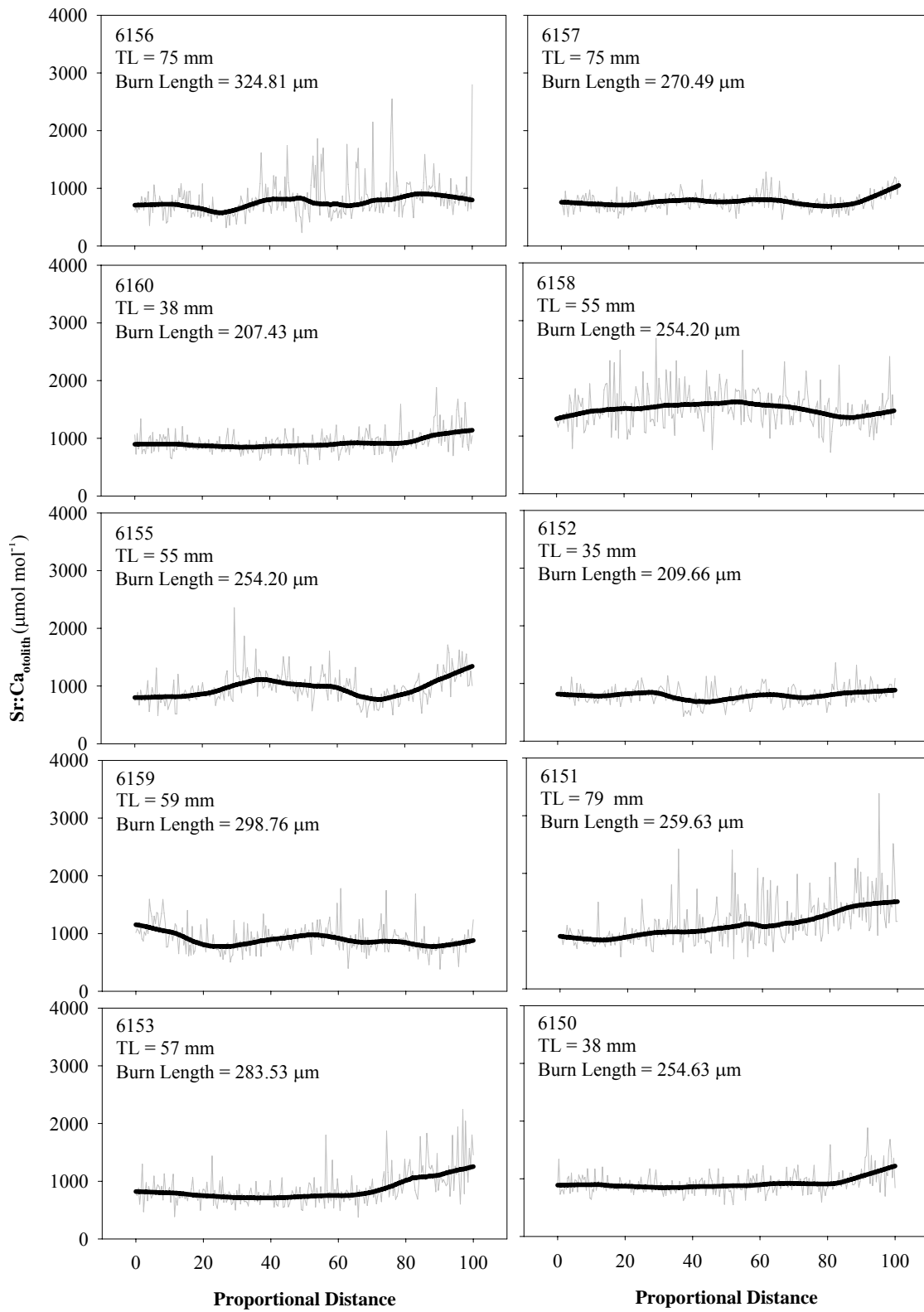


Figure 14. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year class collected from Gravine Island (Middle Region) in Spring 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

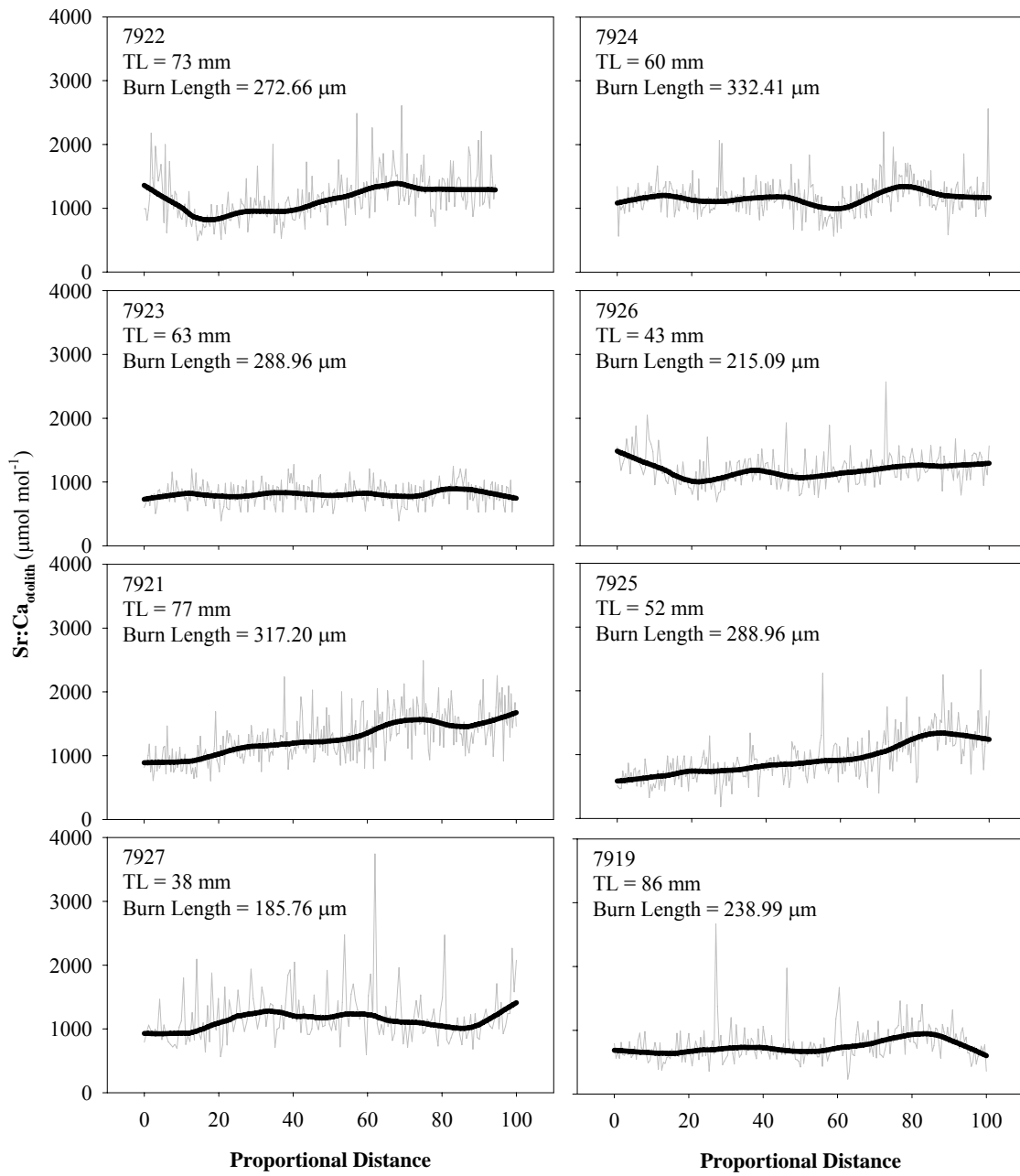


Figure 15. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year class collected from Crab Creek (Middle Region) in Spring 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.



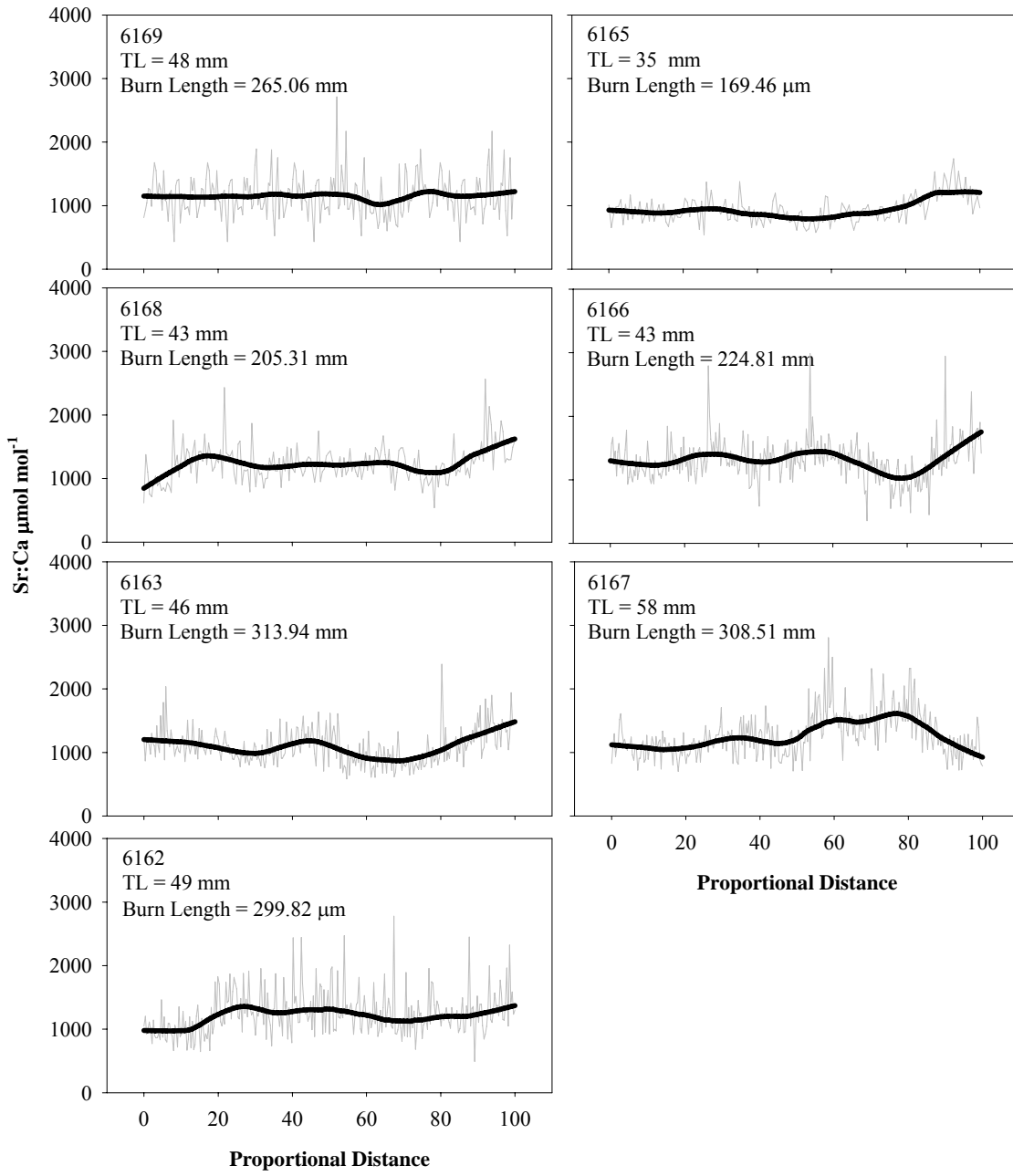


Figure 16. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year class collected from Crab Creek (Middle Region) in Spring 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

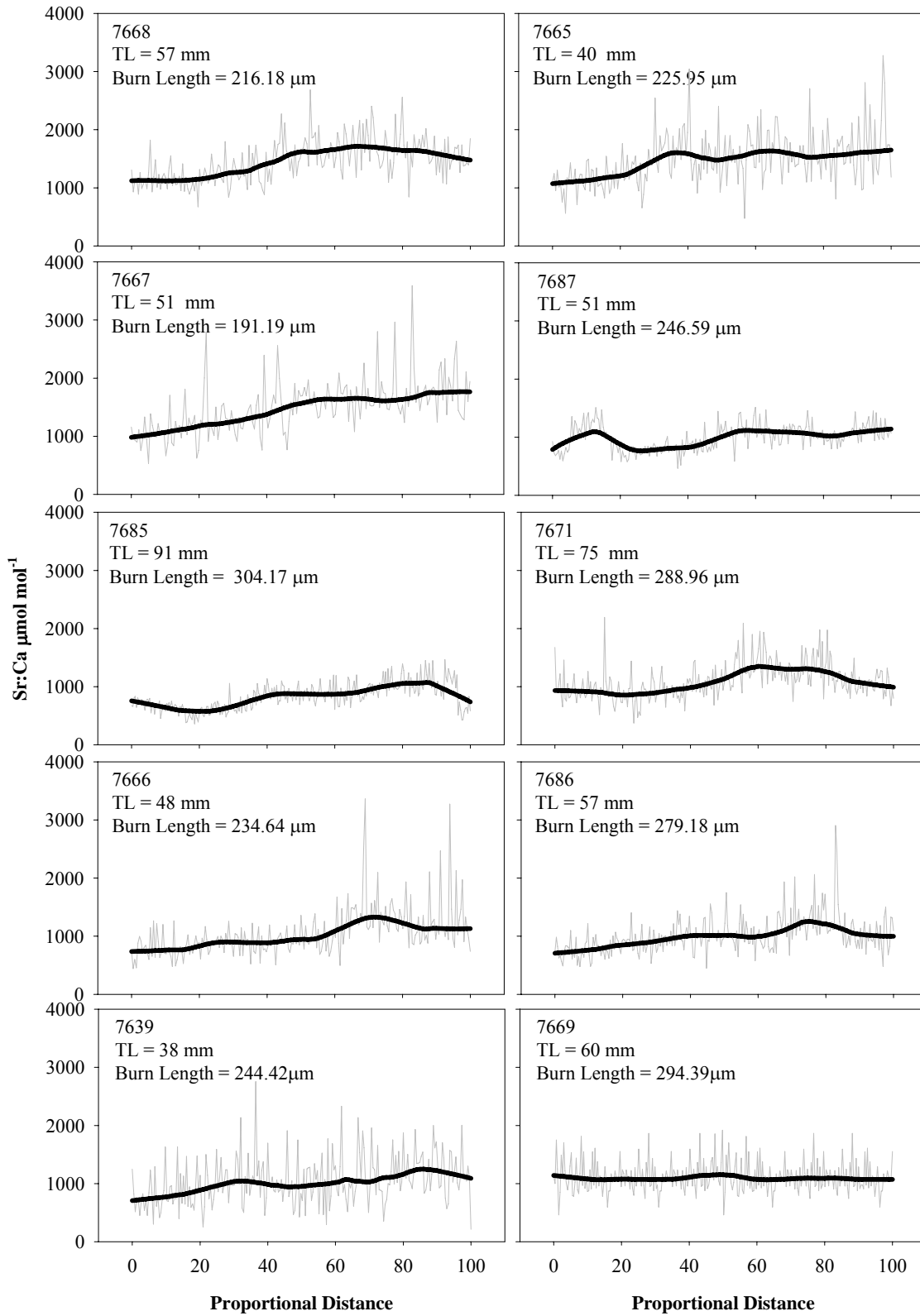


Figure 17. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year class collected from Bay Minette (Downstream Region) in Spring 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

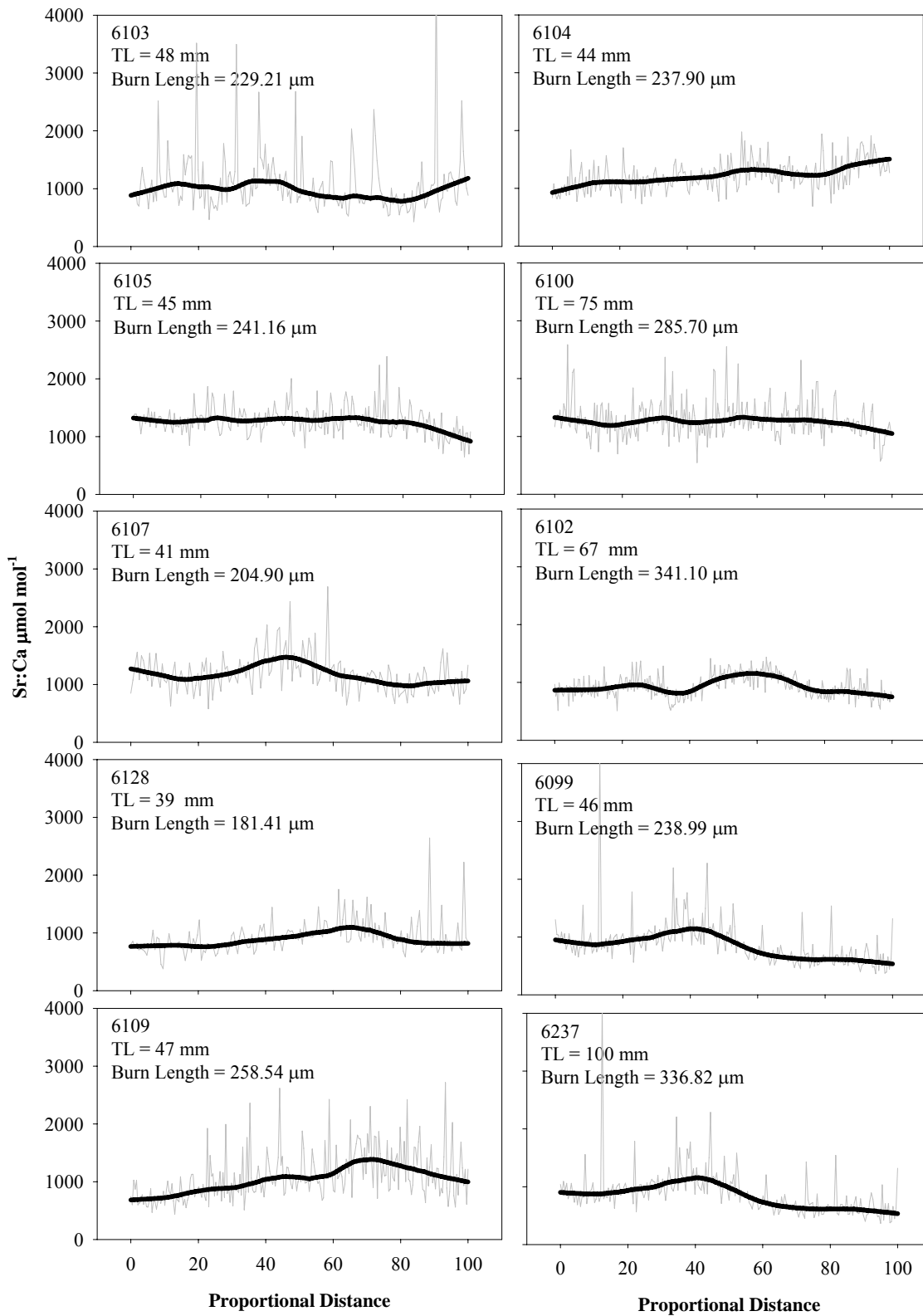


Figure 18. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year class collected from Bay Minette (Downstream Region) in Spring 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

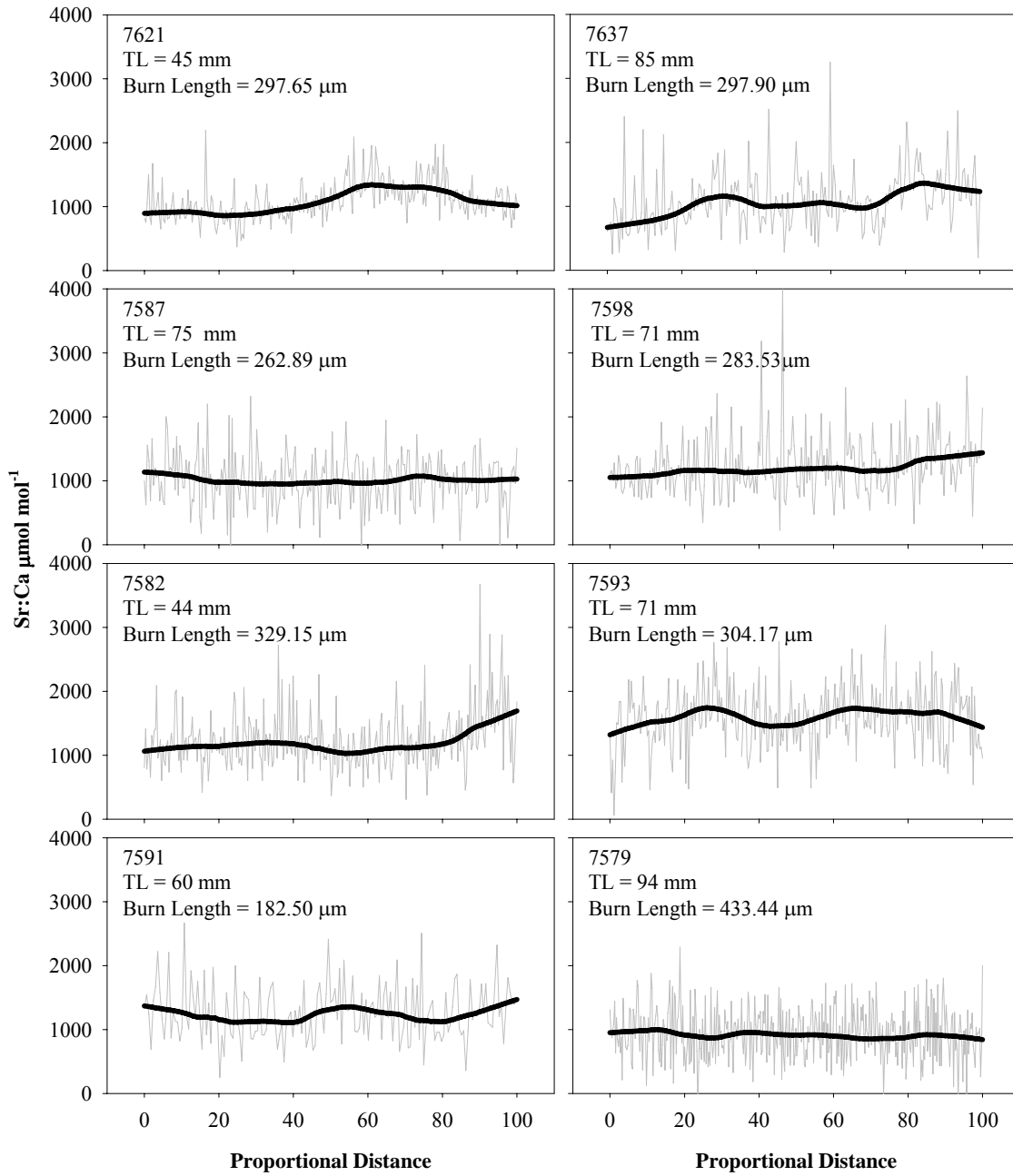


Figure 19. Life history plots of otolith Sr:Ca for age-0 largemouth bass from the 2004 year class collected from D'Olive Bay (Downstream Region) in the Spring of 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively. Dashed line represents the 1500 ppm reference line between marine and freshwater environments.



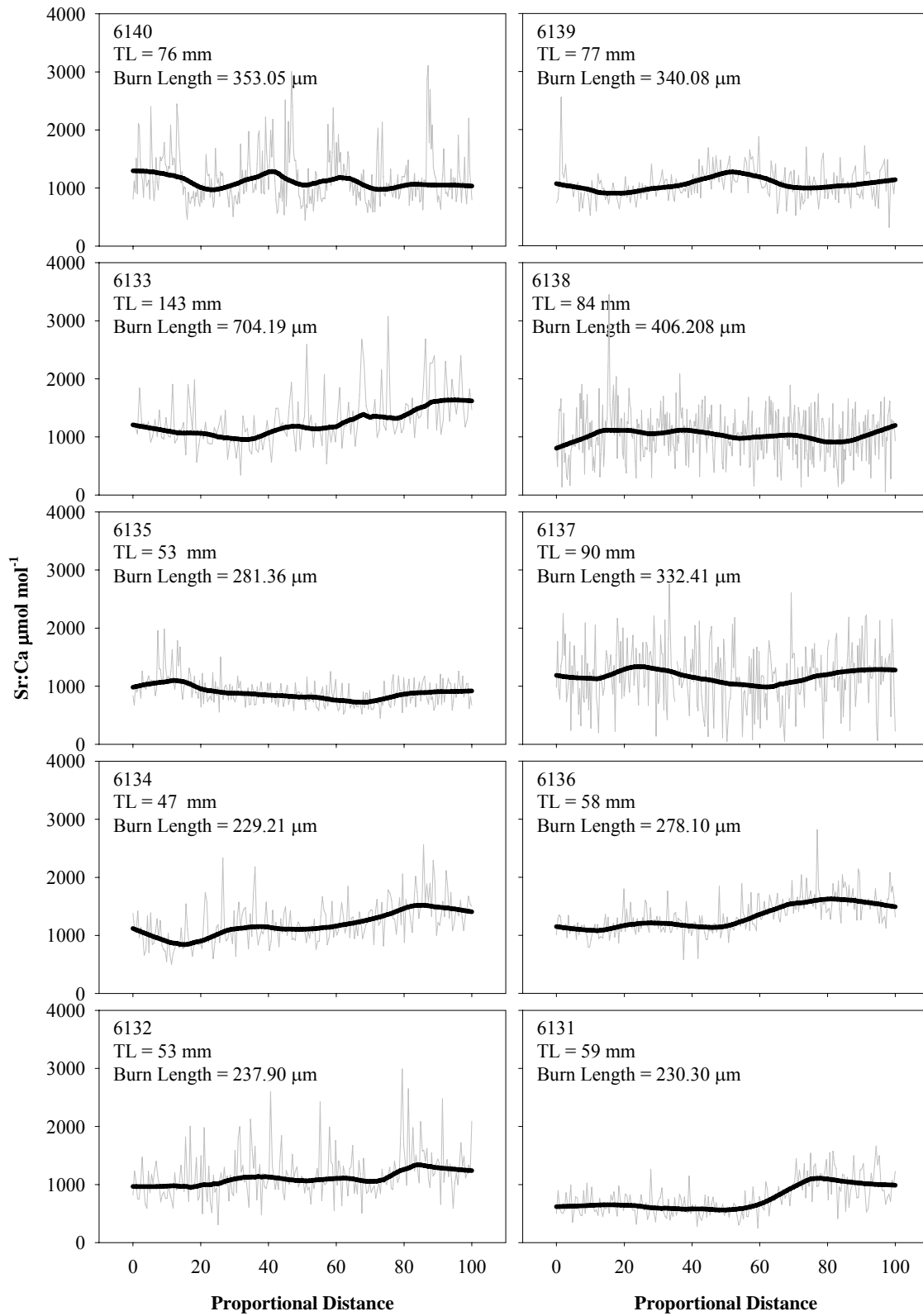


Figure 20. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year-class collected from D'Olive Bay (Downstream Region) in Spring 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

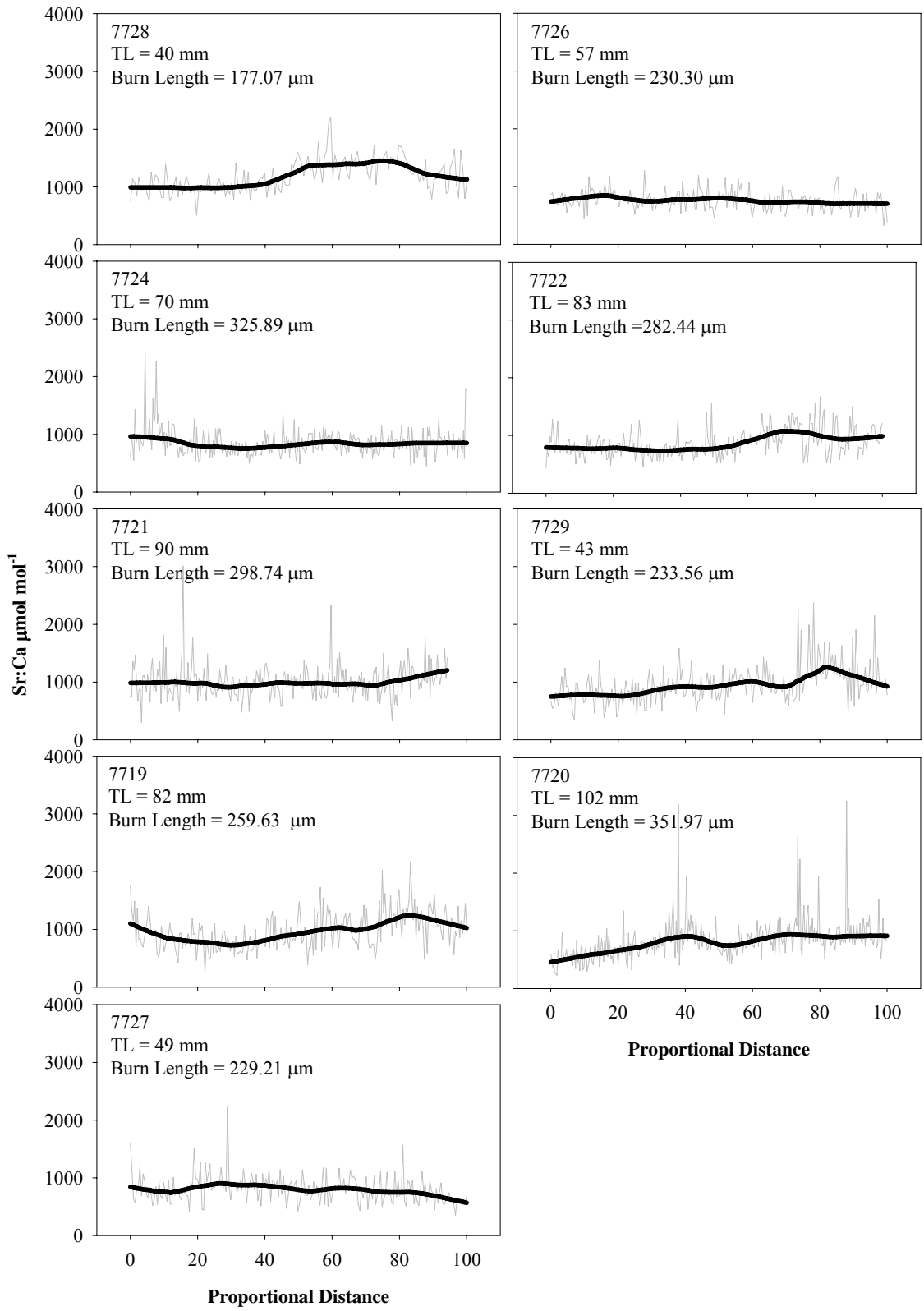


Figure 21. Profile analysis using mean ( $\pm$  95% C.I.) smoothed profiles for upstream (shaded triangles), middle (open circles), and downstream (shaded circles) age-0 largemouth bass collected in the spring. Dashed lines represent the interval along the proportional distance axis at which the *post hoc* comparisons (shown at the top of the graph) are relevant. Letters at top followed by an arrow indicate that the groups differ significantly from the core to the otolith edge (Scheffe's;  $P \leq 0.05$ )

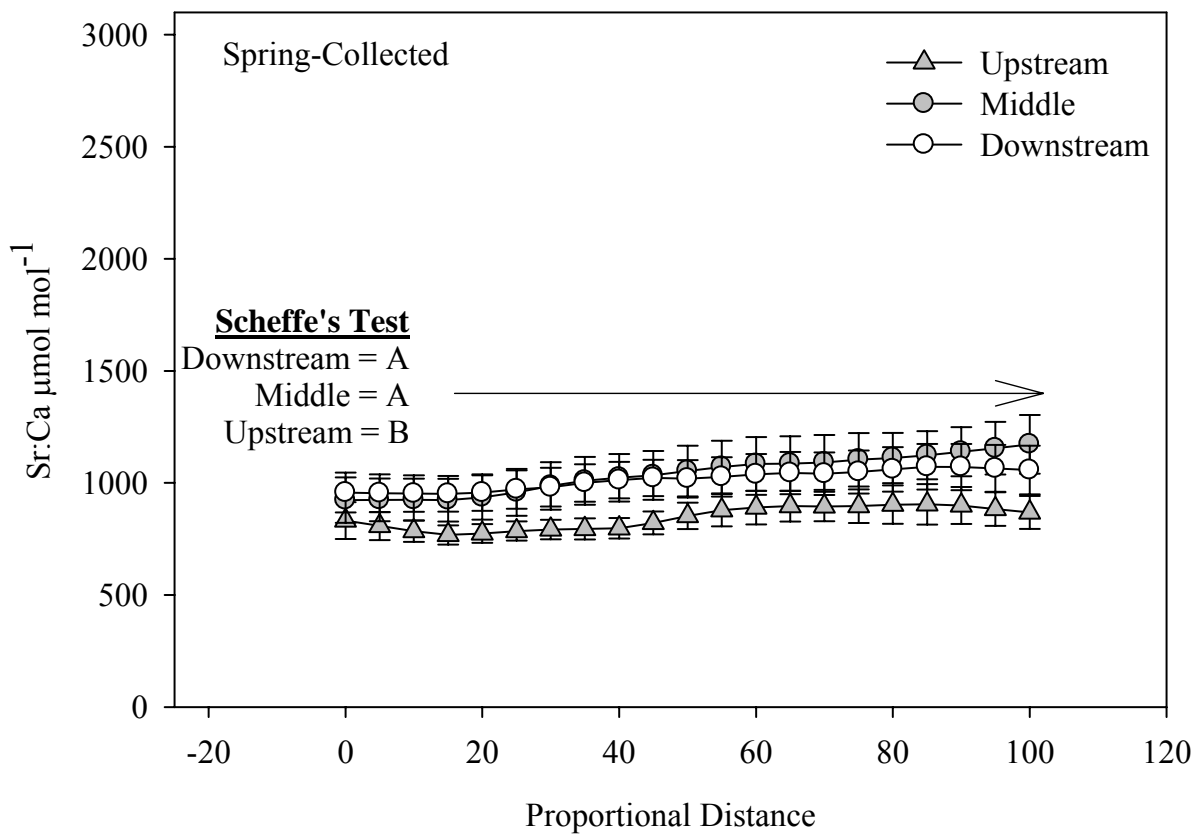


Figure 22. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year-class collected from Dennis Lake (Upstream Region) in Fall 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

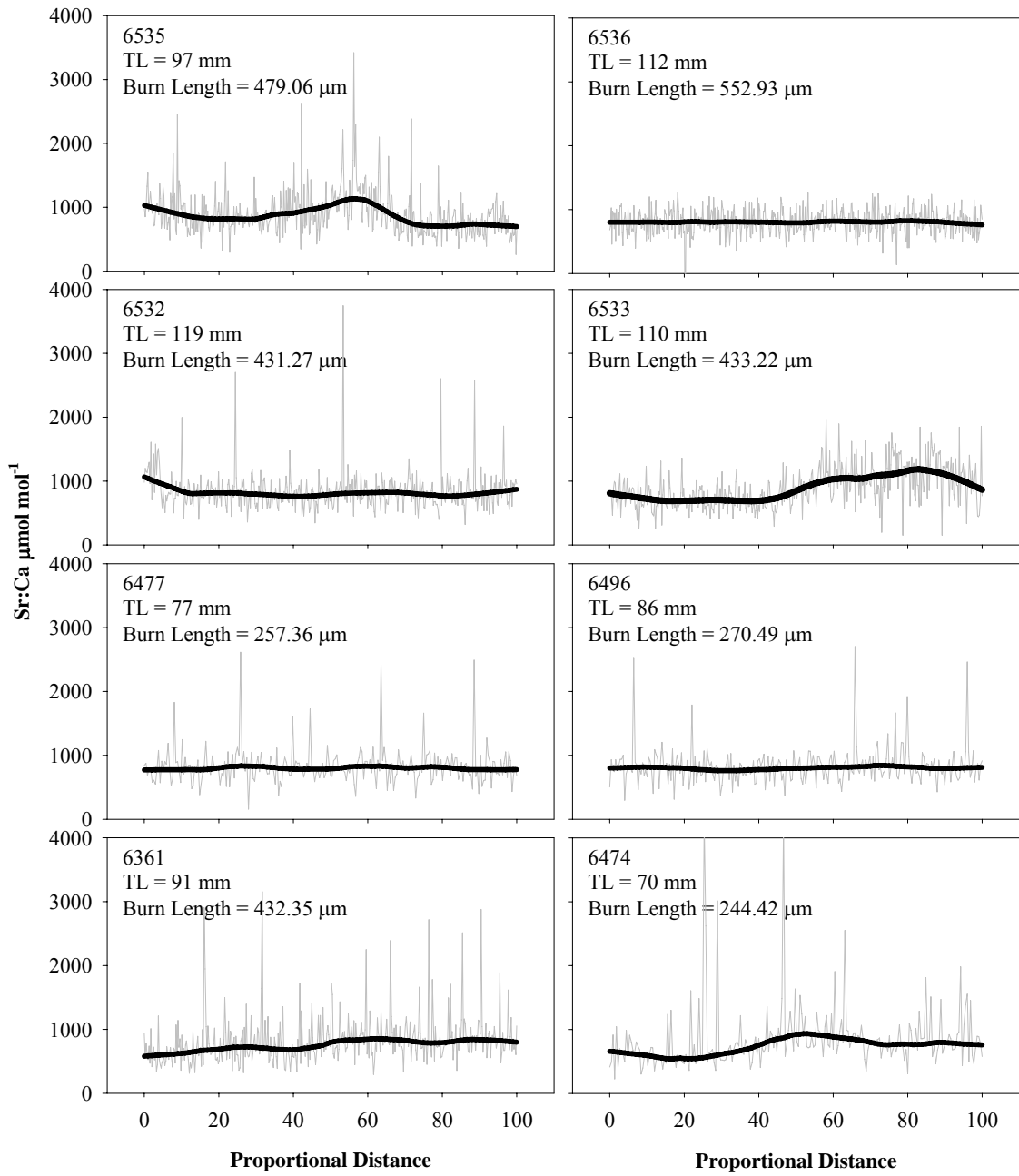


Figure 23. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year-class collected from Dennis Lake (Upstream Region) in Fall 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.



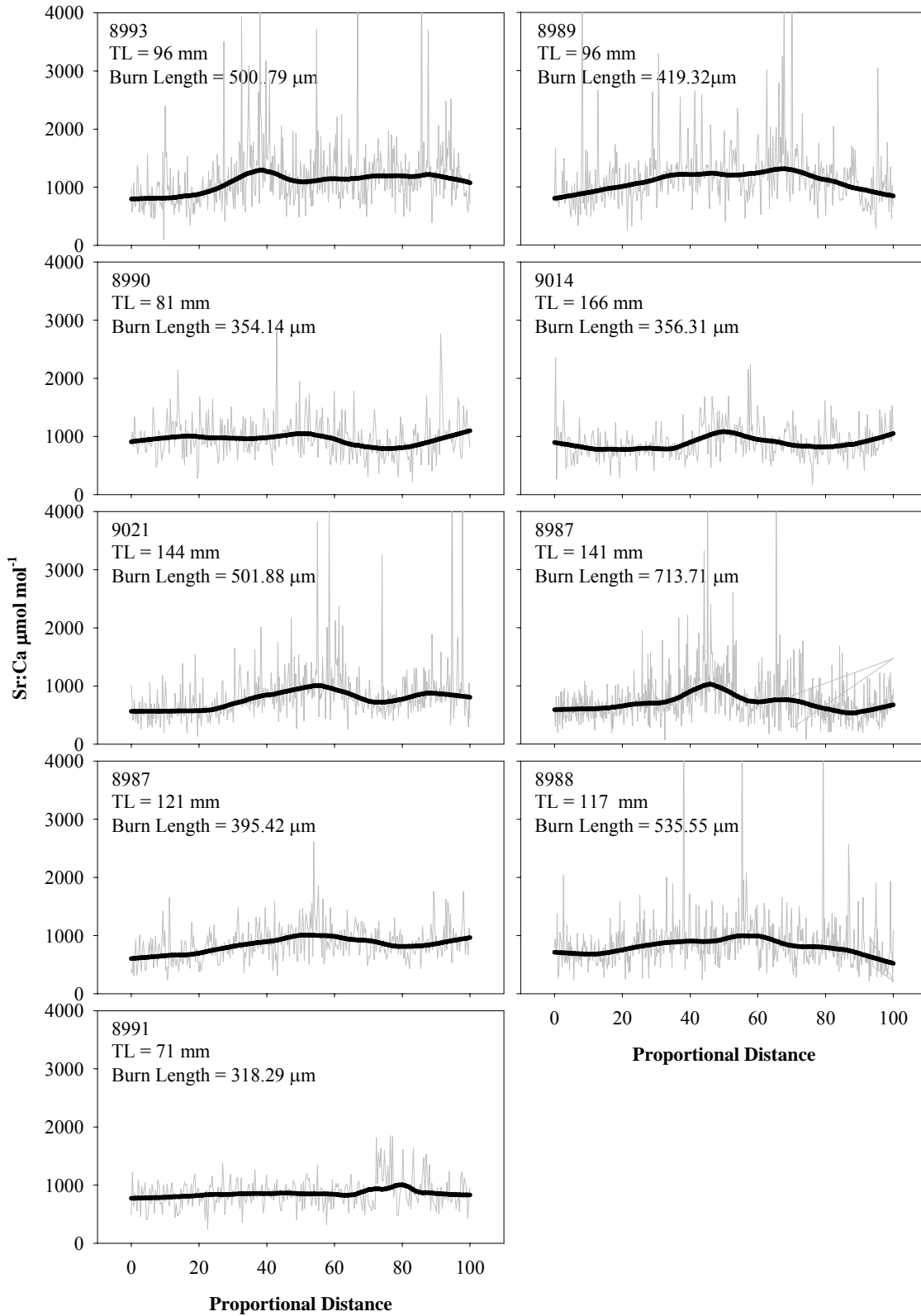


Figure 24. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year-class collected from McReynolds Lake (Upstream Region) in Fall 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

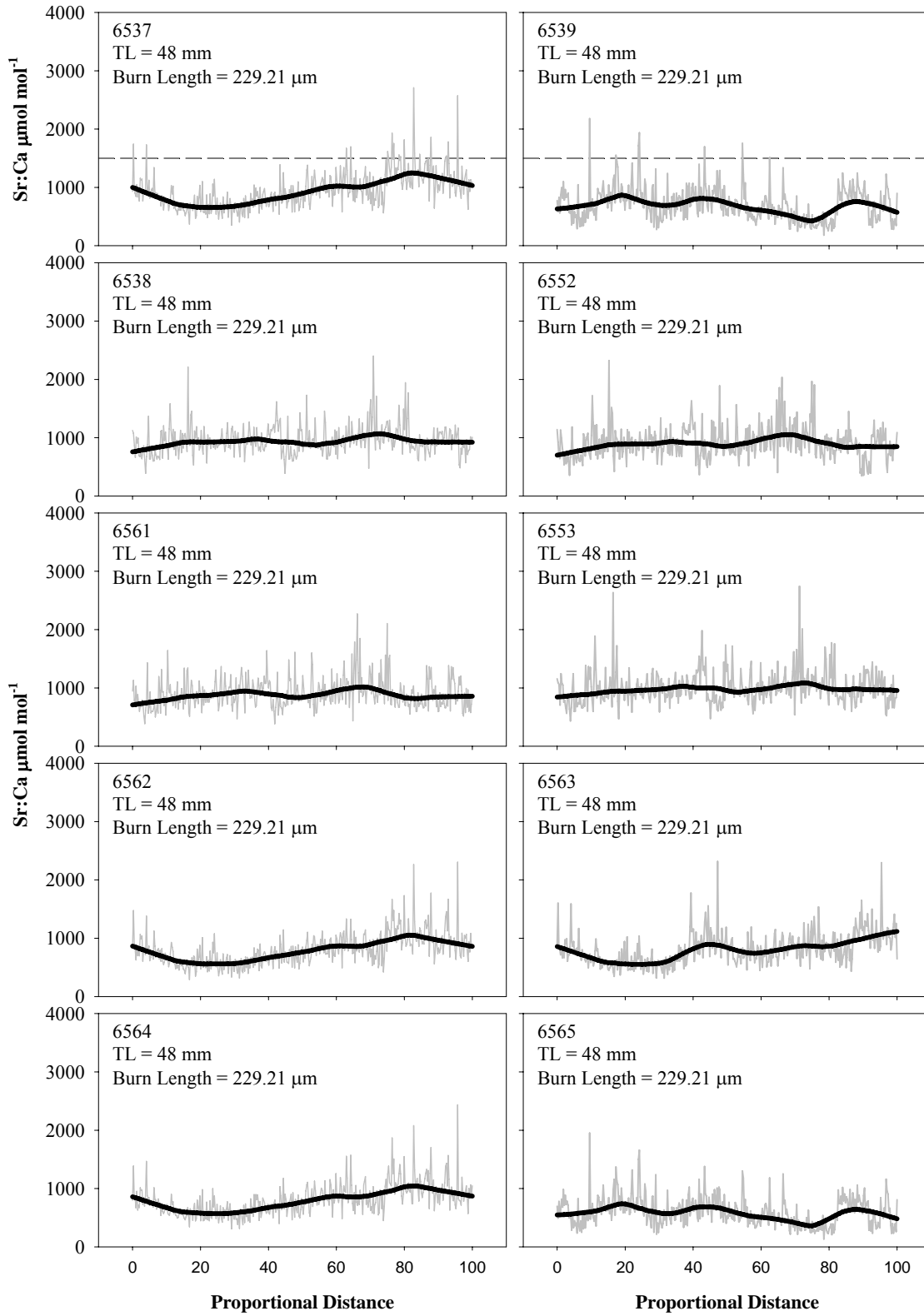


Figure 25. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year-class collected from McReynolds Lake (Upstream Region) in Fall 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

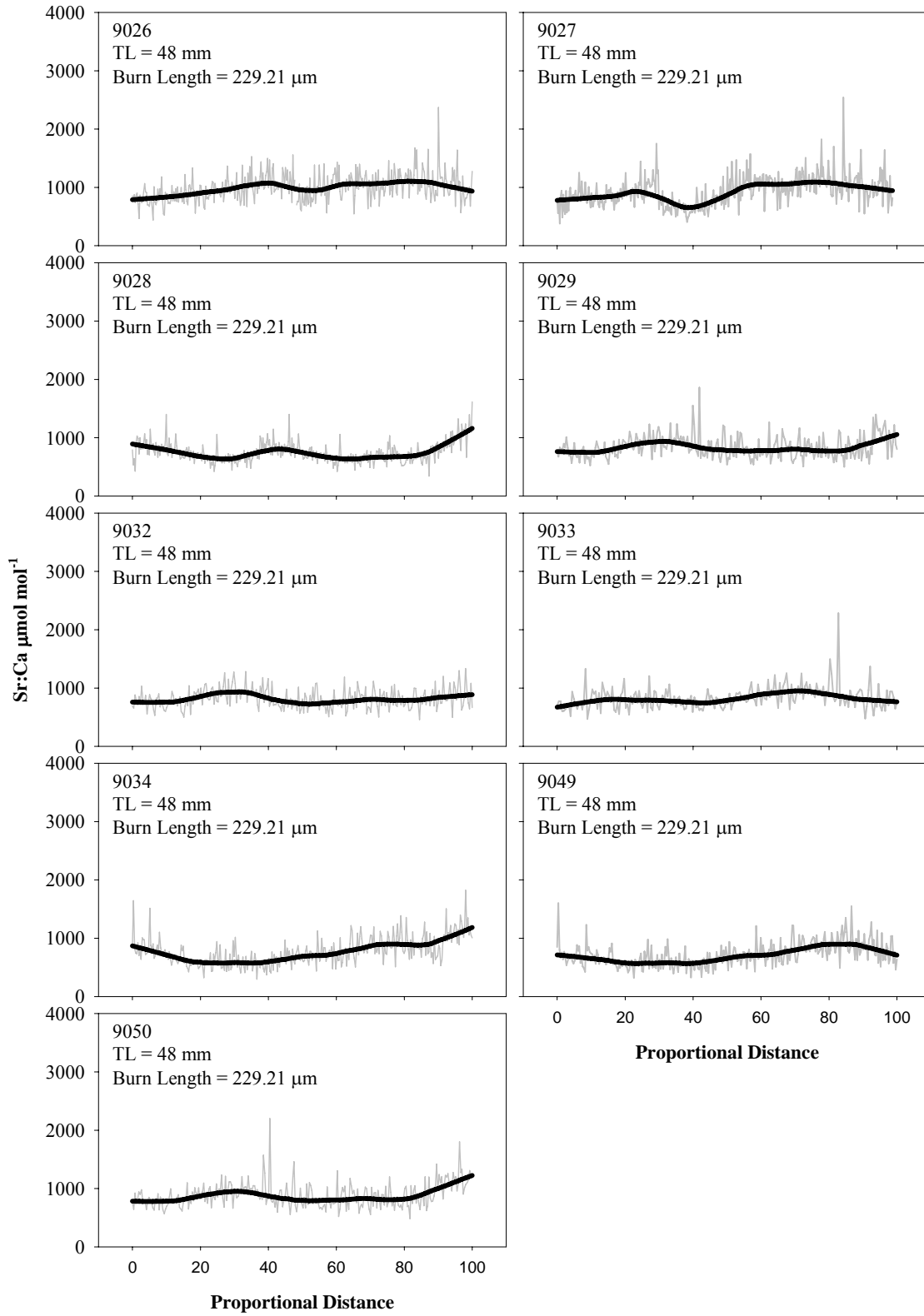


Figure 26. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year-class collected from Gravine Island (Middle Region) in Fall 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

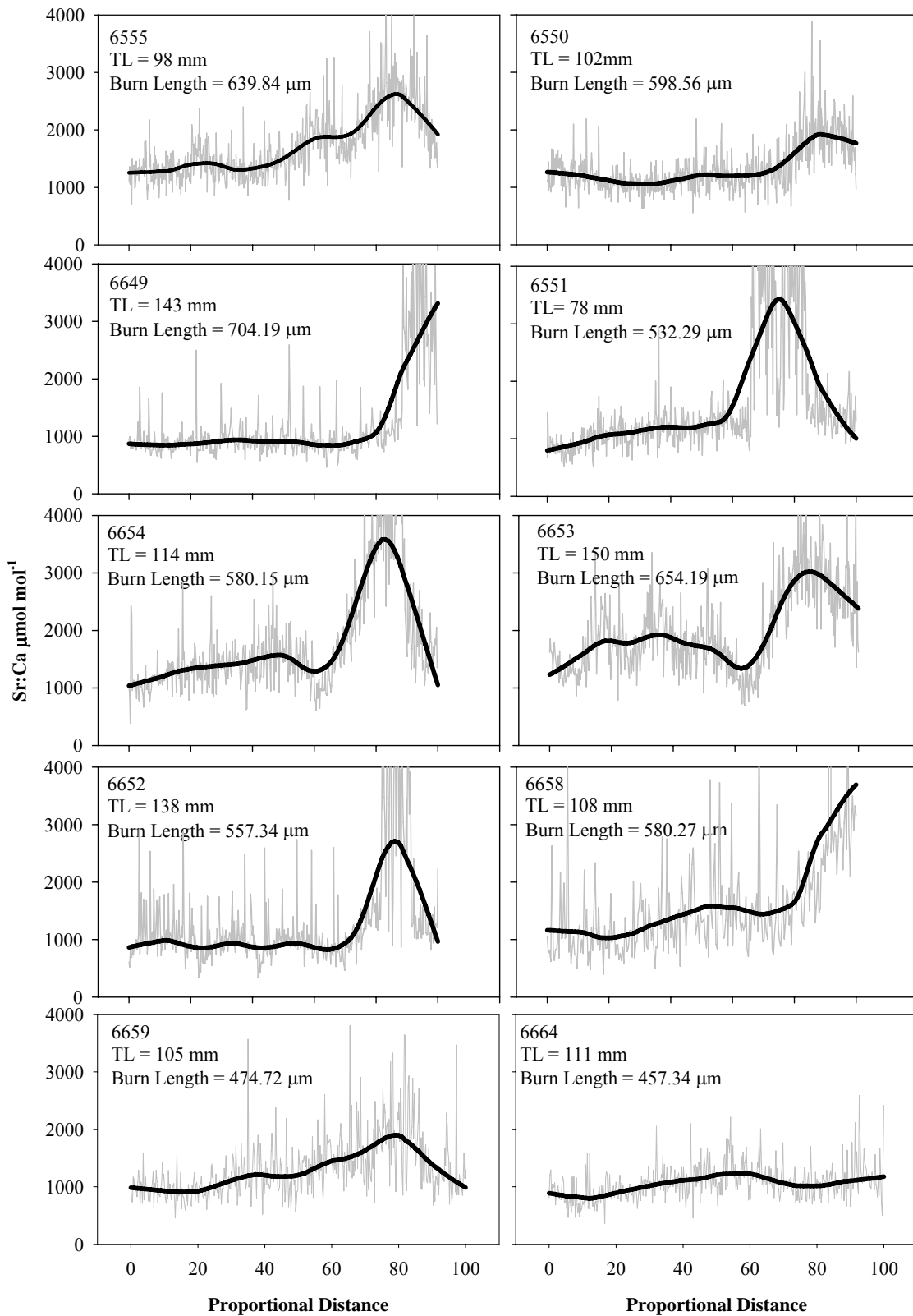


Figure 27. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year-class collected from Gravine Island (Middle Region) in Fall 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.



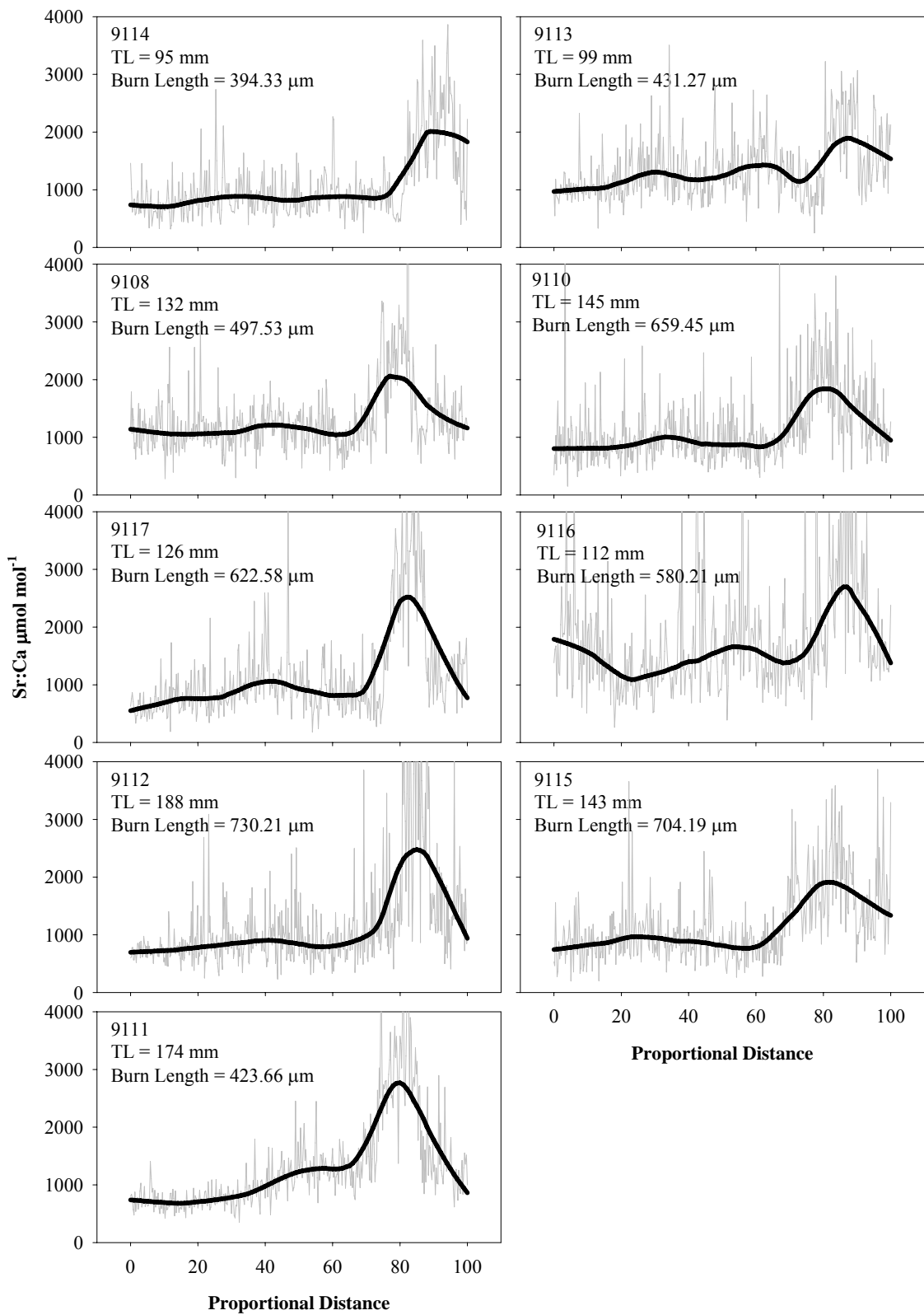


Figure 28. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year-class collected from Crab Creek (Middle Region) in Fall 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

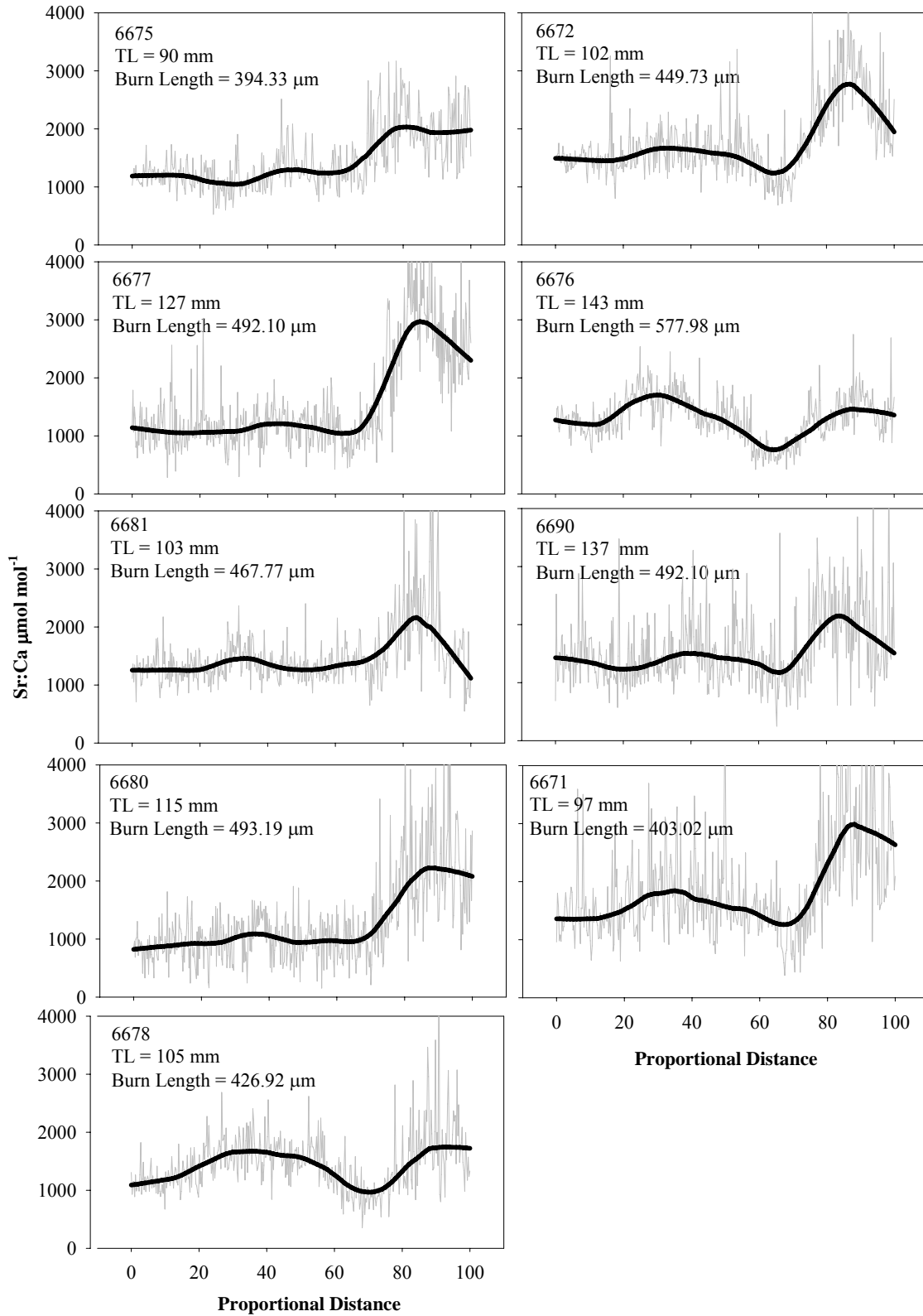


Figure 29. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year-class collected from Crab Creek (Middle Region) in Fall 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

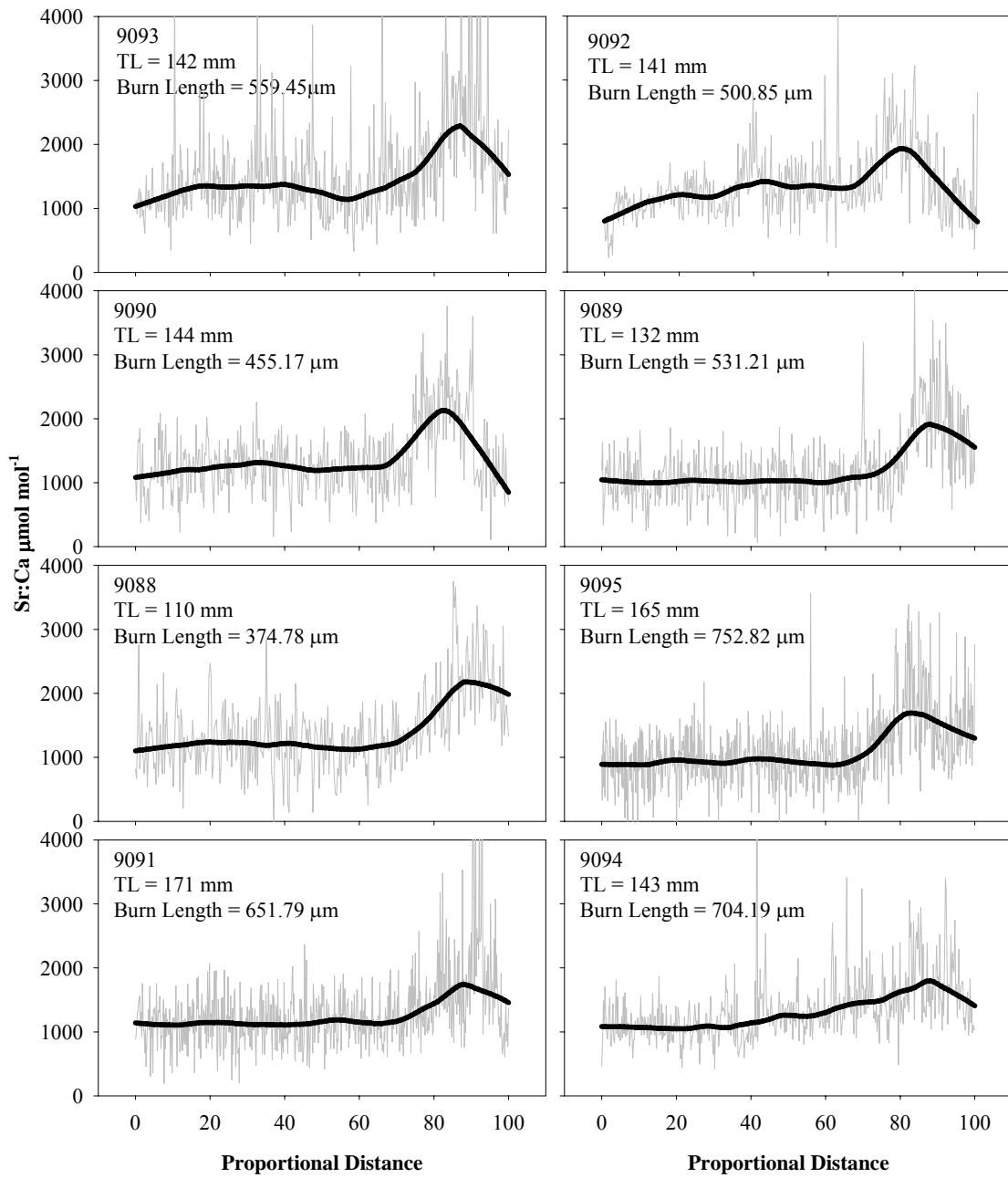


Figure 30. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year-class collected from Bay Minette (Downstream Region) in Fall 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

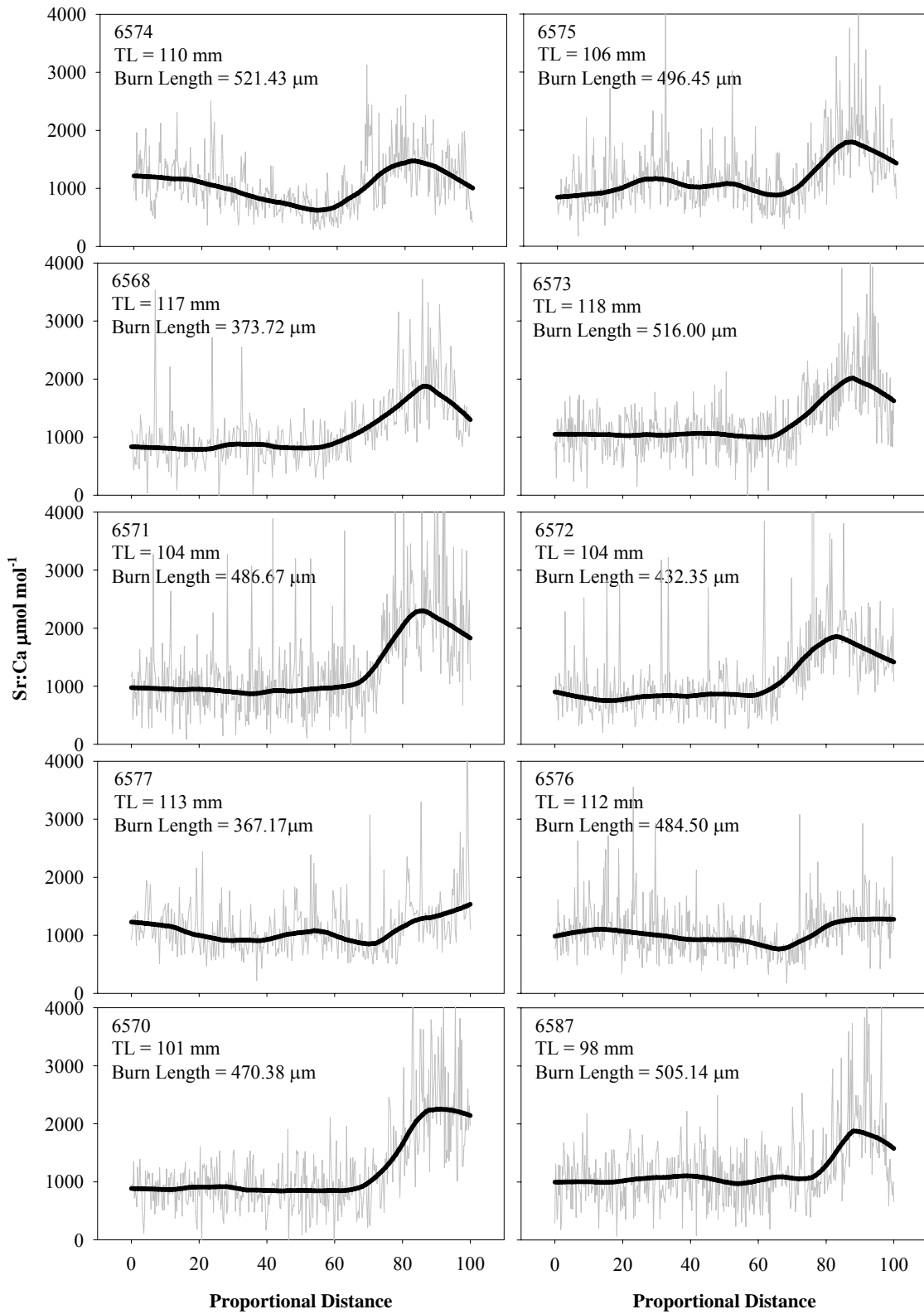


Figure 31. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year-class collected from Bay Minette (Downstream Region) in Fall 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.



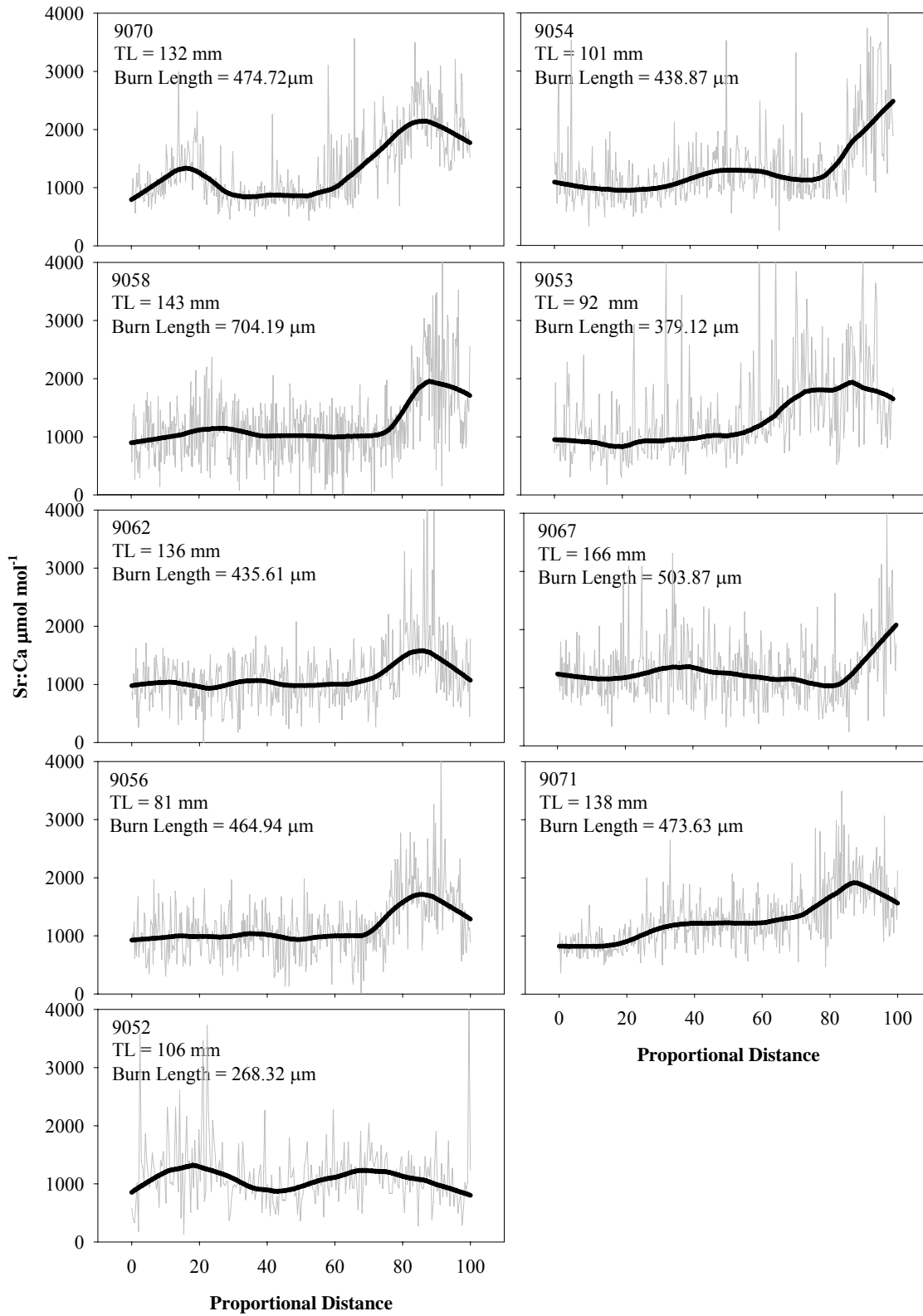


Figure 32. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2004 year-class collected from D'Olive Bay (Downstream Region) in Fall 2004. Gray and black plots are raw data and LOWESS smoothed data, respectively.

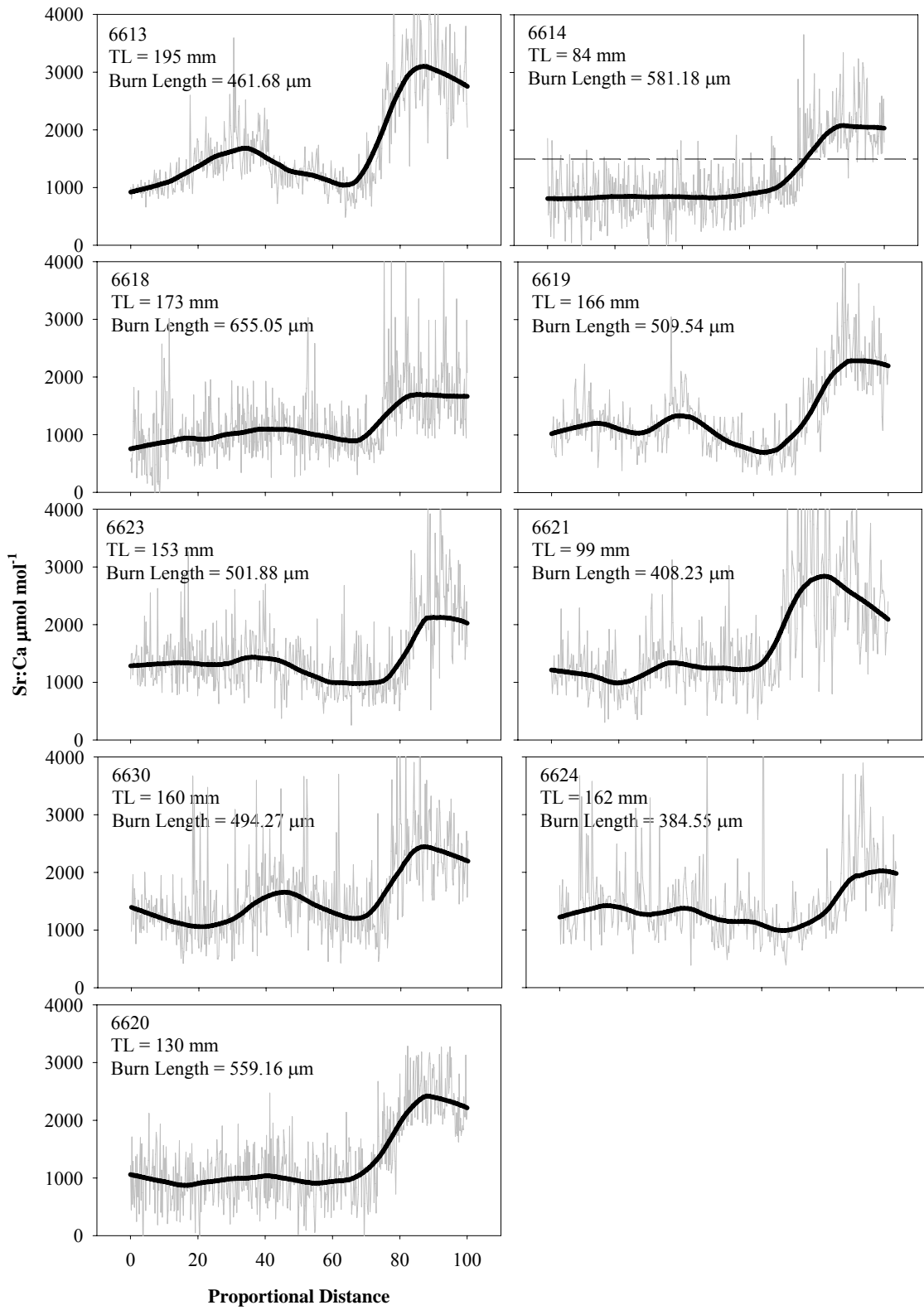


Figure 33. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 largemouth bass from the 2005 year-class collected from D'Olive Bay (Downstream Region) in Spring 2005. Gray and black plots are raw data and LOWESS smoothed data, respectively.

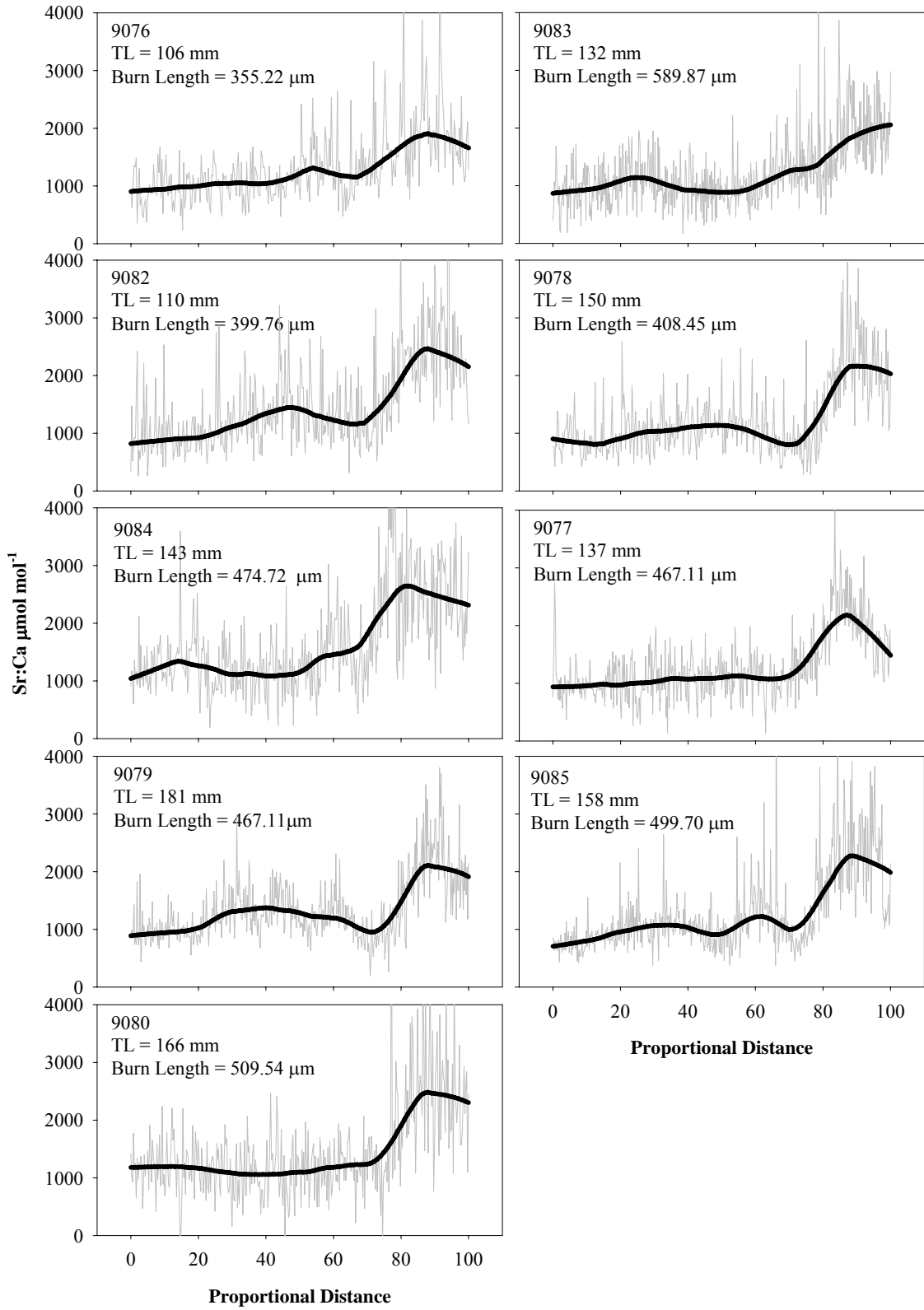


Figure 34. Profile analysis using mean ( $\pm$  95% C.I.) smoothed profiles for upstream (shaded triangles), middle (open circles), and downstream (shaded circles) age-0 largemouth bass collected in the fall. Dashed lines represent the interval along the proportional distance axis at which the *post hoc* comparisons (shown at the top of the graph) are relevant. Groups with different letters differ significantly (Sheffe's;  $P \leq 0.05$ ).

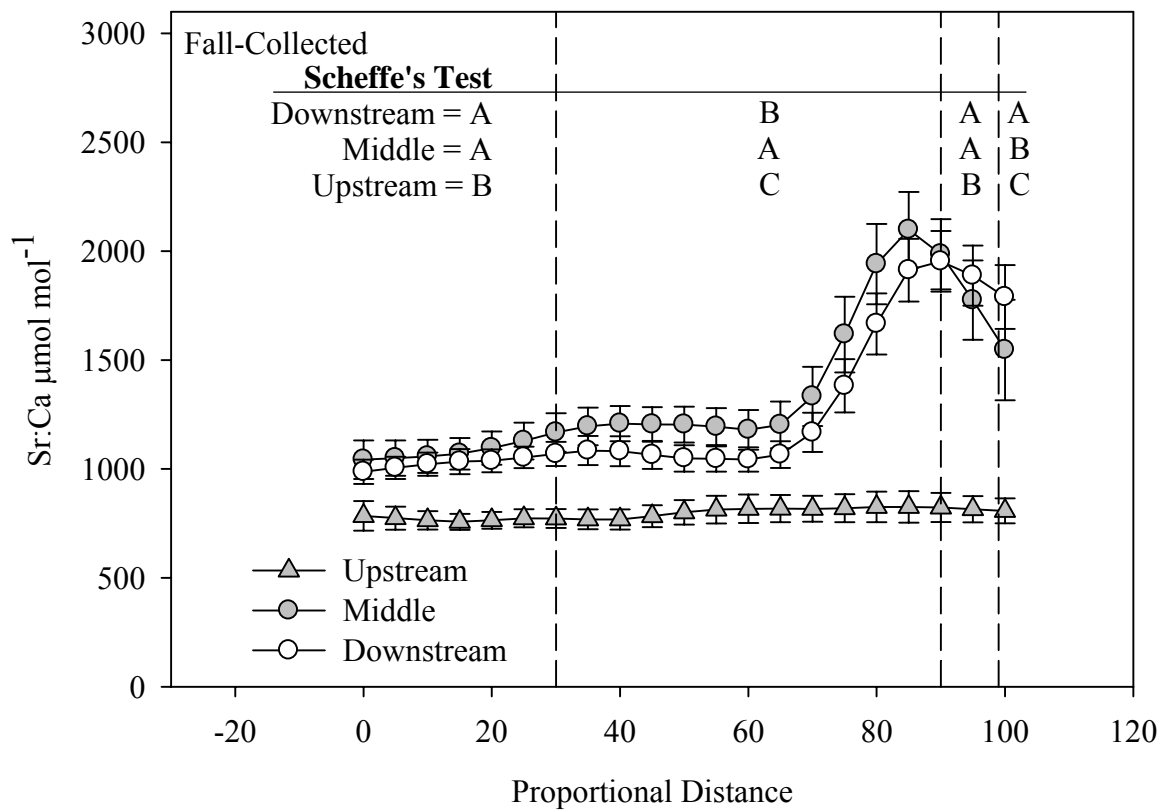


Figure 35. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2004 year-class collected from Dennis Lake (Upstream Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.



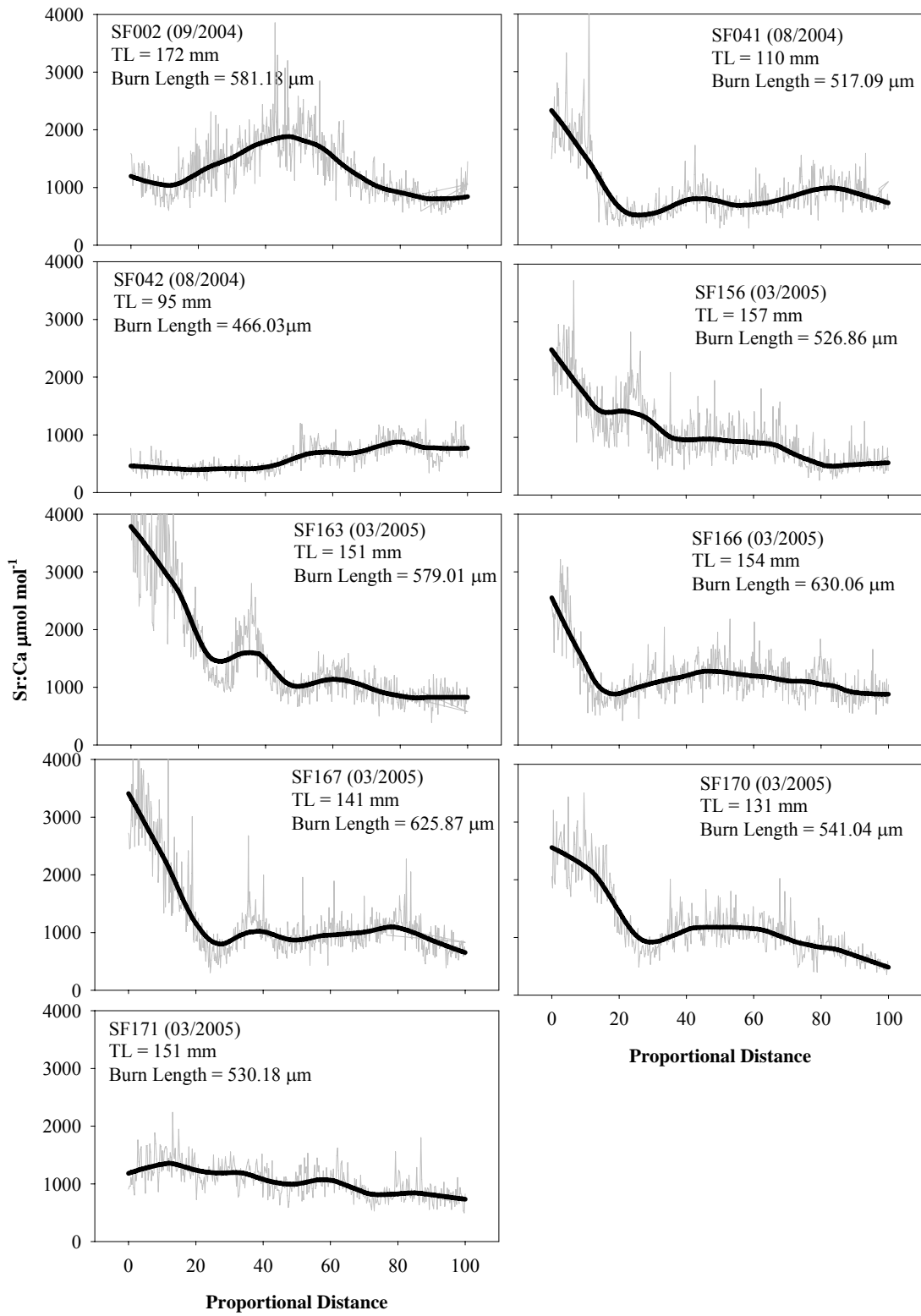


Figure 36. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2005 year-class collected from Dennis Lake (Upstream Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.

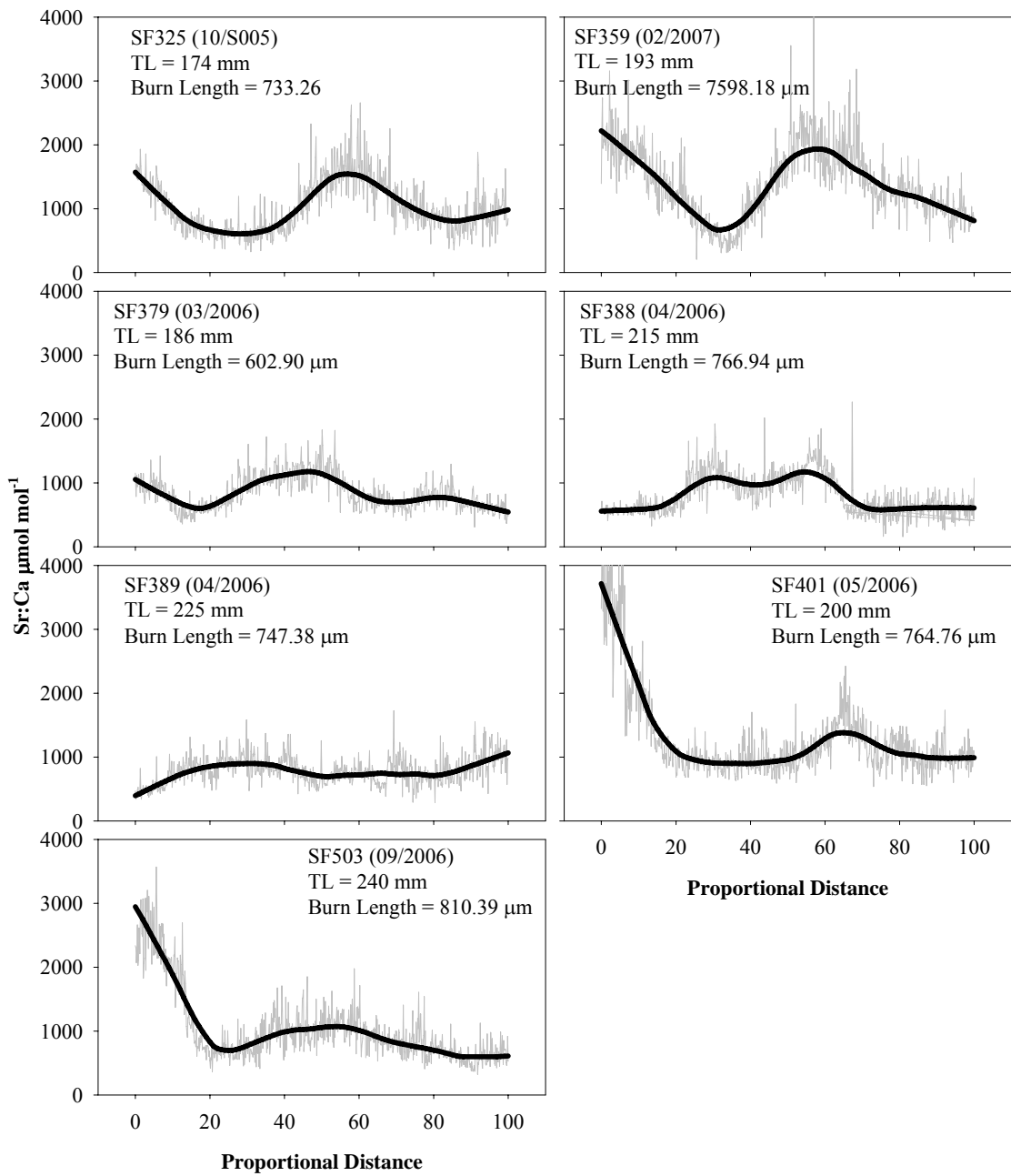
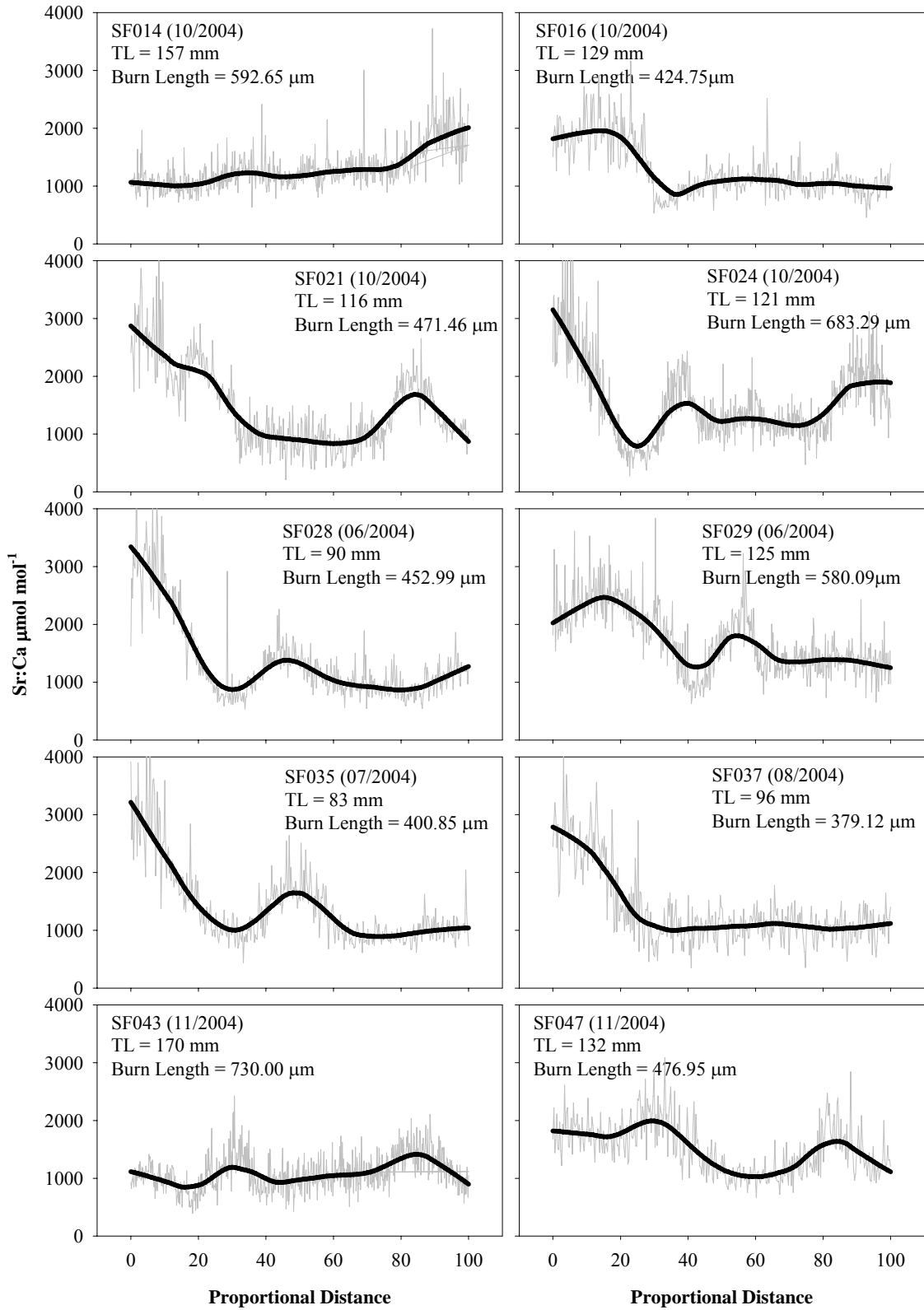


Figure 37. Life history plots of otolith Sr:Ca from age-0 southern flounder from the 2004 year class collected from Gravine Island. Plots are arranged in order by month of capture. Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.



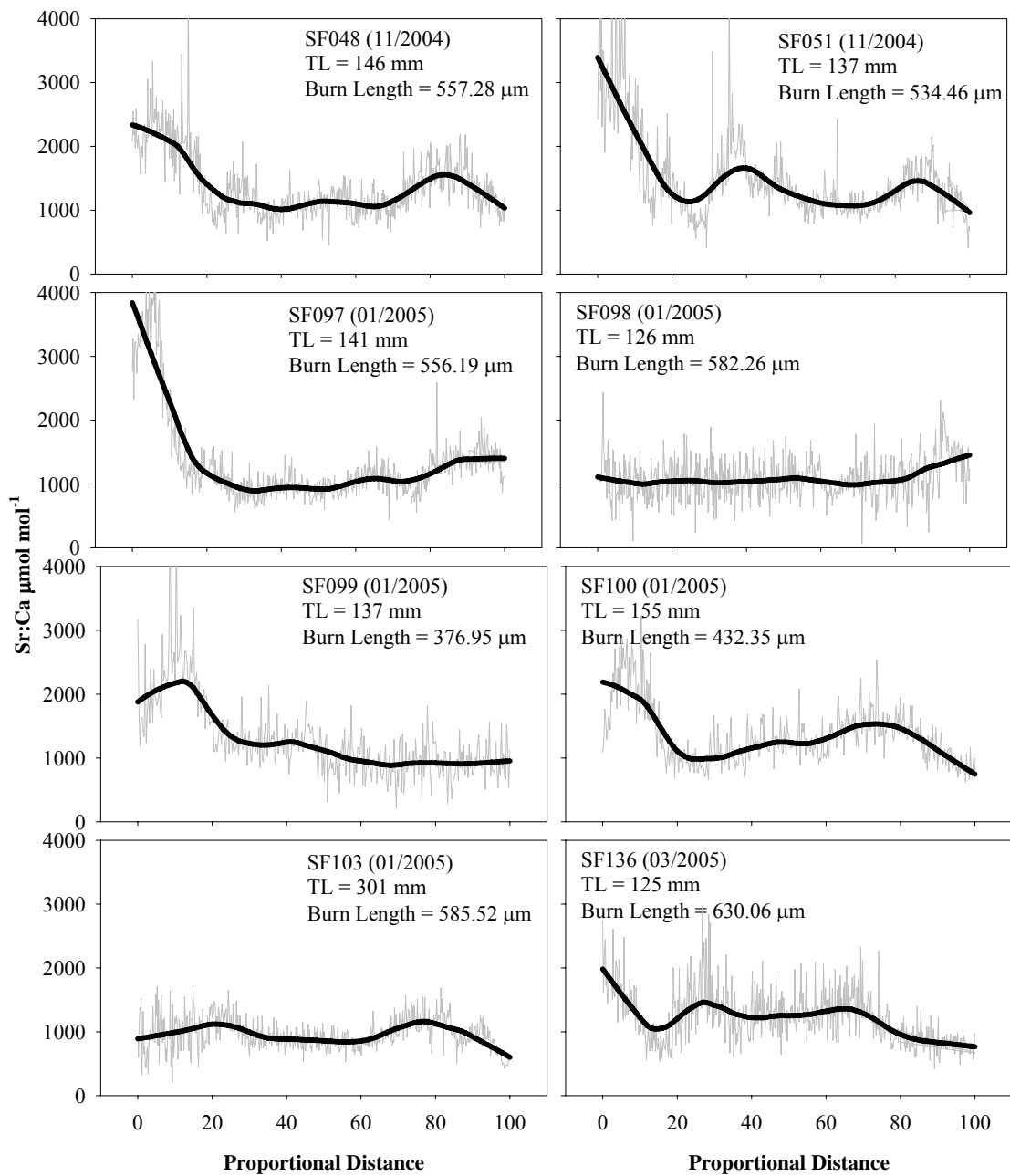
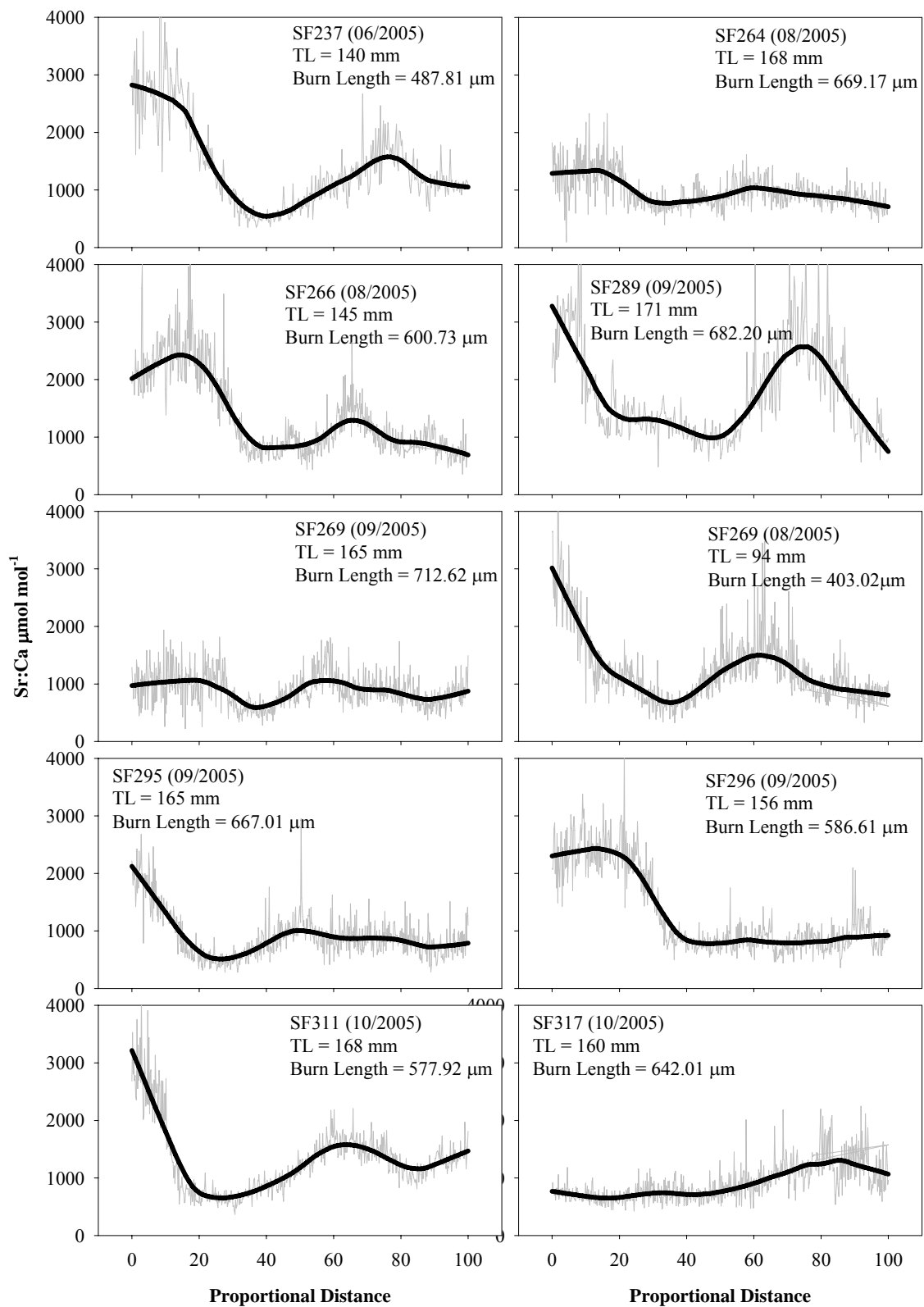


Figure 38. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2005 year-class collected from Gravine Island (Middle Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.





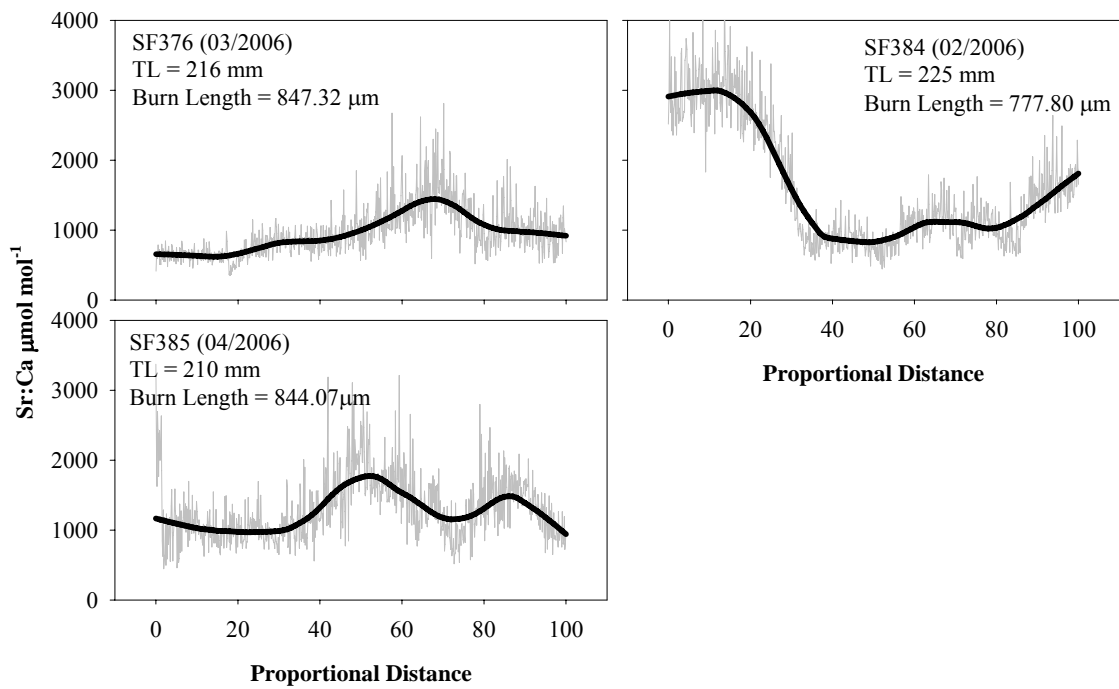


Figure 39. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2004 year-class collected from Crab Creek (Middle Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.

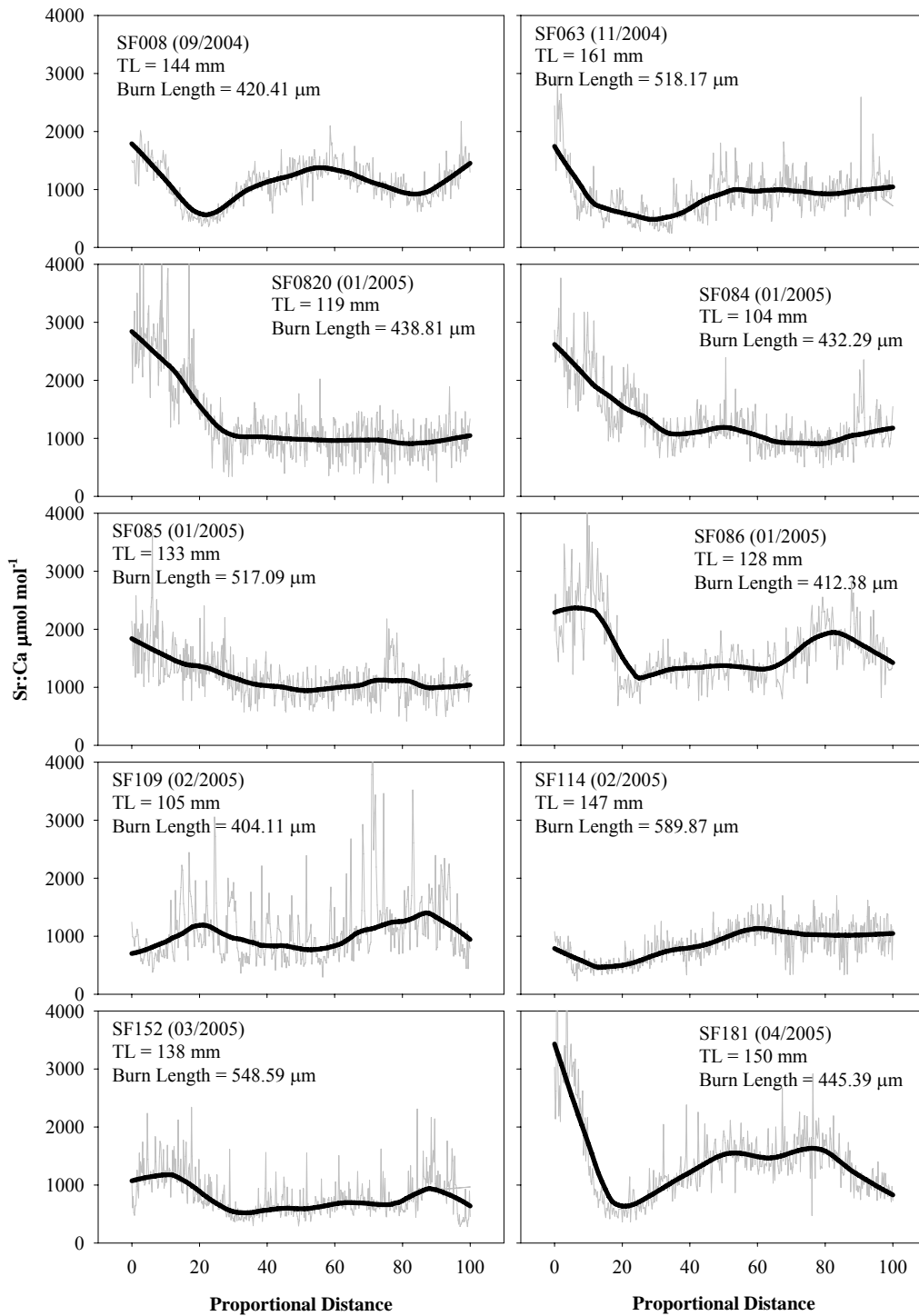


Figure 40. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2005 year-class collected from Crab Creek (Middle Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.

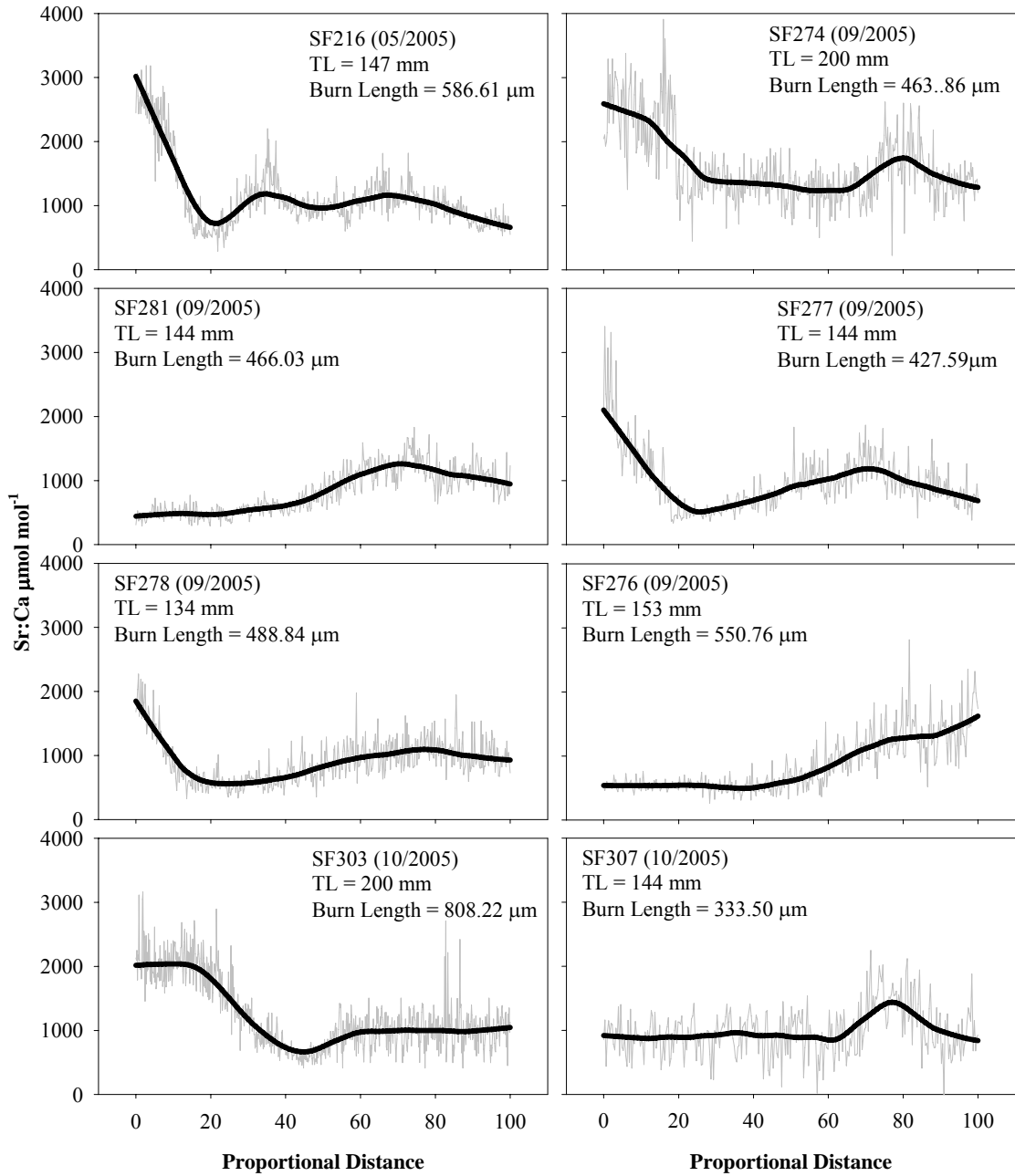


Figure 41. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2004 year-class collected from Bay Minette (Downstream Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.

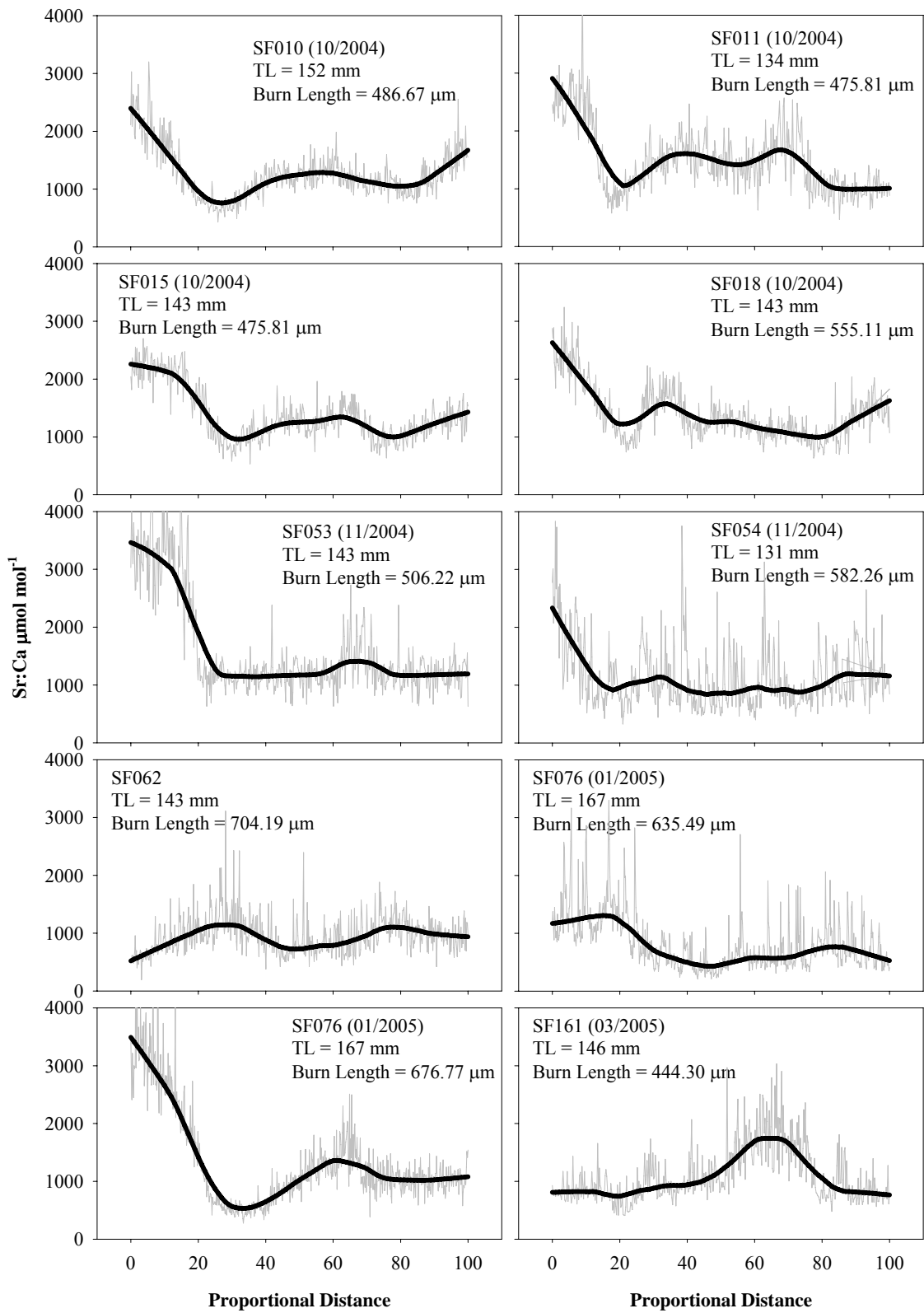


Figure 42. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2005 year-class collected from Bay Minette (Downstream Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.



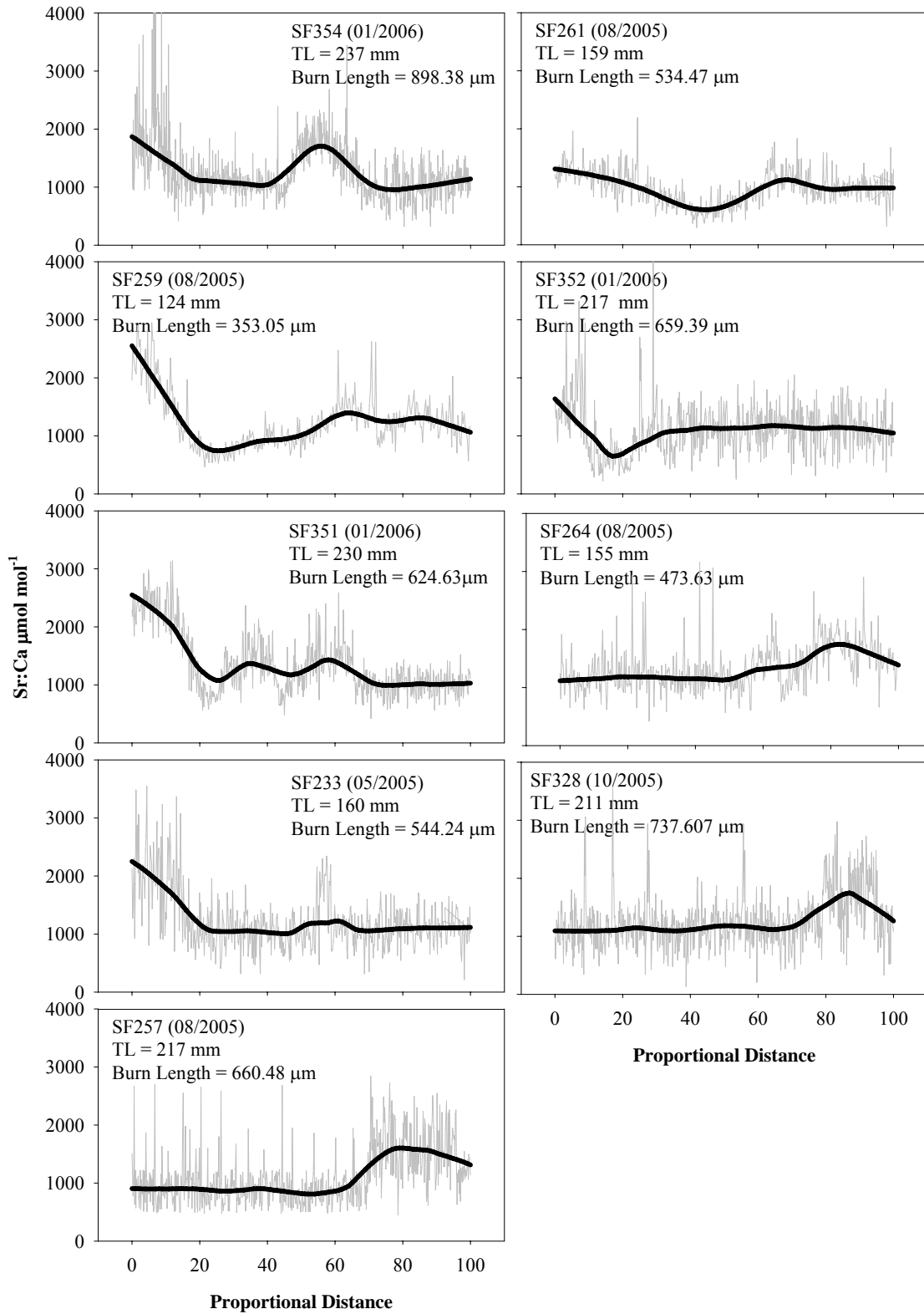


Figure 43. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2004 year-class collected from D'Olive Bay (Downstream Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.

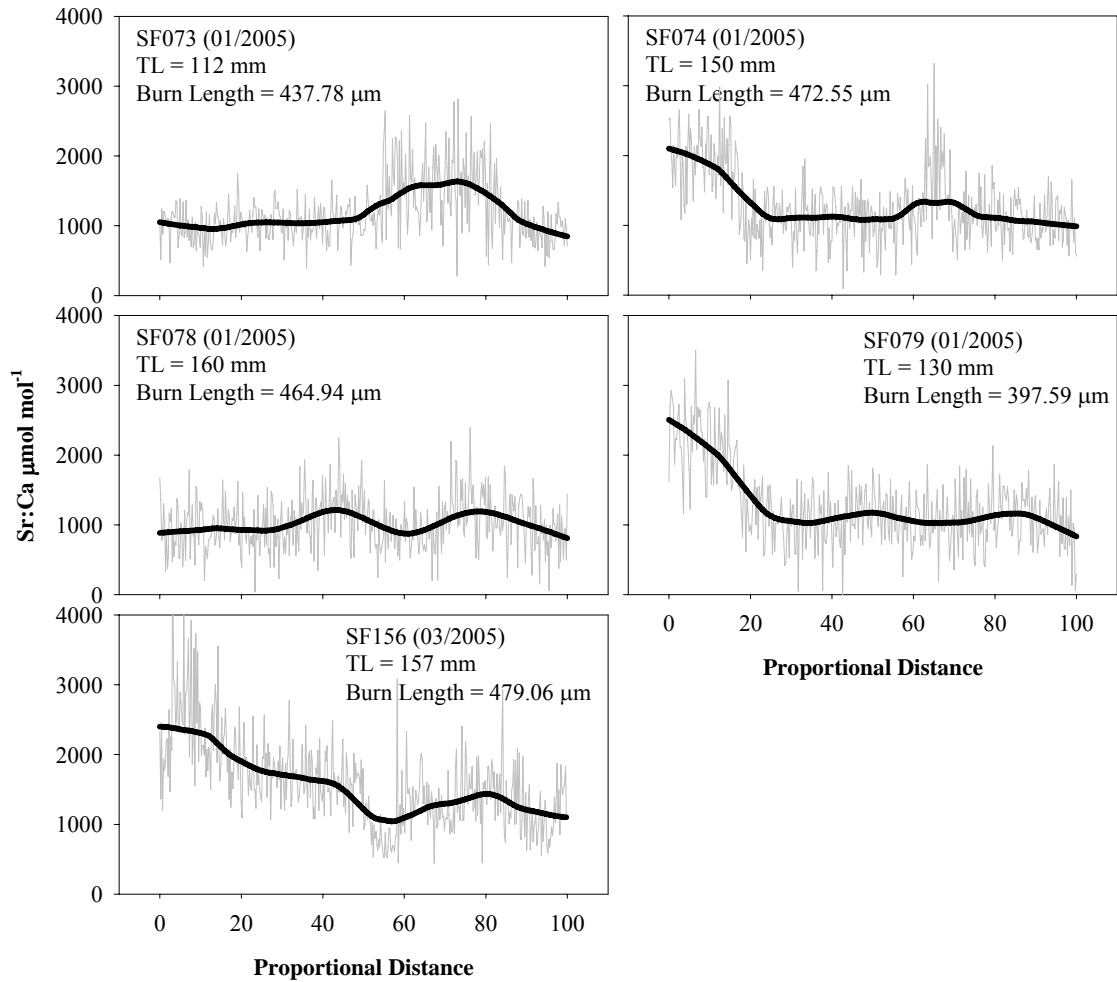


Figure 44. Individual Sr:Ca<sub>otolith</sub> profiles for age-0 southern flounder from the 2005 year-class collected from D'Olive Bay (Downstream Region). Gray and black plots are raw data and LOWESS smoothed data, respectively. Plots are arranged in order by month of capture.

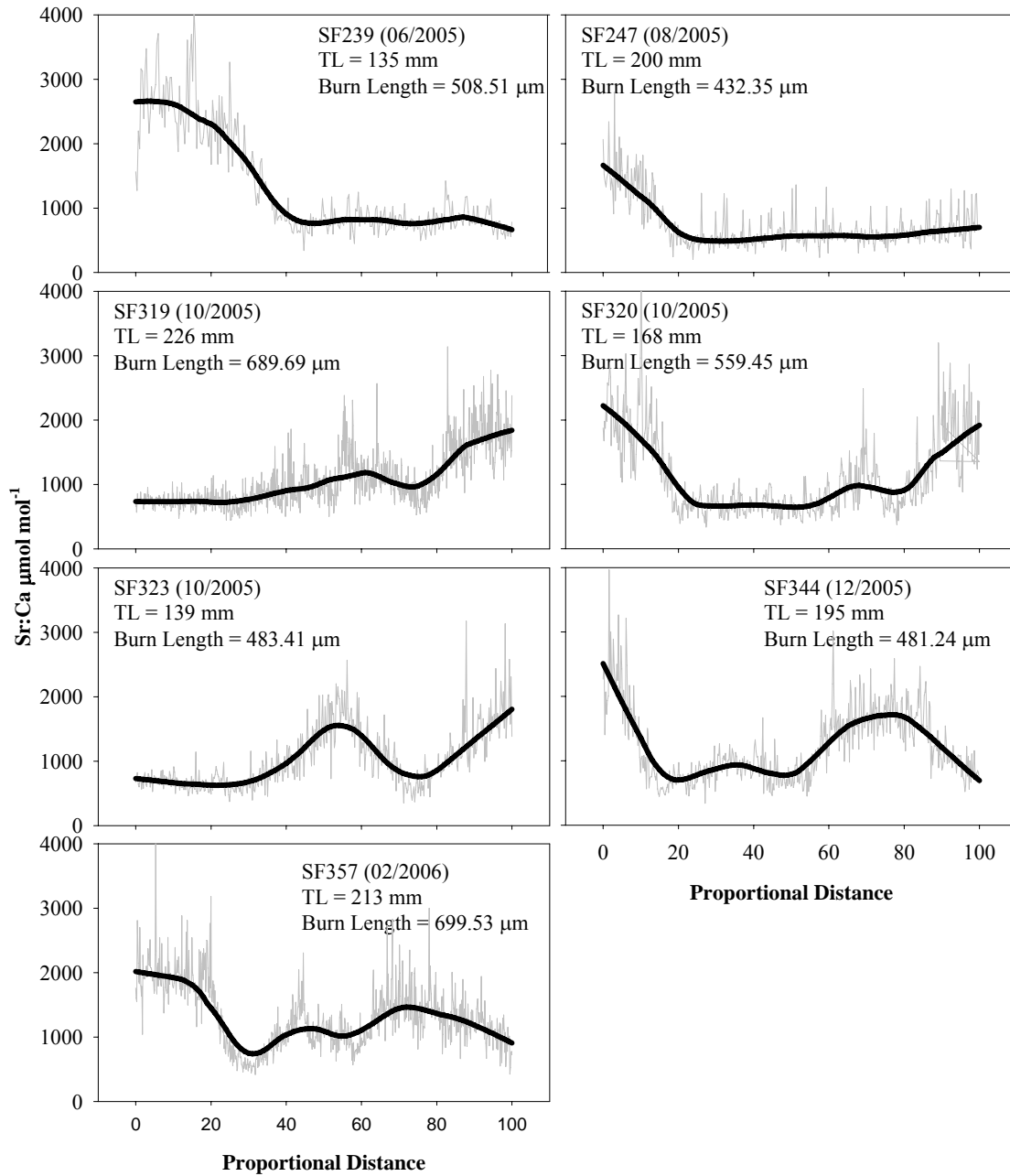


Figure 45. Mean (+SE) element concentrations of core and edge region of spring and fall-collected age-0 largemouth bass from the Mobile Tensaw Delta in 2004.

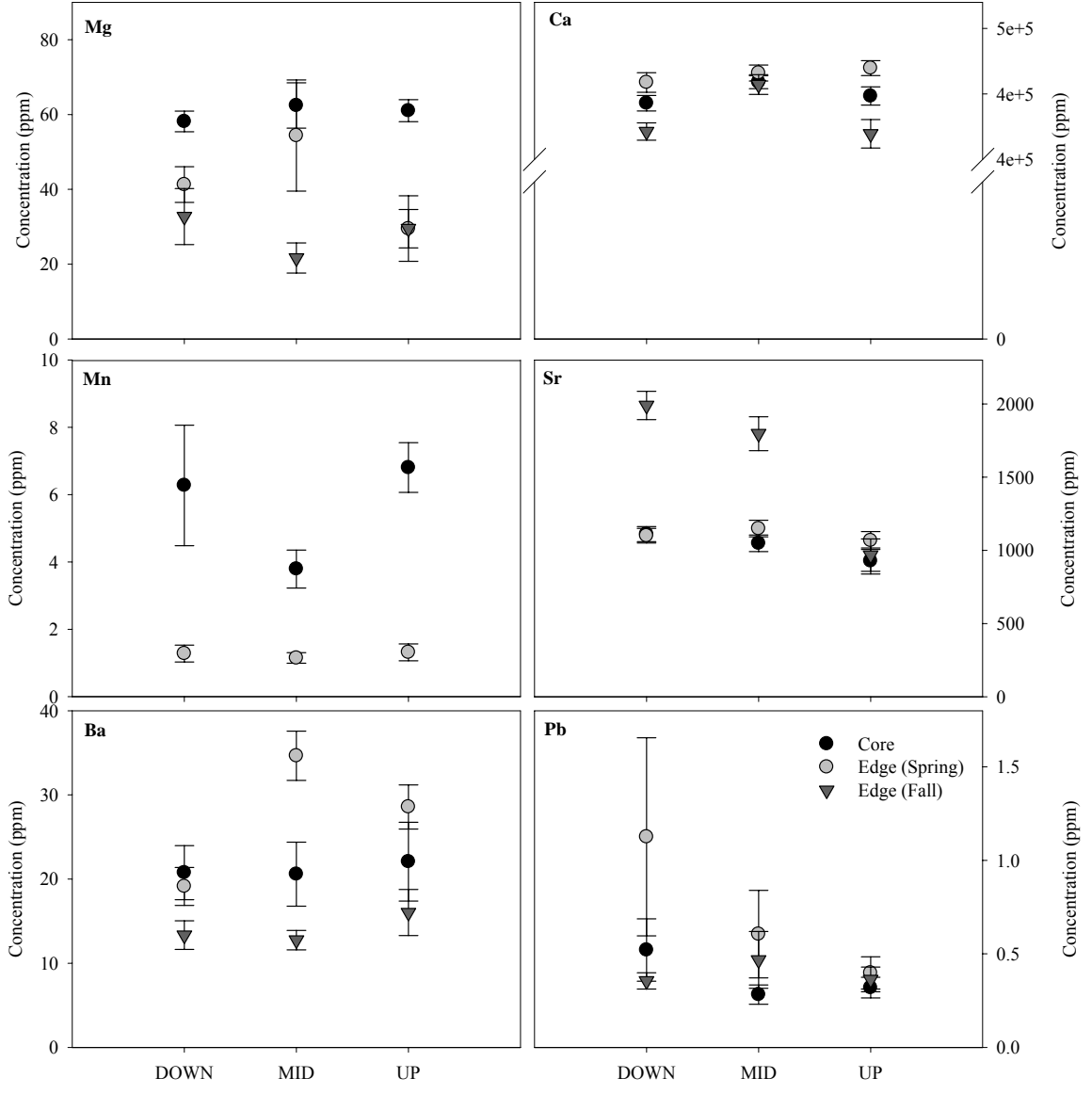


Figure 46. Mean (+SE) element concentrations of core and edge region of spring and fall-collected age-0 largemouth bass from the Mobile Tensaw Delta in 2005.



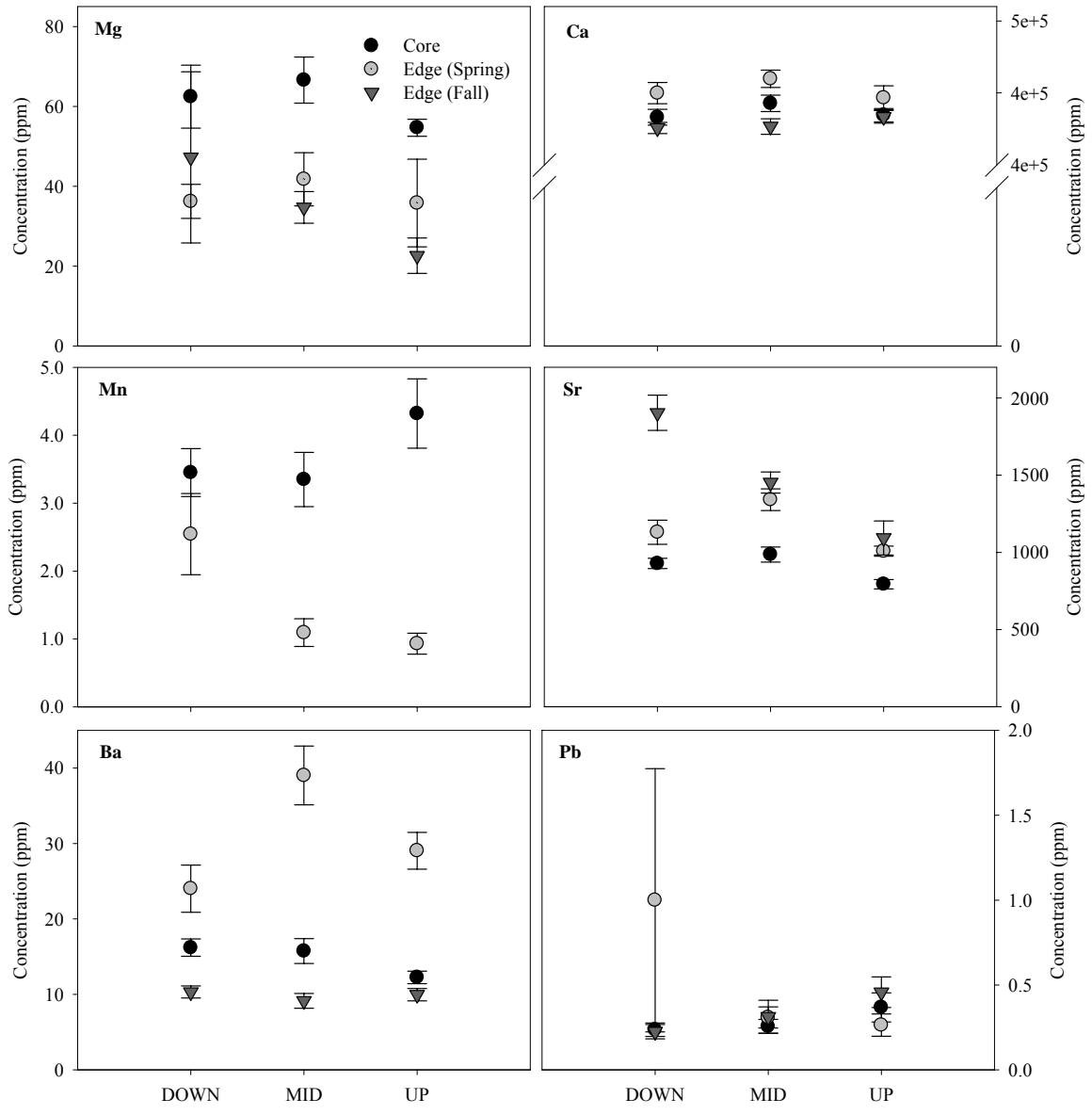


Figure 47. Linear discriminant function analysis of root 1 versus root 2 for age-0 largemouth bass otoliths (cores from 2004 year class only) collected from three regions in the Mobile Tensaw Delta. Solid, small-dashed, and large-dashed lines are 95 % confidence ellipses upstream (UP), middle (MID), and downstream (DOWN) collection regions, respectively.

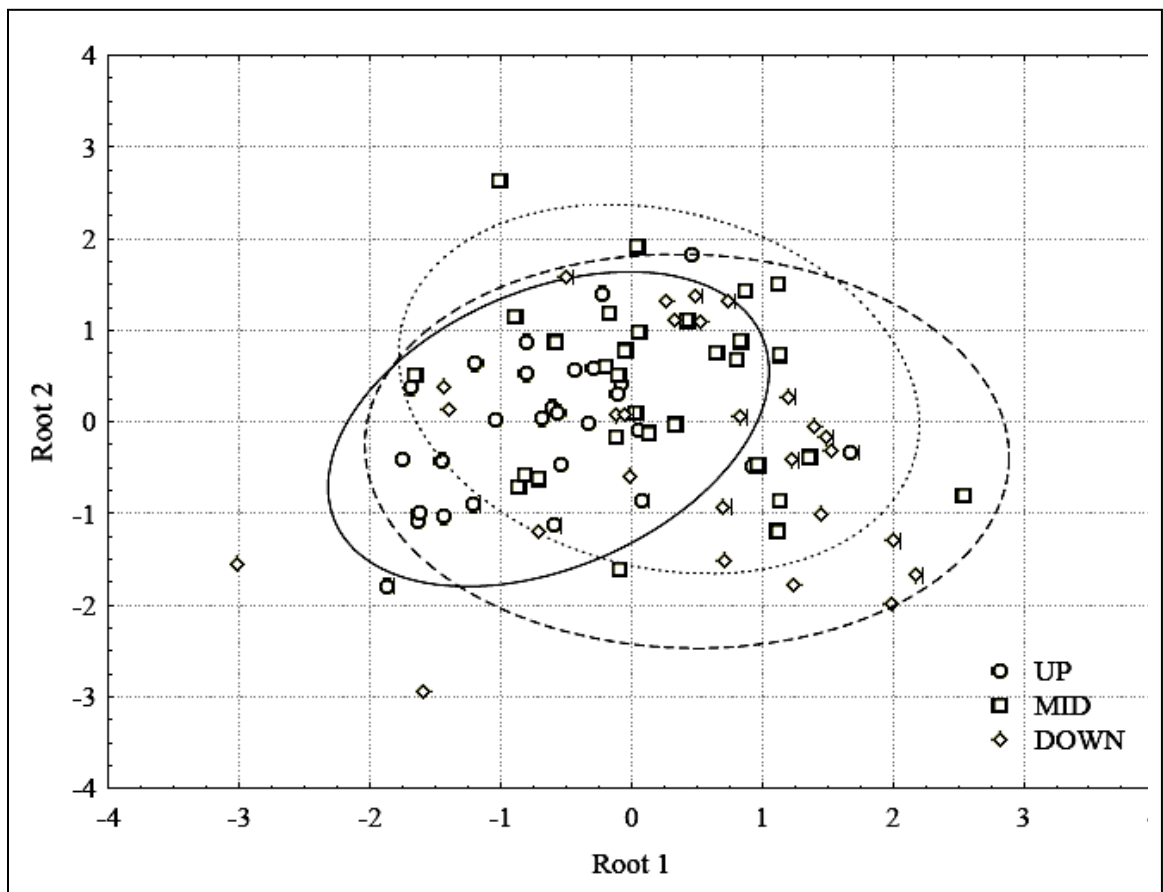


Figure 48. Linear discriminant function analysis of root 1 versus root 2 for age-0 largemouth bass otoliths (cores from 2005 year class only) collected from three regions in the Mobile Tensaw Delta. Solid, small-dashed, and large-dashed lines are 95 % confidence ellipses upstream (UP), middle (MID), and downstream (DOWN) collection regions, respectively.

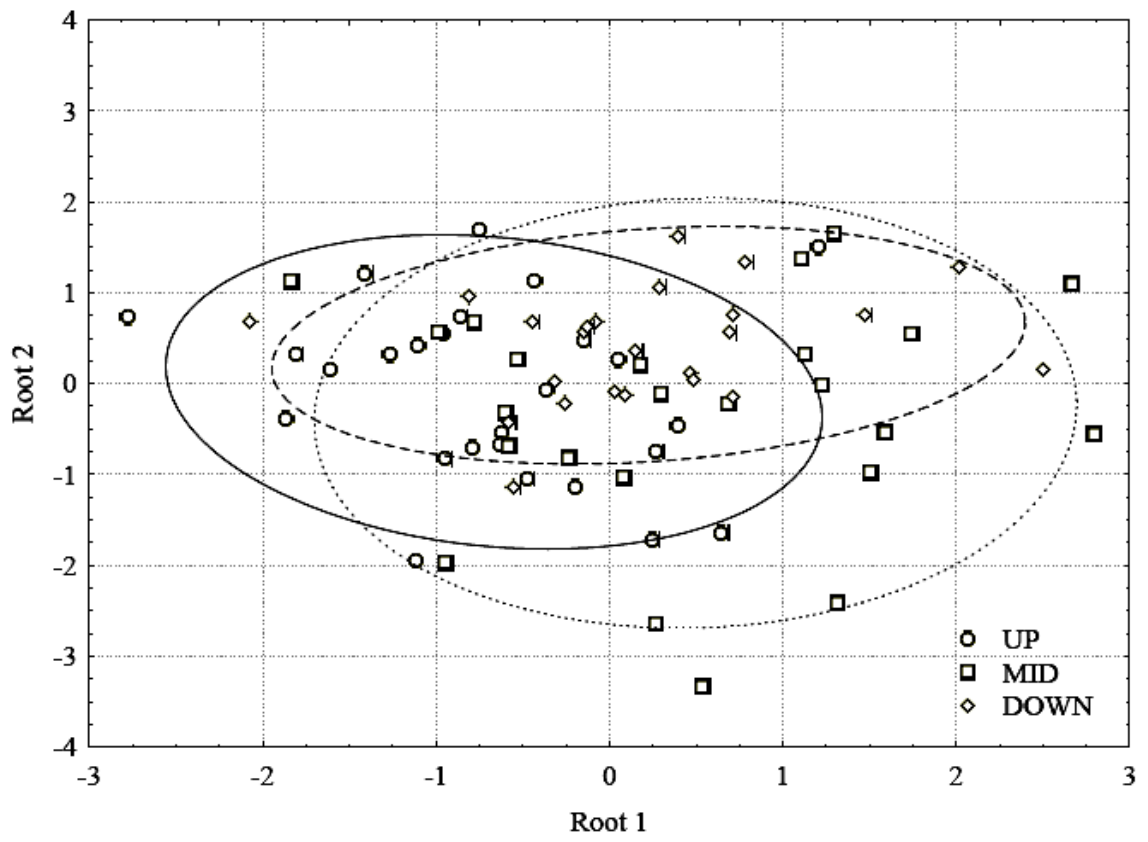


Figure 49. Linear discriminant function analysis of root 1 versus root 2 for age-0 largemouth bass otoliths (edges from 2004 year class only) collected in the spring from three regions in the Mobile Tensaw Delta. Solid, small-dashed, and large-dashed lines are 95 % confidence ellipses upstream (UP), middle (MID), and downstream (DOWN) collection regions, respectively.

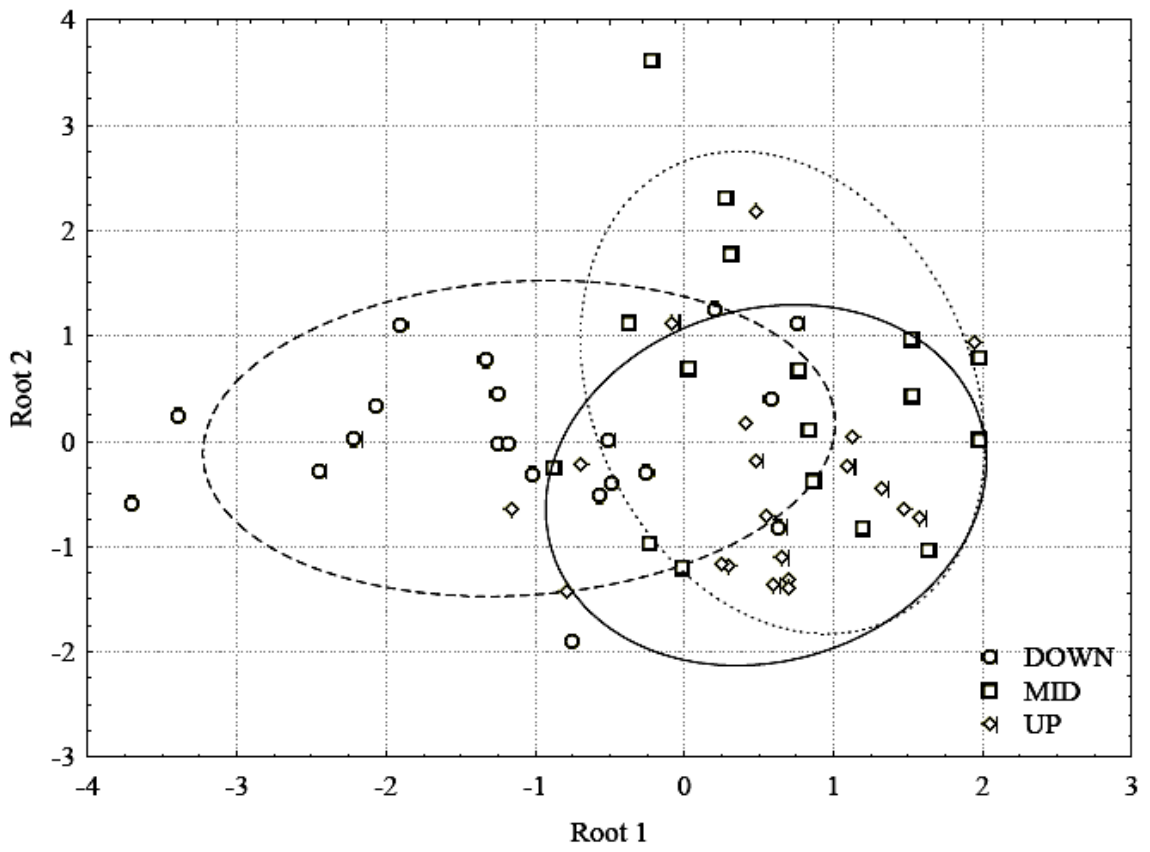


Figure 50. Linear discriminant function analysis of root 1 versus root 2 for age-0 largemouth bass otoliths (edges from 2005 year class only) collected in the spring from the Mobile Tensaw Delta. Solid, small-dashed, and large-dashed lines are 95 % confidence ellipses upstream (UP), middle (MID), and downstream (DOWN) collection regions, respectively.



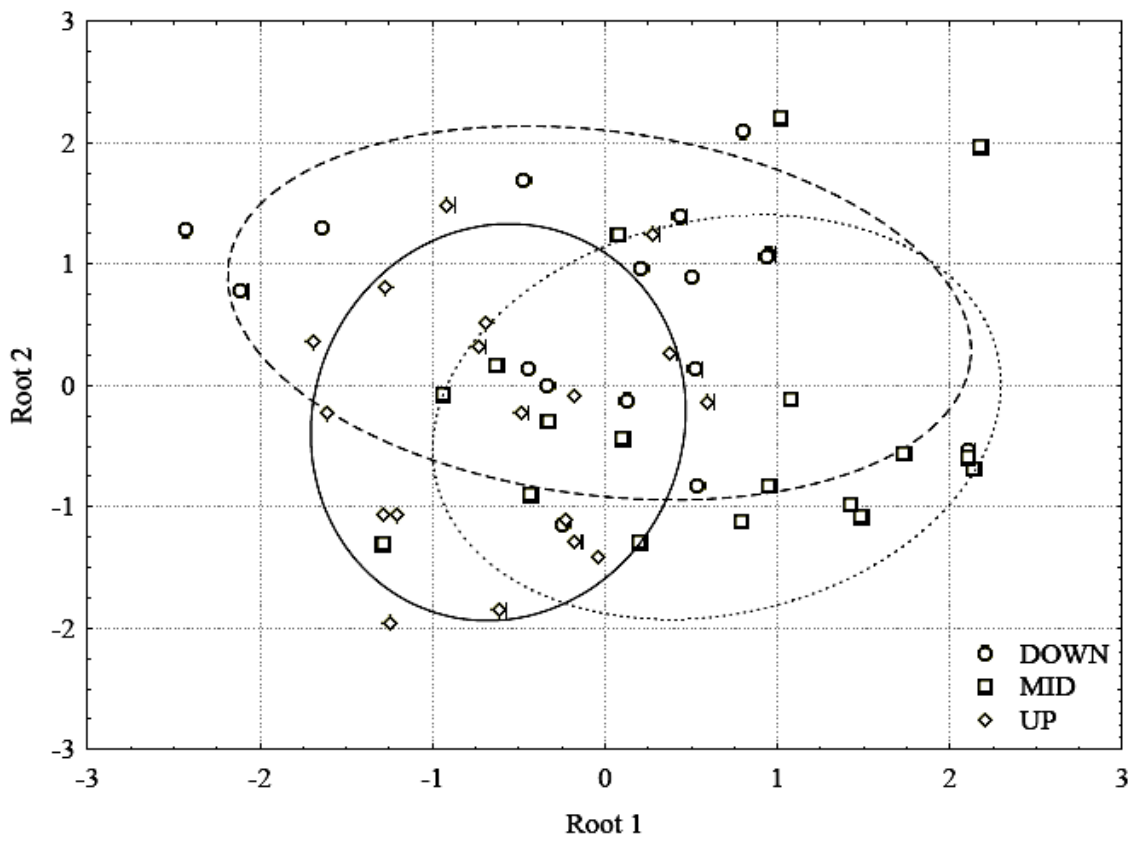


Figure 51. Linear discriminant function analysis of root 1 versus root 2 for age-0 largemouth bass otoliths (edges from 2004 year class only) collected in the fall from the Mobile Tensaw Delta. Solid, small-dashed, and large-dashed lines are 95 % confidence ellipses upstream (UP), middle (MID), and downstream (DOWN) collection regions, respectively.

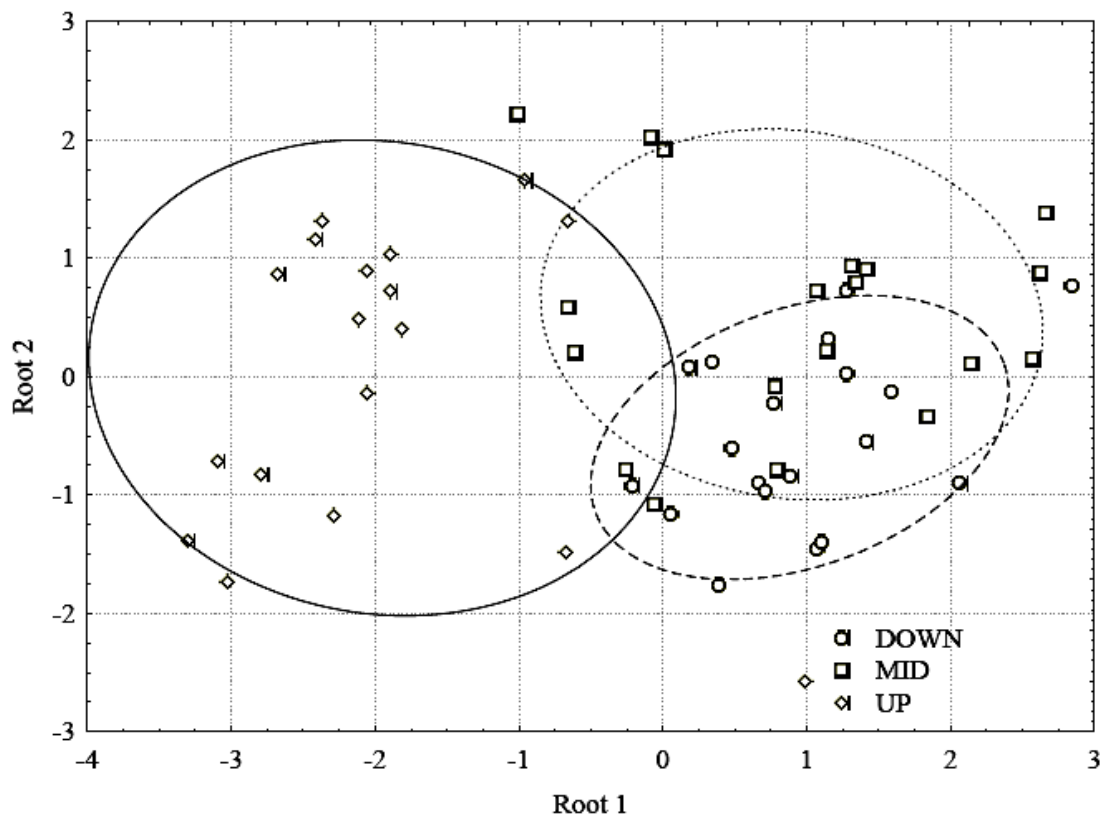


Figure 52. Linear discriminant function analysis of root 1 versus root 2 for age-0 largemouth bass otoliths (edges from 2005 year class only) collected in the fall from the Mobile Tensaw Delta. Solid, small-dashed, and large-dashed lines are 95 % confidence ellipses upstream (UP), middle (MID), and downstream (DOWN) collection regions, respectively.

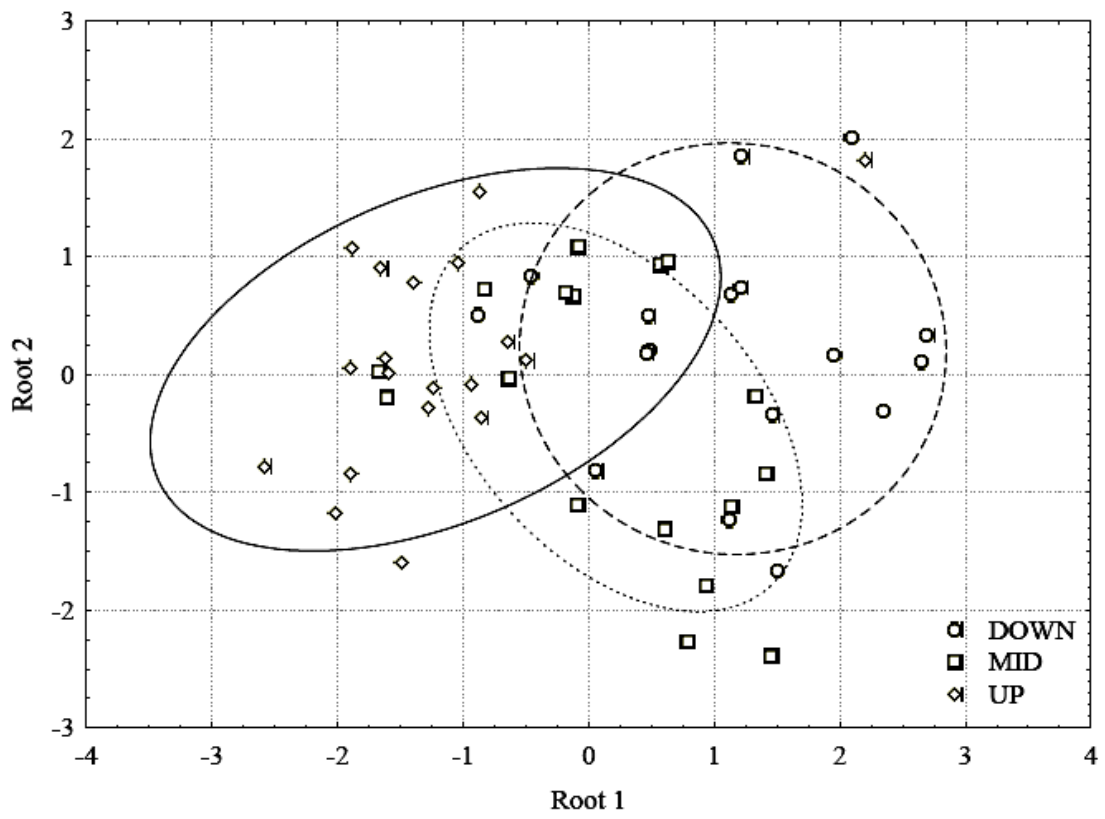


Figure 53. Mean ( $\pm$ SE) dissolved oxygen (A), water temperature (B), and salinity (C) among treatment levels and time periods for the experiment. Means with the same letter or a single bar did not differ significantly among treatment levels or periods (Tukey's HSD;  $P \leq 0.01$ ).

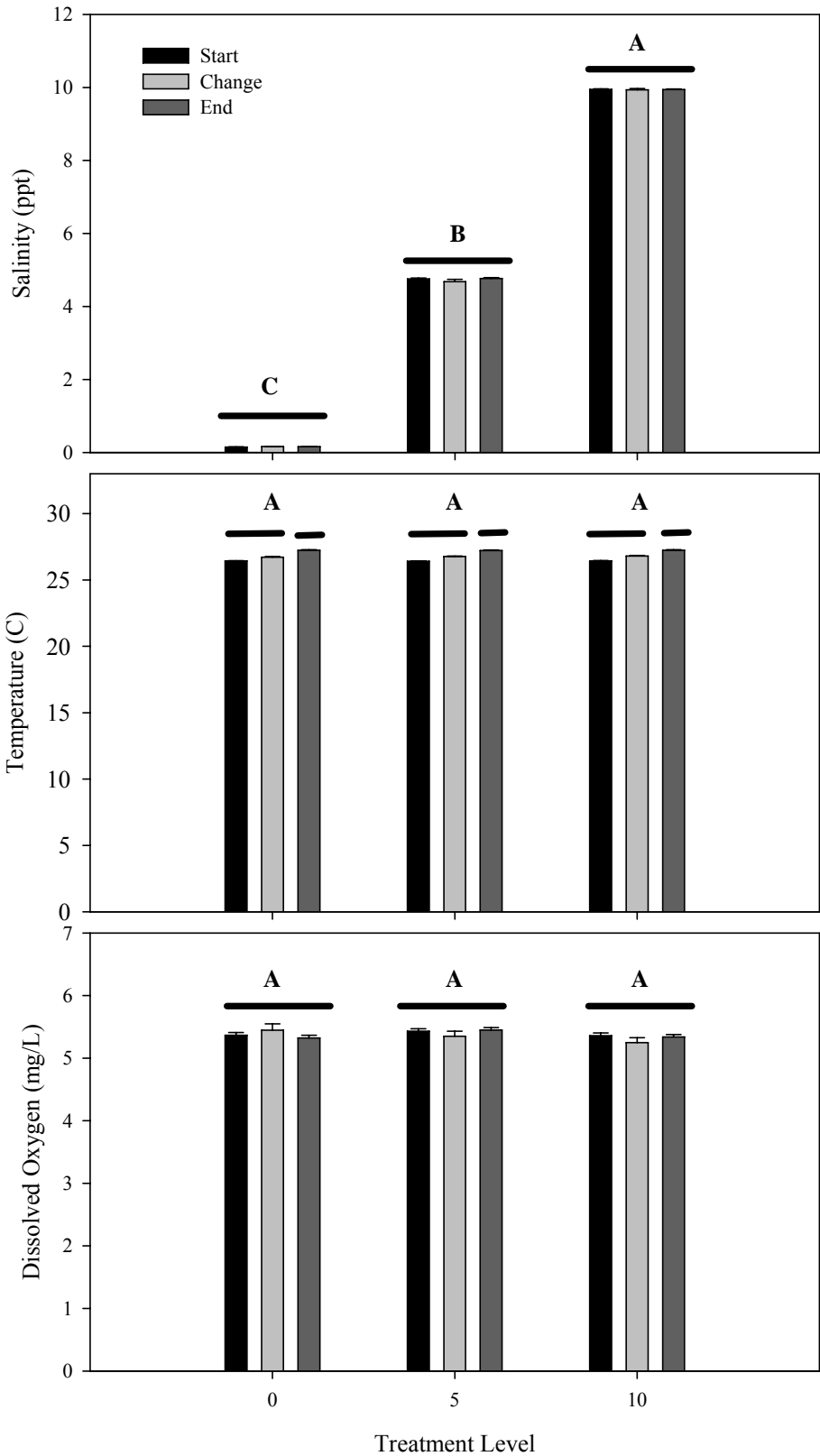


Figure 54. Mean Sr:Ca<sub>water</sub> ( $\pm$ SE) among treatment levels and time periods for the experiment. Means with the same letter or a single bar did not differ significantly among treatment levels or periods (Tukey's HSD;  $P \leq 0.01$ ).



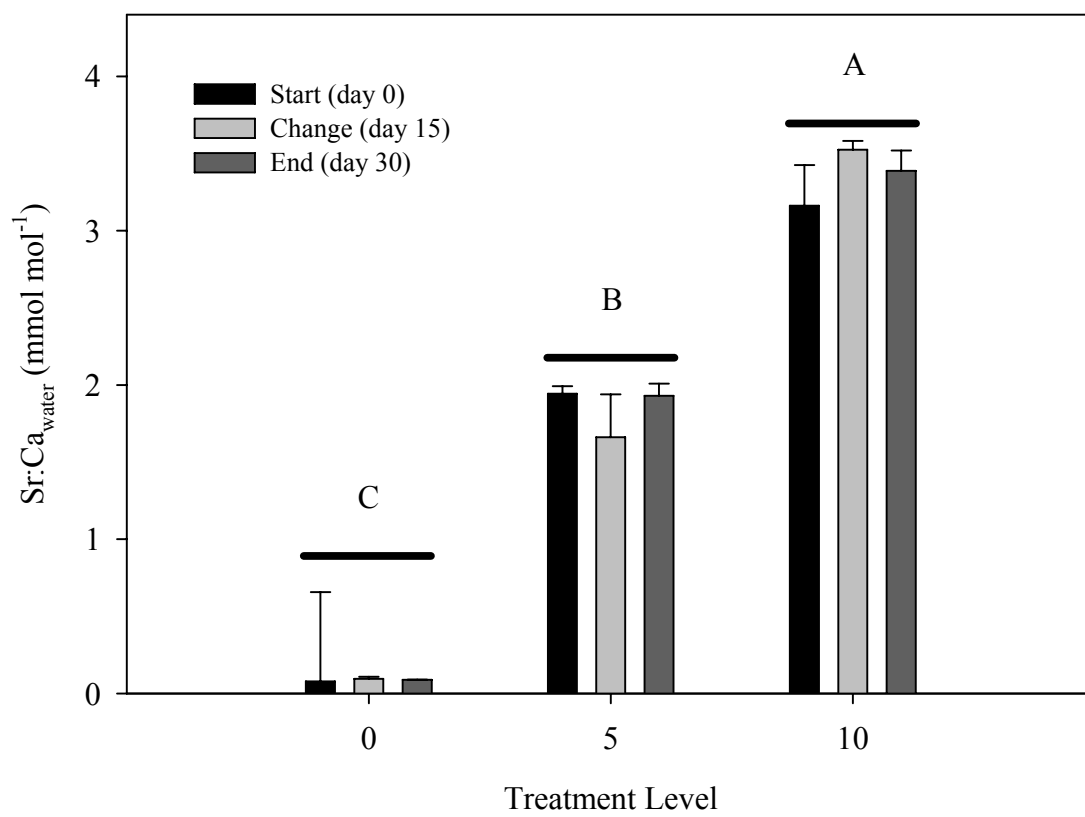


Figure 55. Smoothed Sr:Ca profiles of individual largemouth bass from the “0 - 0” (A), “0 - 5” (B), “0 - 10” (C), “5 - 5” (D), and “10 - 10” treatments. Locally weighted scatterplot smoothing (LOWESS) was conducted at a 0.25 span. Each data point in the Proportional Distance (X-axis) is a proportion of the overall laser burn length for each individual fish. The vertical dashed line at 50% represents day 15 when the changeover occurred and points 0 and 100 represent the start and end of the experiment, respectively.

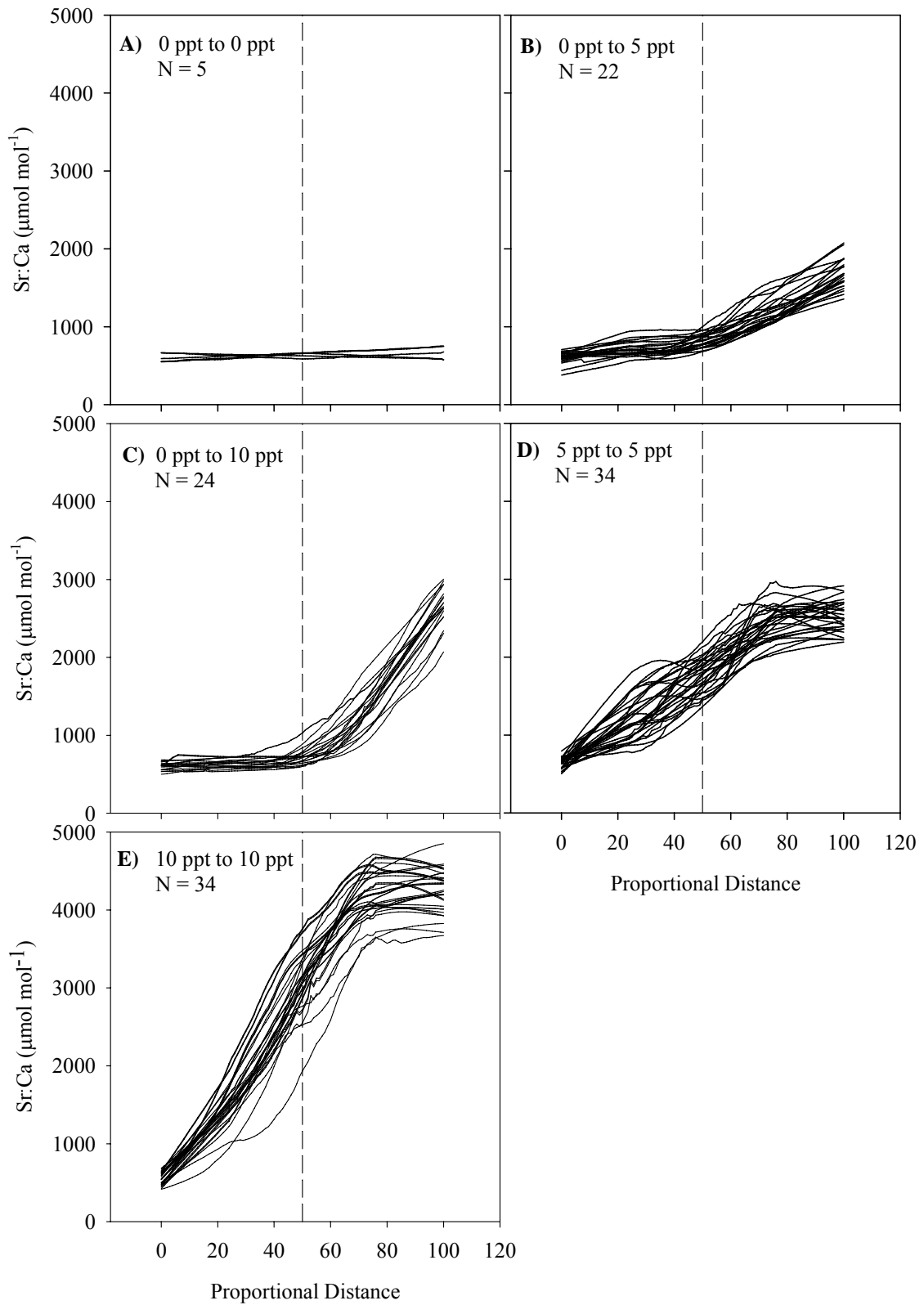


Figure 56. Mean ( $\pm$ SE) slope (A) and saturation levels (B) for the 5 treatment groups from the experiment. Means with the same letter are not significantly different (Tukey's HSD;  $P < 0.01$ ).

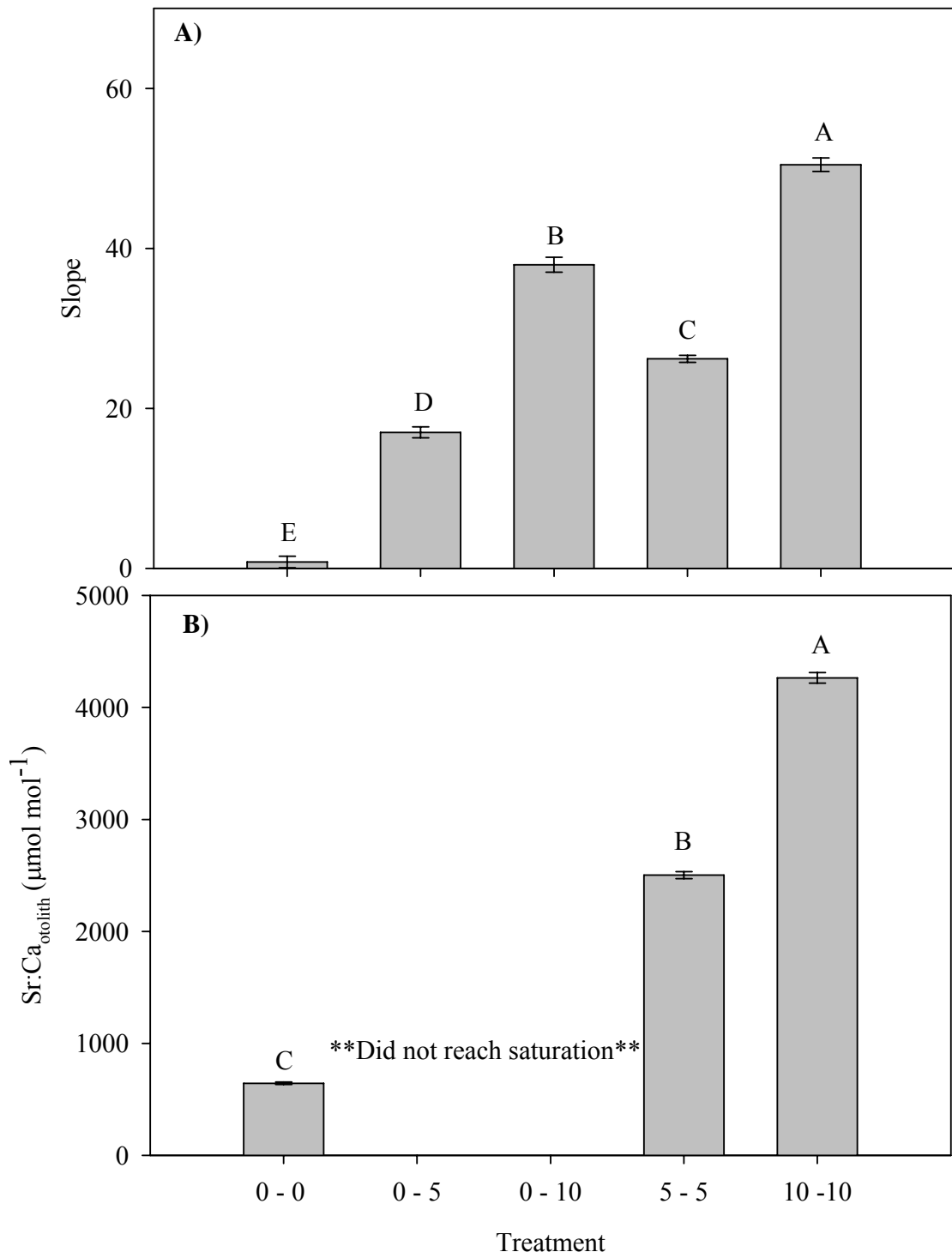


Figure 57. Profile analysis using mean ( $\pm$  95 % C.I.) smoothed profiles for the “0-0” (closed diamonds), “0 - 5” (open triangles), “0 - 10” (open circles), “5 - 5” (closed triangles), and “10 - 10” (closed circles) groups. Dashed lines represent the interval along the proportional distance axis that the *post hoc* comparisons (shown at the top of the graph) are relevant. Groups with different letters are significantly different (SNK;  $P \leq 0.0005$ ).

