MERCURY BIOACCUMULATION PATTERNS IN TWO

ESTUARINE SPORTFISH POPULATIONS

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# MERCURY BIOACCUMULATION PATTERNS IN TWO ESTUARINE SPORTFISH POPULATIONS

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MERCURY BIOACCUMULATION PATTERNS IN TWO

ESTUARINE SPORTFISH POPULATIONS

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# VITA

Troy Mason Farmer, son of Bill and Mickey Farmer, was born on April 10, 1982 in Birmingham, Alabama. He graduated from Pelham High School in Pelham, Alabama in 2000. He received his Bachelor of Science in Fisheries Management from Auburn University in May, 2004 and began working as a Research Assistant in the Department of Fisheries and Allied Aquacultures in June 2004. As a Research Assistant, he worked in Dr. Carol Johnston's Ichthyology laboratory and then for Dr. Dennis DeVries and Dr. Rusty Wright in the Ireland Center's Aquatic Ecology Group. In June 2005, he married Karen Berry, daughter of Mark and Angie Berry and Elise and Larry Musick. In January 2006, he entered the Graduate School at Auburn University in the Department of Fisheries and Allied Aquacultures. He will work towards his PhD in Evolution, Ecology, and Organismal Biology at The Ohio State University.

THESIS ABSTRACT

MERCURY BIOACCUMULATION PATTERNS IN TWO ESTUARINE SPORTFISH POPULATIONS

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Mercury (Hg) is a harmful bioaccumulative heavy metal to which humans are exposed primarily through fish consumption. Several consumption advisories for mercury have been issued for fishes along the US Atlantic and Gulf coasts, including the Mobile-Tensaw River Delta in coastal Alabama. Mercury cycling in estuaries is complex and little is known about the extent of mercury bioaccumulation in the ecologically diverse fishes in coastal areas such as the Mobile-Tensaw Delta, Alabama. Using traditional tissue analysis techniques combined with otolith microchemistry, diet analysis, age and growth analysis, and bioenergetics modeling, I investigated seasonal and spatial trends of mercury accumulation in largemouth bass, *Micropterus salmoides*, and southern flounder, *Paralichthys lethostigma*, inhabiting the Mobile-Tensaw Delta.

Age-normalized largemouth bass mercury tissue concentrations increased significantly from downstream to upstream locations with little seasonal variation. Largemouth bass collected at both upstream and downstream locations exceeded common minimum consumption and no consumption advisory levels. Microchemistry of largemouth bass otoliths indicated that individuals did not migrate between upstream and downstream regions in the Mobile-Tensaw Delta. Diet analysis showed that largemouth bass at downstream locations foraged on lower trophic levels than those upstream and bioenergetics analysis confirmed that this difference was the primary factor responsible for lower largemouth bass Hg accumulation downstream.

Southern flounder mercury tissue concentrations were uniform across the sample area and were lower than those of largemouth bass. No southern flounder had Hg tissue concentrations that exceeded common minimum consumption advisory levels. Microchemistry analysis of southern flounder otoliths indicated a highly variable migratory life history across salinity gradients. This migratory life history was likely responsible for similar Hg tissue concentrations across the Mobile Delta for southern flounder. Differences in Hg bioaccumulation between largemouth bass and southern flounder were not the result of trophic differences between species but rather the result of faster growth rates in southern flounder. For both largemouth bass and southern flounder a negative relationship existed between salinity exposure and Hg tissue concentration, although there was considerable variability in this relationship. Initial efforts to detect mercury directly in the otoliths of both species as a way to measure lifetime Hg accumulation trends were not successful.

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# I. INTRODUCTION: FACTORS AFFECTING MERCURY BIOACCUMULATION IN ESTUARINE FISH POPULATIONS

Mercury (Hg) is a bioaccumulative heavy metal that is a neurotoxin and has the potential to bioaccumulate in the upper trophic levels of aquatic food webs (USEPA 1997). Humans are primarily exposed to Hg through fish consumption (USDHHS 1999). Within the U.S., the majority of fish that is consumed is of marine origins, however freshwater and estuarine species also account for a large portion of total fish consumption (USEPA 2002). Estuaries, in particular, serve as critical habitat for marine, estuarine and freshwater species (Boesch and Turner 1984; Ruiz et al. 1993). Therefore, understanding how estuarine environments affect Hg bioaccumulation in fish populations has important human health implications.

Within estuaries, many abiotic and biological factors that affect Hg accumulation in fish can vary spatially and temporally, sometimes dramatically (Mason et al. 1999, 2006; Lambertsson and Nilsson 2006; Canário et al. 2007). While there has been considerable study of the abiotic processes controlling Hg bioaccumulation in estuarine environments (Mason et al. 1999; Benoit et al. 1999, 2001; Lambertsson and Nilsson 2006; Skyllberg et al. 2007; Delaune et al. 2008) much less attention has focused on how variation in biological variables can affect Hg accumulation in estuarine fishes. In non-contaminant related studies, fish diets (Peer et al. 2006; Norris 2007; Hajisamae and

Ibrahim 2008; Reum and Essington 2008) and growth rates (Peer et al. 2006; Norris2007) have been shown to vary considerably across estuarine salinity gradients. Biological variables such as diet and growth have a strong effect on Hg bioaccumulation in fish (McCrimmon et al. 1983, Rodgers et al. 1996, Hall et al. 1997, Stafford et al. 2004). Therefore, in order to fully understand the dynamics of Hg bioaccumulation in estuarine fish populations these biological variables should be quantified along with Hg concentrations in target species (Simoneau et al. 2005).

However, quantifying these dynamic variables is only a first step in understanding their effect on Hg bioaccumulation in fish. In order to conclusively link abiotic and biological processes to contaminant accumulation, knowledge of the past environments experienced by a fish is also needed (Leah et al. 1991; Peterson et al. 1996; Paller et al. 2005; Fowlie et al. 2008). Once this is known, a correlation between past environment and Hg contamination can be drawn (Geffen et al. 2003; Mason et al. 2006; Lochet et al. 2008). Additionally, if abiotic and biological variables are quantified within certain environments, then a more complete understanding of Hg bioaccumulation can be attained by relating those variables to observed Hg concentrations.

An increasingly common tool used to determine previous environments experienced by an individual fish is otolith microchemistry. Recent advances in the field of otolith microchemistry have led to widespread use of elemental signatures in fish otoliths as markers to determine previous environments experienced by an individual (Elsdon et al. 2008). Elemental signatures in otoliths can be used as natural tags and have many advantages over applied tags (e.g., pit tags, archival tags, artificial otolith marks). Natural tags are applied early in life, are permanent, and can be related to age (Elsdon et

al. 2008). Specifically, certain elements (i.e., strontium, barium, and other elements) in fish otoliths have proven effective indicators of salinity exposure across a variety of systems and species (Secor et al. 1995; Thorrold et al. 1997; Whitledge et al. 2007; Lowe 2007; Milton et al. 2008).

The Mobile-Tensaw River Delta, Alabama offers an ideal environment in which to quantify and assess the effect of these abiotic and biological variables on Hg accumulation. Situated along the northern Gulf of Mexico, this system experiences high levels of atmospheric Hg deposition (NADP 2006) and as a result several advisories are issued for Hg in piscivorous fish species within its waters (ADPH 2007). Within this system, diet and growth rates of piscivorous fish species vary across a seasonal salinity gradient (Peer et al. 2006; Norris 2007). Also, a recent study (Lowe 2007) demonstrated the value of an otolith microchemistry approach to indicate salinity exposure in age-0 individuals of two fish species inhabiting this system.

Largemouth bass *Micropterus salmoides* and southern flounder *Paralichthys lethostigma* are recreationally and commercially important species that occur in coastal rivers and estuaries throughout the U.S Atlantic and Gulf coasts (Swingle and Bland 1974; Tucker 1985; Odum 1988; McEachran and Fechhelm 2005). Here, I study Hg accumulation in these two species across a seasonal salinity gradient in the Mobile-Tensaw River Delta, Alabama. In chapter II, I examine spatial and seasonal trends in Hg tissue concentrations in these two species. I also study the effect of abiotic and biological variables on Hg accumulation across a seasonal salinity gradient. In chapter III, I use otolith microchemistry to examine lifetime salinity exposure in individuals of both species. This approach allowed me to quantify individual salinity exposure and compare

Hg accumulation between individuals that inhabited freshwater environments and those that inhabited estuarine environments. Through this combination of approaches, I quantify how abiotic and biological variables influence mercury accumulation in fishes across an estuarine salinity gradient.

# II. MERCURY BIOACCUMULATION PATTERNS IN TWO ESTUARINE FISH POPULATIONS

## INTRODUCTION

Mercury (Hg) is a naturally occurring heavy metal whose various chemical forms have been linked to neurological, developmental, and reproductive disorders in humans (USDHHS 1999). Mercury exposure is particularly harmful to the developing human brain, and therefore children and women of reproductive age are strongly advised to avoid exposure (USDHHS 1999). Consumption of contaminated fish is the primary pathway through which humans are exposed to Hg (USEPA 1997).

In the U.S., as of 2006, 48 out of 50 states had consumption advisories due to Hg concentrations in fish (USEPA 2007). These advisories covered 38% of the total lake surface area and 26% of the total river length in the U.S (USEPA 2007). Also, virtually all of the U.S. Atlantic (92%) and Gulf of Mexico (100%) coastlines had advisories for selected marine fish species due to mercury contamination (USEPA 2007). In addition to having adverse effects on human health, Hg levels in fish have decreased the economic, recreational, nutritional, and cultural benefits provided by fisheries (USEPA 1997).

Mercury contamination of fish is a natural occurrence that has been exacerbated by human activities (Stafford et al. 2004). Atmospheric deposition (from a combination of local, regional, and global sources) is the primary source of Hg for most aquatic

ecosystems with two thirds of the atmospheric Hg released from anthropogenic sources (Mason et al. 1994). The majority of atmospheric Hg is inorganic but may be transformed by natural processes into methylmercury (MeHg) when it is bound with a methyl ($CH_3$) group (USEPA 1997). Anerobic sulfate-reducing bacteria have been found to be the primary source of Hg methylation in most aquatic ecosystems (Gilmour et al. 1992; Zillioux et al. 1993). A suite of abiotic and biotic factors affect methylation by sulfate-reducing bacteria. Specifically, low pH (Watras et al. 1998; Scheuhammer and Graham 1999; Kelly et al. 2003) and high levels of organic matter and sulfates (Lambertsson and Nilsson 2006) have been found to enhance methylation.

In freshwater systems, Hg methylation is generally limited by sulfate availability (Branfireun et al. 1999). In contrast, sulfate in marine systems is essentially unlimited. Consequently, this results in high sediment sulfide concentrations in marine systems which tend to form mercury sulfide (HgS) complexes and inhibit the formation of methylmercury (Benoit et al. 1999, 2001). Recent studies have indicated that coastal areas comprising estuaries and brackish waters may constitute "hot spots" for Hg methylation due to the abundant sulfates combined with ideal conditions for methylation (Lambertsson and Nilsson 2006). Because coastal areas generally support substantial recreational and commercial fisheries, it is important that processes controlling MeHg bioaccumulation and biomagnification in coastal fish communities are understood (Rolfhus et al. 2003).

Rodgers (1996) cites four processes as key to controlling Hg accumulation in fish: Hg supply, Hg methylation, trophic interactions, and bioaccumulation. As previously discussed, there has been considerable attention paid in the scientific literature to abiotic

(i.e., pH, dissolved oxygen, dissolved organic carbon, etc.) and microbial (e.g., sulfate reducing bacteria) factors controlling supply and methylation of Hg. However, there has been less investigation into ecological factors that influence trophic transfer and bioaccumulation of Hg (Simoneau et al. 2005), particularly in estuarine environments.

Generally, total Hg, MeHg, and the percent Hg occurring as MeHg increase with increasing trophic levels (Cabana and Rasmussen 1994; Mason et al. 1999). This trophic transfer of MeHg has been well documented in a variety of freshwater and marine systems (Mason and Sullivan 1997; Atwell et al. 1998; Mason et al. 2000; Stafford et al. 2004; Bank et al. 2007). Estuarine environments, with high diversities of marine, freshwater and estuarine invertebrate and vertebrate species (Peterson and Ross 1991), have complex food webs (e.g., Norris 2007). Within these complex food webs, transfer of Hg to recreational and commercial fish species likely has a great deal of variability due to foraging behavior. Therefore, understanding trophic dynamics within estuarine systems is key to identifying pathways through which Hg bioaccumulates in fish species of interest.

In addition to directly influencing fish Hg levels, fish diet may also regulate Hg levels indirectly through growth rate. Bioenergetic simulations and field studies have shown that Hg concentration is inversely related to growth (Thomann 1989; Rodgers 1996; Stafford and Haines 2004; Simoneau et al. 2005). Understanding variations in growth within and among species in estuarine environments will therefore be important in determining species and areas at risk for elevated Hg accumulation.

As previously noted, abiotic and biological processes controlling supply, methylation, trophic transfer of MeHg and bioaccumulation are complex. In an estuarine

setting, spatial and temporal variation in abiotic and biotic factors affecting methylation, trophic transfer, and fish bioenergetics increase the numbers of complex factors that affect these processes. Consequently, the effect of these complex factors on MeHg concentrations of piscivorous fish in estuarine environments is largely unknown and difficult to predict.

Largemouth bass *Micropterus salmoides* and southern flounder *Paralichthys lethostigma* are commercially and recreationally important species that occur in coastal rivers and estuaries throughout the U.S Atlantic and Gulf Coasts (Swingle and Bland 1974; Tucker 1985; Odum 1988; McEachran and Fechhelm 2005). In this study, I focus on largemouth bass and southern flounder distributed across a seasonal salinity gradient in a tidal-river estuary along the northern Gulf of Mexico. The goals of the study were to:

1) Quantify temporal and spatial trends in tissue Hg concentration of both species.
2) Correlate diet and growth rate with temporal and spatial trends in tissue Hg concentration.
3) Use a bioenergetics approach to quantify the effect of diet and growth rate on Hg accumulation for each species.
4) Determine the probability that a fish of a given size will exceed mercury advisory limits.

# METHODS

## Site Information

The Mobile-Tensaw River Delta (hereafter referred to as the Mobile Delta) forms at the confluence of the Alabama and Tombigbee rivers and ends at the mouth of Mobile Bay. It is roughly 55 km in length and 10-15 km wide (Swingle et al. 1966). In terms of discharge (~2,000 $m^3$/second), it ranks as the fourth largest from U.S. watersheds (USGS 2002). While most delta systems are wide areas at the mouths of rivers where flows slow and sediments are deposited, the Mobile Delta is characterized by its narrow width and high ratio of freshwater inflow to estuary area (Wallace 1996). During periods of low flow, the Delta can experience salt water intrusion extending north to the confluence of the Alabama and Tombigbee rivers (Swingle 1971). Annual variation in flows through the Delta dictates the timing and the magnitude of the salinity intrusion.

The Mobile Delta is part of the larger Mobile-Alabama River Basin (MARB), which drains a diverse physiographic region and experiences mercury contamination primarily from non-point source atmospheric deposition (Bonzongo and Lyons 2004). Past studies in the MARB indicate coastal plain regions in the watershed have higher aqueous levels of MeHg than upland regions (Bonzongo and Lyons 2004; Warner et al. 2005). These findings are supported by a growing body of literature that shows enhanced methylation of Hg in coastal regions (Rolfhus et al. 2003; Lambertsson and Nilsson 2006). In addition to potential for enhanced methylation in this region, it is likely that

high levels of atmospheric deposition may be acting as a source of Hg to the region. In 2004, the National Deposition Monitoring Program's site in Spanish Fort, Alabama recorded the highest total wet Hg deposition (22.8 $\mu g/m^2$) in the U.S. (NADP 2005). In addition to atmospheric deposition, the Mobile Delta region has two historic point source releases of Hg (Olin Chemical Corp. in McIntosh, Alabama and Stauffer Chemical Company near Bucks, Alabama, NOAA 1990).

These high levels of Hg input coupled with factors that enhance Hg methylation translate into high concentrations of MeHg in fishes. Evidence of this can be found in Alabama Department of Public Health's consumption advisories. In 2007, thirty advisories were issued in Alabama due to mercury concentrations in freshwater fish. Twenty-six of these advisories were issued for waters in the coastal plain region with five of these issued for waters within the Mobile Delta (ADPH 2007).

**Species of Interest**

Largemouth bass is a popular sportfish species across the U.S. (Philipp and Ridgway 2002) and particularly in the Mobile Delta (Armstrong et al. 2000). They can tolerate low salinity and populations are present in estuaries along the U.S. Atlantic and Gulf coasts (Swingle and Bland 1974; Tucker 1985; Odum 1988). Typically an apex predator, largemouth bass has the potential, due to its trophic position, to bioaccumulate high levels of Hg (Lange et al. 1993, 1994). Measured Hg tissue levels in largemouth bass can range from very low (<0.1 ppm) to extremely high (>10 ppm) (Rumbold and Fink 2006). Of the twenty-six mercury advisories in the Alabama coastal plain, twenty-four were issued for, or included, largemouth bass (ADPH 2005).

Southern flounder is also an important sportfish species along the U.S. Gulf Coast and also of commercial importance as incidental catch in shrimp trawls (Regan and Wingo 1985). Adults can generally be found in bays and estuaries along the Gulf of Mexico from June through November and move offshore to spawn between October and December (Stokes 1977). Southern flounder has a unique life history which gives it direct contact with bottom sediments that may contain Hg levels elevated above those in surface waters (Geffen et al. 2003). However, there are currently no advisories issued for southern flounder in Alabama (ADPH 2007).

**Collection of Biological Samples in the Field**

Largemouth bass and southern flounder were collected monthly from January 2005 – November 2006 with pulsed DC electrofishing (7.5 GPP, 7500 watt, Smith-Root, Vancouver, Washington). Largemouth bass were collected at four sites in 2005 (McReynold's Lake [ML], Gravine Island [GI], Bay Minette [BM], and D'Olive Bay [DB]). In 2006, largemouth bass were collected from the same four sites along with two additional sites (Tensaw Lake [TN] and Big Bayou Canot [BB]) (Figure 1). Sites were classified as upstream or downstream based on past measurements of salinity (Peer 2004, Norris 2007, Lowe 2007). In spring (March-May) and fall (August-October) of both years, ten largemouth bass from across a size range (150-500 mm) at each site were returned to the lab for muscle tissue Hg analysis. Additional largemouth bass were collected in July and October 2006 from an upstream (TN) and downstream (DB) site for muscle tissue analysis to determine the fraction of Hg occurring as MeHg. For age and growth analysis, all largemouth bass collected in September and October of each year

were returned to the lab. Largemouth bass not retained for Hg analysis or age and growth collections were weighed, measured, and their diets removed using an acrylic tube (Van Den Avyle and Roussel 1980) before being released. Diets collected in the field were held on ice and returned to the lab for analysis.

Southern flounder were collected at four sites (Dennis Lake [DL], GI, BM, and DB) in 2005 and 2006, three of which were also sites for largemouth bass collections (GI, BM, and DB) (Figure 1). In addition to monthly electrofishing, trawling was conducted for southern flounder sites in the fall of 2005 using a 4.9 m head rope otter trawl (6.4-mm bar mesh wings and body and 3.2-mm bar mesh cod end). Fish from across a size range (100-450 mm) were collected, and all southern flounder collected during these months were held on ice and returned to the lab. Muscle tissue was analyzed for total Hg for southern flounder collected during fall of 2005 (August-October) and 2006 (September-October). Additionally southern founder collected in September and October of each year were used for age and growth analysis. From December 2005 - June 2006 southern flounder were collected from three sites (DB, GI, DL) for determination of the fraction of Hg occurring as MeHg.

During July 2007, small bluegill *Lepomis macrochirus* (40 – 120 mm total length) were collected by electrofishing from a downstream (BM) and upstream (TN) site for whole body Hg and MeHg analysis. Juvenile blue crab *Callinectes sapidus* (30 – 70 mm carapace width) were collected with baited crab traps (20 mm mesh; Custer and Mitchell 1992) and modified minnow traps (6 mm mesh) at a downstream (DB) and upstream (DL) site monthly, from May – August 2007, for whole body Hg and MeHg analysis. All largemouth bass, southern flounder, bluegill, and blue crab collected for Hg or MeHg

determination were rinsed with ambient water, placed in polyethylene bags and stored on ice (following USEPA 2000) for transport to the lab and processing.

**Collection of Abiotic Samples in Field**

Dissolved oxygen (mg/L; YSI 550A meter) was measured monthly at fixed sites at 1 m intervals from the surface to the bottom. Also, pH was measured at the surface (Oakton pHTestr 30) and 1 m above the bottom at each site from May 2007 to March 2008 as a measure of methylation potential (Bonzongo and Lyons 2004). Temperature loggers (Onset Computer Corp.) were placed 1 m below average low tide mark at each site and set to record at 2-hour intervals. Data loggers (Solonist Levelogger 3001 ®) placed at 1 m below average low tide mark recorded salinity (ppt) readings every 30 minutes at two sites (Figure 1).

**Processing of Samples in the Laboratory**

All largemouth bass and southern flounder returned to the lab were weighed (nearest g) and measured (nearest mm total length). All procedures for the removal of tissue followed USEPA (2000). Five to ten g of lateral anterior dorsal muscle tissue were removed from largemouth bass and southern flounder using a stainless steel scalpel and weighed (nearest 0.01 g). After each sample was collected, the stainless steel scalpel was washed thoroughly with a solution of soap and distilled ionized ultra filtered water (DIUF), followed by a rinse with a weak Nitric acid (2%) DIUF solution, and then rinsed three times with DIUF water. Tissue samples from each individual fish were placed in a Whirl-Pak® bag and frozen until analysis. Otoliths were removed from each fish using

Teflon coated forceps and placed in 30% $H_2O_2$ for 30 seconds to clean all tissue and organic matter from the outside of the otolith. After a rinse in DIUF, otoliths were dried and stored in polyethylene vials. Stomachs of each individual were removed and stored in 50% ethyl alcohol until analyzed. Beginning in October 2005, the sex of each largemouth bass was determined by visual inspection of the gonads.

Bluegill samples were returned to the lab, weighed, measured, and sorted into 10-mm size groups. Bluegill were frozen on dry ice and individuals were homogenized using a mortar and pestle, which was wrapped in polypropylene plastic that was changed after each fish. Equal weights from each individual were then combined into a single composite sample for each length group. This composite sample was then re-homogenized (USEPA 2000). Composite samples of four individuals were processed from the upstream site (TN) for 40, 60, 80, 100, and 120 mm length groups. This size range generally covered the average range of sunfish prey consumed by adult largemouth bass and southern flounder in the Mobile Delta (personal observation). Due to low catch rates of bluegill at downstream sites only two composite samples were processed from this region. Composite samples of four individuals were processed for the 90 mm length group and 2 individuals were processed for the 100 mm length group from the downstream site (BM).

Blue crab samples were returned to the lab, weighed, measured, and sorted into 10-mm size groups based on carapace width. Blue crab samples were processed in the same manner as bluegill samples (USEPA 2000). Composite samples of four crabs from the downstream site (DB) were processed for 30, 40, 50, 60 and 70 mm carapace width groups. While composite samples of four crabs from the upstream site (DL) were

processed for 30, 40, 50, and 70 mm carapace width groups, only 3 crabs were processed for the 60 mm carapace width group. Generally, largemouth bass and southern flounder consume blue crab < 70 mm carapace in width, and while they do consume blue crab smaller than 30 mm, blue crab of these sizes were not collected by my sampling gear.

An equipment blank was collected with reagent grade water from the bluegill and blue crab processing to evaluate the potential for Hg contamination of these samples. All bluegill and blue crab composite samples together with the equipment blank were placed in acid washed glass jars and shipped on wet ice overnight to a chemical laboratory for processing.

**Mercury Analysis**

Frozen largemouth bass and southern flounder tissue samples were shipped overnight on dry ice to the Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor (Ontario, Canada). At GLIER, the samples were digested in a sulfuric and nitric acid solution and processed for total Hg by atomic absorption spectrometry vapor generation following CAEAL (Canadian Association of Environmental Analytical Laboratories) certified procedures (Haffner and Barette 2004). Quality assurance/quality control procedures involved the processing of blind replicate samples, laboratory blanks, water control samples, in house reference material, and three certified reference samples (lobster hepatopancreas [BT-Luts1], dogfish liver [BT-Dolt3], and dogfish muscle [BT-Dorm2], National Research Council of Canada, Ottawa, Ontario). All quality assurance/quality control procedures were within control limits (Table 1). The instrument detection limit (three times the standard deviation of the

calibration blank) was 0.05 μg/g and the reporting limit (two times the instrument detection limit) was 0.1 μg/g on a dry weight basis.

Additional largemouth bass and southern flounder tissue samples, along with bluegill and blue crab samples, were shipped to Frontier Geosciences (Seattle, WA) for determination of the percent of Hg occurring as MeHg. Samples were digested in a sulfuric and nitric acid solution and analyzed by cold vapor atomic fluorescence spectrometry for determination of total Hg. To measure MeHg, samples were digested in a basic methanol solution and analyzed by cold vapor gas chromatography atomic fluorescence spectrometry (EPA draft method 1630 modified) (Frontier Geosciences unpublished data). Quality assurance/quality control for Hg and MeHg analysis included processing of laboratory blanks, laboratory control samples, certified reference materials (BT-Dorm2), replicate sample analysis, and spiked replicate sample analysis. All quality assurance/quality control procedures for Frontier Geosciences were within acceptable limits (Table 2). The average reporting limit for total Hg and MeHg (mean ± SD) were 0.008 ± 0.005 μg/g and 0.013 ± 0.005 μg/g (wet weight) respectively.

The equipment blank comprising reagent water was analyzed at Frontier Geosciences for total Hg by cold vapor atomic fluorescence spectrometry and MeHg by cold vapor gas chromatography atomic fluorescence spectrometry (EPA draft method 1630 modified) (Frontier Geosciences unpublished data). The reporting limit for total Hg and MeHg in water was 0.001 μg/L and 0.0001 μg/L.

## Otolith Aging

Otoliths were aged for all largemouth bass and southern flounder processed for Hg analysis and those collected in the fall for age-and-growth analysis. Largemouth bass had a single otolith selected at random, while for southern flounder the right otolith was consistently selected due to asymmetry between right and left otoliths in *Paralichthys* species (Sipe and Chittenden 2001; Fisher and Thompson 2004). Otoliths from both species were mounted in epoxy resin. A transverse section through the core of each otolith was removed with a low-speed diamond blade Isomet® saw (South Bay Technologies, San Clemente, California). Transverse core sections were mounted on petrographic slides with thermoplastic cement and polished with 320-, 600-, and 800- grit paper until the core appeared smooth. Two independent readers aged each otolith by counting annuli. A third reader independently aged any otolith on which the first two readers disagreed. If this reader was in agreement with either one of the fist two readers, the age on which they agreed was used. If the third reader did not agree with either previous reader, all three readers discussed and came to consensus on the age, or discarded the otolith from analysis.

## Diet Analysis

Monthly diet samples from largemouth bass and southern flounder collected in 2005 and 2006 were used in diet analysis. I identified stomach contents of largemouth bass and southern flounder to species for fish and genus for macroinvertebrates and crustaceans. Appropriate measurements were taken from each identified prey item to allow for back-calculation of the original biomass (wet weight) of each prey item from

measurement of hard parts or other indices (Bowen 1996). Dietary proportions (% by weight) were calculated for individual fish and then averaged across fish instead of pooling diets across all fish (Krebs 1989).

To assess seasonal, spatial, and ontogenetic shifts in largemouth bass diet composition, diet proportions were calculated for four fish size groups based on total length (100-230 mm, 231-310 mm, 311-361 mm, and 361 mm and larger; these groups corresponded to age ranges modeled in bioenergetic simulations). The average proportion (by weight) of vertebrates and invertebrates consumed by largemouth bass in upstream and downstream regions was calculated seasonally for each size group, combining data from 2005-2006. Due to low sample sizes and similarity of southern flounder diets throughout the Mobile Delta (personal observation), diets were combined across dates to assess seasonal and ontogenetic shifts in southern flounder diet composition. Additionally, southern flounder diets collected at nearby locations in the Mobile Delta during 2005 and 2006 were included to increase sample size (Crab Creek N=44, Justine's Bay N=1, Bayou Sara N=1; McReynold's Lake N=1, and Tensaw Lake N=1). Southern flounder diet proportions were calculated for three total length size groups (100-240 mm, 241-400 mm, and 400 mm and larger).

Diets of southern flounder (at all sites) and largemouth bass (at upstream and downstream regions) were combined across seasons and years for analysis of prey types. Each prey item was assigned to one of six groupings for vertebrates or one of five groupings for invertebrates. These groupings were broad and generally at the family level, although some contained multiple families of vertebrates or invertebrates. Average proportions (by weight) for each of these groups were found for each size class of

southern flounder (at all sites) and largemouth bass (by region). The relationship between prey size and predator total length was also evaluated. Measures of prey size were total length (mm) for fish prey, carapace width (mm) for crab prey, and uropod length (mm) for shrimp prey.

## Statistical Analysis

### *Total Hg and MeHg Relationships*

For largemouth bass, southern flounder, blue crab, and bluegill, the percent of total Hg occurring as methylmercury was determined for each sample. One-way analysis of covariance (ANOVA) was used to determine if the percent of total Hg occurring as methylmercury differed between upstream and downstream regions (SAS version 9.1, PROC ANOVA; SAS Institute 2003).

### *Selection of Best Regressor for Hg*

Largemouth bass and southern flounder total length, wet weight, and age were evaluated with stepwise multiple regression (SAS v. 9.1, SAS Institute Cary, N.C.) to find the single best predictor of Hg. The assumptions of linear regression (linearity, normally distributed error and constant variance) were evaluated with residual analysis to investigate the need for variable transformation.

### *Largemouth Bass Spatial, Seasonal and Gender Analysis*

Largemouth bass total tissue Hg concentrations from 2005 and 2006 were combined to test for spatial, seasonal and gender influences on Hg-to-size relationship.

These variables were evaluated by using two separate analyses of covariance (ANCOVA) models (SAS version 9.1, PROC GLM; SAS Institute 2003). This technique unites ANOVA and regression techniques (Littell et al. 2002). A primary assumption of ANCOVA is that slopes fitted separately to each of the covariate groups are equal. This assumption was tested with two, three, and four-way interaction terms. If interaction terms were not significant ($\alpha > 0.05$; indicating slopes within covariate groups were equal), they were dropped from the model using backwards selection ($\alpha = 0.05$) and a classic ANCOVA was performed using significant main effects. If interaction terms were significant, they remained in the ANCOVA model and pair-wise comparisons of slopes determined which slopes were responsible for significant differences (ESTIMATE; SAS Institute 2003).

In the first ANCOVA model, the effect of gender was evaluated after accounting for any age, spatial, and/or seasonal effects on tissue Hg concentrations. In the second model, seasonal and spatial effects were evaluated after accounting for the effect of age. Two separate ANCOVA models were necessary because gender was not identified for all largemouth bass during the study.

After all non-significant terms were eliminated from both models, estimated Hg concentrations were then taken from across the size range of interest for largemouth bass at ages 1, 3, and 6 (or roughly 200 mm, 320 mm, and 410 mm respectively) using least square means (LSMEANS; SAS Institute 2003). These point-estimates quantified the influence of significant main effects while allowing for the effects of any significant interaction terms to be evaluated (Littell et al. 2002).

*Southern Flounder Spatial Analysis*

No seasonal analysis was conducted for southern flounder, given that they were only collected during fall of each year. Southern flounder collections for 2005 and 2006 were combined in a single ANCOVA to test for spatial differences after accounting for the effect the Hg-to-size relationship. Estimated Hg concentrations were determined from the final ANCOVA model for southern flounder at ages 1 and 3 (240 mm and 400 mm respectively) using least square means.

*Effect of Growth*

Mean total length and mean weight at age were calculated for upstream and downstream largemouth bass and all southern flounder collected in fall 2005 and 2006. Von Bertalanffy growth equations were fitted to largemouth bass total length-at-age data to compare growth between regions. Due to the absence of older (age-3 +) individuals, von Bertalanffy growth equations could not be calculated for southern flounder, although mean total length and mean weight were calculated for those ages sampled.

For both largemouth bass and southern flounder, residuals from total length vs. Hg regression (contamination equation) were regressed against negative residuals from the age vs. total length regression (growth equation) following the procedure of Stafford and Haines (2001). All southern flounder were characterized by a single growth and contamination equation. However largemouth bass were divided by region for this analysis. If the plot of residuals generated a significant negative regression, this was interpreted as evidence supporting growth biodilution (Stafford and Haines 2001).

*Diet Analysis*

Measurements of hard parts and standard lengths of diet items were transformed into total length using regressions developed from samples collected in the field (Appendices A and B). Total length measurements of diet items were transformed into biomass through length : weight regressions, which were used from the literature when available (Smock 1980; Pace and Orcut 1981; Benke et al. 1999; Peer 2004; Norris 2007). If needed, length : weight regressions were developed from samples collected in the field.

*Bioenergetics*

I used a bioenergetics approach (Fish Bioenergetics v. 3.0 – Contaminants Mode, University of Wisconsin Seagrant Institute, Hanson et al. 1997) to determine the effect of diet and growth rate on Hg contamination in largemouth bass and southern flounder. A bioenergetics model is a mass balance model in which energy consumed (C) is balanced by total metabolism (R + A +S), waste losses (F + U) and growth ($\triangle$B + G):

$$C = (R + A + S) + (F + U) + (\triangle B + G)$$

where R is respiration, A is active metabolism, S is specific dynamic action, F is egestion, U is excretion, $\triangle$B is somatic growth and G is gonad production. In Fish Bioenergetics 3.0, consumption and metabolic energy costs are temperature and size dependent functions and waste loss is a constant proportion of consumption. Physiological parameters required for the Fish Bioenergetics model have been previously developed for largemouth bass (Rice et al. 1983) and southern flounder (Burke and Rice 2002).

Mercury uptake was modeled with the gross assimilation efficiency and constant elimination model in Fish Bioenergetics 3.0. This model assumes that mercury intake is entirely through food intake (uptake from water represents < 0.1% of the Hg in fish; Becker and Bingham 1995, Hill et al. 1996) and that elimination rates are entirely dependent on body size. The change in mercury concentration per unit time was modeled as:

$$dX_{Pred}/dt = C * [X]_{Prey} * X_{ae} - Clearance$$

where C was the mass of prey consumed per unit time, $[X]_{Prey}$ was the mean concentration of mercury in the prey, and $X_{ae}$ is the gross assimilation efficiency of mercury from prey. Clearance was modeled as:

$$Clearance = Mass^{\zeta} * X_{Pred} * K_{Cl}$$

where $\zeta$ accounts for the effect of body size on elimination rates and $K_{Cl}$ is the clearance rate in g/day. Rodgers (1994) used $X_{ae} = 0.8$, $\zeta = -0.58$, and $K_{Cl} = 0.029$ to model mercury elimination in yellow perch and lake trout. However, Van Walleghem et al. (2007) found that the $K_{Cl}$ used by Rodgers (1994) overestimated clearance rates by as much as six times. Van Walleghem et al. (2007) estimated that a $K_{Cl} = 0.0048$ was more accurate and therefore this value was used in simulations.

For modeling purposes, the numerous prey items consumed by largemouth bass and southern flounder in this study were simplified into four prey groups: fish, crab, shrimp, and other invertebrates. Caloric densities and Hg concentrations for prey groups were derived from laboratory processing of samples and from literature values (Tables 3-5).

Before conducting model simulations, Hg muscle tissue concentrations measured in largemouth bass and southern flounder had to be converted to whole body Hg concentration. The equation:

$$y = 0.35 + 0.92\,x$$

for all species from Goldstein et al. (1996) was used where $y$ is the $\log_e$ of tissue Hg concentration, $x$ is the $\log_e$ of whole body Hg concentration.

Three model simulations were run using the Fish Bioenergetics v. 3.0 software. In the first simulation, largemouth bass from an upstream and downstream site were modeled for 365 days (October 1 through September 30 of the following year) from age 1.5 - 2.5. Largemouth bass were modeled with Hg concentrations and diet data from McReynold's Lake (upstream) and D'Olive Bay (downstream) along with regional growth rate and mean daily temperature data (Table 3). In the second simulation, McReynold's Lake and D'Olive Bay largemouth bass were modeled from ages 2.5 – 6.5, again with site-specific Hg concentration and diet data along with regional growth and temperature data (Table 4). Finally, southern flounder were modeled from age 1.5 – 2.5 with Hg concentration, diet, and growth data from across the Mobile Delta, and downstream temperature data (Table 5).

Each of the three simulations discussed above consisted of two steps: model fitting and model comparison. In model fitting, simulations were run as described above and the final Hg values were compared to the age-specific mean Hg concentration ± 95% confidence intervals for a specific site. If the final Hg concentration predicted by the model was not within the 95% confidence interval for the site, the model was re-run and prey Hg concentrations for all diet groups were adjusted until predicted end Hg

concentrations agreed with observed data, as in Harris and Bodaly (1998). Modifications to prey Hg concentrations for each simulation are presented in Tables 3-5.

Model comparison followed model fitting. This procedure was slightly different for largemouth bass and southern flounder simulations. For the two largemouth bass simulations (age 1.5 - 2.5 and age 2.5 - 6.5), beginning weight (g) and whole body Hg concentration (μg/g wet) were averaged from upstream and downstream simulations (Tables 3, 4). This caused any differences present at the end of a simulation to be the result of the variables parameterized and not pre-existing differences. For southern flounder, a single model simulation characterized the entire Mobile Delta, therefore, no averaging of beginning weight or Hg concentrations was required.

To complete model comparison for largemouth bass, sensitivity analysis was conducted within age-specific simulations. Sensitivity analysis substituted single variables (e.g., diet proportions) between sites in each age-specific model while holding all other variables (e.g. prey Hg concentration, growth, and temperature) constant. In this manner, the effect of each site or regionally-specific variable on Hg accumulation in largemouth bass could be determined. For model comparisons with southern flounder (modeled from age 1.5 – 2.5; model incorporated diet, growth, and temperature data from throughout the delta), largemouth bass variables were averaged from McReynold's Lake (upstream) and D'Olive Bay (downstream) to obtain values representative of an overall average for the Mobile Delta (Table 5). These largemouth bass variables were then substituted into the southern flounder model to determine the effect of species-specific diet and growth rate differences on Hg accumulation.

*Size Class Risk Assessment.*

I used logistic regression (PROC GENMOD DIST=BINOMIAL; SAS Institute 2003) to determine the probability that largemouth bass of specific total lengths would exceed Alabama Department of Public Health (ADPH) Hg advisory levels of 0.3 and 1.0 μg/g wet weight. Above the 0.3 μg/g level, no consumption is advised for women of childbearing age and children (< 15 years old). For everyone else, the 0.3 μg/g level corresponds to two meals per month and the 1.0 μg/g level corresponds to one meal every two months (ADPH 2007). Probabilities of exceeding both of these levels were determined for upstream and downstream regions.

In conjunction with this analysis, the effect of a theoretical 356 mm (14 inch) minimum length limit on the probability that a harvestable size largemouth bass would exceed consumption advisory levels was evaluated. Also, current harvest practices of anglers in the Mobile Delta (Alabama Dept. of Conservation and Natural Resources 2006, unpublished data) were evaluated to determine the probability that anglers are targeting largemouth bass that exceed advisory levels.

# RESULTS

## Abiotic Measurements

Salinity in the Mobile Delta was seasonally and spatially stratified with the highest levels occurring at downstream sites in the late summer and fall of 2005 and 2006 (Figure 2). Salinity was elevated (4-16 ppt) at D'Olive Bay from August-December in 2005 and June-December in 2006. Salinity at Gravine Island was consistently below 2 ppt throughout the study period with the exception of three brief events, which were correlated with hurricane and storm surges (Figure 2). The two peaks in salinity at Gravine Island in 2005 were associated with the storm surges of Hurricanes Katrina and Rita. Monthly measurements of salinity at the two other downstream sites (Bay Minette and Big Bayou Canot) followed similar trends as those observed at D'Olive Bay (Appendix C), and those at the three remaining upstream sites (Dennis Lake, McReynold's Lake and Tensaw Lake) were consistently < 2 ppt. Average mean daily temperature (Figure 3) was moderately warmer downstream from February – May, but was similar between regions during all other months. Hypolimnetic anoxic conditions ( < 2.0 mg/L dissolved oxygen) were observed at all sites during the duration of the study with the exception of Bay Minette and D'Olive Bay (Figure 4). Surface and bottom pH (Figure 5) were generally similar among sites, with the majority of values ranging in the neutral range from 7 - 8.5.

**Total Hg and MeHg Relationships**

Methylmercury accounted for a majority of the total Hg in the muscle tissue of both largemouth bass (mean ± SD; 80 ± 12%) and southern flounder (87 ± 20%). Methylmercury also accounted for virtually all of the MeHg in whole body bluegill (100 ± 17%) and blue crab (86 ± 10%) samples. The percent of total Hg occurring as MeHg did not differ between upstream and downstream regions for largemouth bass (ANOVA $F_{1,17}$=0.95, P=0.34), southern flounder (ANOVA $F_{1,9}$=0.16, P=0.70), blue crab (ANCOVA $F_{1,9}$=0.30, P=0.60), or bluegill (ANOVA $F_{1,6}$=0.85, P=0.40). However, the fraction of Hg occurring as MeHg decreased slightly with increasing Hg concentration in tissue samples from largemouth bass and southern flounder (Figure 6). Such decreases were not as obvious in whole body samples of bluegill or blue crab (Figure 7).

**Best Regressor for Hg**

Age was the best predictor of Hg concentration in both largemouth bass (Mallows $C_P$=6.00, $R^2$=0.76) and southern flounder (Mallow's $C_P$=1.51, $R^2$=0.28). Age did not violate any of the assumptions of linear regression when regressed against largemouth bass and southern flounder Hg and, therefore, did not require transformation for analysis. One largemouth bass processed for total Hg was not assigned an age due to disagreement among readers over a poorly-calcified otolith. In all, 196 largemouth bass and 41 southern flounder processed for total Hg at GLIER had ages assigned to them and were subsequently used in ANCOVA analysis.

**Spatial, Seasonal, and Gender Analysis**

Overall, there was a strong bioaccumulative effect, with age explaining the majority of the variation in Hg concentrations (ANCOVA $F_{1,195} = 676.51$, $P < 0.0001$; Figure 8). Largemouth bass at all sites exceeded 0.3 μg/g, the minimum advisory level for Hg in Alabama and numerous other states. Mercury concentrations differed spatially (ANCOVA $F_{5,191} = 13.02$, $P < 0.0001$), however, slopes of the Hg*age relationship did not differ among sites (ANCOVA $F_{5,191} = 1.28$, $P = 0.28$; Figure 8), indicating least squared means could be used to assess differences between sites. These point estimates indicated Hg concentrations were lowest at the most downstream site (DB) and increased steadily moving upstream, reaching the highest concentration at the most upstream site in the study area (TN; Figure 9). Season of collection also had a significant effect on largemouth bass Hg concentration (ANCOVA $F_{1,195} = 5.31$, $P = 0.02$) with Hg concentrations slightly elevated in the spring (0.035 μg/g) over those in the fall.

While gender did not have an overall effect on largemouth bass Hg concentration (ANCOVA $F_{1,134} = 1.40$, $P = 0.24$), the slope of the Hg*age regression was different for male and female largemouth bass (ANCOVA $F_{1,134} = 6.19$, $P = 0.01$; Figure 10) with older female largemouth bass accumulating more Hg than males (Figure 10).

Southern flounder had lower muscle tissue Hg concentrations than largemouth bass, with no individuals > 0.3 μg/g Hg. Southern flounder Hg concentrations did not vary among sites (ANCOVA $F_{3,37} = 0.32$, $P = 0.81$), therefore, a single Hg*age regression was used to find point estimates for Hg concentrations at ages 1.5 and 3 (Figure 11). Age-1.5 southern flounder (0.16 ± 0.01 μg/g Hg; mean ± 95% confidence limit) had Hg levels similar to age-1 largemouth bass at DB, BB and GI. However,

predicted age-3 southern flounder Hg concentrations (0.23 ± 0.03 μg/g Hg) were significantly lower than those for age-3 largemouth bass at all sites (Figure 9). Residual analysis indicated that final ANCOVA models fit observed data well and did not violate assumptions of normality.

**Growth**

Overall largemouth bass growth rates were similar between regions for largemouth bass (Figure 12). There were no differences in the slopes (ANCOVA $F_{1,17}$ = 0.72 P = 0.41) of the mean total length to $\log_{10}$ age regression for each region. However, despite this overall similarity, age-specific differences in growth existed. Age-1 largemouth bass at downstream sites were significantly larger than those at upstream sites (Figure 12). However, this growth difference disappeared after age-1. Age-1 southern flounder were similar to age-1 largemouth bass downstream in terms of mean total length. However, by age-2, southern flounder were significantly larger than both downstream and upstream largemouth bass (Figure 12). This faster growth in southern flounder could help explain lower Hg concentrations in southern flounder relative to largemouth bass.

Growth biodilution of Hg appeared to be occurring in largemouth bass at both upstream and downstream sites (Figure 13). However, the negative relationship between growth and Hg concentration appeared to be much stronger at downstream sites (slope = -0.84) than upstream sites (slope = -0.46). Growth also explained a greater amount of the variation in Hg concentrations downstream ($R^2$ = 0.35) as compared to upstream ($R^2$ =

0.13). Mercury concentration did not appear to be related to southern flounder growth (Figure 14).

**Prey Mercury Concentrations**

Bluegill had elevated whole body Hg concentrations above those measured in blue crabs (Figure 15). While composite samples of 40 mm bluegill and blue crab had similar Hg concentrations (~0.01 μg/g), larger bluegill bioaccumulated Hg while larger blue crab did not. There also appeared to be spatial differences in bluegill Hg concentrations, with those upstream having higher Hg concentrations than those downstream. However, this comparison was limited in scope due to the low samples sizes of bluegill collected at downstream sites. Blue crab Hg concentration did not differ between upstream and downstream sites (Figure 15).

**Diet Analysis**

Largemouth bass at upstream sites generally consumed a higher proportion of vertebrates than those at downstream sites (Figure 16). Seasonal foraging trends were evident with increased vertebrate consumption occurring during fall and winter at both upstream and downstream sites (Figure 16). At downstream sites, the lowest proportions of vertebrates were consistently consumed in summer across size classes (Figure 16). While ontogenetic trends in foraging were not immediately obvious upstream, the smallest (100-230 mm) and largest (360 mm +) largemouth bass downstream consumed a higher proportion of vertebrates than adults of intermediate sizes (Figure 16).

The dominant invertebrates consumed by upstream and downstream largemouth bass were crab (primarily blue crab or fiddler crab, *Uca longissignalis*) and shrimp species (primarily white shrimp *Penaeus setifer* or grass shrimp *Palaemonetes pugi*; Figure 17). Sunfish (family Centrarchidae) along with other identified fish species and unidentified fish dominated the vertebrate portion of largemouth bass diets. With the shrimp prey as the exception, sizes of prey items consumed generally did not differ between upstream and downstream regions for largemouth bass (Figure 18). Larger shrimp were more common in the diets of largemouth bass downstream than those upstream (Figure 18). Sizes of prey consumed by largemouth bass generally increased with increasing total length for largemouth bass.

Southern flounder displayed dramatic increases in vertebrate consumption with size (Figure 19). While smaller (100-240 mm) southern flounder had diets that were dominated by invertebrates, larger southern flounder (401+ mm) diets contained virtually 100% vertebrates. Seasonally, vertebrate consumption increased throughout the year in small and medium-sized (241-400 mm) southern founder. As with largemouth bass, crab and shrimp species were the dominant invertebrate consumed by southern flounder (Figure 20) while vertebrate proportions primarily comprised sunfish, topminnow (family Fundulidae), goby (families Gobiidae and Eleotridae) and other unidentified fish species (Figure 19). However, while southern flounder and largemouth bass consumed similar prey items, the size range of prey selected by southern flounder were much smaller than those selected by largemouth bass (Figure 18).

**Bioenergetic Simulations**

Bioenergetics models from both species were calibrated with differing prey Hg concentrations until predicted Hg concentrations fell within the 95% confidence intervals of observed values (Figures 21, 23, 25). With the exception of upstream largemouth bass modeled from age 2.5 - 3.5 (Table 4), Hg concentrations in prey species in each simulation had to be increased above those predicted from my data (Figure 15) and from the literature (Custer and Mitchell 1992) in order for model predictions to match observed values (Tables 3-5). Increases in prey Hg concentrations required for largemouth bass ranged from 20 - 100% (Tables 3 - 4). Generally, downstream largemouth bass simulations required greater increases in prey Hg concentrations than upstream simulations (Tables 3 - 4). Southern flounder required an 80% increase in prey Hg concentrations for model fitting (Table 5).

Model comparisons showed that young adult (age 1.5 - 2.5) largemouth bass upstream reached higher Hg concentrations than those downstream, even when weights and Hg concentrations were identical at the start of simulations (Figures 21). These differences were largely the result of differences in diet composition and prey Hg concentrations between upstream and downstream largemouth bass (Figure 22). Upstream largemouth bass consumed a larger proportion of fish prey and overall, prey concentrations were predicted to be higher upstream than downstream (Table 3). Slower growth rates in upstream largemouth bass (Table 3) also contributed to higher Hg accumulation upstream (Figure 22), although growth accounted for much less of the difference than diet composition and prey Hg concentration. Temperatures downstream

were slightly more conducive to higher Hg accumulation in largemouth bass than upstream temperatures (Figure 22).

Older adult (age 2.5 – 6.5) largemouth bass upstream also reached higher Hg concentrations than those downstream when starting conditions (weight and Hg concentration) were identical (Figure 23). These higher largemouth bass Hg concentrations were attained upstream despite the fact that largemouth bass growth and water temperature were more conducive to Hg accumulation downstream (Figure 24). For older largemouth bass, the effects of diet composition and prey Hg concentrations were entirely responsible for observed differences in largemouth bass Hg concentrations between regions (Figure 24). Of these two factors which drove differences, diet composition appeared to have a greater effect on largemouth Hg accumulation than prey Hg concentrations (Figure 24).

Southern flounder (ages 1.5 – 2.5) had much lower Hg accumulation than either upstream or downstream largemouth bass of the same ages (Figures 21, 25). These differences in Hg concentration were due to differences in growth and caloric densities between species and occurred despite the fact that largemouth bass diet composition and prey Hg concentrations had a negative effect on southern flounder Hg concentrations (Figure 26). Slower growth rate (0.50 g/day) and higher caloric density (1,200 calories/g) in largemouth bass had a positive effect on southern flounder (1.58 g/day, 1,025 calories/g) Hg accumulation (Figure 26). Of these two variables, growth was responsible for a majority of the differences between species (Figure 26). This indicated that if southern flounder had growth rates similar to largemouth bass and continued to have

southern flounder diet composition; they would have higher Hg concentrations than largemouth bass (Figure 26).

Across both species, bioenergetics modeling predicted that the majority of Hg accumulation occurred in the summer when mean daily temperatures were highest (Figure 27). Largemouth bass at upstream and downstream sites experienced sharp declines in mean daily weight increments throughout the summer, which correlated with increased rates of Hg accumulation (Figure 27). While southern flounder also experienced increased Hg accumulation rates in the summer, they did not experience any noticeable declines in daily weight increments during the summer months (Figure 27).

**Size Class Risk Assessment by Region**

Probabilities that a largemouth bass of a given size would exceed the minimum (0.3 μg/g) Alabama Department of Public Health (ADPH) advisory level were greater for similarly sized largemouth bass at upstream sites than downstream sites (Figure 28). Probabilities increased at both upstream and downstream sites as largemouth bass increased in size. Sizes at which largemouth bass reached 50% and 75% probabilities of exceeding the minimum advisory level were significantly smaller for upstream largemouth bass (Figure 28). At upstream sites, virtually all largemouth bass larger than 300 mm exceeded the minimum advisory level whereas downstream largemouth bass had to reach 375 mm before this occurred (Figure 28).

Furthermore, it appears that a theoretical 356 mm (14 inch) minimum length limit would virtually guarantee that all largemouth bass of harvestable sizes would have high probabilities of exceeding the minimum advisory level at both upstream (~95%) and

downstream (~80%) sites (Figure 28). While the median size (300 mm) of largemouth bass harvested by Mobile Delta anglers in recent years is below 356 mm (ADNCR 2006), largemouth bass of this size still had > 75% probability of exceeding the minimum advisory level upstream and > 50% probability downstream (Figure 28). This indicates that current harvest practices likely put Mobile Delta anglers at risk of consuming fish which exceed the minimum ADPH advisory level.

Conversely, the probability of largemouth bass exceeding the more stringent 1.0 μg/g advisory levels was very low for all but the largest individuals. Largemouth bass < 300 mm upstream and 350 mm downstream had zero chance of exceeding this advisory level (Figure 29). At upstream sites, a 450 mm largemouth bass had ~ 25% chance of exceeding the 1.0 μg/g advisory level, whereas a 500 mm largemouth bass had slightly > 50% probability of exceeding this level. At downstream sites, even the largest largemouth bass (500 mm) for which probabilities were calculated had < 25% chance of exceeding the higher advisory level. Current harvest practices indicate that anglers are taking few largemouth that could possibly exceed 1.0 μg/g Hg.

# DISCUSSION

## Methylmercury

Methylmercury contributed the majority of largemouth bass and southern flounder total Hg (>80% for both species) and virtually all (100%) of blue crab and bluegill total Hg. For largemouth bass and southern flounder these findings generally agree with those of Bloom (1992), which were that essentially all Hg in upper trophic level predators is MeHg. However, these findings contrast with recent work that found lower % MeHg in estuarine and coastal fish populations (Baeyens et al. 2003, Mason et al. 2006).

Overall concentrations and the percent of MeHg in whole body bluegill samples were similar to those reported in bluegill muscle tissue from Chesapeake Bay (Mason et al. 2006) and other freshwater ecosystems (Becker and Bingham et al. 1995; Sveinsdottier and Mason 2005). Concentrations of MeHg in juvenile blue crabs were similar to those reported in other studies (Palmer and Presley 1996, Mason et al. 2006). However, the percent of MeHg in blue crabs from the Mobile Delta was much greater than those previously documented (55 ± 19% for Chesapeake Bay; Mason et al. 2006, 63% in Lavaca Bay, Texas; Evans and Engle 1994). In a freshwater setting, Mason et al. (2000) showed that virtually all of the Hg in predatory insects and insectivorous fish occurred as MeHg. Typically, juvenile blue crabs are opportunistic omnivores that feed on locally and seasonally available benthic invertebrates and plant detritus (Ryer 1987,

Dittel et al. 2006). Therefore, elevated percent of MeHg in blue crabs from the Mobile Delta could indicate a diet base that is higher in invertebrate content and lower in detritus than other coastal ecosystems.

High proportions of total Hg occurring as MeHg in bluegill and blue crab (which are both common prey items for largemouth bass and southern flounder in the Mobile Delta) help to explain why largemouth bass and southern flounder tissue samples had high proportions of MeHg across the Mobile Delta. If spatial differences existed for either prey species, or if blue crabs had a lower percent of MeHg than bluegill, then spatial differences in the percent of MeHg as total Hg in largemouth bass and southern flounder would have also been expected.

**Best Predictor of Hg**

Age has been found to be the best predictor of Hg concentrations for many species in a variety of ecosystems (Lange et al. 1993, Stafford et al. 2004, Cai et al. 2004, Haxton and Findlay 2008). I also found it to be the best regressor of largemouth bass and southern flounder Hg levels in the Mobile Delta. Age is likely better than other size indices (i.e., length or weight) because it is collinear with two factors that help predict Hg concentrations; time of exposure and size of the organism.

**Spatial, Seasonal and Gender Specific Trends**

Total Hg concentrations measured in largemouth bass in this study (0.07 - 1.19 μg/g) were similar to those measured in largemouth bass in other estuarine areas along the Gulf of Mexico (Ache et al. 2000 [mean=0.46 μg/g, range=0.01 – 1.6 μg/g], Lewis

and Chancy 2008 [mean=0.43 μg/g]). However, unlike past studies in the region, I examined largemouth bass Hg concentrations across a seasonal salinity gradient. Across this salinity gradient, age-standardized Hg concentrations in largemouth bass increased from downstream sites to upstream sites and were roughly 20-30% higher upstream than downstream. Downstream sites at which largemouth bass had the lowest age standardized Hg concentrations experienced elevated salinity while upstream sites with higher largemouth bass Hg concentrations were typically < 2 ‰ salinity for the duration of the study (Appendix C).

Other studies in coastal ecosystems have also found that Hg concentrations in fish were negatively related to increasing salinity. Largemouth bass and prey species sampled from the central San-Joaquin River Delta in California were found to be lower in Hg than those from more upstream tributaries in this region (Davis et al. 2008). This trend existed despite the fact that sediments in the central delta had higher sediment MeHg concentrations than those upstream (Davis et al. 2008). Gilmour and Riedel (2000) found that largemouth bass in Chesapeake Bay and its tributaries had lower Hg concentrations than largemouth bass collected from coastal plain freshwater reservoirs in Maryland. They hypothesized that atmospheric deposition of Hg was likely similar between these two environments and that lower methylation potential in estuarine areas may be responsible for lower Hg concentrations.

More recently, Mason et al. (2006) also reported low percentages of total Hg occurring as MeHg in striped bass, largemouth bass, and white perch, caught in Chesapeake Bay and surrounding tributaries. These levels were much lower than were typically found in nearby reservoirs (Sveinsdottir and Mason 2005). They noted these

differences were likely the result of lower MeHg levels in estuarine waters than in fresh water reservoirs (Mason et al. 2006). While I found upstream largemouth bass typically had elevated Hg levels above those downstream, overall largemouth bass Hg concentrations in this study were not lower than largemouth bass from upstream regions of Mobile-Alabama River Drainage (Warner et al. 2005 [mean=0.45 μg/g, range=0.0.2 - 2.8 μg/g]) and had percentages of MeHg that were also similar to largemouth bass from the Black Warrior River, near Tuscaloosa, Alabama (83%; Bonzongo and Lyons 2004).

Within the Mobile Alabama River Basin, the ratio of THg : MeHg in water samples was higher in Mobile Bay and the downstream areas of the Mobile Delta than in inland waters (Bonzongo and Lyons 2004). However, despite this elevated ratio of THg : MeHg, the overall levels of THg and MeHg were low for downstream areas of the Mobile Delta and Mobile Bay. Total Hg was found to be positively related with total suspended solids (TSS) in the Mobile Alabama River Basin, and, therefore, was prone to removal from the water column. Bonzongo and Lyons (2004) measured high TSS along with elevated THg in the Alabama near the confluence with the Tombigbee River (which marks the start of the Mobile Delta). Downstream, the lower Mobile Delta and Mobile Bay had low TSS and low total Hg in water samples. Therefore, the authors hypothesized that the upstream Mobile Delta acted as a sediment trap, receiving higher deposition of TSS along with total Hg than downstream areas. Spatial patterns in bluegill Hg levels support this hypothesis of higher total Hg upstream, but the lack of a spatial pattern in blue crab Hg concentrations does not.

I also found largemouth bass collected in the spring had significantly higher Hg concentrations than those collected in the fall after accounting for the effect of age and

spatial trends in Hg concentrations (as indicated by ANCOVA modeling). However, while significant, this seasonal difference was fairly small (0.035 μg/g) given the high degree of variability in Hg concentrations within largemouth bass of similar sizes. Past studies have indicated warmer sediment temperatures (Ramlal et al. 1993), and seasonal flooding of wetlands (Kelley et al. 1997) resulting in anoxic in conditions (Gilmour et al. 1992) typically occur in the spring and may create environments where MeHg production is increased. Along the northern Gulf of Mexico coast and in the Mobile Delta in particular, spring is typically a time of increased temperatures and high freshwater inflow into estuaries (Stout 1984, Peer 2005), which can flood wetland habitats and possibly create conditions favorable for methylation. However, few studies have documented seasonal differences in Hg concentrations of edible fish tissues (Foster et al. 2000). Unlike other contaminants, such as trace organochlorines (e.g. PCBs, DDTs, and chlordane), which bind to lipids in fish and therefore may have strong seasonality in their concentrations with regard to periods of lipid loss in fish (Greenfield et al. 2005), MeHg is typically more stable with regard to lipid loss as it binds to sulfhydryl groups in proteins (Yoon et al. 2005).

Additionally, largemouth bass spawn during spring months (Philipp and Ridgway 2002) and spawning activity has been shown to correlate with increased Hg concentrations in some fish species (Lochet et al. 2008). Theoretically, increased consumption in response to increased energetic costs along with decreases in body condition could both lead to increased Hg levels during reproduction (Lochet et al. 2008). Given that largemouth bass in this study were collected over a period of two to three months in the spring and then grouped for seasonal analysis, more targeted sampling pre-

and post spawning could better elucidate any relationship between spawning and Hg concentration. Given that male and female largemouth bass also have gender-specific spawning behaviors and energetic costs associated with spawning (Adams et al. 1982), any relationship may also be gender specific.

While there were no overall differences between male and female largemouth bass observed in this study, ANCOVA analysis indicated that the relationship between Hg and age was different between genders, with older females having slightly elevated Hg over males of the same age. As female largemouth bass have been shown to reach larger sizes at age than male largemouth bass (Lange et al. 1994), this would appear to contradict the growth biodilution hypothesis. However, growth rates in older (age-5+) largemouth bass are very low in the Mobile Delta (Norris 2007, this study). Other studies have noted that as age increases, the effect of growth on Hg bioccumlation declines (Stafford and Haines 2001). If the effect of growth was minimal and these larger females consumed larger and more contaminated prey than smaller males, they would be expected to have higher Hg concentrations. A similar trend was shown for walleye, *Stizostedion vitreum*, males and females when Hg was regressed against age (Henderson et al. 2003). However, largemouth bass in Florida lakes were found to have the opposite trend with larger females having lower Hg than males of the same age (Lange et al. 1994).

Southern flounder tissue Hg concentrations in the Mobile Delta were similar to those in other estuarine systems along the Gulf of Mexico (Ache et al. [mean=0.26 μg/g, range=0.01 – 1.2 μg/g]). Southern flounder exhibited no spatial patterns and was uniformly low in Hg concentration throughout the Mobile Delta. No southern flounder

collected exceeded the 0.3 μg/g Hg advisory level. While age-1.5 southern flounder had Hg concentrations that were similar to largemouth bass of the same age at downstream sites, by age-2.5 southern flounder had Hg concentrations that were well below those of largemouth bass of similar ages at all sites. This finding was similar to that from Lavaca Bay, Texas, in which southern flounder had the lowest Hg concentrations among all piscivorous fish species analyzed for Hg (Sager 2002). Due to the fact that southern flounder were only collected in the fall of each year and gender could not be consistently determined, no seasonal or gender-specific analyses were conducted for this species.

**Hg Concentration in Prey Species**

Of the two prey species for which I measured Hg concentration, bluegill had higher concentrations than blue crab. While the uptake, transfer, and storage of Hg among the organs and tissue in invertebrates and fishes has been found to be different, Mason et al. (2000) concluded that the majority of differences in Hg between trout and crayfish were the result of Hg concentrations in food resources and not physiological differences in Hg accumulation mechanisms. Furthermore, in a laboratory setting, blue crab were found to accumulate Hg to the same concentration as that in food, even when concentrations in food were elevated significantly over most environmentally-relevant levels (Evans et al. 2000). Therefore, differences in bluegill and blue crab whole body Hg concentrations in this study are likely the result of differing Hg levels in food resources. These differences in foraging can likely be explained by life history attributes of each species. Bluegill is known to be an opportunistic invertivore (Wootton 1988) and was found to feed primarily on water column-derived prey in an estuarine setting

(VanderKooy et al. 2000). Juvenile blue crab is known to be an omnivore that feeds on a variety of benthic invertebrates and plant detritus (Dittel et al. 2006).

Mercury concentrations increased with size in bluegill, likely indicating that prey with increasing levels of Hg were consumed by larger individuals. Conversely Hg concentrations in blue crab did not show increases with size within the size range of crabs that I collected. This suggests that mercury concentrations in blue crab diets remained fairly constant over the size range processed for Hg. Additionally, I found limited evidence that bluegill Hg concentration may be lower downstream compared to upstream. These inter- and intra-species trends in Hg concentration have important implications for largemouth bass and southern flounder Hg bioaccumulation.

**Largemouth bass and southern flounder diets**

Diet has been shown to be the primary pathway for Hg incorporation into fish tissues (Hall et al. 1997). In a laboratory setting, fish exposed to Hg only in water accumulated 1000 times less Hg than fish exposed to Hg in water and food indicating that water accounts for less than 0.01% of the Hg accumulated in fish tissue (Trudel and Rasmussen 2001). Generally, total Hg, MeHg, and the percent of Hg occurring as MeHg increase with increasing trophic levels (Cabana and Rasmussen 1994, Mason et al. 1999). This trophic transfer of MeHg has been well documented in a variety of freshwater and marine systems (Mason and Sullivan 1997, Atwell et al. 1998, Mason et al. 2000, Stafford et al. 2004, Bank et al. 2007). Therefore, the Hg concentration in predators will likely be largely dependent on the Hg concentrations in prey species and the selection of those prey species.

In this study, I analyzed the proportion (weight based) of vertebrates and invertebrates in largemouth bass and southern flounder diets as a rough indicator of trophic level. Vertebrate prey species were typically fish while invertebrate prey species were primarily blue crabs and shrimp species. Therefore, increased consumption of vertebrates was viewed as evidence of foraging on higher trophic levels than invertebrates. Mercury concentrations in bluegill and blue crab supported this assumption.

Largemouth bass diet proportions appeared to indicate that upstream largemouth bass were foraging on higher trophic levels than downstream largemouth bass. This supports the spatial trends I observed in largemouth bass tissue. Spatial trends in diet composition of adult largemouth bass appear to be consistent across years, as similar trends in vertebrate and invertebrate consumption were observed in a previous study of largemouth bass diets in the Mobile Delta (Norris 2007).

Southern flounder exhibited dramatic increases in the proportion of vertebrates consumed with increasing size. Within the range of southern flounder collected, diet composition transitioned from complete invertebrate consumption (dominated by small shrimp species) in the smallest individuals to complete piscivory in the largest individuals collected. This transition to piscivory has been shown as a common trait of adult southern flounder throughout their range (Fox and White 1969, Fitzhugh et al. 1996, Wright et al. 1993). However, this dramatic increase in piscivory did not correlate with a dramatic increase in Hg concentrations.

One possible reason for this lack of a relationship between increasing vertebrate consumption and Hg concentration in southern flounder were the sizes of prey consumed.

While largemouth bass at both upstream and downstream sites consumed larger fish, crab, and shrimp as they increased in size, larger southern flounder above 200 mm did not appear to consume large prey with increasing body size.

There was also a seasonal trend in vertebrate consumption within largemouth bass. Higher proportions of vertebrates were consumed in the fall and winter than in the spring and summer across upstream and downstream sites. This trend could offer a possible explanation of slightly elevated Hg concentration in largemouth bass collected in the spring over those collected in the fall.

**Growth Differences**

In addition to directly affecting fish Hg levels, diets of fish may also regulate Hg levels as an indirect function of growth. While the selection of prey species determines the amount of Hg passed up the food chain to piscivorous fish, it also determines the caloric intake that a fish can allocate to energetic costs (e.g. activity costs, metabolic costs, and growth; Wootton 1998). Bioenergetics simulations have shown that Hg contamination is inversely related to growth with faster growing fish having lower Hg levels than slower growing fish of the same size (Thomann 1989, Rodgers 1996). Therefore, the effect of prey species on bioaccumulation of Hg will be a combination of their caloric content as well as their Hg concentration. Theoretically, the prey species that will have the lowest potential for Hg bioaccumulation are those with high caloric content and low Hg concentrations.

However, while theory and bioenergetics simulations suggest growth biodilution of Hg should occur, this theory is based largely on the assumption that all other factors

affecting Hg accumulation will be equal (Trudel and Rasmussen 2006). As this is seldom the case in natural systems, there has been limited empirical evidence of biodilution in field studies (Doyon et al 1998, Stafford and Haines 2001, Simoneau et al. 2005). Conversely, a laboratory experiment showed that faster growing fish had higher mercury levels than slower growers (Dutton et al. 1997). This lack of evidence supporting growth biodilution is likely due to numerous factors (e.g., allocation of energy between activity costs and growth rates, assimilation efficiencies, energy densities of prey and predator species) that may cause Hg contamination to be either positively or negatively related to changes in growth (Trudell and Rasmussen 2006).

I found growth differences between upstream and downstream largemouth bass and also between southern flounder and largemouth bass. For largemouth bass, von Bertalanffy growth parameters were similar between regions, but differences in mean total length-at-age existed for specific ages. I found that age - 1.5 largemouth bass were significantly larger downstream than upstream, however, this growth advantage was not evident for older ages. Peer et al. (2006) found that growth of age-0 largemouth bass was significantly faster at downstream sites in the Mobile Delta. It appears that this growth advantage is sustained through the first year of life and that faster growth at downstream sites is also consistent across years in the Mobile Delta (Norris 2007). This faster growth of young largemouth bass downstream could lead to greater growth biodilution of Hg downstream as compared to upstream sites. Largemouth bass also exhibited large differences with southern flounder mean length at age. Given these large differences between species, growth may be a potential factor contributing to lower Hg accumulation in southern flounder compared to largemouth bass.

While growth appeared to have the potential to influence Hg concentrations between regions and species, it clearly had an effect within regions for largemouth bass. Residual analysis showed that faster growth was correlated with lower Hg concentrations in largemouth bass at both upstream and downstream sites. However, the biodilutive effect of growth on Hg concentrations appeared to be greater at downstream sites. This analysis indicated that faster growth had a general negative impact on Hg concentration as largemouth bass of all ages and both genders were included in this analysis. Together with other recent studies, this finding adds to the list of species for which biodilution has been documented. Using the same technique as described here, Stafford and Haines (2001) found that growth had a negative relationship ($R^2$=0.26) with Hg in age 4 - 12 smallmouth bass. Simoneau et al. (2005) demonstrated a positive relationship between age at 350 mm and Hg concentration in walleye from 12 Canadian lakes. Finally, Stafford and Haines (2004) found that growth had a significant negative effect on Hg concentrations of lake trout *Salvelinus namaycush* and lake whitefish *Coregonus clupeaformis* in Flathead Lake, Montana.

Findings of growth biodilution highlight the importance of knowing the age of fish used in contaminant analysis (Stafford and Haines 2001). In studies which only relate Hg to the size of fish, it is assumed that growth rates are either equal among fish, or that they do not affect Hg concentration. The negative relationship between growth and Hg concentration in largemouth bass in this study, as well as findings from other studies, indicate that this is not always the case.

**Bioenergetic Simulations**

Bioenergetic models have been used in the past to study Hg accumulation in a variety of species and settings and are particularly useful due to their use of common units of energy to describe Hg uptake and elimination (Rodgers 1994). In particular, bioenergetic models are useful tools to distinguish between the effects of diet and growth on Hg accumulation (Rodgers 1996). For the purposes of this study I adjusted prey Hg concentrations until final predicted Hg concentrations of largemouth bass and flounder met observed vales. Fitting of models was necessary because the purpose of their use in this study was not to predict Hg concentrations but rather to explain observed trends with regard to variables that were quantified (i.e. diet, growth rate, and temperature). Such an approach has been used previously where only predator Hg concentration was known (Harris and Bodaly 1998).

Largemouth bass and southern flounder prey Hg concentrations had to be increased considerably in order for model predictions to meet observed Hg concentrations. Several possible reasons for this exist. Largemouth bass and southern flounder consumed a diet that varied greatly and contained a large number of vertebrate and invertebrate species. Diet parameterization in models was simplified and may have excluded important sources of Hg in the diets of these species. It is also possible that the bioenergetics models used did not adequately quantify the metabolic costs of largemouth bass or southern flounder inhabiting an estuarine environment. If there are increased metabolic or activity costs associated with increased salinities, particularly for largemouth bass, increased consumption could be required in order to meet energetic demands. The fact that greater proportional increases in largemouth bass prey Hg

concentrations were required downstream than upstream supports this possibility. Finally it should be noted that reproduction was not simulated in these bioenergetic simulations. This could also result in the need to increase prey Hg concentrations. Energetic requirements for reproduction are not well understood and, therefore, they are often omitted from bioenergetic studies (Ney 1993). Nevertheless, despite the fact that this modeling required substantial increases in prey Hg concentrations, it still offers the best available tool with which to investigate the relative contributions of growth, diet, and temperature on Hg accumulation within and among species.

Based on modeling results, most of the difference between upstream and downstream largemouth bass was accounted for by diet differences between the regions. For both younger (age 1.5 - 2.5) and older (age 2.5 - 6.5) largemouth bass, diets with higher proportions of fish prey and elevated prey Hg levels over those downstream were responsible for greater Hg accumulation upstream. For younger largemouth bass, while faster growth downstream did contribute to lower Hg concentrations, the effects of diet and prey Hg concentration greatly outweighed those of growth. In older largemouth bass, growth had the opposite effect with growth favoring increased accumulation downstream and promoting dilution of Hg upstream. Again, the effects of diet and prey Hg concentrations greatly outweighed those of growth.

As Stafford and Haines (2001) noted, the effect of growth dilution on Hg concentration may be minimal when variation in diet is large. It is likely that growth had greater influence on largemouth bass Hg accumulation within regions, as was shown with residual analysis, when diets were more similar between those fish being compared.

Furthermore, growth likely has a decreasing effect on Hg concentration with age as growth rates decline in older fish (Stafford and Haines 2004).

Despite the minimal effect of growth on largemouth bass Hg accumulation, growth (in combination with decreased caloric density in southern flounder as compared to largemouth bass) appeared to be the driving factor causing southern flounder to have lower Hg concentrations than largemouth bass. Diets of southern flounder from across the Mobile Delta were actually predicted to be more conducive to Hg accumulation than largemouth bass diets averaged across all sites. This result stands in contrast to that from the largemouth bass model simulations. Despite fairly large differences in diet between southern flounder (fish = 78%, invertebrates = 22% by weight) and largemouth bass (fish =38%, invertebrates = 62% by weight) in this analysis, growth was able to offset any greater accumulation potential in southern flounder due to diet. Therefore it appears that growth can result in biodilution even when sufficient differences in diet exist so long as the magnitude of the difference in growth is large, as it was in this case (southern flounder = 1.5 g/day, largemouth bass = 0.5 g/day).

Finally, while seasonal analysis was not the goal of bioenergetic simulations (and thus seasonally specific diet proportions were not parameterized), it is interesting to note that these models predicted summer to be the primary time for Hg bioaccumulation in both species. This is in contrast to the small increase in Hg concentrations observed for largemouth bass in the spring.

**Size Class Risk Analysis**

In issuing Hg advisories, adequately quantifying the risk of consuming fish that vary in Hg concentration with size is difficult (McClain et al. 2006). Due to budgetary constraints most states issue advisories based on small sample sizes of fish (McClain et al. 2006). However, due to the high degree of variability in Hg concentration with size, the potential exists for states to either over or under estimate the risk of consuming a species based on an inadequate sample size (McClain 2006). When fish from across a size range are processed for Hg, and sample sizes are adequate, logistic regression can provide a useful tool to evaluate the potential of a fish at a given size to exceed Hg levels of interest. It also proved helpful in evaluating the impact of a theoretical minimum length limit as it pertained to Hg and fish size.

Results from this analysis indicated that a theoretical 356 mm minimum length limit on largemouth bass in the Mobile Delta would not be advisable from a public health perspective. Also, referencing creel survey data to the logistic regression allowed for assessment of the probability that current angler harvest practices were targeting fish that exceeded Hg levels of interest (in this case 0.3 μg/g and 1.0 μg/g). However, creel data should be interpreted with caution as the degree to which anglers consume harvested largemouth bass is unknown and may be a size dependent relationship, with a higher proportion of smaller individuals consumed than larger individuals.

# CONCLUSIONS

In general, Hg concentrations were similar to those observed in inland areas of the Mobile Alabama River Basin for largemouth bass (Bonzongo and Lyons 2004, Warner et al. 2005) and across the northern Gulf of Mexico coast for largemouth bass and southern flounder (Ache et al. 2006, Lewis and Chancy 2008) indicating the likely source of Hg to the Mobile Alabama River Basin and surrounding area is atmospheric deposition. However, across a seasonal salinity gradient largemouth bass Hg concentrations were highest in freshwater areas while southern flounder had consistently low Hg concentrations across the study area. Several ecological factors, including diet and growth, appeared to influence these trends. Diets of largemouth bass explained regional differences in largemouth bass Hg concentrations and differences in growth explained variation within upstream and downstream regions where diets were similar. Differences between largemouth bass and southern flounder were largely the results of growth rate differences between species. Given these conditions, if predictions for increased drought and subsequent decreased river discharge are correct for the southeastern United States (Hanson and Weltzin 2000), the potential exists for decreased Hg accumulation in upstream largemouth bass in response to drought conditions.

Human heath implications from this work include that largemouth bass, particularly larger individuals, from the Mobile Delta should be consumed with caution. Southern flounder appeared low in Hg concentration in regard to Hg advisory levels.

Minimum length limits as a tool to improve the quality of the Mobile Delta largemouth bass fishery should also include consideration that such advisories would likely increase human exposure to Hg through consumption of larger largemouth bass.

# III. USING OTOLITHS TO UNDERSTAND MERCURY BIOACCUMULATION PATTERNS IN ESTUARINE FISH

## INTRODUCTION

Otoliths, the inner ear bones of fish, are primarily composed of calcium carbonate in the aragonite crystal form (Carlström 1963). Recently, techniques have been developed that use the microchemistry of otoliths as a record of the environment (temperature, salinity, dissolved oxygen, metals exposure) experienced by a fish throughout life (Campana et al. 2000). Relating the environmental condition to elemental and isotopic signatures in the otolith relies on 3 assumptions: 1) otoliths are not susceptible to dilution or resorption 2) the growth of these tissues continues throughout life (Gunn et al. 1992; Thresher et al. 1994; Campana 2001) and 3) the concentration of elements in the otolith is functionally related to the concentration in the water (Elsdon et al. 2008). Therefore, otoliths can keep a record of the chemical effects on the calcium-protein matrixes throughout a fish's life (Gunn et al. 1992; Thresher et al. 1994).

Otolith microchemistry analysis has been used in a variety of applications. For example, strontium (Sr) and barium (Ba) have been used to reconstruct migration pathways (Campana 1999; Kraus and Secore 2004) and trace-element signature (usually Sr) has been used to detect anadromy (Kalish 1989; Halden et al. 1995; Secor et al.

1995). Otolith microchemistry has also been used as a natural tag for stock discrimination (Edmonds et al. 1991; Gunn et al. 1992; Campana et al. 1994; Thresher et al. 1994; Thorrold et al. 2001; Secor et al. 2002; Brazner et al. 2004). The Mobile-Tensaw River Delta, Alabama (hereafter referred to as the Mobile Delta) experiences high levels of atmospheric Hg deposition, which translates into high levels of Hg in fish (Chapter II). In estuarine environments, salinity is a strong factor affecting abiotic and biotic processes that control Hg methylation and trophic transfer (Chapter II). However, studying the effect of salinity on Hg accumulation in estuarine fish species is often confounded by movement of fish across salinity gradients (on diel, seasonal, or annual cycles [Hagan and Able 2008]) and dynamic salinity patterns, which can vary in response to numerous factors (i.e., tidal stage, wind direction, freshwater inflow into the estuary). Otolith microchemistry provides a tool capable of determining the salinity exposure of individual fish. Specifically, Sr concentrations in otoliths have been used as indicators of salinity exposure in numerous systems for many different species (Secor et al. 1995; Thorrold et al. 1997; Whitledge et al. 2007; Lowe 2007; Milton et al. 2008).

Additionally, heavy metals in otoliths have seen limited use as proxies to indicate pollution gradients (Grady et al. 1989; Dove and Kingsford 1998; Geffen et al. 1998; Geffen et al. 2003; Milton and Chenery 2001). Results in this area have been variable depending on metal and species (Hanson and Zdanowicz 1999; Swearer et al. 1999). In the past, mercury (Hg) has been detected in otoliths of fish exposed to high levels of Hg in the laboratory (Geffen et al. 1998) but has not been detected in otoliths of fish exposed to environmentally-relevant levels of Hg (Geffen et al. 2003). Quantifying Hg in fish

otoliths would allow for the unique time keeping ability of otoliths to be used to more fully understand the temporal dynamics of Hg accumulation.

Southern flounder and largemouth bass are both resident piscivores in the Mobile Delta. Despite living in similar habitats, largemouth bass accumulate far higher levels of Hg than do southern flounder (Chapter II). Part of the explanation for this difference between the species might include patterns of movement that might expose the species differentially to Hg. While southern flounder is known to have a migratory life history across estuarine salinity gradients (Stokes 1977), it has also been hypothesized that largemouth bass may migrate to upstream freshwater areas in response to increased salinity (Swingle and Bland 1969; Meador and Kelso 1989). This assumption is based largely on largemouth bass preference for salinity < 3 ‰ and the lethal effects that occur when largemouth bass are exposed for extended durations to salinity > 12 ‰ (Meador and Kelso 1989). However, more recent studies (Norris et al. 2005; Lowe 2007) have indicated adult and juvenile largemouth bass may not migrate to freshwater areas in response to elevated salinities. Determination of past salinity experienced by individuals of these species would allow for greater understanding of how the abiotic and biotic factors which accompany salinity influence contaminant accumulation.

In this study, I quantified trace element concentrations across core-edge transects in the otoliths of largemouth bass and southern flounder from the Mobile Delta. Total Hg muscle tissue concentration was known for each largemouth bass and southern flounder (see Chapter II) processed for otolith trace element data. Water samples from throughout the Mobile Delta were also processed for trace elements to relate elemental concentrations at differing salinities to those measured in the otoliths of both species.

Otolith and water trace element data were analyzed to determine if trace element concentrations could be used to indicate lifetime salinity exposure in this system and thereby help to assess if exposure to salinity was related to Hg tissue concentration. Additionally, other trace elements in the otoliths of both species were evaluated to determine if a suite of elements helped to predict elevated Hg tissue concentrations. Finally, the possibility of detecting Hg directly in the otoliths of each species as an indicator of lifetime accumulation trends was explored with Fs LA-ICPMS.

# METHODS

## Field Collection

Procedures for collection of largemouth bass and southern flounder in the field and return to the laboratory were described in Chapter II. In addition to collections of fish from within the Mobile Delta, largemouth bass were also collected in September of 2005 from the Tombigbee River adjacent to Olin Basin (approximately 15 km north of our sampling area). This site is adjacent to the Olin Corporation's chloralkali plant in McIntosh, Alabama, which is a historical point source for Hg releases (NOAA 1990). These fish were used to determine if Hg could be detected directly in the otolith.

Water samples for determination of trace elements were collected prior to and during elevated salinity in the Mobile Delta in 2005 and 2006. Samples were collected at the same sites where fish collection occurred (Figure 1, Chapter II) in June, August and October of 2005 and in March and October of 2006. Samples were taken 1 m below the surface with a Van Dorn sampler, filtered through a 0.45 μm glass-fiber filter, and acidified with 1.25 mL of high grade nitric acid in the field (USEPA 1996). Water samples were stored in acid-washed polypropylene bottles (125 mL). Samples were transported to the lab on ice and refrigerated until being shipped to the chemical laboratory for determination of trace metal content. Salinity was measured (YSI 30 meter) in 1 m increments from the surface to bottom of the water column as water samples were collected.

**Laboratory Processing**

Removal, cleaning, selection, sectioning, mounting and polishing of otoliths from largemouth bass and southern flounder followed those steps outlined in Chapter II. After polishing, eight otoliths were mounted per 27 x 44 mm glass slide. Upon arrival at the Great Lakes Institute of Environmental Research (GLIER) laboratory in Windsor, Ontario, Canada, slides of otoliths were sonicated in an ULTRAsonik cleaner (model 57X; Ney Dental, Inc., Bloomfield Connecticut) for 10 minutes. After sonication, otoliths were rinsed three times with Milli-Q water and allowed to dry for 24-48 hours in a Class-100 clean room under a laminar-flow hood (Ludsin et al. 2006).

**Processing otoliths for trace element data**

Otolith ablations were conducted at GLIER using a LA-ICPMS unit equipped with a high energy (>3.5mJ / pulse), ultrafast femtosecond laser (Integra-C by Quantronics, East Setauket, New York; Pulse rate: 100 Hz; Energy: 0.075mJ/pulse; Beam width: 24 - 29 μm) coupled to a Thermo-Elemental X7 ICPMS. Straight line ablations were run from the otolith core to either the dorsal or ventral edge of the otolith parallel to the sulcus. Ablation speed for all otoliths was consistently ~ 5 μm/s. In all, 17 isotopes representing 13 elements were quantified for each otolith ($^{7}$Li, $^{25}$Mg, $^{43}$Ca, $^{44}$Ca $^{55}$Mn, $^{57}$Fe, $^{85}$Rb, $^{86}$Sr, $^{88}$Sr, $^{111}$Cd, $^{118}$Sn, $^{120}$Sn, $^{137}$Ba, $^{138}$Ba, $^{139}$La, $^{140}$Ce, $^{208}$Pb). Due to the potential for isotopic interferences, multiple isotopes were analyzed for key elements of interest. To provide an external standard, control for instrument drift, and obtain precision (coefficient of variation [CV], the standard deviation divided by the mean) for element measurements, a glass reference standard (National Institute of Standards and

Technology [NIST] 610) with known elemental concentrations was analyzed twice before and after every eight otolith ablations. In order to determine a limit of detection (LOD) for elemental data from each otolith, the background (argon [Ar]) carrier gas was analyzed for 60 s prior to each ablation. To calculate the LOD the formula:

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

was used where $\sigma_{bgd}$ is the standard deviation of the background level for each element; $S$ is the mean sensitivity for each element (counts $s^{-1}$ per unit concentration); $Y$ is the ablation yield standardized for each element against the reference material; $N_{bgd}$ and $N_{samp}$ are replicate determinations used to integrate the background and ablation signals. (Longerich et al 1996). Essentially, the LOD was three standard deviations above the background level for a given element. In order for an element to be determined reliable and subsequently included in analysis, it needed to be above the LOD for 90% of the otoliths for a species and be measured precisely (CV > 10%; Ludsin 2006).

In order to explore the possibility of detecting Hg directly in the otoliths of largemouth bass, additional work was conducted at GLIER. A Hg standard for Fs LA-ICPMS was created by soaking a glass fiber filter pad in known concentrations of Hg, beryllium (Be), Mg, Ca, Sr, indium (In), and thallium (Tl). This standard was ablated twice before and after every eight otolith ablations to provide and external standard, correct for instrumental drift, and obtain precision measurements for elements of interest. Otoliths from largemouth bass collected from the Tombigbee River were processed for 10 isotopes representing 7 elements ($^{9}Be$, $^{26}Mg$, $^{44}Ca$, $^{84}Sr$, $^{88}Sr$, $^{115}In$, $^{200}Hg$, $^{202}Hg$, $^{203}Tl$, and $^{205}Tl$).

**Data Processing and Analysis**

Isotopic counts per second were converted to parts per million using the formula from Ludden et al. (1995). A detailed description of this formula is given in Lowe (2007). Parts per million measurements of all isotopes were quantified every 0.299 s. Calcium was used as the internal standard to correct for ablation yield, as it comprises a constant in the majority of otoliths (Campana 1999). For those isotopes that met the criteria for analysis, concentrations are reported as element concentrations (ppm).

In order to facilitate comparison of transect elemental data between fish of differing ages and year classes, elemental data were scaled to years for each fish. This was accomplished by measuring the total distance of each otolith ablation as well as the distance between the core and each succeeding annuli the ablation crossed. Using the ablation speed recorded for each transect, elemental data scaled in time increments (every 0.299 s) were re-scaled to distance increments. Assuming the distance between annuli represented 365 days, as did the distance between the core and the edge of the first annuli, distance measurements were converted to years (This procedure also assumed that growth and elemental incorporation into the otolith was constant throughout the year.)

When conversions were made, the age at capture corresponded to the last elemental measurement at the otoliths edge. Hatch dates were assumed to be April 1 for largemouth bass (DeVries and Frie 1996; Peer 2006) and January 1 for southern flounder (Fitzhugh et al. 1996; Glass 2006). For example, an age-3 largemouth bass from the 2003 year class captured on April 30, 2006 would have elemental data scaled from 2003.00 at the center of the core to 2006.08 at the edge (30 days / 365 days per year = 0.08 years). Whereas an age-3 southern flounder collected on April 30, 2006 would have

elemental data scaled from 2003.00 in the core to 2006.33 (120 days / 365 days per year = 0.33 years).

For elements that met criteria for analysis, element concentrations in otoliths and water samples were also examined to determine if any elements were useful as indicators of salinity exposure. Element concentrations in water samples were regressed against salinity measured at 1 m depth concurrent with their collection. The same elements in water samples were also regressed against element concentrations measured at the edge of otoliths (means of transect data spanning the last 30 days or 0.08 years; ~ 20 microns) from fish that were collected on the same day as water samples.

For each element that could be used to indicate salinity exposure, a lifetime mean was calculated as the average of the transect data across the entire otolith. Mean element concentrations were tested for differences among sites using a one-way analysis of variance (SAS version 9.1, PROC ANOVA; SAS Institute 2003). For those elements with significant ($\alpha = 0.05$) differences among sites, Tukey's studentized multiple range test (SAS Institute 2003) was used to compare mean otolith element concentrations among all six sites for largemouth bass and four sites for southern flounder.

Finally, otolith elemental transect data were plotted against year for each individual largemouth bass and southern flounder for visual comparison. Transect profile plots were grouped by site and year class in order to determine if patterns indicating salinity exposure were similar among fish that were aged to the same year class and captured at the same site. Plots were also compared among sites for each species to evaluate the potential for salinity exposure.

To determine if certain elements in the otoliths of either species helped to predict the magnitude of Hg accumulation, Pearson correlation coefficients determined if certain elements were related to Hg tissue concentrations in largemouth bass and southern flounder. However, because incorporation of certain elements into otoliths is influenced by age (Chittaro et al. 2006), which is also a key factor affecting Hg accumulation in fish (particularly in largemouth bass [see Chapter II]), a modeling approach was also used to determine if certain elements helped predict Hg accumulation after accounting for the effect of age. A variety of statistical techniques (Akaike's Information Criterion [AIC; Akaike 1974] stepwise selection [Mallows' $C_P$; Mallows 1973], model sums of squares error, and $R^2$ values) was used to determine the model with the best fit to the observed data while minimizing the number of parameters used.

# RESULTS

## Otolith elements meeting criteria for analysis

Six isotopes representing four elements met the criteria for analysis for largemouth bass otoliths ($^{25}$Mg, $^{57}$Fe, $^{86}$Sr, $^{88}$Sr, $^{137}$Ba, and $^{138}$Ba). The same six isotopes in southern flounder otoliths also met criteria for analysis, as did $^{55}$Mn (Table 6). If not for low LODs, many more elements could have been included in analysis, as all elements quantified with Fs-LA-ICPMS were measured precisely (CV < 10%; Table 6).

Of the isotopes that met criteria for analysis, four isotopes representing two elements, were highly correlated with one another in both largemouth bass ($^{86}$Sr and $^{88}$Sr: $r = 0.93$, $P < 0.0001$ ; $^{137}$Ba and $^{138}$Ba: $r = 0.99$, $P < 0.0001$) and southern flounder ($^{86}$Sr and $^{88}$Sr: $r = 0.91$, $P < 0.0001$ ; $^{137}$Ba and $^{138}$Ba: $r = 0.99$, $P < 0.0001$) otoliths. Therefore, $^{86}$Sr and $^{137}$Ba were subsequently dropped from analysis for both species.

## Elements as indicators of salinity exposure

Several element concentrations (ppm) in water samples had positive linear relationships with salinity (Mg, Ca, Sr, Ba; Figure 30). However, when these elements were standardized to Ca (which also had an increasing relationship with salinity), only Mg:Ca and Sr:Ca had definable positive relationships with respect to increasing salinity (Figure 31). Conversely Ba:Ca and Fe:Ca were inversely related with increasing salinity. For both of these elements, the majority of the variation occurred at salinity < 1 ‰ (Two

dimensional Klomogrov-Smirnov tests [2DKS, Garvey et al. 1998]) with very little variation occurring at salinities > 1 ‰ (Figure 31). Manganese:Ca ratios did not have a significant relationship within the range of salinity measure in the Mobile Delta (Figure 31).

Of the four elements in water samples that had definable (increasing or decreasing) relationships with salinity, only Sr had a consistent and significant ($\alpha = 0.05$) relationship between water and otolith edge concentrations across species and years (Figures 32). While relationships differed between years of collection, measurements of Sr at the edges of largemouth bass otoliths had a positive relationship with water Sr concentrations at the time of collection in both October 2005 (Figure 32A) and 2006 (Figure 32C). The fact that largemouth bass spanning a wide range of ages were used for this analysis (Table 7) indicates that Sr should be expected to reflect water concentrations throughout the lifetime of largemouth bass. Strontium concentrations in southern flounder otoliths were also positively correlated with Sr water concentrations for samples collected in October 2005 (Figure 32B). Due to low sample sizes in 2006, this analysis was only conducted for 2005 southern flounder collections.

Barium had a weak positive relationship between the otolith edge concentrations and water samples of largemouth bass collected in the fall of 2006 ($Log_{10}$ [Ototlith Sr] = 1.65 + 0.63 * $Log_{10}$ [Water Sr], $P = 0.02$, $R^2 = 0.21$), however this relationship was not evident in largemouth bass and southern flounder otoliths collected in 2005 ($P > 0.3$). No other elements had significant positive or negative relationships between otolith edge concentrations and water concentrations ($P > 0.05$).

**Otolith Transect Data**

Transect profile plots of Sr concentrations in largemouth bass otoliths displayed two consistently different patterns across the Mobile Delta. Individuals collected from upstream sites had consistently low Sr concentrations (typically below 1,500 ppm) across the entire otolith transect while individuals collected from downstream sites had much higher variation in Sr levels (Figure 33). Downstream largemouth bass typically displayed a single peak of Sr between each otolith annulus.

While southern flounder exhibited the same two patterns seen in largemouth bass otoliths (consistently low Sr across the otolith, and oscillatory patterns with a single annual increase), they also displayed patterns that differed from those in largemouth bass (high Sr in core then low Sr across the otolith; Figure 34). Unlike largemouth bass, in which a single distinct pattern in Sr concentration was seen for each region, southern flounder Sr otoliths from within a region varied considerably (Figure 34). Some southern flounder collected downstream had Sr patterns that were consistently low across the otolith while other individuals collected at upstream sites had oscillatory patterns of Sr across their otoliths (Figure 34).

Plots of other elements (Mg, Mn, Ba) revealed that their concentrations across the otolith were likely the result of ontogenetic changes. In largemouth bass otoliths, Mg and Ba were both elevated in the core compared to the outer edges of the otolith (Figure 35). In southern flounder otoliths Mg, Mn, and Ba also were elevated in the core (Figure 36). Typically, elements that are physiologically regulated or that have ontogenetic patterns in otoliths are poor indicators of environmental conditions experienced by fish (Geffen et al. 1998, Halden et al. 2000, Chittaro et al. 2006).

**Mean Element Concentrations in Otoliths**

When overall mean Sr concentrations in otoliths were examined across sites for largemouth bass, concentrations were significantly lower at upstream sites than downstream sites (Figure 37). Mean Sr concentrations in downstream otoliths did not differ significantly across sites, but they were significantly higher and more variable than at the upstream sites. Gravine Island largemouth bass had otolith Sr concentrations that were intermediate between those of upstream and downstream sites (Figure 37).

Other mean element concentrations also differed among sites for largemouth bass (Table 8). Barium concentrations had a weak increasing trend from upstream to downstream, with McReynold's Lake being significantly lower than Bay Minette and D'Olive Bay. McReynold's Lake largemouth bass otoliths also had significantly lower mean Mg concentrations than D'Olive Bay (Table 8).

Southern flounder mean element concentrations in otoliths differed little among sites (Table 8). This lack of difference in mean Sr concentrations derives from the variety of patterns seen across sites in southern flounder otoliths. Mean Ba concentrations were the only concentrations that differed spatially for southern flounder, with Bay Minette being significantly higher than D'Olive Bay. However, Ba in the otoliths of upstream fish was intermediate to and not different from Ba concentrations in the otoliths of downstream southern flounder (Table 8).

Mean concentrations for all elements, with the exception of Fe (one-way ANOVA $F_{1,251} = 1.11$, $P = 0.29$), were significantly different between species. Strontium concentrations were slightly higher in largemouth bass otoliths (one-way ANOVA $F_{1,252} = 7.61$, $P = 0.006$) while Ba concentrations (one-way ANOVA $F_{1,252} = 521.54$, $P <$

0.0001) and Mg concentrations (one-way ANOVA $F_{1,252} = 92.02$, $P < 0.0001$) were higher in southern flounder otoliths.

**Relationships between otolith elements and Hg concentrations**

Concentrations of all of the elements in otoliths that met criteria for analysis were correlated with Hg accumulation in largemouth bass (Mg [$r = -0.43$, $P < 0.0001$], Fe [$r = 0.31$, P , 0.0001], and Ba [$r = -0.37$, $P < 0.0001$]). However, of these elements, all three were also correlated with largemouth bass age (Mg [$r = -0.52$, $P < 0.0001$], Fe [$r = 0.39$, $P < 0.0001$], Ba [$r = -0.37$, $P < 0.0001$], Figure 34) which is an important factor affecting Hg bioaccumulation in this species (see Chapter II). After accounting for the effect of age on largemouth bass Hg accumulation, only Sr concentrations were useful in predicting Hg concentrations in largemouth bass tissue (Table 9). For younger largemouth bass (age $\leq 2.5$) there was a negative relationship between tissue Hg concentrations and mean otolith Sr concentrations (Figure 39); however this relationship was absent in older (age $\geq 3$) largemouth bass ($P = 0.59$, $R^2 = 0.003$, $N = 104$).

For southern flounder, only Sr ($r = -0.33$, $P = 0.02$) and Fe ($r = 0.33$, $P = 0.02$) concentrations in otoliths were correlated with Hg accumulation. Of these two elements, only Fe was correlated with age ($r = 0.40$, $P = 0.005$). Strontium concentrations in southern flounder were not correlated with age and were also selected along with age, as the best predictor of Hg concentrations for this species (Table 10). Across all ages, southern flounder Hg tissue concentrations had a negative relationship with mean otolith Sr concentrations (Figure 39). This relationship was strongly influenced by a single individual that had an elevated mean Sr concentration (3,294 ppm Sr). However, when

this individual was removed from the analysis, a weak relationship between mean Sr concentration and Hg tissue concentration remained ($P = 0.056$, $R^2 = 0.08$, $N = 47$).

**Detecting Hg in Otoliths**

Initial efforts to detect Hg directly in the otoliths of both species were unsuccessful. A standard developed for measuring Hg by laser ablation proved to be effective in quantifying Hg with Fs-LA-ICPMS. However, after the standard was removed from the sample cell, background levels of $Hg^{200}$ and $Hg^{202}$ remained elevated (~ 20,000 counts per second) as a result of residual Hg in the ICPMS system. Background levels for both Hg isotopes were eventually lowered (~ 2,000 counts per second) but I was still unable to detect Hg in the otoliths of either species above background levels.

# DISCUSSION

## Movement patterns in relation to salinity

All of the elements that met the criteria for analysis in otoliths of both species in this study have been commonly detected in the otoliths of other freshwater and marine species (Campana 1999; Whitledge et al. 2007; Elsdon et al. 2008). Of the four elements in largemouth bass and five elements in southern flounder that met criteria for analysis, only Sr was an acceptable indicator of salinity in both species. This was due to the fact that water Sr concentrations had an increasing relationship with salinity and that otolith edge Sr concentrations had a definable relationship with water Sr concentrations. This relationship between otolith edge and water Sr concentrations was present in spite of highly variable daily salinity fluctuations (Figure 2; Chapter II) and an approximate 21-day time lag for largemouth bass otoliths to reach saturation under constant water Sr concentrations (Lowe 2007).

Other elements in the otoliths of largemouth bass (Mg and Ba) and southern flounder (Mg, Mn, and Ba) were highest in the core and declined as distance from the core increased. This pattern was observed across sites and could possibly indicate an ontogenetic effect favoring incorporation of these elements early in life. Typically, elements that are physiologically regulated or that have ontogenetic patterns in otoliths are poor indicators of environmental conditions experienced by fish (Geffen et al. 1998, Halden et al. 2000, Chittaro et al. 2006).

Otolith Sr concentrations have been found to be a reliable indicator of salinity in a variety of species and systems (Milton et al. 2000; Krause and Secore 2004; Whitledge et al. 2007; Elsdon et al. 2008). Specifically for largemouth bass, lab experiments have shown that Sr in the otolith was a valid indicator of water Sr concentrations (Lowe 2007). Strontium transect profile plots across otoliths have also been used to recreate salinity exposure throughout life for numerous species (Milton et al. 2000; Rooker et al. 2004; McCulloch et al. 2005; Brenkman et al. 2007) and they were used in a similar manner in this study.

In largemouth bass transect profile plots, virtually all downstream largemouth bass had a single Sr peak between each annulus, whereas upstream largemouth bass had consistently low Sr across the otolith. As I found otolith Sr concentrations to be a reliable predictor of salinity exposure in largemouth bass, this Sr peak in the otoliths of downstream largemouth bass likely indicated that these fish resided through the annual influx of salinity into the lower Mobile Delta. The absence of elevated Sr concentrations in the otoliths of upstream largemouth bass indicates that these individuals were not experiencing the annual salinity increases that largemouth bass at downstream sites were experiencing. Mean Sr concentrations in largemouth bass otoliths also supported this conclusion.

Southern flounder exhibited a variety of patterns in Sr transect profile plots within upstream and downstream regions of the Mobile Delta. Oscillatory Sr patterns in the otoliths of southern flounder collected at upstream sites indicated that these southern flounder had experienced periods of elevated salinities. Southern flounder displaying this Sr pattern may have recently moved to upstream areas after residing in downstream areas

of the Mobile Delta. Conversely, southern flounder collected downstream with consistently low Sr concentration may have recently moved to downstream areas after inhabiting freshwater upstream areas. The mix of these patterns between upstream and downstream regions indicates that southern flounder are likely moving between upstream and downstream regions of the Mobile Delta. Mean Sr concentrations in southern flounder otoliths were not significantly different between regions, likely due to the mix of Sr patterns across regions.

Transect profile plots and mean concentrations of Sr in largemouth bass and southern flounder otoliths suggest two different life history strategies with regard to movement patterns within the Mobile Delta. Whereas largemouth bass were sedentary and appeared to remain within either upstream or downstream regions throughout life, southern flounder moved between regions of the Mobile Delta. For southern flounder in particular, there appeared to be variable movement patterns.

These movement patterns for adult largemouth bass and southern flounder support and build upon previous research conducted on these species in the Mobile Delta. Norris et al. (2005) assessed adult largemouth bass movement in the Mobile Delta using acoustic telemetry and external tagging of largemouth bass. Over a two year study there was little indication that largemouth bass downstream migrated to upstream areas in response to elevated salinities. However, peak salinities at 1 meter depth reached only 2‰ during the telemetry portion of the study and, therefore, uncertainty remained if salinities had reached a threshold high enough to initiate movement of adult largemouth bass (although salinity during the external tagging portion of the study reached 9.3 ‰ at 1 meter depth).

Lowe (2007) used otolith microchemistry to analyze Sr concentrations in otoliths of age-0 largemouth bass and southern flounder from the Mobile Delta and thereby determine salinity exposure. Age-0 largemouth bass downstream appeared to reside through salinity increases while those upstream appeared to reside in year round freshwater environments. Age-0 southern flounder exhibited a variety of Sr patterns in the otolith with some individuals having high Sr in the core and others having low Sr in the core. Lowe (2007) also determined that some age-0 southern flounder collected downstream lacked the increase in Sr at the otolith edge that was present for virtually all age-0 largemouth bass collected downstream.

My results from otolith microchemistry conducted on adult largemouth bass and southern flounder confirm the findings of Norris et al. (2005) and Lowe (2007). This work in combination with that of Norris et al. (2005) and Lowe (2007) offers solid evidence that largemouth bass do not migrate to upstream freshwater areas in response to increased salinity in downstream areas of the Mobile Delta. If downstream largemouth bass were moving to freshwater areas during times of elevated salinity and then returning to downstream areas during freshwater periods, as suggested by previous investigators (Swingle and Bland 1974; Meador and Kelso 1990), then I should have seen more largemouth bass with consistently low Sr across the otolith collected downstream. Furthermore, southern flounder appear to have variable early life habitat choices that include residence in freshwater areas in addition to estuarine and marine waters.

Understanding these species-specific movement patterns has important implications with regard to contaminant accumulation. Due to limited movement of largemouth bass between regions, Hg concentrations could be related to specific variables

measured within regions (i.e., diet, growth, temperature, etc.). For instance, I found that regional differences in diet were the primary factor driving higher Hg accumulation in upstream largemouth bass than those downstream (Chapter II). Spatial patterns in Hg accumulation that resulted from a lack of movement between regions indicated that regionally-specific Hg advisories may be needed for some species across salinity gradients in estuaries. In contrast, other species, such as southern flounder, that have a more migratory life history strategy across salinity gradients may be well suited for system wide Hg advisories.

**Salinity and Hg tissue accumulation**

Both largemouth bass and southern flounder exhibited a negative relationship between otolith Sr concentration and Hg tissue concentration. While this relationship only existed for age-2.5 and younger largemouth bass, it was present for all ages of southern flounder collected (ages 0.5 - 3.5). It is also important to note that elevated Sr concentration in the otolith has no direct physiological link to decreased Hg accumulation. However, it does indicate that for both species, residing in regions with increased salinity had a negative relationship to Hg accumulation. This effect could be due to the effect of salinity on biological factors such food web dynamics and growth rates (Chapter II) or potential abiotic effects on methylation potential (Gilmore and Riedel 2000).

While this negative effect of salinity on Hg accumulation could be inferred from spatial trends in largemouth bass Hg tissue concentrations, otolith microchemistry confirmed a sedentary life history for this species in an estuarine environment and

allowed for a conclusive correlation to be drawn between salinity exposure and Hg concentrations. Due to the migratory life history of southern flounder, no relationship between salinity exposure and Hg concentrations could be inferred from tissue data alone. Otolith microchemistry confirmed a highly variable migratory life history for southern flounder and at the same time allowed for an individual's salinity exposure to be related to Hg concentrations. These findings for largemouth bass and southern flounder offer examples of how otolith microchemistry can be used as a tool to indicate the effect of specific environments on contaminant accumulation.

Previous studies have also used otolith microchemistry analysis to relate residence in specific environments to contaminant accumulation. Mason et al. (2006) used Sr:Ca ratios in the otoliths of striped bass *Morone saxatilis* from Chesapeake Bay to indicate if individuals had migratory (i.e., high salinity exposure) or resident (i.e., low salinity exposure) life histories and relate these movement patterns to MeHg tissue concentrations. Striped bass with resident life histories tended to have higher MeHg tissue concentrations than those with more migratory life histories, although there was considerable variability in this relationship. These findings concur with our results that those individuals with higher salinity exposure throughout life will have lower Hg concentrations than those individuals with lower salinity exposure. In a similar manner, Geffen et al. (2003) used heavy metals in the otoliths of plaice *Pleuronectes platessa* and whiting *Merlangius merlangus* from the Irish Sea to indicate residence in areas of sewage dumping and found increased residence in these areas correlated with increased concentrations of Hg in the tissue.

**Detecting Hg in Otoliths**

Quantifying contaminants directly in otoliths could allow for the unique time keeping ability of otoliths to be used to more fully understand the temporal dynamics of contaminant accumulation. Several studies have previously attempted to quantify heavy metals in otoliths as indicators of contaminant burdens (Grady et al. 1989; Dove and Kingsford 1998; Geffen et al. 1998; Milton and Chenery 2000) with mixed results. In this study, I was not able to detect Hg directly in the otoliths of either species with Fs-LA-ICP-MS. While previous research has indicated that Hg can be incorporated into otoliths at very high levels of Hg exposure (Geffen et al. 1998), it has been difficult to detect Hg in the otoliths at environmentally-relevant levels of exposure (Geffen et al. 2003).

# CONCLUSIONS

I was able to determine that otolith Sr concentration was a valid indicator of salinity exposure in both largemouth bass and southern flounder. This allowed for the region of residence to be determined for individuals of both species within the Mobile Delta. This, in turn, allowed for regionally-specific ecological variables (i.e., diet, growth rates and temperature) that were measured during the course of this study to be linked to Hg accumulation for fish inhabiting those regions (Chapter II). I also found that increased salinity exposure (as indicated by elevated otolith Sr concentrations) was negatively related to Hg tissue concentrations in both largemouth bass and southern flounder. Initial efforts to detect Hg in the otoliths of largemouth bass and southern flounder as an indicator of lifetime Hg accumulation were unsuccessful.

These findings have important implications for understanding mercury bioaccumulation in estuarine fish populations and also for issuing consumption advisories for these dynamic systems. The finding that salinity exposure was negatively correlated with Hg accumulation suggests that there are marine environmental variables (chemical, physical, and/or biological) that lead to lower Hg bioaccumulation in fish. Furthermore, for species such as largemouth bass that have sedentary life histories, Hg accumulation may be related to regionally-specific variables and, therefore, regional or even sub-watershed specific Hg advisories may be appropriate. For species which have migratory life histories, system wide advisories may be necessary.

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# TABLES

Table 1. Measures of accuracy (mean values) and precision (coefficient of variation - CV) of methods used by Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor to determine total Hg in tissue samples processed for largemouth bass and southern flounder from the Mobile-Tensaw River Delta, AL. (NRCC - National Research Council of Canada standard reference material)

| | N | Mean CV ± SE | Mean Value ± SD | Control Limits |
|---|---|---|---|---|
| Duplicates | 6 | 0.07 ± 0.02 | - | -1.24 to 2.28 |
| Instrument Control | 3 | - | 126.1 ± 1.7 | 108.2 to 140.6 |
| NRCC BT-Cnt | 2 | - | 1.38 ± 0.13 | 1.23 to 1.52 |
| NRCC BT-Dolt3 | 4 | - | 4.49 ± 0.36 | 2.69 to 5.99 |
| NRCC BT-Dorm2 | 4 | - | 5.57 ± 0.14 | 3.85 to 7.15 |
| NRCC BT-Luts1 | 2 | - | 0.30 ± 0.08 | 0.07 to 0.21 |

Table 2. Measures of accuracy (% recovery) and precision (relative % difference) of methods used by Frontier Geosciences, Inc. to determine total and methyl Hg in tissue samples processed for largemouth bass, southern flounder and prey items from the Mobile-Tensaw River Delta, AL.

| | N | Mean Relative % Difference ± SE | Mean % Recovery ± SE | Control Limit |
|---|---|---|---|---|
| **Total Mercury** | | | | |
| Certified Reference Material | 6 | - | 100 ± 1.4% | 70-130% |
| Matrix Spike | 6 | - | 91.6 ± 1.1% | 70-130% |
| Duplicates | 3 | 3.4 ± 1.7% | - | 25% |
| Certified Reference Material Duplicate | 3 | 3 ± 1.4% | - | 25% |
| Matrix Spike Duplicate | 3 | 3.2 ± 2.1% | - | 25% |
| **Methylmercury** | | | | |
| Certified Reference Material | 6 | - | 91 ± 1.6% | 70-130% |
| Matrix Spike | 6 | - | 92.2 ± 2.3% | 70-130% |
| Duplicates | 3 | 10.8 ± 8% | - | 25% |
| Certified Reference Material Duplicate | 3 | 4.3 ± 2.2% | - | 25% |
| Matrix Spike Duplicate | 3 | 3.7 ± 2.5% | - | 25% |

Table 3. Inputs for fish bioenergetics model v. 3.0 for largemouth bass modeled from ages 1.5 - 2.5. Simulations ran for 365 days from October 1 at age 1.5 - September 30 at age 2.5. All data are from this study unless noted otherwise. (Prop: proportion of diet by weight, Cals: calories/g wet wt., Hg: $Hg^P$: Prey Hg [µg/g wet wt.] corresponding to mean total length of prey items consumed, $Hg^V$: Hg concentration [µg/g wet wt.] needed to fit model, WB: Whole body Hg concentration µg/g wet wt.).

| Variable | Largemouth bass Upstream | Largemouth bass Downstream |
|---|---|---|
| Caloric Density | 1,200 cals/g | 1,200 cals/g |
| Begin - End Size | 160 - 334 g | 251 - 443 g |
| Growth Rate | 0.47 g/day | 0.53 g/day |
| Diet: | | |
| Fish: Prop-Cals-$Hg^V$ ($Hg^P$) | 0.59-1000-0.048 (0.04) | 0.17-1000-0.06 (0.04) |
| Crab: Prop-Cals-$Hg^V$ ($Hg^P$) | 0.20-595-0.012 (0.01) | 0.51-595-0.015 (0.01) |
| Shrimp: Prop-Cals-$Hg^V$ ($Hg^P$) | 0.07-800-0.036 (0.03[*]) | 0.15-800-0.045 (0.03[*]) |
| Inverts: Prop-Cals-$Hg^V$ ($Hg^P$) | 0.14-698-0.024 (0.02[†]) | 0.17-698-0.03 (0.02[†]) |
| Assimilation Efficiency[‡] | 80% | 80% |
| Depuration Rate[§] | 0.0048 g/day | 0.0048 g/day |
| Beginning Hg WB (Tissue) | 0.215 (0.346) | 0.103 (0.176) |
| Mean Daily Temp | Figure 3 - Upstream | Figure 3 - Downstream |
| Comparison Modeling Data: | | |
| Beginning Weight (g) | 206 | 206 |
| Beginning Hg WB | 0.159 | 0.159 |

[*]Custer and Mitchell 1992 [†]Average Shrimp and Crab Hg

[‡]Trudel and Rasnmussen 2001; Rodgers 1996 [§] Van Walleghem et al. 2007

Table 4. Inputs for fish bioenergetics model v. 3.0 for largemouth bass modeled from ages 2.5 - 6.5. Simulations ran for 1,460 days from October 1 at age 2.5 - September 30 at age 6.5. All data are from this study unless noted otherwise. (Prop: proportion of diet by weight, Cals: calories/g wet wt., $Hg^P$: Prey Hg [µg/g wet wt.] corresponding to mean total length of prey items consumed, $Hg^V$: Hg concentration [µg/g wet wt.] needed to fit model, WB: Whole body Hg concentration [µg/g wet wt.]).

| Variable | Largemouth bass Upstream | Largemouth bass Downstream |
|---|---|---|
| Caloric Density | 1,200 cals/g | 1,200 cals/g |
| Begin - End Size | 334 - 1544 g | 443 - 1160 g |
| Growth Rate | 0.83 g/day | 0.49 g/day |
| Diet Item (Days): | Prop - Cals - $Hg^V$ ($Hg^P$) | Prop - Cals - $Hg^V$ ($Hg^P$) |
| Fish (1 - 365) | 0.62-1000-0.06 (0.06) | 0.34-1000-0.06 (0.04) |
| Fish (366 - 1460) | 0.79-1000-0.112 (0.08) | 0.27-1000-0.08 (0.04) |
| Crab (1 - 365) | 0.08-595-0.01 (0.01) | 0.34-595-0.015 (0.01) |
| Crab (365 - 1460) | 0.08-595-0.014 (0.01) | 0.58-595-0.02 (0.01) |
| Shrimp (1 - 365) | 0.06-800-0.03 (0.03*) | 0.14-800-0.045 (0.03*) |
| Shrimp (366 - 1460) | 0-0-0 (0.03*) | 0-0-0 (0.03*) |
| Inverts (1 - 365) | 0.24-698-0.02 (0.02†) | 0.18-698-0.03 (0.02†) |
| Inverts (366 - 1460) | 0.13-698-0.028 (0.02†) | 0.15-698-0.04 (0.02†) |
| Assimilation Efficiency‡ | 80% | 80% |
| Depuration Rate§ | 0.0048 g/day | 0.0048 g/day |
| Beginning Hg WB(Tissue) | 0.283 (0.432) | 0.175 (0.278) |
| Mean Daily Temp | Figure 3 - Upstream | Figure 3 - Downstream |
| Comparison Modeling Data: | | |
| Beginning Weight (g) | 389 | 389 |
| Beginning Hg WB | 0.229 | 0.229 |

*Custer and Mitchell 1992 †Average Shrimp and Crab Hg

‡Trudel and Rasmussen 2001; Rodgers 1996 §Van Walleghem et al. 2007

Table 5. Inputs for fish bioenergetics model v. 3.0 for southern flounder modeled from ages 1.5 - 2.5. Simulations ran for 365 days from October 1 at age 1.5 - September 30 at age 2.5. All data are from this study unless noted otherwise. (Prop: proportion of diet by weight, Cals: calories/g wet wt., Hg: $Hg^P$: Prey Hg [µg/g wet wt.] corresponding to mean total length of prey items consumed, $Hg^V$: Hg concentration [µg/g wet wt.] needed to fit model, WB: Whole body Hg concentration µg/g wet wt.). Largemouth bass variables shown were averages for upstream and downstream regions of the Mobile Delta and were used only for sensitivity analysis.

| Variables | Southern flounder | Largemouth bass |
|---|---|---|
| Caloric Density | 1025 cals/g* | 1200 cals/g |
| Begin - End Size | 160 - 735 g | - |
| Growth Rate | 1.58g/day | 0.50 g/day |
| Diet: | | |
| Fish: Prop-Cals-$Hg^V$ ($Hg^P$) | 0.78-1000-0.054 (0.03) | 0.38-1000-0.054 |
| Crab: Prop-Cals-$Hg^V$ ($Hg^P$) | 0.06-595-0.018 (0.01) | 0.36-595-0.014 |
| Shrimp: Prop-Cals-$Hg^V$ ($Hg^P$) | 0.14-800-0.054 (0.03[†]) | 0.11-800-0.041 |
| Inverts: Prop-Cals-$Hg^V$ ($Hg^P$) | 0.02-698-0.036 (0.02[‡]) | 0.15-698-0.027 |
| Assimilation Efficiency[§] | 80% | - |
| Depuration Rate[‖] | 0.0048 g/day | - |
| Beginning Hg WB (Tissue) | 0.099 (0.164) | - |
| Mean Daily Temp | Figure 3 - Downstream | - |

*Burke and Rice 2002 [†]Custer and Mitchell 1992 [‡]Average Shrimp and Crab Hg
[§]Trudel and Rasmussen 2001; Rodgers 1996 [‖]Van Walleghem et al. 2007

Table 6. Isotopes quantified during trace element analysis of largemouth bass and southern flounder otoliths. Element limit of detection (LOD μg/g) is the mean LOD averaged for all runs. Coefficient of variation (CV) was determined from NIST 610 data from each experiment and is presented here as the overall average. The percent of otoliths above LOD (%>LOD) for each species is given for each isotope. Bold values indicate those elements which met our criteria for inclusion in model selection.

| Isotope | Element LOD | CV (%) | Largemouth bass (%>LOD) N=206 | Southern flounder (%>LOD) N=48 |
|---|---|---|---|---|
| $^{7}$Li | 1.27 | 1.9 | 0 | 0 |
| $^{25}$Mg | 2.41 | 1.2 | **100** | **100** |
| $^{55}$Mn | 0.44 | 1.4 | 72.8 | **100** |
| $^{57}$Fe | 37.95 | 9.5 | **99.5** | **100** |
| $^{65}$Cu | 1.1 | 2.1 | 3.4 | 2.1 |
| $^{66}$Zn | 0.58 | 3.5 | 44.7 | 37.5 |
| $^{85}$Rb | 0.22 | 2.4 | 48.5 | 64.6 |
| $^{86}$Sr | 1.41 | 1.3 | **100** | **100** |
| $^{88}$Sr | 0.12 | 1.4 | **100** | **100** |
| $^{89}$Y | 0.03 | 1.5 | 0 | 0 |
| $^{111}$Cd | 0.17 | 4.9 | 2.9 | 4.2 |
| $^{118}$Sn | 0.15 | 4.9 | 69.9 | 52.1 |
| $^{120}$Sn | 0.12 | 2.3 | 78.6 | 58.3 |
| $^{133}$Cs | 0.11 | 2.7 | 0 | 0 |
| $^{137}$Ba | 0.09 | 2.7 | **100** | **100** |
| $^{138}$Ba | 0.03 | 1.4 | **100** | **100** |
| $^{139}$La | 0.01 | 1.4 | 1.5 | 0 |
| $^{140}$Ce | 0.01 | 1.6 | 3.4 | 8.3 |
| $^{208}$Pb | 0.02 | 3.2 | 63.5 | 65.3 |

Table 7. Number, age ranges (years) and mean total length (mm) of largemouth bass and flounder used to relate otolith edge element:Ca ratios to water element:Ca ratios. Values in parentheses are standard errors. Values without a letter in common were significantly different among sites for each species (One-way ANOVA, Tukey's studentized multiple range test, $\alpha$=0.05).

| | Southern flounder 2005 | | | Largemouth bass 2005 | | | Largemouth bass 2006 | | |
|---|---|---|---|---|---|---|---|---|---|
| Site | N | Ages | Mean TL | N | Ages | Mean TL | N | Ages | Mean TL |
| Tensaw Lake | - | - | - | - | - | - | 11 | 1.5 - 6.5 | 336 (27)[a] |
| McReynold's Lake | - | - | - | 5 | 1.5 - 6.5 | 349 (38)[a] | 6 | 2.5 - 9.5 | 397 (28)[a] |
| Dennis Lake | 4 | 1 | 225 (15)[a] | - | - | - | - | - | - |
| Gravine Island | 1 | 2 | 417 (.)[b] | 4 | 2.5 - 4.5 | 322 (19)[a] | 5 | 2.5 - 7.5 | 358 (39)[a] |
| Big Bayou Canot | - | - | - | - | - | - | 5 | 1.5 - 5.5 | 306 (33)[a] |
| Bay Minette | 3 | 1 | 230 (16)[a] | 4 | 2.5 - 5.5 | 352 (29)[a] | 6 | 1.5 - 7.5 | 367 (34)[a] |
| D'Olive Bay | 4 | 1 | 242 (16)[a] | 5 | 1.5 - 3.5 | 335 (13)[a] | 9 | 1.5 - 3.5 | 318 (15)[a] |

Table 8. Sample size (N) and mean total length (TL [mm]) of largemouth bass and southern flounder collected from the Mobile Delta in spring (March-May) and fall (Aug-Oct) of 2005 and 2006. Mean Mg, Mn, Fe, and Ba concentrations (ppm) for each site are given for both species. Values in parenthesis are standard errors. Values without a letter in common were significantly different among sites for each species (One-way ANOVA, Tukey's Studentized multiple range test, $\alpha$=0.05).

| Site | N | TL | $^{25}$Mg | $^{55}$Mn | $^{57}$Fe | $^{138}$Ba |
|---|---|---|---|---|---|---|
| **Largemouth bass** | | | | | | |
| Tensaw Lake | 29 | 329 (16)$^{a}$ | 15.13 (0.98)$^{ab}$ | - | 114.7 (9.19)$^{a}$ | 12.24 (0.62)$^{bc}$ |
| McReynold's Lake | 38 | 326 (14)$^{a}$ | 12.98 (0.43)$^{b}$ | - | 117.7 (7.42)$^{a}$ | 10.47 (0.58)$^{c}$ |
| Gravine Island | 39 | 314 (12)$^{a}$ | 14.12 (0.47)$^{ab}$ | - | 111.8 (6.45)$^{a}$ | 13.72 (0.52)$^{ab}$ |
| Big Bayou Canot | 19 | 317 (18)$^{a}$ | 14.58 (0.91)$^{ab}$ | - | 119.9 (9.47)$^{a}$ | 13.17 (0.64)$^{abc}$ |
| Bay Minette | 36 | 331 (15)$^{a}$ | 13.60 (0.50)$^{ab}$ | - | 117.7 (5.85)$^{a}$ | 15.95 (0.80)$^{a}$ |
| D'Olive Bay | 44 | 303 (10)$^{a}$ | 15.20 (0.45)$^{a}$ | - | 120.2 (6.47)$^{a}$ | 13.78 (0.48)$^{ab}$ |
| **Southern flounder** | | | | | | |
| Dennis Lake | 12 | 293 (27)$^{a}$ | 19.73 (1.23)$^{a}$ | 10.79 (2.60)$^{a}$ | 109.7 (6.72)$^{a}$ | 52.20 (5.36)$^{ab}$ |
| Gravine Island | 13 | 336 (27)$^{a}$ | 20.06 (1.35)$^{a}$ | 11.87 (2.43)$^{a}$ | 121.5 (16.64)$^{a}$ | 54.09 (6.98)$^{ab}$ |
| Bay Minette | 7 | 258 (26)$^{a}$ | 18.51 (1.10)$^{a}$ | 15.35 (3.74)$^{a}$ | 141.4 (18.65)$^{a}$ | 67.99 (4.27)$^{a}$ |
| D'Olive Bay | 16 | 314 (19)$^{a}$ | 20.46 (1.35)$^{a}$ | 12.28 (3.68)$^{a}$ | 130.0 (14.65)$^{a}$ | 35.46 (3.73)$^{b}$ |

Table 9. Model selection results for otolith element concentrations used in conjunction with age to predict mercury accumulation in largemouth bass (N=204) from the Mobile Delta. K = the number of parameters in the model. Mallows' $C_P$, Akaike's Information Criterion (AIC), Sums of Squares Error (SSE) and $R^2$ are all statistics evaluating the goodness of fit for each model. Bold values indicate the best model selected based by fit statistics.

| K | $R^2$ | $C_P$ | AIC | SSE | Variables |
|---|---|---|---|---|---|
| 1 | 0.75 | 21.89 | -842.97 | 3.29 | Age |
| 1 | 0.19 | 519.47 | -602.46 | 10.64 | Mg |
| 1 | 0.13 | 570.40 | -588.45 | 11.39 | Ba |
| **2** | **0.77** | **3.66** | **-860.47** | **2.99** | **Age Sr** |
| 2 | 0.76 | 17.34 | -847.08 | 3.20 | Age Ba |
| 2 | 0.75 | 23.38 | -841.43 | 3.28 | Age Fe |
| 3 | 0.77 | 3.71 | -860.45 | 2.96 | Age Mg Sr |
| 3 | 0.77 | 4.40 | -859.75 | 2.97 | Age Sr Ba |
| 3 | 0.77 | 5.61 | -858.52 | 2.99 | Age Fe Sr |
| 4 | 0.77 | 4.01 | -853.44 | 2.96 | Age Mg Sr Ba |
| 4 | 0.77 | 4.86 | -852.56 | 2.97 | Age Mg Fe Sr |
| 4 | 0.77 | 5.42 | -81.99 | 2.98 | Age Fe Sr Ba |
| 5 | 0.77 | 6.00 | -851.45 | 2.96 | Age Mg Fe Sr Ba |

Table 10. Model selection results for otolith element concentrations used in conjunction with age to predict mercury accumulation in southern flounder (N=48) from the Mobile Delta. K = the number of parameters in the model. Mallows' $C_P$, Akaike's Information Criterion (AIC), Sums of Squares Error (SSE) and $R^2$ are all statistics evaluating the goodness of fit for each model. Bold values indicate the best model selected by fit statistics.

| K | $R^2$ | $C_P$ | AIC | SSE | Variables |
|---|---|---|---|---|---|
| 1 | 0.26 | 2.61 | -306.58 | 0.07 | Age |
| 1 | 0.11 | 11.79 | -297.95 | 0.09 | Fe57 |
| 1 | 0.11 | 12.01 | -297.76 | 0.09 | Sr88 |
| **2** | **0.31** | **1.16** | **-308.27** | **0.069** | **Age Sr** |
| 2 | 0.28 | 3.13 | -306.14 | 0.072 | Age Ba |
| 2 | 0.28 | 3.33 | -305.92 | 0.072 | Age Fe |
| 3 | 0.34 | 1.34 | -308.34 | 0.066 | Age Fe Sr |
| 3 | 0.33 | 2.10 | -307.47 | 0.067 | Age Mn Sr |
| 3 | 0.32 | 2.69 | -306.81 | 0.068 | Age Sr Ba |
| 4 | 0.34 | 3.17 | -306.54 | 0.066 | Age Fe Sr Ba |
| 4 | 0.34 | 3.21 | -306.50 | 0.066 | Age Mn Fe Sr |
| 4 | 0.34 | 3.32 | -306.37 | 0.066 | Age Mg Fe Sr |
| 5 | 0.35 | 5.03 | -304.71 | 0.065 | Age Mg Mn Fe Sr |
| 5 | 0.34 | 5.12 | -304.60 | 0.066 | Age Mn Fe Sr Ba |
| 5 | 0.34 | 5.15 | -304.56 | 0.066 | Age Mg Fe Sr Ba |
| 6 | 0.35 | 7.00 | -303.21 | 0.002 | Age Mg Mn Fe Sr Ba |

FIGURES

Figure 1. Map of the Mobile-Tensaw River Delta, Alabama with locations, names, and abbreviations for seven sampling sites and locations of salinity loggers shown (✦). TN, DL, ML, and GI were classified as upstream sites while BB, BM and DB were classified as downstream sites. Largemouth bass collection sites in 2005 were ML, GI, BM, and DB and TN, ML, GI, BB, BM, and DB in 2006. Southern flounder collection sites in 2005 and 2006 were DL, GI, BM, and DB.

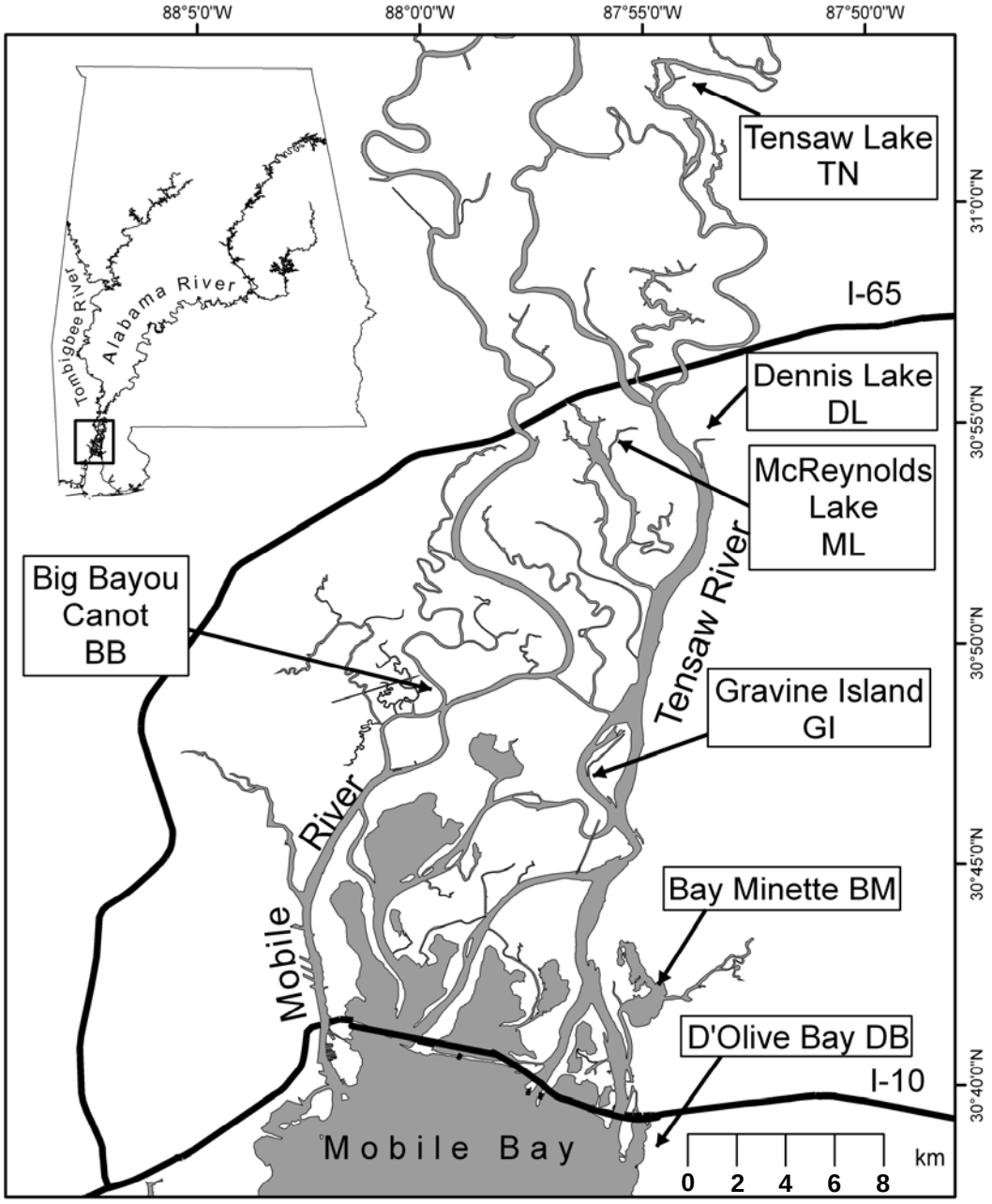

88°5'0"W
88°0'0"W
87°55'0"W
87°50'0"W
31°0'0"N
30°55'0"N
30°50'0"N
30°45'0"N
30°40'0"N
Tensaw Lake
TN
I-65
Dennis Lake
DL
McReynolds
Lake
ML
Big Bayou
Canot
BB
Tensaw River
Gravine Island
GI
Mobile River
Bay Minette BM
D'Olive Bay DB
I-10
Mobile Bay
km
0 2 4 6 8
Tombigbee River
Alabama River

Figure 2. Mean daily salinity (——) measured by salinity loggers and discrete monthly measurements (●) of salinity measured at 1 meter depths at Gravine Island and D'Olive Bay from January 2005- December 2006.

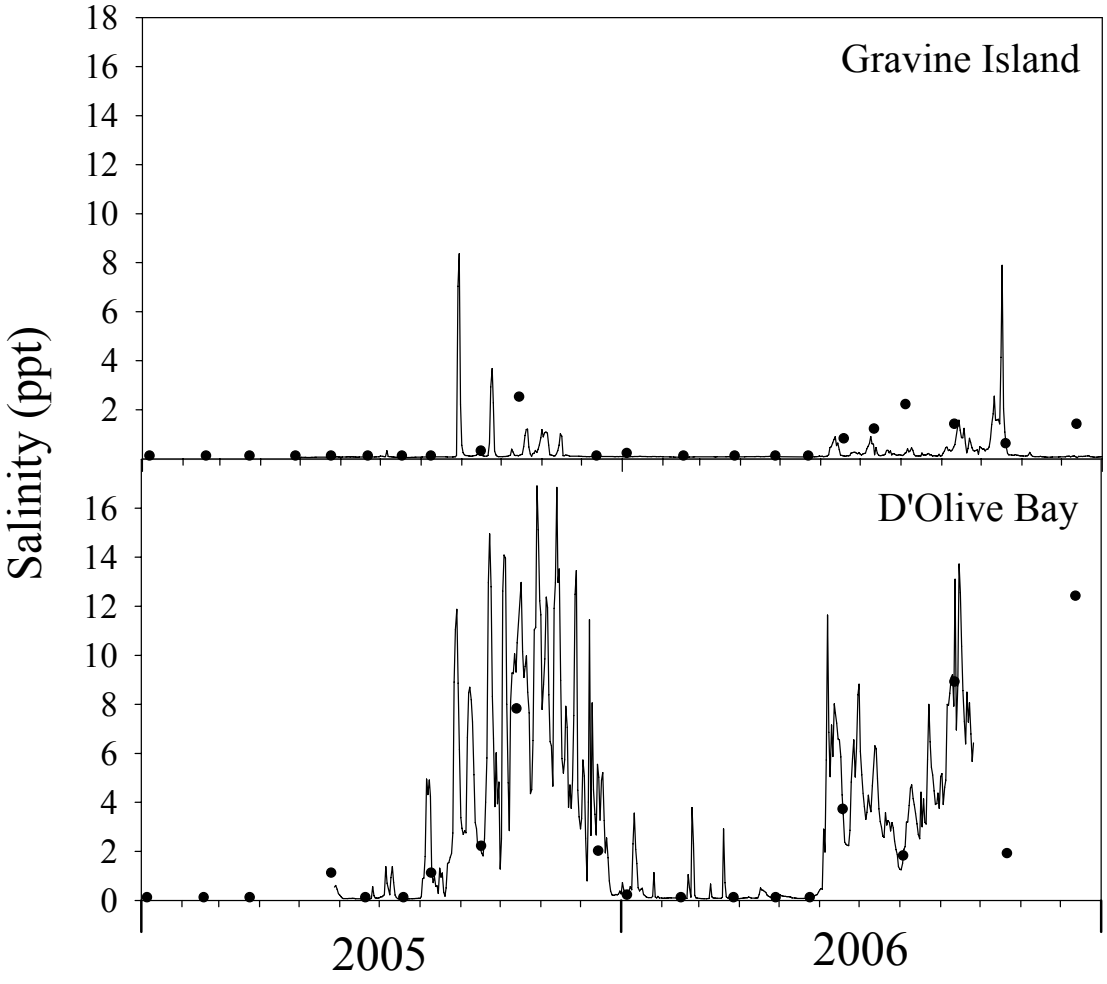

Salinity (ppt)
Gravine Island
D'Olive Bay
18
16
14
12
10
8
6
4
2
0
2005
2006

Figure 3. Mean daily temperature for depicted for an entire year at upstream and downstream sites. Measurements of temperature were made at all seven sites from 2002-2006 every 2-hours by Hobo® temperature loggers at 1 m below mean low tide depth.

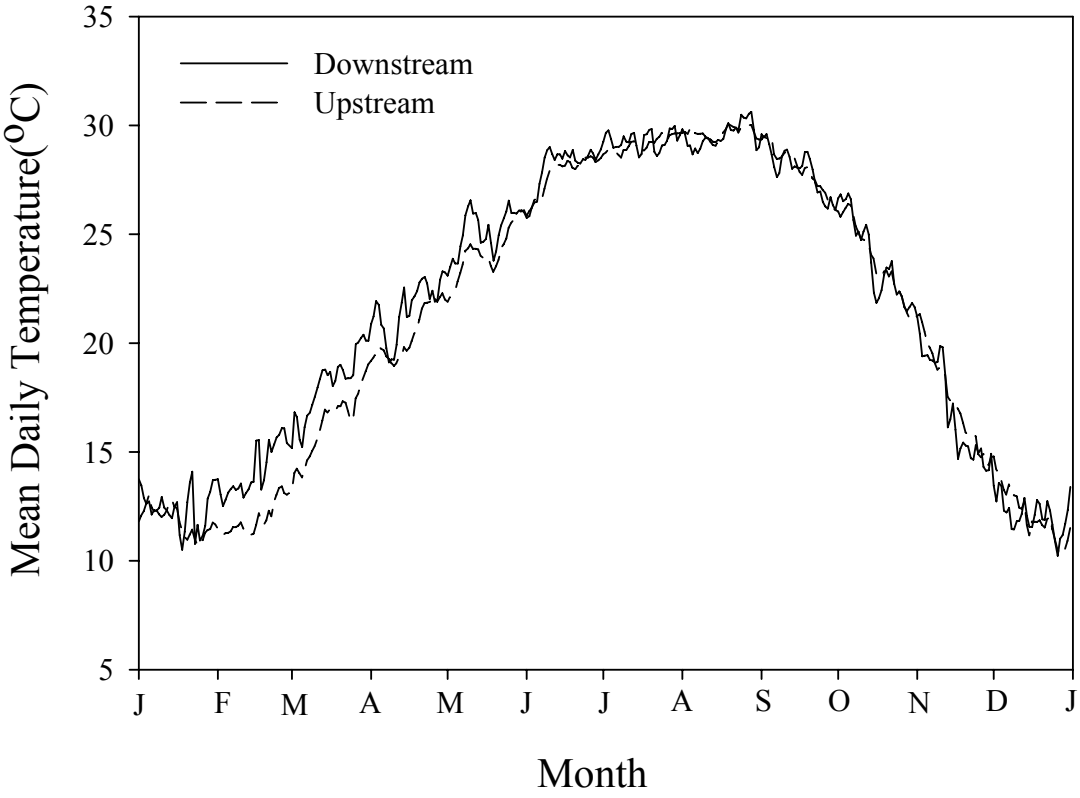

Downstream
Upstream
Mean Daily Temperature(°C)
35
30
25
20
15
10
5
J
F
M
A
M
J
J
A
S
O
N
D
J
Month

Figure 4. Surface (○) and bottom (●) dissolved oxygen at all seven sampling sites measured monthly from January 2005 – December 2006.

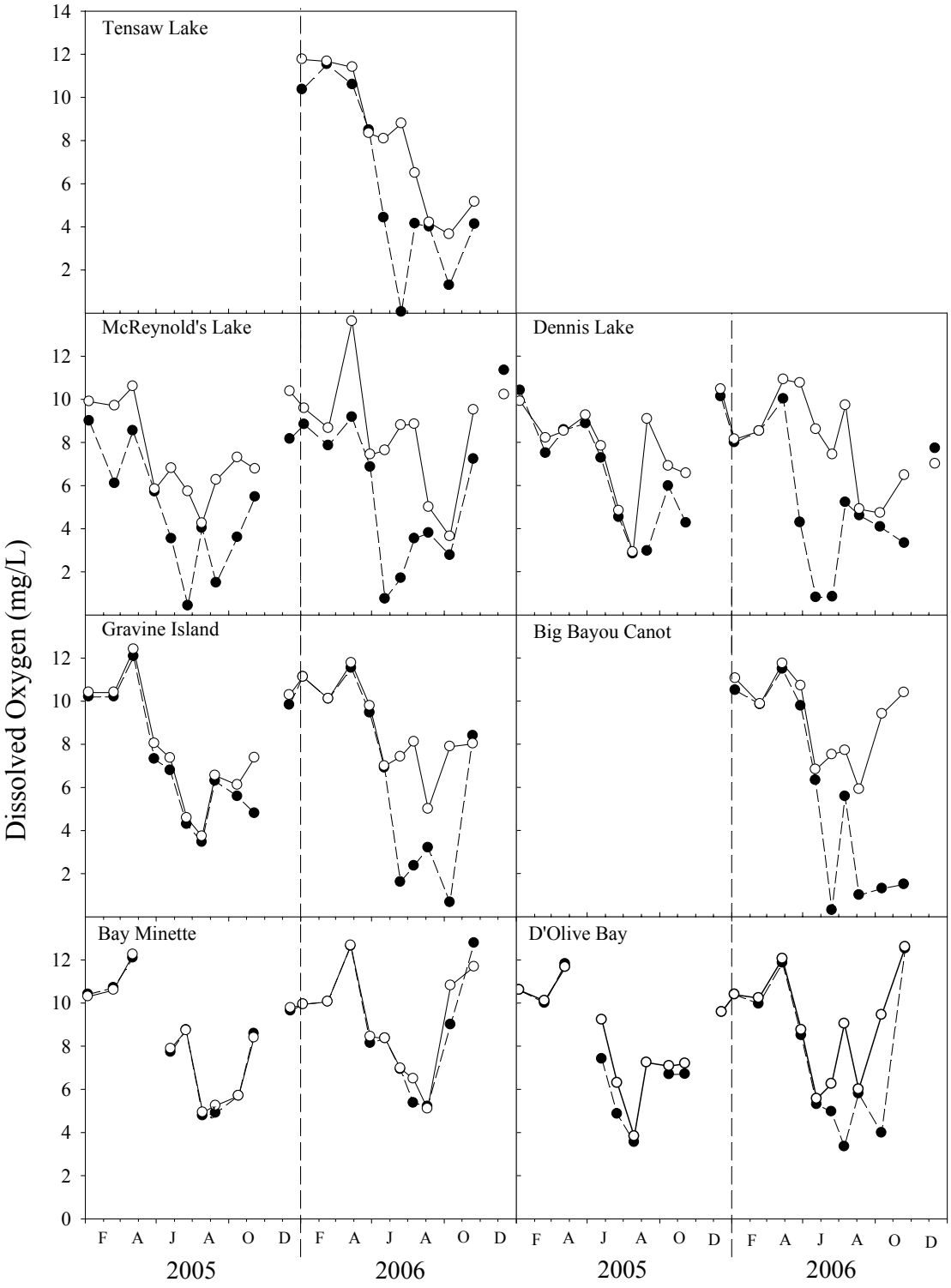

Tensaw Lake
McReynold's Lake
Dennis Lake
Gravine Island
Big Bayou Canot
Bay Minette
D'Olive Bay
Dissolved Oxygen (mg/L)
0
2
4
6
8
10
12
14
F A J A O D
2005
2006

Figure 5. Surface (○) and bottom (●) pH measured at all seven sampling sites from May 2007 – March 2008.

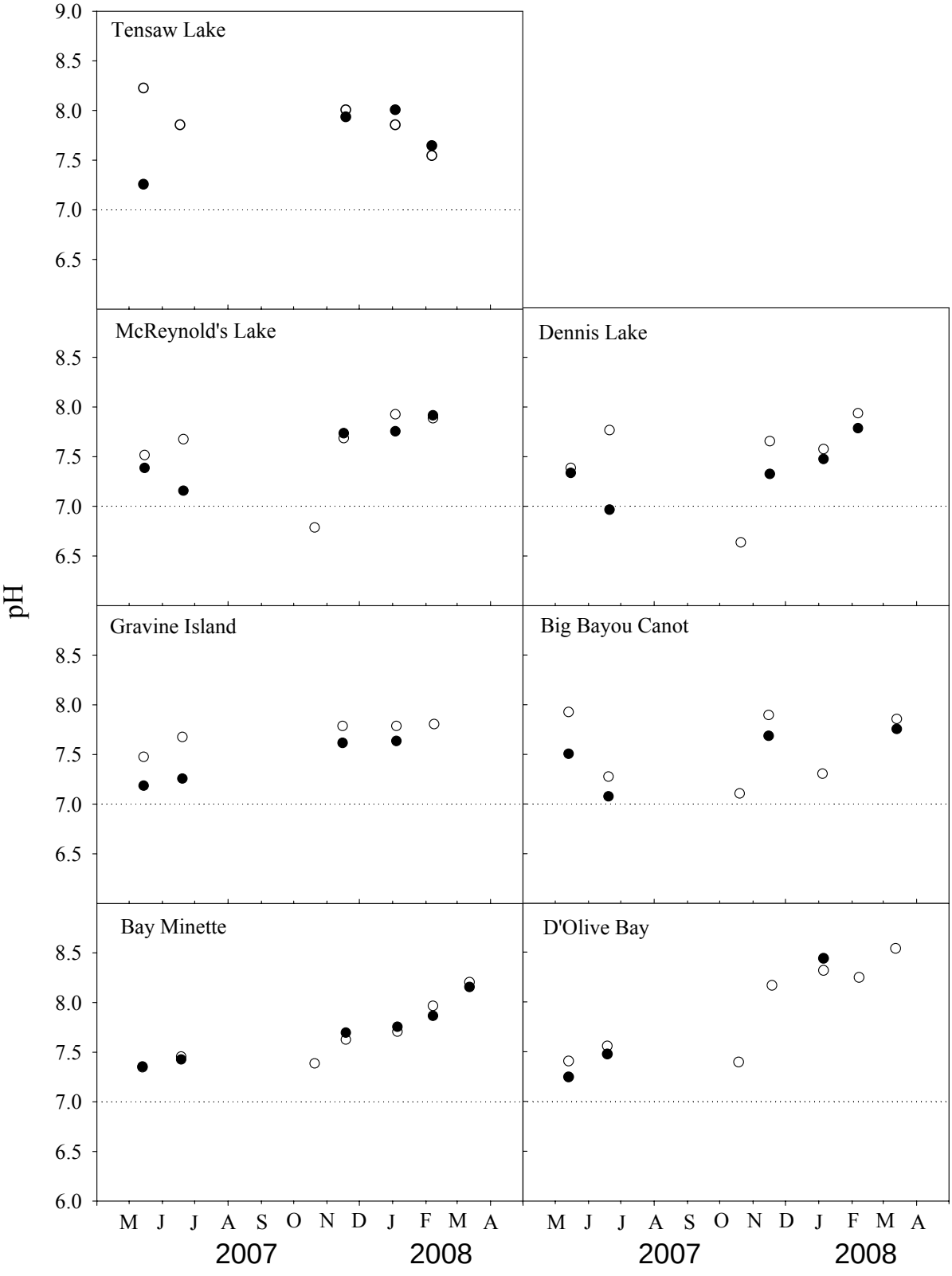
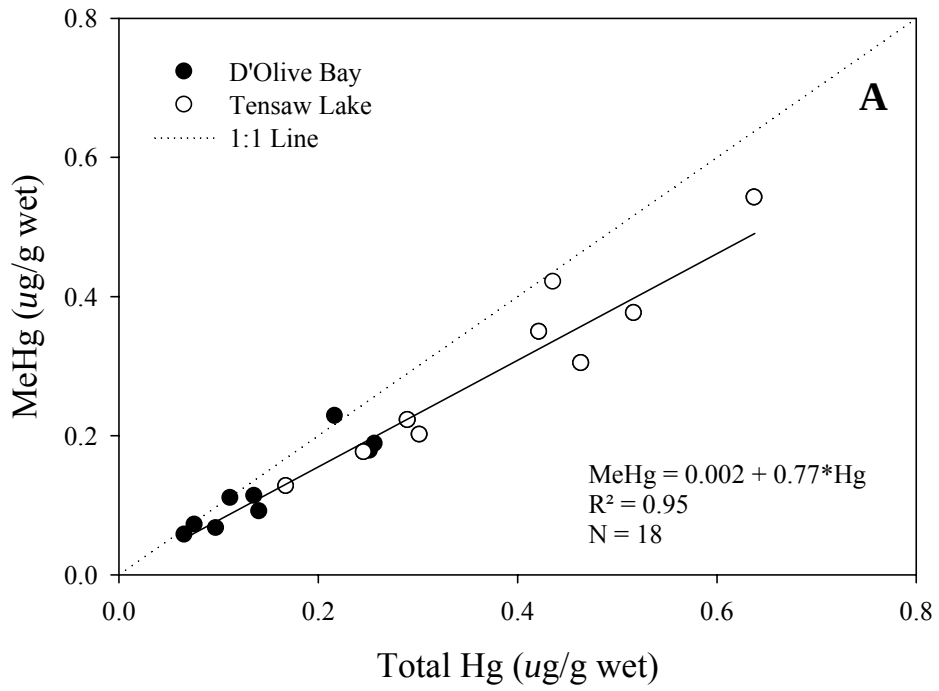

Tensaw Lake
McReynold's Lake
Dennis Lake
Gravine Island
Big Bayou Canot
Bay Minette
D'Olive Bay
pH
9.0
8.5
8.0
7.5
7.0
6.5
6.0
M J J A S O N D J F M A
2007
2008

Figure 6. Relationship between total mercury (Hg) and methylmercury (MeHg) in A) largemouth bass and B) southern flounder collected in 2006 from the Mobile Delta. The dashed line indicates the expected 1:1 ratio of total mercury : methylmercury.


A
D'Olive Bay
Tensaw Lake
1:1 Line
MeHg = 0.002 + 0.77*Hg
R² = 0.95
N = 18
MeHg (ug/g wet)
Total Hg (ug/g wet)
0.0
0.2
0.4
0.6
0.8

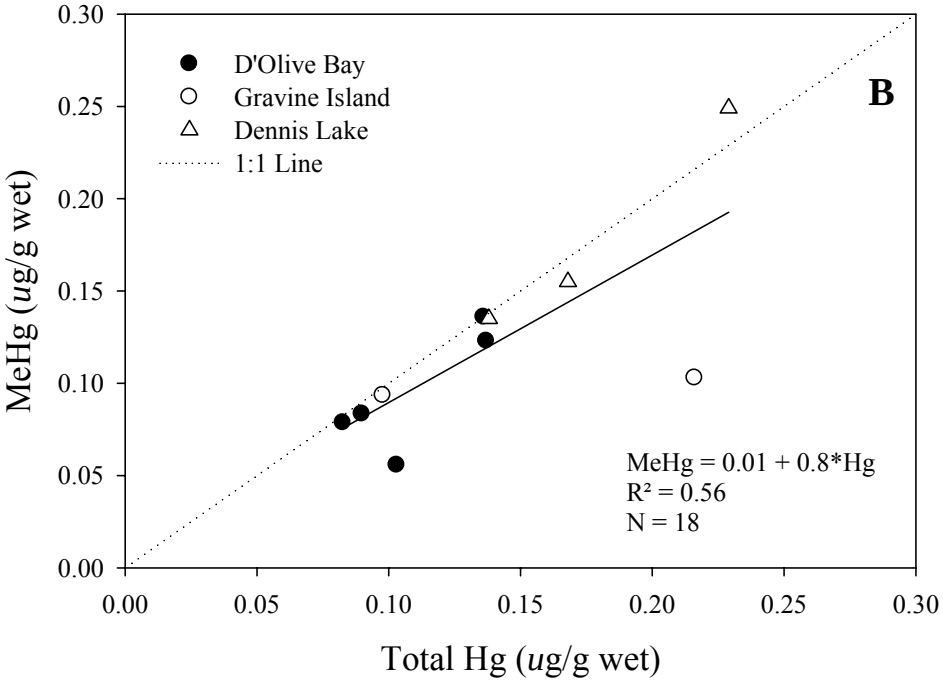
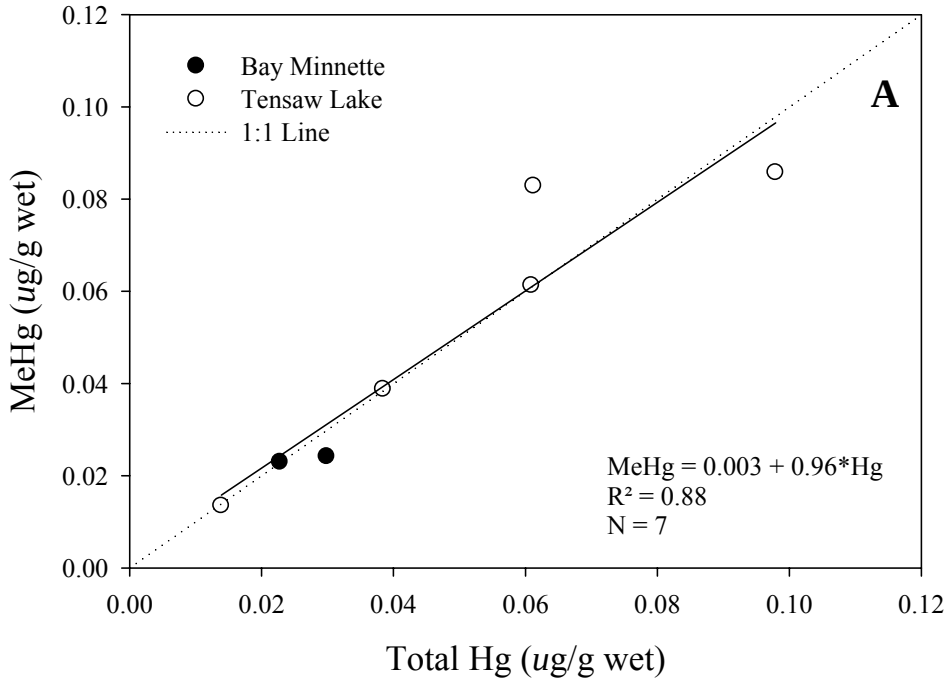

B
D'Olive Bay
Gravine Island
Dennis Lake
1:1 Line
MeHg = 0.01 + 0.8*Hg
R² = 0.56
N = 18
MeHg (ug/g wet)
Total Hg (ug/g wet)
0.00
0.05
0.10
0.15
0.20
0.25
0.30

Figure 7. Relationship between total mercury (Hg) and methylmercury (MeHg) in A) bluegill and B) blue crab collected in 2007 from the Mobile Delta. The dashed line indicates the expected 1:1 ratio of total mercury : methylmercury.


Bay Minnette
Tensaw Lake
1:1 Line
A
MeHg = 0.003 + 0.96*Hg
R² = 0.88
N = 7
MeHg (ug/g wet)
Total Hg (ug/g wet)
0.00
0.02
0.04
0.06
0.08
0.10
0.12

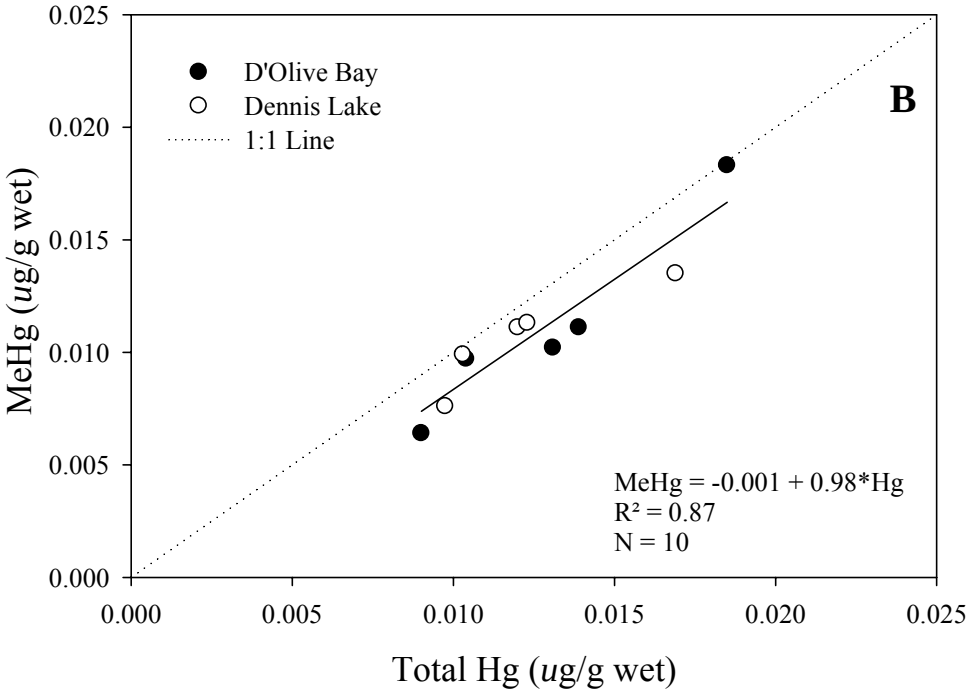

D'Olive Bay
Dennis Lake
1:1 Line
B
MeHg = -0.001 + 0.98*Hg
R² = 0.87
N = 10
MeHg (ug/g wet)
Total Hg (ug/g wet)
0.000
0.005
0.010
0.015
0.020
0.025

Figure 8. Relation between total mercury (Hg) and largemouth bass age at six sites in the Mobile-Tensaw River Delta. Solid line is the regression line; dotted lines are 95% confidence intervals. Largemouth bass were collected during (○) spring 2005, (●) fall 2005, (△) spring 2006, and (▲) fall 2006. Dashed lines indicate 0.3 μg/g (- - -) and 1.0 (– – -) μg/g advisory levels used by many state and federal agencies to issue limited and no consumption advisories.

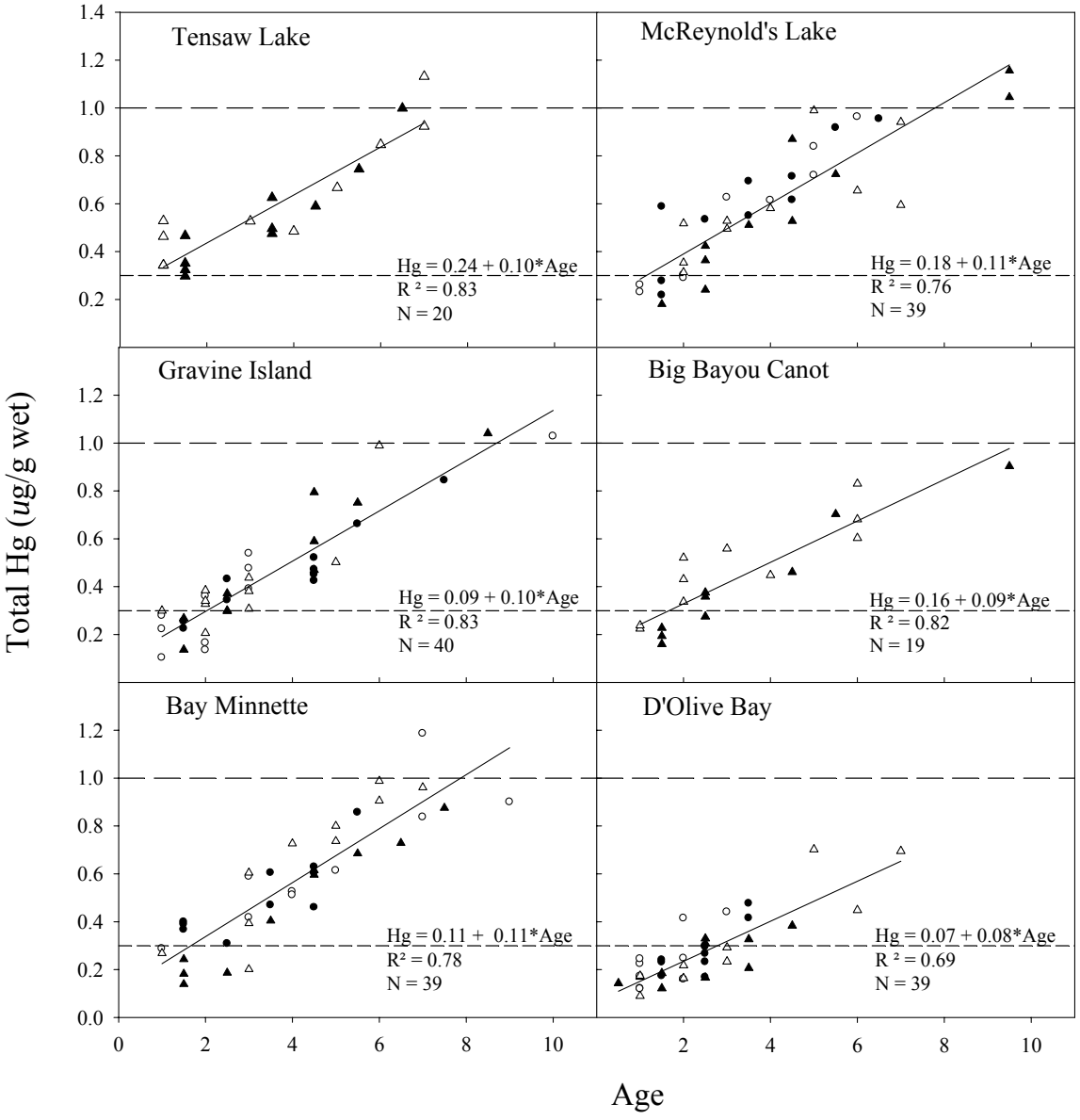

Tensaw Lake
Hg = 0.24 + 0.10*Age
R ² = 0.83
N = 20
McReynold's Lake
Hg = 0.18 + 0.11*Age
R ² = 0.76
N = 39
Gravine Island
Hg = 0.09 + 0.10*Age
R ² = 0.83
N = 40
Big Bayou Canot
Hg = 0.16 + 0.09*Age
R ² = 0.82
N = 19
Bay Minnette
Hg = 0.11 + 0.11*Age
R² = 0.78
N = 39
D'Olive Bay
Hg = 0.07 + 0.08*Age
R ² = 0.69
N = 39
Total Hg (ug/g wet)
Age

Figure 9. Estimated mean total mercury (Hg) concentrations and 95% confidence intervals for A) age-6, B) age-3, and C) age-1 largemouth bass (●) and southern flounder (○) collected in 2005 and 2006 at seven sites. The dotted line indicates the 0.3 μg/g advisory level. Lowercase letters indicate means that are significantly different after Bonferroni correction for multiple comparisons.

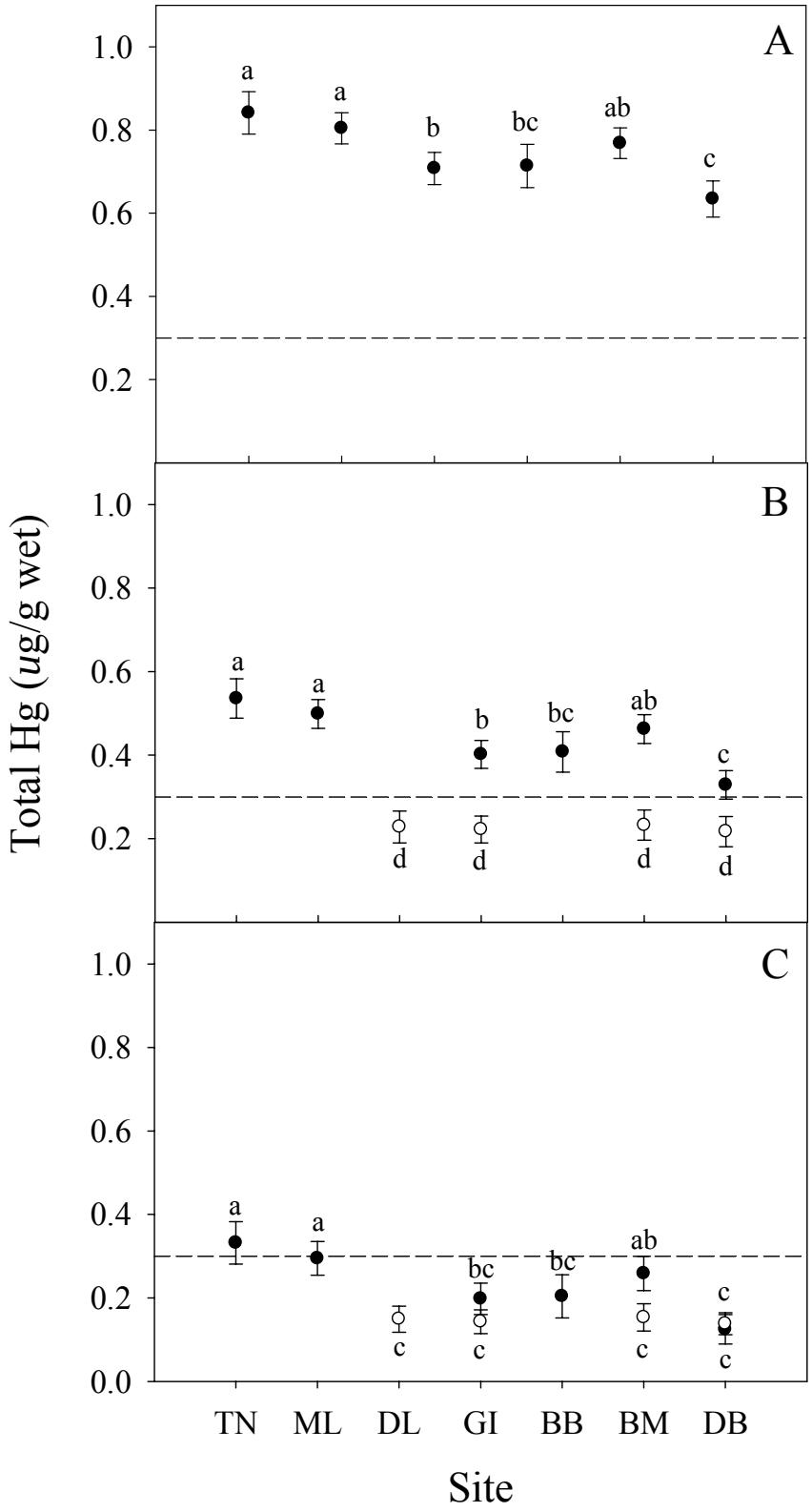

A
B
C
Total Hg (ug/g wet)
1.0
0.8
0.6
0.4
0.2
0.0
a
a
b
bc
ab
c
d
TN
ML
DL
GI
BB
BM
DB
Site

Figure 10. Relationship between total mercury (Hg) and age for male (N=71) and female (N=74) largemouth bass collected at all six sites from fall 2005 and spring and fall of 2006.

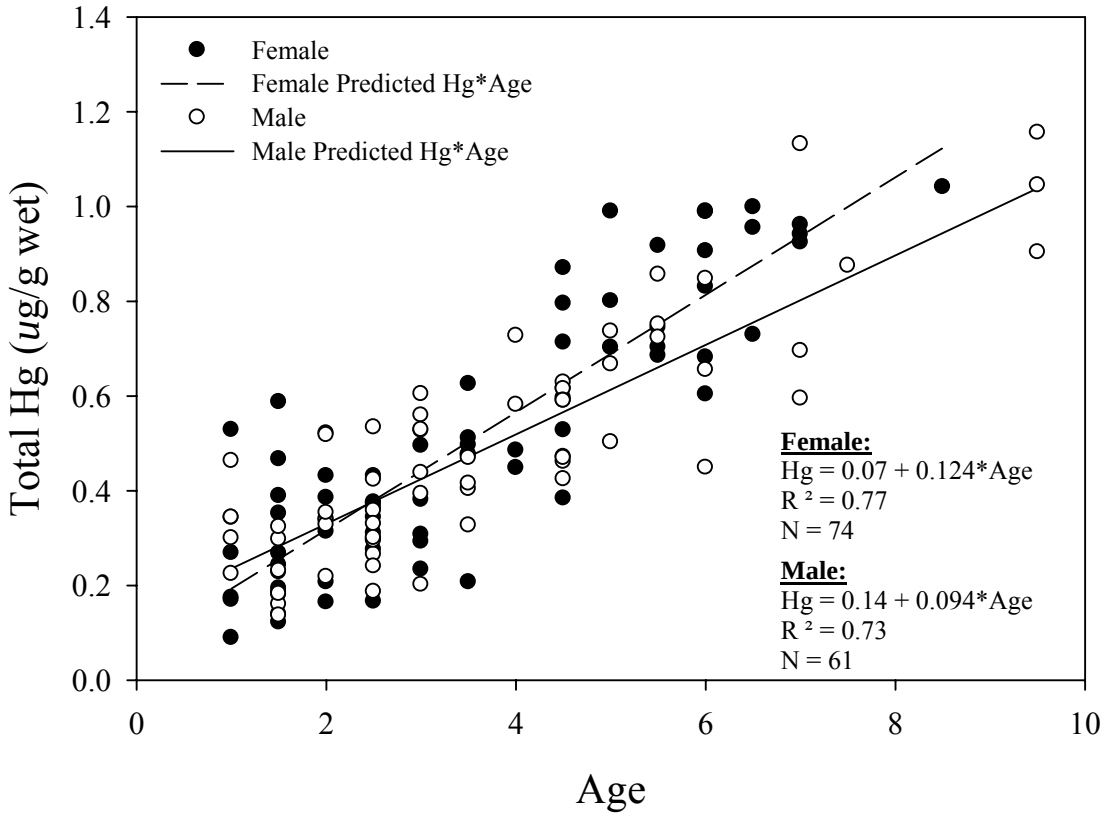

Female
Female Predicted Hg*Age
Male
Male Predicted Hg*Age
Female:
Hg = 0.07 + 0.124*Age
R ² = 0.77
N = 74
Male:
Hg = 0.14 + 0.094*Age
R ² = 0.73
N = 61
Total Hg (ug/g wet)
Age
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
2
4
6
8
10

Figure 11. Relation between total mercury (Hg) and age for southern flounder collected at four sites in the Mobile-Tensaw Delta in the fall of 2005 and 2006. Solid line is regression line; dotted lines are 95% confidence intervals. Dashed lines indicate 0.3 µg/g (- - -) and 1.0 (– – -) µg/g advisory levels used by many state and federal agencies to issue limited and no consumption advisories.

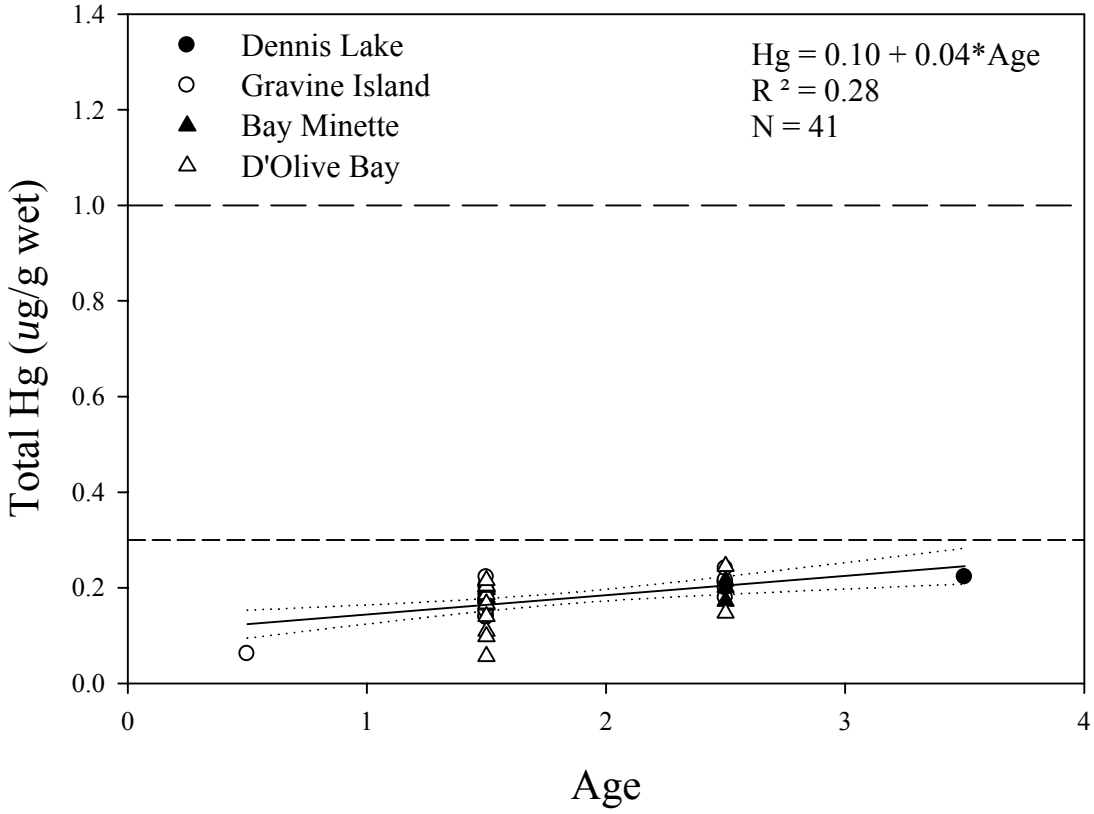

Dennis Lake
Gravine Island
Bay Minette
D'Olive Bay
Hg = 0.10 + 0.04*Age
R ² = 0.28
N = 41
Total Hg (ug/g wet)
Age
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4

Figure 12. Mean total length (MTL) at age for upstream (○) and downstream (●) largemouth bass (± standard error) and southern flounder (△) collected throughout the Mobile-Tensaw River Delta in 2005 and 2006. Von Bertalanfy growth equations are plotted for upstream (- - - -) and downstream (——) largemouth bass. (* largemouth bass MTL's were significantly different between upstream and downstream regions; ** southern flounder MTL were significantly different from all largemouth bass MTL at a given age.)

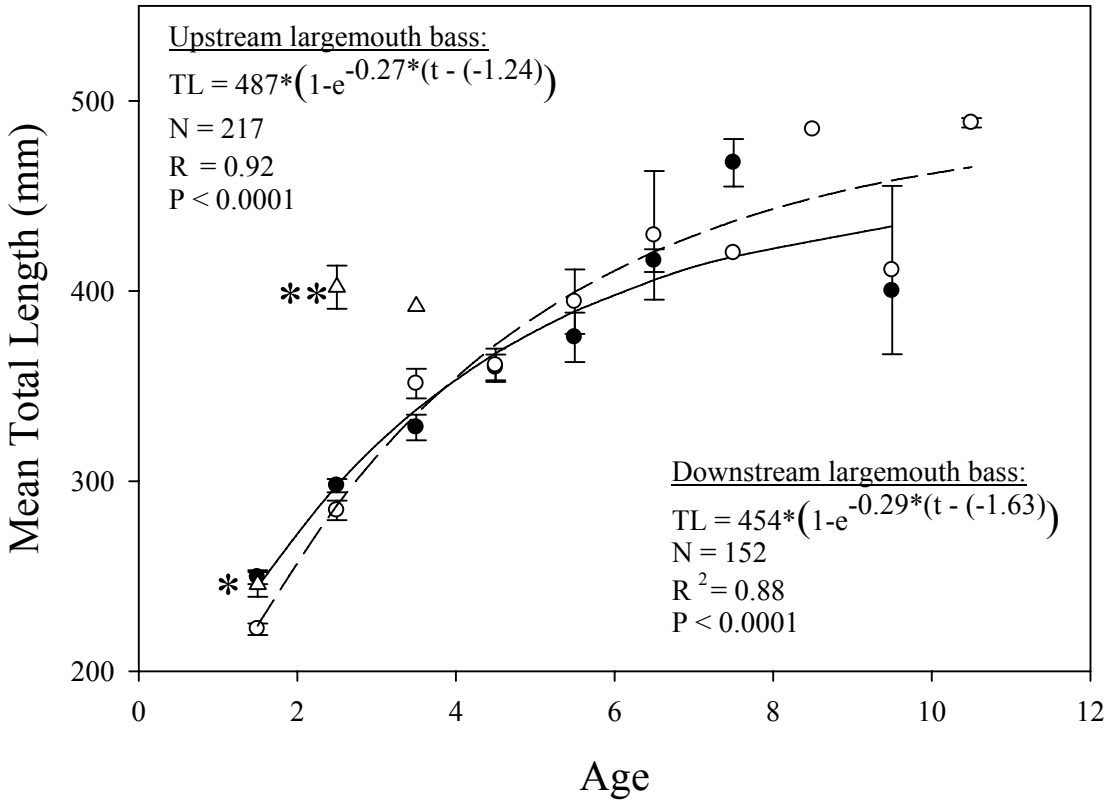

Upstream largemouth bass:
TL = 487*(1-e-0.27*(t - (-1.24)))
N = 217
R = 0.92
P < 0.0001
Downstream largemouth bass:
TL = 454*(1-e-0.29*(t - (-1.63)))
N = 152
R 2 = 0.88
P < 0.0001
Mean Total Length (mm)
Age
200
300
400
500
0
2
4
6
8
10
12

Figure 13. Negative residuals from the $Log_{10}$(age) vs $Log_{10}$(total length) regression (Growth axis) plotted against residuals from the $Log_{10}$(Hg) vs $Log_{10}$(total length) regression (Hg concentration axis) for largemouth bass from A) upstream and B) downstream sites.

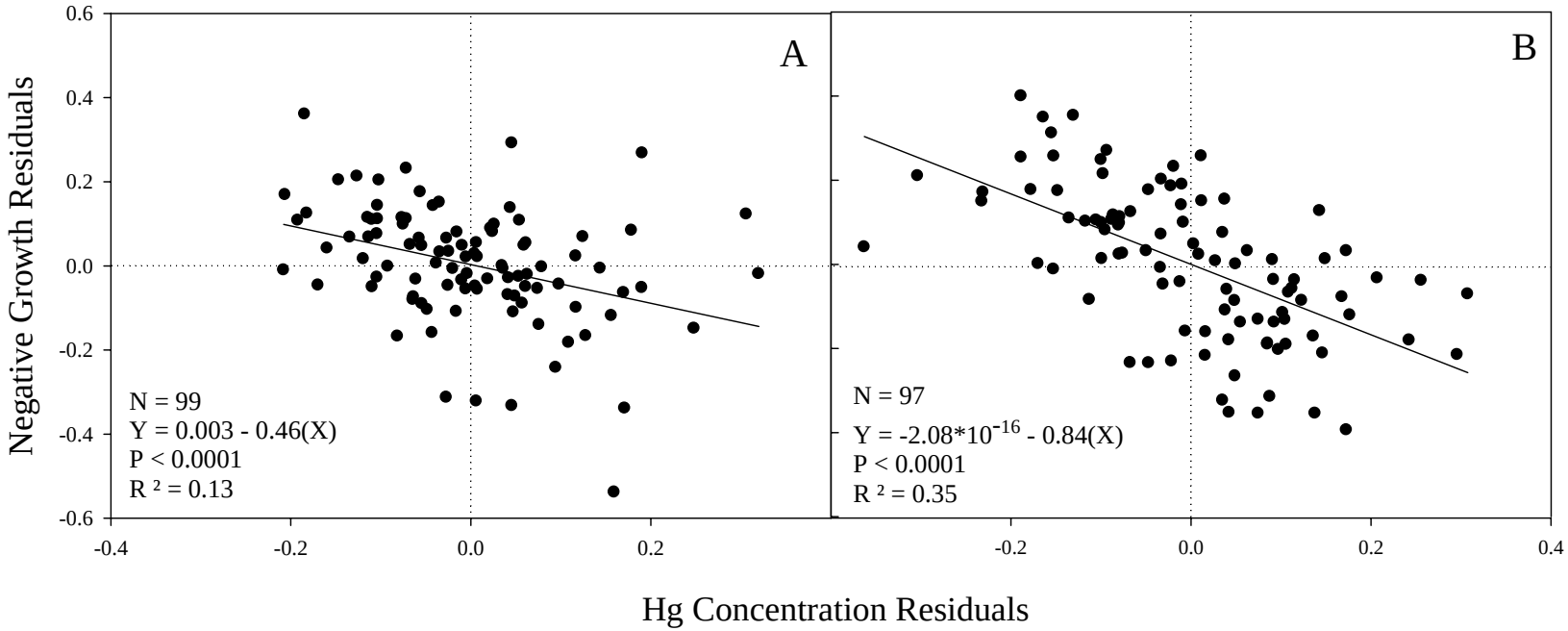

A
B
Negative Growth Residuals
Hg Concentration Residuals
N = 99
Y = 0.003 - 0.46(X)
P < 0.0001
R ² = 0.13
N = 97
Y = -2.08*10-16 - 0.84(X)
P < 0.0001
R ² = 0.35
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4

Figure 14. Negative residuals from the $Log_{10}$(age) vs $Log_{10}$(total length) regression (Growth axis) plotted against residuals from the $Log_{10}$(Hg) vs $Log_{10}$(total length) regression (Hg concentration axis) for southern flounder from all sites.

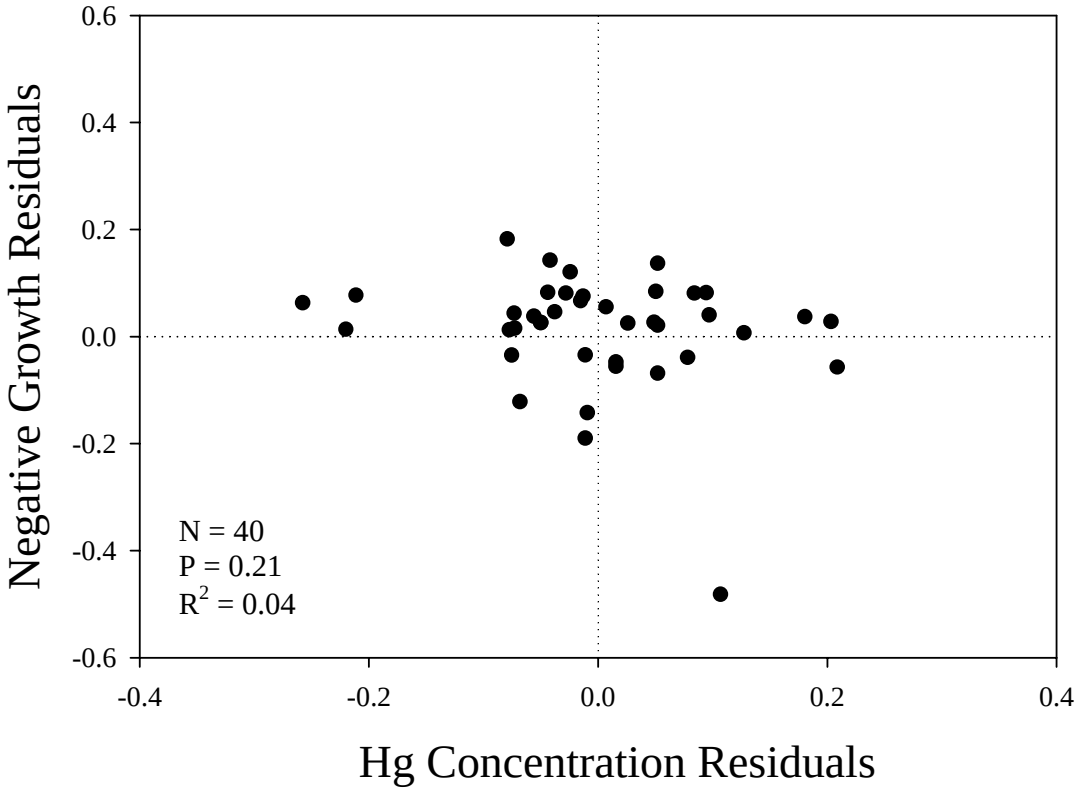

0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
Negative Growth Residuals
Hg Concentration Residuals
N = 40
P = 0.21
R2 = 0.04

Figure 15. Whole body total mercury (Hg) concentration for bluegill (total length) and blue crab (carapace width) from upstream (Tensaw Lake – bluegill; Dennis Lake – blue crab) and downstream (Bay Minette – bluegill; D'Olive Bay – blue crab) sites. Each sample was a composite of 2-4 individuals per size group. Samples were collected in the spring and summer of 2007.

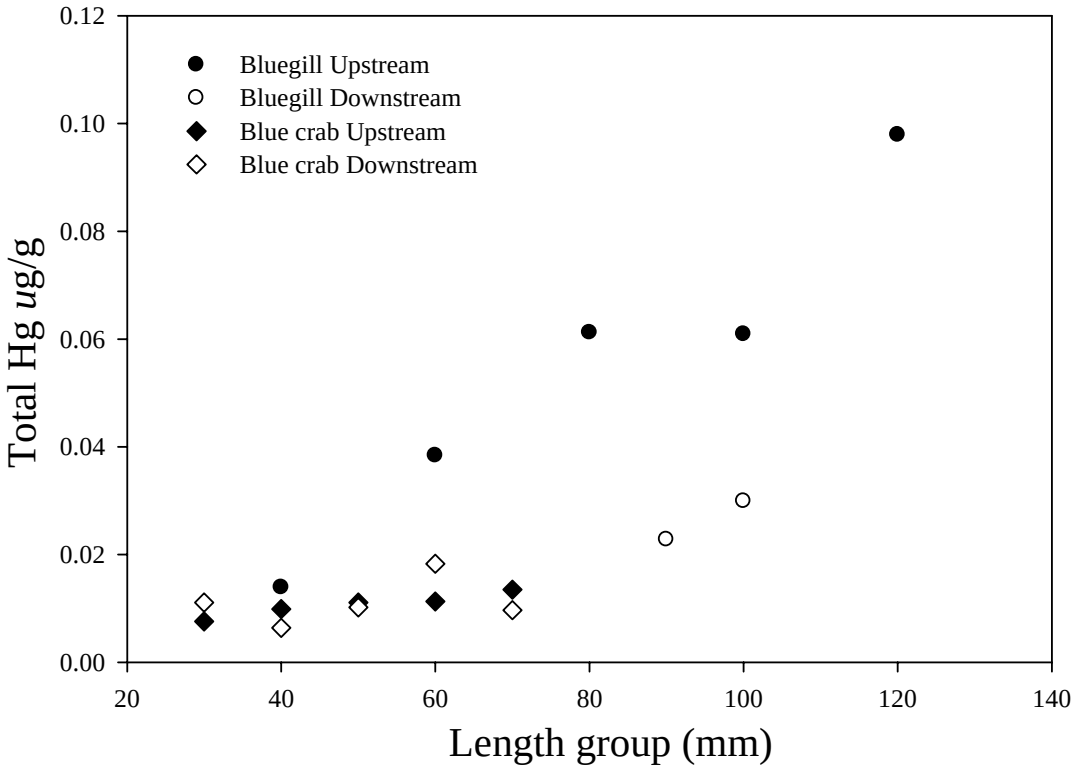

Bluegill Upstream
Bluegill Downstream
Blue crab Upstream
Blue crab Downstream
Total Hg ug/g
0.12
0.10
0.08
0.06
0.04
0.02
0.00
20
40
60
80
100
120
140
Length group (mm)

Figure 16. Average proportional biomass of vertebrates (■) and invertebrates (▭) consumed by largemouth bass (A. 100-230 mm B. 231-310 mm C. 311-360 mm D. 360+ mm) at upstream and downstream sites in 2005-2006. N's are the number of largemouth bass with prey items identified in stomachs (WI=Winter: January-March, SP=Spring: May-June, SU=Summer: July-August, FA=Fall: September-December)

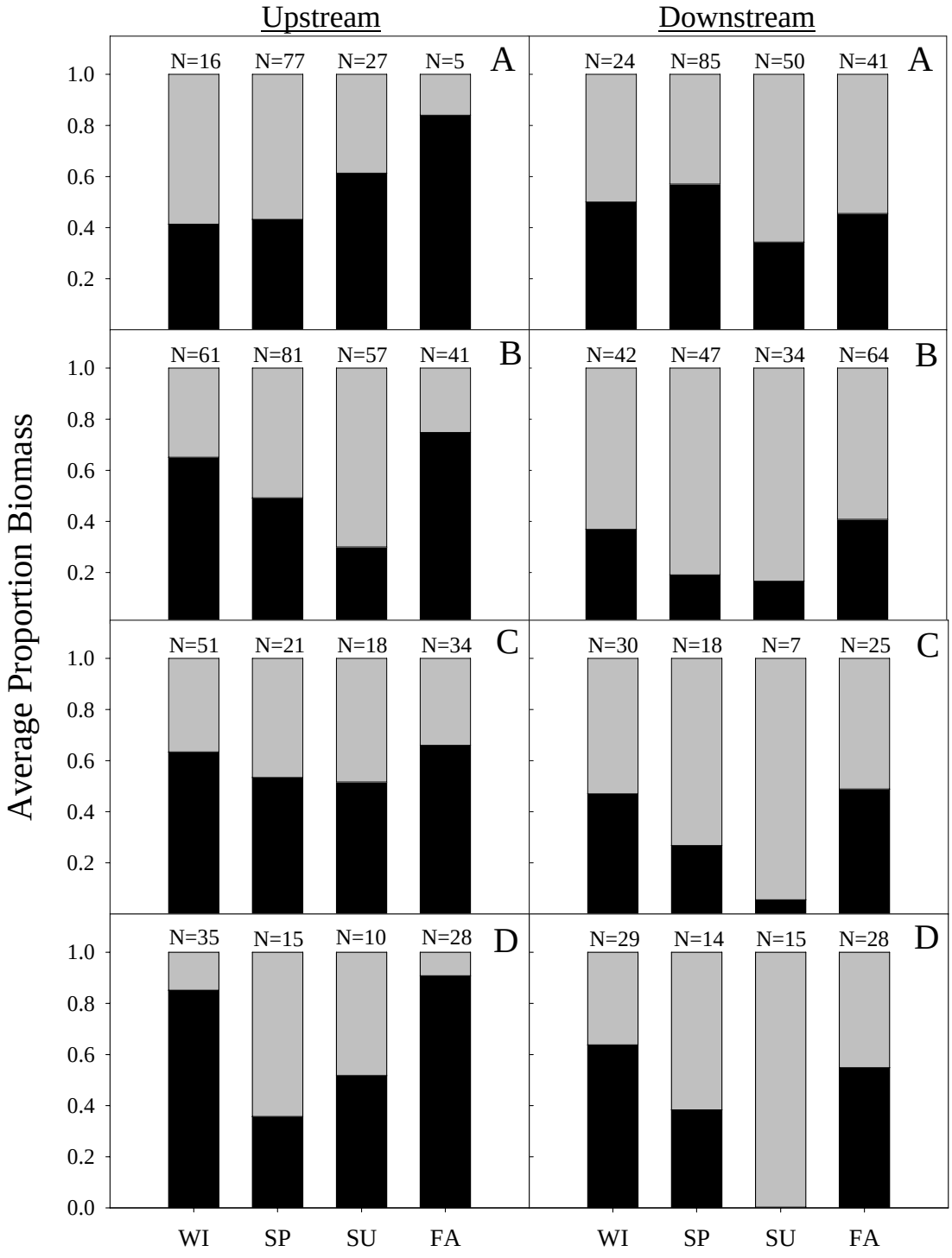

Upstream
Downstream
Average Proportion Biomass
A
B
C
D
N=16
N=77
N=27
N=5
N=24
N=85
N=50
N=41
N=61
N=81
N=57
N=41
N=42
N=47
N=34
N=64
N=51
N=21
N=18
N=34
N=30
N=18
N=7
N=25
N=35
N=15
N=10
N=28
N=29
N=14
N=15
N=28
0.0
0.2
0.4
0.6
0.8
1.0
WI
SP
SU
FA

Figure 17. Average proportional biomass of diet groups consumed by four size classes of largemouth bass at A) upstream and B) downstream sites in 2005-2006. N' are the number of largemouth bass with prey items identified in stomachs. (SUNF = Centrarchidae species, GOBY = Gobiidae and Eleotridae species, CLUP = Clupeidae species, FUND = Fundulidae species, FISH = other or unidentified fish species, VERT = non-fish vertebrates, CRAB = Primarily *Callinectes sapidus* (blue crab) or *Uca longissignalis* (fiddler crab), SRMP = Primarily *Penaeus setifer* (white shrimp) or *Palaemonetes pugi* (grass shrimp), CRAY = Procambarus species, CRUS = Unidentified crustaceans, IVRT = Other invertebrates - primarily insects)

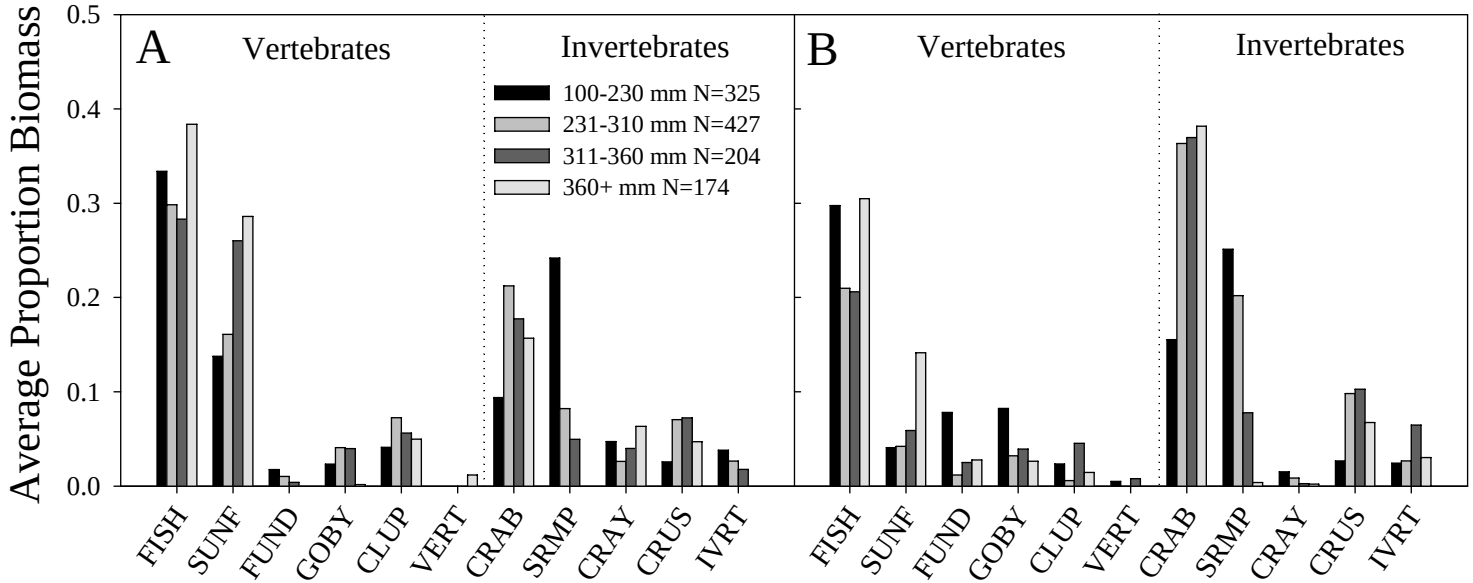

Average Proportion Biomass
0.5
0.4
0.3
0.2
0.1
0.0
A
Vertebrates
Invertebrates
B
Vertebrates
Invertebrates
100-230 mm N=325
231-310 mm N=427
311-360 mm N=204
360+ mm N=174
FISH
SUNF
FUND
GOBY
CLUP
VERT
CRAB
SRMP
CRAY
CRUS
IVRT

Figure 18. Length of individual prey items (total length of fish species, carapace width of crab species and uropod length of shrimp species) plotted against the total length of largemouth bass and southern flounder that consumed them. Data for each species are summed across all sites in 2005-2006. (N's denote number of prey items measured in the stomachs of each grouping.)

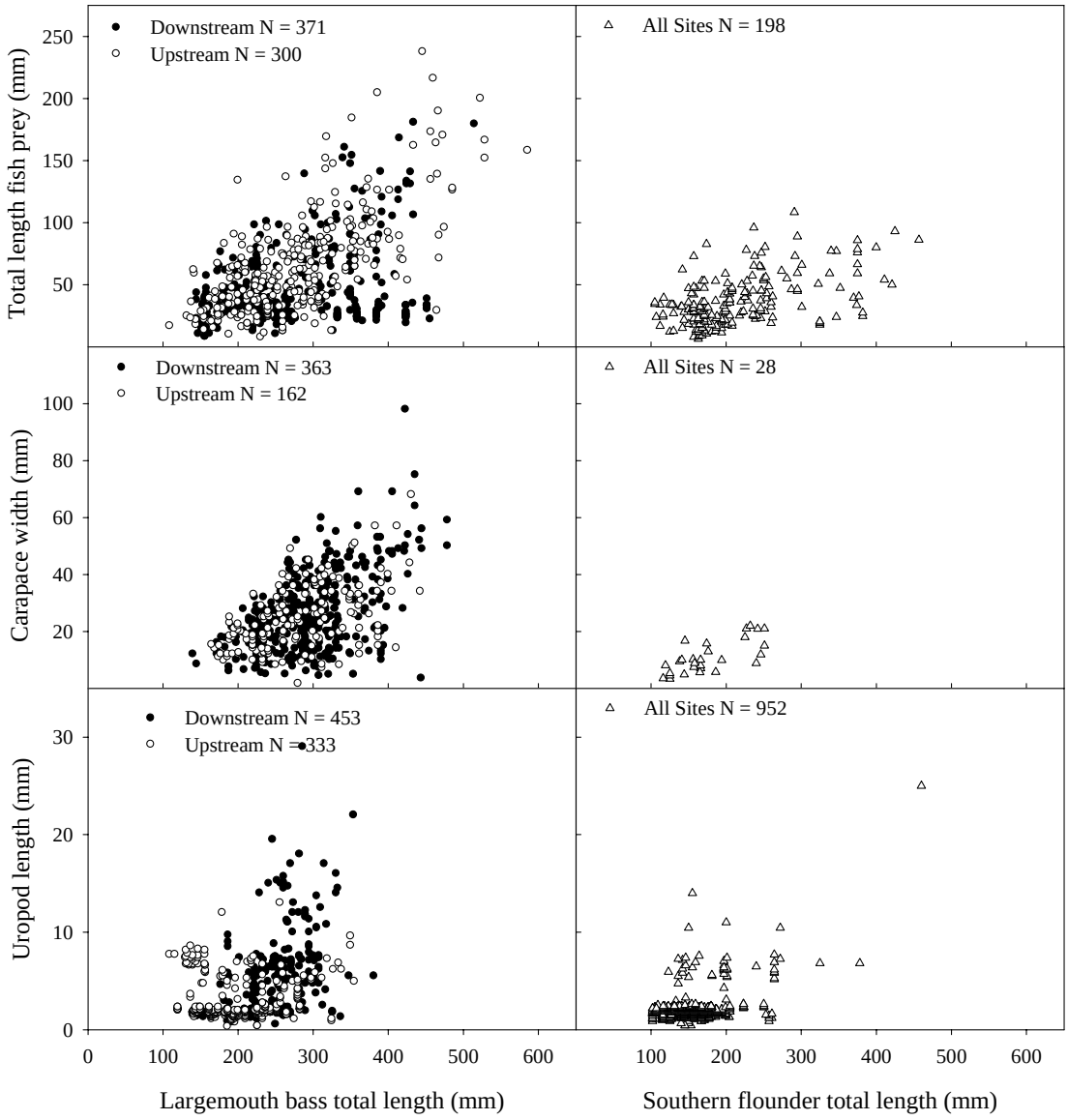

Downstream N = 371
Upstream N = 300
All Sites N = 198
Downstream N = 363
Upstream N = 162
All Sites N = 28
Downstream N = 453
Upstream N = 333
All Sites N = 952
Total length fish prey (mm)
Carapace width (mm)
Uropod length (mm)
Largemouth bass total length (mm)
Southern flounder total length (mm)

Figure 19. Average proportional biomass of vertebrates (■) and invertebrates (▭) consumed by southern flounder in three size groups A) 100-240 mm B) 240-400 mm C) 401+ mm across upstream and downstream sites in 2005-2006. N's are the number of southern flounder with prey items identified in diets. (WI=Winter: January-March, SP=Spring: May-June, SU=Summer: July-August, FA=Fall: September-December)

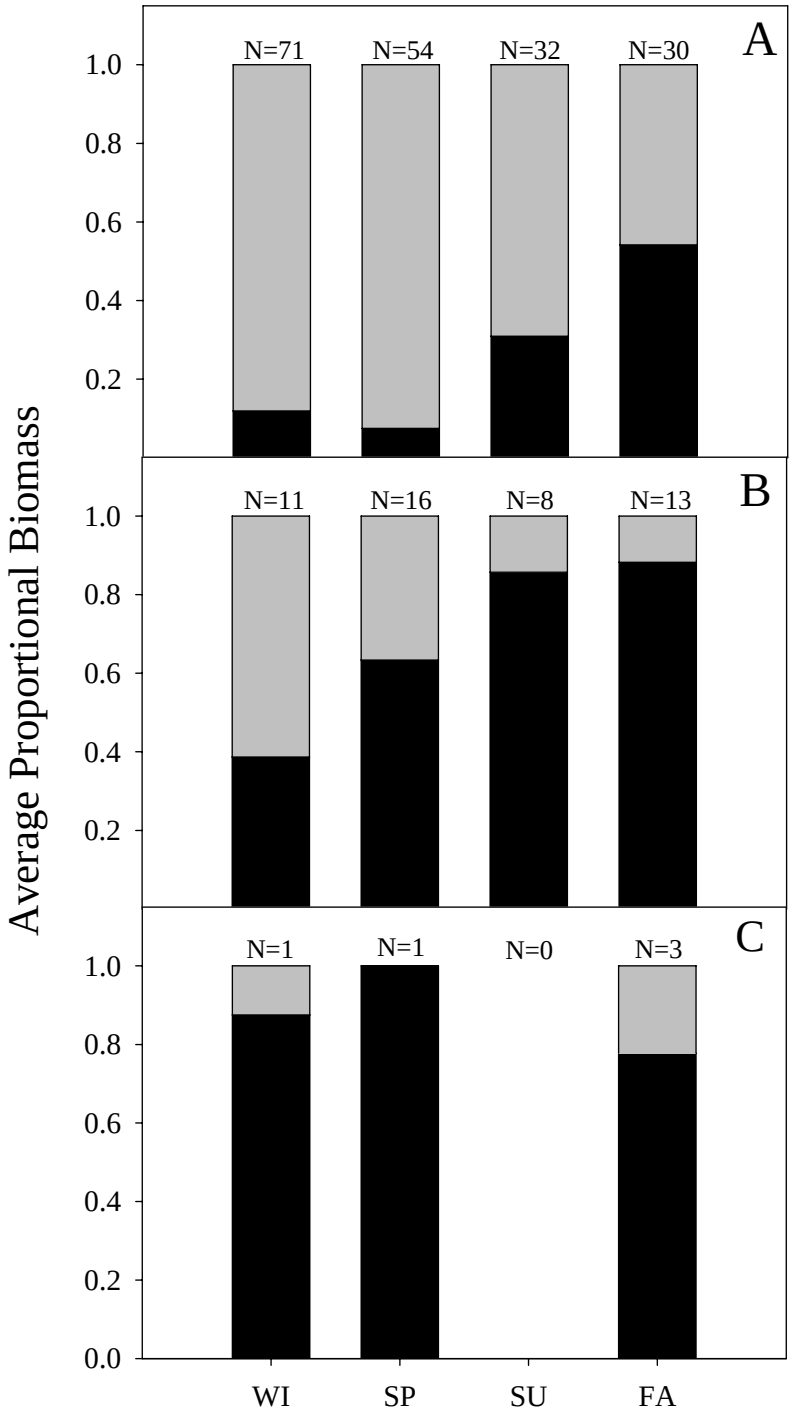

A
B
C
N=71
N=54
N=32
N=30
N=11
N=16
N=8
N=13
N=1
N=1
N=0
N=3
1.0
0.8
0.6
0.4
0.2
0.0
Average Proportional Biomass
WI
SP
SU
FA

Figure 20. Average proportional biomass of diet groups consumed by three size classes of southern flounder across all sites in 2005-2006. N's are the number of southern flounder with prey items identified in diets. (SUNF = Centrarchidae species, GOBY = Gobiidae and Eleotridae species, CLUP = Clupeidae species, FUND = Fundulidae species, FISH = other or unidentified fish species, VERT = non-fish vertebrates, CRAB = Primarily *Callinectes sapidus* (blue crab) or *Uca longissignalis* (fiddler crab), SRMP = Primarily *Penaeus setifer* (white shrimp) or *Palaemonetes pugi* (grass shrimp), CRAY = *Procambarus* species, CRUS = Unidentified crustaceans, IVRT = Other invertebrates - primarily insects)

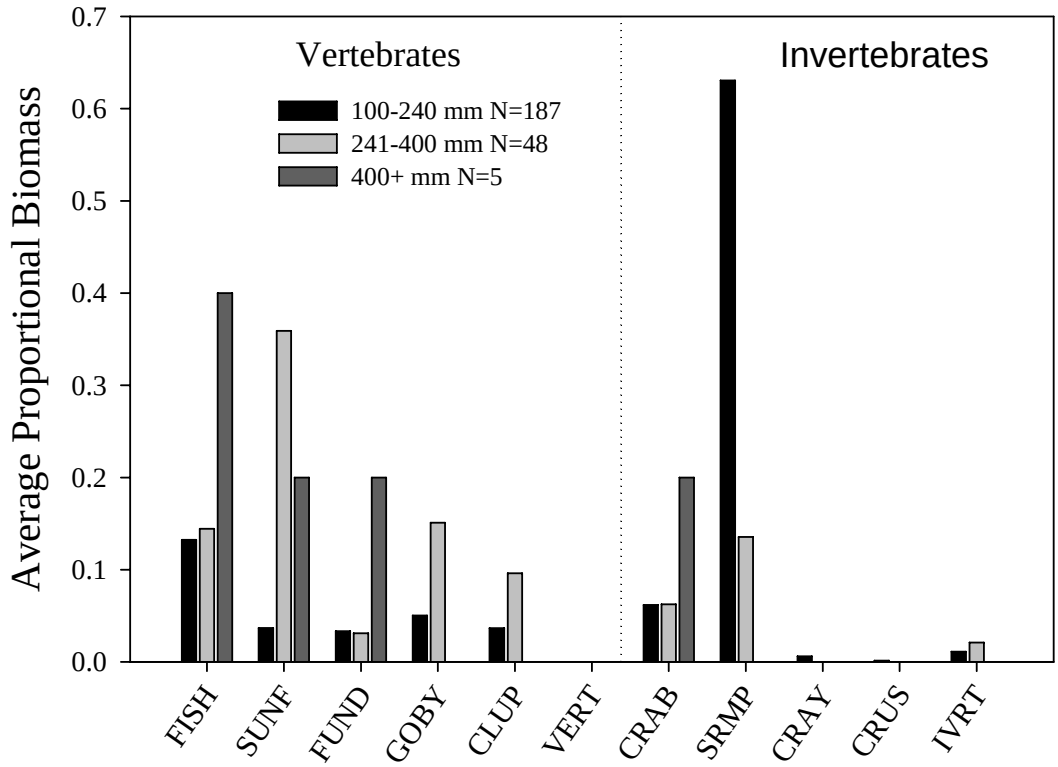

Average Proportional Biomass
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Vertebrates
Invertebrates
100-240 mm N=187
241-400 mm N=48
400+ mm N=5
FISH
SUNF
FUND
GOBY
CLUP
VERT
CRAB
SRMP
CRAY
CRUS
IVRT

Figure 21. A) Model fitting for bioenergetics models simulating Hg accumulation by McReynold's Lake (upstream) and D'Olive Bay (downstream) largemouth bass from age 1.5 – 2.5. Lines indicate model predicted Hg concentrations on a daily time step while circles with error bars represent observed mean Hg concentrations (± 95 % CI) at each site for given ages. B) Model comparison with equal starting weights and Hg concentrations for McReynold's Lake and D'Olive Bay largemouth bass. Simulations began on October 1 and concluded on Sept 30 of the following year. Seasons are - fall: age 1.5 – 1.75; winter: age 1.75 – 2.0; spring: age 2.0 – 2.25; summer: age 2.25 – 2.5.

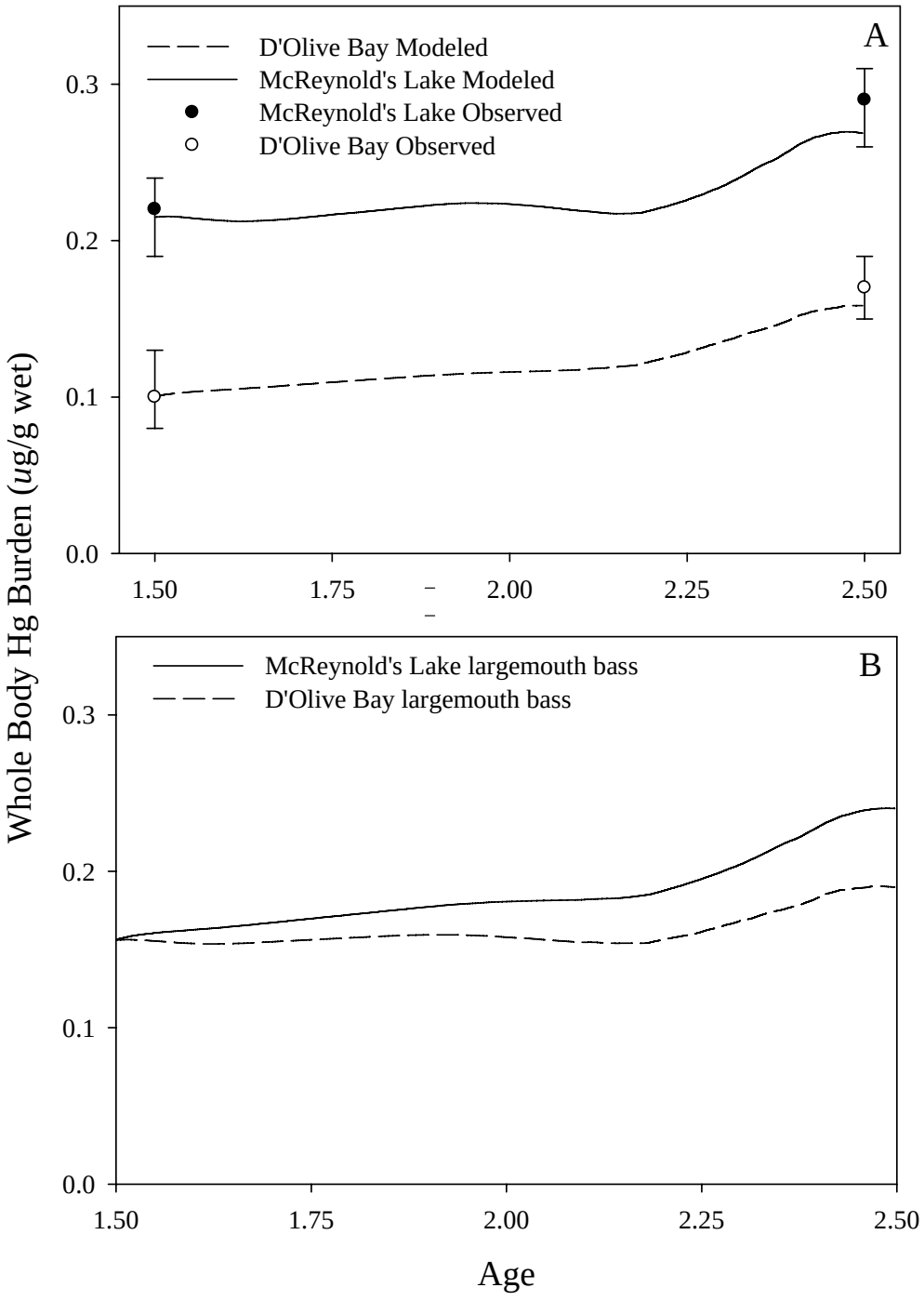

A
D'Olive Bay Modeled
McReynold's Lake Modeled
McReynold's Lake Observed
D'Olive Bay Observed
0.3
0.2
0.1
0.0
1.50
1.75
2.00
2.25
2.50
B
McReynold's Lake largemouth bass
D'Olive Bay largemouth bass
0.3
0.2
0.1
0.0
1.50
1.75
2.00
2.25
2.50
Whole Body Hg Burden (ug/g wet)
Age

Figure 22. Percent of the total difference in whole body Hg concentration from comparison simulations for largemouth bass from McReynold's Lake (upstream) and D'Olive Bay (downstream) that were attributed to each of the four variables (diet proportions, prey Hg concentration, growth and temperature) that differed between sites. To determine the effect of a single variable, each of the four variables were substituted (one at a time) into the opposing site model, while all other variables remained unchanged. The resulting difference was divided by the total difference observed between the two site simulations to obtain the percent of total difference that each term was responsible for.

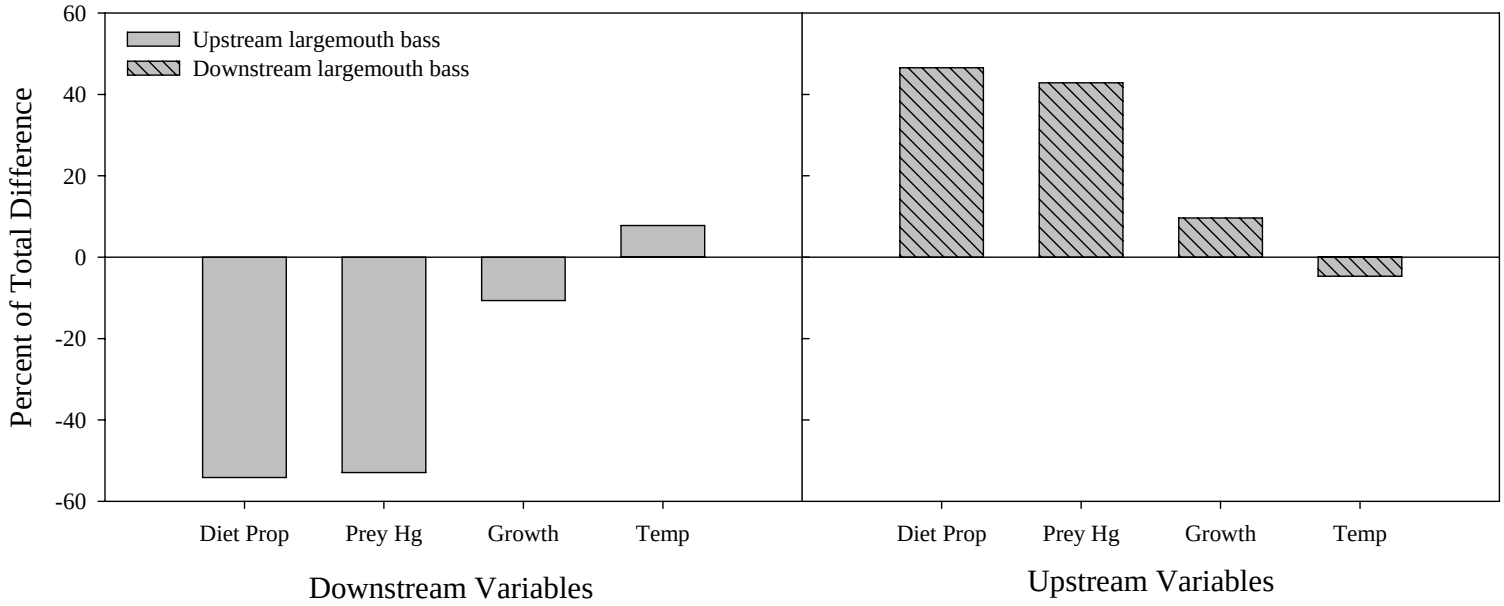

Upstream largemouth bass
Downstream largemouth bass
Percent of Total Difference
60
40
20
0
-20
-40
-60
Diet Prop
Prey Hg
Growth
Temp
Downstream Variables
Diet Prop
Prey Hg
Growth
Temp
Upstream Variables

Figure 23. A) Model fitting for bioenergetics models simulating Hg accumulation by McReynold's Lake (upstream) and D'Olive Bay (downstream) largemouth bass from age 2.5 – 6.5. Lines indicate model predicted Hg concentrations on a daily time step while circles with error bars represent observed mean Hg concentrations (± 95 % CI) at each site for given ages. B) Model comparison with equal starting weights and Hg concentrations for McReynold's Lake and D'Olive Bay largemouth bass. Simulations began on October 1 and concluded on Sept 30 four years later. Whole ages (i.e. 3.0, 4.0, etc.) represent April 1$^{st}$ of each year. Ages ending in 0.5 represent October 1$^{st}$ of each year.

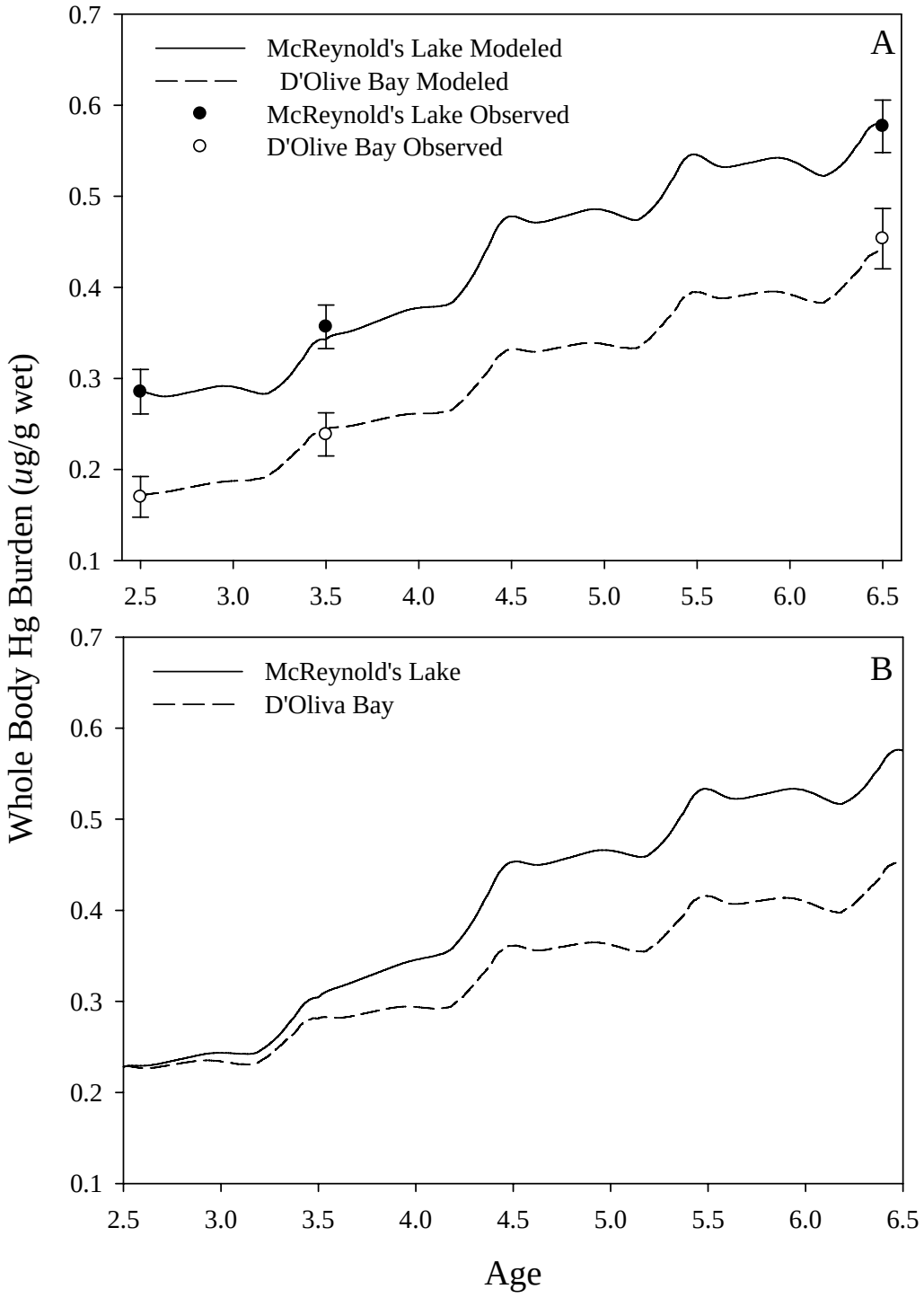

A
McReynold's Lake Modeled
D'Olive Bay Modeled
McReynold's Lake Observed
D'Olive Bay Observed
B
McReynold's Lake
D'Oliva Bay
Whole Body Hg Burden (ug/g wet)
Age
0.1
0.2
0.3
0.4
0.5
0.6
0.7
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5

Figure 24. Percent of the total difference in whole body Hg concentration for largemouth bass from McReynold's Lake (upstream) and D'Olive Bay (downstream) that were attributed to each of the four variables (diet proportions, prey Hg concentration, growth and temperature) that differed between site-specific simulations. To determine the effect of a single variable, each of the four variables were substituted (one at a time) into the opposing site model, while all other variables remained unchanged. The resulting difference was divided by the total difference observed between the two site simulations to obtain the percent of total difference that each term was responsible for.

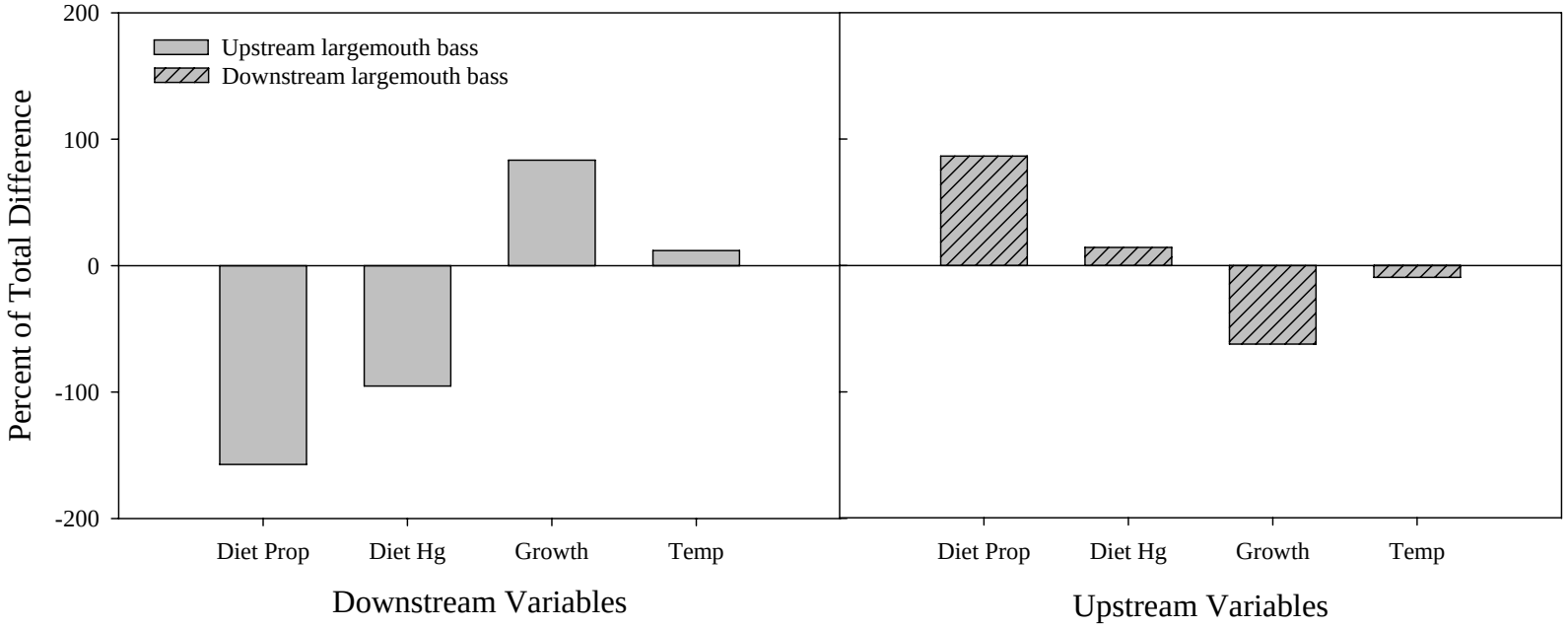

Upstream largemouth bass
Downstream largemouth bass
Percent of Total Difference
200
100
0
-100
-200
Diet Prop
Diet Hg
Growth
Temp
Downstream Variables
Diet Prop
Diet Hg
Growth
Temp
Upstream Variables

Figure 25. Model fitting for bioenergetics models simulating Hg accumulation by southern flounder throughout ages 1.5 – 2.5 throughout the Mobile Delta. Lines indicate model predicted Hg concentrations on a daily time step while circles with error bars represent observed mean Hg concentrations (± 95 % CI) at each site for given ages. Simulations began on October 1 and concluded on Sept 30 of the following year. Seasons are - fall: age 1.5 – 1.75; winter: age 1.75 – 2.0; spring: age 2.0 – 2.25; summer: age 2.25 – 2.5.

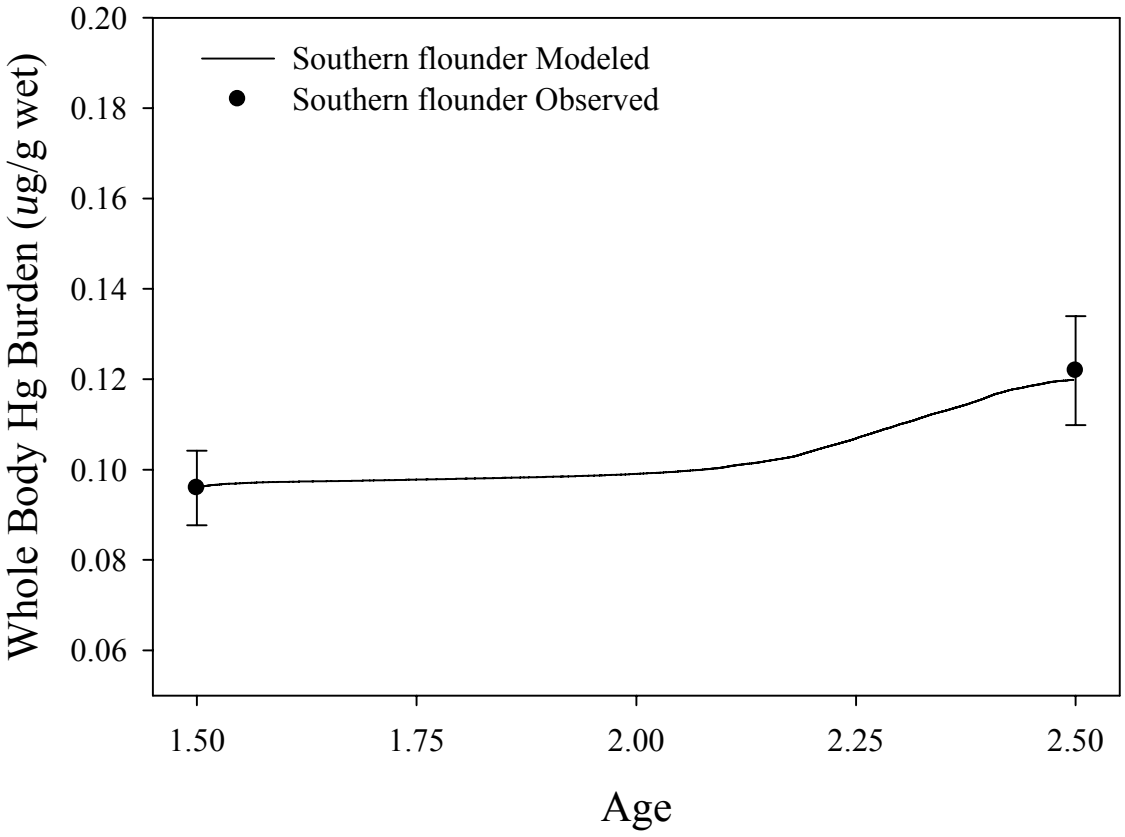

Southern flounder Modeled
Southern flounder Observed
Whole Body Hg Burden (ug/g wet)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
1.50
1.75
2.00
2.25
2.50
Age

Figure 26. The proportional change in whole body Hg concentration for southern flounder attributed to each of the four variables (diet proportions, prey Hg concentration, growth and temperature) that differed between southern flounder and largemouth bass simulations. To determine the effect of a single largemouth bass variable, each of the four variables were substituted (one at a time) into the southern flounder model, while all other southern flounder variables remained unchanged. The resulting difference was divided by the end Hg concentration in the model fitting to obtain the proportional change.

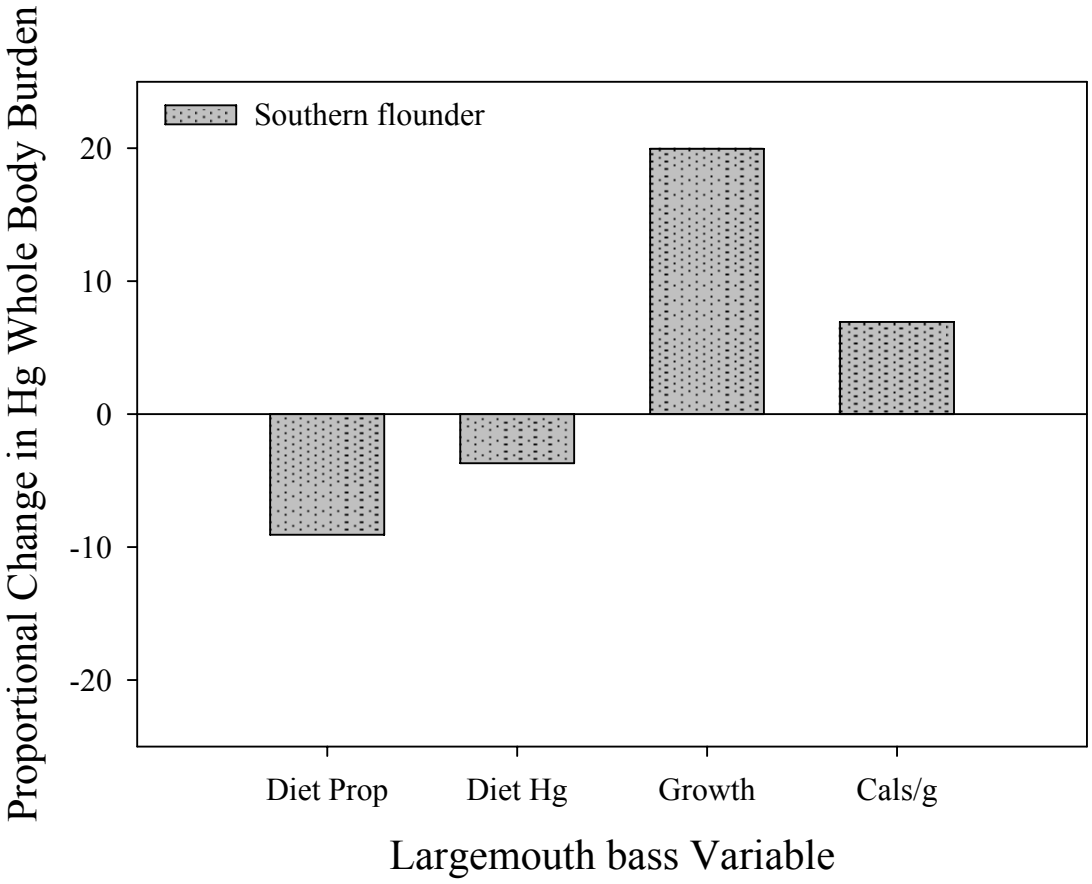

Proportional Change in Hg Whole Body Burden
Southern flounder
20
10
0
-10
-20
Diet Prop
Diet Hg
Growth
Cals/g
Largemouth bass Variable

Figure 27. Whole body Hg concentration (μg/g wet) and mean daily weight increment (g/day) predicted by bioenergetics model fitting runs for largemouth bass (upstream and downstream) and southern flounder modeled for 365 days from ages 1.5 – 2.5. Mean daily temperature (°C) is also shown. Seasons are - fall: age 1.5 – 1.75; winter: age 1.75 – 2.0; spring: age 2.0 – 2.25; summer: age 2.25 – 2.5.

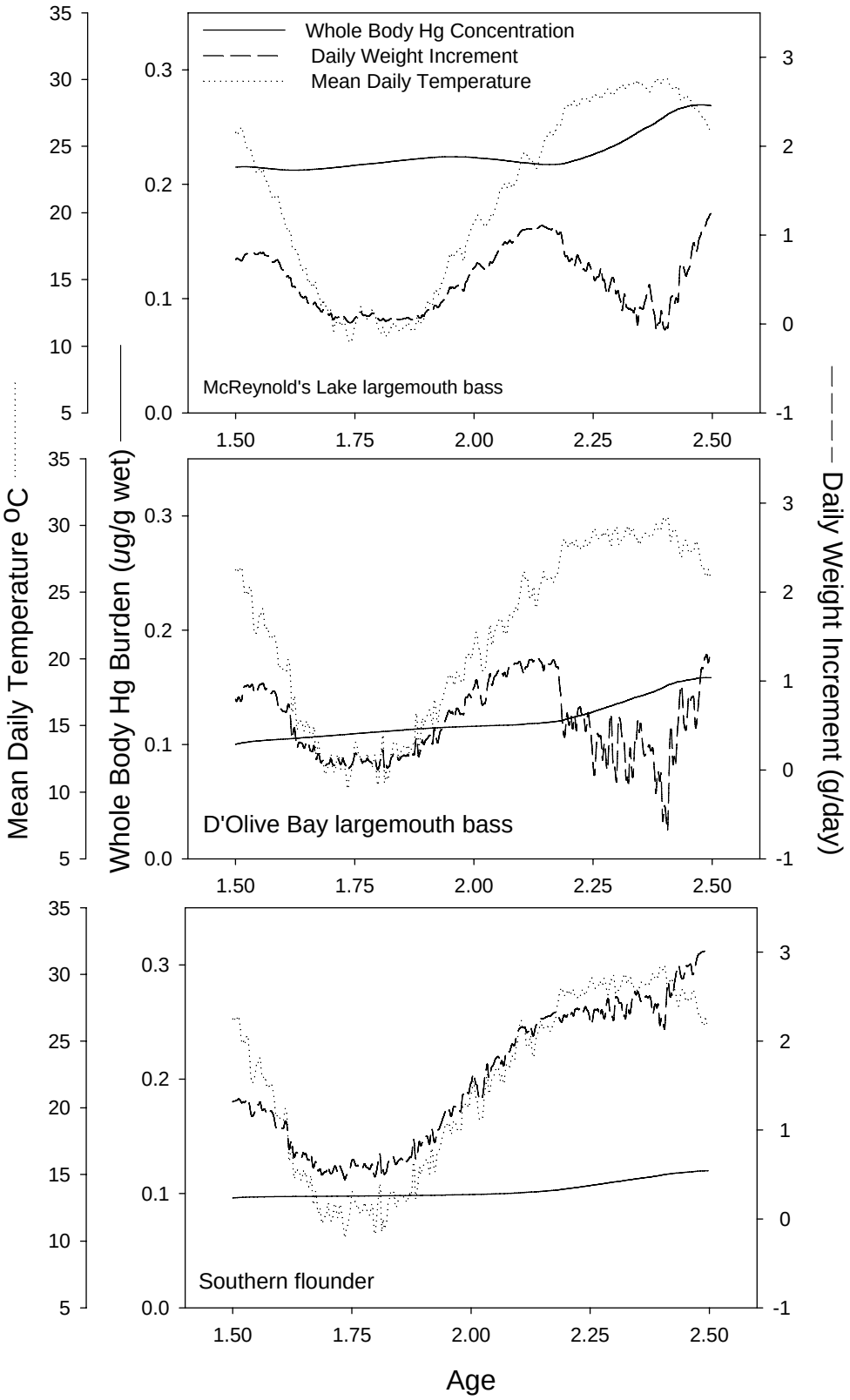

Whole Body Hg Concentration
Daily Weight Increment
Mean Daily Temperature
McReynold's Lake largemouth bass
D'Olive Bay largemouth bass
Southern flounder
Mean Daily Temperature °C
Whole Body Hg Burden (ug/g wet)
Daily Weight Increment (g/day)
Age

Figure 28. Probability that a largemouth bass of a given size from upstream and downstream locations will exceed the common 0.3 μg/g minimum advisory level. Dotted lines surrounding the probability plot are 95% confidence intervals. Solid straight lines are shown to aid in referencing size class to probabilities for each region. * indicates size ranges for a given probability that had 95% confidence intervals that did not overlap between regions. The size distributions of largemouth bass harvested for the entire Mobile Delta are also shown (data averaged from creel surveys conducted in 2004 and 2006; Alabama Dept. Conservation and Natural Resources 2006, unpublished data). The dotted line indicates a theoretical 356 mm minimum length limit for largemouth bass.

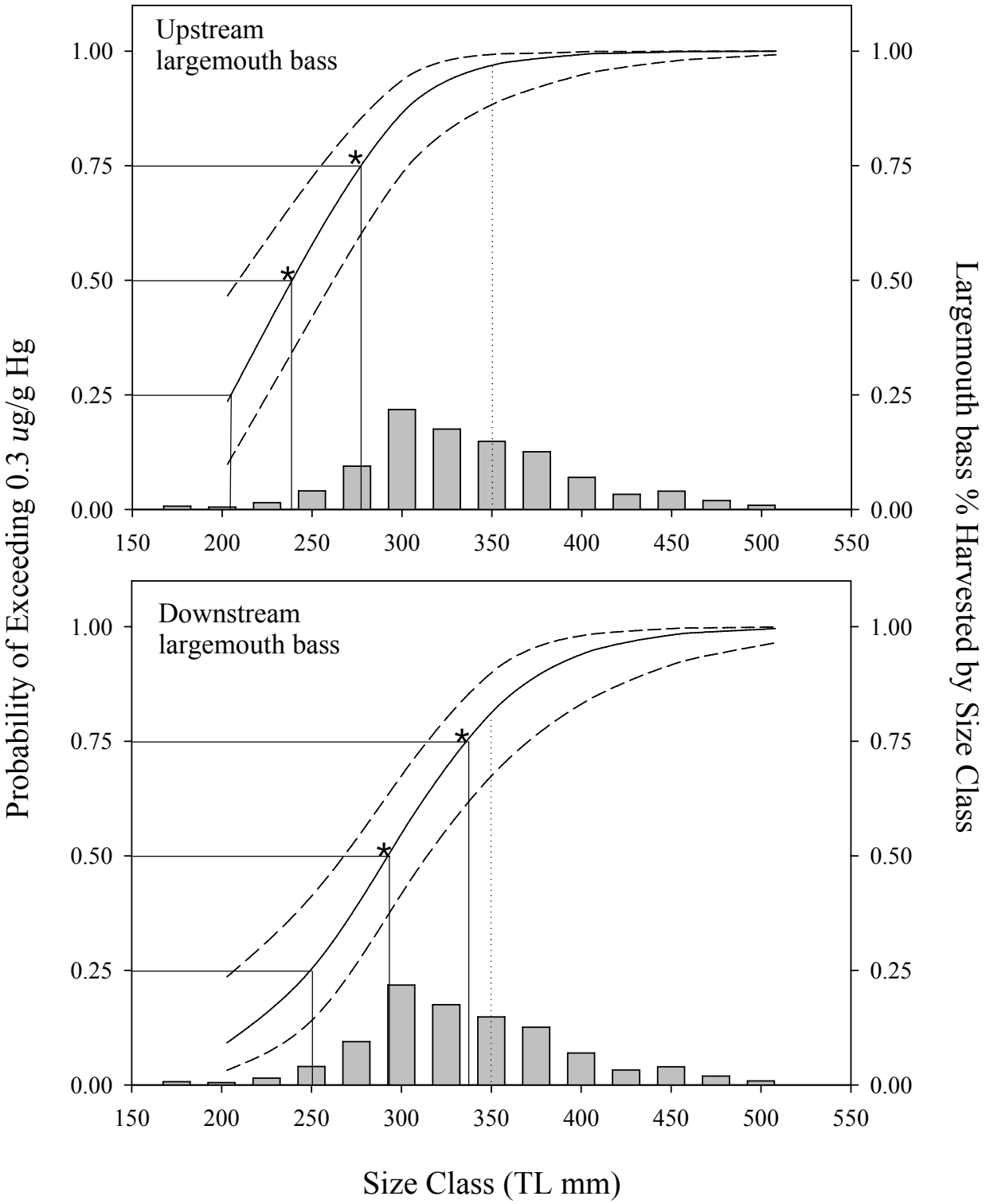

Upstream
largemouth bass
Downstream
largemouth bass
Probability of Exceeding 0.3 ug/g Hg
Largemouth bass % Harvested by Size Class
Size Class (TL mm)
1.00
0.75
0.50
0.25
0.00
150
200
250
300
350
400
450
500
550

Figure 29. Probability that a largemouth bass of a given size from upstream and downstream locations will exceed the common 1.0 μg/g minimum advisory level. Dotted lines surrounding the probability plot are 95% confidence intervals. Solid straight lines are shown to aid in referencing size class to probabilities for each region. * indicates size ranges for a given probability that had 95% confidence intervals that did not overlap between regions. The size distributions of largemouth bass harvested for the entire Mobile Delta are also shown (data averaged from creel surveys conducted in 2004 and 2006; Alabama Dept. Conservation and Natural Resources 2006, unpublished data). The dotted line indicates a theoretical 356 mm minimum length limit for largemouth bass.

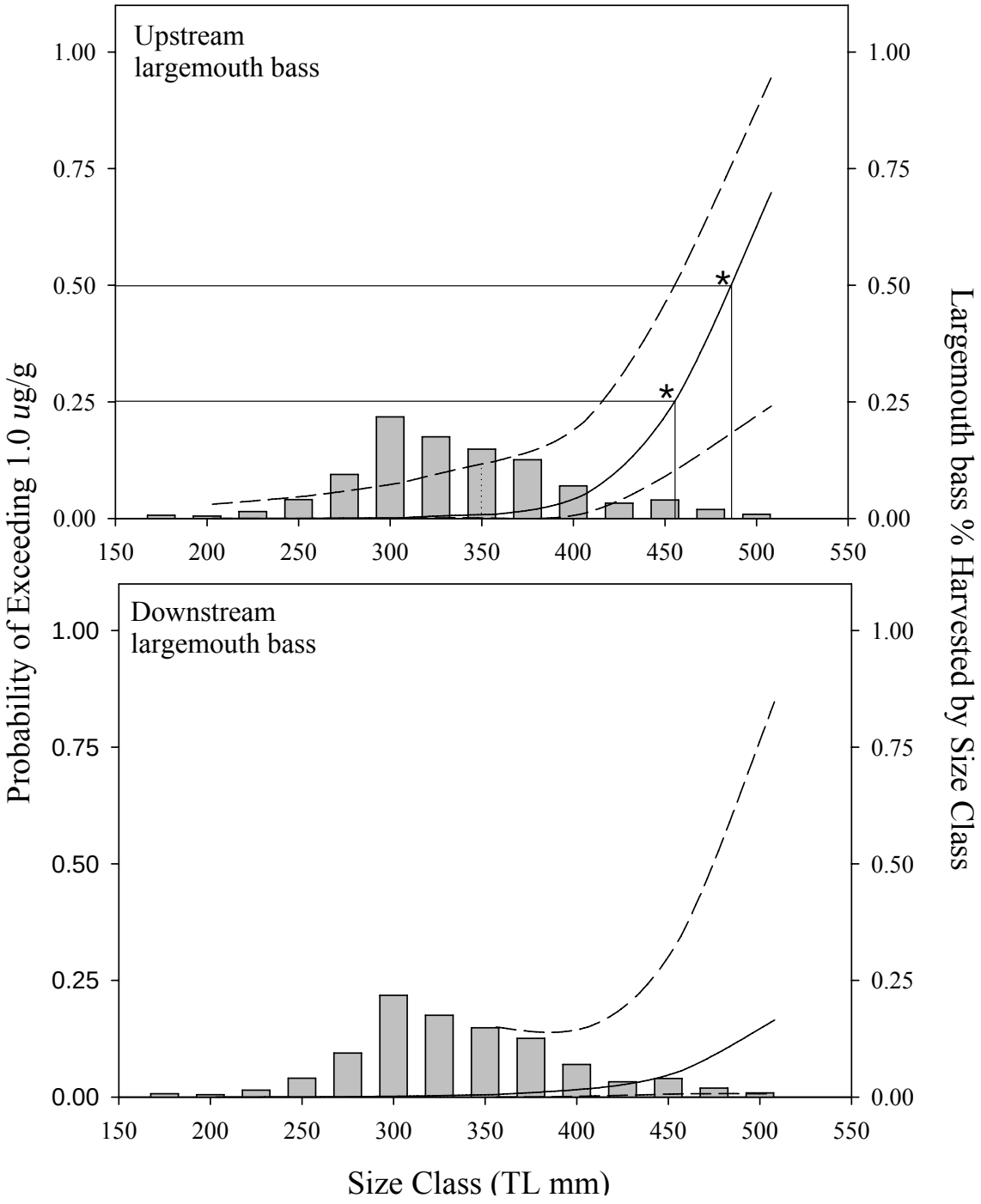

Upstream
largemouth bass
Downstream
largemouth bass
Probability of Exceeding 1.0 ug/g
Largemouth bass % Harvested by Size Class
Size Class (TL mm)
1.00
0.75
0.50
0.25
0.00
150
200
250
300
350
400
450
500
550
*
*

Figure 30. Concentrations (ppm) of six elements (corresponding to those which met criteria for analysis in largemouth bass and southern flounder otoliths) in water samples collected from the Mobile Delta, regressed against salinity measured at 1 m at the time of collection. Samples were collected in June, August, and October of 2005 and in March and October of 2006. Linear regression and two-dimensional Klomogrov-Smirnov (2DKS) tests are used to describe the distributions ($\diamondsuit$ indicates values producing $D_{BKS}$).

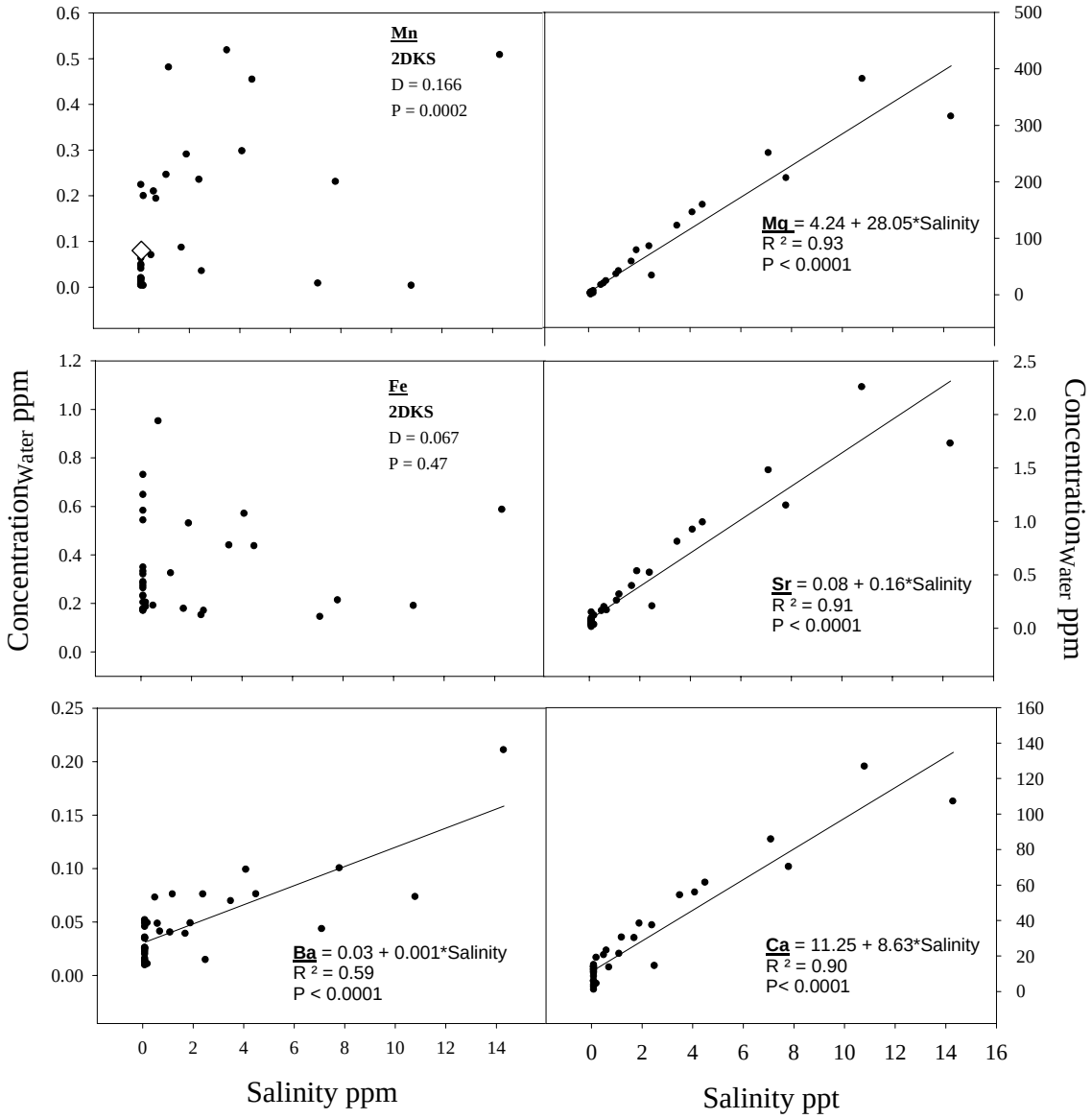

Mn
2DKS
D = 0.166
P = 0.0002
Mg = 4.24 + 28.05*Salinity
R ² = 0.93
P < 0.0001
Fe
2DKS
D = 0.067
P = 0.47
Sr = 0.08 + 0.16*Salinity
R ² = 0.91
P < 0.0001
Ba = 0.03 + 0.001*Salinity
R ² = 0.59
P < 0.0001
Ca = 11.25 + 8.63*Salinity
R ² = 0.90
P< 0.0001
Concentration water ppm
Concentration water ppm
Salinity ppm
Salinity ppt

Figure 31. Element:Ca ratios of six elements (corresponding to those which met criteria for analysis in largemouth bass and southern flounder otoliths) in water samples collected from the Mobile Delta regressed against salinity measured at 1 m at the time of collection. Samples were collected in June, August, and October of 2005 and in March and October of 2006. Linear regression and two-dimensional Klomogrov-Smirnov (2DKS) tests are used to describe the distributions (◇ indicates values producing $D_{BKS}$).

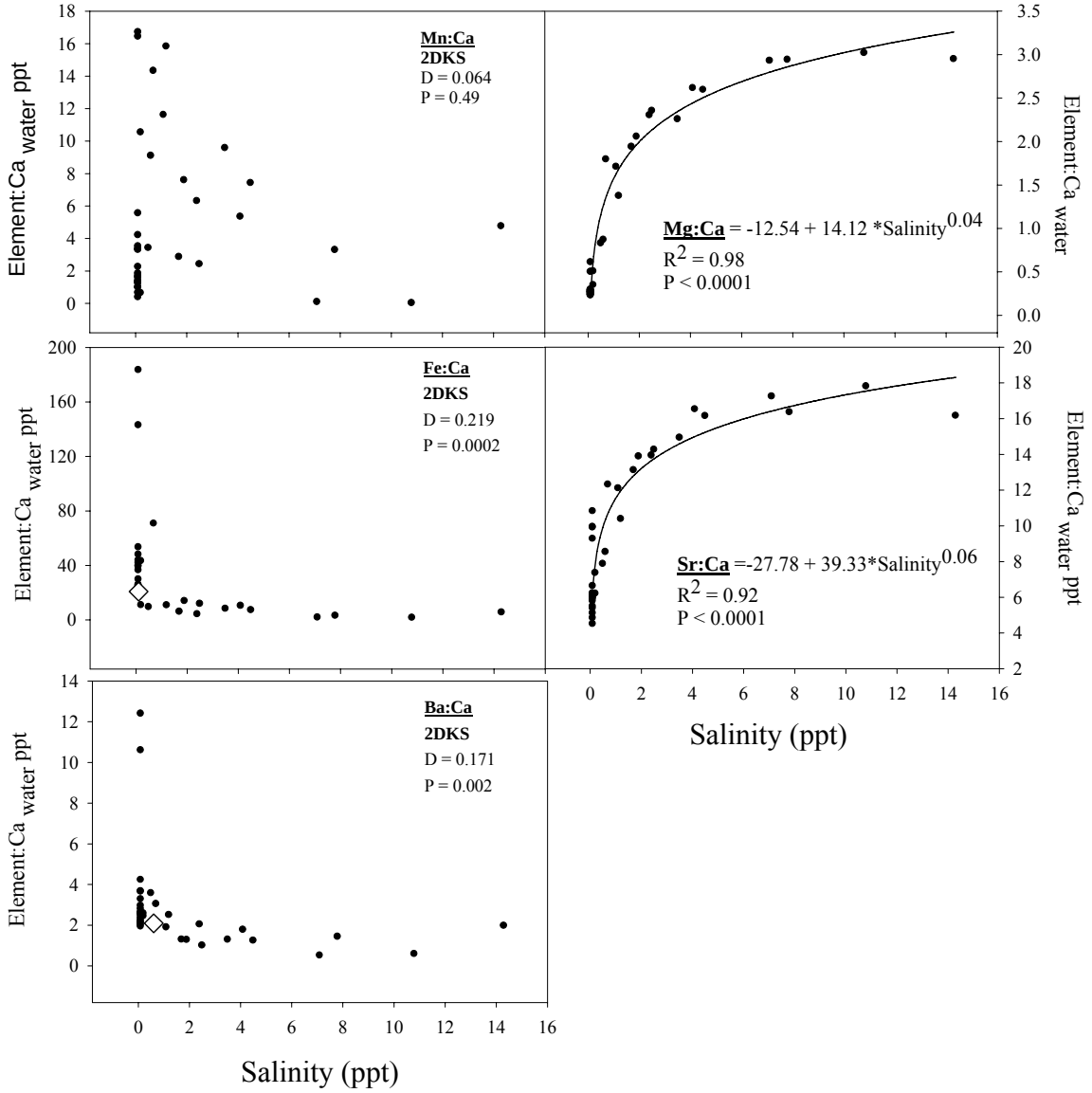

Mn:Ca
2DKS
D = 0.064
P = 0.49
Mg:Ca = -12.54 + 14.12 *Salinity^0.04
R2 = 0.98
P < 0.0001
Fe:Ca
2DKS
D = 0.219
P = 0.0002
Sr:Ca =-27.78 + 39.33*Salinity^0.06
R2 = 0.92
P < 0.0001
Ba:Ca
2DKS
D = 0.171
P = 0.002
Element:Ca water ppt
Salinity (ppt)

Figure 32. $Log_{10}$ transformed strontium (Sr) concentrations (ppm) measured at the edge (approximately the last 20 microns) of A) largemouth bass and B) southern flounder otoliths from fish collected in October 2005 and C) largemouth bass collected in October 2006. Values are regressed against $Log_{10}$ transformed Sr concentrations (ppm) in water samples taken concurrently with fish collection. Water Sr values used for Dennis Lake, Gravine Island, and Bay Minette were averaged from water samples collected during early (Oct 3-4, 2005) and mid October (Oct 14-16, 2005).

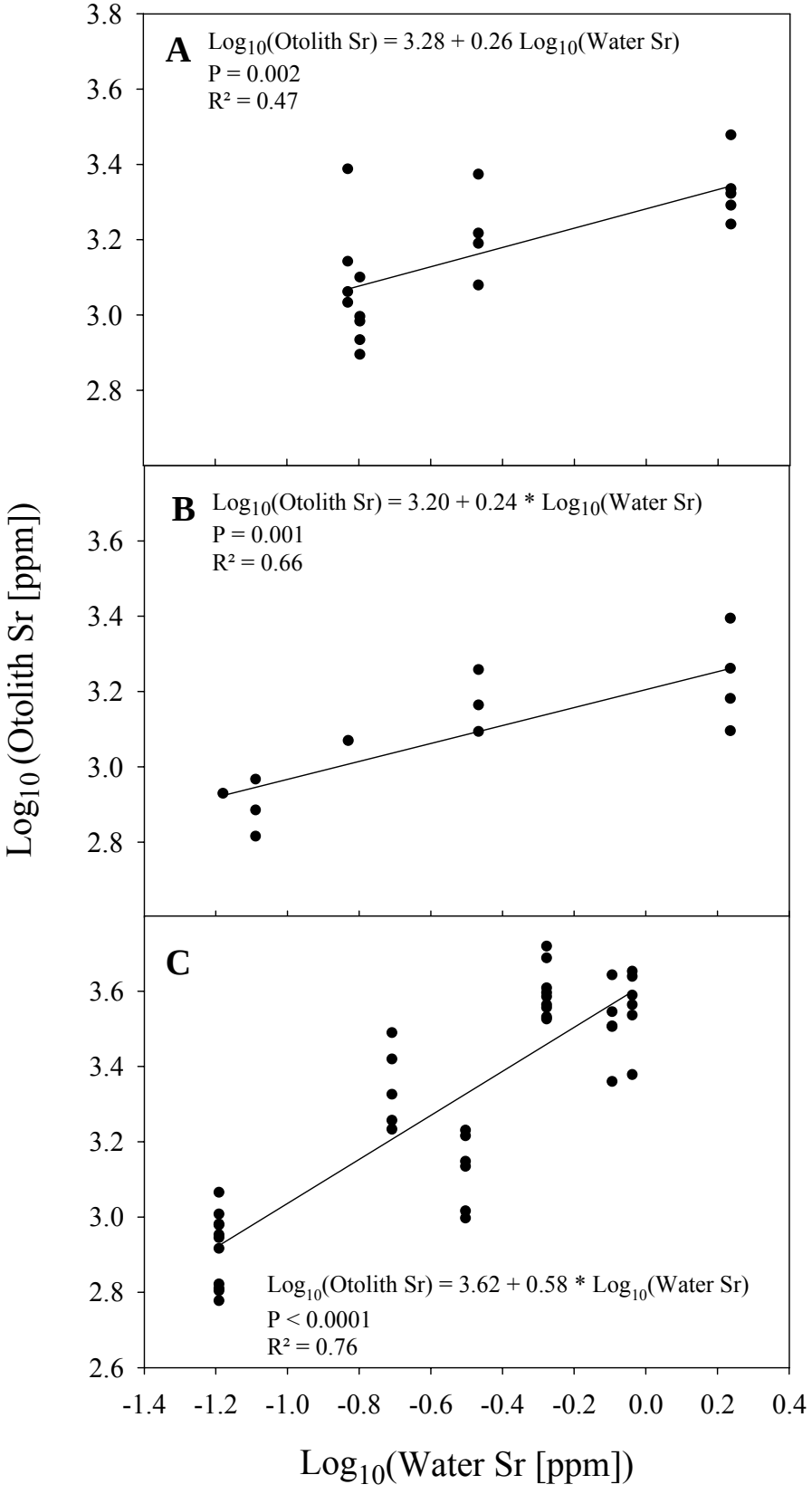

A
Log10(Otolith Sr) = 3.28 + 0.26 Log10(Water Sr)
P = 0.002
R² = 0.47
B
Log10(Otolith Sr) = 3.20 + 0.24 * Log10(Water Sr)
P = 0.001
R² = 0.66
C
Log10(Otolith Sr) = 3.62 + 0.58 * Log10(Water Sr)
P < 0.0001
R² = 0.76
Log10 (Otolith Sr [ppm])
Log10(Water Sr [ppm])

Figure 33. Transect profile plots of otolith strontium (Sr) for upstream and downstream largemouth bass. Site, month and year of collection, and age of each largemouth bass are given. Years along x-axis indicate location of each annulus and are assumed to indicate April 1$^{st}$ of each year.

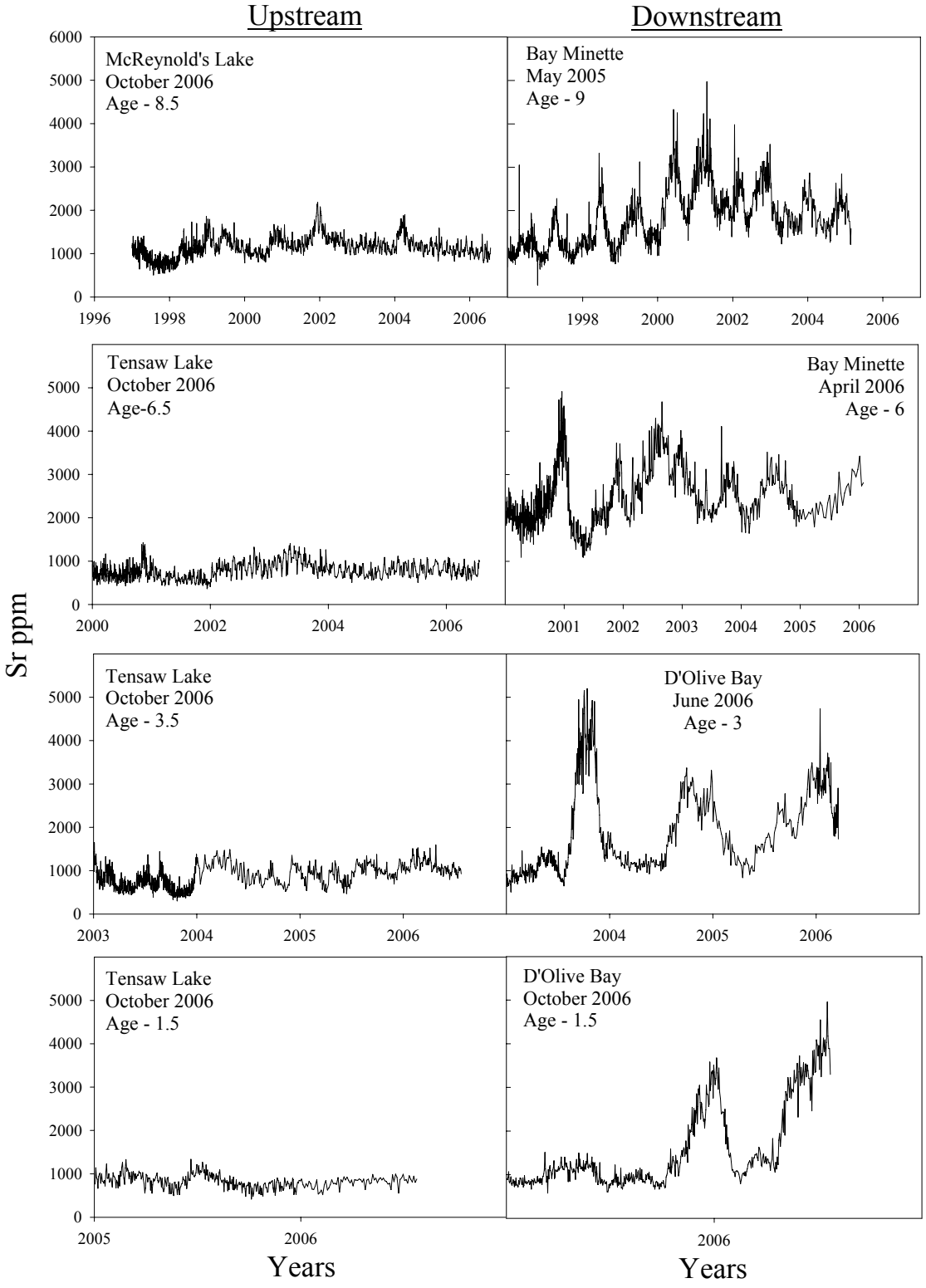

Upstream
Downstream
McReynold's Lake
October 2006
Age - 8.5
Bay Minette
May 2005
Age - 9
Tensaw Lake
October 2006
Age-6.5
Bay Minette
April 2006
Age - 6
Tensaw Lake
October 2006
Age - 3.5
D'Olive Bay
June 2006
Age - 3
Tensaw Lake
October 2006
Age - 1.5
D'Olive Bay
October 2006
Age - 1.5
Sr ppm
Years
Years

Figure 34. Strontium (Sr) concentrations across otolith transects from six southern flounder collected at upstream and downstream sites. Site, month and year of collection, and age of each individual are given. Years along x-axis indicate location of each annulus and are assumed to indicate January 1$^{st}$ of each year.

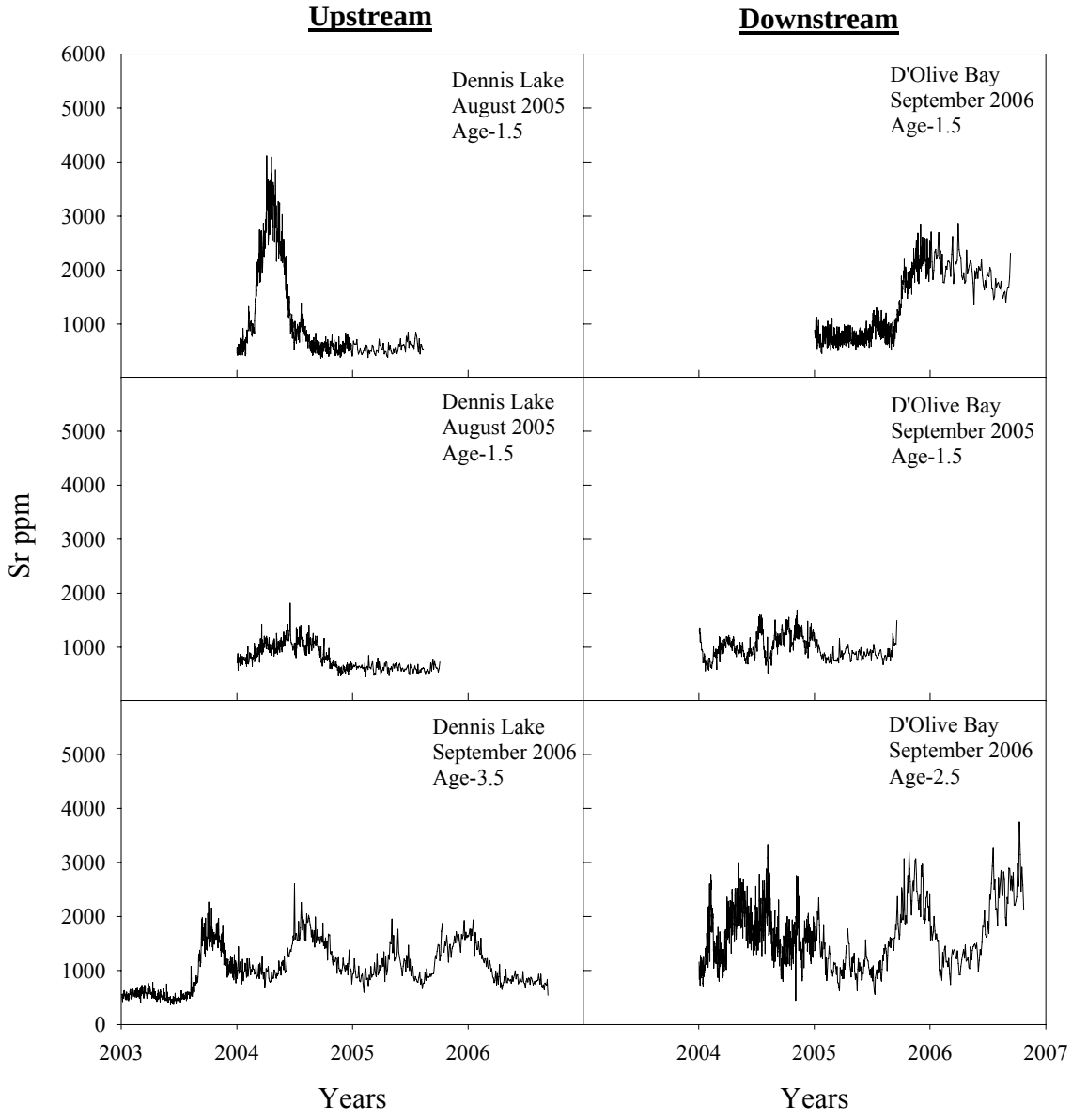

Upstream
Downstream
Dennis Lake
August 2005
Age-1.5
D'Olive Bay
September 2006
Age-1.5
Dennis Lake
August 2005
Age-1.5
D'Olive Bay
September 2005
Age-1.5
Dennis Lake
September 2006
Age-3.5
D'Olive Bay
September 2006
Age-2.5
Sr ppm
Years
Years

Figure 35. Transect profile plots of magnesium (Mg) and barium (Ba) concentrations across otolith transects for largemouth bass collected at Tensaw Lake (Upstream) and largemouth bass collected at D'Olive Bay (Downstream). Site, month and year of collection, and age of each individual are given. Years along x-axis indicate location of each annulus and are assumed to indicate April 1$^{st}$ of each year.

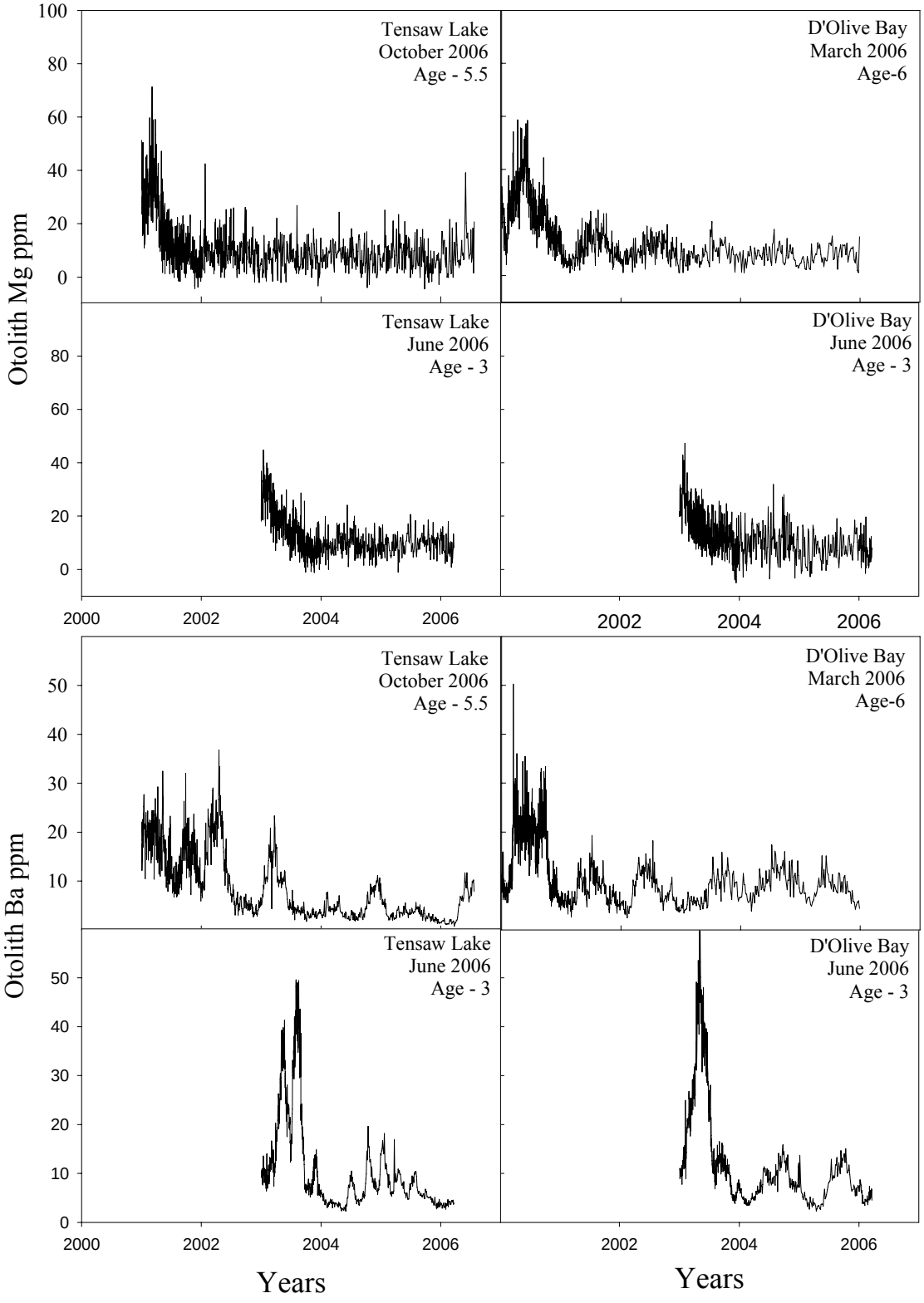

Otolith Mg ppm
Otolith Ba ppm
Tensaw Lake
October 2006
Age - 5.5
D'Olive Bay
March 2006
Age-6
Tensaw Lake
June 2006
Age - 3
D'Olive Bay
June 2006
Age - 3
Years
Years

Figure 36. Magnesium (Mg), manganese (Mg), and barium (Ba) concentrations across the otolith of an age-2.5 southern flounder collected at D'Olive Bay on October 22, 2006. Years along x-axis indicate location of each annuli and are assumed to indicate January 1st of each year.

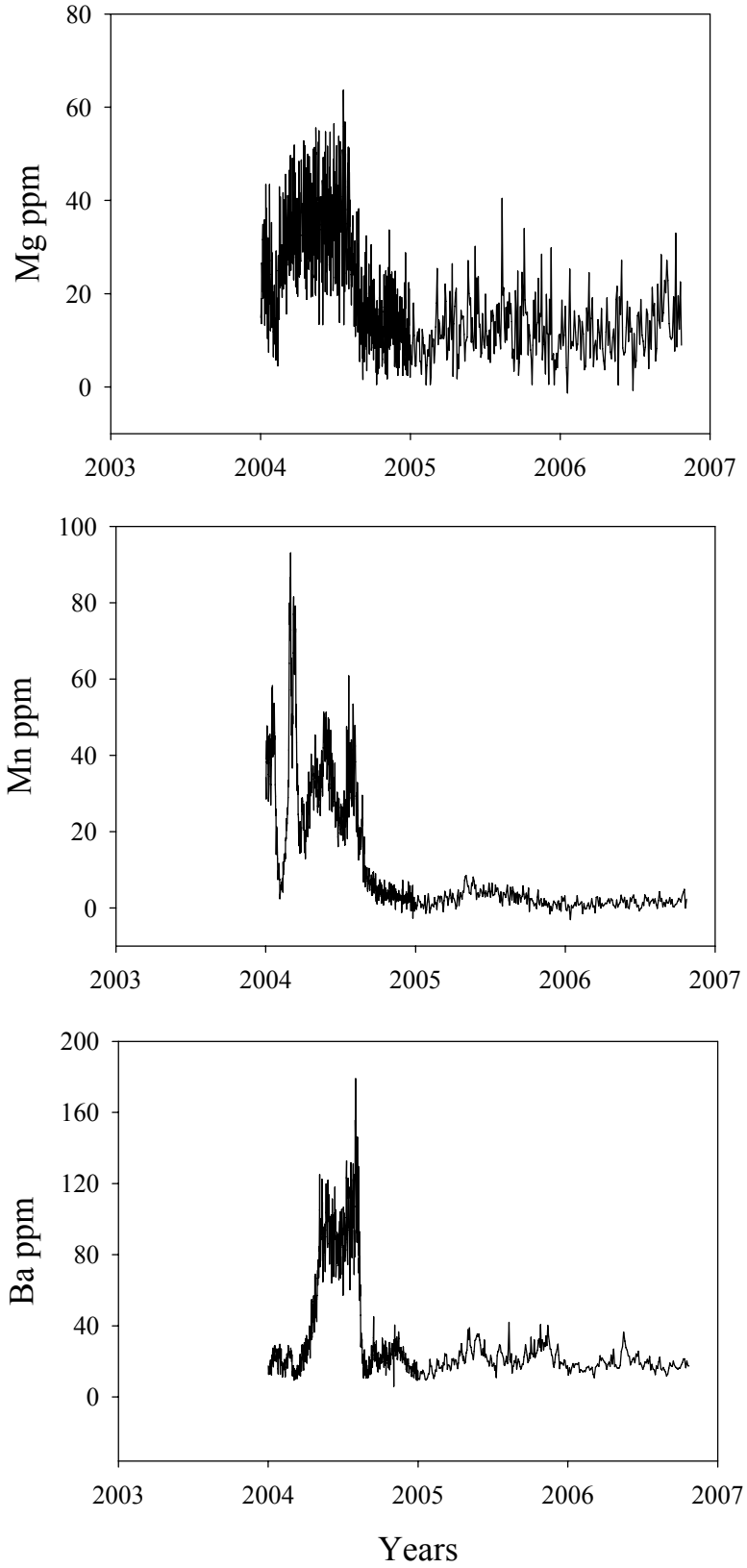

Mg ppm
80
60
40
20
0
2003
2004
2005
2006
2007
Mn ppm
100
80
60
40
20
0
2003
2004
2005
2006
2007
Ba ppm
200
160
120
80
40
0
2003
2004
2005
2006
2007
Years

Figure 37. Mean strontium (Sr) concentrations (ppm) for otoliths of all largemouth bass collected from each site. Strontium concentrations were averaged across the entire otolith for each largemouth bass. An average otolith Sr concentration was then calculated for all largemouth bass from each specific site. N indicates the number of largemouth bass with otolith data at each site. Error bars represent 1 SE.

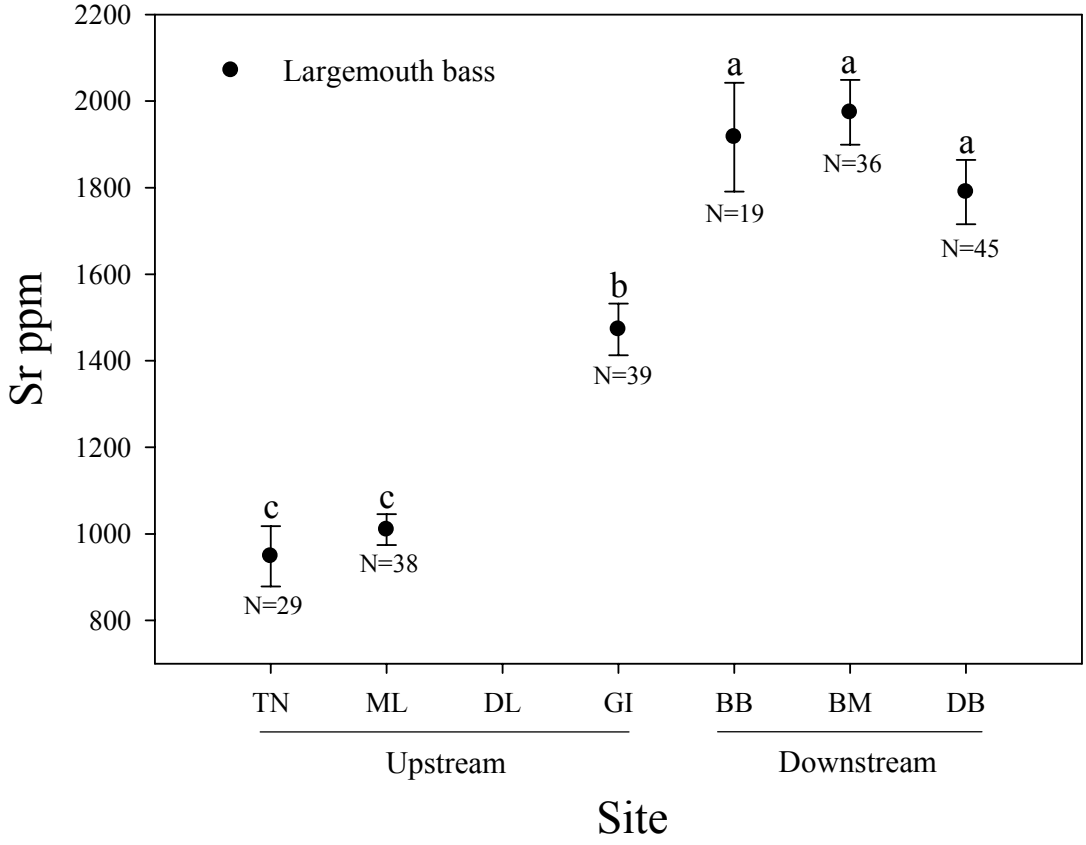

Largemouth bass
Sr ppm
2200
2000
1800
1600
1400
1200
1000
800
a
a
a
b
c
c
N=19
N=36
N=45
N=39
N=29
N=38
TN
ML
DL
GI
BB
BM
DB
Upstream
Downstream
Site

Figure 38. Mean strontium (Sr) concentrations (ppm) for otoliths of all southern flounder collected from each site. Strontium concentrations were averaged across the entire otolith for each southern flounder. An average otolith Sr concentration was then calculated for all southern flounder from each specific site. N's indicate the number of southern flounder with otolith data at each site. Error bars represent standard error.

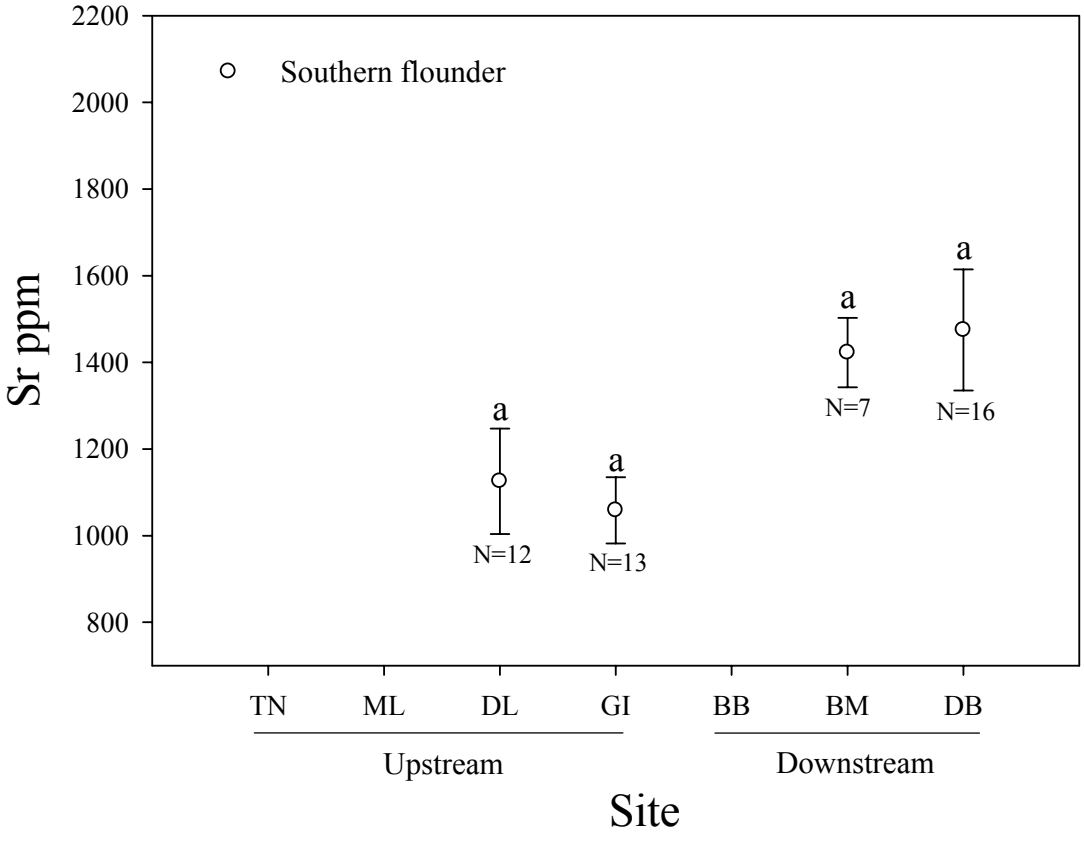
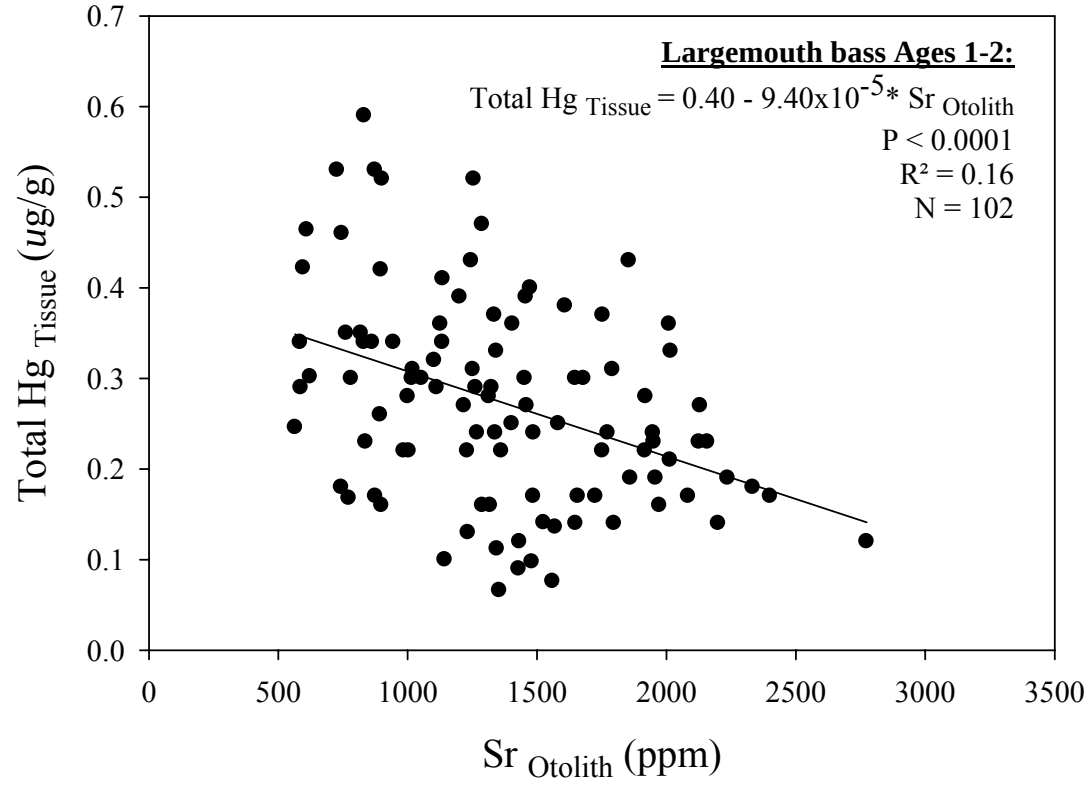
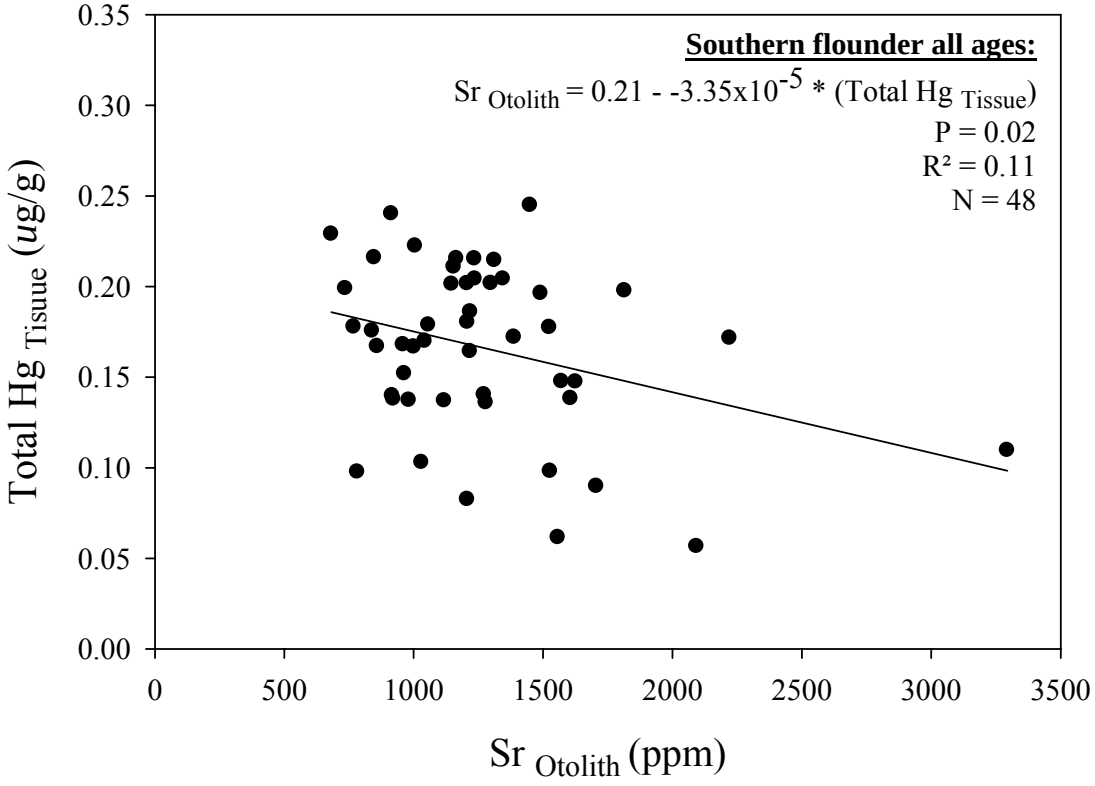

Southern flounder
Sr ppm
2200
2000
1800
1600
1400
1200
1000
800
a
a
a
a
N=12
N=13
N=7
N=16
TN
ML
DL
GI
BB
BM
DB
Upstream
Downstream
Site

Figure 39. Total Hg (µg/g wet weight) regressed against mean otolith Sr concentration (ppm) for largemouth bass age-2.5 and younger and all ages for southern flounder.


Largemouth bass Ages 1-2:
Total Hg Tissue = 0.40 - 9.40x10-5* Sr Otolith
P < 0.0001
R² = 0.16
N = 102
Total Hg Tissue (ug/g)
Sr Otolith (ppm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
500
1000
1500
2000
2500
3000
3500


Southern flounder all ages:
Sr Otolith = 0.21 - -3.35x10-5 * (Total Hg Tissue)
P = 0.02
R² = 0.11
N = 48
Total Hg Tisuue (ug/g)
Sr Otolith (ppm)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0
500
1000
1500
2000
2500
3000
3500

# APPENDICES

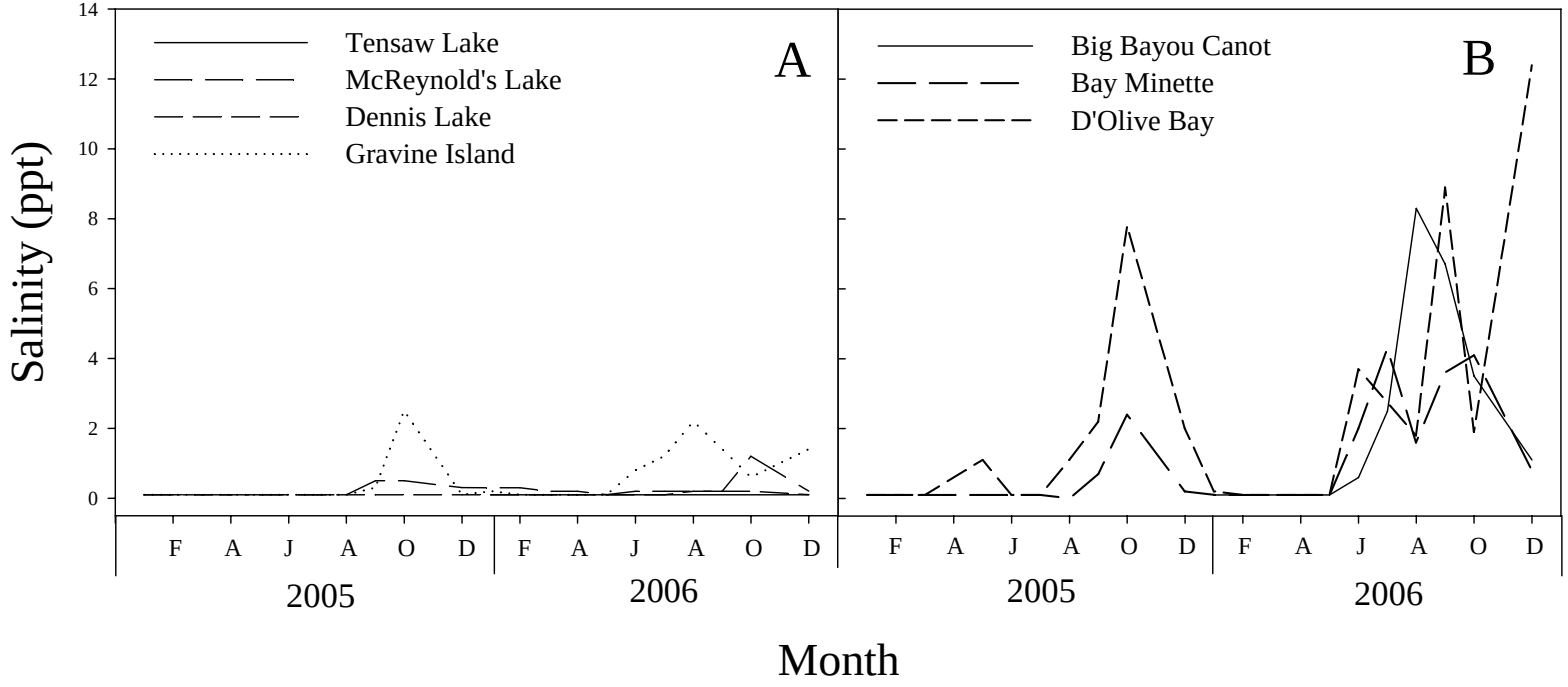
Appendix A. Intercept and slope values describing the relationship between otolith length (mm) and total length (mm) for fish species from the Mobile Delta. Equations are in the form: $\log_{10}(\text{total length}) = a + b * \log_{10}(\text{otolith length})$. Species and size ranges are those found common in diets of largemouth bass and southern floudner from the Mobile Delta.

| **Species** | **Otolith size range (mm)** | **b** | **a** | **N** | **$R^2$** |
|---|---|---|---|---|---|
| *Anchoa mitchelli* (bay anchovy) | 0.2 - 2.3 | 0.39 | 1.67 | 27 | 0.93 |
| *Brevoortia patronus*(gulf menhaden) | 0.3 - 2.8 | 1.13 | 1.65 | 44 | 0.91 |
| *Cynoscion nebulosus* (speckled seatrout) | 5.6 - 8.0 | 1.05 | 1.25 | 8 | 0.98 |
| *Dorosoma petenense* (threadfin shad) | 1.6 - 3.2 | 1.36 | 1.51 | 34 | 0.86 |
| *Fundulus grandis* (gulf killifish) | 1.3 - 2.2 | 1.16 | 1.60 | 8 | 0.94 |
| *Labidesthes sicculus* (brook silverside) | 0.5 - 1.6 | 0.90 | 1.71 | 22 | 0.97 |
| *Leiostomus xanthurus* (spot) | 2.0 - 6.3 | 1.26 | 1.24 | 44 | 0.95 |
| *Lepomis gulosus* (warmouth) | 1.8 - 8.1 | 0.86 | 1.39 | 24 | 0.95 |
| *Lepomis macrochirus* (bluegill) | 1.4 - 6.5 | 1.14 | 1.31 | 75 | 0.95 |
| *Lepomis microlophus*(redear sunfish) | 2.2 - 6.8 | 0.92 | 1.47 | 48 | 0.88 |
| *Lepomis miniatus* (redspotted sunfish) | 1.6 - 5.7 | 1.07 | 1.33 | 50 | 0.96 |
| *Menidia beryllina* (inland silverside) | 0.4 - 1.95 | 0.90 | 1.71 | 22 | 0.97 |
| *Microgobius gulosus* (clown goby) | 1.3 - 2.3 | 1.24 | 1.40 | 7 | 0.97 |
| *Mircropterus salmoides*(largemouth bass) | 0.4 - 0.95 | 0.71 | 1.46 | 10 | 0.95 |
| *Mugil cephalus* (striped mullet) | 2.6 - 6.1 | 1.24 | 1.28 | 9 | 0.95 |
| *Notripos texanus* (weed shinner) | 0.7 - 1.0 | 0.72 | 1.79 | 8 | 0.80 |
| *Notropis candidus* (silverside shiner) | 0.3 - 1.2 | 0.76 | 1.80 | 27 | 0.89 |
| *Sciaenops ocellatus* (red drum) | 4.0 - 4.9 | 1.87 | 0.74 | 11 | 0.96 |

Appendix B. Intercept and slope values describing the relationship between standard length (mm) and total length (mm) for fish species from the Mobile Delta. Equations are in the form: $\log_{10}$(total length) = a + b*$\log_{10}$(standard length). Species and size ranges are those common in diets of largemouth bass and southern flounder from the Mobile Delta.

| Species | Standard length range (mm) | b | a | N | $R^2$ |
|---|---|---|---|---|---|
| *Anchoa mitchelli* (bay anchovy) | 20 - 66 | 1.02 | 0.06 | 27 | 0.99 |
| *Brevoortia patronus* (gulf menhaden) | 19 - 130 | 1.04 | 0.01 | 46 | 1.00 |
| *Dorosoma petenense* (threadfin shad) | 34 - 147 | 0.99 | 0.12 | 37 | 0.99 |
| *Fundulus grandis* (gulf killifish) | 48 - 91 | 1.00 | 0.08 | 10 | 0.99 |
| *Fundulus notatus* (blackstriped topminnow) | 35 - 56 | 0.97 | 0.13 | 10 | 0.97 |
| *Labidesthes sicculus* (brook silverside) | 26 - 77 | 1.03 | 0.02 | 22 | 1.00 |
| *Leiostomus xanthurus* (spot) | 31 - 153 | 1.04 | 0.02 | 46 | 1.00 |
| *Lepomis gulosus* (warmouth) | 38 - 151 | 0.96 | 0.17 | 27 | 1.00 |
| *Lepomis macrochirus* (bluegill) | 26 - 257 | 0.99 | 0.12 | 75 | 1.00 |
| *Lepomis microlophus* (redear sunfish) | 40 - 154 | 1.01 | 0.08 | 53 | 1.00 |
| *Lepomis miniatus* (redspotted sunfish) | 25 - 126 | 1.02 | 0.06 | 53 | 1.00 |
| *Menidia beryllina* (inland silverside) | 17 - 92 | 1.00 | 0.08 | 36 | 1.00 |
| *Microgobius gulosus* (clown goby) | 30 - 60 | 1.07 | 0.00 | 7 | 1.00 |
| *Micropogonias undulates* (atlantic croaker) | 22 - 79 | 1.02 | 0.07 | 22 | 0.99 |
| *Moxostoma poecilurum* (blacktail redhorse) | 107 - 156 | 0.95 | 0.20 | 11 | 0.99 |
| *Mugil cephalus* (striped mullet) | 58 - 157 | 1.03 | 0.03 | 9 | 0.98 |
| *Notemigonus crysoleucas* (golden shiner) | 35 - 181 | 0.98 | 0.14 | 7 | 0.99 |
| *Notropis candidus* (silverside shiner) | 21 - 76 | 0.99 | 0.10 | 34 | 0.99 |
| *Sciaenops ocellatus* (red drum) | 67 - 99 | 0.99 | 0.11 | 11 | 1.00 |

Appendix C. Monthly salinity measured a 1 m depth at A) four upstream sites and B) three downstream sites from January 2005 through December 2006.


Salinity (ppt)
14
12
10
8
6
4
2
0
Tensaw Lake
McReynold's Lake
Dennis Lake
Gravine Island
A
Big Bayou Canot
Bay Minette
D'Olive Bay
B
F A J A O D F A J A O D
2005
2006
F A J A O D F A J A O D
2005
2006
Month