

**Residency and Habitat Utilization of Southern Flounder,
Paralichthys lethostigma, in a North Carolina Coastal Watershed**

by Michael Ryan Spidel

July 23, 2009

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ABSTRACT

In 2008 the commercial harvest of southern flounder *Paralichthys lethostigma* had a landed value of 5.6 million dollars, making southern flounder the most profitable commercial finfish fishery in North Carolina. However, southern flounder has recently been listed as over-fished in North Carolina. Soft-bottom habitats in coastal rivers and estuaries are critical to young southern flounder and are being threatened by dredging operations, shoreline development, mining operations and bottom disturbing fishing gear. In the North Carolina Southern Flounder Fishery Management Plan (FMP) the North Carolina Division of Marine Fisheries (NCDMF) highlighted the importance of protecting and improving critical fish habitat for southern flounder to increase growth, survival, and reproductive success. However, there has been a limited amount of research devoted to determining and identifying the importance of critical freshwater (0 – 0.5 ppt) habitat for the southern flounder fishery in North Carolina. Southern flounder are known to utilize coastal rivers, but the duration of their residency is unknown. Residency and use of freshwater habitats by

southern flounder was examined using fatty acid profiles and total mercury levels of muscle tissue, and otolith microchemistry. Southern flounder were collected using fishery independent techniques in 2007 and 2008 from three locations in coastal North Carolina: the Tar and Pamlico Rivers, and Pamlico Sound. Results indicated that 74% of southern flounder collected in the Tar River were resident, pre-spawning fish. Fatty acid profiles of resident southern flounder had low *n*-3:*n*-6 ratios ($\bar{x} = 2.01$, $SD = 0.40$), elevated total mercury levels ($\bar{x} = 0.105$, $SD = 0.034$), and low otolith strontium concentrations confirming residency in a freshwater environment. Age-0 and older sub-adult southern flounder were commonly found utilizing flat, shallow muddy-sand or sandy banks in the curves of the river. Total mercury levels were elevated in southern flounder residing in freshwater, but were substantially below the human consumption advisory guideline of 0.4 mg/kg. Reduced growth and low abundance are several indicators that coastal rivers are not optimal habitat for southern flounder, but should be considered as important, secondary habitat.

**Residency and Habitat Utilization of Southern Flounder,
Paralichthys lethostigma, in a North Carolina Coastal Watershed**

A Thesis

Presented To

The Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Masters of Science in Biology

by

Michael Ryan Spidel

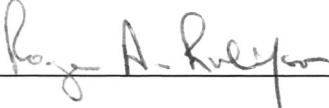
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RESIDENCY AND HABITAT UTILIZATION OF SOUTHERN FLOUNDER,
PARALICHTHYS LETHOSTIGMA, IN A NORTH CAROLINA COASTAL WATERSHED

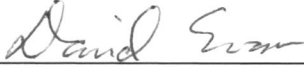
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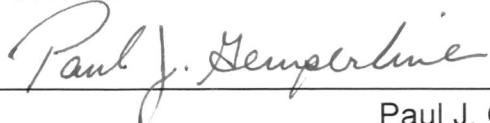
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ACKNOWLEDGMENTS

I thank Dr. Rulifson for his advice, support, encouragement, and trust throughout my time at East Carolina. Thank you to my committee members for the support, editorial comments, and time that you provided to my thesis. I thank Dr. Overton for the time and expertise that he shared with me conducting SAS and growth analyses. I thank Dr. Evans and the NOAA Center for Coastal Fisheries and Habitat Research for the time and lab supplies donated for the mercury analyses, and Drs. Kohler and Trushenski, Southern Illinois University – Carbondale, for the fatty acid analyses. I am indebted to Dr. Wuensch of East Carolina for his help, effort, and time that he devoted to me with several statistical analyses. Also, I thank Dr. Halden, University of Manitoba, for conducting the otolith microchemistry.

Thanks also to Bob Barwick and Justin Homan from the NCWRC for providing useful data for this project. I also thank Chris Batsavage from the NCDMF for his time in training me on reading flounder otoliths and the extra effort that he spent reading controversial otoliths for me. This research would not have been possible without funding received from a North Carolina Fisheries Resource Grant; special thanks to Charlie Van Salisbury from Engelhard, N.C. for his participation in the project. Also, I thank East Carolina University's Institute for Coastal Science and Policy for lab space, equipment, and logistical support throughout the project.

Thanks to all of the ECU fisheries students for their time and effort that they devoted to my research. Specifically, I thank John Mohan, Jillian Osborne, Cecilia Krahforst, Ken Riley, Jennifer Cudney-Burch, Kevin Hart, Becky Deehr, Samantha Binion, and Garry Wright. In closing, I thank my wife, Jennifer for the countless hours and weekends that she spent with me in the necropsy room, and the continued support and love that she provided throughout. I also thank my family for the support and encouragement that you have shown for me through all of my collegiate studies; without your faith in me this would have not been possible.

Matthew 4: 18-20

TABLE OF CONTENTS

LIST OF TABLES.....	vi
LIST OF FIGURES.....	viii
CHAPTER 1: INTRODUCTION.....	1
Critical Habitat.....	1
Objectives and Hypothesis.....	2
Southern Flounder.....	3
Comparative Analyses.....	5
Condition Indices.....	5
Fulton's Condition Factor.....	5
Relative Condition Factor.....	6
Liver-Somatic Index (Hepatosomatic Index)...	7
Micro-technology Analysis.....	9
Fatty Acids.....	9
Mercury.....	10
Otoliths.....	12
CHAPTER 2: METHODOLOGY.....	14
Study Area.....	14
Field Collections.....	15
Tar River.....	15
Pamlico River and Pamlico Sound.....	17
Laboratory Methodology.....	18
Growth and Condition.....	19
Fatty Acids.....	19
Mercury.....	21
Otoliths.....	21
Aging.....	21
Microchemistry.....	22

Statistical Analysis.....	24
CHAPTER 3: RESULTS.....	27
Otolith Microchemistry.....	27
Fatty Acids.....	27
Mercury.....	29
Growth and Condition.....	30
Sex Ratio.....	30
Growth.....	30
Condition.....	31
CHAPTER 4: DISCUSSION.....	34
Freshwater Residency.....	34
Otolith Microchemistry.....	34
Fatty Acids.....	35
Mercury.....	36
Freshwater Habitat.....	37
Growth and Condition.....	38
Conclusions and Recommendations.....	40
LITERATURE CITED.....	43
APPENDIX A: SOUTHERN FLOUNDER FISH DATA.....	84
APPENDIX B: SOUTHERN FLOUNDER TOTAL MERCURY DATA.....	102
APPENDIX C: LIPID EXTRACTION PROCEDURES	105
APPENDIX D: PREPARATION OF FATTY ACID METHYL ESTERS	106

LIST OF TABLES

1. Tar River, North Carolina electrofishing section.....	51
2. Tar River, North Carolina electrofishing locations in 2007 and 2008 ...	52
3. Pamlico River, North Carolina gill netting locations	53
4. Sampling protocol for the Milestone DMA-80 direct mercury analyzer	55
5. Total mercury analysis of 91 southern flounder from three size classes (small, median, large) from the Tar River, Pamlico River, and Pamlico Sound, North Carolina.....	56
6. Southern flounder analyzed by LA-ICP-MS for otolith microchemistry..	57
7. Linear regression parameter estimates of log transformed total length and weight for southern flounder collected from the Tar River, Pamlico River, and Pamlico Sound, North Carolina in 2007 and 2008.....	58
8. Otolith microchemistry classification matrix based on strontium levels of southern flounder collected in 2008 from the Tar River (TR), Pamlico River (PR) and Pamlico Sound (PS), North Carolina.....	59
9. Southern flounder freshwater residency classification matrix based upon fish collected from the Tar River, North Carolina in 2007 and 2008..	61
10. Lipid, PUFA, and <i>n</i> -3: <i>n</i> -6 of southern flounder collected in the spring and fall of 2008 from the Tar River, Pamlico River, and Pamlico Sound, North Carolina.....	62
11. Fatty acid content (mean percent $\pm$ SD) of white muscle tissue of southern flounder from spring 2008 and fall 2008, collected from three	

locations in North Carolina.....	63
12. Pearson's correlation analysis of total mercury and total length for southern flounder collected in 2007 and 2008 from the Tar River, Pamlico River, and Pamlico Sound, North Carolina.....	64
13. Frequency table of all southern flounder collected during this study from the Tar River, Pamlico River, and Pamlico Sound, North Carolina in 2007 and 2008 by location and sex.....	65
14. Von Bertalanffy growth parameters for southern flounder collected from the Tar River, Pamlico River and Pamlico Sound, North Carolina in 2007 and 2008	66
15. Literature review of n -3: n -6 from various habitats.....	67
16. Total mercury content (mg/kg) of harvestable size (≥ 355 mm TL) southern flounder captured in a North Carolina coastal river and estuarine system in 2008.....	68
17. Sediment analysis of benthic habitat utilized by southern flounder collected in the Tar River.....	69
18. Diet analysis of southern flounder collected in 2007 and 2008 from the Tar River, Pamlico River and Pamlico Sound, North Carolina.....	70

LIST OF FIGURES

1. Map of sampling locations: Tar River, Pamlico River, Pamlico Sound North Carolina.....	71
2. 1 min X 1 min sampling grid of Pamlico River, North Carolina flounder gill netting locations.....	72
3. Southern flounder otoliths immersed in water for aging viewed under an Olympus SZX16 microscope with an Olympus DP71 digital camera for imaging.....	73
4. Southern flounder otoliths mounted in 25-mm Plexiglas ring mounts.....	74
5. Section and polished southern flounder otolith with LA-ICP-MS transect and ages identified to verify whole otolith aging.....	75
6. Omega-3 to omega-6 ratios of southern flounder collected in the spring and fall months of 2008 from the Tar River, Pamlico River and Pamlico Sound.....	76
7. Mean total mercury levels for three size classes of southern flounder from the Pamlico River, Pamlico Sound, and Tar River.....	77
8. Southern flounder collected from the Tar River, North Carolina in 2008 by electrofishing.....	78
9. Histogram of southern flounder ages captured by location. Southern flounder were represented from the Tar River (September 2007– November 2008), Pamlico River (May 2008 – November 2008), and Pamlico Sound (May 2008 – November 2008).....	79

10. Strontium transects of three southern flounder from the Tar River, Pamlico River and Pamlico Sound.....	80
11. Strontium transect of a southern flounder collected near Washington, NC with corresponding salinities measured from USGS station 02084472.....	81
12. Von Bertalanffy growth curve of female southern flounder collected in 2008 by gill netting in Pamlico River, North Carolina.....	82
13. Von Bertalanffy growth curve of female southern flounder collected in 2007 and 2008 by electrofishing in the Tar River, North Carolina.....	83

CHAPTER 1: INTRODUCTION

Critical Habitat

Soft-bottom habitats in coastal rivers and estuaries are critical to young southern flounder *Paralichthys lethostigma*. These habitats are being threatened by dredging operations, shoreline development, mining operations and bottom disturbing fishing gear (Street et al. 2005). In the North Carolina Southern Flounder Fishery Management Plan (FMP), the North Carolina Division of Marine Fisheries (NCDMF) highlighted the importance of protecting and improving critical fish habitat for southern flounder to increase growth, survival, and reproductive success (NCDMF 2005). However, there has been limited research devoted to determining and identifying the importance of critical freshwater (0 – 0.5 ppt) habitat for the southern flounder fishery in North Carolina. Burke et al. (1991) suggested that age-0 southern flounder inhabit freshwater habitats, but sampling targeting southern flounder typically occurs in salinities >2.0 ppt. Therefore, southern flounder using freshwater habitats are not being sampled. Electrofishing conducted by North Carolina Wildlife Resources Commission (NCWRC) biologists and personnel at East Carolina University (ECU) has collected southern flounder in the Tar River from Washington to Rocky Mount, North Carolina. Angler creel surveys also report that flounder have been angled, but not necessarily targeted, in the Tar River (Homan et al. 2006). Tar River is not the only coastal river in NC in which southern flounder are caught. Reports from biologists and fishermen from the Roanoke, Neuse, and Cape Fear rivers,

NC, indicate that southern flounder also inhabit these coastal rivers. Not only are southern flounder utilizing freshwater habitats in North Carolina, but they are also exhibiting this behavior in Georgia and Alabama (Dahlberg 1972; Farmer 2008). If southern flounder use coastal rivers as sub-adults and adults, then it is important to document the habitat utilized and their duration of residency within the coastal river ecosystem. This documentation will provide a better understanding of habitat utilization and residence time of sub-adult and adult southern flounder in freshwater habitats.

Objectives and Hypothesis

The objectives of this study were:

1. To identify critical coastal freshwater habitats utilized by southern flounder by using the Tar-Pamlico River basin as a model.
2. To compare growth and condition of southern flounder from freshwater (Tar River) to mesohaline and polyhaline habitats of the Pamlico River and Pamlico Sound for the same age class and sex.
3. To determine the extent of freshwater residency in one portion of the southern flounder population in North Carolina by fatty acid analysis, total mercury analysis, and otolith microchemistry.

I hypothesized that:

1. Southern flounder found in freshwater (<0.5 ppt) would be utilizing similar benthic habitat types to that utilized in oligohaline and mesohaline areas.

2. Mercury levels of southern flounder would be higher in freshwater habitats than those found in estuarine habitats.
3. Southern flounder collected from freshwater will have lower omega-3 to omega-6 fatty acid ratios compared to flounder collected from mesohaline (5 - 18 ppt) and polyhaline (18 - 30 ppt) habitats.
4. Southern flounder collected from freshwater will have Sr:Ca concentrations less than 1000 ppm that are indicative of freshwater residency.

Southern Flounder

Southern flounder range from Virginia to Florida and along the Gulf of Mexico from Florida to Texas; however, they do not occupy the tropical waters around the tip of Florida (Daniels 2000; Monaghan and Armstrong 2000; Takade-Heumacher and Batsavage 2009). Southern flounder are a benthic piscivorous fish that preys primarily on bay anchovy *Anchoa mitchilli*, species of Sciaenidae, and other fishes (Fitzhugh et al. 1996). They can be found in numerous habitat types including coastal rivers, estuaries, inlets, and marine environments ranging in salinities from 0 to 36 parts per thousand (ppt) (Dahlberg 1972; Daniels 2000). Adult southern flounder in North Carolina migrate from estuaries to the continental shelf of the Atlantic Ocean in the fall for winter spawning (Monaghan and Armstrong 2000). Warlen and Burke (1990) determined that spawning of southern flounder occurs from November until February off of North Carolina's coast because larval southern flounder were collected in the Newport River

estuary (mean age = 31 days) from December until March with a peak in early February. Because the peak ingress occurs in early February, the birth date is assumed to be January 1 in stock assessment analyses (Takade-Heumacher and Batsavage 2009). Southern flounder eggs hatch offshore in high salinity waters (>32 ppt), which is required for the flotation of eggs (Smith et al. 1999). Not only is high salinity water in the marine environment necessary for egg buoyancy, but its inshore flow to nearshore estuarine waters is critical for the hatching success of the eggs. Once southern flounder larvae have reached metamorphosis near 50 days post hatch, they have high survival (97%) in salinity as low as 5 ppt (Smith et al. 1999).

After spawning, some adult southern flounder return to high salinity portions of the estuaries in the months of March and April, while others remain offshore (Monaghan 1996; Taylor et al. 2008; Takade-Heumacher and Batsavage 2009). The NCDMF has reported that the sex ratio of southern flounder harvested in North Carolina is highly skewed with females comprising 75% of age 0-2 fish, 97% of age 3-4, and 100% of age 5+ fish (Monaghan and Armstrong 2000). Maximum size and age is greater for females than for males in the North Carolina southern flounder fishery due to sexual dimorphic growth (Daniels 2000; Monaghan and Armstrong 2000). Females can achieve a maximum age of 9 years, but the males do not typically live past 4 years (Zhao et al. 1998; Takade-Heumacher and Batsavage 2009).

Comparative Analysis

Condition Indices

Condition indices have been established as a tool in fisheries science to measure the fitness or condition of fish. Two condition indices commonly used in fisheries management are Fulton's condition factor, and a liver-somatic index (Murphy and Willis 1996; Pope and Kruse 2007). Both of these indices have their limitations and advantages. Incorporating environmental data with these indices increases the precision with which one can assess the condition of fish (Bulow et al. 1978; Adams and McLean 1985).

Fulton's Condition Factor. Fulton's condition factor is named after Thomas Wemyss Fulton (1855-1929), a native of Edinburgh, U.K., who studied fisheries of the North Sea and other aspects of fisheries biology and science (Nash et al. 2006). The term "Fulton's Condition Factor" was officially introduced into the literature by Ricker (1975) with the equation, $K = W/L^3$ (Nash et al. 2006). What is most commonly used today by fisheries scientists is the equation $K = 100 \cdot W/L^3$, where W = wet weight and L = length in centimeters (Froese 2006). In addition, Murphy and Willis (1996) described Fulton's condition factor as $K = 100,000 \cdot W/L^3$ when millimeters and grams are used for length and weight measurements, respectively. Fulton was the first to state that this ratio will vary spatially and seasonally, and by reproductive state. He described what is known as today as allometric growth (Froese 2006).

Many people have been critical of Fulton's condition factor (LeCren 1951; Cone 1989,1990; Kurkilahti et al. 2002). One concern is that comparisons between populations using K-values is impossible due to morphological variation; comparisons should be limited to fish from the same length class, the same spatial and temporal area, and caught by the same gear type. Also, there should be no comparison between sexes because of differences in gonadal growth between males and females (LeCren 1951; Murphy and Willis 1996; Kurkilahti et al. 2002; Froese 2006). Fulton's condition factor does not measure fish condition, but measures the shape of the fish by comparing an observed weight with a hypothetical fish (LeCren 1951; Cone 1989,1990; Kurkilahti et al. 2002). What Fulton's condition factor can estimate is the excess weight gained or lost over several weeks to months; so it is not an immediate response indicator of environmental conditions (Hoque et al. 1998).

Relative Condition Factor. A better indicator of condition is the relative condition factor, known in fisheries science as K_r . Relative condition factor compares the actual weight of a fish to a standard predicted by the weight – length regression that uses the coefficients of the population from which the fish was collected (LeCren 1951; Pope and Kruse 2007). Relative condition factor is calculated as $K_r = (W / W') * 100$, where W is the actual weight of a fish, and W' is the predicted weight using the weight-length regression coefficients that are based on $\log_{10}$ transformed data (LeCren 1951, Pope and Kruse 2007). LeCren (1951) concluded that K_r measures the deviation of an individual fish

from the population average, whereas K is measuring the deviation from a hypothetical "ideal" fish. Due to the underlying principles of regression dynamics, the population K_n will average 100. This allows testing the null hypothesis that a subset of a population differs significantly from the population mean (H_0 : The mean K_n of location $X = 100$). Evaluating the latest weight – length regression coefficients provided by the 2009 North Carolina stock assessment on southern flounder, it is appropriate to use K_n for comparative purposes because they are growing faster than the "ideal" fish (Takade-Heumacher and Batsavage 2009).

Liver-Somatic Index (Hepatosomatic Index). The liver of an organism is used as an indicator of growth because excess fat, water and glycogen are stored in the liver during periods of high energy intake (Heidinger and Crawford 1977). When a fish is taking in excess food, the liver stores some of it in the form of glycogen, which is used at later times when the fish is stressed. This makes the liver swell and increases its overall weight. Liver condition is an index of the wet weight of the liver divided by the wet weight of the fish (Heidinger and Crawford 1977). The equation for the liver-somatic index (LSI) is:

$$LSI = \left(\frac{\text{wet weight of liver (g)} - \text{gallbladder}}{\text{wet weight of fish (g) (including liver)} - \text{wet weight gonads (g)}} \right) \times 100$$

Overall, the LSI is best suited for determining weekly to monthly changes in growth rates of fish (Hoque et al. 1998). The LSI does not change over a range of various body weights; therefore, the LSI is a good indicator of condition for all sizes of fish (Delahunty and de Vlaming 1980). However, the LSI is better

suited for non-sexually mature fish because gonadal development utilizes energy reserves that are stored in the liver (Adams and McLean 1985). Adams and McLean (1985) found that the LSI predicted growth rates better in age 1 largemouth bass *Micropterus salmoides* versus age 2 largemouth bass, which expend more energy producing gonads. This leads to a false understanding of the fish's condition due to the declining LSI value. The LSI is positively correlated with daily food intake and energy storage, so therefore is a more sensitive indicator of growth than the relatively slow indicator of condition factors (Heidinger and Crawford 1977; Bulow et al. 1978).

Because the LSI is a relatively quick responder of the intercellular growth rate it can be used as an indicator of stress to the fish. Adams and McLean (1985) stated that the liver is sensitive to environmental toxic stressors and that fluctuations in the LSI could serve as an indicator for toxic stress faster than a concurrent change in length or mass. Hoque et al. (1998) supported this concept by subjecting fish to hydrogen sulfide (H_2S) for six weeks and found significant decreases in the LSI, but not in the condition factor. Bulow et al. (1978) warned that LSIs should not be used as a stand alone indicator of growth, but it should be used in conjunction with other growth indices. Multiple growth indices and environmental factors should be considered together. The LSI can be influenced by variables such as consumption rate, water temperature, seasonality, and reproductive variation throughout the year and between sexes (Heidinger and Crawford 1977; Bulow et al. 1978; Adams and McLean 1985).

Micro-technology Analysis

Fisheries scientists have used several technologies to help determine the migration patterns of fish. These technologies include (1) analysis of white muscle tissue for fatty acid content to determine the diet composition of the species (freshwater food web versus estuarine or marine food web) (Gallagher et al. 1991; Karahadian et al. 1995; Paramore and Rulifson 2001); (2) evaluating total mercury levels in fish to determine a length of residency in freshwater (Leah et al. 1992, Mason et al. 2006, Kim and Rochford 2008); and (3) analysis of otolith microchemistry (Secor 1992; Secor et al. 1995, Chesney et al. 1998, Campana 1999, Paramore and Rulifson 2001, Campana et al. 2007, Taylor et al. 2008).

Fatty Acids. In marine fish, the primary constituents of fatty acid profiles are omega-3 (*n*-3) long-chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (20:5 *n*-3, EPA) and docosahexaenoic acid (22:6 *n*-3, DHA) (Steffens 1997). Not only are these long chain PUFA necessary for biochemical, cellular and physiological functions in fish (Sargent et al. 1999), but there is evidence that the human consumption of these PUFA have cardiovascular benefits (Stansby 1984; Simopoulos 1991; Kris-Etherton et al. 2003; Sidhu 2003). The healthy consumption of fish high in *n*-3 PUFA have been reported to decrease the risk of heart arrhythmias and thrombosis, lower cholesterol levels, decrease hypertension, and reduce the risk of coronary heart

disease (Stansby 1984; Simopoulos 1991; Kris-Etherton et al. 2003; Sidhu 2003).

A fish diet comprised of a marine-based food web will be higher in n -3 fatty acids, than a fish from a freshwater environment (Stansby 1967, Karahadian et al. 1995, Steffens 1997, Paramore and Rulifson 2001). In addition, fish that feed in freshwater food webs will have higher concentrations of omega-6 (n -6) fatty acids than fish feeding in a marine based food web (Steffens 1997).

Therefore, a fish harvested from a freshwater environment should have a lower n -3 to n -6 ratio (n -3: n -6) than fish of the same species harvested from estuarine or marine habitats. Stansby (1967) reported that coho salmon *Oncorhynchus kisutch* fingerlings captured in freshwater rivers had high levels of linoleic acid (18:2 n -6) and low levels of EPA and DHA when compared to ocean caught mature salmon, which had high levels of EPA and DHA but low levels of linoleic acid. Freshwater fish will have a n -3: n -6 ranging from 1 – 4, whereas marine species will range from 5 – 10 (Steffens 1997). By analyzing the fatty acid profiles of white muscle tissue, specifically the n -3: n -6, I was able to identify what food web they were feeding in to determine the duration of freshwater residency.

Mercury. Southern flounder is a species that can spend its juvenile and sub-adult life stages in coastal rivers, which are known to have high levels of mercury. High mercury levels of piscivorous species in coastal rivers of North Carolina are attributed to two main causes. Atmospheric deposition related to anthropogenic activities is a major source of excess mercury (Facemire et al.

1995, Rolfhus and Fitzgerald 1995, Downs et al. 1998, Simoneau et al. 2005). A secondary contributor in eastern North Carolina can be attributed to rich-organic sediments and sulfate reduction, which can aid in the methylation of mercury into methyl mercury (CH_3Hg), the toxic form of mercury that accumulates in organisms (St. Louis et al. 1994). Methyl mercury accumulates in the muscle tissue of fish and contributes to >95% of the total mercury in most fish (Bloom 1992). Because CH_3Hg largely represents the total mercury in fish, it is an adequate surrogate for measuring CH_3Hg to measure total mercury levels (Bloom 1992).

Mercury bioaccumulation occurs at a faster rate in fish that feed higher in the food web because their food is generally higher in mercury; however, these species typically have the highest demand in the fish market. The North Carolina Division of Public Health labels “flounder” as a fish that is low in CH_3Hg and can be consumed without serious effects (Williams 2006). However, this attribute is based on the results from testing flounder from Florida and not from North Carolina estuaries or coastal rivers. Although “flounders” are not considered to have mercury concentrations hazardous for consumption, no studies have been conducted to determine the total content or rate at which southern flounder accumulate mercury in coastal North Carolina.

Mercury levels have been used to establish a length of residency for individual fish in a particular habitat (Radtke and Morales-Nin 1989; Riget et al. 2000; Mason et al. 2006; Kim and Rochford 2008). By comparing mercury levels

between fish of a single species, it is possible to determine if a group of fish is residing in a particular habitat or if that fish exhibits migratory behavior. Kim and Rochford (2008) concluded that CH₃Hg levels in brown trout *Salmo trutta* could be used as an indicator of freshwater residency. The authors used otolith microchemistry (strontium: calcium) to support that CH₃Hg was low in trout that had lived at sea a portion of its life, while the trout with elevated MeHg had completely resided in freshwater.

Otoliths. Determining the elemental composition of otoliths acquired over the lifetime of the fish is used by fisheries scientists to classify an individual fish to a specific habitat during a portion of its life (Secor 1992; Secor et al. 1995; Chesney et al. 1998, Campana 1999, Paramore and Rulifson 2001, Campana et al. 2007, Taylor et al. 2008). Otoliths are the inner ear bones of fish that are calcified bony structures comprised of a mixture of inorganic and organic matter, primarily calcium carbonate (CaCO₃) in the aragonite crystal form (Carlström 1963). As a fish grows, a new layer of calcium and other trace elements from the environment are deposited in the otolith (Campana 1999). Trace elements such as strontium (Sr) are incorporated into the aragonite matrix as impurities in place of Ca (Kinsman and Holland 1969, Secor 1992).

Migrations through various salinity environments can be traced by analyzing elemental ratios embedded into the otolith over short periods or the lifetime of a fish (Secor 1992, Secor et al. 1995, Chesney et al. 1998, Campana 1999, Morris et al. 2003, Campana et al. 2007). Strontium to calcium ratios

(Sr:Ca) are commonly utilized because strontium levels are easy to detect and are positively correlated with salinity (Secor et al. 1995; Chesney et al. 1998; Campana 1999). Strontium concentrations in otoliths can be used to distinguish various occupied habitats (Radtke and Morales-Nin 1989; Secor 1992; Secor et al. 1995; Secor et al. 2001; Kraus and Secor 2004; Kellison and Taylor 2007).

In this study, I combined condition factors with predictable migratory analyses to determine freshwater residency by southern flounder in a portion of its range. Southern flounder were collected from three habitats varying in salinity profiles and compared using growth, condition, fatty acid profiles, mercury content, and strontium concentrations. The Tar River was used as a model for southern flounder residency for North Carolina coastal watersheds.

CHAPTER 2: METHODOLOGY

Study Area

The Tar-Pamlico River system is a major contributor to the Albemarle-Pamlico Sound Ecosystem (APSE), the second largest estuarine system in the United States (Figure 1) (Giese et al. 1985; Mallin et al. 2000; Reed et al. 2004). The Tar and Pamlico rivers are the same river with the narrower portion west of US Highway 17 in Washington, NC, designated as the Tar River, and east of US Highway 17 in Washington, NC, designated as the Pamlico River (Pease et al. 2007). The Tar River is divided into the lower Tar and upper Tar regions by the Rocky Mount dam (Pease et al. 2007); this study examined the lower Tar region only. The lower Tar River has a narrow, winding channel typical of a coastal plain river with little elevation gradient. Much of the lower Tar River basin is low-lying agriculture and forested land (Pease et al. 2007). The Tar River is typically freshwater (<0.5 parts per thousand (ppt)) until the interface of the Pamlico River; however, during draught conditions a salt wedge can migrate upstream reaching Grimesland, NC (~7.5 km upstream) (Copeland et al. 1984; Giese et al. 1985).

The Pamlico River is a shallow ($\bar{x} = 3.3$ m), wind-driven estuary with little tidal influence (Copeland et al. 1984; Giese et al. 1985). The Pamlico River is classified as partially oligohaline (0.5 ppt – 5 ppt) in the upstream sections and mesohaline (5 ppt – 18 ppt) in the middle section (Copeland et al. 1984). Deep-water sediments are composed of anoxic mud with shallow shoreline sediments consisting primarily of sand (Copeland et al. 1984). Pamlico River frequently

incurs hypoxic conditions during the summer months due to eutrophication, stratification, and limited volume exchange (Giese et al. 1985).

Pamlico Sound is connected to the Atlantic Ocean by three openings (Ocracoke, Hatteras, and Oregon Inlets) along the Outer Banks that border the sound to the east (Giese et al. 1985). Pamlico Sound covers approximately 5300 km² and contains approximately 26 billion m³ of water (Giese et al. 1985). Depths are relatively shallow with an average of 4.4 m, and a maximum of 7.6 m. Pamlico Sound has little tidal influence except along the Outer Banks. It is mostly a wind-driven system because of its large mass of water and limited access to the ocean (Giese et al. 1985; Reed et al. 2004). Pamlico Sound is defined as polyhaline habitat with salinities typically >18 ppt.

Field Collections

Tar River

Southern flounder were collected from September 2007 – November 2008 in the lower Tar River from Rocky Mount, NC (35° 56.748 N, 77° 39.861 W) to Grimesland, NC (35° 34.483 N, 77° 10.578 W). Electrofishing was conducted by ECU personnel using a stratified-random sampling design. The river was divided into eight sections beginning at the rapids downriver of NC-SR 1250 in Rocky Mount, NC and ending at NC-SR 1565 in Grimesland, NC, averaging 15.3 km per section (Table 1). The sections were numbered, chosen at random, and two or three sections were sampled per month during the summer (May-August). The same sections were revisited during the fall (September-November).

Electrofishing was typically conducted using one netter and one boat operator. During an electrofishing sampling trip, four 1800-s shocking sessions (not spatially overlapping) were held in random locations within the one pre-assigned section (water depth dependent). Problems with low flow and boating access limited the sampling regime to areas that were accessible to the electrofishing equipment (Table 2). The electrofishing boat was a 5.5-m aluminum jon-boat with a 60 hp Yamaha[®] two-stroke outboard motor. The electrofishing unit was a Smith Root Incorporated 2.5-GPP electrofishing rig with a 5.5-hp Honda generator using two stainless steel arrays. Both DC and AC currents, with high voltage (1000 volts), were used and amperage was limited to 2 – 3 A. Sampling typically occurred in < 2.0 m depth. Flounder in tetany could be observed and collected in shallow waters. Each fish was given a unique identification number, placed on ice and returned to the laboratory for analysis. The GPS location was recorded for each fish collected.

Sampled habitats were documented by recording the location and depth and visually assessing the substrate. Sediment samples were collected using a 7.62-cm PVC corer from each point of capture and analyzed for sediment texture composition. Local water quality parameters were recorded for the surface and bottom of the water column (dissolved oxygen (mg/L), % saturation of oxygen, temperature (°C), salinity (ppt), conductivity (μS or mS), pH, and secchi depth (cm)). Capture locations were mapped in conjunction with environmental

parameters using GPS coordinates and a GIS mapping program (ArcMap[®] v.9.2).

Pamlico River and Pamlico Sound

Pamlico River and Pamlico Sound were sampled from May 1, 2008 – November 30, 2008. Pamlico River was sampled for flounder from the US Highway 17 bridge at Washington (35° 32.550 N, 77° 3.700 W) to a boundary designated by the mouths of North Creek (35° 25.000 N, 76° 40.000 W) and South Creek (35° 21.000 N, 76° 42.000 W). This area was divided into three sections and then sampled each night in randomly chosen blocks selected from a 1 min x 1 min grid within the pre-selected section (Figure 2). From May 1 until November 30, 2008 two sections were sampled randomly each month by fishing one section for two consecutive nights (Table 3). Nets were placed at depths of 0.3 m – 1.2 m one hour prior to sunset and retrieved one hour after sunrise the following morning. Sub-adult and adult flounders were harvested with gill nets resembling typical commercial flounder gill net. Each of the three 200-yd (182.9-m) gill nets had four mesh sizes ranging from 5.25-in (13.34-cm) stretch mesh to 6.0-in (15.24-cm) stretch mesh. For juvenile flounder capture, two 100 ft (30.5-m) experimental style gill nets with four mesh sizes ranging from 3.0-in (7.62-cm) stretch mesh to 5.0-in (12.7-cm) stretch mesh were randomly set adjacent to two of the 200-yd (182.9-m) nets. In addition to the ambient sea and weather conditions, the same habitat and water quality parameters were recorded as in the freshwater sampling regime.

The northern portion of Pamlico Sound was sampled by a commercial fisherman using his traditional ecological knowledge (TEK) of the flounder fishery to achieve the highest catch per unit effort (CPUE). The commercial fisherman used the same design of commercial and experimental gill nets as those described for the Pamlico River sampling. The fisherman set the nets one hour prior to sunset and retrieved the nets one hour after sunrise the following morning. He set and retrieved the nets for two consecutive nights once each month from May to November 2008. The same habitat and water quality parameters were recorded as described above. Ambient sea and weather conditions, and GPS coordinates were recorded as well. All flounder collected were given a unique identification number, placed on ice, and then frozen until laboratory examination. For otolith microchemistry the immediate removal of otoliths is best, but freezing and removing the otoliths at a later date is the next best alternative (Proctor and Thresher 1998).

Laboratory Methodology

Once the specimens were returned to the laboratory, all flounder were identified to species by counting the number of gill rakers on the first gill arch (Ginsburg 1952). Total length (mm), weight (g), liver weight (g), gonad weight (g), development stage and sex were recorded. Stomach contents were identified to family (if possible) and identifiable contents weighed. Scales were removed from the flounder and two white muscle tissue samples were frozen at -20°C for no longer than 6 months before analysis (one for Hg analysis, one for

fatty acid analysis). Sagittal otoliths were surgically removed from the brain case, extraneous tissue was removed, and then they were rinsed with 70% ethanol and stored in 70% ethanol in 1.5-mL microcentrifuge tubes for later examination.

Growth and Condition

Growth rates were compared using Von Bertalanffy growth coefficients (L_{∞} , K , and t_0) and length-weight relationships ($W = aL^b$; $\log(W) = \log(a) + b * \log(L)$). Condition indices used included relative condition factor (K_n), and liver-somatic index (LSI).

Fatty Acids

Tissue samples for fatty acid analysis were collected by filleting a white-muscle portion from the dorsal side, directly behind the visceral opening at the lateral line, with skin and scales attached (Gallagher et al. 1991). All utensils and cutting boards were thoroughly rinsed between samples. Individual samples were labeled and stored in storage bags and frozen at -20°C until analysis. Individual fish were selected post-hoc to compare fatty acid profiles for the three sampling locations during the spring (24 March 2008 – 6 June 2008) and fall (1 September 2008 – 31 October 2008).

Samples were freeze-dried for 48 h in a Labconco Freeze Dryer 5 with a Welch Duo-Seal Vacuum Pump at -50°C. Skin was removed, and the remaining dried muscle was then ground with a food processor (Model DLC-1BCH, Cuisinart®, Cuisinart, Stamford, CT) to a homogeneous mixture for analysis

(Karahadian et al. 1995). Samples were then transferred to sterile, 50-mL polypropylene, conical centrifuge tubes and net tissue mass was determined (nearest 0.001 g), and stored at -20°C until analysis. Samples were transported on dry ice to the Fisheries and Illinois Aquaculture Center at Southern Illinois University for fatty acid analysis.

Crude lipid content was determined using the Folch method and retained for subsequent fatty acid analysis (Folch et al. 1957) (Appendix A). Fatty acid methyl esters (FAME) were prepared by acid-catalyzed transmethylation for 16 h at 50°C as described by Christie (1982) (Appendix B). The FAME was separated using a Shimadzu GC-17A gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a flame ionization detector (FID) fitted with a permanently bonded polyethylene glycol, fused silica capillary column (Omegawax 250, 30 m × 0.25 mm i.d., 0.25 µm film). A volume of 1.0 µL of resuspended FAME was injected into the gas chromatograph using helium as the carrier gas (30 cm/s, 205°C) at an injection temperature of 250°C. A split injection technique (100:1) was used with the following temperature protocol: 50°C held for 2 min, increased to 220°C at 4°C/min, and held at 220°C for 15 min. External standards were used to identify individual FAME (Supelco 37 Component FAME Mix, PUFA-1, and PUFA-3; Supelco, Bellefonte, PA, USA). All solvents used were of HPLC grade and obtained from Sigma Diagnostics Inc. (St. Louis, MO, USA) (Lane et al. 2006).

Mercury

Tissue samples for mercury analysis were collected by filleting a white-muscle portion from the upper side, directly behind the visceral opening. All utensils and cutting boards were thoroughly rinsed between samples. Individual samples were labeled, stored separately and frozen until analysis at -20°C.

The total mercury content of the sample was determined in cooperation with Dr. David Evans at the NOAA Center for Coastal Fisheries and Habitat Research in Beaufort, NC using a Milestone DMA-80 direct mercury analyzer. United States Environmental Protection Agency (1998) method 7473 was used for the methodology of the mercury testing using a subsample of muscle tissue no greater than 0.2 g in size (Table 4). Ninety-one samples were analyzed representing three size classes from each location. The size classes were comprised of fish that represented small, median, and large fish to test if there was a correlation with total mercury and total length (Table 5). Lobster hepatopancreas (TORT-2) (0.27 ± 0.06 mg/kg Hg); spiny dogfish *Squalus acanthias* liver (DOLT-3) (3.37 ± 0.14 mg/kg Hg); and 5% HCl-Hg solution (5 ppm Hg) were used as reference standards. Total mercury levels in southern flounder were compared from each location to identify resident behavior among those captured from the Tar River.

Otoliths

Aging. All otoliths were aged whole with reflected light by placing the concave side up in a black petri dish while immersed in water (Fitzhugh et al.

1996, Sipe and Chittenden 2001) (Figure 3). Otoliths were examined under an Olympus SZX16 research stereo microscope in conjunction with an Olympus DP72 digital camera. Digital calibrated images were captured and analyzed with ImagePro Plus (v. 6.2.1). Otoliths were randomly aged twice without knowledge of sex or size to minimize bias. Once the otoliths were aged twice, the two independent samples were compared; those otoliths with ages not in agreement were aged a third time independently. Otoliths that were aged a third time were compared with the previous ages and were assigned the age with the concurring ages. The development of annuli in southern flounder otoliths varies from March to June in North Carolina. Therefore readers knew the collection date so they could decide whether to bump the fish to the next year of age. This variability can be explained by differences in growth rates, water temperatures, and age (Chris Batsavage NCDMF, personal communication).

Microchemistry. Forty-one otoliths representing five age classes from the three habitats were sent to the mass spectrometry laboratory at the University of Manitoba, Winnipeg, Canada, for elemental analysis using laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (Table 6). Otoliths sent for micro-analysis were removed from 70% ethanol solution, dried for two h in a drying oven, and placed in new 1.5-mL microcentrifuge vials. Previous studies report that Sr levels in otoliths are unaffected by handling and storage media, including ethanol preservation (Milton and Chenery 1998, Proctor and Thresher 1998, Hedges et al. 2004).

Each otolith was prepared for LA-ICP-MS by rinsing in distilled water and then drying for 24 hours in a laminar flow hood. Otoliths were embedded in epoxy resin (Buehler Epoxicure), and a 2-mm thick dorso-ventral transverse section was removed, including the core, using a low speed, diamond blade (15.2 cm x 0.5 mm) on an Isomet saw (Buehler 1000). The 2-mm sections were re-embedded with the same epoxy resin in 25-mm diameter Plexiglas ring mounts (usually five sections per mount) (Figure 4). Sections were then polished simultaneously by hand using 30- μ m and 9- μ m Buehler diamond polishing suspensions. One micron and 0.05- μ m polishing suspensions were used on a polishing wheel to reveal the core and achieve a completely flat and smooth surface. All mounts were cleaned in an ultrasonic bath with ultrapure water between polishes.

Otolith elemental isotopes were quantified using a Thermo Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd:YAG laser. LA-ICP-MS operating parameters included a 30- μ m beam size and 2- μ m/s scan speed. The elemental isotopes that were quantified were: ^{44}Ca , ^{25}Mg , ^{88}Sr , ^{138}Ba , ^{55}Mn , ^{63}Cu , ^{66}Zn , and ^{208}Pb . These isotopes were selected because they tend to reduce molecular interference and can be used for habitat characterization. Calcium (56 wt% CaO) was used as an internal standard and the external calibration was completed using NIST 610 glass to maintain instrument analytical precision. Laser scans were initiated beyond the nucleus, through the core, and along the longest axis of otolith growth to the outer edge. The data software GLITTER was

used to quantify elemental concentrations and detection limits. Isotopic measurements were converted to ppm using Microsoft Excel and plotted versus distance traveled by the laser microprobe (μm).

Once the LA-ICP-MS analysis was conducted the embedded otoliths were returned to ECU personnel. Images of the scanned otoliths were taken using the Olympus SZX16 research stereo microscope, and measurements of the annuli were taken along the laser transects. All sectioned otoliths that were used for LA-ICP-MS were aged to validate the aging technique of using whole otoliths (Figure 5).

Statistical Analysis

Several procedures were used in SAS (version 9.1[©]) and SAS Enterprise Guide (version 4.1[©]): PROC CORR, PROC GLM, PROC NLIN, PROC REG, and PROC TTEST. Parametric tests were used where skewness was minimal or when data transformation was used to reduce skewness ($\log_{10}(X)$, $\sqrt{(x)}$ = positive; x^2 = negative). The power maintained with parametric testing and robust tests (t-test) was needed due to low sample sizes from the Tar River. The t-test procedure (SAS PROC TTEST) was used to test for sexual dimorphic growth rates, differences between sexes (fatty acids, mercury), and differences between seasons (fatty acids) using a two sample test for unequal variances for each location. Tukey's studentized range test was used for testing differences of means in all quantitative comparisons that met the necessary assumptions (Tar River vs Pamlico River vs Pamlico Sound). Bartlett's test was used to confirm

homogeneity of variance in all analyses of variance. An alpha level of 0.05 was used for all tests of significance.

Length at age data were used in the non-linear procedure (SAS PROC NLIN) to estimate von Bertalanffy growth curve parameters (L_{∞} - asymptotic length, k – growth coefficient, t_0 - theoretical time in which length would equal zero) by creating iterations to reduce the sum of squares to a minimum. Ages were converted to fractional ages using the date of capture (in Julian days) divided by 365, plus the number of annuli on the otolith. This calculation uses the assumption of a January 1 birth date.

A liver somatic index (LSI) was compared for age 1 females from the month of September by using the ANOVA procedure (SAS PROC ANOVA). By applying these limitations, variability was reduced due to gonadal growth, temporal variation due to somatic growth, and increased power because of larger sample sizes. The LSI data met the assumptions for using an ANOVA, including normality (skewness = 0.57) and homoscedasticity (Bartlett's Test for Homogeneity, $\chi^2_{(2)} = 4.57$, $p = 0.1018$).

Female southern flounder from the months of September, October, and November were used for the analysis of Kn to limit variation due to growth and temporal variability. The weight-length regression parameters from the entire population were used as the predicted length-specific weight. However, when conducting the analysis of variance (ANOVA) the assumption of homogeneity of variance was violated (Bartlett's Test for Homogeneity, $\chi^2_{(2)} = 8.55$, $p = 0.0139$);

therefore, no statistical comparisons were made. Since the variance among the locations was not equal, I tested the null hypothesis, using a one-sample *t*-test, that the mean relative condition factor of each group did not differ significantly from the population average ($\mu = 100$).

An analysis of covariance (ANCOVA) was used to compare the total length and weight regression parameters by collection location. Tests of parallelism and intercepts were used to investigate the covariate of location. Log transformed total length and weight data were used with the regression procedure (SAS PROC REG). I first tested for significant differences in the log transformed regression parameter estimates of total length and weight between sexes in each location. Tar River and Pamlico Sound parameter estimates of intercept and slope were significantly different between sexes ($p < 0.05$) (Table 7); therefore, I continued the analysis by testing sexes separately. Log transformed data met the assumptions of linearity, normality, and homoscedasticity.

The correlation procedure (SAS PROC CORR) was used to test for a significant correlation between total length and total mercury content.

CHAPTER 3: RESULTS

Otolith Microchemistry

Otolith microchemistry results reveal that southern flounder exhibit habitat and site fidelity once they have completed their larval migration. Southern flounder were assigned to a broad habitat category using strontium concentration profiles over the lifetime of the fish: freshwater (<1000 ppm) (Farmer 2008), estuary (1000 – 2500 ppm) (Taylor et al. 2008), or marine (>2500 ppm) (Taylor et al. 2008). Incremental categories of birth - 0.5 years, 0.5 years - first annuli, first annuli to second annuli, and etcetera were used to designate utilized habitats during those associated periods (Table 8).

Approximately 74% (14/19) of southern flounder that were captured in freshwater were designated as resident fish. Back calculations of the last 6 months were made using images of scanned otoliths with measurements, a measurement of laser transects, and the capture date. Strontium values were averaged over this period (300- μ m laser transect distance) to help classify southern flounder habitat use. Mercury content, n -3: n -6, and strontium concentrations were used to classify southern flounder collected in the Tar River as resident or transient fish (Table 9).

Fatty Acids

The three locations studied had significantly different quantities of poly-unsaturated fatty acids (PUFA), percent lipid in muscle tissue, and omega 3:omega 6 ratios (Table 10). For all three variables the sexes were not

significantly different ($p > 0.05$) in either season. Therefore sexes were combined for all tests thereafter. Pamlico River fish were significantly greater in the spring for PUFA ($t_{(18)} = 3.28$, $p = 0.0041$) and $n-3:n-6$ ($t_{(11)} = 3.07$, $p = 0.0106$) than in the fall (Table 11). Because of the difference in the two variables for Pamlico River fish among the seasons, all further analyses were compared within a single season.

Total PUFA quantities in muscle tissues were significantly greater in Pamlico River flounder during the spring months. Total PUFA data met the assumption of homogeneity of variance using Bartlett's Test during both seasons (spring: $\chi^2_{(2)} = 0.4558$, $p = 0.7962$; fall: $\chi^2_{(2)} = 0.5610$, $p = 0.7554$). Southern flounder collected from Pamlico River ($\bar{x} = 48.95$, $SD = 3.87$, $n = 10$) during the spring months had significantly greater quantities of PUFA than southern flounder collected from both the Tar River ($\bar{x} = 42.42$, $SD = 4.57$, $n = 7$) and Pamlico Sound ($\bar{x} = 42.74$, $SD = 4.57$, $n = 12$). There was no significant difference between the total PUFA of the Tar River and Pamlico Sound southern flounder during the spring. Southern flounder collected during the fall months showed no significant difference in their total PUFA levels among the locations.

Tar River flounder had significantly greater lipid content than flounder from Pamlico River and Pamlico Sound during the spring months. Percent lipid data met the assumption of homogeneity of variance using Bartlett's Test during both seasons (spring: $\chi^2_{(2)} = 1.2530$, $p = 0.5345$; fall: $\chi^2_{(2)} = 0.9946$, $p = 0.6082$). Southern flounder collected from the Tar River during the spring months ($\bar{x} =$

3.09, $SD = 0.82$, $n = 6$) had significantly greater lipid content than Pamlico River ($\bar{x} = 1.96$, $SD = 0.53$, $n = 10$) and Pamlico Sound fish ($\bar{x} = 2.15$, $SD = 0.64$, $n = 12$). There was no significant difference in the lipid content between Pamlico River and Pamlico Sound flounders during the spring. Southern flounder collected during the fall months showed no significant difference in lipid content among the locations.

Southern flounder collected during the spring and fall from the Tar River had significantly lower n -3: n -6 ratios than flounder collected from Pamlico River or Pamlico Sound. Omega-3 to omega-6 ratio data met the assumption of homogeneity of variance using Bartlett's Test during both seasons (spring: $\chi^2_{(2)} = 5.0892$, $p = 0.0785$; fall: $\chi^2_{(2)} = 4.3748$, $p = 0.1122$). During the spring months all locations were significantly different from one another, whereas in the fall only Tar River flounder were significantly lower than Pamlico River and Pamlico Sound fish (Figure 6). When each location was compared individually by season, only Pamlico River flounder had a significantly greater ($t_{9,92} = 3.67$, $p = 0.0044$) n -3: n -6 ratio in the spring ($\bar{x} = 6.10$, $SD = 1.19$) than in the fall.

Mercury

Total mercury levels were significantly higher in flounder collected from the Tar River compared to Pamlico River and Pamlico Sound flounder (Figure 7). A significant correlation between total mercury content and total length was revealed at all locations. Differences between sexes were not significant ($p > 0.05$) at any location, and therefore sexes were combined for all tests.

Southern flounder total mercury content was significantly and positively correlated with total length (Table 12). Correlation analysis was conducted with mercury content and LSI and K_n values and no significant correlations were reported.

Growth and Condition

Sex Ratio

Female southern flounder dominated the catches in all three areas comprising 82.1% of the total collected (Table 13). A total of 811 southern flounder were collected -- 20 from Tar River (Figure 8), 364 from Pamlico River, and 427 from Pamlico Sound -- resulting in female sex percentages of 65.0%, 91.5%, and 74.9%, respectively. Females dominated the catches of harvestable size southern flounder (>355 mm TL) -- Tar River = 80.0%, Pamlico River = 99.4%, Pamlico Sound = 97.7%. The age distribution of southern flounder collected from the sampling locations was composed of sub-adult and young adults (Figure 9).

Growth

Sexual dimorphic growth of southern flounder was evident only in fish captured from Pamlico River and Pamlico Sound. For all locations combined, females ($\bar{x}$ = 353.0 mm, SD = 50.6 mm) had a significantly greater mean total length than males ($\bar{x}$ = 304.4 mm, SD = 37.3 mm), $t_{(240)} = 12.6$, $p < 0.0001$. However, when sorted by location, flounder from Tar River did not exhibit significant sexual dimorphic growth, $t_{(14)} = 1.64$, significant at $p = 0.06$. Both

Pamlico River ($p < 0.0001$) and Pamlico Sound ($p < 0.0001$) exhibited sexual dimorphic growth and all further growth analyses were conducted for each sex separately.

The non-linear regression analysis of the total length of southern flounder using fractional ages was different among the three locations (Table 14). The regression model for the Tar River was not significant for males ($F_{(2,3)} = 0.66$, $p = 0.5779$) and no comparisons were made.

Condition

The condition of southern flounder determined by the LSI was similar for Tar River and Pamlico River fish, but Pamlico Sound fish had significantly lower values. Southern flounder collected from the Tar River ($\bar{x} = 1.69$, $SD = 0.17$, $n = 4$) and Pamlico River ($\bar{x} = 1.43$, $SD = 0.40$, $n = 85$) had LSI values that were significantly greater than Pamlico Sound ($\bar{x} = 1.11$, $SD = 0.33$, $n = 64$). However, there was not any significant difference between Tar River and Pamlico River fish.

Female southern flounder collected in Pamlico River during the fall exhibited a greater relative condition factor (K_n) than those collected from Pamlico Sound and Tar River. Pamlico River relative condition factor was significantly greater than the population mean ($\bar{x} = 102.1$, $t_{(178)} = 2.94$, $p = 0.0037$) and Pamlico Sound was significantly less than the population mean ($\bar{x} = 97.4$, $t_{(100)} = 3.39$, $p = 0.0010$). The Tar River relative condition factor was not

significantly different from the population mean ($\bar{x} = 100.4$, $t_{(4)} = 0.17$, $p = 0.8763$).

The ANCOVA shows that female southern flounder for Pamlico River and Pamlico Sound are in better condition than southern flounder in Tar River. To test for differences between the locations (sub-populations) in each sex, I conducted an analysis of covariance. An ANCOVA was used for females in the general linear model (SAS PROC GLM) using untransformed total lengths (skewness = -0.134) and square root transformed weights (skewness = 0.265). The slopes of the three sub-populations, when controlling for length, were not significantly different for the female group ($F_{(2,615)} = 2.05$, $p = 0.13$). A test of intercepts concluded that the three sub-populations have different intercepts ($F_{(2,617)} = 18.67$, $p < 0.0001$).

An ANCOVA conducted separately for males and revealed that male southern flounder from Pamlico Sound are in better condition than males from the Tar River. The same ANCOVA model was used to test for differences between locations (sub-populations) in males using untransformed total lengths (skewness = -0.574) and square root transformed weights (skewness = -0.367). The slopes of the three sub-populations were statistically significantly different ($F_{(2,124)} = 3.21$, $p = 0.0439$) indicating that incremental weight gain for a given increase in length is different among the three sub-populations. A *t*-test using the least significant difference criterion indicated that Pamlico River and Pamlico

Sound are similar, and Pamlico River and Tar River are similar, but Pamlico

Sound and Tar River are significantly different at the 0.05 level.

CHAPTER 4: DISCUSSION

Freshwater Residency

Otolith Microchemistry

Strontium concentrations of southern flounder collected from the Tar River were indicative of freshwater residency. An average strontium concentration over the last 6 months of life was used to classify residency in the freshwater environment. Only the last 6 months were used due to the collections containing primarily 1+ age fish (95%). However, a 3-year old female was collected that immigrated to the freshwater environment during its first year of life and resided there until collected (Figure 10).

Pamlico River and Pamlico Sound are considered mesohaline and polyhaline environments respectively, but seasonal fluctuations can occur (Giese et al. 1985). Strontium levels of southern flounder from Pamlico River and Pamlico Sound did not differ enough to distinguish the two habitats (Figure 10). Therefore, the two habitats were combined as estuarine habitat (Table 8).

Otolith microchemistry indicates that southern flounder exhibit high site fidelity once they have completed their larval immigration. Southern flounders examined in this study maintained consistent strontium concentrations after the first year of life suggesting that their movements are localized. Variation in strontium concentration that would normally indicate movement can be explained by fluctuating salinities that coincide with seasonal weather patterns (Figure 11). Strontium concentrations gave no indication that southern flounder captured in

the estuarine habitats were post-spawn individuals. Taylor et al. (2008) used otolith microchemistry to conclude that southern flounder that returned to the estuary after spawning stayed in high salinity habitats adjacent to inlets.

Fatty Acids

Fatty acid profiles of southern flounder collected from the Tar River were indicative of a freshwater food web with a n -3: n -6 range of 1.57 – 3.20. Steffens (1997) reported that n -3: n -6 ratios of freshwater fish range from 1 – 4 (Table 15). Compared to southern flounder collected from the estuaries (Pamlico River = 5.45; Pamlico Sound = 4.05), Tar River flounder had significantly lower n -3: n -6 ratios ($\bar{x}$ = 2.02) more equivalent to other freshwater species. This supports the conclusion that southern flounder exhibit resident behaviors as pre-spawning individuals in the coastal rivers of North Carolina.

Not only was the n -3: n -6 ratio indicative of a freshwater lifestyle, but the individuals fatty acids further provided evidence of freshwater residency. Southern flounder collected from the Tar River had elevated levels of linoleic acid (18:2 n 6) and DHA (22:6 n 3) levels half that of the estuarine flounder (Table 11). These results are in agreement with the findings of Stansby (1967) that compared ocean-caught and river-caught Coho salmon.

Southern flounder collected from Pamlico River during the spring months (24 March 2008 – 6 June 2008) were significantly higher in total PUFA and n -3: n -6 ratios than during the fall months (1 September 2008 – 31 October 2008). Taylor et al. (2009) found synchrony in the juvenile abundance of spot

Leiostomous xanthurus, pinfish *Lagodon rhomboides*, and southern flounder in North Carolina estuaries. The spring arrival of these juvenile estuarine dependent prey species could have a fatty acid profiles indicative of a marine food web, ultimately increasing the total PUFA levels of predatory flounder and the $n-3:n-6$ springtime ratio.

Mercury

Total mercury levels were significantly higher in southern flounder collected from the Tar River; however, levels were still considerably lower than the consumption advisory level for North Carolina fishes (0.4 mg/kg) (Table 16). Mercury has been used by other investigators to establish patterns of residency (Radtke and Morales-Nin 1989; Mason et al. 2006; Kim and Rochford 2008), but is more effective when other habitats are used for comparative purposes. Several studies have reported that fish of the same species will have a larger Hg burden in freshwater systems when compared to estuarine or marine systems (Riget et al. 2000; Mason et al. 2006; Rochford and Kim 2008). In this study Hg levels were nearly two-fold higher in the Tar River when compared to estuarine locations.

Mercury levels increased with length. All three habitats had significant positive correlations indicative of the bioaccumulation of mercury over the lifetime of the fish. Larger fish are preferred by recreational and commercial anglers; however, they have higher mercury levels. In North Carolina the largest southern flounder are caught in the ocean near-shore or offshore on wrecks, but have

substantially lower Hg burdens than pelagic species that feed higher in the food chain (Williams 2006). This study confirms that southern flounder in North Carolina are relatively low in mercury, and can be recommended as a species that can be consumed safely.

Freshwater Habitat

Collection efforts of southern flounder in freshwater indicate that coastal watersheds should be considered important secondary habitat. In my study southern flounder were collected in low numbers because our sampling techniques were inefficient, so collections underestimated the population. Flounders escaped as often as those collected (Table 2).

Fish captured within the Tar River by electrofishing were located in shallow water (<1.0 m deep) on coarse sand, fine sand, and muddy substrates. I quantified sediment samples from the harvest location by calculating the percent composition of sand, silt, and clay (Table 17). This sediment composition allows the flounder to burrow into the sediment to conceal itself from prey. During the collections I noticed that flounder in the Tar River were located on flat, shallow areas on the inside of a curve in the river. I hypothesize that flounder are utilizing this habitat for feeding due to the current pushing prey past them. Flounder are ferocious ambush predators in other habitats they utilize currents or tides to deliver prey to them.

Few of the southern flounder collected in the freshwater habitat had stomach contents, but those that were feeding consumed small centrarchids and

cyprinids. These findings in conjunction with the total mercury levels and fatty acid profiles confirm that southern flounder residing in Tar River have adapted to a freshwater environment. However, reduced growth seems to be a consequence of living in this secondary habitat.

Growth and Condition

Females dominated the catch (82.1%) during this study. This was not unexpected because the NCDMF 2009 southern flounder stock assessment indicated that the harvestable population is heavily skewed towards females (Takade-Heumacher and Batsavage 2009). The percentage of female southern flounder collected in this study >355 mm (14" commercial size limit) agrees with the NCDMF 2009 stock assessment that females dominate the commercial and recreational harvest. The sex ratio of all southern flounder collected from the Tar River (65.0%) was less skewed than the other locations (Pamlico River = 91.5%, Pamlico Sound = 74.9%). One factor might be reduced gear bias in electrofishing versus gill netting. The gill nets used for the collections in the estuaries were representative of a commercial gill net that is specifically sized to minimize bycatch of under-sized fish. Therefore, slower growing males should have been underrepresented in estuarine collections.

Growth was measured using Von Bertalanffy growth parameters. However, the non-linear regression model was not significant for males in the Tar River because of low sample sizes and homogeneity among ages. Female southern flounder grew the fastest in Pamlico River ($K = 0.176$) (Figure 12). This

parameter indicates that southern flounder were growing very rapidly and at a faster rate than the NCDMF 2009 southern flounder stock assessment reported. However, the catch was dominated by fast growing age-1 fish and the model only included four age classes (0,1,2,3), whereas the stock assessment used ages representative of the entire life span (0-10). Female southern flounder from the Tar River had slower growth rates as indicated by a high growth coefficient ($K = 1.387$) and a short maximum theoretical length ($L_{\infty} = 456$ mm) (Figure 13). However, the non-linear regression model resulted in broad confidence limits indicating that this model should be used with caution (CI_{95} : $L_{\infty} = 354.4, 556.8$; $K = 0.139, 2.635$).

Southern flounder collected from the Tar River were in equivalent condition compared to Pamlico River. Liver somatic index values were significantly greater in Pamlico Sound, but did not differ from Pamlico River compared to the Tar River. The K_n of Tar River southern flounder was not significantly different from the population mean. This suggests that southern flounder utilizing the Tar River are in good condition and their condition is not being suppressed by utilizing this habitat. The ANCOVA for females suggests that the three sub-populations gain weight incrementally in a similar fashion; however, females from Pamlico River and Pamlico Sound were consistently heavier at a given length than females from the Tar River. One possibility for the differences in weight or condition could be differences in prey availability and the quality of prey. The prey base that southern flounder were consuming in the

freshwater environment was mainly small centrarchids and cyprinids. In contrast, the southern flounder collected from estuarine locations were feeding primarily on sciaenids, pinfish, and shrimp *Penaeus* spp. (Table 18). Another potential hypothesis that could explain the differences in weight are the additional osmoregulatory cost associated with residing in freshwater. By devoting more energy to maintain their osmoregulation in the freshwater, less energy is available for somatic growth.

Conclusions and Recommendations

The majority (74%) of southern flounder collected in the freshwater environment are residing there until time to migrate offshore to spawn. Collections of young, immature flounder suggest that coastal rivers in North Carolina are important, secondary sub-adult habitat. Coastal rivers should be considered secondary habitat because of low abundance and non-optimal growth. Although secondary, it is important habitat because it is utilized during the sub-adult life stage. The freshwater habitat has fewer natural predators and very little fishing mortality potentially increasing survival of sub-adult flounder. Other coastal rivers in North Carolina should be evaluated and compared to determine the importance of freshwater habitat to the North Carolina flounder fishery.

The utilization of freshwater habitats by southern flounder is known (Dahlberg 1972, Farmer 2008), but the mechanism that introduces larvae or juveniles to this habitat is yet to be determined. Southern flounder could

immigrate into the Tar River by following tides as far inland as Greenville, NC (~95 km from Pamlico Sound) (Giese et al. 1985). In conjunction to utilizing tides, the benthic species could follow a salt-wedge upriver, become acclimated to the environment, and exhibit site fidelity as they do in other habitats.

The Tar River and other coastal rivers in North Carolina are affected by urbanization, which can contribute to excess nutrients, pollution, sedimentation, and degradation of habitat (Street et al. 2005). Using Hg as an indicator of residency for southern flounder is a result of urbanization and increased anthropogenic effects on our ecosystems. Thankfully, total mercury levels in southern flounder, North Carolina's most profitable finfish fishery, are substantially below consumption advisory standards.

Southern flounder otoliths exhibited lower than expected strontium levels at the core. However, early in life there often was a spike in the strontium level that could possibly coincide with metamorphosis, benthic settlement, in-shore immigration, and consequently a diet change. The physiological processes of the elemental profile before, during, and after metamorphosis must shift to levels not observed in the surrounding environment. Exactly when strontium levels in the otoliths match those of the environment is not known in southern flounder and should be further investigated.

Recommendations for further research include a telemetry tracking study to help further evaluate the importance of freshwater habitats. In conjunction with a tracking study, a reward-tagging study should be conducted to evaluate

the importance of southern flounder that utilized freshwater to the North Carolina fishery.

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Table 1. Tar River, North Carolina electrofishing sections. Sections were separated on the proximity to the nearest boat ramp, sections averaged 15.3 river km.

Section	Boat ramp	City	Section	River km	GPS Coordinates	
					North	West
1	Dunbar Bridge	Rocky Mount	Upriver to rapids	~ 18.2	35 56.437	-77 39.3080
2	Bells Bridge	Tarboro	Upriver to Dunbar Bridge	~ 20.6	35 55.676	-77 32.9725
3	City Park	Tarboro	North to Bells Bridge	~ 17.3	35 53.676	-77 31.728
4	Old Sparta	Pine Tops	North to City Park	~ 12.3	35 47.427	-77 32.926
5	Falkland	Falkland	North to Old Sparta	~ 13.5	35 41.707	-77 29.395
6	Falkland	Falkland	South to 264 W	~ 10.0	35 41.707	-77 29.395
7	Town Commons	Greenville	264 W to 264 E	~ 12.1	35 36.9322	-77 22.0695
8	Port Terminal	Greenville	264 E to SR 1565	~ 18.5	35 35.8483	-77 18.8383

Table 2. Tar River, North Carolina electrofishing locations in 2007 and 2008. One electrofishing session lasted approximately 1800 shocking seconds with typically one boat operator and one netter. Date format = MM/DD/YYYY.

Sessions	Date	Boat Ramp	Section	Flounder	Comments
4	9/14/2007	Port Terminal	8	3	3 not collected
4	10/5/2007	Transters Creek	*	0	high conductivity
3	11/9/2007	Town Commons	7	0	
3	12/18/2007	Port Terminal	8	0	
3	1/24/2008	Town Commons	7	1	
4	2/25/2008	Town Commons	7	1	3 not collected
3	3/24/2008	Port Terminal	8	5	9 not captured
3	4/15/2008	Tarboro	3	0	
4	5/12/2008	Falkland	5	0	2 not collected
2	6/9/2008	Tarboro	4	3	3 not collected
4	6/26/2008	Port Terminal	8	0	3 not collected
3	7/8/2008	Tarboro	3	1	Turbid water
4	7/17/2008	Town Commons	7	0	2 not collected
1	8/1/2008	Falkland	6	0	1 not collected
1	8/15/2008	Dunbar Bridge	1	0	low water conditions
3	9/4/2008	Town Commons	7	0	4 not collected
*	9/10/2008	Grimesland	8	1	NCWRC
*	9/11/2008	Grimesland	8	1	NCWRC
*	9/12/2008	Grimesland	8	3	NCWRC
1	9/18/2008	Dunbar Bridge	1	0	
2	10/10/2008	Falkland	5	1	equipment problems
3	10/23/2008	Port Terminal	8	0	1 not collected
3	11/25/2008	Tarboro	4	0	
3	11/26/2008	Falkland	6	1	
Total =				21	31

*Missing data, fish were collected cooperatively by the North Carolina Wildlife Resources Commission.

Table 3. Pamlico River, North Carolina gill netting locations. Three large mesh and two small mesh flounder gill nets were set overnight in randomly chosen locations from May 2008 through November 2008. Date format = MM/DD/YYYY

Net	Set		Pick-up		GPS Coordinates		Flounder
	Date	Time	Date	Time	Degrees N	Degrees W	
1	5/13/2008	1804	5/14/2008	800	35 29.966	-76 48.516	7
2	5/13/2008	1837	5/14/2008	902	35 27.091	-76 49.465	3
3	5/13/2008	1902	5/14/2008	950	35 27.457	-76 52.345	1
1	6/3/2008	1817	6/4/2008	825	35 22.175	-76 42.746	4
2	6/3/2008	1836	6/4/2008	910	35 22.549	-76 43.838	2
3	6/3/2008	1914	6/4/2008	1003	35 25.012	-76 40.388	3
1	6/4/2008	1827	6/5/2008	1007	35 22.944	-76 45.423	2
2	6/4/2008	1849	6/5/2008	915	35 25.261	-76 45.190	3
3	6/4/2008	1930	6/5/2008	822	35 25.569	-76 42.501	5
1	6/15/2008	1751	6/16/2008	803	35 28.616	-76 59.785	1
2	6/15/2008	1812	6/16/2008	850	35 29.054	-77 0.911	2
3	6/15/2008	1830	6/16/2008	920	35 30.795	-77 0.645	11
1	6/16/2008	1830	6/17/2008	800	35 29.718	-76 57.448	4
2	6/16/2008	1908	6/17/2008	905	35 27.920	-76 58.761	5
3	6/16/2008	1934	6/17/2008	1000	35 30.473	-77 01.396	1
1	7/1/2008	1823	7/2/2008	743	35 22.664	-76 43.603	0
2	7/1/2008	1900	7/2/2008	836	35 25.205	-76 42.210	0
3	7/1/2008	1930	7/2/2008	910	35 24.830	-76 40.953	0
1	7/2/2008	1750	7/3/2008	751	35 22.666	-76 44.313	1
2	7/2/2008	1816	7/3/2008	842	35 25.535	-76 44.653	3
3	7/2/2008	1836	7/3/2008	935	35 25.113	-76 41.907	1
1	7/13/2008	1745	7/14/2008	737	35 28.447	-76 56.694	23
2	7/13/2008	1817	7/14/2008	940	35 30.826	-77 0.586	8
3	7/13/2008	1847	7/14/2008	1056	35 31.674	-77 3.011	6
1	7/17/2008	1830	7/18/2008	645	35 26.141	-76 57.721	8
2	7/17/2008	1855	7/18/2008	745	35 26.859	-76 58.283	14
3	7/17/2008	1920	7/18/2008	900	35 28.421	-76 56.298	12
1	8/3/2008	1915	8/4/2008	832	35 25.445	-76 46.103	1
2	8/3/2008	1938	8/4/2008	927	35 23.668	-76 48.264	2
3	8/3/2008	2011	8/4/2008	1032	35 22.589	-76 43.866	1

Table 4. Sampling protocol for the Milestone DMA-80 direct mercury analyzer. Ninety-one southern flounder white muscle tissue samples were analyzed for total mercury. This protocol is used for each series of samples when starting the machine for the day. Operations take approximately 6 minutes each.

Position	Order of operations
1	blank
2	200 ng check standard
3	blank
4	200 ng reference standard
5	blank
6	soy flour standard
7	soy flour standard + 200 ng reference standard
8	blank
9	TORT-2 (Lobster reference material)
10	DOLT-3 (Dogfish reference material)
11	blank
12	method blank
13-24	samples
25	200 ng check standard
26	blank
27	blank
28-38	samples
39	200 ng check standard
40	blank

Table 5. Total mercury analysis of 91 southern flounder from three size classes (small, median, large) from the Tar River, Pamlico River, and Pamlico Sound, North Carolina. Total mercury was measured with a Milestone DMA-80 direct mercury analyzer using EPA method 7473 (Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry).

Location	<i>n</i>	Size	Total length (mm) $\bar{x} \pm SD$	Hg (mg/kg) $\bar{x} \pm SD$
Tar River	9	small	227 ± 20.7	0.083 ± 0.052
Tar River	6	median	326 ± 28.9	0.126 ± 0.033
Tar River	5	large	387 ± 36.3	0.122 ± 0.037
Pamlico River	12	small	229 ± 24.2	0.028 ± 0.011
Pamlico River	12	median	318 ± 39.2	0.028 ± 0.009
Pamlico River	12	large	422 ± 89.4	0.047 ± 0.019
Pamlico Sound	12	small	241 ± 16.5	0.051 ± 0.016
Pamlico Sound	12	median	339 ± 23.4	0.045 ± 0.013
Pamlico Sound	11	large	414 ± 67.4	0.068 ± 0.016

Table 6. Southern flounder analyzed by LA-ICP-MS for otolith microchemistry. Age was determined by aging whole otoliths. TL = Total length, date format = MM/DD/YYYY.

Location	ID	Capture Date	Sex	TL (mm)	Age
Pamlico River	113	6/4/2008	M	327	2
Pamlico River	122	6/5/2008	M	329	2
Pamlico River	236	7/13/2008	M	337	2
Pamlico River	255	7/17/2008	M	355	2
Pamlico River	382	9/10/2008	F	485	2
Pamlico River	399	9/10/2008	M	351	2
Pamlico River	524	10/2/2008	F	482	2
Pamlico River	1006	9/10/2008	M	332	2
Pamlico River	4005	9/10/2008	F	466	2
Pamlico River	398	9/10/2008	F	516	3
Pamlico River	506	10/2/2008	F	479	3
Pamlico River	515	10/2/2008	F	525	3
Pamlico Sound	164	5/18/2008	M	350	2
Pamlico Sound	243	6/20/2008	M	363	2
Pamlico Sound	265	6/20/2008	F	457	2
Pamlico Sound	305	7/17/2008	F	453	2
Pamlico Sound	310	7/17/2008	M	364	2
Pamlico Sound	346	7/18/2008	M	353	2
Pamlico Sound	367	7/18/2008	F	465	2
Pamlico Sound	393	7/18/2008	M	363	2
Pamlico Sound	607	10/10/2008	F	461	2
Pamlico Sound	4039	8/17/2008	M	355	2
Pamlico Sound	307	7/17/2008	F	456	3
Pamlico Sound	314	7/17/2008	F	505	4
Tar River	104	1/24/2008	F	245	1
Tar River	105	2/25/2008	M	254	1
Tar River	106	3/24/2008	M	213	1
Tar River	107	3/24/2008	F	233	1
Tar River	108	3/24/2008	F	271	1
Tar River	109	3/24/2008	F	213	1
Tar River	110	3/24/2008	F	215	1
Tar River	111	6/9/2008	F	355	1
Tar River	112	6/9/2008	M	367	1
Tar River	122	6/9/2008	F	346	1
Tar River	150	10/10/2008	M	333	1
Tar River	161	11/26/2008	F	372	1
Tar River	201	9/10/2008	F	392	1
Tar River	202	9/12/2008	F	332	1
Tar River	203	9/12/2008	F	351	1
Tar River	204	9/12/2008	F	320	1
Tar River	205	9/11/2008	M	256	1
Tar River	2A	4/18/2007	M	220	1
Tar River	140	7/8/2008	F	447	3

Table 7. Linear regression parameter estimates of log transformed total length and weight for southern flounder collected from the Tar River, Pamlico River, and Pamlico Sound, North Carolina in 2007 and 2008.

Location	Sex	N	Intercept		Slope		ANOVA statistics	
			<i>a</i>	<i>CI</i> ₉₅	<i>b</i>	<i>CI</i> ₉₅	<i>r</i> ²	<i>p</i>
Tar River	F	13	-5.724	-6.130, -5.318	3.319	3.156, 3.482	0.9945	< 0.0001
Tar River	M	7	-6.324	-7.102, -5.546	3.580	3.257, 3.903	0.9939	< 0.0001
Pamlico River	F	309	-5.015	-5.237, -4.793	3.046	2.959, 3.133	0.9388	< 0.0001
Pamlico River	M	30	-5.034	-5.631, -4.438	3.055	2.812, 3.298	0.9595	< 0.0001
Pamlico Sound	F	299	-5.617	-5.788, -5.447	3.274	3.208, 3.341	0.9690	< 0.0001
Pamlico Sound	M	93	-6.396	-6.870, -5.922	3.592	3.402, 3.782	0.9394	< 0.0001

Table 8. Otolith microchemistry classification matrix based on strontium levels of southern flounder collected in 2008 from the Tar River (TR), Pamlico River (PR) and Pamlico Sound (PS), North Carolina. Classifications were based on strontium concentrations: >2500 ppm = marine (1), 1000-2500 ppm = estuary (2), <1000 ppm = freshwater (3).

Location	ID	Capture Date	Sex	Age						Capture Location
				0	0.5	1+	2+	3+	4+	
TR	104	1/24/2008	F	2	3					3
TR	107	3/24/2008	F	3	1					3
TR	108	3/24/2008	F	3	3					3
TR	109	3/24/2008	F	1	2					3
TR	110	3/24/2008	F	2	2					3
TR	111	6/9/2008	F	2	3	3				3
TR	122	6/9/2008	F	2	3	3				3
TR	140	7/8/2008	F	1	2	3	3	3		3
TR	161	11/26/2008	F	2	2	3				3
TR	201	9/10/2008	F	2	3	3				3
TR	202	9/12/2008	F	2	3	3				3
TR	203	9/12/2008	F	2	3	3				3
TR	204	9/12/2008	F	2	3	3				3
TR	105	2/25/2008	M	2	3					3
TR	106	3/24/2008	M	1	2					3
TR	112	6/9/2008	M	2	3	3				3
TR	150	10/10/2008	M	1	2	3				3
TR	205	9/11/2008	M	1	2	3				3
PR	382	9/10/2008	F	2	2	2				2
PR	398	9/10/2008	F	2	2	2	2	2		2
PR	506	10/2/2008	F	2	2	2	2	2		2
PR	515	10/2/2008	F	1	2	2	2	2		2
PR	524	10/2/2008	F	1	2	2	2			2
PR	4005	9/10/2008	F	1	2	2	2			2
PR	113	6/4/2008	M	2	2	2	2			2
PR	122	6/5/2008	M	1	2	2	2			2
PR	236	7/13/2008	M	2	2	2	2			2
PR	255	7/17/2008	M	2	2	2	2			2
PR	399	9/10/2008	M	2	2	2	2			2
PR	1006	9/10/2008	M	2	2	2	2			2
PS	265	6/20/2008	F	1	2	2	2			2
PS	305	7/17/2008	F	2	2	2	2			2
PS	307	7/17/2008	F	1	2	2	2	2		2
PS	314	7/17/2008	F	2	2	2	2	2	2	2
PS	367	7/18/2008	F	2	2	2	2			2

Table 8. Continued.

Location	ID	Capture Date	Sex	Age						Capture Location
				0	0.5	1+	2+	3+	4+	
PS	607	10/10/2008	F	1	2	2	2			2
PS	164	5/18/2008	M	2	2	2	2			2
PS	243	6/20/2008	M	2	2	2	2			2
PS	310	7/17/2008	M	1	2	2	2			2
PS	346	7/18/2008	M	2	2	2	2			2
PS	393	7/18/2008	M	2	2	3	2			2
PS	4039	8/17/2008	M	1	2	2	2			2

Table 9. Southern flounder freshwater residency classification matrix based upon fish collected from the Tar River, North Carolina in 2007 and 2008. Total mercury (mg/kg), $n\text{-}3:n\text{-}6$, and strontium concentration (ppm) was used to classify southern flounder collected from the Tar River as residents or transients. Total mercury values $> 2\text{ SD}$ above the mean total mercury level for Pamlico River (0.066) = resident; $n\text{-}3:n\text{-}6 \leq 3.000$ = resident; strontium concentrations ≤ 1000.0 = resident. Classification of residency was only assigned to individuals that exhibited 100% of the characteristics.

ID	Sex	Age	Total Hg (mg/kg)	$n\text{-}3:n\text{-}6$	Strontium (ppm)	Classification
104	F	1	0.086	*	703.3	resident
107	F	1	0.070	2.077	2350.2	transient
108	F	1	0.070	*	608.1	resident
109	F	1	0.049	3.194	1733.6	transient
110	F	1	0.072	2.161	1358.5	transient
111	F	1	0.094	*	534.2	resident
122	F	1	0.106	1.932	535.3	resident
140	F	3	0.186	1.894	621.7	resident
161	F	1	0.120	2.137	655.0	resident
201	F	1	0.116	2.082	595.3	resident
202	F	1	0.143	1.566	584.5	resident
203	F	1	0.131	1.605	589.5	resident
204	F	1	0.164	1.674	656.8	resident
105	M	1	0.081	*	610.0	resident
106	M	1	0.062	1.985	1255.0	transient
112	M	1	0.096	1.897	697.4	resident
150	M	1	0.143	1.956	718.5	resident
205	M	1	0.094	*	1025.9	transient
2A	M	1	0.208	*	465.2	resident

*Asterisk indicates data not measured.

Table 10. Lipid, PUFA, and n -3: n -6 of southern flounder collected in the spring and fall of 2008 from the Tar River, Pamlico River, and Pamlico Sound, North Carolina. Mean values are given, standard deviations are in parenthesis. Season 1 = spring, season 2 = fall; TL = total length. PUFA = total polyunsaturated fatty acids; n -3: n -6 = total omega 6 fatty acids divided by the total omega 3 fatty acids.

Location	Sex	Season	n	TL - mm	% Lipid	PUFA	n -3: n -6
Tar River	M	1	2	290.0 (108.9)	2.50 (1.04)	41.28 (1.31)	1.94 (0.06)
	F	1	5	290.8 (103.2)	3.98 (1.46)	42.87 (5.47)	2.25 (0.54)
	M	2	1	333.0	3.57	38.55	1.96
	F	2	5	353.4 (29.2)	2.39 (0.84)	42.53 (2.32)	1.81 (0.27)
Pamlico River	M	1	2	289.0 (93.3)	2.17 (0.51)	47.80 (5.53)	5.63 (1.88)
	F	1	8	352.4 (31.7)	1.91 (0.55)	49.24 (3.80)	6.21 (1.26)
	M	2	2	341.5 (13.4)	2.58 (0.31)	42.11 (0.47)	5.03 (0.21)
	F	2	14	409.3 (82.7)	1.84 (0.76)	44.26 (3.73)	4.72 (0.57)
Pamlico Sound	M	1	6	355.5 (9.6)	2.02 (0.45)	43.18 (2.74)	3.81 (0.43)
	F	1	6	444.8 (58.1)	2.27 (0.81)	42.30 (6.16)	4.29 (1.02)
	M	2	2	277.5 (21.9)	2.68 (0.01)	44.65 (2.24)	3.68 (1.65)
	F	2	2	414.5 (65.7)	2.42 (0.83)	39.87 (3.09)	4.47 (0.03)

Table 11. Fatty acid content (mean percent $\pm$ SD) of white muscle tissue of southern flounder from spring 2008 and fall 2008, collected from three locations in North Carolina. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

Fatty acid	Tar River		Pamlico River		Pamlico Sound	
	Spring (n= 7)	Fall (n= 6)	Spring (n= 10)	Fall (n= 16)	Spring (n= 12)	Fall (n= 4)
13:0	0.20 $\pm$ 0.06	0.31 $\pm$ 0.10	0.40 $\pm$ 0.07	0.33 $\pm$ 0.08	0.30 $\pm$ 0.07	0.36 $\pm$ 0.11
14:0	2.11 $\pm$ 0.69	1.52 $\pm$ 0.39	1.19 $\pm$ 0.49	1.43 $\pm$ 0.53	1.72 $\pm$ 0.65	1.60 $\pm$ 0.79
15:0	0.66 $\pm$ 0.18	0.55 $\pm$ 0.05	0.75 $\pm$ 0.10	0.89 $\pm$ 0.27	1.14 $\pm$ 0.91	1.31 $\pm$ 0.46
15:1	*	0.07 $\pm$ 0.02	0.09 $\pm$ 0.04	0.06 $\pm$ 0.01	0.08 $\pm$ 0.06	0.11 $\pm$ **
16:0	23.91 $\pm$ 1.75	25.61 $\pm$ 0.65	25.09 $\pm$ 0.85	27.29 $\pm$ 1.45	26.13 $\pm$ 1.17	26.59 $\pm$ 1.24
16:1n7	6.43 $\pm$ 2.13	4.64 $\pm$ 1.62	2.77 $\pm$ 1.16	2.54 $\pm$ 1.02	3.96 $\pm$ 1.48	3.48 $\pm$ 1.41
16:2n4	0.40 $\pm$ 0.16	0.22 $\pm$ 0.11	0.18 $\pm$ 0.06	0.10 $\pm$ 0.04	0.17 $\pm$ 0.14	0.18 $\pm$ 0.05
16:3n4	0.25 $\pm$ 0.11	0.12 $\pm$ 0.02	0.19 $\pm$ 0.08	0.10 $\pm$ 0.05	0.32 $\pm$ 0.19	0.28 $\pm$ **
17:0	0.75 $\pm$ 0.23	0.73 $\pm$ 0.08	0.80 $\pm$ 0.12	0.91 $\pm$ 0.16	0.79 $\pm$ 0.16	1.04 $\pm$ 0.05
17:1	0.40 $\pm$ 0.15	0.36 $\pm$ 0.20	0.65 $\pm$ 0.19	0.70 $\pm$ 0.13	1.11 $\pm$ 0.54	0.94 $\pm$ 0.51
18:0	6.10 $\pm$ 0.83	6.99 $\pm$ 0.79	7.52 $\pm$ 0.45	7.60 $\pm$ 0.50	7.40 $\pm$ 0.35	7.55 $\pm$ 0.78
18:1n7	4.12 $\pm$ 0.66	3.62 $\pm$ 0.69	2.52 $\pm$ 0.35	2.09 $\pm$ 0.40	2.78 $\pm$ 0.35	2.56 $\pm$ 0.58
18:1n9	11.89 $\pm$ 1.92	12.66 $\pm$ 1.35	8.18 $\pm$ 2.18	10.89 $\pm$ 1.50	10.65 $\pm$ 1.98	11.04 $\pm$ 2.20
18:2n6	4.99 $\pm$ 1.37	5.47 $\pm$ 0.70	1.19 $\pm$ 0.34	1.39 $\pm$ 0.40	1.26 $\pm$ 0.42	1.47 $\pm$ 1.33
18:3n3	1.83 $\pm$ 0.89	3.37 $\pm$ 2.36	0.19 $\pm$ 0.10	0.47 $\pm$ 0.40	0.28 $\pm$ 0.34	0.47 $\pm$ 0.60
18:3n4	0.21 $\pm$ 0.05	0.14 $\pm$ 0.03	0.29 $\pm$ 0.05	0.22 $\pm$ 0.05	0.17 $\pm$ 0.04	0.16 $\pm$ 0.05
18:3n6	0.30 $\pm$ 0.08	0.26 $\pm$ 0.09	0.16 $\pm$ **	0.09 $\pm$ 0.03	0.16 $\pm$ 0.10	0.17 $\pm$ 0.02
18:4n3	0.37 $\pm$ 0.21	0.58 $\pm$ 0.40	0.88 $\pm$ 0.24	0.96 $\pm$ 0.64	0.77 $\pm$ 0.32	0.69 $\pm$ 0.38
20:0	0.19 $\pm$ 0.04	0.20 $\pm$ 0.03	0.20 $\pm$ 0.07	0.22 $\pm$ 0.05	0.30 $\pm$ 0.08	0.21 $\pm$ 0.07
20:1n9	0.21 $\pm$ 0.09	0.20 $\pm$ 0.04	0.23 $\pm$ 0.07	0.30 $\pm$ 0.09	0.27 $\pm$ 0.09	0.22 $\pm$ 0.09
20:2	0.52 $\pm$ 0.06	0.50 $\pm$ 0.07	0.40 $\pm$ 0.11	0.52 $\pm$ 0.13	0.31 $\pm$ 0.05	0.34 $\pm$ 0.08
20:3n3	0.44 $\pm$ 0.15	0.61 $\pm$ 0.33	0.13 $\pm$ 0.02	0.19 $\pm$ 0.07	0.17 $\pm$ 0.07	0.23 $\pm$ 0.05
20:3n6	0.49 $\pm$ 0.10	0.50 $\pm$ 0.03	0.22 $\pm$ 0.04	0.28 $\pm$ 0.03	0.30 $\pm$ 0.05	0.25 $\pm$ 0.06
20:4n3	0.44 $\pm$ 0.11	0.42 $\pm$ 0.20	0.25 $\pm$ 0.09	0.36 $\pm$ 0.12	0.30 $\pm$ 0.12	0.23 $\pm$ 0.15
20:4n6	7.30 $\pm$ 1.50	8.41 $\pm$ 2.83	5.56 $\pm$ 1.37	5.83 $\pm$ 0.90	6.76 $\pm$ 1.30	6.78 $\pm$ 1.28
20:5n3	4.60 $\pm$ 0.99	2.57 $\pm$ 0.52	3.99 $\pm$ 0.65	3.18 $\pm$ 0.32	4.25 $\pm$ 0.76	3.23 $\pm$ 0.70
22:5n3	4.49 $\pm$ 0.20	3.33 $\pm$ 0.57	3.50 $\pm$ 0.62	3.46 $\pm$ 0.70	4.75 $\pm$ 0.39	3.73 $\pm$ 0.95
22:6n3	15.78 $\pm$ 5.11	15.54 $\pm$ 3.73	32.31 $\pm$ 4.84	27.22 $\pm$ 4.00	23.09 $\pm$ 4.75	24.55 $\pm$ 5.09
24:1n9	0.52 $\pm$ 0.07	0.63 $\pm$ 0.21	0.76 $\pm$ 0.09	0.80 $\pm$ 0.10	0.67 $\pm$ 0.08	0.80 $\pm$ 0.13
Σ n-3	28.03 $\pm$ 4.72	26.44 $\pm$ 1.50	41.17 $\pm$ 4.00	35.65 $\pm$ 3.23	33.58 $\pm$ 4.38	33.13 $\pm$ 3.69
Σ n-6	13.08 $\pm$ 1.40	14.64 $\pm$ 2.27	6.98 $\pm$ 1.32	7.55 $\pm$ 0.91	8.46 $\pm$ 1.34	8.58 $\pm$ 2.45
n-3:n-6	2.16 $\pm$ 0.47	1.84 $\pm$ 0.25	6.10 $\pm$ 1.30	4.76 $\pm$ 0.54	4.05 $\pm$ 0.79	4.07 $\pm$ 1.06
SFA	33.92 $\pm$ 1.85	35.92 $\pm$ 1.31	35.92 $\pm$ 0.94	38.67 $\pm$ 1.47	37.78 $\pm$ 1.66	38.67 $\pm$ 1.23
MUFA	23.57 $\pm$ 4.33	22.13 $\pm$ 3.11	15.12 $\pm$ 3.44	17.33 $\pm$ 2.80	19.47 $\pm$ 3.68	19.07 $\pm$ 4.31
PUFA	42.42 $\pm$ 4.57	41.87 $\pm$ 2.63	48.95 $\pm$ 3.87	43.99 $\pm$ 3.55	42.74 $\pm$ 4.57	42.26 $\pm$ 3.53

*No mean because there was no representation of that fatty acid in the sample. **No standard deviation because only one fish in the sample contained that fatty acid.

Table 12. Pearson's correlation analysis of total mercury and total length for southern flounder collected in 2007 and 2008 from the Tar River, Pamlico River, and Pamlico Sound, North Carolina.

Location	<i>n</i>	TL (mm)	Hg (mg/kg)	<i>r</i>	<i>p</i>
		$\bar{x} \pm SD$	$\bar{x} \pm SD$		
Tar River	20	296 ± 73.1	0.105 ± 0.047	0.5484	0.0123
Pamlico River	36	323 ± 98.0	0.034 ± 0.016	0.4861	0.0027
Pamlico Sound	36	328 ± 80.8	0.054 ± 0.017	0.3865	0.0199

Table 13. Frequency table of all southern flounder collected during this study from the Tar River, Pamlico River, and Pamlico Sound, North Carolina in 2007 and 2008 by location and sex. (*n* and row %)

Location	Female	Male	Total
Tar River	14 66.7%	7 33.3%	21 2.6%
Pamlico River	333 91.5%	31 8.5%	364 44.8%
Pamlico Sound	320 74.9%	107 25.1%	427 52.6%
Total	667 82.1%	145 17.9%	812

Table 14. Von Bertalanffy growth parameters for southern flounder collected from the Tar River, Pamlico River and Pamlico Sound, North Carolina in 2007 and 2008. Statewide parameters provided by the 2009 NCDMF southern flounder stock assessment (Takade-Heumacher and Batsavage 2009).

Location	Sex	N	L_{∞}	K	T_0
Tar River	F	13	456	1.387	0.628
Pamlico River	F	308	748	0.176	-2.096
Pamlico Sound	F	298	420	0.761	-0.662
North Carolina	F	*	699	0.284	-0.761
Tar River ¹	M	6	324	3.335	0.798
Pamlico River	M	93	356	1.452	0.705
Pamlico Sound	M	30	366	1.247	0.555
North Carolina	M	*	381	0.803	-0.011

¹The non-linear regression model was not significant for males from the Tar River. * N was not provided by the southern flounder stock assessment.

Table 15. Literature review of $n-3:n-6$ from various habitats.

Species	Location	Habitat	$n-3:n-6$	Source
Chinook salmon, <i>Oncorhynchus tshawytscha</i>	Lake Michigan, US	freshwater	2.4	Honeyfield et al. 2008
Southern flounder, <i>Paralichthys lethostigma</i>	Tar River, NC	freshwater	2.0	this study
Striped bass, <i>Morone saxatilis</i>	Liberty Reservoir, Md	freshwater	2.5	Karahadian et al. 1995
Southern flounder	Charleston, SC	*	5.8	Gooch et al. 1987
Southern flounder	Pamlico Sound, NC	estuary	4.1	this study
Southern flounder	Pamlico River, NC	estuary	5.3	this study
Striped anchovy, <i>Anchoa hepsetus</i>	Charleston Harbor, SC	estuary	6.9	Recks and Seaborn 2008
Striped bass	Upper Chesapeake Bay, Md	estuary	5.7	Karahadian et al. 1995
Striped mullet, <i>Mugil cephalus</i>	Charleston Harbor, SC	estuary	4.0	Recks and Seaborn 2008
Atlantic mackerel, <i>Scomber scombrus</i>	Atlantic Coast, US	marine	4.7	Gruger et al. 1964 in Steffens 1997
Atlantic menhaden, <i>Brevoortia tyrannus</i>	Atlantic Coast, US	marine	9.1	Gruger et al. 1964 in Steffens 1997
Chum salmon, <i>Oncorhynchus keta</i>	Pacific Coast, US	marine	6.7	Gruger et al. 1964 in Steffens 1997
Pacific herring, <i>Clupea pallasii</i>	Pacific Coast, US	marine	10.5	Gruger et al. 1964 in Steffens 1997

* Exact harvest location not defined in article.

Table 16. Total mercury content (mg/kg) of harvestable size (≥ 355 mm TL) southern flounder captured in a North Carolina coastal river and estuarine system in 2008. North Carolina's fish advisory action level is 0.4 mg/kg CH₃Hg.

Location	<i>n</i>	Total length (mm) $\bar{x} \pm SD$	Total Mercury (mg/kg) $\bar{x} \pm SD$
Tar River	5	395 $\pm$ 36	0.129 $\pm$ 0.037
Pamlico River	13	424 $\pm$ 80	0.040 $\pm$ 0.019
Pamlico Sound	15	401 $\pm$ 61	0.057 $\pm$ 0.020
Combined	33	408 $\pm$ 66	0.060 $\pm$ 0.035

Table 17. Sediment analysis of benthic habitat utilized by southern flounder collected in the Tar River. A 7.62-cm pvc sediment corer was used to collect sediment samples. Samples were quantified by percent composition using a simplified graduated cylinder method.

Fish	Latitude (°N)	Longitude (°W)	River KM	% Sand	% Silt	% Clay	Sediment designation
103	35.59647	-77.30760	27	88	10	2	sand
104	35.61815	-77.39163	36	88	13	0	sand
106	35.60552	-77.32413	29	64	34	2	sandy loam
107	35.59787	-77.30817	27	99	1	0	sand
108	35.59865	-77.30675	27	97	3	0	sand
109	35.59795	-77.30193	27	97	3	0	sand
110	35.59800	-77.29947	26	92	5	3	sand
111	35.89133	-77.53705	76	0	98	2	silt
112	35.89330	-77.53383	76	100	0	0	sand
122	35.89412	-77.52855	76	0	96	4	silt
140	35.90255	-77.50007	80	100	0	0	sand
150	35.69922	-77.49082	51	93	7	0	sand
161	35.66878	-77.46755	47	100	0	0	sand

Table 18. Diet analysis of southern flounder collected in 2007 and 2008 from the Tar River, Pamlico River and Pamlico Sound, North Carolina. Stomach contents were identified to the nearest species (if identifiable) or family. Numbers given are percentages of identifiable stomach contents of those fish with stomach contents, quantities (*n*) are given in parenthesis.

Prey item	Location		
	Tar River	Pamlico River	Pamlico Sound
Sciaenids	0.0	72.2 (39)	31.3 (15)
Pinfish, <i>Lagodon rhomboides</i>	0.0	5.6 (3)	27.1 (13)
Shrimp, <i>Penaeus</i> spp.	0.0	3.7 (2)	20.8 (10)
Menhaden, <i>Brevoortia tyrannus</i>	0.0	11.1 (6)	0.0
Anchovies, <i>Anchoa</i> spp.	0.0	7.4 (4)	8.3 (4)
Striped mullet, <i>Mugil cephalus</i>	0.0	0.0	2.1 (1)
Blue crab, <i>Callinectes sapidus</i>	0.0	0.0	6.3 (3)
Squid	0.0	0.0	4.2 (2)
Cyprinids	66.6 (4)	0.0	0.0
Centrarchids	33.3 (2)	0.0	0.0



Figure 1. Map of sampling locations: Tar River, Pamlico River, Pamlico Sound North Carolina. Purple star = Greenville, NC; yellow star = Washington, NC; red star = Engelhard, NC.

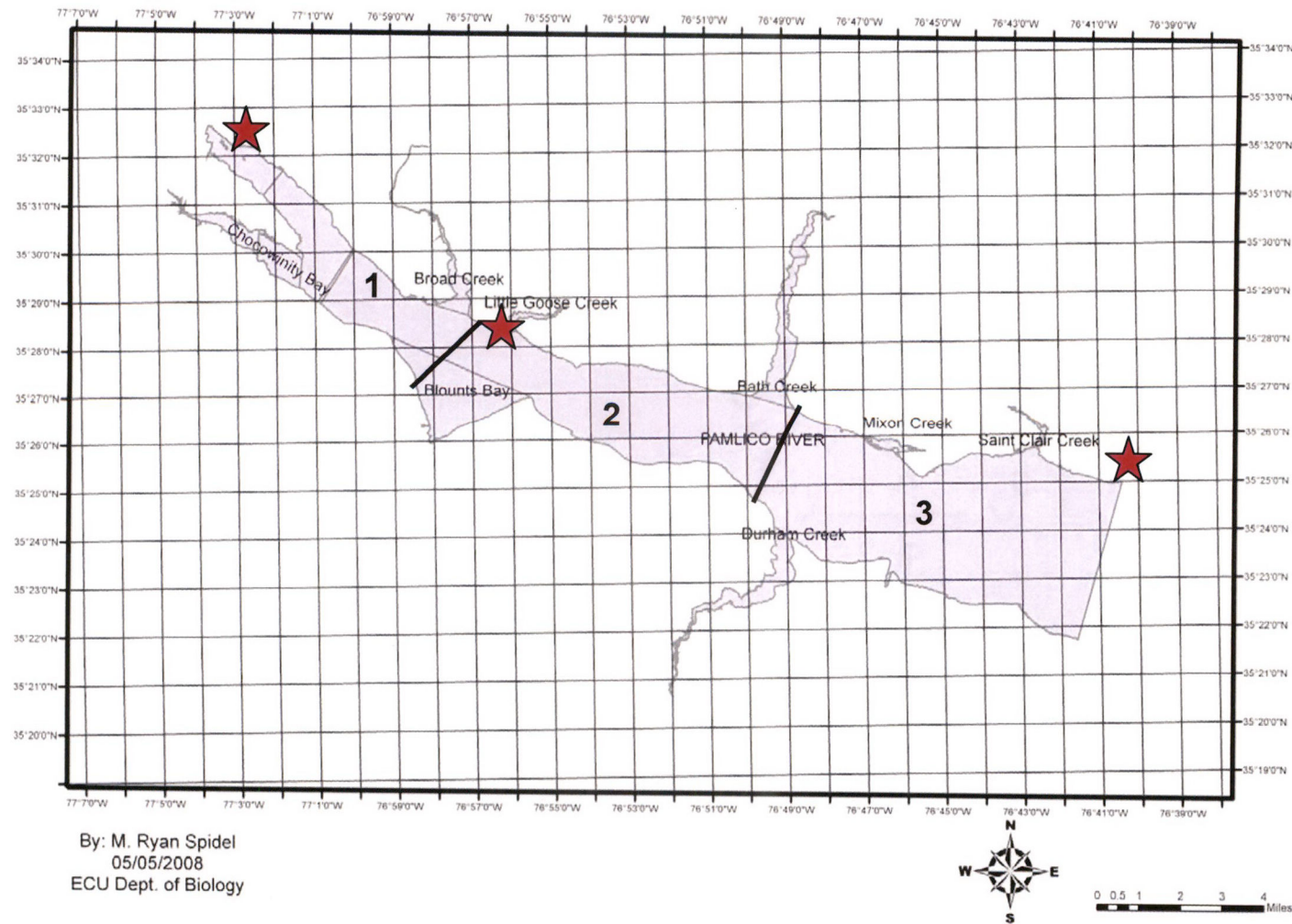


Figure 2. 1 min X 1 min sampling grid of Pamlico River, North Carolina flounder gill netting locations. Sampling locations were randomly selected from the above map; one section (1-3) would be fished overnight with 3 large mesh and 2 small mesh flounder gill nets placed in randomly selected blocks (1 large mesh net per block). Stars = boat ramps used in each section.

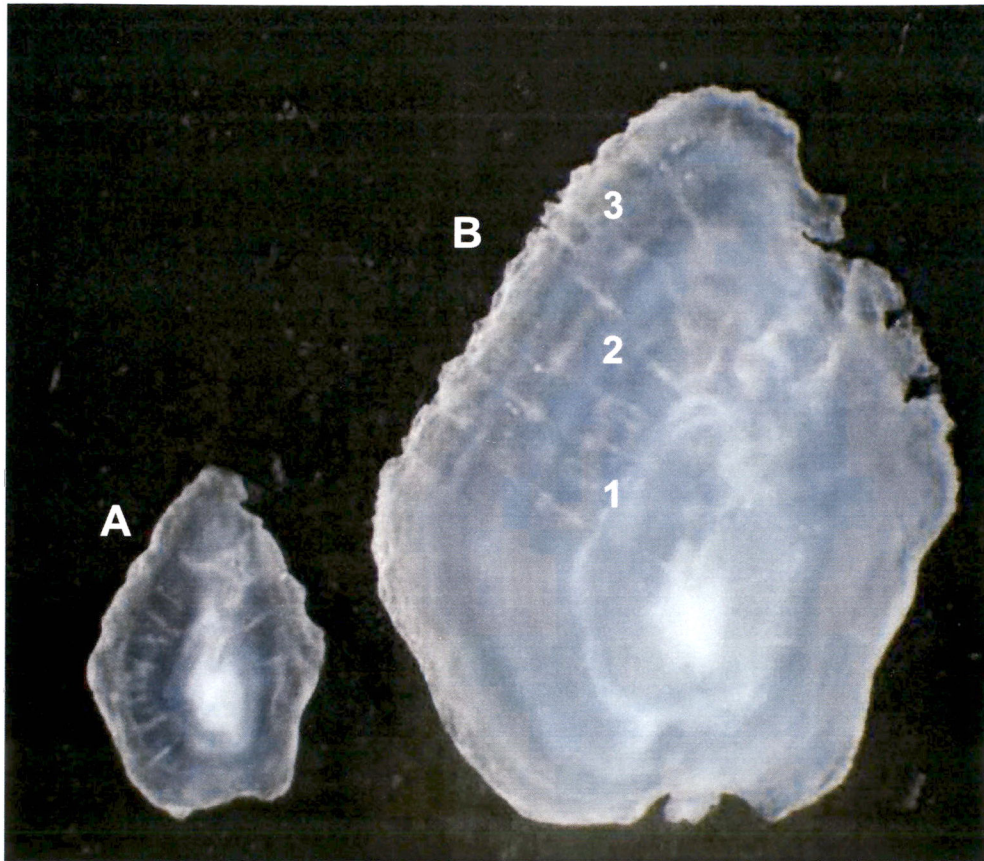


Figure 3. Southern flounder otoliths immersed in water for aging viewed under an Olympus SZX16 microscope with an Olympus DP71 digital camera for imaging. Otolith A = age-0, otolith B = age-3.



Figure 4. Southern flounder otoliths mounted in 25-mm Plexiglas ring mounts. Sectioned otoliths were mounted in ring mounts to be polished prior to conducting LA-ICP-MS. Usually 5 otoliths were embedded per ring mount.

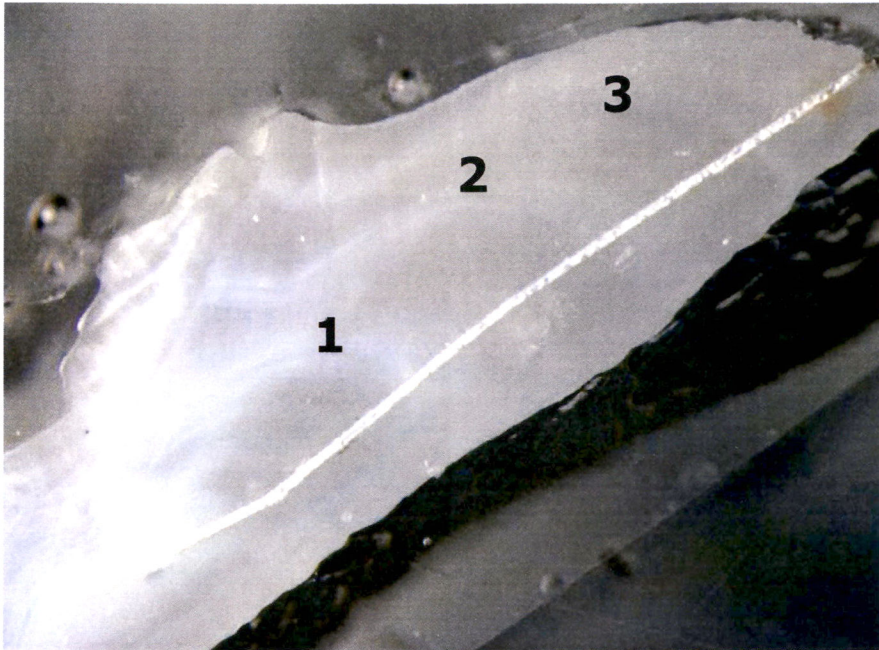


Figure 5. Section and polished southern flounder otolith with LA-ICP-MS transect and ages identified to verify whole otolith aging.

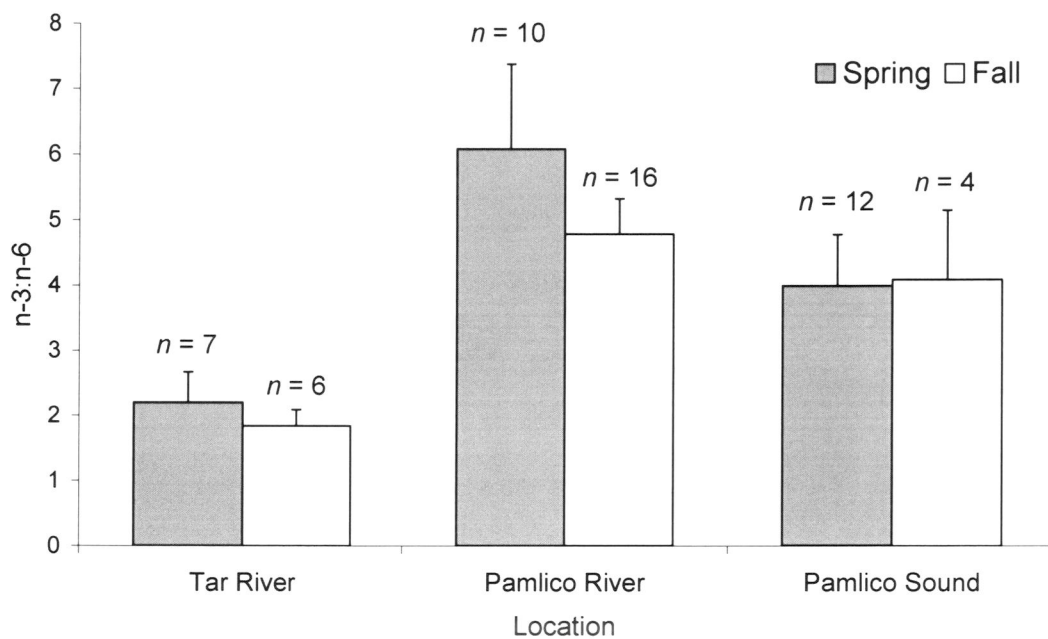


Figure 6. Omega-3 to omega-6 ratios of southern flounder collected in the spring and fall months of 2008 from the Tar River, Pamlico River and Pamlico Sound. Error bars are one standard deviation above the mean.

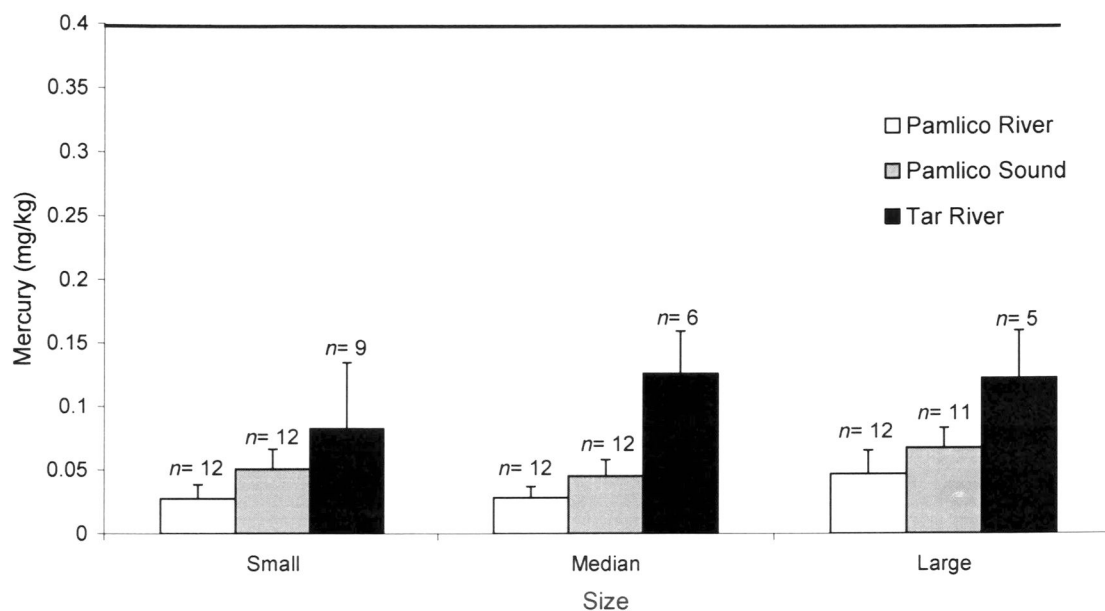


Figure 7. Mean total mercury levels for three size classes of southern flounder from the Pamlico River, Pamlico Sound, and Tar River. The line at 0.4 is the consumption advisory level for mercury in North Carolina provided by the North Carolina Department of Health and Human Services (Williams 2006). Error bars are one standard deviation above the mean.

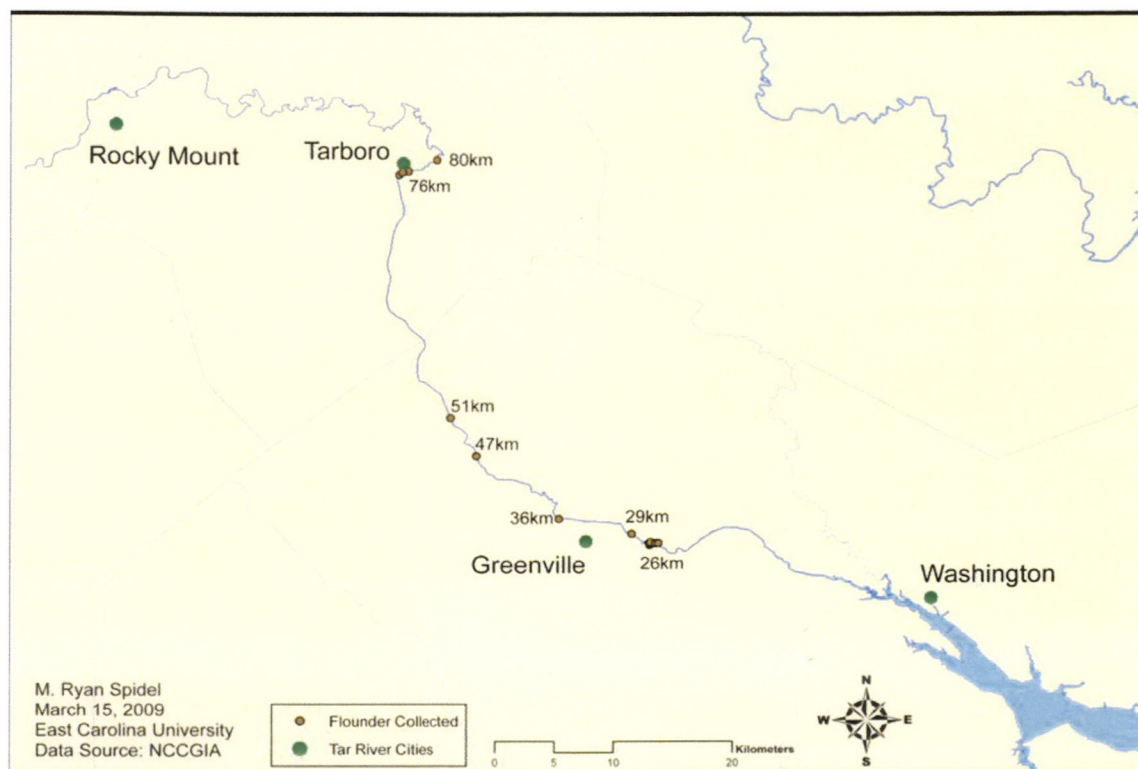


Figure 8. Southern flounder collected from the Tar River, North Carolina in 2008 by electrofishing.

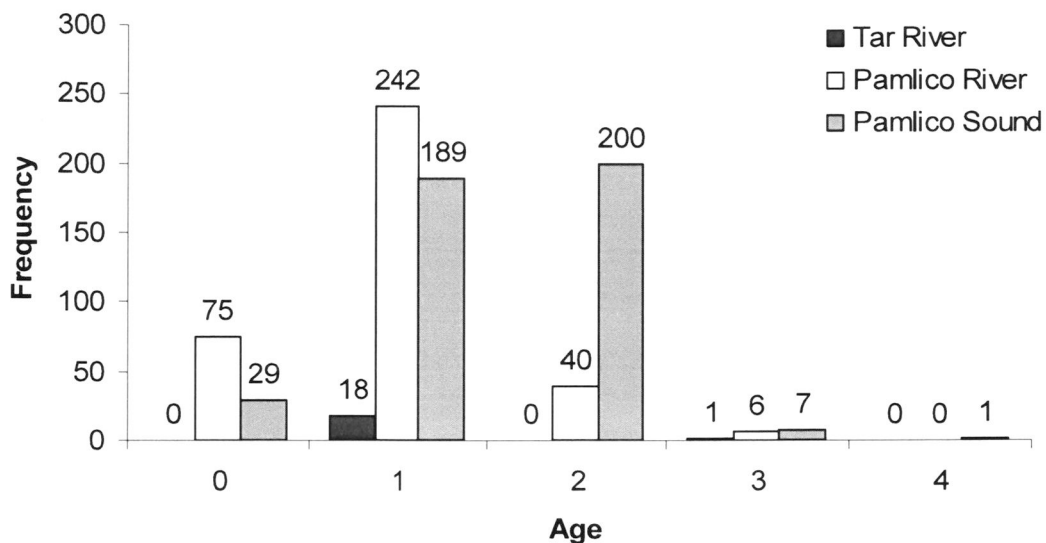


Figure 9. Histogram of southern flounder ages captured by location.

Southern flounder were represented from the Tar River (September 2007 – November 2008), Pamlico River (May 2008 – November 2008), and Pamlico Sound (May 2008 – November 2008). Southern flounder were aged by examining whole otoliths. Numbers on top of bars = n .

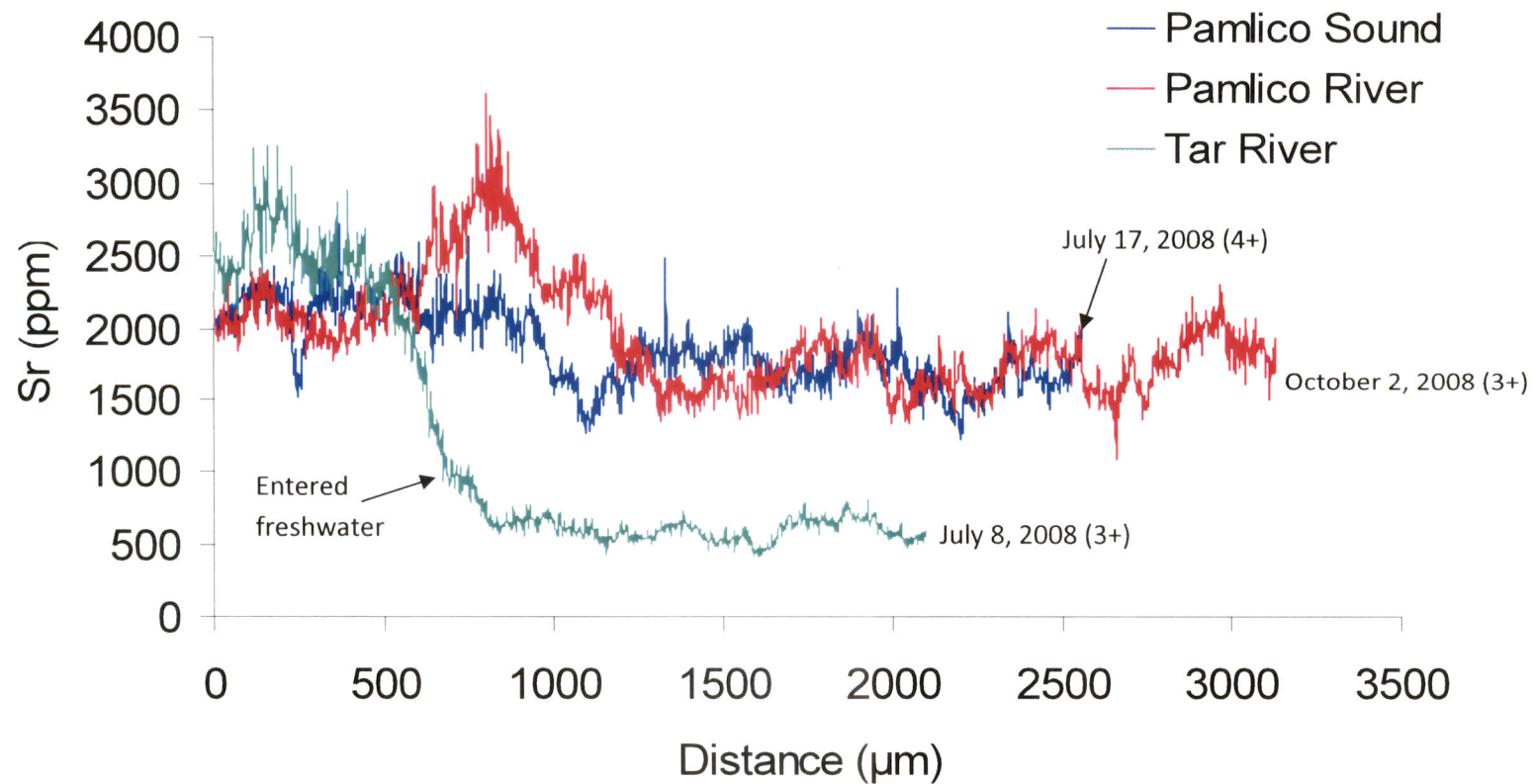


Figure 10. Strontium transects of three southern flounder from the Tar River, Pamlico River and Pamlico Sound. Fish identification numbers: Tar River = 140 (3+ y old), Pamlico River = 515 (3+ y old), Pamlico Sound = 314 (4+ y old). Labeled dates are dates of capture (age in parenthesis); distance is the measure of the transect from the core of the otolith to the edge along its longest axis.

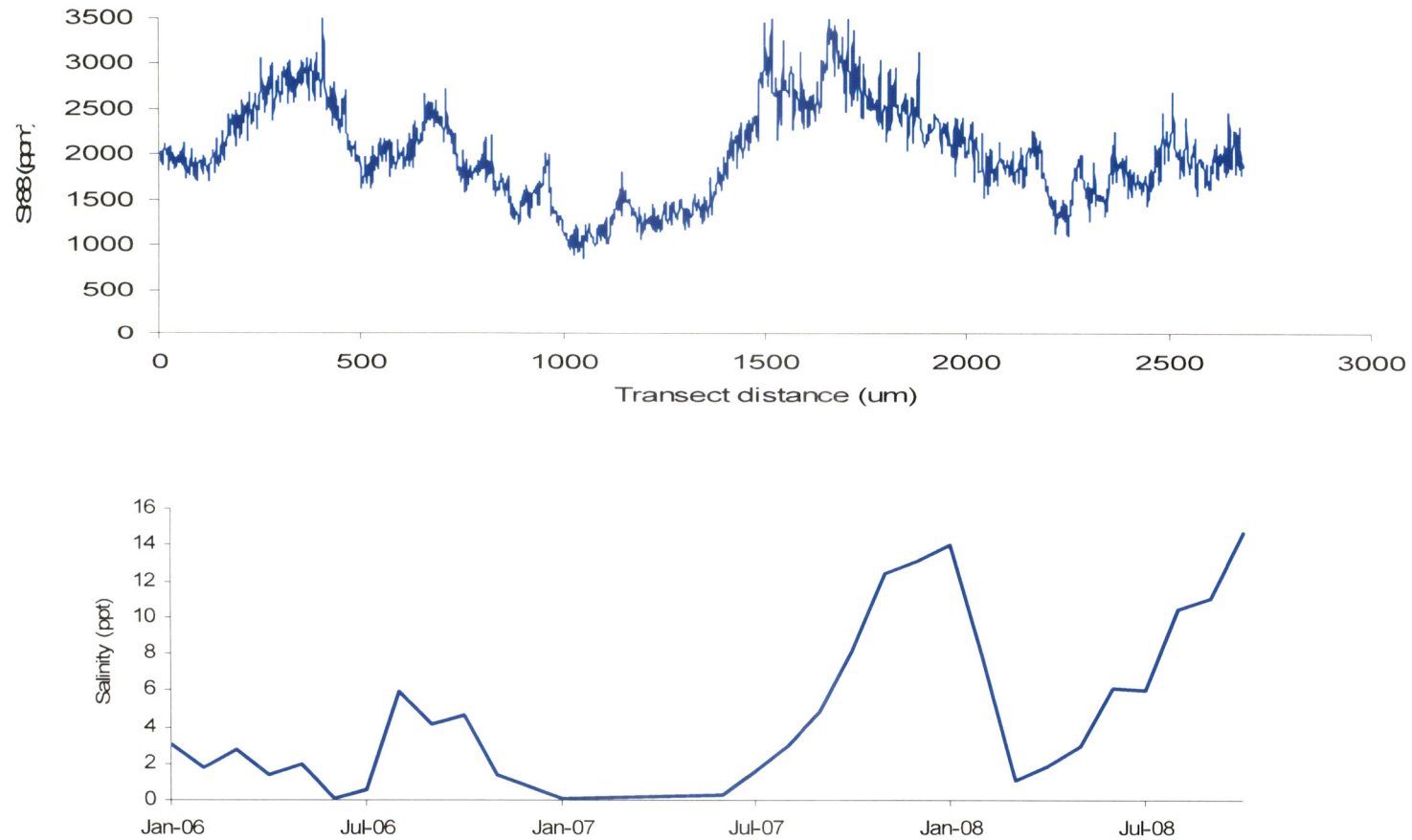


Figure 11. Strontium transect of a southern flounder collected near Washington, NC with corresponding salinities measured from USGS station 02084472. Birth date was calculated using measurements of the laser transect.

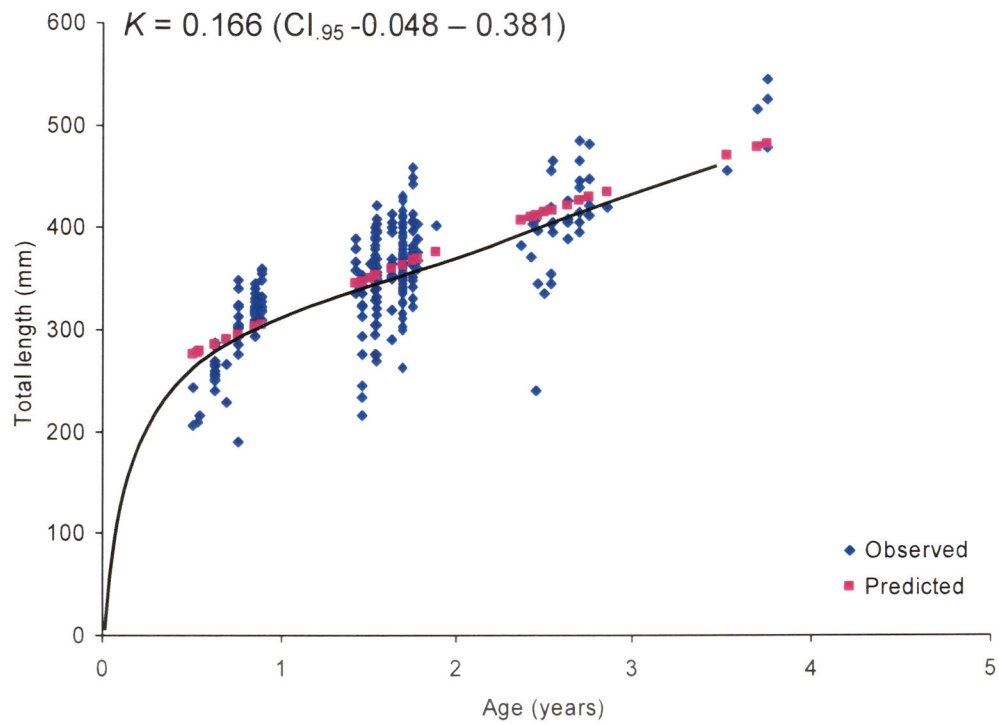


Figure 12. Von Bertalanffy growth curve of female southern flounder collected in 2008 by gill netting in Pamlico River, North Carolina.

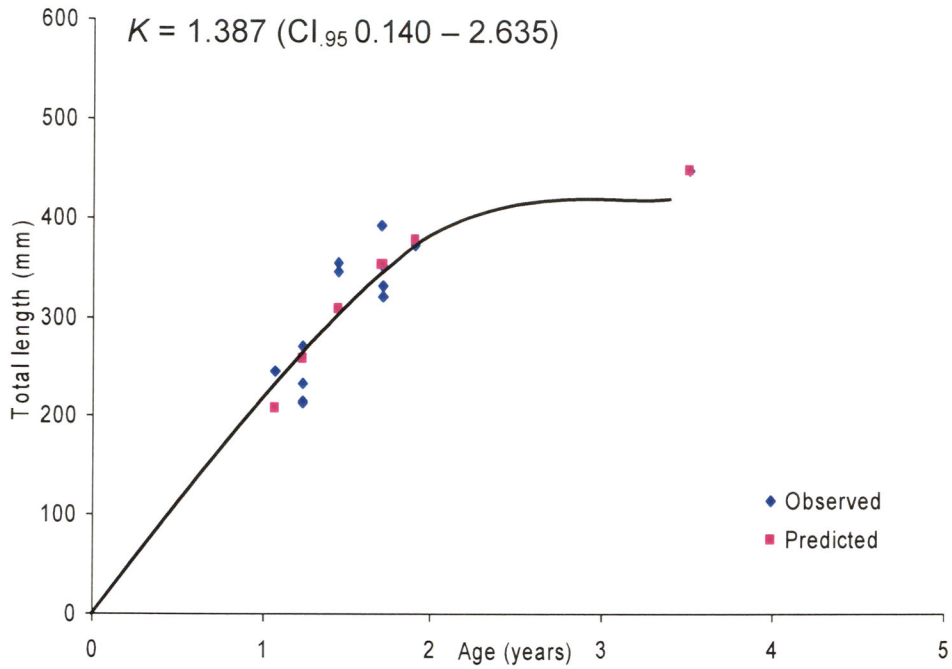


Figure 13. Von Bertalanffy growth curve of female southern flounder collected in 2007 and 2008 by electrofishing in the Tar River, North Carolina.

APPENDIX A: SOUTHERN FLOUNDER FISH DATA

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PR	110	5/13/2008	F	383	752.87	2	1.05	11.30	*	*
PR	112	6/4/2008	F	372	674.56	2	1.03	9.82	1.46	3.38
PR	114	6/4/2008	F	336	488.78	1	1.02	6.24	1.28	1.12
PR	115	6/4/2008	F	390	848.15	1	1.12	9.90	1.17	2.72
PR	116	6/4/2008	F	345	575.6	1	1.11	6.22	1.08	1.76
PR	118	6/4/2008	F	366	540.98	1	0.87	3.92	0.73	1.34
PR	119	6/4/2008	F	358	549.59	1	0.94	4.64	0.85	1.84
PR	120	6/4/2008	F	380	628.71	1	0.90	5.30	0.85	1.82
PR	121	6/5/2008	F	346	509.54	1	0.97	5.46	1.08	1.74
PR	123	6/5/2008	F	344	406.78	1	0.79	2.24	0.55	1.08
PR	124	6/5/2008	F	403	872.38	2	1.05	11.18	1.29	2.98
PR	150	6/15/2008	F	408	811.22	2	0.94	11.10	1.37	2.70
PR	151	6/15/2008	F	313	377.67	1	0.98	2.52	0.67	1.48
PR	152	6/15/2008	F	350	549.5	1	1.01	10.16	1.86	1.94
PR	155	6/15/2008	F	240	173.46	2	1.01	1.92	1.11	0.44
PR	156	6/15/2008	F	216	111.36	1	0.89	0.92	0.83	0.08
PR	158	6/15/2008	F	245	191.54	1	1.05	D	*	0.34
PR	159	6/15/2008	F	275	230.94	1	0.89	D	*	0.48
PR	160	6/15/2008	F	233	142.86	1	0.91	1.40	0.98	0.24
PR	162	6/15/2008	F	323	414.1	1	0.97	3.22	0.78	1.24
PR	164	6/16/2008	F	233	150.04	1	0.95	1.22	0.81	0.22
PR	166	6/16/2008	F	398	688.55	2	0.86	5.38	0.78	2.48
PR	167	6/16/2008	F	345	487.06	2	0.94	4.52	0.93	1.76
PR	168	6/16/2008	F	293	332.31	1	1.05	3.36	1.01	1.18
PR	169	6/16/2008	F	343	542.23	1	1.06	7.98	1.48	1.80
PR	170	6/16/2008	F	336	487.52	1	1.02	6.30	1.30	1.20
PR	171	6/16/2008	F	324	462.68	1	1.08	5.18	1.12	0.96
PR	172	6/16/2008	F	335	459.04	1	0.97	4.56	1.00	1.90
PR	173	6/16/2008	F	353	548.36	1	0.98	4.20	0.77	1.70
PR	175	7/2/2008	F	243	180.71	0	1.01	2.16	1.20	0.06
PR	177	7/2/2008	F	335	484.31	2	1.02	6.13	1.27	1.54
PR	178	7/2/2008	F	365	625.39	1	1.01	7.60	1.22	2.18
PR	179	7/2/2008	F	206	108.94	0	1.01	1.50	1.38	0.10
PR	201	7/13/2008	F	350	528.26	1	0.97	7.06	1.34	1.52
PR	202	7/13/2008	F	403	897.39	2	1.08	18.08	2.02	3.24
PR	203	7/13/2008	F	373	721.23	1	1.09	14.84	2.07	2.84
PR	204	7/13/2008	F	339	497.69	1	1.01	4.66	0.94	1.50
PR	205	7/13/2008	F	305	327.43	1	0.92	2.90	0.89	1.20
PR	206	7/13/2008	F	329	404.31	1	0.90	D	*	0.60
PR	207	7/13/2008	F	364	578.02	1	0.94	6.48	*	1.52
PR	208	7/13/2008	F	365	623.26	1	1.01	7.16	1.15	2.24
PR	209	7/13/2008	F	295	397.91	1	1.23	6.60	1.66	1.12
PR	210	7/13/2008	F	380	701.85	1	1.01	D	*	2.94
PR	211	7/13/2008	F	360	624.86	1	1.06	6.50	1.04	2.13
PR	212	7/13/2008	F	383	678.45	1	0.95	7.04	1.04	2.66

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PR	213	7/13/2008	F	391	838.57	1	1.10	14.30	1.71	3.32
PR	214	7/13/2008	F	363	577.45	1	0.95	7.68	1.33	2.14
PR	215	7/13/2008	F	347	607.07	1	1.15	6.30	1.04	2.26
PR	216	7/13/2008	F	383	785.09	1	1.10	14.28	1.83	3.44
PR	217	7/13/2008	F	400	820.41	1	1.01	10.02	1.23	3.04
PR	218	7/13/2008	F	355	601.44	2	1.06	7.42	1.24	2.06
PR	219	7/13/2008	F	363	678.02	1	1.12	11.40	1.69	2.30
PR	220	7/13/2008	F	356	621.19	1	1.09	7.86	1.27	2.00
PR	221	7/13/2008	F	356	628.54	*	1.10	10.92	1.74	2.18
PR	222	7/13/2008	F	209	146.06	0	1.29	D	*	0.12
PR	224	7/13/2008	F	455	1184.61	3	0.98	11.80	1.00	4.32
PR	225	7/13/2008	F	396	764.33	1	0.97	D	*	3.18
PR	226	7/13/2008	F	420	977.19	2	1.03	12.80	1.32	3.96
PR	227	7/13/2008	F	455	1070.54	2	0.89	12.58	1.18	4.22
PR	228	7/13/2008	F	390	749.85	1	0.99	6.12	0.82	2.32
PR	229	7/13/2008	F	330	469.49	1	1.03	4.22	0.90	1.16
PR	230	7/13/2008	F	360	581.72	1	0.98	6.40	1.10	2.38
PR	231	7/13/2008	F	275	261.46	1	1.00	2.44	0.94	0.50
PR	233	7/13/2008	F	295	290.1	1	0.90	2.90	1.00	0.64
PR	234	7/13/2008	F	370	671.7	1	1.04	6.06	0.90	2.00
PR	235	7/13/2008	F	345	506.09	2	0.97	3.80	0.75	2.00
PR	237	7/13/2008	F	360	544.71	1	0.92	6.24	1.15	1.66
PR	238	7/17/2008	F	215	114.12	0	0.93	0.86	*	*
PR	240	7/17/2008	F	350	573.85	1	1.06	8.46	1.48	1.88
PR	241	7/17/2008	F	333	462.76	1	0.99	5.14	1.11	1.26
PR	242	7/17/2008	F	362	588.72	1	0.98	5.98	1.02	1.60
PR	243	7/17/2008	F	351	588.36	1	1.07	7.86	1.34	2.80
PR	244	7/17/2008	F	350	549.05	1	1.01	6.64	1.21	1.64
PR	245	7/17/2008	F	310*	408.9	1	*	5.36	1.31	1.00
PR	247	7/17/2008	F	348	580.06	1	1.09	9.04	1.56	1.54
PR	248	7/17/2008	F	370*	699.54	1	*	12.92	1.85	2.58
PR	249	7/17/2008	F	270	628.53	1	2.55	8.50	1.36	2.62
PR	250	7/17/2008	F	370	669.68	1	1.04	7.92	1.19	2.22
PR	251	7/17/2008	F	408	986.42	1	1.14	15.10	1.54	4.02
PR	252	7/17/2008	F	390	805.75	1	1.07	12.42	1.55	2.26
PR	253	7/17/2008	F	395	835.83	2	1.06	10.36	1.25	4.70
PR	254	7/17/2008	F	321	431	1	1.03	5.02	1.17	1.08
PR	256	7/17/2008	F	357	583.5	1	1.01	8.34	1.43	1.68
PR	257	7/17/2008	F	353	636.58	1	1.14	7.68	1.21	1.96
PR	258	7/17/2008	F	362	593.9	1	0.99	6.74	1.14	1.86
PR	259	7/17/2008	F	271*	330.74	1	*	3.38	*	*
PR	260	7/17/2008	F	398	885	1	1.10	13.92	1.58	3.66
PR	261	7/17/2008	F	370	697.96	1	1.09	11.72	1.68	1.96
PR	262	7/17/2008	F	404	760.67	1	0.90	6.22*	*	3.30
PR	263	7/17/2008	F	275	718.18	1	2.76	13.90	1.94	2.22
PR	264	7/17/2008	F	332	442.22	1	0.96	4.02	0.91	1.24

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PR	265	7/17/2008	F	395	846.78	1	1.08	14.30	1.70	3.20
PR	267	7/17/2008	F	390	731.7	1	0.97	7.96	1.09	3.22
PR	268	7/17/2008	F	314	404.02	1	1.04	3.94	0.98	1.04
PR	269	7/17/2008	F	305	358.54	1	1.00	4.18	1.17	0.74
PR	270	7/17/2008	F	328	475.15	1	1.07	5.32	1.12	1.96
PR	271	7/17/2008	F	341	507.5	1	1.01	6.66	1.32	2.08
PR	272	7/17/2008	F	406	815.55	2	0.96	7.90	0.97	4.06
PR	272	7/17/2008	F	421	959.73	1	1.01	12.22	1.28	3.42
PR	273	7/17/2008	F	465	1254.12	2	0.97	16.02	1.28	5.18
PR	303	9/10/2008	F	250*	195.97	0	*	D	*	0.18
PR	304	9/10/2008	F	228	151.85	0	1.03	D	*	0.10
PR	306	8/17/2008	F	406	932.16	1	1.09	17.58	1.89	3.84
PR	307	8/17/2008	F	427	1005.58	2	1.01	14.26	1.42	4.14
PR	308	8/17/2008	F	406	876.5	2	1.03	15.74	1.80	3.56
PR	309	8/17/2008	F	255	214.51	0	1.04	2.38	1.11	0.20
PR	310	8/17/2008	F	256	197.81	0	0.94	2.18	1.10	0.22
PR	311	8/17/2008	F	250	182.63	0	0.94	2.56	1.40	0.08
PR	312	8/17/2008	F	406	921.12	1	1.08	15.20	1.66	4.32
PR	313	8/17/2008	F	396	814.97	1	1.03	11.68	1.44	3.06
PR	314	8/17/2008	F	409	1016.55	2	1.16	14.38	1.42	4.66
PR	316	8/17/2008	F	414	914.85	1	1.01	12.24	1.34	4.16
PR	317	8/17/2008	F	320	405.74	1	0.98	6.18	1.53	1.96
PR	318	8/17/2008	F	287	311.84	0	1.05	3.62	1.16	0.44
PR	319	8/17/2008	F	283	275.24	0	0.97	2.86	1.04	0.80
PR	320	8/17/2008	F	390	695.6	2	0.92	6.22	0.90	3.02
PR	321	8/17/2008	F	395	809.63	1	1.03	10.30	1.28	3.64
PR	322	8/17/2008	F	353	459.87	1	0.83	4.66	1.02	2.18
PR	323	8/17/2008	F	352	530.65	1	0.96	10.32	1.95	2.66
PR	324	8/17/2008	F	353	528.33	1	0.95	5.72	1.09	1.76
PR	325	8/17/2008	F	252	185.8	0	0.93	2.04	1.10	0.14
PR	326	8/17/2008	F	369	680.71	1	1.07	10.52	1.55	3.48
PR	328	8/17/2008	F	240	160.4	0	0.93	1.86	1.16	0.16
PR	329	8/17/2008	F	257	203.87	0	0.96	2.56	1.26	0.26
PR	340	8/17/2008	F	260	220.34	0	1.00	3.10	1.41	0.26
PR	341	8/17/2008	F	266	244.45	0	1.04	3.68	1.51	0.52
PR	342	8/17/2008	F	350	601.13	1	1.11	11.34	1.89	2.60
PR	343	8/17/2008	F	400	880.98	1	1.08	12.96	1.48	3.18
PR	345	8/17/2008	F	265	260.65	0	1.12	3.42	1.31	0.32
PR	346	8/17/2008	F	352	470.24	1	0.85	9.34	2.00	2.34
PR	347	8/17/2008	F	266	228.11	0	0.97	3.16	1.39	0.28
PR	349	8/17/2008	F	270	237.35	0	0.96	2.48	1.05	0.26
PR	350	8/17/2008	F	259	223.67	0	1.03	2.72	1.22	0.24
PR	351	8/17/2008	F	291	409.09	1	1.32	3.30	0.81	1.22
PR	352	8/17/2008	F	365	609.55	1	0.99	8.38	1.38	1.86
PR	360	9/10/2008	F	337	539.71	1	1.12	6.08	1.13	1.86
PR	361	9/10/2008	F	405	984.02	1	1.16	17.98	1.84	5.08

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PR	362	9/10/2008	F	404	978.15	1	1.16	15.58	1.60	4.18
PR	363	9/10/2008	F	385	841.45	1	1.16	22.08	2.63	3.32
PR	364	9/10/2008	F	410	938.82	1	1.07	20.38	2.18	4.78
PR	365	9/10/2008	F	432	994.01	1	0.96	13.00	1.31	4.48
PR	366	9/10/2008	F	351	572.26	1	1.04	M	*	2.60
PR	367	9/10/2008	F	354*	535.53	1	*	D	*	1.06
PR	368	9/10/2008	F	405	880.62	1	1.04	D	*	4.36
PR	369	9/10/2008	F	337	478.3	1	0.99	D	*	1.90
PR	370	9/10/2008	F	349	544.76	1	1.01	9.00	1.66	2.34
PR	371	9/10/2008	F	341*	482.42	1	*	D	*	1.38
PR	372	9/10/2008	F	388*	898.74	2	*	21.80	2.44	4.26
PR	373	9/10/2008	F	366*	781.94	1	*	10.10	1.30	3.64
PR	374	9/10/2008	F	339*	498.91	1	*	7.44	1.50	1.80
PR	375	9/10/2008	F	355*	638.04	1	*	11.38	1.79	3.40
PR	376	9/10/2008	F	339	471.84	1	0.96	7.22	1.54	2.20
PR	378	9/10/2008	F	310*	465.37	1	*	D	*	1.48
PR	379	9/10/2008	F	393	739.6	1	0.96	13.22	1.80	3.74
PR	380	9/10/2008	F	266	190.71	0	0.81	D	*	0.16
PR	381	9/10/2008	F	403	836.34	1	1.00	13.16	1.58	4.16
PR	382	9/10/2008	F	485	1335.54	2	0.91	27.76	2.09	6.94
PR	384	9/10/2008	F	275*	294.77	1	*	M	*	0.52
PR	385	9/10/2008	F	405	925.41	2	1.09	11.62	1.26	5.26
PR	386	9/10/2008	F	405	926.96	1	1.09	14.76	1.60	4.38
PR	387	9/10/2008	F	401	830.92	1	1.01	D	*	4.52
PR	388	9/10/2008	F	365*	638	1	*	10.02	1.58	2.90
PR	389	9/10/2008	F	355	618.25	1	1.09	10.56	1.72	2.94
PR	390	9/10/2008	F	353	513.01	1	0.92	6.48	1.27	2.14
PR	391	9/10/2008	F	327	417.38	1	0.95	5.74	1.38	1.56
PR	392	9/10/2008	F	363	540.56	1	0.89	D	*	1.92
PR	393	9/10/2008	F	403	740.87	1	0.89	8.82	1.20	3.08
PR	394	9/10/2008	F	371	674.84	1	1.04	15.30	2.28	3.70
PR	395	9/10/2008	F	363	564.32	1	0.93	9.62	1.72	4.10
PR	396	9/10/2008	F	352*	428.82	1	*	M	*	2.32
PR	397	9/10/2008	F	367	607.25	1	0.97	8.04	1.33	2.30
PR	398	9/10/2008	F	516	1566.15	3	0.88	18.04	1.16	9.04
PR	400	9/10/2008	F	346	555.52	1	1.06	D	*	2.42
PR	401	9/10/2008	F	365	678.54	1	1.10	10.94	1.62	3.18
PR	402	9/10/2008	F	371	587.91	1	0.91	9.74	1.66	1.96
PR	403	9/10/2008	F	396	774.46	2	0.98	13.22	1.72	3.72
PR	404	9/10/2008	F	527*	2016.87	3	*	42.74	2.13	10.02
PR	405	9/10/2008	F	391	775.34	1	1.02	14.50	1.88	4.34
PR	406	9/10/2008	F	386	769.34	1	1.05	13.16	1.72	3.24
PR	407	9/10/2008	F	313*	401.91	1	*	D	*	1.20
PR	408	9/10/2008	F	350	763.87	1	1.41	D	*	3.86
PR	409	9/10/2008	F	365*	713.16	1	*	14.40	2.03	2.66
PR	410	9/10/2008	F	446	1209.57	2	1.06	25.18	2.09	5.92

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PR	411	9/10/2008	F	345*	667.98	1	*	12.76	1.92	3.94
PR	412	9/10/2008	F	363	588.35	1	0.97	M	*	2.96
PR	500	10/2/2008	F	407	958.96	1	1.12	19.30	2.02	3.84
PR	501	10/2/2008	F	294	314.75	0	0.99	3.90	1.24	0.42
PR	502	10/2/2008	F	421	1016.68	2	1.07	15.32	1.51	5.28
PR	503	10/2/2008	F	447	1227.43	2	1.07	25.48	2.09	7.52
PR	504	10/2/2008	F	405	826.83	1	0.98	13.70	1.67	4.58
PR	505	10/2/2008	F	383	675	1	0.94	5.22	0.78	2.74
PR	506	10/2/2008	F	479	1590.29	3	1.13	30.94	1.96	9.32
PR	507	10/2/2008	F	376	627.53	1	0.93	5.14	0.82	1.98
PR	508	10/2/2008	F	313	371.96	0	0.96	4.04	1.09	0.32
PR	509	10/2/2008	F	342	472.47	1	0.93	5.94	1.26	1.64
PR	510	10/2/2008	F	352	564.95	1	1.02	8.50	1.51	1.78
PR	511	10/2/2008	F	323	389.69	1	0.92	3.10	0.80	0.86
PR	512	10/2/2008	F	364	575.95	1	0.94	6.54	1.14	2.04
PR	513	10/2/2008	F	323	376.64	0	0.89	5.18	1.38	0.76
PR	514	10/2/2008	F	331	461.38	1	1.01	7.30	1.59	1.64
PR	515	10/2/2008	F	525	2023.7	3	1.08	59.82	2.97	11.68
PR	516	10/2/2008	F	383	812.27	1	1.14	15.48	1.92	3.92
PR	517	10/2/2008	F	443	964.64	1	0.87	9.10	0.95	4.46
PR	518	10/2/2008	F	305	370.59	0	1.04	4.50	1.22	0.56
PR	519	10/2/2008	F	269*	230.51	0	*	1.40	0.61	0.30
PR	520	10/2/2008	F	348	555.76	0	1.04	7.84	1.41	1.32
PR	521	10/2/2008	F	286	298.31	0	1.02	3.20	1.07	0.46
PR	522	10/2/2008	F	459	1363.92	1	1.10	40.86	3.03	13.44
PR	523	10/2/2008	F	303	360.55	0	1.03	3.58	1.00	0.84
PR	524	10/2/2008	F	482	1621.82	2	1.13	48.58	3.02	11.82
PR	525	10/2/2008	F	375	673.92	1	1.01	12.68	1.89	2.46
PR	526	10/2/2008	F	348	511.94	1	0.96	7.74	1.52	2.24
PR	527	10/2/2008	F	302	336.7	0	0.97	4.70	1.40	0.78
PR	528	10/2/2008	F	398	840.49	1	1.05	16.40	1.96	4.34
PR	529	10/2/2008	F	412	1004.54	2	1.13	21.96	2.20	7.18
PR	530	10/2/2008	F	303*	383.21	0	*	5.98	1.56	0.78
PR	531	10/2/2008	F	380	691.7	1	0.99	9.58	1.39	3.46
PR	532	10/2/2008	F	305	376.82	0	1.06	5.34	1.42	0.58
PR	533	10/2/2008	F	313	391.59	0	1.01	5.54	1.42	0.96
PR	534	10/2/2008	F	315*	420.95	0	*	6.38	1.52	0.68
PR	535	10/2/2008	F	390*	863.02	1	*	15.92	1.85	4.32
PR	536	10/2/2008	F	340	524.35	0	1.05	8.58	1.64	1.18
PR	537	10/2/2008	F	276	260.46	0	0.99	3.30	1.27	0.32
PR	538	10/2/2008	F	325	415.39	0	0.96	5.52	1.33	0.74
PR	540	10/2/2008	F	413	912.57	1	1.02	17.90	1.97	5.84
PR	541	10/2/2008	F	380	664.5	1	0.95	3.20	0.48	2.10
PR	542	10/2/2008	F	370	627.48	1	0.98	6.24	1.00	2.00
PR	543	10/2/2008	F	449	1248.62	1	1.08	17.32	1.39	6.78
PR	544	10/2/2008	F	370	641.25	1	1.00	9.18	1.44	2.48

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PR	545	10/2/2008	F	189	66.41	0	0.80	0.48	*	*
PR	546	10/2/2008	F	545	2288.01	3	1.09	44.16	1.94	15.84
PR	601	10/15/2008	F	390	704.95	1	0.93	7.70	1.10	3.30
PR	602	10/15/2008	F	376	688.9	1	1.02	12.56	1.83	3.32
PR	603	10/15/2008	F	403	840.95	1	1.01	14.62	1.75	3.50
PR	604	10/15/2008	F	368	613.91	1	0.97	9.60	1.57	2.70
PR	605	10/15/2008	F	360	619.95	1	1.05	14.34	2.34	5.84
PR	606	10/15/2008	F	278*	442.7	1	*	5.36	1.21	1.40
PR	701	11/19/2008	F	333	408.15	0	0.87	4.18	1.03	0.90
PR	702	11/19/2008	F	402	690.61	1	0.83	5.86	0.85	2.68
PR	703	11/19/2008	F	323	413.5	0	0.97	6.30	1.53	0.50
PR	704	11/19/2008	F	307	355.49	0	0.98	5.82	1.64	0.52
PR	705	11/19/2008	F	360	248.56	0	0.42	13.76	5.58	1.78
PR	706	11/19/2008	F	309	399.85	0	1.08	5.28	1.32	0.64
PR	707	11/19/2008	F	358	544.87	0	0.94	10.32	1.90	1.26
PR	708	11/19/2008	F	318	393.34	0	0.97	5.58	1.42	0.70
PR	709	11/19/2008	F	348	538.54	0	1.01	11.40	2.12	0.92
PR	710	11/19/2008	F	327	423.21	0	0.96	D	*	0.74
PR	711	11/19/2008	F	355	497	0	0.88	7.86	1.59	1.84
PR	712	11/19/2008	F	319	421.53	0	1.03	8.06	1.92	0.82
PR	713	11/19/2008	F	307	342.86	0	0.94	4.40	1.29	0.62
PR	714	11/19/2008	F	355	551.15	0	0.97	9.98	1.81	1.08
PR	715	11/19/2008	F	332	472.35	0	1.02	9.74	2.07	0.68
PR	1001	9/10/2008	F	365	573.3	1	0.93	D	*	2.68
PR	1002	9/10/2008	F	312	285.16	1	0.75	2.62	0.92	0.52
PR	1003	9/10/2008	F	356	596.92	1	1.04	9.86	1.66	2.32
PR	1004	9/10/2008	F	371	605.62	1	0.93	D	*	2.48
PR	1005	9/10/2008	F	383	682.54	1	0.96	10.16	1.49	2.44
PR	1007	9/10/2008	F	336	449.41	1	0.94	5.60	1.25	1.50
PR	1008	9/10/2008	F	379	672.47	1	0.97	D	*	2.80
PR	1009	9/10/2008	F	303	342.5	1	0.98	D	*	1.10
PR	1010	9/10/2008	F	362	550.09	1	0.91	6.44	1.18	2.10
PR	1011	9/10/2008	F	375	703.51	1	1.05	10.66	1.52	3.60
PR	1013	9/10/2008	F	372	684.34	1	1.05	D	*	1.86
PR	1014	9/10/2008	F	367	523.53	1	0.83	D	*	1.86
PR	1015	9/10/2008	F	352	529.6	1	0.96	4.54	0.86	1.94
PR	1016	9/10/2008	F	355	552.15	1	0.97	7.26	1.32	3.00
PR	1017	9/10/2008	F	360	571.26	1	0.97	D	*	2.52
PR	1018	9/10/2008	F	350	552.2	1	1.02	8.50	1.55	2.12
PR	1019	9/10/2008	F	352	461.22	1	0.83	5.50	1.20	1.76
PR	1020	9/10/2008	F	303*	343.28	1	*	2.62	0.77	0.82
PR	1021	9/10/2008	F	340	461.89	1	0.93	3.40	0.74	1.52
PR	1023	9/10/2008	F	345	473.54	1	0.91	4.18	0.89	2.02
PR	1024	9/10/2008	F	351	522.69	1	0.95	D	*	1.54
PR	2002	9/10/2008	F	379	665.55	1	0.96	5.22	0.79	3.62
PR	2003	9/10/2008	F	364	619.01	1	1.01	9.38	1.52	3.06

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PR	2004	9/10/2008	F	366	604	1	0.97	7.76	1.29	2.68
PR	2005	9/10/2008	F	370	642.84	1	1.00	12.40	1.94	2.82
PR	2006	9/10/2008	F	412	882.81	1	0.99	D	*	1.92
PR	2007	9/10/2008	F	335	406.44	1	0.86	3.00	0.74	1.24
PR	2008	9/10/2008	F	262	195.89	1	0.87	2.78	1.42	0.04
PR	2009	9/10/2008	F	392	697.66	1	0.91	8.48	1.22	4.12
PR	2010	9/10/2008	F	342	477.04	1	0.94	5.68	1.20	2.22
PR	2011	9/10/2008	F	430	1052.62	1	1.04	19.06	1.82	5.44
PR	2014	9/10/2008	F	355	507.21	1	0.89	D	*	1.90
PR	2015	9/10/2008	F	361	589.46	1	0.99	8.64	1.47	2.56
PR	2016	9/10/2008	F	416	865.01	1	0.94	11.32	1.32	4.54
PR	3003	9/10/2008	F	354	521.28	1	0.93	7.08	1.36	2.24
PR	3004	9/10/2008	F	300	281.85	1	0.83	2.36	0.84	0.86
PR	3005	9/10/2008	F	316	357.96	1	0.90	3.64	1.02	0.98
PR	3006	9/10/2008	F	326	380.03	1	0.87	3.84	1.01	1.04
PR	3007	9/10/2008	F	350	565.58	1	1.04	6.90	1.23	3.32
PR	3008	9/10/2008	F	395	815.01	1	1.04	13.74	1.70	5.02
PR	3009	9/10/2008	F	380	763.15	1	1.09	14.64	1.93	4.58
PR	3010	9/10/2008	F	373	697.11	1	1.06	D	*	2.80
PR	3011	9/10/2008	F	357	570.43	1	0.99	12.20	2.15	2.76
PR	3012	9/10/2008	F	377	655.2	1	0.96	9.76	1.50	3.06
PR	3013	9/10/2008	F	415	856	2	0.94	19.46	2.29	5.10
PR	4001	9/10/2008	F	360	617.45	1	1.04	7.84	1.28	2.60
PR	4002	9/10/2008	F	427	1013.69	1	1.02	18.72	1.86	5.14
PR	4003	9/10/2008	F	395	680.64	1	0.87	8.72	1.29	2.86
PR	4004	9/10/2008	F	345	463.65	1	0.89	D	*	1.70
PR	4005	9/10/2008	F	466	1199.41	2	0.92	14.68	1.23	6.42
PR	4008	9/10/2008	F	316	403.82	1	1.02	D	*	0.94
PR	4009	9/10/2008	F	367	606.44	1	0.97	D	*	2.78
PR	4010	9/10/2008	F	440	1094.44	2	1.00	D	*	5.56
PR	4011	9/10/2008	F	386	729.66	1	1.00	7.48	1.03	3.52
PR	4012	9/10/2008	F	311	331.54	1	0.88	D	*	0.84
PR	4013	9/10/2008	F	385	647.63	1	0.89	10.08	1.56	2.92
PR	4014	9/10/2008	F	345	515.39	1	0.99	7.12	1.39	1.58
PR	6010	11/6/2008	F	323	447.36	0	1.05	9.04	2.02	0.68
PR	6020	11/6/2008	F	346	525.19	0	1.00	11.80	2.25	1.02
PR	6030	11/6/2008	F	327	452.24	0	1.02	8.00	1.77	1.10
PR	6040	11/6/2008	F	317	381.75	0	0.95	4.20	1.10	0.58
PR	6050	11/6/2008	F	340	473	0	0.95	8.48	1.80	0.92
PR	6060	11/6/2008	F	321	398.32	0	0.95	7.42	1.87	0.60
PR	6070	11/6/2008	F	322	473.71	0	1.12	9.02	1.91	0.82
PR	6080	11/6/2008	F	345	541.49	0	1.04	8.18	1.51	0.94
PR	6090	11/6/2008	F	340	499.68	0	1.01	9.52	1.91	1.10
PR	6100	11/6/2008	F	328	446.41	0	1.00	5.34	1.20	0.86
PR	6110	11/6/2008	F	323	457.7	0	1.08	10.42	2.28	0.96
PR	6120	11/6/2008	F	304	394.82	0	1.12	7.96	2.02	0.90

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PR	6130	11/6/2008	F	314	409.81	0	1.05	8.70	2.13	0.82
PR	6140	11/6/2008	F	420	1022.77	2	1.08	34.10	3.36	8.66
PR	6150	11/6/2008	F	293	331.82	0	1.05	6.76	2.04	0.38
PR	6160	11/6/2008	F	335	468.45	0	0.99	13.54	2.90	0.78
PR	6170	11/6/2008	F	331	477.7	0	1.04	9.88	2.07	0.80
PR	6180	11/6/2008	F	306	390.7	0	1.08	9.34	2.39	0.70
PR	6190	11/6/2008	F	320	407.02	0	0.98	7.22	1.78	0.60
PR	6200	11/6/2008	F	340	513.13	0	1.03	12.00	2.34	1.14
PR	6210	11/6/2008	F	314	394.18	0	1.01	2.96	0.75	1.14
PR	6220	11/6/2008	F	303	309.46	0	0.88	4.08	1.32	0.38
PR	6230	11/6/2008	F	310	394.79	0	1.05	7.20	1.83	0.70
PR	6240	11/6/2008	F	318	429.99	0	1.06	7.62	1.78	1.02
PR	113	6/4/2008	M	327	480.12	2	1.08	9.66	2.01	0.30
PR	122	6/5/2008	M	329	471.05	2	1.04	9.64	2.05	0.38
PR	154	6/15/2008	M	223	139.76	1	1.01	1.18	0.84	0.10
PR	161	6/15/2008	M	225	140.46	1	0.99	1.56	1.11	0.18
PR	176	7/2/2008	M	263	225.95	1	0.99	2.62	1.16	0.03
PR	223	7/13/2008	M	271	268.44	1	1.07	3.18	1.18	*
PR	236	7/13/2008	M	337	447.98	2	0.92	7.52	1.68	0.22
PR	239	7/17/2008	M	276	300.68	1	1.14	3.88	1.29	0.10
PR	246	7/17/2008	M	288	300.86	1	1.00	3.64	1.21	0.12
PR	255	7/17/2008	M	355	582	2	1.02	7.86	1.35	0.24
PR	266	7/17/2008	M	253*	213.38	1	*	2.14	1.00	0.06
PR	301	9/10/2008	M	283	280.11	1	0.98	D	*	*
PR	302	9/10/2008	M	261	237.62	1	1.07	2.82	1.19	0.08
PR	305	8/17/2008	M	324	451.32	2	1.05	7.46	1.65	0.10
PR	315	8/17/2008	M	267	230.55	1	0.97	3.36	1.46	0.02
PR	327	8/17/2008	M	287	297.39	1	1.00	6.20	2.09	0.08
PR	344	8/17/2008	M	280	318.17	1	1.15	4.72	1.48	0.04
PR	348	8/17/2008	M	292	355.03	1	1.13	5.12	1.44	0.18
PR	377	9/10/2008	M	252	200.27	1	1.00	1.70	*	*
PR	383	9/10/2008	M	273	256.04	1	1.00	D	*	0.08
PR	399	9/10/2008	M	351	525.67	2	0.95	10.62	2.02	0.42
PR	413	9/10/2008	M	260	238.2	1	1.08	D	*	0.10
PR	539	10/2/2008	M	288	295.12	1	0.98	2.74	0.93	0.06
PR	1006	9/10/2008	M	332	449.67	2	0.97	D	*	0.34
PR	1012	9/10/2008	M	274	225.97	1	0.87	2.58	1.14	0.02
PR	2001	9/10/2008	M	265	203.62	1	0.87	1.18	0.58	0.02
PR	2012	9/10/2008	M	263	225.74	1	0.99	D	*	0.12
PR	2013	9/10/2008	M	295	315.86	1	0.98	D	*	0.20
PR	3001	9/10/2008	M	281	249.1	1	0.89	2.68	1.08	0.10
PR	3002	9/10/2008	M	293	289.88	1	0.91	6.26	2.16	0.10
PR	4006	9/10/2008	M	260	206.81	1	0.94	D	*	*
PS	102	5/17/2008	F	387	724.15	2	1.01	7.36	1.02	2.66
PS	103	5/17/2008	F	345	515.05	2	1.05	5.74	1.12	1.56
PS	104	5/17/2008	F	358	541.48	2	0.97	4.48	0.83	1.96

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	106	5/17/2008	F	351	516.47	1	0.99	6.40	1.24	1.56
PS	108	5/17/2008	F	376	667.41	2	1.02	6.64	1.00	2.48
PS	110	5/17/2008	F	348	519.54	2	1.02	4.70	0.91	1.50
PS	112	5/17/2008	F	330	420.84	2	0.99	4.00	0.95	1.46
PS	113	5/17/2008	F	376	624.8	1	0.96	5.60	0.90	1.96
PS	114	5/17/2008	F	361	502.99	1	0.88	4.60	0.92	1.28
PS	116	5/17/2008	F	343	519.2	2	1.07	4.74	0.92	2.22
PS	117	5/17/2008	F	356	613.51	2	1.12	7.78	1.27	2.60
PS	118	5/17/2008	F	398	760.38	3	0.97	10.54	1.39	2.92
PS	119	5/17/2008	F	361	683.24	1	1.20	10.32	1.51	1.42
PS	120	5/17/2008	F	353	553.62	1	1.04	8.48	1.54	1.34
PS	121	5/17/2008	F	370	654.85	2	1.06	8.16	1.25	1.86
PS	123	5/17/2008	F	410	976.41	2	1.13	20.66	2.12	3.82
PS	125	5/17/2008	F	320	435.7	1	1.13	6.86	1.58	1.04
PS	126	5/17/2008	F	300	334.63	1	1.07	3.36	1.01	0.66
PS	127	5/17/2008	F	360	537.58	1	0.95	5.00	0.93	1.76
PS	128	5/17/2008	F	343	542.94	2	1.12	8.16	1.51	1.48
PS	129	5/17/2008	F	357	548.46	2	1.00	4.22	0.77	2.08
PS	130	5/17/2008	F	357	570.11	2	1.03	5.62	0.99	2.20
PS	134	5/17/2008	F	333	452.07	2	1.03	3.50	0.78	1.48
PS	135	5/17/2008	F	373	640.49	2	1.01	9.82	1.54	1.92
PS	136	5/17/2008	F	360	569.04	2	1.00	6.42	1.13	2.48
PS	138	5/17/2008	F	360	527.52	1	0.93	4.84	0.92	1.38
PS	151	5/18/2008	F	303	350.43	1	1.09	2.88	0.82	0.94
PS	153	5/18/2008	F	347	525.3	1	1.05	5.16	0.99	1.50
PS	154	5/18/2008	F	337	468.26	2	1.03	3.04	0.65	1.66
PS	155	5/18/2008	F	365	663.3	1	1.12	8.30	1.26	3.44
PS	156	5/18/2008	F	322	427.33	1	1.09	3.94	0.93	1.64
PS	159	5/18/2008	F	345	571.51	1	1.16	8.23	1.44	1.54
PS	162	5/18/2008	F	339	474.61	2	1.02	D	*	1.08
PS	163	5/18/2008	F	336	461.74	2	1.02	3.90	0.85	1.50
PS	165	5/18/2008	F	360	559.4	2	0.99	7.28	1.31	1.92
PS	168	5/18/2008	F	355	605.01	1	1.12	7.32	1.21	2.12
PS	169	5/18/2008	F	275	252.1	1	1.08	2.46	0.98	0.56
PS	170	5/18/2008	F	321	443.9	1	1.14	4.60	1.04	0.99
PS	173	5/18/2008	F	356	500.01	1	0.92	3.40	0.68	1.34
PS	176	5/18/2008	F	410	838.37	2	0.97	12.92	1.55	3.56
PS	178	5/18/2008	F	373	581.14	1	0.91	4.78	0.82	1.58
PS	179	5/18/2008	F	345	492.43	1	1.00	3.54	0.72	1.34
PS	181	5/18/2008	F	355	542.02	1	1.00	5.30	0.98	1.54
PS	183	5/18/2008	F	405	776.6	2	0.93	10.36	1.34	3.68
PS	184	5/18/2008	F	371	636.19	1	1.02	5.30	0.84	1.74
PS	185	5/18/2008	F	387	757.38	1	1.06	8.62	1.14	2.50
PS	201	6/19/2008	F	365	562.81	2	0.95	4.40	0.78	2.24
PS	204	6/19/2008	F	343	463.85	2	0.96	5.06	1.09	1.36
PS	206	6/19/2008	F	383	712.71	2	1.03	6.72	0.95	2.38

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	207	6/19/2008	F	391	716.98	2	0.97	6.26	0.88	2.60
PS	210	6/19/2008	F	360	618.73	2	1.09	6.12	0.99	2.64
PS	212	6/19/2008	F	376	705.65	2	1.08	9.26	1.32	2.34
PS	214	6/19/2008	F	372	636.71	2	1.01	9.32	1.47	2.05
PS	215	6/19/2008	F	380	626.69	2	0.93	10.32	1.65	2.12
PS	216	6/19/2008	F	365	533.05	2	0.90	4.70	0.88	1.76
PS	217	6/19/2008	F	373	547.93	1	0.86	3.80	0.70	1.68
PS	218	6/19/2008	F	386	643.83	1	0.90	6.36	0.99	2.28
PS	219	6/19/2008	F	362	599.12	1	1.04	7.98	1.34	1.68
PS	220	6/19/2008	F	373	654.55	2	1.03	10.98	1.68	2.22
PS	221	6/19/2008	F	361	623.21	1	1.09	12.00	1.93	1.98
PS	222	6/19/2008	F	350	538.67	1	1.04	7.86	1.46	1.28
PS	223	6/19/2008	F	340	463.88	1	0.99	D	*	1.94
PS	225	6/19/2008	F	382	664.06	2	0.97	8.66	1.31	2.30
PS	227	6/19/2008	F	310*	437.8	1	*	5.84	1.34	0.96
PS	228	6/19/2008	F	416	883.74	2	0.97	10.98	1.25	4.08
PS	229	6/19/2008	F	355	546.85	1	1.01	4.40	0.81	1.56
PS	232	6/20/2008	F	342	479.21	1	1.00	5.60	1.17	1.08
PS	235	6/20/2008	F	316	380.22	2	1.03	4.64	1.22	1.40
PS	236	6/20/2008	F	305	306.86	1	0.93	3.60	1.18	0.80
PS	237	6/20/2008	F	345	419.17	1	0.85	3.02	0.72	1.20
PS	238	6/20/2008	F	345	466.64	1	0.95	7.08	1.52	1.22
PS	239	6/20/2008	F	354	496.39	2	0.93	5.12	1.03	1.52
PS	240	6/20/2008	F	416	891.27	3	0.98	12.94	1.46	4.38
PS	241	6/20/2008	F	421	906.94	2	0.96	16.52	1.83	2.78
PS	246	6/20/2008	F	391*	845.28	2	*	10.88	1.29	4.04
PS	247	6/20/2008	F	367*	626.78	1	*	6.56	1.05	2.04
PS	248	6/20/2008	F	443	1089.73	2	0.98	12.36	1.14	5.54
PS	249	6/20/2008	F	388	724.66	2	1.00	9.86	1.37	3.10
PS	250	6/20/2008	F	414	811.11	3	0.91	7.78	0.96	3.50
PS	251	6/20/2008	F	388	645.74	2	0.89	7.74	1.20	2.72
PS	252	6/20/2008	F	387	656.65	2	0.91	7.04	1.08	2.72
PS	253	6/20/2008	F	450	1093.23	2	0.93	11.38	1.05	5.44
PS	254	6/20/2008	F	345*	527.43	1	*	6.94	1.32	1.46
PS	255	6/20/2008	F	370	621.73	2	1.00	8.08	1.30	2.14
PS	257	6/20/2008	F	346	480.52	2	0.97	4.56	0.95	1.82
PS	258	6/20/2008	F	312	357.8	1	1.01	3.32	0.93	1.04
PS	259	6/20/2008	F	328	494.6	2	1.18	3.64	0.74	1.76
PS	260	6/20/2008	F	325	417.58	2	1.03	4.68	1.13	1.60
PS	261	6/20/2008	F	410	871.98	3	1.01	13.12	1.51	4.16
PS	262	6/20/2008	F	332	430.07	2	0.99	5.28	1.23	1.92
PS	263	6/20/2008	F	342	450.82	2	0.94	5.34	1.19	1.60
PS	264	6/20/2008	F	335*	519	2	*	4.49	0.87	2.64
PS	265	6/20/2008	F	457	1246.93	2	1.01	30.56	2.46	4.76
PS	268	6/20/2008	F	385	718.33	2	1.02	7.30	1.02	2.39
PS	269	6/20/2008	F	296*	357.17	2	*	2.38	0.67	1.22

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	270	6/20/2008	F	330	437.82	2	1.03	4.84	1.11	1.38
PS	271	6/20/2008	F	337	498.87	2	1.09	6.38	1.28	1.80
PS	272	6/20/2008	F	371	631.38	1	1.01	7.08	1.12	1.95
PS	275	6/20/2008	F	310	342.23	2	0.99	2.60	0.76	1.54
PS	301	7/17/2008	F	410	867.35	2	1.00	14.10	1.63	3.36
PS	302	7/17/2008	F	380	560.28	2	0.83	4.62	0.83	2.28
PS	303	7/17/2008	F	412	857.68	2	0.97	15.78	1.85	3.38
PS	304	7/17/2008	F	415	905.65	2	1.00	17.52	1.94	3.16
PS	305	7/17/2008	F	453	1280.2	2	1.07	23.98	1.88	4.72
PS	306	7/17/2008	F	425*	956.7	2	*	10.20	1.07	4.10
PS	307	7/17/2008	F	456	1166.08	3	0.95	13.74	1.18	4.12
PS	308	7/17/2008	F	375	598.93	1	0.93	3.88	0.65	2.70
PS	309	7/17/2008	F	367*	718.93	2	*	D	*	2.24
PS	311	7/17/2008	F	311*	546.93	1	*	7.60	1.39	1.68
PS	313	7/17/2008	F	311	322.73	1	0.92	D	*	0.84
PS	314	7/17/2008	F	505	1652.46	4	0.96	19.08	1.16	10.98
PS	315	7/17/2008	F	408	935.32	1	1.10	18.84	2.02	3.12
PS	316	7/17/2008	F	373	730.08	1	1.15	16.14	2.22	2.33
PS	317	7/17/2008	F	375	621.3	1	0.96	D	*	2.44
PS	318	7/17/2008	F	404	860.79	2	1.04	17.92	2.09	3.78
PS	319	7/17/2008	F	385	812.47	1	1.15	11.26	1.39	2.54
PS	320	7/17/2008	F	405	786.8	2	0.94	16.20	2.07	3.20
PS	321	7/17/2008	F	370	625.45	2	1.01	9.88	1.59	2.20
PS	322	7/17/2008	F	393	763.98	1	1.01	13.54	1.78	2.70
PS	323	7/17/2008	F	377	643.55	1	0.98	11.50	1.79	2.48
PS	324	7/17/2008	F	365*	729.65	1	*	13.10	1.80	2.84
PS	325	7/17/2008	F	371	639.55	2	1.02	D	*	2.54
PS	326	7/17/2008	F	383	675.15	2	0.97	9.22	1.37	2.22
PS	327	7/17/2008	F	392	712.65	2	0.95	12.90	1.82	2.16
PS	328	7/17/2008	F	384	678.31	1	0.97	14.84	2.19	2.14
PS	329	7/17/2008	F	355*	608.5	1	*	9.08	1.50	1.62
PS	334	7/17/2008	F	383	676.22	2	0.97	7.48	1.11	2.28
PS	335	7/17/2008	F	388	722.7	*	1.00	14.56	*	D
PS	336	7/17/2008	F	417	936.08	2	1.02	20.00	2.15	4.02
PS	337	7/17/2008	F	366	637.88	1	1.07	12.20	1.92	2.99
PS	340	7/17/2008	F	404	870.42	2	1.05	D	*	3.40
PS	341	7/17/2008	F	351	545.79	2	1.05	D	*	1.78
PS	345	7/17/2008	F	358*	596.57	2	*	D	*	2.30
PS	347	7/18/2008	F	316	474.99	2	1.29	D	*	1.80
PS	348	7/18/2008	F	315	399.95	1	1.09	4.98	1.25	1.06
PS	350	7/18/2008	F	332	474.21	1	1.09	D	*	1.08
PS	352	7/18/2008	F	368	628.33	2	1.03	D	*	2.26
PS	353	7/18/2008	F	525	1739.08	3	0.89	26.86	1.55	8.44
PS	354	7/18/2008	F	350	536.93	1	1.04	5.74	1.07	1.82
PS	355	7/18/2008	F	424	1016.15	2	1.05	17.28	1.71	3.56
PS	356	7/18/2008	F	375	645.7	1	1.00	9.22	1.43	2.16

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	357	7/18/2008	F	376	642.59	2	0.98	D	*	2.44
PS	358	7/18/2008	F	335	461.27	2	1.03	D	*	1.92
PS	360	7/18/2008	F	402	749.87	2	0.92	D	*	2.46
PS	361	7/18/2008	F	325	437.28	1	1.08	D	*	1.38
PS	362	7/18/2008	F	361	746.3	1	1.31	14.70	1.98	2.48
PS	364	7/18/2008	F	372	634.63	2	1.01	12.30	1.95	2.66
PS	365	7/18/2008	F	376	653.36	2	1.00	7.92	1.22	2.74
PS	366	7/18/2008	F	376	621.43	2	0.95	D	*	2.82
PS	367	7/18/2008	F	465	1388.6	2	1.06	20.10	1.45	6.00
PS	370	7/18/2008	F	345	452.14	2	0.92	D	*	1.40
PS	374	7/18/2008	F	365	712.45	2	1.20	14.50	2.04	3.30
PS	376	7/18/2008	F	305	321.37	1	0.98	D	*	1.12
PS	377	7/18/2008	F	282	273.19	1	1.07	M	*	*
PS	380	7/18/2008	F	368	561.26	2	0.92	5.86	1.05	2.10
PS	381	7/18/2008	F	346	471.27	2	0.95	4.58	*	*
PS	382	7/18/2008	F	339	474.37	1	1.02	4.58	0.97	1.08
PS	384	7/18/2008	F	380	653.89	2	0.97	D	*	3.04
PS	385	7/18/2008	F	326	434.86	1	1.06	5.44	1.25	1.20
PS	386	7/18/2008	F	392	650.5	2	0.87	5.68	0.88	2.42
PS	387	7/18/2008	F	420	998.89	2	1.06	16.50	1.66	4.52
PS	388	7/18/2008	F	403	801.95	2	0.98	11.26	1.41	2.98
PS	389	7/18/2008	F	355	522.8	2	0.97	8.62	1.65	1.94
PS	392	7/18/2008	F	384	598.43	1	0.86	D	*	1.86
PS	394	7/18/2008	F	308	338.03	1	0.99	3.02	0.90	1.06
PS	395	7/18/2008	F	412	792.45	3	0.90	D	*	3.40
PS	396	7/18/2008	F	317	347.89	1	0.93	2.40	0.69	1.04
PS	397	7/18/2008	F	400*	840.93	1	*	D	*	3.42
PS	398	7/18/2008	F	353*	644.48	2	*	D	*	2.76
PS	399	7/18/2008	F	387	652.8	1	0.91	D	*	2.30
PS	400	7/18/2008	F	390	687.07	2	0.93	8.26	1.21	2.66
PS	401	7/18/2008	F	445*	1088.82	2	*	16.66	1.54	4.12
PS	404	7/18/2008	F	370	592.72	2	0.96	8.02	1.36	2.12
PS	405	7/18/2008	F	317	392.82	1	1.05	3.66	0.93	1.00
PS	406	7/18/2008	F	362	571.4	2	0.99	7.80	1.37	2.18
PS	408	7/18/2008	F	367	565.36	1	0.94	5.38	0.96	2.12
PS	410	7/18/2008	F	358	547.06	2	0.98	5.36	0.98	1.92
PS	411	7/18/2008	F	423	881.49	2	0.92	11.98	1.37	4.50
PS	501	9/21/2008	F	400	778.96	1	0.97	10.12	1.31	4.84
PS	502	9/21/2008	F	362	565.38	1	0.98	3.18	0.56	2.24
PS	503	9/21/2008	F	373	633.13	1	1.00	6.78	1.07	2.16
PS	504	9/21/2008	F	375	622.3	1	0.96	4.84	0.78	2.98
PS	505	9/21/2008	F	403	814.96	2	0.99	17.44	2.15	4.68
PS	506	9/21/2008	F	337	473.38	1	1.04	5.06	1.07	1.74
PS	507	9/21/2008	F	350	484.45	1	0.94	4.00	0.83	1.58
PS	508	9/21/2008	F	350	530.64	1	1.03	8.52	1.61	2.46
PS	510	9/21/2008	F	218	109.79	0	1.00	0.78	0.71	0.02

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	511	9/21/2008	F	307	329.59	1	0.98	2.80	0.85	0.90
PS	512	9/21/2008	F	340	464.64	1	0.99	6.56	1.42	1.38
PS	513	9/21/2008	F	278	251.23	0	1.03	1.88	0.75	0.26
PS	514	9/21/2008	F	358	691.76	1	1.24	11.56	1.68	3.20
PS	515	9/21/2008	F	373	677.71	1	1.07	8.28	1.23	2.06
PS	516	9/21/2008	F	365	619.57	1	1.05	D	*	2.24
PS	517	9/21/2008	F	356	554.21	1	1.01	6.26	1.13	1.80
PS	518	10/3/2008	F	386	685.63	1	0.96	8.86	1.30	2.38
PS	519	10/3/2008	F	391	669.43	1	0.90	7.64	1.15	2.28
PS	520	10/3/2008	F	365	596.33	1	1.01	4.22	0.71	1.62
PS	522	10/3/2008	F	326	402.33	1	0.98	3.68	0.92	1.48
PS	524	10/3/2008	F	365	545.81	1	0.92	5.64	1.04	2.12
PS	525	10/3/2008	F	363	552.17	1	0.95	4.92	0.89	1.70
PS	527	10/3/2008	F	371	593.33	1	0.95	8.64	1.46	2.12
PS	528	10/3/2008	F	348	442.47	1	0.87	5.58	1.27	1.50
PS	528	10/3/2008	F	379	604.85	1	0.90	6.98	1.16	1.96
PS	529	10/3/2008	F	395	751.33	1	0.98	8.30	1.11	2.90
PS	530	10/3/2008	F	448	1212.51	2	1.05	27.56	2.29	7.60
PS	531	10/3/2008	F	365	637.61	1	1.08	7.64	1.20	2.90
PS	532	10/3/2008	F	350	507.87	1	0.98	7.42	1.47	1.44
PS	533	10/3/2008	F	335	459.12	1	1.03	4.84	1.06	1.04
PS	534	10/3/2008	F	359	582.72	1	1.04	5.44	0.94	2.02
PS	535	10/3/2008	F	337*	509.28	1	*	7.70	1.52	2.50
PS	536	10/3/2008	F	360	536.67	1	0.95	6.06	1.13	2.02
PS	537	10/3/2008	F	295	322.59	0	1.09	3.68	1.14	0.42
PS	538	10/3/2008	F	324	370.6	1	0.92	3.58	0.97	1.20
PS	539	10/3/2008	F	305	327.32	1	0.99	1.72	0.53	0.62
PS	540	10/3/2008	F	267	235.72	0	1.11	2.28	0.97	0.28
PS	542	10/3/2008	F	265	188.7	1	0.91	0.92	0.49	0.56
PS	546	10/3/2008	F	284	252.85	0	0.97	2.08	0.82	0.42
PS	601	10/10/2008	F	273	230.95	0	1.01	1.58	0.69	0.60
PS	603	10/10/2008	F	288	269.75	0	0.99	D	*	0.36
PS	604	10/10/2008	F	349	509.65	1	1.00	6.72	1.32	1.66
PS	605	10/10/2008	F	354	560	1	1.04	4.38	0.78	1.68
PS	606	10/10/2008	F	350	455.15	1	0.88	3.76	0.83	1.20
PS	607	10/10/2008	F	461	1359.2	2	1.07	34.14	2.53	9.02
PS	608	10/10/2008	F	394	832.36	2	1.09	15.40	1.86	5.42
PS	609	10/10/2008	F	392	786.61	1	1.05	14.04	1.79	4.02
PS	610	10/10/2008	F	361	593.93	1	1.04	5.34	0.90	2.50
PS	612	10/10/2008	F	305	304.92	0	0.93	2.84	0.93	0.46
PS	613	10/10/2008	F	340	435.5	1	0.93	4.22	0.97	1.26
PS	616	10/10/2008	F	367	717.11	2	1.19	11.44	1.60	3.66
PS	617	10/10/2008	F	325	313.35	1	0.77	1.72	0.55	0.82
PS	619	10/10/2008	F	335	436.06	1	0.97	2.98	0.69	1.08
PS	620	10/10/2008	F	330	392.26	1	0.92	3.84	0.98	1.18
PS	621	10/10/2008	F	335	406.96	1	0.91	3.60	0.89	0.98

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	622	10/10/2008	F	398	936.32	1	1.19	20.50	2.20	5.94
PS	624	10/10/2008	F	368	604.28	1	0.99	7.78	1.29	2.58
PS	625	10/10/2008	F	363	578.58	1	0.99	6.34	1.10	2.24
PS	626	10/10/2008	F	410	1005.03	2	1.16	23.60	2.36	6.24
PS	627	10/10/2008	F	357	559.01	1	1.01	8.88	1.60	2.48
PS	628	10/10/2008	F	350	486.32	1	0.94	5.06	1.04	1.36
PS	629	10/10/2008	F	374	618.3	1	0.96	7.94	1.29	2.86
PS	630	10/10/2008	F	255	194.79	0	1.06	1.88	0.97	0.20
PS	633	10/11/2008	F	266	185.51	0	0.88	0.82	0.44	0.20
PS	636	10/11/2008	F	372	581.27	1	0.92	5.16	0.89	1.78
PS	638	10/11/2008	F	380	675.29	1	1.00	8.64	1.28	2.86
PS	639	10/11/2008	F	300	275.88	1	0.88	2.42	0.88	0.68
PS	640	10/11/2008	F	293	250.36	1	0.87	1.42	0.57	0.62
PS	641	10/11/2008	F	272	210.01	0	0.93	1.86	0.89	0.20
PS	642	10/11/2008	F	366	557.55	1	0.93	7.30	1.31	2.02
PS	643	10/11/2008	F	274	234.19	0	1.01	1.32	0.56	0.30
PS	644	10/11/2008	F	402	764.65	1	0.94	13.46	1.77	3.98
PS	646	10/11/2008	F	436	1105.83	1	1.04	18.42	1.67	5.54
PS	647	10/11/2008	F	396	726.48	1	0.94	10.08	1.40	4.52
PS	648	10/11/2008	F	355	554.79	1	1.03	7.22	1.31	2.16
PS	649	10/11/2008	F	285	258.02	0	0.98	2.32	0.90	0.52
PS	652	10/11/2008	F	361	545.49	2	0.95	11.44	2.11	3.64
PS	654	10/11/2008	F	277	222.45	1	0.93	1.16	0.52	0.72
PS	655	10/11/2008	F	272	211.16	0	0.93	1.34	0.64	0.30
PS	656	10/11/2008	F	280	215.94	0	0.87	1.84	0.85	0.30
PS	657	10/11/2008	F	254	177.82	0	0.98	1.20	0.68	0.18
PS	659	10/11/2008	F	355	479.93	1	0.89	4.48	0.94	1.62
PS	660	10/11/2008	F	293	259.58	0	0.90	1.38	0.53	0.48
PS	701	10/31/2008	F	353	520.94	1	0.98	5.58	1.07	1.40
PS	703	10/31/2008	F	300	331.06	0	1.06	3.90	1.18	0.48
PS	704	10/31/2008	F	323	420.47	1	1.06	D	*	1.54
PS	705	10/31/2008	F	321	400.24	1	1.03	6.18	1.55	1.26
PS	706	10/31/2008	F	278	231.31	0	0.95	2.06	0.89	0.24
PS	707	10/31/2008	F	315	339.02	1	0.93	3.94	1.17	1.26
PS	708	10/31/2008	F	340	481.59	0	1.03	6.98	1.45	1.32
PS	709	10/31/2008	F	357	619.82	1	1.12	7.52	1.22	2.10
PS	710	10/31/2008	F	365	615.97	1	1.04	9.36	1.52	1.88
PS	711	11/1/2008	F	365	616.56	1	1.04	9.96	1.62	2.02
PS	712	11/1/2008	F	270	184.08	0	0.83	0.78	0.42	0.24
PS	713	11/1/2008	F	268	211.35	0	0.98	0.92	0.44	0.26
PS	714	11/1/2008	F	313	301.99	1	0.84	3.92	1.30	0.82
PS	715	11/1/2008	F	403	839.52	1	1.02	19.88	2.39	7.28
PS	717	11/1/2008	F	259	173.82	0	0.90	1.68	0.97	0.24
PS	718	11/1/2008	F	277	210.46	0	0.88	2.94	1.40	0.20
PS	719	11/1/2008	F	246	153.37	0	0.94	1.00	0.65	0.16
PS	721	11/1/2008	F	288	268.96	1	0.99	2.56	0.95	0.74

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	723	11/1/2008	F	307	343.51	0	1.02	2.48	0.72	0.56
PS	4002	8/16/2008	F	342	477.99	2	1.00	7.90	1.66	1.92
PS	4006	8/16/2008	F	323	479.53	1	1.21	6.39	1.34	1.38
PS	4008	8/16/2008	F	383	748.07	1	1.08	12.48	1.68	3.60
PS	4009	8/16/2008	F	332	407.8	1	0.94	3.28	0.81	1.50
PS	4010	8/16/2008	F	382*	768.16	2	*	15.48	2.02	3.52
PS	4011	8/16/2008	F	343	516.85	1	1.07	9.32	1.81	1.96
PS	4014	8/16/2008	F	391	816.96	1	1.10	17.44	2.14	3.56
PS	4016	8/16/2008	F	347	513.83	1	1.02	5.88	1.15	1.58
PS	4017	8/16/2008	F	335	467.77	1	1.05	D	*	1.58
PS	4018	8/16/2008	F	382	794.38	2	1.15	18.50	2.34	3.32
PS	4019	8/16/2008	F	377	663.45	2	1.01	11.96	1.81	2.42
PS	4020	8/16/2008	F	243	158.3	1	1.01	1.34	0.85	0.28
PS	4022	8/16/2008	F	344	536.01	1	1.10	9.82	1.84	1.08
PS	4023	8/16/2008	F	347*	624.8	2	*	8.78	1.41	3.52
PS	4026	8/16/2008	F	360	586.7	2	1.04	8.06	1.38	2.32
PS	4027	8/16/2008	F	370	643.03	2	1.04	11.86	1.85	2.56
PS	4028	8/16/2008	F	232	139.97	0	1.04	D	*	0.16
PS	4030	8/17/2008	F	227	143.34	0	1.15	1.36	0.95	0.06
PS	4032	8/17/2008	F	363	579.86	1	1.00	8.54	1.48	2.40
PS	4033	8/17/2008	F	301	326.07	1	1.03	D	*	0.74
PS	4034	8/17/2008	F	396	739.22	1	0.96	10.98	1.49	3.14
PS	4036	8/17/2008	F	360	546.03	1	0.96	6.82	1.25	2.14
PS	4037	8/17/2008	F	340	446.4	1	0.95	4.26	0.96	1.00
PS	4038	8/17/2008	F	346	556.3	2	1.12	10.30	1.86	2.40
PS	4040	8/17/2008	F	392*	815.26	2	*	14.94	1.84	2.88
PS	4041	8/17/2008	F	389*	819.68	2	*	13.78	1.69	2.66
PS	4042	8/17/2008	F	357	603.2	2	1.09	10.34	1.72	2.36
PS	4043	8/17/2008	F	362	579.89	2	1.01	8.92	1.55	2.70
PS	4044	8/17/2008	F	335	475.51	1	1.06	8.54	1.80	2.00
PS	4045	8/17/2008	F	373	667.28	1	1.05	9.88	1.49	2.08
PS	4046	8/17/2008	F	246	161.67	0	0.99	2.14	1.32	0.06
PS	4047	8/17/2008	F	323*	216.3	1	*	1.48	0.69	0.30
PS	4050	8/17/2008	F	335	427.73	1	0.96	5.48	1.29	1.44
PS	4051	8/17/2008	F	323	478.5	1	1.20	6.00	1.26	1.40
PS	4052	8/17/2008	F	305	349.45	1	1.06	D	*	0.86
PS	4053	8/17/2008	F	313	368.06	1	1.03	3.72	1.01	0.84
PS	4054	8/17/2008	F	360	567.15	2	1.00	9.60	1.70	2.34
PS	4056	8/17/2008	F	375	707.08	1	1.09	6.64	0.94	3.28
PS	4057	8/17/2008	F	347	558.88	1	1.11	D	*	2.28
PS	363A	7/18/2008	F	390	718.16	2	0.98	12.86	1.80	2.68
PS	101	5/17/2008	M	335	451.92	2	0.96	6.90	1.53	0.10
PS	105	5/17/2008	M	307	349.65	2	1.01	4.22	1.21	0.20
PS	107	5/17/2008	M	326	425.35	2	0.99	6.38	1.50	0.14
PS	109	5/17/2008	M	337	531.5	2	1.10	8.78	1.65	0.30
PS	111	5/17/2008	M	331	444.34	2	0.98	6.30	1.42	0.12

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	115	5/17/2008	M	311	377.92	2	1.05	3.22	0.85	0.16
PS	122	5/17/2008	M	336	458.16	2	0.96	4.94	1.08	0.18
PS	124	5/17/2008	M	340	503.31	2	1.01	9.88	1.96	0.24
PS	132	5/17/2008	M	298	381.49	2	1.23	7.20	1.89	0.30
PS	133	5/17/2008	M	308	355.61	2	1.02	3.30	0.93	0.14
PS	137	5/17/2008	M	331	450.15	2	1.00	4.94	1.10	0.44
PS	150	5/18/2008	M	341	532.6	2	1.06	7.02	1.32	0.28
PS	152	5/18/2008	M	313	400.96	2	1.08	4.44	1.11	0.34
PS	160	5/18/2008	M	268	240.08	2	1.13	3.24	1.35	0.22
PS	161	5/18/2008	M	326	492.46	2	1.15	8.18	1.66	0.39
PS	164	5/18/2008	M	350	581.31	2	1.05	10.92	1.88	0.34
PS	166	5/18/2008	M	313	382.46	2	1.03	5.58	1.46	0.44
PS	171	5/18/2008	M	318	374.3	2	0.96	4.88	1.30	0.28
PS	172	5/18/2008	M	308	375.31	2	1.07	5.78	1.54	0.18
PS	174	5/18/2008	M	332	441.5	2	0.97	5.72	1.30	0.24
PS	175	5/18/2008	M	335	470.27	2	1.00	6.00	1.28	0.30
PS	177	5/18/2008	M	332	520.14	2	1.14	9.23	1.78	0.30
PS	180	5/18/2008	M	310	377.93	2	1.06	4.24	1.12	0.18
PS	182	5/18/2008	M	330	446.89	2	1.00	6.90	1.54	0.12
PS	202	6/19/2008	M	327	448.59	2	1.04	7.16	1.60	0.13
PS	203	6/19/2008	M	343	532.67	2	1.04	7.82	1.47	0.16
PS	205	6/19/2008	M	345	459.75	2	0.88	8.84	1.92	0.14
PS	208	6/19/2008	M	330	459.35	2	1.03	5.66	1.23	0.12
PS	209	6/19/2008	M	337	511.86	2	1.06	10.66	2.08	0.16
PS	211	6/19/2008	M	335	563.33	2	1.19	9.06	1.61	0.18
PS	213	6/19/2008	M	348	608.11	2	1.12	12.56	2.07	0.22
PS	224	6/19/2008	M	283	243.68	2	0.95	2.02	0.83	0.04
PS	226	6/19/2008	M	349	519.96	2	0.95	6.02	1.16	0.10
PS	230	6/19/2008	M	213	100.86	1	1.09	0.72	0.71	0.02
PS	233	6/20/2008	M	311	391.01	2	1.08	6.30	1.61	0.08
PS	234	6/20/2008	M	333	356.23	2	0.77	3.76	1.06	0.04
PS	242	6/20/2008	M	303*	300.54	2	*	4.60	1.53	0.04
PS	243	6/20/2008	M	363	595.15	2	0.94	10.56	1.77	0.15
PS	244	6/20/2008	M	302*	385.36	2	*	9.72	2.52	0.14
PS	245	6/20/2008	M	347	522.57	2	0.98	9.84	*	*
PS	256	6/20/2008	M	340*	544.63	2	*	10.09	1.85	0.08
PS	266	6/20/2008	M	336	556.3	2	1.17	9.14	1.64	0.14
PS	267	6/20/2008	M	295	321.03	2	1.07	3.22	1.00	0.14
PS	273	6/20/2008	M	334	465.41	2	1.00	7.36	1.58	0.04
PS	274	6/20/2008	M	233	135.91	1	1.06	1.36	1.00	0.03
PS	310	7/17/2008	M	364	623.15	2	0.98	D	*	*
PS	312	7/17/2008	M	345*	699.68	2	*	15.30	2.19	0.14
PS	330	7/17/2008	M	335	511.53	2	1.08	12.33	2.41	0.14
PS	331	7/17/2008	M	339*	504.15	2	*	9.26	1.84	0.17
PS	339	7/17/2008	M	348	525.54	2	0.97	D	*	D
PS	342	7/17/2008	M	346	542.96	2	1.02	D	*	*

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	344	7/17/2008	M	342	501.62	2	0.99	10.90	2.17	0.12
PS	346	7/18/2008	M	353	545.63	2	0.96	11.84	2.17	0.16
PS	349	7/18/2008	M	340	324.25	2	0.65	D	*	0.18
PS	351	7/18/2008	M	431*	478.37	2	*	D	*	0.14
PS	359	7/18/2008	M	330	473.89	2	1.06	14.54	3.07	0.16
PS	368	7/18/2008	M	350	527.01	2	0.95	9.62	1.83	0.10
PS	371	7/18/2008	M	327	409.94	2	0.95	D	*	*
PS	372	7/18/2008	M	343	517.45	2	1.01	D	*	0.18
PS	375	7/18/2008	M	340*	607.8	2	*	13.82	2.27	0.12
PS	378	7/18/2008	M	322	482.14	2	1.18	11.36	2.36	0.12
PS	379	7/18/2008	M	291	354.09	2	1.24	4.94	1.40	0.20
PS	390	7/18/2008	M	330	418.37	2	0.94	4.62	1.10	0.22
PS	391	7/18/2008	M	320	404.41	2	1.01	6.96	1.72	0.16
PS	393	7/18/2008	M	363	530.16	2	0.84	D	*	D
PS	402	7/18/2008	M	268	198.7	1	0.94	1.68	0.85	0.08
PS	403	7/18/2008	M	322	405.47	2	0.99	4.82	1.19	0.16
PS	409	7/18/2008	M	327	434.1	2	1.00	5.22	1.20	0.10
PS	509	9/21/2008	M	269	210.7	1	0.98	1.72	*	*
PS	523	10/3/2008	M	271	204.94	1	0.93	1.28	*	*
PS	543	10/3/2008	M	265	184.35	1	0.91	0.84	*	*
PS	544	10/3/2008	M	263	208.79	1	1.05	1.90	0.91	0.06
PS	545	10/3/2008	M	262	188.1	1	0.96	2.06	*	*
PS	547	10/3/2008	M	257	180.6	1	0.99	1.24	0.69	0.08
PS	602	10/10/2008	M	279	235.69	1	0.96	0.76	*	*
PS	611	10/10/2008	M	293	281.72	1	0.97	2.26	0.80	0.08
PS	614	10/10/2008	M	272	200.8	1	0.90	0.88	*	*
PS	623	10/10/2008	M	283	236.84	1	0.92	1.72	0.73	0.06
PS	632	10/11/2008	M	316	408.76	1	1.07	5.72	1.41	2.22
PS	634	10/11/2008	M	290	253.87	1	0.90	2.40	0.95	0.08
PS	635	10/11/2008	M	268	187.96	1	0.89	1.54	*	*
PS	645	10/11/2008	M	262	211.52	1	1.08	2.14	1.01	0.38
PS	650	10/11/2008	M	272	206.15	1	0.92	1.48	*	*
PS	651	10/11/2008	M	283	222.53	1	0.86	1.90	0.85	0.02
PS	658	10/11/2008	M	253	162.54	1	0.94	1.10	*	*
PS	661	10/11/2008	M	297	266.74	1	0.87	2.30	*	*
PS	702	10/31/2008	M	327	427.64	1	0.99	6.04	1.41	0.20
PS	722	11/1/2008	M	265	192.34	1	0.95	D	*	*
PS	4001	8/16/2008	M	271	225.7	1	1.02	2.34	1.04	0.03
PS	4003	8/16/2008	M	290	283.29	1	1.01	2.96	1.05	0.08
PS	4004	8/16/2008	M	310*	475.75	2	*	8.98	1.89	0.16
PS	4005	8/16/2008	M	323	454.41	2	1.10	7.78	1.71	0.26
PS	4007	8/16/2008	M	320*	553.26	2	*	15.12	2.73	0.24
PS	4012	8/16/2008	M	320	420.72	2	1.05	4.96	1.18	0.24
PS	4013	8/16/2008	M	310	415.79	2	1.16	8.26	1.99	0.24
PS	4015	8/16/2008	M	307	290.26	2	0.84	3.82	1.32	0.20
PS	4021	8/16/2008	M	300*	364.27	1	*	5.42	1.49	0.16

APPENDIX A: CONTINUED

Location	ID	Date	Sex	TL (mm)	Weight (g)	Age (y)	Kn	Liver (g)	LSI	Gonad (g)
PS	4024	8/16/2008	M	345*	627.17	1	*	10.78	1.72	0.44
PS	4025	8/16/2008	M	335	523.04	2	1.11	9.50	1.82	0.18
PS	4029	8/17/2008	M	325	474.33	2	1.12	10.62	2.24	0.14
PS	4031	8/17/2008	M	337	477.8	2	0.99	6.82	1.43	0.22
PS	4035	8/17/2008	M	311	355.1	2	0.98	6.24	1.76	0.06
PS	4039	8/17/2008	M	355	550.68	2	0.95	9.76	1.77	0.24
PS	4048	8/17/2008	M	308	318.06	1	0.91	D	*	0.08
PS	4049	8/17/2008	M	327*	557.18	2	*	13.32	2.39	0.36
PS	4055	8/17/2008	M	323*	499.36	2	*	9.98	2.00	0.12
PS	363B	7/18/2008	M	275*	367.69	2	*	D	*	0.12
TR	104	1/24/2008	F	245	177.42	1	0.91	*	*	*
TR	107	3/24/2008	F	233	133.4	1	1.02	*	*	2.40
TR	108	3/24/2008	F	271	213.75	1	1.05	*	*	2.68
TR	109	3/24/2008	F	213	105.25	1	0.96	*	*	1.68
TR	110	3/24/2008	F	215	96.48	1	1.08	*	*	1.36
TR	111	6/9/2008	F	355	605.15	1	0.91	11.86	1.97	1.86
TR	122	6/9/2008	F	346	488	1	1.03	5.30	1.09	1.26
TR	140	7/8/2008	F	447	1123.81	3	1.05	21.46	1.92	7.04
TR	161	11/26/2008	F	372	645.11	1	1.00	9.96	1.55	2.92
TR	201	9/10/2008	F	392	821.18	1	0.93	14.44	1.77	4.02
TR	202	9/12/2008	F	332	431.97	1	1.02	8.20	1.91	1.62
TR	203	9/12/2008	F	351	497.82	1	1.06	D	*	2.40
TR	204	9/12/2008	F	320	388.89	1	1.00	6.02	1.55	1.68
TR	105	2/25/2008	M	254	183.12	1	0.76	*	*	*
TR	106	3/24/2008	M	213	106.64	1	0.48	*	*	*
TR	112	6/9/2008	M	367	707.62	1	1.96	16.98	2.40	0.20
TR	150	10/10/2008	M	333	531.31	1	1.53	11.80	2.22	0.92
TR	205	9/11/2008	M	256	189.38	1	0.78	D	*	0.06
TR	1A	10/2/2007	M	197	72.44	*	0.39	*	*	*
TR	2A	4/18/2007	M	220	128.8	1	0.52	*	*	*

Location: PR = Pamlico River, PS = Pamlico Sound, TR = Tar River

Date: MM/DD/YYYY

TL = total length, * = damaged caudal fin

Kn: Relative condition factor

Liver: D = decomposed, M = missing from fish

* = missing data

APPENDIX B: SOUTHERN FLOUNDER TOTAL MERCURY DATA

Location	ID	Date	Sex	Age (y)	Total length (mm)	Weight (g)	Total Hg (ppm)
PR	546	10/2/2008	F	3	545	2288.0	0.0319
PR	515	10/2/2008	F	3	525	2023.7	0.0353
PR	398	9/10/2008	F	3	516	1566.2	0.0554
PR	382	9/10/2008	F	2	485	1335.5	0.0906
PR	524	10/2/2008	F	2	482	1621.8	0.0462
PR	506	10/2/2008	F	3	479	1590.3	0.0451
PR	218	7/13/2008	F	2	355	601.4	0.0282
PR	389	9/10/2008	F	1	355	618.3	0.0424
PR	711	11/19/2008	F	0	355	497.0	0.0208
PR	714	11/19/2008	F	0	355	551.2	0.0158
PR	1016	9/10/2008	F	1	355	552.2	0.0310
PR	2014	9/10/2008	F	1	355	507.2	0.0308
PR	255	7/17/2008	M	2	355	582.0	0.0479
PR	399	9/10/2008	M	2	351	525.7	0.0367
PR	236	7/13/2008	M	2	337	448.0	0.0593
PR	1006	9/10/2008	M	2	332	449.7	0.0631
PR	122	6/5/2008	M	2	329	471.1	0.0326
PR	113	6/4/2008	M	2	327	480.1	0.0190
PR	327	8/17/2008	M	1	287	297.4	0.0280
PR	301	9/10/2008	M	1	283	280.1	0.0178
PR	3001	9/10/2008	M	1	281	249.1	0.0365
PR	344	8/17/2008	M	1	280	318.2	0.0278
PR	239	7/17/2008	M	1	276	300.7	0.0210
PR	1012	9/10/2008	M	1	274	226.0	0.0409
PR	302	9/10/2008	M	1	261	237.6	0.0233
PR	413	9/10/2008	M	1	260	238.2	0.0278
PR	4006	9/10/2008	M	1	260	206.8	0.0486
PR	377	9/10/2008	M	1	252	200.3	0.0308
PR	304	9/10/2008	F	0	228	151.9	0.0162
PR	161	6/15/2008	M	1	225	140.5	0.0454
PR	154	6/15/2008	M	1	223	139.8	0.0353

APPENDIX B: CONTINUED

Location	ID	Date	Sex	Age (y)	Total length (mm)	Weight (g)	Total Hg (ppm)
PR	156	6/15/2008	F	1	216	111.4	0.0275
PR	238	7/17/2008	F	0	215	114.1	0.0171
PR	222	7/13/2008	F	0	209	146.1	0.0191
PR	179	7/2/2008	F	0	206	108.9	0.0138
PR	545	10/2/2008	F	0	189	66.4	0.0253
PS	353	7/18/2008	F	3	525	1739.1	0.0808
PS	314	7/17/2008	F	4	505	1652.5	0.1014
PS	607	10/10/2008	F	2	461	1359.2	0.0738
PS	265	6/20/2008	F	2	457	1246.9	0.0430
PS	307	7/17/2008	F	3	456	1166.1	0.0730
PS	310	7/17/2008	M	2	364	623.2	0.0627
PS	243	6/20/2008	M	2	363	595.2	0.0537
PS	393	7/18/2008	M	2	363	530.2	0.0552
PS	127	5/17/2008	F	1	360	537.6	0.0470
PS	136	5/17/2008	F	2	360	569.0	0.0445
PS	138	5/17/2008	F	1	360	527.5	0.0275
PS	165	5/18/2008	F	2	360	559.4	0.0420
PS	210	6/19/2008	F	2	360	618.7	0.0325
PS	536	10/3/2008	F	1	360	536.7	0.0409
PS	4039	8/17/2008	M	2	355	550.7	0.0705
PS	346	7/18/2008	M	2	353	545.6	0.0624
PS	164	5/18/2008	M	2	350	581.3	0.0663
PS	107	5/17/2008	M	2	326	425.4	0.0379
PS	161	5/18/2008	M	2	326	492.5	0.0387
PS	4029	8/17/2008	M	2	325	474.3	0.0522
PS	4005	8/16/2008	M	2	323	454.4	0.0519
PS	403	7/18/2008	M	2	322	405.5	0.0519
PS	379	7/18/2008	M	2	291	354.1	0.0780
PS	545	10/3/2008	M	1	262	188.1	0.0417
PS	645	10/11/2008	M	1	262	211.5	0.0706
PS	547	10/3/2008	M	1	257	180.6	0.0520

APPENDIX B: CONTINUED

Location	ID	Date	Sex	Age (y)	Total length (mm)	Weight (g)	Total Hg (ppm)
PS	658	10/11/200	M	1	253	162.5	0.0377
PS	719	11/1/2008	F	0	246	153.4	0.0579
PS	4046	8/17/2008	F	0	246	161.7	0.0454
PS	4020	8/16/2008	F	1	243	158.3	0.0507
PS	274	6/20/2008	M	1	233	135.9	0.0336
PS	4028	8/16/2008	F	0	232	140.0	0.0490
PS	4030	8/17/2008	F	0	227	143.3	0.0883
PS	510	9/21/2008	F	0	218	109.8	0.0482
PS	230	6/19/2008	M	1	213	100.9	0.0360
TR	140	7/8/2008	F	3	447	1123.8	0.1855
TR	201	9/10/2008	F	1	392	821.2	0.1158
TR	161	11/26/200	F	1	372	645.1	0.1202
TR	112	6/9/2008	M	1	367	707.6	0.0959
TR	111	6/9/2008	F	1	355	605.2	0.0935
TR	203	9/12/2008	F	1	351	497.8	0.1305
TR	122	6/9/2008	F	1	346	488.0	0.1057
TR	150	10/10/200	M	1	333	531.3	0.1431
TR	202	9/12/2008	F	1	332	432.0	0.1434
TR	204	9/12/2008	F	1	320	388.9	0.1635
TR	108	3/24/2008	F	1	271	213.8	0.0699
TR	205	9/11/2008	M	1	256	189.4	0.0940
TR	105	2/25/2008	M	1	254	183.1	0.0811
TR	104	1/24/2008	F	1	245	177.4	0.0856
TR	107	3/24/2008	F	1	233	133.4	0.0702
TR	110	3/24/2008	F	1	215	96.5	0.0723
TR	106	3/24/2008	M	1	213	106.6	0.0624
TR	109	3/24/2008	F	1	213	105.3	0.0485

Location: PR = Pamlico River, PS = Pamlico Sound, TR = Tar River

Date: MM/DD/YYYY

APPENDIX C: LIPID EXTRACTION PROCEDURES

Reagents:

Chloroform/methanol (2:1)	2 volumes chloroform
1 volume methanol	Chloroform/methanol (2:1) + BHT
0.01 g BHT in 100 mL	chloroform/methanol (2:1)
0.88% Potassium Chloride _{aq}	0.88 g KCL in 100 mL nanopure H ₂ O
Sodium sulfate (anhydrous)	

PROCEDURE

- Freeze-dry tissue samples and pulverize using tissue grinder
 - Adipose tissue cannot be freeze-dried effectively—extract these tissues intact, or homogenize in chloroform/methanol (step 4)
- Label an appropriate number of large (50 mL) centrifuge tubes
- Weigh out tissue sample, recording the mass to at least 2 decimal places, and transfer to large centrifuge tube
 - For liver samples, use ~0.1-0.2 g
 - For fillet samples, use ~0.2-0.5 g
 - For adipose samples, use <0.1 g, wet weight
- Add 12 mL of chloroform/methanol to the sample and vortex well to mix.
- Place samples in the freezer (-20C) for 1 hour
- Add 0.25 volumes of 0.88% KCl to the sample, vortex well to mix, return to the freezer for 5 minutes
 - 3 mL of 0.88% KCl per sample, assuming 12 mL of chloroform/methanol)
- Centrifuge samples at 1500 RPM for 5 minutes
 - Program #3, using rotor #243 on the refrigerated centrifuge
- Label and weigh an appropriate number of 16 mL, glass, screw-cap vials, recording the mass to 4 decimal places
- Prepare sodium sulfate filters, by layering 2 cm of sodium sulfate (anhydrous) on a small amount of kimwipe in a pasteur pipet.
- Transfer bottom layer from centrifuged samples to labeled glass vials through the sodium sulfate filters.
- Evaporate solvent under ultra-pure nitrogen (UPN) at 45C to dryness, then dessicate *in vacuo* for a minimum of 1 hour (overnight preferred)
- Re-weigh vials, recording mass to 4 decimal places.
 - If proceeding directly to methylation, return vials to dessicator until ready
 - If storing samples long-term prior to methylation (not recommended), re-dissolve lipid in chloroform/methanol + BHT to a concentration of 10 mg/mL, blanket with UPN, and store in freezer (-20C).

APPENDIX D: PREPARATION OF FATTY ACID METHYL ESTERS (ACID-CATALYZED TRANSMETHYLATION)

Reagents:

Methylating reagent	1 mL H ₂ SO ₄ in 100 mL methanol
Toluene (HPLC grade)	2% potassium biocarbonate _{aq}
2 g KHCO ₃ in 100 mL nanopure H ₂ O	Isohexane/diethyl ether (1:1)
1 volume isohexane	1 volume diethyl ether

PROCEDURE

13. If lipid is suspended in solvent, evaporate to dryness under ultra-pure nitrogen (UPN)
 - a. If desired, 23:0 free fatty acid can be added to the samples at 10% of the total lipid mass as a recovery standard
 14. Add 1 mL of toluene, followed by 2 mL of the methylating reagent to crude lipid samples
 - a. If the samples are in culture tubes, add the toluene, vortex, and transfer to labeled 16 mL, glass, screw-cap vials prior to adding the methylating reagent
 15. Flush the vials with UPN and cap
 16. Incubate the vials for 16 hours at 50°C in the water bath
 17. Remove the vials from the water bath and cool for 5 minutes
 18. Add 2 mL of 2% KHCO₃, followed by 3-5 mL of the iso-hexane diethyl ether
 19. Cap the vials and shake gently, pausing frequently to vent CO₂ produced Vortex after sample is stable
 20. Centrifuge the tubes at 1500 RPM for 2 minutes using the fixed-angle rotor centrifuge
 21. Label and weigh an appropriate number of glass vials, recording the mass to 4 decimal places
 - a. Use 16 mL, screw-cap vials for samples extracted using the general method
 - b. Use 5 mL glass culture vials for sample extracted using the micro-procedure
 22. Transfer the upper layer from the centrifuged samples to the pre-weighed vials.
 23. Add an additional 3-5 mL of isohexane/diethyl ether to the sample and shake, vortex, and centrifuge as before.
 24. Remove upper layer as before and add to previous in glass vial
 25. Evaporate solvent under UPN at 45°C to dryness, then dessicate *in vacuo* for a minimum of 1 hour (overnight preferred)
 26. Re-weigh vials and resuspend fatty acid methyl esters in isohexane at a concentration of 10 mg/mL.
- Transfer samples to GC vials and store in freezer (-20°C) until GC analysis.