Natal Origin of Central Southern Management Area, North Carolina
Striped Bass, Inferred from Otolith Microchemistry

by

Jeffrey M. Dobbs

April, 2013

Director of Thesis: Dr. Roger A. Rulifson

Major Department: Department of Biology

Abstract

Striped bass (*Morone saxatilis*) is an important commercial and game fish throughout North Carolina coastal waters. These fish have spawning populations present in all of the state's coastal rivers, however populations south of the Albemarle Sound in North Carolina have rarely been studied. These populations lie within North Carolina's immense Central Southern Management Area (CSMA). The CSMA stretches from the northernmost point of Pamlico Sound in the north down to the South Carolina border in the south. There are three main watersheds in the CSMA: the Tar/Pamlico River, the Neuse River, and the Cape Fear River. These rivers have spawning populations of striped bass, yet very few age 0 fish have been collected to support this in recent years. My study investigated the natal origin of CSMA striped bass through the use of water and otolith elemental analyses. Surface water samples and environmental data were collected once per month from 15 sample sites throughout the CSMA from May 2011 to July 2012. Two additional sample ponds from Edenton National Fish Hatchery were sampled once per week for two weeks in April 2012. Samples were analyzed for concentrations of calcium (Ca), strontium (Sr), magnesium (Mg), barium (Ba), and manganese (Mn) using an inductively-coupled plasma optical emission spectrometer. Concentrations were recorded as element to Ca ratios to account for the role of Ca in otolith deposition. Salinity

differed significantly by location, while temperature and dissolved oxygen differed significantly by month and season. Only temperature differed significantly by year. All measured elements were consistently detected at every sample site. All measured elements differed significantly by location, but only Mn differd by month, season, and year. A multivariate classification of samples to their river of origin yielded only 42% success. When the classification was narrowed to include only one low salinity sample site per river, samples were classified to their sample site of origin with 82% accuracy. Fish (N=251) were collected from the Neuse and Tar/Pamlico rivers from April 2011 to April 2012. Fish total length (TL), total weight (TW), gonad weight, and liver weight were collected to calculate relative weight (Wr), liver somatic index (LSI), and gonadal somatic index (GSI). Otoliths were removed for ageing and elemental analysis. Elemental analysis was conducted by measuring concentrations of Sr, Mg, Mn, and Ba in the natal origin region of the otolith using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Condition of fish collected in the Neuse and Tar/Pamlico was sub-optimal. Fish GSI follow predictable yearly cycles, but were maturing a full year earlier than Roanoke River striped bass. Using the Sr signature from otolith elemental analysis, 88.4% of fish originated from the hatchery. Fish determined to be of natural origin were classified to their river of origin with 58.0% accuracy, and to their management area of origin with 84.0% accuracy. This study suggests that striped bass has become a put and take fishery in the Neuse and Tar/Pamlico rivers.

Natal Origin of Central Southern Management Area, North Carolina Striped Bass, Inferred from Otolith Microchemistry

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

by

Jeffrey M. Dobbs

April, 2013

Natal Origin of Central Southern Management Area, North Carolina
Striped Bass, Inferred from Otolith Microchemistry

by

Jeffrey M. Dobbs

APPROVED BY:

DIRECTOR OF
DISSERTATION/THESIS: _____
(Roger A. Rulifson, PhD)

COMMITTEE MEMBER: _____
(Anthony S. Overton, PhD)

COMMITTEE MEMBER: _____
(D. Reide Corbett, PhD)

COMMITTEE MEMBER: _____
(Norman M. Halden, PhD)

CHAIR OF THE DEPARTMENT
OF BIOLOGY: _____
(Jeff S. McKinnon, PhD)

DEAN OF THE
GRADUATE SCHOOL: _____
Paul J. Gemperline, PhD

ACKNOWLEDGEMENTS

List of Tables

List of Figures

Chapter 1: Introduction

Striped Bass as a Species

Striped bass (*Morone saxatilis*) is an anadromous fish that spends the majority of its adult life in the high salinity environment of the ocean, but returns to freshwater to spawn. However, populations south of Albemarle Sound are considered endemic riverine populations (McIlwain 1980; Morris et al. 2003). Striped bass have a natural distribution along North America's Atlantic coast, from Florida to the Gulf of St. Lawrence, and in the Gulf of Mexico, but have been introduced elsewhere (Klein-MacPhee 2002; Woodroffe 2012). They are most prevalent from North Carolina to Massachusetts (Merriman 1941). The largest and most important contingent of Atlantic coast striped bass is the Hudson River stock. These fish are highly migratory and make up the majority of the Atlantic migratory stock (Wirgin et al. 1993). North Carolina's Albemarle Roanoke stock is the third largest, behind only the Chesapeake Bay and Hudson River stocks.

Striped bass is a long-lived species, with a maximum age of nearly 30 years. They can grow to nearly 2 m in length and weigh upwards of 50 kg, but individuals >20 kg are rarely recorded (Robins and Ray 1986). Striped bass, like most anadromous species, are assumed to return to natal waters to spawn as adults (Thorrold et al. 1998a). They are iteroparous broadcast spawners that make yearly migrations up rivers to spawn as reproductive adults. In North Carolina, females normally mature at age 3 or 4, but can first reproduce as late as 6 years of age depending on environmental conditions (Olsen and Rulifson 1992; Boyd 2011). After maturation females can spawn every subsequent year, though skip spawning has been hypothesized as a result of striped bass longevity (Secor 2008). Females from the Albemarle Sound and Roanoke

River first spawn between 3 and 6 years with over 90% sexually mature by age 4 (Boyd 2011).

As females age and grow, their fecundity increases. Studies conducted on Roanoke River striped

bass have found that age 3 females produced approximately 175,000 to 300,000 eggs while the

oldest fish that were collected during the studies (16 years old) produced approximately

3,000,000 to 5,000,000 eggs (Olsen and Rulifson 1992; Boyd 2011). Water temperature and

increasing day length are the key factors in determining when reproductively active striped bass

will spawn. In the Roanoke River, this typically occurs when water temperatures reach 18-23 ˚C

in the spring (Rulifson and Manooch 1993; Beasley and Hightower 2000).

Striped Bass Management in North Carolina

North Carolina's estuarine striped bass make up one of the most important recreational

and commercial fisheries in North Carolina (Burgess and Bianchi 2004; NCDMF 2011).

Populations of striped bass are managed as distinct unit stocks in North Carolina. A unit stock is

a fisheries management unit used to denote a population or group of populations of fish that are

reproductively isolated (Kutkuhn 1981). These stocks are managed by the North Carolina

Division of Marine Fisheries (NCDMF), and North Carolina Wildlife Resources Commission

(NCWRC) through the implementation of three distinct management areas. Each management

area has its own regulations regarding total allowable catch (TAC) for commercial and recreation

fisheries, as well as bag and size restrictions for the recreational fisheries. The Albemarle Sound

Management Area (ASMA) encompasses Albemarle Sound and is North Carolina's most

valuable estuarine striped bass management area with 125,000 kgs of TAC per year in its

commercial fishery (NCDMF 2004). The Roanoke River Management area encompasses the

Roanoke River. This management area, combined with the ASMA, is home to North Carolina's

largest stock of striped bass; the Albemarle Roanoke stock (A/R stock). Because of the A/R stock's size and economic value, the vast majority of research on striped bass in the state has been devoted to these management areas (Olsen and Rulifson 1992; Rulifson and Manooch 1993; Haeseker et al. 1996; Beasley and Hightower 2000; Mohan et al. 2012).

The Central Southern Management Area (CSMA) striped bass stock makes up a smaller, yet important portion of the resident fish population and directed fishery for the state (NCDMF 2004). Despite the area's significant striped bass population, successful recruitment to the fishery of these populations remains undocumented. The CSMA consists of three primary watersheds: the Tar/Pamlico River, Neuse River and Cape Fear River systems. These watersheds support an important recreational fishery with substantial economic impact. There are spawning populations of striped bass in each of these watersheds, and larvae and eggs have been collected to support this (Beasley and Hightower 2000; Burdick and Hightower 2006). However, few juveniles have been collected in abundance index surveys to show evidence of successful recruitment (Barwick et al. 2009). This lack of data necessitates research to determine if spawning is successful; i.e., whether the subadults and adults from the CSMA are recruiting from these watersheds, from hatchery raised stocked fish, or from outside the CSMA. The NCWRC has an extensive stocking program for the CSMA watersheds since 1980 (Woodroffe 2012). The number, origin and growth phase at stocking of the fish has changed several times since the inception of the program. Since 2004, the NCWRC releases 100,000 phase II (12-20 cm TL) fingerling striped bass per year into each of the three main CSMA rivers (NCDMF 2004; NCDMF 2011). Phase II fish are used in the stocking program so as not to obscure the results of juvenile abundance index surveys (NCDMF 2004).

The total allowable catch (TAC) per year is currently split evenly between recreational and commercial fisheries. The North Carolina estuarine striped bass fisheries management plan from (FMP) 2004 states that in the Albemarle Sound management area, the TAC is currently 125,000 kg for the commercial fishery, and 125,000 kg for the recreational fishery. In the much smaller Central Southern Management Area (CSMA) stock, the TAC for the commercial harvest is 11,000 kg. There is no TAC for the recreational fishery in the CSMA; instead it is controlled by a slot limit (46 to 56 cm TL) and daily bag limit of two fish. On average the yearly recreational harvest exceeds the commercial harvest in the CSMA, but the recreational harvest and catch per unit effort has been declining in recent years (NCDMF 2011). In response, the NCDMF has instituted a harvest moratorium in the Cape Fear River and its tributaries. Commercially harvested striped bass are a by-catch only fishery in North Carolina with extensive restrictions on size of fish, gear type, quantity of catch, and location in which they can be caught. According to 2011 NCDMF catch statistics, 227,154 kg of striped bass valued at $1,220,542 were commercially harvested in North Carolina waters in 2010 (NCDMF 2004; NCDMF 2011).

Trace Element Analysis

Stock identification and delineation are key problems in modern fisheries management (Kutkuhn 1981; Begg et al. 1999). Unit stocks are managed independently of one another; therefore, for fisheries management to be effective, separate stocks of fish must be identified and delineated. Historically, mark recapture studies, morphology and meristics, as well as physiological and behavioral characteristics have been implemented to identify stocks with varying success (Gillanders 2001). Recent advancements in elemental analysis have created a

way to identify and delineate stocks, and determine fish movement patterns and origin, based on concentrations of trace elements in water and calcified structures (Campana and Fowler 1994; Campana 1999; Gillanders 2001). The elemental signature of the water is compared to the chemical composition of calcified structures, such as otoliths, to determine if they are similar. Otoliths, sometimes called earstones, are paired calcified structures found in the inner ears of bony fish. There are three sets of otoliths: the sagittae, lapilli, and asterisci. Their primary functions are for hearing and orientation within the water column. Otoliths are thought to form shortly after hatching, but current research suggests that striped bass otoliths begin to form before the larva even hatches (B. Elking, East Carolina University, personal communication). They are composed of a crystalline aragonite structure over a protein matrix. The aragonite layers are permanently deposited in discrete increments as the fish grows. The chemical composition of these layers is proportional to that of the ambient water inhabited by the fish at the time of deposition (Campana 1999). These discrete layers create a temporal record of the water inhabited by the fish. Of the three pairs of otoliths in bony fish, the largest (saggita), is most commonly used for ageing and microchemical analysis. The core, or primordium, of the otolith corresponds to the earliest larval period, and perhaps a portion of time spent in the egg, of a fish's life. Thus the microchemistry of the core of the otolith can be used as a means of inferring natal origin of fish (Campana and Fowler 1994).

In order to determine the origin of fish based on otolith microchemistry, water chemistry must be stable over time. Temporally stable water chemistry would allow comparison of fish otolith chemistry outside the year in which the water chemistry was determined. In contrast, varying water chemistry with time limits the comparison of otolith microchemistry among years. If variation is at a small enough scale (e.g. months or season), otolith microchemistry may not be

appropriate for determining origin of fish. The consistency of watershed signatures within the CSMA watersheds has never been evaluated. Mohan et al. (2012) found that water chemistry within the A/R management area watersheds was stable over a three-month period. To determine if the watershed chemical signatures are consistent over seasons and years, a similar study of water chemistry over time should be conducted.

The choice of elements used in the elemental analysis of water and otoliths are key to establishing a signature for a watershed. There are a wide array of elements that are used in the analysis. However, several elements in the alkaline earth metal group are most useful because they are chemically homologous to calcium (Ca) in their bonding affinity and can substitute for Ca in the aragonite crystalline lattice (Campana 1999). Manganese (Mn), in addition to the three alkaline earth metal elements, strontium (Sr), magnesium (Mg), and barium (Ba), are commonly used to discriminate estuarine habitats (Thorrold et al. 1998b; Gillanders and Kingsford 2000; Mohan et al. 2012). Other elements such as lead (Pb), copper (Cu), lithium (Li), and cesium (Cs) as well as stable isotopes of carbon (C), oxygen (O) and Sr are also useful in identifying a chemical signature of water (Morris et al. 2003; Walther and Thorrold 2006; Halden and Friedrich 2008). Morris et al. (2003) classified 79% of striped bass caught in the Neuse River as Neuse River fish by using a discriminant analysis of concentrations of Mn, iron (Fe), bromine (Br), zinc (Zn), Cu, and Sr. Concentrations of trace elements in water must be compared as element:Ca ratios to account for the role of Ca in otolith deposition (Campana 1999). In contrast, absolute values can be recorded for otolith concentrations if Ca is used as an internal standard to monitor laser ablation yield. This is possible because the entire otolith is comprised of the mineral aragonite, so the Ca concentrations are assumed to remain constant (Halden and Friedrich 2008).

The North Carolina estuarine striped bass FMP is currently undergoing revisions. In order for the new FMP to be successful the origin of Central Southern Management Area striped bass must be determined. If the majority of striped bass collected from the Neuse and Tar rivers originate from CSMA watersheds, this would show that the reproductive adults within the CSMA are spawning successfully and their offspring are recruiting to the fishery. This would have a substantial impact on future management plans. The need for future stocking should be reevaluated. If the majority of fish within the CSMA are stocked or immigrants from the A/R stock, then the stocking programs currently in place will need to be reevaluated. The success of the FMP is essential for protecting the economically important striped bass fishery in North Carolina.

Goal and Objectives

The goal of this study was to determine the origin of striped bass within the CSMA. To accomplish this, the study was broken into three sections.

1. Examine the water chemistry and water quality of the three main CSMA rivers. The objectives for section one were: 1) to collect water samples and environmental data from 15 sample sites from the three major river systems and their tributaries within the CSMA; 2) to use chemical analysis of the water samples to establish unique elemental fingerprint for each watershed; and 3) to assess temporal variability of watershed elemental fingerprint.

2. Examine the elemental analysis from the core to the first annulus of CSMA striped bass otoliths. The objectives of section two were: 1) to collect striped bass from the Neuse and

Tar rivers each calendar season for one year; 2) to identify elemental fingerprints of natal origin by analyzing the area of the otolith representing the first year of life; 3) to compare proportions of different natal origins to determine stock structure; and 4) to assess temporal variability of elemental fingerprints by comparing fish collected during this study to fish collected by North Carolina Wildlife Resources Commission in previous years.

3. Examine condition and growth of adult CSMA striped bass. The objectives for section three were: 1) to calculate various condition factors including gonadal somatic index (GSI), Fulton's condition factor (K-factor), relative weight (Wr), and liver somatic index (LSI) for each fish collected; 2) to compare condition factors of striped bass collected from the CSMA to striped bass collected elsewhere in North Carolina; and 3) to compare age length frequencies of fish collected in the CSMA to striped bass collected elsewhere in North Carolina.

References

Barwick, R. D., J. M. Homan, and C. D. Thomas. 2009. Investigating striped bass recruitment in the Neuse River, North Carolina. Proceedings of the Annual Conference / Southeastern Association of Fish and Wildlife Agencies 63:161-165.

Bath, G. E., S. R. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. Geochimica Et Cosmochimica Acta 64(10):1705-1714.

Beasley, C., and J. E. Hightower. 2000. Effects of a low-head dam on the distribution and characteristics of spawning habitat used by striped bass and American shad. Transactions of the American Fisheries Society 129(6):1316-1330.

Begg, G. A., K. D. Friedland, and J. B. Pearce. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. Fisheries Research 43(1):1-8.

Boyd, J. B. 2011. Maturation, fecundity, and spawning frequency of the Albemarle/Roanoke striped bass stock. Master's Thesis. East Carolina University, Greenville, N.C.

Brown, M.,L., and B. Murphy R. 1991. Standard Weights (Ws) for striped bass, white bass, and hybrid striped bass. North American Journal of Fisheries Management 11:451-467.

Burdick, S., and J. E. Hightower. 2006. Distribution of spawning activity by anadromous fishes in an Atlantic slope drainage after removal of a low-head dam. Transactions of the American Fisheries Society 135(5):1290-1300.

Burgess, C. C., and A. J. Bianchi. 2004. An economic profile analysis of the commercial fishing industry of North Carolina including profiles for state-managed species. North Carolina Department of Environment and Natural Resources, North Carolina Division of Marine Fisheries License and Statistics Section, Report NA16FW1543, Morehead City, NC.

Campana, S. E., and A. J. Fowler. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 51(9):1942-1950.

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

Cone, R. 1989. The need to reconsider the use of condition indexes in fishery science. Transactions of the American Fisheries Society 118(5):510-514.

De Stefano, C., C. Foti, A. Gianguzza, and S. Sammartano. 1998. The single salt approximation for the major components of seawater: association and acid-base properties. Chemical Speciation and Bioavailability 10(1):27-30.

Dorval, E., C. M. Jones, R. Hannigan, and J. v. Montfrans. 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Canadian Journal of Fisheries and Aquatic Sciences 64(3):411-411.

Elsdon, T. S., B. K. Wells, S. E. Campana, B. M. Gillanders, C. M. Jones, K. E. Limburg, D. H. Secor, S. R. Thorrold, and B. D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology: An Annual Review 46:297-330.

Elsdon, T. S., and B. M. Gillanders. 2006. Temporal variability in strontium, calcium, barium, and manganese in estuaries: Implications for reconstructing environmental histories of fish from chemicals in calcified structures. Estuarine, Coastal and Shelf Science 66(1):147-156.

Gentry, D. E. 2006. Evaluation of striped bass, *Morone saxatilis*, condition in coastal North Carolina using physiological and condition indices. Master's Thesis. East Carolina University, Greenville, NC.

Giese, G. L., H. B. Wilder, G. G. Parker, and North Carolina. Dept. of Natural Resources and Community Development. 1979. Hydrology of major estuaries and sounds of North Carolina. U.S. Geological Survey, Water Resources Division, Report 79-46., Raleigh, N.C.

Gillanders, B. M., and M. J. Kingsford. 2000. Elemental fingerprints of otoliths of fish may distinguish estuarine 'nursery' habitats. Marine Ecology-Progress Series 201:273-286.

Gillanders, B. 2001. Trace metals in four structures of fish and their use for estimates of stock structure. Fisheries Bulletin 99:410-419.

Guay, C. K., and K. Kenison Falkner. 1998. A survey of dissolved barium in the estuaries of major Arctic rivers and adjacent seas. Continental Shelf Research 18(8):859-882.

Haeseker, S. L., J.T. Carmichael, and J.E. Hightower. 1996. Summer distribution and condition of striped bass within Albemarle Sound, North Carolina. Transactions of the American Fisheries Society 125(5):690-704.

Halden, N. M., and L. A. Friedrich. 2008. Trace-element distributions in fish otoliths: natural markers of life histories, environmental conditions and exposure to tailings effluence. Mineralogical Magazine 72(2):593-593.

Hassler, W. W., N. L. Hill, J. T. Brown. 1981. The status and abundance of striped bass, *Morone saxatilis*, in the Roanoke River and Albemarle Sound, North Carolina, 1956-1980. N.C. Dept. of Natural Resources and Community Development, Division of Marine Fisheries, Raleigh.

Heinimaa, S. 2004. Seasonal changes of liver glycogen content and condition factor of wild Atlantic salmon parr in a sub‑arctic river. Ecology of Freshwater Fish 13(4):323-326.

Klein-MacPhee, G. 2002. Striped bass/*Morone saxatilis*(Walbaum 1792). Pages 377-389 *in* B. B. Collette, and G. Klein-MacPhee, editors. Bigelow and Schroeder's Fishes of the Gulf of Maine. Smithsonian Institution Press, Washington, DC.

Kraus, R. T., and D. H. Secor. 2004. Incorporation of strontium into otoliths of an estuarine fish. Journal of Experimental Marine Biology and Ecology 302(1):85-106.

Kutkuhn, J. H. 1981. Stock definition as a necessary basis for cooperative management of Great Lakes fish resources. Canadian Journal of Fisheries and Aquatic Sciences 38(12):1476-1478.

Limburg, K. E., and D. I. Seigel. 2006. The hydrogeochemistry of connected waterways: The potential of linking geology to fish migrations. Northeastern Geology and Environmental Sciences 28(3):254-265.

Lin, J., L. J. Pietrafesa, J. S. Ramus, and H. W. Paerl. 2007a. Water quality gradients across Albemarle-Pamlico Estuarine System: seasonal variations and model applications. Journal of Coastal Research 23(1):213-229.

Lin, S., C. Chang, Y. Iizuka, and W. Tzeng. 2007b. Salinities, not diets, affect strontium/calcium ratios in otoliths of *Anguilla japonica*. Journal of Experimental Marine Biology and Ecology 341(2):254-263.

Mallin, M. A. 1991. Zooplankton abundance and community structure in a mesohaline North Carolina estuary. Estuaries 14(4):481-488.

Mallin, M. A., L. B. Cahoon, M. R. McIver, D. C. Parsons, and G. C. Shank. 1999. Alternation of factors limiting phytoplankton production in the Cape Fear River estuary. Estuaries 22(4):825-836.

Mallin, M. A., and H. W. Paerl. 1992. Effects of variable irradiance on phytoplankton productivity in shallow estuaries. Limnology and Oceanography 37(1):54-62.

McIlwain, T. D. 1980. Striped bass in coastal waters, south Atlantic and Gulf. Pages 37-43 *in* H. Clepper, editor. Marine recreational fisheries, Volume 5. Sport Fishing Institute, Washington, D.C.

Merriman, D. 1941. Studies on the striped bass (*Roccus saxatilis*) of the Atlantic coast. Fisheries Bulletin 50(35):1-77.

Mohan, J. A., R. A. Rulifson, D. R. Corbett, and N. M. Halden. 2012. Validation of oligohaline elemental otolith signatures of striped bass by use of in situ caging experiments and water

chemistry. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 4(1):57-70.

Mohan, J. 2009. Habitat utilization of juvenile striped bass Morone saxatilis in Albemarle Sound inferred from otolith and water chemistries. Master's Thesis. East Carolina University, Greenville, NC.

Morris, A. W. 1966. Studies of major and trace components of sea-water. Doctoral Dissertation. The University of Liverpool, Liverpool, United Kingdom.

Morris, J. A., R. A. Rulifson, and L. H. Toburen. 2003. Life history strategies of striped bass, Morone saxatilis, populations inferred from otolith microchemistry. Fisheries Research 62(1):53-63.

NCDMF. 2011. North Carolina license and statistics section summary statistics of license and permit program, commercial trip ticket program, marine recreational fishery statistics survey, and striped bass creel survey in the Central and Southern Management Area. North Carolina Division Of Marine Fisheries and North Carolina Wildlife Resources Commission, Report 390, Raleigh, North Carolina.

NCDMF. 2004. North Carolina estuarine striped bass fishery management plan. North Carolina Division of Marine Fisheries and North Carolina Wildlife Resources Commission, Report 375, Morehead City, North Carolina.

North Carolina Division of Water Resources and Engineering. 1952. Hydrologic data on the Roanoke and Tar River basins, 1871-1945. United States Geological Survey, Report 931439, Raleigh, North Carolina.

North Carolina Division of Water Resources and Engineering. 1947. Hydrologic data on the Neuse river basin, 1866-1945. United States Geological Survey, Report 835550, Raleigh, North Carolina.

Odum, H. T. 1951. The stability of the world strontium cycle. Science 114(2964):407-411.

Olsen, E.J., and R. A. Rulifson. 1992. Maturation and fecundity of Roanoke River-Albemarle Sound striped bass. Transactions of the American Fisheries Society 121(4):524-537.

Paramore, L.M., and R.A. Rulifson. 2001. Dorsal coloration as an indicator of different life history patterns for striped bass within a single watershed of Atlantic Canada. Transactions of the American Fisheries Society 130(4):663-674.

Quinby-Hunt, M. S., and K. K. Turehian. 1983. Distribution of elements in sea water. Eos, Transactions American Geophysical Union 64(14):130.

Rätz, H., and J. Lloret. 2003. Variation in fish condition between Atlantic cod (*Gadus morhua*) stocks, the effect on their productivity and management implications. Fisheries Research 60(2):369-380.

Robins, C. R., and C. Ray. 1986. A Field Guide to Atlantic Coast Fishes : North America. Houghton Mifflin, Boston.

Rulifson, R. A., and C. S. Manooch III. 1993. Roanoke River Water Flow Committee report for 1991-1993. Albemarle-Pamlico Estuarine Study, Report no. 93-18., Raleigh, NC.

Secor, D. H., and J. M. Dean. 1992. Comparison of otolith-based back-calculation methods to determine individual growth histories of larval striped bass, *Morone saxatilis*. Canadian Journal of Fisheries and Aquatic Sciences 49(7):1439-1454.

Secor, D. H., A. Henderson-Arzapalo, and P. M. Piccoli. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? Journal of Experimental Marine Biology and Ecology 192(1):15-33.

Secor, D. 2008. Influence of skipped spawning and misspecified reproductive schedules on biological reference points in sustainable fisheries. Transactions of the American Fisheries Society 137(3):782-789.

Sundby, B., L. G. Anderson, P. O. J. Hall, Å Iverfeldt, M. M. R. van der Loeff, and S. F. G. Westerlund. 1986. The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface. Geochimica Et Cosmochimica Acta 50(6):1281-1288.

Thorrold, S. R., C. M. Jones, S. E. Campana, and J. W. McLaren. 1998a. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). Limnology and Oceanography 43(8):1826-1835.

Thorrold, S.R., C.M. Jones, P.K. Swart, and T.E. Targett. 1998b. Accurate classification of juvenile weakfish, *Cynoscion regalis*, to estuarine nursery areas based on chemical signatures in otoliths. Marine Ecology Progress Series 173:253-265.

Todd, M. J., G. Vellidis, R. R. Lowrance, and C. M. Pringle. 2009. High sediment oxygen demand within an instream swamp in southern Georgia: implications for low dissolved oxygen levels in coastal blackwater streams. Journal of the American Water Resources Association 45(6):1493-1507.

Tomás, J., S. Augagneur, and E. Rochard. 2005. Discrimination of the natal origin of young‑of‑the‑year Allis shad (*Alosa alosa*) in the Garonne–Dordogne basin (south‑west France) using otolith chemistry. Ecology of Freshwater Fish 14(2):185-190.

U.S. Fish and Wildlife. 1978. Development of fishes of the Mid-Atlantic Bight : an atlas of egg, larval, and juvenile stages. Department of the Interior, United States Fish and Wildlife Service, Office of Biological Services, Washington, District of Columbia.

Vasconcelos, R. P., P. Reis-Santos, V. Fonseca, M. Ruano, S. Tanner, M. J. Costa, and H. N. Cabral. 2009. Juvenile fish condition in estuarine nurseries along the Portuguese coast. Estuarine, Coastal and Shelf Science 82(1):128-138.

Volk, E., A. Blakley, S. L. Schroder, and S. M. Kuehner. 2000. Otolith chemistry reflects migratory characteristics of Pacific salmonids: Using otolith core chemistry to distinguish maternal associations with sea and freshwaters. Fisheries Research 46(1-3):251-266.

Walther, B.D., and S.R. Thorrold. 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. Marine Ecology-Progress Series 311:125-130.

Warner, R. R., S. E. Swearer, J. E. Caselle, M. Sheehy, and G. Paradis. 2005. Natal trace-elemental signatures in the otoliths of an open-coast fish. Limnology and Oceanography 50(5):1529-1542.

Wells, B.K., B.E. Rieman, J.L. Clayton, D.L. Horan, and C.M. Jones. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 132(3):409-424.

Wirgin, I., L. Maceda, J.R. Waldman, and R.N. Crittenden. 1993. Use of mitochondrial-DNA polymorphisms to estimate the relative contributions of the Hudson River and Chesapeake Bay striped bass stocks to the mixed fishery on the Atlantic coast. Transactions of the American Fisheries Society 122(5):669-684.

Woodroffe, J. R. 2012. Historical ecology of striped bass stocking in the southeastern United States. Master's Thesis. East Carolina University, Greenville, N.C.

Woods, T. L. 2000. Geochemical tracers of groundwater movement between the Castle Hayne and associated coastal plain aquifers. Water Resources Research Institute of The University of North Carolina, Report 70148, Greenville, NC.

Woods, T. L., P. D. Fullagar, R. K. Spruill, and L. C. Sutton. 2000. Strontium isotopes and major elements as tracers of ground water evolution: example from the Upper Castle Hayne Aquifer of North Carolina. Ground Water 38(5):762-771.

Zapf, D. H. 2012. River herring nursery habitat in Albemarle Sound, North Carolina, inferred from otolith microchemistry. Master's Thesis. East Carolina University, Greenville, NC.

Chapter 2: Surface Water Chemistry of Central Southern Management Area Tributaries

Abstract

An elemental analysis of Central Southern Management Area (CSMA) watersheds in coastal North Carolina was conducted to assess the feasibility of using otolith microchemical analysis to determine the natal origin of CSMA striped bass (*Morone saxatilis).* Surface water samples and environmental data were collected once per month from 15 sample sites throughout the CSMA from May 2011 to July 2012. Two additional sample ponds from Edenton National Fish Hatchery were sampled once per week for two weeks in April 2012. Samples were analyzed for concentrations of calcium (Ca), strontium (Sr), magnesium (Mg), barium (Ba), and manganese (Mn) using an inductively-coupled plasma optical emission spectrometer. Concentrations were recorded as element to Ca ratios to account for the role of Ca in otolith deposition. Salinity differed significantly by location, while temperature and dissolved oxygen differed significantly by month and season. Only temperature differed significantly by year. All measured elements were consistently detected at every sample site. All measured elements differed significantly by location, but only Mn varied by month, season, and year. A multivariate classification of samples to their river of origin yielded only 42% success. When the classification was narrowed to include only one low salinity sample site per river, samples were classified to their sample site of origin with 82% accuracy. The variability of elemental concentrations between sites, and their stability over time, makes the use of otolith microchemistry to determine natal origin appropriate, even between different year classes of fish.

Introduction

The spatial variability of water chemistry is driven by a complex array of factors. Geological variation between watersheds, salinity, and temperature are three of the most important factors in water chemistry variation in estuarine river systems (Limburg and Siegel 2006). The spatial variability of water chemistry in these systems makes them well suited for otolith elemental analysis.

Water chemistry provides the basis for the interpretation of otolith microchemical analysis for natal origin determination (Thorrold et al. 1998a; Warner et al. 2005). The spatial heterogeneity of water chemistry creates unique chemical signatures through variation in the concentrations of elements, isotopes, and other chemical compounds. These unique signatures are used to characterize water from different geographical areas. The concentrations of some chemical components of water are transferred to fish otoliths in similar proportions as the water in which the fish inhabits (Campana 1999). The elements strontium (Sr), barium (Ba), and manganese (Mn) are appropriate for otolith chemical analysis because they are taken up in similar proportion to the water (Thorrold et al. 1998a; Campana 1999; Dorval et al. 2007; Mohan et al. 2012). Mg is also a common element used in otolith elemental analysis, although the relationship between Mg:Ca in the otolith and water is poorly understood (Campana 1999; Dorval et al. 2007; Mohan et al. 2012).

This study focuses on the natal origin of Central Southern Management Area (CSMA) striped bass. Otolith microchemistry is an effective tool in discriminating natal origin in fish (Tomás et al. 2005; Dorval et al. 2007). In order to establish elemental signatures for different watersheds of origin within the otolith, variation in water chemistry between suspected watersheds of origin must first be established. This study examined variation in Sr:Ca, Mg:Ca,

16

Mn:Ca, and Ba:Ca ratios between the Tar/Pamlico, Neuse, Northeast Cape Fear, Black, Pungo, Trent, and Cape Fear Rivers as well as the rearing ponds at the Edenton National Fish Hatchery (ENFH). The ENFH is the origin of all striped bass stocked into the Tar and Neuse Rivers.

Methods

Site Description

The Tar/Pamlico, Neuse and Cape Fear rivers are the primary watersheds of the CSMA. The Tar River Basin is the smallest of three river systems that lie completely in North Carolina. It originates in the piedmont plateau and flows approximately 190 km to its junction with the Pamlico River (North Carolina Division of Water Resources and Engineering 1952). The Pamlico River is the name given to the 60 km long estuarine section of the Tar River from the confluence with Tranters Creek to where the river flows into Pamlico Sound. The Neuse River Basin is the second largest river system that lies completely in North Carolina. It originates in the piedmont plateau and is 290 km long, although, from the head of its longest tributary to its confluence with Pamlico Sound the Neuse stretches 480 river km (Rkm) in length (North Carolina Division of Water Resources and Engineering 1947a). The Cape Fear River is the largest river system that lies completely in North Carolina, and, like the Tar/Pamlico and Neuse rivers, it originates in the piedmont plateau and terminates in the coastal plains. The Cape Fear watershed is nearly 320 km in length and encompasses over 22,000 km^2 (North Carolina Division of Water Resources and Engineering 1947b).

The Tar/Pamlico, Neuse, and Cape Fear rivers all flow through the piedmont and coastal plains regions of North Carolina. The piedmont region geology is comprised of granites,

gneisses, schists, and slates of various Paleozoic ages, consolidated sedimentary rocks of the Triassic age, and covered in a layer of soil and weathered rock of variable thickness (North Carolina Division of Water Resources and Engineering 1947b). The Coastal plains region geology is considerably different than that of the piedmont region. The coastal plains region consist of bedrock similar to the piedmont, but is covered in a thick unconsolidated, and semi-consolidated sediment and rock layer that thickens from west to east. This layer of unconsolidated and semi-consolidated formations consists of sand, clay, marl, shale, and limestone, with occasional sandstone belts (North Carolina Division of Water Resources and Engineering 1947b).

Sample Areas and Water Collection

Water was sampled from 15 locations within the Tar/Pamlico, Neuse, Black, Northeast Cape Fear, Trent, Pungo, and Cape Fear rivers (Figure 1) as well as from the Phase I and Phase II rearing ponds at the ENFH. The Phase I ponds are filled with surface water from a small, nearby, freshwater creek. This water is used because of its high abundance of zooplankton, which is the food source of juvenile striped bass until they are transferred into the Phase II ponds (about 1 month). The Phase II ponds are filled with water from a deep, low salinity well (2-3 ppt) that draws from the upper Castle Hayne aquifer. Hatchery water samples were collected from sample ponds once per week for two weeks in April 2012. River sample sites were selected to cover a transect from completely fresh upstream areas of the rivers to mesohaline areas near the mouth when salinity gradients were present. Taking samples over such a long transect facilitated spatial variability of chemical signatures. Sample sites closest to the mouth of a river were labeled as site A. Each subsequent upstream site within the same river was labeled B, C, and D. Rivers with only one sample site had only site A; rivers with two sites had sites A and B, and so

on up to the four samples sites (A, B, C, D) utilized in the Neuse and Tar rivers. River water samples were collected from each sample site once per month from May 2011 until July 2012 when possible. Hurricane Irene and other heavy rain events prevented many sites from being sampled in August 2011. All samples at a single river were taken on the same day, and all rivers were sampled on consecutive days when possible. Weather did not permit this on several occasions. Sampling throughout the year enabled analysis of temporal variability on monthly, seasonal and yearly (spawning season only) scales.

Water sample collection and chemical characterization was done using the same methods and protocols used by Mohan et al. (2012) and Zapf (2012). Sub-surface water samples were collected from each site at a depth of 80 cm. Bottom sample sites were also collected, but due to missing data for many bottom samples, and hypoxic zones at the bottom of several sample sites, only surface samples were utilized for analysis in my study. Striped bass are known to actively avoid these bottom hypoxic zones, and larvae and young juveniles primarily reside in surface waters to feed on zooplankton (Coutant 1985). Furthermore surface water has been shown to be adequate for comparison in otolith natal origin studies (Mohan et al. 2012). A Masterflex[®] peristaltic pump collected and filtered water inline with Whatman glass microfibre filters (GF/D; 1.5 um and GF/F; 0.7 um) into new 125-ml high-density fluorinated Nalgene bottles that were rinsed with three sample volumes before collection. Water samples were placed on ice for transport to the laboratory, where they were acidified to pH < 2 with trace metal grade nitric acid. Each sample was then filtered using a 0.2-μm syringe filter (Supor) to remove particulate fractions, but retain colloidial and dissolved fractions. Ambient water quality parameters of temperature (˚C), salinity (ppt), and dissolved oxygen (mg/L) were recorded with each sample. Samples were stored at 4 ˚C until subjected to elemental analysis.

Water Elemental Analysis

　　Water sample analysis used a method very similar to that described by Mohan et al. (2012). Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES model: Perkins/Elmer - Optima 2100 DV) was used to measure elemental concentrations of Ca (ppm), Mg (ppm), Sr (ppb), Ba (ppb), and Mn (ppb) using external standards and calibration methods described hereafter. A stock standard solution (1000 ppm in 2% NHO3) for each element was diluted to create an element specific calibration curve with five standards (lowest low, low, medium, high, highest high). The combined stock solution was analyzed prior to samples to achieve a calibration curve with an r^2 of at least 0.999, and quality control checks requiring >90% and <110% recovery were issued every nine samples. Samples were diluted with 18.5 Ω ultrapure water by one of two factors depending on the salinity of the sample. This prevented malfunctions of the ICP-OES associated with high dissolved solids content (>1 ppt) in the samples. For salinities 0<10 ppt a 10x dilution was used. For salinities ≥10 ppt a 20x dilution was used (no sample had a salinity exceeding 22.5 ppt). All elemental water chemistry was reported as element to Ca ratios to account for the role of Ca in otolith deposition.

Statistical Analysis

　　Normality of all variables was visually assessed using Q-Q plots. Significant differences in water temperature, dissolved oxygen, salinity, and ratios of Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca between sampling locations, months, seasons, and years were determined using Kruskal-Wallis and Wilcoxon tests because data was found to be non-parametric. The effects of water

temperature, dissolved oxygen, and salinity on elemental ratios were examined by plotting the elemental ratios against the aforementioned environmental parameters. Water samples were classified to their river and site by their Sr:Ca, Ba:Ca, Mn:Ca, and Mg:Ca ratios using linear (ldfa) and quadratic discriminate function analyses (qdfa).

Results

Environmental Variables

Dissolved oxygen and salinity differed significantly between locations (Table 1). Temperature and dissolved oxygen differed significantly between months and seasons, while only temperature differed significantly between years (Table 1). Mean dissolved oxygen was highest in the Pungo River (mean = 8.37 mg/L, SD = 1.75), and lowest in the Black River (mean = 4.57 mg/L, SD = 2.01). Mean salinity was highest in the Pungo River (mean = 13.42 ppt, SD = 2.85), and lowest in the Black River (mean = 0.10 ppt, SD = 0.00) and Northeast Cape Fear River (mean = 0.17 ppt, SD = 0.18). Mean temperature was highest in the summer during the months of July and August, and lowest in the winter during the months of January and February (Figure 2). Mean dissolved oxygen was highest in the winter during the months of January and February, and lowest in the summer during the months of August and September (Figure 3). Mean temperature was highest in 2011 (mean = 29.39° C, SD = 1.93), and lowest in 2012 (mean = 26.53° C, SD = 4.01).

Elemental Variables

Mg, Sr, and Ba were detected consistently in all rivers and sample sites, but several

individual samples did not contain Ba. Mn was detected consistently in all rivers and sample sites except for the ENFH low salinity well pond. Mg, Sr, Ba, and Mn differed significantly between rivers (Table 1). Mn differed significantly between months, seasons, and years (Table 1). Mean Mg:Ca was highest in the Pungo, and lowest in the Northeast Cape Fear and Black (Figure 5). Mean Sr:Ca was highest in the ENFH low salinity well pond, and the lowest was in the Northeast Cape Fear (Figure 5). The highest mean Sr:Ca in a river occurred in the Pungo (Figure 5). Mean Ba:Ca was highest in the Black, and lowest in the Pungo (Figure 5). Mean Mn:Ca in sites where it was detected was highest in the Cape Fear, and lowest in the Pungo (Figure 5). Mean Mn:Ca was highest in summer during the month of September, and lowest in winter, although the lowest monthly mean Mn:Ca occurred in November (Figure 6). Mean Mn:Ca was highest in 2011 (mean = 0.0058, SD = 0.0172), and lowest in 2012 (mean = 0.0026, SD = 0.0053).

No element to Ca ratio showed any relationship to water temperature (Figure 7). Mg:Ca showed a weak positive relationship with dissolved oxygen (r=0.459), while Ba:Ca (r= -0.390) and Mn:Ca (r=-0.464) showed weakly negative relationships, and Sr:Ca exhibited no relationship (Figure 7). Mg:Ca was strongly correlated with salinity (r=0.833), while Sr:Ca exhibited a weaker (r=0.433) correlation (Figure 7). Ba:Ca showed a strong negative correlation to salinity (r=-0.584), while Mn:Ca exhibited a weaker negative (r=-331) correlation (Figure 7).

Mg:Ca, Sr:Ca, Ba:Ca, and Mn:Ca were all used in water sample classification because they were consistently detected in all sample sites (except Mn in Edenton well pond). Multivariate means differed significantly between rivers (Pillai's trace statistic: F=13.13, df=860, p<0.0001) for a linear discriminant function analysis combining sample sites in order to classify by river (Figure 8). Classification rates ranged from 100.0% (Pungo) to 8.3% (Neuse) (Table 2).

Total classification rate was 42.0%. Most misclassifications occurred because of variability in salinity within rivers. Rivers with less variability in salinity had the best classification rates (Table 2). Because of low classification success in the whole river discriminant analysis, a quadratic discriminant function analysis of only sample sites where juvenile striped bass are likely to be found was conducted. Sample sites were chosen using the criteria of mid-river, slow moving, and low salinity because this has been shown to be common late larvae to early juvenile striped bass habitat (Hassler et al. 1981). Only one sample site per river was used to reduce misclassification. Multivariate means differed significantly between sample sites (Pillai's trace statistic: $F=9.86$, $df=336$, $p<0.0001$) (Figure 9). Classification rates ranged from 100.0% (Neuse C, and Black A) to 56.3% (Trent A) (Table 3). Total classification rate was 82.22%. Most misclassifications occurred to sample sites in close geographic proximity.

Discussion

Environmental Variables

Dissolved Oxygen

Dissolved oxygen differed significantly by location, month, and season. Dissolved oxygen followed previously observed patterns; highest at well mixed, mesohaline sites (i.e., Neuse A) (Giese et al. 1979), and lowest in slow moving black-water sites (i.e., Black A) (Todd et al. 2009). Dissolved oxygen closely followed temperature, as was also observed by Mohan et al. (2012) and Zapf (2012) for Albemarle Sound tributaries. As water temperature decreased into winter, dissolved oxygen increased, and when temperature rose to its maximum during the summer, dissolved oxygen significantly declined. The only deviation to this pattern occurred

after Hurricane Irene in August and September 2011. The large rainfalls triggered hypoxic events throughout the CSMA. Dissolved oxygen was also linked to Mn concentrations. During hypoxic events Mn is released from sediments (Sundby et al. 1986). This phenomenon facilitated dissolved oxygen minimums to coincide with Mn maximums (Figure 6).

Salinity

Salinity differed significantly by location. Although salinity did not differ significantly on any measured time scale, mean salinity did make a noticeable decline in August and September 2011 following large rainfalls associated with Hurricane Irene (Figure 4). Salinity followed the same longitudinal gradient that was observed by Mohan et al. (2012) and Zapf (2012). The longitudinal gradient of salinity was facilitated by the output of freshwater from the rivers in the sample area (Lin et al. 2007a). Low salinity samples were collected at upstream, western locations of their respective rivers, while higher salinity samples were collected from downstream, eastern locations. The observed variability of salinities in the sample rivers was largely driven by the orientation and number of the sample sites in each river. The Tar/Pamlico and Neuse Rivers had five sample sites each encompassing transects of over 70 river km (Rkm), and average salinity ranges of over 17 ppt in each river. In contrast, the Trent, Black, and Northeast Cape Fear rivers had only one sample site, significantly reducing the variability in their salinity. The disparity in sample sites between rivers had significant consequences when analyzing rivers as a whole. Salinity is a leading factor in concentrations of Sr (Secor et al. 1995), Ba (Elsdon and Gillanders 2006), and Mg in estuarine waters (Thorrold et al. 1998a; Dorval et al. 2007). Samples from rivers with high variability in salinity (i.e., Neuse) tended to discriminate to rivers with low variability (i.e., Pungo) that matched the salinity of the sample.

This led to very low correct classification rates in the LDFA. In order to alleviate the confounding effect of salinity, samples sites of similar salinity were used for the QDFA.

Temperature

Temperature differed significantly on all temporal scales, but not by location. These results are consistent with Mohan et al. (2012) and Zapf (2012), who found that temperature of Albemarle Sound watersheds varied by month, but not by location. Water temperature followed expected yearly cycles for temperate regions of warming in the summer and cooling in the winter. Temperature showed no relationship with any of the measured element to Ca ratios. It did, however, correlate to dissolved oxygen with the relationship described previously.

Elemental Variables

Magnesium

Mg:Ca differed significantly by location, but not by any measured time scale. Unlike Mohan et al. (2012) and Zapf's (2012) research in the Albemarle Sound and its tributaries, Mg was detected consistently at all sample sites in this study. Mg is a major component of seawater (Morris 1966; De Stefano et al. 1998), and should thus be present in all water with some level of seawater mixed in (i.e., estuarine water with >0.1 ppt). The association of Mg with seawater is the driving force for the strong positive correlation between Mg:Ca and salinity observed in my study (Quinby-Hunt and Turehian 1983; Dorval et al. 2007). In contrast to Mohan et al. (2012) and Zapf (2012), Mg was also detected at all upstream, freshwater sites in my study. This may be indicative of different water chemistry patterns in CSMA watersheds as compared to Albemarle

Sound watersheds. Despite the value of Mg as a discriminator for water classification, its use in otolith microchemistry work is suspect at best (Campana 1999; Dorval et al. 2007). The relationship between Mg:Ca levels in water and otoliths has been shown to be unpredictable (Campana 1999; Dorval et al. 2007; Mohan et al. 2012).

Strontium

Sr:Ca differed significantly between locations, but not by any time scale measured in this study. The highest mean river Sr:Ca was found in the Pungo, and highest mean sample site Sr:Ca ratios were found in Neuse A and Pungo A samples. The lowest mean river Sr:Ca ratios were found in the Black and Northeast Cape Fear and lowest mean sample site Sr:Ca ratios were found in Black A, Northeast Cape Fear A, and Neuse D. Upstream, freshwater sites tended to have lower Sr:Ca, while downstream mesohaline sites tended to have higher Sr:Ca. This agrees well with previous research that has shown that Sr correlates to salinity in a relationship similar to Mg (Odum 1951; Mohan et al. 2012; Zapf 2012), with several important exceptions (Woods et al. 2000; Limburg and Seigel 2006; Zapf 2012).

Sr concentrations in freshwater can be higher than what might be expected based upon sample salinity (Limburg and Seigel 2006) because Sr can be incorporated into water through weathering of igneous rocks or limestones (Odum 1951). This is a particularly intriguing fact to this study. The Phase II pond water at the ENFH is sourced from deep low-salinity wells that draw from the upper Castle Hayne Aquifer. The Castle Hayne Aquifer is a well indurated limestone aquifer with Sr:Ca ranging from approximately 0.005 to 0.100 (Woods 2000). Seawater contains both Sr and Ca in a fairly consistent ratio of 0.0185 (Quinby-Hunt and

Turehian 1983). Water samples from the Phase II ponds at ENFH have a mean Sr:Ca of 0.0261 (SD = 0.0025). This is significantly higher than the ocean ratio, but well within the range observed by Woods (2000). This extremely high Sr:Ca ratio relative to other samples, paired with Sr:Ca tendency to be conserved in otolith deposition (Secor et al. 1995; Campana 1999) provides a unique and unintended tag in the otoliths of all fish reared in this water. In addition to the Castle Hayne aquifer fed ponds that had the unusually high Sr, salinity pattern, there were two anomalous Sr:Ca readings in Cape Fear B samples. The anomalous samples had Sr:Ca ratios of 0.040 and 0.046. These levels are more than double that of full strength seawater, but still fall within the range reported by Woods (2000) for the Upper Castle Hayne. This could be evidence of groundwater seeps as was hypothesized by Zapf (2012). These samples were taken during a period just prior to Hurricane Irene when eastern North Carolina was experiencing a drought. This could have allowed for groundwater drainage to make up a higher than normal proportion in the watershed as has been observed before in the Tar River (O'Driscoll et al. 2008).

The unique levels of Sr:Ca ratios at the hatchery ponds, and the variation between sample sites coupled with its conservative deposition pattern in otoliths (Secor et al. 1995; Campana 1999; Lin et al. 2007b) make it an ideal elemental signature for use in otolith microchemistry in the CSMA.

Barium

Like Mg:Ca and Sr:Ca, the Ba:Ca ratio differed significantly by location but not by any time scale measured. Ba followed the pattern described by Guay and Falkner (1998), who found that Ba had a strong inverse relationship with salinity in North American watersheds. However,

concentrations were highly variable in freshwater. The Ba:Ca ratio was highest in freshwater, upstream sample sites (i.e., Neuse D and Black A) and lowest in mesohaline, downstream sample sites (i.e., Neuse A and Cape Fear A). Although Ba:Ca tended to follow an inverse relationship with salinity, freshwater sample sites differed significantly. This is not surprising because the Ba:Ca ratio has been shown to differ between freshwater rivers (Wells et al. 2003; Limburg and Seigel 2006). Ba:Ca ratio variation among sample sites, even those with similarly low salinity, its stability over months, seasons, and years, and its conservative nature when deposited in otoliths make Ba:Ca an excellent elemental ratio for use in discriminating natal origin of fish through otolith microchemistry in the CSMA.

Manganese

Mn:Ca ratios differed significantly by location, month, season, and year. Mean Mn:Ca was highest in the Cape Fear and lowest in the Northeast Cape Fear. Increased Mn concentration in water has been linked to reducing conditions in sediment associated with anoxic conditions (Sundby et al. 1986). This agreed well with the findings of this study because high Mn:Ca ratios tended to occur at low salinity, upstream, black-water sites (Neuse D and Cape Fear B) where dissolved oxygen tends to be very low (Todd et al. 2009). The trend was further followed for sites with low Mn:Ca. Downstream mesohaline sites (i.e., Neuse A and Pungo A) had the lowest mean Mn:Ca ratio and tended to have higher dissolved oxygen because they are well mixed (Giese et al. 1979; Lin et al. 2007a). Mean Mn:Ca ratios were highest immediately following Hurricane Irene. The Hurricane caused massive rainfalls that triggered large-scale anoxic and hypoxic events throughout eastern North Carolina watersheds. This single event was responsible for the majority of variation in Mn:Ca between months, seasons, and years observed in this study

(Figure 6). Although Mn:Ca differed significantly at all time scales measured in this study, it still

has good potential for use in otolith microchemistry. Significant spikes in Mn:Ca could be

markers for large-scale anoxic or hypoxic events and lend a time scale to otolith microchemistry

data.

Discriminant Analysis

Unlike Mohan (2012) and Zapf (2012), all elements (Ca, Mg, Sr, Ba, Mn) examined were

detected consistently at all sample sites. This allowed for multivariate classifications to include

all elements. Despite including four element to Ca ratios, sample sites were only classified to

their river of collection 42% of the time. This was due to high variability in salinity within river

sample sites. Salinity is a major factor effecting the variability of Mg:Ca, Sr:Ca, and Ba:Ca

ratios. Samples from rivers with sample sites in a large range of salinities (i.e., Neuse and

Pamlico) were most commonly misclassified. This occurred because the samples were classified

to rivers with few sample sites (i.e., Northeast Cape Fear and Pungo), whose mean element to Ca

ratios best matched the sample. In order to account for this, a discriminant analysis was done

comparing all sample sites. The classification rates were nearly as low as the river of collection

classification because neighboring sample sites in rivers were very similar. Despite the low

classification rates in some rivers, the ENFH low salinity well pond samples were classified

correctly with 100% accuracy due to the water's uniquely high Sr:Ca ratio. To alleviate

misclassifications to adjacent sites, one sample site from each river known to have a spawning

population of striped bass was selected. Low salinity, mid river, slow moving sites were selected

for each river because this has been shown to be common nursery areas for late larvae and early

juvenile striped bass (Hassler et al. 1981). Despite similar salinities between sites, an 82.22%

classification rate was achieved from a quadratic discriminant function analysis. Most misclassifications still occurred to neighboring sites (i.e., Trent A to Neuse C). Sites tended to group by geographic location on the canonical correspondence plot; the northern sites Pamlico C, Neuse C and Trent A in one group, and the southern sites Cape Fear B Northeast Cape Fear A and Black A in the other.

Conclusions

Mg:Ca, Sr:Ca, Mn:Ca, and Ba:Ca ratios were detected consistently at all sites and differed significantly between rivers. Classification of sample sites to river of origin was only correct 42% of the time. When the confounding factors of salinity gradient and adjacent sample sites were corrected for by selecting one sample site from each river, samples were correctly classified to their site of origin with 82% accuracy. Mg:Ca, Sr:Ca, and Ba:Ca ratios did not differ by months, seasons, or years. Mn:Ca ratios differed significantly by all measured time scales, but these differences were primarily driven by the anoxic and hypoxic events associated with Hurricane Irene. Overall the elemental concentrations within the study rivers are temporally stable. This demonstrates that elemental analysis may be appropriate for classifying CSMA striped bass to their natal origin using otolith microchemistry. The stability of the elemental concentrations in the water between years makes it feasible to compare origin between different year classes of fish.

References

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

Coutant, C. C. 1985. Striped bass, temperature, and dissolved oxygen: a speculative hypothesis for environmental risk. Transactions of the American Fisheries Society 114:31-61.

De Stefano, C., C. Foti, A. Gianguzza, and S. Sammartano. 1998. The single salt approximation for the major components of seawater: association and acid-base properties. Chemical Speciation and Bioavailability 10(1):27-30.

Dorval, E., C. M. Jones, R. Hannigan, and J. v. Montfrans. 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Canadian Journal of Fisheries and Aquatic Sciences 64(3):411-411.

Elsdon, T. S., and B. M. Gillanders. 2006. Temporal variability in strontium, calcium, barium, and manganese in estuaries: Implications for reconstructing environmental histories of fish from chemicals in calcified structures. Estuarine, Coastal and Shelf Science 66(1):147-156.

Giese, G. L., H. B. Wilder, G. G. Parker, and North Carolina. Dept. of Natural Resources and Community Development. 1979. Hydrology of major estuaries and sounds of North Carolina. U.S. Geological Survey, Water Resources Division, Report 79-46., Raleigh, N.C.

Guay, C. K., and K. Kenison Falkner. 1998. A survey of dissolved barium in the estuaries of major Arctic rivers and adjacent seas. Continental Shelf Research 18(8):859-882.

Hassler, W. W., N. L. Hill, J. T. Brown. 1981. The status and abundance of striped bass, *Morone saxatilis*, in the Roanoke River and Albemarle Sound, North Carolina, 1956-1980. N.C. Dept. of Natural Resources and Community Development, Division of Marine Fisheries, Raleigh.

Limburg, K. E., and D. I. Seigel. 2006. The hydrogeochemistry of connected waterways: The potential of linking geology to fish migrations. Northeastern Geology and Environmental Sciences 28(3):254-265.

Lin, J., L. J. Pietrafesa, J. S. Ramus, and H. W. Paerl. 2007a. Water quality gradients across Albemarle-Pamlico Estuarine System: seasonal variations and model applications. Journal of Coastal Research 23(1):213-229.

Lin, S., C. Chang, Y. Iizuka, and W. Tzeng. 2007b. Salinities, not diets, affect strontium/calcium ratios in otoliths of Anguilla japonica. Journal of Experimental Marine Biology and Ecology 341(2):254-263.

Mohan, J. A., R. A. Rulifson, D. R. Corbett, and N. M. Halden. 2012. Validation of oligohaline elemental otolith signatures of striped bass by use of in situ caging experiments and water chemistry. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 4(1):57-70.

Morris, A. W. 1966. Studies of major and trace components of sea-water. Doctoral Dissertation. The University of Liverpool, Liverpool, United Kingdom.

North Carolina Division of Water Resources and Engineering. 1952. Hydrologic data on the Roanoke and Tar River basins, 1871-1945. United States Geological Survey, Report 931439, Raleigh, North Carolina.

North Carolina Division of Water Resources and Engineering. 1947a. Hydrologic data on the Neuse river basin, 1866-1945. United States Geological Survey, Report 835550, Raleigh, North Carolina.

North Carolina Division of Water Resources and Engineering. 1947b. Hydrologic data on the Cape Fear river basin, 1820-1945. United States Geological Survey, Report 835551, Raleigh, North Carolina.

O'Dricoll, M. A., D. J. Mallinson, and P. K. Johnson. 2008. Surface water/ground water interactions along the Tar River, NC. Water Resources Research Institute of The University of North Carolina, Report 70215, Greenville, NC.

Odum, H. T. 1951. The stability of the world strontium cycle. Science 114(2964):407-411.

Quinby-Hunt, M. S., and K. K. Turehian. 1983. Distribution of elements in sea water. Eos, Transactions American Geophysical Union 64(14):130.

Secor, D. H., A. Henderson-Arzapalo, and P. M. Piccoli. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? Journal of Experimental Marine Biology and Ecology 192(1):15-33.

Sundby, B., L. G. Anderson, P. O. J. Hall, Å Iverfeldt, M. M. R. van der Loeff, and S. F. G. Westerlund. 1986. The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface. Geochimica Et Cosmochimica Acta 50(6):1281-1288.

Thorrold, S. R., C. M. Jones, S. E. Campana, and J. W. McLaren. 1998. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). Limnology and Oceanography 43(8):1826-1835.

Todd, M. J., G. Vellidis, R. R. Lowrance, and C. M. Pringle. 2009. High sediment oxygen demand within an instream swamp in southern Georgia: implications for low dissolved oxygen levels in coastal blackwater streams. Journal of the American Water Resources Association 45(6):1493-1507.

Tomás, J., S. Augagneur, and E. Rochard. 2005. Discrimination of the natal origin of young‑of‑the‑year Allis shad (*Alosa alosa*) in the Garonne–Dordogne basin (south‑west France) using otolith chemistry. Ecology of Freshwater Fish 14(2):185-190.

Warner, R. R., S. E. Swearer, J. E. Caselle, M. Sheehy, and G. Paradis. 2005. Natal trace-elemental signatures in the otoliths of an open-coast fish. Limnology and Oceanography 50(5):1529-1542.

Wells, B.K., B.E. Rieman, J.L. Clayton, D.L. Horan, and C.M. Jones. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 132(3):409-424.

Woods, T. L. 2000. Geochemical tracers of groundwater movement between the Castle Hayne and associated coastal plain aquifers. Water Resources Research Institute of The University of North Carolina, Report 70148, Greenville, NC.

Woods, T. L., P. D. Fullagar, R. K. Spruill, and L. C. Sutton. 2000. Strontium isotopes and major elements as tracers of ground water evolution: example from the Upper Castle Hayne Aquifer of North Carolina. Ground Water 38(5):762-771.

Zapf, D. H. 2012. River herring nursery habitat in Albemarle Sound, North Carolina, inferred from otolith microchemistry. Master's Thesis. East Carolina University, Greenville, NC.

Table 1. – Results of Kruskal-Wallis and Wilcoxon tests examining the statistical differences in Temperature (˚C), Dissolved Oxygen (mg/L), Salinity (ppt), and element to Ca ratios between locations, months, seasons, and years from water samples collected from the Neuse, Tar/Pamlico, and Cape Fear rivers from May 2011 to July 2012. For each one-way analysis α was set at 0.05. Asterisks represent statistically significant p values.

Variable	Effect	chi-squared	df	p
Temperture	Location	4.27	14	0.9936
	Month	190.3621	11	<.0001*
	Season	171.8413	5	<.0001*
	Year	23.3388	1	<.0001*
Dissolved Oxygen	Location	56.3588	14	<.0001*
	Month	103.8767	11	<.0001*
	Season	92.0386	5	<.0001*
	Year	3.2201	1	0.0727
Salinity	Location	187.7404	14	<.0001*
	Month	12.5335	11	0.3249
	Season	2.3437	5	0.7998
	Year	1.7863	1	0.1814
Mg/Ca	Location	180.0709	14	<.0001*
	Month	6.8631	11	0.8101
	Season	0.78	5	0.9783
	Year	0.6167	1	0.4323
Mn/Ca	Location	124.6554	14	<.0001*
	Month	29.8923	11	0.0016*
	Season	22.1872	5	0.0005*
	Year	7.5266	1	0.0061*
Sr/Ca	Location	161.6814	14	<.0001*
	Month	4.8304	11	0.9391
	Season	1.5887	5	0.9026
	Year	0.4138	1	0.5201
Ba/Ca	Location	168.237	14	<.0001*
	Month	17.5713	11	0.0921
	Season	4.366	5	0.498
	Year	0.0587	1	0.8086

Table 2. – Results of linear discriminant function analysis used to classify all water samples to river of collection. Rivers were abbreviated as follows: Cape Fear River (Cape Fear), Edenton National Fish Hatchery Phase I pond (ENFH), Edenton National Fish Hatchery Phase II pond (ENFH SW), Northeast Cape Fear River (NECF), Neuse River (Neuse), Tar/Pamlico River (Pamlico), Pungo River (Pungo), and Trent River (Trent).

Location	N	Black River	Cape Fear	ENFH	ENFH SW	NECF	Neuse	Pamlico	Pungo	Trent	% Correct
Black River	14	11	0	2	0	1	0	0	0	0	78.6
Cape Fear	24	2	7	3	2	2	0	0	8	0	29.2
ENFH	4	0	1	3	0	0	0	0	0	0	75.0
ENFH SW	9	0	0	0	9	0	0	0	0	0	100.0
NECF	16	2	0	0	0	14	0	0	0	0	87.5
Neuse	60	7	1	2	0	4	5	2	38	1	8.3
Pamlico	51	6	1	3	0	1	6	6	27	1	11.8
Pungo	30	0	0	0	0	0	0	0	30	0	100.0
Trent	16	0	0	0	0	2	3	0	2	9	56.3

Table 3. – Results of quadratic discriminant function analysis used to classify selected mid-river water samples to sample site of collection. Samples sites used the same river abbreviations as Table 2 with site letter added.

Location	N	Black River A	Cape Fear B	NECF A	Neuse C	Pamlico C	Trent A	% Correct
Black River A	14	14	0	0	0	0	0	100.0
Cape Fear B	16	4	10	2	0	0	0	62.5
NECF A	16	0	1	15	0	0	0	93.8
Neuse C	15	0	0	0	15	0	0	100.0
Pamlico C	13	0	0	0	2	11	0	84.6
Trent A	16	1	1	0	5	0	9	56.3
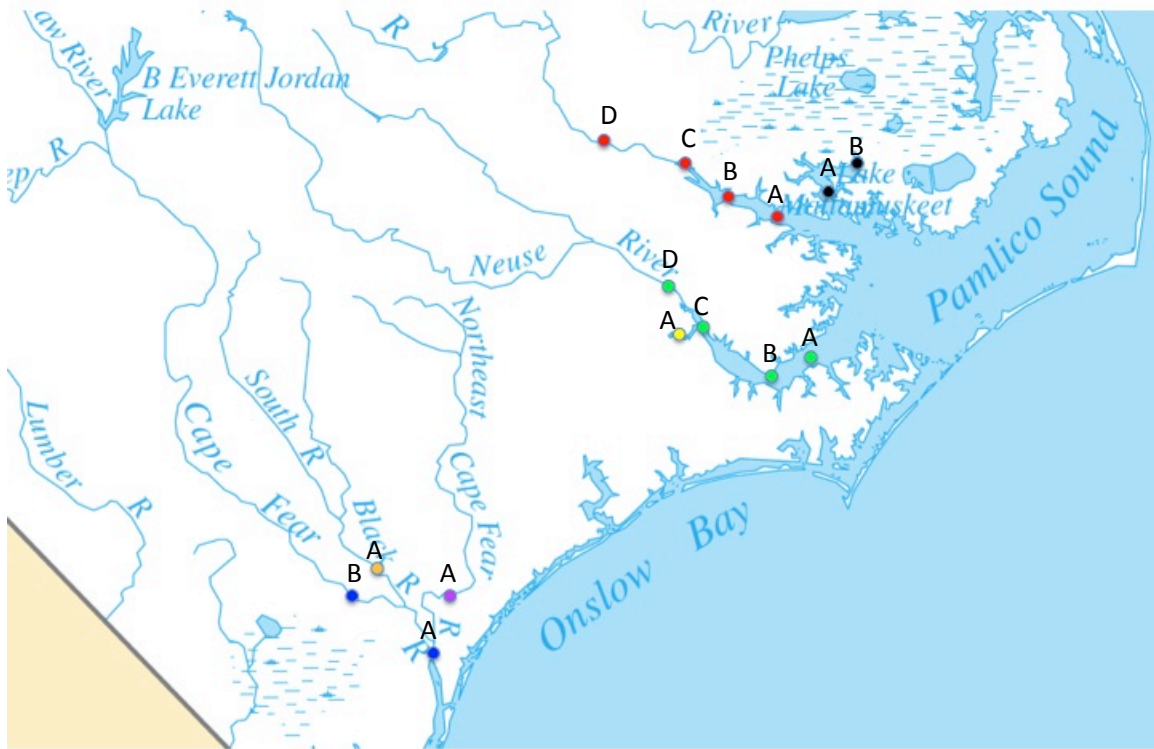
Figure 1. – Map of study area. Water sample sites marked with color coded circles: red; Tar/ Pamlico River, black; Pungo River, green; Neuse River, yellow; Trent River, blue; Cape Fear River, orange; Black River, and purple; Northeast Cape Fear River (Map from USGS).
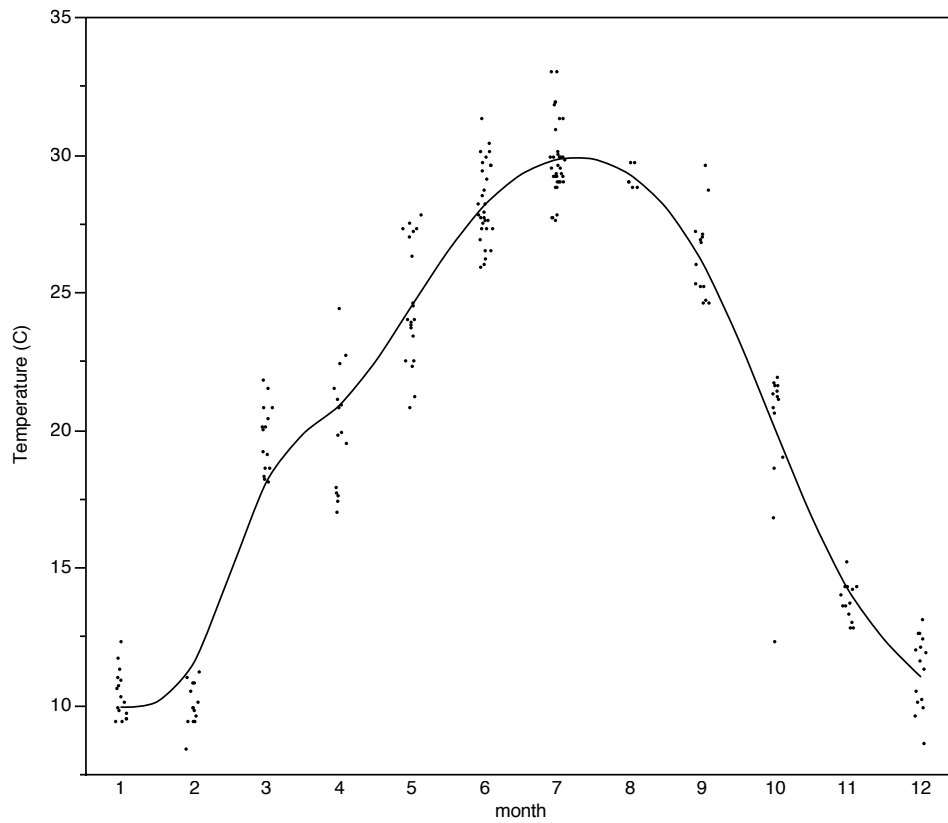
Figure 2. – Scatter plot with smoothed trend line showing variation in mean water temperature (˚C) over months from May 2011 to July 2012 (all stations combined).
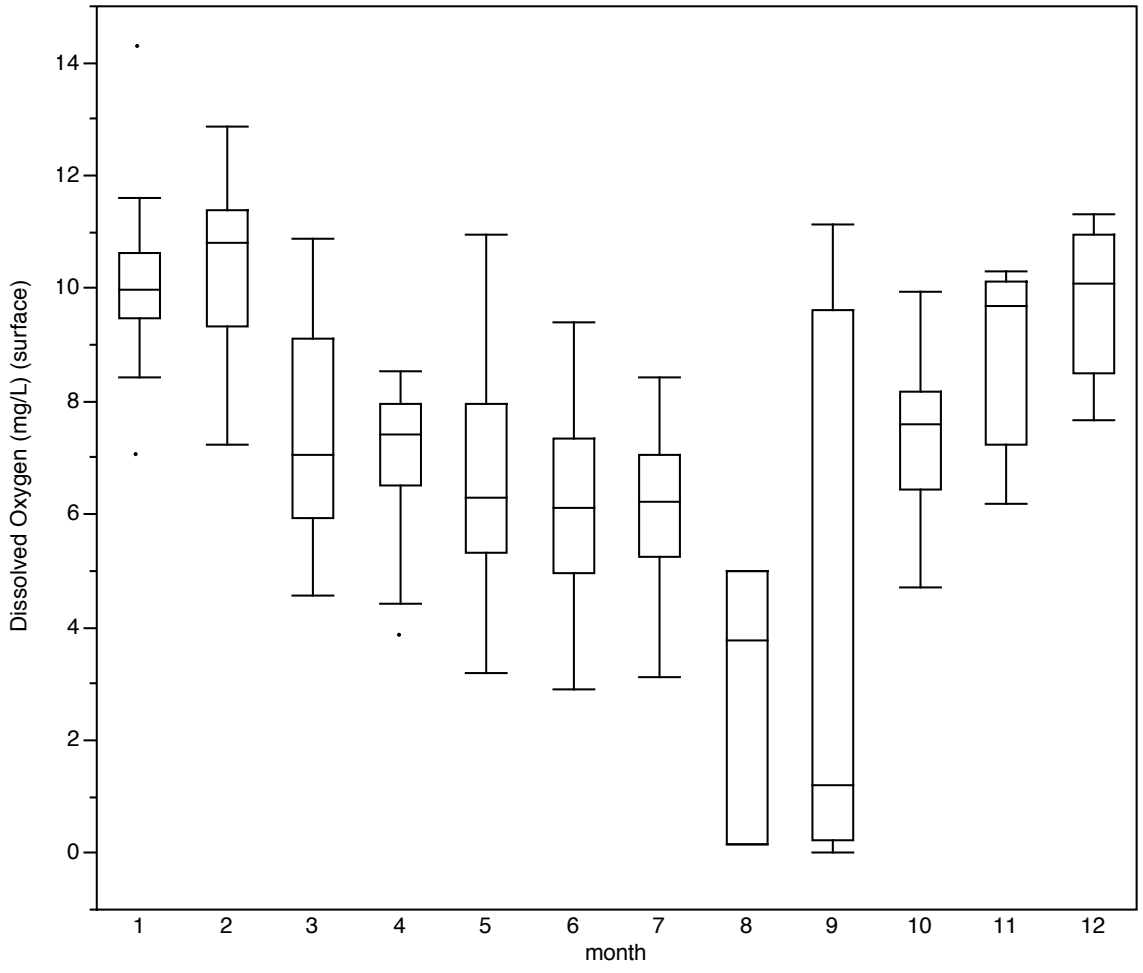
Figure 3. – Box and whisker plot showing dissolved oxygen concentrations in the Neuse, Tar/Pamlico, and Cape Fear rivers from May 2011 to July 2012 (all stations combined). Horizontal line within box = median; lower and upper edges of box = 25th and 75th percentiles; ends of whiskers = 10th and 90th percentiles; points = outliers.
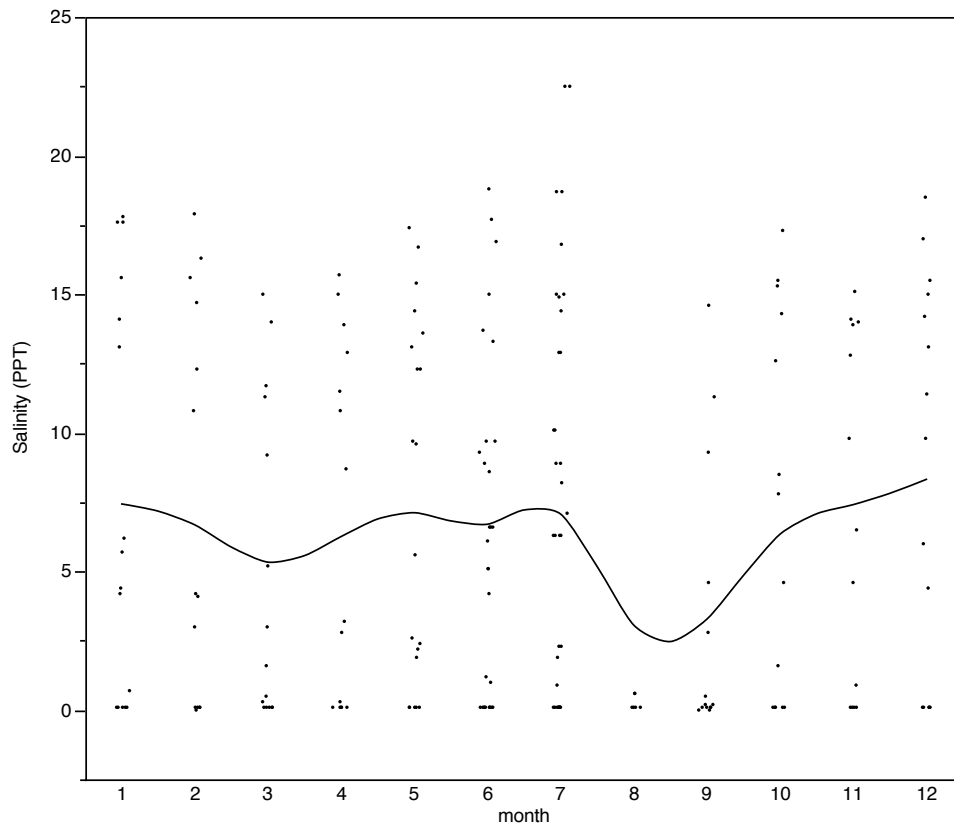
Figure 4. – Scatter plot with smoothed trend line showing variation in mean salinity (ppt) over months from May 2011 to July 2012 (all stations combined).
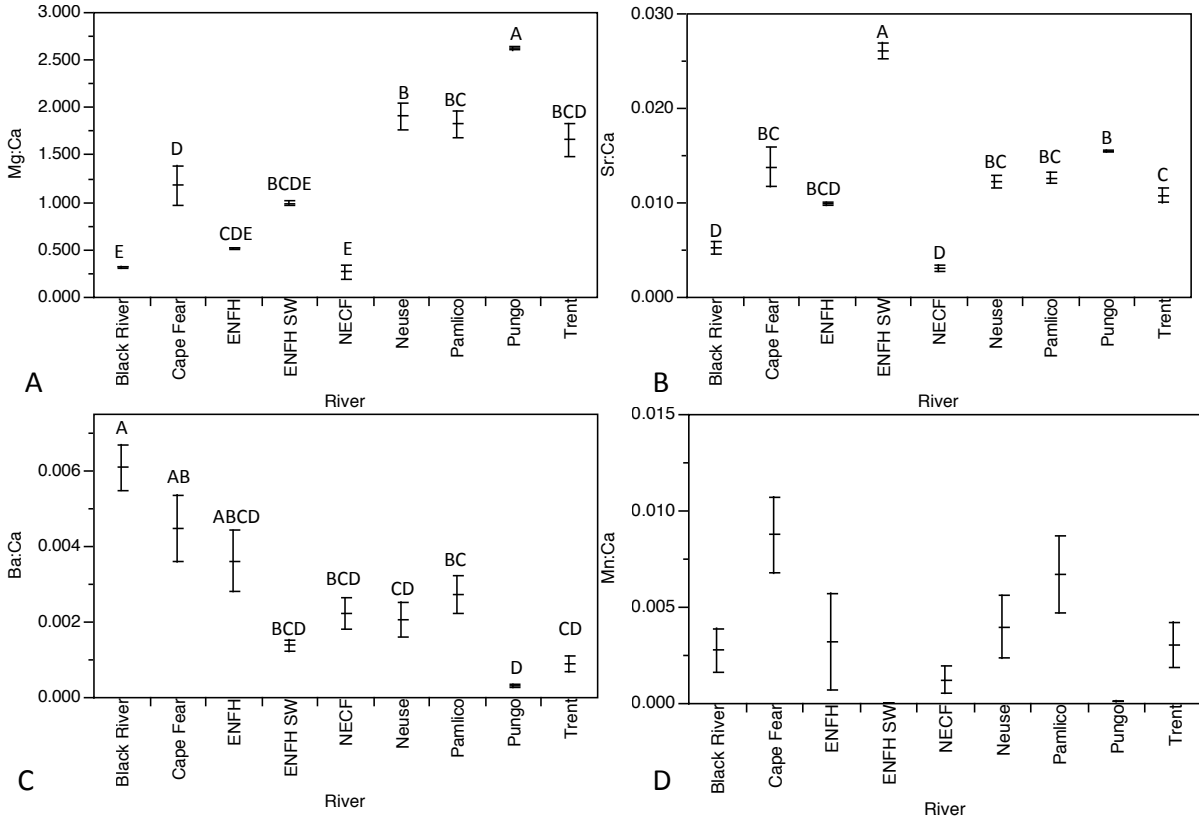
Figure 5. – Mean (± SE) Mg:Ca (A), Sr:Ca (B), Ba:Ca (C). and Mn:Ca (D) of the 7 rivers and 2 hatchery ponds sampled in this study from May 2011 to July 2012. In graphs A, B, and C differing letters (above bars) indicate significant differences between rivers (Tukeys HSD tests after Kruskal–Wallis test).
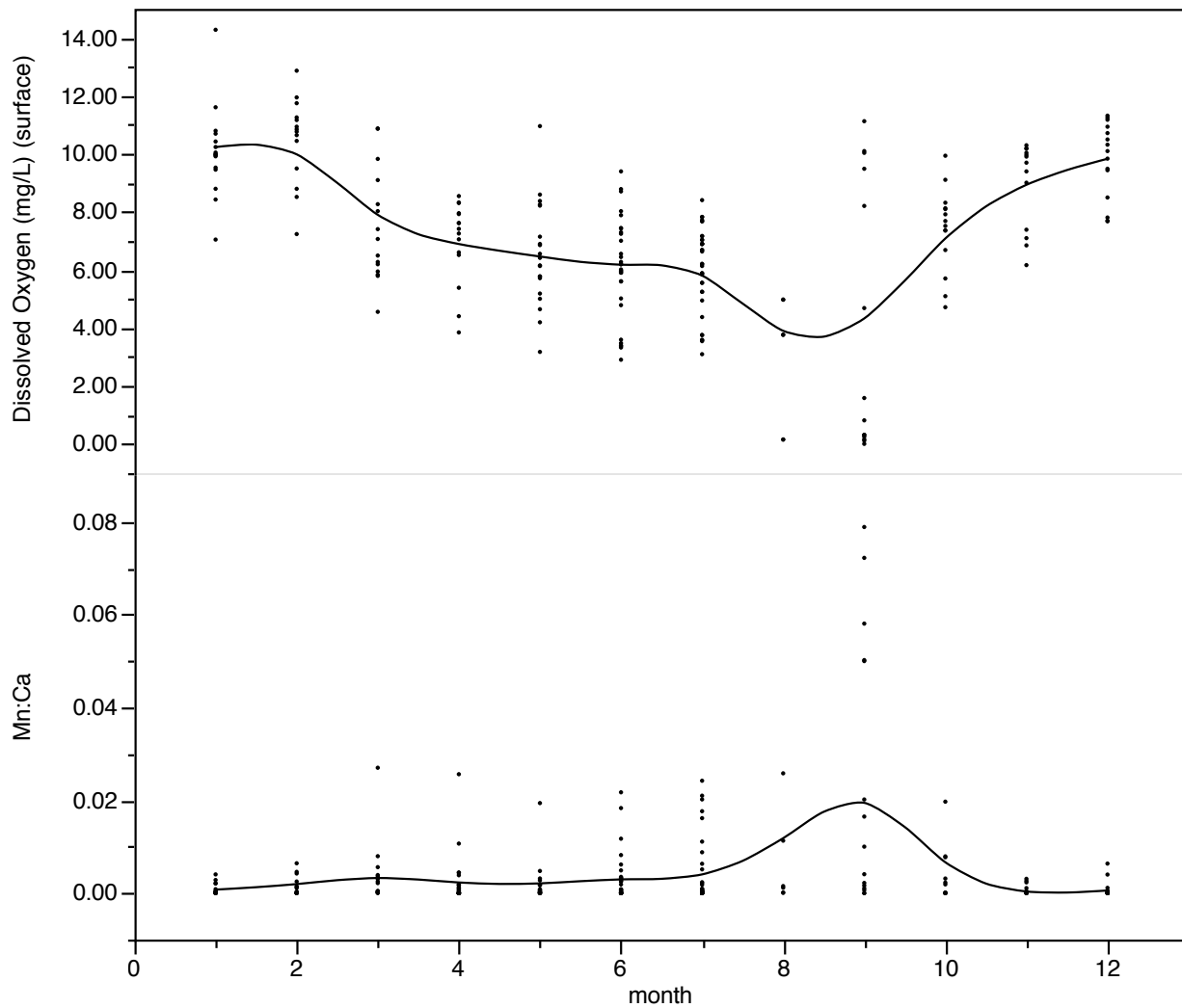
Figure 6. – Scatter plot with smoothed trend line showing variation in mean Mn:Ca and dissolved oxygen (mg/L) over months from May 2011 to July 2012 (all stations combined).
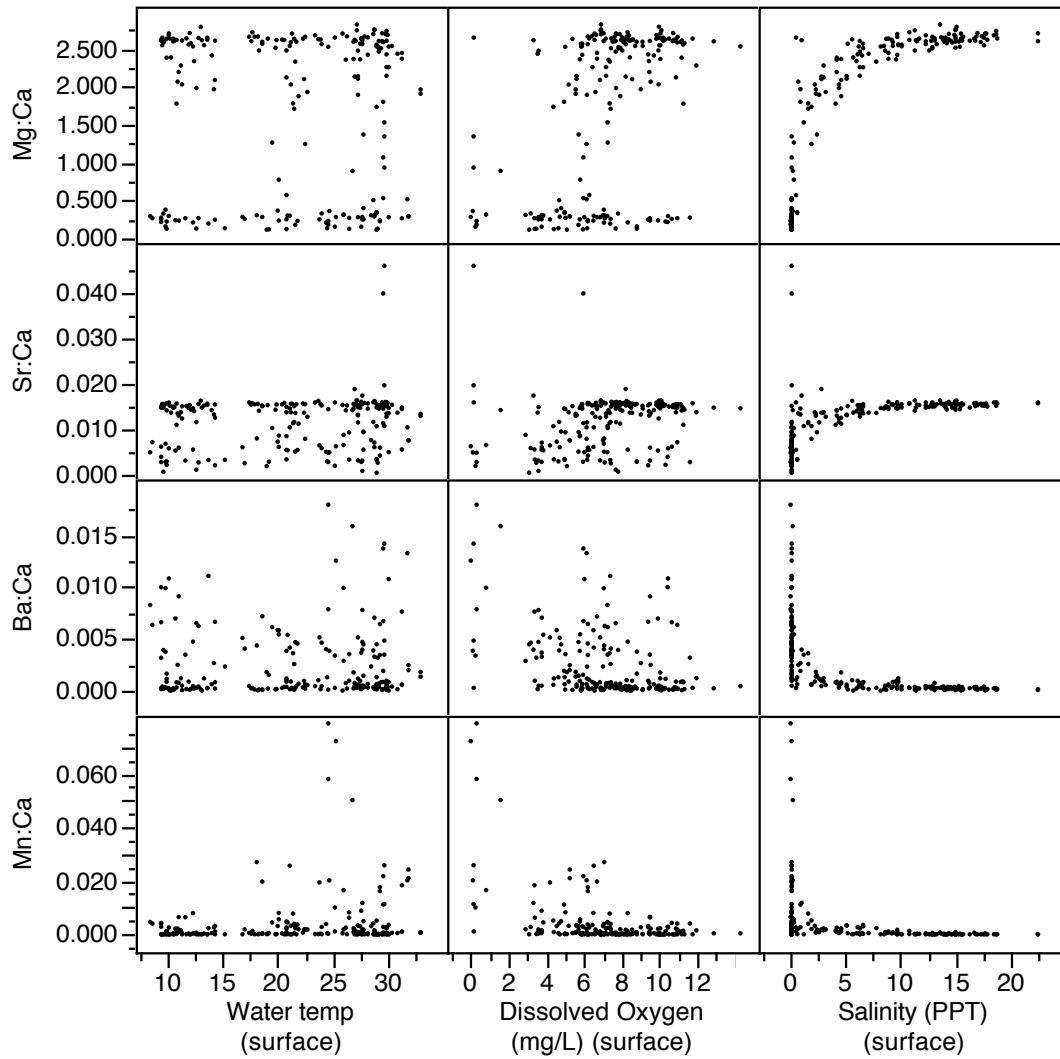
Figure 7. – Scatterplot matrix showing relationships between temperature (˚C), dissolved oxygen (mg/L), and salinity (ppt), and element to Ca ratios (all stations combined).
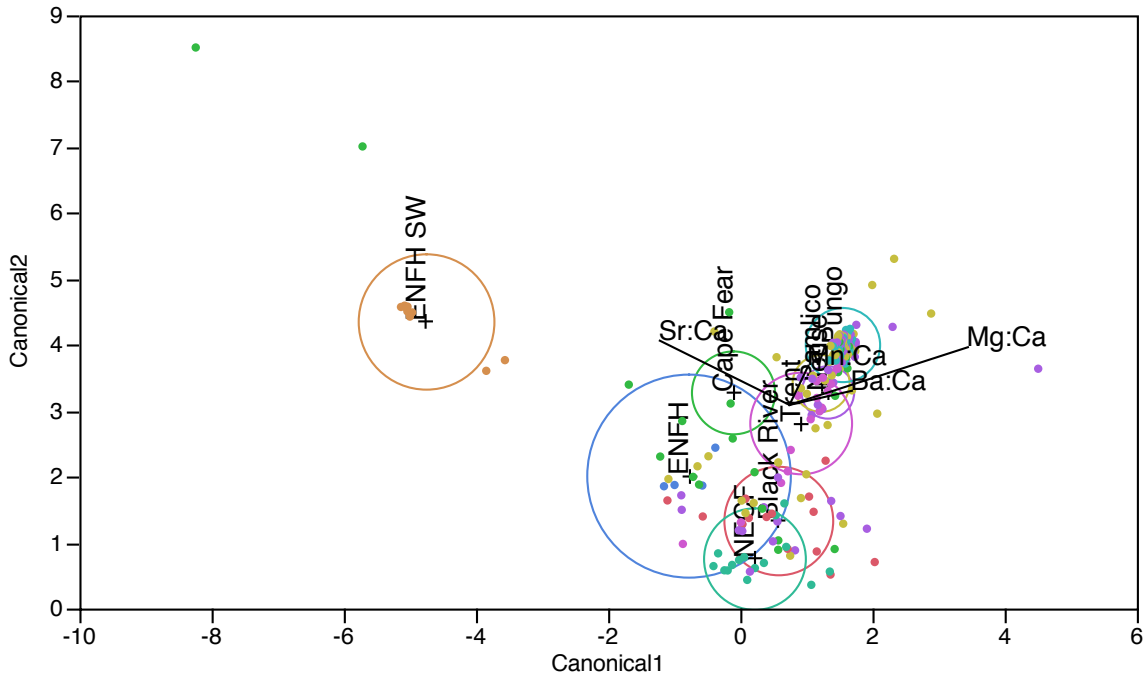
Figure 8. – Canonical correspondence plot showing results from linear discriminant function analysis to classify water samples to river (all stations) or hatchery pond of collection using Mg:Ca, Sr:Ca, Ba:Ca, and Mn:Ca. Tar/Pamlico (Pamlico)= tan, Neuse (Neuse)= purple, Pungo (Pungo)= teal, Trent (Trent)= fuchsia, Cape Fear (Cape Fear)= light green, Northeast Cape Fear (NECF)= dark green, Black (Black River)=red, Edenton National Fish Hatchery freshwater (ENFH)= blue, and Edenton National Fish Hatchery low salinity well (ENFH SW)= orange. Group centroids are marked with (+), circles represent 95% confidence circle for each river or pond.
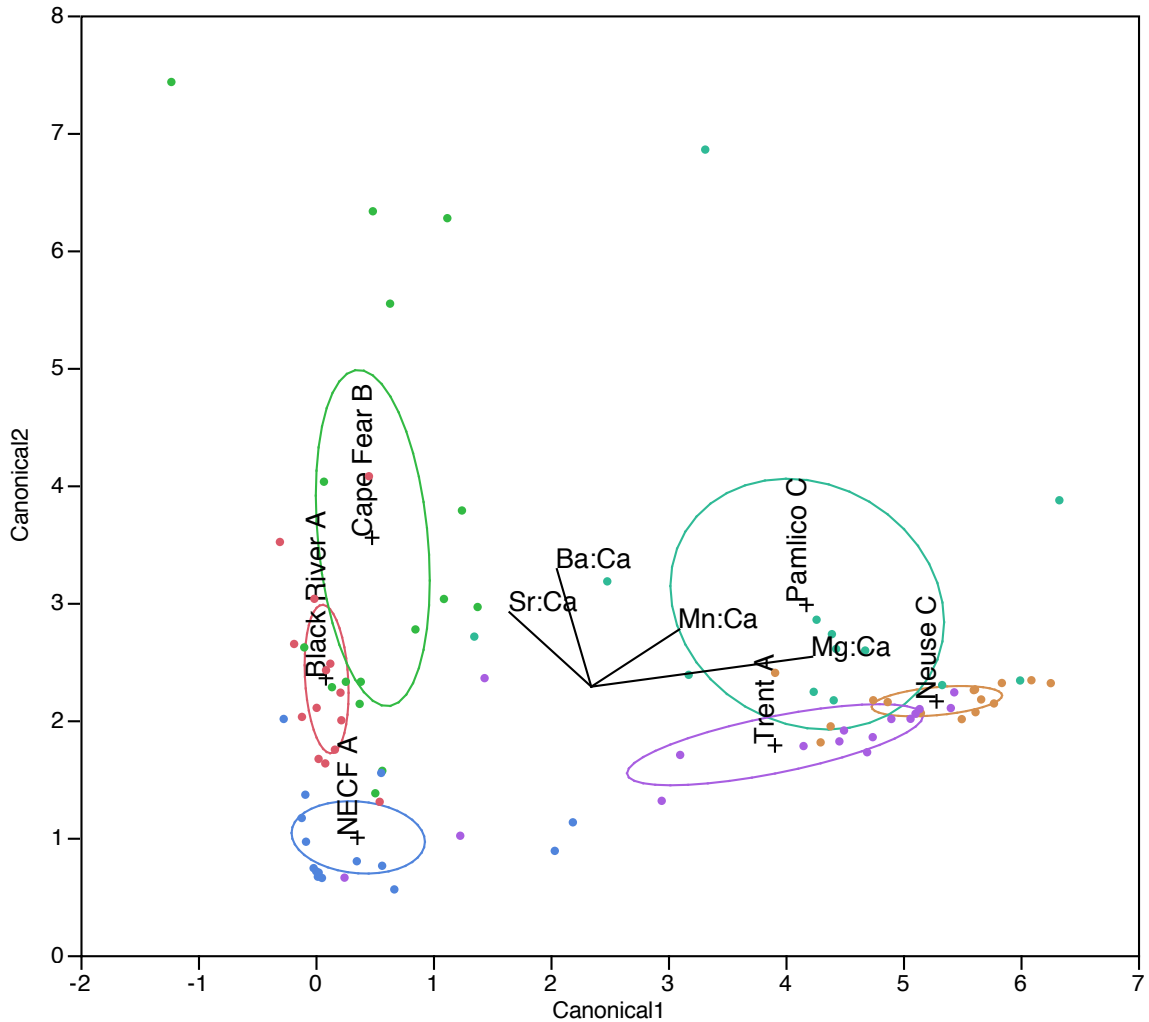
Figure 9. - Canonical correspondence plot on selected stations (one per river) showing results from quadratic discriminant function analysis to classify water samples to sample site of collection using Mg:Ca, Sr:Ca, Ba:Ca, and Mn:Ca. Neuse C (Neuse C)= orange, Pamlico C (Pamlico C)= teal, Trent A (Trent A)= purple, Cape Fear B (Cape Fear B)=green, Black A (Black River A)= red, and Northeast Cape Fear A (NECF A)= blue. Group centroids are marked with (+), ellipses represent 95% confidence ellipses for each sample site.

Chapter 3: Otolith Microchemistry of Central Southern Management Area Striped Bass to Determine Natal Origin

Abstract

Striped bass (*Morone saxatilis*) is an important commercial and game fish throughout North Carolina coastal waters. These fish have spawning populations in all of the state's coastal rivers; however, populations south of the Albemarle Sound have rarely been studied. These populations lie within North Carolina's immense Central Southern Management Area (CSMA). The CSMA stretches from the northernmost point of Pamlico Sound in the north all the way to the South Carolina border in the south. There are three main watersheds in the CSMA: the Tar/Pamlico River, the Neuse River, and the Cape Fear River. These rivers have spawning populations of striped bass, yet very few age 0 fish have been collected in recent years to support this. My study investigated the natal origin of CSMA striped bass through the use of otolith elemental analysis. Fish (N=251) were collected from the Neuse and Tar/Pamlico rivers from spring 2011 to spring 2012. Fish total length (TL), total weight (TW), gonad weight, and liver weight were collected to calculate relative weight (Wr), liver somatic index (LSI), and gonadal somatic index (GSI). Otoliths were removed for ageing and elemental analysis. Elemental analysis was conducted by measuring concentrations of strontium (Sr), magnesium (Mg), manganese (Mn), and barium (Ba) in the natal origin region of the otolith using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Condition of fish collected in the Neuse (Wr=83.3) and Tar/Pamlico (Wr=86.3) were found to be sub-optimal (Wr=97.0±5.0). Fish GSI follow predictable yearly cycles, but were maturing a full year earlier than Roanoke River striped bass. Using the Sr signature from otolith elemental analysis, 88.4% of fish originated

from the hatchery. Fish determined to be of natural origin were classified to their river of origin

with 58.0% accuracy, and to their management area of origin with 84.0% accuracy. The high

level of hatchery origin fish suggests that striped bass has become a put-and-take fishery in the

Neuse and Tar/Pamlico rivers.

Introduction

Stock identification and delineation are key problems in modern fisheries management

(Kutkuhn 1981; Begg et al. 1999). A stock is a fisheries management unit used to denote a

population or group of populations of fish that are reproductively isolated (Kutkuhn 1981). Unit

stocks are managed independently of one another; therefore, for fisheries management to be

effective, separate stocks of fish must be identified and delineated. Historically, mark recapture

studies, morphology and meristics, as well as physiological and behavioral characteristics have

been implemented to identify stocks with varying success (Gillanders 2001). Over the past 30

years otolith microchemistry has emerged as a reliable method for stock identification and

delineation (Campana and Fowler 1994; Gillanders 2001).

Some isotopes, chemicals, and elements are found in the otoliths of fish in a similar

proportion as the water in which the fish inhabits (Campana 1999; Elsdon and Gillanders 2006;

Dorval et al. 2007; Elsdon et al. 2008). These trace chemicals are incorporated into the otolith as

it is deposited in discrete, permanent layers. The correlation between water and otolith chemistry

in combination with the permanent layers within the otolith's aragonite structure allows the

otolith to serve as a proxy for fish habitat over time (Halden and Friedrich 2008). The elements

strontium (Sr), barium (Ba) (Bath et al. 2000), and manganese (Mn) have been shown to be

appropriate for otolith chemical analysis because they are taken up in similar proportion to the water (Thorrold et al. 1998a; Campana 1999; Dorval et al. 2007; Mohan et al. 2012). Magnesium (Mg) is also a common element used in otolith elemental analysis, although the relationship between Mg:Ca in the otolith and water is poorly understood (Campana 1999; Dorval et al. 2007; Mohan et al. 2012). Fish from the same stock should exhibit similar otolith chemical patterns, while fish from different stocks should show divergent chemical patterns.

Natal origin is a key factor when examining stock structure. It provides insight into the degree of separation or mixing of a particular stock. It can also reveal key habitats for juvenile fish and the importance of different subpopulations within a stock. Natal origin can be determined by examining the microchemistry of the core region in the otolith (Thorrold et al. 1998b; Bath et al. 2000; Tomás et al. 2005; Dorval et al. 2007).

Otolith microchemistry can help determine natal origin, mixing rates of stocks, and the proportions of different subpopulations within a stock. However, it cannot shed light on the causation of these trends. Condition of fish can provide an explanation for one region or subpopulation's prominence over another. Condition can be a valuable tool to assess habitat quality (Vasconcelos et al. 2009) and production of a population (Rätz and Lloret 2003). Condition of a fish can be assessed through a variety of condition indices. Fulton's condition factor (K) has been used to assess condition of many fish species (Gentry 2006). Condition factor is based upon a fish's weight at a given length. Due to the assumption of isometric growth in its calculation, comparison between fish of different size is not practical in species, such as striped bass, that exhibit allometric growth (Cone 1989). Relative weight (Wr) is a more reliable index for examining condition because it uses a species-specific standard weight (Ws) that takes the

allometric growth of fish into account. Relative weight has been used to measure condition of striped bass in North Carolina by several studies (Haeseker et al. 1996; Gentry 2006).

Physiological indices can also be implemented to assess the overall health of a fish. Liver somatic index (LSI) is the ratio of liver weight to body weight. As fish feed beyond what is required for their basal metabolic rate, they store energy in the form of glycogen in their liver. As the fish amasses glycogen reserves, their LSI increases. Therefore, a fish with high LSI should be in good condition because it has a surplus of energy. Gonadal Somatic Index (GSI) is another physiological index used to examine energy allocation for reproductive investment. GSI is simply the ratio of gonad weight to body weight. As fish prepare to spawn they invest great amounts of energy into gonadal development. This energy allocation can be observed in the increase in gonad weight leading up to spawning. Because gonadal development is so energy intensive, GSI should have a negative correlation with LSI. This would account for decreases in LSI leading up to spawning that do not necessarily indicate a decrease in fish condition.

This study focuses on the natal origin of Central Southern Management Area (CSMA) striped bass. The CSMA consists of "All internal Coastal, Joint and contiguous Inland waters of North Carolina south of a line from Roanoke Marshes Point across to Eagle Nest Bay to the South Carolina State line" as defined by the North Carolina Division of Marine Fisheries (NCDMF) (2004) (Figure 1). The CSMA consists of three primary watersheds: the Tar/Pamlico River, Neuse River and Cape Fear River systems. These watersheds support an important recreational fishery with substantial economic impact. There are spawning populations of striped bass in each of these watersheds, and larvae and eggs have been collected to support this (Beasley and Hightower 2000; Burdick and Hightower 2006). Yet, few juveniles have been collected in abundance index surveys to show evidence for successful recruitment (Barwick et al.

49

2009). Since 1980, the North Carolina Wildlife Resources Commission (NCWRC) has implemented an extensive stocking program for the CSMA watersheds to augment the low natural recruitment (Woodroffe 2012). The number, origin and growth phase at stocking of the fish has changed several times since the inception of the program. Since 2004 the NCWRC releases 100,000 phase II (12-20 cm) fingerling striped bass per year into each of the three main CSMA rivers (NCDMF 2004). All fish stocked into the Tar and Neuse rivers originate from the Edenton National Fish Hatchery (ENFH). The larval and early juvenile (phase I) striped bass are held in ponds of surface water from a nearby creek because of the high abundance of zooplankton. Once the fish reach approximately 2.5 to 6.0 cm TL they are transferred to the facility's phase II ponds. These ponds are filled with water from a deep well that draws from the Upper Castle Hayne Aquifer because the water is abiotic and reduces infectious outbreaks of the fish (S. Jackson, ENFH, personal communication).

In order for striped bass in the CSMA to be managed effectively, the source of the striped bass populations residing within it must be determined. Otolith chemistry is an appropriate tool to establish natal origin of these fish since it has been previously shown that these watersheds have unique elemental fingerprints that are stable over months, seasons, and years (Dobbs 2013, Chapter 2).

The goal of this study was to determine the origin of striped bass within the CSMA. The objectives were to : 1) collect striped bass from the Neuse and Tar rivers each calendar season for one year; 2) identify elemental fingerprints of natal origin by analyzing the area of the otolith representing the first year of life; 3) compare proportions of different natal origins to determine stock structure; 4) assess temporal variability of elemental fingerprints by comparing fish collected during this study to fish collected by NCWRC in previous years; 5) calculate various

condition factors including gonadal somatic index (GSI), Fulton's condition factor (K-factor), relative weight (Wr), and liver somatic index (LSI) for each fish collected; 6) compare condition factors of striped bass collected from the CSMA to striped bass collected elsewhere in North Carolina; and 7) compare age length frequencies of fish collected in the CSMA to striped bass collected elsewhere in North Carolina.

Methods

Site Description

The Tar/Pamlico and Neuse rivers are the primary tributaries of the Pamlico Sound. The Tar River Basin is the smallest of three river systems that lie completely in North Carolina. It originates in the piedmont plateau and flows approximately 190 km to its junction with the Pamlico River (North Carolina Division of Water Resources and Engineering 1952). The Pamlico River is the name given to the 60-km long estuarine section of the Tar River from the confluence with Tranters Creek to where the river flows into Pamlico Sound. The Neuse River Basin is the second largest river system that lies completely in North Carolina, behind only the Cape Fear River Basin. The Neuse basin originates in the piedmont plateau and is 290 km long; however, from the head of its longest tributary to its confluence with Pamlico Sound the Neuse stretches 480 river km (Rkm) in length (North Carolina Division of Water Resources and Engineering 1947). The Tar/Pamlico, and Neuse rivers range from oligohaline in upstream sections to mesohaline where they mix with the Pamlico Sound. The estuarine sections of the Neuse and Pamlico are well mixed, wind driven systems (Mallin 1991; Mallin and Paerl 1992).

Fish and Biological Sample Collection

Fish were collected only from the Neuse and Tar/Pamlico rivers. No fish were collected from the Cape Fear River due to budget constraints. A goal of at least 20 fish per calendar season from spring 2011 to spring 2012 for the Neuse and Tar/Pamlico rivers was set for this study. Fish were provided by the NCDMF independent gillnet survey, the NCWRC electrofishing survey, the ENFH brood stock collection, and through independent hook and line sampling, seining, and electrofishing. The NCDMF gillnet survey provided fish throughout the year from the estuarine portions of the Pamlico and Neuse rivers. The NCWRC electrofishing surveys were only conducted during the spring in the freshwater upstream portions of the Neuse and Tar rivers. Most of the NCWRC collections were from the striped bass spawning grounds near Tarboro in the Tar River and Goldsboro in the Neuse River. The ENFH fish were obtained from the NCWRC electrofishing survey and were provided for this study after spawning. The independent hook and line sampling, seining and electrofishing were conducted throughout the year as a means to augment collections provided by the state. Archived otoliths from Neuse River electrofishing collections in 2009 and 2010 were also provided by the NCWRC to validate the stability of signatures detected through trace elemental analysis. Otolith data from 10 fish collected in the Roanoke River in 2010 was provided by a similar study to determine the Roanoke River natal origin signature.

All fish collected were either processed immediately (ENFH samples), or promptly delivered to the laboratory at East Carolina University on ice. Fish total length (TL in mm), and total weight (TW, g) were measured prior to dissection to calculate relative weight and for use with age length analysis. Relative weight was used instead of Fulton's condition factor because

relative weight uses a species-specific growth calculation. This enables comparison of condition between age classes and populations.

Relative weight was calculated using the formula:

$$Wr = W/Ws * 100,$$

where W is the total weight (g) of the fish, and W_s is the length-specific standard weight for striped bass. The equation used to calculate standard weight is:

$$log10\ (Ws) = a + b * log10\ (TL),$$

where TL is total length (mm), and a and b are species-specific constants. The length-specific standard weight for striped bass was calculated using the equation developed by Brown and Murphy (1991). The equation to calculate W_s for striped bass is:

$$log10\ (Ws) = -4.924 + 3.007\ log10\ (TL).$$

Livers were excised and weighed (0.01 g) for calculation of LSI. LSI was calculated using the formula:

$$LSI = (Wliver\ /\ W)\ x\ 100,$$

where Wliver is the total weight of the liver and W is the total weight of the fish.

Gonads were sexed and weighed to the nearest 0.01 g for calculation of GSI. GSI was calculated using the formula:

$$GSI = (Wgonads\ /\ W)\ x\ 100,$$

where Wgonads is the total weight of the gonads and W is the total weight of the fish.

Theoretical length at age was estimated by fitting observed length at age data to a Von

Bertalanffy growth model. The Von Bertalanffy growth model uses the formula:

$$Lt = L\infty \ (1 - e^{-k \ (t \ - \ t0)});$$

where Lt is length (TL) at age (year), L∞ is the theoretical asymptotic length (TL), k is the

growth constant, t is age in years, and t_0 is theoretical age zero length.

Sagittal otoliths were removed with non-metallic forceps to prevent contamination in

trace element analysis, rinsed to remove remaining tissue, and placed in 15 mL microcentrifuge

polypropylene vials under a negative pressure hood for drying. When possible, the left otolith

was used for both trace element analysis and ageing, although there is no significant difference

between left and right otoliths (Mohan 2009). Otoliths were used in the ageing of each specimen

to ensure accuracy. Otoliths are assumed to be 100% accurate, while scales have been shown to

underestimate the true age for striped bass 10 years and older (Paramore and Rulifson 2001) and

over estimate the age of striped bass 3 years and younger (Boyd 2011). Otoliths were aged twice

by two independent agers to ensure accuracy. When discrepancies occurred, a third independent

reader was used to resolve it.

Otolith Preparation and Analysis

Otolith preparation and analysis were conducted using the same protocols as Mohan et al.

(2012) and Zapf (2012). After removal and drying, the otolith samples (n=281) selected for trace

elemental analysis were sent to the University of Manitoba, Winnipeg, Canada. Once there, the

otoliths were embedded in an epoxy resin (Buehler Epoxicure), and 2-mm thick dorso-ventral

transverse sections, including the cores, were cut using a diamond blade Isomet saw (Buehler

646) at low speed. The dorso-ventral section exposes annuli with a steeply dipping geometry into the plane of the section, allowing scanning laser beam to resolve individual annuli (Halden and Friedrich 2008). The cut sections were then re-embedded in 25-mm diameter, Plexiglass ring mounts. The orientation and identity of each section within each ring mount was recorded for sample reference. To expose the nucleus region and otolith core, sections were ground down using 320-, 400-, and 600-grit wet sandpaper and then ultrasonically cleaned for 2 min. Scratches on the otolith surface were removed by polishing with Buehler diamond polishing suspensions (9 μm and 0.05 μm) on a polishing wheel to achieve a completely smooth surface required for laser ablation. Polished, mounted otoliths were given a final ultrasonic cleaning with ultrapure water and then digitally photographed to create an illustrated reference for analysis.

Elements were quantified using a Thermo-Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd-YAG laser. Operating parameters for laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) included: 15-μm beam size; 2 μms^{-1} scan speed; repetition rate 20 Hz; and 75% power, using low resolution (R = 300) mode. The isotopes counted were ^{44}Ca, ^{25}Mg, ^{88}Sr, ^{138}Ba, ^{55}Mn, ^{63}Cu, ^{66}Zn, and ^{208}Pb. Calcium (as 56 wt. % CaO) was used as the internal standard to monitor ablation yield. NIST 610 glass was used for external calibration and to monitor any instrument drift. Laser scans were initiated before the nucleus, on the shorter axis of the sulcal groove, and continued through the core and along the longest axis of otolith growth to the outer edge. The intensity (in counts per second) for elements was converted to ppm using a Macro written in Microsoft Excel and plotted versus distance across the otolith corresponding to the laser scan.

Natal origin of fish was divided into two main categories: fish that originated from the ENFH, and fish naturally spawned in the rivers. The classification of fish to one of these two

groups was based on otolith chemistry from the entire first year of life. The key feature to distinguish hatchery fish from natural fish was the presence of a consistent, abnormally high (>5000 ppm) [88]Sr concentration throughout the majority of the first year of life. The abnormally high Sr concentrations are a result of extremely high Sr:Ca ratios found in ENFH rearing ponds (Dobbs 2013, Chapter 2). All fish containing this marker were designated as hatchery origin. All other patterns were designated as naturally spawned fish.

Fish classified as naturally spawned were further analyzed to determine watershed of origin. For this, an area of the otolith corresponding to the larval and earliest juvenile period was investigated. This area had to exclude any maternal input that would affect otolith chemistry, while still representing the earliest period of the fish's life before it would have migrated out of its natal watershed.

Otolith Chemistry during pre-hatch and early larval periods reflects maternal chemistry (Volk et al. 2000). Once a fish hatches, the only remaining maternal influence is the yolk sac. In order for maternal influence to be eliminated from the otolith analysis, the area of the otolith corresponding to the yolk-sac larval period must be discarded. The largest size larval striped bass with any remaining yolk sac is 7 mm TL, or 6.44 mm standard length (SL) (U.S. Fish and Wildlife 1978). Secor and Dean (1992) determined that larval striped bass SL to otolith diameter could be described with the following equation:

$$SL = 4.160 + (0.0239 * otolith\ diameter),$$

where otolith diameter is in μm, and SL is in mm. Using this equation, a 6.44-mm SL larval striped bass should have an approximately 96 μm diameter otolith. Therefore elemental data more than 48 μm from the core should represent a post yolk-sac larva. An additional 10-μm

buffer zone was added to this to assure no maternal contribution was included. A 48-µm area

beyond the buffer zone was analyzed to determine the natal origin signature in an otolith.

Statistical Analysis

Physical and Biological Factors of Fish

Normality of all variables was visually assessed using Q-Q plots. Wilcoxon and Kruskal-

Wallis tests were used to examine differences Wr, LSI, and GSI by river, season, year, and year

class because all variables were found to be non-parametric. Wilcoxon tests were also used to

examine differences in Wr from fish collected in the Tar/Pamlico River in my study to fish

collected in the Tar/Pamlico River from Gentry's (2006) study to examine long term trends. Von

Bertalanffy growth model was fit using MS Excel and results were validated using JMP.

Elemental analysis

Chi square analysis was used to examine differences in proportion of hatchery fish by

river and year of collection. When significant differences were found, an analysis of means for

proportion was used to identify which years differed significantly. Wilcoxon and Kruskal-Wallis

tests were used to examine differences in the mean concentrations of Sr, Mg, Mn, and Ba in the

natal origin region of the otolith by season, river of capture, management area, and year class.

When significant differences were detected between elemental concentrations, Tukey's HSD was

used to examine which locations differed significantly. Visual assessments of Sr signatures were

used to distinguish hatchery fish from naturally spawned fish. Quadratic discriminant function

analysis (QDFA) was used to assess how elemental concentrations in the natal origin region of

the otolith could be used to classify fish to the river of origin and management area of origin.

Pillai's trace statistic was used to examine differences in multivariate means between rivers and

management rivers (JMP ® 2012).

Results

Catch Data

A total of 135 striped bass were collected from the Tar/Pamlico River, and 116 from the

Neuse River from spring 2011 to spring 2012 (Table 1). The greatest number of fish were

collected in spring 2012 from the Tar/Pamlico River, and the fewest were collected in fall 2011

from the Neuse River (Table 1). Striped bass collected ranged from age 0 to 7 years in the

Tar/Pamlico, and ages 1 to 8 years in the Neuse (Figure 2). The most prominent age class in the

Tar/Pamlico was 4 year-olds, and 3 year-olds in the Neuse, while the least common age class

was age 0 in the Tar/Pamlico, and age 8 in the Neuse (Figure 2).

Physical Characteristics and Condition Indices

The specific Von Bertalanffy equation for the fish collected from the Neuse River was:

$$Lt = 669.71 \ (1 - e^{-0.4980 \ (t - 0.0)}).$$

The specific Von Bertalanffy equation for the fish collected from the Tar/Pamlico River was:

$$Lt = 639.21 \ (1 - e^{-0.6123 \ (t - 0.0)}).$$

Wr, LSI, and GSI differed significantly by season and year class (Table 2). Wr and LSI differed significantly by year, while only Wr differed significantly by river (Table 2). Mean Wr was highest during fall 2011 in the Tar/Pamlico, and spring 2011 in the Neuse, while lowest during summer 2011 in the Tar/Pamlico, and fall 2011 in the Neuse (Figure 5). Overall mean Wr was higher in the Tar/Pamlico than the Neuse (Figure 5). Mean LSI was highest in winter 2012, and lowest in spring 2012 (Figure 6). Mean GSI was highest in spring 2011, and lowest in summer 2011, and fall 2011 (Figure 7). Spring 2012 GSI was excluded from analysis because all fish were post spawn. Mean spring GSI was highest in 8 year-old fish, and lowest in 2 year-old fish (Figure 8).

Elemental Data

Hatchery Fish

Hatchery fish were identified by the unique Sr signature in the otolith. The hatchery Sr signature was characterized by variable maternal contribution at the core, low strontium during the phase I region of growth that quickly rises to extremely high Sr (>5000 ppm) over the entire phase II region of growth, followed by a precipitous drop-off in strontium concentration caused by a stocking into low salinity mid-river sites (Figure 10). Hatchery fish made up the majority of all seasonal collections for my study, with the highest percentage of hatchery fish occurring during fall 2011 in the Neuse (100.0%) and Tar (100.0%) rivers, while the lowest percentage occurred during summer 2011, and spring 2011 in the Tar River (80.0%), and spring 2012 in the Neuse River (84.3%) (Table 4). Hatchery fish make up 88.2±5.5% of striped bass in the

Tar/Pamlico River, 88.6±5.9% in the Neuse River, and 88.4±4.0% of all striped bass in the Neuse and Tar/Pamlico rivers (±95% confidence interval) (Table 4).

The percentage of hatchery fish differed significantly among years in the Neuse River, but not in the Tar/Pamlico. Fish collected in 2010 (47.4% hatchery) by the NCWRC (n=19), was the only collection year to differ significantly from the mean percentage of hatchery fish in the Neuse River (Figure 11).

Natural Fish

Mean element concentrations in the natal origin region of the otolith differed significantly by season of collection, river of collection, management area of collection, and year class (Table 5). Mean Ba and Mn in otoliths differed significantly by management area of collection, while only Ba differed significantly by river of collection; however, variability of Mn in otoliths nearly met significance by river (p=0.0539), and Sr nearly differed significantly by management area (p=0.0653) (Table 5). Despite not varying significantly in the Wilcoxon test, Sr differed significantly by management area in the Tukey HSD (Figure 12). Sr differed significantly by year class, and Mg differed significantly by season of collection (Table 5). Mean Sr concentrations were highest in the Roanoke River, while lowest in the Tar/Pamlico and Neuse rivers (Figure 13). Ba concentrations were highest in the Roanoke River, and lowest in the Tar/Pamlico River (Figure 13). Sr was highest in the ASMA, and lowest in the CSMA (Figure 12). Ba was highest in the ASMA, and lowest in the CSMA (Figure 12).

Classification

Sr, Mg, Mn, and Ba were used in natal origin classification because they were all found consistently in all fish and water sample sites (Dobbs 2013, chapter 2). Multivariate means differed significantly between rivers (Pillai's trace statistic: F= 2.4833, df=90, p=0.0176) for a QDFA classifying the elemental signature in the natal origin region of the otolith to river of capture (Figure 14). Classification rates ranged from 37.5% for the Neuse, to 80.0% for the Roanoke (Table 6). Overall classification rate was only 58.0%. Most misclassifications occurred between the Neuse and Tar rivers. Because of low overall classification rates, and the majority of misclassifications occurring between rivers in the same management area in the river discriminant analysis, a QDFA of management areas was conducted. Multivariate means differed significantly between management areas (Pillai's trace statistic: F=5.6541, df=45, p=0.0009) for a QDFA classifying the elemental signature in the natal origin region of the otolith to management area of capture (Figure 15). Classification rates ranged from 80.0% in the ASMA, to 85.0% in the CSMA. The overall classification rate was 84.0% (Table 7).

Discussion

Physiological and Condition Indices

LSI

LSI is used as a way of evaluating energy storage in fish. When fish eat in excess of their metabolic needs, they store the remaining energy in their livers in the form of glycogen. LSI has been shown to increase seasonally as water temperature decreases (Heinimaa 2004). This inverse relationship is due to a decrease in metabolic rate as temperatures drop and possibly because of

greater prey availability. In my study LSI varied by season, year, and year-class, but not by location (Table 2). It was highest in the winter 2011, and lowest in summer 2011 and spring 2012 (Figure 6). The increase in LSI during fall and winter and decrease during summer agrees well with the established seasonal temperature to LSI relationship. The curiously low spring 2011 mean LSI, however, does not fit this trend. Spring striped bass usually have high LSI due to cool water temperatures and abundant food (Gentry 2006). However, spring LSI rarely exceeds winter LSI because a tremendous amount of energy is channeled into gonad development and spawning migration in the spring, which leaves very little excess to store as glycogen in the liver.

The disparity in spring LSI values in my study is due to sampling bias. In spring 2011, all of the fish were collected pre-spawn on or near the spawning grounds. These fish had finished gorging themselves all winter and early spring, and were in peak condition waiting to spawn. The majority of fish collected during spring 2012 were provided by the ENFH. These fish were spent broodstock for the stocking program. They had been kept in spawning tanks for up to one week prior to my collection and analysis. During the time spent in the spawning tanks the fish were not provided with any food. This period of starvation, coupled with the high energy consumption of spawning and the stressful conditions in the tanks, was adequate to deplete the glycogen stores the fish had built up prior to capture.

GSI

GSI is an indirect way of evaluating energy expenditure for gonad development. GSI is expected to vary seasonally in accordance with the spawning schedule of the particular fish species. Striped bass are spring spawners, so they should have highest GSI values in the spring

that precipitously drop off post spawn in the summer, where they remain low for a period of reproductive rest into mid fall. At this point, the fish once again begin to allocate large amounts of energy to gonad development for spawning the next spring. This energy allocation is observed as increasing GSI through fall and winter, leading into peak GSI during the spring spawning season. GSI calculations from my study follow this expected pattern well with highest GSI during spring 2011, and lowest during summer 2011 (Figure 7). Similarly to LSI measurements in my study, there was a significant difference between spring 2011 and spring 2012 GSI values. This difference was due to the same sampling bias as the disparity in yearly LSI. All of the fish collected in spring 2011 were collected on or near the spawning grounds. These individuals were all pre-spawn and, subsequently, had peak GSI values. In contrast, the majority of the fish collected during spring 2012 were from the ENFH. These fish were only provided for this study after they had been completely spawned out for the states stocking program. This significantly reduced the GSI of these fish. Because of this sampling bias, spring 2012 GSI data were excluded from analysis and yearly GSI variation was not investigated.

Olsen and Rulifson (1992), and Boyd (2011) found that striped bass in the Roanoke River and Albemarle Sound had substantial increases in fecundity as fish aged. The number and size of eggs increased as mature females grew older. The GSI results from this study agreed well with the findings of Olsen and Rulifson (1992), and Boyd (2011). GSI differed significantly by age, with GSI increasing as fish grew older (Table 2) (Figure 8). The largest increase in GSI occurred between ages 2 and 3 (Figure 8). GSI rose from around 1 to over 5 in one year. This enormous increase, in conjunction with the absence of age 0 and 1 fish collected on the spawning grounds suggests that most striped bass sexually mature by age 3 in the Neuse and Tar/Pamlico rivers. This is one full year sooner than in the Roanoke River (Olsen and Rulifson 1992; Boyd 2011).

The truncated age structure from intense recreational fishing in the Neuse and Tar/Pamlico rivers may be selecting for early maturation in these populations. Striped bass produce more, higher quality (i.e, larger) eggs as they grow. The large number of small reproductive adults that are probably producing low numbers of low quality eggs, may be an important factor in why there has been so little evidence for juvenile recruitment in the Neuse and Tar/Pamlico rivers in recent years.

Wr

Wr varied by river, season, year, and year-class (Table 2). Overall, mean Wr was higher in the Tar/Pamlico than in the Neuse River (Figure 5). The seasonal trends in Wr agreed well with the findings of Gentry (2006). Mean Wr was highest in the spring and lowest in the winter. However, much like LSI and GSI, Wr differed significantly between spring 2011 and spring 2012 because of the same sampling bias described above. Wr was abnormally high during summer 2011 in the Neuse River (84.3), where only spring 2011 (88.2) had a higher mean Wr. This was most likely due to the large schools of Atlantic menhaden that pods of striped bass were feeding on in the Neuse River during summer 2011. This behavior was observed consistently throughout the summer. The prevalent prey allowed the fish to maintain a higher than expected condition despite the increased metabolic effects, associated with warm summer conditions. It is important to note that even the highest seasonal mean Wr in either river (93.1 during fall 2011 in the Tar/Pamlico) only exceeded the minimum Wr for good condition by a small margin. Brown and Murphy (1991) established the 75th percentile (97±5 units) as the benchmark for good condition. This was never achieved during any season in the Neuse, and only during the spring and fall of 2011 in the Tar/Pamlico. The low Wr observed in Neuse and

Tar/Pamlico river fish could be evidence for sub-optimal water quality or habitat in these rivers. This, in turn, may be a possible cause of the low juvenile recruitment observed in these rivers.

The low Wr observed in my study over a 5-season period does not appear to be an aberration in yearly norms. Mean Wr of fish collected in spring 2011 from my study, and spring 2005 from Gentry (2006) were the only seasonal mean Wrs to differ significantly between the two studies (Table 3). Gentry (2006) found mean Wr to be highest in winter 2005 (90.33), and lowest in summer 2005 (74.26) (Figure 10). Overall mean Wr was not significantly different between Gentry's (2006) study (86.37) and this study (85.08) (Figure 9). Because few fish collected during my study appeared gaunt or sickly, a reevaluation of Brown and Murphy's (1991) benchmark for good condition may be necessary for CSMA striped bass.

Elemental Data

Magnesium

The use of Mg in otolith elemental analysis has always been somewhat controversial. Mg uptake into the otolith appears to be more physiologically regulated than reflective ambient water chemistry (Campana 1999; Wells et al. 2003; Dorval et al. 2007). Although Mg tends to be directly proportional to salinity gradients, this relationship is seldom observed in otolith chemistry (Dorval et al. 2007; Mohan et al. 2012). The otolith Mg concentration patterns in this study were similarly difficult to characterize. Mg:Ca ratios in CSMA rivers were highly correlated to salinity (Dobbs 2013, chapter 2), however, natal origin region of the otolith Mg concentrations only differed significantly by month of collection, and not by location (Table 5). Mg concentrations were slightly higher in CSMA, the higher salinity system fish, than ASMA

fish, although this difference was not significant. Despite its confusing relationship of water to otolith chemistry, the inclusion of Mg in discriminant function analyses boosted their classification accuracy, if only slightly, so Mg was included in natal origin classification for my study.

Manganese

Mn exhibits a much more reliable pattern of proportional uptake from water to otolith than Mg (Dorval et al. 2007; Mohan et al. 2012). Increased Mn levels in water are associated with redox conditions in sediment caused by anoxic conditions (Sundby et al. 1986), therefore elevated levels of Mn in the otolith should be indicative of anoxic conditions in the fish's environment. Otolith Mn concentrations only differed significantly between management areas in a Wilcoxon test (Table 5), but not a Tukey's HSD (Figure 12). The difference between the Mn in management areas probably would have been significant in the Tukey's HSD if the sample sizes were increased. The ASMA fish had higher mean Mn than the CSMA fish (Figure 12). This agrees well with Mohan (2009), and Zapf (2012) who found consistently high Mn:Ca ratios in the Perquimans and Roanoke rivers in the ASMA.

Strontium

Sr is the most widely utilized element for otolith elemental analysis. It is deposited in the otolith in similar proportion to the ambient water (Campana 1999; Bath et al. 2000; Walther and Thorrold 2006; Elsdon and Gillanders 2006). It varies predictably in water along a salinity gradient (Elsdon and Gillanders 2006; Lin et al. 2007), but also has significant variability in

completely freshwater (Wells et al. 2003; Kraus and Secor 2004). Strontium was the key element for discriminating hatchery fish from naturally spawned fish (Figure 10). The high Sr:Ca ratio in the ENFH phase II ponds provided a unique natural tag to identify the hatchery fish. In natural fish, Sr in the natal origin region only differed significantly by year class in the Wilcoxon test, but differed significantly by management area using Tukey's HSD.

Barium

Ba has been shown to be excellent choice for otolith elemental analysis because it is taken up into the otolith in similar proportion to the ambient water (Bath et al. 2000; Walther and Thorrold 2006; Mohan et al. 2012; Zapf 2012). Ba:Ca ratios in the water are inversely related to salinity (Guay and Kenison Falkner 1998), and, similar to Sr, there is significant variability in freshwater systems (Wells et al. 2003; Limburg and Seigel 2006). Mean Ba concentrations differed significantly between rivers and management area in the natal origin region of natural fish. Ba was much higher in Roanoke River fish than Neuse and Tar/Pamlico rivers. The Neuse and Tar/Pamlico river fish had very similar mean Ba concentrations.

Hatchery Signature

The hatchery signature for fish from the ENFH was determined through a visual assessment of the Sr signature throughout the first year of life (Figure 10). The Sr concentrations within the maternal contribution region were quite variable. This area gets its name from the element concentrations near the core of the otolith that reflect the chemistry of the egg (Volk et al. 2000). The egg is formed inside the mother and thus reflects her physiological chemistry at

the time of egg development. The otoliths begin to develop prior to hatching (B. Elking, East Carolina University, personal communication) so the only source of elements for deposition comes from the egg itself during otolith formation. Once hatched, the larvae rely completely on the egg for nourishment for several days. Otolith deposition during this time is greatly influenced by egg chemistry until the larva is post egg-sac and has begun to take up ambient water chemistry in its otoliths (Volk et al. 2000).

Sr levels as low as 500 ppm or as high as 5000 ppm were observed in the maternal contribution region (Figure 10). The variability is most likely a factor of the mothers' migration patterns prior to spawning. If the mother was an ocean migrator then she would have developed her eggs in the high Sr environment of the ocean. This would impart high Sr concentrations into the yolk and albumen of the eggs, leading to high strontium in the core. If the mother was a resident fish, she would have developed her eggs in the low to moderate Sr environment of the river. This would cause low to moderate Sr concentrations in the maternal contribution region.

The chemistry in the phase I region of otoliths was very consistent. The phase I region gets its name from the phase I growth ponds at the ENFH. Fish are reared in these ponds from time of first feeding (3 days old) until the fish reach approximately 2.5 to 6 cm, which takes 1-2 months. The ponds are filled with completely fresh surface water from a nearby creek. This water has a very low Sr:Ca ratio (Dobbs 2013, chapter 2). This low salinity is reflected in the otolith with concentrations ranging from 1000 to 2000 ppm (Figure 10).

Once the juveniles reach 2.5 to 6 cm, they are transferred to phase II growth ponds. The fish are reared in these ponds until they are released into their destined river of stocking the following winter (about 8 months). The water in these ponds is sourced from a deep well that

draws from the Upper Castle Hayne Aquifer. This water has been found to have an extremely high Sr:Ca ratio (Dobbs 2013, chapter 2) consistent with what is expected from the Upper Castle Hayne (Woods 2000). The region of otolith deposition corresponding to this growth period has been labeled the phase II region for my study. The phase II region is characterized by the highly elevated Sr concentrations that reflect the pond waters' high Sr:Ca ratio (Figure 10). The Sr concentration in this section remains quite consistent over the entire region, although, there is considerable variation between fish. The Sr concentrations ranged from 5000 to 9000 ppm for this region.

Possibly the most revealing characteristic of the hatchery signature is the stocking event region. This region is characterized by the precipitous drop in Sr concentrations associated with the fish being stocked into mid-river sites in the Neuse or Tar/Pamlico rivers (Figure 10). This steep drop occurs in less than 500 µm just prior to the first annulus. The stocking sites have relatively low Sr:Ca ratios (Dobbs 2013, chapter 2) that translate to Sr concentrations around 2500 ppm in the otolith. After the stocking event, the otolith begins to reflect ambient river chemistry, and the Sr begins to fluctuate like natural fish signatures.

Natural Fish Classification

Natural fish were classified to their river and management area of capture using the concentrations of Mg, Ba, Sr, and Mn in the natal origin region of the otolith. Classification of fish should indicate the natal origin signature for the river or management area of their collection because Neuse and Tar/Pamlico river striped bass are considered endemic riverine populations (Morris et al. 2003). Fish classified as Roanoke River fish should provide a natal origin signature

for the Roanoke River because these fish are believed to home to their natal river to spawn, and

all Roanoke fish were collected on the spawning grounds. The natal origin signatures of natural

fish assigned to the Neuse and Tar/Pamlico rivers can only be assumed because no ground-

truthing for these signals was available. Misclassifications represent either mixing, or similarities

in watershed chemistry. The elemental signatures of CSMA watersheds have been shown to be

stable over time (Dobbs 2013, chapter 2); therefore, the comparison of the otolith elemental

chemistry of fish from a variety of year-classes was conducted. Fish were only classified to their

river of collection 58% of the time using a QDFA. However, this does not appear to be because

of high mixing rates between systems. The majority of misclassifications occurred between the

Neuse and Tar/Pamlico rivers. This misclassification pattern is similar to a QDFA previously

conducted that classified water samples to their river of collection (Dobbs 2013, chapter 2).

These rivers are adjacent to each other in the CSMA, and their multivariate means were not

significantly different (Figure 14). The similarity in elemental signature is most likely due to

their close geographic association. When management areas were compared using a QDFA, the

classification success rate rose to 84%. The misclassifications in this discrimination could be

indicative of mixing between the two management areas, although, only two misclassifications

occurred within the 95% confidence ellipse of the other management area (Figure 15).

The classification of fish to their natal river is extremely important in order to understand

mixing rates between management areas. The populations within these management areas are

managed separately and the stocks are assumed to be isolated. My study shows preliminary

evidence that mixing rates of natural fish are low between the CSMA and ASMA, but the results

are quite inconclusive. In order to distinguish the river of origin of the naturally spawned striped

bass residing in the CSMA, more juveniles need to be collected in these rivers, or an in-situ

caging experiment similar to Mohan (2012) needs to be conducted to ground-truth the signal. Furthermore, juveniles from the same year class need to be collected from various ASMA watersheds to establish natal origin signatures for all of North Carolina's striped bass populations.

Stock Structure

No fish older than 8 years or longer than 800 mm (TL) were collected in my study. The majority of fish collected on the spawning grounds ranged from 2-4 years old, which is significantly younger than what Boyd (2011) found in the Roanoke River. In addition to younger average spawning age, the fish from the Neuse and Tar/Pamlico rivers had a much faster growth rate, and much lower asymptotic length than fish in the Roanoke River according to their Von Bertalanffy growth curves (Boyd 2011).

The striped bass populations in the Neuse, and Tar/Pamlico rivers are comprised mainly of hatchery fish ($p<0.0001$) (Table 4). Natural fish make up only 11.6±4.0% (95% confidence interval) of striped bass in the Neuse and Tar/Pamlico Rivers. This indicates that the stocking program in these rivers is vital to maintaining the economically important recreational fishing in this region. Of the small percentage of naturally spawned fish in these rivers, it appears the majority are originating from within the CSMA, although this study was unable to elucidate whether there was mixing between the Neuse and Tar/Pamlico rivers.

The populations of striped bass in the Neuse and Tar/Pamlico rivers appear to be growing much more quickly, and maturing a full year earlier than ASMA striped bass (Figures 3, 4, and

8). The lowered age at maturity may be a product of the intense recreational fishing pressure in these rivers.

Conclusions and Recommendations

My study has shown otolith elemental analysis to be an appropriate method for determining natal origin of striped bass in the CSMA. Through unintended natural tags, the hatchery contribution to the fishery was determined. Otolith chemistry was also able to distinguish management areas, even without a way of ground-truthing the signals.

The striped bass populations in the Tar/Pamlico and Neuse rivers are predominantly of hatchery origin. This means that these watersheds rely heavily on the NCWRC stocking program to support their primarily put-and-take striped bass fisheries. The small proportion of natural fish that are present in these watersheds appear to be originating in them, but some unknown factor, possibly water quality, or overfishing, is preventing the historical natural populations in these rivers from recovering. The striped bass collected in these rivers show unusual growth and maturation patterns, and tend to be in sub-optimal condition. This provides evidence that some factor or group of factors is negatively affecting these populations.

In order to maintain the current level of recreational and commercial fishing in the Neuse and Tar rivers, the current stocking program must be continued. However, if the state wishes to restore naturally reproducing populations of striped bass to these rivers, fishing pressure on these populations of striped bass needs to be significantly reduced. This could be accomplished through a larger minimum size, smaller slot limit, lower bag limit, a shorter season, a

combination of any of these approaches, or a complete moratorium, similar to the Cape Fear River.

In order to determine the river of origin for CSMA striped bass, juveniles need to be collected from nursery areas or a caging experiment needs to be conducted to ground truth the natal origin signatures. In order to elucidate possible causes for the low abundance of natural fish in CSMA watersheds, an evaluation of strategic habitat for juvenile striped bass needs to be conducted. Land use, and its effects on water quality in CSMA watersheds needs to be investigated to determine if it plays a role in the poor recruitment of striped bass in CSMA watersheds. Fishing mortality rates need to be investigated to determine if overfishing is occurring.

References

Barwick, R. D., J. M. Homan, and C. D. Thomas. 2009. Investigating striped bass recruitment in the Neuse River, North Carolina. Proceedings of the Annual Conference / Southeastern Association of Fish and Wildlife Agencies 63:161-165.

Bath, G. E., S. R. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. Geochimica Et Cosmochimica Acta 64(10):1705-1714.

Beasley, C., and J. Hightower. 2000. Effects of a low-head dam on the distribution and characteristics of spawning habitat used by striped bass and American shad. Transactions of the American Fisheries Society 129(6):1316-1330.

Begg, G. A., K. D. Friedland, and J. B. Pearce. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. Fisheries Research 43(1):1-8.

Boyd, J. B. 2011. Maturation, fecundity, and spawning frequency of the Albemarle/Roanoke striped bass stock. Master's Thesis. East Carolina University, Greenville, N.C.

Brown, M.,L., and B. Murphy R. 1991. Standard Weights (Ws) for striped bass, white bass, and hybrid striped bass. North American Journal of Fisheries Management 11:451-467.

Burdick, S., and J. Hightower. 2006. Distribution of spawning activity by anadromous fishes in an Atlantic slope drainage after removal of a low-head dam. Transactions of the American Fisheries Society 135(5):1290-1300.

Campana, S. E., and A. J. Fowler. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 51(9):1942-1950.

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

Cone, R. 1989. The need to reconsider the use of condition indexes in fishery science. Transactions of the American Fisheries Society 118(5):510-514.

Dorval, E., C. M. Jones, R. Hannigan, and J. V. Montfrans. 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Canadian Journal of Fisheries and Aquatic Sciences 64(3):411-411.

Elsdon, T. S., B. K. Wells, S. E. Campana, B. M. Gillanders, C. M. Jones, K. E. Limburg, D. H. Secor, S. R. Thorrold, and B. D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology: An Annual Review 46:297-330.

Elsdon, T. S., and B. M. Gillanders. 2006. Temporal variability in strontium, calcium, barium, and manganese in estuaries: implications for reconstructing environmental histories of fish from chemicals in calcified structures. Estuarine, Coastal and Shelf Science 66(1):147-156.

Gentry, D. E. 2006. Evaluation of striped bass, *Morone saxatilis*, condition in coastal North Carolina using physiological and condition indices. Master's Thesis. East Carolina University, Greenville, NC.

Gillanders, B. 2001. Trace metals in four structures of fish and their use for estimates of stock structure.

Guay, C. K., and K. Kenison Falkner. 1998. A survey of dissolved barium in the estuaries of major Arctic rivers and adjacent seas. Continental Shelf Research 18(8):859-882.

Haeseker, S. L., J.T. Carmichael, and J.E. Hightower. 1996. Summer distribution and condition of striped bass within Albemarle Sound, North Carolina. Transactions of the American Fisheries Society 125(5):690-704.

Halden, N. M., and L. A. Friedrich. 2008. Trace-element distributions in fish otoliths: natural markers of life histories, environmental conditions and exposure to tailings effluence. Mineralogical Magazine 72(2):593-593.

Heinimaa, S. 2004. Seasonal changes of liver glycogen content and condition factor of wild Atlantic salmon parr in a sub‑arctic river. Ecology of Freshwater Fish 13(4):323-326.

Kraus, R. T., and D. H. Secor. 2004. Incorporation of strontium into otoliths of an estuarine fish. Journal of Experimental Marine Biology and Ecology 302(1):85-106.

Kutkuhn, J. H. 1981. Stock definition as a necessary basis for cooperative management of Great Lakes fish resources. Canadian Journal of Fisheries and Aquatic Sciences 38(12):1476-1478.

Limburg, K. E., and D. I. Seigel. 2006. The hydrogeochemistry of connected waterways: The potential of linking geology to fish migrations. Northeastern Geology and Environmental Sciences 28(3):254-265.

Lin, S., C. Chang, Y. Iizuka, and W. Tzeng. 2007. Salinities, not diets, affect strontium/calcium ratios in otoliths of *Anguilla japonica*. Journal of Experimental Marine Biology and Ecology 341(2):254-263.

Mallin, M. A. 1991. Zooplankton abundance and community structure in a mesohaline North Carolina estuary. Estuaries 14(4):481-488.

Mallin, M. A., and H. W. Paerl. 1992. Effects of variable irradiance on phytoplankton productivity in shallow estuaries. Limnology and Oceanography 37(1):54-62.

Mohan, J. A., R. A. Rulifson, D. R. Corbett, and N. M. Halden. 2012. Validation of oligohaline elemental otolith signatures of striped bass by use of in situ caging experiments and water chemistry. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 4(1):57-70.

Mohan, J. 2009. Habitat utilization of juvenile striped bass *Morone saxatilis* in Albemarle Sound inferred from otolith and water chemistries. Master's Thesis. East Carolina University, Greenville, NC.

Morris, J. A., R. A. Rulifson, and L. H. Toburen. 2003. Life history strategies of striped bass, *Morone saxatilis*, populations inferred from otolith microchemistry. Fisheries Research 62(1):53-63.

NCDMF. 2004. North Carolina estuarine striped bass fishery management plan. North Carolina Division of Marine Fisheries and North Carolina Wildlife Resources Commission, Report 375, Morehead City, North Carolina.

North Carolina Division of Water Resources and Engineering. 1952. Hydrologic data on the Roanoke and Tar River basins, 1871-1945. United States Geological Survey, Report 931439, Raleigh, North Carolina.

North Carolina Division of Water Resources and Engineering. 1947. Hydrologic data on the Neuse river basin, 1866-1945. United States Geological Survey, Report 835550, Raleigh, North Carolina.

Olsen, E.J., and R. A. Rulifson. 1992. Maturation and fecundity of Roanoke River-Albemarle Sound striped bass. Transactions of the American Fisheries Society 121(4):524-537.

Paramore, L.M., and R.A. Rulifson. 2001. Dorsal coloration as an indicator of different life history patterns for striped bass within a single watershed of Atlantic Canada. Transactions of the American Fisheries Society 130(4):663-674.

Rätz, H., and J. Lloret. 2003. Variation in fish condition between Atlantic cod (*Gadus morhua*) stocks, the effect on their productivity and management implications. Fisheries Research 60(2):369-380.

Secor, D. H., and J. M. Dean. 1992. Comparison of otolith-based back-calculation methods to determine individual growth histories of larval striped bass, *Morone saxatilis*. Canadian Journal of Fisheries and Aquatic Sciences 49(7):1439-1454.

Sundby, B., L. G. Anderson, P. O. J. Hall, Å Iverfeldt, M. M. R. van der Loeff, and S. F. G. Westerlund. 1986. The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface. Geochimica Et Cosmochimica Acta 50(6):1281-1288.

Thorrold, S. R., C. M. Jones, S. E. Campana, and J. W. McLaren. 1998a. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). Limnology and Oceanography 43(8):1826-1835.

Thorrold, S., C. Jones, P. Swart, and T. Targett. 1998b. Accurate classification of juvenile weakfish, *Cynoscion regalis*, to estuarine nursery areas based on chemical signatures in otoliths. Marine Ecology Progress Series 173:253-265.

Tomás, J., S. Augagneur, and E. Rochard. 2005. Discrimination of the natal origin of young‑of‑the‑year Allis shad (*Alosa alosa*) in the Garonne–Dordogne basin (south‑west France) using otolith chemistry. Ecology of Freshwater Fish 14(2):185-190.

U.S. Fish and Wildlife. 1978. Development of fishes of the Mid-Atlantic Bight : an atlas of egg, larval, and juvenile stages. Department of the Interior, United States Fish and Wildlife Service, Office of Biological Services, Washington, District of Columbia.

Vasconcelos, R. P., P. Reis-Santos, V. Fonseca, M. Ruano, S. Tanner, M. J. Costa, and H. N. Cabral. 2009. Juvenile fish condition in estuarine nurseries along the Portuguese coast. Estuarine, Coastal and Shelf Science 82(1):128-138.

Volk, E., A. Blakley, S. L. Schroder, and S. M. Kuehner. 2000. Otolith chemistry reflects migratory characteristics of Pacific salmonids: Using otolith core chemistry to distinguish maternal associations with sea and freshwaters. Fisheries Research 46(1-3):251-266.

Walther, B.D., and S.R. Thorrold. 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. Marine Ecology-Progress Series 311:125-130.

Wells, B.K., B.E. Rieman, J.L. Clayton, D.L. Horan, and C.M. Jones. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 132(3):409-424.

Woodroffe, J. R. 2012. Historical ecology of striped bass stocking in the southeastern United States. Master's Thesis. East Carolina University, Greenville, N.C.

Woods, T. L. 2000. Geochemical tracers of groundwater movement between the Castle Hayne and associated coastal plain aquifers. Water Resources Research Institute of The University of North Carolina, Report 70148, Greenville, NC.

Zapf, D. H. 2012. River herring nursery habitat in Albemarle Sound, North Carolina, inferred from otolith microchemistry. Master's Thesis. East Carolina University, Greenville, NC.

Table 1. – Number of fish collected from the Tar/Pamlico and Neuse rivers each season from spring 2011 to spring 2012.

River	Spring 2011	Summer 2011	Fall 2011	Winter 2012	Spring 2012	Total
Tar/Pamlico	15	20	21	19	60	135
Neuse	25	13	9	18	51	116
Total	40	33	30	37	111	251

Table 2. – Results of Wilcoxon and Kruskal-Wallis tests examining the statistical difference in Wr, LSI, and GSI between river, season, year and year class of fish collected from the Neuse and Tar/Pamlico rivers from Spring 2011 to spring 2012. For each one-way analysis α was set at 0.05. Asterisks represent statistically significant p values. [1] Only fish capture in spring season were used to examine GSI between year classes.

Variable	Effect	chi-square	df	p
Wr	River	7.5221	1	0.0061*
	Season	41.2937	4	<.0001*
	Year	14.1477	1	0.0002*
	Year Class	24.1555	8	0.0022*
LSI	River	0.0020	1	0.9645
	Season	42.8061	4	<.0001*
	Year	19.1369	1	<.0001*
	Year Class	33.1529	8	<.0001*
GSI	River	0.4659	1	0.4949
	Season	95.0428	4	<.0001*
	Year	3.7137	1	0.054
	Year Class[1]	22.5751	6	0.0010*

Table 3. – Results of Wilcoxon test examining the statistical differences in Wr of striped bass collected from the Tar/Pamlico River between results from my study and Gentry (2006). For each one-way analysis α was set at 0.05. Asterisks represent statistically significant p values.

Comparison	chi-square	df	p
Spring 2005 vs. Spring 2011	4.1848	1	0.0408*
Spring 2005 vs. Spring 2012	0.6876	1	0.4070
Summer 2005 vs. Summer 2011	1.6662	1	0.1968
Winter 2005 vs. Winter 2012	0.1140	1	0.7357

Table 4. – Number and percent of hatchery and natural striped bass collected from the Neuse and Tar/Pamlico rivers for each season from spring 2011 to spring 2012. * 2 fish collected during spring 2011 from the Neuse River were never analyzed.

Season	River	Hatchery	% Hatchery	Natural	% Natural	Total
Spring 2011	Tar/Pamlico	12	80.0	3	20.0	15
	Neuse*	21	91.3	2	8.7	23
Summer 2011	Tar/Pamlico	16	80.0	4	20.0	20
	Neuse	12	92.3	1	7.7	13
Fall 2011	Tar/Pamlico	21	100.0	0	0.0	21
	Neuse	9	100.0	0	0.0	9
Winter 2012	Tar/Pamlico	18	94.7	1	5.3	19
	Neuse	16	88.9	2	11.1	18
Spring 2012	Tar/Pamlico	52	86.7	8	13.3	60
	Neuse	43	84.3	8	15.7	51
Total		220	88.4	29	11.6	249

Table 5. – Results of Kruskal-Wallis and Wilcoxon tests examining the statistical differeneces in Sr, Mg, Mn, and Ba concentrations of the natal origin region of otoliths from natural fish between seasons, rivers, management areas, and year classes. For each one-way analysis α was set at 0.05. Asterisks represent statistically significant p values. [1] Season and year class examininations were limited to CSMA fish only.

Variable	Effect	chi-squared	df	p
Sr	Season[1]	8.4165	5	0.1347
	River	3.4339	2	0.1796
	Management Area	3.3976	1	0.0653
	Year Class[1]	15.5278	6	0.0165*
Mg	Season[1]	12.4193	5	0.0295*
	River	0.8213	2	0.6632
	Management Area	0.0212	1	0.8843
	Year Class[1]	8.1357	6	0.2283
Mn	Season[1]	2.7716	5	0.7351
	River	5.8422	2	0.0539
	Management Area	3.8594	1	0.0495*
	Year Class[1]	10.291	6	0.1129
Ba	Season[1]	8.1318	5	0.1491
	River	14.858	2	0.0006*
	Management Area	14.6847	1	0.0001*
	Year Class[1]	9.3575	6	0.1545

Table 6. – Results of quadratic discriminant function analysis used to classify natal origin region of the otolith elemental signatures of natural fish to river of origin.

Location	N	Neuse	Roanoke	Tar	% Correct
Neuse	24	9	3	12	37.5
Roanoke	10	1	8	1	80.0
Tar	16	3	1	12	75.0

Table 7. – Results of quadratic discriminant function analysis used to classify natal origin region of the otolith elemental signatures of natural fish to management area of origin. Management areas were abbreviated as follows: Albemarle Sound Management Area (ASMA), Central Southern Management Area (CSMA).

Location	N	ASMA	CSMA	% Correct
ASMA	10	8	2	80
CSMA	40	6	34	85
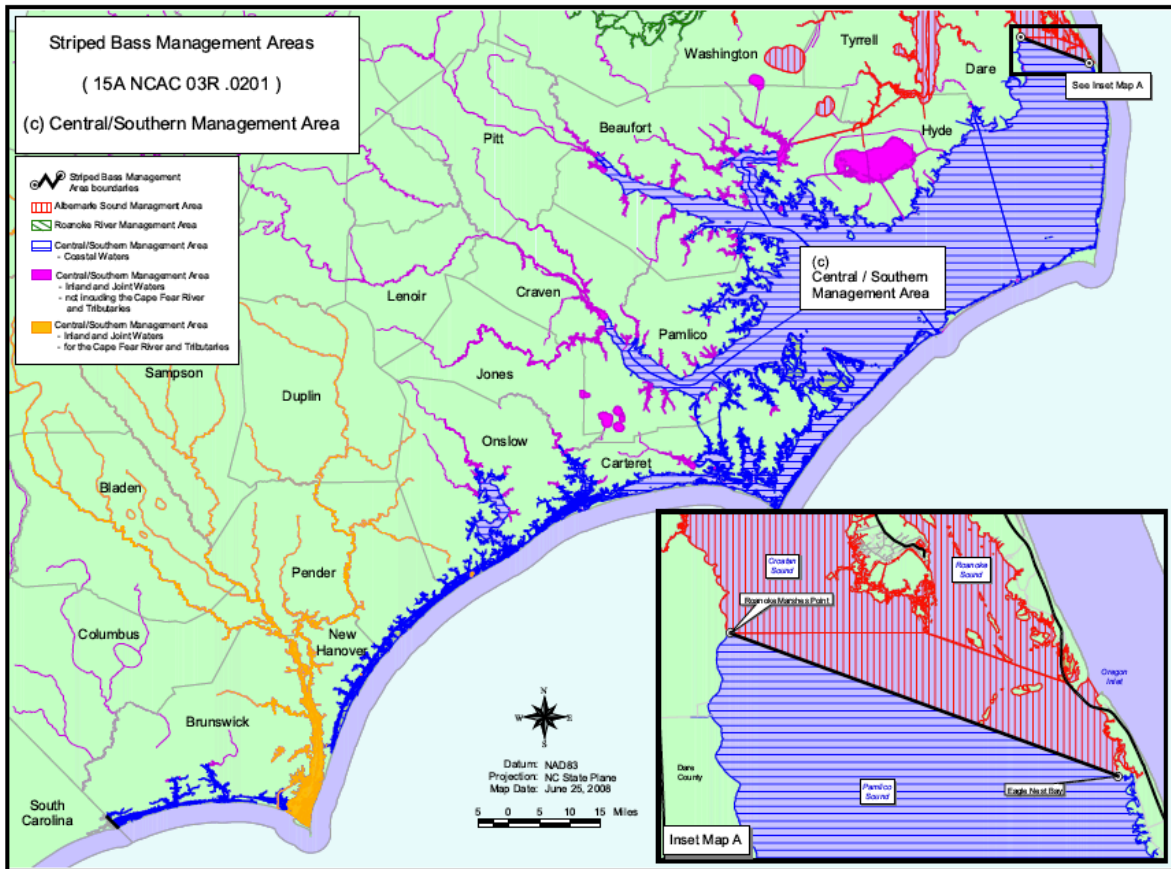
Figure 1. – Map of North Carolina Department of Environment and Natural Resources'
Central Southern Management Area. (Map from NCDENR)
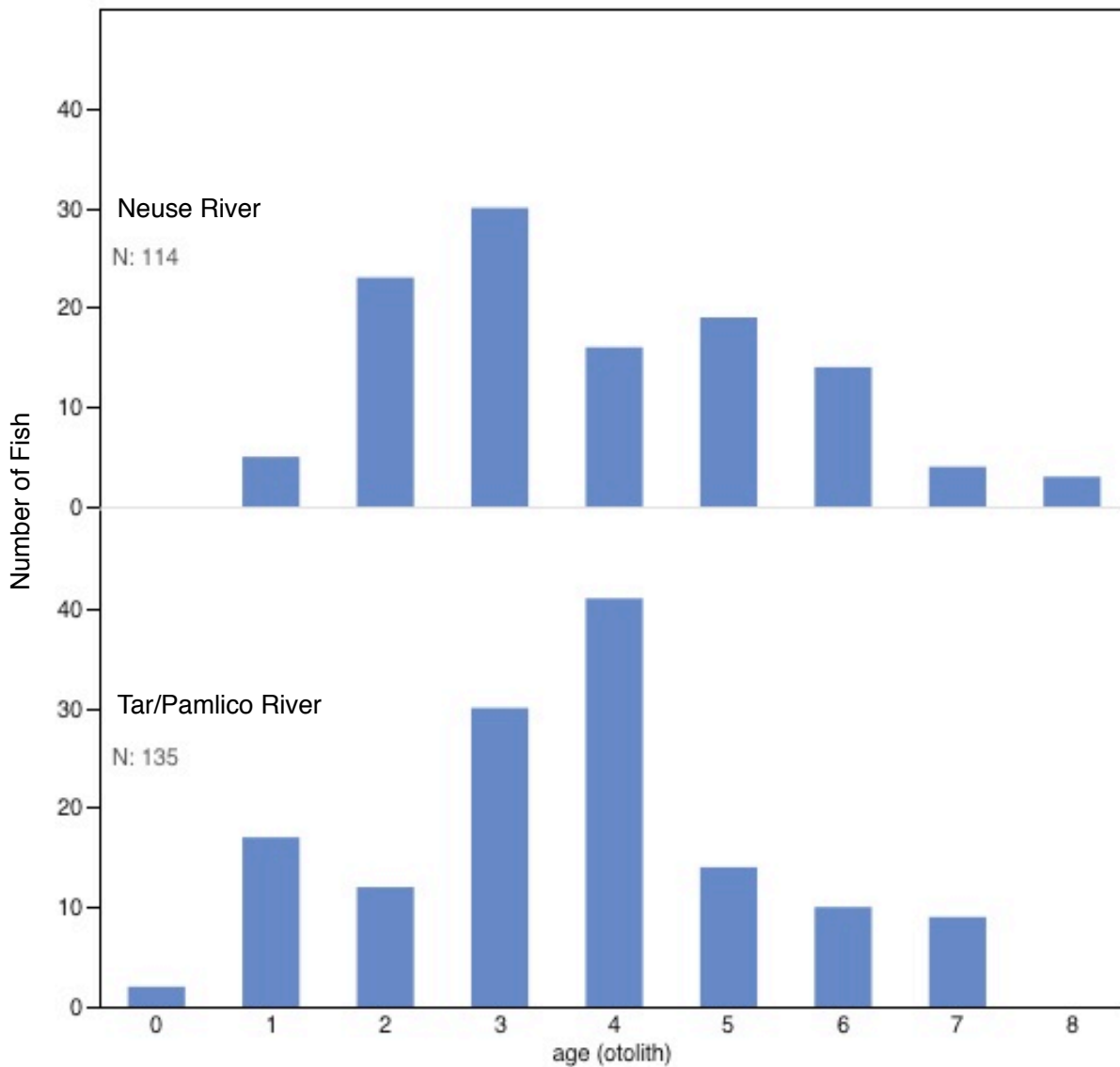
Figure 2. – Histogram of ages of fish age at captured for the Neuse and Tar/Pamlico rivers.
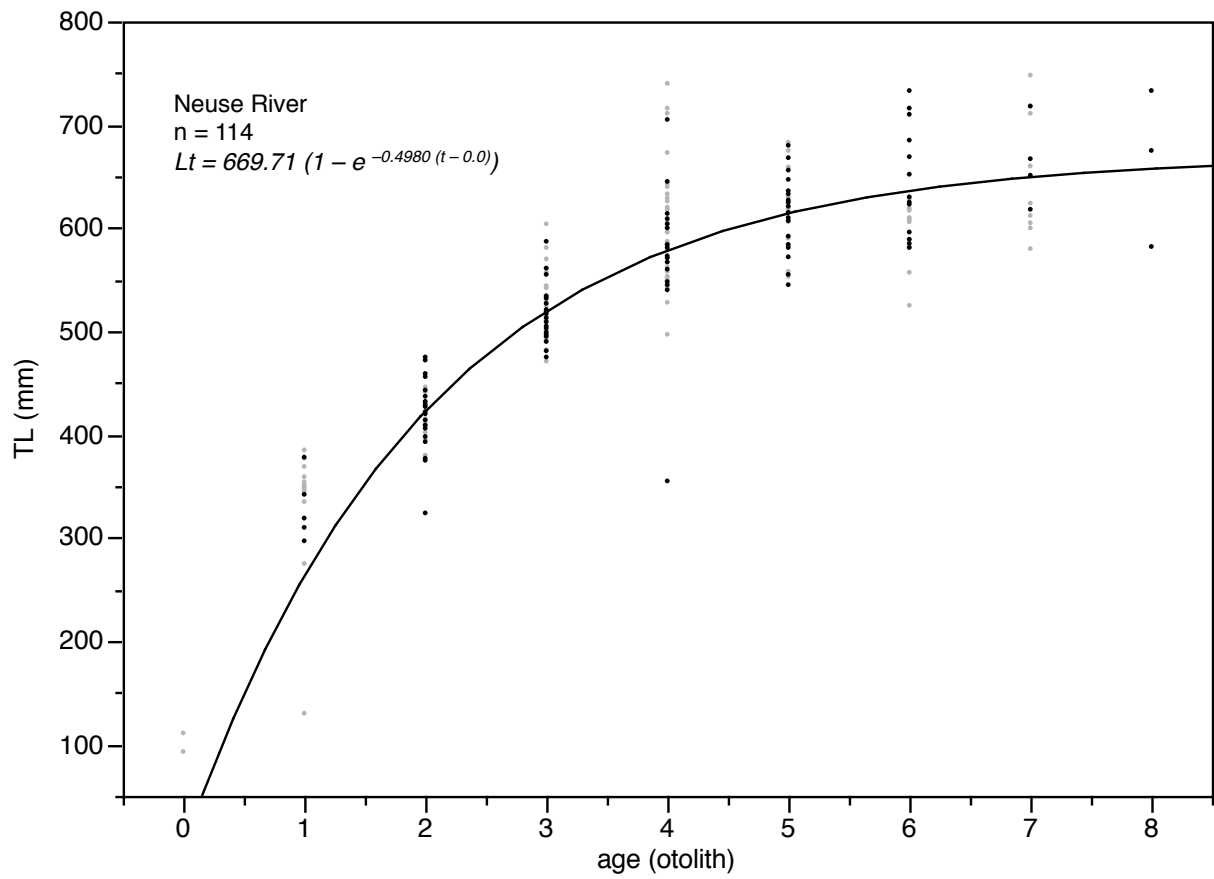
Figure 3. –Von Bertalanffy growth curve for Neuse River fish collected from spring 2011 to spring 2012.
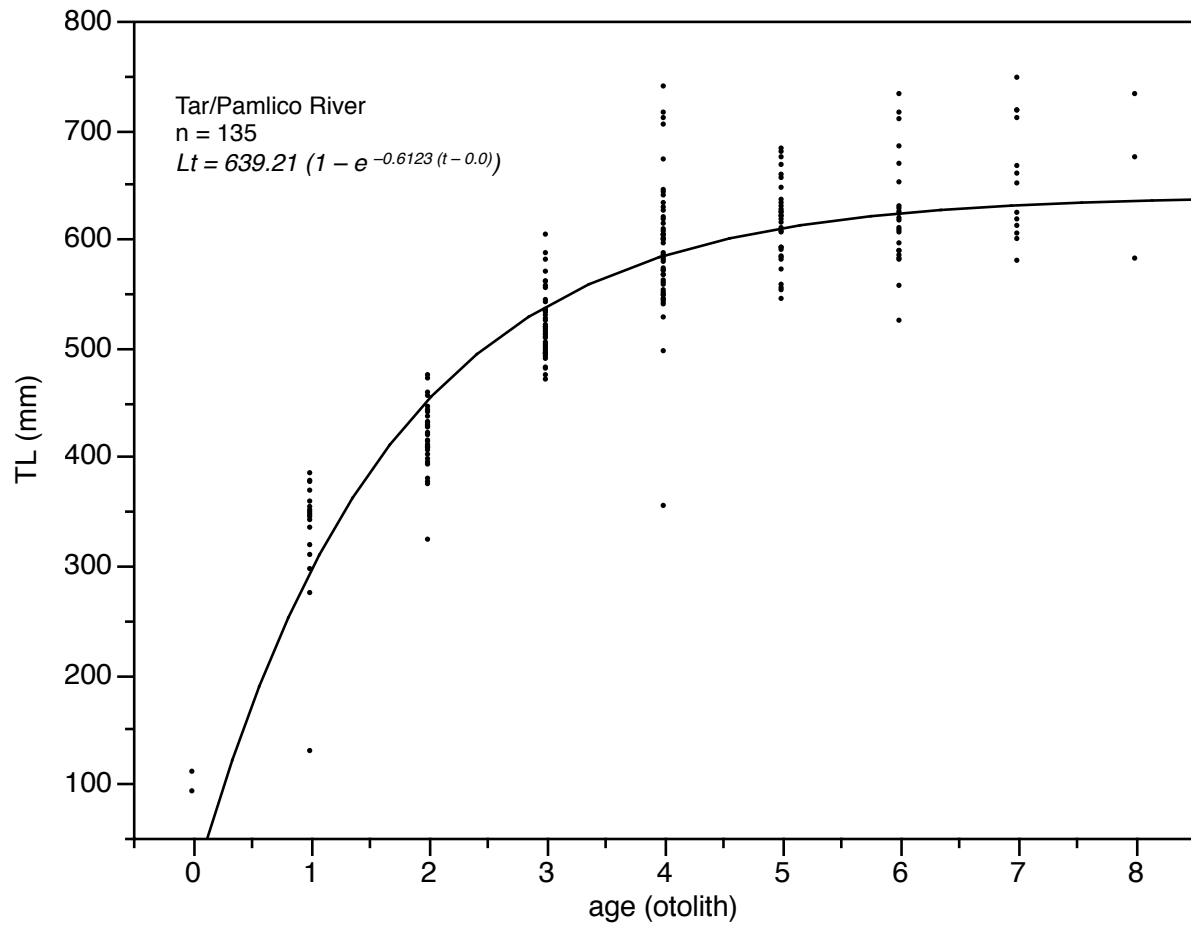
Figure 4. – Von Bertalanffy growth curve for Tar/Pamlico River fish collected from spring 2011 to spring 2012.
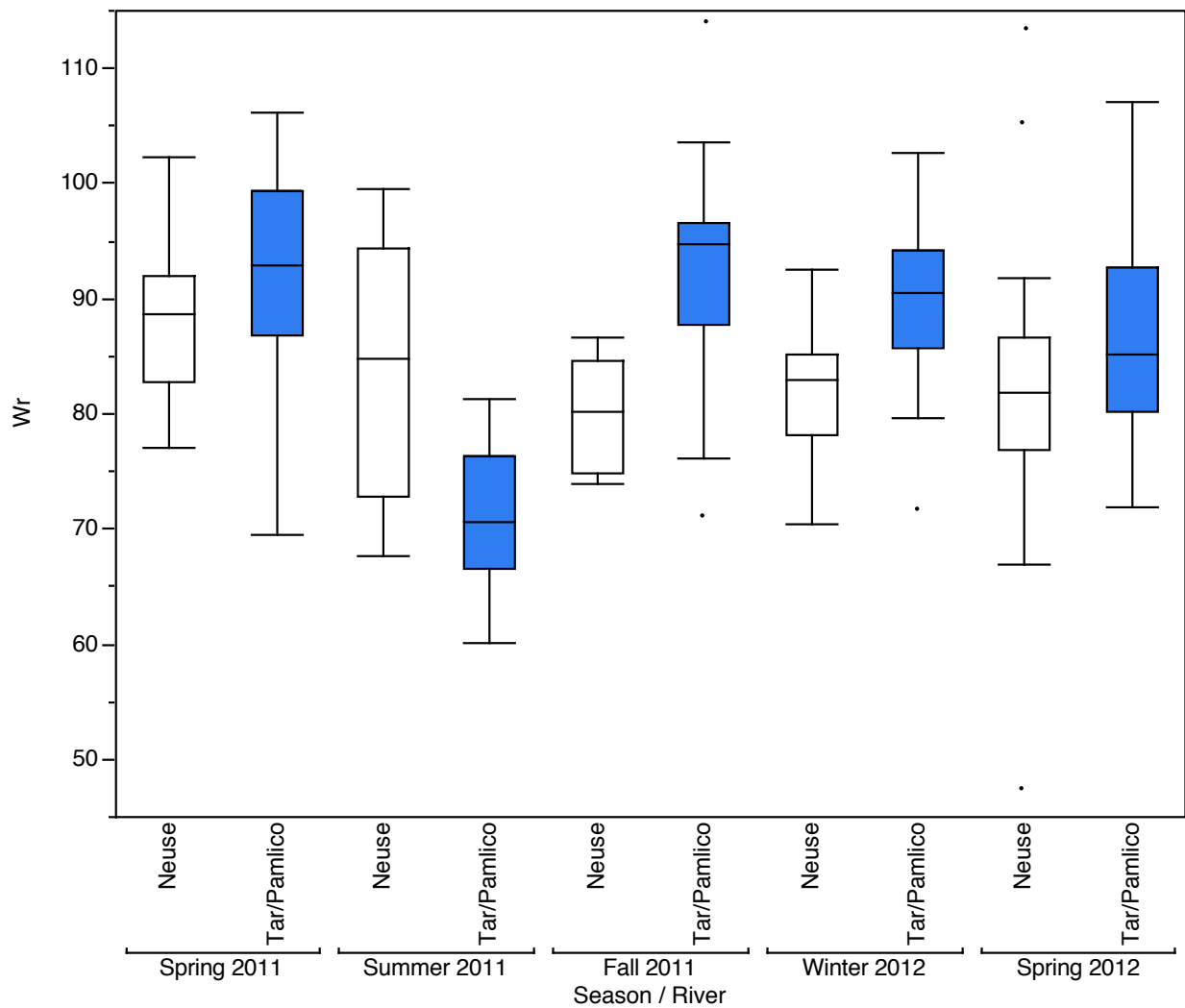
Figure 5. – Box and whisker plots showing Wr of striped bass collected from the Neuse and Tar/Pamlico rivers from spring 2011 to spring 2012. Blue box plots represent Tar/Pamlico River, and white box plots represent Neuse River. Horizontal line within box = median; lower and upper edges of box = 25th and 75th percentiles; ends of whiskers = 10th and 90th percentiles; points = outliers.
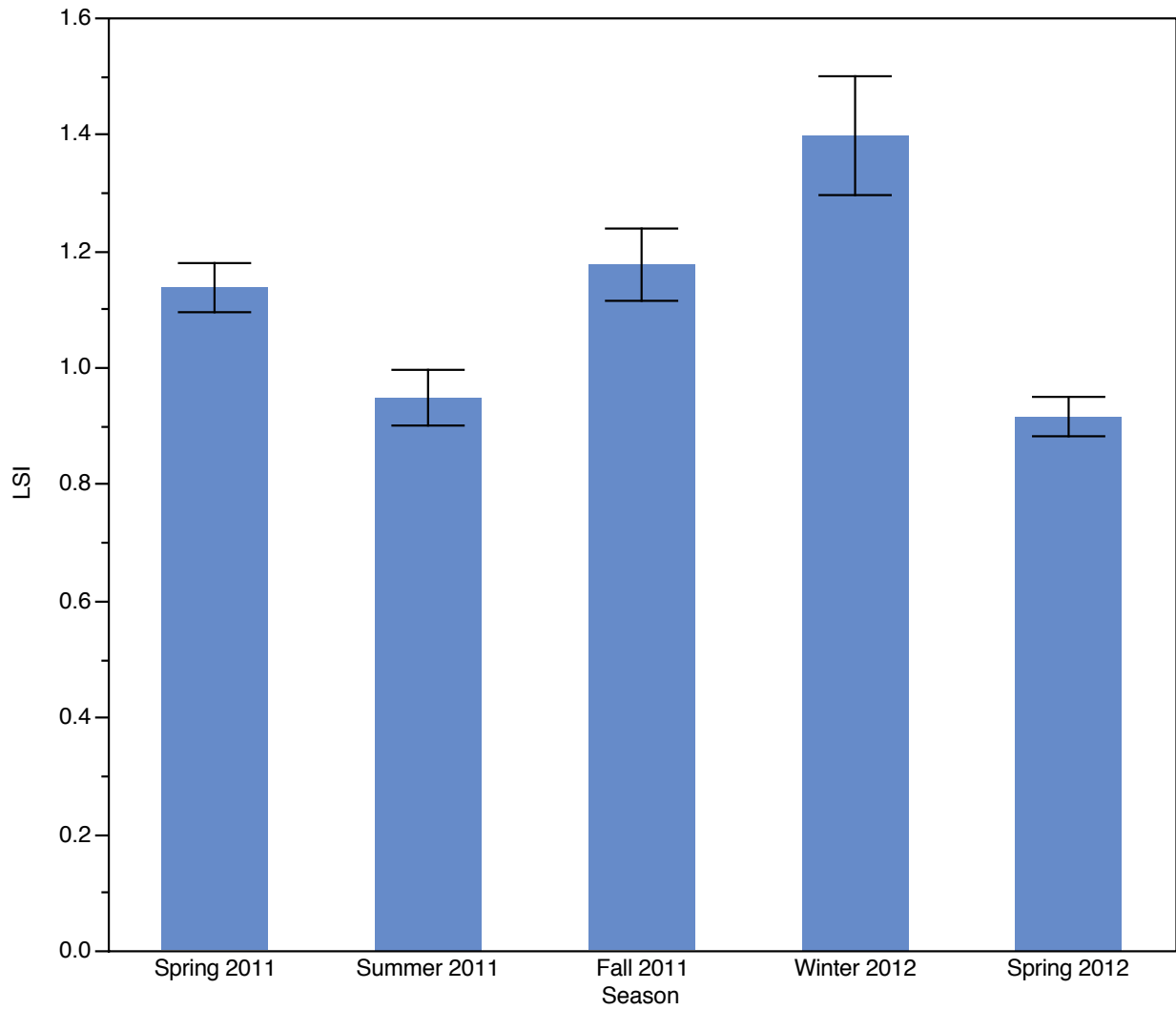
Figure 6. – Mean (± S.E.) LSI by season for fish collected from Neuse (n=90) and Tar/Pamlico (n=78) rivers.
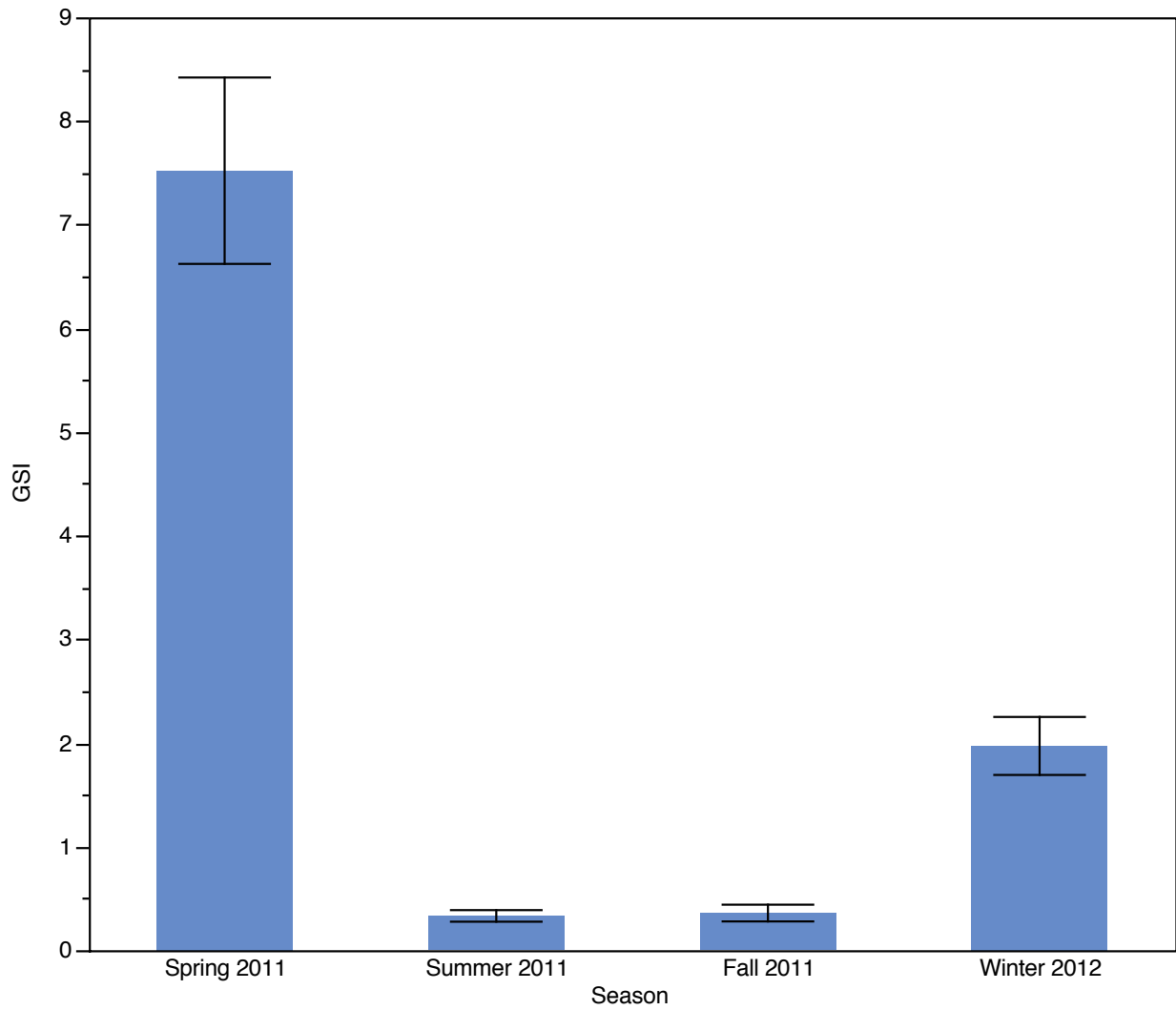
Figure 7. – Mean (± S.E.) GSI by season for fish collected in the Neuse (n=56) and Tar/Pamlico (n=54) rivers from spring 2011 to winter 2012.
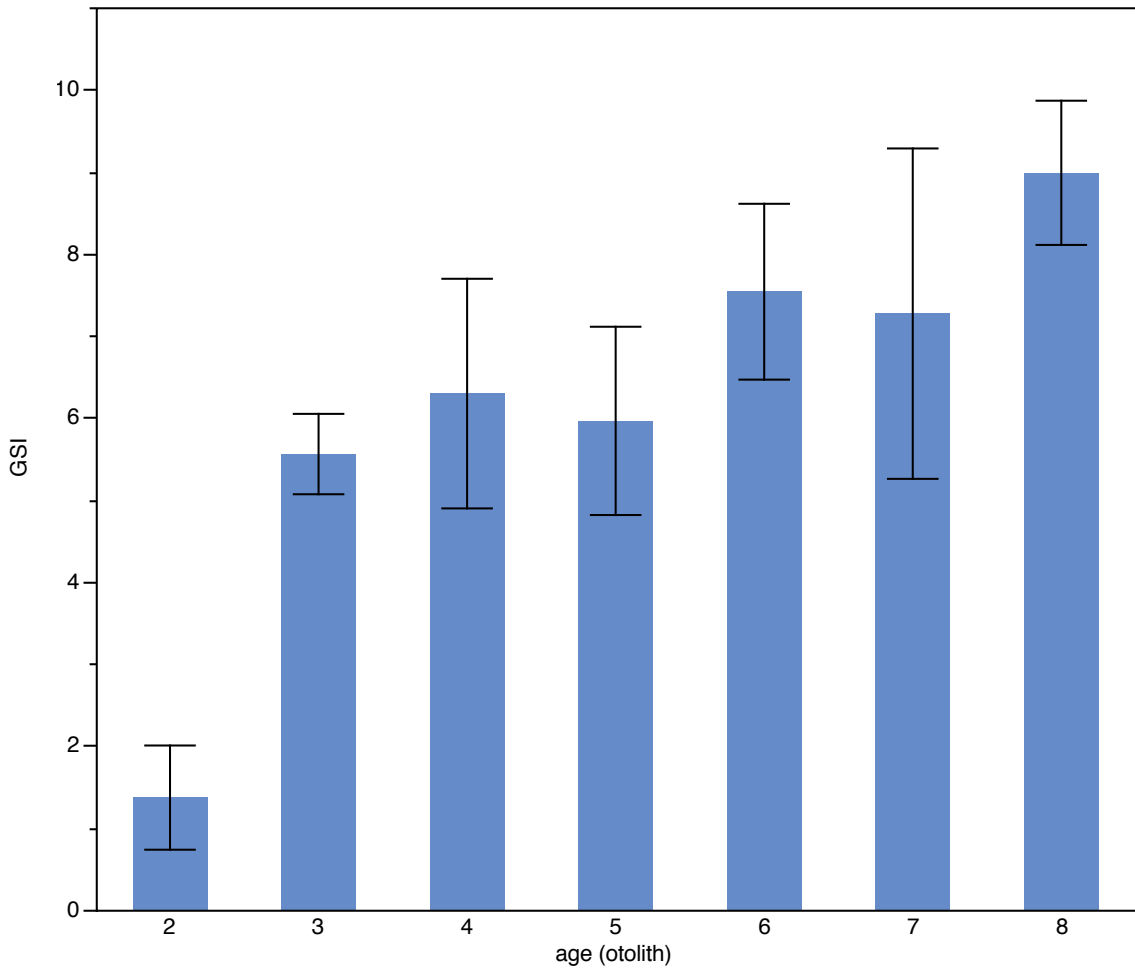
Figure 8. – Mean (±) GSI by age for fish collected in the Neuse and Tar/Pamlico rivers.
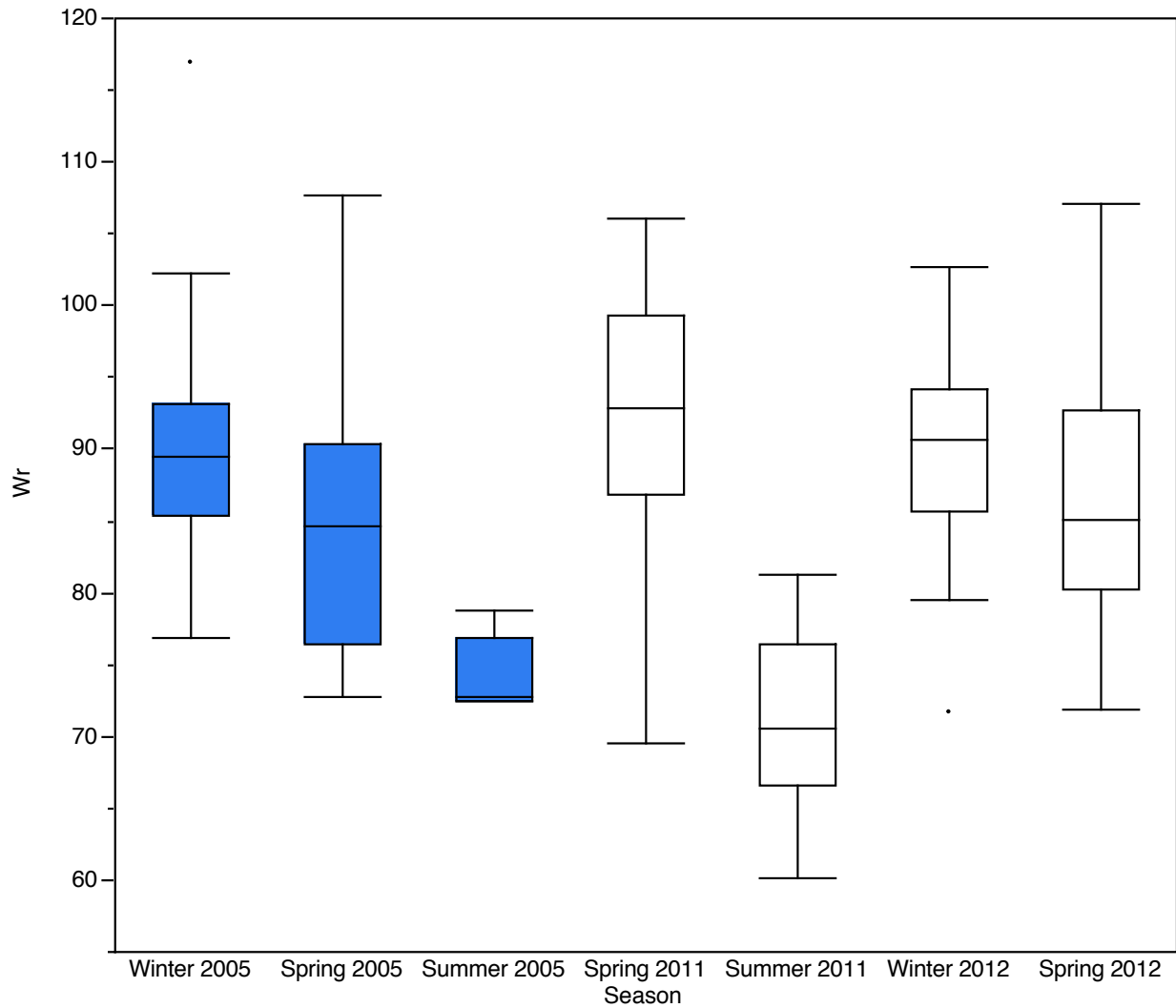
Figure 9. – Box and whisker plots comparing Wr of Tar/Pamlico fish collected from my study (n=113) with fish collected in Gentry (2006) (n=49). Blue box plots represent Gentry (2006), and white box plots represent this study. Horizontal line within box = median; lower and upper edges of box = 25th and 75th percentiles; ends of whiskers = 10th and 90th percentiles; points = outliers.
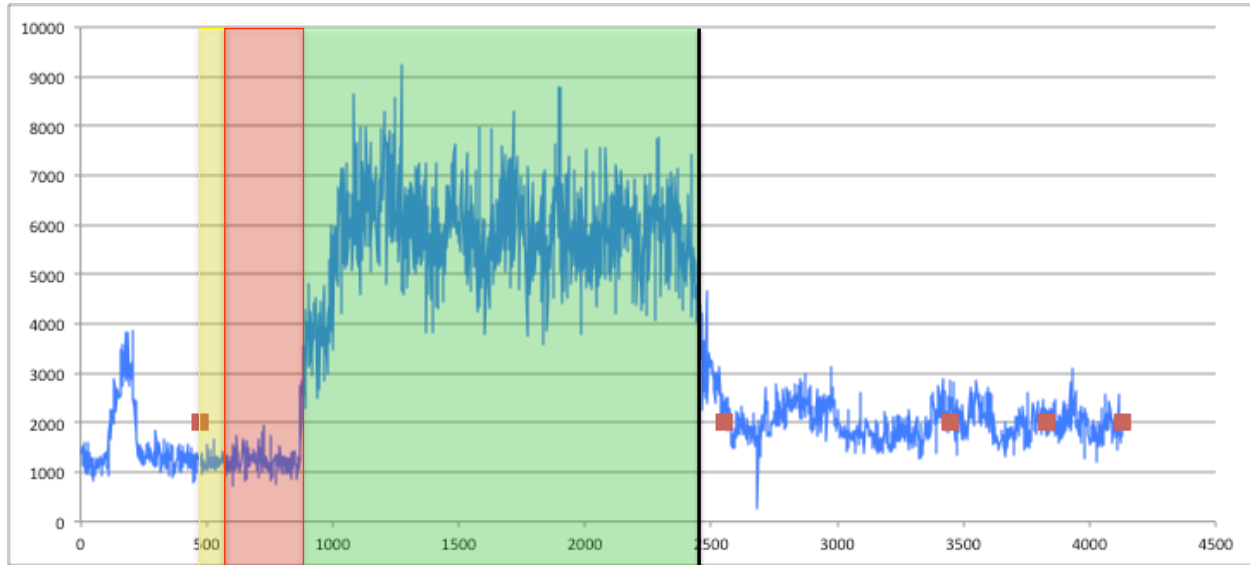
Figure 10. – Prototypical hatchery fish Sr signature. Red squares represent otolith focus (farthest left) and annuli, yellow area represents maternal contribution period, red area represents phase I period, green area represents phase II period, and black line represents stocking event.
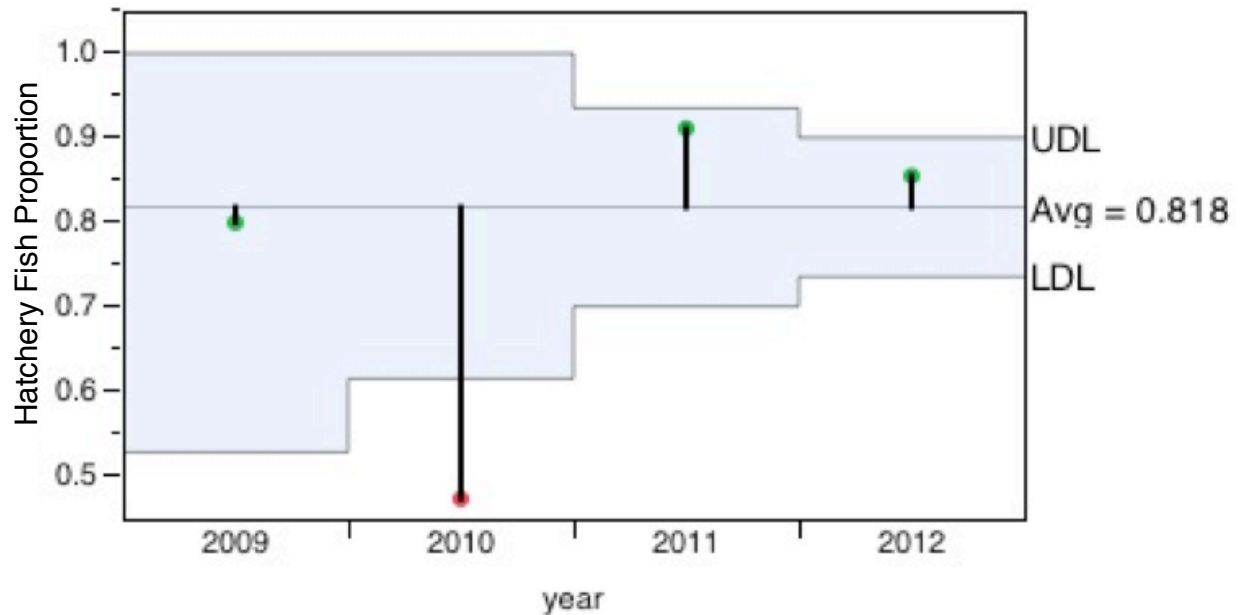
Figure 11. – Analysis of means for proportion of hatchery fish. Years 2009 and 2010 are samples previously collected by the NCWRC, while 2011 and 2012 are fish collected from my study. Dots represent yearly proportion means, horizontal line represents total proportion mean (0.818), vertical lines represent difference from total proportion mean, red dots represent statistically different proportion, shaded area represents decision limit for test, UDL is the upper decision limit, LDL is the lower decision limit.
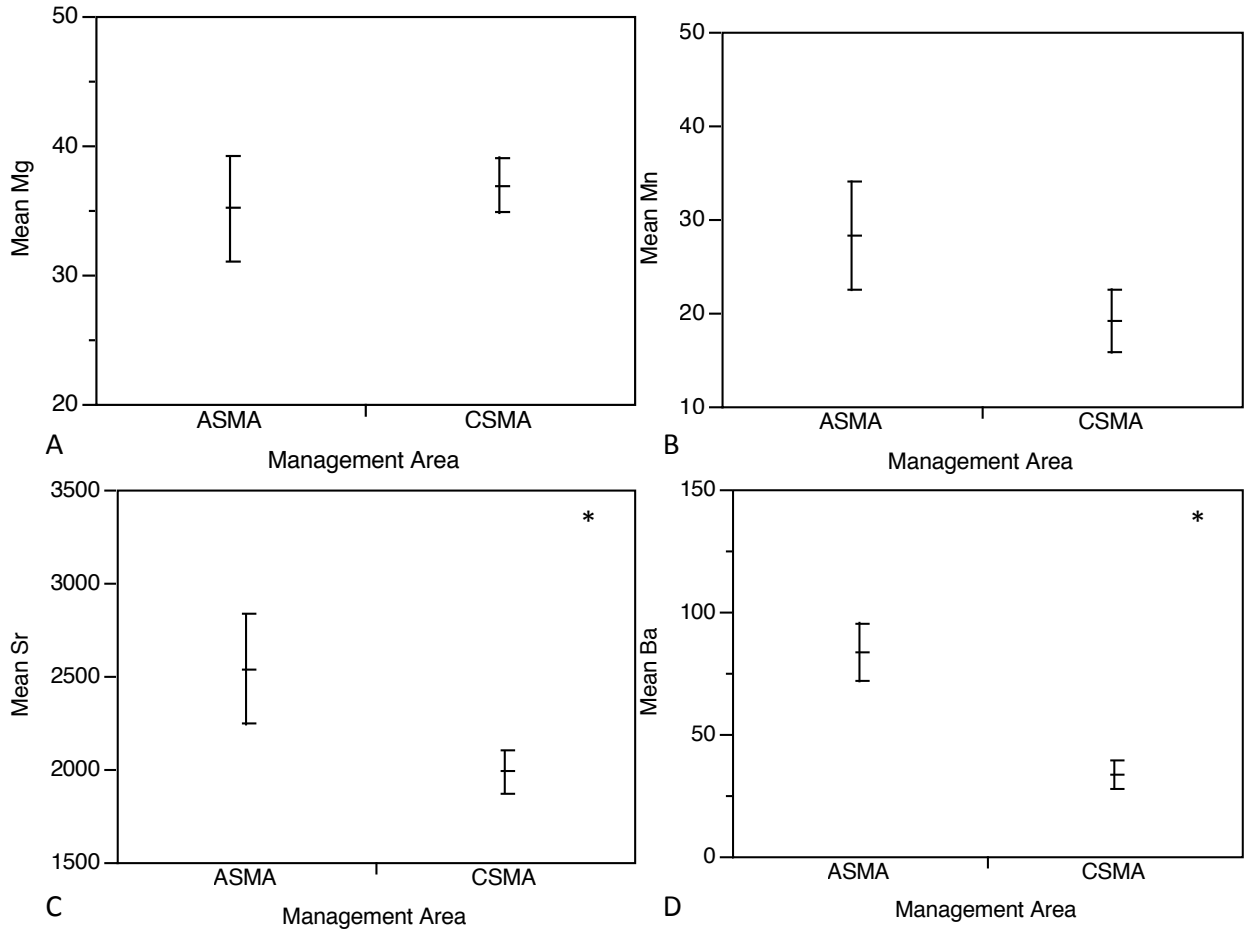
Figure 12. - Mean (± S.E.) Mg (A), Mn (B), Sr (C), and Ba (D) in the natal origin region of the otoliths of striped bass collect from the CSMA and ASMA. Asterisks represent element concentrations that are significantly different.
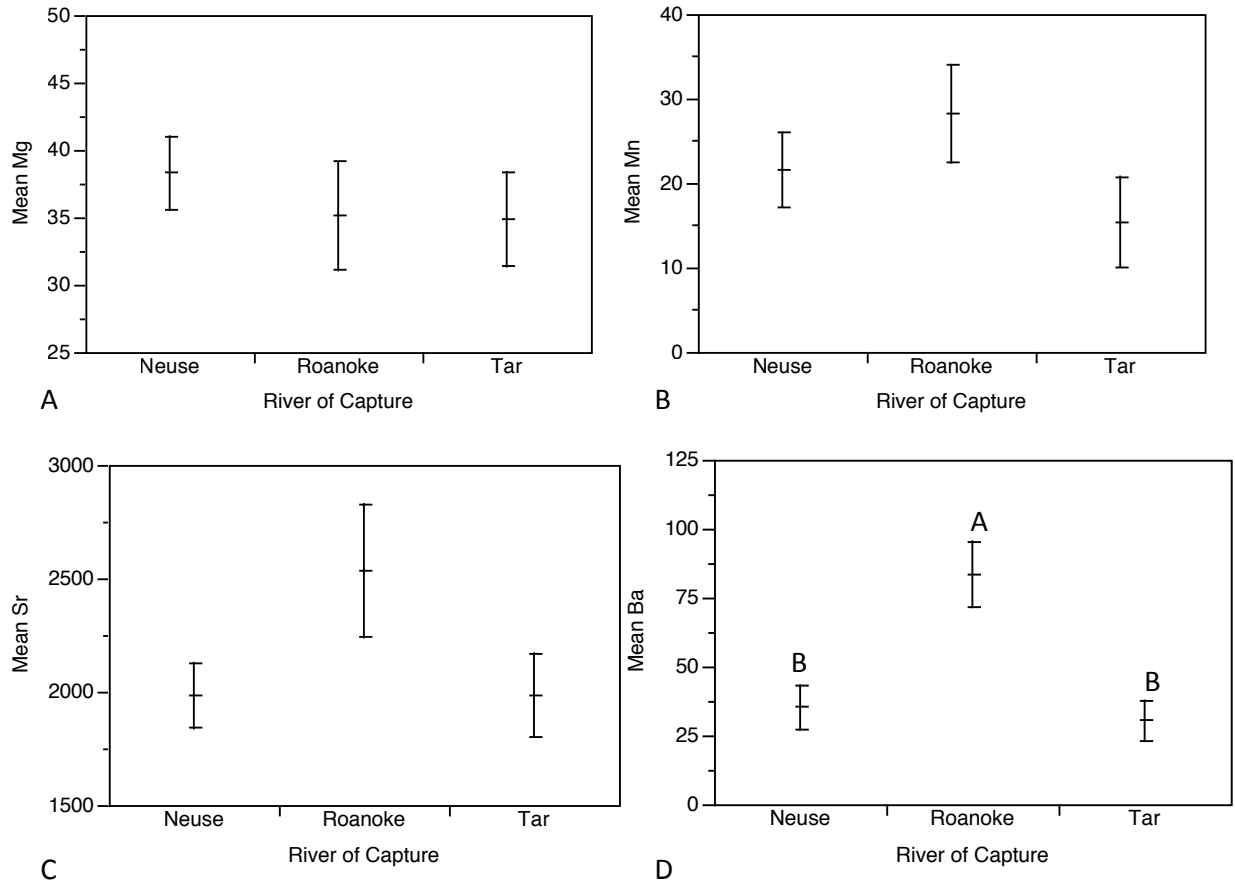
Figure 13. – Mean (± S.E.) Mg (A), Mn (B), Sr (C), and Ba (D) in the natal origin region of the otoliths of striped bass collect from the Neuse, Tar/Pamlico (Tar), and Roanoke rivers. In graph D differing letters (above bars) indicate significant differences between rivers (Tukeys HSD tests after Kruskal–Wallis test).
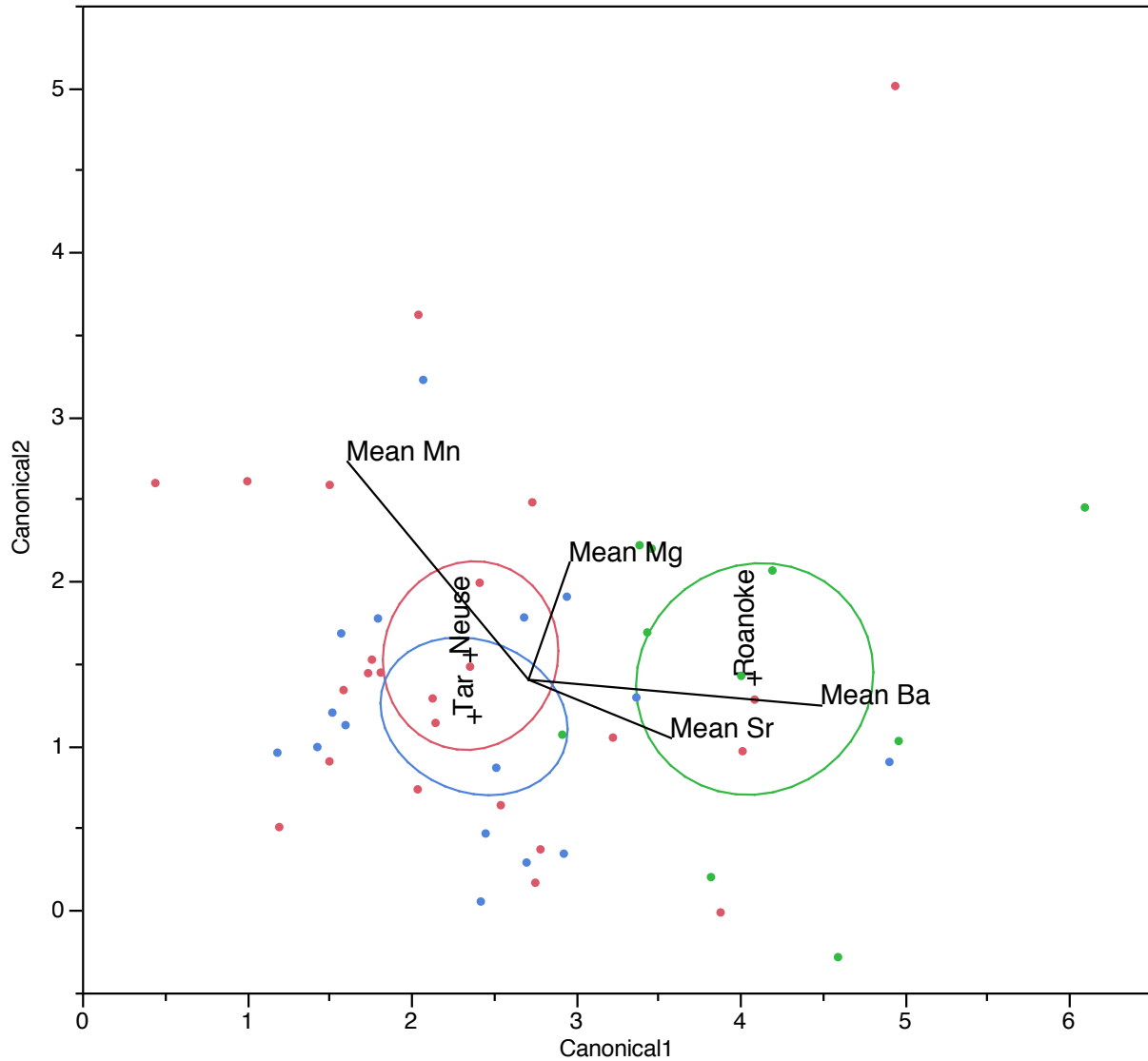
Figure 14. – Canonical correspondence plot showing results from quadratic discriminant function analysis to classify elemental signatures in the natal origin region of the otolith to river of origin using Sr, Mg, Mn, and Ba. Tar/Pamlico (Tar)= blue, Neuse (Nesue)= red, and Roanoke (Roanoke)= green. Group centroids are marked with (+), ellipses represent 95% confidence ellipses for each sample site.
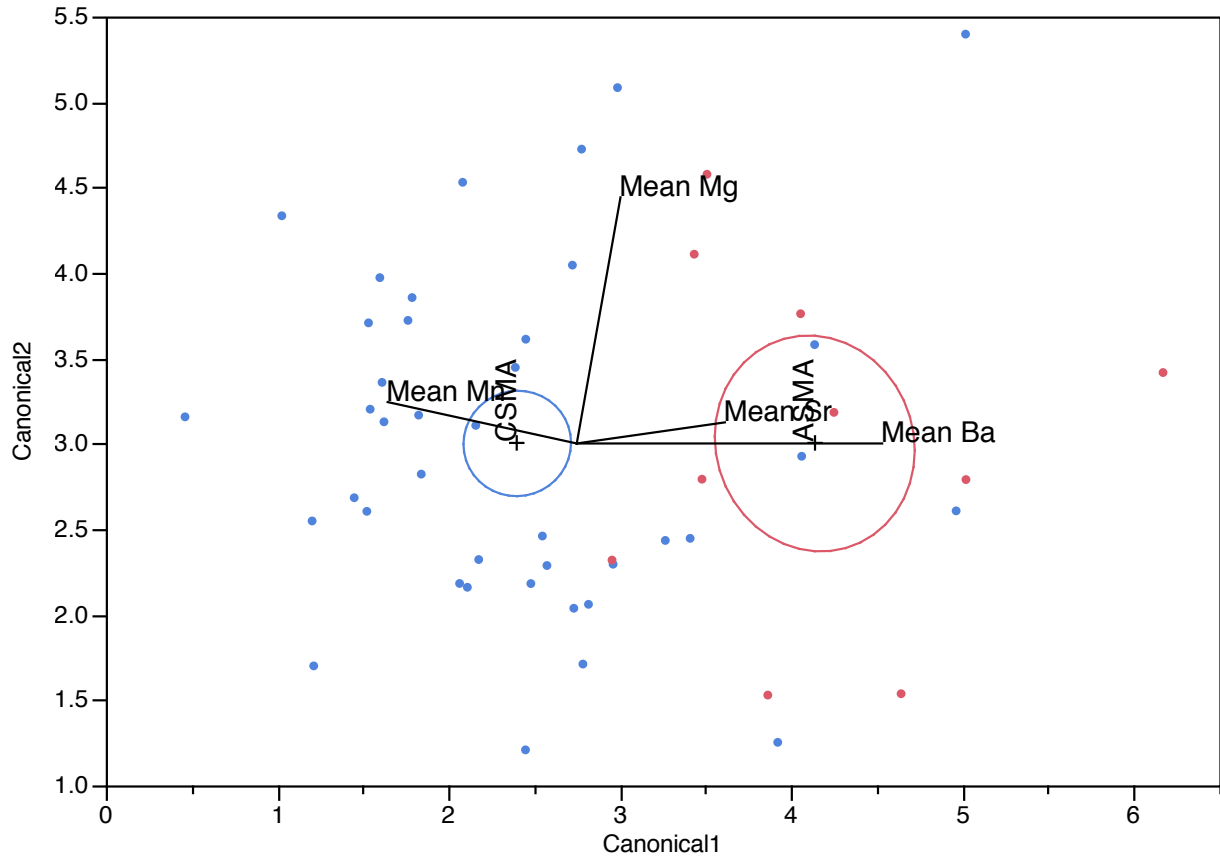
Figure 15. – Canonical correspondence plot showing results from quadratic discriminant function analysis to classify elemental signatures in the natal origin region of the otolith to management area of origin using Sr, Mg, Mn, and Ba. CSMA= blue, and ASMA= red. Group centroids are marked with (+), ellipses represent 95% confidence ellipses for each sample site.

East Carolina University.
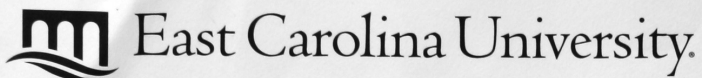
Animal Care and
Use Commitee

212 Ed Warren Life
Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

July 15, 2011

North Carolina Marine
  Resources Fund

The following application submitted to the North Carolina Marine Resources Fund was
reviewed and approved by this institution's Animal Care and Use Committee:

Title of Application: "Origin of Central Southern Management Area Striped Bass Using
                         Otolith Chemistry, and Recommendations for Fishery
                         Management"

Name of Principal Investigator: Roger Rulifson, Ph.D.

Name of Institution: East Carolina University

Date of Approval: July 15, 2011

This institution is fully accredited by AAALAC and has an Animal Welfare Assurance on
file with the Office of Laboratory Animal Welfare. The Assurance Number is A3469-01.

Sincerely yours,

Scott E. Gordon, Ph.D.
Chairman, Animal Care and Use Committee

SEG/jd

*East Carolina University is a constituent
institution of the University of North
Carolina. An equal opportunity university.*