Movements of North Carolina Striped Bass, *Morone saxatilis*, Inferred through Otolith Microchemistry

by

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Abstract

Striped Bass, *Morone saxatilis*, is an anadromous, recreationally and commercially important fish species found throughout the U.S. Atlantic east coast, whose migrations have been intensely studied. A review of the relevant literature on Striped Bass migrations revealed that the paradigm of Striped Bass migration along the U.S. Atlantic coast should be updated, as new information has shown that in addition to the Chesapeake Bay and Hudson River, the Roanoke River, NC, Delaware River, and possibly the Shubenacadie River, Nova Scotia, Canada, may also contribute fish to the mixed Atlantic Migratory Stock. The needs for an updated delineation of stocks that contribute to the Atlantic Migratory Stock and determination of inshore migrations and habitat use were identified as additional avenues for further research. In effort to answer the latter question, the inshore movements and potential mixing of North Carolina stocks of adult Striped Bass from separate management areas (Albemarle Sound Management Area, ASMA, and Central Southern Management Area, CSMA) were determined using otolith microchemistry. Trace element ratios (strontium:calcium, Sr:Ca; barium:calcium, Ba:Ca; magnesium:calcium, Mg:Ca; and manganese:calcium, Mg:Ca) measured through inductively coupled plasma optical emission spectrometry (ICP-OES) from ASMA and CSMA water samples were used to

determine that each management area had different water chemistries through linear discriminant function analysis (LDFA), allowing for discrimination of otolith chemistries of fish from different management areas. Adult otolith elemental concentrations of Sr, Ba, Mg, and Mn, measured using laser ablation inductively couple plasma mass spectroscopy, of fish from separate management areas were compared using linear discriminant function analysis, which determined that little mixing of adult fish occurred between the two management areas, except in years of high abundance of ASMA fish, in which those fish would migrate to the CSMA. The same methods were used on CSMA fish determined to be of hatchery or wild origin by Dobbs (2013) to determine that CSMA hatchery and wild fish use different habitat during their sub-adult and early adult lives, but similar habitat as they aged. Finally, otolith microchemistry was used in an attempt to determine if the Roanoke River, NC (ASMA) contributed fish to the Atlantic Migratory Stock, as North Carolina Division of Marine Fisheries (NCDMF) tag returns indicated the Roanoke River was contributing significant numbers of fish. Otolith Sr:Ca ratios were used to determine anadromous migrations, as otolith Sr:Ca is directly correlated to ambient salinity. Results did not agree well with NCDMF tagging, as many larger fish exhibited resident Sr:Ca profiles, whereas NCDMF tag returns indicated that most, larger (>800 mm total length, L_T) fish were anadromous and undertook long distance migrations. It is possible that the Roanoke River harbors discrete resident and anadromous contingents of large, adult Striped Bass.

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Introduction

Striped Bass (*Morone saxatilis*) is a long-lived (>30 years) highly migratory, generally anadromous, commercially and recreationally sought after fish that is found throughout the eastern Atlantic coasts of Canada and the United States (Merriman 1941; Setzler et al. 1980). An anadromous species is one that lives its adult life in the ocean and enters the fresh water estuaries to spawn. Some anadromous species spawn once and die (semelparous), like salmon, while others will spawn many times during their adult lives (iteroparous), like shad, sturgeon, and Striped Bass. Once the eggs hatch, the larvae and juveniles live in the estuaries until they reach adulthood and migrate to the ocean. Striped Bass are considered to be iteroparous (Setzler et al. 1980). Striped Bass are reproductively mature between ages 3-6, though females may mature later than males (Setzler et al.1980; Olsen and Rulifson 1992; Boyd 2011).

Because of their commercial and recreational importance it is paramount that effective management of the Atlantic Migratory Stock continues. Total commercial and recreational landings of the Atlantic Migratory Stock (stock in this paper will refer to distinct management units, which are not necessarily genetically distinct populations) were estimated to be 13,600 metric tons in 2010 (ASMFC 2011). The stock is currently not overfished, nor is overfishing occurring, however catch rates and population estimates have declined in each of the last several years (ASMFC 2013). Effective management of this stock is difficult because of their highly migratory nature, which places them under the jurisdiction of several different state and federal agencies, as well as two different countries. States especially may manage their stocks

differently, even though there may be significant mixing of fish from different geographical areas in a single stock. The need for accurate stock identification is paramount in order to correctly allocate catch for different fisheries, to identify spawning and nursery habitats, and to develop appropriate management models (Begg et al. 1999).

The large-scale spatial and temporal migrations of the Atlantic Migratory Stock of Striped Bass have been well characterized (Chapoton and Sykes 1961). Between the years 1955-1959, 478 Striped Bass between the weights of 2.7-24.5 kg were tagged in the Chesapeake Bay (n=300), Roanoke River in North Carolina (n=97), and North Carolina coast (n=81). Tag recaptures occurred off of the North Carolina coast in the late winter and spring, on the spawning grounds in the Chesapeake Bay in the spring and as far north as the coast of Massachusetts in the summer and fall, thus establishing that the Atlantic Migratory Stock moves along the coast northward toward Massachusetts and the Gulf of Maine during the spring and summer and southward toward the northeastern North Carolina coast during the fall and winter. Boreman and Lewis (1987) confirmed this notion, when they consolidated all Striped Bass tagging data into one database and determined that migration was northward in the spring-summer and southward in the fall-winter. They also found that Striped Bass populations occurring in riverine systems south of Cape Hatteras were endemic to their natal rivers.

Another unknown aspect of the Atlantic Migratory Stock was the relative contribution of different Striped Bass stocks along the coast. It was generally considered that the Chesapeake Bay, Hudson River, and the Roanoke River populations were the major contributors to the Atlantic Migratory Stock, but it was not known which river was the largest contributor (Figure 1.1). In order to answer this question Berggren and Lieberman (1977) measured the morphological characteristics of adult Striped Bass taken from the spawning grounds of these

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three estuarine systems. Assuming that adults taken from the spawning ground were native to those waters (Mansueti 1961; Nichols and Miller 1967), they compared the morphological characteristics of the fish collected on the spawning grounds to the those of adult fish collected along the Atlantic coast from Cape Hatteras to Maine. Using quadratic discriminant function analysis (QDFA), 75% of fish were correctly classified. It was determined that the Chesapeake stock was the major contributor to the migratory stock while the Hudson and Roanoke Rivers were minor contributors. However, the Hudson River stock contributed a majority of fish to those collected off the coasts of Long Island and New Jersey, and the Roanoke stock contributed a significant number of fish collected off of the North Carolina coast and somewhat surprisingly, the Maine coast.

Boreman and Lewis (1987) echoed these results. In their meta-analysis of tag return data they found that the majority of the Atlantic Migratory Stock came from the Chesapeake Bay while the Hudson and Roanoke Rivers were minor contributors. Furthermore, they showed that the Hudson River stock moved solely northeastward during its summer and fall migration. In further support of Berggren and Lieberman (1977), Wirgin et al. (1993) used the polymorphisms of microsatellite DNA in Striped Bass from the Hudson River and Chesapeake Bay to determine that the Hudson River contributed a majority of fish to the Atlantic Migratory Stock sampled off of the eastern coast of Long Island, NY. Caution should be used when interpreting their results, as there was much debate about the ability of this type of analysis to determine relative contribution, as individual watersheds may have had several genetically distinct groups of fish (Stellwag et al. 1994). A recent analysis of composition of Atlantic Migratory Stock by Waldman et al. (2012) indicated a return dominance by the Chesapeake Bay stock, as it had been rebuilt since its population crash (ASMFC 2013). Some recent evidence suggests the Delaware

Bay and Canadian waters contribute fish to the Atlantic Migratory Stock (Waldman and Wirgin 1994; Nemerson and Able 2003).

It would seem like the movements of Striped Bass are well documented, which begs the question: why is a review of Striped Bass migration warranted? The last major review of the movements of Striped Bass stocks contributing to the Atlantic Migratory Stock occurred almost 30 years ago, in 1987. Since then, the Atlantic Migratory Stock has recovered from its well-documented crash (ASMFC 2013) and new tracking techniques, namely acoustic telemetry, otolith microchemistry, and DNA analysis, have allowed investigators and managers to determine what had not been well represented in the literature until the early 1990s: the origin and movements of fish at the edge of the range of the Atlantic Migratory Stock, the oceanic movements of juvenile and sub-adult fish, and the fine scale movements of members of the Atlantic Migratory Stock when they enter estuarine systems. This review will (1) summarize the recent advances in knowledge of the movements of Striped Bass from each of the major estuarine systems that contribute to the Atlantic Migratory Stock, (2) document coastal migrations of juvenile and sub-adult fish, (3) describe new techniques used to investigate movements of Striped Bass once they enter estuaries and (4) provide a way forward for researchers looking to completely characterize the life history the fish that comprise the Atlantic Migratory Stock.

Movements of Major Estuarine Stocks Contributing to the Atlantic Migratory Stock

Chesapeake Bay

One of the most studied estuarine systems with fish contributing to the Atlantic Migratory Stock is the Chesapeake Bay (Figure 1.1A). In fact, results of the early studies were

the basis for the current paradigm of Striped Bass migration: larger Striped Bass are more likely to undertake long distance, oceanic migrations, than smaller Striped Bass (Mansueti 1961; Nichols and Miller 1967). These early studies also concluded that very small percentages of Striped Bass in the Chesapeake Bay migrated to the ocean. This created a conundrum. Berggren and Lieberman (1977) clearly demonstrated that the vast majority of fish that make up the Atlantic Migratory Stock originated in the Chesapeake Bay. If this was the case, then how could only small percentages of fish be leaving the Chesapeake?

Kohlenstein (1981) attempted to resolve this paradox. By reanalyzing much of the same tagging data used in the 1960s he was able to make several important conclusions. He determined that the majority of fish were tagged in the upper reaches of the Potomac River, and that most of those fish there were males age 2-4. Most of the females were tagged in the mouth of the Bay, and that they were also age 2-4. Part the answer was due to the sample composition. If the earlier studies determined that larger, older fish were more likely to migrate, then it would stand to reason that samples comprised of mostly smaller younger fish would not show very many long distance migrations. He then analyzed the tag returns based upon sex and age of the fish and found that males were less likely to participate in long distance migrations at any age, especially if they were caught in upper parts of the Potomac River. Fifty percent of age-3 females migrated out of the Bay and joined the migratory stock. He speculated that if there were strong year classes, then migrations of females might occur as a function of population density as well.

In order to determine the validity of Kohlenstein's (1981) hypothesis with more current data, Dorazio et al. (1994) tagged 9,500 fish 40-100 cm L_T from various well-known spawning areas in the Chesapeake Bay. Tag returns supported Kohlenstein (1981). As fish increased in

size it was very likely that they would be ocean migrants. Sex-specific differences were also observed, as females were more likely than males to migrate to the ocean. The observed difference in migration between sexes was explained by the difference in size males and females attain at the same ages. Because females were larger than males at a given age it was more likely that females would migrate.

Secor (1992) employed a novel technique in order to accurately determine the amount of anadromy in Striped Bass within the Chesapeake Bay: otolith microchemistry. Certain trace elements from ambient water are incorporated into otoliths, and the ratios of trace elements have been shown to reflect elemental ratios in the water (Campana 1999). These trace elemental ratios are signatures of a body of water, and when incorporated into otoliths are a powerful tool used to identify separate stocks or separate migratory contingents of one population (Campana and Thorrold 2001). In their review, Secor and Rooker (2000) determined there was very strong relationship between increased water salinity and increased Sr:Ca ratios in several different species of fish, including Striped Bass.

Secor (1992) used the ratio of Sr:Ca within otoliths to determine anadromous migrations of fish. By using a wavelength dispersive electron microprobe, transects from the focus to leading edge of otoliths from large female, Chesapeake Bay Striped Bass assumed to be anadromous and large female Santee-Cooper River, SC Striped Bass assumed to be riverine, Sr:Ca ratios were measured and plotted against the age (determined from the otoliths) of each fish. By using a previously determined standard that high otolith Sr denotes anadromy, he showed that all of the Chesapeake fish were indeed anadromous, while all of the South Carolina fish were riverine. This study showed that otolith microchemistry could be a valuable instrument

in determining movements of fish, though a drawback of this study were the small sample sizes used due to the cost of the analysis.

In 2007, in an effort to use otolith microchemistry to determine migrations of adult Striped Bass in the Chesapeake Bay, Secor and Piccoli (2007) collected 40 male and 82 female Striped Bass. Using the 4000 ppm Sr threshold, their results determined that 50-75% of females aged 7-13 exhibited anadromy, and that significant numbers, though not a majority, of male Striped Bass between the ages of 4-13 exhibited oceanic migrations. These results somewhat contradicted Dorazio et al. (1994), who determined that almost no males exhibited oceanic migrations. The authors speculated that based on earlier work by Merriman (1941), increased migrations may have been a result of poor water quality or density dependence, as record numbers of Striped Bass had been recorded in the Bay in the years preceding the study.

Using DNA microsatellite analysis Gauthier et al. (2013) were not only able to determine the proportion of the Atlantic Migratory Stock that was from Chesapeake Bay fish, but also the contribution of Chesapeake Striped Bass to reproduction in non-natal river systems. One hundred fourteen fish from the Hudson River, Delaware River, Chesapeake Bay, Roanoke River, NC, and the Santee-Cooper River, SC were used to determine if each system contained unique, reproducing populations of Striped Bass. They found that each system did contain unique populations. Evidence of gene flow between the Hudson and Chesapeake was found with the flow going from the Chesapeake to the Hudson, especially when the Chesapeake exhibited dominant year classes. The authors suggested the Chesapeake Bay stock also contributed to a small amount of genetic material to all of the estuarine systems studied. Additionally they were able to confirm that the Chesapeake Bay stock contributed the vast majority of the fish to the Atlantic Migratory Stock.

Delaware Bay

Historically the Delaware Bay and its main tributary, the Delaware River (Figure 1.1B) had its own stock of Striped Bass that may have contributed to the Atlantic Migratory Stock (Merrimen 1941). Chittenden (1971) investigated the abundance of Striped Bass in the Delaware River by collecting fish at six sites from New York to Pennsylvania. Chittenden (1971) reported catching no fish at any of his sites during the two years of extensive sampling. He postulated that habitat destruction was the main cause of his failure to catch a single fish. The tidal fresh water areas of the river, which were important spawning and nursery grounds, had poor water quality conditions, with low dissolved oxygen being a primary factor.

In recent years, there has been steadily increasing Striped Bass recruitment indices in the Delaware Bay (Rago et al. 1992; Weisberg et al. 1996). Waldman and Wirgin (1994) used comparisons of mitochondrial DNA to determine if the higher numbers of fish were a result of migration of fish from the Hudson River or Chesapeake Bay, or if they were members of an expanded Delaware River stock that was once thought to be extirpated. Samples of 191 juvenile Striped Bass were taken from the Delaware River. Juveniles were taken because the authors wanted to ensure the fish they were sampling had the highest likelihood of originating from the Delaware River. Mitochondrial DNA from the Delaware River fish was compared to that of fish from the Hudson River and Chesapeake Bay. Their results showed that most juveniles collected from the Delaware Bay had unique mitochondrial DNA, which meant the Delaware River had a reestablished reproducing population of Striped Bass that was likely an expansion of a small, historical population (Waldman and Wirgin 1994).

Hudson River

The most studied Striped Bass stock contributing to the Atlantic Migratory Stock other than the Chesapeake Bay stock is the Hudson River stock (Figure 1.1C). Clark (1968) tagged Striped Bass in the Long Island Sound, New York Harbor, and other coastal waters of Connecticut and coastal New Jersey year round between 1959-1963 to determine migrations routes of the fish found there, assuming all of the fish were native to the Hudson River. Tag returns supported an idea that fish in the same area exhibited differential migration, and formed migratory contingents. His tag returns suggested that the Hudson River could be a major spawning area, as many tag returns there occurred during the spring. Secor et al. (2001) determined definite biases within Clark's study as tagging intensity differed between areas (areas with the most tagged fish showed the highest number of returns), and the origin of tagged Striped Bass could not be determined because the Chesapeake Bay also contributes large numbers of fish to the Atlantic Migratory Stock (Berggren and Lieberman 1978; Boreman and Lewis 1987; Wirgin et al. 1993). Clark's study was and still is significant as it was one of the first studies that tried to determine the movements of Hudson River Striped Bass and gave rise to the idea that one population may have separate migratory components or subgroups (i.e., "contingents").

With the movements of Striped Bass originating from the Hudson River still undetermined, but with evidence suggesting that the Hudson made up a portion of the Atlantic Migratory Stock (Berggren and Lieberman 1978), McLaren et al. (1981) performed a tagging study where all of the fish were collected on the spawning grounds in the Hudson River so that the river of origin for all tagged fish was known (i.e., assumed to be native to the Hudson River).

Over 5,000 age 2 fish and older were tagged during the springs of 1976 and 1977. Their returns indicated that fish generally undertook oceanic migrations, generally moving northeastward along the coast from the river, but migrations greater than 50 km were rare, even for older larger fish. Age, length, and sex of fish were not correlated with increased migration distance. These results directly contradicted the paradigm of Chesapeake Bay Striped Bass migrations that older, larger fish were more likely to migrate long distances. As with many tagging studies, the number of returns could have been influenced by fishing effort and selectivity.

Based on evidence suggesting that the Hudson River stock may have contributed more fish to the Atlantic Migratory Stock (Fabrizio 1987), Waldman et al. (1990) performed a comprehensive tagging study in which almost 30,000 Striped Bass were tagged in the Hudson River to determine the long-range migrations of the population. Migrations of larger fish were significantly longer than those of smaller fish, and a higher proportion of fish in the 200-400 mm range were migrating longer distances than reported in previous studies. One fish was recovered near Cape Hatteras, NC, which supported the notion that large Hudson River fish would join the mixed stock that overwinters off the North Carolina coast. Waldman et al. (1990) found no evidence supporting Clark's (1968) hypothesis of differential migrations of the same population. They hypothesized that Clark concluded that fish of different size classes were different contingents, and he did not realize the migrations of one population may relate to size. Waldman et al. (1990) also postulated that the observed increase in the number of fish undertaking long migrations could have been a result of the increasing abundance of the Hudson River stock and the simultaneous decline of the Chesapeake Bay population. Indeed, population estimates of Chesapeake Striped Bass during the early-mid 1980s supported this assertion (ASMFC 2013).

Dorazio et al. (1994) also tagged fish in the Hudson River (n=3,000) and reported results similar to Waldman et al. (1990).

Using a novel technique in the analysis of Hudson River Striped Bass migration, Secor and Piccoli (1996) used otolith microchemical Sr:Ca ratios to evaluate the migration patterns of 25 male and 25 female Striped Bass. Similar methods of analysis to Secor's studies of Striped Bass in the Chesapeake Bay were used to determine anadromy. The authors demonstrated that Striped Bass had migrated earlier than age 3 in the Hudson River, in contrast to contemporary findings in the Chesapeake Bay. But, similar to the Chesapeake, females were more likely to undertake long distance ocean migrations, and larger and older fish were more likely to migrate as well. Interestingly, he reported that fish collected at the same time and place were more likely to demonstrate similar migration patterns, providing some evidence to support Clark's (1968) stock contingent hypothesis.

Interested in investigating the possibility of stock contingency further, Secor et al. (2001) again used otolith microchemistry to determine the movements of Striped Bass in the Hudson River. The ratios Ba, Sr, Mg, Mn, K, and Na to Ca were used to classify contingents of fish. Between four and nine fish each were collected in fresh, brackish and salt water. Their whole otolith signals were determined, and fish were classified using discriminate function analysis. The fish were classified 100% correctly as resident, mesohaline, or oceanic migrants. For example, all fish caught in freshwater were classified as resident fish based upon the concentration of elements in their otoliths. Results showed that there was strong evidence of migratory contingents in the Hudson River.

Using a similar technique to Secor et al. (2001), but with a larger sample size, Zlokovitz et al. (2003) again employed otolith microchemistry to determine possible migratory contingents

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of Striped Bass in the Hudson River. Based on the predicted salinities in which fish lived, four separate migratory contingents were identified: long term residents in the upper Hudson River, long term residents in the lower Hudson River and western Long Island Sound, coastal habitat use, and mid-life habitat shift from fresh to salt water. This study served to reinforce the once rejected hypothesis of different migratory contingents within the same population.

As it had been established that the Hudson River population had different migratory contingents, Wingate and Secor (2007) investigated the specific movement of the resident population of Striped Bass. Using acoustic telemetry, 12 fish assumed to be resident were implanted with acoustic tags. Passive listening arrays were installed in the upper and lower parts of the Hudson River and recorded the date and time each fish passed. Fish conformed to seasonal migration documented in the literature. Fish migrated downstream during the fall and overwintered in the brackish lower Hudson River. Fish migrated upstream in the spring and summer to spawn and feed.

Contributors at the Edges of the Migratory Range

Roanoke River, NC

Berggren and Lieberman (1978) demonstrated that in some places the Roanoke River population of Striped Bass significantly contributed to the Atlantic Migratory Stock. Trent and Hassler (1968) observed large spawning events, but the Roanoke contribution has largely been ignored in the literature. As a result movements of fish to, from, and within this system have not been well studied (Figure 1.1D).

Haeseker et al. (1996) was one of the first studies to determine movements of Striped Bass during the summer in the Roanoke River and the associated Albemarle Sound system. He

was spurred by the recent loss of stock abundance during the 1980s as a result of overfishing and habitat destruction (Rulifson 1992; USFWS 1992). Seventy-eight fish were tagged with ultra sonic tags, and acoustic listening arrays were constructed at several sites in the Roanoke River-Albemarle Sound system. Additionally, more than 600 fish were caught, weighed, and measured to determine their condition. Fish in poor condition were found throughout the sound and most commonly in deeper water in the western sound or around structure during the summer as result of the very high water temperatures (>30°C). No fish moved outside of the system.

Carmichael et al. (1998) used acoustic telemetry to characterize the timing and duration of spawning run migrations of the Roanoke River population. Seventy-eight fish were tagged between 1993 and 1994 and receiving arrays were used along the length of the Roanoke River. Twenty-nine of the tagged fish migrated upstream to spawn in the spring of 1994, and 14 migrated upriver in the spring of 1995 at water temperatures of 17-18°C. Migrations, on average, took one week to complete. Males stayed on the spawning grounds for slightly more than 20 days in both years, while females stayed on the spawning grounds for 10 days in both years. These findings were supported by previous research suggesting differential residence time for males and females in other estuarine systems and in other species of anadromous fish.

While much was being learned about the movement of fish within the Roanoke River-Albemarle Sound system, little was known about their possible anadromy and contribution to the Atlantic Migratory Stock. Only very recently have these questions been investigated. Boyd (2011) used otolith Sr:Ca ratios of 288 fish collected from the spawning grounds on the Roanoke River to determine that approximately 23% of adult Striped Bass from the Roanoke River-Albemarle Sound in North Carolina were anadromous. He used the threshold of 4000 ppm Sr in the otoliths to determine anadromy.

In support of Boyd's findings, Callihan et al. (2014) also determined adult Striped Bass migration in the Roanoke River-Albemarle Sound system through analysis of tag returns of fish tagged between the years of 1991-2008. More than 42,000 fish were tagged in the Roanoke River-Albemarle Sound system during this period. Tag returns indicated that up until 1997, when the stock was depleted, very few fish migrated out of the system (<4%). Between 1997-2008, with the stock rebuilt, between 15-31% of fish were recaptured outside of North Carolina waters annually. More than 90% of tagged fish >800 mm total length (TL) were recaptured in ocean waters, with most (78%) coming from as far away as coastal New Jersey to Cape Cod. Just 47% of fish 600-799 mm in TL were recaptured in the Albemarle Sound. Fish 350-600 mm TL were most likely to be resident to the Albemarle Sound, but in years with high stock abundance they were likely to migrate to neighboring NC estuarine waters. This study demonstrated that when at high levels of abundance, the Roanoke and Albemarle stock of Striped Bass exhibited population level migrations similar to those of the Chesapeake Bay and Hudson River, where long-distance oceanic migrations were more likely due to fish size.

Canadian Waters

Any review of the movements of Striped Bass originating in Canada should start with Rulifson and Dadswell (1995). Their paper reviewed all of the relevant literature regarding life history, population characteristics, and movements of Striped Bass in Atlantic Canada up to that point. They identified nine rivers with unique populations of Striped Bass: the St. Lawrence River (where the population was believed to be extirpated), the Nepisiguit River (part of the Chaler Bay system), the western Gulf of St. Lawrence Rivers: Tabusintac, Miramichi, Kouchibougauc, and Richibucto, the outer Bay of Fundy system which includes the Saint John

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and Annapolis Rivers, and the Shubenacadie-Stewiacke River in the inner Bay of Fundy (Figure 1.2). They used the results of tagging, morphometric and meristic analysis, mitochondrial DNA analysis, and parasite community analysis to determine that the Bay of Fundy Rivers at varying times contained native fish and fish from the Atlantic Migratory Stock, and that some fish from the Bay of Fundy rivers joined the Atlantic Migratory Stock in their summer migrations to northeastern U.S. waters. Adults from all systems seemed to overwinter in upriver freshwater habitat. Fish from the Gulf of Saint Lawrence system all appeared to be residents.

One of the important studies influencing Rulifson and Dadswell (1995) was a study of Striped Bass tag returns during the mid-1980s from the Bay of Fundy by Rulifson et al. (2008). They investigated the composition of the mixed stock of Striped Bass that use the bay as foraging ground by analyzing tag returns. In the years 1985 and 1986 over 1,300 fish were tagged in the inner Bay of Fundy. Generally, the most fish were tagged in the late summer and early fall. Returns over the next two years indicated a portion of Striped Bass overwintered in the freshwater around the Bay of Fundy and another portion traveled south along the coast of the U.S. to overwinter. This study clearly indicated that fish from both the U.S. and Canada use the Bay of Fundy as summer foraging habitat.

Wirgin et al. (1993b) used differences in mitochondrial DNA to determine the uniqueness of the Shubenacadie River (part of the Bay of Fundy system), Miramichi and Tubasintac River (part of the Gulf of St. Lawrence system) populations. The results of the DNA analysis were then compared to Striped Bass DNA from the Hudson River and Chesapeake Bay populations to determine if any mixing between U.S. and Canadian Striped Bass was occurring. They found that the Shubenacadie River fish differed from the Miramichi and Tubasintac Rivers, indicating a high degree of reproductive isolation between the Bay of Fundy system and the Gulf of St.

Lawrence system. All three Canadian populations discriminated from the U.S. populations, indicating unique Canadian and U.S. stocks. These results did not preclude the possibility of mixing of Canadian and U.S. fish during the summer coastal migrations, but strongly indicated there was little if any interbreeding between U.S. and Canadian fish.

Continuing their investigation of Striped Bass genetics in Canada, Wirgin et al. (1995) attempted to determine the composition of the mixed Striped Bass stock aggregating in the Bay of Fundy, and St. John and Shubenacadie Rivers during the summer using mitochondrial DNA analysis. Ninety-seven percent of the fish collected in the St. John River were of U.S. origin, while <10% of fish collected in the Shubenacadie River were of U.S. origin. The results suggested that the Bay of Fundy and its associated river systems provide important summer foraging grounds for Atlantic Migratory Stock Striped Bass. The St. John River population appeared to be effectively extirpated, while the Shubenacadie River population was small but stable.

Interestingly, the Shubenacadie River population exhibits two different color morphotypes: those with a black dorsal coloration and those with green dorsal coloration. Paramore and Rulifson (2001) investigated each of the color morphs to examine the possible differences in life histories between them by analyzing the differences in diet, gonadal fatty acids, and otolith microchemistry. Fish with green dorsal coloring showed a high Sr:Ca ratio after year 1, indicating anadromy, while fish with black dorsal coloring, showed low Sr:Ca after year 1, indicating residency. Green-backed fish also had diets characteristic of ocean residence while black-backed fish had diets reflecting riverine residence. Finally, gonadal fatty acid differences indicated that green-backed fish had high levels of omega-3 fatty acids, signifying oceanic migrations, while black-backed fish had high levels of omega-6 fatty acids, signifying residency.

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These results showed that Striped Bass from a Canadian system could contain separate migratory contingents like those documented in the Hudson River.

Coastal Movements of Juvenile and Sub-adult Striped Bass

The historical paradigm suggests that only large, adult Striped Bass join the Atlantic Migratory Stock; however, recent evidence suggests that small, young fish may also take part in coastal migrations. Robichaud-LeBlanc et al. (1998) and Robinson et al. (2004) published a series of studies that examined the distribution of young-of-year (YOY) Striped Bass in the Miramichi River, which is part of the Gulf of St. Lawrence estuarine system. Robichaud-LeBlanc et al. (1998) found through intensive beach seine and trawl surveys that YOY abundance increased in downstream, brackish parts of the river in the late summer as the small fish grew. The same research group attempted to determine the genetic relatedness of YOY fish observed in the late summer in the Kouchibougauc and Richibucto rivers to test the hypothesis that fish observed there were migrant age-0 fish from the Miramichi River. Fish collected in each river were of the same genetic makeup to those found on the spawning grounds in the Miramichi River, indicating that fish spawning in the Miramichi River in the spring migrated downstream and into non-natal rivers, a migration of 35-55km over the span of a few months (Robinson et al. 2004).

Able et al. (2012) investigated a similar phenomenon of age-0 and juvenile dispersal to non-natal estuaries in coastal New Jersey. Small fish <20 cm L_T were observed in beach seines, trawls, and acoustic tag returns in estuaries in New Jersey. There is no known spawning population of Striped Bass in New Jersey waters (Collette and Klein-MacPhee 2002). The authors speculated that small fish from the Hudson River and Delaware Bay emigrated to New Jersey estuaries in search of suitable habitat. Patrick (2010) reported seeing abnormally high

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otolith Sr before the first year annulus in adult Striped Bass caught in the Roanoke River in North Carolina in 2004 and 2005, hypothesizing that young fish migrated to the ocean for short periods of time before finding suitable habitat in the Albemarle Sound. Able et al. (2012) would seem to support Patrick's (2010) findings, but few age-0 fish have been observed in North Carolina coastal waters (Able et al. 2012), so Patrick's (2010) interpretations need to be investigated further.

Using tag returns Mather et al. (2009) investigated the movements of juvenile and sub-adult (400-500 mm L_T) Striped Bass tagged in the non-natal summer foraging waters of the Plum Island Estuary (PIE) in coastal northern Massachusetts. Their tag returns indicated that juvenile and sub-adult fish were likely to undergo long migrations to the south in the fall and winter before returning to Massachusetts' waters by the summer to feed.

Mather et al. (2010) not only documented movement of sub-adult fish, but also described several different migratory routes for these smaller fish. Fish were tagged with acoustic tags during the summer in PIE, and acoustic listening arrays were erected in coastal Long Island and Delaware Bay estuarine waters. Half of the fish tagged were detected during the winter in the Long Island Sound. The other half was detected in the Delaware Bay estuary.   Some fish that traveled to Delaware were also detected initially in the Long Island Sound, but many apparently did not travel through the sound and took a more direct route to the Delaware Bay. This study demonstrated the complexity of migratory paths that even smaller Striped Bass take during their coastal migrations. It is possible that since these fish likely originated from different systems, it might be expected that they make different fall coastal migrations.

Local Movements of Striped Bass

Recent advances in tracking technology have allowed investigators to pinpoint local migrations of Striped Bass once they enter the estuaries. Nemerson and Able (2003) investigated the local movements of Age-0 Striped Bass in the coastal creeks of the Delaware coast, sampling fish with otter trawls. Age-0 fish were abundant, with high reported catch per unit effort (CPUE). Movement appeared to be associated with salinity regimes. The small, young fish preferred oligohaline conditions.

Ng et al. (2007) implanted adult fish (483-953 mm) with acoustic tags and tracked them with a mobile hydrophone to determine specific habitat preferences within the Mullica River-Great Bay estuary in southern New Jersey. A parallel study, (Able and Grothues 2007), indicated that seasonal movements into and out of the bay were related to the migrations of the Atlantic Migratory Stock. Individual fish would return to the same exact locations in the bay year after year, signifying those locations as habitat worthy of protection. In general Striped Bass preferred deep water near shorelines. Fish exhibited extreme site fidelity, even when travelling to non-natal waters. This type of small-scale measurement would not have been possible with fixed acoustic arrays (Ng et al. 2007).

Again, using acoustic telemetry, Pautzke et al. (2010) were able to determine habitat use in the PIE, Massachusetts. Between 2005 and 2006, 60 fish were tagged with acoustic tags. Fish were shown to stay in the estuary for 66 and 72 days for each respective year of the study. Striped Bass preferred to stay in the middle Plum Island Sound and the lower Rowley River during their stay in PIE. As many other studies have shown, fish considered seasonal residents (residence of at least 30 days) split into two contingents. One contingent seemed to forage in the middle Plum Island Sound, while the other preferred the lower Rowley River.

Conclusion: an Updated Paradigm

The Old Paradigm

The currently accepted view of Striped Bass migrations along the Atlantic coast is as follows: Striped Bass migrate to their natal streams in the spring to spawn. They then migrate downstream and into the ocean to summer foraging habitat off of the coast of Massachusetts to the Bay of Fundy, Canada. In the fall they generally migrate south to overwinter in the coastal waters of northeastern North Carolina and Virginia. Long distance migrations of Striped Bass in all the systems that have stocks contributing to the Atlantic Migratory Stock are more likely to occur as fish become more abundant and as they grow larger. The Chesapeake Bay is the largest contributor of fish to the Atlantic Migratory Stock, although as a result of the Chesapeake Bay stock crash in the 1970s and 80s, the Hudson River stock may have been the major contributor for a short time during the late 1980s and early 1990s (Table 1.1). This view is simple to understand and seemingly makes management of the coastal migratory stock easier. However, as the stock has rebounded from its crash (ASMFC 2013), it seems other coastal watersheds may be contributing significant numbers of fish. As a result management practices of Striped Bass and the paradigm of Striped Bass migration should be updated.

The New Paradigm

Much of what has been gleaned from the migrations of adult Striped Bass from the Atlantic Migratory Stock on the basis of tag returns in the Chesapeake Bay from 1960-1990 still

holds true today, but the updated paradigm adds some layers of complexity (Table 1.1). As the

Roanoke River stock has recovered from its own population crash in the late 1980s, evidence

strongly suggests that it is contributing more fish to the Atlantic Migratory Stock. Other recent

findings suggest that the Shubenacadie River also contributes fish to the Atlantic Migratory

Stock. The Delaware Bay has a small, unique population, but its contribution is unknown.

Many systems, such as the Roanoke River, Hudson River, and Shubenacadie River populations,

likely have unique resident and anadromous contingents. This means that stock assessments of

the coastal migratory stock should not count all fish produced in those systems. Observations of

age-0 and juvenile Striped Bass in non-natal streams suggest that some small young fish also

undertake long coastal migrations in search of suitable habitat.

Future Research

Much is known about the migrations of Striped Bass in the Atlantic Migratory Stock, but

there is much still to be understood. The timing and routes of migration will need to be

monitored as sea temperatures continue to increase as a result of climate change (IPCC 2014).

With the increased contribution of fish from the Roanoke River, Shubenacadie River, and

possible contribution of fish from Delaware Bay, the relative contribution of all possible

contributing systems to the Atlantic Migratory Stock should be reevaluated. The percentage of

anadromous fish in each possible contributing system should also be determined. A combination

of techniques can be used to determine this. Conventional tagging, along with otolith

microchemistry, could be compared to Gauthier et al. (2013)'s analysis of Atlantic Migratory

Stock nuclear DNA to definitively show which stocks contribute to the Atlantic Migratory Stock.

In addition to being able to discriminate fish from different areas, otolith microchemistry would

be useful because otoliths provide a record of migration throughout a fish's life. The use of these techniques together would be powerful. Any future stock assessment of the Atlantic Migratory Stock should count fish from the Roanoke and Shubenacadie Rivers. Acoustic and conventional tagging, along with otolith microchemistry, could also be used to determine the fine scale movements and habitat preferences of Striped Bass within estuaries, which will be crucial as people continue to develop coastal areas, affecting Striped Bass habitat. The possible oceanic migrations of age-0 and juveniles must be understood, as their migrations may indicate high population density or poor habitat quality in their natal streams. This thesis will investigate the inshore movements, potential mixing and oceanic migrations of separate Striped Bass stocks in North Carolina using otolith microchemistry.

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Tables

Table 1.1. A table comparing the currently accepted paradigm of Striped Bass coastal migration to the updated paradigm argued for in this paper. The studies supporting each paradigm are listed.

Pre/Mid-Crash Paradigm (Current)	
Studies	Paradigm
Berggren and Lieberman (1977) Kohlenstein (1981) Boreman and Lewis (1987) Wirgin et al. (1993) Dorazio et al. (1994)	The Chesapeake Bay and Hudson River stocks are the only contributors to the Atlantic Migratory Stock. Fish undertake long distance ocean migrations only when they reach adulthood.

Post Crash Paradigm (Updated)	
Studies	Paradigm
Rulifson and Dadswell (1995) Wirgin et al. (1995) Paramore and Rulifson (2001) Rulifson et al. (2008) Boyd (2009) Callihan et al. (2014) Mather et al. (2009) Mather et al. (2010) Patrick (2010) Able et al. (2012) Gauthier et al. (2013)	While the Chesapeake Bay and Hudson River stocks contribute the majority of fish to the Atlantic Migratory Stock, other systems such as the Roanoke River and the Bay of Fundy estuaries do contribute small but significant numbers of fish to the stock. Juvenile and sub-adult fish may also participate in coastal migrations, possibly due to density dependent factors.

Figures
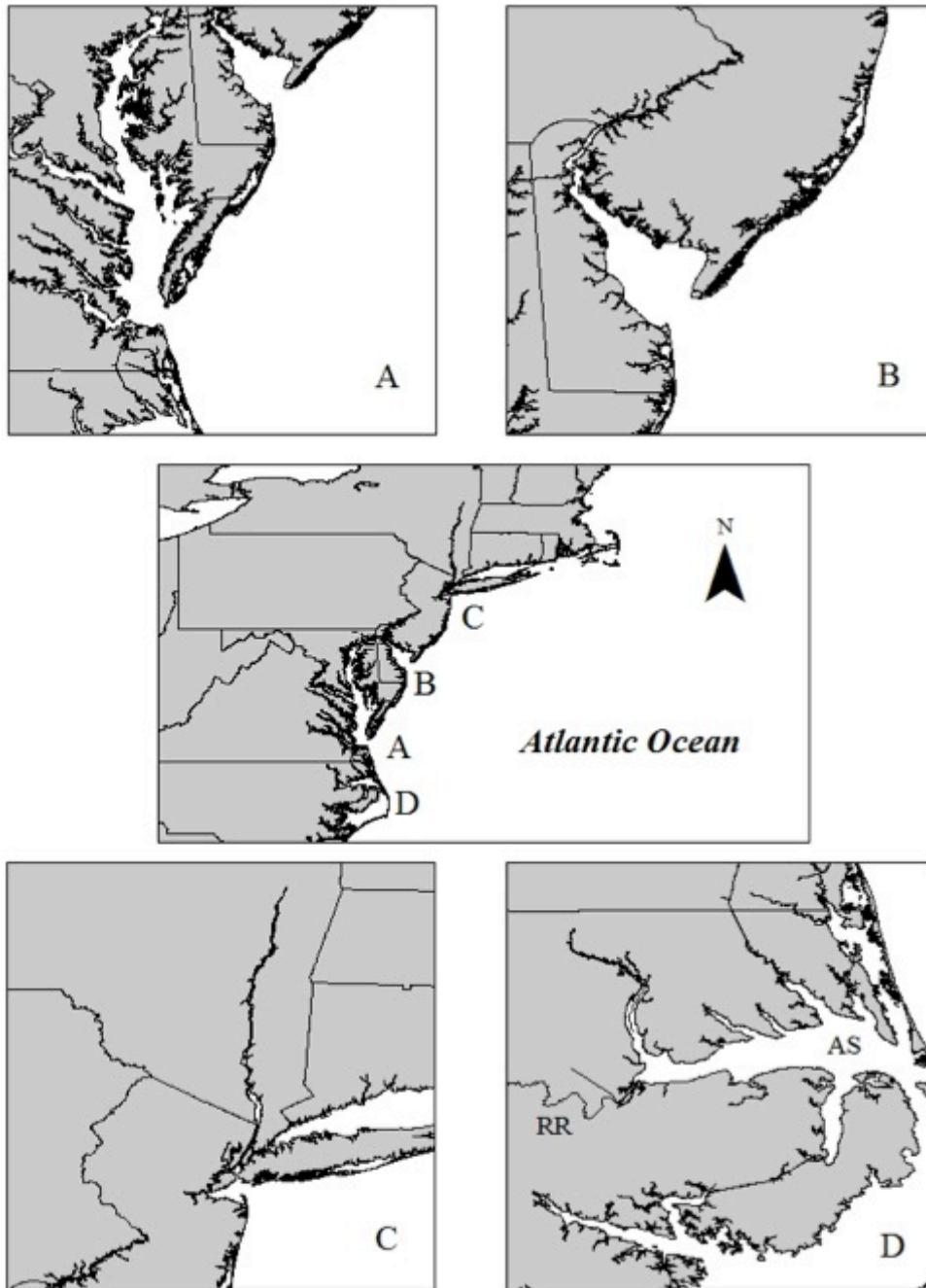
Figure 1.1. Maps of the U.S. watersheds known to support Striped Bass populations that contribute fish to the Atlantic Migratory Stock. A. Chesapeake Bay B. Delaware Bay C. Hudson River D. Roanoke River (RR)-Albemarle Sound (AS).
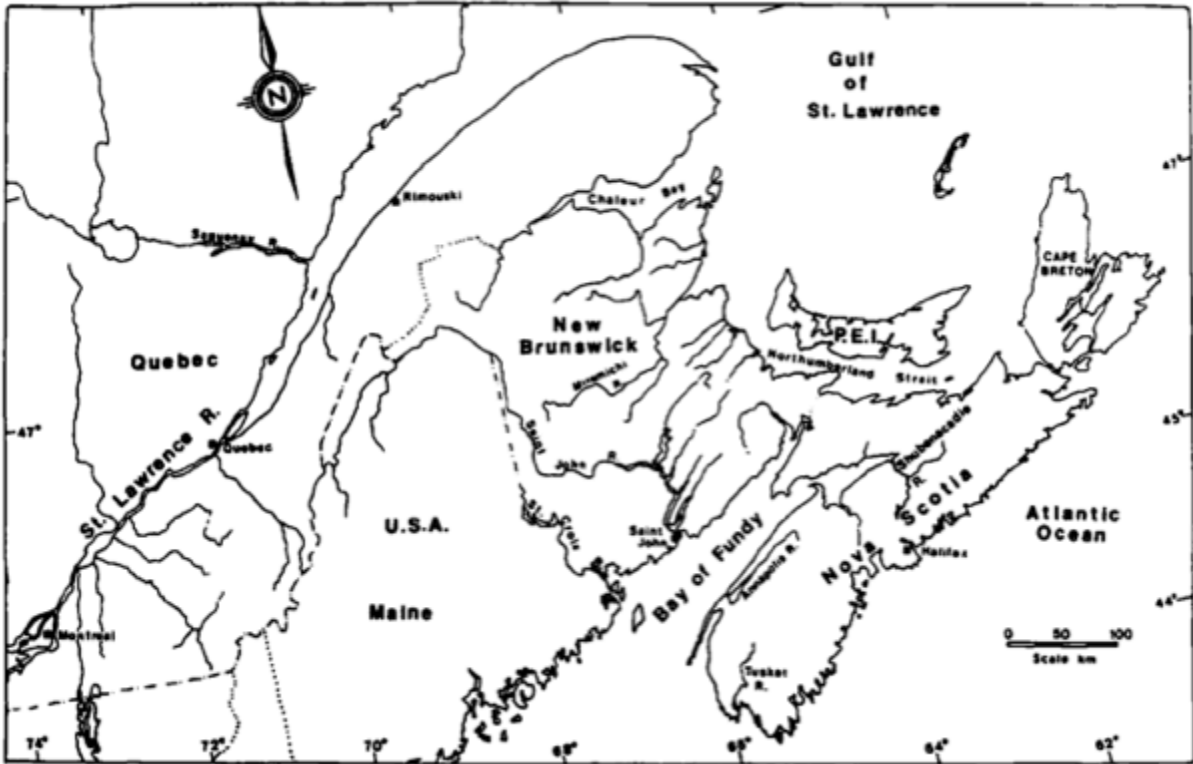
Figure 1.2. A map of Atlantic Canada, with the major rivers and estuarine systems known to produce Striped Bass labeled. This map is from Rulifson and Dadswell (1995) and is reproduced in this paper with their permission

Chapter 2. Migration of Striped Bass, *Morone saxatilis*, Between Separate Management Areas in Inshore Waters of North Carolina

Dan Zurlo

Abstract

With three independently managed Striped Bass, *Morone saxatilis,* stocks, management of Striped Bass in North Carolina is challenging. In this study Striped Bass from two of the three major management areas in North Carolina, the Albemarle and Roanoke River Management Area (ASMA) and Central/Southern Management Area (CSMA), were used to analyze stock migration patterns and delineate the two stocks. Otolith microchemistry was used as a proxy for migration patterns as otoliths incorporate trace elemental signatures of the watersheds the fish inhabit. The elements Sr, Ba, Mg, and Mn were used to determine elemental signatures. Water chemistry analyzed via ICP-OES determined differences between water of the ASMA and CSMA. Otolith microchemistry was analyzed using LA-ICP-MS. Adult otolith data and yearly adult otolith data were compared between stocks to determine migration between them. Otolith chemistry also was compared between CSMA hatchery and wild fish to determine migration patterns of both groups. Linear discriminant function analysis (LDFA) revealed high correct classification rates of water chemistry between management areas. Linear discriminant function analysis (LDFA) of adult whole and yearly otolith data showed high correct classification rates of fish of different management areas, and poor classification rates between hatchery and wild CSMA fish. Because little migration between management areas occurs, the ASMA and CSMA should continue to be managed as separate stocks. Hatchery augmentation of the CSMA stock

should continue, but fishing mortality must be reduced to allow recovery of the possibly depleted stock, as it exhibits many of the defining traits of a stock undergoing heavy mortality.

Introduction

Background

The Atlantic Striped Bass, *Morone saxatilis*, is an anadromous, highly migratory species (Setzler et al. 1980; Boreman and Lewis 1987). They also represent a significant commercial and recreational fishery, totaling $45 million in catch revenue and fishing expenditures in 2011 in North Carolina (NCDMF and NCWRC 2013). Because of the complex migrations of this species, state, regional, and federal management plans can be complex.

One technique that has been used to better understand Striped Bass migrations is the chemical analysis of trace elements in otoliths. Otoliths are inner ear bones present in teleost fish that serve for hearing and to orient the fish. The bones are made up of an aragonitic $CaCO_3$-protein matrix, which is deposited in discrete permanent daily growth rings, and can be used by fisheries biologists to age fish (Campana and Nielson 1985).

Once incorporated into the otoliths the ratios or concentrations of trace elements have been shown to reflect the trace elemental ratios in ambient waters (Campana 1999). These trace elements are signatures of a body of water, and when incorporated into otoliths are a powerful tool used to identify separate fish stocks or separate migratory contingents of one fish population (Campana and Thorrold 2001). In this paper a fish stock is defined as a discrete management unit that may comprise one or more spawning populations. Campana and Gagne (1995)

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demonstrated that analysis of trace elemental otolith microchemistry of whole otoliths was able to discriminate between different Canadian stocks of Atlantic Cod, *Gadus morhua*.

Interpreting otolith chemical signals becomes more difficult when a stock is anadromous. Under normal conditions, the otolith Sr:Ca ratio directly correlates to water salinity allowing anadromy to be determined (Secor 1992; Secor and Rooker 2000). Generally, otolith Sr concentrations are higher in saltwater and lower in freshwater. The anadromy of one of the two most important Striped Bass stocks contributing to the Atlantic Migratory Stock, the Chesapeake Bay stock, was confirmed using ratios of Sr:Ca in yearly growth rings of otoliths (Secor et al. 1995). Using larger sample sizes than previous studies, Secor and Piccoli (2007) were able to more accurately determine rates of anadromy of Chesapeake Bay Striped Bass using Sr:Ca ratios. Roughly 75% of female Chesapeake Bay Striped Bass, aged 7-13, were anadromous. Significant numbers, but not a majority, of adult male fish were also anadromous. Through otolith microchemistry of yearly growth rings, anadromy of the Striped Bass stock in the Hudson River was also confirmed. The stock was shown to have three separate contingents: a resident contingent with a low Sr:Ca ratio and a high Ba:Ca ratio, a mesohaline contingent with intermediate Sr:Ca ratios, and a fully anadromous contingent with high Sr:Ca ratios (Secor et al. 2001).

Sr:Ca otolith ratios in yearly growth rings also corroborated the hypothesis that dorsal coloring patterns in the population of Striped Bass in the Shubenacadie River, Nova Scotia reflected either anadromy or residency (Paramore and Rulifson 2001). Fish with green dorsal coloring, which previously were believed to be anadromous, showed high otolith Sr:Ca ratios after year 1, while fish with black dorsal coloring, which were believed to be resident, showed low otolith Sr:Ca values (Paramore and Rulifson 2001). Boyd (2011) was able to use Sr:Ca

ratios to determine that approximately 22% of adult Striped Bass collected in the Albemarle

Sound in North Carolina were anadromous.

Otolith microchemistry can also reflect residency in different estuarine habitats,

especially when the trace metals Manganese (Mn), Strontium (Sr), Barium (Ba), and Magnesium

(Mg) are used (Thorrold et al. 1998; Gillanders and Kingsford 2000). Morris et al. (2003) was

able to discriminate Striped Bass populations using trace elemental analysis of the natal regions

of Striped Bass otoliths. The Neuse and Roanoke Rivers in North Carolina and Stewiacke River

in Nova Scotia were used in the study. Striped Bass were matched to their rivers of natal origin;

the Neuse River population (88% discrimination) and Stewiacke River population (79%

discrimination) had the best discrimination, however fish from the Roanoke River were correctly

classified just 47% of the time, perhaps due to introgression of fish from other neighboring North

Carolina estuaries or from the Atlantic Migratory Stock (Morris et al. 2003). Zapf (2012) was

able to discriminate the specific tributaries of natal origin within the Albemarle Sound

populations of blueback herring, *Alosa aestivalis*, and alewife, *A. pseudoharengus*, by using trace

elemental data in the primordial regions of the otoliths (central part of the otolith formed within

days of hatching) as a proxy for water chemistry and then comparing them to primordia of adult

otoliths. Thus, critical habitat areas for juveniles were identified and could be compared to

Strategic Habitat Areas (SHAs) designated by the state of North Carolina (NCDMF 2007).

Certain geological and environmental conditions can complicate this seemingly

straightforward analysis. Howland et al. (2009) reported anomalous levels of otolith Sr in a

freshwater contingent of Salmonid Sheefish, *Stenodus leucichthys*, with Sr levels being 10x

greater than expected in a purely freshwater fish. In fact the anadromous contingent had lower

Sr levels than the freshwater contingent. They hypothesized that the unique geology of the

Mackenzie River watershed, Canada, comprised of limestone and dolomite, contributed to the abnormally high Sr levels in the water, causing the high Sr levels in the otolith. Patrick (2010) also reported seeing abnormally high otolith Sr before the first annulus in otoliths of adult Striped Bass caught in the Roanoke River in North Carolina in 2004 and 2005. He hypothesized that at age-0, these fish migrated to the ocean for very short periods of time. But the high Sr may have been due, again, to unique hydrological conditions as upwelling from the Sr rich Upper Castle Hayne Aquifer (Woods et al. 2000) could have caused spikes in otolith Sr. These data highlight how crucial it is to have as much information as possible about the geological, environmental, and anthropogenic inputs into a watershed when doing this type of analysis.

Water temperature may also affect otolith element uptake, as ambient temperature can speed or slow the biochemical pathways that assimilate different elements (Townsend et al. 1992), or affect the kinetics of the crystallography of the otolith (Nielson and Christoffersen 1982). However in a meta-analysis of otolith chemistry literature, Campana (1999) found that ambient water temperature would have small and insignificant effect on uptake. A more recent review of otolith chemistry literature did acknowledge that temperature would affect element uptake, but concluded that the effect would not hinder the applications of elemental analysis (Elsdon et al. 2008).

Temporal variability in watershed elemental signatures can have perhaps the largest confounding effect on trace otolith elemental analysis. Depending upon the area being studied, ambient chemistry can vary or be relatively stable (Elsdon et al. 2008). Campana et al. (2000) showed that otolith elemental signatures were stable for up to three years in the fully marine Atlantic cod, *Gadus morhua*, suggesting chemistry of the water they inhabited was stable. Reis-Santos et al. (2012) suggested that while ambient elemental concentrations varied within years

and seasons, the amount of variation did not hinder their ability to use otolith chemistry to classify juvenile fish to their estuary of origin in estuaries along the Portuguese coast. Mohan et al. (2012) suggested that concentrations were stable for up to 3 months during the late summer and early fall in the Albemarle Sound, North Carolina. Dobbs (2013) analyzed water chemistry (ratios of Sr, Ba, Mn, and Mg to Ca) of several different rivers of the Central Southern Management Area (CSMA), North Carolina and found mixed results. Some elements varied seasonally, some over the course of a year, and some did not vary at all. Stable water chemistry would allow for otolith comparison to water chemistry data in years other than the year in which the water chemistry was determined. If the chemistry were unstable throughout the year then analysis of otolith data would be severely restricted. Any otolith chemistry study must include validation of the relative temporal stability of ambient chemistry.

The state of North Carolina has three separately managed stocks of Striped Bass, each with unique migration patterns, so delineating each stock can be challenging. Two areas jointly managed by the North Carolina Division of Marine Fisheries (NCDMF) and North Carolina Wildlife Resources Commission (NCWRC) are the Albemarle-Roanoke Management Area (ASMA and RRMA) and the Central/Southern Management Area (CSMA). The third North Carolina stock, the Atlantic Ocean Migratory Stock, is managed at the federal level (NCDMF and NCWRC 2013). NCWRC manages all inland populations. Delineation between fish of the ASMA and CSMA must be established in order to more efficiently manage the stocks of Striped Bass in North Carolina. Stock delineation is the first step in managing any stock, so that correct catch limit and population models can be implemented (Begg et al. 1999).

In addition, the NCWRC has stocked the CSMA with 100,000-300,000 Striped Bass 25-75 mm in total length annually since 2001 (NCWRC, unpublished data). The brood source of

those hatchery fish is the Roanoke River, in the ASMA. Dobbs (2013) showed that fish stocked in the CSMA from the Watha State Fish Hatchery had distinct and abnormally higher Sr signals in the natal portion of their otoliths, and that >85% of CSMA fish were of hatchery origin. So far, hatchery augmentation of the CSMA stock has been unsuccessful in improving poor juvenile recruitment to the fishery. Understanding movements of hatchery and wild fish would help managers determine the influence of the current stocking program on the possibly depleted CSMA stock, whose status is undetermined, but does exhibit signs of a stock undergoing heavy mortality, such as poor recruitment and a truncated age structure (Homan et al. 2013; NCDMF and NCWRC 2013). It is possible that because fish stocked in the CSMA are from Roanoke River brood stock, stocked fish may not be migrating to the spawning grounds of the CSMA. This would limit their reproductive success, meaning NCWRC hatchery augmentation efforts have done little to improve the CSMA stock.

Currently, work is ongoing to determine the natal origin of fish of each of the stocks of Striped Bass in North Carolina. Dobbs (2013) showed that Striped Bass that in the CSMA likely originated there. However, little is known about adult migration between management areas. Callihan et al. (2014) provided compelling evidence that large, old Striped Bass from the ASMA were very likely to be anadromous, based on tag return data. It also suggested that some smaller, younger sub-adult fish had migrated from the ASMA to the CSMA (Callihan et al. 2014). Scattered tag-return data suggest that adult fish of the CSMA are primarily resident to the CSMA (NCDMF and NCWRC 2013).

Goals

The goal of this study was to determine through the use of otolith microchemistry if mixing of Striped Bass was occurring between the ASMA and CSMA. A secondary aim of this study was to determine the migratory habits of hatchery and wild fish of the CSMA using otolith microchemistry.

Delineation of the ASMA and CSMA stocks was determined by first analyzing water chemistry of the two areas. I hypothesized that based on previous work in our lab, each watershed would have unique water chemistries. If water chemistry between the two areas was different and did not vary temporally, then differences in otolith chemistry between fish of the two areas would be a function of residency of fish to their respective management areas. Otolith chemistries between all ASMA and CSMA fish were compared in order to ascertain differences. Also, I hypothesized, that adult fish would be largely resident to their respective management areas, based on NCDMF and NCWRC tag returns. Yearly otolith chemistry of co-occurring year classes in the ASMA and CSMA were compared to more accurately determine if any migration between the two management areas has occurred. This method eliminates much of the temporal variation in water chemistry that could confound efforts to classify ASMA and CSMA fish.

Otolith chemistry between CSMA hatchery and wild fish were compared to determine if those two groups of fish have different migratory patterns. I hypothesized that wild and hatchery reared CSMA Striped Bass occupy different habitats and therefore, have different otolith elemental signatures. Yearly otolith chemistry of wild and hatchery CSMA fish was compared to further illuminate the migratory habits of hatchery and wild fish.

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Methods
Study Area and Water Sample Collection

Dobbs (2013), Hughes (unpublished) and Knight (unpublished) collected water samples

from upstream and downstream sites from rivers in both the ASMA (n=434) and the CSMA

(n=312) during 2010, 2011, and 2012 (Figure 2.1 and Figure 2.2). Samples were taken from

upstream and downstream sites in order to identify spatial differences in water chemistry

between oligohaline and mesohaline portions of the rivers. Rivers in the ASMA included the

Roanoke, Chowan, Perquimans, Little, Pasquotank, North, Alligator, Scuppernong, and the

Currituck Sound. Upstream sites were labeled B and downstream sites were labeled A (Figure

2.1). Rivers in the CSMA included the Tar/Pamlico, Pungo, Trent, Neuse, Cape Fear, Northeast

Cape Fear, and Black (Figure 2.2). Downstream sites were labeled A and upstream sites were

labeled B. In the Neuse and Tar/Pamlico Rivers, water samples were collected at four sites with

the most downstream site labeled as A and each subsequent upstream site labeled B, C, and D

(Figure 2.2).

Fish Collection

Sample methods for ASMA fish were described by Boyd (2011). Briefly, adult fish were

collected during the spring in the western Albemarle Sound by the NCDMF during the

independent gillnet survey (IGNS) (n=237) and from the Roanoke River, near the spawning

grounds via electroshocking by the NCWRC and NCDMF (n=216). Samples were collected

from March-May 2009 and 2010 during the spawning period. A total of 453 fish were collected.

Sample methods for CSMA fish were described in Dobbs (2013). Briefly, adult fish were

sampled by Dobbs (2013) from the Tar/Pamlico and Neuse Rivers by rod and reel, gillnets, and

electroshocking year round during 2011 and 2012. Tag returns indicated that few fish migrate

out of the Cape Fear River (NCDMF and NCWRC 2013), so fish were not collected from rivers

of the Cape Fear system. NCDMF staff provided samples year round from the estuarine parts of

the Neuse and Pamlico Rivers from their gillnet survey, and NCWRC provided fish from the

spawning grounds of the Tar River near Tarboro and Neuse River near Goldsboro during the

springs of 2011 and 2012. The Edenton National Fish Hatchery (ENFH) provided post spawn

fish used as brood fish. A total of 281 fish were collected throughout the course of sampling.

CSMA fish were humanely sacrificed by Dobbs (2013): ECU IACUC Assurance Number

A3469-01. The number of fish collected in each year class is summarized in Table 2.8.

Water Sample Analysis

Concentrations of Sr, Ca, Mg, Mn, and Ba were determined in water samples of the

ASMA (Figure 2.1) and CSMA (Figure 2.2) via inductively coupled plasma optical emission

spectroscopy (ICP-OES). For exact ICP-OES methods see Mohan et al. (2012). Briefly,

samples were collected in the field at from both the surface and bottom of the water column

using a peristaltic pump and whatman filters, then stored at 4°C until analysis. A Perkin Elmer

inductively coupled plasma (ICP) optical emission spectrometer (OES) (Optima 2100 DV) was

used to measure elemental concentrations of Ca (ppm), Mg (ppm), Sr (ppb), Ba (ppb), and Mn

(ppb). Samples were diluted with 10 parts of ultrapure water (18.5Ω) to one or two parts of

sample, depending on salinity, to prevent damage to the ICP-OES machine. To create an element

specific calibration curve, a stock standard solution (1,000 mg/L in 2% HNO3) for each element

was used with five standards (lowest low, low, medium, high, and highest high) and analyzed

before the samples to attain a r^2 of $\geq$0.999. Quality control checks requiring greater than 90%

recovery were issued after every nine samples. To account for the role of Ca in otolith element

uptake, the concentration of each element was divided by the concentration of Ca in order to normalize concentrations from water samples (Mohan et al. 2012).

Otolith Preparation and Analysis

ASMA fish in this study were the same fish analyzed by Boyd (2011). CSMA fish used in this study were the same fish analyzed by Dobbs (2013). Methods for otolith preparation were the same as those in Mohan et al. (2012) and Dobbs (2013). Briefly, sagittal otoliths were removed using plastic forceps, cleaned with deionized water and stored and dried in 1.5 mL microcentrifuge tubes. All 281 CSMA otoliths and a subsample of 288 ASMA otoliths, representing all collection areas and year classes, were sent to the University of Manitoba for trace elemental analysis. Once in Canada, otoliths were embedded in Buehler Epoxicure epoxy resin. 2-mm thick dorso-ventral transverse sections, including cores, were cut using a diamond blade Isomet saw (Bueler 646) at low speed (Halden and Friedrich 2008). Sections were ground down using 320-, 400-, and 600- grit sandpaper to expose the otolith core. They were then ultrasonically cleaned for 2 minutes. Otolith surface scratches were removed using Buehler diamond polishing suspensions (9 μm and 0.05 μm) on polishing wheels to achieve smooth otolith surfaces. Polished otoliths were cleaned ultrasonically and digitally photographed.

Methods for otolith elemental analysis were the same as those in Mohan et al. (2012) and Dobbs (2013). Briefly, otolith element concentrations were measured with a Thermo-Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd-YAG laser. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) operation parameters comprised: a 15 μm beam size; $2 \mu m s^{-1}$ scan speed; repetition rate of 20 Hz; and 75% power using low resolution (R=300) mode. ^{44}Ca, ^{25}Mg, $^{88}Sr/ ^{86}Sr$, ^{138}Ba, ^{55}Mn, ^{63}Cu, ^{66}Zn, and ^{208}Pb were the

isotopes counted in the analysis. Calcium (56 wt. % CaO) was used as the internal standard to monitor ablation yield. NIST 610 glass was used for external calibration and to monitor instrumental drift. Each laser scan of the otolith began before the nucleus, on the shorter axis of the sulcal groove and went through the nucleus and continued along the longest axis of otolith growth until reaching the outer edge. Elements were measured in counts per second and converted to ppm using a macro, written for Microsoft Excel, and plotted versus distance across the otolith corresponding to the laser scan (Mohan et al. 2012; Dobbs 2013). Otoliths were aged using two independent readers; a third reader resolved discrepancies.

Statistical Analysis: Water Chemistry

Mean elemental ratios of Mg, Sr, Ba, and Mn to Ca in water samples collected in 2011 and 2012 were compared between management areas using Wilcoxon and Kruskal-Wallis tests to determine if spatial or temporal differences in water chemistry existed between management areas. Linear discriminant function analysis (LDFA) was used to compare multivariate means of elemental ratios of water samples collected in the ASMA and CSMA. Ba:Ca ratios were Log_{10} transformed for the LDFA as they were log normally distributed. Water samples collected from rivers in the Cape Fear River system were excluded from the analysis, as no fish were collected from that system. A bootstrapped approach was used to validate results of the LDFA comparing water chemistries, in which a number generator randomly assigned water chemistries to different management areas, and repeated the LDFA. This was repeated ten times, in a manner similar to Mohan et al. (2012).

Statistical Analysis: Otolith Chemistry

Only otoliths of adult and sub-adult fish (>age-2) were selected for this analysis. A total of 235 otoliths were selected from the ASMA and 189 were selected from the CSMA. Table 2.8 gives the number of fish in each year class analyzed in this study. Mean Mg, Sr, Mn, and Ba concentrations of all fish were compared to determine differences in the adult otolith chemistry between management areas using Wilcoxon and Kruskal-Wallis tests. Linear discriminant function analysis (LDFA) was used to compare multivariate adult otolith elemental means and to classify all ASMA and CSMA fish. Yearly otolith chemistry was compared of those ASMA and CSMA fish in the same year class. Significant multivariate differences and high LDFA classification rates should indicate low or non-existent mixing of fish between management areas.

Similar methods were used to differentiate hatchery and wild fish of the CSMA. Using fish determined to be hatchery and wild by Dobbs (2013), mean Mg, Sr, Mn, and Ba concentrations were compared between the groups to determine differences in migration patterns using Wilcoxon and Kruskal-Wallis tests. LDFA was used to compare multivariate means of hatchery and wild fish and classify them. To further understand the migratory patterns of these two groups, LDFA was used to compare multivariate means and classify fish at 2-5. As with the comparisons of ASMA and CSMA fish, significant multivariate differences and high LDFA classification rates would indicate low or non-existent mixing of hatchery and wild fish. A bootstrapped approach was used to validate results of the LDFAs comparing otolith chemistries, in which a number generator randomly assigned otolith chemistries to different management areas and repeated the LDFA. This was repeated ten times, in a manner similar to Mohan et al. (2012).

Results

Water Chemistry

Mg:Ca, Sr:Ca, Mn:Ca, and Ba:Ca ratios were used to classify water samples between management areas because those elements were detected at all sites in both management areas. Both the Wilcoxon and Kruskal-Wallis tests showed that all mean element to calcium ratios varied significantly between management areas (Table 2.1). All ASMA water chemistry ratios were significantly higher than CSMA ratios. For exact mean ratios for each management area see Table 2.2. Mean ASMA Mg:Ca was 2.59, and mean CSMA Mg:Ca was 2.04 (Figure 2.3A). Mean ASMA Sr:Ca was 0.016, and mean CSMA Sr:Ca was 0.013 (Figure 2.3B). Mean ASMA Mn:Ca was $4.1*10^{-3}$, and mean CSMA Mn:Ca was $3.5*10^{-3}$ (Figure 2.3C). Mean ASMA Ba:Ca was $5.9*10^{-3}$, and mean CSMA Ba:Ca was $2.6*10^{-3}$ (Figure 2.3D). Elemental means reflect samples taken in both 2011 and 2012.

Wilcoxon and Kruskal-Wallis tests revealed that some elemental ratios varied temporally within management areas (Table 2.2). Between 2011 and 2012, mean ASMA Sr:Ca differed significantly, while Mg:Ca, Mn:Ca, Ba:Ca did not significantly differ. Mean ASMA Sr:Ca ratios were 0.0156 (2011) and 0.0164 (2012). Between 2011 and 2012 mean CSMA Mn:Ca and Ba:Ca varied, while mean Mg:Ca and Sr:Ca did not. Mean CSMA Mn:Ca ratios were $4.96*10^{-3}$ (2011) and $1.84*10^{-3}$ (2012) and mean CSMA Ba:Ca ratios were $3.15*10^{-3}$ (2011) and $2.02*10^{-3}$ (2012). Only significantly different ratios are presented. All ambient elemental concentrations were measured in mg/L.

LDFA was able to significantly discriminate multivariate mean water chemistry between the ASMA and CSMA (Pillai's Trace: F=225.7368, df=4, p<0.0001) (Table 2.4 and Figure 2.4). Overall classification of water chemistry samples was 87%. ASMA samples were classified with

88% accuracy and CSMA samples were classified with 87% accuracy (Table 2.4 and Figure 2.4).

Multivariate analyses reflect samples taken in both 2011 and 2012. Bootstrapping showed

classification rates for each management area were similar to initial classification rates (85-90%

correct classification). Overall Classification rates were also very similar (85-90%).

Otolith Chemistry: ASMA vs. CSMA

Wilcoxon and Kruskal-Wallis tests indicated that all otolith elemental concentrations

varied significantly between management areas (Table 2.3). Mean ASMA Mg was 11.15 ppm

(Figure 2.5A), and mean ASMA Sr was 2654.2 ppm (Figure 2.5B). Mean ASMA Mn was 2.59

ppm (Figure 2.5C), and mean ASMA Ba was 20.15 ppm (Figure 2.5D). Within CSMA fish,

mean otolith Mg was 12.59 ppm (Figure 2.5A), and mean Sr was 2066.31 ppm (Figure 2.5B).

Mean CSMA Mn was 1.35 ppm (Figure 2.5C), and mean CSMA Ba was ppm (Figure 2.5D).

LDFA revealed that multivariate means of otoliths differed significantly between

management areas (Pillai's Trace: F=113.9, df=4, p<0.0001) (Figure 2.6). Overall classification

of Striped Bass otolith chemistry to management area of collection was 87.5%. ASMA Striped

Bass otoliths classified at 83% to the ASMA. Classification of CSMA Striped Bass otoliths to

the CSMA was 93% (Table 2.5). Bootstrapping showed classification rates for each

management area were similar to initial classification rates (80-90% correct classification).

Overall Classification rates were also very similar (85-90%).

LDFA also revealed significant mean multivariate canonical differences in yearly otolith

chemistry between ASMA and CSMA of the same year class (Appendix B). ASMA fish were

generally classified at a lower rate than CSMA fish. Of note were the significant mean

multivariate canonical differences identified by LDFA between ASMA and CSMA fish of the

2005 and 2006 year classes at ages two, three, and four (Table 2.6 and Figure 2.7). LDFA indicated significant mean multivariate canonical differences between ASMA and CSMA fish of the 2005 year class at age-3 (Pillai's Trace: F=2.565, df=4, p<0.0426) and age-4 (Pillai's Trace: F=3.231, df=4, p<0.0153) (Table 2.6 and Figure 2.7A and 2.7B). At age-3 overall classification was 68%. ASMA classification was 65%, and CSMA classification was 87% (Table 2.6). At age-4 overall classification was 71%. ASMA classification was 69% and CSMA classification was 89% (Table 2.6). The classification rates of the 2005-year class were notably lower than most other classification rates between ages of co-occurring year classes.

LDFA also indicated significant mean multivariate canonical differences ASMA and CSMA fish of the 2006-year class at age-2 (Pillai's Trace: F=61.81 df=4, p<0.0001) and age-3 (Pillai's Trace: F=39.2263, df=4, p<0.0001). At age-2 overall classification was 93.5%. ASMA classification was 88% and CSMA classification 98.1%. At age-3 overall classification was 90%. ASMA classification was 83%, and CSMA classification was 96%. Age-3 of the 2005-year class and age-2 of the 2006 occurred in the same year, 2008, yet fish of the 2005-year class showed lower classification. The same is true for age-4 of the 2005-year class and age-3 of the 2009-year class. Fish at those ages in those year classes occurred in the year 2009, yet again fish of the 2005-year class showed lower classification (Table 2.6, Figure 2.7 C and D.).

Otolith Chemistry: CSMA Hatchery vs. Wild

Wilcoxon and Kruskal-Wallis tests showed that all otolith elemental concentrations, except for Sr varied significantly between hatchery and wild CSMA fish (Table 2.4 and Figure 2.8). Mean Mg of hatchery and wild fish were statistically different at 12.55 and 12.84 ppm respectively (Figure 2.8A). Mean Sr of hatchery and wild fish were statistically similar at 2070.6

and 2044.30 ppm respectively (Figure 2.8B). Mean Mn of hatchery and wild fish were statistically different at 1.21 and 2.02 ppm respectively (Figure 2.8C). Mean Ba of hatchery and wild fish were statistically different at 17.07 and 14.86 ppm respectively (Figure 2.8D).

LDFA revealed that the multivariate canonical means of hatchery and wild fish differed significantly (Pillai's Trace: F=4.0359, df=4, p<0.0037) (Figure 2.9). Overall classification was 72.5% (Table 2.5). Hatchery fish were classified correctly 77% of the time, but wild fish were classified correctly 51% of the time.

In an effort to determine the cause of high rates of discrimination between hatchery and wild fish, LDFA was used to discriminate hatchery and wild fish by age. Ages two, three, four, and five were the ages used in the analysis. Fish at age-2 showed significant mean canonical multivariate differences and were classified with 73% accuracy (Pillai's Trace: F=4.8511, df=4, p<0.0010) (Table 2.7 and Figure 2.10A). Hatchery fish were classified 76% correctly, and wild fish were classified with 58% accuracy (Table 2.7). Fish at age-3 showed no significant mean multivariate canonical differences and were classified with 71% accuracy (Pillai's Trace: F=2.1916, df=4, p<0.0716) (Table 2.7 and Figure 2.10B). Hatchery fish were classified with 76% accuracy, but wild fish were classified with 45% accuracy (Table 2.7). Fish at age-4 showed no significant mean multivariate canonical differences and were classified with 50% accuracy. (Pillai's Trace: F=0.3851, df=4, p=0.8190) (Table 2.7 and Figure 2.10C). Hatchery fish were classified with 43% accuracy, and wild fish were classified with 56% accuracy (Table 2.7). Fish at age-5 showed no significant mean multivariate canonical differences and were classified with 59% accuracy. (Pillai's Trace: F=0.1383, df=4, p<0.9675) (Table 2.7 and Figure 2.10D). Hatchery fish were classified with 62% accuracy, and wild fish were classified with 37.5% accuracy (Table 2.7).

Discussion

Water Chemistry

My results show that while some elemental ratios vary yearly within management areas (Sr:Ca in the ASMA and Mn:Ca and Ba:Ca in the CSMA) all elemental ratios still varied significantly between management areas and were higher in the ASMA than the CSMA. That such differences exist between the management areas is both fortuitous and curious. Dobbs (2013) examined the same CSMA water chemistry samples that were analyzed in this study. He examined differences in them by river, month, season, and year of collection. All elemental ratios differed significantly by rivers in the CSMA but were temporally stable over years. Zapf (2012) and Mohan et al. (2012) both examined ASMA water chemistry. They both found that water chemistry differed significantly between rivers of the ASMA, but that the chemistry of individual rivers was stable seasonally, and possibly yearly. Because such differences between management areas exist, differentiation of otolith chemistry between ASMA and CSMA fish would mean that fish from the different management areas likely do not mix.

My results showed some temporal variation of water chemistry within management areas, but that variation did not hinder efforts to determine spatial differences between the two, as evidenced by 87% overall correct classification between the ASMA and CSMA using LDFA (Figure 2.4). There was one factor that likely reduced classification of water chemistry in the ASMA. Sr:Ca differed within the ASMA between 2011 and 2012. There are two possible explanations for this change. The first is a possible change in salinity between the two years, as Sr:Ca ratios are directly correlated to salinity (Odum 1951; Mohan et al. 2012). A change in salinity would alter the ambient Sr:Ca ratios. The second is possible upwelling of Sr rich water

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from the Upper Castle Hayne Aquifer, a limestone aquifer known to contain high levels of Sr (Woods et al. 2000). Significant yearly differences in mean CSMA Mn:Ca and Ba:Ca ratios did not appear to affect classification rates of CSMA water chemistry. The high rates of classification achieved using Mg, Sr, Mn, and Ba:Ca ratios to classify water chemistry support Mohan et al. (2012)'s assertion that these ratios are valid for use in discriminating watersheds of North Carolina.

Otolith Chemistry: ASMA vs. CSMA Adult Otolith Analysis

Results of the Wilcoxon and Kruskal-Wallace tests indicated that all elemental concentrations in the adult portions of otoliths differed significantly between ASMA and CSMA fish (Figure 2.5). In fact, like mean water chemistry elemental ratios, the mean elemental concentrations in otoliths were greater in the ASMA than the CSMA, with the exception of Mg, which was greater in CSMA fish (Figure 2.5). This result, along with high classification rates obtained from LDFA of all ASMA and CSMA fish (overall 88% classification) suggests that there was little mixing of fish between the ASMA and CSMA (Figure 2.6). Sources of misclassification were likely a combination of variation in water chemistry, migration of fish between management areas, or introgression of fish from neighboring estuarine systems, like the Chesapeake Bay.

It is remarkable that such high rates of classification between the ASMA and CSMA were observed, given that fish from the ASMA ranged in age from 3 to 16, and fish from the CSMA ranged in age from 3 to 8 representing a large timescale in which variations in water chemistry could have made differentiating fish from the two management areas difficult. A similar study using whole otolith signatures to delineate stocks of Atlantic Cod, *Gadus morhua*,

also found high levels of differentiation between stocks (Campana and Gagne 1995). However, Atlantic Cod are fully marine fish, and the chemistry of seawater tends to be temporally conserved (Sturrock et al. 2012). While low amounts of temporal variation in estuarine water chemistry were observed in the Albemarle-Pamlico estuarine system, as well as other systems (Wells et al. 2003; Dorval and Jones 2005; Mohan et al. 2012; Dobbs 2013), most water chemistries were observed at most for only one year. Results of this otolith analysis suggest that between North Carolina estuaries, water chemistries remain stable enough to allow differentiation of otolith chemistries of fish occurring in adjacent estuaries over long periods of time, conceivably as long as 10 years.

Perhaps this is not surprising, given that the Albemarle-Pamlico Sound estuarine system is largely wind driven and not tidally driven. The inlets are small and scattered, limiting seawater exchange in the system (Bowden and Hobbie 1977; Copeland et al. 1983; Pietrafesa et al. 1996). Because of the low seawater exchange rate it might be expected that water chemistry, and consequently otolith chemistry, remains stable for long periods of time in the Albemarle-Pamlico Sound system. Studies analyzing water chemistry in tidally driven estuaries have found high degrees of temporal variance (Hatje 2003; Elsdon and Gillanders 2006).

Otolith Chemistry: ASMA vs. CSMA Yearly Otolith Analysis

In an attempt to provide a clearer picture of the delineation between the ASMA and CSMA fish, yearly otolith data were compared between ASMA and CSMA fish of the same year class (2004-2007). Overall, LDFA classification rates reflected those of the whole otolith LDFA (Figure 2.5; Appendix B), and ASMA fish were consistently misclassified at a higher rate, which may have been the result of temporally variable ASMA water chemistry or migration of ASMA

fish to the CSMA or other systems. However, there were some ages within the 2005-year class that showed some lower rates of classification that were likely not just caused by variation in water chemistry. At ages 3 and 4 of the 2005-year class, which represent the years 2008 and 2009, overall classification rates of both management areas were 68% and 71%, respectively. ASMA fish were classified correctly just 65% (age 3, 2008) and 68% (age 4, 2009) of the time (Table 2.6 and Figure 2.7A and 2.7B). Both overall and ASMA classification rates at ages 2 and 3 of the 2006-year class, which also represent the years 2008 and 2009, may help to explain the low overall and ASMA classification rates the 2005-year class. At ages 2 and 3 of the 2006-year class, overall classification was 93.5% and 90% respectively. ASMA fish were classified correctly 88% (age 2, 2008) and 83% (age 3, 2009) of the time (Table 2.6 and Figure 2.7A and 2.7B).

Water chemistry cannot be the only source of variation causing misclassification, as fish occurring at the same time from the 2005 and 2006-year classes from the same management area had such different rates of classification. Water chemistry or environmental variation should cause similar, high rates of variation in otolith chemistry in all fish inhabiting a management area at a given time, thereby affecting all year classes of fish, not just one specific year class. Therefore, the observed large differences in misclassification must have been caused by migration between management areas or introgression of fish from neighboring coastal estuarine systems.

It is likely that fish from the ASMA migrated to the CSMA and back to the ASMA before they were collected. CSMA fish are largely resident based on results of the CSMA tag returns (NCDMF and NCWRC 2013). In addition, tag returns of >42,000 fish tagged in the ASMA on the spawning grounds near Weldon, NC and in the western Albemarle Sound,

indicated younger sub-adult and adult fish were more likely to migrate from the ASMA to the CSMA as a result of high density of conspecifics (Callihan et al. 2014). If migration of sub-adult and young adult fish from the ASMA to the CSMA is related to high density of conspecifics, then high rates of misclassification of otolith chemistries between the two management areas should be observed only in highly abundant year classes. The year-1 juvenile abundance of 2005-year class fish in the ASMA was estimated to be over 600,000 fish in the most recent assessment of the ASMA stock. This was the highest number of juvenile fish recorded during the time frame of the assessment (1982-2008) (Takade-Heumacher 2010). The combined evidence of the otolith signature, tag return, and juvenile abundance estimates point to no option other than that sub-adult and young adult ASMA fish are more likely to migrate to the CSMA during periods of high abundance of conspecifics.

Otolith Chemistry: CSMA Hatchery vs. Wild

As Dobbs (2013) showed, >85% of CSMA fish collected for this study were of hatchery origin. My study aimed to further elucidate sub adult and adult migration patterns of the same fish analyzed by Dobbs. Analysis of adult otolith chemistry of hatchery and wild fish revealed that mean elemental concentrations between the two groups varied significantly. Furthermore, LDFA showed a high overall successful (72.5%) classification rate between the two groups (Table 2.5 and Figure 2.9). This finding is noteworthy, because it could indicate that current CSMA stocking programs have not increased juvenile recruitment, or improved population structure and condition indices (Dobbs 2013; Homan et al. 2013). Since hatchery and wild fish otolith signatures were different (i.e., using different habitat), then many hatchery fish may not

use the spawning grounds or hatchery fish outcompete wild fish for better habitat or a combination of those scenarios.

However, a more likely scenario causing differences in otolith signatures between adult hatchery and wild fish may be more benign. LDFA of yearly otolith data of all adult year classes within the CSMA (2004-2009) showed that hatchery and wild fish discriminated well for ages 2 and 3 and poorly for ages 4 and 5 (Table 2.7 and Figure 2.10). Hatchery fish were classified at higher rates than wild fish (Table 2.7). Because yearly otolith deposition rates are related to somatic growth rate and younger fish grow more quickly and accrete otolith material more quickly than older fish, ages 2 and 3 dominated the sample, causing overall classification to reflect ages 2 and 3 (Kline 1990). I hypothesize that the trend in high classification rates of hatchery fish at younger ages and low classification rates at older ages may indicate that hatchery fish stay in and around the areas where they were stocked initially (Goose Creek State Park at Dinah's Landing near Washington, NC on the Tar-Pamlico River and the Bridgeton boat access near New Bern, NC on the Neuse River) (Dockendorf, NCWRC, personal communication) as age-0 fish. As they become older and larger, the hatchery fish moved throughout the system, mixing with the wild, naturally occurring fish.   The wild (naturally occurring) fish may not have classified well because they may have been dispersed throughout the CSMA for their entire lives. It is not likely that temporal variation in water chemistry had a large influence on the classifications because fish representing all year classes were successfully classified by age. Temporal variation in water chemistry would lower classification rates at all ages, not just the older ages.

Conclusions and Recommendations

While certain ambient elemental ratios in the environment varied over time within the ASMA and CSMA, that amount of variation did not confound efforts to differentiate water chemistry between the two areas. The water chemistries of the different management areas were unique, allowing for comparison of otolith chemistries between management areas. Otolith chemistries of ASMA and CSMA fish were unique as well, following the results of water chemistries. Results indicate that little migration of sub-adult and adult Striped Bass occurs between the two systems, however fish were more likely to introgress to the CSMA in years of high sub-adult and young adult abundance in the ASMA. Hatchery and wild fish of the CSMA likely spend the first two to three years of their lives in separate habitats but then mix together as they grow older.

The ASMA and CSMA should continue to be managed as separate stocks, but when strong year classes in the ASMA are observed, managers should expect an increased level of migration from the ASMA to the CSMA. Tagging efforts should continue in both management areas in order to monitor rates of exchange.

It is difficult to determine the efficacy of the CSMA stocking program through adult otolith analysis. My results do not suggest significant differences in migratory patterns between hatchery and wild fish. Because there are so many more hatchery fish, they may be outcompeting wild fish for resources. The CSMA stock exhibits many characteristics of a highly stressed, overfished stock (truncated age structure, poor conditions indices, low juvenile recruitment) (Homan et al. 2013; NCDMF and NCWRC 2013), so augmenting the population through stocking should continue. Fishing effort must also be reduced in order to afford the

CSMA stock the opportunity to recover its losses. The quality of habitat in the CSMA must also

be investigated, as poor habitat may also be causing poor recruitment.

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Tables

Table 2.1. Results of Kruskal-Wallis and Wilcoxon tests examining the spatial differences in element to Ca ratio between ASMA and CSMA water chemistry samples collected in 2011 and 2012 not including rivers in the Cape Fear River System. For each one-way analysis, α was set at 0.05. Asterisks (*) represent significant p-values.

| Water elemental ratio | Number of samples | | Chi-square | df | Confidence level (p) |
	ASMA	CSMA			
Mg/Ca	434	312	99.945	1	<0.0001*
Sr/Ca	434	312	117.948	1	<0.0001*
Mn/Ca	434	312	40.314	1	<0.0001*
Ba/Ca	434	312	185.308	1	<0.0001*

Table 2.2. Results of Kruskal-Wallis and Wilcoxon tests examining the temporal differences in element to Ca ratio from ASMA and CSMA water chemistry samples between the years 2011 and 2012 not including rivers in the Cape Fear River System. For each one-way analysis, α was set at 0.05. Asterisks (*) represent significant p-values.

Management area	Number of samples 2011	2012	Elemental ratio	Chi-square	df	Confidence level (p)
ASMA	287	147	Mg/Ca	0.0468	1	0.829
	287	147	Sr/Ca	5.291	1	0.021*
	287	147	Mn/Ca	2.972	1	0.095
	287	147	Ba/Ca	1.382	1	0.230
CSMA	168	144	Mg/Ca	0.436	1	0.509
	168	144	Sr/Ca	0.001	1	0.971
	168	144	Mn/Ca	4.221	1	0.039*
	168	144	Ba/Ca	4.826	1	0.028*

Table 2.3. Results of Kruskal-Wallis and Wilcoxon tests examining the statistical differences in mean Mg, Sr, Mn, and Ba ratios between ASMA and CSMA otolith chemistry samples. For each one-way analysis, α was set at 0.05. Asterisks (*) represent significant p-values.

| Otolith element | Number of samples | | Chi-square | df | Confidence level (p) |
	ASMA	CSMA			
Mg	235	189	18.86	1	<0.0001*
Sr	235	189	220.86	1	<0.0001*
Mn	235	189	13.07	1	0.0003*
Ba	235	189	17.11	1	<0.0001*

Table 2.4. Results of Kruskal-Wallis and Wilcoxon tests examining the statistical differences in mean Mg, Sr, Mn, and Ba ratios between CSMA hatchery and wild otolith chemistry samples. For each one-way analysis, α was set at 0.05. Asterisks (*) represent significant p-values.

| Otolith element | Number of samples | | Chi-square | df | Confidence level (p) |
	Hatchery	Wild			
Mg	158	31	0.0310	1	0.8603
Sr	158	31	0.2146	1	0.6432
Mn	158	31	9.7151	1	0.0018*
Ba	158	31	3.5271	1	0.0604

Table 2.5. Results of the LDFA (water chemistry) and LDFA (otolith chemistry) of water samples and Striped Bass otolith samples collected in the ASMA and CSMA. Results of the LDFA comparing the hatchery and wild fish from the CSMA are also shown. Percentages reflect classification of ASMA fish to the ASMA and CSMA fish to the CSMA, and hatchery fish as hatchery and wild fish as wild. Asterisks (*) represent significant multivariate differences between the ASMA and CSMA. Alpha was set to 0.05.

Type of sample	Classification group	Number of samples	Percent correctly classified	Overall percent classification	Multivariate confidence level (p)
Water Chemistry	ASMA	434	88.0	87.0	<0.0001*
	CSMA	312	87.0		
ASMA vs. CSMA Otolith Chemistry	ASMA	235	83.0	87.5	<0.0001*
	CSMA	189	93.0		
CSMA Hatchery vs. Wild Otolith Chemistry	Hatchery	158	77.0	72.5	<0.0037*
	Wild	31	51.0		

Table 2.6. Results of the LDFAs classifying Striped Bass to management area of collection based on otolith microchemistry. Age two, three, and four fish from the 2005 and 2006 are shown in this table. Percentages reflect classification of ASMA fish to the ASMA and CSMA fish to the CSMA. Asterisks (*) represent significant multivariate differences between the ASMA and CSMA. Alpha was set to 0.05.

Management area of collection	Number of samples	Year class	Age	Year	Percent correctly classified	Overall classification	Multivariate confidence level (p)
ASMA	92	2005	3	2008	65.0	68.0	0.0426*
CSMA	15				87.0		
ASMA	92	2005	4	2009	68.0	71.0	0.0153*
CSMA	15				87.0		
ASMA	41	2006	2	2008	88.0	94.0	<0.0001*
CSMA	52				98.0		
ASMA	41	2006	3	2009	83.0	90.0	<0.0001*
CSMA	52				96.0		

Table 2.7. Results of the LDFAs classifying CSMA Striped Bass as hatchery or wild based on otolith microchemistry. Percentages reflect classification of hatchery fish as hatchery and wild fish as wild. Asterisks (*) represent significant multivariate differences between the hatchery and wild fish. Alpha was set to 0.05.

Hatchery/Wild	Number of samples	Age	Percent correctly classified	Overall classification	Multivariate confidence level (p)
Hatchery	158	2	76.0	73.0	<0.0100*
Wild	31		58.0		
Hatchery	158	3	76.0	71.0	0.0716
Wild	31		45.0		
Hatchery	108	4	51.0	50.0	0.8190
Wild	23		43.0		
Hatchery	17	5	37.5	59.0	0.9675
Wild	8		62.5		

Table 2.8. Number of fish examined each year class from the ASMA and CSMA collected in 2009 and 2010 (ASMA) and 2011 and 2012 (CSMA).

Year Class	Number of samples ASMA	Number of samples CSMA
1994	2	-
1996	2	-
1997	2	-
1998	2	-
1999	1	-
2000	24	-
2001	11	-
2002	7	-
2003	6	1
2004	28	8
2005	92	15
2006	41	52
2007	17	23
2008	-	68
2009	-	22
Total	235	189

Figures

Figure 2.1. A map of the ASMA. Water sampling locations are marked. Site A marks the watershed mouth, and site B marks upstream locations beyond the influence of salinity.
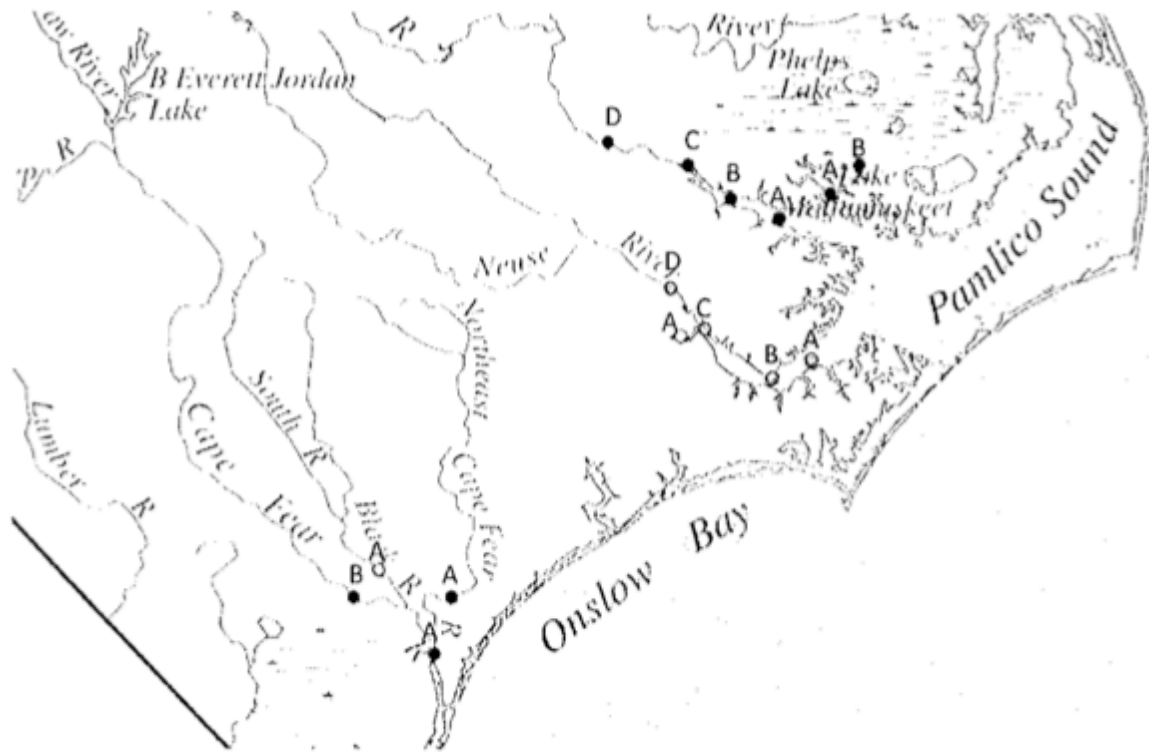
Figure 2.2. A map of the CSMA. Water sample locations are marked. This map is a USGS map, and used in Dobbs (2013). It is used here with his permission.
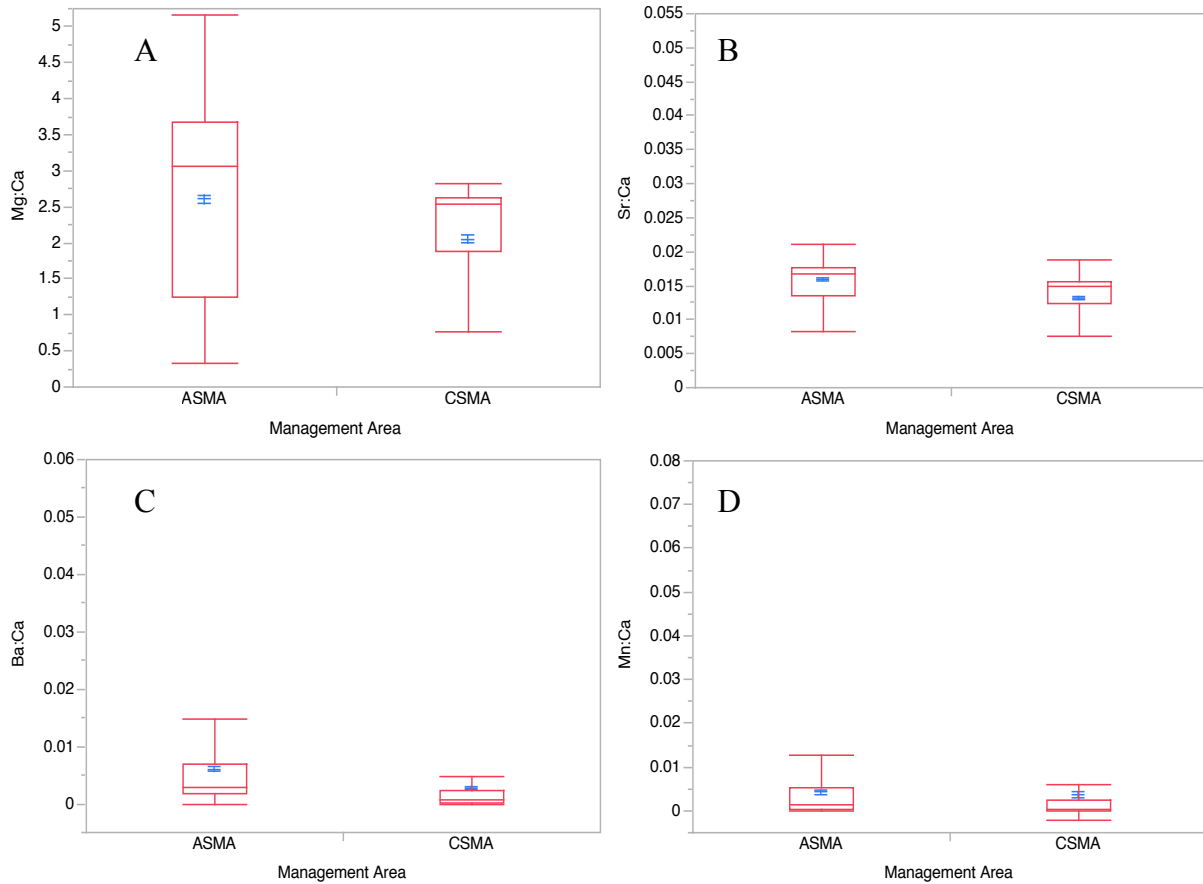
Figure 2.3. Box plots of Mg:Ca (A), Sr:Ca (B), Ba:Ca (C), and Mn:Ca (D) of water chemistry samples collected from the ASMA and CSMA in North Carolina in 2011 and 2012, not including rivers in the Cape Fear River system. The end lines of the box plots show the distribution of 95% of the data. The central boxes show the range of the 25th and 75th percentile of data. Lines at the center of the box plots show the median of the data. Mean (± SE) of all elemental ratios is also shown. All mean elemental ratios varied significantly between management areas and were higher in the ASMA than the CSMA.
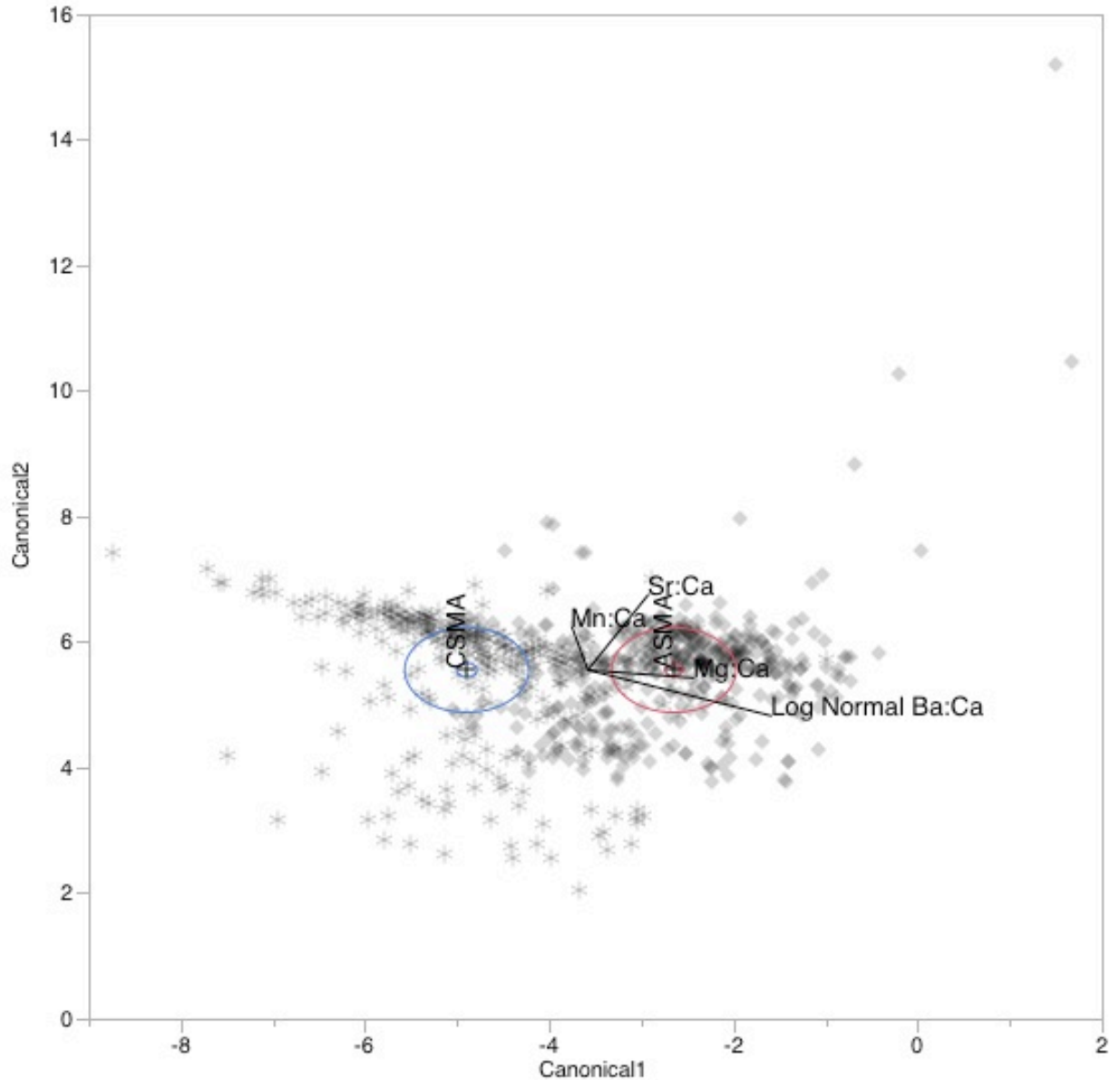
Figure 2.4. Plot of first two canonical variates obtained using LDFA to classify water chemistry samples from the ASMA (♦) and CSMA (*) to their management areas of collection. Ba:Ca ratios were log_{10} transformed as the Ba:Ca data were log normally distributed. Group centroids (+) are surrounded by ellipses representing 95% confidence intervals (the smaller ellipse). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar.
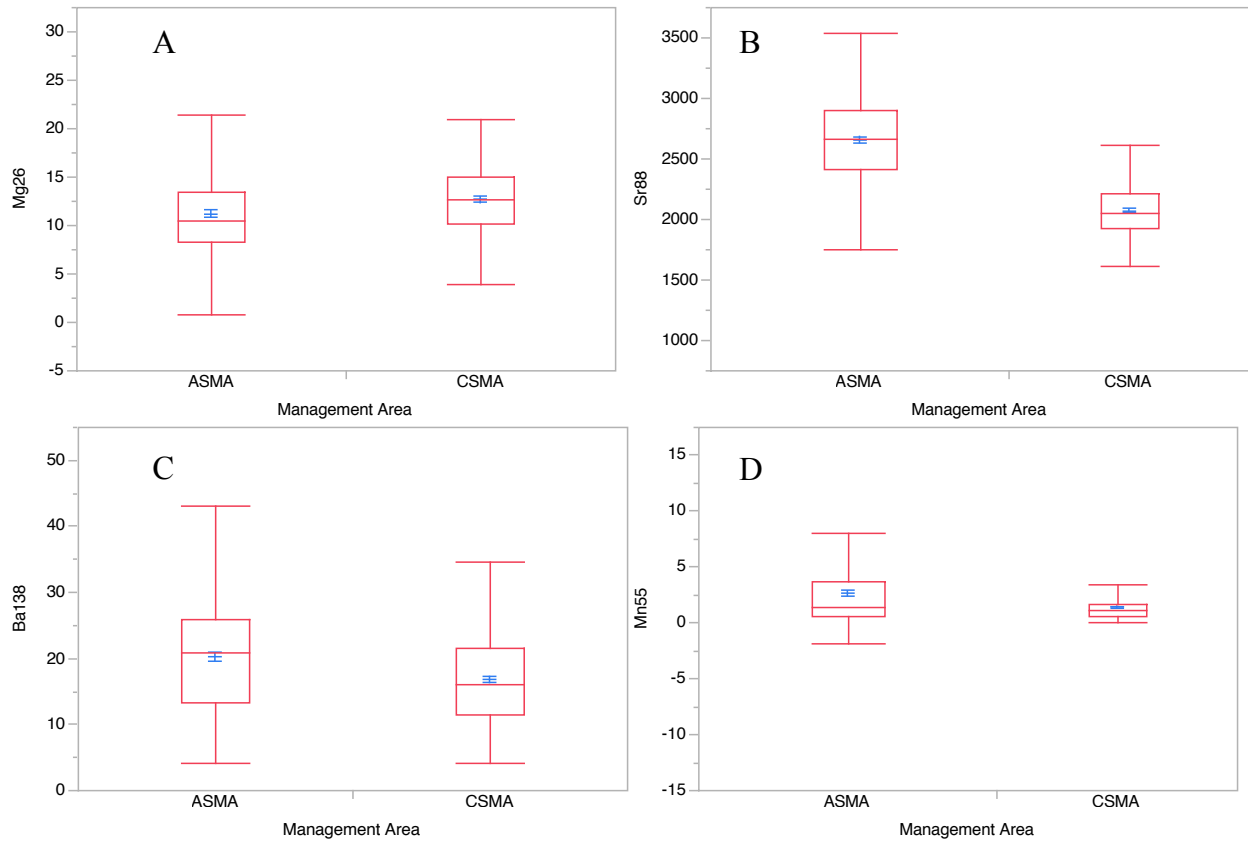
Figure 2.5. Box plots of Mg (A), Sr (B), Ba (C), and Mn (D) of adult otolith chemistry samples collected from the ASMA and CSMA in North Carolina. The end lines of the box plots show the distribution of 95% of the data. The central boxes show the range of the 25th and 75th percentile of data. Lines at the center of the box plots show the median of the data. Mean (± SE) of all elemental ratios is also shown. All mean elemental ratios varied significantly between management areas.
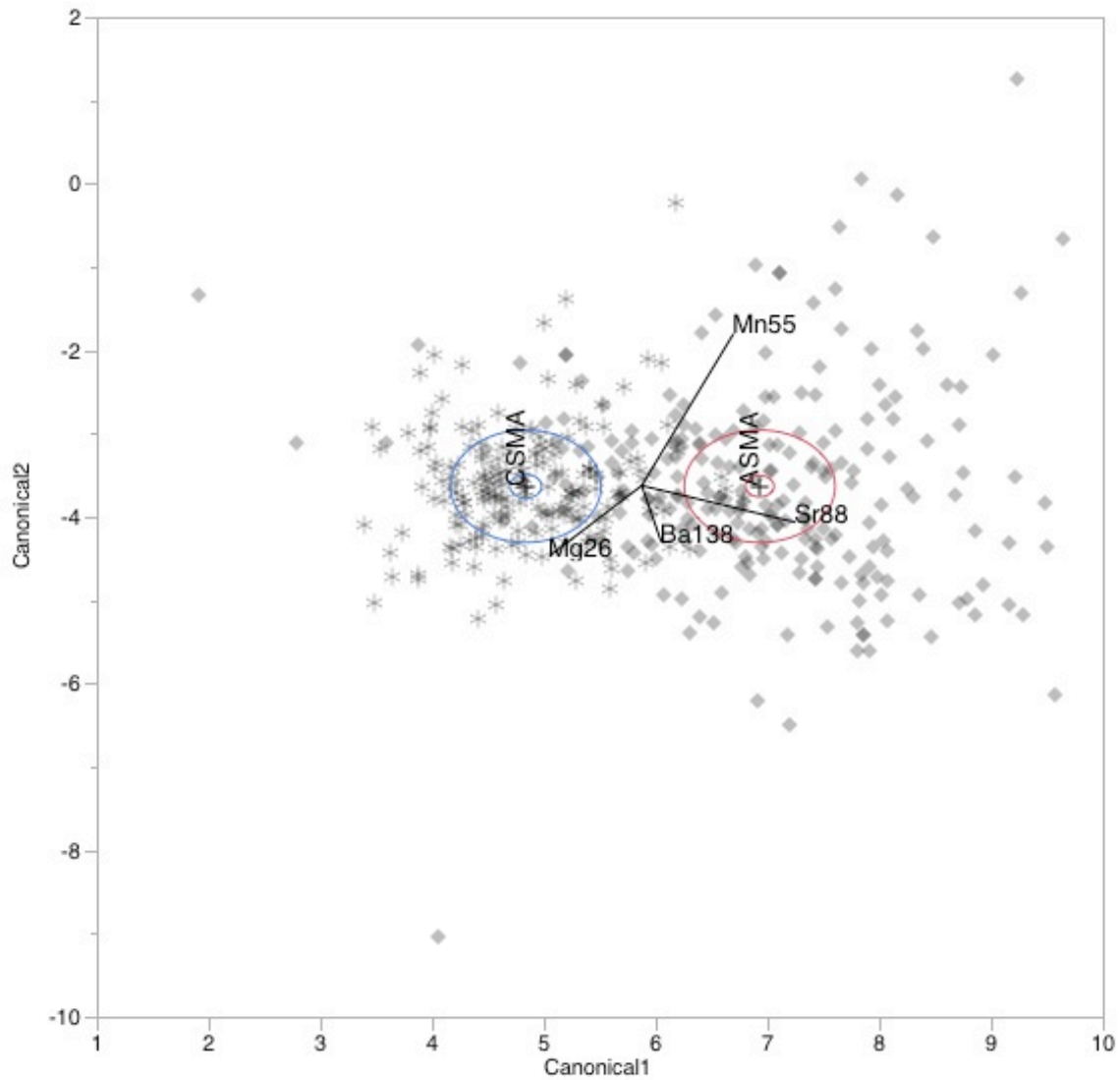
Figure 2.6. Plot of the first two canonical variates obtained using LDFA to classify adult Striped Bass otolith microchemical signals collected from the ASMA (♦), and CSMA (*). Group centroids (+) are surrounded by ellipses representing 95% confidence intervals (the smaller ellipse). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar.
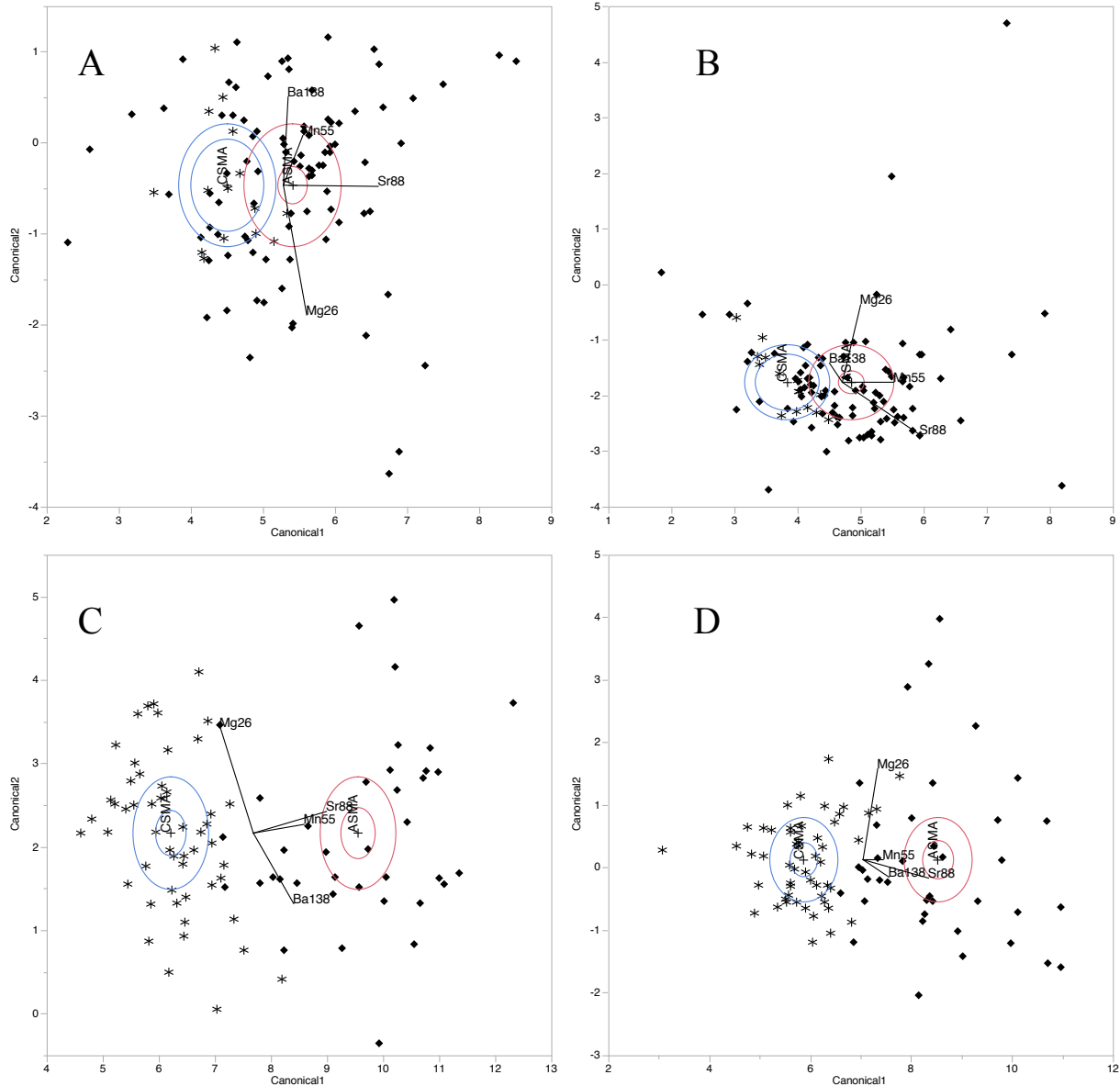
Figure 2.7. (A) represents the LDFA classifying age 3 of 2005 year class adult Striped Bass from the ASMA (♦) and CSMA (*). (B) represents the LDFA classifying age 4 of 2005 year class adult Striped Bass from the ASMA (♦) and CSMA (*). (C) represents the LDFA classifying age 2 of 2006 year class adult Striped Bass from the ASMA (♦) and CSMA (*). (D) represents the LDFA classifying age 3 of 2006 year class adult Striped Bass from the ASMA (♦) and CSMA (*). (A) and (C) represent the year 2008, while (B) and (D) represent the year 2009. Group centroids (+) are surrounded by ellipses representing 95% confidence intervals (the smaller ellipse). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar.
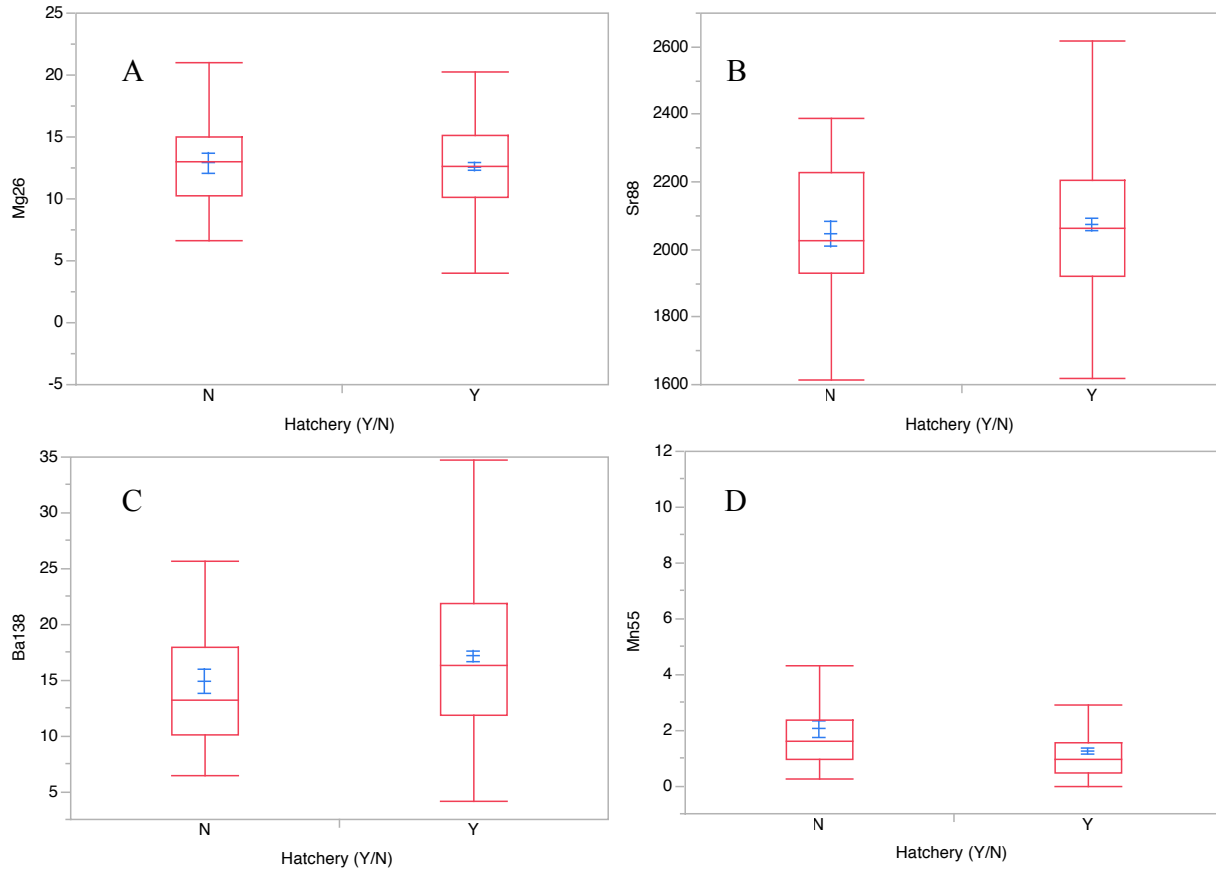
Figure 2.8. Box plots of Mg (A), Sr (B), Ba (C), and Mn (D) of adult otolith chemistry samples collected from the hatchery and wild CSMA fish in North Carolina in 2011 and 2012, not including rivers in the Cape Fear River system. The end lines of the box plots show the distribution of 95% of the data. The central boxes show the range of the 25th and 75th percentile of data. Lines at the center of the box plots show the median of the data. Mean (± SE) of all elemental ratios is also shown. All mean elemental ratios varied significantly between management areas.
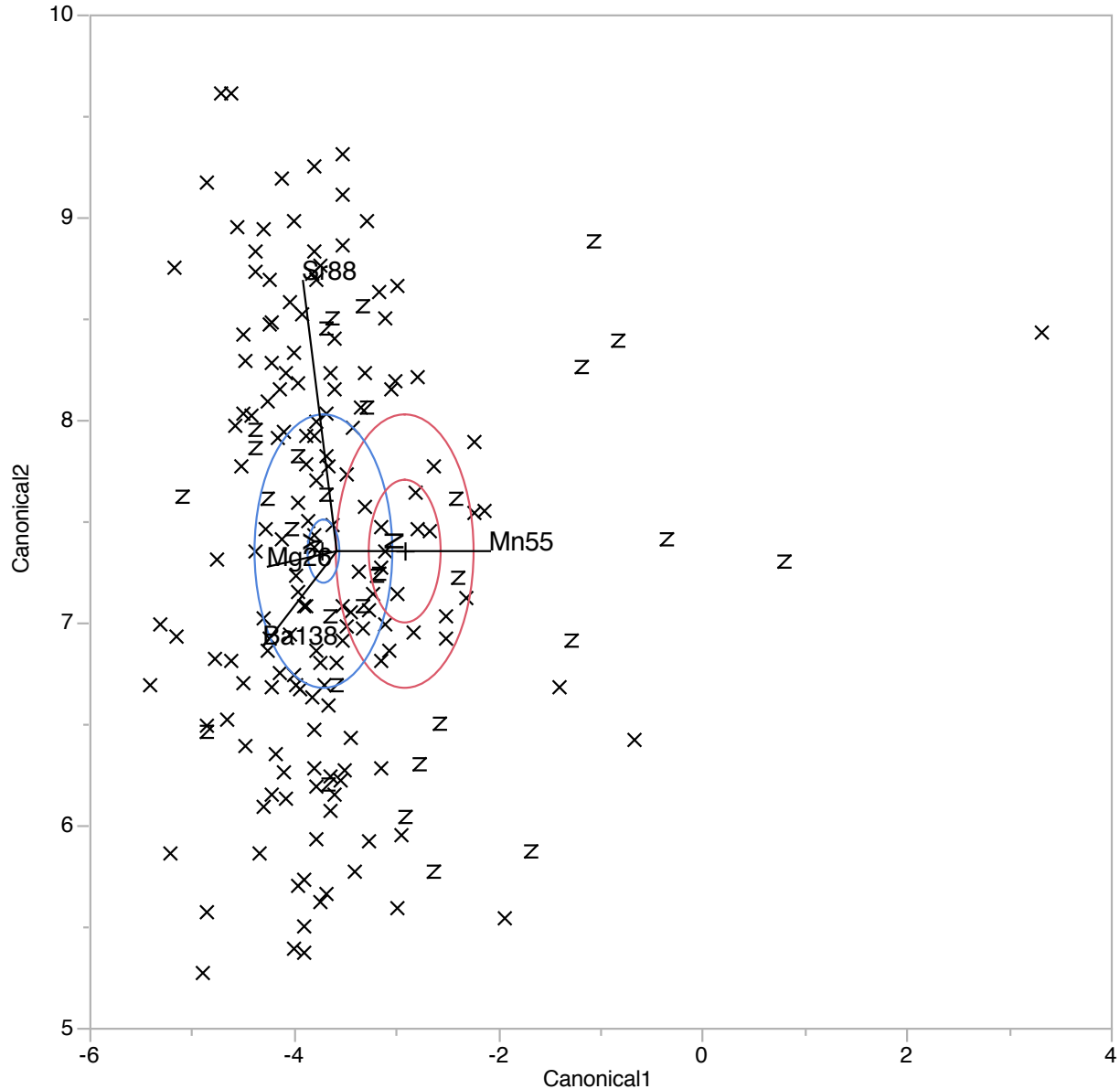
Figure 2.9. Plot of the first two canonical variates obtained using LDFA to classify CSMA Striped Bass adult otolith microchemical signals as hatchery (blue contours, x) and non-hatchery (red contours, z). Group centroids (+) are surrounded by ellipses representing 95% confidence intervals (the smaller ellipse). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar.
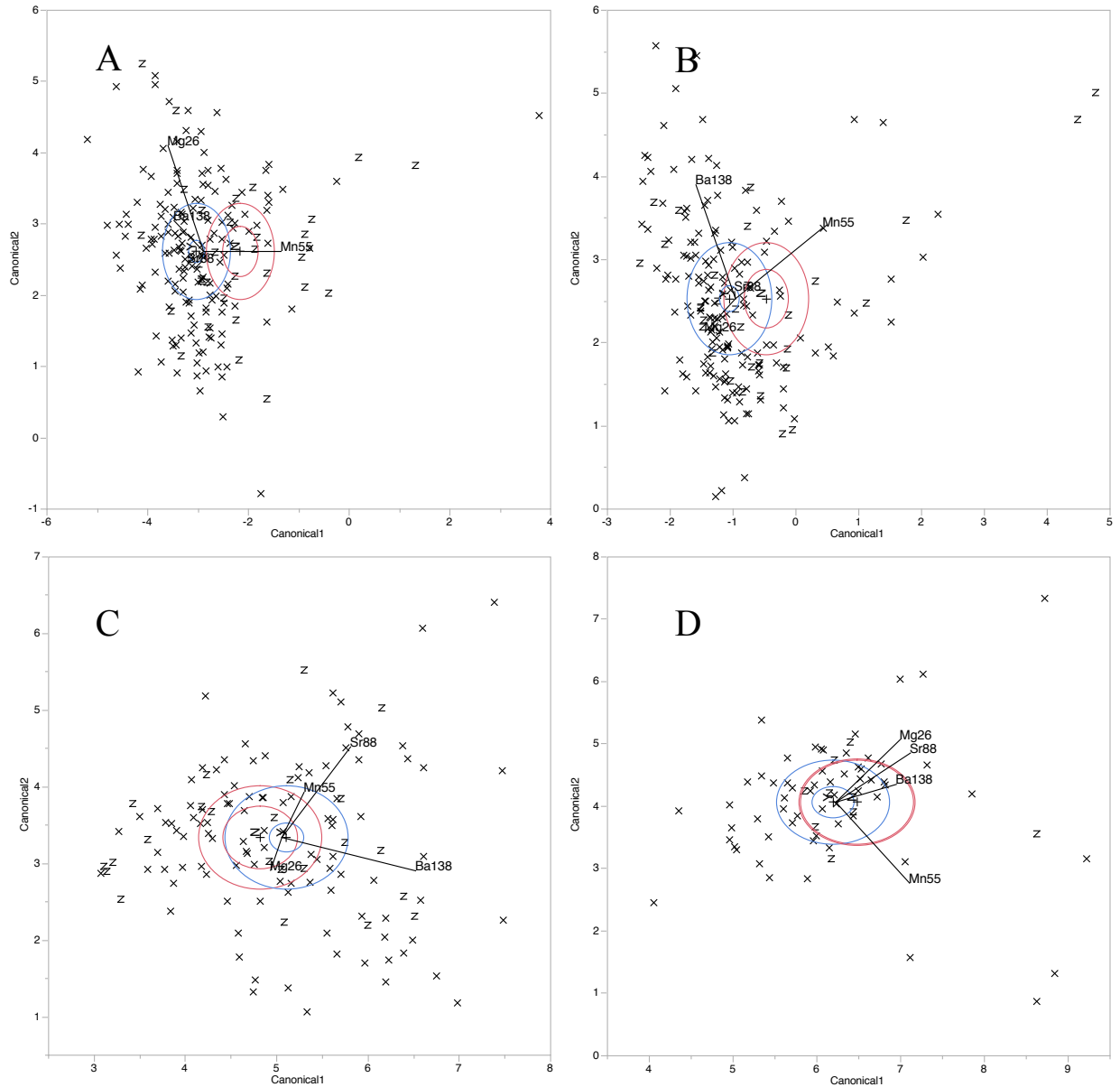
Figure 2.10. (A) represents the LDFA classifying age 2 of CSMA adult Striped Bass as hatchery reared (x, blue) and wild (z, red). (B) represents the LDFA classifying age 3 of CSMA adult Striped Bass as hatchery reared (x, blue) and wild (z, red). (C) represents the LDFA classifying age 4 of CSMA adult Striped Bass as hatchery reared (x, blue) and wild (z, red). (D) represents the LDFA classifying age 5 of CSMA adult Striped Bass as hatchery reared (x, blue) and wild (z, red). Group centroids (+) are surrounded by ellipses representing 95% confidence intervals (the smaller ellipse). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar.

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Appendix A.

The mean depths (m) and locations (lat/long) of all of the water sample locations from which samples were analyzed in this study.

Management Area	River	Site	Latitude/Longitude	Mean depth (m)
ASMA	Alligator	A	35° 53.666' N 76° 00.665' W	4.04
		B	35° 45.721' N 76° 01.357' W	3.51
	Chowan	A	36° 03.293' N 76° 41.072' W	3.41
		B	36° 16.155' N 76° 40.871' W	3.86
	Little	A	36° 09.148' N 76° 13.750' W	2.72
	North	A	36° 11.092' N 75° 54.636' W	3.09
		B	36° 18.511' N 75° 58.285' W	2.13
	Pasquotank	A	36° 11.406' N 76° 04.082' W	2.90
		B	36° 17.839' N 76° 13.030' W	5.44
	Perquimans	A	36° 07.525' N 76° 20.603' W	2.93
		B	36° 11.480' N 76° 28.001' W	1.65
	Roanoke	A	35° 55.493' N 76° 42.051' W	6.27
		B	35° 52.111' N 76° 47.171' W	3.16

| Appendix A cont.				
Management Area	River	Site	Latitude/Longitude	Mean depth (m)
	Scuppernong	A	35° 56.524' N 76° 19.037' W	2.75
		B	35° 53.208' N 76° 16.218' W	5.39
CSMA	Neuse	A	35° 00.539' N 76° 40.137' W	6.64
		B	34° 57.295' N 76° 48.167' W	3.69
		C	35° 05.745' N 77° 01.744' W	3.09
		D	35° 12.600' N 77° 07.321 W	4.85
	Pamlico	A	35° 24.262' N 76° 43.773' W	5.07
		B	35° 27.495' N 76° 55.328' W	5.19
		C	35° 32.549' N 77° 03.620' W	3.37
		D	35° 36.901' N 77° 21.059' W	1.04
	Pungo	A	35° 26.305' N 76° 34.599' W	5.40
		B	35° 31.717' N 76° 29.160' W	4.25
	Trent	A	35° 04.245' N 77° 05.434' W	2.60

Appendix B.

Full results from LDFA of yearly otolith data of ASMA and CSMA fish in co-occurring year classes
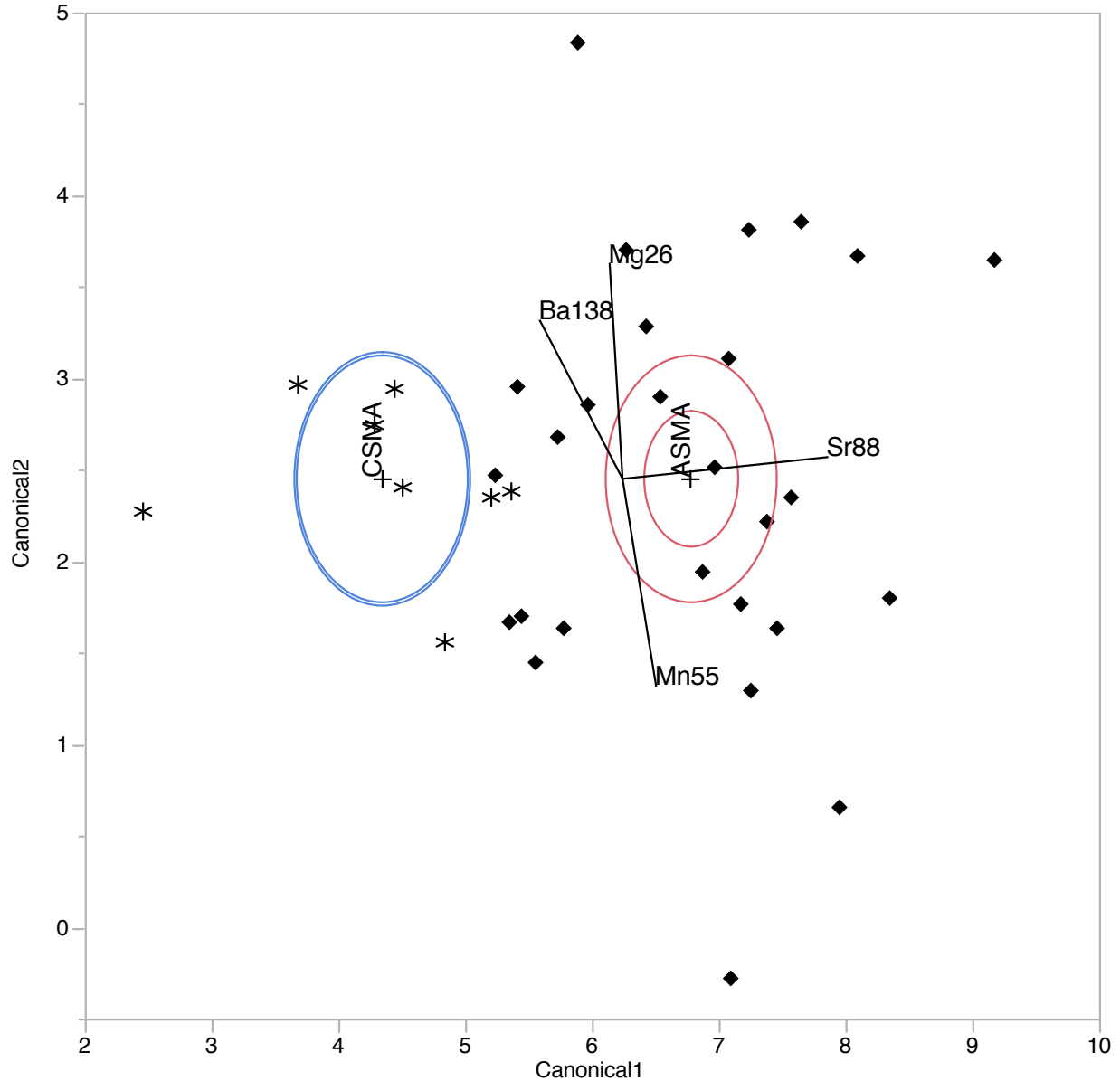Appendix B cont. LDFA Comparing 2004 age 2 fish from the ASMA and CSMA
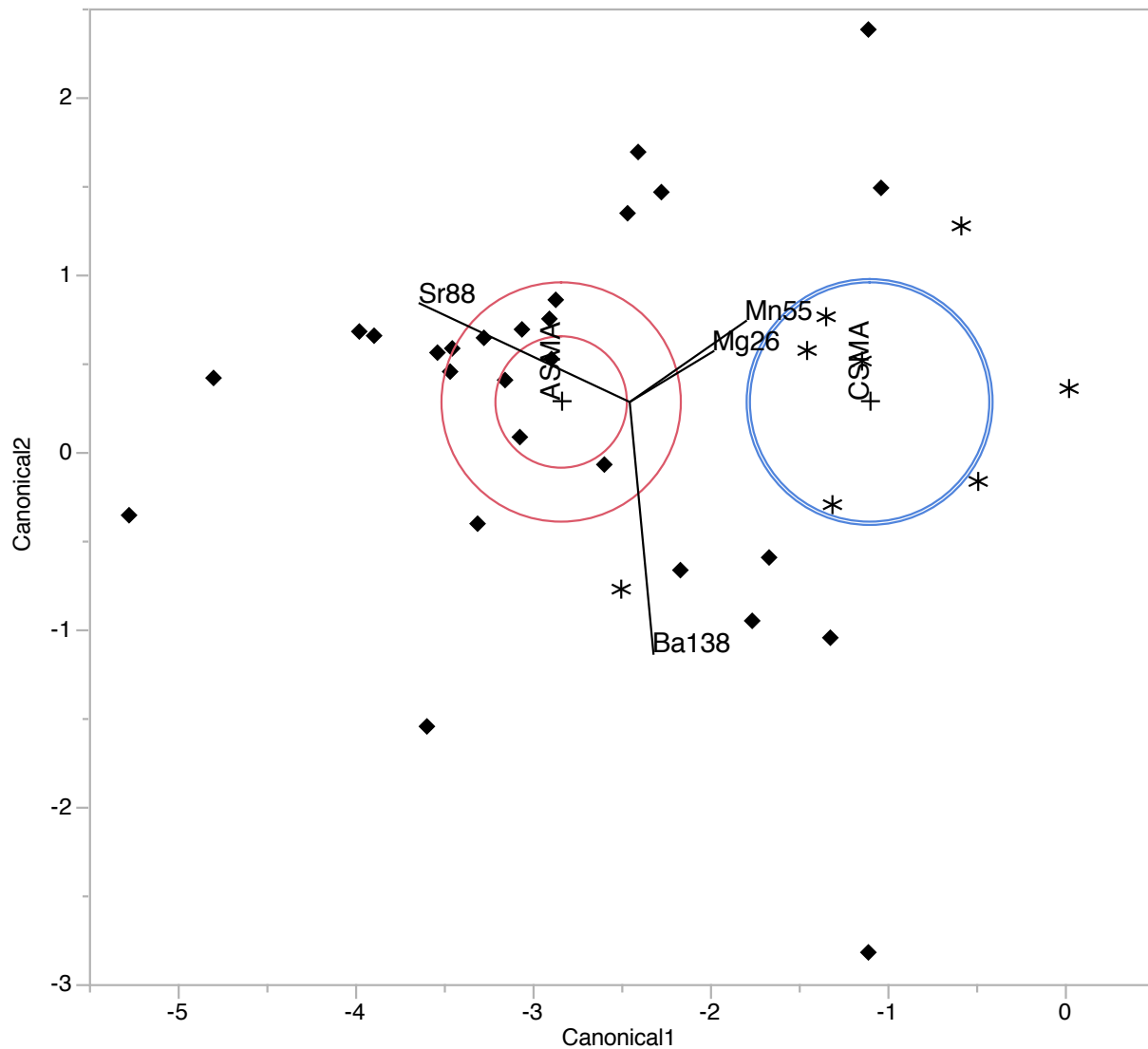
Group centroids (+) are surrounded by ellipses representing 95% confidence intervals (the smaller ellipse). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=8, ASMA=28; Overall classification: 80%; CSMA classification: 87.5%, ASMA classification: 78.5 %. Pillai's Trace: F=4.29, df=4, p<0.0001

Appendix B cont. LDFA comparing age 3 fish from 2004 year class
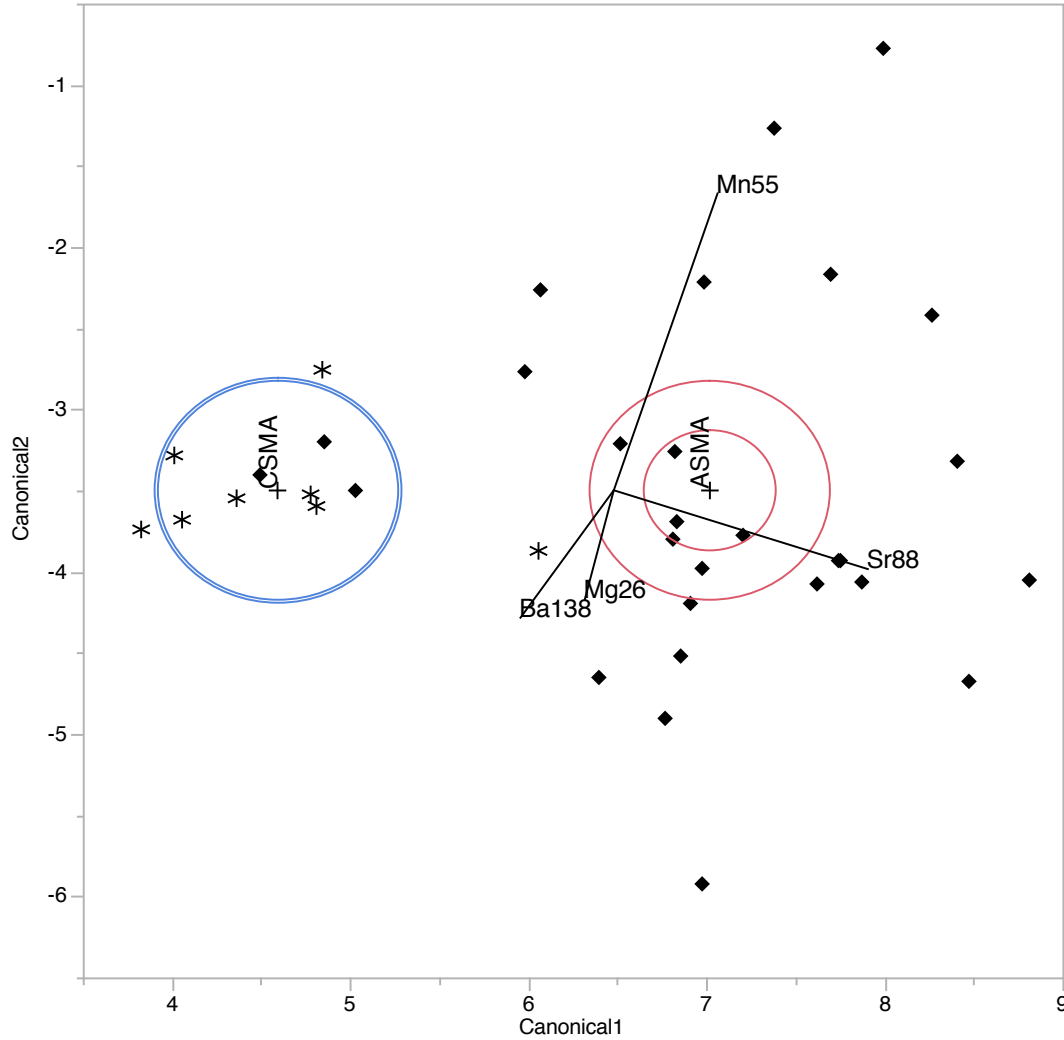
Group centroids (+) are surrounded by ellipses representing 95% confidence intervals (the smaller ellipse). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=8, ASMA=28; Overall classification: 89%; CSMA classification: 87.5%, ASMA classification: 89%. Pillai's Trace: F=8.33, df=4, p<0.0001

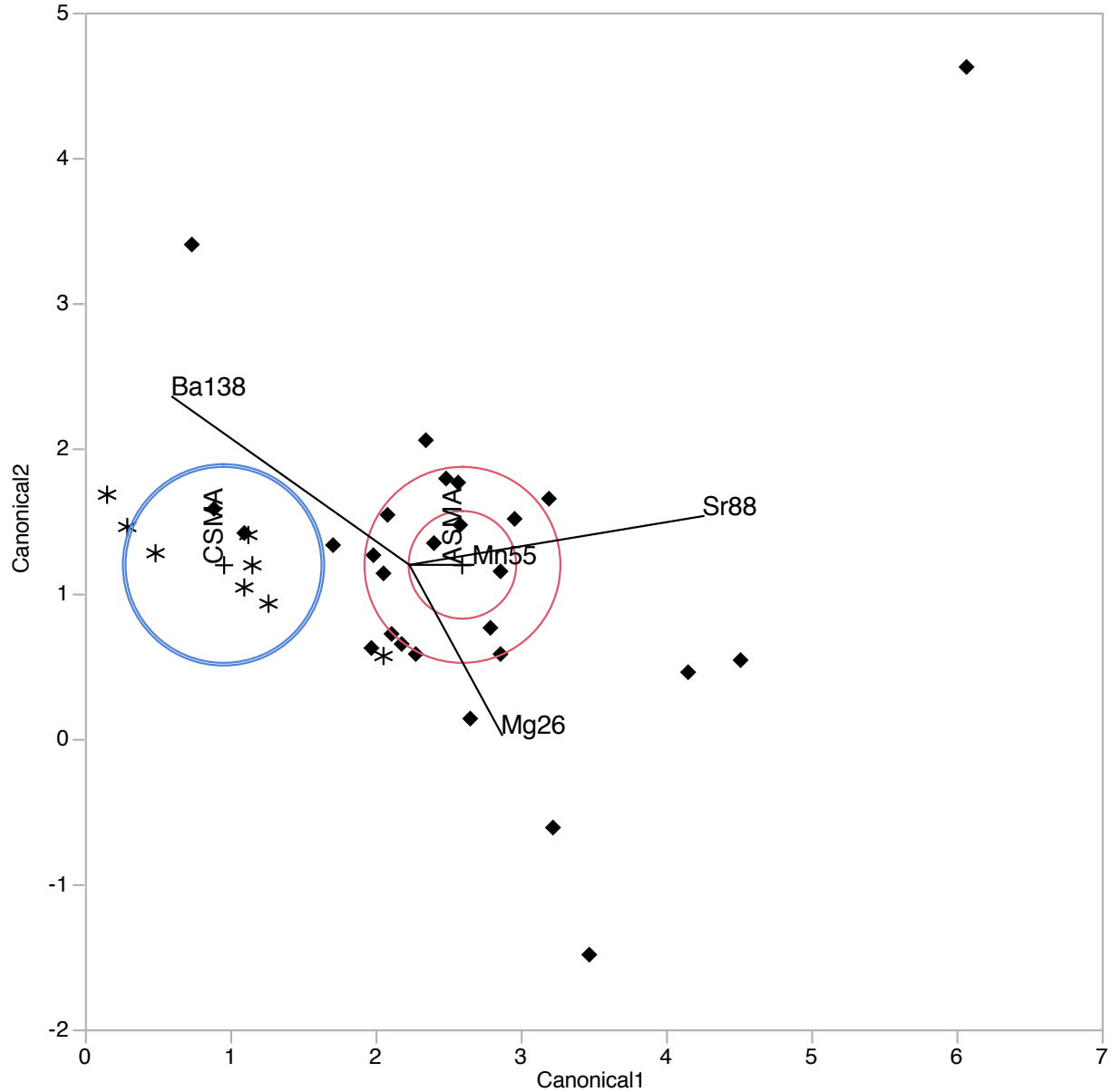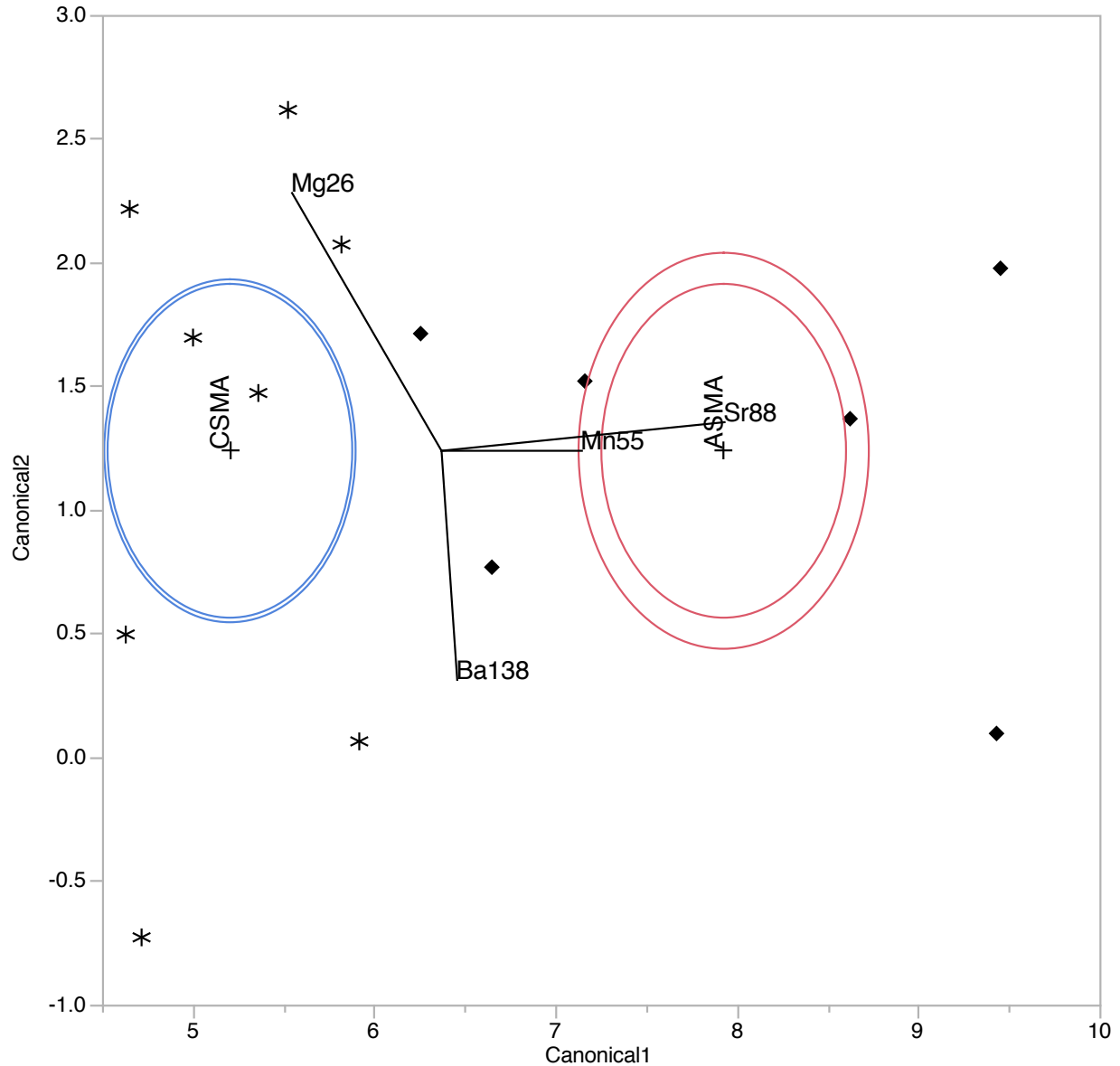Appendix B cont. LDFA comparing age 4 fish from 2004 year class

Group centroids (+) are surrounded by ellipses representing 95% confidence intervals (the smaller ellipse). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar.CSMA=8, ASMA=28; Overall classification: 86%; CSMA classification: 100%, ASMA classification: 82%. Pillai's Trace: F=8.42, df=4, p<0.0001

Appendix B cont. LDFA comparing age 5 fish from 2004 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=8, ASMA=28; Overall classification: 86%; CSMA classification: 87.5%, ASMA classification: 86%. Pillai's Trace: F=0.0120, df=4, p<0.0001

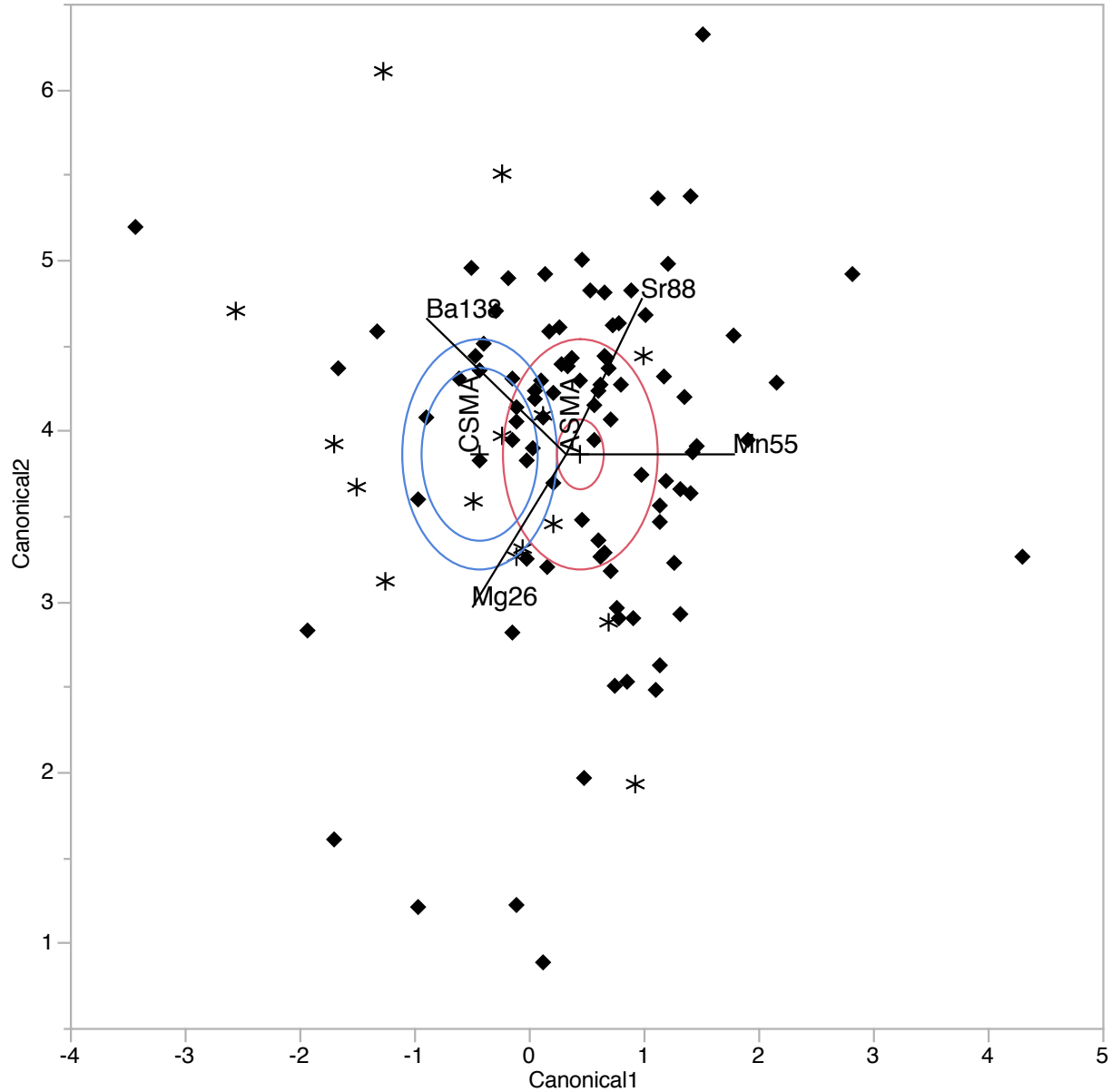Appendix B cont. LDFA comparing age 6 fish from 2004 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=8, ASMA=6; Overall classification: 93%; CSMA classification: 100%, ASMA classification: 83%. Pillai's Trace: F=4.77, df=4, p<0.0243

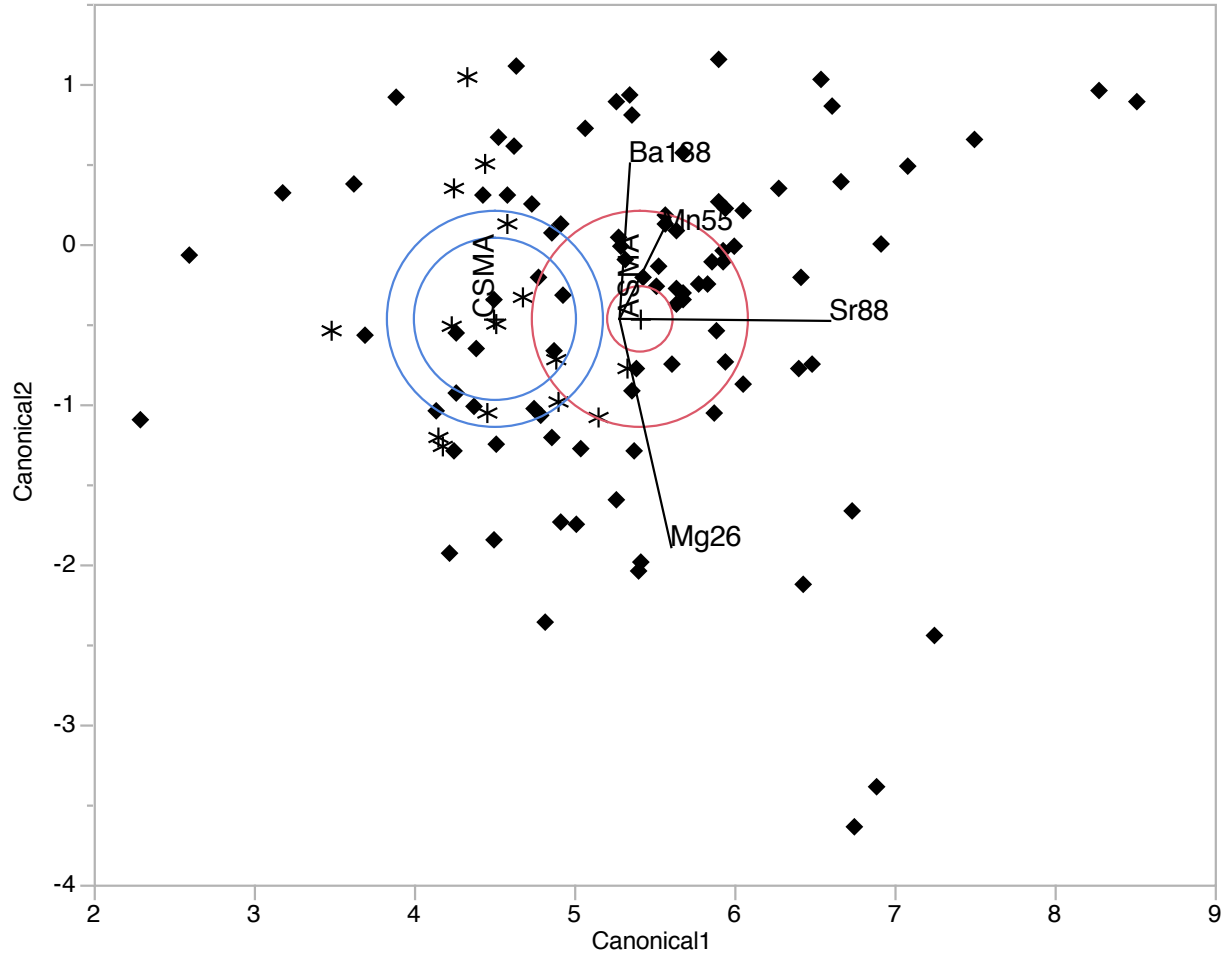Appendix B cont. LDFA comparing age 2 of fish from 2005 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar.   CSMA=15, ASMA=92; Overall classification: 73%; CSMA classification: 67%, ASMA classification: 74%. Pillai's Trace: F=2.413, df=4, p<0.0538

Appendix B cont. LDFA comparing age 3 of fish from 2005 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=15, ASMA=93; Overall classification: 69%; CSMA classification: 87%, ASMA classification: 65%. Pillai's Trace: F=2.565, df=4, p<0.0426

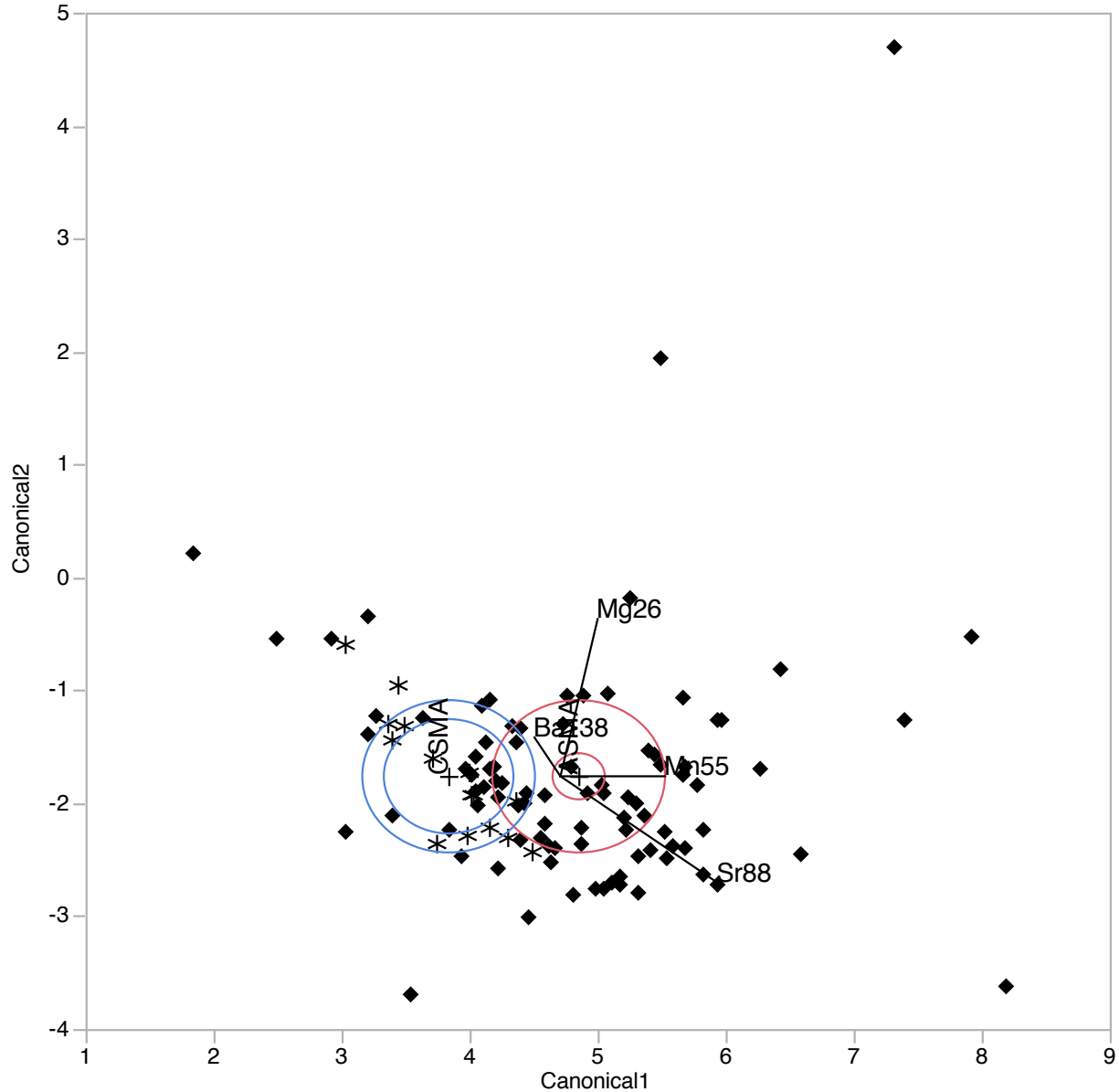Appendix B cont. LDFA comparing age 4 of fish from 2005 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=15, ASMA=92; Overall classification: 71%; CSMA classification: 87%, ASMA classification: 68%. Pillai's Trace: F=3.23, df=4, p<0.0153

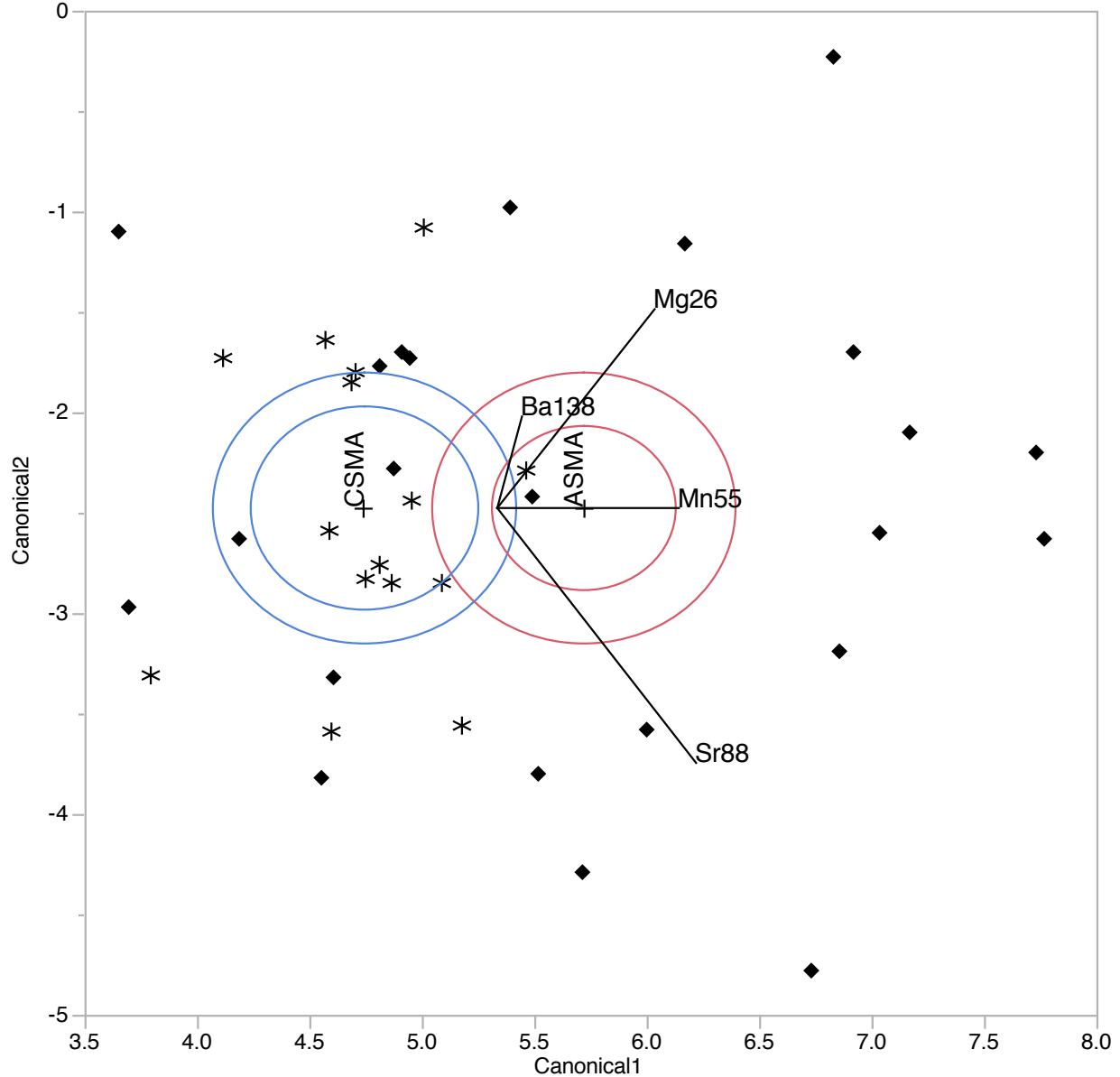Appendix B cont. LDFA comparing age 5 of fish from 2005 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=15, ASMA=23; Overall classification: 74%; CSMA classification: 93%, ASMA classification: 61%. Pillai's Trace: F=1.98, df=4, p<0.1206

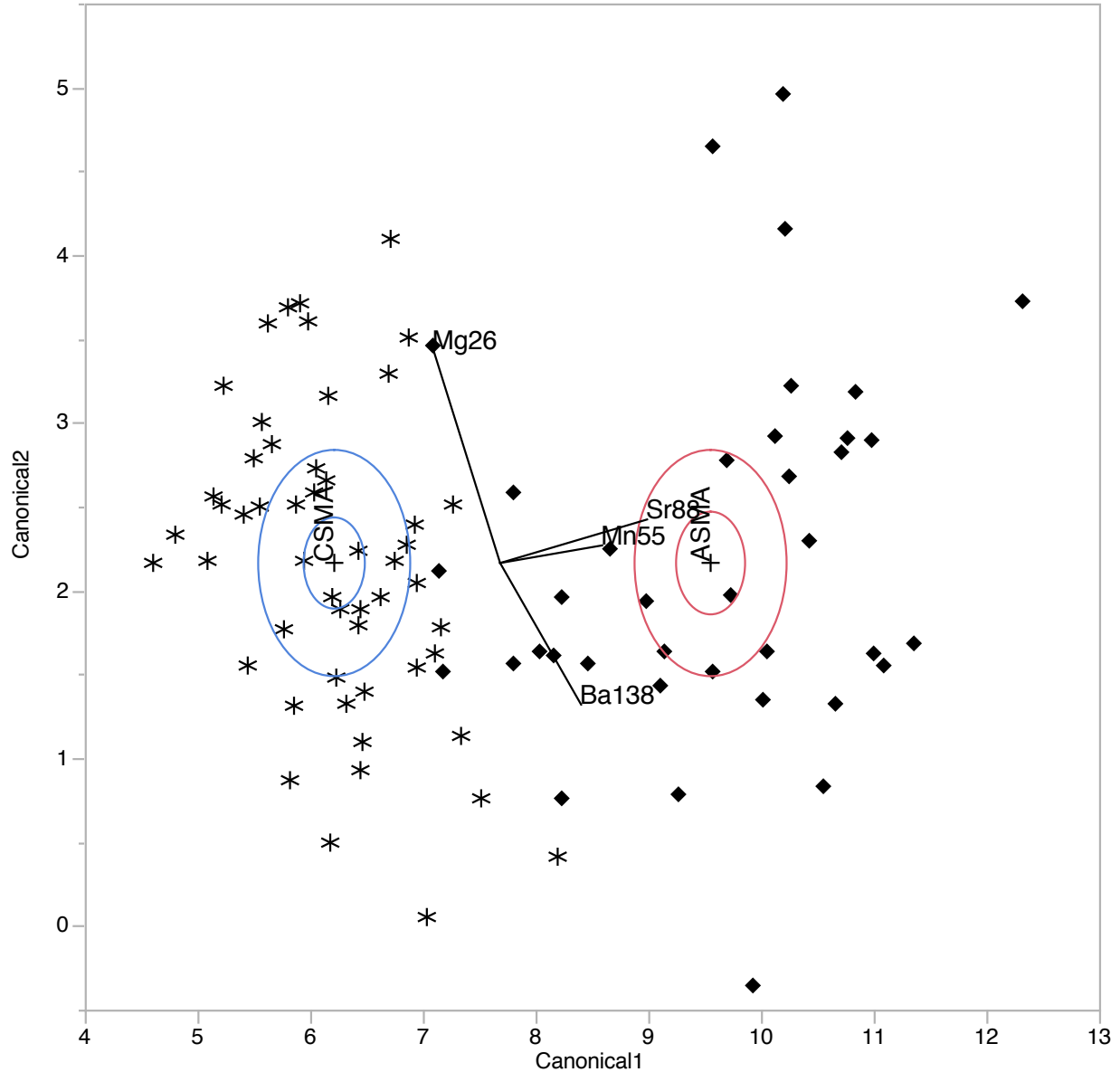Appendix B cont. LDFA comparing age 2 of fish from 2006 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=41, ASMA=52; Overall classification: 94%; CSMA classification: 98%, ASMA classification: 88%. Pillai's Trace: F=61.81, df=4, p<0.0001

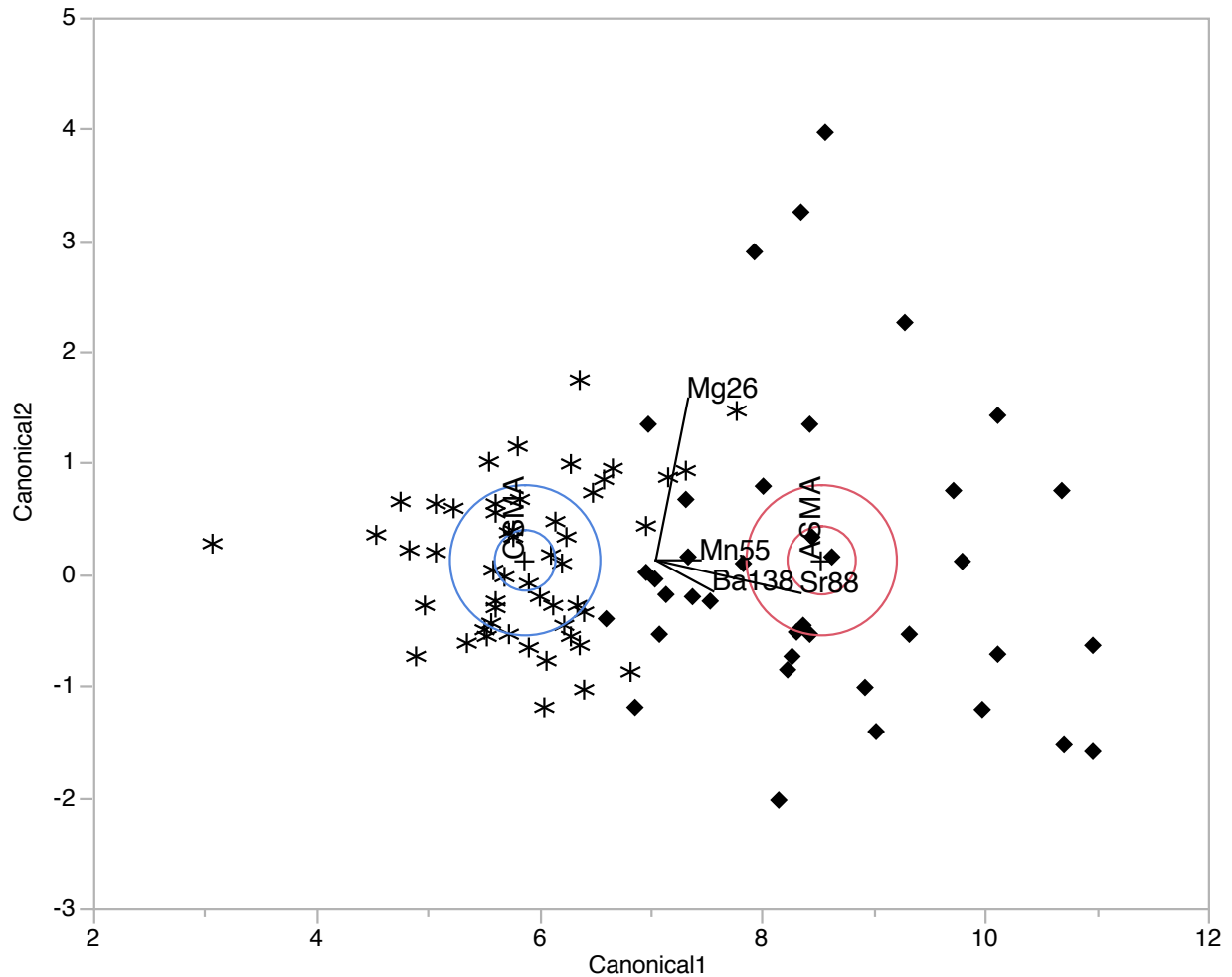Appendix B cont. LDFA comparing age 3 of fish from 2006 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=52, ASMA=41; Overall classification: 90%; CSMA classification: 96%, ASMA classification: 83%. Pillai's Trace: F=39.22, df=4, p<0.0001

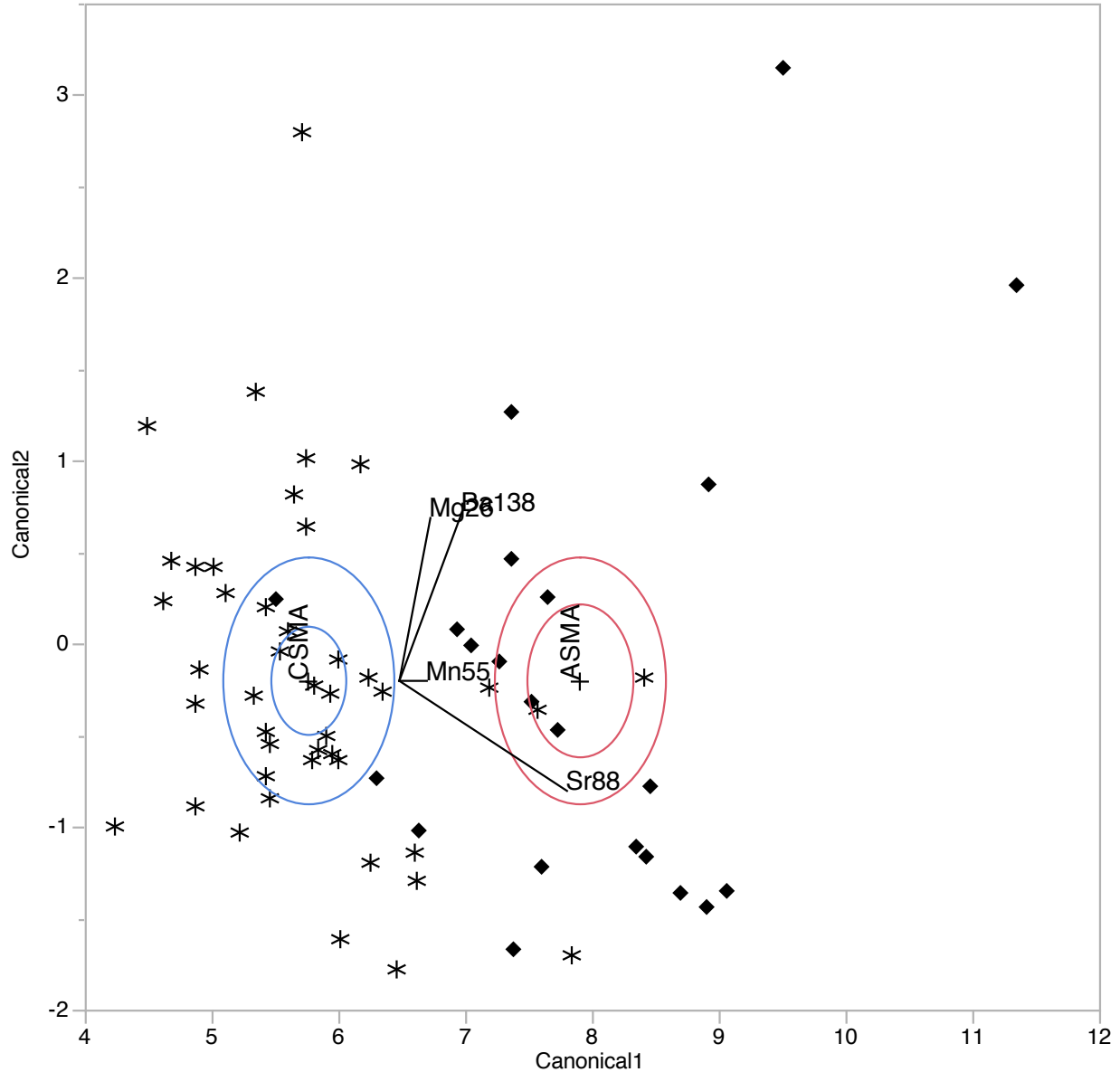Appendix B cont. LDFA comparing age 4 of fish from 2006 year class

Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=44, ASMA=22; Overall classification: 89%; CSMA classification: 91%, ASMA classification: 86%. Pillai's Trace: F=208.9885, DF=4, P<0.0001

Appendix B cont. LDFA comparing age 2 of fish from 2007 year class
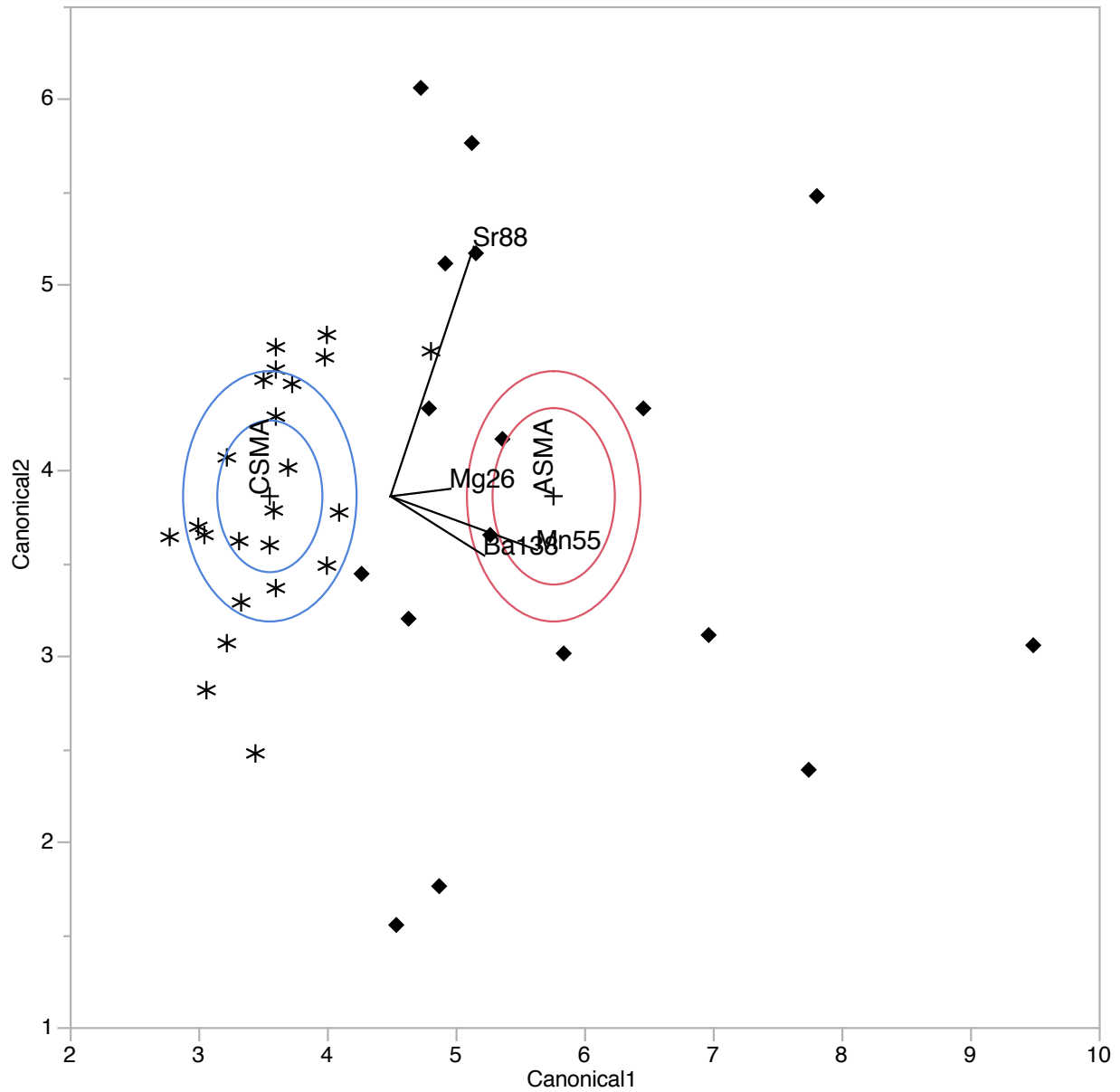
Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=23, ASMA=17; Overall classification: 92.5%; CSMA classification: 96%, ASMA classification: 88%. Pillai's Trace: F=21.71, df=4, p<0.0001
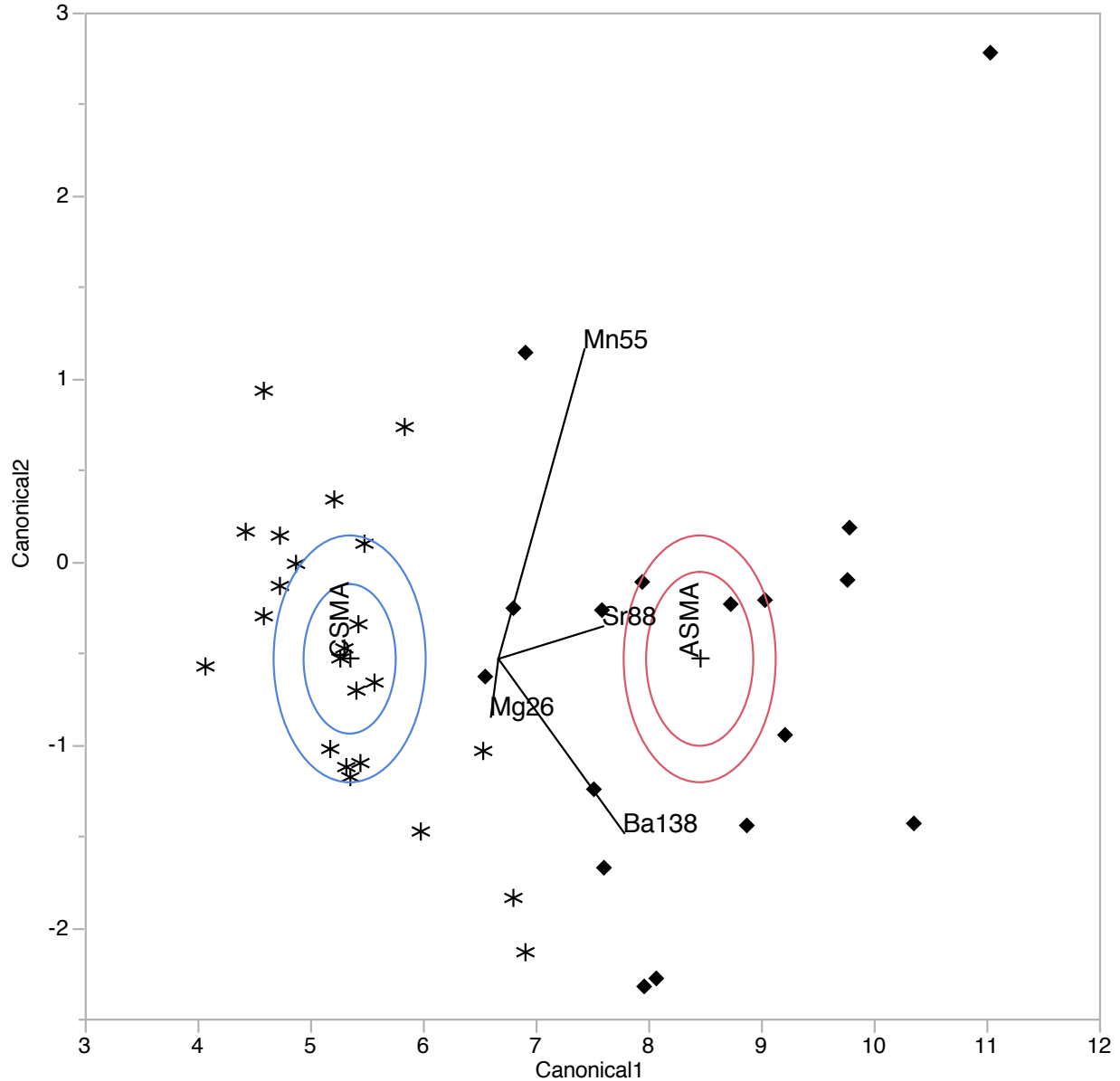
Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar. CSMA=23, ASMA=17; Overall classification: 90%; CSMA classification: 96%, ASMA classification: 82%. Pillai's Trace: F=10.96, df=4, p<0.0001

Chapter 3. A Comparison of Anadromy Rates of Striped Bass, *Morone saxatilis,* Inferred through Otolith Microchemistry and Tag Return Data: Do Separate Methods for Determining Striped Bass Migration Patterns Produce Similar Results

Abstract

The relative contribution of Atlantic Striped Bass, *Morone saxatilis*, from different watersheds to the Atlantic Migratory Stock is a well-studied problem. To implement effective management strategies delineation of the separate contributing stocks is necessary. Traditionally, the Chesapeake Bay has been thought to contribute the largest number of fish, with the Hudson River and Roanoke River-Albemarle Sound system contributing the second and third most fish, respectively. Results of the North Carolina Division of Marine Fisheries (NCDMF) tagging study indicate that as the Roanoke River population has been rebuilt from its crash during the 1980s, it is contributing more fish to the Atlantic Migratory Stock than in the past. Also, using otolith Sr concentrations, Boyd (2011) determined migration patterns of Striped Bass collected on the Roanoke River spawning grounds and estimated that 22% were anadromous. The goal of my study was to determine if the percentage of anadromy of fish examined in Boyd's (2011) study increased with total length (L_T), as NCDMF tagging data indicated that longer fish were more likely to enter ocean habitats. Boyd's (2011) fish were arranged into the same size classes that the NCDMF used (<600 mm, 600-799 mm, and ≥800 mm L_T) for their tag return determinations. Logistic regressions were run on Boyd's (2011) fish to determine if L_T had any effect on the frequency of residency or anadromy, as indicated by Sr concentrations in the otoliths. The percentage and number of anadromous and resident fish of each size class were

tabulated and then compared to the NCDMF tag return data. Results of the logistic regression

indicated that L_T had little effect on frequency of anadromy or residency as suggested by otolith

Sr concentrations. More, smaller fish (<600 mm L_T) were anadromous than NCDMF tag returns

indicated. Even more interesting was that ~5% of larger fish (≥800 mm L_T) were anadromous, in

contrast to NCDMF tag returns which indicated that almost all larger fish were anadromous. The

difference of anadromy rates among the smaller fish could be explained by the small possibility

of in salinity changes in the Albemarle Sound, causing fish to have high otolith Sr levels

normally associated with ocean going migrations, or to a more likely reporting bias in the

tagging study in which smaller fish would not have been caught in the ocean due to the large

minimum harvestable length. Differences in anadromy rates of larger fish may explained by the

low number of those fish whose otoliths were analyzed. The NCDMF tagging study did not

analyze tags returned from the spawning grounds the year after fish were released. Because so

many of the larger fish exhibited resident Sr profiles in their otoliths, it is likely that the Roanoke

River population has discrete anadromous and resident contingents.

Introduction

Background

The relative contribution of Striped Bass to the Atlantic Migratory Stock from different

watersheds has long been studied. Indeed, migration of the species along the U.S. and Canadian

Atlantic coasts may be one of the most studied aspects of life history of any fish species, save for

the various Atlantic and Pacific species of salmon. Generally, Striped Bass of the Atlantic

Migratory Stock migrate north in the summer to foraging habitat off the New England coast after migrating upstream to spawn in their various natal watersheds in the spring. They will then migrate south to overwintering grounds off the coast of southeastern Virginia and northeastern North Carolina during the fall and winter (Boreman and Lewis 1987). Recent data indicate that Delaware River stock is also contributing some fish (Waldman and Wirgin 1994).

Striped Bass have cultural and economic significance for the region. It is the state fish of Maryland, and the combined recreational and commercial Striped Bass catch along the Atlantic Coast was 26.6 million lbs. (NMFS 2012 and NMFS 2012b). Because Striped Bass have such long distance migrations, spanning several states and two countries, delineation of the stocks contributing to the migratory stock is necessary as Striped Bass are managed differently in different areas. Stock delineation is the basis for any fishery management plan as it allows for catch to be correctly allocated, nursery and juvenile habitats to be identified, and proper stock assessment models to be developed (Begg et al. 1999).

Three watersheds along the U.S. Atlantic coast are known to contribute Striped Bass to the Atlantic Migratory Stock: the Chesapeake Bay, Hudson River and Roanoke River, NC (Berggren and Lieberman 1977). The Chesapeake Bay is thought to contribute the largest number of fish, with the Hudson River contributing the second most, and the Roanoke River, NC the least (Berggren and Lieberman 1977; Boreman and Lewis 1987; Gauthier et al. 2013).

Although evidence exists that the Roanoke River did contribute fish to the Atlantic Migratory Stock, its contributions have largely been ignored in the literature. Results of Roanoke River Striped Bass tagged on the spawning grounds at Weldon, NC during the springs of 1959-1977 indicated that almost no fish migrated out of the system, and so were considered to be a population endemic to the Roanoke River-Albemarle Sound system (Hassler et al. 1981).

However, most fish tagged by Hassler et al. (1981) were <600 mm total length (L_T), and fish that size would be less likely to be anadromous (Dorazio et al. 1994). More recent analysis of North Carolina Division of Marine Fisheries (NCDMF) tag returns by Callihan et al. (2014) has indicated that the Roanoke River does contribute more fish to the Atlantic Migratory Stock than in the past, as the stock has increased in abundance since its crash in the 1980s (Callihan et al. 2014). Over 42,000 fish of all size classes were collected though electroshocking and gillnetting and tagged. Tags returns of almost all fish ≥800 mm total length (L_T) were recovered in the Atlantic Ocean along the coasts of New Jersey to Maine. Most fish over 600 mm L_T were found to leave the Albemarle Sound system as well (Callihan et al. 2014).

Boyd (2011) corroborated these results when he used otolith microchemistry to determine that roughly 22% of Striped Bass sampled on the spawning grounds of the Roanoke River were considered to be anadromous, using a criterion of a concentration of 4000 ppm or more of Sr in their otoliths. Those with less than 4000 ppm of otolith Sr were considered to be resident. The threshold of 4000 ppm of Sr was a previously determined standard used to establish Striped Bass anadromy in other systems, such as the Chesapeake Bay, Hudson River, and Shubenacadie River, Canada, as otolith Sr was shown to be directly correlated with salinity (Secor 1992; Secor and Rooker 2000; Zlokovitz et al. 2003; Paramore and Rulifson 2001; Secor and Piccoli 2007). Boyd (2011) did not, however, determine if L_T influenced the anadromy of the fish he sampled.

Additionally, using otolith Sr:Ca ratios Secor et al. (2001) and Zlokovitz et al. (2003) showed that the Hudson River Striped Bass population had unique migratory contingents, where fish collected in one area of the Hudson River tended to stay in that area, no matter what size they were (i.e., fish collected in freshwater exhibited Sr:Ca profiles of a freshwater resident, fish collected in estuarine water exhibited Sr:Ca profiles of an estuarine resident, and fish collected in

fully saline water exhibited Sr:Ca profiles of anadromous fish undertaking long distance ocean migrations). It may be that the Roanoke River stock also exhibits similar patterns. This will be investigated using otolith microchemistry.

Goals

The purpose of this study was to determine if the migration patterns of Roanoke River or Albemarle Sound Management Area (ASMA) Striped Bass inferred from two separate methods (conventional tagging and otolith microchemistry) were similar. This was accomplished by a reexamination of Boyd's (2011) fish to ascertain if the incidence of anadromy increased as L_T increased. Results of this study were compared to results of tagging done by the NCDMF, which showed that increasing L_T was the most significant factor causing long distance migrations of Striped Bass from the Roanoke River. I hypothesized that anadromy and residency rates inferred through otolith microchemistry would be similar to those shown by NCDMF tag return data. If otolith chemistry indicated large, ASMA Striped Bass were resident, than otolith chemistry from those fish was compared to otolith chemistry of adult North Carolina Central Southern Management Area (CSMA) fish to determine if large, resident ASMA fish primarily used the ASMA or CSMA for year round habitat. Based on the results of chapter 2 of this thesis it was expected that large, adult, resident ASMA fish would primarily use habitat in the ASMA (Zurlo 2014, Chapter 2).

Methods

Sample Collection

For detailed methods on sample collection, see Zurlo (2014, Chapter 2). Briefly, adult Striped Bass were collected on the well-described spawning grounds by the North Carolina Wildlife Resources Commission (NCWRC) on the Roanoke River near Weldon, NC via electroshocking (n=216) and in the western Albemarle Sound through the Independent Gillnet Survey done by the NCDMF (n=237). The Roanoke River-Albemarle Sound system in northeastern North Carolina, also known as the Albemarle Sound Management Area (ASMA), is shown in Figure 3.1. Fish were collected year round from the CSMA during 2011-2012 (Dobbs 2013) rod and reel, gillnets, and electrofishing (n=281).

Otolith Preparation and Analysis

For detailed methods of otolith analysis, see Zurlo (2014, Chapter 2). Briefly, sagittal otoliths were removed using plastic forceps, cleaned with deionized water, and stored and dried in 1.5-mL microcentrifuge tubes. A subsample of 288 ASMA otoliths, and all CSMA otoliths, were sent to the University of Manitoba for trace elemental analysis. Only otoliths of CSMA fish of the 2005-year class were analyzed in this study, as they were a year class with a large number of adults, whose otolith chemistries were easily comparable to adult ASMA fish. In Canada, otoliths were embedded in Buehler Epoxicure epoxy resin. 2mm thick dorso-ventral transverse sections, including cores, were cut using a diamond blade Isomet saw (Bueler 646) at low speed (Halden and Friedrich 2008). Sections were ground down using 320-, 400-, and 600-grit sandpaper to expose the otolith core. They were then ultrasonically cleaned for 2 minutes. Otolith surface scratches were removed using Buehler diamond polishing suspensions (9 μm and

108

0.05 μm) on polishing wheels to achieve smooth otolith surfaces. Polished otoliths were cleaned

ultrasonically and digitally photographed.

Methods for otolith elemental analysis are the same as those used by Mohan et al. (2012)

and Dobbs (2013). Briefly, otolith element concentrations were measured with a Thermo-

Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd-YAG laser. Laser ablation

inductively coupled plasma mass spectrometry (LA-ICP-MS) operation parameters involved: a

15 μm beam size; $2\mu ms^{-1}$ scan speed; repetition rate of 20 Hz; and 75% power using low

resolution (R=300) mode. ^{44}Ca, ^{25}Mg, ^{88}Sr, ^{138}Ba, ^{55}Mn, ^{63}Cu, ^{66}Zn, and ^{208}Pb were the isotopes

measured in the analysis. Calcium (56 wt. % CaO) was used as the internal standard to monitor

ablation yield. NIST 610 glass was used for external calibration and to monitor instrumental

drift. Laser scans began before the nucleus, on the shorter axis of the sulcal groove and went

through the nucleus and continued along the longest axis of otolith growth until reaching the

outer edge. Elements were measured in counts per second and converted to ppm using a macro

written for Microsoft Excel and plotted versus distance across the otolith corresponding to the

laser scan (Mohan et al. 2012; Dobbs 2013). Two independent readers aged otoliths. A third

reader resolved discrepancies. Distances between yearly annuli were measured using Image Pro

Plus 5.1 software, similar to Boyd (2011).

Migration Patterns and Statistical Analysis

Boyd (2011) designated fish as anadromous by using otolith Sr levels. Fish >age-2 were

designated as anadromous if otolith Sr levels >4000 ppm at any point in their lives were (Figure

3.5) and those with otolith Sr levels <4000 ppm were determined to be resident (Figure 3.6). A

logistic regression was done for all fish and for each of the size classes (<600, 600-799, and

≥800 mm L_T) used by Callihan et al. (2014) to determine if anadromy was related to L_T. A

frequency histogram showing the number of anadromous and resident fish compared L_T was

used to further determine if size was related to anadromy. A table comparing migration patterns

determined in this study and those determined through NCDMF tagging was used to show the

relative frequency of anadromy and residency among three different size classes (<600, 600-799,

and ≥800 mm L_T) of fish. 284 fish were used for the length analyses, as that was the number of

fish whose total lengths were measured and whose otoliths were taken.

In order to determine if larger fish (≥800 mm L_T) designated as resident by Boyd (2011)

moved to other NC inshore waters, linear discriminant function analysis (LDFA) was used to

compare the annual otolith chemistry of all large (≥800 mm L_T) resident ASMA Striped Bass to

adult. The year in which the fish occurred was determined by adding otolith age to the year class

of the fish.

Results

Boyd (2011) determined that 22% (64 fish) of his fish were anadromous, and ~78% (224

fish) were resident. I reanalyzed Boyd's (2011) results by comparing purported anadromous and

resident fish to their L_T to determine if there was any relationship between anadromy and size

reported by Callihan et al. (2014) for the NCDMF tagging study.

Results of the logistic regression analysis for all fish showed that L_T explained <1% of

the variation observed between resident and anadromous fish (r^2=0.0093), and that increasing L_T

was insignificantly positively correlated with increased frequency of anadromy (Chi-square=2.56

df=1, p<0.1093). The logistic regression analysis for fish <600 mm showed that L_T explained

7.9% of the variation observed between resident and anadromous fish ($r^2=0.0787$), and that increasing L_T was significantly correlated with increased frequency of anadromy (Chi-square=16.49 df=1, p<0.0001). The logistic regression analysis for fish 600-799 mm showed that L_T explained <0.2% of the variation observed between resident and anadromous fish ($r^2=0.0015$), and that increasing L_T was insignificantly correlated with frequency of anadromy (Chi-square=0.077 df=1, p<0.7811). The logistic regression analysis for fish ≥800 mm showed that L_T explained 100% of the variation observed between resident and anadromous fish ($r^2=1.000$), and that increasing L_T was significantly correlated with frequency of anadromy (Chi-square=7.83, df=1, p<0.0051). These results are summarized in Table 3.1. The frequency histogram showed 82.0% (<600 mm L_T) to be resident. The percentage of anadromous fish increased as L_T increased at 600-799 mm L_T, to 30.2%. After 800 mm L_T, 18 (94.2%) fish were found to be resident (Figure 3.2). These results are also summarized in Table 3.2.

Results of the LDFA of the adult ASMA (n=17) and CSMA (n=15) annual otolith chemistry comparisons showed high classification rates. The two groups had significant differences in mean multivariate canonical values in 2007 (Pillai's Trace: F=18.3309, df=4, p<0.0001). Overall correct classification in 2007 was ~91%, while ASMA classification was ~94%; and CSMA classification was ~87% (Figure 3.4A). The two groups also had significant multivariate differences for 2008 (Pillai's Trace: F=23.1023, df=4, p<0.0001). Overall correct classification for 2008 was ~97%; while ASMA classification was ~100%, and CSMA classification was ~94% (Figure 3.4B). For 2009, again the two groups had significant multivariate differences (Pillai's Trace: F=24.7675 df=4, p<0.0001). Overall correct classification for 2009 was 100%; ASMA classification was 100%, and CSMA classification was 100% (Figure 3.4C). Results of this analysis are summarized in Table 3.3.

Discussion

Agreement between Otolith Data and NCDMF Tag Returns

The migratory patterns inferred using otolith microchemistry in this study do not agree well with the results of tagging found by the NCDMF, and the cause of the disagreement is unclear. The logistic regression of otolith data indicated that L_T was poorly correlated with frequency of anadromy. There were significant correlations in the smallest and largest size classes, but a low amount of variation was explained in the smallest size class, and the correlation was so strong in the largest size class because almost all fish in that size class were resident, meaning that the regression predicted that any fish over 800 L_T would be resident.

While overall anadromy rates (22%) found by Boyd (2011), agreed with NCDMF tag return data (15-31% annually) discrepancies were found when fish were broken into size classes. The NCDMF found that fish under 600 mm L_T were almost exclusively resident, with migration to other North Carolina estuaries occurring during years of high abundance (Callihan et al. 2014). My study indicated that 20% of <600 mm fish had Sr profiles typically interpreted to be anadromous fish. For the second size class, roughly half of the NCDMF Striped Bass recaptures between 600-799 mm L_T migrated out of the Albemarle Sound to other North Carolina inshore waters or into ocean waters, often to the Chesapeake Bay. My study indicated that 30% of fish in the second size class were anadromous based on otolith Sr. The differences in migration rates in this size class is large (~20%) but strongly suggests that these fish do undergo ocean migration. Almost all NCDMF tagged fish over 800 mm L_T were not only anadromous, but many fish traveled hundreds, if not thousands of kilometers (Callihan et al. 2014). Just 1 of 19 fish ≥800 mm analyzed in my study exhibited otolith Sr that denoted an anadromous lifestyle (Table 1).

Discrepancies in the Migrations Patterns of Smaller Size Classes of Fish

The differences in migratory patterns of the smallest fish are stark, and the question must be asked: What could cause these discrepancies? The first explanation that comes to mind is sampling error. Sampling techniques in this study and the NCDMF tagging efforts were very similar. In both studies fish were sampled using electroshocking on the spawning grounds of the Roanoke River during the spring, when fish of all sizes would be available for collection. Past Striped Bass migration studies have been biased because only smaller fish were collected (Mansueti 1961; Nichols and Miller 1967; Hassler et al. 1981), but that is not a possibility here, as both the present study and the NCDMF tagging study collected fish of all size classes. It is unlikely that sampling error is the reason for the discrepancies seen.

One possible source of error in the tagging study was recapture bias created by size targeting. In inshore waters along the coast the minimum harvestable size is L_T is 457 mm. Tagging studies rely on fishermen to report what they catch, and if they can only catch certain sizes of fish, it is likely they will only report certain sizes of fish. The authors of the NCDMF tagging study did provide evidence suggesting that this type of bias did not have a large effect of the results of the tag return data. They referenced Haeseker et al. (1996), in which 26 acoustically-tagged Striped Bass <600 mm L_T were released in the Albemarle Sound during the summer months, and all but one remained resident to the Sound, supporting their assertion that fish of that size were not migratory, but Callihan et al. (2014) did not sample in the ocean to ensure recapture bias did not influence their study.

Incorrect interpretation of high otolith Sr levels could be a cause of misinterpretation in my study, causing a resident fish to be improperly classified as anadromous. High Sr levels could be a result of a spike in salinity in the Albemarle Sound or a result of Albemarle Sound's

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unique geological factors. Twenty percent of smaller fish (<600 mm L_T) exhibited anadromous Sr profiles. The Albemarle Sound exhibits wide salinity changes at the extreme eastern end, which could also confound otolith Sr analysis of fish from the system (Mohan et al. 2012). In age-0 Salmonid Sheefish, *Stenodus leucichthys*, high otolith Sr was observed, likely due to the unique geology of the study area (Howland et al. 2009). The Albemarle Sound may have a unique hydrology, as Sr-rich water from the Upper Castle aquifer might be upwelling into the system. As a result high Sr could be observed in Striped Bass otoliths if they use these cooler aquifer waters as a refuge (Woods et al. 2000). However, such upwelling events within the sound have not been documented.

Several recent fishery independent studies have shown that sub-adult and small adult Striped Bass can undertake long distance ocean migrations, making it possible that some smaller fish tagged by the NCDMF could have migrated and not been reported or not recaptured. Mather et al. (2010) documented an abundance of Striped Bass 400-500 mm L_T in the Plum Island Estuary (PIE) in coastal northern Massachusetts. There is no known spawning population there. Forty-six fish were acoustically tagged, and three-quarters of them exhibited long distance migrations south in the fall. Patrick (2010) observed extremely high values of Sr levels in age-0 and age-1 Striped Bass otoliths collected in the Roanoke River, suggesting the small fish may have migrated to the ocean. It is also possible the Sr observed in Patrick's (2010) fish were hatchery fish (Dobbs 2013). However, Able et al. (2012) supported Patrick's (2010) findings, as they observed fish <200 mm L_T utilizing coastal estuaries in New Jersey. There is no known spawning population of Striped Bass in New Jersey (Collette and Klein-MacPhee 2002), so those fish must have migrated from far off natal waters. Reporting bias could account for the lack of smaller, migratory Striped Bass observed in the NCDMF tagging study.

Discrepancies between the Migration Patterns of the Largest Fish

That just 1 of 19 Striped Bass ≥800 mm L_T in this study had otolith Sr profiles consistent with those of an anadromous fish is peculiar. These results are similar to Patrick (2010) who analyzed Roanoke River Striped Bass for otolith Sr and found that none of the 115 fish >6 years of age exhibited otolith Sr signifying marine migrations. It is possible that due to the small number of larger fish collected in my study; the small number of larger anadromous fish observed is a result of randomness. Indeed, the compelling NCDMF tagging results, and results of many other studies, including otolith microchemistry studies, have indicated that most fish that large should be anadromous and migratory. Secor and Piccoli (2007) used otolith Sr concentrations to show that 50-75% of the adult, female Striped Bass aged 7-13 they collected in the Chesapeake Bay were very likely to be migratory. Tagging studies of Striped Bass in the Chesapeake Bay also reflected those conclusions (Kohlenstein 1981; Dorazio et al. 1994).

Perhaps with a larger sample size, more, larger anadromous fish would have been observed in this study. Another explanation may be that large, anadromous fish may not return to the spawning grounds. Callihan et al. (2014) did not count tag returns later than 10 months at liberty in order to demonstrate that large ASMA Striped Bass were highly migratory. What they did not do was determine if those larger, migratory fish returned to the spawning grounds, as those returning fish would have been observed at 12 months at liberty. More detailed ASMA Striped Bass tagging data in the most recent NCWRC and NCDMF Striped Bass fishery management plan (FMP) shows that 25% of tag returns from fish >711 mm L_T occurred in the Roanoke River spawning grounds between 1995 and 2009 (NCWRC and NCDMF 2013). In addition, unpublished NCDMF tagging data indicated that of the 526 Striped Bass tagged on the

spawning grounds, 21 Striped Bass ≥800 mm were recovered (4.8% recovery rate) on the

spawning grounds between 2005-2011, and just 4 were returned after one year at liberty

(NCDMF unpublished). That so few tag returns occurred in the Roanoke River could have been

because only a small percentage of Striped Bass returned to the spawning grounds yearly,

indicating that Striped Bass skip spawn. However, it is just as likely that the tag returns were

influenced by poor luck in recapturing large fish, so more ASMA otoliths are needed, and

ASMA fish should be acoustically tagged on the spawning grounds so their migrations can be

tracked. But, it is possible that based on results of this study, in which 18 of 19 Striped Bass on

the Roanoke River were resident based on otolith Sr, and the additional tag return data from the

NCDMF, that primarily large resident fish return to the spawning grounds, as just one migratory

fish was observed using otolith microchemistry, and few were observed through tag returns.

If true, this has significant implications for management of ASMA Striped Bass. Large

ASMA Striped Bass may not be returning to the spawning grounds of the Roanoke River

because of intense fishing mortality once they reach the Atlantic Ocean. Currently, contributions

of ASMA fish to the Atlantic Migratory Stock are not counted by the ASMFC when the status of

the migratory stock is determined (ASMFC 2013). This may cause fishing pressure to

disproportionately affect the ASMA stock. High fishing mortality may also have caused zero of

the 103 anadromous Green Sturgeon, *Acipenser medirostris*, tagged on the spawning grounds of

the Rogue River, Oregon to return in successive years (Erickson and Webb 2007).

A more speculative explanation is that global warming is causing ocean waters through

which large ASMA Striped Bass would migrate in order to return to the ASMA and ultimately

the spawning grounds of the Roanoke River, to warm so much that ASMA Striped Bass cannot

move through them, creating a thermal barrier. Indeed, average sea surface temperatures have

risen 0.1°C per decade in the last 40 years (IPCC 2014). This might cause large Striped Bass to stay away from the ASMA and join other stocks, like the Chesapeake Bay or Hudson River stock after leaving the system. Warming waters within the ASMA may also be causing large fish to stay away as temperatures in the summer already routinely reach 28°C during the summer (Giese et al. 1985).

That large, anadromous fish may not return to the spawning grounds often or at all explains why they were not observed by my study, but it does not explain where the resident fish observed could reside. Results of the LDFAs comparing yearly otolith chemistry of large adult ASMA Striped Bass to adult CSMA fish suggested that large resident ASMA fish in this study primarily resided in the ASMA (Figures 3.4 and 3.5). However, it was not possible to determine where in the ASMA these large fish reside.

Resident fish could remain in the upriver portions of the Roanoke River after spawning and not be observed, as neither the NCDMF nor North Carolina Wildlife Resources Commission (NCWRC) survey heavily there during times of year other than the spring, fishing pressure would be lighter there after the spawning run in the spring, and fishermen may not have been targeting large fish the because the minimum legal size in the ASMA is 457 mm L_T.

The NCDMF would not have observed larger resident fish through course of their tagging study, as only tag returns that occurred between 14 days and 10 months at liberty were counted. If a large fish resident to the Roanoke was tagged, it could have eluded capture as a result of reduced fishery independent surveys and lighter fishing pressure, when the spring spawning is not occurring. If it were caught on the spawning grounds the next year, it would not have counted as a tag return.

Because so many resident fish were observed, it seems likely that ASMA Striped Bass have unique migratory contingents in which discrete portions of the population are migratory, and some are resident. This would mean that certain fish would not migrate, no matter how large of a size they attain. There is precedent for the contingent phenomenon, as two studies analyzed otolith Sr levels of Hudson River Striped Bass and indicated that there were discrete migratory contingents: an upper Hudson River group, a lower Hudson River group, a Long Island Sound group, and a group that went from fresh to saltwater. Many large Striped Bass were found to live their lives completely in upriver, freshwater areas (Secor et al. 2001; Zlokovitz et al. 2003), contrary to results found by tagging studies done on Hudson River Striped Bass (Waldman et al. 1990; Dorazio et al.1994).

This explanation may seem improbable, but there must be a reason such low otolith Sr was observed in most large fish in this study. If anything, fish found to have higher otolith Sr levels in my study may have been falsely determined to be anadromous. But no study has reported seeing anomalously low otolith Sr in Striped Bass that were migratory. It is highly unlikely that this study is the first to do so, as the full weight of the literature indicates that Striped Bass migrating to the ocean from any system would almost certainly exhibit high levels of otolith Sr.

Conclusions and Recommendations

The migratory patterns of Striped Bass collected from the Roanoke River inferred through otolith microchemistry in this study do not agree well with those seen from the results of NCDMF tag returns. The present study observed 20% of fish <600 mm L_T to be migratory, while the NCDMF tagging data indicated almost none went to the ocean. Reporting bias may

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have been a reason no tags from smaller fish were recovered outside of North Carolina waters, and unique Albemarle Sound hydrology or salinity fluctuations may have caused them to exhibit Sr levels that would indicate anadromy.

Just 1 of 19 fish >800 mm L_T (~5%) exhibited anadromy based on otolith Sr profiles, while almost all fish tagged by NCDMF were migratory. This may be due to low sample size, limiting the number of anadromous fish observed or that large, migratory ASMA fish do not return to the system as a result of heavy fishing pressure, while in the Atlantic Ocean, skip spawning, or warming ocean or ASMA waters preventing large Striped Bass from returning. However, this must be investigated further with a larger sample size of otoliths from large ASMA fish. ASMA fish should also be acoustically tagged on the Roanoke River spawning grounds so their migrations can be tracked on the various acoustic arrays along the U.S. Atlantic coast. Because so many residents were observed it is likely that the Roanoke River population has unique resident and anadromous migratory contingents. The resident fish may not have been observed by the NCDMF due to the conditions of the tagging study, reduced fishery independent surveys, and fishing pressure on the upper parts of the Roanoke River when the Striped Bass are not spawning.

The results of this study do not invalidate the results of the NCDMF tag returns. It is still likely that the Roanoke River contributes significant numbers of fish to the Atlantic Migratory Stock, and that fishery regulations of that stock should reflect the contributions of the Roanoke River population. It is not known how many fish from the Roanoke River become part of the migratory stock, and the results of this study suggest that not all fish ≥800 mm L_T should be counted as contributors. Thus, it is critical that the percentage of the Roanoke population that is resident and anadromous be determined so that accurate assessments of the Atlantic Migratory

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Stock can be made, and so that regulations of the Atlantic Migratory Stock do not adversely affect the Roanoke River population. The possible effects of warming ocean and Albemarle Sound waters on the migrations of ASMA Striped Bass should also be investigated.

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Tables

Table 3.1. Results of the logistic regression analysis correlating L_T to frequency of anadromy and residency for all fish and the various size classes. Asterisks (*) represent significant correlations.

Size Class (mm L_T)	Number of returns	Correlation coefficient (r)	Statistical confidence level (p)
All fish	284	0.0964	0.1093
<600	222	0.280	0.0001*
600-799	43	0.0387	0.7811
≥800	19	1.000	0.0051*

Table 3.2. A table showing the percentage of anadromous and resident fish in the ASMA determined in this study through otolith microchemistry vs. NCDMF tag returns.

| Size class (mm L_T) | Number of returns | | Migratory pattern (A/R) | | | |
| | | | % Anadromous | | % Resident | |
	This Study	NCDMF Tagging	This Study	NCDMF Tagging	This Study	NCDMF Tagging
<600	222	1040	18.0	<8.0	82.0	>92.0
600-799	43	102	30.2	53.0	69.8	47.0
≥800	19	55	5.2	98.0	94.8	2.0

Table 3.3 Results of the LDFAs classifying Striped Bass to management area of collection based on otolith microchemistry. Combined yearly otolith chemistry of adult Striped Bass occurring in the years 2007-2009 is shown in this table. Percentages reflect classification of ASMA fish to the ASMA and CSMA fish to the CSMA. Asterisks (*) represent significant multivariate differences between the ASMA and CSMA. Alpha was set to 0.05.

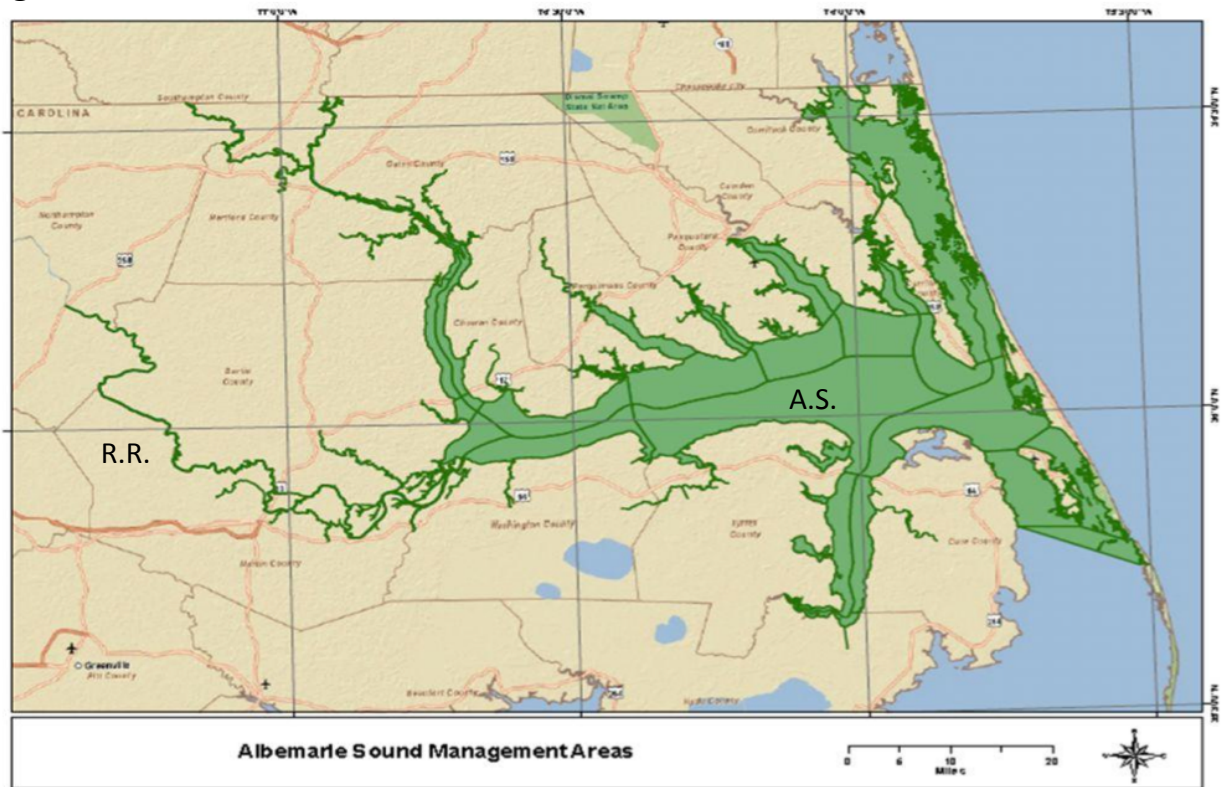| Management area of collection	Number of samples	Year	Percent correctly classified	Overall classification	Multivariate confidence level (p)
ASMA	18	2007	94	91	<0.0001*
CSMA	15		87		
ASMA	18	2008	100	97	<0.0001*
CSMA	15		93		
ASMA	18	2009	100	100	<0.0001*
CSMA	15		100		

Figures

Figure 3.1. A map the Roanoke River-Albemarle Sound system. The Roanoke River is denoted as R.R., and the Albemarle Sound is denoted as A.S. This figure is from Boyd (2011).
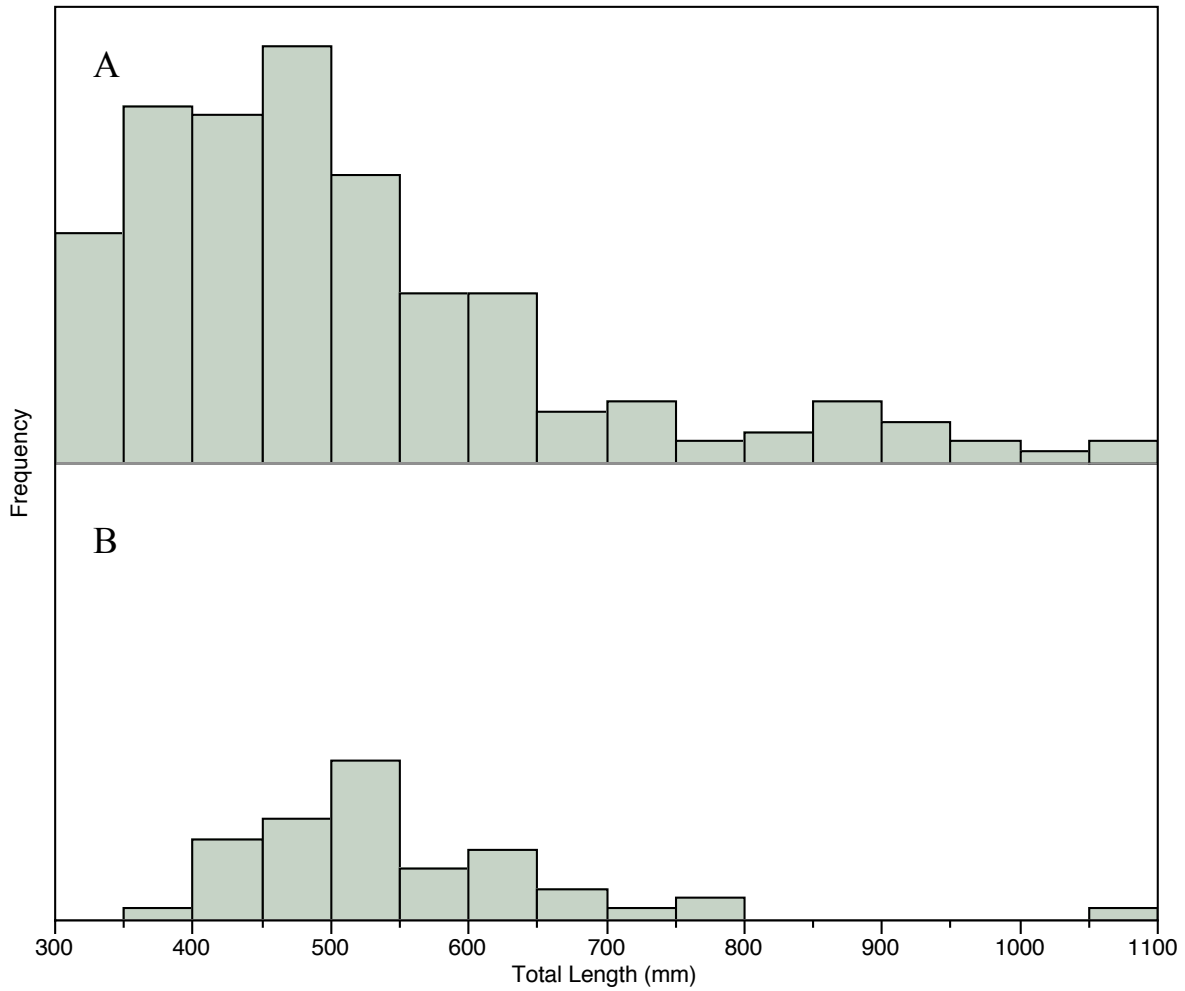
Figure 3.2. Frequency histogram showing the total lengths of the number of fish determined to be anadromous (n=64) and resident (n=224) (Boyd 2011) to the ASMA by size class. Resident fish make up the top half of the figure and anadromous fish make up the bottom half of the figure. A represents resident fish and B represents anadromous fish.
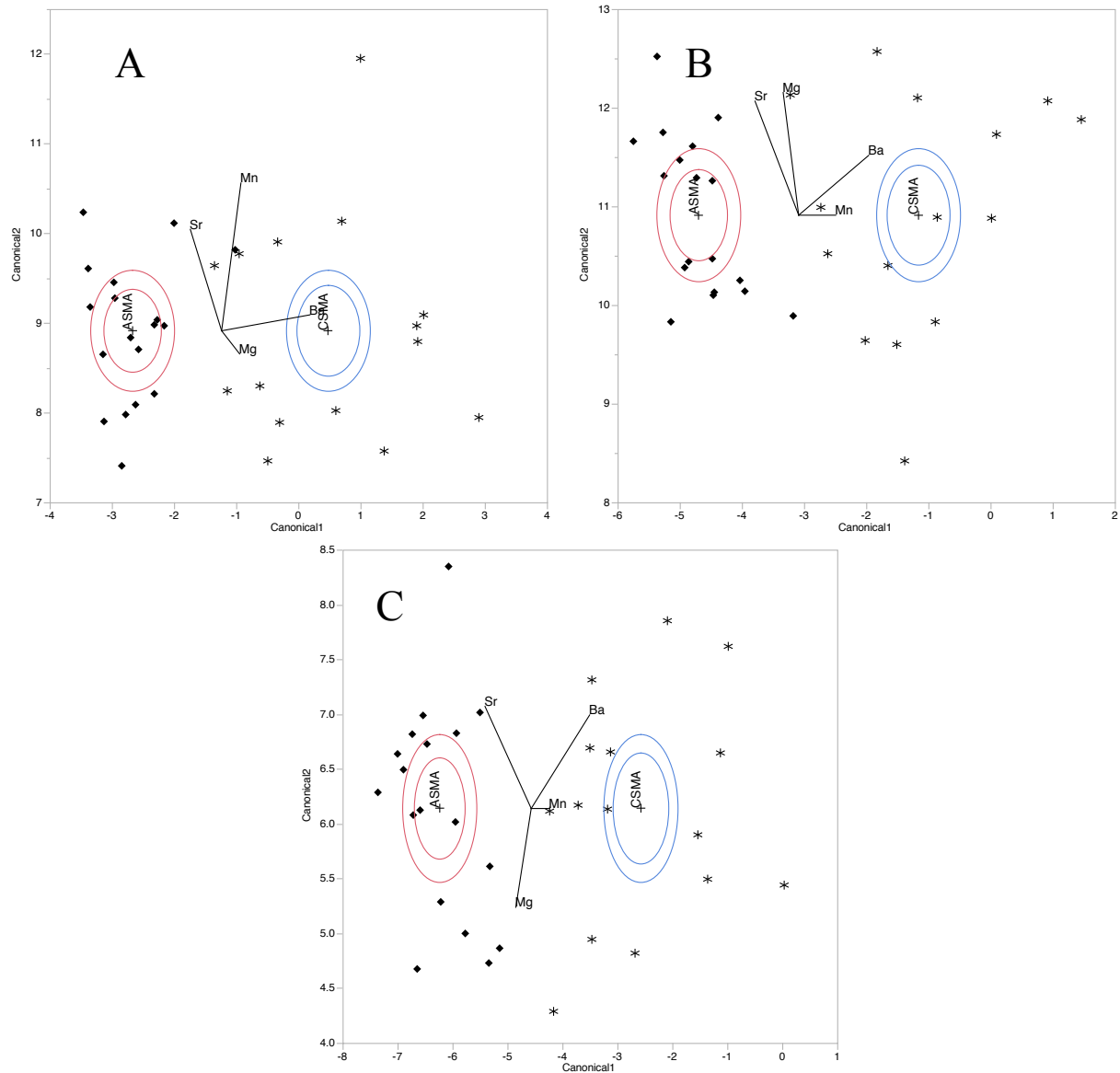
Figure 3.3. Results of the first two canonical variates obtained using LDFA to compare annual otolith chemistry of large (>800 mm L_T) ASMA Striped Bass to adult CSMA Striped Bass of the 2005 year class in years that those fish co-occurred: (A) 2007; (B) 2008; (C) 2009. ASMA fish collected in 2009 were excluded from (C), as there was a small amount of data from fish collected that year. Group centroids are surrounded by ellipses representing 95% confidence intervals (smaller ellispes) and 50% contours (larger ellipses). The multivariate mean would fall in the smaller ellipse 95 times if the analysis were run 100 times. The larger ellipses represent 50% contours in which 50% of the data points from each management area would fall. Points falling in the overlap of the 50% contours are similar.
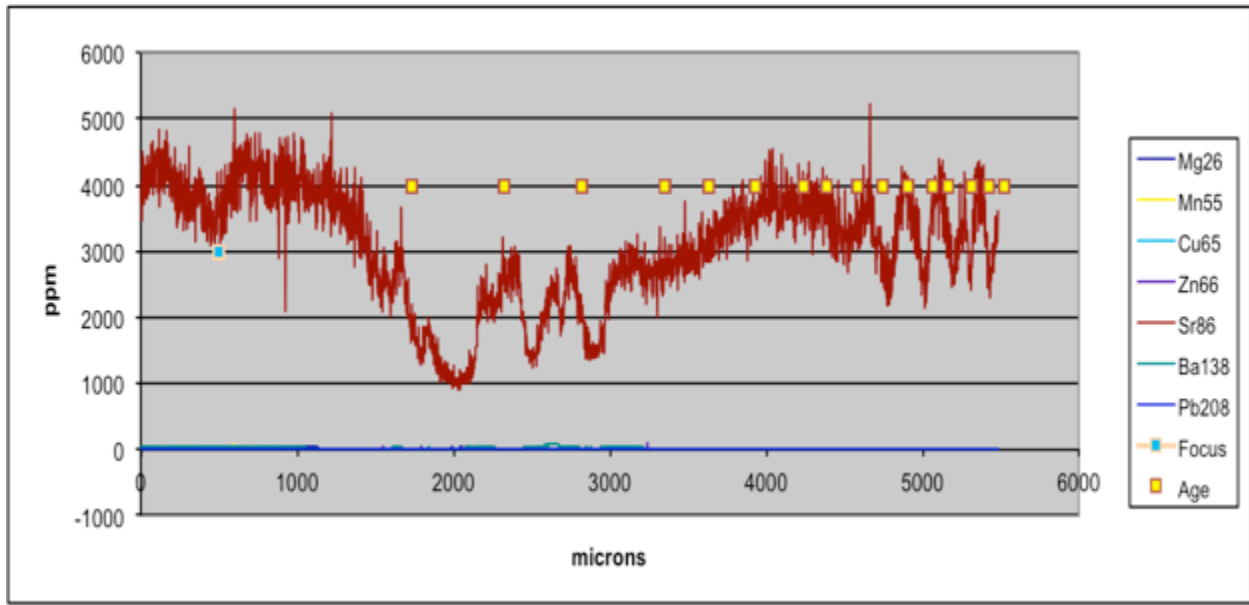
Figure 3.4. Strontium profile of a large anadromous ASMA fish. This fish was 15 years old. The x-axis represents distance along the otolith in micrometers. The y-axis represents strontium concentration in parts per million. The focus of the otolith is marked with an unshaded square, and the fish's age is marked by shaded squares. This is ASMA fish 10-637.
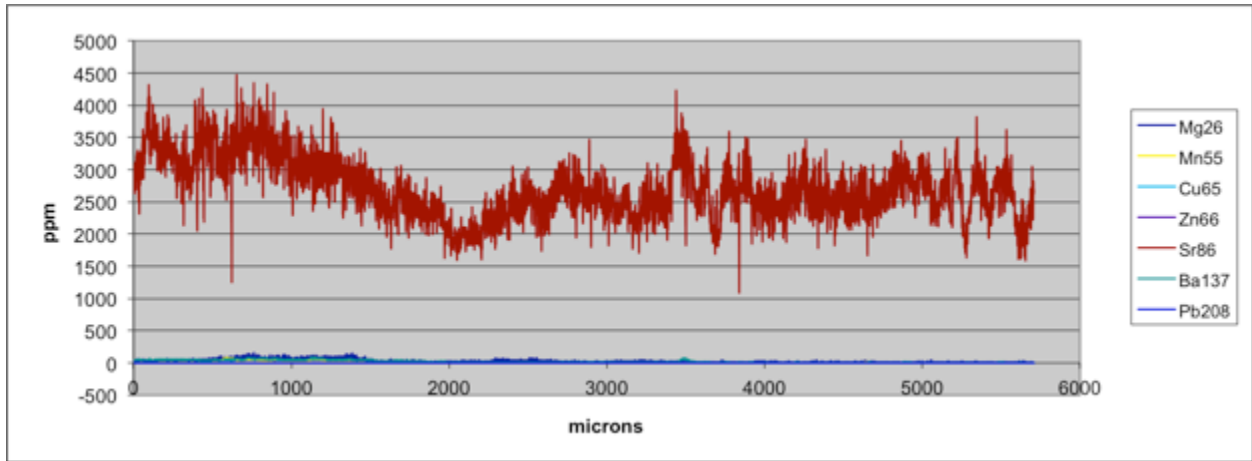
Figure 3.5. Strontium profile of a large, adult resident fish. This fish was 13 years old. The x-axis represents distance along the otolith in micrometers. The y-axis represents strontium concentration in parts per million. This is ASMA fish 10-671.