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Evaluation of Fish Scale Chemistry for Determining Habitat Associations

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EVALUATION OF FISH SCALE CHEMISTRY FOR DETERMINING HABITAT ASSOCIATIONS

by

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ABSTRACT

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This is the first work examining the utility of scale chemistry for determining natal origins and habitat associations. I quantified a relationship of scale chemistry to water chemistry, quantified geographic variation in scale chemistry, and evaluated stability of scale chemistry through maturation. Scale chemistry accurately reflected trace element composition of the water in which fish had lived. Juvenile spot (*Leiostomus xanthurus*) were held in four concentrations of Sr, Cd, and Ba maintained at either 20°C or 25°C, for 42 days. Strontium:Ca, Cd:Ca, and Ba:Ca levels in scales were linearly related to environmental concentrations while temperature had no effect. These results suggested scale chemistry could reflect differences related to habitat use by fishes. To test this, Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca levels in scales from juvenile weakfish (*Cynoscion regalis*) from five estuaries along the Atlantic coast were measured. Significant variability in multivariate elemental signatures was found among estuaries and between collections from 1996 and 1997. Linear discriminant function analysis was used to classify individual juvenile weakfish to natal estuary with ~65% accuracy. Results from simulated learning and test samples derived from the juvenile data indicated a maximum likelihood (ML) procedure could estimate proportions of juveniles from each natal

estuary with ~90-95% accuracy. Inter-annual variability in the trace element signatures meant fish could not be accurately classified to natal estuary based on signatures collected from juvenile fish in a different year. Trace element levels in scales were significantly correlated with otolith concentrations from the same fish in both studies, suggesting that similar processes control both scale and otolith chemistries. Finally, using natal-estuary signatures, natal location of adult weakfish collected in Pamlico Sound, Chesapeake Bay, and Delaware Bay was estimated with ML. Composition of adults was estimated similarly by otolith and scale chemistries. However, these data suggested that scale chemistry was not stable after the juvenile period possibly due to continued crystallization. In all, at least some trace elements in scales reflect levels in the ambient environment, and may be useful for quantifying life-history characteristics of individual fish. However, caution is required when applying the technique to adults as elemental signatures may degrade.

To my Grandmother who shows I come from good stock and to my wife, Chrissann, who will ensure continued good recruitment.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

GENERAL INTRODUCTION

Measures of growth, survival, and reproduction can be different between fish stocks of the same species and, therefore, stocks must be discriminated and managed, when necessary, as independent units (Campana and Casselman 1993; Ruzzante et al. 1996). If growth and mortality rates vary greatly from one stock to another, then similar fishing pressures on these stocks can have drastically different effects (Edmonds et al. 1989). It is possible, then, that when stocks mix and fishing occurs, those stocks most sensitive to fishing pressure suffer detrimental effects (Ruzzante et al. 1996). Such detrimental effects include restricted genetic variation, range alteration, and reduced fecundity. Further, identification of the individual stocks of a population can be useful to assess the recruitment success, importance of individual spawning stocks and nursery areas, and exploitation rates for each stock. With this knowledge, management priorities can be properly assigned. It is, therefore, important to determine whether a population is homogeneous or composed of a collection of stocks that have varying characteristics.

Beyond the implications to fisheries, the determination of stock structure can be used to understand dispersal, population ecology, and metapopulation structure of a species (Thorrold et al. 1997). Understanding the range, amount of mixing, and genetic stability of a stock can give the researcher needed information to evaluate recruitment processes, population stability, and evolutionary pressures that have led to past and current population structure. In general, an understanding of population structure can be

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This dissertation follows the format of Transactions of the American Fisheries Society.

applied to predicting how populations react to biotic and abiotic circumstances along their entire range across a landscape of varied habitats.

A stock can be defined as a single interbreeding group with complete or nearly complete reproductive isolation from the remaining members of the species. However, stocks can also be defined as manageable units where estimates of growth, mortality, and fecundity are useful in predicting the persistence of the group. The difference between these definitions is their temporal resolution. For instance, reproductively isolated and, therefore, genetically distinct stocks are formed over thousands of years where there has been time for portions of a population to develop dissimilar vital rates. For management purposes, however, the population is broken into manageable units based on differences between stocks that may be the result of recent environmental events. Typically, managers are interested in identifying spawning stocks, their important nursery areas, and the further contributions of each to the entire population, all of which rely on the detection of variation in the population that may be caused by more recent or variable events.

Various techniques can identify stocks at different levels of resolution. For instance, studies based on morphometric and meristic differences often assume long-term changes to the population or inherent plasticity of genotypes along an environmental gradient. Similarly, techniques based on detecting genetic variation (i.e., allozyme and mitochondrial) require that evolutionary processes have occurred. In fact the length of time required for geographic separation of stocks to be reflected in mtDNA is 1,000 to 10,000 years (Thorrold et al. 1996). Further, even a very low exchange between stocks is sufficient to prevent identifiable differentiation of the stocks within the population (Nolan

et al. 1991; Mills and Allendorf 1996). Genetic variation can also be restricted if population bottlenecks occur (Bentzen et al. 1989; Nolan et al. 1991; Graves et al. 1992; Epifanio et al. 1995). It is possible that the smaller groups within a population are different in one or more life-history parameters even though they are apparently genetically similar. Although differentiation has not occurred, there may still be the need to manage the stocks as individual units. If stocks are defined as manageable units, they must be detectable with great resolution so that the importance of specific spawning stocks, spawning grounds, and nursery areas can be evaluated. In fact, even determining generational differences in population structure can assist in managing a fishery over time as it can be used to better understand recruitment processes and environmental effects on the population.

Artificial and natural tags can assist in determining population structure with high resolution. Tagging studies are valuable for determining spawning stock, nursery grounds and recruitment, but large numbers of tags must be applied and recaptured (Nichols 1960; Westrheim et al. 1992) . Natural tags such as parasites can reduce the effort needed for a study (Lester et al. 1988; Dailey and Vogelbein 1991) when enough fish are infected for them to be detected. However, it is rare that parasites are sufficiently site-specific for stock management. The elemental components of bony structures can also act as a natural tag. Importantly, elemental tags are available on every fish throughout its entire life and each fish is affected independently by its environment. Therefore, elemental analysis of bony structures has recently played a vital role in stock discrimination and life-history parameter estimation. Using elemental analysis, researchers have been able to identify nursery areas, residential areas, and migratory

routes. Managers have used these determinations to assess the life-history parameters of each stock as they relate to population ecology and to maintaining sustainable fisheries.

The Use of Otolith Chemistry

For trace element analysis to yield meaningful results that can be used for discriminating stocks and/or habitat use, there must be a correlation between habitat characteristics and hardpart elemental composition. To date, the majority of research to evaluate what determines elemental composition in hardparts has been performed on otoliths. Hence, a review of the factors affecting the elemental components of otoliths can be helpful toward understanding the factors that control the elemental components of other hardparts such as scales. Water chemistry controls as much as 75% of the Ca and 88% of the Sr in otoliths (Farrel and Campana 1996). Otolith formation and chemistry has been shown experimentally to be the product of numerous interacting processes including water temperature, salinity, and ontogeny, with temperature having the greatest effect after water composition (Fowler et al. 1995a, 1995b). It is possible that a given stock can be distinguishable from other stocks by identifying specific elemental signatures (Campana et al. 1994) formed for each water mass (defined by chemistry, temperature and salinity among other things). Thresher et al. (1994), however, suggest that the chemical composition of otoliths may be somewhat less dependent on environmental conditions. Instead, regional differences in otolith composition may be reflective of genetic differences or are set by environmental influences early in life that are maintained through subsequent life. Somewhat in support of Thresher et al., Gallaher and Kingsford (1996) demonstrated that Sr:Ca ratios in otoliths did not decrease, as

expected, with increasing temperature (19°C - 28°C). Further, an increase in Sr obtained from food caused a slight increase of otolith Sr:Ca concentrations (Gallaher and Kingsford 1996). In part, the elemental contents of otoliths may also be controlled by diet. Regardless of what most strongly controls otolith elemental composition "...if otolith elemental components (the elemental 'fingerprint') reflect, in some way, the characteristics of the environment the elemental fingerprint of the otolith nucleus could serve as a natural marker of the fish populations hatched at different sites" (Campana et al. 1994). As long as there is site-dependent variation in the elemental composition, a researcher can use otoliths to determine stock structure, nursery areas, stock exploitation rates, and migrations.

For twenty years researchers have applied these concepts and techniques to otoliths to differentiate stocks. In a study designed to determine the relationship of age and size to elements in the otoliths of a pelagic fish, Papadopolou et al. (1980) stated that "... fish otoliths could serve as storage sites of certain elements of biological and/or radioecological importance." Edmonds et al. (1989) examined concentrations of trace elements in otoliths of pink snapper (*Chrysophrys auratus*) from seven locations along the western coast of Australia and determined 84 % of specimens could be correctly classified to location. Edmonds et al. used trace element analysis to discriminate stocks of orange roughy (*Hoplostethus atlanticus*) in 1991, and in 1992 distinguished stocks of yellow-eye mullet (*Aldrichetta forsteri*). Campana et al. (1994) successfully assigned Atlantic cod (*Gadus morhua*) to five separate nursery areas. Juvenile walleye pollock (*Theragra chalcogramma*) were traced to five locations in the Gulf of Alaska with 60 to 80% accuracy (Severin et al. 1995). Trace element analysis has also been used to

evaluate the importance of estuary regions for recruitment to coral reefs (Gillanders and Kingsford 1996). In the freshwater environment, lake herring (*Coregonus artedi*) in Lake Superior were assigned to their different spawning areas in the lake (Bronte et al. 1996).

A few studies report limitations to these techniques. For example, Hoff and Fuiman (1995) present experimental results from tests on red drum (*Sciaenops ocellatus*) where diet, temperature, and salinity were manipulated. Despite highly significant correlations there was a high degree of variation in concentrations of each element within individual otoliths produced under constant conditions. Therefore, they showed that precise reconstruction of environmental history using these methods was impractical. Proctor et al. (1995) attempted to determine the number of spawning areas and diversity of migration routes of blue fin tuna (*Thunnus thynnus*), but could not because variation in elemental fingerprints was minimal due to homogeneity of environment. Despite these limitations, trace element analysis has been shown to work well for stock delineation in a variety of environments on multiple species of fish with different life histories.

Beyond stock and residence identification, trace element analysis can also be applied to gaining previously unattainable information about the fish's life history. Secor (1992) and Secor et al. (1995) examined Sr:Ca ratios in striped bass (*Morone saxitilis*) otoliths to evaluate migratory schedules. They found that otolith microchemistry reflected the environmental history of the individual across an estuary gradient of salinity. The time of metamorphosis from leptocephali to glass eel (*Anguilla japonica*) was estimated from otolith increments where the width and Sr:Ca ratios changed drastically and indicated arrival time to estuaries (Cheng and Tzeng 1996). Kalish (1990) demonstrated that otolith chemical analysis could be used to distinguish progeny of

anadromous and non-anadromous salmonids. These studies, in addition to ones used to directly identify stocks, spawning grounds, and nursery areas, clearly demonstrate the broad range of information that can be attained by examining the elemental composition of otoliths. It seems possible that other hardparts such as scales could yield similar results. Importantly, scale collection is nonlethal and easier to conduct. Therefore, in an effort to reduce cost and fish mortality, I evaluate the usefulness of scale chemistry for examination of habitat use.

The Use of Scale Chemistry

Scale structure and development

The scale is made of two layers, the fibrillary plate and the bony layer. The fibrillary plate, located on the proximal side of the scale, is largely uncalcified and without vascular canals (van Oosten 1957; Fouda 1979). The distal bony layer is an organic framework impregnated with calcium phosphate (hydroxyapatite) and calcium carbonate (aragonite; van Oosten 1957). The fibrillary plate varies in thickness, being very thin at the anterior and thickening at the focus while the bony layer has a constant thickness (Fouda 1979). Generally, the scale is 41 to 84% protein and the rest is mineral (van Oosten 1957).

The development of the scale has been examined by histology (van Oosten 1957; Fouda 1979) and electron microscopy (Fouda 1979). There are three types of cells involved in the development of scales: osteoblasts associated with formation of the osteoid zone at the anterior edge of the scale, osteoblasts at the surface of the osseous layer responsible for thickening the osseous layer and formation of circuli, and fibroblasts

(specifically scleroblasts; Zylberberg et al. 1992) associated with formation of the fibrillary plate (Fouda 1979). First, a follicle forms around a scale pocket and produces the osteoblasts (van Oosten 1957). Then an osteoid zone matrix of amorphous ground substance with dispersed collagen fibers is produced which acts as a template for calcification (Fouda 1979). The osteoblasts then cause the osteoid tissue to become impregnated with calcium (van Oosten 1957; Fouda 1979). Subsequently, the fibrillary plate appears as a thin sheet between the bony layer and the lower layer of the osteoblasts (van Oosten 1957). The development of the fibrillary plate occurs as fibroblasts adjacent to the marginal osteoblasts secrete a layer of small collagen fibers (Fouda 1979). Later, the fibroblasts disappear and the fibrillary plate remains between the scale and the connective tissue (van Oosten 1957). Scale growth continues through life by accretion at the margin of the surface layer and deposition of the thin fibrous layer below it (van Oosten 1957). Because bony deposits occur at the periphery faster than the fibrillary plate grows, the bony layer extends beyond the fibrillary plate during growth (van Oosten 1957). Additionally, growth and calcification of the bony layer is at the margin, so that the bony layer has a uniform thickness but the fibrillary plate grows in length and thickness (Fouda 1979). Incidently, regenerated scales extend their length from secretion of osteogenic cells at both ends until the scale pocket is filled (Fouda 1979).

Trace element analysis of scales

For scales to be useful, differences in the physical and chemical environment must be reflected in the scale, much the same way as found in the otolith. The scale can be intentionally marked by increasing Sr concentration in the ambient environment (Yamada and Mulligan 1982; Yamada and Mulligan 1987). In fact, a doubling or tripling

of Sr concentrations in the ambient water can produce a corresponding two- and three-fold increase in scale Sr levels (Snyder et al. 1992). van Coillie and Rousseau (1974) demonstrated that in polluted waters excess metal from water can replace Ca in the scales. Capitalizing on this, they were able to differentiate elemental tags in scales of fish from two rivers (one polluted and the other not). Scales have also been shown experimentally to include higher concentrations of induced lanthanide tags than either otoliths or vertebrae from the same fish (Ennevor and Beames 1993). Thus, there is experimental evidence that the levels of an element in the environment can, to an extent, control elemental concentration in scales. Also, some elements (F, Sr, Zn, Ca) are assimilated into the bone matrix more readily as temperature increases (Gauldie et al. 1990, 1991), which I argue by pointing to the results of Wells et al 2000a, reflected differential growth. Importantly, a starvation study indicated that fish underfed for 20 and 30.5 weeks showed no record of deprivation on the scales nor did checks form during the same period (Bilton and Robins 1971). Further, there was an indication of scale resorbtion (Bilton 1975). Thus, undernourished fish may not have sufficient scale growth for the scale to be a useful indicator of environment because periods of migration and spawning may not be recorded.

It is also essential that the elemental tag persist over time. While the otolith is not reworked over the life of the fish and its tag remains stable (Campana et al. 1994), there is little research evaluating the stability of a scale tag. Ennevor and Beames (1993) showed that lanthanide tags induced on juvenile salmonids remained on the scale for at least 10.5 months. Yamada and Mulligan (1982, 1987) showed a Sr tag remained at the core of scales from returning coho salmon (*Oncorhynchus kisutch*) which were tagged

when they were still at the hatchery, thus the tag was identifiable after maturation and migration. Parenthetically, when analysis of the whole scale was used there was no difference between control and tagged groups (Yamada and Mulligan 1982) indicating a need for loci-specific analysis of the scale. In all, evidence suggests that tags initially present on scales remain over long periods of time.

Analysis of trace elements in scales has been used for purposes such as identification of poached fish and stock structure. Some of the earliest work was done by Bagenal et al. (1973) who used Sr:Ca ratios in scales to differentiate brown trout (*Salmo trutta*) from sea trout (*Salmo trutta*). Because Sr is more available in the marine environment, its presence differentiated the sea-run trout. Yamada et al. (1979) Sr-tagged hatchery-reared coho salmon and later observed the tag in scales. To identify poached freshwater striped bass in the commercial catch, Belanger et al. (1987) used scale Sr levels to differentiate fresh from saltwater fish. Coutant and Chen (1993) examined Sr spikes at the primordia of young striped bass and suggested that increased Sr spikes indicated marine parentage or estuarine rearing. These authors also profess the expanded use of laser ablation mass spectrometry for the high resolution, loci-specific, and extensive analysis of trace elements in the scale. Finally, Pender and Griffin (1996) examined the population structure of barramundi (*Lates calcarifer*) using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Theirs is one of the earliest attempts at examining many elements of the scale simultaneously.

I use laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to describe the scale elemental tags from weakfish (*Cynoscion regalis*) from five estuaries along the Atlantic coast. Loci-specific analysis is crucial as any technique that

analyzes the entire scale will dilute the core tag and integrate the elemental signals from all the environments that the fish has experienced (Yamada and Mulligan 1982). Energy-dispersive and wavelength-dispersive electron microprobes (EPMA) have been used to examine specific areas of the otolith (Mulligan et al. 1987; Kalish 1990; Secor 1992; Rieman et al. 1994; Thresher et al. 1994) and it is likely that these techniques could also work with scales. EPMA, however, has high detection limits (> 100 μg/g) so that only 6 major and minor elements have been quantified in the otolith (Na, Sr, K, Ca, S, Cl; Gunn et al., 1992). The proton microprobe, while more sensitive than EPMA, still has detection limits that are close to the concentration of trace elements found in otoliths (> 1 μg/g; Sie and Thresher 1992; Thorrold et al. 1997) and may be similarly restrictive for analyzing scales. Recently, ICP-MS has been used on otoliths with great success (Edmonds et al. 1991; Campana and Gagné 1994; Fowler et al. 1995a, 1995b) and can offer similar success for scales (Coutant and Chen 1993). The technique provides excellent analytical power for trace element analysis, with extremely low detection limits (0.1-0.01 μg/g; Houk 1986), wide dynamic range (ng/g to %), high sample throughput, and isotopic discrimination (Henshaw et al. 1989; Colodner et al. 1994). Potentially, ICP-MS can distinguish fish on spatial scales ranging from km (Dove et al. 1996) to hundreds of km (Campana et al. 1994; Campana and Gagné 1994). LA-ICP-MS uses a narrow beam to probe solid samples. The resulting vaporized material is swept by argon or helium gas into a plasma torch and analyzed by mass spectrometry (Thorrold et al. 1997).

Controlled experimentation

A controlled experiment is needed to estimate the effects of the environment on the assimilation of scale elements. Through experimentation, the importance of temperature (Radtke 1989; Townsend et al. 1992; Fowler et al. 1995a, 1995b), salinity (Fowler et al. 1995a, 1995b), diet (Gallaher and Kingsford 1996), and ambient elemental concentration (Farrell and Campana 1996) have been evaluated for their importance in controlling otolith elemental concentrations. Such research is limited for scales (Gauldie et al. 1990, 1991). While scales share similarities with otoliths (bony deposits, growth through life, and circuli), drastic dissimilarities could hamper the use of scales for complex trace element analysis (resorbtion, composition mostly of hydroxyapatite rather than aragonite, less mineral material, more plasticity of scale growth, and exposure to contamination even at the scale core). Because of these dissimilarities, scales need to be tested under controlled conditions to judge their usefulness for trace element analysis. I have performed an experiment to evaluate the effects of ambient concentration of elements on the concentrations in the scale to determine if scale elements can reflect the fish's environment.

Between-site variability in scale elemental tags for juveniles

If scales have and maintain nursery-specific tags they can be used in the same way as otoliths to determine nursery areas of individual fish later in life (Mulligan et al. 1987; Edmonds et al. 1989; Edmonds et al. 1991; Edmonds et al. 1992; Campana et al. 1994; Thresher et al. 1994; Severin et al. 1995; Bronte et al. 1996; Gillander and Kingsford 1996). If this is the case, individual juveniles on and off their nursery grounds could be assigned to their respective nursery areas by using scale core elemental tags.

Adults, however, can be assigned to nursery areas only if it has been demonstrated that the tag is maintained through maturation and migration.

Testing stability of the scale tag after maturation

Stability of the elemental tag at the scale core through maturation must be assessed. If it is stable, site fidelity can be evaluated by collecting spawning adults. Using the elemental tag at the otolith core as a more stable tag to determine a fish's nursery area, the elemental tag at the scale core can be judged against the previously determined scale tag from fish of the same nursery area. If the tags on scales show no or only minor alterations they can be used to assign adults to their natal grounds.

Characteristics of a species well suited to evaluate scale elemental tags

To test the usefulness of scales for trace element analysis it is important to choose a species with a life history that is likely to have between-site variability of elemental tags. The fish must have a low threshold to starvation so that mortality occurs before any scale resorbtion takes place, hence, scales from living fish will have grown through the fish's entire life. The species must exist over a large geographic range that includes various environmental conditions. It is also essential that there are discrete nursery areas with no mixing of juveniles between areas. If nursery areas are not isolated, juvenile mixing will limit between-area variability in the elemental tags. I show that weakfish is an appropriate species to be used to evaluate the usefulness of scale trace element analysis for determining natal origins.

Chapter Objectives

As trace element analysis becomes a more common approach for describing population structure, habitat use, and stock identification, there is a need for easier and less expensive data collection. While otoliths have offered a great deal of information, their collection is time consuming and expensive. Additionally, it is obviously a lethal approach. Scales can be collected easily by the researcher or possibly the fisher. Therefore, scale collection and analysis may offer a nonlethal and relatively inexpensive approach to trace element analysis of bony structures.

This dissertation is divided into three separate manuscripts. The first manuscript (Chapter II) describes the relationship between elemental composition of the ambient environment and the elemental composition of juvenile spot (*Leiostomus xanthurus*) scales. The second manuscript (Chapter III) describes the elemental signatures in the scales of juvenile weakfish (*Cynoscion regalis*) along the U. S. Atlantic coast and demonstrates that they vary significantly. The third manuscript (Chapter IV) estimates the stability of the elemental signatures in the scales and determines the natal origins of adult weakfish.

CHAPTER II

INCORPORATION OF STRONTIUM, CADMIUM, AND BARIUM IN JUVENILE SPOT SCALES REFLECTS WATER CHEMISTRY

Introduction

The elemental composition of fish bony structures such as fin rays, scales, and otoliths may be useful natural markers of population structure and habitat associations. Such elemental tags are available on every fish throughout their lives as each fish is affected independently by its environment. Therefore, elemental analysis of bony structures can play a vital role in stock discrimination and the estimation of life history parameters. Geochemical signatures in otoliths have been used to determine nursery area residency, gain information on parentage, and to estimate larval dispersal pathways and adult migration routes (reviewed by Campana 1999). Analyses of trace elements in scales have been used to identify poached fish (Belanger et al. 1987), understand stock structure (Pender and Griffin 1996), determine marine residency (Bagenal et al. 1973), and identify nursery areas (Coutant and Chen 1993). Importantly, scales offer a non-lethal approach that is not an option with otoliths, and thus may be particularly useful when applied to rare or endangered species.

Before scales can be used to reconstruct environmental histories of individual fish, it is necessary to validate the assumption that trace elements in scales are deposited in proportion to dissolved concentrations in the ambient environment. To date, only qualitative observation and experimentation have been performed to describe the relationship. Yamada et al. (1979) and Yamada and Mulligan (1982, 1987) demonstrated

that increasing Sr concentration in the environment marks the scale. Doubling or tripling of Sr concentrations in the water has been shown to produce a corresponding two- and three-fold increase in scale Sr levels (Snyder et al. 1992). van Coillie and Rousseau (1974) demonstrated that in polluted waters excess metal ions from water (Cr, Mn, Co, Ni, Sr, Ag, Cd, Cs, Hg, and Pb) replaced Ca in scale apatite. Ennevor and Beames (1993) exposed juvenile salmonids to elevated levels of lanthanum, cerium and samarium by adding each of the three elements to the water in which the fish were reared. Resulting levels of the elements in scales were higher than in either otoliths or vertebrae, suggesting that scales may better reflect trace element concentrations in the environment.

Accurate descriptions of the relationship between scale and water chemistry, as modified by physical variables such as temperature, requires detailed knowledge of environmental exposures of individual fish. This is most easily achieved under controlled laboratory conditions. For instance, laboratory experiments have documented the effects of temperature (Radtke 1989; Townsend et al. 1992; Fowler et al. 1995a, 1995b), salinity (Fowler et al. 1995a, 1995b), diet (Gallaher and Kingsford 1996) and environmental element concentration (Farrell and Campana 1996; Bath et al. 2000) on otolith composition. Such research is, however, limited for scales (Gauldie et al. 1990, 1991; Mugiya et al. 1991).

In this study, I examined the relationship between Sr, Cd, and Ba levels in seawater and the resultant incorporation of these elements in the scales of juvenile spot, *Leiostomus xanthurus*. Strontium and Ba are commonly assayed in otoliths and scales as they can be used to indicate movement in and out of an estuary as well as discriminate between different estuaries (Wells et al. 2000b). In oceans Cd follows a nutrient-type

distribution with much greater concentrations in deep water than in shallow water such that it can be used to indicate exposure to upwelling waters. Further, anthropogenic inputs in rivers and estuaries can lead to very high concentrations of Cd. By maintaining the fish in the laboratory, I was able to manipulate and quantify exposures to both water temperatures and dissolved concentrations of the elements. I was also able to compare and contrast scale chemistry with that of otoliths from the same fish, which have been reported elsewhere (Bath et al. 2000).

Methods

Experimental conditions

Spot were spawned on November 22, 1997 at the National Marine Fisheries Service Laboratory (Beaufort, NC, USA) in a flow-through natural sea water system (30‰ salinity), assuring larvae were from the same brood stock and of known age. Thirty-five days after hatching, the larvae were transported to a rearing laboratory and transferred to 24 acid washed high-density polyethylene tanks (20L capacity) in a clean facility equipped with a positive flow air filter (0.2 μm) system. The fish were acclimated to artificial seawater (20‰, "Instant Ocean") for four days and were raised under experimental conditions for 42 days. Enriched *Artemia* were fed to the fish for the first two weeks of the experiment, and thereafter fish were fed an artificial diet. Mortality over the experiment was low with an average of seven fish used from each tank for scale analyses (Table 1). Any fish that died before the designated end of the experiment were not used in any analyses.

TABLE 1. – Summary of average temperature (T), pH, dissolved Sr:Ca (mmol/mol), Cd:Ca (μmol/mol), Ba:Ca (μmol/mol), standard length (SL), number of fish from each tank used, and the number of pooled samples which were analyzed from each tank.

Tank	T (°C)	pH	Sr:Ca	Cd:Ca	Ba:Ca	SL	No. fish	No. pooled samples
1	20.8	8.00	15.21	19.53	151.16	24.59	9	3
2	25.2	8.05	17.40	22.41	71.41	20.21	6	3
3	25.2	8.00	16.03	14.84	25.48	27.46	11	3
4	20.3	7.97	15.17	26.37	23.90	23.05	5	3
5	20.6	7.96	22.36	26.97	138.83	24.06	4	3
6	20.4	8.02	17.68	18.16	72.30	25.27	1	1
7	24.8	8.01	22.74	29.79	148.14	23.84	8	3
8	25.2	7.98	17.88	30.33	20.85	24.02	11	3
9	25.1	8.00	22.47	12.18	22.93	22.52	9	3
10	20.3	7.95	12.73	22.44	155.85	24.39	9	3
11	20.4	7.91	12.54	20.01	228.09	25.83	10	3
12	25.4	8.04	22.50	49.75	70.21	23.00	10	3
13	20.3	7.97	17.79	48.49	22.98	24.42	5	3
14	20.3	7.94	22.55	53.65	215.56	24.46	9	3
15	25.1	8.04	12.75	29.31	211.75	21.89	5	2
16	25.0	8.03	15.23	24.78	144.87	22.88	5	3
17	24.7	8.02	13.03	46.67	74.27	----	----	----
18	25.5	7.98	18.29	11.49	231.45	24.41	9	3
19	20.3	8.00	17.81	12.01	75.09	25.71	7	3
20	24.9	8.00	13.39	49.66	142.93	23.61	5	3
21	19.7	7.98	15.00	50.14	70.96	24.78	7	3
22	20.4	8.01	12.60	32.56	222.20	23.15	6	3
23	24.5	8.01	15.02	22.73	24.88	24.17	8	3
24	20.0	7.98	22.80	14.39	23.19	26.56	8	3

Triplicate experimental tanks within each temperature treatment (20 °C and 25 °C) were randomly assigned one of four levels of Sr:Ca corresponding to baseline, 1.2x, 1.4x, and 1.8x baseline levels, and Ba:Ca corresponding to baseline, 3x, 6x, and 10x baseline levels. Mean concentration levels for Cd:Ca over the experiment were baseline, 1.5x, 2x, and 3.5x baseline. After the first three weeks I doubled the spiked levels of Cd:Ca due to difficulties maintaining dissolved concentrations in the tanks. Baseline concentrations for each element were determined by inductively coupled plasma mass spectrometry (ICP-MS) assays of the experimental artificial seawater, at the correct salinity, before the start of the experiment (Table 1). The spiked waters were prepared by adding appropriate amounts of standard solutions (SPEX) of $SrCl_2$, $CdCl_2$, and $BaCl_2$ to each of the tanks.

Water chemistry

To maintain water quality and spike levels in the tanks, water was changed at 50% volume daily. Temperature, salinity, and pH were measured throughout the experiment and water samples were analyzed from each tank at weekly intervals, including the first and last day of the experiment. Water samples were filtered through a 0.22 μm cellulose nitrate membrane filter, acidified with trace metal grade 12N HCl to pH 2, and stored frozen acidified for subsequent analysis (Bath et al. 2000). In all data analyses, the average of each tank's six weekly values was used (Table 1).

I encountered two difficulties using artificial seawater in the experiment. First, Sr:Ca ratios in the "Instant Ocean" salts that I used were slightly higher (12mmol/mol) than those of typical seawater (8.5-9 mmol/mol). Because dilution would only lower

absolute Sr levels and would not change Sr:Ca ratios in the water I could do little to lower this value. Therefore, the highest Sr:Ca values in this experiment were 2.5x that of normal seawater. I did not face this problem with baseline and spiked Ba:Ca levels which spanned a range (23 –230 μmol/mol) that would commonly be encountered by estuarine-dependent fish along the east coast of the United States (Coffey et al. 1997). Cd:Ca levels were higher than those from pristine environments but were possible for polluted waters. Second, our decision to increase spiked levels of Cd half way through the experiment, to achieve better separation among treatments, meant that within-tank variances of Cd:Ca were considerably higher than for either Sr:Ca or Ba:Ca levels. The increased variance in water chemistry was also, in all probability, manifested in concomitant within-tank variability in scale Cd:Ca ratios.

Scale collection

One hundred sixty seven fish were used for scale analysis. Scales were removed from the entire fish with acid washed glass probes and were washed twice with Milli-Q water. The scales were stored dry in acid washed low-density polyethylene vials prior to the ICP-MS analyses. To ensure sufficient scale material for trace element analysis, scales from individual fish within a tank were pooled into three randomly assigned groups and analyzed as a pooled sample. This practice maintained much of the within-tank variance, while still yielding enough scale material to obtain adequate results. Pooled scale sample weights ranged from 0.4 - 6.5 mg with a mean weight of 1.6 mg.

Scale analysis

Elemental analyses of scales were conducted using ICP-MS, with quantification using a combination of isotope dilution (Sr, Cd, and Ba) and internal standardization

(Ca). Scales were dissolved in high purity nitric acid (Seastar Chemicals Inc.) containing enriched isotopes (^{87}Sr, ^{112}Cd, and ^{137}Ba) and an internal standard (^{45}Sc). These isotopes, along with ^{48}Ca, ^{88}Sr, ^{114}Cd, and ^{138}Ba, were then monitored in the samples for quantification purposes. I confirmed that all isotopes were free of isobaric interferences using high resolution ICP-MS (Thorrold and Shuttleworth 2000). Blank values based on analysis of high purity nitric acid were less than 100 μg/g for Ca, less than 1 μg/g for Sr, less than 0.1 μg/g for Cd, and less than 0.05 μg/g for Ba. ICP-MS operating conditions are shown in Table 2. Scale samples were blocked by tank so that a sample from each of the 24 tanks was assayed in turn. The order of tanks within each of the 3 blocks was randomized. Elemental concentrations were standardized to Ca because the elements examined are likely to substitute for Ca in the bone matrix (van Coillie and Rousseau 1974; Campana 1999). The incorporation of Sr, Cd, and Ba ions into the hydroxyapatite will be, therefore, dependent upon the ratios of these elements to Ca in the depositional fluid and not the absolute concentration of the elements themselves.

Data analysis

Least-squares regression was used to evaluate the significance of our treatments and assimilation relationships. Elemental results from each tank were averaged and used in the statistical analyses. Otolith chemistry from the same fish as used in the scale analyses were also determined, and reported elsewhere (Bath et al. 2000). I compared the chemistry of scales and otoliths from the juvenile spot using Pearson's correlation analysis after averaging both otolith and scale data across each of the 24 tanks. Data from one tank (temp = 25 °C, Sr = 1x, Cd = 3.5x, and Ba = 3x) were removed because of either

TABLE 2. – Inductively coupled plasma mass spectrometry (ICP-MS) system operating conditions.

General:	
Instrument	ICP-MS
Make	Turner Scientific (Finnegan/MAT)
Model	Sola
R. f. power	1500 W
Sample gas flow rate	0.8 mL/min
Nebulizer gas flow rate	0.9 L/min
Auxiliary gas flow rate	1.2 L/min
Coolant flow rate	15 L/min
For the Ca and Sr run:	
Detector	Multiplier
Channels per atomic mass unit	8
Passes per scan	16
Dwell time	8 ms
Number of scans	1
Resolution	30
For the Cd and Ba run:	
Detector	Faraday
Channels per atomic mass unit	8
Passes per scan	4
Dwell time	64 ms
Number of scans	5
Resolution	30

measurement error or sample contamination, reducing the number of replicates for this treatment to two.

Variances in mean concentrations in water and scales among the four Sr:Ca and Cd:Ca treatments were equal and values were normally distributed. However, variances in the Ba:Ca treatments increased with increasing spike levels, which also appeared to be reflected in scale chemistry. Unfortunately natural log transformations of the data did not result in equal variances nor did they improve the distribution of residuals. Given the difficulties generally associated with transformations before regression analyses (Underwood 1997), I have presented both linear and curvilinear models for the Ba:Ca data.

Results

Juvenile spot collected from the experiment had standard lengths (SL) that ranged from 18.0-32.6 mm with an overall mean of 24.2 mm. Standard lengths were similar between the temperature groups (*t*- test; df = 21; $P = 0.055$; Table 1). While these values were on the threshold of being statistically significant, mean lengths for the 20 °C (SL = 24.7 ± 0.3 SE) and 25 °C (SL = 23.5 ± 0.5 SE) treatments were only 1 mm (or ~5%) different, suggesting that temperature had little measurable effect on fish growth. Growth rate differences did not, therefore, contribute significantly to variability in scale chemistry between temperature treatments.

Water chemistry

Results from a fixed factorial analysis of variance (ANOVA) showed all three element:Ca ratios increased significantly with spiking levels (ANOVA; df = 3, 15; P = 0.0001; Figure 1). Also, element:Ca ratios were not influenced by temperature (ANOVA; df = 1, 15; $P > 0.05$).

Trace element incorporation

All three element:Ca concentrations in the scales were significantly correlated with ambient levels in the tanks (Figure 2). Results from analysis of covariance (ANCOVA) showed temperature had no influence on the incorporation of Sr, Cd, or Ba (ANCOVA, df = 1, 20, $P > 0.05$), and was therefore dropped as a treatment effect in all subsequent statistical analyses.

Sr:Ca concentrations in scales ($[Sr:Ca]_{scale}$) were significantly correlated with tank concentrations ($[Sr:Ca]_{water}$), and described by the following least-squares regression equation ($r^2 = 0.81$, $P = 0.0001$):

$$[Sr:Ca]_{scale} = 0.16\ (\pm 0.02\ SE)\ [Sr:Ca]_{water} + 0.06\ (\pm 0.29\ SE).$$

The intercept was not significantly different from zero and constraining the line through the origin also resulted in a slope of 0.16. Despite higher variation within individual tanks, Cd:Ca ratios in scales were also significantly correlated with Cd:Ca levels in the ambient tank waters, albeit with a relatively low coefficient of variation. The least-squares linear regression equation for these data ($r^2 = 0.18$, $P = 0.0395$) was given by

$$[Cd:Ca]_{scale} = 0.44\ (\pm\ 0.20\ SE)\ [Cd:Ca]_{water} + 18\ (\pm\ 6\ SE).$$

The intercept of the line was significantly greater than zero, due, at least in part, to increased variability at low Cd:Ca values in both ambient water and scales.

Barium incorporation in scales was highly correlated with ambient Ba levels in the tanks. Least-squares regression described a linear relationship between $[Ba:Ca]_{water}$ and $[Ba:Ca]_{scale}$ ($r^2 = 0.91$, $P = 0.0001$) and was given by

$$[Ba:Ca]_{scale} = 0.20\ (\pm\ 0.01\ SE)\ [Ba:Ca]_{water} + 11\ (\pm\ 2\ SE).$$

As with the Cd data, the intercept of the regression line was significantly greater than zero. A curvilinear function also fit ($r^2 = 0.95$, $P = 0.0001$) and was given by

$$\log_e [Ba:Ca]_{scale} = 0.61\ (\pm\ 0.03\ SE)\ [Ba:Ca]_{water} + \log_e (0.69 \pm 0.14\ SE).$$

The fit of the curvilinear model ($r^2 = 0.95$) was only slightly better than the linear model ($r^2 = 0.91$) and did not improve the distributions of the model residuals. Therefore, for reasons of parsimony, only the linear function is presented graphically (Figure 2).

Comparison of scale and otolith chemistry

Finally, I correlated Sr:Ca and Ba:Ca ratios in scales with the same ratios quantified in otoliths from the same fish, after first averaging data within tanks. The Cd:Ca concentrations in many otoliths were below detection limits and therefore could not be compared to scale data. Both Sr:Ca and Ba:Ca ratios from scales were highly

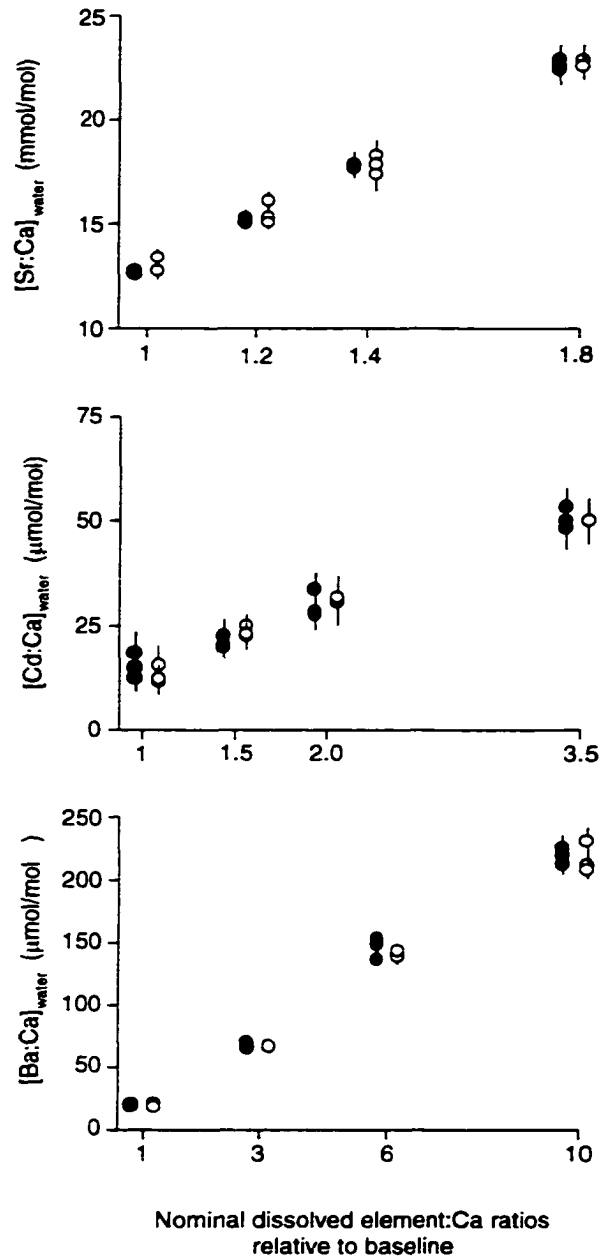

FIGURE 1. – The mean water element:Ca concentration of each tank plotted against the treatment concentration groups (± 1 SE) at 20°C (solid circles) and 25°C (open circles) for Sr:Ca, Cd:Ca, and Ba:Ca. The x-axis shows the four different concentration treatments represented as relative multiples of the baseline concentrations.

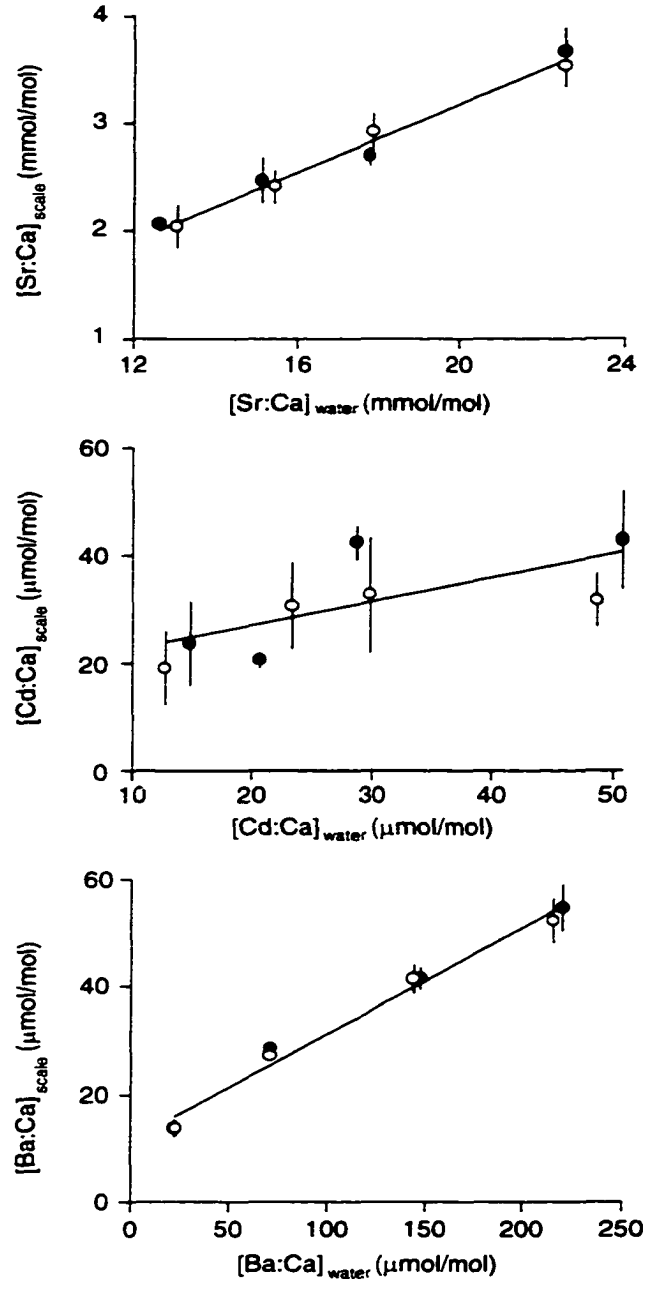

FIGURE 2. – Mean element:Ca concentrations in the scales from the eight treatment groups are plotted against actual element:Ca concentrations in the water (± 1 SE) at 20°C (solid circles) and 25°C (open circles) for Sr:Ca, Cd:Ca, and Ba:Ca. Lines represent the least-squares regressions.

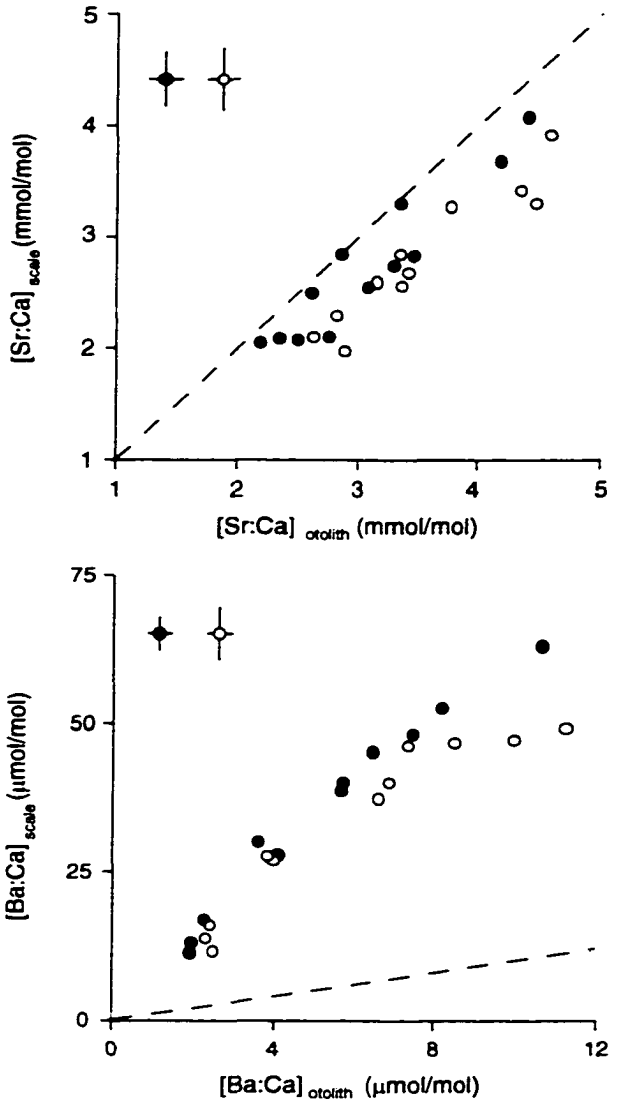

FIGURE 3. – A comparison of the average scale element:Ca concentration from each tank to the average otolith element:Ca concentration from the same tank at 20°C (solid circles) and 25°C (open circles) for Sr:Ca and Ba:Ca. The dashed line indicates a 1:1 relationship between scales and otoliths. The average SEs across all tanks within each temperature group is shown for otoliths and scales

correlated with values in otoliths (Figure 3), with correlation coefficients of 0.91 and 0.94 respectively. Sr:Ca values were of similar magnitude in scales and otoliths with otoliths having only slightly greater concentrations. In contrast, Ba:Ca ratios were approximately 6-fold higher in scales than in otoliths. Furthermore, the Ba:Ca relationship between scales and otoliths was somewhat curvilinear. This indicated that the uptake of Ba:Ca in scales may be non-linear, especially at the higher temperature treatment.

Discussion

I have shown that Sr:Ca, Cd:Ca, and Ba:Ca concentrations in spot scales reflect differences in elemental concentrations of the ambient environment. This, in turn, indicates that water chemistry is a major determinant of at least a subset of the elemental composition of scales. It may, therefore, be possible to reconstruct past environmental histories, and in particular, ambient dissolved Sr and Ba concentrations, of individual fish based on geochemical analyses of scales. For instance, movement of fish from marine (high Sr, low Ba) waters to estuarine (low Sr, high Ba) should be accurately recorded by scale chemistries (e.g. Coutant and Chen 1993). Similarly, these data strengthen our conclusion that geographic differences in scale geochemistry of juvenile weakfish (*Cynoscion regalis*) are a function of exposure to waters with different chemical characteristics (Wells et al. 2000b).

Temperature was not important in determining Sr:Ca, Cd:Ca, or Ba:Ca ratios in scales. While considerable debate continues over the influence of temperature on otolith chemistry (Campana 1999), there has been little discussion of the possible influences of temperature on scale chemistry. Bath et al. (2000) found that, unlike scales, Sr:Ca ratios

in otoliths from the same fish used in the present study were related to temperature. This dichotomy may help determine where the temperature effect in otolith Sr:Ca ratios is generated. Strontium from the ambient water that is subsequently deposited in scales and otoliths is transported to depositional sites by the blood plasma (Takagi et al. 1989; Kalish 1991). Therefore, any temperature-dependent fractionation between Sr and Ca cannot be occurring at the interface between either the branchial or intestinal membrane and the ambient water, or within the blood plasma. Fractionation at either of these locations would have resulted in a significant temperature effect on scale Sr:Ca ratios in our experiment because scales receive ions directly from the blood plasma (Takagi et al 1989). It follows that temperature affects Sr:Ca ratios in otoliths either by influencing the transfer of Sr across the endolymphatic membrane, or by determining the rate at which Sr ions substitute for Ca ions in the aragonite lattice. Nonetheless, reconstruction of Sr concentrations in waters based on scales rather than otoliths has an advantage because variations in Sr:Ca ratios can be attributed unambiguously to differences in water chemistry, and not temperature.

The slope of the relationship between $[Sr:Ca]_{scale}$ and $[Sr:Ca]_{water}$ provides an estimate of the partition coefficient (D_{Sr}) between ambient water and the scale, where $[Sr:Ca]_{scale} = D_{Sr}\ [Sr:Ca]_{water}$ with the intercept forced through the origin. I found a value of 0.16, which is similar to the value for otoliths from the same fish ($D_{Sr} \sim 0.19$, Bath et al. 2000). It may be wrong to conclude, however, that Sr has a comparable affinity for scale hydroxyapatite and otolith aragonite based on these data. Scale apatite incorporates elements directly from the blood plasma while otoliths incorporate elements from the endolymphatic fluid. Kalish (1991) noted that Sr:Ca concentrations are, in fact,

higher in blood plasma than in the endolymphatic fluid. If this were also the case with juvenile spot, it follows that Sr substitutes more easily for Ca in otolith aragonite than in scale apatite. Given results from a comparison of Sr:Ca values in scales and otoliths from field-caught weakfish which were strikingly similar to comparisons made here Wells et al. (2000b) came to the same conclusion.

A rough estimate of the Ba:Ca partition coefficient for scale apatite, based on the slope of the relationship between $[Ba:Ca]_{scale}$ and $[Ba:Ca]_{water}$, was 0.20. Surprisingly, this value is much larger than the otolith partition coefficient (~ 0.06, Bath et al. 2000). The absence of any data on Ba levels in the blood plasma or endolymphatic fluid of fish meant I could not determine if the increased affinity for Ba in scales compared to that for otoliths was occurring during uptake or transport in the blood plasma, or at the depositional surface. The relationship between $[Ba:Ca]_{scale}$ and $[Ba:Ca]_{water}$ was also noteworthy because the intercept of the relationship did not pass through the origin. This implies that Ba will be present in the scale even when there is no Ba in the water. Although such a result would obviously seem illogical in an inorganic system, fish scales contain significant quantities of protein along with hydroxyapatite, which may also be a source of Ba. The organic component of scales may have contributed the excess Ba, and I suspect that the regression line would have gone through the origin if the protein material had been removed before the chemical assays. The alternative hypothesis is that the relationship between $[Ba:Ca]_{scale}$ and $[Ba:Ca]_{water}$ is curvilinear rather than linear, since a curvilinear relationship would, by definition, pass through the origin. Although I presented the results of a linear regression for easier comparison among earlier studies, there was little difference between this relationship and a curvilinear least-squares

regression. Interestingly, the relationship between otolith and scale Ba:Ca ratios appeared to be approaching an asymptote, especially in the 25 °C tanks. However, a comparison of Ba:Ca values in scales and otoliths from weakfish caught at varied environments along the U.S. Atlantic coast showed no indication of being asymptotic through the data up to scale values of 300 μmol/mol (Wells et al. 2000b).

While the relationship between $[Cd:Ca]_{scale}$ and $[Cd:Ca]_{water}$ was not very precise, Cd:Ca ratios were similar in scales and water. This contrasts with otoliths, in which it is probably safe to assume that the partition coefficient is somewhat less than 0.0001 (Campana 1999). It is possible that the endolymphatic fluid does not accumulate Cd ions to the same extent as blood plasma – Campana (1999) reported that Cd concentrations in blood plasma may be 3000x that of the ambient environment. However, this would require that the endolymphatic membrane be able to actively discriminate against Cd ions when the branchial and intestinal membranes apparently cannot. Alternatively, Cd may substitute more easily for Ca in hydroxyapatite than in aragonite. Indeed, Cd:Ca in scales can be 35 - 55% that of Ba:Ca and 22-30% that of Sr:Ca (van Coillie and Rousseau 1974). The organic component of the scales may also have contributed to the high total Cd levels. This may, in turn, explain the variability in the relationship between $[Cd:Ca]_{scale}$ and $[Cd:Ca]_{water}$, since it is unlikely that the concentration of organically-bound Cd would be a function of water chemistry.

The results from this work are limited to nonessential elements that are able to substitute for Ca in the hydroxapatite matrix of scales. Our results will, in all likelihood, not apply to physiologically controlled elements (e.g. Na, K, Mg, Cl, P, Cu, and S; Campana 1999) as their levels in blood plasma is more a function of active regulation by

the fish than it is of environmental concentration. Interestingly, Mugiya et al. (1991) found that of ten elements examined (Al, Cd, Ba, Mn, Fe, Ni, Cu, Zn, Pb, and Sr) in scales and otoliths, only Sr and Ba were deposited in proportion to availability in the environment for both hardparts.

If scale geochemistry is to be used as a proxy for past environmental conditions, material in scales must remain unmodified after deposition. Although otoliths are generally considered to be metabolically inert after crystallization, few studies have examined the stability of geochemical signatures in scales. There is evidence that scales may cease to grow, or even be resorbed, during times of physiological stress (Bilton and Robins 1971; Bilton 1975). However, Yamada and Mulligan (1982) reported that induced Sr tags in scales remained constant for at least 3 years and suggested that, once Sr was incorporated into the scale, it became metabolically inert. Studies of other hydroxyapatite structures have suggested that such metabolic reworking may also be rare, at least for those elements commonly assayed in scales (Wells et al. 2000b). For instance, Rosenthal (1963) found that Sr in fin rays had a biological half-life of at least 2.5 years. More recently, Veinott and Evans (1999) noted minor and trace element measurements (Mg, Mn, Sr, and Ba) of fin rays made 2 years apart remained highly correlated (r values for all elements were ≥ 0.88). Thus while more research is clearly needed on the stability of scale chemistry, it appears that the half-lives of at least some elements in scales may approach those of the same elements in otoliths.

The use of scales to identify elemental signatures offers certain advantages and disadvantages over using elemental signatures in otoliths. Scale analysis offers a non-lethal alternative to otoliths, and scales are generally easy to collect, archive and prepare

for analysis. However, scales are not present on fish during most of the larval period, and therefore are not suited to questions of larval dispersal (e.g. Swearer et al. 1999). Scales are typically more common in sediment cores and institutional collections than otoliths. Further, time periods represented by circuli on the scale can be assayed without grinding because each circulus is exposed throughout a fish's life. This, in turn, eliminates the possibility of contamination from the grinding material.. Although elemental concentrations in scales were generally higher than otoliths, I found that elemental concentrations in otoliths were more tightly correlated with ambient water chemistry than were scales (Bath et al. 2000). Finally, the observations that otoliths are metabolically inert, even under extreme stress (Campana and Neilson 1985), suggests that otoliths will remain the structure of choice for elemental and isotopic analyses when available. However, providing elemental signatures remain sufficiently stable throughout the fish's life, scales may offer a non-lethal alternative for determining retrospective information of environmental conditions experienced by individual fish. This information could then be used as natural tags of population structure, habitat use, and migration pathways, in a similar way that such information is currently used in otolith studies.

CHAPTER III

GEOGRAPHIC VARIATION IN TRACE ELEMENT COMPOSITION OF JUVENILE WEAKFISH SCALES

Introduction

Description of past environments experienced by fish through chemical analyses of scales has a long history in fisheries ecology. Early work by Bagenal et al. (1973) used Sr:Ca ratios in scales as a tag of marine residency in brown trout (*Salmo trutta*). Belanger et al. (1987) showed that poached freshwater striped bass (*Morone saxatilis*) could be identified in the commercial catch by using Sr levels in scales. van Coillie and Rousseau (1974) demonstrated that in polluted waters excess metals replaced Ca in the scales, and were able to differentiate elemental signatures in scales of fish from two rivers (one polluted and the other not). Yamada and Mulligan (1982, 1987) observed a Sr mark in the core of scales from returning coho salmon (*Oncorhynchus kisutch*) that were tagged while still at the hatchery, thus indicating the elemental tag was identifiable even after maturation and migration. Coutant and Chen (1993) examined Sr spikes at the primordia of young striped bass scales and suggested that increased Sr levels indicated marine parentage or estuarine rearing. These authors suggested that more research using loci-specific analyses of trace elements in scales appeared to be warranted based on these results.

Recently, the development of new analytical instrumentation has enhanced the information potentially available from calcified structures such as scales. Probe-based

techniques such as proton-induced x-ray emission (PIXE) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) offer the ability to sample specific parts of individual scales with limits of detection less than 100 μg/g (Campana et al. 1997). In particular, LA-ICP-MS has been used for elemental analysis of otoliths to distinguish stock structure and understand life history (Campana et al. 1994; Fowler et al. 1995a, 1995b; Geffen et al. 1998), and may have similar applicability to scales. The technique is useful for spatial analysis of trace elements as it combines the low detection limits (0.1-0.01 μg/g ; Houk 1986), wide dynamic range (ng/g to %), and isotopic discrimination (Henshaw et al. 1989; Colodner et al. 1994) of conventional ICP-MS with spatial resolution of < 10 μm (Wang et al. 1994).

The objective of the present study was to quantify trace element signatures in scales from juvenile weakfish, *Cynoscion regalis*, along the Atlantic coast of the United States. I was interested primarily in these signatures as natural tags of the natal estuary of juvenile weakfish. Weakfish have a life history that is well suited for promoting differences in elemental signatures in the scale along a geographic range. Adults follow a seasonal migration moving south and offshore during the fall and winter, and north and inshore during the spring and summer spawning season (Nesbit 1954; Wilk 1979). Adults spawn along their entire range in estuarine and near-shore waters in the same areas used as larval and juvenile nursery grounds. The larvae and juveniles then remain in their respective nursery areas through spring and summer by using selective tidal stream transport (Rowe and Epifanio 1994), thus reducing any mixing among estuaries. In the fall juveniles are believed to migrate from estuarine waters to overwintering grounds off the North Carolina coast (Wilk 1979). Therefore, trace element signatures in

scales may be used to identify the estuarine nursery areas and hence to quantify the contribution of each estuary to the adult fishery.

Methods

An otter trawl was used to collect weakfish from five estuarine systems from Georgia to New York (Figure 4) in 1996 and 1997. Multiple sites within each estuary were sampled to quantify within-estuary variation in elemental signatures of the scale. The sites were chosen to best represent the estuary and sample sizes were approximately equal between sites within the estuaries. All fish were stored on ice in the field and were frozen upon arrival in the lab. Otoliths were extracted from each fish for chemical analysis (1996 data is presented in Thorrold et al. 1998; 1997 data is unpublished) before scales were removed. Sagittal otoliths were removed with glass probes, cleaned with Milli-Q water, ultrasonically cleaned for 5 min, rinsed in ultra-pure hydrogen peroxide, triple rinsed in Milli-Q water, and air dried. Whole otoliths were then dissolved in ultrapure nitric acid for subsequent determination of element:Ca ratios using isotope dilution ICP-MS.

Using glass probes scales were taken, whenever possible, from the caudal region and were cleaned of organic material and contaminants in a class-100 clean room. Following three to five minutes of sonicating in 3% ultra-pure hydrogen peroxide, scales went through two rounds of cleaning with an electric rotary toothbrush and glass probes. Cleaned scales were dried with the fibrillary plate down on glass slides. Once scales adhered to the slide they were rinsed with Milli-Q water, dried again, and stored on slides in conical vials. Scales were removed from slides and placed on mounting tape for laser

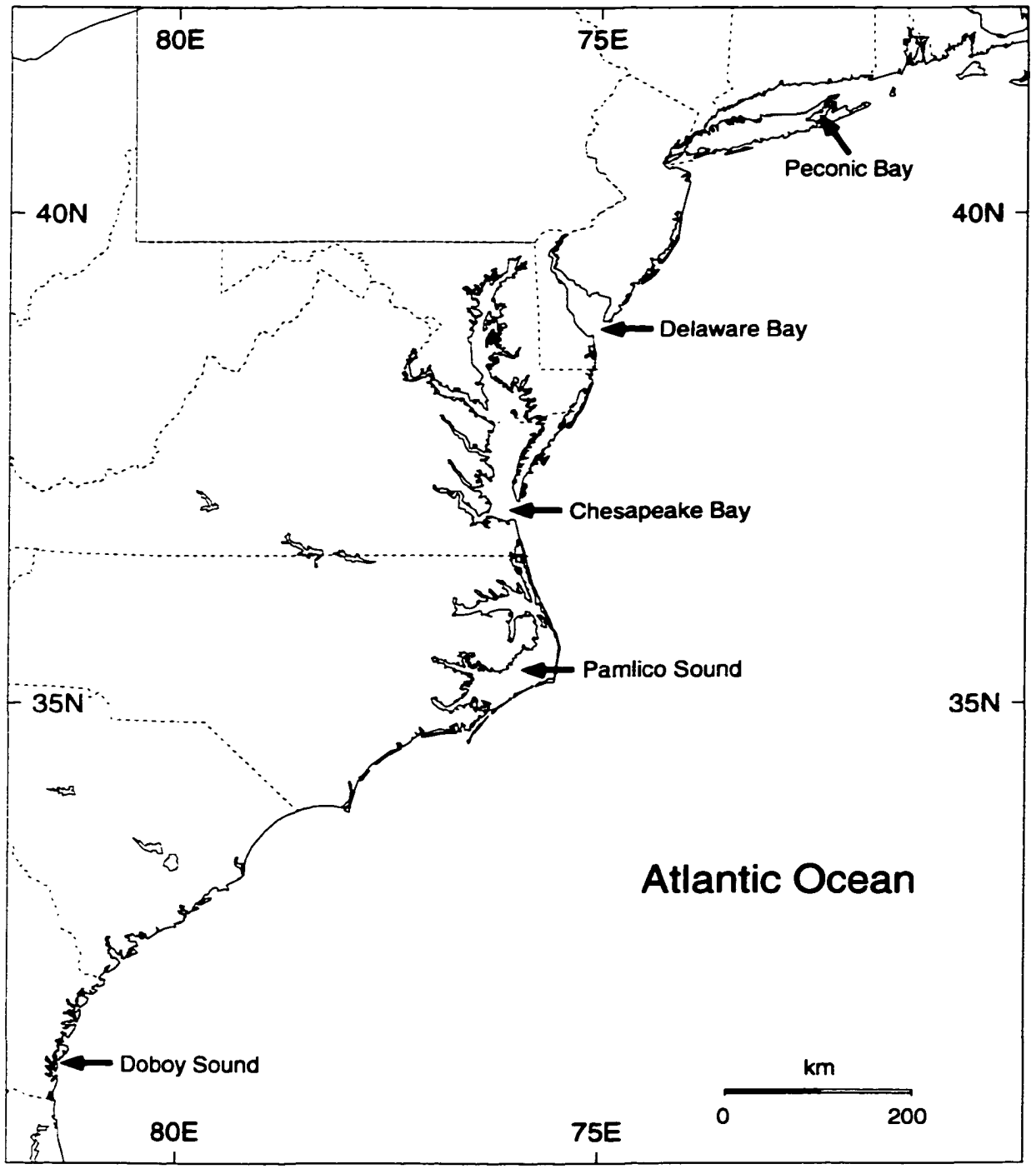

FIGURE 4. – Five estuaries on the Atlantic Coast were sampled for juvenile weakfish, *Cynoscion regalis*: Doboy Sound, Pamlico Sound, Chesapeake Bay, Delaware Bay, and Peconic Bay during July to September 1996 and August to October 1997.

ablation. All equipment that came directly or indirectly in contact with the scales was acid washed.

A preliminary analysis indicated that sonicating scales in 3% ultra-pure hydrogen peroxide (adapted from Whaley 1991) reduced the variability in trace element concentrations in the scales. Scales from six fish were cleaned with two methods. One method included sonicating the scale in 3% hydrogen peroxide followed by two rounds of cleaning and the alternative method included two rounds of cleaning without sonicating the scale. Clean scales were sampled along a transect from the core to the anterior margin and values from the transects were averaged. Trace element concentrations of sonicated scales were not significantly different than those which were not sonicated (paired *t*-test, $P > 0.05$), but B:Ca, Mg:Ca, Mn:Ca, Zn:Ca, Sn:Ca, Ba:Ca and Pb:Ca ratios in scales which were sonicated had on average 52% lower standard errors. Additionally, mean counts of B:Ca, Mg:Ca, Mn:Ca, Sn:Ca, Ba:Ca, and Pb:Ca were 22% lower for sonicated scales. I attribute the reduced variance and mean element:Ca counts to a more consistent and efficient removal of organic debris from the scale.

Scale analysis

LA-ICP-MS was used to analyze one scale each from 146 fish in 1996 and 125 fish in 1997. LA-ICP-MS operating conditions (Table 3) were similar to those in Thorrold et al. (1997). To account for instrument drift I assayed NIST 612 standard one to three times during each day of scale ablation. Scales were sampled in blocks of five with one randomly chosen fish from each estuary. Ten isotopes were counted in 1996 (^{11}B, ^{25}Mg, ^{48}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{112}Cd, ^{138}Ba, and ^{208}Pb) of which seven were

TABLE 3. – Operating conditions of the laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) system used for analysis of weakfish scales.

ICP-MS	
Acquisition mode	Scan
Acquisition time	60s
Coolant gas flow rate	1.4 l/min
Auxiliary gas flow rate	1.4 l/min
Carrier gas flow rate	1.6 l/min
Dwell time	320 ms
Channels/amu	20
Mass range	6 – 208
Laser probe	
Laser type	Nd: YAG pulse
Laser mode	Q-switched
Flashlamp voltage	500 – 520 V
Frequency	266 nm
Repetition rate	2 Hz
Beam size	~ 10 μm
Crater size	~ 50 μm
Focus condition	Focus

above detection limits (^{25}Mg, ^{48}Ca, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, and ^{208}Pb). In 1997 five isotopes were counted (^{25}Mg, ^{48}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba). Both ^{66}Zn and ^{208}Pb were dropped from the 1997 analyses due to high variances in 1996 samples. Limits of detection were calculated as mean blank values plus three standard deviations ($N = 333$). Isotopic counts were converted to elemental intensities by multiplying percent natural occurrence of the isotope. All data were standardized to Ca to account for variability in laser energy and the weight of ablated material. Finally, standardized values were converted to molar ratios based on repeated measurements of NIST 612 glass that were made throughout the scale analysis. Certified values of each of the elements in the glass were used to calibrate intensities measured by the ICP-MS during the course of the assays.

An area of approximately 0.3 mm^2 was rastered on each scale. The laser ablated material from both the osseous layer and the fibrillary plate, starting one to five circuli anterior to the primordia and covering approximately 20 – 30 days of growth toward the anterior edge of the scale. Because first scale growth begins at about 26 days old in juvenile weakfish (Szedlmayer et al.1991), the average raster covered the a region that corresponded to ages 27-57 days.

To examine elemental variation across scales, five positions on six scales were analyzed with the laser from the core to the anterior margin. Three fish (approximately 55 days old) were used from both Georgia and Chesapeake Bay to ensure that any trends could not be attributed to site-specific environmental variation. Seven isotopes were quantified (^{25}Mg, ^{48}Ca, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, and ^{208}Pb) and then converted to

elemental counts by multiplying by the percentage of natural abundance. Counts were then standardized to Ca.

Data analysis

A mixed model analysis of variance (ANOVA) was used to test for statistically significant differences in trace element concentrations among and within estuaries, and between years. Within-estuary differences were accounted for by nesting sites (random term) within estuarine locations (fixed term). Residual analysis was used to test for assumptions of homogeneity of variances and normal distribution of errors (Winer 1971). To meet these assumptions it was necessary to log*e* transform Ba:Ca and Mn:Ca ratios. Additionally, the elements were entered into a MANOVA and canonical discriminant analysis (CDA, CANDISC procedure of SAS) to characterize the multivariate elemental signatures of the scales. I assumed that the compliance of the univariate data with ANOVA assumptions meant that the MANOVA assumptions (equal variance-covariance matrices and multivariate normality of errors) were also met. Bootstrapped 95% confidence ellipses (1000 iterations) were calculated around the class means of the first two canonical variates to determine where the significant differences among estuaries detected by MANOVA were located. Finally, linear discriminant function analysis (LDFA, DISCRIM procedure of SAS) was used to quantify the accuracy of classification of individuals to their nursery grounds based on the elemental signatures of the scales. The cross-validation algorithm in the SAS Inst. (1989) DISCRIM procedure, which uses a jack-knife technique, was used to determine classification accuracy.

Results

Juvenile weakfish collected throughout the sampling locations ranged in size from approximately 30 to 125 mm SL (Table 4). There were no significant differences in standard length among estuaries, although there were significant differences among sites within estuaries (Table 5). Regression analysis found no significant relationship between any element:Ca ratios and standard length in either 1996 or 1997. Finally, I examined patterns of variation in the elemental composition along transects from the center to the edge of scales from Doboy Sound and Chesapeake Bay. A repeated measures ANOVA showed no significant trends in Mg:Ca, Ba:Ca or Sr:Ca across the transect from the scale center to the anterior margin (ANOVA; df = 4,1; $P > 0.10$ for each variable). However, Mn:Ca varied significantly across the transect (ANOVA; df = 4, 1; Transect Location $P = 0.0013$). Scales from Doboy Sound fish had higher Mn:Ca concentrations at the second transect position from the center (approximately 35 days old) while Chesapeake Bay scales showed no trend (ANOVA; df = 4,1; Transect Location*Estuary $P = 0.0019$).

Trace element analysis

Analysis of variance found significant differences in Mg:Ca and Mn:Ca ratios among sites within estuaries, among estuaries, and between years (Figure 5; Table 6). The estuary*year interaction was non-significant for both elements. Unplanned comparisons among estuaries using Duncan's multiple range test found that these differences were relatively subtle (Table 6). In fact, the more conservative Tukey's test results showed no separation between estuaries for Mg:Ca. The ANOVA for both Sr:Ca and Ba:Ca ratios found significant differences among estuaries and between years. In

TABLE 4. – Sample collection dates, sample sizes, mean standard lengths (SL), and standard errors (SE) for juvenile weakfish in 1996 and 1997.

Estuary	Date Collected	*N*	Mean SL	SE
Doboy Sound	July 16–17,1996	30	79.1	3.80
	Aug. 18, 1997	22	64.7	2.85
Pamlico Sound	July 30–Aug.1,1996	28	68.2	4.20
	Aug. 20-21, 1997	29	59.9	3.12
Chesapeake Bay	July 10, 1996	29	60.2	2.20
	Aug 6 - Sept. 10, 1997	27	65.6	1.75
Delaware Bay	Sept. 20, 1996	29	82.3	4.51
	Oct. 14 - 31, 1997	28	66.6	4.17
Peconic Bay	July 8, 1996	30	67.6	1.63
	Aug 11-25, 1997	19	55.7	3.82

TABLE 5. – Analysis of variance table demonstrating that there is no significant difference in standard length between the samples from the five estuaries. Note that the denominator for the estuary *F* approximation is the site(estuary) term, whereas the error term is the denominator in all other *F* approximations.

Source	Mean Sq.	*F*	Num. df	*P*
Year	5098.52	17.88	1	0.0001
Estuary	2368.43	2.07	4	0.1775
Year*estuary	993.81	3.48	4	0.0086
Site(estuary)	1146.22	4.02	8	0.0002

both cases the estuary*year interaction was also significant, indicating that the two effects were not independent. A plot of mean values for each estuary in 1996 and 1997 showed, however, that the patterns among estuaries were reasonably consistent between years for both elements (Figure 6). Pamlico Sound showed the lowest Sr:Ca ratios in both years, with little difference between the other four estuaries.

Analysis of the multivariate trace element signatures generally confirmed the results from the individual ANOVA (Table 7). The presence of a significant year*estuary interaction meant, however, that further analyses of the multivariate signatures had to be conducted separately for each year. Differences among estuaries were visualized using canonical variate analysis (CDA). For 1996, bootstrapped 95% confidence ellipses around class means on the first two canonical variates overlapped for Doboy Sound and Delaware Bay but not for the remaining three estuaries (Figure 7). Confidence ellipses for all five estuaries were clearly separated in 1997. Although, as noted earlier, a significant estuary*year interaction was present in the MANOVA, the results of the CDA analysis for the two years were quite similar. In 1996, Peconic Bay and Pamlico Sound separated from the other estuaries on the first canonical variate (CV1) and from each other on the second canonical variate (CV2). Also in 1996, Doboy Sound, Chesapeake Bay, and Delaware Bay formed a grouping with little separation on CV1 but Chesapeake Bay separated from the other two on CV2. In 1997, Pamlico Sound separated from all the other estuaries on CV1. Peconic Bay and Chesapeake Bay

TABLE 6. – Individual analysis of variance (ANOVA) tables for each element quantified in juvenile weakfish scales from estuaries along the U.S. Atlantic coast that was used in the multiple ANOVA. Note that the denominator for the estuary *F* approximation is the site(estuary) term, whereas the error term in the denominator in all other *F* approximations. Unplanned Duncan's multiple-range tests (DMR) were used to determine significant estuary differences for Mg:Ca and Mn:Ca concentrations. the error term for all significance tests was the site(estuary) error term. The multiple-range test was done using log-transformed Mn:Ca values, as was the case for ANOVA; however, actual mean values are shown in table results, and log-transformed values are in parentheses. Means for both Mg:Ca and Mn:Ca concentrations are in mmol/mol. Estuary abbreviations are as follows: DS, Doboy Sound; PA, Pamlico Sound; CB, Chesapeake Bay; DE, Delaware Bay; and GP, Peconic Bay. Values for mean concentration without letter in common are significantly different ($P < 0.05$).

Element:Ca	Source	Mean Square	*F*	Numerator df	*P*
Mg:Ca	Year	25996.45	60.74	1	0.0001
	Estuary	4910.48	4.74	4	0.0296
	Year*estuary	892.42	2.09	4	0.0832
	Site(estuary)	1036.47	2.42	8	0.0155
DMR	DE	GP	DS	CB	PA
Mean Conc.	85.25 z	84.17 z	78.86 zy	71.42 zy	67.03 y
Mn:Ca	Year	25.10	113.30	1	0.0001
	Estuary	9.1388	8.11	4	0.0064
	Year*estuary	0.4403	1.99	4	0.0969
	Site(estuary)	1.1263	5.08	8	0.0001
DMR	GP	PA	CB	DE	DS
Mean Conc.	1.62 z	1.20 zy	1.13 zy	0.76 yx	0.50 x
	(0.37)	(-0.03)	(-0.09)	(-0.44)	(-0.84)
Sr:Ca	Year	0.5307	11.54	1	0.0008
	estuary	1.4327	6.76	4	0.0111
	Year*estuary	0.1310	2.85	4	0.0245
	Site(estuary)	0.2120	4.61	8	0.0001
Ba:Ca	Year	0.8323	3.58	1	0.0597
	Estuary	10.8311	17.92	4	0.0005
	Year*estuary	1.5042	6.46	4	0.0001
	Site(estuary)	0.6044	2.60	8	0.0096

TABLE 7. – Results from multivariate analysis of variance of elements:Ca quantified in juvenile weakfish scales from estuaries along the U.S. Atlantic coast; Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca were dependent variables. All significance tests used Pillai's trace statistic. Note, that the denominator for the estuary *F* approximation is the site(estuary) term, whereas the error term is the denominator in all other *F* approximations.

Source	Value	*F*	Numerator df	Denominator df	*P*
Year	0.4890	59.81	4	250	0.0001
Estuary	2.6576	3.96	16	32	0.0005
Year*estuary	0.2502	4.22	16	1012	0.0001
Site(estuary)	0.4104	3.62	32	1012	0.0001

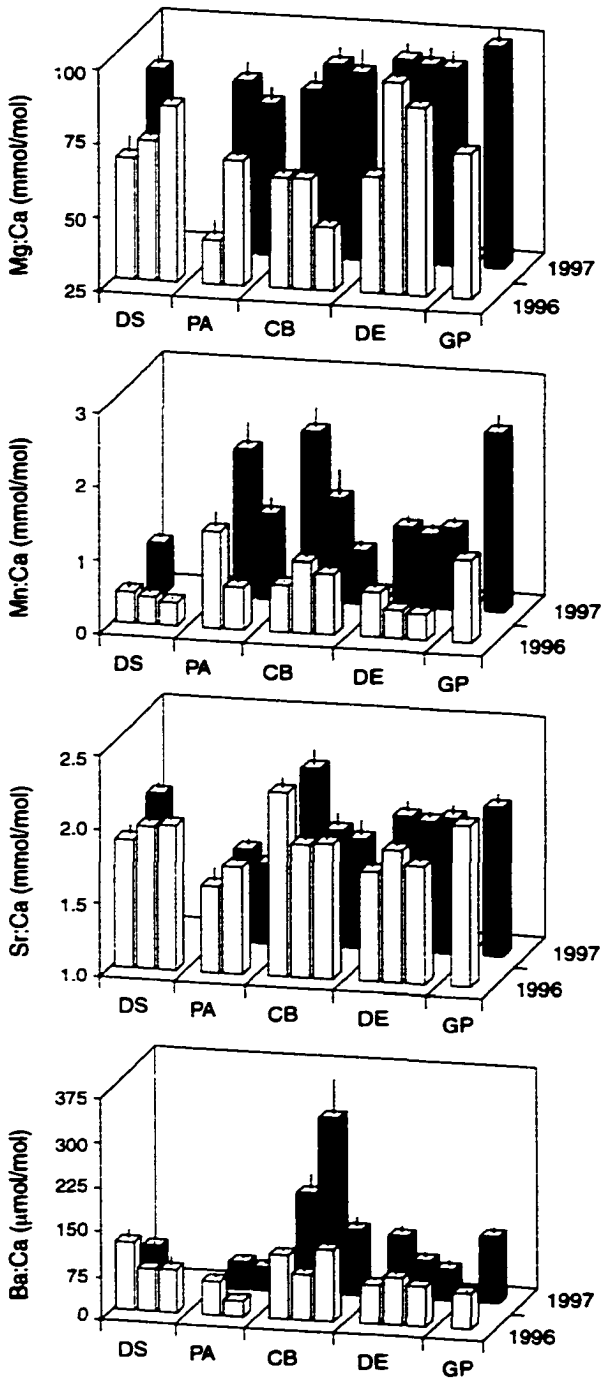

FIGURE 5. – Mean concentrations of four element:Ca ratios (+1 SE) for sites within Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP) in 1996 and 1997.

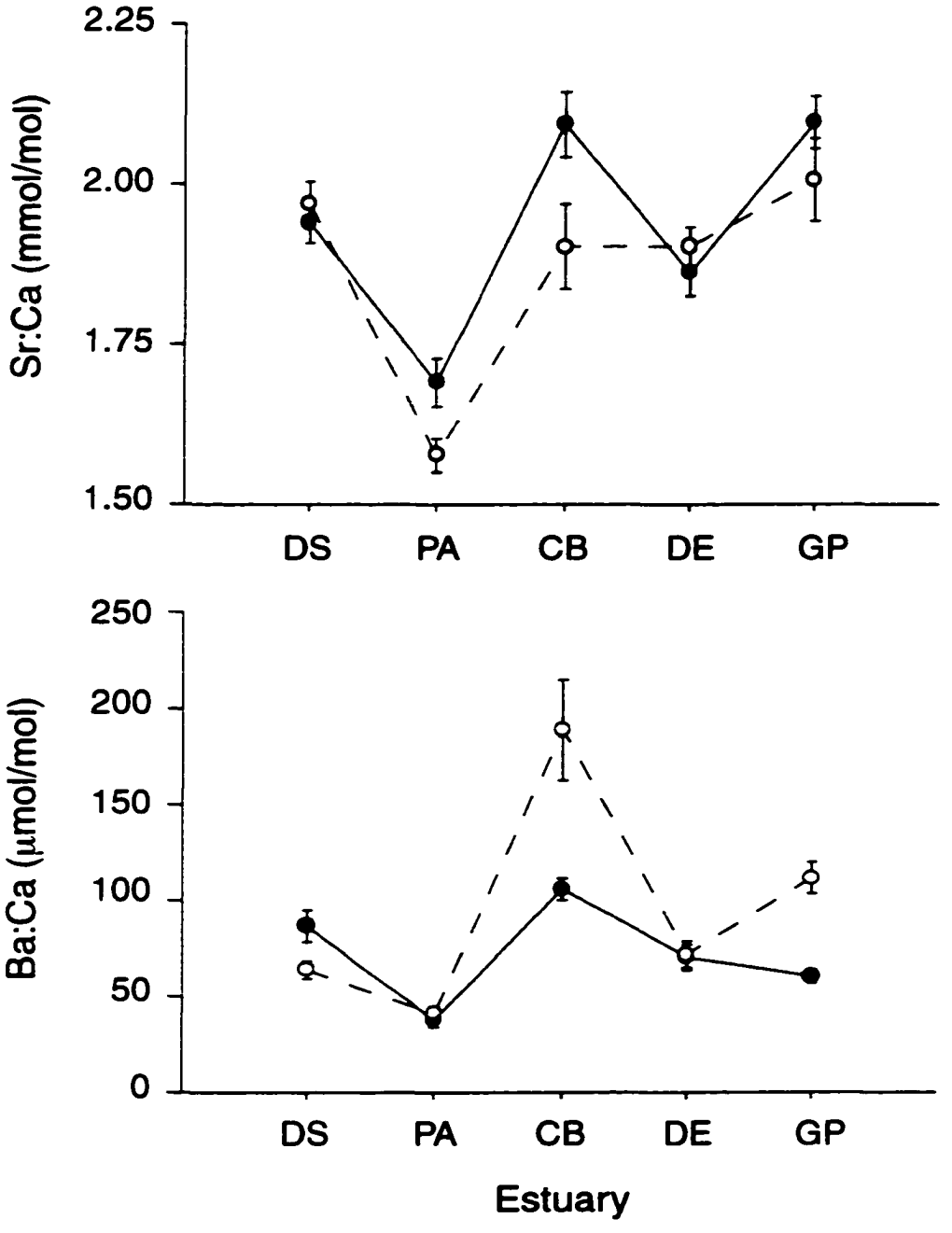

FIGURE 6. – Mean concentrations of Sr:Ca and Ba:Ca (±1 SE) for Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP) in 1996 (solid circles) and 1997 (open circles).

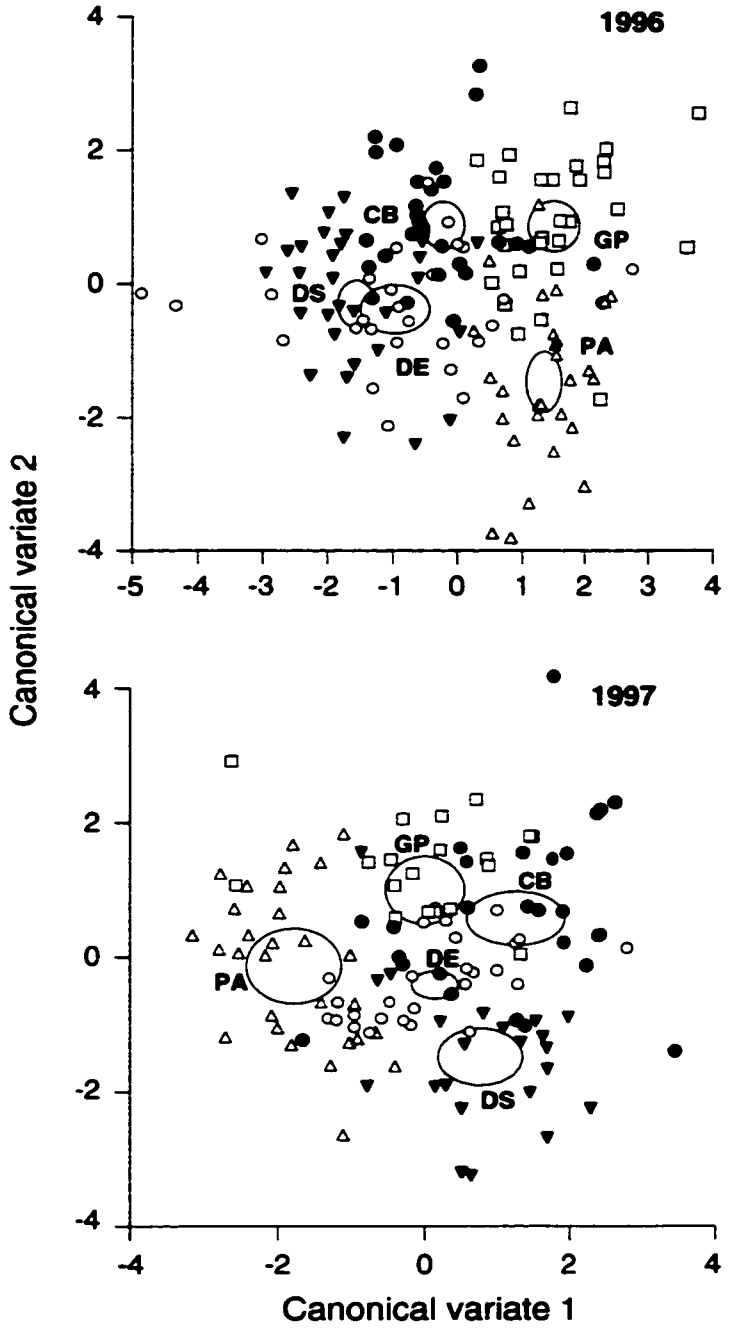

FIGURE 7. – Plot of canonical variates one and two, summarizing multivariate separation of scale elemental signatures from juvenile *Cynoscion regalis* from Peconic Bay (open squares), Delaware Bay (open circles), Chesapeake Bay (filled circles), Pamlico Sound (open triangles) Doboy Sound (filled inverse triangles) in 1996 and 1997. Ellipses represent bootstrapped 95% confidence areas around estuary means for each variate.

separated from one another slightly but significantly on CV1. Delaware Bay and Doboy Sound separated each other and from Peconic Bay and Chesapeake Bay on CV2. Canonical coefficients from CV1 in 1996 contrasted Mn:Ca concentrations (positive value) with Ba:Ca (negative value) and contrasted Mn:Ca concentrations (negative value) with Ba:Ca and Sr:Ca concentrations (positive value) in 1997. In both years canonical coefficients from CV2 were positive for each of the four elements, indicating that all four variables increased positively along this axis.

Linear discriminant function analysis (LDFA) was used to determine if the differences in elemental signatures among estuaries were sufficient to classify individuals accurately to their respective natal estuaries. In 1996 classification accuracy using the cross-validation approach ranged from 38%, for fish from Delaware Bay, to 86%. for individuals from Pamlico Sound, with a total accuracy of 67% (Table 8). In 1997 classification accuracy using the cross-validation approach ranged from 41%, for fish from Chesapeake Bay, to 83% for individuals from Pamlico Sound, with a total accuracy of 65% (Table 8). Finally, LDFA was used to determine if trace element signatures from one year were sufficient to classify accurately individuals from the other year to their respective natal estuaries. The LDFA from 1996 accurately classified approximately 46% of 1997 fish, but only 34% of the fish from 1996 were accurately classified using 1997 fish (Table 9). Classification accuracy ranged from 11% for Doboy Sound to 95% for Peconic Bay using the 1996 to classify 1997 fish and from 7% for Chesapeake Bay and Peconic Bay to 93% for Doboy Sound using the 1997 to classify 1996 fish (Table 9).

TABLE 8. – Results of linear discriminant function analysis (LDFA) for classifying juvenile weakfish to natal estuary based on trace element signatures in scales. Values indicate cross-validation accuracy (%) with Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca as dependent variables. Samples came from Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE) and Peconic Bay (GP).

Sample source	Procedure	DS	PA	CB	DE	GP
		1996 cross-validation accuracy (%)				
DS (N = 30)	LDFA	**67**	3	10	20	0
PA (N = 28)	LDFA	0	**86**	4	0	11
CB (N = 29)	LDFA	10	3	**72**	3	10
DE (N = 29)	LDFA	31	14	14	**38**	3
GP (N = 30)	LDFA	0	10	13	3	**73**
		1997 cross-validation accuracy (%)				
DS (N = 22)	LDFA	**73**	0	0	22	5
PA (N = 29)	LDFA	7	**83**	0	7	3
CB (N = 27)	LDFA	11	4	**41**	19	26
DE (N = 28)	LDFA	4	14	29	**54**	0
GP (N = 19)	LDFA	0	11	11	6	**72**

TABLE 9. – Results of LDFA analysis classifying juvenile weakfish to natal estuary based on trace element signatures in scales. A model parameterized with data from 1996 was used to classify fish collected in 1997, and a model parameterized with data from 1997 was used to classify fish collected in 1996. Values indicate accuracy (%) with Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca as dependent variables. Samples came from Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE) and Peconic Bay (GP). Values in bold type indicate correct allocations.

		Classified (%)				
Sample source	Model year (Classified year)	DS	PA	CB	DE	GP
DS	96(97)	**11**	7	21	29	32
	97(96)	**93**	0	7	0	0
PA	96(97)	0	**66**	0	0	34
	97(96)	32	**61**	0	7	0
CB	96(97)	0	4	**36**	32	28
	97(96)	83	7	**7**	0	3
DE	96(97)	0	3	7	**23**	67
	97(96)	83	3	14	**0**	0
GP	96(97)	0	5	0	10	**95**
	97(96)	63	7	0	23	**7**

Trace element concentrations in scales and otoliths were compared by averaging trace element data from fish within a site, and then calculating Pearson's correlation coefficients between these mean site values. Scales generally had higher levels of trace elements than otoliths, although the magnitude of the difference was element-specific. For instance, Ba:Ca ratios in scales were approximately three times higher than in otoliths, while Mg:Ca ratios in scales were over two orders of magnitude higher than in otoliths (Figure 8). The one exception to this trend was Sr:Ca ratios, which were slightly higher in otoliths than in scales. Despite large differences in magnitude, data from scales and otoliths were highly correlated for three of the four elements. Correlation coefficients for Mn:Ca ($r = 0.66$, $N = 22$, $P = 0.0009$), Sr:Ca ($r = 0.72$, $N = 22$, $P = 0.0002$), and Ba:Ca ratios ($r = 0.89$, $N = 22$, $P = 0.0001$) were all highly significant. There was no apparent relationship between scales and otoliths for Mg:Ca ratios among years – indeed, there was evidence that the two were negatively correlated in 1996 ($r = -0.82$, $N = 12$, $P = 0.0011$), although in 1997 the data were positively, although not significantly, correlated ($r = 0.42$, $N = 10$, $P = 0.2241$). Correlations between elemental concentrations in scales and otoliths of individual fish were also significant. Correlation coefficients for Mn:Ca ($r = 0.55$, $N = 253$, $P = 0.0001$), Sr:Ca ($r = 0.59$, $N = 253$, $P = 0.0001$), and Ba:Ca ratios ($r = 0.72$, $N = 253$, $P = 0.0001$) were all highly significant. In 1996 Mg:Ca ratios between scales and otoliths was negatively correlated ($r = -0.39$, $N = 134$, $P = 0.0001$) and in 1997 they were no significant relationship ($r = -0.01$, $N = 119$, $P = 0.8728$). Because different time periods were sampled for scales and otoliths, with

scales representing a shorter duration, correlation coefficients calculated for individual fish were lower than when element concentrations were averaged over sites.

Discussion

Trace element signatures in scales of juvenile weakfish showed significant variability within and among estuaries, and between years. Further, despite the among-site variation, signatures were sufficiently distinct to allow individual fish to be classified to natal estuary with approximately 65-67% accuracy. Although error rates are probably too high for adults to be classified to estuarine nursery areas individually, this level of accuracy is often sufficient to allow the estimation of stock proportions in mixed populations. For instance, Beacham et al. (1996) demonstrated that stock composition could be accurately estimated with an individual classification accuracy of 65% using maximum likelihood techniques (e.g. Fournier et al. 1984). A significant disadvantage of the scale chemistry technique is that, unlike genetic approaches, significant inter-annual variability in the trace element signatures implies that a continual monitoring program from year to year would be necessary to examine multiple cohorts in a population.

Chemical analyses of calcified structures in fish have concentrated recently, if not historically, on otoliths (e.g. Campana et al. 1997; Thorrold et al. 1998). In general, however, trace element concentrations are higher in biogenic apatites such as bone (Hamada et al. 1995), opercula (Moreau et al. 1983) and scales (Moreau et al. 1983; Pender and Griffin 1996) than in biogenic aragonite such as otoliths (Campana and Gagné 1994). In this study, I found that three of the four elements assayed (Mg, Mn and Ba) had considerably higher concentrations in scales than otoliths. Trace element

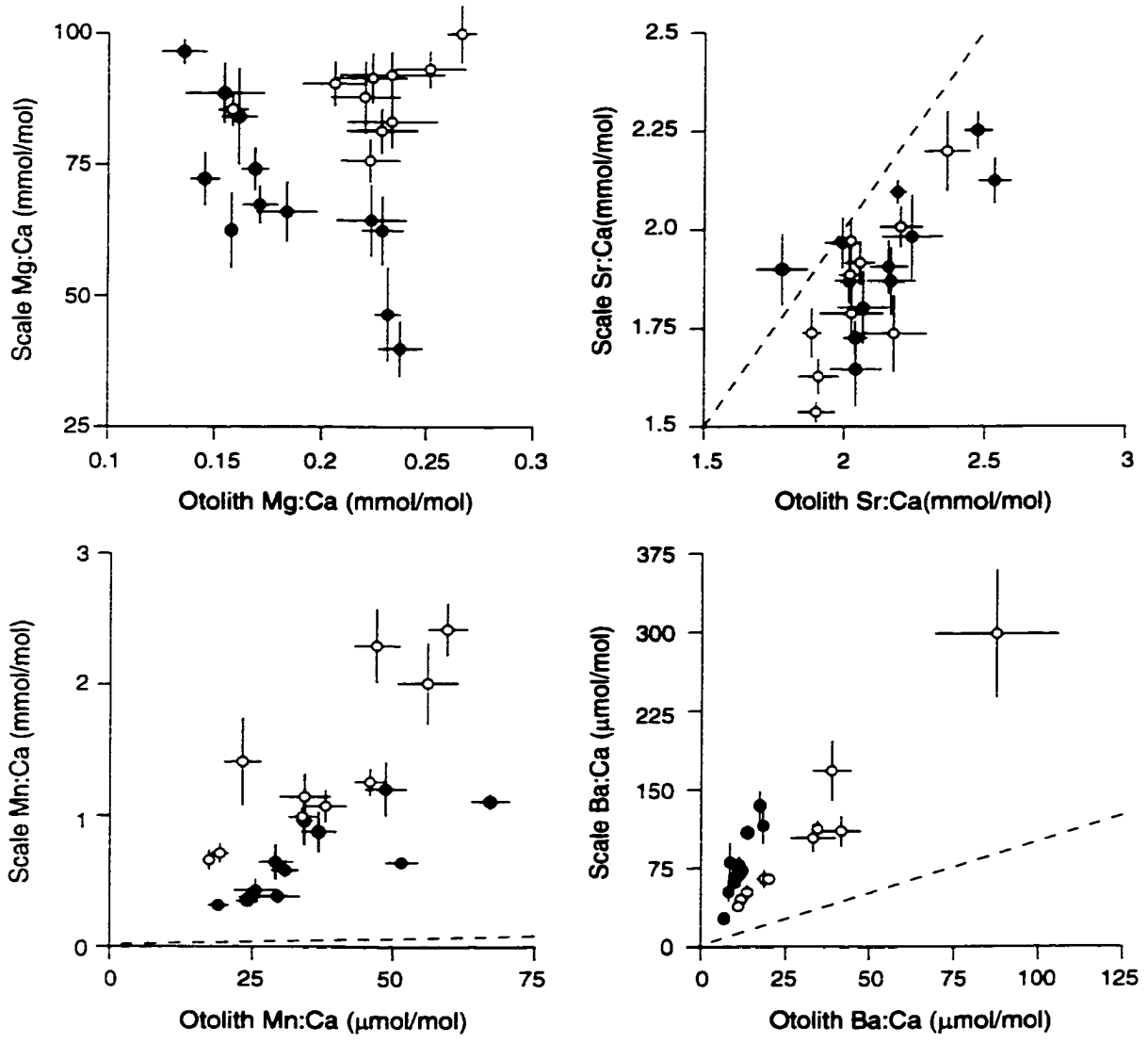

FIGURE 8. – A comparison of mean scale element:Ca concentrations to mean otolith concentrations (±1 SE) for each site sampled in 1996 (solid circles) and 1997 (open circles). The dashed line indicates 1:1 relationship between otoliths and scales.

concentrations in biogenic hydroxyapatite can, however, vary greatly. For instance, Mn concentrations in tooth enamel and mammalian and avian bone range from far below scale and otolith concentrations (Losee et al. 1974; Hamada et al. 1995) to double the concentration in otoliths (Lane and Peach 1997). More importantly in the context of the present study, synthetic and biological apatite trace element concentrations are positively correlated to the environmental concentrations in which they crystallize (Legeros et al. 1977). Trace element concentrations in the scale apatite, therefore, reflect those in the blood plasma (Takagi et al. 1989; Bereiter-Hahn and Zylberberg 1993). Otolith aragonite is precipitated from endolymphatic fluid in the sacculus which also receives Ca and other elements from the blood plasma (Kalish 1991). The high correlations between Mn:Ca, Sr:Ca and Ba:Ca ratios in scales and otoliths suggest that both are recording changes in blood plasma chemistry similarly, albeit at different absolute levels. The degree to which the blood plasma tracks changes in the trace element chemistry of the environment will, in turn, determine the degree to which trace element chemistry in either scales or otoliths is reflective of environmental conditions experienced by individual fish.

The Sr:Ca ratios in scales were comparable to levels in otoliths. Sr substitutes for Ca in apatite and aragonite in an amount relative to the concentration of the environment in which crystallization occurs (Legeros et al. 1977; Kalish 1991). Scale apatite incorporates elements from the blood plasma while otoliths incorporate elements from the endolymphatice fluid. Given that Sr:Ca concentrations are higher in the plasma than in the endolymph (Kalish 1991) it follows that Sr must substitute for Ca more easily in otolith aragonite than in scale apatite. In all, these data suggest that Sr incorporation into

scales and otoliths is in proportion to levels in the environment, although the Sr distribution coefficient for otolith aragonite appears to be larger than for scale apatite.

There is some evidence that the elemental composition of scales, unlike that of otoliths, may change through time. This is primarily due to the multi-layer structure of scales. Scales consist of a distal layer, composed of an organic framework impregnated with hydroxyapatite (van Oosten 1957; Hamada and Mikuni 1990) and a proximal layer that is an uncalcified fibrillary plate (van Oosten 1957; Fouda 1979). An osseous layer is also formed at the margin where a framework of collagen is built and subsequently crystallized with hydroxyapatite (van Oosten 1957; Fouda 1979; Hamada and Mikuni 1990). Elemental concentrations in a given area of a scale change due to increasing calcification of hydroxyapatite in the osseous layer through time. For instance, the ratio of Mg and P to Ca in hydroxyapatite crystals typically decreases through the life of an individual scale (Fouda 1979; Burnell et al. 1980; Gedalia et al. 1982; Bigi et al. 1992; Aoba et al. 1992). Scales are also often lost and regenerated so complete time periods can be missing. During times of physiological stress scales may cease to grow (Bilton and Robins 1971) or may even be resorbed (Bilton 1975). Clearly, caution must be taken before using scales exclusively to describe population patterns and movements as any site-specific tags may be altered after fish leave their natal estuary. Future research will address the question of tag stability by comparing trace element signatures from scale and otolith cores of adult weakfish.

The use of scales to identify nursery-specific elemental signatures does offer advantages over using those in otoliths. Scale collection is a non-lethal alternative to otoliths, which is beneficial for rare or endangered fishes and regulated species with catch

restrictions such as weakfish. Scales are also easy to collect and prepare for analysis. Further, each time period represented on the scale can be assayed without grinding because scale growth is at the margin and each circulus is exposed throughout life. This, in turn, eliminates the possibility of contamination from the grinding material. The elements I chose for analysis were bone loving and have little presence in the fibrillary plate, however, analysis of scales from the adult population should be restricted to a shallow depth into the scale. Also, elemental concentrations in scales were generally higher than in otoliths which reduces counting errors and increases precision in the ICP-MS analyses, especially for those elements in otoliths that are near limits-of-detection of the instrument in otoliths (Campana et al. 1997).

I have demonstrated that scales can be analyzed with LA-ICP-MS to determine nursery-specific elemental signatures. This method is comparable to elemental analysis of otoliths in juveniles. In the future I will evaluate the stability of the tag as the scale grows. It may be possible to offer a non-lethal method of determining natal estuary identification through the life of the fish.

CHAPTER IV

EVALUATION OF SCALE CHEMISTRY STABILITY FOR A MIGRATORY FISH

Introduction

Measures of growth, survival, and reproduction can be different between stocks of the same species and when this occurs stocks must be discriminated and managed as independent units (Campana and Casselman 1993; Ruzzante et al. 1996). Identification of individual stocks in the population is necessary to assess the recruitment success, importance of individual spawning stocks and nursery areas, and exploitation rates for each stock. With this knowledge, management practices can be initiated that maintain stock variability. Without the proper management, however, those stocks most sensitive to fishing pressure will suffer detrimental effects within mixed-stock fisheries (Ruzzante et al. 1996) such as restricted genetic variation, range alteration, and reduced fecundity. Beyond the implications to fisheries, the determination of stock structure within a population can be used to understand dispersal, population ecology, and metapopulation structure of a species (Thorrold et al. 1997). It is the purpose of this research to determine if scale chemistry is stable and can be used to estimate the proportion of adult fish from each natal estuary.

Hardpart chemistry is becoming more commonly used to identify natal origins (e.g. Pender and Griffin 1996; Campana 1999). These methods are specifically important in cases such as weakfish (*Cynoscion regalis*) for which analysis of allozyme, mtDNA, and microsatelite DNA have failed to distinguish separate stocks. Indeed, methods based

on genetics are dependent on the long-term effects of complete or nearly complete separation, whereas hardpart chemistry relies on differences in the environment (Wells et al. 2000a; Bath et al. 2000) and identifying representative differences in fish hardparts at a given age and location. Obviously, otolith methods are lethal, require mutilation of the catch, and are not well represented in historic collections. Also, regulated fishes are often released, fishers are reluctant to allow the dissection of their catch, and typically archival collections are made up of scales not otoliths. Of course, hardpart chemistry can only give information about current population structure, whereas, genetics offers a historical picture.

Elemental signatures quantified from juvenile weakfish scales based on Mg:Ca, Sr:Ca, Mn:Ca, and Ba:Ca were sufficient to classify individual juveniles to their natal estuaries with 65% accuracy using linear discriminant function analysis (LDFA; Wells et al. 2000b). By identifying these same signatures at the scale core, adults could potentially be allocated to their natal estuaries. Better, I use a maximum likelihood (ML) procedure to determine the contribution of each natal estuary to the adult sample. Using ML, chum salmon (*Oncorhynchus keta*, Fournier 1984), sockeye salmon (*Oncorhynchus nerka*; Wood et al. 1989), chinook salmon (*Oncorhynchus tshawytscha*, Millar 1990) stock compositions have been evaluated. In each case, genetics, morphometrics, and/or meristics were used as distinguishing features of the stocks. This is the first study to use ML procedures to examine stock composition using elemental signatures in scales.

The degree of usefulness of scale chemistry is dependent on the relative stability of the elemental signatures to the age when determination of natal estuary is desired. While the otolith is considered to be stable (Campana 1999), scale chemistry has not been

properly tested. During times of increased calcium demand or depletion scales are vulnerable to resorbtion (Bilton 1975). Such times include migration, spawning, and fasting (Bilton and Robins 1971; Bilton 1975). Further, scales may continue to crystallize after circuli are initially formed (Fouda 1979). To examine the stability of the elemental signature described on the scales of juvenile weakfish through maturation, I considered the otolith tag to be stable over time and compared the signature at the core of the scale to that at the otolith core from the same individual fish. Previous studies support that scale and otolith elemental signatures are highly correlated in juveniles (Wells et al. 2000a, 2000b). Correlation between scales and otolith will be evaluated through maturation.

I will evaluate the classification of juveniles (LDFA) using both scale and otolith chemistries. This comparison will show the baseline variation between the two methods as the true natal estuaries are known. Following, I will examine the individual allocations (LDFA) estimated from scale and otolith chemistries. Finally, scale and otolith chemistries will be used to determine the natal-estuary contribution to a sample of adults and the estimated proportional contribution by each hardpart will be compared. The ML procedure will be used for this comparison.

Methods

Quantification of the natal-estuary elemental signatures

Elemental signatures in scales and otoliths were quantified for juvenile weakfish from five estuaries along the Atlantic coast (Doboy Sound, Pamlico Sound, Chesapeake Bay, Delaware Bay, and Peconic Bay) representing the 1996 and 1997 cohorts (Thorrold

et al. 1998; Wells et al. 2000b). One nonregenerated scale from each fish was cleaned before examination with laser ablation inductively coupled mass spectrometry (LA-ICP-MS; Wells et al 2000b). One otolith from each fish was also cleaned of contaminants and analyzed using isotope dilution ICP-MS following methods in Thorrold et al. (1998). In 1996, 146 juveniles were examined for scale chemistry and 274 for otolith chemistry. In 1997, 125 juveniles were examined for scale chemistry and 397 for otolith chemistry. Both scale and otolith chemistry were examined for 118 fish in 1996 and 125 fish in 1997.

Adult data collection

Collections of adults were made June trough September 1998 from the Pamlico Sound (long-haul seine), Chesapeake Bay (pound net), and Delaware Bay (10m otter trawl). Fish 18 cm to 36 cm total length were targeted as they represented a large portion of the 1- and 2-year-old fish (Lowerre-Barbieri 1995). Using a stainless steel knife scales and otoliths were removed from each fish. Also, ovaries were preserved in 10% formalin, soaked for 24 hours in tap water and stored in 70% ethanol. Ovarian sections were then embedded in paraffin, sectioned to 5 μm, and stained with hematoxylin and eosin Y for reproductive staging. Ages were determined using otoliths (Lowerre-Barbieri et al. 1994).

Scales and otoliths from adults were prepared for trace element analysis. Scales were sonicated for five minutes in 3% ultra-pure hydrogen peroxide followed by two rounds of washing with an acid-washed electric rotary toothbrush and Milli-Q water. Scales were secured on petrographic slides with mounting tape for analysis with LA-ICP-MS. To expose otolith core material for both ageing and chemical analysis, sections (~

500 μm) were taken using a diamond blade on a low-speed isomet saw with deionized water as a coolant. Otolith sections were mounted on petrographic slides with *krazy*® glue and were polished with aluminum oxide paper to make the juvenile growth period visually apparent and provide a smooth surface for laser ablation. Mounted otoliths were triple rinsed with Milli-Q water, scrubbed with an acid-washed nylon bristle brush, sonicated in Milli-Q water for five minutes, rinsed with dilute ultrapure nitric acid, triple rinsed with Milli-Q water, and dried under a laminar flow fume hood and stored in plastic bags until analysis with LA-ICP-MS.

Chemical Analysis

Laser ablation sector field ICP-MS was used to analyze scales and otoliths (Table 10; Thorrold and Shuttleworth 2000). Ablated scale material was swept with helium into an aspirated standard solution and the combined material was analyzed. This procedure alleviated the need for matrix matching with reference standards and took advantage of the more precise solution-based methods (Campana 1999). A 0.3 mm^2 raster was ablated from the scale core toward the posterior edge. The depth of the raster typically did not penetrate the full thickness of the scale. A raster covering an area representing a slightly greater range of growth was ablated for each otolith using similar ICP-MS conditions. Five isotopes were quantified in both scales and otoliths (^{25}Mg, ^{48}Ca, ^{55}Mn, ^{86}Sr, and ^{138}Ba). Limits of detection were calculated as mean blank values plus three standard deviations. Isotopic counts were converted to elemental intensities by multiplying

TABLE 10. – Operating conditions of the sector field inductively coupled plasma mass spectrometry system (ICP-MS) used for analysis of weakfish scales and otoliths in this study.

ICP-MS	
Model	Finnigan MAT Element2
R.f. power	1200W
Coolant gas flow rate (Ar)	13.4 L/min
Auxilary gas flow (Ar)	0.65 L/min
Carrier gas flow (He)	0.4 L/min
Time of analysis	~ 2 min/sample
Laser probe	
Laser type	Nd: YAG pulse
Frequency	266 nm
Repetition rate	2 Hz
Scan speed	50 μm/s
Spot size	40 μm

percent natural occurrence of the isotopes. All data were standardized to Ca to account for variability in laser energy and weight of ablated material. Finally, standardized values were converted to molar ratios. Both scales and otoliths were analyzed in blocks of six with one randomly chosen fish from each estuary and each age.

Data analysis

Stability of the elemental signature at the scale core through maturation was evaluated relative to the otolith. Values of Sr:Ca, Ba:Ca and Mn:Ca in the scales of juveniles were significantly correlated to the elemental concentrations in the otoliths from the same fish (r = 0.59, 0.72 and 0.55 respectively; Wells et al. 2000b). Magnesium:Ca values were not significantly correlated. In this study, correlations of raw element:Ca concentrations at the core of scales and otoliths were compared for 1- and 2-year-old fish from the 1997 and 1996 cohorts. Also, using LDFA individual juveniles were classified to their natal estuaries with scale chemistry and the similarity of classifications was compared to classifications from otolith chemistry. This comparison estimated the amount of baseline variation before any change to the signatures was possible. This analysis was an extension of that in Wells et al (2000b). Finally, once the scale data were transformed to meet statistical assumptions and the data were rescaled, the proportion of the 1- and 2-year-old fish from each natal estuary was estimated with ML using scale and otolith data from juvenile and adult fish for which both scale and otolith chemistry had been evaluated. Mg:Ca, Sr:Ca, Ba:Ca, and Mn:Ca values were used to define the elemental signatures. Confidence limits were placed around the estimated differences in natal contributions by bootstrapping adult samples 1000 times.

Before determining the contribution of each natal estuary to the adult sample the elemental data were transformed and standardized. Residual analysis was used to test assumptions of homogeneity of variances and normality for the elemental data from juveniles (Winer 1997). To meet these assumptions it was necessary to log*e* transform Ba:Ca and Mn:Ca ratios for the juvenile scale data (Wells et al. 2000b) and, therefore, concentrations from the adults were also transformed before the mixed-origin compositions and associated errors were estimated. All assumptions were met for otolith data (Thorrold et al. 1998). After transforming the data to meet statistical assumptions, the element:Ca concentrations in scales and otoliths from juveniles and adults were standardized $[(x_k - x_k\text{bar})/ sd_k]$ before estimating natal origins of the adults. Specifically, juveniles from 1996 and 1997 cohorts were scaled independently as were 1- and 2-year-old fish. This practice accounted for possible differences due to ICP-MS techniques and for apparent differences in absolute element:Ca concentrations between the age groups.

The mixed-origin composition of adults was estimated with a ML procedure developed by D. Naik (Old Dominion University, Department of Mathematics and Statistics, Appendix 1) using SAS's interactive matrix language (SAS/IML, SAS 1989). Elemental signatures in the scales and otoliths of juvenile weakfish from known origin were used as a reference set representing elemental signatures from the five examined estuaries. Using the reference set, parameters of the prior probability distribution were estimated and with these values individuals from the adult set were assigned posterior probabilities of having come from each of the five represented natal estuaries. Following the classification of the adult set, ML estimates for the prior probability distributions were recalculated using data from both the reference set and the classified adult set.

Individuals from the adult set were then again assigned posterior probabilities of having come from each of the natal estuaries based on the recalculated ML values for the discriminant function. This iterative procedure of classification followed by recalculation of the prior probability distribution for the discriminant function continued until the difference between the classification results of the current run and the previous run was less than 10^{-4}. Using a data set with unknown composition to build a more powerful discriminant function was a novel approach different from other reported methods (e.g. - Fournier et al 1984; Millar 1987).

First, before actually using ML on the adult data set, I estimated how robust the ML procedure was to my data. Error in allocation was evaluated with simulations on the juvenile data sets. By doing these simulations, I could evaluate the effect of variation in the reference set and adult set. Using scale data from juveniles, reference and test sets for each cohort were generated from each cohort's data set by removing the estuary identifier from 10, 20, 30, 40, 50, and 60% of the observations. In doing this, test sets were randomly selected without replacement and the remaining observations were used as a reference set. Sampling without replacement ensured that there were no common observations in the test and reference sets. With such a small data set this was important. Further, because my data sets were small, confidence limits for the error were conservative. For instance, it was possible that fish important to defining the estuary-specific signatures were removed from the reference set. Error in estimation of the composition of the test set was determined as the average of 1000 random samples. Further, using scale data sets, I simulated the possible situation in which one of the natal estuaries was not represented in the test set and the rather extreme case in which the

mixed population was made up entirely of individuals from one natal estuary. For these simulations an initial random selection of 40% of the juveniles were allocated to the test set. This proportion (40%) was chosen to ensure that the test set was a large enough percentage of the reference set after removing the appropriate fish from specified natal estuaries. Juvenile otolith data was also used to judge the accuracy of the ML results. Using otoliths, only the case in which the test set composition was similar to the reference set was examined. In this simulation 30% (approximately the relative proportion of adults to juveniles in later simulations) of the data were allocated to the test set and estuary-specific error was determined as the average of 1000 random samples.

Results

Two-year-old (N= 45) weakfish were collected from the 1996 cohort and 1 year-old (N = 46) weakfish were collected from the 1997 cohort for both scale and otolith chemical analysis (Table 11). The sex ratios for 1- and 2-year-old fish used were 50:50 and 46:54 (F:M) respectively. All females used for analyses showed evidence of spawning or preparing to spawn. For each cohort, adults collected from the three separate estuaries were combined and the contribution of each natal estuary was estimated.

In otoliths, magnitudes of the four element:Ca values were unchanged after maturation, however, magnitudes changed within scales. Magnesium:Ca and Mn:Ca values in scales from adults were a fraction of the values observed for juveniles (Figures 9 and 10). Barium:Ca and Sr:Ca values were of similar magnitude between the different

TABLE 11. – Adult weakfish from the 1996 and 1997 cohorts were collected from Pamlico Sound (PA), Chesapeake Bay (CB), and Delaware Bay (DE) during June to September 1998. Age, collection date, sample size of complete adult scale data (N_a), sample size of complete adult otolith data set (N_b), mean total lengths (TL_a and TL_b) and standard errors (SE_a and SE_b) are shown for each group of data. Also, N_a represents the number of fish for which both scales and otoliths were analyzed.

				Scale			Otoliths		
Location	Cohort	Age	Date	N_a	TL_a	SE_a	N_b	TL_b	SE_b
PA	1996	2	Jul 1 - Sep 1	15	24.4	0.28	51	23.5	0.16
CB	1996	2	Jun 16 - Aug 16	13	25.1	0.37	44	25.3	0.28
DE	1996	2	Jun 25 - Jul 20	17	26.0	0.36	58	26.1	0.19
PA	1997	1	Jul 1 - Sep 1	17	20.7	0.37	58	20.8	0.21
CB	1997	1	Jun 16 - Aug 16	15	21.2	0.19	51	21.3	0.12
DE	1997	1	Jun 25 - Jul 20	14	21.4	0.23	48	21.3	0.13

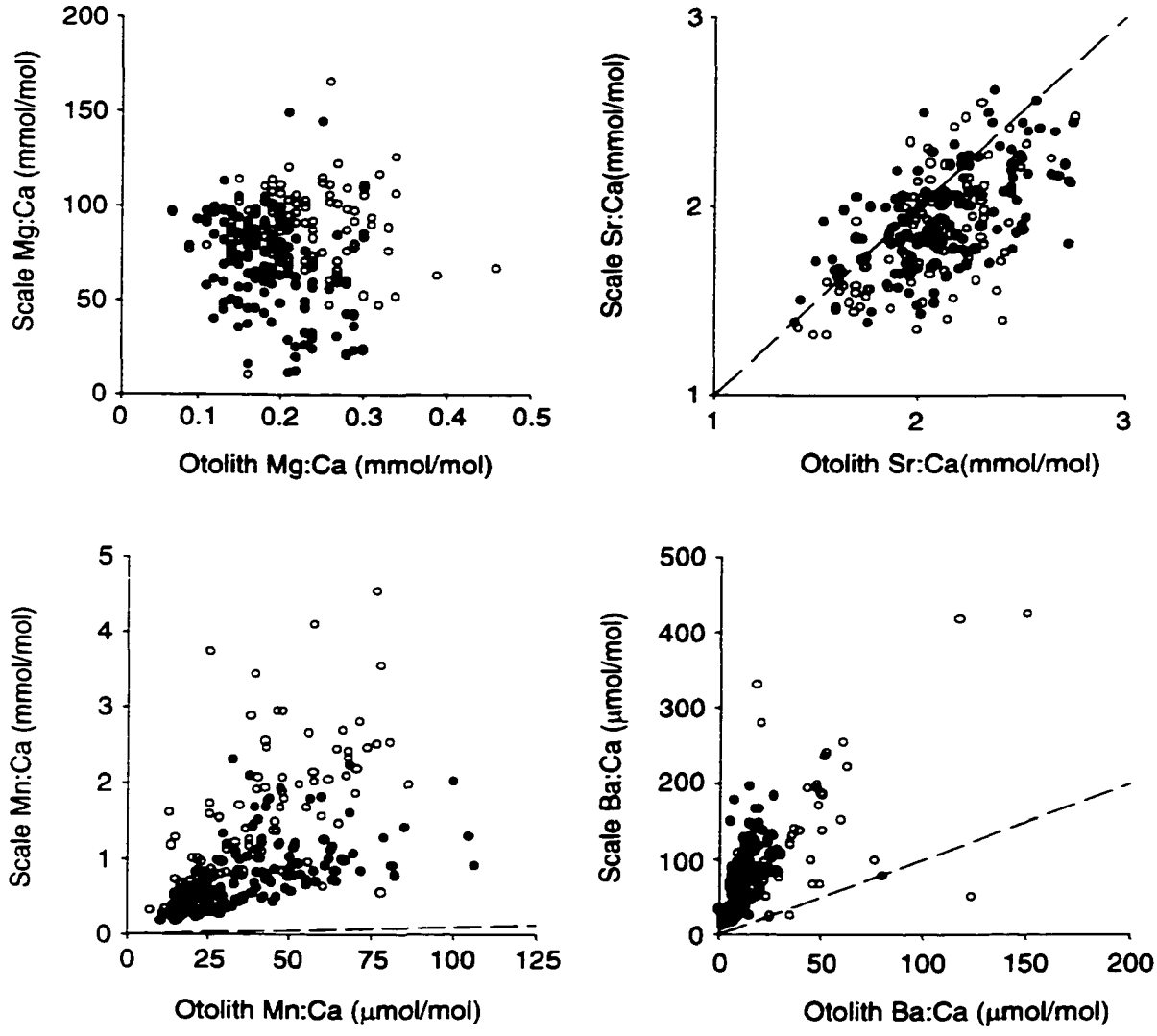

FIGURE 9. Individual juvenile *Cynoscion regalis* element:Ca values in scales and otoliths from the 1996 (solid circles) and 1997 (open circles) cohorts. The dashed line indicates a 1:1 relationship between otoliths and scales.

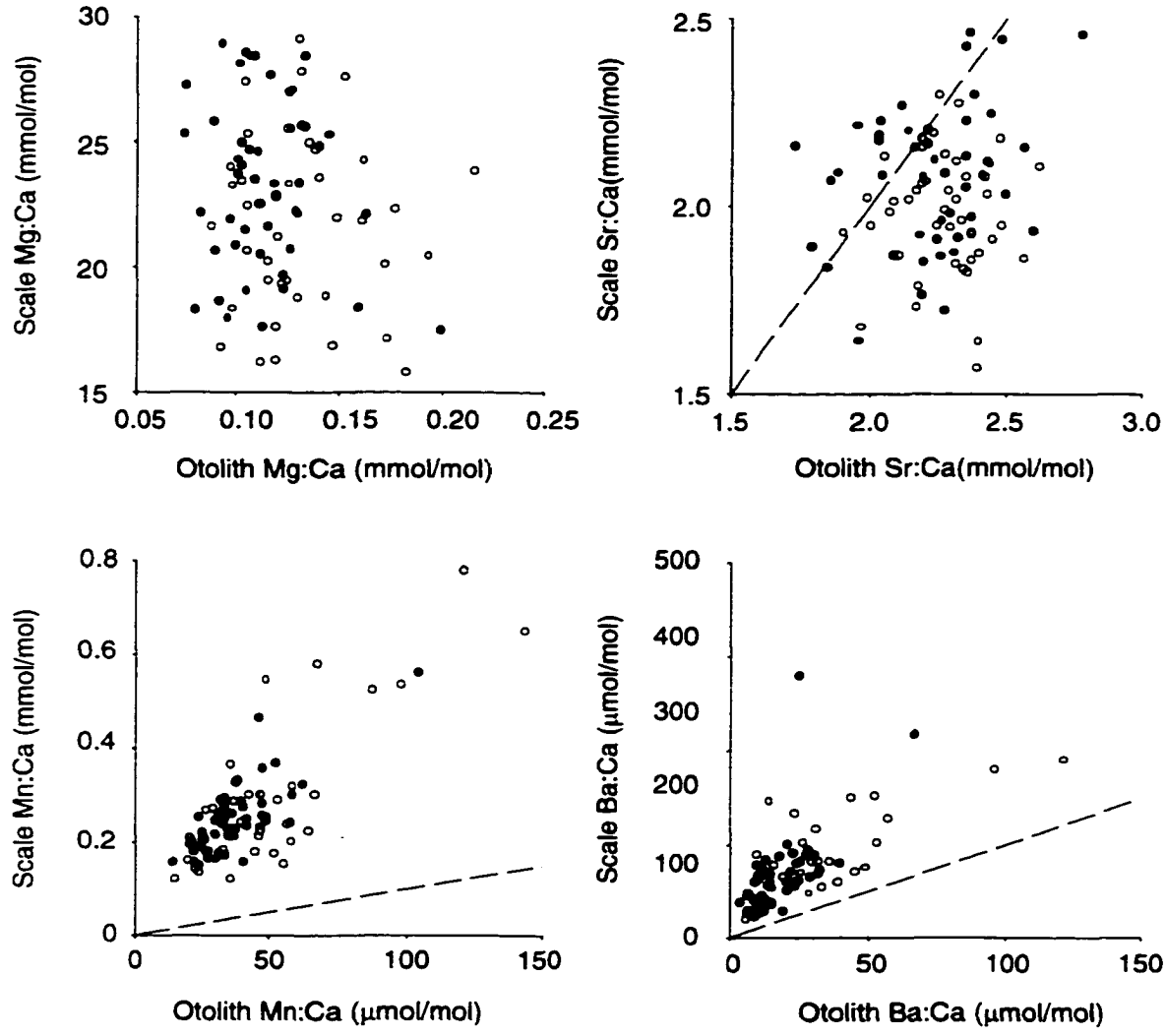

FIGURE 10. Element:Ca values in scales and otoliths of individual adult *Cynoscion regalis* collected in 1998 representing the 1996 (solid circle) and 1997 (open circles) cohorts. The dashed line indicates a 1:1 relationship between otoliths and scales.

ages. Importantly, Mn:Ca and Ba:Ca values maintained a similar relationship between scales and otoliths through maturation.

Correlation between element:Ca concentrations in scales and otoliths indicated that relative Mn:Ca and Ba:Ca concentrations in scales remained stable after maturation. Further, the higher values represented by the 1997 cohort for Mn:Ca and Ba:Ca remained relatively high through the years suggesting a certain conservation of the elemental signature (Figures 9 and 10). Strontium:Ca correlations, however, were reduced. For juveniles, concentrations of Sr:Ca, Mn:Ca, and Ba:Ca in scales were significantly correlated to values in otoliths (r = 0.55, 0.59, and 0.72 respectively; N = 252). Manganese:Ca values in the scales remained highly correlated to otolith values from 1- (r = 0.88, N = 46, P = 0.0001) and 2-year-olds (r = 0.77, N = 45, P = 0.0001). Barium:Ca correlations were also correlated for 1- (r = 0.77, N = 46, P = 0.0001) and 2-year-olds (r = 0.62, N = 45, P = 0.0001). Notice, one Ba:Ca value was much higher in the scale relative to the otolith (Figure 10) and when removed the correlation was increased (r = 0.79, N = 44, P = 0.0001). Values of Sr:Ca in scales and otoliths were not correlated for 1- (r = 0.18, N = 46, P = 0.2375) or for 2-year-olds (r = 0.28, N = 45, P = 0.0607). Mg:Ca values were not significantly related in any comparison and therefore correlation was not an appropriate method to examine stability of Mg:Ca.

Individual allocations (LDFA) of juvenile weakfish to their natal origins using scale and otolith chemistries shows the classification accuracies were similar at the time when signatures were first quantified. Using the entire scale and otolith juvenile data sets the allocations were similar using each hardpart's chemistry and between cohorts with average accuracy ranging from 62-67% (Table 12). Tests were also similar when only

TABLE 12. – Results of linear discriminant function analysis for classifying juvenile weakfish to natal estuary based on trace element signatures in scales and otoliths. Values indicate cross-validation accuracy (%) with Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca as dependent variables. Samples came from Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE) and Peconic Bay (GP).

Sample source	Hardpart	DS	PA	CB	DE	GP
		1996 cross-validation accuracy (%)				
DS	Scale (N = 30)	**67**	3	10	20	0
	Otolith (N = 53)	**57**	4	7	32	0
PA	Scale (N = 28)	0	**86**	4	0	11
	Otolith (N = 54)	4	**50**	13	26	7
CB	Scale (N = 29)	10	3	**72**	3	10
	Otolith (N = 53)	7	10	**66**	9	8
DE	Scale (N = 29)	31	14	14	**38**	3
	Otolith (N = 58)	15	17	7	**59**	2
GP	Scale (N = 30)	0	10	13	3	**73**
	Otolith (N = 56)	0	5	11	0	**84**
		1997 cross-validation accuracy (%)				
DS	Scale (N = 22)	**73**	0	0	22	5
	Otolith (N = 56)	**95**	0	3	2	0
PA	Scale (N = 29)	7	**83**	0	7	3
	Otolith (N = 70)	19	**37**	0	23	21
CB	Scale (N = 27)	11	4	**41**	19	26
	Otolith (N = 105)	19	4	**55**	15	7
DE	Scale (N = 28)	4	14	29	**54**	0
	Otolith (N = 90)	28	13	7	**45**	7
GP	Scale (N = 19)	0	11	11	6	**72**
	Otolith (N = 76)	0	8	7	10	**75**

fish for which both scale and otolith data were collected (Table 13). Tests of homogeneity on the main diagonals of Table 13, which represented correct classifications, demonstrated scale and otolith chemistries yielded equally accurate classification for the 1996 cohort ($\chi^2 = 5.24$, df = 4, $P = 0.264$) but were different for the 1997 cohort ($\chi^2 = 19.44$, df = 4, $P = 0.0001$). Differences with the 1997 cohort were attributed to scale chemistry yielding an accuracy that was double that from otolith chemistry for classifying Pamlico Sound fish and being 15% less accurate for Chesapeake Bay. Some differences in allocation were noted when specific individuals were examined (Table 14). Only 58% of individuals were similarly allocated from the 1996 cohort and only 53% from the 1997 cohort. A symmetry test (Bowker 1948) showed there was no significant pattern in differences for the 1996 cohort ($\chi^2 = 12.90$, df = 10, $P > 0.05$) yet there was a pattern for the 1997 cohort ($\chi^2 = 22.53$, df = 10, $P < 0.05$). This asymmetry was not obviously apparent. The natal origins of individual adults were also estimated using LDFA (juvenile reference set) and there were no patterns in the dissimilar allocations for 1- or 2-year-olds ($P > 0.05$) yet similar allocation occurred only 33% for 1-year-olds and 29% for 2-year-olds (Table 15).

Before estimating natal contributions to the adult sample with ML, error in allocation due to the variability of my data was evaluated with simulations using the scale and otolith Mg:Ca, Sr:Ca, Mn:Ca, and Ba:Ca data for juveniles. Using 1996 cohort signatures as a reference set and 1997 cohort signatures as the test set, absolute average error was 10% with Chesapeake Bay percentage being underestimated 15% and Peconic Bay contribution overestimated 17% (Table 16). Using 1997 juvenile collections as a reference set and 1996 collections as the test set, absolute average error was 12% with

TABLE 13. – Results of linear discriminant function analysis for classifying juvenile weakfish to natal estuary based on trace element signatures in scales and otoliths for which element data was available for both. Values indicate cross-validation accuracy (%) with Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca as dependent variables. Samples came from Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE) and Peconic Bay (GP).

Sample source	Hard part	DS	PA	CB	DE	GP
		1996 cross-validation accuracy (%)				
DS (N = 26)	Scale	**61**	4	12	23	0
	Otolith	**39**	19	15	27	0
PA (N = 26)	Scale	0	**81**	4	0	11
	Otolith	4	**69**	0	12	15
CB (N = 26)	Scale	11	0	**81**	4	4
	Otolith	4	4	**73**	15	4
DE (N = 29)	Scale	28	14	14	**41**	3
	Otolith	17	17	17	**49**	0
GP (N = 27)	Scale	0	11	7	0	**82**
	Otolith	0	11	7	0	**82**
		1997 cross-validation accuracy (%)				
DS (N = 18)	Scale	**78**	0	0	22	0
	Otolith	**89**	0	0	11	0
PA (N = 28)	Scale	7	**78**	0	11	4
	Otolith	18	**36**	0	18	28
CB (N = 27)	Scale	15	15	**37**	11	22
	Otolith	11	4	**52**	22	11
DE (N = 28)	Scale	4	11	28	**57**	0
	Otolith	21	11	4	**57**	7
GP (N = 17)	Scale	0	0	6	6	**88**
	Otolith	0	0	23	6	**71**

TABLE 14. – Individual juvenile allocations based on otolith and scale chemistries for the juveniles captured in 1996 and 1997. Linear discriminant function analysis was used and the reference sets were from representative cohorts. Estuaries shown are Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP). Bold values indicate similar allocations.

1996 (N = 134)					
Scale Allocation:	Otolith Allocation				
	DS	PA	CB	DE	GP
DS	**12**	.	5	12	.
PA	.	**20**	1	3	7
CB	1	2	**19**	4	2
DE	5	6	.	**9**	.
GP	.	4	3	1	**18**
1997 (N = 118)					
Scale Allocation:	Otolith Allocation				
	DS	PA	CB	DE	GP
DS	**17**	.	2	2	.
PA	2	**13**	1	6	7
CB	1	1	**8**	9	2
DE	**8**	3	1	**10**	3
GP	.	.	6	2	**14**

TABLE 15. – Individual adult allocations based on otolith and scale chemistries for the juveniles captured in 1996 and 1997. Linear discriminant function analysis was used and the reference sets were from representative cohorts. Estuaries shown are Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP). Bold values indicate similar allocations.

1996 (N = 45)					
Scale Allocation:	Otolith Allocation				
	DS	PA	CB	DE	GP
DS	**4**	2	2	2	3
PA	1	**4**	1	1	1
CB	.	3	**1**	.	1
DE	1	3	.	**3**	.
GP	.	5	6	.	**1**
1997 (N = 46)					
Scale Allocation:	Otolith Allocation				
	DS	PA	CB	DE	GP
DS	**2**	1	.	4	.
PA	3	**1**	1	5	1
CB	3	1	**1**	4	.
DE	4	1	1	**7**	.
GP	.	1	.	1	**4**

TABLE 16. – The errors from using one year's data as a reference (Ref.) set and the other as a test set are shown with 1996 reference and 1997 test shown as 96-97. The error in allocation for Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP) is given.

	Estuary					
Ref.-Test	DS	PA	CB	DE	GP	Average
96-97	- 3	8	-15	-7	17	10
97-96	- 13	- 4	29	- 8	-4	12

Chesapeake Bay percentage overestimated by 29% and Doboy Sound contribution underestimated 13% (Table 16). These results indicated that reference and test sets should be of the same cohort. Therefore, cohort-specific analyses were performed for determining the proportion of adult fish from each estuary.

Using simulations on juvenile elemental signature data from scales, the composition of the test set was determined with only 4 to 7% error when 10 to 60% of the individuals were allocated to the test set (Table 17). Error estimates between cohorts were similar. Estimates of error were also evaluated by estuary when the test set consisted of 30% of the total data set and had a similar composition as the reference set (Table 18). The 30% proportion was chosen because it was similar to that for adults (test) to juveniles (reference) in later simulations. The 1996 simulations showed Delaware Bay was underestimated by 2% with a total average absolute error of only 5%. Using 1997 data, error ranged between –3% and +3 %, with Delaware Bay being overestimated and Chesapeake Bay underestimated with a total average absolute error rate of 5%. These results were reasonable given that signatures from Delaware Bay fish, although significant, were central to the other estuarine signatures for both the 1996 and 1997 cohorts (Figure 7; Wells et al. 2000b). For remaining simulations, I used 40% of the data as the test set and any modifications were made on that test set such that its stock composition was different from the reference set. By using 40% I was confident that enough fish were in the test set to allow for a reasonable estimate of stock composition.

The removal of one estuary from the test set, leaving the other four, demonstrated that the composition could still be estimated with only 6 to 9% absolute average error (Table 19). Examining simulations on the 1996 cohort, error was highest (8%) when

TABLE 17. – The robustness of the maximum likelihood procedure to the data was tested by simulations of the juvenile weakfish data. Average absolute errors by year with test sample as a specified proportion of the entire juvenile scale data set were calculated as the mean of 1000 random samples. The 95% confidences were calculated by 1000 random samples of the data. The column headings represent the proportional size of the test set (i.e. -- 30% of entire data set was allocated to the test set and 70% to the reference set).

	Proportion of the test set					
Cohort	10%	20%	30%	40%	50%	60%
1996	7	5	5	4	4	5
(N = 146)	(2, 13)	(2, 9)	(2, 9)	(2, 8)	(2, 8)	(2, 8)
1997	7	5	5	5	5	5
(N = 125)	(3, 14)	(2, 10)	(2, 9)	(2, 9)	(2, 9)	(2, 9)

TABLE 18. – Shown are average errors by year and estuary based on the mean of 1000 random samples using a test set which was 30% of the total juvenile weakfish scale data and a reference set which was 70%. The 95% confidence limits were calculated by 1000 random samples of the data. Each grand average was the mean of the absolute errors calculated for each of the 1000 random samples for all estuaries. The estuaries are labeled as Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP).

	Estuary					
Cohort	DS	PA	CB	DE	GP	Average
1996	-1	1	1	-2	1	5
(N = 146)	(-13, 11)	(-7, 10)	(-12, 12)	(-16, 10)	(-8, 11)	(2, 9)
1997	0	1	-3	3	-1	5
(N = 125)	(-12, 10)	(-9, 11)	(-16, 10)	(-11, 16)	(-12, 16)	(2, 9)

TABLE 19. – Once the test set was generated, which was 40% of the total juvenile weakfish scale data, fish from a chosen estuary were removed from the test set. Row titles indicate the estuary that was removed and columns show the average error of the percentage estimate of each estuary's contribution to the test set calculated as the mean of 1000 random samples. The 95% confidence limits were determined by 1000 random samples of the data. Each grand average was the mean of the absolute errors calculated for each of the 1000 random samples for all estuaries. The estuaries are labeled as Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP).

1996 averaged misallocation (%)						
Removed:	DS	PA	CB	DE	GP	Average
DS	13 (7, 19)	1 (-10, 10)	-2 (-15, 10)	-11 (-23, 11)	-1 (-13, 14)	8 (4, 12)
PA	-1 (-14, 12)	7 (5, 18)	0 (-14, 12)	-3 (-18, 11)	-3 (-12, 6)	6 (3, 11)
CB	-4 (-17, 8)	1 (-8, 9)	13 (7, 20)	-7 (-20, 6)	-3 (-13, 7)	7 (4, 11)
DE	-9 (-19, 0)	1 (-10, 8)	-3 (-16, 8)	14 (8, 20)	-1 (-12, 10)	8 (4, 11)
GP	-1 (-16, 12)	-2 (-9, 7)	-3 (-15, 8)	-4 (-19, 11)	10 (4, 18)	6 (3, 10)
1997 average misallocation (%)						
Removed:	DS	PA	CB	DE	GP	Average
DS	10 (4, 17)	0 (-12, 12)	-4 (-18, 9)	-4 (-19, 12)	-2 (-14, 8)	7 (3, 11)
PA	-2 (-14, 11)	10 (4, 16)	-4 (-19, 11)	-1 (-19, 16)	-3 (-15, 8)	7 (4, 12)
CB	-3 (-15, 8)	-1 (-13, 10)	11 (5, 19)	-2 (-18, 13)	-5 (-15, 4)	7 (4, 11)
DE	-4 (-14, 6)	-3 (-14, 7)	-10 (-23, 1)	20 (12, 29)	-3 (-15, 9)	9 (6, 13)
GP	-1 (-12, 10)	-1 (-13, 10)	-6 (-18, 7)	0 (-16, 15)	8 (3, 14)	6 (3, 10)

Doboy Sound or Delaware Bay fish were removed from the test set. Simulations with the 1997 cohort showed error was greatest when Delaware Bay fish were removed from the test set (9%). An examination of canonical variate plots for both years supports these findings (Figure 7). In 1996, Delaware Bay and Doboy Sound fish had similar signatures and in 1997 Delaware Bay signatures was more distinct but still central relative to the other four estuaries. Figure 7 also demonstrates that Pamlico Sound and Peconic Bay signatures were the most separated from the groups and consequently could be estimated with little error. When the test set was made up entirely by one estuary, the composition of the test set could be estimated with only 11 to 27% absolute average error (Table 20). Using data from the 1996 and 1997 cohort, error was greatest when the test set was made up entirely of Delaware Bay fish. Another examination of Figure 7 supports these findings.

Simulations using the 1996 and 1997 juvenile otolith data, which used the complete juvenile otolith data set, indicated the absolute average error was 4% for determining stock composition of the test set (Table 21). These errors were similar to those found using scale data. Using either scale or otolith data, the simulation results indicated that it was possible to estimate the stock composition of adults with little error whether or not the compositions of the test and reference sets were similar.

The natal-estuary contributions to the mature 1996 and 1997 adults were estimated similarly by scale and otolith chemistries. Those otoliths with matching scale data were used in addition to randomly selected otolith data (from a larger data set) such that the proportion of adult fish caught at different estuaries was equal to the data for

TABLE 20. – Once the test set was generated, which was 40% of the total juvenile weakfish scale data, fish from all estuaries but one were removed from the test set. Row titles indicate the estuary that was left in the test set and columns show the average error of the percentage estimate of each estuary's contribution to the test set calculated as the mean of 1000 random samples. The 95% confidence limits were determined by 1000 random samples of the data. Each grand average is the mean of the absolute errors calculated for each of the 1000 random samples for all estuaries. The estuaries are labeled as Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP).

1996 averaged misallocation (%)						
Remaining:	DS	PA	CB	DE	GP	Average
DS	-52 (-67, -34)	4 (0, 13)	14 (5, 24)	33 (18, 47)	1 (0, 4)	21 (13, 27)
PA	1 (0, 3)	-24 (-45, -8)	6 (1, 13)	3 (4, 7)	14 (3, 32)	10 (3, 18)
CB	15 (6, 28)	4 (0, 13)	-49 (-64, -33)	16 (6, 27)	14 (3, 29)	20 (13, 26)
DE	34 (19, 51)	10 (1, 21)	14 (5, 25)	-66 (-78, -53)	8 (1, 17)	27 (21, 31)
GP	1 (1, 3)	11 (2, 24)	17 (5, 31)	5 (6, 12)	-34 (-54, -16)	14 (6, 22)
1997 averaged misallocation (%)						
Remaining:	DS	PA	CB	DE	GP	Average
DS	-43 (-64, -24)	4 (0, 12)	6 (1, 13)	29 (15, 45)	4 (2, 12)	17 (10, 25)
PA	6 (1, 16)	-28 (-50, -8)	1 (0, 3)	14 (4, 30)	7 (0, 19)	11 (3, 20)
CB	12 (2, 21)	8 (1, 19)	-54 (-74, -33)	18 (7, 31)	16 (4, 35)	21 (13, 30)
DE	16 (5, 29)	14 (4, 31)	24 (8, 42)	-60 (-73, -43)	6 (2, 11)	24 (17, 29)
GP	2 (0, 8)	12 (1, 31)	16 (3, 32)	20 (8, 35)	-50 (-72, -26)	20 (10, 29)

TABLE 21. – Using otolith chemistry, the average errors by year and estuary using a test set which was 30% of the total juvenile weakfish otolith data and a reference set which was 70% are shown as the means of 1000 random samples. The 95% confidence limits were calculated by1000 random samples of the data. Each grand average is the mean of the absolute errors calculated for each of the 1000 random samples for all estuaries. The estuaries are labeled as Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP).

	Estuary					
Cohort	DS	PA	CB	DE	GP	Average
1996	-2	0	1	2	-1	4
(N = 274)	(-11, 6)	(-10, 9)	(-7, 9)	(-9, 12)	(-6, 4)	(1, 7)
1997	4	-1	-6	2	1	4
(N = 397)	(-1, 10)	(-8, 6)	(-13, 1)	(-6, 10)	(-6, 6)	(1, 7)

scales (Table 11; compare N_a to N_b). This was important as the ML procedure accuracy was not independent of true stock composition. Composition was also estimated with the complete scale data sets and the estimated compositions derived from otolith and scale chemistries were compared with tests of homogeneity. Using scale chemistry the contribution of natal estuaries to the adult samples was estimated similarly (df = 4) as when estimated with otolith chemistry for the 1996 cohort ($\chi^2 = 8.79$, $P = 0.067$) and 1997 cohort ($\chi^2 = 0.75$, $P = 0.945$, Table 22). Also, estimated proportions of fish from each natal estuary were similar (df = 4, $P > 0.05$) when only fish for which both scale and otolith chemistry was determined (both reference and test sets) were used to estimate stock composition ($\chi^2 = 7.88$ for 1996 cohort, $\chi^2 = 3.33$ for 1997 cohort, Table 23). Notably, however, using scale chemistry, the contribution of 2-year-olds from Pamlico Sound and Chesapeake Bay to the adult sample was underestimated and Peconic Bay fish were overestimated, relative to otolith-based estimates (Tables 22 and 23).

Using fish for which both scale and otolith chemistry was determined (Table 11; N_a) the difference in proportion estimates from each natal estuary was determined as the average of 1000 estimates for bootstrapped adult sets (Difference in allocation = Allocation using scale chemistry - Allocation using otolith chemistry). Differences for 1-year-old fish ranged between 6 and -6% (Table 24). For 2-year-old fish the difference was greatest for fish allocated by otolith chemistry to Peconic Bay origin (15%). Further, Pamlico Sound and Chesapeake Bay contributions were underestimated 7%. Notice, for 1-year-old fish bootstrapped 95% confidence intervals encompassed zero for all estuaries and for 2-year-olds the confidence intervals encompassed zero for all but Peconic Bay (Figure 11).

TABLE 22. – Estimated contribution by natal estuary to the 1- and 2-year-old weakfish collected from Pamlico Sound, Chesapeake Bay, and Delaware Bay in 1998 estimated by scale and otolith chemistries. All fish for which otolith data were available were used after truncating to match the same proportions as the scale data (Table 2; N_b). Also, all fish for which scale data were available were used (Table 2; N_a). Also shown are χ^2 values for comparisons between allocations from scale and otolith chemistry (df = 4 for all tests). The estuaries are labeled as Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP)

		Estuary						
Part	Cohort	DS	PA	CB	DE	GP	χ^2	P
Scale	1996	19	20	15	18	28		
							8.79	0.067
Otolith	1996	17	27	23	21	12		
Scale	1997	14	21	20	29	16		
							0.75	0.945
Otolith	1997	15	21	18	33	13		

TABLE 23. – Estimated contribution by natal estuary to the 1- ($N = 46$) and 2-year-old ($N = 45$) weakfish collected from Pamlico Sound, Chesapeake Bay, and Delaware Bay in 1998 estimated by scale and otolith chemistries. Only juvenile and adult fish for which both scale and otolith chemistry was measured were used. Also shown are χ^2 values for comparisons between allocations from scale and otolith chemistry (df = 4 for all tests). The estuaries are labeled as Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP)

		Estuary						
Part	Cohort	DS	PA	CB	DE	GP	χ^2	P
Scale	1996	18	20	14	20	28		
							7.88	0.100
Otolith	1996	17	27	20	23	13		
Scale	1997	12	24	19	31	14		
							3.33	0.504
Otolith	1997	17	22	13	37	11		

TABLE 24. – Estimated differences in estimated contribution by natal estuary (Difference = Scale allocation - Otolith allocation) to the 1- and 2-year-old weakfish collected from Pamlico Sound, Chesapeake Bay, and Delaware Bay in 1998 estimated by scale and otolith chemistries. Only juvenile and adult fish for which both scale and otolith chemistry was measured were used. Estimated differences are averaged difference percentages of 1000 bootstrapped adult samples. The 95% confidence limits were calculated based on 1000 bootstrapped samples. Number of juveniles in the reference set (N_a) and adults in the test sets (N_b) are shown. The estuaries are labeled as Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP)

			Estuary				
Cohort	N_a	N_b	DS	PA	CB	DE	GP
1996	134	46	2	-7	-7	-3	15
			(-4, 9)	(-17, -4)	(-15, 1)	(-9, 3)	(4, 27)
1997	118	45	-5	2	6	-6	3
			(-14, 2)	(-9, 14)	(0, 14)	(-16 3)	(-2, 10)

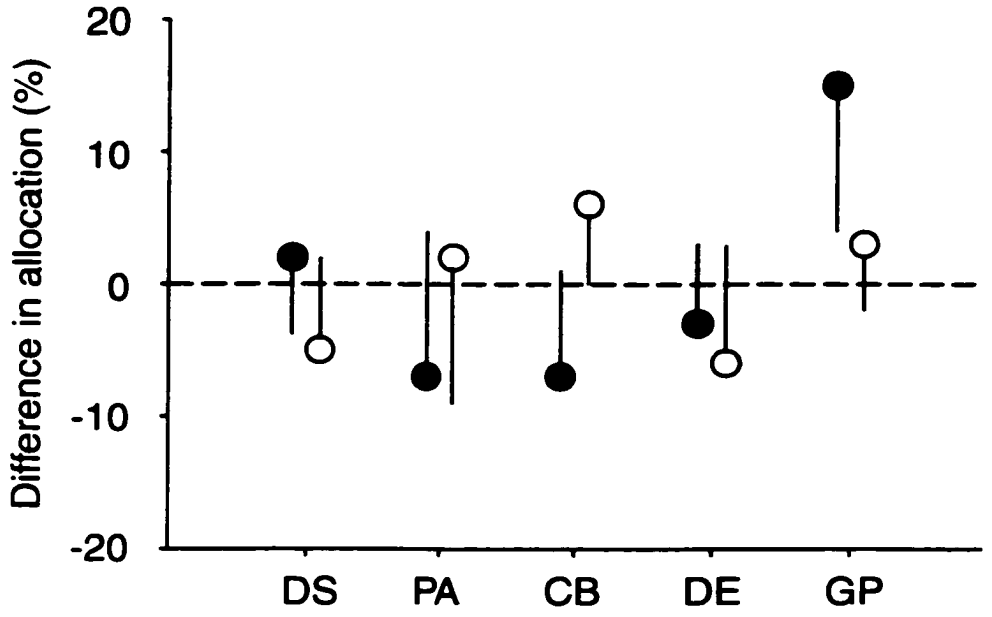

FIGURE 11. The contribution of each natal estuary to the adult sample was estimated using scale and otolith chemistry for the 1996 (solid circles) and 1997 (open circles) cohorts. Shown are the differences in estimated proportions using the two hardpart chemistries (Difference in allocation = Allocation using scale chemistry - Allocation using otolith chemistry). The 95% confidence interval toward the zero reference line was calculated from 1000 bootstrapped adult mixed stocks. Estuaries represented are Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP).

The individual allocation results reveal the distribution of different allocations. Firstly using ML, similarities of individual allocations from scale and otolith chemistries were slightly increased over LDFA with 41% and 31% of the fish similarly allocated for 1- and 2-year-olds (Table 25). Still, the results of individual classifications were generally similar to those from LDFA (Table 15). Individual differences in allocation between the hardpart chemistries for 1-year-old fish had no clear pattern (Table 25). However, 2-year-old fish allocated to Pamlico Sound and Chesapeake Bay origin with otolith chemistry were classified differently using scale chemistry (Table 25) -- a pattern that was not noted for juveniles (Table 14). Specifically, the majority of fish allocated to Pamlico Sound origin with otolith chemistry were allocated to Delaware Bay and Peconic Bay with scale chemistry and almost all of the fish allocated to Chesapeake Bay with otolith chemistry were allocated to Peconic Bay using scale chemistry. These determinations, however, are based on assigning a probability of 1.0 to the maximum posterior probability estimated by the ML procedure. Importantly, the average maximum posterior probabilities for scale assignments were all greater than 0.20 (Table 26). This indicated the allocations were not random.

Examination of these results in light of the multivariate signatures described for juvenile scales shows a pattern of slight change in the scale chemistry after maturation. Canonical variates 1 (CV1) and 2 (CV2) describing the juvenile scale chemistry data encompassed 93% and 89% of the variation for the 1996 and 1997 cohort signatures respectively. The coefficients on CV1 and CV2 can be used to understand the differences between signatures from the five natal estuaries (Table 27). For the 1996 cohort Ba:Ca (-) and Mn:Ca (+) were most influential for CV1 and Sr:Ca (+) and

TABLE 25. – Individual adult allocations to natal location based on otolith and scale chemistries for the adults captured in 1998. Reference sets from representative cohorts were used. Estuaries shown are Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP). Bold values indicate similar allocations.

			1996 ($N = 45$)		
Scale Allocation:			Otolith Allocation		
	DS	PA	CB	DE	GP
DS	**3**	1	1	4	.
PA	1	**4**	1	1	1
CB	.	2	.	.	.
DE	1	6	.	**3**	.
GP	.	5	7	.	**4**
			1997 ($N = 46$)		
Scale Allocation:			Otolith Allocation		
	DS	PA	CB	DE	GP
DS	**2**	1	.	1	.
PA	2	**1**	1	7	1
CB	.	1	**2**	3	.
DE	5	2	1	**10**	.
GP	.	1	.	1	**4**

TABLE 26. – Average maximum posterior probabilities for fish allocations from scale chemistry shown for both similar and dissimilar allocations given by otolith chemistry based on adults captured in 1998. Also shown are the average maximum posterior probabilities of assignment to each estuary based on scale chemistry. Reference sets from representative cohorts were used. Estuaries shown are Doboy Sound (DS), Pamlico Sound (PA), Chesapeake Bay (CB), Delaware Bay (DE), and Peconic Bay (GP). Bold values indicate similar allocations.

1996 ($N = 45$)						
Scale Allocation:	Otolith Allocation					
	DS	PA	CB	DE	GP	Average
DS	**0.78**	0.30	0.67	0.63	.	0.65
PA	0.63	**0.83**	0.69	0.99	0.46	0.76
CB	.	0.38	.	.	.	0.38
DE	0.43	0.40	.	**0.50**	.	0.53
GP	.	0.84	0.65	.	**0.82**	0.75
1997 ($N = 46$)						
Scale Allocation:	Otolith Allocation					
	DS	PA	CB	DE	GP	Average
DS	**0.61**	0.38	.	0.87	.	0.62
PA	0.80	**0.88**	0.68	0.74	0.74	0.76
CB	.	0.44	**0.83**	0.78	.	0.74
DE	0.53	0.62	0.51	**0.59**	.	0.57
GP	.	0.94	.	0.48	**0.88**	0.82

TABLE 27. – Standardized canonical coefficients for canonical variate 1 (CV1) and 2 (CV2) based on elemental signatures in scales of juveniles from 1996 and 1997 (Wells et al. 2000b).

	1996 Cohort		1997 Cohort	
Element:Ca :	CV1	CV2	CV1	CV2
Mg:Ca	-0.11	0.48	0.04	0.47
Sr:Ca:	0.31	0.73	0.59	-0.69
Mn:Ca	1.31	0.52	-1.01	0.93
Ba:Ca	-1.05	0.63	1.19	0.77

Ba:Ca (+) for CV2. First, an examination of CV1 and CV2 values plotted against one another for each individual juvenile (Figure 7) demonstrated that Delaware Bay and Doboy Sound signatures were similar. Dissimilar adult allocations, therefore, were expected between the two, with fish from one allocated to the other somewhat randomly. Interestingly, 30% of 2-year-olds allocated to Pamlico Sound using otolith chemistry were allocated to Peconic Bay and Delaware Bay each with scale chemistry while only 13% and 19% of the juveniles allocated to Pamlico Sound with otoliths were allocated to Peconic Bay and Delaware Bay respectively. In fact, only 22% of the 2-year-olds allocated to Pamlico Sound with otoliths were allocated similarly to scales compared to 63% for juveniles. This was a clear indication of alteration of the elemental composition after maturation. Also, 78% of the 2-year-olds allocated to Chesapeake Bay with otolith chemistry were allocated to Peconic Bay with scale chemistry (Table 25) which was not noted for juveniles (11%). Only an increase in Sr:Ca in the scale could cause both Chesapeake Bay and Pamlico Sound fish (according to otolith chemistry) to be allocated to Peconic Bay. Pamlico Sound and Peconic Bay elemental signatures in the scale were separated on CV2 (Figure 7) with Pamlico Sound having had fish with much lower Sr:Ca values and slightly lower Ba:Ca values (Wells et al. 2000b). Chesapeake Bay and Peconic Bay elemental signatures separated on CV1 with Chesapeake Bay having had fish with higher Ba:Ca, slightly lower Mn:Ca, and similar Sr:Ca. Magnesium:Ca had little influence on separating the juvenile estuarine signatures (Wells et al. 2000b).

Importantly, Sr:Ca correlations between scales and otoliths were lower after maturation which may have been caused by a change in relative Sr:Ca concentrations. However, Ba:Ca and Mn:Ca values in scales remained highly correlated to those in

otoliths. Strontium:Ca values for 2-year-old fish (mean = 2.078) were significantly higher than as juveniles (mean = 1.94, df = 98.6, T = -3.86, P = 0.0002) and less variable (df = 145, 44, F = 1.84, P = 0.0202). Also, log*e* transformed values of Ba:Ca were not significantly different between age groups. Further, a relative increase in Mn:Ca could account for fish allocated with otolith chemistry to Chesapeake Bay having been allocated to Peconic Bay (higher Mn:Ca; Table 6) using scale chemistry. However, that so many fish allocated to Pamlico Sound with otolith chemistry were allocated to Peconic Bay (higher Sr:Ca) and Delaware Bay (lower Mn:Ca and higher Sr:Ca; Table 6) with scale chemistry removed a logical argument that Mn:Ca relative concentrations changed (examine CV1 and CV2 coefficients; Table 27). Rather, Chesapeake Bay signatures were most similar to Peconic Bay signatures (Figure 7) and a minor change in Sr:Ca could cause different allocations toward the upper right quadrant of Figure 7 (Table 27) representing the Peconic Bay signature. In all, scales from the 1996 cohort showed evidence of increased relative Sr:Ca values while Ba:Ca and Mn:Ca values remained stable.

Canonical variates 1 and 2 were less helpful in understanding the differences for 1-year-old fish from the 1997 cohort. Manganese:Ca (-) and Ba:Ca (+) were the most highly loaded variables on CV1 and Mn:Ca (+) and Ba:Ca (+) on CV2 (Table 26). Delaware Bay and Doboy Sound fish were most often allocated differently for the 1-year-old group. Given that the Delaware Bay signatures were central to the other four estuary signatures it was likely that they would be allocated poorly. Generally, differences in allocation for the 1997 cohort were distributed somewhat randomly. Interestingly, for the 1997 cohort the difference in the mean Sr:Ca value in adults relative to juveniles was

similar to the difference noted for the 1996 cohort. The mean Sr:Ca value for 1-year-old fish (mean = 1.99) was greater than juveniles (mean = 1.85, df = 138.4, T = -3.89, P = 0.0002) and was less variable (df = 124, 45, F = 3.01, P= 0.0001).

Discussion

Estimated natal-estuary contributions to the adult sample were the same when estimated by scale and otolith chemistries. This result is promising and demonstrates that scale chemistry may be useful for estimating stock structure even though minor relative alterations to the scale chemistry were noted. Indeed, results for adults were somewhat similar to those found from juvenile simulations -- a time when the signature was first quantified. Only when individual allocations were examined was a change in the elemental signature of the scale noted. Further, both Mn:Ca and Mg:Ca values were much lower in the adults and there was an indication that Sr:Ca increased. There are two reasonable causes for this alteration of the elemental signatures. The first is founded on a change in Ca demand during the reproductive season. The second argues that the scale continues the process of crystallization after the fish leaves its natal estuary.

During gonadal development Ca demand is relatively high for both males and females compared to the resting period (Mugiya and Watabe 1977; Kalish 1991). Experimentation has shown that injections of estradiol lead to mobilization of Ca from the scale to the blood plasma preferentially to other hardparts (Mugiya and Watabe 1977). Kalish (1991) also showed an increase of Ca in the blood plasma during the spawning season. He also demonstrated that the Sr:Ca ratios increased during the time of gonadal maturation and drastically decreased at peak gonadal development. For the Ca

demand during spawning to cause increased Sr:Ca and decreased Mn:Ca and Mg:Ca in the scale, elements would have to be selectively resorbed. While Sr may be more stable in the scale than Ca (Norris et al. 1963) little is understood about the stability of Mn and Mg related to spawning activity. That Ba:Ca remained constant indicated either that Ba is being removed from the scale equally to Ca or was untouched in the fibrous layer.

More likely, the scale continued to crystallize after the juvenile period. Given the findings of Fouda (1979) showing P:Ca increased from the scale core to the anterior margin, the scale continued to crystallize to a certain degree after bony material was initially laid down. The ratio of Mg and P to Ca in hydroxyapatite crystals typically decreases as the hydroxyapatite forms (Fouda 1979, Burnell et al. 1980, Gedalia et al. 1982, Bigi et al. 1992, Aoba et al. 1992). I noted increasing Mg:Ca values along an ablated transect from the core to the anterior margin. Although this trend was not significant when examined by a repeated measures analysis of variance (Wells et al. 2000b), it was an indication of continuing crystallization along the transect. Once I accept that crystallization occurs, the changes in the other three elements can be explained. Strontium:Ca in the scale increased because as the fish left the estuarine nursery areas they moved into the marine system in which there were higher Sr:Ca levels. The absolute values of Mn:Ca decreased because Mn^{+2} is not available in the more oxidizing marine environment where Mn^{+4} dominates. Finally, Ba:Ca must have been concentrated in the fibrous layer of the scale (Wells et al. 2000a), and thus remained unaffected by crystallization. In support, I noted considerably higher values of Ba:Ca in samples of ablated material from the fibrillary plate. Because the fibrous layer is not calcified, even

a small amount of Ba present yields high Ba:Ca values. Therefore, if the raster depth was not relatively consistent Ba:Ca varied substantially and inversely to Sr:Ca.

Manganese:Ca and Ba:Ca values in scales remained highly correlated to those in otoliths. While the scale may continue to crystallize, Mn:Ca was neither added nor removed. It follows that elements present in the juvenile environment that are not present in the adult environment may remain useful as only the absolute value of Mn:Ca changes. From this an estimate of Ca addition can be made because a reduction of approximately 70% in Mn:Ca values indicated that Ca nearly tripled in the scale after the juvenile period. By remaining more abundant in the fibrous layer Ba:Ca may be less affected by crystallization but may be affected instead by the more vascular nature of the fibrillary plate (Fouda 1979). Drastic differences were not expected for Sr:Ca as its value is not so obviously different between the estuarine and marine environments.

The ML procedure used in this study was shown to be accurate, precise, and robust. Further, the ML procedure outperformed LDFA in estimating proportional contribution of each natal estuary and performed somewhat better for individual classifications. When the reference set and the test set had similar true composition the error in estimation was minimal for both scale and otolith data. By randomly selecting the test set and then determining the natal-estuary contributions, I examined the possible sampling error. However, in randomly allocating the test set without replacement, I also estimated error in defining the reference set. Given my variable data and limited sample size, the success of the procedure was especially notable. The ML also preformed reasonably well when the test set was grossly dissimilar to the reference set. The decreased precision of allocation when the two sets were dissimilar was caused less by

the ML accuracy and was more a product of the inherent overlap of the data in which individuals from various estuaries overlapped signatures. This was clearly demonstrated with Delaware Bay fish generally being estimated with the most error.

The error estimates that were generated should not be used to account for bias when evaluating adult stock structure. At first this seems logical but these errors are based on specific true compositions and on specific reference sets. I cannot make prior assumptions about the true stock structure of the adult population. Further, I cannot assume that all possible signatures present in the adult collections were referenced in the reference set. In fact, the error I estimated using the juvenile set may only represent the best case scenario.

Care must be taken when sampling the scale as the laser ablates easily through the calcified layer and relatively deeply into the fibrillary plate. As the depth increases, Sr:Ca decreases and Ba:Ca increases. If crater depth is maintained relatively constant, as in this study, this should not affect the analysis. Also, because signatures were based on relative amounts of the elements and standardized values were used, this was not a cause of any of the apparent degradation of the signature. In the future it may be better, however, to sample the entire depth of the scale (Pender and Griffin 1996). In doing this, element:Ca values for those elements present only in the boney layer will be diluted but can still be used as relative concentrations. However, those elements represented mostly in the fibrous layer will be overestimated and relative concentrations between groups may not remain constant as the fibrous layer thickens as the fish ages.

In all, scale chemistry does not appear to be stable following the juvenile period, it may be too easy to induce highly variable results, and individual allocations were

different from those estimated using otolith chemistry. Nonetheless, estimated proportions of adults from each natal estuary were similar when using the two hardpart chemistries. Importantly, I showed that there was great variation in otolith and scale allocation when juveniles of known origin were used to determine classification accuracy. Therefore, much of the variation in adult classifications was due to initial (baseline) variation.

To understand fully the question of stability more analyses should be performed. A controlled study should be performed to examine how elemental:Ca concentrations relate to scale layers sampled. More abundant transect data may expose some of the temporal differences in scale crystallization. Most importantly, scales should be examined from the same individual fish captured at different time periods to judge stability directly. Specifically, I want to examine repeated measures at different ages to determine fully the time when calcification stops and signatures become stable. Perhaps the juvenile fish I used were too young to have stable signatures and, had I waited to collect them at the end of the nursery period, tag degradation would have been negligible. It may also be possible that scale chemistry is altered in a predictable fashion such that any degradation could be corrected. Lastly, Thorrold et al. (1998) noted an increase of ~25% in accurate classification when $\delta^{13}C$ and $\delta^{18}O$ values were analyzed in addition to trace elements in otoliths. Alone, otolith trace elements led to slightly lower classification accuracies than those from scale chemistry. It may be best to include isotope data when available especially for fish sampled along a latitudinal gradient. Little, however, is understood about isotopic incorporation in the scale and it was cost prohibitive to examine this for the work presented here.

CHAPTER V

SUMMARY

I have shown that changes in the environment can directly affect the elemental signatures in the scale (Chapter II). This finding confirms that the scale signature is a result of the environment in which the fish resided. Therefore, it follows that differences in environmental conditions within natal regions can be represented on the scale as I demonstrated with juvenile weakfish along the Atlantic coast. Further, I showed that the relationship between scale and environmental conditions is linear for two commonly assayed elements (Sr and Ba) such that subtle differences in the water chemistry can be reconstructed with good precision. Unlike otoliths, Sr:Ca levels in the scale were not affected by temperature. Therefore, differences observed in Sr:Ca levels in the scale are unambiguously caused by differences in water chemistry. I also helped to elucidate some of the processes which lead to temperature dependent Sr:Ca levels in the otoliths. As growth was equal between temperature treatments, differences in otolith Sr:Ca levels must be attributed to transfer of ions into the endolymphatic fluid or onto the aragonite itself (temperature dependent affinity). Cadmium:Ca was not as clear a relationship but can be considered an element which would act as a good binary indicator (presence and absence) of the environmental conditions experienced by fish. This is not true for otoliths which rarely have detectable levels of Cd within them.

Correlations between Sr and Ba signatures in scales and otoliths were very high (~ 80-90%; Chapters II and III) and were also significant for Mn (~ 66%; Chapter III). This gives evidence that scales may be a useful surrogate for otoliths for reconstruction of

environmental conditions experienced by the fish. Further, scales generally had higher concentrations of Cd, Ba, Mn, and Mg (Chapters II and III) such that chemical analysis can be performed with less expensive methods with higher limits of detection (rather than ICP-MS). The higher concentrations in the scales also reduces counting errors compared to otoliths, thus increasing precision of the assays.

Significant differences in the elemental signatures of scales existed between juvenile weakfish from five estuaries along the Atlantic coast (Chapter III). This indicates that the differences in environmental conditions along the Atlantic coast can be recorded within scale material and later used to identify natal estuaries. The signatures also varied between the years sampled, indicating that continued monitoring of signatures for each cohort may be needed. Classification procedures (LDFA) indicated that the signatures were significantly different enough to classify individual juveniles with ~ 65% accuracy (Chapter III). In 1996, cross-validated classification accuracy ranged from 38% for Delaware Bay, to 86% for Pamlico Sound, with an overall accuracy of 67%. Classification accuracy in 1997 ranged from 41% for Chesapeake Bay to 83% for Pamlico Sound, with an overall accuracy of 65%. Inter-annual variability in the trace element signatures meant that fish could not be accurately classified to natal estuary based on signatures collected from juvenile fish in a different year.

I demonstrated, with simulations on the juvenile data, that the proportions of juveniles from each natal estuary could be estimated with ~ 90-95% accuracy (Chapter IV) using a new ML procedure. When scale chemistry was used to determine the natal-estuary contribution to adults the results were similar to otolith allocations. However, I also showed that the elemental signatures in the scale degraded over time. I argued that

this degradation was due to continued crystallization of the scale after the juvenile period. In fact, I postulated that Ca abundance in the scale nearly tripled. Interestingly, however, Mn:Ca and Ba:Ca in scales and otoliths remained highly correlated after maturation (~80% and ~70% respectively). This indicated that these elements, unlike Sr and Mg, remained stable for defining the elemental signature. The fact that they remained stable indicated that Mn was only diluted due to crystallization and Ba was left untouched in the fibrillary plate. Further research will address fully these questions of stability. It is likely that a predictable pattern of degradation in scale chemistry can be used to correct for the alteration. This is especially true given the results of Chapter II in which assimilation curves were developed. If these relationships are used in conjunction with information about the environment in which fish reside and additional information about when the scale becomes stable, I believe that alterations will have a negligible effect on understanding stock structure and movements.

REFERENCES

Aoba, T., E. C. Moreno, and S. Shimoda. 1992. Competitive adsorption of magnesium and calcium ions onto synthetic and biological apatites. Calcified Tissue International 51:143–150.

Bagenal, T. B., F. J. H. Mackereth, and J. Heron. 1973. The distinction between brown trout and sea trout by the strontium content of their scales. Journal of Fish Biology 5:555-557.

Bath, G. E., S. R. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 2000. Sr and Ba uptake in otoliths of marine fish. Geochima et Cosmochimica Acta 64:1705-1714.

Beacham, T. D., R. E. Withler, and T. A. Stevens. 1996. Stock identification of chinook salmon (*Oncorhynchus tshawytscha*) using minisatellite DNA variation. Canadian Journal of Fisheries and Aquatic Sciences 53:380-394.

Belanger, S. E., D. S. Cherry, J. J. Ney, and D. K. Whitehurst. 1987. Differentiation of freshwater versus saltwater striped bass by elemental scale analysis. Transactions of the American Fisheries Society 116:594-600.

Bentzen, P., G. G. Brown, and W. C. Leggett. 1989. Mitochondrial DNA polymorphism, population structure, and life-history variation in American shad (*Alosa sapidissima*). Canadian Journal of Fisheries and Aquatic Sciences 46:1446 - 1454.

Bereiter-Hahn, J. and L. Zylberberg. 1993. Regeneration of teleost fish scale. Comparative Biochemical Physiology 105A:625–641.

Bigi, A., E. Foresti, R. Gregorini, A. Ripamonti, N. Roveri, and J. S. Shah. 1992. The role of magnesium on the structure of biological apatites. Calcified Tissue International 50:439-444.

Bilton, H. T. 1975. Factors influencing the formation of scale characters. International North Pacific Fishery Commission Bulletin 32:102–108.

Bilton, H. T. and G. L. Robins. 1971. Effects of starvation, feeding, and light period on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*). Journal of the Fisheries Research Board of Canada 28:1749–1755.

Bowker, A. H. 1948. A test for symmetry in contingency tables. Journal of the American Statistical Association, 43:572-574.

Bronte, C. R.,R. J. Hasselberg, J. A. Shoesmith, and M. H. Hoff. 1996. Discrimination among spawning concentrations of Lake Superior lake herring based on trace element profiles in sagittae. Transactions of the American Fisheries Society 125:852-859.

Burnell, J. M., E. J. Teubner, and A. G. Miller. 1980. Normal maturational changes in bone matrix, mineral, and crystal size in the rat. Calcified Tissue International 31:13-19.

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

Campana, S. E. and J. M. Casselman. 1993. Stock discrimination using otolith shape analysis. Canadian Journal of Fisheries and Aquatic Sciences 50:1062-1083.

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

Campana, S. E. and J. A. Gagné. 1994. Cod stock discrimination using ICPMS elemental assays of otoliths. Pages 671-691. *In*: Secor, D. H., J. M. Dean, S. E.Campana, editors. New developments in fish otolith research. University South Carolina Press, Columbia, South Carolina.

Campana, S. E., and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences. 42: 1014-1032.

Campana S.E., S. R. Thorrold, C. M. Jones, D. Gunther, M. Tubrett, H. Longerich, S. Jackson, N. M. Halden, J. M. Kalish, P. Piccoli, H. de Pontual, H. Troadec, J. Panfili, D. H. Secor, K. P. Severin, S. H. Sie, R. Thresher, W. J. Teesdale, and J. L. Campbell. 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Canadian Journal of Fisheries and Aquatic Sciences 54:2068-2079.

Cheng, P. W. and W. N. Tzeng. 1996. Timing of metamorphosis and estuarine arrival across the dispersal range of the Japanese eel, *Anguilla japonica*. Marine Ecological Progress Series 131:87-96.

Coffey, M. F., F. Dehairs, O. Collette, G. Luther, T. Church, and T. Jickells. 1997. The behaviour of dissolved barium in estuaries. Estuarine, Coastal and Shelf Science 45:113-121.

Colodner, D., V. Salters, and D. C. Dockworth. 1994. Ion sources for analysis of inorganic solids and liquids by MS. Analytical Chemistry 66:1079A-1089A.

Coutant, C. C. and C. H. Chen. 1993. Strontium microstructure in scales of freshwater and estuarine striped bass (*Morone saxitilis*) detected by laser ablation mass spectrometry. Canadian Journal of Fisheries and Aquatic Sciences 50:1318-1323.

Dailey, M. D., and W. K. Vogelbein. 1991. Parasite fauna of three species of Antarctic whales with reference to their use as potential stock indicators. Fishery Bulletin 89:355-365.

Dove, S. G., B.M. Gillanders, and M. J. Kingsford. 1996. An investigation of chronological differences in the deposition of trace metals in the otoliths of two temperate reef fishes. Journal of Experimental Marine Biology 205:15-33.

Edmonds, J. S., N. Caputi, and M. Morita. 1991. Stock discrimination by trace element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. Australian Journal of Marine and Freshwater Research 42:383-389.

Edmonds, J. S., R. C. J. Levanton, N. Caputi, and M. Morita. 1992. Trace elements in the otoliths of yellow-eye mullet (*Aldrichetta forsteri*) as an aid to stock identification. Fisheries Research 13:39-51.

Edmonds, J. S., M. J. Moran, N. Caputi, and M. Morita. 1989. Trace elemental analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australian waters. Canadian Journal of Fisheries and Aquatic Sciences 46:50-54.

Ennevor, B. C. and R. M. Beames. 1993. Use of lanthanide elements to mass mark juvenile salmonids. Canadian Journal of Fisheries and Aquatic Sciences 50:1039-1044.

Epifanio, J. M., P. E. Smouse, C. J. Kobak, and B. L. Brown. 1995. Mitochondrial DNA divergence among populations of American shad (*Alosa sapidissima*): how much variation is enough for mixed stock analysis? Canadian Journal of Fisheries and Aquatic Sciences 52:1688-1702.

Farrell, J., and S. E. Campana. 1996. Regulation of calcium strontium deposition in the otoliths of juvenile tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology 115A:103-109.

Fouda, M. M. 1979. Studies on scale structure in the common goby *Pomatoschistus microps* Kroyer. Journal of Fish Biology 15:173-183.

Fournier D. A., T. D. Beacham, B. E. Riddell, and C. A. Busack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Canadian Journal of Fisheries and Aquatic Sciences 41:400–408.

Fowler, A. J., S. E. Campana, C. M. Jones, and S. R. Thorrold. 1995a. Experimental assessment of the effect temperature and salinity on elemental composition of otoliths using solution-based ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 52:1421-1430.

Fowler, A. J., S. E. Campana, C. M. Jones, and S. R. Thorrold. 1995b. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Canadian Journal of Fisheries and Aquatic Sciences 52:1431-1441.

Gallaher, N. K. and M. J. Kingsford. 1996. Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: an experimental investigations. Journal of Fish Biology 48:174-186.

Gauldie, R. W.,G. Coote, I. F. West, and R. L. Radtke. 1990. The influence of temperature on the flourine and calcium composition of fish scales. Tissue & Cell 22:645-654.

Gauldie, R. W., I. F. West, and G. Coote. 1991. Seasonal and environmental codes in the chemistry of the scales of the hoki *Macruronus novaezelandiea*. Tissue & Cell 23:489-503.

Gedalia, I., I. Mayer, J. Giron, and D. Deutsch. 1982. Fluoride deposition in the bones of rats determined by fluoride and X-ray diffraction analysis. Archives of Oral Biology 27:823–825.

Geffen, A. J., N. J. G. Pearce, and W. T. Perkins. 1998. Metal concentrations in fish otoliths in relation to body composition after laboratory exposure to mercury and lead. Marine Ecology Progress Series 165:235-245.

Gillanders, B. M. and M. J. Kingsford. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Marine Ecological Progress Series 141:13-20.

Graves, J. E., J. R. McDowell, and M. L. Jones. 1992. A genetic analysis of weakfish, *Cynoscion regalis*, stock structure along the mid-Atlantic coast. Fishery Bulletin 90:469-475.

Gunn, J. S., I. R. Harrowfield, C. H. Proctor, and R. E. Thresher. 1992. Electron probe microanalysis of fish otoliths - evaluation of techniques for studying age and stock discrimination. Journal of Experimental Marine Biology and Ecology 158:1-36.

Hamada, M. and A. Mikuni. 1990. X-ray diffraction analysis of sardine scale ash. Nippon Suisan Gakkaishi 56:947–951.

Hamada, M., T. Nagai, N. Kai, Y. Tanoue, H. Mae, M. Hashimoto, K. Miyoshi, H. Kumagai, and K. Saeki. 1995. Inorganic constituents of bone of fish. Fisheries Science 61:517-520.

Henshaw, J. M., E. M. Heithmar, and T. A. Hinners. 1989. Inductively coupled plasma mass spectrometric determination of trace elements in surface waters subject to acid deposition. Analytical Chemistry 61:335-342.

Hoff, G. R. and L. A. Fuiman. 1995. Environmentally induced variation in elemental composition of red drum *(Sciaenops ocellatus)* otoliths. Bulletin of Marine Science 56:578-591.

Houk, R. S. 1986. Mass spectrometry of inductively coupled plasmas. Analytical Chemistry 58:97A-105A.

Kalish, J. M. 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fishery Bulletin 88:657-666.

Kalish, J. M. 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus.* Marine Ecology Progress Series 74:137–159.

Lane, D. W. and D. F. Peach. 1997. Some observations on the trace element concentrations in human dental enamel. Biological Trace Element Research 60:1–11.

Legeros, R. Z., M. A. Miravite, G. B. Quirolgico, and M. E. J. Curzon. 1977. The effect of some trace elements on the lattice parameters of human and synthetic apatites. Calcified Tissue Research Supplement 22:362–367.

Lester, R. J. G., K. B. Sewell, A. Barnes, and K. Evans. 1988. Stock discrimination or orange roughy, *Hoplostethus atlanticus*, by parasite analysis. Marine Biology 99:137-143.

Losee, F. L., M. E. J. Curzon, and M. F. Little. 1974. Trace element concentrations in human enamel. Archives of Oral Biology 19:467-470.

Lowerre-Barbieri, S. K., M. E. Chittenden Jr., and L. R. Barbieri. 1995. Age and growth of weakfish, *Cynoscion regalis*, in the Chesapeake Bay region with a discussion of historical changes in maximum size. Fishery Bulletin. 93:643-656.

Lowerre-Barbieri, S. K., M. E. Chittenden Jr., and C. M. Jones. 1994. A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. Fishery Bulletin 92:555-568.

Millar, R. B. 1990. Comparison of methods for estimating mixed stock fishery composition. Canadian Journal of Fisheries and Aquatic Sciences. 47:2235-2241.

Mills, L. S. and F. W., Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10:1509-1518.

Moreau, G., C. Barbeau, J. J. Ferenette, J. Saint-Onge, and M. Simoneau. 1983. Zinc, manganese and strontium in opercula and scales of brook trout (*Salvelinus fontinalis*) as indicators of lake acidification. Canadian Journal of Fisheries and Aquatic Sciences 40:1685–1691.

Mugiya, Y., I., Hakomori, and K., Hatsutori. 1991. Trace metal incorporation into otoliths and scales in the goldfish, *Carassius auratus*. Comparative Biochemistry and Physiology 99C:327-331.

Mugiya, Y. and N. Watabe. 1977. Studies on fish scale formation and resorption - II. Effect of estradiol on calcium homeostasis and skeletal tissue resorption in the goldfish, Carassius auratus, and the killifish, Fundulus heteroclitus. Comparative Biochemical Physiology. 57A:197-202.

Mulligan, T. J., F. D. Martin, R. A. Smucker, and D. A. Wright. 1987. A method of stock identification based on the elemental composition of striped bass *Morone saxitilis* (Walbaum) otoliths. Journal of Experimental Marine Biology and Ecology 114:241-248.

Nesbit, R. A. 1954. Weakfish migration in relation to conservation. U.S. Department of Interior Special Scientific Report: Fisheries Number115.

Nichols, P. R. 1960. Homing tendency of American shad, *Alosa sapidissima*, in the York River, Virginia. Chesapeake Science 1:200-201.

Nolan, K., J. Grossfield, and I. Wirgin. 1991. Discrimination among Atlantic coast populations of American shad (*Alosa sapidissima*) using mitochondrial DNA. Canadian Journal of Fisheries and Aquatic Sciences 48:1724-1734.

Norris, W. P., W. Chavin, and L. S. Lombard. 1963. Studies of calcification in a marine teleost. Annals of the New York Academy of Science. 109:312-336.

Papadopoulou, C., G. D. Kanias, and E. Kassimati. 1980. Trace element content in fish otoliths in relation to age and size. Marine Pollution Bulletin 11:68-72.

Pender, P. J. and R. K. Griffin. 1996. Habitat history of barramundi *Lates calcarifer* in a North Australian river system based on barium and strontium levels in scales. Transactions of the American Fisheries Society 125:679-689.

Proctor, C. H., R. E. Thresher, J. S. Gunn, D. J. Mills, I. R. Harrowfield, and S. H. Sie. 1995. Stock structure of the southern bluefin tuna, *Thunnus maccoyii*: an investigation based on probe microanalysis of otolith composition. Marine Bulletin 122:511-526.

Radtke, R. L. 1989. Strontium-calcium concentration ratios in fish otoliths as environmental indicators. Comparative Biochemistry and Physiology 92A:189-193.

Rieman, B. E., D. L. Myers, and R. L. Nielsen. 1994. Use of otolith microchemistry to discriminate *Oncorhynchus nerka* of resident and anadromous origin. Canadian Journal of Fisheries and Aquatic Sciences 51: 68 - 77.

Rosenthal, H. L. 1963. Uptake, turnover and transport of bone seeking elements in fishes. Annals of the New York Academy of Sciences. 109:278-293.

Rowe, P. M. and C. E. Epifanio. 1994. Tidal stream transport of weakfish larvae in Delaware Bay, USA. Marine Ecology Progress Series 110:105-114.

Ruzzante, D. E., C. T. Taggart, D. Cooke, and S. G. Goddard. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Canadian Journal of Fisheries and Aquatic Sciences 53:634-645.

SAS Institute. 1989. SAS/STAT user's guide version 6, volume 1. SAS, Cary, North Carolina.

Secor, D. H. 1992. Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay striped bass, *Morone saxitilis*. Fishery Bulletin 90:798-806.

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

Severin, K. P., J. Carroll, and B. L. Norcross. 1995. Electron microprobe analysis of juvenile walley pollock, *Theragra chalcogramma*, otoliths from Alaska: a pilot stock separation study. Environmental Biology of Fishes 43:269-283.

Sie, S. H. and R. E. Thresher. 1992. Micro-PIXE analysis of fish otoliths: methodology and evaluation of first results for stock discrimination. International Journal of PIXE 2:357-379.

Snyder, R. J., B. A. McKeown, K. Colbow, and R. Brown. 1992. Use of dissolved strontium in scale marking of juvenile salmonids: effects of concentration and exposure time. Canadian Journal of Fisheries and Aquatic Sciences 49:780-782.

Swearer, S. E., J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral-reef fish. Nature 402: 799-802.

Szedlmayer, S. T., K. W. Able, J. A. Musick, and M. P. Weinstein. 1991. Are scale circuli deposited daily in juvenile weakfish, *Cynoscion regalis*? Environmental Biology of Fishes 31:87-94.

Takagi, Y. T. Hirano, and J. Yamada. 1989. Scale regeneration of tilapia (*Oreochromis niloticus*) under various ambient and dietary calcium concentrations. Comparative Biochemical Physiology 92A:605-608.

Thorrold, S.R., J. E. Graves, and C. M. Jones. 1996. Application of otolith elemental fingerprinting and microsatellite DNA markers as natural tags of young-of-the-year weakfish (*Cynoscion regalis*) along the U. S. Atlantic coast. Proposal to Sea Grant, Virginia Graduate Marine Science Consortium.

Thorrold, S. R., C. M. Jones, and S. E. Campana. 1997. Response of otolith microchemistry to environment variations experienced by larval and juvenile Atlantic Croaker (*Micropogonias undulatus*). Limnology and Oceanography 42:102-111.

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

Thresher, R. E., C. H. Proctor, J. S. Gunn, and I. R. Harrowfield. 1994. An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae). Fishery Bulletin 92:817-840.

Townsend, D. W., R. L. Radtke, S. Corwin, and D. A. Libby. 1992. Strontium:calcium ratios in juvenile Atlantic herring, *Clupea harengus* L., otoliths as a function of water temperature. Journal of Experimental Marine Biology and Ecology 160:131-140.

Thorrold, S. R., and S. Shuttleworth. 2000. In situ analysis of trace elements and isotope ratios in fish otoliths using laser ablation sector field ICP-MS. Canadian Journal of Fisheries and Aquatic Sciences. 57: 1232-1242.

Underwood, A. J. 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, New York.

van Coillie, R. and A. Rousseau. 1974. Composition minerale des ecailles du *Catostomus commersoni* issu de deux milieux differents: etude par microscopie electronique and analytique. Journal of the Fisheries Research Board of Canada 31:63-66.

van Oosten, J. 1957. The skin and scales. Pages 207-244. *In* M. E. Brown, editor. The physiology of fishes. Academic Press, New York.

Veinott, G. I., and Evans, R. D. 1999. An examination of elemental stability in the fin ray of white sturgeon with laser ablation sampling - inductively coupled plasma - mass spectrometry (LAS-ICP-MS). Transactions of the American Fisheries Society 128:352 -361.

Wang, S., R. Brown, and D. J. Gray. 1994. Application of laser ablation-ICPMS to the spatially resolved micro-analysis of biological tissue. Applied Spectroscopy 48:1321-1325.

Wells, B. K., G. E. Bath, S. R. Thorrold, and C. M. Jones. 2000a. Incorporation of strontium, cadmium, and barium in juvenile spot, *Leistomus xanthurus*, reflects water chemistry. Canadian Journal of Fisheries and Aquatic Sciences. 57:2122-2129.

Wells, B. K., S. R. Thorrold, and C. M. Jones. 2000b. Geographic variation in trace element composition of juvenile weakfish scales. Transactions of the American Fisheries Society 129:889-900.

Westrheim, S. J., W. H. Barss, E. K. Pikitch, and L. F. Quirollo. 1992. Stock delineation of Dover sole in the California-British Columbia region, based on tagging studies conducted during 1948 - 1979. North American Journal of Fisheries Management 12:172-181.

Whaley, R. A. 1991. An improved technique for cleaning fish scales. North American Journal of Fisheries Management 11:234-236.

Wilk, S. J. 1979. Biological and fisheries data of weakfish, *Cynoscion regalis* (Bloch and Schneider). NOAA Technical Series Report 21, NMFS Sandy Hook Lab., Highlands, New Jersey.

Winer B. 1971. Statistical principles in experimental design, 2nd edition. McGraw-Hill, New York.

Wood, C. C., D. T. Rutherford, and S. McKinnell. 1989. Identification of sockeye salmon (Oncorhynchus nerka) stocks in mixed-stock fisheries in British Columbia and southeast Alaska using biological markers. Candian Journal of Fisheries and Aquatic Sciences. 46:2108-2120.

Yamada, S. B. and T. J. Mulligan. 1982. Strontium marking of hatchery reared coho salmon, *Oncorhynchus kisutch* Walbaum, identification of adults. Journal of Fish Biology 20:5-9.

Yamada, S. B. and T. J. Mulligan. 1987. Marking nonfeeding salmonid fry with dissolved strontium. Canadian Journal of Fisheries and Aquatic Sciences 44:1502-1506.

Yamada, S. B., T. J. Mulligan, and S. J. Fairchild. 1979. Strontium marking of hatchery-reared coho salmon (*Oncorhynchus kisutch*, Walbaum). Journal of Fish Biology 14:267-275.

Zylberberg, L., J. Bonaventure, L. Cohen-Solal, D. J. Hartman, and J. Bereiterhahn. 1992. Organization and characterization of fibrillar collagens in fish scales in situ and in vitro. Journal of Cellular Science 103:273-285.

APPENDIX 1

MAXIMUM LIKELIHOOD SAS PROGRAM

```
title1 'D. Naik: A SAS Program for Discriminant Analysis';
title2 'assuming Mixture of Multivariate Normal Model';
title3 'and using Both Classified and Unclassified Data';

*The IML code;
proc iml;
use tstdata;

*Create data matrix using the sas data set;
read all var {MG_CA,MN_CA,SR_CA,BA_CA} into x;

*Create a vector containing information on group allocation of the
observations;
*The group variable is assumed to have numbers 1,2,..  as the
populations;
read all var {group} into g;

* Specify the number of groups as k= ;
k=5;

n=nrow(x);
p=ncol(x);

phat=j(k,1,0);
xbar=j(k,p,0);

*Determine the number of classified observations in each group;
m=j(k,1,0);
mc=0;
do i=1 to k;
  do j=1 to n;
    if g[j]=i then do;
    m[i]=m[i]+1;
    xbar[i,]=xbar[i,]+x[j,];
    end;
  end;
xbar[i,]=xbar[i,]/m[i];
mc=mc+m[i];
end;

*This matrix will be used later in the iteration steps;
```

```
xbarm=diag(m)*xbar;

*Identify and store the unclassified observations;
xun=j(n-mc,p,0);
ind=0;
do j=1 to n;
  if g[j]=. then do;
  ind=ind+1;
  xun[ind,]=x[j,];
  end;
end;

*Sample mean vector and variance covariance matrix using all the data;
xbarbar=(1/n)*j(1,n,1)*x;
qu=i(n)-(1/n)*j(n,n,1);
s=(1/(n))*t(x)*qu*x;

*Inverse of s to use later for the calculation of Sigma inverse;
si=inv(s);

*Initial Values;
do j=1 to k;
  phat[j]=m[j]/(mc);
end;

b1hat=j(k,p,1);
oldb1hat=j(k,p,0);
count=0;

*Start the iteration process;
do while(max(abs(b1hat-oldb1hat))>0.0001);
oldb1hat=b1hat;

count=count+1;
dphat=diag(sqrt(phat));

*Computation of Sigma inverse;
C=dphat*(xbar-j(k,1,1)*xbarbar);
smati=inv(i(k)-c*si*t(c));
siginv=si+si*t(c)*smati*c*si;

*Computation of b1hat and b0hat;
b1hat=xbar*siginv;
b0hat=(-0.5)*vecdiag(xbar*t(b1hat));

* Computation of Posterior probabilities;
```

```
px=j(k,n-mc,0);

do i=1 to n-mc;
  do j=1 to k;
    px[j,i] = b0hat[j]+b1hat[j,]*t(xun[i,]);
    px[j,i]=exp(px[j,i])*phat[j];
  end;
end;

do i=1 to n-mc;
const=0;
  do j=1 to k;
    const=const+px[j,i];
  end;
  do j=1 to k;
    px[j,i]=px[j,i]/const;
  end;
end;
*print px;
* Computation of phat;
do j=1 to k;
  phat[j]=(sum(px[j,])+m[j])/n;
end;

*print phat;

* Computation of mu_j (Xbar);
xbar=xbarm+px*xun;
do j=1 to k;
  xbar[j,]=xbar[j,]/(n*phat[j]);
end;

end; /* End of the iterations */

* Determining the proportion of unclassified observations
classified to each of the k populations;

*the method that follows can be used to make razor edge decisions;
* about where individuals should be allocated;

/*
*n_class=j(k,n-mc,0);
*do i=1 to n-mc;
*  do j=1 to k;
*    if px[j,i]=max(px[,i]) then n_class[j,i]=1;
*  end;
```

```
*end;
*
*p_class=j(k,1,0);
*do j=1 to k;
*  p_class[j]=sum(n_class[j,])/(n-mc);
*end;
*/


*what follows is the equation for determining the;
*composition of the unclassified data by using all;
* probabilities;


p_class=j(k,1,0);
do j=1 to k;
  p_class[j]=sum(px[j,])/(n-mc);
end;

*on a very rare occasion two percent at most;
* the calculation of variable smati above;
*becomes so large the program blows up;
* to account for this I have eliminated those observations;
*I do not know IML so I eliminated them outside of IML;
** as a delete statement;

/*
print 'k Posterior probabilities are written in k rows';
print px;
print 'Number of iterations';
print count;

print 'The estimated probabilities: phat';
print phat;

print 'The proportion of unclassified observations';
print 'classified to the k populations';
print p_class;
*/
```

APPENDIX 2

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1

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Wells, B. K., C. M. Jones, and S. R. Thorrold. 1998. ID-ICP-MS analysis of scales from laboratory reared juvenile spot *(Leiostomus xanthurus)* to examine the effects of ambient elemental concentrations on the elemental signature of the scale. Virginia Graduate Marine Science Consortium (R/MG-99-01) through the Virginia Sea Grant. $6,825.00

PUBLICATIONS:

Wells, B. K., S. R. Thorrold, and C. M. Jones. 2000. Geographic variation in trace element composition of juvenile weakfish scales. *Transactions of the American Fisheries Society*. 129:889 - 900.

Wells, B. K., G. E. Bath, S. R. Thorrold, and C. M. Jones. 2000. Incorporation of strontium, cadmium, and barium in juvenile spot (*Leiostomus xanthurus*) scales reflects water chemistry. *Canadian Journal of Fisheries and Aquatic Sciences*. 57: 2122 - 2129.

Jones, C. M. and B. Wells. Yield-per-recruit analysis for black drum, *Pogonias cromis*, along the East Coast of the U.S. and management strategies for the Chesapeake Bay. *Fishery Bulletin*. In Press.

Jones, C. M. and B. Wells. 1998. Age, growth, and mortality of black drum. *Pogonias cromis*, in the Chesapeake Bay. *Fishery Bulletin* 96: 451 – 461.