Summer 2012

Assessing Movement of Fish Through Spectral Analysis of Otolith Life History Scans

Renee Reilly Hoover
Old Dominion University

ASSESSING MOVEMENT OF FISH THROUGH SPECTRAL ANALYSIS OF OTOLITH LIFE HISTORY SCANS

by

Reneé Reilly Hoover
B.S. May 2007, The Pennsylvania State University
M.S. August 2009, Old Dominion University

A Dissertation Submitted to the Faculty of Old Dominion University in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

OCEANOGRAPHY

OLD DOMINION UNIVERSITY
August 2012

Approved by:

Cynthia M. Jones (Director)

Chester E. Grosch (Member)

Norou Diawara (Member)

ABSTRACT

ASSESSING MOVEMENT OF FISH THROUGH SPECTRAL ANALYSIS OF OTOLITH LIFE HISTORY SCANS

Reneé Reilly Hoover
Old Dominion University, 2012
Director: Dr. Cynthia M. Jones

The ability to accurately measure movement timing across environmental gradients is fundamental for testing hypotheses in marine ecology that deal with ingress, egress, and migration of fish. Timing and patterns of movement have been estimated using life-history scans of the chemical signatures encoded in fish otoliths (ear stones). I provide a quantitative approach to examining life history scan data using spectral analysis, which retrospectively measures the movement timing for individual fish. Sagittal otoliths from juvenile Atlantic croaker (*Micropogonias undulatus*) and adult black sea bass (*Centropristis striata*) were sampled using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS).

For Atlantic croaker, spectral analyses of the data estimate the timing of ingress at 68 days on average using strontium and 85 days using barium. Based on the inflection points of their nonlinear mixing curves, these data reveal entry and subsequent movement up-estuary. Moreover, I use spectrally-derived estimates to show that growth rates did not drive ingress timing for these samples. These data thus lend no support to the critical-size hypothesis in this instance.

I additionally hypothesized that the three-dimensional structure of otoliths could produce sampling artifacts in the results of laser ablation scans. To test this hypothesis,

I ablated two trenches of different depths on each otolith, performing spectral analyses on these data to investigate the effects of ablation depth, including differences in periodicities and temporal variability between trenches. The means of the two trench depths were significantly ($t=114.25$, $p<0.0001$). From shallow to deep trenches, variance decreased. Peaks or poles are very useful indicators of the frequency and periodicity (seasonal effect) when working with spectral densities. In this case, peaks in spectral density shifted in absolute value an average of 32 days. These results highlight the necessity of considering the depth of ablation when conducting life history scans.

I examined the periodic movement of a coastal fish population by quantifying the inshore-offshore migration patterns in black sea bass. I sampled adult black sea bass from two portions of the population's residency as a means of making inferences about this expected life history pattern, using differences in space as a proxy for differences in time. I estimated the movement timing between inshore and offshore environments using the aforementioned spectral analysis techniques. Mean timing of this periodic movement was estimated at 365 days using strontium and 455 days using barium. The otolith edge chemistry between the inshore and offshore samples was significantly different in both strontium ($t=33.48$, $p<0.0001$) and barium ($t=7.59$, $p<0.0001$), with offshore samples consistently exhibiting much higher barium signals. These results offer preliminary evidence to the hypothesis of barite-rich waters being supplied to the offshore coastal shelf via submarine canyon upwelling processes.

Dedicated to my husband, Benjamin, with more gratitude than my words will ever be able to express.

ACKNOWLEDGEMENTS

I would like to thank my dissertation committee: Dr. Cynthia M. Jones, Dr. Chester E. Grosch, and Dr. Norou Diawara, for their endless guidance and patience. My thanks also go to my advisory committee, made up of Dr. Cynthia M. Jones, Dr. Norou Diawara, and Dr. Alexander Bochdansky, for bringing me through my coursework and the doctoral candidacy experience.

I will be forever indebted to my advisor and mentor, Dr. Cynthia M. Jones for this degree. Never have I met a more dedicated scientist, teacher, or mentor. Dr. Jones brings her commitment to all three of these objectives to bear every single day. Though I worry about her reaction to my saying so, her impact on my life in fact defies quantification.

Dr. Norou Diawara has spent countless hours answering what surely must have felt like the most inane questions to me. I thank him from the bottom of my heart for understanding both my frustrations and joys in learning new statistical methods and applications. It is through Dr. Diawara that I really began to learn statistics; while that is a lifelong process, the foundations upon which I will build are strong thanks to him.

Without question, this body of work would not have been possible without Dr. Chester E. Grosch. Always willing to help me and always insightful, I am so thankful to have ever had the chance to meet Dr. Grosch, let alone work with him. He is truly one of the most intelligent and gracious people I know.

The group of individuals, past and present, at the Center for Quantitative Fisheries Ecology has become a second family to me. To Dr. Hongsheng Liao, Dr. William E. Persons, Dr. Stacy K. Beharry, James Davies, Jason Ferguson, Kristen Anstead, Michael Schmidtke, and Antranik Kajajian, Jessica Gilmore, Rebekah Joyce, and Brandy Thompson: thank you. Not only did each of you literally help me in the completion of this dissertation, be it sectioning otoliths or listening to my concerns, but also, coming to the lab was always easy with your bright faces there to cheer me on.

Dr. Jason J. Schaffler has been helpful to so many of the students at our lab. To list the aspects of my degree that Dr. Schaffler helped me with would be to outline the dissertation itself, and then some. Literally every step of the way, he was there to offer advice, instruction, and support. I sincerely thank him for his tremendous help and heart.

A special thanks to Dr. Benjamin Cuker and the Hall-Bonner Fellowship; through Dr. Cuker's programs I was able to develop a very special network of colleagues in the aquatic sciences. Also through his programs, I was afforded the opportunity to travel to several scientific meetings, as well as an understanding of how the business of science operates.

The new community of colleagues I have gained during my first year of employment at the Virginia Marine Resources Commission deserves their own book of thanks, mostly for their patience with me throughout this time. I am so humbled by their kindness and acceptance of me, and their support throughout this final year of my doctoral degree. I look forward to many more years of fulfilling work together.

Though not included in this dissertation, the research that I completed in my first two years at ODU was very much a part of my doctoral experience. From that work, I would like to specifically thank Jacques van Montfrans and Deb Parthee at the Virginia Institute of Marine Science, Dr. Robert F. Dias, Dr. Hussein Abdulla, William Thompson, Michael Lane, and David Franklin for all of their help.

I am so thankful to Sabrina Reilly for her unfailing wisdom, support, and perspective, to Debra and John Kwiterovich-Hoover for being there always, to Idalia Robles for checking in on me always, and to my parents for, just about everything, always. Thank you.

I thank the Department of Ocean, Earth, and Atmospheric Sciences at Old Dominion University for the opportunity to complete this degree. This research was funded in part by a grant from the National Science Foundation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Background

Describing the abundance and distribution of species lies at the core of ecological research. Two key aspects that dictate abundance and distribution of species are the dispersal and movement of individuals. Particularly in fisheries ecology, these dynamics are difficult to quantify. The success of tagging studies to estimate movement has been limited for a number of reasons, especially in the case of larval and juvenile fish. Applying tags to fish is logistically difficult, financially exhaustive, and recapture rates tend to be very low. Additionally, to obtain spatially descriptive data requires the use of either acoustic or archival tags (see Sibert and Nielsen, 2001). While the development of such tags in recent years has rapidly advanced, the fundamental logistic issues remain. As a result, the use of natural tags has grown in popularity in the areas of population dynamics and quantitative fisheries ecology.

Fish otoliths (ear stones) have proven valuable as natural tags, allowing discrimination among populations or stocks (Kalish 1990; Thresher et al. 1994), tracking dispersal and retention (Secor and Rooker 2000), as well as habitat use patterns (Tanner et al. 2012). The following work focuses on using otoliths as a recorder of fish movement through various environments. The otolith has a chronological record of the age of fish, in addition to a chemical signature which in some way describes the environment the fish experienced, making it an extremely valuable tool in fisheries science. By understanding the timing of ingress in larval and

juvenile fish, I can more accurately predict patterns of species abundance. Likewise, our knowledge of adult movement and migration patterns directly impacts fisheries science and management, by directly allowing for estimation and description of fish/angler interactions, while also providing distribution information that is used in stock assessment modeling.

Biochemistry of otoliths

Fish otoliths are biological recorders of age, past environment, and life history. Otoliths are generally composed of calcium carbonate ($CaCO_3$), in the form of aragonite, precipitated in a proteinaceous matrix (Degens et al. 1969). As a fish grows, a layer of calcium carbonate accretes onto the otolith each day (Panella 1980), allowing fisheries scientists to determine ages and growth rates of individuals (Campana and Neilson 1985). Not only does the physical size of the otolith increment reflect somatic growth, but the otolith's composition additionally reflects the ambient water chemistry.

This characteristic is due to the crystalline structure of the otolith. As each increment forms, some calcium ions are replaced by minor and trace elements, such as strontium or barium (Dorval et al. 2005). These and other cations can enter the bloodstream through either digestion (i.e. food sources) or by crossing the branchial membranes (Campana 1999). The ions must then travel to the inner ear and across a membrane into the endolymph, where they are available for crystallization (Walther and Thorrold 2006). As such, the concentration of these elements can reflect both dietary and environmental sources of the ions that eventually precipitate out of solution and are deposited on the surface of the otolith. Importantly, the otolith is also

metabolically inert, ensuring that patterns incorporated into the otolith are preserved throughout the life of the fish (Campana and Neilson 1985; Campana 1999).

Aside from the cations which enter the inorganic matrix, other markers and information are contained chronologically in the otolith. Stable isotope ratios of carbon and oxygen reveal patterns in temperature, and other trace metals and rare earth elements indicate changes in geochemistry, diet (Sanchez-Jerez et al. 2002), and metabolism. Additionally, the growth of the fish is also recorded in the size and structure of the otolith increments. The pattern of changes in minor and trace elements, stable isotope ratios, and growth can all be used to infer changes in water masses to which the fish was exposed.

Development of otolith chemistry

Otoliths have been used to study various topics in fisheries over the last century. The breadth of trace element otolith chemistry work has grown tremendously in relatively recent times, with the number of published articles increasing by at least an order of magnitude in the past 5 years. From 1992 to 2002, on the order of 70 articles regarding otolith chemistry were published, in the following decade, from 2002 to 2012, this number increased nearly 5 fold to over 340 articles. This increase in the number of studies using otolith chemistry has led to unparalleled advances in our understanding of fisheries ecology.

Strontium has been most effective in reconstructing movements of anadromous and catadromous fishes (Kalish 1990; Limburg 1995; Secor et al. 2001; Chang et al. 2004) due to the predictable changes in water strontium with salinity (*see reviews*:

Campana, 2005; Gillanders, 2005; Campana et al, 1997). In general, increasing salinity corresponds with increased strontium concentration and decreased barium concentration, with corresponding changes in otolith chemistry (Martin et al. 2004; Zimmerman 2005; Martin and Wuenschel 2006; Macdonald and Crook 2010). However, these relationships have been shown to be somewhat spatially-specific, again, particularly with respect to otolith uptake and deposition (*see, e.g., the strontium review by* Secor and Rooker, 2000). Moreover, the effects of other drivers such as diet and temperature on strontium incorporation into otoliths (Fowler et al. 1995) continue to be explored (Webb et al. 2012). All of this work has yielded a tremendous amount of insight into the movement of fish populations and the technical progress in measuring otolith chemical concentrations has developed rapidly. Unfortunately, one aspect of this research that has not fully developed is the application of appropriate statistical techniques for analyzing life history scan data.

When conducting life history scans on otoliths, one obtains a series of measurements of the chemical composition of the otolith from the core, which represents when the fish was hatched, to the edge, which represents its date of capture (Campana, 2005). While life history scan data have greatly increased our understanding of migration patterns and links among populations, their analyses have been largely qualitative (e.g., Humphreys, 2006; Tzeng, 2003). Assertions of differences among data must be supported by appropriate statistical tests, albeit these methods have been better explored in the theoretical and applied statistics literature.

The most common methods of statistical analysis in fisheries literature, however, are parametric. In the case of life history scans, employing these methods

may violate the underlying assumptions necessary for their use. In the otolith chemistry literature, parametric tests are seen, which assume independence of observations, being applied to data that are clearly autocorrelated (Chang et al. 2004; FitzGerald et al. 2004; Limburg and Elfman 2010).

Autocorrelation is the similarity between observations as a function of the time separating them. While autocorrelation can be used as a tool to find repeating patterns in certain cases, if unaccounted for, it makes statistical tests less stringent. For example, the classical regression model includes assumptions about the independence of its error terms. If the error terms are correlated with their own values, parameter estimates will be. Consequently, one could incorrectly reject the null hypothesis with a probability greater than the nominal significance level. A thorough discussion of the effects of autocorrelation is found in the climate and meteorological literature (Trenberth 1984; Ebisuzaki 1997; Thiébaux and Zwiers 1997; Wunsch 1999).

Data that have a natural temporal order, such as life history scans, can be statistically described as time series. Time series analyses make use of the fact that data observed close together in time will be more strongly related than those farther apart. Doing so accounts for the fact that the data are inherently autocorrelated, as is clearly the case with life history data. The chemical signature found in a given growth band of an otolith will be more similar to the preceding band than one formed weeks or months earlier. While there are several applications that may be used to analyze time series data, I concentrate on spectral analysis.

Spectral analysis estimates the spectral density function, or spectrum, of a given time series (Chatfield 2004). A spectral point of view is particularly helpful when

examining multivariate stationary processes (Brockwell and Davis 2002), because drawing even simple conclusions about the overall changes and differences in multivariate datasets often proves difficult. Examining the spectrum of our time series allows us to separate statistically significant periodic components from the noise inherently present in most empirical data.

Applying spectral analysis

The following studies introduce the application of spectral analysis to the time series obtained from life history scans of otoliths. The approach makes use of the full suite of information contained within these scans, including the spatio-temporal components of the data along with the elemental concentrations. The process of spectral analysis is computationally complex, but conceptually has been clearly analogized by Priestley (1982).

To paraphrase her analogy, the nature of spectral analysis can be related to our own visual perception of color. The quantitative measure of color is generally discussed in terms of light frequency. When viewing an object, human eyes observe the strength of the light it is reflecting, as well as its color. Since monochromatic light is rarely seen, a mixture of different frequencies usually makes up the observed colors. Human eyes are not very good spectrometers, however, as one can only give a rough approximation of the constituent colors (e.g. t-shirt appears "blueish"). To quantify how much of red light or blue light is making up the observed color, something that will separate the components is needed, such as a prism. This is in essence the process that spectral analysis achieves.

By applying spectral analysis to an otolith chemistry signal, I obtain measures for the periodic components which make up the observed signal. If there is a significant change in otolith chemistry, there will be a significant peak in the spectral density of the signal at that frequency or period. Just as each color has a corresponding frequency in the light analogy above, the periodic movements of fish will have a certain associated frequency. Because the otolith contains a temporal scale, I can relate this significant periodic component back to the age of the fish, revealing the timing of movement in a quantitative way.

Objectives

The following dissertation research develops the application of a new quantitative method for analyzing life history scan data. Three main questions are addressed through this work:

1) Is spectral analysis an appropriate tool for analyzing otolith life history scan data?
2) Does the depth of ablation affect trace element results of life history scans obtained by laser ablation inductively coupled plasma mass spectrometry?
3) Can spectral analysis of life history scans determine variations in movement patterns of fish from different water masses along the Atlantic coast?

Applications

Atlantic croaker

To answer the above questions, the following work will focus on applications to two species. The first species to which I apply the method is Atlantic croaker, *Micropogonias undulatus*. Atlantic croaker is a demersal species found in saline to brackish waters along the coast from South America to Nova Scotia; they are one of the most abundant fishes along the southeastern coast of the United States (Murdy et al. 1997). In the Mid-Atlantic populations, such as those in the Chesapeake Bay and Pamlico Sound, their life history strategy is to spawn offshore; their larvae are advected inshore through physical currents (Barbieri et al. 1994).

Because of this life history pattern, Atlantic croaker is an ideal case study for our investigations. The well-documented movement from off- to onshore habitats (Warlen and Burke 1990; Hettler 1998) create a strong change in the most commonly analyzed habitat markers of otolith strontium and barium. Using this expected shift in environmental markers, I am able to gauge the success of our method in accurately estimating ingress timing using life history scans. I estimated the ages and growth patterns Atlantic croaker using the growth curves as conversion factors to translate the life history scan data from a temporal reference of the laser to that of the fish.

I then explored the effects of laser ablation itself on the raw otolith chemistry data, as well as ingress timing estimates. I study the depth of laser ablation, a dimension which has not previously been discussed in the otolith chemistry literature, along with the temporal integration which may occur in the horizontal plane of the life history scan. I also investigate the ways in which laser ablation may impact different environmental markers (strontium versus barium), considering the different types of information each element contains.

Black sea bass

The second species for our applications is black sea bass, *Centropristis striata*. This species is a protogynous hermaphrodite that occurs all along the Atlantic coast and into the Gulf of Mexico. Fisheries scientists have inferred the migratory behavior of black sea bass from state and federal trawl surveys, as well as the patterns of targeted commercial and recreational fisheries (Drohan et al. 2007). The species spawns from April through November; by late fall, juveniles move offshore to spend their first winter in the continental shelf waters, and then return to their estuaries in the spring (Musick and Mercer 1977; Able et al. 1995). The seasonal timing of the inferred inshore-offshore migration suggests that movement may be triggered by a change in the environment, such as temperature (Moser and Shepherd 2009).

This recurrent pattern of movement provides a second type of otolith chemistry signal to investigate, that of a repeated oscillation. Whereas the Atlantic croaker juveniles exhibited one major shift in environment, the sea bass could potentially

move across their environmental gradient as many times as they are years old. The following study will investigate whether black sea bass are in fact moving from the onshore to the offshore environment in a seasonal manner.

The well-established recreational and commercial black sea bass fisheries that target offshore habitats in the winter months would suggest that these fish are using submarine canyons as a habitat type. While the effects of submarine canyons on water circulation patterns has been well-documented (She and Klinck 2000), their subsequent effects on fish assemblages is less understood. I will use these samples to examine whether sea bass from the Mid-Atlantic bight are using canyon systems during their winter movements offshore. Through this application, I begin to explore the hypothesis that persistent upwelling in submarine canyons produce areas of increased productivity for coastal fish species.

I address the three objective questions in Chapters II, III, and IV of the following dissertation, along with a summary of their findings, significance, and future directions provided in Chapter V.

CHAPTER II

ESTUARINE INGRESS TIMING AS REVEALED BY SPECTRAL ANALYSIS OF OTOLITH LIFE HISTORY SCANS

Introduction

The ability to accurately measure the timing of migration is fundamental in testing hypotheses in marine ecology that deal with ingress, egress, and general movement of fish populations. For example, the critical-size hypothesis (Houde 1997), which links an animal's ability to enter an estuary to its size and age, requires knowledge of both the mean and variance of the population's size or age upon ingress, the time of estuarine entry. Understanding variability in the timing of ingress is especially essential to evaluate the importance of nursery habitats in the recruitment of larval or juvenile fish in bays and estuaries. The critical-size hypothesis (*see review*: Sogard (1997)) has been supported by results in several species, but particularly well in salmon (Beamish and Mahnken 2001; Beamish et al. 2004; Farley Jr. et al. 2005). While these studies have increased our knowledge of complex drivers of recruitment dynamics, some of the key elements to testing the hypothesis itself lack quantifiable estimates, such as the timing of ingress.

Both the timing and patterns of movement in larval and juvenile fish are often estimated from scans of the chemical signatures encoded in their otoliths (Thorrold et al. 1998; Secor et al. 2001). The effectiveness of reconstructing these environmental histories from otoliths has been demonstrated convincingly (Kalish 1990; Limburg

1995; Secor et al. 2001; Chang et al. 2004; Jones and Campana 2009), especially with strontium (*see review*: Gillanders (2005)). However, while the technical progress in measuring otolith chemical concentrations has increased rapidly, application of appropriate statistical techniques for analyzing such data is still developing.

When conducting life history scans on otoliths, one obtains a series of measurements tracing the chemical composition from the core, when the fish was hatched, to the edge, which represents its date of capture (Campana 2005). Analyses of these data have been largely qualitative and graphical or, when parametric, limited to ANOVA (Tsukamoto and Arai 2001; Morris et al. 2003; Ben-Tzvi et al. 2007). Although on some level, all analyses are ad hoc, qualitative analyses suffer from being fully subjective in addition. Moreover, for life history scans, employing commonly used parametric methods such as ANOVA violates underlying assumptions because of the intrinsic autocorrelation of scan data. While autocorrelation can be used as a tool to find repeating patterns, if unaccounted for, it compromises statistical analyses because it violates the fundamental assumption of independence (Trenberth 1984; Ebisuzaki 1997; Thiébaux and Zwiers 1997; Wunsch 1999). In contrast, time series analyses account for the inherent autocorrelation in data that have a natural order, as with life history scan data. For example, when using otolith chemistry to measure movement, the signature found in one growth band will be more similar to the preceding band than to one formed several days earlier. A number of applications have been used to analyze time series data, specifically in testing hypotheses that require classification analyses. Fablet et al. (2007) reconstructed fish life histories using unsupervised signal processing methods in a Bayesian framework. Later, Daverat et al. (2011) applied this

same method in three catadromous species, revealing colonization tactics. Finally, zoning algorithms were used by Hedger et al. (2008) to classify otolith chemistry sequences.

While each of these techniques is an interesting use of life history scan data, they each have inherent assumptions about the data to achieve classification. I chose to concentrate on revealing the timing of ingress using a time series application that is particularly suited to the questions that arise in testing life history migration hypotheses: spectral analysis.

Spectral analysis estimates the spectral density function, or spectrum, of a given time series (Chatfield 2004). Examining the spectrum of a time series allows us to separate statistically significant periodic components exhibited by our data from the noise that is intrinsic in most empirical data. The strength of this approach is that it simply asks where significant changes in otolith chemistry occur, without specifying a model for the movement pattern itself.

Spectral analysis is not new to the field of ecology as a whole; for example, Trancart et al. (2011) applied Maximum Entropy Spectral Analysis (MESA) to the swimming activity of thinlip mullet. I introduce the application of spectral analysis to the time series data obtained from life history scans of otoliths, which, to our knowledge, has not been used on these data before. I use the ingress of juvenile Atlantic croaker (*Micropogonias undulatus*) to estuarine nurseries to demonstrate the ability of this method to distinguish and quantify changes in elemental concentrations in an otolith life history scan and to test the critical-size hypothesis. Specifically, I relate the peaks in spectral density of multiple elements to measure the timing of

ingress. Atlantic croaker spawn offshore in the late fall and juveniles move inshore from sea- to brackish-water approximately three months after peak spawning occurs (Warlen and Burke 1990). To estimate the timing of this movement, our approach makes use of the full suite of information contained within life history scans, including the chronology of environmental markers. Perhaps most importantly, I demonstrate the value of spectral analyses in providing a proper statistical tool to test migration hypotheses. Testing for differences in the distributions of ingress timing for populations from diverse locations, or for the presence of annual migration through environmental gradients becomes possible with quantifiable estimates for movement.

Materials and methods

Sample preparation and analysis

I sampled sagittal otoliths of juvenile Atlantic croaker (*Micropogonias undulatus*) collected from the Pamlico River in North Carolina (n=14), after standard length (SL) had been measured (57.5±9.4 mm). One otolith from each fish was chosen at random (left or right) and analyzed for trace element chemistry, while the second sagittal otolith was used for age and growth rate analyses. Otoliths ranged in diameter from 1064-1891μm. Otoliths were prepared for trace element analysis in a class 100 clean room using the Center for Quantitative Fisheries Ecology standard clean room protocols (Dorval et al. 2005). I cleaned the samples of any attached tissue and rinsed with ultrapure hydrogen peroxide (H_2O_2) and ultrapure Milli-Q water. Otoliths were affixed to microscope slides with trace-element free silicone adhesive, sectioned through the core, then polished using 30μm and 9μm lapping paper, ensuring a smooth

surface for laser ablation. Otoliths were then sonicated in ultrapure Milli-Q water and allowed to dry.

I conducted trace element analysis using the MAT Element 2 ICP-MS (Finnigan) at Old Dominion University. The ICP-MS was coupled to a 266 mm Nd:YAG laser, with spot size of 20μm, speed of $5\mu m \cdot s^{-1}$, power of 45%, producing a trench depth of 80μm. Instrument details are given in Jones and Chen (2003). I ablated a line from the core of the otolith to the edge, following the methods described by Thorrold et al. (1997). I analyzed otolith calcium (^{48}Ca), strontium (^{86}Sr) and barium (^{138}Ba) scanned in low-resolution mode (Chen and Jones 2006) on each of the 14 samples, creating 28 individual elemental profiles. I converted the raw data to element-to-calcium molar ratios.

Transverse thin sections were cut from the otoliths for ageing. Sections were mounted and polished to reveal the core for ageing of daily growth bands. Daily ages were obtained following the methods for juvenile Atlantic croaker of Nixon and Jones (1997). Daily increment widths, used for growth rate estimation, were obtained using ImagePro Version 5.0.1 software. Daily ages and increments were measured on the same sample.

Data analysis

Because of the inherently temporal nature of otolith chemistry, I related the significant features in the elemental spectra to time-specific events in an individual fish life history. One issue that arises when interpreting a laser scan is data spacing. Because the laser samples otoliths at a constant speed, the resulting data are distributed

equally in both laser-space and laser-time. Most fish do not grow at a constant rate, however, as is well demonstrated by many growth models (e.g., Nixon and Jones 1997; Schaffler et al. 2009a) for Atlantic croaker. The number of data points contained within each time increment, therefore, varies.

To convert evenly spaced laser data to non-linear fish growth, I translated the distance the laser traveled across the otolith into the amount of growth completed by the individual fish at each time step. Doing so allowed for a direct calculation of the fish's age for each otolith chemistry data point, which results in assignment of fractional age to each scan point. To appropriately model the change in increment widths over time, I first fit a growth curve to the fish size data. The Gompertz growth model (shown below) provided a good fit to the data (Figure 1),

$$y = A_0 e^{\left(e^{b_0}\left(\frac{e^{b_1 x}-1}{b_1}\right)\right)} \quad (1)$$

where y is the attained growth, A_0 is the value of the growth function at age 0, b_0 is the slope of the logarithm of the relative growth rate at age 0, and b_1 is the slope of the logarithm of the relative growth rate (*adapted from* Song and Kuznetsova (2003), *see also* Nixon and Jones (1997), and Gossett et al. (2007)).

After fitting individual growth models, I obtained parameter estimates for each fish, then back-calculated the fractional ages, using the formula below.

$$x = \frac{ln\left(\frac{b_1 \times ln\left(\frac{y}{A_0}\right)}{e^{b_0}} + 1\right)}{b_1} \quad (2)$$

where all parameters are as above, and x is the fractional age at each increment in laser-time (Figure 1).

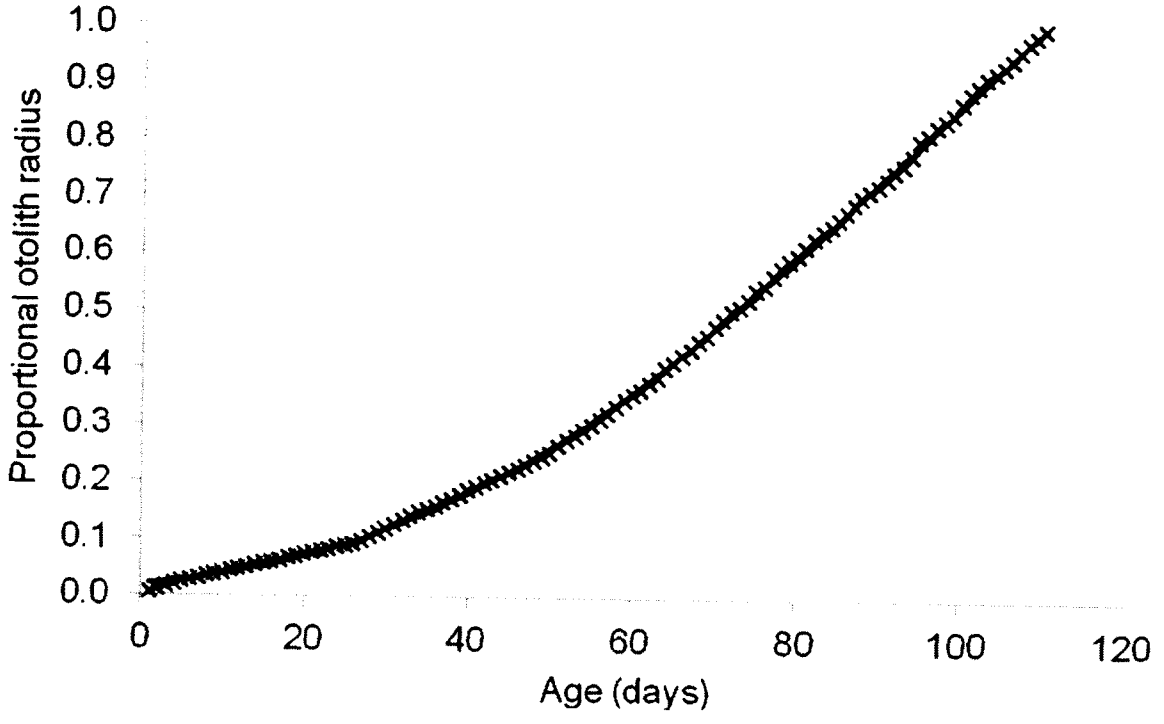

Fig. 1. Example plot of the fitted retrospective otolith radius data (Sample (j)). The solid line shows the predicted cumulative otolith radius and the cross-hatch line shows the observed otolith radius data, using the Gompertz model.

As a result, the raw data (Figure 2a) were spaced with respect to the increment width of each day (Figure 2b).Aside from correcting data spacing, the parameters from this model were also used in investigating potential relationships between the timing of ingress and the growth rates of individual fish.

After I converted laser data into fractional fish ages, the converted data were non-uniformly spaced (Figure 2). I therefore interpolated the data with the commonly used cubic spline to a spacing of 0.2 days (i.e., $\Delta t = 0.2$). Finally, because of the inherent noise in LA-ICPMS data, I applied a low-pass filter to remove the components of the series not adding information to our analysis, with the cutoff frequency (f) at $f\Delta t = 0.3$. The cutoff frequency sets the boundary of the filter, such that the frequencies greater than $f\Delta t = 0.3$ are not passed through the filter, hence "low-pass".

Detrending

To conduct spectral analyses on the interpolated data, I applied the general time series techniques of detrending and normalizing before calculating our spectral estimates (Brockwell and Davis 2002). These techniques are best described through a statistical example. Suppose our data set $\{X(j\Delta t) \equiv X_j\}$ for $j = 1, 2, \ldots, N$ with a mean $\bar{X}$ and variance σ_X^2, represents the time series of Ba:Ca measurements through time. The spectrum $S(f)$ is an estimate of the distribution of the signal "energy", with f as the frequency. Here f lies in the range $0 < f < f_{Ny} \equiv \frac{1}{2\Delta t}$, the Nyquist frequency$(f_{Ny})$. Because the spectrum is symmetric about $f = 0$,

$$2\int_0^{f_{Ny}} S(f)df = \sigma_X^2. \quad (3)$$

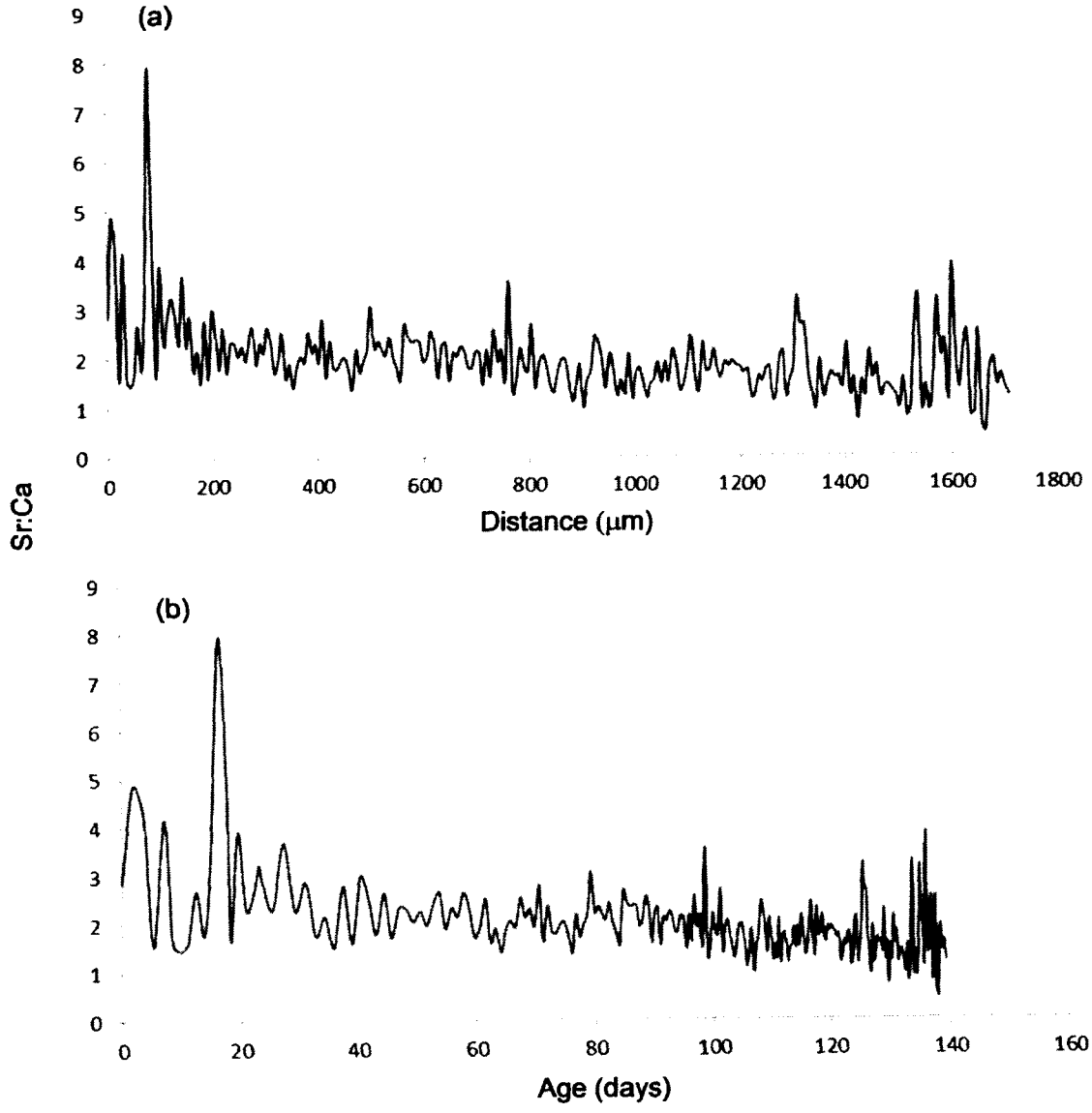

Fig. 2. (*a*) Sr:Ca versus distance (*b*) Sr:Ca versus age of the fish.

If X_j contains a trend, a large fraction (in some cases most) of the spectral energy will reside at low frequencies, i.e., at or in the vicinity of $f = 0$. This large peak in energy density at low frequencies obscures our ability to see the variation of $S(f)$ at higher frequencies in the plot of spectral density (Figure 3). For example, in our case I sought to determine whether or not there are any significant spectral "peaks" indicating a significant periodic component in our data. Presence of the low frequency peak caused by the trend made it difficult to separate these higher frequency peaks from background noise. I plotted the scan data to observe its main features and trends were apparent reflecting the well-studied transit from offshore to inshore (e.g., the decreasing trend in strontium seen in Figure 2). Uncovering the trend which is both clearly observed in the data and well-known to the life history of this fish, however, does not advance our knowledge of its ingress timing.

Therefore, the trend was modeled by fitting either a linear

$$X_j = A_1 + B_1 t_j \tag{4}$$

or quadratic function

$$X_j = A_2 + B_2 t_j + C_2 t_j^2 \tag{5}$$

to the data for each sample. The coefficients of the fit were found by the least squares method. The fitted trend curve was then subtracted from the original data. The criterion for determining if the trend was significant was computation of the variance before and after the fit, and use of an F-test to determine if the variances were significantly different for each individual. Of the 28 trend fits this entailed calculating, 17 were quadratic trends (p-values ranging from <0.0001 to 0.0119) and 11 were linear trends

(p-values ranging from <0.0005 to 0.025). Only those trends which were significant at the α=0.05 level were removed. I caution that detrending must be done carefully, and with knowledge of the species life history, to ensure no loss of valuable information occurs.

Standardization

Suppose I now have more than one time series, i.e., Ba:Ca measurements for Sample 1 = X and Ba:Ca concentrations for Sample 2 = Y. I standardized the data to have variables with means of zero, variances of 1, and

$$\int_0^{f_{Ny}} S_X(f)df = \int_0^{f_{Ny}} S_Y(f)\,df = \frac{1}{2} \tag{6}$$

(see example Figure 4). With standardization, the relative magnitudes of significant peaks in the respective spectra of the standardized data, $S_X(f)$ and $S_Y(f)$, are an accurate measure of the relative fraction of the total energy in each of the peaks. Therefore, the relative magnitude of peaks in the S_Y spectrum can be compared to those in the S_X spectrum, solving the problems of differing spectral variability.

Spectral estimate

Fourier methods are the basis of nearly all spectral estimation methods. A fundamental limitation of all such methods is that the frequency of resolution is fixed, i.e., spectral estimates are obtained for a set of N frequencies

$$\frac{f_k}{f_{Ny}} = \frac{k}{N}; k = 0, 1, 2, \ldots, N-1. \tag{7}$$

The Maximum Entropy Method (MEM) (Ulrych and Bishop 1975, Priestley 1982, Press et al. 1994, Lee et al. 2011) is a technique that allows spectral estimation for any

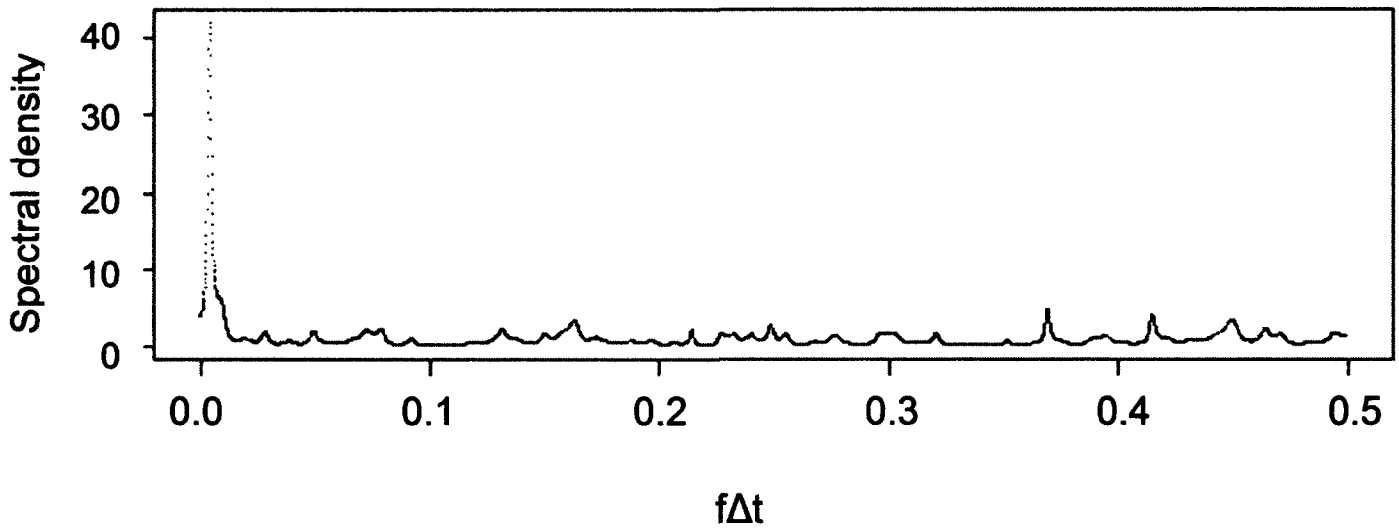

Fig. 3. Example spectrum of raw data for strontium over all fΔt from zero to f_{Ny}.

arbitrary set of frequencies between zero frequency and the Nyquist frequency (half the sampling frequency).

In Information Theory, entropy is a measure of the uncertainty associated with a random variable (Ihara 1993). The principle of maximum entropy states that the probability distribution which best represents the current state of knowledge is the one with the largest information-theoretical entropy. The MEM therefore, is based on choosing a spectral estimate $\hat{h}(\omega)$ which is such that the "entropy",

$$E = \int_{-\pi}^{\pi} log_e\{\hat{h}(\omega)\}d\omega, \tag{8}$$

is maximized, subject to the constraints,

$$E = \int_{-\pi}^{\pi} \hat{h}(\omega)e^{-i\omega r}d\omega = \hat{R}(r), \qquad r = 0, \pm 1, \dots, \pm k, \tag{9}$$

where k is a given integer and the $\{\hat{R}(r)\}$ are the sample autocovariances. I seek the functional form of $\hat{h}(\omega)$that maximizes E, subject to the constraints that the first k Fourier coefficients of $\hat{h}(\omega)$ must match exactly the first k sample autocovariances (Priestley 1982).

The MEM is particularly good, as compared to Fourier methods, at resolving spectral peaks at relatively low frequencies, because it is calculating the spectral estimate for an autoregressive model that has been fit to the data, rather than calculating the spectral density of the data themselves. As such, the MEM is able to estimate the spectral density of a single or even partial oscillation. The MEM is based on fitting the N data values, the $\{X_j\}$, with an autoregressive model of order $M(AR(M)$ process, $M < N$). An autoregressive process is said to be of order M if

$$X_t = \alpha_1 X_{t-1} + \cdots + \alpha_M X_{t-M} + Z_t \tag{10}$$

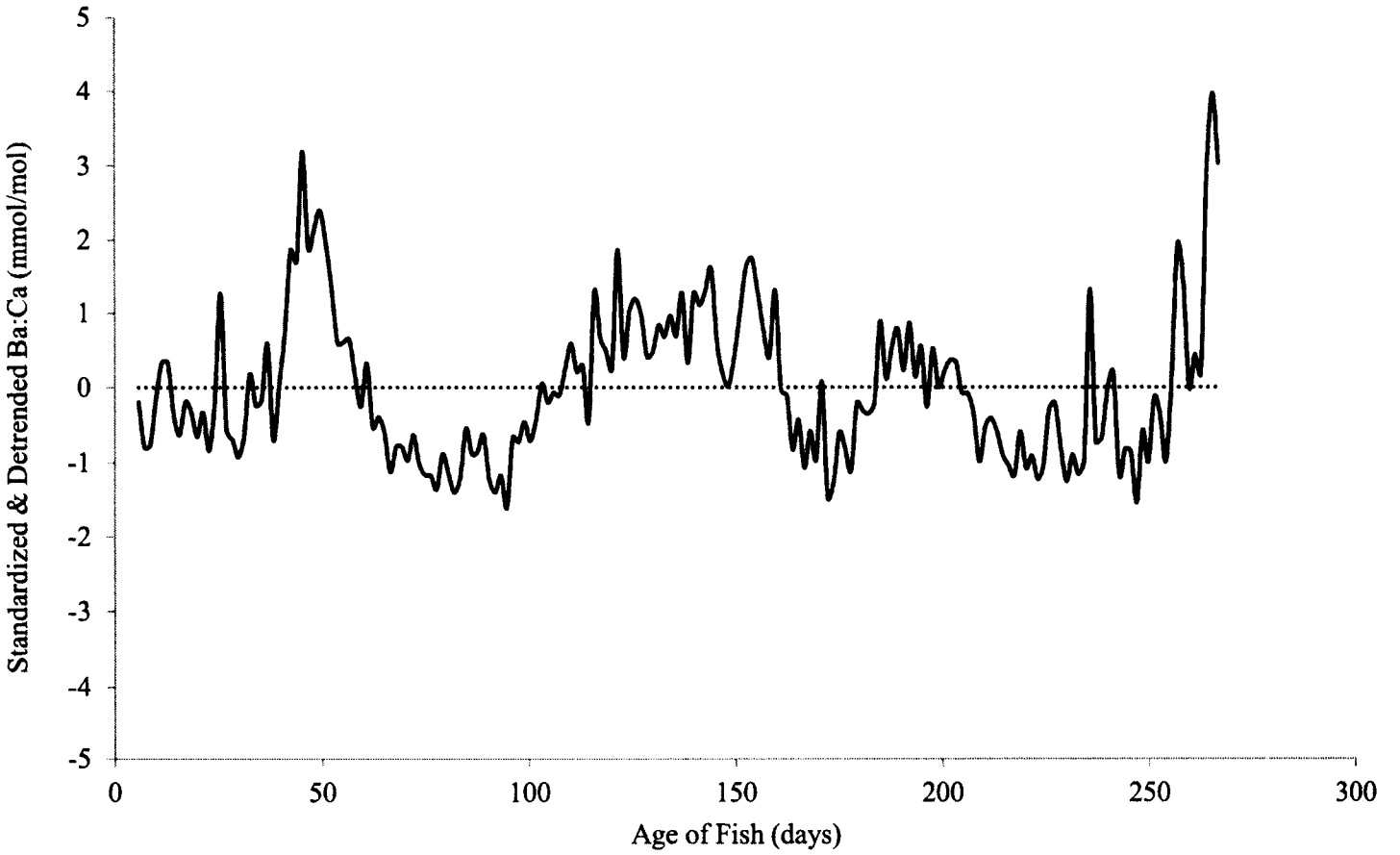

Fig. 4. Example of the standardized and detrended barium data.

where a_i are coefficients and Z_t are random variates, with mean of zero and standard deviation σ. Here, X_t is regressed on past values of X_t rather than independent variables, hence the model is autoregressive. The MEM spectral estimator is a function of the continuously varying frequency f with $(0 \leq f \leq f_{Ny})$, and is found using the formula for the spectrum of the $AR(M)$ process as a function of f.

The spectra presented here were computed using the algorithm for MEM given in Press, et al. (1994). The spectral estimates were obtained for 5,000 values of f in $(0, f_{Ny})$ and equivalently for $f\Delta t$ in $(0, \frac{1}{2})$. A test of the numerical accuracy of the calculation is to numerically integrate the spectrum and compare the results to $\frac{1}{2}$. In all cases, the error was < 2% (Table 1).

Confidence limits

The confidence limits of the spectral estimates depend on two factors, the null hypothesis as to form of the assumed noise spectrum, and the number of degrees of freedom (dof) of the spectral estimate. The null hypothesis often involves the assumption that the $\{X_j\}$ are white noise. For the calculated spectra, I observed that all of the spectral peaks found were at relatively low frequencies, so I used a stronger null hypothesis for the confidence limits: the $\{X_j\}$ was red noise as generated by an $AR(1)$ process. Fewer spectral peaks will be significant when assuming an $AR(1)$ error structure than the more general $AR\left(\frac{N}{2}\right)$, making these confidence limits more conservative. If $\{X_j\}$ was generated by an $AR(1)$ process, then it follows that the

constant $a = \rho(1)$, the autocorrelation coefficient of the $\{X_j\}$ at lag 1, and the normalized spectrum of this $AR(1)$ process, $S_{AR(1)}$is

$$S_{AR(1)}(f) = \frac{1-a^2}{1-2a\cos(\theta)+a^2} \tag{11}$$

where $\theta = 2\pi f \Delta t$ with $0 \leq f\Delta t \leq \frac{1}{2}$ (Priestley 1982).

I computed the value of $\rho(1)$ of the detrended and standardized time series of each data set and the basic red noise spectrum as above. The 0.99 confidence limits for this spectrum were assumed to be those of the chi-squared distribution with the dof of the computed MEM spectrum (Vecer and Herman 2011). In all cases for the spectral estimates, M was chosen to be $N/2$ so the process was an $AR(N/2)$ process with

$$dof = \frac{N}{M} = 2 \tag{12}$$

for these spectra. Thus the confidence limits for the calculated MEM spectrum are those of the chi-squared 0.99 limits, with $dof = 2$, for the $S_{AR(1)}$spectrum. Peaks that are significant represent changes in elemental composition that reflected life-history, environment, or habitat change. The highest peak in the spectral density plot represented the most significant periodic component of the series.

While both barium and strontium are obtained from the same scan, I analyzed each element to obtain univariate elemental signals, each containing its own unique information. I chose to analyze these data in a univariate setting because both strontium and barium offer different information pertaining to the timing of movement up-estuary, and I can assume that the frequencies based on these two variables are independent. Such an assumption will be tested by comparing the ingress timing

Table 1. Differences between numerical integrations of spectra and 0.5 (test of numerical accuracy of spectral estimates).

Sample	Sr	% Difference	Ba	% Difference
1	0.497	0.27	0.497	0.27
2	0.498	0.19	0.498	0.20
3	0.512	-1.20	0.498	0.20
4	0.497	0.27	0.498	0.18
5	0.499	0.15	0.498	0.16
6	0.499	0.12	0.499	0.11
7	0.498	0.20	0.498	0.20
8	0.497	0.27	0.497	0.27
9	0.498	0.22	0.498	0.18
10	0.498	0.20	0.498	0.20
11	0.498	0.25	0.498	0.25
12	0.498	0.20	0.498	0.20
13	0.498	0.20	0.498	0.20
14	0.498	0.25	0.498	0.25

estimates for the same individual using each marker separately, and again in Chapters 3 and 4 of this dissertation.

To test the critical-size hypothesis, I selected the highest peak in spectral density for each individual, and regressed the time (in days) of that peak against the growth rate of each individual. Were the critical-size hypothesis to be supported by our data, I would expect the fastest growing individuals to have the earliest timing of ingress relative to slower-growing fish.

Results

I analyzed 14 juvenile Atlantic croaker ranging in age from 78 days to 145 days old (mean = 121.5 days, 1SD = 18.62 days) and standard length (SL) ranged from 41 to 70 mm (mean = 56.6 mm, 1SD = 8.61 mm).

Using the Gompertz growth model (Equation 1), I found the mean growth rate was 0.0115 $mm \cdot day^{-1}$ (1SD = 0.004). Growth curves showed consistently good fits to the observed data (e.g., Figure 1). The mean parameter estimates were A_0 = 0.0279, b_0 = -2.939, and b_1 = -0.0111, with average proportional standard errors being 4.996%, 1.049%, and 6.195% for A_0, b_0, and b_1, respectively.

Mean Sr:Ca (Figure 5a) and Ba:Ca (Figure 5b) plots demonstrate the overall trends in the raw data. The barium signal shows the expected pattern of low to high concentrations as the fish move from a higher to lower salinity environment. Conversely, strontium decreases over time. For each individual, either a linear or quadratic trend was fit to the data for the detrending step.

I calculated the spectral density and confidence limits (α=0.01) of strontium (Figure 6) for each fish. The most significant (highest) peaks in strontium spectral density were fairly uniform in their frequency distribution across ages (Figure 7a), with a range of 29 to 111 days for all samples.

The spectral density and confidence limits (α=0.01) for barium (Figure 8) showed a slightly negatively skewed frequency distribution of peaks (Figure 7b). The range in most significant peaks for barium was 26 to 95 days, with 43% occurring near 85 days.

Secondary peaks were present, however, and I noted that a significant peak occurring at some point between 50 to 95 days was present in 57% of the strontium spectra and 93% of the barium spectra. Additionally, none of the samples' peak densities occurred at an age of less than 26 days.

I plotted the highest peak in spectral density against the absolute value of the fish growth rates (Figure 9a) and found no linear relationship for either strontium (R^2=0.1564, p=0.1617; RMSE=24.0057) or barium (R^2=0.1251, p=0.2147; RMSE=18.2095). However, plotting the highest peak in spectral density against fish age (Figure 9b) did reveal a relationship with barium (R^2=0.8523, p<0.001; RMSE=7.4819) but not strontium (R^2=0.0368, p=0.5111; RMSE=25.6503). I report these results only as an example of the types of tests that could be conducted using these data.

Discussion

Our results produced a quantitative point estimate for the timing of migration for each individual fish. I account for the inherent autocorrelation of scan data, while at

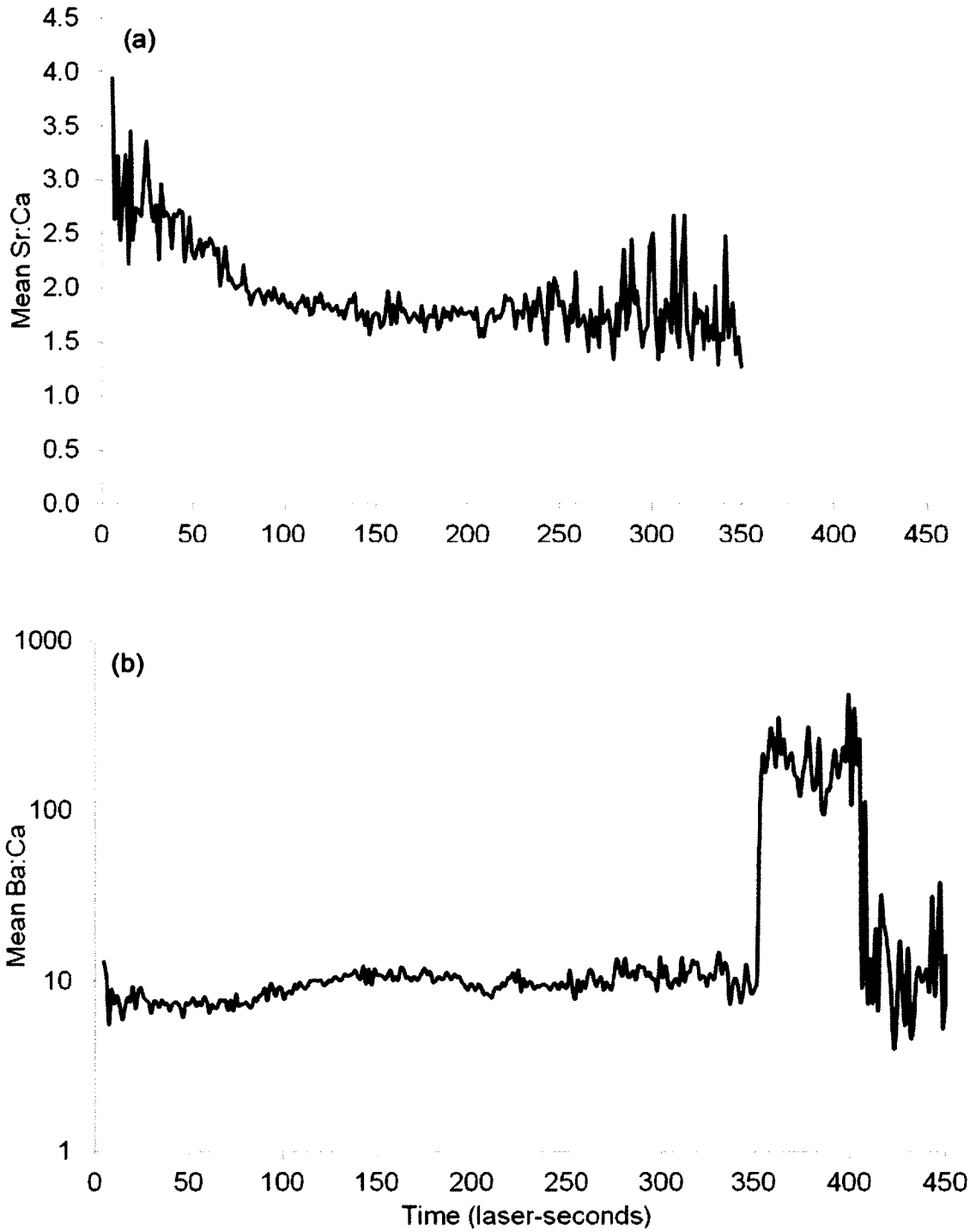

Fig. 5. (*a*) Mean Sr:Ca versus laser scan time (seconds) (*b*) mean Ba:Ca versus laser scan time (seconds).

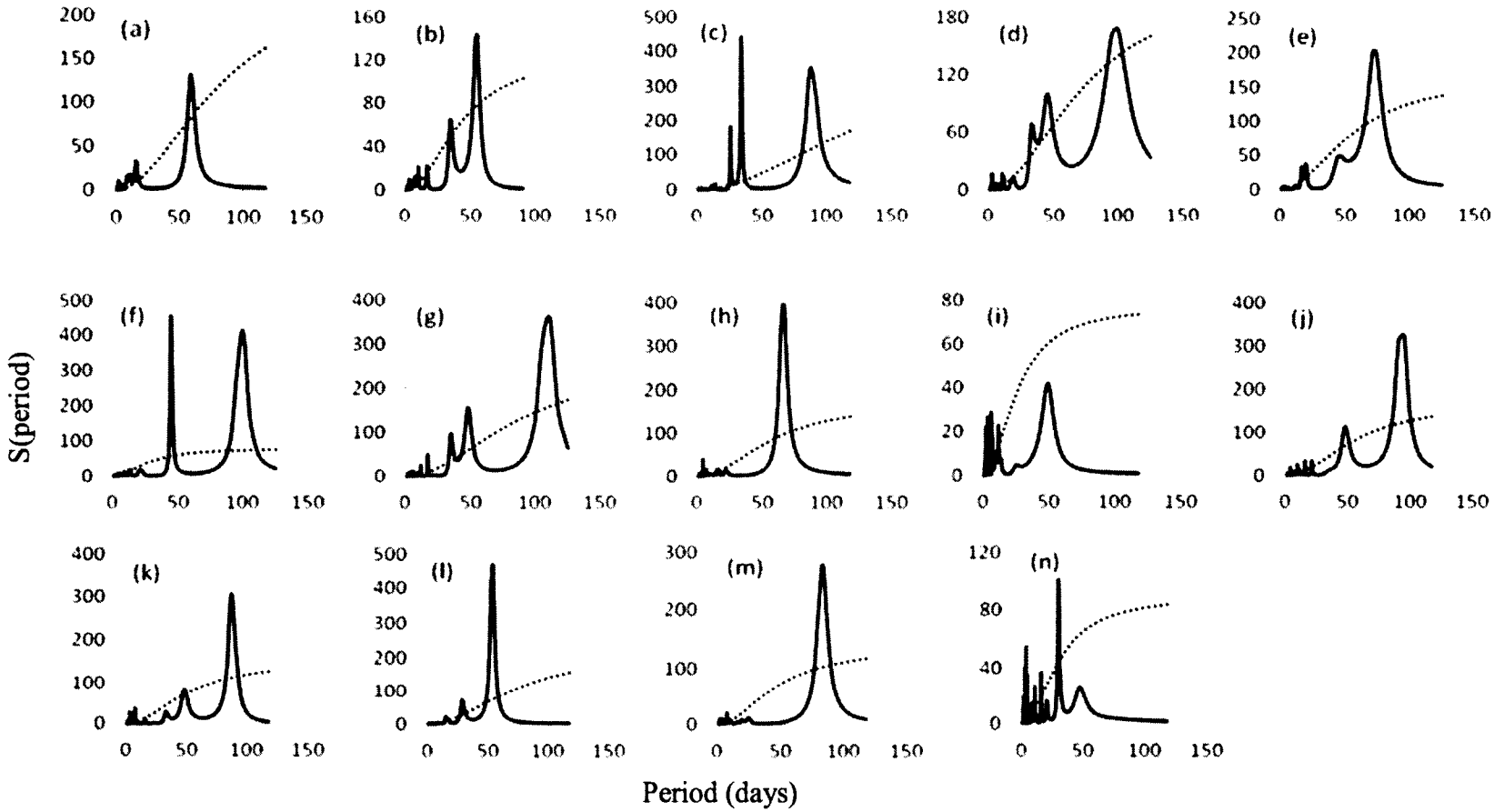

Fig. 6. Spectral density plots for strontium (n=14). The x-axis is the periodicity of the signal in days, the y-axis is the strength of the signal (as measured by energy density). The solid lines indicate the spectral density and the dotted lines represent the 99% confidence limit. Those peaks exceeding the confidence limit represent significant periodic components, and the magnitude of each peak is related to its level of significance, i.e., the highest peaks are the most significant components.

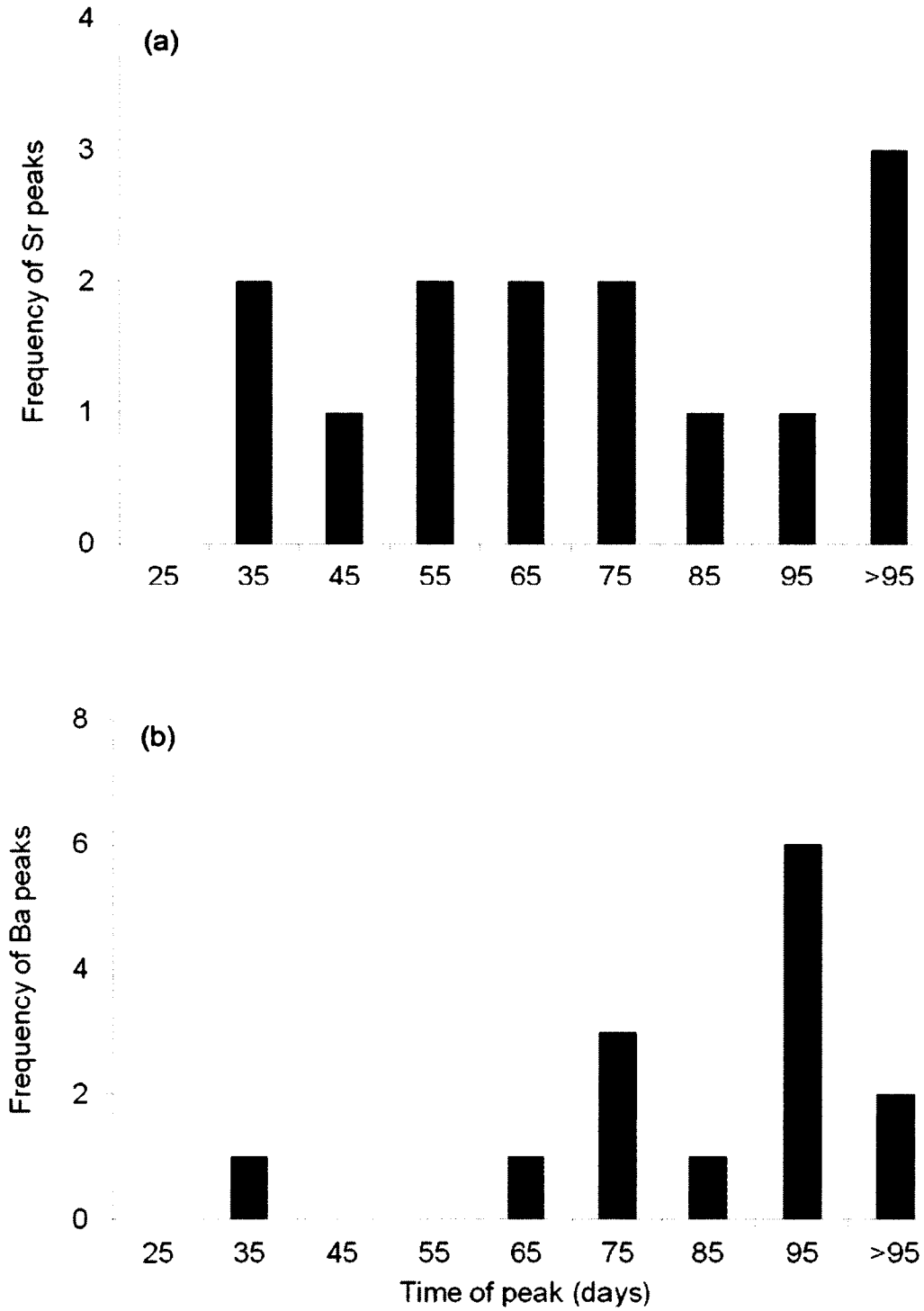

Fig. 7. Frequency distribution of the most significant spectral density peaks for (*a*) strontium and (*b*) barium.

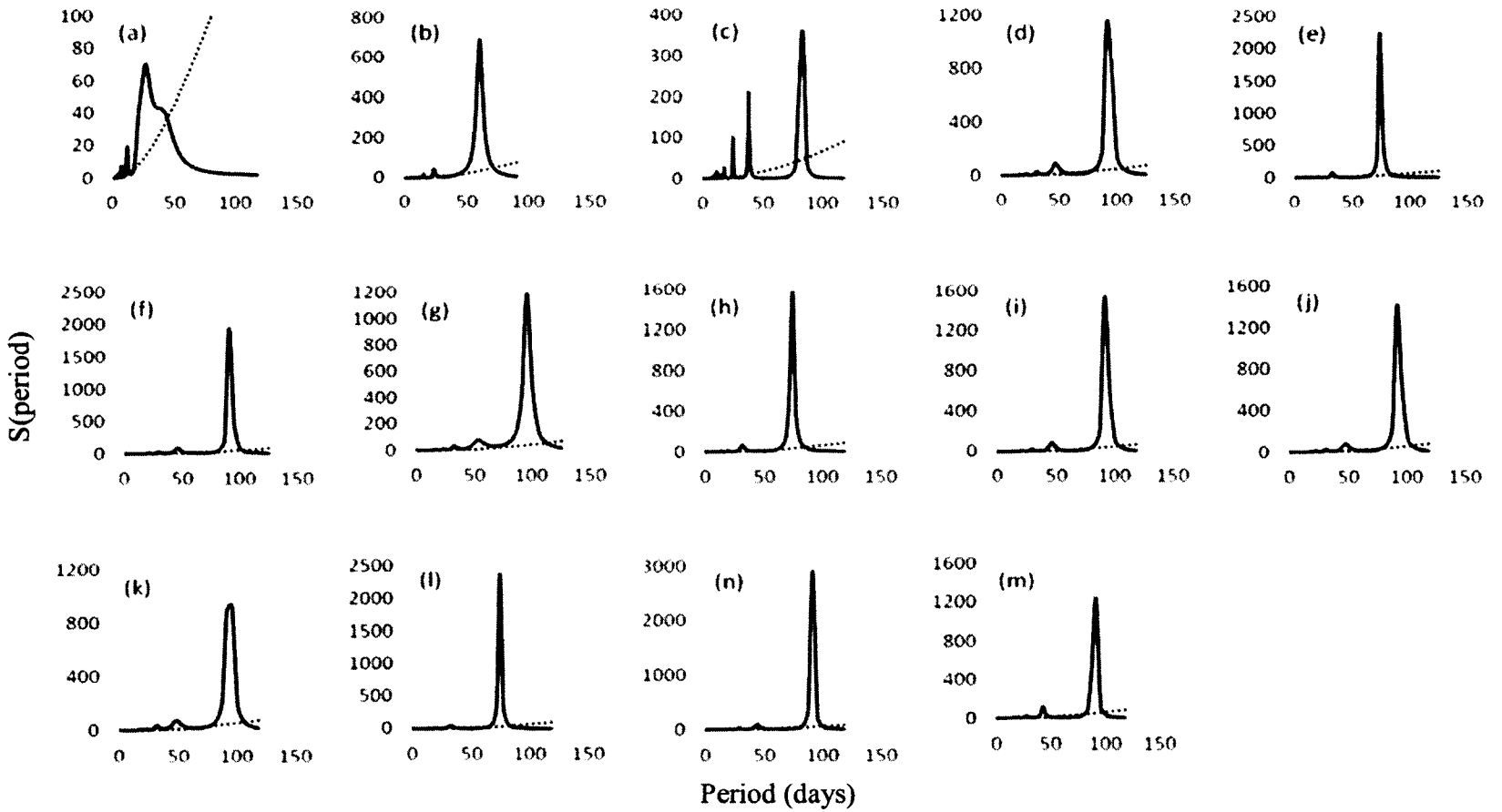

Fig. 8. Spectral density plots for barium (n=14). The x-axis is the periodicity of the signal in days, the y-axis is the strength of the signal (as measured by energy density). The solid lines indicate the spectral density and the dotted lines represent the 99% confidence limit. Those peaks exceeding the confidence limit represent significant periodic components, and the magnitude of each peak is related to its level of significance, i.e., the highest peaks are the most significant components. Note the consistent highly significant peak at approximately 50-90 days.

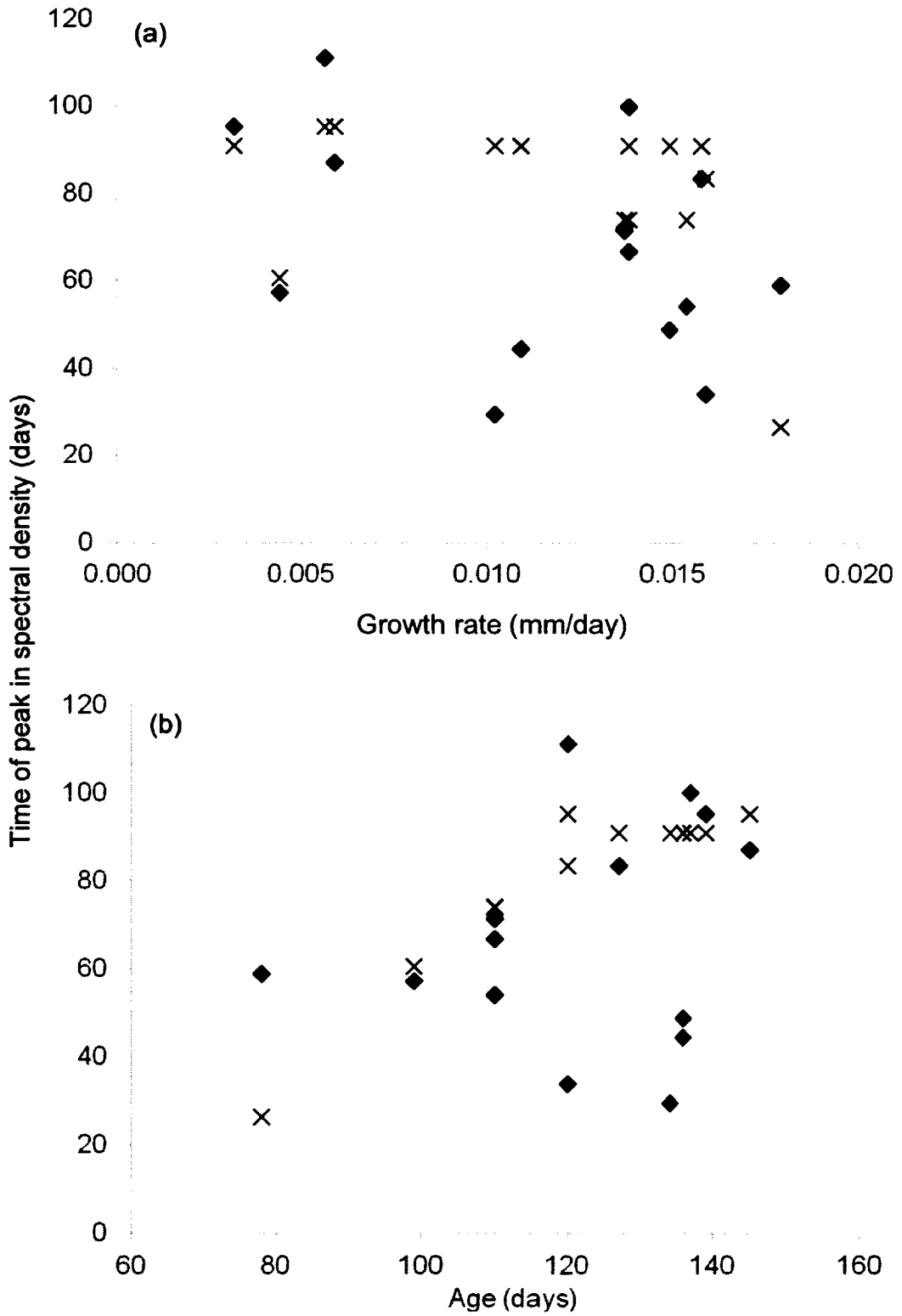

Fig. 9. (*a*) Peak in spectral density (estimated time of ingress) versus the fish growth rate (Equation 1) and (*b*) peak in spectral density (estimated time of ingress) versus the fish age (Equation 2) for both strontium (diamonds) and barium (cross-hatches).

the same time fully employing the spatial and temporal information contained in each time series. The point estimates I generate are unique in that they measure the point in time with the highest probability of transition from one habitat to the next.

Qualitative assessments might estimate the inception point of the change, the midpoint, or even the end of the change, before a new elemental equilibrium is reached. Our method consistently provides a quantitative measure of both when the change had the highest probability of occurring and whether that change was significant. These results have important implications for understanding migratory patterns and environmental histories of fish species. I can now use this method to accurately determine the timing of fish movement across environmental gradients.

Non-integration of elemental concentrations in the field could be a source of bias for certain questions, but in this case, I seek to determine the relative changes in elemental concentrations, not their absolute values. Walther and Thorrold (2006) showed 83% and 98% of the strontium and barium, respectively, deposited in otoliths are derived from the ambient water chemistry for marine species. Additionally, Bath et al. (2000) sampled laboratory-reared sciaenids and showed barium to be an excellent marker of the physicochemical properties of an individual's environment, with no biological overprint. They also showed strontium to be a good recorder of salinity, but cautioned the need for considering temperature effects in tandem with strontium when crossing latitudinal gradients. To better contextualize our samples' otolith chemistry with the water chemistry in our system, however, I looked to previous work conducted in the same drainage basin by Dorval et al. (2007) and Hannigan et al. (2010).

Considering the mixing curves and the well-documented life history of this species (Hoskin 2002; Schaffler et al. 2009a), I was able to draw meaningful conclusions from the significant peaks produced by our spectral estimation. Significant peaks in spectral density can occur in two ways. The first is when a significant change in the original series occurs, as is the case with croaker ingress. This change in the original series can be conceptualized as a step function, with a single large shift in the signal. Because I am using MEM, a sufficiently large oscillation emerges in the calculated spectrum, which in turn appears as a peak in the spectral density. When the change is significant, there will be a peak in the spectral density even if it only occurred once in the record. Consequently, if a croaker had lived to 500 days and ingressed only once, I would still be able to discern that a significant change in the otolith chemistry had occurred at 85 days.

The second way significant peaks in the spectral density occur is when consistent changes take place on a periodic basis, such as a fish moving on- and offshore over annual time scales. The periodic nature of such a change would appear as a peak in the spectral density at a certain period, indicating the most important periodic component of the data. The height of the peak indicates the significance of that periodic component, which is why I chose the highest peak as the strongest indicator of the transition from one state to another. The width of the peak, however, indicates the uncertainty about a given periodic component. The peak's shape tells us how consistent a given periodic component was, i.e., if the peak were broad, there may be variability around that annual time signal, if it were narrow, the annual movement is

likely to have been very consistent through years. Much the same as a probability density, the more narrow a peak, the greater the certainty about the estimate.

Additionally, secondary peaks occur. The secondary peak, which occurs at one half of the period of the highest peak, is actually a harmonic of the signal. This secondary peak appears in the spectral density as a fundamental in the Fourier expansion of the signal. Because I evaluated the spectral density over 5,000 frequencies, far more peaks appear than would be present at coarser frequency scales. These secondary peaks are not biologically relevant and I henceforth refer to the most significant peaks for our estimates of ingress timing.

A potential use for these ingress timing estimates is as predictors for the timing of movement, against which I can test hypotheses of observed movement and migration patterns. Based on barium, I show that peaks are occurring at approximately 85 days in fish that have a mean age of 121.5 days. As a result, this periodic component can occur only once over the organism's entire life span. A second oscillation would occur only at 180 days or more, and none of the fish collected were over 145 days old. Therefore, these peaks in spectral density suggest that the most significant periodic component was in fact a single change in otolith chemistry. Studies showing a two to three month ingress time for these species (Warlen and Burke 1990; Hettler 1998; Hoskin 2002) suggest this significant change in otolith chemistry is a direct result of ingress to the estuary.

This change is clearly seen in the plots of the raw element-to-calcium ratios of both the strontium and barium life history scans (e.g., Figure 2b). Knowing that the peak in spectral density lies near 85 days, I plainly observe a change in both the slope

of the curve and the variability of the signal at that point. Even if this large change only occurs once, it will appear as an oscillation after detrending. I see the corresponding peak in spectral densities plot near 85 days. Assessing these features visually would be not only subjective, but also likely inaccurate. Additionally, changes in elemental concentrations seldom show such unambiguous changes through time; estimating timing of ingress loses objectivity in the qualitative setting. Using spectral analysis allows us to obtain an objective measure of the timing of ingress events through life history scans.

The advantage of this approach is that I obtain objective measures for inherently noisy data, according to an unbiased criterion. In a subjective approach, e.g., visually estimating timing of ingress from a life history scan, the results are subject to reinterpretation from one researcher to another. This lack of objectivity allows for the introduction of unknown, and unknowable, bias. Conversely, the spectral approach offers complete repeatability, and a means to estimate its bias.

The power of examining our estimates for all fish samples together is that I create a single frequency distribution. In essence, the frequency distributions that are seen in the data produce a probability distribution of the timing of ingress for the population. This distribution can be refined with increased sample size. By modeling such a distribution, however, I infer the timing of a population's movement across an environmental gradient from an objective basis, while understanding the potential for bias. Given a distribution function, I would obtain many measures about the population's behavior, including the mean time of ingress, the variability of ingress timing across individuals, and the overall shape of the distribution.

Powerful insight is gained once the underlying distribution of the sample data is revealed. If the distribution is uniform, as seen for strontium, the data suggest that time of ingress is not dependent on size (i.e., competency to settle). To further illustrate the value of these statistically derived estimates, I plotted the strontium and barium spectral peaks against fish growth rates and age, thus testing the critical-size hypothesis (*as shown in* Beamish and Mahnken (2001); Cowan and James (1988); Heintz and Vollenweider (2010)). Were the expected relationship to be present, I could conclude that the fastest growing fish ingress at an earlier age. The absence of any relationship between age at time of ingress and growth rate implies these two theoretically related metrics may in fact be independent. These data, although limited, suggest Atlantic croaker ingress likely occurs on a time scale of two to three months after spawning. That this timing occurs regardless of growth rate points to physical processes, such as wind patterns and currents, as determining ingress patterns for this species, and does not support the critical-size hypothesis in this case. To accurately test such a hypothesis on the population level, however, a much larger sample size of fish would be necessary.

The distribution of peaks for barium was slightly negatively skewed, with a large number of individuals migrating into brackish water at older ages, and very few moving at ages less than 60 days. Again, most individuals exhibited a peak in spectral density on the order of two to three months of age. That the peaks in both strontium and barium occur at 50 to 90 days is no coincidence. Atlantic croaker begin spawning near Cape Hatteras in early September, with spawning activity peaking in October and declining by late December (Morse 1980). Both Warlen and Burke (1990) and Hettler

(1998) show larval croaker ingress on the time scale of approximately 3 months in North Carolina estuaries. Additionally, Hoskin (2002) demonstrates similar results for croaker in her work specifically in Pamlico Sound. Our results strongly support these studies. However, the strength of our approach lies in our ability to retrospectively examine survivors of the ingress process and quantitatively link environmental forcing effects with the ingress process, such as the mixing curves of the elements with respect to salinity and temperature.

When interpreting the results of both strontium and barium together, the mixing curves of the water masses through which the fish traverse must be considered. Although both strontium and barium have been shown to be incorporated proportionally to ambient water chemistry (Bath et al. 2000), the inflection of the concentration of each of these elements occurs at different salinities, as shown by Dorval (2004) and Dorval et al. (2007) for this drainage basin. This pattern is seen in the lag in timing of barium as compared to strontium. I surmise that strontium peaks, which consistently estimate earlier ingress times, reflect arrival to the estuarine environment, while barium peaks reflect subsequent movement up-estuary. Further, our results call for the investigation of barium as a potential marker in addition to the more commonly used strontium. In certain chemical environments, one element may prove more informative than the other. Although I advocate the univariate analysis of elements for that case of estimating ingress timing, I am also aware that there is great value of multivariate retrospective classification, such as the approach of Fablet et al. (2007).

Because the most significant peak for all samples fell above 26 days, these data support a natural threshold for timing of ingress, as was also seen in Nixon and Jones (1997). Being able to discern these changes proves quite useful in characterizing the movement of the local population. Given information from other areas, I could then use strong statistical tools such as the Kruskal-Wallis one-way analysis of variance to test whether distributions between locations were equal. By performing this test, I would see whether a mean difference in the timing of ingress was present without assuming the sample came from a normal distribution.

Considering our limited sample size, I proceed with caution in applying these interpretations, as any population-scale conclusions necessitate broader sampling, and the purpose of this work was to demonstrate the method and its value in testing hypotheses. Additionally, I emphasize that this method could reflect changes in chemical environment that are either linked to salinity or other environmental gradients. Differing otolith chemistry signatures might result from behavioral changes, as well, such as switching from a pelagic to a benthic or demersal life history (Hare et al. 2005). An advantage of this technique is that it discriminates not only which events are more distinct in the environmental past, but also uncovers potentially important components of the life history not previously revealed. While I have shown the power of this technique in detecting such changes, I also caution that life history must always be considered to understand the causes for the patterns revealed. In our case, the general trend showed a well-documented ingress from saltwater to the estuarine environment, consistent with previous results (Warlen and Burke 1990; Hettler 1998), however, and because I had a well-studied life history pattern I was able to evaluate the

more nuanced dynamics of timing. The advantage here is I am able to calculate these estimates retrospectively, eliminating the need for intensive in situ sampling as done for this species by Hare and colleagues (Hare et al. 2005).

The unique signatures associated with various water masses are reflected in croaker otolith chemistries (Schaffler et al. 2009b). With our new application of spectral analysis, I now anticipate applying these techniques to fish from various locations to compare not only timing, but also overall patterns of ingress in both juvenile and adult samples.

CHAPTER III

ESTABLISHING THE IMPORTANCE OF ABLATION DEPTH IN LIFE HISTORY SCANS OF OTOLITHS

Introduction

Fish otoliths have proven powerful tools for providing information about population movements and structure (Campana 1999; Thorrold et al. 2001). As with any developing technique, however, caution must be exercised in advancing such applications. The intrinsic limitations of using otoliths as natural tags have not yet been fully addressed or understood. The following study focuses on how to deal with the issues that arise in life history scan methods from the three-dimensional nature of otolith morphology.

To convey the ability to estimate age and growth using otoliths, fisheries scientists often rely on conceptual models to describe the methods of analyzing fish life histories. The most common conceptual model to describe the morphology of otoliths is that of tree rings. Considering the long history of silviculture and ageing of trees with annuli, this conceptual model for ageing otoliths and extracting information from their increments is practical. With the tree ring concept, each cross-section has concentric layers; a cut made at the top of the structure theoretically contains the same number of layers as a cut made through the center (Figure 10a). Otoliths, however, have layers which curve around and underneath, much more like an onion. A slice taken from the top of the onion will not necessarily have the same number of rings as a

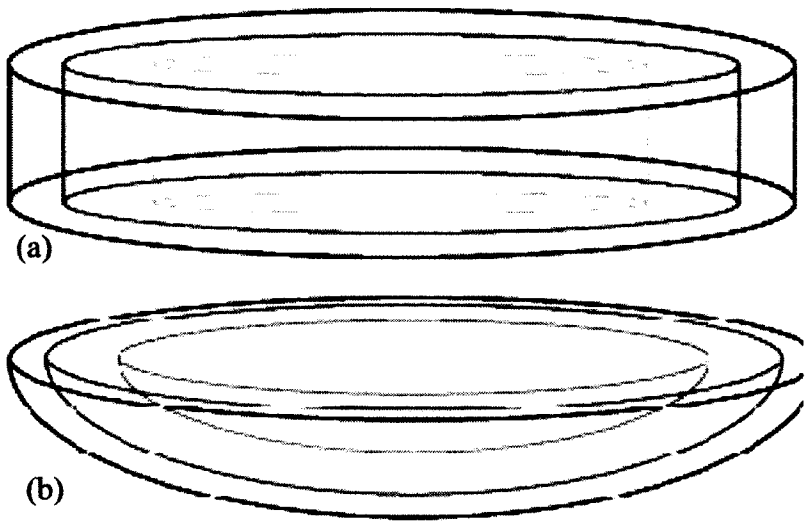

Fig. 10. (*a*) Section of a tree-like structure, where cuts at the top, middle and bottom of the structure all contain the same number of rings (*b*) half of an onion-like structure (otolith), where layers curve around and underneath. A cut at the top or bottom will not contain the same number of rings as a cut in the middle.

slice taken through the center. Figure 10b illustrates an otolith half in the typical preparation for use in a life history scan. By changing the conceptual diagram from a cylinder (i.e., a tree) to a sphere, I emphasize a third dimension of the otolith: depth.

For certain areas of otolith research, morphology may not play an important role; when conducting life history scans, however, it may be significant. The scan itself can occur at considerable depth within the otolith depending on the method used. If the depth of ablation is not considered and accounted for in these analyses, the inherent assumption is that ablation depth will not influence chemical signatures. While this assumption is valid for surface scans using wave-dispersive microprobes, with depths of 1-3μm (Zlokovitz et al. 2003), it may be violated if the scan occurs at depth. For example, certain laser scans may ablate as deep as 300μm (Jones and Chen 2003). Thus, depth may be an issue if a scan is being conducted on an entire otolith half, as is often the case (Ben-Tzvi et al. 2008; Hale and Swearer 2008; Standish et al. 2008).

One widely used method for life history scans of otoliths is Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS), which involves ablating a trench into the otolith across its increments. Using this method, one obtains a scan from the beginning of the fish's life, at the core of the otolith, through to the date of capture, at the edge. The dotted horizontal line in Figure 10 demonstrates a cross-section of this trench. When a deeper trench is made, however, one may be ablating material not only across layers, but also through them. Doing so incorporates more growth increments than desired, as is seen by the solid line in Figure 11.

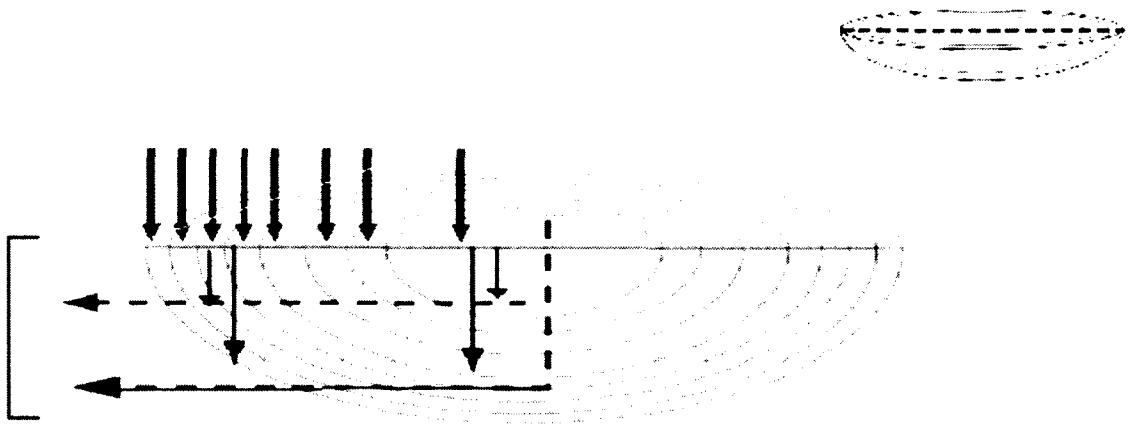

Fig. 11. Stylized cross-section of an otolith half. Dashed line represents a shallow laser ablation trench; solid line represents deeper trench.

Despite increasing popularity of laser ablation to obtain life history scans, previous studies have largely ignored the depth of ablation. In a sample of twelve recent LA-ICPMS otolith chemistry papers, only one (Kemp et al. 2011) reported laser ablation depth in its methods. In a few studies, such as Ruttenberg et al. (2005), ablation depth was indirectly accounted for by pulsing the laser in a series of small, discrete pits at the otolith core. The goal of these studies was not to complete a life history scan, however, the laser was instead used to isolate and characterize the juvenile portion of the otolith for natal habitat identification.

The lack of attention to laser ablation depths is a potential cause for concern in current otolith chemistry investigations. Specifically, fisheries ecology studies have reported lag effects in strontium uptake, as well as saturation levels in otolith uptake (Elsdon and Gillanders 2005b; Macdonald and Crook 2010; Miller 2011). While these studies help us to comprehend the link between ambient strontium levels and otolith elemental concentrations, it is a concern that some of these results could be sampling artifacts in how the life history scans of otoliths were performed, and the "lag effect" not in fact real. The concept of delayed uptake, by as much as two weeks, runs counter to studies in the physiological literature that demonstrate uptake of radio-labeled elements onto the otolith within several hours (Mugiya and Tanaka 1995; Skov et al. 2001). Moreover, if a scan were taken at an ablation depth that crossed many increments of growth in the vertical plane, the signal could be an integration of several life history stages, with no quantitative measure of the level of integration, or the bias that such integration would introduce. By examining the conceptual models used in

this area of otolith research, i.e., 2-dimensional tree rings versus 3-dimensional onions, a new level is reached in understanding how the mechanics of laser ablation sampling might affect otolith chemistry results.

The effect of ablating through layers on trace element signatures has not yet been investigated experimentally. The purpose of this study is to examine how the depth of ablation affects elemental chemistry results. I expect that the distance between the polished surface and the bottom of the ablation trench will change the number of increments sampled, causing more recent layers to be sampled with deeper ablation depths. I tested the null hypothesis that depth of ablation will not influence chemical signatures through a comparison between shallow and deep otolith chemistry signatures using LA-ICPMS. The second physical aspect of LA-ICPMS which has not been investigated is the effect of the integration of the horizontal (x and y) plane across ages of the fish due to the laser spot size. I preliminarily explore this concept and how it might act as an additional smoothing function on otolith chemistry data.

Because of the longitudinal nature of these data, I analyzed the results using the time series techniques described by Hoover et al. (2012). Doing so makes use of the full environmental chronology contained in each life history scan, while maintaining the temporal resolution and accuracy of our data analysis.

Materials and Methods

I performed the elemental analysis using sagittal otoliths of juvenile Atlantic croaker (*Micropogonias undulatus*). Atlantic croaker are found in demersal, brackish,

and marine habitats, from 43°N - 37°S. The fish I analyzed were a subsample of individuals from the Pamlico River in North Carolina captured during April and May of 1997 (n=14).

One sagittal otolith from each fish was chosen at random (left or right) for elemental analysis, and the second otolith was used for ageing. I prepared all otoliths for trace element analysis in a class 100 clean room, cleaning the samples of any attached tissue and rinsing in ultrapure hydrogen peroxide (H_2O_2) and ultrapure Milli-Q water. Trace element analysis followed the methods described in Hoover et al. (2012). Based on the results of Hoover et al (2012) I concluded that barium provided the best marker to asses effects of laser depth on resulting element concentrations. The two elements analyzed were barium (^{138}Ba) and calcium (^{48}Ca) scanned in low-resolution mode (Taylor 2001; Chen and Jones 2006). I converted the raw data to element-to-calcium molar ratios.

The following depth model was developed (Jones and Chen 2003) for the MAT Element 2 ICP-MS (Finnigan) at Old Dominion University's Laboratory for Isotope and Trace Element Chemistry:

$$Crater\ Depth = 19 + 1.4 * Power - 0.4 * Travel\ Speed/\mu m/s. \quad (13)$$

I used this equation to determine the power levels necessary to obtain our desired trench depths (Table 2). I ablated a line from the core of the otolith towards the edge to obtain series of the trace element signatures over the fish's life. Two trenches were made directly adjacent and parallel to one another along the transect. To obtain two

different depths, holding speed ($5\mu ms^{-1}$) and spot size ($20\mu m$) constant, I used 45% and 100% power to obtain scans at $80\mu m$ and $160\mu m$, respectively. These depths were verified microscopically.

I cut transverse thin sections from the otoliths for age and growth analyses. Sections were mounted and polished to reveal the core for ageing of daily growth increments. I measured daily increment widths using ImagePro Version 5.0.1 software. The daily ages and increments were measured on the same sample, and used in subsequent growth rate estimation to transform the life history scan data into a temporal scale in terms of the days in a given fish's life (Hoover et al. 2012).

Because the data obtained from these analyses are time series, applying traditional parametric statistics to compare the two ablation depths would not be appropriate. I therefore conducted a spectral analysis following the methods in Hoover et al. (2012) to quantitatively analyze the effects of trench depth on elemental concentrations. I used a low-pass filter to remove the components of the series not adding information to our analysis, with the cutoff at $f\Delta t = 0.3$. Significant trends were removed with either a linear

$$X_j = A_1 + B_1 t_j \tag{14}$$

or quadratic function

$$X_j = A_2 + B_2 t_j + C_2 t_j^2. \tag{15}$$

Table 2. Operational settings for the Finnigan Element 2 Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICPMS).

Operational Setting	80μm Depth	160 μm Depth
Spot Size	20 μm	20 μm
Speed	$5\mu ms^{-1}$	$5\mu ms^{-1}$
Power	45%	100%

I tested for the significance of the trend component by calculating the variance before and after the fit and using an F test to decide if the variances were significantly different.

Subsequently, I standardized the data by dividing each series by its standard deviation, which allowed us to compare series with widely varying levels of stochasticity. I calculated spectral density estimates using the Maximum Entropy Method (MEM) as described by Press et al. (1994). Details of the application of above methods are described in Hoover et al. (2012).

To compare the overall means between the two trenches, I performed both a paired t-test and a mixed-effects ANOVA. The mixed-effects ANOVA is appropriate in this case because I am dealing with both fixed and random effects. The fixed effect is the trench depth, since our sampling fraction for that variable is one (I am sampling the entire population of possible depths, because I was only interested in shallow versus deep trenches). The random effect is the individual, since the sampling fraction is less than one; I am analyzing some subsample of the entire population of Atlantic croaker being considered.

I also examined the maximum peak in spectral density for both depths for each element. The highest peak in spectral density represents the most significant frequency component of the signal, and consequently measures the point in time with the highest probability of transition from one habitat (saline) to the next (estuarine). Thus, I use the peak in spectral density as a proxy for the timing of ingress to the estuary. I compared

and tested for significant differences in the timing of ingress among the two depths using a paired t-test in SAS version 9.2.

Finally, I can model the number of layers that will be incorporated for a given depth by assuming a known shape to approximate the morphology of the otolith. The most generalized conceptual model of otolith growth would be a sphere. The oversimplified example of a sphere dramatically underestimates the number of increments ablated and is grossly conservative; however, I use it to illustrate the conceptual point directly. I measured the width of the increments when ageing the fish. By assuming spherical growth, I can calculate the quantity in the vertical plane that corresponds to a given increment width in the horizontal plane. Again, I make this example bearing well in mind that in real otoliths, more material accumulates in the horizontal plane than the vertical.

For a conservative example, I conducted our analysis on a sciaenid with fast-growing otoliths; our mean otolith increment width for the first day outside of the core is 8μm. Assuming the otolith's morphology to be a sphere with constant increment width, a trench depth of 160μm would exceed our first increment of growth by 20 times. Understanding that most otoliths are not spherical, but rather more elliptical in shape and that increment widths are not constant, it can easily be seen that our vertical axis is even further compressed with respect to the horizontal plane, which incorporates more recent material.

The surface area of a general (tri-axial) ellipsoid is

$$S = 2\pi c^2 + \frac{2\pi ab}{sin\varphi}(E(\varphi,k)sin^2\varphi + F(\varphi,k)cos^2\varphi), \quad (16)$$

where

$$\varphi = arc\cos\left(\frac{c}{a}\right), k^2 = \frac{a^2(b^2-c^2)}{b^2(a^2-c^2)}, a \geq b \geq c \quad (17)$$

given $a, b,$ and c are the semi-principal axes of the ellipsoid and $F(\varphi,k)$, $E(\varphi,k)$ are incomplete elliptic integrals of the first and second kind, respectively.

Therefore, given an increment width, c, along the same transect used for ablation, I can solve for the depth, a, that the layer runs around and underneath. If the ablation depth exceeds that amount, the scan results will be incorporating at least that much material at a given point in time along the laser transect.

Each increment is measured in the horizontal plane, and therefore those measurements can be used to describe the b and c measurements, accounting for the unequal increment widths between years. The amount of material (or number of increments) ablated in the vertical plane, then, is equal to c in the above equation.

Results

I analyzed two trench depths, one shallow (80μm) and one deep (160μm), for two elements (^{138}Ba and ^{48}Ca) on 14 fish. Fish ranged in size from 41 to 70mm standard length, with ages ranging from 78 to 145 days. Otoliths ranged in diameter from 1064-1891μm.

Mean plots of raw Ba:Ca data for both shallow (dashed line) and deep (solid line) scans are shown in Figure 12. The expected trend from low to high barium concentrations, as the fish moves from saline to estuarine waters, appears clearly. Shallow barium values ranged from 7.29 (±6.54) mmol/mol at the core to 10.23 (±7.44) mmol/mol at the edge at the time of capture. The deep trench ranged in barium values from 7.47 (±6.55) at the core to 11.20 (±6.99) mmol/mol at the edge. The paired t-test showed overall significant differences between mean shallow and deep barium values ($t=114.25$, $p<0.0001$) within each fish.

As a result of the averaging effect of ablating through layers, deep scans consistently exhibit less variable results as compared to shallow scans on the same individual. I noted that the highest peaks and lowest troughs in raw element-to-calcium molar ratios always appeared in the shallow trench data, as is seen in Figure 12. The overall variance of the data diminished dramatically from the shallow (SD=34.919 mmol/mol) to deep (SD=4.944 mmol/mol) barium data.

I conducted spectral analysis on the shallow and deep data using Ba:Ca for each sample. The highest peak in spectral density represents the most significant periodic component of a life history scan signal, in this case representing the timing of transition between the marine and estuarine environments (i.e., ingress timing). Results of the spectrally-derived ingress times, and differences between shallow and deep estimates are summarized in Table 3, along with the overall means and their associated standard deviations.

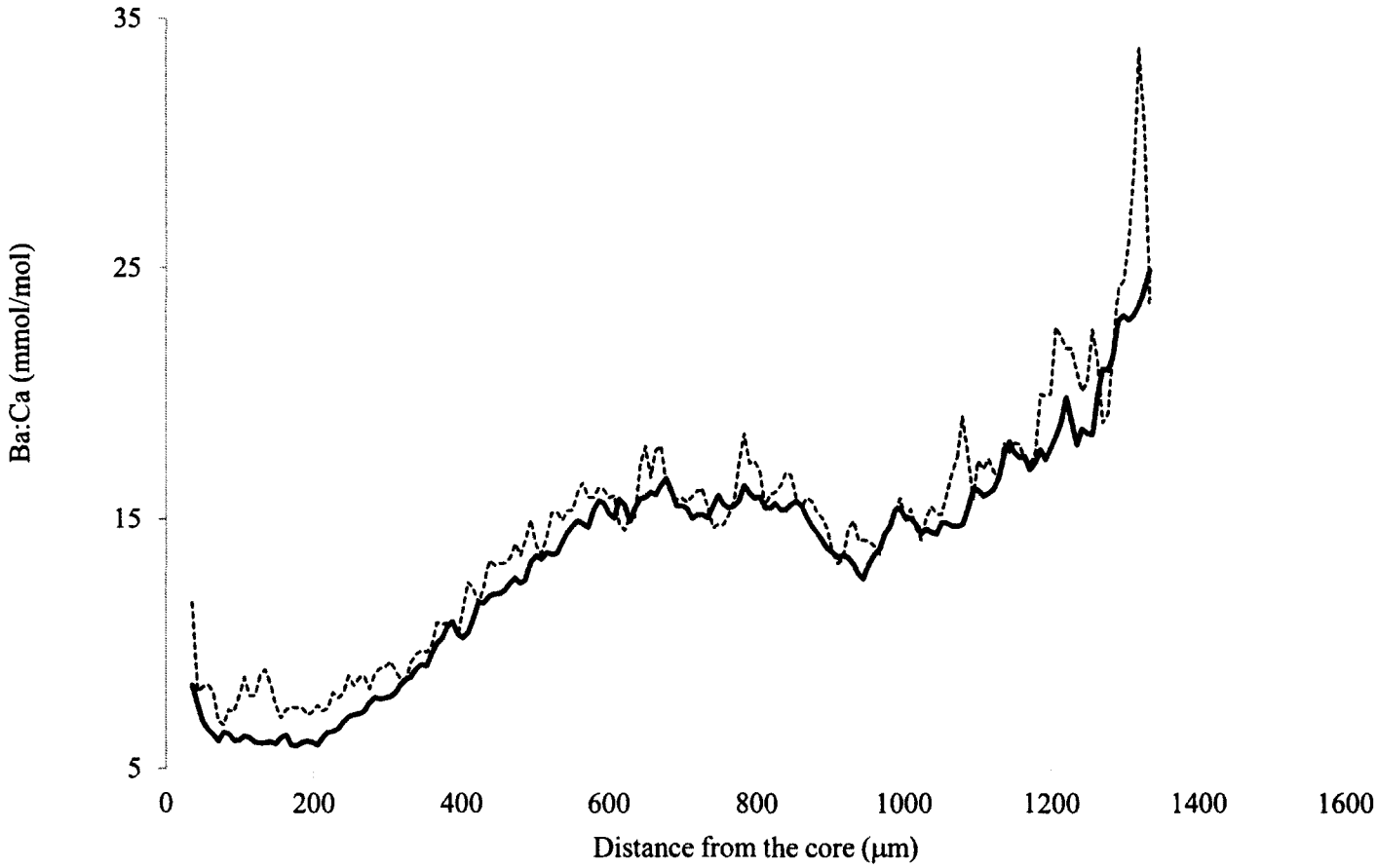

Fig. 12. Mean Ba:Ca ratios plotted against distance from the core of the otolith to the edge for all samples (n=14). The dotted line is the shallow (80μm) trench and the solid line is the deep (160μm) trench.

The spectral density plots for barium are shown in Figure 13 with both shallow (dashed) and deep (solid line) data for each individual. The estimated ingress timing was significantly different between shallow and deep data using the paired t-test ($t=6.14$, $p<0.0001$). The mixed-effects ANOVA also showed significant differences for the fixed effect of depth for barium ($F=18.46$, $p=0.0002$). Figure 14 displays the resultant distribution of ingress times estimated by the shallow and deep trenches for barium. Note the difference in shapes between the shallow and deep distributions, with a negatively skewed shape for the shallow relative to the deep ingress times.

Additionally, I note that there is a lag in the overall timing of ingress as estimated using the shallow ablation depth data when compared to the ingress timing from the deep data. The mean lag in estimated ingress timing comparing shallow with respect to deep data was 32.00 ± 19.5 days.

Discussion

Accurately quantifying the minor and trace elements is the fundamental objective of many otolith life history scan studies. While not all life history scan investigations use laser ablation methods, there is a predominance of this method in the otolith chemistry literature (Campana et al. 1994; Thorrold et al. 1997; Jessop et al. 2012). There have been several successful studies investigating whether differences in life history are recorded in strontium signals (Waight et al. 2002; Milton and Chenery 2003), but note that the wide use of LA-ICPMS also results from its ability to detect a broad array of minor and trace elements in trace and ultra-trace concentrations (Marklevitz et al. 2011; Olley et al. 2011; Tanner et al. 2012).

Table 3. Spectrally-derived estimates of ingress timing (days).

Sample	Age	Ba - Shallow	Ba - Deep	Difference
1	78	26	16	10
2	99	61	30	30
3	120	83	36	47
4	137	91	91	0
5	110	74	31	43
6	136	91	74	17
7	120	95	48	48
8	110	74	69	5
9	136	91	56	35
10	139	91	45	45
11	145	95	83	12
12	110	74	28	46
13	127	91	43	47
14	134	91	29	61
Mean	121.5	80.6	48.6	32.0
SD	18.6	18.7	22.9	19.5

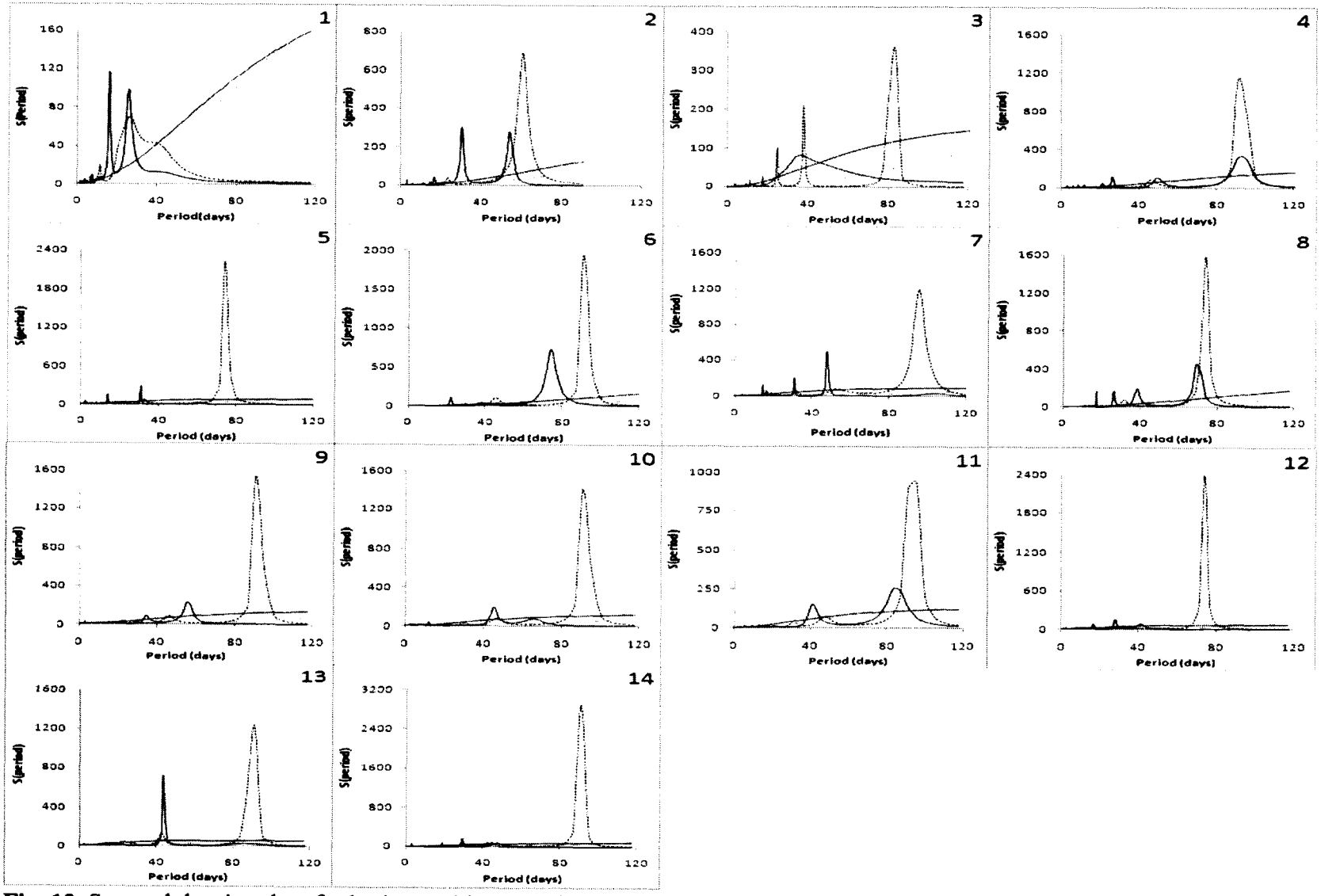

Fig. 13. Spectral density plots for barium, with spectral density on the y-axis and period (in days) on the x-axis.

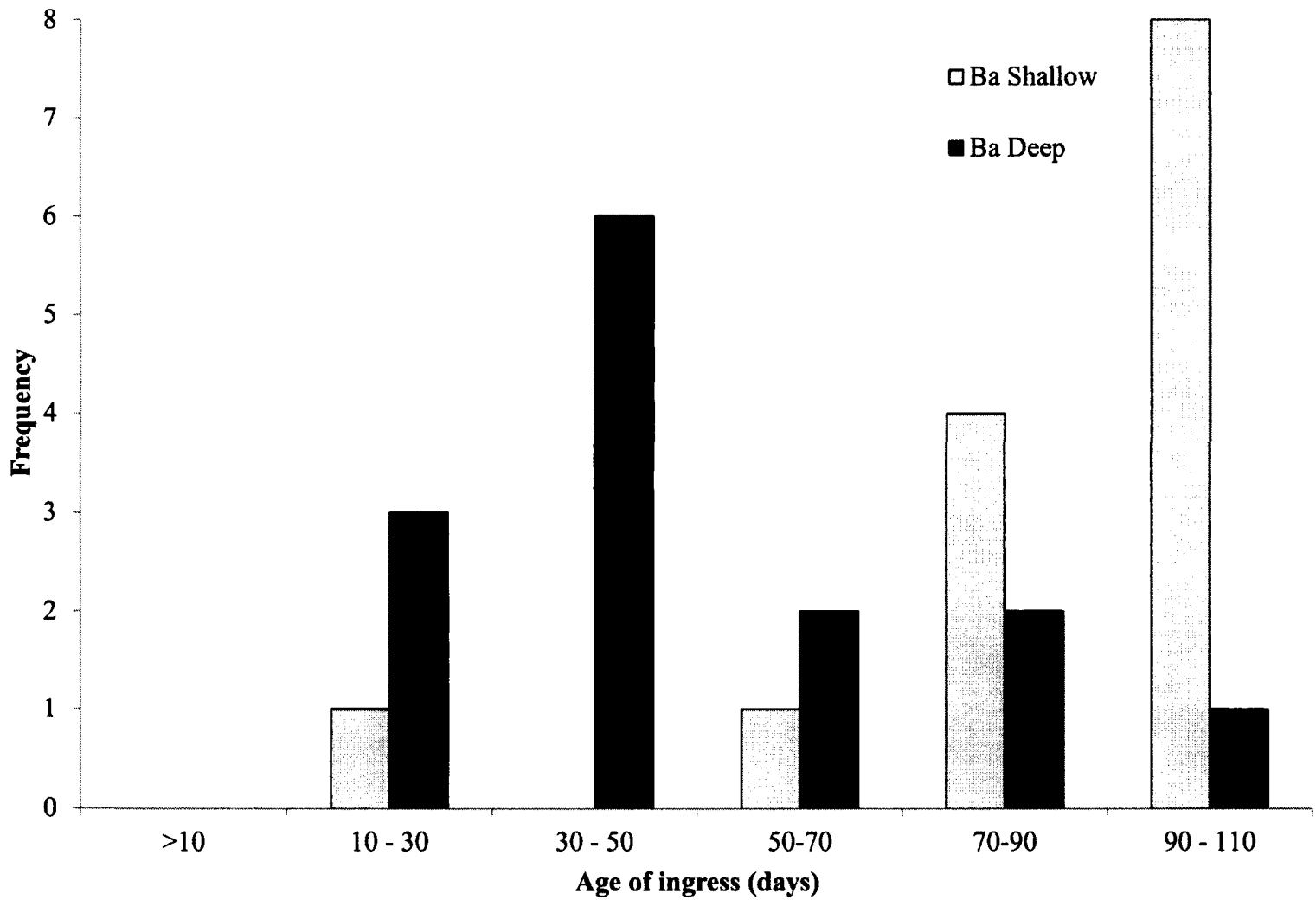

Fig. 14. Distribution of estimated ingress timing based on barium values using both shallow (gray) and deep (black) data.

I demonstrate that when using laser ablation, the depth of the transect significantly affects chemical signatures obtained from life history scans of otoliths. Using LA-ICPMS to conduct life history scans, I directly compared chemical signatures from two different ablation depths. I saw a significant difference in raw element:calcium molar ratios between the two trench depths for barium. These results illustrate the critical role of depth in understanding the spatial heterogeneity of elemental signatures across the otolith when analyzed using laser ablation.

Comparing the spatial differences between the two trenches, the visible smoothness of deeper trenches is consistent among all samples. This smoothing effect is likely due to the fact that a deeper trench samples through more layers, so each data point in that signal integrates over more growth bands, rather than a single incremental layer. In effect, the trace elemental signature is being averaged by the surrounding layers. This is reflected in the decrease in variance seen in the deeper layers of our samples that is typified in the decreased variance seen in a moving average estimate.

By using the spectral approach in Hoover et al. (2012) I further investigated the effects of ablation depth on these data by estimating the timing of ingress for each individual. I hypothesized that the overall effect across a known salinity gradient would be to shift the ingress timing, as calculated through the spectral analysis of the barium signals. Barium exhibited significant differences in the estimated timing of ingress, with a skew towards earlier ingress time using the deeper trench data. This skew demonstrates that barium-richer layers were prematurely incorporated into putatively "early" sampling and, thus, anticipates movement.

In addition to the vertical integration resulting from the depth of laser ablation, the second means of integration that physically results from this sampling method is in the horizontal (x, y) plane due to the spot size of the laser. Though not directly quantified, I note the importance of considering increment width relative to the spot size of the laser, particularly with larval and juvenile fish, and those fishes with small or thin otolith morphologies. Given the relatively large size of juvenile Atlantic croaker otoliths and wide increment widths, the spot size of 25μm likely integrated on the order of a single day, as our mean increment width for all samples was 17 (±6) μm. Considering that this species is a fast-growing sciaenid, I stress that any generalizations concerning integration across or through increments are conservative in nature. In an otolith with narrower increment widths, however, the integration over several days in the horizontal plane could present a similar integration problem to the one I describe in the vertical (z) plane in Figure 11.

These two cases of integration demonstrate important potential impacts of the physical aspects of laser ablation on life history scan results, particularly in studies investigating the timing of fish movement through environmental gradients. Those studies which attempt to reconstruct environmental histories using LA-ICPMS data require deliberate consideration of ablation dimensions in their analysis. Papers in the otolith chemistry literature suggest a lag effect in the incorporation of strontium (Elsdon and Gillanders 2005b) and barium (Miller 2011) may be occurring. These studies report up to a two-week lag in the incorporation of elements into the otolith. Moreover, these results strongly contrast with the historical physiology literature which

reports almost immediate incorporation of minor and trace elements into the otolith microchemistry (Farrell and Campana 1996). As follows from the work of Mugiya et al. (1991), establishing the incorporation of trace metals into otoliths, as well as the biophysiological studies conducted by Payan et al. (2004), and the precipitation kinetics investigation of Romanek and Galudie (1996), the incorporation of trace elements into the otolith should occur on the order of 24-48 hours. Even more specifically, Borelli et al. (2003) conclude that CaCO3 precipitation should occur when saturation is reached, at the end of the night.

These physiological studies offer no support for the presence of neither a lag effect in uptake, nor an organ where strontium and barium are stored to be released later. The discrepancy between the physiological and ecological studies raises concerns for whether lag effects are in fact present in the otolith chemistry. Conceivably the most parsimonious explanation could be that the lags seen in laser ablation studies are sampling artifacts and could result from an effect as simple as ablation depth.

I hypothesize that the discrepancy seen in our data when examining ingress timing is itself a powerful indicator of the effect of ablation depth on otolith chemistry results. Our data show different timing of element uptake, depending on ablation depth. Specifically, our data show higher barium concentrations sooner than would be expected, as fish move from lower to higher concentrations and barium-estimated ingress time differ by approximately two weeks in the deeper trench data. Whether the elemental change occurs earlier or later than it should in reality will depend on whether the concentration gradient is positive or negative and on the shape of the otolith. The

limitations of these techniques continue to evolve and be investigated (De Vries et al. 2005; Ben-Tzvi et al. 2007; Elsdon et al. 2008).

While several issues exist in obtaining accurate microchemical data from otoliths, our study has particular implications for morphological considerations. If the otolith being analyzed by a technique that samples deeply is ovoid in shape, then material will be accumulated around the otolith fairly evenly as the fish grows. In this case, the morphology provides a somewhat muted sensitivity to ablation depths because growth layers are more uniform. In contrast, otoliths that are long and thin, pose more of a problem for life history scans using LA-ICPMS because for a given depth, more growth layers are present to be ablated and this is exacerbated at the edges of these otoliths. Because of this morphology, the danger of ablating through more layers instead of only across them is much greater. Unfortunately, some of the world's most valuable fish have long, thin otolith morphologies, making them more susceptible to microchemistry measurement errors and consequently, conclusions drawn from these analyses.

Note that in whole otolith dissolution, integration occurs over the entire life history of the fish, obtaining a single set of chemical signatures. Because this technique uses the entire otolith, no individual sampling of the substructural layers occurs and morphology therefore is not a problem. While particularly useful in elemental fingerprinting, solution-based otolith chemistry studies lose all of the temporal information the otolith contains through dissolution. Both methods contain unique sets of information functional in answering different ecological questions.

Such limitations on otolith chemistry investigations with laser- and solution-based ICPMS argues for the use of surface-sampling instruments such as the wave-dispersive microprobe (Secor et al. 1998). However, this approach is not without its limitations. Although the microprobe provides estimates of strontium, other important elements such as barium are below detections limits for most otolith samples (Campana et al. 1997). While strontium can be valuable in evaluating some types of movement during the life history in embayments, other elements such as barium may be more useful in riverine and estuarine systems (Elsdon and Gillanders 2005a; Feutry et al. 2011).

Fish otoliths are widely recognized as valuable natural recorders of life history information. While our ability to obtain information from these structures continues to increase, the inherent limitations of both our sampling techniques and data analyses must be acknowledged. The value of increasing our understanding of those limitations lies in our future ability to predict the exact distribution function and compensate for inherent restrictions in our analyses. Since the data acquired from otolith microchemistry are used to identify nursery habitat, larval dispersal trajectories, and migratory routes, otolith chemistry studies have the potential to impact fisheries management directly. With this knowledge base and a precautionary approach, fisheries scientists may comprehend ever-higher levels of the complex and intricate systems they seek to analyze and manage.

CHAPTER IV

EVALUATING MOVEMENT PERIODICITIES IN A COASTAL FISH POPULATION

Introduction

Quantifying movement rates and patterns is fundamental to assessing stock structure and connectivity in marine populations. However, this information is often lacking in many data-poor fisheries where a scarcity of basic life history information exists. For example, black sea bass (*Centropristis striata*) are a protogynous hermaphrodite distributed along the Atlantic coast of North America and into the eastern Gulf of Mexico that support intense recreational and commercial fisheries (Mercer and Moran 1989). Both commercial and recreational landings have varied over time with no trend (Shepherd et al. 1994) and roughly account for similar proportions of the fishery. Further, throughout recent history both Atlantic coast stocks of black sea bass have suffered from excessive exploitation rates (Shepherd 2009; Logbo et al. 2011). Despite the fishery's inherent value, much of the life history of this species remains poorly understood, in contrast to other species.

Black sea bass are currently managed as three distinct stocks: Gulf of Mexico, South Atlantic (ranging from Cape Canaveral, Florida to Cape Hatteras, North Carolina), and Mid-Atlantic (Musick and Mercer 1977; Bowen and Avise 1990; Shepherd 1991). Although these management definitions exist, there is considerable uncertainty in the distinction of the two Atlantic coast stocks. This confusion has arisen because of the limited genetic differentiation of the two Atlantic coast stocks,

and the assumed migratory behavior within the Mid-Atlantic stock. The fishery for this stock is seasonally localized in that the majority of landings occur near-shore in the warmer months and offshore during winter (Shepherd et al. 1994), despite near-uniformly distributed fishing effort through space and time. The fishery operates this way presumably because of an annual migratory pattern that arose in response to temperature variations in either space or time. Regardless of the mechanism, tagging results largely confirm this cyclical migratory behavior in black sea bass (Moser and Shepherd 2009). Beyond this relatively recent tagging study, however, the commonly accepted notion of an inshore-offshore movement pattern for this population has not been empirically established.

The expected seasonal movements between in- and offshore environments set up a periodic shift in the chemical environment through which these fish migrate. Considering the extensive literature describing the recording of environmental histories in otolith chemistry, one might expect that this seasonal movement would be reflected in the trace element profiles of black sea bass otoliths. However, quantification of such changes in otolith chemistry has, to date, largely focused on fish species that cross much more drastic environmental gradients, e.g., (Elsdon and Gillanders 2003; Chang et al. 2004); *see review in* (Elsdon et al. 2008). The otolith chemistry signal of such movement is assumed to be more pronounced in a fish moving through a strong salinity gradient, e.g., from an estuary to the open ocean, as seen in much of the otolith chemistry literature (*see review*: Gillanders, 2005). The drivers of change in ambient water chemistry, however, clearly are not limited to

salinity. Moreover, modest gradients are widely known to exist in the coastal and open ocean environments. These gradients are the results of a myriad number of physical oceanographic processes, such as upwelling (Patterson et al. 1999; Ashford et al. 2011), surface circulation (Ashford et al. 2005; Schaffler et al. 2009a), and thermohaline circulation patterns (Ashford et al. 2010), and have resulted in distinct otolith chemistries.

While a number of studies have attempted to test whether spatial variation in otolith chemistry is sufficient to discriminate among spatially discrete subpopulations, our study proposes a slightly different focus. I collected fish from different portions of the black sea bass population's residency as a means of making inferences about their life history patterns, using differences in space as a proxy for differences in time. Fish collected in the inshore environment represent the population's summer habitat use, while fish in the offshore environment reflect the winter habitat use of this population. I seek to determine whether the ambient conditions the fish inhabited, although in the same type of coastal Atlantic water mass, might be identifiable through their otolith chemistry. I hypothesize that there will be significant differences in the inshore and offshore chemistries. Sufficient differences between these two groups will set up a model through which I can examine the timing of the population's movement between the inshore and offshore environments.

The second question this study attempts to address is whether two of the most common environmental markers used in otolith chemistry, strontium and barium, differ in the information they each convey regarding the timing of movement for this

population. I examine this question by estimating the timing of movement using each marker separately, and then comparing those estimates within each individual. To obtain the estimates for timing of movement, I applied a statistical approach which is relatively new to life history scans (Hoover et al. 2012).

Recent publications in the otolith chemistry literature have demonstrated the breadth of analyses which may be employed to analyze such environmental histories of fish, providing quantitative advances to earlier, more optical approaches, e.g., (Morris Jr et al. 2005). Hedger et al. (2008) used zoning algorithms to classify otolith life history scans into fish environmental histories. Both Daverat et al. (2011) and Fablet et al. (2007) reconstructed fish life histories using unsupervised signal processing methods in a Bayesian framework. Additionally, repeated-measures analysis of variance (ANOVA) (Nielsen and Munk 2004), as well as multivariate analysis of variance (MANOVA) (Swearer et al. 2003) have been applied. A recent method that is particularly suited to assessing the actual movement based on life history scan data, however, is the spectral analysis described by Hoover et al. (2012). I applied this method in our analysis as a means of examining whether black sea bass are moving across the continental shelf in a periodic manner.

The potential influence of physical processes, such as coastal upwelling patterns, has received less attention in the otolith chemistry literature than movement of the fish through salinity gradients or across water masses. I hypothesized that the edge chemistries, representing the portion of life history just before capture, would differ significantly for individuals caught in the inshore waters as compared with those

caught from offshore coastal waters, where upwelling processes may play a significant role in the abundance and distribution of various minor and trace elements. I expect a significant difference in the inshore and offshore barium edge chemistries because of known upwelling (Shen et al. 1992; Patterson et al. 1999; She and Klinck 2000) near canyon systems, where black sea bass are commonly found in winter. I do not expect any differences in the strontium edge chemistries; the inshore and offshore environments do not differ significantly in salinity, the major driver of strontium in otoliths (Bath et al. 2000). However, temperature is another major factor affecting strontium incorporation in otoliths, and its confounding effects must be considered along with salinity (Martin et al. 2004).

To test these hypotheses, I conducted life history scans using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) and then conducted spectral analysis on the data. The spectral analysis techniques in Hoover et al. (2012) were originally applied to life history scans of otoliths to estimate the timing of ingress into an estuary. Because the fish examined in that study were juvenile Atlantic croaker (*Micropogonias undulatus*), the change in otolith elemental chemistry was a single shift from the marine to the estuarine environment. In the offshore environment, however, changes in the otolith chemistry may be much more subtle. For example, Schaffler et al. (2009b) demonstrated there are no significant differences in larval Atlantic croaker otolith chemistry among in- and off-shore locations in the Mid-Atlantic Bight, but there were differences in otolith chemistry between the Mid-Atlantic Bight and South-Atlantic Bight. In the following study, I designed the

investigations to test the null hypothesis that there would be no significant differences in otolith chemistry between black sea bass from inshore versus offshore waters. I first demonstrate differences in the otolith chemistry of black sea bass collected in- and off-shore and then I examine the timing of inshore-offshore movement as estimated by the spectral density of the life history scans of fish from each location.

Materials and Methods

Sample preparation and analysis

To first test whether inshore and offshore waters were sufficiently different as to exhibit oscillations in otolith chemistry, I sampled black sea bass from two areas of the population's residency. In 2010, I collected individuals from the inshore environment of Point Judith, Rhode Island during the summer, and the offshore environment of the Norfolk Canyon off of the coast of Virginia in the winter. I analyzed a total of 26 otolith samples (n=13 from inshore, n=13 from offshore). Sagittal otoliths were removed and chosen at random, left or right, for trace element analysis. Transverse sections were taken from the otoliths in the sagittal plane. Each section was mounted in a thermoplastic adhesive which had been tested to ensure absence of the trace elements of interest. All annular increment widths were measured using Image Pro Version 6.2 software.

Samples were then prepared for laser ablation ICP-MS analysis; details are outlined in Ashford et al. (2010). Final sample processing was performed in a class 100 clean room. The mounted otolith sections were sonicated in ultrapure Milli-Q water

and dried under a laminar-flow hood. I conducted the elemental analyses using a Thermo Finnigan Element 2 double-focusing sector-field inductively coupled plasma mass spectrometer (ICP-MS) located at the Plasma Mass Spectrometry Facility at Woods Hole Oceanographic Institute (Woods Hole, Massachusetts). Samples were ablated using the New Wave Merchantek UP-193 laser ablation system and a microflow nebulizer (Elemental Scientific, Inc., Omaha, Nebraska). Otoliths were analyzed for ^{48}Ca, ^{88}Sr, and ^{138}Ba and are reported as molar ratios to ^{48}Ca using a laser speed of 10 $\mu m \cdot s^{-1}$, with a laser spot size of 25 μm and power at 70%, producing a crater of approximately 25 μm in width and approximately 35 μm deep (Jones and Chen 2003).

Data Analysis

The laser ablation data were scaled to the age of the fish by first calculating the age of the fish in days, and then applying a fractional age to each laser data point. Because of the nature of LA-ICPMS data, this means the amount of time between each data point was specifically calculated for every individual.

The periodic components of the strontium and barium signals were estimated using the spectral analysis techniques described in Hoover et al. (2012). Briefly, the data were first detrended by fitting either a linear

$$X_j = A_i + B_i t_j \tag{18}$$

or quadratic function

$$X_j = A_i + B_i t_j + C_i t_j^2, \quad (19)$$

where X_j is the original series, t_j is the time, and A_i, B_i, and C_i, are the coefficients ($j = 1, \ldots, 26;\ i = 1, 2$). Because of the operational settings of the LA-ICPMS at Woods Hole, no low-pass filter was necessary in this case.

Subsequently, I standardized the data by removing the mean and dividing by the standard deviation, to account for differing variability among samples. Spectral estimates were obtained using the maximum entropy method (MEM) (Ulrych and Bishop 1975, Priestley 1982, Press et al. 1994) and were computed using the algorithm given in Press et al. (1994) over 5000 frequencies. To test the numerical accuracy of the calculation, I integrated the spectra and compared the results with ½. In all cases, the error was <2%.

I calculated the 0.99 confidence limits for the calculated MEM spectrum, which are those of the χ^2 with the degrees of freedom of the computed spectrum. The 99% confidence limit was chosen to ensure conservative results. Peaks that fall above the confidence limit are statistically significant periodic components of the original signal (and their associated harmonics), representing changes in the elemental otolith composition at that time. The height of the spectral density peak corresponds to the strength of that component, i.e., the highest peak is the most significant periodicity in the data.

I interpret these peaks as the quantitative estimate of the periodicity of the inshore-offshore movement. To examine the presence of a periodic inshore-offshore

movement, I combined the highest peaks in spectral density for each individual to form a frequency distribution. The shapes of these distributions offer insight into the variability of the movement timing, not only within the inshore and offshore groups, but also between them.

To demonstrate that differences in otolith chemistry exist between in- and offshore environments I used a t-test to compare the edge chemistry of fish collected in each habitat. I first conducted Levene's test for homogeneity of variance. Finally, a paired t-test was used to test for differences between overall timing estimates between strontium and barium as elemental markers within an individual.

Results

Fish ranged in size from 300 to 500 mm for the inshore samples. Mean age of the inshore samples was 2.85±0.69 years old, which is equal to about 34 months. Offshore samples ranged in size from 300 to 500 mm and had a mean age of 2.92±0.76 years, which is approximately 35 months.

A representative plot of the raw element-to-calcium ratios for a summer-inshore sample demonstrated the relatively flat strontium signal (Figure 15a) and decreased barium levels at the edge (Figure 15b). The barium signal shows an oscillatory pattern at approximately 200 days. In general, for both inshore and offshore samples, the strontium signal showed little variation through time relative to the mean strontium value. For both inshore and offshore samples, barium exhibited strong oscillations through time, with clearly repeating patterns of alternating high and low

barium levels relative to the mean of each given sample. The strontium signal for a representative fish from the offshore sample (Figure 16a), shows the relatively stable strontium signal through time, with the barium exhibiting increased concentration at the edge (Figure 16b).

Although within an individual, the signal appeared stable through time, I sought to examine differences between the inshore and offshore. The mean edge chemistry strontium signature was 2.33±0.29 mmol/mol for inshore fish and 2.77±0.35 mmol/mol for offshore fish. These strontium edge chemistries were significantly different ($t=33.48$, $p<0.0001$), and the data met the assumption of homogeneity of variance by Levene's test for the inshore ($p=0.2896$) and offshore ($p=0.6381$) samples. The mean edge chemistry barium signature was 1.84±1.23 mmol/mol for inshore fish and 4.36±2.02 mmol/mol for offshore fish. These barium edge chemistries were significantly different ($t=7.59$, $p<0.0001$). Together, these differences in edge chemistries indicate that the in- and off-shore environments differ significantly in their chemical properties and these differences may be useful for reconstructing migratory histories.

The results of the spectral analysis provided estimates for the timing of movement through the environmental gradient from off- to inshore and back. The highest peak in spectral density, corresponding to the data in Figure 15a, occurs at approximately 225 days when examining the strontium signal (Figure 17). The spectral density for the barium signal shown in Figure 15b shows the most significant peak at 714 days (Figure 18). For the representative offshore sample in Figure 16a, the highest

peak in spectral density for strontium occurs at 725 days (Figure 19) and at 390 days for barium (Figure 22) corresponding with the data in Figure 16b. I interpret these peaks in spectral density as the estimated periodic movement of the fish across the environmental gradient.

The mean estimated timing of movement for inshore fish is 298.1±162.2 days using strontium, and 464.06±205.167 days using barium. The offshore fish moved across gradients at an older age, with a mean age of 431.9 ±159.0 days using strontium, and 444.9 ±176.4 days using barium. Note that although the mean times for barium were overall longer, they were also more variable. The movement timing between inshore and offshore black sea bass was significantly different when examining timing using strontium (χ^2=4.0027, p=0.0454), but not when using barium (χ^2=0.0947, p=0.7582).

The spectral analysis data met the assumptions of normality using the Shapiro-Wilk test for normality (W=0.9415, p=0.1458). The distributions of movement timing estimates using strontium for both inshore and offshore fish are shown in Figure 21. The distributions for estimated timing of movement using barium for both inshore and offshore fish are in Figure 22. A paired t-test showed no significant differences in the estimated timing of movement when using strontium versus barium for inshore fish (t=-2.16, p-value=0.0517) and offshore fish (t=-0.20, p=0.8415). Therefore, the data can therefore be pooled and examined together to form frequency distributions for the timing of movement across the population. Note that the distribution for strontium is

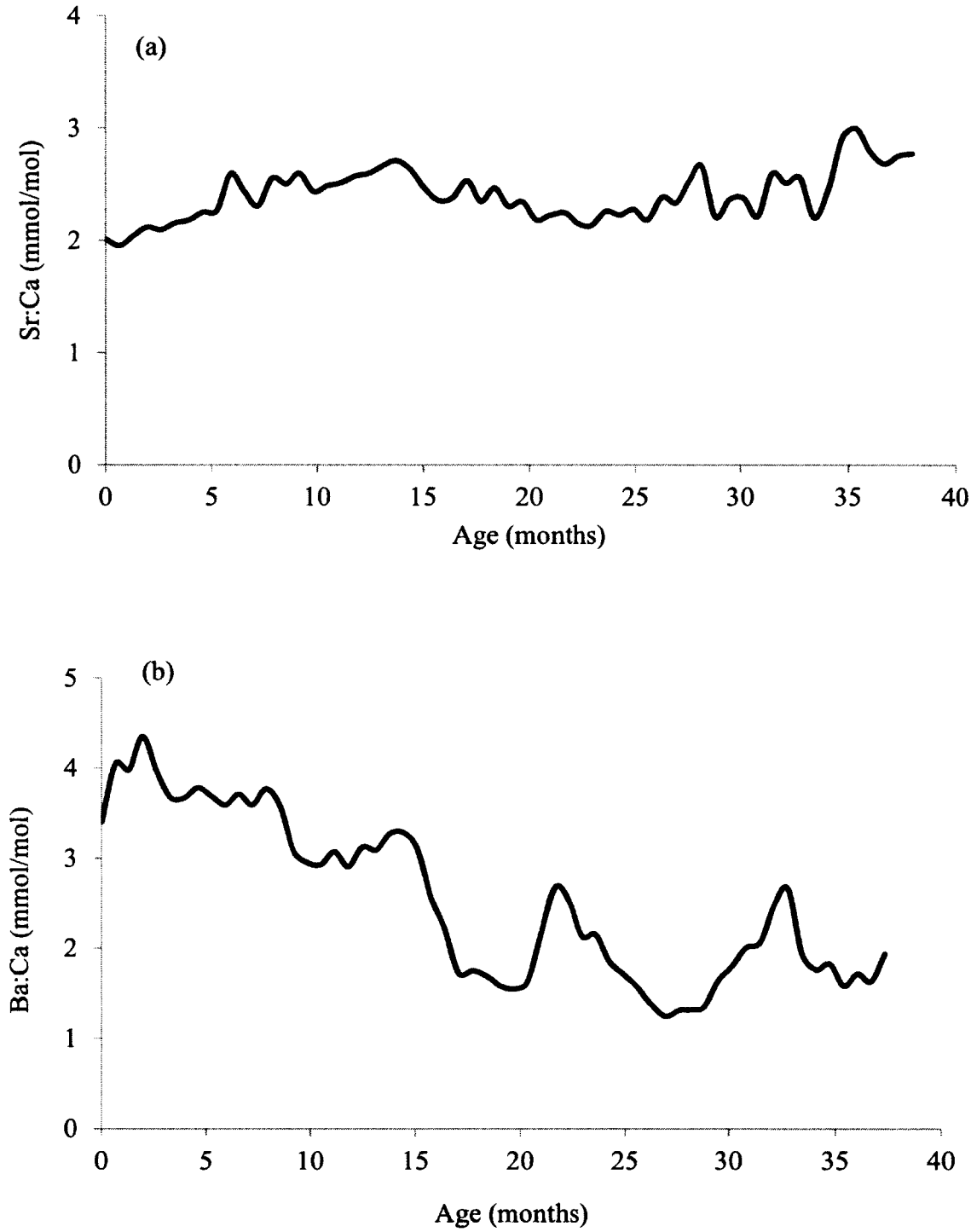

Fig. 15. (*a*) Raw Sr:Ca and (*b*) Ba:Ca molar ratios for a representative individual from the inshore sample.

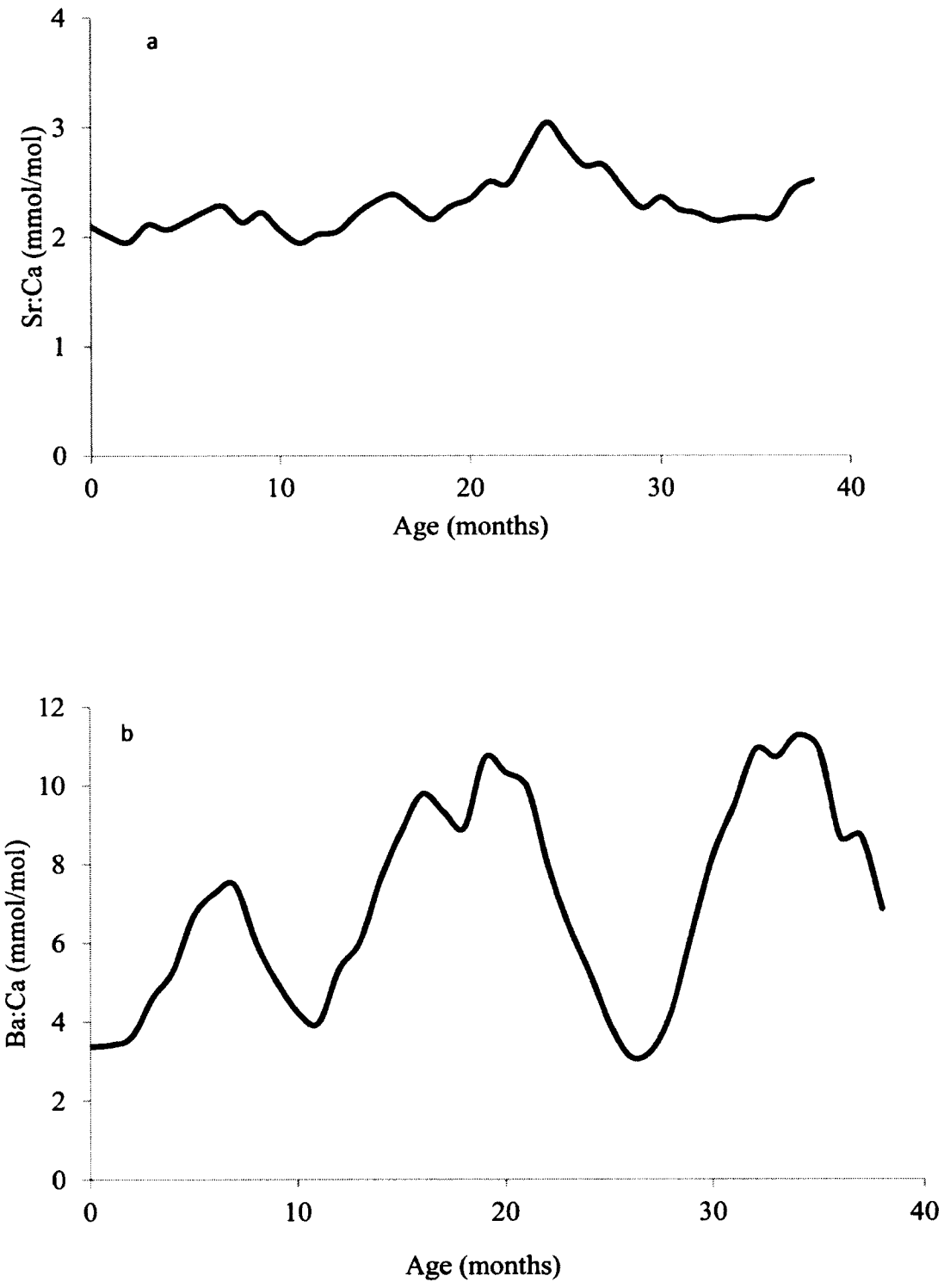

Fig. 16. (*a*) Raw Sr:Ca and (*b*) Ba:Ca molar ratios for a representative individual from the offshore sample.

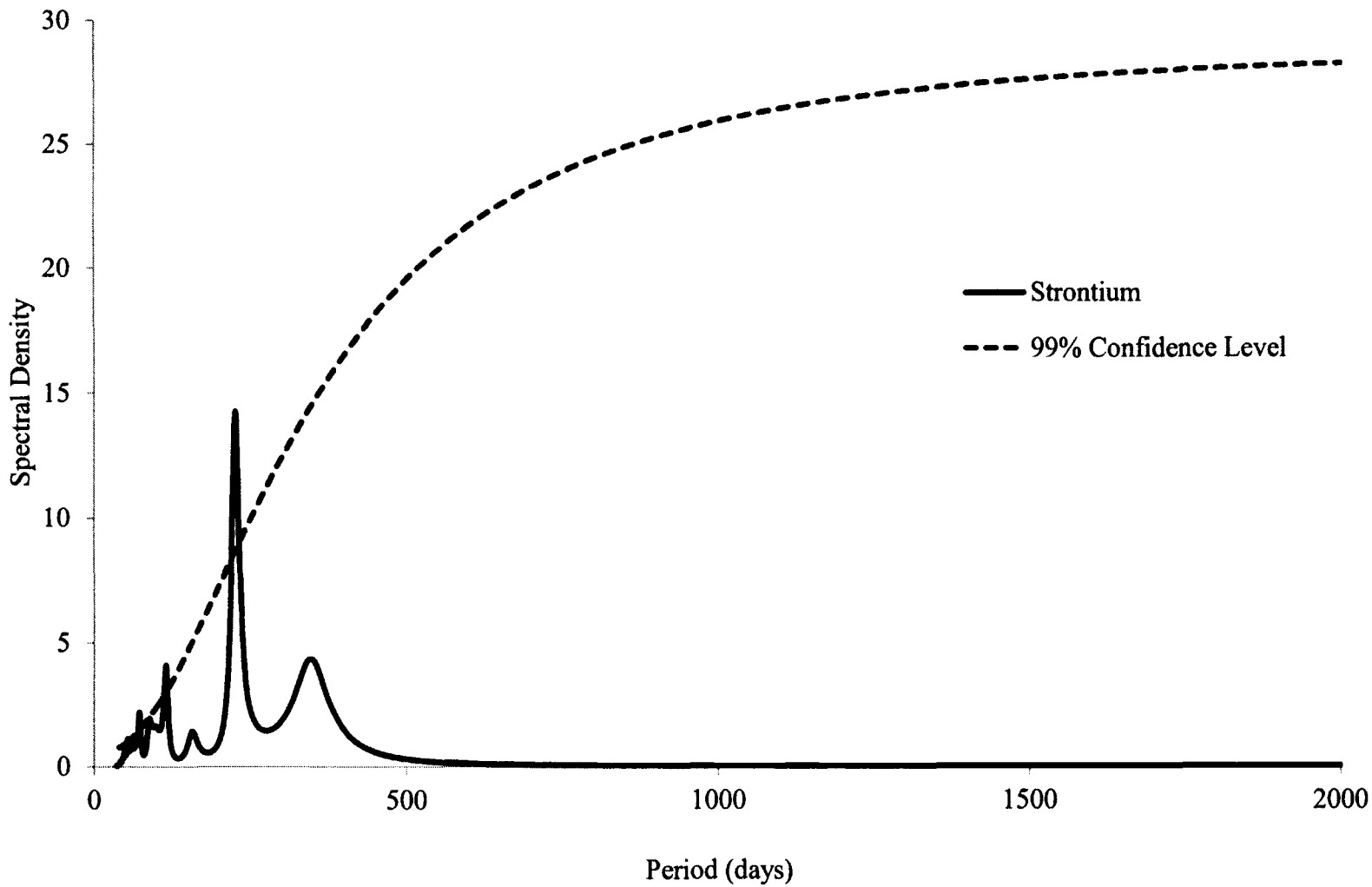

Fig. 17. Spectral density estimates for the representative inshore sample using strontium, showing peak in spectral density at 225 days (7.5 months).

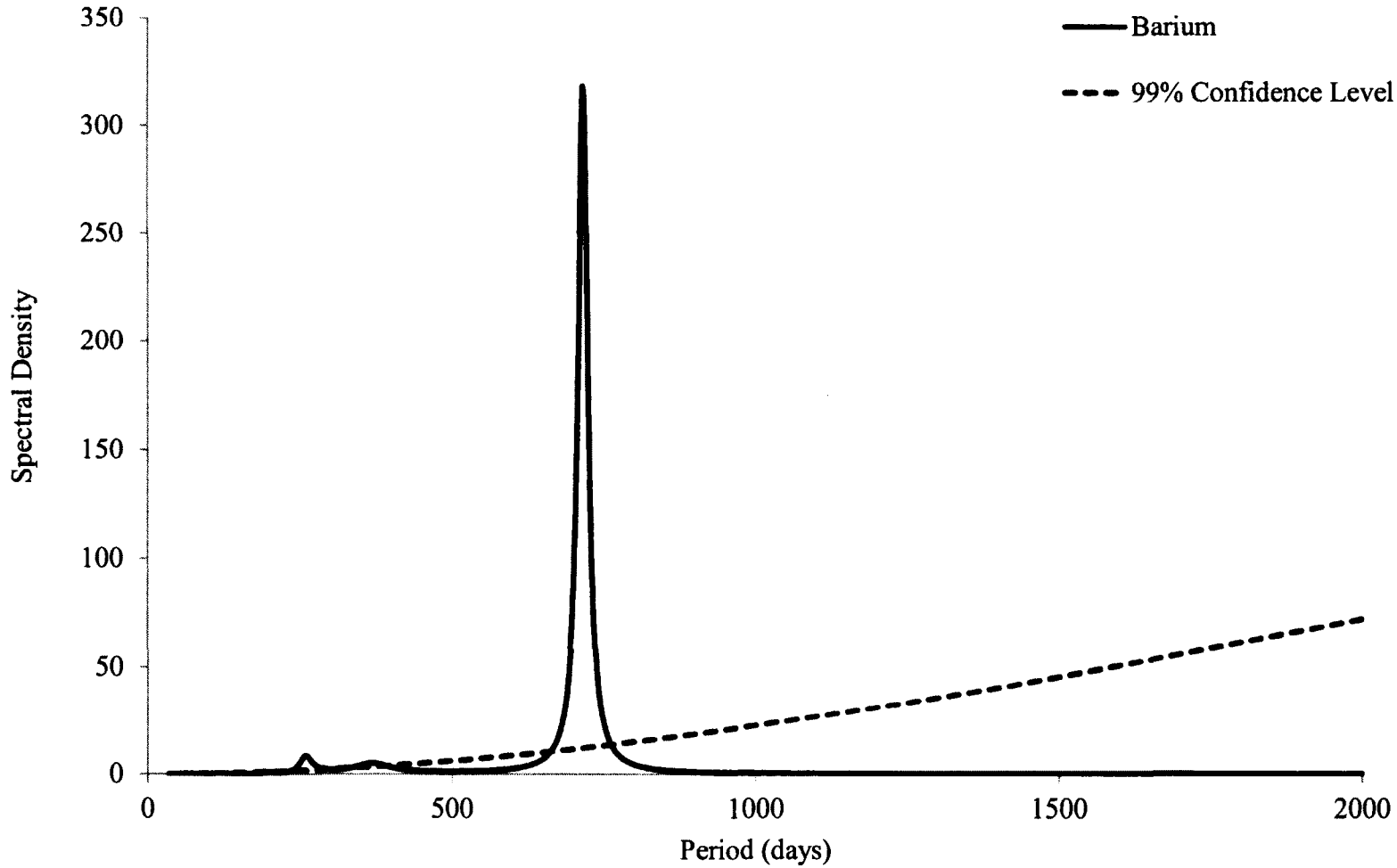

Fig. 18. Spectral density estimates for the representative inshore sample using barium, showing peak in spectral density at 714 days (23.8 months).

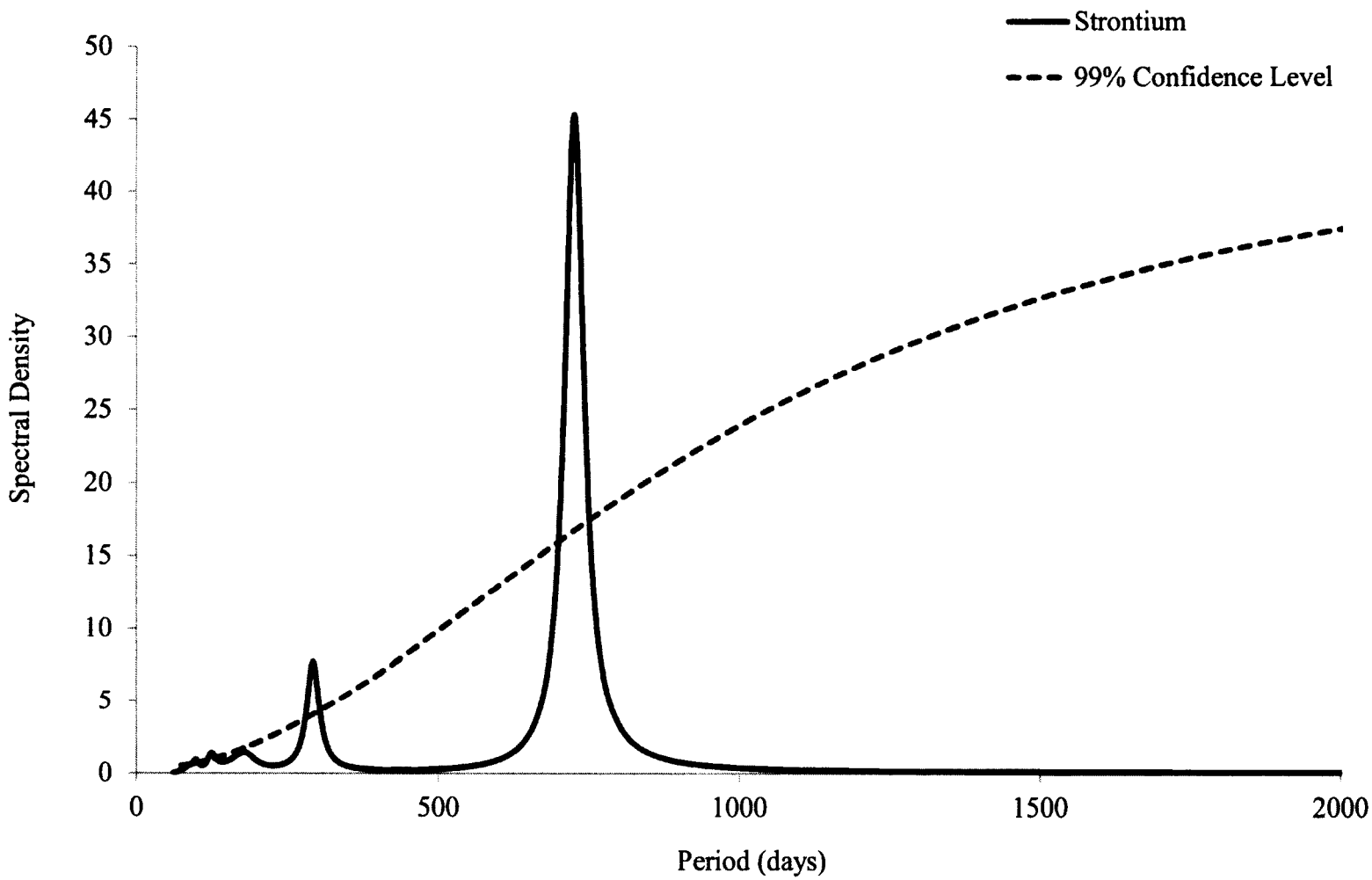

Fig. 19. Spectral density estimates for the representative offshore sample using strontium, showing peak in spectral density at 725 days (24.2 months).

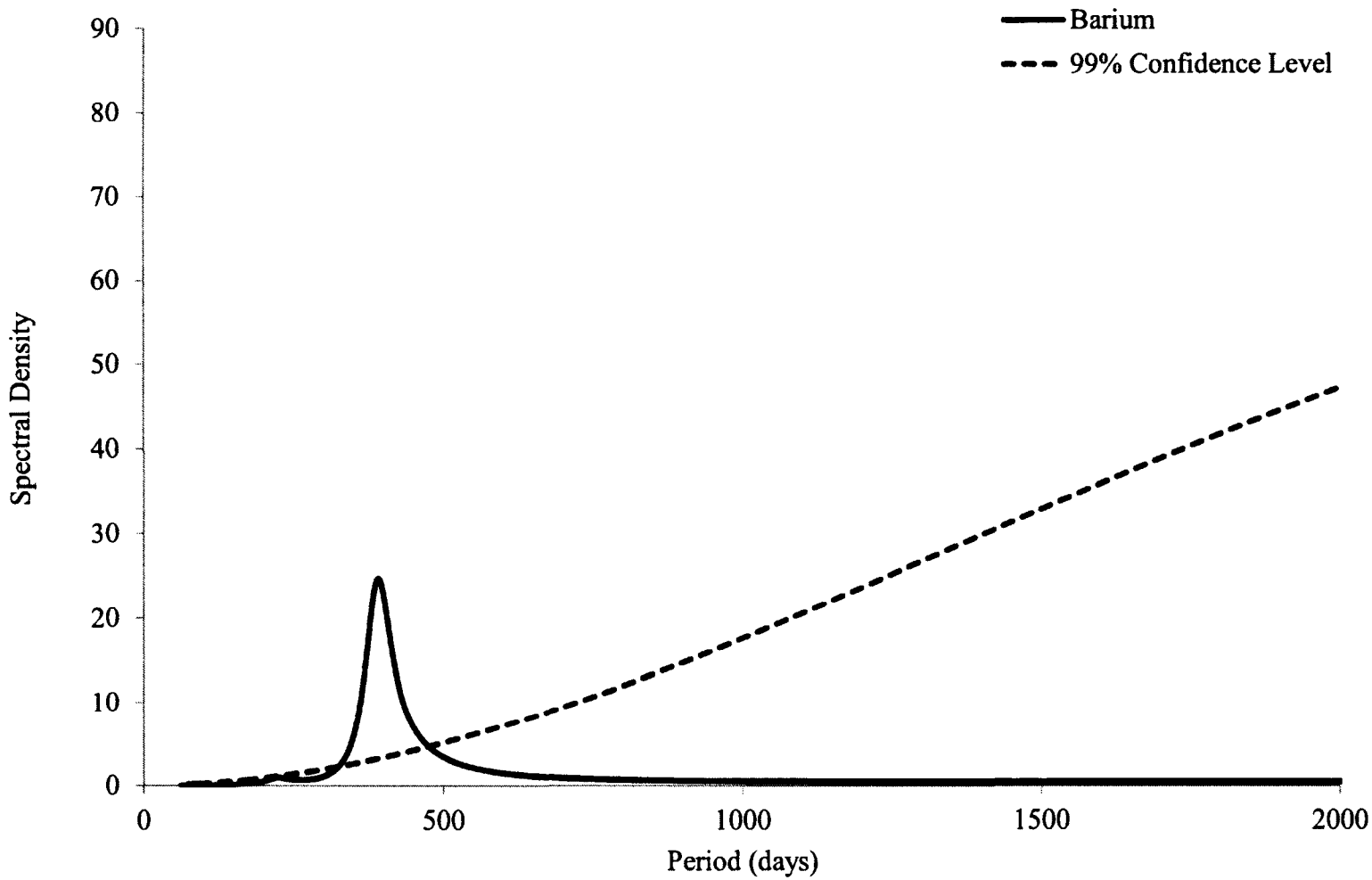

Fig. 20. Spectral density estimates for the representative offshore sample using barium, showing peak in spectral density at 390 days (13.0 months).

positively skewed as compared with barium (i.e., the movement timing estimates using strontium are slightly earlier as compared with barium).

Discussion

While several studies have demonstrated the ability for otolith chemistry to track extremely different water masses (i.e., from fully marine to fully estuarine or brackish waters), I evaluated differences in chemistry that were expect to be more subtle in the coastal shelf waters. Our results demonstrate that inshore-offshore movement in black sea bass is fully detectable using otolith life history scans. I hypothesized that the difference in the inshore and offshore habitats would result in differing otolith chemistry signatures; our results support this hypothesis through significantly different edge chemistries of samples collected in the inshore and offshore environments. A subsequently critical ecological question is what type of environment would induce such changes in the otolith chemistry signatures. Despite occupying what appear to be physically similar water masses, I show that black sea bass from separate parts of the population's residency (inshore and offshore environments) have significantly different otolith chemistry signatures at their edges.

I examined both strontium and barium, but found the most information regarding habitat use was revealed in the barium chemistries. The barium chemistry at the edge of the otolith (representing the portion of life history close to and up to the time of capture) is a proxy for the patterns of habitat use in this case. For example, the fish were collected from the offshore area represent this species' winter (offshore) habitat use. The

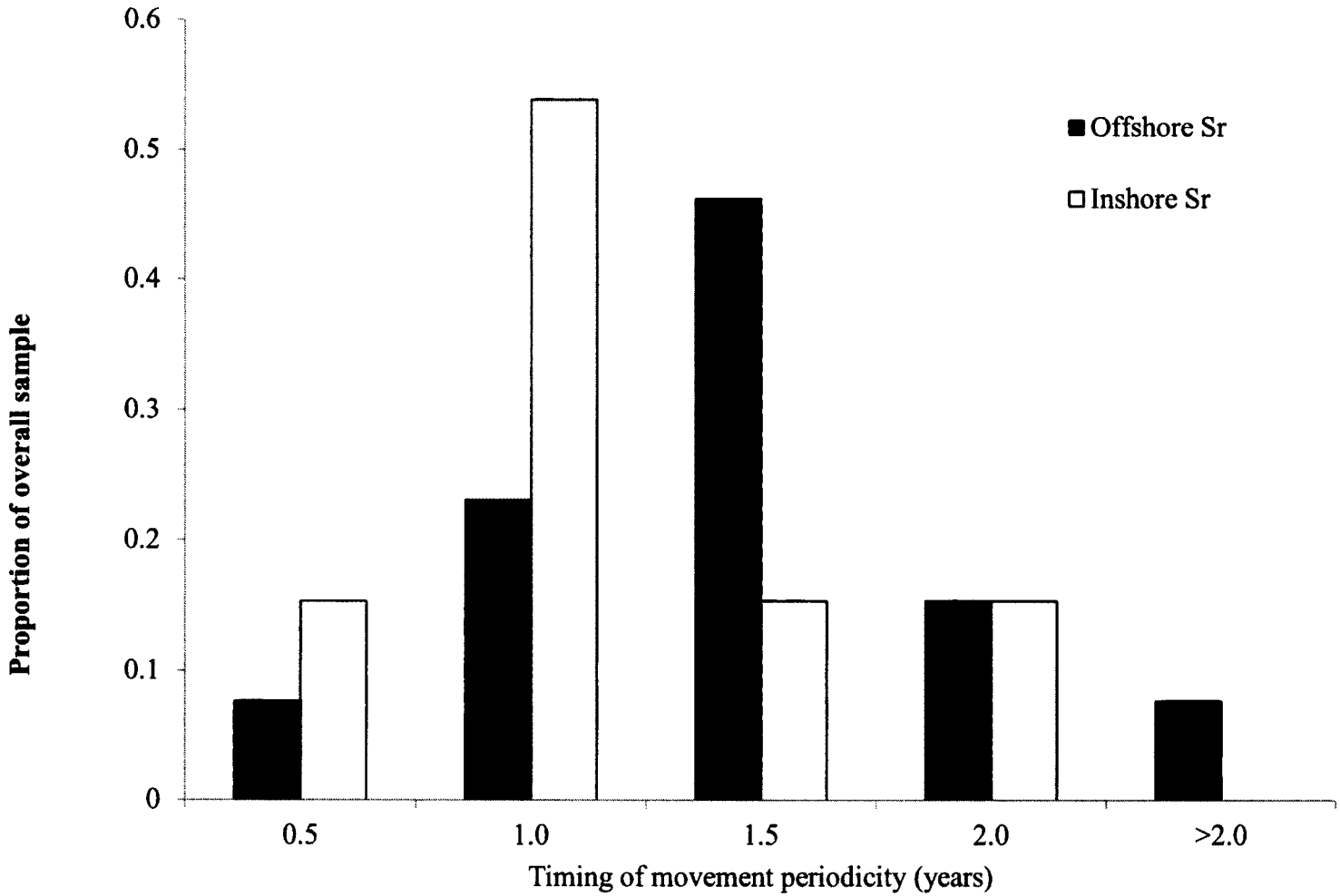

Fig. 21. Distribution of timing of movement for inshore (gray) and offshore (black) fish using strontium.

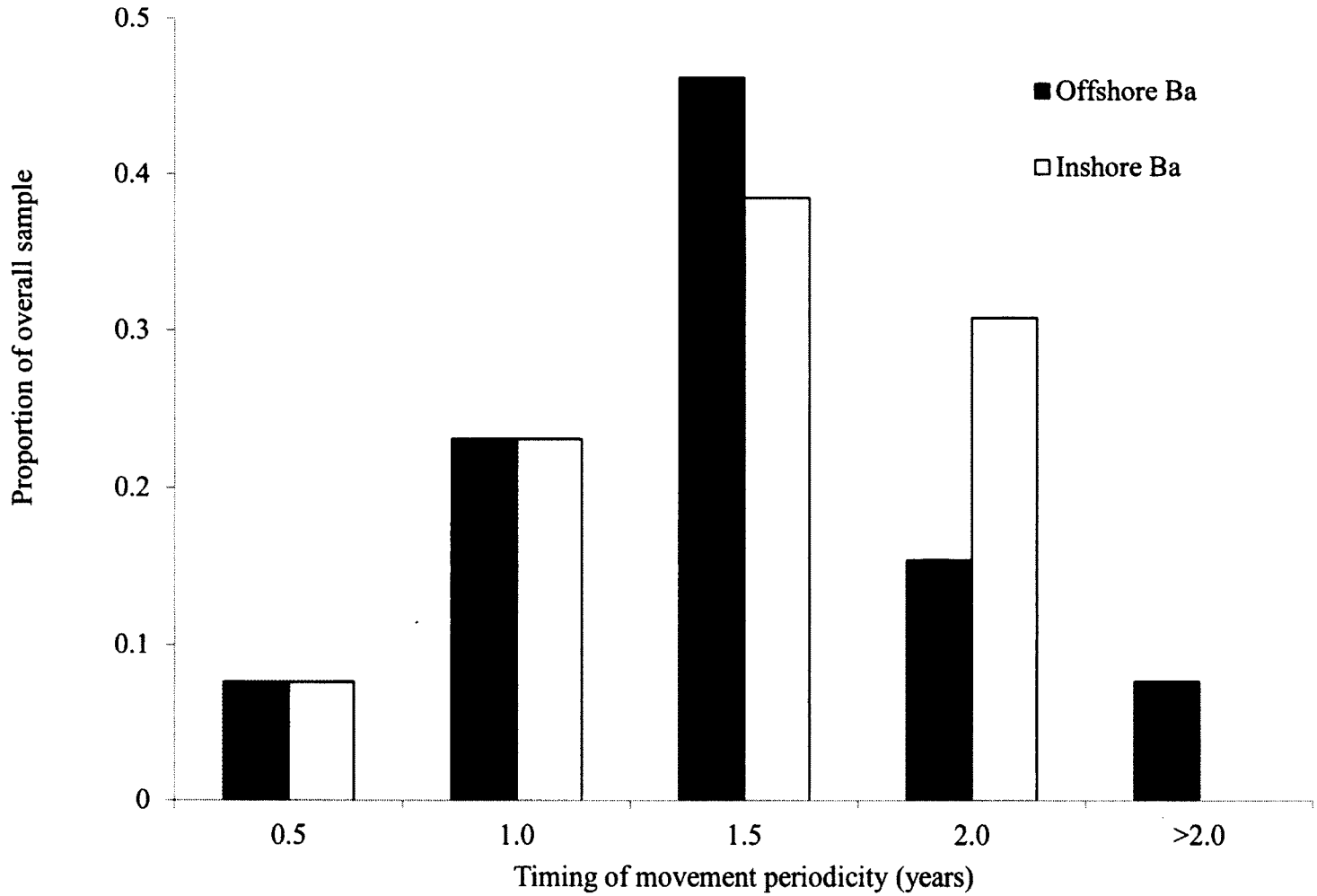

Fig. 22. Distribution of timing of movement for inshore (gray) and offshore (black) fish using barium.

interesting aspect of the edge chemistry of these samples is that they show a significantly higher barium signal than the edge chemistry of inshore fish. Significantly high barium signals would not normally be expected in oceanic waters, as higher barium concentrations are traditionally associated with a freshwater signal in the otolith chemistry literature, e.g., (Secor et al. 2001). Yet, other research has shown that barite-rich waters may in fact be delivered to the surface through upwelling processes (Ashford et al. 2011). Although barium has a nutrient-type distribution in seawater, in surface waters it has been shown to be sourced both from riverine input as well as coastal upwelling (Shen et al. 1992; Patterson et al. 1999).

The significant differences in edge chemistry, particularly for barium, between inshore fish and offshore fish support the hypothesis that these fish are moving off the coastal shelf into an upwelling environment. Our results support the hypothesis that black sea bass move not only to the shelf edge, but in fact make use of submarine canyon systems. The flow near submarine canyons has been established as a considerable source of upwelling in coastal shelf systems, transporting water across the shelf break in areas with "left bounded" flow, i.e., coast is to the left looking the direction of flow (She and Klinck 2000). As a result, canyon systems have been shown to be highly productive upwelling areas for fish assemblages (Edwards et al. 2008).

While the main driver of strontium dynamics tends to be salinity in the estuarine and marine settings (Bath et al. 2000), significant effects of temperature (Miller 2011; Webb et al. 2012), diet (Engstedt et al. 2012), and growth rates (Walther et al. 2010) have also been demonstrated. The overall trend components of our

strontium signals are increasing through time. Considering the relatively small changes in salinity these black sea bass experience, our data most likely demonstrate one of these less commonly attributed drivers of strontium dynamics.

Additionally, I show that spectral analysis of these life history scans provides quantitative estimates of the timing of movement from the offshore to inshore environment. This more recent approach to life history scan data is particularly suited to assessing movement; I applied this method in our analysis as a means of examining whether black sea bass are in fact moving on and offshore in a periodic manner. While the previous tagging study showed a simple inshore-offshore movement, our application quantitatively assesses the timing of that movement. To our knowledge, this is the first successful application of spectral analysis to a recurring periodic otolith chemistry signal. Moreover, I emphasize that a tagging study, by its very nature, will only be able to confirm the movement patterns of those individuals who are completing the migratory behavior. The individuals who do not return will not be represented in the tagging samples, and therefore will remain unknown. The advantage of the otolith chemistry is that all individuals contain this natural tag, and as such, a proper random sample of the population can be taken to appropriately estimate the proportion of individuals exhibiting this migratory behavior.

Black sea bass are expected to make the movement to the offshore environment during the winter season (Moser and Shepherd, 2009). The mean age of our samples was approximately 3 years, and the mean estimated movement timing using barium was 1.25 years. This result indicates a significant periodic component in these data

occurs at approximately 15 months, when using barium as the habitat marker. As such, our data strongly support the expected winter offshore movement. For example, a typical fish that was spawned in the late summer months, e.g., August, would be expected to make this offshore movement for the first time in November of its second year. I note that when examining the timing of movement for individuals, rather than the entire sample, there is some loss of generality, since the estimates are for each individual, rather than the population as a whole. Conversely I fully caution that there will always be variability in the timing of movement when examining the population as a whole. This variability can be examined explicitly, however, through the frequency distributions of estimated movement times. The first aspect of estimate variability lies within a given sample group (inshore or offshore). Differences in the timing of movement are apparent, with an earlier movement in the inshore fish, as estimated using strontium. The second source of variability I need to examine is the difference in the estimated timing of movement between strontium and barium.

The paired t-test on the estimated timing of movement showed significant differences when using strontium, but not when using barium. These conflicting results for the two environmental markers cautions against analyzing life history scan data in an overly-simple framework. More than one environmental marker may need to be considered when attempting to reconstruct timing of movement events. I caution, however, that a relatively small sample sizes such as ours limit the power of such tests. By applying the spectral methods described by Hoover et al. (2012), I was able to discriminate among the results obtained by the two markers and find there are

significantly different types of information contained in each. There were significant differences in the mean timing of movement as estimated by the two markers, with barium estimating later movement timing than strontium in both inshore and offshore fish. Strontium did show significant differences between inshore and offshore samples, demonstrating that although samples from the two areas in the population's residency are assumed to return the same coastal pool during spawning, in fact, the separate areas may be experiencing very distinct water masses and migratory behaviors.

Nonetheless, the concept of inconsistency among habitat use markers has recently been explored in the case of American eels by Jessop et al. (2012). Our results highlight the need for comparison among several environmental markers to spatially contextualize otolith chemistry signatures. Particularly when considering the differences in not only the mean estimates of timing of movement, but also the variability of the signatures themselves, I see that different elements contain diverse forms and quality of information.

Because the inshore fish were collected in a more northern location, the winter temperatures they experience occur earlier in the year relative to the offshore fish. As a result, I would expect the movement timing for the summer-inshore habitat fish to be slightly sooner than that of the winter-offshore fish. I see this pattern confirmed in the distribution of movement times as estimated through both strontium and barium.

The use of fish from separate portions of the black sea bass population's residency allowed us to examine differences in space as a proxy for differences in time for this population. I now know that the subtle differences in otolith chemistry of fish

in the inshore and offshore environments are significantly different. As such, I can further demonstrate that the otolith chemistry oscillations within an individual, particularly in the barium signatures, represent a periodic movement between the inshore and offshore environments. Our results draw attention to the differences in estimates of movement timing acquired through different environmental markers.

Finally, the high barium levels in the edge chemistry of fish caught in the offshore region demonstrate that the population is making use of submarine canyon systems, which are known transporters of barite-rich waters. The importance of offshore canyon systems to marine fishes has not yet been fully investigated, and may prove a significant driver in many species' population dynamics.

CHAPTER V

CONCLUSIONS

Identifying the movement dynamics of fish populations plays a central role in our understanding and proper management of many marine, freshwater, and diadromous species. A broad array of fisheries issues, such as recruitment dynamics, growth processes, and even harvester interactions, rely on accurate information regarding the movement of fish populations through time and space. Using otolith life history scan data, I developed quantitative methods for estimating the timing of movement for fish that travel across environmental gradients using spectral analysis.

I demonstrated that spectral analysis is a viable tool for analyzing otolith life history scan data. Our first study estimated the timing of ingress for an estuarine-dependent species. The thoroughly documented life history pattern of Atlantic croaker (*Micropogonias undulatus*), which transition from the marine to the estuarine environment as juveniles, made them an ideal candidate for our investigations. Our results produced quantitative point estimates for the timing of migration for each individual in our sample. I then pooled these estimates to form frequency distributions for the overall sample. Such distributions, when expanded to an appropriate sample size (e.g., on the order of 30 fish for a given location), offer a great potential for examining the variability and behavior of ingress timing estimates at the population level. Narrow distributions of ingress timing would indicate a population whose

ingress timing was temporally restricted, whereas broad or uniformly distributed timing estimates might imply a more variable timing of ingress.

The importance of such information lies in its ability to inform us of various life history strategies. Because croaker had such a broadly dispersed timing of ingress across individuals, I can infer that the evolutionary driver of ingress for this species may not be dependent on the size or age of the individual fish. In our case, these results do not lend support to such ecological theories as the critical size hypothesis, but more likely speak to physical drivers, such as currents, as determining ingress patterns for this species. Using these methods, the breadth of information that can be accessed through otolith life history scans is truly revealed.

Along with our contributions, various quantitative methods for analyzing otolith life history scan data will surely continue to develop as investigators seek answers to new questions in fisheries science. Likewise, I expect technological advances in physically measuring otolith elemental composition to continue, as well. Our investigations into the sampling effects of laser ablation, however, beget caution in the appropriate application of such sampling methods. I investigated the impacts of laser ablation on otolith chemistry data, finding that the three dimensional morphology of otolith significantly impacts results obtained from these types of life history scans . Our results suggest that both the depth of the ablation trench and the width of the laser spot size should be considered when conducting otolith life history scans. Despite this conclusion, I have found very few studies consider the integrating effects of laser ablation depth or spot size when conducting life history scans on otoliths.

Through these first two studies, I moved from the more theoretical investigations of developing quantitative methods for analyzing life history scan data into the more functional study of how laser ablation affects results. In the final investigation, I fully implemented our method using the quantitative approach in the first study, with the technical considerations of the second, to explore the inshore-offshore dynamics of a coastal fish population. The putative movement of black sea bass (*Centropristis striata*) had not been empirically validated outside of a relatively recent tagging study. Our approach offered a means of examining whether the inshore-offshore dynamics of this population were: (1) testable through otolith chemistry; (2) present in the sampled population; and (3) caused by the existing physical features of the environment that the fish inhabit. This last point lead us to an understanding of how the physical oceanography in and around submarine canyons might affect barium signals in fish that frequently traverse such environmental gradients. Our results supported our hypothesis that such movements were testable through otolith chemistry, and consequently, that the inshore-offshore movement could in fact be confirmed in individual black sea bass, and for the population as a whole.

To our knowledge, these are the first applications of spectral analysis to otolith life history scan data. Although preliminary steps have been made in the application of Bayesian reconstruction, as well as various classification techniques, ours is the first application of a method to obtain quantitative estimates of fish movement timing through otolith chemistry. One of the next steps in developing these methods will be to

more fully investigate the remaining environmental information contained within the otolith.

While our work focused on the two most common environmental markers used in the current otolith chemistry literature, strontium and barium, future studies should seek to evaluate the information from other commonly sampled variables, such as minor and trace elements (e.g., Mg, Mn, Rb, Yt, Li), stable isotope ratios of carbon and oxygen, and growth parameters. I suggest applying a spectral analysis to these other metrics, where applicable, and further investigating the analysis of the spectrally derived periodic components of these variables.

The timing of ingress and movement are of critical importance to several ecological questions. I anticipate that the natural cyclic nature of many variables contained within the otolith will reveal periodic information that will be useful in the understanding of fish population dynamics. I would expect those variables which are metabolically controlled to be most likely to contain periodic signals. These are all questions whose answers will provide insight to the continually evolving landscape of fisheries ecology.

REFERENCES

Able, K., M. Fahay, and G. Shepherd. 1995. Early life history of black sea bass, *Centropristis striata*, in the mid-Atlantic Bight and a New Jersey estuary. Fishery Bulletin **93**:429-445.

Ashford, J., M. La Mesa, B. A. Fach, C. Jones, and I. Everson. 2010. Testing early life connectivity using otolith chemistry and particle-tracking simulations. Canadian Journal of Fisheries and Aquatic Sciences **67**:1303-1315.

Ashford, J., R. Serra, J. C. Saavedra, and J. Letelier. 2011. Otolith chemistry indicates large-scale connectivity in Chilean jack mackerel (*Trachurus murphyi*), a highly mobile species in the Southern Pacific Ocean. Fisheries Research **107**:291-299.

Ashford, J. R., C. M. Jones, E. Hofmann, I. Everson, C. Moreno, G. Duhamel, and R. Williams. 2005. Can otolith elemental signatures record the capture site of Patagonian toothfish (*Dissostichus eleginoides*), a fully marine fish in the Southern Ocean? Canadian Journal of Fisheries and Aquatic Sciences **62**:2832-2840.

Barbieri, L. R., M. Chittenden Jr, and S. K. Lowerre-Barbieri. 1994. Maturity, spawning, and ovarian cycle of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters. Fishery Bulletin **92**:671-685.

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

Beamish, R., C. Mahnken, and C. Neville. 2004. Evidence that reduced early marine growth is associated with lower marine survival of coho salmon. Transactions of the American Fisheries Society **133**:26-33.

Beamish, R. J. and C. Mahnken. 2001. A critical size and period hypothesis to explain natural regulation of salmon abundance and the linkage to climate and climate change. Progress in Oceanography **49**:423-437.

Ben-Tzvi, O., A. Abelson, S. D. Gaines, M. S. Sheehy, G. L. Paradis, and M. Kiflawi. 2007. The inclusion of sub-detection limit LA-ICPMS data, in the analysis of otolith microchemistry, by use of a palindrome sequence analysis(PaSA). Limnology and Oceanography: Methods **5**:97-105.

Ben-Tzvi, O., M. Kiflawi, S. D. Gaines, M. Al-Zibdah, M. S. Sheehy, G. L. Paradis, and A. Abelson. 2008. Tracking recruitment pathways of *Chromis viridis* in the Gulf of Aqaba using otolith chemistry. Marine Ecology Progress Series **359**:229.

Borelli, G., N. Mayer-Gostan, P. Merle, H. D. Pontual, G. Boeuf, D. Allemand, and P. Payan. 2003. Composition of biomineral organic matrices with special emphasis on turbot (*Psetta maxima*) otolith and endolymph. Calcified Tissue International **72**:717-725.

Bowen, B. and J. Avise. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: influence of zoogeographic factors and life-history patterns. Marine Biology **107**:371-381.

Brockwell, P. J. and R. A. Davis. 2002. Introduction to time series and forecasting. Springer Verlag.

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

Campana, S. E. 2005. Otolith science entering the 21st century. Marine and Freshwater Research **56**:485-495.

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. 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, H. Depontual, H. Troadec, J. Panifili, D. H. Secor, K. P. Severin, S. H. Sie, R. Thresher, W. J. Teesdale, and 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 couplied plasma mass spectrometry. Canadian Journal of Fisheries and Aquatic Sciences **54**:2068-2079.

Chang, C. W., Y. Iizuka, and W. N. Tzeng. 2004. Migratory environmental history of the grey mullet *Mugil cephalus* as revealed by otolith Sr: Ca ratios. Marine Ecology Progress Series **269**:277-288.

Chatfield, C. 2004. The analysis of time series: an introduction. CRC press.

Chen, Z. and C. M. Jones. 2006. Simultaneous determination of 33 major, minor, and trace elements in juvenile and larval fish otoliths by high resolution double focusing sector field inductively coupled plasma mass spectrometry *in* 2006 Winter Conference on Plasma Spectrochemistry, Tucson, Arizona.

Cowan, J. and H. James. 1988. Age and growth of Atlantic croaker, *Micropogonias undulatus*, larvae collected in the coastal waters of the northern Gulf of Mexico as determined by increments in saccular otoliths. Bulletin of Marine Science **42**:349-357.

Daverat, F., J. Martin, R. Fablet, and C. Pécheyran. 2011. Colonisation tactics of three temperate catadromous species, eel *Anguilla anguilla*, mullet *Liza ramada* and flounder *Plathychtys flesus*, revealed by Bayesian multielemental otolith microchemistry approach. Ecology of Freshwater Fish.

De Vries, M. C., B. M. Gillanders, and T. S. Elsdon. 2005. Facilitation of barium uptake into fish otoliths: influence of strontium concentration and salinity. Geochimica et Cosmochimica Acta **69**:4061-4072.

Degens, E. T., W. G. Deuser, and R. L. Haedrich. 1969. Molecular structure and composition of fish otoliths. International Journal on Life in Oceans and Coastal Waters **2**:105-113.

Dorval, E. 2004. Relating water and otolith chemistry in Chesapeake Bay, and their potential to identify essential seagrass habitats for juveniles of an estuarine-dependent fish, spotted seatrout (*Cynoscion nebulosus*). Doctoral. Old Dominion University, Norfolk.

Dorval, E., C. M. Jones, R. Hannigan, and J. Montfrans. 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Canadian Journal of Fisheries and Aquatic Sciences **64**:411-424.

Dorval, E., C. M. Jones, R. Hannigan, and J. Van Montfrans. 2005. Can otolith chemistry be used for identifying essential seagrass habitats for juvenile spotted seatrout, *Cynoscion nebulosus*, in Chesapeake Bay. Marine and Freshwater Research **56**:654-653.

Drohan, A., J. Manderson, and D. Packer. 2007. Essential fish habitat source document: Black sea bass, *Centropristis striata*, life history and habitat characteristics. NOAA Tech Memo NMFS NE **200**:68.

Ebisuzaki, W. 1997. A method to estimate the statistical significance of a correlation when the data are serially correlated. American Meteorological Society **10**:2147-2153.

Edwards, K., J. Hare, and F. Werner. 2008. Dispersal of black sea bass (*Centropristis striata*) larvae on the southeast US continental shelf: results of a coupled vertical larval behavior–3D circulation model. Fisheries Oceanography **17**:299-315.

Elsdon, T. S. and B. Gillanders. 2005a. Consistency of patterns between laboratory experiments and field collected fish in otolith chemistry: an example and applications for salinity reconstructions. Marine and Freshwater Research **56**:609-617.

Elsdon, T. S. and B. Gillanders. 2005b. Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. Marine Ecology Progress Series **285**:233-243.

Elsdon, T. S. and B. M. Gillanders. 2003. Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. Reviews in Fish Biology and Fisheries **13**:219-235.

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

Engstedt, O., P. Koch-Schmidt, and P. Larsson. 2012. Strontium (Sr) uptake from water and food in otoliths of juvenile pike (*Esox lucius L.*). Journal of Experimental Marine Biology and Ecology **418**:69-74.

Fablet, R., F. Daverat, and H. De Pontual. 2007. Unsupervised Bayesian reconstruction of individual life histories from otolith signatures: case study of Sr: Ca transects of European eel (*Anguilla anguilla*) otoliths. Canadian Journal of Fisheries and Aquatic Sciences **64**:152-165.

Farley Jr, E. V., J. M. Murphy, M. D. Adkison, L. B. Eisner, J. H. Helle, J. H. Moss, and J. Neilsen. 2005. Critical-size, critical-period hypothesis: an example of the relationship between early marine growth of juvenile Bristol Bay sockeye salmon, subsequent marine survival, and ocean conditions. BASIS 2004: Salmon and marine ecosystems in the Bering Sea and adjacent waters:46.

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

Feutry, P., P. Keith, C. Pécheyran, F. Claverie, and T. Robinet. 2011. Evidence of diadromy in the French Polynesian Kuhlia malo (Teleostei: Percoidei) inferred from otolith microchemistry analysis. Ecology of Freshwater Fish **20**:636-645.

FitzGerald, J. L., S. R. Thorrold, K. M. Bailey, A. L. Brown, and K. P. Severin. 2004. Elemental signatures in otoliths of larval walleye pollock (*Theragra chalcogramma*) from the northeast Pacific Ocean.

Fowler, A. J., S. E. Campana, C. M. Jones, and S. R. Thorrold. 1995. 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.

Gillanders, B. M. 2005. Otolith chemistry to determine movements of diadromous and freshwater fish. Aquatic Living Resources **18**:291-300.

Gossett, J., P. Simpson, P. Casey, L. Whiteside-Mansell, R. Bradley, and C. H. Jo. 2007. Growing growth curves using PROC MIXED and PROC NLMIXED. University of Arkansas for Medical Sciences, Little Rock.

Hale, R. and S. E. Swearer. 2008. Otolith microstructural and microchemical changes associated with settlement in the diadromous fish *Galaxias maculatus*. Marine Ecology Progress Series **354**:229-234.

Hannigan, R., E. Dorval, and C. Jones. 2010. The rare earth element chemistry of estuarine surface sediments in the Chesapeake Bay. Chemical Geology **272**:20-30.

Hedger, R. D., P. M. Atkinson, I. Thibault, and J. J. Dodson. 2008. A quantitative approach for classifying fish otolith strontium: calcium sequences into environmental histories. Ecological Informatics **3**:207-217.

Heintz, R. A. and J. J. Vollenweider. 2010. Influence of size on the sources of energy consumed by overwintering walleye pollock (*Theragra chalcogramma*). Journal of Experimental Marine Biology and Ecology **393**:43-50.

Hettler, W. F. 1998. Abundance and size of dominant Winter-immigrating fish larvae at two inlets into Pamlico Sound, North Carolina. Brimleyana **25**:144-155.

Hoover, Reneé R., Cynthia M. Jones, and Chester E. Grosch. 2012. Estuarine ingress timing as revealed by spectral analysis of otolith life history scans. Canadian Journal of Fisheries and Aquatic Sciences.

Hoskin, S. 2002. Recruitment Variability of Atlantic Croaker, *Micropogonias undulatus*, with Observations on Environmental Factors. Old Dominion University.

Houde, E. 1997. Patterns and trends in larval-stage growth and mortality of teleost fish. Journal of Fish Biology **51**:52-83.

Ihara, S. 1993. Information theory for continuous systems. World Scientific Pub Co Inc.

Jessop, B. M., C. H. Wang, W. N. Tzeng, C. F. You, J. C. Shiao, and S. H. Lin. 2012. Otolith Sr: Ca and Ba: Ca may give inconsistent indications of estuarine habitat

use for American eels (Anguilla rostrata). Environmental Biology of Fishes:1-15.

Jones, C. M. and Z. Chen. 2003. New techniques for sampling larval and juvenile fish otoliths for trace-element analysis with laser-ablation sector-field inductively-coupled plasma mass spectrometry (SF-ICP-MS). *in* 26th Annual Larval Fish Conference. Institute of Marine Research, Postboks 1870 Nordnes, N-5817, Bergen, Norway.

Jones, J. B. and S. E. Campana. 2009. Stable oxygen isotope reconstruction of ambient temperature during the collapse of a cod (*Gadus morhua*) fishery. Ecological Applications **19**:1500-1514.

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

Kemp, J., S. E. Swearer, G. P. Jenkins, and S. Robertson. 2011. Otolith chemistry is more accurate than otolith shape in identifying cod species (genus *Pseudophycis*) in the diet of Australian fur seals (*Arctocephalus pusillus doriferus*). Canadian Journal of Fisheries and Aquatic Sciences **68**:1732-1743.

Lee, S., I. Vonta, and A. Karagrigoriou. 2011. A maximum entropy type test of fit. Computational Statistics & Data Analysis.

Limburg, K. E. 1995. Otolith strontium traces environmental history of subyearling American shad, *Alosa sapidissima*. Marine Ecology Progress Series **119**:25-35.

Limburg, K. E. and M. Elfman. 2010. Patterns and magnitude of Zn: Ca in otoliths support the recent phylogenetic typology of Salmoniformes and their sister groups. Canadian Journal of Fisheries and Aquatic Sciences **67**:597-604.

Logbo, S. C., M. Errigo, S. Atlant, E. Hiltz, J. Byrd, and S. Carolin. 2011. SEDAR25-DW23.

Macdonald, J. I. and D. A. Crook. 2010. Variability in Sr: Ca and Ba: Ca ratios in water and fish otoliths across an estuarine salinity gradient. Marine Ecology Progress Series **413**:147-161.

Marklevitz, S. A. C., B. J. Fryer, D. Gonder, Z. P. Yang, J. Johnson, A. Moerke, and Y. E. Morbey. 2011. Use of otolith chemistry to discriminate juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from different wild populations and hatcheries in Lake Huron. Journal of Great Lakes Research **37**:698-706.

Martin, G. B., S. R. Thorrold, and C. M. Jones. 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). Canadian Journal of Fisheries and Aquatic Sciences **61**:34-42.

Martin, G. B. and M. J. Wuenschel. 2006. Effect of temperature and salinity on otolith element incorporation in juvenile gray snapper Lutjanus griseus. Marine Ecology Progress Series **324**:229-239.

Mercer, L. P. and D. Moran. 1989. Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (South Atlantic). Black Sea Bass. DTIC Document.

Miller, J. 2011. Effects of water temperature and barium concentration on otolith composition along a salinity gradient: Implications for migratory reconstructions. Journal of Experimental Marine Biology and Ecology.

Milton, D. A. and S. R. Chenery. 2003. Movement patterns of the tropical shad hilsa (*Tenualosa ilisha*) inferred from transects of 87Sr/86Sr isotope ratios in their otoliths. Canadian Journal of Fisheries and Aquatic Sciences **60**:1376-1385.

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

Morris Jr, J. A., R. A. Rulifson, J. A. Babaluk, P. G. May, and J. L. Campbell. 2005. Use of micro-PIXE to investigate otolith Sr distributions of the anadromous striped bass, *Morone saxatilis*. X-Ray Spectrometry **34**:301-305.

Morse, W. W. 1980. Maturity, spawning, and fecundity of Atlantic croaker, Micropogonias undulatus, occurring north of Cape Hatteras, North Carolina. Fishery Bulletin, Seattle **78**:190-195.

Moser, J. and G. R. Shepherd. 2009. Seasonal distribution and movement of black sea bass (*Centropristis striata*) in the Northwest Atlantic as determined from a mark-recapture experiment. Journal of Northwest Atlantic Fishery Science **40**: 17-28.

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

Mugiya, Y. and S. Tanaka. 1995. Incorporation of water-borne strontium into otoliths and its turnover in the goldfish *Carassius auratus*: effects of strontium concentrations, temperature, and 17β-estradiol. Fisheries Science **61**:29-35.

Murdy, E. O., R. S. Birdsong, and J. A. Musick. 1997. Fishes of Chesapeake Bay. Smithsonian Institution Press, Washington and London.

Musick, J. A. and L. P. Mercer. 1977. Seasonal distribution of black sea bass, *Centropristis striata*, in the Mid-Atlantic Bight with comments on the ecology and fisheries of the species. Transactions of the American Fisheries Society **106**:12-25.

Nielsen, R. and P. Munk. 2004. Growth pattern and growth dependent mortality of larval and pelagic juvenile North Sea cod, *Gadus morhua*. Marine Ecology-Progress Series **278**:261-270.

Nixon, S. W. and C. M. Jones. 1997. Age and growth of larval and juvenile Atlantic croaker, *Micropogonias undulatus*, from the Middle Atlantic Bight and estuarine waters of Virginia. Fishery Bulletin **95**:773-784.

Olley, R., R. G. Young, G. P. Closs, E. A. Kristensen, T. O. Bickel, N. A. Deans, L. N. Davey, and S. M. Eggins. 2011. Recruitment sources of brown trout identified by otolith trace element signatures. New Zealand Journal of Marine and Freshwater Research **45**:395-411.

Panella, G. 1980. Growth patterns in fish sagittae. Pages 519-560 *in* D. C. Rhoads and R. A. Lutz, editors. Skeletal growth of aquatic organisms: biological records of environmental change. Plenum Press, New York.

Patterson, H., S. Thorrold, and J. Shenker. 1999. Analysis of otolith chemistry in Nassau grouper (*Epinephelus striatus*) from the Bahamas and Belize using solution-based ICP-MS. Coral Reefs **18**:171-178.

Payan, P., H. De Pontual, G. Bœuf, and N. Mayer-Gostan. 2004. Endolymph chemistry and otolith growth in fish. Comptes Rendus Palevol **3**:535-547.

Press, W. H., S. A. Teukolsky, W. T. Vetterling, and B. P. Flannery. 1994. Numerical Recipes in Fortran. Cambridge University Press, Cambridge.

Priestley, M. B. 1982. Spectral Analysis and Time Series. Academic Press, New York.

Romanek, C. and R. Gauldie. 1996. A predictive model of otolith growth in fish based on the chemistry of the endolymph. Comparative Biochemistry and Physiology Part A: Physiology **114**:71-79.

Ruttenberg, B. I., S. L. Hamilton, M. J. H. Hickford, G. L. Paradis, M. S. Sheehy, J. D. Standish, O. Ben-Tzvi, and R. R. Warner. 2005. Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. Marine Ecology-Progress Series **297**.

Sanchez-Jerez, P., B. M. Gillanders, and M. J. Kingsford. 2002. Spatial variability of trace elements in fish otoliths: comparison with dietary items and habitat constituents in seagrass meadows. Journal of Fish Biology **61**:801-821.

Schaffler, J. J., C. S. Reiss, and C. M. Jones. 2009a. Patterns of larval Atlantic croaker ingress into Chesapeake Bay, USA. Marine Ecology Progress Series **378**:187-197.

Schaffler, J. J., C. S. Reiss, and C. M. Jones. 2009b. Spatial variation in otolith chemistry of Atlantic croaker larvae in the Mid-Atlantic Bight. Marine Ecology Progress Series **382**:185-195.

Secor, D. H., T. Ohta, K. Nakayama, and M. Tanaka. 1998. Use of otolith microanalysis to determine estuarine migrations of Japanese sea bass, *Lateolabrax japonicus*, distributed in Ariake Sea. Fisheries science: FS **64**:740-743.

Secor, D. H. and J. R. Rooker. 2000. Is otolith strontium a useful scalar of life cycles in estuarine fishes? Fisheries Research **46**:359-371.

Secor, D. H., J. R. Rooker, E. Zlokovitz, and V. S. Zdanowicz. 2001. Identification of riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerprints. Marine Ecology Progress Series **211**:245-253.

She, J. and J. M. Klinck. 2000. Flow near submarine canyons driven by constant winds. Journal of Geophysical Research **105**:28671-28694.

Shen, G. T., J. E. Cole, D. W. Lea, L. J. Linn, T. A. McConnaughey, and R. G. Fairbanks. 1992. Surface ocean variability at Galapagos from 1936–1982: Calibration of geochemical tracers in corals. Paleoceanography **7**:563-588.

Shepherd, G. 1991. Meristic and morphometric variation in black sea bass north of Cape Hatteras, North Carolina. North American Journal of Fisheries Management **11**:139-148.

Shepherd, G. R. 2009. Black sea bass 2009 stock assessment update. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center.

Shepherd, G. R., M. Terceiro, and U. S. N. M. F. Service. 1994. The summer flounder, scup, and black sea bass fishery of the Middle Atlantic Bight and southern New

England waters. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Sibert, J. R. and J. L. Nielsen. 2001. Electronic tagging and tracking in marine fisheries. Electronic tagging and tracking of marine fisheries. Edited by JR Sibert and JL Nielsen. Kluwer Academic Publishers, Dordrecht, The Netherlands:1-6.

Skov, C., P. Grønkjær, and C. Nielsen. 2001. Marking pike fry otoliths with alizarin complexone and strontium: an evaluation of methods. Journal of Fish Biology **59**:745-750.

Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Science **60**:1129-1157.

Song, C. Q. and O. M. Kuznetsova. 2003. Fitting Gompertz Nonlinear Mixed Model to infancy growth data with SAS version 8 Procedure NLMIXED. Pages 1-8. Merck & Co., Inc., Rahway, New Jersey.

Standish, J. D., M. Sheehy, and R. R. Warner. 2008. Use of otolith natal elemental signatures as natural tags to evaluate connectivity among open-coast fish populations. Marine Ecology Progress Series **356**:259-268.

Swearer, S. E., G. E. Forrester, M. A. Steele, A. J. Brooks, and D. W. Lea. 2003. Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblage. Estuarine, Coastal and Shelf Science **56**:1111-1123.

Tanner, S., P. Reis-Santos, R. Vasconcelos, S. França, S. Thorrold, and H. Cabral. 2012. Otolith geochemistry discriminates among estuarine nursery areas of Solea solea and S. senegalensis over time. Marine Ecology Progress Series **452**:193-203.

Taylor, H. E. 2001. Inductively coupled plasma-mass spectrometry: practices and techniques. Academic Pr.

Thiébaux, H. J. and F. W. Zwiers. 1997. The interpretation and estimation of effective sample size. Journal of Climate and Applied Meteorology **23**:800-811.

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

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

Thorrold, S. R., C. Latkoczy, P. K. Swart, and C. M. Jones. 2001. Natal homing in a marine fish metapopulation. Science **291**:297.

Thresher, R., C. Proctor, J. Gunn, and I. Harrowfield. 1994. An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a Southern Temperate Groundfish, *Nemadactylus-Macropterus* (Cheilodactylidae). Fishery Bulletin **92**:817-840.

Trancart, T., P. Lambert, E. Rochard, F. Daverat, C. Roqueplo, and J. Coustillas. 2011. Swimming activity responses to water current reversal support selective tidal-

stream transport hypothesis in juvenile thinlip mullet, *Liza ramada*. Journal of Experimental Marine Biology and Ecology **399**:120-129.

Trenberth, K. E. 1984. Some effects of finite sample size and persistence on meteorological statistics. Part I: Autocorrelations. Monthly Weather Review **112**:2359-2368.

Tsukamoto, K. and T. Arai. 2001. Facultative catadromy of the eel Anguilla japonica between freshwater and seawater habitats. Mar Ecol Prog Ser **220**:265-276.

Ulrych, T. J. and T. N. Bishop. 1975. Maximum entropy spectral analysis and autoregressive decomposition. Reviews of Geophysics and Space Physics **13**:183-200.

Vecer, J. and P. Herman. 2011. Maximum entropy analysis of analytically simulated complex fluorescence decays. Journal of Fluorescence **21**:873-881.

Waight, T., J. Baker, and D. Peate. 2002. Sr isotope ratio measurements by double-focusing MC-ICPMS: techniques, observations and pitfalls. International Journal of Mass Spectrometry **221**:229-244.

Walther, B. D., M. J. Kingsford, M. D. O'Callaghan, and M. T. McCulloch. 2010. Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. Environmental Biology of Fishes **89**:441-451.

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

Warlen, S. M. and J. S. Burke. 1990. Immigration of larvae of fall/winter spawning marine fishes into a North Carolina estuary. Estuaries **13**:453-461.

Webb, S. D., S. H. Woodcock, and B. M. Gillanders. 2012. Sources of otolith barium and strontium in estuarine fish and the influence of salinity and temperature. Marine Ecology-Progress Series **453**:189-199.

Wunsch, C. 1999. The interpretation of short climate records, with comments on the North Atlantic and Southern Oscillations. Bulletin of the American Meteorological Society **80**:245-255.

Zimmerman, C. E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. Canadian Journal of Fisheries and Aquatic Sciences **62**:88-97.

Zlokovitz, E. R., D. H. Secor, and P. M. Piccoli. 2003. Patterns of migration in Hudson River striped bass as determined by otolith microchemistry. Fisheries Research **63**:245-259.

VITA

Reneé Reilly Hoover was born on October 12, 1984 in New York, New York. She is the second child of William and Laura Reilly. Reneé was awarded a full tuition academic scholarship to the Pennsylvania State University to pursue a Bachelor of Science Degree in Environmental Resources Management. She earned her Bachelor's degree along with minors in Marine Science and Spanish in 2007. Reneé was accepted as a Hall-Bonner Scholar and enrolled in Old Dominion University's Master of Science in Ocean and Earth Sciences degree program (4600 Elkhorn Avenue, Norfolk, Virginia 23529) in August 2007; she obtained her Master's degree in August 2009. Her major professor was Dr. Cynthia M. Jones at the Center for Quantitative Fisheries Ecology (800 West 46th Street, Norfolk, Virginia 23508). Reneé continued her work with Dr. Jones, enrolling as a doctoral student in the Oceanography degree program in August 2009. Prior to completing her Doctoral degree in August 2012, Reneé was offered a position as a Fisheries Management Specialist in the Plans and Statistics Department of the Virginia Marine Resources Commission in Newport News, Virginia, where she is currently employed. Reneé lives in Chesapeake, Virginia with her husband Benjamin K. Hoover, Esq.