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Habitat use and trophic structure of Atlantic tarpon (*Megalops atlanticus*) inferred from geochemical proxies in scales

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Habitat use and trophic structure of Atlantic tarpon (*Megalops atlanticus*) inferred from geochemical proxies in scales

by

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Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Marine Science

The University of Texas at Austin

May 2016

Dedication

This work is dedicated to my mother, Brenda Seeley, father, Jeffrey Seeley, and brother, Robert Seeley, for exposing me to all depths of the marine environment (pun intended).

Acknowledgements

I would like to thank my advisor, Dr. Benjamin Walther, and thesis committee members, Dr. Bryan Black and Dr. Lee Fuiman. I would also like to thank the past and present members of the Walther Lab including, John Mohan, Skye Woodcock, and Kyle Logan. Very special thanks to Marcus Poffenberger for providing opportunities to conduct fieldwork, as well as providing the majority of scale samples for this project. Thanks are also extended to the Texas State Aquarium for providing opportunities to sample, the Project Tarpon fishing tournament out of Port O'Connor, TX, as well as, to several members of the Fisheries and Mariculture Laboratory, including Cindy Faulk, Jeff Kaiser, Rene Lopez, and my fellow graduate students. Trace element analysis was conducted at the University of Texas at Austin Jackson School of Geosciences with the help of Dr. Nathaniel Miller. Stable isotope analysis was conducted at the University of California Davis Stable Isotope Facility. Funding for this research was provided by the Saltwater Fisheries Enhancement Association, the Texas State Aquarium, the Rotary Club of Corpus Christi (Harvey Weil Sportsman Conservationist Award), and a Coastal Conservation Association Tuition Fellowship.

Abstract

Habitat use and trophic structure of Atlantic tarpon (*Megalops atlanticus*) inferred from geochemical proxies in scales

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The University of Texas at Austin, 2016

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Atlantic tarpon, *Megalops atlanticus*, are highly migratory euryhaline predators that occupy different habitats throughout life. Atlantic tarpon are known to inhabit oligohaline waters, although the frequency and duration of movements across estuarine gradients into these waters are poorly known. This species supports over a two billion dollar industry within the Gulf of Mexico and is currently listed as vulnerable by the International Union for the Conservation of Nature (IUCN). Analysis of trace element and stable isotope compositions of growth increments in fish scales is a non-lethal method for reconstructing migrations across estuaries in vulnerable species. We analyzed Atlantic tarpon scales from the Texas coast to validate this method using inductively coupled plasma mass spectrometry (ICP-MS) for trace elements and isotope ratio mass spectrometry (IR-MS) for stable isotope ratios. Multiple scales and otoliths were taken from the same individual to confirm the consistency of elemental and isotopic uptake

within the same individual and between structures. Results show that scale Sr/Ca and $\delta^{13}C$ are effective proxies for salinity, while increases in $\delta^{15}N$ are consistent with known trophic shifts throughout life history. Patterns of elemental concentrations and isotope values across scales within an individual were consistent with each other. Scale and otolith transects contained the same overarching trend with comparable shifts in elemental concentrations across growth increments in the two structures. Migratory contingents, or groups within distinct populations that exhibit different patterns of habitat use and movement across salinity gradients, were identified. The distribution of contingents indicated that migratory behavior is highly variable, with some, but not all fish transiting estuarine gradients into oligohaline waters. Yet, the majority of individuals sampled exhibited early life residency in oligohaline waters. This work demonstrates the use of low salinity habitats by Atlantic tarpon. Our validation of the methods for analyzing scales will provide novel opportunities to monitor fish migrations across salinity gradients.

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Introduction

Atlantic tarpon (*Megalops atlanticus*) support a recreational fishery from Virginia to Texas, which is worth upwards of $6 billion. The town of Tarpon, Texas was renamed Port Aransas, Texas in 1910 and was once considered the Atlantic tarpon capital of the world (Holt et al. 2005). In the 1960s, the Atlantic tarpon fishery in Texas began to collapse presumably due to overfishing, lack of regulations, habitat degradation, and low freshwater inflow. Until recently, there have been few efforts to help conserve and monitor Texas Atlantic tarpon populations. Now, anglers in Texas can retain only one fish per year over 2.03 meters. Currently, Atlantic tarpon abundances along the Texas coast are much lower than historic levels (Adams et al., 2014). The lack of full recovery has spurred interest in Atlantic tarpon migration, habitat use, trophic structure, and life history across the Texas coastal bend.

Researchers throughout the Gulf of Mexico have focused on answering questions about Atlantic tarpon life history through tagging, genetics, diet, and growth studies (Guindon et al., 2015; Jud et al., 2011; Luo et al., 2008; Zerbi, 2001). Results from studies such as these are used to develop appropriate management practices for conservation of the species (Adams et al., 2014). A patchwork of state-by-state management in the Gulf of Mexico has allowed Atlantic tarpon to thrive in certain regions but not others. In addition to Texas, it is legal for recreational anglers to harvest Atlantic tarpon of a designated size throughout Louisiana, Mississippi, and Alabama. Over the years, steps have been taken to assist conservation of the species by implementing larger minimum size limits, smaller bag limits, and/or requiring a harvest

tag. However, in Florida, Atlantic tarpon are strictly a catch-and-release fishery, with managers even restricting which size fish can be removed from the water after capture (Bonefish and Tarpon Trust, Fishing Regulation List, Personal Communication). The management practices in Florida, in conjunction with the exceptional near shore flats habitat, have allowed Atlantic tarpon to thrive. As a result, Atlantic tarpon in Florida are the most protected and abundant of anywhere in the United States (Adams et al., 2014). Unfortunately, in Mexico there is no size limit on the many fish that can be harvested, and because Atlantic tarpon migrations often cross exclusive economic zone boundaries, this is a major conservation concern. In the near future, managers need to work closely to develop international regulations to protect Atlantic tarpon throughout their range.

In addition to improving conservation efforts for Atlantic tarpon by developing and implementing appropriate regulations, it is necessary to monitor changes in essential habitats that support the fishery. These changes include altered freshwater inflows and damming, because Atlantic tarpon occupy different habitats during their lifetime (Adams et al., 2014; Crabtree et al., 1995). Previous research has shown that the variety of habitats occupied spans freshwater rivers, mangrove assemblages, and open ocean (Rickards, 1968). Crabtree et al. (1997) noted that Atlantic tarpon are found in freshwater lakes and rivers when younger, but are most often targeted by anglers in the coastal and estuarine regions, as these individuals are typically larger. Yet, Luo et al. (2008) recorded a fully mature Atlantic tarpon migrating from Eastern Florida coastal waters up the St. Lucie River and back. During this excursion, estimated salinities experienced ranged from 2-35 psu. As these habitats change due to natural and anthropogenic perturbations,

access to prey, nursery habitats, and migratory routes may be affected. This will potentially lead to a decline in Atlantic tarpon population abundances or cause a shift in habitat utilization patterns. If these shifts in habitat use patterns lead to sub-optimal for feeding, growth or reproduction, then the sustainability of the recreational fishery could be impaired.

The wide array of habitats used by Atlantic tarpon has led to questions regarding the importance of specific habitats at different times of life. Research is now focusing on the duration of residency in low salinity habitats, the frequency of low salinity occupancy, the frequency of migrations across salinity gradients, and the shifts in trophic level between multiple habitats. Answering these questions will provide necessary insight into Atlantic tarpon behavior at different life stages and will allow researchers to identify where conservation efforts should be focused.

CHAPTER 1: High resolution profiles of elements in Atlantic tarpon (*Megalops atlanticus*) scales obtained via cross-sectioning and laser ablation ICP-MS: a literature survey and novel approach for scale analyses

Published as: Seeley, M., Miller, N., & Walther, B. (2015). High resolution profiles of elements in Atlantic tarpon (*Megalops atlanticus*) scales obtained via cross-sectioning and laser ablation ICP-MS: a literature survey and novel approach for scale analyses. *Environmental Biology of Fishes*, *98*(11), 2223–2238. http://doi.org/10.1007/s10641-015-0443-z

Sample collection and preparation, method development, data collection and analysis, and writing were conducted by Matthew Seeley. Method development and data collection were conducted by Nathaniel Miller. Project development, funding, and writing were conducted by Benjamin Walther.

INTRODUCTION

Methods for tracking fish migration and habitat use often employ conventional tagging approaches with the use of acoustic telemetry arrays, pop-up satellite tags, and/or dart tags (Luo and Ault 2012; Meyer et al. 2010; O'Toole et al. 2011). An alternative approach is by assaying chemical signatures within calcified structures such as otoliths using in-situ probe-based methods such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to quantify lifetime variation in chemical constituents that can reveal movements across environmental gradients (Campana and Thorrold 2001; Gillanders 2005; Walther and Limburg 2012). Otoliths have been particularly useful for reconstructing movements across major salinity gradients such as those traversed by diadromous species, given that concentrations of dissolved elements such as strontium (Sr) and barium (Ba) co-vary with salinity and are incorporated into otoliths largely in proportion to their ambient concentration (Elsdon et al. 2008). Nonetheless, otolith extraction is a lethal process limited to species not listed as threatened or endangered and in catch-and-release fisheries.

An alternative nonlethal method of studying migration and habitat use is the geochemical analysis of scales. Scales share several properties with otoliths such as incremental growth and the incorporation of some of the same chemical constituents from water or diet (Kennedy et al. 2000; Wells et al. 2000a; Wells et al. 2003a). Unlike otoliths, which grow through radial accretion of aragonite ($CaCO_3$) layers, fish scales are bipartite structures comprised of: (1) a well-calcified (calcium phosphate) external layer that is underlain by (2) a composite basal plate, consisting of layers of poorly-calcified collagen (Hutchinson and Trueman 2006). Therefore, scales can be potentially assayed for inorganic (e.g. Ba:Ca and Sr:Ca) and organic (e.g. $\delta^{13}C$ and $\delta^{15}N$) proxies by targeting either the external layer or the basal plate. One complexity for scale analysis is that the well-calcified external layer grows radially, while the poorly calcified basal plate grows via the addition of new layers that accrete underneath prior layers as well as extending outward at the leading edge (Hutchinson and Trueman, 2006). This means, in the basal plate new increments underplate older increments at the core of the scale extending to the edge. Therefore, scale collagen analyses from the basal plate provide a weighted temporal average of isotopic compositions, with the weighting dependent on proportional underplating biased towards the more recently formed collagen (Hutchinson and Trueman 2006; Ramsay et al. 2012). For this reason, researchers using stable isotope ratios typically homogenize entire scales or subsample the exterior growing edge of scales to avoid the complexity of underplating (but see Woodcock and Walther, 2014). Since the mineralized external layer is thought to involve negligible underplating during growth (Kerr and Campana 2014), chemical time-series accompanying physiological

development of the external layer are expected to be recorded radially similar to otoliths. If true, chemical time-series for the external layer may be established from traverses across scale exteriors or in polished cross sections. Scales that can be sampled in cross section provide a potentially new approach to quantify element:Ca concentrations across all new concentrically accreted layers. The ability to pursue this method depends on the thickness of the external well-calcified layer relative to the spatial resolution of analytical instrumentation, given that the external layer of fish scales can vary greatly in thickness across species (Table 1). For example, Ikoma et al. (2003) found the thickness of the external layer from red seabream *Pagrus major* to range from 3-4 μm while more robust scales can have external layers that are up to 250 μm in thickness such as Atlantic tarpon *Megalops atlanticus*. Provided scales are thick enough to allow targeted sampling of the external layer in cross-section, assays of the elemental composition of this surficial calcified region across growth increments could provide more direct life history profiles of previous water chemistries encountered by migratory fish compared to stable isotope ratios, as well as the opportunity to compare otoliths and scales from the same individuals.

Over the last four decades, many workers have obtained elemental compositions of scales for a diverse suite of goals ranging from marking, stock identification, and tracking movements between fresh and marine waters (see Table 2 for a survey of studies quantifying elements in scales). In our survey, we focus on efforts to assay elemental compositions and exclude studies using organic stable isotope ratios (e.g. $\delta^{15}N$ and $\delta^{13}C$) in scales, which have been reviewed elsewhere (Trueman and Moore 2007; Trueman et

al. 2012). A significant proportion of these studies relied on solution-based elemental analyses using a variety of analytical techniques, such as flame emission photometry (FEP; Ophel and Judd 1968), atomic absorption spectroscopy (AAS; e.g. Bagenal et al. 1973; Moreau and Barbeau 1979), thermal ionization mass spectrometry (TIMS; Kennedy et al. 2000), and inductively coupled plasma mass spectrometry (ICP-MS; e.g. Gillanders 2001; Wells et al. 2000a; Woodcock and Walther 2014). These techniques are largely based on whole-scale digests (or multiple pooled scale digests from one or several individuals), or digests of subsamples from specific scale growth regions. The majority of scale sampling studies in recent years have relied on *in situ* solid sampling approaches using techniques such as electron microprobe (Courtemanche et al. 2005; Courtemanche et al. 2006) and laser ablation ICP-MS (e.g. Borcherding et al. 2008; Flem et al. 2005; Wells et al. 2000b). Most of these analyses have quantified elemental concentrations from specified regions, such as the anterior growing edge or the interior core representing juvenile life, as well as discrete spot transects across scales.

Two studies have examined scale composition in cross-section using EMP techniques (Courtemanche et al. 2005; Holá et al. 2011). While EMP is non-destructive and has a high spatial resolution, it is labor-intensive and not conducive to high-throughput sampling. However, EMP may be an alternative analytical option for scales with narrow calcified layers that are thinner than LA-ICP-MS spot diameters (Table 1). Only three studies have performed continuous line transects across entire scales using LA-ICP-MS, all of which have been conducted via top-down analyses of the external layer (Clarke et al. 2007; Holá et al. 2011; Wolff et al. 2013). However, this top-down

approach to continuous transects can be difficult to implement for species with large scales with significant surface irregularities and substantial thickness variations that yield focusing problems for a laser-based instrument. Additionally, the stratigraphic consistency of what the laser is actually sampling may be relatively unknown. For example, the external layer of scales is more often subject to contamination and likely to incorporate environmental or sample processing contaminants than interiors. In addition, post-depositional ion exchange between the surface of the external calcified layer and surrounding waters could potentially alter the surficial elemental composition after movement into elementally distinct habitats (e.g. fresh versus marine waters). A cross-sectional approach for scales with thick calcified layers would avoid sample roughness issues inherent in a top-down approach and allow the transect to be placed at a depth below the surface that may be less subject to post-depositional ion exchange.

Here, we present a new method of cross-sectional analyses of large scales that allows high-throughput and high resolution continuous transects of elemental concentrations in scales with a thick external layer. This method provides for consistent sampling of the same layer throughout the scale that can be documented by follow-up SEM studies to confirm sampling location. We evaluate the quality of elemental data obtained with this approach for a suite of potential proxy elements to assess the nature of signals recorded in the calcified external layer. Our study species is Atlantic tarpon (*Megalops atlanticus*), a highly migratory elopiform with a broad distribution across the North Atlantic, Caribbean and the Gulf of Mexico (Adams et al. 2014). This species occupies a wide range of habitats including marine, hypersaline and fresh waters at

multiple ontogenetic stages (Luo et al. 2008; Zerbi et al. 2001). The life history of Atlantic tarpon begins with an offshore spawning event. Pelagic leptocephalus larvae hatch and move inshore with the help of currents where they metamorphose into juvenile fish. Juvenile recruitment at this stage most often occurs in estuaries and brackish streams. As the juveniles grow into sub adults some individuals make movements across salinity gradients back to fully marine waters (Adams et al. 2014; Rickards 1968; Stein et al. 2012; Wade 1962; Zerbi et al. 2001). These variable movements may be recorded by proxy element (Ba, Sr) concentration changes in otoliths and scale external layers, enabling researchers to decipher movement/migration strategies that may help with management of this socioeconomically important species. Atlantic tarpon are currently listed as vulnerable under the International Union for the Conservation of Nature (IUCN), so it is of great interest to develop a nonlethal sampling method for a species that requires conservation.

METHODS

Study species

As a popular sport fishery and given current historic lows in population, Atlantic tarpon are prosecuted primarily as catch-and-release in the United States. There is thus significant interest in using non-lethal methods to characterize habitat use patterns across salinity gradients (Adams et al. 2014). Further, this species was chosen for this study because of its very large scales, which can exceed 6cm in diameter for adult fish. Atlantic tarpon scales develop shortly after metamorphosis from leptocephalus larvae into juvenile fish at approximately 30-40 mm standard length, and are retained for the

duration of the fish's life assuming they are not shed due to stress (Harrington 1958). The external layer of these scales vary substantially in thickness from core to edge when mounted flat on a level substrate, meaning that top-down continuous laser ablation transects of this layer is not practical because the laser has a limited focal range for consistent ablations.

In a recent study on elemental and stable isotope values in Atlantic tarpon scales, Woodcock & Walther (2014) used solution-based methods of scale subsamples from different scale growth increments to describe general patterns of habitat use at the population and species levels. For that study, three scale subsections of approximately 1-2 mm^2 were sampled from the core, middle and outer edge of each scale and dissolved in 4 ml ultra-high purity Aristar Ultra® nitric acid for solution-based inductively coupled plasma mass spectrometry (SB-ICP-MS) quantification of Sr/Ca, Ba/Ca, Mg/Ca, and Mn/Ca ratios. Patterns in ontogenetic movement across salinity gradients were evident from the Sr/Ca and Ba/Ca ratios, given known mixing dynamics of these constituents in local estuaries (Walther and Nims 2015). For the present study, we re-analyzed two scales from Woodcock and Walther (2014) in cross section by LA-ICP-MS as described below.

Scale preparation and analysis

Scale collection and processing procedures are detailed in Woodcock and Walther (2014). Briefly, scales were collected by recreational anglers along the Texas coast, and supplied estimated length, weight, capture location, and date of capture. The two scales selected for this study were both removed from Atlantic tarpon landed near Port

O'Connor, Texas. Capture dates and weights were September 9th, 2012 and 36 kilograms (hereafter referred to as Tarpon 1) and September 8th, 2012 and 41 kilograms (Tarpon 2). Scales were sonicated in ultrapure water for 5 minutes and then scrubbed clean of any tissue with a soft bristled brush in a class-100 laminar flow hood. Samples were then flattened between two panels of glass and dried overnight.

Flattened and dried scales received transverse cuts from the edge to the core in two regions making a 90 degree angle, which allowed for the removal of an entire quadrant (Figure 1.1C). Each quadrant was embedded in epoxy with the previously unexposed midpoint of the cut scale submerged not touching the other scales. The following day when samples were dry, scales were longitudinally sectioned into a 1-2 mm thin section exposing the 2.7 cm and 2.5 cm transects for Tarpon 1 and Tarpon 2 respectively (Figure 1.1D). Finally, the epoxy section was polished on an 8-inch polishing pad (Beta Diamond Products, Inc.) with 0.3 then 0.05-micron alumina grit for imaging by environmental scanning electron microscopy (ESEM) and laser ablation analysis. Images of cross-sectioned scales were taken using an XL30 ESEM in the Department of Geological Sciences at the University of Texas at Austin to determine the thickness and surface morphology of the external layer and basal plate within the scales. Semi-quantitative estimates of relative elemental weight percents for each layer were quantified by an energy dispersive x-ray (EDX) attached to the ESEM.

LA-ICP-MS analysis

Elemental concentrations in the external layer of sample scales (in cross section) were measured by LA-ICP-MS at the University of Texas at Austin (Jackson School of

Geosciences) using a New Wave Research UP193-FX fast excimer (193nm wavelength, 4-6 ns pulse width) laser system coupled to an Agilent 7500ce ICP-MS. The laser system is equipped with a large format, two-volume cell, for direct sampling of the ablation plume with fast (<1s) washout times to minimize spatial carryover. Laser ablation parameters optimized from test scans of scale cross sections were 25μm/s line traverses using a 35μm spot, 20% power, 10 Hz repetition rate, and a He cell flow of 300 mL/min (Table 1.3). Laser energy densities (fluences) obtained for the analytical session averaged 3.2 J/cm^2 with <3.8% variation. Prior to analyses, transects were preablated (20% power, 10 Hz, 50μm spot, 40μm/s scan speed) as a precaution to remove external contaminants. The ICP-MS operated at an RF power of 1600 W and an average Ar carrier flow of 1.23 L/min. Oxide production rates as monitored by ThO/Th for NIST 612 were ≤ 0.18%. The quadrupole time-resolved method involved measurement of 10 masses (^{25}Mg, ^{26}Mg, ^{31}P, ^{42}Ca, ^{43}Ca, ^{55}Mn, ^{88}Sr, ^{137}Ba, ^{138}(Ba, La, Ce), and ^{208}Pb) using 25 ms integration times. The analytical sampling period of 0.2702 s, equivalent to a reading every 6.755 μm, corresponds to 92.5% measurement time.

Time-resolved intensities were converted to concentration (ppm) equivalents using Iolite software (Univ. Melbourne), using ^{43}Ca as the internal standard. Additionally, calculations for the limits of detection were performed using Iolite software based on Longerich et al. (1996). Baselines were determined from 60 s gas blank intervals measured while the laser was off and all masses were scanned by the quadrupole. USGS MAPS-4, a synthetic modern bone standard, was used as the primary reference standard for all analytes. Recoveries (relative 1s deviations versus GeoREM preferred values)

among analytes for secondary standards run as unknowns against the primary standard were typically better than 91% for NIST 612 and 94% for NIST 610. Laser traverses were oriented above and parallel to the base of the external calcium phosphate layer (in cross section, Fig. 1.1) beginning in the proximal core of the scale and ending at the distal edge of the scale. Element concentrations were calculated assuming a concentration of 39.89% Ca in the sampled outer calcium phosphate layer, corresponding to an assumed composition of $Ca_{10}(PO_4)_6(OH)_2$. For each analyte quantified from Tarpon 1 and Tarpon 2 scales we calculated the range, mean, and median of observed analyte values as well as and the percent of data points in scales that fell above the limits of detection derived from either NIST612 and NIST610.

RESULTS

The semi-quantitative estimates of the relative weight percent of Ca in the surface layer (Spectra 1-4) were 16-19%, while the Ca weight percent of the basal plate (Spectrum 5) was 2% (Table 1.4). The thickness of the calcified external layers ranged 200-250 μm in the interior region of the scales, and tapered to 45-110 μm at the anterior growing edges. This meant the laser beam (spot diameter of 35 μm) was fully contained within the calcified external layers for the entire cross-sectional transects. Accuracies (recoveries) and precisions (residual standard deviations, RSD) were high for all analytes with the exception of P (Table 1.5). Concentrations of most elements (^{25}Mg, ^{31}P, ^{42}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba) in both scales were above LODS for 100% of the acquired data, whereas 54-67% of acquired ^{208}Pb data in the scale from Tarpon 1 was above the LODs. The scale from Tarpon 2 had 95-98% of acquired ^{208}Pb above the LODs (Table 1.6).

Representative continuous transects from core to edge of the two scales are shown for Sr/Ca, Ba/Ca, and Mn/Ca (Figure 1.2). Individual patterns within the 2 scales were evident, particularly for Sr/Ca, which showed a major and abrupt transition for Tarpon 2, but not for Tarpon 1.

DISCUSSION

A survey of the previous four decades of scale elemental chemistry research shows the wide diversity of goals, methods, instruments and analytes explored by researchers working in fresh, marine, estuarine and diadromous scenarios (Table 1.2). Of the 49 studies surveyed, 21 used dissolution-based analyses of whole or partial scales, while the remaining 28 quantified analytes via analytical methods permitting *in situ* sampling of scale surfaces. Nearly half (12) of *in situ* studies used surface spots, lines or rasters using LA-ICP-MS, EMP, PMP, SEM, XEDS, or XMA to target a specific region of the scales, such as the core to resolve juvenile growth periods or edges to assess the most recently accreted material. Ten of the *in situ* studies used LA-ICP-MS, EMP, PMP, or XMA to sample across entire scales as transects of spots, with variable spot diameters and sampling densities. Finally, only four studies (excluding the present work) acquired continuous transects across scales, and all three acquired these transects via LA-ICPMS top-down surface analyses.

Only two "*in-situ*" studies have examined scale composition in cross-section. Holá *et al*. (2011) used EMP to identify differences in elemental abundances between the calcified external layer and the basal plate, showing a concentration of many elements (Mg, Ba, Sr) in the surface layer. In a study more directly comparable to this one,

Courtemanche *et al*. (2005) used EMP to compare transects of Sr/Ca between surface and cross-sectional analyses, which revealed comparable oscillations in elements, although the cross-sectional approach yielded higher precisions. Our approach used the same cross-sectional method of Courtemanche et al. (2005) but instead used LA-ICP-MS and for a wider suite of analytes. While WD-EMP offers some advantages over LA-ICP-MS such as non-destructiveness and higher spatial resolution (<3 μm), a LA-ICP-MS method offers higher throughput, lower detection limits, wide elemental coverage and avoids the need for extensive sample preparation such as carbon coating. The 35-μm laser spot size selected was amply sufficient for resolving spatial differences in large scales for species such as Atlantic tarpon. In addition, ICP-MS instruments, now common in academic and government research labs, are likely to be the most readily accessible instrument for quantifying elements in scales. Scale size, architecture, and the particular ecological question to be answered should ultimately dictate the choice of method and instrument, but we suggest that the cross-sectional LA-ICP-MS approach presented here may be applicable to species with robust scales for related research problems.

The thickness of the external layers of scales can vary over two orders of magnitude across species (Table 1.1), which raises two important issues to consider when deciding whether to use a cross-sectional analytical approach as presented here. The first issue is operational, given that the calcified layer must be of sufficient depth to contain the diameter of the probe spot size for the entire length of the desired transect. Currently, typical LA-ICP-MS spot diameters used for assays of elements such as Sr and Ba in calcified tissues are on the order of 25-50 μm, meaning this method can be applied to

species such as Atlantic tarpon and common carp *Cyprinus carpio*, but not those with very thin scales such as red seabream *Pagrus major* or sheepshead minnow *Cyprinodon variegatus*. Depending on the shape and length of a scale, LA-ICP-MS may be able to analyze scales with external layers <10 μm when a 5-μm tall rectangular aperture (e.g. 5x50 μm, equivalent to ~17-μm spot) is used as opposed to a larger circular spot. Thinner scales may also be mapped in cross-section using higher-resolution techniques such as EMP, although this method does not allow the high throughput of ICP-MS. Researchers must therefore take care to ensure that the external layer in their scales is of sufficient thickness and the elements of interest must be above the method detection limit to allow a cross-sectional approach before opting for this method.

The second issue with regards to external layer thickness concerns the potential for post depositional ion exchange to significantly alter the chemical composition at the surface. Unlike aragonitic otoliths, the calcified portion of scales is composed of calcium orthophosphate in the form of hydroxyapatite, and therefore has similar chemical properties and crystal structures to bones and tooth enamel (Boanini et al. 2010). Divalent cations such as Sr and Ba may substitute for Ca in the crystal lattice, although such substitutions can induce structural distortion and alter solubility and thermal stability (Terra et al. 2009; Wang and Nancollas 2008). Sr in particular has a high affinity for bone, and Sr-doping can cause rapid uptake and incorporation in bone, driven primarily by ionic exchange rather than ionic substitution (Dahl et al. 2001). This ionic exchange is reversible in Ca-rich solutions, leading to decreases in bone Sr content following cessation of Sr doping (Dahl et al. 2001). For fish ecologists seeking to use

calcified layers of scales for reconstructing migrations, post-depositional ionic exchange has the potential to alter compositions of previously accreted material and lead to incorrect migratory reconstructions. For instance, diadromous fishes that accrete a freshwater signature in the core of their scale prior to migrating to marine waters later in life may incorporate elevated Sr into the scale interior via ion exchange, resulting in a scale that appears to contain no early freshwater residence period. Given that ion exchange will primarily affect the hydrated upper surface of the calcified layer in direct contact with water (Cazalbou et al. 2005), this issue may be avoided by directing probes to the lower portion of calcified layers that are presumably less subject to post-depositional alteration. Again, the ability to avoid the potentially altered upper portion of a calcified layer depends on the thickness of the external layer relative to the sampling probe resolution. To our knowledge, such ion exchange processes in fish scales have not been experimentally evaluated, although clearly such experiments should be undertaken to ensure that assumptions about elemental stability in scales are accurate (Trueman 2013). Thus, for both operational and post-depositional exchange reasons, researchers should give careful consideration to the thickness of the calcified layer in scales from their species before pursuing a cross-sectional approach.

The range of applications for scale elemental chemistry was also evident from our survey. The earliest known study by Ophel and Judd (1968) demonstrated that fish reared on diets rich in Sr incorporated retained elevated Sr concentrations in their scales up to one year after marking. This was observed to be a potentially valuable tool for identifying hatchery-reared fish, which has been verified by a handful of subsequent

studies using either diet or water elemental spiking (Ennevor and Beames 1993; Snyder et al. 1992; Woodcock et al. 2013). A dominant use of scale chemistry, particularly for freshwater species and the freshwater life history stage of anadromous species, is identifying spatial differences in multivariate elemental signatures that could subsequently be used to identify natal origins. This application is directly analogous to a majority of otolith chemistry publications, and has been investigated with scales of species including brook trout *Salvelinus fontinalis* (Moreau et al. 1983), Atlantic salmon *Salmo salar* (Flem et al. 2005; Kennedy et al. 2000), brown trout *Salmo trutta* (Ramsay et al. 2012; Ramsay et al. 2011), and westslope cutthroat trout *Oncorhyncus clarki lewisi* (Muhlfeld et al. 2005; Wells et al. 2003a). Other researchers have capitalized on elemental mixing relationships with salinity (primarily Sr) to identify fishes that have undergone migrations between marine and fresh waters (e.g. Belanger et al. 1987; Castonguay and FitzGerald 1982; Courtemanche et al. 2006; Coutant and Chen 1993; Pender and Griffin 1996). Much of this work assumes that the primary source of the assayed analytes derives from dissolved ions in water.

In the most comprehensive evaluation of scale elemental chemistry as an otolith analogue, Wells and colleagues employed a suite of laboratory and field-based studies to evaluate relationships between water and scale chemistry, as well as the temporal stability of geographical differences. Experimental manipulation of water chemistry demonstrated significant linear relationships between water and scales for Sr/Ca, Cd/Ca, and Ba/Ca, with minimal influence of temperature (Wells et al. 2000a). These results were consistent with field-based studies that found significant linear relationships

between water and scale Sr/Ca and Ba/Ca, but not for Mg/Ca (Wells et al. 2003a). The authors point out that the latter result may be expected given that elements such as Mg may be under stronger physiological regulation and therefore decoupled from ambient water concentrations. Finally, although scale elemental signatures can perform comparably in their ability to discriminate geographic origins, multivariate elemental signatures are not necessarily static across time meaning repeated sampling may be necessary to evaluate stock composition (Wells et al. 2000b; Wells et al. 2003b). Many of these issues are of similar concern to otolith chemistry approaches (Elsdon et al. 2008; Sturrock et al. 2012), and workers wishing to pursue scale analysis take similar precautions in interpreting chemical profiles.

As evident from the literature survey and results presented here for analysis of Atlantic tarpon scales, a variety of elements are readily quantifiable in fish scales. This includes elements that are commonly used as proxies for lifetime salinity histories in otolith chemistry analysis, such as Sr and Ba (Walther and Limburg 2012) or redox-sensitive elements such as Mn used to reconstruct hypoxia exposure (Limburg et al. 2015). These elements were consistently above limits of detection in Atlantic tarpon scales and were quantified with high recoveries and analytical precision, thereby comparing favorably to laser-based quantification of these elements in otoliths (Geffen et al. 2013; Thorrold and Shuttleworth 2000). The majority of prior studies using scale chemistry to answer ecological questions have used these elements, and Sr in particular, to reconstruct movements across salinity gradients and geographical differences in habitat residence in fresh waters overlying unique geologies. We anticipate continued interest in

employing these environmental proxies in scales to answer questions about migration and habitat use, although we urge the community to pursue experimental validations of elemental uptake, incorporation and stability analogous to the work done with otoliths to date (Elsdon et al. 2008). Of particular concern with scales is the degree to which resorption or ionic exchange may alter chemical composition of the external layer subsequent to accretion, rendering the structure a less permanent record than an otolith. In this respect, comparisons between recorded profiles in otoliths and scales from the same individuals would be instructive in addition to experimental manipulations of water chemistry and conditions. Finally, an ongoing issue in the field of otolith chemistry that is equally relevant to scales is the role of physiology in controlling elemental composition of structures. Although elements such as Sr and Ba may be effective recorders of dissolved ambient water composition of these elements, other elements in biogenic structures such as Mg, P, or Pb may be primarily determined by physiological status and strongly regulated by the organism, rendering them ineffective proxies for water composition (Sturrock et al. 2015; Woodcock et al. 2012). Thus, although we quantified Mg and to a lesser extent P in Atlantic tarpon scales, the utility of these elements remains in question pending further experimental evaluation of the relative influence of environment versus physiology on their incorporation dynamics.

Future research on Atlantic tarpon migration and habitat use can apply scale chemistry analysis to assess variability in these movement patterns across salinity gradients that have not already been assessed using conventional tagging approaches. Pop-up archival transmitting (PAT) tags implanted on Atlantic tarpon from Florida and

throughout the rest of the Gulf of Mexico have shown Atlantic tarpon utilize freshwater habitats to fully marine waters on an individual level (Luo et al. 2008). Additionally, some individuals will traverse hundreds of kilometers throughout a seasonal basis (Crabtree 1995). This nonlethal scale sampling approach will provide advantageous results on the ecology and life history of Atlantic tarpon with minimal handling. Many questions related to Atlantic tarpon behavior and ecology still remain such as geographic variability in migratory behaviors, whether freshwater habitat use is required, and the degree of individual variability in habitat use patterns. Using this scale cross-sectional elemental analysis method will provide researchers with an opportunity to address these questions with a method that is complementary to PAT tagging efforts, due to the ability to quantify shifts in salinity across environmental gradients. Furthermore, this method provides extensive life history data from squamation to capture that cannot be acquired via PAT tags. Ultimately, combining these methods in the future may allow researchers to quantify almost complete ontogenetic patterns.

More work is clearly necessary to further validate the efficacy of scales being used as alternative analogues to otoliths. Experimental manipulations of water properties such as salinity and redox state should be manipulated to evaluate uptake, incorporation and stability of elements in scales in different conditions. Scales and otoliths from the same individuals need to be sampled to compare their respective concentrations and transects. Variability among scales from the same individual in different regions on fish (anterior, posterior, dorsal, ventral) needs to be evaluated to confirm that location of scale removal is not a confounding factor Additionally, how signals vary from core-to-edge

with transect orientation across scales (in plan and cross section) should be evaluated in the same scale to determine if an optimal transect orientation should be standardized for future studies. Finally, validation of complete scale elemental records and periodicities are ultimately required to confirm the usage of scales as a nonlethal alternative structure to otoliths for addressing important ecological questions.

CHAPTER 1 TABLES

Table 1.1. Survey of variations in depths of total scales (TS), external layers (EL) and basal plates (BP). Values were obtained from the literature as directly reported measurements or estimated from published images that contained accurate scale bars. Large ranges in some cases indicate depth variations between scale edges (thinner) and cores (thicker). All depths are in μm.

Citation	Species	TS depth	EL depth	BP depth
This paper	*Megalops atlanticus*	65-500	45-250	20-250
Ikoma *et al*., 2003	*Pagrus major*	4-6	3-4	1-2
Olson and Watabe, 1980	*Cyprinodon variegatus*	-	1.6-6	-
Gauldie *et al*., 1991a	*Pseudocyttus masculatus*	20	-	-
Courtemanche *et al*., 2005	*Salvelinus fontinalis*	18-43	6-18	12-25
Hola *et al*., 2011	*Cyprinus carpio*	120-170	50-100	70

Table 1.2. Literature survey of elemental assays in scales. Studies that focused solely on organic stable isotope ratios in scales (e.g. $\delta^{15}N$ and $\delta^{13}C$) were excluded from this survey. Studies are organized according to whether scales were dissolved or probed in situ. The primary purpose of each study is listed, although some studies had multiple aims. The primary water system of interest is listed; where diadromous fishes were assayed with an exclusive focus on the freshwater signature within scales, these studies were categorized as "fresh". All analytes reported as measured above LODS are listed.

Citation	Species	System	Purpose	Analytes	Method	Instrument†
Dissolution-based studies						
Gillanders, 2001	*Parma microlepis*	Marine	Compare structures	Ba, Mn, Pb, Sr	Whole scales	ICP-MS
Pouilly *et al*., 2014	multiple	Fresh	Compare structures, geographical diffs.	$^{87}Sr/^{86}Sr$	Whole scales	TIMS
Vazquez *et al*., 2015	*Odontesthes bonairensis*	Fresh	Quantify analytes	Cr, Mn, Zn	Whole scales	ICP-OES
Moreau *et al*., 1983	*Salvelinus fontinalis*	Fresh	Geographical diffs.	Ca, Mg, Mn, Sr, Zn	Whole scales	AAS
Kennedy *et al*., 2000	*Salmo salar*	Fresh	Geographical diffs.	Sr (isotopes)	Whole scales	TIMS
Moreau and Barbeau, 1979	*Coregonus clupeaformis*	Diad.	Geographical diffs., Identify diadromy	Ca, Mg, Sr	Whole scales	AAS
Belanger *et al*., 1987	*Morone saxatilis*	Diad.	Identify diadromy	Al, Ba, Ca, Fe, K, Mg, Mn, Na, Sr, Zn	Whole scales	AAS
Castonguay and FitzGerald, 1982	*Salvelinus fontinalis*	Diad.	Identify diadromy	Sr	Whole scales	AAS

Table 1.2 Continued

Bagenal *et al*., 1973	*Salmo trutta*	Diad.	Identify diadromy	Sr	Whole scales	AAS
Pender and Griffin, 1996	*Lates calcarifer*	Diad.	Identify diadromy	Al, Ba, Ca, Ce, Fe, La, Mg, Mn, Na, Nd, Sr, Zn	Whole scales	AAS, ICP-MS, ICP-AES
Eek and Bohlin, 1997	*Salmo trutta*	Diad.	Identify diadromy	Sr	Whole scales	AAS
Adey *et al*., 2009	*Salmo salar*	Diad.	Identify hatchery fish	Ag, Ba, Cd, Ce, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Rb, Sm, Sr, Th, Ti, U, V, Zn, Zr	Whole scales	ICP-MS
Ennevor and Beames, 1993	*Oncorhynchus kisutch*	Fresh	Marking	Ce, La, Sm	Whole scales	ICP-MS
Snyder *et al*., 1992	*Oncorhynchus mykiss*	Fresh	Marking	Sr	Whole scales	ICP-MS
Ophel and Judd, 1968	*Carassius auratus*	Fresh	Marking	Sr	Whole scales	FEP
Wells *et al*., 2000a	*Leiostomus xanthurus*	Estuarine	Uptake dynamics	Ba, Ca, Cd, Sr	Whole scales	ICP-MS
Bijvelds *et al*., 1996	*Oreochromis mossambicus*	Fresh	Uptake dynamics	Mg	Whole scales	ICP-AES
Aydın and Tokalıoğlu, 2015	multiple	Marine	Quantify analytes	Ag, As, Cd, Co, Fe, Mn, Ni, Pb, Se	Subsampled scales	ICP-MS
Woodcock and Walther, 2014	*Megalops atlanticus*	Diad.	Identify diadromy	Ba, Ca, Mg, Mn, Sr	Subsampled scales	ICP-MS

Table 1.2 Continued

Gausen and Berg, 1988	*Salmo salar*	Fresh	Identify hatchery & diadromy	Sr	Subsampled scales	AAS
Yamada and Mulligan, 1982	*Oncorhynchus kisutch*	Diad.	Marking	Sr	Subsampled scales	AES
In situ solid sampling studies						
Wells *et al*., 2003b	*Cynoscion regalis*	Estuarine	Geographical diffs., temporal diffs.	Ba, Ca, Mg, Mn, Sr	Surface raster, core region	LA-ICP-MS
Wells *et al*., 2003a	*Oncorhyncus clarki lewisi*	Fresh	Geographical diffs.	Ba, Ca, Mg, Mn, Sr	Surface raster, edge region	LA-ICP-MS
Muhlfeld *et al*., 2005	*Oncorhyncus clarki lewisi*	Fresh	Geographical diffs.	Ba, Ca, Mg, Mn, Pb, Sr	Surface raster, core and edge	LA-ICP-MS
Lapi and Mulligan, 1981	*Oncorhynchus nerka*	Fresh	Geographical diffs.	Ba, Ca, Mg, Na, P, Sr, Zn	Surface raster, core region	SEM+XEDS
Flem *et al*., 2005	*Salmo salar*	Fresh	Geographical diffs.	Ba, Ca, Cr, Fe, Li, Mg, Mn, Pb, Sr, Zn	Surface raster, core region	LA-ICP-MS
van Coillie and Rousseau, 1974	*Catostomus commersoni*	Fresh	Geographical diffs., Pollution monitoring	Ag, Al, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Sr, Zn	Surface raster, whole scale	EMP
Gauldie *et al*., 1991a	multiple	Marine	Quantify analytes	C, Ca, Fl, O	Surface scan, entire scale	PMP
Ramsay *et al*., 2011	*Salmo trutta*	Fresh	Geographical diffs.	Ba, Co, Cu, Li, Mg, Mn, Pb, Si, Sr, U, Zn	Surface spots, edge	LA-ICP-MS

Table 1.2 Continued

Ramsay *et al*., 2012	*Salmo trutta*	Fresh	Geographical diffs.	Ba, Mg, Mn, Sr	Surface spots, edge	LA-ICP-MS
Ramsay *et al*., 2015	*Salmo trutta*	Fresh	Geographical diffs.	Mn, Sr	Surface spots, edge	LA-ICP-MS
Woodcock *et al*., 2013	*Sciaenops ocellatus*	Marine	Marking	Ba (isotopes)	Surface spots, edge	LA-ICP-MS
van Coillie and Rousseau, 1975	multiple	Fresh	Quantify analytes	Ca, Cu, Fe, K, Mg, Na, Zn	Surface spots, random	EMP
Mugiya *et al*., 1991	*Carassius auratus*	Fresh	Uptake dynamics	Al, Ba, Cd, Cu, Fe, Mn, Ni, Pb, Sr, Zn	Surface spots, random	XMA
Campbell *et al*., 2015	*Oncorhynchus tshawytscha*	Diad.	Compare structures, Identify diadromy	Sr	Surface spots, scale transects	LA-ICP-MS
Coutant and Chen, 1993	*Morone saxatilis*	Diad.	Identify diadromy	Sr	Surface spots, scale transects	LA-ICP-MS
Courtemanche *et al*., 2006	*Salvelinus fontinalis*	Diad.	Identify diadromy	Ca, Sr	Surface spots, scale transects	EMP
Borcherding *et al*., 2008	*Coregonus oxyrinchus*	Diad.	Identify diadromy	Ca, Sr	Surface spots, scale transects	LA-ICP-MS
Wang *et al*., 1994	Salmonid (sp. unknown)	Diad.	Marking	Ca, Sr	Surface spots, scale transects	LA-ICP-MS
Wells *et al*., 2000b	*Cynoscion regalis*	Estuarine	Geographical diffs.	B, Ba, Ca, Cd, Cu, Mg, Mn, Pb, Sr, Zn	Surface spots, scale transects; core raster	LA-ICP-MS
Saur and Watabe, 1989	*Fundulus heteroclitus*	Estuarine	Pollution monitoring	Ca, Cd, Co, Cu, Mn, Ni, Pb, Sr, Zn	Surface spots, scale transects	XMA

Table 1.2 Continued

Holá *et al*., 2009	multiple	Fresh	Archaeology	As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, V, Zn	Surface spots, scale transects	LA-ICP-MS, EMP
Kalvoda *et al*., 2009	multiple	Fresh	Diagenesis effects	As, Ba, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr, V, Zn	Surface spots, scale transects	LA-ICP-MS, EMP
Farrell *et al*., 2000	*Thymallus arcticus*	Fresh	Pollution monitoring	As, Cd, Hg, Mg, Ni, Pb, Sb, Se, Zn	Surface spots, scale transects	LA-ICP-MS
Gauldie *et al*., 1991b	*Macruronus novaezelandiae*	Marine	Age validation	Ca, Fl, Sr, Zn	Surface spots, scale transects	PMP
Courtemanche *et al*., 2005	*Salvelinus fontinalis*	Diad.	Identify diadromy	Ca, Sr	Surface spots and cross-sectioned spots, transects across scales	EMP
Holá *et al*., 2011	*Cyprinus carpio*	Fresh	Quantify analytes	Ca, Fe, Mg, Mn, P, Pb, Sr, Zn	Continuous transects across scales; Depth profile; Cross-section scan (EMP)	LA-ICP-MS, SB-ICP-MS, EMP
Wolff *et al*., 2013	*Chasmistes liorus*	Fresh	Identify hatchery fish	Sr (bulk & isotopes), Ca	Continuous transects across scales; Surface raster, core and edge;	LA-ICP-MS
Clarke *et al*., 2007	*Thymallus arcticus*	Fresh	Geographical diffs.	Ba, Ca, Mg, Mn, Sr	Continuous transects across scales	LA-ICP-MS

Table 1.2 Continued

†Abbreviatons. SB-ICP-MS: solution-based ICP-MS; LA-ICP-MS: laser ablation ICP-MS; AAS: atomic absorption spectroscopy; TIMS: thermal ionization mass spectrometry; OES: optical emission spectroscopy; AES: atomic emission spectroscopy; SB-ICP-AES: solution-based ICP atomic emission spectroscopy; FEP: flame emission photometry; SEM: scanning electron microscopy; XEDS: energy dispersive X-ray spectroscopy; EMP: electron microprobe; PMP: proton microprobe; XMA: X-ray microanalysis.

Table 1.3. Laser parameters used for sample analysis.

Laser Parameters	
Laser energy output	20%
Repetition Rate	10 Hz
Spot Size	35 μm
Laser Speed	25 μm/s
He Flow	300 ml/min
Ar Flow	1.23 L/min

Table 1.4. Semi-quantitative elemental weight percent within five different sampling locations across a cross-section of an Atlantic tarpon scale (see spectra positions in Figure 1). Spectra 1-4 represent increasing depths below the exterior within the external layer. Spectra 5 derives from the basal plate.

Position	C	O	Ca	P
Spectrum 1	39.6	31.8	19.1	8.4
Spectrum 2	42.6	33.7	16.1	6.8
Spectrum 3	43.7	30.3	18.5	7.2
Spectrum 4	42	33	16.7	7.1
Spectrum 5	65.5	30.2	2	0.8

Table 1.5. Concentrations (in ppm), percent recoveries, limits of detection (LOD, in ppm) and residual standard deviations (RSD, in percent) of quantified analytes using either NIST612 or NIST610 (n = 9 each) certified reference materials standardized against MAPS4. Concentration, recovery and LOD values are means ± standard deviations. Recoveries are calculated relative to GeoREM preferred values (http://georem.mpch-mainz.gwdg.de).

Analyte	Concentration (ppm)	Recovery (%)	LOD	RSD (%)
NIST612				
^{25}Mg	68 ± 5.1	86 ±6.3	1.2 ± 0.5	6.3
^{26}Mg	69 ± 5.1	90 ±4.1	3.8 ± 0.8	4.1
^{31}P	46 ± 6.9	46 ±71.3	45.6 ± 11	71.3
^{42}Ca	85048 ± 715	99 ±3.12	609.3 ± 119.3	3.12
^{55}Mn	38.7 ± 0.9	97 ±2.4	1.3 ± 0.3	2.4
^{88}Sr	78.4 ± 0.2	104 ±2.2	0.1 ± 0.2	2.2
^{137}Ba	39.3 ± 0.9	90 ±3.8	<0.1	3.8
^{138}Ba	39.3 ± 0.10	95 ±2.3	<0.1	2.3
^{208}Pb	38.57 ± 0.2	98 ±5.3	0.1 ± 0.0	5.3
NIST610				
Analyte	**Concentration (ppm)**	**Recovery (%)**	**LOD**	**RSD (%)**
^{25}Mg	432 ± 29	100 ±3.3	1.1 ± 0.3	3.3
^{26}Mg	433 ± 29	100 ±2.1	3.2 ± 0.4	2.1
^{31}P	413 ± 46	59 ±7.9	43.2 ± 10.2	7.9
^{42}Ca	81475 ± 1429	100 ±1.1	563.6 ± 60.7	1.1
^{55}Mn	444 ± 14	93 ±2.8	1.2 ± 0.2	2.8
^{88}Sr	515.5 ± 1	103 ±1.9	<0.1	1.9
^{137}Ba	452 ± 9	90 ±3.2	0.1 ± 0.1	3.2
^{138}Ba	453 ± 9	94 ±2.7	<0.1	2.7
^{208}Pb	426 ± 1	94 ±5.1	0.1 ± 0.0	5.1

Table 1.6. Summary of analyte values relative to ^{43}Ca quantified in transects across each tarpon scale. Values shown are the range (minimum to maximum), average (± standard deviation, S.D.), median, and the percent of data points above the limit of detection derived from NIST612 and NIST 610 reference materials. Units are μmol/mol for Mn, Ba, Pb and mmol/mol otherwise.

Tarpon 1 Analyte	**Range**	**Mean (S.D.)**	**Median**	**NIST612 % Above LOD**	**NIST610 % Above LOD**
^{25}Mg	16.8-55.8	20.1 (2.9)	19.4	100	100
^{26}Mg	16.7-55.1	20.4 (2.9)	19.6	100	100
^{31}P	300.4-455.2	367.3 (9.9)	366.6	100	100
^{42}Ca	912.4-1027.4	992.8 (11.2)	992.9	100	100
^{55}Mn	8.8-93.0	24.9 (11.8)	23.5	100	100
^{88}Sr	2.1-3.2	2.5 (0.1)	2.5	100	100
^{137}Ba	0.2-21.5	3.5 (2.5)	2.8	100	100
^{138}Ba	0.5-18.4	3.5 (2.5)	2.8	100	100
^{208}Pb	0-1.3	0.1 (0.1)	0.1	54	67

Tarpon 2 Analyte	**Range**	**Mean (S.D.)**	**Median**	**NIST612 % Above LOD**	**NIST610 % Above LOD**
^{25}Mg	16.4-48.1	21.0 (4.6)	19.4	100	100
^{26}Mg	16.6-46.7	21.2 (4.5)	19.7	100	100
^{31}P	339.8-583.6	380.2 (32.7)	369.9	100	100
^{42}Ca	882.8-1184.8	992.0 (16.5)	991.0	100	100
^{55}Mn	4.0-81.1	23.8 (14.1)	18.4	100	100
^{88}Sr	0.7-2.5	1.1 (0.7)	0.9	100	100
^{137}Ba	0.4-34.2	4.4 (2.9)	4.2	100	100
^{138}Ba	0.8-24.0	4.4 (2.6)	4.2	100	100
^{208}Pb	0-34.4	0.6 (2.0)	0.2	95	98

CHAPTER 1 FIGURES

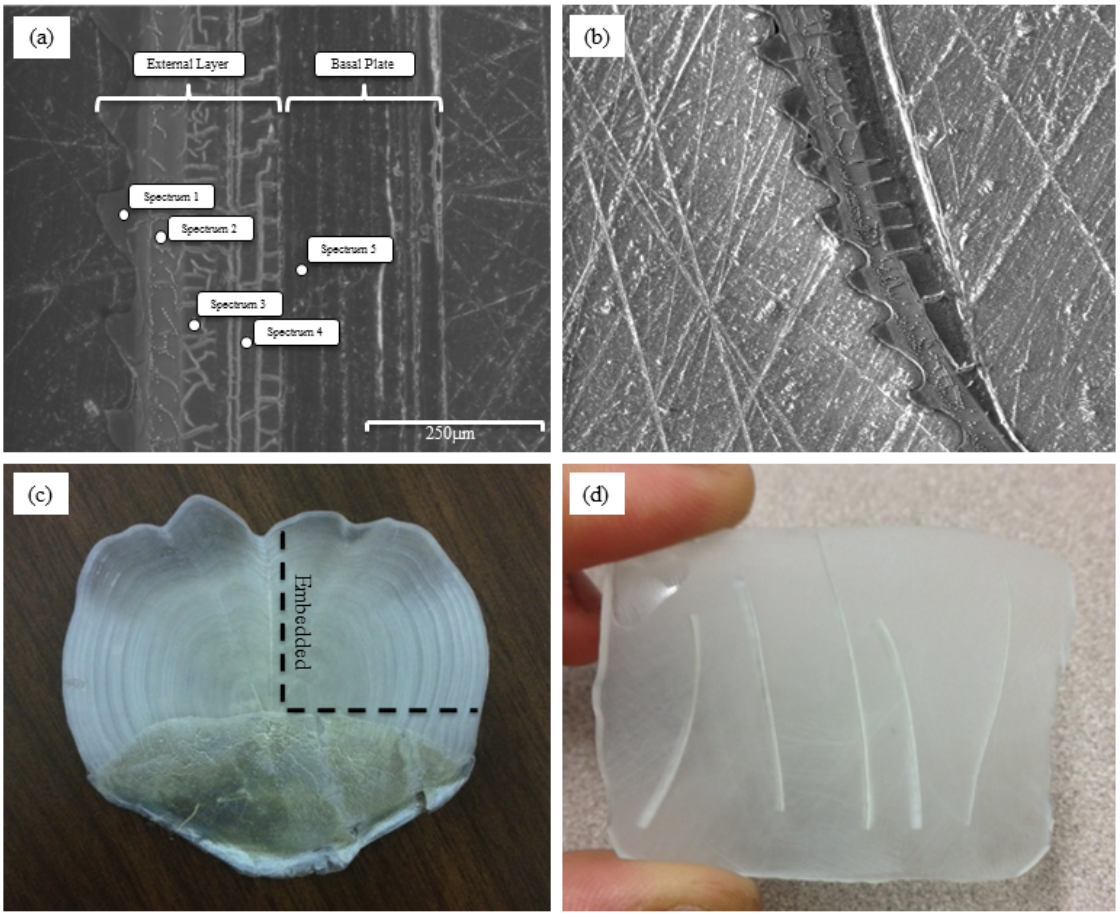

Figure 1.1. Photographs depicting cross-sectional composition and sample preparation procedure. A. ESEM image of an Atlantic tarpon scale in cross section showing sampled spectra. B. ESEM image of an Atlantic tarpon scale in cross section. C. Atlantic tarpon scale set to be cut and embedded in epoxy. D. Scales embedded in epoxy prepared for laser ablation analysis.

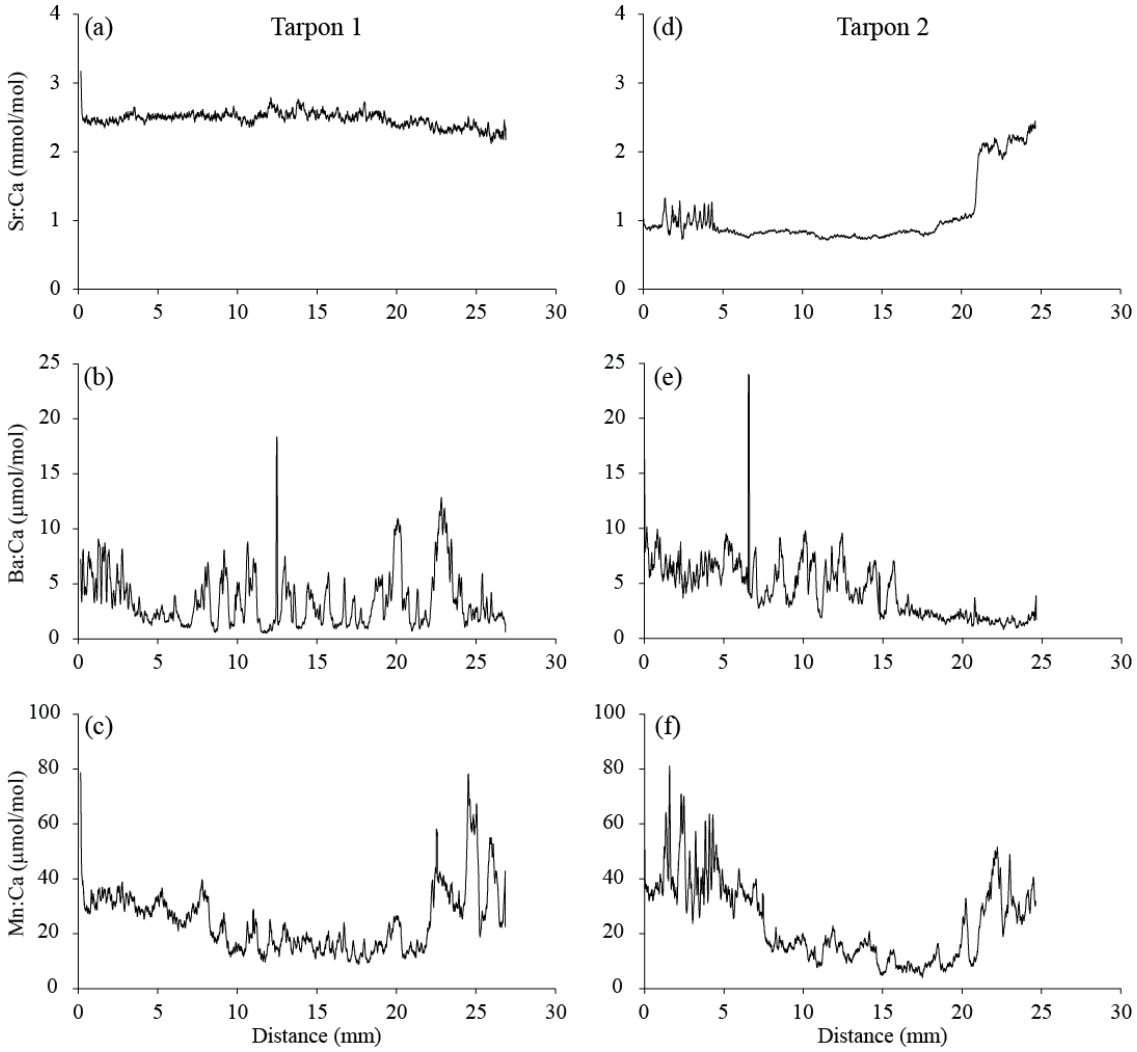

Figure 1.2. Representative continuous scale transects of Sr:Ca, Ba:Ca, and Mn:Ca for Tarpon 1 (a-c) and Tarpon 2 (d-f).

CHAPTER 2: Relationships between Atlantic tarpon scales and otoliths using elemental and isotopic proxies

INTRODUCTION

Many commercially and recreationally important fish species are highly migratory and display species-specific site fidelity in a variety of habitats throughout life. In the Gulf of Mexico, the Texas coast offers a variety of habitats that suits the life histories of many euryhaline species (Palmer et al. 2013). These habitats range from riverine, estuarine and mangrove flats, to coastal and offshore environments. Euryhaline species use these habitats in different ways, both spatially and temporally. One common theme between some euryhaline species is the use of inshore estuarine habitats as nursery grounds and use of coastal and offshore environments as spawning grounds (Gelwick et al. 2001; Holt, 2008; Luo & Ault, 2012).

Tracking migrations of fishes across habitats and salinity gradients is important to better understand the life histories of migratory species. Currently, a variety of tracking methods are employed to achieve this goal. These techniques include, but are not limited to, dart tags, telemetry, satellite tags, and natural chemical tags (Hearn et al., 2014; Walther & Limburg, 2012; Wilson et al., 2015). Of the aforementioned techniques, only natural chemical tags are capable of providing almost complete life history data regardless of where the fish travels (Elsdon et al., 2008).

Otolith microchemistry quantifies the incorporation of elements from the surrounding medium into the structure (Campana, 1999). Otoliths are composed of a calcium carbonate matrix and grow by the continuous accretion of new material onto the outer surface (Campana & Thorrold, 2001). When properly sectioned and polished,

growth rings are visible from the core to the edge of the otolith spanning the entire life of the fish (Campana et al. 2009). Certain elements dissolved in the water, such as strontium (Sr), are incorporated into otoliths proportional to their abundance in the water (Kraus & Secor, 2004), although factors such as fish growth in certain life stages and temperature can influence the amount of ions incorporated into the precipitated material (Brown & Severin, 2009). Sr/Ca concentrations vary across salinity gradients, typically ranging from high in marine and estuarine environments to low in oligohaline environments, depending on the geological composition of riverine systems (Walther & Limburg, 2012). Although rivers may contain elevated Sr concentrations that are similar to marine values, this is rare and not observed in freshwater systems in the south Texas coastal bend (Walther & Nims, 2015). Provided that information about the direction and shape of the mixing relationship between Sr/Ca and salinity is known, movements of fish across salinity gradients can therefore be reconstructed using the composition of incrementally accreted biogenic structures such as otoliths.

Although otolith chemistry is an increasingly popular technique to quantify fish migration, otolith extraction is lethal and therefore less desirable when working with protected species or species in catch-and-release fisheries. A nonlethal alternative to an otolith chemistry approach is non-lethal removal of scales and quantifying the chemical composition of their increments (Adey et al. 2009). There are several properties that scales share with otoliths, such as incremental growth and incorporation of some of the same chemical constituents from water or diet (Holá et al. 2011; Secor, 1999). In contrast to otoliths, scales are composed of a well-calcified external layer underlain by an organic

hydroxyapatite matrix (basal plate). Therefore, scales can be assayed for both inorganic (e.g., Sr/Ca) and organic (e.g., $\delta^{13}C$ and $\delta^{15}N$) proxies (Seeley et al. 2015). Fortunately, Sr/Ca and $\delta^{13}C$ complement each other as salinity tracers because Sr/Ca ratios typically increase with salinity (Walther & Limburg, 2012) and $\delta^{13}C$ values of dissolved inorganic carbon (DIC) typically increase as salinity increases (Fry, 2002). Values of $\delta^{15}N$ in tissues are frequently used to quantify organismal trophic level due to its roughly 3‰ increase with each increase in trophic level (Vander Zanden & Rasmussen, 2001). One complexity for scale chemistry is that the well-calcified external layer increases in area, but not thickness, while the poorly calcified basal plate varies in thickness (Hutchinson & Trueman, 2006). This means that in the basal plate new increments underlie older increments at the interior of the scale. Therefore, scale collagen analyses from the basal plate provide integrated isotope signals biased towards the more recently formed collagen. However, if the difference in isotope ratio values between freshwater and marine endmembers is sufficiently large, movements across salinity gradients could still be observed despite underplating bias. Additionally, the degree of underplating can vary across different species with different sized scales (Figure 2.1; Hutchinson & Trueman, 2006; Ramsay et al. 2012). The combined analysis of the elemental and isotope ratios from the two different portions of the scale also allows comparison between chemical proxies for salinity to verify of a shift likely to represent movements into oligohaline water.

For this study, Atlantic tarpon (*Megalops atlanticus*) was chosen as a model species due to their highly migratory behavior, movement across salinity gradients on the

Texas coast, and unusually large scales, which exceed 6 cm in diameter (Adams et al., 2014; Wade, 1962). Squamation occurs shortly after metamorphosis from a leptocephalus larva at approximately 30-40 mm standard length and less than 1 year old. Scales are then retained for the duration of the fish's life unless they are lost through damage or shed due to stress (Harrington, 1958; Wade, 1962). In contrast, otoliths begin development during embryonic stages, and thus contain pre-metamorphic information at the interior of the structure (Elsdon et al., 2008). Therefore, comparing transects from otoliths and scales will not contain the same amount of life history.

In order to use scales as non-lethal alternatives to otoliths, it is important to determine whether scale elemental concentrations (Sr/Ca) and isotope values ($\delta^{13}C$ and $\delta^{15}N$) display similar patterns to otoliths across salinity gradients and in trophic structure. Unlike otoliths, there are hundreds of scales on most fish, so it must be confirmed that scales from the same region on an individual display the same chemical trends in concentrations and isotope values across scale life histories. To address these questions, I quantified the patterns of variation in Sr/Ca, $\delta^{13}C$, and $\delta^{15}N$ across life-history transects of multiple scales from the same individual to test consistency. Next, I quantified patterns of Sr/Ca across life-history transects of otoliths and scales from the same individuals to test consistency between the two structures. Finally, I quantified the relationship between water concentrations of dissolved elements (Sr/Ca, Ba/Ca, Mg/Ca, Mn/Ca) and scales from fish that were resident in the Texas State Aquarium (TSA) to determine elemental uptake dynamics. Results of this work verify the utility and reliability of scales as non-lethal analogs of otoliths for reconstructing migratory and dietary histories.

METHODS

Sample Collection and Preparation

Scales and otoliths were collected from Fall 2013 to Fall 2015 along the Texas coastal bend. Local anglers, directors of fishing tournaments, and the Texas State Aquarium donated the sampled scales. Each donated sample arrived in a water resistant envelope with recorded capture location, angler-estimated weight or length of fish, and angler name. Anglers were instructed to remove scales from the just above the lateral line under the dorsal fin for all sampled fish. In total, I collected multiple scales (2-4 scales per individual) from the dorsal region of 8 individual Atlantic tarpon from Port Aransas (n = 2 fish), Matagorda Bay (n = 4), and the Texas State Aquarium (n = 2), totaling 22 scales. I also opportunistically collected eight otoliths from Port Aransas (n = 6) and the Texas State Aquarium (n = 2) with paired scales. All fish ranged in size from 8.7 to 127.0 cm standard length.

Scales were cleaned, dried, and flattened following the methods outlined by Woodcock and Walther (2014). Scales were then subsampled for two analyses: (1) laser ablation to quantify elements along a continuous transect across each scale and (2) stable isotope ratio ($\delta^{13}C$ and $\delta^{15}N$) measurement from discrete subsamples removed along a core to edge transect of each scale. For laser ablation analyses, a rectangular strip of scale measuring 8 mm wide was removed from the core to the edge along the longest growth axis of each scale. This core-to-edge rectangle therefore contained all increments from onset of squamation (core) through capture (edge) from each sampled fish. Four to five dissected rectangles were placed adjacent to each other between two pieces of 3M

Scotch® Heavy Duty Mounting Tape (double-sided) on the long edge, standing 8 mm high. One of the two pieces of double-sided tape was then adhered down in a silicone 7.4 cm x 4.8 cm x 2.5 cm mold. Otoliths were also placed in molds for embedding. The scales and otoliths were then embedded in EpoxiCure ® Epoxy Resin, mixed with EpoxiCure ® Epoxy Hardener in a 5:1 ratio (Buehler, Lake Bluff, Illinois, USA). After 24 h, scales were sectioned in 1-mm sections along the horizontal core-to-edge axis to yield a cross-section of rectangular strip with the calcified surface layer exposed for analysis. Otoliths were sectioned along the transverse plane. Both structures were sectioned using a Buehler Isomet™ low speed saw with a diamond-wafering blade (Buehler, Lake Bluff, Illinois, USA). Sections were then mounted on a microscope slide using the same epoxy hardener mix for scales and Crystal Bond® 509 (Ft. Washington, Pennsylvania, USA) for otoliths. Finally, scales and otoliths were polished using 12- then 8-µm aluminum oxide discs on a Buehler MetaServ 250 Grinder-Polisher until the external layer and basal plate were clearly visible on the scales and growth rings visible on the otoliths.

Stable isotope scale preparation was conducted after results were already analyzed for the majority of the elemental data. The second longest edge of the same scales was identified, which was most often 90 degrees to the previously dissected rectangular laser ablation transect. Subsamples 1 mm x 1 mm, weighing between 0.5 mg and 2 mg, were cut out using ceramic scissors at predetermined distances on the newly identified transect. I used the life-history transects of Sr/Ca obtained across scales to identify locations of interest for subsampling to determine stable isotope values corresponding to major shifts

in elemental concentrations. Subsamples were removed from the core and the edge of each scale to encompass the scale life history. The number of intermediate subsamples was determined based on the size of each scale and ranged from three to ten. For scales where Sr/Ca transect data were not yet available, subsamples were spaced evenly from the core to the edge.

Water Collection and Preparation

Water samples were collected monthly from the tank containing Atlantic tarpon at the TSA over an 8-month span from March to October 2015 with the exception of April. We also sampled the aquarium's wet lab to test municipal water that is occasionally added to the Atlantic tarpon tank for salinity control. All water samples were taken with polytetrafluorethylene (PTFE) syringes and then passed through 0.45-µm and 0.20-µm PTFE filters in sequence. Water was stored in 30-mL LDPE bottles and fixed with 2% trace metal grade nitric acid. All materials used to collect and store water were acid-washed to remove metal contamination prior to use. Fixed water samples were refrigerated at 1.7 °C until analysis.

Element Analysis in Scales and Otoliths

Analyses were conducted in two different sessions, one in Spring 2015 and the other in Fall 2015. Elements (^{26}Mg, ^{31}P, ^{43}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba) were quantified at the University of Texas Jackson School of Geosciences. Samples were analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). A New Wave Research UP193-FX fast excimer (193 nm wavelength, 4–6 ns pulse width) laser system

was coupled to an Agilent 7500ce ICP-MS for scale transect analyses (Seeley et al. 2015). Preablation was conducted on all samples prior to analysis with a spot size larger than final ablation spot size to remove any surface contaminants. For scales, we consistently sampled from core to edge within the external layer, following the methods outlined by Seeley et al. (2015).

The majority of scales from wild and TSA fish were analyzed in cross section with laser ablation of a transect from core to edge solely within the exposed surficial calcified layer. However, scales from the five juveniles captured in Port Aransas were too small to execute the cross-sectioning technique used for larger scales, and were instead mounted flat directly onto slides with the calcified surface layer on top. For these juvenile scales, the laser ablated a top-down (lateral to medial) transect from core to edge.

Laser ablation parameters were 25 μm s^{-1} line traverses using a 25-μm spot, 30% power, and 10-Hz repetition rate. Laser energy densities (fluences) obtained for the analytical session ranged from 2.5-3.25 J cm^{-2}. Certified reference materials, NIST 612, NIST 610, and MAPS4 bracketed scale runs, while ^{43}Ca was used as an internal standard. In Spring 2015, the percent residual standard deviation for scale analyses for the element of interest, Sr, was Sr = 2.13 and 2.32 for NIST 612 and 610 against MAPS4, respectively. In Fall 2015, the percent residual standard deviation for scale analyses was Sr = 4.51 and 6.67 for NIST 612 and 610 against MAPS4, respectively.

For otoliths, a transect was ablated from one edge of the otolith to the other across the longest growth axis and transiting through the otolith core to obtain a palindromic life history transect of elemental concentrations. Otolith elemental transects were

subsequently truncated to obtain only the core-to-edge elemental profile across the longest half of the palindromic transect. Laser ablation parameters for otolith analyses were 5 μm $^{-1}$s line traverses using a 25-μm spot, 25% power, and 10-Hz repetition rate. Fluences for this analytical session also ranged from 2.5-3.25 J cm^{-2}. Certified reference materials, NIST 612, NIST 610, and MACS3 bracketed otolith runs, while ^{43}Ca was used as an internal standard. In Fall 2015, the percent residual standard deviation for otolith analyses was Sr = 4.83 and 3.52 for NIST 612 and 610 against MACS3, respectively.

All otolith transects displayed the same initial pattern from high to low Sr/Ca over the first 400 μm from the core outwards. This distance on the transect was assumed to represent the larval phase of Atlantic tarpon in high salinity water prior to migration inshore, metamorphosis and squamation. Data for all otolith transects were then trimmed by 400 μm to remove the putative pre-squamation portion before comparison of elemental transects between otoliths and scales.

During trace element analyses, outlier values were occasionally observed within transects. These points were due to analytical error as the laser spot drifted out of the external layer and into the epoxy or basal plate of the scale. When this occurred, the elemental value for these regions were removed and data were interpolated to replace the missing portions.

Stable Isotope Analysis (δ^{13}C and δ^{15}N) in Scales

Following sample preparation for trace elements, stable isotope analyses (δ^{13}C and δ^{15}N) were also conducted in two different sessions, one in Spring 2015 and the other in Fall 2015. Scale subsamples were encapsulated in tin and analyzed at University of

California, Davis, Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). In Spring 2015, samples were run against four reference materials (Nylon: $\delta^{15}N = -10.31$, $\delta^{13}C = -27.72$; Bovine liver: $\delta^{15}N = 7.72$, $\delta^{13}C = -21.69$; Glutamic acid (USGS-41): $\delta^{15}N = 47.6$, $\delta^{13}C = 37.63$; and Glutamic acid: N = 9.52%, $\delta^{15}N = -6.8$, C = 40.81%, $\delta^{13}C = -16.65$). In Fall 2015, samples were run against four reference materials (Nylon: $\delta^{15}N = -10.31$, $\delta^{13}C = -27.72$; Bovine liver: $\delta^{15}N = 7.72$, $\delta^{13}C = -21.69$; Glutamic acid: N = 9.52%, $\delta^{15}N = -6.8$, C = 40.81%, $\delta^{13}C = -16.65$; and Enriched Alanine: $\delta^{15}N = 41.13$, $\delta^{13}C = 43.02$).

Water Analysis

Water samples were analyzed at the University of Texas Jackson School of Geosciences. Elements (^{26}Mg, ^{55}Mn, ^{88}Sr, and ^{138}Ba) were analyzed using an Agilent 7500ce quadrupole inductively coupled plasma mass spectrometer (ICP-MS) run in solution mode. Depending on salinity, samples were diluted prior to analysis by either 10 or 100x using 2% nitric acid to achieve less than 200 ppm total dissolved solids. Instrument drift was monitored using (n = 6) samples spiked with an internal standard solution. Spike recoveries (mean ± SD) were: $^{26}Mg = 0.89 \pm 0.06$, $^{55}Mn = 1.02 \pm 0.01$, $^{88}Sr = 0.99 \pm 0.01$, and $^{137}Ba = 0.99 \pm 0.01$. Calculation of a partition coefficient for scales from the TSA was quantified to examine the proportion of dissolved elements incorporated into the TSA scales (Morse & Bender, 1990), using the equation $D_{element} = [(Element/Ca)_{scale}] / [(Element/Ca)_{water}]$. To calculate this partition coefficient, elemental values from the outer 1000 μm of each scale transect, representing the most recently

accreted material, was averaged across the six scale samples from the TSA to represent recent scale elemental ratios within the tanks. The calculated water elemental ratios were averaged within each month, excluding July, and across the eight months sampled. The month of July was excluded from this average due to exceptionally low salinities during the sampling day that were not consistent with the other salinities measured and therefore assumed to be an anomalous event. The calculated partition coefficient for aquarium-reared Atlantic tarpon was compared to coefficients calculated in other studies for scales and otoliths to determine the comparability of the structures with respect to incorporation of elements from the water.

Statistics

Statistical analyses were performed using SigmaPlot 13. Twenty-one separate analyses of covariance (ANCOVA) were run to test whether proxy values or concentrations were consistent from multiple scales (2 to 4 scales from the same fish) within an individual. The twenty-one tests encompassed the three proxies for seven of eight sets of multiples. One set was not sampled because the discrete stable isotope transect comprised only three subsamples. Tests were run between $\delta^{13}C$ and $\delta^{15}N$ with $\delta^{15}N$ as the covariate, $\delta^{13}C$ and Sr/Ca with $\delta^{13}C$ as the covariate, and Sr/Ca and $\delta^{15}N$ with $\delta^{15}N$ as the covariate. Individual scales within each multiple were used as the main effect for each test.

To compare mean shifts within a scale transect a regime-shift algorithm was applied. The algorithm identified significant changes in mean proxy values, while excluding outliers, using a sequential t-test (Rodionov, 2004). The algorithm was set with

a 0.05 significance level, a cut-off length of 1000 cells or approximately 5000 μm, and a Huber's weight parameter of 1. Also, qualitative comparisons were made between scale transects within an individual and between scales and otoliths from the same individual. To facilitate comparisons, scale transect lengths were converted to proportional distances given that transects in different scales were not precisely the same length. Proxy values were also normalized to a mean of 0 and a standard deviation of 1 for scale-otolith comparisons in subadult and older fish. When qualitatively comparing otoliths to scales, otolith transect distances were log-transformed for all subadult and adult fish, as a logarithmic relationship is present when comparing otolith size to the fork length of the same fish. Juvenile fish otolith transects remained on a linear distance scale, as a linear relationship between otolith size and fish fork length was present. Finally, qualitative comparisons were made between the TSA partition coefficients for scales in relation to partition coefficients for otoliths from other studies.

RESULTS

Scales Within an Individual

There were no significant differences in either the interactions or the slopes of Sr/Ca between multiple scales from the same fish when using either $\delta^{15}N$ or $\delta^{13}C$ as a covariate within scales ($P > 0.05$) for six of the seven fish for which multiple scales were available. The exception was one fish (Fish #052) that had a significant difference in the interaction for Sr/Ca vs. $\delta^{13}C$ and Sr/Ca vs. $\delta^{15}N$. Comparisons of $\delta^{13}C$ vs. $\delta^{15}N$ were all not significantly different ($P > 0.05$) for the interaction. Therefore, with one exception all

non-regenerated scales from an individual contained the same patterns in elemental and isotope ratios across increments (Figure 2.2).

Qualitative comparisons were made using the Rodionov Sequential Regime Shift Detection Software for eight different sets of multiple scales (Figure 2.3). All scales from the same individual exhibited similar shifts in Sr/Ca values. However, different numbers of shifts were calculated for certain scales (Table 1). Three of the eight fish with multiple scales had the same number of shifts. The other five fish were all within two shifts of each other.

Visual comparisons were also made of Sr/Ca and $\delta^{13}C$ vs. distance and $\delta^{15}N$ vs. distance between scale multiples of an individual (Figure 2.4). All transects yielded similar patterns within a fish, with shifts in $\delta^{13}C$ occurring simultaneously with shifts in Sr/Ca.

Scales and Otoliths Within an Individual

Qualitative comparisons were made between a scale and an otolith from 8 fish (juveniles to adults) using normalized Sr/Ca against proportional distance along the transect. Paired transects (scale and otolith) consistently exhibited similar trends, with small discrepancies in timing of observed shifts (Figure 2.5).

Regenerated Scales

Scale regeneration was observed in one scale from Fish 061 (Matagorda, 58 kg) and one scale from Fish 062 (Matagorda, 18 kg) (Figure 2.6). Concentrations of Sr/Ca in regenerated scales showed distinct patterns that differed substantially from Sr/Ca patterns

in other scales from the same fish. Values of $\delta^{13}C$ and $\delta^{15}N$ yielded substantially different patterns across transects for Fish 061. For Fish #062, $\delta^{13}C$ values were different between regenerated and non-regenerated scales while $\delta^{15}N$ values were consistent among all scales from that individual (Figure 2.7).

TSA Water and Scale Partition Coefficients

Water Sr/Ca values from the TSA ranged from 3.58-4.55 (mmol mol^{-1}) from March 2015 to October 2015, excluding the month of July (1.96 mmol mol^{-1}). The municipal water values ranged from 1.56-2.85 mmol mol^{-1} (Figure 2.8). The partition coefficients calculated from scale and water samples collected in the TSA were D_{Sr} = 0.53, D_{Mg} = 0.01, D_{Mn} = 0.07, and D_{Ba} = 0.1 (Table 2.2). The D_{Sr} was within the range of partition coefficient values reported in the literature for otoliths, thus supporting the conclusion that scales and otoliths incorporate chemical constituents from water in roughly the same order of magnitude.

DISCUSSION

The use of fish scales in elemental and isotopic studies has been increasing recently (Seeley et al., 2015; Trueman et al., 2012). Scales can be used as an additional structure or nonlethal alternative to otoliths for identifying habitat use and trophic structure. Researchers most often use either the left or right sagittal otolith for analyses. With scales, hundreds of scales are available from an individual. Therefore, it is imperative to know that scales from the same general region on a fish exhibit the same elemental and isotopic life-history patterns.

Seven of eight scale multiples analyzed from individual Atlantic tarpon contained a sufficient number of discrete subsamples to compare elemental and isotopic proxies. These included samples from Port Aransas (n =1), Matagorda Bay (n =4), and TSA (n =2). Comparing Sr/Ca to $\delta^{13}C$ and $\delta^{15}N$ consistently yielded similar slopes and patterns between multiple scales within an individual. As expected, these relationships were consistent for the majority of scales within an individual. The one exception was from an Atlantic tarpon analyzed from Matagorda Bay containing two scale samples that were significantly different in the interaction between slopes for $\delta^{13}C$ ($P = 0.006$) and $\delta^{15}N$ ($P = 0.002$). This difference was most likely due to Sr/Ca as the dependent variable. Qualitatively, the two scale transects appear to have different initial shifts during the first ~25% of analysis. At this region of the scale external layer, cracks were present in the structure. The cracks led the laser spot to ablate half on the scale and half in the epoxy, ultimately altering initial concentrations of Sr/Ca. This analytical error resulted in two major "shifts," ultimately yielding statistically significant differences in the two transects. In contrast, the comparison between $\delta^{13}C$ and $\delta^{15}N$ was not statistically significant for any of the scale multiples from individual Atlantic tarpon. Therefore, after excluding a scale that appeared to have superficial laser-disrupting blemishes, all scales within an individual contained the same patterns. The similarities between proxy analyses within a scale indicated that any non-regenerated scale removed from the dorsal region would yield comparable patterns in the elemental and isotope proxies that are of greatest interest for reconstructing life histories.

Comparisons between scales within the same individual were made qualitatively using the regime shift algorithm. Of the eight multiples analyzed, only three of the fish had an equal number of shifts across all of its scales. The other five fish had a difference of either one or two significant shifts across the transect. The different numbers of significant shifts were either due to a small range of values across an entire transect, slight analytical noise, or small, yet significant variation in scale chemical composition along the transects. The variation in shifts between scales within an individual simply represents the expected natural variability in chemical composition across each structure, and are not due to the presence or absence of a major shift indicative of a movement across a substantial salinity gradient.

Elemental and isotopic transects of scales within an individual most often represent very similar concentrations and values. Variability that occurs between scales within an individual may be due to the size of the scales, from environmental exposure, and/or due to scale underplating. Underplating was hypothesized to play a major role in altering the collection of isotopic scale life-history data. But, since $\delta^{13}C$ shows the same sequential patterns across transects as Sr/Ca, underplating appears to be a minor source of bias that does not prevent assessment of isotopic variability within interior scale increments. It should be noted that the size and structure of Atlantic tarpon scales are likely important in minimizing the degree of underplating bias, and more severe underplating bias could be more problematic for species that have smaller scales.

The timing and magnitude of shifts in Sr/Ca observed for otolith and scale transects within an individual were present in all subadult and adult Atlantic tarpon. As

increases or decreases in Sr/Ca occurred in the scales, they also occurred in the otoliths, although at different locations along the transects. The differences in timing of shifts leads to two proposed explanations: First, the fixed distance from the core along otolith transects that was truncated to remove presquamation life history may need to be reevaluated using a larger sample size representing multiple size classes. Second, otoliths and scales grow at different rates and at different times. Accretion rates may vary between the two structures due to exposure to the external environment and/or the physiological barriers that need to be passed for circuli to be laid down (Thorrold et al. 1997). These processes together introduce variability in the timing of chemical shifts across structures that lead to misalignment of transects and hamper the ability to assess comparability. Ultimately, these possible explanations will require experimental validation to identify the periodicities of growth in both structures.

Three scales were analyzed from fish 061 from Matagorda Bay weighing 59 kg. The two non-regenerated scales (B and C) exhibited consistent patterns with increases in concentrations and values for Sr/Ca, $\delta^{13}C$, and $\delta^{15}N$ from low to high across the scale transect. Regeneration was present in scale A. Deviations were observed in all proxies for scale A, with consistent concentrations and values across the scale transect. The central approximately 70% of the scale lacked circuli indicating that regeneration obliterated the early life Sr/Ca and $\delta^{13}C$ records of shifts across salinity gradients. When the scale regenerated and resumed normal accretion, the fish was residing in waters with higher Sr/Ca. $\delta^{15}N$ values were constantly high throughout the scale transect and differed significantly from the other two proxies. $\delta^{15}N$ increased from 12.18 to 15.22 ‰, an

increase of ~3.0 ‰, which is commonly reported between an animal and its diet (Vander Zanden & Rasmussen, 2001; Davenport & Bax, 2002). Therefore, this elevated $\delta^{15}N$ for the entirety of the scale was likely due to a shift in diet and ultimately trophic level prior to scale regeneration. Additionally, regeneration represented about 58% of the scale in one of two scales from fish 062 from Matagorda Bay, weighing 18 kg. We observed similar concentrations of Sr/Ca and values of $\delta^{13}C$ as observed in fish 061 with shifts across salinity gradients from low to high, but consistent life-history values of $\delta^{15}N$ across the two scales. The major shifts in $\delta^{15}N$ values occurred after regeneration, and thus both regenerated and non-regenerated scales exhibited comparable profiles in $\delta^{15}N$ values. Therefore, regeneration is not reflected in an altered $\delta^{15}N$ profile even though it is evident in Sr/Ca and $\delta^{13}C$ values.

To better understand the relationships between scales and otoliths, it was necessary to confirm uptake dynamics of elements from the water. Water samples collected from the TSA had a small range (0.96 Sr/Ca (mmol/mol)). A small range is to be expected as the TSA actively pumps in water from Corpus Christi Bay and closely manages salinity by addition of municipal water or salt. At times, coastal waters in South Texas may become hypersaline (Palmer et al., 2013), so it is important to note that the tank is diluted with low salinity water. The salinity of the tank at TSA remained fairly constant at around 30 psu since 2008. This is representative of marine water and thus, should contain a Sr/Ca value of approximately 8.5 mmol/mol (De Villiers, 1999). Yet, the average Sr/Ca value during sampling for this study was 4.20 mmol/mol, excluding July. We attribute this discrepancy in Sr/Ca values to the use of municipal water (1.18

Sr/Ca mmol/mol) to adjust salinity. Additionally, we do not know the chemical composition or Sr/Ca value of the agent that was added to increase salinity when needed.

Sr/Ca measured at scale edges from the TSA was on average 2.22 mmol/mol. This is comparable to that observed in wild marine fish (2.34 Sr/Ca mmol/mol). Yet, the dissolved water Sr/Ca value within the TSA was lower than the marine water value by 4.3 mmol/mol. With lower ambient Sr/Ca waters, the aquarium scale Sr/Ca value was initially expected to be lower than that observed in wild marine-resident fish. One potential explanation for this discrepancy is the possibility of elevated Sr/Ca in the dietary items on which captive fish were fed. Dietary contribution of Sr/Ca may be of significant influence on scale Sr/Ca when ambient water strontium concentrations are low (Doubleday et al., 2013; Woodcock & Walther 2014). The TSA Atlantic tarpon were fed a consistent diet while in the aquarium, consisting of capelin, krill, herring, and a gel mixture in an unknown quantity and frequency. This is a predominantly marine diet that is most likely higher in Sr/Ca than the water in which the fish were residing. For example, capelin Sr/Ca has been recorded to reach 9.8 mmol/mol (Davoren et al., 2015) and likely is a major contributing factor to the elevation of Sr/Ca in TSA Atlantic tarpon scales (Kalvoda et al., 2009; Woodcocket al., 2013).

Understanding the water chemistry throughout the Texas Bays and TSA is essential for comparing uptake ratios between different calcified structures. The calculated TSA partition coefficient for scales is within the observed range of other partition coefficients for Sr/Ca in otoliths from marine species (0.2-1.62 for $D_{Sr/Ca}$) (Brown & Severin, 2009). This provides support for the notion that scales incorporate

chemical constituents in roughly the same proportion as their abundance in the water. Do scales and otoliths convey the same information? Observed differences between scales and otoliths from the same individual may have been due to differences in the pathway through which chemical constituents pass to build scales and otoliths. Future research should focus on identifying the dynamics of elemental uptake between scales and otoliths and validation of the periodicity of scale growth compared to other calcified structures.

CHAPTER 2 TABLES

Table 2.1. Number of regime shifts quantified between scales within an individual using the regime shift detection algorithm. A dash indicates that scale could not be sampled or did not exist. "R" indicates a regenerated scale.

	Regime Shifts			
Scale	*A*	*B*	*C*	*D*
052	3	-	2	-
057	2	2	2	1
061	R	4	3	-
062	2	2	R	-
063	2	2	2	-
068	2	3	4	-
069	4	2	2	-
070	1	1	1	-

Table 2.2. Survey of D_{Sr} identified for calcified structures on a variety of different species.

Stucture	**Species**	**Partition Coefficient**	**Source (Author)**
Aragonite	Hematypic Coral	1.0-1.2	Plummer and Busenberg 1987
Otolith	*Pseudophycis barbatus*	0.18 ± 0.04	Kalish 1991
Bivalve	*Mercenaria mercenaria*	0.23-0.31	Stecher et al. 1996
Otolith	*Leiostomus xanthurus*	0.18-0.21	Bath et al. 2000
Otolith	*Leiostomus xanthurus*	0.19	Wells et al. 2000
Otolith	*Oncorhyncus clarki lewisi*	0.4	Wells et al. 2003
Scale	*Oncorhyncus clarki lewisi*	0.34	Wells et al. 2003
Hydroxyapatite	Calcium phosphate	0.01-0.45	Balter and Lecuyer 2004
Otolith	*Pagrus auratus*	0.08	Hamer and Jenkins 2007

Table 2.2 Continued

Otolith	*Platycephalus bassensis*	0.1	Hamer and Jenkins 2007
Otolith	32 sp.	0.2-1.62	Brown and Severin 2009
Otolith	*Gadus macrocephalus*	0.38-0.6	DiMaria et al. 2010
Otolith	*Macquariua novemaculeata*	~0.3-0.38	Macdonald and Crook 2010
Otolith	*Acanthochromis polyacanthus*	0.05-0.09	Walther et al. 2010
Otolith	*Mallotus villosus*	0.4-1.2	Davoren et al. 2015
Scale	*Megalops atlanticus*	*0.53*	*This Study*

CHAPTER 2 FIGURES

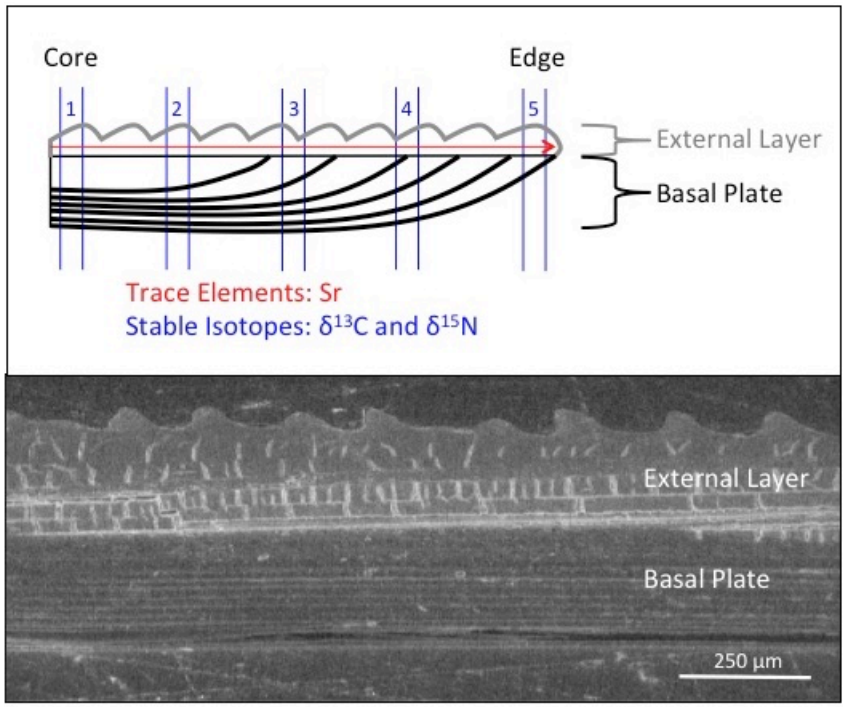

Figure 2.1. (Top) Schematic of a cross-section of a scale, exposing the surface external layer underlain by the basal plate. Grey humps in the external layer represent individual circuli. Black lines in the basal plate represent underplating where core materials are biased towards edge materials. Red arrow represents the elemental transect run via LA-ICP-MS. Blue vertical lines and numbers represent the discrete isotopic transect run via IR-MS (Redrawn from Hutchinson and Trueman 2006). (Bottom) ESEM image of a middle section of a scale showing the external layer (light grey) underlain by the basal plate (darker grey).

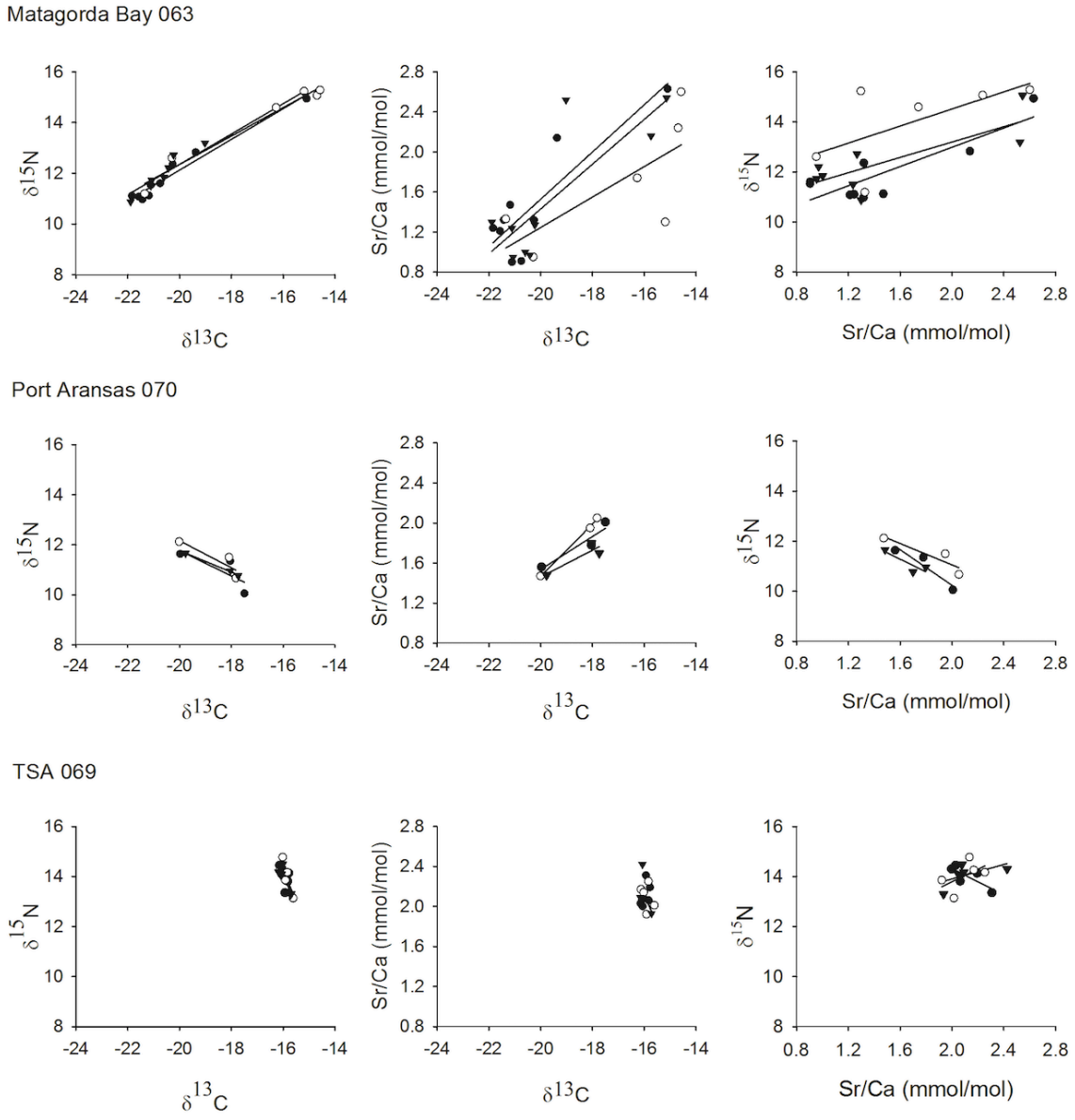

Figure 2.2. Regressions demonstrate consistency among multiple scales from individual fish for all proxies. Representative samples are present from all three sampling locations. Open circles represent scale A, closed circles represent scale B, triangles represent scale C.

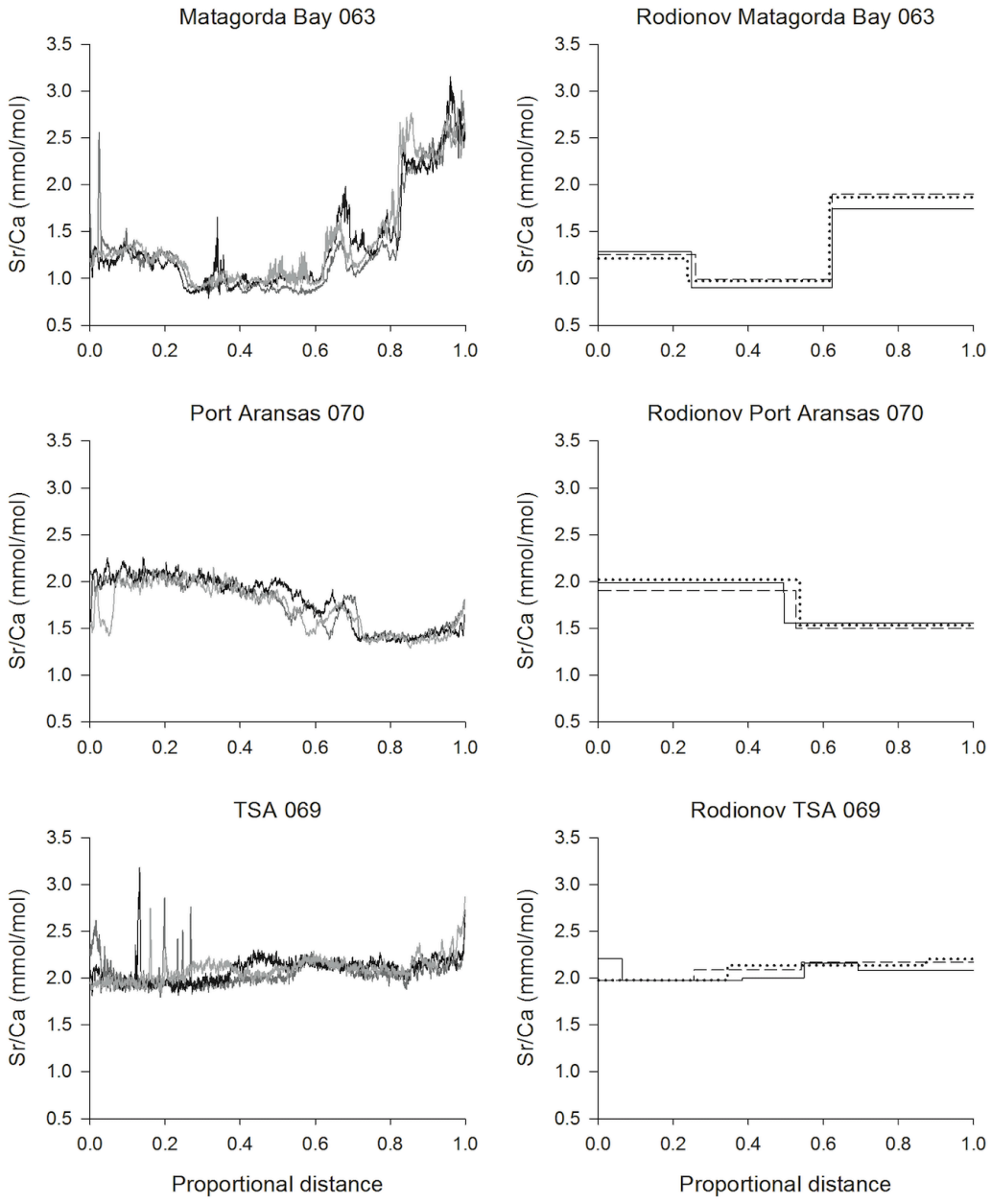

Figure 2.3. Representative Sr/Ca (mmol/mol) transects of three scales from the same individual, spanning all sampling locations (left). Dark grey transects represent scale A, black transects represent scale B, light grey transects represent scale C. Rodionov regime shift index mean transects for three scales from the same individuals (right). Solid transect represents scale A, dotted line represents scale B, and dashed line represents scale C.

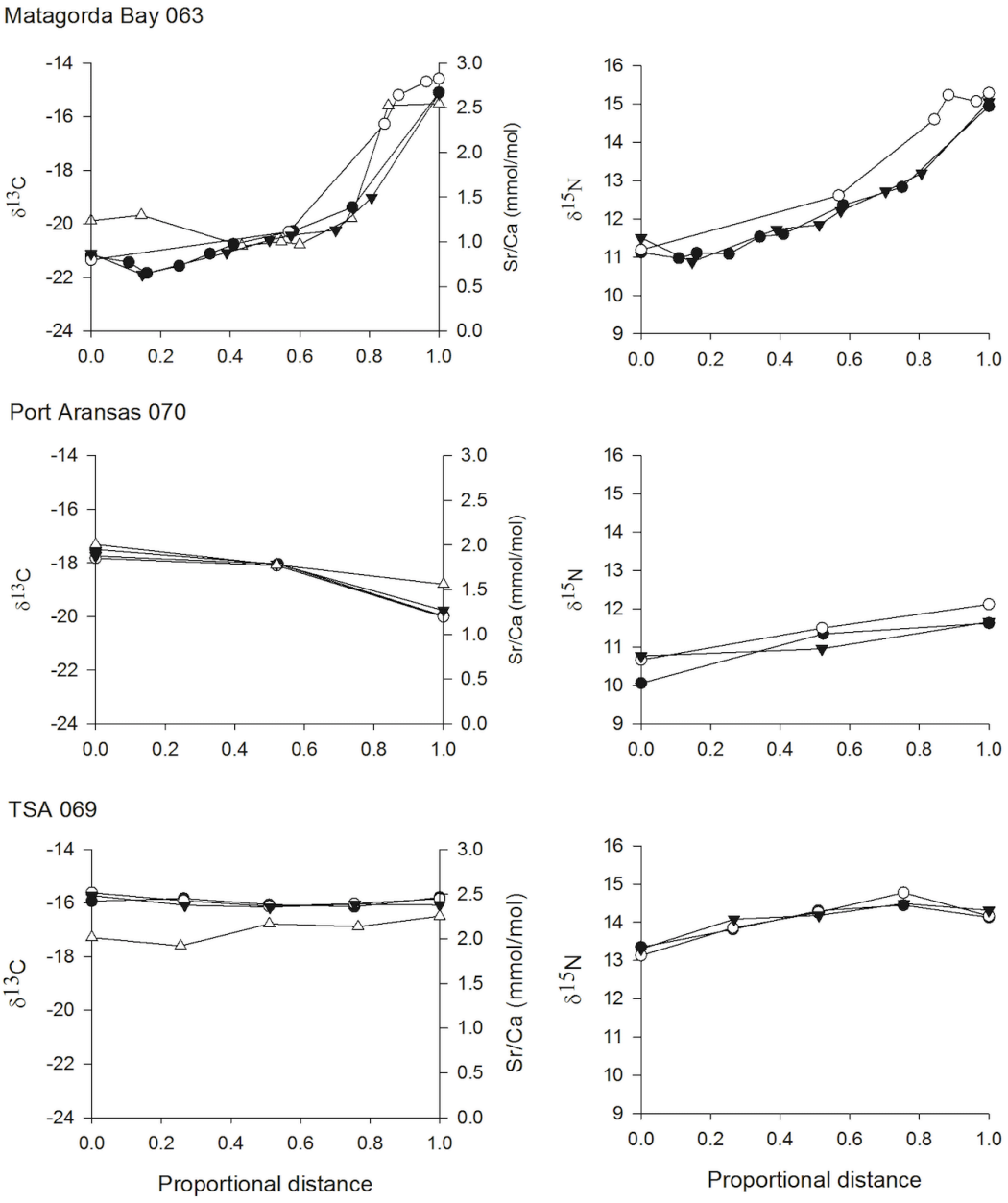

Figure 2.4. Representative $\delta^{13}C$ transects for three scales (closed circles are scale A, open circles are scale B, and closed triangles are scale C) and Sr/Ca (mmol/mol) of one representative scale (open triangles) from the same individual, spanning all sampling locations (left). Representative $\delta^{15}N$ transects of three scales from the same individual, spanning all sampling locations (right).

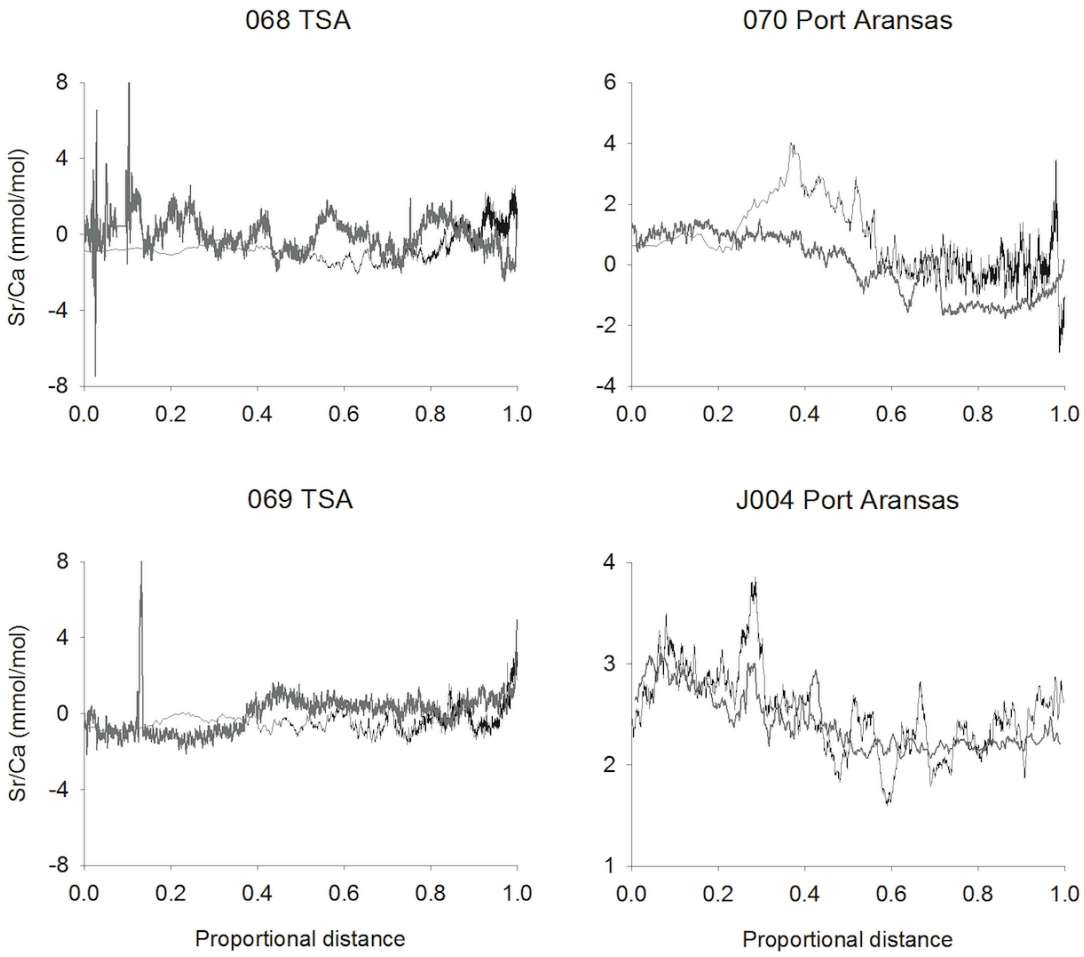

Figure 2.5. Scale (grey) and otolith (black) Sr/Ca (mmol/mol) transects from the same individual. Samples 068, 070, and 069 are mature adults with log-transformed distance transects for otoliths and linear transects for scales. Sample J004 is a juvenile with linear transects for both structures. All transects are shown on a proportional distance.

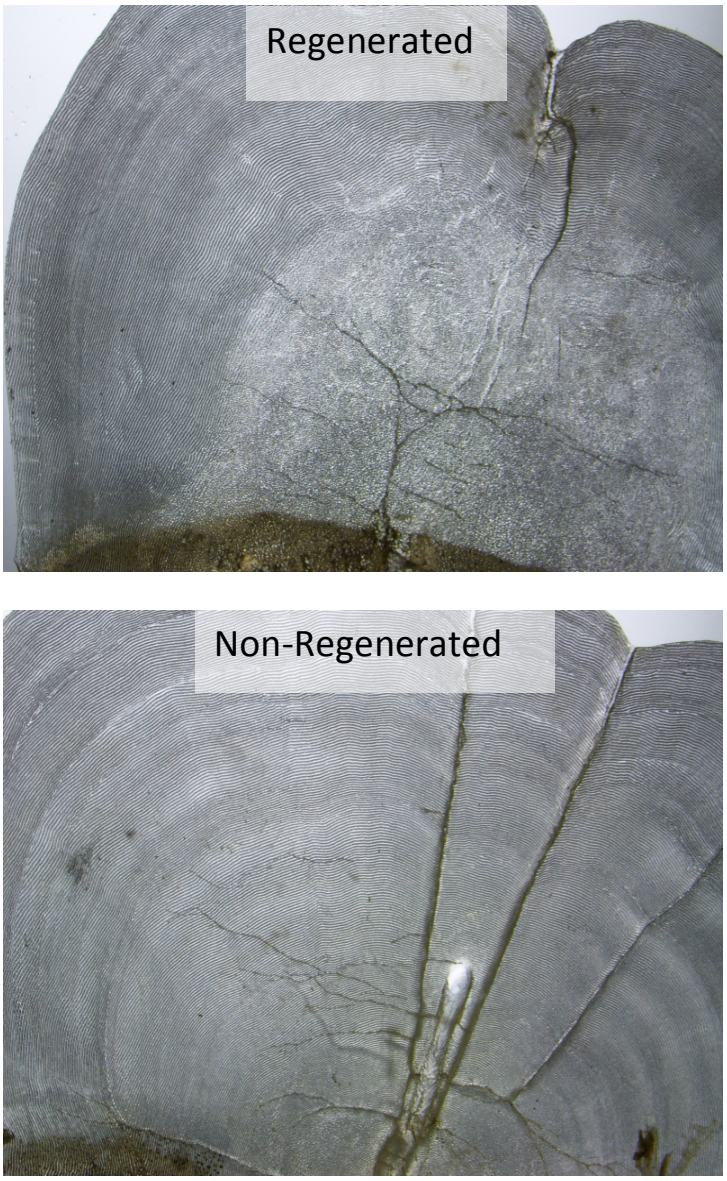

Figure 2.6. Microscope image of a regenerated (top) and non-regenerated scale (bottom)

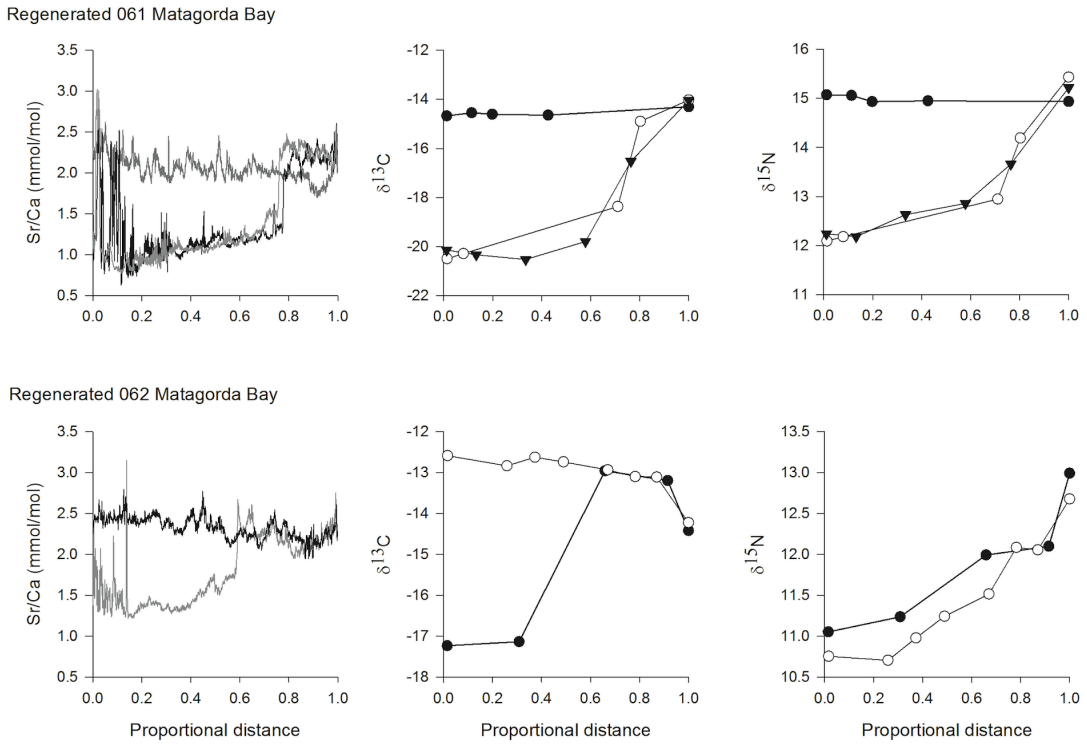

Figure 2.7. Sr/Ca (mmol/mol), $\delta^{13}C$, $\delta^{15}N$ transects of multiple scales from the same individual showing scale regeneration. Dark grey transects represent scale A, black transects represent scale B, light grey transects represent scale C. For the top three graphs, closed circles represent scale A, open circles represent scale B, triangles represent scale C. For the bottom three graphs, open circles represent scale B and closed circles represent scale C.

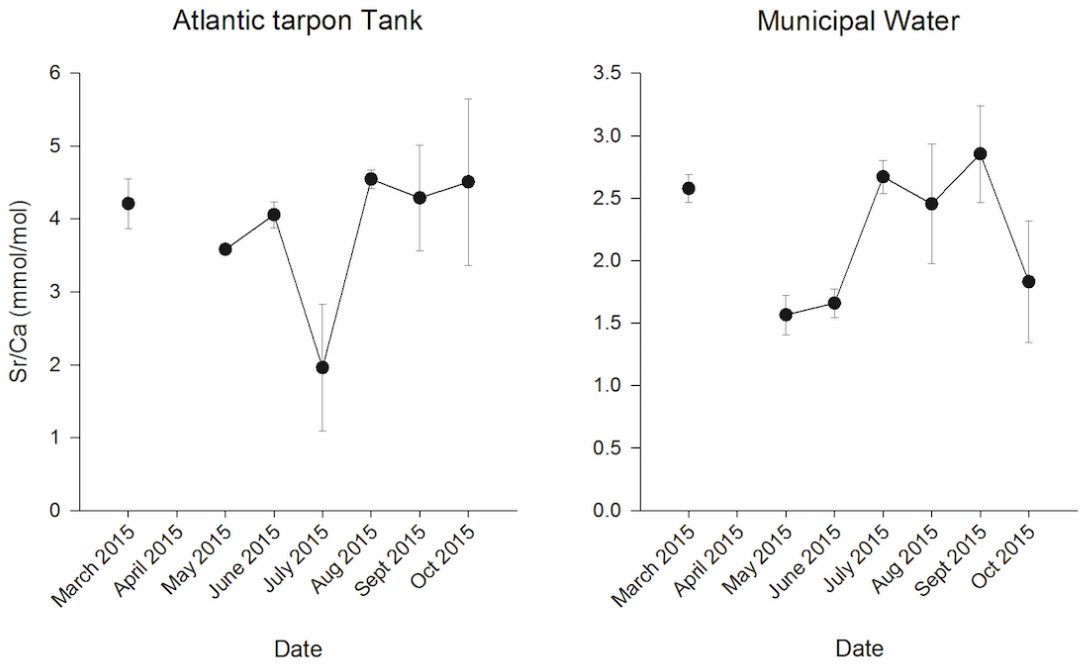

Figure 2.8. Sr/Ca in water samples collected from the Texas State Aquarium tank (left) and wet lab to represent municipal water (right) over 8 months from March-October, excluding April.

CHAPTER 3: Oligohaline habitat use and trophic structure in Atlantic tarpon, inferred from scale chemistry

INTRODUCTION

Understanding habitat use by highly migratory fishes is essential for effective management and conservation (Hobson, 1999). Yet, use of multiple habitats throughout life makes tracking certain species more difficult. Atlantic tarpon (*Megalops atlanticus*) are highly migratory euryhaline predators which inhabit subtropical to tropical waters throughout the Eastern and Western Atlantic Ocean (Wade, 1962). Their migratory habit and ability to tolerate a wide range of salinities provides opportunities to occupy widely separated and diverse habitats (Rickards, 1968). Questions still remain about the timing and duration of migrations and the frequency of movements across salinity gradients into chemically distinct habitats.

Atlantic tarpon spawn offshore or in inshore areas where currents can transport eggs offshore (Wade, 1962). Fertilized eggs hatch into leptocephalus larvae that grow to 18-30 mm in length before metamorphosis. At this stage leptocephalus prey upon phytoplankton and small species of zooplankton and swim toward near shore habitats where they eventually metamorphosis into a juvenile (Rickards, 1968; Crabtree et al., 1995). Near shore habitats for juveniles consist of mangrove assemblages, marshes, rivers, and bays where they prey upon copepods, aquatic insects, and small fish (Crabtree et al. 1995; Harrington, 1958; Wade, 1962). These fish most often remain in near shore nursery habitats that range from fresh water to fully marine salinities, until they become reproductively mature at a fork length of 120 cm and approximately 18.2 kg (Ault & Luo, 2013; Crabtree et al., 1997). In these coastal environments Atlantic tarpon of all sizes

prey upon striped mullet (*Mugil cephalus*), silversides (*Atherinidae*), marine catfish (*Bagre marinus*), ladyfish (*Elops saurus*), ribbonfish (*Trichiurus lepturus*), and crustaceans (Jud et al., 2011; Luo & Ault, 2012; Wade, 1962). Once mature, most Atlantic tarpon make offshore migrations to spawn from May through August. Reproductively mature Atlantic tarpon after spawning, display variability in habitats and salinity selection (Crabtree, 1995).

Luo et al. (2008) noted that fully mature Atlantic tarpon migrate into oligohaline waters or remained resident in coastal marine waters. Life histories of Atlantic tarpon may become more clear by recognizing contingents: groups within populations that exhibit different patterns of habitat use and movement across salinity gradients (Secor, 1999). Each contingent may be unique in its migratory behavior, site fidelity, timing, and duration of habitat use (Kerr & Secor, 2010). Identifying contingents among individual populations has been shown to enhance an understanding of fish migration, stock assessments, and conservation (Chapman et al., 2012; Nims & Walther, 2014; Walther & Limburg, 2012).

Identifying contingents can be done via tagging populations of fishes. This is important when it comes to managing highly migratory fish that use different habitats over their life history for variable durations. Management plans may be able to focus on specific contingents when developing regulations. Luo & Ault (2012) used pop-up archival transmitting (PAT) tags to monitor vertical depth and thermal habitat utilization of Atlantic tarpon. Guindon et al. (2015) used DNA fingerprinting to track individual Atlantic tarpon movement and recapture off the Florida coast. Other techniques that

could potentially be applied to Atlantic tarpon are dart tags, acoustic telemetry, mark recapture, isotopic tissue analysis, and natural chemical tags (Elsdon et al., 2008; Guindon et al., 2015; Meyer et al., 2010; Trueman et al., 2012)

Of the aforementioned techniques, only natural chemical tags can provide information on almost the entire life history of wild fish (Elsdon et al., 2008; Trueman et al., 2012). Specifically, otolith geochemistry is commonly used to monitor fish migration across salinity gradients (Campana, 1999). Otolith geochemical analyses are conducted by quantifying chemical constituents in the water, such as Sr/Ca, that have been incorporated into otolith circuli during accretion. Sr/Ca concentrations vary across salinity gradients, with concentrations typically higher in marine and lower in oligohaline environments (Walther & Nims, 2015). Unfortunately, otolith extraction is a lethal process and should not be conducted on Atlantic tarpon because they are currently listed as a vulnerable species (IUCN 2000).

Unlike otoliths, scales begin to develop once juveniles reach 30-40 mm standard length and approximately 2-4 months old. Scales that have not been removed or shed grow for the entire life of the fish, exceeding 6 cm in diameter if located near the lateral line beneath the dorsal fin (Harrington, 1958). Scales share many properties with otoliths that allow them to be used as a nonlethal analog. Both structures have incremental growth and incorporate chemical constituents from the surrounding environment (Holá et al., 2009; Wells et al. 2000). Unlike otoliths, scales are a bipartite structure composed of an external layer underlain by a basal plate. The external layer is well calcified and can be assayed for inorganic chemicals (e.g., Sr/Ca), while the basal plate is a hydroxyapatite

matrix that can be assayed for organic constituents (e.g., $\delta^{13}C$ and $\delta^{15}N$) (Seeley et al. 2015). Sr/Ca and $\delta^{13}C$ are complementary proxies for salinity since they vary similarly across salinity gradients, and sampling of $\delta^{13}C$ may further distinguish between coastal estuarine and offshore systems where Sr/Ca may be less informative. Sr/Ca ratios increase from low to high salinity in most environments (Walther & Limburg, 2012), while dissolved inorganic carbon (DIC) increases across most salinity gradients from estuarine to offshore environments (Fry, 2002).

Additionally, trophic structure can be interpreted from $\delta^{15}N$ values due to an approximate 3‰ enrichment with each trophic level (Peterson & Fry, 1987). Sequential analysis of subsamples taken across scale increments can reveal changes in trophic position (Vander Zanden & Rasmussen, 2001). An increase in trophic level is expected as these fish increase in size due to shifts in diet from small invertebrates to larger fish (Woodcock & Walther, 2014). Shifts in tissue $\delta^{15}N$ may also be associated with movements across salinity gradients that are not dictated solely by ontogenetic trophic increases. For example, if a fish residing in marine waters consumes mid-level trophic position prey with high $\delta^{15}N$ values and subsequently migrates into brackish water, the only available prey may be lower trophic level crustaceans and small fishes. If this is the case, older migratory fish may exhibit a decrease in $\delta^{15}N$ values coinciding with their movement from high to low salinity due to altered prey availability in new habitats. In addition, researchers need to consider whether the individuals actually shifted their diet to lower trophic level prey or to prey at the same trophic level that have a different $\delta^{15}N$ value due to salinity gradients in baseline isotope signatures. Bishop (2012) showed that

particulate organic matter (POM) $\delta^{15}N$ shifts from approximately 11 to 0 ‰ as salinity increases from 0 to 35 psu in the Mission and Aransas Rivers to the Bays. In this case, fish that migrate from marine to fresh water habitats that remain feeding at the same trophic level could exhibit increases in tissue $\delta^{15}N$ values due to an elevated isotope baseline rather than ontogenetic trophic increases. These phenomena may occur in combination, adding complexity for interpretations of tissue $\delta^{15}N$ values with respect to trophic position of migratory species

Underplating is a common phenomenon in scale growth that needs to be considered when interpreting shifts in scale chemistry. Underplating occurs as collagen is added to the bottom portion of the basal plate furthest form the external layer. The degree of underplating can range from minimal to severe, depending on the age of the fish and the size of the scale (Hutchinson and Trueman, 2006). This means earlier portions of life history interpreted from isotope ratios in organic material (e.g. $\delta^{13}C$ and $\delta^{15}N$) may be biased by more recently accreted material. Therefore, shifts in isotopic values between samples within a scale likely underestimate the actual ontogenetic changes experienced by the fish. Fortunately, the surface external layer of scales grows by accretion of inorganic material on the outer edge and has minimal overlap with earlier points in time (Trueman and Moore, 2007). This allows for unbiased quantification of life-history changes in elemental proxies.

Using scales as a nonlethal alternative to otoliths can identify patterns of habitat use and trophic structure. Current research is focusing on identifying patterns of movement (contingents) into oligohaline waters. The purposes of this study are to: (1)

identify contingents and oligohaline habitat use by Atlantic tarpon using elemental and isotopic proxies and (2) assess whether movements across salinity gradients are accompanied by shifts in trophic position. Together, these analyses provide novel insight into Atlantic tarpon migratory behavior and trophic structure that will inform conservation efforts.

METHODS

Scale Collection and Preparation

Atlantic tarpon scales were opportunistically collected along the Texas coast during fishing tournaments and donated by local anglers. Scales were placed in water resistant envelopes on which estimated length, weight, and capture location were recorded. From 2013-2015, scales were collected from 31 individual Atlantic tarpon from three different locations. Collections occurred in Port Aransas (n = 7, with 2 collected in 2013, 4 in 2014, and 1 in 2015), Matagorda Bay (n = 22, with 9 in 2013 and 13 in 2014), and the Texas State Aquarium (TSA) (n = 2, with 2 collected in 2015). TSA fish were sampled as non-migratory controls. One Atlantic tarpon collected from the TSA had been in captivity for the majority of its life (5-10 years), while the other was caught offshore and kept in captivity for the last 10-15 years of its life. Fish ranged from 0.91-90.72 kg in weight. Individuals were considered sexually immature when < 18.2 kg (n = 11) and sexually mature at > 18.2 kg (n = 18).

Scale preparation for elemental analysis followed the methods outlined by Woodcock and Walther (2014) and Seeley et al. (2015) and are the same as those

presented in Chapter 2 of this study. Briefly, scales were cut, sectioned, polished, and mounted to a microscope slide to create a core to edge transect for laser ablation.

Stable isotope preparation was conducted after elemental analysis was complete to allow for the confirmation of identified movements via elemental analysis with stable isotopes. A subsample transect was created by cutting 2-10 subsamples between 0.5-2 mg from the core to the edge. Subsamples were always removed from the core and the edge, but the number of intermediate samples depended on the size of the scale and the amount of measured variability that occurred during the elemental analysis.

Trace Element (Sr/Ca) Analysis

Sampling was conducted in Spring 2015 and Fall 2015 at the University of Texas Jackson School of Geosciences. Elements (^{43}Ca and ^{88}Sr) were analyzed using LA-ICP-MS. The ICP-MS worked in conjunction with a New Wave Research UP193-FX fast excimer (193 nm wavelength, 4–6 ns pulse width) laser system coupled to an Agilent 7500ce ICP-MS (Seeley et al. 2015). Preablation was conducted on all samples prior to remove any potential surface contaminants. Ablation occurred from the core to edge within the external layer following the methods outlined by Seeley et al. (2015).

Laser ablation parameters were 25 μm s^{-1} line traverses using a 25-μm spot, 30% power, and 10-Hz repetition rate. Laser energy densities (fluences) obtained for the analytical session ranged from 2.5-3.25 J cm^{-2}. Certified reference materials, NIST 612, NIST 610, and MAPS4 bracketed scale runs, while ^{43}Ca was used as an internal standard. In Spring 2015, the percent residual standard deviation for scale analyses for the element of interest, Sr, was Sr = 2.13 and 2.32 for NIST 612 and 610 against MAPS4,

respectively. In Fall 2015, the percent residual standard deviation for scale analyses was Sr = 4.51 and 6.67 for NIST 612 and 610 against MAPS4, respectively.

Occasionally, high magnitude points were observed on specific transects. These points were due to analytical error as the laser spot drifted out of the external layer and into the epoxy or as the laser ablated across a crack in the scale. Data interpolation was conducted when high frequency points were observed.

Stable Isotope ($\delta^{13}C$ and $\delta^{15}N$) Analysis

Analysis of $\delta^{13}C$ and $\delta^{15}N$ values from scale subsamples was conducted in Spring 2015 and Fall 2015. Each sample was encapsulated in tin and analyzed at University of California, Davis, Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). In Spring 2015, samples were run against four reference materials (Nylon: $\delta^{15}N = -10.31$, $\delta^{13}C = -27.72$; Bovine liver: $\delta^{15}N = 7.72$, $\delta^{13}C = -21.69$; Glutamic acid (USGS-41): $\delta^{15}N = 47.6$, $\delta^{13}C = 37.63$; and Glutamic acid: N = 9.52%, $\delta^{15}N = -6.8$, C = 40.81%, $\delta^{13}C = -16.65$). In Fall 2015, samples were run against four reference materials (Nylon: $\delta^{15}N = -10.31$, $\delta^{13}C = -27.72$; Bovine liver: $\delta^{15}N = 7.72$, $\delta^{13}C = -21.69$; Glutamic acid: N = 9.52%, $\delta^{15}N = -6.8$, C = 40.81%, $\delta^{13}C = -16.65$; and Enriched Alanine: $\delta^{15}N = 41.13$, $\delta^{13}C = 43.02$).

Water Analysis, Uptake Validation, and Thresholds

The proportion of dissolved ambient element concentrations in water that are taken up and incorporated into scales is defined by the partition coefficient *D*, calculated

here as $D_{Sr/Ca} = [(Sr/Ca)_{scale}] / [(Sr/Ca)_{water}]$. This $D_{Sr/Ca}$ is then used to estimate expected scale Sr/Ca values in oligohaline waters given known measured ambient Sr/Ca values. To calculate this $D_{Sr/Ca}$, the exterior portion (last 1000 μm) of Sr/Ca transects in scales from the 22 fish captured in marine environments was averaged to represent expected scale Sr/Ca values in marine habitats and parameterize the numerator of the $D_{Sr/Ca}$ equation. Scales from fish captured in estuarine habitats or that contained a major shift in Sr/Ca values within the exterior 1000 μm of their transects were excluded from this calculation. The denominator of the $D_{Sr/Ca}$ equation was parameterized with the globally homogeneous Sr/Ca value of 8.54 mmol/mol (De Villiers, 1999). This value is stable in marine waters worldwide due to the large reservoir and long residence time (2-5 million years) of Sr in the ocean (Walther & Limburg 2012), and therefore a robust value to use as a marine endmember.

Mean values of Sr/Ca in the oligohaline (0-5 psu) portions of coastal streams and rivers in the Texas coastal bend region were analyzed by Walther and Nims (2015) and used here to develop a threshold for expected scale Sr/Ca values for Atlantic tarpon inhabiting oligohaline waters. Water Sr/Ca values reported by Walther & Nims (2015) were averaged, excluding reported values from water samples with >5 psu. This average oligohaline endmember value was multiplied by the $D_{Sr/Ca}$ obtained above to calculate the expected scale Sr/Ca threshold value below which oligohaline residence was indicated. In order to assess the robustness of this threshold to variations in the oligohaline endmember, alternative thresholds were calculated using the average plus 1, 1.5 and 2 times the standard deviation of oligohaline Sr/Ca values reported by Walther & Nims

(2015).

Statistics

Least squares linear regressions were performed between $\delta^{15}N$ and $\delta^{13}C$, $\delta^{13}C$ and Sr/Ca, and $\delta^{15}N$ and Sr/Ca, to determine whether there were significant relationships between the chemical tracers. Slopes for a representative group of random fish within the larger sample that had similar ranges of proxy values, which were 0.42 for $\delta^{15}C$ vs. $\delta^{13}N$, 4.38 for $\delta^{13}C$ vs. Sr/Ca, and 0.87 for $\delta^{15}N$ vs. Sr/Ca had similar values to the pooled slopes (0.31, 3.70, and 1.46, respectively) and thus, justified pooling. When identifying the proportion of time spent above or below a salinity or trophic threshold, immature and mature Atlantic tarpon were analyzed separately and in aggregate. Significant shifts in elemental values were identified by a regime-shift detection algorithm (Rodionov, 2004) followed by a sequential t-test. The algorithm was set with a 0.05 significance level, a cut-off length of 1000 cells or approximately 5000 μm, and a Huber's weight parameter of 1. When more than one adjacent point differed significantly based on the variance and predetermined significance level (P), a shift was identified and a new moving average was applied until the next shift occurred. Once the algorithm identified all shifts in a transect, mean values between shifts (regimes) were calculated (Turner & Limburg, 2012). Each regime value was then compared to the calculated oligohaline thresholds to determine whether the regime was within oligohaline waters (at or below the threshold) or in meso-polyhaline waters (above the threshold). For the purposes of this chapter, meso-polyhaline water encompasses any salinity ranging from > 5-30 psu regardless of being near shore, coastal, or offshore. The proportion of each transect spent above or

below the threshold as well as the number of shifts across the threshold was then calculated for each scale. These calculations were made separately using the average oligohaline threshold in addition to the average plus or minus 1, 1.5, or 2 standard deviations of water endmember values. Distributions of the individual shifts and transect proportions spent in oligohaline waters obtained based on the seven different thresholds were compared to determine sensitivity of results to threshold choice.

We then quantified $\delta^{13}C$ and $\delta^{15}N$ total ranges in each transect from the core to the edge, as well as the difference between the edge and core values. Comparisons were made between the shifts in both isotopes for each value to identify whether temporal shifts occurred in midlife, early life, or later life. This comparison helped determine whether the majority of movements made by individuals occurred once they reached sexual maturity (early-midlife).

RESULTS

Proxies, Partition Coefficients, and Thresholds

There were significant positive linear relationships ($P < 0.001$) for $\delta^{15}N$ vs. $\delta^{13}C$, $\delta^{13}C$ vs. Sr/Ca, and $\delta^{15}N$ vs. Sr/Ca (Figure 3.1). The calculated Sr/Ca partition coefficient for all wild scales was $D_{Sr} = 0.27$. This value was then applied to the mean oligohaline water Sr/Ca concentrations of 5.23 ± 1.23 mmol/mol measured by Walther and Nims (2015) to obtain an oligohaline threshold and limits at different tolerance levels (Table 3.1).

Contingents

When all wild fish were averaged, 42 ± 34% of scale transects was below the oligohaline threshold. The portion was 30 ± 26% for mature fish and 63 ± 38% for immature fish. For a threshold of minus 1 SD, pooled fish had 27 ± 34%, mature was 15± 21%, and immature was 46± 43% below the oligohaline threshold. For a threshold of minus 1.5 SD, pooled fish had 17 ± 31%, mature was 6 ± 15%, and immature was 36± 43% below the oligohaline threshold. For a threshold of minus 2 SD, pooled fish had 7 ± 22%, mature was 0 ± 0%, and immature was 19 ± 34% below the oligohaline threshold. Only the mean and lower error bound are reported here to identify lower Sr/Ca limits since a higher SD approaches known marine Sr/Ca values in scales similar to where fish were captured. Overall, much of the variability in the percentage of transect below the oligohaline threshold appears to be associated with sexual maturity, as immature fish are spending a longer percentage of their lives in oligohaline waters (Figure 3.2).

Within all wild fish transects four general contingents were present based on the presence and sequence of movement into oligohaline waters. Fish were classified as non-migrant marine (NM), non-migrant oligohaline (NO), migrant oligohaline-marine (OM), or migrant marine-oligohaline-marine (MOM) depending on the number and sequence of significant shifts across the threshold or consistency above/below the threshold (Figure 3.3). The timing of these movements did not affect the characterization of each migration pattern, so fish were classified in similar contingents regardless of whether a migratory shift occurred early or late in the transect provided the sequence was the same. Eight fish were classified as NM, four were NO, twelve were OM, and five were MOM. The non-

migratory control TSA fish were characterized as NM, as expected given their long-term residence in marine aquaria (Figure 3.4). The distribution of transects below the oligohaline threshold characterized using the mean, mean plus 1 SD, and mean minus 1 SD threshold is presented in Figure 3.5. Sixteen of 17 fish made their first significant movement across a threshold in the first 75% of their scale life histories, while only one fish had a significant shift in the last 25% (Figure 3.6). When fish shifted into oligohaline waters across the elemental threshold, the lowest recorded $\delta^{15}N$ value most often coincided within one subsample of the greatest $\delta^{13}C$ value. The exception to this pattern was a fish (070) from Port Aransas that displayed movement from high to low salinity, but not significantly across the oligohaline threshold. That fish did not have the lowest $\delta^{15}N$ value within one subsample of the greatest $\delta^{13}C$ value.

Temporal shifts

Total range (maximum minus minimum subsample per transect for $\delta^{13}C$ and $\delta^{15}N$) values were most often different than edge minus core (edge subsample minus core subsample for $\delta^{13}C$ and $\delta^{15}N$ per transect) values. Excluding the TSA fish, 20 of 29 for $\delta^{13}C$ and 18 of 29 for $\delta^{15}N$ had different total ranges than edge minus core values.

An overall mean increase was observed for total range and edge minus core values for both $\delta^{13}C$ and $\delta^{15}N$. Differences were observed in both size classes (Figure 3.7). The majority of the intermediate scale transect excursions in $\delta^{15}N$ that are greater than the edge minus core comparison are present in the mature fish (Figure 3.8).

TSA scale transects were consistently flat above the oligohaline threshold. Both the Sr/Ca and $\delta^{13}C$ values were marine-type across the entire transects. The total range in

the $\delta^{13}C$ transect was 1.30‰ and $\delta^{15}N$ was 1.27‰. The edge minus core increase in the $\delta^{13}C$ transect was -0.99‰ and $\delta^{15}N$ was 0.77‰.

DISCUSSION

Scales have been used in a variety of studies to quantify elemental and isotopic signatures to track fishes, measure uptake dynamics, and compare structures (Seeley et al., 2015; Trueman et al., 2012). These studies demonstrate the efficacy of scales as a nonlethal alternative structure to otoliths. To enhance the use of scales to track fish life histories, partition coefficients need to be calculated to estimate the rate of elemental uptake from the water into scales. Fortunately, deviations from the generally accepted marine Sr/Ca value of 8.5 mmol/mol in water rarely occur (De Villiers, 1999). This constant marine endmember allows for quantification of uptake ratios of Sr/Ca in most marine environments. Sr/Ca values vary significantly across salinity gradients. The sampled water chemistry for Sr/Ca from 2010 and 2011 by Walther and Nims (2015) ranged from 3.02-7.51 mmol/mol. This wide range was due to the underlying geology, long residence time of Sr, and limited sources of Sr/Ca in terrestrial environments. When interpreting Sr/Ca concentrations in scales, it must be understood that it is likely not feasible to identify differences between estuarine nursery habitats and the marine environment. This is particularly important for migrating fishes along the southern Texas coast because of the presence of hypersaline estuaries. Therefore, the oligohaline water data with significantly lower Sr/Ca values (Walther and Nims 2015) were used to create an oligohaline threshold that could be distinguished from meso-polyhaine waters. Unfortunately, if the Atlantic tarpon examined here resided in estuarine nursery habitats

having salinities > 5 psu, our elemental technique will not distinguish estuarine from fully marine habitats. Sampling of $\delta^{13}C$ values, which increase from coastal to offshore environments (Fry, 2002), was used to help distinguish between these two systems. Therefore, the positive linear relationship between $\delta^{13}C$ and Sr/Ca suggests that both constituents can be used as salinity tracers. However, $\delta^{13}C$ values may not be as strongly correlated with Sr/Ca in high salinity estuarine environments.

Using the generally accepted marine Sr/Ca endmember value and the oligohaline water sampling data from Walther and Nims (2015), we obtained a threshold value to categorize movement with respect to salinity. To accurately determine how much natural variability in the threshold would be realistic, predicted thresholds must be compared to corresponding water Sr/Ca values observed in likely oligohaline habitats near the Gulf of Mexico. The water chemistry encompassing the upper and lower limits 1 SD of the mean identified by Walther and Nims (2015) were within the range of all measured water bodies. The values were exceeded on both ends of the standard deviation by two samples. At mean plus 1.5 standard deviations the water threshold excluded the two highest Sr/Ca values, the North Floodway and the Arroyo Colorado. At mean minus 1.5 standard deviations the threshold excluded the lowest Sr/Ca value, the Aransas River in 2010. At mean plus and minus 2 standard deviations, all sampling sites were included and resulted in Sr/Ca values that were not realistic for the local riverine systems. Water Sr/Ca samples analyzed by Brown and Severin (2009) for the Mississippi River were identified at approximately 2 mmol/mol, which is lower than all samples Walther and Nims (2015) used to create the threshold. This value, which was calculated from 252 samples,

provides a robust representation of Gulf of Mexico Sr/Ca as the Mississippi River Watershed spans a large portion of the continental United States. Since this value is lower than that of the sampled sites in Texas, the mean and lower limits of 1 SD provide a more realistic estimation of Sr/Ca thresholds for the Gulf of Mexico. In addition, any increases to the threshold can be compared to the Sr/Ca values from the edge of scales taken from fish captured in marine waters to identify when the threshold no longer accurately distinguishes marine-captured fish.

For scales, as the 1.43 threshold was increased by 1 standard deviation (0.34 Sr/Ca (mmol/mol)) all edge values were above the threshold, meaning they were still accurately identified as marine-captured. At mean plus 1.5 standard deviations, only 1 fish was incorrectly identified as captured in oligohaline waters. Finally, at mean plus 2 standard deviations, 3 fish were incorrectly categorized as being captured in oligohaline waters. Given that varying the threshold beyond 1 standard deviation begins to introduce unrealistic thresholds for both the oligohaline and marine endmembers in the system, threshold variation appears to be most likely between one standard deviation of measured water values reported by Walther & Nims (2015). Thus, assessments of contingent patterns should be made considering the potential for oligohaline endmember variability of that magnitude.

Using a scale Sr/Ca threshold of 1.43 ± 0.34 (1 SD) mmol/mol allowed for the identification of one contingent within TSA fish and a variety of contingents within wild Atlantic tarpon. No migrations were evident in the TSA fish as these individuals were captured in marine waters and transferred to the TSA where they were consistently

contained in marine salinities. When salinities in the tank dropped due to external storm events, they were artificially returned to marine levels. Similarly, trophic structure remained fairly constant for both TSA fish since they were fed a constant marine-type diet for the duration of their captive lives.

We identified significant variation in oligohaline habitat use by wild fish with the threshold at the upper and lower limits of 1 SD of the mean. The number of individuals that spent 0% of their scale life histories in oligohaline waters decreased from 14 at mean minus 1 SD to 4 at mean plus 1 SD. Thus, even when the oligohaline threshold is decreased to its most conservative value (mean minus 1 SD), approximately half of the sampled wild fish (15 out of 29 individuals) moved into oligohaline waters at some point during their lives. The duration of residence in oligohaline waters varied substantially across individuals, ranging from less than 25% of the scale transect to upwards of 75% of the transect. This result indicates that oligohaline residence is a common and important occurrence in the lives of many, though not all, Atlantic tarpon. Clearly, there is considerable individual variability in Atlantic tarpon migratory behavior, particularly with respect to oligohaline habitat use. My findings are consistent with previous studies that concluded that juveniles inhabit wide ranges of salinities (Rickards, 1968), and the results presented here extend that conclusion to include variability in subsequent life history stages.

Sample sizes for wild fish were too small to separate geographic location. Therefore, temporal patterns of the sequence of oligohaline habitat use were used to classify individual contingents. It is important to note that all scale samples were from

fish captured in marine waters. This was corroborated by edge values of Sr/Ca, as no contingents migrating from meso-polyhaline environments and back to oligohaline waters were present. The few individuals that we assigned to the NO contingent most likely did not exhibit a marine signature at the edge of the scale because they were likely recent migrants to meso-polyhaline waters and did not have sufficient time to incorporate a meso-polyhaline signature in the edge of the scale. Ultimately, the classified contingents showed that oligohaline habitats were consistently being used by younger Atlantic tarpon prior to marine migrations, and were also often visited by putatively reproductively mature Atlantic tarpon. Among all scale samples, 12 individuals were assigned to the OM contingent. Of these, seven were greater than 18.2 kg and deemed sexually mature. Of the five individuals less than 18.2 kg, four of them were > 15.88 kg and may have been sexually mature out-migrating individuals even though they are slightly below the designated maturity threshold.

We observed that 16 of 17 migrating Atlantic tarpon made their first significant shift across the oligohaline threshold in the first 75% of scale transects. Smaller fish that were present in oligohaline waters exhibited a greater percentage of scale transects in these waters compared to reproductively mature individuals that were out of low salinity habitats for many years. Since scale development does not occur until these fish are approximately 30-40 mm, an initial migration as leptocephali from offshore to inshore cannot be identified. The first significant migration that we observed was most likely the shift from oligohaline to meso-polyhaline waters when these fish were nearing sexual maturity and heading offshore to spawn.

When an Atlantic tarpon inhabits oligohaline waters, $\delta^{13}C$ value should be lower, based on how $\delta^{13}C$ values are expected to shift across salinity gradients (Fry, 2002). The majority of the sampled fish were in these low salinity waters early in their scale life histories using these habitats as nursery grounds. The $\delta^{15}N$ values paired with the $\delta^{13}C$ subsamples increased as fish moved from low to high salinity. This relationship may be due to the increase in trophic level as these fish increase in size and shift diet coincident with shifts across salinity gradients. However, exceptions to the expected increase were observed in two fish from Matagorda Bay, both weighing more than 68 kg and longer than 190 cm, as MOM contingents. Initial $\delta^{13}C$ and $\delta^{15}N$ transects started higher than the average and they decreased over time, indicating a migration into low salinity and a shift in trophic position of diets or $\delta^{15}N$ baseline. The shift in the $\delta^{15}N$ baseline is unlikely, however, given that $\delta^{15}N$ of POM is higher in low salinities, which is contradictory to the observed pattern in scale $\delta^{15}N$ of these fish that moved into oligohaline waters. Eventually the paired subsamples near the edge of the scale increased to values comparable to the core of the scale. This increase indicated a migration back across a salinity gradient into marine waters and a shift in trophic level back to a marine diet. Thus, these two representative fish support the idea that $\delta^{15}N$ can be a trophic tracer since the gradient in baseline $\delta^{15}N$ is not consistent with the salinity gradient the fish crossed and the direction of $\delta^{15}N$ values changed (Bishop, 2012 and Walther & Nims, 2015). The pattern of $\delta^{13}C$ and $\delta^{15}N$ tracking each other was only observed in 2 of 5 of the fish that were in the MOM contingent. This may be due to the individuals feeding at different trophic levels in the new habitats. Prey of juvenile Atlantic tarpon had been identified in

diet and growth studies (Jud et al., 2011; Wade, 1962), yet diet studies of subadult and adult Atlantic tarpon are lacking. Therefore, shifts in $\delta^{15}N$ value across the scale life history of these fish is either Atlantic tarpon changing diet or migrating into a region with a different baseline $\delta^{15}N$ value.

Temporal increases in $\delta^{13}C$ and $\delta^{15}N$ from the core to edge were expected as fish move offshore to spawn and are assumed to shift their diets to a higher trophic level. Comparisons between the differences in total range values and edge minus core were not always consistent. This is because the total range values varied between fish likely due to individuals migrating at different times. Since these values were not consistent, we can conclude that changes in trophic level are not solely ontogenetic and that a shift in midlife, such as prey availability in low salinity or different $\delta^{15}N$ baselines may be biasing $\delta^{15}N$ values. Additionally, scale underplating within the basal plate has the potential to alter isotopic values.

The paired scale stable isotope samples need to be interpreted given the potential for scale underplating, which biases stable isotope sampling towards the most recently accreted material. Stable isotope sampling from scales for time-resolved analyses of diet or environmental conditions across discrete time periods was originally thought to be impossible due to scale underplating (Hutchinson & Trueman, 2006). Even though increments may be underestimated, the method still provides an estimate of minimum migratory and trophic shifts across life-history stages. This can be seen in the mean range of observed values from the core to the edge (i.e., $\delta^{13}C$: -19.49 to -15.97 and $\delta^{15}N$: 12.04 to 15.07) of the scale as well as the consistency between isotopic and elemental proxies

(e.,g. $\delta^{13}C$ and Sr/Ca). Atlantic tarpon scales may be an exception to interpreting stable isotope results in regard to underplating, due to their exceptionally large size. Evidence of underplating was present when comparing the core to edge values for the mature and immature individuals. The immature individuals increased in $\delta^{15}N$ values from 11.20-14.13 ‰, while $\delta^{15}N$ increased from 12.56-15.64 ‰ in mature individuals. This is a much smaller range than would be expected if underplating did not occur. Since mature individuals were much older and larger, we would expect a higher edge $\delta^{15}N$ value compared to immature individuals. The core of mature individuals may be higher due to underplating as the greater $\delta^{15}N$ value at the edge is contributing to the elevated core value.

The increase in PON $\delta^{15}N$ from oligohaline to marine waters (Bishop, 2012) supports the hypothesis that the change in $\delta^{15}N$ is actually trophic and not a baseline shift in these systems, since some fish shift $\delta^{15}N$ as they move from meso-polyhaline to oligohaline waters. $\delta^{15}N$ values of PON present in waters surrounding Atlantic tarpon collection locations were in the range of approximately 2-8 ‰ (Mooney & McClelland, 2012). The mean maximum $\delta^{15}N$ edge value quantified from mature Atlantic tarpon scales was 15.64 ‰. This is associated with an increase of approximately 3-5 trophic levels, assuming a 3 ‰ increase as the baseline, which as expected, places Atlantic tarpon near the top of the local food web (Dorado et al., 2012; Winemiller et al., 2007).

Using scale chemistry offered a nonlethal sampling approach to quantifying shifts in elemental ratios and isotopic values over the life of a fish, from scale formation to capture date. Based on previous water chemistry analyses and an understanding of how

these elements and isotopes vary across salinity gradients and through food webs in Texas waters, I identified four contingents, the percentage of life in oligohaline habitat, and shifts in trophic level. Each contingent identified (NM, NO, OM, MOM) exhibited movement patterns previously observed in Atlantic tarpon studies throughout the species range (Crabtree et al., 1995; Rickards, 1968; Skye H. Woodcock & Walther, 2014). The variety of contingents suggests that within a Texas population of Atlantic tarpon, individual variability is quite common. Residency in oligohaline habitat has previously been noted for juveniles as well as mature Atlantic tarpon (Jud et al., 2011; Luo et al., 2008); however, the timing of this transition had not yet been defined. This study concludes that oligohaline habitat use is not obligatory and that individuals show variability in the frequency and duration of their use of oligohaline waters. The consistent evidence of oligohaline habitat use from this study may indicate that there is a population of Atlantic tarpon that recruit to Texas rivers and estuaries. However, because all fish for this study were captured in marine waters, the nursery oligohaline signature present in most fish may be from known spawning populations on the West coast of Florida or Mexico that migrated along the coast to Texas. These hypotheses about migratory connectivity would need to be addressed through genetic studies, with population specific markers and could eventually be validated by sampling oligohaline sites along the Texas coast for juvenile Atlantic tarpon.

Movements into oligohaline habitat vary among individual Atlantic tarpon, yet they are not obligate migrations. Residency in oligohaline habitat until near sexual maturity may be consistent across regions and will be important in conservation efforts

that focus on protecting coastal nursery habitats. It is unclear why few individual adult Atlantic tarpon transects indicate movement back into oligohaline waters after sexual maturity. These migrations to low salinity may be due to avoiding predation from larger predators, the availability of prey, and/or a shallower/warmer environment to inhabit. This should be investigated further and validated through conventional tagging approaches (satellite tags).

CHAPTER 3 TABLES

Table 3.1. Sr/Ca (mmol/mol) thresholds for oligohaline habitat.

Oligohaline Thresholds	
Average	1.43
+1 SD	1.77
-1 SD	1.10
+1.5 SD	1.94
-1.5 SD	0.93
+2 SD	2.11
-2 SD	0.76

Table 3.2. Proportion (%) of scale transect below different threshold levels (inferred oligohaline habitat). Total (n =29), Mature (n = 18), Immature (n = 11). Results exclude TSA fish.

		Sr/Ca Threshold (mmol/mol)						
Life Stage	Mean weight (Kg)	Mean	+1 SD	-1 SD	+1.5 SD	-1.5 SD	+2 SD	-2 SD
Total	35.4	42± 34	54 ± 32	27 ± 34	65 ± 28	17 ± 32	75 ± 25	7 ± 22
Mature	50.2	30 ± 26	42 ± 26	15 ± 21	51 ± 23	6 ± 15	65 ± 24	0 ± 0
Immature	11.5	63 ± 38	73 ± 32	46 ± 43	88 ± 20	36 ± 43	93 ± 16	19 ± 34

CHAPTER 3 FIGURES

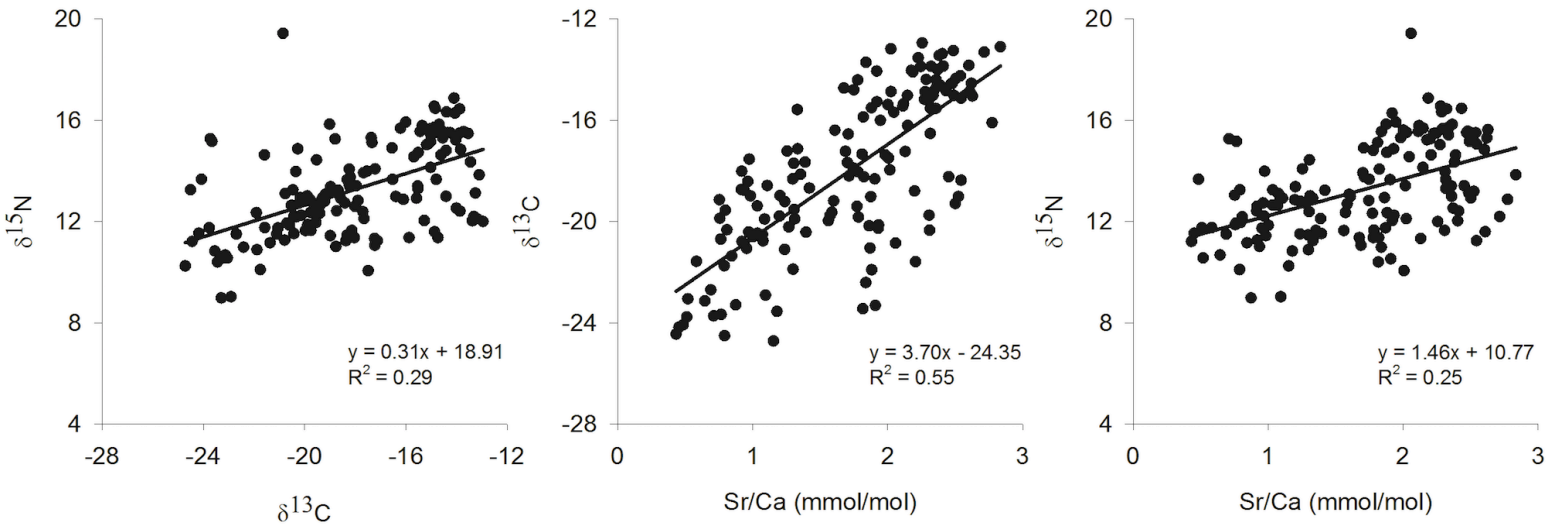

Figure 3.1. Linear regressions showing the relationships between three chemical proxies in scales for salinity conditions (Sr/Ca, $\delta^{13}C$) and trophic dynamics ($\delta^{15}N$).

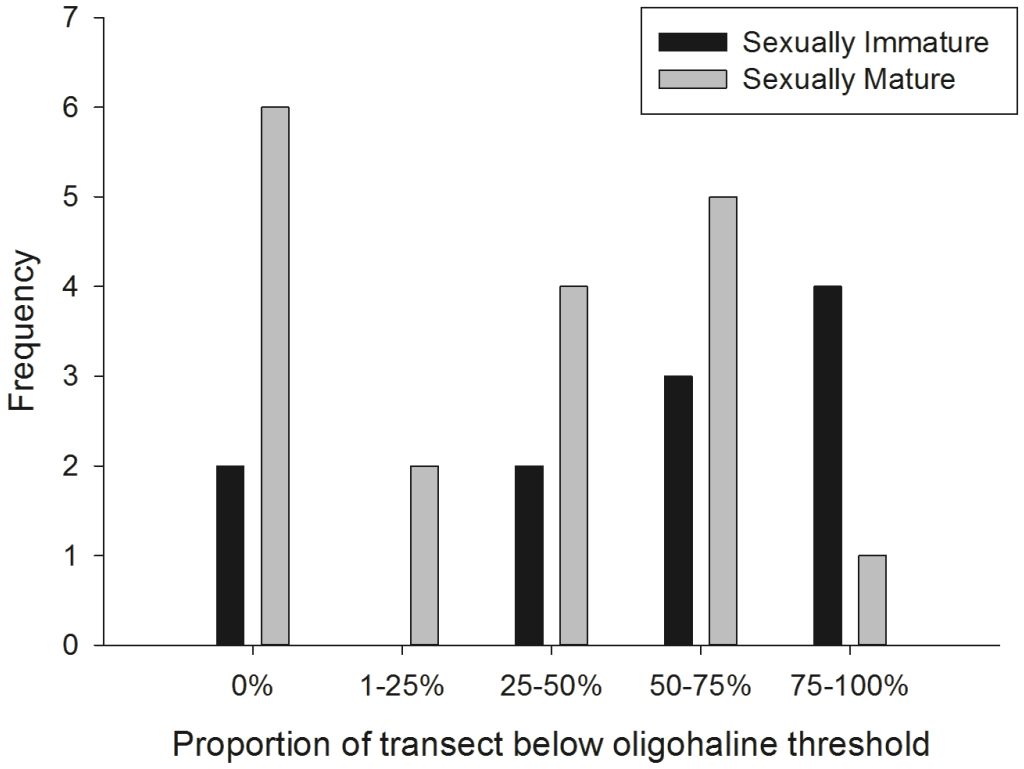

Figure 3.2. Frequency histogram based on sexual matutity grouped by the proportion of individual Sr/Ca life history below the average oligohaline threshold.

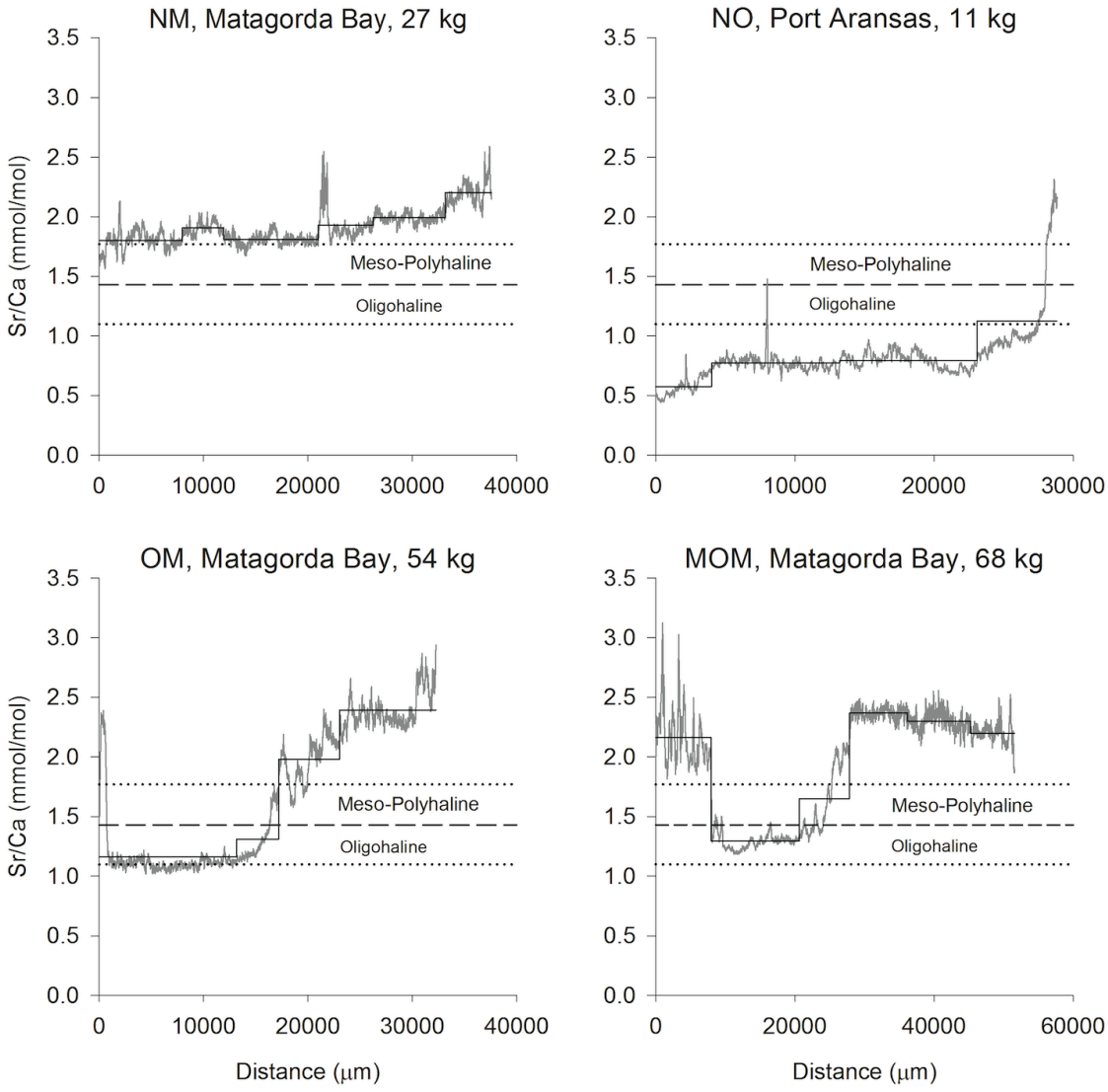

Figure 3.3. Representative Sr/Ca scale transects of four Atlantic tarpon (gray line) and Sr/Ca regime shift (black line). The dashed line represents the mean oligohaline Sr/Ca threshold and the dotted lines represent the mean threshold ± 1 SD.

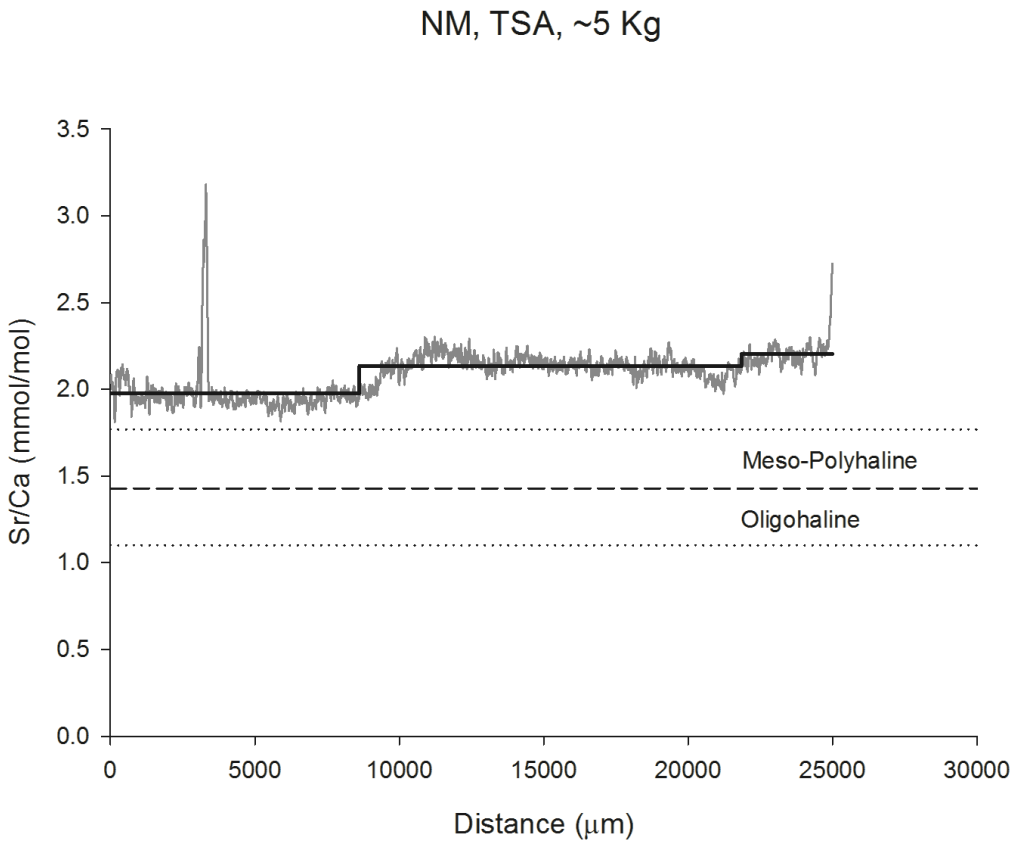

Figure 3.4. Texas State Aquarium Atlantic tarpon scale transect (gray line) and Sr/Ca regime shift (black line). The dashed line represents the mean oligohaline Sr/Ca threshold and the dotted lines represent the mean threshold ± 1 SD.

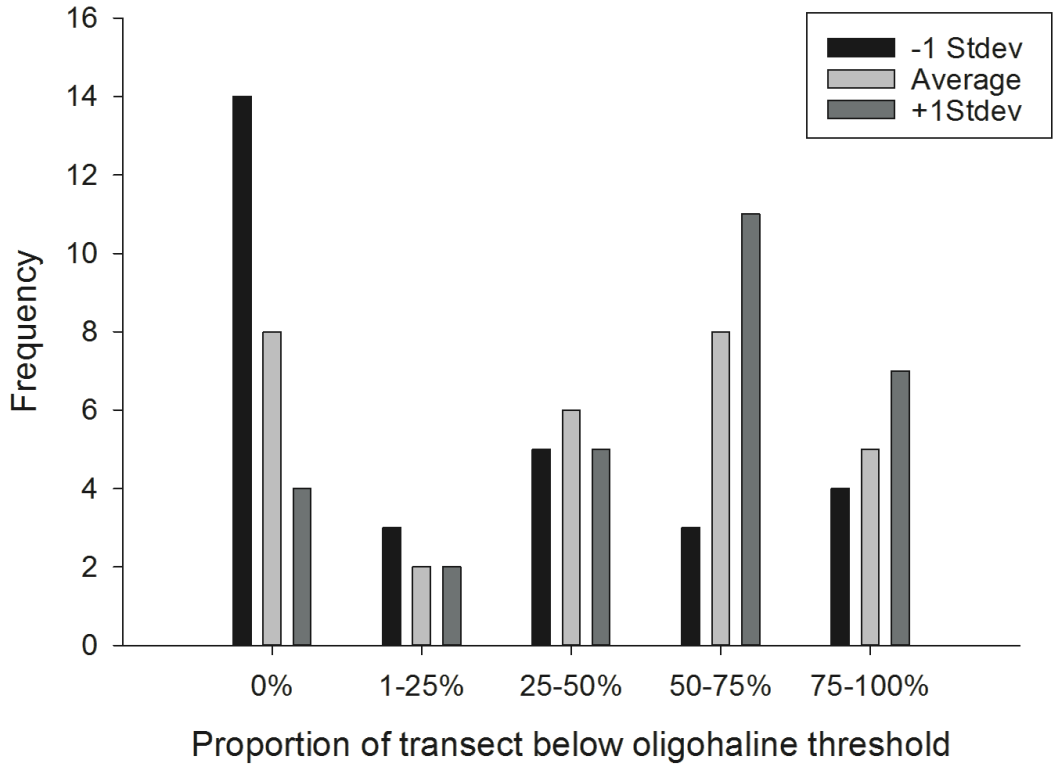

Figure 3.5. Frequency of individuals below the average oligohaline threshold and ± 1 SD grouped by the proportion of their Sr/Ca transect.

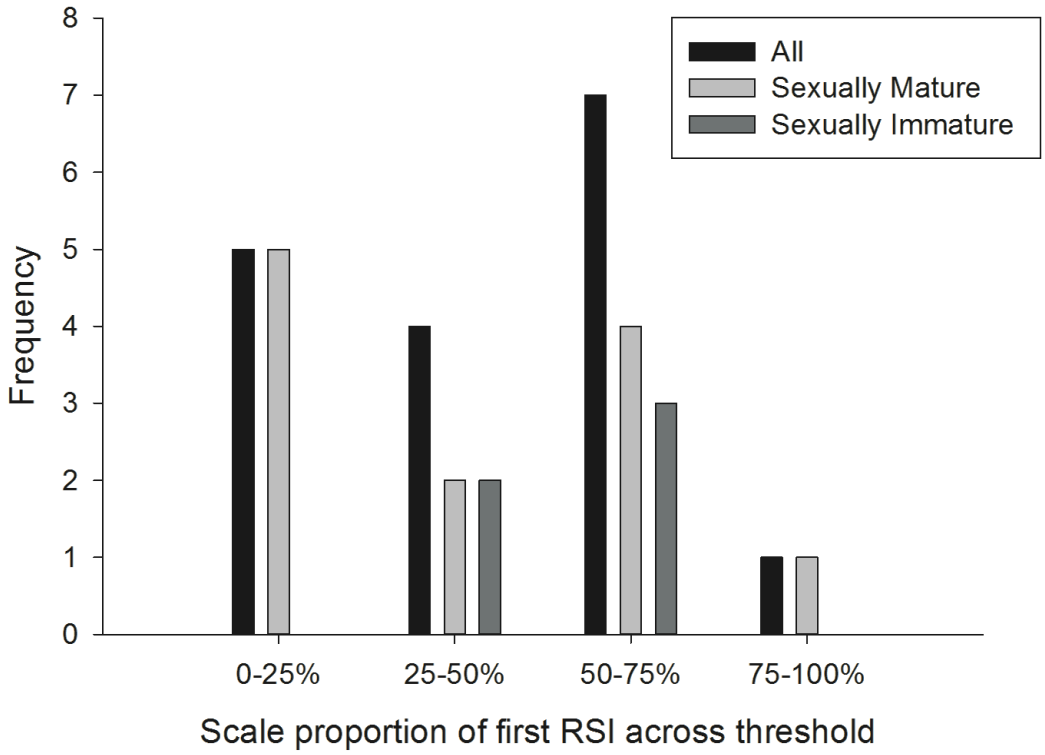

Figure 3.6. Frequency of individuals based on sexual maturity grouped by when individuals made their first significant migration across the average oligohaline threshold using the Regime Shift Index (RSI).

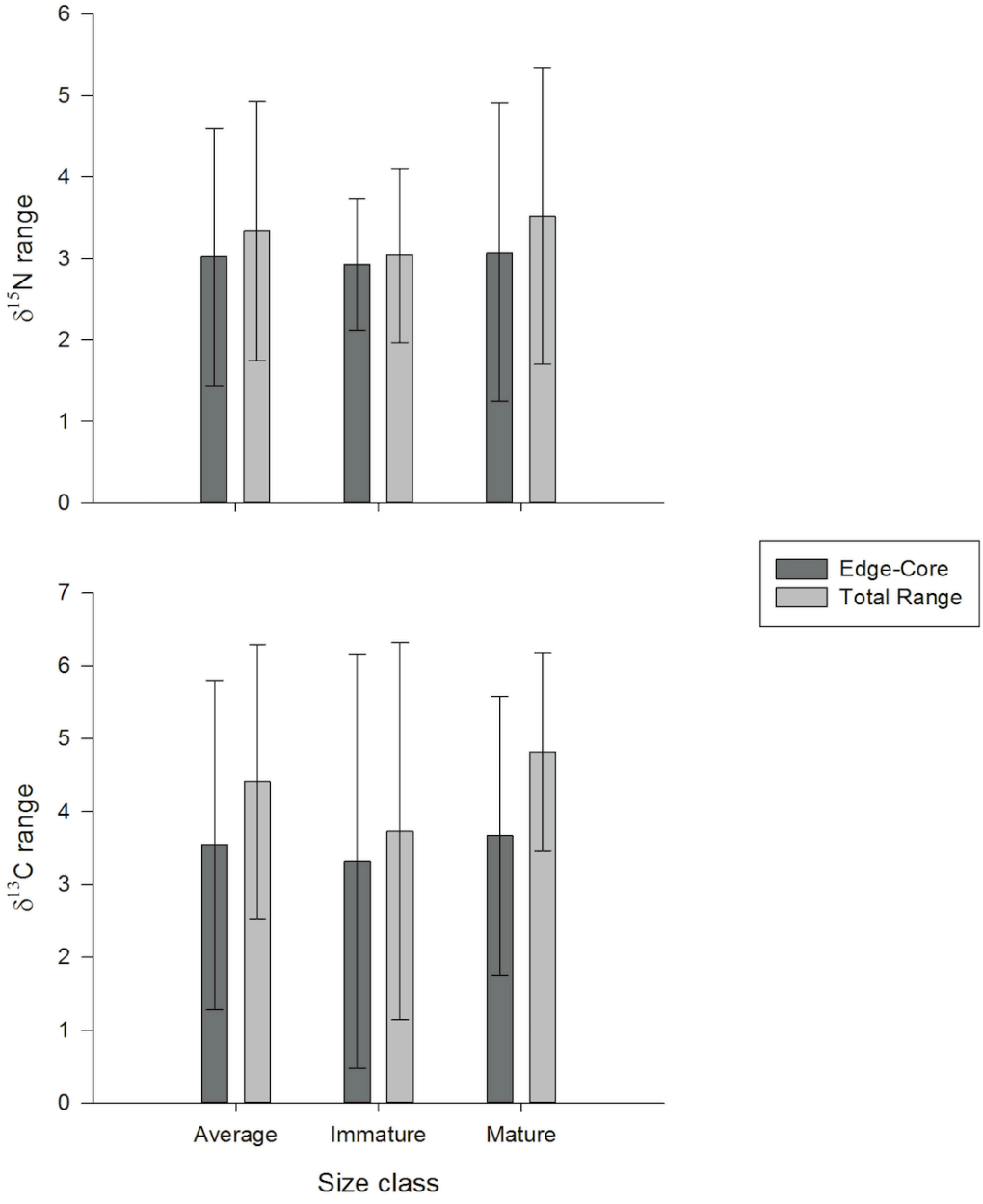

Figure 3.7. Stable isotope (mean ± SD) $\delta^{15}N$ edge-core and total range values from each transect averaged across all, immature, and mature Atlantic tarpon.

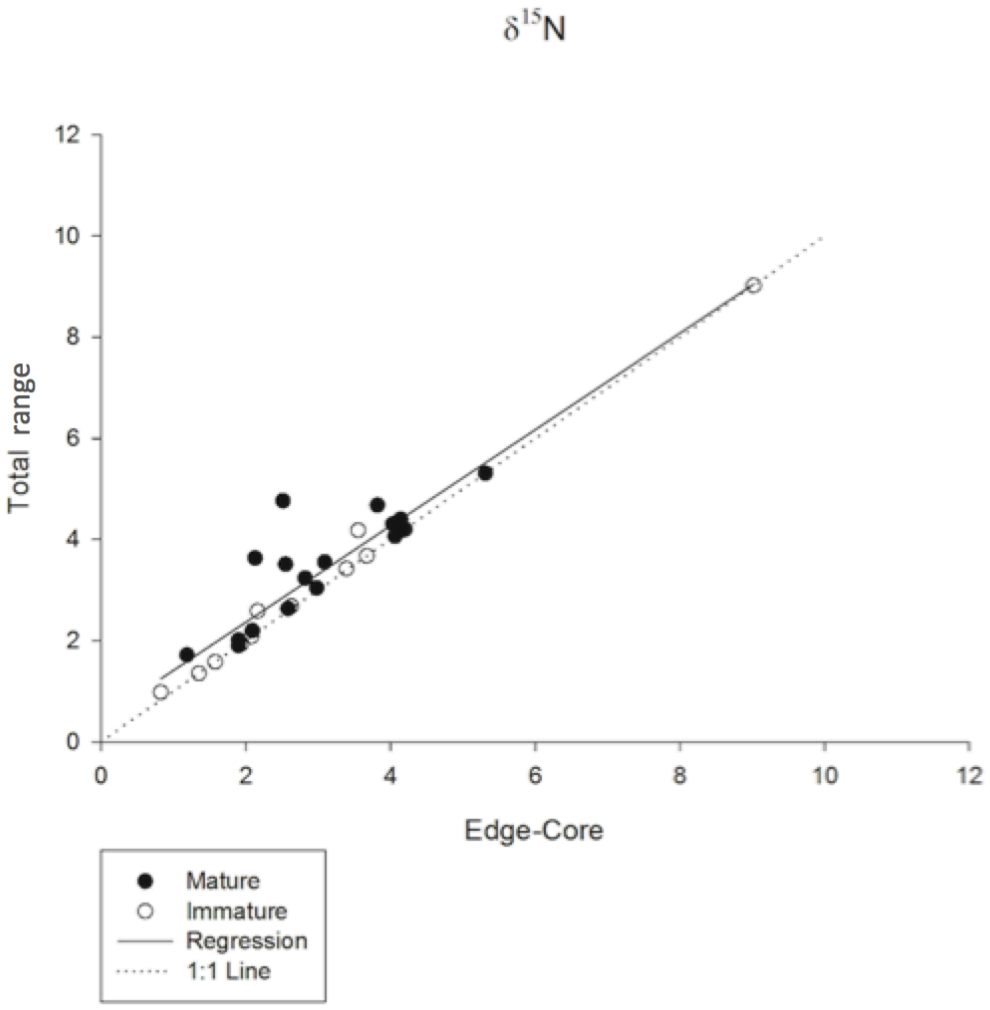

Figure 3.8. Comparison between edge minus core and total range values of stable isotope (mean ± SD) $\delta^{15}N$.

References

Adams, A. J., Horodysky, A. Z., Mcbride, R. S., Guindon, K., Shenker, J., Macdonald, T. C., … Carpenter, K. (2014). Global conservation status and research needs for tarpons (Megalopidae), ladyfishes (Elopidae) and bonefishes (Albulidae). *Fish and Fisheries*, *15*(May 2012), 280–311. http://doi.org/10.1111/faf.12017

Adey, E. a., Black, K. D., Sawyer, T., Shimmield, T. M., & Trueman, C. N. (2009). Scale microchemistry as a tool to investigate the origin of wild and farmed Salmo salar. *Marine Ecology Progress Series*, *390*, 225–235. http://doi.org/10.3354/meps08161

Ault, J. S., & Luo, J. (2013). A reliable game fish weight estimation model for Atlantic tarpon (Megalops atlanticus). *Fisheries Research*, *139*, 110–117. http://doi.org/10.1016/j.fishres.2012.10.004

Balter, V., & Lécuyer, C. (2004). Determination of Sr and Ba partition coefficients between apatite and water from 5°C to 60°C: A potential new thermometer for aquatic paleoenvironments. *Geochimica et Cosmochimica Acta*, *68*(3), 423–432. http://doi.org/10.1016/S0016-7037(00)00453-8

Brown, R. J., & Severin, K. P. (2009). Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 1790–1808. http://doi.org/10.1139/F09-112

Bath, G. E., Thorrold, S. R., Jones, C. M., Campana, S. E., McLaren, J. W., & Lam, J. W. H. (2000). Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta*, *64*(10), 1705–1714. http://doi.org/10.1016/S0016-7037(99)00419-6

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

Campana, S. E., & Thorrold, S. R. (2001). Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences*, *58*, 30–38. http://doi.org/10.1139/f00-177

Chapman, B. B., Hulthen, K., Brodersen, J., Nilsson, P. A., Skov, C., Hansson, L. A., & Bronmark, C. (2012). Partial migration in fishes: Causes and consequences. *Journal of Fish Biology*, *81*(2), 456–478. http://doi.org/10.1111/j.1095-8649.2012.03342.x

Crabtree, R. E. (1995). Relationship between lunar phase and spawning activity of tarpon, Megalops atlanticus, with notes on the distribution of larvae. *Bulletin of Marine Science*, *56*(3), 895–899.

Crabtree, R. E., Cyr, E. C., Chacón Chaverri, D., McLarney, W. O., & Dean, J. M. (1997). Reproduction of tarpon, Megalops atlanticus, from Florida and Costa Rican waters and notes on their age and growth. *Bulletin of Marine Science*, *61*(2), 271–285.

Crabtree, R. E., Cyr, E. C., & Dean, J. M. (1995). Age and growth of tarpon , Mega / ops at / anticus , from South Florida waters *.

Davenport, S. R., & Bax, N. J. (2002). A trophic study of a marine ecosystem off southeastern Australia using stable isotopes of carbon and nitrogen. *Canadian*

Journal of Fisheries and Aquatic Sciences, *59*(3), 514–530. http://doi.org/10.1139/f02-031

Davoren, G. K., Woloschiniwsky, C. S. A., Halden, N. M., & Wang, F. (2015). Does otolith chemistry indicate the natal habitat of Newfoundland capelin Mallotus villosus? *Journal of Experimental Marine Biology and Ecology*, *464*, 88–95. http://doi.org/10.1016/j.jembe.2014.10.025

De Villiers, S. (1999). Seawater strontium and Sr/Ca variability in the Atlantic and Pacific oceans. *Earth and Planetary Science Letters*, *171*(4), 623–634. http://doi.org/10.1016/S0012-821X(99)00174-0

Dorado, S., Rooker, J. R., Wissel, B., & Quigg, A. (2012). Isotope baseline shifts in pelagic food webs of the Gulf of Mexico. *Marine Ecology Progress Series*, *464*, 37–49. http://doi.org/10.3354/meps09854

Doubleday, Z. A., Izzo, C., Woodcock, S. H., & Gillanders, B. M. (2013). Relative contribution of water and diet to otolith chemistry in freshwater fish. *Aquatic Biology*, *18*(3), 271–280. http://doi.org/10.3354/ab00511

Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., Limburg, K. E., … Walther, B. D. (2008). Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. *Oceanography and Marine Biology: An Annual Review, Vol 46*, *46*(2), 297–+. Retrieved from <Go to ISI>://000256878700007

Fry, B. (2002). Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. *Estuaries*, *25*(2), 264–271. http://doi.org/10.1007/BF02691313

Gelwick, F. P., Senol, A., Arrington, A., & Winemiller, K. (2001). Fish Assemblage Structure in Relation to Environmental Variation in a Texas Gulf Coastal Wetland. *Estuaries*, *24*(2), 285–296.

Guindon, K., Neidig, C., Tringali, M., Gray, S., King, T., Gardinal, C., & Kurth, B. (2015). An overview of the tarpon genetic recapture study in Florida – a citizen science success story. *Environmental Biology of Fishes*, *98*(11), 2239–2250. http://doi.org/10.1007/s10641-015-0440-2

Harrington, R. (1958). Morphometry and Ecology of Small Tarpon, Megalops atlantica Valenciennes from Transitional Stage Through Onset of Scale Formation. *Copeia*, *1958*(1), 1–10.

Hearn, A. R., Chapman, E. D., Singer, G. P., Brostoff, W. N., LaCivita, P. E., & Klimley, a. P. (2014). Movements of out-migrating late-fall run Chinook salmon (Oncorhynchus tshawytscha) smolts through the San Francisco Bay Estuary. *Environmental Biology of Fishes*, *97*(8), 851–863. http://doi.org/10.1007/s10641-013-0184-9

Hobson, K. a. (1999). Tracing origins and migration of wildlife using stable isotopes: A review. *Oecologia*, *120*, 314–326. http://doi.org/10.1007/s004420050865

Holá, M., Kalvoda, J., Bábek, O., Brzobohatý, R., Holoubek, I., Kanický, V., & Skoda, R. (2009). LA-ICP-MS heavy metal analyses of fish scales from sediments of the Oxbow Lake Certak of the Morava River (Czech Republic). *Environmental*

Geology, *58*, 141–151. http://doi.org/10.1007/s00254-008-1501-z
Holá, M., Kalvoda, J., Nováková, H., Škoda, R., & Kanický, V. (2011). Possibilities of LA-ICP-MS technique for the spatial elemental analysis of the recent fish scales: Line scan vs. depth profiling. *Applied Surface Science*, *257*, 1932–1940. http://doi.org/10.1016/j.apsusc.2010.09.029
Holt, G. J., Holt, S. A., & Frank, K. T. (2005). What can historic tarpon scales tell us about the tarpon fishery collapse in Texas? *Contributions in Marine Science*, *37*, 66–77.
Holt, S. a. (2008). Distribution of Red Drum Spawning Sites Identified by a Towed Hydrophone Array. *Transactions of the American Fisheries Society*, *137*(2), 551–561. http://doi.org/10.1577/T03-209.1
Hutchinson, J. J., & Trueman, C. N. (2006). Stable isotope analyses of collagen in fish scales: Limitations set by scale architecture. *Journal of Fish Biology*, *69*, 1874–1880. http://doi.org/10.1111/j.1095-8649.2006.01234.x
Jud, Z. R., Layman, C. A., & Shenker, J. M. (2011). Diet of age-0 tarpon (Megalops atlanticus) in anthropogenically-modified and natural nursery habitats along the Indian River Lagoon, Florida. *Environmental Biology of Fishes*, *90*(3), 223–233. http://doi.org/10.1007/s10641-010-9734-6
Kalish, J. M. (1991). Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod Pseudophycis barbatus. *Marine Ecology Progress Series*, *74*(2-3), 137–159. http://doi.org/10.3354/meps075137
Kalvoda, J., Novák, M., Bábek, O., Brzobohatý, R., Holá, M., Holoubek, I., … Škoda, R. (2009). Compositional changes in fish scale hydroxylapatite during early diagenesis; An example from an abandoned meander. *Biogeochemistry*, *94*, 197–215. http://doi.org/10.1007/s10533-009-9319-7
Kerr, L. A., & Secor, D. H. (2010). Latent effects of early life history on partial migration for an estuarine-dependent fish. *Environmental Biology of Fishes*, *89*(3), 479–492. http://doi.org/10.1007/s10641-010-9664-3
Kraus, R. T., & Secor, D. H. (2004). Incorporation of strontium into otoliths of an estuarine fish. *Journal of Experimental Marine Biology and Ecology*, *302*(1), 85–106. http://doi.org/10.1016/j.jembe.2003.10.004
Luo, J., & Ault, J. S. (2012). Vertical movement rates and habitat use of Atlantic tarpon. *Marine Ecology Progress Series*, *467*, 167–180. http://doi.org/10.3354/meps09957
Luo, J., Ault, J. S., Larkin, M. F., & Barbieri, L. R. (2008). Salinity measurements from pop-up archival transmitting (PAT) tags and their application to geolocation estimation for Atlantic tarpon. *Marine Ecology Progress Series*, *357*, 101–109. http://doi.org/10.3354/meps07288
Macdonald, J. I., & Crook, D. A. (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. http://doi.org/10.3354/meps08703
Meyer, C. G., Papastamatiou, Y. P., & Holland, K. N. (2010). A multiple instrument approach to quantifying the movement patterns and habitat use of tiger (Galeocerdo

cuvier) and Galapagos sharks (Carcharhinus galapagensis) at French Frigate Shoals, Hawaii. *Marine Biology*, *157*, 1857–1868. http://doi.org/10.1007/s00227-010-1457-x

Mooney, R. F., & McClelland, J. W. (2012). Watershed Export Events and Ecosystem Responses in the Mission-Aransas National Estuarine Research Reserve, South Texas. *Estuaries and Coasts*, 1–18. http://doi.org/10.1007/s12237-012-9537-4

Morse, J. W., & Bender, M. L. (1990). Partition coefficients in calcite: Examination of factors influencing the validity of experimental results and their application to natural systems. *Chemical Geology*, *82*(C), 265–277. http://doi.org/10.1016/0009-2541(90)90085-L

Nims, M. K., & Walther, B. D. (2014). Contingents of Southern Flounder from Subtropical Estuaries Revealed by Otolith Chemistry. *Transactions of the American Fisheries Society*, (July 2014), 37–41. http://doi.org/10.1080/00028487.2014.892535

Palmer, T. a., Montagna, P. a., & Kalke, R. D. (2013). The effects of opening an artificial tidal inlet on hydrography and estuarine macrofauna in Corpus Christi, Texas. *Environmental Monitoring and Assessment*, *185*(7), 5917–5935. http://doi.org/10.1007/s10661-012-2995-0

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, *18*, 293–320. http://doi.org/10.1146/annurev.es.18.110187.001453

Plummer, L. N., & Busenberg, E. (1987). Thermodynamics of Aragonite-Strontianite Solid-Solutions - Results From Stoichiometric Solubility At 25-Degrees-C and 76-Degrees-C. *Geochimica Et Cosmochimica Acta*, *51*, 1393–1411 St – thermodynamics of aragonite–strontium.

Ramsay, A. L., Milner, N. J., Hughes, R. N., & McCarthy, I. D. (2012). Fish scale ?? 15N and ?? 13C values provide biogeochemical tags of fish comparable in performance to element concentrations in scales and otoliths. *Hydrobiologia*, *694*, 183–196. http://doi.org/10.1007/s10750-012-1143-8

Rickards, W. L. (1968). Ecology and Growth of Juvenile Tarpon Megalops Atlanticus in a Georgia Salt Marsh. *Bulletin of Marine Science*, *18*(869), 220–&. Retrieved from <Go to ISI>://A1968B020000011

Rodionov, S. N. (2004). A sequential algorithm for testing climate regime shifts. *Geophysical Research Letters*, *31*(9), n/a–n/a. http://doi.org/10.1029/2004GL019448

Secor, D. H. (1999). Specifying divergent migrations in the concept of stock: The contingent hypothesis. *Fisheries Research*, *43*, 13–34. http://doi.org/10.1016/S0165-7836(99)00064-8

Seeley, M., Miller, N., & Walther, B. (2015). High resolution profiles of elements in Atlantic tarpon (Megalops atlanticus) scales obtained via cross-sectioning and laser ablation ICP-MS: a literature survey and novel approach for scale analyses. *Environmental Biology of Fishes*, *98*(11), 2223–2238. http://doi.org/10.1007/s10641-015-0443-z

Stecher, H. A., Krantz, D. E., Lord, C. J., Luther, G. W., & Bock, K. W. (1996). Profiles of strontium and barium in Mercenaria mercenaria and Spisula solidissima shells.

Geochimica et Cosmochimica Acta, *60*(18), 3445–3456. http://doi.org/10.1016/0016-7037(96)00179-2
Thorrold, S. R., Jones, C. M., & Campana, S. E. (1997). Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (Micropogonias undulatus). *Limnology and Oceanography*, *42*(1), 102–111. http://doi.org/10.4319/lo.1997.42.1.0102
Trueman, C. N., Mackenzie, K. M., & Palmer, M. R. (2012). Identifying migrations in marine fishes through stable-isotope analysis. *Journal of Fish Biology*, *81*, 826–847. http://doi.org/10.1111/j.1095-8649.2012.03361.x
Trueman, C. N., & Moore, A. (2007). CHAPTER 10 Use of the Stable Isotope Composition of Fish Scales for Monitoring Aquatic Ecosystems, 145–161.
Turner, S. M., & Limburg, K. E. (2012). Comparison of Juvenile Alewife Growth and Movement in a Large and a Small Watershed. *Marine and Coastal Fisheries*, *4*(1), 337–345. http://doi.org/10.1080/19425120.2012.675974
Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in d15N and d13C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*(8), 2061–2066. http://doi.org/10.4319/lo.2001.46.8.2061
Wade, R. a. (1962). the Biology of the Tarpon, Megalops Atlanticus, and the Ox-Eye, Megalops Cyprinoides, With Emphasis on Larval Development. *Bulletin of Marine Science O the Gulf and Caribbean*, (424).
Walther, B. D., Kingsford, M. J., O'Callaghan, M. D., & McCulloch, M. T. (2010). Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. *Environmental Biology of Fishes*, *89*(3), 441–451. http://doi.org/10.1007/s10641-010-9661-6
Walther, B. D., & Limburg, K. E. (2012). The use of otolith chemistry to characterize diadromous migrations. *Journal of Fish Biology*, *81*, 796–825. http://doi.org/10.1111/j.1095-8649.2012.03371.x
Walther, B. D., & Nims, M. K. (2015). Spatiotemporal Variation of Trace Elements and Stable Isotopes in Subtropical Estuaries: I. Freshwater Endmembers and Mixing Curves. *Estuaries and Coasts*, 754–768. http://doi.org/10.1007/s12237-014-9881-7
Wells, B. K., Bath, G. E., Thorrold, S. R., & Jones, C. M. (2000). Incorporation of strontium, cadmium, and barium in juvenile spot (*Leiostomus xanthurus*) scales reflects water chemistry. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(1974), 2122–2129. http://doi.org/10.1139/cjfas-57-10-2122
Wilson, S. G., Jonsen, I. D., Schallert, R. J., Ganong, J. E., Castleton, M. R., Spares, A. D., … Block, B. A. (2015). Tracking the fidelity of Atlantic bluefin tuna released in Canadian waters to the Gulf of Mexico spawning grounds. *Canadian Journal of Fisheries and Aquatic Sciences*, *72*(11), 1700–1717. http://doi.org/10.1139/cjfas-2015-0110
Winemiller, K. O., Akin, S., & Zeug, S. C. (2007). Production sources and food web structure of a temperate tidal estuary: Integration of dietary and stable isotope data. *Marine Ecology Progress Series*, *343*, 63–76. http://doi.org/10.3354/meps06884
Woodcock, S. H., Grieshaber, C. A., & Walther, B. D. (2013). Dietary transfer of

enriched stable isotopes to mark otoliths, fin rays, and scales. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(3), 1–4. http://doi.org/10.1017/S0016756897007061

Woodcock, S. H., & Walther, B. D. (2014). Trace elements and stable isotopes in Atlantic tarpon scales reveal movements across estuarine gradients. *Fisheries Research*, *153*, 9–17. http://doi.org/10.1016/j.fishres.2014.01.003

Zerbi, a. (2001). Growth of juvenile tarpon in Puerto Rican Estuaries. *ICES Journal of Marine Science*, *58*, 87–95. http://doi.org/10.1006/jmsc.2000.0992

Zerbi A, Aliaume C, Joyeux JC (2001) Growth of juvenile tarpon in Puerto Rican estuaries. ICES J Mar Sci 58:87-95 doi:10.1006/jmsc.2000.0992

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