Developing and applying elemental composition of shark vertebrae as a tool for quantifying life history characteristics over ontogeny

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DEVELOPING AND APPLYING ELEMENTAL COMPOSITION OF SHARK VERTEBRAE AS A TOOL FOR QUANTIFYING LIFE HISTORY CHARACTERISTICS OVER ONTOGENY

by

Heather M. Christiansen

A Thesis
Submitted to the Faculty of Graduate Studies through Environmental Science in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

2011

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DEVELOPING AND APPLYING ELEMENTAL COMPOSITION OF SHARK VERTEBRAE AS A TOOL FOR QUANTIFYING LIFE HISTORY CHARACTERISTICS OVER ONTOGENY

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September 20, 2011
I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, undertaken under the supervision of Dr. Brian Fryer as follows: Chapter 2 contains material from a manuscript entitled “Validating elemental profiles in white shark, Carcharodon carcharias, vertebrae as a marker of size-based habitat-use and migration patterns using Fs-LA-ICP-MS” that has been rejected by PLoS ONE and will be resubmitted to Marine Biology. This manuscript is co-authored by H.M. Christiansen, N.E. Hussey, A.T. Fisk, S.P. Wintner, G. Cliff, S.F.J. Dudley, S.A. Rush, and B.J. Fryer. Chapter 3 contains material from a manuscript entitled “Verifying the age of white sharks, Carcharodon carcharias, using calcium profiles in vertebral centra as determined by Fs-LA-ICP-MS” that will be submitted in the near future. This manuscript is co-authored by H.M. Christiansen, N.E. Hussey, S.P. Wintner, G. Cliff, S.F.J. Dudley, A.T. Fisk, M.A. MacNeil, and B.J. Fryer. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of co-authors was primarily through the assistance with data analysis and statistical procedures as well as helping with revising early drafts.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis. I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

II. Declaration of Previous Publication

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ABSTRACT

In response to the need for life history data for sharks, the use of femtosecond laser ablation inductively coupled plasma mass spectrometry (Fs-LA-ICP-MS) was investigated as a novel method to investigate elemental profiles within white shark (Carcharodon carcharias) vertebrae. It was determined that (i) a suite of elements are present within the vertebrae above detection limits and (ii) elements were not reworked within the vertebral matrix. These elemental profiles are likely reflective of the individual’s environment and basic information about habitat use, movement, and migration patterns throughout ontogeny can be determined. Calcium profiles within the vertebrae were analyzed as an alternative method to age sharks. The ages for Ca-profiles were ~3 to 4 times higher than previous methods using X-radiography, the growth rate was greatly decreased and age of maturity was increased 3 to 4 times. Fs-LA-ICP-MS is an important tool to expand knowledge on life history characteristics of elasmobranchs.
DEDICATION

I dedicate this thesis to my parents, Timothy and Theresa McCann; I would not be where I am today without all your love and support and to my husband, Daniel Christiansen, for always believing in me.
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I would like to thank my supervisor, Dr. Brian Fryer for his guidance and support throughout this process. I would also like to thank Dr. Nigel Hussey for his continuous advice, insights, and feedback. I thank my committee members Dr. Joel Gagnon and Dr. Trevor Pitcher for their helpful comments and advice. Thank you to Dr. Aaron Fisk for his guidance throughout this project. I would like to thank Zhaoping Yang and Mohamed Shaheen for their assistance with the Fs-LA-ICP-MS and Sharon Lackie for her support and knowledge on the SEM. Thank you also to Alice Grgicak-Mannion for the GIS map of South Africa. My thanks go to Dr. Lisa Natanson from NOAA for helping with age estimation. I express my gratitude to the KwaZulu-Natal Shark Board laboratory staff for undertaking the required sampling. Thank you to M. Naicker and D. McMohan at KwaZulu-Natal hospital and to L. Lang-Lawrence at CML Healthcare for their assistance in X-raying the shark vertebrae. Finally, I would like to my officemates for their endless support and encouragement.
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1.1 Introduction

Currently, populations of elasmobranchs are declining worldwide. This is due to many factors including fishing, by-catch, habitat destruction and pollution (Fowler et al. 2005). Species that spend periods of their life in the pelagic realm and migrate outside of Exclusive Economic Zones (EEZ) are especially vulnerable to commercial fishing operations; 32% of pelagic sharks and rays are listed as threatened on the International Union for Conservation of Nature (IUCN) Red List (Camhi 2008). Many of these threatened elasmobranchs are large predators that feed at the top of the food web and are believed to exert top-down control (Stevens et al. 2000, Ferretti et al. 2010). In addition, these large species are typically highly mobile (Stevens et al. 2000, Dulvy et al. 2008), and exert influence over multiple environments within the marine ecosystem, the scale of the effect being species-, size- and sex-specific (Hussey et al. 2011). Large-scale removal of these apex predators therefore has serious implications for the structure and stability of the entire marine food web (Stevens et al. 2000, Frank et al. 2005, Heithaus et al. 2008).

In response to increased awareness of declining populations of elasmobranch species, the Food and Agriculture Organization of the United Nations (FAO) developed an international plan of action for the conservation and management of sharks in 1999. As a part of this plan it was recommended that individual nations create their own national plan of action (NPOA). However, since that time few member countries have enacted a NPOA leading to a paucity of information on the conservation and
management status of sharks worldwide (FAO 2008). It was further recommended that nations that have not created a NPOA should focus on determining critical life history information (e.g. age, habitat use, movement and migration patterns) on primary species of concern, including those on the CITES appendices (FAO 2008).

Determining these key characteristics in large marine predators, particularly sharks, is often difficult. Habitat usage, movement, and migration patterns of sharks have primarily been monitored through passive, acoustic and satellite tags. While these tags provide high resolution data on individual movement and diving patterns, the data provided is a snapshot of the individual’s life. A recent review by Speed et al. (2010) highlights the need to quantify long term habitat changes over ontogeny and any changes associated with sex and size as being vital to aid in conservation and management efforts.

Age determination is the basis for determining growth rates, age at maturity and life span, which are all important metrics to conservation and management efforts (Cortés 1998). While age determination has been the topic of many studies on sharks and other elasmobranchs (see reviews by Cailliet and Goldman, (2004), Cailliet et al. (1986), Cailliet et al., (2006)), the age for few species have been validated. For most species, there is an annual periodicity to growth ring formation (one opaque band and one translucent band form per year). Thus the number of growth rings counted represent the age of the individual. However, annual periodicity must be validated on a species to species basis and without this validation it is difficult to create an accurate management strategy for that species.

Given the need to determine key life history characteristics of large pelagic sharks and the difficulty of obtaining this information through currently available methods, I
investigated the use of femtosecond laser ablation inductively coupled plasma mass spectrometry (Fs-LA-ICP-MS) as a tool to analyze elemental profiles within shark vertebrae. To determine if Fs-LA-ICP-MS is a suitable method to investigate these characteristics, I determined baseline concentrations on a suite of elements and investigated any patterns in the elemental profiles within and between individuals.

In order to investigate the relationship between elemental profiles and environment due to seasonal changes accurate age estimates are required. Additionally, accurate age estimates are required to investigate habitat use for each life stage. To verify current age estimates obtained through X-radiography (X-ray) analysis I analyzed the variation of calcium (Ca) within the vertebral centra across ontogeny.

**Biomineralization**

In many species, biomineralized structures have helped to elucidate information on age and key life history characteristics. Biomineralized structures (e.g. fish otoliths, mollusc shells, corals, and elasmobranch vertebrae) are formed by the incorporation of elements from the surrounding environment into a growing structure (Weiner and Dove 2003), providing information on a variety of characteristics over ontogeny.

Elemental profiles contained within fish otoliths, a well-studied biomineralized structure, have been used to identify natal streams, discriminate between stocks, determine habitat preference, and identify movement patterns over ontogeny (Campana 1999, Elsdon et al. 2008). Several elements have predictable relationships between environmental concentrations and concentrations within the otoliths, which allow researchers to elucidate information on these life history characteristics. For example, the strontium: calcium (Sr:Ca) ratio within fish otoliths has been validated as an indicator of
fluctuating salinity within the habitat of the fish (Secor and Rooker 2000). Bath et al. (2000), determined that elemental uptake in otoliths of spot (*Leiostomus xanthurus*) were reflective of the surrounding environment for both strontium and barium. Using the elemental profiles of potassium, strontium, manganese and barium in American shad (*Alosa sapidissim*) otoliths, Thorrold et al. (1998) were able to identify natal streams of adult fish based on the juvenile portion of the otoliths. While many studies have focused on the differences between fresh and saltwater environments, Chittaro et al. (2004) were able to distinguish between mangrove and coral reef habitat for the French grunt (*Haemulon flavolineatum*) using the microchemistry of otoliths, showing the utility of this method at a smaller spatial scale within an entirely marine system. In addition, fish otoliths, have been used in age determination and validation studies (Pannella 1971, Campana and Neilson 1985), based upon banding patterns within the otoliths caused by differences in somatic growth.

*Elasmobranch vertebrae: an under-utilized biomineralized structure*

Elasmobranch vertebrae are also biomineralized structures, however, unlike fish otoliths, which are aragonite, vertebrae are composed of hydroxyapatite and are secreted incrementally (Ridewood 1921, Moss 1977). The vertebrae consist of cartilage, which is primarily proteoglycans in a meshwork of collagen and mineralized areas (Zhang et al. 2009). For many species, it has been hypothesized that two growth bands (one opaque and one translucent) combine to form one growth ring (Cailliet and Goldman 2004). It has been determined for several species, such as gray reef shark (*Carcharhinus amblyrhynchos*), common thresher shark (*Alopias vulpinus*), spiny dogfish (*Squalus acantbias*), and thornback ray (*Raja clavatai*), that opaque bands are more highly
mineralized and have higher Ca levels, while translucent bands are less mineralized and have lower Ca levels (Holden and Vince 1973, Jones and Geen 1977, Cailliet and Radtke 1987). It is believed that most species will follow this pattern of mineralization (the angel shark (*Squatina califorinca*) being one exception, Natanson and Cailliet (1990)).

Many researchers have attempted to apply the same techniques used in determining life history characteristics in fish otoliths to elasmobranch vertebrae. While there are similarities between the two structures there are also key differences in composition and formation, which may complicate the blanket use of the same technique applied to fish otoliths in vertebrae. In order to utilize the chemical composition as a means of determining life history characteristics, several assumptions must be addressed. First, there must be no reworking of the vertebral centra (i.e. elements are not exchanged within the system once they have been laid down) and second, the elements contained within the vertebral centra must be above detection limits of the analytical system and be reflective of the surrounding environment.

The metabolic stability of the vertebral centra has been questioned to this point. Campana et al. (2002) determined the organic component of the vertebrae was stable through the use of bomb radiocarbon dating. However, they did not prove that the inorganic portion was stable as well. The stability of the inorganic component has been questioned throughout the literature often with conflicting results. Clement (1992) provided a literature review and investigated mineralized tissues from five species of sharks and concluded that vertebral centra grow by apposition without resorption. Preziosi et al. (2006) investigated four species of sharks in Australian waters with spinal deformities and also found no distortion in the calcification of individual vertebrae.
Several studies however, have found opposite results. Welden et al. (1987) attempted to use radiometric dating to age four species of elasmobranchs, but the results were inconclusive, which was attributed to possible reworking of the vertebral centra; a spinal deformity in a sand tiger shark (Carcharias taurus) was found to have reworking occurring on five deformed vertebrae (Heupel et al. 1999); evidence of resorption after a trauma to the vertebrae has also been documented in the school shark (Galeorhinus galeus) (Officer et al. 1995), at the time of the study there was no reworking occurring and a portion of the vertebrae that was cracked was found to not have undergone reworking. This provided evidence that reworking was limited and used to restore function to the vertebrae. No evidence was found for continued reworking and it was concluded that it was not a part of normal physiological growth or Ca regulation. Since there is no conclusive evidence to support or deny reworking, it must be determined whether reworking is occurring before analyzing the elemental composition of the vertebral centra.

*Elemental composition of vertebral centa*

Few studies have analyzed the elemental concentration of whole elasmobranch vertebrae (Eisler 1984, Vas 1987, Vas et al. 1990). Vas et al. (1990) reported a decrease in metal concentrations with an increase in size. This conclusion, however, was based on concentration per total weight of the vertebral centrum. In addition, the vertebral centra were not serially sampled, and comparisons were made based on differences between individuals only. These results, therefore, may not truly represent ontogenetic shifts occurring within individuals and patterns between individuals may be due to differences in life history, such as sex, migration, and habitat usage. While Hale et al. (2006) used
LA-ICP-MS to investigate the composition of round stingray (*Urobatis halleri*) vertebrae throughout ontogeny, they did not relate the elemental profiles to the environment. no conclusions were made on the relationship between elemental concentrations in the vertebrae and of the external environment.

*Age validation and verification*

Age validation has been attempted in many species of sharks through several methods such as tag/recapture data (Cailliet et al. 1992), chemical marking utilizing oxytetracycline (OTC) (Holden and Vince 1973, Wintner and Cliff 1999, Skomal and Natanson 2003, Smith et al. 2003) and using bomb radiocarbon (Campana et al. 2002, Kerr et al. 2006). Age verification has also been accomplished through the use of X-ray spectrometry (Jones and Geen 1977) and electron microprobe (Cailliet and Radtke 1987). Using these techniques there were clear peaks and troughs in both Ca and phosphorus values. These peaks and valleys coincided with the opaque (peak) and translucent (troughs) bands, which have enabled researchers to age these animals by counting their bands. This same principle of Ca and phosphorus peaks occurring in opaque bands and troughs occurring in translucent bands was the basis of the Hale et al. (2006) study verifying optically determined ages for round stingrays up to age 5.

*Femtosecond Laser Ablation Inductively Coupled Plasma Mass Spectrometry*

Several methods such as energy dispersive electron microprobe, wavelength dispersive electron microprobe and proton induced x-ray emissions can potentially be used to determine the elemental composition of vertebral centra (Campana et al. 1997). LA-ICP-MS has been favored over these methods due to its ability to analyze a suite of elements over small spatial scales. Additionally, using Fs-LA-ICP-MS there are minimal
thermal and matrix effects and detection limits of elements are decreased, allowing more elements with lower concentrations to be analyzed (Shaheen et al. 2008). By utilizing Fs-LA-ICP-MS, I will analyze a continuous transect from the focus (beginning of development) to the outer edge (end of the individual’s life). The elemental profiles collected will provide valuable information, which to this point has not been collected at the individual level.

Study species

White sharks (*Carcharodon carcharias*) are large marine predators, which are currently listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List and are listed on Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II. In addition, white sharks are protected in coastal waters of Australia, Croatia, European Union, Maldives, Malta, Mexico, Montenegro, Namibia, New Zealand, South Africa, and the United States of America (Camhi 2008). Unfortunately, the development of region specific conservation and management strategies for the white shark is difficult due to a lack of life history data or more specifically data on geographic-specific life history strategies over ontogeny. This is due in part to their large size, the scale of the environment they inhabit, the relatively low encounter rates outside of known aggregation sites and their protected status.

Much research has been undertaken in recent years to improve our knowledge of many of these important characteristics of white sharks including data on reproduction (Francis 1996, Saidi et al. 2005), diet and trophic level (Estrada et al. 2006, Kerr et al. 2006, Hussey et al. 2011) and movement and migration (Boustany et al. 2002, Bonfil et
The habitat and migration patterns of white sharks have been well documented in the eastern Pacific using both photo identification (Domeier and Nasby-Lucas 2007) and archival tagging studies (Boustany et al. 2002, Dewar et al. 2004, Weng et al. 2007, Domeier and Nasby-Lucas 2008, Jorgensen et al. 2010). These studies have determined that white sharks spend several months in coastal waters, and then make large-scale seasonal migrations to an offshore region outside the EEZ. Bonfil et al. (2010) found that white sharks from Chatham Islands, New Zealand behaved similarly to white sharks from the Eastern Pacific ocean, spending several months around the islands before making directed migrations offshore. In contrast, white sharks in South Africa and Australia are most active in inshore regions on the continental shelf with occasional offshore movements including a documented trans-oceanic migration (Bonfil et al. 2005, Bruce et al. 2006). It is likely that geographically separated populations of white sharks exhibit different behavioral patterns and will, therefore, require region-specific management plans. For most regions, it is unknown at what age offshore migrations occur and at what frequency. In order to create an accurate and effective management plan for this species, more information about its basic life history characteristics is needed.

Vertebral centra samples used in this thesis were obtained from incidentally caught white sharks in beach protection nets off the coast of KwaZulu-Natal, South Africa. No sharks were sacrificed for this study. Following the KwaZulu-Natal Sharks Board (KZNSB) tag and release protocols, all live sharks found in the nets were tagged and released. Permission was granted by the KZNSB to use the shark samples and all
samples were exported in accordance with the CITES requirements (CITES South African Permit No. 106627).

**Rationale and objectives**

Using Fs-LA-ICP-MS and elemental profiles obtained from white shark vertebral centra, we can investigate whether reworking is occurring based on comparisons of elemental profiles of individuals of different age, sex and sizes. Furthermore, elemental profiles can be analyzed to determine if patterns in the profiles exist within individuals and between individuals. If these patterns exist, we can then begin linking vertebral profiles to environmental elemental data thereby inferring habitat usage, movement, and migration of the individual throughout ontogeny.

A more robust method is currently needed to determine if the banding patterns (opaque and translucent bands being indicative of one year of growth) observed through X-ray analysis are correct, as ages for white sharks have yet to be validated. Using Fs-LA-ICP-MS, a continuous transect from the focus to the edge of the vertebral centra is analyzed providing continuous data throughout ontogeny. There will be one peak and one trough in the Ca data for each year of growth. By determining the number and location of peaks and troughs, a second method of verifying opaque and translucent bands is available. If X-ray analysis is accurate, the number of peaks and troughs in the Ca profile will correspond to the number of opaque and translucent bands observed, respectively.

My thesis consists of four objectives as follows to investigate the suitability of Fs-LA-ICP-MS as a tool to investigate habitat usage, movement, migration, and age of white sharks:
1. Determine baseline concentration and detection limits on a suite of elements within the vertebral centra of the white shark using Fs-LA-ICP-MS. (Chapter 2)

2. Investigate the extent of reworking (if any) within the vertebral centra of the white shark using Fs-LA-ICP-MS and elemental profiles. (Chapter 2)

3. Preliminary investigations relating elemental profiles within vertebral centra to external environmental characteristics. (Chapter 2)

4. Using normalized Ca data, determine peaks and troughs to verify current aging techniques in white sharks. (Chapter 3)

The implications of these results will be discussed in Chapter 4.
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analysis of vertebrae in reconstructing ontogenetic feeding ecology in white

and opportunities for improving the monitoring of shark fisheries and trade. FAO


CHAPTER II

VALIDATING ELEMENTAL PROFILES IN WHITE SHARK, CARCHARODON CARCHARIAS, VERTEBRAE AS A MARKER OF SIZE-BASED HABITAT-USE AND MIGRATION PATTERNS USING FS-LA-ICP-MS

2.1 Introduction

There is considerable concern over the status of global shark populations, but incomplete life history data for many species complicates the formulation of effective management options (Ellis et al. 2005, Dulvy et al. 2008). In response to documented declines in regional shark populations (Stevens et al. 2000, Dulvy et al. 2008), the Food and Agriculture Organization of the United Nations (FAO) recommended that individual nations focus on determining critical life history information for species of concern (FAO 2008).

Knowledge of habitat use, movement and migration patterns of sharks are key parameters required for developing both regional and global management and conservation plans (Kinney and Simpfendorfer 2009, Knip et al. 2010, Speed et al. 2010). To date, standard tag-recapture programs (Kohler and Turner 2001, Hussey et al. 2009), acoustic (Holland et al. 1999, Heupel and Hueter 2001) and satellite tags (Eckert and Stewart 2001, Boustany et al. 2002) have been used with varying degrees of success to determine these parameters. While the more technologically advanced tags provide high-resolution data on individual movement and diving patterns, this method is often labor intensive, expensive, and restricted in deployment time (typically 6 to 12 months). New methodological approaches are, therefore, required to elucidate information on species-specific habitat and movement profiles of individual animals over ontogeny (Speed et al. 2010).
Long-term changes in habitat use, movement, and migration patterns have been monitored in aquatic species through the use of elemental profiles conserved in biomineralized structures. Similarly, the elemental profiles conserved in shark vertebral centra may provide unique tools to assess these parameters. Biomineralization is the process whereby elements from the surrounding environment are incorporated into the hard structures of an organism (e.g., fish otoliths, mollusc shells, statoliths), establishing an elemental history throughout ontogeny (Weiner and Dove 2003). Profiles of a number of elements in fish otoliths have been widely used to identify natal streams for adult fish, habitat preference, stock discrimination, connectivity between sub-populations, movement patterns over ontogeny and to validate pre-existing aging techniques (see reviews by (Campana 1999, Elsdon et al. 2008)). Experimental work has determined that the uptake of both strontium (Sr) and barium (Ba) in fish otoliths reflect that of the fish’s environment (Bath et al. 2000). Thus, the strontium: calcium (Sr:Ca) concentration ratio profile within fish otoliths can serve as an indicator of fluctuating salinity and water temperature conditions (see reviews by (Secor and Rooker 2000, Martin et al. 2004, Brown and Severin 2009)). This method has also been shown to be effective at discriminating habitat at a range of spatial scales within entirely marine systems (Chittaro et al. 2004, Hamer et al. 2006).

To validate the use of elemental profiles within the vertebral centra of sharks as a tool for assessing habitat use, movement, and migration patterns, it is necessary to first establish that the vertebral centra matrix, and the associated elements, is stable. Essentially, there needs to be validation that there is no reworking or exchange of Ca and other elements throughout ontogeny. Although Welden et al. (1987) suggested that the
inorganic portion of shark vertebrae was reworked based on the inability of the $^{210}\text{Pb}$: $^{210}\text{Po}$ isotopes to age four shark species, Campana et al. (2002) determined that the organic component of the vertebral centra was stable based on Δ$^{14}\text{C}$ profiles.

To date, few studies have utilized the elements contained in the calcified cartilage of elasmobranchs to investigate life history characteristics. In a preliminary study, Edmonds et al. (1996) were able to differentiate regional gummy shark ($\text{Mustelus antarcticus}$) populations in south-western Australia using the elemental composition of jaw cartilage. More recently, in an effort to validate the counting of growth rings (opaque and translucent bands) as an accurate method of aging, Hale et al. (2006) examined the incremental chemistry of elasmobranch vertebral centra utilizing laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Results indicated that Ca peaks within the vertebral centra were closely correlated with the opaque bands, which are formed during periods of high mineralization (summer).

Several analytical methods are available to determine the elemental profiles in biomineralized structures. LA-ICP-MS is favored over most methods such as energy dispersive electron microprobe, wavelength dispersive electron microprobe, and proton induced X-ray emission, for its ability to be effective at analyzing a suite of elements over small spatial scales with good precision (Campana et al. 1997). Additionally, by using the newly available femtosecond laser ablation inductively coupled plasma mass spectrometry (Fs-LA-ICP-MS), analyses are less matrix dependant, as finer particle sizes are generated with minimal thermal effects (Shaheen et al. 2008). These conditions decrease the required detection limits of elements, enabling previously undetected elements (due to low concentration) to be detected.
To determine if the vertebral centra matrix and associated elements are stable and to assess the utility of Fs-LA-ICP-MS in determining elemental profiles in shark vertebral centra, we examined nine white sharks (*Carcharodon carcharias*) (5 male and 4 female) ranging in size from 184 to 380 cm precaudal length (PCL) sampled from KwaZulu-Natal, South Africa. The white shark is an apex predator (McCosker 1985) currently listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List (IUCN 2011) and in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II. The aim of this study was two-fold, (i) to establish baseline data on a suite of elements measurable within shark vertebral centra and their associated detection limits using Fs-LA-ICP-MS, and (ii) to determine the extent of reworking (if any) by examining and comparing elemental concentration and deposition patterns across ontogeny and between individuals of different sizes. We hypothesize: i) that patterns of elements across the vertebral centra of the sharks will vary between different elements; and ii) that elemental patterns in the vertebral centra of small (juvenile) sharks will be conserved in the juvenile portion of the vertebral centra from larger individuals, confirming that reworking of vertebral material is not taking place.

2.2 Methods

*Vertebrae preparation and aging*

Vertebral centra were obtained from white sharks incidentally caught in beach protection nets along the coast of KwaZulu-Natal between 1992 and 2008 (Figure 2.1). All white sharks sampled were found deceased on capture in the nets and no sharks were sacrificed for this study. All live net-caught white sharks are tagged and released according to the KwaZulu-Natal Sharks Board tag and release protocols. For more
information on the netting operation see Cliff and Dudley (2011). Permission to use white shark vertebral centra samples was granted by the KwaZulu-Natal Sharks Board and samples were exported in strict accordance with the CITES requirements (CITES South African Permit No. 106627). On arrival at the KwaZulu-Natal Sharks Board laboratory, shark mortalities were sexed and PCL and maturity were recorded. PCL was measured as the straight-line distance from the tip of the snout to the precaudal notch as defined by Cliff et al. (1988). Maturity was assessed based on criteria described by Bass et al. (1973). Vertebral centra were excised anterior to the first dorsal fin from five males and four females and immediately stored frozen (Table 2.1). Prior to analysis, samples were cleaned of excess tissue and the following measurements were taken: dorsal diameter, lateral diameter, and vertebral depth (Wintner and Cliff 1999). X-radiographs were then taken of the whole centra, in a horizontal plane, using a Philips Optimus Bucky Diagnost TH X-ray unit. The CR cassettes used were Kodak Directview CR with Kodak GP Storage Phosphor screens. The CR reader used was a Kodak Directview CR975. Ages were determined by counting band pairs (consisting of one opaque and one translucent band). To determine individual band widths, measurements from the focus (mid-point of the notochordal remnant) to the birth mark and to the edge of each opaque and translucent band were made on the X-ray images using Image-Pro Discovery (version 4.5.1.29 © 2002). Pixel measurements were then converted to millimeter measurements using the above-defined measurements of each vertebral centrum.

Vertebral centra were oven dried for 48 hrs at 40°C and 0.6 mm thick bow-tie sections (Figure 2.2) were cut from each vertebral centra (corresponding to where X-ray measurements were made) using an IsoMet® low speed diamond saw (Beuhler-Whitby,
Ontario, Canada). The corpus calcareum was then separated from the intermedialia using a rotary cutter, after which no metal instruments were used to reduce risk of contamination. Each sample was sonicated in Milli-Q water for 20 minutes and dried for 24 hours in a class 100 laminar flow hood. After drying, each sample was fixed to a microscope slide, using Crystal Bond 509, and stored in a covered petri dish.

**Preliminary calcium study**

When using LA-ICP-MS, an internal standard is required to correct for sampling rate and to enable the calculation of element concentrations along the transect of ablated material (Longerich et al. 1996). To determine the variation in calcium (Ca) present in white shark vertebral centra, and to identify if Ca could be used as a reliable internal standard, two preliminary investigations were undertaken; (i) the corpus calcareum from one vertebral centrum was analyzed by scanning electron microscope with energy dispersive spectroscopy (SEM-EDS). SEM-EDS measurements were made in 12 box sections along a line scan from the inner edge (focus) to the outer edge of the corpus calcareum (Figure 2.3) and; (ii) Powdered samples were hand drilled from predetermined opaque and translucent bands (by X-ray analysis) for age 1 (3 samples for each band), age 5 (2 samples for each band) and age 13 (1 sample each for each band) had Ca concentrations determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). An analysis of variance (ANOVA) was performed to determine if there was a statistical difference in Ca concentrations between opaque and translucent bands. The mean Ca concentration in the corpus calcareum of white shark vertebral centra derived from these two methods was used as the internal standard for Fs-LA-ICP-MS analysis (see results below).
The elemental concentrations of each corpus calcareum were analyzed using Fs-LA-ICP-MS at the Great Lakes Institute for Environmental Research, University of Windsor. This system utilizes a Quantronix Integra C® femtosecond laser with a Ti:sapphire laser based on the Chirped Pulse Amplification technique for sampling. The ablated material was carried to the Thermo Electron X7-II ICP-MS® by argon (Ar) gas. The laser instrumental conditions were as follows: repetition rate 100 Hz; energy 0.2 mJ/pulse; pin hole diameter 2.5mm; and laser ablation spot size 20µm. For further details of the analytical system see Shaheen et al. (2008)

To calibrate the instrument and to correct for drift, two analyses of a trace element-doped glass standard (National Institute of Standards and Technology, NIST 610) were run before and after each sample set. Each sample was pre-ablated over the complete sampling transect with the laser to remove any residual surface contamination. Data acquisition consisted of a 60 s gas background followed by sample ablation (2800s to 5500s). To obtain background-corrected signals, the acquired background analyte mean intensity (counts s⁻¹) of the gas blank was subtracted from the sample analyte signal intensity (counts s⁻¹) obtained during ablation of the sample. The center of the corpus calcareum was ablated in a continuous transect on an automated microscope stage that moved at constant speeds of 4.3 to 7.1µm s⁻¹, depending on the orientation of the sample.

In total, nineteen elements (twenty-four separate isotopes) including lithium (Li), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca) manganese (Mn), copper (Cu), zinc (Zn), rubidium (Rb), strontium (Sr), yttrium (Y), tin (Sn), cesium (Cs), barium (Ba), lanthanum (La), cerium (Ce), praseodymium (Pr), lead (Pb), and uranium (U) were
analyzed by Fs-LA-ICP-MS (Table 2.2). These elements were selected because they have previously been used as chemical tracers for determining movement and habitat (e.g. Sr and Ba) or are monoisotopic and have extremely low detection limits (e.g. Y, Cs, Pr). As this study constitutes the first attempt, to our knowledge, to document trace element concentrations in white shark vertebral centra over ontogeny using Fs-LA-ICP-MS, we did not wish to eliminate any potentially useful markers. To account for the natural variation in isotopic abundances, concentrations of the three lead isotopes ($^{206}$Pb, $^{207}$Pb, and $^{208}$Pb) were averaged. Precision was determined by percent relative standard deviation (%RSD) as based on the NIST 610 (Table 2.2). Elements were considered above detection limits if they were more than 3σ above the gas blank (Longerich et al. 1996).

Raw data were processed using Plasma Lab® software and in-house written macros for Microsoft® Excel® to integrate data and standardize elemental counts to internal standards. Every 683 ms, a sample cycle (consisting of measurements of all 24 isotopes) was completed, and five of these sampling cycles were integrated to provide an elemental profile. Each data point, therefore, represents the average composition of the corpus calcareum over a length of 14.7 to 24.2 µm (depending on the microscope stage scanning speed). To account for variation in the performance of the laser and the ICP-MS, each elemental concentration is presented as a ratio to calcium (herein referred to as element: Ca ratio) ($^{43}$Ca was measured as the internal standard) (Campana et al. 1997). Outliers were removed using the interquartile range method whereas any data points greater than three times the interquartile range were removed (Tukey 1977). To reduce any confounding influence of the maternal elemental signatures, data from the pre-birth
period (i.e. developing embryo stage, as indicated by any data points prior to the angle change) were also discarded. Data were smoothed using a 25-point running average and the mean, minimum, and maximum elemental concentrations for the nine sharks (using the mean Ca concentration of 16.9%) were calculated for each isotope measured (Table 2.3).

Statistical analysis

Percentage frequency of occurrence ($\%F_o$) for each element and per shark was determined as the presence of a detectable signal at each sampling point across the length of the corpus calcareum and was calculated as:

$$\%F_o = \frac{E_i \times 100}{E_T}$$

where $E_i$ is the number of times the individual element was above detection limit across the corpus calcareum and $E_T$ is the total number of data points sampled for the individual.

Several elements (Sr, Ba, Pb, and U) had high percentage frequency of occurrence and were further analyzed to examine the oscillation patterns, consisting of high points (peaks), low points (troughs), and the straight line distance between these two values (amplitude) of individual elements throughout ontogeny and between different sized individuals. Considering the documented relationship between temperature and Sr incorporation rates in other biomineralized structures, and that white sharks, (i) spend proportionally more time off-shore (Bonfil et al. 2005, Weng et al. 2007) and (ii) begin deeper dive profiles with increasing size (Bonfil et al. 2005, Weng et al. 2007, Bonfil et al. 2010), we examined the change in the Sr:Ca profile using least squares linear regressions for each individual shark.
To determine if statistically significant changes in elemental profiles occurred in each vertebral centrum, a split moving window analysis (SMW) was conducted for each element profile in R (R Core Development Team 2010). Following Cornelius and Reynolds (1991), a window of one hundred data points was placed at the beginning of each data series. The window was then split into two halves and the element profile was averaged for each half. The windows were then shifted by one data point, and the analysis was repeated until the end of the dataset was reached creating an elemental dissimilarity profile for each shark (SMW dissimilarity profile). To compare averages obtained along the element profiles, the Euclidean distance between the two halves was calculated. To determine the statistical significance of any peaks in the resulting SMW dissimilarity profiles, signifying dissimilarities between the window halves, a Monte Carlo simulation was used. The Monte Carlo simulation consisted of randomizing the position of each data vector along the series and calculating the SMW dissimilarity profile. Each randomization was repeated 1000 times and a mean dissimilarity was calculated for each window mid-point position. The variance of each element profile was determined by calculating a weighted expected mean across all nine sharks and comparing it to the mean value obtained from the Monte Carlo simulations. These variance estimates were then pooled across all sharks and values of 1, 2, and 3 times the pooled standard deviation were used to determine significance of the SMW dissimilarity profiles. The SMW dissimilarity profiles obtained for the Ba:Ca ratios were then compared to the seasonal band data to determine if significant changes coincided with opaque (summer) and translucent (winter) bands.
2.3 Results

Preliminary calcium analysis

The percentage of Ca in the corpus calcareum as determined by SEM-EDS ranged from 14 to 21% with a mean of 17.5 ± 0.65 (mean ± SE). The Ca percentage determined by ICP-OES in vertebral centra varied from 9.3 to 25% in opaque bands and from 5.5 to 20% in translucent bands. An ANOVA including age as a random factor found no significant difference in Ca concentrations between opaque and translucent bands (Band: $F_{1, 6} = 0.08, p = 0.82$; Age: $F_{1, 6} = 0.01, p = 0.94$ and Band-age interaction: $F_{1, 6} = 1.94, p = 0.21$). A mean concentration Ca (as determined by the two methods) of 16.9% was used as the internal standard for Fs-LA-ICP-MS calculations.

Elemental analysis

Nineteen elements including major (P, K, Ca), minor (Mg, Zn, Sr), trace (Li, Mn, Cu, Rb, Sn, Cs, Ba, Pb, U), and rare earth elements (Y, La, Ce, Pr) were detected within the corpus calcareum of the white shark vertebral centra. The %RSD for the NIST 610 standard was determined to be acceptable (below 10%) for all elements except P and K (they have exceedingly high background levels); these elements were therefore excluded from further analysis (Table 2.2). The 16 elements with acceptable %RSD were also above detection limits for our analytical conditions and the Fs-LA-ICP-MS system (Table 2.3). Element concentrations for all 9 individuals tested were variable, ranging from a maximum of 2200 ± 8.4 ppm for Mg to a minimum of 0.011 ± 0.00041 ppm for La (Table 2.3).

The percentage frequency of occurrence of each element varied between individuals (Table 2.4). For Mg, Mn, Zn, Sr, Ba, and U, the percentage frequency of
elemental occurrence was greater than 95% in all individuals of different sizes, while the occurrence of Cu, Rb, Y, Sn, Cs, and Ce were more variable (Figure 2.4). For less abundant elements (Cu, Rb, Y, Sn, Cs, and Ce), the percentage frequency of occurrence varied between individuals in terms of both total abundance and the location of occurrence (Figure 2.4).

There was a significant negative linear relationship between the Sr:Ca ratio and distance from the focus of the vertebral centrum (as a proxy for animal size) (Figure 2.5). The Sr:Ca ratio variability that was explained by the regression increased with increasing size of animal (Figure 2.5). In addition to the negative linear Sr:Ca ratio relationship; SMW dissimilarity profiles for each individual shark showed there were also significant oscillations occurring throughout ontogeny.

The U:Ca ratio and SMW dissimilarity profiles followed oscillatory patterns and had statistically significant peaks and troughs throughout ontogeny for all individuals, but the amplitude and level of significance of these oscillations increased beyond approximately 18 to 23mm from the focus of the vertebral centrum (equating to ~200cm PCL) (Figure 2.6). While there was intra-individual variation in the actual U:Ca ratio, the above pattern was conserved between individuals of different sizes, i.e. the U:Ca ratio patterns in small (juvenile) animals were the same in the juvenile section of large animals (Figure 2.6).

The Pb:Ca ratio also followed an oscillatory pattern, however, no systematic pattern was observed between individuals by size, sex or season (Figure 2.7). The Pb:Ca SMW dissimilarity profile showed varying levels of statistical significance between individuals in addition to large intra-animal variation throughout ontogeny (Figure 2.7).
Statistically significant oscillations were evident for the Ba:Ca SMW dissimilarity profile with peaks of the Ba:Ca ratio occurring in both opaque bands (summer) and translucent bands (winter) (Figure 2.8). In addition, there was an overall decrease in the Ba:Ca ratio with increasing size of animal.

2.4 Discussion

While the element uptake in otoliths of teleost fish is well documented and a commonly applied approach to investigate various aspects of fish biology, little is known about element dynamics in shark vertebral centra. This study provides the first comprehensive elemental analysis of shark vertebral centra using an ultra-high resolution and highly sensitive Fs-LA-ICP-MS system. The capability of the Fs-LA-ICP-MS system to detect a suite of elements at a range of concentrations above detection limits, and evidence that reworking of the vertebral material is not taking place (see below), indicates that these profiles can be used to determine species-specific habitat, movement and migration patterns of sharks at the individual and population level.

Calcium levels in white shark vertebral centra

Calcium is commonly used as the internal reference standard for LA-ICP-MS of otoliths, due to its high abundance, which provides increased precision and reproducibility (Craig et al. 2000). In white shark vertebral centra, we found no systematic variation in Ca levels between seasonal bands or across the length using SEM (i.e. ontogeny) of the corpus calcareum, indicating the suitability of Ca as an internal standard for this species. This is in contrast to other studies investigating Ca concentrations in shark and stingray vertebral centra, where oscillatory patterns have been found (Cailliet and Radtke 1987, Hale et al. 2006). The two methods used to
quantify Ca in white shark vertebral centra, ICP-OES and SEM-EDS, were in agreement, but given the reported Ca variation between species, further work is required to determine inter-species corpus calcareum Ca concentration profiles and, therefore, the general applicability of Ca as an internal standard for shark vertebral centra. Irrespective of calcium concentrations, to account for any actual variation in Ca concentrations (whether specific to seasonal bands or variation in the hydroxyapatite matrix of shark vertebral centra) all element concentrations in the white shark vertebral centra were presented as a ratio to Ca. This approach should be viewed as standard when presenting elemental profiles from shark vertebral centra.

*Elements in the corpus calcareum of white shark vertebral centra*

A total of nineteen elements were recorded above the detection limits of the Fs-LA-ICP-MS system in the corpus calcareum of white shark vertebral centra. While Hale et al. (2006) were able to detect six of the elements (Mg, P, Ca, Sr, Ba, and Pb) in the vertebral centra of the round stingray using LA-ICP-MS, they were unable to detect several low concentration elements, such as Mn, Cu, Rb, Y, Ce, La, Ce, Pr, and U, that were detected in this study. Elemental profiles of rare earth and trace elements such as Na, Rb, Ce, Cu, Pb, Li, Cs, and Se have been proven to be unique indicators of mineral deposits and pollution, and have been used to identify specific habitat usage patterns for several species of fish (Friedrich and Halden 2008, Halden and Friedrich 2008). These newly-detected elements in shark vertebral centra have the potential to become unique tracers for determining specific habitat and movement patterns within the marine environment but may require the use of high resolution and ultra-sensitive systems such as the Fs-LA-ICP-MS.
Do sharks rework Ca and elements in their vertebrae?

Our results provide strong evidence that there is minimal, if any, reworking of elements in the vertebral centra of sharks. Firstly, the location of the low percentage frequency of occurrence elements (Li, Cu, Rb, Y, Ce, La, and Cs) was not equally distributed across the vertebral centra within individuals. If reworking of the vertebral centra matrix was occurring, particularly among elements, it would be expected that the location of occurrence would be equally distributed reflecting the amount of reworking that has occurred.

Secondly, we observed peaks and troughs in element: Ca ratios that showed sized-based variation within individual white sharks. The differences in the relative element: Ca ratio patterns of individuals across ontogeny, and yet with consistent patterns in select elements across individuals, provides strong evidence that Ca and the other elements are not reworked and that element profiles reflect the animal’s environment. If reworking were occurring, elemental profiles of older individuals would reflect the amount of reworking and the magnitude and presence of peaks and troughs would differ from younger individuals. For example, there was a consistent pattern of increasing amplitude of U:Ca ratios and increasing level of significance with increasing distance from the center of the vertebral centra. The magnitude of the U:Ca ratio peaks early in life (i.e. smaller amplitude oscillations relative to amplitude oscillations after 18 to 23mm) was conserved in vertebral centra from both old and young animals. This systematic increase in the amplitude of oscillations with size may indicate that individuals switch habitat at a similar size and that larger white sharks (~>200 cm PCL) utilize a specific habitat on a seasonal basis where U is present in higher concentrations. White sharks begin to
incorporate marine mammals in the diet at approximately 200cm PCL (equating to ~18 to 23 mm from focus of vertebral centra) (Hussey et al. 2011) and the increase in amplitude of the U:Ca ratio may indicate that the habitat switch is related to diet.

For other elements, for example the Pb:Ca ratios, there was no consistent pattern between the nine individuals and significance levels from SMW analysis were variable. Moreover, the actual concentrations of Pb:Ca ratios were highly variable within and between individuals. Welden et al. (1987) reported that radiometric aging using $^{210}\text{Pb}$ was not a valid technique due to the non-constant uptake of lead and the vertebral centra matrix possibly undergoing reworking. The inter- and intra- individual variation in our results, however, support non-constant uptake of lead with little to no reworking. The variations observed in Pb:Ca ratios are likely not due to reworking, but due to spatial and temporal variation in Pb sources. The concentration of Pb in the marine environment is affected by proximity to anthropogenic sources, particularly leaded gasoline (Wu and Boyle 1997, Boyle et al. 2005). Unleaded gasoline was introduced in South Africa in 1996 with leaded gasoline phased out by 2006 (State of the Air Report 2009). The reduction of Pb from anthropogenic sources would affect ambient lead levels in the marine environment, and the uptake of Pb within the vertebral centra.

The oscillating patterns of element: Ca ratios found in our study were similar to the oscillations of Ca and P reported by Cailliet and Radtke (1987) in the vertebral centra from grey reef (Carcharhinus amblyrhynchos) and common thresher (Alopias vulpinus) sharks, that were used to validate aging techniques. Furthermore, tetracycline and oxytetracycline (OTC) markers in shark vertebrae have been used to validate aging techniques in several elasmobranchs (Wintner and Dudley 2000, Smith et al. 2003,
Cailliet and Goldman 2004) including the white shark (Wintner and Dudley 2000). OTC is an antibiotic, which binds to calcium at the site of active mineralization when injected into the animal and is retained throughout the life of the tissue, thus validating annual growth ring deposition (Frost et al. 1961). If the calcium contained within the vertebral centra was being remodeled, the optical detection of the OTC would be reduced (Frost et al. 1961). Many elements in biomineralized structures substitute for calcium in the crystalline structure; hence if the calcium is not reworked then any elements that substitute for calcium are also likely to be stable.

*Using elemental profiles to aid in determining habitat, movement, and migration patterns*

Our results suggest that the environmental influence on elemental incorporation into elasmobranch vertebral centra is similar to that of otoliths. For example, elements that do not consistently occur across the biomineralized structure or elements that have distinct changes in their profile can indicate when the individual was in a specific environment (where that element naturally occurs) (i.e. the increase in U:Ca ratio ~18 to 23mm from the focus of the vertebral centra). Palace et al. (2007) were able to discern at what point in ontogeny rainbow trout (*Oncorhynchus mykiss*) migrated to an environment contaminated by coal mining runoff by the presence/absence of selenium in their otoliths. Using differences in elemental profiles from blue fin tuna (*Thunnus maccoyii*) otoliths, habitat shifts between the larval stage and the spawning (between southern Java and north-western Australia) and feeding (open ocean) grounds in adult fish were determined (Wang et al. 2009).

The Sr:Ca ratio in biomineralized structures such as corals (Beck et al. 1992, Alibert and McCulloch 1997) and otoliths (Bath et al. 2000, Martin et al. 2004) has been
determined to have a positive relationship with temperature. The decline of the Sr:Ca ratio indicates that larger sharks experience a lower mean ambient water temperature than juvenile animals. This decline in mean ambient temperature is in agreement with catch data and satellite tag studies in South Africa that show young of the year and juvenile sharks predominantly use continental shelf habitat (Cliff et al. 1996, Bonfil et al. 2005, Dicken 2008) in the Eastern Cape, while larger sharks reside at more temperate seal colonies in the Western Cape, make oceanic migrations and exhibit more pronounced deep-diving profiles (Bonfil et al. 2005). Significant oscillatory patterns in the Sr:Ca ratio, however, also support known coastal patrolling patterns between temperate and tropical waters off South Africa (Bonfil et al. 2005).

Increased Ba:Ca is indicative of areas of nutrient-rich upwelling (Wolgemut and Broecker 1970). Sea surface temperatures and the Ba:Ca ratios of corals from the Galapagos Islands were compared and peaks in the Ba:Ca ratio corresponded with periods of cold nutrient-rich upwelling and troughs corresponded with warm, nutrient-poor waters over the period of thirteen years (Lea et al. 1989). While the location of peaks in the Ba:Ca ratios in the white shark were variable between opaque and translucent bands, the consistent oscillatory pattern may suggest seasonal movements between areas of high and low productivity. Along South Africa’s coast, there are two predominating currents; the Benguela and the Agulhas current. The Benguela current carries cold nutrient-rich water along the southwestern coast (Shannon 1985, Griffiths et al. 2010) and has prolonged periods of intense upwelling with high biological production (Andrews and Hutchings 1980). The Agulhas current carries nutrient-poor tropical waters southward along the eastern coast (Walker 1986, Griffiths et al. 2010) and close to
shore along KwaZulu-Natal (Bang 1970, Schumann 1987). The observed oscillations in
the Ba:Ca ratio are consistent with white sharks patrolling along the South African coast
from the nutrient-rich temperate waters off the Western Cape to the less productive
tropical waters (Bonfil et al. 2005). Additionally, inter-individual variation in the
seasonal occurrence of Ba:Ca peaks and troughs indicate variable movement strategies
within the population, which is supported by previous tagging data (Bonfil et al. 2005).
The observed decline in the Ba:Ca ratio identifies that larger sharks may be spending
proportionally more time offshore or frequenting seal colonies in the Western Cape
during periods of low productivity (Bustamante et al. 1995, Ferreira and Ferreira 1996).

Summary

Fs-LA-ICP-MS analysis of white shark vertebral centra was successfully used to
determine ontogenetic elemental profiles of a suite of minor, trace, and rare earth
elements. Our data provide strong evidence that the elemental concentrations in the
inorganic portion of the vertebral centra are stable, which is vital to further the
application of the LA-ICP-MS technique for sharks and other elasmobranchs. Specific
elemental concentrations (Sr, U, and Ba) in all nine white sharks showed oscillatory
patterns related to animal size and season, thus demonstrating their utility to track habitat,
movement and migration patterns. Future work will aim to determine environment
 elemental data to generate elemental-scapes, similar to isoscapes (Bowen et al. 2010,
Hobson et al. 2010), which will assist in interpreting the vertebral centra element data.
Controlled experiments in elasmobranchs, such as those examining the relationship
between environmental element concentrations and elemental concentrations within
otoliths (Bath et al. 2000, Milton and Chenery 2001, Elsdon and Gillanders 2003) will
also verify element uptake relationships in shark vertebral centra. Considering the availability of shark vertebrae in archived collections, LA-ICP-MS analysis has the potential to examine population level variation in habitat, movement, and migration strategies of threatened and difficult-to-study species complementing the more sophisticated spatial tracking methodologies. In addition, there is the potential of archived samples being used for retrospective analysis to determine if population declines and anthropogenic disturbance to the marine environment have modified behavioral patterns over time.
2.5 References


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### Table 2.1

<table>
<thead>
<tr>
<th>Shark ID</th>
<th>Date Caught (mm/dd/yyyy)</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Precaudal Length (cm)</th>
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<td>09/25/2003</td>
<td>F</td>
<td>1.5</td>
<td>184</td>
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<td>06/07/2008</td>
<td>F</td>
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<td>SAL06010</td>
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<td>F</td>
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<td>ZIN06012</td>
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<tr>
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<td>06/04/1992</td>
<td>M</td>
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</table>

Table 2.1- Date of collection, sex, age and size of the nine white sharks sampled from South Africa and used in the analysis of element profiles across vertebral centra by Fs-LA-ICP-MS.
Table 2.2-Isotopes measured in nine white shark vertebral centra with dwell time (time spent analyzing each isotope used in Fs-LA-ICP-MS analysis) and the mean elemental detection limit and percent relative standard deviation (RSD) based on the NIST 610 standard.

<table>
<thead>
<tr>
<th>Isotope Measured</th>
<th>Dwell Time (ms)</th>
<th>Typical Detection Limit (ppm)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>10</td>
<td>99</td>
<td>110</td>
</tr>
<tr>
<td>$^{39}$K</td>
<td>10</td>
<td>210</td>
<td>24</td>
</tr>
<tr>
<td>$^{41}$Ca</td>
<td>10</td>
<td>260</td>
<td>N/A</td>
</tr>
<tr>
<td>Minor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{25}$Mg</td>
<td>10</td>
<td>13</td>
<td>1.5</td>
</tr>
<tr>
<td>$^{66}$Zn</td>
<td>10</td>
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<tr>
<td>$^{67}$Zn</td>
<td>40</td>
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<td>5.8</td>
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<tr>
<td>$^{86}$Sr</td>
<td>10</td>
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<tr>
<td>$^{88}$Sr</td>
<td>10</td>
<td>1.1</td>
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<td>4.8</td>
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<tr>
<td>$^{55}$Mn</td>
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<td>0.41</td>
<td>0.76</td>
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<td>$^{63}$Cu</td>
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<td>$^{120}$Sn</td>
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<td>$^{133}$Cs</td>
<td>40</td>
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<td>$^{137}$Ba</td>
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<tr>
<td>$^{138}$Ba</td>
<td>40</td>
<td>0.013</td>
<td>1.3</td>
</tr>
<tr>
<td>$^{206}$Pb</td>
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<td>0.013</td>
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<tr>
<td>$^{207}$Pb</td>
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<td>0.015</td>
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<tr>
<td>$^{208}$Pb</td>
<td>40</td>
<td>0.011</td>
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<tr>
<td>$^{238}$U</td>
<td>40</td>
<td>0.0010</td>
<td>2.0</td>
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<tr>
<td>Rare Earth</td>
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<tr>
<td>$^{89}$Y</td>
<td>40</td>
<td>0.0070</td>
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</tr>
<tr>
<td>$^{139}$La</td>
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<td>0.0038</td>
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</tr>
<tr>
<td>$^{140}$Ce</td>
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<td>1.6</td>
</tr>
<tr>
<td>$^{141}$Pr</td>
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<td>0.0037</td>
<td>1.5</td>
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<tr>
<td>Isotope Measured</td>
<td>Mean Concentration ± SE (minimum concentration-maximum concentration)</td>
<td>Isotope Measured</td>
<td>Mean Concentration ± SE (minimum concentration-maximum concentration)</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------------------------------------------</td>
<td>-----------------</td>
<td>---------------------------------------------------------------------</td>
</tr>
<tr>
<td>Minor Trace</td>
<td></td>
<td>Trace</td>
<td></td>
</tr>
<tr>
<td>²⁵Mg</td>
<td>2200 ± 8.4 (150-5200)</td>
<td>⁷Li</td>
<td>4.8 ± 0.17 (4.5-5.3)</td>
</tr>
<tr>
<td>⁶⁰Zn</td>
<td>63 ± 0.36 (5.6-210)</td>
<td>⁵³Mn</td>
<td>2.3 ± 0.019 (0.41-19)</td>
</tr>
<tr>
<td>⁶⁷Zn</td>
<td>56 ± 0.32 (5.4-180)</td>
<td>⁶⁵Cu</td>
<td>4.0 ± 0.052 (1.0-23)</td>
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<tr>
<td>⁸⁶Sr</td>
<td>1100 ± 1.6 (490-1700)</td>
<td>⁸³Rb</td>
<td>1.2 ± 0.032 (0.57-6.0)</td>
</tr>
<tr>
<td>⁸⁸Sr</td>
<td>1100 ± 1.4 (290-1800)</td>
<td>¹²⁰Sn</td>
<td>0.63 ± 0.16 (0.26-7.9)</td>
</tr>
<tr>
<td>Rare Earth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>⁸⁹Y</td>
<td>0.013 ± 0.0016 (0.0070-0.80)</td>
<td>¹³⁷Ba</td>
<td>1.9 ± 0.0078 (0.20-5.5)</td>
</tr>
<tr>
<td>¹³⁹La</td>
<td>0.011 ± 0.00041 (0.0038-0.051)</td>
<td>¹³⁸Ba</td>
<td>1.9 ± 0.0071 (0.47-4.7)</td>
</tr>
<tr>
<td>¹⁴⁰Ce</td>
<td>0.015 ± 0.0015 (0.0043-0.70)</td>
<td>²⁰⁶Pb</td>
<td>0.41 ± 0.0053(0.013-3.4)</td>
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<tr>
<td>¹⁴¹Pr</td>
<td>0.055 ± 0.014 (0.0037-0.94)</td>
<td>²⁰⁷Pb</td>
<td>0.39 ± 0.0049 (0.015-3.2)</td>
</tr>
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<td></td>
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<td>²⁰⁸Pb</td>
<td>0.40 ± 0.0051(0.011-3.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean Pb</td>
<td>0.40 ± 0.0051 (0.018-3.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>²³⁸U</td>
<td>0.066 ±0.00035 (0.0027-0.21)</td>
</tr>
</tbody>
</table>

Table 2.3-Mean ± standard error, minimum, and maximum elemental concentration (ppm) in the nine white shark vertebral centra sampled from South Africa
<table>
<thead>
<tr>
<th>Element</th>
<th>Mean ± SE (Minimum-Maximum) Percentage Frequency of Occurrence</th>
<th>Element</th>
<th>Mean ± SE (Minimum-Maximum) Percentage Frequency of Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor</td>
<td></td>
<td>Trace</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>98 ± 0.62 (95-100)</td>
<td>Li</td>
<td>2.8 ± 1.2 (0-8)</td>
</tr>
<tr>
<td>Zn</td>
<td>99 ± 0.32 (97-100)</td>
<td>Mn</td>
<td>99 ± 0.45 (97-100)</td>
</tr>
<tr>
<td>Sr</td>
<td>99 ± 0.31 (98-100)</td>
<td>Cu</td>
<td>79 ± 12 (4-100)</td>
</tr>
<tr>
<td>Rb</td>
<td></td>
<td>Rb</td>
<td>23 ± 6.2 (0.3-55)</td>
</tr>
<tr>
<td>Y</td>
<td>13 ± 4.4 (1-43)</td>
<td>Sn</td>
<td>58 ± 8.1 (28-97)</td>
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<td>La</td>
<td>7 ± 2.9 (0.2-29)</td>
<td>Cs</td>
<td>6 ± 4.5 (0-41)</td>
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<tr>
<td>Ce</td>
<td>14 ± 4.7 (1-44)</td>
<td>Ba</td>
<td>99 ± 0.32 (97-100)</td>
</tr>
<tr>
<td>Pr</td>
<td>2 ± 1.9 (0.2-7)</td>
<td>Pb</td>
<td>97 ± 1.3 (87-100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U</td>
<td>99 ± 0.39 (97-100)</td>
</tr>
</tbody>
</table>

Table 2.4- Mean ± standard error, minimum and maximum element percentage frequency of occurrence for nine white shark vertebral centra sampled from South Africa.
Figure 2.1-Map of KwaZulu-Natal, South Africa. Sample region. lines indicates approximate location of beach protection nets in KwaZulu-Natal. The netted region is currently comprised of 38 individual net installations (see Cliff and Dudley 2011 for specific details on locations).
Figure 2.2-Representative picture of bow-tie section from vertebral centra. Line indicates transect ablated by femtosecond laser ablation inductively coupled plasma mass spectrometry and common terms used to describe vertebral centra.
Figure 2.3-Combined images from SEM-EDS. Transect across the vertebrae starting at the focus of the corpus calcareum (pre-natal) extending to the outer edge (i.e. point of death). Boxes indicate areas of SEM-EDS analysis.
Figure 2.4-Percentage frequency of occurrence of all detected elements across the corpus calcareum of the vertebral centra of six white sharks. Black dots represent location of element ratio occurrence (Element: Ca ratio). Elements are listed with percent frequency of occurrence across the entire vertebrae (excluding pre-natal region). Elements are ranked from least abundant to most abundant for each individual shark.
Figure 2.5-Sr: Ca ratio (left graphs) as determined by Fs-LA-ICP-MS for four white sharks. Gray dashed lines indicate least square linear regression with associated F, $R^2$, and p-values. Dissimilarity profiles (right graphs) obtained from split moving window analysis. Dashed lines indicate 1, 2, and 3 times the standard deviation.
Figure 2.6-Dissimilarity profiles (top graphs) obtained from split moving window analysis and corresponding U: Ca ratio as determined by Fs-LA-ICP-MS (bottom graphs) for three white sharks. Dashed lines indicate 1, 2, and 3 times the standard deviation. Vertical gray line indicates point of increasing amplitude of U: Ca oscillations.
Figure 2.7-Dissimilarity profiles (top graphs) from split moving window analysis and corresponding Pb: Ca ratio as determined by Fs-LA-ICP-MS (bottom graphs) for three white sharks. Dashed lines indicate 1, 2, and 3 times the standard deviation. Note different y-axis scale for each graph (bottom).
Figure 2.8-Dissimilarity profiles (top graphs) from split moving window analysis and corresponding Ba:Ca ratio as determined by Fs-LA-ICP-MS (bottom graphs) for three white sharks. Dashed lines indicate 1, 2 and 3 times the standard deviation as determined above. Gray bars indicate location of opaque (summer) bands and white bars indicate location of translucent (winter) band.
CHAPTER III

VERIFYING THE AGE OF WHITE SHARKS, CARCHARODON CARCHARIAS,
USING CALCIUM PROFILES IN VERTEBRAL CENTRA AS DETERMINED BY
FS-LA-ICP-MS

3.1 Introduction

Conservation of threatened or endangered species is systematically complicated by either data deficiencies or limitations for primary life history parameters, which are critical for constructing appropriate management plans (Heppell et al. 2000). Typically, relative population sizes of threatened species limit biological sampling, confounding data on basic demographic parameters, for example, age-specific length, weight, fecundity, vulnerability to exploitation, maturity status, individual and population growth rates, and mortality rates; data which are the fundamental tenets for management (Walters and Martell 2004). In many instances, accurate assessments of these parameters are even difficult to obtain for large mega fauna that capture the general public’s attention and are identified as flagship species for conservation (Zhan et al. 2006, Blake et al. 2007, Robbins et al. 2011).

Age is one of the most important life history parameters required for effective management plans. This is especially true for marine populations where growth patterns are influenced by the environment and with incomplete knowledge of age structure it is more difficult to predict how a change in environment will effect a species population (Campana and Thorrold 2001). The use of models to predict the effects of environmental change on a population is also growing, and it has been shown that using age-structure as a component of these models is important to obtain accurate results (Botsford et al. 2011).
Determining the age and growth of an organism can be carried out in several ways, including; using known ages to calculate growth rates (Shrader et al. 2006), mark and recapture studies (Clear et al. 2000) or by using calcified structures as recorders of time, such as mammalian teeth (Goren et al. 1987) or teleost otoliths (Secor et al. 1995). Unfortunately, many aging methods, including those that are well established, are not always conclusive, have variability around estimates, and effectiveness often varies across species (Wilson 2003, Cailliet and Goldman 2004). One of the most intensely used and evaluated methods is the aging of teleost fish using otoliths (Campana and Thorrold 2001). Otoliths are ear stones that consist of a unique chemical and physical structure that are laid down in daily and annual rings that can be counted to age individual fish. However, differences in the formation of these rings across species have led to erroneous estimates of age with consequences for management (Beamish and McFarlane 1995). For example, the orange roughy (*Hoplostethus atlanticus*) was fished intensively in New Zealand and Australia, to the point of collapse. The management plan for this species was based on an estimated life expectancy of 20 to 30 years based on otoliths. It was later determined that the otolith method was flawed and that they can live to over 100 years (Smith et al. 1995).

Elasmobranch populations worldwide have decreased in recent years (Stevens et al. 2000, Dulvy et al. 2008) but for effective management there remains a pressing need to quantify and/or verify age structure for many species. Elasmobranchs, however, lack many of the hard parts (otoliths and scales) that are commonly used in fish aging. Therefore other calcified structures such as dorsal spines and vertebrae have been used (Gallagher and Nolan 1999, Watson and Smale 1999, Avsar 2001, Clarke et al. 2002).
Elasmobranch vertebral centra are biomineralized structures containing hydroxyapatite (Ridewood 1921, Moss 1977). The incremental growth of this crystalline structure creates a banded pattern, alternating periods of high mineralization (opaque bands) and low mineralization (translucent bands), which are hypothesized to represent annual periodicity (one opaque band and one translucent band combine to form one growth ring) forming the basis for many aging techniques (see for example, Cailliet and Goldman (2004)). Typically, X-radiography (X-ray) or using stains that bind to Ca (e.g. alizarin red, silver nitrate, and crystal violet) (see review by Cailliet and Goldman (2004)) are used to enhance optical detection of the differences in calcification (i.e. opaque and translucent bands).

While all elasmobranch vertebrae have the same basic framework, the crystalline structure and percent mineralization varies by species (Ridewood 1921, Applegate 1967). Consequently verification (confirming ages obtained with other indeterminate methods) and validation (proving accuracy of ages with a determinate method (Cailliet 1990)) techniques are required to determine if annual ring formation occurs on a species by species basis (see Cailliet and Goldman (2004) for review). Although annual periodicity has been validated in several species (Campana et al. 2002, Smith et al. 2003, Natanson et al. 2006), for others such as the angel shark (*Squatina californica*), it has been determined that growth ring formation was not relative to time but rather to somatic growth (Natanson and Cailliet 1990).

Validating the age of elasmobranchs can be accomplished either through the injection of oxytetracycline (OTC), which binds to the active site of calcification in the vertebral centra and can be used to quantify time at liberty (Wintner and Dudley 2000,
Natanson et al. 2002, Smith et al. 2003, Booth et al. 2011), or bomb radiocarbon dating (Campana et al. 2002, Natanson et al. 2006, Kneebone et al. 2008). Both of these methods have limitations, however, for example injection of OTC requires the recapture of the animal and radiocarbon dating requires individuals to have growth bands formed before 1965 (Campana et al. 2002).

Verifying the age of elasmobranchs can be achieved by quantifying the calcium (Ca) concentration through ontogeny, through the use of X-ray spectrometry (Jones and Geen 1977) or electron microprobe (Cailliet and Radtke 1987). Recently more advanced techniques, such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) have become available (Hale et al. 2006). If this method can be validated, by confirming results obtained by OTC or bomb radiocarbon, LA-ICP-MS would not be limited by requiring recapture or samples from a specific time period. Additionally, femtosecond LA-ICP-MS is now available, which is less matrix dependant and creates minimal thermal effects with smaller particle sizes allowing for higher sensitivity of the system (Shaheen et al. 2008).

White sharks (Carcharodon carcharias) may be one of the most recognizable shark species in the world, however, there are still many gaps in our knowledge of their basic biology including validated ages. Previous attempts to validate age using OTC (Wintner and Cliff 1999) and bomb radiocarbon (Kerr et al. 2006) were inconclusive. A recent study by Tanaka et al. (2011) indicated rapid growth (55 to 75 cm yr⁻¹) and early maturity (males-4 years and females-7 years). Given that white sharks are high trophic level predators, these rates seem to contradict life history characteristics, such as slow growth and late maturity typically associated with K-selected life history strategies.
(Cortés 2000, Frisk et al. 2001). Additionally, there is evidence from photo-identification studies indicating previous size-at-age estimates are incorrect (Anderson et al. 2011). Since ages of white sharks have yet to be validated, it is possible that previous studies using X-ray analysis have underestimated the age for this species and therefore determined faster growth rates and lower ages of maturity.

In an attempt to verify previous age and growth as determined by X-ray analysis, we used FS-LA-ICP-MS to quantify the Ca levels across the vertebral centra of 56 white sharks incidentally captured off South Africa and compared these with ages generated by conventional X-ray methods. In addition, Ca derived growth rates of white sharks were modeled and compared against mark-and-recapture data to evaluate the various aging methods for this important species.

3.2 Methods

Vertebrae preparation

Vertebral centra were obtained from white sharks incidentally caught in beach protection nets off the coast of KwaZulu-Natal between 1991 and 2009. Beach protection nets and drumlines are installed in 27 locations along the shoreline, approximately 400m offshore, in water depths of 10 to 14m. The nets are serviced by the KwaZulu-Natal Sharks Board (KZNSB). For specific details regarding the KZNSB netting and service operations, refer to Cliff and Dudley (2011). Following KZNSB service protocols, all sharks found live in the nets are tagged and released. No white sharks were actively killed for the purpose of this research; all animal mortalities were a result of net capture. At the KZNSB laboratory (Umhlanga Rocks, Durban), fork length (FL) defined as the straight-line distance from the tip of the snout to the fork of the
caudal fin, sex and maturity stage (based upon criteria described in Bass et al. (1973)) were recorded for each individual animal. For one individual, an accurate measurement of FL was not taken at the time of capture. To determine the FL for this individual, centrum radius was regressed against FL for 55 individuals and the equation used to determine FL based on known centrum radii relationships (Wintner and Cliff 1999).

Data from 56 individuals (31 female and 25 male) were analyzed, ranging in size from 139 to 487 cm FL and included three mature males and one mature female (Table 3.1). Vertebral centra were excised anterior to the first dorsal fin and stored frozen (-20°C). Frozen vertebrae were thawed, connective tissue was removed, and measurements of dorsal and lateral diameter, height, and birth diameter (change in angle signifying change in growth rate from in utero to free swimming) were taken. Vertebral centra were then oven dried for 48 hours at 40°C.

X-ray age analysis

Based on previous studies, X-ray analysis of the whole centrum was used to determine the number of growth rings visible (Cailliet et al. 1985, Wintner and Cliff 1999). Whole vertebrae were X-rayed in two groups. For group one (n = 10), X-ray analysis was performed in South Africa. X-rays were taken of the whole centra, in a horizontal plane, using a Philips Optimus Bucky Diagnost TH X-ray unit. The CR cassettes used were Kodak Directview CR with Kodak GP Storage Phosphor screens. The CR reader used was a Kodak Directview CR975. To determine individual band widths, measurements from the focus (mid-point of the notochordal remnant) to the birth mark and to the edge of each opaque and translucent band were made on the X-ray images using Corel Draw (see Wintner and Cliff (1999)). Pixel measurements were then
converted to millimeter measurements using the above-defined measurements of each vertebral centrum. For group 2 (n = 46), whole centrum were X-rayed at CML Healthcare, Windsor, Ontario, Canada. Digital measurements were made following the same protocol as above using Image-Pro Discovery (version 4.5.1.29 © 2002). For both groups, an experienced reader determined age by counting band pairs (consisting of one opaque and one translucent band) as indicated by light and dark banding patterns on the X-ray image following standard procedures (Cailliet et al. 1985, Wintner and Cliff 1999).

**Fs-LA-ICP-MS analysis**

A bow-tie section (0.6mm thick) was cut from the central section of each vertebral centra using an IsoMet® low speed diamond saw (Beuhler-Whitby, Ontario, Canada). The corpus calcareum was then separated from the intermedialia using a rotary cutter. Samples were sonicated in Milli-Q water for 20 minutes then dried for 24 hours in a class 100 laminar flow hood. Using Crystal Bond 509, each sample was attached to a glass slide.

Calcium concentration within the corpus calcareum was analyzed using Fs-LA-ICP-MS at the Great Lakes Institute for Environmental Research, University of Windsor. This system utilizes a Quantronix Integra C® femtosecond laser with a Ti: sapphire laser based on the Chirped Pulse Amplification technique for sampling. Argon gas carried the ablated material to the Thermo Electron X7-II ICP-MS® analyzer. The laser instrumental conditions were as follows: repetition rate 100 Hz; energy 0.103 mJ/pulse; pin hole diameter 2.5mm; and laser ablation spot size 30µm. For additional information on system parameters see Shaheen et al. (2008) and Christiansen et al. (Chapter 2).
Currently, there is no matrix-matched standard for elasmobranch vertebrae, however, using the Fs-LA-ICP-MS system greatly reduces the effects of non-matching matrixes (Shaheen et al. 2008). As such, two analyses of a trace element-doped glass standard (National Institute of Standards and Technology, NIST 612) were run before and after each sample set to calibrate the instrument. Furthermore, an otolith standard, FEBS-1, was created by pressing the powdered standard into a pellet and replicate analyses using Fs-LA-ICP-MS of this standard for strontium concentrations showed no significant difference from accepted values (measured = 2131 ppm, accepted =2059 ppm). These results indicated that NIST 612 was a suitable standard for this study.

Due to the difficulty of cleaning the porous vertebral section using traditional methods, each sampling transect was pre-ablated with the laser to remove any surface contamination. A gas background was run for 60s prior to each data acquisition period, which consisted of 1440s to 5840s, depending on the length of the sample and speed of ablation (5 to 7.11 µm s⁻¹). Background-corrected signals were obtained by subtracting the acquired background analyte mean intensity (counts s⁻¹) of the gas blank from the sample analyte signal intensity (counts s⁻¹).

Calcium data were normalized by factoring in the instrument sensitivity for each run as determined by the NIST 612 standard. Normalized Ca data were plotted against the distance from the focus (any data collected from before the measured angle change was removed, so that only peaks that occurred after birth were counted). Since Ca concentrations are expected to vary with season (one Ca peak and one Ca trough per year), peaks and troughs of each individual’s Ca profile were counted by two readers (herein Ca-R). Prior to counting all 56 individuals, six animals were randomly selected.
for inter-lab calibration to define peak and trough criteria. To investigate any bias and precision in the counts between readers, average percent error (APE), D (Beamish and Fournier 1981) and coefficient of variation (V) (Chang 1982) were determined. Any individuals with an APE greater than 20% were discarded. To further investigate if any bias between readers existed, an age-bias plot was calculated (Campana et al. 1995).

**Model Parameters**

To provide a statistically unbiased estimate of the number of Ca peaks within each vertebral centrum, we modified a standard peak-finding algorithm used in mass-spectrometry (herein Ca-M; O'Haver (2011)). Specifically, we searched through a moving window of each Ca profile to detect peaks where the smoothed first derivative crossed zero and then estimated the peak location using least-squares regression given a second-order polynomial. Three parameters were used to model the first derivative of each Ca profile: slope threshold, which specifies a minimum acceptable slope at the zero crossing; smoother width, which determines the width of the smoothing window applied; and fit width, which specifies the number of data points used to estimate peak locations. These parameters were calibrated using Ca profiles from the two smallest and two largest individuals, with the resulting fits examined by eye, to evaluate model fit. We also explored the range of values surrounding calibrated parameters to understand their sensitivity.

**Comparison of methods**

The results from the three aging methods (X-ray analysis, Ca-R, and Ca-M) were compared using standard growth functions. First, a three parameter von Bertalanffy
growth function (VBGF) (von Bertalanffy 1938) was fit to length-at-age data using the following equation;

\[ L_t = L_\infty (L_\infty - L_0) e^{(-kt)} \]  

1. Where \( L_t \) = length at age \( t \)  
   \( L_\infty \) = maximum theoretical length  
   \( k \) = rate at which \( L_\infty \) is reached  
   \( L_0 \) = size at birth  
   \( t \) = age

A two-parameter VBGF was then constructed with \( L_0 \) constrained to 122cm (smallest confirmed free swimming individual (Casey and Pratt 1985)). Additionally, the Gompertz growth function (GGF) (Ricker 1975) was fitted to length-at-age data derived from each aging method. The Gompertz equation is as follows;

\[ L_t = L_0 e^G (1 - e^{(-kt)}) \]  

2. Where \( L_t \) = length at age \( t \)  
   \( L_\infty = L_0 e^G \) = maximum theoretical length  
   \( k \) = rate at which \( L_\infty \) is reached  
   \( L_0 \) = size at birth  
   \( t \) = age

This model was also run as a two parameter GGF with \( L_0 \) constrained to 122cm.

To determine which model had the best fit, several criteria were used including; Akaike’s Information Criterion (AIC) (Akaike 1973):

\[ \text{AIC} = -2(\text{LL}) + 2p \]  

3. Where \( \text{LL} \) = maximum log-likelihood for each model  
   \( p \) = number of model parameters

and the Bayesian Information Criterion (BIC):

\[ \text{BIC} = -2(\text{LL}) + p \ln(n) \]  

4. Where \( n \) = sample size.

Additionally, coefficients of determinations \( (r^2) \) and MSE were analyzed.
3.3 Results

Age analysis

X-ray analysis indicated that the age of the 56 individual white sharks ranged from a minimum of 0.5 years to a maximum of 16.5 years. In contrast, ages determined using the Ca-R and Ca-M methods ranged from 2 to 48 and 2 to 60 years, respectively (Figure 2.1). For males, the youngest and oldest shark determined by all three methods was the same individual (Table 3.2). For females, the youngest shark varied between methods, while the oldest shark was the same individual (Table 3.2).

For the Ca-R method, reader 2 systematically counted more peaks than reader one (Figure 2.2). Agreement between readers on the number of counted peaks occurred 19.6% of the time, 48.2% were within 1 peak, and 73.2% were within 3 peaks; thus the mean number of peaks from the two readers was used for all further analysis. The APE for the mean Ca-R method for 6 individuals was greater than 20%, consequently these data were discarded; for the remaining data (n=50) the APE was 6.6%, D was 0.066, and V was 0.093.

Maturity

Of the mature individuals, the minimum age-at-maturity was found to vary between methods from 6 years old (X-ray) to 24 years old (Ca-R) and 33 years old (Ca-M) for males and from 16.5 years old (X-ray) to 48 years old (Ca-R) and 60 years old (Ca-M) for females (Table 3.1). There was variability in size-at-maturity for males resulting in an individual that was 8 years old (X-ray), 29 years old (Ca-R), and 37 years old (Ca-M) being immature.

Model parameter
Ca peak determination using Ca-M was influenced most by slope threshold and smoother width. Changing the values of these two parameters greatly impacted the number of peaks detected by the model. The slope threshold was determined to be optimal (creating optimal numbers of peaks for both young and old animals) at 0.00002; the smoother width was determined to be optimal at 30 data points. The fit width was not as influential on determining number of peaks and was determined to be optimal at 40 data points.

**Growth curves and parameters**

The 3-parameter VBGF model had the lowest AIC, MSE and highest $r^2$ for the X-ray generated ages, providing the best fit to the data (Table 3.3). The 2-parameter VBGF model had the lowest AIC, BIC, MSE and highest $r^2$ for both the Ca-R and Ca-M generated ages, indicating best fit (Table 3.3). The growth curves fit for the Ca-R and Ca-M produced age-at-size estimates much higher than by X-ray analysis (Figure 2.3). The smallest $L_\infty$ obtained was from X-ray counts, Ca-M was the next largest and Ca-R estimated the largest $L_\infty$ (Table 3.4).

**Growth rates**

Estimated growth rates of the white shark varied between the three methods (as indicated by k values from growth functions -Table 3.4). The Ca-R and Ca-M methods predicted growth rates of ~9 and ~7 cm\textperiodcentered yr\textsuperscript{-1}, until approximately age 17. After age 17 growth rates gradually decreased to 2 cm\textperiodcentered yr\textsuperscript{-1} at age 48 (Ca-R) and 60 (Ca-M). X-ray generated ages estimated the fastest growth rates, >20 cm\textperiodcentered yr\textsuperscript{-1} until age 6 and > 10 cm\textperiodcentered yr\textsuperscript{-1} until age 13. By age 16 growth slowed to 7 cm\textperiodcentered yr\textsuperscript{-1}. 
3.4 Discussion

By using Fs-LA-ICP-MS, we were able to determine the Ca concentrations along a continuous transect of the corpus calccareum and as expected, obvious troughs and peaks in Ca concentrations were evident for all 56 white sharks. Through quantifying Ca peaks in the vertebral centra of white sharks using Fs-LA-ICP-MS, age estimates were considerably higher, more than three times those determined by traditional X-ray methods. Fs-LA-ICP-MS estimated ages and growth rates were more consistent with age and size estimates determined from mark-and-recaptured and photo-identified white sharks, indicating a much older age at maturity and larger maximum size for this species. Redefining age and growth parameters has serious implications for the management and conservation of this threatened species.

The amount of Ca within shark vertebral centra is influenced by several factors including environment, diet, growth, age, and body condition (Jones and Geen 1977, Natanson 1993, Goldman 2005). Predominantly, Ca has been shown to vary seasonally (high Ca concentrations in summer-opaque bands, and low Ca concentrations in winter-translucent bands), which has formed the basis for visual counting of bands to age sharks (Cailliet et al. 1986, Cailliet and Goldman 2004). Variations in Ca concentrations across the vertebral centra have been previously measured using X-ray spectrometry (Jones and Geen 1977), an electron microprobe (Cailliet and Radtke 1987) and LA-ICP-MS (Hale et al. 2006). In these studies, the number and location of Ca peaks were equal to the number and location of opaque bands, verifying optically-determined ages (Jones and Geen 1977, Cailliet and Radtke 1987, Hale et al. 2006). For the white shark, based on one peak and one trough per annum, the Ca-peak counting methods determined much
older ages than the optical X-ray method. As X-ray analysis is dependent on the contrast produced by differences in the amount of calcification to enhance opaque (as white) and translucent (as dark) bands, it is likely that X-ray analysis is not sensitive enough to discriminate the fine scale Ca concentration changes occurring within the vertebral centra of white sharks.

Basic ecosystem dynamics have been used since Elton (1927) used the pyramid of numbers as an approach to determine relationships between organisms based on feeding ecology. Based on this premise, apex predators such as white sharks should have relatively low population sizes, with slow growth and reproduction rates. Growth rates for white sharks determined using the Ca peak method were slower than previously reported growth rates obtained by X-ray analysis; South Africa ranging from 26 cm•yr\(^{-1}\) for one growth ring to 12 cm•yr\(^{-1}\) for 12 to 13 growth rings (Wintner and Cliff 1999), while juvenile white sharks caught off Japan had growth rates of 55 cm•yr\(^{-1}\) for males and 75 cm•yr\(^{-1}\) for females (Tanaka et al. 2011). Given these fast growth rates, white sharks would mature quickly and the rate of population increase would be high under the assumption that young white sharks have low mortality rates. This contradicts known population sizes for this species (Cliff et al. 1996a, Strong et al. 1996, Chapple et al. 2011), indicating the Ca methods likely provide a more accurate estimate of age and growth rate. Additionally, Ca-R and Ca-M growth rates were more comparable to data from tag and recaptured animals (Table 3.5). Although Tanaka et al. (2011) suggested that the high growth rates for Japanese white sharks were realistic given similar growth observed in an aquarium held shark, this animal was fed a constant daily diet and its growth was not considered typical of wild individuals (Kerr et al. 2006).
Estimates of size for a given age were much smaller for the Ca peak methods than previous studies; the oldest aged individuals using X-ray analysis were a 15 year old female (433 cm FL) and a 13 year old male (404 cm FL) (Cailliet et al. 1985), whereas a 433 cm FL individual would have been approximately 42 (Ca-R) or 61 (Ca-M) and a 404 cm FL individual would have been approximately 35 (Ca-R) or 50 (Ca-M). Recently, photo-identification was used to identify individual white sharks off the coast of California over a period of 22 years (Anderson et al. 2011). Five individual white sharks were identified multiple times over a 16 to 22 year period. As the age at first sighting was unknown this provides a minimum age for each individual. The size at last sighting of these individuals was, 316 cm FL -16 years (2 individuals), 316 cm FL-19 years, 276 cm FL-20 years and 395 cm FL-22 years (Anderson et al. 2011). These minimum size-at-age estimates provide strong evidence that previous age and growth estimates of white sharks using X-ray analysis are incorrect and that the Ca peak data provide a more realistic estimate.

The maximum size of white sharks is commonly reported as 640 cm TL (Bigelow and Schroeder 1948), however, Castro (2011) found that this measurement was unreliable and reported a maximum of 600 cm TL. Mollet et al. (1996) calculated the size of two individual white sharks using morphometric measurements of pectoral fins, jaws, and teeth and reported based on the mean TL that the animals were in the size range of 460 to 700 cm TL and 530 to 820 cm TL, respectively. Converting these sizes to FL using the equation determined by Cliff et al. (1996b), the maximum confirmed size according to Castro (2011) is 527 cm FL and the range is from 403 to 720 cm FL (Mollet et al. 1996). The growth parameters determined from the Fs-LA-ICP-MS Ca peak methods fall within
this range. The $L_\infty$ value for X-ray analysis, however, was lower than the confirmed maximum size and at the lower end of the maximum size range.

The smallest shark analyzed (140 cm FL) was estimated to be 2 or 4 years old using the Ca-R and Ca-M methods, respectively, but still possessed an umbilical scar (stage 5). Viviparous sharks are born with an umbilical scar, which can be used to age individuals, but it generally heals within the first year of life (Duncan and Holland 2006, Hussey et al. 2009). It would seem highly unlikely that a 2- or 4-year-old white shark would have a stage 5 umbilical scar. Consequently, either the Ca method provides an inaccurate age estimate for young white sharks (< 2 years old) or the relationship between Ca profiles and age is more complex than previously determined (Jones and Geen 1977, Cailliet and Radtke 1987) and other factors such as diet, photoperiod, temperature, and environment may be more influential on Ca deposition than previously thought. Two controlled studies conducted on the little skate (*Leucoraja erinacea*) determined that temperature (Natanson 1993) and varying temperature with photoperiod (Sagarese and Frisk 2010) had no effect on band formation. Additional controlled experiments are required to accurately determine what influences Ca deposition in the vertebral centra of white sharks.

Considering there was only one mature female in our sample set, it is possible that the age-at-maturity is lower than determined by our data. However, there were two large immature females in this sample set, a 405 cm FL individual determined to be 13 years (X-ray), 36 years (Ca-R) and 49 years (Ca-M) and a 422 cm FL individual determined to be 12 years (X-ray), 35 years (Ca-R), and 45 years (Ca-M), indicating that minimum age-at-maturity is likely above these ages. Size and age-at-maturity have been found to be
variable for male white sharks in the western North Atlantic with animals maturing between 201 to 403 cm FL (Pratt 1996). This variation could explain the range in size-at-maturity of the males in our sample set (Table 3.1).

Using X-ray analysis, Wintner and Cliff (1999) determined males mature at 8 years old, while Tanaka et al. (2011) determined males matured at 4 years old and females at 7 years old. The Ca peak method determined that age-at-maturity of the white shark would increase 3 to 4 times that determined by X-ray analysis. The X-ray derived age-at-maturity estimates are similar to lower trophic level abundant predators such as the black tip shark (*Carcharhinus limbatus*) which matures at 6 years old (males) and 7 years old (females) (Wintner and Cliff 1996). While age at 50% maturity for the shortfin mako (*Isurus oxyrinchus*) was estimated at 8 years old (males) and 18 years old (females) (Natanson et al. 2006), indicating they mature later than white sharks (using X-ray determined ages). White sharks are high trophic level predators and, therefore, would be considered to have a *K*-selected life history strategy (Cortés 2000, Stevens et al. 2000, Frisk 2001) with a late age-at-maturity, which is supported by the Ca peak determined ages. Few confirmed mature individuals have been aged to date: 3 males (Wintner and Cliff 1999), 3 males and 2 females (Tanaka et al. 2011), and 3 males and 1 female (this study); in order to accurately determine the minimum age-at-maturity of white sharks, more samples will be required.

Species with young age-at-maturity are able to recover from over exploitation more quickly than species with late age-at-maturity (Smith et al. 1998). Using previous X-ray age-at-maturity estimates one would predict that white shark populations would be relatively abundant in regions where they have been protected from sport and commercial
fishing (South Africa and California). In contrast, a first population size estimate for white sharks off South Africa predicted 1279 individuals (Cliff et al. 1996a), while a more recent Bayesian analysis estimated the population of adults and sub-adult white sharks off the Californian coast numbered 219 individuals (Chapple et al. 2011). These population estimates are similar to those obtained in the Spencer Gulf, Australia (192 individuals)(Strong et al. 1996) before sport and commercial fishing were prohibited. These low population size estimates would not be representative of a species with a fast growth rate and low age-at-maturity following protection, further supporting the slower growth rates and later age-at-maturity determined by the Ca peak method.

Although the Ca peak method estimated growth rates, age at maturity, and maximum size that are in agreement with known ages and growth rates obtained from photo-identification and tag and recapture studies, there is still a need to validate counting peaks in Ca profiles as a method to determine age in sharks. To validate the Ca peak method, future work should undertake Fs-LA-ICP-MS analysis of bomb radiocarbon-validated vertebral centra and vertebral centra taken from individuals injected with OTC. Aside from white sharks, the Ca method could also be validated for other elasmobranch species, providing a method that is not limited by the need for animals that lived during a specific time period or requiring recapture after injection with OTC.

We were unable to verify X-ray analysis as an accurate technique to age white sharks; however, the agreement of the Ca peak method with known growth rates, photo-identification observations, and estimated low population levels indicates that Fs-LA-ICP-MS provides a realistic estimate of age and growth. These revised life history parameters for the white shark would indicate a low rate of population increase and high
vulnerability to exploitation. As white sharks are currently listed as vulnerable on the IUCN Red List and on Appendix II of CITES, current management strategies may need to be re-evaluated. Recent studies have indicated white sharks are wide ranging and spend extended periods of time offshore (Bonfil et al. 2005, Domeier and Nasby-Lucas 2008, Bonfil et al. 2010, Jorgensen et al. 2010) where they are not protected. This combined with a lack of knowledge of important habitat (e.g. nursery grounds (Ferreira and Ferreira 1996, Dewar et al. 2004)) indicate further measures may be required to fully protect this species and aid in recovery.
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Table 3.1-Largest immature individual and mature individuals for each sex showing overlap in size and maturity. Ages are shown determined by the three methods including X-radiography (X-ray), peaks in calcium profile as determined by readers (Ca-R), and peaks in calcium profile as determined by model (Ca-M).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Mature</th>
<th>Size (FL cm)</th>
<th>X-ray</th>
<th>Ca-R</th>
<th>Ca-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>N</td>
<td>397</td>
<td>8</td>
<td>29</td>
<td>37</td>
</tr>
<tr>
<td>M</td>
<td>Y</td>
<td>316</td>
<td>6</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>M</td>
<td>Y</td>
<td>360</td>
<td>8.5</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td>M</td>
<td>Y</td>
<td>415</td>
<td>13.5</td>
<td>37</td>
<td>51</td>
</tr>
<tr>
<td>F</td>
<td>N</td>
<td>405</td>
<td>13</td>
<td>36</td>
<td>49</td>
</tr>
<tr>
<td>F</td>
<td>Y</td>
<td>487</td>
<td>16.5</td>
<td>48</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td></td>
<td></td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>---------</td>
<td>---------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>X-ray</td>
<td>Ca-M</td>
<td>Ca-R</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>FL (cm)</td>
<td>0.5</td>
<td>4</td>
<td>2</td>
<td>149.2</td>
</tr>
<tr>
<td>Lowest age</td>
<td>0.5</td>
<td>4</td>
<td>2</td>
<td>149.2</td>
<td>0.5(166)</td>
</tr>
<tr>
<td>Highest age</td>
<td>13.5</td>
<td>46</td>
<td>34</td>
<td>415</td>
<td>16.5</td>
</tr>
<tr>
<td>Smallest animal</td>
<td>0.5</td>
<td>4</td>
<td>2</td>
<td>149.2</td>
<td>1</td>
</tr>
<tr>
<td>Largest animal</td>
<td>13.5</td>
<td>46</td>
<td>34</td>
<td>415</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Table 3.2-Minimum and maximum age and fork length (FL) as determined by the three methods including X-radiography (X-ray), peaks in calcium profile as determined by readers (Ca-R), and peaks in calcium profile as determined by model (Ca-M). The size for the lowest age female varied with method and FL for each individual is represented within parenthesis.
<table>
<thead>
<tr>
<th></th>
<th>AIC</th>
<th>BIC</th>
<th>MSE</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>von Bertalanffy 3-parameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>534.20</td>
<td>540.28</td>
<td>772.26</td>
<td>0.89</td>
</tr>
<tr>
<td>Ca-R</td>
<td>438.01</td>
<td>443.74</td>
<td>352.17</td>
<td>0.95</td>
</tr>
<tr>
<td>Ca-M</td>
<td>516.70</td>
<td>522.78</td>
<td>565.02</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>von Bertalanffy 2-parameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>535.24</td>
<td>539.30</td>
<td>800.32</td>
<td>0.88</td>
</tr>
<tr>
<td>Ca-R</td>
<td>436.89</td>
<td>440.71</td>
<td>350.96</td>
<td>0.95</td>
</tr>
<tr>
<td>Ca-M</td>
<td>514.74</td>
<td>518.79</td>
<td>554.94</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>Gompertz 3-parameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>536.16</td>
<td>542.24</td>
<td>799.83</td>
<td>0.88</td>
</tr>
<tr>
<td>Ca-R</td>
<td>440.43</td>
<td>446.16</td>
<td>369.64</td>
<td>0.95</td>
</tr>
<tr>
<td>Ca-M</td>
<td>516.80</td>
<td>522.88</td>
<td>566.05</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>Gompertz 2-parameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>X-ray</td>
<td>542.47</td>
<td>546.52</td>
<td>910.56</td>
<td>0.86</td>
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<tr>
<td>Ca-R</td>
<td>444.01</td>
<td>447.84</td>
<td>404.71</td>
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</tr>
<tr>
<td>Ca-M</td>
<td>515.41</td>
<td>519.46</td>
<td>561.63</td>
<td>0.92</td>
</tr>
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</table>

Table 3.3-Comparison of Akaike’s Information Criterion (AIC), Bayesian Information Criterion (BIC), mean square error (MSE) and $r^2$ for each growth function and for the three methods including X-radiography (X-ray), peaks in calcium profile as determined by readers (Ca-R), and peaks in calcium profile as determined by model (Ca-M).
<table>
<thead>
<tr>
<th></th>
<th>$L_\infty$</th>
<th>$k$</th>
<th>$L_0$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>von Bertalanffy 3 Parameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>515 ± 56</td>
<td>0.102 ± 0.028</td>
<td>142 ± 11</td>
<td>56</td>
</tr>
<tr>
<td>Ca-R</td>
<td>946 ± 321</td>
<td>0.012 ± 0.006</td>
<td>130 ± 9</td>
<td>50</td>
</tr>
<tr>
<td>Ca-M</td>
<td>670 ± 142</td>
<td>0.014 ± 0.005</td>
<td>120 ± 11</td>
<td>56</td>
</tr>
<tr>
<td><strong>von Bertalanffy 2 Parameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>462 ± 25</td>
<td>0.145 ± 0.018</td>
<td></td>
<td>56</td>
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<tr>
<td>Ca-R</td>
<td>774 ± 115</td>
<td>0.016 ± 0.004</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Ca-M</td>
<td>691 ± 105</td>
<td>0.014 ± 0.003</td>
<td></td>
<td>56</td>
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<tr>
<td><strong>Gompertz 3 Parameter</strong></td>
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<td></td>
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</tr>
<tr>
<td>X-ray</td>
<td>468</td>
<td>0.173 ± 0.031</td>
<td>151 ± 9</td>
<td>56</td>
</tr>
<tr>
<td>Ca-R</td>
<td>621</td>
<td>0.036 ± 0.006</td>
<td>140 ± 7</td>
<td>50</td>
</tr>
<tr>
<td>Ca-M</td>
<td>517</td>
<td>0.034 ± 0.006</td>
<td>129 ± 9</td>
<td>56</td>
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<tr>
<td><strong>Gompertz 2 Parameter</strong></td>
<td></td>
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<td></td>
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<tr>
<td>X-ray</td>
<td>418</td>
<td>0.268 ± 0.022</td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Ca-R</td>
<td>521</td>
<td>0.050 ± 0.004</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Ca-M</td>
<td>494</td>
<td>0.038 ± 0.003</td>
<td></td>
<td>56</td>
</tr>
</tbody>
</table>

Table 3.4 Growth parameters for all growth models with standard error for each parameter (all measurements are in cm and represent FL) for each method including X-radiography (X-ray), peaks in calcium profile as determined by readers (Ca-R), and peaks in calcium profile as determined by model (Ca-M). $L_\infty$ represents maximum theoretical length, $k$ is the rate at which $L_\infty$ is reached, $L_0$ is the size at birth, and $n$ is the sample size.
<table>
<thead>
<tr>
<th>Type of Study</th>
<th>Initial Size (cm)</th>
<th>Location</th>
<th>Time at liberty (days)</th>
<th>Growth (cm)</th>
<th>Growth rate (cm•yr⁻¹)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tag/ recapture</td>
<td>245</td>
<td>South Africa</td>
<td>135</td>
<td>3</td>
<td>8</td>
<td>Dunlop and Mann (2011)</td>
</tr>
<tr>
<td>Tag/ recapture</td>
<td>150</td>
<td>South Africa</td>
<td>366</td>
<td>15</td>
<td>15</td>
<td>Cliff et al. (1996)</td>
</tr>
<tr>
<td>Tag/ recapture</td>
<td>164</td>
<td>South Africa</td>
<td>57</td>
<td>3</td>
<td>19</td>
<td>Dunlop and Mann (2011)</td>
</tr>
<tr>
<td>Tag/ recapture</td>
<td>176</td>
<td>South Africa</td>
<td>573</td>
<td>34</td>
<td>22</td>
<td>Dunlop and Mann (2011)</td>
</tr>
<tr>
<td>Tag/ recapture</td>
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<td>South Africa</td>
<td>429</td>
<td>30</td>
<td>25</td>
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</tr>
<tr>
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<td>942</td>
<td>69</td>
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</tr>
<tr>
<td>Tag/ recapture</td>
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<td>South Africa</td>
<td>940</td>
<td>69</td>
<td>27</td>
<td>Dunlop and Mann (2011)</td>
</tr>
<tr>
<td>Captive</td>
<td>152</td>
<td>California, USA</td>
<td>198</td>
<td>42</td>
<td>78</td>
<td>Kerr et al. (2006)</td>
</tr>
</tbody>
</table>

Table 3.5-Known growth rates of white sharks from previous tag/recapture and captive studies.
Figure 3.1—Calcium profiles as determined by Fs-LA-ICP-MS for three white sharks. Y-axis is normalized calcium concentration. X-axis is the distance from the focus. A. Female- 139 cm FL. B. Male-232 cm FL. C. Female 487cm FL. Black X’s indicate identified peak; profiles on left are peaks detected by CA-R, profiles on right are peaks detected by Ca-M.
Figure 3.2-Age bias plot of Ca profile identified peaks between Reader 1 and Reader 2. Black line indicates one to one line.
Figure 3.3-Optimal growth curves from this study (X-ray-short dashed black, Ca-M-solid black, Ca-R long dashed black) along with growth curves from California (gray solid line), South Africa (short dashed gray line) and Japan (longer dashed gray line). Black x’s indicate minimum age for photographically identified individuals from California. Lengths have been converted to fork length for all studies.
CONCLUSIONS AND RECOMMENDATIONS

4.1 Summary

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used successfully in analyzing teleost fish otoliths to determine a wide range of life history characteristics through ontogeny, including stock identification (Campana et al. 1994), habitat use (Chittaro et al. 2004), natal origin (Thorrold et al. 1998), and connectivity between populations (Gillanders 2002). This method can provide data on multiple elements for the entire life of an individual that is cost-effective, provides more complete information and is less labor-intensive than traditional methods, such as mark-recapture. Researchers investigating life history characteristics of elasmobranchs using LA-ICP-MS are presented with unique challenges, as compared to teleosts, because elasmobranchs lack otoliths, which have been proven to be metabolically inert and the relationship between environment and incorporation of many elements into otoliths have been validated and quantified. However, the vertebrae of elasmobranchs have potential to be used in a manner similar to otoliths, as this structure is biomineralized, and grows incrementally creating growth rings annually for many species (Cailliet and Goldman 2004). The primary goal of this research was to explore the utility of femtosecond LA-ICP-MS (Fs-LA-ICP-MS) to determine elemental concentrations within white shark (Carcharodon carcharias) vertebral centra as a means to quantify life history characteristics of elasmobranchs.

Chapter two focused on methodological aspects, establishing which elements could be detected and their concentration relative to Ca. Additionally, the variation across ontogeny and among individuals, in the corpus calcareum of 9 white shark
vertebral centra was analyzed. A major objective was to determine if elements are stable (i.e. no remixing occurs over time) within the vertebral centra. This is a critical assumption that needs to be validated if elemental profiles in vertebrae are to be used as a tool for life history characterization. First, it was determined that a suite of elements including major, minor, trace, and rare earth elements were present within the corpus calcareum at detectable levels, indicating the suitability of the Fs-LA-ICP-MS technique. Secondly, by examining the elemental profiles through split moving window analysis (SMW) it was determined that elemental profiles between individuals of different sex and size were conserved, indicating that no remixing/reworking of elements occurred throughout ontogeny. By establishing that the vertebral matrix is stable, the relationship between the elements within the vertebral centra and the environment the animal was in at time of formation can be explored and used to assess life history characteristics over ontogeny. Elements, such as strontium (Sr), barium (Ba), lead (Pb), and uranium (U), have the potential to provide information on the environment a shark inhabits and how this changes with increasing animal size (e.g. temperature, inshore vs. offshore movement).

Preliminary investigations revealed trends within and between individuals allowing basic information on habitat use to be elucidated. There was a significant negative linear relationship between Sr and increasing distance from the focus for all individuals possibly indicating a decrease in mean ambient temperature as the individual increases in size. The Ba profiles indicated there was cyclic variation throughout ontogeny, which was correlated with season, indicating possible seasonal movements. The U profiles were cyclic as well; however, amplitude of these variations increased
approximately 18 to 23 mm from the focus indicating a possible habitat shift associated with diet. While Pb profiles had no significant trend between individuals, it is believed the differences occur due to anthropogenic influences (e.g. proximity to areas where leaded gasoline was used).

In chapter three, the calcium (Ca) profiles in the corpus calcareum of 56 individual white sharks were examined using Fs-LA-ICP-MS as a method to verify ages obtained by X-ray analysis. Validated methods for aging sharks are critical for proper management and conservation; however, ages for many species remain unknown. Optically determined ages have been verified for several species using the variation in Ca concentration where a peak in Ca is associated with an opaque band and a trough is associated with a translucent band (Jones and Geen 1977, Cailliet and Radtke 1987, Hale et al. 2006). In this study, Ca concentrations were determined using Fs-LA-ICP-MS where each Ca peak was considered to represent one year, and were counted by readers and statistical models. Each sample was also aged using whole centrum X-ray analysis, which is currently the preferred method for aging white sharks (Cailliet et al. 1985, Wintner and Cliff 1999).

Ca peaks produced estimates of age (2 to 60 yrs) that were much greater than those generated by X-rays (0.5 to 16.5 yrs), and the age of maturity varied from 6 yrs (X-ray) to 24 yrs (Ca-R) and 33 yrs (Ca-M) for males and from 16.5 yrs (X-ray) to 48 yrs (Ca-R) and 60 yrs (Ca-M). Growth curves were fit using age and size data for all three methods of aging. The von Bertalanffy growth function (VBGF) with fixed birth size provided the best fit of the Ca-peak data, for both the readers and statistical models, whereas the 3-parameter VBGF provided the best fit for the X-ray data. Juvenile growth
rates calculated for Ca-R (~9 cm\(\text{yr}^{-1}\)) and Ca-M (~7 cm\(\text{yr}^{-1}\)) were much lower than growth rates calculated from X-ray analysis (>20 cm\(\text{yr}^{-1}\)).

4.2 Implications and Future Work

Chapter 2

Using Fs-LA-ICP-MS, it was determined that the vertebral matrix was stable and a suite of elements were present above detection limits in the vertebral centra of white sharks. These two findings indicate that Fs-LA-ICP-MS is a suitable method for investigating life history characteristics such as habitat usage, movement, and migration patterns in white sharks, and that this method is applicable to the study of other elasmobranch species. The stability of the elements in the shark vertebral centra is currently a major source of debate, and has likely limited the application of element profiles of vertebral centra as a tool for assessing life history characteristics. Given the lack of data on life history for elasmobranchs, concerns about the worldwide decline of many species, and the difficulties associated with working with elasmobranchs, Fs-LA-ICP-MS analysis of vertebrae may prove to be an invaluable tool, providing data and insights that contribute directly to the conservation and management of elasmobranchs and ecosystems in general.

Although, my research provides evidence that elements are stable in elasmobranchs, there is still a need for further validation of this method. If element profiles are to be applied to understanding life history characteristics, then the relationship between element levels and those of the elasmobranchs environment (food and water) need to be quantified. Thus, controlled laboratory studies should be conducted to address these unknowns and also to determine the influence that
environmental factors can have on the incorporation of elements into the vertebral centra. Controlled studies have investigated many aspects of elemental incorporation into teleost otoliths, such as the effects of temperature (Martin et al. 2004, Martin and Thorrold 2005), salinity (Secor and Rooker 2000, Martin et al. 2004), exposure time (Elsdon and Gillanders 2005) and influence of diet on incorporation rates (Farrell and Campana 1996, Walther and Thorrold 2006). While it is possible to undertake controlled experiments with small teleost species, it is not feasible for the white shark and other large elasmobranchs. However, since vertebral mineralization differs between species there may be a species-specific effects on incorporation rates, so caution must be used when applying a single incorporation rate across species.

Until controlled studies can be conducted on large sharks, researchers will have to combine knowledge of known habitats (obtained from tagging studies or sightings) with elemental data for that life stage. Choosing a shark species with a simpler and very well characterized life history to examine Fs-LA-ICP-MS would be a welcome step in the development of this tool for elasmobranchs. Additionally, using known environmental data, environmental-scapes, similar to isoscapes (Bowen et al. 2010, Hobson et al. 2010), could be created to help interpret element data. Development of environmental-scapes would allow researchers to compare unknown elemental profiles obtained from vertebral centra to a known environment.

Using Fs-LA-ICP-MS to investigate life history characteristics has several advantages, for example, archived and museum-collected specimens can also be used, which will allow researchers to gain the maximum knowledge possible without taking any further samples. Additionally, by using archived samples collected over a large time
period, changes at the population level in habitat use and migration over time can be analyzed. This method has a vast potential to aid in answering many questions that remain about white sharks such as habitat use throughout ontogeny (i.e. are there specific nurseries, what is the overlap in juvenile and adult habitat), migration patterns and how they differ throughout ontogeny, and differentiating between stocks. The data provided by this technique could complement data obtained from other methods currently in use (e.g. tag-recapture, photo-identification, and satellite tags) to assist in the formulation of effective conservation and management plans.

White sharks are a wide-ranging species that encounter multiple environments throughout ontogeny (e.g. coastal, offshore, and deepwater) (Dewar et al. 2004, Bonfil et al. 2005, Bruce et al. 2006, Weng et al. 2007), thus they present unique challenges when trying to create effective management plans. White sharks often reside in areas outside of the economic exclusion zones (EEZ) of a country (Bonfil et al. 2005, Bruce et al. 2006, Weng et al. 2007, Domeier and Nasby-Lucas 2008, Bonfil et al. 2010, Jorgensen et al. 2010), which leaves animals vulnerable to unregulated exploitation. In countries where fishermen are not required to land sharks with their fins attached, it is likely that white sharks are caught as by-catch are finned and sold (Dulvy et al. 2008). White sharks are also known to spend significant periods of time as seasonal residents in a specific area (Domeier and Nasby-Lucas 2008, Bonfil et al. 2010). Using environmental-scapes created for these known locations and correlating them to the elemental profile will indicate how often throughout ontogeny the individual was in that habitat.

Specific nursery areas for many of the white shark populations are currently unknown. The newborn portion of the elemental profiles could be compared between
individuals to determine if there are similarities, possibly indicating similar habitat. Few pregnant females have been caught; leaving many questions remaining about the environment they inhabit (Francis 1996, Uchida et al. 1996, Cliff et al. 2000). The elemental profiles prior to the angle change contain data that are influenced by the maternal signature. By determining the influence of the mother’s external environment on the developing pup’s signature, insight into the mother’s environment can be gained. Determining the habitat of pregnant females has important implications for protecting this important life-stage.

Chapter 3

Age is a key life history characteristic as it influences many other metrics, such as age-at-maturity, growth rates, and longevity. The history of fisheries management includes many cases where incorrect aging parameters have been used, resulting in negative impacts on the population levels of a species (Campana 2001). Over estimates of population size and underestimation of fishing mortality can also lead to the collapse of populations, as exhibited by Canadian populations of Atlantic cod (Gadus morhua). Unsustainable catch limits were set and after years of intense fishing, 6 of the 7 cod populations in the Canadian Atlantic had collapsed (Myers et al. 1997).

The white shark is currently listed as vulnerable on the IUCN Red List and on CITES Appendix II, and thus there is a need for accurate age estimates. Additionally, many countries (including Australia, South Africa, and the United States of America) have given full protection to white sharks (Camhi 2008). These preemptive measures were determined to be necessary based on limited knowledge of life history characteristics and low preliminary population estimates (California-219 (adults and sub-
The age estimates obtained in our study indicate individuals mature 3 to 4 times later than previously thought. Additionally, growth rates are much lower than previously determined (approximately 1/2 previous estimates for South African populations (Wintner and Cliff 1999) and 1/5 estimates for Japanese populations (Tanaka et al. 2011)). These large differences in age estimates along with low population estimates indicate the current CITES and IUCN listings may not be stringent enough to protect this species.

Similar to using elemental profiles to determine habitat, controlled studies should be conducted (see above for difficulties associated with large sharks) to evaluate if peaks and troughs in the Ca profile are annual. Changes in mineralization may be caused by a variety of different factors including; seasonal changes, food availability, temperature fluctuations, migration or environmental conditions (Jones and Geen 1977, Natanson 1993, Goldman 2005); controlled studies will allow researchers to determine the extent that mineralization is affected. Additionally, studies should be conducted using vertebrae from species that have their ages validated by injection of oxytetracycline (OTC). This will allow researchers to determine if the peaks in Ca coincide with the time at liberty. Having a larger sample size, which includes more mature animals and young-of-the-year animals, would also increase the confidence in the calculated growth curves.

Importantly, this method can be used not only for white sharks but also for other species of sharks, particularly endangered species and ones designated as data deficient by the IUCN. For species where ages have been validated through other methods such as
OTC and bomb radiocarbon, analyzing Ca profiles will further validate the Ca aging technique. For species that have yet to be aged due to difficulty in visually counting bands, this method provides a simple and accurate alternative.
4.3 References


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