Calcified Structures: The Use of Metal Signatures to Understand Life History and Estimate Age in Lake Sturgeon *Acipenser fulvescens*

by

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Abstract
Understanding population structure in fisheries management is dependent on knowledge of a variety of factors, such as age structure and age at maturation of individuals in that population. Lake Sturgeon are a large freshwater fish found throughout North America’s inland lakes and rivers. Although Lake Sturgeon populations were once numerous, anthropogenic pressure has led to the majority of them being designated as endangered, threatened, or of special concern. Due to their current status, efforts to conserve and restore populations are extensive. In this thesis, I investigated two distinct themes regarding the age structure of Lake Sturgeon, using the chemical composition of their pectoral fin rays. The first experimental chapter focused on testing a relatively new aging approach that uses the annular periodicity in fin ray microchemistry to assist with traditional aging. For this chapter, I compared age precision and accuracy measurements across three age assignment methods (two chemical methods and the traditional visual method) to determine if this new approach could provide true age estimates for known-age fish. Results indicated that both accuracy and precision measurements were higher when using the elemental concentrations to assist with traditional aging ultimately supporting the method as a supplementary aging technique. Throughout the second experimental chapter, I examined the morphological and chemical composition of pectoral fin rays across life stages in a closed population of Lake Sturgeon to determine if physiological influences related to onset of sexual maturity (OSM) could be identified. Results indicated that levels of Ba, Pb, Mn, Mg, and Zn increased after OSM while growth zone width decreased. Using this information, a random forest model was established to discriminate year-specific signatures to before or after OSM. The model could reliably discriminate signatures to before or after OSM with high success (98.8%), suggesting that elemental signatures and growth zone width may prove useful in determining sexual maturity status in Lake Sturgeon. Using the microchemistry of calcified structures to age fish or assess life history status related to reproduction may have the potential to significantly advance current fisheries management strategies that primarily rely on the chemistry of structures to assess environmental impacts.
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Chapter 1: Thesis Introduction

General Introduction

Fisheries management strategies often involve visual or chemical examination of bony structures found in fish. Often referred to as calcified structures due to their chemical composition, otoliths, fin rays, or scales can provide fisheries managers with insight into important population parameters related to individual age, growth rate, diet, or habitat use (Maceina et al. 2007; Allen et al. 2009; Ferenbaugh et al. 2009; Rugg, et al. 2014; Smith and Kwak, 2014; Hessenauer et al. 2018). Understanding the age of individual fish and the age at which they reach maturity is crucial for effectively managing fisheries populations since they can provide valuable insight into stock composition related to fish health, life history, and productivity (Campana 2001; Maceina et al. 2007; Chen et al. 2022). However, there is an overall lack of research regarding the use of elemental concentrations (chemical composition) in structures to both age fish or examine physiological changes such as the onset of sexual maturity. Using the chemical composition of structures to help aid in age assignment is continuously evolving (Heimbrand et al. 2020; Brophy et al. 2021; Frey 2022). Likewise, using elemental signatures to examine physiological change in fish has also proven useful in recent years although it is not commonly practiced (Sturrock et al. 2014; Sturrock et al. 2015; Grammer et al. 2017). The use of chemical signatures in structures could be expanded to obtain valuable information on stock-composition. Throughout this thesis, I explored two separate topics related to Lake Sturgeon conservation using pectoral fin ray trace elemental concentrations quantified via Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). This research aimed to identify and evaluate elemental concentrations in pectoral fin rays of Lake Sturgeon to potentially aid in age assignment as well as to identify individual sexual maturation status. The overall goal of the study was to develop new ways to better manage current stocks using structures that are already commonly removed for other purposes. This chapter will serve as an introduction into current biomineral microchemistry applications, including their potential to aid in fish age estimation, as well as to examine physiological changes that fish may experience throughout their lifetime. Using microchemistry to age or examine physiology in fish is quite new to science and could greatly benefit current fisheries management practices that strictly use the chemistry of structures to examine environmental influences alone.
Literature Review

Elemental Incorporation Pathways in Calcified Structures

Calcified structures are primarily composed of calcium carbonate or hydroxyapatite (CaCO3 in otoliths; Ca5(PO4)3(OH) in fin rays and scales) but they can also frequently contain other similar elements (i.e., Ba, Cu, Fe, Mg, Mn, Sr, Zn) that are substituted for calcium in trace amounts. The incorporation of elements into calcified structures is obtained through specific pathways. Depending on whether the element came from the water column or from dietary ingestion, their ions may enter the bloodstream through branchial or gastrointestinal (GI) absorption (Campana 1999). During uptake across the gills, elements usually enter the blood through passive transport mechanisms including diffusion, which is triggered by the water to blood electrochemical gradient (Evans et al. 1999; Wood 2011). Essential elements (i.e., Ca, Cu, Fe, Mg, Mn, Zn) may also be taken up through their respective transporters (i.e., ECaC, Ctr1, SLC30, SLC39) as well as DMT1 (the divalent metal transporter) (Mims and Prchal 2005; Garrick et al. 2006; Wood 2011). Since non-essential elements (i.e., Au, Ba, Cd, Pb, Sr) are not required for biological function, specific transporters have not evolved, but they are rather taken up by other transporters through a process known as molecular or ionic mimicry (Bridges and Zalups 2005). Similar uptake mechanisms allow ions ingested by diet to enter the blood through the GI tract. Once in the blood, elements can be transferred throughout the body via red blood cells (RBC), bound to plasma proteins, or a combination of the two. In otoliths, ions from the blood enter the endolymph, in which the otoliths are bathed, and then ultimately integrate into the calcified material (Pracheil et al. 2014). Fin spines and rays experience mineralization similar to otoliths and vertebrae (Kerr and Campana 2014; Tzadik et al. 2017) with elements from the blood diffusing into the extracellular fluid surrounding the bone where they either replace calcium on the hydroxyapatite matrix or become trapped within the collagenous fibrils of the organic matrix (Tillett et al. 2011; Kerr and Campana 2014). Essential elements are more tightly regulated compared to non-essential elements throughout the pathway from uptake to mineralization and therefore the organism has greater homeostatic control over their internal concentrations (Wood 2011; Loeppky 2021).
Assigning ages to fish is an essential component of fisheries management that allows biologists to estimate important demographic parameters such as growth rate, maximum age, and age at maturity (Campana 2001; Maceina et al. 2007). There are a variety of calcified structures that can be collected from fish in order to assign ages with most species having a specific structure that results in the best age estimate for them. Currently, otoliths, fin rays, scales, opercules, cleithra, and vertebrae have been used to age fish; but most species use otoliths as the preferred aging structure since they typically provide the most accurate age estimates (Campana and Neilson 1985; Brennan and Cailliet, 1989; Jackson et al. 2007; Rugg et al. 2014; Tzadik et al. 2017). Fisheries managers usually assign ages to fish by identifying and counting visual growth bands across structures that are presumed to be deposited annually (Campana and Neilson 1985). Growth bands, often referred to as annuli, are formed due to a difference in growth deposition between periods of cold and warm seasons, which affects nutrient uptake (Roussow 1957; Tzadik et al. 2017). The innermost portion of the structure, known as the “core”, is presumed to represent the first year of life whereas the outermost area of the structure is thought to represent the last year before the structure was removed. As a result, an age can be estimated by simultaneously counting paired growth bands across the structure.

Seasonal variations can occur in the assimilation of trace elements into fish’s calcified structures. As such, the chemical composition of calcified structures has proven useful to assist with the typical aging process in fish. This potential new strategy, for age validation, uses annular periodicity in the microchemistry of structures. The main premise of these investigations is that changes in the chemical composition of structures correspond with seasonality in growth formation which may be as a result of seasonal differences in the individuals habitat, metabolism, or diet. Historically, elemental oscillations corresponding with visually identified growth bands were identified for a variety of fish species (Red Emperor Lutjanus sebae: Seyama et al. 1991; Arctic Char Salvelinus alpinus: Halden et al. 2000; Brown Trout Salmo trutta, Atlantic Salmon Salmo salar: Limburg et al. 2001; Lake Trout Salvelinus namaycush: Halden and Friedrich 2008; Northern Pike Esox lucius: Friedrich and Halden 2010). In recent years, researchers have used this information to assist with the traditional aging method in an attempt to assign better age estimates for Eastern Baltic Cod Gadus morhua (Hüssy et al. 2015; Heimbrand
et al. 2020; Hüsey et al. 2021), Atlantic Bluefin Tuna *Thunnus thynnus* (Siskey et al. 2016), European Hake *Merluccius merluccius* (Brophy et al. 2019), White Anglerfish *Lophius piscatorius* (Brophy et al. 2021), Black Sea Bass *Centropristis striata* and Atlantic Monkfish *Lophius americanus* (Frey 2022). This process has been referred to as “chemical aging” and usually involves interpreting the different areas of element deposition across structures whether it be by visual inspection (Heimbrand et al. 2020) or the use of a quantitative approach (Hüssy et al. 2015; Siskey et al. 2016; Brophy et al. 2019; Hüsey et al. 2021; Frey 2022).

**The use of Microchemistry of Calcified Structures to Examine Life History**

In addition to aging, some of these structures (notably otoliths and fin rays) have been utilized to evaluate past events that a fish may have experienced in their ambient environment. Due to the correlation between trace element concentrations in structures and elemental levels in the individual's ambient environment at the time of uptake, calcified structures can act as internal recorders of past chemical exposure (Veinott et al. 1999; Campana 1999). Studies that analyze elemental signatures in calcified structures typically aim to better understand specific environmental changes that an individual may have previously encountered, such as diadromous migration, habitat use, or pollutant exposure (Veinott et al. 1999; Veinott and Evans 1999; Clarke et al. 2007; Allen et al. 2009; Nelson et al. 2013; Sellheim et al. 2017; Tzadik et al. 2017). Researchers are able to accomplish this, by comparing elemental levels of annuli to that of the surrounding water chemistry to determine the age when a fish is in a certain environment. For example, several studies have examined elemental compositions of calcified structures (specifically Sr:Ca ratios) to understand past movements between marine and freshwater environments (e.g., White Sturgeon *A. transmontanus*: Veinott et al. 1999; Inconnu *Stenodus leucichthys*: Howland et al. 2001; Beluga *Huso huso*, Russian sturgeon *A. gueldenstaedtii* and Starry Sturgeon *A. stellatus*: Jarić et al 2010; Brown Trout *Salmo trutta*: Taal et al. 2017). Strontium (Sr) concentrations are generally higher in marine environments and therefore Sr:Ca ratios can provide evidence of past marine, estuarine, or freshwater exposure based on element uptake across the gills. Sr has also proven useful to examine small-scale habitat movements since it is strongly correlated with salinity in freshwater environments (Kafemann et al. 2000; Ziegeweid et al. 2021). Furthermore, metals like Sr and Co from ingested hooks have been identified in structures (Alós et al. 2017) and exposure to heavy metal pollution such as Cd, Pb
and Ni from the surrounding environment has been detected in structures providing evidence of habitat degradation for certain fish-stocks (Selleslagh et al. 2016). Additionally, fin ray cross sections have been used to distinguish between hatchery vs naturally reared individuals by biologically marking fish through immersion in known concentrations of rare stable isotopes of a given element (Smith and Whitledge 2010; Carriere et al. 2016; Loeppky et al. 2020).

Physiological Influences on Elemental Concentrations in Calcified Structures

Microchemistry studies commonly assume that elemental levels found in calcified structures directly reflect the ambient environment with few outside influences. Still, physiological processes in the body may also contribute to elemental signatures. Indeed, the chemical composition of calcified structures has been observed to differ between species, populations, sex, and life stages, indicating that physiological processes may have an overall effect that can outweigh environmental signals (Sturrock et al. 2014 and references within). Non-essential elements (i.e., Au, Ba, Cd, Pb, Sr) are more likely to reflect the ambient environment compared to essential elements (i.e., Ca, Cu, Fe, Mg, Mn, Zn) that are actively regulated in the body. Reproductive effort, in particular, appears to have an effect on elemental signatures (Kalish 1991; Granzotto et al. 2003; Clarke and Friedland 2004; Sturrock et al. 2015), notably in Sr and Zn concentrations. Sr is the most commonly reported element in biomineral microchemistry studies because it acts as an environmental tracer of salinity, but studies have shown an increase in Sr levels after the onset of sexual maturity, particularly in marine species (Striped Bass Morone saxatilis: Secor and Rooker 2000; Cockney Snapper Pagrus auratus: Fowler et al. 2005; American Eel Anguilla rostrata: Jessop et al. 2008; Brazilian Flathead Percophis brasiliensis: Avigliano et al. 2015; Australian Salmon Arripis trutta: Hughes et al. 2016; Atlantic Bluefin Tuna Thunnus thynnus: Siskey et al. 2016; Reef Ocean Perch Helicolenus percoides: Grammer et al. 2017). Furthermore, Sr levels have also been reported to spike during spawning periods in Bearded Rock Cod Pseudophycis barbatus (Kalish 1991), Grass Goby Zosterisessor ophiocephalus (Granzotto et al. 2003), Atlantic Salmon Salmo salar (Clarke and Friedland 2004), and European Plaice Pleuronectes platessa (Sturrock et al. 2015). The effect of reproductive effort on Zn concentrations is far less documented but there has been a report of a decrease in Zn concentrations during spawning in sexually mature female European Plaice (Sturrock et al. 2015). Unfortunately, literature regarding physiological influences on element
deposition in calcified structures is lacking with few studies actually examining elements that are thought to be physiologically regulated (Hüssy et al 2020). Even so, elemental concentrations (specifically Ca, Sr, Mn, Cu, Zn, Se, and Pb) in blood plasma have been shown to be highly influenced by growth and maturation status in fish (Hüssy et al. 2020 and reference therein). Because of this, it is reasonable to assume that elemental deposition in calcified structures would be subject to change, especially in fish that invest dramatically in gametic development.

**Study Species: The Lake Sturgeon *Acipenser fulvescens***

Lake Sturgeon are a large, freshwater fish endemic to North America’s inland waters. Current populations range from Hudson Bay to the southern United States with some populations found as far south as the state of Mississippi. Found in both riverine and lacustrine environments, Lake Sturgeon serve as important biological components of the aquatic systems they reside in. Historically, Lake Sturgeon populations were abundant, but due to anthropogenic pressure, most are now listed as endangered, threatened, or of special concern (Bruch et al. 2016). The history of fishing for Lake Sturgeon dates as far back as the 1870s. Originally, their carcasses and meat were commercially harvested to create fuel and gelatin as well as to be consumed as a food source which ultimately led to rapid population declines (Ferguson and Duckworth 1997). In addition to overharvesting pressure, populations suffer from man-made barriers that block important spawning habitats along with habitat degradation caused by logging and farming practices (Peterson et al. 2006; COSEWIC 2017; Moore et al. 2021). Due to their unique biology, population recovery is slow, exacerbating their need for conservation. In contrast to species with much shorter life spans, it can take an individual Lake Sturgeon up to 15-25 years to reach sexual maturity, depending on their sex (Bruch et al. 2016; COSEWIC 2017). Therefore, for the first two decades of their life, a sturgeon may not be contributing to the next generation and population growth. Furthering this issue, once sexually mature, individuals spawn sporadically, with males usually spawning every 1 to 2 years and females every 2 to 7 years, which results in less annual recruitment compared to species that spawn annually (COSEWIC 2017).
Thesis Objectives

Chapter 2: Using Seasonal Oscillations in Fin Ray Microchemistry to Chemically Age Lake Sturgeon *Acipenser fulvescens*

Pectoral fin rays are the most utilized aging structure in sturgeon since sacrifice of the fish is not required during removal. Unfortunately, age validation and/or precision studies for sturgeon have reported unreliable age estimates across taxa, specifically as fish increase in age (Pallid Sturgeon *Scaphirhynchus albus*: Hurley et al. 2004; Shovelnose Sturgeon *Scaphirhynchus platyrhynchus*: Whiteman et al. 2004; White Sturgeon: Rien and Beamesderfer 1994). Currently, age validation studies for Lake Sturgeon also yield unfavorable results, with most studies reporting low accuracy and precision in age estimates after fish reach a certain length (~100cm TL) (Bruch et al. 2009; Hessenauer et al. 2018). Assignment of inaccurate ages to fish could substantially alter our understanding of important population metrics such as growth rate estimates, age at maturity, year-class strength, and possible recruitment (Paragamian and Beamesderfer 2003). The recent development of chemically assigning ages to fish has shown promise when applied to species and/or populations that are often challenging to age traditionally. However, this application has never been used to assist with aging in sturgeon. In this chapter, I examined the potential of chemically assigning ages to Lake Sturgeon using trace elemental oscillations found in pectoral fin rays. A total of 94 known-age individuals (ages 5-21) were aged in order to test the hypothesis that chemical ages could provide more accurate and precise age estimates when compared to using the traditional method alone. Specific objectives were to (1) establish population-specific elements useful for assigning chemical ages across geographic locations, (2) to develop a statistical method to assign ages without reader-bias, and (3) to compare accuracy and precision measurements for three age assignment methods (two chemical age estimation methods and the traditional visual method).

Chapter 3: Using Fin Ray Elemental Signatures and Growth Zone Width to Estimate Onset of Sexually Maturity in a Closed Population of Lake Sturgeon *Acipenser fulvescens*

Current biomineral microchemistry studies focus solely on examining environmental changes an individual might experience throughout life such as habitat movement or pollutant exposure. What is less common is using elemental signatures in structures to predict
physiological changes in the animal, such as gamete development and sexual maturation status. Sturgeon are broadcast spawners, meaning that females release many thousands of eggs into the water column that are then fertilized externally with the coordinated release of sperm from the males (Marshall and Bolton 2007). This reproductive strategy requires significant investment in gamete production from both males and females which could potentially lead to a difference in growth or element deposition in structures after the onset of sexual maturity. Since existing studies on microchemistry in sturgeon do not account for the effects of reproductive effort, the interpretation of life history may be flawed. Additionally, using the chemical composition of structures to examine physiological influences in sturgeon may prove useful, especially if signatures could be used to identify important life history events such as individual maturation. In this chapter, I examined pectoral fin ray elemental and morphological signatures across life stages in a closed population of Lake Sturgeon. I hypothesized that reproductive effort would influence morphological and chemical signatures in fin rays of Lake Sturgeon. Specifically, I predicted that growth zone width would decrease, and essential elemental concentrations would decrease at the onset of sexual maturation. My overall goals of this chapter were to (1) determine if the onset of sexual maturity in Lake Sturgeon influenced element deposition or growth zone formation across pectoral fin rays (2) to develop a model to discriminate year-specific signatures to before or after onset of sexual maturity using the information from the first objective and (3) to test the discrimination model across distinct populations of Lake Sturgeon to determine its potential use to identify onset of sexual maturity across populations.
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Chapter 2: Using Seasonal Oscillations in Fin Ray Microchemistry to Chemically Age Lake Sturgeon *Acipenser fulvescens*

2.1 Abstract

Fin rays are the most common aging structure for Lake Sturgeon *Acipenser fulvescens* but tend to underestimate the true age in older slow-growing individuals (> age 14). Current aging practices involve counting opaque and translucent bands (known as annuli) along the structure that are presumed to represent different seasonal zones. Oscillations of certain trace elements corresponding with annuli have been seen across a variety of fish species with patterns continuing to the marginal edge of the structure. This study explores fin ray microchemistry patterns in Lake Sturgeon of known age (n = 94, age 5-21) to determine their potential use as an alternative or supplementary aging technique. Elements of interest were chosen for age determination analysis by examining the relationship between profile minima and maxima with visual growth zones. Fish were assigned ages using three different methods (1) traditional-interpretation: counting visible growth zones along the structure, (2) chemical-interpretation: interpretation of seasonal variations in elemental profiles along with the visual growth zones, and (3) statistical model: multivariate multiple changepoint analysis with finite differencing using raw elemental profiles. Mean coefficient of variation in estimated age was 11.4% for the traditional interpretation method and 4.04% for the chemical-interpretation method. Mean absolute differences between age estimates and known age were higher for the traditional-interpretation method than for the chemical-interpretation method (Wilcoxon’s signed rank, \( P < 0.001 \)), indicating that that the chemical-interpretation method led to more accurate age estimates. Ages estimated using the statistical model were able to correctly classify 100% of the samples ±1 year in one of the two populations examined but was not able to classify samples from the second population. Our results suggest that chemical aging techniques may be able to provide more reliable age estimates for Lake Sturgeon. Further work is required to determine the applicability of the model to assign ages to fish across populations and structures.
2.2 Introduction

In fisheries management, the use of calcified structures for age and growth analysis has been commonly practiced (Jackson, et al. 2007; Koch and Quist 2007; Maceina et al. 2007; Rugg, et al. 2014; Hessenauer et al. 2018; Branigan, et al. 2019). Age estimates provide managers with a way to assess important population dynamics (such as changes in stock size, mortality rate, mature individuals, and possible recruitment) in order to develop polices to protect and enhance stock-structure (Campana 2001; Maceina et al. 2007). The traditional aging method involves counting annual growth bands along the structure that represent different seasonal zones. Typically, narrow growth bands are presumed to be formed during the winter months due to inactivity and limited feeding whereas wide bands are presumed to be formed during the summer months when water temperature is high (Roussow 1957). Variation in precision and accuracy of age estimates must be at an acceptable level in order for management strategies to be implemented appropriately (Campana 2001). For managers to receive valuable information, choosing the appropriate ageing method and/or structure is essential. As such, age accuracy and among-reader precision has been assessed across structures and taxa to optimize species-specific aging methods (Svedang et al. 1998; Long & Fisher 2001; Campana 2001; Nuevo et al. 2004; O’Connell 2020 and references therein). Currently, otoliths are the most widely accepted aging structure in fish and have been used to provide age estimates for over a century (Reibisch 1899). Fin spines and rays, scales, opercules, cleithra, and vertebrae can also be utilized but are less common throughout the literature (Campana & Neilson 1985; Brennan & Cailliet, 1989; Jackson et al. 2007; Rugg et al. 2014; Tzadik et al. 2017).

In addition to aging, calcified structures can be used to understand past life history events in fish by examining the microchemistry within. Trace elements from the ambient environment (e.g., Mg^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Ba^{2+}, Sr^{2+}, Pb^{2+}) can become incorporated into calcified structures through the process of osteogenesis or protein synthesis (Tzadik et al. 2017), ultimately creating a historical record of past or recent environmental conditions (Veinott et al. 1999; Campana 1999). During osteogenesis, trace elements become trapped in the inorganic component of calcified structure when substituted for calcium (Ca^{2+}) in calcium carbonate crystals (CaCO_3) of otoliths or the hydroxyapatite matrix (Ca_{10}(PO_4)_6(OH)_2) of dermal bone (teleost scales, fin spines and rays) (Tzadik et al. 2017). While during protein synthesis, trace elements are incorporated
into the organic component of the structure, commonly referred to as the proteinaceous matrix (Tzadik et al. 2017).

Biomineralization in hard structures is element-specific and can be under physiological or environmental control (Hüssy et al. 2015). Elemental concentrations have been seen to oscillate seasonally in various fish species with elemental peaks occurring on annuli associated with high growth and minimum concentrations present on the narrow winter zones of the structure (Arctic Char *Salvelinus alpinus*: Halden et al. 2000; Brown Trout *Salmo trutta* and Atlantic Salmon *Salmo salar*: Limburg et al. 2001; Lake Trout *Salvelinus namaycush*: Halden and Friedrich 2008; Northern Pike *Esox lucius*: Friedrich and Halden 2010). Using this information, the microchemistry within hard structures has been utilized to assist in aging fish that are difficult to age traditionally (Hüssy et al. 2015; Siskey et al. 2016; Heimbrand et al. 2020; Brophy et al. 2019; Brophy et al. 2021; Hüssy et al. 2021, Frey 2022). This technique has been referred to as ‘chemical aging’ and involves interpretation of areas of high and low element deposition (trace element maxima/minima). Although this specific application is quite new to science, hard structure chemistry (i.e., cosmogenic radionuclide and radiocarbon dating) has been used in the past to validate age estimates of difficult-to-age species (Heimbrand et al. 2020). Access to known-age fish is crucial when developing a new method for age estimation and current chemical age applications encourage the use of known-age individuals to further validate the method (Hüssy et al. 2015; Heimbrand et al. 2020).

The Lake Sturgeon *Acipenser fulvescens* is one of the largest freshwater fish species residing in North America (exceeding 100kg; Stewart and Watkinson 2004). Belonging to the family Acipenseridae, it is one of 27 species of sturgeon, known for their cartilaginous skeleton, five rows of bony scutes, and prehistoric ancestry. Over time, anthropogenic influences have led to declines in Lake Sturgeon populations resulting in differing statuses (i.e., endangered, threatened, or of special concern) throughout much of its range (Bruch et al. 2016). Due to late-maturation and intermittent spawning, only a small portion of a population spawns each year leading to slow species recovery. Currently, declining factors can be linked to overexploitation, increased water pollution, habitat loss, and man-made barriers that limit migration to important spawning locations (Bruch et al 2016; COSEWIC 2017).
Due to their dwindling status, conservation efforts have been at a high to protect and restore sturgeon (Acipenseridae) populations across North America. Pectoral fin rays (hereafter ray/rays) are the most commonly used structure to age sturgeon species as they can be removed non-lethally. Age validation and/or precision studies have used rays to age both juvenile and adult Lake Sturgeon (Rossiter et al. 1995; Bruch et al. 2009; Hessenauer et al. 2018; Izzo et al. 2021), Shovelnose Sturgeon Scaphirhynchus platorynchus (Whiteman et al. 2004; Jackson et al. 2007; Rugg et al. 2014), Atlantic Sturgeon Acipenser oxyrinchus (Balazik et al. 2012; Stewart et al. 2015; Dunton et al. 2016), Pallid Sturgeon Scaphirhynchus albus (Hurley et al. 2004; Killgore et al. 2007; Koch et al. 2011), Green Sturgeon Acipenser medirostris (Nakamoto and Kisauki 1995), White Sturgeon (Brennan and Cailliet 1989; Rien and Beamesderfer 1994; Paragamian and Beamesderfer 2003), Shortnose Sturgeon Acipenser brevirostrum (Usvyatsov et al. 2012; Gragson and Fox 2022), and Gulf Sturgeon Acipenser oxyrinchus desotoi (Baremore and Rosati 2014). Other structures such as dorsal scutes, dorsal rays, opercules, cleithra, and otoliths have been used but are not as commonly accepted (Brennan & Cailliet 1989; Jackson et al. 2007; Rugg et al. 2014). Unfortunately, rays have been found to provide unpromising age estimates in most sturgeon species with studies reporting low precision for within-reader and between-reader estimates (Pallid Sturgeon Scaphirhynchus albus: Hurley et al. 2004; Shovelnose Sturgeon Scaphirhynchus platorynchus: Whiteman et al. 2004; White Sturgeon: Rien & Beamesderfer 1994). Age validation studies involving techniques to assign known ages to fish have also shown unpromising results, specifically for older slow-growing individuals. Hessenauer et al. (2018) suggests only using rays to age Lake Sturgeon less than 100-cm in total length (TL). Bruch et al. (2009) found a similar cut off range at 100.7cm TL as rays from larger individuals underestimated known ages. High energy demands after onset of sexual maturity may influence growth zone formation causing structures to become unreadable later in life.

Annual oscillations of certain trace elements in fin rays may provide a way to estimate ages of older Lake Sturgeon when distinct bands are no longer visible. In this study, we explored the possibility of chemically aging Lake Sturgeon using elemental profiles found in the pectoral fin ray of known-age individuals. Objectives were (1) to identify useful elements for chemical age estimation across different regions (Winnebago and Manitoba), (2) develop a statistical model/method to assign chemical ages with limited bias using elemental profiles determined
from objective one and (3) compare accuracy and among-reader precision of three aging methods (traditional-interpretation, chemical-interpretation, and statistical-model).

2.3 Methods

Study Area and Sample Collection

Rays from known-aged hatchery-reared Lake Sturgeon were captured during surveys throughout northern Manitoba and the Lake Winnebago System (Wisconsin, USA). Rays were examined via microchemistry analysis to determine whether elemental profiles found in rays can be used to establish an additional or alternative ageing procedure for sturgeon. Two study regions were chosen to examine chemical aging applicability across age classes and geographical locations.

Region 1: Sea Falls (Manitoba, Canada)

Hatchery-reared Lake Sturgeon \(n = 54, \text{ age} 5-8\) were captured via gillnet in Sea Falls, Manitoba between 2012-2015 (Figure 2.1; Table 2.1). Fish were raised at the Grand Rapids Fish Hatchery in Grand Rapids, Manitoba (53°11'04.4"N 99°15'40.7"W) and were stocked at 18-months of life. Individuals were assigned a known age using Passive Integrated Transponder (PIT) tags present at recapture (capture year-hatch year = age). In addition to ray removal, each fish was measured to the nearest mm (total length [TL] and fork length [FL]) and weighed to the nearest gram. All rays from this population were used in a previous study examining elemental signatures during hatchery residence for identification of hatchery-reared Lake Sturgeon (Loeppky et al. 2020).

Region 2: The Lake Winnebago System (Wisconsin, USA)

Hatchery-reared Lake Sturgeon \(n = 40, \text{ ages} 12-21\) were harvested in the Lake Winnebago System, Wisconsin USA between 2020-2022 (Table 2.1). All rays from this population were provided by the Wisconsin Department of Natural Resources. Hatchery reared Lake Sturgeon were raised at the Wild Rose Fish Hatchery in Wild Rose, Wisconsin, (44°11'32.7"N 89°15'03.4"W) and were stocked as fall fingerlings between 2002-2011. Individuals were assigned a known age using Passive Integrated Transponder (PIT) tags present at harvest in 2020 and 2022 (harvest year-hatch year = age). All rays were collected from
Wisconsin Department of Natural Resources staff during the annual spear harvest at a designated registration station (Figure 2.2). In addition to ray removal, each fish was measured to the nearest mm (TL and FL) and weighed to the nearest gram.

**Fin Ray Preparation**

**Manitoba Population**

Rays were prepared by Alison Loeppky (Loeppky et al. 2020) following procedures outlined in Smith and Whitledge (2011). At room temperature, rays were embedded in epoxy resin (5:1 EpoxicureTM resin to EpoxicureTM hardener) and allowed to harden for 24 hours or more. After hardening, rays were sectioned to 1mm at the articulating process using a low-speed saw (ISOMET™). Sections were individually polished by hand using dampened silicon carbide paper, set in 2.54cm rings (n = 5-7 rays/ring) and covered in epoxy to harden. After the epoxy was cured, the surface of the rings was once again polished to eliminate any contamination and to expose rays for analysis.

**Wisconsin Population**

Rays were prepared at the University of Manitoba in a similar manner to the Manitoba population outlined above. First, rays were embedded in epoxy resin and allowed to cure for at least 24hr at 25°C. Rays were then sectioned to 1mm at the articulating process and polished by hand with silicon carbide paper. After polishing, rays were set in 2.54cm rings (n = 1-4 rays/ring) and covered with epoxy. After hardening, the surface was polished to expose the rays and rinsed with ethanol before being stored in Kimwipes™ until analysis.

**Ray Elemental Analysis**

Following standard protocols, rays were analyzed for element deposition via Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA ICP-MS, Perkin-Elmer DRC II) in a similar fashion to Loeppky et al. (2020 and 2021). Epoxy rings were placed individually in the ablation chamber. Line transects (longest axis from core to marginal edge) for each ray were selected and pre-ablated (30-µm beam size, 100 µm/s scan speed, 10-Hz repetition rate, and 60% energy) to once again remove surface contamination. Following pre-ablation, the transect line was re-ablated (30-µm spot size, 5-µm/s scan speed, 20-Hz repetition rate, and 80% energy.
output) with the following trace elements selected to be quantified: As, Ba, Cd, Co, Cu, Fe, Li, Mg, Mn, Pb Zn, S, Se, and Sr. To account for any changes in instrument drift throughout the analyses, glass standards (NIST 610, National Institute of Standards and Technology, Gaithersburg, MD, USA) were ablated before and after each ring. To clear the chamber of past residue, gas blanks were run before each ablation for a total of 30 seconds. Rays were imaged before and after LA-ICP-MS using a polarizing microscope (Nikon Eclipse 50i) allowing the entire scan line and visual growth zones to be visible. When transect lines were too long to be contained within a single image, multiple images were captured and stitched together (Affinity Photo™ imaging software).

Igor Pro graphing software (Iolite version 3.7 package) was used for data reduction (Paton et al., 2011). Calcium (\(^{43}\)Ca) in counts per second (CPS) was used as an internal standard to adjust for variations in ablation yield (i.e., cracks, holes, slope of sample) and baseline concentrations from gas blanks were used to calculate detection limits (LOD) for each trace element. Any value below LOD was not significant and was removed from elemental profiles used in future analysis. Any elements with >25% of their measures below LOD were also removed. Therefore, the final data set consisted of 6 elements (Ba, Mg, Mn, Zn, Sr, and Pb).

Profile Analysis

To determine which profiles to use in chemical age assessments across populations, elemental profile analysis was completed on all 5-year-old hatchery fish from the Manitoba population (\(n = 18\)) and a random subsample (\(n = 15\)) of rays from the Wisconsin population, following similar methods to Hüsey et al. (2015). First, elemental profiles were overlaid on top of images to identify and locate the winter zones along the scan lines. Winter zones were identified for the entire profile in 5-year-old fish but were only identified for the first 5 winter annuli in older Wisconsin individuals since profile analysis along the whole scan line is not necessary. Growth is often specific to the individual so in order to allow comparisons across individuals in each region, relative distance was calculated for each growth zone by dividing each value by the length of its corresponding yearly zone. Element concentrations can also vary between individuals thus relative element concentrations were calculated by dividing each value by the average of the entire profile. Profiles were then Loess smoothed (span = 0.1) by element for each individual and then averaged across individuals in regard to the relative distance of each.
growth zone. Results were plotted along with the location of reader identified winter zones for comparison (Figure 2.3; Figure 2.4) (Hüssy et al. 2015). Elements oscillating seasonally corresponding with visual growth zones were identified and used in future chemical aging methods outlined below. Elemental profiles of Mn, Mg, Zn, and Ba were chosen to age Manitoban fish while profiles of Mn, Mg, Zn, and Pb were selected to assign ages to adult fish from the Wisconsin population (Figure 2.3; Figure 2.4). It is important to note that all Manitoban fish had hatchery signatures due to their time spent in well water, significantly altering the chemistry in the first year of life. Because of this, rays from this population (Region 1) were manipulated to remove the area of the profile corresponding with hatchery residence. Therefore, all profiles correspond to known age minus one-year.

**Aging**

*Interpretation: Visual (Traditional) and Chemical (Novel)*

To compare accuracy and precision for within-reader and between-reader agreement rays were aged twice by three independent readers using the traditional method of counting visible growth zones along the structure. Separately, elemental profiles for each ray were laid over images and analyzed twice by the same readers who were instructed to assign ages based on interpretation of the seasonal variations in the profiles along with the visual growth zones (Heimbrand et al. 2020). The order of rays was randomized between readings to avoid potential bias and readers had no previous knowledge of fish ID, length at capture, or ages estimated by other readers. Traditional aging was completed first and readers were required to wait at least one week before assigning ages using the chemical method. All readers were provided a training set for both visual and chemical aging methods before assigning ages to fish in this study. Both training sets were set up in a way to provide similar instructions to the readers to limit overall bias between method accuracy. Since all readers had highly limited (< 20 known age structures previously read) to no aging experience, they were considered to have the same experience level for both methods.

*Chemical Model*

In addition to the two estimation methods based on reader interpretation, a model was developed in R to assign ages to fish using the raw elemental profiles simultaneously (R Core
Team 2022). First, the profiles were segmented using a multivariate multiple change point analysis from the ECP package (Matteson and James 2014; James and Matteson 2014; Function: e.agglo). This changepoint analysis detects changes along the profiles using hierarchical agglomerative clustering. It was developed by James and Matteson (2014), and the thorough method is outlined therein. To start, each observation is assigned to its own segment and then adjacent segments merge together continuously ultimately maximizing the goodness of fit statistic. Once this is done, the profile is divided into its respected segments with changepoints at the start of each segment. Data points in the same segment are more similar to one another compared to the points in other segments. Therefore, when using this technique on elemental profiles that fluctuate seasonally, the function segments the profile into areas of high elemental concentrations (peak segments) and areas of low elemental concentrations (valley segments).

After the changepoints were calculated, the valley segments were filtered out and used to age the fish as they should correspond to the number of winter zones along the profile. To filter out the valley segments, the diff and sign functions were utilized to difference each observation and assign each changepoint to its respected sign (-1 or +1) to determine if the changepoint was on a negative, or positive slope (Hüssy et al. 2015). A valley segment is defined as the area in between a change point on a negative slope followed by a changepoint on a positive slope. Ultimately, due to the time of the year the fish were sampled, the fish were assigned an age based on the number of valley segments in the profile plus 1 year. Figure 2.5 illustrates an example of elemental profiles used to assign ages to a 7-year-old juvenile fish. The black vertical dashed lines correspond to reader identified winter zones while the horizontal arrows indicate valley segments identified by the chemical model.

**Accuracy, Precision, and Bias**

All statistical analyses were conducted in R statistical software 4.2.2 (R Core Team, 2022). Percent agreement between each method and known age was calculated at exact agreement, ±1 year, and ±2 years (Hurley et al. 2004; Izzo et al. 2020). Percent agreement was also calculated for all 3 levels to measure between-reader and within-reader agreement. Within-reader agreement was calculated as the percent agreement between the first and second readings for each reader, and between-reader agreement was calculated as the percent agreement between readings from all combinations of reader pairs.
Using the FSA package (Ogle et al. 2022; Function: agePrecision), the precision of age estimates was measured for each interpretation method using Chang’s coefficient of variation (CV) (Chang 1982):

\[
CV_j = 100\% \times \sqrt{\frac{\sum_{i=1}^{p} \frac{x_{ij} - x_j^2}{R - 1}}{x_j}}
\]

In this equation \(X_{ij}\) was the \(i\)th age determination of the \(j\)th fish, \(X_j\) was the mean age of the \(j\)th fish, and \(R\) was the number of times each fish was aged (Kadri et al. 2013; Dehghani et al. 2015; Izzo et al. 2021). Therefore, a lower CV estimate is related to higher precision. To further measure accuracy, the absolute difference between age estimates and known age was calculated and averaged across readers for both methods to obtain mean absolute disagreement (MAD) for each sample (Crane et al. 2020).

Using the FSA and ggplot2 packages (Ogle et al. 2022; Function: ageBias | Wickham 2016; Function: ggplot), age bias plots were created to visually evaluate bias across age estimates (Campana et al. 1995). Separate age bias plots were constructed to compare age estimates between-readers, between methods, and between all age estimates compared to known age for each method. All plots were created and visually evaluated in a relatively similar fashion to O’Connell (2020), the most thorough paper currently available regarding aging Lake Sturgeon, to allow comparison between reader bias results.

Statistical Analysis

Utilizing the stats package (R Core Team 2022; Function: wilcox.test), paired sample Wilcoxon’s signed rank tests were implemented to examine differences in CV and MAD for ages estimated using each interpretation method (Izzo et al. 2021). Because current literature does not suggest aging Lake Sturgeon > 100cm TL (~age 15) all analyses were completed across all ages as well as to examine differences across two separate age classes (Class 1: < 15 years, Class 2: ≥ 15 years). Non-parametric techniques were required since the data did not satisfy the assumptions of normality and homogeneity of variance, even after log transformation. For all statistical tests, significance was accepted at \(\alpha = 0.05\).
2.4 Results

Profile Analysis

Distinct annular oscillations of Mg, Mn, Zn, and Ba were chosen to chemically assign ages to rays from the Manitoba population, while profiles of Mg, Mn, Zn, and Pb were chosen to assign ages for rays taken from the Lake Winnebago System. These elements were chosen as profiles were observed to correspond with visually identified annuli with highest element concentrations found between winter zones (Figure 2.3; Figure 2.4). Elemental concentrations of Pb and Sr did not follow visually identified growth zones across the structure in rays from Sea Falls and were not used to chemically assign ages to fish in that population. Elemental concentrations of Ba and Sr did not follow visually identified growth zones across the structure in rays from the Lake Winnebago System and were not used to chemically assign ages to fish in that population.

Accuracy, Precision, and Bias

When both age groups were examined simultaneously (n = 94), the paired Wilcoxon signed-rank test indicated that overall accuracy between age estimates and known age was higher for the chemical-interpretation method (MAD = 0.92, SD = 1.52) compared to the traditional-interpretation method (MAD = 1.45, SD = 1.66) (Wilcoxon’s signed rank, P < 0.001). When comparing age classes separately, accuracy between age estimates and known age for both age class 1 (n = 55) and age class 2 (n = 39) were also higher for the chemical interpretation method (MAD = 0.22, SD = 0.30; MAD = 1.92, SD = 1.95) when compared to the traditional aging method (MAD = 0.73, SD = 0.52; MAD = 2.47, SD = 2.14) (Wilcoxon’s signed rank, P < 0.001) indicating that results were significant for both before and after age 15 even when examined separately (Table 2.2). The chemical interpretation method versus known age had the greatest agreement, with 56.1% exact agreement, 77.8% within 1 year, and 85.3% within 2 years, compared to the traditional interpretation method, which had 37.1% exact agreement, 66.7% within 1 year, and 80.8% within 2 years. Ages estimated using the traditional method differed from known ages by +7 to -12 years, whereas the chemical method produced estimates that ranged from +4 to -8 years. Differences between known-age agreement for both age classes and specific readers are outlined below (Table 2.3). Known age agreement for the chemical model was at 65% exact agreement and 100% within 1 year for rays sampled from Sea Falls, Manitoba.
The model did not run the same way on samples from the Wisconsin population and therefore estimates were not reported. Details describing this issue are outlined in the discussion.

Age bias plots indicated that estimates made using the chemical method were normally closer to the 1:1 agreement line compared to estimates made using the traditional method, although these differences were not great (Figure 2.6). Reader 1 tended to slightly underage rays after age 8 using the traditional method though estimates across all ages were still relatively close to the 1:1 agreement line. When chemically aging rays, reader 1 tended to slightly underestimate ages starting at age 18. Reader 2 tended to have similar results across methods starting to underage at age 18 using the traditional method and age 19 using the chemical method. Reader 3 tended to overestimate rays from younger fish when assigning ages traditionally, with underaging beginning at age 18. When assigning ages using the chemical method, reader 3 began to underestimate ray ages at 18 but ages from younger fish were closer to or within the 1:1 agreement line (Figure 2.6).

Precision of age estimates varied between aging methods. The paired Wilcoxon signed-rank test \((n = 94)\) indicated that among-reader precision was higher for the chemical interpretation method \((\text{CV} = 4.04\%, \text{SD} = 4.07)\) compared to the traditional aging method \((\text{CV} = 11.14\%, \text{SD} = 8.12)\) (Wilcoxon’s signed rank, \(P < 0.001\)). When comparing age classes separately, precision of age estimates among-readers for both age class 1 \((n = 55)\) and age class 2 \((n = 39)\) were also higher for the chemical interpretation method \((\text{CV} = 3.54\%, \text{SD} = 4.82; \text{CV} = 4.75\%, \text{SD} = 2.58)\) when compared to the traditional aging method \((\text{CV} = 13.26\%, \text{SD} = 9.21; \text{CV} = 8.13\%, \text{SD} = 5.01)\) (Wilcoxon’s signed rank, \(P < 0.001; P < 0.001\)) indicating that results were significant for both before and after age 15 when examined separately. Between-reader agreement was highest for the chemical interpretation method, with 60.5% exact agreement, 88.3% within 1 year, and 97.3% within 2 years, compared to 34.7% exact agreement, 67.9% within 1 year, and 84.1% within 2 years for the traditional interpretation method. Likewise, within-reader agreement for the chemical interpretation method also showed the greatest agreement, with 69.2% perfect agreement, 92.2% within 1 year, and 98.7% within 2 years, compared to the traditional interpretation method, which had 45.1% exact agreement, 79.1% within 1 year, and 90.4% within 2 years. Differences between reader agreement for both age classes and specific reader combinations are outlined below (Table 2.4 and Table 2.5).
Between reader age-bias plots indicated that estimates were similar across readers for both methods. Although, when assigning ages traditionally reader 3 tended to have slightly greater age estimates in rays less than 10 compared to readers 1 and 2 (Figure 2.7). Between method age bias plots indicated that age estimates using the chemical method for reader 1 were similar to ages estimated using the traditional method across all ages while age estimates between methods were similar for reader 2 until approximately age 22. Reader 3 tended to have lower age estimates when using the chemical method for ages 8-10 but then estimates were similar until approximately age 21.

2.5 Discussion

Results indicated that ages estimated using the chemical method were more accurate when compared to ages estimated using the traditional method. In total, 60.1% of readings were in correct agreement with known age, when using the chemical method, compared to 39.9% when using the traditional method. When chemical profiles were available, readers had an easier time distinguishing certain growth zones that may have been incorrectly counted when solely relying on identifying visual zones using ray images alone. Readers noted that growth zones were usually more widely spaced near the fin ray core becoming more closely packed towards the marginal end of the structure, although this was not always the case. A small subsample of rays had areas with less growth followed by wider bands indicating that narrow zones may sometimes occur sporadically across the structure. A common issue leading to inaccuracies in age estimates seemed to be a result of readers mistaking multiple growth zones as one due to growth zone crowding. Like previous studies, distinguishing the first growth zone may have also led to higher inaccuracies in traditional ages specifically in rays sampled from Sea Falls, Manitoba (n = 54, ages 5-8). Samples from this location had hatchery signatures (Loeppky et al. 2020) and therefore when assigning ages chemically, the first year of life (first growth zone) was able to be identified without reader bias which may have led to higher classification success.

Known age agreement was higher for ages estimated using the chemical method but both methods resulted in >80% agreement within ±2 years which is similar or higher when compared to other sturgeon studies: 89% ±2 years for Hurley et al. (2004; Gulf Sturgeon) and 75% ±2 years for Izzo et al. (2021; Lake Sturgeon). Age bias plots suggested that under aging seemed to be the most common issue leading to inaccuracies in age estimates specifically at or around age
18 for both methods although there was still some under-aging occurring in younger years. Rossiter et al. (1995) reported similar results with age accuracies declining at age 18 in Lake Sturgeon also resulting from under-aging. Other studies for Lake Sturgeon expect deviations from known age to occur at ~100 cm TL which was around age 14 for the population being examined (Bruch et al. 2009). This could occur for a variety of different reasons, but most researchers suggest that this may coincide with onset of sexual maturity which may increase energy demands to other areas of the body ultimately resulting in a decrease in energy allocated to somatic growth and growth zone formation in calcified structures. This is feasible, as Lake Sturgeon are known to invest greatly in gamete development due to the large number of eggs/sperm required during broadcast spawning. However, this investment is most likely elevated in females and the point of deviation from known age may be sex-specific.

Accuracy results for the chemical model were low when all rays were used; however, separating rays by population indicated that the model could potentially be used to estimate ages of Lake Sturgeon from Manitoba (65% correct, 100% within ±1 year). Elemental oscillations from this population were distinct and clear and the model had limited issues segmenting areas of high or low elemental concentrations leading to high classification success. Profiles from the Wisconsin population were noticeably less clear. Elemental profiles were often more uneven or patchy, and areas of high elemental concentrations or low were less divisible leading to low classification success. The mechanisms controlling element deposition in calcified structures are marginally understood with current reports documenting highly inconsistent or uninformative results (Hüssy et al. 2020, and references therein). Oscillations in elemental concentrations may be as a result of extrinsic or intrinsic factors or a mixture of the two. In this study, the elements primarily considered under physiological control (Mg, Mn, Zn, and Pb) are likely to be subject to seasonal variation due to food supply or metabolic processes (Halden et al. 2000). Temperature affects the metabolic rate of organisms (Hüssy et al. 2020), which may result in differences in elemental deposition between seasons, especially for fish in temperate climates. Elements strongly linked to dietary sources may show seasonality as a result of high to low nutrient uptake throughout the year. Cool winter periods are associated with inactivity and limited feeding whereas warmer, summer periods are often associated with increased feeding and rapid growth (Halden et al. 2000). The mechanisms controlling seasonality in elements primarily under environmental control (presumably Ba in this study), are even less understood, but may correlate
with temperature or salinity variation throughout the year (Hüssy et al 2020). Hence it is believed that issues relating to model classification success will most likely be population specific and therefore this method will need to be evaluated on other populations in order to determine its ability to accurately assign ages to fish without bias.

Results indicated that age estimates from the chemical method were more precise when compared to ages estimated using the traditional method. When compared to the range of CV values reported in other sturgeon studies (7.8%, Rien and Beamesderfer 1994; 4.8% Stevenson and Secor 1999; 8.2%, Jackson et al. 2007; 1.8%, Balazik et al. 2012; 5.6%, Stewart et al. 2015; 3.8%, Dunton et al. 2016), CV for the traditional method (11.34%) was on the higher end while CV for the chemical method (4.04%) was more moderate. Both CV values were reasonable when compared to studies examining across reader precision in Lake Sturgeon specifically (17%, O’Connell 2020; 12.4%, Izzo et al. 2021), with CV for the chemical method quite low, suggesting that elemental profiles may result in higher precision when aging Lake Sturgeon. When chemically aging rays, 62.1% of samples were assigned the same age by two readers while only 35.5% were assigned the same age by two readers when aging traditionally. There are no current studies reporting within-reader or between-reader agreement in Lake Sturgeon but values for this study were comparable to reports for other sturgeon studies. Within-reader agreement was higher for ages estimated using the chemical method but both methods resulted in >90% agreement within ±2 years which is similar when compared to what has been documented for Gulf Sturgeon (78.2%, Hurley et al. 2004) and Shovelnose Sturgeon (100%, Jackson et al. 2007). Between-reader agreement was also higher for ages estimated using the chemical method but both methods resulted in >80% agreement within ±2 years which is similar when compared to what has been reported for Shovelnose Sturgeon (81%, Jackson et al. 2007) but lower than what has been reported in Gulf Sturgeon (100%, Hurley et al 2004).

Age bias plots indicated that readings were similar between readers across all ages and methods. Results of between-reader age bias plots visually demonstrated that readers assigned ages similarly since most observations fell within the 1:1 agreement line (Figure 2.7). Age estimates compared to known age were also similar across readers with most observations reasonably assigned until under aging began (Figure 2.6) indicating that reader bias was not greatly affecting accuracy results. In contrast, O’Connell (2020), found that reader experience
was extremely important to their study as reader bias was evident. Specifically, readers with the most amount of Lake Sturgeon aging experience assigned higher and more accurate ages compared to the reader with the least amount of Lake Sturgeon aging experience (O’Connell 2020). All readers for our study had the same level of experience (highly limited to none) for both methods which most likely led to similar results across readers furthering the argument that reader experience is critical in assigning accurate and precise age estimates. Because of this, the use of a quantitative approach, such as the model described in this study, could help to limit systematic biases that may result from reader experience.

Even though ray age estimates may not be reliable in older Lake Sturgeon as previous studies have suggested, when using rays to age sturgeon, the use of elemental profiles to assist in age estimation may result in more precise and accurate results. For populations showing distinct annual oscillations coinciding with growth zones, the chemical model may provide a way to assign ages without reader bias but when oscillations are less clear, the model will likely assign inaccurate estimates and should be avoided. Further work is required to determine the applicability of the model to assign ages to fish across populations and structures.

2.6 Acknowledgements

Authors would like to thank Alison Loeppky for assistance and guidance with fin ray preparation, Panseok Yang for assistance with LA-ICP-MS operation, and Mathew Thorstensen for guidance with R programming. Manitoba fin rays were provided by Manitoba Hydro and the Keeyask Hydropower Limited Partnership. Wisconsin fin rays were provided by the Wisconsin Department of Natural Resources. Funding for this research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC)/ Manitoba Hydro Industrial Research Chair awarded to WGA and Science Enhancement of Grant Stipends support from the Faculty of Science at the University of Manitoba to AT. We are grateful to have been able to conduct this work on the University of Manitoba campus which is located on the original lands of the Anishinaabeg, Cree, Oji-Cree, Dakota, and Dene peoples, and on the homeland of the Métis Nation.
### 2.7 Tables

Table 2. Sampling locations and sample information for hatchery fish used in this study. Total length and mass were taken at recapture. Class 1 = known ages < 15, Class 2 = known ages ≥ 15. Age was determined by referencing PIT tag number.

<table>
<thead>
<tr>
<th>Age Class</th>
<th>Sea Falls, Manitoba</th>
<th>Winnebago System, Wisconsin</th>
<th>( n )</th>
<th>Total Length (mm)</th>
<th>Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>54</td>
<td>1</td>
<td>55</td>
<td>885 ± 73</td>
<td>3598 ± 952</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(680-1024)</td>
<td>(1720-5225)</td>
</tr>
<tr>
<td>Class 2</td>
<td>0</td>
<td>39</td>
<td>39</td>
<td>1200 ± 136</td>
<td>10180 ± 4872</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(993-1554)</td>
<td>(4944-27715)</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>40</td>
<td>95</td>
<td>1012 ± 186</td>
<td>6129 ± 4466</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(680-1554)</td>
<td>(1720-27715)</td>
</tr>
</tbody>
</table>

**Note:** Values of total length and mass are given as mean ± standard deviation with the range of values in parentheses below.
Table 2.2. Results of Wilcoxon signed rank tests comparing CV and MAD for ages estimated for each interpretation method. Descriptive statistics of mean and standard deviation are included. Class 1 = estimates for ages < 15, Class 2 = estimates for ages ≥ 15, All = estimates for both age classes. CV is an estimation of precision while MAD is an estimation of accuracy.

<table>
<thead>
<tr>
<th></th>
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<th>Chemical</th>
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<th>95% Confidence Interval</th>
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<td></td>
<td>N</td>
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<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>CV</td>
<td></td>
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<tr>
<td>Class1</td>
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<td>13.26</td>
<td>9.21</td>
<td>3.54</td>
<td>4.82</td>
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<td>Class2</td>
<td>39</td>
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<td>5.01</td>
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<td>All</td>
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<td>11.14</td>
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<td>MAD</td>
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<td>0.73</td>
<td>0.52</td>
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<td>All</td>
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<td>1.45</td>
<td>1.66</td>
<td>0.92</td>
<td>1.52</td>
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Table 2. 3. Known-age agreement for both reader interpretation aging methods for all hatchery fish \((n = 94, \text{ages} \ 5-21)\). Age Class 1 = known age < 15, Age Class 2 = known age ≥ 15.

<table>
<thead>
<tr>
<th>Method</th>
<th>Known-Age Agreement (%)</th>
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<th>Reader 1</th>
<th>Reader 2</th>
<th>Reader 3</th>
<th>Mean</th>
<th>Age Class 2</th>
<th>Reader 1</th>
<th>Reader 2</th>
<th>Reader 3</th>
<th>Mean</th>
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<td></td>
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<tr>
<td>Within 1 year</td>
<td>75.5 53.6 31.8 53.6</td>
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<td>21.8</td>
<td>23.1</td>
<td>16.7</td>
<td>20.5</td>
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<td>Within 2 years</td>
<td>98.2 87.3 64.5 83.3</td>
<td></td>
<td>55.1</td>
<td>51.3</td>
<td>43.6</td>
<td>50.0</td>
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<tr>
<td></td>
<td>99.1 98.2 82.7 100.0</td>
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<td>65.4</td>
<td>61.5</td>
<td>57.7</td>
<td>61.5</td>
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<td>Exact Agreement</td>
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<td>100.0 100.0 97.3 99.1</td>
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<td>Within 2 years</td>
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<td>73.1</td>
<td>65.4</td>
<td>70.5</td>
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Table 2.4. Within-reader agreement for both reader interpretation aging methods for all hatchery fish \( (n = 94, \text{ages 5-21}) \). Age Class 1 = known age < 15, Age Class 2 = known age ≥ 15.

<table>
<thead>
<tr>
<th>Method Classification</th>
<th>Within-Reader Agreement (%)</th>
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<tr>
<td>Exact Agreement</td>
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<td>45.5</td>
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<td>23.1</td>
<td>51.3</td>
<td>17.9</td>
<td>30.8</td>
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<tr>
<td>Within 1 year</td>
<td>94.5</td>
<td>96.4</td>
<td>70.9</td>
<td>87.3</td>
<td>74.4</td>
<td>84.6</td>
<td>53.8</td>
<td>70.9</td>
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<td>Within 2 years</td>
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<td>98.2</td>
<td>94.5</td>
<td>97.0</td>
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<td>89.7</td>
<td>71.8</td>
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<tr>
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<td>87.3</td>
<td>90.9</td>
<td>92.1</td>
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<td>51.3</td>
<td>41.0</td>
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<td>89.7</td>
<td>79.5</td>
<td>85.5</td>
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<tr>
<td>Within 2 years</td>
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<td>100</td>
<td>100</td>
<td>97.4</td>
<td>100</td>
<td>94.9</td>
<td>97.4</td>
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</table>
Table 2. Between-reader agreement for both reader interpretation aging methods for all hatchery fish ($n = 94$, ages 5-21). Age Class 1 = known age < 15, Age Class 2 = known age ≥ 15. Cross 1 = Reader 1 x Reader 2, Cross 2 = Reader 1 x Reader 3, Cross 3 = Reader 2 x Reader 3.

<table>
<thead>
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<th>Between-Reader Agreement (%)</th>
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<td>Age Class 1</td>
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<td>Cross 2</td>
<td>Cross 3</td>
<td>Mean</td>
<td>Age Class 2</td>
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<td>Traditional Exact Agreement</td>
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<td>30.9</td>
<td>31.4</td>
<td>40.5</td>
<td>30.8</td>
<td>24.4</td>
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<td>Within 1 year</td>
<td>88.2</td>
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<td>72.0</td>
<td>75.0</td>
<td>56.4</td>
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<td>Within 2 years</td>
<td>98.2</td>
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<td>83.6</td>
<td>88.3</td>
<td>90.4</td>
<td>75.0</td>
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<td>Chemical Exact Agreement</td>
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<td>90.0</td>
<td>81.2</td>
<td>50.0</td>
<td>33.3</td>
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<td>Within 1 year</td>
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<td>94.6</td>
<td>97.3</td>
<td>86.5</td>
<td>73.1</td>
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<tr>
<td>Within 2 years</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>96.8</td>
<td>92.9</td>
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</tbody>
</table>
2.8 Figures

Figure 2.1. Map of the study area of Sea Falls, Manitoba.
Figure 2. Map of the study area of the Lake Winnebago System, Wisconsin, USA: including Lake Winnebago, Lake Poygan, Lake Butte des Morts, and Lake Winneconne. Black stars represent WDNR stations where pectoral fin rays were collected during annual winter harvest in 2020 and 2022.
Figure 2. 3. Time series plot comparing elemental profiles to winter identified annuli of pectoral fin rays collected from Lake Sturgeon sampled from Sea Falls, Manitoba (Region 1). Plots depict relative elemental profiles of Ba, Mg, Mn, Pb, Sr, and Zn (mean ± s.d., n = 18) averaged across multiple individuals in regard to the relative distance of each annulus. Black lines represent elemental profile means with the grey shaded region corresponding to the standard deviation. Horizontal bars correspond to the location of reader identified winter zones.
Figure 2. 4. Time series plot comparing elemental profiles to winter identified annuli of pectoral fin rays collected from Lake Sturgeon sampled from the Lake Winnebago System, Wisconsin (Region 2). Plots depict relative elemental profiles of Ba, Mg, Mn, Pb, Sr, and Zn (mean ± s.d., n = 15) averaged across multiple individuals in regard to the relative distance of each annulus. Black lines represent elemental profile means with the grey shaded region corresponding to the standard deviation. Horizontal bars correspond to the location of reader identified winter zones.
Figure 2.5. Raw elemental profiles of a 7-year-old, 914mm TL, juvenile Lake Sturgeon captured in Sea Falls, Manitoba in 2014. Due to hatchery signatures, the first year of life was removed from all profiles for chemical age estimation. Horizontal arrows indicate valley segments identified by the chemical age model corresponding to winter zones along the profile. Vertical lines represent winter zone location consensus from chemical age readers. Thus, this fin ray was aged to 6 years old (# of winter zones + 1) for both chemical age methods ultimately corresponding to known age of the profile.
Figure 2. 6. Age-bias plots comparing known-age agreement for Lake Sturgeon \((n = 94)\) collected in Sea Falls, Manitoba and the Lake Winnebago System, Wisconsin from 2012-2022. Plots on the left (A, C, E) represent ages estimated from the traditional interpretation method compared to known age and plots on the right (B, D, F) represent ages estimated from the chemical interpretation method compared to known age. Each panel depicts mean (points) and 95% confidence intervals of ages estimated on the y-axis to corresponding known ages on the x-axis. The dashed gray lines represent 1:1 agreement in age estimates and age estimates are considered statistically similar when CI’s overlap the line.
Figure 2. 7. Age-bias plots comparing between-reader agreement for Lake Sturgeon \((n = 94)\) collected in Sea Falls, Manitoba and the Lake Winnebago System, Wisconsin from 2012-2022. Plots on the left (A, C, E) represent ages estimated from the traditional interpretation method and plots on the right (B, D, F) represent ages estimated from the chemical interpretation method. Each panel depicts mean (points) and 95% confidence intervals (CIs) of ages estimated by the reader on the y-axis to corresponding age estimated by the reader on the x-axis. The dashed gray lines represent 1:1 agreement in age estimates and age estimates are considered statistically similar when CI’s overlap the line.
Figure 2. Age-bias plots comparing between-method agreement for Lake Sturgeon \( n = 94 \) collected in Sea Falls, Manitoba and the Lake Winnebago System, Wisconsin from 2012-2022. The plot depicts mean ages in pectoral fin ray estimates from the traditional interpretation method (y-axis) in relation to ages estimated using the chemical interpretation method (x-axis) for three independent readers. Each panel depicts mean (points) and 95% confidence intervals of ages estimated on the y-axis to corresponding age estimates on the x-axis. The dashed gray lines represent 1:1 agreement in age estimates and age estimates are considered statistically similar when CI’s overlap the line.
2.9 References


Chapter 3: Using Fin Ray Elemental Signatures and Growth Zone Width to Estimate Onset of Sexually Maturity in a Closed Population of Lake Sturgeon *Acipenser fulvescens*

3.1 Abstract

Understanding individual age at sexual maturation for long-lived species like sturgeon would provide insight into potential year-class strength and recruitment dynamics allowing for more effective management practices. Calcified structures have been used to predict past life history events in fish, with most studies focusing on the morphology or elemental signatures found in otoliths, pectoral fin rays, or scales. These applications are built around the assumption that trace element incorporation is strongly correlated to environmental availability at time of uptake, but physiological factors related to metabolic rate, reproductive status, health status or ontogeny may also contribute to elemental signatures. This study aims to analyze the chronology of trace elemental profiles as well as changes in annual growth formation across Lake Sturgeon pectoral fin rays to determine potential onset of sexual maturity (OSM). Elemental signatures (measured via LA-ICP-MS analysis) and growth zone width were compared before and after presumed OSM and used to build a classification model to discriminate year-specific signatures before or after OSM across distinct populations. Random forest (RF) was used, and all possible combinations of variables were examined to determine optimal accuracy in classification assignment. Elemental profiles were relatively constant throughout the core and early years but began to visually increase towards the middle to end of the fin ray on average, at age 26 in females and 15 in males. Levels of Ba, Pb, Mn, Mg, and Zn were found to increase after OSM while Cu remained the same across life stages. RF classification demonstrated an overall accuracy of 98.8% across populations. Ba was the most important variable related to classification success followed by zone width, Pb, Mn, Mg, Zn, Cu, and Sr. Future work will focus on developing predictive models to investigate if elemental signatures and growth zone width can be used to predict the exact year of OSM in Lake Sturgeon.
3.2 Introduction

Calcified structures have been used to predict past life history events in fish, with most studies focusing on elemental signatures found in otoliths, fin rays, or scales. These applications are known as biomineral microchemistry studies and are built around the assumption that trace element incorporation is strongly correlated to environmental availability at the time of uptake. Specifically, that elemental concentrations remain relatively constant throughout the pathway from uptake to deposition in tissues, ultimately providing a historical record of past or recent environmental exposure (Campana 1999). Because of this, most applications examine habitat-specific elemental signatures such as diadromous migration (Allen et al. 2009; Smith and Kwak, 2014), pollutant exposure (Nelson et al. 2015; Daros et al. 2022), diet (Ferenbaugh et al., 2009), natal origin, (Loeppky et al. 2020; Stewart et al. 2021; Mikheev et al., 2022), or habitat use (Arai et al., 2003; Feitosa et al., 2020). However, the physiology of the animal may also contribute to elemental signatures (Sturrock et al. 2015; Hüsey et al. 2020), as studies report certain otolith elemental concentrations to be species or sex-specific (Reis-Santos et al. 2008; Chang and Geffen 2012; Sturrock et al. 2015).

Various elemental pathways are responsible for incorporating elements into calcified structures, with ions entering the bloodstream through the GI tract or branchial system depending on the mineral's origin (i.e., diet or ambient water) (Campana 1999). Once absorbed by the blood, elements diffuse into the extracellular fluid surrounding the structure and can ultimately replace calcium within the organic crystal of the hard structure (Tillett et al. 2011; Kerr and Campana 2014). Internal factors such as metabolic rate, reproductive status, or ontogeny may influence elemental deposition due to changes in protein binding along the pathway from uptake to tissue incorporation (Hüsey et al. 2020). Specifically, energy demands related to spawning and maturation status may lead to a differential deposition of elements in calcified structures after the onset of sexual maturity in individuals that invest dramatically in gamete production when spawning. Reproductive effort can be an exhaustive activity and has been linked to changes in elemental signatures coinciding with spawning in Bearded Rock Cod Pseudophycis barbatus (Kalish 1991), Grass Goby, Zosterisessor ophiocephalus (Granzotto et al. 2003), Atlantic Salmon Salmo salar (Clarke and Friedland 2004), and European Plaice Pleuronectes platessa (Sturrock et al., 2015). Specifically, Sr and Zn concentrations in European Plaice otoliths have been found to be significantly correlated to the gonadal somatic index in sexually mature females.
(Sturrock et al. 2015). Other elements such as Ca, Mg, Mn, Cu, Se, and Pb are also assumed to be under some type of physiological control related to development and maturation, with Se and Cu also potentially related to reproductive effort (Martinho et al. 2020; Hüssy et al. 2021).

In order to examine if physiological influences related to reproductive effort are present in calcified structures, it is crucial to choose a study population that does not experience a dramatic change in ambient water chemistry during spawning events since external influences must be limited, such as, a population that does not migrate large distances but still invests a significant amount of energy into gamete development. For this reason, this study involved a closed population of broadcast spawning Lake Sturgeon *Acipenser fulvescens* that has been closely monitored since 2001. Lake Sturgeon are one of the largest freshwater fish species in North America, known to significantly invest in gamete development due to the amount of eggs/sperm required during broadcast spawning. An adult female may even mature as many as 500,000 to 1,000,000 eggs in preparation for spawning (Scott and Crossman 1998), which requires significant investment in advance of the spawn. This investment in gamete development, particularly when the individual first matures, may lead to differential deposition of elements in calcified structures related to sexual maturity status.

In this study, we examined elemental concentrations in pectoral fin rays (hereafter, rays) of Lake Sturgeon from a closed population and compared them to rays collected from populations that are geographically distinct. We hypothesized that reproductive effort related to onset of sexual maturity (hereafter, OSM) would result in a detectable difference in the elemental composition or morphology across rays. Our overall goal was to quantify elemental signatures before and after presumed OSM and build a classification model using this data to predict sexual maturity status in Lake Sturgeon. Specific objectives were to (1) compare elemental signatures and growth zone width before and after OSM (2) develop a model to discriminate year-specific signatures and zone width to before or after OSM in a closed population; (3) determine the model’s ability to correctly predict maturity status in rays from the same population; and (4) determine the model’s ability to correctly predict maturity status in rays across geographically distinct populations.
3.3 Methods

Data Sets

Dataset 1: Black Lake Population

Rays for this study were split into two distinct data sets (Table 3.1). Dataset 1 consisted of rays collected from the Black Lake Sturgeon Population in Michigan, USA. All rays were collected from known sexually mature individuals of known sex, verified by either gamete release upon capture and/or genetic sex markers from dorsal fin clip analysis (Kanefsky et al. 2022). The Black Lake Sturgeon Population was chosen as the target population for this study since individuals are confined to a small geographic range with dams present both upstream and downstream of the lake they inhabit year-round (Figure 3.1). The total available habitat for the population consists of 8 km downstream in the Lower Black River, 11 km upstream in the Upper Black River, and 40.99 km² within Black Lake (Smith and Baker 2005). Since 2001, adults have been captured annually during the spring spawning survey. The use of passive integrated transponder tags (PIT tags) have been implemented along with Radio Frequency Identification (RFID) tags and antennas that allow the individual to be scanned and identified upon capture allowing confirmation of sex and sexual maturity status based on past survey documentation.

Dataset 2: Seven Other Geographically Separated Populations

Dataset 2 consisted of ray samples available from a reference collection at the University of Manitoba. Individuals greater than 33 (estimated age) were assumed to be sexually mature and therefore were selected for this study (COSEWIC 2006). This dataset consisted of 20 rays from seven distinct populations that were geographically separated from the Black Lake population. These rays were sampled from 1999-2007 in Churchill River Manitoba (n = 1), Kiskittogisu Lake, Manitoba (n = 2), Nelson River, Manitoba (n = 9), Norman Dam, Ontario (n = 1), Sturgeon Falls, Ontario (n = 3), Whitedog River, Ontario (n = 1), and the Lake Winnebago System, Wisconsin (n = 3) (Figure 3.2).

Fin Ray Preparation and Elemental Analysis

Ray preparation and elemental analysis for samples from the Black Lake population (Dataset 1) are outlined below. All other rays (Dataset 2) were used in previous studies and
background information for these studies as well as ray sampling, preparation, and details of Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) settings can be found in Appendix A.

Rays were prepared for Laser Ablation Inductively Coupled Plasma-Mass Spectrometry following procedures outlined in Smith and Whitledge (2011) and Loeppky et al. (2020). Rays were laid out in rows, set in epoxy resin, and cured for at least 24 hrs under a laminar flow hood. Using a BUEHLER ISOMET™ low-speed saw (0.3mm blade thickness), rays were sectioned to 1mm at the articulating process and polished by hand using dampened silicon carbide paper (30-µm, 9-µm, and .3-µm). After surface polishing, rays were set in 2.54cm rings (n =1-2 rays/ring) and covered in epoxy to cure overnight. The surface of the rings was polished (Buehler Meta SERV 250 grinder-polisher) to expose rays. All rings were rinsed with distilled water and ethanol and stored in Kimwipes™ until analysis.

Rays were scanned (LA ICP-MS, Perkin-elmer DRC II) in a similar fashion to Loeppky et al. (2020 and 2021). Epoxy rings were placed separately in the ablation chamber and line transects for each ray were created to quantify elemental concentrations along the longest axis of the structure from the first growth zone to the marginal edge. All line transects were pre-ablated (30-µm beam size, 100 µm/s scan speed, 10-Hz repetition rate, and 60% energy) to remove surface contamination before final quantification. Transect lines were then ablated (30-µm beam size, 5-µm/s scan speed, 20-Hz repetition rate, and 80% energy) outward from the core with the following trace elements selected to be quantified: Ba, Ca, Cu, Fe, Li, Mg, Mn, Pb Zn, S, Se, and Sr. Thirty second gas blanks were run before each ablation to clear residue between samples. To account for possible instrument drift, glass standards (NIST 610, National Institute of Standards and Technology, Gaithersburg, MD, USA) were ablated (110-s scan time, 30-m beam size, 5-Hz repetition rate, and 65% energy) before and after each ring. After ablation, rays were imaged using a polarizing microscope (Nikon Eclipse 50i) (2-20x magnification) to ensure the entire transect line and corresponding growth zones were distinguishable for future age assessments.

Using Igor Pro graphing software (Iolite version 3.7), data reduction was performed to correct for changes in ablation yield during analysis. An internal standard was determined using Ca (43Ca) in counts per second (CPS). For each ray, baseline concentrations measured from gas blanks were used to calculate limits of detection (LOD) for each trace element. Any value falling
below LOD was not significant and was therefore removed. Elements with >25% of their measures below LOD and that did not show any transient increase across the scan line were removed (Croghan and Egeghy, 2003). Therefore, the final data set consisted of 7 elements (Mg, Mn, Cu, Zn, Sr, Ba, Pb).

Data Preparation

Element concentrations can often vary between individuals and populations due to ambient environment availability and individual-specific physiological influences. Due to issues regarding differences in elemental concentrations between individuals and locations, relative elemental concentrations were calculated for each ray by dividing each value by the average over the entire profile (Hüssy et al. 2015). To examine if OSM has an influence on elemental concentrations present in rays, the scan-line portions (µm) for each individual growth zone were determined to attain year-specific elemental signatures (outlined below). Two readers worked together simultaneously to identify each growth zone (a white opaque band followed by a dark translucent band) and determine the corresponding scan line portion for each year. Elemental profiles were laid over ray images to help aid in growth zone determination since seasonal oscillations of certain elements may provide more accurate age estimates in Lake Sturgeon (Taylor et al. Submitted).

To obtain year-specific signatures before and after presumed OSM mean elemental concentrations and growth zone width were calculated for each year before and after age 15 in males and 20 in females. Though the exact year the fish became sexually mature is unknown, a standard age was chosen based on current documented age ranges for OSM in Lake Sturgeon populations (COSEWIC 2017). For rays collected from individuals without confirmed sex (Dataset 2; n = 20) presumed OSM was set at 20, the same as females.

Statistical Analysis

All statistical analyses were conducted in R statistical software 4.2.2 (R Core Team, 2022). Even after log transformation, data did not meet the assumptions of normality and homogeneity of variance to warrant parametric analysis, therefore non-parametric methods were used. Utilizing the stats package (R Core Team 2022; Function: wilcox.test), paired sample Wilcoxon’s signed-rank tests were used to test for differences in each individual variable before
and after OSM. Using the vegan and ggplot2 packages (Oksanen et al. 2022; Function: metaMDS | Wickham 2016; Function: ggplot), a nonmetric multidimensional scaling (nMDS) ordination plot was created to visually evaluate similarities (or dissimilarities) between before and after OSM for both datasets. Mean concentrations for all elements and growth zone width were also calculated before and after OSM to summarize variable variation for both categories.

In total 78 rays were collected and successfully analyzed from the Black Lake Population (Dataset 1), and these were randomly split (70:30) to create training \( n = 55 \) and test samples \( n = 24 \). In order to build a classification model to discriminate year-specific signatures to before or after the OSM, random forest (RF) was implemented on the training sample \( n = 55 \) using the randomForest package (Liaw and Wiener 2002; Function: randomForest). This function has a built-in cross-validation method that uses random sampling with replacement to calculate an overall Out-of-Bag classification error rate (Breiman, 2001). The 8 predictor variables in the final data set (Ba, Cu, Pb, Mn, Mg, Sr, Zn, and growth zone width) were used to analyze a total of 255 potential formula combinations. The model with the best classification success (lowest Out-of-Bag classification error rate) was then chosen to be used on the test samples from Dataset 1 \( n = 24 \) and Dataset 2 \( n = 20 \). All packages allowing for mixed effect random forest (merf: Hajjem et al. 2012; MixRF: Wang and Chen 2016; MEml: Ngufor et al. 2019) currently cannot account for a categorical response variable and therefore were not able to be used in this study.

Because the exact age at OSM was not known for samples in the test dataset, the chosen model was run on signatures for the last growth zone as well as year 5 to determine the model’s ability to correctly discriminate year-specific signatures to before or after OSM. Therefore, each ray from the test sample had two year-specific signatures tested; one to represent before OSM and one to represent after OSM. All rays used in the test dataset were from known sexually mature individuals or those that had an estimated age of ≥33 and therefore the last growth band was believed to represent a signature after OSM (COSEWIC 2006). Likewise, there has been no documentation of Lake Sturgeon either male or female being sexually mature at age five and the variables calculated for this year were believed to represent a signature before OSM. The “mean decrease in accuracy”, which measures classification accuracy deprivation when a variable is excluded from the analysis (averaged across all trees), was used to quantify variable importance for the RF model (Breiman, 2001; Wright et al. 2018). Utilizing the ULT package (Maugoust
2022; Function: multkw) a multivariate extension of the nonparametric Kruskal-Wallis test (He et al. 2017) was used to quantify dissimilarity in mean elemental concentrations and zone width among all possible combinations before and after OSM to further determine potential variable importance. For all statistical tests, significance was accepted at $\alpha = 0.05$.

3.4 Results

Variable Differences Before and After OSM

Elemental trends in rays were seen across individuals for both datasets. When assigning scan-line portions to corresponding growth zones, readers identified visual elemental increases occurring across certain profiles (Figure 3.3; Figure 3.4). Elemental levels were relatively constant throughout the core and early years but began to visually increase towards the middle to end of rays. This elemental increase was seen to occur on average at age 26 in females and 15 in males. Readers determined the age at increase by first agreeing when it occurred for each individual and matching it to its corresponding age. The elements that increased varied by individual but usually occurred in Ba, Mg, Mn, Pb, and Zn.

Likewise, for both datasets, values of Ba, Mg, Mn, and Pb were significantly higher after OSM while growth zone width was significantly lower (Figure 3.5; Table 3.2). Sr values did not show clear patterns across datasets with values significantly higher after OSM for Dataset 1 but statistically similar before and after OSM in Dataset 2. Zn values also did not show clear patterns across datasets with values significantly higher after OSM for Dataset 1 ($P < 0.001$) but only slightly higher after OSM for Dataset 2 ($P = 0.04$). Cu values were similar before and after OSM across datasets showing no differences before and after OSM. The nMDS ordination plot showed full dissociation of ray signatures and growth zone width before and after OSM with clear overlap between both datasets indicating that variables for each category were similar across geographic locations (Figure 3.6).

Random Forest Classification Success

Classification accuracy for the training samples was highest when all variables were included in the model with an overall Out-of-Bag error rate of 12.87%. The final model (using all 8 predictor variables) demonstrated an overall classification accuracy of 98.8% when run on the test samples, correctly classifying 100% of ray signatures from the same population as
training samples (Dataset 1) and 97.5% of ray signatures from geographically separated populations (Dataset 2). There was only one observation that was misassigned being classified as after OSM rather than before OSM (Table 3.3).

According to the multivariate Kruskal-Wallis analysis performed on all variables simultaneously, ray elemental signatures and growth zone width were significantly different in both datasets before and after OSM (H = 170.9, df = 8, P < 0.001), indicating the usefulness of all variables for model discrimination. Ba, Pb, and zone width were found to be the three most important model discriminators since they had the lowest p-value when multivariate Kruskal-Wallis was performed on all three variable combinations (H = 167.7, df = 3, P < 0.001). Results of “mean decrease in accuracy” measurements further validated this finding indicating that Ba was the most important variable related to classification success (70.6%), followed by zone width (60.7%), Pb (40.6%), Mn (39%), Mg (25.3%), Zn (22.7%), Cu (19.7%), and Sr (11.5%) (Figure 3.7).

3.5 Discussion

Discrimination Success

Random forest models based upon ray elemental signatures and growth zone width successfully discriminated year-specific signatures to before or after OSM in Lake Sturgeon from eight distinct populations with an overall classification accuracy of 98.8%. Discrimination success was highest when all variables were included in the model, but the three most discriminatory variables were Ba, Pb, and growth zone width. Based on the three most discriminatory variables, RF classification success was still high at 96.3%. The results of the RF classification model were further supported by visual inspection of the nMDS ordination plot, which revealed clear dissociation of ray signatures and growth zone width before and after OSM with a definite overlap between the two datasets indicating that changes to signatures were comparable across geographic locations. These findings suggest that OSM may influence elemental and morphological signatures across rays in Lake Sturgeon.

Variable Differences Before and After OSM

Reproductive effort, leading to a proliferation in gamete production may inhibit or decelerate somatic growth which was reflected in the annual deposition width on rays. Growth...
zone width was one of the most important variables for discrimination success as it was found to significantly decrease after OSM which is consistent with previous research as numerous studies have reported a decrease in otolith or ray growth zone formation after OSM for a variety of fish species (Burbot *Lota lota*: Pulliainen and Korhonen 1994; European plaice *Pleuronectes platessa*: Rijnsdorp and Storbeck 1995; Orange Roughy *Hoplostethus atlanticus*: Francis and Horn 1997; Atlantic Cod *Gadus morhua*: Denechaud et al. 2021; European Sprat *Sprattus sprattus*: Reglero and Mosegaard 2006). This phenomenon has also been documented in sturgeon with studies using growth zone width to help identify OSM in female Persian Sturgeon *Acipenser persicus*, Starry Sturgeon *Acipenser stellatus* (Bakhshalizadeh et al. 2017), and Lake Sturgeon (Roussow 1957).

Certain elements seemed to be under physiological control related to OSM. Specifically, levels of Ba, Mg, Mn, Pb, and Zn were higher after OSM and were seen to visually increase towards the end of the fin ray, proportionate with current documented age ranges of OSM in Lake Sturgeon. Age at sexual maturity in Lake Sturgeon is sex-specific and can vary by population but usually occurs later in females (~15-30) than in males (~12-20) (COSEWIC 2017). Elemental increases visually identified across structures were found to occur on average at age 24 in females (range 14-34) and 15 in males (range 9-21) suggesting that elements may begin to increase at or at least around the same time as OSM. In contrast, Cu levels remained the same across life stages and, thus, may not be affected by sexual maturity status.

Factors that could cause element deposition in hard structures to change after OSM are currently unknown. Sturgeons have not yet been studied for the effects of maturation on ray elemental concentrations and reports regarding otolith mineralization in other species have been highly inconsistent or uninformative (Hüssy et al 2020). According to available research, the direct mechanistic basis for changes in otolith concentration of Ba, Mg, Mn or Cu following sexual maturation is unknown (Hüssy et al. 2020); but when examining element concentrations in otoliths from female European Plaice, Sturrock et al. (2015) found a relationship between sexual maturation and otolith element concentrations where increases in otolith Sr and Zn were significantly correlated with gonadal somatic index. Furthermore, evidence for the effects of growth rate on otolith elemental concentrations has been well documented as otolith Mg, Zn, and Cu concentrations are reported to be positively correlated with fish growth. Whereas Ba, Mn,
and Sr show a conflicting relationship to growth since positive, negative, and non-significant relationships have been reported (Hüssy et al. 2020 and references therein).

The effect of OSM on elemental concentrations in sturgeon might be attributed to Ca requirements throughout the reproductive cycle. Ca is an essential element required for a variety of physiological processes related to reproduction in fish, such as the formation of vitellogenin (a yolk precursor protein that facilitates oocyte development) in females as well as for the regulation of sperm motility in males (Allen et al. 2009; Bondarenko et al. 2017). In addition, Ca is present in both male and female gametes, suggesting an increase in Ca requirements over the course of the reproductive cycle. Furthermore, total plasma Ca was reported to increase significantly during reproduction in sexually mature females and to increase marginally in males (Allen et al. 2009). This investment, which causes the rerouting of Ca for progenitive use, may result in a decrease of available Ca during the calcification of hard structures. Indeed, scute/scale quality (i.e., size and toughness) has been seen to diminish in older slow-growing members of the Acipenser family (Allen et al. 2009 and references therein). Divalent cations (e.g., Mg$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Mn$^{2+}$, Ba$^{2+}$, Sr$^{2+}$, Cu$^{2+}$, Pb$^{2+}$), such as those seen to increase after OSM in this study, replace Ca randomly within the crystalizing structure. As a consequence, a decrease in ray percent Ca could result in higher replacement by other similar divalent cations which would be reflected in higher elemental concentrations.

Sr and Cu were the least important discriminators in the RF model. Sr levels for this study varied by geographic location indicating its importance as an environmental tracer not as strongly influenced by OSM. Indeed, Sr is the most common and often only element examined in adult sturgeon microchemistry studies since it is considered an environmental indicator allowing both large and small-scale habitat differentiation. Surprisingly, levels of Cu, a divalent cation, were found to remain the same across life stages which was not expected based on the results of other similar elements. As a cofactor for several enzymes, Cu is one of the most important essential elements in fish. Highly regulated in the body, fish require Cu for a variety of intercellular processes related to brain and red blood cell development, cholesterol and glucose metabolism, and the development of bone tissue. Ambient water Cu concentrations were extremely low compared to other essential elements indicating that Cu may be more reflective of dietary sources rather than environmental, but this assumption has not been validated (Table 3.4).
Interestingly, Cu concentrations were also extremely low compared to other essential elements (Mn, Mg, and Zn) incorporated in the rays (Table 3.5). Reasons why this may occur, could be related to environmental availability or to just how vital this element is to the body, which may result in it being more highly regulated both before and after OSM. Similarly, Pb concentrations were also relatively low, but Pb is not regulated as strictly as Cu since it is considered a non-essential element and therefore it is possible that a decrease in Ca may lead to higher replacement. In fact, Ba and Pb, the only two non-essential elements measured (excluding Sr), were the two most important elemental discriminators in the RF model, indicating that non-essential elements may experience the most change after OSM. This is most likely related to the fact that they are unregulated and are therefore more readily available during calcification after OSM.

Other Sturgeon Microchemistry Studies

Due to their dwindling status, several biomineral microchemistry studies have been performed on sturgeon populations in order to assess important population dynamics and implement policies to improve stock structure. The majority of studies that have analyzed sturgeon rays through microchemistry have concentrated on Sr variation in an attempt to distinguish habitat use, with other elements often left out of the analysis (Green Sturgeon: Allen et al. 2011; Pallid Sturgeon: Phelps et al. 2012; Lake Sturgeon and Shortnose Sturgeon: Phelps et al. 2016; White Sturgeon: Sellheim et al. 2017; Lake Sturgeon: Ziegeweid et al. 2021). Studies that reported on other elements examined specific time points and did not report differences between early and late life stages (Beluga, Russian Sturgeon, and Stellate Sturgeon: Jarić et al. 2010; Atlantic Sturgeon: Balazik et al. 2012; Gulf Sturgeon: Gunn et al. 2019; Persian Sturgeon: Bakhshalizadeh et al. 2021; and Lake Sturgeon: Loeppky et al. 2021). Thus, to our knowledge this is the first study to document variations between Ba, Cu, Mg, Mn, and Zn between adult and juvenile sturgeon.

Conclusion and Future Directions

This study is the first description of elemental variation in rays coinciding with OSM for any species. Results indicated that sexual maturity may influence element deposition and growth zone width in calcified structures. When using the microchemistry of calcified structures to interpret important population metrics, one should consider the effects of physiological
influences, specifically in species that are known to invest dramatically throughout the reproductive cycle. It is possible for elemental data to be misinterpreted if physiological influences override elemental signals, ultimately resulting in poor management of the fishery. Though the reasons for changing elemental concentrations remain unclear, this study suggests that year-specific signatures in rays can be a potential tool for identifying OSM in long-lived species such as Lake Sturgeon. Future work is required to further validate this study to determine its potential use on other sturgeon species and populations.

3.6 Acknowledgements

Rays for Dataset 1 were provided by the Black Lake Sturgeon Rearing Facility in partnership with Michigan State University and the Michigan Department of Natural Resources. We especially thank Jessie Hanson, Baylee Moser, Jacob Kimmel, Joseph Reidy, Alex Florian, Mike Diefenbach, Gary Michaud, Maxwell Majinska, Emily Barkley, Grace Wagner, Abby Kessler, Signe VanDrunen, Douglas Larson, and Kim Scribner for assistance with field sampling, Allison Loeppky with fin ray preparation, Panseok Yang for LA-ICP-MS analysis, and members of the Anderson Lab for assistance in growth zone determination. Authors would also like to acknowledge Mathew Thorstensen for guidance with R programming. Rays for Dataset 2 were provided by Norm Halden. Funding for this research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC)/Manitoba Hydro Industrial Research Chair awarded to WGA and Science Enhancement of Grant Stipends support from the Faculty of Science at the University of Manitoba to AT. We are grateful to have been able to conduct this work on the University of Manitoba campus which is located on the original lands of the Anishinaabeg, Cree, Oji-Cree, Dakota, and Dene peoples, and on the homeland of the Métis Nation.
3.7 Tables

Table 3. 1. Sample information for Lake Sturgeon \((n = 98)\) used in this study. Pectoral fin rays were collected from a geographically closed population (Dataset 1) and seven other geographically separated populations (Dataset 2). Total length and mass were measured at time of pectoral fin ray removal.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Sample Years</th>
<th>Age Range</th>
<th>Total Length (cm)</th>
<th>Mass (kg)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2021-2022</td>
<td>(15-62)</td>
<td>157.9 ± 16.4</td>
<td>33 ± 13</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(119-187)</td>
<td>(13-60)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1999-2007</td>
<td>(34-58)</td>
<td>144.3 ± 11.2</td>
<td>24 ± 8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(121-160)</td>
<td>(15-48)</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Values of total length and mass are given as mean ± standard deviation with the range of values in parentheses below.
Table 3.2. Results of statistical analysis (Wilcoxon signed rank test) comparisons of pectoral fin ray mean elemental concentrations and growth zone width before and after onset of sexual maturity in Lake Sturgeon (n = 98) from a geographically closed population (Dataset 1) and seven other geographically separated populations (Dataset 2). Mean ± standard deviation measurements are shown.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dataset 1</th>
<th></th>
<th></th>
<th></th>
<th>Dataset 2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before OSM</td>
<td>After OSM</td>
<td>P</td>
<td>Before OSM</td>
<td>After OSM</td>
<td>P</td>
<td>Before OSM</td>
<td>After OSM</td>
</tr>
<tr>
<td>Ba</td>
<td>0.87 ± 0.08</td>
<td>1.51 ± 0.45</td>
<td>&lt;0.001</td>
<td>0.91 ± 0.08</td>
<td>1.23 ± 0.19</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.99 ± 0.09</td>
<td>1.03 ± 0.28</td>
<td>0.654</td>
<td>0.97 ± 0.09</td>
<td>1.05 ± 0.19</td>
<td>0.154</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>0.98 ± 0.02</td>
<td>1.10 ± 0.06</td>
<td>&lt;0.001</td>
<td>0.97 ± 0.02</td>
<td>1.06 ± 0.06</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.86 ± 0.10</td>
<td>1.56 ± 0.50</td>
<td>&lt;0.001</td>
<td>0.84 ± 0.09</td>
<td>1.33 ± 0.17</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>0.99 ± 0.02</td>
<td>1.03 ± 0.05</td>
<td>&lt;0.001</td>
<td>1.01 ± 0.05</td>
<td>0.98 ± 0.09</td>
<td>0.114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.94 ± 0.05</td>
<td>1.25 ± 0.29</td>
<td>&lt;0.001</td>
<td>0.97 ± 0.08</td>
<td>1.08 ± 0.16</td>
<td>0.039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.72 ± 0.24</td>
<td>2.00 ± 0.90</td>
<td>&lt;0.001</td>
<td>0.68 ± 0.17</td>
<td>1.68 ± 0.42</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone Width</td>
<td>234.3 ± 98.4</td>
<td>97.9 ± 16.35</td>
<td>&lt;0.001</td>
<td>235.5 ± 102.9</td>
<td>104.7 ± 37.6</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Relative elemental concentrations were calculated for each pectoral fin ray by dividing each measurement by the average over the entire profile.
Table 3. 3. Confusion matrix for RF classification success of year-specific signatures to before or after onset of sexual maturity derived from Lake Sturgeon pectoral fin rays from a geographically closed population (Dataset 1, $n = 24$) and seven other geographically separated populations (Dataset 2, $n = 20$).

<table>
<thead>
<tr>
<th></th>
<th>Dataset 1</th>
<th></th>
<th>Dataset 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted</td>
<td>Correctly</td>
<td>Predicted</td>
<td>Correctly</td>
</tr>
<tr>
<td>Actual</td>
<td>Before</td>
<td>After</td>
<td>Actual</td>
<td>Before</td>
</tr>
<tr>
<td>Before</td>
<td>24</td>
<td>0</td>
<td>Before</td>
<td>19</td>
</tr>
<tr>
<td>After</td>
<td>0</td>
<td>24</td>
<td>After</td>
<td>0</td>
</tr>
<tr>
<td>Overall Class. Rate</td>
<td>100%</td>
<td>Overall Class. Rate</td>
<td>97.5%</td>
<td></td>
</tr>
</tbody>
</table>

Note: Two observations for every pectoral fin ray were tested in the model to classify both a before and after onset of sexual maturity signature for each fish.
Table 3. Mean elemental concentrations for water chemistry data collected across Black Lake, Michigan (Dataset 1) in May 2021. Water samples were taken across 10 sample points distributed across the lake approximately 1 meter above the benthic zone. Total metals in the water were quantified by CRC-ICP-MS (ALS Environmental Laboratory; Winnipeg, Manitoba).

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (mg.L-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba</td>
<td>0.007 ± 0.002</td>
</tr>
<tr>
<td>Ca</td>
<td>48.56 ± 15.41</td>
</tr>
<tr>
<td>Cu</td>
<td>0.0015 ± 0.001</td>
</tr>
<tr>
<td>Mg</td>
<td>28.69 ± 11.71</td>
</tr>
<tr>
<td>Mn</td>
<td>0.0041 ± 0.003</td>
</tr>
<tr>
<td>Pb</td>
<td>0.0002 ± 0.0002</td>
</tr>
<tr>
<td>Sr</td>
<td>0.078 ± 0.028</td>
</tr>
<tr>
<td>Zn</td>
<td>0.0017 ± 0.0010</td>
</tr>
</tbody>
</table>
Table 3.5. Pectoral fin ray mean elemental concentrations (± standard deviation) for Lake Sturgeon (*n* = 98) from a geographically closed population (Dataset 1: Location 1) and seven other geographically separated populations (Dataset 2: Locations 2 to 8).

<table>
<thead>
<tr>
<th>Location</th>
<th>Region</th>
<th>$^{137}$Ba</th>
<th>$^{63}$Cu</th>
<th>$^{25}$Mg</th>
<th>$^{55}$Mn</th>
<th>$^{88}$Sr</th>
<th>$^{66}$Zn</th>
<th>$^{208}$Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Black Lake</td>
<td></td>
<td>19.4</td>
<td>1.28</td>
<td>4401.4</td>
<td>27.1</td>
<td>113.4</td>
<td>158.6</td>
<td>2.14</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.7</td>
<td>1.03</td>
<td>613.8</td>
<td>16.4</td>
<td>11.2</td>
<td>61.0</td>
<td>3.03</td>
</tr>
<tr>
<td>2 Churchill River</td>
<td></td>
<td>51.1</td>
<td>0.83</td>
<td>3031.2</td>
<td>24.9</td>
<td>190.0</td>
<td>69.4</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.9</td>
<td>0.60</td>
<td>291.7</td>
<td>10.3</td>
<td>33.5</td>
<td>20.1</td>
<td>0.25</td>
</tr>
<tr>
<td>3 Kiskittogisu Lake</td>
<td></td>
<td>52.8</td>
<td>0.74</td>
<td>3036.5</td>
<td>34.4</td>
<td>198.6</td>
<td>106.8</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.7</td>
<td>0.42</td>
<td>295.7</td>
<td>20.0</td>
<td>24.3</td>
<td>33.0</td>
<td>0.54</td>
</tr>
<tr>
<td>4 Nelson River</td>
<td></td>
<td>50.4</td>
<td>0.91</td>
<td>3268.5</td>
<td>22.9</td>
<td>184.6</td>
<td>87.9</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.4</td>
<td>0.52</td>
<td>478.8</td>
<td>13.5</td>
<td>25.9</td>
<td>31.3</td>
<td>0.66</td>
</tr>
<tr>
<td>5 Norman Dam</td>
<td></td>
<td>23.0</td>
<td>0.50</td>
<td>3473.9</td>
<td>21.2</td>
<td>100.9</td>
<td>122.1</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.77</td>
<td>0.29</td>
<td>550.9</td>
<td>8.75</td>
<td>13.6</td>
<td>31.4</td>
<td>3.13</td>
</tr>
<tr>
<td>6 Sturgeon Falls</td>
<td></td>
<td>21.8</td>
<td>0.60</td>
<td>3186.6</td>
<td>37.3</td>
<td>96.5</td>
<td>133.7</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.41</td>
<td>0.28</td>
<td>384.5</td>
<td>16.1</td>
<td>9.24</td>
<td>53.1</td>
<td>1.76</td>
</tr>
<tr>
<td>7 Whitedog River</td>
<td></td>
<td>17.7</td>
<td>1.13</td>
<td>3437.9</td>
<td>27.5</td>
<td>108.0</td>
<td>111.5</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
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<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.07</td>
<td>0.48</td>
<td>285.0</td>
<td>13.1</td>
<td>11.8</td>
<td>37.7</td>
<td>0.48</td>
</tr>
<tr>
<td>8 Lake Winnebago System</td>
<td></td>
<td>17.0</td>
<td>3.96</td>
<td>4519.8</td>
<td>17.6</td>
<td>115.3</td>
<td>225.1</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>±</td>
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<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>

**Note:** Elemental concentrations are expressed in ppm = μg/g.
3.8 Figures

Figure 3.1. Map of the study area in the Upper Black River and Black Lake, Michigan, USA (Dataset 1, \( n = 78 \)). Alverno Dam, Kleber Dam, and the adult spawning grounds are shown.
Figure 3. 2. Map of all study areas. Site 1 = Churchill River, Manitoba ($n = 1$), site 2 = Nelson River, Manitoba ($n = 9$), site 3 = Kiskittogisu Lake, Manitoba ($n = 2$), site 4 = Whitedog River, Ontario ($n = 1$), site 5 = Norman Dam, Ontario ($n = 1$), and site 6 = the Lake Winnebago System, Wisconsin ($n = 3$), site 7 = Black Lake, Michigan ($n = 78$), and site 8 = Sturgeon Falls, Ontario ($n = 3$). Dataset 1 = site 7. Dataset 2 = sites 1-6, 8.
Figure 3. Raw elemental profiles (pectoral fin ray) of a 37-year-old, 1710mm TL, female Lake Sturgeon captured in the Upper Black River, Michigan in 2021. Vertical lines represent the point of elemental increase identified by reader consensus (age 26).
Figure 3.4. Raw elemental profiles (pectoral fin ray) of a 36-year-old, 1409mm TL, Lake Sturgeon (sex unknown) captured in the Nelson River, Manitoba in 1999. Vertical lines represent the point of elemental increase identified by reader consensus (age 23).
Figure 3.5. Box plots presenting comparisons of pectoral fin ray mean elemental concentrations and growth zone width before and after onset of sexual maturity in Lake Sturgeon (n = 98) from a geographically closed population (Dataset 1) and seven other geographically separated populations (Dataset 2). Median values are indicated by a horizontal bar within each box. Vertical lines above and below each box represent 5 and 95 percentiles. Comparisons were performed by Wilcoxon signed rank test. Symbols: * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001. Note: Relative elemental concentrations were calculated for each pectoral fin ray by dividing each measurement by the average over the entire profile.
Figure 3. Nonmetric multidimensional scaling (nMDS) ordination plot showing elemental and morphometric variation among Lake Sturgeon pectoral fin rays \((n = 98)\) before and after presumed onset of sexual maturity from a geographically closed population (Dataset 1) and seven other geographically separated populations (Dataset 2). Each dot represents an individual value and the relative distance between two points represents the similarity (or dissimilarity) between them. Stress = 0.092 indicating that the plot provides a “great” representation in reduced dimensions.
Figure 3. 7. Random forest (RF) variable importance plot. The mean decrease in accuracy measures classification accuracy deprivation when a variable is excluded from the RF analysis (averaged across all trees). A higher value indicates the importance of that variable in predicting onset of sexual maturity status (before vs. after) in Lake Sturgeon pectoral fin rays.
3.9 References


https://doi.org/10.1023/A:1010933404324


Croghan, C., and P. Egeghy. 2003. Methods of dealing with values below the limit of detection using SAS. Presented at Southeastern SAS User Group, St. Petersburg, FL.


Maugoust, J. 2022. _ULT: Useful Little Things_. R package version 0.0.0.9000.


Chapter 4: Thesis Conclusions and Synthesis

Thesis Discussion

Throughout this thesis, I examined two areas of conservation for Lake Sturgeon. In the first experimental chapter (chapter 2), I set out to discover a new way to assign ages using elemental oscillations across pectoral fin rays, the most common structure removed for age and growth studies, whereas in the second experimental chapter (chapter 3), I wanted to quantify useful variables for sexual maturation identification using elemental signatures and growth zone width of pectoral fin rays. Both chapters had favorable results showing promise of the use of pectoral fin rays to both age and identify maturity in sturgeon.

In chapter 2, our results demonstrated that elemental oscillations may prove useful when assigning ages to sturgeon. Specifically, accuracy (MAD) and precision (CV) measurements were higher for ages estimated using elemental oscillations compared to ages assigned using the traditional method alone (chemical interpretation method: MAD = 0.92, CV = 4.04%; traditional interpretation method: MAD = 1.45, CV = 11.14%). However, when comparing our results to those of previous studies, our ages were still unpromising in older fish (Bruch et al. 2009; Hessenauer et al. 2018). Notably, all aging techniques (across methods) began to underestimate the true age of the individual at around age 18 indicating that OSM may inhibit both annulus and elemental clarity. Because of this, aging sturgeon using pectoral fin rays continues to be challenging after fish reach a certain age. Nonetheless, our results imply that using elemental oscillations to assist with aging in younger fish or other species, that are hard to traditionally age, may prove useful.

Interestingly, elemental profile analysis, which was used to identify useful elements for aging, established that elemental oscillations may vary by location (Manitoba: Ba, Mg, Mn, and Zn; Wisconsin: Mg, Mn, Pb, and Zn) therefore being population-specific. Oscillation clarity also varied by population indicating that some populations may be easier to chemically age than others. Likewise, this finding proved significant when trying to assign ages using the quantitative approach (multivariate multiple change point analysis with finite differencing). For example, the model was not able to be successfully run on profiles from Wisconsin, which had patchy elemental oscillations. After further examination of the model, it was believed that low success was related to oscillation clarity rather than the increasing age of the fish/increasing length of the
profiles. To test this assumption, the model was run on an older reference dataset of rays from Nelson River, Manitoba, and Black Lake, Michigan (ages 20-24). Results reinforced this assumption, with 100% classification success within ±2 years of visually identified growth zones. This model is the first of its kind to assign chemical ages using the raw elemental profiles showing promise for developments in chemical age models in this field.

In chapter 3, the high discrimination success of year-specific signatures to before or after OSM (98.8% across populations) suggested that the chemical composition and microstructure of pectoral fin rays may prove useful in identifying maturity status in Lake Sturgeon. Statistical analysis supported the discrimination model's findings, with divalent cations (specifically Ba, Mg, Mn, Pb, and Zn) found to be significantly higher after OSM and growth zone width found to be significantly lower. Additionally, elements were found to visually increase at documented ages of OSM in Lake Sturgeon, suggesting that the change in signatures may be visually identifiable. This new discovery suggests that fin ray chemistry could potentially be used to estimate an age of when each fish reaches sexual maturity. Furthermore, since the age at OSM in sturgeon is sex-specific (males 12-20: females 15-30; COSEWIC 2017) this increase may also prove useful in identifying sex for individuals on the marginal ends of their range. Currently, an ultrasound or surgical biopsy of the internal gonads is required to determine maturity status in sturgeon (Wildhaber et al. 2007; Chapman and Van Eenennaam 2008; Webb et al. 2017). Although this process has become less invasive over time, using the pectoral fin ray to identify OSM could result in less stress during handling of the fish, especially if the fin ray is already being removed for age and growth analysis. In addition, if year-specific signatures could be used to identify maturity status, as this paper suggests, it may be possible to expand this method to use the second fin ray in place of the fin spine, for less invasive removal (Baremore and Rosati 2014; Izzo et al. 2021). All of the samples used in this chapter had the second pectoral fin ray attached, allowing for the implementation of further analysis to determine its discrimination success. This structure could result in not only a less invasive procedure but also less time and money spent on ray prep and elemental analysis. Furthermore, glass-slides could be utilized to limit cost and allow for larger sample sizes if desired.

Although the reason for the elemental increase is not completely understood, it may be possible that other trace elements are able to substitute for fin ray calcium at a faster rate due to
Ca demands throughout the reproductive cycle (Allen et al. 2009). Non-essential elements (specifically Ba and Pb) seemed to have the greatest change after OSM indicating that non-essential elements may have the opportunity to replace Ca at a higher rate compared to essential elements that may be rerouted to other areas of the body during reproduction (i.e., Mg, Mn, Zn) (Volkoff and Sydney 2018; Dawood et al. 2021; Studer et al. 2022). Although the results of this study are promising, more work is required to determine the cause of the elemental increase in order to validate its use to estimate maturity status. If this is a result of fin ray Ca reduction associated with maturation and/or spawning, other species that exhibit similar Ca requirements may also yield comparable results.

**Final Conclusions**

My results cast a new light on the use of microchemistry in sturgeon, ultimately opening up the door for new conservation opportunities. Knowing individual age and the age at OSM could allow managers to better predict year class strength and potential population growth. Most sturgeon microchemistry studies strictly examine Sr and Ba levels to examine habitat use, therefore other elements found useful in this study (specifically Mg, Mn, Pb, and Zn) could be added to future analysis in an attempt to identify OSM or help estimate fish age. Additionally, a new discrimination model could be developed using the point of elemental increase to discriminate year-specific signatures to before or after OSM. A model using this increase will most likely result in higher discrimination success in year-specific signatures closest to the actual point of OSM. The chemical age model could also be expanded to be tested on other species and structures, specifically otoliths in species that allow removal. Otoliths are chemically inert and will most likely warrant better results since they do not experience material resorption which may alter fin ray growth and elemental clarity.

As opposed to Lake Sturgeon that are strictly freshwater species, other North American sturgeon species are anadromous, meaning that they can move between salt and freshwater environments. This difference in life history strategy may influence elemental signatures (most likely for Sr and Ba). Consequently, future studies investigating other sturgeon species and/or populations are required to further validate both methods outlined in this thesis. Even so, this study implies that new applications for fin ray microchemistry are possible and could greatly benefit fisheries management strategies in the future.
References


Appendix

Table 1. Background information and typical laser parameters used for LA-ICP-MS analysis of pectoral fin rays used for Chapter 2 (Dataset 2).

<table>
<thead>
<tr>
<th>Background Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reason For Removal: Age and growth analysis as well as to examine regional trace elemental differences.</td>
</tr>
<tr>
<td>Sample Years: 1999-2007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Typical Laser Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beam Size: 30-µm</td>
</tr>
<tr>
<td>Scan Speed: 2-µm/s</td>
</tr>
<tr>
<td>Repetition Rate: 20-Hz</td>
</tr>
<tr>
<td>Energy Output: 100%</td>
</tr>
<tr>
<td>Elements Quantified: Al, As, Ba, Cr, Cu, Mg, Mn, Ni, Pb, Rb, Sr, and Zn</td>
</tr>
<tr>
<td>Glass Standard: NIST 610 National Institute of Standards and Technology, Gaithersburg, MD, USA</td>
</tr>
</tbody>
</table>
Table 2. Supplementary material regarding where to access R code and data presented throughout this thesis. All files/scripts can be accessed through the available GitHub link.

<table>
<thead>
<tr>
<th>Supplementary Material</th>
<th>GitHub Link:</th>
<th>Brief Description:</th>
</tr>
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<tbody>
<tr>
<td>GitHub Link:</td>
<td><a href="https://github.com/AlainaTaylor/Lake_Sturgeon_Microchemistry_R">https://github.com/AlainaTaylor/Lake_Sturgeon_Microchemistry_R</a></td>
<td></td>
</tr>
<tr>
<td>Brief Description:</td>
<td>A Git repository for scripts regarding analyses and data presented in this thesis. Brief descriptions are available for each separate folder, within their corresponding readme files.</td>
<td></td>
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</tbody>
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