INVESTIGATING THE ENVIRONMENTAL INFLUENCES ON ELEMENTAL UPTAKE AND DEPOSITION IN HARD STRUCTURES OF STURGEON TO IDENTIFY NATAL ORIGIN AND RECONSTRUCT LIFE HISTORY

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Abstract

Habitat-specific elemental signatures form when divalent cations such as strontium (Sr^{2+}) are substituted for calcium (Ca^{2+}) in the calcified hard structures of fishes (e.g., otoliths, fin rays, vertebrae) allowing for the retroactive interpretation of spatial and temporal changes in environmental life history. The ability to accurately reconstruct life histories of fish using microchemistry techniques relies on predictable responses to changes in the environment. As such, the overarching objectives of this thesis were to 1 – Examine how the environment influences the processes involved in the elemental pathway from initial uptake to crystallization in hard structures and 2 – Validate ways can we use this knowledge to make improved inferences on the ecology of sturgeon utilizing hard part microchemistry. To do this, I applied a multidisciplinary approach to describe the effect the environment (i.e., ambient water chemistry, temperature, pH) on the initial uptake, partitioning, and biomineralization of otoliths and fin rays in larval and juvenile Lake Sturgeon, *Acipenser fulvescens*, and White Sturgeon, *A. transmontanus*, followed by the application of microchemistry techniques to validate the use of elemental signatures in fin rays to determine natal origin (i.e, hatchery versus wild-spawned) and habitat use of juvenile and adult Lake Sturgeon. Data presented here indicate the initial uptake and deposition of Sr^{2+} into hard structures of larval Lake Sturgeon are inversely related to ambient calcium (Ca^{2+}) concentrations and positively correlated to environmental temperature. Additionally, temperature, but not pH, had a significant effect on the biomineralization of sturgeon otoliths, independent of ontogeny, indicating the determination of otolith polymorph composition in sturgeons is controlled by both intrinsic and extrinsic factors. Microchemistry of fin rays collected from wild-caught juvenile and adult Lake Sturgeon of both known and unknown origin reflected the heterogeneity of water chemistry profiles in the environment. Development and validation of a novel analytical technique using multiple elements allowed for strong predictions of habitat use on a fine spatial scale. This thesis has made a significant contribution to our understanding of fish hard part structures and techniques developed will contribute to improved management strategies for the conservation of Lake Sturgeon and other migratory fishes.

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Chapter 1: Thesis Introduction

1.1 General Introduction

Successful management and conservation of any species requires an in-depth knowledge of the stability and resilience of local populations through the understanding of connectivity and population structure (Gillanders 2002; Pracheil et al. 2014; Thorrold et al. 2001). The ability to assess connectivity relies on the identification of an individual's natal origin and migration patterns that then informs and directs conservation strategies such as stock enhancement programs and monitoring the effects of environmental stressors on populations (Thorrold et al. 2001). Interpreting the life history of fish, however, can be difficult due to the challenges and cost of conducting mark-recapture studies on large numbers of individuals that mix to inhabit potentially expansive geographic ranges (Davoren and Halden 2014; Thorrold et al. 2001). As an alternative to artificial tags, fisheries managers around the world frequently conduct microchemistry analyses to assess elemental signatures deposited in calcified hard structures of fish (e.g., otoliths, fin rays, scales, vertebrae) as they provide a chronological record of an individual's environmental life history (Campana 2005; Secor et al. 1995). The ability to make strong ecological inferences from elemental signatures, however, is limited due to a lack of an in-depth understanding of the physiological and physical processes of element uptake and deposition in the hard structures of fishes. This thesis integrates techniques from the life, geological, and physical sciences to provide a holistic view of how we might interpret chemical signatures deposited in fish hard structures, vastly improving the ecological inferences we can make from these life history recorders.

1.2 Literature Review

Many chemical elements including, but not limited to, strontium (Sr^{2+}), barium (Ba^{2+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), and zinc (Zn^{2+}) are present in minute amounts in the environment and, thus, are considered trace elements. Depending on the physical landscape of a given habitat, these trace elements exist in varying concentrations related to geologic availability and/or anthropogenic input (Pracheil et al. 2014). Because fish tightly regulate their internal solute and water balance, the concentration of elements in the ambient environment are often directly reflected in the calcified hard parts of fishes thus forming habitat specific elemental signatures (Campana 1999). The pathway from elemental incorporation to deposition, however, is complex and involves a series of regulated barriers (Campana 1999; Loewen et al. 2016). For freshwater fishes, the initial step in this pathway begins with the uptake of ions into the blood plasma primarily via the gills (Allen et al. 2011; Campana 1999). Once in the blood stream, elements are transported to specific tissues such as in the endolymph for otolith crystallization, or bone for ossification in fins (Campana 1999; Carriere et al.

2016; Loeppky and Anderson 2020). Elemental discrimination can occur in varying degrees at any of these barriers, in processes that are likely species and element specific (Campana 1999). As such, it is important to have a thorough understanding of these processes in order to make accurate inferences on the environmental life histories of individuals using elemental signatures in hard structures.

The most considerable of the elemental barriers for freshwater fishes occurs at the initial gill-water interface (Campana 1999). Indeed, transport mechanisms specific to essential elements that are required for biological function such as calcium (Ca^{2+}) exist to selectively uptake these ions from the aqueous environment (Allen et al. 2011; Flik and Verbost 1993; Perry and Wood 1985). For non-essential elements like Sr^{2+} and Ba^{2+}, however, the direct homeostatic control over these elements has not evolved (Chowdhury and Blust 2001). As a result, they are incorporated via Ca^{2+} transport mechanisms due to their similar chemical properties (i.e., atomic radius, valence charge; Chowdhury and Blust 2012; Loewen et al. 2016; Mann 1988). Nonetheless, because of the essential nature of Ca^{2+} for biological function, there is a higher affinity for Ca^{2+} resulting in competitive inhibition against most other ions that might exist in a natural aqueous environment (Chowdhury et al. 2000; Wood 2012). As a consequence, uptake of elements such as Sr^{2+} are inversely related to the availability of Ca^{2+} in the environment (Chowdhury et al. 2000; Loeppky and Anderson 2020). Additionally, the rate at which this uptake occurs and subsequent transport across additional biological membranes is influenced by environmental variables (e.g., temperature, salinity, pH, dissolved oxygen) highlighting the complexity of elemental uptake in fishes.

Once incorporated, these elements are transported to sites of biomineralization providing the elemental backbone of hard structures such as otoliths and fin rays (Campana 1999). The crystallization of biominerals occurs when the amount of free energy required to transition from a supersaturated solution to a crystal is met (De Yoreo and Vekilov 2003; Ruiz-Agudo et al. 2011). Otoliths are such calcified structures that form by the accumulation of biominerals in the inner ear of teleost and chondrostean fishes and function as hearing and balance systems (Campana 1999; Secor et al. 1995). Despite their widespread use in fisheries research (e.g., species identification, habitat movements), relatively little is known about the mechanisms of otolith biomineralization in fishes. These paired structures are primarily composed of crystalized polymorphs of calcium carbonate ($CaCO_3$) in the form of calcite, aragonite, and vaterite deposited on a proteinaceous matrix, of which the thermodynamic stability of each polymorph decreases, respectively (Campana 1999; De Yoreo and Vekilov 2003; Pracheil et al. 2017). As such, otolith polymorph composition is determined by both intrinsic (i.e.,

genetic) and extrinsic (i.e., environment) factors (Coll-Llado et al. 2018; Sollner et al. 2003). Understanding how these variables influence the phenotypic plasticity of otolith polymorphs has important implications for conservation as changes to the proportion of polymorphs in otoliths, as a result of climate change or manipulation of rearing environments in aquaculture, can alter the form and function of otoliths, potentially impacting the behavioural performance of fishes (Coll-Llado et al. 2018; Reimer et al. 2016; Reimer et al. 2017).

Fin rays are also biomineralized hard structures in fish and are composed of ossified bone in the form of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) deposited on an organic collagen matrix (Akiva et al. 2015; Omelon et al. 2009). In contrast to otoliths, fin rays can be sampled non-lethally and have the ability to regenerate when injured (Allen et al. 2018), therefore making them attractive alternatives for studying species that are considered endangered or at-risk (Pracheil et al. 2019). Habitat specific elemental signatures in these structures form when divalent cations are substituted for Ca^{2+} on the surface of hard structures thus producing a permanent record of environmental life history. Microchemistry analyses of otoliths and fin rays are frequently used to retroactively interpret both broad- and fine-scale habitat use of fishes with varying life history characteristics such as oceanodromous, diadromous, and potadromous species (Pracheil et al. 2019). Regulated by a periodic environmental cycle, visible rings (i.e., annuli) associated with seasonal growth on both otoliths and fin rays form throughout the life of fish serving as useful timekeepers of temporal events experienced by the individual (Campana 1999; Pracheil et al. 2014). Typically used for age and growth studies, these annuli serve as reference points that when paired with elemental profiles in the environment, provide information on key movements at specific life history stages (e.g., natal origin, nursery habitats, migration movements; Kalish 1989). Importantly, the efficacy of microchemistry studies relies on water chemistry profiles that differ on the spatial and temporal scale that is of interest to the research questions being posed. Environmental factors such as the geology (i.e., bedrock composition), hydrology (i.e., groundwater vs. surface water), and physical characteristics (i.e., flow and/or floodplain input) of a specific area are all factors that contribute to elemental profiles in the water and, thus, will determine whether habitat specific elemental signatures can be distinguished (Pracheil et al. 2014).

Elemental signatures in hard structures are also valuable tools used in hatchery marking studies. While there are a variety of physical marking techniques available (i.e., passive, electronic), biological tags have been of specific interest in recent years due to their ability to successfully mass mark individuals in a cost-effective manner (Drenner et al. 2012). Specifically, stable isotope marking

involves manipulating the natural isotopic ratio of particular elements, whether intentionally or unintentionally, in order to create a unique elemental signature in the hard structures of specific cohorts of fish (e.g., Smith and Whitledge 2011; Thorrold et al. 2001; Woodcock et al. 2011). This technique provides a reliable and long-lasting tag that requires minimal effort and no individual handling of fish (Woodcock et al., 2011). Thus, mass groups of fish can be marked at once with minimal stress providing a low-cost tagging method for hatcheries and fishery programs. The ability to mark groups of individuals with unique elemental signatures is beneficial for tracking the survivorship of hatchery cohorts post-release, ultimately aiding in determining the success of hatchery restocking programs.

1.3 Study Species

Sturgeons are part of the phylogenetically ancient group of Acipenserid fishes. Having evolved approximately 200 million years ago, sturgeon are often considered "living fossils" as their biology can provide insight into the evolution of evolutionarily more modern fish (Bemis et al. 1997; Shen et al. 2020). Of the 25 extant species of sturgeons, nine are endemic to North America and can be found in habitats ranging from coast to coast. The Lake Sturgeon, *Acipenser fulvescens*, is the only exclusively freshwater sturgeon species in North America and occupies the Great Lakes, Hudson Bay, and Mississippi River drainage basins. Population decline has been rapid and severe over the past 150 years due to extreme harvesting and habitat loss resulting in Lake Sturgeon populations in Manitoba being listed as endangered or threatened (COSEWIC 2017). Consequently, increasing focus has been placed on recovering Lake Sturgeon populations, which include gaining a further understanding of the biology and ecology of the species in order to optimize conservation strategies and increase the success of restocking programs. Stock enhancement has been conducted with Lake Sturgeon in Manitoba since 2008, however, the success of the program has been challenging to assess due to the difficulty of evaluating the survivorship of hatchery-reared individuals post-release. In order to ensure conservation efforts are successful, accurately identifying and tracking fish from different hatchery reared cohorts is essential (Warren-Myers et al. 2015a). Although there are a variety of marking techniques available to hatcheries (e.g. fin clipping, DNA analysis, coded wire tags, PIT tags) most come with substantial costs and produce tags that may not be reliable long-term (Warren-Myers et al. 2015b). As an alternative to physical tags, elemental signatures recorded in the pectoral fin rays of Lake Sturgeon are becoming increasingly popular to both mark individuals and track habitat movement (e.g., Carriere et al. 2016; Phelps et al. 2017; Smith and Whitledge 2011). Most microchemistry studies, however, only consider the final elemental signature when making inferences on life history characteristics. In order to utilize

this technique to its full potential, and be able to interpret fine scale habitat movements, it is important to understand the species-specific processes involved in elemental uptake and deposition in hard structures of Lake Sturgeon.

1.4 Thesis Objectives

The ability to reconstruct environmental life histories of fish using elemental signatures in hard structures relies on predictable responses to changes in the environment. As such, the overarching objective of my thesis was to investigate how the environment influences the processes involved in the elemental pathway from initial uptake to crystallization in the hard structures of Lake Sturgeon to facilitate improved modeling of life history strategies. In Chapter 2, I examined how the environment influences the physiological process of Sr^{2+} uptake in age-0 Lake Sturgeon by conducting a series of lab-based flux experiments. Michaelis-Menten substrate inhibition models were then used to test the hypotheses that 1) environmental temperature and 2) ambient Ca^{2+} concentration would influence uptake and subsequent partitioning of Sr^{2+} in Lake Sturgeon tissues. In Chapter 3 I described the crystallization of sturgeon otoliths and examined their percent polymorph composition in response to both ontogenetic development and the effect of environmental pH and temperature. Here I tested the hypotheses that 1) otolith polymorph composition would vary throughout larval ontogeny and 2) environmental temperature and pH would influence the precipitation of $CaCO_3$ polymorphs. I then utilized these observations in Chapter 4 to make ecological inferences on the environmental life histories of several populations of juvenile and adult Lake Sturgeon throughout the province of Manitoba. Specifically, I developed novel microchemical analytical techniques to test the hypothesis that habitat specific chemical signatures would reflect changes in habitat use throughout the life history of individuals. Lastly, in Chapter 5 I investigated the application of chemical marking, both intentional and unintentional, of hatchery reared fish to better asses the success of hatchery restocking programs. Here, I tested the hypothesis that the manipulation of the rearing environment would produce a distinguishable mark in the fin ray chemical profile, allowing us to identify fish post hatchery release. This thesis, which is synthesized in Chapter 6, incorporates physiological flux studies with physical determination of crystal structure, as well as ecological modeling and applied marking techniques to provide improved understanding of the effects of the environment on elemental deposition in Lake Sturgeon hard structures; principles that can be applied to and tested on numerous fishes. Overall, this research provides the fundamental knowledge to greatly improve life history determination of Lake Sturgeon and,

thus, contribute to more informed management strategies for the conservation of this species throughout its North American range.

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Chapter 2: Environmental influences on uptake kinetics and partitioning of strontium in age-0 Lake Sturgeon *Acipenser fulvescens*: Effects of temperature and ambient calcium activities

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2.1 Abstract

Non-essential elements like Sr^{2+} are incorporated via Ca^{2+} transport proteins due to their similar chemical properties and are substituted for Ca^{2+} in hard structures of fishes. Few studies have investigated the uptake kinetics of non-essential elements or the effect the ambient environment has on uptake rates. We tested the hypothesis that temperature and environmental Ca^{2+} free ion activity would influence uptake and subsequent deposition rates of Sr^{2+} in the fin rays of Lake Sturgeon, *Acipenser fulvescens*. Michaelis-Menten substrate inhibition models were used to measure the kinetics of Sr^{2+} uptake on Lake Sturgeon larvae that were exposed to varying temperature and Ca^{2+} free ion activities. Sr^{2+} influx increased at higher temperatures (maximum J_{maxSr}=56.5 pmol.g^{-1}.h^{-1}) and decreased when larvae were exposed to increasing free ion activities of Ca^{2+} (minimum J_{maxSr}=6.4 pmol.g^{-1}.h^{-1}) indicating Ca^{2+} has an inhibitory effect on Sr^{2+} influx. Additional studies on the partitioning of Sr^{2+} into tissues were conducted to determine whether the environment influences the rate at which Sr^{2+} is deposited in the hard structures of larval Lake Sturgeon. Results identified Sr^{2+} was in fact preferentially accumulated in fin rays and partitioning was significantly affected by temperature and Ca^{2+} free ion activity providing, for the first time, an understanding of the underlying physiological mechanisms involved in elemental uptake and deposition of non-essential metals in sturgeons.

2.2 Introduction

Elemental signatures recorded in calcified hard structures of fishes (e.g., otoliths, fin rays, scales) reflect changes in ambient water chemistry and are frequently used by fisheries researchers and managers to interpret fine-scale habitat movements of individuals (Campana 1999, 2005; Pracheil et al. 2014). Despite their widespread use in marine, diadromous, and freshwater populations, much fewer studies comparatively have investigated the physiological mechanisms involved in elemental incorporation. Of these studies, none have paired Michaelis-Menten uptake kinetics to the subsequent deposition of non-essential metals into fish hard structures. Strontium (Sr^{2+}) concentrations in particular are used to identify natal origin and evaluate key life history movements including river migration and transitions from freshwater to saltwater environments (and vice versa) in a multitude of species providing the ability to fill knowledge gaps traditional tagging methods simply cannot (e.g., Allen et al. 2009b; Allen et al. 2018a; Brown and Severin 2009; Brown et al. 2007; Kennedy et al. 2002; Phelps et al. 2017; Phelps et al. 2012; Tsunagawa and Arai 2008). The interpretation of Sr^{2+} signatures, however, are often made on the assumption that Sr^{2+} concentrations in hard structures are proportional to the concentration of Sr^{2+} in the ambient environment (Bath et al. 2000). Thus far studies examining the mechanistic uptake of Sr^{2+} in fish has, to the authors knowledge, only been conducted on a single teleost species, the Common Carp, *Cyprinus carpio* (i.e.,Chowdhury and Blust 2001a, 2001b; Chowdhury et al. 2000) requiring researchers to make broad assumptions on potentially species-specific mechanisms. In order to make more accurate inferences of life history movements based on elemental signatures in hard structures, studies investigating the mechanistic uptake and subsequent deposition of non-essential trace elements are necessary.

Strontium is an alkaline earth metal with similar chemical properties (i.e., atomic size, valence charge) to that of calcium (Ca^{2+}) and is present primarily as a free metal ion with relatively high bioavailability in freshwater systems (Chowdhury and Blust 2012; Loewen et al. 2016; Mann 1988). Not required for biological function, Sr^{2+} is considered a non-essential element and, thus, direct homeostatic control over extra- and intra-cellular concentrations has not evolved (Loewen et al. 2016; Pors Nielsen 2004). Therefore, Sr^{2+} is likely incorporated via epithelial calcium channels (ECaC) primarily found in gill tissue, although the gastrointestinal tract and skin have also been identified as important uptake sites (Allen et al. 2011; Flik and Verbost 1993; Genz et al. 2014; Liao et al. 2007b; Perry and Wood 1985; Shahsavarani 2006; Wood and Shuttleworth 1995). In mammals, two isoforms of Ca^{2+} channels exist, ECaC1 (TRPV5) and ECaC2 (TRPV6), both exhibiting ion permeability to divalent cations (den Dekker

et al. 2003). In teleosts and chondrosteans, however, only one isoform has been identified that is more closely related to the ECaC2 gene of mammals (Allen et al. 2011; Liao et al. 2007a; Qiu and Hogstrand 2004). Despite the potential for Sr^{2+} to replace Ca^{2+}, the affinity for Ca^{2+} remains higher due to the evolved nature of ECaC for Ca^{2+} transport because of its necessity for biological function (Chowdhury and Blust 2012; Wood 2012). Once incorporated, Sr^{2+} is assumed to be preferentially accumulated in bony tissues whereby it is substituted for Ca^{2+} in the calcium carbonate ($CaCO_3$) or hydroxyapatite ($Ca_{10}(PO_4)_8(OH)_2$) matrix of calcified hard structures forming habitat specific elemental signatures (Carriere et al. 2016; Chowdhury and Blust 2012; Konovalenko et al. 2016; Loewen et al. 2016; Yankovich 2009).

The rate at which trace elements, including Sr^{2+}, are incorporated into fish is influenced by environmental variables such as temperature, salinity, pH, dissolved oxygen, and ambient water chemistry (Campana 1999; Chowdhury and Blust 2001b; Chowdhury et al. 2000). In freshwater fishes, temperature and water hardness are among the greatest influences on elemental uptake rates (Campana 1999). Water hardness is determined by the concentration of multivalent cations (e.g. Ca^{2+}, Mg^{2+}) in the water. When exposed to environments with low ambient Ca^{2+} concentrations (i.e. soft water) whole body Ca^{2+} concentration has been shown to decrease in a variety of fishes (Allen et al. 2011; Chou et al. 2002; Flik et al. 1986). As a result, fish will compensate by increasing Ca^{2+} uptake in part through adjusting the density of mitochondrial rich cells (MRC's) on the gills in order to maintain internal Ca^{2+} concentrations, particularly at early life history stages when Ca^{2+} demand for rapid growth is especially high (Chou et al. 2002; Genz et al. 2014; Hwang et al. 1996; Wood 2012). Because Sr^{2+} is competing for binding sites on Ca^{2+} transport proteins, Sr^{2+} accumulation is typically inversely related to the concentration of Ca^{2+} in the ambient environment whereby Sr^{2+} accumulation is highest when environmental Ca^{2+} concentrations are low (Chowdhury and Blust 2001a; Chowdhury and Blust 2012).

As poikilotherms, fish generally lack physiological mechanisms to control internal temperature (Hazel 1993; Hazel 1997), therefore, uptake of essential elements is highly dependent on the ambient environmental temperature (Chowdhury and Blust 2001b; Kulikov 1973; Polikarpov 1966). In particular, temperature influences enzyme activity and metal uptake by impacting the function of carrier proteins, ion channels, and transport pumps (Chowdhury and Blust 2001b). In addition, an increase in temperature can also influence the solution chemistry of alkaline earth metals rendering them more soluble and, thus, readily incorporated by aquatic organisms (Hazel 1993). While the relationship between environmental variables and the interpretation of elemental signatures in hard structures has

been well documented and widely used in ecological studies (e.g., Campana 1999; DiMaria et al. 2010; Elsdon and Gillanders 2002; Elsdon and Gillanders 2004; Loeppky and Davoren 2018; Martin and Thorrold 2005; Miller 2009) it is uncommon for studies to couple this with the underlying species-specific mechanisms influencing the kinetics of trace element incorporation.

A species of particular interest in freshwater systems is the Lake Sturgeon, *Acipenser fulvescens*, a long-lived fish (~80-100 years) of conservation concern throughout its North American range. Environmental life histories of this species are largely unknown and difficult to determine using traditional tagging methods, particularly at early life history stages. Interpreting elemental signatures recorded in the fin rays of sturgeons has become an increasingly utilized technique to determine natal origin and migration patterns (e.g., Allen et al. 2009b; Phelps et al. 2017; Phelps et al. 2012). Understanding how the environment influences the uptake and deposition rates of Sr^{2+} in Lake Sturgeon will, for the first time, offer insight into the uptake kinetics of non-essential elements in sturgeons and provide key information for researchers to make more accurate inferences on habitat use based on Sr^{2+} signatures in the hard structures of Lake Sturgeon. In this study, we tested the hypothesis that environmental temperature and Ca^{2+} concentration would influence Sr^{2+} uptake and deposition in fin rays of Lake Sturgeon. We first investigated the rate at which Ca^{2+} and Sr^{2+} are incorporated separately in the uninhibited process in 30 days post hatch (dph) Lake Sturgeon when acclimated to five different ambient Ca^{2+} free ion activities ranging from 0.1 to 5.0 $mmol.L^{-1}$. Results from this experiment were then used to calculate kinetic constants for the Michaelis-Menten substrate inhibition model to investigate the effects ambient Ca^{2+} concentration and temperature have on uptake rates of waterborne Sr^{2+}. Finally, Sr^{2+} accumulation was measured in several tissues to identify partitioning of the isotope once incorporated into the individual fish. As in teleosts, we predicted that Ca^{2+} would exhibit an inhibitory effect on Sr^{2+} uptake whereby Sr^{2+} influx would be inversely related to the concentration of Ca^{2+} in the environment.

2.3 Methods

2.3.1 Experimental Animals

All fish used in these experiments were the artificially produced progeny from wild-spawning adult Lake Sturgeon captured from the Winnipeg River in Manitoba, Canada (53°11’04.4”N, 99°15’40.7”W). Milt and eggs collected from at least three males and two females were mixed to fertilize eggs and allowed to incubate (~5-7 days) in the Animal Holding Facility at the University of Manitoba, Winnipeg, Canada. Hatched larvae were transferred and reared in multiple 11-litre tanks

(~200 fish/tank) supplied with a constant flow of dechlorinated city of Winnipeg tap water maintained at ~15°C. At the onset of exogenous feeding (~14 dph), fish were fed brine shrimp nauplii (*Artemia salina*) to satiation three times daily until the initiation of each experiment. By 30 dph, when experiments were initiated, pectoral fins are clearly visible and apparent fin ray supports can be identified as described in Zhang and Dang (2014). All procedures conducted on fish were approved by the animal care committee at the University of Manitoba permit# F15-007 in accordance with guidelines established by the Canadian Council for Animal Care.

2.3.2 Experiment 1: Determination of Sr^{2+} and Ca^{2+} uptake rates

At 30 days dph, Lake Sturgeon were haphazardly sampled from holding tanks by dipnet and transferred at equal densities to one of five Ca^{2+} treatments (0.1, 0.5, 1.0, 1.5, and 5.0 $mmol.L^{-1}$) in triplicate 3 L acclimation aquaria (15 tanks total). The treatment environments were created following recipes as previously described in Allen et al. (2011) and Genz et al. (2014) using 0.11 $mmol.L^{-1}$ NaCl, 0.022 $mmol.L^{-1}$ $MgSO_4 \bullet 7H_2O$, 170 nM Na_2HPO_4, and the desired experimental concentration of $CaCl_2 \bullet 2H_2O$ added to deionized water. Treatment water was then adjusted to a pH of 7 using $NaHCO_3$ and chilled to 15°C prior to being added to treatment tanks. Water samples were collected from each experimental treatment and measured for metal content at ALS laboratories in Winnipeg, Canada using Inductively Coupled Plasma Collision Cell Mass Spectroscopy following EPA method 200.2 / 6020B. The equilibrium concentrations and activities of free Ca^{2+} and other metal ions in the treatment waters were then calculated using Visual MINTEQ (ver. 3.1; Sweden, 2013). Because metals are only bioavailable to fish in the form of free ion levels in the aqueous environment, experimental conditions are presented as free metal ion activity rather than total metal concentration throughout the text. Fish continued to be fed artemia nauplii twice daily to satiation. To maintain water quality, all fish waste and uneaten artemia were removed and a 50% water change with the corresponding treatment water was conducted 30 minutes after each feeding. Aeration was continually provided to each tank and nitrite, nitrate, and ammonia levels were monitored weekly to ensure suitable water conditions were sustained throughout the acclimation period. Fish were held at 15°C in a temperature-controlled room and a 12h:12h light:dark photoperiod was set during the one-week acclimation period. Actual temperatures and pH were monitored multiple times daily using a YSI handheld muliparameter instrument (Xylem Analytics, Ohio, USA) to ensure constant temperature and pH levels were held throughout the experimental period.

Following acclimation, fish were randomly selected from each acclimation tank and transferred into individual flux chambers with a minimum mass:volume ratio of 1 g:10 ml (Wood 1992) to measure whole body Ca^{2+} or Sr^{2+} influx. Flux chambers were comprised of 20 mL glass scintillation vials with opaque lids to reduce light exposure during flux trials and minimize stress on the individual. The diameter and height of the vials measured 28 x 61 mm, respectively allowing the fish to move freely within the chambers. A small hole was drilled into the lid of each vial so that a capillary tube could be placed into the vial to aerate each chamber. To measure whole body Ca^{2+} influx in the uninhibited process, 40 fish from each acclimated Ca^{2+} treatment were randomly selected by dipnet and immediately transferred into an individual flux chamber filled with 10 mL of either 0.1, 0.5, 1.0, 1.5, or 5.0 $mmol.L^{-1}$ Ca^{2+} treatment water as measured by free ion activity and prepared the same way as described above (n=8 fish/acute Ca^{2+} treatment/acclimated Ca^{2+} treatment, total fish = 200). Fifteen minutes prior to adding fish to the chambers, 75 $kBq.L^{-1}$ of radioactive $^{45}CaCl_2$ was added to each flux chamber allowing the isotope to equilibrate. Fish were held in the flux chambers at 15°C for a total of eight hours. A pilot study was conducted to determine the amount of time fish should be left in the flux chambers to ensure linear accumulation of the isotope as measured by disappearance of the isotope from the water sampled every hour over an 8-hour period. A schematic of the experimental design is provided in the supplementary materials (Figure 2.1). After the experimental trial, fish were immediately euthanized in an overdose of MS-222 buffered with equal concentrations of $NaHCO_3$ (250 ppm) and rinsed in tracer free water. Each fish was blotted dry and weighed before being digested separately in 500 µL of 70% HNO_3 at room temperature for 24-hours then neutralized with an equal volume of 8 N NaOH. The entire digested sample was then mixed with scintillation fluid (Ultima Gold AM, PerkinElmer, Waltham, MA, USA) and read for five minutes on a β-scintillation counter (LS 6500, Beckman Coulter, Mississauga ON, Canada) to measure the activity of ^{45}Ca in each fish. A study using similar techniques with Lake Sturgeon (Genz et al. 2013) did not observe any quenching in the measured samples and, as such, we did not test for quenching in the present study.

To measure whole body Sr^{2+} influx in the uninhibited process, 120 fish from each acclimated Ca^{2+} treatment were selected by dipnet and immediately transferred into individual flux chambers filled with 10 mL of either 0.5, 1.0, 5.0, 50.0, or 500 $\mu mol.L^{-1}$ of Sr^{2+} as measured by free ion activity at one of three Ca^{2+} free ion activities (0.03, 0.3, 3.0 $mmol.L^{-1}$, n=8 fish/acute Ca^{2+} treatment/acute Sr^{2+} treatment/acclimated Ca^{2+} treatment, total fish = 600). Fish were exposed to minimal levels of Ca^{2+} in the flux chambers in order to maintain their survival over the experimental trial period as well as to

extrapolate the kinetic constants used in the subsequent competitive inhibition models by simultaneously fitting the results to a single non-linear model. Acute treatment water was created the same way as described above with the addition of $SrCl_2 \bullet 2H_2O$ to obtain the desired Sr free ion activity. Water samples were collected and analyzed for free metal activity as described above. Fifteen minutes prior to adding fish to the chambers, 2 $kBq.L^{-1}$ of radioactive $^{85}SrCl_2$ was added to each flux chamber and allowed to equilibrate. The flux trials and sample preparation were conducted as described above. Each sample was read for five minutes on a γ-counter (Wizard^2, PerkinElmer) to measure the activity of ^{85}Sr in each fish. Unlike β scintillation counters that measure light photons in the sample vial, the γ-counter converts γ-rays to light photons in the NaI crystal and, therefore, minimises the effect of quenching.

Whole body Ca^{2+} and Sr^{2+} influx (j in $nmol.h^{-1}.g^{-1}$) was then calculated using the following equation (Eqn. 1):

$$j = \frac{CPM_{total}}{SA \times T \times M} \quad (1).$$

where CPM_{total} is the calculated amount of radioactivity in counts per minute (CPM) for the entire fish, SA is the specific activity of either ^{45}Ca or ^{85}Sr and accounted for the free ion activity of cold element in each vial, T is the duration of the experiment in hours, and M is the mass of the fish in grams. Results from these experiments were then used to model the kinetics of Ca^{2+} and Sr^{2+} uptake in age-0 Lake Sturgeon using the following Michaelis-Menten equation (Eqn 2; Weiss 1996) as outlined in Chowdhury and Blust (2001b):

$$j_I = \frac{J_{max} \times (S_I)}{[K_m + (S_I)]} \quad (2).$$

where j_I is the calculated uptake rate ($nmol.h^{-1}.g^{-1}$) of the metal ion in question (i.e. I = Ca^{2+} or Sr^{2+}), J_{max} is the apparent maximum saturation, S is the substrate concentration of I ($mmol.L^{-1}$) and K_m is the apparent half saturation constant.

2.3.3 Experiment 2: Inhibitory effect of ambient Ca^{2+} concentration and temperature on Sr^{2+} uptake

The acclimation period of this experiment was set up in a similar way as described in Experiment 1 where 30 dph Lake Sturgeon were transferred and equally distributed into one of five Ca^{2+} treatments (0.1, 0.5, 1.0, 1.5, and 5.0 $mmol.L^{-1}$) in duplicate 3 L acclimation aquaria in three temperature controlled environmental chambers. All chambers were initially set to 15°C then gradually adjusted to experimental temperatures of 12, 15, or 18°C over the course of two days. Once all chambers achieved their respective experimental temperature the acclimation period began. Feeding, water changes, and monitoring were conducted as described in Experiment 1.

After an acclimation period of one week, 40 fish from each acclimated Ca^{2+} treatment were randomly selected by dipnet and immediately transferred into individual flux chambers filled with 10 mL of either 0.1, 0.5, 1.0, 1.5, 5.0 mmol.L^{-1} $CaCl_2$ at their respective acclimation temperatures (n=8 fish/acute Ca^{2+} treatment/acclimated Ca^{2+} treatment/temperature treatment, total fish = 600). Fifteen minutes prior to adding fish to the chambers, 2 kBq.L^{-1} of radioactive $^{85}SrCl_2$ was added to each flux chamber and allowed to equilibrate. Flux chambers were held in a water bath (Julabo, F25-MC, Allenton, PA, USA) set to 12, 15, or 18°C to maintain a constant temperature over eight hours. The flux trials and sample preparation were conducted as described in Experiment 1 to measure the activity of ^{85}Sr in each fish. Whole body uptake rates of Sr^{2+} were calculated using Eqn. 1. To model the inhibitory effect ambient Ca^{2+} had on Sr^{2+} uptake, the following Michaelis-Menten substrate inhibition equation (Eqn 3; Weiss 1996) was used as described in Chowdhury and Blust (2001a, 2001b):

$$j_{Sr} = \frac{J_{maxSr} \times (Sr)}{[K_{mSr} + (Sr)] \times \left[1 + \frac{(Ca)}{K_{iCa}}\right]} \quad (3).$$

where j_{Sr} is the whole body uptake rate of Sr^{2+} (pmol.g^{-1}.h^{-1}), J_{maxSr} is the maximum uptake rate of Sr^{2+}, (Sr) and (Ca) are the concentrations of each element in the flux chamber (mmol.L^{-1}), K_{mSr} is the true K_m for Sr^{2+} uptake in the uninhibited process at each acclimation Ca^{2+} treatment (calculated in Experiment 1), and K_{iCa} is the inhibitor constant for the effect of Ca^{2+} on Sr^{2+} uptake.

2.3.4 Experiment 3: Determination of Sr^{2+} accumulation in tissues

The acclimation period and flux trials of this experiment were conducted as described in Experiment 2 with the exception of there being just three acclimation and acute Ca^{2+} concentrations (0.1, 1.5, 5.0 mmol.L^{-1}, n = 8 fish/acute Ca^{2+} treatment/acclimated Ca^{2+} treatment/acclimated temperature treatment, total fish = 216). The Ca^{2+} concentrations in this experiment were chosen because of the consistent results observed in Experiments 1 and 2. Following the flux trials, fish were euthanized, rinsed, dried and weighed as described in Experiment 1. Each fish was then immediately dissected to collect both left and right whole pectoral fins, skin, and muscle tissue. Each tissue and the remaining body were then weighed and placed separately in 2 mL vials. Dissecting tools were cleaned in a water and soap mixture then rinsed in tracer free water and patted dry three times between each tissue dissection to avoid contamination between samples. Tissue samples were then digested and counted for ^{85}Sr activity as described in Experiment 1. The activity of Sr^{2+} (CPM) present in each tissue was then transformed to measure the amount of Sr^{2+} present per mass of tissue (accumulation) using the following equation (Eqn 4; Carriere et al. 2016):

$$\text{Accumulation} = \frac{\left[\left(\frac{\text{CPM}}{60}\right) / \text{M}\right]}{\text{SA}} \quad (4).$$

where CPM is the measured amount of ^{85}Sr activity in each tissue, 60 is the factor used to convert CPM to Becquerel, M is the mass of tissue, and SA is the specific activity of Sr^{2+} and accounted for the free ion activity of cold Sr^{2+} in the vial.

2.3.5 Statistical Analyses

All analyses and modelling were conducted in Prism (GraphPad Software, version 8.4.1). The data was first examined separately for normality using the Shapiro-Wilk's W test and for homogeneity of variance using the Levine's test. Results from Experiments 1 and 2 were fitted to models using nonlinear regression techniques (i.e. Michaelis-Menten kinetics). Analysis of variance (ANOVA) was used to evaluate significant differences ($p<0.05$) of uptake rates among experimental treatments or independent variables (i.e. ambient Ca^{2+} free ion activity, temperature). For Experiment 3, mean (± s.e.m.) Sr^{2+} accumulation in tissues was compared using a one-way ANOVA. Post-hoc Tukey HSD tests were performed to identify which factor levels were significantly different. To test whether Sr^{2+} accumulation in each tissue differed significantly among environmental treatments, a general linear model was used with temperature (°C), acclimated and acute Ca^{2+} free ion activity ($mmol.L^{-1}$) as fixed independent factors.

In all experiments, values greater than ± two standard deviations from the mean were considered outliers and removed from the data set.

2.4 Results

2.4.1 Experimental parameters

Mean temperature (± s.e.m.) within each environmental chamber were maintained at 12.6 ± 0.1°C, 15.0 ± 0.1 °C, and 17.9 ± 0.1 °C for the 12, 15, and 18°C treatments, respectively, while average pH (± s.e.m.) for all experimental waters was 7.11 (± 0.005). Measured free metal activity in the prepared acclimation and acute treatment waters is presented in Table 1.

1.4.2 Experiment 1

The average mass (± s.e.m.) of all fish used in this experiment was 0.12 ± 0.002 g. Both whole body Ca^{2+} and Sr^{2+} uptake rates for age-0 Lake Sturgeon were positively correlated with substrate free ion activity (i.e. acute Ca^{2+} or Sr^{2+} free ion activity, $mmol.L^{-1}$) in the uninhibited process (Figure 2.2). The Michaelis-Menten models for Ca^{2+} and Sr^{2+} uptake were globally fit to the data by simultaneously fitting the results from each acclimated Ca^{2+} treatment. The kinetic parameter estimates (i.e. j_{max}, K_m, r^2)

are presented in Table 2.2. The Michaelis-Menten model for Ca^{2+} influx accounted for 75% of the variation in the data indicating it was able predict the maximum saturation rate (j_{max}) and half saturation constant (K_m) for Ca^{2+} uptake with reasonable success. The K_m for Sr^{2+} was substantially greater than that of Ca^{2+} indicating the affinity for Ca^{2+} is higher in the uptake process. Acclimated Ca^{2+} free ion activity had a significant effect on both the maximum saturation rate (j_{max}, $F_{(4, 590)}$=14.7, p<0.0001) and K_m ($F_{(4, 590)}$=10.68, p<0.0001) when the Michaelis-Menten model for Sr^{2+} uptake in the uninhibited process was globally fit to three acute Ca^{2+} free ion activities (Figure 2.3, Table 2.3). Indeed, both kinetic parameters significantly decreased as acclimated Ca^{2+} free ion activity increased suggesting the abundance of Ca^{2+} transporters that have a lower affinity for Sr^{2+} increase when there is less available Ca^{2+} in the environment.

2.4.3 Experiment 2

The average mass (± s.e.m.) of all fish used in this experiment was 0.07 ± 0.001 g. Whole body Sr^{2+} uptake rates of age-0 Lake Sturgeon were influenced by both ambient Ca^{2+} free ion activity and temperature (Figure 2.4). Indeed, Sr^{2+} uptake decreased significantly in increasing ambient Ca^{2+} free ion activities ($F_{4,584}$=37.4, p<0.0001) and increased significantly with increasing temperature ($F_{2,584}$=44.3, p<0.0001). The K_m values from the uninhibited process (Table 2.3) were used to constrain the kinetic constants (K_{mSr}) for each acclimated Ca^{2+} treatment in the Michaelis-Menten substrate inhibition model (Figure 2.5). The kinetic parameter estimates are presented in Table 2.4. The maximum velocity of Sr^{2+} uptake (j_{maxSr}) significantly increased with increasing temperature ($F_{2,576}$=49.92, p<0.0001) and decreased with increasing acclimated Ca^{2+} concentration ($F_{4,576}$=60.40, p<0.0001) indicating that the uptake process is both temperature and substrate dependent. While the inhibitor constant for the effect of Ca^{2+} concentration on Sr^{2+} uptake (K_{iCa}) was also significantly influenced by both temperature ($F_{2,576}$=23.23, p=<0.0001) and acclimated Ca^{2+} free ion activity ($F_{4,576}$=7.75, p<0.0001) the trends are more variable. Indeed, K_{iCa} values were greatest at 15°C in both the 1.5 and 5.0 $mmol.L^{-1}$ acclimated Ca^{2+} treatments indicating, perhaps not surprisingly, that Ca^{2+} had the greatest inhibitory effect on Sr^{2+} when ambient Ca^{2+} free ion activities were high. The greater values of K_{mSr} in comparison to K_{iCa} identify there is a higher affinity for Ca^{2+} further supporting the inhibitory effect Ca^{2+} has on Sr^{2+} uptake in age-0 Lake Sturgeon. *2.4.4 Experiment 3*

The average mass (± s.e.m.) of each tissue measured in this experiment was 1.2 ± 0.03 mg, 4.0 ± 0.10 mg, 4.5 ± 0.09 mg, and 46.9 ± 4.42 mg for fin rays, skin, muscle, and remaining body tissue, respectively. Strontium accumulation was highest in the fin rays of age-0 lake sturgeon in comparison to

skin, muscle, and remaining body tissue ($F_{3, 820}$=399.5, $p<0.0001$) regardless of temperature, acclimated Ca^{2+} or acute Ca^{2+} free ion activity treatments indicating there is strong partitioning of Sr^{2+} in hard tissues (Figure 2.6). Statistical results of the general linear models whereby experimental treatments are considered as fixed effects (i.e. temperature, acclimated Ca^{2+}, acute Ca^{2+}) are presented in Table 2.5. Most notably, temperature and acute Ca^{2+} free ion activity had a significant effect on Sr^{2+} accumulation in all tissues measured indicating Sr^{2+} partitioning is both temperature and substrate dependent, however, acclimated Ca^{2+} concentration had no effect on Sr^{2+} accumulation in tissues except for muscle.

2.5 Discussion

Results from the present experiments are, to our knowledge, the first time Michaelis-Menten modelling of non-essential elements has been paired with subsequent tissue partitioning and deposition in any fish species. This data provides integral information for researchers using Sr^{2+} signatures in hard structures to interpret habitat use by fishes. While there have been no studies investigating specific molecular transport mechanisms of Sr^{2+} in fishes to date, we present evidence that suggests Sr^{2+} enters Lake Sturgeon via Ca^{2+} transport systems as has been observed in other fish species (Chowdhury et al. 2000). Both Sr^{2+} and Ca^{2+} are alkaline earth metals that share similar chemical properties allowing Sr^{2+} passive entry through Ca^{2+} selective channels on the apical surface of MRCs primarily in gill epithelium (Chowdhury and Blust 2012; Shahsavarani 2006). The ions are actively transported into the blood across the basolateral membrane presumably via Ca^{2+} specific transport proteins such as Ca^{2+}-ATPase and/or Na^{2+}/Ca^{2+} exchanger where competitive inhibition is likely to occur due to the high affinity for Ca^{2+} (Chowdhury and Blust 2012; Wood 2012). Indeed, Chowdhury et al. (2000) observed Ca^{2+} inhibited Sr^{2+} uptake in common carp suggesting shared binding sites in the transport system (Chowdhury and Blust 2012). Results from the present experiments indicate Ca^{2+} also acts as a competitive inhibitor of Sr^{2+} uptake in age-0 Lake Sturgeon.

Despite the Michaelis-Menten curve not reaching a plateau for Ca^{2+} uptake in the uninhibited process (Figure 2.2A), indicating saturation of the transport mechanisms was not met, the model still accounted for 75% of the variation and, thus, was able to predict the maximum saturation rate and affinity with reasonable success. This is consistent with findings presented by Chowdhury and Blust 2000). As such, we infer that the higher affinity for Ca^{2+} presented in Experiment 1 indicates transport proteins are preferentially binding Ca^{2+} over Sr^{2+}. Calcium is an essential element required to maintain a multitude of physiological processes particularly during periods of rapid growth such as early development at the larval life stage in fishes (Flik et al. 1995; Hwang et al. 1996; Wood and

Shuttleworth 1995). During early life history, fish larvae tightly regulate their internal Ca^{2+} levels resulting in compensatory uptake in environments of reduced water hardness and thus low levels of Ca^{2+} in order to avoid potentially adverse effects on growth and survival (Chen et al. 2003; Genz and Anderson 2019; Hwang et al. 1996). Age-0 Lake Sturgeon require Ca^{2+} in particular for the development of bony scutes during the first few weeks of growth after hatch (Genz and Anderson 2019; Loewen et al. 2016). Indeed, Allen et al. (2011) observed Ca^{2+} uptake in larval and juvenile Lake Sturgeon increased when fish were exposed to low Ca^{2+} environments (0.1 $mmol.L^{-1}$). While we observed a positive correlation with Ca^{2+} uptake and acute Ca^{2+} concentration in this study (Figure 2.2A), the highest uptake rates were observed in fish that were acclimated to the lowest Ca^{2+} concentrations (0.1 and 0.5 $mmol.L^{-1}$) demonstrating the fish in these treatments displayed compensatory uptake likely due to a lack of available Ca^{2+} during the acclimation period.

Acclimated Ca^{2+} concentration also had a significant effect on whole body Sr^{2+} uptake rates in the uninhibited process (Figure 2.3, Table 2.3). Indeed, both the apparent j_{max} and K_m for Sr^{2+} uptake were highest in low acclimated Ca^{2+} environments (0.1, 0.5 $mmol.L^{-1}$) suggesting the abundance of ECaC transporters increases and their affinity for Sr^{2+} decreases in order to facilitate Ca^{2+} uptake as compensation for the lack of available Ca^{2+} in the environment and to maintain internal Ca^{2+} homeostasis. In addition, the change in affinity observed in our study implies there may be a secondary isoform of ECaC present in age-0 Lake Sturgeon. To date, only one ECaC isoform has been identified in teleosts (Qiu and Hogstrand 2004). Allen et al. (2011) observed Lake Sturgeon ECaC had a shorter amino acid structure compared to teleosts placing Lake Sturgeon ECaC between amphibians and modern fishes. In mammals, two isoforms of ECaC exist and while both isoforms allow the transport of divalent cations in addition to Ca^{2+} (i.e. Sr^{2+}, Ba^{2+}, Mn^{2+}), the relative permeability of ECaC1 to other elements is significantly higher in comparison to ECaC2 (den Dekker et al. 2003). In mice, the expression of ECaC1/2 in the digestive system is regulated by the concentration of dietary Ca^{2+} (den Dekker et al. 2003; Hoenderop et al. 2002). Furthermore, Hoenderop et al. (2002) demonstrated an increase in the expression of ECaC1, the more permeable of the two isoforms, in mice fed a Ca^{2+} enriched diet. The switch between a high abundance and lower affinity for Sr^{2+} transport in high Ca^{2+} environments to a high abundance and low affinity for Sr^{2+} transport in low Ca^{2+} environments in our study suggests a similar isoform switch may have occurred in the Lake Sturgeon. Further investigation of the molecular expression of ECaC transport proteins are required in order to fully examine the potential of multiple ECaC isoforms being present in Lake Sturgeon.

Results from modeling Michaelis-Menten substrate inhibition kinetics identified Sr^{2+} uptake is both temperature and substrate dependent (Table 2.4, Figure 2.5). Values reported for j_{maxSr} follow similar trends as in the uninhibited process whereby acclimating age-0 Lake Sturgeon to low Ca^{2+} environments increases the apparent maximum uptake rate of Sr^{2+} despite the reduced affinity for Sr^{2+}. This is facilitated by the lower inhibitory effect of Ca^{2+} on Sr^{2+} uptake (K_{iCa}) observed in low Ca^{2+} environments. As the concentration of Ca^{2+} in the environment increases, Sr^{2+} appears to be less successful at competing for binding sites. Indeed, Ca^{2+} had the greatest inhibitory effect not surprisingly when fish were acclimated to high Ca^{2+} environments resulting in low whole-body Sr^{2+} influx rates (Figure 2.4). These results further support the evidence that Ca^{2+} acts as an inhibitor of Sr^{2+} uptake as was observed in the Common Carp (Chowdhury et al. 2000) and is similar to the inhibitory effect Ca^{2+} has on other divalent cations (Comhaire et al. 1994; Spry and Wood 1988). While temperature appeared to have a lesser effect on K_{iCa} values within each acclimated Ca^{2+} treatment, a clear linear increase in J_{maxSr} values with temperature was observed indicating the maximum saturation of Sr^{2+} uptake is temperature dependent. Temperature is known to accelerate physiological processes, in particular those involved in catalytic systems such as ion transport (Chowdhury and Blust 2001b; Hochachka and Somero 2002). Temperature effects the kinetic energy available at active binding sites thus increasing the rate of reactions and subsequent uptake rates of ions (Hazel and Prosser 1974). In addition, temperature influences the solution chemistry of metals making ion transport rates in aquatic poikilotherm organisms, like fishes, particularly susceptible to changes in ambient temperature conditions (Chowdhury and Blust 2001b).

Despite the impact temperature has on ion transport mechanisms, the correlation between temperature and Sr^{2+} concentrations in fish hard structures, which has been investigated in depth in both marine and freshwater species, has resulted in variable positive, negative, and non-significant correlations (e.g., Bath et al. 2000; Campana 1999; Elsdon and Gillanders 2002; Fowler et al. 1995; Gillanders et al. 2010; Loeppky and Davoren 2018). The majority of these studies, however, only examined final deposition concentrations of Sr^{2+} and did not measure initial incorporation rates. Elemental pathways from the external environment to crystallization are multi-step processes with several barriers along the way, each of which are likely species specific both in terms of the element and the organism (Campana 1999). While understanding the influence the external environment has on elemental uptake is crucial in order to make accurate inferences on life history movements based on elemental signatures in hard structures, pairing this information with the final partitioning of elements

within an individual provides the full scope of the kinetic uptake and deposition process. Indeed, Konovalenko et al. (2016) found that the concentration of Sr^{2+} in tissues of multiple fish species was inversely related to the electrical conductivity of both freshwater and brackish water systems and, thus, the concentration of metals such as Ca^{2+} indicating substrate concentration has an effect on the partitioning of elements into tissues. In addition, Carriere et al. (2016) identified $Sr^{2+}$was preferentially accumulated in the fin rays of juvenile Lake Sturgeon, data which is supported by the present study. We identified that even under variable conditions of ambient Ca^{2+} and temperature, this trend is conserved in larval Lake Sturgeon whereby Sr^{2+} accumulation was significantly highest in the fin rays of age-0 Lake Sturgeon in comparison to other tissues measured (Figure 2.6).

At the initiation of the acclimation period in our study, the pectoral girdles of the 30 dph Lake Sturgeon larvae were clearly defined with apparent fin ray supports. According to Zhang & Dang (2014), rudimentary pectoral fins become evident as early as ~2 dph in Lake Sturgeon with bony rays beginning to develop at 14 dph (Harkness and Dymond 1961). Indeed, Davis et al. (2004) described a similar developmental timeline of White Sturgeon, *Acipenser transmontanus,* pectoral girdles where the structures first become visible at 14 days post fertilization (i.e. ~4 dph) with the fin rays becoming visible at 12 dph. During this early developmental stage, Ca^{2+} demand is extensive and likely primarily dominated by the cellular processes that are required to form bony structures such as vertebrae, scutes and fin spines (Genz and Anderson 2019). In comparison to teleosts, internal Ca^{2+} stores are limited in sturgeons largely due to a cartilaginous skeleton and ganoid as opposed to placoid scales (Allen et al. 2011). While we did not measure calcification rates of Lake Sturgeon fin rays in our study, Sweeney et al. (2020) identified the fin rays of White Sturgeon begin to calcify at ~20 dph becoming 50% and 95% calcified by ~32 and 72 dph, respectively. Given the similar description of ontogenetic development outlined in Zhang & Dang (2014) to that of White Sturgeon in Davis et al. (2004) and Sweeney et al. (2020), data suggests Lake Sturgeon fin ray calcification rates would be comparable to those of White Sturgeon. As such, the fin rays of larvae in our study would not have been fully calcified and would have been in a period of rapid and active development likely attributing to the increased sequestering of Sr^{2+} in fin rays in our study in comparison to the other tissues that were measured.

Strontium accumulation in fin rays was significantly influenced by acute Ca^{2+} free ion activity and followed an inverse relationship with Ca^{2+} free ion activity in the water, similar to initial whole body influx of Sr^{2+}. These results are consistent with distinctive changes in Sr^{2+} concentrations that are observed in the fin rays of anadromous Green Sturgeon (*A. medirostrus*) when individuals transition

from freshwater nursery habitats to saline environments where ambient Sr:Ca ratios are 2-3.4 orders of magnitude higher than in freshwater systems (Allen et al. 2009b). When anadromous fishes transition to saline environments, major physiological adaptations are required in order to maintain osmo- and ionoregulation in a process known as smoltification for salmonids (Hoar 1988). Indeed, Allen et al. (2009a) observed MRCs in gill tissue of Green Sturgeon increased in size and abundance when exposed to saline environments likely influencing Sr^{2+} uptake rates and subsequent deposition of Sr^{2+} in fin rays. In addition, differences in Sr:Ba concentration increase ~40-70 x in saline environments resulting in less competition between the two divalent cations for Ca^{2+} transport mechanisms (Allen et al 2009a).

Temperature also influenced Sr^{2+} partitioning in all tissues measured further highlighting the dependence of these physiological mechanisms to temperature in fishes. Interestingly, there was a 4x increase in accumulation at 18°C in comparison to both the 12°C and 15°C treatments. Temperature is known to effect growth and metabolic rates of fishes with both increasing exponentially with temperature (Jobling 1997). While fish mass is accounted for in the tissue accumulation equation, increased growth rate would impact crystallization rates and, thus, the calcification of hydroxyapatite crystals, the ossified mineral composing fin rays. Fin rays form when calcium phosphate ($Ca_3(PO4)_2$) crystals mineralize on a collagen protein matrix (Allen et al. 2018b; Mahamid et al. 2010). The nucleation and growth of these crystals is impacted by temperature by manipulating the solubility of minerals and changing their chemical potential, or free energy response, therefore increasing the quantity and size of crystals being precipitated (Dove et al. 2003). With more crystals present, this increases the opportunity for Sr^{2+} to replace Ca^{2+} in the $Ca_3(PO4)_2$ backbone of hydroxyapatite fin rays thus attributing to the increased accumulation of Sr^{2+} observed in our study.

In summary, Ca^{2+} has an inhibitory effect on whole body Sr^{2+} uptake rates of age-0 Lake Sturgeon and is described using the Michaelis-Menten substrate kinetics model. Notably, the change in affinity for Sr^{2+} when acclimated to high and low ambient Ca^{2+} free ion activities suggests there may be more than one isoform of ECaC present in Lake Sturgeon. Additional research investigating the presence of a secondary ECaC gene in Lake Sturgeon could provide key information on the evolution of Ca^{2+} uptake channels in vertebrates. Furthermore, our results indicate the final deposition of Sr^{2+} into fin rays is temperature and substrate dependent in the eight-hour period of our experiments and was particularly sensitive to increased temperatures likely attributing to calcification rates of hydroxyapatite. Combined, these results highlight the complexity of pathways non-essential elements are required to take from initial incorporation to final deposition further emphasizing the need to consider the collective

effect of environmental variables in order to make accurate inferences on life history characteristics of fishes, in particular at early life history stages, based on elemental signatures recorded in their hard structures. These results will provide researchers and fisheries managers with important in-depth knowledge to formulate models to match predicted habitats of known temperature or water hardness based on concentrations of Sr^{2+} in fin rays ultimately aiding in conservation strategies of populations.

2.6 Acknowledgements

The authors thank the animal holding staff at the University of Manitoba for assistance in care and maintenance of the fish used in flux trials in addition to J. Ali, H. Amjad, L. Belding, F. Bjornson, C. Brandt, M. Earhart, I. Isinguzo, J. Kroeker, J. MacPherson, K. Merks, C. Wiwchar, G. Yoon, and M. Yusishen. Further, North/South consultants Inc. is thanked for their assistance in fish capture. Thanks also to S. McKinnon in the Department of Chemistry at the U of M for guidance with Michaelis-Menten substrate kinetics modelling. Funding for this research was provided to WGA by NSERC/Manitoba Hydro Industrial Research Chair and NSERC Discovery grant (05348-15) and to ARL by NSERC PGS D.

2.7 Tables

Table 2.1. Experimental conditions for (a) acclimated and acute calcium (Ca^{2+}) and (b) acute strontium (Sr^{2+}) treatment waters including mean (± s.e.m.) temperature (°C), pH, and free metal activity ($mmol.L^{-1}$).

a. Acclimated and acute Ca^{2+} experimental parameters								
Experimental Treatment		**Measured Parameter**		**Free Metal Activity**				
Ca^{2+} ($mmol.L^{-1}$)	Temperature (°C)	Temperature (°C)	pH	Ca^{2+} ($mmol.L^{-1}$)	Sr^{2+} ($mmol.L^{-1}$)	Na^{+} ($mmol.L^{-1}$)	Mg^{2+} ($mmol.L^{-1}$)	K^{+} ($mmol.L^{-1}$)
0.1	12	12.3 ± 0.2	7.11 ± 0.01	0.1579	0.0016	1.2293	0.1410	0.0300
	15	14.8 ± 0.2	7.10 ± 0.01	0.1578	0.0016	1.2291	0.1409	0.0300
	18	18.1 ± 0.1	7.12 ± 0.02	0.1576	0.0016	1.2288	0.1407	0.0300
0.5	12	12.4 ± 0.2	7.12 ± 0.02	0.5887	0.0035	1.6600	0.1602	0.0599
	15	15.1 ± 0.2	7.09 ± 0.01	0.5881	0.0035	1.6596	0.1600	0.0599
	18	18.0 ± 0.1	7.12 ± 0.01	0.5875	0.0034	1.6592	0.1598	0.0599
1.0	12	12.3 ± 0.2	7.13 ± 0.01	0.9974	0.0067	1.1354	0.1263	0.0412
	15	14.9 ± 0.1	7.11 ± 0.02	0.9969	0.0067	1.1351	0.1262	0.0412
	18	18.0 ± 0.1	7.12 ± 0.01	0.9963	0.0067	1.1348	0.1261	0.0412
1.5	12	12.6 ± 0.2	7.17 ± 0.03	1.6123	0.0182	0.8586	0.1177	0.0837
	15	15.1 ± 0.2	7.15 ±0.03	1.6105	0.0182	0.8584	0.1176	0.0836
	18	17.8 ± 0.1	7.13 ± 0.01	1.6084	0.0181	0.8581	0.1174	0.0836
5.0	12	12.7 ± 0.1	7.12 ± 0.01	4.8201	0.0542	0.2783	0.0960	0.2328
	15	15.3 ± 0.1	7.11 ± 0.01	4.8105	0.0541	0.2781	0.0958	0.2327
	18	17.9 ± 0.2	7.11 ± 0.01	4.8000	0.0539	0.2780	0.0956	0.2326
b. Acute Sr^{2+} experimental parameters								
0.5	15	15.3 ± 0.2	7.11 ± 0.01	0.9291	0.4964	1.8170	0.1526	0.0505
1.0	15	15.3 ± 0.2	7.13 ± 0.01	1.3246	0.7086	1.7694	0.1458	0.0499
5.0	15	15.3 ± 0.2	7.09 ± 0.03	5.6229	4.5744	1.6288	0.0986	0.0452
50.0	15	15.3 ± 0.2	7.11 ± 0.02	32.7930	51.2190	1.4398	0.0601	0.0400
500.0	15	15.3 ± 0.2	7.10 ± 0.01	364.3220	498.3400	3.6367	2.4481	0.1010

Table 2.2. Kinetic parameter estimates for calcium (Ca^{2+}) and strontium (Sr^{2+}) whole body uptake in the uninhibited process for age-0 Lake Sturgeon derived from the Michaelis-Menten substrate kinetics model (Eqn. 2) where j_{max} is the apparent maximum saturation rate, K_m is the half saturation constant, and r^2 is the coefficient of determination for the global non-linear fitting curves.

Parameter	**Ca^{2+}**	**Units**	**Sr^{2+}**	**Units**
j_{max}	3525	$nmol.g^{-1}.h^{-1}$	56.8	$pmol.g^{-1}.h^{-1}$
K_m	38.7	$mmol.L^{-1}$	255.2	$mmol.L^{-1}$
r^2	0.75		0.97	

Table 2.3. Kinetic parameter estimates (± s.e.m.) of strontium (Sr^{2+}) whole body uptake in the uninhibited process for age-0 Lake Sturgeon when acutely exposed to three Ca^{2+} concentrations (0.01, 0.1, 1.0 mmol.L^{-1}) in individual flux chambers. Values are derived from the Michaelis-Menten substrate kinetics model (Eqn. 2) where j_{max} is the apparent maximum saturation rate, K_m is the half saturation constant, and r^2 is the coefficient of determination for the global non-linear fitting curves. The K_m values presented here were used as the kinetic constants (K_{mSr}) for the subsequent Michaelis-Menten substrate inhibition model (Eqn. 3). Statistical results from one-way ANOVAs indicated acclimated Ca^{2+} concentration had a significant effect on the apparent j_{max} and K_m values ($p<0.05$).

Acclimated Ca^{2+} (mmol.L^{-1})	**j_{max} (pmol.g^{-1}.h^{-1})**	**K_m (mmol.L^{-1})**	**r^2**	**n**
0.1	50.5 (±1.5) ^{a}	79.88 (±8.5) ^{a}	0.94	120
0.5	51.5 (±1.6) ^{a}	96.94 (±11.1) ^{a}	0.96	119
1.0	40.5 (±1.2) ^{b}	44.93 (±5.1) ^{b}	0.93	117
1.5	40.4 (±1.7) ^{b}	50.45 (±7.7) ^{b}	0.88	120
5.0	42.1 (±1.1) ^{b}	38.33 (±4.1) ^{b}	0.94	119

^{a,b} indicate results for post-hoc Tukey HSD tests among values within the same column. Values not sharing the same letter are significantly different ($p<0.05$).

Table 2.4. Kinetic parameter estimates (± s.e.m.) of strontium (Sr^{2+}) whole body uptake for age-0 Lake Sturgeon when acclimated to five calcium (Ca^{2+}) concentrations and three temperature environments. Values are derived from the Michaelis-Menten substrate inhibition model (Eqn. 3) where j_{maxSr} is the apparent maximum velocity of Sr^{2+} uptake if left uninhibited, K_{mSr} is the true K_m for Sr^{2+} uptake and was constrained based on values derived in the uninhibited process at each acclimated Ca^{2+} concentration (table 2), K_{iCa} is the inhibitor constant for the effect of Ca^{2+} concentration on Sr^{2+} uptake and r^2 is the coefficient of determination for the non-linear fitting curves. Statistical results from two-way ANOVAs indicated both acclimated Ca^{2+} concentration and temperature had a significant effect on the apparent j_{max} and K_{iCa} values ($p<0.05$).

Acclimated Ca^{2+} ($mmol.L^{-1}$)	**Temperature (°C)**	**j_{maxSr} ($pmol.g^{-1}.h^{-1}$)**	**K_{mSr} ($mmol.L^{-1}$)**	**K_{iCa} ($mmol.L^{-1}$)**	**r^2**	**n**
0.1	18	56.5 (±6.0) ^{a}	79.88	6.8 (±1.1) ^{a}	0.81	38
	15	31.5 (±3.1) ^{b}	79.88	7.9 (±1.2) ^{a}	0.82	40
	12	15.7 (±1.4) ^{c}	79.88	9.7 (±1.3) ^{ab}	0.80	39
0.5	18	27.8 (±1.9) ^{a}	96.94	9.0 (±0.8) ^{a}	0.89	40
	15	22.4 (±2.2) ^{b}	96.94	10.1 (±1.4) ^{b}	0.78	40
	12	15.1 (±1.1) ^{c}	96.94	9.8 (±1.1) ^{ab}	0.85	38
1.0	18	17.6 (±1.0) ^{a}	44.93	12.2 (±1.1) ^{a}	0.92	40
	15	13.9 (±1.1) ^{b}	44.93	15.4 (±1.9) ^{b}	0.86	39
	12	10.8 (±0.5) ^{c}	44.93	13.9 (±0.9) ^{a}	0.93	40
1.5	18	15.9 (±1.7) ^{a}	49.91	14.1(±2.3) ^{a}	0.77	40
	15	12.3 (±1.1) ^{b}	49.91	19.6 (±2.6) ^{b}	0.79	40
	12	8.9 (±4.1) ^{b}	49.91	13.9 (±0.9) ^{a}	0.93	40
5.0	18	11.8 (±0.9) ^{a}	37.98	16.3 (±1.9) ^{a}	0.84	39
	15	5.9 (±0.3) ^{b}	37.98	21.7 (±1.7) ^{b}	0.86	38
	12	6.4 (±0.6) ^{b}	37.98	13.7 (±1.9) ^{a}	0.72	40

^{a,b,c} indicate results for post-hoc Tukey HSD tests among temperature treatments within the same column. Values not sharing the same letter are significantly different ($p<0.05$).

Table 2.5. Statistical results of general linear models for strontium accumulation in each tissue sample. P-values <0.05 (bolded) indicate significant differences among treatments.

Tissue	**Treatment**	**F**	**df_{error}**	**f-ratio**	**p-value**
Fin Ray	Temperature	2	200	314.7	**<0.0001**
	Acclimated Ca^{2+}	2	200	0.3	0.74
	Acute Ca^{2+}	2	200	50.9	**<0.0001**
Skin	Temperature	2	202	7.9	**0.0005**
	Acclimated Ca^{2+}	2	202	1.4	0.25
	Acute Ca^{2+}	2	202	4.2	**0.02**
Muscle	Temperature	2	201	4.6	**0.01**
	Acclimated Ca^{2+}	2	201	3.4	**0.03**
	Acute Ca^{2+}	2	201	7.5	**0.0008**
Remaining	Temperature	2	190	199.4	**<0.0001**
Body	Acclimated Ca^{2+}	2	190	1.1	0.32
	Acute Ca^{2+}	2	190	56.7	**<0.0001**

2.8 Figures

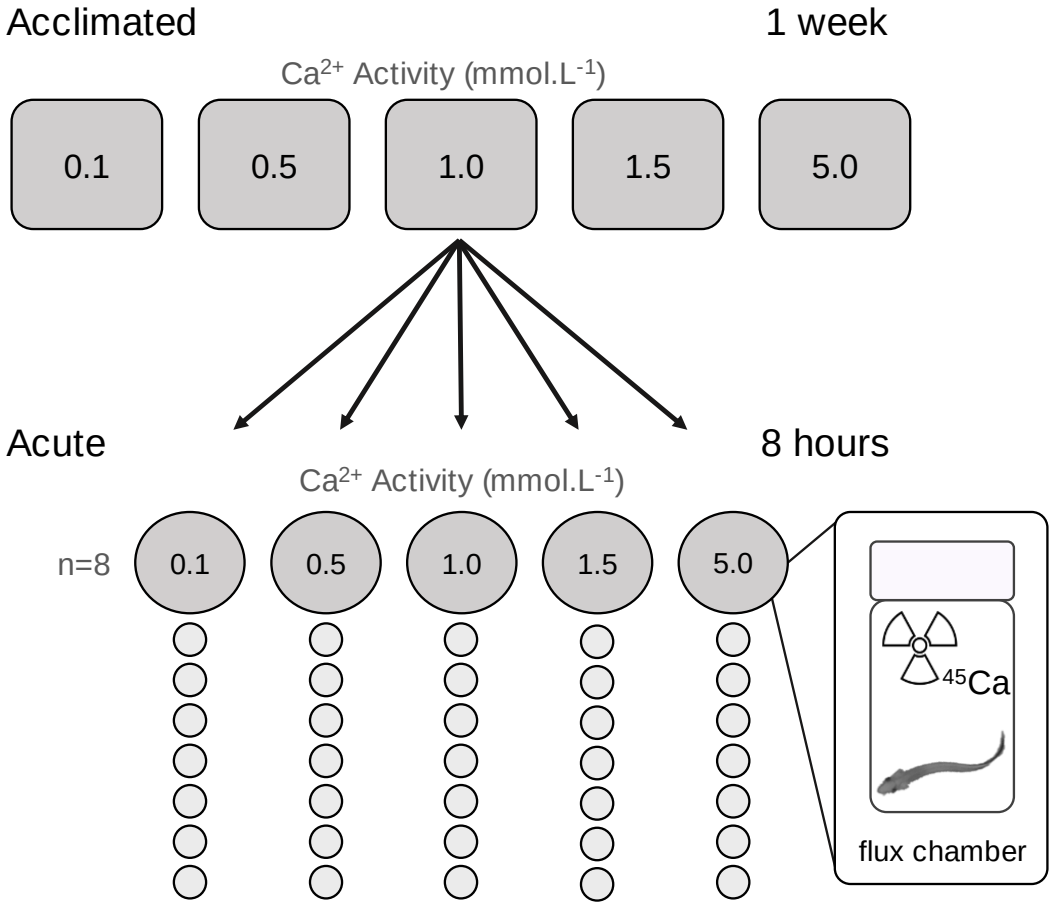

Figure 2.1. Schematic of Experiment 1 measuring calcium (Ca^{2+}) uptake in the uninhibited process whereby 30 days post fertilization (dpf) Lake Sturgeon were first acclimated for one week to 0.1, 0.5, 1.0, 1.5 or 5.0 mmol.L^{-1} Ca^{2+} treatment water at 15°C. Following the acclimation period, 40 fish from each Ca treatment were acutely transferred into individual flux chambers filled with 10 mL of 0.1, 0.5, 1.0, 1.5 or 5.0 mmol.L^{-1} Ca^{2+} treatment water (n=8/acute treatment) in addition to 75 kBq.L^{-1} of radioactive $^{45}CaCl_2$. Flux chambers were aerated and held at the acclimation temperature for the entirety of the 8 hr experimental trials. All additional experiments were set up similarly substituting acute treatments and/or temperature regimes.

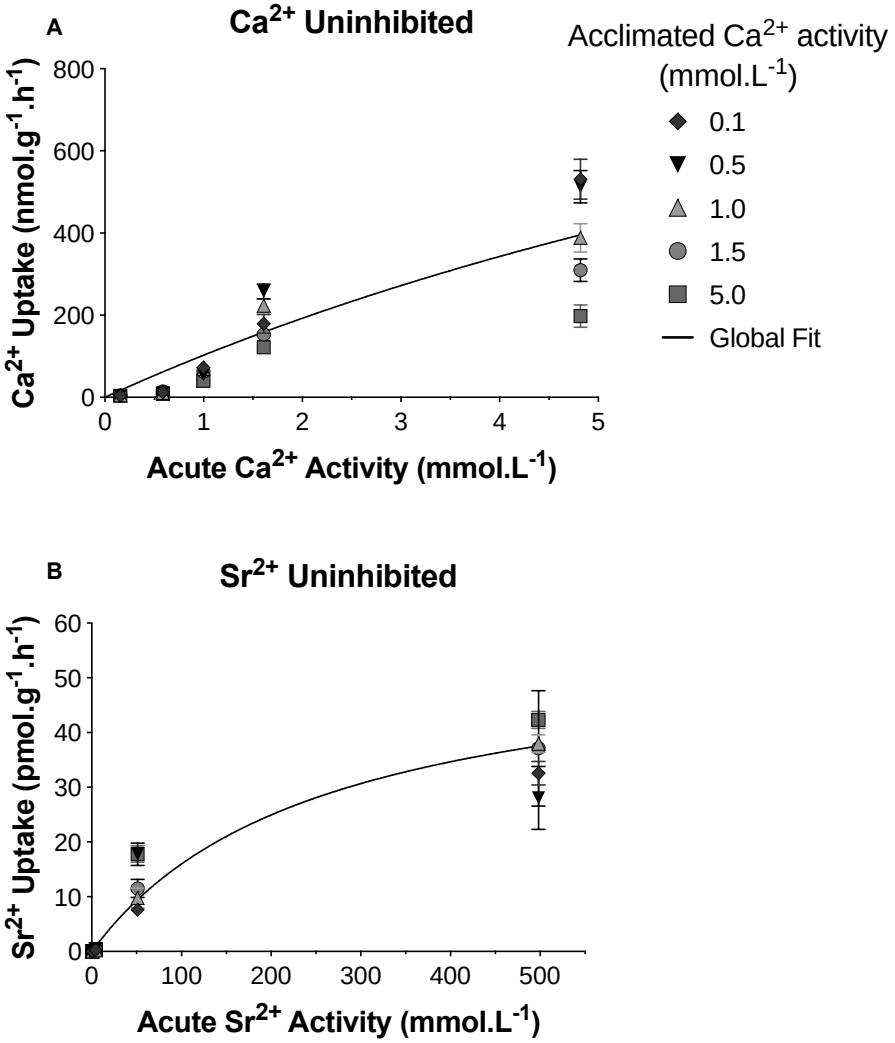

Figure 2.2. (A) Effect of acute calcium (Ca^{2+}) free ion activity ($mmol.L^{-1}$) on whole body Ca^{2+} uptake ($nmol.g^{-1}.h^{-1}$) in the uninhibited process of 30 days post hatch (dph) Lake Sturgeon (A. fulvescens) acclimated to five Ca^{2+} free ion activities after 8h exposure to ^{45}Ca. (B) Effect of acute strontium (Sr^{2+}) free ion activity ($mmol.L^{-1}$) on whole body Sr^{2+} uptake ($pmol.g^{-1}.h^{-1}$) in the uninhibited process of 30 dph Lake Sturgeon acclimated to different Ca^{2+} free ion activities after 8h exposure to ^{85}Sr and 0.03 $mmol.L^{-1}$ Ca^{2+}. Minimal Ca^{2+} free ion activity in the chambers was required in order to maintain survival during the flux trial. Symbols represent means ± s.d. for each acclimated Ca^{2+} treatment (N=8). The solid lines are global non-linear regression predictions by the Michaelis-Menten model (Eqn. 2) representing uptake kinetics across all five acclimated Ca^{2+} free ion activity treatments.

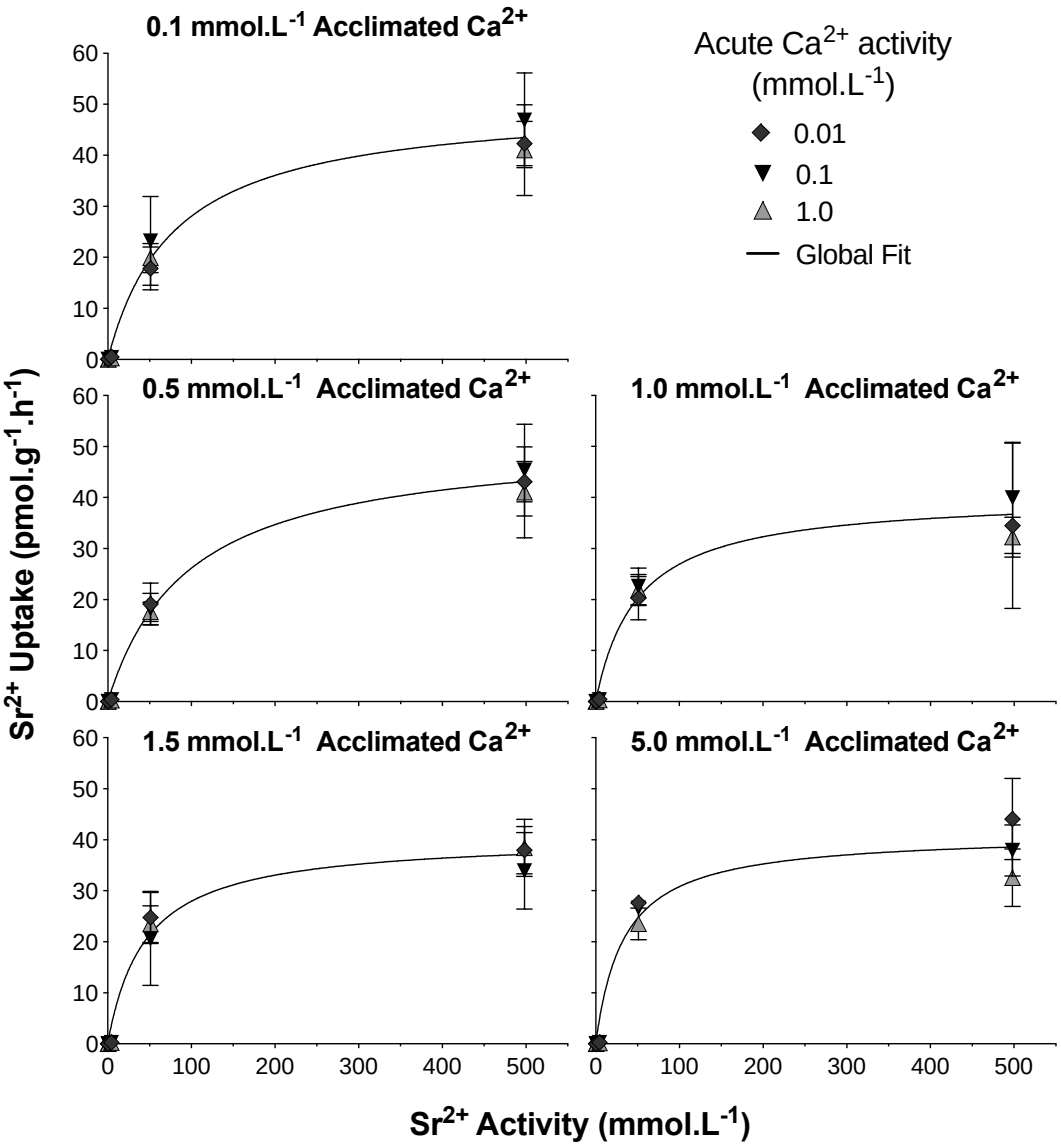

Figure 2.3. Whole body strontium (Sr^{2+}) uptake rates (pmol.g^{-1}.h^{-1}) of 30 days post hatch (dph) Lake Sturgeon (*A. fulvescens*) acclimated to five calcium (Ca^{2+}) free ion activities (0.1, 0.5, 1.0, 1.5, 5.0 mmol.L^{-1}) after 8h exposure to ^{85}Sr at varying acute concentrations of Sr^{2+}. Symbols represent means ± s.d. for each acute Ca^{2+} treatment (N=8). The solid lines are global non-linear regression predictions by the Michaelis-Menten model (Eqn. 2) representing uptake kinetics across all three acute Ca^{2+} free ion activity treatments and were used to determine K_{mSr} values for the subsequent substrate inhibition models.

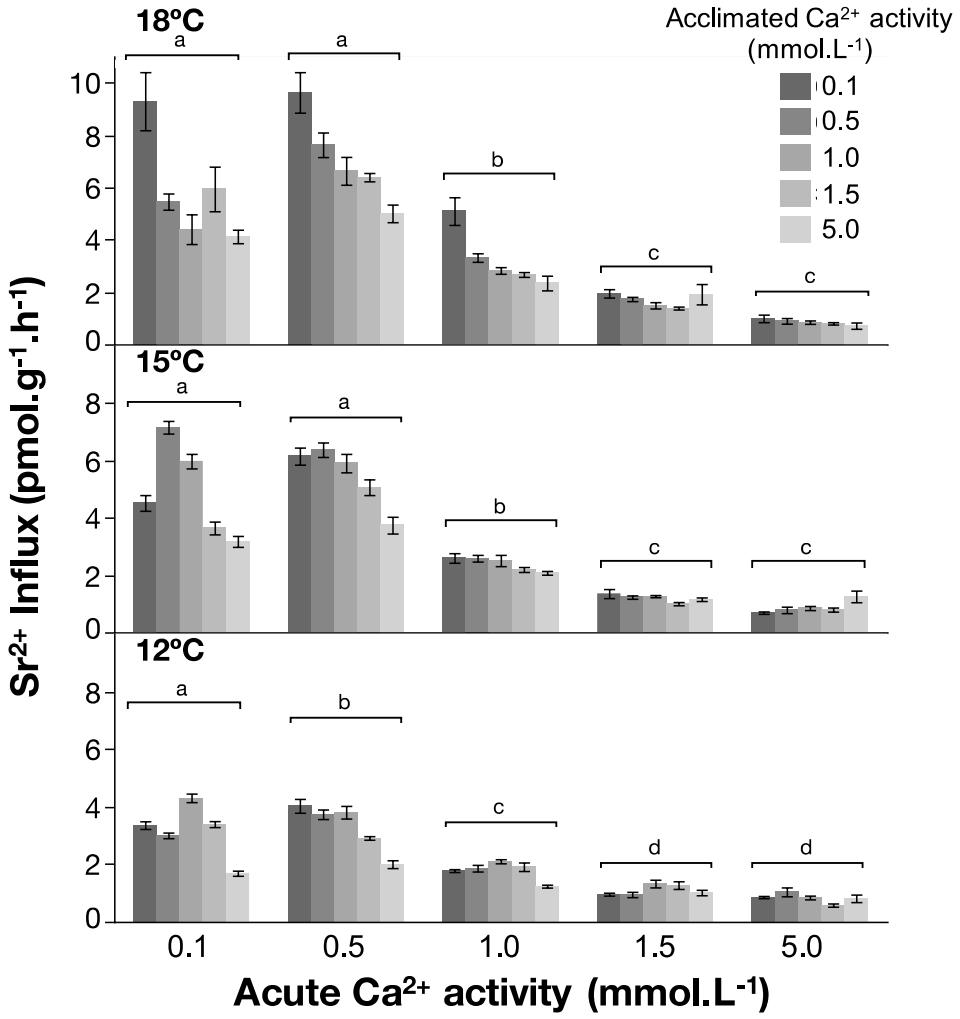

Figure 2.4. Whole body strontium (Sr^{2+}) influx ($pmol.g^{-1}.h^{-1}$) of 30 days post hatch (dph) Lake Sturgeon (*A. fulvescens*) acclimated to five Ca^{2+} free ion activities (0.1, 0.5, 1.0, 1.5, 5.0 $mmol.L^{-1}$) and three temperatures (°C) after acute exposure to ^{85}Sr and varying Ca^{2+} free ion activities ($mmol.L^{-1}$) for 8h. Data are means ± s.e.m. (N=8). Both temperature and acute Ca^{2+} free ion activity had a significant effect on Sr^{2+} influx (ANOVA, $P<0.05$). Lowercase letters indicate differences in Sr^{2+} accumulation in fin rays among acute Ca^{2+} treatments for each temperature treatment. Treatments not sharing the same letter are significantly different ($P=<0.05$).

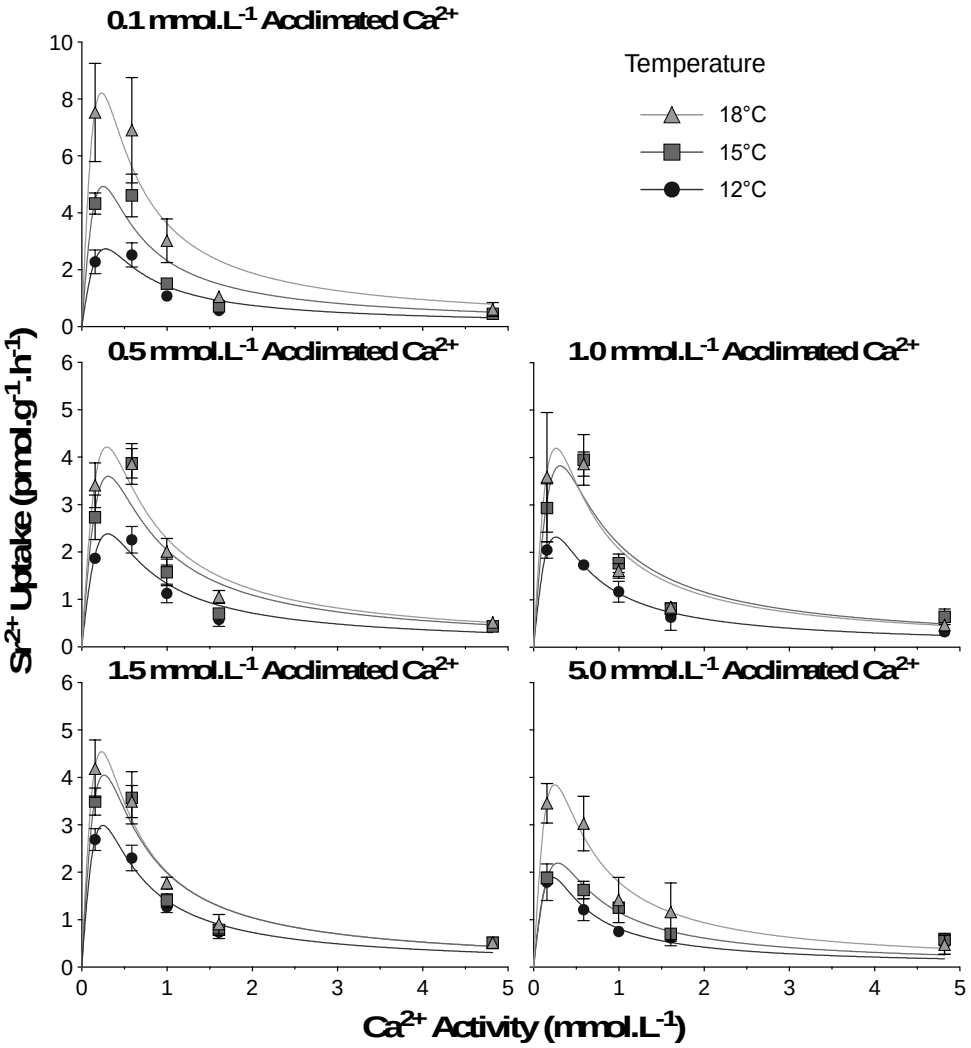

Figure 2.5. Whole body strontium (Sr^{2+}) uptake rates (pmol.g^{-1}.h^{-1}) of 30 days post hatch (dph) Lake Sturgeon (*A. fulvescens*) acclimated to five calcium (Ca^{2+}) free ion activities (0.1, 0.5, 1.0, 1.5, 5.0 mmol.L^{-1}) and three temperature treatments (12, 15, 18°C) after 8h exposure to ^{85}Sr at varying acute free ion activities of Ca^{2+}. Symbols represent means ± s.d. for each temperature treatment (N=8). The solid lines are non-linear regression predictions by the Michaelis-Menten substrate inhibition model (Eqn. 3) for each temperature treatment.

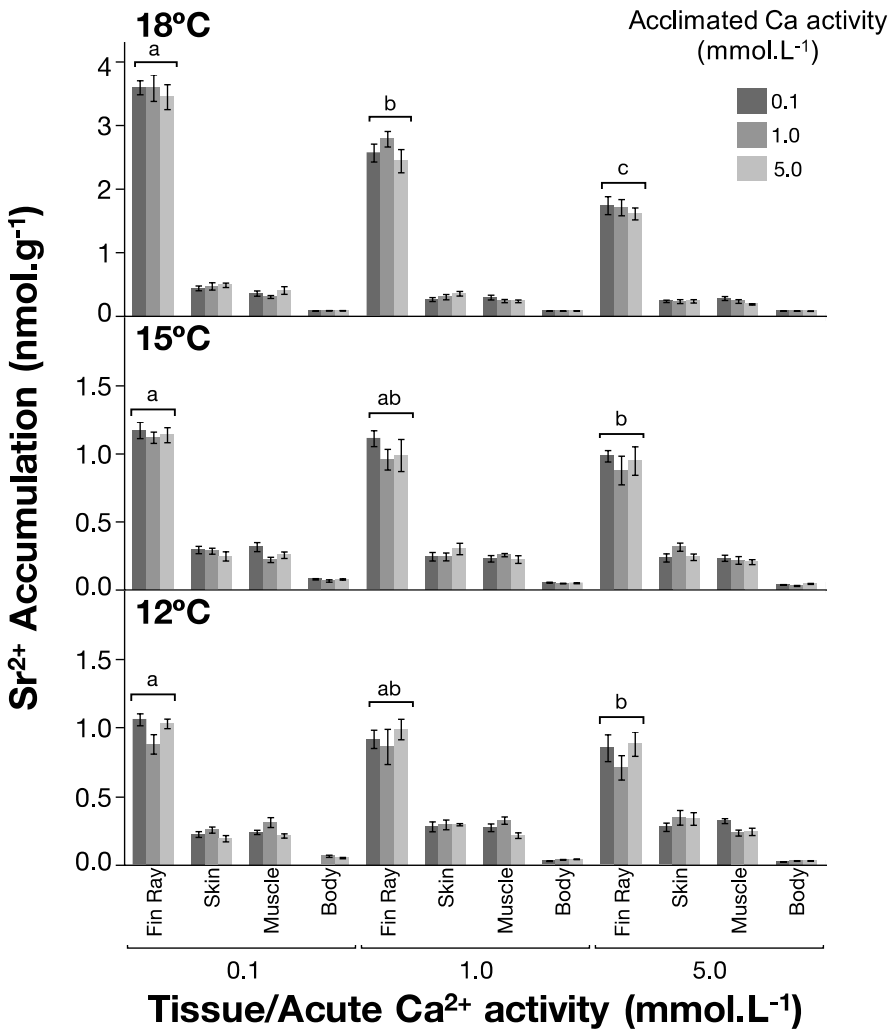

Figure 2.6. Accumulation of strontium (Sr^{2+}, nmol.g^{-1}) into tissues (fin ray, skin, muscle, remaining body) of 30 days post hatch (dph) Lake Sturgeon (*A. fulvescens*) acclimated to three Ca^{2+} free ion activities (0.1, 1.0, 5.0 mmol.L^{-1}) and three temperatures (12, 15, 18°C) after acute exposure to ^{85}Sr and varying Ca^{2+} free ion activity (mmol.L^{-1}) for 8h. Data are means ± s.e.m. (N=8). Accumulation of Sr^{2+} into fin rays was significantly higher than other tissues in all environmental treatments (ANOVA, P=<0.05). Lowercase letters indicate differences in Sr^{2+} accumulation in fin rays among acute Ca^{2+} treatments for each temperature treatment. Treatments not sharing the same letter are significantly different (P=<0.05).

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Chapter 3: Otolith polymorph composition in sturgeons: Influence of ontogenetic development and the environment

This manuscript has been published in the journal Scientific Reports. As the lead author on the manuscript I was responsible for the experimental design of the ontogeny experiment, data collection and analysis, and primary writing and revision of the manuscript. My co-author L.D. Belding designed and conducted the climate change experiment and A.R. Quijada-Rodriguez collected the blood pH data. As well, J.D. Morgan provided White Sturgeon samples from VIU and B.M. Pracheil and B.C. Chakoumakos provided analytical tools and guidance at ORNL. Finally, W.G. Anderson provided insight and direction to the overall review and design of the experiments as well as editorial revision on multiple drafts of the manuscript. All co-authors participated in revisions of the final manuscript drafts prior to submission.

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3.1 Abstract

Changes to calcium carbonate ($CaCO_3$) biomineralization in aquatic organisms is among the many predicted effects of climate change. Because otolith (hearing/orientation structures in fish) $CaCO_3$ precipitation and polymorph composition are controlled by genetic and environmental factors, climate change is predicted to affect the phenotypic plasticity of otoliths. We examined combined effects of ontogeny, temperature, and pH on the precipitation of otolith polymorphs (aragonite, vaterite, calcite) in two species of sturgeon, Lake Sturgeon, (*Acipenser fulvescens*) and White Sturgeon (*A. transmontanus*), using quantitative X-ray microdiffraction. Both species showed similar fluctuations in otolith polymorphs with a significant shift in the proportions of vaterite and aragonite in sagittal otoliths coinciding with the transition to fully exogenous feeding. To identify environmental influences on otolith morphology, Lake Sturgeon larvae were reared in varying temperature (16/22ºC) and pCO_2 (1000/2500 µatm) environments for six months. Otolith area significantly increased in fish raised in elevated temperature and induced the precipitation of large single calcite crystals. Interestingly, pCO_2 had no effect on size or polymorph composition of otoliths despite blood pH exhibiting a mild alkalosis, which is contrary to what has been observed in several studies on marine fishes. These results suggest climate change will affect otolith polymorph composition during early development.

3.2 Introduction

Organisms that produce biogenic minerals through the process of biomineralization are widespread throughout the plant and animal kingdoms (Lowenstam 1989). While the specific mechanisms and products of biomineralization are diverse among taxa, common approaches for regulating mineral production can be observed across phyla (De Yoreo and Vekilov 2003). The function of biomineralized materials among organisms is also extensive. For example, some mollusks secrete hard calciferous shells that protect the organism, while mineralized bone materials provide the structural framework of the vertebrate skeleton (Lowenstam 1989). Additionally, mobile organisms use calcified structures in their vestibular system, such as otoliths in fish —calcium carbonate ($CaCO_3$) structures located in the inner ears— to perceive gravity and detect linear and angular acceleration (Campana 1999). These calcifying organisms face particular challenges with climate change, especially in aquatic environments, where acidification results in a decline in the availability of carbonates (CO_3^{2-}), thus impacting the ability of the individual to precipitate $CaCO_3$ structures (Gattuso and Buddemeier 2000). Consequences on the processes of biomineralization in response to climate change depend on several factors specific to the organism, including biochemical pathways, physiological mechanisms, and the ecology of the organism (Ries et al. 2009).

In fishes, relatively little is known about the actual biogenesis and crystal growth mechanisms of otoliths resulting in a disparate gap in the fundamental understanding of otolith biomineralization compared to the number of studies that have examined their practical use for estimating age, growth, and habitat use (Campana 1999; Gauldie 1993). Otoliths can be made of any of the three most common crystalline polymorphs of $CaCO_3$ (i.e., aragonite, vaterite, calcite), each of which have varying unit cell densities and lattice structures (Chakoumakos et al. 2016; Christy 2017; Gauldie 1993). Aragonite has the highest crystal growth rate (Gauldie and Nelson 1988), which is conducive for creating heavy, dense, and constantly growing otoliths (Schulz-Mirbach et al. 2018). As such, the otoliths of most fishes, particularly teleosts, are primarily composed of aragonite (Carlstrom 1963; Schulz-Mirbach et al. 2018). Conversely, the otoliths of more primitive fishes, including acipenserids, have been classified for decades as being comprised entirely of the least dense, and structurally rarer polymorph vaterite (Gauldie 1993; Schulz-Mirbach et al. 2018). Recent studies, however, demonstrated that otoliths of adult Lake Sturgeon, *Acipenser fulvescens*, contained significant proportions of calcite (~18-35%) in addition to vaterite (Pracheil et al. 2017). As well, otoliths of age-0 Lake Sturgeon have been shown to be primarily composed of aragonite (>60%) along with vaterite (Loeppky et al. 2019). These results

indicate that species in the earliest branch of Actinopterygii have the capacity to precipitate any form of $CaCO_3$ polymorph, a mechanism of otolith biomineralization in fishes that previously was thought to only have evolved after the separation of teleosts from chondrostean (i.e., sturgeons, paddlefish) and holostean (i.e, gars, bowfins) lineages (Carlstrom 1963; Pracheil et al. 2019).

Otolith polymorph composition appears to be both phylogenetically implicit and controlled by phenotypic responses to biological or environmental changes (Gauldie 1993; Pracheil et al. 2019). The organic matrix, which is made up of proteins and amino acids, serves as a template for crystal nucleation controlling the polymorphism of $CaCO_3$ crystallites (Kitano and Hood 1965; Thomas et al. 2019). In particular, otolin-1, a collagenous protein that stabilizes the mineral and organic compounds on the surface of otoliths, provides the framework for subsequent calcification during otolith growth (Murayama et al. 2005). Indeed, the presence of different compounds in the matrix (e.g., citrate, malate, pyruvate), which are controlled by protein metabolism, were identified to reduce calcification rates favouring the formation of calcite (Kitano and Hood 1965). Additionally, the induced downregulation of *starmaker*, a gene responsible for regulating crystal growth, resulted in a switch from aragonite to calcite precipitation in zebrafish, *Danio rerio* (Sollner et al. 2003), suggesting the molecular control over matrix forming proteins, which can change throughout ontogeny, is an important factor in determining otolith polymorph composition (Thomas et al. 2019).

The environment can also impact otolith biomineralization. The classical process of $CaCO_3$ crystallization is driven by the change in free energy required to transition from a supersaturated solution to a crystal (De Yoreo and Vekilov 2003; Ruiz-Agudo et al. 2011). The amount of free energy required to crystallize each polymorph decreases from calcite to aragonite to vaterite and, thus, the thermodynamic stability of each polymorph also decreases, respectively (Dalas et al. 1988; De Yoreo and Vekilov 2003). As such, temperature and environmental pH have direct impacts on supersaturation states and can lead to changes in the rate of crystallization and the precipitation of varying polymorphs of $CaCO_3$ (Ruiz-Agudo et al. 2011). Furthermore, when fish are exposed to environments with elevated carbon dioxide (pCO_2), resulting in a decrease in environmental pH, a series of coordinated physiological responses occur to maintain internal pH gradients (Brauner and Baker 2009); potentially influencing the solution chemistry of the endolymph (Ishimatsu et al. 2008) and subsequently otolith morphology and/or crystal polymorph composition (reviewed in Holmberg et al. 2019). Few studies, however, have examined the effects of freshwater acidification on otolith development in fishes, none of which have been conducted on sturgeons. Freshwater systems regularly experience fluctuating

temperature and environmental pCO_2 due to the variation in sources of carbon from geologic dissolution, anthropogenic runoffs, decaying of organic matter, and seasonal variability (Hasler et al. 2018). As global temperatures and atmospheric pCO_2 levels continue to rise, fluctuations in temperature and pH in freshwater environments are predicted to increase in both scale and frequency likely having a significant impact on the organisms that occupy these habitats (Ficke et al. 2007; Hasler et al. 2018). As such, it is important to understand how elevated temperature and pCO_2 might impact the morphology and development of freshwater fish otoliths, particularly at the vulnerable early life history stages when fish are typically more sensitive to environmental change (Bignami et al. 2013; Munday et al. 2011).

Studies examining otolith polymorph composition have to-date focused primarily on the impacts of ocean acidification in marine fishes (listed in Holmberg et al. 2019), while to our knowledge, the combined effects of pH, temperature and/or ontogenetic development have not been empirically tested in any freshwater fish. In this study, we sought to provide novel insight on otolith phenotypic responses to both intrinsic (i.e., ontogeny) and extrinsic (i.e., environment) variables and how they relate to species-specific responses of otolith morphology in sturgeons. Specifically, we tested the hypotheses that 1. otolith polymorph composition varies throughout ontogeny of Lake and White Sturgeon and 2. water temperature and pH influence size and polymorphism of otoliths independent of ontogeny in juvenile Lake Sturgeon. To do this, percent polymorph composition was examined using X-ray microdiffraction throughout larval development in two sturgeon species, both of which are of critical concern throughout their North American ranges: Lake Sturgeon (*A. fulvescens*), a potadromous species that occupies the Great Lakes, Hudson Bay, and Mississippi River basins; and White Sturgeon (*A. transmontanus*), a semi-anadromous species occupying the Sacramento-San Joaquin, Columbia, and Fraser River drainages. To investigate the influence the environment has on otolith morphology, otoliths collected from age-0 Lake Sturgeon were analyzed after long-term exposure (six months) to varying temperature and pCO_2 conditions that were chosen to mimic the projected changes increasing global temperature and atmospheric CO_2 may have on freshwater systems in the natural range of Lake Sturgeon. Blood pH was also measured at the time of sampling to provide insight into the physiological mechanisms that influence otolith precipitation.

3.3 Methods

All procedures conducted on laboratory-reared fish were approved by the Animal Care Committee at the University of Manitoba (Permit F15-007) and Vancouver Island University (Permit AUP# 2015-01-H) in accordance with guidelines established by the Canadian Council on Animal Care.

Otolith samples were transported to Oak Ridge National Laboratory (ORNL) in Tennessee, USA for analysis under CITES permit #18CA0004FONHQ.

3.3.1 Experiment 1 – Ontogenetic effects

To investigate changes in otolith polymorph composition throughout ontogenetic development of sturgeons, lab-reared fish used in this experiment were the artificially fertilized progeny of both Lake Sturgeon and White Sturgeon. Lake Sturgeon gametes were collected from wild-spawning adult fish captured in the Winnipeg River, Manitoba, Canada. Fertilization and initial seeding of eggs was conducted in the Animal Holding Facility at the University of Manitoba in Winnipeg, Canada following techniques described in Loeppky et al. (2019). Briefly, eggs from two females were mixed separately with the milt of at least three males then incubated in MacDonald tumbling jars. After ~7-10 days, hatched larvae were transferred to multiple 11-litre flow through tanks supplied with dechlorinated City of Winnipeg tap water held at 16ºC with a 12h:12h light to dark photoperiod. Fish were first introduced to freshly hatched brine shrimp nauplii (*Artemia salina*) starting at 10 days post hatch (dph) and fully transitioned to exogenous feeding by 20 dph. At 50 dph fish were then introduced to chironomid larvae (i.e., bloodworms) over the course of several days before being fed solely a bloodworm diet. Throughout larval rearing fish were fed to satiation three times daily at which point all uneaten food and waste was removed from the tanks.

White Sturgeon gametes were collected from resident adult males and females at the International Centre for Sturgeon Studies at Vancouver Island University in Nanaimo, British Columbia, Canada. The broodstock for these fish originated from the Lower Fraser River in Vancouver, BC. After ~8-11 days of egg incubation, hatched larvae were transferred to 2,000 L tanks supplied with a constant flow of dechlorinated and UV-sterilized Nanaimo municipal freshwater held at 14ºC while photoperiod was determined by an external light sensor. Fish were first introduced to a commercial starter feed (EWOS #0, Cargill Incorporated, Minneapolis, MN, USA) at 5 dph with initial feeding observed at 12 dph and full transition to exogenous feeding occurring at 17 dph. At 49 dph, larger commercial feed was gradually introduced (EWOS #1), although nutritional composition of the feed remained the same. Throughout larval rearing fish were fed to satiation using 24-hr belt feeders with all uneaten food and waste being removed each day.

Both species were sampled on the same schedule throughout larval ontogeny corresponding to their respective times of hatch whereby eight larvae were randomly selected from multiple rearing tanks every two days from 12 to 20 days post hatch (dph) and then every 7 days until 69 dph (n = 88

fish/species). At each sampling point, larvae were randomly selected and immediately euthanized in an overdose of tricane methanesulphonate (MS-222) buffered with equal volumes of sodium bicarbonate, then transferred to 20 mL glass scintillation vials filled with 95% ethanol for preservation until analysis. All otolith dissections were conducted at the University of Manitoba following protocols outlined in Loeppky et al. (2019). A dissecting microscope (Olympus SZXY7) mounted with polarized lenses (Olympus SZX-PO) was used to aid with the visualization of otoliths during removal. To collect both the left sagittal and lapilli otoliths, heads were removed at the base of the opercula then sectioned dorsal medially through the sagittal plane from between the occipital cavities to the base of the skull to expose the semicircular canals where the otoliths are housed. Dissected otoliths were then transferred to microscope slides that had a 1 cm^2 5x5 grid covered with a piece of double-sided tape to organize otoliths for transportation to Oak Ridge National Laboratory in Tennessee, USA for analyzation via X-Ray microdiffraction (μXRD). Quantitative phase analysis to determine the percent polymorph composition by weight of each otolith was conducted in the GSAS II open-source software package (Toby and Von Dreele 2013) using the Rietveld method (Rietveld 1969).

3.3.2 Experiment 2 – Environmental effects

To investigate the effects of environmental temperature and pH on otolith size and polymorph composition of age-0 Lake Sturgeon, fish were reared from egg incubation for six months in varying temperature and pCO_2 treatments (Table 3.1). Fertilized Lake Sturgeon eggs, collected as described in Experiment 1, were equally distributed into four McDonald tumbling jars (~1L/jar) assigning the fish to one of the following four experimental treatments: Control (15ºC, 1000 μatm pCO_2), elevated pCO_2 (15ºC, 2500 μatm pCO_2), elevated temperature (22ºC, 1000 μatm pCO_2) or elevated temperature + pCO_2 (22ºC, 2500 μatm pCO_2). Each treatment was supplied water from separate header tanks corresponding to their pCO_2 levels. The header tanks were injected with a controlled amount of CO_2 regulated by an IKS Aquastar system equipped with pH monitoring electrodes (IKS Aquastar Computer Systeme GmbH, Germany). Given limitation on space and resources required to maintain aquaria, fish were reared in a single large aquarium per treatment. Care was taken to ensure other environmental variables (i.e., light, density, feeding) were consistent among tanks. At the onset of hatching, larvae were transferred to 170 L fibreglass flow-through aquaria and reared in the same assigned experimental treatments for 150 days at which point temperature in all four treatment tanks was dropped to 3ºC (0.5ºC/day) to simulate an overwintering period (experimental pCO_2 levels were maintained). After 40 days in the overwintering conditions, 8 fish per treatment (n = 32 fish total) were randomly sampled and

immediately euthanized in an overdose of MS-222 buffered with equal volumes of sodium bicarbonate (250 $mg.L^{-1}$) in their respective treatment water to maintain experimental CO_2 tensions.

To account for differences among experimental rearing treatments, total body length (mm), fish mass (g) and blood pH were measured. Because of the small size of fish, blood samples were collected by severing the tail of euthanized fish to expose the caudal sinus and drawing blood using a heparinized capillary tube. The blood was then ejected from the capillary tube using a syringe and transferred into 1.5 mL vials. Blood samples were floated in a 3°C water bath (Julabo F25-MC, Allenton, PA, USA) and pH was measured with a thermostated micro pH electrode (InLab Micro, Mettler Toledo, Ohio, USA). To correct for gas exchange that may have occurred during the expelling of the blood from the capillary tube, larger Lake Sturgeon (3 years) were acclimated to the same experimental pCO_2 levels for three weeks at 3ºC in duplicate 170 L tanks (n = 6 fish/treatment). Fish were lightly anesthetized in an anesthetic bath that was created using 150 $mg.L^{-1}$ MS-222 buffered with equal volumes of sodium bicarbonate in their respective treatment waters. Blood samples were drawn from the caudal sinus using a syringe then transferred to a vial so that blood pH could be measured immediately. A capillary tube was then used to draw a small sample of blood then expelled using a syringe to mimic the measuring of the age-0 fish. The percent change in blood pH from the syringe method and capillary method was consistent across treatments and thus was used to correct the original blood pH data collected from the experimental fish using the capillary method.

The left sagittal otolith was then removed from each fish and imaged for size comparison at 10x magnification using a dissecting microscope (Olympus SZXY7). Otolith area was calculated using Fiji for ImageJ software (http://rsb.info.nih.gov/i) by converting images to 8-bit then adjusting the threshold to isolate the outline of the otolith. The function Analyze Particles was then used to calculate the area of the otolith. Otoliths were then transferred to ORNL and analyzed via µXRD as described above.

3.3.3 Statistical Analyses

All analyses were conducted using SAS statistical software in JMP Pro® version 15.2.1. Percent composition of otolith polymorph were logit transformed prior to conducting analyses. For all data sets, response variables were first examined separately for normality using the Shapiro-Wilk W test and for homogeneity of variance using the Levene's Test. In Experiment 1, only vaterite and aragonite were present in otoliths thus polymorph proportion in sagittal and lapilli otoliths (response variables, % weight) were examined using general linear models (GLMs) with species and developmental stage (dph) as fixed independent variables along with their interactions. Post-hoc Tukey HSD tests were then

conducted to determine which factor levels were different. In Experiment 2, regression analyses examining otolith area (mm^2) and fish length (mm) and body mass (g) were determined using linear mixed models. Additionally, normalized differences (z-score) in otolith area (mm^2), fish length (mm), and mass (g) among experimental treatments were tested using GLMs with treatment as fixed independent factos examining temperature (°C) and pCO_2 (µatm) and their interaction followed by post-hoc Tukey HSD tests to determine which factor levels were different. To examine differences in vaterite, aragonite, and calcite composition (response variables, % weight) as well as blood pH, GLMs were used with temperature and pCO_2 as fixed independent factors as well as their interactions.

3.4 Results

3.4.1 Experiment 1

Environmental parameters (i.e., temperature, pH, alkalinity, hardness) during the rearing period for Lake and White Sturgeon are presented in Table 3.1. Both species had varying proportions of both vaterite and aragonite present in sagittal and lapilli otoliths that followed similarly changing patterns (Figure 3.1). Statistical differences in polymorph composition among species and ontogenetic development are presented in Table 3.2. Notably, percent aragonite was significantly higher in the sagittal otoliths of Lake Sturgeon compared to White Sturgeon while lapilli otoliths were not statistically different between species. Additionally, developmental stage (i.e., dph) had a significant effect on both sagittal and lapilli otolith polymorph composition with peak aragonite precipitation in sagittal otoliths occurring at 20 dph in both species, coinciding with the onset of exogenous feeding.

3.4.2 Experiment 2

Environmental parameters (i.e., temperature, pH, alkalinity, pCO_2) measured throughout the rearing and overwintering period are presented in Table 3.1. Otolith area was positively linearly related to both fish length and fish mass (Figure 3.2) with each variable being significantly influenced by experimental rearing temperature (Table 3.2). In fact, all variables had the highest values in treatments that experienced elevated temperatures during the summer/fall rearing period (Figure 3.3). This is most prominently observed in the size differences in otoliths presented in Figure 3.4a. Interestingly, calcite precipitation was present only in otoliths collected from fish reared in elevated temperature treatments (i.e, temperature, temperature + pCO_2; Figure 3.4c, 3.5) with temperature being the only environmental variable that had a significant effect on polymorph precipitation (Table 3.2). This is reflected in the diffraction patterns of otoliths from the elevated temperature and elevated temperature + pCO_2 treatments where spots are clearly visible indicating the presence of large single crystals (Figure 3.4b).

Despite not having an effect on otolith polymorph composition, environmental pCO_2 significantly influenced blood pH with fish reared in elevated pCO_2 treatments exhibiting a mild alkalosis (i.e., increase in blood pH; Table 3.3).

3.5 Discussion

While variation in otolith polymorph composition in response to acidification is relatively well described in several marine teleosts, this is the first time, to the authors knowledge, the effects of ontogeny, temperature, and pH on the polymorphism of otoliths has been experimentally manipulated providing important information on the formation of acipenserid otoliths at the larval life stage. Early growth and development of specific structures or organs has long lasting effects on the phenotype of the structure in all organisms, not least fishes. Given otoliths are considered an inert tissue, once the matrix and crystal polymorphs are deposited, there is no reabsorption of the otolith material (Campana 1999). Therefore, the development of otoliths set during early ontogeny may have significant effects on future hearing and orientation abilities, and, thus, the subsequent survival of the individual as these senses are required for habitat choice, predator avoidance, and foraging. Here we have described changes in polymorph composition in the otoliths of Lake and White Sturgeon during early ontogeny and demonstrate significant effects of the environment on otolith development in age-0 Lake Sturgeon.

3.5.1 Ontogenetic effect

By examining the early ontogeny of otoliths in Lake Sturgeon and White Sturgeon, we followed the progression of otolith formation in two species with disparate life history characteristics at their juvenile and adult stages. In the wild, spawning for each species occurs in freshwater habitats and, thus, the development of eggs and larvae occurs in broadly comparable environments. Both sagittal and lapilli otoliths collected from Lake and White Sturgeon showed similarly varying proportions of percent vaterite and aragonite throughout the developmental period resulting in significant differences in overall mean percent polymorph composition reported between species in sagittal otoliths only although there was a significant interaction between species and developmental stage (Figure 3.1, Table 3.2). During the rearing period, both species were held independently in constant conditions, however, the environmental parameters experienced by each species were different. Temperature was higher during larval rearing for Lake Sturgeon and although not explicitly measured in this study, City of Winnipeg and Nanaimo water quality reports (winnipeg.ca; nanaimo.ca) indicate differences in total metal content, alkalinity, and water hardness between sources (Table 3.1). Water hardness and alkalinity in particular were considerably different whereby Lake Sturgeon were reared in moderately hard and alkaline water

while White Sturgeon were reared in soft and lower alkalinity water. Both measurements reflect the amount of carbonates present in the system, which in turn affects the development of larval fishes, in particular their calcified structures (Blanksma et al. 2009). Additionally, temperature impacts the solubility of ions and has been observed to increase metal uptake and deposition mechanisms in Lake Sturgeon (Loeppky and Anderson 2020). Consequently, with the higher availability of Ca^{2+} and carbonate ions, changes in supersaturation in the endolymph of Lake Sturgeon may have been affected thus impacting the crystallization rates of the otoliths and increasing the precipitation of the denser polymorph aragonite.

The similarities observed in the patterns of percent vaterite and aragonite changes throughout ontogeny among both species, with relatively low variation (Figure 3.1), suggest genetic control of $CaCO_3$ polymorph crystallization in sturgeons. The organic matrix, which controls the nucleation, shape, and orientation of crystallites, forms when otolith soluble-matrix proteins are secreted into the endolymph by specialized cells in the saccular epithelium (Takagi and Takahashi 1999). As such, changes in the metabolism of key amino acids affects the synthesis of proteins that regulate the rate of calcification and, thus, the polymorphism of $CaCO_3$ crystals (Murayama et al. 2005; Nagasawa 2013; Takagi and Takahashi 1999). Interestingly, peak aragonite composition in sagittal otoliths occurred simultaneously at 20 dph in both species, coinciding with the complete transition to exogenous feeding, at which point vaterite precipitation increased. At hatch, sturgeon rely on yolk sac reserves that are high in amino acids as sources of metabolic fuel, which are essential for the growth and development of larvae (Houlihan et al. 1989). As such, during yolk resorption, free amino acid availability, necessary for protein synthesis, steadily diminishes until first feeding (Ronnestad et al. 1999). This in turn would affect the rate of protein synthesis in the saccular membrane thus impacting the composition of the otolith organic matrix within the saccule (Houlihan et al. 1989). A similar occurrence was observed in multiple marine species where diet correlated with changes in otolith shape attributed to variation in the quantity and composition of proteins in prey (Mille et al. 2016).

The difference in protein content of the diet provided to both species at first feeding may also have been a factor in the overall mean differences in vaterite proportions observed in the sagittal otoliths throughout development. Lake Sturgeon larvae were initially fed a diet of artemia nauplii, which contain ~60% crude protein (reedmariculture.com), while White Sturgeon were fed EWOS commercial aquaculture feed containing ~54% crude protein (cargill.com). This difference in protein content, along with other essential nutrients (e.g., lipids, carbohydrates and minerals), affects the proportion of water-

soluble (associated with mineral phase) and -insoluble (associated with structural foundation for crystal growth) proteins that are synthesized (Asano and Mugiya 1993). One such protein is otolin, a chemically unique protein specific to otoliths that is characterized by a high abundance of acidic amino acids (Asano and Mugiya 1993; Nagasawa 2013). These acidic amino acids contribute to otolith mineral formation by concentrating Ca^{2+} ions and CO_3^{2-} molecules thus inducing supersaturation of the system, which is required for crystallization to occur (Asano and Mugiya 1993; De Yoreo and Vekilov 2003). As such, the amount of otolin present influences the probability of crystal nucleation and consequently the growth of otoliths (De Yoreo and Vekilov 2003; Nagasawa 2013). The lapilli otoliths of both species followed similar trends of increasing aragonite composition until 48 dph. After 48 dph, increased precipitation of aragonite continued in White Sturgeon, while a switch to vaterite precipitation is clearly demonstrated in Lake Sturgeon lapilli otoliths. Interestingly, it was during this transition period (50 dph) that Lake Sturgeon larvae were introduced to bloodworms, which contain a minimum crude protein of only 6% (hikari.com). This transition was gradual whereby the proportion of bloodworms was increased by 10% every two days and as such the residual effects on otolith formation were likely delayed. Overall these results suggest that diet may have a significant influence on matrix proteins that control $CaCO_3$ precipitation in sturgeons, however, further investigation of protein expression in the saccule is needed to confirm this hypothesis.

Despite such large proportions of aragonite being present in all otoliths at the larval stages of sturgeon development in this study, as well as in Loeppky et al. (2019), no aragonite was reported in the adult Lake Sturgeon otoliths measured by Pracheil et al. (2017) when examined via neutron diffraction. As sturgeon age and grow, and consequently their otoliths grow, vaterite and calcite appear to be the primary $CaCO_3$ polymorphs being precipitated. As such, the proportion of aragonite that is crystallized during the larval stages eventually falls below the detectable limits of Rietveld refinement in the larger mass otoliths of adults.

3.5.2 Environmental effects

The effects of the environment on otolith morphology have thus far focused primarily on teleosts (e.g., Coll-Llado et al. 2018; Holmberg et al. 2019; Munday et al. 2011). In this study, we examined for the combined effects of temperature and pH on otolith size and polymorph composition in non-teleost fish. Indeed, otolith size was significantly influenced by rearing treatment and was positively correlated with both fish length and mass (Figure 3.2). This is consistent with other fish species where, in general, otolith growth is directly proportional to the somatic growth of the individual (Lombarte and Lleonart

1993). Perhaps not surprisingly, temperature was the driving factor in the size differences among experimental rearing environments with otolith area, fish length, and mass being highest in the elevated temperature treatments (Figure 3.3). This is particularly evident in the size differences in otoliths sampled among treatments (Figure 3.4a).

Interestingly, calcite precipitation was only observed in the otoliths also sampled from fish reared in the elevated temperature treatments (Figure 3.5). This is reflected in the X-ray diffraction patterns of these otoliths, which displayed spots (i.e., single-crystal Bragg peaks) whereas finer grained vaterite and aragonite produced Debye-Scherrer rings (Figure 3.4b). The spots are indicative of relatively large single-crystal calcite grains (>100 µm up to mm size) implying a lower nucleation rate as compared to aragonite and vaterite, suggesting temperature had an effect on the crystallization process in the otoliths of the fish in our study. Vaterite is a precursor to denser aragonite and calcite $CaCO_3$ polymorphs, the pathways of which are controlled by both thermodynamic and kinetic reactions (Konopacka-Łyskawa et al. 2020). Therefore, increasing temperature, increases the rate of these reactions by raising the kinetic energy available to the system. Temperature also influences the free energy response of minerals by affecting their chemical potential and solubility making Ca^{2+} and CO_3^{2-} more readily available to be transported by enzymes, the rate of which is also increased by temperature, in the saccular epithelium (Kwan et al. 2020) thus increasing $CaCO_3$ accretion and the growth rate of otoliths (Mosegaard and Titus 1987). Additionally, the precipitation of large single calcite crystals may have been controlled by differential expression of otolith matrix proteins in the saccular membrane. Given the strong connection of biomineralized structures with organic matrices that act as templates for crystallization, the organic matrix in otoliths is presumably the main controller of the orientation and size of crystallites being formed (Nagasawa 2013; Thomas et al. 2019). Temperature directly influences protein synthesis and, thus, impacts the composition of proteins in the saccule. Similarly, the proportion of water-soluble and -insoluble proteins were disparately affected by temperature increases in the otoliths of juvenile cod, *Gadus morhua*, impacting the density and, thus, microstructure of their otoliths (Hüssy et al. 2004). Regardless of the mechanism, calcite precipitation appears to be temperature dependent in age-0 Lake Sturgeon.

Despite pCO_2 reportedly having an influence on the otolith morphology of multiple marine teleosts (e.g., Bignami et al. 2013; Coll-Llado et al. 2018; Munday et al. 2011; Shen et al. 2016), environmental pCO_2 (and therefore pH) had no effect on otolith size or polymorph composition in our study. Acid-base regulation is a critical physiological process as inappropriate maintenance will

influence the overall performance of the individual by impacting the functionality of proteins and other essential macromolecules (Shartau et al. 2017). Sturgeons are considered to be particularly tolerant to low pH environments and are able to tightly regulate intra- and extra-cellular acid-base balance (Baker et al. 2009; Shartau et al. 2017). These highly sophisticated physiological mechanisms likely evolved to historical extremely high pCO_2 environments when global climates more closely resembled tropical aquatic systems (~250-300 million years ago; Baker et al. 2009; Ultsch 1996). Interestingly, the blood pH measurements collected at the time of sampling indicated the Lake Sturgeon in our study exhibited a mild alkalosis when compensating for the elevated levels of pCO_2 in the environment (Table 3.3). This is evidenced by the significant increase in blood pH (i.e., more basic) in comparison to fish that were reared in control pCO_2 environments. The mild alkalosis observed in our study may have been a factor of the chronic exposure to elevated pCO_2 that the age-0 Lake Sturgeon experienced as typically an initial rapid acidosis in fishes (decrease in blood pH) is gradually corrected by metabolic alkalosis to return blood to a normal pH (Baker et al. 2009). As such, it is likely that for the majority of time during development, endolymph pH would have been buffered against high external pCO_2 conditions. Although not statistically significant, the variability in otolith polymorph composition was reduced in the pCO_2 treatment suggesting greater control over $CaCO_3$ precipitation (Figure 3.5). Had the fish in our study been exposed to greater differences in environmental pCO_2 there likely would have been greater impacts on otolith morphology. While the interaction between the environment and the specific mechanisms of phenotypic expression of otolith polymorphs remains unclear, our results suggest temperature, over pH, plays a primary role in the phenotypic plasticity of otolith crystallization in Lake Sturgeon.

3.5.3 Conclusions

Overall, this study provides a novel demonstration of the interplay between biological and environmental controls on otolith phenotypes. We showed that otolith polymorph composition varies similarly throughout early Lake and White Sturgeon ontogeny, suggesting otolith polymorph composition is under genetic control and may also be correlated to diet at this life stage. On the other hand, temperature also influenced otolith phenotypic plasticity, increasing otolith size and inducing the precipitation of large single calcite crystals.

Our findings have several implications for fish conservation. Information on polymorph composition through early ontogeny is important for locating the initial seeding of sturgeon otoliths, which may help with identification of the core, or first formed, region in otoliths of adult fish. The

otolith core contains elemental signatures of the natal areas of fish and can be useful for identifying critical habitats in need of protection (Pracheil et al. 2014). The consequences of variation in polymorph composition on the behavioural performance of sturgeons (i.e., hearing, orientation, foraging) remains unknown. Fish reared in aquaculture settings are typically subjected to controlled environments where conditions are markedly different to those experienced by wild conspecifics. Despite this, the release of hatchery-reared fish is a commonly used conservation technique, often applied to increase sturgeon stocks. Fish hatcheries often encourage enhanced growth by feeding enriched diets, increasing water temperatures, and using continuous light to encourage rapid growth rates (Reimer et al. 2017). The increased growth rate in hatchery reared fish likely impacts otolith matrix proteins resulting in the abnormal crystallization of otoliths in farmed fish and has been shown to result in hearing impairment in farmed salmonids (Reimer et al. 2017). For conservation hatcheries, where the primary goal is to produce offspring that are capable of survival post hatchery release and contribute to future populations (Flagg et al. 1999), it is important to ensure rearing practices do not have adverse effects on the development of individuals. If changes in otolith size and composition (affecting density) have adverse effects on the hearing and navigation capabilities of sturgeons, this may impact the survivability of stocked individuals once introduced into wild environments.

To understand the underlying processes of otolith morphology and $CaCO_3$ polymorph determination, investigating the molecular mechanisms involved in protein expression in the saccule is essential. While these mechanisms are described to some degree in teleosts (e.g., Lundberg et al. 2015; Murayama et al. 2002; Sollner et al. 2004), the equivalent mechanisms in acipenserids are unknown. Sturgeon are primitive fishes often considered living fossils, suggesting that evolutionary change in this group is relatively slow. Given their phylogenetically more distant status, identifying the proteins involved in the synthesis of the organic matrix in chondrosteans, and the mechanisms involved in their expression, will offer new insights into the evolution of hearing and balance structures among fish species and potentially higher vertebrates.

3.6 Acknowledgements

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3.7 Tables

Table 3.1. Mean (±s.e.m.) environmental rearing conditions within each rearing location in Experiment 1 (ontogenetic effects) and rearing period in Experiment 2 (environmental effects).

Experiment 1 – Ontogenetic effects

Rearing Location	Species	Temperature (°C)	pH	Alkalinity ($mg.L^{-1}$)	Hardness ($mg.L^{-1}$)
Winnipeg, MB	Lake Sturgeon	16.3 (±0.08)	7.75*	68.3*	80.7*
Nanaimo, BC	White Sturgeon	13.8 (±0.01)	7.58*	32.1*	32.0*

Experiment 2 – Environmental effects

Rearing Period	Treatment	Temperature (°C)	pH	Alkalinity ($mEQ.L^{-1}$)	pCO_2 (µatm)
Summer/Fall (30-160 dph)	Control	15.6 (±0.03)	7.78 (±0.07)	1219.8 (±19.18)	1096.9 (±136.55)
	pCO_2	15.7 (±0.06)	7.44 (±0.06)	1225.4 (±13.02)	2644.2 (±109.54)
	Temperature	22.4 (±0.58)	8.00 (±0.03)	1230.7 (±12.16)	1019.7 (±41.90)
	Temperature + pCO_2	22.5 (±0.58)	7.44 (±0.08)	1213.8 (±10.40)	2365.3 (±116.64)
Overwinter (220-260 dph)	Control	2.9 (±0.10)	7.64 (±0.10)	1204.7 (±18.23)	1041.2 (±46.30)
	pCO_2	2.9 (±0.17)	7.04 (±0.06)	1198.3 (±15.28)	2594.0 (±78.10)
	Temperature	2.8 (±0.09)	7.48 (±0.06)	1214.0 (±79.09)	1088.6 (±81.68)
	Temperature + pCO_2	2.9 (±0.12)	7.07 (±0.03)	1204.3 (±24.26)	2648.4 (±117.82)

* Indicates measurements were obtained from City of Winnipeg and Nanaimo water quality reports (winnipeg.ca; nanaimo.ca)

Table 3.2. Statistical results of general linear models from both the ontogenetic and environmental effects experiments. In Experiment 1, species (i.e., Lake Sturgeon, White Sturgeon) and developmental stage (i.e., days post hatch, dph) were fixed independent variables. In Experiment 2, response variables were % polymorph composition (i.e., vaterite, aragonite, calcite), normalized (z-score) otolith area (mm^2), fish length (mm), fish mass (g), and blood pH with experimental temperature (°C) and pCO_2 (µatm) as fixed independent variables. P-values <0.05 indicate significant differences and are bolded.

Experiment 1 – Ontogenetic effects

Response Variable	Independent Variable	df	df_{error}	F ratio	p-value
Sagittae	Species	1	129	32.62	**<.0001**
(% aragonit/vaterite	dph	10	129	15.33	**<.0001**
composition)	Species x dph	10	129	3.54	**0.0004**
Lapillus	Species	1	103	0.01	0.89
(% aragonit/vaterite	dph	8	103	10.59	**<.0001**
composition)	Species x dph	8	103	6.42	**<.0001**

Experiment 2 – Environmental effects

Response Variable	Independent Variable	df	df_{error}	F ratio	p-value
Vaterite	Temperature	1	28	1.30	0.26
	pCO_2	1	28	0.23	0.64
	Temperature x pCO_2	1	28	1.56	0.22
Aragonite	Temperature	1	28	0.07	0.79
	pCO_2	1	28	0.003	0.96
	Temperature x pCO_2	1	28	0.37	0.55
Calcite	Temperature	1	28	10.09	**0.004**
	pCO_2	1	28	1.89	0.18
	Temperature x pCO_2	1	28	2.00	0.17
Otolith area	Temperature	1	28	11.19	**0.002**
	pCO_2	1	28	2.52	0.12
	Temperature x pCO_2	1	28	0.54	0.47
Fish length	Temperature	1	28	67.51	**<.0001**
	pCO_2	1	28	0.02	0.89
	Temperature x pCO_2	1	28	3.31	0.08
Fish mass	Temperature	1	28	48.89	**<.0001**
	pCO_2	1	28	0.65	0.43
	Temperature x pCO_2	1	28	2.09	0.16
Blood pH	Temperature	1	28	4.07	0.053
	pCO_2	1	28	31.01	**<.0001**
	Temperature x pCO_2	1	28	0.21	0.65

Table 3.3. Mean (± s.e.m.; n = 8/treatment) blood pH measurements of age-0 Lake Sturgeon, *Acipenser fulvescens*, at sampling (260 days post hatch) within each rearing treatment in Experiment 2 (i.e., control [15ºC, 1000 µatm pCO_2], pCO_2 [15ºC, 2500 µatm pCO_2], temperature [22ºC, 1000 µatm pCO_2], temperature + pCO_2 [22ºC, 2500 µatm pCO_2]).

Rearing Treatment	Blood pH
Control	7.78 (±0.03) ^{a}
pCO_2	7.96 (±0.03) ^{b}
Temperature	7.84 (±0.02) ^{a}
Temperature + pCO_2	7.98 (±0.03) ^{b}

^{a,b} indicate results for post-hoc Tukey HSD tests among treatments. Values not sharing the same letter are significantly different ($p<0.05$).

3.8 Figures

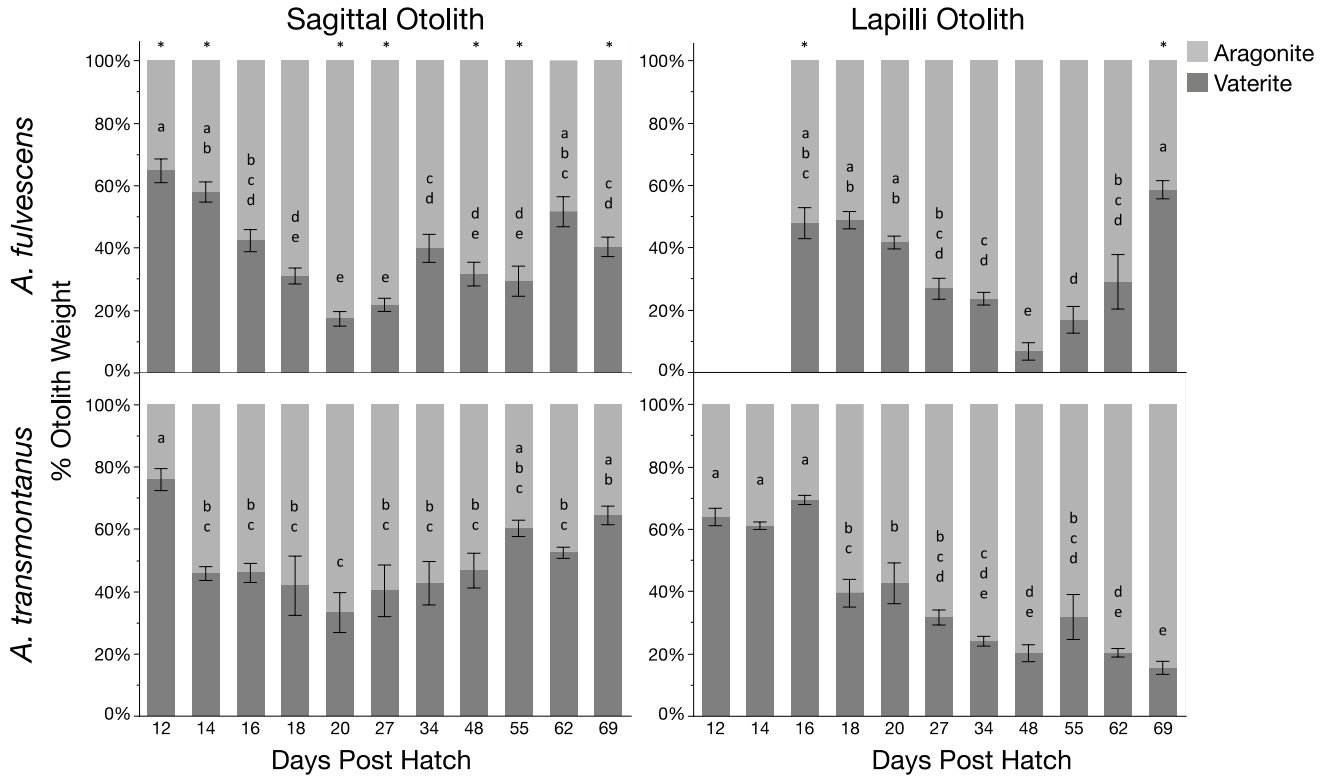

Figure 3.1. Mean (± s.e.m., n = 8 otoliths) percent polymorph composition (i.e., aragonite, vaterite) by weight of sagittal and lapilli otoliths collected throughout the ontogenetic development (i.e., days post hatch, dph) of Lake Sturgeon, *Acipenser* fulvescens, and White Sturgeon, *A. transmontanus*. Stars (*) indicate significant differences between species among dph while letters indicate results of post-hoc Tukey HSD tests where developmental stages not sharing the same letter are significantly different. Lapilli otoliths of Lake Sturgeon are not included at 12 and 14 dph because they were too small to remove. Note the measured values are proportions thus the error bars apply to both aragonite and vaterite.

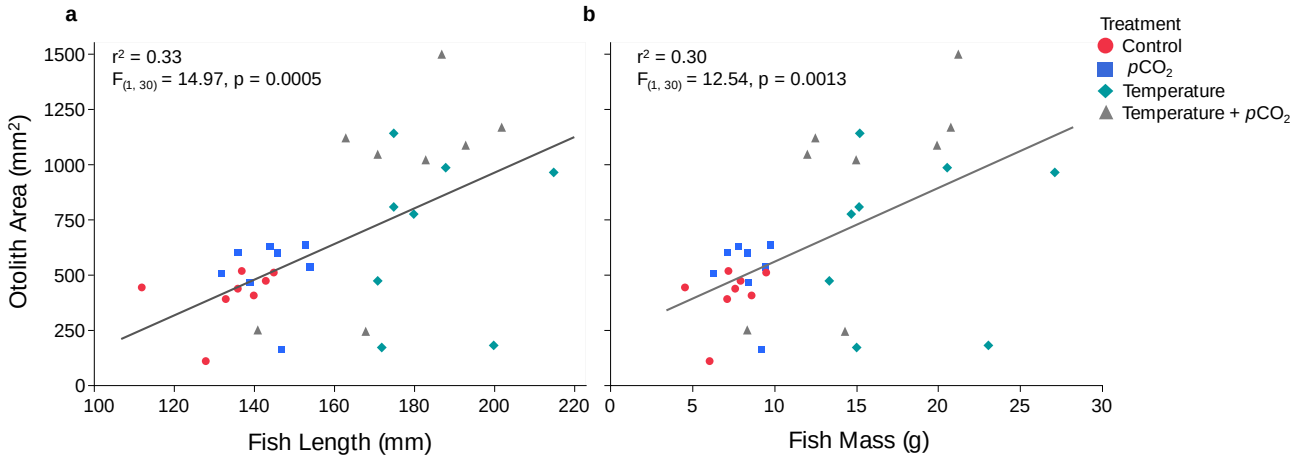

Figure 3.2. Regression analyses examining the relationship of otolith area (mm^2) and (a) fish length (mm) and (b) fish mass (g) with 95% confidence intervals shaded in grey. Rearing treatment is indicated by red circles (control, 15ºC, 1000 µatm pCO_2), blue squares (pCO_2, 15ºC, 2500 µatm pCO_2), teal diamonds (temperature, 22ºC, 1000 µatm pCO_2), and grey triangles (temperature + pCO_2, 22ºC, 2500 µatm pCO_2). The coefficient of determination and significance are presented for each regression.

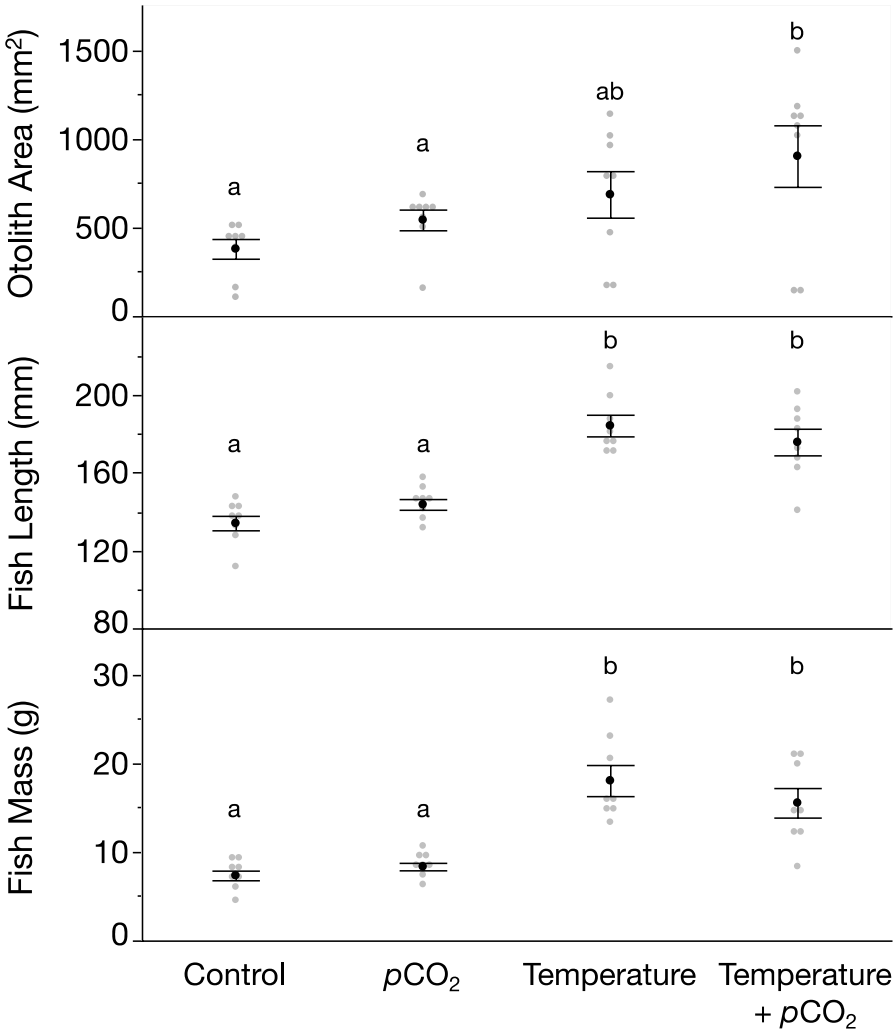

Figure 3.3. Mean (±s.e.m.) otolith area (mm^2), fish length (mm), and fish mass (g) within each experimental treatment (i.e., control [15ºC, 1000 µatm pCO_2], pCO_2 [15ºC, 2500 µatm pCO_2], temperature [22ºC, 1000 µatm pCO_2], temperature + pCO_2 [22ºC, 2500 µatm pCO_2]). Grey points represent measurements for each individual (n = 8/treatment) and are jittered on the x-axis for visualization. Treatments not sharing the same letter are significantly different (ANOVA, $p<0.05$).

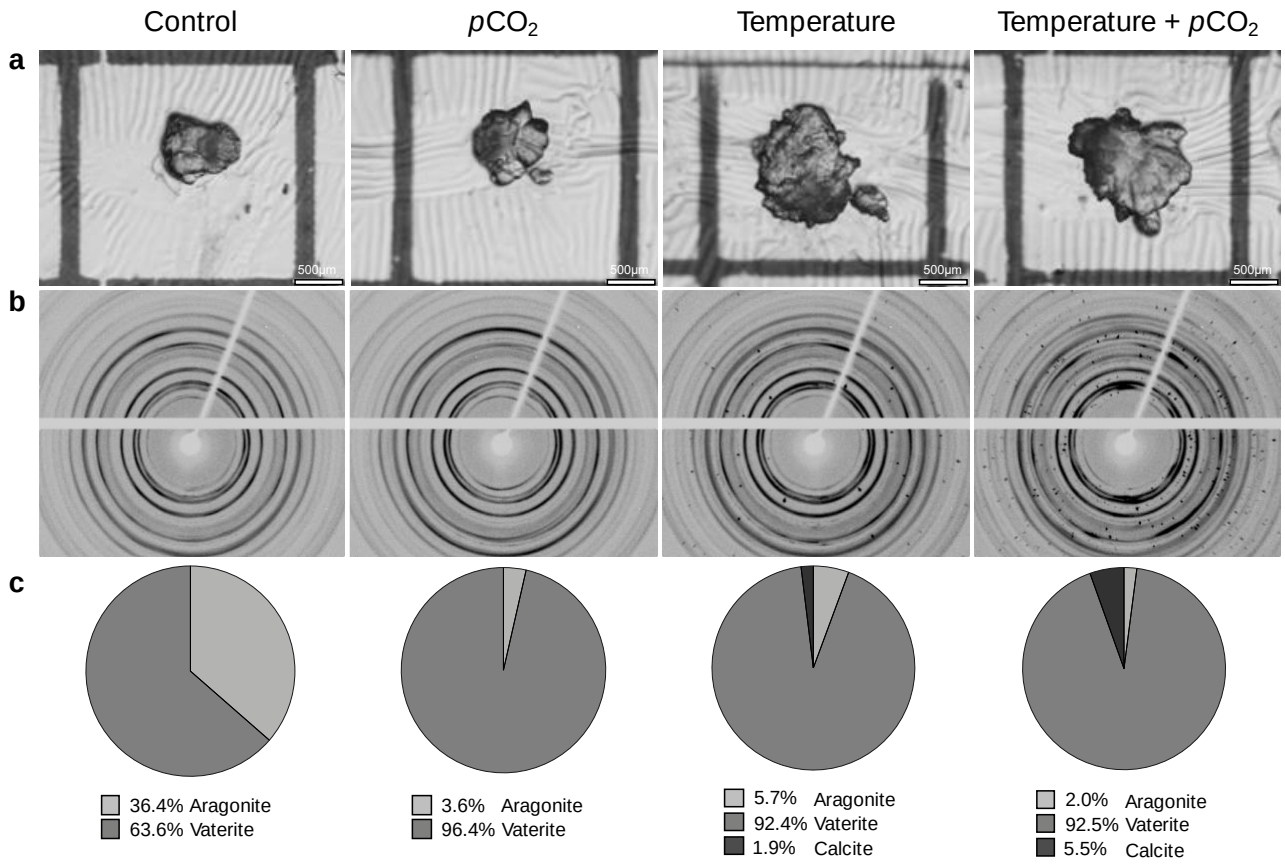

Figure 3.4. Examples of age-0 Lake Sturgeon, *Acipenser fulvescens*, otolith polymorph quantification results from single individuals within each experimental treatment (i.e., control [15ºC, 1000 µatm pCO_2], pCO_2 [15ºC, 2500 µatm pCO_2], temperature [22ºC, 1000 µatm pCO_2], temperature + pCO_2 [22ºC, 2500 µatm pCO_2]) including (**a**) images of sagittal otoliths at 10x magnification, (**b**) Debye-Scherrer diffraction patterns collected using X-ray microdiffraction, and (**c**) percent polymorph composition (i.e., aragonite, vaterite, calcite) by otolith weight.

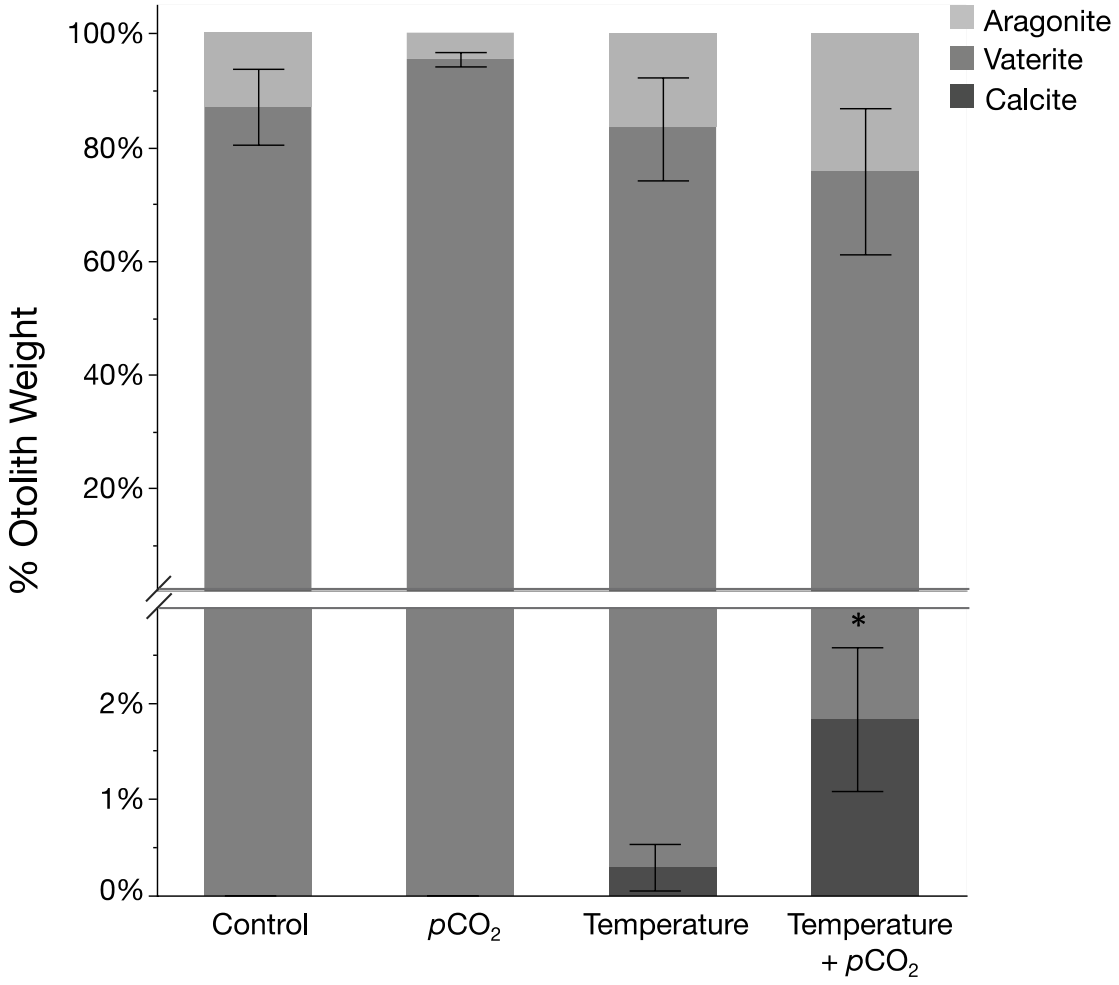

Figure 3.5. Mean (± s.e.m., n = 8 otoliths) percent polymorph composition (i.e., aragonite, vaterite, calcite) by weight of sagittal otoliths collected from age-0 Lake Sturgeon, *Acipenser fulvescens*, within each experimental treatment (i.e., control [15ºC, 1000 µatm pCO_2], pCO_2 [15ºC, 2500 µatm pCO_2], temperature [22ºC, 1000 µatm pCO_2], temperature + pCO_2 [22ºC, 2500 µatm pCO_2]). Note the break in y-axis corresponds to a change in scale. * indicates calcite composition is significantly different among experimental treatments (ANOVA, <0.05).

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Chapter 4: Field validation of fin ray microchemistry as a tool to assess lifelong habitat use of Lake Sturgeon, *Acipenser fulvescens*, on small spatial scales

This manuscript will be submitted to Methods in Ecology and Evolution and was coauthored with my advisor and collaborators. As the lead author on the project I was responsible for the data collection, I developed the novel analytical techniques, wrote and revised the manuscript. My co-authors S.M. Backhouse and C.A. McDougall provided the fin ray samples as well as feedback on the analyses and manuscript drafts. Additionally, W.G. Anderson provided insight and guidance to the overall review and design of the project as well as editorial revision on multiple drafts of the manuscript.

Loeppky A.L., Backhouse S.M., McDougall C.A., and Anderson W.G. Field validation of fin ray microchemistry as a tool to assess lifelong habitat use of Lake Sturgeon, *Acipenser fulvescens*, on small spatial scales

4.1 Abstract

Identifying the historical movements of migratory fishes has important implications for managing stocks and implementing conservation strategies. Physical tagging methods exist to monitor the fine-scale habitat use of individuals; however, these techniques typically only provide a snapshot of information during the period of which the fish has been outfitted with the tag. As an alternative to physical tagging, chemical signatures in hard structures (e.g., otoliths, fin rays) have proven useful to identify spatial and temporal movements of fish throughout their entire lifespan. To make accurate inferences on historical movements using element signatures, it's necessary to validate whether habitat-specific changes in elemental concentrations reflect those that are recorded in hard structures on the spatial scale of interest. Here, we first examined water chemistry profiles throughout our study area using data collected as part of a long-term aquatic monitoring program. To validate whether significant shifts in elemental signatures recorded in the fin rays of juvenile and adult Lake Sturgeon (*Acipenser fulvescens*) reflected variability in water chemistry, we compared spatial and temporal shifts in combined elemental fin ray concentrations of both hatchery-stocked and wild-spawned individuals to known habitat movements on relatively small spatial scales (<40km) in four geographically distinct sampling regions. Multivariate analyses examining combined concentrations of trace elements were used to distinguish known movements of recaptured hatchery-stocked individuals with high success (87.5-100%) corroborating fin ray microchemistry with habitat specific elemental signatures in our study region. In particular, elemental signatures reflected hatchery residency and known movements post release. Given this, we were able to apply this technique to investigate the movements of Lake Sturgeon of unknown origin to make inferences on lifetime movements. Examining microchemistry in reference collections of fin rays collected from Lake Sturgeon of both known and unknown origin provided us with the unique opportunity to validate this technique in a field setting.

4.2 Introduction

Understanding connectivity and spatial movements of fishes is essential for studying population dynamics, managing stocks, and monitoring the effects of environmental stressors, all of which contribute to implementing conservation strategies including the design of stock enhancement programs (Gillanders 2002; Pracheil et al. 2014). In freshwater systems, the fragmentation of rivers due to the construction of dams and diversions, although economically important, have adverse effects on the ecosystems they disrupt (Barbarossa et al. 2020). Fragmentation can limit migration and larval dispersal of fishes, resulting in a lack of gene flow, ultimately contributing to population decline and increasing the threat of endangerment (Dudgeon et al. 2006; Pracheil et al. 2019). As such, it is particularly important to asses connectivity and movements of populations that are affected by river fragmentation in order to identify critical habitats and design effective and biologically relevant species recovery plans.

The ability to assess connectivity relies on the identification of an individual's natal origin and habitat movements throughout its lifetime. Traditionally, fisheries researchers rely on mark-recapture and/or telemetry studies to track migration, which require the physical tagging of fish (Pollock et al. 2004; Skalski et al. 2009). Although these studies are typically successful at identifying habitat use, they often only offer a snapshot of information during the period of which the individual has been outfitted with a tag (Pine et al. 2003; Pracheil et al. 2014). Most of these physical tags also come with substantial financial and handling costs and are subject to malfunction or retention issues and, thus, may not be reliable long-term (Skalski et al. 2009). Alternatively, the emergence of elemental analysis of calcified structures has provided a powerful tool for reconstructing spatial and temporal movements of fish in a variety of environments (e.g., Phelps et al. 2012; Pracheil et al. 2014; Thorrold et al. 2001). Elemental signatures, representative of discrete changes in the ambient water chemistry, form when a combination of trace metals and stable isotopes are incorporated from the surrounding environment into the calcified surface of hard structures (e.g., otoliths, fin ray, scales, vertebrae) creating a chronological record representative of the fish's environmental life history (Campana 1999; Tzadik et al. 2017).

Associating changes in elemental composition across these structures provides the ability to reconstruct life history patterns retrospectively (Campana 2005). Otoliths in particular are commonly used as they are inert structures that constantly grow throughout the entire lifetime of the fish (Campana 1999). As such, trace elemental signatures in otoliths have been used to identify habitat use and large-scale migration movements (e.g., D'Avignon and Rose 2013; Elsdon and Gillanders 2002, 2003) and are particularly successful at pinpointing the transition between marine and freshwater environments in

diadromous fishes (e.g., Brown et al. 2007; Gillanders 2005). Elemental signatures in fin rays have also been used to make similar inferences on the environmental life history, or habitats in which the fish has resided, including identifying natal origin (e.g., Allen et al. 2009b; Phelps et al. 2012) and differentiating between wild and stocked individuals (e.g., Loeppky et al. 2020; Rude et al. 2014). Unlike otoliths, fin rays are part of the endoskeleton bone structure and have the ability to regenerate when injured (Allen et al. 2018b). In fact, Allen et al. (2018b) identified Lake Sturgeon fin rays were able to completely regrow after one year post removal. As such, fin rays can be sampled non-lethally, which is beneficial when studying species whose populations are considered to be at-risk (Allen et al. 2009b; Pracheil et al. 2014). One consideration when examining fin rays of fishes is the potential for resorption by the individual under periods of high-stress (e.g., reproduction, smoltification; Persson et al. 1997; Weiss and Watabe 1979). Because fin rays are composed of hydroxyapatite ($Ca_5(PO_4)_3(OH)$), they can be subject to re-modeling (resorption and/or deposition) depending on the calcium demands of the fish (Witten and Huysseune 2009). If bone remodeling has occurred, the area of secondary bone can be visually identified and is typically accompanied by accompanied by soft connective tissues and vascular canals (Tzadik et al., 2017). Nonetheless, fin ray microchemistry studies have proven useful to track spatial and temporal migration patterns in juvenile and adult sturgeons (e.g., Allen et al. 2009b; Allen et al. 2018a; Phelps et al. 2017; Phelps et al. 2012; Pracheil et al. 2019; Sellheim et al. 2017) providing important ecological information that is typically difficult to assess given the broad migration patterns and long life span of this group of fish.

Microchemistry studies typically operate on the assumption that hard part chemistry is proportional to water chemistry (Bath et al. 2000). Because elements don't exist independently in the environment, it is important to consider how combined elemental profiles might influence the final signatures that are deposited in hard structures when making inferences on habitat use, rather than simply considering each element individually. As osmoregulators, sturgeons tightly control the movement of solutes in order to maintain homeostasis (Wood 2012). For essential metals like calcium (Ca), magnesium (Mg), manganese (Mn), and zinc (Zn), which are required for biological function, specific transport mechanisms exist to selectively uptake these ions from the aqueous environment (Crichton 2008; Loewen et al. 2016). Conversely, for non-essential elements like strontium (Sr), barium (Ba), and lead (Pb), the direct homeostatic control over extra- and intracellular concentrations has not evolved (Loewen et al. 2016; Pors Nielsen 2004). Due to their chemical similarities (e.g., atomic size, valence charge), these elements are likely inadvertently incorporated via Ca transport mechanisms

where they are then substituted for Ca on the biomineralizing surface of calcified hard structures (Loewen et al. 2016; Mann 1988). Because of the essential nature of Ca however, there is a higher affinity for Ca to be incorporated by the organism, resulting in the competitive inhibition of these non-essential elements (Chowdhury et al. 2000). Indeed, Loeppky and Anderson (2020) identified Sr uptake and partitioning in tissues of larval Lake Sturgeon, *Acipenser fulvescens*, is inversely related to ambient Ca concentrations highlighting the complexity of elemental pathways for non-essential elements.

Despite this, relatively few studies have utilized combined elemental concentrations recorded in hard structures to assess migratory movements of fishes (e.g., Pan et al. 2020; Pracheil et al. 2019), none of which have validated this technique in a field setting paired with water chemistry analysis performed at fine spatial scales. In this study we sought to investigate the validity of utilizing combined elemental signatures in fin rays to assess fine-scale habitat movements of Lake Sturgeon. Lake Sturgeon are a long-lived (~80-100 years), potadromous fish that are considered threatened or endangered throughout their North American range (COSEWIC 2017). In Manitoba, Canada, population declines have been rapid and severe over the past 150 years due to extreme harvest and the construction of hydroelectric dams and flow diversions causing fragmentation of key Lake Sturgeon habitats. As such, recovery efforts in Manitoba have placed increased focus on gaining a further understanding of the biology and ecology of the species in order to optimize conservation strategies and improve the success of hatchery restocking programs. Identifying key life history movements of this species in Manitoba, however, has been difficult given their long lifespan and logistical challenges often faced by monitoring programs.

To assess whether microchemistry of fin rays could be used to reconstruct fine-scale movements for Lake Sturgeon, we first examined water chemistry profiles among habitats of interest to determine whether differences were large enough and on a fine enough scale to distinguish among habitats. We then examined a reference collection of juvenile and adult Lake Sturgeon (ages 2-23 years) of both known and unknown origin sampled in four geographically distinct regions located ~100-500 km apart in Northern Manitoba. Elemental signatures in pectoral fin rays (Sr, Ba, Mg, Mn, Zn and Pb) were quantified via Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA ICP-MS) and compared to water chemistry profiles to make inferences on lifetime habitat movements. The ability to accurately identify and trace movements of large numbers of Lake Sturgeon, or any migratory fish, by retroactively interpreting elemental signatures recorded in their fin rays will provide key information on natal origin, nursery habitat, and/or migration movements that are currently largely unknown to fisheries managers.

4.3 Methods

4.3.1 Water Chemistry

To investigate whether habitat-specific elemental signatures recorded in Lake Sturgeon fin rays, we first examined water chemistry differences at 14 sampling sites throughout Northern Manitoba (Figure 4.1). Water chemistry samples were collected as part of the Coordinated Aquatic Monitoring Program (CAMP) at quarterly intervals throughout the year from 2008 to 2016. When water sampling locations at specific Lake Sturgeon monitoring sites where the fin rays in this study were collected were not available, the next closest sampling sites were chosen from the reference data collection to give general water chemistry profiles in the areas of interest. Surface samples were collected by hand then preserved using nitric acid and sent to an environmental analytical laboratory for analysis (CAMP, 2017). A selection of both essential and non-essential elements (i.e., Ca Sr, Ba, Mg, Mn, Zn, Pb) were chosen for examination to determine which elements would be of interest in fin rays based on significant differences among water sampling sites.

4.3.2. Study Area

To examine whether naturally occurring elemental signatures in the pectoral fin rays of Lake Sturgeon can be used as a tool to identify fine-scale spatial differences in habitat use, a reference collection of 291 fin rays were examined via microchemistry analysis. The fin rays were collected from a range of known hatchery-stocked and putative wild-spawned juvenile and adult Lake Sturgeon sampled during gill-net surveys in Northern Manitoba from 2012 to 2019 (Table 4.1). Four study regions were defined to examine habitat-specific differences in elemental signatures of geographically distinct Lake Sturgeon populations (Figure 4.1).

4.3.3 Region 1 – Upper Nelson

Fin rays were collected from known hatchery stocked fish as determined by the presence of PIT tags applied prior to release, that were recaptured at one of three sampling sites located ~30km apart (i.e., Little Playgreen Lake, Pipestone Lake, Sea Falls; Figure 4.2). These fish were all residents of the Grand Rapids Fish Hatchery (GRFH) in Grand Rapids, Manitoba for ~18 months prior to stocking. Fish from the 2007 and 2013 hatchery cohorts were released in Little Playgreen then recaptured 5-8 years later either in the same location or in Pipestone Lake. Juvenile Lake Sturgeon sampled in 2015 at Sea Falls were known hatchery released fish that were also stocked in Sea Falls in 2014. By examining fin rays with information on the release and capture locations, we were able to determine reference points of

known habitats, which allowed for the determination of temporally significant and habitat specific elemental signatures recorded in fin rays.

4.3.4 Region 2 – Lower Nelson

Fin rays in this region were collected from both recaptured hatchery fish reared at the GRFH as described above and wild-spawned individuals in either Stephens or Gull Lake (Figure 4.3). Sites are located ~40km apart and are connected by the Nelson River. Construction of the Keeyask generating station on the Nelson River between the two sites began in 2014 and is set to be completed in 2021. During construction, access between the lakes was available and thus hatchery fish from the 2016 cohort that were stocked in Gull Lake were recaptured downstream in Stephens Lake from 2017 to 2019. To compare habitat-specific elemental signatures between Gull and Stephens Lake, and assess the timing of movements between sites, fin rays collected from putative wild fish captured in both Gull and Stephens were also examined.

4.3.5 Region 3 – Lower Churchill

Lake Sturgeon in this region are known to congregate at the Churchill River confluence during spawning, however, it is largely unknown whether these individuals are residents of the area or are potential migrants into an isolated remnant population on the Churchill River (Figure 4.4). To assess the life history of individuals captured at the confluence, fin rays collected in 2014 and 2015 from juvenile and adult Lake Sturgeon were examined. Given the known sampling location, significant shifts in elemental signatures across the entire life span of each individual were examined in order to assess whether fish were residents of the habitat or had migrated to the confluence prior to sampling.

4.3.6 *Region 4 – Hudson Bay Estuary*

Both the Nelson and Hayes river discharge into Hudson Bay near York Factory, Manitoba (Figure 4.5). Despite monitoring of populations in this region being limited, there is evidence that tagged Lake Sturgeon travel between the Nelson and Hayes rivers requiring them to venture into the Hudson Bay. The prevalence and frequency of this migratory movement, however, is unknown. To investigate whether fin ray microchemistry can determine whether Lake Sturgeon use the Hudson Bay to migrate between the two rivers, fin rays from two populations of sturgeon sampled in the Nelson and Hayes river were examined.

4.3.7 *Fin Ray Microchemistry*

All fin ray samples were analyzed for elemental quantification of Sr, Ba, Mg, Mn, Zn, and Pb following methods outlined by Loeppky et al. (2020). Briefly, 1 mm sections were cut using an Isomet

low-speed saw then buffed and polished using ultra fine grit silicon carbide paper. Multiple sectioned fin rays were then set in epoxy ring molds (n = 4-12 fin rays per ring) and prepared for analysis via Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA ICP-MS; Perkin-Elmer DRC II). Prior to and following the scanning of each new ring, a reference glass material (NIST 610) was ablated to provide the external standard and to correct for instrument drift during analysis. Laser line transects were ablated from the primordial core region of the fin ray, representing fin ray growth in the natal habitat, laterally to the outer edge of the structure, which is associated with fin ray growth prior to sampling. A 30 s gas blank was run between each fin ray sample in order to ensure any material from the previous fin ray had been cleared and to determine background concentrations of each element. The following laser parameters were used for the analyses: 30 µm spot size, 10 µm/s scan speed, 80% energy, 20 Hz repetition rate, and 15 ms dwell time. Once ablated, each fin ray was imaged using a dissecting microscope (Olympus SZX) ensuring the entire scan line and annuli were clearly visible.

Data reduction was conducted in Igor Pro graphing software with Iolite (version 2.21) for LA ICP-MS. Internal standards were determined using ^{43}Ca counts per second (CPS) and used to account for changes in ablation volume. Calcium concentration was set to 27% based on values reported for other sturgeon microchemistry studies examining hydroxyapatite material (e.g., Allen et al. 2009b; Phelps et al. 2017). The raw elemental profiles were then plotted against scan distance (µm) by multiplying scan time by 10 (i.e., scan speed) then overlaid on top of the images of the fin rays, aligning the ablation transect with total scan distance (Figure 4.6). Annual sections of fin ray growth were visually identified by the presence of light and dark growth bands to isolate elemental profiles within each annulus. Additionally, three regions of each fin ray were defined to assess mean elemental signatures (i.e., combined concentrations of Sr, Ba, Mg, Mn, Zn, Pb) at key life history moments providing consistent benchmarks of habitat use. The core region was determined as the portion of growth from the nucleus, or start of the scan line, to the initiation of the first growth band while the end region was the portion from the last growth band to the outer edge of the fin ray. The period of growth between the core and end regions were defined as the mid region (Figure 4.6).

4.3.7 *Data Analysis*

All analyses were conducted using SAS statistical software in JMP Pro® (version 15.2.1). Yearly mean water chemistry concentrations (i.e., Ca, Sr, Ba, Mg, Mn, Zn, Pb; response variables) within each sampling site were first examined separately for normality using the Shapiro-Wilk W test and for homogeneity of variance using the Levene's test and results identified that the majority of

elements at each site met the underlying assumptions of parametric statistics. Sampling sites that had elements that were not normally distributed were examined visually using normal-quantile (Q-Q) plots and were determined to only moderately deviate from the normal distribution thus results of parametric statistics are presented. To assess whether water chemistry within each sampling region is temporally stable, differences in yearly mean elemental concentrations (i.e., Ca, Sr, Ba, Mg, Mn, Zn, Pb) were tested using one-way ANOVAs with sampling sites as nested random factors. Additionally, quadratic discriminant function analyses (qDFA) were performed where all response variables (i.e., Ca, Sr, Ba, Mg, Mn, Zn, Pb) were combined to determine whether unique elemental signatures in the water could be distinguished among sampling sites.

In fin rays, mean elemental concentrations (i.e., Sr, Ba, Mg, Mn, Zn, Pb; response variables) in the core, mid, and end fin ray regions within each sampling site were first examined separately for normality and homogeneity of variance as described above. Results identified the majority of response variables met the underlying assumptions of parametric statistics. Similar to the water chemistry data, response variables that were non-normal were determined to only moderately deviate from the normal distribution by visually examining Q-Q plots, thus, parametric statistics were used. Because the Lake Sturgeon examined in this study are not expected to mix between regions due to both natural (e.g., large rapids) and anthropogenic (e.g., hydroelectric dams) barriers, analyses were focused on examining differences among sampling sites within each region. Univariate analyses were first conducted to determine whether there were differences in mean fin ray elemental concentrations in the end annulus, representing elemental concentrations that were presumably acquired at or near the sampling site, using one-way ANOVAs with sampling site as the predictor variable followed by post-hoc Tukey HSD tests to identify which factor levels were different. To examine combined elemental signatures in fin rays, a series of qDFAs were performed where response variables (i.e., mean Sr, Ba, Mg, Mn, and Zn concentrations) were combined to investigate whether elemental signatures could be distinguished among known residency in specific habitats within each sampling region. The independent variables for each sampling region were as follows: in the upper and lower Nelson regions (Region 1 and 2), differences in elemental signatures in both the fin ray regions within each sampling site and isolated in the end annuli among sampling sites were examined separately to validate that habitat specific fin ray signatures could be distinguished given the known residency in specific habitats corresponding to life history stages of these individuals (i.e., hatchery rearing period, release and recapture sites). In the lower Churchill river region (Region 3), differences among fin ray regions were examined to determine

whether fish captured at the Churchill river confluence were residents or migrants. In the Hudson Bay estuary region (Region 4), to assess whether distinctive fin ray signatures between the Nelson and Hayes rivers exist, differences in combined elemental signatures between sampling sites in the end annuli were tested.

4.4 Results

Results are primarily presented in the tables and figures sections of this chapter. Ranges in trace element concentrations in the water chemistry samples at each sampling site in the study area are depicted in Figure 4.1. Mean element concentrations were consistent among years for the majority of elements examined in all sampling regions (Table 4.2), indicating water chemistry is largely temporally stable in the study area. Detailed water chemistry profiles within each sampling region, including statistical results of ANOVAs for individual elements and qDFA analyses of combined elemental signatures, are presented in Figures 4.2-5. Despite significant differences in individual elements between sampling sites, the multivariate qDFA models indicated combined elemental profiles were unique among and within each sampling region. In fin rays, univariate analyses examining differences in individual elements in the end fin ray region among sampling sites are presented in Table 4.2 with the majority of elements being significantly different in each region. When elements were considered together as combined elemental signatures, all models were statistically significant and had high predictive capacities for determining either sampling site or fin region (76-100%) in all sampling regions examined. Results of the qDFAs including model significance and classification success within each sampling region are presented in Figures 4.7-12. Notably, in the upper and lower Nelson regions (Region 1 and 2), differences in elemental signatures among fin regions were consistent with patterns of known habitat use particularly in the core region of stocked individuals where low Mn profiles were indicative of hatchery residency. In the lower Churchill river (Region 3), the majority of fin ray samples had distinctly different elemental signatures between the core and end fin region indicating Lake Sturgeon sampled at the confluence are migrants to the area. Finally, in the Hudson Bay estuary (Region 4), elemental signatures in the end annulus were significantly different between the Nelson and Hayes rivers indicating river residency can be determined using multivariate microchemistry techniques.

4.5 Discussion

Habitat specific elemental signatures form when trace elements dissolve from the bedrock or are introduced to the environment by anthropogenic inputs, which are then reflected in the hard structures of fishes (Pracheil et al. 2014). The success of microchemistry studies relies on two primary factors: 1 –

water chemistry in the habitats of interest must differ reliably, and 2 – the individual must maintain residency in these habitats long enough for the differences to be reflected in the calcified matrix of hard structures (Campana 1999; Pracheil et al. 2014). When met, these elemental signatures can be related to growth bands in otoliths and fin rays providing meaningful temporal benchmarks to retrospectively interpret the life history of the individual (e.g., natal origin, migration). In our study, water chemistry in the sampling regions reflected the heterogeneous bedrock landscape in the study areas (Peck et al. 2000). As a result, combined elemental concentrations, or elemental signatures, recorded in the fin rays of Lake Sturgeon were both spatially and temporally significant. Indeed, the multivariate models used were able to successfully distinguish fin ray region (i.e., core, mid, end) and sampling site with high classification success (>75%) in all sampling regions that were investigated. Conversely, when elements were considered individually, significant differences among fin region and sampling site were variable and, as such, were more difficult to interpret and relate to habitat movements. These results highlight the increased predictive capacity that is gained when elemental signatures are tested rather than exploring elements independently in fin rays. By examining groups of Lake Sturgeon with known life histories, we were able to validate that significant shifts in combined elemental signatures corresponded to the timing of known habitat movement patterns. Detailed interpretations of each sampling region are discussed in the following subsections.

4.5.1 Region 1 – Upper Nelson river

Lake Sturgeon conservation strategies in the upper Nelson river have included hatchery restocking programs combined with annual monitoring of populations through field surveys. Prior to stocking, fish from the GRFH are implanted with a PIT and/or floy tag so that if recaptured, they are identified by referencing the tag number and, thus, their general life history can be monitored. Examining fin rays sampled from recaptured hatchery stocked fish in this relatively small spatial region was ideal for comparing patterns of known habitat movement to elemental signatures in a field setting.

When differences in habitat-specific elemental concentrations in the end annulus were tested separately (Figure 4.7a-f), representing concentrations of trace elements that were deposited in fin rays following the last overwintering period to the time immediately prior to capture, significant differences among sampling sites were observed in all elements that were measured, however, significance trends were variable. Despite this, qDFA models were able to classify Lake Sturgeon into sampling site with high success (96.5%; Figure 4.7g). Notably, Sr and Ba were the elements that had the strongest scoring coefficients related to canonical 1 indicating that they were the elements driving the differences in fin

ray signatures. As non-essential elements, direct control over these ions does not exist, and thus, variation in the concentrations of Sr and Ba deposited in calcified hard structures tend to more closely reflect variability in the surrounding environment and, as such, are commonly used in microchemistry studies (Campana 1999; Farrell and Campana 1996). It is interesting, however, that despite higher concentrations of both elements being present in water downstream in Cross Lake, patterns of deposition of Sr and Ba in fin rays are opposite to one another. Given the similar chemical properties of Sr and Ba to Ca, it is likely that these elements are taken in through Ca transport channels on gill epithelium (Chowdhury and Blust 2001; Loeppky and Anderson 2020). Higher affinities for different elements will determine the uptake rates when competing for binding sites (Wood and Shuttleworth 1995). As a result, the relationship of all three ions in the water will determine the rate at which they are incorporated and subsequently deposited further highlighting the necessity to consider combined signatures of elements to make more accurate inferences on habitat use. The high predictive capacity offered by multi-element signatures in the end fin ray region allowed subsequent analyses of migration patterns to be interpreted with confidence.

All individuals that were examined in this sampling region were of known hatchery origin, thus, the fin ray signatures in the core region can definitively be related to water chemistries experienced during the hatchery residency period (~18 months, Figure 4.8). This stark difference in hatchery signatures is attributed to redox reactions in the well water supplied to the GRFH where these fish were reared from egg incubation resulting in markedly low concentrations of Mn in fin rays (Loeppky et al. 2020). After stocking, these fish were recaptured years later either at their same stocking location of Sea Falls or Little Playgreen Lake, or downstream in Pipestone Lake (Figure 4.2). The overlap in elemental signatures observed between the mid and end fin ray regions, and reduced classification success into fin region, in the Pipestone Lake fish suggests movement downstream occurred relatively quickly after stocking. Similar patterns, however, were observed in Sea Falls where, despite fish having been stocked and recaptured in the same location, distinctive signatures between the mid and end fin regions were observed. At this early life stage, fish growth and development is occurring rapidly, resulting in different demands for Ca and other essential elements required to form bony structures (e.g., scutes, vertebrae, fin spines; Blanksma et al. 2009; Genz et al. 2014). As such, it is possible that ontogenetic development plays a role in elemental incorporation and deposition at this life history stage, resulting in the subtle variation in elemental signatures between the mid and end fin ray regions. Given the rapid growth rate at this early life stage, it is likely that elemental incorporation and deposition is happening at an increased

rate suggesting that elemental signatures may be able to reflect even more subtle habitat movements as residence time required to incur the signature would be reduced.

4.5.2 *Region 2 – Lower Nelson river*

Until recently, passage between Gull and Stephens Lake in the lower Nelson river was largely undisturbed. With the construction of the Keeyask generating station beginning in 2014, passage between the two lakes has become fragmented (Figure 4.3). Historical Lake Sturgeon movement between Gull and Stephens Lake, however, is unknown. The ability to assess habitat movement between these sites using microchemistry relies on the ability to detect differences in habitat specific signatures that are located <40km apart. Comparing the timing of movement of known hatchery stocked fish in Gull Lake that were later captured in Stephens Lake, to those of resident fish to either location, provided the ability to validate whether fin ray microchemistry is a potentially valuable technique to investigate historical movement of Lake Sturgeon in this area; which could contribute to future conservation efforts such as the implementation of a fish passage at the generating station.

Multivariate models examining elemental signatures in the end fin ray region, representative of presumptive elemental accumulation incurred at Gull or Stephens prior to the time of capture, provided strong evidence that distinctive signatures in either habitat could be reliably distinguished with high predictive success (91.8%; Figure 4.9). The high predictive capacity for classifying individuals into sampling site can likely be attributed to the strong differences observed in water chemistry in this region (Figure 4.3). Even at these relatively close water sampling distances, significant differences in all elements except Ba were observed both up and downstream of the Keeyask generating station. Despite concentrations of Zn and Pb being significantly different between water sampling sites, differences in fin ray concentrations of these elements did not vary and in fact were also non-significant (Table 4.2). The uptake of these ions has been attributed to both the environment and trace dietary inputs in some teleost fish (Odzak and Zvonaric 1995; Van Campenhout et al. 2009). Additionally, given the essential nature of Zn, elemental uptake is under homeostatic control (Loewen et al. 2016). As a result, the environmental input may have a lesser role in the uptake of certain elements, which would then be reflected in the microchemistry of hard structures (Marohn et al. 2009; Sturrock et al. 2014). Regardless, the high predictive capacity allowed by the multi-elemental analysis in the end fin ray region suggests inferences of movement between Gull and Stephens lake can made.

The apparent differences in elemental signatures when examined among fin ray regions in each sampling site could be successfully attributed to the known life histories of the fish in this sampling

region (Figure 4.10). Indeed, Lake Sturgeon that were captured in Stephens Lake were either known stocked fish that were released in Gull Lake, or wild-spawned fish that were presumably residents to the area. The marked separation of the core fin ray region of the hatchery fish is consistent with the known differences in signatures deposited during hatchery residency as described above. Additionally, the separation observed in the mid and end fin ray regions, and the high classification success (97.4%), suggests these fish may have remained in Gull Lake long enough to incur differences in elemental signatures prior to movement upstream of the Keeyask generating station to Stephens Lake where they were recaptured. In contrast, the reduced classification successes among fin regions (75.6-81.4%) suggests the wild-spawned fish were residents to either Gull or Stephens Lake resulting in similar elemental signatures across the entire fin ray scan. The consistencies in elemental patterns and known habitat movement provide strong evidence that historical movements of Lake Sturgeon between Gull and Stephens Lake can be determined using this technique. The ability to assess frequency of movements will provide important information for fisheries managers when assessing the impacts of the construction of the generating station and aid in determining the appropriate conservation efforts in this region.

4.5.3 Region 3 – Lower Churchill

The construction of dams, reservoirs, and/or diversions for flood protection or hydroelectric power have significant effects on water levels and flow regimes (Barbarossa et al. 2020). This can be particularly detrimental to suitable Lake Sturgeon spawning habitats as they require areas of high flow, such as at the base of rapids or waterfalls to spawn. The portion of the lower Churchill River near the Little Churchill River confluence (Figure 4.4) has been particularly affected by the construction of the Churchill River diversion, which has greatly reduced water levels and flow in the area, especially during dry years (gov.mb.ca 2012). Despite this, there appears to be a self-sustaining Lake Sturgeon stock that utilizes this reach of the river, however, the range of the population is unknown and as such the habitats on which the stock relies is currently undetermined (gov.mb.ca 2012).

Water chemistry sampling in this particular region was limited and thus a broad range of sampling sites was examined in order to determine the potential for habitat specific elemental signatures to be incorporated. In general, water chemistry was relatively homogeneous throughout the sampling region with the concentrations of Ca and Mn being the only elements that differed significantly among sampling sites (Figure 4.4). Interestingly, the geology in this region is also relatively homogeneous compared to the other sampling regions (Peck et al. 2000), which likely contributed to the reduced

variability in water chemistry that was observed. Because of this, interpretations of habitat use among individuals was limited to identifying significant shifts in elemental signatures among fin regions to determine whether fish were residents or migrants into this remnant population on the Churchill river. In order to determine where fish have migrated from, further water sampling is required. Despite this, multivariate models examining elemental signatures throughout the lifespan of juvenile and adult Lake Sturgeon provided strong evidence that distinctive signatures could be reliably distinguished among fin ray regions (Figure 4.11). The separation between fin regions, with no overlap occurring between the core and end regions, and high classification success (95.5%) suggests the individuals that were sampled at the confluence were migrants to the area prior to sampling. Interestingly, there was more variation in end annuli signatures despite all fish being sampled at the same location. This may be because timing of movement into the confluence area was variable among individuals and, thus, signatures in this fin region may also include migration movements prior to sampling although Sellheim et al. (2017) did identify that subtle changes in ambient Sr concentrations could be detected in the fin rays of White Sturgeon, *A. transmontanus*, in as little as 11 days. Further, given the different age classes of the fish that were examined, there could be an influence of ontogenetic development or cohort differences. With finer scale sampling of habitat specific water chemistry profiles, inferences on migration movements could likely be determined providing key information on crucial habitats for this population.

4.5.4 Region 4 – Hudson Bay Estuary

Although the Nelson and Hayes Rivers are not connected, both rivers discharge into the Hudson Bay near York Factory (Figure 4.5). At present, Lake Sturgeon populations in the two rivers are treated as separate management units as it was thought that the brackish water in the Hudson Bay estuary was a physiological barrier given this species has not adapted to survive in saline environments (gov.mb.ca 2012). Previously tagged fish in the lower Nelson, however, have in fact been recaptured upstream in the Hayes River system requiring these fish to have ventured into Hudson Bay in order to access this habitat (gov.mb.ca 2012). Identifying the frequency and prevalence of this movement and, thus, the connectivity between the two populations has important management implications and will influence conservation efforts in this region.

In anadromous Green Sturgeon, *A. medirostrus*, stark changes in Sr and Ba concentrations in fin rays have been identified to correspond to the timing of movement into marine habitats (e.g., Allen et al. 2009a; Allen et al. 2009b). This is attributed to 1 – marked differences in trace element concentrations in marine and freshwater environments and 2 – changes in uptake associated with the physiological

mechanisms required to undergo a transition for life in a marine environment (Allen et al. 2009a). Because Lake Sturgeon are not adapted to marine environments, it is unlikely that when they transition between the Nelson and Hayes, they spend significant time in the brackish water. Instead they likely move quickly into and out of the estuary potentially riding on the tides. As such, a similar mark to anadromous fishes, whereby migrations between marine and freshwater habitats can be clearly distinguished, is to be present in the fin rays of Lake Sturgeon. Instead, transitions between the Nelson and the Hayes are more likely to be identified by changes in elemental signatures associated to the differences in water chemistries in the two habitats. Indeed, all elements except for Zn showed marked differences between the two sampling sites resulting in 100% classification success based on combined elemental signatures in each habitat (Figure 4.5). These differences in the habitat specific elemental signatures are reflected in the end fin ray region signatures, representing elemental accumulation presumably associated with the location of capture, whereby distinct differences were observed among all individuals measured with no overlap between the two sampling sites (Figure 4.12). These results suggest that if Lake Sturgeon in this region are transitioning between the Nelson and Hayes Rivers, known site specific elemental signatures should be distinguishable if these individuals are residing in either habitat for an extended time.

4.5.6 *Conclusions*

Results of this study illustrated that examining combined elemental signatures in the fin rays of Lake Sturgeon provides a marked advantage over comparing independent elements as evidenced by the increased predictive capacity of the multivariate models. While this technique is becoming more widely used in marine environments, expanding the use of this technique in freshwater environments provides fisheries managers and researchers with the capacity to examine habitat use on multiple species. In our study, examining reference collections of both known-stocked and wild-spawned fish provided a unique opportunity to validate this technique in a field setting within multiple geographically separated populations given stocking location and some movement data was available. The ability to retrospectively reconstruct historical movements of sturgeons using fin ray microchemistry offers a powerful non-lethal tool for investigating ecological questions that have traditionally been difficult to assess in this long-lived group of fish. Importantly, this technique could be used to identify natal rearing environments, detect important nursery habitats at vulnerable early life history stages, or track migration movements on fine spatial scales, to improve fishery management regimes and environmental protection plans.

4.6 Acknowledgments

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4.7 Tables

Table 4.1. The number of fin rays analyzed at each sampling site within the upper and lower Nelson River, lower Churchill River, and Hudson Bay Estuary in Northern Manitoba, Canada. Hatchery or wild classification refers to natal source and was determined by the presence or absence of a PIT tag applied prior to stocking. Age was determined by referencing the PIT tag number and/or by counting annuli in sectioned fin rays.

Region	Sampling Site	*n* hatchery	*n* wild	Age (years)
Upper Nelson	Pipestone Lake	16	-	2-4
	Sea Falls	52	-	2-7
	Little Playgreen Lake	19	-	1-2
Lower Nelson	Gull Lake	-	60	3-8
	Stephens Lake	15	24	2-3
Lower Churchill	Churchill River Confluence	-	47	5-23
Hudson Bay Estuary	Nelson River	-	40	4-20
	Hayes River	-	19	2-16

Table 4.2. Statistical results for fixed effects tests examining the temporal stability of water chemistry between 2008 and 2016 within each sampling region.

Sampling Region	Element	df	df_{error}	F ratio	p-value
Upper Nelson	Ca	8	14	1.83	0.17
	Sr	8	14	2.34	0.09
	Ba	8	14	2.95	**0.04**
	Mg	8	14	3.29	**0.03**
	Mn	8	14	2.41	0.08
	Zn	8	14	1.28	0.18
	Pb	8	14	1.44	0.27
Lower Nelson	Ca	6	5	0.77	0.63
	Sr	6	5	0.58	0.74
	Ba	6	5	2.09	0.12
	Mg	6	5	0.38	0.86
	Mn	6	5	0.98	0.53
	Zn	6	5	1.49	0.40
	Pb	6	5	1.58	0.34
Lower Churchill	Ca	8	12	2.19	0.13
	Sr	8	12	2.25	0.12
	Ba	8	12	0.5	0.46
	Mg	8	12	2.53	0.09
	Mn	8	12	1.51	0.27
	Zn	8	12	10.7	**0.03**
	Pb	8	12	2.55	0.07
Hudson Bay Estuary	Ca	7	7	0.60	0.77
	Sr	7	7	0.69	0.69
	Ba	7	7	0.66	0.72
	Mg	7	7	0.89	0.57
	Mn	7	7	3.70	0.07
	Zn	7	7	1.01	0.51
	Pb	7	7	6.14	**0.02**

Table 4.3. Statistical results of one-way ANOVAs examining differences in mean fin ray elemental concentrations in the end fin ray region among sampling sites. The Lower Churchill region was not included in the analysis because only one sampling site was examined.

Sampling Region	Element	df	df_{error}	F ratio	p-value
Upper Nelson	Sr	2	83	40.0	**<.0001**
	Ba	2	83	50.5	**<.0001**
	Mg	2	83	5.2	**0.007**
	Mn	2	83	3.9	**0.02**
	Zn	2	83	13.5	**<.0001**
	Pb	2	83	18.9	**<.0001**
Lower Nelson	Sr	1	96	8.9	**0.003**
	Ba	1	96	0.5	0.46
	Mg	1	96	20.0	**<.0001**
	Mn	1	96	17.3	**<.0001**
	Zn	1	96	10.7	**0.002**
	Pb	1	96	0.2	0.65
Hudson Bay Estuary	Sr	1	55	331.5	**<.0001**
	Ba	1	55	76.1	**<.0001**
	Mg	1	55	68.5	**<.0001**
	Mn	1	55	0.1	0.79
	Zn	1	55	37.0	**<.0001**
	Pb	1	55	11.9	**0.001**

4.8 Figures

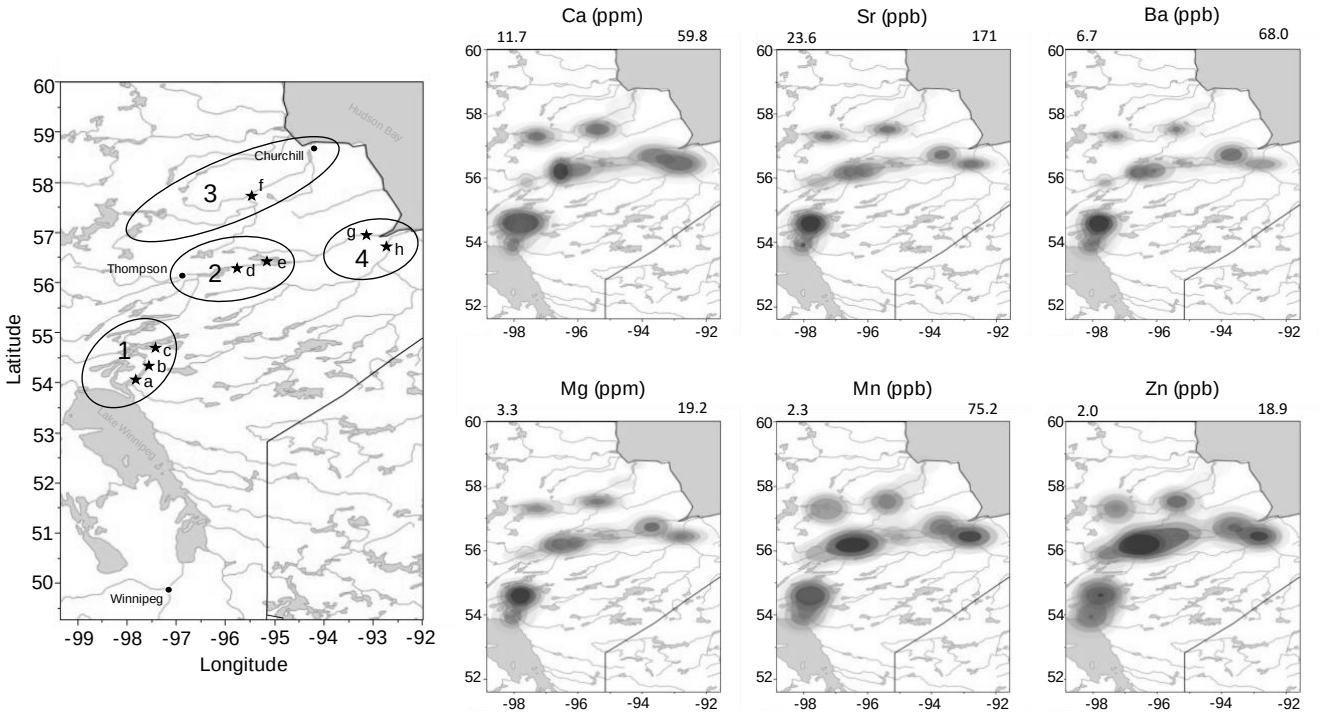

Figure 4.1. Heat maps depicting water chemistry data collected by the Coordinated Aquatic Monitoring Program (CAMP) in Northern Manitoba from 2008 – 2016 including calcium, strontium, barium, magnesium, manganese, and zinc. Shaded ellipses represent Lake Sturgeon sampling regions (1 – Upper Nelson; 2 – Lower Nelson; 3 – Lower Churchill; 4 – Hudson Bay Estuary) with stars indicating sampling sites (a – Little Playgreen Lake; b – Sea Falls; Pipestone Lake; d – Gull Lake; e – Stephens Lake; f – Churchill River confluence; g – Nelson River; h – Hayes River). Detailed sampling sites and water chemistry maps by region can be found in Figures 4.2-5.

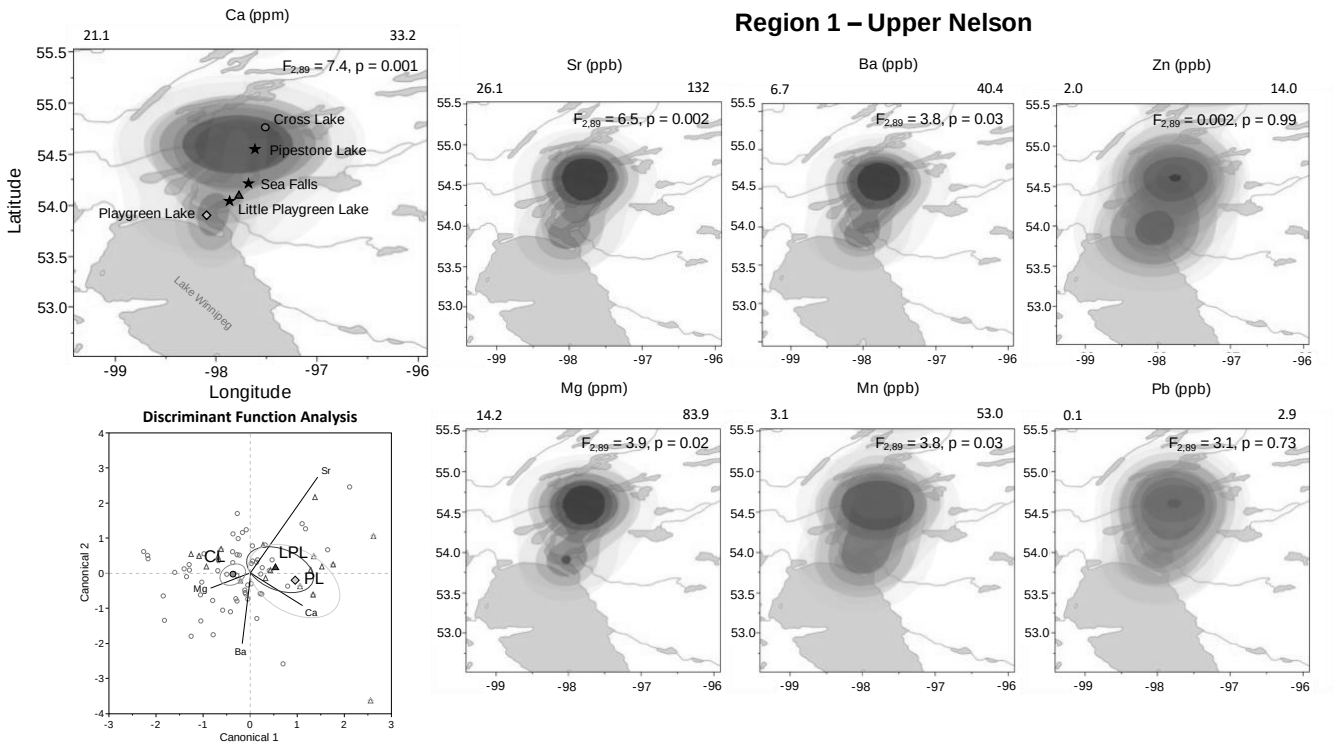

Figure 4.2. Heat maps depicting water chemistry data collected by the Coordinated Aquatic Monitoring Program (CAMP) at Cross Lake (CL, circles), Little Playgreen Lake (LPL, triangles), and Playgreen Lake (PL, diamonds) in the Upper Nelson river region from 2008 – 2016 including calcium (Ca, ppm), strontium (Sr, ppb), barium (Ba, ppb), zinc (Zn, ppb), magnesium (Mg, ppm), manganese (Mn, ppb), and lead (Pb, ppb). Statistical differences (ANOVA) among water sampling sites are presented for each element. Stars indicate Lake Sturgeon, *Acipenser fulvescens*, fin ray sampling locations. The quadratic Discriminant Function Analysis (qDFA) depicts combined elemental profiles of each water sample (open symbols) and the overall mean elemental profile at each water sampling location denoted by closed symbols on the qDFA biplot. Ellipses represent 95% confidence intervals. MANOVA results indicate combined elemental profiles of Ca, Sr, Ba, and Mg among sampling sites were statistically significant (Wilk's λ, $F_{8,164} = 2.65$, $p = 0.009$) and classification success into water sampling site was moderately high (60.3%).

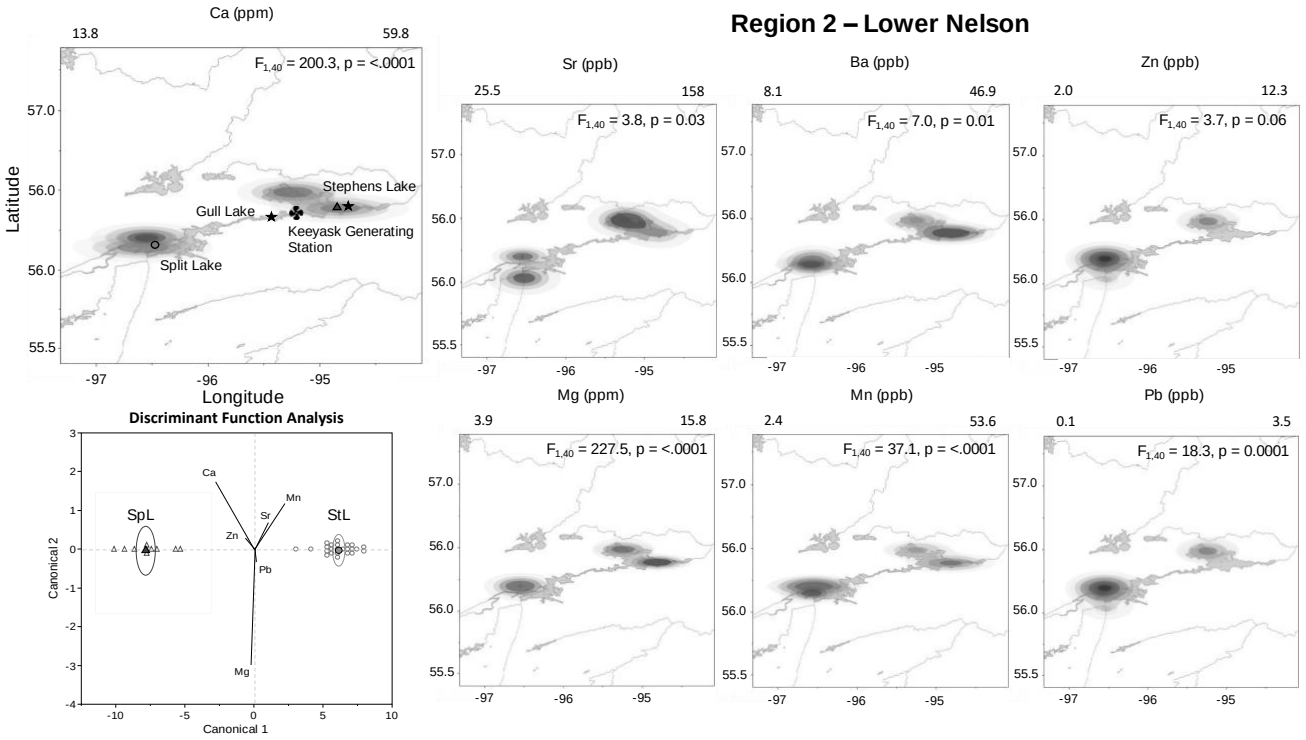

Figure 4.3. Heat maps depicting water chemistry data collected by the Coordinated Aquatic Monitoring Program (CAMP) at Split Lake (SpL, circles) and Stephens Lake (StL, triangles) in the Lower Nelson river region from 2009 – 2016 including calcium (Ca, ppm), strontium (Sr, ppb), barium (Ba, ppb), zinc (Zn, ppb), magnesium (Mg, ppm), manganese (Mn, ppb), and lead (Pb, ppb). Statistical differences (ANOVA) among water sampling sites are presented for each element. Stars indicate Lake Sturgeon, *Acipenser fulvescens*, fin ray sampling locations. The quadratic Discriminant Function Analysis (qDFA) depicts combined elemental profiles of each water sample (open symbols) and the overall mean elemental profile at each water sampling location denoted by closed symbols on the qDFA biplot. Ellipses represent 95% confidence intervals. MANOVA results indicate combined elemental profiles of Ca, Sr, Ba, Mg and Mn among sampling sites were significantly different (Wilk's λ, $F_{5,32}$ = 134.5, p = <.0001) and classification success into water sampling site was high (100%).

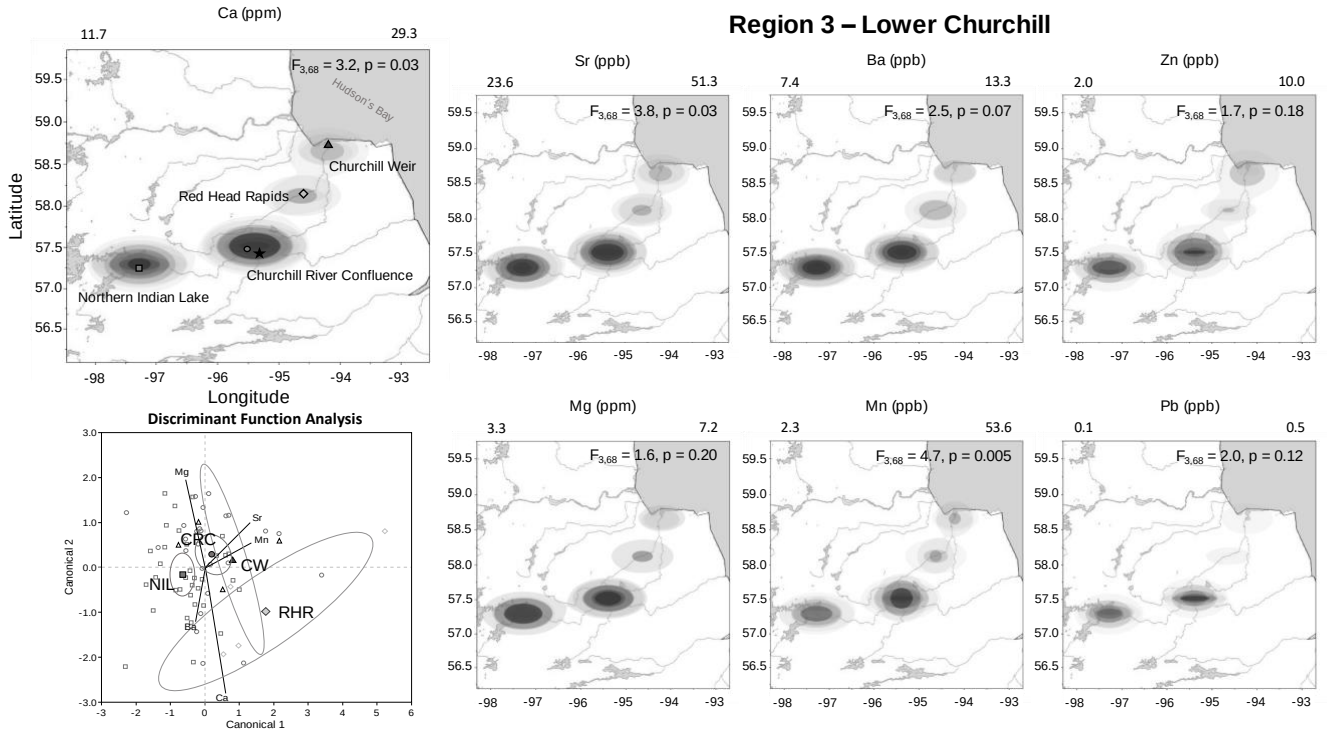

Figure 4.4. Heat maps depicting water chemistry data collected by the Coordinated Aquatic Monitoring Program (CAMP) at Northern Indian Lake (NIL, squares), Churchill River Confluence (CRC, circles), Red Head Rapids (RHR, diamonds), and Churchill Weir (CW, triangles) in the Lower Churchill river region from 2008 – 2016 including calcium (Ca, ppm), strontium (Sr, ppb), barium (Ba, ppb), zinc (Zn, ppb), magnesium (Mg, ppm), manganese (Mn, ppb), and lead (Pb, ppb). Statistical differences (ANOVA) among water sampling sites are presented for each element. Stars indicate Lake Sturgeon, *Acipenser fulvescens*, fin ray sampling locations. The quadratic Discriminant Function Analysis (qDFA) depicts combined elemental profiles of each water sample (open symbols) and the overall mean elemental profile at each water sampling location denoted by closed symbols on the qDFA biplot. Ellipses represent 95% confidence intervals. MANOVA results indicate combined elemental profiles of Ca, Sr, Ba, Mg and Mn among sampling sites were significantly different (Wilk's λ, $F_{10,120}$ = 3.0, p 0.002) and classification success into water sampling site was relatively high (71.6%).

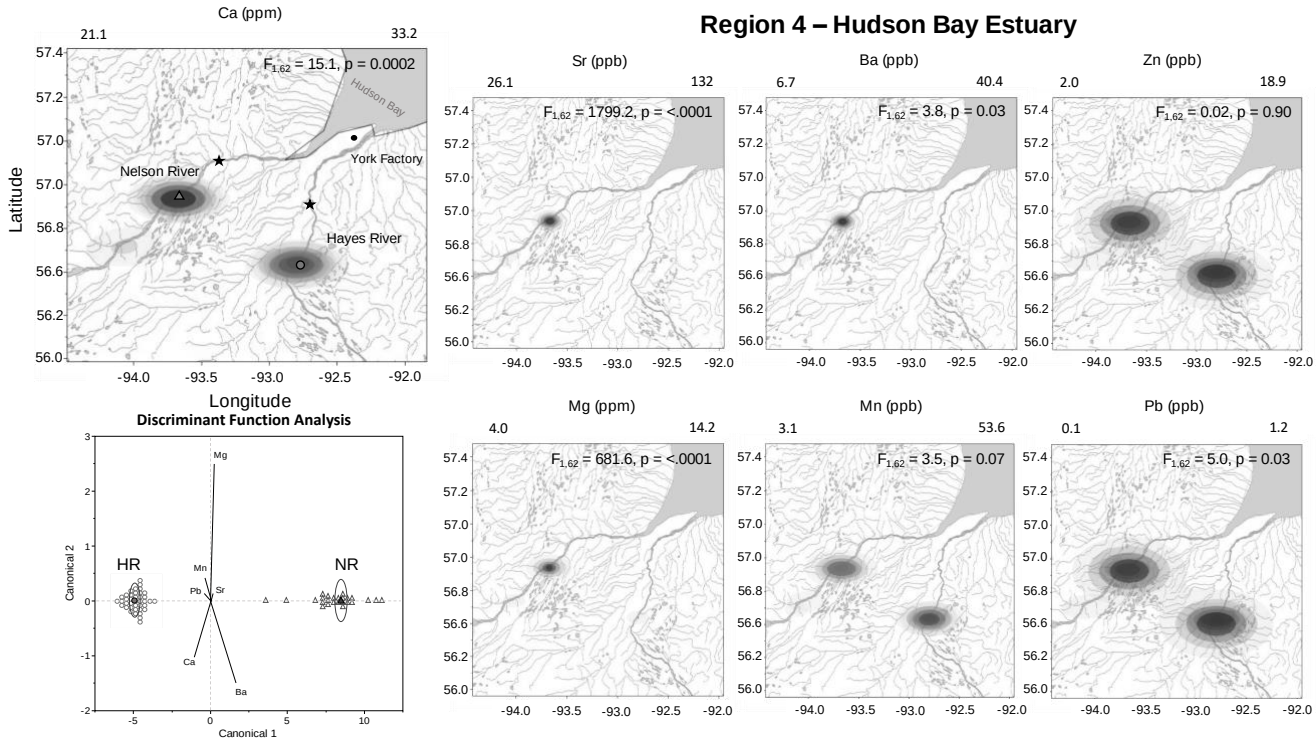

Figure 4.5. Heat maps depicting water chemistry data collected by the Coordinated Aquatic Monitoring Program (CAMP) in the Nelson River (NR, triangles) and Hayes River (HR, circles) in the Hudson Bay estuary region from 2009 – 2016 including calcium (Ca, ppm), strontium (Sr, ppb), barium (Ba, ppb), zinc (Zn, ppb), magnesium (Mg, ppm), manganese (Mn, ppb), and lead (Pb, ppb). Statistical differences (ANOVA) among water sampling sites are presented for each element. Stars indicate Lake Sturgeon, *Acipenser fulvescens*, fin ray sampling locations. The quadratic Discriminant Function Analysis (qDFA) depicts combined elemental profiles of each water sample (open symbols) and the overall mean elemental profile at each water sampling location denoted by closed symbols on the qDFA biplot. Ellipses represent 95% confidence intervals. MANOVA results indicate combined elemental profiles of Ca, Sr, Ba, Mg and Mn among sampling sites were significantly different (Wilk's λ, $F_{6,57}$ = 405.9, p = <.0001) and classification success into water sampling site was high (100%).

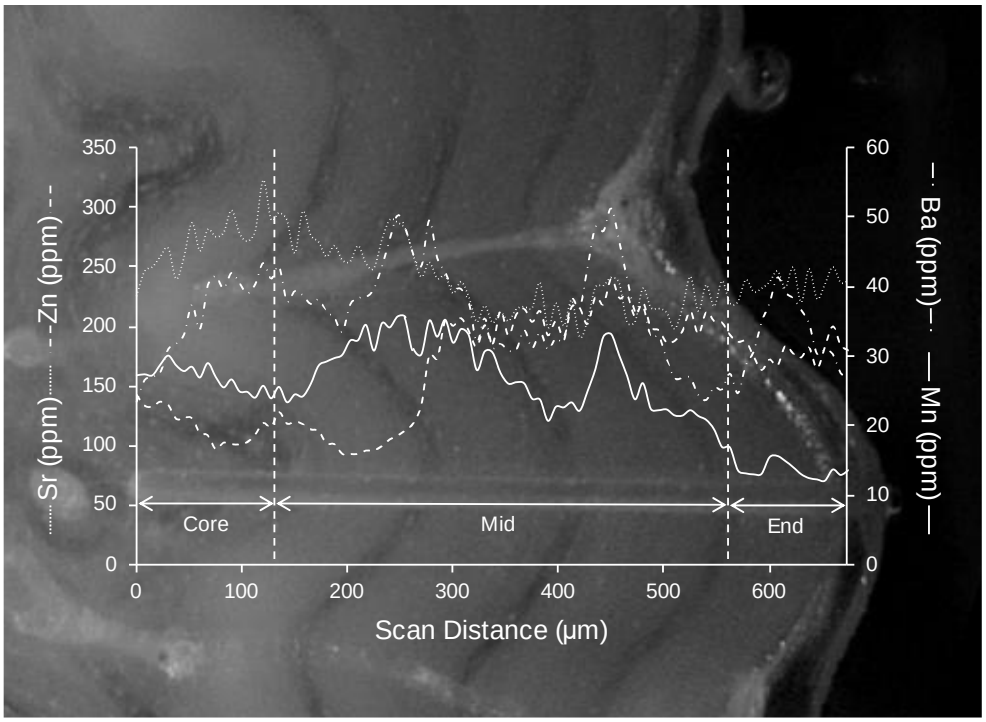

Figure 4. 6. Sectioned Lake Sturgeon pectoral fin ray aged 7 years with raw elemental scan lines measuring concentrations of strontium (Sr), zinc (Zn), barium (Ba), and manganese (Mn) overlaid on top of the laser ablation transect. Fin ray regions (core, mid, end) were determined by visually identifying the initiation of the first and last growth bands (vertical dashed lines).

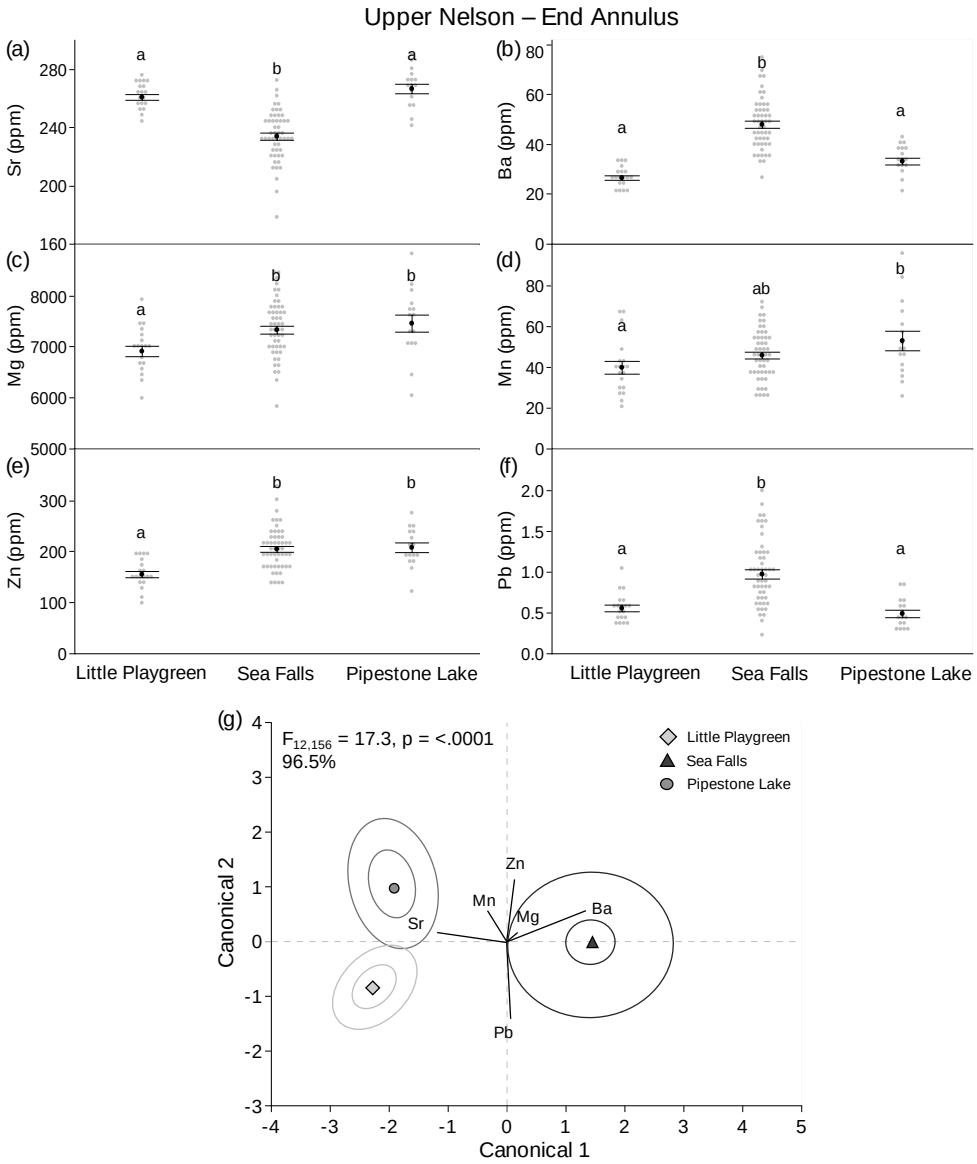

Figure 4.7. Mean (± s.e.m., black points) concentrations of (a) strontium, (b) barium, (c) magnesium, (d) manganese, (e) zinc, and (f) lead in the end pectoral fin ray region of juvenile Lake Sturgeon captured in the upper Nelson. Grey points represent single fin rays and are jittered on the x-axis for visualization. Sites not sharing the same letter are significantly different (p=<0.05). Combined elemental concentrations (g) are presented in the canonical biplot for the quadratic discriminant function analysis (qDFA) grouping Lake Sturgeon into sampling locations (predictor variables). Open symbols represent single individuals while closed symbols are the combined overall mean from each sampling site with inner and outer ellipses representing the 95 and 50% contours, respectively. Standardized scoring coefficients (black lines) represent the canonical weight of each element. Statistical differences of the qDFA (Wilk's λ) and classification success (%) are presented on the biplot.

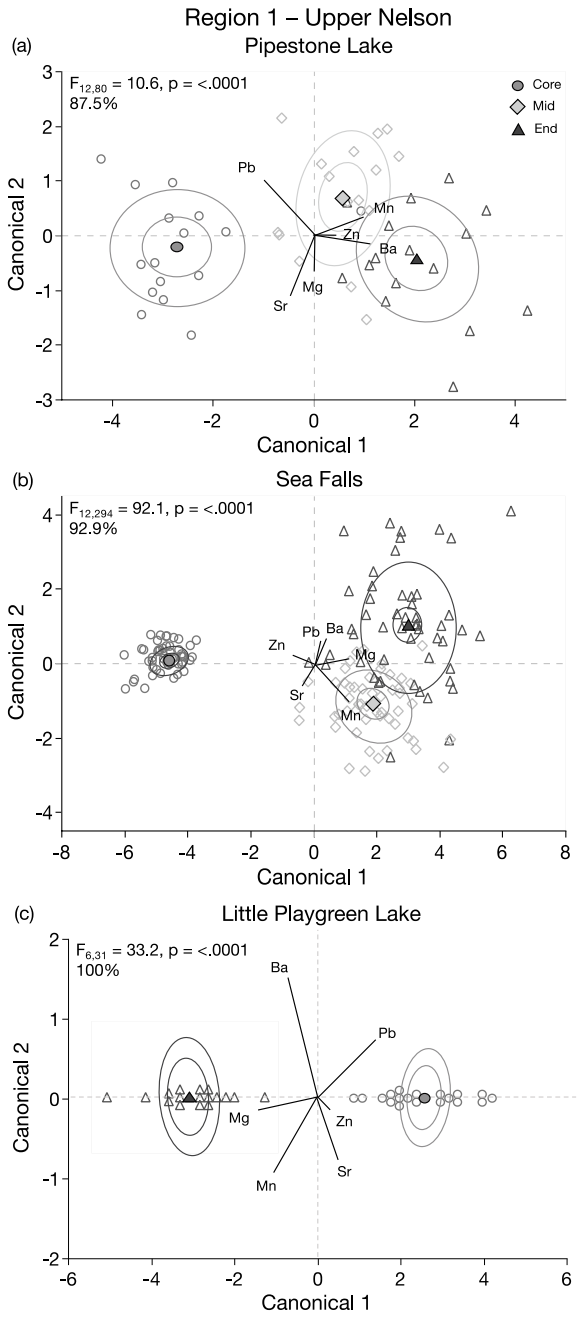

Figure 4.8. Canonical biplots for the quadratic discriminant function analyses (qDFAs) grouping combined elemental signatures by fin region (predictor variables) within (a) Pipestone Lake, (b) Sea Falls, and (c) Little Playgreen Lake in the upper Nelson. Open symbols represent single individuals while closed symbols are the combined overall mean from each sampling site with inner and outer ellipses representing the 95 and 50% contours, respectively. Standardized scoring coefficients (black lines) represent the canonical weight of each element. Statistical results of the qDFAs (Wilk's λ) and classification success (%) are presented on each biplot.

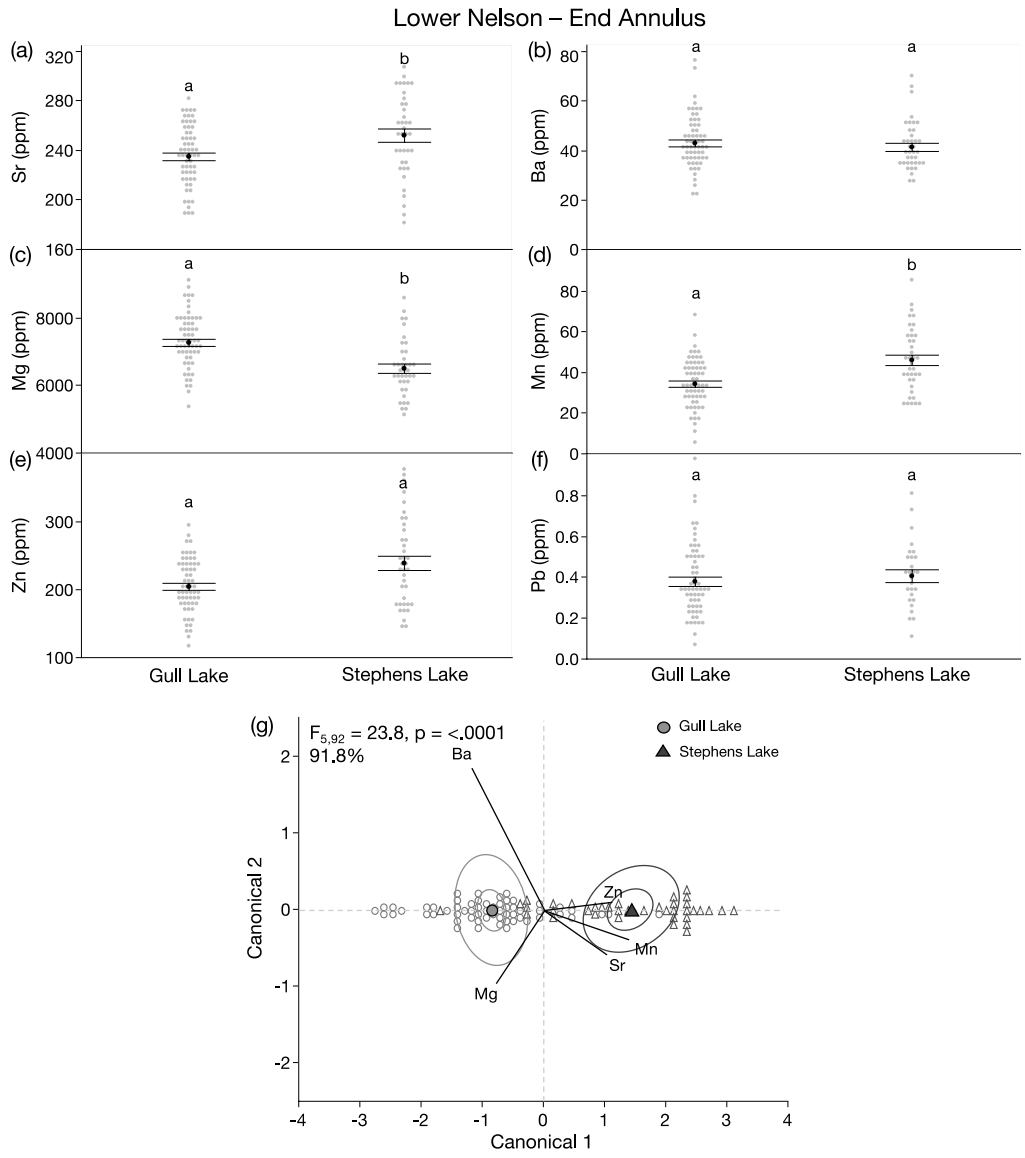

Figure 4.9. Mean (± s.e.m., black points) concentrations of (a) strontium, (b) barium, (c) magnesium, (d) manganese, (e) zinc, and (f) lead in the end pectoral fin ray region of juvenile Lake Sturgeon captured in the lower Nelson. Grey points represent single fin rays and are jittered on the x-axis for visualization. Sites not sharing the same letter are significantly different (p=<0.05). Combined elemental concentrations (g) are presented in the canonical biplot for the quadratic discriminant function analysis (qDFA) grouping Lake Sturgeon into sampling locations (predictor variables). Open symbols represent single individuals while closed symbols are the combined overall mean from each sampling site with inner and outer ellipses representing the 95 and 50% contours, respectively. Standardized scoring coefficients (black lines) represent the canonical weight of each element. Statistical differences of the qDFA (Wilk's λ) and classification success (%) are presented on the biplot.

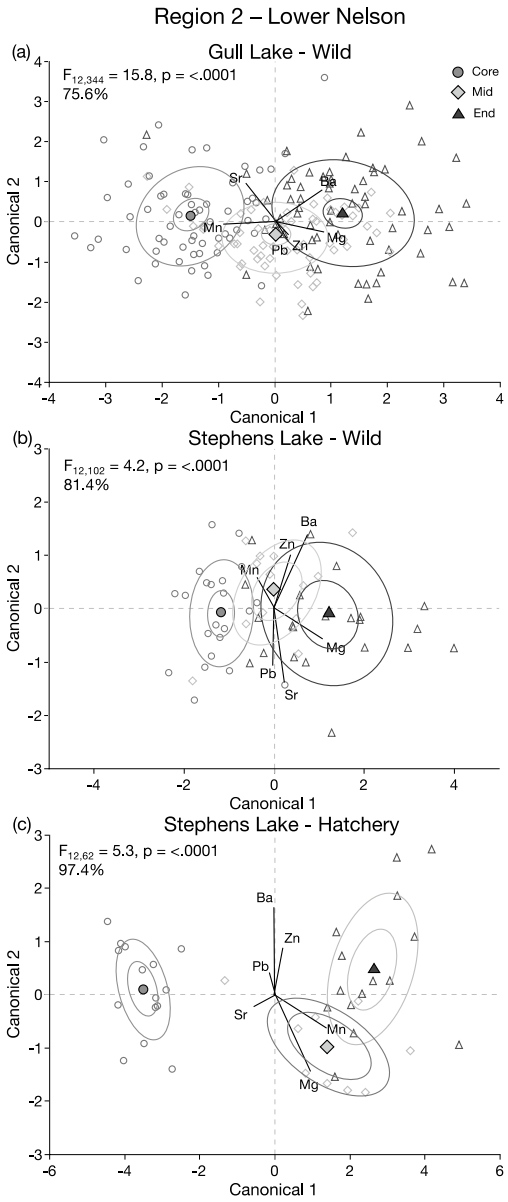

Figure 4.10. Canonical biplots for the quadratic discriminant function analyses (qDFAs) grouping combined elemental signatures by fin region (predictor variables) within (a) hatchery stocked fish in Gull Lake, (b) wild-spawned and (c) hatchery stocked fish in Stephens Lake in the lower Nelson. Open symbols represent single individuals while closed symbols are the combined overall mean from each sampling site with inner and outer ellipses representing the 95 and 50% contours, respectively. Standardized scoring coefficients (black lines) represent the canonical weight of each element. Statistical results of the qDFAs (Wilk's λ) and classification success (%) are presented on each biplot.

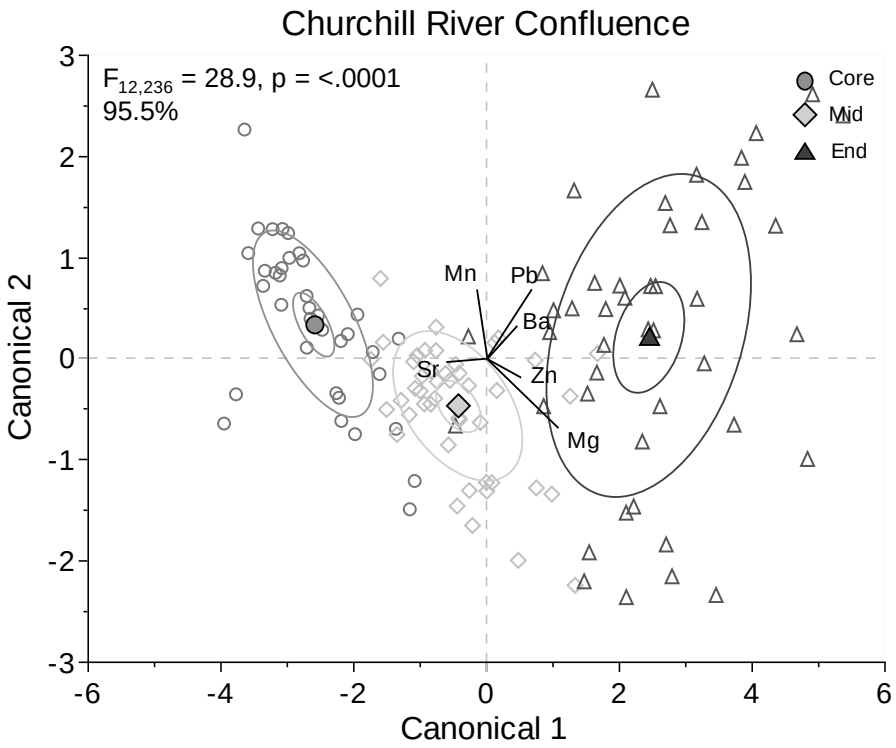

Figure 4.11. Canonical biplot for the quadratic discriminant function analysis (qDFA) grouping combined elemental signatures by fin region (predictor variables) in fin rays from Lake Sturgeon sampled at the Churchill River Confluence in the lower Nelson river. Open symbols represent single individuals while closed symbols are the combined overall mean from each sampling site with inner and outer ellipses representing the 95 and 50% contours, respectively. Standardized scoring coefficients (black lines) represent the canonical weight of each element. Statistical results of the qDFAs (Wilk's λ) and classification success (%) are presented on each biplot.

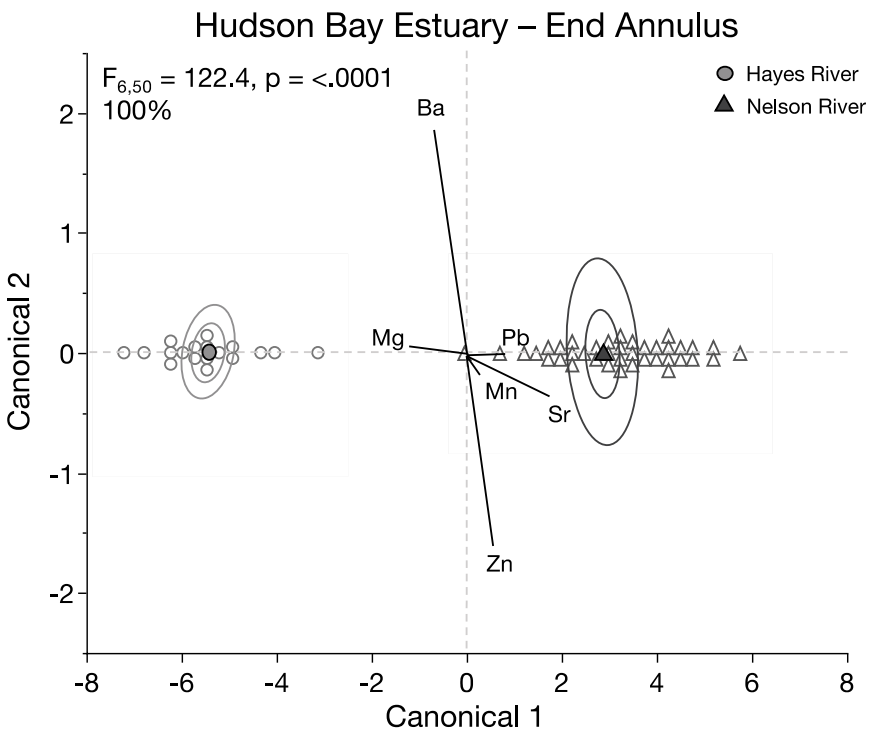

Figure 4.12. Canonical biplot for the quadratic discriminant function analysis (qDFA) grouping combined elemental signatures by fin region (predictor variables) in fin rays from Lake Sturgeon sampled in the Hayes and Nelson River in the Hudson Bay estuary. Open symbols represent single individuals while closed symbols are the combined overall mean from each sampling site with inner and outer ellipses representing the 95 and 50% contours, respectively. Standardized scoring coefficients (black lines) represent the canonical weight of each element. Statistical results of the qDFAs (Wilk's λ) and classification success (%) are presented on each biplot.

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Chapter 5: Identification of hatchery-reared Lake Sturgeon *Acipenser fulvescens* using natural elemental signatures and stable isotope marking of fin rays

This manuscript has been published in the North American Journal for Fisheries Management. As the lead author I was responsible for the experimental design, data collection and analysis, and writing and revision of the manuscript. My co-author C.A. McDougall provided the fin ray samples as well as feedback on the manuscript drafts. Additionally, W.G. Anderson provided insight and guidance to the overall review and design of the experiments as well as editorial revision on multiple drafts of the manuscript.

Loeppky A.L., McDougall C.A. and Anderson W.G. (2020) Identification of hatchery-reared Lake Sturgeon *Acipenser fulvescens* using natural elemental signatures and stable isotope marking of fin rays. North American Journal of Fisheries Management, DOI: 10.1002/nafm.10372

5.1 Abstract

Stock enhancement programs often involve the introduction of hatchery reared fish into wild environments with the goal of increasing the abundance of a particular stock. To measure the success of stocking efforts for depressed populations, hatchery-reared individuals must be distinguishable from naturally spawned fish, potentially many years post-release. Biological tags are attractive due to their ability to batch mark whole cohorts in a cost-effective manner, with minimal disruption to hatchery procedures, or handling stress inflicted on individuals during the marking process. Elemental marking may occur naturally or involves deliberately manipulating the concentration of specific elements to create recognizable elemental signatures in the hard structures of fish (e.g., fin rays, otoliths). In this study, elemental signatures in the fin rays of known hatchery-released Lake Sturgeon *Acipenser fulvescens* (age 1-7 years) were quantified via LA ICP-MS to assess whether the ambient water chemistry in a groundwater fed hatchery would create an elemental signature that facilitated discrimination of hatchery reared individuals from those naturally spawned in the wild. The concentration of divalent trace elements, particularly manganese, within the first growth band of hatchery-reared fish were significantly different than wild conspecifics, allowing us to accurately classify hatchery- versus wild-spawned individuals with 99% success. In addition, we conducted a preliminary experiment to test the validity of two separate 24-hour immersions in ^{86}Sr and ^{137}Ba 30 days apart to induce multiple combination marks in the fin rays of juvenile Lake Sturgeon. Fin rays collected 60 days post-second immersion were analyzed for isotopic ratios via LA ICP-MS and results indicated elemental marking at both time points was achieved with 100% success. Induction of a combination of isotopic signatures could provide hatcheries the ability to track the success of families or stocking groups within a single year-class or across multiple year-classes

5.2 Introduction

Stock enhancement programs are practiced globally in both marine and freshwater environments and typically involve introduction of hatchery-reared fish into wild environments with the goal of increasing the abundance of a particular stock (Travis et al. 1998; Molony et al. 2003; Woodcock et al. 2011b). While the majority of fish hatcheries in the United States operate using surface water withdrawals, the proportion of groundwater sourced hatcheries has increased from 19% in 2010 to 21% in 2015 (Maupin et al. 2010; Dieter et al. 2015). The main differences between groundwater and surface water is often water chemistry (Winter et al. 1998). One of the primary determinants of water chemistry is the composition of geological materials in the drainage basin where the water is sourced, which can influence geochemical characteristics including, but not limited to, acid-base reactions, precipitation and dissolution of minerals, and oxidation-reduction (redox) reactions (Winter et al. 1998). These processes interact to influence the concentration of dissolved minerals in the water thus creating unique elemental profiles in each water source. As such, fish reared in hatcheries supplied with groundwater can be exposed to distinctly different water chemistries compared to wild conspecifics that have been exposed only to surface water.

In order to measure the success of stocking efforts, hatchery-reared individuals need to be distinguishable from naturally spawned fish, typically for many years post-release (Taylor et al. 2005). There are a variety of marking techniques available that include passive, electronic, and biological tagging each with their own distinct advantages and disadvantages (Pine et al. 2009; Drenner et al. 2012; Cadrin et al. 2014; Warren-Myers et al. 2018). As an alternative to physical tags, elemental signatures in hard structures (i.e., otoliths, fin rays, vertebrae, eye lenses) of fish have proven useful as natural markers to reconstruct environmental life histories and identify the natal origin or nursery habitats of individuals (Thorrold et al. 2001; Tzadik et al. 2017b; Warren-Myers 2018). Naturally occurring trace elements (e.g. Sr^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}) are incorporated by fish from the surrounding aquatic environment and are substituted for calcium (Ca^{2+}) in hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) in bone material or calcium carbonate ($CaCO_3$) in otoliths due to their similar chemical properties (i.e., atomic size, valence charge, Campana 1999; Loewen et al. 2016; Tzadik et al. 2017a). The unique abundance and combination of elements from different environments form an otolith/fin ray ‘signature’ and provide a chronological record that can be used to reconstruct the environmental life history of an individual throughout various life stages (Campana 1999; Elsdon and Gillanders 2003).

Fish actively regulate their internal solute and water balance and, thus, the microchemistry of their calcified structures tend to reflect ambient water chemistry (Campana 1999; Bath et al. 2000; Phelps et al. 2016). As such, variation in elemental concentrations in the ambient environment alters the deposition of elemental signatures in hard structures resulting in a biological tag (Wolff et al. 2013). Natural differences in ambient water chemistry reflected in the elemental signatures accumulated in otoliths and fin rays during hatchery rearing were used to successfully identify the origin of known-hatchery muskellunge *Esox masquinongy* (Rude et al. 2014), and unmarked hatchery- and wild-reared June sucker *Chasmistes liorus* (Wolff et al. 2013). Alternatively, stable isotope marking involves deliberately manipulating the natural isotopic ratio of particular elements (e.g. $^{88}Sr/^{86}Sr$, $^{138}Ba/^{137}Ba$), which typically show little variation in the natural environment to intentionally induce an elemental mark (MacLellan and Fargo 1995; Thorrold et al. 2001; Munro et al. 2008; Woodcock et al. 2011a,b, Smith and Whitledge 2011; Warren-Myers et al. 2015a,b,c, Carriere et al. 2016; Mirali et al. 2017). These techniques provide a reliable and long-lasting tag that requires minimal effort and no handling of individual fish (Woodcock et al. 2011b). Thus, mass groups of fish can be tagged at once, providing a cost-effective marking method for hatchery and fishery programs without significantly altering hatchery procedures in comparison to implementing physical tags in individuals (Mirali et al. 2017).

Unlike otoliths, fin rays can be removed non-lethally making them attractive for assessing at risk populations (Bruch et al. 2009; Collins et al. 2013; Wolff et al. 2013). As such, fin rays are commonly removed from sturgeon species to assess age and growth (Cuerrier 1951; Rien and Beamesderfer 1994; Bruch 1999; 2009; Parsons et al. 2003; Koch et al. 2008; McDougall et al. 2018; Steffensen and Hammel 2018) as well as to ascertain information on habitat use and movement (Veinott and Evans 1999; Arai et al. 2002; Allen et al. 2009; 2018b; Phelps et al. 2012; 2017; Pracheil et al. 2014; 2019). Fin rays are the structural components of fish fins and are primarily composed of calcium phosphate ($Ca_3(PO_4)_2$) mineralized as hydroxyapatite (Tzadik et al. 2017). The identification and manipulation of stable isotopic signatures in the fin rays of sturgeon species has been proven useful as a technique to batch mark hatchery-reared individuals (Smith and Whitledge 2011a,b, Carriere et al. 2016; Mirali et al. 2017). For example, Mirali et al. (2017), reported juvenile Persian Sturgeon *Acipenser persicus* fin rays were successfully marked when immersed in ^{137}Ba for just 24 hours. Despite the potential for resorption of fin ray material during periods of prolonged nutritional stress or spawning events, induced concentrations of ^{86}Sr remained detectable in the fin rays of sturgeon species 120 to 550 days post

marking (Smith and Whitledge 2011; Carriere et al. 2016) although the long-term retention of elemental signatures (> 2 years) in fin rays of long-lived species, such as Lake Sturgeon has yet to be ascertained.

Typically, immersion marking studies assess the efficacy of mass marking individuals with a single isotope, but the ability to mark groups of hatchery-reared fish with multiple unique elemental signatures has potential benefits in relation to the assessment of stock enhancement programs. For example, it could allow for cost-effective delineation of offspring from different families or hatchery rearing/stocking strategies within the same year-class and across multiple year-classes. Indeed, Woodcock et al. (2011a) were able to successfully mark the otoliths of larvae with up to 15 different isotopic signatures implementing multiple unique marks to individuals from a single cohort. However, the validity of inducing multiple elemental marks in the fin rays of sturgeon species has not yet been tested.

The Lake Sturgeon *A. fulvescens* is a freshwater potamadromous sturgeon species occupying the Great Lakes, Hudson Bay, and Mississippi River drainage basins (Scott and Crossman 1992). Severe and rapid population declines commenced ~150 years ago due to overexploitation, coupled with habitat impacts (Harkness and Dymond 1961; Houston 1987; Scott and Crossman 1992; Bruch et al. 2016). In 2017, the Committee for the Status of Wildlife in Canada (COSEWIC) recommended Lake Sturgeon in Designatable Unit 2 (i.e., Saskatchewan-Nelson River) be listed as endangered under the Species at Risk Act (SARA; COSEWIC 2017). Lake Sturgeon stocking on the Nelson River, Manitoba began in 1994 (MCWS 2012). Contemporary evaluation of stocking success has focused on the recapture of individuals marked with passive integrated transponder (PIT) tags prior to release into the wild at age-1 (McDougall et al. 2014; McDougall et al. in review). While there currently is minimal evidence of contribution by hatchery fish released in the Nelson River as larvae or age-0 (McDougall et al. in review), utilizing biological tags to batch mark hatchery-reared individuals based on naturally occurring differences in ambient water chemistry and/or deliberate induction of isotopic signatures could allow post-hoc assessment of wild versus stocked origin in situations where PIT tagging in the hatchery prior to release was not possible (e.g., fish were too small to receive PIT tags) or in cases where tag loss was suspected.

The main objective of this study was to assess whether naturally occurring elemental signatures measured within the first growth band (i.e., period of growth from hatch to the first annulus which corresponds to overwinter growth cessation) of Lake Sturgeon fin rays could be used to successfully discriminate between hatchery-reared and wild-spawned individuals, several years post-release.

Analysis focused on elemental signature distinctness and persistence. In addition, a preliminary study was conducted where we investigated the effectiveness of multiple 24-hour immersions in known concentrations of the rare stable isotopes of Strontium (^{86}Sr) and Barium (^{137}Ba) to implement combination marks on a single cohort.

5.3 Methods

5.3.1 Hatchery versus wild classification

Pectoral fin rays were examined from both known hatchery-reared (n=153, age 1-7 years) and putative wild Lake Sturgeon (n=80, age 2-8 years) captured during gillnet surveys of five different sections of the Nelson River between 2012 and 2017 (Figure 5.1, Table 5.1). All hatchery fish were definitively known to be such based on the presence of PIT tags applied in the Grand Rapids Fish Hatchery in Grand Rapids, Manitoba (53°11'04.4"N, 99°15'40.7"W) prior to fish being released in the wild following ~1.5 years of hatchery residence (overwinter rearing followed by release the next fall). Fish age was determined by referencing hatchery cohort year, or in the case of putative wild individuals (lacking hatchery PIT tags), by counting annuli. Samples collected from wild-caught fish were done so under Manitoba Sustainable Development Scientific Collection Permits 16-12, 50-12, 16-14, 18-14, 10-15, 17-15, and 09-17.

5.3.2 Immersion Marking

A preliminary marking study was conducted to validate whether unique isotopic signatures could be distinguished in the fin rays of age-0 Lake Sturgeon after multiple 24-hour immersions in $^{86}SrCO_3$ and $^{137}BaCO_3$. Fish used in this series of experiments were the artificially reared progeny of wild spawning adult Lake Sturgeon captured by gill net downstream of Pointe du Bois on the Winnipeg River (50°18'06.6"N, 95°32'29.5"W) in Manitoba. To simulate hatchery rearing environments and to achieve the goal of mass marking groups of fish, 200 randomly selected fish were transferred and equally distributed into two 170 L experimental flow through tanks constantly aerated and supplied by a continuous flow of dechlorinated City of Winnipeg tap water held at 16°C, the original source of which is Shoal Lake, Manitoba/Ontario (headwaters of the Winnipeg River). At 100 days post fertilization (dpf), the flow to each tank was shut off and 100 $\mu g.L^{-1}$ of either $^{86}SrCO_3$ or $^{137}BaCO_3$ was added separately to each of the treatment tanks. $^{86}SrCO_3$ and $^{137}BaCO_3$ are sparingly soluble in water of near neutral pH, thus marking solutions were prepared by first dissolving the carbonates in concentrated HCl then diluted using distilled water. The pH of each solution was then adjusted to 7 using $NaHCO_3$. The

volume of dissolved $^{86}SrCO_3$ or $^{137}BaCO_3$ needed to achieve the desired marking concentration was calculated and added to each tank.

Fish remained in the spiked water for 24 hours during which the water flow to each tank remained off and food was temporarily withheld. Forced air was provided to each tank using an air stone to maintain water quality. After the 24-hour immersion period the flow was turned back on allowing the isotopes to be flushed from the holding tanks. Feeding was resumed and fish continued to grow under normal holding conditions. Thirty days later, the same process was repeated this time exposing fish to the opposite element for 24 hours, therefore fish were exposed to each element only once at each immersion event. Fish that were immersed in ^{86}Sr first and ^{137}Ba second will be labeled SrBa and fish immersed in the opposite treatment will be labeled BaSr hereafter. At 190 dpf, 20 individuals from both immersion treatments were randomly sampled by dipnet and immediately euthanized via an overdose of MS-222 buffered with equal volumes of sodium bicarbonate (250 ppm). Ten individuals that had not been exposed to any immersion treatments were also sampled at this time to obtain baseline control values for $^{88}Sr/^{86}Sr$ and $^{138}Ba/^{137}Ba$ ratios from the aquarium water. The left pectoral fin ray was removed from each fish and air dried prior to sectioning. All procedures conducted on lab reared fish were approved by the animal care committee at the University of Manitoba permit# F15-007 in accordance with guidelines established by the Canadian Council for Animal Care.

5.3.3 Fin Ray Elemental Analysis

All fin rays were prepared for elemental analysis via Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA ICP-MS, *Perkin-elmer DRC II*) at the University of Manitoba following the procedure described in Smith & Whitledge, (2011). First, fin rays were set in an epoxy resin and sectioned (1 mm) at the articulating process using an ISOMET^{TM} low-speed saw. Sectioned samples were then polished with silicon carbide paper until the core was exposed. Multiple fin ray sections were then mounted in epoxy rings and once again polished and buffed until the entire surface of each ring was smooth and even. Epoxy rings (n=5-12 fin rays/ring) were placed separately in the ablation cell. Ablation was first performed on a standard reference glass material (NIST 610, National Institute of Standards and Technology, Gaithersburg, MD, USA) to calibrate the LA ICP-MS and to provide an external standard. A line transect analysis technique was used to quantify trace elemental concentrations (i.e., hatchery mark: Sr, Ba, Mg, Mn, Zn, immersion mark: $^{88}Sr/^{86}Sr$, $^{138}Ba/^{137}Ba$) whereby a lateral line along the longest axis of the sectioned fin rays was ablated from the core to the outer edge ensuring a consistent and distinguishable signature corresponding with each growth band. Prior to ablating each fin

ray sample, a 30 s gas blank was run to ensure the previous sample had been cleared and to determine background concentrations for each trace element to estimate detection limits. The following laser parameters were used for the analyses: 30 μm spot size, 10 μm/sec scan speed, 80% energy, 20 Hz repetition rate, and a dwell time of 15 ms for all elements analyzed. After ablating all fin rays in a ring, the reference glass material was once again ablated to correct for any changes during analysis. Ablated fin ray sections were then imaged so that the entire laser ablation transect and growth bands were clearly visible.

Data reduction was performed using *Igor Pro* graphing software with an *Iolite* version 2.21 package for LA ICP-MS. Calcium (^{43}Ca) counts per second (CPS) were used to determine an internal standard to correct for changes in ablation volume. Average concentration (± s.e.m) of Sr, Ba, Mg, Mn, and Zn by growth band (i.e., within each growth increment) was then calculated by measuring the scan length (μm) of each growth band using *ImageJ* pixel measuring software. For known hatchery fish, elemental signatures produced by the first growth band of the fin ray (i.e., between the nucleus and the annuli corresponding with the fish's first winter in the wild) represent the hatchery signature. For the immersion marking experiment, line transects were smoothed using a 10-point running average similar to Munro et al. (2008) and Smith and Whitledge (2011) prior to calculating the mean (± s.e.m) concentration of ^{88}Sr, ^{86}Sr, ^{138}Ba, and ^{137}Ba across the ablation transect. The entire ablation transect was used because fish in this experiment were <1 year of age and, thus, any significant deviations in ^{88}Sr/^{86}Sr and ^{138}Ba/^{137}Ba ratios could confidently be considered a result of the immersion marking events.

5.3.4 Data Analysis

Concentrations of each trace element (response variables, ppm) were examined separately for normality using the Shapiro-Wilk's W test, and for homogeneity of variance using the Levene's test. Response variables did not meet the underlying assumptions of parametric statistics, even after log transformation, therefore non-parametric (Wilcoxon Signed-rank test, Quadratic Discriminant Function Analysis (qDFA)) tests were conducted. For the hatchery versus wild classification experiment, based on known hatchery fish, mean elemental concentrations from the first growth band (which corresponded with the 1.5 year period of hatchery residence wherein water temperatures facilitated sustained growth, even during winter) were used to quantify signatures accumulated during their hatchery-rearing period. Mean elemental concentrations from the second growth band observed in hatchery reared fish were used to quantify signatures accumulated during the first season of wild-residence. For putative wild fish, mean elemental concentrations associated with first and second growth bands were calculated.

Differences in mean elemental concentration (i.e., Sr, Ba, Mg, Mn, Zn, continuous response variables, ppm) between initial source (i.e., hatchery versus wild) and first and second growth bands of both known hatchery and putative wild fish were assessed using a Wilcoxon Signed-rank test. In addition, a qDFA was performed where all response variables were combined to determine whether individual Lake Sturgeon could be reliably identified as hatchery or wild fish based on fin ray chemical signatures present in the first band of growth.

For the immersion experiment, average elemental ratios of $^{88}Sr/^{86}Sr$ and $^{138}Ba/^{137}Ba$ were calculated across the entire ablation transect of each fin ray. Due to the small size of the fin rays analyzed, holes in the fin ray material were difficult to avoid with the ablation transect. As such fin rays that had significant portions of the scan line with ^{43}Ca CPS values of 0 typically resulted in erroneous isotopic ratios of $^{88}Sr/^{86}Sr$ and $^{138}Ba/^{137}Ba$. Therefore, mean elemental ratios that were greater than two standard deviations from the mean were considered outliers and removed from the data set (BaSr: n = 2, SrBa n = 3). Because of the lack of true replication, fish in each marking treatment tank (i.e., Control, BaSr, SrBa) were considered as replicates (i.e., pseudo-replicates) in the statistical analyses. Response variables (i.e., $^{88}Sr/^{86}Sr$,$^{138}Ba/^{137}Ba$) met the underlying assumptions of parametric statistics, therefore, single-factor ANOVAs were performed. Fish were considered to be successfully marked if the $^{88}Sr/^{86}Sr$ and $^{138}Ba/^{137}Ba$ ratios were >5 SD from the mean of the control. Marking success was then calculated as the number of successfully marked fin rays per total fin rays analyzed in each treatment. Fish were only exposed to each element once at either the first immersion timepoint at 100 dpf or the second immersion timepoint at 130 dpf indicating. Therefore, differences in mean $^{88}Sr/^{86}Sr$ and $^{138}Ba/^{137}Ba$ ratios (i.e., continuous response variables) among marked and control fish were assessed using a single-factor ANOVA. A post-hoc Tukey HSD test was performed to identify which factor levels were significantly different.

5.4 Results

5.4.1 Hatchery versus Wild Classification

Concentrations of all trace elements analyzed in the first growth band of the hatchery fish were significantly different than those from putative wild fish, regardless of capture location (Figure 5.2). The concentration of Mn within the first growth band of the hatchery fish was consistently low (majority <10 ppm) relative to wild fish, but concentrations within the second growth band were similar (Figure 5.3 A, B). Indeed, mean Mn concentrations in the first growth band of hatchery released fish caught in Stephens Lake (n=20) were significantly lower than for wild fish caught in the same location in the same

year (n=20, Z=5.26, p=<0.0001). Timing of the marked increase observed for hatchery fish was consistent with when these fish were release from the hatchery and remained clearly distinguishable even four years after hatchery release (Figure 5.3 A, C). Conversely, there were no significant differences in average Mn concentrations in the second growth band for hatchery and putative wild individuals caught in Stephens Lake (Z=1.39, p=0.16). Statistically significant differences among means were also observed for all other trace elements analyzed (with the exception of Sr, which showed no significant differences among the first and second growth bands for hatchery fish), although there was more overlap in the data distributions than there was for Mn (Figure 5.4 A). When all trace elements were combined within the first growth band, fish could successfully be classified into source (i.e., wild or hatchery, $F_{5,219}$=234.18, p=<0.0001, Figure 5.5) with a strong classification success of 99.1% regardless of sampling site (number misclassified: 2/225 fish). Both fish that were misclassified by the qDFA model were hatchery fish from the 2013 cohort captured in the Sea Falls sampling location; these two fish were erroneously predicted as wild origin. When location was removed as a potential confounding source of variation, and only the fish captured in Stephen's Lake were considered, classification success was 100% ($F_{5,33}$=44.86, p=<0.0001).

5.4.2 Immersion Marking

Average $^{88}Sr/^{86}Sr$ and $^{138}Ba/^{137}Ba$ ratios in the fin rays of control fish (n=10) were 8.54 (±0.02 s.e.m) and 6.45 (±0.02 s.e.m), respectively (Figure 5.5, 5.6). Both of these values are similar to the natural isotopic ratios of $^{88}Sr/^{86}Sr$ (8.48, Munro et al. 2008; Smith and Whitledge 2011) and $^{138}Ba/^{137}Ba$ (6.38, Munro et al. 2009) in freshwater. Immersing juvenile Lake Sturgeon in 100 $\mu g.L^{-1}$ of $^{86}SrCO_3$ or $^{137}BaCO_3$ at 100 dpf and then in the opposite element at 130 dpf for 24-hours resulted in a significant decrease in fin ray $^{88}Sr/^{86}Sr$ and $^{138}Ba/^{137}Ba$ ratios (Sr: $F_{2,43}$=26.47, p=<0.0001, Ba: $F_{2,43}$=16.38, p=<0.0001) at both time points and successfully implemented two combination elemental marks (i.e., Sr then Ba and Ba then Sr). Sixty days after the final immersion period average $^{88}Sr/^{86}Sr$ ratios were 7.87 (±0.06 s.e.m) and 7.99 (±0.05 s.e.m) and $^{138}Ba/^{137}Ba$ ratios were 5.29 (±0.14 s.e.m) and 5.34 (±0.13 s.e.m) in the BaSr and SrBa treatments, respectively. Marking success was 100% for both elements regardless of timing.

5.5 Discussion

Results from the hatchery versus wild classification experiment identified differences in all trace elements measured within the first growth band of Lake Sturgeon pectoral fin rays between known hatchery and putative (now confirmed) wild-origin fish captured in the Nelson River, Manitoba, despite

there being no intentional manipulation of the ambient water chemistry. These differences are most likely attributed to the different water chemistries (i.e., ambient Sr, Ba, Mg, Mn, and Zn concentration) observed in the groundwater supplied to the Grand Rapids Fish Hatchery (Manitoba Water Services Board 2013) and surface water sources where fish were captured in the wild (Coordinated Aquatic Monitoring Program 2017, Table2). Although there was a significant amount in overlap in concentrations of Sr, Ba, Mg, and Zn of individual fin rays, combining all trace elements in the first growth band to predict hatchery versus wild-spawned individuals was successful with a high predictive capacity (99.1%), which was largely driven by the distinct differences in Mn concentrations in the first growth band of hatchery reared Lake Sturgeon. Results from the qDFA confirmed that the vast majority of hatchery-released fish can be accurately identified post-release using elemental signatures in the first growth band of Lake Sturgeon pectoral fin rays.

Manganese (Mn) was of particular interest in the present study as Lake Sturgeon caught in Stephens Lake showed Mn concentrations in the first growth band of hatchery fish to be nearly 14 times lower on average, and with minimal overlap, than in the first growth band of wild conspecifics regardless of sampling location suggesting strong discriminatory power from this single variable alone (Figure 5.3B). In support, Mn was weighted the highest in the qDFA model indicating it is the most strongly associated covariate with the canonical variable in the positive direction (i.e., hatchery origin, Figure 5.5). Such distinct differences in fin ray Mn concentrations can be attributed to the divergent ambient water chemistries between the hatchery ground water source and wild surface water elemental concentrations. Indeed, Mn concentrations were negligible (<0.0003 ppm) in the groundwater supplying the hatchery (Table 5.2), which can likely be attributed to redox reactions occurring in the well. The hardness of the water before treatment is known to be very high (270 ppm) and the pH is basic (7.67) rendering Mn from the fractured limestone aquifer to be less soluble ultimately decreasing the amount of available dissolved Mn in the water (Grenthe et al. 1992; McMahon et al. 2011; Manitoba Water Services Board 2013). The lack of dissolved/available Mn is reflected in the low concentrations of Mn measured in the fin rays of hatchery fish during their time in the hatchery. Further, Mn concentrations in the second growth band of hatchery-reared fish, representing signatures accumulated post-release, were significantly elevated relative to the first growth band, likely as a function of higher Mn available in Nelson River water (Table 5.2). These distinct and consistent differences in Mn concentration in the pre- and post-release regions of hatchery-reared Lake Sturgeon fin rays provide a reliable biological tag that

can be used to classify origin of wild-caught fish, potentially negating the need to physically mark individual fish.

Similar trends are observed with all other trace elements measured in this study with the exception of strontium (Sr). Interestingly, Sr concentrations in the fin rays of hatchery reared fish showed no significant differences among the first and second growth bands (i.e., pre-/post-release) despite there being double the concentration of ambient Sr in the Nelson River relative to the Grand Rapids Hatchery groundwater source (Table 5.2). Fin ray Sr concentrations in the first growth band of wild-spawned fish were, however, significantly lower than hatchery-reared individuals. This negative relationship between ambient and fin ray Sr concentrations is inconsistent with previous research conducted on Green Sturgeon, *A. medirostris*, where fin ray Sr concentrations were positively correlated with ambient Sr concentrations related to increasing salinity (Allen et al. 2009). Changes in Sr concentrations in the otoliths of teleost fish, in relationship to salinity and environmental Sr concentrations, have been studied in depth and are commonly used to identify transitions from freshwater to saline environments (e.g., Limburg et al. 1995; Secor and Rooker 2000; Zimmerman et al. 2005; Nelson et al. 2018). The opposite trends observed in this study could be attributed to ambient Ca concentrations measured in the groundwater and surface water sources affecting uptake kinetics of waterborne Sr through competitive inhibition (Chapter 2).

Strontium, Ba, and Ca are alkaline earth metals with similar elemental properties, such as valence charge and atomic radii (Mann 2001; Loewen et al. 2016). Due to these chemical properties, Sr is likely incorporated into the organism via Ca transport proteins (McKim 1994) and is assumed to experience the same variations in uptake rate in relationship to changes in environmental variables, such as temperature (Loewen et al. 2016). Calcium acts as a competitive inhibitor on Sr at Ca transport proteins (McKim 1994), which have a higher affinity for Ca, resulting in an inverse relationship between ambient Ca concentration and Sr uptake as observed in Common Carp, *Cyprinus carpio* (Chowdhury and Blust 2000) and juvenile Lake Sturgeon (Loeppky et al. *unpublished*). Based on these assumptions, it could be hypothesised that the same result should have been observed for Ba in the fin rays analyzed in this study, however, the opposite trend was observed. Importantly, multiple biological barriers exist during the movement of ions from the environment into calcified tissues (Campana 1999). These barriers can be both ion- and tissue-specific resulting in accumulation and fractionation of elements in the organism (Campana 1999; Loewen et al. 2016). Indeed, Carriere et al. (2016) observed Sr to be preferentially accumulated in the fin rays of age-0 Lake Sturgeon. Conversely, Ba accumulation

appeared to be relatively evenly distributed amongst muscle, vertebrae, and fin ray (Carriere et al. 2016; Loewen et al. 2016) perhaps attributing to the greater variation of Ba concentration, and ultimately the opposite trend reported in the fin rays of wild caught Lake Sturgeon from this study.

Tag persistence is an important factor in determining the efficacy of fisheries marking techniques (Cadrin et al. 2014; Warren-Myers et al. 2018). This is of particular importance for long-lived species, such as Lake Sturgeon, where managers need to be able to track the survivorship of hatchery-released individuals over many years (Taylor et al. 2005). The persistence of the hatchery induced mark observed in this study appears to be promising. Indeed, hatchery fish that were aged at five to seven years retained the clearly detectable unique hatchery signature in their first growth bands (e.g., Figure 5.3C). This is consistent with hatchery stocked Muskellunge where hatchery specific Sr:Ca signatures were retained at least seven years after stocking into wild environments (Rude et al. 2014). Growth bands and annuli (i.e., non-growth bands, typically corresponding with overwinter growth cessation) can become more compressed as the fish ages, particularly at the onset of sexual maturity (Francis and Horn 1997; Bruch et al. 2009) making it difficult sometimes to identify the age of long-lived individuals. However, the first growth band tends to remain relatively broad in comparison to subsequent bands that form as fish age. As such, the elemental signatures incurred during hatchery rearing should remain identifiable in the pectoral fin rays of Lake Sturgeon throughout their adult life.

Results from the preliminary immersion marking experiment indicate that it is possible to successfully implement multiple distinguishable marks in the pectoral fin rays of juvenile Lake Sturgeon by immersing individuals in 100 $\mu g.L^{-1}$ of $^{137}BaCO_3$ or $^{86}SrCO_3$ for just 24-hours resulting in 100% marking success at 190 dph. These results are consistent with studies that have previously examined isotopic marking in sturgeon fin rays (Smith and Whitledge 2011; Mirali et al. 2017; Carriere et al. 2016) in addition to otoliths in various teleosts (Ennevor and Beames 1993; Munro et al. 2008; Woodcock et al. 2011b). Indeed, we were able to implement marks of similar values as researchers that immersed their fish in lower concentrations of isotopes for longer periods of time. For example, fin ray $^{88}Sr/^{86}Sr$ ratios in this study were similar to values reported in fin rays from Lake Sturgeon that were immersed for ten days in 25 $\mu g.L^{-1}$ of $^{86}SrCO_3$ (Smith and Whitledge 2011). However, fin ray $^{138}Ba/^{137}Ba$ ratios from marked fish in our study did not decrease to the same degree as $^{138}Ba/^{137}Ba$ ratios reported in Mirali et al. (2017) where juvenile Persian Sturgeon were immersed in similar concentrations of $^{137}BaCO_3$ for 24-hours. The higher temperature of 26°C in the Persian Sturgeon study compared to 16°C in our study is the most likely cause. Temperature influences binding and transport kinetics of all

proteins including Ca transport proteins (Chowdhury and Blust 2001), as such, an increase in temperature during immersion would increase uptake of the less abundant isotope further manipulating the isotopic ratio of the trace element implementing a stronger mark in the 24-hour period in the Persian Sturgeon. Although using a lower concentration of isotope may be less expensive initially, longer immersion times require fish to be maintained in stagnant water conditions with periodic water changes (containing isotope), which may disrupt hatchery routines and can cause physiological stress on the individual resulting in potential adverse effects on the health and survivorship of the fish being marked, therefore, keeping immersion time to a minimum is not only safer for the fish but also more convenient for the hatchery.

In this preliminary study, we report for the first time multiple distinguishable isotopic marks in the fin rays of juvenile Lake Sturgeon using a combination of environmentally rare Sr and Ba isotopes, successfully implementing two unique marks to groups of fish from the same cohort. In theory, applying different combinations of the isotopes at the same timepoints would allow the potential of creating up to nine unique marks (e.g., SrBa, BaSr, SrSr, BaBa, XSr, XBa, SrX, BaX, XX, where X is a blank mark with no isotope being added at the immersion event). Validating additional marking timepoints would increase the amount of unique marking combinations possible to identify different groups of fish within the same cohort. Indeed, Woodcock et al. (2011a) reported the uptake and successful marking of otoliths in larval Murray Cod with 15 distinctive stable isotope combinations. From a management perspective, uniquely marking multiple families, experimental groups and/or cohorts of fish reared in a conservation aquaculture setting could be useful for the purpose of assessing the effectiveness of the rearing environment and/or variation in survival among fish stocked. While the combination marks in this preliminary study were identifiable 190 dpf, the long-term persistence of these marks need to be validated. However, based on previous studies examining other species and elements, as well as the persistence of the naturally induced hatchery signature in the hatchery versus wild study, there is no reason to suspect the deposition of the Sr and Ba signatures accumulated over 24 hours in this study would not persist for years after the mark was introduced.

Despite the widespread use of fin rays by fisheries management organizations for the purposes of estimating important biological parameters such as growth and age-at-maturity, some concerns have been raised regarding potentially adverse effects of removing the pectoral fin ray from sturgeons. Most notably, Stefensen and Hammel (2018) found the apparent survival of sub-adult Pallid Sturgeon *Scaphyrynchus albus* was negatively impacted by fin ray removal. Several studies conducted on other

sturgeon species, however, have shown there are no adverse effects on swimming performance (e.g., Shovelnose Sturgeon *S. platorynchus* Parsons et al. 2003; White Sturgeon *A. transmontanus* Nguyen et al. 2016; Siberian Sturgeon *A. baerii* Nguyen et al. 2017) or growth and survival (Shortnose Sturgeon *A. brevirostrum* and Atlantic Sturgeon *A. oxyrichus oxyrinchus* Collins and Smith 1996). In addition, Allen et al. (2018a) confirmed the complete regeneration of the pectoral fin ray of juvenile Atlantic Sturgeon was achieved after 12 months of healing. As such, fin rays should be visually inspected for signs of bone regrowth and vasculature to ensure area of interest on the fin ray hasn't been reabsorbed (Tzadik et al., 2017). With specific reference to Lake Sturgeon (the focus of our study), recent mark-recapture studies (wherein all juvenile Lake Sturgeon captured have fin rays removed for ageing) have produced annual survival estimates of 0.99 for two well-studied populations (McDougall et al. 2017; McDougall et al. in review), a level which would be incompatible with even a very low rate of delayed sampling mortality. All things considered, the biological information gained from the non-lethal sampling of fin rays from most sturgeon species outweighs the potential negative effects fin ray removal might have, and as such we suspect that most management organizations will continue to collect sturgeon fin rays as a matter of course. Availability of fin rays is unlikely to be a limiting factor in the utility of the methods described; indeed, perhaps the most significant caveat associated with analyzing elemental signatures in fin rays relates to the specialized skills and equipment required to conduct the work, and therefore the resulting "per fish analyzed" cost. The methods described herein are therefore unlikely to be a complete replacement for physical tagging, but could have widespread utility as a secondary fail-safe method, capable of resolving uncertainties about the origin of an individual when other methods cannot be used for example when fish are too small to receive a PIT tag, or when tag loss is suspected.

5.6 Acknowledgements

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5.7 Tables

Table 5.1. Sampling locations within the upper and lower Nelson River where pectoral fin rays used in the hatchery versus wild classification experiment were sampled from Lake Sturgeon caught in annual surveys in Manitoba, Canada. Source was determined by the presence/absence of PIT on the captured individual. Cohort was determined by referencing the PIT tag number, or in the case of wild fish, by counting the annuli in the sectioned fin rays.

Sampling Region	Sampling Location	Source	Cohort	Age (years)	n
Upper Nelson	Pipestone Lake	Hatchery	2013	2-4	17
	Little Playgreen	Hatchery	2013	1-2	19
	Sea Falls	Hatchery	2007	5-7	57
	Sea Falls	Hatchery	2013	2	40
Lower Nelson	Stephens Lake	Hatchery	2015	2	20
	Stephens Lake	Wild	2016	2-3	20
	Gull Lake	Wild	2008	3-8	60

Table 5.2. Concentrations of trace elements ($mg.L^{-1}$) measured in samples collected from groundwater used at the Grand Rapids Fish Hatchery, where released hatchery Lake Sturgeon were reared for 1 year prior to being stocked into wild environments (see Appendix F in Manitoba Water Services Board 2013). Surface water samples were collected at 0.3 m depth and averaged (mean ± s.e.m) from multiple locations within both the upper and lower Nelson River (CAMP 2017). Asterisks indicate average surface water concentrations in the upper Nelson River that were significantly different from those in the lower Nelson River ($P \leq 0.05$).

Element	Groundwater (Hatchery)	Surface water (Upper Nelson River)	Surface water (Lower Nelson River)
Ca	1.42	31.08 ± 0.44	28.65 ± 1.18
Sr	0.005	0.11 ± 0.003	0.06 ± 0.003*
Ba	0.001	0.04 ± 0.0008	0.02 ± 0.001*
Mg	0.32	12.39 ± 0.26	7.62 ± 0.29*
Mn	<0.0003	0.02 ± 0.0006	0.02 ± 0.001
Zn	<0.005	0.005 ± 0.0004	0.005 ± 0.0003

5.8 Figures

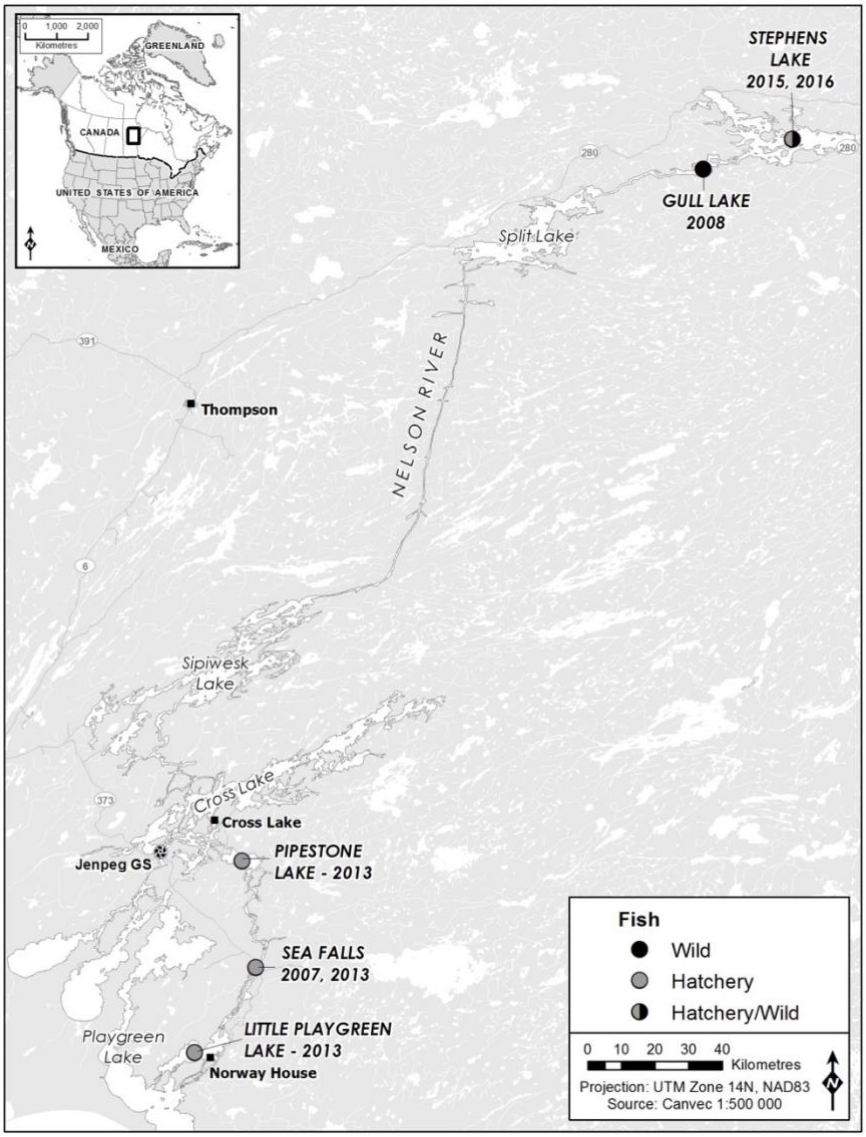

Figure 5.1. Map of study area where fin rays from Lake Sturgeon A. Fulvescens (age 2-7) were collected in the upper and lower Nelson River in Manitoba, Canada. Black circles represent putative wild fish and grey circles represent known hatchery fish. Circles that are both black and grey represent sampling sites where both known hatchery and putative wild fish were caught. Years represent the cohort fish belong to.

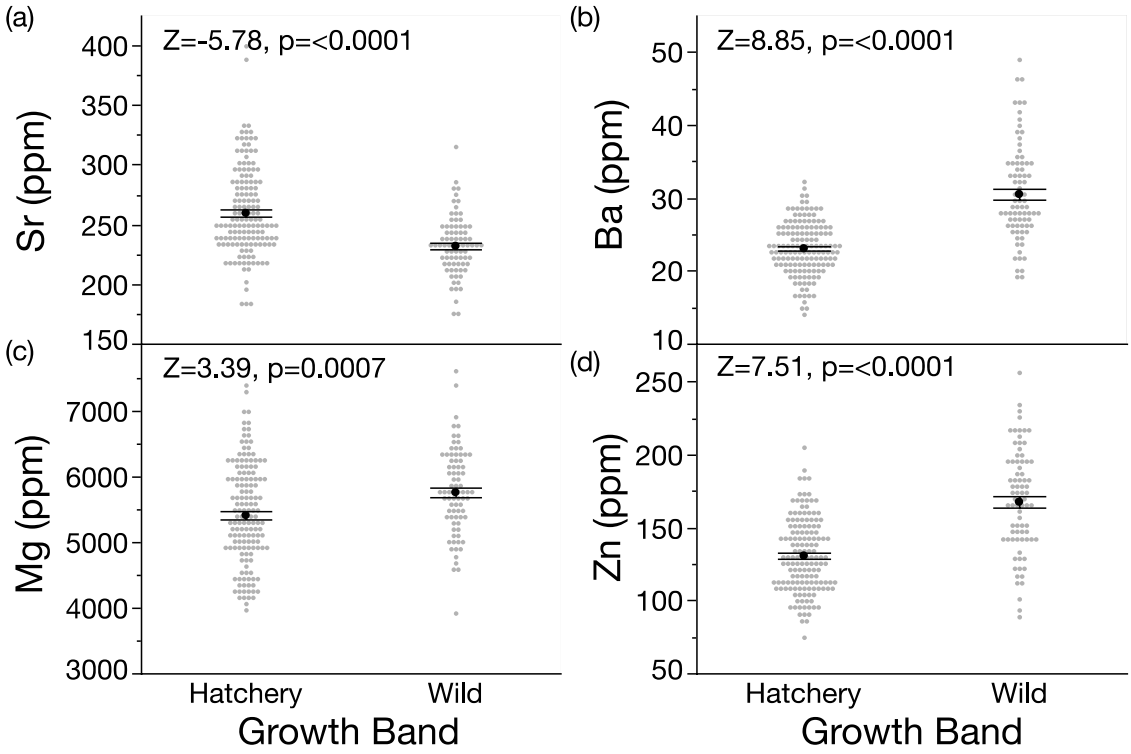

Figure 5.2. Mean (± s.e.m) fin ray concentrations (black points; ppm = µg/g) of (a) Sr, (b) Ba, (c) Mg, and (d) Zn in the first growth band (i.e., between the fin ray nucleus and the annulus corresponding with the first period of growth cessation) from known hatchery-released and putative wild Lake Sturgeon caught during annual surveys at various locations in Manitoba. Gray points represent the average elemental concentrations of individual fin rays and are jittered on the x-axis for visualization (hatchery: n = 149; wild: n = 79). Statistical results of Wilcoxon signed-ranks tests examining differences in mean fin ray trace element concentrations between fish sources (i.e., hatchery and wild) are shown; P-values less than 0.05 indicate significant differences.

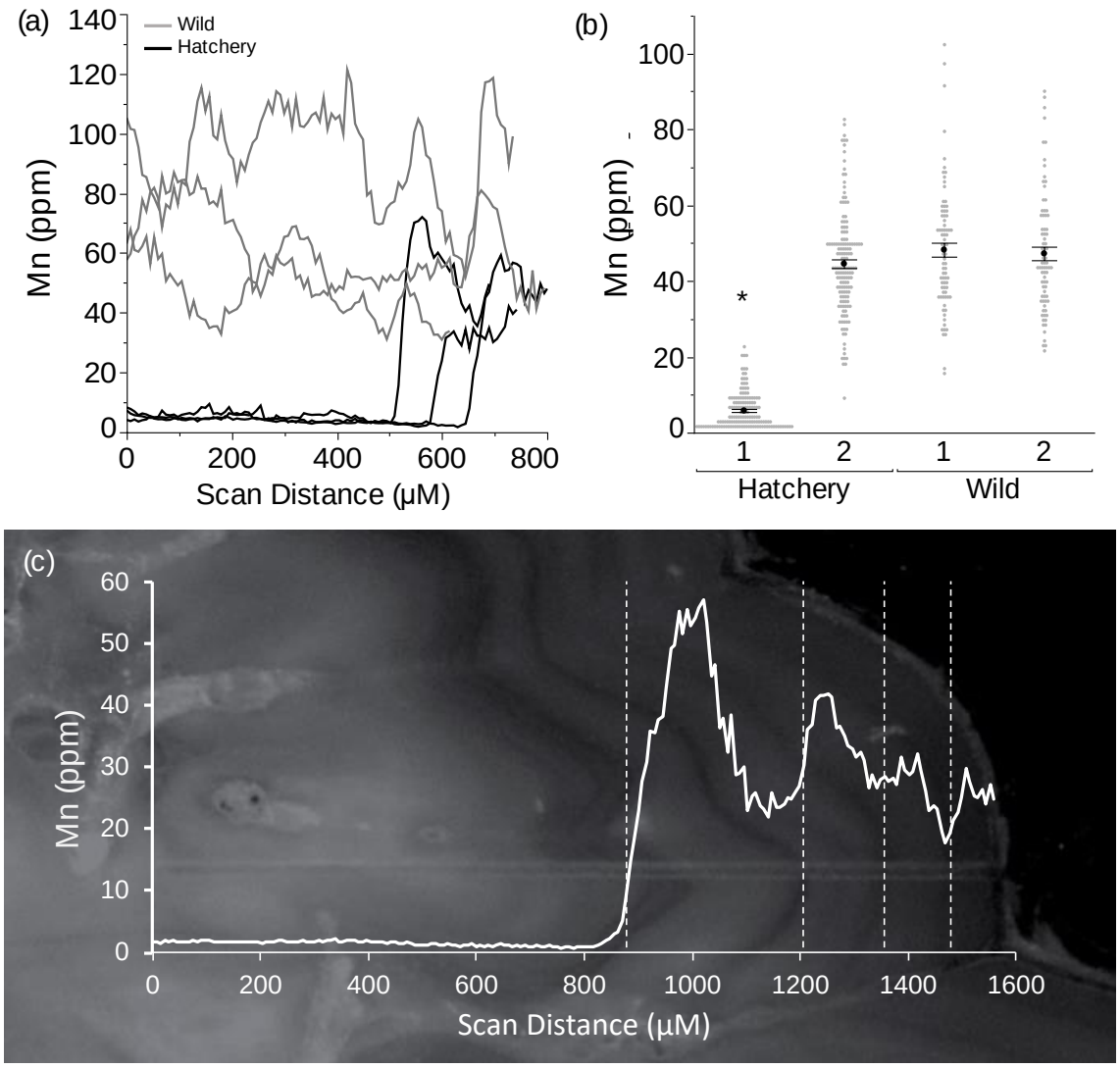

Figure 5.3. (a) Scan profiles for Mn concentrations (ppm = µg/g) in the fin rays of three putative wild Lake Sturgeon (gray lines) and three known hatchery-released Lake Sturgeon (black lines) caught in Stephens Lake, Manitoba. The sharp increases in Mn concentration in the hatchery profiles (~500–650 µm) correspond to the time when individuals were released from the Grand Rapids Fish Hatchery, Manitoba. (b) Mean (± s.e.m) Mn concentrations in the first and second growth bands (i.e., 1 and 2) from known hatchery-released and putative wild Lake Sturgeon caught during annual surveys at various locations in Manitoba are shown. Gray points represent the average Mn concentrations of individual fin rays and are jittered on the x-axis for visualization (hatchery: n = 149; wild: n = 79). The asterisk indicates that the mean Mn concentration is significantly different from other values ($P < 0.05$). Mean fin ray Mn concentrations within the first growth band were significantly different between fish sources (i.e., known hatchery and putative wild; Wilcoxon signed-ranks test: $Z = 8.85$, $P < 0.0001$); concentrations were also

significantly different between the first and second growth bands of recaptured known hatchery fish, representing signatures that accumulated before and after release from the hatchery ($Z = -11.60$, $P < 0.0001$). (c) The scan profile for Mn along the fin ray ablation transect from a 5-year-old, known hatchery-released Lake Sturgeon captured in Pipestone Lake, Manitoba, is depicted. The distinct Mn signature accumulated during the hatchery rearing period within the first growth band (0–800 μm) coincides with when this individual was released from the hatchery. The persistence of the natural hatchery mark was clearly identifiable even 4 years after the fish was released from the hatchery. Dashed vertical lines represent the leading edge of each growth band.

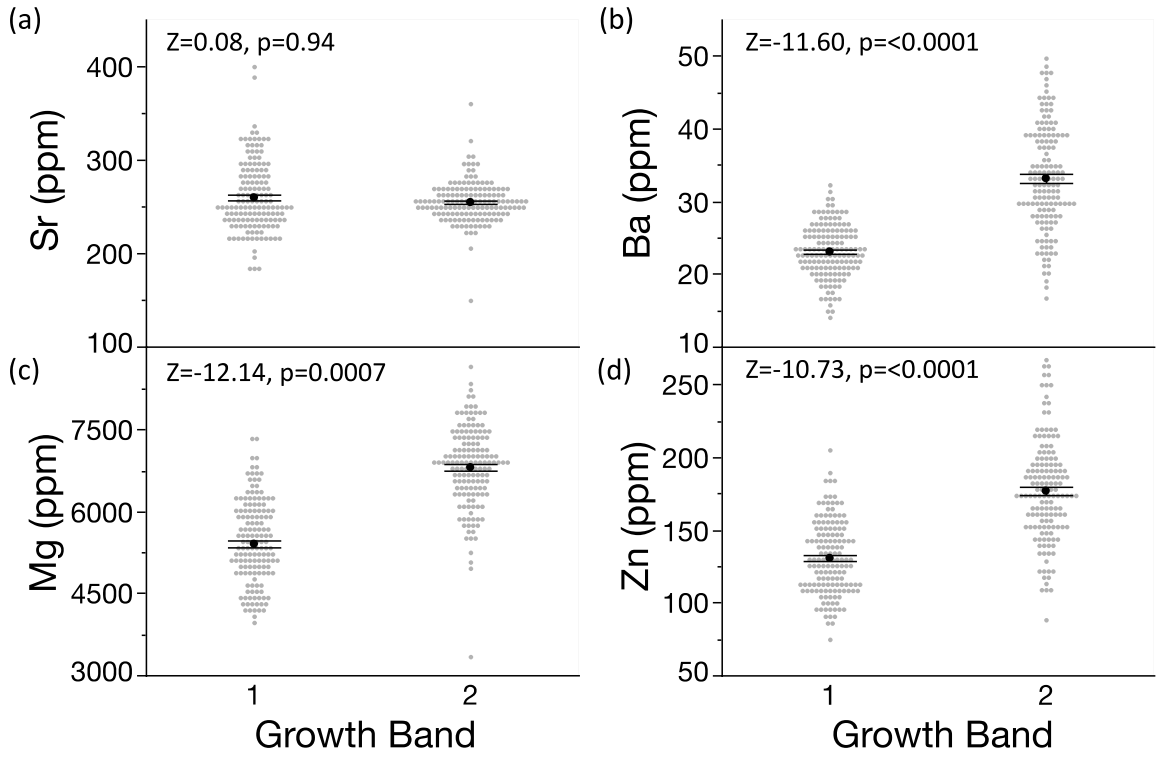

Figure 5.4. Mean (± s.e.m) fin ray concentrations (black points; ppm = µg/g) of (a) Sr, (b) Ba, (c) Mg, and (d) Zn in the first and second growth bands (i.e., 1 and 2), representing signatures accumulated before and after release from the Grand Rapids Fish Hatchery, for known hatchery-released Lake Sturgeon caught during annual surveys at various locations in Manitoba. Gray points represent the average elemental concentrations of individual fin rays and are jittered on the x-axis for visualization (n = 149). Statistical results of Wilcoxon signed-ranks tests examining differences in mean fin ray trace element concentrations between the first and second growth bands are shown; P-values less than 0.05 indicate significant differences.

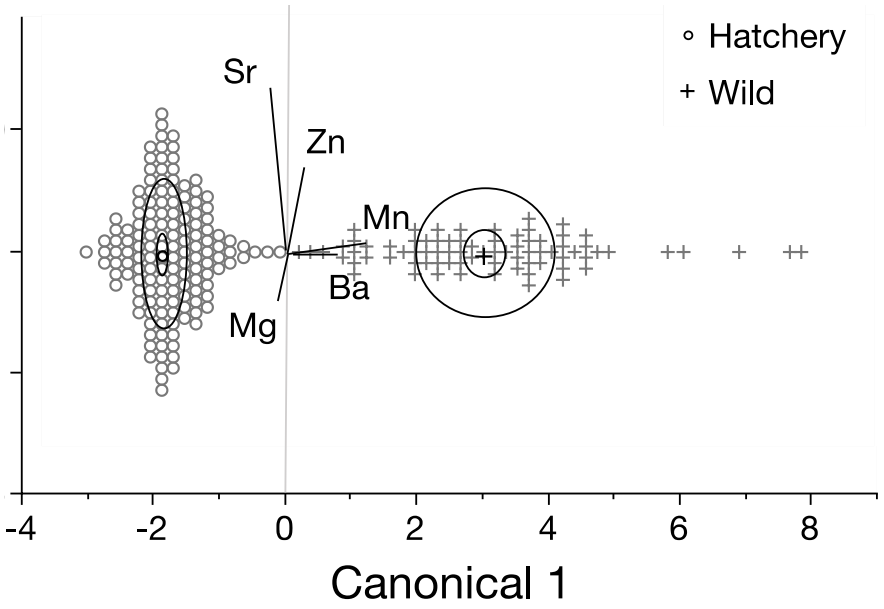

Figure 5.5. Canonical plot for the quadratic discriminant function analysis grouping Lake Sturgeon from all sampling locations into source (i.e., predictor variables; hatchery or wild) based on combined concentrations of Sr, Ba, Mg, Mn, and Zn, forming "elemental signatures." Because there are only two predictor categories (wild and hatchery), there is only a single canonical variable; however, to highlight data distributions, points are jittered on the y-axis. Gray symbols represent average elemental signatures of each individual from the hatchery source (open circles) and wild source (plus [+] symbols). Symbols in black represent the overall mean from each source, with the inner ellipses representing the 95% confidence intervals and the outer ellipses representing the 50% contours, containing approximately half of the observations of each predictor variable. Standardized scoring coefficients (black lines) represent the canonical weight of each element. Position on the x-axis represents the degree of association corresponding to canonical 1 (Sr = −0.29; Ba = 0.47; Mg = −0.09; Mn = 0.90; Zn = 0.18).

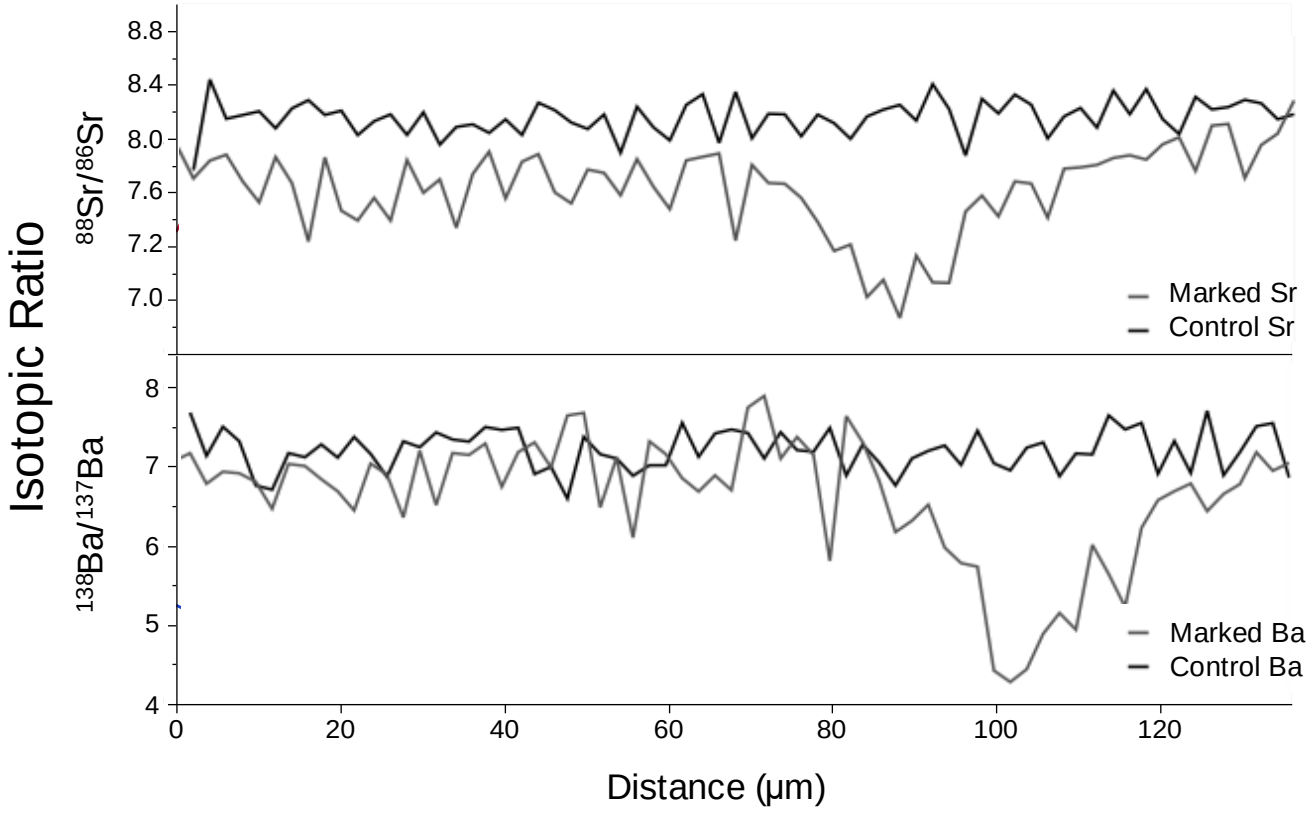

Figure 5.6. Strontium (Sr) and barium (Ba) scan profiles from a juvenile Lake Sturgeon that was first immersed in water spiked for 24 h with known concentrations of $^{86}SrCO_3$ at 100 d post fertilization (dpf) and again in $^{137}BaCO_3$ at 130 dpf (grey lines) compared to an individual in the control treatment that was not exposed to any isotope (black lines).

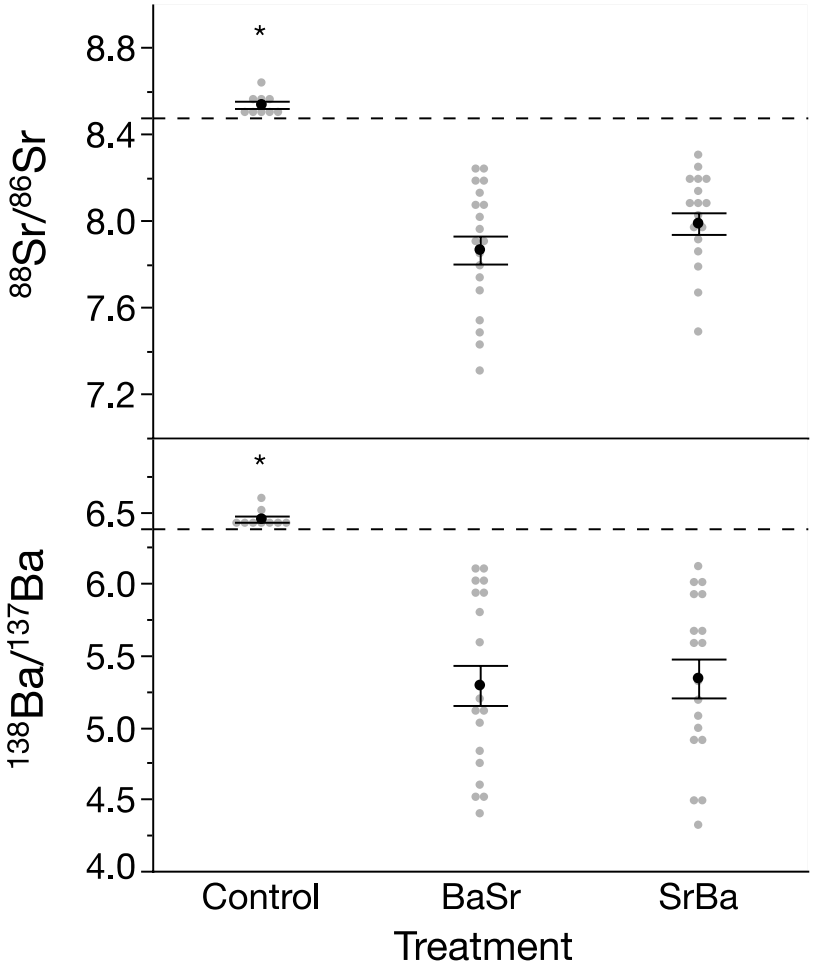

Figure 5.7. Average (± s.e.m) isotopic ratios (black points) across the entire scan line for pectoral fin rays sampled from Lake Sturgeon reared in ambient water (control) and ambient water spiked for 24 h with known concentrations of either 137BaCO3 or 86SrCO3 at 100 d post fertilization (dpf) and again in the opposite isotope at 130 dpf (i.e., BaSr and SrBa treatments). Gray points represent average elemental concentration of individual fin rays and are jittered on the x-axis for visualization (n = 45). The dashed lines represent the natural 88Sr/86Sr and 138Ba/137Ba ratios in freshwater. Asterisks indicate average 88Sr/86Sr or 138Ba/137Ba ratios that are significantly different from other values ($P < 0.05$).

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Chapter 6: Thesis Conclusions and Synthesis

6.1 Thesis Summary

My thesis sought to improve our understanding of the effects the environment has on the processes involved in the elemental pathway from initial uptake to crystallization in the calcified hard structures of sturgeon. Secondly, with these data we would improve the ecological inferences we are able to make from these life history recorders. Three critical questions were central to the theme of this thesis: 1 – What are the physiological and physical processes involved in the biomineralization of hard structures, 2 – How does the environment influence these processes, and 3 – In what ways can we use this knowledge to make improved inferences on the ecology of fish? By applying an integrative and multidisciplinary approach to investigating these questions, I was able to provide a holistic view of how we can interpret chemical signatures that are deposited in the hard structures of Lake Sturgeon, *Acipenser fulvescens*, to make more informed predictions of lifelong habitat use.

Specifically, I described uptake of strontium (Sr) into larval Lake Sturgeon, in particular examining how the ambient environment influences the kinetics of Sr incorporation and deposition in calcified hard structures (Chapter 2). This was only the second time the process of Sr uptake kinetics had been examined in fish, and the first time in an acipenserid, providing important empirical data to support the use of Sr as an indicator of environmental change in fish hard structures. I also experimentally examined the crystallization of otoliths in two species of sturgeon providing novel information on how both intrinsic (ontogeny) and extrinsic (environment) variables influence the phenotypic plasticity of otolith crystallization (Chapter 3). This was the first time the combined effects of pH, temperature, and ontogenetic development have on otolith biomineralization have been quantitatively tested in any freshwater fish species. The validity of microchemistry techniques, examining combined elemental signatures recorded in fin rays, was then assessed to determine the efficacy of this technique to retrospectively determine habitat use of individuals (Chapter 4). By utilizing multivariate models to assess habitat use in juvenile and adult Lake Sturgeon of both known and unknown origin, I was able to significantly increase the predictive capacity of microchemistry techniques, compared to examining elements individually, corroborating significant shifts in fin ray signatures and provide meaningful spatial and temporal information on the movements of fish. Finally, the application of microchemistry analysis for identifying hatchery-stocked versus wild-spawned Lake Sturgeon was investigated (Chapter 5). Here I determined that fin ray microchemistry has the capacity to successfully identify stocked fish with 99% accuracy significantly increasing our confidence in identifying hatchery fish using biological tags. Importantly, results from these studies will aid in interpreting the life history of Lake Sturgeon,

particularly during early life history stages, improving fishery management regimes and environmental protection plans for this culturally important species.

6.2 Thesis Discussion

Microchemistry studies operate on the assumption that elemental chemistries in the environment are transferred to the calcified hard structures of fish, such as otoliths and fin rays, where they are retained throughout the lifetime of the individual providing a chronological record of environmental life history. The availability of trace elements in the environment depends greatly on the geologic make up and hydrology of the habitat (Pracheil et al. 2014). As well, anthropogenic input from runoffs or bedrock disturbances can influence the concentration of elements and isotopes, all of which contribute to overall water chemistry profiles (Friedrich and Halden 2010). This spatial heterogeneity in water chemistry is a critical factor in determining whether habitat specific signatures in the hard parts of fish can be used to retrospectively determine the environmental life history of individuals. Additionally, environmental variables including, but not limited to, temperature and/or pH can influence both the solubility of elements in the environment and the physiological mechanisms involved in both the uptake and biomineralization of these calcified structures, highlighting the complexity of the elemental pathway from incorporation to deposition (Campana 1999). As such, it is important to have a thorough understanding of these mechanisms in order to make the most accurate inferences possible on the environmental life histories of individuals using elemental signatures in hard structures.

6.2.1 *Processes of Uptake and Biomineralization*

Elements are building blocks required by all living organisms and, thus, their homeostatic regulation is crucial to ensure the survival of individuals (Hazel 1993). The pathway of elements from the environment to biomineralization of hard structures requires a series of regulated barriers that involves transfer from the aqueous environment into the blood plasma, followed by the partitioning of elements to tissues, and finally crystallization on the surface of calcified structures (Campana 1999). Each barrier is controlled by physiological mechanisms that are both specific to the element and the organism. Until now, the examination of the pathways of elemental incorporation of non-essential elements, to the biomineralization of hard structures, had not been described in depth in a sturgeon species.

For freshwater fishes like Lake Sturgeon, movement of most elements into and out of the fish occurs across the gills. As a non-essential element, strontium (Sr^{2+}) is not required for biological function and, as such, specific transport mechanisms do not exist to selectively uptake this ion from the

environment. The inverse relationship of Sr^{2+} uptake to ambient concentrations of calcium (Ca^{2+}) provides strong evidence that Sr^{2+} is taken in through epithelial Ca^{2+} channels (ECaC) in larval Lake Sturgeon (Chapter 2). Importantly, the essential nature of Ca^{2+} for biological function, such as the biomineralization of calcified structures, resulted in a higher affinity for Ca^{2+} over Sr^{2+} and, thus, the competitive inhibition of Sr^{2+} at uptake sites. The ability of Sr^{2+} to be taken in through Ca^{2+} transport channels is attributed to the chemical similarities of these elements including valence charge and atomic radii (Chapter 2). As such, other non-essential elements of similar chemical properties (e.g., Ba^{2+}, Pb^{2+}) are likely to share parallel uptake mechanisms. Because the direct homeostatic control over these ions does not exist, these elements tend to be more reflective of variation in the ambient environment, which lends to their utility for interpreting habitat specific elemental signatures.

Once incorporated, the partitioning of elements to different tissues results in varying proportions of elements dependent on the function and/or demand of the tissue. For mineralized hard parts, Ca^{2+} provides the elemental backbone of the calcium carbonate ($CaCO_3$) or hydroxyapatite ($Ca_5(PO_4)_3(OH)$) matrix of otoliths and fin rays, respectively. As such, demand for Ca^{2+} in these tissues is high and the partitioning of Ca^{2+}, and Ca^{2+} like elements, tends to reflect this. When the partitioning of Sr^{2+} in the tissues of larval Lake Sturgeon was examined, preferential accumulation of the element was observed in the fin rays and was inversely related once again to ambient Ca^{2+} concentrations (Chapter 2). This knowledge instills further confidence that Sr^{2+} concentrations recorded in fin rays are reflective of subtle changes in environmental chemistries and suggests Sr^{2+} signatures can be used to make ecological inferences on habitat use.

The final step in the elemental pathway occurs at the calcifying surface of biomineralized structures. Despite the widespread use of otoliths in fisheries research, there is a disparate gap in the understanding of the mechanisms involved in otolith biomineralization compared to the number of studies that have utilized these structures for practical purposes (e.g., species identification, habitat use). Examining otolith polymorph composition (i.e., aragonite, vaterite, calcite) during larval development provided for the first-time information on the initial seeding of sturgeon otoliths (Chapter 3). Made up of acidic amino acids and glycoproteins, the organic matrix plays a key role in determining the shape and growth of crystallites by providing a three-dimensional scaffold. The observed similarities in otolith polymorph variation between both Lake Sturgeon and White Sturgeon (*A. transmontanus*), with distinctive shifts coinciding with the transition to exogenous feeding during early ontogeny, suggests there is intrinsic genetic control over otolith crystallization in sturgeon (Chapter 3). Despite key proteins

involved in otolith formation having been identified in several teleosts, the equivalent mechanisms in acipenserids have yet to be identified. Given the phylogenetically distant status of sturgeons, identifying the molecular mechanisms involved in the synthesis of matrix forming proteins in this group of fish could offer new insights into the evolution of otoliths among more modern fishes and potentially higher vertebrates.

6.2.2 *Environmental Influences*

As ectotherms, fish generally lack the physiological mechanisms to regulate their internal temperature and, as such, the rate at which many of these mechanisms occur is typically directly related to the ambient temperature of the environment. This is particularly true for the kinetics of enzyme reactions where an increase in temperature will also increase the rate of the reaction. Indeed, the kinetics of waterborne Sr^{2+} in larval Lake Sturgeon was positively correlated with temperature whereby both initial Sr^{2+} uptake and accumulation in fin rays increased when fish were exposed to higher environmental temperatures (Chapter 2). Furthermore, given temperature increases the solubility of elements in the environment, and thus their availability to fish and other aquatic organisms, the combination of both increased substrate and uptake should be considered when making inferences on habitat use. This is beneficial if intentional marking of fish using stable isotopes is attempted as increasing the temperature in the environment can reduce time and concentrations of isotopes required to instill an identifiable mark (Chapter 5). As a result, the amount of isotope and associated cost required to mark groups of fish is significantly reduced.

The process of biomineralization is also impacted by changes in the ambient environment. Given $CaCO_3$ crystallization is driven by the change in free energy required to transition from a supersaturated solution to a crystal, both temperature and environmental pH have the ability to alter supersaturation states leading to changes in the precipitation of different polymorphs (Chapter 3). As climate change continues to impact aquatic environments, it is important to consider how future changes in temperature and CO_2 will influence the crystallization of fish otoliths. Having evolved some 200 million years ago, sturgeon have become particularly well adapted to fluctuating environments. Despite this, prolonged exposure to elevated temperature, but not pH, induced the precipitation of relatively large single calcite crystals, the density of which is greater than the predominate vaterite polymorphs observed in most adult sturgeon otoliths (Chapter 3). The impacts changes in otolith $CaCO_3$ polymorph precipitation have on the functionality of these critical structures in sturgeons, however, remains unknown. If otolith density alters the oscillation and amplitude of otoliths, changes in otolith polymorph composition may have

adverse effects on the behavior of fish by altering sound perception and/or orientation abilities. This should be an important consideration for conservation hatcheries as fish reared in these settings are often exposed to increased temperatures and enhanced diets in an effort to increase growth rates prior to stocking.

Changes in otolith polymorph composition potentially have important implications for interpreting elemental signatures in otoliths as well. Each polymorph varies in crystal lattice structure and, thus, the density and Ca^{2+} per unit volume of each polymorph also varies with aragonite being the most dense and vaterite being the least. As a result, changes in otolith density in relation to the different polymorphs results in higher affinity for trace elements of similar chemical properties such as Sr^{2+} and/or Ba^{2+} to be substituted for Ca^{2+} in the $CaCO_3$ matrix of otoliths. This may be exacerbated by increased uptake of these elements, particularly in environments of elevated temperature where increased temperature also induces the precipitation of abnormal otoliths (Chapter 2,3). At present, studies investigating otolith chemistry typically assume polymorph composition is homogeneous across the entire otolith, which may result in the erroneous interpretation of habitat movements if areas of abnormal otoliths are scanned without also considering polymorph composition. Further, examination of otolith formation and polymorph composition in fishes should be quantified in order to ensure interpretations of elemental concentration analyses in relation to habitat movements are accurate.

6.2.3 *Ecological Inferences*

Data presented here have increased our understanding of element uptake and deposition in fish hard structures, which can be used to make improved inferences on the environmental life history of Lake Sturgeon using elemental signatures recorded in the fin rays of juvenile and adult fish. Examination of a robust reference collection of fin rays sampled from Lake Sturgeon of both known and unknown life histories provided a unique opportunity to validate this technique in a field setting (Chapter 4,5). The success of this validation was largely due to the heterogeneity of the water chemistry in the study regions (Chapter 4). Despite the relatively small spatial scales, significant differences in elemental profiles could be distinguished in all areas of interest, which can be attributed to the variable bedrock composition and hydrology among these habitats. Most prominently were the differences in water chemistry profiles observed between the ground water source that supplies the Grand Rapids Fish Hatchery (GRFH) and the surface water chemistry where fish were stocked (Chapter 5). In particular, manganese (Mn) concentrations were substantially lower in the hatchery water due to redox reactions occurring in the groundwater source where oxygen levels are reduced. This resulted in markedly low Mn

profiles in fin rays (<10 ppm) that could be visually identified in the raw scan line data and was consistent with hatchery residency times allowing for the classification of hatchery versus wild-spawned fish with 99.1% success (Chapter 5). This biologically induced tag provides an alternative option for reliably identifying hatchery-stocked fish, which will ultimately aid in assessing the success of Lake Sturgeon restocking programs in Manitoba.

Importantly, this distinctive hatchery signature that consistently aligned with the initiation of the first growth band allowed for the definitive confirmation that differences in elemental signatures recorded in fin rays could be attributed to both spatial and temporal changes in habitat use (Chapter 4,5). To further justify this, elemental signatures of recaptured hatchery-stocked fish, and thus individuals with known life histories, were compared to those of wild-spawned individuals. When patterns of elemental differences were examined separately, variability among sampling sites made inferences on habitat use difficult to interpret. Because elements do not exist independently in the environment, and interact with one another during both initial uptake and deposition (Chapter 1), combined elemental signatures were assessed to determine whether significant shifts in elemental profiles corresponded to known changes in habitat residency (Chapter 4). The increased predictive capacity that was attained by comparing combined elemental profiles, rather than individual elements, highlights the efficacy of this technique for examining lifelong habitat use in migratory fishes.

The success of this technique for interpreting the environmental life history of fishes provides fisheries researchers and managers an opportunity to investigate the ecology of sturgeons that has traditionally been difficult to determine given the long lifespan and logistical challenges of conducting monitoring programs in remote habitats. This is particularly valuable when identifying natal rearing environments or assessing important nursery habitats at vulnerable early life history stages. Most physical tagging techniques come with substantial costs and require the individual handling of each fish and ultimately only provide a snapshot of information during the period of which the fish has been outfitted with the tag. Given fish are constantly taking in elements and depositing them in their hard structures, these biological tags provide the opportunity to retrospectively assess habitat use across the entire lifespan. Further, the efficacy of this technique using pectoral fin rays over otoliths offers the added benefit of being able to acquire samples non-lethally, which is important when examining species that are considered to be at risk. Ultimately, this technique could be used to improve fishery management regimes and environmental protection plans not only for Lake Sturgeon in Manitoba, but any migratory fish species that resides in, and moves between, areas of variable water chemistry.

6.3 Concluding Statements

Results from these studies provide an overall improved understanding of the elemental pathway from initial uptake to deposition in hard structures of fish. Understanding the underlying physiological mechanisms involved in elemental incorporation and biomineralization, and how they are influenced by environmental conditions, will allow for improved interpretation of elemental signatures deposited in these life history recorders. Specifically, this research will lead to more informed management strategies for the conservation of Lake Sturgeon, not only in Manitoba, but throughout their North American range. By incorporating techniques from multiple scientific disciplines, including physiological flux studies, physical determination of crystal structures, ecological modeling, and applied marking techniques, this thesis provides a significant contribution specifically to fisheries research and population management. Broadly, this research offers contributions to advancements in scientific knowledge related to fish biology on multiple levels by providing a mechanistic understanding of elemental uptake and deposition to decipher ecological information on a macroscopic scale.

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