The Use of Otolith Micro-chemical Techniques to Examine Trace Element Residence Time, Migration, and Population Discrimination of Teleost Fishes in the Canadian Polar North

By

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ABSTRACT

Studying calcium carbonate (otoliths) and calcium phosphate (fins, scales, bones) hard structure chemistry has numerous applications in the fisheries field for both freshwater and marine environments. The overall thesis objectives were: 1.) to provide an integrated and multidisciplinary approach to understanding the incorporation of trace elements and isotopes into biomineralized hard structures, and 2.) to apply this multidisciplinary perspective in the examination of element marking, stock discrimination, and migration in teleost fish species found within the Canadian Polar North. Varying physiological mechanisms within fishes control the uptake of essential and non-essential trace elements and isotopes during biomineralization processes. Essential life elements such as zinc and magnesium are controlled by their own uptake regulation systems whereas non-essential elements such as strontium and barium are controlled primarily by calcium uptake at the gills driven by internal calcium homeostasis. Secondarily, environmental trace elements compete with calcium and with each other for uptake at the gills. The ability of certain hard structures such as bones, fins, and scales to remobilise calcium and associated calcium-like elements, plays a role in the prolonged high concentrations of strontium that were observed in otolith marking of Greenland Halibut, *Reinhardtius hippoglossoides*. High doses of strontium-chloride resulted in a prolonged expulsion of excess strontium. Strong associations of Dolly Varden Char, *Salvelinus malma malma*, with groundwater allowed discrimination of populations among studied river systems using otolith strontium and barium, and strontium isotopes. Calculation of otolith strontium freshwater baselines allowed for a quantitative method to examine migration histories of Arctic Char, *S. alpinus*, in Canada

and western Greenland. Migration seaward was related to ease of access to estuary and marine habitats. Easy access to estuaries resulted in migration at a young age and small size whereas longer rivers resulted in a delay of migration to older ages and larger sizes. Understanding the role of fish physiology in association with calcium homeostasis provided a stronger basis for understanding the incorporation and presence of trace elements and isotopes found within biomineralized hard structures. These studies underscore the utility of microchemical studies for elucidating biological phenomena, thus linking the aspects of biology, physiology, and geology.

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Chapter 1

Dissertation Introduction

1.1 General Introduction

This thesis is developed as an interdisciplinary bridge between several fields of research including fish biology, fish physiology, biomineralization, environmental chemistry, geological sciences, and otolith microchemistry. Otoliths are biologically made mineralised structures that share traits with geological crystals. Otoliths incorporate environmental information relevant to biological aspects of fish life histories, thus recovery of such information may provide insights to key biological phenomena. Commonality between otoliths and crystals and the processes by which they are formed links biological and geological sciences. The approach used herein advances our current state of knowledge in the examination of elements and isotopes in biologically made mineral structures. Linkages to the relevant disciplines are provided throughout the thesis and specifically in this introductory chapter. Further, an overall approach is described and a structure and organization is provided for the thesis.

1.2 Otolith Biomineralization

Advances in research techniques allow us to measure environmental change and changes in the biodemographics of species more readily than ever before. Often we can infer environmental parameters (past and present) experienced by aquatic species through examination of biologically derived minerals (biominerals) that have a calcium-based origin (Mann 2001, Bäuerlein 2007). Biominerals often occur in the form of teeth, bones and most significantly for this work as otoliths or “ear stones” in fishes. Biominerals incorporate trace elements and associated isotopes into their calcium carbonate or calcium phosphate structures (Mann 2001, Bäuerlein 2007). Once

incorporated into an otolith, elements remain unchanged over time (Mann 2001, Bäuerlein 2007). For example, the discovery of archaeological biominerals has allowed for reconstructions of ocean temperatures from past climatic regimes (Grimes et al. 2003, Surge and Walker 2005, Kastelle et al. 2011). Presently biominerals, specifically otoliths and associated trace elements and isotopes, have been applied to understanding migratory movements, thermal habitat preferences of juvenile fish species, diets, and fisheries stock structuring (Campana 1999, De Pontual and Geffen 2002, Allemand et al. 2008). The incorporation of elements and isotopes into biominerals is intrinsically tied to fish physiology (Sturrock et al. 2014, 2015, **Chapter 2**). The functions of the trace elements in life processes (Crichton 2008, **Chapter 2**), and differences among ambient environments (Allemand et al. 2008, **Chapters 2 and 3**), both allow for great possibilities to understanding habitat uses and migratory behaviours in fish species (**Chapters 4 and 5**).

1.2.1 *Otolith Function and Formation*

Otoliths exist as paired structures found within the midbrain region of teleost fish. They function as balance and sound detection systems in teleost fish similar to those of otoconia found within the human ear/hearing system (Popper et al. 2005, Lundberg et al. 2006). Specifically, otoliths are used to maintain equilibrium and excite nerve cells sensitive to pressure, gravity, angular movement and sound vibration. Three semi-circular canals each hold paired otolith structures named asteriscus, sagitta, and lapillus (Popper et al. 2005). Otolith formation is acellular; each otolith structure is encompassed in an endolymphatic sac (utriculus, sacculus and lagena respectively)

and each otic sac holds an otolith oriented over kinocilia of the macula, fixed by the otolithic membrane into which the kinocilia project (Popper and Lu 2000, Wright et al. 2002, Popper et al. 2005).

All otoliths continually grow (i.e., accrete new calcium-carbonate material) during the lifetime of a fish, exhibit an absence of reabsorption, and incorporate elemental and isotopic signals from the surrounding ambient environment and/or diet (Panfili et al. 2002, Allemand et al. 2008). Variation in annular and daily accretions, likely due to changes in the underlying organic matrix, provides a time line that allows for age estimation (via alternating translucent and opaque zonation) and a time series for the analysis of trace element and isotope incorporation.

Otolith formation is a multi-stage process involving compartmentalization (i.e., extracellular-endolymphatic sac) and highly controlled regulation of ion pathways (Ibsch et al. 2004a, b). Ions initially enter fish through barriers such as gill and intestine epithelia in association with enzymatic activities vital to life processes (Marshall and Grosell 2006, Crichton 2008) such as osmoregulation and calcium homeostasis (Chowdhury and Blust 2001). Once internal to the fish body, ions are then able to enter the blood and influence transport mechanisms involved in internal ionoregulation. The otolithic epithelium and endolymph serve as direct control mechanisms of ion transport for biomineralization of the otolith. The otolith itself once formed is composed of >90% calcium carbonate and ~0.01-10 % organic components and trace elements (Borelli et al. 2003a, Payan et al. 2004a, b).

Three different polymorphs of calcium carbonate minerals exist as biominerals: calcite, aragonite, and vaterite, all of which have the same composition (Allemand et al.

2008). Calcite and aragonite are the two most thermodynamically stable structures and are deposited most extensively as biologically derived minerals (Mann 2001, Allemand et al. 2008). Calcite should be favoured for deposition due to the ease of physiochemical precipitation of the crystal morph (Takagi 2002, Allemand et al. 2008). However, the aragonite crystal morph is most commonly deposited in teleosts indicating biological control over mineral polymorph deposition (Allemand et al. 2008, Tohse et al. 2009). The vaterite crystal polymorph is most commonly found in asteriscus otolith pairs and occasionally in sagittal otoliths (Takagi 2002). The sagittal otolith formed of aragonite is the most studied of all biologically derived mineral structures due to its generally larger size in comparison to the two other otolith structures (De Pontual and Geffen 2002).

Aside from biological processes, geochemical conditions must be considered in mineral formation such as the saturation coefficient (S_a) of the ions (Payan et al. 2004a, b, Takagi et al. 2005, Allemand et al. 2008). The equation:

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3$$

describes the ionic formation of calcium carbonate ($CaCO_3$). The solubility product of the crystal (K_{sa}) is significant in this process since S_a is defined by:

$$S_a = \left[Ca^{2+}\right] \bullet \left[CO_3^{2-}\right] \div K_{Sa}$$

When S_a is >1 the endolymph is supersaturated with ions and calcium carbonate can precipitate spontaneously, whereas S_a <1 results in no precipitation of calcium carbonate (Payan et al. 2004a, Allemand et al. 2008). Within teleosts, endolymph is generally known to be a highly supersaturated fluid with a S_a of ~2-3 (Payan et al. 2004a, Allemand et al. 2008) suggesting precipitation of calcium carbonate can occur

readily. Approximately 8 times more calcium is required in otolith calcification than is presented in the contents of the endolymph pool during mineralization, suggesting continuous mechanisms to re-supply the endolymph to a super-saturated state (Allemand et al. 2008).

Inorganic carbon predominantly exists as bicarbonate (HCO_3^-) under the normal pH of biological fluids (7-7.5) and can influence calcification in biological systems. Thus this equation best represents calcification in the otolith:

$$Ca^{2+} + HCO_3^- \longrightarrow CaCO_3 + H^+$$

The extra H^+ ion must be actively removed for mineralization to occur, influencing the calcification process (Payan et al. 2004a, Allemand et al. 2008).

The mechanism for transport of calcium ions (Ca^{2+}) into the endolymph is thought to occur via paracellular channels with passive movement across the saccular epithelium (Payan et al. 2002, Ibsch et al. 2004a, b) although other mechanisms of transcellular active transport have also been suggested (Tohse and Mugiya 2001). Calcium precipitates at junctions between macular epithelial cells supporting paracellular transport mechanisms (Payan et al. 2002, Ibsch et al. 2004b).

The activities of calcium ions (Ca^{2+}) depend on total concentration in the endolymph, pH of the fluid, nature and concentration of binding proteins and the presence of competing elements and inhibitors such as magnesium ions (Mg^{2+}) (Payan et al. 2002, Borelli et al. 2003b). Alternatively, bicarbonate (HCO_3^-) concentrations appear to be controlled by transporters (Cl^-/HCO_3^- exchangers and anhydrase) from blood and proton elimination (Na^+/H^+ exchangers) to blood by saccular epithelial cells (Mann 2001, Allemand et al. 2008).

Models for mineralization suggest that there are three compartments that need consideration. First is the epithelium. It must synthesize and secrete organic precursors and regulating compounds directly on the mineralization site (Mann 2001, Allemand et al. 2008). The epithelium must ensure a supply of the ionic precursors at sufficient concentrations as well as being able to actively remove hydrogen ions (H^+) during calcium carbonate formation (Ibsch et al. 2004a, b). Second is the pericrystalline fluid (endolymph) in which the components for biomineralization exist (Mann 2001, Allemand et al. 2008). Finally, the biomineral itself is formed in three-dimensional organization of the organic matrix in which the calcium carbonate crystals become embedded (Mann 2001, Allemand et al. 2008). Secretion of protein precursors into endolymphatic fluid is energy demanding and hypothesized to have links to fish metabolism (Hüssy 2008).

1.2.2 *Otolith Organic Matrix*

The organic components are the underlying matrix upon which calcium carbonate is deposited in biological derived minerals, a distinguishing characteristic in comparison to environmentally formed minerals. This underlying biologically formed matrix is continuous over the entire mineral surface and influences trace element incorporation, isotopic discrimination, nucleation, orientation inhibition, crystal nature, growth regulation, and the structure and morphology of the forming mineral (Mann 2001, Allemand et al. 2008). Much control over calcification is thought to occur within the organic matrix with glycoproteins, proteoglycans and glycolipids playing roles in mineral formation (Söllner et al. 2003, 2004).

The organic matrix in otoliths is made up of approximately 50% proteins, 30% proteoglycans and 20% collagens (Allemand et al. 2008). The organic matrix itself is complex containing both fully derived proteins relevant for deposition as well as protein precursors that are modified during calcification of the otolith. Several proteins, generally having increased proportions of acidic residues, have been identified as playing significant roles in calcium carbonate deposition (e.g., otolin-1 and starmaker) (Borelli et al. 2001, Söllner et al. 2003, 2004). The major protein components of the organic matrix are otolin-1, starmaker and OMP-1 (Murayama et al. 2002, 2005). On a daily scale, only a small fraction of organic precursors in the endolymph are used in the matrix formation of the underlying lattice of otolith growth thus a constant excess of daily requirements for mineralization is available in the endolymph (Allemand et al. 2008).

1.2.3 *Spatial Distribution of Endolymph Components*

Mineral growth is thought to take place at the proximal surface of the otolith adjacent to the sensory epithelium (Beier et al. 2004, Ibsch et al. 2004a). The surrounding endolymph from which mineralization occurs is composed of an acellular medium secreted by the saccular epithelium (Ibsch et al. 2001, Payan et al. 2002). The proximal and distal zones relative to the orientation of the otolith show a lack of uniformity in endolymph composition in proteins such as proteoglycans, collagen and ionic parameters (Ibsch et al. 2001, Payan et al. 2002).

The gradient of organic components in the endolymph suggests spatial organization of otolith growth along determinate axes (Allemand et al. 2008). The proximal region of the endolymph is composed of macula and large ionocytes arranged

in a meshwork around the macula and faces the proximal otolith face; it is significant for the incorporation of organic compounds (Allemand et al. 2008). The distal region of the endolymph is composed of squamous (flat scale-like) cells and small ionocytes and surrounds the distal zone (sulcus side) of the otolith (Allemand et al. 2008). The proximal zone facing the macula corresponds to the concave shape of the otolith due to the greater calcium carbonate growth rate than on the distal side (convex) (Payan et al. 2004a). Correspondingly, factor-retarding compounds (FRC, i.e., inhibitory proteins) are highest in regions of greatest calcium carbonate growth (proximal zone). The proximal region of the otolith endolymph is known to be more acidic than the distal region containing higher levels of proteins, phosphate and magnesium (Payan et al. 2004a). In the distal region of the otolith endolymph, potassium and total carbon dioxide (CO_2) levels are found to be in higher concentration.

The carbonic anhydrase (CAH) enzyme, thought to be involved in otolith calcification is not distributed evenly on the otolith surface (Beier et al. 2006). Top arrangement of CAH-rich areas in saccular macula may be responsible for calcification of areas of sagittae centrifugally and centripetally from the sulcus region (sulcus forming) (Beier et al. 2006).

1.2.4 *Trace Element Incorporation*

To exist, all life forms require elements and the formation of organic and inorganic compounds. In total about 25 elements are required by living organisms (Crichton 2008). Essential elements (to form carbon containing compounds) include carbon, nitrogen, hydrogen, oxygen, phosphorous and sulphur (Crichton 2008). Elements such

as sodium, potassium, calcium and magnesium are essential for life processes such as ionoregulation, ion gradients, and structural and redox reactions (Marshall and Grosell 2006, Crichton 2008). Other elements such as cobalt, copper, zinc, and iron exist in trace levels within living forms and are dietary requirements (Crichton 2008). Zinc, specifically, plays an important role in enzyme functions (Lee et al. 2015) and has a catalytic role in association to carbonic anhydrase (Eide 2006, Crichton 2008)

Biological use of elements is important in the biomineralization process since their physiological regulation and purpose to a fish are significantly different, and thus they may exist in concentrations higher or lower than ambient environmental levels (Bäuerlein et al. 2007, Sturrock et al. 2015). Elements such as sodium, chlorine and potassium are all used in osmoregulation processes, in the formation of ion gradients, and in active ion-removal pumps (Crichton 2008). The endolymph, for example, has higher concentrations of potassium [K+] and lower concentrations of sodium [Na+] compared to other bodily fluids such as plasma (Allemand et al. 2008). These essential elements may be incorporated into the otolith in association with the organic matrix (**Chapter 2**) rather than in association with the calcium carbonate minerals. Other elements such as strontium (Sr), lead (Pb) and barium (Ba) are taken up through calcium channels as a result of similar ionic sizes and valences (De Pontual and Geffen 2002, Allemand et al. 2008, Chowdhury and Blust 2001, 2011). Thus, strontium can easily and readily substitute for calcium in calcium carbonate (aragonite) biomineralization processes (Mann 2001, De Pontual and Geffen 2002, Allemand et al. 2008, Omelon et al. 2009). Strontium concentrations in otoliths are found to have a direct relationship to primarily ambient calcium environmental conditions (**Chapter 2**).

The comparisons among trace elements in different crystal polymorphs (i.e., variation in size of the site and bond arrangement and geometry) are invalid. Aragonite favours strontium insertion in replacement of calcium during deposition whereas other elemental substitutions are less stable (Markwitz et al. 2000, Mann 2001, Doubleday et al. 2014). Vaterite favours magnesium insertion (Melançon et al. 2005, 2008, 2009). Strontium, lead and barium are isostructural with aragonite, whereas other trace elements appear to form labile carbonates whose concentrations are influenced by the chemistry of the surrounding endolymph (Markwitz et al. 2000, Mann 2001, Melançon et al. 2005, 2008, 2009, Doubleday et al. 2014). Ingestion of strontium and barium from ambient water and post-ingestion (i.e., excretion, expulsion, and elimination) remain in a stable ratio with calcium, most probably due to calcium-associated enzymes and the inability of paracellular channels to distinguish between calcium and either strontium or barium (Nachshen and Blaustein 1982, Markwitz et al. 2000, Chowdhury and Blust 2001, 2011).

1.2.5 *Trace Elements as Biological Tracers*

Insights into habitat use and life histories for numerous fish species have been examined using calcium-based hard structures such as otoliths analyzed for trace elements and isotopes (Campana 1999, Campana and Thorrold 2001). The most extensive knowledge base regarding hard structure microchemical and isotope analyses is the identification of habitat switching in teleosts (Campana 1999, 2005, Kennedy et al. 2000, Gillanders 2005, Elsdon et al. 2008, Mulfeld et al. 2012). Anadromy (i.e., the movement of fish between fresh and marine waters) is strongly

inferred by the correlation of otolith strontium (Sr) to environmental calcium through the calculation of ratios between strontium and calcium (Brown and Severin 2009). Otolith trace element studies on Chinook Salmon (*Oncorhynchus tshawytscha*), Coho Salmon (*O. kisutch*), Sockeye Salmon (*O. nerka*), Rainbow Trout (*O. mykiss*), Arctic Char (*Salvelinus alpinus,* **Chapter 5**), White Sturgeon (*Acipenser transmontanus*), and Green Sturgeon (*A. medirostris*) consistently infer movement of individuals from freshwater habitats into marine or coastal waters (Veinott et al. 1999, Zimmerman 2005, Allen et al. 2009).

The examination of otolith strontium isotopic ratios of fishes confined within freshwater systems has allowed inference of patterns of habitat switching within watersheds. The success of these techniques is highlighted by studies on Atlantic Salmon (*Salmo salar*) in tributaries of the Connecticut River (Kennedy et al. 2000, 2002), Cutthroat Trout (*O. clarki*) in the Flathead River drainage of the Rocky Mountains (Muhlfeld et al. 2012), Dolly Varden Char (*S. malma malma*) for the North Slope, Yukon and Northwest Territory (**Chapter 4**), and for Chinook Salmon within Bristol Bay, Alaska (Brennan et al. 2015b). Groundwater hydrology and underlying geological conditions play a role in determining local environmental strontium isotopic ratios that are reflected in otolith composition (**Chapter 4**, Hegg et al. 2013, Brennan et al. 2014, 2015a, 2015b).

Migrations of fishes between coastal areas or marine habitats are sometimes observed in marine systems. Otolith and environmental concentrations of trace elements such as strontium in these systems are more complex. Campana et al. (1994) and Tanner et al. (2013) demonstrated differential habitat use during life history of

Atlantic cod (*Gadus morhua*) and Common Sole (*Solea solea*) for marine and tidal estuary regions, respectively. However, a multiple element and isotopic suite was needed as increased difficulty was observed in linking otolith microchemistry to individual water masses within the study area. Additionally, environmental strontium/calcium was found to drive otolith strontium/calcium in anadromous and freshwater species but not in marine species (Brown and Severin 2009).

There is a lack of complete understanding of the connectivity of fish physiological control mechanisms and the presence of substituted trace elements in biominerals (Sturrock et al. 2012, 2014). These observations are inconsistent with theoretical expectations and have led to the consideration of the primary pathways for element uptake and deposition in biominerals. The relationship between strontium deposition in the otolith of fishes, and environmental strontium and calcium, explain the underlying mechanism for this relationship within all freshwater fish (**Chapter 2**). In parallel, this led to investigation of how trace elements (i.e., strontium) are retained after artificial marking events (**Chapter 3**) which connects elevated otolith (i.e., calcium carbonate) strontium post-marking to the remobilisation of calcium and mimicking elements from calcium phosphate hard structures (**Chapter 2**). Limitations for the application of these techniques and inferences made in the life history of fishes are demonstrated in the multidisciplinary approach taken in this thesis (**Chapter 2)**.

1.2.6 *Analytical Instrumentation*

The instruments used for analyzing minerals, trace elements and isotopes are common in geological sciences. Several analytical machines are available to analyze trace

elements and isotopes within otoliths (Reed 2005, Sylvester and Raeside 2001). Specifically, three types of instruments are referred to, and two were used extensively in this research to collect trace element or isotopic information on otoliths: electron microprobe (EMPA), proton induced x-ray emission (PIXE), and laser ablation-inductively coupled plasma-mass spectrometer (LA-ICP-MS). Both EMPA and PIXE are non-destructive X-ray based techniques that require flat, polished surfaces for the best analytical outcomes (Campbell et al. 1995, Reed 2005). In EMPA, an electron beam is used to bombard the sample so X-rays whose wavelengths are characteristic of an element are emitted for quantification (Reed 2005). Detection limits for the EMPA are not as low in comparison to other instrumentation. However, when element concentrations are relatively high in the natural environment and in the elementally dosed fish as observed in the Greenland Halibut (*Reinhardtius hippoglossoides*) marking study (**Chapter 3**), the use of the scanning electron microscopy as part of EMPA for backscatter electron (BSE) imaging and the small beam size for line scans provided optimal results. The BSE images showed variation in the grey scale dependent upon the element concentration studied. To include additional samples in **Chapter 5**, archived PIXE data were used in parallel to LA-ICP-MS analytical outcomes. The PIXE uses an ion beam (in this case protons) to interact with a sample where X-rays specific to an element are measured during atomic interactions (Campbell et al. 1995, Halden et al. 1995). Otolith trace element concentrations collected from PIXE and LA-ICP-MS instruments are shown to be similar (Campana et al. 1997, N. Halden *unpublished data*). LA-ICP-MS is, by comparison with EPMA and PIXE, a destructive technique that can analyze both elements and isotopic ratios (single collector and multi-collector) from

solid-state samples (Sylvester and Raeside 2001). LA-ICP-MS uses a laser beam to ablate the surface of the sample into aerosol-sized particles that are transported to the secondary excitation source of the ICP-MS instrument for ionization (Sylvester and Raeside 2001). The excited ions in the plasma torch are moved to the mass spectrometer for elemental and isotopic analyses (Sylvester and Raeside 2001). LA-ICP-MS has the lowest detection limits of the three instruments (typically 1 ppb) in comparison to the EMPA (100 ppm under ideal conditions) and PIXE (1-10 ppm) (Sylvester and Raeside 2001, Reed 2005, Potts et al. 2012). LA-ICP-MS techniques have been used to analyze otoliths in **Chapters 2, 4,** and **5** of the thesis. Trace element and isotope data were resolved both spatially and across time scales in each chapter to the appropriate levels to provide for the best detection limits and spatial resolution of the data.

1.3 Thesis Objectives and Significance of Research

The overall thesis objectives are: 1.) to provide an integrated, multidisciplinary approach to understanding the incorporation of trace elements and isotopes into biomineralized hard structures and 2.) to apply this multidisciplinary perspective in the examination of element marking, stock discrimination, and migration in teleost fish species found within the Canadian Polar North. In this approach, calcium uptake at the gills of fish, and environmental calcium concentrations relative to calcium homeostasis of a fish are addressed to enhance the understanding of uptake kinetics and competition of trace elements at the environment/gill interface. The new perspective developed in **Chapter 2**, provides insights into the underlying mechanisms of trace elements and isotopes

incorporated into hard structures such as otoliths. The remaining thesis chapters apply this knowledge allowing for stronger quantitative analyses in answering fisheries questions regarding element marking, stock discrimination, and anadromy.

Chapter 2: This chapter provides an integrated and multidisciplinary understanding of trace element and isotope incorporation in calcium carbonate (otoliths) and calcium phosphate (fins, scales, and bones) biomineralization.

New insight into the underlying mechanisms for the presence of trace elements and isotopes in biominerals results from multidisciplinary integration of knowledge. Data provided for the review suggest that trace element incorporation into biominerals is driven primarily by hypo- and hyper-calcemic environmental conditions. Secondarily, the kinetics of competition between environmental calcium and non-essential elements mimicking calcium define what is observed in trace element incorporation into a biomineral structure. Essential elements, on the other hand, have uptake processes that are likely influenced by hormones and enzyme function. Notably, understanding the role of calcium phosphate for remobilising calcium (and calcium mimic elements) through the action of osteoclast-like cells provides insights into isotope fractionation observed in fins, scales and bones but not for otoliths (calcium carbonates). This internal remobilisation of calcium after being deposited in calcium phosphate structures is interconnected to transport kinetics and the retention of otolith strontium in post-marking studies (**Chapter 3**). These new insights into trace element and isotope incorporation allow for increased precision and data interpretation when using trace elements and

isotopes for application in marking (**Chapter 3**), stock discrimination (**Chapter 4**), and inferring migratory patterns of fishes (**Chapter 5**).

Chapter 3: The objectives for this chapter were to characterise strontium in left and right sagittal otoliths of elementally marked Greenland Halibut to: (1) locate the strontium mark; (2) determine strontium uptake and retention time post-marking; (3) examine dose responses of strontium-chloride; and, (4) define fish growth and associated otolith accretion rates.

Chapter three provides quantification of uptake and retention times of strontium post-marking in association to otolith accretion, fish growth rates and dose-responses of elementally marked Greenland Halibut. Significance of the research outcomes highlights long strontium retention periods post-marking. This may provide explanation and assistance to interpreting elemental profiles of long-lived species that migrate frequently between marine and freshwater environments over relatively short time frames. These long retention periods suggest that short time frames between natural marking events (i.e., movement into high calcemic environments such as marine waters) may not allow for complete expulsion of strontium when migratory fish such as char (*Salvelinus*) species move back into low calcemic environments (fresh water) for overwintering and/or reproduction. In addition, element storage mechanisms such as calcium phosphate hard structures and their ability to remobilise calcium (and calcium mimic elements) (discussed in **Chapter 2**) will further create prolonged strontium retention within fish. Within the study, otolith accretion occurred along different axes in the left and right otolith structures. Otolith accretion rates were similar between left and

right structures along the edge region; however, accretion over the nucleus in the left proximal region was slower than that of the edge regions. Strontium uptake took anywhere from 17 to 242 days and was strongly influenced by otolith accretion rate and dose. Slow accretion resulted in a longer time to reach maximal strontium concentrations. Similarly, high doses of strontium took longer to reach maximal strontium concentrations. The estimated return-to-background duration (328-1518 days) is much longer than that reported in other studies.

Chapter 4: The chapter objective was to examine elemental and isotopic composition of archived otoliths for their utility in discriminating Dolly Varden Char populations of the YT North Slope and northwestern NT, Canada.

This chapter demonstrates the close linkage of Dolly Varden Char to perennial groundwater sources in the freshwater stages of their life cycle through otolith trace elements and strontium isotopes. Perennial groundwater during winter months contributes the majority of ice-free habitats needed by Dolly Varden Char for spawning and over-wintering. Discrimination among Dolly Varden Char populations inhabiting YT North Slope and northwestern NT river systems was highly successful (94.44% correct classification). Non-essential elements and isotopes (i.e., strontium, $^{87}Sr/^{86}Sr$ ratios and Ba) were the most significant discriminators although removal of other elements reduced discriminatory power in the model (discussed in **Chapter 2**). These unique elemental identifiers can be used as a tool for fisheries managers in understanding mixed-stock fisheries stock composition. Models are currently being expanded to include Alaskan river systems and research is in development to determine stock

composition of the Canadian Beaufort Sea coastal mixed-stock fishery. This study continues to provide advice to government, co-management organisations, Gwich'in communities, and Inuvialuit communities on the management of Dolly Varden Char subsistence harvests in the Inuvialuit Settlement Region and the Gwich'in Settlement Region.

Chapter 5: The chapter objective was to use otolith microchemistry to understand the anadromy behaviours of Arctic Char populations at the edges of their eastern North American and western Greenland geographic range.

The significance of this chapter is the methodological quantification of migration histories and the first examination of large-scale behaviours associated with anadromy in northeastern North American and western Greenland populations of Arctic Char. To develop methodologies for quantifying migration histories, environmental calcium in association with freshwater (lakes) baseline otolith strontium was examined (discussed in **Chapter 2**). In turn, it was demonstrated that High Arctic chars have strong associations to estuaries and coastal habitats once they have migrated seaward. Variation in anadromy was notable among populations. A new finding for the northern-most range of Arctic Char (i.e., Lake A and Clements Markham Lake) was migration seaward at a young age and small size. Chars utilised low strontium waters, indicative of coastal estuaries, throughout their lifetime and are facultative in their use of these estuarine habitats. In contrast, southern-most Arctic Char from Lac Davidson, delayed migration seaward until achieving older ages and larger sizes. They utilised high strontium waters indicative of less dependence on estuarine waters within the first year

of migration and all subsequent years thereafter. They are continuous migrators once this behaviour commences. Interesting anomalies and variation were noted for other High Arctic Lakes. For example, Lake Buchanan is the only High Arctic Lake where continuous migration is prevalent. Further, a subset of Arctic Char from Taserssuit Lakes likely over-winter in the associated estuary. This study provides a basis for future studies on Canadian Arctic Char anadromy in association to freshwater productivity and accessibility to marine environments. This also sets the foundation for monitoring changes in migratory behaviours driven by climate variability and change.

1.4 Structure of the Thesis

This thesis is structured in chapters that represent independent publishable primary journal articles. In addition, an overall introduction and conclusion are provided. Specifically, **Chapter 1** provides an introduction to the thesis, demonstrating the connectivity of all the chapters as part of the thesis. It outlines each chapter's objectives and significance, and provides the structure of the thesis. **Chapter 2** provides a review and comprehensive discussion of biomineralization and trace element incorporation in fishes. **Chapters 3 to 5** provide applications of otolith trace element and isotope techniques in the fisheries field through element marking, stock discrimination, and inference of migration histories. Finally, **Chapter 6** provides concluding statements for the thesis.

1.5 References

Allen, P. J., Hobbs, J. A., Jr., J. J. C., Eenennaam, J. P. Van, and Doroshov, S. I. 2009.

Using Trace Elements in Pectoral Fin Rays to Assess Life History Movements in Sturgeon: Estimating Age at Initial Seawater Entry in Klamath River Green Sturgeon. Trans. Am. Fish. Soc. **138**: 240–250. doi: 10.1577/T08-061.1.

Allemand, D., Mayer-Gostan, N., De Pontual, H., Boeuf, G., and Payan, P. 2008. Fish Otolith Calcification in Relation to Endolymph Chemistry. *In* Handbook of Biomineralization. Wiley-VCH Verlag GmbH. pp. 291–308. doi: 10.1002/9783527619443.ch17.

Bäuerlein, E., Behrens, P., and Epple, M. 2007. Handbook of biomineralization. Wiley-VCH.

Beier, M., Anken, R. H., and Rahmann, H. 2004. Calcium-tracers disclose the site of blomineralization in inner ear otoliths of fish. Adv. Space Res. **33**(8): 1401-1405.

Beier, M., Anken, R., and Hilbig, R. 2006. Sites of calcium uptake of fish otoliths correspond with macular regions rich of carbonic anhydrase. Adv. Space Res. **38**(6): 1123-1127.

Borelli, G., Guibbohni, M. E., Mayer-Gostan, N., Priouzeau, F., De Pontual, H., Allemand, D., Puverel, S., Tambutte, E., and Payan P. 2003a. Daily variations of endolymph composition: relationship with the otolith calcification process in trout. J. Exp. Biol. **206**(15): 2685-2692.

Borelli, G., Mayer-Gostan, N., Merle, P. L., De Pontual, H., Boeuf, G., Allemand, D., and Payan, P. 2003b. Composition of biomineral organic matrices with special emphasis on turbot (*Psetta maxima*) otolith and endolymph. Calcif. Tissue Int. **72**(6): 717-725.

Borelli, G., Mayer-Gostan, N., De Pontual, H., Boeuf, G., and Payan, P. 2001. Biochemical relationships between endolymph and otolith matrix in the trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Calcif. Tissue Int. **69**(6): 356-364.

Brennan, S. R., Fernandez, D. P., Mackey, G., Cerling, T. E., Bataille, C. P., Bowen, G. J., and Wooller, M. J. 2014. Strontium isotope variation and carbonate versus silicate weathering in rivers from across Alaska: Implications for provenance studies. Chem. Geol. **389**: 167–181. doi: http://dx.doi.org/10.1016/j.chemgeo.2014.08.018.

Brennan, S. R., Fernandez, D. P., Zimmerman, C. E., Cerling, T. E., Brown, R. J., and Wooller, M. J. 2015a. Strontium isotopes in otoliths of a non-migratory fish (slimy sculpin): Implications for provenance studies. Geochim. Cosmochim. Ac. **149**: 32–45. doi: http://dx.doi.org/10.1016/j.gca.2014.10.032.

Brennan, S. R., Zimmerman, C. E., Fernandez, D. P., Cerling, T. E., McPhee, M. V, and Wooller, M. J. 2015b. Strontium isotopes delineate fine-scale natal origins and migration histories of Pacific salmon. Sci. Adv. **1**: e1400124.

Brown, R. J., and Severin, K. P. 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. Can. J. Fish. Aquat. Sci. **66**: 1790–1808. doi: 10.1139/F09-112.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. **188**: 263–297.

Campana, S. E. 2005. Otolith science entering the 21st century. Mar. Freshw. Res. **56**:

485. doi: 10.1071/MF04147.

Campana, S. E., Fowler, A. J., and Jones, C. M. 1994. Otolith Elemental Fingerprinting for Stock Identification of Atlantic Cod (*Gadus morhua*) Using Laser Ablation ICPMS. Can. J. Fish. Aquat. Sci. **51**: 1942–1950. doi: 10.1139/f94-196.

Campana, S. E., and Thorrold, S. R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. **58**: 30–38. doi: 10.1139/f00-177.

Campana, S. E., Thorrold, S. R., Jones, C. M., Günther, D., Tubrett, M., Longerich, H., Jackson, S., Halden, N. M., Kalish, J. M., Piccoli, P., De Pontual, H., Troadec, H., Panfili, J., Secor, D. H., Severin, K. P., Sie, S. H., Thresher, R., Teesdale, W. J., and Campbell, J. L. 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Can. J. Fish. Aquat. Sci. **54**: 2068–2079. doi: 10.1139/f97-117.

Campbell, J. L., Teesdale, W. J., and Halden, N. M. 1995. Theory, practice and application of micro-PIXE analysis and element-distribution maps. Can. Mineral. **33**: 279–292.

Chowdhury, M. J., and Blust, R. 2001. A Mechanistic Model for the Uptake of Waterborne Strontium in the Common Carp (*Cyprinus carpio* L.). Environ. Sci. Technol. **35**: 669–675. doi: 10.1021/es000142t.

Chowdhury, M. J., and Blust, R. 2011. 7 - Strontium. *In* Homeostasis and Toxicology of Non-Essential Metals. *Edited by* A. C. M. Wood, A. Farrell and C. Brauner. Academic Press. pp. 351–390. doi: http://dx.doi.org/10.1016/S1546-

5098(11)31029-1.

Crichton, R. R. 2008. Biological Inorganic Chemistry. *Edited by* R.R. Crichton. Elsevier, Amsterdam.

De Pontual, H., and Geffen, A. J. 2002. Otolith microchemistry. *In* Manual of Fish Sclerochronology. *Edited by* J. Panfili, H. De Pontual, H. Troadec, and P. J. Wright. Ifremer-IRD, Brest, France. pp. 243–303.

Doubleday, Z. A., Harris, H. H., Izzo, C., and Gillanders, B. M. 2014. Strontium Randomly Substituting for Calcium in Fish Otolith Aragonite. Anal. Chem. **86**: 865–869. American Chemical Society. doi: 10.1021/ac4034278.

Eide, D. J. 2006. Zinc transporters and the cellular trafficking of zinc. Biochim. Biophys. Acta **1763**: 711–722. doi: http://dx.doi.org/10.1016/j.bbamcr.2006.03.005.

Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., and Limburg, K. E. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. **46**: 297–330.

Gillanders, B. M. 2005. Otolith chemistry to determine movements of diadromous and freshwater fish. Aquat. Living Resour. **18**: 291–300. doi: 10.1051/alr:2005033.

Grimes, S. T., Mattey, D. P., Hooker, J. J., and Collinson, M. E. 2003. Paleogene paleoclimate reconstruction using oxygen isotopes from land and freshwater organisms: the use of multiple paleoproxies. Geochim. Cosmochim. Ac. **67**(21): 4033-4047.

Halden, N. M., Campbell, J. L., and Teesdale, W. J. 1995. PIXE analysis in mineralogy and geochemistry. Can. Mineral. **33**: 293–302.

Hegg, J. C., Kennedy, B. P., and Fremier, A. K. 2013. Predicting strontium isotope variation and fish location with bedrock geology: Understanding the effects of geologic heterogeneity. Chem. Geol. **360–361**: 89–98. doi: http://dx.doi.org/10.1016/j.chemgeo.2013.10.010.

Hüssy, K. 2008. Otolith accretion rates: Does size really matter? J. Exp. Mar. Bio. Ecol. **362**: 131–136. doi: 10.1016/j.jembe.2008.06.016.

Ibsch, M., Anken, R. H., Vohringer, P., and Rahmann, H. 2001. Vesicular bodies in fish maculae are artifacts not contributing to otolith growth. Hear. Res. **153**(1-2): 80-90.

Ibsch, M., Anken, R., Beier, M., and Rahmann, H. 2004a. Endolymphatic calcium supply for fish otolith growth takes place via the proximal portion of the otocyst. Cell Tissue Res. **317**(3): 333-336.

Ibsch, M., Anken, R. H., and Rahmann, H. 2004b. Calcium gradients in the fish inner ear sensory epithelium and otolithic membrane visualized by energy filtering transmission electron microscopy (EFTEM). Adv. Space Res. **33**(8): 1395-1400.

Kastelle, C. R., Helser, T. E., Black, B. A., Stuckey, M. J., Gillespie, C., McArthur, J. D., Little, D., Charles, K. D., and Khan, R. S. 2011. Bomb-produced radiocarbon validation of growth-increment crossdating allows marine paleoclimate reconstruction. Palaeogeogr. Palaeoclimatol. Palaeoecol. **311**(1-2): 126-135.

Kennedy, B. P., Blum, J. D., Folt, C. L., and Nislow, K. H. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Can. J. Fish. Aquat. Sci. **57**: 2280–2292. doi: 10.1139/f00-206.

Kennedy, B. P., Klaue, A., Blum, J. D., Folt, C. L., and Nislow, K. H. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Can. J. Fish. Aquat.

Sci. **59**(6): 925-929 doi: 10.1139/f02-070.

Lee, S. R., Noh, S. J., Pronto, J. R., Jeong, Y. J., Kim, H. K., Song, I. S., Xu, Z., Kwon, H. Y., Kang, S. C., Sohn, E. H., Ko, K. S., Rhee, B. D., Kim, N., and Han, J. 2015. The Critical Roles of Zinc: Beyond Impact on Myocardial Signaling. Korean J Physiol. Pharmacol. **19**: 389–399.

Lundberg, Y. X. W., Zhao, X., and Yamoah, E. N. 2006. Assembly of the otoconia complex to the macular sensory epithelium of the vestibule. Brain Res. **1091**: 47-57.

Mann, S. 2001. Biomineralization: principles and concepts in bioinorganic materials chemistry. Oxford University Press.

Markwitz, A., Grambole, D., Herrmann, F., Trompetter, W. J., Dioses, T., and Gauldie, R. W. 2000. Reliable micro-measurement of strontium is the key to cracking the life-history code in the fish otolith. Nucl. Instr. Meth. Phys. Res. **168**(1): 109-116.

Marshall, W. S., and Grosell, M. 2006. Ion transport, osmoregulation, and acid-base balance. *In* The physiology of fishes. CRC Press Boca Raton. pp. 177–230.

Melançon, S., Fryer, B. J., Gagnon, J. E., and Ludsin, S. A. 2008. Mineralogical approaches to the study of biomineralization in fish otoliths. Mineral. Mag. **72**: 627–637. doi: 10.1180/minmag.2008.072.2.627.

Melançon, S., Fryer, B. J., Ludsin, S. A., and Gagnon, J. E. 2005. Effects of crystal structure on the uptake of metals by lake trout (*Salvelinus namaycush*) otoliths. Can J. Fish. Aquat. Sci. **2619**: 2609–2619. doi: 10.1139/F05-161.

Melançon, S., Fryer, B. J., and Markham, J. L. 2009. Chemical analysis of endolymph and the growing otolith: fractionation of metals in freshwater fish species. Environ.

Toxicol. Chem. **28**: 1279–87. doi: 10.1897/08-358.1.

Muhlfeld, C. C., Thorrold, S. R., McMahon, T. E., and Marotz, B. 2012. Movements in a River Network Using Strontium Isoscapes. Can. J. Fish. Aquat. Sci. **69**: 1129–1130. doi: 10.1139/f2012-049.

Murayama, E., Herbomel, P., Kawakami, A., Takeda, H., and Nagasawa, H. 2005. Otolith matrix proteins OMP-1 and Otolin-1 are necessary for normal otolith growth and their correct anchoring onto the sensory maculae. Mech. Dev. **122**: 791–803. doi: 10.1016/j.mod.2005.03.002.

Murayama, E., Takagi, Y., Ohira, T., Davis, J. G., Greene, M. I., and Nagasawa, H. 2002. Fish otolith contains a unique structural protein, otolin-1. Eur. J. Biochem. **269**: 688–696. doi: 10.1046/j.0014-2956.2001.02701.x.

Nachshen, D. A., and Blaustein, M. D. P. 1982. Influx of calcium, strontium, and barium in presynaptic nerve endings. J. Gen. Physiol. **79**: 1065–1087. doi: 10.1085/jgp.79.6.1065.

Omelon, S., Georgiou, J., Henneman, Z. J., Wise, L. M., Sukhu, B., Hunt, T., Wynnyckyj, C., Holmyard, D., Bielecki, R., and Grynpas, M. D. 2009. Control of Vertebrate Skeletal Mineralization by Polyphosphates. PLoS One **4**: e5634. doi: 10.1371%2Fjournal.pone.0005634.

Panfili, J., De Pontual, H., Troadec, H., and Wrigh P. J. 2002. Manual of Fish Sclerochronology. Ifremer, France. Available from http://archimer.ifremer.fr/doc/00017/12801/.

Payan, P., Borelli, G., Priouzeau, F., De Pontual, H., Boeuf, G., and Mayer-Gostan, N. 2002. Otolith growth in trout *Oncorhynchus mykiss*: supply of Ca^{2+} and Sr^{2+} to the saccular endolymph. J. Exp. Biol. **205**(17): 2687-2695.

Payan, P., De Pontual, H., Boeuf, G., and Mayer-Gostan, N. 2004a. Endolymph chemistry and otolith growth in fish. C. R. Palevol. **3**(6-7): 535-547.

Payan, P., De Pontual, H., Edeyer, A., Borelli, G., Boeuf, G., and Mayer-Gostan, N. 2004b. Effects of stress on plasma homeostasis, endolymph chemistry, and check formation during otolith growth in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **61**(7): 1247-1255.

Popper, A. N., Ramcharitar, J., and Campana, S. E. 2005. Why otoliths? Insights from inner ear physiology and fisheries biology. Mar. Freshwater Res. **56**(5): 497-504.

Popper, A. N., and Lu, Z. M. 2000. Structure-function relationships in fish otolith organs. Fish. Res. **46**(1-3): 15-25.

Potts, P. J., Bowles, J. F., Reed, S. J. B., and Cave, M. R. (*Editors*). 2012. The Mineralogical Society Series 6: Microprobe techniques in the earth sciences. *In* 2nd edition. Chapman & Hall, London, UK.

Reed, S. J. B. 2005. Electron microprobe analysis and scanning electron microscopy in geology. *In* 2nd edition. Cambridge University Press, Cambridge, UK.

Söllner, C., Burghammer, M., Busch-Nentwich, E., Berger, J., Schwarz, H., Riekel, C., and Nicolson, T. 2003. Control of crystal size and lattice formation by starmaker in otolith biomineralization. Science **302**: 282–6. doi: 10.1126/science.1088443.

Söllner, C., Schwarz, H., Geisler, R., and Nicolson, T. 2004. Mutated otopetrin 1 affects the genesis of otoliths and the localization of Starmaker in zebrafish. Dev. Genes

Evol. **214**: 582–590. doi: 10.1007/s00427-004-0440-2.

Sturrock, A. M., Trueman, C. N., Darnaude, A. M., and Hunter, E. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? J. Fish Biol. **81**: 766–95. doi: 10.1111/j.1095-8649.2012.03372.x.

Sturrock, A., Trueman, C., Milton, J., Waring, C., Cooper, M., and Hunter, E. 2014. Physiological influences can outweigh environmental signals in otolith microchemistry research. Mar. Ecol. Prog. Ser. **500**: 245–264. doi: 10.3354/meps10699.

Sturrock, A. M., Hunter, E., Milton, J. A., Eimf, Johnson, R.C., Waring, C. P., and Trueman, C. N. 2015. Quantifying physiological influences on otolith microchemistry. Methods Ecol. Evol. **6**: 806–816. doi: 10.1111/2041-210X.12381.

Surge, D., and Walker, K. J. 2005. Oxygen isotope composition of modern and archaeological otoliths from the estuarine hardhead catfish (*Ariopsis felis*) and their potential to record low-latitude climate change. Palaeogeogr. Palaeoclimatol. Palaeoecol. **228**(1-2): 179-191.

Sylvester, P., and Raeside, R. (*Editors*). 2001. Laser ablation ICPMS in the earth sciences, principles and applications Volume 29. *In* Mineralogical Association of Canada, Shortcourse. Minerological Association of Canada, St. John's, Newfoundland.

Takagi, Y. 2002. Otolith formation and endolymph chemistry: a strong correlation between the aragonite saturation state and pH in the endolymph of the trout otolith organ. Mar. Ecol. Prog. Ser. **231**: 237-245.

Takagi, Y., Tohse, H., Murayama, E., Ohira, T., and Nagasawa, H. 2005. Diel changes in endolymph aragonite saturation rate and mRNA expression of otolith matrix proteins in the trout otolith organ. Mar. Ecol. Prog. Ser. **294**: 249-256.

Tanner, S. E., Reis-Santos, P., Vasconcelos, R. P., Thorrold, S. R., and Cabral, H. N. 2013. Population connectivity of *Solea solea* and *Solea senegalensis* over time. J. Sea Res. **76**: 82–88. doi: http://dx.doi.org/10.1016/j.seares.2012.11.005.

Tohse, H., Saruwatari, K., Kogure, T., Nagasawa, H., and Takagi, Y. 2009. Control of Polymorphism and Morphology of Calcium Carbonate Crystals by a Matrix Protein Aggregate in Fish Otoliths. Cryst. Growth Des. **9**(11): 4897-4901.

Tohse, H., and Mugiya, Y. 2001. Effects of enzyme and anion transport inhibitors on in vitro incorporation of inorganic carbon and calcium into endolymph and otoliths in salmon *Oncorhynchus masou*. Comp. Biochem. Phys. A **128**(1): 177-184.

Veinott, G., Northcote, T., Rosenau, M., and Evans, R. D. 1999. Concentrations of strontium in the pectoral fin rays of the white sturgeon (*Acipenser transmontanus*) by laser ablation sampling - inductively coupled plasma - mass spectrometry as an indicator of marine migrations. Can. J. Fish. Aquat. Sci. **56**: 1981–1990. doi: 10.1139/f99-120.

Wright, P. J., Panfili, J., Morales Nin, B., and Geffen, A. J. 2002. Types of calcified structures. *In* Manual of Fish Sclerochronology. *Edited by* J. Panfili, H. de Pontual, H. Troadec and P. J. Wright. Ifremer-IRD coedition, Brest, France. pp. 31-88.

Zimmerman, C. E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity : experimental validation for juvenile salmonids. Can. J. Fish. Aquat. Sci. **62**: 88–97. doi: 10.1139/F04-182.

Chapter 2

Literature Review

Linking Physiology and Biomineralization Processes to Ecological Inferences on the Life History of Fishes

This manuscript has been submitted to the journal of Comparative Biochemistry and Physiology Part A. It has been accepted for publication. This manuscript has been coauthored with my thesis committee and a fellow graduate student. I have been the lead on the manuscript. Specifically I was responsible for the majority of the writing, integrating interdisciplinary material and revisions for the manuscript. My co-authors provided writing assistance on a minority of the manuscript sections, insight to the overall review, and provided editorial and review comments on multiple drafts of the manuscript. This paper, including two figures created by B. Carriere, is reproduced with the permission of my coauthors.

Loewen, T. N., Carriere B., Reist, J. D., Halden, N. M., and Anderson, W. G. (*in press*). Linking physiology and biomineralization processes to ecological inferences on the life history of fishes. Comp. Biochem. Phys. A. doi: 10.1016/j.cbpa.2016.06.017.

2.1 Abstract

Biomineral chemistry is frequently used to infer life history events and habitat use in fishes; however, significant gaps remain in our understanding of the underlying mechanisms. Here we have taken a multidisciplinary approach to review the current understanding of element incorporation into biomineralized structures in fishes. Biominerals are primarily composed of calcium-based derivatives such as calcium carbonate found in otoliths and calcium phosphates found in scales, fins and bones. By focusing on non-essential life elements (strontium and barium) and essential life elements (calcium, zinc and magnesium), we attempt to connect several fields of study to synergise how physiology may influence biomineralization and subsequent inference of life history. Data provided in this review indicate that the presence of non-essential elements in biominerals of fish is driven primarily by hypo- and hyper-calcemic environmental conditions. The uptake kinetics between environmental calcium and its competing mimics define what is ultimately incorporated in the biomineral structure. Conversely, circannual hormonally driven variations likely influence essential life elements like zinc that are known to associate with enzyme function. Environmental temperature and pH as well as uptake kinetics for strontium and barium isotopes demonstrate the role of mass fractionation in isotope selection for uptake into fish bony structures. In consideration of calcium mobilisation, the action of osteoclast-like cells on calcium phosphates of scales, fins and bones likely plays a role in fractionation along with transport kinetics. Additional investigations into calcium mobilisation are warranted to understand differing views of strontium, and barium isotope fractionation between calcium phosphates and calcium carbonate structures in fishes.

2.2 Introduction

In the present day, the fisheries biology research community directly applies biomineralized structure microchemistry to habitat and life history determination particularly for freshwater to saline water movements (Campana 1999). Although there is much success in these applications, limitations also exist suggesting insufficient understanding of element presence in biominerals (Sturrock et al 2012, 2014). Initial examination of how each structure is formed on a biogeochemical level provides the basic understanding of hard structure biomineralization (Mann 2001, Bäuerlein et al. 2007). Calcium carbonate and calcium phosphate hard structures have different chemical properties and affinities for element substitution and insertion in association to the protein matrix during the biomineralization process (Mann 2001, De Pontual and Geffen 2002, Bäuerlein et al. 2007, Doubleday et al. 2014). The building block for all these structures involves calcium, and its mobilisation and remobilisation within a fish (Schönbörner et al. 1979, Weiss and Watabe 1979, Mann 2001, Allemand et al. 2008). Element presence within hard structures can be viewed in terms of essential and non-essential need for life processes (Crichton 2008). This qualification plays a large role in the function of the element once available internally to an organism and before incorporation in biominerals and subsequent measurement (Crichton 2008). Physiologically, an organism can then control the presence of elements, particularly divalent cations, either indirectly through calcium uptake mechanisms for non-essential elements or directly for essential life elements (Nachshen and Blaustein 1982, Bijvelds et al. 1998, Mann 2001, Eide 2006, Wang and Berlin 2007, Omelon et al. 2009, Van

Campenhout et al. 2009, Zhou and Clapham 2009, Chowdhury and Blust 2011, Feng 2011, Swenson et al. 2014, Lee et al. 2015). This further leads to isotopic discrimination based on energetics in uptake pathways (Kedem and Essig 1965, Sharp 2007). By bringing the research fields of fisheries biology, biogeochemistry, and physiology together, a new perspective to the current state of element applications to biomineralized structures is highlighted suggesting a multi-disciplinary approach to the research questions in this field.

Living organisms selectively extract and take up elements from their environment and incorporate them into tightly controlled functional biological structures. These biologically derived minerals (biominerals) act as protective structures, aid in movement, feeding, buoyancy, vision, gravity sensing and sound detection, and allow for storage of key elements (Mann 2001, Bäuerlein et al. 2007). Of particular relevance to this review is the storage of ~99% of the whole-body fraction of calcium (Ca^{2+}) in biomineralized structures such as otoliths, scales, fins and bones within a teleost (Flik et al. 1986).

Calcium is a fundamental element forming the foundation of biominerals, as well as being essential for other physiological functions such as muscle fibre contraction, intracellular messaging, and reproduction (Crichton 2008). As a consequence calcium concentration in both intracellular and extracellular fluid environments is tightly controlled at the level of the cell and whole body so that normal physiological function can be maintained (Flik et al. 1984, 1995, 1996, Bijvelds et al. 1995).

In teleost fish, homeostatic regulation of calcium is a primary function of the gill (Herrmann-Erlee and Flik 1989, Perry et al. 1992, Flik and Verbost 1993), and secondarily the intestinal epithelia (Flik and Verbost 1993, Wilson and Grosell 2003)

with the opercular epithelia playing a minor role (Marshall et al. 1992, McCormick et al. 1992). Within the gills, mitochondrial rich cells (MRC) or ionoregulatory cells and pavement or respiratory cells, have both been shown to be involved in calcium uptake (Flik et al. 1995). Evidence in some fish species of gastrointestinal uptake is observed (Flik et al. 1990, Flik and Verbost 1993, Allen et al. 2011, Genz et al. 2013), for example in Atlantic cod (*Gadus morhua*), intestinal calcium uptake was estimated at 30% of the total uptake (Sundell and Björnsson 1988). Although the renal system is not recognised as an uptake mechanism from the environment, it does play a role in internal calcium homeostasis by excreting excess ions when necessary, such as periods of high environmental calcium (e.g., seawater environment) and/or increased dietary calcium intake (Flik et al. 1996).

Calcium is stored in organisms in the form of calcium carbonate ($CaCO_3$) and hydroxyapatite ($Ca_5(PO_4)_3(OH)$). Calcium carbonate found within the hearing systems of mammals and fish (otoconica in mammals analogous to otoliths in fish) is a calcium sink due to the lack of resorbing qualities of the structure once formed (Allemand et al. 2008). Conversely, bone-like structures (bones, fins, scales) can be reabsorbed which results in a remobilisation of calcium in the ionic form – Ca^{2+}. One well-documented mode for the reabsorption of calcium from bone in mammals is through the dissolution of bone stores by osteoclasts (Schönbörner et al. 1979, Weiss and Watabe 1979).

In the following review we focus on teleost and chondrostei fish in particular and examine the incorporation of strontium, barium, magnesium and zinc into biominerals. Each of these elements shares a commonality in that they are all, like calcium, divalent

cations in solution (Campana 1999, De Pontual and Geffen 2002, Allemand et al. 2008). Strontium and barium are non-essential and typically considered trace elements that substitute directly for calcium in biomineralization of the crystalline inorganic structure (Campana 1999, De Pontual and Geffen 2002, Allemand et al. 2008, Doubleday et al. 2014) and have been extensively used to infer various life history, habitat, chemical marking and biological characteristics of freshwater and marine fishes (Campana 1999, Elsdon and Gillanders 2003, 2006, Elsdon et al. 2008, Brown and Severin 2009). Magnesium (bulk element) and zinc (trace dietary requirement) are essential elements also used to infer life history traits, but are more likely to be incorporated in the protein organic compartment of the biomineral in otoliths although magnesium can also be directly substituted for calcium in hydroxyapatite mineralisation (Mann 2001, Crichton 2008, Omelon et al. 2009). Given the different end fates of these two groups of substituting elements in biominerals, strontium and barium are expected to be governed principally by regulatory drivers of calcium (Mann 2001), whereas, the magnesium and zinc concentrations are governed by their own regulatory processes (Crichton 2008). When all these elements are considered together, stronger inferences regarding physiological mechanisms of element incorporation into biomineralized structures can be made.

2.2.1 *Microchemistry of Calcium-Based Hard Structures*

To date the most extensive knowledge base regarding hard structure microchemical and isotope analyses is the identification of habitat switching in teleosts (Campana

1999, 2005, Kennedy et al. 2000, Gillanders 2005, Elsdon et al. 2008, Muhlfeld et al. 2012). Anadromy (i.e., the movement of fish between fresh and marine waters) is often inferred by otolith strontium/calcium ratios because otolith and environmental strontium/calcium ratios tend to be positively correlated and elevated in marine compared to freshwater environments (Brown and Severin 2009). Specific studies on a number of salmonids, White Sturgeon (*Acipenser transmontanus*), and Green Sturgeon (*A. medirostris*) have reported movement of individuals from freshwater habitats into marine or coastal waters through otolith or fin ray element analyses (Veinott et al. 1999, Zimmerman 2005, Allen et al. 2009).

Examination of otolith strontium isotopic ratios of fishes confined within freshwater systems has also allowed for inference of patterns of habitat switching at micro-scales within watersheds. The success of these techniques is highlighted by studies on Atlantic Salmon (*Salmo salar*) in tributaries of the Connecticut River (Kennedy et al. 2000, 2002), Chinook Salmon (*Oncorhynchus tshawytscha*) in the California Central Valley (Barnett-Johnson et al. 2008), Cutthroat Trout (*O. clarki*) in the Flathead River drainage of the Rocky Mountains (Muhlfeld et al. 2012), and for Chinook Salmon within Bristol Bay, Alaska (Brennan et al. 2015b). Groundwater hydrology and underlying geological conditions are instrumental in determining local environmental strontium isotopic ratios that are reflected in otolith composition (Hegg et al. 2013, Brennan et al. 2014, 2015a, 2015b). However, this approach is inappropriate in purely marine fishes where the isotopic ratio of environmental strontium ($^{86}Sr/^{87}Sr$ of 0.70918 +/- 0.00006) is constant due to long residence times of strontium and relatively short turnover rate of the worlds oceans (Faure and Mensing 2005).

In marine systems, migrations between coastal areas or marine habitats are sometimes observed although otolith and environmental concentrations of strontium in these systems are more complex. Campana et al. (1994) and Tanner et al. (2013) demonstrated differential habitat use during life history of Atlantic cod and Common Sole (*Solea solea*) for marine and tidal estuary regions, respectively but a multiple element analysis was needed as increased difficulty was observed in linking otolith microchemistry to individual water masses within the study area. Further, examination of multiple freshwater, anadromous and marine species (n = 81) concluded that environmental strontium/calcium drives otolith strontium/calcium in anadromous and freshwater species but not in marine species (Brown and Severin 2009).

In recent years, microchemical and isotopic analysis of biominerals has been frequently used in biological research (>1900 primary articles published based on the search criteria in Google scholar linked to the University of Manitoba's library databases of "fish" and "otolith" or "fin" or "scale" and "microchemistry" or "trace element" or "isotope"). Despite this large body of primary literature, a lack of complete understanding between the connectivity of fish physiological control mechanisms and the presence of elements in biominerals is evident (Sturrock et al. 2012, 2014). While most deviations from the expected relationship between otolith and environmental strontium/calcium have been reported for marine species (Brown and Severin 2009, Sturrock et al. 2012, 2014), there are also instances in freshwater systems. For example, we have observed strontium profiles (**Figure 2.1**) that suggest lake-dwelling Arctic Char, (*Salvelinus alpinus*), are migrating between marine and freshwater environments, which is highly unlikely given the presence of a large waterfall preventing

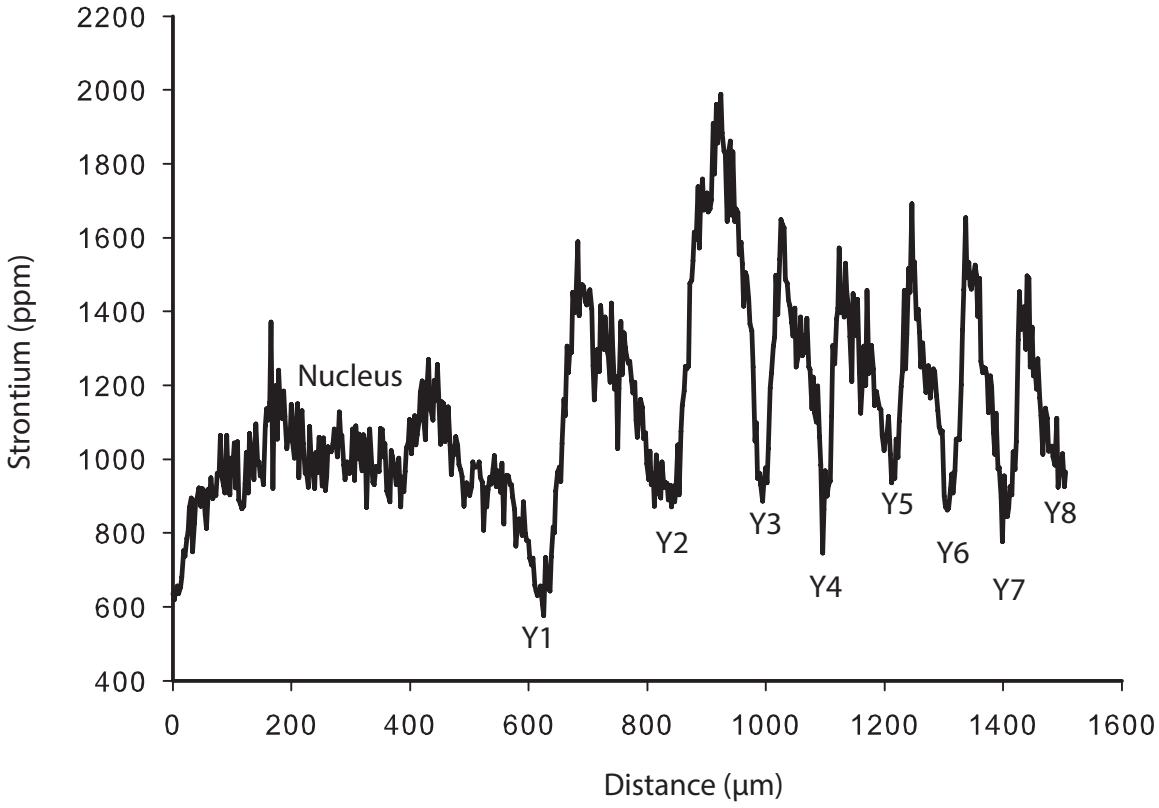

Figure 2.1: Labrador Arctic Char (lake-dwelling) otolith strontium concentration (ppm) from the nucleus region to the outer edge with annuli (years) indicated by ($\mathbf{Y}_i$). Otolith preparation and analytical methods are provided *in* Loewen et al. (2015).

return from the marine or estuarine environments to the freshwater lake or any tidal influence on the freshwater ecosystem. Further, we observe otolith strontium concentration ranging between 174 and 1367 parts per million (ppm) from species captured in freshwater environments (**Figure 2.2**) across the Canadian and western Greenland Arctic regions. Thus the questions become: 1.) what causes these large variations in the freshwater strontium signature observed in the otoliths of freshwater resident fishes in stable environments and 2.) how should we examine the relationship between strontium and calcium to best interpret the differences observed in biominerals? These observations are inconsistent with theoretical expectations and have led us to consider the primary pathways for element uptake and deposition in biominerals. Understanding the physiological uses of calcium specifically associated with interactions with other elements and biomineralization provides increased understanding of the biomineralization process. Further, it demonstrates some limitations of the application of these techniques and inferences made in the life history of fishes.

2.3 Biomineralizing Structures in Fish

Bones, teeth, fins, scales and otoliths are calcium-derived hard-structures in fish that incorporate elements and their isotopes during formation. Of these structures otoliths, fins, and scales are predominantly used to infer habitat switching and life history events within fishes through microchemical and isotopic analyses (Begg et al. 2005, Campana 1999, Feyrer et al. 2007, Veinott et al. 1999, Woodcock et al. 2013). The affinity for elements to become adsorbed to the surface of the mineral forming region, substituted

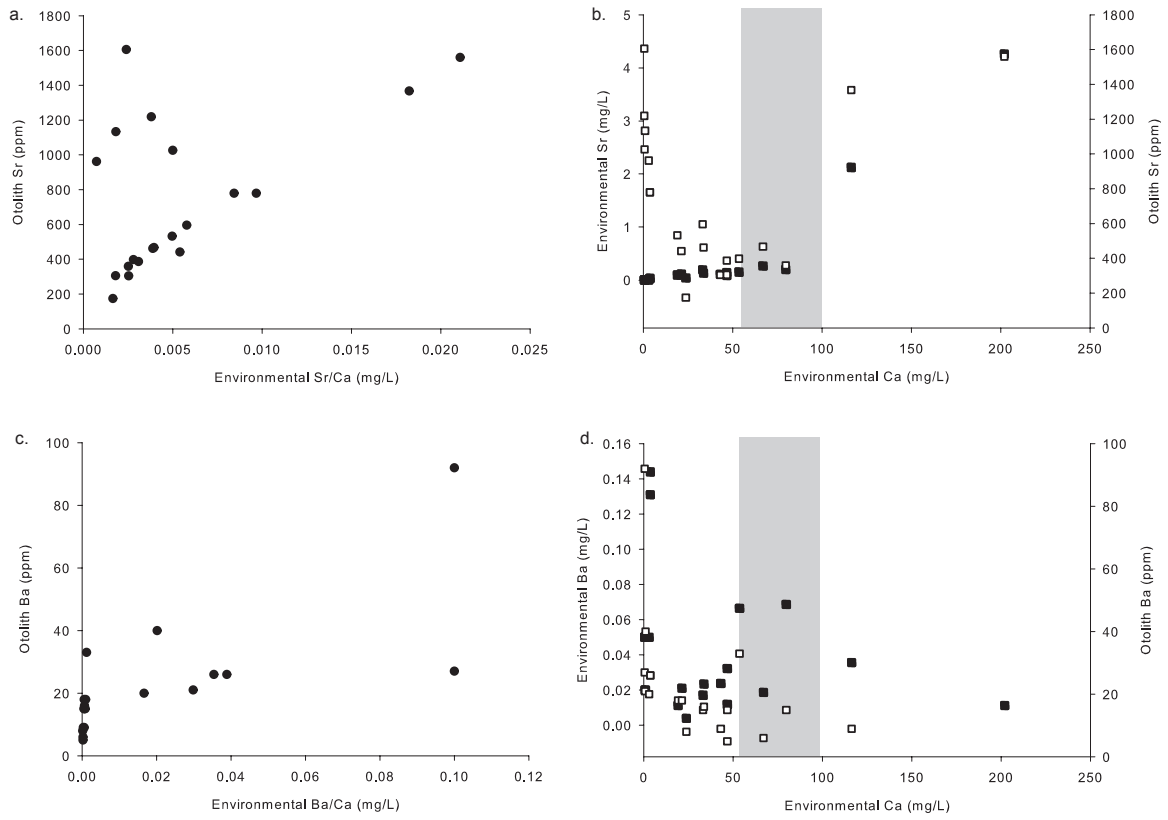

Figure 2.2: The relationships of environmental strontium/calcium (Sr/Ca mg/L), environmental barium/calcium (Ba/Sr mg/L) to otolith strontium (ppm) and barium (ppm) found in part **a.** and **c.** of the figure. The relationships of environmental calcium to environmental strontium, and barium found in part **b.** and **d.** of the figure where the right axis represents the relationship of (**b.**) otolith strontium and (**d.**) otolith barium to environmental calcium. The black filled squares represent the relationship between environmental calcium to environmental strontium and barium and the open squares represent the otolith strontium and barium values in relationship to environmental calcium. The corresponding values (Otolith Sr, Otolith Ba, environmental Sr, environmental Ba) for 202 mg/L of environmental calcium represent a seawater environment. The grey shaded region indicates the general internal regulation of

calcium within fish (64-96 mg/L). Methodology for the analytical techniques used to calculate otolith element values is provided *in* Loewen et al. (2015).

for calcium, or attached to the underlying organic matrix of the forming mineral (Allemand et al. 2008, De Pontual and Geffen 2002) relates to the different types of calcium minerals and their isoforms (see below).

2.3.1 *Otoliths*

Otoliths or 'ear stones' within the inner ear, used for balance and orientation by fish, are composed of calcium carbonate ($CaCO_3$). Characteristics such as continuous growth throughout a fish's lifetime, daily and annular changes in growth, incorporation of persistent microchemicals, and the absence of resorbing mechanisms in the endolymphatic sac surrounding the otoliths have made these structures ideal for studies of habitat use and life history, particularly in teleosts. Teleosts have 3 paired structures of otoliths: asteriscus, lapillus and sagitta. The sagittal otoliths are generally the largest of the pairs in most teleost species and the most studied (Campana 1999, 2005, Campana and Thorrold 2001). Six different isoforms of calcium carbonate biominerals exist, all having similar composition: calcite, aragonite, vaterite, calcium carbonate monohydrate, calcium carbonate hexahydrate, and amorphous calcium carbonate. Calcite and aragonite are the most thermodynamically stable isoforms and are deposited most extensively in biominerals (Mann 2001, Allemand et al. 2008). Calcite should be favoured for deposition due to its physiochemical properties (Takagi 2002); however, aragonite is most commonly deposited in lapillus and sagittal otolith pairs of teleost fish. Vaterite is less commonly observed and is generally found in asteriscus otolith pairs. This variation among otoliths strongly indicates differential physiological

control of the calcium carbonate isoform expressed (Takagi 2002, Tohse et al. 2009, Allemand et al. 2008).

The organic components comprise the underlying matrix upon which calcium carbonate is deposited in otoliths. This underlying biologically formed matrix is continuous over the entire mineral surface and influences element incorporation, nucleation, orientation inhibition, crystal nature, growth regulation, and the structure and morphology of the forming mineral (Mann 2001). Much of the control over calcification is thought to occur within the organic matrix with glycoproteins, proteoglycans and glycolipids playing roles in mineral formation (Söllner et al. 2003).

The otolith organic matrix is made up of approximately 50% proteins, 30% proteoglycans and 20% collagens (Allemand et al. 2008). The organic matrix itself is complex containing both fully derived proteins for deposition as well as protein precursors that are modified during calcification of the otolith. The major protein components of the organic matrix are otolin-1, starmaker and OMP-1 (Murayama et al. 2002, 2005).

Otolin-1 and starmaker protein both contain a high proportion of acidic amino acid residues, suggesting promotion of calcium carbonate deposition (Borelli et al. 2001, Söllner et al. 2003). On a daily scale, only a small fraction of organic precursors in the endolymph are used in the matrix formation of the underlying lattice of otolith growth. Thus a constant excess of proteinaceous material is available in the endolymph for mineralization (Allemand et al. 2008). Water-insoluble proteins (collagen components) are the structural foundation for calcium carbonate crystal growth whereas the water-

soluble components (polysaccharides, lipoproteins, glycoproteins) are thought to provide a lattice to aid in regulating crystal growth (Hüssy 2008).

The protein starmaker is a major controlling factor of structural formation in the otolith (Kapłon et al. 2008, 2009) and has been identified as playing a significant role in crystal choice and otolith biomineralization processes of zebrafish (*Danio rerio*). Starmaker influences the size of calcium carbonate crystals and crystal polymorph selection and secretion of the protein is controlled by the trans-membrane protein otopetrin (Otop1) (Kapłon et al. 2009). The Otop1 transporter gene is expressed at the initiation of otolith seeding and is only found in hair cells during otolith growth. Otop1 in zebrafish otoliths was shown to play a role in the localization and aggregation of matrix proteins. During vestibular organ development Otop1 is thought to regulate the ionic environment of the otolith membrane (Hughes et al. 2004, Söllner et al. 2004). Similarly the protein otoconin-1 (Otoc1) is thought to play a role in the nucleation process of calcium carbonate mineralisation of zebrafish otoliths (Petko et al. 2008).

Starmaker aids in the radial organization of the protein skeleton around the zebrafish otolith core (Kapłon et al. 2009). Söllner et al. (2003) found that the absence of starmaker resulted in severe alteration of otolith morphology (star-shaped otoliths, pure crystalline calcite) and a switch from aragonite isoform to a calcite isoform of the inorganic matrix. These studies suggest that the regularly spaced acidic groups in the 3 dimensional component of the β sheets in the peptide structure could serve as a template for the binding of divalent ions leading to crystal formation. By alternating serine and aspartic acid residues in the starmaker β sheet, selection of aragonite over

calcite occurs. Starmaker, once bound, may act to inhibit or regulate crystal growth at surface regions of the otolith.

Otolin-1 and starmaker together are contained in nuclei of sagittae as an aggregate and contribute to the formation of the aragonite polymorph (Kapłon et al. 2008). It is unknown whether these proteins contribute to nucleation of vateritic otoliths such as the asteriscus. The organic matrix composition in vateritic and aragonitic Atlantic Cod otoliths varies significantly despite similarities in mineralogy and microstructure (Dauphin and Dufour 2003). Variability in the soluble organic matrix is further noted among fish species. Similarities exist in the key protein components such as starmaker and otolin but variability is found in molecular weights of components within the complexes, the protein/carbohydrate ratios, and within carbohydrates (Dauphin and Dufour 2003).

Tohse et al. (2008) suggested that proteins and polysaccharides play important roles in the formation of phases and or morphs of the crystals deposited on the organic matrix of Rainbow Trout, (*O. mykiss*). They identified a novel protein otolith matrix macro-protein 64 (OMM-64) and described it as a similar protein to starmaker demonstrating that this protein forms ring shape complexes with otolin-1 to promote deposition of aragonite crystals (Tohse et al. 2008, 2009). Interestingly aragonite deposition does not occur when only one of these proteins is present; therefore, both proteins interact to induce crystal deposition. The microenvironment created by both proteins to cause crystal deposition is unknown.

Murayama et al. (2000) first identified the protein otolith matrix protein-1 (OMP-1) belonging to the transferrin family of proteins in the otolith of Rainbow Trout and Chum

Salmon (*O. keta*). Using knockout zebrafish models, Murayama et al. (2002, 2005) suggested that OMP-1 (zOMP-1) was required for normal otolith growth and that otolin-1, a collagenous type protein, is part of the fibrous structure that attaches the otolith to hair cells, and is excreted into the endolymph from the marginal zone of sensory epithelium near the transitional epithelium.

Proteoglycans are keratin-like proteins that exhibit anionic properties whose role in calcification is not well understood. Proteoglycans may be used as a stimulatory factor by increasing ionized calcium at the endolymph-crystal interface (Addadi et al. 1989, Khan and Falcone 1997, Borelli et al. 2003, Allemand et al. 2008).

Inhibition of calcium carbonate deposition occurs in the organic matrix of an otolith as a means to control the calcification process due to the super saturation state of ionic precursors (Ca^{2+} and HCO_3^-) in the endolymph. Inhibitory factors likely act directly on crystal growth to inhibit calcification. Guibbolini et al. (2006) proposed that levels of factor retarding crystallization (FRC) in endolymph could indicate the thickness of aragonite deposition during calcification and may further explain reduction of otolith growth in turbot (*Psetta maxima)* and Rainbow Trout observed after starvation or stress. FRC inhibition occurs when proteins in solution co-precipitate with the structural proteins of the matrix (Guibbolini et al. 2006).

2.3.2 *Fins and Scales*

Fins and elasmoid scales are part of the endoskeleton boney structure of teleost fish. Both are of mesodermal origin (Lee et al. 2013, Mongera and Nüsslein-Volhard 2013, Shimada et al. 2013) and have the capacity of regeneration when injured (Akimenko et

al. 2003, Sire and Géraudie 1984, Sire et al. 1997). They differ from otoliths due to the expression of calcium-phosphate mineralisation in the form of hydroxyapatite ($Ca_{10}(PO_4)_6X_2$) where X is mostly fluorine (F) or hydroxide (OH) (De Vrieze et al. 2012, Metz et al. 2012) in lieu of calcium carbonate. Osteoblast- and osteoclast-like cells are present in fin rays and scales (referred to *in scales* as scleroblasts and scleroclasts but are thought to be analogous to osteoblasts and osteoclasts in bony material) for the creation of new bony materials and the reorganisation and remobilization of calcium, respectively (Akiva et al. 2015, Omelon et al. 2009, Onozato and Watabe 1979, Sire et al. 1997). Acellular and cellular bony tissues found in teleosts also express these cell types for calcium management. Cellular bones have osteocytes contained within their organic matrix and remobilisation predominantly occurs via multi-nucleated osteoclasts. Osteocytes are absent within the organic matrix in acellular bones and remobilisation of calcium is thought to generally occur via mono-nucleated osteoclasts (Kranenbarg et al. 2005, Witten and Huysseune 2009). The remobilisation of calcium aids in the maintenance of calcium homeostasis and other physiological needs of the organism such as reproduction, smoltification and stress response (Abbink et al. 2004, Fjelldal et al. 2006, Persson et al. 1997, 1998a, 1998b).

Hydroxyapatite is the thermodynamically stable end-phase of calcium phosphates in the bone matrix (Christoffersen et al. 1989, Johnsson and Nancollas 1992, Omelon et al. 2009). It is the only calcium phosphate mineral phase stable at neutral and basic pH (Cheng 1985, Glimcher 2006, Lijima 1991). A precursor amorphous calcium phosphate (ACP) phase has been documented in zebrafish fin rays (Mahamid et al. 2008, 2010, 2011). The ACP phase transforms to octacalcium

phosphate (OCP) and then undergoes hydrolysis to form the final stage of mineralisation to carbonated hydroxyapatite (HAP) (Akiva et al. 2015, Christoffersen et al. 1989, Kobayashi et al. 2014, Omelon et al. 2009).

Hydroxyapatite is deposited onto an organic matrix of collagen I type during bone (fin) and scale formation. Protein structures, associated with osteoblast macrophages such as alkaline phosphatase (ALP), osteocalcin or bone γ-carboxyglutamic acid protein (BGP), phosphatase orphan 1, osteonectin, bone-morphogenetc proteins (BMPs), transcription factors (*osterix* and *Runx2a*), matrix proteins (collagen I alpha), and sclerostin are known to play an important role in tissue and bone mineralisation (Hartmann 2009, Kang et al. 2008, Karsenty 2008, Lehane et al. 1999, Mertz et al. 2012, Nishimoto et al. 1992). Runx2 and osterix are major transcription factors that control osteoblast commitment and differentiation (Hartmann 2009, Karsenty 2008). Phosphatase orphan 1, responsible for providing phosphate in nucleation of crystal growth, is known to also contribute to bone formation (Stewart et al. 2006).

ALP and its enzymatic activity of tissue non-specific alkaline phosphatase (TNAP) is the best-known and well-established protein associated with bone formation and osteoblast activity (Estêvão et al. 2011, Persson et al. 1995, Witten 1997, Witten et al. 2001) and is thought to free phosphates required for mineralisation (Huitema et al. 2012, Omelon and Grynpas 2008, Pombinho et al. 2004) and play a role in the breakdown of pyrophosphates, a strong inhibitor of bone mineralization (Huitema et al. 2012, Omelon and Grynpas 2008). Within Common Carp (*Cyprinus carpio*) scales, ALP activity is found predominantly in the hyposquamal cells (De Vrieze et al. 2010). Presence of ALP is detected in the episquamal region but its activity is at a reduced

rate. Within Gilt-head Bream (*Sparus aurata*) fin and bony tissue, ALP activity has been detected in osteoblast macrophages (Pombinho et al. 2004). Additionally, the gene Entpd5 expressed in the osteoblasts of fish is a crucial contributor to bone mineralisation in zebrafish through phosphate homeostasis (Huitema et al. 2012).

Osteocalcin (bone Gla protein), matrix Gla protein (MGP), and osteonectin proteins are also abundant in vertebrate bone mineralising regions. In vertebrates, osteocalcin is found to be one of the few osteoblast-specific proteins and is implicated in bone formation and calcium homeostasis (Estêvão et al. 2011, Lee et al. 2007, Mertz et al. 2012, Nishimoto et al. 1992). Osteocalcin and MGP are thought to be negative regulators of mineralisation (Ducy et al. 1996, Hunter et al. 1996, Luo et al. 1997, Pombinho et al. 2004, Roy and Nishimoto 2002). Specifically, osteocalcin is linked to inhibition of crystal maturation (Boskey et al. 1998), whereas MGP is a general inhibitor of tissue calcification in cartilage and the vascular systems (Boström 2000). Osteonectin on the other hand is a regulator of bone mineralisation, tissue remodelling and cell growth. It is secreted from osteoblasts during the bone formation process. Osteonectin is present in zebrafish scales (Hong et al. 2011, Mertz et al. 2012), and medaka (*Oryzias latipes*)*,* zebrafish, and goldfish *(C. auratus*) bones (Estêvão et al. 2011, Renn and Winkler 2010, Renn et al. 2006).

Tartrate-resistant acid phosphatase (TRAcP), Cathepsin K, and matrix metalloproteinase (MMP-2 and MMP-9) are known protein markers for osteoclast activity in vertebrates (Minkin 1982, Witten and Huysseune 2009, Witten et al. 2001). In scales, these proteins are always localized to the episquamal-mineralised layer. TRAcP activity functions in resorption pits of the osteoclasts. The required acidic pH for

optimised function of TRAcP occurs via HCl production (De Vrieze et al. 2010, 2011). TRAcP is also thought to play a role in osteoclast migration by weakening the interaction of proteins that bind the osteoclast to the collagen matrix (De Vrieze et al. 2010, 2011). TRAcP activity, measured through PCR and histochemistry, is observed to occur in and around multi-nucleated osteoclast sites in zebrafish scales and bones (De Vrieze et al. 2010, 2011, Persson et al. 1995, 1999, Witten et al. 2000, 2001).

Cathepsin K is a cysteine proteinase that is predominantly found in the osteoclast and is thought to degrade the bone collagen matrix and has high proteolytic activity (Metz et al. 2012). De Vrieze et al. (2010) demonstrated cathepsin K localisation in zebrafish and Common Carp scales via histochemistry. Other proteins such as matrix metalloproteinase (MMP-2 and MMP-9) are thought to play a role in the remodelling of the scale plate during early scale regeneration as well as play a role in bone matrix degradation and scale matrix turn-over (Metz et al. 2012, 2014).

2.3.3 *Calcium Mobilisation Rates*

Of particular relevance to the use of biominerals as references to life history events is calcium mobilisation that is found to occur at different rates among different storage structures. Thus the ability to remobilise these elements and the rate of its occurrence must be considered when examining various structures to infer life history events. Goldfish and Rainbow Trout elasmoid scales take up calcium the fastest and subsequently serve as the first source of calcium remobilisation, likely followed by fins and finally bones in teleosts (Persson et al. 1997, Shinozaki and Mugiya 2000). This is indicative that scales under most circumstances are not the best choice for element

analysis. Goldfish otoliths display a time delay in uptake and slower mineralization in comparison to all the phosphate derived biominerals in bony or bony-like structures and become a calcium sink, since they lack the remobilising capacity once formed (Ichii and Mugiya 1983, Shinozaki and Mugiya 2000).

2.4 Essential and Non-essential Elements and their Presence in Hard Structures

Life forms require both organic and inorganic elements to exist. In total about 25 elements are required by life forms (Crichton 2008). Essential organic elements include carbon, nitrogen, hydrogen, oxygen, phosphorous and sulphur (Crichton 2008). Inorganic elements such as sodium, potassium, calcium and magnesium are bulk elements essential for life processes, whereas elements such as cobalt, copper, zinc and iron exist as trace elements but are nonetheless essential (Crichton 2008).

In fishes, biological use of elements should be acknowledged in the biomineralization process since their regulation and purpose are significantly different than those of non-essential life elements. The bulk elements essential to life, such as sodium, chlorine and potassium, are all used to maintain ion and water balance and ensure the appropriate electrochemical gradient across cell membranes. Consequently these ions are highly regulated with specific transport proteins providing the appropriate extracellular environment for optimal cell function (Marshall and Grosell 2006, Crichton 2008). This environment can vary depending on what organ or tissue is being examined. In the case of teleost fish otoliths, the endolymph has higher concentrations of potassium [K+] and lower concentrations of sodium [Na+] compared to plasma (Allemand et al. 2008).

Physiologically the bulk element, magnesium, interacts with numerous enzymes and loosely binds amino acids (Crichton 2008). As a result it has the ability to modulate activity of these enzymes and processes. This is observed in Swenson et al.'s (2014) study on magnesium modulation of actin binding in smooth muscle of the rat. Further, magnesium plays a role in the conductivity of excitable membranes by binding to the calcium channels and modulating their activities. This is seen in rat ventricular myocytes as increasing extracellular concentrations of magnesium cause a change in voltage gated calcium channel gating kinetics (Wang and Berlin 2007).

In aragonite otoliths, magnesium is thought to interact with collagen to change the structure of collagen molecules and induce aragonite and vaterite formation during otolith biomineralization (Feng 2011). In hydroxyapatite, magnesium along with sodium and strontium has kinetically driven roles during biomineralization. They can substitute with cations, anions such as fluoride, polyatomic anion carbonates, and with hydroxyl in the crystal matrix (Mann 2001, Omelon et al. 2009).

Essential trace elements such as zinc are incorporated into both calcium carbonate and hydroxyapatite structures. Zinc has been found to incorporate into over 300 enzymes (Lee et al. 2015) and is known to have a structural and catalytic role in association with carbonic anhydrase (Eide 2006, Crichton 2008). In association with bony structures, we can infer zinc is most likely incorporated with the organic matrix rather than with the calcium carbonate or hydroxyapatite minerals during biomineralization due to poor efficiency kinetics for the replacement of minerals in both otolith and hydroxyapatite bone structures (Mann 2001), and its role in enzyme function (Lee et al. 2015).

Nonessential elements such as strontium, lead and barium are most likely transported across epithelial cells by way of calcium transport proteins given their similar atomic size and valence charge (Mann 2001). It has been demonstrated in mammals, that supra-physiological concentrations of strontium inhibit the release of the calcium regulatory hormone parathyroid hormone (Brown et al. 1990) and replaces calcium in blood clotting and muscular contraction but with lesser effects (Pors Nielsen 2004). The lowered efficacy of strontium at stimulating these physiological events speaks to its role and competitive ability in comparison to calcium. There is evidence, however, that strontium has an effect on physiological processes. Jensen et al. (1996), Ammann and Rizzoli (2003), and Ammann et al. (2007) showed a correlation between strontium content of bone and bone strength in mammals. Studies have observed that the administration of strontium decreases symptoms of osteoporosis in humans (Shorr and Carter 1952, McCaslin and Janes 1959, Marie 1996, Reginster et al. 2005, 2012). After the administration of strontium, decreased bone resorption and increased bone ossification is observed. No reported correlation is observed between strontium deficiency and osteoporosis (Zhang et al. 2002) and negative health effects of strontium deficiency are undetected (Pors Nielsen 2004). Due to the lack of negative effects from strontium deficiency, it is classified as a non-essential element (Chowdhury and Blust 2011). This further indicates an absence of evolutionary pressure on uptake of strontium from the environment and the need to develop direct homeostatic control over extracellular and intracellular concentrations (Pors Nielsen 2004). Barium has no known positive biological role but has known toxic effects. Barium ions can block potassium channels, causing negative effects in excitable tissues (Frank and Rohani 1982). It is

not known if strontium has a similar effect despite the similarities of these elements in the ionic form.

In aragonite and hydroxyapatite biomineralization processes, strontium, lead and barium are known to substitute for calcium (Mann 2001, De Pontual and Geffen 2002, Allemand et al. 2008, Omelon et al. 2009) and it is these substitution end products within the biominerals that have been measured with element signatures being used to infer life history events, such as migration between environments, stock discrimination or exposure to contaminants (Elsdon and Gillanders 2003, 2006, Melançon et al. 2005, 2008, 2009, Elsdon et al. 2008, Brown and Severin 2009).

Biomineral isoforms (aragonite, vaterite or calcite) account for some variation in elemental insertion of calcium carbonate structures. Thus, comparisons among elements in different crystal morphologies are invalid. During calcium deposition, aragonite favours strontium insertion in replacement of calcium (Markwitz et al. 2000, Mann 2001, Doubleday et al. 2014). Alternatively, vaterite favours magnesium insertion in replacement of calcium (Melançon et al. 2005, 2008). As mentioned previously, strontium, magnesium and sodium are favoured for substitution with cations, anions, polyatomic anion carbonates; and hydroxyl in the crystal matrix, in the hydroxyapatite biomineralization process (Mann 2001, Omelon et al. 2009).

Recent studies have begun to implicitly test element and isotope incorporation with the physiological state of individual fish. Results of these studies suggest physiological control over element fractionation and partitioning (barium, copper, lead, lithium, manganese, magnesium, potassium, rubidium, selenium, strontium, and zinc) is significant for marine fish such as European Plaice (*Pleuronectes platessa*) (Sturrock et

al. 2014, 2015). Growth rate and/or sex were shown to play a role in otolith element concentrations such as strontium, barium, potassium, magnesium, and lithium when examined in conjunction with environmental availability. Other elements such as zinc and manganese had positive relationships to changes with environmental salinity. This demonstrates a complex relationship between otolith element incorporation and the external environment. Magnesium, barium and strontium are all members of the alkaline earth metal group and as such share similar chemical properties. Due to differences in their role in biological systems there are, however, differences in the ion transport processes.

2.5 Substitution, Transport Kinetics for Movement and Binding Affinity in Trace Elements

Non-essential elements such as strontium, barium and lead are present in organisms and tissues as their ionic form is sufficiently close to ions such as Ca^{2+} and Mg^{2+} that their movement across biological membranes is predictable and they are often incorporated into biological matrices in place of Ca^{2+}. Indeed approximately 50 trace elements are present in calcium-based structures (De Pontual and Geffen 2002, Sturrock et al. 2012). The presence of non-essential elements in biological tissues does not normally impede physiological function as the free forms of these elements are present in the environment at, often orders of magnitude, lower in concentration in comparison to the essential elements (Bäuerlein et al. 2007). Nonetheless, the ability of the ionic forms of non-essential elements to compete with calcium transport processes

and functional roles plays a large part in their presence and availability for subsequent biomineralization (Mann 2001, Bäuerlein et al. 2007, Crichton 2008).

2.5.1 *Physiological Control of Magnesium*

For fish, homeostatic regulation of any essential element between the fish and the environment is regulated at three primary sites: the gills, the gastrointestinal tract and the kidneys. In Mozambique Tilapia (*Oreochromis mossambicus*), the primary route of entry for magnesium was via the intestine when dietary sources were high. However, when dietary sources were low, intestinal uptake of magnesium increased in efficiency such that overall uptake rates were equivalent to those of the gill. Further, renal and extra-renal excretion of magnesium decreased when dietary magnesium was reduced (Bijvelds et al. 1998). Interestingly, Bijvelds et al. (1996) reported an increased abundance of chloride cells in the gills of Mozambique Tilapia after the fish was introduced to a low magnesium diet.

At the cellular level there are several known transport proteins that play a role in the uptake and excretion of magnesium. One group of transporters that has been linked to magnesium uptake in fruit flies (*Drosophila*) and vertebrates consists of the TRPM6 and TRPM7 proteins. It appears that on its own TRPM7 acts as a calcium transport protein, however, when present with TRPM6 the affinity for magnesium of both channels is greater than the affinity for calcium (Runnels et al. 2002, Monteilh-Zoller et al. 2003). Despite an initial report that TRPM6 could function as a magnesium transporter alone, there is evidence to suggest that TRPM6 requires the presence of TRPM7 to function in mammals and frogs (*Xenopus*) (Chubanov et al. 2004, Voets et al.

2004). Modulation of the function between calcium transport and magnesium transport is likely through the presence of the TRPM6 protein which is only found in select cell types, whereas TRPM7 has been observed in all cell types studied (Chubanov et al. 2004).

Claudin proteins found in tight junctions between epithelia cells are also involved in the cellular uptake of magnesium. Claudin-16 has been found to play a role in allowing magnesium to pass through the paracellular pathway in the proximal tubules of human kidneys (Konrad et al. 2006). There is also evidence of a human sodium-magnesium exchanger that uses the sodium gradient created by sodium-potassium ATPase to help shuttle magnesium ions out of the cell (Standley and Standley 2002). The gene SLC41A1 that codes for the sodium-magnesium exchanger has been found in the Japanese Pufferfish (*Takifugu rubripes*) and Megafugu (*T. obscurus*) (Islam et al. 2013). Genes found in both species were localized to kidney tissue specifically the proximal tubules (Islam et al. 2013). This, coupled with evidence from mammalian kidney cells that the transport protein is located on the apical surface (Islam et al. 2013), may indicate a role in the resorption of magnesium from the urine.

The MagT1 protein has also been shown to play a role in magnesium uptake. In knockdown studies, a decreased concentration of magnesium was present in teleost embryos lacking the MagT1 protein (Zhou and Clapham 2009). MagT1 also plays a role in teleost fish, shown by the effects of a knockdown on zebrafish embryos causing death of the embryos (Zhou and Clapham 2009).

2.5.2 *Physiological Control of Zinc*

As with magnesium, the uptake route of zinc depends on the availability of zinc from both the environment and dietary sources (Van Campenhout et al. 2009). When environmental zinc is highly available there is proportionally less zinc taken up by the intestine, however, if the environmental concentrations of zinc are low the majority of zinc uptake occurs across the intestine of the Common Carp (Van Campenhout et al. 2009).

After uptake zinc travels throughout the plasma to target tissues either as a free ion or bound to metalloproteins such as metallothionein (Coyle et al. 2002). Unlike magnesium, zinc is not commonly stored in large organs such as bone. Zinc(II) can, however, be stored in bone as it forms a divalent cation in solution and can replace calcium in the bone crystal matrix, but this is only a small percentage and cannot be considered storage to the same extent as magnesium or calcium (Hambidge et al. 1986). Because of this, loss of zinc via excretion routes must be minimized to ensure proper concentrations of zinc in extracellular and intracellular fluids. Despite the need for homeostatic control of zinc in extracellular and intracellular fluids there is little evidence thus far of control mechanisms. Administration of interleukin-1 in rats caused a decrease in extracellular concentration of zinc (Cousins and Leinart 1988). Cousins and Leinart (1988) also observed an increase in metallothionein production in rats after interleukin-1 injection. An increase in the carrier protein could explain the decrease in zinc concentrations observed. Further to this Müntzing et al. (1977) found that removal of rat testes decreased the uptake of zinc in the dorsolateral prostate, and upon injection of estradiol saw a two-fold increase in the uptake of zinc in this tissue. Müntzing et al. (1977) also found a possible role of prolactin as it affected uptake rates

of zinc in the dorsolateral prostate. However, the effects of prolactin, removal of testes, or application of estradiol were not seen in uptake of zinc by the ventral prostate (Müntzing et al. 1977), suggesting a very tissue specific role of these hormones in zinc homeostasis. More recently it has been observed that increased dietary zinc is related to decreases in expression of zinc transporters in the jejenum of pigs and increased production of metallothionein, which suggests control to inhibit high concentrations of zinc entering the circulation (Martin et al. 2013).

Examination of zinc transport in fish cells is limited so here we briefly discuss some general aspects of zinc cellular transport. In mammals zinc transporters belong to the ZIP or CDF family (Eide 2006). The human ZIP protein or ZIP2 has been shown to be energy independent and relies on bicarbonate gradients to bring zinc into the cell (Gaither and Eide 2001). The CDF family, or cation diffusion facilitator, is a group of transporters that are not specific for zinc and allow multiple cations across the plasma membrane down concentration gradients and out of the cell (Eide 2006). In zebrafish embryos, the expression of specific zinc transporters ZnT1, ZnT5 and ZIP10 and the carrier protein metallothionein was positively correlated with environmental zinc concentrations but not for ZIP3 suggesting a strong link between the mammalian zinc transporters and the homeostatic regulation of zinc in fish (Zheng et al. 2008).

2.5.3 *Physiological Control of Strontium*

There is no evidence thus far of a specific strontium transport protein in vertebrates. Strontium is thought to act as a calcium mimic by replacing calcium through calcium uptake mechanisms (Chowdhury and Blust 2011). Presumably this occurs simply

through diffusion gradients and substitutions. In consideration of strontium and calcium relative abundances, strontium enters the organism at a much lower rate than calcium, which may suggest selectivity and/or competition between these elements for uptake (Chowdhury and Blust 2011). The calcium uptake mechanism in teleost fish will be briefly discussed given its role in strontium uptake.

Calcium in teleosts is taken up predominately across the gills (Flik et al. 1995). However, in some fish species there is evidence of gastrointestinal uptake (Flik et al. 1990, Flik and Verbost 1993, Allen et al. 2011, Genz et al. 2013). The current model of intestinal calcium uptake in teleost fish is similar to the mammalian model where there are both transcellular and paracellular pathways in which calcium or strontium will pass across the epithelia cell layer. Transcellular transport is mainly facilitated by passive diffusion of calcium (or strontium) through the epithelial calcium channels or transient receptor potential channels (TRPV5 and TRPV6) on the apical cell membrane. Intracellular calcium concentrations are maintained at low concentrations by the presence of binding proteins in combination with sarcoendoplasmic reticulum calcium ATPase (SERCA) actively pumping calcium into the sarcoendoplasmic reticulum out of the cell cytosol (Peng et al. 2003, Hoenderop et al. 2004). Calcium (or strontium) is then removed from the cell on the basolateral membrane via the sodium-calcium exchanger (NCX), or via plasma membrane calcium ATPase (PMCA) (Hildmann et al. 1982, Flik et al. 1990, van Baal et al. 1996, Larsson and Nemere 2002). NCX is powered by the inward sodium gradient created by sodium-potassium ATPase. In contrast, ATP is used by PMCA to move calcium (or strontium) out into the extracellular fluid against its own concentration gradient.

Support for the hypothesis that strontium movement across epithelial cells is facilitated by calcium transport mechanisms was shown in isolated synaptosomes from the rat brain using radioactive strontium and calcium isotopes. Influx of both strontium and calcium was reported with calcium permeability typically being twice that of strontium (Nachshen and Blaustein 1982). In the Common Carp uptake of calcium and strontium was competitive and through similar routes as influx rates were influenced by concentrations of the opposing ion (Chowdhury and Blust 2001).

As there is no specific strontium uptake machinery there is also no specific homeostatic regulation of strontium in extracellular and intracellular fluids. Instead strontium is subject to the hormonal control of calcium uptake (Chowdhury and Blust 2011) and remobilisation from hydroxyapatite bony structures (Witten and Huysseune 2009).

2.5.4 *Physiological Control of Barium*

Barium, in parallel to strontium, is assumed to be a calcium mimic. It enters the organism via the calcium uptake mechanism described previously and has a higher V_{MAX} (the maximum reaction rate that is observed at saturating substrate concentrations) in mammalian nerve cells than calcium but a much lower binding affinity to the shared transport protein (Nachshen and Blaustein 1982).

2.6 Evidence of Endocrine Regulation of Otolith, Scale and Fin Formation

Calcium homeostasis is controlled through hypo- and hyper-calcemic hormones such as stanniocalcin, calcitonin, estrogen, melatonin, calcitrol, retinoic acid, cortisol, growth

hormone, prolactin, parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP). These hormones can be up- or down-regulated during periods of stress (Abbink et al. 2004, Stolte et al. 2008), natural circadian rhythms (Abbink et al. 2008), vitellogensis and reproduction (Persson et al. 1997, Suzuki et al. 2000), smolting events, and osmoregulation (Mayer et al. 1997a, 1997b, García-Allegue et al. 2001). In fish, stanniocalcin is a hypocalcemic hormone that works to inhibit the influx of calcium from water at the gill and intestinal interface (Verbost et al. 1993, Wagner et al. 1998).

Calcitonin is a hypocalcemic hormone that has inhibitory effects on bone calcium resorption in both mammals and fish. Within mammals and fish, it is known to lower calcium serum levels by inhibiting resorption by direct action on osteoclast activity and increasing ALP production in osteoblasts (Chakrabarti and Mukherjee 1993, Oughterson et al. 1995, Wimalawansa 1997). Calcitonin in fish is associated with reproductive physiology due to a positive relationship with estrogen levels. It is also thought to play a role in daily calcium homeostasis associated with balancing calcium following a meal. In goldfish it was demonstrated that calcitonin acted on scale osteoclasts to suppress activity of this cell type and keep plasma calcium constant post feeding (Suzuki et al. 2000). Within Mozambique Tilapia bone and scales, calcitonin showed stimulated bone growth (Yoshikubo et al. 2005). Whereas, calcitonin in Spotted Catfish (*Corydoras puntatus*) caused hypocalemia and suppressed both plasma TRAcP and ALP activities along with the reduction in urinary excretion of hydroxyproline (Mukherjee et al. 2004).

Melatonin (*N*-acetyl-5methotrytamine) is a light sensitive hormone, secreted from the pineal gland and retina of all vertebrates, known to influence circadian and cirannual

biorhythms (Davis 1997, Dubocovich and Markowska 2005). No storage mechanisms for melatonin exist within the pineal gland and thus levels of this hormone directly reflect synthesis activity. In association with biominerals, melatonin influences calcium homeostasis by acting upon both osteoclast and osteoblast activities. Within goldfish scales, down-regulation of both TRAcP and ALP by melatonin was observed (Suzuki and Hattori 2002). Production of melatonin is stimulated by increasing plasma calcium levels and inhibited following hypocalcemia conditions. Reproduction, osmoregulation, photoperiod and water temperature in fishes can all act to interfere with melatonin synthesis (Mayer et al. 1997a, García-Allegue et al. 2001, Kulczykowska 2001). Melatonin has been demonstrated to play a role in development and timing of parr-smolt transformation in Atlantic Salmon (Porter et al. 1998).

Within otoliths, the circadian cycle, and thus melatonin production has been shown to influence accretion. Variation from day to night in endolymph characteristics such as pH and total [CO_2] has been observed. Proximal calcification can occur under low alkaline endolymph pH (~7.4, saturation coefficient ~1) allowing for smaller concentrations of ions to reach the saturation state of aragonite and induce biomineralization (Payan et al. 2004). Protein and collagen are favoured and reach maximum concentrations during the day to form the organic matrix (Guibbolini et al. 2006), although variation cannot be explained by otolith accretion since <1% of the proteins formed are used in the daily formation of the otolith matrix (excess). Ca^{2+} and HCO_3^- concentrations over the course of the night-time period increase, reaching a maximum at the end of night/early morning to induce an increase in aragonite saturation

in the proximal endolymph (Mugiya et al. 1981, Guibbolini et al. 2006, Allemand et al. 2008).

Calcitriol and retinoic acid are both active metabolites created by vitamins D and A respectively. They are known to aid in vision, growth, reproduction, embryological development, and calcium and phosphate homeostasis (Lock et al. 2007, Ørnsrud et al. 2009). Calcitriol is a hypercalcemic hormone important to bone formation and in stimulating intestinal calcium absorption within fish (Lock et al. 2007). Retinoic acid and calcitriol act together elevating osteoblast activity thereby increasing bone formation and supressing the action of osteoclasts (Wendelaar Bonga et al. 1983, Ørnsrud et al. 2009). Chronic exposure has been shown to increase bone formation in rats but also impair mineralisation processes (Wronski et al. 1986). Further, calcitriol has been shown to inhibit and induce the expression of collagen-I mRNA expression depending on factors such as species, osteoblast differentiation stage and duration of exposure (Van Leeuwen et al. 2001). Within humans and rats these hormones induce osteocalcin, MGP, osteopontin and ALP expression (Van Leeuwen et al. 2001). Calcitriol in fish increased mRNA expression of epithelial calcium channels in gills and intestinal epithelia (Fenwick et al. 1984). Further the administration of calcitriol stimulated the uptake of zinc in Rainbow Trout (Qiu et al. 2007). Production of retinoic acid is known to interfere with osteoblast activities by inhibiting the expression of osteocalcin mRNA in mouse osteoblasts, reducing collagen mRNA synthesis in rat and chicken osteoblasts and increasing collagenase mRNA expression and collagen degradation in rats (Dickson and Walls 1985, Kim and Chen 1989, Varghese et al. 1994, Cohen-Tanugi and Forest 1998). Within Atlantic Salmon, dietary levels of retinoic

acid can cause skeletal deformities by interfering with plasma calcitriol and associated bone physiology (Ørnsrud et al. 2002). Growth hormone along with calcitrol, melatonin and prolactin is involved with smoltification, stimulating growth and improving hypo-osmoregulatory capability for life in a marine environment (Lock et al. 2007).

Estradiol is known to effect bone metabolism by regulating osteoblast activity during teleost reproduction as well as playing a role in osteoblast activity and differentiation in scale regeneration (Yoshikubo et al. 2005). In addition, estradiol is a reproductive hormone that enhances vitellogenin, a major component of egg protein (Norberg et al. 1989). Calcium and calcium mimics are remobilised from bony structures and diverted into egg production during egg development. The administration of estradiol-17β (E_2) within fish is known to induce vitellogenin synthesis and the binding of calcium ions to vitellogenin (Abbink and Flik 2007, Persson et al. 1997). Within Rainbow Trout E_2 was shown to mobilize calcium from scales, due to osteoclast activity and enhance intestinal and kidney reabsorption (Persson et al. 2000). Prolactin is also known to stimulate vitellogensis in fish (Flik et al. 1994).

Thyroid hormones (T_3 and T_4) in vertebrates are associated with control of growth, development and metabolism (Brent et al. 1991, Epstein and Brent 1994). In Indo-Pacific Tarpon (*Megalops cyprinoides*) and Mozambique Tilapia exogenously administered T_3 was found to disrupt the production and transport of ions from the otolith saccular epithelium into the endolymph, influencing endolymph alkalinity (pH=8). Hyper-thyroidism was demonstrated to cause an increase in otolith growth whereas hypo-thyroidism retarded or completely inhibited otolith growth (Shiao and Hwang 2004, Shiao et al. 2008).

Glucocorticoids, such as cortisol and corticosterone are involved in the endocrine stress response, growth, metabolism, reproduction and immunity in vertebrates whereas the mineralocorticoid, aldosterone plays a role in mineral regulation within mammals but is absent in fish (Mommsen et al. 1999, Stolte et al. 2008). Instead glucocortiocid and mineralocorticoid actions of cortisol in teleosts are facilitated through the receptors at the target site (Bury et al. 2003). Increased cortisol secretion in Rainbow Trout was observed during hypocalcemic stress allowing for calcium homeostasis by inducing the uptake of environmental calcium at the gills (Flik and Perry 1989). Further, cortisol can stimulate zinc signalling pathways and expression of metallothioneins and ZnT1 in Rainbow Trout gill epithelial cells (Bury et al. 2008).

Parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP) are both present in teleosts (Parsons et al. 1978, Abbink and Flik 2007). These hormones are hypercalcemic in nature and are involved in the regulation of calcium uptake from the environment, regulating calcium resorption from scales in bone formation, blood clotting, regulation of enzymatic processes, modulating permeability and excitability of plasma membranes (Rotllant et al. 2005, Bevelander et al. 2006, Abbink and Flik 2007). PTH and PTHrP both suppress osteonectin gene expression in teleosts (Guerreiro et al. 2001, Nakajima et al. 2002, Anjos et al. 2005). In Sea Bream larvae, PTHrP has the ability to stimulate whole body calcium influx and reduce calcium efflux (Abbink and Flik 2007). Further, PTHrP levels reflect not only calcium but phosphate and magnesium elemental availability in teleosts although the exact role of mineral handling is unknown (Abbink and Flik 2007). PTHrP was correlated to both calcitrol and estrogen levels within Sea Bream suggesting interactive effects of these

hormones in the regulation of calcium metabolism (Bevelander et al. 2006). Specifically, E_2 was shown to stimulate secretion of PTHrP into the blood preceding a rise in plasma calcium levels (Abbink and Flik 2007) further PTHrP was shown to stimulate cortisol release in a dose-dependent manner (Rotllant et al. 2005).

2.7 Application of Isotope Analysis and Isotopic Fractionation to Biomineral Studies

Preferential uptake of light versus heavy element isotopes through physiological processes has been observed in experimental studies. Light isotopes are often preferred due to the thermodynamic properties resulting in an energetically less expensive passage through non-specific ion transport pathways (Kedem and Essig 1965). However, isotopic fractionation can be mass dependent or mass independent. Mass dependent fractionation defines the rate of separation and its correlation to difference in mass between the isotopes, whereas mass independent fractionation shows no correlation between the two (Sharp 2007). Elements such as carbon, nitrogen and oxygen have been extensively investigated for isotopic discrimination factors (Schoeninger and DeNiro 1984, Kalish 1991b, Thorrold et al. 1997, Tohse and Mugiya 2001, Godiksen et al. 2010).

Fractionation events in the uptake of isotopes can cause a deviation of isotopic ratios away from the hypothetical ratios. Failure to determine the presence of isotopic fractionation could lead to misinterpretation of data. If the ratio has been modified by isotopic fractionation and there is no correction, researchers may observe spurious differences. However, the effects of isotopic fractionation when known can be used to

elucidate true differences. There has been evidence of isotopic fractionation in several elements in biological systems (Schoeninger and DeNiro 1984, Kalish 1991a, Thorrold et al. 1997, Godiksen et al. 2010). Notably element isotope discrimination factors differ between tissue and biomineral types as discussed below.

2.7.1 *Magnesium and Zinc Isotopes*

Magnesium isotopes have not been examined in teleost fish bony structures at present; however, studies have been conducted on other organisms to understand magnesium isotope fractionation. Magnesium ratios between the stable isotopes $^{26}Mg/^{24}Mg$ are correlated with dietary choices of the species (Martin et al. 2015). Herbivores had lower $^{26}Mg/^{24}Mg$ ratios when compared to omnivores. Magnesium fractionation may possibly occur in the formation of chlorophyll (Black et al. 2006, Martin et al. 2015) or due to an enrichment of ^{26}Mg from muscle rather than bone (Martin et al. 2015). Black et al. (2008) found that there was a preferential incorporation of heavier magnesium isotopes in a mass dependent fashion in wheat (*Triticum aestivum* L.). Further, there has been evidence of magnesium isotopic fractionation in several species of deep-sea corals (Yoshimura et al. 2011) where preferential precipitation of lighter magnesium isotopes in high magnesium calcite was observed in the 15 species examined. Isotopic fractionation was temperature dependent and allowed for the determination of environmental temperatures experienced by the corals. However, Yoshimura et al. (2011) also observed that in aragonite deposited by related species of coral there was no such preferential deposition of lighter magnesium isotopes. Evidence for both the

fractionation of magnesium isotopes in calcite and the lack of fractionation in aragonite was also seen in foraminifera species (Chang et al. 2004).

Zinc isotopic fractionation has not been examined in teleost fish bony structures at present; however, studies have been conducted on plants and other vertebrates. Evidence of partitioning of the zinc isotopes in several plant species has been observed with preferential accumulation of lighter isotopes (Deng et al. 2014). The authors found that the preferential accumulation in Alpine Pennygrass (*Noccaea caerulescens*) of lighter zinc isotopes could be due to the rapid uptake of zinc into the root causing isotope diffusion in the rhizosphere. They further observed a competitive effect of zinc concentrations on nickel fractionation. This result suggests that other ions may influence the fractionation of isotopes of ions from another element. The environmental concentration of zinc has also been observed to affect zinc isotope fractionation. Smolders et al. (2013) observed that soils deficient in zinc preferentially accumulated heavier isotopes of zinc.

There is also evidence of differences in zinc isotope ratios between trophic levels (Jaouen et al. 2013). Jaouen et al. (2013) found that partitioning of the metals throughout the body explains some of difference observed. However, Jaouen et al. (2013) suggest that the difference seen in isotopic ratios may be due to differences in uptake between carnivores and herbivores; this explanation has yet to be tested.

2.7.2 *Strontium Isotopes*

Natural strontium isotopic ratios can vary depending on the geographic location. The differences in aquatic strontium isotopic ratios in different geographic locations are due

to weathering of the underlying rock and their strontium isotopic ratios (Horton et al. 1999, Kennedy et al. 2000, Hegg et al. 2013, Brennan et al. 2014). Strontium ratios vary in the rock depending on the age and concentrations of strontium and rubidium; radioactive ^{87}Rb undergoes nuclear decay and becomes ^{87}Sr (Faure and Powell 1972). Due to this natural phenomenon, the determination of the organism's origin by examining the strontium ratio in their otoliths, bones or teeth has been highly successful (Font et al. 2015, Hartman et al. 2015, Loewen et al. 2015, Valentine et al. 2015). This technique has been used to determine the origin of ancient humans (Font et al. 2015, Hartman et al. 2015, Valentine et al. 2015) and fish such as, Slimy Sculpin (*Cottus cognatus*), Dolly Varden Char (*S. malma malma*), Trahira (*Hoplias malabaricus*), Aracu (*Schizodon fasciatus*), and Chinook Salmon (Barnett-Johnson et al. 2008, 2010, Hobson et al. 2010, Pouilly et al. 2014, Brennan et al. 2015a, Loewen et al. 2015).

Evidence for partitioning of strontium isotopes in biological tissue is observed in several studies where the $^{87}Sr/^{86}Sr$ ratio has been observed to change when strontium is incorporated into biological tissue. This process has been quantified (Krabbenhöft et al. 2009) and therefore the data can be corrected. However, research has shown no fractionation in strontium isotopes from dietary sources (Blum et al. 2001, Flockhart et al. 2015). Further studies are required to determine its effectiveness in the application of identifying differences between trophic levels (Sillen et al. 1995, Blum et al. 2000, 2001).

Stable strontium isotopes have been applied as a marking technique for bony structures in fish. This technique has been successfully used to mark the otoliths and/or fin rays of several fish species including: Lake Sturgeon (*Acipenser fulvescens*), Golden

Perch (*Macquaria ambigua*), Atlantic Salmon, and Murray Cod (*Maccullochella peelii*) (Smith and Whitledge 2011, Woodcock et al. 2011, de Braux et al. 2014, Warren-Myers et al. 2015). The stable isotope marking technique changes the isotopic ratio in the tissues of the marked organism by placing them in an environment with a manipulated ratio.

Fractionation may occur in the incorporation of strontium from the environment into the bone crystal. In juvenile Lake Sturgeon administered with the rare Sr isotope (^{86}Sr) at a dose of 2mg.kg^{-1} ^{86}Sr in Sturgeon Ringers by intramuscular injection the $^{88}Sr/^{86}Sr$ ratio differed in muscle, fin ray and vertebrae as measured by solution based inductively coupled mass spectrometry (**Figure 2.3**). Further the ratio of these two isotopes departed from the natural abundance of the strontium isotopes suggesting the potential for mass-dependent fractionation in Lake Sturgeon bony tissues; however, further research is required to verify these findings.

2.7.3 *Barium Isotopes*

Barium isotopes have been shown to be an effective marking tool for both fish and cephalopods (Thorrold et al. 2006, Munro et al. 2008, Williamson et al. 2009, Pecl et al. 2011). Despite the effectiveness of using the stable isotope of barium as a marking technique there has been little study of the underlying mechanisms involved with barium uptake and incorporation in fish perhaps due to the inhibitory nature of this ion on potassium transport. Evidence of barium stable isotopic fractionation in the natural environment is observed in globally collected barium samples that significantly differed in their isotopic signatures (Von Allmen et al. 2010). In addition, using similar methods

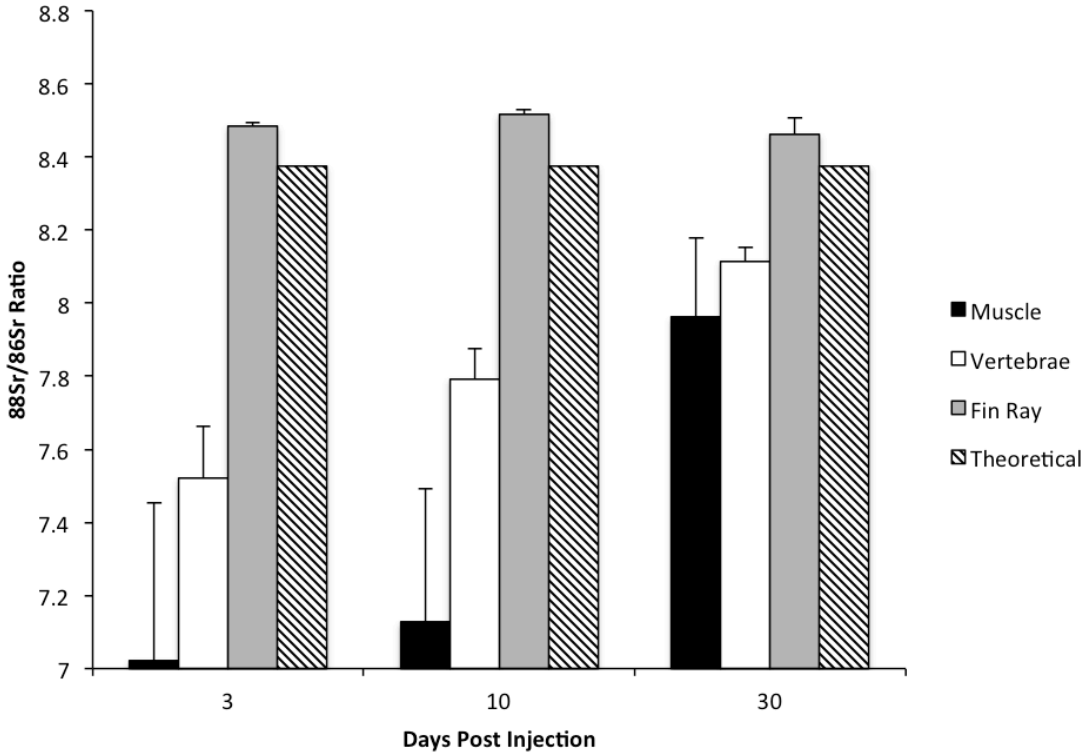

Figure 2.3: $^{88}Sr/^{86}Sr$ ratio in juvenile Lake Sturgeon muscle (black bars), vertebrae (open bars) and fin ray (hatched bars) following administration of 2mg.kg^{-1} ^{86}Sr in Lake Sturgeons Ringers by intramuscular injection. Tissue was removed 3, 10 or 30 days post administration and digested in nitric acid and then hydrogen peroxide. $^{88}Sr/^{86}Sr$ ratio was determined from solution based inductively coupled plasma mass spectrometry on the resultant fluid digests. Methods are provided *in* Carriere et al. (2016).

as described above for Sr an intramuscular injection of a 2mg.kg^{-1} dose of ^{137}Ba in Lake Sturgeon appeared to result in a mass-dependent fractionation of barium isotopes in muscle, fin ray and vertebrae, as the corrected ratios of ^{138}Ba/^{137}Ba were lower than the predicted values from barium's natural abundance (**Figure 2.4**). Similar to the case with strontium isotopes described previously, calcium remobilisation may also provide explanation into the possible mass-dependent fractionation in Lake Sturgeon hydroxyapatite boney tissues.

2.8 Perspectives and Conclusions

The application of elements in biominerals led several research groups to link elemental presence to salinity in determining life history events such as migration timing. In examination of data from Arctic freshwater systems (**Figure 2.2**) large fluctuations and variations exist among locations in otolith elements such as strontium, a key element used to examine migration histories and habitat use in fish. Brown and Severin (2009) indicate that otolith strontium/calcium is driven by environmental strontium/calcium but highlight that anomalies exist in this relationship. Indeed **Figure 2.2a,** alongside a growing body of literature, further demonstrates these anomalies and our lack of complete understanding of the mechanisms regulating element incorporation in otoliths. We contend that the missing piece in many cases is the lack of physiological information on calcium uptake and variation between freshwater and saline water calcium and other elements (Campana 1999, 2005, Elsdon et al. 2008, Brown and Severin 2009). A switch from freshwater to seawater environments changes the environmental calcium available to fish for calcium homeostasis as well as the

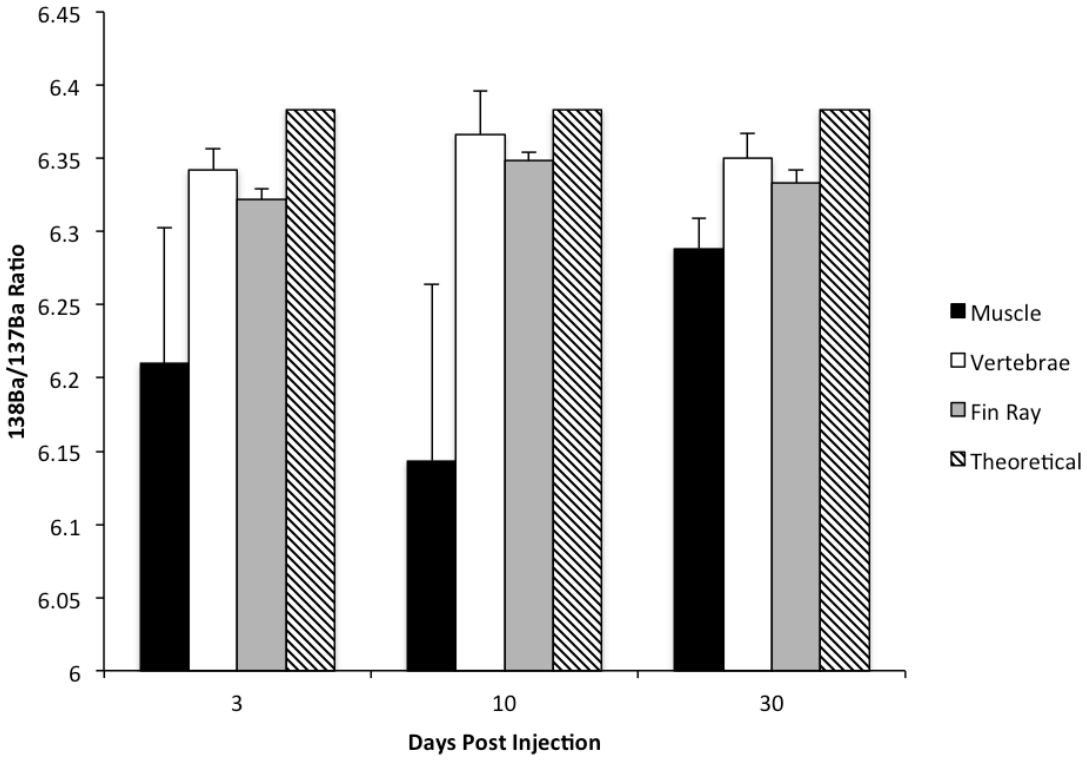

Figure 2.4: $^{138}Ba/^{137}Ba$ ratio in juvenile Lake Sturgeon muscle (black bars), vertebrae (open bars) and fin ray (hatched bars) following administration of 2mg.kg^{-1} ^{137}Ba in Lake Sturgeons Ringers by intramuscular injection. Tissue was removed 3, 10 or 30 days post administration and digested in nitric acid and then hydrogen peroxide. $^{138}Ba/^{138}Ba$ ratio was determined from solution based inductively coupled plasma mass spectrometry on the resultant fluid digests. Methods are provided *in* Carriere et al. (2016).

associated concentrations of elements and isotopes examined in biominerals. In examination of whole body calcium homeostasis of Sea Bream larvae, Guerreiro et al. (2004) found that overall environmental calcium concentration was the main factor in calcium uptake whereas salinity played only a minor role. In support, studies by Sturrock et al. (2015) on marine fish demonstrate the importance of linking physiology to biomineral processes. Variation in elements within Sturrock et al.'s (2014, 2015) studies of marine fish indicate that physiological variables influence incorporation rates when environmental conditions are relatively stable. In this review, we present a multidisciplinary approach that examines the present application of element and isotopic techniques, the biogeochemical processes of hard structure mineralisation of otoliths, fins and scales, the physiological uptake processes, and isotope discrimination. This broad perspective allows for increased understanding of element presence in hard structure biomineralization within fish and thus provides a stronger basis for future fine-scale data interpretation in the examination of life history events and habitat use of fish.

The key to understanding element presence and concentration in hard structures initially lies with understanding calcium homeostasis. Environmental calcium entry to a fish is controlled at the gills and intestine. Hormones such as stanniocalcin and PTHrP are up- or down-regulated to maintain strict control of calcium metabolism (Verbost et al. 1993, Wagner et al. 1998, Abbink and Flik 2007). In vertebrates, the majority of calcium (~99%) is bound within calcium phosphate and/or calcium carbonate structures (Flik et al. 1986). Calcium is remobilised from calcium phosphate structures through osteoclast-like action in fish. Once incorporated into calcium carbonate structures, it is no longer available for remobilisation. During periods of vitellogensis and reproduction,

growth, circadian rhythms, and circannual rhythms, various hormones such as melatonin, retinoic acid, estrogen, and calcitrol can act on calcium homeostasis to either suppress osteoblast-like activity to remobilise calcium or alternatively suppress osteoclast-like activity and enhance bone growth (Mayer et al. 1997a, Persson et al. 1997, Suzuki et al. 2000, García-Allegue et al. 2001, Abbink et al. 2004, 2008, Stolte et al. 2008).

Understanding functionality of an element to an organism's life processes is crucial when applying its presence to life history events. The ability of non-essential life elements such as strontium, and barium and some essential life elements such as magnesium in calcium phosphates to replace calcium in the mineral structure during formation allows us to measure life history events such as migration. These non-essential life elements have an affinity to substitute for calcium due to similar chemical properties (Mann 2001, Bäuerlein et al. 2007). No known mechanism is present to specifically control their presence within an organism and thus they are available for non-specific incorporation into an organism solely via indirect control of calcium uptake mechanisms and to a lesser extent zinc and magnesium (Chowdhury and Blust 2011). Essential elements such as zinc and magnesium have their own distinctive uptake mechanisms independent of calcium (Runnels et al. 2002, Monteilh-Zoller et al. 2003, Martin et al. 2013). Both are thought to be taken up through dietary consumption and less so through environmental concentrations (Bijvelds et al. 1998, Van Campenhout et al. 2009). We hypothesize that there is a seasonal change in the protein matrix underlying calcium carbonate accretion due to the strong association of zinc to melatonin, calcitriol (Vitamin D) (Qiu et al. 2007) and cortisol (Bury et al. 2008) and the

likelihood of zinc's association with the protein matrix. Indirect evidence of zinc's strong association to hormones and annual cycling is observed in the zinc-otolith profiles of several salmonid species (Halden et al. 2000, Limburg and Elfman 2010).

As indicated in our review, calcium homeostasis is most likely the controlling factor to the presence of non-essential elements. It is then logical to infer that kinetics and competition for entry through calcium channels is significant to their presence within an organism and thus their concentration in biomineral structures. Epithelial calcium transport channels have an energetic preference to calcium in their uptake (Chowdhury and Blust 2011). Competition for these sites is likely dependent on environmental concentrations of both calcium and its mimics in relationship to the fish's internal calcium concentration maintained at ~2-3 $mmol^{-1}$ (or 64-96 mg/L) (Abbink et al. 2004). From our data in **Figure 2.2b** we can infer that average calcium levels within fresh water (19.10 to 72.50 mg/L) resulted in less strontium taken up as observed in otolith strontium concentrations (178-598 ppm). In this case calcium may outcompete strontium for uptake and availability for incorporation into biominerals. Where calcium concentrations are deficient in fresh water (0.05-3.70 mg/L calcium), more strontium may be incorporated as observed in otolith strontium (780-1606 ppm). Strontium may be a better competitor with calcium in more hypocalcemic conditions as the calcium transport mechanisms may be enhanced to maintain internal calcium concentrations. In comparison to most freshwater systems movement to sea water (>202 mg/L) would result in an exponentially larger amount of environmental calcium relative to other divalent cations. Once in seawater environments, fish must adjust to hypercalcemic conditions and combat the passive movement of calcium into the organism along

concentration gradients. In teleosts internal levels of calcium are maintained by excretion of the ion by intestinal and renal routes. Hypothetically, environmentally derived calcium ions could enter at higher rates and thus increases of non-essential element substitution could follow. Changes in strontium otolith concentrations are the net result of fish that have moved from freshwater to seawater environments (**Figure 2.5)**.

In examination of barium, a similar trend exists as observed in strontium but clearly indicates more complex competitive interactions between calcium and barium (**Figure 2.2d**). Understanding the environmental strontium (or barium) and calcium relationships within fresh water as described can provide a strong baseline for the investigation of migration movements of fish from fresh to sea water and prevent erroneous interpretations of high fresh water baseline values of otolith strontium observed in some systems.

In order to standardise and determine strontium within biomineral structures it is necessary to examine strontium/calcium ratios as part of the internal calculation (Craig et al. 2000) before applying an external standard such as NIST 610, 612 or MACS-3 to obtain an absolute strontium or barium concentration (Mokgalaka and Gardea-Torresdey 2006, Sylvester 2008, Chen et al. 2011, Jochum et al. 2012). These ratios are imperative since calcium-based structures are analysed and the elements in question are predominantly present due to their ability to substitute for calcium (Mann 2001, Doubleday et al. 2014). Strontium or barium will covary with calcium once internal to a fish. By independently examining environmental calcium and secondarily environmental strontium or barium relative to the presence of otolith strontium,

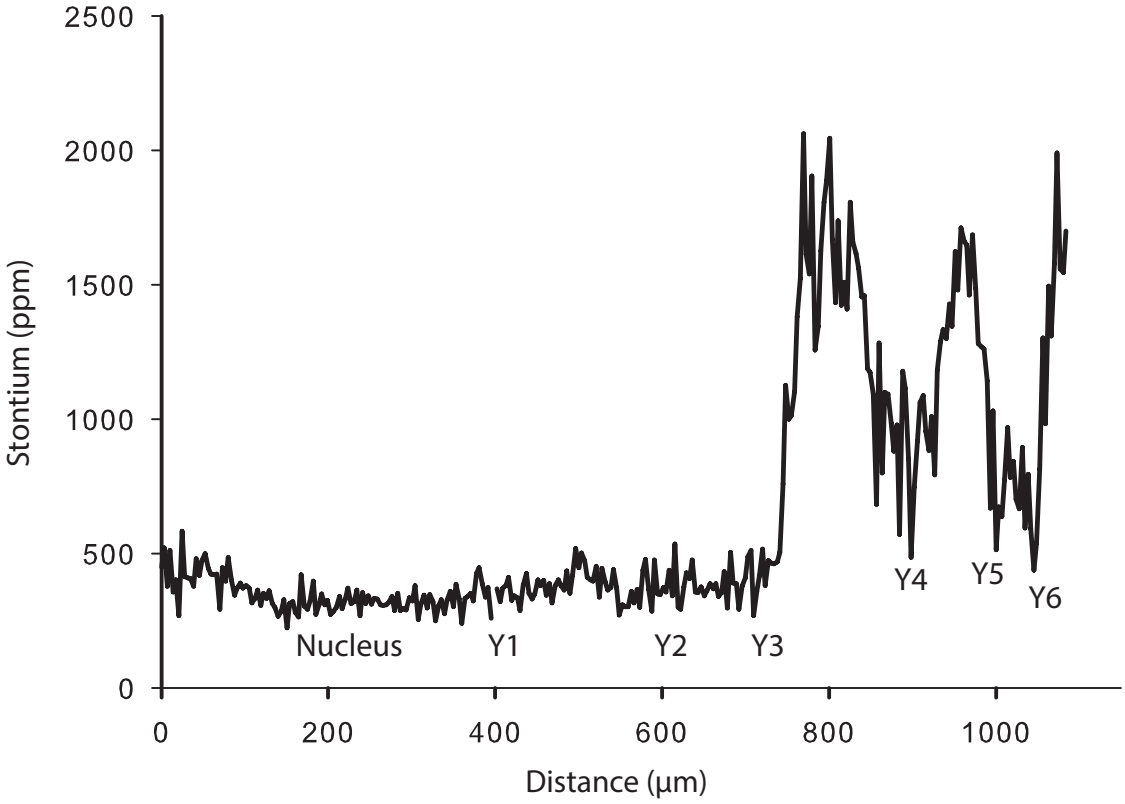

Figure 2.5: A profile of otolith strontium of a migrating Dolly Varden Char from the Rat River, NT (otolith preparation and analytical methods provided *in* Loewen et al. 2015) where (Y_i) indicates the annuli. Migration commenced at the age of 3 years.

underlying physiological mechanisms can be inferred as discussed above. Calcium concentration gradients from the environment to internal fish calcium homeostasis will dominate physiological condition (hyper-, balanced- or hypo-calcemic environments). Secondarily, reporting of environmental strontium allows for a better understanding of competition between strontium or barium and calcium at the gills under different calcemic conditions. This is observed in the end product of biomineralization. In support, Phillis et al. (2011) noted that otolith strontium/calcium ratios did not vary with salinity for Striped Bass (*Morone saxatilis*) in San Francisco Estuary, California. Calcium concentration in the fresh water flowing into the estuary was the primary factor controlling the response of otolith strontium/calcium ratios to salinity.

Environmental conditions, such as temperature and pH would further play a role in uptake kinetics of an element. Current investigations on temperature effects on element otolith presence are inconclusive (Fowler et al. 1995, Bath et al. 2000, Elsdon and Gillanders 2002), although these studies were not focused on calcium transport mechanisms, and did not examine environmental calcium independent of strontium/calcium ratios, thus are lacking key components. Another layer of complexity that warrants further experimentation is the phylogenetic perspective on element uptake. Initial studies demonstrate element variation among species found within similar environmental conditions (Limburg and Elfman 2010, Chang and Geffen 2013). Noteworthy, zinc concentrations affected biological zinc isotopic fractionation and magnesium isotopic fractionation was affected by temperature, due to their effects on transport kinetics (Yoshimura et al. 2011, Deng et al. 2014). Further research to examine the combined effect of element and isotopic fractionation in relation to

temperature, transport kinetics and ultimately incorporation into biominerals is warranted.

Isotopic fractionation is commonly reported for elements essential to life such as oxygen, carbon and nitrogen (Schoeninger and DeNiro 1984, Kalish 1991b, Thorrold et al. 1997, Tohse and Mugiya 2008, Godiksen et al. 2010). In examination of nonessential element fractionation of barium and strontium, our data show some tissues may fractionate more than others. Understanding calcium remobilisation in calcium phosphate hard structures within fish may provide explanation to this occurrence. In goldfish it has been demonstrated that scales are the quickest source of calcium mobilisation followed by fins and bony tissue (Persson et al. 1997, Shinozaki and Mugiya 2000). Otoliths are the last place of deposition for calcium and are considered a calcium sink (Ichii and Mugiya 1983, Shinozaki and Mugiya 2000), thus the observed otolith $^{86}Sr/^{87}Sr$ ratios are thought to be a direct relationship to naturally occurring environmental ratios (Barnett-Johnson et al. 2008, 2010, Hobson et al. 2010, Brennan et al. 2015a, 2015b, Loewen et al. 2015). Elements and their associated ions and isotopes previous to the marking periods could be released via osteoclast-like action and be available for re-incorporation after or during the marking period resulting in lower than expected isotopic ratios in Lake Sturgeon hydroxyapatite boney tissues. Mass dependent fractionation observed in the calcium phosphate bony structures of Lake Sturgeon to calcium carbonates are likely different as a result of these remobilisation rates in association to isotopic fractionation. Slight differences may exist between bony tissues (**Figures 2.3 and 2.4**) and should be considered when choosing a tissue for analysis. Further investigations of fin ray and otolith isotope ratios in the same species

would allow for the comparison of variation of isotopic fractionation among bony structures.

Remobilisation rates of calcium phosphate bony structures may also lead to loss of an element mark on a quicker time scale due to possible fractionation of the isotopes incorporated. When the isotopic mark is decreased in the ratio so too may be the rate of fractionation. Further investigations to examine signal retention in calcium phosphate bony structures should be examined in element marking studies.

A multidisciplinary approach to examining biomineralization in hard structures increases our understanding of underlying mechanisms to element and isotope incorporation. The importance of calcium is highlighted for non-essential elements (strontium and barium) whereas essential elements (magnesium and zinc) are regulated by their own uptake mechanisms. This strong emphasis on physiological maintenance particularly in terms of fish calcium homeostasis allows for new perspectives moving forward. Outcomes are stronger interpretations and understanding of hard structure elements and isotopes to elucidate the life histories of fish. Specifically, this contributes greatly to studies on estuarine habitat use by fish where mixed fresh and marine waters create unique calcium environments for element and isotopic uptake. Future experimental research is needed to examine hard structure non-essential element and isotopes in various calcemic environments. This will provide a better understanding of physiological responses to phylogeny, ontogeny, stress, and temperature for element competition with calcium during uptake.

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2.10 References

Abbink, W., Bevelander, G. S., Rotllant, J., Canario, A. V. M., and Flik, G. 2004. Calcium handling in *Sparus auratus*: effects of water and dietary calcium levels on mineral composition, cortisol and PTHrP levels. J. Exp. Biol. **207**: 4077–4084.

Abbink, W., and Flik, G. 2007. Parathyroid hormone-related protein in teleost fish. Gen. Comp. Endocrinol. **152**: 243–251. doi: http://dx.doi.org/10.1016/j.ygcen.2006.11.010.

Abbink, W., Kulczykowska, E., Kalamarz, H., Guerreiro, P. M., and Flik, G. 2008.

Melatonin synthesis under calcium constraint in gilthead sea bream (*Sparus auratus* L.). Gen. Comp. Endocrinol. **155**: 94–100. doi: http://dx.doi.org/10.1016/j.ygcen.2007.03.002.

Addadi, L., Berman, A., Oldak, J. M., and Weiner, S. 1989. Structural and Stereochemical Relations Between Acidic Macromolecules of Organic Matrices and Crystals. Connect. Tissue Res. **21**: 127–135. doi: 10.3109/03008208909050003.

Akimenko, M. A., Marí-Beffa, M., Becerra, J., and Géraudie, J. 2003. Old questions, new tools, and some answers to the mystery of fin regeneration. Dev. Dyn. **226**: 190–201. doi: 10.1002/dvdy.10248.

Akiva, A., Malkinson, G., Masic, A., Kerschnitzki, M., Bennet, M., Fratzl, P., Addadi, L., Weiner, S., and Yaniv, K. 2015. On the pathway of mineral deposition in larval zebrafish caudal fin bone. Bone **75**: 192–200. doi: http://dx.doi.org/10.1016/j.bone.2015.02.020.

Allemand, D., Mayer-Gostan, N., De Pontual, H., Boeuf, G., and Payan, P. 2008. Fish Otolith Calcification in Relation to Endolymph Chemistry. *In* Handbook of Biomineralization. Wiley-VCH Verlag GmbH. pp. 291–308. doi: 10.1002/9783527619443.ch17.

Allen, P. J., Hobbs, J. A., Jr., J. J. C., Eenennaam, J. P. Van, and Doroshov, S. I. 2009. Using Trace Elements in Pectoral Fin Rays to Assess Life History Movements in Sturgeon: Estimating Age at Initial Seawater Entry in Klamath River Green Sturgeon. Trans. Am. Fish. Soc. **138**: 240–250. doi: 10.1577/T08-061.1.

Allen, P. J., Weihrauch, D., Grandmaison, V., Dasiewicz, P., Peake, S. J., and Anderson, W. G. 2011. The influence of environmental calcium concentrations on

calcium flux, compensatory drinking and epithelial calcium channel expression in a freshwater cartilaginous fish. J. Exp. Biol. **214**: 996–1006.

Ammann, P., Badoud, I., Barraud, S., Dayer, R., and Rizzoli, R. 2007. Strontium ranelate treatment improves trabecular and cortical intrinsic bone tissue quality, a determinant of bone strength. J. Bone Miner. Res. **22**: 1419–1425.

Ammann, P., and Rizzoli, R. 2003. Bone strength and its determinants. Osteoporos. Int. **14**: 13–18.

Anjos, L., Rotllant, J., Guerreiro, P. M., Hang, X., Canario, A. V. M., Balment, R., and Power, D. M. 2005. Production and characterisation of gilthead sea bream (*Sparus auratus*) recombinant parathyroid hormone related protein. Gen. Comp. Endocrinol. **143**: 57–65.

Barnett-Johnson, R., Pearson, T. E., Ramos, F. C., Grimes, C. B., and MacFarlane, R. B. 2008. Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. Limnol. Oceanogr. **53**: 1633–1642. doi: 10.4319/lo.2008.53.4.1633.

Barnett-Johnson, R., Teel, D., and Casillas, E. 2010. Genetic and otolith isotopic markers identify salmon populations in the Columbia River at broad and fine geographic scales. Environ. Biol. Fish. **89**: 533–546. doi: 10.1007/s10641-010-9662-5.

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

Bäuerlein, E., Behrens, P., and Epple, M. 2007. Handbook of biomineralization. Wiley-

VCH.

Begg, G. A., Campana, S. E., Fowler, A. J., and Suthers, I. M. 2005. Otolith research and application: current directions in innovation and implementation. Mar. Freshw. Res. **56**: 477. doi: 10.1071/MF05111.

Bevelander, G. S., Hang, X., Abbink, W., Spanings, T., Canario, A. V. M., and Flik, G. 2006. PTHrP potentiating estradiol-induced vitellogenesis in sea bream (*Sparus auratus*, L.). Gen. Comp. Endocrinol. **149**: 159–165. doi: http://dx.doi.org/10.1016/j.ygcen.2006.05.016.

Bijvelds, M., Heijden, A., Flik, G., Verbost, P., Kolar, Z., and Bonga, S. 1995. Calcium pump activities in the kidneys of *Oreochromis mossambicus*. J. Exp. Biol. **198**: 1351–1357.

Bijvelds, M. C., Flik, G., Kolar, Z., and Wendelaar Bonga, S. 1996. Uptake, distribution and excretion of magnesium in *Oreochromis mossambicus*: dependence on magnesium in diet and water. Fish Physiol. Biochem. **15**: 287–298. doi: 10.1007/BF02112355.

Bijvelds, M. J., Velden, J. A., Kolar, Z. I., and Flik, G. 1998. Magnesium transport in freshwater teleosts. J. Exp. Biol. **201**: 1981–1990.

Black, J. R., Epstein, E., Rains, W. D., Yin, Q., and Casey, W. H. 2008. Magnesium-Isotope Fractionation During Plant Growth. Environ. Sci. Technol. **42**: 7831–7836. doi: 10.1021/es8012722.

Black, J. R., Yin, Q., and Casey, W. H. 2006. An experimental study of magnesium-isotope fractionation in chlorophyll-a photosynthesis. Geochim. Cosmochim. Ac. **70**: 4072–4079. doi: http://dx.doi.org/10.1016/j.gca.2006.06.010.

Blum, J. D., Taliaferro, E. H., and Holmes, R. T. 2001. Determining the sources of calcium for migratory songbirds using stable strontium isotopes. Oecologia **126**: 569–574. doi: 10.1007/s004420000550.

Blum, J. D., Taliaferro, E. H., Weisse, M. T., and Holmes, R. T. 2000. Changes in Sr/Ca, Ba/Ca and $^{87}Sr/^{86}Sr$ ratios between trophic levels in two forest ecosystems in the northeastern USA. Biogeochemistry **49**: 87–101.

Borelli, G., Mayer-Gostan, N., Merle, P. L., De Pontual, H., Boeuf, G., Allemand, D., and Payan, P. 2003. Composition of biomineral organic matrices with special emphasis on turbot (*Psetta maxima*) otolith and endolymph. Calcif. Tissue Int. **72**: 717–25. doi: 10.1007/s00223-001-2115-6.

Borelli, G., De Pontual, H., Boeuf, G., and Payan, P. 2001. Biochemical Relationships Between Endolymph and Otolith Matrix in the Trout (*Oncorhynchus mykiss*) and Turbot (*Psetta maxima*). Calcif. Tissue Int. **69**: 356–364. doi: 10.1007/s00223-001-2016-8.

Boskey, A. L., Gadaleta, S., Gundberg, C., Doty, S. B., Ducy, P., and Karsenty, G. 1998. Fourier transform infrared microspectroscopic analysis of bones of osteocalcin-deficient mice provides insight into the function of osteocalcin. Bone **23**: 187–196. doi: http://dx.doi.org/10.1016/S8756-3282(98)00092-1.

Boström, K. I. 2000. Cell differentiation in vascular calcification. Z. Kardiol. **89**: S069–S074. doi: 10.1007/s003920070102.

Brennan, S. R., Fernandez, D. P., Mackey, G., Cerling, T. E., Bataille, C. P., Bowen, G. J., and Wooller, M. J. 2014. Strontium isotope variation and carbonate versus silicate weathering in rivers from across Alaska: Implications for provenance

studies. Chem. Geol. **389**: 167–181. doi: http://dx.doi.org/10.1016/j.chemgeo.2014.08.018.

Brennan, S. R., Fernandez, D. P., Zimmerman, C. E., Cerling, T. E., Brown, R. J., and Wooller, M. J. 2015a. Strontium isotopes in otoliths of a non-migratory fish (slimy sculpin): Implications for provenance studies. Geochim. Cosmochim. Ac. **149**: 32–45. doi: http://dx.doi.org/10.1016/j.gca.2014.10.032.

Brennan, S. R., Zimmerman, C. E., Fernandez, D. P., Cerling, T. E., McPhee, M. V, and Wooller, M. J. 2015b. Strontium isotopes delineate fine-scale natal origins and migration histories of Pacific salmon. Sci. Adv. **1**: e1400124.

Brent, G. A., Moore, D. D., and Larsen, R. P. 1991. Thyroid hormone regulation of gene expression. Annu. Rev. Physiol. **53**: 17–35.

Brown, E. M., El-Hajj Fulihan, G., Chen, C. H. U. J., and Kifor, O. 1990. A Comparison of the Effects of Divalent and Trivalent Cations on Parathyroid Hormone Release, 3′,5′-Cyclic-Adenosine Monophosphate Accumulation, and the Levels of Inositol Phosphates in Bovine Parathyroid Cells. Endocrinology **127**: 1064–1071. doi: 10.1210/endo-127-3-1064.

Brown, R. J., and Severin, K. P. 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. Can. J. Fish. Aquat. Sci. **66**: 1790–1808. doi: 10.1139/F09-112.

Bury, N. R., Chung, M. J., Sturm, A., Walker, P. A., and Hogstrand, C. 2008. Cortisol stimulates the zinc signaling pathway and expression of metallothioneins and ZnT1 in rainbow trout gill epithelial cells. Am. J. Physiol. Regul. Integr. Comp. Physiol.

294: R623–R629.

Bury, N. R., Sturm, A., Le Rouzic, P., Lethimonier, C., Ducouret, B., Guiguen, Y., Robinson-Rechavi, M., Laudet, V., Rafestin-Oblin, M. E., and Prunet, P. 2003. Evidence for two distinct functional glucocorticoid receptors in teleost fish. J. Mol. Endocrinol. **31**: 141–156. doi: 10.1677/jme.0.0310141 .

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. **188**: 263–297.

Campana, S. E. 2005. Otolith science entering the 21st century. Mar. Freshw. Res. **56**: 485. doi: 10.1071/MF04147.

Campana, S. E., Fowler, A. J., and Jones, C. M. 1994. Otolith Elemental Fingerprinting for Stock Identification of Atlantic Cod (*Gadus morhua*) Using Laser Ablation ICPMS. Can. J. Fish. Aquat. Sci. **51**: 1942–1950. doi: 10.1139/f94-196.

Campana, S. E., and Thorrold, S. R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. **58**: 30–38. doi: 10.1139/f00-177.

Carriere, B., Gillis, D., Halden, N., and Anderson, G. 2016. Strontium metabolism in the juvenile Lake Sturgeon, *Acipenser fulvescens* (Rafinesque, 1817), and further evaluation of the isotope as a marking tool for stock discrimination. J. Appl. Ichthyol. **32**: 258–266. doi: 10.1111/jai.13019.

Chakrabarti, P., and Mukherjee, D. 1993. Studies on the Hypocalcemic Actions of Salmon Calcitonin and Ultimobranchial Gland Extracts in the Freshwater Teleost *Cyprinus carpio*. Gen. Comp. Endocrinol. **90**: 267–273. doi: http://dx.doi.org/10.1006/gcen.1993.1081.

Chang, V. T. C., Williams, R. J. P., Makishima, A., Belshawl, N. S., and O'Nions, R. K. 2004. Mg and Ca isotope fractionation during $CaCO_3$ biomineralisation. Biochem. Biophys. Res. Commun. **323**: 79–85. doi: http://dx.doi.org/10.1016/j.bbrc.2004.08.053.

Chang, M. Y., and Geffen, A. J. 2013. Taxonomic and geographic influences on fish otolith microchemistry. Fish Fish. **14**: 458–492. doi: 10.1111/j.1467-2979.2012.00482.x.

Chen, L., Liu, Y., Hu, Z., Gao, S., Zong, K., and Chen, H. 2011. Accurate determinations of fifty-four major and trace elements in carbonate by LA–ICP-MS using normalization strategy of bulk components as 100%. Chem. Geol. **284**: 283–295. doi: http://dx.doi.org/10.1016/j.chemgeo.2011.03.007.

Cheng, P. T. 1985. Octacalcium phosphate formationin vitro: Implications for bone formation. Calcif. Tissue Int. **37**: 91–94. doi: 10.1007/BF02557685.

Chowdhury, M. J., and Blust, R. 2001. A Mechanistic Model for the Uptake of Waterborne Strontium in the Common Carp (*Cyprinus carpio* L.). Environ. Sci. Technol. **35**: 669–675. doi: 10.1021/es000142t.

Chowdhury, M. J., and Blust, R. 2011. 7 - Strontium. *In* Homeostasis and Toxicology of Non-Essential Metals. *Edited by* A. C. M. Wood, A. Farrell and C. Brauner. Academic Press. pp. 351–390. doi: http://dx.doi.org/10.1016/S1546-5098(11)31029-1.

Christoffersen, J., Christoffersen, M. R., Kibalczyc, W., and Andersen, F. A. 1989. A contribution to the understanding of the formation of calcium phosphates. J. Cryst. Growth **94**: 767–777. doi: http://dx.doi.org/10.1016/0022-0248(89)90102-4.

Chubanov, V., Waldegger, S., Schnitzler, M. Y. M., Vitzthum, H., Sassen, M. C., Seyberth, H. W., Konrad, M., and Gudermann, T. 2004. Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesemia with secondary hypocalcemia. Proc. Natl. Acad. Sci. **101**: 2894–2899. doi: 10.1073/pnas.0305252101.

Cohen-Tanugi, A., and Forest, N. 1998. Retinoic acid suppresses the osteogenic differentiation capacity of murine osteoblast-like 3/A/1D-1M cell cultures. Differentiation **63**: 115–123.

Cousins, R. J., and Leinart, A. S. 1988. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J. **2**: 2884–2890.

Coyle, P., Philcox, J. C., Carey, L. C., and Rofe, A. M. 2002. Metallothionein: the multipurpose protein. Cell. Mol. Life Sci. C. **59**: 627–647. Birkhäuser Verlag. doi: 10.1007/s00018-002-8454-2.

Craig, C. A., Jarvis, K. E., and Clarke, L. J. 2000. An assessment of calibration strategies for the quantitative and semi-quantitative analysis of calcium carbonate matrices by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). J. Anal. At. Spectrom. **15**: 1001–1008. Royal Society of Chemistry.

Crichton, R. R. 2008. Biological Inorganic Chemistry. *Edited by* R. R. Crichton. Elsevier, Amsterdam.

Dauphin, Y., and Dufour, E. 2003. Composition and properties of the soluble organic matrix of the otolith of a marine fish: *Gadus morhua* Linne, 1758 (Teleostei, Gadidae). Comp. Biochem. Physiol. A **134**: 551–561. doi: http://dx.doi.org/10.1016/S1095-6433(02)00358-6.

Davis, F. C. 1997. Melatonin: Role in Development. J. Biol. Rhythm. **12**: 498–508. doi: 10.1177/074873049701200603.

De Braux, E., Warren-Myers, F., Dempster, T., Fjelldal, P. G., Hansen, T., and Swearer, S. E. 2014. Osmotic induction improves batch marking of larval fish otoliths with enriched stable isotopes. ICES J. Mar. Sci. **71**: 2530–2538.

Deng, T. H. B., Cloquet, C., Tang, Y. T., Sterckeman, T., Echevarria, G., Estrade, N., Morel, J. -L., and Qiu, R. -L. 2014. Nickel and Zinc Isotope Fractionation in Hyperaccumulating and Nonaccumulating Plants. Environ. Sci. Technol. **48**: 11926–11933. doi: 10.1021/es5020955.

De Pontual, H., and Geffen, A. J. 2002. Otolith microchemistry. *In* Manual of Fish Sclerochronology. *Edited by* J. Panfili, H. De Pontual, H. Troadec, and P. J. Wright. Ifremer-IRD, Brest, France. pp. 243–303.

De Vrieze, E., Heijnen, L., Metz, J. R., and Flik, G. 2012. Evidence for a hydroxyapatite precursor in regenerating cyprinid scales. J. Appl. Ichthyol. **28**: 388–392. doi: 10.1111/j.1439-0426.2012.01989.x.

De Vrieze, E., Metz, J. R., Von den Hoff, J. W., and Flik, G. 2010. ALP, TRAcP and cathepsin K in elasmoid scales: a role in mineral metabolism? J. Appl. Ichthyol. **26**: 210–213. doi: 10.1111/j.1439-0426.2010.01407.x.

De Vrieze, E., Sharif, F., Metz, J. R., Flik, G., and Richardson, M. K. 2011. Matrix metalloproteinases in osteoclasts of ontogenetic and regenerating zebrafish scales. Bone **48**: 704–712. doi: http://dx.doi.org/10.1016/j.bone.2010.12.017.

Dickson, I., and Walls, J. 1985. Vitamin A and bone formation. Effect of an excess of retinol on bone collagen synthesis in vitro. Biochem. J. **226**: 789–795.

Doubleday, Z. A., Harris, H. H., Izzo, C., and Gillanders, B. M. 2014. Strontium Randomly Substituting for Calcium in Fish Otolith Aragonite. Anal. Chem. **86**: 865–869. doi: 10.1021/ac4034278.

Dubocovich, M., and Markowska, M. 2005. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine **27**: 101–110. doi: 10.1385/ENDO:27:2:101.

Ducy, P., Geoffroy, V., and Karsenty, G. 1996. Study of Osteoblast-Specific Expression of One Mouse Osteocalcin Gene: Characterization of the Factor Binding to OSE2. Connect. Tissue Res. **35**: 7–14. doi: 10.3109/03008209609029169.

Eide, D. J. 2006. Zinc transporters and the cellular trafficking of zinc. Biochim. Biophys. Acta **1763**: 711–722. doi: http://dx.doi.org/10.1016/j.bbamcr.2006.03.005.

Elsdon, T. S., and Gillanders, B. M. 2002. Interactive effects of temperature and salinity on otolith chemistry : challenges for determining environmental histories of fish. Can. J. Fish. Aquat. Sci. **59**: 1796–1808. doi: 10.1139/F02-154.

Elsdon, T. S., and Gillanders, B. M. 2003. Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. Mar. Ecol. Prog. Ser. **260**: 263–272.

Elsdon, T. S., and Gillanders, B. M. 2006. Temporal variability in strontium, calcium, barium, and manganese in estuaries: Implications for reconstructing environmental histories of fish from chemicals in calcified structures. Estuar. Coast. Shelf Sci. **66**: 147–156. doi: 10.1016/j.ecss.2005.08.004.

Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., and Limburg, K. E. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences.

Oceanogr. Mar. Biol. **46**: 297–330.

Epstein, F. H., and Brent, G. A. 1994. The molecular basis of thyroid hormone action. N. Engl. J. Med. **331**: 847–853.

Estêvão, M. D., Silva, N., Redruello, B., Costa, R., Gregório, S., Canário, A. M., and Power, D. 2011. Cellular morphology and markers of cartilage and bone in the marine teleost *Sparus auratus*. Cell Tissue Res. **343**: 619–635. doi: 10.1007/s00441-010-1109-y.

Faure, G., and Mensing, T. M. 2005. Isotopes: principles and applications. John Wiley & Sons Inc.

Faure, G., and Powell, J. L. 1972. Strontium isotope geology. Minerals, rocks and inorganic materials. Mon. Ser. Theor. Exp. Stud. **5**: 188.

Feng, Q. 2011. Molecular Biomineralization. *Edited by* W. E. G. Müller. Springer Berlin Heidelberg, Berlin, Heidelberg. doi: 10.1007/978-3-642-21230-7.

Fenwick, J. C., Smith, K., Smith, J., and Flik, G. 1984. Effect of various vitamin D analogs on plasma calcium and phosphorus and intestinal calcium absorption in fed and unfed American eels, *Anguilla rostrata*. Gen. Comp. Endocrinol. **55**: 398–404. doi: http://dx.doi.org/10.1016/0016-6480(84)90010-8.

Feyrer, F., Hobbs, J., Baerwald, M., Sommer, T., Yin, Q., Clark, K., May, B., and Bennett, W. 2007. Otolith Microchemistry Provides Information Complementary to Microsatellite DNA for a Migratory Fish. Trans. Am. Fish. Soc. **136**: 469–476. doi: 10.1577/T06-044.1.

Fjelldal, P. G., Lock, E. -J., Grotmol, S., Totland, G. K., Nordgarden, U., Flik, G., and Hansen, T. 2006. Impact of smolt production strategy on vertebral growth and

mineralisation during smoltification and the early seawater phase in Atlantic salmon (*Salmo salar*, L.). Aquaculture **261**: 715–728. doi: http://dx.doi.org/10.1016/j.aquaculture.2006.08.008.

Flik, G., Fenwick, J. C., Kolar, Z., Mayer-Gostan, N., and Wendelaabonga, S. E. 1986. Effects of Low Ambient Calcium Levels on Wholebody Ca^{2+} Flux Rates and Internal Calcium Pools in the Freshwater Cichlid Teleost, *Oreochromis Mossambicus*. J. Exp. Biol. **120**: 249–264.

Flik, G., Klaren, P. H. M., Schoenmakers, T. J. M., Bijvelds, M. J. C., Verbost, P. M., and Bonga, S. E. W. 1996. Cellular Calcium Transport in Fish: Unique and Universal Mechanisms. Physiol. Zool. **69**: 403–417.

Flik, G., and Perry, S. F. 1989. Cortisol stimulates whole body calcium uptake and the branchial calcium pump in freshwater rainbow trout. J. Endocrinol. **120**: 75–82.

Flik, G., Rentier-Delrue, F., and Wendelaar Bonga, S. E. 1994. Calcitropic effects of recombinant prolactins in *Oreochromis mossambicus*. Am. J. Physiol. Regul. Integr. Comp. Physiol. **266**: R1302–R1308.

Flik, G., Schoenmakers, T. M., Groot, J., van Os, C., and Wendelaar Bonga, S. E. 1990. Calcium absorption by fish intestine: The involvement of ATP-and sodium-dependent calcium extrusion mechanisms. J. Membr. Biol. **113**: 13–22. doi: 10.1007/BF01869601.

Flik, G., and Verbost, P. M. 1993. Calcium transport in fish gills and intestine. J. Exp. Biol. **184**: 17–29.

Flik, G., Verbost, P. M., and Wendelaar Bonga, S. E. 1995. Calcium transport processes in fishes. Fish Physiol. **14**: 317–342.

Flik, G., Wendelaar Bonga, S. E., and Fenwick, J. C. 1984. Ca^{2+}-dependent phosphatase and Ca^{2+}-dependent ATPase activities in plasma membranes of eel gill epithelium—III. Stimulation of branchial high-affinity Ca^{2+}-ATPase activity during prolactin-induced hypercalcemia in American eels. Comp. Biochem. Physiol. B **79**: 521–524. doi: http://dx.doi.org/10.1016/0305-0491(84)90359-6.

Flockhart, D. T. T., Kyser, T. K., Chipley, D., Miller, N. G., and Norris, D. R. 2015. Experimental evidence shows no fractionation of strontium isotopes ($^{87}Sr/^{86}Sr$) among soil, plants, and herbivores: implications for tracking wildlife and forensic science. Isotopes Environ. Health Stud. **51**: 372-381.

Font, L., van der Peijl, G., van Leuwen, C., van Wetten, I., and Davies, G. R. 2015. Identification of the geographical place of origin of an unidentified individual by multi-isotope analysis. Sci. Justice **55**: 34–42.

Fowler, A. J., Campana, S. E., Thorrold, S. R., and Jones, C. M. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Can. J. Fish. Aquat. Sci. **52**: 1431–1441.

Frank, G. B., and Rohani, F. 1982. Ba^{2+} ions block K^+-induced contractures by antagonizing K^+-induced membrane depolarization in frog skeletal muscle fibres. Can. J. Physiol. Pharmacol. **60**: 47–51. doi: 10.1139/y82-006.

Gaither, L. A., and Eide, D. 2001. Eukaryotic zinc transporters and their regulation. *In* Zinc Biochemistry, Physiology, and Homeostasis SE - 5. *Edited by* W. Maret. Springer Netherlands. pp. 65–84. doi: 10.1007/978-94-017-3728-9_5.

García-Allegue, R., Madrid, J. A., and Sánchez-Vázquez, F. 2001. Melatonin rhythms in European sea bass plasma and eye: influence of seasonal photoperiod and water

temperature. J. Pineal Res. **31**: 68–75. doi: 10.1034/j.1600-079X.2001.310110.x.

Genz, J., Carriere, B., and Anderson, W. G. 2013. Mechanisms of calcium absorption by anterior and posterior segments of the intestinal tract of juvenile lake sturgeon. Comp. Biochem. Physiol. Part A **166**: 293–301. doi: http://dx.doi.org/10.1016/j.cbpa.2013.06.033.

Gillanders, B. M. 2005. Otolith chemistry to determine movements of diadromous and freshwater fish. Aquat. Living Resour. **18**: 291–300. doi: 10.1051/alr:2005033.

Glimcher, M. J. 2006. Bone: Nature of the Calcium Phosphate Crystals and Cellular, Structural, and Physical Chemical Mechanisms in Their Formation. Rev. Mineral. Geochemistry **64**: 223–282. doi: 10.2138/rmg.2006.64.8.

Godiksen, J. A., Svenning, M. A., Sinnatamby, R. N., Dempson, J. B., Borgstrøm, R., and Power, M. 2010. Stable isotope-based determinations of the average temperatures experienced by young-of-the-year Svalbard Arctic charr (*Salvelinus alpinus* (L.)). Polar Biol. **34**: 591–596. doi: 10.1007/s00300-010-0907-8.

Guerreiro, P. M., Fuentes, J., Flik, G., Rotllant, J., Power, D. M., and Canario, A. V. M. 2004. Water calcium concentration modifies whole-body calcium uptake in sea bream larvae during short-term adaptation to altered salinities. J. Exp. Biol. **207**: 645–653.

Guerreiro, P. M., Fuentes, J., Power, D. M., Ingleton, P. M., Flik, G., and Canario, A. V. M. 2001. Parathyroid hormone-related protein: a calcium regulatory factor in sea bream (*Sparus aurata* L.) larvae. Am. J. Physiol. Integr. Comp. Physiol. **281**: R855–R860.

Guibbolini, M., Borelli, G., Mayer-Gostan, N., Priouzeau, F., De Pontual, H., Allemand,

D., and Payan, P. 2006. Characterization and variations of organic parameters in teleost fish endolymph during day–night cycle, starvation and stress conditions. Comp. Biochem. Physiol. Part A **145**: 99–107. doi: http://dx.doi.org/10.1016/j.cbpa.2006.05.003.

Halden, N. M., Mejia, S. R., Babaluk, J. A., Reist, J. D., Kristofferson, A. H., Campbell, J. L., and Teesdale, W. J. 2000. Oscillatory zinc distribution in Arctic char (*Salvelinus alpinus*) otoliths: The result of biology or environment ? Fish. Res. **46**: 289–298.

Hambidge, K., Casey, C. E., and Krebs, N. F. 1986. Zinc. *In* Trace Elements in Human and Animal Nutrition., 5th edition. *Edited by* W. Mertz. Academic Press Inc., Orlando Florida. pp. 1–137.

Hartman, G., Hovers, E., Hublin, J. J., and Richards, M. 2015. Isotopic evidence for Last Glacial climatic impacts on Neanderthal gazelle hunting territories at Amud Cave, Israel. J. Hum. Evol. **84**: 71–82. doi: http://dx.doi.org/10.1016/j.jhevol.2015.03.008.

Hartmann, C. 2009. Transcriptional networks controlling skeletal development. Curr. Opin. Genet. Dev. **19**: 437–443. doi: http://dx.doi.org/10.1016/j.gde.2009.09.001.

Hegg, J. C., Kennedy, B. P., and Fremier, A. K. 2013. Predicting strontium isotope variation and fish location with bedrock geology: Understanding the effects of geologic heterogeneity. Chem. Geol. **360–361**: 89–98. doi: http://dx.doi.org/10.1016/j.chemgeo.2013.10.010.

Herrmann-Erlee, M. P. M., and Flik, G. 1989. Regulation of calcium and phosphate. Vertebr. Endocrinol. **3**: 211.

Hildmann, B., Schmidt, A., and Murer, H. 1982. Ca^{++}-transport across basal-lateral

plasma membranes from rat small intestinal epithelial cells. J. Membr. Biol. **65**: 55–62. doi: 10.1007/BF01870469.

Hobson, K. A., Barnett-Johnson, R., and Cerling, T. 2010. Using isoscapes to track animal migration. *In* Isoscapes. Springer Netherlands pp: 273–298. doi: 10.1007/978-90-481-3354-3.

Hoenderop, J. G. J., Nilius, B., and Bindels, R. J. M. 2004. Calcium Absorption Across Epithelia. Physiol. Rev. **85**: 373–422.

Hong, Y. J., Choi, Y. W., Myung, K. B., and Choi, H. Y. 2011. The Immunohistochemical Patterns of Calcification-related Molecules in the Epidermis and Dermis of the Zebrafish (*Danio rerio*). Ann Dermatol **23**: 299–303.

Horton, T. W., Chamberlain, C. P., Fantle, M., and Blum, J. D. 1999. Chemical weathering and lithologic controls of water chemistry in a high-elevation river system: Clark's Fork of the Yellowstone River, Wyoming and Montana. Water Resour. Res. **35**: 1643–1655.

Hughes, I., Blasiole, B., Huss, D., Warchol, M. E., Rath, N. P., Hurle, B., Ignatova, E., David Dickman, J., Thalmann, R., Levenson, R., and Ornitz, D. M. 2004. Otopetrin 1 is required for otolith formation in the zebrafish *Danio rerio*. Dev. Biol. **276**: 391–402. doi: http://dx.doi.org/10.1016/j.ydbio.2004.09.001.

Huitema, L. F. A., Apschner, A., Logister, I., Spoorendonk, K. M., Bussmann, J., Hammond, C. L., and Schulte-Merker, S. 2012. Entpd5 is essential for skeletal mineralization and regulates phosphate homeostasis in zebrafish. Proc. Natl. Acad. Sci. **109**: 21372–21377. doi: 10.1073/pnas.1214231110 .

Hunter, G., Hauschka, P., Poole, A., Rosenberg, L., and Goldberg, H. 1996. Nucleation

and inhibition of hydroxyapatite formation by mineralized tissue proteins. Biochem. J. **317**: 59–64.

Hüssy, K. 2008. Otolith accretion rates: Does size really matter? J. Exp. Mar. Bio. Ecol. **362**: 131–136. doi: 10.1016/j.jembe.2008.06.016.

Ichii, T., and Mugiya, Y. 1983. Comparative Aspects of Calcium Dynamics in Calcified Tissues in the Goldfish *Carassius auratus*. Nippon Suisan Gakk. **49**: 1039–1044. doi: 10.2331/suisan.49.1039.

Islam, Z., Hayashi, N., Yamamoto, Y., Doi, H., Romero, M. F., Hirose, S., and Kato, A. 2013. Identification and proximal tubular localization of the Mg^{2+} transporter, Slc41a1, in a seawater fish. Am. J. Physiol. - Regul. Integr. Comp. Physiol. **305**: R385–R396.

Jaouen, K., Pons, M. L., and Balter, V. 2013. Iron, copper and zinc isotopic fractionation up mammal trophic chains. Earth Planet. Sci. Lett. **374**: 164–172. doi: http://dx.doi.org/10.1016/j.epsl.2013.05.037.

Jensen, J. E. B., Larsen, M. M., Kringsholm, B., Pritzl, G., and Sørensen, O. H. 1996. Measurement of Trace Elements in Bone by ICP-MS. *In* Therapeutic Uses of Trace Elements SE - 50. *Edited by* J. Nève, P. Chappuis, and M. Lamand. Springer US. pp. 297–301. doi: 10.1007/978-1-4899-0167-5_50.

Jochum, K. P., Scholz, D., Stoll, B., Weis, U., Wilson, S. A., Yang, Q., Schwalb, A., Börner, N., Jacob, D. E., and Andreae, M. O. 2012. Accurate trace element analysis of speleothems and biogenic calcium carbonates by LA-ICP-MS. Chem. Geol. **318–319**: 31–44. doi: http://dx.doi.org/10.1016/j.chemgeo.2012.05.009.

Johnsson, M. S. A., and Nancollas, G. H. 1992. The Role of Brushite and Octacalcium

Phosphate in Apatite Formation. Crit. Rev. Oral Biol. Med. **3**: 61–82. doi: 10.1177/10454411920030010601.

Kalish, J. 1991a. ^{13}C and ^{18}O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. Mar. Ecol. Prog. Ser. **75**: 191–203. doi: 10.3354/meps075191.

Kalish, J. M. 1991b. Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). Mar. Biol. **110**: 37–47. doi: 10.1007/BF01313090.

Kang, S. W., La, W. G., Kang, J., Park, J. H., and Kim, B. S. 2008. Bone morphogenetic protein-2 enhances bone regeneration mediated by transplantation of osteogenically undifferentiated bone marrow-derived mesenchymal stem cells. Biotechnol. Lett. **30**: 1163–1168. doi: 10.1007/s10529-008-9675-8.

Kapłon, T. M., Michnik, A., Drzazga, Z., Richter, K., Kochman, M., and Ożyhar, A. 2009. The rod-shaped conformation of Starmaker. Biochim. Biophys. Acta - Proteins Proteomics **1794**: 1616–1624. doi: http://dx.doi.org/10.1016/j.bbapap.2009.07.010.

Kapłon, T. M., Rymarczyk, G., Nocula-Ługowska, M., Jakób, M., Kochman, M., Lisowski, M., Szewczuk, Z., and Ożyhar, A. 2008. Starmaker Exhibits Properties of an Intrinsically Disordered Protein. Biomacromolecules **9**: 2118–2125. doi: 10.1021/bm800135m.

Karsenty, G. 2008. Transcriptional Control of Skeletogenesis. Annu. Rev. Genomics Hum. Genet. **9**: 183–196. doi: 10.1146/annurev.genom.9.081307.164437.

Kedem, O., and Essig, A. 1965. Isotope Flows and Flux Ratios in Biological Membranes. J. Gen. Physiol. **48**: 1047–1070. doi: 10.1085/jgp.48.6.1047.

Kennedy, B. P., Blum, J. D., Folt, C. L., and Nislow, K. H. 2000. Using natural strontium

isotopic signatures as fish markers: methodology and application. Can. J. Fish. Aquat. Sci. **57**: 2280–2292. doi: 10.1139/f00-206.

Kennedy, B. P., Klaue, A., Blum, J. D., Folt, C. L., and Nislow, K. H. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Can. J. Fish. Aquat. Sci. **59**(6): 925-929 doi: 10.1139/f02-070.

Khan, K. M. F., and Falcone, D. J. 1997. Role of Laminin in Matrix Induction of Macrophage Urokinase-type Plasminogen Activator and 92-kDa Metalloproteinase Expression. J. Biol. Chem. **272**: 8270–8275. doi: 10.1074/jbc.272.13.8270.

Kim, H. T., and Chen, T. L. 1989. 1, 25-Dihydroxyvitamin D3 interaction with dexamethasone and retinoic acid: effects on procollagen messenger ribonucleic acid levels in rat osteoblast-like cells. Mol. Endocrinol. **3**: 97–104.

Kobayashi, K., Anada, T., Handa, T., Kanda, N., Yoshinari, M., Takahashi, T., and Suzuki, O. 2014. Osteoconductive Property of a Mechanical Mixture of Octacalcium Phosphate and Amorphous Calcium Phosphate. ACS Appl. Mater. Interfaces **6**: 22602–22611. doi: 10.1021/am5067139.

Konrad, M., Schaller, A., Seelow, D., Pandey, A. V, Waldegger, S., Lesslauer, A., Vitzthum, H., Suzuki, Y., Luk, J. M., Becker, C., Schlingmann, K. P., Schmid, M., Rodriguez-Soriano, J., Ariceta, G., Cano, F., Enriquez, R., Jüppner, H., Bakkaloglu, S. A., Hediger, M. A., Gallati, S., Neuhauss, S. C. F., Nürnberg, P., and Weber, S. 2006. Mutations in the Tight-Junction Gene Claudin 19 (CLDN19) Are Associated with Renal Magnesium Wasting, Renal Failure, and Severe Ocular Involvement. Am. J. Hum. Genet. **79**: 949–957. doi: http://dx.doi.org/10.1086/508617.

Krabbenhöft, A., Fietzke, J., Eisenhauer, A., Liebetrau, V., Böhm, F., and Vollstaedt, H.

2009. Determination of radiogenic and stable strontium isotope ratios ($^{87}Sr/^{86}Sr$; $\delta^{88/86}Sr$) by thermal ionization mass spectrometry applying an $^{87}Sr/^{84}Sr$ double spike. J. Anal. At. Spectrom. **24**: 1267–1271.

Kranenbarg, S., van Cleynenbreugel, T., Schipper, H., and van Leeuwen, J. 2005. Adaptive bone formation in acellular vertebrae of sea bass (*Dicentrarchus labrax* L.). J. Exp. Biol. **208**: 3493–3502.

Kulczykowska, E. 2001. Responses of circulating arginine vasotocin, isotocin, and melatonin to osmotic and disturbance stress in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. **24**: 201–206. doi: 10.1023/A:1014079917644.

Larsson, D., and Nemere, I. 2002. Vectorial Transcellular Calcium Transport in Intestine: Integration of Current Models. J. Biomed. Biotechnol. **2**: 117–119.

Lee, N. K., Sowa, H., Hinoi, E., Ferron, M., Ahn, J. D., Confavreux, C., Dacquin, R., Mee, P. J., McKee, M. D., Jung, D. Y., Zhang, Z., Kim, J. K., Mauvais-Jarvis, F., Ducy, P., and Karsenty, G. 2007. Endocrine Regulation of Energy Metabolism by the Skeleton. Cell **130**: 456–469. doi: http://dx.doi.org/10.1016/j.cell.2007.05.047.

Lee, R. T. H., Thiery, J. P., and Carney, T. J. 2013. Dermal fin rays and scales derive from mesoderm, not neural crest. Curr. Biol. **23**: R336–R337. doi: http://dx.doi.org/10.1016/j.cub.2013.02.055.

Lee, S. R., Noh, S. J., Pronto, J. R., Jeong, Y. J., Kim, H. K., Song, I. S., Xu, Z., Kwon, H. Y., Kang, S. C., Sohn, E. -H., Ko, K. S., Rhee, B. D., Kim, N., and Han, J. 2015. The Critical Roles of Zinc: Beyond Impact on Myocardial Signaling. Korean J. Physiol. Pharmacol. **19**: 389–399.

Lehane, D. B., McKie, N., Russell, R. G. G., and Henderson, I. W. 1999. Cloning of a

Fragment of the Osteonectin Gene from Goldfish, *Carassius auratus*: Its Expression and Potential Regulation by Estrogen. Gen. Comp. Endocrinol. **114**: 80–87. doi: http://dx.doi.org/10.1006/gcen.1998.7237.

Lijima, S. 1991. Synthesis of carbon nanotubes. Nature **354**: 56–58.

Limburg, K. E., and Elfman, M. 2010. Patterns and magnitude of Zn:Ca in otoliths support the recent phylogenetic typology of Salmoniformes and their sister groups. Can. J. Fish. Aquat. Sci. **67**: 597–604. doi: 10.1139/F10-014.

Lock, E. J., Ørnsrud, R., Aksnes, L., Spanings, F. A. T., Waagbø, R., and Flik, G. 2007. The vitamin D receptor and its ligand 1α,25-dihydroxyvitamin D3 in Atlantic salmon (*Salmo salar*). J. Endocrinol. **193**: 459–471. doi: 10.1677/JOE-06-0198.

Loewen, T. N., Reist, J. D., Yang, P., Koleszar, A., Babaluk, J. A., Mochnacz, N., and Halden, N. M. 2015. Discrimination of northern form Dolly Varden Char (*Salvelinus malma malma*) stocks of the North Slope, Yukon and Northwest Territories, Canada via otolith trace elements and $^{87}Sr/^{86}Sr$ isotopes. Fish. Res. **170**: 116–124. doi: http://dx.doi.org/10.1016/j.fishres.2015.05.025.

Luo, G., Ducy, P., McKee, M. D., Pinero, G. J., Loyer, E., Behringer, R. R., and Karsenty, G. 1997. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature **386**: 78–81. doi: 10.1038/386078a0.

Mahamid, J., Aichmayer, B., Shimoni, E., Ziblat, R., Li, C., Siegel, S., Paris, O., Fratzl, P., Weiner, S., and Addadi, L. 2010. Mapping amorphous calcium phosphate transformation into crystalline mineral from the cell to the bone in zebrafish fin rays. Proc. Natl. Acad. Sci. **107:** 6316–6321. doi: 10.1073/pnas.0914218107.

Mahamid, J., Sharir, A., Addadi, L., and Weiner, S. 2008. Amorphous calcium

phosphate is a major component of the forming fin bones of zebrafish: Indications for an amorphous precursor phase. Proc. Natl. Acad. Sci. **105**: 12748–12753. doi: 10.1073/pnas.0803354105.

Mahamid, J., Sharir, A., Gur, D., Zelzer, E., Addadi, L., and Weiner, S. 2011. Bone mineralization proceeds through intracellular calcium phosphate loaded vesicles: A cryo-electron microscopy study. J. Struct. Biol. **174**: 527–535. doi: http://dx.doi.org/10.1016/j.jsb.2011.03.014.

Mann, S. 2001. Biomineralization: principles and concepts in bioinorganic materials chemistry. Oxford University Press.

Marie, P. J. 1996. Effects of Strontium on Bone Tissue and Bone Cells. *In* Therapeutic Uses of Trace Elements SE - 48. *Edited by* J. Nève, P. Chappuis, and M. Lamand. Springer US. pp. 277–282. doi: 10.1007/978-1-4899-0167-5_48.

Markwitz, A., D. Grambole, F. Herrmann, W. J. Trompetter, T. Dioses, and R. W. Gauldie. 2000. Reliable micro-measurement of strontium is the key to cracking the life-history code in the fish otolith. Nucl. Instr. Meth. Phys. Res. **168**(1): 109-116.

Marshall, W. S., Bryson, S. E., and Wood, C. M. 1992. Calcium transport by isolated skin of rainbow trout. J. Exp. Biol. **166**: 297–316.

Marshall, W. S., and Grosell, M. 2006. Ion transport, osmoregulation, and acid-base balance. *In* The physiology of fishes. CRC Press Boca Raton. pp. 177–230.

Martin, J. E., Vance, D., and Balter, V. 2015. Magnesium stable isotope ecology using mammal tooth enamel. Proc. Natl. Acad. Sci. **112**: 430–435. doi: 10.1073/pnas.1417792112.

Martin, L., Lodemann, U., Bondzio, A., Gefeller, E. M., Vahjen, W., Aschenbach, J. R.,

Zentek, J., and Pieper, R. 2013. A High Amount of Dietary Zinc Changes the Expression of Zinc Transporters and Metallothionein in Jejunal Epithelial Cells in Vitro and in Vivo but Does Not Prevent Zinc Accumulation in Jejunal Tissue of Piglets. J. Nutr. **143**: 1205–1210. doi: 10.3945/jn.113.177881.

Mayer, I., Bornestaf, C., and Borg, B. 1997a. Melatonin in non-mammalian vertebrates: Physiological role in reproduction? Comp. Biochem. Physiol. A **118**: 515–531. doi: http://dx.doi.org/10.1016/S0300-9629(96)00468-9.

Mayer, I., Bornestaf, C., Wetterberg, L., and Borg, B. 1997b. Melatonin Does Not Prevent Long Photoperiod Stimulation of Secondary Sexual Characters in the Male Three-Spined Stickleback *Gasterosteus aculeatus*. Gen. Comp. Endocrinol. **108**: 386–394. doi: http://dx.doi.org/10.1006/gcen.1997.6985.

McCaslin Jr, F. E., and Janes, J. M. 1959. The effect of strontium lactate in the treatment of osteoporosis. *In* Proceedings of Staff Meetings of the Mayo Clinic. pp. 329–334.

McCormick, S. D., Hasegawa, S., and Hirano, T. 1992. Calcium uptake in the skin of a freshwater teleost. Proc. Natl. Acad. Sci. **89**: 3635–3638. doi: 10.1073/pnas.89.8.3635.

Melançon, S., Fryer, B. J., Gagnon, J. E., and Ludsin, S. A. 2008. Mineralogical approaches to the study of biomineralization in fish otoliths. Mineral. Mag. **72**: 627–637. doi: 10.1180/minmag.2008.072.2.627.

Melançon, S., Fryer, B. J., Ludsin, S. A., and Gagnon, J. E. 2005. Effects of crystal structure on the uptake of metals by lake trout (*Salvelinus namaycush*) otoliths. Can. J. Fish. Aquat. Sci. **2619**: 2609–2619. doi: 10.1139/F05-161.

Melançon, S., Fryer, B. J., and Markham, J. L. 2009. Chemical analysis of endolymph and the growing otolith: fractionation of metals in freshwater fish species. Environ. Toxicol. Chem. **28**: 1279–87. doi: 10.1897/08-358.1.

Mertz, E. L., Facchini, M., Pham, A. T., Gualeni, B., De Leonardis, F., Rossi, A., and Forlino, A. 2012. Matrix Disruptions, Growth, and Degradation of Cartilage with Impaired Sulfation. J. Biol. Chem. **287**: 22030–22042. doi: 10.1074/jbc.M110.116467.

Metz, J. R., Leeuwis, R. H. J., Zethof, J., and Flik, G. 2014. Zebrafish (*Danio rerio*) in calcium-poor water mobilise calcium and phosphorus from scales. J. Appl. Ichthyol. **30**: 671–677. doi: 10.1111/jai.12513.

Metz, J. R., de Vrieze, E., Lock, E. J., Schulten, I. E., and Flik, G. 2012. Elasmoid scales of fishes as model in biomedical bone research. J. Appl. Ichthyol. **28**: 382–387. doi: 10.1111/j.1439-0426.2012.01990.x.

Minkin, C. 1982. Bone acid phosphatase: Tartrate-resistant acid phosphatase as a marker of osteoclast function. Calcif. Tissue Int. **34**: 285–290. doi: 10.1007/BF02411252.

Mokgalaka, N. S., and Gardea‐Torresdey, J. L. 2006. Laser Ablation Inductively Coupled Plasma Mass Spectrometry: Principles and Applications. Appl. Spectrosc. Rev. **41**: 131–150. doi: 10.1080/05704920500510703.

Mommsen, T., Vijayan, M., and Moon, T. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fisher. **9**: 211–268. doi: 10.1023/A:1008924418720.

Mongera, A., and Nüsslein-Volhard, C. 2013. Scales of fish arise from mesoderm. Curr.

Biol. **23**: R338–R339. doi: http://dx.doi.org/10.1016/j.cub.2013.02.056.

Monteilh-Zoller, M. K., Hermosura, M. C., Nadler, M. J. S., Scharenberg, A. M., Penner, R., and Fleig, A. 2003. TRPM7 Provides an Ion Channel Mechanism for Cellular Entry of Trace Metal Ions. J. Gen. Physiol. **121**: 49–60. doi: 10.1085/jgp.20028740.

Mugiya, Y., Watabe, N., Yamada, J., Dean, J. M., Dunkelberger, D. G., and Shimizu, M. 1981. Diurnal rhythm in otolith formation in the goldfish, *Carassius auratus*. Comp. Biochem. Physiol. A Physiol. **68**: 659–662. doi: http://dx.doi.org/10.1016/0300-9629(81)90374-1.

Muhlfeld, C. C., Thorrold, S. R., McMahon, T. E., and Marotz, B. 2012. Movements in a River Network Using Strontium Isoscapes. Can. J. Fish. Aquat. Sci. **69**: 1129–1130. doi: 10.1139/f2012-049.

Mukherjee, D., Sen, U., Bhattacharyya, S. P., and Mukherjee, D. 2004. The effects of calcitonin on plasma calcium levels and bone metabolism in the fresh water teleost *Channa punctatus*. Comp. Biochem. Physiol. A **138**: 417–426. doi: http://dx.doi.org/10.1016/j.cbpb.2004.04.006.

Munro, A. R., Gillanders, B. M., Elsdon, T. S., Crook, D. A., and Sanger, A. C. 2008. Enriched stable isotope marking of juvenile golden perch (*Macquaria ambigua*) otoliths. Can. J. Fish. Aquat. Sci. **65**: 276–285. doi: 10.1139/f08-010.

Müntzing, J., Kirdani, R., Murphy, G. P., and Sandberg, A. A. 1977. Hormonal control of zinc uptake and binding in the rat dorsolateral prostate. Invest. Urol. **14**: 492–495.

Murayama, E., Herbomel, P., Kawakami, A., Takeda, H., and Nagasawa, H. 2005. Otolith matrix proteins OMP-1 and Otolin-1 are necessary for normal otolith growth and their correct anchoring onto the sensory maculae. Mech. Dev. **122**: 791–803.

doi: 10.1016/j.mod.2005.03.002.

Murayama, E., Okuno, A., Ohira, T., Takagi, Y., and Nagasawa, H. 2000. Molecular cloning and expression of an otolith matrix protein cDNA from the rainbow trout, *Oncorhynchus mykiss*. Comp. Biochem. Physiol. B **126**: 511–520. doi: http://dx.doi.org/10.1016/S0305-0491(00)00223-6.

Murayama, E., Takagi, Y., Ohira, T., Davis, J. G., Greene, M. I., and Nagasawa, H. 2002. Fish otolith contains a unique structural protein, otolin-1. Eur. J. Biochem. **269**: 688–696. doi: 10.1046/j.0014-2956.2001.02701.x.

Nachshen, D. A., and Blaustein, M. P. 1982. Influx of calcium, strontium, and barium in presynaptic nerve endings. J. Gen. Physiol. **79**: 1065–1087. doi: 10.1085/jgp.79.6.1065.

Nakajima, A., Shimoji, N., Shiomi, K., Shimizu, S., Moriya, H., Einhorn, T. A., and Yamazaki, M. 2002. Mechanisms for the Enhancement of Fracture Healing in Rats Treated With Intermittent Low-Dose Human Parathyroid Hormone (1–34). J. Bone Miner. Res. **17**: 2038–2047.

Nishimoto, S. K., Araki, N., Robinson, F. D., and Waite, J. H. 1992. Discovery of bone gamma-carboxyglutamic acid protein in mineralized scales. The abundance and structure of *Lepomis macrochirus* bone gamma-carboxyglutamic acid protein. J. Biol. Chem. **267**: 11600–11605.

Norberg, B., Björnsson, B. T., Brown, C. L., Wichardt, U. -P., Deftos, L. J., and Haux, C. 1989. Changes in plasma vitellogenin, sex steroids, calcitonin, and thyroid hormones related to sexual maturation in female brown trout (*Salmo trutta*). Gen. Comp. Endocrinol. **75**: 316–326.

Omelon, S., Georgiou, J., Henneman, Z. J., Wise, L. M., Sukhu, B., Hunt, T., Wynnyckyj, C., Holmyard, D., Bielecki, R., and Grynpas, M.D. 2009. Control of Vertebrate Skeletal Mineralization by Polyphosphates. PLoS One **4**: e5634. doi: 10.1371%2Fjournal.pone.0005634.

Omelon, S. J., and Grynpas, M. D. 2008. Relationships between Polyphosphate Chemistry, Biochemistry and Apatite Biomineralization. Chem. Rev. **108**: 4694–4715. doi: 10.1021/cr0782527.

Onozato, H., and Watabe, N. 1979. Studies on fish scale formation and resorption. Cell Tissue Res. **201**: 409–422. doi: 10.1007/BF00236999.

Ørnsrud, R., Graff, I. E., Høie, S., Totland, G. K., and Hemre, G. 2002. Hypervitaminosis A in first-feeding fry of the Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. **8**: 7–13.

Ørnsrud, R., Lock, E. J., Glover, C.N., and Flik, G. 2009. Retinoic acid cross-talk with calcitriol activity in Atlantic salmon (*Salmo salar*). J. Endocrinol. **202**: 473–482.

Oughterson, S. M., Munoz-Chapuli, R., De Andres, V., Lawson, R., Heath, S., and Davies, D. H. 1995. The Effects of Calcitonin on Serum Calcium Levels in Immature Brown Trout, *Salmo trutta*. Gen. Comp. Endocrinol. **97**: 42–48. doi: http://dx.doi.org/10.1006/gcen.1995.1004.

Parsons, J. A., Gray, D., Rafferty, B., and Zanelli, J. M. 1978. Evidence for a hypercalcaemic factor in the fish pituitary immunologically related to mammalian parathyroid hormone. *In:* Endrocrinology of Calcium Metabolism. *Edited by* D. H. Copp, and R. V. Talmage, Excerpta Media, Amsterdam pp. 111–114.

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

10.1016/j.crpv.2004.07.013.

Pecl, G. T., Tracey, S. R., Danyushevsky, L., Wotherspoon, S., Moltschaniwskyj, N. A., and Gillanders, B. 2011. Elemental fingerprints of southern calamary (*Sepioteuthis australis*) reveal local recruitment sources and allow assessment of the importance of closed areas. Can. J. Fish. Aquat. Sci. **68**: 1351–1360. doi: 10.1139/f2011-059.

Peng, J. B., Brown, E. M., and Hediger, M. A. 2003. Apical Entry Channels in Calcium-Transporting Epithelia. Physiology **18**: 158–163.

Perry, S. F., Goss, G. G., and Fenwick, J. C. 1992. Interrelationships between gill chloride cell morphology and calcium uptake in freshwater teleosts. Fish Physiol. Biochem. **10**: 327–337. doi: 10.1007/BF00004482.

Persson, P., Björnsson, B. T., and Takagi, Y. 1999. Characterization of morphology and physiological actions of scale osteoclasts in the rainbow trout. J. Fish Biol. **54**: 669–684.

Persson, P., Johannsson, S. H., Takagi, Y., and Björnsson, B. T. 1997. Estradiol-17β and nutritional status affect calcium balance, scale and bone resorption, and bone formation in rainbow trout, *Oncorhynchus mykiss*. J. Comp. Physiol. B **167**: 468–473. doi: 10.1007/s003600050098.

Persson, P., Shrimpton, J. M., McCormick, S. D., and Björnsson, B. T. 2000. The presence of high-affinity, low-capacity estradiol-17β binding in rainbow trout scale indicates a possible endocrine route for the regulation of scale resorption. Gen. Comp. Endocrinol. **120**: 35–43.

Persson, P., Sundell, K., Björnsson, B. T., and Lundqvist, H. 1998a. Gonadal Maturation, Calcium Metabolism, Osmoregulatory Ability, and Plasma Hormones

during Spawning Migration of Atlantic Salmon. Ann. N. Y. Acad. Sci. **839**: 440–441. doi: 10.1111/j.1749-6632.1998.tb10826.x.

Persson, P., Sundell, K., Björnsson, B. T., and Lundqvist, H. 1998b. Calcium metabolism and osmoregulation during sexual maturation of river running Atlantic salmon. J. Fish Biol. **52**: 334–349. doi: 10.1111/j.1095-8649.1998.tb00801.x.

Persson, P., Takagi, Y., and Björnsson, B. 1995. Tartrate resistant acid phosphatase as a marker for scale resorption in rainbow trout, *Oncorhynchus mykiss*: effects of estradiol-17β treatment and refeeding. Fish Physiol. Biochem. **14**: 329–339. doi: 10.1007/BF00004071.

Petko, J. A., Millimaki, B. B., Canfield, V. A., Riley, B. B., and Levenson, R. 2008. Otoc1: A novel otoconin-90 ortholog required for otolith mineralization in zebrafish. Dev. Neurobiol. **68**: 209–222. doi: 10.1002/dneu.20587.

Phillis, C. C., Ostrach, D. J., Ingram, B. L., and Weber, P. K. 2011. Evaluating otolith Sr/Ca as a tool for reconstructing estuarine habitat use. Can. J. Fish. Aquat. Sci. **68**: 360–373. doi: 10.1139/F10-152.

Pombinho, A., Laizé, V., Molha, D., Marques, S. P., and Cancela, M. L. 2004. Development of two bone-derived cell lines from the marine teleost *Sparus aurata*; evidence for extracellular matrix mineralization and cell-type-specific expression of matrix Gla protein and osteocalcin. Cell Tissue Res. **315**: 393–406. doi: 10.1007/s00441-003-0830-1.

Pors Nielsen, S. 2004. The biological role of strontium. Bone **35**: 583–588. doi: http://dx.doi.org/10.1016/j.bone.2004.04.026.

Porter, M. J. R., Randall, C. F., Bromage, N. R., and Thorpe, J. E. 1998. The role of

melatonin and the pineal gland on development and smoltification of Atlantic salmon (*Salmo salar*) parr. Aquaculture **168**: 139–155. doi: http://dx.doi.org/10.1016/S0044-8486(98)00345-7.

Pouilly, M., Point, D., Sondag, F., Henry, M., and Santos, R. V. 2014. Geographical Origin of Amazonian Freshwater Fishes Fingerprinted by $^{87}Sr/^{86}Sr$ Ratios on Fish Otoliths and Scales. Environ. Sci. Technol. **48**: 8980–8987. doi: 10.1021/es500071w.

Qiu, A., Glover, C. N., and Hogstrand, C. 2007. Regulation of branchial zinc uptake by 1α,25-(OH)2D3 in rainbow trout and associated changes in expression of ZIP1 and ECaC. Aquat. Toxicol. **84**: 142–152. doi: http://dx.doi.org/10.1016/j.aquatox.2007.02.020.

Reginster, J. Y., Kaufman, J. M., Goemaere, S., Devogelaer, J. P., Benhamou, C. L., Felsenberg, D., Diaz-Curiel, M., Brandi, M. L., Badurski, J., Wark, J., Balogh, A., Bruyère, O., and Roux, C. 2012. Maintenance of antifracture efficacy over 10 years with strontium ranelate in postmenopausal osteoporosis. Osteoporos. Int. **23**: 1115–1122. doi: 10.1007/s00198-011-1847-z.

Reginster, J. Y., Seeman, E., De Vernejoul, M. C., Adami, S., Compston, J., Phenekos, C., Devogelaer, J. P., Curiel, M. D., Sawicki, A., Goemaere, S., Sorensen, O. H., Felsenberg, D., and Meunier, P. J. 2005. Strontium Ranelate Reduces the Risk of Nonvertebral Fractures in Postmenopausal Women with Osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) Study. J. Clin. Endocrinol. Metab. **90**: 2816–2822. doi: 10.1210/jc.2004-1774.

Renn, J., and Winkler, C. 2010. Characterization of collagen type 10a1 and osteocalcin

in early and mature osteoblasts during skeleton formation in medaka. J. Appl. Ichthyol. **26**: 196–201.

Renn, J., Winkler, C., Schartl, M., Fischer, R., and Goerlich, R. 2006. Zebrafish and medaka as models for bone research including implications regarding space-related issues. Protoplasma **229**: 209–214. doi: 10.1007/s00709-006-0215-x.

Rotllant, J., Redruello, B., Guerreiro, P. M., Fernandes, H., Canario, A. V. M., and Power, D. M. 2005. Calcium mobilization from fish scales is mediated by parathyroid hormone related protein via the parathyroid hormone type 1 receptor. Regul. Pept. **132**: 33–40. doi: http://dx.doi.org/10.1016/j.regpep.2005.08.004.

Roy, M. E., and Nishimoto, S. K. 2002. Matrix Gla protein binding to hydroxyapatite is dependent on the ionic environment: calcium enhances binding affinity but phosphate and magnesium decrease affinity. Bone **31**: 296–302. doi: http://dx.doi.org/10.1016/S8756-3282(02)00821-9.

Runnels, L. W., Yue, L., and Clapham, D. E. 2002. The TRPM7 channel is inactivated by PIP2 hydrolysis. Nat. Cell Biol. **4**: 329–336. doi: http://dx.doi.org/10.1038/ncb781.

Schoeninger, M. J., and DeNiro, M. J. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim. Cosmochim. Ac. **48**: 625–639.

Schönbörner, A. A., Boivin, G., and Baud, C. A. 1979. The mineralization processes in Teleost fish scales. Cell Tissue Res. **202**: 203–212. doi: 10.1007/BF00232235.

Sharp, Z. 2007. Principles of stable isotope geochemistry. Pearson Education Upper Saddle River, NJ.

Shiao, J., and Hwang, P. 2004. Thyroid hormones are necessary for teleostean otolith growth. Mar. Ecol. Prog. Ser. **278**: 271–278.

Shiao, J. C., Wu, S. M., Hwang, Y. P., Wu, D. P., and Hwang, P. P. 2008. Evaluation of thyroid-mediated otolith growth of larval and juvenile tilapia. J. Exp. Biol. **211**: 1919–26. doi: 10.1242/jeb.013748.

Shimada, A., Kawanishi, T., Kaneko, T., Yoshihara, H., Yano, T., Inohaya, K., Kinoshita, M., Kamei, Y., Tamura, K., and Takeda, H. 2013. Trunk exoskeleton in teleosts is mesodermal in origin. Nat. Commun. **4**: 1639. doi: http://dx.doi.org/10.1038/ncomms2643.

Shinozaki, F., and Mugiya, Y. 2000. Effects of salmon calcitonin on calcium deposition on and release from calcified tissues in fed and starved goldfish *Carassius auratus*. Fish. Sci. **66**: 695–700. doi: 10.1046/j.1444-2906.2000.00110.x.

Shorr, E., and Carter, A. C. 1952. The usefulness of strontium as an adjuvant to calcium in the remineralization of the skeleton in man. Bull. Hosp. Joint Dis. **13**: 59.

Sillen, A., Hall, G., and Armstrong, R. 1995. Strontium calcium ratios (Sr/Ca) and strontium isotopic ratios ($^{87}Sr/^{86}Sr$) of *Australopithecus robustus* and *Homo sp*. from Swartkrans. J. Hum. Evol. **28**: 277–285.

Sire, J. Y., Allizard, F., Babiar, O., Bourguignon, J., and Quilhac, A. 1997. Scale development in zebrafish (*Danio rerio*). J. Anat. **190**: 545–561. doi: 10.1046/j.1469-7580.1997.19040545.x.

Sire, J. Y., and Géraudie, J. 1984. Fine structure of regenerating scales and their associated cells in the cichlid *Hemichromis bimaculatus* (Gill). Cell Tissue Res. **237**: 537–547. doi: 10.1007/BF00228438.

Smith, K. T., and Whitledge, G. W. 2011. Evaluation of a stable-isotope labelling technique for mass marking fin rays of age-0 lake sturgeon. Fish. Manag. Ecol. **18**: 168–175. doi: 10.1111/j.1365-2400.2010.00771.x.

Smolders, E., Versieren, L., Shuofei, D., Mattielli, N., Weiss, D., Petrov, I., and Degryse, F. 2013. Isotopic fractionation of Zn in tomato plants suggests the role of root exudates on Zn uptake. Plant Soil **370**: 605–613. doi: 10.1007/s11104-013-1655-7.

Söllner, C., Burghammer, M., Busch-Nentwich, E., Berger, J., Schwarz, H., Riekel, C., and Nicolson, T. 2003. Control of crystal size and lattice formation by starmaker in otolith biomineralization. Science **302**: 282–6. doi: 10.1126/science.1088443.

Söllner, C., Schwarz, H., Geisler, R., and Nicolson, T. 2004. Mutated otopetrin 1 affects the genesis of otoliths and the localization of Starmaker in zebrafish. Dev. Genes Evol. **214**: 582–590. doi: 10.1007/s00427-004-0440-2.

Standley, P. R., and Standley, C. A. 2002. Identification of a functional Na^{+}/Mg^{2+} exchanger in human trophoblast cells. Am. J. Hypertens. **15**: 565–570. doi: http://dx.doi.org/10.1016/S0895-7061(02)02272-0.

Stewart, A. J., Roberts, S. J., Seawright, E., Davey, M. G., Fleming, R. H., and Farquharson, C. 2006. The presence of PHOSPHO1 in matrix vesicles and its developmental expression prior to skeletal mineralization. Bone **39**: 1000–1007. doi: http://dx.doi.org/10.1016/j.bone.2006.05.014.

Stolte, E. H., de Mazon, A. F., Leon-Koosterziel, K. M., Jesiak, M., Bury, N. R., Sturm, A., Savelkoul, H. F. J., van Kemenade, B. M. L. V., and Flik, G. 2008. Corticosteroid receptors involved in stress regulation in common carp, *Cyprinus*

carpio. J. Endocrinol. **198**: 403–417. doi: 10.1677/JOE-08-0100.

Sturrock, A. M., Trueman, C. N., Darnaude, A. M., and Hunter, E. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? J. Fish Biol. **81**: 766–95. doi: 10.1111/j.1095-8649.2012.03372.x.

Sturrock, A., Trueman, C., Milton, J., Waring, C., Cooper, M., and Hunter, E. 2014. Physiological influences can outweigh environmental signals in otolith microchemistry research. Mar. Ecol. Prog. Ser. **500**: 245–264. doi: 10.3354/meps10699.

Sturrock, A. M., Hunter, E., Milton, J. A., Eimf, Johnson, R. C., Waring, C. P., and Trueman, C. N. 2015. Quantifying physiological influences on otolith microchemistry. Methods Ecol. Evol. **6**: 806–816. doi: 10.1111/2041-210X.12381.

Sundell, K., and Björnsson, B. T. 1988. Kinetics of Calcium Fluxes Across the Intestinal Mucosa of the Marine Teleost, *Gadus Morhua*, Measured Using an In Vitro Perfusion Method. J. Exp. Biol. **140**: 171–186.

Suzuki, N., and Hattori, A. 2002. Melatonin suppresses osteoclastic and osteoblastic activities in the scales of goldfish. J. Pineal Res. **33**: 253–258. doi: 10.1034/j.1600-079X.2002.02953.x.

Suzuki, N., Suzuki, T., and Kurokawa, T. 2000. Suppression of osteoclastic activities by calcitonin in the scales of goldfish (freshwater teleost) and nibbler fish (seawater teleost). Peptides **21**: 115–124. doi: http://dx.doi.org/10.1016/S0196-9781(99)00181-3.

Swenson, A. M., Trivedi, D. V, Rauscher, A. A., Wang, Y., Takagi, Y., Palmer, B. M., Málnási-Csizmadia, A., Debold, E. P., and Yengo, C. M. 2014. Magnesium

modulates actin binding and ADP release in myosin motors. J. Biol. Chem. **289**: 23977–23991.

Sylvester, P. J. 2008. Matrix effects in laser ablation ICP-MS. Laser Ablation ICP-MS Earth Sci. Curr. Pract. Outst. Issues (P. Sylvester, ed.). Mineral. Assoc. Canada. Short Course Ser. **40**: 67–78.

Takagi, Y. 2002. Otolith formation and endolymph chemistry: a strong correlation between the aragonite saturation state and pH in the endolymph of the trout otolith organ. Mar. Ecol. Prog. Ser. **231**: 237–245. doi: 10.3354/meps231237.

Tanner, S. E., Reis-Santos, P., Vasconcelos, R. P., Thorrold, S. R., and Cabral, H. N. 2013. Population connectivity of *Solea solea* and *Solea senegalensis* over time. J. Sea Res. **76**: 82–88. doi: http://dx.doi.org/10.1016/j.seares.2012.11.005.

Thorrold, S. R., Campana, S. E., Jones, C. M., and Swart, P. K. 1997. Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. Geochim. Cosmochim. Ac. **61**: 2909–2919.

Thorrold, S. R., Jones, G. P., Planes, S., and Hare, J. A. 2006. Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. Can J. Fish. Aquat. Sci. **63**: 1193–1197. doi: 10.1139/F06-048.

Tohse, H., and Mugiya, Y. 2001. Effects of enzyme and anion transport inhibitors on in vitro incorporation of inorganic carbon and calcium into endolymph and otoliths in salmon *Oncorhynchus masou*. Comp. Biochem. Physiol. A **128**: 177–184. doi: http://dx.doi.org/10.1016/S1095-6433(00)00287-7.

Tohse, H., and Mugiya, Y. 2008. Sources of otolith carbonate: experimental determination of carbon incorporation rates from water and metabolic CO_2, and

their diel variations. Aquat. Biol. **1**: 259–268. doi: 10.3354/ab00029.

Tohse, H., Saruwatari, K., Kogure, T., Nagasawa, H., and Takagi, Y. 2009. Control of Polymorphism and Morphology of Calcium Carbonate Crystals by a Matrix Protein Aggregate in Fish Otoliths. Cryst. Growth Des. **9**: 4897–4901. doi: 10.1021/cg9006857.

Tohse, H., Takagi, Y., and Nagasawa, H. 2008. Identification of a novel matrix protein contained in a protein aggregate associated with collagen in fish otoliths. FEBS J. **275**: 2512–2523. doi: 10.1111/j.1742-4658.2008.06400.x.

Valentine, B., Kamenov, G. D., Kenoyer, J. M., Shinde, V., Mushrif-Tripathy, V., Otarola-Castillo, E., and Krigbaum, J. 2015. Evidence for Patterns of Selective Urban Migration in the Greater Indus Valley (2600-1900 BC): A Lead and Strontium Isotope Mortuary Analysis. PloS One **10**: e0123103.

van Baal, J., Raber, G., de Slegte, J., Pieters, R., Bindels, R. J. M., and Willems, P. H. G. M. 1996. Vasopressin-stimulated Ca^{2+} reabsorption in rabbit cortical collecting system: effects on cAMP and cytosolic Ca^{2+}. Pflügers Arch. **433**: 109–115. doi: 10.1007/s004240050255.

Van Campenhout, K., Bervoets, L., Redeker, E. S., and Blust, R. 2009. A kinetic model for the relative contribution of waterborne and dietary cadmium and zinc in the common carp (*Cyprinus carpio*). Environ. Toxicol. Chem. **28**: 209–219. doi: 10.1897/08-136.1.

Van Leeuwen, J., Van Driel, M., Van Den Bemd, G., and Pols, H. A. P. 2001. Vitamin D control of osteoblast function and bone extracellular matrix mineralization. Crit. Rev. Eukaryot. Gene Expr. **11:** 1-3.

Von Allmen, K., Böttcher, M. E., Samankassou, E., and Nägler, T. F. 2010. Barium isotope fractionation in the global barium cycle: First evidence from barium minerals and precipitation experiments. Chem. Geol. **277**: 70–77.

Varghese, S., Rydziel, S., Jeffrey, J. J., and Canalis, E. 1994. Regulation of interstitial collagenase expression and collagen degradation by retinoic acid in bone cells. Endocrinology **134**: 2438–2444.

Veinott, G., Northcote, T., Rosenau, M., and Evans, R. D. 1999. Concentrations of strontium in the pectoral fin rays of the white sturgeon (*Acipenser transmontanus*) by laser ablation sampling - inductively coupled plasma - mass spectrometry as an indicator of marine migrations. Can. J. Fish. Aquat. Sci. **56**: 1981–1990. doi: 10.1139/f99-120.

Verbost, P., Flik, G., Fenwick, J., Greco, A. M., Pang, P. T., and Wendelaar Bonga, S. 1993. Branchial calcium uptake: possible mechanisms of control by stanniocalcin. Fish Physiol. Biochem. **11**: 205–215. doi: 10.1007/BF00004568.

Voets, T., Nilius, B., Hoefs, S., van der Kemp, A. W., Droogmans, G., Bindels, R. J., and Hoenderop, J. G. 2004. TRPM6 Forms the Mg^{2+} Influx Channel Involved in Intestinal and Renal Mg^{2+} Absorption. J. Biol. Chem. **279**: 19–25. doi: 10.1074/jbc.M311201200.

Wagner, G. F., Haddad, M., Fargher, R. C., Milliken, C., and Copp, D. H. 1998. Calcium Is an Equipotent Stimulator of Stanniocalcin Secretion in Freshwater and Seawater Salmon. Gen. Comp. Endocrinol. **109**: 186–191. doi: http://dx.doi.org/10.1006/gcen.1997.7010.

Wang, M., and Berlin, J. R. 2007. Voltage-dependent modulation of L-type calcium

currents by intracellular magnesium in rat ventricular myocytes. Arch. Biochem. Biophys. **458**: 65–72. doi: http://dx.doi.org/10.1016/j.abb.2006.10.022.

Warren-Myers, F., Dempster, T., Fjelldal, P.G., Hansen, T., Swearer, S. E., and Fisk, A. 2015. Immersion during egg swelling results in rapid uptake of stable isotope markers in salmonid otoliths. Can. J. Fish. Aquat. Sci. **72**: 722–727.

Weiss, R. E., and Watabe, N. 1979. Studies on the biology of fish bone. III. Ultrastructure of osteogenesis and resorption in osteocytic (cellular) and anosteocytic (acellular) bones. Calcif. Tissue Int. **28**: 43–56. doi: 10.1007/BF02441217.

Wendelaar Bonga, S. E., Lammers, P. I., and van der Meij, J. C. A. 1983. Effects of 1, 25-and 24, 25-dihydroxyvitamin D_3 on bone formation int he cichlid teleost *Sarotherodon mossambicus*. Cell Tissue Res. **228**: 117-126.

Williamson, D., Jones, G., and Thorrold, S. 2009. An experimental evaluation of transgenerational isotope labelling in a coral reef grouper. Mar. Biol. **156**: 2517–2525. doi: 10.1007/s00227-009-1276-0.

Wilson, R. W., and Grosell, M. 2003. Intestinal bicarbonate secretion in marine teleost fish—source of bicarbonate, pH sensitivity, and consequences for whole animal acid–base and calcium homeostasis. Biochim. Biophys. Acta Biomembr. **1618**: 163–174. doi: http://dx.doi.org/10.1016/j.bbamem.2003.09.014.

Wimalawansa, S. J. 1997. Amylin, Calcitonin Gene-Related Peptide, Calcitonin, and Adrenomedullin: A Peptide Superfamily. Crit. Rev. Neurobiol. **11**: 167–239.

Witten, P. E. 1997. Enzyme histochemical characteristics of osteoblasts and mononucleated osteoclasts in a teleost fish with acellular bone (*Oreochromis*

niloticus, Cichlidae). Cell Tissue Res. **287**: 591–599. doi: 10.1007/s004410050782.

Witten, P. E., Hansen, A., and Hall, B. K. 2001. Features of mono- and multinucleated bone resorbing cells of the zebrafish *Danio rerio* and their contribution to skeletal development, remodeling, and growth. J. Morphol. **250**: 197–207. doi: 10.1002/jmor.1065.

Witten, P. E., and Huysseune, A. 2009. A comparative view on mechanisms and functions of skeletal remodelling in teleost fish, with special emphasis on osteoclasts and their function. Biol. Rev. **84**: 315–346. doi: 10.1111/j.1469-185X.2009.00077.x.

Witten, P. E., Villwock, W., Peters, N., and Hall, B. K. 2000. Bone resorption and bone remodelling in juvenile carp, *Cyprinus carpio* L. J. Appl. Ichthyol. **16**: 254–261. doi: 10.1046/j.1439-0426.2000.00233.x.

Woodcock, S. H., Gillanders, B. M., Munro, A. R., Crook, D. A., and Sanger, A. C. 2011. Determining mark success of 15 combinations of enriched stable isotopes for the batch marking of larval otoliths. North Am. J. Fish. Manag. **31**: 843–851.

Woodcock, S. H., Grieshaber, C. A., and Walther, B. D. 2013. Dietary transfer of enriched stable isotopes to mark otoliths, fin rays, and scales. Can. J. Fish. Aquat. Sci. **70**: 1–4. doi: 10.1139/cjfas-2012-0389.

Wronski, T. J., Halloran, B. P., Bikle, D. D., Globus, R. K., and Morey-Holton, E. R. 1986. Chronic Administration of 1,25-Dihydroxyvitamin D_3: Increased Bone but Impaired Mineralization. Endocrinology **119**: 2580–2585. doi: 10.1210/endo-119-6-2580.

Yoshikubo, H., Suzuki, N., Takemura, K., Hoso, M., Yashima, S., Iwamuro, S., Takagi,

Y., Tabata, M. J., and Hattori, A. 2005. Osteoblastic activity and estrogenic response in the regenerating scale of goldfish, a good model of osteogenesis. Life Sci. **76**: 2699–2709. doi: http://dx.doi.org/10.1016/j.lfs.2004.10.063.

Yoshimura, T., Tanimizu, M., Inoue, M., Suzuki, A., Iwasaki, N., and Kawahata, H. 2011. Mg isotope fractionation in biogenic carbonates of deep-sea coral, benthic foraminifera, and hermatypic coral. Anal. Bioanal. Chem. **401**: 2755–2769. doi: 10.1007/s00216-011-5264-0.

Zhang, Y., Li, D., Zhuang, G., Cheng, F., Zhang, G., Wang, Z., and Xia, J. 2002. Investigation of the elemental distribution in iliac crests of female New Zealand rabbits using NAA. Biol. Trace Elem. Res. **86**: 65–72.

Zheng, D., Feeney, G. P., Kille, P., and Hogstrand, C. 2008. Regulation of ZIP and ZnT zinc transporters in zebrafish gill: zinc repression of ZIP10 transcription by an intronic MRE cluster. Physiol. Genomics **34**: 205–214.

Zhou, H., and Clapham, D. E. 2009. Mammalian MagT1 and TUSC3 are required for cellular magnesium uptake and vertebrate embryonic development. Proc. Natl. Acad. Sci. **106**: 15750–15755. doi: 10.1073/pnas.0908332106.

Zimmerman, C. E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity : experimental validation for juvenile salmonids. Can. J. Fish. Aquat. Sci. **62**: 88–97. doi: 10.1139/F04-182.

Chapter 3

Strontium Uptake and Expulsion in Otoliths: A Case Study of Greenland Halibut (*Reinhardtius hippoglossoides*) Assessing Element Marking, Otolith Growth and Tag-Recapture Studies within Natural Environments

This manuscript has been coauthored with my adviser and collaborators. I have been the lead on the manuscript. Specifically I was responsible for the study design, experimental analysis, writing, and revisions for the manuscript. My co-authors provided insight to the overall review of the study, conducted field-based aspects of the study, and provided editorial and review comments on multiple drafts of the manuscript.

Loewen T.N., Treble, M. A., Wastle, R., Reist, J. D., Sidhu, R., Sünksen, K., Jørgensen, O. A., and Halden, N. M. Strontium uptake and expulsion in otoliths: A case study of Greenland Halibut (*Reinhardtius hippoglossoides*) assessing element marking, otolith growth and tag-recapture studies within natural environments.

3.1 Abstract

Elemental marking and recapture techniques were used to document fish growth and otolith accretion rates, and characterise strontium (Sr) uptake and retention periods for Greenland Halibut (*Reinhardtius hippoglossoides)*. Two different concentrations of a strontium-chloride ($SrCl_2$) solution were injected into the intraperitoneal cavity prior to releasing the fish back into their natural environment. Mean fish growth rate was 2.19 $cm.yr^{-1}$ for all 18 individuals or 6.31 $cm.yr^{-1}$ with the elimination of one individual experiencing negative growth. Otolith accretion occurred along different axes in the left and right otolith structures. The right otolith accretion occurred in an anterior-ventral direction, whereas the left grew in posterior-ventral, anterial-dorsal, and proximal directions. The accretion rates were similar between left and right structures along the edge region whereas accretion over the nucleus in the proximal region was slower. Strontium uptake took anywhere from 17 to 242 days and was strongly influenced by otolith accretion rate and dose. In the initial stages of strontium retention, and during the estimated return-to-background, only dose level played a role in strontium retention. The estimated return-to-background varied from 328-1518 days and one individual in our study did not return to background levels. Storage mechanisms, temperature and growth rates of fish likely contribute to the extended strontium retention period post-marking. Knowing that retention periods post-marking can be long in duration may assist others when interpreting elemental profiles of long-lived species that migrate frequently between marine and freshwater environments on relatively short timeframes. Further research to examine element storage mechanisms and their remobilisation in

the context of fish physiology will aid our understanding of element incorporation into otoliths.

3.2 Introduction

The use of physical and chemical tags in fisheries science has become a valuable tool for assessing abundance, age, growth, age validation, mortality, stock identification, movement/migration, stocking success, and mixed-population analysis (Cadrin 2005, Munro et al. 2008, Williamson et al. 2009, Huelga-Suarez et al. 2013). In marine environments, the likelihood of tag recovery is low due to the spatial extent involved and migratory capabilities of fish. As a result, large numbers of fish must be tagged to ensure post-marking recapture success (Cadrin 2005, Williamson et al. 2009, Albert and Vollen 2014).

Strontium (Sr) in the form of strontium-chloride ($SrCl_2$) has become the most commonly used elemental tag in fisheries research (Hernaman et al. 2000, Gillanders 2009, Hobbs et al. 2012) due to the strong affinity for strontium to be incorporated into bony structures within organisms. Although strontium is a non-essential element for life and therefore is not directly regulated physiologically by an organism (Chowdhury and Blust 2011), it is thought to augment bone calcium (Ca) through stimulating Ca^{2+} receptors to reduce fracture rates and act as an antioxidant (Nielsen 2004, Radzki et al. 2009). Strontium regulation is indirect through essential life element uptake mechanisms, specifically calcium as a result of similar chemical properties such as ionic radius and charge (Chowdhury and Blust 2011). Competition with calcium for entry pathways occurs at every level from the external environment to internal pathways,

including the endolymphatic fluid surrounding the otolith where it is available for otolith biomineralization. The endpoint for strontium in organisms, moreover, is bony structures with only 5% found in soft tissues (Chowdhury 2001, Smith et al. 2005, Yankovich 2009). In fish, calcium and therefore strontium is generally controlled by the branchial epithelium and to a less extent the gastrointestinal tract and skin (Chowdhury and Blust 2011). During intraperitoneal-based marking, initial uptake routes for strontium are bypassed as the $SrCl_2$ is injected directly into the body cavity. The fish then must work to regain homeostasis. This process can be indirectly observed by the examination of strontium within the bony structures of the organism such as the otolith. Otoliths are formed of calcium carbonate and are inert upon the completion of biomineralization (Allemand et al. 2008, Doubleday et al. 2014) unlike other calcium-formed structures such as fins, bones, scales and cartilage (Weiss and Watabe 1978, Mahamid et al. 2011). Injection of high concentration $SrCl_2$ into fish results in an elemental "check" or mark in the otolith detectable by scanning electron-microscope backscatter electron imagery or similar techniques (Hernaman et al. 2000, Courtney and Severin 2007, Gillanders 2009). New calcium-carbonate accretion, post-$SrCl_2$ marking, can then be measured and estimates can be calculated for daily accretion in the otolith, since both marking and re-capture dates are known.

Greenland Halibut (*Reinhardtius hippoglossoides),* the species used within the study, is a commercially important deepwater flatfish that inhabits Arctic and sub-Arctic waters in both the North Pacific and North Atlantic oceans. The species is highly migratory in nature and has been the subject of several tagging studies (Bowering 1984, Boje 2002, Peklova et al. 2012, Albert and Vollen 2014). Evidence suggests Greenland

Halibut move from spawning to feeding grounds on an annual basis (Godø and Haug 1989, Jørgensen 1997, Bowering and Nedreaas 2000, Albert 2003). In offshore areas during winter, Greenland Halibut are thought to migrate to greater depths (>1100m), whereas during the spring to autumn they are known to occur in greater densities at depths of <1000m with juveniles utilising shallow shelf habitats to feed (200-400m) (Godø and Haug 1989, Jørgensen 1997, Bowering and Nedreaas 2000, Albert 2003). Genetic homogeneity is observed (Vis et al. 1997, Roy et al. 2015) leading researchers to define the stock as a single spawning complex (Godø and Haug 1989, Jørgensen 1997, Bowering and Nedreaas 2000, Albert 2003). Within the first year of life, during settlement to demersal habitats, juvenile Greenland Halibut undergo a metamorphosis that includes craniofacial restructuring (Wang et al. 2011). Although there is no formal agreement on age validation techniques, bomb radiocarbon otolith assays, oxytetracycline otolith marking studies, and Floy © tagging data suggest that this species is long-lived and slow growing (Gregg et al. 2006, Treble et al. 2008, Albert et al. 2009). In order to further investigate age determination methods and migration patterns for Greenland Halibut in Baffin Bay and Davis Strait, a mark-recapture study was designed that utilized both external Floy © tags and strontium marked otoliths.

The purpose of our study is to characterise strontium in left and right sagittal otoliths of elementally marked Greenland Halibut to: (1) locate the strontium mark; (2) determine strontium uptake and retention time post-marking; (3) examine dose responses of $SrCl_2$; and, (4) define fish growth and associated otolith accretion rates.

3.3 Materials and Methods

Elemental marking of Greenland Halibut occurred in NAFO Divisions 0A and 1AB (Baffin Bay and Northwest Greenland, Northwest Atlantic Ocean) during October of 2007, 2008, and 2009. Greenland Halibut were caught in an aluminum capture box (2.4 m x 1.0 m x 1.0 m; modelled after Holst and McDonald 2000) attached to the cod end of an otter trawl towed along the sea floor at depths between 700-950 m for approximately 15 minutes by the Greenland Institute of Natural Resources Research Vessel Paamiut. The net and capture box were hauled back to the surface at half the normal speed, 40 m of wire.min^{-1}. The capture box allowed the fish to be retained in sea water during haul back and while on deck, thus protecting them from net abrasion and minimizing the stress of capture. Each fish was measured for total length (cm) and either a low dose (20mg) or high dose (100 mg) per kg body weight of $SrCl_2$ hexahydrate ($SrCl_2 \cdot 6H_2O$) was injected into the intraperitoneal cavity (Hüssy et al. 2009). From 2007-2009, 3092 Greenland Halibut were marked with the low dose of $SrCl_2$ and 1239 with the high dose. The fish were tagged externally with plastic Floy © tags before release back into the sea. Re-capture of marked fish occurred opportunistically in the commercial offshore fishery. Fishers returned otolith pairs, date of capture, total length (cm) and the tag number to Greenlandic or Canadian fisheries authorities for a monetary reward. Otoliths from 21 fish that had been marked with $SrCl_2$ and subsequently re-captured were available to our study. Of the 21 fish recaptured, marked strontium profiles were identified in otoliths of 18 fish.

3.3.1 *Electron Microprobe Analysis*

Otoliths were cleaned with distilled water to remove biological material, dried for 24 hours, and placed into individual Lucite rings with the proximal surface facing upward, back-filled with epoxy resin and left to harden for 3-4 days. Due to the concave/convex nature of both left and right otoliths, each whole otolith was positioned in the Lucite ring to best expose areas of the otolith typically used for age analysis (**Figure 3.1**). A Buehler MetaServ® 2000, variable speed grinder-polisher with 30 µm lapping film and 0.05 µm diamond paste were used respectively to expose and polish targeted areas. Care was taken to ensure proper exposure to the otolith edges. A Leica DFC490 camera and M125 dissecting microscope were used to capture images of the otoliths prior to electron microprobe analysis. Embedded and polished otolith disks were ultra-sonically cleaned in distilled water for five minutes, dried for several days, and carbon coated. Upon completion of whole otolith analysis, cross-sections through the nucleus of the left otolith were cut with a Buehler® slow speed saw along the dorso-ventral axis. Left and right otoliths grow asymmetrically. Unlike the right otolith, the left otolith grows around the nucleus in the proximal plane. As a result, the left cross-section is one of the methods used for age determination (Gregg et al. 2006). Preparation procedures for the cross-sectioned otoliths were identical to those of the whole otoliths (embedding, polishing and carbon coating processes described previously).

A Cameca SX100 electron microprobe was used to obtain back-scattered electron (BSE) images and x-ray maps, as well as line-scans measuring the intensity of the strontium signal as counts per second (cps) versus distance across the otolith (µm). Electron beam conditions were set at 15 KeV accelerating voltage with a current of 100 nA for backscatter electron (BSE) imaging and 200 nA for line-scan and x-ray mapping.

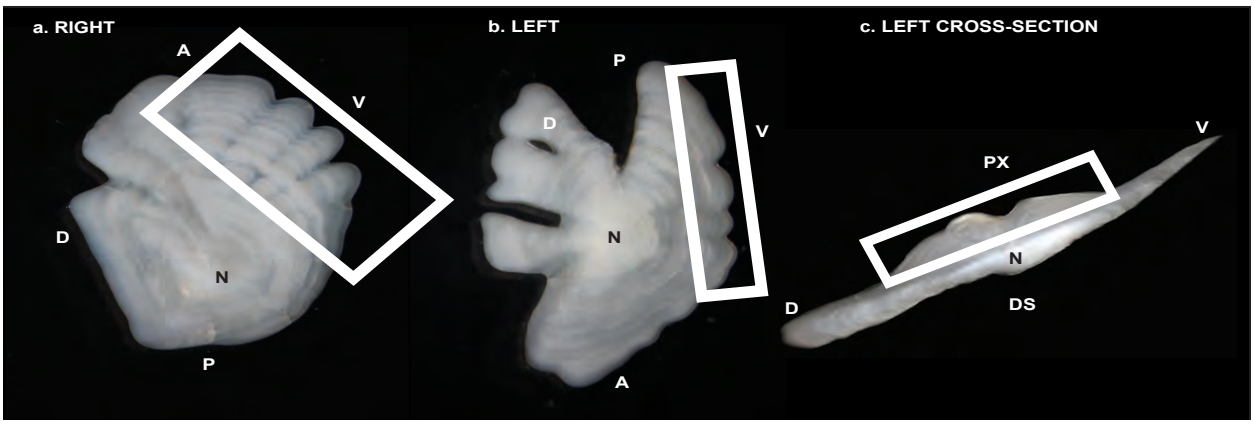

Figure 3.1: The regions of interest for electron microprobe analysis **(a.)** distal surface of the right, **(b.)** distal surface of the left and, **(c.)** proximal region of the left cross-section are highlighted by the white box on each otolith. Orientation for each otolith is provided: **A** (anterior), **P** (posterior), **D** (dorsal), **V** (ventral), **PX** (proximal) and **DS** (distal). The nucleus (**N**) is labelled on all otolith structures. These regions are regularly used in age determination for the species.

Thallium acid phthalate (TAP) crystals were used for x-ray mapping images and line-scan profiles using the strontium L_α X-ray line. X-ray mapping and BSE imaging identified the line-scan locations. Line-scans were run from the pre-marking area of the otolith to the post-mark outer edge intersecting the strontium mark at a 90° angle. Line profiles were collected in scanning mode, with a dwell time of 0.3 second, beam current energy of 15KeV and beam current of 200 nA. Multiple line-scans were run for each sample to encompass variation in otolith accretion over the scanned area.

3.3.2 *Strontium Uptake and Signal Retention*

The uptake of strontium, following the $SrCl_2$ injection, was considered to begin when there was a continual increase of >50 cps over the ambient background count rate for each line-scan. The end point of each strontium line scan was determined by a sharp decrease in the count rate at the otolith edge. Average otolith accretion per day (OAR) was determined by dividing the total line-scan distance (μm**)** by the number of days from marking to re-capture. Two to four line-scans with the highest rates of accretion per sample were chosen for further detailed analysis.

Total days of strontium uptake (SU), also referred to as the marking period, were determined by measuring the number of days from the start of strontium marking through to the maximum count rate (**Figure 3.2**). The start of strontium retention (SR1), the period of time where excess strontium was still available to be incorporated into otoliths, was determined by measuring the number of days from the maximum count rate to the inflection point (IP) in the data marking a change in slope of the count rate. The IP was determined by the use of an iterative R code program designed to find the

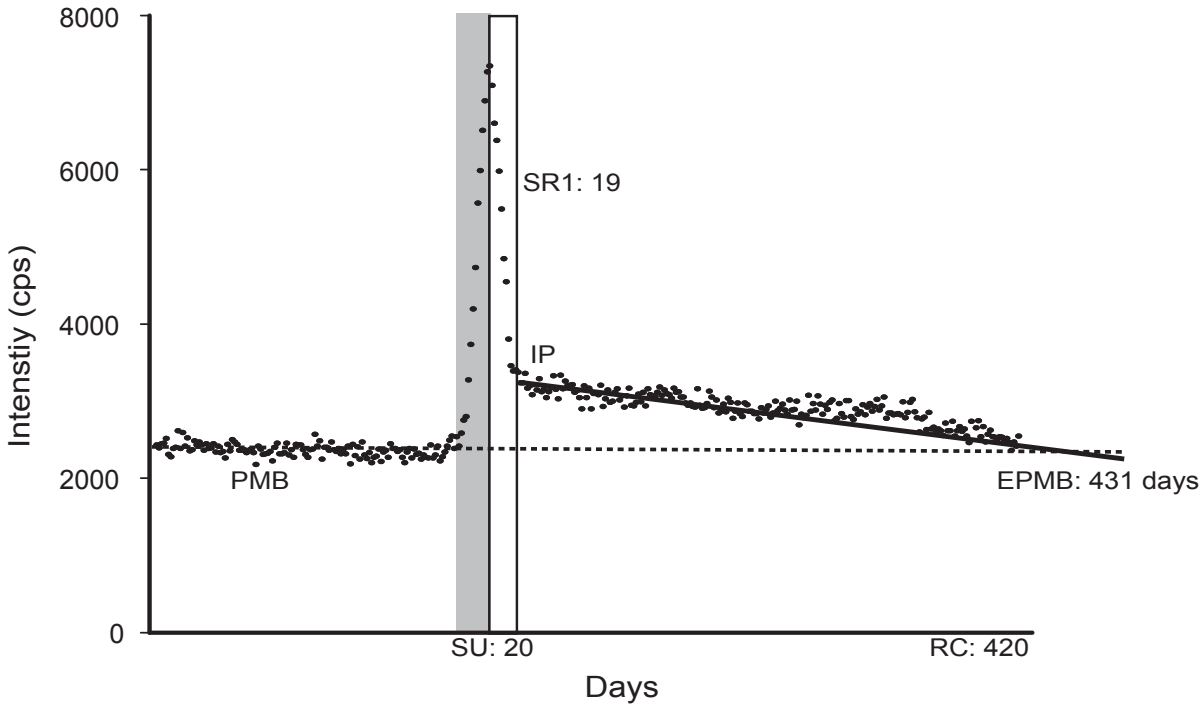

Figure 3.2: A representative line-scan image used in analysis. The pre-marking background (**PMB**) is represented by the horizontal dashed line. The grey box highlights the initial strontium uptake period (**SU**; 20 days). The first phase of strontium retention (**SR1**; 19 days) is indicated by the open box. The inflection point (**IP**) represents the commencement of the second phase of strontium retention that ends at recapture (**RC;** 420 days). The linear regression model represented by the solid line (R-sq = 0.79, slope = -2.24, intercept = 3351), estimates the return to pre-marking background (**EPMB**; 431 days).

best fit of a linear regression of the data points from the maximum value in the uptake curve to the end point of the data. The best-fit linear regression model was also used to estimate the return-to-background levels (EPMB) (i.e., pre-marking) when R-square (Rsq) was >0.60. On samples where the recapture period was extensive (i.e., >600 days), only the first 450 days were used in the linear regression analysis for the return to EPMB. Finally, the means of the line scans (n=2-4) for SU, SR1, and EPMB in association to otolith growth rates, fish growth rates, size at capture, and mark-recapture period were summarised for each otolith per sample.

3.3.3 Fish Growth and Otolith Accretion

Linear regression analysis examined which variables were associated with fish growth during the time of marking. The linear regression model is:

$$\boldsymbol{FGR \sim RC + SM + Interaction + Error},$$

where the fish growth rate (***FGR***) is determined by the independent variables of the mark-recapture period (***RC***) and the size-at-marking (***SM***). Overall mean fish growth rate was calculated twice: for all available fish where the strontium mark was identified (n=18) and with one sample (fish 506566; **Table 3.1**) eliminated due to large values of negative growth (4 cm) that may have occurred due to measurement error. Since it is not definitively known why this fish experienced negative growth, both fish growth estimates are reported.

Table 3.1: Fish growth rates (**FGR**; $cm.day^{-1}$), are listed for individual fish (**SNO**) along with dose level (**SrD**), size at marking (**SM**; cm), and recapture period (**RC**, days). **HD** represents high dosed fish (100 mg/kg body weight) and **LD** represents low dosed fish (20 mg/kg body weight).

SNO	SrD	SM	FGR	RC
506566	HD	67	-0.0189	212
006402	HD	54	-0.0045	220
615354	HD	52	-0.0043	235
001287	LD	43	0.0027	367
000716	LD	45	0.0000	368
000777	LD	47	-0.0027	368
000788	LD	37	0.0050	402
000059	LD	42	0.0024	413
000538	LD	43	0.0072	417
000026	LD	43	0.0048	420
000727	LD	45	0.0000	423
002179	LD	58	0.0050	600
629798	HD	46	0.0000	614
638081	HD	46	0.0032	620
584647	HD	41	0.0091	656
003275	HD	52	-0.0040	996
001574	LD	52	0.0026	1138
003017	LD	40	0.0929	1382

Analysis of variance (ANOVA) was used to examine otolith accretion. The ANOVA model is:

$$\textit{OAR} \sim \textit{SrD} + \textit{OS} + \textit{Interaction} + \textit{Error},$$

where otolith accretion rate (**OAR**) is examined by the categorical variables of $SrCl_2$ Dose (**SrD**), and otolith structure (**OS**). $SrCl_2$ dose had two categorical classifications of high or low dose response.

The relationship between otolith accretion rates and fish growth rates was examined using linear regression analysis. Due to differences of otolith accretions rates for type of structure, two separate linear regressions were completed: 1.) left and right whole otolith accretion data combined, and 2.) cross-section otolith accretion data.

In exploratory stages of statistical analysis, SU (discounting categorical groups) was initially plotted against otolith accretion rate and a power relationship was observed where $SU=8.5008OAR^{-0.816}$ and an Rsq of 0.80. In subsequent analysis data were normalised using the model:

$$\textit{log(SU)} \sim \textit{log(OAR)} + \textit{SrD} + \textit{OS} + \textit{Interaction} + \textit{Error.}$$

A similar approach was used to examine the start of SR1. In this case, data were normalized to reduce heteroscedasticity and modelled using linear regression. The model is:

log(SR1) ~ log(OAR) + SrD + OS + Interaction + Error.

The EPMB was initially examined via linear regression in a similar fashion as SU and SR1. A weak model fit (adjusted (adj.) Rsq = 0.22) was found between the estimated return to background and otolith accretion rates. Normalizing the data reduced the linear regression model fit to an adj. Rsq of 0.14 therefore an ANOVA was examined categorical variables in the model:

EPMB~ SrD + OS + Interaction + Error.

When possible, the EPMB was ground-truthed by the examination of samples where the mark-recapture period was longer than the estimated value (n=3 individuals).

3.4 Results

Three otolith pairs for the study were found to have no strontium marking and were removed from further analysis. Strontium marks were clearly identifiable in BSE images in all remaining samples examined and x-ray mapping further verified the strontium mark at a higher resolution (**Figure 3.3**). BSE images were used as reference maps for identifying the mark in the initial stages of the project.

3.4.1 *Fish Growth and Otolith Accretion*

Fish growth rates ranged from -0.019 to 0.009 cm.day^{-1} for a range of fish sizes at marking of 37-67 cm (**Table 3.1**). Overall mean growth was 0.006 cm.day^{-1} (+/- 0.062

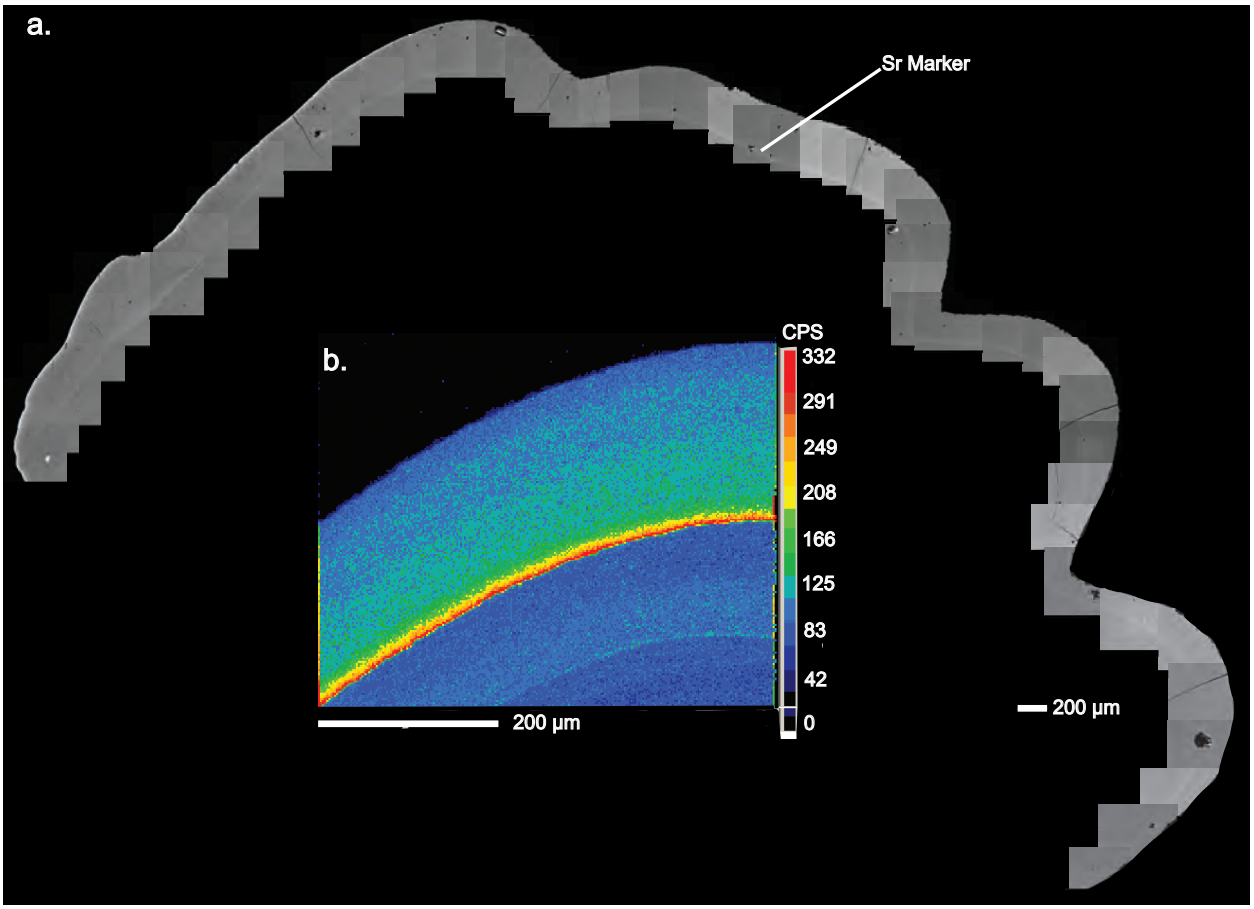

Figure 3.3: A BSE image representative of a whole right otolith. The strontium mark is visible as a lightened line in the grey scale image **(a.)** around the otolith edge. An x-ray map **(b.)** is shown inset, for a sub-region within the BSE imaged region. The yellow to red colour indicates the higher count intensities of strontium over the area identified as the strontium mark within the otolith.

standard deviation (sd)) or 2.19 cm.yr^{-1} for all 18 fish and 0.017 cm.day^{-1} (+/- 0.040 sd) or 6.31 cm.yr^{-1} with the one individual with large negative growth eliminated. Fish size at the time of marking significantly affected fish growth ($p < 0.001$) where small fish generally had increased growth in comparison to larger individuals. There was no significant relationship between the number of days from marking to recapture (212-1382 days; $p = 0.072$). The interaction effect of fish marking size with the mark to recapture period may affect fish growth rates ($p = 0.050$; overall model: $F_{3, 14} = 10.89$, residual standard error (rse) 0.0038, (adj. Rsq = 0.64)). Notably, some of the largest fish had both the highest negative growth and shortest mark to recapture periods (**Table 3.1**).

Otolith accretion rates (**Table 3.2**) were significantly different with respect to the type of structure examined ($p = 0.009$) but did not differ in relationship to $SrCl_2$ dose ($p = 0.728$). The mean accretion values in the left (0.225 μm.day^{-1}, +/- 0.138 sd) and right (0.239 μm.day^{-1}, +/- 0.144 sd) otoliths were similar, but differed from the cross-section (0.107 μm.day^{-1}, +/- 0.053 sd) indicating that the proximal region of the left cross-section accretes material at a slower rate than that seen on the edges of the right and left otoliths (**Figure 3.1**).

Wide variations in otolith growth occurred in comparison to fish growth rate (**Figure 3.4**). Weak relationships between otolith accretion rates and fish growth rates were observed for left and right (Rsq = 0.12), and cross-sectioned (Rsq = 0.04) otolith structures.

3.4.2 *Strontium Uptake and Signal Retention*

Table 3.2: Otolith accretion rate (**OAR**; µm.day^{-1}) is listed for individual fish (**SNO**) and the associated type of structured examined (**TS**) along with the number of line scans (**LS**) used. The structures examined are the left whole otolith edge (**LW**), the right whole otolith edge (**RW**) and the proximal zone over the left otolith nucleus in cross-sectional view (**XS**). Strontium uptake (**SU**, days), initial signal retention (**SR1**; days) and estimated pre-marking background (**EPMB**; days) are provided for each individual and structure type where N/A represents no data available. Standard deviation associated to each data value is provided in parentheses.

SNO-TS	LS	OAR	SU	SR1	EPMB
506566-LW	4	0.5608 (0.0975)	18 (04)	38 (31)	328 (016)
RW	3	0.2327 (0.0076)	29 (09)	60 (38)	375 (015)
XS	4	0.1572 (0.0154)	42 (09)	25 (10)	405 (076)
006402-LW	4	0.1038 (0.0080)	42 (05)	39 (04)	387 (023)
RW	3	0.0933 (0.0079)	46 (10)	30 (12)	565 (025)
XS	4	0.1335 (0.0089)	37 (07)	22 (03)	611 (029)
615354-LW	4	0.1516 (0.0166)	35 (04)	70 (48)	1147 (720)
RW	4	0.2580 (0.0195)	26 (04)	67 (07)	662 (067)
XS	2	0.0170 (0.0023)	158 (12)	158 (12)	N/A
001287-LW	3	0.1903 (0.0253)	34 (03)	62 (46)	557 (071)
RW	4	0.4496 (0.0285)	13 (03)	8 (02)	489 (025)
XS	4	0.0758 (0.0074)	63 (09)	41 (44)	744 (342)
000716-RW	4	0.2178 (0.0379)	17 (03)	14 (04)	478 (030)
XS	2	0.1221 (0.0339)	30 (08)	12 (08)	501 (028)
000777-LW	3	0.0350 (0.0103)	175 (15)	N/A	N/A
RW	4	0.0264 (0.0052)	210 (26)	N/A	N/A
XS	3	0.0317 (0.0052)	242 (62)	N/A	N/A
000788-LW	4	0.3311 (0.0065)	21 (03)	16 (06)	462 (034)
RW	4	0.1657 (0.0139)	41 (10)	14 (03)	451 (011)
XS	3	0.1401 (0.0259)	35 (13)	12 (02)	464 (036)
000059-LW	4	0.1240 (0.0114)	37 (05)	32 (08)	589 (081)
RW	3	0.1679 (0.0828)	34 (15)	75 (54)	647 (077)
XS	3	0.0970 (0.0067)	55 (03)	44 (13)	426 (276)
000538-LW	3	0.3172 (0.0539)	22 (03)	22 (22)	340 (024)

RW	4	0.1823 (0.0310)	35 (06)	45 (11)	362 (053)
XS	4	0.0649 (0.0150)	60 (11)	49 (11)	483 (054)
000026-LW	4	0.2119 (0.0470)	25 (07)	22 (10)	475 (064)
RW	4	0.5134 (0.0989)	18 (08)	32 (09)	660 (014)
000727-LW	4	0.1412 (0.0423)	33 (06)	20 (06)	563 (158)
RW	3	0.1482 (0.0484)	23 (01)	36 (13)	589 (031)
XS	2	0.1697 (0.0206)	16 (01)	32 (26)	608 (210)
002179-RW	2	0.1844 (0.0305)	20 (01)	43 (10)	664 (006)
629798-LW	4	0.0492 (0.0314)	192 (52)	128 (49)	905 (058)
RW	4	0.0484 (0.0019)	147 (28)	203 (44)	1065 (130)
XS	4	0.0345 (0.0045)	177 (25)	170 (37)	1016 (136)
638081-LW	4	0.2986 (0.1138)	57 (28)	105 (15)	778 (046)
RW	4	0.3610 (0.0530)	26 (03)	86 (29)	1093 (197)
XS	4	0.1872 (0.0168)	45 (10)	140 (26)	1464 (064)
584647-XS	4	0.1520 (0.0086)	42 (09)	136 (82)	902 (085)
003275-LW	3	0.2950 (0.0595)	31 (03)	137 (67)	1426 (238)
RW	3	0.4144 (0.0087)	20 (01)	142 (17)	1518 (043)
XS	3	0.1318 (0.0366)	47 (13)	51 (43)	1281 (303)
001574-LW	4	0.1993 (0.0126)	27 (09)	46 (10)	882 (044)
003017-LW	4	0.3615 (0.1206)	21 (05)	11 (04)	530 (019)
RW	4	0.3667 (0.2152)	31 (17)	7 (05)	535 (022)
XS	3	0.0955 (0.0224)	59 (01)	60 (41)	561 (242)

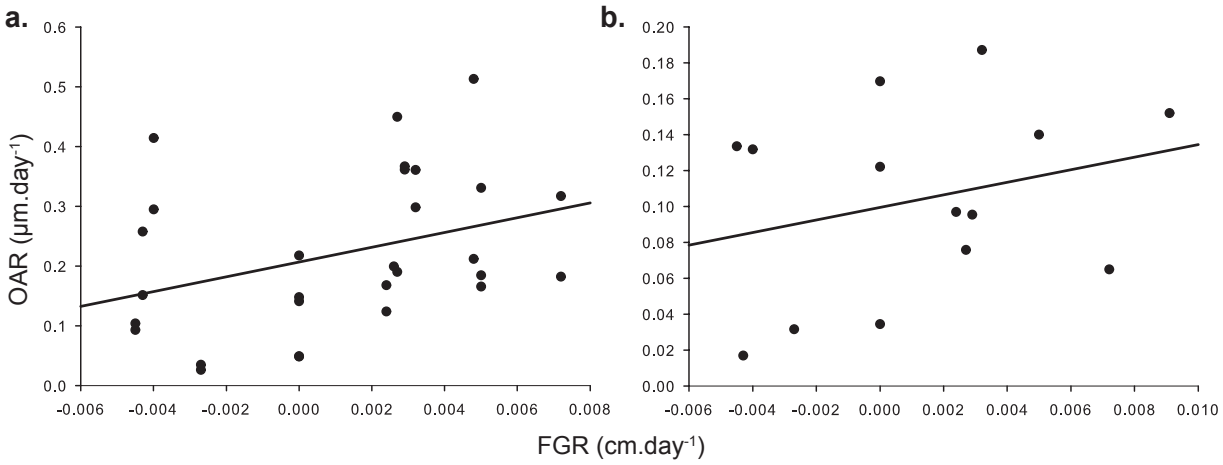

Figure 3.4: The linear regression relationship between fish growth rate (**FGR**) and otolith accretion rate (**OAR**) for the right and left whole otolith edge region (Rsq = 0.12, slope = 12.349, intercept = 0.2069) **(a.)** and the proximal growth over the nucleus in a cross-section of the left otolith (Rsq = 0.08, slope = 3.496, intercept = 0.0995) **(b.)**.

Linear regression ($F_{7, 38}$ = 29.83, rse = 0.1382, adj. Rsq = 0.82) analysis to examine strontium uptake days (SU) demonstrated that otolith growth ($p < 0.001$) and $SrCl_2$ dosage ($p = 0.016$) played a role in the initial stages of uptake. Strontium uptake was similar for all otolith structures examined ($p > 0.219$). Slower growing otoliths took longer to reach maximum incorporation (**Figure 3.5a**). High doses of $SrCl_2$ also increased the number of days to reach maximum incorporation over all otolith accretion values. No interaction effects are observed in the linear regression model between otolith accretion, $SrCl_2$ dose and type of otolith structure.

A linear relationship ($F_{7, 35}$ = 5.94, rse = 0.2829, adj. Rsq = 0.45) was observed in the period of initial strontium retention (SR1) to otolith growth (**Figure 3.5b**). The $SrCl_2$ dose ($p = 0.001$) influenced the initial signal retention period where the higher dose took a longer time to reach a point of stability. Otolith growth ($p = 0.628$) and type of otolith structure ($p > 0.205$) along with interactive effects did not play a role in the SR1 period.

Estimated return to background was influenced by $SrCl_2$ dose ($p < 0.001$) where, for higher doses of $SrCl_2$, fish took more days to return to the estimated strontium pre-marking background levels in comparison to lower dosed otoliths. In ground truthing our estimates for return to pre-marking backgrounds, three samples were available for analysis, all from the low dose group (sample numbers: 001574, 000538, 003017). For all otolith structure types, samples 001574 and 000538 returned to background levels within the range provided in the estimate +/- the sd. Fish (sample 003017), captured 1382 days after the marking event, reached pre-marking background levels around 550 days in the right otolith structure then established what appears to be new

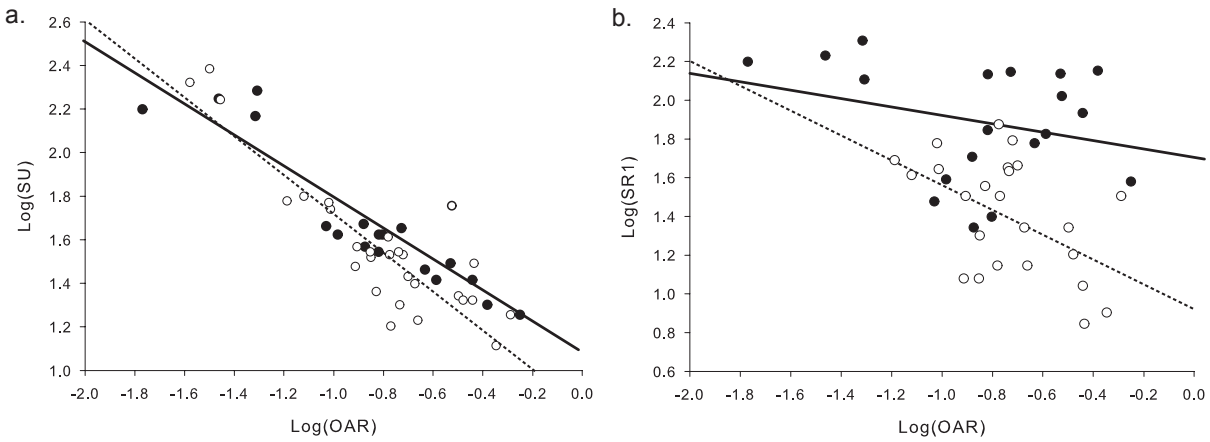

Figure 3.5: The relationships between otolith accretion rate (**Log(OAR)**) and the strontium uptake (**Log(SU)**) accounting for dose is shown in panel **(a.)**. Low dosed fish had an Rsq of 0.83, a slope of -0.8947, and an intercept of 0.8225 while the high dosed fish had an Rsq of 0.82, a slope of -0.7247, and an intercept of 1.0665. The linear relationships between otolith accretion rate (**Log(OAR)**) and the initial strontium retention period (**Log(SR1)**) is shown in panel **(b.)**. Low dosed fish had an Rsq of 0.26, a slope of -0.641, and an intercept of 0.9362 while the high dosed fish had an Rsq of 0.08, a slope of -0.2164, and an intercept of 1.7063. Low dosed fish (20 mg/kg wet body weight) are represented with the dashed line and open circles. High dosed fish (100 mg/kg we body weight) are represented by the solid line and filled circles.

background conditions thereafter (**Figure 3.6a**). In the left otolith structure no return to pre-marking background levels was observed (**Figure 3.6b**).

3.5 Discussion

In order to fully understand strontium marking characteristics and their potential meaning, it is important to first examine the broad context of otolith accretion and fish growth. Daily otolith accretion rates and associated fish growth rates can be influenced by factors such as stress, reproduction, seasonal energy storage and feeding (Kalish 1989, Geffen 1992, Campana 2005, DiMaria et al. 2010). These factors affect the physiology through calcium biomineralization and re-mobilisation within a fish (Weiss and Watabe 1978, Mahamid et al. 2011) and can influence strontium uptake and retention in the longer term. Not only is the fish marked with a natural environmental tracer (strontium) it also encounters strontium from its surrounding environment. Parallel to Geffen (1992), fish used in our study were reared in a natural environment post-tagging to provide the best possible way to understand strontium marking against a natural background; fish growth and associated otolith accretion given that the environmental conditions after the stress of marking were similar to that previous.

3.5.1 *Fish Growth and Otolith Accretion*

Greenland Halibut otoliths are asymmetrical in shape suggesting differences in overall otolith accretion axes between left and right-paired structures. Within our study, daily otolith accretion rates overall were similar between left and right structures when examined whole. However, the directionality of accretion between the left and right

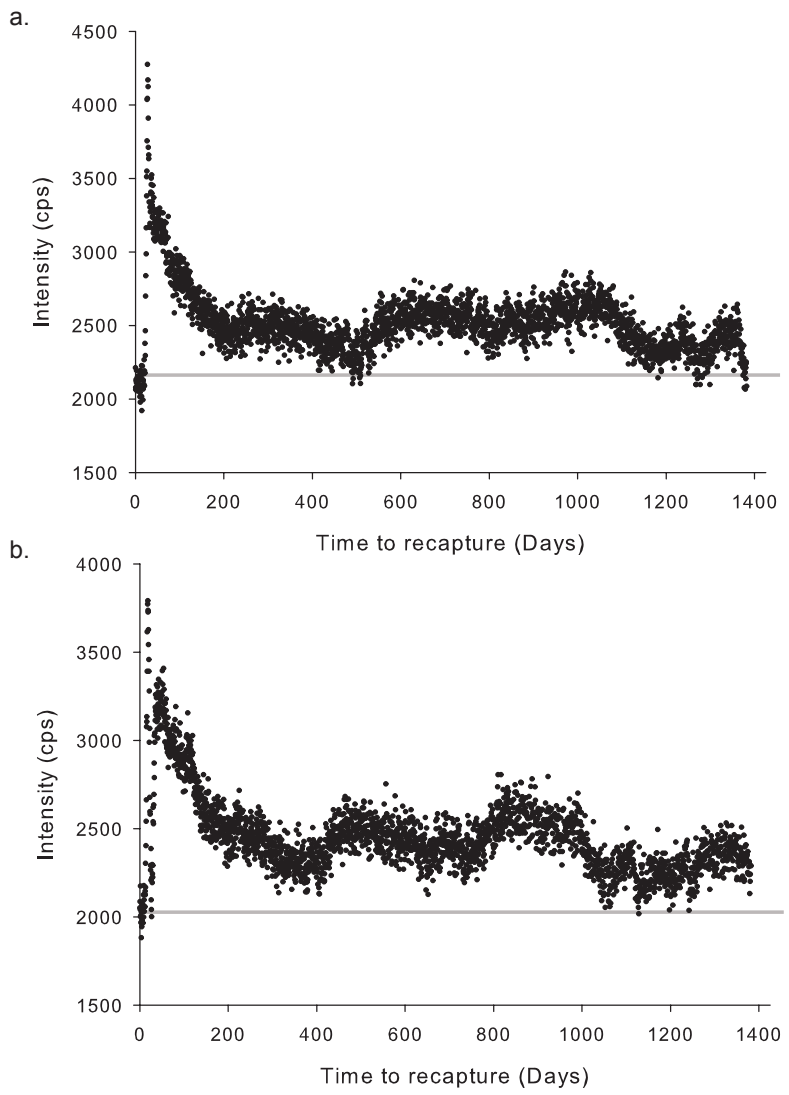

Figure 3.6: Representative ground truthed plots for sample number 003017 out at sea for 1382 days. The time to recapture is on the x-axis in days and strontium intensity in counts per second on the y-axis. The right whole otolith **(a.)** reaches pre-marking background levels at 550 days post-marking and thereafter establishes a new background norm. The left whole otolith **(b.)** appears to establish a new background norm post-marking. The grey line represents the average pre-marking background levels not shown in the graphic.

otolith varied; the right otolith grew in the anterior-ventral direction whereas the left otolith grew in a posterior-ventral direction as well as in the proximal direction. This second dimension of accretion, in the proximal zone of the cross-sectional view in the left otolith, is slower than that of the edge regions. The line scan data and associated BSE images of the proximal area, showed accretion was irregular, more so than was observed in the ventral areas examined on the left and right otolith edges. This difference in the directionality of the active growth zones between the left and right otoliths is likely due to differences in the physical space available for accretion since the left eye does not migrate beyond the dorsal ridge during metamorphosis. Further, given the heterogeneity in organic and ionic components of the endolymph observed in the proximal and distal zones of a bilaterally symmetrical otolith (Allemand et al. 2008), both a restricted and contorted physical space may affect the arrangement of otolith precursors, particularly the protein matrix that influences otolith growth and shape.

Greenland Halibut growth can be influenced by several factors such as life stage, stress, reproduction, and physical environment (Godø and Haug 1989, Miller et al. 1991, Morgan et al. 1997, Godø and Haug 1999, Morgan et al. 2013). In this study, fish size at marking played a significant role in fish growth rates over the time of the study. Some larger fish (>47 cm) had zero or negative growth, suggesting a resorption of tissues and/or no growth, whereas smaller individuals generally had positive growth. Size at maturation for Greenland Halibut found in Baffin Bay and Davis Strait is variable. Simonsen and Gundersen (2005) found that only females >80 cm were mature while Harris et al. (2009) found the length at 50% maturity varied between 62 cm and 84 cm for females and between 39 and 50 cm for males. Although, sex information is missing

for most of the samples used within the study, we infer that there is a mix of mature and immature fish of both sexes used in the study.

Stress induced during tagging, measured by the rate of mortality for long-line caught Greenland Halibut was relatively low (7.1 %) (Simonsen and Treble 2003). The rate of mortality for fish caught with the new aluminum box capture technique has not been studied. However, use of this box is thought to significantly reduce the stress of capture and improve survival rates for trawl caught fish (Holst and McDonald 2000). The post-marking period was extensive for most individuals, spanning from 212-1385 days, providing sufficient time in most cases for recovery to stress levels experienced before marking. Greenland Halibut growth rates in this study were noticeably variable with a mean of 2.19 $cm.yr^{-1}$ for individuals 37-67 cm (or 6.31 $cm.yr^{-1}$ for individuals 37-58 cm), higher than the results for oxytetracycline marking and Floy © tag studies conducted by Albert et al. (2009) (0.80 $cm.yr^{-1}$ for individuals 40 cm to 60 cm) and Treble et al. (2008) (<2 $cm.yr^{-1}$ for individuals 50 cm to 80 cm).

Measurements of pre- and post-marking individuals were taken in centimeters. Given the low or extremely slow growth observed for this species smaller scale length measurements such as millimeters should be considered, especially for larger fish. Alternatively, secondary measures of weight and body depth may need to be applied to further understand growth for this species. As in many other species, Greenland Halibut may demonstrate allometric growth (i.e., body depth or girth) as opposed to isometric growth (i.e., length) (Froese 2006). This would be observed as an increase in morphometric measures of body depth over size as fish grow larger. Despite this concern and suggestion of additional measures, a strong general observation of positive

growth in smaller individuals in comparison to negative growth in larger individuals out at sea for extensive periods of time suggests that this trend is likely accurate in representing changes of growth rates over the juvenile period for the species.

A large variation of otolith accretion values is observed over the range of fish growth suggesting the possibility of fast and slow otolith accretion groupings. The exploratory examination of otolith growth in comparison to fish growth yielded results that warrant further investigation beyond the scope of this study.

3.5.2 *Strontium Uptake and Signal Retention*

There are two phases of strontium substitution within an otolith to be addressed independently: 1. uptake and 2. retention. The uptake phase commences from the time of the intraperitoneal injection to time of maximum strontium incorporation into the otolith. This study indicated that the time of maximal strontium uptake occurred from 17 days to 242 days. For laboratory-reared Yellow Perch (*Perca flavescens*), the marking uptake period was approximately 27 days (Melançon 2008). The availability of strontium for otolith incorporation, through intraperitoneal injection bypasses the normal routes through Ca^{2+} uptake channels of the branchial epithelium and gastrointestinal tract (Chowdhury and Blust 2011). Instead strontium is absorbed through Ca^{2+} associated channels of peritoneal cells and into soft tissues and blood from the body cavity. The fish must then work to regain homeostasis by expelling strontium or its homologous counter-part calcium. This process is indirectly observed through the examination of otolith strontium uptake (uncontrolled dose response) and the subsequent retention period.

Within our study, otolith growth and dosage played a role in the otolith strontium uptake phase as reflected by the variation in the number of days to reach maximum uptake. The otolith uptake rate is considered a power relationship. Slower growing otoliths took a longer time to incorporate strontium to maximal levels up to a specified accretion value and thereafter played less of a role to the number of days of maximal incorporation. Similarly high dose individuals took longer to reach maximal strontium otolith incorporation levels. In the context of the broad scale literature on otolith biomineralization, the delayed response can be accounted for in terms of the turnover time for otolith precursors in surrounding endolymphatic fluids (Payan et al. 2002, Allemand et al. 2008). We infer that these otolith precursor reserves for slower growing otoliths would experience an initial delay in taking up the injected strontium, less turnover, and longer residence time once present in the endolymphatic fluid. Replacement would occur on an as-needed basis because biomineralization proceeds with calcium (and therefore strontium) entering the endolymphatic fluid by diffusion, likely via extracellular pathways (Payan et al. 2004, Allemand et al. 2008). A delay in the detection of the strontium injection would further suggest that the slower growing otoliths actually take longer than our estimates to reach maximal strontium uptake. This delay cannot be controlled in the present study design. We also expect that individuals would require longer periods of time to regain homeostasis with the application of the higher strontium dose; observed here as both a prolonged period in otolith strontium uptake and longer retention period.

Accretion in symmetrical otoliths occurs along the outer edges of the proximal surface with inhibitors slowing accretion on the distal surface (Allemand et al. 2008). In

Greenland Halibut where the left eye migrates as far as the dorsal ridge, asymmetry is observed in the otoliths. The right otolith grows in the anterior-ventral direction and the nucleus is not in a central position. The left otolith has a centrally located nucleus but growth occurs in two dimensions: along the edge region and over the nucleus on the proximal size only (**Figure 3.1**). Accretion, in our study, along the left and right otolith edges was similar and occurred at a faster rate than over the nucleus in the left otolith. Within the same otolith, slow accretion rates over the nucleus region resulted in a longer uptake period and higher accretion rates along the edge resulted in shorter number of uptake days. Individual element and protein precursor gradients within the endolymphatic fluid on either side of the left otolith appear to be strongly conservative and asynchronous in their turnover rates during the biomineralization process. Allemand et al. (2008) similarly found evidence that the proximal and distal endolymphatic fluid is functionally separated even though spatially connected.

After maximal uptake of strontium is achieved, the fish began the process of expelling the excess from its body. There are two relevant aspects of strontium retention: the initial phase of expulsion where instability and rapid release of strontium is exhibited (SR1), and upon reaching an inflection point the second phase is characterised by the predictable release of strontium to estimate a return to background levels (EPMB). A decoupling of otolith growth from strontium retention is observed during the rapid release of strontium (SR1) although a weak linear relationship is still present. During this phase, we infer that physiological control mechanisms play the strongest role in strontium availability for biomineralization. A rapid expulsion rate overcomes the otolith growth relationship initially observed in the strontium uptake

phase. In addition, higher saturation rates of strontium during dosing still play a role in strontium retention with high dose individuals taking longer to expel strontium as observed in this study. When we further examine the estimated return to background, no relationship is observed in otolith accretion rates. As expected, only a relationship with dose is observed. In ground-truthing our estimates of return-to-background, it is notable that some lower dosed individuals returned to their previous background state while others do not. Based upon preliminary findings, these fish appear to have established new background equilibria, higher than that prior to marking. Increased observation of ground-truthed estimates are required before drawing any strong conclusions, however, this is difficult given the low return rate of otoliths from natural settings.

The estimated return-to-background levels (328-1518 days) were much longer than found for Yellow Perch (140 days) (Melançon 2008). In addition to dose, the extended strontium retention period, as discussed below in terms of physiology and homeostasis can also be influenced by temperature, the composition of external environments, strontium storage mechanisms, and metabolism/growth rates. Temperature can affect the kinetics of competition with calcium and thus the incorporation of strontium into the otolith ($CaCO_3$) structure (Elsdon and Gillanders 2002, Martin et al. 2004, Marohn et al. 2011). This relationship is reported as positive (Kalish 1989, Arai et al. 1995, Bath et al. 2000), non-existent (Gallahar and Kingsford 1996), or inversely correlated (Radtke et al. 1990, Tzeng 1994) across studies. We conclude from these studies that environmental conditions, regardless of the exact relationship, will influence the retention time of calcium (and therefore strontium) within

the endolymphatic sac as well as incorporation into the otolith itself. Greenland Halibut are a deep-water, bottom-dwelling species that experience low temperatures (between 0 - 4 °C) over their lifespan in the study area (Jørgensen 1997, Treble 2015). Individuals are known to undertake migrations, likely between rearing and spawning grounds but large temperature differences between regions are not reported (Jørgensen 1997).

The elevated strontium concentrations observed in the otolith likely occurred in other calcium storage sites within bony structures of the fish such as fins, bones, scales, and cartilage. Williamson et al. (2009) and Melançon (2008) noted increased levels of barium and strontium, respectively, in the bones they studied but not in any other tissues or fluids examined. This would suggest that strontium is remobilised from bone tissues over time and available to other calcium-based hard structures such as otoliths to keep strontium elevated over background levels. In support, Loewen et al. (*in press*) provides a strong basis of calcium remobilisation (and therefore strontium) in calcium-phosphate bony structures. Otoliths, once formed, are inert and thus do not share this characteristic (Allemand et al. 2008, Doubleday et al. 2014). Other factors, such as life stage (i.e., growth potential) could affect the length of the retention period and the release of excess strontium from internal storage mechanisms (Sadovy and Severin 1994). Interaction amongst low temperature, storage mechanisms and slow-growth within Greenland Halibut (>37 cm in size) likely contributes to the retention of strontium above background levels for extensive periods of time and for at least one individual in our study, background levels were not reached and a new base-line seems to have been established.

3.6 Summary

Greenland Halibut elemental marking and recapture was used to document otolith accretion, fish growth rates and characterise the uptake and retention period of strontium post-marking. Otolith accretion occurred along different axes in the left and right otolith structures. The right otolith accretion occurred in an anterior-ventral direction whereas the left grew in posterior-ventral, anterial-dorsal, and proximal directions. The accretion rates were similar between left and right structures along the edge region; however, accretion over the nucleus in the left proximal region was slower than that of the edge regions. The mean fish growth rate (2.19 $cm.yr^{-1}$ for all 18 individuals 37-67 cm; 6.31 $cm.yr^{-1}$ for 17 individuals 37 to 58 cm) was slightly higher than that reported in the literature for Greenland Halibut. The examination of otolith accretion in comparison to fish growth demonstrated a large variation of otolith accretion values over fish growth rates. The possibility of fast and slow otolith accretion groupings warrants further investigation outside the scope of the study. Strontium uptake took anywhere from 17 to 242 days and was strongly influenced by otolith accretion rate and dose. A power relationship was observed between the otolith accretion rate and strontium uptake. Slow accretion resulted in a longer time to reach maximal strontium concentrations. Similarly, high doses of strontium took longer to reach maximal strontium concentrations. In the initial stages of strontium retention, and during the estimated return-to-background, only dose level plays a role in signal retention time. The estimated return-to-background (328-1518 days) is much longer than that reported in other studies. Storage mechanisms, temperature and growth rates of fish likely contribute to the extended strontium-retention period post-marking. Knowledge of long

retention periods post-marking may assist in interpreting elemental profiles of long-lived species that migrate frequently between marine and freshwater environments over relatively short timeframes. Further research to examine element storage mechanisms and their remobilisation in the context of fish physiology will aid our understanding of element incorporation into otoliths.

3.7 Acknowledgements

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3.8 References

Albert, O. T. 2003. Migration from nursery to spawning area in relation to growth and maturation of Greenland Halibut (*Reinhardtius hippoglossoides*) in the Northeast Arctic. J. Northw. Atl. Fish. Sci. **31**: 113–125.

Albert, O. T., Kvalsund, M., Vollen, T., and Salberg, A. B. 2009. Towards accurate age determination of Greenland Halibut. J. Northw. Atl. Fish. Sci. **40**: 81–95. doi: 10.2960/J.v.40.m659.

Albert, O. T., and Vollen, T. 2014. A major nursery area around the Svalbard archipelago provides recruits for the stocks in both Greenland halibut management areas in the Northeast Atlantic. ICES J. Mar. Sci. **72**: 872–879.

Allemand, D., Mayer‐Gostan, N., De Pontual, H., Boeuf, G., and Payan, P. 2008. Fish otolith calcification in relation to endolymph chemistry. *In* Handbook of Biomineralization: Biological aspects and structure formation. *Edited by* E. Baeuerlien. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. pp. 291–308.

Arai, N., Sakamoto, W., and Maeda, K. 1995. Analysis of trace elements in otoliths of Red Sea Bream *Pagrus major*. Fish. Sci. **61**: 43–47. doi: 10.2331/fishsci.61.43.

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

Boje, J. 2002. Intermingling and seasonal migrations of Greenland halibut (*Reinhardtius hippoglossoides*) populations determined from tagging studies. Fish. Bull. **100**: 414-422.

Bowering, W. R. 1984. Migrations of Greenland Halibut, *Reinhardtius hippoglosoides*, in the Northwest Atlantic from tagging in the Labrador Region. J. Northw. Atl. Fish. Sci. **5**: 85-91.

Bowering, W. R., and Nedreaas, K. H. 2000. A comparison of Greenland halibut (*Reinhardtius hippoglossoides* (Walbaum)) fisheries and distribution in the Northwest and Northeast Atlantic. Sarsia **85**: 61–76. doi: 10.1080/00364827.2000.10414555.

Cadrin, S. X., Friedland, K. D., and Waldman, J. R. (*Editors*). 2005. Stock Identification Methods: Applications in Fishery Science. Elsevier Academic Press, Burlington, MA.

Campana, S. E. 2005. Otolith science entering the 21st century. Mar. Freshw. Res. **56**: 485-495. doi: 10.1071/MF04147.

Chowdhury, M. J., and Blust, R. 2001. A mechanistic model for the uptake of waterborne strontium in the Common Carp (*Cyprinus carpio* L.). Environ. Sci. Technol. **35**: 669–675. doi: 10.1021/es000142t.

Chowdhury, M. J., and Blust, R. 2011. Chapter 7: Strontium. *In* Homeostasis and Toxicology of Non-Essential Metals. *Edited by* A. P. Farrell, C. J. Brauner, and C. M. Wood. Academic Press. pp. 351–390. doi: http://dx.doi.org/10.1016/S1546-5098(11)31029-1.

Courtney, D. L., and Severin, K. P. 2007. Validation of otolith increment daily periodicity in captive juvenile sablefish (*Anoplopoma fimbria*) experimentally immersed in strontium chloride ($SrCl_2$). Fish. Res. **83**: 246–252. doi: 10.1016/j.fishres.2006.10.013.

DiMaria, R. A., Miller, J. A., and Hurst, T. P. 2010. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. Environ. Biol. Fish. **89**: 453–462. doi: 10.1007/s10641-010-9665-2.

Doubleday, Z. A., Harris, H. H., Izzo, C., and Gillanders, B. M. 2014. Strontium randomly substituting for calcium in fish otolith aragonite. Anal. Chem. **86**: 865–869. doi: 10.1021/ac4034278.

Elsdon, T. S., and Gillanders, B. M. 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. Can. J. Fish. Aquat. Sci. **59**: 1796–1808. doi: 10.1139/F02-154.

Froese, R. 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. **22**: 241–253. doi: 10.1111/j.1439-0426.2006.00805.x.

Gallahar, N., and Kingsford, M. 1996. Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: an experimental investigation. J. Fish Biol. **48**: 174–186. doi/10.1111/j.1095-8649.1996.tb01111.x/abstract.

Geffen, A. J. 1992. Chapter 6: Validation of Otolith Increment Deposition Rate. *In* Otolith microstructure examination and analysis. *Edited by* D. K. Stevenson and S. E. Campana. Can. Spec. Publ. Fish. Aquat. Sci **117**. pp. 101–113.

Gillanders, B. 2009. Chapter 13: Tools for Studying Biological Marine Ecosystem Interactions-Natural and Artificial Tags. *In* Ecological Connectivity among Tropical Coastal Ecosystems. *Edited by* I. Nagelkerken. Springer Netherlands. pp. 457–492. doi: 10.1007/978-90-481-2406-0_13.

Godø, O. R., and Haug, T. 1989. A review of the natural history, fisheries, and management of Greenland halibut (*Reinhardtius hippoglossoides*) in the eastern Norwegian and Barents Seas. ICES J. Mar. Sci. **46**: 62–75. doi: 10.1093/icesjms/46.1.62 .

Godø, O. R., and Haug, T. 1999. Growth rate and sexual maturity in Cod (*Gadus morhua*) and Atlantic Halibut (*Hippoglossus hippoglossus*). J. Northwest Atl. Fish. Sci. **25**: 115–123.

Gregg, J. L., Anderl, D. M. and Kimura, D. K. 2006. Improving the precision of otolith-based age estimates for Greenland halibut (*Reinhardtius hippoglossoides*) with preparation methods adapted for fragile sagittae. Fish. Bull. **104**: 643–648.

Harris, L. N., Treble, M. A. and Morgan, M. J. 2009. An update of maturity for Greenland halibut from trawl surveys of NAFO Subarea 0 with emphasis on Division 0A. Northwest Atlantic Fisheries Organization SCR Doc. **09/025**. 12 pp.

Hernaman, V., Munday, P. L., and Schla, M. L. 2000. Validation of otolith growth-increment periodicity in tropical gobies. Mar. Biol. **137**: 715–726.

Hobbs, J., Castillo, G., Tigan, G., Lindberg, J., Ikemiyagi, N., and Ramos, G. 2012. Tagging the next generation: validation of trans-generational chemical tagging for an endangered fish. Environ. Biol. Fish. **95**: 463–468. doi: 10.1007/s10641-012-0034-1.

Holst, J. C., and McDonald, A. 2000. FISH-LIFT: a device for sampling live fish with trawls. Fish. Res. **48**: 87-91.

Huelga-Suarez, G., Fernández, B., Moldovan, M., and García Alonso, J. I. 2013. Detection of transgenerational barium dual-isotope marks in salmon otoliths by means of LA-ICP-MS. Anal. Bioanal. Chem. **405**: 2901–9. doi: 10.1007/s00216-012-6452-2.

Hüssy, K., Nielsen, B., Mosegaard, H., and Clausen, L. 2009. Using data storage tags to link otolith macro- structure in Baltic cod *Gadus morhua* with environmental conditions. Mar. Ecol. Prog. Ser. **378**: 161–170. doi: 10.3354/meps07876.

Jørgensen, O. A. 1997. Movement Patterns of Greenland Halibut, *Reinhardtius hippoglossoides* (Walbaum), at West Greenland, as Inferred from Trawl Survey Distribution and Size Data. J. Northw. Atl. Fish. Sci. **21**: 23–37.

Kalish, J. M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J. Exp. Mar. Bio. Ecol. **132**: 151–178. doi: 10.1016/0022-0981(89)90126-3.

Kennedy, J., Hedeholm, R. B., Gundersen, A. C., and Boje, J. 2014. Estimates of reproductive potential of Greenland halibut (*Reinhardtius hippoglossoides*) in East Greenland based on an update of maturity status. Fish. Res. **154**: 73–81. doi: http://dx.doi.org/10.1016/j.fishres.2014.02.009.

Loewen, T. N., Carriere B., Reist, J. D., Halden, N. M., and Anderson, W. G. (*in press*). Linking physiology and biomineralization processes to ecological inferences on the life history of fishes. Comp. Biochem. Phys. A doi: 10.1016/j.cbpa.2016.06.017.

Mahamid, J., Addadi, L., and Weiner, S. 2011. Crystallization pathways in bone. Cells Tissues Organs **194**: 92–97. doi: http://www.karger.com/DOI/10.1159/000324229.

Marohn, L., Hilge, V., Zumholz, K., Klügel, A., Anders, H., and Hanel, R. 2011. Temperature dependency of element incorporation into European eel (*Anguilla anguilla*) otoliths. Anal. Bioanal. Chem. **399**: 2175–2184. doi: 10.1007/s00216-010-4412-2.

Martin, G. B., Thorrold, S. R., and Jones, C. M. 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). Can. J. Fish. Aquat. Sci. **61**: 34–42. doi: 10.1139/F03-143.

Melançon, S. 2008. Mechanisms controlling the microchemistry and composition of fish otoliths. PhD. Dissertation. University of Windsor, Windsor, ON, Canada. pp iii-146.

Miller, J. M., Burke, J. S., and Fitzhugh, G. R. 1991. Early life history patterns of Atlantic North American flatfish: Likely (and unlikely) factors controlling recruitment. Netherlands J. Sea Res. **27**: 261–275. doi: http://dx.doi.org/10.1016/0077-7579(91)90029-Z.

Morgan, M. J., and Bowering, W. R. 1997. Temporal and geographic variation in maturity at length and age of Greenland halibut (*Reinhardtius hippoglossoides*) from the Canadian Northwest Atlantic with implications for fisheries management. ICES J. Mar. Sci. **54**: 875–885. doi: 10.1006/jmsc.1996.0200.

Morgan, M. J., Garabana, D., Rideout, R. M., Román, E., Pérez-Rodríguez, A., and Saborido-Rey, F. 2013. Changes in distribution of Greenland halibut in a varying environment. ICES J. Mar. Sci. **70**: 352–361. doi: 10.1093/icesjms/fss179 .

Munro, A. R., Gillanders, B. M., Elsdon, T. S., Crook, D. A., and Sanger, A. C. 2008. Enriched stable isotope marking of juvenile golden perch (*Macquaria ambigua*) otoliths. Can. J. Fish. Aquat. Sci. **65**: 276–285. doi: 10.1139/f08-010.

Payan, P., Borelli, G., Priouzeau, F., De Pontual, H., Boeuf, G., and Mayer-Gostan, N. 2002. Otolith growth in trout *Oncorhynchus mykiss*: supply of Ca^{2+} and Sr^{2+} to the saccular endolymph. J. Exp. Biol. **205**: 2687–2695. http://www.ncbi.nlm.nih.gov/pubmed/12151374.

Payan, P., De Pontual, H., Edeyer, A., Borelli, G., and Boeuf, G. 2004. Effects of stress on plasma homeostasis, endolymph chemistry, and check formation during otolith growth in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **61**: 1247–1255. doi: 10.1139/F04-059.

Peklova, I., Hussey, N. E., Hedges, K. J., Treble, M. A., and Fisk, A. T. 2012. Depth and temperature preferences of the deepwater flatfish Greenland halibut *Reinhardtius hippoglossoides* in an Arctic marine ecosystem. Mar. Ecol. Prog. Ser. **467**: 193-205.

Pors Nielsen, S. 2004. The biological role of strontium. Bone **35**: 583–588. doi: http://dx.doi.org/10.1016/j.bone.2004.04.026.

Radtke, R. L., Townsend, D. W., Folsom, S. D., and Morrison, M. A. 1990. Strontium:calcium concentration ratios in otoliths of herring larvae as indicators of environmental histories. Environ. Biol. Fish. **27**: 51-61.

Radzki, R.P., Bienko, M., Filip, R., Albera, E., and Kankofer, M. 2009. Effect of strontium ranelate on femur densitometry and antioxidative/oxidative status in castrated male rats. Scand. J. Lab. Anim. Sci. **36**: 193–201.

Roy, D., Hardie, D. C., Treble, M. A., Reist, J. D., and Ruzzante, D. E. 2014. Evidence supporting panmixia in Greenland halibut (*Reinhardtius hippoglossoides*) in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **71**: 763-774.

Sadovy, Y., and Severin, K. P. 1994. Elemental Patterns in Red Hind (*Epinephelus guttatus*) Otoliths from Bermuda and Puerto Rico Reflect Growth Rate, Not Temperature. Can. J. Fish. Aquat. Sci. **51**: 133–141. doi: 10.1139/f94-015.

Simonsen, C. S., and Gundersen, A. C. 2005. Ovary development in Greenland halibut *Reinhardtius hippoglossoides* in West Greenland waters. J. Fish. Biol. **67**: 1299-1317.

Simonsen, C. S., and Treble, M. A. 2003. Tagging Mortality of Greenland Halibut *Reinhardtius hippoglossoides* (Walbaum). J. Northw. Atl. Fish. Sci. **31**: 373–385.

Treble, M. A. 2015. Report on Greenland halibut caught during the 2014 trawl survey in Divisions 0A and 0B. Northwest Atlantic Fisheries Organization SCR Doc. **15/030**. 35 pp.

Treble, M. A., Campana, S. E., Wastle, R. J., Jones, C. M., and Boje, J. 2008. Growth analysis and age validation of a deepwater Arctic fish, the Greenland halibut (*Reinhardtius hippoglossoides*). Can. J. Fish. Aquat. Sci. **65**: 1047–1059. doi: 10.1139/F08-030.

Tzeng, W. N. 1994. Temperature effects on the incorporation of strontium in otolith of Japanese eel, *Anguilla japonica*. J. Fish Biol. **45**: 1055–1066. doi: 10.1111/j.1095-8649.1994.tb01072.x.

Vis, M. L., Carr, S. M., Bowering, W. R., and Davidson, W. S. 1997. Greenland halibut (*Reinhardtius hippoglosoides*) in the North Atlantic are genetically homogeneous. Can. J. Fish. Aquat. Sci. **54**(8): 1813-1821.

Wang, X., Tan, Y., Sievers, Q., Sievers, B., Lee, M., Burrall, K., and Schreiber, A. M. 2011. Thyroid hormone-responsive genes mediate otolith growth and development during flatfish metamorphosis. Comp. Biochem. Phys. A **158**: 163–168. doi: 10.1016/j.cbpa.2010.10.014.

Weiss, R. E., and Watabe, N. 1978. Studies on the biology of fish bone-I. Bone resorption after scale removal. Comp. Biochem. Phys. A **60**: 207–211. doi: http://dx.doi.org/10.1016/0300-9629(78)90233-5.

Williamson, D., Jones, G., and Thorrold, S. 2009. An experimental evaluation of transgenerational isotope labelling in a coral reef grouper. Mar. Biol. **156**: 2517–2525. doi: 10.1007/s00227-009-1276-0.

Yankovich, T. L. 2009. Mass balance approach to estimating radionuclide loads and concentrations in edible fish tissues using stable analogues. J. Environ. Radioact. **100**: 795–801. doi: http://dx.doi.org/10.1016/j.jenvrad.2009.05.001.

Chapter 4

Discrimination of Northern Form Dolly Varden Char (*Salvelinus malma malma*) Stocks of the North Slope, Yukon and Northwest Territory, Canada via Otolith Trace Elements and $^{87}Sr/^{86}Sr$ Isotopes

This manuscript has been published in the journal of Fisheries Research. This manuscript has been coauthored with my adviser and collaborators. I have been the lead on the manuscript. Specifically I was responsible for the study design, experimental analysis, writing, aspects of the field collection (water sampling) and revisions for the manuscript. My co-authors provided insight to the overall review of the study, conducted most of the field-based parts of the study, and provided editorial and review comments on multiple drafts of the manuscript. This paper is reproduced with the permission from Elsevier (copyright by the primary author is retained for publication in a thesis dissertation) and my coauthors.

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4.1 Abstract

Determination of unit-stock structure and the assignment of individuals from mixed groups to stock-of-origin is important to conservation and management of Arctic riverine fishes. Northern form Dolly Varden Char (*Salvelinus malma malma*) are closely linked to perennial groundwater sources during the freshwater stages of their life cycle. This close connectivity provides an ideal circumstance for stock discrimination using archived sagittal otoliths (1986-2007) collected from the river drainages of the study area (Firth River, Yukon Territory (YT), Babbage River, YT, Big Fish River, Northwest Territories (NT), Rat River, NT, and Vittrekwa River, NT). Laser ablation-ICP-MS techniques were employed to measure $^{87}Sr/^{86}Sr$ isotopes and trace elements (Sr, Ba, S, Zn, Mg, P) from dorso-ventral otolith thin sections. Random forest model, learning algorithm and tree classification provided 94.4% classification success for individual fish to the river systems (putative stocks). Misclassifications within the model occurred among the Vittrekwa, Babbage, and Rat river drainages. Strontium, $^{87}Sr/^{86}Sr$ ratio and Ba were the most significant discriminators within the model, although removal of the remaining elements reduced overall discriminatory power. Microchemical analyses of archived otoliths provided high classification and discrimination success thus can be used as a tool for fisheries managers in developing effective management and conservation plans regarding mixed-stock fisheries for this species.

4.2 Introduction

Northern form Dolly Varden Char (*Salvelinus malma malma*) are distributed in high-gradient river systems along the Alaskan and Yukon Territory (YT) North Slope and

western Northwest Territories (NT) region of the Western Arctic. The species exhibits three ecotypes and life history variants (inland isolates, anadromous, residual male), all of which are closely associated with rivers with perennial groundwater flows for key life history events including spawning, egg development, rearing of juveniles and overwintering (Craig,1989, Krueger et al. 1999, Mochnacz et al. 2010, Reist and Sawatzky 2010, Brown et al. 2014). In mountain streams where perennial groundwater inflow is absent, river flow ceases in the winter due to complete freezing of the water column to the riverbed, making these habitats unsuitable for Dolly Varden Char during parts of the year. Accordingly, individual populations are thought to be closely associated with river systems having perennial groundwater sources.

Anadromous Arctic populations of Dolly Varden Char migrate from overwintering rivers to coastal margins of the Beaufort Sea for summer feeding (Krueger et al. 1999, Brown et al. 2014), where individuals from multiple stocks mix. Winter marine conditions in Arctic waters obligate some salmonids such as Dolly Varden Char to return to fresh water to over-winter. Being iteroparous, coastal groups of Dolly Varden Char consist of juvenile fish (smolts and larger), ripening adult spawners of the year, and resting adults. Although philopatry to natal streams appears to be high (see below), considerable wandering among non-natal over-wintering sites may also occur.

The habitat associated with perennial groundwater is a primary limiting factor for abundances of individual Dolly Varden river/migratory populations (Sandstrom et al. 2001, Mochnacz et al. 2010, Brown et al. 2014). In recent years, due to environmental change (changes in precipitation, permafrost melt) and also fishing pressure, Dolly Varden Char population numbers have become a concern to management and

conservation organisations within Canada such as the Fisheries Joint Management Committee, Fisheries and Oceans Canada, Committee on the Status of Endangered Wildlife in Canada and local communities (COSEWIC 2010, Gallagher et al. 2010, Reist and Sawatzky 2010).

The close association of Dolly Varden Char to streams with perennial groundwater sources during adult and early life stages allows the study of stock discrimination among various populations within the region. Past tagging and genetic studies suggest high adult over-wintering and spawning philopatry of Dolly Varden Char on the YT North Slope and northwestern NT. Reist (1989) and Harris et al. (2015) identified genetically discrete Dolly Varden Char stocks in the Firth, Babbage, Big Fish, Vittrekwa and Rat rivers. Similarly, Crane et al. (2005) confirmed genetically distinct Alaskan Dolly Varden Char stocks. These and other studies such as Krueger et al. (1999) suggest inter-drainage exchange (of spawning individuals) is infrequent. A more extensive exchange among systems in southeastern Alaska is observed specifically between over-wintering adults and post-smolt juveniles (Armstrong 1974, Armstrong and Morrow 1980). Adult Dolly Varden Char from the Sagavanirktok River drainage area (Alaskan North Slope) predominantly over-wintered in natal systems whereas a small number of post-smolt juveniles (~15%) strayed in over-wintering habitat from their natal river of origin (Krueger et al. 1999). Brown et al. (2014) found that 20.5% of radio-tagged Dolly Varden Char in the Hulahula River strayed to other river systems for the purpose of overwintering and potentially reproduction. A tagging study (DeCicco 1997) in contrast suggested a lack of natal over-wintering site fidelity in the Wulik River and Noatak River region, northwestern Alaska. This finding was most observed for summer-

spawning Dolly Varden Char; whereas, fall-spawning individuals demonstrated philopatry to over-wintering sites within natal tributaries after spawning. These tagging studies (DeCicco 1992,1997) also exemplified large-scale migrations >1690 km (Alaska to Russia) for a small number of Dolly Varden Char. DeCicco (1997) hypothesised that the tagged Dolly Varden Char were of Russian origin overwintering in Alaska then returning to natal rivers in the Anadyr River region, Russia to spawn.

Presently, mixed stocks in the coastal environment are targeted in subsistence fisheries at Shingle Point, and Herschel Island (YT North Slope, Canada), and within Alaskan waters by aboriginal and Inuvialuit community members (Papik et al. 2003, Pedersen and Linn 2005). Fisheries occur where anadromous Dolly Varden Char congregate and mix along the coast to feed during summer months (DeCicco 1997, Fechhelm et al. 1997). Krueger et al. (1999) suggested that approximately 20% of the fish in the Endicott Canal (Prudhoe Bay), Alaskan North Slope, captured by aboriginal fishers, were of Canadian origin. Likewise, unpublished genetic data (Bajno and Reist, Fisheries and Oceans Canada, Winnipeg) from Herschel Island and Shingle Point suggest an Alaskan component to the Canadian mixed-stock coastal fishery. This subsistence fishery holds historical and cultural significance for Inuvialuit and Gwich'in from communities such as Aklavik, Inuvik and Fort McPherson (Gwich'in Elders 1997, Haszard and Shaw 2000, Papik et al. 2003). Emphasis on understanding the origins of fish in the mixed-stock fishery is highlighted in the development of management plans by aboriginal and Inuvialuit communities and governing bodies for the fish stocks in the YT North Slope and northwestern NT.

Various approaches have been used to identify the origin of stocks in mixed-stock fisheries including genetics and otolith elements and isotopes (Cadrin 2000, Barnett-Johnson et al. 2010). It is known that otoliths incorporate trace elements from ambient environments into the calcium carbonate structure as well as the associated organic matrix. Elements such as strontium (Sr) and barium (Ba) are known to substitute directly for calcium (Ca) in calcium-carbonate structures due to similarities in valence structures and charge of atoms (Mann 2001, De Pontual and Geffen 2002). It has been well recognized that Sr, a non-essential element for life processes, is a proxy for environmental signals within the environment (Farrell and Campana 1996). Likewise, studies on Ba parallel those of Sr where otolith element accretion occurred in relation to element concentrations in ambient environments (Bath et al. 2000, Elsdon and Gillanders, 2002, 2003, 2004, Walther and Thorrold 2009). Other elements, commonly incorporated into stock discrimination studies such as lithium (Li), lead (Pb), manganese (Mn) and magnesium (Mg), have been shown to increase discriminatory power in stock-of-origin identifications (Swearer et al. 2003, Brown 2006, Swan et al. 2006, Hamer and Jenkins 2007, Standish et al. 2008, Arkhipkin et al. 2009). Specifically, within freshwater systems, Schaffler and Winkelman (2008), Veinott and Porter (2005) and Wells et al. (2003) determined stock-of-origin of Striped Bass (*Morone saxatilis*), Westslope Cutthroat Trout (*Oncorhyncus clarki lewisi*) and Atlantic Salmon (*Salmo salar*), respectively based on suites of trace elements from otoliths.

Elements such as sodium (Na), potassium (K), phosphorous (P), sulphur (S), Mn, Mg, iron (Fe), copper (Cu), and zinc (Zn) are essential in life processes such as ionic gradients, osmoregulation, redox reactions and structural function thus are regulated in

fish through various cellular pathways (Mann 2001, Crichton 2008). Their substitution into the otolith calcium-carbonate structure is thus indirect, more complex and less understood. Essential life elements are useful in stock discrimination studies despite the complexity of their presence in the otolith (Campana1999, 2005a, 2005b). This is possible assuming the otolith element concentrations between groups of fish and/or their environments vary significantly.

The use of $^{87}Sr/^{86}Sr$ isotope ratios is ideal for use in stock discrimination studies due to lack of fractionation by biological processes including the incorporation into otoliths (Kennedy et al. 2000, 2002). Ratios of $^{87}Sr/^{86}Sr$ remain relatively constant over ecological timescales due to the long half-life of ^{87}Rb ($4.92x10^{10}$ years), thus different $^{87}Sr/^{86}Sr$ in otoliths reflect distinct watershed lithologies (Hobbs et al. 2005). Further, the $^{87}Sr/^{86}Sr$ ratios found within an otolith are not physiologically regulated and therefore provide a precise geochemical signal of stock of origin.

Within the YT North Slope and northwestern NT region, geological and hydrological differences have been identified among the various river systems inhabited by Dolly Varden Char (Clark and Lauriol 1997, Clark et al. 2001, Lacelle et al. 2004). Stability of the environmental geochemical signals over a longer time scale (~25 years) is due to perennial spring water within each of the studied rivers (Clark et al. 2001, Utting et al. 2012). Given that rivers from this region have distinct trace elements and $^{87}Sr/^{86}Sr$ ratios which provide good proxies for river-specific “fingerprints”, successful assignment of Dolly Varden Char captured in the coastal mixed-stock fishery back to natal streams is possible. In addition, previous studies by Campbell et al. (2002) and Outridge et al. (2002) demonstrated the feasibility of using total Sr and $^{87}Sr/^{86}Sr$ ratio

resolution within the study region. The purpose of this study was to examine elemental and isotopic composition of archived otoliths for their utility in discriminating Dolly Varden Char populations of the YT North Slope and northwestern NT.

4.3 Materials and Methods

4.3.1 *Study Area*

Dolly Varden Char inhabiting river drainage systems of the YT North Slope and northwestern NT, fed by perennial spring sources and documented as habitat, are the focus of the study: 1.) Firth River (tributary Joe Creek), 2.) Babbage River (tributary Fish Hole Creek), 3.) Big Fish River (tributary Little Fish Creek), 4.) Rat River (tributary Fish Creek), and 5.) Vittrekwa River (**Figure 4.1**). Populations from these systems consist of mixtures of migratory anadromous and non-migratory riverine and residual life history forms of Dolly Varden Char (Reist and Sawatzky 2010).

4.3.2 *Field Collection*

An extensive scientific collection program for Dolly Varden Char took place on these rivers from 1986-2001. During this time, Dolly Varden Char were captured via electrofishing, seining, angling and gillnetting typically at sites near to spawning locations within each studied river. Biological information (including life history form), morphological measurements, tissue samples and sagittal otoliths were collected from fishes captured and archived. From 2002-2012, due to declines in population

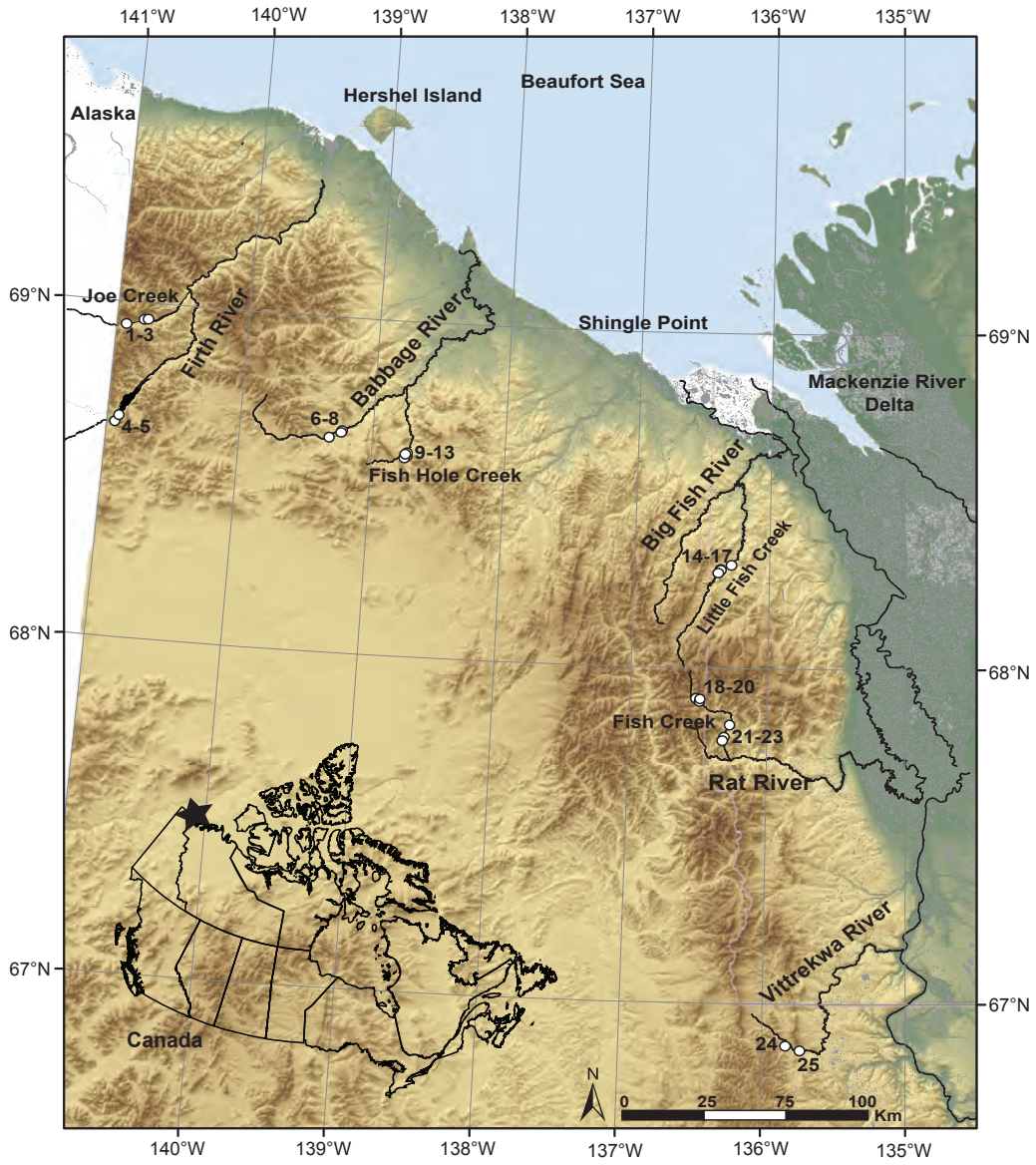

Figure 4.1: A map of the Yukon North Slope and northwestern Northwest Territories region showing study rivers: Vittrekwa River, Rat River (tributary Fish Creek), Big Fish River (tributary Little Fish Creek), Babbage River (tributary Fish Hole Creek) and Firth River (tributary Joe Creek) and water collection sites (white circles, refer to **Table 4.1**) Shingle Point and Herschel Island are areas of aboriginal subsistence fishing (mixed-stocks) along the Canadian inshore coast.

abundances, a moratorium on most Dolly Varden Char scientific sample collection occurred across the region with the exception of a small number (<20 individuals) of Dolly Varden Char captured in the Vittrekwa River between 2006-07.

In 2009-2013 an intensive water sample collection occurred in areas where perennial springs were previously identified and during periods of decreased runoff (winter or fall). Water sample collection occurred specifically in sections of the river where Dolly Varden Char were known to congregate for overwintering and/or spawning (see Mochnacz et al. 2010 for in-depth information on Dolly Varden Char habitat use of the North Slope rivers). For the purpose of this study, water samples were examined for trace elements, and $^{87}Sr/^{86}Sr$ ratios. All trace element data available from water collections and isotope data are reported and used as a proxy for each river system.

4.3.3 *Otolith Selection*

Unlike several marine regions, hydrological studies within the region suggested long-term stability in the trace elemental composition of the water in the river systems (Clark et al. 2001, Utting et al. 2012). As a result, sagittal otoliths were selected from the overall archive (1986-2007), for individual fish (including anadromous, isolate and residual life history forms) ranging from reproductive adults (>6 yrs.) to pre-smolt juveniles (between 1-4 yrs.), from each river system. Anadromous (adults and post-smolt juveniles) individuals (>5 yrs.) in a non-reproductive life stage were eliminated from the selection process due to the potential of over-winter straying. Samples were selected at random to represent time periods (1986-2007) of collection and encompassed sampling within both the main river system and associated tributaries

where Dolly Varden Char were believed to overwinter and/or reproduce. Sample sizes for each river system and their associated tributary are as follows: Firth River (tributary Joe Creek) n= 40, Rat River (tributary Fish Creek) n=35, Vittrekwa River n=21, Big Fish River (Little Fish Creek) n= 38, and Babbage River (tributary Fish Hole Creek) n=49.

4.3.4 *Otolith Preparation and Micro-Chemical Analysis*

Otoliths were ultra-sonically cleaned with distilled water and allowed to dry, embedded in Cold Cure or Buhler Epothin epoxy resin with the sulcus facing upward and allowed to harden for 2-3 days. A Leica dissection microscope with crosshair graticule capabilities was used to dorso-ventrally mark the otolith over the nucleus region in a transverse direction and sectioned with a Buhler Isomet slow-speed saw. Otolith sections were re-embedded with epoxy resin into a Lucite disk and polished with diamond grit paste (30 µm, 9 µm, 6 µm). Final polishing was done on a polishing wheel with 0.1µm aluminum oxide paste. Otoliths were imaged and ultrasonically cleaned in distilled water prior to analysis.

A Thermo-Finnigan Element2 HR-ICP-MS coupled with a Merchantek LUV213 laser system in the Geological Sciences Department at the University of Manitoba, Winnipeg was used to analyse trace elements along the dorso-ventral transect on the otolith-exposed surface. A 17-element suite was initially developed for analysis. To account for potential interference issues between specific elements/isotopes, the 17-element suite was broken into two groups: low-resolution analysis group (^{7}Li, ^{9}Be, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{88}Sr, ^{137}Ba, ^{43}Ca) and a medium-resolution analysis group (^{24}Mg, ^{27}Al, ^{28}Si, ^{31}P, ^{32}S, ^{39}K, ^{55}Mn, ^{56}Fe, ^{44}Ca). Each elemental resolution group was analysed

individually over the same region (from the otolith core region to the ventral outer lobe intersecting all visible annuli). To allow for analysis on the same region of the otolith, a beam size of 30 μm was used at a scanning speed of 2 μm/sec at 10 Hz with a fluence of 3.5-4 J/cm^2. The NIST610 glass standard was run at the start, every hour thereafter, and at the end of each disk (each disk holds between 6-10 samples). All elemental data were internally standardized to calcium content in pure aragonite (40.02 wt%) then externally to NIST610 standard reference material. Standard deviations (2 σ**)** and detection limits for each element were calculated with the software program Iolite (Paton et al. 2011).

Otolith $^{87}Sr/^{86}Sr$ isotope ratios were measured on a Nu Plasma multi-collector ICP-MS coupled with a Photon Machines G2 Excimer laser ablation system in the W.M. Keck Collaboratory for Plasma Spectrometry at Oregon State University, Corvallis. Analyses followed the general methods described by Miller and Kent (2009) and Zimmerman et al. (2013). To correct for krypton and rubidium interferences and to monitor for Ca argide and dimer formation, a method described by Woodhead et al. (2005) was followed. For all otoliths the calculated contribution of ^{87}Rb to the measured total mass 87-peak intensity was negligible (<0.1%). $^{84}Sr/^{86}Sr$ ratios for all analyses were 0.05653 ± 0.00014 (2 se; n = 147).

A deep-sea marine gastropod collected from the Gulf of Mexico (Miller and Kent 2009, Miller et al. 2011) was used as a within-lab marine carbonate standard for $^{87}Sr/^{86}Sr$ that falls within the range for modern day seawater (0.70918). Gastropod measurements throughout the analytical session were $^{87}Sr/^{86}Sr$ = 0.70943 ± 0.00004 (2 se; n = 147). The small difference between our corrected value for the gastropod from

laser ablation and the solution measurement is normal for this instrument (Miller and Kent 2009, Miller et al. 2011, Zimmerman et al. 2013) and as a result, a small correction is applied to $^{87}Sr/^{86}Sr$ measured in otoliths. As an additional check of this correction, a pressed pellet of USGS MACS-3 synthetic calcium carbonate standard with a known isotopic ratio (0.70759 ± 0.000025) was analysed. Repeat analysis (n = 107) of this material throughout the analytical session returned a corrected value of $^{87}Sr/^{86}Sr$ = 0.70764 ± 0.00002 (2 se). A laser spot size ranging from 65-85 µm was used with a repetition rate of 10 Hz, and a scan speed of 5 µm/sec for all measurements. Line scan profiles were run from the core of the otolith to the outer edge of the first annulus for each individual sample parallel to transects created in trace element analysis. For all analyses, the gastropod standard was run every two samples.

4.3.5 *Water Collection and Analysis*

Water collection (2009-2013) occurred at each river during the late winter (March) in open reaches of the river thought to be associated with overwintering habitat and in the fall (September-October) in spawning reaches of the river. For trace element analysis, nitric acid-washed polyethylene bottles (250 ml) were rinsed at the sample location with water three times then filled to the top. Additionally, 0.5-1.0 ml nitric acid (HNO_3) was added to preserve the water until analysis. Trace elements were analysed by ALS laboratories, Winnipeg, MB using an ICP-MS (http://www.alsglobal.com accessed September 12, 2016). To collect water for the purpose of $^{87}Sr/^{86}Sr$ isotope analysis, HNO_3 acid-washed polyethylene bottles (250 ml) were rinsed at the sample location with water three times then filled to the top. Further, 0.5-1 ml hydrochloric acid (HCL)

was added to preserve the water until laboratory analysis then later filtered pre-analysis. $^{87}Sr/^{86}Sr$ isotope samples were analysed by the Department of Earth Sciences, Carleton University, Ottawa, ON with a Thermo Finnigan Triton TI thermal ionization-MS (similar to the techniques described in Parras et al. 2012).

4.3.6 *Data Analysis*

Otolith images (post-laser ablation) were overlaid with trace element data and aligned with annular signals observed within the otolith structure using Image-Pro Plus (Ver. 6.3 2008). An iterative R code (R Foundation for Statistical Computing, Ver. 3.1.1, 2014) was used to bin element data from each annulus and calculate descriptive parameters such as maximum, minimum, mean and median. Mean element values for the first year of the fish's life were used in further analyses and all other year-element data (after first annulus) were excluded. In a few cases (n=15), a clear migration away from hatch areas occurred within the first year of life (observed as a ~500 ppm increase in Sr within the year 1 annulus). In these situations, otolith core data were used avoiding the primordial (nucleus) region to avoid the residual maternal signature (Rieman et al. 1994, Veinott et al. 2013). All element and isotopic data were collected from the ventral lobe of the otolith for consistency purposes. Several elements were excluded from statistical analysis including: Li, Be, Ni, Cu, Al, Si, K, Mn, and Fe due to concentrations close to and below the limits of detection or to issues with interferences resulting in unusable data, specifically K.

Random forest analysis, using Statistica software (Ver. 12, 2013), was used to classify river of origin, risk of error, and ranking of element and isotope importance. This

analysis has been shown to provide better predictive capability in otolith element and isotopic studies where data demonstrated heteroscedasticity (Mercier et al. 2011). For an in-depth description of random forest analysis see Biau (2012), Breiman (2001), and Strobl et al. (2009). The random forest model was iteratively run, adjusting the child node parameter to tune the model and calculate the lowest risk of error. Misclassification costs were equal to all categories within the analysis with prior probabilities estimated. The predictor variable was set at 3, following Breiman's calculation (2001). The main data set was divided at random into a training set (70%), used to build the model and a testing set (30%) for testing the model. The stopping parameter was adjusted to 2, allowing for fewer cases in each split than the minimum number (5) of cases used to determine if splitting was to occur. The percentage decrease in training error was set to 2%.

4.4 Results

4.4.1 *Water Analysis*

Water collection analysis of winter habitat and reproductive habitat reaches from 2009-2013 (**Table 4.1**) shows isotopic and total Sr differences among river systems and tributaries within larger catchment reaches. Sr isotopic values varied over the study region at large and within the main river system as well as associated tributaries. Trace element differences were observed, specifically Sr, Mg, and Ba, among the river systems of the study. Trace elements that were below the detection limit or eliminated from random forest model are not shown in **Table 4.1**.

Table 4.1: Water chemistry and $^{87}Sr/^{86}Sr$ isotope ratios (2009-2013) collected on the studied rivers. Location (**LOC**) for all rivers corresponds with sample collection location on the study map (**Figure 4.1**) and associated river name: Joe Creek (**JC**), Firth River (**FR**), Babbage River (**BR**), Fish Hole Creek (**FHC**), Little Fish Creek (**LFC**), Fish Creek (**FC**), and Vittrekwa River (**VR**). Temperature (**T**) is reported in degrees Celsius and total element concentrations are reported in mg/L. Date refers to the timing of the collection of the water sample. Data not available are reported as **n/a** and values below the detection limit are reported as **bdl**. Standard error was between 0.000008-0.000021 for the $^{87}Sr/^{86}Sr$ isotopic ratios.

LOC	RIVER	DATE	$^{87}Sr/^{86}Sr$	T	Ba	Mg	Na	Sr
1	JC	Mar-09	0.709504	0.10	0.016	6.38	0.300	0.122
2	JC	Mar-09	0.708556	1.40	0.010	5.25	0.210	0.170
3	JC	Mar-09	0.708698	0.47	0.010	5.64	0.220	0.141
4	FR	Mar-09	0.708658	0.31	0.021	5.86	0.470	0.273
5	FR	Mar-09	0.708350	0.58	0.016	5.45	0.320	0.257
6	BR	Mar-09	0.709162	3.86	0.020	9.42	0.310	0.112
7	BR	Mar-09	0.709320	2.54	0.021	8.83	0.310	0.109
8	BR	Mar-09	0.709209	3.12	0.023	8.34	0.650	0.134
9	FHC	Mar-13	n/a	2.33	0.031	10.20	0.528	0.083
10	FHC	Mar-09	0.710639	2.59	0.043	11.70	0.380	0.080
11	FHC	Sep-09	0.710977	3.50	0.033	10.70	n/a	0.089
12	FHC	Mar-09	0.711116	1.40	0.025	9.62	0.440	0.076
13	FHC	Mar-10	0.711061	3.10	0.028	9.84	0.494	0.093
14	LFC	Mar-13	0.715871	5.44	0.038	27.20	581.000	2.490
15	LFC	Mar-13	0.715980	9.80	0.034	24.00	584.000	2.590
16	LFC	Mar-10	0.715971	5.20	0.034	29.30	622.000	2.310
17	LFC	Sep-09	0.713002	7.53	0.036	45.70	148.000	1.100
18	FC	Mar-13	0.712802	1.62	0.078	18.90	11.100	0.236
19	FC	Mar-09	0.711903	3.36	0.067	16.50	8.030	0.186
20	FC	Mar-09	0.712949	4.15	0.021	11.40	11.200	0.143
21	FC	Mar-09	0.711911	0.33	0.064	15.10	8.140	0.179
22	FC	Sep-09	0.712017	n/a	0.056	15.10	8.790	0.201

23	FC	Mar-09	0.712911	0.29	0.021	11.80	11.500	0.129
24	VR	Sep-09	0.712833	n/a	0.071	16.90	4.400	0.167
25	VR	Sep-09	0.712689	n/a	0.062	15.10	n/a	0.134

4.4.2 *Random Forest Model*

The misclassification rate for the random forest model stabilized at 160 trees to produce an overall misclassification rate of 2.99 % (± 1.47 se) for the training data set and 5.66 % (± 3.17 se) for the test data set (**Figure 4.2**). The single classification tree shown in **Figure 4.3** demonstrates the splitting and voting process that contributed to the overall forest output and associated error of misclassification calculation. In the case of this tree, six non-terminal child nodes were needed before reaching a final classification with seven terminal nodes. The number of terminal and non-terminal nodes varies amongst other individual trees in the model.

Variables contributing the most to the classification were mean Sr (100%), the $^{87}Sr/^{86}Sr$ ratio (96%) and mean Ba (95%). Removal of any one of the lower ranking elements (S (63%), Mg (58%), Zn (48%), P (46%)) resulted in significant increases of misclassification (15.38% ± 5.0 se, 11.54% ± 4.43 se, 13.46% ± 4.73 se, 11.54% ± 4.43 se respectively) with all model parameters remaining constant, suggesting that they also play an important role in the learning algorithm and tree classification process.

The classification matrix of observed and predicted values defined where misclassifications occurred within the random forest model (**Table 4.2**). Dolly Varden Char from the Firth and Big Fish rivers were consistently classified to the correct river grouping whereas a small number of misclassifications occurred amongst Dolly Varden Char from the Vittrekwa, Rat and the Babbage rivers. Specifically, within the Baggage River group, 3 misclassifications (1 Vittrekwa River and 2 Rat River) were observed out of the total sample size of 50 resulting in 94.00% correct classification within the group. Two misclassifications were observed in the Vittrekwa River (1 Babbage River and

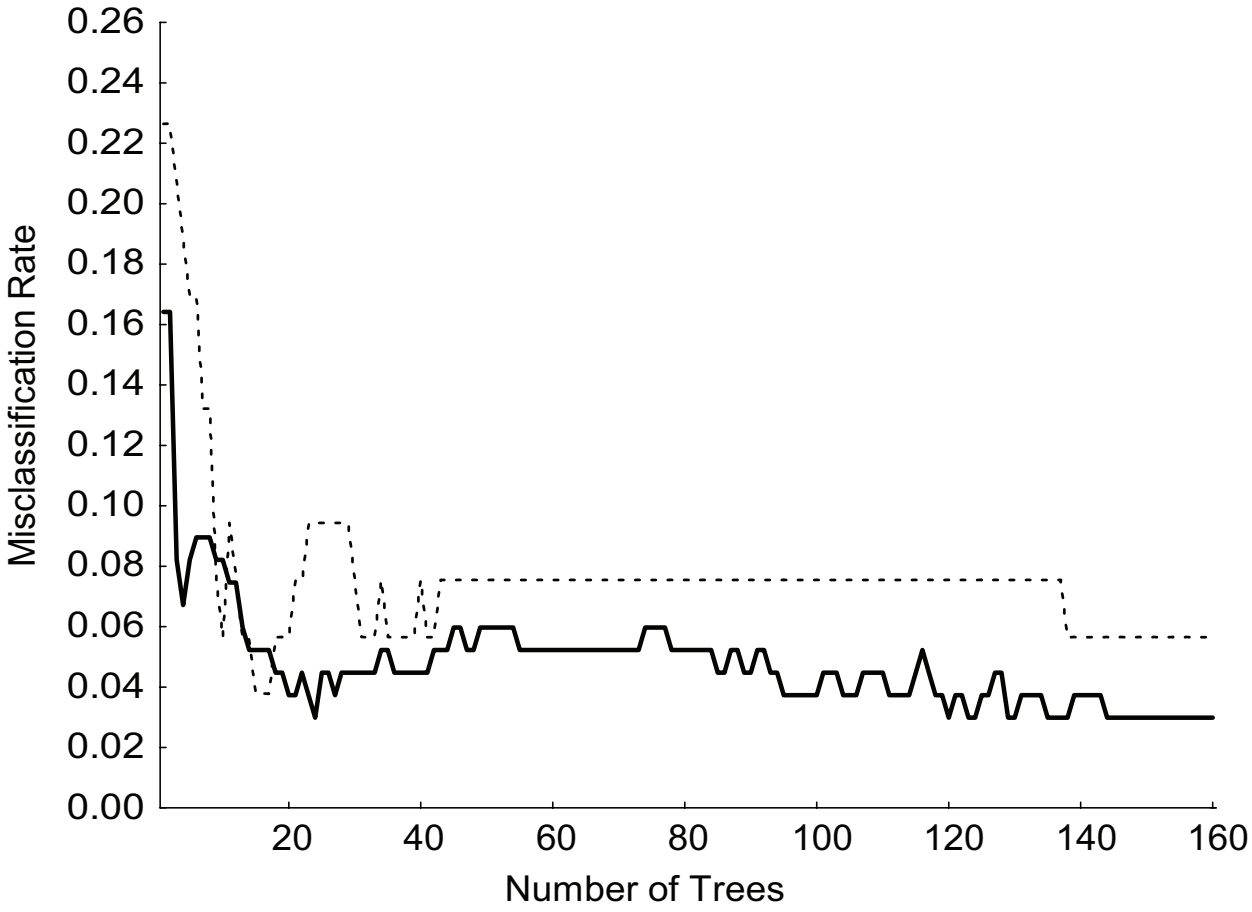

Figure 4.2: Misclassification rate of the trained data (solid line) and test data (dashed line) for the random forest. The misclassification rate stabilized at 160 trees and at 2.98 % (± 1.47 se) for the trained data and 5.66% (± 3.17 se) for the test data.

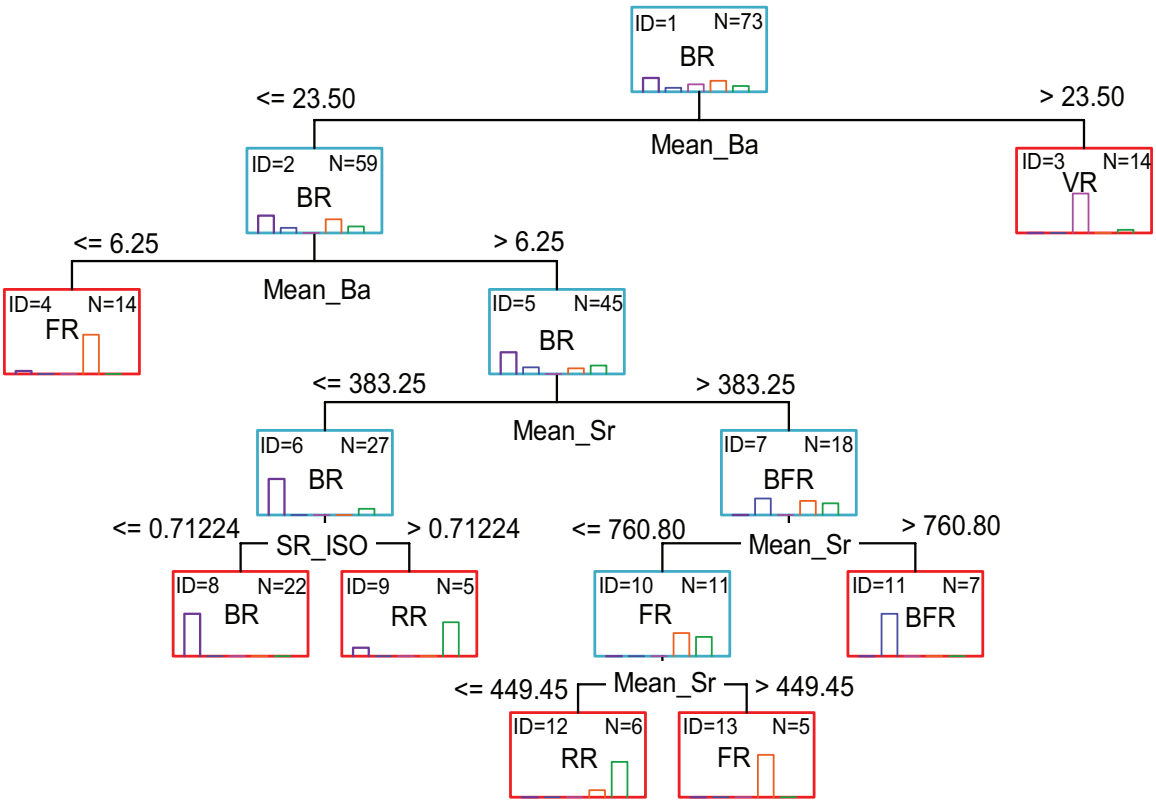

Figure 4.3: A tree classification example that contributes to the overall building of the forest (160 trees). The blue squares indicate non-terminal child nodes (6) and the red squares indicate terminal nodes (7). The bars within each square indicates voting for classification groups depending on the node split (mean element or $^{87}Sr/^{86}Sr$ ratio (**Sr_ISO**) and the specific splitting value) where, from left to right, purple represents Babbage River (**BR**), blue represents Big Fish River (**BFR**), pink represents Vittrekwa River (**VR**), orange represents the Firth River (**FR**) and green represents the Rat River (**RR**).

Table 4.2: The random forest classification matrix for all 160 trees of observed and predicted group classification expressed as numbers and percentages. The group classifications represented within the model are the Babbage River (**BR**), Big Fish River (**BFR**), Vittrekwa River (**VR**), Firth River (**FR**) and Rat River (**RR**).

Observed	Predicted BR	Predicted BFR	Predicted VR	Predicted FR	Predicted RR
BR	47		1		2
	94.00%	0.00%	2.00%	0.00%	4.00%
BFR		38			
	0.00%	100.00%	0.00%	0.00%	0.00%
VR	1		19		1
	4.76%	0.00%	90.48%	0.00%	4.76%
FR				40	
	0.00%	0.00%	0.00%	100.00%	0.00%
RR	1		1		32
	2.94%	0.00%	2.94%	0.00%	94.12%

1 Rat River) out of the total sample size of 21 resulting in 90.48% correct classification within the group and two misclassifications were also observed in the Rat River (1 Babbage River and 1 Vittrekwa River) resulting in 94.12% correct classification within the group.

4.5 Discussion

From our results, it is observed that there are 5 discrete stocks of Dolly Varden Char from the YT North Slope and northwestern NT. Both essential and non-essential elements in life processes were successfully used for stock discrimination where distinctive differences were observed in the riverine aquatic environments in our study area (Sr, $^{87}Sr/^{86}Sr$ isotopes, Ba and Mg). These elements are regularly used in stock discrimination studies and in the case of our study Sr, $^{87}Sr/^{86}Sr$ ratios and Ba were the most significant contributors to otolith-based discrimination in the model.

The current study further supports the long-term stability of otolith $^{87}Sr/^{86}Sr$ and the associated waterborne strontium isotopes as found in other studies such as Walther and Thorrold's (2009) investigations of American Shad (*Alosa sapidissima*). Variability in elemental inclusion and importance in otolith discriminatory studies is evident. Wells et al. (2003) found the suite of Sr, Ba, Mg, and Mn provided the best discriminatory power for otolith trace elements, however, Swearer et al. (2003) found a wider range of elements (Mn, Cu, Zn, Sr, Ba, Pb) helpful in discrimination. Within this study, elements such as Mn and Cu were near analytical limits of detection in otoliths and at low, constant levels in water samples from the river drainages thus unusable for discrimination. Some essential life elements, regulated through physiological processes,

such as S, P and Zn, aided the discrimination within this study but exhibited low differences in the water chemistry. These elements had measurable differences among otoliths and removal of the elements from the model, reduced our discriminatory power, suggesting that they are important to the overall differentiation of fish from the river systems. From the perennial groundwater spring characteristics within the river systems, we infer that physiological conditions and metabolic rates for regulation of ions would be distinct for each river drainage (Clark et al. 2001, Mochnacz et al. 2010), resulting in the variation we observed in S, Zn and P within our otoliths. For example, the Big Fish River originates from perennial springs of deeper origins than that of the other river systems resulting in higher temperatures and high concentrations of total dissolved solids (Clark et al. 2001). Larger egg size, higher frequency of spawning events, high mortality rates, a reduced age of male maturation and a shorter life span have been observed in Dolly Varden Char from the Big Fish River population (Sandstrom 1995, Reist and Sawatzky 2010, Stewart et al. 2010, Gallagher et al. 2013). Similar characteristics have not been observed in the other populations, which suggests that intra-specific variation occurs among river drainages in this region (Reist and Sawatzky 2010, Stewart et al. 2010).

Within our random forest model, the ability to identify misclassifications was possible. It was observed that the Firth and Big Fish river drainages were classified correctly 100% of the time, whereas misclassifications occurred among the remaining river systems of the study at a low rate. This result is paralleled by the hydrology of the region. Hydrological studies indicate that the Big Fish River is fed by deep thermal groundwater springs (Clark et al. 2001, Mochnacz et al. 2010) as observed in higher

water temperatures, high Na and associated Sr thus making it unique amongst the river drainages of the study. The $^{87}Sr/^{86}Sr$ isotopic ratios for the Big Fish River, ranging from 0.714 - 0.716, are the highest in the area and distinguish this river. These values are distinct from seawater (0.70918), and would be more consistent with the waters having equilibrated with underlying bedrock and/or geothermal springs. All remaining rivers within the study have non-thermal perennial springs that are deeper than the active groundwater layer but are not geothermally heated, within their drainage basins (Clark and Lauriol 1997, Mochnacz et al. 2010). The Firth River has $^{87}Sr/^{86}Sr$ isotopic ratios of 0.708 – 0.709, close to that of seawater, and juvenile igneous rocks. The Rat River values are intermediate between these previous values suggesting the possibility of a mixed source between seawater or juvenile rocks and geothermal waters. At the Babbage River, the value of 0.709 is again consistent with juvenile rocks or seawater, but the elevated values at Fish Hole Creek, a tributary of the Babbage River, would indicate a spatially restricted source where mixing had affected one tributary. The Vittrekwa River has intermediate isotopic values that fall within the middle range. The few misclassifications observed using the random forest classification system were seen in samples from the rivers with intermediate isotopic values.

We grouped river systems and associated tributaries to understand identification of Dolly Varden Char to their river drainage. This resulted in high discriminatory power as suggested above, although distinct intra-river differences in $^{87}Sr/^{86}Sr$ ratios and trace elements were observed between perennial groundwater sources within tributaries and their associated main river systems. Dolly Varden Char intra-river drainage movements could be further investigated in the future to provide detailed information regarding their

usage preferences for spawning habitat perennial springs within the Firth, Babbage, and Big Fish river drainages similar to methods used by Barnett-Johnson et al. (2008) that associated spawning tributaries with landscape geology. Since multi-year data are available for each river system, documentation of preferential shifts for perennial groundwater spring usage for reproduction within specified rivers is feasible.

Although the use of archival otoliths for stock discrimination is less common, our results of high overall discriminatory power (94.44%) indicate that it is feasible under conditions of relative environmental stability as supported by genetic analysis (Harris et al. 2015), and water element and isotope analyses. Dolly Varden Char within the study area demonstrate strong connectivity between individual stocks and perennial groundwater springs (Mochnacz et al. 2010). Genetic analysis, done in parallel with this study, established the long-term structuring and differentiation in YT North Slope and northwestern NT Dolly Varden Char populations with low rates of straying in reproductive individuals between river drainages (Harris et al. 2015). Although not undertaken in the context of this study, the degree of straying could be investigated with otolith microchemical techniques by examination of the non-spawning fish from each river system. Close associations of Dolly Varden Char to perennial springs are exemplified by a radio-telemetry study that identified four perennial springs used by Dolly Varden Char for overwintering in the Hulahula River drainage, Alaska, restricting the entire population to approximately 12 km of the river for winter survival (Brown et al. 2014). Habitat surveys of known spawning and overwintering reaches associated with a perennial groundwater spring for each river within the study region suggest that <5% of the overall catchments are available to fish in critical periods (Mochnacz et al. 2010).

The success of archival otoliths for stock discrimination can aid the examination of past and present mixed-stock fishery composition of the Canadian Beaufort Sea coastal fishery. Due to differences in sample archive availability between otoliths and genetic tissues, the use of both techniques in conjunction with each other can provide a longer timeframe for the mixed-stock fishery composition. Northern form Dolly Varden Char have been listed as an 'at-risk' species within Canada due to their heavy reliance on perennial springs for survival, the potential impacts of climate change to the fish populations, lower population levels within some rivers of the region and continued subsistence harvesting (Harwood 2001, Stephenson 2003, Harwood et al. 2009, COSEWIC 2010, Gallagher et al. 2010, Reist and Sawatzky 2010). Fishery management plans are a necessity for the overall health and monitoring of the Dolly Varden Char populations. Presently, the mixed-stock coastal harvesting generally removes the greatest numbers of Dolly Varden Char from Canadian North Slope region <1000 kg annually (Gallagher et al. 2013, Fisheries and Oceans Canada, unpublished harvest data 2011-2014). Management of the fisheries must include the understanding of stock composition on an annual basis with various techniques such as genetic and otolith analyses. Future research directions, based on the positive outcomes of our study, couple genetic and otolith techniques to address these fishery management issues within the region and provide a greater understanding of coastal Dolly Varden Char stock structure (past and present) in the coastal Canadian Beaufort Sea. As part of this process, both otoliths and genetic information from river drainages in western Alaska that experience similar freshwater constraints for overwintering and reproduction, such as the Kongakut, Canning and Hulahula, will be incorporated into the

unit stock analysis to provide a stronger interpretation of the Canadian mixed-stock fishery composition.

4.6 Summary

Dolly Varden Char are closely linked to perennial groundwater sources in the freshwater stages of their life cycle. The perennial groundwater in winter months contributes the majority of ice-free habitats needed by Dolly Varden Char for spawning and over-wintering. This close connection of Dolly Varden Char to groundwater provides an ideal situation for stock discrimination studies utilizing mineralised tissues that capture trace element and isotopic signatures from the surrounding environment. Geochemical signals in sagittal otoliths were used to discriminate among Dolly Varden Char populations inhabiting YT North Slope and northwestern NT river systems. The random forest model provided 94.44% classification success for fish from the different river systems of the study. Strontium, $^{87}Sr/^{86}Sr$ ratios and Ba were the most significant discriminators although removal of other elements reduced discrimination power in the model. The unique elemental identifiers can be used as a tool for fisheries managers in understanding mixed-stock fisheries stock composition. Models can be developed to determine stock composition of the Canadian Beaufort Sea coastal mixed-stock fishery.

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4.8 Reference

Arkhipkin, A. I., Schuchert, P. C., and Danyushevsky, L. 2009. Otolith chemistry reveals fine population structure and close affinity to the Pacific and Atlantic oceanic spawning grounds in the migratory southern blue whiting (*Micromesistius australis australis*). Fish. Res. **96**(2-3): 188-194.

Armstrong, R. H. 1974. Migration of Anadromous Dolly Varden (*Salvelinus malma*) in Southeastern Alaska. Fish. Res. Board Can. **31**(4): 435-444.

Armstrong, R. H., and Morrow, J. E. 1980. The Dolly Varden charr, *Salvelinus malma*. *In* Charrs: Salmonid Fishes of the Genus *Salvelinus*. *Edited by* Balon, E. K. Dr. W. Junk, The Hague, The Netherlands. pp. 99–140.

Barnett-Johnson, R., Pearson, T. E., Ramos, F. C., Grimes, C. B., and MacFarlane, R. B. 2008. Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. Limnol. Oceanogr. **53**(4): 1633-1642.

Barnett-Johnson, R., Teel, D., and Caillas, E. 2010. Genetic and otolith isotopic markers identify salmon populations in the Columbia River at broad and fine geographic scales. Env. Biol. Fish. **89**: 533-546.

Bath, G. E., Thorrold, S. R., Jones, C. M., Campana, S. E., McLaren, J. W., and Lam, J. W. H. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. Geochim. Cosmochim. Ac. **64**(10): 1705-1714.

Biau, G. 2012. Analysis of a random forests model. J. Mach. Learn. Res. **13**: 1063–1095. Retrieved from http://dl.acm.org/citation.cfm?id=2188385.2343682

Breiman, L. 2001.Random forests. Mach. Learn. **45**: 5-32.

Brown, J. A. 2006. Classification of juvenile flatfishes to estuarine and coastal habitats based on the elemental composition of otoliths. Estuar. Coast. Shelf S. **66**: 594-611.

Brown, R. J., Loewen, M. B., and Tanner, T. L. 2014. Overwintering locations, migrations, and fidelity of radio-tagged Dolly Varden in the Hulahula River, Arctic National Wildlife Refuge, 2007–09. Arctic **67**(2): 149–158.

Cadrin, S. X., Friedland, K. D., and Waldman, J. R. (Eds.). 2005. Stock Identification Methods: Applications in Fishery Science. Elsevier Academic Press, Burlington, MA.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. **188**: 263-297.

Campana, S. E. 2005a. Otolith elemental composition as a natural marker of fish stocks. *In* Stock identification methods: applications in fishery science. *Edited by* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, Elsevier Academic Press, Burlington, MA. pp. 227-245.

Campana, S. E. 2005b. Otolith science entering the 21st century. Mar. Freshwater Res. **56**(5): 485-495.

Campbell, J. L., Babaluk, J. A., Cooper, M., Grime, G. W., Halden, N. M., Nejedly, Z. Rajta, I., and Reist, J. D. 2002. Strontium distribution in young-of-the-year Dolly Varden otoliths: potential for stock discrimination. Nucl. Instrum. Meth. B **189**:185-189.

Clark, I. D., and Lauriol, B. 1997. Aufeis of the Firth River basin, Northern Yukon Canada: insights into permafrost hydrogeology and karst. Arctic Alpine Res. **29**(2): 240-252.

Clark, I. D., Lauriol, B., Harwood, L., and Marschner, M. 2001. Groundwater contributions to discharge in a permafrost setting, Big Fish River, NWT, Canada. Arct. Antarct. Alp. Res. **33**(1): 62-69.

COSEWIC 2010. COSEWIC assessement and status report on Dolly Varden *Salvelinus malma malma* (Western Arctic populations) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 65 p.

Craig, P. C. 1989. An introduction to anadromous fishes in the Alaskan Arctic USA. Biological Papers of the University of Alaska **24**: 27-54.

Crane, P. A., Viavant, T., and Wenburg, J. K. 2005. Overwintering patterns of Dolly Varden *Salvelinus malma* in the Sagavanirktok River in the Alaskan North Slope inferred using mixed-stock analysis. Alaska Fisheries Technical Report **84**, US Fish and Wildlife Service.

Crichton, R. R. 2008. Biological inorganic chemistry: an introduction. Elsevier, Belgium.

De Pontual, H., and Geffen, A. J. 2002. Otolith microchemistry. *In* Manual of fish sclerochronolgy. *Edited by* J. Panfili, , H. De Pontual, H.Troadec, and P. J.Wright, Ifremer-IRD coedition, Brest, France. pp. 245-301.

DeCicco, A. L. 1992. Long-distance movements of anadromous Dolly Varden between Alaska and the USSR. Arctic **45**(2): 120-123.

DeCicco, A. L. 1997. Movements of postmolt anadromous Dolly Varden in northwestern Alaska. *In* American Fisheries Society Symposium; Fish ecology in arctic North America. *Edited by* J. B. Reynolds pp. 175-183.

Elsdon, T. S., and Gillanders, B. M. 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. Can. J. Fish. Aqu. Sci. **59**(11): 1796-1808.

Elsdon, T. S., and Gillanders, B. M. 2003. Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri.* Mar. Ecol. Prog. Ser. **260**: 263-272.

Elsdon, T. S., and Gillanders, B. M. 2004. Fish otolith chemistry influenced by exposure to multiple environmental variables. J. Exp. Mar. Biol. Ecol. **313**: 269-284.

Farrell, J., and Campana, S. E. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus.* Comp. Biochem. Physiol. **115A**(2): 103-109.

Fechhelm, R. G., Bryan, J. D., Griffiths, W. B., and Martin, L. R. 1997. Summer growth patterns of northern Dolly Varden (*Salvelinus malma*) smolts from the Prudhoe Bay region of Alaska. Can. J. Fish. Aquat. Sci. **54**: 1103-1110.

Gallagher, C. P., Roux, M. J., Howland, K. L., and Tallman, R. F. 2010. Synthesis of biological and harvest information used to assess populations of northern form Dolly Varden (*Salvelinus malma malma*) in Canada Part II: Big Fish River. DFO Can. Sci. Advis. Sec. Res. Doc. **2010/115**. pp. i-51.

Gallagher, C.P., Howland, K., Harris, L., Banjo, R., Sandstrom, S., Loewen, T., and Reist, J. 2013. Dolly Varden (*Salvelinus malma malma*) from the Big Fish River: abudnance estimates, effective population size, biological characteristics, and contribution to the coastal mixed-stock fishery. DFO Can. Sci. Advis. Sec. Res. Doc. **2013/059**. pp. v-46.

Gwich'in Elders 1997. Gwich'in words about the land. Gwich'in Renewable Resource Board, Inuvik. pp. 161-164

Hamer, P. A., and Jenkins, G. P. 2007. Comparison of spatial variation in otolith chemistry of two fish species and relationships with water chemistry and otolith growth. J. Fish Biol. **71**(4): 1035-1055.

Harris, L., Banjo, R., Gallagher, C. P., Koizumi, I., Johnson, L. K., Howland, K. L., Taylor, E. B., and Reist, J. D. 2015. Life-history characteristics and landscape attributes of genetic variation, gene flow and fine-scale population structure in

northern Dolly Varden (*Salvelinus malma malma*) in Canada. Can. J. Fish. Aquat. Sci. **72**: 1477-1493. doi: 10.1139/cjfas-2015-0016.

Harwood, L. A. 2001. Status of anadromous Dolly Varden (*Salvelinus malma*) of the Rat River, Northwest Territories, as assessed through community-based sampling of the subsistence fishery, August-September 1989-2000. DFO Can. Sci. Advis. Sec. Res. Doc. **2001/090**. pp. v-46.

Harwood, L. A., Sandstrom, S., and Linn, E. 2009. Status of anadromous Dolly Varden (*Salvelinus malma*) of the Rat River, Northwest Territories, as assessed through sampling of the subsistence fishery (1995-2007). Can. Man. Report Fish. Aquat. Sci. **2891**: i-52.

Haszard, S., and Shaw, J. 2000. Rat River biodiversity, cultural and historical assessment 00-01. Gwich'in Renewable Resource Board, Inuvik. pp. v-85.

Hobbs, J. A., Yin, Q., Burton, J., and Bennett, W A. 2005. Retrospective determination of natal habitats for an estuarine fish with otolith strontium isotope ratios. Mar. Freshwater Res. **56**(5): 655-660.

Image-Pro® Plus, Ver. 6.3 2008, Media Cybernetics, Inc. Bethesda, MD

Kennedy, B. P., Blum, J. D., Folt, C. L., and Nislow, K. H. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Can. J. Fish. Aquat. Sci. **57**(11): 2280-2292.

Kennedy, B. P., Klaue, A., Blum, J. D., Folt, C. L., and Nislow, K. H. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Can. J. Fish. Aquat. Sci. **59**: 925-929.

Krueger, C. C., Wilmot, R. L., and Everett, R. J. 1999. Stock origins of Dolly Varden collected from Beaufort Sea coastal sites of Arctic Alaska and Canada. Trans. Am. Fish. Soc. **128**: 49-57.

Lacelle, D., Bjornson, J., Lauriol, B., Clark, I. D., and Troutet, Y. 2004. Segregated-intrusive ice of subglacial meltwater origin in retrogressive thaw flow headwalls, Richardson Mountains, NWT, Canada. Quaternary Sci. Rev. **23**(5-6): 681-696.

Mann, S. 2005. Biomineralization: Principles and concepts in bioinorganic materials chemistry. Oxford University Press, Bristol, United Kingdom.

Mercier, L., Darnaude, A. M., Bruguier, O., Vasconcelos, R. P., Cabral, H. N., Costa, M. J., Lara, M., Jones, D. L., and Mouillot, D. 2011. Selecting statistical models and variable combinations for optimal classification using otolith microchemistry. Ecol. App **21**(4): 1352–64.

Miller, J. A., and Kent, A. J. R. 2009. The determination of maternal run time in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) based on Sr/Ca and $^{87}Sr/^{86}Sr$ within otolith cores. Fish. Res. **95**(2-3): 373–378.

Miller, J. A., Butler, V. L., Simenstad, C. A., Backus, D. H., Kent, A. J. R., and Gillanders, B. 2011. Life history variation in upper Columbia River Chinook salmon (*Oncorhynchus tshawytscha*): a comparison using modern and ~500-year-old archaeological otoliths. Can. J. Fish. Aquat. Sci. **68**(4): 603–617.

Mochnacz, N. J., Schroeder, B. S., Sawatzky, C. D., and Reist, J. D. 2010. Assessment of northern Dolly Varden, *Salvelinus malma malma* (Walbaum, 1792), habitat in Canada. Can. Man. Report Fish. Aquat. Sci. **2926**: v-48.

Outridge, P. M., Chenery, S. R., Babaluk, J. A., and Reist, J. D. 2002. Analysis of geological Sr isotope markers in fish otoliths with subannual resolution using laser ablation-multicollector-ICP-mass spectrometry. Environ. Geol. **42**(8): 891-899.

Papik, R., Marschke, M., and Ayles, G. B. 2003. Inuvialuit traditional ecological knowledge of fisheries in rivers west of the Mackenzie River in the Canadian Arctic., Inuvik. pp. v-22.

Parras, A., Dix, G.R., and Griffin, M. 2012. Sr-isotope chronostratigraphy of Paleogene–Neogene marine deposits: Austral Basin, southern Patagonia (Argentina). J. South Am. Earth Sci. **37**: 122–135. doi: http://dx.doi.org/10.1016/j.jsames.2012.02.007.

Paton, C., Hellstrom, J., Paul, B., Woodhead, J., and Hergt, J. 2011. Iolite: Freeware for the visualisation and processing of mass spectrometric data. J. Anal. At. Spectrom. **26**(12): 2508-2518.

Pedersen, S. A., and Linn A. 2005. Kaktovik 2000-2002 subsistence fishery harvest assessment 01-101. U.S. Fish and Wildlife Service, Fairbanks. p.p. v-66.

R Foundation for Statistical Computing, Ver. 3.1.1, 2014, Vienna, Austria

Reist, J. D. 1989. Genetic structuring of allopatric populations and sympatric life history types of charr, *Salvelinus alpinus/malma*, in the Western Arctic, Canada. Physiol. Ecol. Japan **1**: 405-420.

Reist, J. D., and Sawatzky C. D. 2010. Diversity and distribution of Chars, Genus Salvelinus, in northwestern North America in the context of northern Dolly

Varden (*Salvelinus malma malma* (Walbaum 1792)). Can. Sci. Ad. Secret. Res. Doc. **014**: i-23.

Rieman, B. E., Myers, D. L., and Nielsen, R. L. 1994. Use of Otolith Microchemistry to Discriminate *Oncorhynchus nerka* of Resident and Anadromous Origin. Can. J. Fish. Aquat. Sci. **51**(1): 68–77.

Sandstrom, S. J., Chetkiewicz, C. B., and Harwood, L. A. 2001. Overwintering habitat of juvenile Dolly Varden (*Salvelinus malma*) (W.) in the Rat River, NT as determined by radio telemetry. Can. Sci. Ad. Secret. Res. Doc. **2001/092**: i-17.

Sandstrom, S. J. 1995.The effect of overwintering site temperature on energy allocation and life history characteristics of anadromous female Dolly Varden char (*Salvelinus malma*), from northwestern Canada. MSc. dissertation. University of Manitoba, Winnipeg MB.

Schaffler, J. J., and Winkelman, D. L. 2008. Temporal and spatial variability in otolith trace-element signatures of juvenile striped bass from spawning locations in Lake Texoma, Oklahoma-Texas. Trans. Am. Fish. Soc. **137**(3): 818-829.

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

Statistica®, Ver. 12, 2013, StatSoft Inc. Tulsa, OK

Stephenson, S. A. 2003. Local and scientific observations of Dolly Varden (*Salvelinus malma*) (W.) in the Big Fish River, Northwest Territories, Canada: 1995-2002. Can. Man. Report Fish. Aquat. Sci. **2644**: i-20.

Stewart, D. B, Mochnacz, N. J., Reist, J. D., Carmicheal, T. J., and Sawatzky, C. D. 2010. Fish life history and habitat use in the Northwest Territories: Dolly Varden (*Salvelinus malma*). Can. Man. Report Fish. Aquat. Sci. **2915**: i-63.

Strobl, C., Malley, J., and Tutz, G. 2009. An introduction to recursive partitioning: rationale, application, and characteristics of classification and regression trees, bagging, and random forests. Psychol. Met. **14**(4): 323–48.

Swan, S. C., Geffen, A. J., Morales-Nin, B., Gordon, J. D. M., Shimmield, T., Sawyer, T., and Massuti, E. 2006. Otolith chemistry: an aid to stock separation of *Helicolenus dactylopterus* (bluemouth) and *Merluccius merluccius* (European hake) in the Northeast Atlantic and Mediterranean. ICES J. Mar. Sci. **63**(3): 504-513.

Swearer, S. E., Forrester, G. E., Steele, M. A., Brooks, A. J., and Lea, D. W. 2003. Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblage. Estuar. Coast. Shelf S. **56**(5-6): 1111-1123.

Utting, N., Lauriol, B., Mochnacz, N., Aeschbach-Hertig, W., and Clark, I. 2012. Noble gas and isotope geochemistry in western Canadian Arctic watersheds: tracing groundwater recharge in permafrost terrain. Hydrogeol. J. **21**(1): 79–91.

Veinott, G., and Porter, R. 2005. Using otolith microchemistry to distinguish Atlantic salmon (*Salmo salar*) parr from different natal streams. Fish. Res. **71**: 349-355.

Veinott, G., Westley, P. A. H., Purchase, C. F., and Warner, L. 2013. Experimental evidence simultaneously confirms and contests assumptions implicit to otolith microchemistry research. Can. J. Fish. Aquat. Sci. **71**(3): 356–365.

Walther, B. D., and Thorrold, S. R. 2009. Inter-annual variability in isotope and elemental ratios recorded in otoliths of an anadromous fish. J. Geochem. Explor. **102**(3): 181-186.

Wells, B. K., Rieman, B. E., Clayton, J. L., Horan, D. L., and Jones, C. M. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. Trans. Am. Fish. Soc. **132**(3): 409-424.

Woodhead, J., Swearer, S., Hergt, J., and Maas, R. 2005. In situ Sr-isotope analysis of carbonates by LA-MC-ICP-MS: interference corrections, high spatial resolution and an example from otolith studies. J. Analyt. At. Spectrom. **20**(1): 22.

Zimmerman, C. E., Swanson, H. K., Volk, E. C., and Kent, A. J. R. 2013. Species and life history affect the utility of otolith chemical composition for determining natal stream of origin for Pacific salmon. Trans. Am. Fish. Soc. **142**(5): 1370–138.

Chapter 5

Arctic Char Anadromy at the Margins of its Geographic Range

This manuscript has been coauthored with my adviser and collaborators. I have been the lead on the manuscript. Specifically I was responsible for the study design, experimental analysis, and writing and revisions for the manuscript. My co-authors provided insight to the overall review of the study, conducted most of the field-based parts of the study, and provided editorial and review comments on multiple drafts of the manuscript.

Loewen, T.N., Halden, N. M., Babaluk, J. A., Power, M., Yang, P., Veillette, J., Vincent, W. Radtke, R., Dempson, B., and Reist, J. D. Arctic Char anadromy at the margins of its geographic range.

5.1 Abstract

Arctic Char (*Salvelinus alpinus*) has an extensive range throughout the circumpolar North, and in North America its distribution spans 38 degrees of latitude, from 45 to 83°N. Our objective in the present study was to determine the migration characteristics of Arctic Char at the northern limit of its range in the High Arctic (Ellesmere Island, Canada; western Greenland), and to compare these characteristics with a reference population (north shore of the St Lawrence River, Canada) towards the southern limit of this species. As an indicator of anadromy, we analysed the strontium concentrations in Arctic Char otoliths using laser ablation-inductively coupled plasma-mass spectrometry. Both fish age- and length- at first migration had a logarithmic relationship with river length in the High Arctic lakes. At the southern site, mean age and size at first migration was 4 (+/- 1 sd) years and 200 (+/- 53 sd) mm, respectively. The High Arctic populations showed high variability in their age at first migration, ranging from young-of-the-year to 11+ years, and in length from ≤50 mm to 504 mm. Arctic Char navigating shorter river systems had lower otolith strontium concentrations indicating the selection of low salinity at first migration. Habitat selection inferred from otolith strontium in subsequent years tended towards higher salinity environments, or longer duration in the sea. Discontinuous migrations were identified in all but one High Arctic Lake populations, while continuous migrations were identified in the southern population.

5.2 Introduction

Species that occur over large spatial ranges often exhibit differences in life history strategies to facilitate survival, adaptation, persistence, and longevity in different

environmental conditions (L'Abée-Lund et al. 1989, Vøllestad and L'Abée-Lund 1994, Garvey and Marschall 2003, Moore et al. 2014). Arctic Char, *Salvelinus alpinus*, is a prime example of a species with an extensive Holarctic range distribution and within North America that exhibits biodiversity within (i.e., intraspecifically) and among populations (Reist et al. 2013). Although this species in North America is primarily considered Arctic by association, its range distribution in North America within cold-water oligotrophic lakes and rivers extends from 45-83°N latitude from temperate lake populations in Maine, USA, to the northern limit of the Canadian High Arctic on Ellesmere Island (Everhart and Waters 1965, Doucett et al. 1999, Babaluk et al. 2002, Klemetsen et al. 2003, Reist et al. 2013). This broad distribution (38 degrees of latitude, >4000 km) of an "Arctic" cold-water fish provides a unique opportunity to examine populations living near the edges of an extensive geographic range. Given that lake productivity likely decreases as a function of latitude north, northern and southern populations are expected to differ in their extent of anadromy and their degree of utilisation of coastal and marine habitats for increased feeding opportunities.

Like most salmonids, Arctic Char are considered primarily diadromous and more specifically anadromous (Johnson 1980, Klemetsen et al. 2003, Reist et al. 2013). Anadromy is the concerted movement of fish from fresh to marine waters for feeding with a subsequent return to natal waters for reproduction and/or overwintering. Classical theories proposed by Gross et al. (1988) and Gross (1997) suggest diadromy evolved due to differential resource availability between fresh water (relatively low) and marine (relatively high) environments. Anadromy may have evolved in northern latitudes due to increased biological productivity of marine resources in comparison with the nutrient-

poor, high latitude fresh waters. Salmoniform migration patterns generally support this resource-associated hypothesis for anadromy, whereas this relationship is not supported for other groups of high latitude fishes such as clupeids and osmerids (McDowall 2008, Dodson et al. 2009, Bloom and Lovejoy 2014). Alternatively, a “safe-site” hypothesis has been proposed suggesting freshwater environments provide less competition and a safe refuge for spawning and rearing of young (Dodson et al. 2009; Bloom and Lovejoy 2014). As a consequence of multiple glacial advances and retreats in northern regions (Power 2002), multiple hypotheses for the evolution of diadromy may be plausible for different family and species groupings globally (McDowall 2008). Means of survival and dispersal would no doubt evolve differently in environments exhibiting a relatively higher degree of instability in thermal niches, fresh water availability, and connectivity over evolutionary timeframes in comparison to those exhibiting relative stability.

In most Arctic Char populations where access to the marine environment is available, partial migration is exhibited (Jonsson and Jonsson 1993, Rikardsen et al. 1997, Klemetsen et al. 2003, Loewen et al. 2009). A portion of the population migrates seaward but a subset does not (Chapman et al. 2012). In these cases, the lake-dwelling (non-migratory) members of the population tend to mature at a smaller size and younger age, although considerable variation is noted within and among populations (Jonsson and Jonsson 1993, Klemetsen et al. 2003, Loewen et al. 2010). In their examination of char biodiversity, Reist et al. (2013) hypothesized that a reduction of migratory behaviour would be observed at the southern edge of the geographic range of the species given the greater availability of food in more productive southern lakes. This

is indirectly supported by climate change studies of Arctic Char that observe a shift towards reduced anadromy within a population when freshwater lake productivity concomitantly increases (Finstad and Hein 2012). Studies presently documented for Arctic Char support the evolution of migration based upon the relative resource availability model initially proposed by Gross et al. (1988). This does not, however, rule out other potential contributory causes.

Rounsefell (1958) noted that anadromy is a series of interrelated traits occurring as a matter of degree relative to an absolute quality expressed by a fish. At the species level, the extent or degree of migration has been examined with the Rounsefell index (Rounsefell 1958) and updated by Quinn and Myers (2005) and Spares et al. (2015a). The use of this index determined that semelparous Chum Salmon (*Oncorhynchus keta)*, Sockeye Salmon (*O. nerka*) and Pink Salmon (*O. gorbuscha*) exhibited the highest degree of anadromy, whereas iteroparous Brook Trout (*S. fontinalis*), Arctic Char and Lake Trout (*S. namaycush*) exhibited the lowest degree of anadromy (Quinn and Myers 2005, Spares et al. 2015a). In a species such as Arctic Char with a wide geographic distribution, the relative resource availability hypothesis for anadromy suggests the index will likely change from High Arctic (high) to southern locations (low). A powerful approach towards evaluating the degree of anadromy is otolith microchemical habitat associations across the lifetimes of individual fish (Walther and Limburg 2012). This allows information to be inferred about the prevalence of migration, age- and size- at first migration, skipped migrations once migration has been initiated, and habitat selection in the marine environment.

Arctic Char migrations have been investigated in greater detail in several regions within the distributional range of the species usually with tagging and telemetry studies (Harwood and Babaluk 2014) and less commonly with otolith microchemistry (Babaluk et al. 1997). Studies have generally found that fish size- and age- at migration is variable and ranges from 150-270 mm (1-12 years) within most studied regions (Nordeng 1961, Finstad et al. 1989a, 1989b, Berg and Berg 1990, Svenning et al. 1992, Jensen 1994, Kristoffersen et al. 1994, Klemetsen et al. 2003, Jensen et al. 2012). Although Arctic Char were previously thought to initiate migrations at >150 mm in length, individuals <80 mm in length can tolerate salinities of <20 PSU, and limited exposure to salinities >20 PSU (Dempson 1993, Gulseth and Nilssen 2000). Arctic Char of small size (<100 mm in fork length) have been observed in estuarine and/or marine habitats along the Labrador coast (Bouillon and Dempson 1989, Dempson 1993), Cumberland Sound, Baffin Island (Loewen et al. 2009, 2010), and Vesturdalsa River, Iceland (Jonsson and Antonsson 2005) while migrating Arctic Char less than 120 mm have been reported in some Norwegian systems (Arnesen et al. 1995; Nilssen and Gulseth 1998). Once having migrated, Arctic Char generally demonstrate a coastal estuarine preference. Movements are found to generally occur <3 km from shorelines (Spares et al. 2012, 2015b, Moore et al. 2016) and movements away from natal rivers often tend to be less than 70 km (Dempson and Kristofferson 1987). Spares et al. (2012) demonstrated that Arctic Char migrate out into fully marine waters with an average return duration to estuaries every 9 days.

Otolith microchemical analysis, specifically the examination of the element strontium (Sr), provides an indirect method from which inferences may be made

regarding migratory histories over the lifetime of anadromous fish (Brown and Severin 2009). High calcium (Ca) is generally observed in fully marine waters whereas freshwater calcium is the primary controlling factor for estuaries where mixed fresh and marine waters provide intermediary calcemic environments. This is reflected in Sr/Ca ratios observed from Striped Bass (*Morone saxatilis*) otoliths (Phillis et al. 2011). Variations between freshwater and marine calcium environmental concentrations are generally extreme, and are reflected in the strontium incorporation into internal calcium-structures such as otoliths (Loewen et al. *in press*). These calcemic environmental differences among fresh, estuarine, and marine waters provide the underlying driver and mechanism inferring Arctic Char habitat use. Our aim in the present study was to use otolith microchemistry in order to compare the anadromous behaviour of Arctic Char near the northern and southern limits of their eastern North American geographic range. Based on the hypotheses and information discussed above, we predicted the following:

1. In extremely resource-limited freshwater environments such as in the High Arctic, Arctic Char should migrate into marine waters as soon as possible given the ability to survive osmotic and thermal challenges. Where nutrients are more available in fresh water such as the southern reaches of the Arctic Char distribution, a delay in first seaward migration is predicted to occur.
2. Upon initiating migratory behaviours, anadromy within Arctic Char populations should be low in the High Arctic lakes. Specifically, on first seaward migration,

Arctic Char should spend time in fresh/marine-mixed waters in comparison to all subsequent migrations.

3. If anadromy is exhibited, migratory behaviour is expected to occur discontinuously across years (i.e., intermittent foregoing of migration opportunities to remain in fresh water) with respect to individual lifetime migration histories in unstable environments (High Arctic). Alternatively, continuous migrations across years (i.e., fixed annual migration seaward once initiated) are more likely to occur in stable environments (southern lakes).

5.3 Materials and Methods

5.3.1 *Study Area*

The study region encompasses the edges of the geographic range of Arctic Char in eastern Canadian and western Greenlandic lake systems. Lac Davidson, Quebec, represents the southern most known location of migratory Arctic Char in North America (Doucette et al.1999). Fish captured near the mouth of the Rivière de la Trinité (49.45°N, 67.43°W) draining to the northern shore of the St. Lawrence River (**Figure 5.1A**) are thought to originate from Lac Davidson, QC (Doucette et al.1999). Samples from the northern distribution of Arctic Char include: Heintzleman Lake, NU, Canada (81.70°N, 66.93°W), Beaufort Lake, NU, Canada (81.9°N, 63.27°W), Taserssiut Lake, Greenland (77.70°N, 69.40°W), Clements Markham Lake, NU, Canada (82.63°N, 68.83°W), Lake A, NU, Canada (83.01°N, 75.44°W), Lake C2, NU, Canada (82.83°N, 77.99°W), Lake Tuborg, NU, Canada (89.01°N, 75.53°W), and Buchanan Lake, NU,

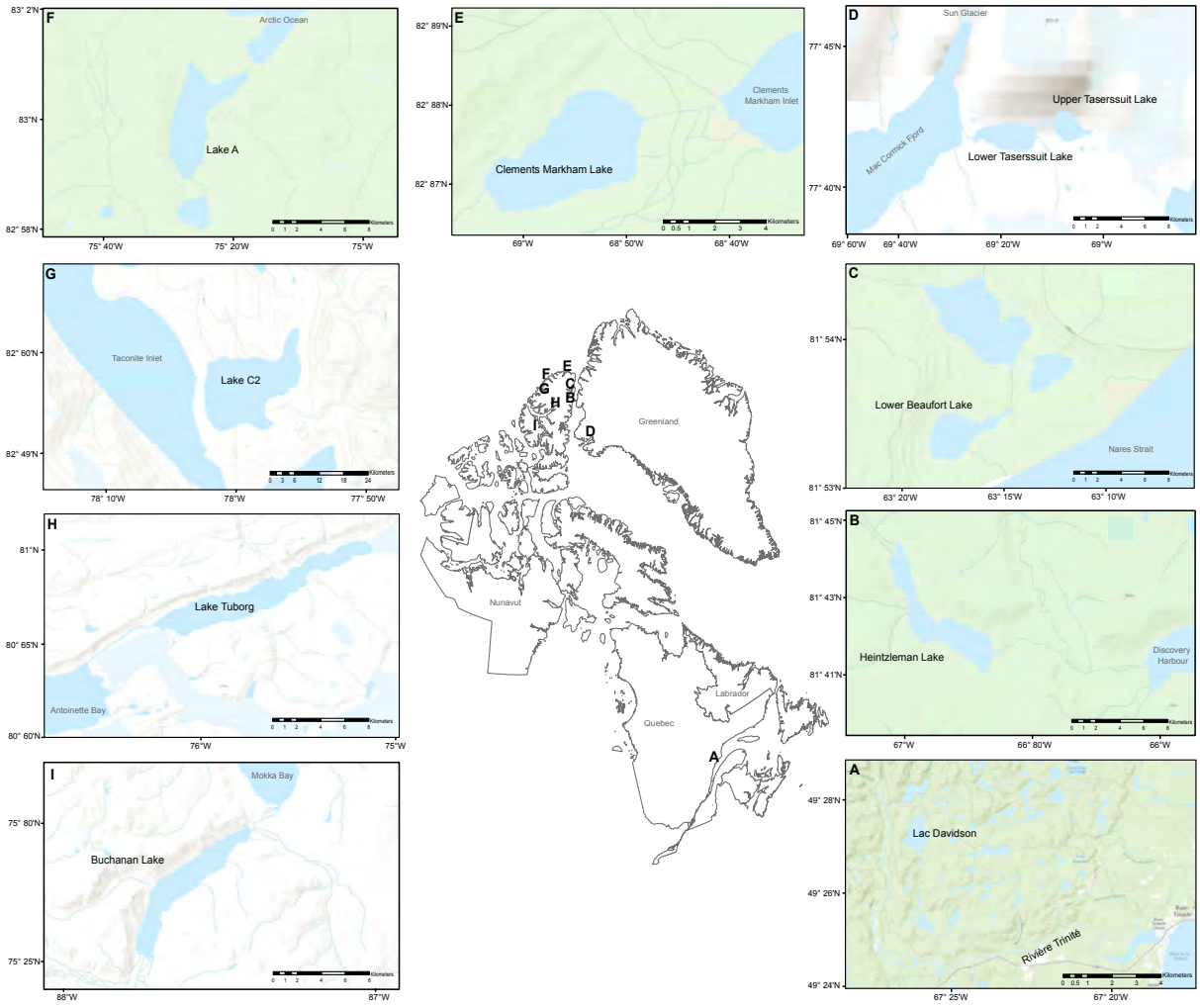

Figure 5.1: A map of the eastern and far northern regions of Canada and Greenland (**A-I**) indicating the location of each lake system studied: Lac Davidson (**A**), Heintzleman Lake (**B**), Lower Beaufort Lake (**C**), Taserssuit Lake (**D**), Clements Markham Lake (**E**), Lake A (**F**), Lake C2 (**G**), Lake Tuborg (**H**), and Buchanan Lake (**I**). The lakes, outlet streams, and nearby marine environment are shown on the inset maps.

Canada (79.47°N, 87.56°W) (**Figure 5.1B-I**). The bottom waters of lakes A, C2 and Tuborg are saline but also anoxic, and Arctic Char are highly unlikely to access these deeper waters. Detailed limnological descriptions of lakes A, C2, and Tuborg can be found in Ludlam (1996), Smith et al. (2004), Veillette et al. (2012) and Vincent et al. (2011), respectively.

5.3.2 *Field Collection*

Arctic Char from Lac Davidson were sampled for otoliths and biological information from an Atlantic Salmon (*Salmo salar*) smolt trap net set annually from May-June (1998-2010) by the Quebec Provincial Government. The smolt trap net was set approximately 1.5 km from the mouth of the Rivière de la Trinité (49.42°N, 67.30°W) to capture out-migrating salmon smolts. It was assumed that these seaward migrating fish originated from Lac Davidson (Doucette et al.1999, Power et al. 2005). Biological, morphological and tissue samples were collected through multi-mesh gillnetting and angling in High Arctic lake systems between 1991-2008 (**Table 5.1;** Babaluk et al. 1997, 2007, 2009b). Otoliths from each collection location were archived. When possible, surface water samples were collected from study lakes and preserved with nitric acid in acid-washed polyethylene bottles to examine total environmental calcium (0.0001 mg/L; Babaluk et al. 2009a).

5.3.3 *Otolith Strontium Analysis*

Table 5.1: Location, year of collection (**Year**), total number of fish captured in the collection (**T.C**), total number of otoliths examined for strontium (**N.Sr**), number of individuals found to have migrated (**N.Mig**), river outlet distance between the lake and the estuary (**R. Len** km), mean fork length (**FL** mm) for individuals found to have migrated, mean lifespan (**LS** years) for individuals found to have migrated, otolith strontium fresh water baseline (**O.Sr** ppm), and environmental calcium concentration (**Ca** mg/L; Babaluk et al. 2009a) for studied Arctic Char. Standard deviations (**sd**) for mean values are provided.

Location	Year	T.C	N.Sr	N.Mig	R. Len	FL (sd)	LS (sd)	O.Sr (sd)	Ca
Davidson	1998-2010	216	59	59	22.0	239 (55)	5 (1)	856 (49)	1.8
Heintzleman	1995, 2001	375	52	7	11.1	295 (50)	10 (2)	464 (37)	33.7
Lower Beaufort	2001	24	20	4	3.0	238 (108)	12 (6)	173 (17)	23.0
Taserssuit	2000, 2001	475	47	46	1.6	208 (140)	15 (4)	779 (151)	3.7
Clements Markham	2001, 2002, 2008	77	71	24	2.7	332 (91)	11 (4)	264 (26)	33.2
A	2002, 2008	32	28	28	0.5	233 (65)	10 (3)	407 (62)	n/a
C2	2006	19	15	15	0.2	348 (55)	11 (3)	356 (53)	n/a
Tuborg	2002, 2003	31	23	19	8.2	513 (95)	15 (2)	441 (22)	21.3
Buchannan	1991	20	17	17	3.1	490 (138)	13 (3)	469 (30)	n/a

Rivieré de la Trinité captured Arctic Char were randomly sampled for otolith analysis. Elsewhere, otoliths were chosen from older or larger individuals to increase the likelihood of detecting migratory individuals for study locations where samples sizes were large. Sizes of mature individuals were examined to aid the selection process (**Table 5.1**).

Otoliths were cleaned with distilled water, dried, embedded in System Three Cold Cure or Buhler Epothin epoxy resins, and allowed to cure. A Leica dissection microscope with crosshair graticule capabilities was used to dorso-ventrally mark the otolith over the nucleus region in a transverse direction, and otoliths were then sectioned with a Buhler Isomet® slow-speed saw. Otolith sections were re-embedded with epoxy resin into a Lucite disk and polished sequentially on aluminum oxide lapping film (30 µm, 9 µm, and 6 µm). Final polishing was completed on a polishing wheel with 0.1µm-diamond grit paste. Otoliths embedded in the Lucite disks were imaged then ultrasonically cleaned in distilled water prior to analysis.

A Thermo-Finnigan Element2 HR- ICP-MS coupled with a Merchantek LUV213 laser system was used to quantify elements along the dorso-ventral transect on the exposed surface. The elements strontium, calcium and zinc were analyzed with a beam size of 25 µm under 20 Hz repetition rate at a scanning speed of 2 µm/s with 3.5-4 J/cm^2 of fluence. A NIST SRM 610 glass standard was run at the start, every hour thereafter, and at the end of each disk (each disk held between 6-10 samples). All elemental data were internally standardized to Ca (40.02 wt %), then externally to the NIST SRM 610. Concentrations, 2 standard deviations and detection limits for each element were calculated with the program Iolite (ver. 2.3.1, Paton et al. 2011).

To increase sample size from Taserssuit Lake, scanning proton microprobe (SPM) data were included in the overall analysis. SPM instrument conditions are reported in Halden et al. (1995, 2000) and Babaluk et al. (1997, 2010). Strontium concentrations between laser ablation-inductively coupled plasma-mass spectrometer and SPM analyses were not significantly different for samples analyzed using both techniques (Campana et al. 1997, N. Halden *unpublished data*).

5.3.4 *Examination of Migration Histories*

To examine strontium concentrations within each annulus, strontium line transect data were overlaid and aligned to annuli with post-laser otolith images using ImagePro Plus (Ver. 6.3 2008). These were also secondarily aligned with annular signals in the zinc line transect data (Halden et al. 2000). An iterative R code (R ver. 3.1.1 2014) was used to bin the strontium data from each annulus and calculate descriptive parameters such as mean, median, maximum, minimum and standard deviation. Bins represented the core region, the outside edge of the core to year one, and consecutive annuli thereafter.

Freshwater baselines for strontium were inferred from the calculated average and standard deviations from sections of the line transect of each run where strontium exhibited its lowest value over the course of an entire year, or from within the core region of the otolith if no maternal effect was visually observed (**Figure 5.2**). In most populations, with the exception of Taserssuit Lake, the values for strontium were lower than those observed in marine waters and were consistent for the freshwater phase over multiple years. As a secondary check of accuracy, the freshwater baselines for migrating fish were compared to individuals within the population that did not migrate (if

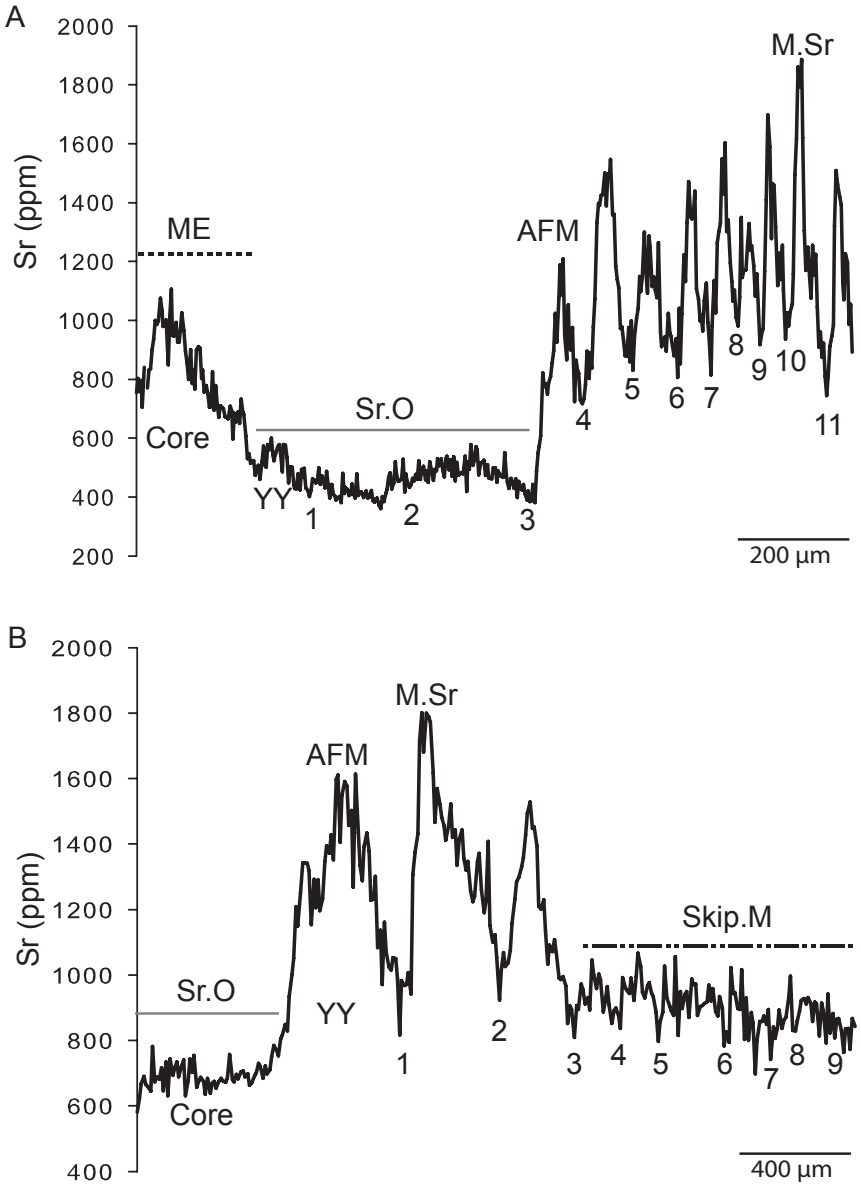

Figure 5.2: Examples of otolith strontium transects from Arctic Char from the various study areas. Strontium transect **A** has a maternal effect (**ME** indicated by the dashed line) within the core region of the otolith and a reduction in strontium as young-of-the-year (**YY**) until the age of 3 years. The otolith strontium fresh water baseline (**Sr.O**, indicated by the solid line) was calculated from YY to 3 (years). At the age of 3 years

this Arctic Char initiated migration (**AFM**). Maximal lifetime strontium (**M.Sr**) concentration occurred at 10+ (years). The lifespan of the fish was 11+ years. Strontium transect **B**, demonstrates AFM as YY and M.Sr occurring in the first year of life. Skipped migrations (**Skip.M** indicated by the dotted line) occurred from age 3 (years) onward. The lifespan of the fish was 9+ years.

available within the data set), and then averaged for all fish analyzed to examine the consistency of the baseline within the population as a whole. Similar to the quantitative means of differentiating a true signal from background in detection limits on most analytical machines (Sylvester and Raeside 2001), a change in habitat (i.e., movement from fresh to estuarine or marine waters) was determined when the maximal value for a peak of strontium within an annulus was three standard deviations above the freshwater baseline for the individual fish.

To determine skipped migration in individual otolith strontium profiles three criteria were examined: 1.) a reduction in strontium in comparison to the values of the previous annuli where migration occurred, 2.) <100 ppm change between maximal and minimal strontium values within a specific annulus, and 3.) low standard deviation <50 ppm for strontium values in an annulus. To secondarily check our criteria, the examination of a graphic for the otolith strontium transect with markers delineating annuli was viewed.

A subset of Taserssuit Lake Arctic Char appeared to have marine/estuarine signatures throughout their lifetime post hatch in comparison to other members of the population. These individuals were captured in the fjord at the mouth of the outlet stream from the lake system and were considered to be part of the Taserssuit Lake population. For the purpose of our analysis, they were considered migratory and examined for age- and size- at first migration, strontium concentrations at first migration, and maximal lifetime strontium, but could not be examined for skipped migration. A freshwater baseline was difficult to determine for these individuals, thus the overall

mean strontium freshwater otolith baseline for the whole lake system was used as a substitute.

Otolith growth in relationship to otolith core diameter was linear (R^2 = 0.45-0.72) for study locations where sample size was >15. Thus, size at first maturation was back-calculated as described in Loewen et al. (2010). Age at first migration, lifespan, maximal lifetime strontium concentration, and skipped migrations were recorded for each individual (**Figure 5.2**). Overall population statistics for each biodemographic datum described above are presented. River length from fresh water to the marine environment was used as a measure for ease of access. Measurements were made from the measuring toolbox of online topographical maps for each lake system of the study (http://atlas.nrcan.gc.ca/toporama/en/index.html accessed March 21, 2016). In association, means and standard deviations were calculated for basic study parameters such as fork length (mm), lifespan (years) and otolith strontium baseline fresh water signature (ppm) for each studied population.

5.3.5 *Age- and Size- at First Migration*

Means and standard deviations of age- and size- at first migration were calculated for each studied lake. Ease of access (river length from fresh to estuarine waters) to marine environments for High Arctic lake systems was examined in relation to age and size at first migration. Lower Beaufort Lake was excluded from the analysis due to low sample number (n = <5). Logarithmic relationships between both age and size at first migration and river length were observed and further normalised as $\log_{10}$ of all parameters.

5.3.6 *Marine Habitat Selection*

Based upon Brown and Severin (2009) and Loewen et al. (*in press*), strontium concentrations infer marine habitat selection by anadromous fishes. Strontium replaces calcium at the gills of fish during uptake processes due to similar chemical affinity (Chowdhury and Blust 2011). Anadromous fishes encounter differences in environmental calcium concentration over their migration from fresh water to marine associated waters. The physiological uptake of calcium and, thereby, strontium varies depending on external concentrations (Chowdhury and Blust 2001, Loewen al. *in press*). Environmental strontium competes with environmental calcium differently depending upon internal homeostasis requirements (Chowdhury and Blust 2011). Higher strontium/calcium concentrations in otoliths once a fish has migrated (similar to that of approximately ≥2000 ppm absolute strontium when applying an external standard as a general guideline) are suggestive of the use of full strength marine environments, whereas intermediate levels are suggestive of the use of fresh water-marine mixed-estuarine waters (Phillis et al. 2011).

Population mean and standard deviations for each studied lake were calculated for peak (maximal) strontium concentration in the annuli associated with first migration. Ease of access to the marine environment and skipped migration accounting for lake system were examined in relation to strontium concentrations at first migration.

Analysis of variance (ANOVA) demonstrated significant differences ($p < 0.05$) between strontium at first migration within the studied lakes and maximal strontium levels over the lifetime of individual fish. As a result, two-tailed t-tests with a confidence

level of 0.95 were performed comparing strontium at first migration to maximal strontium over the lifetime of an individual fish for each studied lake system.

5.3.7 *Skipped Migrations*

Two types of migrations were examined for all migrating individuals from each lake system studied (*see the* migration histories section for criteria in determining skipped migration): 1. discontinuous migrators where individuals intermittently forego migration opportunities to remain in fresh water, and 2. continuous migrators where fixed annual migrations seaward occurred once this behaviour was initiated.

ANOVA was used to examine the relationship between skipped migrations and among lake systems. Tukey's pairwise comparison test was subsequently used to identify significant differences among the lake systems. Additionally, overall percentage of skipping migration was calculated at a population level based upon the presence or absence of skipping on an annual basis by migratory individuals. The groups of discontinuous and continuous migrators within a lake system were delineated as being different from other lakes in the study through Tukey's pairwise comparison tests. Lakes that were similar to each other in the pairwise comparison tests (i.e., not statistically significantly different) were grouped together. The association between age and size at first migration was investigated relative to the percentage of skipped migration for the study lakes that fell within the discontinuous migration group.

5.4 Results

Overall, High Arctic migratory chars had mean lengths that ranged from 208 (+/- 140 standard deviation (sd)) mm in Lower Beaufort Lake, to 513 (+/- 95 sd) mm in Lake Tuborg (**Table 5.1)**. Likewise, High Arctic migratory chars had a mean lifespan (years) that ranged from 10 (+/- 2 sd) years in Heintzleman Lake, to 15 (+/- 2-4 sd) years in Lake Tuborg and Taserssuit Lake (**Table 5.1)**. Migratory chars from Lac Davidson had a mean length of 239 (+/- 55 sd) mm and mean lifespan of 5 (+/- 1 sd) years (**Table 5.1)**. Otolith baseline Sr in the fresh water varied in relationship to environmental calcium (**Table 5.1**, Loewen et al. *in press*). High Arctic river lengths from the freshwater to the marine environment ranged from 0.2 km for Lake C2 to 11.1 km for Heintzleman Lake. Lac Davidson's distance to the marine environment was 22 km (**Table 5.1**).

5.4.1 *Age- and Size- at First Migration*

Age at first migration ranged overall from young-of-the-year (Lake C2) to 11+ years of age (Lower Beaufort Lake) for the High Arctic populations, with high variability notable amongst most of the populations (**Figure 5.3**). For Heintzleman and Lower Beaufort lakes mean age at first migration was 6 (+/- 2 sd) years and 9 (+/- 2 sd) years, respectively, and young-of-the-year were absent in the portion of fish that migrated. Arctic Char in Taserssuit Lakes predominantly migrated as young-of-the-year and showed little variation. Lakes C2 and A had mean ages at first migration of 2 (+/- 2 sd), and 1 (+/- 1 sd) years, respectively. Buchanan, and Clements Markham lakes and Lake Tuborg had mean ages at first migration of 4 (+/- 2 sd), 4 (+/- 3 sd), and 5 (+/- 4 sd) years, respectively. Migration at the southern-most extent of Arctic Char (Lac Davidson)

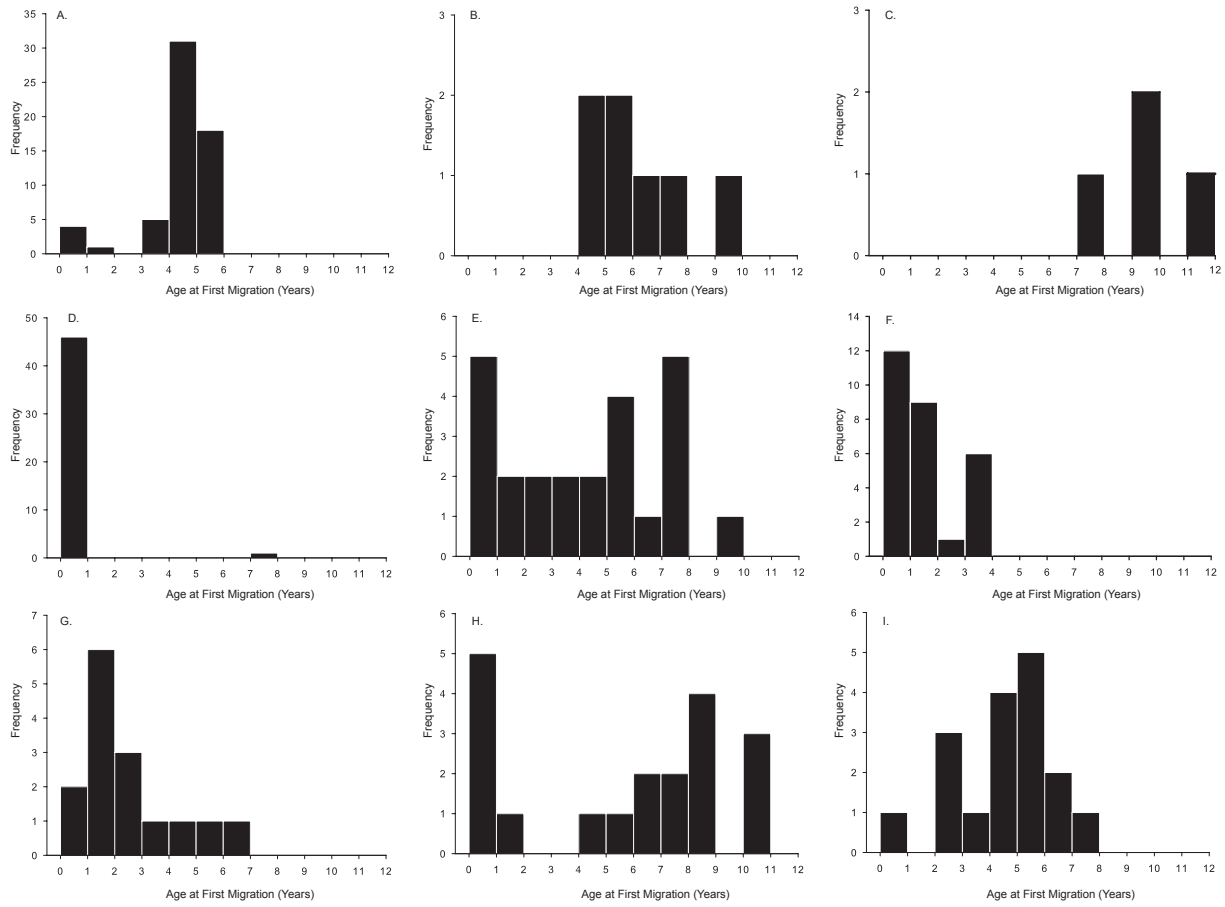

Figure 5.3: Age at first migration (years) histogram plots for Arctic Char from Lac Davidson (**A**), Heintzleman Lake (**B**), Lower Beaufort Lake (**C**), Taserssuit Lake (**D**), Clements Markham Lake (**E**), Lake A (**F**), Lake C2 (**G**), Lake Tuborg (**H**), and Buchanan Lake (**I**).

occurred at the mean age of 4 (+/- 1 sd) years. A small number of Lac Davidson young-of-the-year chars were found to also migrate (**Figure 5.3**).

Length at first migration for individuals ranged overall from ≤50 mm to 504 mm in size for High Arctic populations (**Figure 5.4B-I**). High variability was observed for Clements Markham, C2, Tuborg and Buchanan lakes. Lake Tuborg had the largest size at first migration at 504 mm, whereas almost all individuals in Taserssuit Lake first migrated at a size ≤50 mm with a predominance of individuals migrating at 36 (+/-16 sd) mm. Size at maturation for fish from Heintzleman and Lower Beaufort lakes ranged from 150-300 mm with most individuals migrating at 221 (+/- 45 sd) mm and 255 (+/- 35 sd) mm, respectively. Length at migration in the southern-most location (Lac Davidson) ranged from 50-300 mm with the predominance of individuals migrating at 200 (+/- 53 sd) mm.

River length was logarithmically related to age and fish length at first migration for all High Arctic populations (**Figure 5.5**), although Lower Beaufort Lake was excluded due to small sample size (n=4). Linear regression models, on normalised data, demonstrate significant relationships with river length of both age ($p < 0.003$) and size ($p<0.001$) at first migration ($F = 32.26$, $df = 3, 157$, adjusted (adj) $R^2 = 0.37$; $F = 46.75$, $df = 3, 155$, adj $R^2 = 0.46$, respectively). Interaction between river length and lake system in the linear models ($p<0.001$) indicated that the association of river length to age and size at first migration differs by lake system.

5.4.2 *Marine Habitat Selection*

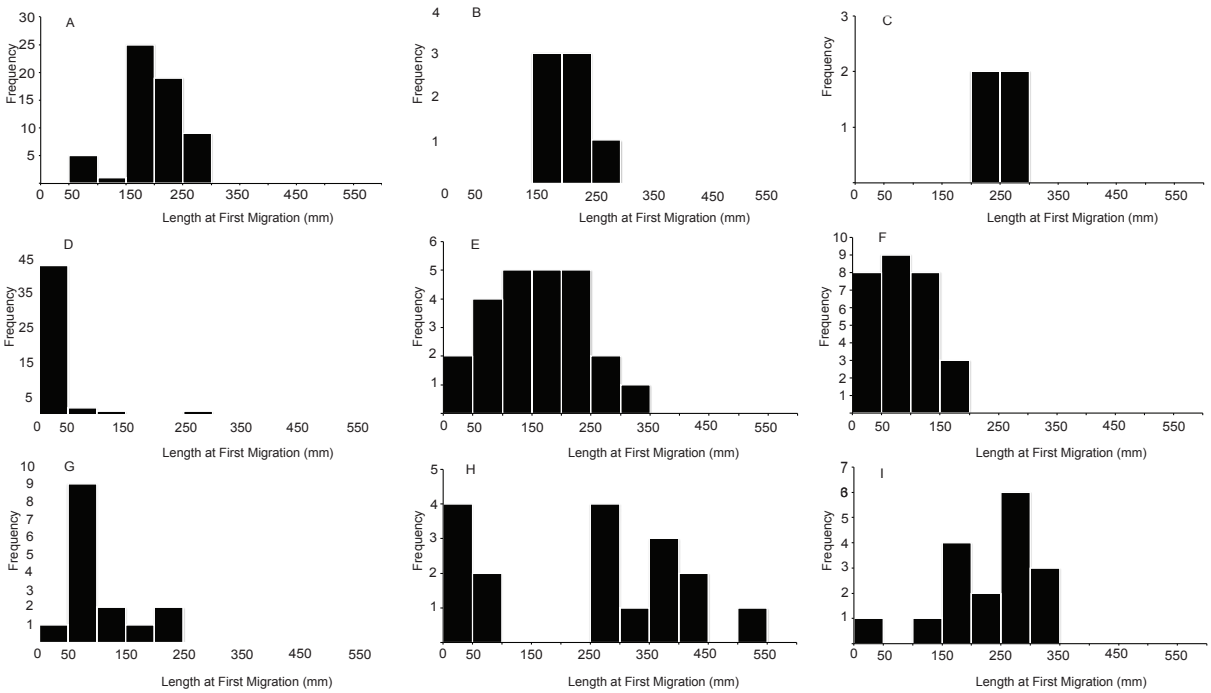

Figure 5.4: Length at first migration (mm) histogram plots for Arctic Char from Lac Davidson (**A**), Heintzleman Lake (**B**), Lower Beaufort Lake (**C**), Taserssuit Lake (**D**), Clements Markham Lake (**E**), Lake A (**F**), Lake C2 (**G**), Lake Tuborg (**H**), and Buchanan Lake (**I**).

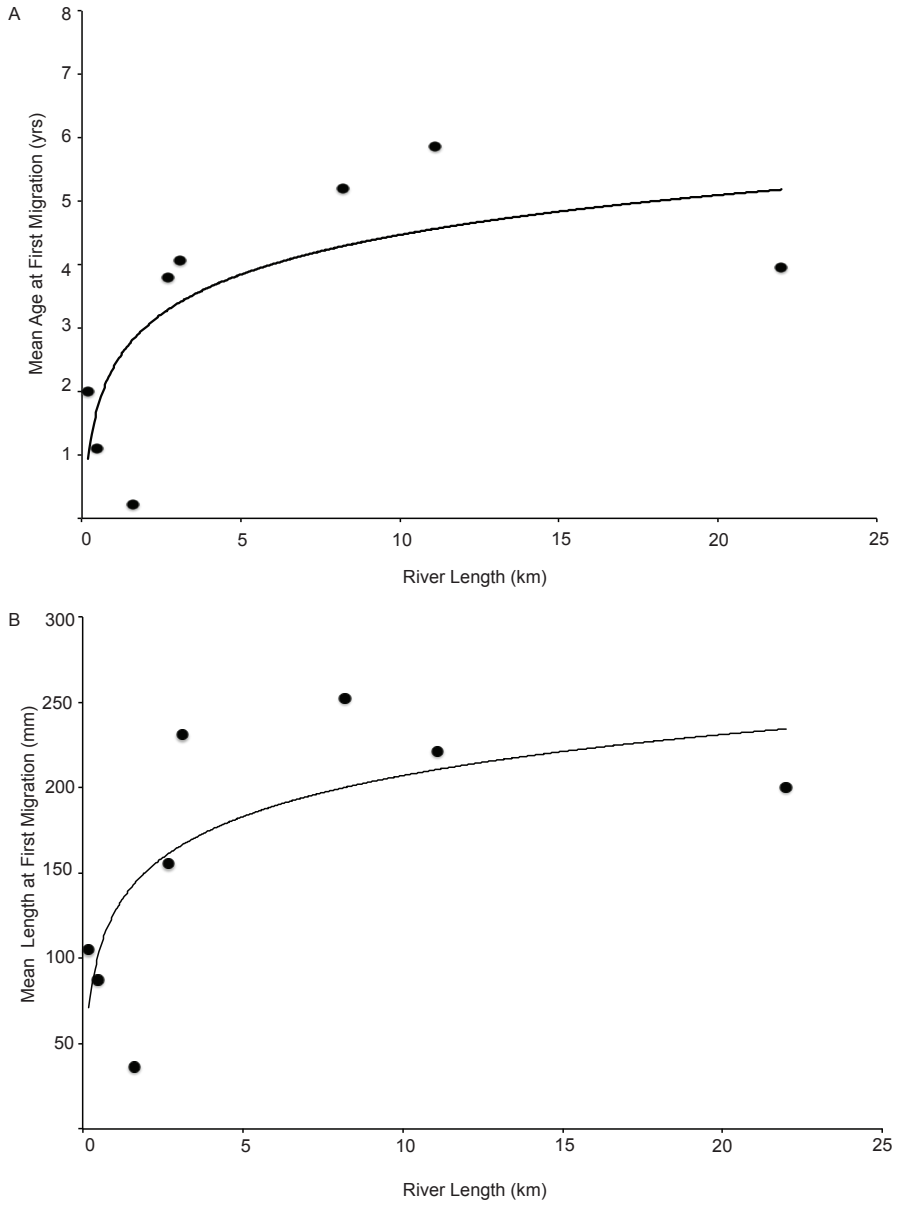

Figure 5.5: The logarithmic relationship between mean age (**A)** is $\gamma = 1.14\ln(x) + 2.41$, where R^2 = 0.61. The logarithmic relationship between length (**B**) at first migration for High Arctic lakes to river length is $\gamma = 41.36\ln(x) + 127.5$, where R^2 = 0.53. Lower Beaufort and Heintzleman lakes were excluded from the relationship due to low sample size.

Variation in the use of mixed estuarine waters is observed among Arctic Char populations in the study (**Table 5.2**). Lower Beaufort Lake chars exhibited the lowest values of otolith strontium in the first migration (634 ppm +/- 149 sd) followed by those from Lake Tuborg (897 ppm +/- 258 sd) and Lake C2 (927 ppm +/- 319 sd). Lake A and Clements Markham fish exhibited intermediate values of strontium in the first migration (1007 ppm (+/- 275 sd) and 1015 ppm (+/- 284 sd), respectively). Char from Buchanan, Heintzleman, Taserssuit lakes, and Lac Davidson exhibited the highest values of strontium in the first migration (1297 ppm (+/- 397 sd), 1424 ppm (+/- 441 sd), 1493 ppm (+/- 360 sd), and 1849 ppm (+/- 242 sd), respectively). Variation among individuals within each population is notable in large standard deviations for each population mean strontium values.

Linear regression analyses suggest significant relationships between river length ($p < 0.001$), skipped migrations ($p = 0.004$) and strontium concentrations at first migration ($F = 32.87$, $df = 5, 185$, adj $R^2 = 0.46$). Interaction between river length ($p<0.001$) and skipped migrations ($p = 0.013$) to lake system indicates that the association with strontium concentrations at first migration differs by lake system. Otolith strontium concentrations at first migration are also linearly related to river length (**Figure 5.6**). Arctic Char navigating shorter river systems tend to have a lower strontium signal at first migration. The exception to this observation is Taserssuit Lake where strontium tends to be high in comparison to other lake systems of similar length. Skipped migrations and lake system interactions are indicative of variation between the continuous and discontinuous behaviours that characterise groupings of char (see below) in relationship to strontium concentrations at first migration. Maximal lifetime

Table 5.2: Two-tailed t-tests (degrees of freedom (**df**) and **p-value**) with a confidence level of 0.95 are presented for each lake system (**Location**). Underlined p-values indicate no significant difference between strontium at first migration relative to maximal lifetime strontium. Mean strontium at first migration (**Sr.AFM**) and maximal lifetime strontium (**Max.Sr**) concentrations are provided for each location. Standard deviations (**sd**) for mean values are provided.

Location	Sr.AFM (sd)	Max.Sr (sd)	df	p-value
Davidson	1849 (242)	1881 (202)	33	0.105
Heintzleman	1424 (441)	1814 (354)	6	0.084
Beaufort	634 (149)	1245 (343)	3	0.087
Taserssuit	1493 (360)	1753 (338)	23	<0.001
Clements Markham	1015 (284)	1394 (281)	46	<0.001
A	1007 (275)	1394 (313)	27	<0.001
C2	927 (319)	1228 (294)	14	0.003
Tuborg	897 (258)	1277 (190)	18	<0.001
Buchanan	1297 (397)	1749 (190)	16	<0.001

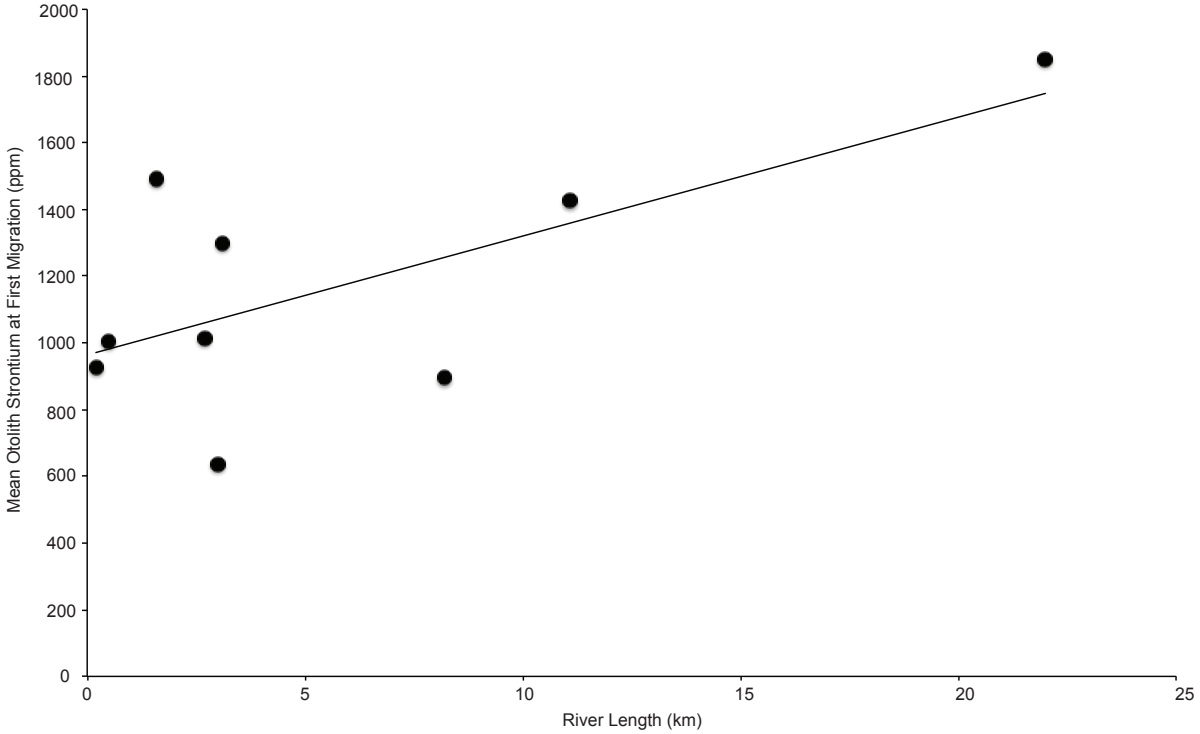

Figure 5.6: The relationship between mean otolith strontium at first migration for all lakes studied to river length was $\gamma = 35.64x + 963.92$, where R^2 = 0.45.

otolith strontium was significantly higher after the first year of migration for all populations with the exception of Lac Davidson, and Heintzleman, and Lower Beaufort lakes (**Table 5.2**).

5.4.3 *Skipped Migrations*

Two-groups, continuous and discontinuous migrators are indicated by the results and are significantly different ($p<0.001$). Tukey's post hoc pairwise comparison identified Lac Davidson and Buchanan Lake as continuous migrators with low occurrences of skipped migrations at 6.8% (n= 4) and 11.8% (n= 2), respectively. Discontinuous migrations were observed in the remaining lakes, ranging from 42.9% (n= 3) in Heintzleman to 80.4% (n= 37) in Taserssuit lakes. There were negative linear relationships between age ($R^2 = 0.71$) and size ($R^2 = 0.81$) at first migration and the percent occurrence of skipping in discontinuous migrating populations (**Figure 5.7**).

5.5 Discussion

5.5.1 *Age- and Size- at First Migration*

Arctic Char migrate into marine waters as soon as possible in life history given the ability to survive osmotic and thermal challenges in the extremely resource limited freshwater environments of the High Arctic. Indeed, the occurrence of young-of-the-year chars and associated small sizes at maturation is observed in migrants from the majority of High Arctic lake systems. Notably age and size at first migration are highly variable among as well as within studied lakes. Arctic Char are known to migrate at small-sizes in various areas (Bouillon and Dempson 1989, Dempson 1993, Arnesen et

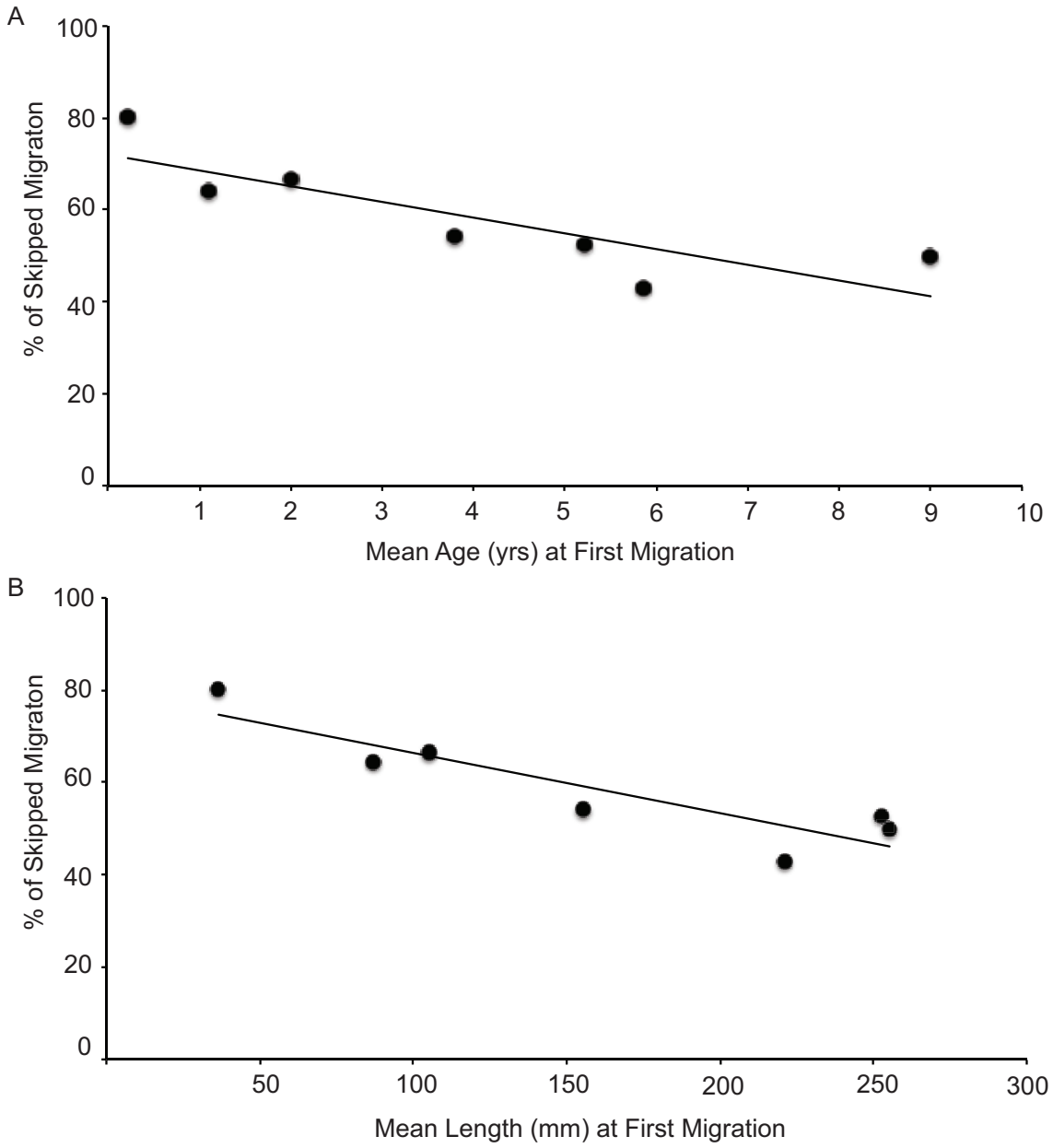

Figure 5.7: The relationship of skipped migration (%) to mean age (**A**) to mean length (**B**) and was $\gamma = -3.41x + 72.01$ where R^2 = 0.70, and $\gamma = -0.13x + 79.45$ where R^2 = 0.81, respectively for Arctic Char lakes grouped as discontinuous migrators.

al. 1995, Nilssen and Gulseth 1998, Jonsson and Antonsson 2005, Loewen et al. 2009, 2010). Studies by Dempson (1993), Gulseth and Nilssen (2000), and Bystriansky et al. (2007) demonstrated the osmoregulatory capacities of <100 mm Arctic Char in estuarine waters and suggested that marine survival was feasible for smaller fish if an estuarine refuge was available. Our study demonstrates the predominance of young (<2 years) and small Arctic Char (<100 mm) that undertake first migrations early in life history. Further, chars from Taserssuit Lake show a preference and predominance of individuals migrating as young-of-the-year and at an associated small size. The absence of young-of-the-year in migratory individuals occurred only in two lake systems, Heintzleman and Lower Beaufort lakes of the High Arctic. Low rates of occurrence were also evident in Buchanan Lake.

A strong relationship between ease of access and age- and length- at first migration was observed in the High Arctic lakes. The “decision” of when to migrate appears to be influenced by proximity and likely ease of access (e.g., river length) to the marine environment. Young and small Arctic Char benefit from migration through increased feeding opportunities in estuaries in comparison to depauperate freshwater habitats, thus can migrate earlier and at a smaller size as observed for some locations examined. Large growth potential is balanced with the difficulty and energetic costs (and presumably risks) associated with accessing higher productive marine environments. Where nutrients are available in fresh water and concomitant productivity is higher, such as the southern areas of Arctic Char distribution, a delay as expected in first migration seaward occurs.

5.5.2 *Marine Habitat Selection*

Upon initiating migratory behaviour, anadromy within High Arctic chars is predominantly restricted to mixed estuary environments. For southern Arctic Char, a seasonal feeding period that is available longer in fresh water allows for a delay in first migration seaward. This concomitantly results in larger body-sizes to facilitate access and movement into higher salinity marine-associated waters.

Ease of access and skipped migrations both demonstrate strong relationships with the type of marine-associated habitat selected. As previously noted ease of access in the High Arctic influences the age and size at first migration. As a result, the physiological capacity of Arctic Char at first migration limits the types of habitat chosen once in marine-associated environments. That is, the use of estuary waters is necessary for successful osmoregulation and survival by early seaward migrating small-bodied Arctic Char (Dempson 1993, Bystriansky et al. 2007).

Skipped migrations, discussed below in the context of continuous or discontinuous migrations, defines the use of “marine” habitat selection. Continuous migrators delay first migration resulting in a larger body-size (>150 mm) when they do migrate. These individuals tend to utilise high strontium environments most likely associated with estuarine waters that are substantively marine influenced. Presumably such habitat use reflects increased osmoregulatory capabilities associated with larger sizes. Lac Davidson and Buchanan Lake exemplify this occurrence. In contrast, discontinuous migrators initiate migrations early in life at a small body size (<150 mm). These individuals tend to utilise low strontium environments most likely associated with

freshened-estuarine waters, likely due to osmoregulatory constraints (e.g., Lakes C2, A, and Taserssuit Lake).

Taserssuit Lake appeared to be an exception to this trend in regards to discontinuous migrators. Young, small-bodied fish from this lake system had encountered estuary-marine waters earlier in life than would be expected. A subset of Arctic Char from this lake had strontium profiles suggestive of over-wintering in the estuary. The fjord in proximity to Taserssuit Lake sits at the head of a glacier that is likely providing a strong constant flow of fresh melt water perhaps throughout the year. In addition, warmer ocean currents influence western Greenland (Holland et al. 2008). This is indicative of overall relatively warmer temperatures in comparison to other High Arctic lake systems considered in the region. Increased fresh water to the fjord and the occurrence of relatively warmer temperatures at a High Arctic location appears to allow for conditions facilitating over-wintering in a refuge within the fjord. Winter estuary use of Arctic Char has also been documented for River Skibotn, Norway (Jensen and Rikardsen 2008). Over-wintering habitat within fresh water in these Norwegian systems is limited and winter estuary temperatures are approximately 5-7 °C and hence vastly different from temperatures in the Canadian Arctic. Thus, a further detailed investigation into life histories found within Taserssuit Lake and associated winter estuary conditions is warranted.

High Arctic chars demonstrate seasonal acclimation to lower strontium environments (inferred use of fresh-estuarine waters) upon initiating migratory behaviour and before utilising increased strontium environments (i.e., the inferred use of estuary, estuary-marine or marine waters). Lower Beaufort and Heintzleman lakes,

however, were exceptions to this finding and demonstrated similarities to the southern counterpart (Lac Davidson) in this study. For southern anadromous Arctic Char, no seasonal acclimation associated with the initiation of migration was exhibited.

Body size is associated with the initial association of Arctic Char to specific habitats. Where size at first migration is small, a strong association to estuaries is exhibited. This association would be a physiological necessity for osmoregulation at a small-body size (<150mm) (Dempson 1993, Bystriansky et al. 2007). The likelihood of survival of small-bodied Arctic Char in fully saline marine waters is low (Dempson 1993, Gulseth and Nilssen 2000). For Heintzleman Lake, Beaufort Lake and Lac Davidson, Arctic Char were associated with higher strontium environments at first migration and at subsequent migrations thereafter suggesting they have strong physiological and osmoregulatory capacities allowing occupancy of fully marine environments. Where a delay in migration is observed and a larger body size is attained, seasonal acclimation is not observed nor needed for marine survival. Thus part of the cost for early migration seaward is a decreased capacity to fully use marine habitats and the necessary presence of estuarine habitats for survival. Seasonal acclimation is inferred for Arctic Char in the initial commencement of migration of small-bodied individuals with their ability to increase the use of marine habitats thereafter.

5.5.3 *Skipped Migrations*

The tendencies of populations to exhibit continuous or discontinuous migration behaviours have not been extensively examined in North American Arctic Char populations. Unique discontinuous (facultative) migration behaviour in Dolly Varden

Char (*Salvelinus malma*), a closely related species from southwestern Alaska, has recently been documented (Bond et al. 2015). A subset of the population migrates early in life to attain larger body size then ceases migration whereas other members of the same population exhibit annual migrations. Flexibility in frequency of migrations provides another means of identifying intra-specific diversity and variation of life history strategies within a species. As hypothesized by Chapman et al. (2011), the degree of environmental stability may play a role in the "decision" to undertake continuous (fixed annual migrations once commenced) or discontinuous (intermittent foregoing of migration opportunities to remain in fresh water) migrations by individuals within a population. Although our data are limited by a small sample size of the overall population, it provides useful information regarding the occurrence of Arctic Char migration behaviour in years after first initiation. Within the study, migratory behaviour was most often found to be discontinuous in all but one High Arctic Lake. This finding is anticipated, as it would allow for increased bet hedging and resilience for the survival and persistence of chars in highly variable Arctic freshwater environments. Arctic Char from Lake Buchanan had low levels of skipped migration once migratory behaviour was initiated. As expected, continuous migrations also occurred in the southern population. Other factors that may affect the how and why a fish migrate are intra-specific competition and resource partitioning within the fresh water itself (e.g., increased predation on small fish from cannibalistic freshwater piscivores), the accessibility of marine environments, and inter-annual variability within the lake systems and warrant further investigation.

In discontinuous migrating populations, relationships were observed between age and size at migration and the degree to which skipped migration occurred. Smaller and younger ages at first migration suggest that Arctic char will have a high likelihood of skipping migration at some point in future years. Arctic Char that exhibited the strongest discontinuous behaviour have relatively easy access to estuary and marine environments. Continuous migrators were of a larger-body size at first migration and experienced longer migrations to access estuary and marine environments. Thus, other factors such as attaining a larger body size to easily acquire freshwater resources as observed in Dolly Varden Char (Bond et al. 2015), and genetic/environmental interactions (Chapman et al. 2011, Pulido 2011) need to be examined in understanding the variability in behaviour. Larger sample sizes and additional analyses are needed to verify the preliminary trends for skipped migration observed within the present study.

5.6 Summary

Rounsefell (1958) in his classification of anadromy examined the degrees and behaviours of migratory salmonids. Arctic Char was classified as having a low degree of anadromy. Telemetry studies by Spares et al. (2012, 2015b) and Moore et al. (2016) support this. Arctic Char were demonstrated to have strong association to estuaries and coastal habitats once fish had migrated seaward. From the examination of Arctic Char otolith strontium to infer migration histories, our study supports the overall association of Arctic Char to estuarine habitats in the High Arctic. Mean otolith strontium was <2000 ppm for both first migrations seaward and lifetime maximal levels in all populations,

suggestive of a high dependence on estuarine waters (Brown and Severin 2009, Phillis et al. 2011).

Although utilization of estuarine habitats is evident, variations exist in anadromous behaviour among populations. At the northern-most range extension such as Lake A and Clements Markham Lake, Arctic Char migrate seaward at a young age and small size. They utilise low strontium waters indicative of coastal estuaries throughout their lifetime and are facultative in their use of these estuarine habitats. In contrast, southern-most migratory Arctic Char from Lac Davidson, delay migration seaward until an older age and larger size. They utilise high strontium waters indicative of less dependence on estuarine waters within the first year of migration and all subsequent years thereafter. They are continuous migrators once this behaviour commences. Variation and anomalies do exist for other High Arctic Lakes. For example, Lake Buchanan is the only High Arctic Lake where continuous migration is prevalent. Further, a subset of Arctic Char from Taserssuit Lakes likely over-winters in the associated estuarine areas perhaps due to continuous input of glacial melt water.

Future research should examine freshwater productivity and access to marine environments to further explore partial migrations within Arctic Char populations. Additional parameters such as river water levels, river gradient, and river water velocity would be needed in models to better estimate ease of access to marine environments (Power and Barton 1987, Hvidsten and Hansen 1988, Kristoffersen 1994). Similarly, measures for productivity such as lake morphology specifically documenting profundal zone habitat (Kristoffersen et al. 1994), intra- and inter-species diversity (Nordeng 1983, Langeland et al. 1991, Reist et al. 2006, 2013) and primary production (Power et al.

2008, Finstad and Hein 2012) will provide stronger understanding of relationships with the occurrence of partial migration for North American and western Greenland Arctic Char populations.

5.7 Acknowledgements

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5.8 References

Arnesen, A. M., Lysfjord, G., and Damsgård, B. 1995. Smolt characteristics of small

first-time mrgrant, and resident Arctric charr, *Salvelinus alpinus* (L.), from a river system in northern Norway. Aquacult. Res. **26**: 809-818.

Babaluk, J. A., Campbell, J. L., Evans, C. L., Halden, N. M., Mejia, S. R., Nejedly, Z., Reist, J. D., and Teesdale, W. J. 2002. Micro-PIXE analysis of strontium in Arctic char, *Salvelinus alpinus*, otoliths from Quttinirpaaq National Park, Nunavut, Canada. Nucl. Instrum. Meth. B **189**: 190–195. doi: 10.1016/S0168-583X(01)01040-0.

Babaluk, J. A., Gantner N., Michaud, M., Muir, D. C. G., Power, M. Reist, J. D., Sinnatamby, R., and Wang, X. 2009a. Chemical analyses of water from lakes and streams in Quttinirpaaq National Park, Nunavut, 2001-2008. Can. Data Rep. Fish. Aquat. Sci. **1217**: v + 24 p.

Babaluk, J. A., Halden, N. M., Reist, J. D., Kristofferson, A. H., Campbell, J. L., and Teesdale, W. J. 1997. Evidence for non-anadromous behaviour of Arctic charr (*Salvelinus alpinus*) from Lake Hazen, Ellesmere Island, Northwest Territories, Canada, based on scanning proton microprobe analysis of otolith strontium distribution. Arctic **50**(3): 224–233.

Babaluk, J. A., Sawatzky, C. D., Wastle, R. J., and Reist, J. D. 2007. Biological data of Arctic charr (*Salvelinus alpinus*) from selected lakes in Quttinirpaaq National Park, Nunavut, 1990-2002. Can. Data Rep. Fish. Aquat. Sci. **1196**: vii + 78 p.

Babaluk, J. A., Wastle, R. J., Johnson, J. D., Troke, B. G., and Reist, J. D. 2009b. Biological data of Arctic Char, *Salvelinus alpinus*, Heintzleman Lake, Quttinirpaaq National Park, Nunavut. Can. Data Rep. Fish. Aquat. Sci. **1216**: v + 27 p.

Babaluk, J. A., Wastle, R. J., and Reist, J. D. 2010. Micro-chemical analysis of Arctic

Char, *Salvelinus alplinus*, otoliths from Ellesmere and Axel Heiberg islands, Nunavut. Can. Data Rep. Fish. Aquat. Sci. **1225**: xiii + 120 p.

Berg, O. K., and Berg, M. 1990. Effects of Carlin tagging on the mortality and growth of anadromous Arctic char, *Salvelinus alpinus* (L.). Aquacult. Res. **21**: 221–228. doi: 10.1111/j.1365-2109.1990.tb00459.x.

Bloom, D. D., and Lovejoy, N. R. 2014. The evolutionary origins of diadromy inferred from a time-calibrated phylogeny for Clupeiformes (herring and allies). Proc. R. Soc. Lond. B Bio. **281**: 20132081.

Bond, M. H., Miller, J. A., and Quinn, T. P. 2015. Beyond dichotomous life histories in partially migrating populations: cessation of anadromy in a long-lived fish. Ecology **96**: 1899–1910. doi: 10.1890/14-1551.1.

Bouillon, D. R., and Dempson, J. B. 1989. Metazoan parasite infections in landlocked and anadromous Arctic charr (*Salvelinus alpinus* Linnaeus), and their use as indicators of movement to sea in young anadromous charr. Can. J. Zool. **67**: 2478–2485. doi: 10.1139/z89-350.

Brown, R. J., and Severin, K. P. 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. Can. J. Fish. Aquat. Sci. **66**: 1790–1808. doi: 10.1139/F09-112.

Bystriansky, J. S., Frick, N. T., Richards, J. G., Schulte, P. M., and Ballantyne, J. S. 2007. Failure to up-regulate gill Na^+, K^+-ATPase alpha-subunit isoform alpha1b may limit seawater tolerance of land-locked Arctic char (*Salvelinus alpinus*). Comp. Biochem. Physiol. A. **148**: 332–338. doi: 10.1016/j.cbpa.2007.05.007.

Campana, S. E., Thorrold, S. R., Jones, C. M., Günther, D., Tubrett, M., Longerich, H., Jackson, S., Halden, N. M., Kalish, J. M., Piccoli, P., De Pontual, H., Troadec, H., Panfili, J., Secor, D. H., Severin, K. P., Sie, S. H., Thresher, R., Teesdale, W. J., and Campbell, J. L. 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Can. J. Fish. Aquat. Sci. **54**: 2068–2079. doi: 10.1139/f97-117.

Chapman, B. B., Brönmark, C., Nilsson, J. -Å., and Hansson, L. A. 2011. The ecology and evolution of partial migration. Oikos **120**: 1764–1775. doi: 10.1111/j.1600-0706.2011.20131.x.

Chapman, B. B., Hulthén, K., Brodersen, J., Nilsson, P. A., Skov, C., Hansson, L., and Brönmark, C. 2012. Partial migration in fishes: causes and consequences. J. Fish Biol. **81**: 456–78. doi: 10.1111/j.1095-8649.2012.03342.x.

Chavarie, L., Dempson, J. B., Schwarz, C. J., Reist, J. D., Power, G., and Power, M. 2010. Latitudinal variation in growth among Arctic charr in eastern North America: evidence for countergradient variation? Hydrobiologia **650**: 161–177. doi: 10.1007/s10750-009-0043-z.

Chowdhury, M. J., and Blust, R. 2001. A mechanistic model for the uptake of waterborne strontium in the Common Carp (*Cyprinus carpio* L.). Environ. Sci. Technol. **35**: 669–675. doi: 10.1021/es000142t.

Chowdhury, M. J., and Blust, R. 2011. 7 - Strontium. *In* Homeostasis and toxicology of non-essential metals. *Edited by* C. M. Wood, A. P. Farrell, and C. J. Brauner. Academic Press. pp. 351–390. doi: 10.1016/S1546-5098(11)31029-1.

Dempson, J. B. 1993. Salinity tolerance of freshwater acclimated, small-sized Arctic charr, *Salvelinus alpinus* from northern Labrador. J. Fish Biol. **43**: 451–462. doi: 10.1111/j.1095-8649.1993.tb00580.x.

Dempson, J. B., and Kristofferson, A. H. 1987. Spatial and temporal aspects of the ocean migration of anadromous Arctic char. Am. Fish. Soc. Symp. **1**: 340–357.

Dodson, J. J., Laroche, J., and Lecomte, F. 2009. Contrasting evolutionary pathways of anadromy in euteleostean fishes. Am. Fish. Soc. Symp. **69**: 63–77.

Doucett, R. R., Power, M., Power, G., Caron, F., and Reist, J. D. 1999. Evidence for anadromy in a southern relict population of Arctic charr from North America. J. Fish Biol. **55**: 84–93. doi: 10.1111/j.1095-8649.1999.tb00658.x.

Everhart, W. H., and Waters, C. A. 1965. Life history of the Blueback trout (Arctic char, *Salvelinus alpinus* (Linnaeus)) in Maine. Trans. Am. Fish. Soc. **94**: 393–397.

Finstad, A. G., and Hein, C. L. 2012. Migrate or stay: terrestrial primary productivity and climate drive anadromy in Arctic char. Glob. Chang. Biol. **18**: 2487–2497. doi: 10.1111/j.1365-2486.2012.02717.x.

Finstad, B., Nilssen, K., and Arnesen, A. 1989a. Seasonal changes in sea-water tolerance of Arctic charr (*Salvelinus alpinus*). J. Comp. Physiol. B **159**(4): 371–378. doi: 10.1007/BF00692408.

Finstad, B., Nilssen, K. J., and Gulseth, O. A. 1989b. Sea-water tolerance in freshwater-resident arctic charr (*Salvelinus alpinus*). Comp. Biochem. Physiol. A **92**: 599–600. doi: 10.1016/0300-9629(89)90372-1.

Garvey, J. E., and Marschall, E. A. 2003. Understanding latitudinal trends in fish body size through models of optimal seasonal energy allocation. Can. J. Fish. Aquat. Sci.

60: 938–948. doi: 10.1139/f03-083.

Gross, M. R. 1997. Evolution of life history and migration in fish. Mem. Fac. Fish. Hokkaido Univ. **44**: 17–21.

Gross, M. R., Coleman, R. M., and McDowall, R. M. 1988. Aquatic productivity and the evolution of diadromous fish migration. Science **239**: 1291–3. doi: 10.1126/science.239.4845.1291.

Gulseth, O. A., and Nilssen, K. J. 2000. The brief period of spring migration, short marine residence, and high return rate of a northern Svalbard population of Arctic char. Trans. Am. Fish. Soc. **129**: 782–796. doi: 10.1577/1548-8659(2000)129<0782:TBPOSM>2.3.CO;2.

Halden, N. M., Babaluk, J. A., Campbell, J. L., and Teesdale, W. J. 1995. Scanning proton microprobe analysis of strontium in an arctic charr, *Salvelinus alpinus*, otolith: implications for the interpretation of anadromy. Environ. Biol. Fish. **43**: 333–339. doi: 10.1007/BF00001166.

Halden, N. M., Mejia, S. R., Babaluk, J. A., Reist, J. D., Kristofferson, A. H., Campbell, J. L., and Teesdale, W. J. 2000. Oscillatory zinc distribution in Arctic char (*Salvelinus alpinus*) otoliths: The result of biology or environment ? Fish. Res. **46**: 289–298.

Harwood, L. A., and Babaluk, J. A. 2014. Spawning, overwintering and summer feeding habitats used by anadromous Arctic Char (Salvelinus alpinus) of the Hornaday River, Northwest Territories, Canada. Arctic **67**(4): 449-461.

Holland, D. M., Thomas, R. H., de Young, B., Ribergaard, M. H., and Lyberth, B. 2008. Acceleration of Jakobshavn Isbrae triggered by warm subsurface ocean waters.

Nat. Geosci. **1**: 659–664. doi: 10.1038/ngeo316.

Hvidsten, N. A., and Hansen, L. P. 1988. Increased recapture rate of adult Atlantic salmon, *Salmo salar* L., stocked as smolts at high water discharge. J. Fish Biol. **32**: 153–154. doi: 10.1111/j.1095-8649.1988.tb05345.x.

Image-Pro® Plus, Ver. 6.3 2008, Media Cybernetics, Inc. Bethesda, MD

Jensen, A. J. 1994. Growth and age distribution of a river-dwelling and a lake-dwelling population of anadromous Arctic Char at the same latitude in Norway. Trans. Am. Fish. Soc. **123**: 370–376. doi: 10.1577/1548-8659(1994)123<0370:GAADOA>2.3.CO;2.

Jensen, A. J., Finstad, B., Fiske, P., Hvidsten, N. A., Rikardsen, A. H., and Saksgård, L. 2012. Timing of smolt migration in sympatric populations of Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and Arctic char (*Salvelinus alpinus*). Can. J. Fish. Aquat. Sci. **69**: 711–723. doi: 10.1139/f2012-005.

Jensen, J. L. A., and Rikardsen, A. H. 2008. Do northern riverine anadromous Arctic charr *Salvelinus alpinus* and sea trout *Salmo trutta* overwinter in estuarine and marine waters? J. Fish Biol. **73**: 1810–1818. doi: 10.1111/j.1095-8649.2008.02042.x.

Johnson, L. 1980. The arctic charr, *Salvelinus alpinus*. *In:* Charrs, Salmonid fishes of the genus *Salvelinus.* Balon, E. K.*,* (*ed.*) Dr. W. Junk Publ. The Hague, Netherland pp. 15–98.

Jonsson, I. R., and Antonsson, T. 2005. Emigration of age-1 Arctic Charr, *Salvelinus alpinus*, into a brackish lagoon. Environ. Biol. Fish. **74**: 195–200. doi: 10.1007/s10641-005-7693-0.

Jonsson, B., and Jonsson, N. 1993. Partial migration: niche shift versus sexual maturation in fishes. Rev. Fish Biol. Fisher. **3**: 348–365. doi: 10.1007/BF00043384.

Klemetsen, A., Amundsen, P. A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F., and Mortensen, E. 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecol. Freshw. Fish **12**: 1–59. doi: 10.1034/j.1600-0633.2003.00010.x.

Kristoffersen, K. 1994. The influence of physical watercourse parameters on the degree of anadromy in different lake populations of Arctic charr (*Salvelinus alpinus* (L.)) in northern Norway. Ecol. Freshw. Fish **3**: 80–91. doi: 10.1111/j.1600-0633.1994.tb00109.x.

Kristoffersen, K., Halvorsen, M., and Jørgensen, L. 1994. Influence of parr growth, lake morphology, and freshwater parasites on the degree of anadromy in different populations of Arctic Char (*Salvelinus alpinus*) in northern Norway. Can. J. Fish. Aquat. Sci. **51**: 1229–1246. doi: 10.1139/f94-123.

L'Abee-Lund, J. H., Jonsson, B., Jensen, A. J., Saettem, L. M., Tor, G., Johnsen, B. O., and Naesje, T. F. 1989. Latitudinal variation in life-history characteristics of sea-run migrant brown trout *Salmo trutta*. J. Anim. Ecol. **58**: 525–542.

Langeland, A., L'Abée-Lund, J. H., Jonsson, B., and Jonsson, N. 1991. Resource Partitioning and Niche Shift in Arctic Charr *Salvelinus alpinus* and Brown Trout *Salmo trutta*. J. Anim. Ecol. **60**: 895–912.

Loewen, T. N., Carriere B., Reist, J. D., Halden, N. M., and Anderson, W. G. (*in press*). Trace element and isotopes in biominerals: what they tell us about physiology and biochemical process in teleost and chondrosteir fish. Comp. Biochem. Physiol. A.

doi: 10.1016/j.cbpa.2016.06.017.

Loewen, T., Gillis, D., and Tallman, R. 2010. Maturation, growth and fecundity of Arctic charr, *Salvelinus alpinus* (L.), life-history variants co-existing in lake systems of Southern Baffin Island, Nunavut, Canada. Hydrobiologia **650**: 193–202. doi: 10.1007/s10750-010-0242-7.

Loewen, T. N., Gillis, D., and Tallman, R. F. 2009. Ecological niche specialization inferred from morphological variation and otolith strontium of Arctic charr *Salvelinus alpinus* L. found within open lake systems of southern Baffin Island, Nunavut, Canada. J. Fish Biol. **75**: 1473–1495. doi: 10.1111/j.1095-8649.2009.02394.x.

Ludlam, S. D. 1996. The comparative limnology of high arctic, coastal, meromictic lakes. J. Paleolimnol. **6**: 111–131. doi: 10.1007/BF00176931.

McDowall, R. M. 2008. Diadromy, history and ecology: a question of scale. Hydrobiologia **602**: 5–14. doi: 10.1007/s10750-008-9290-7.

Moore, J. S., Harris, L. N., Kessel, S. T., Bernatchez, L., Tallman, R. F., and Fisk, A. T. 2016. Preference for near-shore and estuarine habitats in anadromous Arctic char (*Salvelinus alpinus*) from the Canadian high Arctic (Victoria Island, NU) revealed by acoustic telemetry. Can. J. Fish. Aquat. Sci. doi: 10.1139/cjfas-2015-0436.

Moore, J. W., Yeakel, J. D., Peard, D., Lough, J., and Beere, M. 2014. Life-history diversity and its importance to population stability and persistence of a migratory fish: steelhead in two large North American watersheds. J. Anim. Ecol. **83**: 1035–1046. doi: 10.1111/1365-2656.12212.

Nilssen, K. J., and Gulseth, O. A. 1998. Summer seawater tolerance of small-sized Arctic charr, *Salvelinus alpinus*, on Svalbard. Polar Biology **20**: 95-98.

Nordeng, H. 1961. On the biology of char (*Salmo alpinus* L.) in Salangen, North Norway. 1. Age and spawing frequency determined from scales and otoliths. Nytt. Mag. Zool. **10**: 67-123.

Nordeng, H. 1983. Solution to the “Char problem” based on Arctic Char (*Salvelinus alpinus*) in Norway. Can. J. Fish. Aquat. Sci. **40**: 1372–1387. doi: 10.1139/f83-159.

Paton, C., Hellstrom, J., Paul, B., Woodhead, J., Hergt, J., 2011. Iolite: freeware for the visualisation and processing of mass spectrometric data. J. Anal. At. Spectrom. **26**(12), 2508-2518.

Phillis, C. C., Ostrach, D. J., Ingram, B. L., and Weber, P. K. 2011. Evaluating otolith Sr/Ca as a tool for reconstructing estuarine habitat use. **373**: 360–373. doi: 10.1139/F10-152.

Power, G. 2002. Charrs, glaciations and seasonal ice. Environ. Biol. Fish. **64**: 17-35.

Power, G., and Barton, D. R. 1987. Some effects of physiographic and biotic factors on the distribution of anadromous Arctic Char (*Salvelinus alpinus*) in Ungava Bay, Canada. Arctic **40**: 198–203.

Power, M., Dempson, J. B., Reist, J. D., Schwarz, C. J., and Power, G. 2005. Latitudinal variation in fecundity among Arctic charr populations in eastern North America. J. Fish Biol. **67**: 255–273.

Power, M., Reist, J. D., and Dempson, J. B. 2008. Chapter 14: Fish in high-latitude Arctic lakes. *In* Polar lakes and rivers: limnology of Arctic and Antarctic aquatic ecosystems. W. F. Vincent and J. Laybourn-Parry (ed.) Oxford Universtiy Press, New York pp. 249–267.

Pulido, F. 2011. Evolutionary genetics of partial migration – the threshold model of

migration revisited. Oikos **120**: 1776–1783. doi: 10.1111/j.1600-0706.2011.19844.x.

Quinn, T. P., and Myers, K. W. 2005. Anadromy and the marine migrations of Pacific salmon and trout: Rounsefell revisited. Rev. Fish Biol. Fisher. **14**: 421–442. doi: 10.1007/s11160-005-0802-5.

R Foundation for Statistical Computing, Ver. 3.1.1, 2014, Vienna, Austria

Reist, J. D., Power, M., and Dempson, J. B. 2013. Arctic charr (*Salvelinus alpinus*): a case study of the importance of understanding biodiversity and taxonomic issues in northern fishes. Biodiversity **14**: 45–56. doi: 10.1080/14888386.2012.725338.

Reist, J. D., Wrona, F. J., Prowse, T. D., Power, M., Dempson, J. B., King, J. R., and Beamish, R. J. 2006. An overview of effects of climate change on selected arctic freshwater and anadromous fishes. Ambio **35**: 381–7.

Rikardsen, A. H., Svenning, M. -A., and Klemetsen, A. 1997. The relationships between anadromy, sex ratio and parr growth of Arctic charr in a lake in North Norway. J. Fish Biol. **51**: 447–461. doi: 10.1111/j.1095-8649.1997.tb01503.x.

Rounsefell, G. A. 1958. Anadromy in North American Salmonidae. U.S. Fish Wild Serv. Fish. Bull. **58**: 171–185.

Smith, S. V, Bradley, R. S., and Abbott, M. B. 2004. A 300 year record of environmental change from Lake Tuborg, Ellesmere Island, Nunavut, Canada. J. Paleolimnol. **32**: 137–148. doi: 10.1023/B:JOPL.0000029431.23883.1c.

Spares, A. D., Dadswell, M. J., Dickinson, M. P., and Stokesbury, M. J. W. 2015a. A critical review of marine adaptability within the anadromous Salmoninae. Rev. Fish Biol. Fisher. **25**: 503–519. doi: 10.1007/s11160-015-9392-z.

Spares, A. D., Stokesbury, M. J. W., Dadswell, M. J., O'Dor, R. K., and Dick, T. A.

2015b. Residency and movement patterns of Arctic charr *Salvelinus alpinus* relative to major estuaries. J. Fish Biol. **86**: 1754–1780. doi: 10.1111/jfb.12683.

Spares, A. D., Stokesbury, M. J. W., O'Dor, R. K., and Dick, T. A. 2012. Temperature, salinity and prey availability shape the marine migration of Arctic char, *Salvelinus alpinus*, in a macrotidal estuary. Mar. Biol. **159**: 1633–1646. doi: 10.1007/s00227-012-1949-y.

Svenning, M. A., Smith-Nilsen, A., and Jobling, M. 1992. Sea water migration of Arctic char (*Salvelinus alpinus* L.): correlation between freshwater growth and seaward migration, based on back-calculation from otoliths. Nord. J. Freshw. Res. **67***:* 18–26.

Sylvester, P., and Raeside, R. (*Editors*). 2001. Laser ablation ICPMS in the earth sciences, principles and applications Volume 29. *In* Mineralogical Association of Canada, Shortcourse. Minerological Association of Canada, St. John's, Newfoundland.

Veillette, J., Muir, D. C. G., Antoniades, D., Small, J. M., Spencer, C., Loewen, T. N., Babaluk, J. A., Reist, J. D., and Vincent, W. F. 2012. Perfluorinated chemicals in meromictic lakes on the northern coast of Ellesmere Island, High Arctic Canada. Arctic **65**: 245–256.

Vincent, W. F., Fortier, D., Lévesque, E., Boulanger-Lapointe, N., Tremblay, B., Sarrazin, D., Antoniades, D., and Mueller, D. R. 2011. Extreme ecosystems and geosystems in the Canadian High Arctic: Ward Hunt Island and vicinity. Ecoscience **18**: 236-261. doi: 10.2980/18-3-3448.

Vøllestad, L. A., and L'Abée-Lund, J. H. 1994. Evolution of the life history of Arctic charr

Salvelinus alpinus. Evol. Ecol. **8**: 315–327. doi: 10.1007/BF01238281.

Walther, B. D., and Limburg, K. E. 2012. The use of otolith chemistry to characterize diadromous migrations. J. Fish Biol. **81**: 796–825. doi: 10.1111/j.1095-8649.2012.03371.x.

Chapter 6

Dissertation Conclusions and Synthesis

My thesis sought to provide new perspectives on elements and isotopes in calcium-based mineralised hard structures in selected northern fishes and to demonstrate the application to fisheries science. Answering three key questions was central to providing advancement of science in this field: 1. How are elements and isotopes available to fish, 2. Why are elements and isotopes present within fishes and, 3. In what ways can we apply this knowledge to fisheries research? These questions required an integrative and multidisciplinary approach to advance our understanding of biominerals. In the process of addressing these questions, the strength of microchemical analyses, the connection between calcium carbonate (otoliths) and calcium phosphate structures (fins, scales, bones), and the limitations on these were reviewed (**Chapter 2**). Specifically, the utility of microchemical approaches was addressed for inferring the retention period of strontium post-marking (**Chapter 3**), association of fish stocks with specific groundwater habitats for overwintering and reproduction (**Chapter 4**), and fish habitat selection over their lifespan (**Chapter 5**).

The importance of geology and surrounding landscapes underlies the availability of which elements and isotopes are incorporated into fish tissues. Elements and isotopes are naturally occurring in ambient environments due to the underlying geology and geochemical processes. Mineral solubility ultimately controls which elements become available to fish and other aquatic organisms that are consumed by fish. At the broad geographic scale, it was noted that the underlying granite terrane of the Eastern Arctic (Baffin Island, Labrador and Greenland) results in lower concentrations of environmental calcium in comparison to higher concentrations of environmental calcium

from predominantly carbonate terrane of the Western Canadian Arctic and Ellesmere Island (**Chapters 2, 4, 5**).

Elements and isotopes are variable both between and within fresh and marine waters. Within fresh waters such as rivers and lakes that form fish habitats, the movements of water through a landscape (hydrology) and variation in groundwater sources play large roles in element concentrations and isotopic ratios available in ambient environments. Due to their deep crustal origins, thermal groundwater sources, such as those from the Big Fish River, Yukon (**Chapter 4**), increased the concentrations of certain elements and modified isotopic ratios within the ambient aqueous environment. Specifically, strontium isotopes are relatively constant over ecological timescales due to the long half-life of ^{87}Rb ($4.92x10^{10}$ years), thus different $^{87}Sr/^{86}Sr$ ratios in otoliths reflected distinct watersheds and lithologies in the natural environments. In addition, the temperatures of groundwater springs (thermal and non-thermally sourced) modify saturation coefficients and kinetic competition of ions available for uptake to fish. These characteristics then allowed for delineating stocks of Dolly Varden char using otolith microchemistry based upon their associations with particular riverine habitats (**Chapter 4**). Within marine waters, sodium, chlorine, and calcium ions (amongst many others) exist in higher concentrations than they do in fresh water (**Chapters 2, 5**). Environmental calcium concentrations discussed in **Chapter 2** are key to understanding variability of trace elements and isotopes incorporated into biominerals.

Physiological processes within the fish are the key mechanisms determining presence and concentrations of elements and isotopes within fishes. Physiology plays

an important role in the regulation and balance of various elements for life. For fish, physiological regulation occurs predominantly at the gills and intestines. Essential life elements such as carbon, calcium, zinc, and magnesium have controlled pathways for uptake and excretion. These elements can exist in higher or lower concentrations than observed in ambient environments. Essential elements are taken up and excreted to balance internal homeostatic needs. Zinc for example is an important element that is used in over 300 enzyme reactions within fish and is thought to be associated with the protein matrix involved in otolith formation (**Chapter 2**). Elements such as calcium are taken up in large amounts and are known to play important roles in the formation of biomineralized calcium carbonate and calcium phosphate structures within fish (**Chapter 2**). Non-essential-to-life elements such as strontium, barium and lead have no directly controlled pathways for uptake and instead substitute for calcium due to similar ionic valences and radii. Their ability to replace calcium is related to fish calcium homeostasis to maintain life in relationship to environmental calcium (**Chapter 2**). Strontium is more readily taken up when fish are in low calcium environments as observed in the predominantly granite terranes of the Eastern Canadian Arctic, Labrador and Greenland. Since fish in this case are actively working to take up calcium from the environment, strontium is able to successfully compete at the gills for entry. When internal and external calcium environments are at or near equilibrium, calcium outcompetes strontium for entry at the gills. This was observed for fish surrounded by predominantly carbonate terranes of the Western Canadian Arctic and Ellesmere Island regions. In high calcium environments such as marine waters, calcium and strontium are taken up by fish through passive gradient channels in lieu of active uptake routes

and excesses are actively excreted in order to maintain calcium homeostasis. Strontium, due to higher environmental concentrations, is able to gain entry to a fish at higher levels. Based upon calcium homeostasis, using non-essential elements as predictors of life history events in relatively constant calcium environments such as marine waters is limited. Competition between non-essential elements, kinetics, temperature and pH all play a role in uptake of environmental calcium as mediated by fish homeostatic mechanisms. Experimentation to predict and demonstrate these element interactions in relation to calcium environments and fish physiology is needed to further advance this field of research.

Connectivity between calcium carbonates and calcium phosphates in the context of accretion of new materials is evident in the retention of high strontium in artificially marked fish (**Chapter 3**) and in isotopic fractionation studies (**Chapter 2**). Calcium phosphate hard structures have the ability through osteoclasts or "osteoclast-like cells" to break down already formed bony material and remobilise calcium and other divalent cations that mimic calcium. Fishes, however, lack the ability to remobilise calcium from calcium carbonate hard structures. Experiments on isotopic marking in bony structures demonstrated this remobilisation capacity through isotopic fractionation where previously accreted materials are dissolved and mixed with new materials for deposition (**Chapter 2**). This remobilisation capacity of bony tissue has the ability to maintain high levels of strontium internal to the fish and impact strontium concentrations measured in calcium carbonate structures (otoliths). Long strontium-signal retention (>328 days) in Greenland Halibut (*Reinhardtius hippoglossoides*) otoliths post-marking was observed in **Chapter 3**. Preliminary evidence suggested that new background strontium levels in

the fish and thus the otolith are likely to be established post-marking. Understanding strontium uptake and signal retention provides better interpretation in regards to interpreting otolith strontium profiles for anadromous species such as Arctic Char (*Salvelinus alpinus*) and Dolly Varden Char (*S. malma*) that make frequent habitat switches between fresh and marine environments within their lifetimes. Short time frames between fresh and marine water migrations suggest that strontium retention is likely occurring, thus potentially complicating interpretations regarding habitat usage. Further research into strontium uptake and signal retention in hard structures is warranted.

By connecting the fields of fish ecology, fish physiology, and geology, inferences using elements and isotopes in biominerals can be made and constrained regarding biological processes and biodiversity within fishes. The influence of groundwaters on water chemistry, especially for riverine species provided insights into overwintering, reproduction and juvenile rearing habitats within and among river systems. Further, fish use of identifiable river-specific groundwater habitats allowed for stock discrimination among various populations. These fish are susceptible to fisheries when they migrate seaward and have the potential to mix in marine and coastal environments. For Dolly Varden Char, otolith trace elements and strontium isotopic ratios provided high discriminatory power (94.44%) due to their strong associations to particular groundwater chemistries during reproduction and overwintering (**Chapter 4**). Future research will expand the unit stock analyses to closely associated river systems in Alaska, and examine mixed-stock fisheries located at Shingle Point, YK in combination with genetic techniques. Otolith microchemical approaches may also allow for the distinction of

small-scale habitat use within river systems, i.e., groundwater chemistry within main stem and tributary rivers exhibited different elemental and isotopic concentrations. In addition, the large variation between fresh and marine water calcium concentrations and intermediate mixing zones (estuaries) allows for strong inferences of fish life history and habitat use over their lives (**Chapter 5**). Arctic Char from the edges of their geographic extent were compared for ages- and sizes- at first migration, marine habitat selections, and skipped migrations. For High Arctic populations, a predominance of small sized, young-of-the-year was observed in coastal areas; while there was a strong tendency towards fresh-estuarine environments, exceptions for some lake systems are notable. For the southern population a delay in migratory behaviour inferred from older ages and larger sizes of migrants was observed. Moreover, results indicated strong preferences towards marine-estuarine environments for this population. Ease of access, measured as river outlet length from fresh to coastal regions, played a role in determining at what age the fish accessed coastal waters. Skipped migrations exhibited inverse-linear relationships with age- and size- at first migration. Future studies should examine Arctic Char migration biodemographics along a latitudinal gradient from High Arctic to southern-most populations. Including productivity, and additional measures such as river gradient for ease of access to marine environments, will provide a stronger understanding of Arctic Char migrations and strengthen predictions for responses of populations susceptible to productivity changes as a result of climate variability and change.

The new perspectives for element and isotope incorporation into biominerals resulting from the research documented in this thesis will provide integrative and

multidisciplinary advancements for future applications in fisheries science. Based upon these new directions, additional questions arise and warrant further investigation including the following: 1. What are the energetic costs for fish to maintain life in hypo- and hyper- calcemic environments, 2. If differences exist, what physiological adaptations must occur to accommodate a higher energy demand, and 3. How might this impact life histories and intraspecific diversity of fish species such as Arctic Char living in low productivity freshwater environments? Understanding these variations in physiological demand not only will advance our knowledge on element and isotope incorporation into biominerals but will also provide insights into understanding intraspecific biodiversity of species. In turn, such knowledge will facilitate understanding of needed management actions to respond to various anthropogenic stressors that affect northern fish populations.