Life History Reconstruction and Stock Identification of
Sockeye Salmon (*Oncorhynchus nerka*) Using Otolith Trace Element Chemistry

by

Zachary Luke Penney
B.Sc. Sheldon Jackson College, 2004

A Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

in the School of Earth and Ocean Sciences

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ABSTRACT

Recent advances in otolith microchemistry have established that trace element composition can be used to chemically reconstruct fish life history and serve as a stock identification tool. In modern fisheries practices, these two applications are especially pertinent to wild salmon populations, which are difficult to track over large spatial scales and nearly impossible to identify in mixed populations. This project has applied a novel method using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to anadromous sockeye salmon (*Oncorhynchus nerka*) otoliths from four separate watersheds in Sitka, Alaska. Spatial distributions of Li, Mg, Mn, Zn, Sr, and Ba were determined via continuous lateral ablation scans across the diameter of transversely sectioned sagittal otoliths. Time-series data generated from line scan analysis were used to chemically reconstruct sockeye life history, and examine elemental signatures in the core, freshwater, and marine growth regions of otoliths for stock identification purposes.

Chemical profiles of life history showed that Sr, Ba, and to a lesser degree Mg, reflected ambient chemistry, and were effective for tracking sockeye migration from

fresh to marine water. Manganese was also effective for determining migration to fresh and marine water; however, it is believed that diet more than ambient chemistry is the factor controlling uptake. Elements such as Zn and Li provided information related to fish physiology, such as growth and changes in osmoregulation during transitions from low to high salinity environments. Results also showed that several elements were either enriched or depleted in the core of sockeye otoliths. Maternal investments and spatial differences in crystal structure are believed to significantly affect element uptake in otoliths during incubation and early development. Elemental signatures in the otolith core may therefore be inaccurate as an indicator of stock origin. This problem was investigated by isolating core, freshwater, and marine signatures and evaluating individually their ability to correctly classify sockeye otoliths to their natal watersheds using step-wise discriminant function analysis. This demonstrated that freshwater signatures provided the greatest accuracy (91%) for stock ID. Core signatures, which have been used in past stock ID studies, showed poor classification results (68%) for sockeye salmon otoliths. Trace element signatures from the marine growth regions of sockeye otoliths displayed the poorest classification accuracy (52.5%) of the three growth regions. Thus, freshwater signatures are the most effective tool for identifying the origin of wild salmon, even when they far removed from their natal watersheds.

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Acknowledgments

I must start by thanking my supervisor, Kevin Telmer, for all of his time, patience, insight, and friendship, during my time at UVIC. Kevin, you have been an excellent advisor, and have always provided me with intellectual support whenever I needed it. For that I am extremely grateful!

I must also thank the United States Fish and Wildlife Service (USFS) for helping fund this project and my masters thesis. Additionally, I thank the Sitka Tribe of Alaska (STA), and all those who have been affiliated with this project for their support and patience while I finished my degree. These people include: Doug Dobyns, Heather Woody, Jack Lorrigan, Jessica Perkins, and Helen Dangel. Indeed, without the grant writing skills of Doug Dobyns, the funding for this project would have never existed, and neither would have my Masters Thesis. Furthermore, I must thank Doug for his continued interest in this project even after his retirement from STA.

I am indebted to Jody Spence for all of his analytical help with operating the LA-ICP-MS. Even after I ablated the laser cell window, Jody continued to let me operate the laser unaccompanied, and for that I am grateful. Jody I also appreciate your willingness to always give me 5-10 minutes to discuss my project, and lend me feedback whenever I asked for it. Thank you.

I also thank Adrian Clarke for his help with statistics and the intricacies of SPSS, and Mike Sanborn for his extensive knowledge on otolith preparation. Also, thank you to all of my roommates, who have came and went in the 3561 Quadra house: Dennis, Marc, Marina, Tony, Carroll, Iulia, and Sabine, thank you all for your support and friendship, I will miss the good times.

Finally, I am most grateful to Shanna Knight for here love, support, and proof-reading during my thesis. Without you, I may have never finished.

Chapter 1

Introduction

1.1 Age and Growth

The determination of age and growth in teleost fishes has been a continuous endeavor in fisheries science for well over a century. Estimates of age and growth provide information pertinent to fish life history such as growth rates, maturation age, mortality rate, and lifespan (Cailliet et al., 1996). Significant efforts and funding are devoted annually to the estimation of fish age in modern fisheries practices (Campana and Thorrold, 2001). The bulk of age and growth estimates are derived from various calcareous structures that include scales, fin rays, vertebra, and otoliths. Like tree rings, these structures deposit successive increments of growth that can be related to yearly age. These growth increments are commonly known as annuli. The term annuli does not specifically refer to a single annual mark, but distinguishable zones indicative of slow and fast growth periods (Murphy and Willis, 1996). By counting these seasonally distinct zones (annulus) it is possible to estimate the yearly age of fish. However, notably, this is not always a clear case as some species such as Pacific eulachon are

notoriously difficult to age using standard otolith approaches (Clarke et al., in press, Hay & McCarter, 2000).

Of the numerous structures available for aging, most estimates are primarily obtained from scales and sagittal otoliths (Campana and Thorrold, 2001). Both structures provide well-defined annuli for age determination but have distinct advantages and disadvantages. Scales are advantageous because they can be non-lethally collected at large sample sizes. Sagittal otoliths are internally situated in the inner ear and require that fish be sacrificed for extraction. Otolith extraction is much more tedious than scale collection, and limited to a single sagittal pair per fish (Murphy and Willis, 1996). Clearly, scale collection is far easier on both the sampler and the fish making the practice of otolith collection appear needless. However, though scales are adequate as aging tools they do not compare to the continuous chronological record ingrained within otoliths. Skeletal structures, such as scales, may experience greater ranges of aging error than otoliths, especially in long-lived fish (Campana and Thorrold, 2001). Daily growth is not resolvable in scales, vertebra, and fin rays, making otoliths the most precise and chronologically accurate structures for determining fish age (Secor et al., 1996). This characteristic is largely the result of the otoliths seclusion within the highly regulated environment of the inner ear.

1.2 Inner Ear Anatomy and Otolith Function

Otoliths, commonly referred to as ear stones, are found in the inner ears of all teleost fishes. Otoliths are paired accretions of the calcium carbonate polymorph aragonite, located in the fluid-filled labyrinth of the inner ear. The inner ear labyrinth is a

complex system of semicircular canals and vestibules that is bilaterally symmetrical and divided into two distinct regions; the pars superior and pars inferior (Tavolga et al., 1981). The two regions contain three separate vestibules that house three corresponding pairs of otoliths. The pars superior contains the utriculus vestibule and the lapilli otolith pair (Cailliet et al., 1996). The pars inferior contains the remaining two vestibules that consist of the sacculus and lagenus, which contain the sagittal and asteriscus otolith pairs, respectively (Secor et al., 1991). Of the three otolith pairs, the sagittal otoliths are the largest and most frequently used otolith in fisheries science. All further mention of the term "otolith[s]" in this thesis refers to the sagittae, unless otherwise mentioned. A schematic of the teleost inner ear anatomy is shown in figure 1.1.

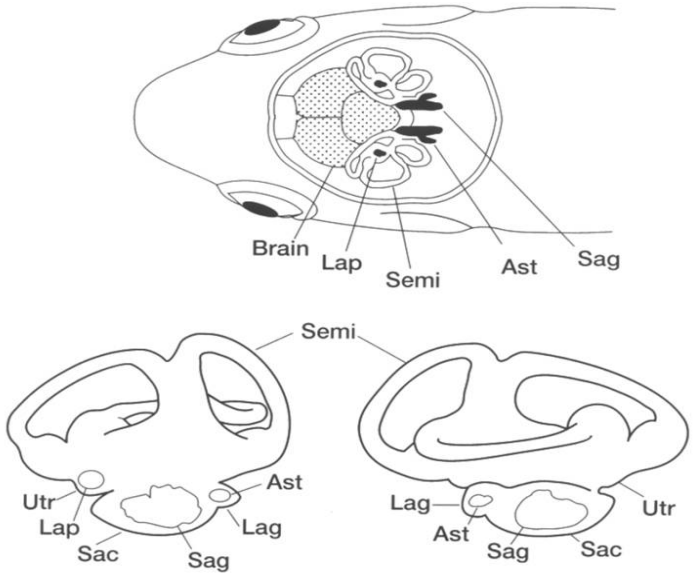

Figure 1.1: Schematic diagram of the teleost inner ear anatomy and otolith position. *Top*, Dorsal view of the cranial cavity showing the position of the brain and otolith pairs. *Bottom*, Medial view of the inner ear labyrinth and location of vestibules and otoliths. Ast, asteriscus; Lag, lagena; Lap, lapillus; Sac, sacculus; Sag, sagitta; Semi, semicircular canal; Utr, utriculus. (Adapted from Campana 2004).

The primary function of otoliths is hearing and balance. Each otolith pair responds to sound vibrations and gravitational forces within their fluid-filled vestibules (Cailliet et al., 1996). Although the exact sensory mechanisms of the inner ear are not entirely certain, otolith pairs either provide specific auditory or vestibular functions (relating to equilibrium). Auditory function is mainly attributed to the sagittae and asterisci otoliths. Sound stimuli are detected during the displacement of water particles encountered as slight vibrations or oscillations to the fish (Moyle and Cech, 1996). Sound vibrations cause the subsequent displacement of the otoliths in their fluid-filled vestibules and stimulate the sensory epithelium or macula. The macula is composed of ciliary bundles of hair-like cells, which when stimulated send a neural response to the auditory portion of the brain (Moyle and Cech, 1996). This process provides teleosts with a sense of hearing. Equilibrium is mainly attributed to the lapilli otolith pair. The maintenance of equilibrium is achieved by similar process used for sound stimuli. During physical movements or changes in orientation, the fluid-filled labyrinth stimulates the ciliary bundles, which transfer the stimuli to the brain. Stimuli are interpreted in the medulla providing fish with a sense of balance during basic motor functions, such as, acceleration, deceleration, or diving (Moyle and Cech, 1996).

1.3 Otolith Composition and Growth

Otolith growth is continuous throughout the entire life of teleost fish. Otoliths are comprised of approximately 95% aragonite, 4% protein matrix, and less than 1% non organic trace impurities (Campana, 2004). Growth is accreted via the precipitation of calcium (Ca^{2+}) and bicarbonate (HCO_3^-) ions from the surrounding endolymphatic media (Murayama et al., 2001). Although deposition is continual, it is not symmetric.

Calcification rates vary with fish age and the pH and temperature in the endolymph with daily additions often ranging from 1 to 20 µm in width (Thorrold et al., 1997, and Campana, 1999). Despite irregularities in daily growth, the continual deposition of aragonite to the otolith provides well-defined increments at yearly, seasonal, and daily time scales (Figure 1.2).

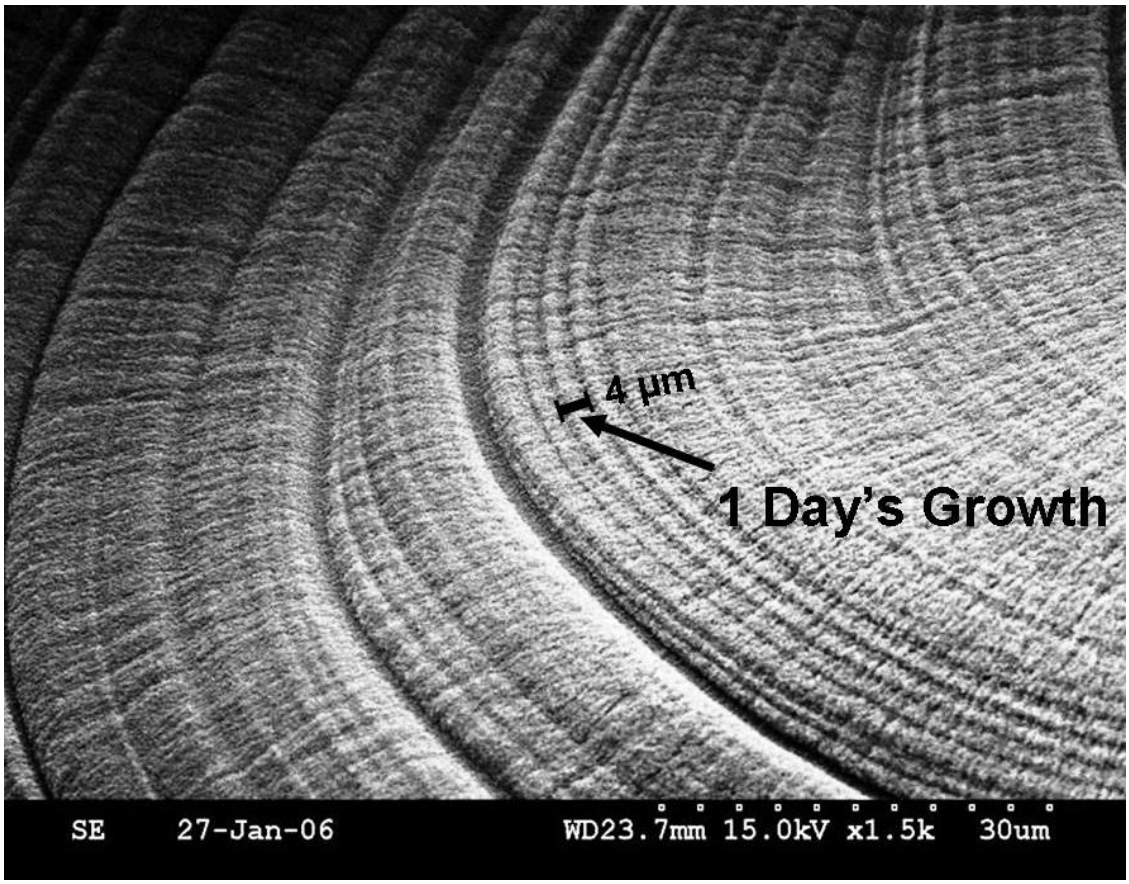

Figure 1.2. Scanning electron microscope image of daily growth from an acid-etched transversely sectioned sockeye otolith taken in the Electron Microscopy Lab at the University of Victoria.

Unlike scales, fin rays, or vertebrae, otoliths have no function in the teleost skeletal system, and are therefore inert to many physiological and metabolic processes (Campana, 1999). In contrast, skeletal structures deposit annuli in proportion to the

somatic growth of the fish, which can be adversely affected by metabolism (Campana and Thorrold, 2001). This intimate relationship to somatic growth makes skeletal structures subject to re-absorption, alteration, and low growth periods. Furthermore, somatic growth gradually decreases as fish increase in age causing later annuli to be smaller and therefore more difficult to analyze. Otoliths growth is less affected by somatic growth, and continually accretes layers of aragonite during the entire life of the fish. As a result, a complete unaltered record of growth is preserved and available for analysis.

1.4 Otolith Microchemistry and Elemental Uptake

In addition to better aging precision versus other calcified structures, otoliths have the ability to record physical and chemical information from the environment. Otoliths share this characteristic with several other well-known biogenic calcitic and aragonitic proxies commonly used in oceanography, such as corals and bivalve shells. Corals and bivalve shell have proven effective in paleoceanography studies for reconstructing physicochemical parameters such as temperature, salinity, and past oceanic production rates (Wyndham et al., 2004, and Carrol et al., 2006). Surprisingly, it is the small percentage of minor and trace elements in otoliths, corals, and bivalve shells that provide the greatest amount of environmental information. Many minor and trace elements are often incorporated into shell, coral, and otolith $CaCO_3$ in proportion to their abundance in the environment providing a record of past ambient chemistry.

However, otoliths are different from coral and bivalve shell in that they are not in contact with the external environment, and are not therefore directly influenced by ambient water chemistry. Elemental uptake in otoliths is under stricter biological and

chemical regulation than that of corals and shells because, trace elements in otolith aragonite must first pass through a series of biological pathways and barriers before reaching the endolymph and being deposited (Kalish, 1989). In general, ions from the environment that end up in otoliths must pass through four interfaces: (1) water, (2) blood plasma, (3) endolymph, and (4) otolith (figure 1.3).

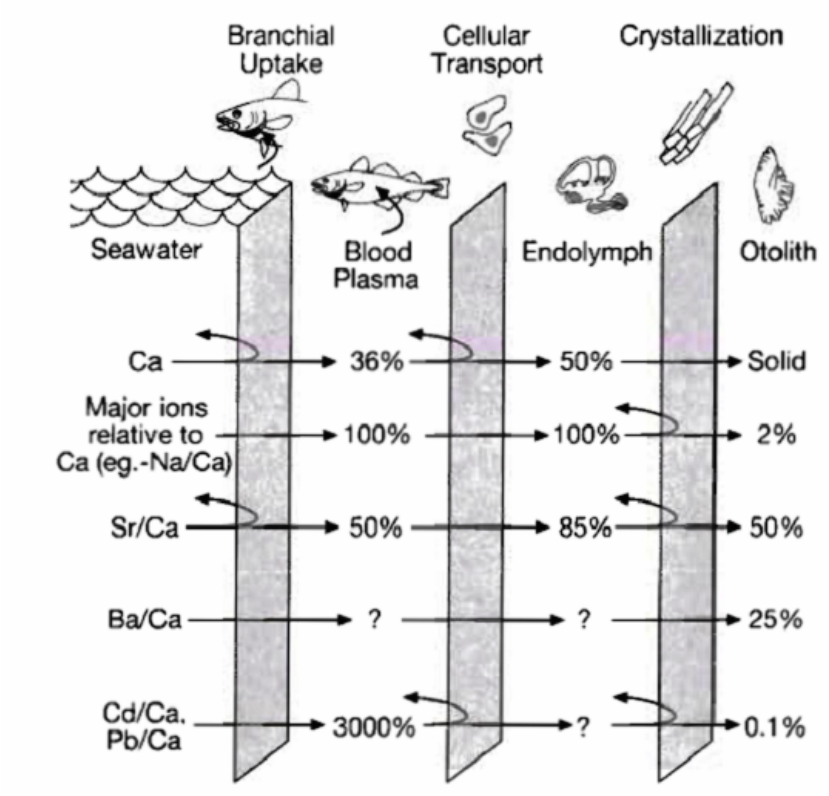

Figure 1.3 Diagram of the route of trace impurities to the otolith (Taken from Campana, 1999).

The incorporation of trace impurities into otolith aragonite is a complex process, and varies between marine, estuarine, and freshwater species. Element discrimination begins at either the water-gill or water-intestinal interface. In freshwater fish the primary route of ions into the bloodstream occurs through the gill-water interface during

respiration. In marine and estuarine fishes the more important pathway is through the intestine via continual consumption of saltwater to maintain osmotic homeostasis (Campana, 1999). In the blood plasma, ion regulation between marine and freshwater fish is similar. The ions may be co-precipitated along with the daily increments of aragonite to become permanently fixed in the otolith.

During calcification, otoliths incorporate minor and trace elements into the aragonite crystal lattice by three possible pathways: (1) Elements with a similar ionic radius to Ca substitute for Ca in the aragonite; (2) ions are trapped within the interstitial spaces of the crystal matrix; or (3) elements are associated with the organic protein matrix (Dove and Kingsford, 1998, Volk et al. 2000, and Miller et al., 2006). Ions captured via the latter two processes are usually lower in abundance than the first and also may not be fixed permanently. Though numerous ions are often present within the endolymphatic fluid only a select few are relatively abundant and easily detected in otolith aragonite. These are dominantly elements from group I and II in the periodic table (Li, K, Mg, Ca, Sr, and Ba) and some transition metals such as manganese (Mn) and zinc (Zn).

To date over 31 elements have been detected in teleost species throughout the world's oceans, lakes, and streams (Campana, 1999). The uptake mechanism for each element is unique and complex, and likely varies between marine, fresh, estuarine, and anadromous fishes. More often than not, many of the elements that have been documented are too infrequent to be reliable proxies. However, several elements have been shown to consistently occur in otolith aragonite as potential indicators of environmental conditions. Strontium (Sr), barium (Ba), magnesium (Mg), Zn, lead (Pb),

Mn, copper (Cu), and iron (Fe) are among the most notable minor and trace elements commonly studied (Edmonds et al. 1989, Campana, 1999, Kraus and Secor, 2004, and Miller et al., 2006). Strontium has received the greatest attention due to its relatively high concentration and its ability to substitute for Ca.

Strontium has a similar valence and radius to Ca, and due to its relative abundance in seawater and chemical behavior is less susceptible to metabolic discrimination than other elements (Kraus and Secor, 2004). Experiments involving Sr enriched water have demonstrated that Sr incorporation is largely a function of ambient chemistry. Schroder et al. (1995) determined that Chum salmon (*Oncorhynchus keta*) immersed in strontium chloride ($SrCl_2$) solutions had significantly higher Sr in their otoliths than control fish in natural conditions. Similar results were supported by Secor et al. (1995), who demonstrated that juvenile striped bass (*Morone saxatilis*) experienced changes in otolith Sr in response to differing salinity gradients. And Telmer et al. (2006) successfully detected Sr peaks in otoliths of juvenile sockeye salmon (*Oncorhynchus nerka*) that had been chemically tagged by short term immersion (6 hours) in an elevated Sr solution. Such findings provide a basis for reconstructing past habitat environments and migrations using otolith microchemistry. Combined with the complete chronological growth record, trace element chemistry provides a novel method and unique information for addressing life history questions in fisheries.

1.5 Purpose of Study

The objectives of this thesis are to utilize otolith trace element chemistry to address two specific fisheries questions involving sockeye salmon (*Oncorhynchus nerka*) from Sitka, Alaska. The first paper, presented in chapter 2, aims to reconstruct the

anadromous life history of sockeye. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) of sockeye otoliths was used to examine migration by utilizing the record of Sr, Ba, Mg, Zn, Mn, and Li preserved in the otoliths. Transitions between fresh, estuarine, and marine water leave distinctive signals in otoliths across the chronological growth sequence. This makes it possible to chemically identify several periods of salmon life history including incubation, emergence, freshwater residence, outmigration, and oceanic residence. Additionally, otolith microchemistry may provide further information related to the distribution and uptake of elements between fresh and marine water.

The second paper, presented in chapter 3, intends to discriminate between four separate sockeye stocks in Sitka, Alaska. LA-ICP-MS analysis is used to characterize natural elemental signatures in the core, freshwater, and marine growth regions of sockeye otoliths returning to Klag, Redoubt, Tumakof, and Salmon Lakes. Each growth region was evaluated using step-wise discriminant function analysis for its ability to classify otoliths to their natal lakes. By identifying natural elemental tags an effective and cost-efficient method may be developed for discriminating between stocks of wild fish.

Chapter 2

Reconstruction of Sockeye Life History and Migration Using High Resolution Time-Series Otolith Chemistry

2.1 Introduction

In the past, fisheries research has primarily used various tagging or marking experiments to follow the migration patterns of wild fish. The ability to track the spatial and temporal movement of fish is important for understanding life history aspects such as habitat use, site fidelity, spawning periods, and gross population movements, all of which are important to fisheries management (Bolle et al., 2005). In modern fisheries, the dominant method for tracking fish migration is through the use of mark/recapture studies. Mark/recapture experiments typically involve physically marking (e.g. fin clip, operculum punch) or inserting either external or internal tags (e.g. T-Bar Anchor Tags, Coded-Wire Tags) into fish as a means for identifying individuals within a given aquatic system. Generally, fish are captured, marked or tagged, released, and eventually recaptured to provide information related to migration. Though effective, mark/recapture studies can be time consuming, expensive, and often yield low recapture rates. More importantly, mark/recapture techniques only provide migratory information related to the date and position of release and recapture. Therefore, mark/recapture methods usually

provide only a fragmented representation of migratory history with periods between recapture being unknown. These informational gaps have been addressed in more recent years with the development of technologically advanced tags that record biotelemetry, such as PIT (passive integrated transmitters) and DST tags (data storage tags).

Advances in electronic tags have proven effective for tracking fish migration at greater temporal and spatial periods than conventional mark/recapture studies. Biotelemetry tags emit a continuous electronic signal, providing researchers with an effective method for locating and following fish movement. Various biotelemetry transmitters have also been fitted with electronic sensors capable of relaying information regarding the fish's environment (e.g. water temperature), and physiology (e.g. heart rate) (Murphy and Willis, 1996). Unfortunately, though biotelemetry is extremely effective at tracking individual migration, it is also expensive, labor-intensive, and cannot be applied during the larval stages of fish life history (Thorrold et al., 1997 and Ruttenberg et al.,. 2005). Perhaps the biggest drawback to biotelemetry is that it is often limited to the more localized movements of specific individuals (Secor et al., 1995). Assumptions based upon the movement of a limited number of tagged fish can be misleading, especially when interpreting the migratory dynamics of a larger population. This is further complicated in fish species not confined to a single aquatic environment. Fish that migrate great distances and traverse numerous systems can be difficult or impossible to track using biotelemetry and conventional tagging methods. To overcome these limitations, recent experiments involving otolith microchemistry have demonstrated that migration can be reconstructed in teleost fish using the trace element composition of otoliths. Unlike physically applied marks and man-made tags, teleost otoliths can

function as both “natural tags” and passive recorders of the environment (Campana, 1999).

Currently, migration reconstruction using otolith microchemistry is dominated by two forms of analysis: (1) solution-based analysis and (2) beam-based analysis. Both methods utilize the chronological growth sequence of the otolith, but greatly differ in their methods of preparation and limits of detection. Solution-based analysis is superior in sensitivity, providing 2-3 orders of magnitude lower detection limits than beam-based approaches (Sanborn and Telmer, 2003). In the past, the use of solution-based analysis has involved bulk analysis of whole otoliths for stock identification. For investigations addressing migration, bulk analysis methods are not feasible. To attain any information related to migration, specific otolith growth regions must be temporally resolved. This is typically accomplished using a process known as micro-milling. Micro-milling removes small quantities of otolith material using fine diameter drills at specific growth regions on the otolith. Generally, multiple regions of the growth record are “milled” for chemical comparisons. Though effective, the resolution of most micro-milling procedures is no better than seasonal (Campana, 1999). Most drills used cannot precisely extract otolith material at defined monthly, weekly, or daily growth increments. To competently investigate fish migration at fine temporal and spatial scales, beam-based analysis is superior. Microprobes can provide a quick and effective method for analyzing otolith microchemistry at temporal scales, which are usually unattainable using micro-milling. As well, detection limits of beam based methods for many of the most useful minor and trace elements are sufficient for precise and continuous detection.

Beam-based analysis utilizes high precision microprobes to sample specific regions of the otolith record. The beam diameters of most modern microprobes range from 2-30 µm, well within the limits of daily growth deposition in most otoliths (Campana, 1999). Currently, three primary types of microprobe exist, which include electron dispersive, energy dispersive, and laser microprobes. Of these, the use of laser ablation inductively coupled plasma mass spectrometry is the superior method in the majority of cases.

Despite the excellent temporal and spatial resolution of modern microprobes, many assays use spot analysis. Spot analysis analyzes discrete spots across the otolith growth record similar to micro-milling. Although, effective for analyzing specific sites on the otolith, spot analysis can be time consuming, and does not take full advantage of the otoliths chronological growth sequence (Sanborn and Telmer, 2003). To fully and accurately reconstruct chemical migration in the otolith, a scan across the entire growth sequence is optimal (Figure 2.1). Here, we utilize full laser ablation line-scans across sockeye (*Oncorhynchus nerka*) otoliths to investigate their life history.

Sockeye salmon follow an anadromous lifecycle, migrating between fresh and marine water to spawn. Over the course of their lifecycle sockeye make only one marine migration, dying shortly after spawning in their natal freshwater systems. The static and predictable nature of their lifecycle allows easy integration with otolith microchemistry. The goal of the following experiment is to characterize the migration of sockeye salmon from four watersheds in Sitka, Alaska. Continuous lateral ablation scans (henceforth termed line scans) were performed across the entire growth axis of transversely sectioned otoliths using laser ablation inductively coupled mass spectrometry (LA-ICP-MS).

Concentrations of strontium (Sr), barium (Ba), magnesium (Mg), lithium (Li), zinc (Zn), and manganese (Mn) were quantified across otoliths to produce complete chemical profiles of life history. The results are used for three outcomes. First, chemical profiles are used to track anadromous migration between fresh and marine water, while also detecting more subtle habitat shifts. Second, ontogenetic changes during sockeye growth and development are investigated. Third, the data is used to assess differing behavior and uptake of Sr, Ba, Mg, Li, Zn, and Mn into aragonite between marine and fresh waters.

In the following section a brief overview of sockeye life history in Southeast Alaska is presented. This section is followed by basic descriptions of the aqueous distributions of Sr, Ba, Mg, Li, Zn, and Mn in marine and freshwaters. By reviewing this information an effective framework will be built for the later interpretation of sockeye otolith trace element composition.

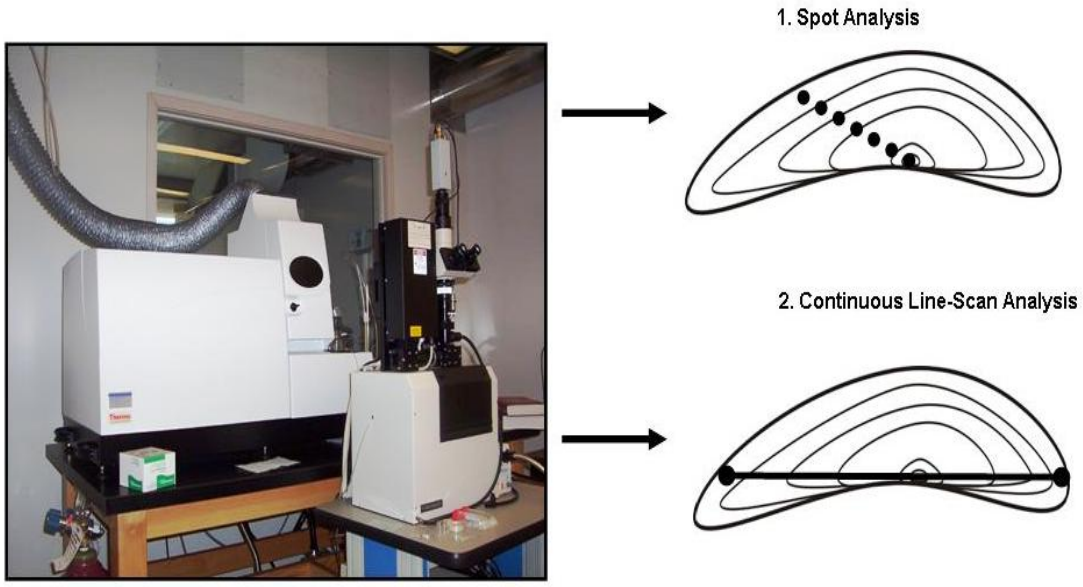

Figure 2.1 *Left*- Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) at the School of Earth and Ocean Sciences, University of Victoria. *Right*- Two beam-based analysis strategies used in otolith microchemistry: 1. Spot Analysis and 2. Continuous Line-Scan Analysis.

2.2 Sockeye Life History

Of the six anadromous salmon species endemic to the west coast of North America, sockeye display the greatest variety in life history patterns (Groot and Margolis, 1991). Anadromy refers to the migration from freshwater to the marine environment, and in the case of Pacific salmon, the eventual return to freshwater to spawn. In general, Pacific salmon anadromy can be described in six basic stages: (1) incubation (2) emergence, (3) freshwater rearing, (4) smolting/outmigration, (5) marine migration, (6) spawning/death (Figure 2.2). Sockeye are no exception, and make only one marine migration during their life cycle. However, though the sockeye lifecycle is similar to all anadromous *Oncorhynchus* sp., dramatic differences of life history exists between populations. Most anadromous populations are adapted to rear in freshwater lakes for at least one year, but some stocks are known to migrate to sea as fry (Thorpe, 1994). One region in particular, where life strategies can substantially differ between sockeye populations occurs along the Alexander Archipelago chain in Southeast Alaska. Here many sockeye stocks follow “lake-type” life histories (i.e. residing in a lake for >1 year), but “sea-type” forms (i.e. emigrate as fry) are known to occur (Thorpe, 1994, and Halupka, et al. 2000).

2.2.1 Spawning

In Southeast Alaska, sockeye return from the ocean to their natal watersheds in early June through August. Spawning typically occurs shortly after re-entry into freshwater from August until early October. Most adults return as 4 and 5 year old fish, but “jack and jill” sockeye are not uncommon in southeast Alaska (Halupka et al., 2000).

The terms “jack and jill” in fisheries refers to sockeye that have spent only one year in the marine environment before returning to spawn.

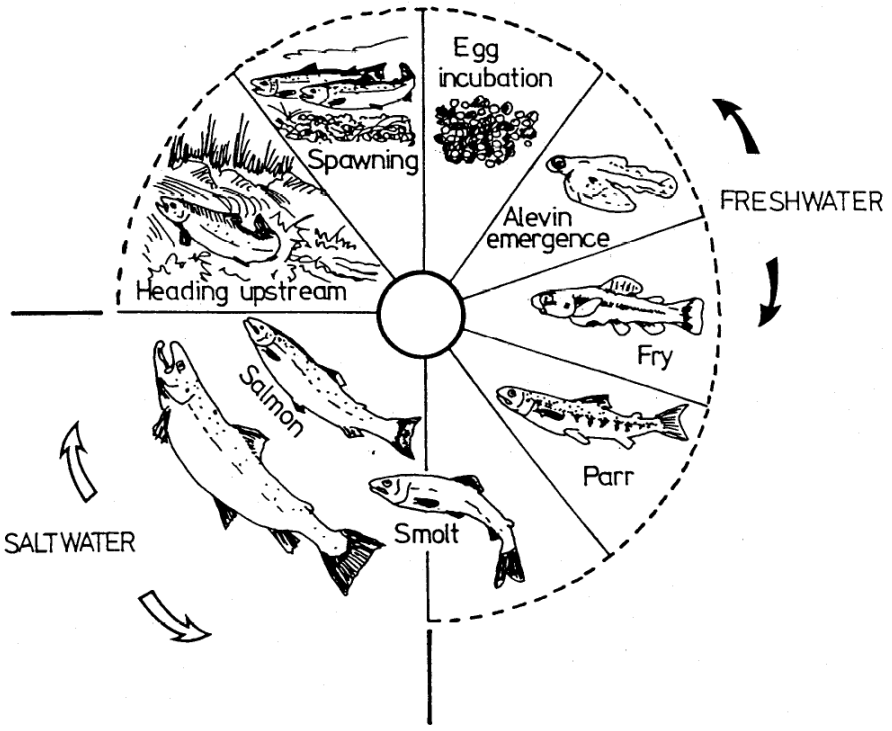

Figure 2.2. General diagram of the anadromous Pacific salmon lifecycle (Taken from Thorpe, 1994).

Spawning takes place along the lake shores and in inlet or outlet streams of most sockeye lakes, especially in areas of upwelling. It has been documented that sockeye avoid lake bottom areas without upwelling, and spawning is heaviest in the areas with the strongest upwelling (Groot and Margolis, 1991). During spawning, female sockeye dig depressions into the substrate using rapid undulations of their caudal fin, and release ~ 2,000 to 2,400 eggs, which are simultaneously fertilized by an accompanying male or males (Groot and Margolis, 1991). The nests are then covered with the dislodged substrate and guarded from competing females and predators until death. The exact

timing of death between males and females has been known to vary, but most sockeye expire within one week after spawning occurs. The decay of post-spawn sockeye is an important flux of marine derived nutrients to lake and riparian ecosystems in Southeast Alaska (Naiman et al., 2002). Marine derived nutrients greatly increase productivity, and subsequently enhance the survival of their progeny.

2.2.2 Incubation and Early Development

Sockeye eggs incubate for approximately 12 weeks after fertilization; which is the longest incubation period of all *Oncorhynchus sp.* (Groot and Margolis, 1991). Embryos hatch into the alevins stage in the late winter months, but alevins will stay submerged in the nest until spring. Hatch rate from the egg to the alevin stage is a function of temperature, which will ultimately vary between lakes and nest sites. In the substrate alevins are sustained via a yolk sac ventrally attached to the abdomen. Alevins gradually absorb this sac as they further develop. Emergence from the nest will coincide with the complete absorption of the sac, which typically occurs in March and April (Groot and Margolis, 1991). At this stage sockeye are now termed fry.

Following emergence, fry disperse to protected rearing areas that provide horizontal and vertical cover from predators where they begin to actively feed on external food sources. Migration between fry hatched from lake shores, inlet, and outlet streams is difficult to track, and likely varies between lakes. However, in general most southeast Alaskan sockeye eventually disperse to the limnetic zones of their lakes (Groot and Margolis, 1991). Unlike salmon species such as Coho and Chinook, which are highly territorial, sockeye group into large schools during freshwater residence. In the limnetic

zone sockeye fry feed on zooplankton, such as ostracods, cladocerans, and copepods, and insects for 1-3 years before smolting (Halupka et al., 2000).

2.2.3 Smolting and Outmigration

The outmigration timing of sockeye smolts in southeast Alaska is not well studied, but existing data indicates outmigration is highly variable between stocks (Halupka et al. 2000). In general, sockeye begin smolting from late April to early June in Southeast Alaska (Halupka et al., 2000). The increase in temperature and photoperiod cause several morphological, physiological, and behavioral changes to occur in sockeye. These changes include activity (predator avoidance), coloration (development of a silvery sheen), shape (slimmer and stream-lined), and tolerance of seawater (osmoregulation) before transitioning to marine water (Groot & Margolis, 1991).

Similar to their behavior in the lakes, sockeye smolts emigrate in large schools to estuaries. Here sockeye reside near shore for several weeks to months feeding and acclimating to the differing salinities before migration to the open ocean (Groot and Margolis, 1991). Thorpe (1994) reported that sockeye smolts in the Situk estuary in Southeast Alaska had completely vacated from the estuary after 3-4 months. It is likely that sockeye near Sitka are similar and migrate to coastal and pelagic areas in the fall following outmigration.

2.2.4 Marine Migration

Southeast sockeye spend 2-3 years in the ocean feeding, before returning to their natal systems to spawn (Halupka et al., 2000). The exact location and distribution of stocks during this period is largely unknown. The factors controlling oceanic migration

are all likely interrelated and include seasonal differences in temperature, salinity, and food availability, as well as the age of the fish (Groot & Margolis, 1991). However, it is food that likely has the largest influence on sockeye movements in marine water. The primary forage base for sockeye in the marine phase includes zooplankton, such as copepods, euphasids, ostracods, crustacean larvae, and occasionally larval fish and squid (Halupka et al., 2000). In search of these specific food items sockeye are likely to follow nutrient-rich and productive waters.

After residing and feeding in the Gulf of Alaska for 2-3 years, Sitka sockeye begin to migrate towards the Southeast Alaska coast. The cues causing sockeye to return to their natal systems to spawn are largely unknown, but factors such as sea surface temperature (SST), freshwater discharge from coastal areas, marine currents, and photoperiod may all have an effect (Hodgson et al., 2006). Sitka sockeye stocks return in May through August to spawn, but may reside within estuaries in close proximity to the outlet of their natal streams before re-entering freshwater. In most systems re-entry corresponds to high water flow periods following precipitation events. Stock escapement data from Klag Lake (2001-2006), Salmon Lake (2001-2006), and Tumakof Lake (2002) show that the highest numbers of sockeye counted past weirs coincided with increased water levels in the outlet streams (Lorrigan et, al. 2003, Conitz et al., 2005, and Tydingco et al., 2006).

2.3 Aqueous Element Distributions

Migration reconstructions of anadromous salmonids using otolith trace element chemistry should be more distinct than species inhabiting chemically homogenous environments, such as solely marine or freshwater fish. This is due to the large physical,

chemical, and biological differences between fresh, estuarine, and marine waters. Salmon traverse all three habitat types during their life history, and this is often discernible in otolith microchemistry.

Elements have specific distributions in the three aquatic environments, and will either exhibit conservative or non-conservative behavior. Conservative behavior is common for the "major" dissolved ions in seawater (Cl^-, Li^+, Na^+, Mg^{++}, Ca^{++}, K^+, and HCO^-_3), which occur at high concentrations and have long replacement times relative to oceanic mixing (inputs = outputs) (Berner and Berner, 1987). In contrast, minor and trace elements, such as Fe, Mn, Ba, and Zn typically behave non-conservatively and are rapidly depleted by biological activity in surface waters (Berner and Berner, 1987). As a result non-conservative elements experience greater temporal and spatial variability than do conservative elements, and they occur generally at lesser concentrations. However, it is important to understand that elements may alternate between conservative and non-conservative behaviors depending on their environment. Barium for example, behaves relatively conservative in freshwater, but non-conservatively in estuaries and marine water. Thus, physical, chemical, and biological influences greatly affect the distribution of elements between fresh, estuarine, and marine water. In the following sections the natural distribution of Sr, Ba, Mg, Li, Zn, and Mn in natural waters and their relation to otolith aragonite is presented.

2.3.1 Alkaline and Alkali Earth Metals

The use of the group 2A alkaline Earth metals has dominated the majority of investigations in otolith microchemistry. This is because Ca is a major constituent in otoliths (40% wt.) and alkaline ions of similar valence and ionic radius can substitute for

Ca in aragonite, as can be seen in figure 2.3 (Radtke et al., 1997, and Volk et al., 2000). The most notable of these ions are Sr, Ba, and Mg, all of which are water soluble and abundant in aqueous environments.

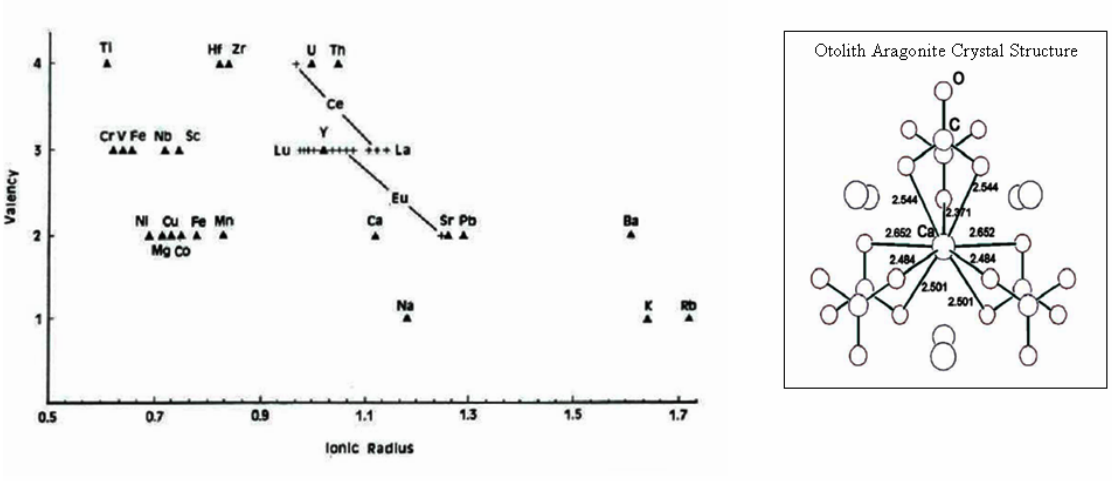

Figure 2.3 Relationship of valency and ionic radius of elements to Ca, and possible substitution in otolith aragonite (Adapted from Lipin et. al. 1989 and Pattanaik, 2004).

As well, these elements vary across environments and so make good tracers. This has been demonstrated in numerous experiments (Shroder et al., 1995; Secor et al., 1995; and Telmer et al., 2006) where increased levels of ambient Sr have positively correlated to increased uptake in otoliths. However, the uptake of alkaline metals is not simply a function of concentration but rather depends on the overall water chemistry. Kraus and Secor (2004), noted that uptake reflects the ratio of a given alkaline ion to calcium (Sr:Ca, Ba:Ca, and Mg:Ca) in the environment. Elements with a similar valence and radius to Ca may act as competitive inhibitors, as all compete for the same uptake pathway. This must be considered when attempting to relate otolith microchemistry to a specific aquatic environment. In Table 2.1, alkaline and alkali otolith values are presented for marine, estuarine, and freshwater fish. These values were compiled by

Campana (1999) from otolith research prior to 1999. Unfortunately, many of the mean values presented are from extremely small sample sizes (e.g. n = 1), and are not necessarily representative.

Element	Marine Species			Freshwater Species			Estuarine Species		
	Mean	SE	N	Mean	SE	n	Mean	SE	n
Ca	380176.0	4981.0	17.0	407232.0	18232.0	2.0	*	*	*
Sr	2137.0	127.0	43.0	698.0	111.0	17.0	1937.0	70.0	4.0
Ba	3.7	0.6	14.0	11.0	2.8	3.0	8.2	2.7	3.0
Mg	27.0	5.8	15.0	32.0	9.2	3.0	33.0	7.5	4.0
Li	1.0	0.3	6.0	0.1	*	1.0	1.0	*	1.0

Table 2.1. Summary of published otolith composition ($\mu g\ g^{-1}$) for alkali and alkaline metals in marine, fresh, and estuarine environments (Campana, 1999). SE- standard error, n-sample size.

2.3.2 Strontium

Strontium is the most frequently used element in otolith microchemistry. Numerous studies have used Sr as a natural tag for stock identification (Kennedy et al., 2000, Veinott and Porter, 2005, and Sohn et al. 2005), mass marking tool (Schroder et al., 1995, and Telmer et al., 2006), and proxy of past environmental conditions (Secor et al., 1995, Volk et al., 2000, Kraus and Secor, 2004; and Elsdon and Gillanders, 2004). It is Sr's ability to reflect ambient chemical conditions that is fundamental for all of its applications. This characteristic has been especially effective for reconstructing migration in anadromous species such as salmon due to the dramatically differing concentration of Sr between fresh, estuarine, and marine waters.

In the marine environment, Sr is a major dissolved component of seawater that follows a conservative behavior. The mean concentration of Sr in the world's oceans is ~8 parts per million (ppm) and does not significantly vary over time (Wadleigh et al.,

1985). Due to Sr's steady state in seawater, characterizing migration in pelagic marine fish using otoliths can be difficult. However, successful migration reconstructions have been achieved in pelagic species that periodically move to coastal and estuarine areas to rear, feed, or spawn. Coastal and estuarine environments often display lower Sr values than the open ocean due to greater freshwater influence. Thorrold et al. (1997) used Sr:Ca ratios to detect the early life history migration of juvenile Atlantic Croaker (*Micropogonias undulates*) in two estuaries on the east coast of the United States. Atlantic Croaker are a demersal species that hatch in offshore spawning areas, but are advected inshore by wind-driven currents. Juvenile Croaker feed and rear in estuarine and coastal areas until they are large enough to migrate offshore. Results indicated that Sr:Ca ratios decreased as larval Croaker were transported from pelagic spawning sites to the lower salinity rearing and feeding areas. The ability to detect such small Sr:Ca differences in fish that inhabit relatively stable chemical environments demonstrate the strong utility of Sr in otolith microchemistry. It only gets easier in anadromous species that travel between fresh, estuarine, and marine environments.

In freshwater, Sr is one order of magnitude lower than the pelagic ocean with a global average of 0.057-0.070 ppm (Wadleigh et al., 1985). Due to its significantly lower value in freshwater, anadromous fish otoliths often illustrate distinct shifts in Sr during transitions between marine and freshwater. For example, Radtke et al. (1997) demonstrated that Sr:Ca otolith values in dianadromous Arctic Char (*Salvelinus alpinus*) in northern Labrador coincided with migration events to and from marine water. Using wavelength dispersive microprobe analysis, it was found that most char migrated to marine water two years after birth, and that char then made seasonal migrations to marine

water over the remainder of their lives. Migration events were evidenced by Sr:Ca peaks in the opaque regions of the polished otoliths, which denote fast growth periods (summer) in temperate and Arctic species. Similar studies have also been directed towards anadromous Pacific salmon, which in comparison to Arctic Char follow a relatively "straightforward" migration history. Pacific salmon species, excluding some steelhead populations, make only one migration to and from the ocean during their life history. Thus, salmon otoliths should exhibit a single defined Sr:Ca increase during outmigration from natal lakes to the Gulf of Alaska. Figure 2.4 displays an idealized model of Sr composition across the life history of an anadromous sockeye salmon. Fresh, estuarine, and marine values were determined using the mean otolith Sr values in table 2.1.

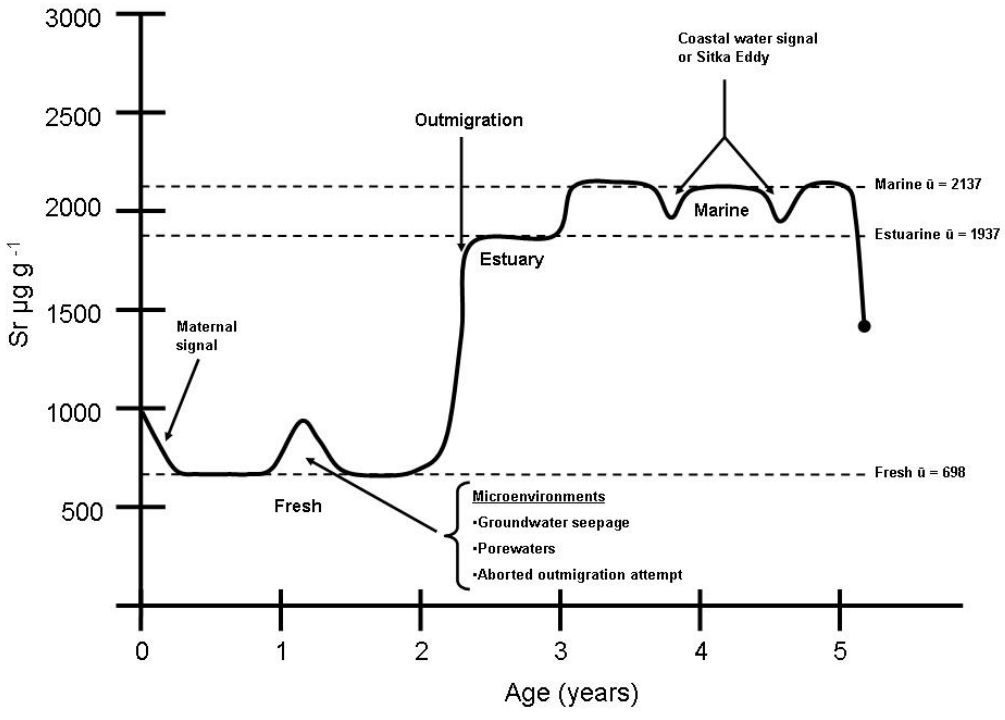

Figure 2.4. Idealized strontium profile of an anadromous sockeye salmon.

2.3.3 Barium

Until recently, otolith microchemistry has largely focused on Sr as the fundamental trace element proxy of ambient chemistry. However, though Sr is indeed a valid environmental proxy, it can be ineffective in migration reconstruction for fish that inhabit regions with relatively static Sr concentrations (Thorrold et al., 1997). In such cases, Ba may be an effective alternative.

Like Sr, Ba ions are divalent cations that can substitute for Ca in otolith aragonite (Elsdon et al., 2005). However, Ba behaves non-conservatively, having a nutrient-like profile in estuarine and marine environments (Colbert and McManus, 2005). Because it is removed from the oceans biologically, Ba has a much shorter residence time (1×10^4 years) than Sr. Therefore its concentrations in the ocean are more variable than Sr. Generally, dissolved Ba is removed by biological uptake in marine surface waters (Li and Chan, 1979). As organisms die and sink, Ba is removed and much of this crystallizes as biogenic barite ($BaSO_4$) with increasing depth (Jacquet et al., 2006, Klump et al., 2001, and Deshairs, 1979). The formation and sedimentation of biogenic barite enriches marine sediments with Ba, where some of it is returned to the deep waters through mineralization (Jacquet et al., 2006). Therefore, Ba is typically depleted in surface waters and relatively enriched in deep waters. During periods of upwelling, as is common along the northwest coast of North America in summer when winds change from southeast to northwest causing offshore Ekman transport, surface waters can periodically become enriched in Ba (Feely and Massoth, 1981). This in turn can import an annual peak in Ba concentrations in otoliths for coastal species, such as eulachon (Clarke et al., 2007).

Aside from upwelling, estuaries are the largest contributors of Ba to marine surface waters, and play an integral role in transforming Ba to a biologically available form. The dominant source of Ba to estuaries is via fluvial input adhered to suspended particulate matter (Guay and Falkner, 1998). During mixing between fresh and marine water, Ba is desorbed from particulate matter through ion exchange with the abundant marine ions such as Na^{+}, Ca^{++}, K^{+}, and Mg^{++} (Hanor & Chan, 1977). As a result, Ba is liberated from particulate matter, leaving estuaries with increased levels of dissolved Ba. However, though dissolved Ba can be high in estuaries it is prone to seasonal fluctuations due to the constantly changing physical, chemical, and biological conditions. Increased or decreased freshwater inputs, temperature, tidal shifts, and biological production all affect Ba in the estuarine environment (Hanor and Chan, 1977, Li and Chan, 1979, Hamer et al., 2006).

In contrast to marine and estuarine systems, freshwater Ba is more conservative. The principle source of Ba in fluvial and lacustrine systems is the weathering of carbonate and other rocks (Jarvie et al., 2000). Few reports have reported the Ba:Ca content in freshwater fish otoliths. In table 2.1 Campana's (1999) compilation of alkali and alkaline values indicates that Ba is on average greater in freshwater fish otoliths than estuarine or marine species. During this experiment the behavior of Ba in otoliths across fresh, estuarine, and marine environments is examined. If Ba is indeed a valid proxy for ambient chemistry it should also be possible to predict its distribution over the course of sockeye salmon life history similar to that of Sr. In figure 2.5 an idealized model of Ba composition across the otolith of an anadromous sockeye salmon is presented. Fresh,

estuarine, and marine values were determined using the mean otolith Ba values in table 2.1.

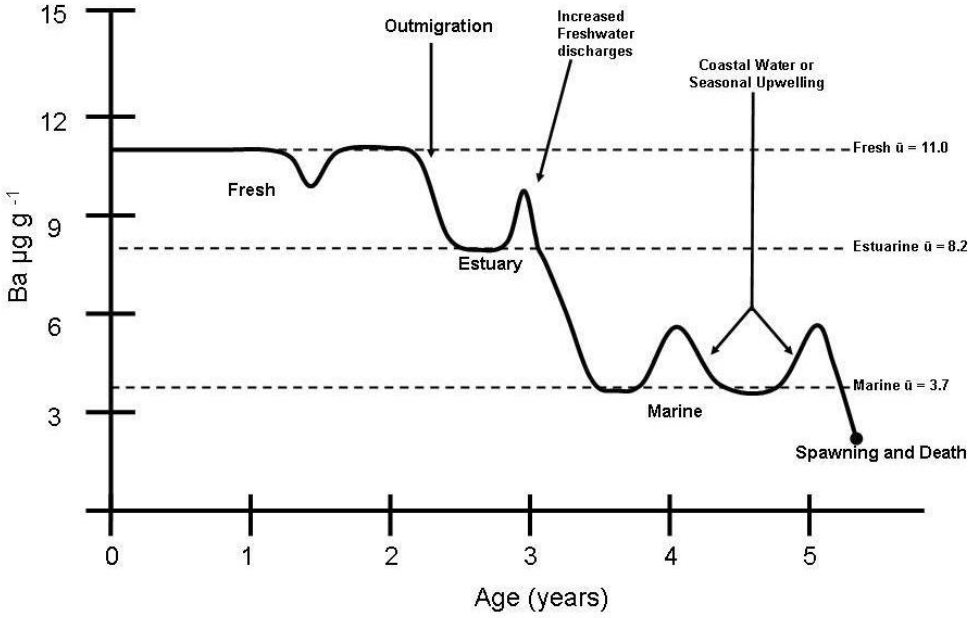

Figure 2.5. Idealized barium profile of an anadromous sockeye salmon.

2.3.4 Magnesium

Like Sr and Ba, Mg is also commonly present in otolith aragonite. As in other alkaline metals, Mg is similar to Ca, and readily substitutes in biogenic carbonates ($Mg_xCa_yCO_3$; where x + y = 1 and x is typically less than 0.01). However, compared to Sr or Ba, the incorporation and uptake of Mg in fish otoliths is complex.

In the marine environment, Mg is a major constituent of seawater, ranking third only behind sodium (Na^+) and Chloride (Cl^-). Due to Mg's high abundance, it follows a conservative behavior in marine water with ambient concentrations remaining stable (Berner and Berner, 1987). In freshwater, Mg concentrations are at least two orders of magnitude lower than in the marine environment (Berner and Berner, 1987). However,

though much lower in freshwater, Mg is still a major dissolved ion. Indeed, fluvial transport is the largest source of Mg to the oceans, occurring primarily through the chemical weathering of Mg-rich silicate minerals, such as amphiboles, pyroxenes, olivine, biotite, and dolomite (Berner and Berner, 1987). Strangely, despite Mg's substantially different distribution between marine and freshwater, otolith chemistry has not been found to reflect environmental differences.

Considering the similarity between Mg and Sr in natural waters it might be expected that Mg would follow similar patterns in fish otoliths. However, this is largely not case. In several studies, no significant differences in Mg have been found to occur between marine, estuarine, or freshwater fish otoliths. Campana, (1999), summarized average Mg values for otoliths representative of marine, fresh, and estuarine species from several past otolith publications, and found that Mg between fresh (32 $\mu g/g^{-1}$), estuarine (33 $\mu g/g^{-1}$) and marine fish (27 $\mu g/g^{-1}$) did not appear to significantly vary. In fact, many freshwater otoliths often contain greater Mg values than marine fish. There are both physiological and thermodynamic explanations for this. It is likely that Mg is under greater physiological control than Sr or Ba due to the relatively greater need of Mg in living organisms (Mader, 2001), and it has long been known that the concentration of Mg in blood is relatively invariant (Campana, 1999). As well, the substitution of Mg into otolith aragonite may not occur in direct proportion to its ambient concentration due to competition by other elements. For example, in fresh waters where ambient Sr is typically low, more precipitation sites may be available for Mg. In such a scenario, Mg in the endolymphatic fluid may be strongly regulated and relatively constant but variations in the other elements in the same fluid may cause Mg to become relatively

enriched or depleted, in turn leading to Mg variations in otolith aragonite that do not correspond to ambient chemistry.

2.3.5 Lithium

Although rarely studied in otolith microchemistry, Li is commonly detected in teleost otoliths. Lithium is a group 1A alkali earth metal that shares a similar mode of inclusion into otolith aragonite as Sr and Ba (Campana, 1999). However, compared to alkaline metals, Li normally occurs at much lower concentrations (< 1.0 $\mu g/g^{-1}$). Unlike ions such as Mg, Li is not an biologically essential element and so is probably less regulated in the blood plasma, and therefore more likely to vary in otoliths in proportion to ambient chemistry. Because of Li's low concentration, detection by ICP-MS has only recently become simple due to the "cool plasma innovation" (Kosler et al., 2001). As a result very little attention has been devoted to Li in otolith microchemistry. .

Lithium is a highly soluble constituent of seawater and it therefore behaves conservatively (Hawthorne and James, 2006). Like other conservative elements, Li concentrations are relatively constant in open marine water, but unlike most dissolved ions transported to the ocean by riverine input, lithium is largely derived from hydrothermal reactions (Hall and Chan, 2004). As a result, lithium values are much lower in freshwater and mix conservatively during transport to estuarine areas (Stoffyn-Egli and Mackenzie, 1984). Although slight, the otolith values between fresh and marine fish do reflect the chemical difference between environments. In table 2.1, the summarized Li otolith values for marine (1.0 $\mu g/g^{-1}$) and estuarine (1.0 $\mu g/g^{-1}$) are relatively higher than freshwater (0.1 $\mu g/g^{-1}$) species. However, at this point Li's role on

otolith chemistry is not much more sophisticated than indicating a shift between fresh and marine water. This may change with improved detection and additional research.

2.3.6 Transition Metals

In addition to the alkali and alkaline elements, various transition metals have also proven effective for life history reconstruction in otolith microchemistry. However, the factors influencing transition metal uptake are not as well understood and their distribution in the natural environment is also more complicated than the group I and II elements due to their lower abundance, lower solubility, and their sensitivity to oxygen concentrations (redox sensitivity). Some elements, such as Zn and Mn are also essential nutrients for the health and growth of organisms, and they are therefore under greater physiological regulation than most of the alkali and alkaline elements. In table 2.2, otolith values of Zn and Mn from otoliths of marine, estuarine, and freshwater species are presented.

Element	Marine Species			Freshwater Species			Estuarine Species		
	Mean	SE	n	Mean	SE	n	Mean	SE	N
Zn	15.6	5.8	15	45.8	18.3	5	9.1	1.1	2
Mn	9.6	5.8	9	11.1	7.5	3	8.8	7.3	2

Table 2.2 Summary of published otolith composition ($\mu g\ g^{-1}$) for zinc and manganese in marine, fresh, and estuarine environments (Campana, 1999). SE- standard error, n- sample size.

Even more confounding is that transition metals can be incorporated in aragonite by three possible ways. First, elements such as Mn, which share a similar ionic radius to Ca, can substitute in aragonite (Miller et al., 2006). Second, elements that have a smaller ionic radius than Ca may occupy the interstitial spaces and crystal defects in the

aragonite. Third, elements such as Zn are thought to be dominantly associated with the 3-4% protein matrix (Miller et al., 2006). Despite the three possible inclusion pathways, most transition metals can still be related to a specific life history phenomenon.

2.3.7 Zinc

Zinc is an essential micronutrient in the growth, reproduction, and immune function of teleost fish (Miller et al.,2006). Fish acquire Zn via branchial uptake and diet, but it is diet that constitutes the majority of Zn in fish. Willis and Sunda (1984), estimated that 78-82% of total Zn accumulation in *Leistomus xanthurus* and *Gambusia affinis* was represented by food intake. Thus, it is likely that Zn is not an accurate proxy of ambient chemistry; rather, Zn is more likely reflective of physiological aspects, such as growth.

In marine and estuarine environments, Zn is non-conservative and follows a nutrient-like profile. Much like Ba, Zn is rapidly depleted from surface waters by biological production and precipitated in organic debris. As a result, Zn distribution in estuarine and marine waters is depleted in surface waters with concentrations gradually increasing with depth (Bruland, 1979). On average, freshwater systems contain higher concentrations of Zn than either estuarine or marine environments. This difference has also been expressed in fish otoliths from the three environments. In table 2.2, it can be observed that marine (15.6 ug/g^{-1}) and estuarine (9.1 $\mu g/g^{-1}$) Zn values are much lower than freshwater (45.8 $\mu g/g^{-1}$) values. However work by Halden et al. (2000), using scanning electron microprobe analysis showed that Zn concentrations in anadromous Artic Char otoliths persisted even after the onset of anadromy. Char otoliths contained

distinctive oscillations of Zn across the annular sequence, which displayed defined peaks during fast growth periods in seawater. These results indicate that fish growth, not ambient chemistry, may be the leading factor controlling Zn uptake into the otolith. We will be able to address this question further via examination of the Zn composition in the fresh and marine growth regions of sockeye otoliths.

2.3.8 Manganese

In addition to Zn**,** Mn is another transition metal that has received interest as an indicator of environmental conditions. However, in contrast to Zn, Mn may actually be less regulated by physiology and more representative of ambient chemistry (Campana, 1999). Manganese has an identical valence (2+) to Ca, as well as a similar ionic radius. This similarity may allow Mn to substitute for Ca in otolith aragonite and be used to indicate past environmental chemistry. Dove et al. (1996) found that Mn composition significantly differed between the otoliths of *Parma microlepis* and *Achoerodus viridus* along temperate reefs. Whole otoliths were digested and analyzed using ICP-MS. Results revealed that *A. viridus* incorporated more Mn than *P microlepis*. This result likely corresponded to *A. viridus* broader range of migration over the course of its life history compared to *P microlepis*. Though perhaps effective in some cases at denoting bulk Mn differences in the otoliths of the two species, Mn may be at least effective for discriminating chemical variation at smaller time scales-providing that its ambient distribution is well documented. Elsdon and Gillanders (2006) noted that Mn varied significantly in estuaries over short temporal time scales of weeks to days. This can be problematic for reconstructing the migration of fish which inhabit highly variable environments, but may also present opportunities for high resolution reconstructions. For

species migrating between significantly differing chemical environments, Mn may also be highly useful. In such cases as the anadromous migration of salmon, Mn may act as a powerful chemical tracer.

In most natural waters Mn also follows a nutrient-like profile, and is non-conservative at low and high salinities (Colbert and McManus, 2005). In many respects, Mn follows the same marine distribution, as Ba and Zn. As a micronutrient Mn is normally depleted from surface waters with concentrations generally increasing with depth. However, in estuaries Mn may behave either conservatively or non-conservatively (Colbert and McManus, 2005). The hydrodynamic and physicochemical mechanisms contributing to Mn behavior in estuaries are diverse and complex, with most processes like redox recycling being beyond the scope of this overview.

In freshwater, Mn is generally conservative and follows a similar distribution to Ba. This distribution appears to also be expressed in fish otoliths. In table 2.2, Mn values are relatively higher in freshwater (11.1 $\mu g/g^{-1}$) otoliths than species inhabiting estuarine (8.8 $\mu g/g^{-1}$) or marine (9.6 $\mu g/g^{-1}$) environments. However, it should be noted that in freshwater Mn tends to occur more abundantly in regions of low oxygen (Davison, 1993). During diagensis of organic matter, Mn is the first common element to be reduced and remobilized when oxygen is consumed. As a result, Mn often becomes enriched near bottom waters. During periods of re-oxidization Mn precipitates onto suspended particles, as well the surfaces of rocks, aquatic plants, and other large debris, such as fallen trees. This distribution may be possible to detect in sockeye otoliths, especially during early development periods when fish are intimately associated with the substrate and protective cover.

2.4 Sampling Sites and Methods

2.4.1 Sampling Sites

Sockeye were sampled from four separate lakes located in Sitka, Alaska. Sitka is situated along the Alexander Archipelago in Southeast Alaska, and is composed of three main islands, including Baranof, Chichagof, and Kruzof. Otoliths and water samples were collected from Tumakof Lake (56° 22.24 N' minutes; 134° W), Redoubt Lake (56° 53 minutes N', 135° W), Salmon Lake (56° 58 minutes N'; 135° W), and Klag Lake (57° 38.5 minutes N'; 136° W). Sitka is located in the Tongass temperate rainforest receiving a mean average of 2180 millimeters of precipitation per year with the largest accumulations occurring during the fall (figure 2.6). Brief descriptions for each lake system, as well as age composition data acquired in past stock assessment studies are given in the following paragraphs.

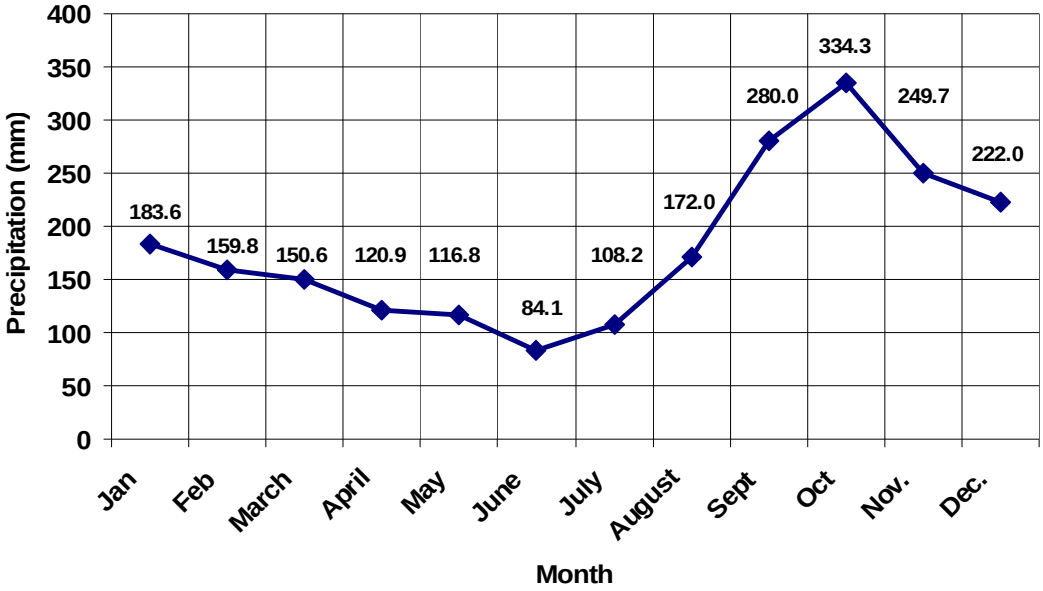

Figure 2.6. Average Annual Precipitation in Sitka, Alaska (1949-2006) sampled at Japonski Island. (Data collected from the Western Data Climate Center).

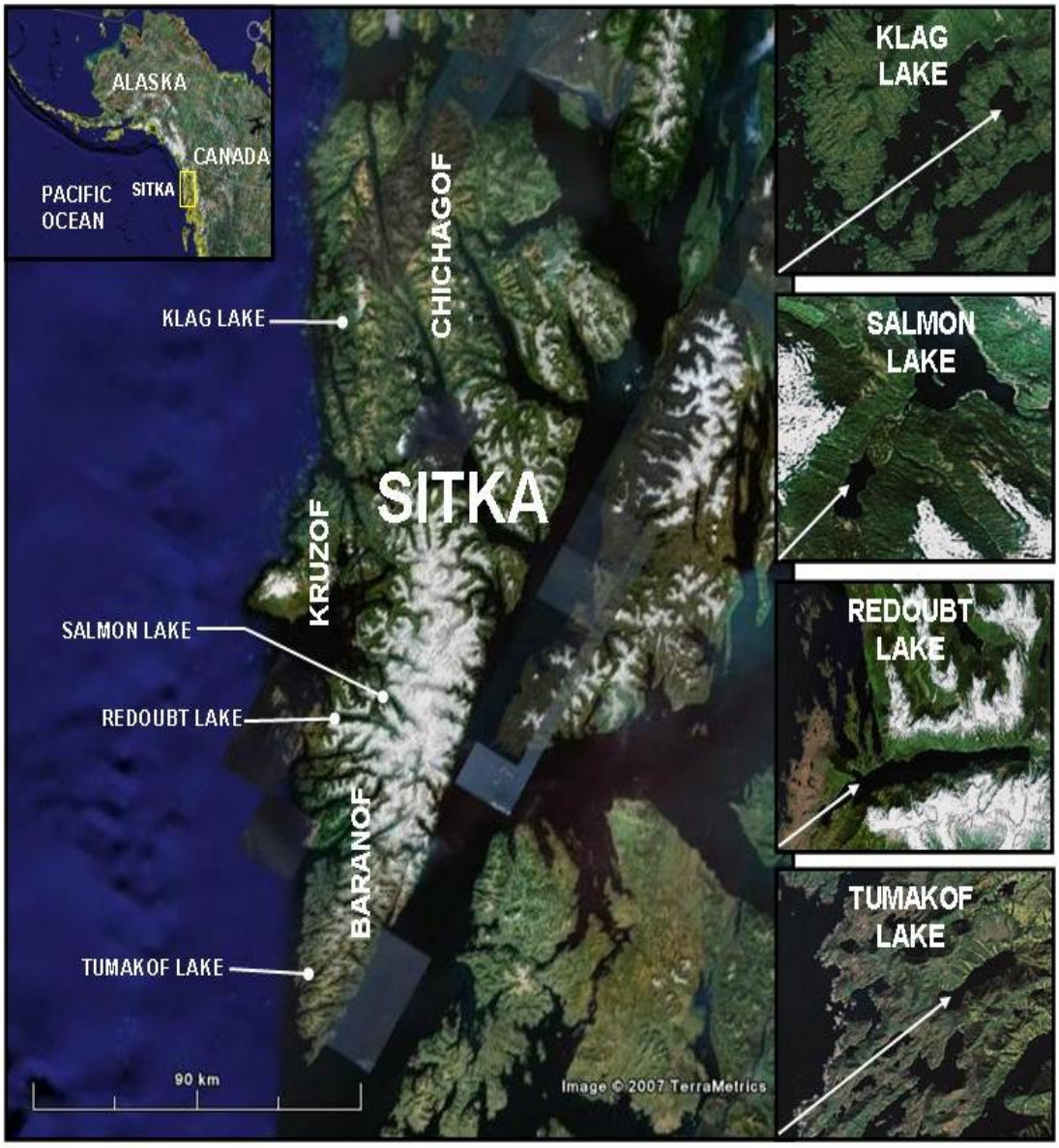

Figure 2.7.Map of Sitka, Alaska and sample sites.

Klag Lake

Klag Bay is located on the west coast of Chichagof Island, and is the most northern of all the sockeye systems sampled for this experiment. Klag or Kleix' was originally under the ownership of Chookaneidi clan of the Sitka Tlingit people, and was regarded as one of the largest sockeye producers in Southeast Alaska (Goldschmidt et al., 1998). Today Klag Bay is still an important subsistence resource to the residents of Sitka, especially during low escapements in systems close to Sitka, such as Redoubt and Necker Bay. Klag Lake receives drainage from approximately 7 square kilometers (km) of sparsely wooded low hills, muskeg, and numerous small shallow ponds (Conitz et al., 2005). Klag Lake is 12 meters (m) above sea level with a surface area of 0.83 km^2, and a maximum depth of 43 m. The lake drains into a single outlet stream at the south end of the lake, which eventually empties into the east side of Klag Bay. The majority of the spawning is believed to occur in the one inlet stream draining into Klag Lake. Interestingly, the inlet stream does not provide "text book" spawning conditions. The spawning area consists of large cobble and immovable bedrock in which spawners simply broadcast their eggs into crevices and spaces between the substrate (Conitz et al., 2005). This may have an affect on the early development history of Klag Bay sockeye, and may be evident in otolith microchemistry.

The primary forage of Klag Lake sockeye is suspected to be larger, slow-moving cladocerans (*Daphnia* sp.) (Conitz et al., 2005). This assumption was based upon limnological sampling of zooplankton on Klag Lake, which found higher abundances of smaller cladoceran species (*Bosmina*) in comparison Daphnia. Aside from sockeye, Klag Lake also supports populations of coho (*O. kisutch*), pink (*O. gorbuscha*), chum (*O.*

keta), steelhead salmon (*O. mykiss*), cutthroat trout (*O. clarki*), Dolly Varden char (*Salvelinus malma*), sculpin (*Cottus species*) and Threespine stickleback (*Gasterosteus aculeatus*) (Conitz et al., 2005).

For the past 6 years, stock assessment studies have been implemented at Klag Lake by the Sitka Tribe of Alaska (STA) and United States Forest Service (USFS). During the summers, field crews counted returning adults using a weir and collected harvest data from subsistence and sport fishers. Crews marked approximately 15-20% of the run for mark-recapture estimates, and randomly sampled 600 adults for length, age, and sex information. These data provide important information regarding the stock structure, especially age composition, using scale annuli. Age classes are designated using the European aging system where residence in fresh and marine water is separated by period (e.g. 1.3 denotes 1 year in fresh water and 3 years in the ocean). Age data obtained from the 2001-2003 indicate that the majority (70%) of returning adults to Klag Lake are 1.2 and 1.3 year-old fish (Conitz et al., 2003). Conitz et al. (2003) speculate that because the majority of sockeye migrate from the lake at age 1.0, juveniles attain adequate growth during their first year due to sufficient zooplankton abundance in Klag lake.

Redoubt Lake

Redoubt Lake is located on the west coast of Baranof Island in Sitka Sound, approximately 11 km south of the city of Sitka. The native name for Redoubt is Kuna, and is claimed by the Kiksadi clan of the Sitka Tlingit people (Goldschmidt et al., 1998). Due to Redoubt's close proximity to the city of Sitka, it sustains the largest direct subsistence and sport harvest of the lakes located on Sitka. Fortunately, Redoubt Lake is

a large lake system that supports one of the largest sockeye returns in southeast Alaska. Recent escapements have exceeded over 40,000 sockeye, but low returns were experienced in 2000 and 2001 with estimated escapements of 3,032 and 2,665, respectively (Geiger, 2003). Sockeye spawn at several locations in Redoubt Lake, including inlet streams as well as several sites along the lake shore. Redoubt Lake also supports populations of pink, coho, steelhead, cutthroat trout, Dolly Varden, and stickleback.

Redoubt is a large meromictic lake, draining an area of 113 km^2, a volume of 231 km^3, and a surface area of about 16.6 km^2 (Geiger, 2003). Because Redoubt Lake is meromictic, it does not turn over seasonally as in dimictic systems. Redoubt Lake has a 100 m freshwater lens on the top surface of the lake. The deep water portion of Redoubt Lake (which has a maximum depth of 266 m) is separated from the cap by a chemocline below which dense anoxic water occurs. The permanent stratification restricts most nutrient recycling in Redoubt Lake. The lack of recycling leaves Redoubt Lake limited in micro and macronutrients important for phyto and zooplankton production and therefore the forage base for juvenile sockeye is likewise limited. Fertilization experiments using nitrogen and phosphorus have been implemented for several years to enhance primary production for rearing sockeye in Redoubt Lake. Interestingly, fertilization will further drive the bottom waters to even greater anoxia by loading it with more organic matter. Unfortunately, very few reports exist on the results of these experiments, but recent large escapements indicate that fertilization efforts may be beneficial for sockeye recruitment.

In addition to the fertilization experiments, the USFS has maintained a weir at the two outlets of Redoubt Lake since 1982, except in 1998. As a result a large catalogue of length, sex, and age data have been collected from this stock. Redoubt Lake experiences a slightly differing age composition than Klag Lake. The bulk of returning adults are dominated by 1.3 and 2.2 age classes (Geiger , 2003). Redoubt Lake also possesses a similar zooplankton community to that of Klag Lake with a large abundance of *Bosmina* sp. Larger cladocerans occur at a lower abundance, which is likely due to predation by sockeye juveniles.

Salmon Lake

Salmon Lake is located on the west coast of Baranof Island at the head of Silver Bay, approximately 15.2 km southeast of the city of Sitka. The lake is 17 m above sea level, and is fed by two inlet streams (Tydingco et al., 2006). Like Redoubt Lake, Salmon Lake was also claimed by the Kiksadi clan of the Sitka Tlingit people (Goldschmidt et al., 1998). Salmon Lake was the smallest system examined during this experiment, with a surface area of ~ 0.44km^2. Annual sockeye escapements are relatively low compared to larger systems such as Redoubt Lake, ranging from 1,000 to 3,000 adults. Spawning occurs at several lake shore areas, but the majority of fish have been observed spawning at the head of Salmon Lake below the largest inlet stream. In addition to sockeye, Salmon Lake supports populations of pink, chum, coho, steelhead, cutthroat trout, Dolly Varden, sculpin sp., and stickleback.

Sockeye stock assessments have been carried out in Salmon Lake since 2001 by the Alaska Department of Fish and Game (ADF & G) and STA. A floating weir was installed at outlet of the lake where crews tag and sample all returning adults for age,

length, and sex data. Results indicated that Salmon Lake experiences greater diversity in age composition than other systems. Data compiled from 2001 to 2003 demonstrated that in 2001 age composition was dominated (37.7%) by 1.3 year-old fish. In 2002, 2.2 year-old sockeye contributed the largest percentage (42.2%), whereas in 2003, age class was dominated (27.5%) by 2.3 year-olds (Tydingco et al., 2006). Limnological data suggests that the zooplankton composition changed in 2001, 2002, and 2003. In 2001 *Cyclop sp.* dominated the largest percentage biomass of zooplankton sampled; however, abundance shifted in 2002 and 2003 with *Bosmina* sp. dominating the species composition (Tydingco et al. 2006). *Bosmina* sp. are not the preferred choice by most sockeye fry, but are usually selected over copepods and calanoids (Geiger, 2003).

Tumakof Lake

Tumakof Lake, also known as Redfish Lake, is located on the southern west coast of Baranof Island and drains into Redfish Bay. Goldschmidt et al., (1998) regards Redfish Bay and Tumakof Lake as one of the most important areas on southern Baranof due to its large sockeye run. Originally named Shee Lanaaxk Gatheeni, Redfish is claimed by the Kiksadi clan of the Sitka Tlingit people (Lorrigan et al., 2003). Due to its greater distance from the city of Sitka, the Tumakof sockeye stock does not undergo consistent subsistence and sport harvests as in Redoubt or Klag Bay.

Tumakof Lake drains an area of approximately 1,062 ha. The lake itself has a maximum depth of 99 m and a surface area of 0.93 km^2 (Lorrigan et al., 2003). Tumakof Lake drains into one outlet stream that is approximately 0.8 km from marine water. Sockeye spawning occurs mainly along the lake shores near the north end of the lake—

the farthest from the ocean. Tumakof Lake also supports small populations of coho, pink, chum, cutthroat trout, Dolly Varden, sculpin *sp.*, and stickleback.

In 2000, an illegal commercial harvest of sockeye occurred in Redfish Bay. The harvest was speculated to have possibly eliminated a significant proportion of returning spawners, and prompted concern in the Sitka community (Lorrigan et al., 2003). In response, the STA, ADF&G, and USFS implemented stock assessment studies in 2002, 2003, and 2004. Prior stock assessments had been conducted in Tumakof during the mid 1900s by ADF&G, but a large landslide near a lower reach of the outlet stream had since significantly changed the morphology of the system. It is therefore possible that life history aspects of this stock may have been altered due to the landslide. Stock assessment followed a plan similar to the method performed in Klag Bay discussed earlier: escapement was estimated using a weir, and returning adults were sampled for age, length, and sex data. A creel census was also conducted for all subsistence and sport users. Limnological sampling was also performed to assess the food base of rearing juvenile sockeye.

The data obtained from the 2002 field season indicates that Tumakof supports a healthy run of sockeye with an escapement over 20,000 before the weir was removed (Lorrigan et al., 2003). Larger returns were also experienced in the 2003 and 2004 field seasons, but these results are not yet published. The dominant age class sampled during 2002 was 2.2 year-old fish followed by 2.3, which in combination represented 82% of the run (Lorrigan et al., 2003). Interestingly, a small proportion of 3.3 fish also returned to Tumakof Lake, which is relatively old for sockeye salmon. It was estimated that 99% of juvenile outmigration occurs at ages 2.0 and 3.0 for Tumakof sockeye (Lorrigan et al.,

2003). Limnological sampling of zooplankton productivity indicated that *Cyclops* sp. were the most abundant species in lake followed by the Cladocerans *Bosmina* sp. and *Daphnia* sp.

2.4.2 Water Collection and Analysis

Water samples were collected at each lake during the spring of 2005. Samples collected from Klag and Tumakof were sampled in the lower reach of each outlet stream, whereas Salmon and Redoubt samples were collected from the actual lake. Samples were not analyzed at the aqueous geochemistry lab at the University of Victoria (UVIC), but sent to the University of Alaska Fairbanks (UAF). Initially water samples were to serve for both this study and a project investigating stable isotopes from the same lake systems. In hindsight, it may have been appropriate for separate water samples to have been collected for this project as the sampling methods and locales, the ions analyzed, and preparation protocols and analysis methods differ between UVIC and UAF.

The method used at the University of Alaska Fairbanks followed standard trace metal clean procedures (Hannigan and Sholkovitz, 2001). Samples were collected in acid-washed PTFE bottles, filtered through a 0.45 mm filter, and acidified using ultra-pure HNO_3 to a pH < 2. Water samples were measured for trace element composition using an Agilent 7500 ICP-MS at the Advanced Instrumentation Laboratory. Precision and accuracy was calculated using linear calibration standards and matrix standards (Nate Bickford, personal communication, 2006). Trace element concentrations for Ca, Mn, Mg, Zn, Sr, and Ba were reduced using Agilent 7500 software following standard methods outlined by EPA 200.8 for the analysis of trace elements (Creed et al., 1994).

2.4.3 Otolith Collection and Extraction

Otolith pairs were collected from adult sockeye in 2003, 2004, and 2005 during the months of July through October. Most sockeye were sampled in marine and estuarine areas within close proximity to the outlets of each lake. Sockeye otoliths from Salmon Lake were only sampled from post-spawn fish due to recent low escapements. It was assumed that all adult sockeye schooled near each lake outlet were produced from that system, though straying is known to occur. A variety of capture methods were used, beach seines, gill-nets, dip-nets, and snagging via rod and reel. Sockeye were killed immediately after capture through single blows to the top of the skull, and bled to reduce excess body fluids. Individuals were then measured from mid-eye to the fork of the tail for length in millimeters (mm), and identified for sex. Most post-spawn sockeye sampled for otoliths in Salmon Lake did not provide viable length measurements due to decomposition.

Otolith extraction was accomplished using the "open the hatch method" as described in the "Manual for Otolith Removal and Preparation for Microstructural Examination" (Secor et al., 1991). The dorsal portion of the cranium was transversely cut to expose the brain. The brain and surrounding tissues were carefully removed using trace-metal grade forceps to reveal the sacculus canals containing the left and right sagittal otolith pairs, respectively (figure 2.7). Sagittae were carefully removed and cleaned of the surrounding endolymphatic sac and residual tissues using fine bristled toothbrushes and Milli-Q water. Otoliths were then dried and stored in labeled acid-washed 1.5 mL polyethylene centrifugal vials. Storage methods differed between the 2003, 2004, and 2005 field seasons. In 2003 and 2004, otoliths were stored between

cotton moistened with distilled water to cushion and maintain a similar hydrostatic environment comparable to the inner ear during transit. This practice was discontinued in 2005, and all otoliths were stored dry without cotton. No significant differences in chemical composition were noticed between the two storage methods after analysis; however, it was noticed that otoliths stored in the moist environment were more prone to damage during preparation.

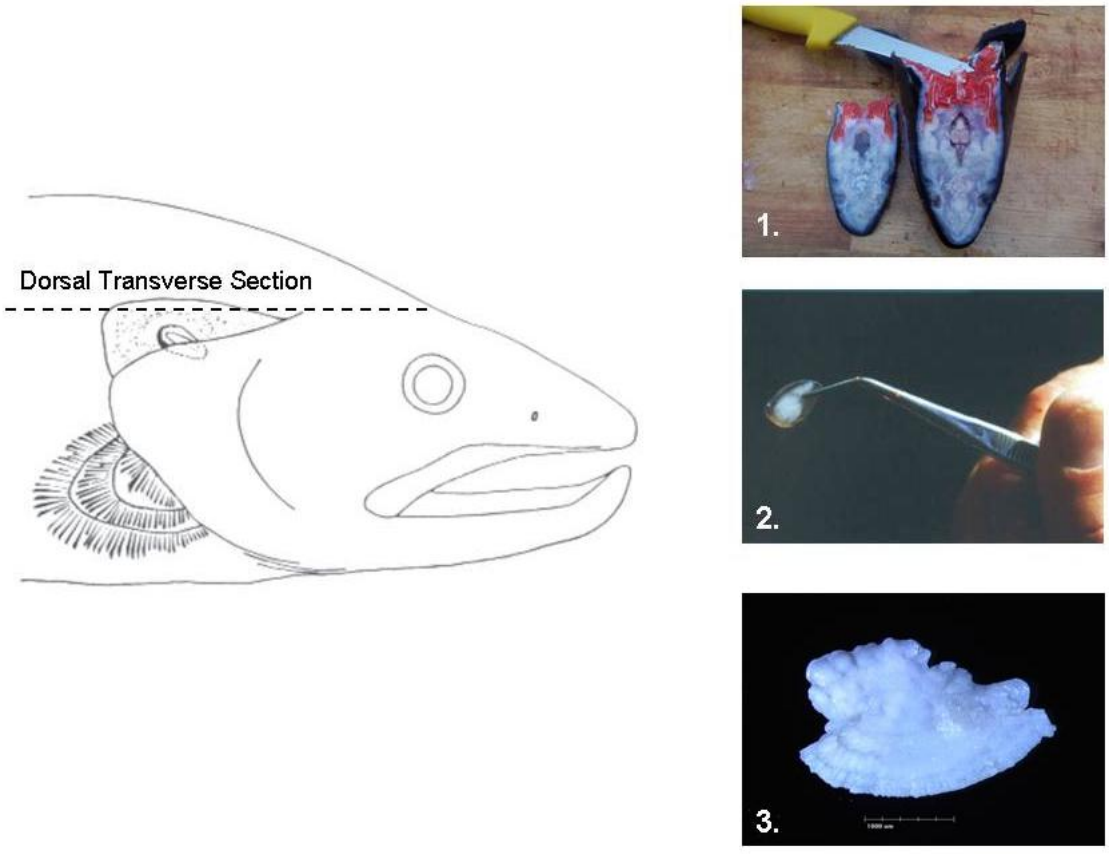

Figure 2.8.Schematic of the “Open the Hatch” otolith extraction method: 1. Dorsal transverse section of a sockeye head 2. Otoliths were pulled from the sacculus canals 3. Otoliths were cleaned of the endolymphatic sac and stored (Adapted from Cailliet et. al. 1986).

In total 243 sagittal otolith pairs were collected during the sampling periods between 2003 and 2005. Only Salmon Lake was sampled in 2003 with a total of 15 otolith pairs collected. In 2004, each system was sampled: Redoubt (42), Salmon (24), Klag (37), and Tumakof Lake (35). In 2005 only Redoubt (30), Klag (30) and Salmon Lake (30) were sampled, thus only one year of Tumakof data exists for this study. These differences were mainly due to constraints on access in these remote areas. Only left otolith pairs were selected for trace element analysis for this experiment. Right sagittal otoliths were sent to the University of Alaska Fairbanks for a separate project investigating stable isotopes of core. Possible comparisons between the two methods may occur in future work.

2.4.4 Otolith Preparation

Otolith preparation for LA-ICP-MS analysis was accomplished using procedures derived from common fisheries aging methods (Cailliet et al., 1986; Murphy and Willis 1996; and Secor et al., 1991). Otoliths were dried overnight under laminar flow hoods to remove excess moisture, and embedded in Beuhler Epoxicure resin (Lake Bluff, IL) sulcus-side down. Embedded otoliths were transversely sectioned using a low speed isomet saw (Markham, Ont.) fitted with a Lapcraft 4”x.004” diamond blade (Powell, OH). Transverse sections were selected due to the concave morphology associated with salmon otoliths. Other common sectioning planes used for aging studies, such as frontal and sagittal sections, can experience significant losses of the outer growth increments during polishing. To prevent this, transverse sections were selected to ensure that all growth increments were retained to fully reconstruct sockeye life history. Otoliths were sectioned approximately 3 mm from the estimated position of the core along the anterior

edge. Sectioned otoliths were re-embedded in ¾ inch acrylic rings positioned with the transverse plane lying flat adhered to industrial strength tape.

Otoliths were polished using a series of several grit (320, 600, and 1200) Carbimet Abrasive Discs (Lake Bluff, IL). Polishing was performed by hand using consistent circular rotations to evenly remove otolith material. Coarser grit discs (320 and 600 grit) were used to remove otolith material quickly and gain close proximity to the core. The finer 1200 grit discs were used to slowly remove otolith material and precisely expose the core. Progress to the core was assessed using a dissecting scope using transmitted light. Otolith mounts were cleaned during transitions each between each gritted disc in Milli-Q water in an ultrasonicator. This step was performed to reduce contamination and the introduction of different sized grit particles between discs. A final polish was applied using a TEXMET 200 0.24µm polishing cloth and Buehler METADI Supreme Polycrystalline Diamond Suspension water base spray (Lake Bluff IL). The final polish provides a mirror smooth surface with little to no surface topography on the otolith surface. This reduces the possibility of contamination by leaving no pores into which contaminating particles can collect. It also enhances the visibility of the annuli and core for aging and identifying the relative position of the first marine entry check (ME) (figure 2.9). The marine entry check is especially important for migration reconstruction in sockeye, as it provides a visually distinct zone separating fresh and marine residence. A final ultrasonicated bath with Milli-Q water was applied to otolith mounts before analysis. Otoliths were then removed from acrylic rings and adhered to glass microscope slides using double-sided tape for analysis.

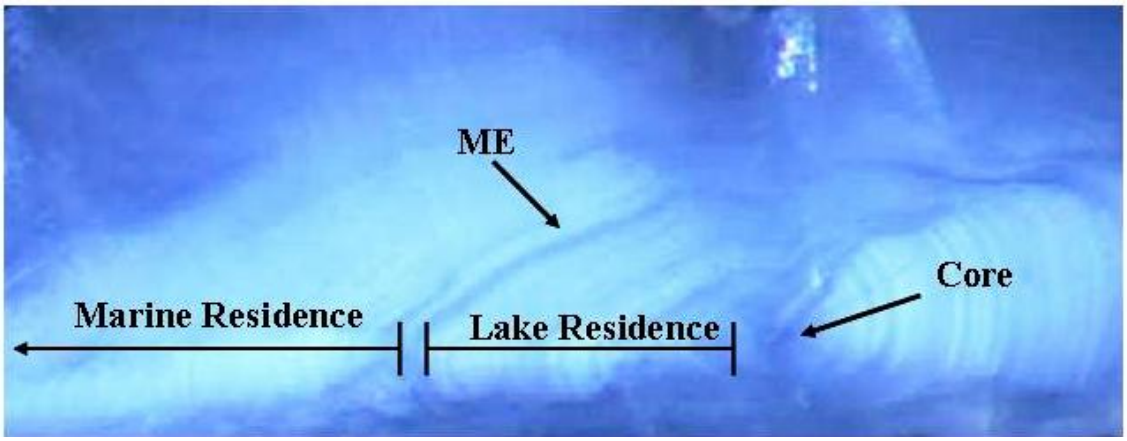

Figure 2.9 Polished transverse section of a sockeye sagittal otolith. The core and marine entry check (ME) are denoted in the otolith microstructure providing clear distinction between fresh and marine residence.

2.4.5 Analysis and Data Acquisition

LA-ICP-MS analysis followed the detailed method outlined by Sanborn and Telmer (2003). Otolith analysis was initially conducted using a VG Elemental PQ II S+ Quadrapole ICP-MS (FISONS Winsford, England). Subsequent analysis were performed on a Thermo X-Series IIX7 Quadrapole ICP-MS (Thermo Electron Corporation, Winsford, England) acquired in early 2006. No significant differences were observed in the data quality produced from either instrument. Laser ablation was performed using a Merchentek Mini-laze II 266nm Nd-YAG laser (Fremont, Ca). Continuous line scans were performed across the full diameter of each otolith at a rate of 0.0024 mm per second (figure 2.10). Scans were started at the dorsal edge of each otolith and directed through the core to the opposing ventral edge.

The Mini-laze II 266nm Nd-YAG laser system operates with a wavelength of 266 nm with a maximum energy output of 4 mJ. During analysis the laser was operated at an output frequency of 20 Hz at 70% power. The energy output ranged from 0.90-2.2 mJ with an average spot size of ~30 µm. Instrument operating conditions (e.g. lens settings,

plasma gas, and carrier gas flow rates, and torch-box position) were optimized prior to each run. The ICP-MS was optimized using National Institute of Standards glass reference material 613 (NIST 613) as an external standard. NIST 613 contains ~50 $\mu g\ g^{-1}$ total trace elements, and was analyzed before and after each scan to correct for instrumental drift. Currently there is no existing matched matrix external reference material for otoliths. This is due to the heterogeneous nature of otoliths—i.e. it is thus far impossible to produce a perfectly homogenous standard with known concentrations in 3D space. Otoliths were measured for signal intensities of ^{43}Ca, ^{86}Sr, ^{137}Ba, ^{24}Mg, ^{55}Mn, ^{66}Zn, and Li^{7} and all abundances were normalized to ^{43}Ca. Ca was used as the internal standard because it is a major component of otoliths and therefore has a consistent and invariant concentration in otolith aragonite. It is 40% molecular weight of aragonite and has variations of less than 1% which are visible in a method that has a precision no better than 3%-discussed later. Variations in the other elements analyzed in this study are much larger by comparison—up to 300% for Sr for example. These data were acquired using peak jumping mode with a dwell time of 10 milliseconds (ms) per element. A 20-25 second gas blank was performed prior to all analysis to collect background intensities. Ablation times for NIST 613 averaged between 30 to 35 seconds. Otolith scans averaged 1300 seconds.

Raw data collected from LA-ICP-MS scans was stored and reduced using VG Thermo Electron PlasmaLab Software 2005 (Version 2.4.1.224, Burlington, On, 2005). Data was converted into individual time-slices" representing 4 second intervals, and transferred into Excel spread sheets. In Excel, mean elemental composition per time slice

for Li, Mg, Mn, Zn, Sr, and Ba were converted into chemical concentration profiles plotted against the distance traversed across the otolith (Δ X position µm).

Because otoliths grow concentrically, line scans tracking from the dorsal to ventral edge produce an almost mirror image chemical profile for either side of the core. In simpler terms, as the laser tracks from the dorsal edge to the core, one chemical profile of life history is attained. A second life history profile is acquired as the laser continues to track from the core out to the opposite ventral edge– from birth to death (figure 2.10). However, opposing chemical profiles on a single otolith will not be completely symmetric. This is generally because otoliths are not perfectly symmetrical in any two dimensions. More specifically, in the transverse sections the distance from the dorsal edge to the core is approximately one third longer than the distance from the ventral edge to the core. This implies that for a laser tracking at constant rate larger quantities of chemical data are acquired from the dorsal edge to core. Thus it was assumed that this portion of the scan (dorsal edge→ core) provided greater spatial and temporal resolution for migration reconstruction and it was therefore universally used in data analysis and interpretation. Yet it should be noted that both sides provide high quality data and provide useful information related to life history. Because scans begin at the perimeter of the dorsal edge, life history profiles also depict life history in reverse. To correct this, profiles were modified to present life history beginning at the core (figure 2.11).

During this study it was important to estimate a temporal resolution for LA-ICP-MS line scans. Converting line scans into “fish time” is complicated because otolith growth is not linear during the course of sockeye life history. Therefore, we have integrated an average value for daily otolith growth with time-series data to attain an

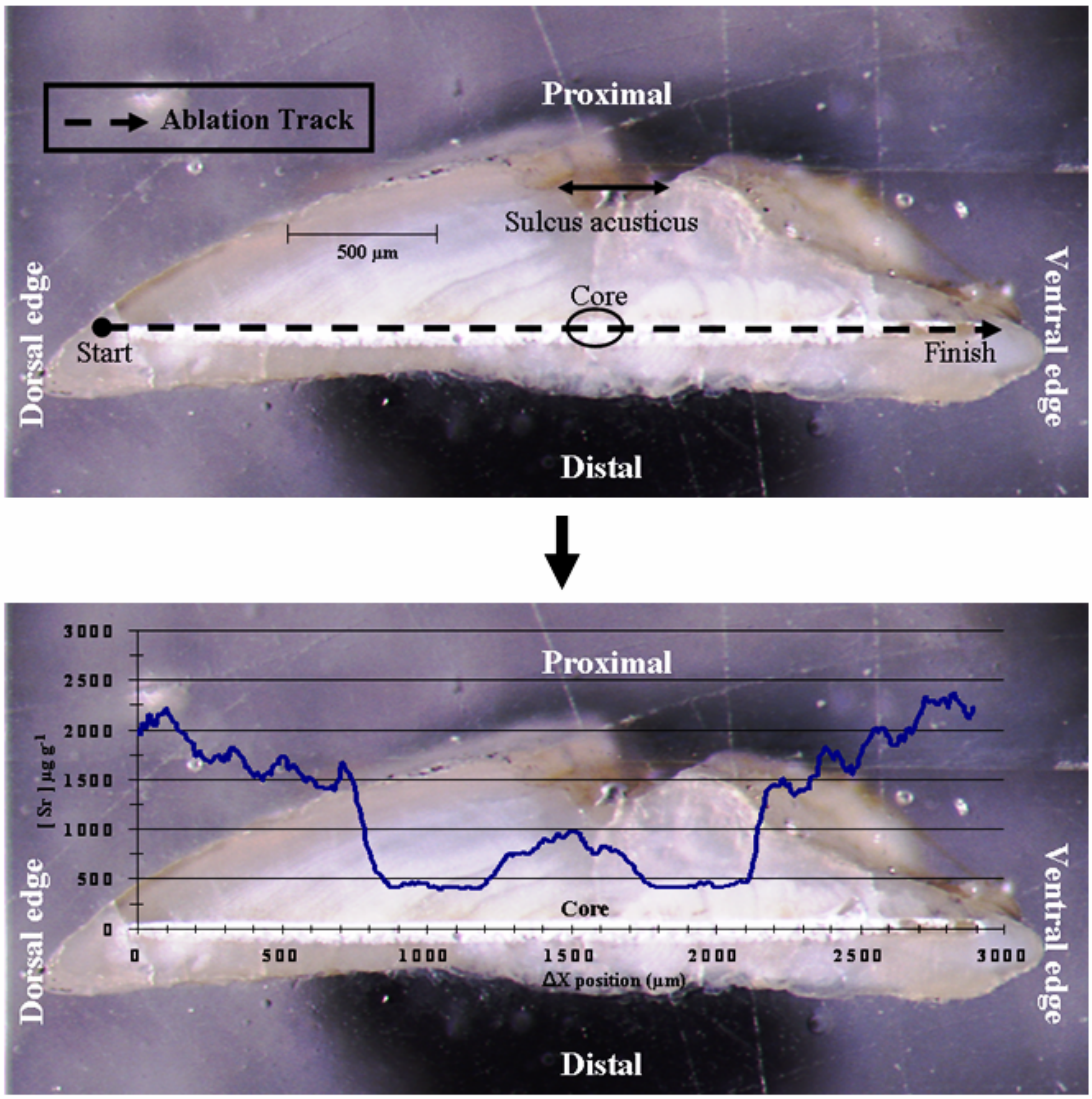

Figure 2.10 *Top:* The laser ablation track of a line scan across a transversely sectioned sockeye otolith from the dorsal edge through the core to the ventral edge. *Bottom:* This style of analysis produces a duplicate life history profile (mirror image)—from death to birth to death. The chemical life history profile in the *bottom figure* is produced from Sr composition ($\mu g\ g^{-1}$).

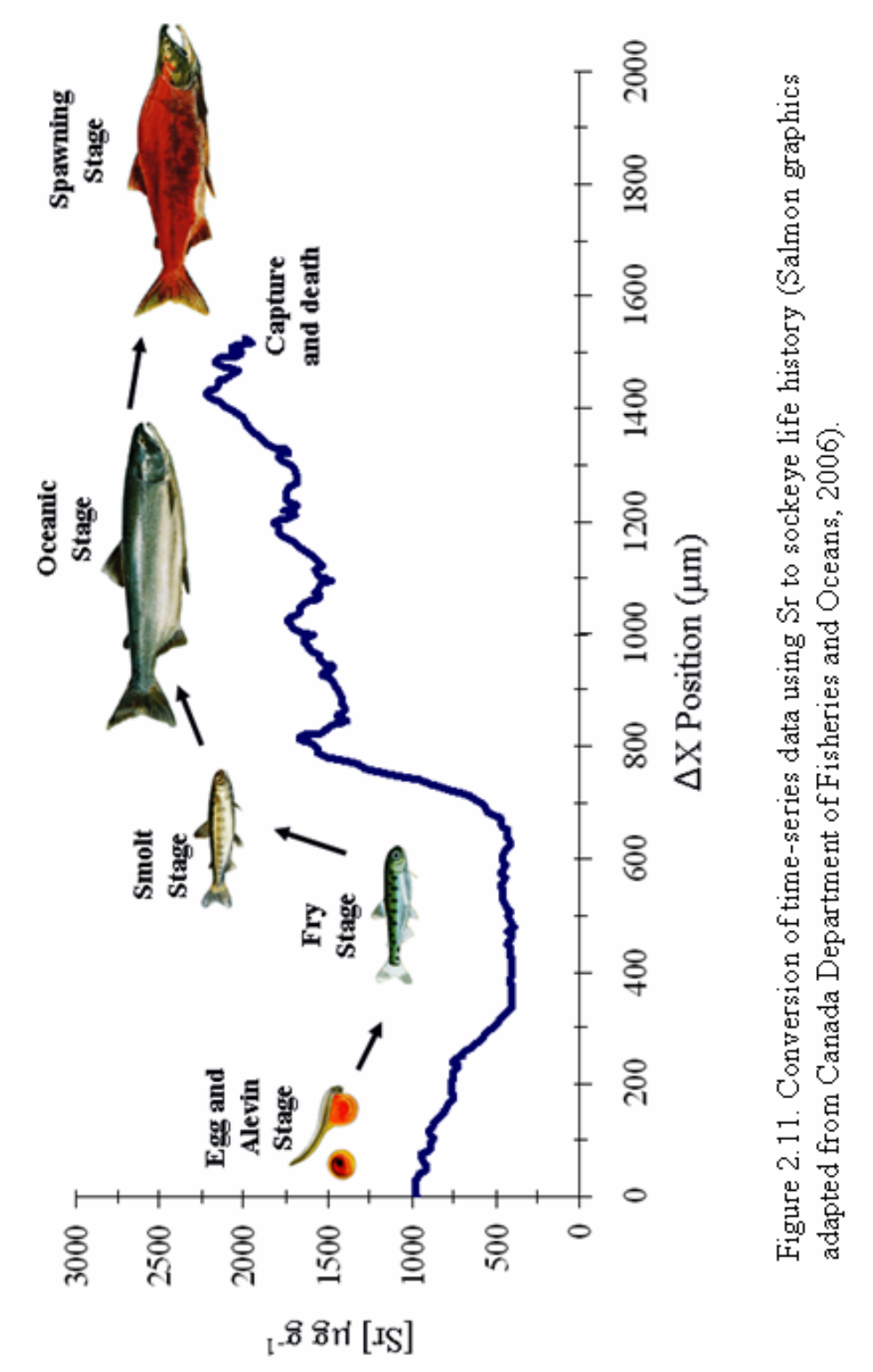

Figure 2.11. Conversion of time-series data using Sr to sockeye life history (Salmon graphics adapted from Canada Department of Fisheries and Oceans, 2006).

estimate of temporal resolution. Otoliths were scanned at rate of 2.4 µm per second, with scan data averaged into 4 second "time-slices." Each time-slice equals an ablation distance of ~ 9.6 µm. The average rate of daily growth deposition onto a salmon otolith was estimated by Sanborn and Telmer (2003) to be approximately 1 to 3 µm in diameter. Based upon this estimation it can be calculated that our line scans have a temporal resolution of approximately 3 to 10 days per time-slice. For this experiment we shall assume daily growth is on average 1 µm/day. For example, a five-year-old sockeye would contain approximately 190 "time-slices" or 1825 µm from the dorsal edge to the core. To check this estimation we evaluated the average distance required to reach the core during line scans. It was calculated that an average distance of 1632 µm was required to reach the core from the dorsal edge. This would translate into 4.5 years of fish life assuming daily growth is 1 µm. This value corresponds to the average age composition of fish (4-5 years total) associated with the four systems from past stock assessment studies.

2.4.6 Statistical Analysis

Analysis of variance (ANOVA) was used to statistically test if elemental uptake in the otoliths was significantly different during sockeye life history. To do this, 10 otoliths were selected from each sockeye population. Line scans that did not completely sample the core or freshwater growth regions of otoliths did not capture the full sockeye life history and were therefore omitted. Time-series data was averaged into values representing the core, fresh, and marine growth regions of the otolith. Each growth region was segregated visually using the core and marine entry check as reference points to determine residence in either fresh or marine water. Elemental data was visually

examined for normality and homogeneity of variance using simple histograms. In order to conform to the assumptions of ANOVA analysis, all elemental data was converted using natural log (ln) transformation. Each growth region of the otolith was compared using a "One-way" ANOVA applying Tukey's Honestly Significant Difference (HSD) post hoc procedure to specifically identify which regions of the otolith were different. All tests were performed at the 95% confidence interval using SPSS™ 11 and 14 statistical software.

2.5 Results

2.5.1 Analytical Precision

To determine the precision of LA-ICP-MS line scans replicate analysis was performed using NIST 613- a certified standard reference material routinely used in LA-ICP-MS analysis. NIST 613 was sampled 13 times at 40 second intervals to determine concentrations of ^{7}Li ^{24}Mg, ^{55}Mn, ^{66}Zn, ^{86}Sr, and ^{137}Ba. Results, expressed a relative standard deviation (RSD), indicating precisions of 3.07%, 0.97%, 0.86%, 2.57%, 0.55%, and 0.60% for each element respectively. A level of precision below 10% is generally regarded as routinely acceptable and so the levels obtained here can be regarded as excellent (Table 2.3).

	Element µg g^{-1}					
	7Li	24Mg	55Mn	66Zn	86Sr	137Ba
Average	39.60	76.58	36.88	36.05	75.66	36.79
STDEV	1.22	0.74	0.32	0.93	0.41	0.22
% RSD	3.07	0.97	0.86	2.57	0.55	0.60

Table 2.3: Precision data obtained from replicate analysis of NIST 613 using LA-ICP-MS lines scans.

In addition to the replicate analysis of NIST 613, a sockeye otolith was sampled to assess the precision and reproducibility of line scans on a heterogeneous target. Four parallel ablation scans were performed on the otolith between two defined growth increments. Scan rate (0.0024 mm/s) and sampling time (100 s) were kept constant to ensure equal distances were ablated for each scan. Results showed good reproducibility between the four scans with expected small variations (figure 2.12). These variations are to be expected because otoliths are heterogeneous and never perfectly symmetrical, and therefore no two trajectories will be identical. This test was done simply to show that the pattern of chemical changes detected by this method is generally reproducible. This is also evident from full line scan profiles which produce mirror image profiles for each side opposite the core—death to birth to death.

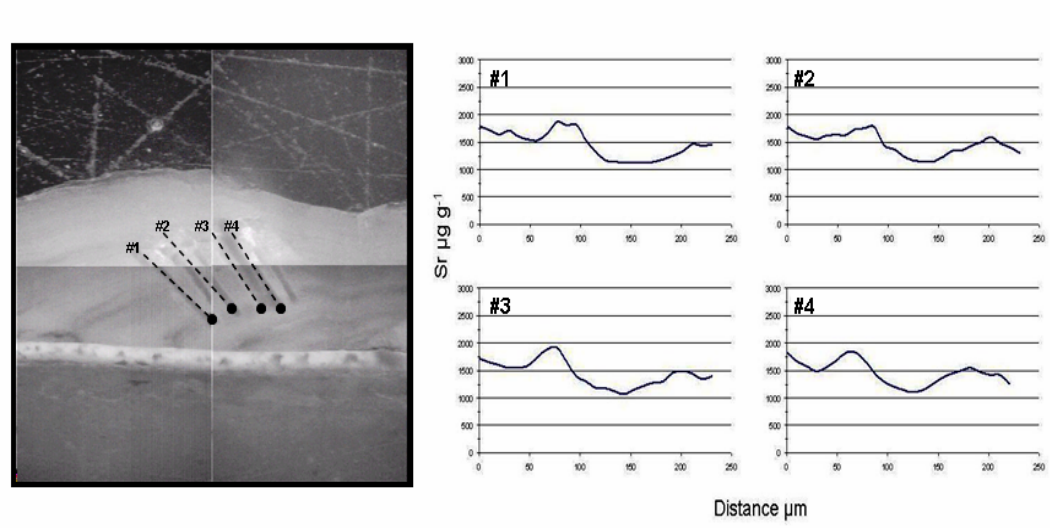

Figure 2.12 Replicate line scans of Sr illustrating expected differences and degree of similarity for profiles across growth bands.

2.5.2 Lake Water Chemistry

Water samples were chemically distinct in at least one element between the four lake systems (Table 2.4). Klag Bay contained the highest concentrations of Mn, but the

lowest concentrations of Ba. Redoubt Lake water was enriched in several elements that included the highest absolute values of Mg, Zn, Sr, and Ba. Salmon Lake demonstrated the highest absolute concentration of Ca of the lakes examined. Tumakof Lake was distinctive due to its relatively low concentrations of several elements, including Ca, Mn, and Sr. Lithium was not analyzed in water samples for any of the four systems.

	Concentration (µg/L)						
Location	Ca	Mn	Mg	Zn	Sr	Ba	Li
Klag Lake	987.00	22.77	339.88	3.02	18.19	2.26	*
Redoubt Lake	1603.00	7.50	2290.50	3.93	25.66	4.67	*
Salmon Lake	2461.00	4.67	292.60	2.45	23.51	2.54	*
Tumakof Lake	339.75	3.34	292.75	2.89	3.05	4.32	*

Table 2.4. Average elemental concentrations (µg/L) measured by ICP-MS for the four sockeye lakes examined. Samples were collected in the spring of 2005. Values are means from duplicate samples.

A common misconception in otolith microchemistry is that absolute water concentrations are the major determinant of otolith uptake. Indeed, otolith variability is a function of water chemistry, but for elements such as Sr and Ba, it is the ratio to calcium that largely governs uptake (Kraus and Secor, 2004). Therefore, it was also important to express the ratio of each element to Ca (Element $_{concentration}$/Ca $_{concentration}$) for all four lakes (Table 2.5). Conversions resulted in vastly differing chemical representations for each system. Tumakof Lake, which initially contained low concentrations for most elements now contained the largest ratios of Ba:Ca and Zn:Ca. However, Sr:Ca in Tumakof Lake remained the lowest of the four lakes. Redoubt Lake contained the highest Mg:Ca value, and the second largest Sr:Ca value. Klag Bay contained the highest Sr:Ca and Mn:Ca ratios of all the systems. Salmon Lake's high Ca content shifted many of its elemental

concentrations to the lowest ratios of the four systems. Salmon Lake contained the lowest values for Ba:Ca, Mg:Ca, Zn:Ca, and Mn:Ca.

	Element:Calcium				
Location	Sr:Ca	Ba:Ca	Mg:Ca	Zn:Ca	Mn:Ca
Klag Lake	0.018	0.002	0.344	0.003	0.023
Redoubt Lake	0.016	0.003	1.429	0.002	0.005
Salmon Lake	0.010	0.001	0.119	0.001	0.002
Tumakof Lake	0.009	0.013	0.862	0.009	0.010

Table 2.5 Average Elemental ratios measured by ICP-MS for the four sockeye lakes examined. Samples were collected in the spring of 2005. Values are means from duplicate samples.

2.5.3 Strontium

Strontium profiles provide an effective means for reconstructing the life history of sockeye. Strontium profiles followed an increasing trend as line scans moved from the core to dorsal edge of sockeye otoliths (figure 2.13). This pattern demonstrates that Sr uptake increases during the marine phase, which agrees with past research showing that salinity is a major factor controlling Sr uptake (Katayama et al. 2000, Kraus and Secor, 2004, Elsdon and Gillanders, 2004, Arai and Hirata, 2006). However, aside from determining the already well-documented transition of sockeye from a low salinity (lake) to high salinity (marine water) environment, Sr profiles also highlight many other environmental shifts.

Otoliths from Salmon and Klag Lake display Sr concentrations in the core that were elevated compared to the freshwater growth regions. No visual differences in Sr composition could be distinguished between the core and freshwater growth regions in Tumakof or Redoubt Lake otoliths. In fact, Sr uptake in freshwater was markedly

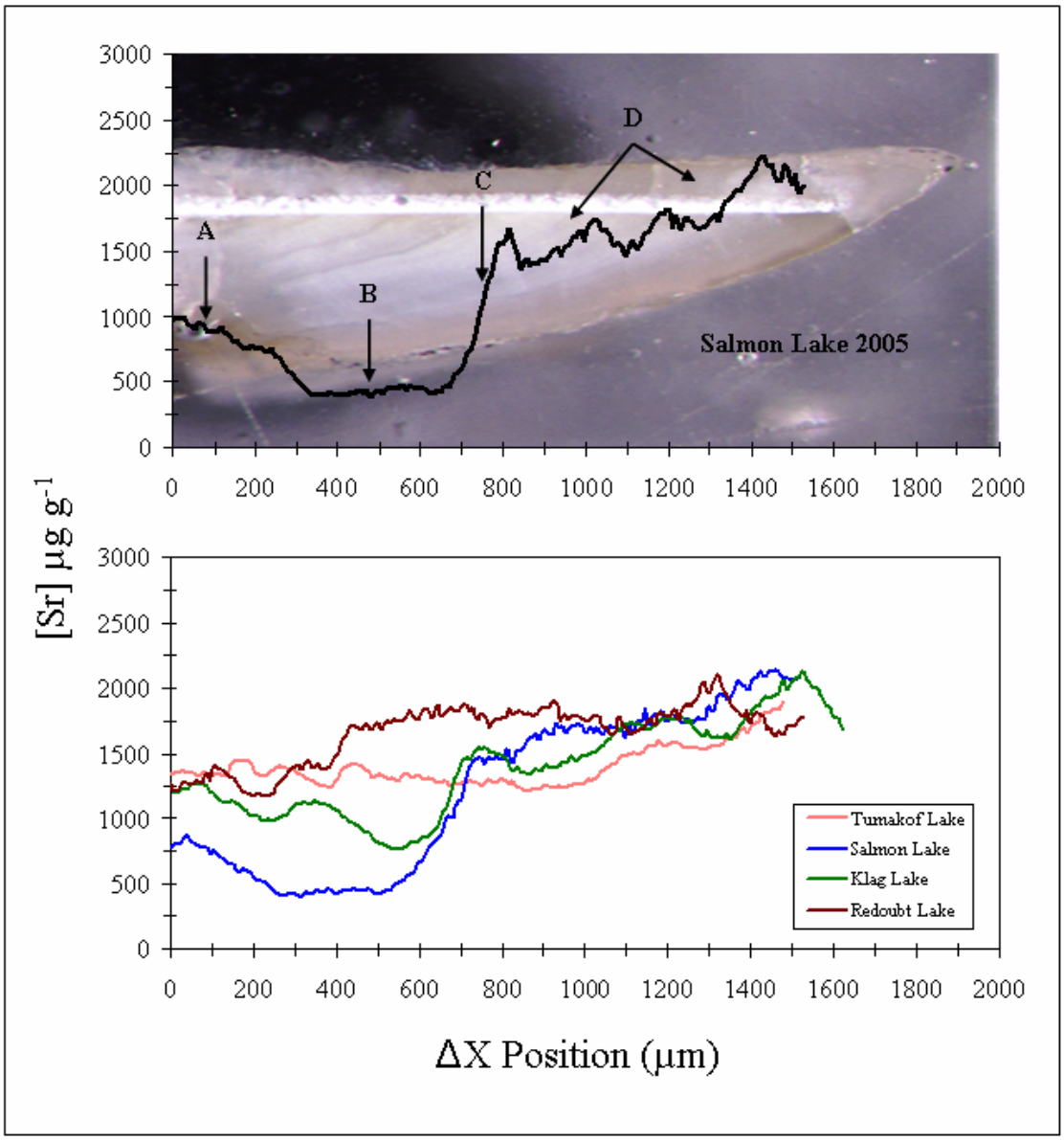

Figure 2.13 Strontium (Sr) profiles produced from line-scan analysis extending from the core (ΔX=0µm) to the dorsal edge (ΔX ≈ 1800 µm). *Top Profile*: Core region with elevated Sr concentration relative to the freshwater signals (A). Freshwater residence observed as stable low Sr concentrations (B). Outmigration denoted by a significant gradient shift in Sr (C). Marine residence denoted by stable high Sr concentrations with slight oscillation possibly indicative of coastal and pelagic marine signals (D). *Bottom Profile*: Comparison of Sr life history profiles between Tumakof, Salmon, Klag and Redoubt Lake.

different between otoliths from the four lakes. Salmon Lake otoliths have invariant and low concentrations of Sr, whereas Sr concentrations in Klag Lake otoliths followed a generally decreasing pattern prior to outmigration. Both Tumakof and Redoubt Lake otoliths contained high Sr concentrations during freshwater residence, often making it difficult to identify the transition from fresh to marine water.

Migration to marine water was clearly marked as defined shifts in Sr from low to high concentrations in Salmon and Klag Lake otoliths, which corresponded to the position of the marine entry check. However, even analysis of the marine entry check did not induce significant shifts in Sr uptake in Redoubt or Tumakof Lake otoliths. Especially in Tumakof Lake otoliths, Sr profiles display only marginal increases in Sr throughout sockeye life history, which indicates that Sr uptake in the lake is not much different than in marine water. This suggests that sockeye habitat in Tumakof Lake is elevated in Sr by at least 3 times—making it equivalent to marine waters –although the water chemistry does not bear out this suggestion (discussed later).

Strontium uptake during the marine phase of life history resulted in similar patterns and concentrations between the four populations. Compared to freshwater systems, Sr concentrations in the ocean are spatially and temporally invariant, providing a natural reference point to examine the reliability of Sr as a proxy of ambient chemistry. However, despite the relatively constant concentration of Sr globally in the ocean Sr profiles are not invariant during the marine residence period. Rather, they gradually increase and exhibit periodic oscillations. Furthermore, the majority of Sr profiles contained a distinctive final increase in Sr:Ca in the late stages of life history, shortly

before capture. This result suggests that salinity is not the only mechanism controlling Sr uptake in these otoliths. (All Sr profiles are located in Appendix I).

To quantitatively assess the observed differences in Sr uptake during sockeye life history an ANOVA analysis was performed. Results demonstrated Sr composition between the core, freshwater, and marine growth regions were significantly different ($P < 0.0001$) in Salmon, Klag, and Redoubt Lake otoliths. No significant differences in Sr uptake could be determined between the 3 growth regions in Tumakof Lake otoliths ($P = 0.062$). A Tukey's post-hoc test further revealed which regions of the otolith were significantly different between the four populations, which is presented in table 2.6.

Dependent Variable	Pair-wise Comparison	Probability
Salmon Lake	core vs. freshwater	P-value < 0.001
	core vs. marine	P-value < 0.001
	freshwater vs. marine	P-value < 0.001
Klag Lake	core vs. freshwater	P-value = 0.040
	core vs. marine	P-value = 0.001
	freshwater vs. marine	P-value < 0.001
Redoubt Lake	core vs. freshwater	P-value = 0.918
	core vs. marine	P-value = 0.000
	freshwater vs. marine	P-value = 0.000
Tumakof Lake	core vs. freshwater	P-value = 0.919
	core vs. marine	P-value = 0.070
	freshwater vs. marine	P-value = 0.150

Table 2.6 P-values calculated using Tukey's post hoc HSD procedure to determine if Sr composition was significantly between the core, freshwater, and marine growth regions of sockeye otoliths. Salmon Lake (n = 10), Klag Lake (n = 10), Redoubt Lake (n = 10), and Tumakof Lake (n = 10).

In both Salmon and Klag Lake, Sr uptake was significantly different in all three growth regions of the otolith. Redoubt Lake displayed no significant differences in Sr

composition between the core and freshwater otolith growth regions. However, both core and freshwater growth in Redoubt otoliths were statistically distinguishable from marine Sr signals. Tukey's post-hoc procedure demonstrated that no significant differences in Sr composition existed between any of the three otolith growth regions in Tumakof Lake sockeye.

2.5.4 Barium

Life history profiles generated from Ba concentrations in sockeye otoliths generally followed an inverse pattern to Sr (Figure 2.14 and 2.15). In the core, Ba concentrations were consistently lower than in the freshwater growth regions of sockeye otoliths. This consistent pattern suggests that Ba uptake is decreased during incubation and early development. Just beyond the core, Ba typically increased to the highest levels and then during the remainder of the freshwater residence, fluctuated. This suggests that ambient Ba concentrations in the lakes vary periodically. Comparatively, freshwater Ba concentrations were similar in Salmon, Klag, and Redoubt Lake otoliths, ranging from 10—20 $\mu g\ g^{-1}$. However, Ba uptake was exceptionally high in Tumakof Lake otoliths, at values normally one order of magnitude greater than the three other sockeye lakes.

Barium is usually depleted in marine surface waters (Hanor and Chan, 1977), and outmigration corresponded with decreases in Ba for the majority of otoliths. In some respects, Ba was a more effective indicator of outmigration than Sr profiles, especially in systems such as Tumakof and Redoubt Lake, which showed little variation in Sr during transitions from fresh and marine water.

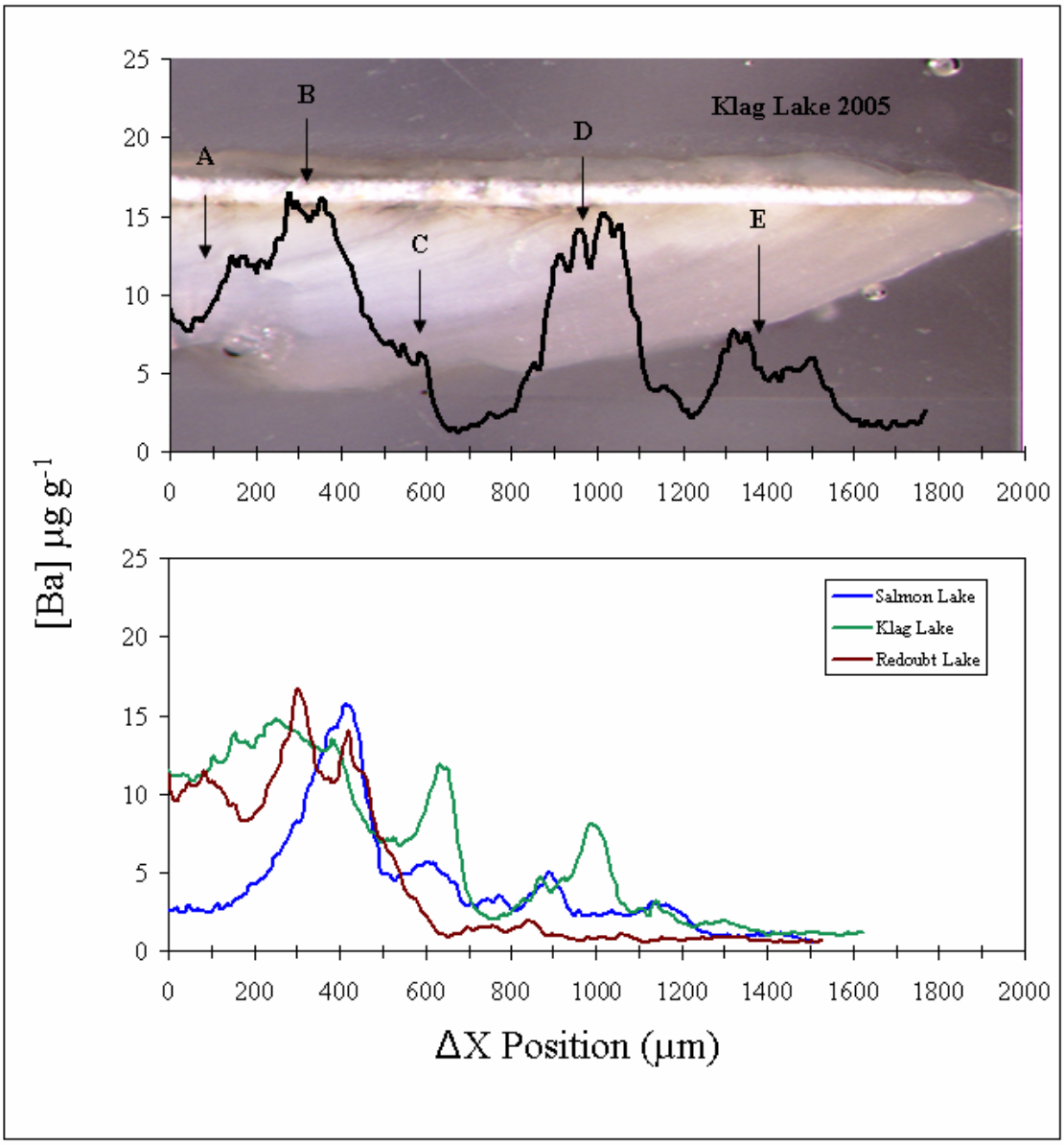

Figure 2.14 Barium life history profiles produced from line-scan analysis extending from the core (ΔX=0µm) to the dorsal edge (ΔX ≈ 1800 µm).*Top Profile*: Core region with a decreased Ba concentration relative to the freshwater signals (A). Freshwater residence displayed a fluctuating pattern in Ba (B). Outmigration denoted by a significant decrease in Ba (C). Early marine residence is denoted by a Ba peak occurring shortly after outmigration (D). Periodic elevations in Ba during later marine growth (E). *Bottom Profile*: Comparison of Ba life history profiles between Salmon, Klag and Redoubt Lake.

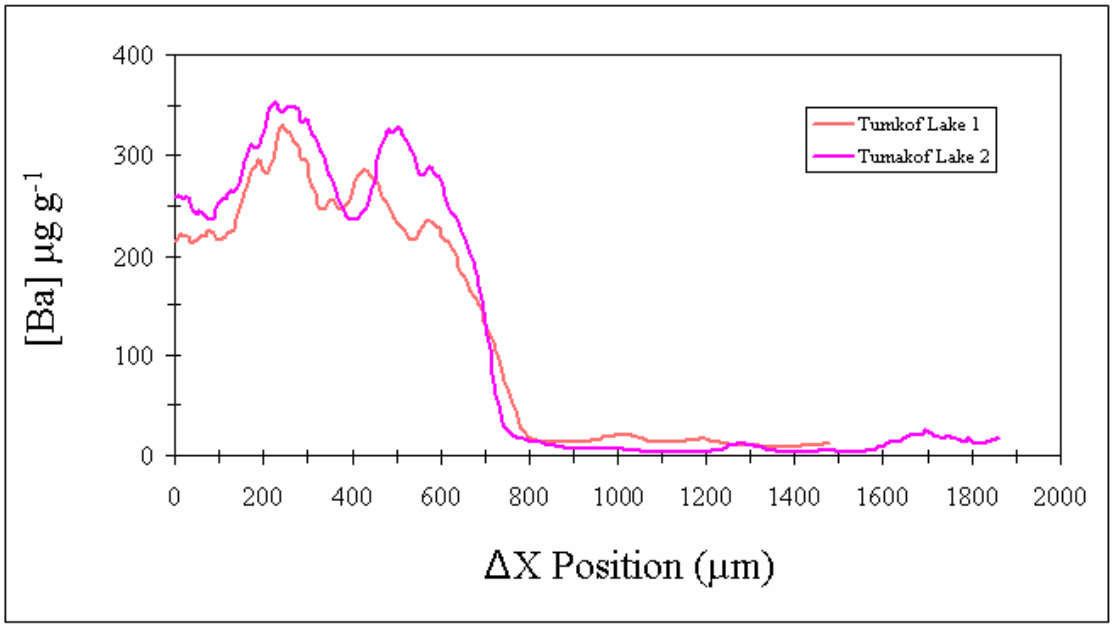

Figure 2.15. Barium life history profiles from Tumakof Lake using line-scan analysis extending from the core (ΔX=0µm) to the dorsal edge (ΔX ≈ 1800 µm).

As well, some sockeye otoliths displayed several distinctive Ba peaks during ocean residence. The most prominent of these peaks occurred shortly after outmigration, probably while fish resided in estuarine and coastal areas. This characteristic was especially evident in Klag Lake otoliths, which often displayed Ba peaks shortly after outmigration that were of similar magnitude to peaks during lake residence. Subsequent to the peaks that followed outmigration, periodic increases in Ba occurred during marine residence. On average, sockeye otoliths displayed 1—3 defined peaks during residence in the ocean, which may possibly be explained by seasonal enrichments of Ba in surface waters due to upwelling nearer to coasts. If Ba peaks are accurate in detecting seasonal upwelling, peaks may possibly have value for determining the marine age of temperate fish species in the northeastern Pacific (Clarke et al., In Press). (All Ba profiles are located in Appendix I).

ANOVA analysis comparing Ba concentrations in the core, fresh, and marine regions of the otolith were significantly different ($P < 0.03$) in all four populations. However, Tukey's post-hoc test revealed that no significant differences could be determined between the core and freshwater growth regions of the otolith in any of the lakes (Table 2.7). On the other hand, marine growth regions were significantly different in Ba composition than those attained in the cores of Klag, Redoubt, and Tumakof Lake otoliths. Marine and core Ba composition were not statistically different in Salmon Lake otoliths. All sockeye otoliths showed significant differences in Ba composition between the freshwater and marine otolith growth periods, which supports past research that Ba uptake between the two environments is different.

Dependent Variable	Pair-wise Comparison	Probability
Salmon Lake	core vs. freshwater	P-value = 0.803
	core vs. marine	P-value = 0.091
	Freshwater vs. marine	P-value = 0.023
Klag Lake	core vs. freshwater	P-value = 0.461
	core vs. marine	P-value < 0.001
	Freshwater vs. marine	P-value < 0.001
Redoubt Lake	core vs. freshwater	P-value = 0.142
	core vs. marine	P-value < 0.001
	Freshwater vs. marine	P-value < 0.001
Tumakof Lake	core vs. freshwater	P-value = 0.430
	core vs. marine	P-value < 0.001
	Freshwater vs. marine	P-value < 0.001

Table 2.7 P-values calculated using Tukey's post hoc HSD procedure to determine if Ba composition was significantly between the core, freshwater, and marine growth regions of sockeye otoliths. Salmon Lake (n = 10), Klag Lake (n = 10), Redoubt Lake (n = 10), and Tumakof Lake (n = 10).

2.5.6 Magnesium

In comparison to Sr and Ba, Mg profiles were not as effective for chemically reconstructing sockeye migration from fresh and marine water. However, profiles did exhibit several distinct characteristics in relation to sockeye life history. Foremost, were the pronounced elevations of Mg in the cores of most otoliths (figure 2.16). Magnesium uptake is clearly higher during the early formation and growth of the otolith, possibly due to a Ca deficiency. Following the initial Mg peak in the core, profiles showed distinctive declines to relatively stable Mg concentrations during freshwater residence.

Migration to the ocean was not obvious from Mg profiles, which typically exhibited gradual increases following the marine entry check. Little evidence has been produced to positively correlate Mg uptake in fish otoliths to ambient chemistry

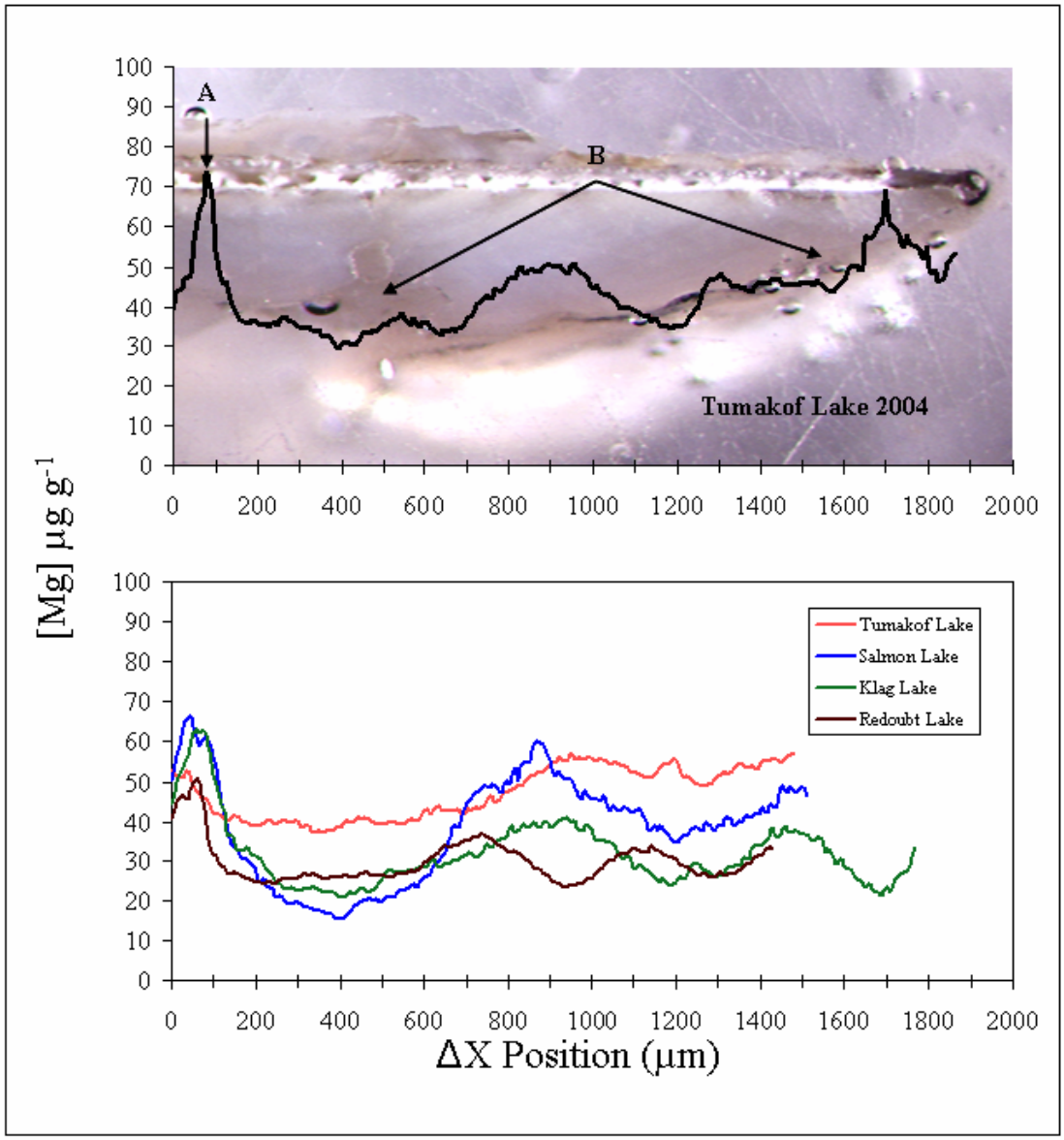

Figure 2.16. Magnesium life history profiles produced from line-scan analysis extending from the core (ΔX=0µm) to the dorsal edge (ΔX ≈ 1800 µm). *Top Profile*: Elevate Mg signals in the otolith core (A). Freshwater and marine residence difficult to discern in life history profiles, but slight changes from low to higher concentrations were observed in some otoliths (B). *Bottom Profile*: Comparison of Mg life history profiles between Tumakof, Salmon, Klag and Redoubt Lake.

(Thorrold et al.,1997, and Campana, 1999). However, although moderate, sockeye otoliths show differences in Mg uptake between fresh and marine water. This behavior is contrary previous assumptions for Mg uptake (Thorrold et al., 1997, Campana, 1999), and suggests that Mg does to some degree reflect ambient chemistry. (Mg profiles are located in Appendix I).

Results from ANOVA analysis showed that otolith Mg composition significantly varied ($P < 0.02$) in all four populations. Tukey's post-hoc procedure revealed that Mg concentrations between the core and freshwater growth regions were significantly different in all otoliths (Table 2.8).

Dependent Variable	Pair-wise Comparison	Probability
Salmon Lake	core vs. freshwater	P-value < 0.001
	core vs. marine	P-value = 0.005
	freshwater vs. marine	P-value = 0.053
Klag Lake	core vs. freshwater	P-value = 0.008
	core vs. marine	P-value = 0.223
	freshwater vs. marine	P-value = 0.287
Redoubt Lake	core vs. freshwater	P-value = 0.001
	core vs. marine	P-value = 0.393
	freshwater vs. marine	P-value = 0.038
Tumakof Lake	core vs. freshwater	P-value = 0.001
	core vs. marine	P-value = 0.998
	freshwater vs. marine	P-value = 0.001

Table 2.8 P-values calculated using Tukey's post hoc HSD procedure to determine if Mg composition was significantly between the core, freshwater, and marine growth regions of sockeye otoliths. Salmon Lake (n = 10), Klag Lake (n = 10), Redoubt Lake (n = 10), and Tumakof Lake (n = 10).

Comparisons between Mg uptake in the fresh and marine growth regions of sockeye otoliths yielded mixed results. In Salmon and Klag lakes no significant difference in Mg was detected, whereas, Redoubt and Tumakof Lake otoliths contained significant differences between the fresh and marine growth regions. Interestingly, three

lakes showed no significant variations in Mg uptake between core and marine otolith growth. Only Salmon Lake otoliths contained significant differences in Mg concentrations between the core and marine growth regions.

2.5.7 Lithium

Of the 5 elements selected for examination, Li contained the lowest detected concentrations during line scan analysis (0.05–3.0 µg g^{-1}.). As a result, Li profiles fluctuated between regions above and below the method detection limit (MDL). However, where it was detectable some consistent patterns emerged. In the core and freshwater growth periods, Li was below MDL. However, distinctive changes in Li uptake occurred while in marine water, which consistently displayed two prominent Li peaks. Most often the first Li peak occurred soon after outmigration from the lake, and provided an effective indicator for identifying outmigration (figure 2.17). However, soon after the initial peak Li concentrations in the otolith typically decreased to levels near MDL—perhaps just higher than freshwater. The second prominent peak was typically detected shortly before capture, prior to re-entry to freshwater to spawn in most otoliths. In some cases, a third marine Li peak was also detected during the middle of marine residence, but occurrences were infrequent. Little is known about Li uptake into otoliths, as it has only been reported in less than 8 papers to date. These results suggest that Li does not accurately reflect ambient chemistry. (Li profiles are located in Appendix I).

At first glance, ANOVA results demonstrate that Li uptake was significantly variable ($P < 0.0001$) during sockeye life history. However, closer inspection using Tukey's post-hoc test revealed that no significant differences existed in Li composition between the core and freshwater growth regions in all four populations (Table 2.9). The

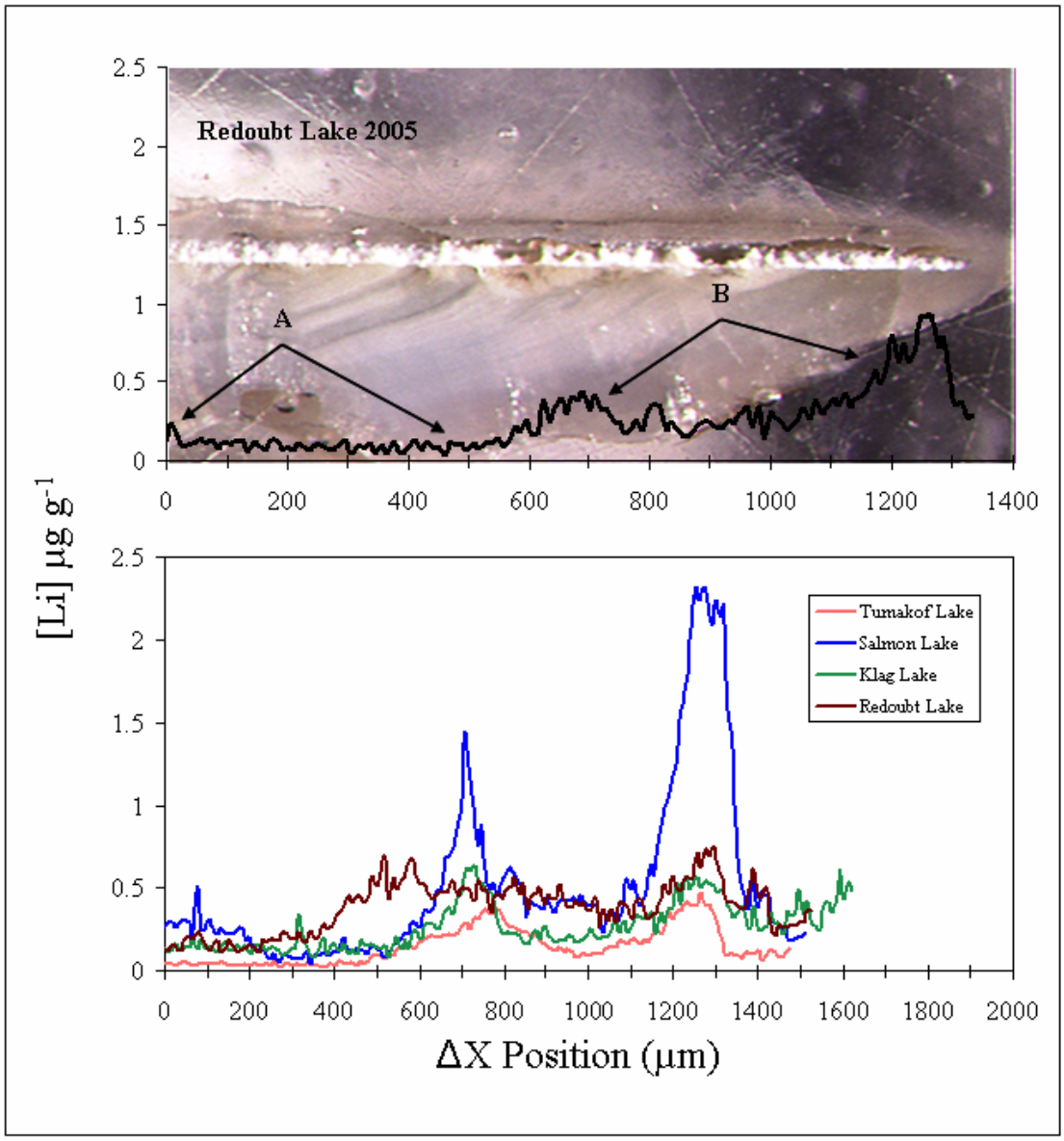

Figure 2.17 Lithium life history profiles produced from line-scan analysis extending from the core (ΔX=0µm) to the dorsal edge (ΔX ≈ 1800 µm). *Top Profile*: No identifiable Li signals in the core or during freshwater residence (A). Two Li peaks consistently occurred while in marine water shortly before transitions from fresh to marine water or vice-versa (B). *Bottom Profile*: Comparison of Li life history profiles between Tumakof, Salmon, Klag and Redoubt Lake.

major difference in Li uptake occurred during marine residence, which was significantly variable from both core and freshwater regions in all four populations.

Dependent Variable	Pair-wise Comparison	Probability
Salmon Lake	core vs. freshwater	P-value = 0.803
	core vs. marine	P-value < 0.001
	freshwater vs. marine	P-value < 0.001
Klag Lake	core vs. freshwater	P-value = 1.000
	core vs. marine	P-value = 0.001
	freshwater vs. marine	P-value = 0.001
Redoubt Lake	core vs. freshwater	P-value = 0.995
	core vs. marine	P-value < 0.001
	freshwater vs. marine	P-value < 0.001
Tumakof Lake	core vs. freshwater	P-value = 0.935
	core vs. marine	P-value < 0.001
	freshwater vs. marine	P-value < 0.001

Table 2.9 P-values calculated using Tukey's post hoc HSD procedure to determine if Li composition was significantly between the core, freshwater, and marine growth regions of sockeye otoliths. Salmon Lake (n = 10), Klag Lake (n = 10), Redoubt Lake (n = 10), and Tumakof Lake (n = 10).

2.5.8 Zinc

Life history profiles produced from Zn were not effective for tracking sockeye migration from fresh to marine water. Zinc profiles followed a highly oscillatory pattern throughout sockeye life history with no dramatic changes occurring during transitions from fresh to marine water (figure 2.18). Core signatures of Zn were inconsistent between sockeye otoliths and displayed both elevated and depleted concentrations.

Outside the core, Zn profiles demonstrated a highly oscillatory pattern from early to mid life history. Otoliths from the four populations did not exhibit any distinctive Zn signatures in fresh or marine water. In fact, Zn concentrations were highly variable between individual otoliths, often ranging from 15-180 $\mu g\ g^{-1}$. This large variability in

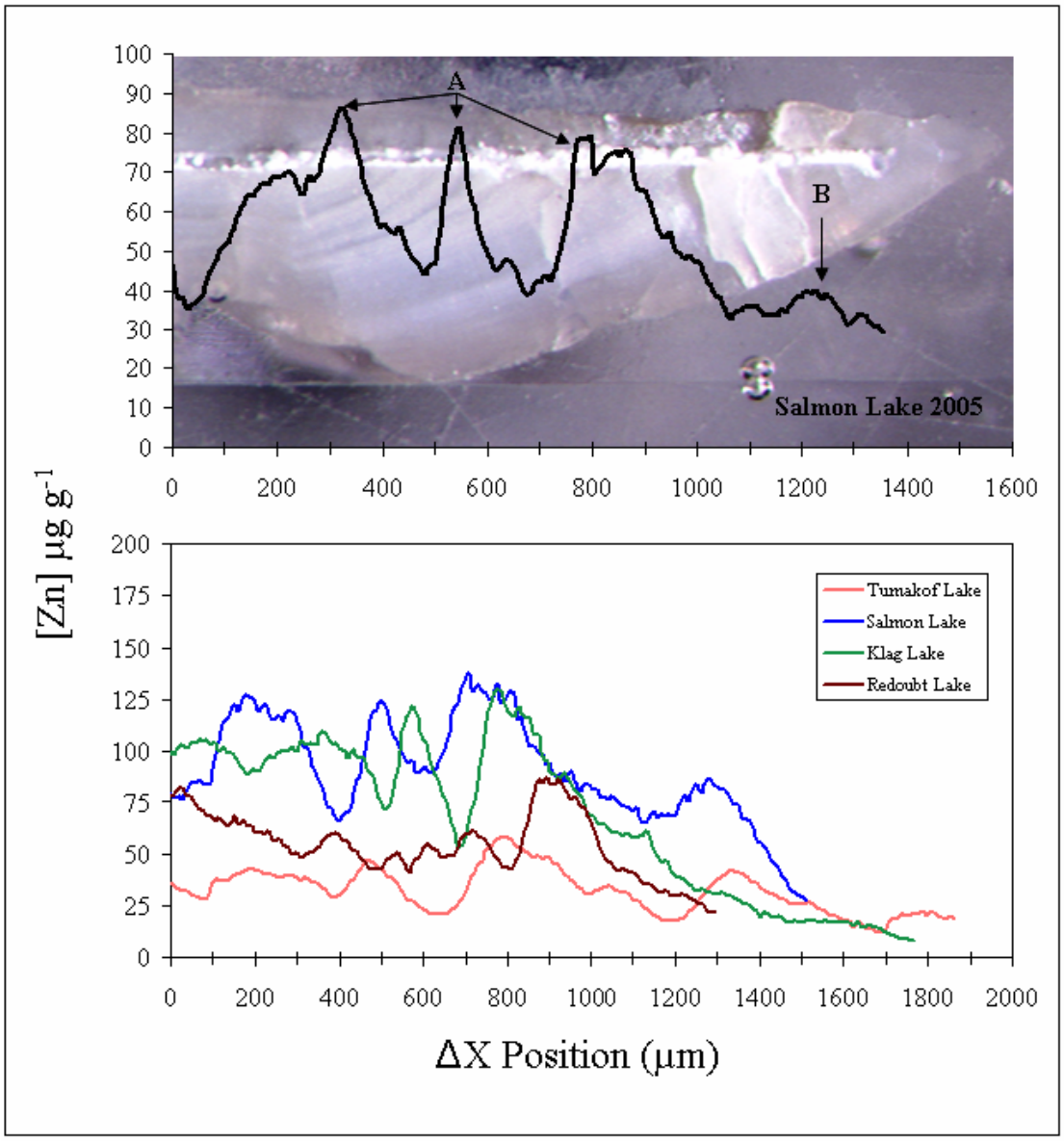

Figure 2.18 Zinc life history profiles produced from line-scan analysis extending from the core (ΔX=0µm) to the dorsal edge (ΔX ≈ 1800 µm). *Top Profile*: Early to mid life history marked by an oscillating pattern of Zn in both fresh and marine water (A). During later life history a generally decreasing pattern in Zn is observed(B). *Bottom Profile*: Comparison of Zn life history profiles between Tumakof, Salmon, Klag and Redoubt Lake.

both environments indicates that Zn is not reflective of ambient chemistry. The most prominent feature of Zn profiles were progressive declines in concentration as sockeye increased in age. Recent research has suggested that the incorporation of Zn into fish otoliths is predominantly associated with the 4% protein matrix, and is more reflective of growth, than of the ambient environment (Miller et al., 2006). The decline of Zn in the late stages of sockeye life history may support this claim, as somatic growth gradually decreases as fish age. (All Zn profiles are located in Appendix I).

Dependent Variable	Pair-wise Comparison	Probability
Salmon Lake	core vs. freshwater	P-value = 0.883
	core vs. marine	P-value = 0.173
	freshwater vs. marine	P-value = 0.368
Klag Lake	core vs. freshwater	P-value = 0.996
	core vs. marine	P-value = 0.310
	freshwater vs. marine	P-value = 0.352
Redoubt Lake	core vs. freshwater	P-value = 1.000
	core vs. marine	P-value = 0.265
	freshwater vs. marine	P-value = 0.262
Tumakof Lake	core vs. freshwater	P-value = 0.757
	core vs. marine	P-value = 0.107
	freshwater vs. marine	P-value = 0.358

Table 2.10 P-values calculated using Tukey's post hoc HSD procedure to determine if Zn composition was significantly between the core, freshwater, and marine growth regions of sockeye otoliths. Salmon Lake (n = 10), Klag Lake (n = 10), Redoubt Lake (n = 10), and Tumakof Lake (n = 10).

Results from ANOVA analysis indicated that no statistical differences could be found in Zn composition during sockeye life history in any of the otoliths. Salmon, Klag, Redoubt, and Tumakof lakes otoliths all contained P-values well beyond the accepted significance level of 0.05 at 0.177, 0.262, 0.201, and 0.119 respectively. Tukey's post-hoc procedure supported ANOVA results, showing no significant difference in Zn composition between any of three regions (Table 2.10).

2.5.9 Manganese

Life history profiles for Mn, exhibited differences between incubation and residence in fresh and marine water (figure 2.19). Manganese concentrations in the core were variable in all four systems. Many otoliths displayed elevated concentrations of Mn in the core, whereas an equivalent number of otoliths contained depleted signals. This suggests that early Mn uptake is complex, and may be affected by a suite of factors, such as ambient chemistry, maternal influences, and possibly differences in early crystal growth.

In freshwater, Mn profiles displayed an oscillating pattern, which typically contained 1—2 defined peaks. Manganese uptake in freshwater was similar between the four populations ranging from approximately 5—15 $\mu g\, g^{-1}$. Analysis of the marine entry check revealed relative declines in Mn as sockeye entered marine water, but is not as effective as Sr or Ba for determining anadromy. For the remainder of marine residence, Mn remained relatively constant at a low concentration showing that that Mn uptake in the ocean was minor. (Mn profiles are located in Appendix I).

ANOVA analysis of the core, freshwater, and marine growth regions indicated that Mn composition in Salmon (P = 0.384), Klag (P = 0.088) and Redoubt Lake (P = 0.711) otoliths was not significantly different. However, sockeye otoliths from Tumakof Lake ($P < .001$) showed that quantitatively, Mn was different in the three growth regions. Tukey's post-hoc procedure supported ANOVA results illustrating that no statistical differences existed between the core, freshwater, and marine growth regions in Salmon, Klag, or Redoubt Lake otoliths (Table 2.11). All regions in Tumakof Lake otoliths were significantly different in Mn concentration.

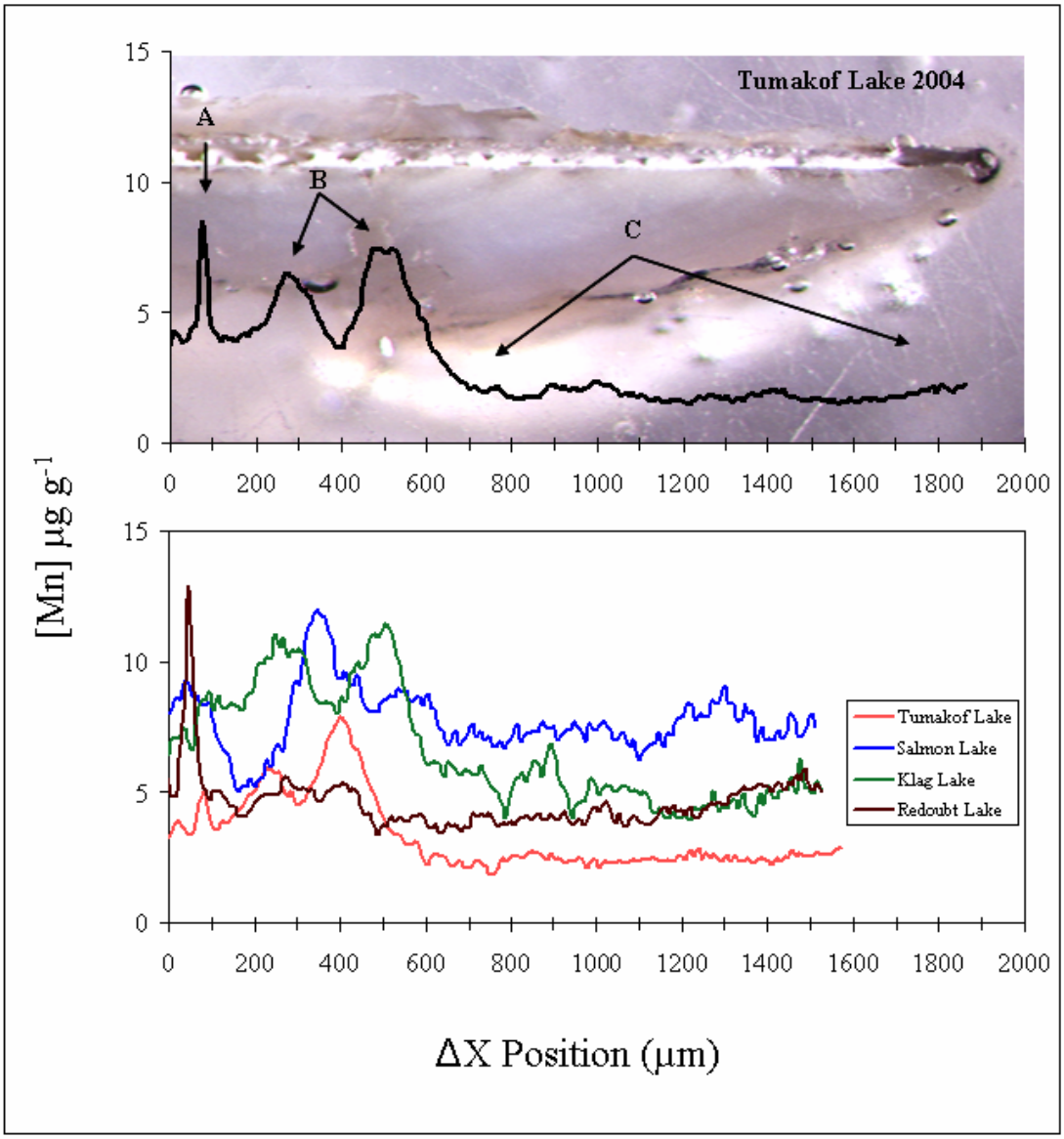

Figure 2.19 Manganese life history profiles produced from line-scan analysis extending from the core (ΔX=0µm) to the dorsal edge (ΔX ≈ 1800 µm). *Top Profile*: Core region containing an elevated Mn signal (A). Freshwater residence displaying two defined Mn peaks prior to outmigration (B). Marine residence displaying a stable low Mn signal (C). *Bottom Profile*: Comparison of Mn life history profiles between Tumakof, Salmon, Klag and Redoubt Lake.

Dependent Variable	Pair-wise Comparison	Probability
Salmon Lake	core vs. freshwater	P-value = 0.611
	core vs. marine	P-value = 0.908
	freshwater vs. marine	P-value = 0.369
Klag Lake	core vs. freshwater	P-value = 0.422
	core vs. marine	P-value = 0.566
	freshwater vs. marine	P-value = 0.073
Redoubt Lake	core vs. freshwater	P-value = 0.948
	core vs. marine	P-value = 0.866
	freshwater vs. marine	P-value = 0.692
Tumakof Lake	core vs. freshwater	P-value = 0.003
	core vs. marine	P-value < 0.001
	freshwater vs. marine	P-value < 0.001

Table 2.11 P-values calculated using Tukey's post hoc HSD procedure to determine if Mn composition was significantly between the core, freshwater, and marine growth regions of sockeye otoliths. Salmon Lake (n = 10), Klag Lake (n = 10), Redoubt Lake (n = 10), and Tumakof Lake (n = 10).

2.6 Discussion

2.6.1 Strontium

Time-series data produced for Sr demonstrated that the anadromous life history of sockeye salmon could be effectively reconstructed using full LA-ICP-MS line scans. Statistical analysis of the core, freshwater, and marine growth periods provided an effective method for detecting differences in Sr uptake during these periods. However, aggregating line scan data in this way limits the temporal resolution of life history to only broad categories, namely (1) incubation and early development, (2) freshwater residence, and (3) marine residence. Using the complete time-series provides more details from the embryonic stage until death making it possible to examine more subtle shifts in Sr uptake than methods that sample only discrete sites on the otolith. Strontium profiles showed

that uptake was dynamic across sockeye life history, but followed similar patterns between otoliths.

The first distinguishable characteristic of Sr in sockeye life history was the presence or absence of elevated concentrations in the core. Past research has shown that elevated concentrations of Sr in the cores of anadromous salmon otoliths are not uncommon (Rieman et al., 1994, Volk et al., 2000, and Arai and Hirata et al., 2005). In fact, otolith core Sr signatures have recently become adapted as an important tool for distinguishing between sympatric anadromous and freshwater-resident salmon populations (Volk et al., 2000). This application in fisheries is possible because maternal Sr signatures are transferred to progeny during egg formation or vitellogenesis, which in anadromous salmon occurs in marine water. Anadromous female salmon derive all nutritional input for egg development from marine sources. Therefore, the early incubation environment of developing embryos is enriched in marine derived elements, especially Sr. As a result, the progeny of anadromous salmon incorporate marine Sr signals during early otolith formation and growth. This phenomenon was demonstrated by Volk et al. (2000), who compared otolith core Sr from experimental crosses of anadromous and freshwater resident coho, sockeye, and chinook (*O. tshawytscha*) salmon. Results revealed that offspring produced by females maturing in seawater had core Sr:Ca values four times greater than salmon that matured in freshwater. Thus, maternal investments of Sr are a conceivable explanation for the observed elevations of Sr in the cores of Salmon and Klag Lake otoliths.

However, profiles also showed that Sr signals were not isolated to the core region, but extended well into the freshwater periods of life history. The persistence of maternal

Sr signals after line scans moved from the core suggests that maternal influence extends beyond embryonic development. The gradual decline of Sr in Salmon and Klag Lake otoliths may be related to the transition of sockeye from the alevin and fry stages. Although free of the egg as alevins, sockeye still derive a large proportion of nutrition from the ventrally attached yolk sac. The yolk sac is remnant of the egg, and so also likely to be enriched in marine nutrients. As alevins develop, the yolk sac is gradually depleted with total adsorption coinciding with emergence from the nest. Thus, as sockeye continue to develop to fry the dependence of maternal endogenous sources gradually lessens, causing gradual declines of Sr in the blood plasma and endolymph available for deposition into the otolith. To test this hypothesis we compared the average duration of maternal Sr signals in life history profiles to the documented average timing of sockeye incubation and development.

Following fertilization, sockeye eggs on average incubate for approximately 12 weeks before hatching (Groot and Margolis, 1991). After hatching alevins typically remain submerged in the nest for an additional 8 to 12 weeks before emerging as fry. Therefore, we can estimate that the total incubation and early development of sockeye salmon occurs over a period of approximately 24 weeks or 6 months. In these otoliths, 6 months of fish time equates to a distance of approximately 170 µm. Examination of the Sr profiles from Salmon and Klag lake otoliths shows that maternal signals often persisted to distances beyond 300µm (~11 months fish time), thus exceeding the estimated duration of incubation and development. This demonstrates that either maternal influences affect Sr uptake (and perhaps other developmental aspects) further into sockeye life history than previously expected; or that the growth cycle reported by

(Groot and Margolis, 1991) is not applicable to these stocks, or that the estimated distance-time relationships for these otoliths is for some unknown reason incorrect. All evidence considered, the first explanation best obeys Ockham's razor. Therefore, it is possible that maternal influences are still operating in sockeye up to 11 months after fertilization.

Despite the observed elevations of Sr in Salmon and Klag Lake otoliths, no discernible differences were detected between the core and freshwater Sr signals in Redoubt and Tumakof Lake otoliths. Probably this is due to the influence of ambient chemistry from lake water. Of the four populations, Redoubt and Tumakof lake otoliths displayed the highest uptake of Sr in freshwater. It is therefore likely that maternal signatures in the core were unidentifiable in Sr profiles due to chemical similarities with the lake water. This same explanation may also explain why Sr profiles were not effective at determining outmigration for these stocks. However, as the levels of Sr in the cores of fish from Salmon and Klag lakes were lower than in fish from Redoubt and Tumakof, this suggests that ambient water chemistry does still influence early incubation to some degree.

Outmigration from fresh to marine water was clearly pronounced as defined shifts from low to high Sr concentrations in Salmon and Klag lake otoliths. This was not the case for Redoubt or Tumakof lake otoliths, which showed little to no changes in Sr indicative of outmigration. Assuming that Sr is reflective of ambient chemistry, Sr profiles produced from Tumakof and Redoubt Lake suggest that uptake in freshwater is slightly less than or equivalent to the marine environment. This was not supported by the water chemistry data, which showed that Klag Lake contained highest Sr:Ca ratio of the

four lakes, and Sr:Ca ratios were lowest in Tumakof Lake. Normally, there is better convergence between water and otolith chemistry, especially for Sr. However, it is possible that the water chemistry samples collected for Tumakof and Redoubt Lake are not reflective of the habitats that sockeye occupy in these systems. Water samples from these lakes were collected from either the lake shore or lower reaches of the outlet streams once. They may have been influenced by dilution from run-off and or limnological stratification whereas sockeye incubation and early life history may have taken place in waters with different chemistry due to groundwater or porewater seepage into the lakes or mixing of the water column.

Although water and otolith chemistry from Redoubt and Tumakof Lake do not correspond, such anomalously high Sr:Ca freshwater values are not uncommon in Sitka, Alaska. Kraus and Secor (2004) reported that several small streams on Sitka contained mean values of Sr:Ca that exceeded seawater. Therefore, such high Sr concentrations during the freshwater phase of sockeye salmon life history are not improbable. This may be especially true in Redoubt Lake, which is meromictic and contains a dense saline bottom layer. It is doubtful sockeye ever venture into the anoxic bottom waters of Redoubt Lake, however exchanges between the chemocline and surface waters do occur. Much like holomictic lakes, the uppermost portion of meromictic lakes can undergo thermal stratification and circulate seasonally (Walker, 1974). Furthermore, physical mixing via wind can also drive periodic circulations in the water column of meromictic lakes. Therefore, it is possible that mixing between the moderately saline chemocline and upper column of Redoubt Lake creates a wide range of salinity gradients throughout the lake. This could conceivably explain the high Sr concentrations observed during

freshwater residence in Redoubt sockeye otoliths. The high Sr concentrations in Tumakof otoliths are not so easily explained. Little to no published data exists or is accessible on past water sampling in Tumakof Lake, aside from the water samples collected for this study. However, analysis of Tumakof Lake otoliths was carried out on two separate dates more than one month apart. Results from both occasions yielded high Sr concentrations during freshwater residency in Tumakof Lake otoliths. Thus, our results imply that sockeye in Tumakof occupy areas containing high Sr:Ca ratios in the lake or outmigrate to marine water immediately after emergence from the nest.

Sea-type (i.e. emigrate as fry) life histories have been documented in several sockeye stocks in Southeast Alaska (Halupka et al. 2000). Typically, sockeye stocks exhibiting sea-type life histories are associated with rivers (e.g. Situk River), and do not usually occur in lake systems. However, the high invariant Sr signals detected in Tumakof otoliths may indicate that sockeye fry migrate directly after emergence and rear in Redfish Bay. Redfish is a large bay containing several eelgrass beds that could provide sockeye fry with excellent rearing habitat. Unfortunately this hypothesis does not agree with past stock assessment data. Estimates from scale annuli in 2002, showed that 99% Tumakof sockeye stayed in the lake for two to three years before outmigration (Lorrigan et al. 2003). These estimates do not imply that Tumakof sockeye follow sea-type life histories, and further supports that Sr:Ca ratios in the lake may be elevated.

In retrospect, although other freshwater otolith studies have relied on single sample collection to characterize ambient stream water chemistry (Clarke et al. (In Press)), and it has been documented by hydrologists that single samples are generally representative of stream waters (Taylor and Hamilton, 1994). In the case of sockeye

lakes, it would be more appropriate to collect multiple water samples from areas of known fish occupancy, as it is possible and appears probable for these two lakes that water chemistry can substantially vary across rearing habitat. In this study, an extensive water chemistry survey was not part of the experimental design, and due to the remoteness and access limitations, performing one would have required considerably more resources.

Strontium uptake in marine water displayed similar patterns and concentrations between the four lakes. As stated earlier, global Sr concentrations (~8 ppm) are relatively invariant in marine water (Wadleigh and Veizer, 1985), however Sr profiles did not entirely reflect this invariant Sr concentration. Rather, life history profiles showed a gradual increase in Sr accompanied by second order periodic oscillations. Early marine residence displayed lower Sr concentrations relative to later marine otolith growth. This characteristic may be due to early residence in estuarine and coastal areas, which are less saline then the pelagic ocean. The later increase in Sr may occur as sockeye eventually migrate from these regions into the Alaskan Gyre to feed. However, many otoliths also displayed periodic oscillations in Sr during marine residence perhaps due to intermittent exposure to alternating high and moderate salinities. Sockeye primarily feed on zooplankton, and likely seek areas of high productivity to feed (Groot and Margolis 1991). It is plausible that sockeye make seasonal movements from pelagic to coastal areas in search of these food items, especially during spring periods when photoperiod and upwelling induce seasonal plankton blooms near the Northeastern Pacific coasts. A similar result was also reported by Sanborn and Telmer (2003) in the otolith of a chum salmon. Using the same method, it was found that the Chum otolith displayed three

yearly oscillations during marine residence. Like sockeye, chum salmon also rely heavily on zooplankton as a food source, and also are likely to make seasonal movements to areas of high productivity to feed.

Another, possibility that may explain these episodic Sr oscillations during marine residence may be sockeye residence in Sitka and Haida Eddies. Haida and Sitka Eddies are large volumes of low salinity, nutrient-rich water formed in late winter from the Queen Charlotte and Alexander Archipelago coasts (Batten and Crawford, 2005). These sea surface anomalies carry large nutrient loads to offshore regions and can be enriched with coastal and pelagic zooplankton species. These eddies could provide sockeye with a large forage base, which they could follow offshore or re-enter annually accounting for the seasonal oscillations in Sr.

Another fascinating aspect of the Sr profiles that occurs during marine residence is the distinct increase in uptake shortly before capture. This final increase does not correspond to expected ambient chemistry of the late stages of sockeye life history. In actuality, this period in the otolith should reflect the return of sockeye to less saline coastal and estuarine areas to spawn, and this would be expected to produce a decline in Sr. However, most otoliths demonstrated brief increases in Sr uptake during this period. It is unlikely that this final increase is caused by ambient chemistry. Other factors that have been shown to affect Sr uptake in the past have included temperature, diet, metabolism, and stress-related events (Kalish, 1992, Campana, 1999). Work by Kalish (1989) showed that there was little evidence to support a linear relationship between temperature and Sr uptake in the otoliths of Australian salmon (*Arripis trutta*) and Blue Grenadier (*Macruronus novaezelandiae*). Campana (1999) noted Sr assimilation into the

otolith via diet can occur during absorption from the intestine, but is miniscule when compared to ambient chemistry. A more logical explanation for the final Sr increase in sockeye otoliths can be related to various changes in metabolism and by stress-induced effects from the formation of gonadal tissue in preparation to spawn.

A factor that has been reported to increase Sr uptake in fish otoliths has been related to the physiological stress associated with gonad formation and spawning. Kalish (1989) reported that in Blue Grenadier, there was a gradual increase in levels of plasma Ca and plasma protein destined exclusively for the gonads prior to spawning. As a result, the level of free diffusible Ca in the endolymph is reduced due to the requirements of Ca in formation of gonads. The competition for Ca would cause a reduction of Ca in the endolymph and cause increased uptake of Sr to the otolith (Kalish, 1992). Considering that the bulk of gonad formation in these particular sockeye occurs in marine water, it is possible that a reduction of Ca in the endolymph could explain the increase of Sr near the end of life.

Another possible effect that may increase Sr uptake during later life history may be related to the differences in otolith surface area versus the volume of endolymphatic fluid in the saccular canal. In the early stages of sockeye development the ratio between the otolith and endolymphatic fluid is large, and therefore the relative size of the reservoir of elements that can substitute in the otolith is large and cannot be easily depleted (Sanborn, 2003). This may also possibly explain the elevations of various other elements in the core in relation to other regions of the otolith. However, over-time the surface area of the otolith gradually increases in relation to the total volume of endolymphatic fluid,

thereby decreasing the relative size of the element reservoir. As a result, depletion of some elements such as Ca may occur more easily, leading to increased uptake of Sr.

2.6.2 Barium

Life history profiles generated using Ba were also effective for reconstructing sockeye life history. Recent investigations into the mechanisms controlling Ba uptake to otolith aragonite have shown that ambient chemistry is a predominant factor (Bath et. al. 2000, Vries et al. 2005, Elsdon and Gillanders, 2006, Hamer et al. 2006). In this study Ba uptake was higher in the freshwater growth period of the otolith compared the marine period which complies with the typical behavior of Ba—that is higher in freshwater systems than in the marine environment (Campana, 1999). However, in addition to identifying gross differences in Ba uptake between fresh and marine habitats, Ba profiles also contain smaller, shorter term shifts reflecting other environmental shifts as follows:

Early Ba concentrations in the core were low relative to the freshwater growth periods. This characteristic may be explained in much the same way as discussed earlier for Sr. Maternal chemistry likely influences early Ba concentrations in developing sockeye embryos. However unlike Sr, Ba is not invariant in the marine environment and is ordinarily depleted in surface waters by biological activity (Hanor and Chan, 1977). Therefore female sockeye are unlikely to incorporate much Ba during egg formation while in marine water. As a result, maternal Ba signals transferred to developing embryos and alevins may be lower in concentration than the ambient levels within the nest or lake. There are no reports describing early Ba uptake in Pacific salmon otoliths, and past investigations involving Ba concentrations in otolith cores have predominantly

involved marine or estuarine species (Bath et al., 2000, Ruttenberg et al., 2005, Vries et al., 2005, Elsdon and Gillanders, 2006, Hamer et al. 2006). Ruttenberg et al. (2005) used LA-ICP-MS to characterize Ba concentrations in the cores of 5 marine and 1 anadromous fish species covering a wide variety of spawning strategies. Results indicated that – contrary to sockeye in this study--Ba was relatively enriched in the core compared to adjacent regions of the otolith in most, but not all species. Similar results were also detected by Clarke et al. (In Press), in the cores of Pacific Eulachon. However, other work by Clarke et al. (2007) using LA-ICP-MS line scan analysis on Artic Grayling otoliths showed that Ba concentrations were depleted in the core. Thorrold et al. (1997), also demonstrated that Ba concentrations in juvenile Atlantic Croaker increased as LA-ICP-MS spot analysis radiated from the core to later regions of growth on the otolith. Perhaps the primary factor causing the wide range of Ba concentrations detected in the cores between fish is related to general differences in life history. For example, residence within a specific water mass (e.g. pelagic ocean, freshwater) prior to spawning may have significant effects on maternal use of Ba during vitellogenesis. Additionally, spawning substrates and incubation periods between species will also greatly affect relative contributions of maternal Ba to the otolith during development. Finally, it is also possible that variations in the physiological requirements for Ca throughout life history could cause Ba substitution into otolith aragonite to vary independently of ambient chemistry – as described earlier for Sr – although the results from this study indicate that this is a secondary process (explained below).

Despite the wide inconsistencies between Ba concentrations in the otolith core, freshwater uptake showed similar results to past otolith research investigating Ba

(Campana, 1999, and Arai and Hirata, 2006). Sockeye otoliths showed that Ba uptake was highest in the freshwater growth period. Unlike surface waters in the marine environment, Ba is more conservative in freshwater and generally at higher concentrations (Hanor and Chan, 1977). Results here for sockeye otoliths broadly support that Ba is reflective of the ambient environment as per earlier work (Bath et al., 2000, Campana, 1999, Vries et al., 2005, Arai and Hirata, 2006, Hamer et al., 2006 and Elsdon and Gillanders, 2006). However, it is evident that Ba is not invariant during fish residence in the lakes as oscillations typically occurred. If Ba is in fact a valid proxy of the ambient environment, this indicates that Ba periodically fluctuates in the lake. This is not unreasonable as Ba can vary dramatically in temperate lakes. During periods of thermal stratification (e.g. summer and winter), Ba often accumulates in the bottom layers of temperate lakes adsorbed to iron (Fe III) and Mn (II) oxides (Finlay et al., 1983, Sugiyama, et al. 1991). However, during mixing periods, as occur during the fall and spring, particulate Ba is re-circulated to surface waters where some of it may desorb, increasing ambient concentrations. Additionally, rainfall can also significantly influence ambient Ba concentrations within temperate lakes via physical and chemical weathering of Ba-bearing rocks and sediments, especially clays (Hanor and Chan, 1977). Run-off following heavy rainfall or snow-melt could carry large sediment loads into the lake, where the sediment can desorb Ba and increase its concentration.

Life history profiles also demonstrated that Ba uptake in freshwater varied between the four populations. Of the four lakes, Tumakof Lake otoliths were most distinctive, containing freshwater Ba concentrations that were at least one order of magnitude larger than Salmon, Klag, or Redoubt otoliths. Interestingly, in contrast to Sr,

water chemistry results for Ba:Ca corresponded to the observed differences in Ba otolith concentrations from the four lakes. Water samples from Tumakof Lake resulted in Ba:Ca ratios that were proportional to the large differences in otolith Ba:Ca between the other lakes. Salmon, Klag, and Redoubt Lake water samples did not have differences in Ba:Ca ratios as dramatic as those for Tumakof Lake.

Despite the differences in Ba concentration in otoliths during freshwater residence, all otoliths showed clear and dramatic declines during outmigration. Overall, Ba profiles provided the most effective indicator of migration from fresh to marine water in this study. Yet, irrespective of the significant decline of Ba during marine entry, life history profiles demonstrated peaks shortly after outmigration. These peaks appear to coincide with residence in estuarine and coastal areas, which are subject to large temporal and spatial fluctuations of Ba (Masson, 2002, Elsdon and Gillanders, 2006, Masson, 2006, and Masson and Cummins, 2007). Estuaries in particular, are an important physical, chemical, and biological interface for the transformation of Ba into biologically available forms. Barium transported into estuaries adhered to suspended particulate matter through fluvial input is desorbed from particulates via ion exchange with abundant marine ions (Guay and Falkner, 1998). Therefore, sockeye holding within estuaries before further migration to pelagic marine water could be exposed to elevated concentrations of dissolved Ba, which may explain the Ba peaks detected shortly after outmigration.

In addition to the Ba peaks occurring after outmigration, many otoliths also displayed Ba increases later in the marine growth period. Given that Ba is on average depleted in marine surface waters, Ba profiles suggest that sockeye are occasionally

exposed to elevated Ba concentrations in the ocean. Similar results were attained by Clarke et al. (In Press) for anadromous Pacific Eulachon, which displayed an oscillating pattern while in marine water. Several possibilities may exist for the periodic Ba elevations in sockeye and eulachon otoliths, which may include temperature mediated effects, seasonal migrations to coastal waters to feed, and seasonal upwelling, with the latter believed to be the most likely (Clarke et al., in press) due to known physical and chemical oceanography of the northeast Pacific ocean (Mason, 2006). Furthermore, Bath et al. (2000) showed that temperature did not significantly affect Ba uptake in otoliths of Black Bream.

The dominant factor controlling upwelling and downwelling along the coast of the northeast in the Gulf of Alaska is wind-forcing. During winter periods, southeast wind patterns promote surface Ekman transport along the coastline, causing downwelling (Feely and Masoth, 1981). In summer, wind direction changes from southeast to northwest, causing a change in water circulation. Northwesterly wind patterns move coastal waters offshore, causing a lowering of sea level, allowing deep cold, nutrient-rich water to upwell along the coast (Davenne and Masson, 2001). Because Ba is enriched in deep marine waters as barite (Guay and Falkner, 1998), summer upwelling likely elevates Ba concentrations in surface waters. This may be further amplified by increased sediment loads carried in fluvial discharge, as warmer temperatures increase snow and glacial-melt from the mountains bordering the northeast Pacific (Feely and Masoth, 1981). Collectively, summer upwelling and increased nutrient availability from freshwater input increases primary productivity in coastal waters, thereby causing a large forage base for sockeye prey items, such as zooplankton. Seasonal migrations of sockeye

to coastal areas to feed would explain the increases of Ba in the otolith during marine residence.

2.6.3 Magnesium

The use of Mg as an indicator of ambient chemistry has produced mixed results throughout the history of otolith microchemistry. Numerous investigations have shown little evidence to support the use of Mg as an effective proxy of the ambient environment (Thorrold et al. 1997, Campana, 1999, Arai, et al. 2006). Magnesium follows a similar aqueous distribution to Sr, and marine concentrations are typically two orders of magnitude higher than freshwater (Berner and Berner, 1987). However, many reports have failed to correlate Mg composition in the otolith with migration between chemically differing habitats. Thorrold et al. (1997) found no consistent differences in Mg uptake in juvenile Atlantic Croaker that migrated from pelagic marine spawning sites to low salinity estuarine areas. Similar results were also presented by Arai et al (2006) in Chum Salmon otoliths, which showed little difference in Mg concentration throughout life history.

The most common explanation for the lack of correspondence between Mg uptake and the ambient environment is physiological regulation. Campana (1999) explained that many of the major dissolved ions, including Mg, were invariant in the blood plasma of both freshwater and marine fish. Because all ion transport to the endolymph occurs by way of blood plasma it is logical that no significant differences would exist between freshwater and marine fish otoliths (Campana, 1999). However, Mg profiles produced from sockeye otoliths in this study did not exhibit homogeneity in uptake between fresh

and marine water. Rather, they show that Mg uptake is most likely regulated by a combination of physiological effects and ambient chemistry.

Line scan analysis consistently detected elevations of Mg in the otolith core; however, unlike Sr and Ba, elevated core Mg signals cannot be explained as a maternal signal. Enriched core concentrations of Mg have also been reported in purely estuarine and marine fish species (Ruttenberg et al. 2005). This indicates that physiological processes during embryonic development not ambient chemistry probably affect early Mg uptake into the otolith. Recent research has suggested that the enrichment of many elements in the otolith core is resultant of spatial variations in the $CaCO_3$ crystal structure (Brophy et al., 2004, Melancon et al., 2005, and Ruttenberg et al., 2005). Although, most sagittal otoliths are dominated by aragonite, regions of calcite and vaterite are also known to occur in other salmonids, such as coho and chinook otoliths (Gauldie et al. 1997, Campana, 1999, and Melancon et al., 2005).

The existence of differing $CaCO_3$ polymorphs in the otolith crystal structure can have substantial impacts on elemental uptake. Melancon et al. (2005) used Raman spectrometry and found that aragonite, vaterite, or a combination of the two occurred in the cores of lake trout (*Salvelinus namaycush*) otoliths. Further chemical analysis using LA-ICP-MS showed that Mg concentrations were 30-fold higher in vaterite dominated cores than those composed of aragonite. Gauldie et al. (1997) showed similar effects on elemental uptake in coho salmon otoliths, which had undergone significant vaterite replacement. Results showed that as vaterite replaced aragonite in the otolith, Mg concentrations increased from trace levels to values exceeding 600 $\mu g\ g^{-1}$. Therefore, it is possible that the elevated Mg signatures detected in the cores of sockeye otoliths are

artifacts of early differences in crystal structure. However, without determining the crystal structure in the otolith, which is difficult on the same spatial scales, relating core signatures to vaterite or calcite is purely speculative.

Regardless, of the factors controlling Mg's occurrence in the core of otoliths the consistent presence of Mg in the core provides an effective chemical "landmark" for accurately determining if the microprobe analysis sampled the earliest regions of otolith growth.

In addition to the detection of elevated Mg concentrations in the core, sockeye otoliths showed that Mg uptake between fresh and marine water varied. This result is contrary to most investigations, which found no significant differences in Mg uptake between fresh, estuarine, or marine areas (Thorrold, et al. 1997, and Arai et al., 2006). Sockeye otoliths displayed that Mg uptake in freshwater was generally lower than marine growth periods. However, the smaller differences between chemistry of the four lakes did not produce significant differences in Mg in the otoliths of the four stocks. For example, Redoubt Lake contained the highest detected Mg concentration and Mg:Ca ratio of the four systems, but otoliths from Redoubt Lake often had the lowest Mg concentrations during freshwater growth periods.

Though Mg uptake in freshwater varied between lakes, most otoliths showed gradual increases in Mg during the transition from fresh to marine water. This behavior shows that, to a certain degree, Mg in sockeye otoliths does correspond to changes in the ambient environment. However, the gradual increase of Mg during marine entry and decrease that frequently occurred during later marine growth periods indicates that Mg is

strongly physiologically regulated by fish in comparison to other elements, such as Sr and Ba.

Other research for other salmon species has produced conflicting results. Arai and Hirata (2006), showed that Mg concentrations in chum salmon otoliths were higher in the freshwater growth zones than those found in marine. This could be explained by the difference in early life strategy between the two species, or it may be a result also of the different methods of analysis employed. Unlike sockeye, chum salmon typically migrate to sea directly after emergence (Groot and Margolis, 1991). As a result, the freshwater growth region in chum salmon otoliths will be significantly smaller than sockeye otoliths, which spend 1-3 years in freshwater. Arai and Hirata (2006) used spot analysis via LA-ICP-MS at 100 µm intervals. This approach may miss the short freshwater transition period of chum fry and unintentionally included a portion of the core which, as discussed earlier, is often elevated in Mg.

In conclusion, Mg in otoliths remains difficult to interpret. The effects of physiology and ambient chemistry remain difficult to separate, and so its utility as a tool for understanding life history, or as an indicator of stock identification likewise remains questionable.

2.6.4 Lithium

Compared to the other elements measured in this experiment, very little is known about Li in otoliths. Lithium concentrations in otolith aragonite typically border near the limits of detection in most beam based elemental assays (< 1.0 µg g^{-1}). Past research investigating Li in fish otoliths have primarily utilized solution-based ICP-MS analysis, due to its lower limits of detection (Campana, 1999 and Sanchez-Jerez et al., 2002).

Unfortunately, solution-based ICP-MS analysis using bulk or micro-milling methods are spatially limited (meaning temporally limited), and provide little information about Li uptake across the otolith growth sequence. This has been remedied in recent years with increased precision and detection limits in modern microprobe analysis. However, the factors controlling Li uptake into otolith aragonite have been rarely studied.

In these sockeye otoliths, Li was consistently detected during marine residence near transitions between fresh and marine water. The proximity of the Li peaks to transitions from fresh to marine water and vice versa suggests that a window opens and closes for Li uptake. This window may be caused by alterations in the sockeye osmoregulatory system as they move between low and high salinity environments. It may be that shifts in major element chemistry of the endolymphatic fluid (Ca, Sr, Mg, pH) that occur during these fresh-marine transitions make conditions for chemical substitution of Li into aragonite momentarily more favorable.

Other research investigating Li uptake in biogenic calcites and aragonites, such as coral skeletons and foraminifera, have shown that the modes of Li inclusion differ between the two polymorphs. Marriott et al. (2004) showed that Li uptake increased in calcites with increasing salinity, while no significant relationship between Li uptake and salinity occurred in aragonite. Marriott et al. (2004) went on to further to explain that Li uptake in calcite is incorporated within the interstitial locations of the crystal lattice, whereas Li directly substitutes for Ca in aragonite. However, this process is likely more complicated in fish otoliths due to their isolation from the external environment. Physiological factors may affect Li uptake into the otolith, although Li has no known biological function (Marriott et al. 2004). More research is needed in the mechanisms

regulating Li in the teleost circulatory system to gain a better understanding of occurrences in otolith aragonite. Regardless, as these are some of the first results reported, there may be potential for Li to be a useful indicator of life history in otoliths—especially as analytical methods will make it increasingly possible to detect it.

2.6.5 Zinc

The use of Zn to reconstruct fish life history has recently become a topic of debate in otolith microchemistry. Past reports have been inconsistent, and either attribute Zn uptake to the availability in the environment (Halden et al., 2000, Arai et al., 2007) or as an indicator of fish growth and physiological development (Miller et al., 2006). The sockeye otoliths in this study indicate that Zn uptake may be a mix of both.

Zinc concentrations in the cores of sockeye otoliths did not follow any discernible pattern, and displayed both depleted and elevated signals. Other research by Arai et al. (2007) showed that Zn signals in the cores chum salmon otoliths were consistently depleted, although as mentioned earlier, the two methods are not directly comparable. Considering that Zn follows a nutrient profile and is depleted in the marine environment relative to freshwater, it is possible that depleted Zn signals in salmon otolith cores are reflective of maternal influences. As with Ba, it is unlikely female salmon accumulate large amounts of Zn from marine water during vitellogenesis. However, the results from sockeye otoliths do not support this hypothesis, and suggest that Zn uptake is highly regulated by fish physiology during early incubation and development.

Zinc uptake was typically highest in the freshwater growth regions of sockeye otoliths. However, Zn uptake did not exhibit significant declines immediately after

outmigration, which would be indicative of low ambient concentrations. Rather, Zn profiles oscillated well into the marine portion of sockeye life history, followed by a gradual decline until death. The same pattern was also detected in adult chum salmon otoliths by Arai et al (2007), which detected gradual Zn declines in the marine environment. These results do not indicate that ambient Zn concentrations were directly responsible for otolith uptake. It is perhaps more likely that the oscillatory pattern of Zn is related to fish growth.

As an alternative to ambient chemistry, fast and slow growth periods may explain the oscillatory pattern of Zn in sockeye otoliths. Willis and Sunda, (1984) determined that food was the predominant source (78-82%) of Zn in teleost fish with any deficiencies being met by increased branchial uptake. In temperate fish species like sockeye, Zn uptake in the otolith would be increased during summer periods when metabolic and feeding rates were high. Decreased Zn concentrations would occur during winter when low temperatures decrease sockeye metabolism and growth. A similar behavior in Zn uptake was found by Halden et al. (2000) in dianadromous Arctic Char otoliths using a scanning proton microprobe. Results displayed that Zn followed an oscillatory pattern with peaks coinciding with fast growth periods, and that growth was fastest during early life history. It was also shown that Zn uptake in char otoliths overlapped with marine migrations, and that Zn gradually declined as char increased in age. The same gradual decreases in Zn were also witnessed in later growth regions of sockeye and chum salmon otoliths (Arai et al., 2007). This behavior is likely indicative of slowed growth rates, which often occurs as teleosts increase in age and metabolism slows (Campana, 1999). This pattern of decreased Zn in later growth regions of sockeye, char, and chum otoliths

corresponding to lower metabolic growth adds further testimony that Zn uptake is mainly a function of growth, and not ambient chemistry.

The link between fish growth and Zn uptake into the otolith has become more provocative in recent years with the discovery that Zn may be more concentrated in the 4-5% protein matrix. Miller et al. (2006) developed a method for extracting otolith proteins without the total disruption of transition metal binding. Results showed that 40-60% of Zn found in whole otoliths was bound in the organic protein matrix. Interestingly, this finding does not coincide with Zn peaks, which occur during fast growth periods in sockeye and char otoliths. If Zn is predominantly sequestered in the protein matrix, it would be expected that Zn peaks would coincide with winter periods denoted by the hyaline high protein/low aragonite regions of the otolith (Gauldie et al. 1987)—dark bands. Here, it seems the empirical evidence supports the growth based uptake model. Further investigation may yet reveal the role of the aragonite versus protein matrix of the otoliths.

2.6.6 Manganese

Manganese is fast becoming a popular tool in otolith microchemistry for reconstructing fish life history. Several studies have linked Mn composition in fish otoliths to increased Mn concentrations in the ambient environment (Bath et al.2000, Sanchez-Jerez et al. 2002, and Arai and Hirata, 2006). Unlike other transition metals such as Zn, Mn has not been shown to be affiliated with the protein matrix (Miller et al., 2006). Manganese can readily substitute for Ca in otolith aragonite due to similarities in valence and ionic radius. Furthermore, Mn is less regulated by fish physiology than

many of the major ions, such as Mg, Na, K, and Cl (Campana, 1999). These characteristics enhance the likelihood that Mn uptake is reflective of environmental availability. Sockeye otoliths in this study demonstrated that Mn was effective for detecting differences in ambient Mn between fresh and marine water, but core signals were variable between otoliths.

Many sockeye otoliths contained elevated Mn concentrations in the core, whereas an equivalent number of otoliths displayed depleted Mn signals in the core. Manganese is generally a non-conservative element in the aqueous environment. In marine water, like Ba, it exhibits a nutrient-like profile that leaves surface waters depleted in dissolved Mn. It is therefore unlikely that female sockeye salmon incorporate much Mn during egg development, and it is to be expected that most otolith cores would contain low Mn concentrations. However, because a substantial proportion of otoliths contained elevated Mn signals in the core, it is unlikely that that maternal influence is a factor. A plausible explanation accounting for the variability in Mn concentrations in the cores of sockeye otoliths may be attributed to ambient concentrations in the nest.

In oxic marine and freshwater, Mn often occurs as Mn oxide coatings (or oxyhydroxide coatings) on particulate matter which ultimately sinks and forms bottom sediments (Sugiyama et al., 1991). During early diagenesis when microbial consumption of organic matter depletes bottom and porewaters in oxygen, these Mn coatings are reduced and dissolved Mn is released to bottom waters, especially in temperate lakes (Davison, 1992). In fish species that utilize substrate for spawning, close associations between developing embryos and elevated concentrations of Mn may exist while incubating in the nest. Though largely considered a closed system to the external

environment, salmon eggs are semi-permeable, and could allow Mn ions to enter the egg (Brophy et al., 2004). This may be especially true for sockeye eggs, which incubate during winter periods when temperature stratification could cause hypoxia to occur in bottom waters. Therefore, differences in spawning locations (e.g. inlet stream, lake shore) could influence early Mn exposure to developing sockeye embryos. However, elevated Mn signals have also been reported numerous species that do not utilize bottom substrates for spawning.

Brophy et al. (2004) demonstrated that elevated signals of Mn were ubiquitous in the cores of demersal spawning Atlantic Herring (*Clupea harengus*), and pelagic spawning Sprat (*Sprattus sprattus*). Similar findings were also produced by Ruttenberg et al. (2005), who reported enriched Mn concentrations in the cores of six different fish species in marine and freshwater. Taken together these results suggest that Mn elevations in otolith cores may not be associated to increased ambient concentrations during incubation.

Another possibility for the elevated concentrations of Mn signals in the cores of sockeye and other teleosts may be related to early differences in the otolith crystal. As discussed earlier for the elevated Mg concentrations in sockeye otolith cores, Mn uptake can significantly vary between different $CaCO_3$ polymorphs. Brophy et al. (2004) noted that Mn has a higher affinity to substitute in calcite than aragonite, and could explain the elevated Mn signals in the otolith cores of Atlantic herring and Sprat. It was also shown by Melancon et al. (2005) that Mn concentrations were 5 times higher in vateritic portions of lake trout otoliths in comparison to aragonite. Unfortunately, it is not possible to determine mineralogy using LA-ICP-MS analysis. A possible solution for determining

if early crystal growth in the otolith core is different than later growth regions of aragonite could be accomplished using micro-milling procedures combined with X-ray diffraction and X-ray absorption fine structure spectroscopy (XAFS). Pattanaik (2005) analyzed the bulk crystal structure of crevalle jack fish (*caranx hippos*) otoliths using XAFS. Analysis showed that mineralization was entirely aragonite, which was evident from a predominant orthorhombic crystal structure. However, considering the small mass of core material in relation to the remainder of otolith, it is possible that the presence of vaterite could be obscured. Perhaps, future work attempting to relate early element enrichments in the otolith core should include XAFS techniques.

Despite the ambiguous nature of early Mn incorporation into the primordial core, differences in Mn uptake between fresh and marine water appear to be related to environmental availability. Sockeye otoliths displayed greater Mn uptake in freshwater relative to marine water, displayed by an oscillating pattern. This pattern may be related to seasonal distributions of Mn in temperate lakes. Like Ba, seasonal turnover can re-circulate Mn sequestered in particulate matter from the anoxic bottom waters of the lake, periodically increasing ambient Mn ions in surface waters.

Comparisons between Mn concentrations from water samples and concentrations observed in sockeye otoliths did not coincide. However, recent research has shown that Mn uptake may not be directly related to ambient chemistry. Sanchez-Jerez et al. (2002), suggested that a trophic transfer of Mn took place into juvenile trumpeter (*Pleates sexlineatus*) residing in sea-grass beds. Results found that Mn concentrations in sea-grass constituents such as detritus and prey items were strongly correlated with concentrations in the otoliths. If Mn concentrations in the otolith are reflective of dietary uptake, it

should be possible to associate Mn signals to specific feeding habitats used during fish life history.

Because, Mn concentrations in freshwater are higher relative to the marine environment, sockeye prey items in freshwater, such as cladocerans and copepods, also contain freshwater Mn signatures. Small suspended particles and organisms in lake water are loci for Mn oxyhydroxide precipitation, and it is possible that sockeye fry ingest significant quantities of Mn through diet. This may explain the decline of Mn in sockeye otoliths after outmigration in addition to ambient water chemistry. Similar results were obtained in the otoliths of catadromous Japanese eels (*Anguilla japonica*) using LA-ICP-MS analysis (Arai and Hirata 2006). Though relationships between water chemistry and trophic transfer were not addressed in eel otoliths, analysis showed that Mn:Ca ratios were significantly higher during freshwater residence than in marine water. Regardless of the source, the consistent pattern of uptake between fresh and marine water shows that Mn uptake is at least a consistent proxy for migrations between marine and freshwater.

2.7 Conclusions

This study set out to accomplish three main goals using LA-ICP-MS line scan analysis. These were: (1) the ability to reconstruct sockeye life history from fresh to marine water using various trace elements in the otolith growth sequence; (2) to examine any elemental shifts possibly related to ontogenetic factors; and (3) to provide understanding on the mechanisms controlling elemental uptake in fresh and marine water. It was possible to address these questions and more.

Chemical life history profiles generated from LA-ICP-MS time-series data provided an effective tool for reconstructing the life of sockeye from birth to death. Other commonly used analysis methods, such as spot analysis, do not utilize the complete chronological chemical record in fish otoliths, and may fail to detect many subtle shifts occurring during fish life history. This problem is avoided using high resolution line scan analysis. Not only was it possible to effectively follow sockeye migration from fresh to marine water, but small temporal shifts in elemental uptake were detected, many of which have not been previously reported.

In this study, 10 representative adult sockeye otoliths were analyzed from each of four separate populations located on Sitka, Alaska. All sockeye sampled followed an anadromous life history, spending 1-3 years in freshwater before migrating into the Gulf of Alaska to feed. LA-ICP-MS line-scans progressed from the dorsal edge through the core to the opposing ventral edge of transversely sectioned sockeye otoliths. Otoliths were measured for signal intensities of ^{43}Ca, ^{86}Sr, ^{137}Ba, ^{24}Mg, ^{55}Mn, ^{66}Zn, and ^{7}Li, and were normalized to ^{43}Ca. Sockeye provided an excellent candidate species for addressing factors controlling elemental uptake into the otolith in both the fresh and marine environments. Results showed that several elements, such as Sr, Ba, and to a certain degree Mg, were effective for tracking migration from fresh to marine water during sockeye life history. Manganese was also effective for determining migration to fresh and marine water, however it is believed that diet, not ambient chemistry is the factor controlling uptake. Elements, such as Zn and Li provided information related to fish physiology, such as growth and changes in osmoregulation during transitions from low to high salinity environments.

The use of Sr, Ba, Mg, Mn, and Li to track sockeye migrations from freshwater and marine habitats has several important applications in fisheries science. In freshwater systems containing both sympatric anadromous and freshwater-resident salmon, it can be difficult to differentiate individuals that migrated from fresh to marine water. Using concentrations of Sr, Ba, Mn, Mg, and possibly Li in the otolith it should be possibly to identify if specific individuals ever migrated to the ocean. However, recent developments have shown that trace element compositions in fin rays can also be used to identify if fish moved between chemically differing habitats (Veinott et al. 1999, and Clarke et al., (In press)). If fins rays are effective at reflecting ambient chemistry, they may provide an effective, non-lethal alternative to otolith sampling.

It was also found that many elements were either enriched or depleted in the core of sockeye otoliths. For elements, such as Sr and Ba it is likely maternal input may control elemental uptake during incubation and early development. These elements may also function as a discriminatory tool in mixed stock systems where sympatric anadromous and fresh-water resident salmon exist. However, for elements such as Mn and Mg, maternal influence is not likely a factor causing early enrichment in the core. It is more likely that physiological and possibly differences in early crystal structure affect the uptake of Mg and Mn. This finding has significant impacts on the practice of using otolith core chemistry for stock identification purposes. If trace element uptake is augmented in the core region by maternal investments and differences in early crystal growth, using core trace element signatures as an indicator of stock origin could be inaccurate.

As occurs in most scientific research, many of results obtained from LA-ICP-MS analysis posed as many questions as answers. It is obvious that many aspects of elemental uptake from the environment into fish otoliths are still unknown. However, this is to be expected considering that fish are complex organisms and are constantly regulating various physiological functions to maintain homeostasis. This is even further complicated in anadromous fish species, such as sockeye, which change osmoregulation twice during life history. However, from this study it is apparent that the field of otolith microchemistry need not be limited to the fish biologist. Otolith microchemistry requires a diverse field of scientific knowledge from disciplines that include but are not limited too, biology, chemistry, physics, geology, geochemistry, mineralogy, limnology, oceanography, and fish ecology. Further collaboration between these numerous disciplines shall only increase our understanding of the otolith as an informational source.

Chapter 3

Stock Identification of Sockeye Salmon (*Oncorhynchus nerka*) Using Otolith Trace Element Chemistry

3.1 Introduction

Sockeye salmon (*Oncorhynchus nerka*) are a prized resource of the indigenous peoples living in the Alexander Archipelago of Southeast Alaska. This was especially true of the Tlingit peoples living on the islands of Chichagof, Kruzof, and Baranof, which compose modern day Sitka. Compared to other salmon species endemic to Sitka, sockeye are the first species to return in large numbers to spawn during late spring and early summer. Annual returns of sockeye provided the Tlingit people with an important source of protein following winter periods. Due to the importance of sockeye as a food resource, subsistence harvests were strictly regulated by differing Tlingit clans to ensure that stocks were not over-fished. It has been noted that "higher status clans had the prestige and responsibility of managing sockeye lakes" from overuse by other clans and early settlers (Conitz et al., 2005). Today sockeye are still an important subsistence resource to the rural communities of Sitka, both indigenous and non-indigenous.

Modern fisheries practices in Alaska give “top priority to the subsistence use of fish resources” (Woodby et al., 2005). However, this was not always the case. From as early as 1812, large commercial harvests of sockeye were documented in Sitka, and a maximum harvest of 3,500,000 sockeye was recorded for all of Southeast Alaska in 1914 (Haluptka et al., 2000). Unfortunately, early fishery management efforts were “weak, poorly funded, and ineffectively enforced” (Woodby et al., 2005). As a result, several sockeye stocks in the Sitka area, as well as other regions of Alaska, were over-exploited and depleted. Declines in returning sockeye had adverse effects on indigenous and non-indigenous subsistence users state-wide. In response to the dramatic declines, the State of Alaska applied greater restrictions on commercial harvests.

As a result of better management practices, most sockeye stocks in Sitka have recuperated to healthy and sustainable populations. However, in many remote sockeye systems, little is still known about stock structure. Recent low escapements in large sockeye systems close to Sitka (e.g. Redoubt Lake 2000-2001) prompted concern among subsistence and sport users that stocks were in decline. In response to the concerns, State, Federal, and Tribal organizations implemented intensive stock assessment studies at several sockeye lakes important to the Sitka community. Lakes of special concern included, but were not limited to: Redoubt, Klag, Salmon, and Tumakof (also known as Redfish) lakes. Stock assessments in Redoubt Lake have been rigorously conducted by the United States Forest Service (USFS) for the past 20 years, with the exception of 1998 (Geiger, 2003). In more recent years, collaborations between the Sitka Tribe of Alaska (STA), Alaska Department of Fish and Game (ADF & G), and USFS have orchestrated

sockeye stock assessment studies at Salmon (2001-2006), Klag (2001-2007), and Tumakof Lakes (2002-2004).

Sockeye stock assessments follow a similar study design for all four lakes. Mark/recapture methods are employed to estimate sockeye escapement in each system. During the spring and summer field seasons, crews maintained fish weirs in the four watersheds, counting returning adults and marking an estimated proportion (e.g. ~ 20%) of returning sockeye using various fin-clips or t-bar Floy™ tags (Lorrigan et al., 2003, Conitz et al., 2005, and Tydingco et al., 2006). Crews also randomly sampled individuals from the returning population for length, weight, sex, and scales. In Klag and Tumakof Lakes, a creel census was also conducted in which crews interviewed subsistence and sport fishers for the number of salmon species harvested, time fished, and gear used. The data collected from these systems has used by fisheries biologists and managers for understanding the stock structure of these sockeye populations, and has supported management practices of these resources.

The sockeye returning to these four lakes are separate stocks as they return to geographically different watersheds and exhibit unique characteristics in run timing and age class (Haluptka et al., 2000). It is, however, difficult for biologists and managers to identify a specific sockeye stock when it is not within close proximity to its natal watershed. Age and length composition and run-timing by themselves are not accurate methods for identifying a specific salmon stock in marine water or in large mixed-stock freshwater systems, such as the Columbia or Fraser Rivers. Poor identification of stocks can impact exploitation decisions, especially in areas where mixed-stock fisheries occur. “Obtaining accurate information on relative stock contribution in most mixed stock

fisheries, and evaluating a fishery's impact on those component stocks" has proven difficult and expensive in the past (Lloyd, 1996). To improve decision making, man-made and natural tags have been applied in fisheries science to discriminate between differing stocks of wild salmon.

Unlike hatchery-produced salmon, it is nearly impossible to differentiate between wild salmon stocks. Hatcheries often apply physical marks (e.g. adipose-clip) or tags (e.g. PIT and Coded Wire Tags) to salmon as a means of identification (Murphy and Willis, 1996, and Kennedy et al., 2000). More recently, hatcheries have also induced thermal and chemical marks in otoliths during early incubation and development (Schroder et al., 1995, Kudzina and Chebanov, 2004, Quinn et al., 2006, and Telmer et al. 2006). This practice is especially important in mass production hatcheries for pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*), where it is not feasible to mark or tag each individual fish. However, physical marks, tags, and induced thermal and chemical marks in otoliths can only be easily achieved in hatchery settings. Attempts to capture and tag wild salmon fry or smolts before migration to the ocean have been successful, but are time-consuming, expensive, risky for the fish, and can only sample a limited number of the population. This has prompted natural tags to become a subject of great interest in fisheries science. A growingly popular method is the use of otolith trace element chemistry to discriminate between disparate salmon stocks.

Otolith microchemistry has shown that trace element signatures incorporated during early life history can be used as an effective natural tag or "fingerprint" for identifying discrete stocks of wild fish (Kalish, 1989, Campana, 1999, Sohn et al., 2005, Veinott and Porter, 2005, and Clark et al., 2007). Otoliths continually deposit increments

of growth during fish life history, and are acellular and metabolically inert. This characteristic allows any elements or compounds deposited onto the growing surface of the otolith to remain permanently fixed throughout fish life history (Campana, 1999). Otoliths are chemically composed of ~ 96% $CaCO_3$ in the form of aragonite, ~4 % organic protein matrix, and < 1% non-organic trace elements (Campana, 2004). Many of the trace elements accumulated into fish otoliths have been found to reflect the environmental chemistry experienced during fish life history. For example, Sr and Ba in the otolith have been shown to reflect ambient water chemistry (Thorrold et al., 1997, Radtke et al., 1998, Bath et al., 2000, and Vries et al. 2005), whereas other elements, such as Zn and Mn, have been shown to reflect physiological factors, including growth and diet (Halden et al., 2000, Sanchez-Jerez et al., 2002, and Miller et al. 2006), albeit new interpretations continue to appear. The otolith's ability to record and retain chemical information from the environment provides a potentially powerful tool for the identification of wild salmon stocks.

Fish stocks may be classified as a group of individuals that share the "same habitats or distribution, and are part of the same gene pool through sexual reproduction of interbreeding individuals within the population" (Sohn et al., 2005). This classification is especially pertinent in anadromous Pacific salmon stocks, which exhibit a life history characteristic known as philopatry or "homing" (Quinn et al., 1999). Homing simply refers to the return of salmon to a geographically specific freshwater system to spawn. Most freshwater lakes and river systems exhibit distinctive chemical signals influenced by the surrounding lithology, which are often reflected in the otolith (Kennedy et al. 2000, and Veinott and Porter, 2005). Since Pacific salmon incubate and rear in

freshwater, it is possible to use trace element signatures acquired in the early growth regions of salmon otoliths to chemically discriminate different stocks of fish.

Two of the most common methods currently used for stock ID utilize elemental signatures from whole dissolved otoliths or the primordial core. Solution-based approaches using whole otoliths for bulk analysis are popular for stock ID due to the ease of preparation, the reduction of error associated with identifying growth increments, and most importantly, lower detection limits than most beam-based assays (Campana, 1999, De Pontual et al., 2000, and Sanborn and Telmer, 2003). However, due to the relative abundance of Sr, Ba, Mg, Zn, and Mn in otoliths, differences between the detection limits of solution-based and beam-based methods are negligible. The major disadvantage of bulk analysis is that elemental signatures are integrated from the entire lifetime of the fish, and do not account for any differences in chemical composition across the chronological growth sequence of the otolith. This aspect is especially important for discriminating between fish stocks like Pacific salmon, which return to specific locations to spawn after having migrated through different chemical environments during their life history. In these instances solution and beam-based analysis of the otolith core have been applied as a direct measure of stock origin (Campana, 1999).

The core represents the earliest growth region of the otolith and provides a defined target for analysis. This approach to stock ID has been at least partially successful in numerous studies (Sie and Thresher, 1992, Severin et al. 1995, Milton et al. 1997, and Thorrold et al. 1997; however, recent findings (Chapter 2) have demonstrated that many elemental signatures in the otolith core are influenced by maternal and physiological factors (Rieman et al., 1994, Volk et al. 2000, Arai et al., 2006, Brophy et

al., 2004, and Ruttenberg et al., 2005), and may be inaccurate as an indicator of stock origin, especially for anadromous salmon. To address this problem, alternative regions of the otolith growth sequence need be examined for stock identification (ID).

In this experiment, trace element concentrations in the core, freshwater, and marine growth regions of sockeye otoliths were examined and compared for stock ID purposes. Sockeye otoliths from four separate lakes in Sitka, Alaska were analyzed using high resolution line scans via laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Each growth region was evaluated separately for its ability to correctly classify sockeye stocks to a specific watershed using step-wise discriminant function analysis.

3.2 Study Sites and Methods

3.2.1 Study Sites, Sampling Methods, Otolith Preparation, and Analysis Procedures

All study sites, sampling methods, otolith preparation, and analysis procedures are the same as described in Chapter 2. However, length data (when available) and annuli estimates from otoliths were also used to examine the age composition and length distribution from each stock. Due to low sockeye escapements in Salmon Lake, all otoliths were collected from post-spawn fish. As a result, no length data was available from Salmon Lake sockeye due to decomposition.

3.2.2 Age Estimation

Sockeye age was visually estimated from transversely sectioned otoliths by counting annuli. Aging methods in this study followed the European system commonly used in Alaska, which separates periods spent in fresh and marine water (e.g. 2.2 denotes

2-years freshwater and 2-years saltwater). However, it is important to note that the European system standardizes all salmon birth dates to January 1st, regardless of date of hatch. For example, a sockeye spawned in the fall of 2000, but hatched in the winter of 2001 is still considered a 0-aged fish from the period of 2001-2002. Therefore, a sockeye denoted as a 2.2 year age class actually refers to a 5 year old fish. Scale data was not available for age validation, therefore length frequency distributions (when available) were used to validate the dominant age classes sampled from each stock.

3.2.3 Data Separation

Time-series data representative of the core, freshwater, and marine growth periods were isolated using chemical life history profiles generated by LA-ICP-MS analysis (Chapter 2). In most cases, Sr concentration profiles provided an effective means for identifying and separating each growth period in sockeye otoliths (figure 3.1). However, in otoliths from Tumakof and Redoubt Lakes, Sr was not always reliable for separating the three growth regions. In these cases, the three growth regions were identified based on a combination of Sr, Ba and Mg profiles.

Barium concentration profiles followed an inverse pattern to Sr in sockeye otoliths, and displayed defined decreases during marine entry (Chapter 2). This pattern provided an effective alternative to Sr for determining residence in the lake and marine water. Core data was separated using Mg, which was consistently elevated in the cores of the otoliths (Chapter 2). Magnesium peaks in the core provided a chemical mark for distinguishing between core and freshwater growth periods in the otolith. Using the combination of these elements, it was possible to separate elemental signatures

representative of each growth period during sockeye life history for subsequent statistical analysis.

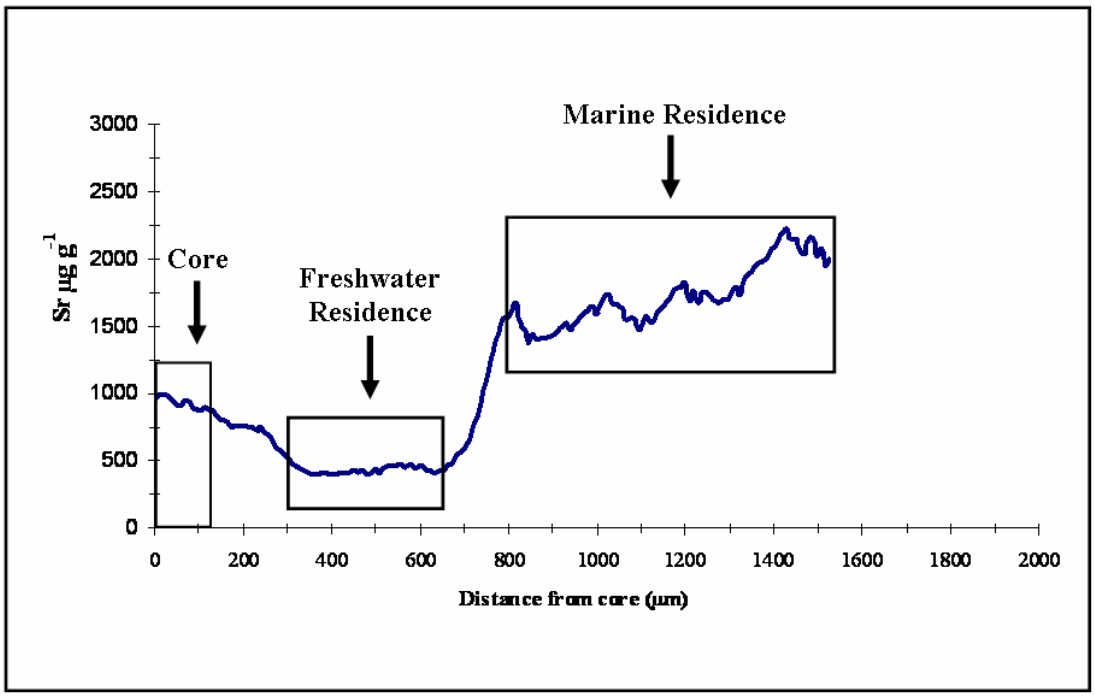

Figure 3.1 Demonstration of how core, freshwater, and marine data were isolated from strontium profiles.

3.2.4 Statistical Analysis

Elemental signatures in the core, freshwater, and marine growth regions (i.e., the average concentration for each region) of sockeye otoliths were differentiated using Step-wise Discriminant Function Analysis (SPSS™ 11 and 14 statistical software). Step-wise discriminant function analysis (DFA) is a statistical technique used to predict group membership, which in this case classifies sockeye to a specific lake system. A multivariate combination of Sr, Ba, Mg, Mn, and Zn were used as predictors to classify sockeye otoliths to a specific lake.

The assumptions used for DFA are similar to those applied in multivariate analysis of variance (MANOVA) (Tabachnick and Fidell, 2001). Unequal sample sizes are acceptable when using DFA, and violations of normality are not fatal. However, data in this study were log (ln) transformed to better approximate a Gaussian distribution. A leave-one-out cross validation classification matrix was used to determine the proportion of correctly classified otoliths. In a leave-one-out cross validation matrix each case is classified by the functions from all cases other than that case (also known as the U-method). The significance of each element (predictor) in DFA was assessed using a Wilks'-Lambda test.

3.3 Results

3.3.1 Age and Length Composition

Age estimates from otolith annuli indicate that 4 and 5 year old sockeye were the dominant age groups sampled from all four lakes (Table 3.1). Salmon Lake otoliths from 2003 and 2005 displayed that 1.2 and 1.3 age classes comprised over 60% of fish sampled. However, in 2004, 1.2 and 2.2 aged fish were the largest age classes comprising 72% of the fish sampled in Salmon Lake. Annuli estimates from Klag Lake otoliths in both 2004 and 2005, showed that the predominant age classes sampled were from 1.2 and 1.3 cohorts, and comprised 79% and 59% of fish sampled respectively. However, in 2005 42% of the otoliths sampled from Klag Lake could not be aged accurately due to difficulties in identifying marine annuli. Age estimates from Redoubt Lake showed that a variety of different age classes were sampled. In 2004, 1.2 and 2.2 age classes comprised 68% of the sockeye sampled for otoliths, whereas in 2005 age

classes were evenly mixed between 1.2, 1.3, 2.1 and 2.2 aged sockeye. Annuli based estimates from Tumakof Lake otoliths showed that 1.3 and 2.2 aged sockeye were the largest age class comprising 59% of the fish sampled.

Salmon Lake 2003									
	Brood Year	**2000**	**1999**	**1998**	**1999**	**1998**	**1997**		
	Age	**1.1**	**1.2**	**1.3**	**2.1**	**2.2**	**2.3**	**unknown**	**All ages**
	Sample Size	1	5	1	0	2	0	0	9
	Percent	11%	56%	11%	0%	22%	0%	0%	100%
Salmon Lake 2004									
	Brood Year	**2001**	**2000**	**1999**	**2000**	**1999**	**1998**		
	Age	**1.1**	**1.2**	**1.3**	**2.1**	**2.2**	**2.3**	**unknown**	**All ages**
	Sample Size	1	5	0	0	3	0	2	11
	Percent	9%	45%	0%	0%	27%	0%	18%	100%
Salmon Lake 2005									
	Brood Year	**2002**	**2001**	**2000**	**2001**	**2000**	**1999**		
	Age	**1.1**	**1.2**	**1.3**	**2.1**	**2.2**	**2.3**	**unknown**	**All ages**
	Sample Size	1	6	7	0	3	0	2	19
	Percent	5%	32%	36%	0%	16%	0%	11%	100%
Klag Lake 2004									
	Brood Year	**2001**	**2000**	**1999**	**2000**	**1999**	**1998**		
	Age	**1.1**	**1.2**	**1.3**	**2.1**	**2.2**	**2.3**	**unknown**	**All ages**
	Sample Size	0	8	7	0	0	1	2	19
	Percent	5%	42%	37%	0%	0%	5%	11%	100%
Klag Lake 2005									
	Brood Year	**2002**	**2001**	**2000**	**2001**	**2000**	**1999**		
	Age	**1.1**	**1.2**	**1.3**	**2.1**	**2.2**	**2.3**	**unknown**	**All ages**
	Sample Size	0	2	5	0	0	0	5	12
	Percent	0%	17%	42%	0%	0%	0%	42%	100%
Redoubt Lake 2004									
	Brood Year	**2001**	**2000**	**1999**	**2000**	**1999**	**1998**		
	Age	**1.1**	**1.2**	**1.3**	**2.1**	**2.2**	**2.3**	**unknown**	**All ages**
	Sample Size	0	6	3	1	9	3	0	22
	Percent	0%	27%	14%	5%	41%	14%	0%	100%
Redoubt Lake 2005									
	Brood Year	**2002**	**2001**	**2000**	**2001**	**2000**	**1999**		
	Age	**1.1**	**1.2**	**1.3**	**2.1**	**2.2**	**2.3**	**unknown**	**All ages**
	Sample Size	2	4	5	3	3	0	0	17
	Percent	12%	24%	29%	18%	18%	0%	0%	100%
Tumakof Lake 2004									
	Brood Year	**2001**	**2000**	**1999**	**2000**	**1999**	**1998**		
	Age	**1.1**	**1.2**	**1.3**	**2.1**	**2.2**	**2.3**	**unknown**	**All ages**
	Sample Size	0	1	5	0	2	1	3	12
	Percent	0%	8%	42%	0%	17%	0%	25%	100%

Table 3.1 Age composition estimated via otolith annuli for sockeye sampled from Salmon, Klag, Redoubt, and Tumakof Lake (2003- 2005).

The average mid eye to fork length measured from sockeye was similar between all four systems. Length data for Klag, Redoubt, and Tumakof Lakes are presented in table 3.2. Length frequency histograms showed that size class corresponded with age estimates from annuli (figure 3.2). In general, 1.3 age class sockeye were larger than 1.2

Watershed (year)	N	Length
Salmon Lake 2003	*	no length data available
Salmon Lake 2004	*	no length data available
Salmon Lake 2005	*	no length data available
Klag Lake 2004	19	535 ± 34.6 (475-590)
Klag Lake 2005	12	524 ± 32.6 (465-580)
Redoubt Lake 2004	22	542 ± 40.2 (475-610)
Redoubt Lake 2005	17	503 ± 60.0 (380-580)
Tumakof Lake 2004	12	543 ± 23.5 (510-575)

Table 3.2 Length data (mid-eye to fork of the tail) for sockeye salmon sampled from all four lakes systems. Length was measured in millimeters from mid-eye to the fork of the tail. Data are presented as mean ± standard deviation and range in parentheses.

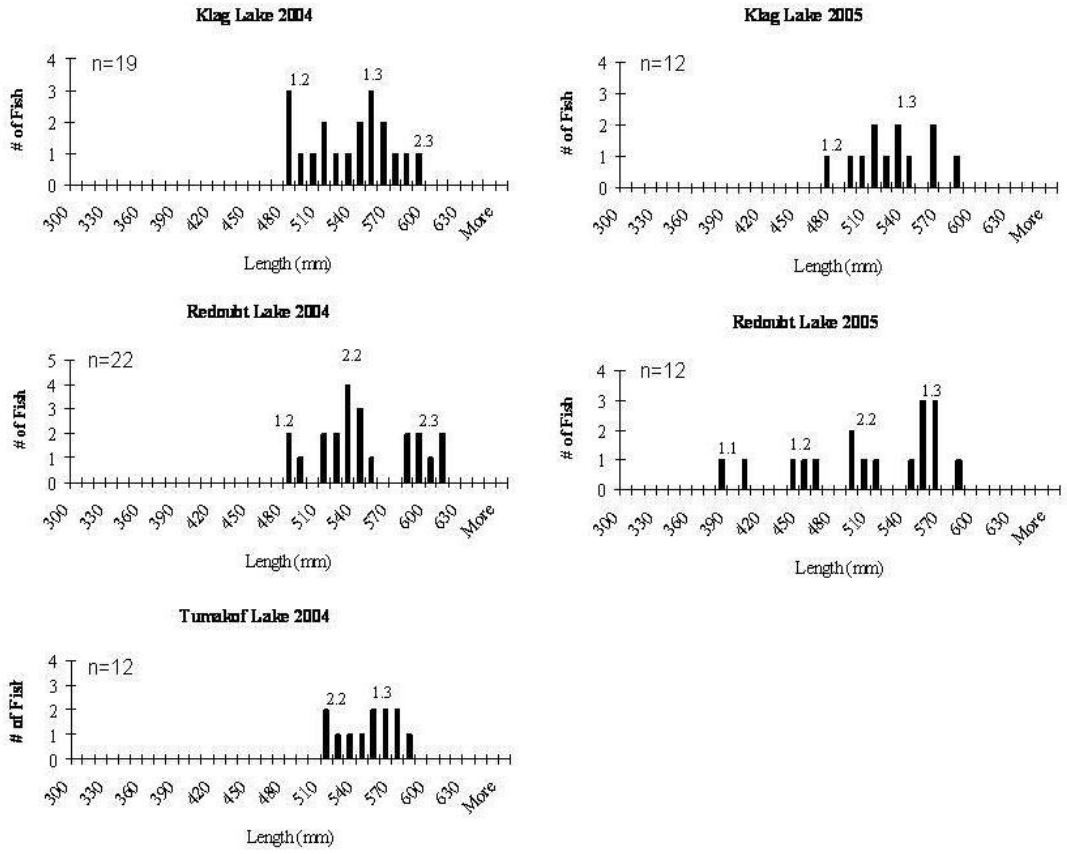

Figure 3.2 Length Frequency histograms Klag, Redoubt, and Tumakof Lake sockeye sampled from 2004-2005.

and 2.2 aged fish, which is most likely due to longer periods spent in the ocean feeding before returning to spawn.

3.3.2 Core Elemental Signatures

Core signatures correctly classified 68% of the sockeye otoliths to their respective lakes (Table 3.3). Of the four watersheds, Tumakof Lake core signatures were the most distinctive and were correctly classified 100% of the time. Core signatures in Salmon Lake otoliths were also distinctive and correctly classified in 87.5% of the cases, while a smaller proportion of otoliths were misclassified to Klag and Redoubt Lake. The greatest errors in classification occurred between Klag and Redoubt otoliths, which greatly overlapped in core chemistry. Over 50% of Klag Lake otoliths were classified to Redoubt Lake, whereas 36% of Redoubt otoliths were classified to Klag Lake. These results demonstrate that core signatures may be ineffective for differentiating these two lakes systems.

Results from the Wilks'-Lambda test revealed that Sr and Ba were the most important elements for separating the four stocks (Table 3.4). Lambda scores vary from 0-1, with 0 meaning group means differ, while values of 1 indicate that group means are the same. The Wilks'-Lambda test revealed that Sr and Ba had scores of 0.661 and Lake and 0.191, respectively. Therefore, the use of Ba core signatures provided the greatest elemental influence for discriminating between the four sockeye stocks. Wilks'-Lambda scores for Mg (0.934), Zn (0.935), and Mn (0.864) show that these elements were not useful for stock classification using core chemistry.

		Assigned Watershed			
Watershed	**N**	**Salmon**	**Klag**	**Redoubt**	**Tumakof**
Salmon Lake	40	87.5%	10%	2.5%	0
Klag Lake	31	6.5%	38.7%	54.8%	0
Redoubt Lake	39	2.6%	35.9%	61.5%	0
Tumakof Lake	12	0	0	0	100%

Table 3.3 The percentage of correct classification determined by discriminant function analysis for sockeye salmon sampled from Salmon, Klag, Redoubt, and Tumakof Lake otolith cores. Cross validation accuracy is expressed as percentage. The elements incorporated into the model were Sr, Ba, Mg, Zn, and Mn.

Element	**Wilks'-Lambda**	**F**	**df1**	**df2**	**Sig.**
Strontium	0.661	20.1	3	118	0.000
Barium	0.191	166.6	3	118	0.000
Magnesium	0.934	2.8	3	118	0.043
Zinc	0.935	2.7	3	118	0.048
Manganese	0.864	6.1	3	118	0.001

Table 3.4 Wilks'-Lambda test statistics displaying the significance of each element in core signatures using discriminant function analysis. Wilks'-Lambda scores varies from 0-1, with 0 meaning groups differ, and 1 meaning all groups are the same. F statistics measure the relative importance of each element in discriminant function analysis.

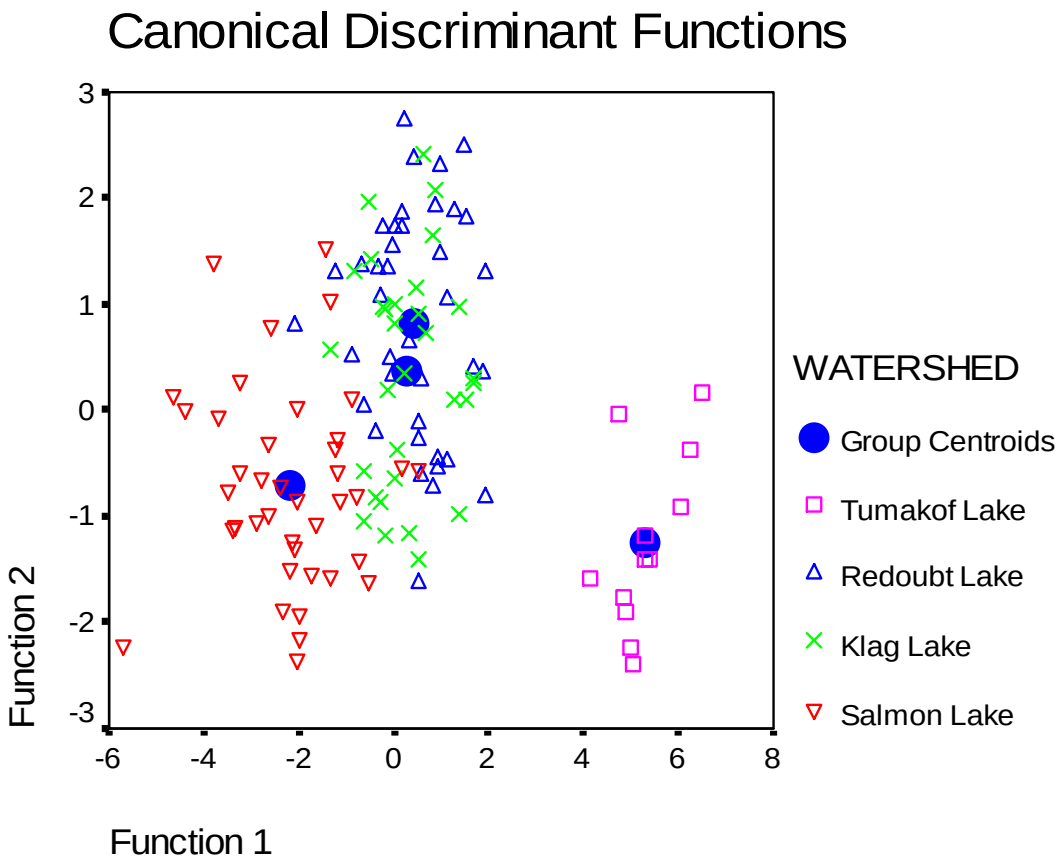

Figure 3.3 Discriminant function analysis plot for core signatures in sockeye otoliths. Elements analyzed were Sr, Ba, Mg, Zn, and Mn. Data were log transformed before statistical analysis. Small symbols are individual fish and large symbols are the group centroids.

3.3.3 Freshwater Elemental Signatures

Freshwater signatures correctly classified 91.0% of sockeye otoliths to their natal watersheds (Table 3.5). Once again, Tumakof Lake otoliths were the most distinctive of the four lakes, and were correctly classified in 100% of the cases. Redoubt and Salmon Lake otoliths also showed distinctive freshwater signatures and were correctly classified over 92% of the time, although some overlap with Klag Lake did exist. Klag Lake otoliths experienced the greatest classification error of the four systems, and were correctly assigned to Klag Lake in 84% of the cases. Results indicate that freshwater signatures in several Klag Lake otoliths shared chemical similarities to Salmon and Redoubt Lake otoliths. Cross-validation analysis showed that out of the 31 Klag Lake otoliths examined, 2 otoliths were incorrectly classified to Salmon Lake, while 3 otoliths were misclassified to Redoubt Lake. These results suggest that Klag Lake sockeye may experience a greater range of chemical environments while rearing in freshwater in comparison to the other stocks. Unlike Tumakof, Salmon, and Redoubt Lakes, Klag Lake is a complex watershed and contains a variety of rearing habitats, which will be discussed later.

The Wilks'-Lambda test demonstrated that Ba and Sr were the most important elements for classifying otoliths to a given system (Table 3.6). As found in core signatures, Ba composition was the most useful element for predicting group membership between stocks, although results also showed that Sr freshwater signatures were also highly significant for classifying sockeye otoliths to their natal lakes. To a lesser extent, freshwater Mg and Mn signatures were also useful to identify stocks; however, the Wilks'-Lambda scores for these elements were relatively large, and so the utility of Mg

		Assigned Stream			
Watershed	**N**	**Salmon**	**Klag**	**Redoubt**	**Tumakof**
Salmon Lake	40	92.5%	7.5%	0	0
Klag Lake	31	6.5%	83.9%	9.7%	0
Redoubt Lake	39	0	7.7%	92.3%	0
Tumakof Lake	12	0	0	0	100%

Table 3.5 The percentage of correct classification determined by discriminant function analysis for sockeye salmon sampled from Salmon, Klag, Redoubt, and Tumakof Lake freshwater signatures. Cross validation accuracy is expressed as percentage. The elements incorporated into the model were Sr, Ba, Mg, Zn, and Mn.

Element	**Wilks'-Lambda**	**F**	**df1**	**df2**	**Sig.**
Strontium	0.203	154.3	3	118	0.000
Barium	0.077	474.3	3	118	0.000
Magnesium	0.762	12.3	3	118	0.000
Zinc	0.966	1.4	3	118	0.425
Manganese	0.882	5.3	3	118	0.002

Table 3.6 Wilks'-Lambda test statistics displaying the significance of each element in freshwater signatures using discriminant function analysis.

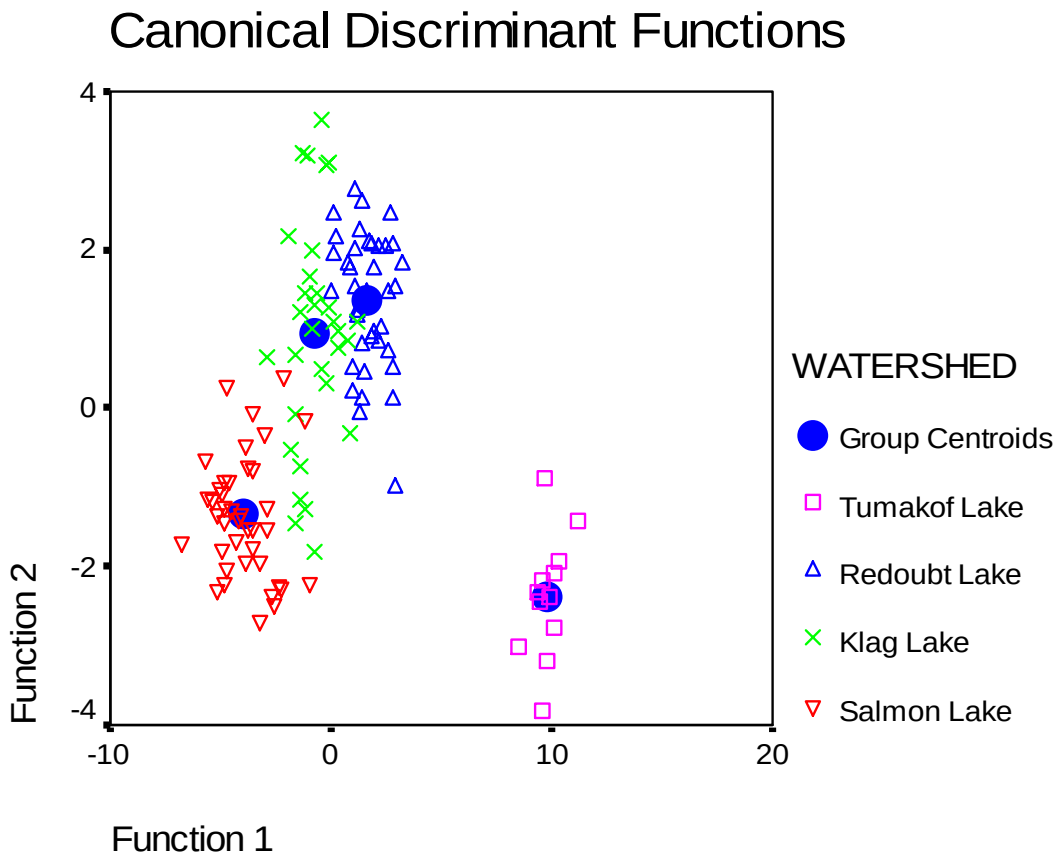

Figure 3.4 Discriminant function analysis plot for freshwater signatures in sockeye otoliths. Elements analyzed were Sr, Ba, Mg, Zn, and Mn. Data were log transformed before statistical analysis. Small symbols are individual fish and large symbols are the group centroids.

and Mn for stock classification is marginal. The Wilks'-Lambda test showed that freshwater Zn signatures did not vary between sockeye otoliths, and were therefore not useful for stock classification.

3.3.4 Marine Elemental Signatures

The use of marine signatures was the least effective method of stock classification in this study. Discriminant function analysis showed that marine signatures correctly grouped sockeye otoliths to their respective watersheds in only 52.5 % of the cases—not much better than a flip of a coin (Table 3.7). This result was expected due to the more homogenous nature of Sr, Ba, Mg, Zn, and Mn in marine waters (Berner and Berner, 1987). However, it was surprising that despite of the invariant chemical composition of marine water, signatures in Redoubt and Tumakof Lake otoliths correctly classified sockeye to their natal systems in 77.5% and 81.8% of the cases, respectively. Possible explanations for this are discussed later.

Results from the Wilks'-Lambda test demonstrated that all elements had relatively little difference in their ability to classify otoliths to a given watershed (Table 3.8). Of the five elements examined, Ba (0.737) and Mn (0.788) displayed the greatest influence in stock classification when using marine signatures. This result may be attributed to the fact that Ba and Mn experience greater variability than Sr or Ba—neither were useful for classifying sockeye otoliths to natal watersheds.

		Assigned Stream			
Watershed	**N**	**Salmon**	**Klag**	**Redoubt**	**Tumakof**
Salmon Lake	40	42.5%	25%	22.5%	10%
Klag Lake	31	22.6%	22.6%	48.4%	6.5%
Redoubt Lake	39	10%	10%	77.5%	2.5%
Tumakof Lake	12	9.1%	0	9.1%	81.8%

Table 3.7 The percentage of correct classification determined by discriminant function analysis for sockeye salmon sampled from Salmon, Klag, Redoubt, and Tumakof Lake marine signatures. Cross validation accuracy is expressed as percentage. The elements incorporated into the model were Sr, Ba, Mg, Zn, and Mn.

Element	**Wilks'-Lambda**	**F**	**df1**	**Df2**	**Sig.**
Strontium	0.915	3.7	3	118	0.015
Barium	0.737	14.0	3	118	0.000
Magnesium	0.842	7.4	3	118	0.000
Zinc	0.850	6.9	3	118	0.000
Manganese	0.788	10.6	3	118	0.000

Table 3.8 Wilks'-Lambda Test statistics demonstrating the significance of each element for classifying sockeye stocks using discriminant function analysis.

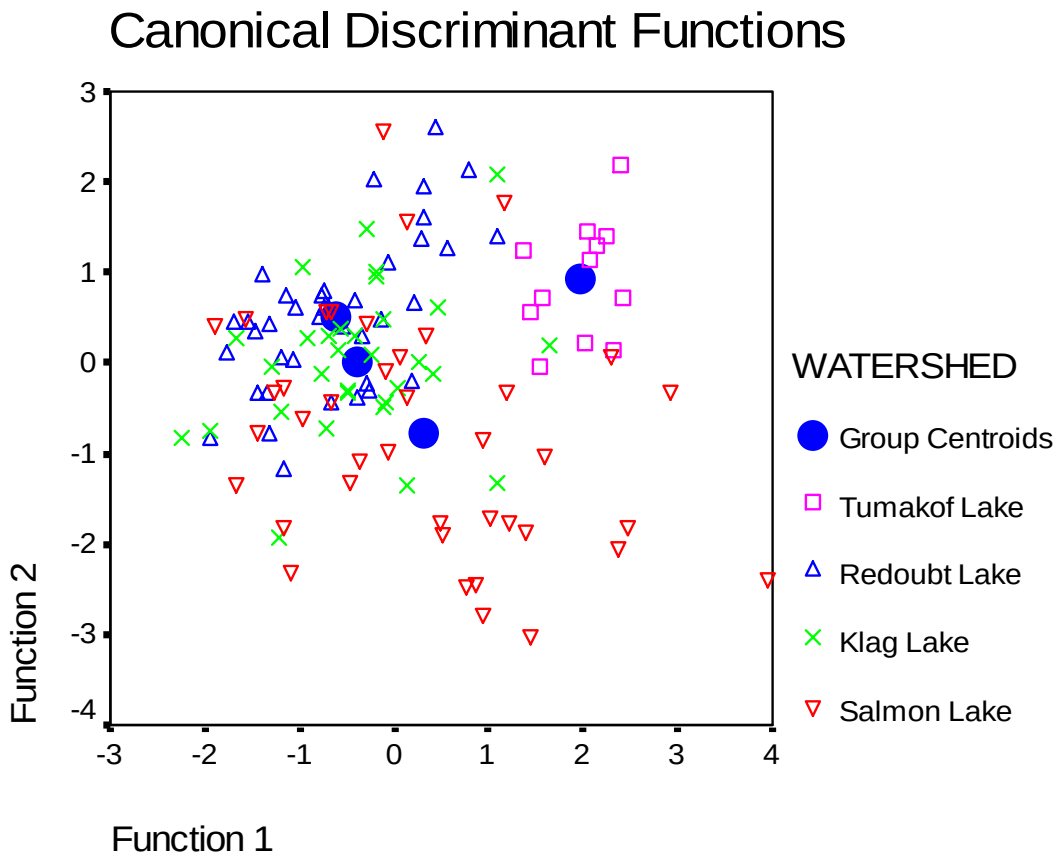

Figure 3.5 Discriminant function analysis plot for marine signatures in sockeye otoliths. Elements analyzed were Sr, Ba, Mg, Zn, and Mn. Data were log transformed before statistical analysis. Small symbols are individual fish and large symbols are the group centroids.

3.4 Discussion

3.4.1 Core Signatures

Despite successful stock ID applications in the past, core signatures displayed poor classification results for sockeye otoliths. A possible reason for this problem may be explained by maternal influences. All of the sockeye sampled for otoliths in this study returned to their natal watersheds with well-developed gonads. This characteristic has been shown to have significant effects in the transfer of marine signatures to the otoliths of developing salmon embryos and alevins. Volk et al. (2000) claim that core signatures in sockeye, coho (*Oncorhynchus kisutch*), and chinook (*Oncorhynchus tshawytscha*) otoliths reflected maternal associations with fresh or marine water. Results revealed that female salmon that matured in seawater had significantly greater Sr:Ca core ratios than females which had matured in freshwater. Additionally, Volk et al. (2000) presented several case studies in systems containing sympatric anadromous and freshwater-resident salmon populations. It was found that high maternal investments of Sr in the core provided a distinguishable mark for separating juveniles spawned from anadromous and non-anadromous parents. Although maternal investments in the otolith core may provide an effective method for determining maternal lineage in systems where non-anadromous and anadromous stocks exist, it adds complexity to the stock identification of purely anadromous populations.

Presently, very little is known about the magnitude to which maternal investments actually affect the core chemistry of anadromous salmon otoliths. Perhaps more importantly, with the exception of Sr, it is not known which elements are specifically affected by maternal investments. Core signatures for other elements, such as Ba, Zn,

Mg, and Mn have yielded mixed results in the past, and have not always reflected maternal associations. Recent research has shown that several elements, including Mn and Mg, are consistently enriched in the cores of freshwater, estuarine, and marine fish otoliths (Brophy et al., 2004, Melancon et al. 2005, and Ruttenberg et al. 2005). At present the reason why some elements but not others are highly enriched in the otolith core is poorly understood. Recent hypotheses have suggested that spatial variations in early crystal growth of otoliths may influence the uptake of specific elements over others (Gauldie et al., 1997, Brophy et al. 2004, Melancon et al. 2005). Alternatively, it has been suggested that increased concentrations of protein and lower concentrations of Ca during early otolith growth may have significant impacts on elemental uptake (Dove et al. 1996, Murayama et al. 2002, and Ruttenberg et al. 2005). Regardless of the cause, it is apparent that the factors controlling elemental uptake in the core are fundamentally different than the other growth regions of the otolith. The results from this study support this assumption, and do not suggest that core signatures are valid indicators of stock origin. Therefore it is advised that alternative growth regions be utilized for analysis when available for stock ID applications. This was done in this study by using the freshwater growth region of sockeye otoliths.

3.4.2 Freshwater Signatures

Of the three growth regions examined, freshwater signatures were the most effective for correctly classifying otoliths to their natal watersheds. Compared to other fish species, sockeye provide a convenient life cycle for stock ID applications using otolith microchemistry. The fact that sockeye spawn and rear in specific natal watersheds makes it possible to use elemental signatures acquired during freshwater residence to

discriminate between different stocks. The basis for this assumption is that water chemistry is typically unique between geographically disparate watersheds due to local variations in bedrock composition, vegetation cover, soil thickness and composition, and precipitation (Berner and Berner, 1987, and Veinott and Porter, 2005). As a result of these localized differences in geochemistry, sockeye otoliths likewise acquire different signatures, which can be used as natural "fingerprints" for stock ID. Our data supports this hypothesis as results from discriminate function analysis showed that the freshwater signatures in Salmon, Klag, Redoubt, and Tumakof Lake sockeye otoliths were chemically distinct.

Though a multivariate combination of five elements was used in this study, Sr and Ba displayed the strongest influence for classifying otoliths to their natal systems. This result was not surprising, as the use of Sr and Ba have proved to be reliable indicators of ambient chemistry (Fowler et al., 1995, Thorrold et al., 1997, et al., 2000, Kraus and Secor, 2004, and Vries et al. 2005). Freshwater signatures of Mg, Mn, and Zn had little to no utility in classifying sockeye to their natal lakes. Presently, the exact mechanisms controlling Mg, Mn, and Zn uptake in the otolith are only speculative, but research indicates that these elements may be under greater metabolic and physiological regulation than Sr or Ba (Thorrold et al., 1997, Campana, 1999, and Halden et al., 2000). As a result, the application of these elements for stock ID may be limited.

Given the negligible influence of Mg, Mn, and Zn in discriminant function analysis, most otoliths in this study could be correctly classified to their natal systems by Sr or Ba composition alone. This was most evident in Tumakof Lake otoliths, which contained Ba concentrations that were nearly one order of magnitude greater than

Salmon, Klag, or Redoubt otoliths (Chapter 2). This distinctive Ba signal contributed to the complete discrimination (100%) of Tumakof otoliths from the other three lakes. Strontium had similar affects in the classification of Salmon and Redoubt Lake otoliths. Although Ba concentrations were similar in the freshwater growth regions of Salmon and Redoubt Lake otoliths, Sr composition was vastly different between the two stocks. Salmon Lake otoliths contained the lowest freshwater Sr concentrations of the four lakes examined, whereas Redoubt Lake otoliths contained some of the highest (Chapter 2). Thus, Sr composition was sufficient for discriminating between these two sockeye stocks. Taken together, Sr and Ba signatures were capable of 100% separation of the Tumakof, Salmon, and Redoubt Lake sockeye stocks.

Of the four stocks sampled, Klag Lake otoliths contained the greatest error (~16%) in stock classification. Discriminant function analysis showed that freshwater signatures in several Klag Lake otoliths could not be differentiated from sockeye returning to either Salmon or Redoubt Lake. This indicates that some sockeye rearing in Klag Lake share similar chemical environments to sockeye produced from Salmon or Redoubt Lakes. Considering the earlier mentioned differences in Sr composition between Salmon and Redoubt otoliths, such an overlap with Klag Lake otoliths is unusual. This overlap suggests that water chemistry in Klag Lake significantly varies in different regions of the lake, and that a certain proportion of juvenile sockeye rear in these areas. This is reasonable considering the variety of rearing habitats available to juvenile sockeye in the Klag lake watershed.

The sockeye sampled in this study are assumed to follow “lake-type” life histories and rear in the limnetic zone of the lake, however, Klag Lake is a complex watershed.

Unlike Salmon, Redoubt, and Tumakof Lakes, which are primarily comprised of one main lake basin, Klag Lake contains several small interconnected lakes and ponds (Figure 3.6). Klag Lake receives drainage from numerous sources, which includes several shallow lakes and ponds, as well as large areas of muskeg (Conitz et al., 2005). The influence of muskeg water can have significant effects on Klag Lake water chemistry, especially salinity. Schwartz and Milne-Home (1982) noted that the standing water of muskegs is less mineralized than most ground or surface waters, but that salinity significantly increases with depth. The seepage of muskeg water into Klag Lake could alter salinity in different regions of Klag Lake, and explain variations in Sr composition between Klag Lake otoliths.

Two important factors that can significantly affect freshwater signatures in anadromous salmon otoliths are that: (1) early freshwater movements by juveniles between watersheds containing different chemistry regimes will influence trace element signatures for stock ID, and (2) any large temporal changes in the geochemistry of a given watershed will cause otolith chemistry to vary over time. The first factor is especially important in salmon stocks that spawn and rear in large fluvial watersheds (e.g. Fraser River) containing multiple tributaries. In species such as chinook and coho salmon, movement in freshwater can be highly variable as fish actively seek habitats providing adequate food supplies, low competition, and protection from predators (Groot and Margolis, 1991). During these migrations juveniles can traverse water masses with significantly different chemistry regimes, which can affect freshwater signatures for stock ID. The effects of freshwater movements on otolith microchemistry were documented by Kennedy et al. (2000) in Atlantic Salmon (*Salmo salar*) otoliths from two tributaries of

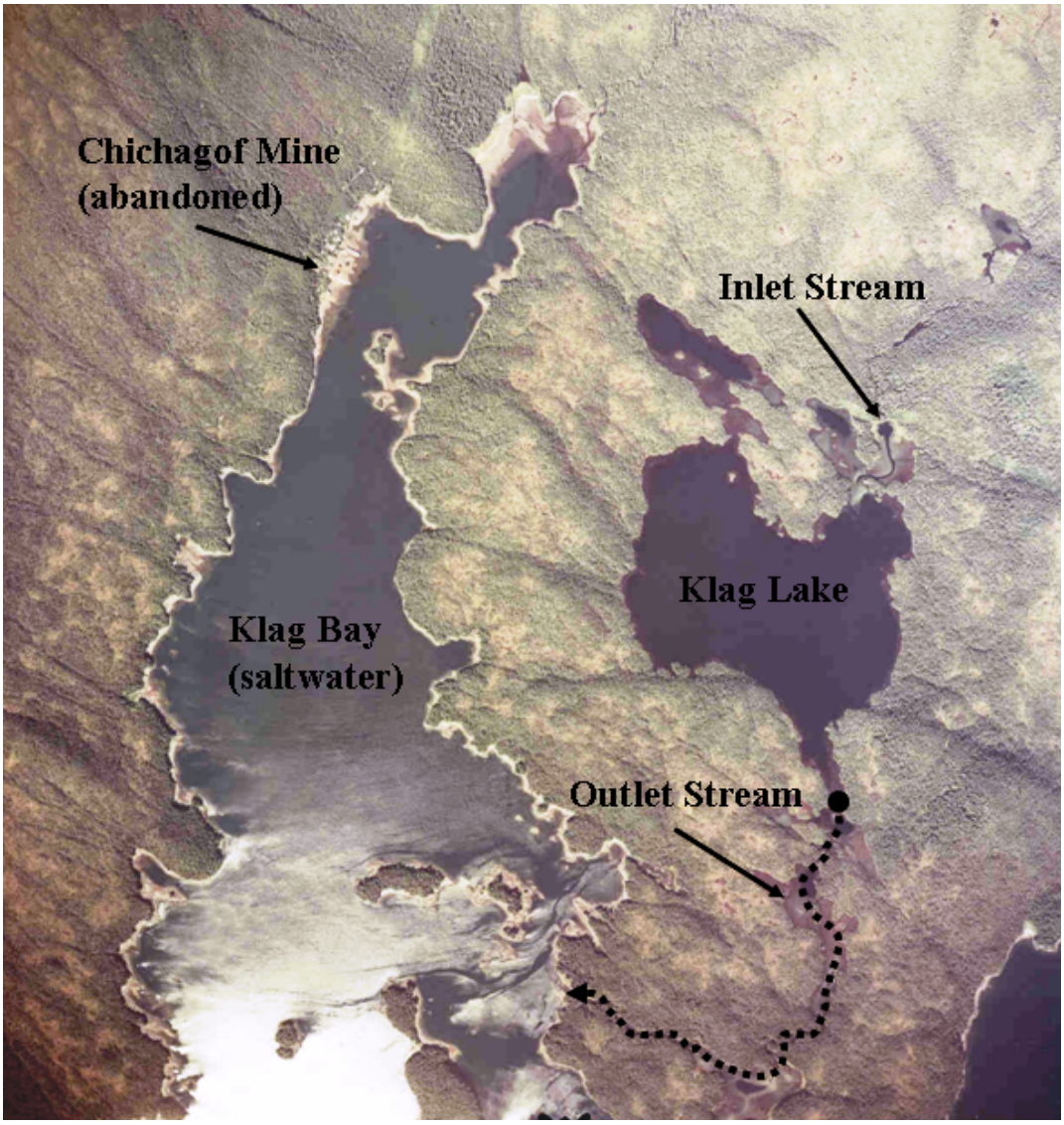

Figure 3.6 Aerial View of the Klag Lake watershed.

the Connecticut River in central and southern Vermont, U.S.A. Using stable Sr isotopes, Kennedy et al. (2000) determined that a small percentage of age-0 fish sampled in the West River basin (<5%) and age-1 fish sampled from the White River (15%) had migrated from their natal tributaries. The movement of these individuals produced a blend of isotopic signals reflective of each exposure to the differing water masses, which resulted in the misrepresentation of the site signature (Kennedy et al. 2000). Although this study used stable isotopes rather than trace element composition, it highlights the fact that large spatial movements in freshwater can affect otolith chemistry for stock ID.

Movements between different watersheds did not occur in the sockeye stocks examined in this study. Compared to large fluvial and lacustrine systems, the sockeye lakes examined are isolated from any connecting watersheds, aside from the outlet streams which empty into marine water. Therefore, with the exception of Klag Lake, sockeye juveniles in Redoubt, Salmon, and Tumakof Lakes are spatially confined within the main lake basin, and do not migrate between different watersheds during freshwater residence.

The second, and perhaps most important factor for stock ID applications of anadromous salmon involving otolith chemistry is that elemental signatures are temporally stable in natal watersheds. Any large temporal variations in water chemistry would also cause otolith chemistry to vary over time and make it impractical to use freshwater signatures as a method for long-term stock ID. Fortunately, large temporal shifts in lake water geochemistry are low in most natural systems, though seasonal variations do frequently occur in temperate and arctic lakes. Seasonal factors such as precipitation, evaporation, biological production, run-off (e.g. spring-summer snow-

melt), and spring and fall turn-over all have effects on the chemical composition of temperate and arctic lakes (Berner and Berner, 1987). Therefore, small variations in trace elemental signatures are unavoidable, and should be expected between different cohorts of anadromous salmon otoliths. In this study, large variations in freshwater signatures do not appear to be a major factor, as the majority of otoliths were correctly classified to their natal lakes, regardless of the year sampled. However, with only 3 years of otolith data for Salmon Lake, 2 years of data from Klag and Redoubt Lake, and 1 year of data from Tumakof Lake, the assumption that otolith signatures are temporally stable for each stock is still premature. Regardless, the results from this study indicate that freshwater signatures in Salmon, Klag, Redoubt, and Tumakof Lake sockeye stocks are distinctive, and can serve as effective tools for stock ID. Further work characterizing and monitoring freshwater signatures from each stock is needed to increase our understanding of the stability of these elemental fingerprints, and how to effectively apply them as a fisheries tool.

3.4.3 Marine Signatures

Time-series data isolated from the marine growth regions of sockeye otoliths demonstrated the poorest (52.5%) classification accuracy of the three growth regions. The chemical composition of seawater is relatively constant compared to freshwater, and variations in total dissolved solids are small (±7%) for well over 95% of the world's oceans (Berner and Berner, 1987). Therefore it was not expected that marine signatures would significantly vary between sockeye otoliths. However, marine signatures correctly classified 77.5% and 81.8% of the otoliths collected from Redoubt and Tumakof Lakes, respectively. This suggests that elemental uptake in these two stocks was distinctive

during marine residence, and suggests that oceanic distribution and migration may be predisposed in Tumakof and Redoubt Lake sockeye populations.

The reasons why marine signatures are distinctive in the majority of Redoubt and Tumakof Lake otoliths is difficult to explain, as the factors influencing oceanic migration of sockeye are complex. Marine migration is controlled by physical, chemical, and biological factors that include currents, water temperature, salinity, food availability, predation, age and size, and maturity stage (Groot and Margolis, 1991, and Hodgson et al., 2006). Because these conditions will ultimately vary, it is not yet possible to determine when and where specific sockeye stocks migrate. Generalized models of sockeye migration have been produced from past tagging, scale-pattern analysis, and parasite studies in the Gulf of Alaska, but these models are limited to broad classifications of regional population movements, and are not stock-specific (Groot and Margolis, 1991, and Halupka et al. 2000). The generalized model of oceanic migration for southeast Alaska sockeye suggests that most stocks intermix and undertake similar circular annual migrations in the Gulf of Alaska north of 46° N (Halupka et al. 2000). Redoubt and Tumakof Lake otoliths showed little overlap with Klag or Salmon Lake otoliths, suggesting that these stocks may follow different migration trajectories during marine residence (Groot and Margolis, 1991, Halupka et. al. 2000). Factors such as marine entry timing and early residence in estuarine and coastal areas will influence the marine signatures of sockeye otoliths, and will be different for each stock. Furthermore, associations with Sitka Eddies generated off of the Alexander Archipelago coast could also affect elemental signatures in different sockeye stocks during marine residence (Chapter 2). However, as mentioned earlier, the factors causing these distinctive marine

signatures in Tumakof and Redoubt Lake otoliths are complex, and any further explanation will be speculative at best. Yet, whatever the case may be, marine signatures are limited in their ability to reliably classify sockeye stocks to their natal watersheds, and therefore should not be considered reliable stock ID tools.

3.5 Conclusion

Comparisons between the core, freshwater, and marine growth regions of sockeye otoliths demonstrated that freshwater signatures provided the greatest accuracy for stock ID. Discriminant function analysis showed that in over 90% of the cases, freshwater signatures correctly classified otoliths to their natal watersheds. Core signatures, which have proven useful in past stock ID studies, showed poor classification results for sockeye otoliths, including significant overlap between Klag and Redoubt Lakes. Since maternal, physiological, and mineralogical factors are known to affect trace element uptake in the cores of anadromous salmon otoliths, it is advised that alternate growth regions (e.g. freshwater) be utilized for stock ID. Trace element signatures from the marine growth regions of sockeye otoliths displayed the poorest classification accuracy of the three growth regions. However, these results were anticipated considering that the chemical composition of the world's oceans is generally invariant, and most sockeye stocks in Southeast Alaska are believed to follow similar marine migrations. However, marine signatures in Redoubt and Tumakof Lake sockeye otoliths were distinctive suggesting that these stocks may deviate from current oceanic migration models predicted for southeast Alaska sockeye stocks.

Though a multivariate combination of five elements were used as predictors in discriminant function analysis, results showed that Sr and Ba were the most significant

elements for stock ID. The uptake of Sr and Ba in fish otoliths has repeatedly shown to occur in proportion to the ambient environment, which in this study provided distinctive freshwater signatures in the four sockeye systems. Elemental concentrations of Mg, Mn, and Zn in the core, freshwater, and marine growth regions displayed little to no influence for separating the four sockeye stocks. Unlike Sr and Ba, Mg, Zn, and Mn uptake are believed to be under greater physiological and metabolic control by the fish, and may not be well suited for stock ID applications.

Though freshwater signatures were successful at discriminating between the four sockeye stocks in this study, two factors that can significantly affect freshwater signatures in anadromous salmon otoliths are that: (1) early freshwater movements by juveniles between watersheds containing different chemistry regimes will influence trace element signatures for stock ID, and (2) any large temporal changes in the geochemistry of a given watershed will cause otolith chemistry to vary over time. Neither of these factors were encountered in this study, as each sockeye lake was isolated from connecting watersheds, and the majority of otoliths were correctly assigned to their natal systems, regardless of the year sampled.

In addition to otolith microchemistry, innovations using genetic markers, namely microsatellite DNA analysis, have been shown to effectively discriminate between different stocks of wild salmon in mixed-stock watersheds (Beacham and Wood, 1999). However, research has also shown that significant genetic heterogeneity can occur within sub-populations of a specific wild salmon stock, especially among groups of fish exhibiting unique run-timing and spawning habitats (Haluptka et al., 2000). Otolith chemistry is not affected by genetic diversity among sub-populations which spawn and

rear in the same watershed. Future work combining otolith chemistry and genetic data will only improve the precision in which fisheries biologists and managers can identify specific wild salmon stocks, and therefore enhance current management plans used for salmon fisheries in the Northeast Pacific.

Further research and development is needed to establish otolith trace element chemistry as a reliable stock ID method before it can be applied as a fisheries management tool. However, the results from this study are promising, and show that otolith trace element chemistry can serve as an effective method for identifying separate stocks of wild salmon. Furthermore, results also showed that marine signatures may provide a tool for determining differences in marine migration between separate sockeye stocks. The identification of wild salmon has become a major preoccupation for fisheries researchers and managers since many wild stocks returning to freshwater systems in the United States and Canada have been drastically reduced, and it is difficult for fisheries managers to regulate the exploitation of endangered stocks when they are intermixed with healthy populations. Otolith trace element chemistry can provide managers with a valuable tool for identifying discreet stocks of salmon, even when they are far removed from their natal systems.

Chapter 4

Summary

A novel and versatile method was developed in this thesis for analyzing the trace element composition of sockeye salmon otoliths. Using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), continuous lateral ablation scans (henceforth line scans) were performed across the entire growth axis of transversely sectioned sagittal otoliths. Applications using full line scans provide better temporal and spatial resolution of trace element composition across the otolith growth sequence than other analysis methods currently used in otolith microchemistry. Full line scans produce a complete chemical record of fish life history (birth to death), and provide a powerful tool for addressing questions in fisheries science. The research presented in this thesis utilized high resolution time-series data generated from line scan analysis to examine two specific fisheries questions involving sockeye salmon returning to Salmon, Klag, Redoubt, and Tumakof lakes in Sitka, Alaska.

In chapter 2, the life history of sockeye salmon was reconstructed using chemical profiles produced from time series data. This experiment set out to accomplish three main goals, which included: (1) the ability to reconstruct sockeye life history from fresh to marine water using various trace elements in the otolith growth sequence; (2) to

examine any elemental shifts possibly related to ontogenetic factors; and (3) to provide understanding on the mechanisms controlling elemental uptake in fresh and marine water.

All sockeye sampled in this study followed an anadromous life history, spending 1-3 years in freshwater before outmigrating into the Gulf of Alaska to feed. LA-ICP-MS line scans progressed from the dorsal edge through the core to the opposing ventral edge of transversely sectioned sockeye otoliths. Otoliths were measured for signal intensities of ^{43}Ca, ^{86}Sr, ^{137}Ba, ^{24}Mg, ^{55}Mn, ^{66}Zn, and ^{7}Li, and were normalized to ^{43}Ca. Sockeye provided an excellent candidate species for addressing factors controlling elemental uptake into the otolith in both the fresh and marine environments. Not only was it possible to effectively follow sockeye migration from fresh to marine water, but small temporal shifts in elemental uptake were detected, many of which have not been previously reported.

Results showed that several elements, such as Sr, Ba, and to a certain degree Mg, were effective for tracking migration from fresh to marine water during sockeye life history. Manganese was also effective for determining migration to fresh and marine water; however, it is believed that diet, not ambient chemistry is the factor controlling uptake. Elements such as Zn and Li provided information related to fish physiology, such as growth and changes in osmoregulation during transitions from low to high salinity environments.

It was also found that many elements were either enriched or depleted in the core of sockeye otoliths. For elements, such as Sr and Ba it is likely maternal input may control elemental uptake during incubation and early development. These elements may also function as a discriminatory tool in mixed stock systems where sympatric

anadromous and fresh-water resident salmon exist. However, for elements such as Mn and Mg, maternal influence is not likely a factor causing early enrichment in the core. It is more likely that differences in early crystal structure affect the uptake of Mg and Mn. This finding has significant impacts on the practice of using otolith core chemistry for stock identification purposes. If trace element uptake is augmented in the core region by maternal investments and differences in early crystal growth, using core trace element signatures as an indicator of stock origin could be inaccurate. This question was examined in chapter 3.

Comparisons between the core, freshwater, and marine growth regions of sockeye otoliths demonstrated that freshwater signatures provided the greatest accuracy for stock ID. Discriminant function analysis showed that in over 90% of the cases, freshwater signatures correctly classified otoliths to their natal watersheds. Core signatures, which have proven useful in past stock ID studies, showed poor classification results for sockeye otoliths, including significant overlap between Klag and Redoubt Lakes. Trace element signatures from the marine growth regions of sockeye otoliths displayed the poorest classification accuracy of the three growth regions. However, these results were anticipated considering that the chemical composition of the world's oceans are generally invariant, and most sockeye stocks in Southeast Alaska are believed to follow similar marine migrations. However, marine signatures in Redoubt and Tumakof Lake sockeye otoliths were distinctive suggesting that these stocks may deviate from current oceanic migration models predicted for southeast Alaska sockeye stocks.

Though a multivariate combination of five elements were used as predictors in discriminant function analysis, results showed that Sr and Ba were the most significant

elements for stock ID. Elemental concentrations of Mg, Mn, and Zn in the core, freshwater, and marine growth regions displayed little to no influence for separating the four sockeye stocks. Unlike Sr and Ba, Mg, Zn, and Mn uptake are believed to be under greater physiological and metabolic control by the fish, and may not be well suited for stock ID applications.

Though freshwater signatures were successful at discriminating between the four sockeye stocks in this study, two factors that should be considered before using freshwater signatures in anadromous salmon otoliths for stock ID are: (1) early freshwater movements by juveniles between watersheds containing different chemistry regimes will influence trace element signatures for stock ID, and (2) any large temporal changes in the geochemistry of a given watershed will cause otolith chemistry to vary over time. Neither of these factors were encountered in this study, as each sockeye lake was isolated from connecting watersheds, and the majority of otoliths were correctly assigned to their natal systems, regardless of the year sampled.

As occurs in most scientific research, many of results obtained from LA-ICP-MS analysis posed as many questions as answers. It is obvious that many aspects of elemental uptake from the environment into fish otoliths are still unknown. However, this is to be expected considering that fish are complex organisms and are constantly regulating various physiological functions to maintain homeostasis. This is even further complicated in anadromous fish species, such as sockeye, which change osmoregulation twice during life history. However, from this study it is apparent that the field of otolith microchemistry need not be limited to the fish biologist. Otolith microchemistry requires a diverse field of scientific knowledge from disciplines that include but are not limited

too, biology, chemistry, physics, geology, geochemistry, mineralogy, limnology, oceanography, and fish ecology. Further collaboration between these numerous disciplines shall only increase our understanding of the otolith as an informational source, and aid in the development of otolith microchemistry as a reliable tool for managing salmon fisheries in the Northeast Pacific.

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Appendices

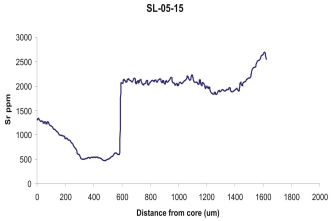
Appendix I: Chemical Life History Profiles (Chapter 2)

Appendix II: Averaged elemental core concentrations of sockeye otoliths determined via LA-ICP-MS time series data. This data has not been transformed.(Chapter 3)

Appendix III: Averaged elemental freshwater concentrations of sockeye otoliths determined via LA-ICP-MS time series data. This data has not been transformed.(Chapter 3)

Appendix IV: Averaged elemental marine concentrations of sockeye otoliths determined via LA-ICP-MS time series data. This data has not been transformed.(Chapter 3).

TL-04-17
TL-04-32
TL-04-03
TL-04-15
TL-04-23
RD-05-03
RD-05-07
RD-05-16
RD-05-11
RD-05-17
KL-05-18
KL-05-13
KL-05-07
KL-05-16
KL-05-01
SL-05-06
SL-05-09
SL-05-11
SL-05-17
SL-05-13
Sr ppm
Distance from core (mm)
Distance from core (um)
Distancefrom core (um)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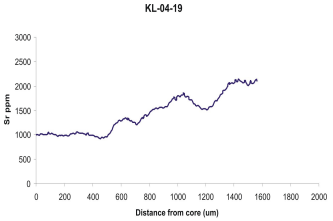
Sr ppm
Distance from core (um)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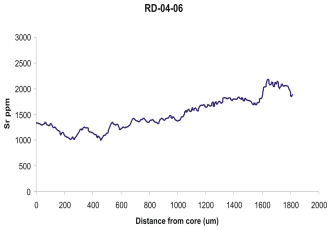
Distance from core (um)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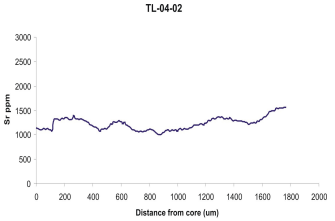
Distance from core (um)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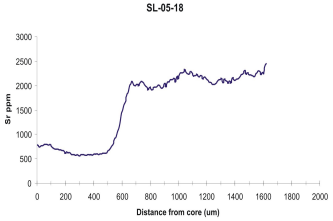
Distance from core (um)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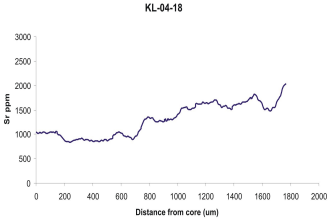
Sr ppm
Distance from core (um)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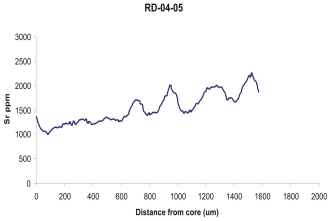
Distance from core (um)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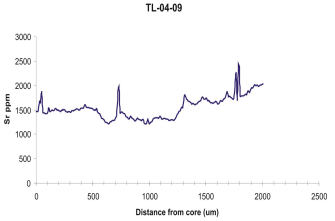
Distance from core (um)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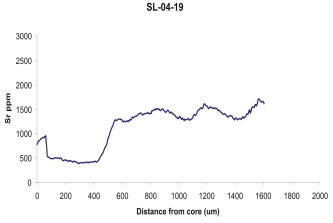
Distance from core (um)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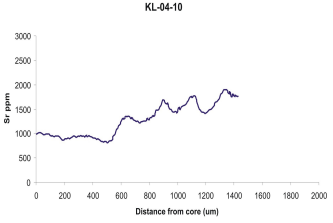
Sr ppm
Distance from core (um)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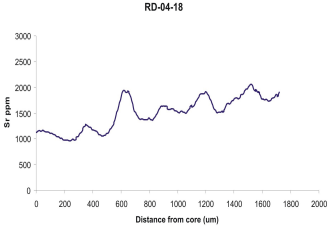
Distance from core (um)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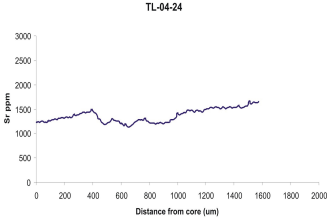
Distance from core (um)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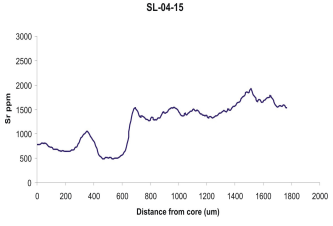
Distance from core (um)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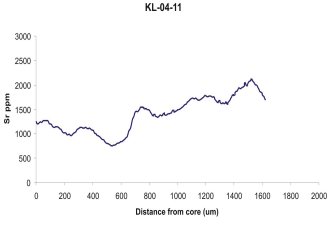
Sr ppm
Distance from core (um)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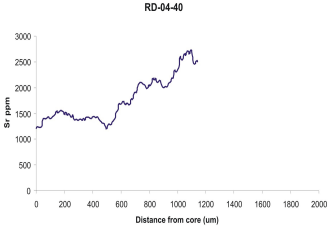
Distance from core (um)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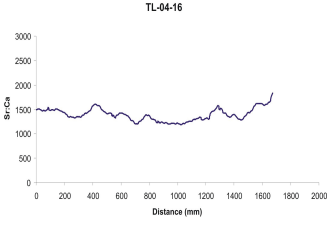
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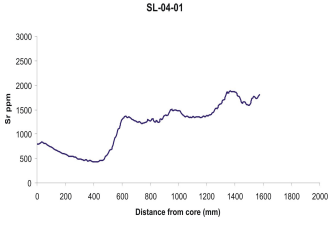
Distance (mm)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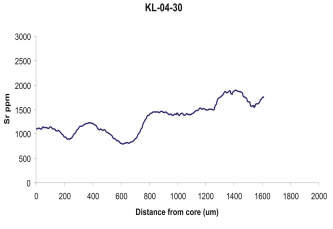
Distance from core (mm)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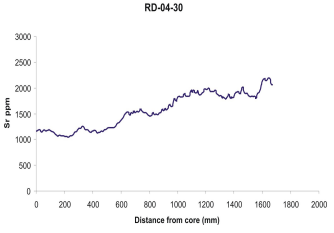
Distance from core (um)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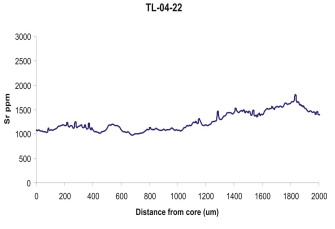
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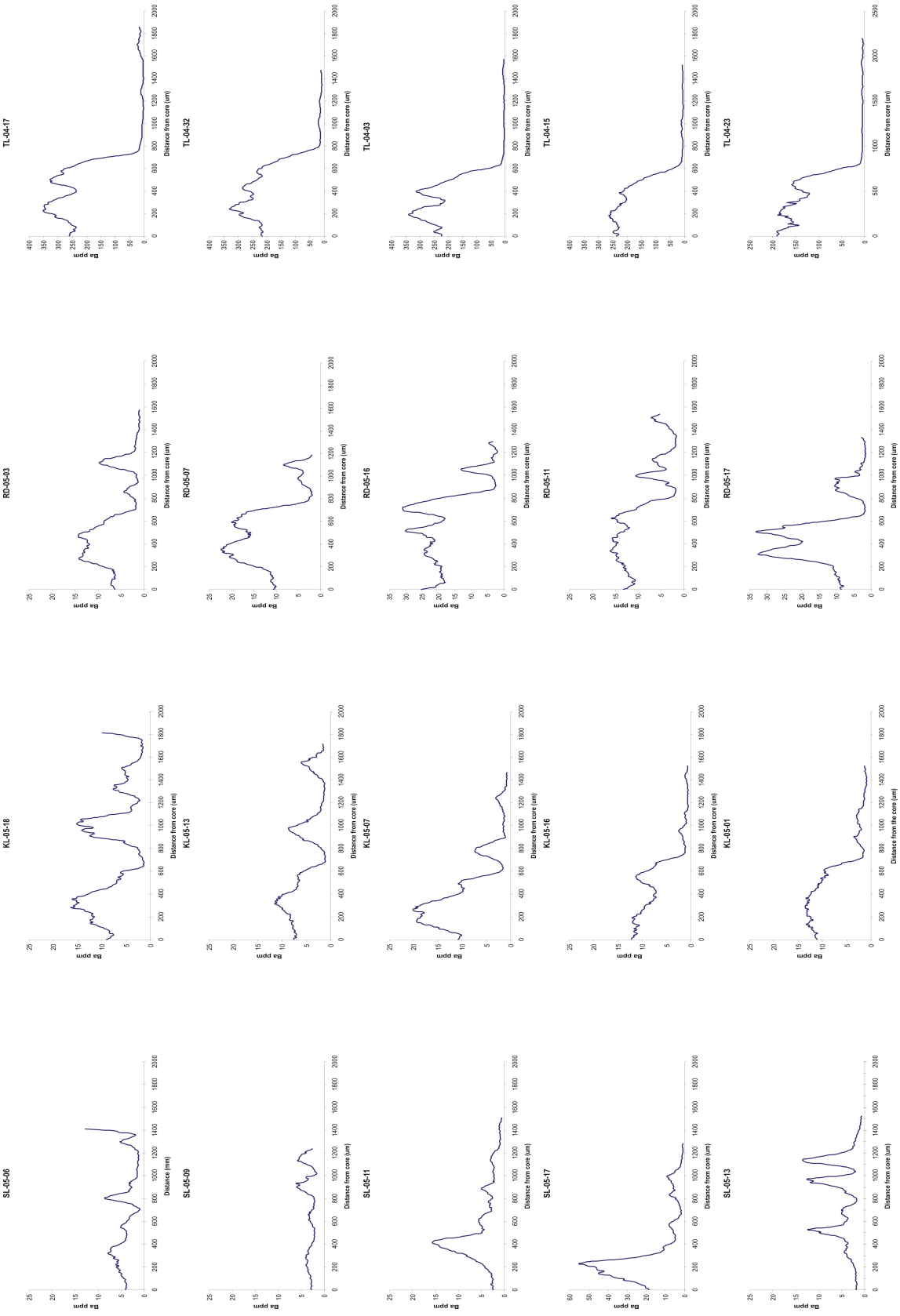
Distance from core (um)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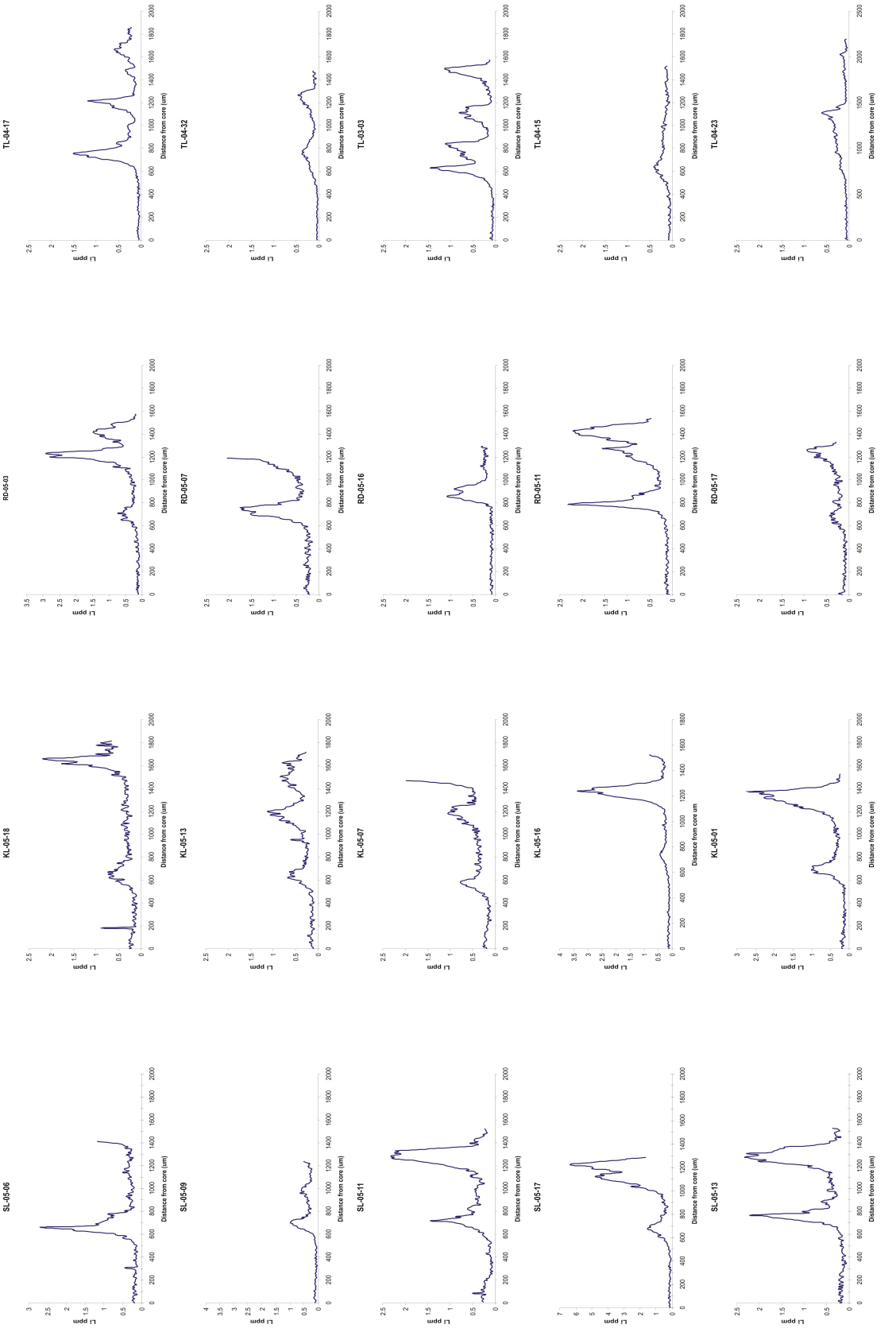
TL-04-15
TL-04-23
RD-05-03
RD-05-07
RD-05-16
RD-05-11
RD-05-17
KL-05-18
KL-05-13
KL-05-07
KL-05-16
KL-05-11
SL-05-06
SL-05-09
SL-05-11
SL-05-17
SL-05-13
Ba ppm
Distance from core (um)
Distance (mm)
Distance from the core (um)

TL-04-02
TL-04-09
TL-04-24
TL-04-16
TL-04-22
RD-04-06
RD-04-05
RD-04-18
RD-04-40
RD-04-30
KL-04-19
KL-04-18
KL-04-10
KL-04-11
KL-04-30
SL-05-15
SL-05-18
SL-04-19
SL-04-15
SL-04-01
Ba ppm
Distance from core (um)

TL-04-17
TL-04-32
TL-04-03
TL-04-15
TL-04-23
RD-05-03
RD-05-07
RD-05-16
RD-05-11
RD-05-17
KL-05-18
KL-05-13
KL-05-07
KL-05-16
KL-05-01
SL-05-06
SL-05-09
SL-05-11
SL-05-17
SL-05-13
Mg ppm
Distance from core (um)

TL-04-02
TL-04-09
TL-04-24
TL-04-16
TL-04-22
RD-04-06
RD-04-05
RD-04-18
RD-04-40
RD-04-30
KL-04-19
KL-04-18
KL-04-10
KL-04-11
KL-05-30
SL-05-15
SL-05-18
SL-04-19
SL-04-15
SL-04-01
Mg ppm
Zn ppm
Distance from core (um)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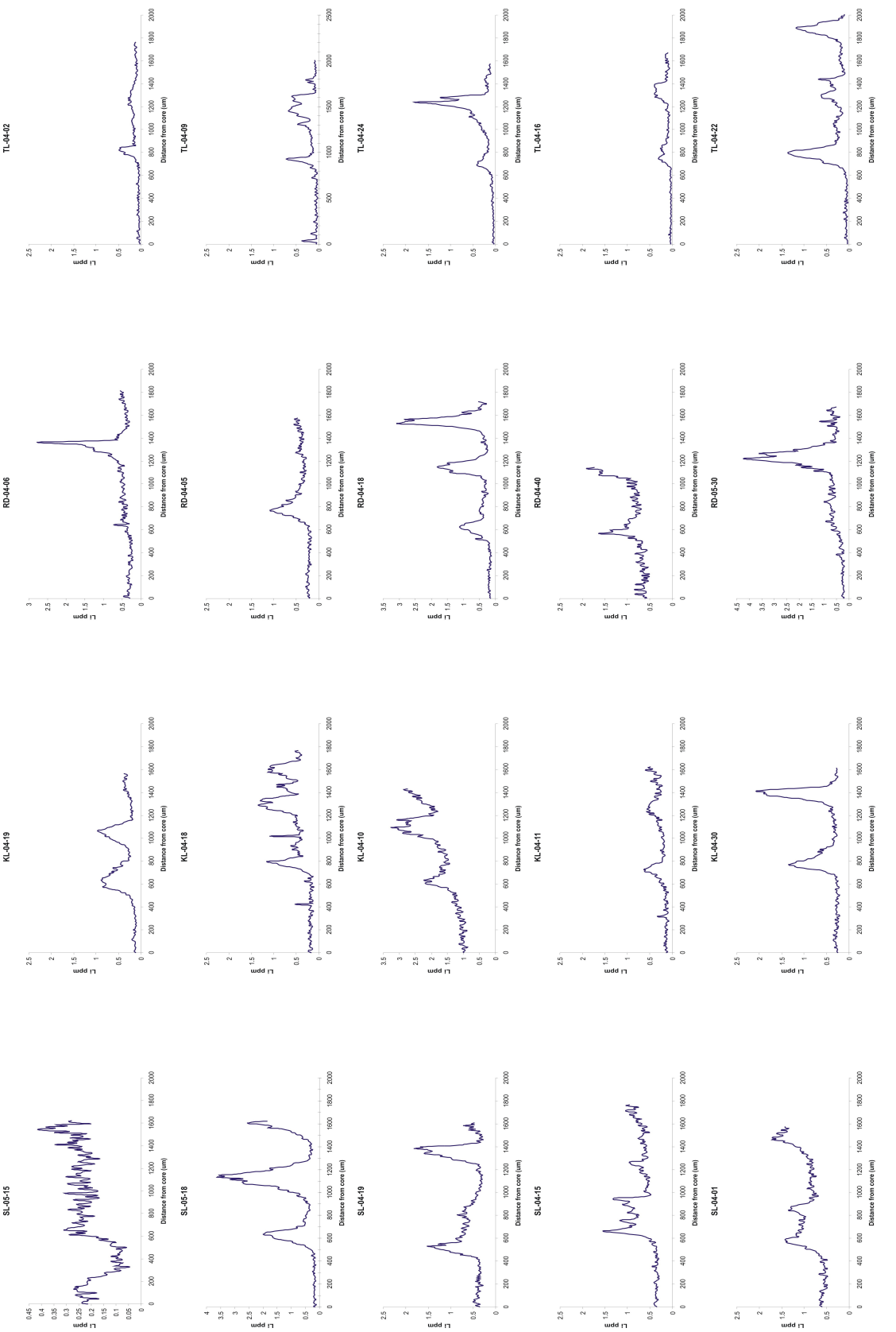
RD-05-03
RD-05-07
RD-05-16
RD-05-11
RD-05-17
TL-04-17
TL-04-32
TL-03-03
TL-04-15
TL-04-23
Li ppm
Distance from core (um)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TL-04-24
TL-04-16
TL-04-22
RD-04-06
RD-04-05
RD-04-18
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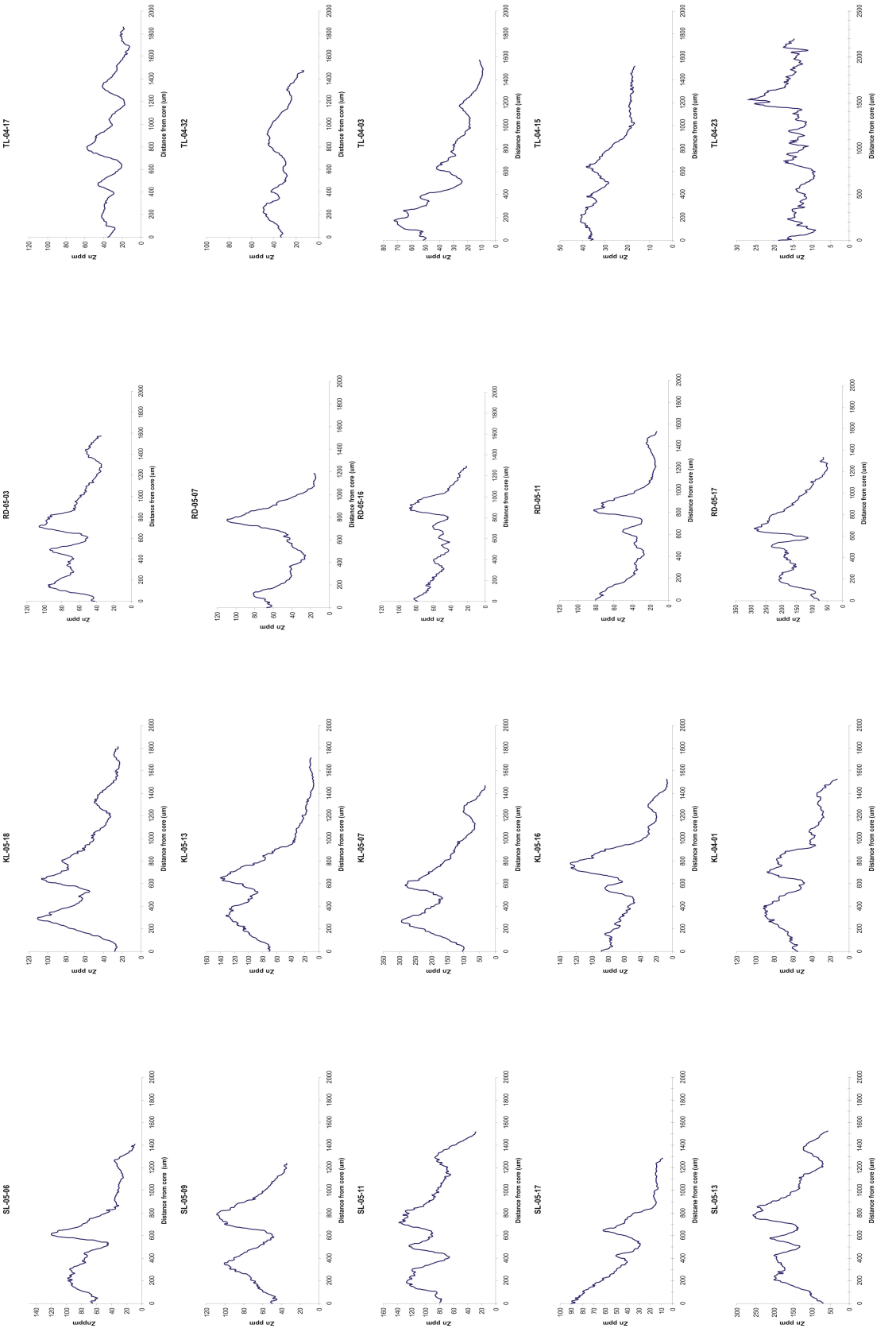
RD-05-30
KL-04-19
KL-04-18
KL-04-10
KL-04-11
KL-04-30
SL-05-15
SL-05-18
SL-04-19
SL-04-15
SL-04-01
L ppm
Distance from core (um)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TL-04-03
TL-04-15
TL-04-23
RD-05-03
RD-05-07
RD-05-16
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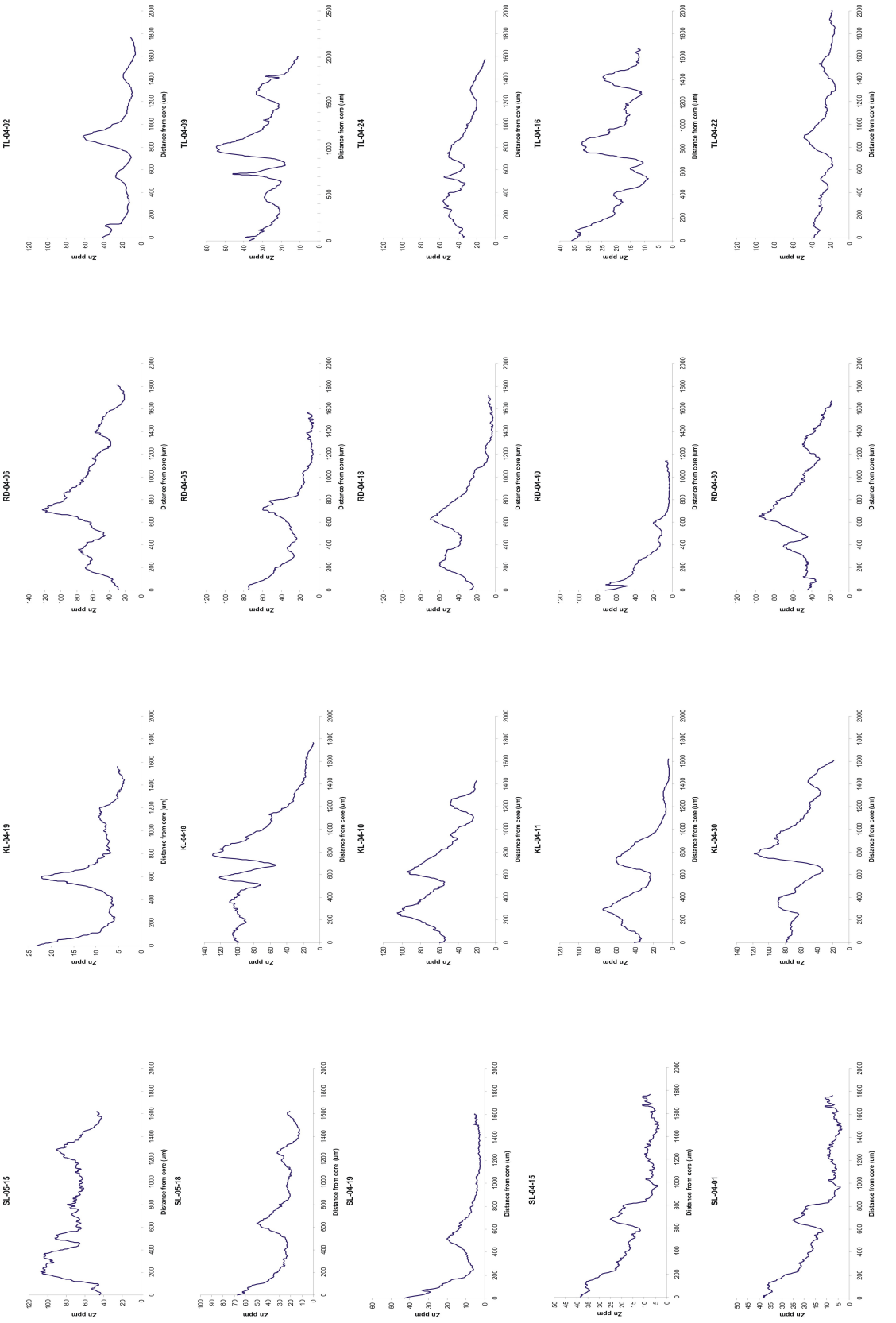
RD-05-17
KL-05-18
KL-05-13
KL-05-07
KL-05-16
KL-04-01
SL-05-06
SL-05-09
SL-05-11
SL-05-17
SL-05-13
Zn ppm
Distance from core (um)


TL-04-02
TL-04-09
TL-04-24
TL-04-16
TL-04-22
RD-04-06
RD-04-05
RD-04-18
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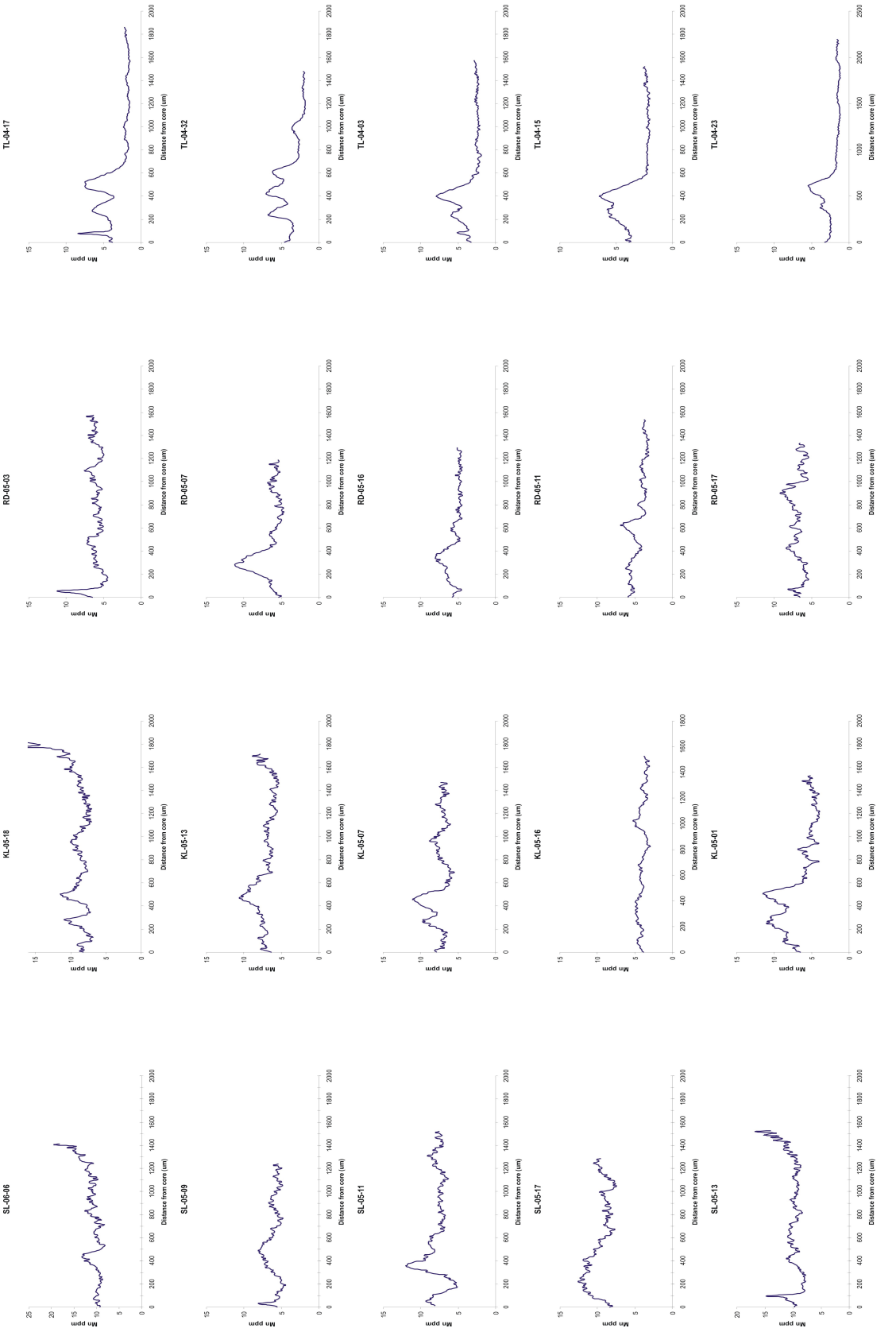
RD-04-30
KL-04-19
KL-04-18
KL-04-10
KL-04-11
KL-04-30
SL-05-15
SL-05-18
SL-04-19
SL-04-15
SL-04-01
Zn ppm
Distance from core (um)


TL-04-17
TL-04-32
TL-04-03
TL-04-15
TL-04-23
RD-05-03
RD-05-07
RD-05-16
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RD-05-17
KL-05-18
KL-05-13
KL-05-07
KL-05-16
KL-05-01
SL-05-06
SL-05-09
SL-05-11
SL-05-17
SL-05-13
Mn ppm
Distance from core (um)

TL-04-02
TL-04-09
TL-04-24
TL-04-16
TL-04-22
RD-04-06
RD-04-05
RD-04-18
RD-04-40
RD-04-30
KL-04-19
KL-04-18
KL-04-10
KL-04-11
KL-04-30
SL-05-15
SL-05-18
SL-04-19
SL-04-15
SL-04-01
Mn ppm
Mg ppm
Distance from core (um)
Distance from the core (um)

Appendix II: Averaged Core Data ($\mu g\ g^{-1}$). (SL-Salmon Lake, KL-Klag Lake, RD-Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
SL-05-07	636.65	4.52	28.64	117.62	5.15
SL-05-02	996.82	9.27	97.42	39.76	9.19
SL-05-16	869.48	2.86	39.55	110.55	7.00
SL-05-01	1101.73	7.41	46.41	41.84	10.22
SL-05-15	766.43	6.00	68.73	60.08	5.86
SL-05-13	941.65	1.81	149.66	89.56	10.79
SL-05-11	792.07	2.57	59.88	81.98	8.59
SL-05-20	744.15	4.21	33.98	66.79	6.49
SL-05-09	735.75	2.83	53.77	50.00	6.05
SL-05-22	528.34	6.81	44.33	62.08	7.71
SL-05-14	478.97	3.87	27.72	106.58	10.72
SL-05-10	656.90	6.09	105.14	87.34	6.77
SL-05-03	572.65	8.02	40.63	91.88	6.28
SL-05-17	714.47	23.97	35.46	84.27	9.23
SL-05-18	780.99	9.55	49.16	58.76	7.66
SL-05-15	1263.18	1.80	45.64	47.25	6.99
SL-05-06	1021.85	4.00	56.88	65.21	9.92
SL-05-08	910.35	3.98	98.72	68.41	7.02
SL-05-21	638.98	5.38	38.06	151.20	7.63
SL-04-01	793.83	2.84	50.38	37.32	4.04
SL-04-15	778.62	3.39	26.24	36.13	2.97
SL-04-10	680.19	7.14	23.33	58.45	4.19
SL-04-19	851.35	5.70	46.77	34.61	8.17
SL-04-05	2174.09	7.78	255.26	28.52	4.81
SL-04-09	1018.63	1.13	23.52	59.00	2.74
SL-04-02	873.34	8.14	26.02	42.04	3.32
SL-04-03	824.06	5.29	36.00	41.34	3.11
SL-04-22	668.24	5.38	13.40	66.69	3.24
SL-04-23	1505.71	6.42	81.34	46.67	10.80
SL-04-16	998.55	9.60	33.67	38.69	2.21
SL-04-18	1085.14	15.95	46.97	47.14	4.63
SL-03-24	1122.05	5.80	18.88	58.45	0.97
SL-03-09	1136.91	4.46	43.20	37.10	1.21

Appendix II: Averaged Core Data ($\mu g\ g^{-1}$) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
SL-03-25	661.50	2.63	16.30	56.41	2.68
SL-03-22	991.88	4.69	31.09	59.35	1.12
SL-03-13	1042.93	10.22	62.06	53.01	4.57
SL-03-19	1163.18	4.99	116.72	50.04	1.75
SL-03-08	752.14	0.93	147.67	52.59	3.37
SL-03-21	1061.09	11.08	57.26	62.12	2.54
SL-03-10	1278.19	6.23	26.31	32.93	2.09
KL-05-18	1461.64	8.46	56.09	28.18	8.35
KL-05-05	1399.18	16.82	45.31	163.28	8.42
KL-05-13	963.35	7.35	29.38	73.66	7.20
KL-05-07	1207.91	11.66	150.76	112.16	7.33
KL-05-06	1768.73	17.77	34.06	75.32	8.12
KL-05-16	1517.45	11.68	28.24	79.66	4.36
KL-05-01	1669.64	11.54	88.04	58.94	7.54
KL-05-12	2150.64	8.17	66.49	39.43	6.74
KL-05-03	1698.55	15.07	46.76	121.45	4.91
KL-05-20	1929.36	14.66	30.77	63.60	7.73
KL-05-14	1283.45	8.75	12.30	9.05	4.32
KL-05-10	1391.55	17.54	15.53	69.92	5.83
KL-04-02	1347.36	27.17	67.58	59.84	6.64
KL-04-08	857.65	16.72	56.37	51.07	13.07
KL-04-30	1116.70	20.29	30.25	75.01	7.84
KL-04-14	884.74	13.13	42.02	54.00	5.03
KL-04-27	1567.73	29.93	50.95	40.45	4.81
KL-04-11	1242.45	11.28	48.75	36.37	2.72
KL-04-09	1273.91	11.25	182.14	53.14	5.50
KL-04-20	1677.64	24.21	46.73	44.08	5.86
KL-04-12	828.52	22.21	46.27	52.14	4.75
KL-04-32	1460.45	29.99	73.83	50.36	6.18
KL-04-15	1482.64	33.03	78.65	45.57	7.13
KL-04-25	994.37	14.90	26.87	19.85	8.91
KL-04-26	947.18	19.67	67.80	63.39	5.33
KL-04-36	869.86	15.39	19.13	33.38	4.33

Appendix II: Averaged Core Data ($\mu g\ g^{-1}$) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
KL-04-10	994.15	16.47	28.56	56.71	6.98
KL-04-35	1088.64	12.55	24.53	31.95	7.35
KL-04-23	805.17	16.41	29.85	58.65	5.67
KL-04-34	744.61	15.59	30.72	39.86	4.69
RD-05-20	1259.64	14.14	22.28	101.49	9.68
RD-05-01	1819.55	22.15	18.91	54.86	7.21
RD-05-10	1607.27	21.72	44.37	58.21	6.27
RD-05-19	1277.45	10.61	25.45	84.84	6.85
RD-05-05	1577.64	12.53	17.62	120.54	9.27
RD-05-02	1518.55	16.75	13.06	28.78	5.19
RD-05-07	1418.00	10.65	35.45	67.87	5.88
RD-05-08	1187.18	11.72	18.37	72.54	3.79
RD-05-13	1562.82	10.53	25.35	53.75	4.67
RD-05-18	1530.09	11.58	23.67	38.78	6.06
RD-05-16	1759.09	20.18	20.41	75.75	5.33
RD-05-11	1341.00	11.50	16.16	74.65	5.42
RD-05-03	1449.55	6.83	54.08	51.96	7.76
RD-05-14	1173.27	4.28	25.46	58.36	4.58
RD-05-17	1313.45	8.87	67.66	90.52	6.95
RD-05-04	1525.73	8.72	48.27	104.60	7.23
RD-05-12	1407.18	10.26	19.97	211.71	6.20
RD-04-19	1149.64	21.89	51.14	31.63	4.15
RD-04-37	826.65	22.18	49.27	66.29	4.29
RD-04-36	1096.82	21.14	69.51	78.62	5.94
RD-04-29	1611.73	27.18	38.16	35.00	9.74
RD-04-15	956.46	30.10	59.06	59.44	8.22
RD-04-20	1070.09	25.17	56.34	57.33	5.92
RD-04-41	1159.83	36.37	53.25	40.42	9.84
RD-04-42	987.84	26.32	52.26	29.31	7.06
RD-04-05	1125.67	13.34	29.68	69.62	5.32
RD-04-33	1044.47	15.71	23.01	41.45	13.16
RD-04-13	1442.09	20.97	30.77	43.87	7.94
RD-04-18	1126.63	9.87	34.36	29.71	4.51
RD-04-12	1106.27	19.08	45.38	32.33	5.36

Appendix II: Averaged Core Data ($\mu g\ g^{-1}$) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
RD-04-40	1322.55	15.85	22.90	60.02	12.92
RD-04-35	1731.27	33.00	46.25	38.15	7.24
RD-04-30	1173.27	19.99	55.70	40.25	7.98
RD-04-09	1314.73	21.75	23.26	47.85	13.49
RD-04-11	1524.82	33.63	49.94	46.68	5.34
RD-04-28	1088.18	41.05	43.78	32.92	4.62
RD-04-01	1135.09	17.99	32.10	45.06	4.48
RD-04-22	1158.27	11.28	39.50	33.43	4.13
RD-04-06	1313.55	16.80	65.40	32.23	8.66
TL-04-17	1164.45	248.30	54.75	31.33	4.97
TL-04-32	1351.82	218.91	49.02	34.07	3.89
TL-04-03	996.61	233.25	65.12	53.20	3.88
TL-04-15	1313.18	235.63	53.44	36.35	3.83
TL-04-23	1242.18	183.25	47.83	13.80	2.73
TL-04-02	1118.09	128.14	42.83	34.91	3.47
TL-04-09	1532.90	349.12	48.62	34.55	5.71
TL-04-24	1241.45	187.28	62.93	36.32	3.58
TL-04-16	1491.91	309.52	41.19	33.95	3.74
TL-04-22	1065.00	215.64	56.84	34.74	3.42
TL-04-18	1542.18	151.95	41.26	96.77	5.16
TL-04-29	1869.91	344.35	38.06	55.60	4.71

Appendix III: Averaged Freshwater Data ($\mu g\ g^{-1}$). (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
SL-05-07	473.97	5.79	14.00	93.43	5.74
SL-05-02	442.17	4.16	34.42	147.13	8.75
SL-05-16	479.73	4.55	25.04	238.18	7.81
SL-05-01	759.53	8.13	19.59	55.66	8.20
SL-05-15	429.98	3.36	26.57	65.22	7.26
SL-05-13	427.05	5.79	34.18	175.41	10.08
SL-05-11	432.80	10.57	18.40	95.61	9.60
SL-05-20	435.18	4.49	38.85	96.91	9.35
SL-05-09	345.45	2.55	26.22	76.64	7.37
SL-05-22	403.71	5.70	52.07	78.22	9.87
SL-05-14	447.02	3.47	18.79	54.79	9.09
SL-05-10	366.78	8.33	28.73	83.50	9.32
SL-05-03	445.38	10.31	21.80	62.09	8.44
SL-05-17	469.95	16.45	17.87	45.85	11.32
SL-05-18	593.04	6.11	30.66	25.78	9.65
SL-05-15	516.52	5.88	13.51	86.44	8.90
SL-05-06	562.30	4.48	22.60	64.95	10.73
SL-05-08	477.28	5.72	20.91	74.41	6.53
SL-05-21	546.23	5.53	19.49	48.36	8.64
SL-04-01	474.71	3.62	20.96	55.76	4.67
SL-04-15	515.14	3.62	21.36	15.28	5.13
SL-04-10	428.68	3.97	19.41	11.65	4.68
SL-04-19	438.78	7.37	20.04	11.11	7.99
SL-04-05	871.98	3.61	50.27	50.49	1.61
SL-04-09	516.21	2.62	19.77	8.47	3.26
SL-04-02	608.24	3.92	16.94	14.37	2.27
SL-04-03	435.06	2.84	18.47	17.77	2.71
SL-04-22	476.15	2.94	15.49	14.91	2.27
SL-04-23	640.53	4.67	22.05	11.45	2.26
SL-04-16	880.34	8.43	32.38	27.21	3.79
SL-04-18	585.91	3.47	19.33	6.50	2.56
SL-03-24	516.99	2.71	13.92	36.83	2.98
SL-03-09	644.11	4.28	31.93	40.39	3.39

Appendix III: Averaged Freshwater Data ($\mu g\ g^{-1}$) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
SL-03-25	517.51	3.24	15.21	45.87	3.66
SL-03-22	538.10	5.77	15.08	13.16	2.65
SL-03-13	469.06	7.40	29.76	22.21	4.58
SL-03-19	501.86	2.77	56.24	27.30	2.48
SL-03-08	504.49	4.34	55.21	27.56	3.31
SL-03-21	563.76	3.10	19.68	13.64	2.71
SL-03-10	655.10	4.94	26.66	9.34	2.66
KL-05-18	1034.97	8.05	23.99	62.92	10.32
KL-05-05	1097.69	11.03	21.52	41.26	10.36
KL-05-13	716.63	6.80	16.39	96.33	9.71
KL-05-07	905.65	10.40	22.76	181.17	10.26
KL-05-06	1443.88	12.52	21.44	43.73	10.67
KL-05-16	1206.90	7.98	18.66	55.11	4.60
KL-05-01	1475.14	11.72	37.74	73.14	9.23
KL-05-12	1621.73	10.04	20.56	103.31	8.81
KL-05-03	1521.19	6.96	44.03	90.32	5.77
KL-05-20	1500.27	7.66	19.05	67.15	6.41
KL-05-14	1211.53	8.89	10.00	17.27	4.35
KL-05-10	1053.74	9.05	12.79	18.61	6.82
KL-04-02	1163.77	19.88	25.19	61.44	8.11
KL-04-08	867.89	15.19	39.74	61.61	17.45
KL-04-30	827.27	9.58	24.55	38.63	8.71
KL-04-14	712.00	8.40	41.21	50.32	5.45
KL-04-27	1107.65	16.32	28.00	61.90	6.73
KL-04-11	813.74	7.89	22.14	25.85	4.65
KL-04-09	1129.42	11.96	58.76	25.07	6.24
KL-04-20	1001.11	10.41	17.64	41.95	6.70
KL-04-12	597.79	9.66	22.96	28.33	5.65
KL-04-32	1040.18	15.08	28.79	65.95	8.86
KL-04-15	917.25	13.28	24.45	39.65	6.78
KL-04-25	1042.71	14.82	27.35	25.29	6.95
KL-04-26	657.84	10.71	25.92	46.33	7.44
KL-04-36	627.42	11.76	17.58	21.29	4.55

Appendix III: Averaged Freshwater Data ($\mu g\ g^{-1}$) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
KL-04-10	900.38	13.04	30.94	80.40	8.00
KL-04-35	1091.56	14.51	22.39	17.02	6.81
KL-04-23	630.35	10.55	18.76	26.24	6.36
KL-04-34	651.84	10.52	20.79	26.22	5.34
RD-05-20	1332.44	15.55	11.32	99.47	6.74
RD-05-01	1781.57	24.94	15.62	44.55	6.77
RD-05-10	1546.77	19.44	26.16	43.72	6.45
RD-05-19	1238.82	11.03	13.60	147.67	4.97
RD-05-05	1399.45	12.11	15.25	120.56	5.98
RD-05-02	1605.78	21.96	10.61	10.29	4.33
RD-05-07	1581.33	17.84	24.54	41.39	7.98
RD-05-08	1403.57	18.38	12.44	12.12	3.90
RD-05-13	1558.25	16.43	18.28	65.30	6.08
RD-05-18	1400.51	12.52	14.95	25.69	7.58
RD-05-16	1645.32	23.07	16.21	53.97	6.05
RD-05-11	1404.47	13.71	13.85	39.05	5.26
RD-05-03	1319.91	11.73	23.92	71.29	6.01
RD-05-14	1518.93	19.79	18.17	49.50	7.06
RD-05-17	1640.27	26.71	16.77	184.14	7.13
RD-05-04	1576.17	22.01	18.54	163.49	6.70
RD-05-12	1494.63	20.70	12.51	64.40	6.21
RD-04-19	1100.48	17.17	22.64	58.98	5.65
RD-04-37	943.83	34.64	33.52	76.61	6.19
RD-04-36	1026.67	20.91	30.88	106.01	6.38
RD-04-29	1577.06	32.66	19.14	33.07	8.21
RD-04-15	1220.30	31.05	22.74	67.11	7.02
RD-04-20	1192.00	26.61	24.88	113.47	8.12
RD-04-41	1178.00	19.77	20.75	9.68	8.42
RD-04-42	1072.91	22.17	23.81	50.91	7.39
RD-04-05	1272.43	26.30	25.33	33.63	7.52
RD-04-33	1147.14	26.20	19.88	43.63	13.70
RD-04-13	1323.27	18.57	19.54	41.53	9.35
RD-04-18	1091.87	16.71	19.60	47.35	3.85
RD-04-12	1133.73	20.41	20.66	36.35	7.32

Appendix III: Averaged Freshwater Data ($\mu g\ g^{-1}$) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
RD-04-40	1413.09	20.10	20.96	26.53	13.73
RD-04-35	1534.88	27.78	23.82	55.26	6.38
RD-04-30	1144.24	20.43	23.09	52.01	9.07
RD-04-09	1148.58	21.01	24.35	45.75	11.75
RD-04-11	1223.74	29.11	24.91	53.17	7.57
RD-04-28	1291.58	25.11	26.06	38.25	3.78
RD-04-01	1027.48	17.71	24.57	20.74	5.78
RD-04-22	1445.56	17.39	32.34	49.66	4.52
RD-04-06	1167.16	24.35	27.03	61.43	8.39
TL-04-17	1276.49	297.35	34.80	36.27	5.33
TL-04-32	1358.10	271.90	39.60	40.67	5.30
TL-04-03	997.14	280.31	45.07	59.14	5.32
TL-04-15	1308.00	231.13	44.54	37.77	5.34
TL-04-23	1203.02	155.94	47.19	13.36	3.08
TL-04-02	1232.38	265.40	35.92	16.91	6.66
TL-04-09	1455.71	326.38	45.84	25.82	6.91
TL-04-24	1335.43	261.45	41.98	46.56	6.27
TL-04-16	1430.18	294.96	38.39	17.51	5.99
TL-04-22	1132.96	297.28	42.66	28.24	5.80
TL-04-18	1749.23	232.03	26.67	120.05	5.93
TL-04-29	1837.32	350.06	34.33	65.96	5.15

Appendix IV: Averaged Marine Data ($\mu g\ g^{-1}$). (SL-Salmon Lake, KL-Klag Lake, RD-Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
SL-05-07	1756.50	4.05	25.03	95.21	3.94
SL-05-02	1629.50	2.26	47.85	109.12	6.99
SL-05-16	1546.10	2.64	28.75	84.07	6.48
SL-05-01	2272.90	1.47	29.27	42.53	8.05
SL-05-15	1544.90	3.08	44.56	70.42	5.90
SL-05-13	1734.95	4.58	38.98	126.89	10.24
SL-05-11	1799.69	2.06	44.62	73.90	7.56
SL-05-20	1568.05	3.46	41.95	60.41	6.55
SL-05-09	1446.88	3.77	41.03	63.76	5.68
SL-05-22	1259.67	2.32	135.30	96.80	7.61
SL-05-14	1483.42	4.36	37.86	93.28	6.87
SL-05-10	1320.89	2.84	65.57	73.74	6.57
SL-05-03	1449.58	4.60	50.51	78.65	7.54
SL-05-17	1465.49	3.77	26.57	21.10	8.84
SL-05-18	2129.07	2.29	31.36	23.07	6.16
SL-05-15	2097.48	13.30	36.80	66.41	8.92
SL-05-06	1606.39	3.52	27.27	32.46	12.10
SL-05-08	2049.90	7.96	35.82	59.49	5.43
SL-05-21	1826.16	2.63	32.19	38.81	8.90
SL-04-01	1473.84	1.60	28.72	15.70	2.93
SL-04-15	1511.33	0.92	29.24	8.16	4.02
SL-04-10	1671.71	1.11	23.20	4.20	2.89
SL-04-19	1408.84	1.95	25.31	5.71	5.46
SL-04-05	2323.36	1.23	25.38	11.07	0.88
SL-04-09	1892.01	2.21	32.89	0.67	0.80
SL-04-02	2006.12	0.93	24.79	0.84	0.56
SL-04-03	1742.43	0.81	27.74	0.68	0.63
SL-04-22	2015.65	2.92	25.34	0.50	0.72
SL-04-23	2046.17	1.24	29.63	1.19	0.07
SL-04-16	2200.20	9.62	37.80	25.86	2.82
SL-04-18	2282.21	4.11	32.96	0.70	0.57
SL-03-24	2125.24	2.18	17.38	3.16	1.36
SL-03-09	2235.20	1.59	40.79	36.72	1.77

Appendix IV: Averaged Marine Data (µg g^{-1}) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
SL-03-25	1975.40	1.30	24.43	19.46	0.85
SL-03-22	2047.38	0.88	21.49	0.42	0.44
SL-03-13	2246.16	1.38	30.64	0.83	0.66
SL-03-19	2087.09	0.82	39.79	7.99	0.88
SL-03-08	1785.70	1.47	43.18	1.45	0.62
SL-03-21	2600.55	3.69	38.98	0.62	0.46
SL-03-10	2263.89	1.39	26.66	1.05	0.57
KL-05-18	1613.67	5.48	31.74	46.12	9.07
KL-05-05	2130.11	3.94	51.45	63.33	7.89
KL-05-13	1418.94	3.16	22.81	36.54	6.54
KL-05-07	1558.21	2.32	30.35	115.71	7.05
KL-05-06	2271.54	3.63	36.71	66.80	7.68
KL-05-16	2200.53	1.07	26.24	40.66	3.80
KL-05-01	2536.01	1.85	45.60	41.96	4.98
KL-05-12	2460.78	3.71	30.79	69.50	5.79
KL-05-03	2458.41	2.97	34.90	70.88	3.78
KL-05-20	2408.17	3.75	26.49	54.89	3.85
KL-05-14	2192.32	1.54	15.90	29.57	3.62
KL-05-10	2121.64	2.71	22.26	20.04	4.26
KL-04-02	2021.07	3.58	29.48	28.86	6.29
KL-04-08	1381.04	2.20	45.39	33.08	29.87
KL-04-30	1577.47	5.17	43.46	23.67	5.26
KL-04-14	1205.03	2.17	60.31	54.94	4.31
KL-04-27	2211.44	4.34	32.76	26.36	4.35
KL-04-11	1668.01	2.63	30.88	17.67	3.03
KL-04-09	1663.63	5.37	81.03	26.34	7.35
KL-04-20	1902.77	6.80	26.44	34.58	3.83
KL-04-12	997.78	2.70	31.35	24.97	4.50
KL-04-32	1720.86	12.14	49.61	61.65	5.84
KL-04-15	1627.75	3.86	32.92	22.73	5.37
KL-04-25	1698.40	3.54	34.98	28.96	5.13
KL-04-26	1380.64	2.97	31.69	18.05	5.93
KL-04-36	1089.02	5.45	28.96	28.52	3.94
KL-04-18	1563.82	3.69	51.72	43.06	6.84

Appendix IV: Averaged Marine Data (µg g^{-1}) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
KL-04-10	1533.02	2.69	36.18	45.90	4.37
KL-04-35	1531.19	5.53	32.98	26.43	5.22
KL-04-23	1053.16	2.84	34.27	32.06	5.46
KL-04-34	1110.96	2.13	29.39	15.78	4.28
RD-05-20	1949.53	4.54	22.00	80.89	5.57
RD-05-01	2576.51	3.48	23.06	14.86	6.28
RD-05-10	2071.09	5.07	41.08	78.93	5.65
RD-05-19	1789.42	1.85	23.75	67.04	4.28
RD-05-05	2026.43	2.96	25.09	79.45	4.71
RD-05-02	2180.03	3.46	22.39	9.92	4.82
RD-05-07	2266.10	4.37	36.68	52.02	5.63
RD-05-08	1769.18	2.32	23.64	26.26	2.66
RD-05-13	2068.17	2.80	33.51	58.54	4.30
RD-05-18	1836.99	2.79	21.55	16.18	5.99
RD-05-16	2041.73	4.55	26.43	49.43	4.79
RD-05-11	2136.09	4.25	23.57	29.29	3.72
RD-05-03	2028.11	2.77	37.49	55.47	6.03
RD-05-14	1958.45	6.00	34.65	71.60	5.33
RD-05-17	1947.09	4.09	31.63	145.36	6.91
RD-05-04	2145.93	3.31	23.06	54.20	5.76
RD-05-12	2277.25	4.15	23.12	162.39	4.85
RD-04-19	1334.57	3.67	28.13	38.07	3.54
RD-04-37	1536.57	6.97	38.31	39.24	4.14
RD-04-36	1880.61	11.60	44.04	56.27	5.73
RD-04-29	1953.65	4.26	30.27	35.48	6.18
RD-04-15	1620.73	14.33	43.13	79.08	8.10
RD-04-20	1541.53	10.03	40.29	76.13	6.64
RD-04-41	1766.89	4.26	31.49	10.35	8.59
RD-04-42	1544.16	4.08	35.50	43.08	7.21
RD-04-05	1757.14	3.03	27.15	14.00	6.23
RD-04-33	1439.69	3.51	25.34	36.92	8.75
RD-04-13	1849.55	2.37	25.31	26.72	6.00
RD-04-18	1673.76	1.58	26.57	18.30	4.45

Appendix IV: Averaged Marine Data ($\mu g\ g^{-1}$) continued. (SL-Salmon Lake, KL-Klag Lake, RD- Redoubt Lake, TL-Tumakof Lake).

Sample Name	Sr	Ba	Mg	Zn	Mn
RD-04-18	1673.76	1.58	26.57	18.30	4.45
RD-04-12	1675.54	4.53	29.95	28.75	4.71
RD-04-40	2173.71	1.81	27.23	4.73	9.52
RD-04-35	1788.60	4.32	31.46	31.52	6.50
RD-04-30	1785.31	10.06	41.58	48.25	11.13
RD-04-09	1753.17	2.58	32.56	22.53	9.58
RD-04-11	1535.16	7.33	38.98	49.54	5.55
RD-04-28	1670.91	2.84	29.91	8.53	3.49
RD-04-01	1559.11	3.06	34.26	21.05	3.20
RD-04-22	2125.84	7.73	37.59	23.54	2.72
RD-04-06	1688.01	8.48	43.03	56.39	7.20
TL-04-17	1447.47	8.45	46.83	28.43	1.84
TL-04-32	1467.75	14.17	52.96	32.55	2.41
TL-04-03	1136.55	7.96	52.73	21.86	2.44
TL-04-15	1540.72	6.82	59.24	21.26	2.21
TL-04-23	1434.69	4.78	50.64	15.65	1.48
TL-04-02	1267.96	4.00	45.59	18.34	2.09
TL-04-09	1639.46	5.03	57.53	28.87	2.56
TL-04-24	1429.02	9.20	47.67	27.55	2.13
TL-04-16	1371.68	5.66	51.49	18.25	3.42
TL-04-22	1355.94	9.26	52.99	24.77	2.27
TL-04-18	1736.35	7.18	34.83	69.50	1.77
TL-04-29	2467.57	5.76	39.63	42.38	1.81