Laser Ablation ICP-MS And Ion Chromatography Method Development For The Analysis Of Fish Otoliths: Applications To Pacific Herring (*Clupea pallasi*) Biology

by

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We accept this thesis as conforming
to the required standard

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Abstract

The goal of this thesis is to develop a method for the chemical discrimination of herring populations by analysis of herring otoliths. A novel approach for delineating populations based on the physical shape of element concentration profiles determined by laser ablation inductively coupled plasma mass spectrometry was conceptualized, and method development steps were followed. This method intends to identify divergence in herring life history, which may be a proxy for population divergence.

A glass sandwich of three National Institute of Standards and Technology (NIST) standard reference materials of varying trace element concentrations was constructed. Element concentration profiles are generated by continuously ablating a line scan across the surface of the glass sandwich. Concentration profiles from a material of known heterogeneity indicate that spatial resolution across concentration gradients is controlled by the magnitude and direction of the change in concentration. Profiles across increasing concentration gradients show better spatial resolution than decreasing gradients by a factor of ~2. For either direction of change, the relationship between the magnitude of the concentration gradient and the spatial resolution is linear, and therefore predictable. The appropriate resolution for any target can therefore be based on the observed range of its concentrations. Spatial resolutions of Sr, Rb, and Pb are similar suggesting that the ablation behaviour of different elements is not a significant control, and therefore a single resolution can realistically be applied to many elements.

A Sr concentration profile from a natural sample (fish otolith) is generated, and the resolutions from the glass sandwich are applied. For the concentrations observed and

the settings and hardware used, a minimum spatial resolution of 50 μm was calculated. Concentration variations at smaller scales can be detected but not quantified. The results of the otolith line scan shows that line scans do provide qualitative data at small spatial scales, which may be useful for characterizing heterogeneous targets.

Due to analytical complications and instrument downtime LA-ICP-MS was not available for quantification of herring otolith chemistry. Instead, ion chromatography was employed, as this is a sensitive, highly precise method for quantifying major cation chemistry. Concentrations of Na, K, Mg, Ca, and Sr in the otoliths of 126 adult Pacific herring sampled from summer feeding areas on the LaPerouse Bank are quantified using ion chromatography. Traditional weighing of small otoliths proved to be imprecise, therefore a robust chemical method for mass determination was developed. The known concentration of calcium in the otolith and the concentration in the digested solution are used to determine the mass of the otolith. This assumes invariant calcium concentration in all otoliths, a valid assumption considering analytical precision. This is a novel approach to determining otolith mass: information critical to quantifying the concentration of elements in the otolith. The dataset is sorted by geographic location, and statistical tools are employed to investigate otolith cation concentrations, and to delineate herring populations.

Concentrations of Na, Mg, and Sr show an inversely proportional relationship with otolith weight, which we theorize is the result of the constant change in the ratio of endolymph volume to otolith surface area. This effect must be removed by normalizing otoliths to a standard weight before the chemistry of otoliths from different populations

can be compared. ANOVA and post-hoc analysis of the weight normalized concentrations of sodium, potassium, magnesium, and strontium show three otolith populations that can be confidently separated by a combination of single element concentrations and element ratios of sodium, potassium, magnesium, and strontium. A two-step process using a combination of concentration and ratio data most easily discriminates all three populations.

The chemical separation of these herring populations confirms that schools of herring are discrete, and that the degree of mixing associated with metapopulation dynamics is not sufficient to homogenize the signals (as is the case with genetic discrimination). This chemical discrimination also provides evidence of differing life histories between herring groups. Herring on the LaPerouse Bank show a high degree of homing, indicating that associations are maintained while on the feeding grounds, as well as during migrations between spawning and feeding areas.

Ion chromatography provides some distinct advantages over inductively coupled plasma mass spectrometry (ICP-MS) techniques. Ion chromatography provides an inexpensive, highly precise method for the quantification of major cations in digested otolith solutions. The low cost, limited infrastructure, and ease of use make ion chromatography a superior technique as a tool for bulk otolith analysis on large scales.

While the experimental design was of small scope, this study illustrates the effectiveness of otolith chemistry to separate herring populations, and suggests that further development in otolith microchemistry can lead to an effective research and management tool for herring on Canada's west coast.

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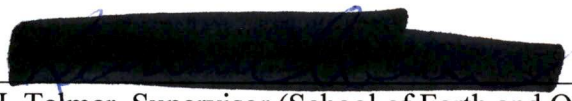

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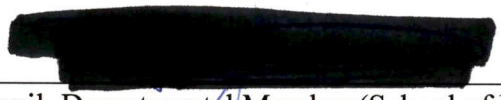

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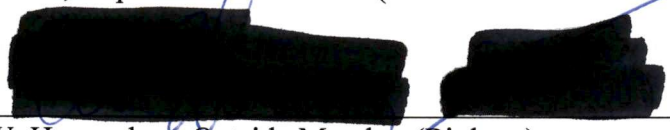

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CHAPTER 1

Introduction

Since the discovery of annular growth rings in otoliths in 1899, and the subsequent discovery of daily growth rings in 1971 (Panella 1971), there has been an explosion of research into the use of otoliths as an information source. Several papers have been published dealing with topics ranging from stock identification (Campana et al. 2000, Thresher 1999), identification of migratory history (Volk et al. 2000, Radtke et al. 1998, Campana et al. 1999), reconstruction of environmental history (Gallahar & Kingsford 1996, Halden et al. 2000, Chesney et al. 1998), and identification of pollution gradients (Hanson & Zdanowicz 1999). Other applications include age determination (Kalish 1993) and chemical marking of batches of fish (Campana 1999).

In the following pages a review of otolith function, growth, composition, and applications are presented. The history, ecology, and management policies of Pacific herring on Canada's west coast are introduced, and two separate papers are presented.

Otoliths

Physiology and function of the Inner Ear

The otolith is an acellular accretion of alternating layers of aragonite and protein that occurs in the labyrinth of the inner ear of all teleost fishes. The labyrinth is bi-laterally symmetrical, except in some flatfish, and is divided into dorsal and ventral sacs, termed the pars superior and pars inferior respectively (Secor et al. in Stevenson & Campana 1992). There are three individual vestibules, each containing an otolith. These

are the sacculus, utriculus, and lagenus, and contain the saccular, utricular, and lagenar otoliths respectively. A schematic diagram of the labyrinth, showing the three vestibules and their associated otoliths is shown in Figure 1.1.

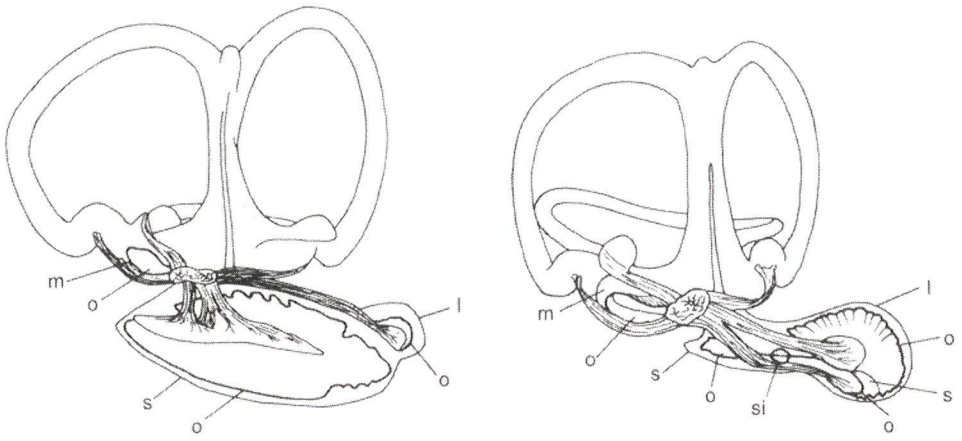


Figure 1.1: Schematic diagram of the labyrinth of teleost fishes. Annotated to show the auditory portion of the ear. Indicated are the positions of the lagena (l), utriculus (m), sacculus (s), the otoliths associated with each of the vestibules (o), and the transverse canal (si). The nerve tissue shown is the auditory portion of the eighth cranial nerve. (From Moyle & Cech 1996)

The otoliths are suspended within the vestibules, bathed in fluid. Adjacent to the otolith is a sensory epithelium (the macula) that is covered in hair cells (ciliary bundles) that come in contact with, and detect movement of the otolith. The ciliary bundles are composed of a kinocilia supported to one side by a group of successively shortened stereocilia causing the ciliary bundle to be asymmetrical in shape (Figure 1.2). Ciliary bundles have the distinction of having both an excitation and inhibitory signal (Figure 1.2). If the kinocilia is bent towards the stereo cilia the result is hyperpolarization of the cell, and an inhibitory signal turns off the cell. If bent away from the stereocilia then the cell becomes depolarized and an excitation signal is the result. If the kinocilia is bent at right angles to the stereocilia there is no response. The options in between follow a cosine

law to the angle of bending (Bone et al. 1995). The physiological polarization of the hair cells make it possible for fish to not only detect sound, but to also determine the direction of its origin.

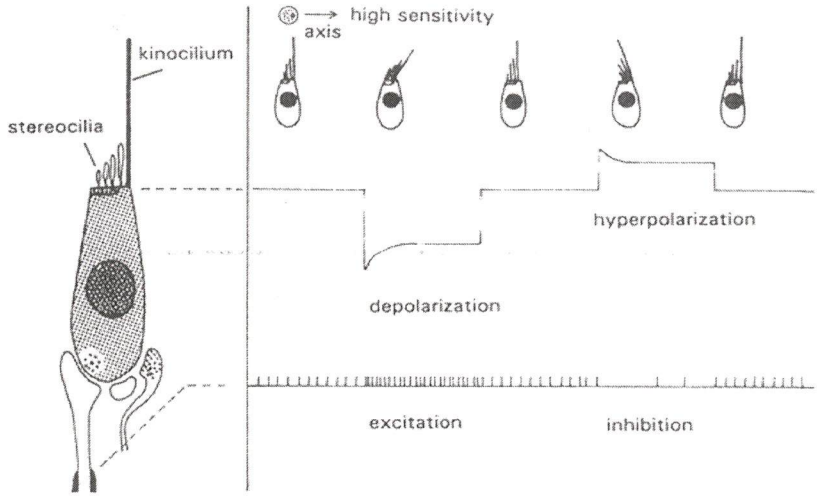


Figure 1.2: Single hair cell depicted on left. To right schematic diagram depicts displacement of hairs along the axis of sensitivity, and the associated excitation or inhibition reactions. *(Taken from Bone et al. 1995)*

As the fish is bombarded with sound stimuli, the entire body of the fish moves with the particle displacement of the fluid medium of which it is a part. The dense otoliths lag behind the particle displacement, and the differential motion between the otoliths and the chambers in which they reside allow the fish to detect sound stimulus (Moyle & Cech 1996). Some species have adapted specialized components that allow them to transform sound pressure into displacement allowing them to detect higher frequencies, at farther distances (Moyle & Cech 1996). These are known as the ostariophysans. The gas chamber of the swim bladder is employed in this transformation in many of the teleost fishes. The space in the swim bladder is far more compressible

than water, and as such will pulsate when exposed to sound stimuli. Specialized structures relay this pulsating to the otolith sac causing particle motion in the vestibules, allowing the fish to detect the sound pressure, in the absence of sound particle motion (Moyle & Cech 1996). A schematic diagram depicting the association between the swim bladder and inner ear is shown in Figure 1.3.

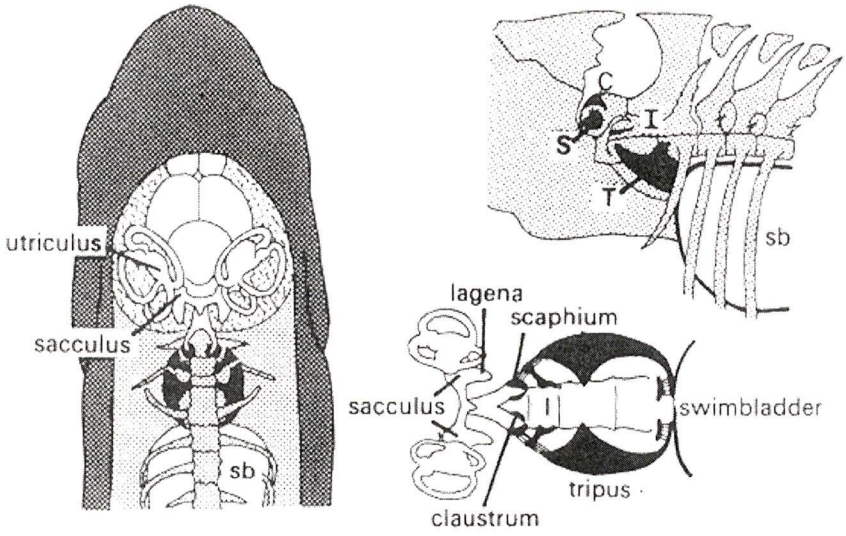


Figure 1.3: Schematic diagram of ostariophysan ear ossicles. Shows the association between the labirynth and swim bladder (SB). Sound pressure is transformed into particle motion by movement of the scaphium (S), claustrum (C), tripus (T), and intercalarium (I). *(Taken from Bone et al. 1995)*

Otolith Growth

The otolith is comprised of alternating layers of calcium carbonate and protein. The calcium carbonate (aragonite) which makes up ~ 96% of the weight of the otolith is precipitated from ions contained in the endolymphatic fluid. This deposition occurs following a chronological pattern, with opaque high growth regions occurring in the summer months, and protein rich low growth regions occurring in the winter months (Campana 1999). The combination of a winter and summer growth band represents one year, and is commonly referred to as an annuli. This chronological record is one of the

features that make the otolith such an attractive research tool for investigation into life history of fishes.

Otolith Composition

The composition of the otolith, as described by Campana (1999), is a relatively pure crystal structure as compared to other structures dominated by calcium carbonate. Campana et al. (1997) found the otolith to contain ~96.2% by weight calcium carbonate, ~1% by weight inorganic impurities, and the remaining ~3% of an organic protein matrix. The composition of otoliths is dominated by carbon, oxygen, and calcium, which make up the crystal matrix but other minor and trace elements may be present in very small quantities (< 10 ppm) (Campana et al. 1997).

Inorganic impurities are likely to occur in one of two ways: (i) a substitutional solid solution – ions of similar size and charge are able to substitute into the crystal matrix taking up a position which would normally be occupied by calcium; (ii) an interstitial solid solution – ions of inorganic impurities are trapped within the interstitial spaces of the crystal matrix (more likely the case for ions of smaller ionic radius). Strontium is a good example of a possible ion for a substitutional solid solution. Strontium has a similar ionic radius and charge to calcium, and would likely replace calcium in the crystal matrix of the otolith.

Contrary to bone, otoliths grow throughout the life of the fish and possess the distinction of being metabolically inert. Once deposited, elements or compounds that are incorporated into the otolith, either held within the matrix or substituting for Ca in the $CaCO_3$, are unlikely to be resorbed or reworked (Campana & Neilson 1985, Campana

1999). This represents a second feature of the otolith that makes it a powerful chemical data recorder.

Another feature of the otolith that makes it a valuable tool is the observation that the $CaCO_3$ and trace elements which make up the otolith are derived mainly from the water in which the fish lives, while elemental composition of the fishes food plays only a minor role in determining the elemental composition of the otolith (Gallahar & Kingsford 1996). The combination of the chronological nature of the otolith, and its ability to serve as a proxy for water chemistry create a continuous record of the fish's environment (a virtual flight data recorder over the history of the fish's life). This attribute can be utilized to explore age, temperature, as well as changes in elemental composition that may occur due to movement through different environments.

Elemental Uptake in Otoliths

The incorporation of elements from the fish's environment into the otolith is likely a highly regulated process for many of the minor elements; however there appears to be little discrimination against the incorporation of trace elements (Campana 1999). The pathway for inorganic element incorporation into the otolith is from the water into the blood plasma via gills or intestine, into the endolymphatic fluid, and finally ions are precipitated from the endolymphatic fluid onto the otolith (Campana 1999). Uptake through the gills is the most important source of inorganic elements for freshwater species, however in marine species the continual drinking of salt water makes the intestine an important mode of transfer for some species. Trace elements are likely assimilated through the intestine in direct proportion to their relative concentration in the

water, however at very limited efficiency (Olsson et al. 1998). Gallahar & Kingsford (1996) manipulated Sr concentration of water and food in two independent experiments using *Girella elevata,* a marine species found in coastal waters of Australia. They found that changes in Sr:Ca ratio were strongly influenced by changes in the ambient water concentration of Sr, while food was shown to play only a small role in the total Sr content of the otoliths.

Elemental discrimination can occur at any of the three interfaces on route to the otolith (Fig 1.4), with discrimination likely being greatest for major and physiologically regulated ions. The presence of inorganic elements in the water does not indicate bioavailability. Factors such as salinity, pH, temperature, and dissolved oxygen content can all potentially influence the bioavailability of elements (Mayer et al. 1994).

Since elements incorporated into the otolith are derived through the fore mentioned system of biological interfaces, it seems unlikely that the otolith would reflect the environmental abundance for metabolically regulated elements. There are several elements however, whose environmental abundance may be accurately reflected in the otolith. Trace elements such as Sr, Zn, Pb, Mn, Ba, and Fe are likely to have abundances in otoliths which reflect environmental condition; these are also elements whose uptake is likely to be less regulated then the major elements, and common salts. Other trace elements such as the rare earths may exhibit similar behaviour, allowing them to be exploited in the analysis of otoliths. Some major cations whose ionic size and charge are similar to Ca are also likely to substitute into the $CaCO_3$ of the otolith.

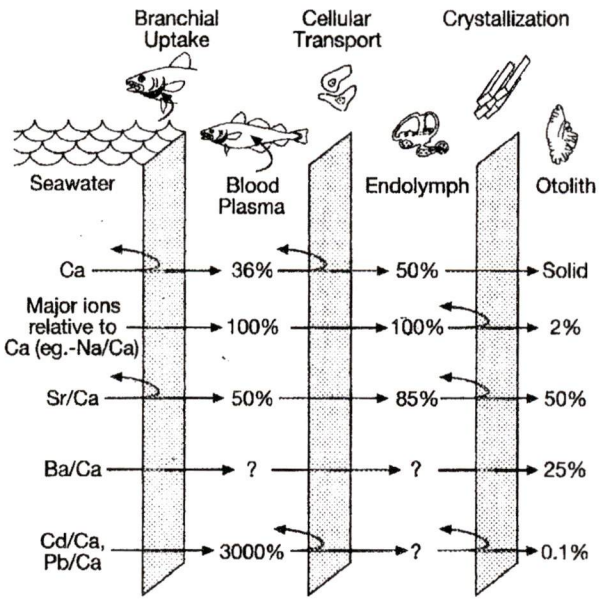


Figure 1.4: Elemental pathways and barriers between seawater and the otolith, with coarse estimates of transfer rates for selected elements at each physiological barrier. Elemental discrimination is greatest for major and physiologically regulated ions (Taken from Campana 1999).

Analysis of Otoliths

Three properties of otoliths have been identified as influential to their utility in fisheries research; (i) otoliths grow throughout the life of the fish and are metabolically inert; (ii) the calcium carbonate and trace elements which make up ~97% of the otoliths weight are derived primarily from the water; (iii) the laminated chronological nature of the otolith. These features allow researchers the flexibility to alter their analytical procedures to best answer the question at hand (Campana 1999). Whole otolith assays are traditionally used to answer questions of stock composition, where the difference in elemental composition of groups of fish (spanning the lifetime of the fish) tells the story (Campana et al. 2000, Kingsford & Gillanders 2000, Patterson et al. 1999, Thorrold et al. 1998). Localized assays take advantage of the chronological nature of the otolith, and are

often used to answer life history questions (e.g. identifying anadromous behaviour) (Radtke et al. 1998, Milton et al. 2000, Arai et al. 2000).

Applications of Otolith Chemistry

Stock Identification

The use of otolith microchemistry has largely been devoted to the field of stock determination. This has emerged as one of the most applicable fields of otolith research, and has focused on three sets of questions; (i) discriminating between marine and freshwater populations; (ii) determining links between natal rivers or estuaries and adult stocks; (iii) assessing population structure of marine fishes (Thresher 1999). While there are some complications with using otolith chemistry as a tool for stock discrimination, it is likely applicable where the environmental differences between stocks are larger then the variation within the stock, and where physiological differences associated with fish size and age have been statistically removed (Campana 1999), or where only larval regions of the otolith are analysed. Use of otoliths to determine differences between freshwater and marine populations has been widely documented (Halden et al. 2000, Radtke et al. 1998), and employs the measurement of Sr:Ca ratios, changes in Sr:Ca ratios are closely linked to movements across salinity gradients.

One of the most robust applications of otoliths in stock characterization is the investigation of mixing, or tracking of stock migration. The strength in this application lies in the fact that the elemental fingerprint is used as a marker of predefined groups, and the fingerprint need not be linked back to its environment (Campana 1999). The success of these natural tags requires three critical assumptions; (i) there must be a characteristic and reproducible chemical signature for each group (if the chemistry of the groups of

interest are not significantly divergent then this application is inappropriate); (ii) all possible groups contributing to the mixture must be identified (if this criterion is not met uncharacterized fish will mistakenly be interpreted as coming from one or more of the reference groups); (iii) the chemistry of the otolith must remain stable over the interval between characterization and mixing. This third point is the basis of the entire method. The investigation into mixing takes advantage of the fact that the size, and composition of the otolith cannot change appreciably over a short period of time (Campana 1999). Once the elemental signature of a source group has been determined this should not change significantly until sufficient otolith growth has occurred to alter the whole otolith chemistry. The time required for sufficient accretion of new otolith to occur means that over short time periods the chemistry of the otolith will be stable.

A study of this type was carried out by Campana et al. (1999) who looked at the migration of over wintering Atlantic cod, *Gadus morhua*, near the mouth of the St. Lawrence River. Using bulk chemistry of whole otoliths determined by isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS) the chemical signature of the four distinct populations known to be present in the over-wintering grounds was determined. Samples from different areas on the over wintering grounds were analysed in order to investigate population structure, distribution and mixing within the over wintering area. The signatures between the sample locations were statistically different suggesting that populations did not exhibit mixing over the migration route or once in the over wintering area. As well, the presence of population specific distributions within the over wintering area were identified and observed to remain the same over successive years. Campana et al (1999) determined that the elemental signatures used in their study

performed well as natural tags, showed good accuracy of classification, and provided information that was not available with either genetic or tagging studies.

The use of otolith microchemistry has also been applied to determine natal environments for adult fish (Thorrold et al. 1998, Kingsford & Gillanders 2000, Milton et al. 1997). This involves relating the elemental fingerprint in the core of the otolith to known or estimated environmental conditions of natal regions. Because of the need for correlation, this application is approached with caution as variation within the nursery area as well as ontogenetic differences may confound results (Campana 1999). When natal regions show sufficient variation however, this is a powerful application and can have implications in current management practices.

Gillanders and Kingsford (2000) used the elemental tag of otoliths from trumpeter (*Pelates sexlineatus*) in order to determine if otoliths of juvenile fish showed variation between and within natal estuaries, and to determine whether the elemental fingerprint that was recorded in the fish was consistent over successive years. In this study juvenile trumpeter were sampled from two locations within seven estuaries along the coast of Australia. They found no significant difference in standard length or otolith weight among estuaries, making ontogenetic variation of elemental uptake of little concern when comparing juveniles of the species. Significant differences in the elemental fingerprint of the otoliths were observed among different estuaries, however large variations of elemental signature within estuaries were also observed, as well as variation over successive years. These data make it difficult to assign fish to a particular estuary, as fingerprints in otoliths showed some overlap among estuaries and years. While this study

showed mixed results, the powerful information held within the otolith is obvious when considering this example.

Reconstruction of Environmental History

Recently otoliths have been used to reconstruct the environmental history, mainly temperature and salinity, of fish (Campana 1999). It was originally thought that Sr:Ca ratios within the otolith would be a useful measurement for detecting changes in temperature of the fish's environment. Campana (1999) states that temperature reconstructions based on Sr:Ca measurements have only been successful in a small number of studies, and the results are often confounded by changes in the salinity of the fish's environment. Gallahar and Kingsford (1996) investigated factors influencing the Sr:Ca ratios in otoliths in a controlled experiment. It was noted that Sr:Ca did not change significantly when water temperatures were elevated from 19° to 28° C. They did however observe an increase in Sr:Ca in the otolith with an increase in ambient Sr concentration of the water. A detectable increase in Sr:Ca was also observed for fish that were fed a Sr enriched diet. These findings confound attempts at reconstruction of thermal history based on Sr:Ca ratios within the otolith. Isotopic systems however have been demonstrated to have a close relationship with temperature (Bath et al. 2000, Thorrold et al. 1997) and should represent a powerful tool for the reconstruction of temperature history.

Characterization of Migratory History

Reconstruction of salinity in the fish's environment can be achieved by employing the Sr:Ca ratio along the growth axis of the otolith. This application has been used

successfully in the study of anadromy for Arctic Charr and (Halden et al. 2000, Radtke et al. 1998) as well as more subtle migration patterns (Campana et al. 1999). The reconstruction of migration pathways makes many of the same assumptions as stock discrimination (based on otolith core analysis). Investigations into migratory history take advantage of the chronological nature of the otolith, and employ multiple analyses along the growth axis of the otolith. This allows for reconstruction of the chemical environment based on age or date (Campana 1999).

Of interest to fish biologists is the life history traits associated with migratory events. This is an area where otolith microchemistry can be used, along with the inherent chronology, to determine such characteristics as age of first migration by looking at otolith microchemistry trajectories. This is a fairly robust application, one that is not easily confounded by ontogenetic variations and where no knowledge of the past environment of the fish is required (Campana 1999). Radtke et al. (1998) employed such a method to determine life history of migration in Arctic charr (*Salvelinus alpinus*). They examined the trajectory of Sr:Ca ratios in the otoliths of anadromous Arctic charr using wavelength dispersive electron microprobe analysis from the centre towards the outer edge of the otolith. Sr:Ca ratios were aliased to the otolith, and the age at divergence from the freshwater signal was determined. Radtke et al. (1998) found that most charr remain in fresh water for at least the first two years of life, at which point they begin to make annual migrations to the sea. It was determined that otoliths clearly recorded habitat shifts associated with migration across a salinity boundary.

Reconstruction of migration history in more homogeneous environments has also been investigated using fish otoliths. Thorrold et al. (1997) looked at the migration of

juvenile Atlantic croaker (*Micropogonias undulates),* a demersal species that moves between coastal waters of the Atlantic and the Gulf of Mexico, and the continental shelf to spawn. Larvae are thought to be transported inland with winds, at which point post larvae migrate to estuarine environments. Sampling of juvenile Atlantic croaker took place at two estuaries, the Neuse River and the Elizabeth River near Cape Hatteras and analysis was done using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Thorrold et al. (1997) observed no significant difference in the core otolith composition from the two locations, and could not reject the hypothesis that Atlantic croaker from the north and south of Cape Hatteras originated from different locations. A significant difference in Ba:Ca ratios was determined in otoliths from the two locations which may indicate difference in water mass residency, but there was insufficient evidence to determine that the observed differences were due to water chemistry.

Campana (1999) warns of some possible stumbling blocks to migratory reconstruction through more homogenous environments based on otolith microchemistry. The interpretation of migration effects reflected in the otolith when dealing with only subtle environmental changes assumes that elemental concentration at any point in the deposition of the otolith accurately reflects environmental concentrations. Differences in deposition and accumulation of elements due to ontogenetic or growth rate are not considered, and assumed to be non-existent. This assumption may limit the ability of otoliths to be accurately interpreted, because these ontogenetic variations of elemental deposition in otoliths have been documented (Campana 1999).

Otoliths as Indicators of Pollution

Metals such as Cu, Cd, & Hg have often been considered a good proxy for the identification of pollution, as many industrial effluents are characterized by relatively high concentrations of these low abundance elements (Campana 1999). Biomonitors as a method of monitoring trace element contamination in aquatic systems are routinely used and have focused on the accumulation of elements in soft tissue of aquatic fauna. Calcified tissues in the monitoring of environmental contaminants have also been used with corals, and bivalves. There has been a similar trend towards the use of calcified structures in fish for monitoring pollution. These include scales, fin rays, bones, and recently otoliths. The use of otoliths as a monitor of pollution has not shown positive applicable results (Campana 1999).

Hanson and Zdanowicz (1999) determined the concentration of Cr, Cu, Mn, and Zn in Atlantic croaker (*Micropogonias undulates)* otoliths from a known pollution gradient in Galveston Bay, Texas. The pollution gradient was present in both the sediments, as well as suspended in the water column. The fish, as well as their prey, would have been exposed to a pollution gradient in all phases (Hanson & Zdanowicz 1999). Liver and whole otolith concentrations of Atlantic croaker sampled along the pollution gradient were compared with sediment concentrations from the sampling sites. There was no increase in hepatic concentrations of either Cu or Zn along the pollution gradient indicating significant metabolic regulation. Also hepatic Cr, Ni, and Mn showed no consistent concentration trends and no correlation to sediment concentrations. Statistical analysis showed a similar result for otolith concentrations of Cu, Cr, Ni, and Mn. No significant difference in otolith concentrations along the pollution gradient was

found. In addition there was no correlation between liver and otolith concentrations, suggesting that metal regulatory processes may be a significant stumbling block in using fish otoliths as a proxy for environmental contamination.

Age Determination

Age validation of fish from otolith microchemistry has been used in many cases to eliminate the subjectivity of visual age determination, and has thus focused primarily on the assumption that incorporation of elements into the otolith will follow annular periodicity (Campana 1999). The periodicity of Sr:Ca within the otolith has been used with some success (Radtke et al. 1998). This periodicity has been attributed to annular cycles in temperature, migration patterns or growth rate. Some studies however, report no clear correlation between the periodicity observed in the otolith chemistry and the visible annuli of the otolith (Campana 1999). Oxygen isotopic periodicity has also been investigated as a means of age determination, but these techniques are no more reliable then visible inspection of annuli (Campana 1999).

Radiometric age determination has seen some success, however the isotopic concentrations to be measured are extremely low. Of the radiometric methods, age determination based on the observance of bomb carbon (C^{14}) is one of the best techniques available. This is based on the enrichment of C^{14} due to nuclear testing which began in the late 1950's (Campana 1999). Therefore the $\Delta\ ^{14}C$ value will give a minimum age for the sample. Samples from the years between 1958 and 1965 are quite sensitive to C^{14}, but samples before or after this time period are less accurate (Campana 1997). However this technique can be used to validate ages derived from more traditional methods. Other

isotopic series have also been employed for age determination of long-lived species, including the $^{238}U \rightarrow ^{210}Pb$ series (Andrews et al. 1999).

Chemical Batch Markers

Chemical marking of fish has been employed for a number of years, and is generally used as a method for tagging large numbers of fish for subsequent identification (Campana 1999). This can provide mark recapture data, as well as age data for released fish. In general chemical markings have been applied through immersion, however, incorporation into the diet has also been successful (Campana 1999). Fluorescent compounds have been used extensively in batch marking, and have many advantages (Campana 1999). These are calcium-binding compounds that are easily introduced into the fish and produce a clear fluorescent mark when viewed with ultraviolet light. They are accumulated in all calcium carbonate structures of the fish including scales, otoliths, and fin rays. The advantage of scales and fin rays is that these structures can be sampled non-lethally (Veinott et al. 1999).

Elemental supplements into the otolith have also been used as a method of marking, however the selectivity of elements incorporated into the otolith as well as analytical limitations should be carefully considered (Campana 1999). Schroder et al. (1995) examined the use of strontium solutions as a method of marking salmon fry. This study used immersion in strontium chloride solutions for a period of 24 hours, which produced easily visible strontium deposition in the otolith under backscattered electron microscopy. Otoliths which were too small for sectioning and subsequent microscopic investigation were analysed by solution ICPMS and were found to have strontium

concentrations 2 orders of magnitude higher than control fish. Immersion of fry in solutions containing 9000 and 1200 ppm Sr for a period of 24 hrs were sufficient to cause increased deposition in the otolith.

Pacific Herring

History of BC's Herring Fishery

Pacific herring have been an important commercially harvested species in British Columbia for over a century with the first recorded commercial catch of 75 tons occurring in 1877. Prior to this, Pacific herring were an important food and bait fish for native communities along British Columbia's Coast. Herring and herring spawn are known to have been used by Pacific coast natives whose settlements have been traced back to 800 BC (Hourston & Haegele 1980). In the early turn of the 20th century an increasing oriental market for dry salted herring drove the fishery to increase its catch to about 30,000 tons, where it remained until the introduction of the purse seine in 1919. The seine increased fishing efficiency, and catches rose to 85,000 tons between 1919 and 1927. A decrease in the market however reduced catches to 30,000 tons by 1934 (Hourston & Haegele 1980). The introduction of the meal and oil industry in 1935 drove increases in the herring fishery, with catches leveling at ~ 100,000 tons between 1938 and 1947, when it jumped to 172,000 tons and remained around 200,000 tons between 1947 and 1966. In the 1962-63 season a record catch of 264,000 tons was taken, followed be a near record catch of 260,000 tons the following year (Hourston & Haegele 1980). Until this point limits on catches had been imposed by market demand, capacity of processing plants, and catching capacity of the vessels, however technological innovations would

increase the efficiency of the fishing fleet masking the limitations the ecology of the herring stocks would impose.

The 1964-65 season took a large catch of 241,000 tons, although fishermen had more difficulty locating schools of fish. Spawn deposition was suppressed in the heavily fished regions, and continued to decline over the next two years (Hourston & Haegele 1980). Also reported was the increase in the proportion of small immature fish in the catch. These warning signs were masked by the increase in fishing efficiency which had the ability to maintain catches at 1960 levels despite dwindling fish stocks. When the downward trend of fish abundance continued for a third year the fishery was closed coast wide in the early portion of the 1967-68 season. This signaled the end of the reduction fishery in British Columbia and for the next four years only traditional food and bait fisheries were permitted to operate.

With decreased pressure from commercial fishing, and favorable oceanic conditions, British Columbia's Pacific herring stocks saw strong year classes in the late 1960s and early 1970s, and recovered by the mid 1970s. This recovery, in combination with an increasing demand for herring roe in the Japanese market, prompted the development of an experimental herring roe fishery in 1971 that was subsequently expanded in 1972 (Environment Canada 1998). In its first season (1971) the herring roe fishery landed 11,000 tons, with catches increasing to ~50,000 tones from 1973 to 1975. The roe herring fishery catch peaked in 1976 at 87,000 tons, and has since declined to ~35,000 tons per year (Environment Canada 1998). The current herring fishery also supports a spawn on kelp (SOK) fishery, which has been in operation since 1975 (Environment Canada 1998). In addition to the roe herring and spawn on kelp fisheries, a

food and bait fishery also operates in British Columbia. The food and bait fishery occurs mainly between November and January intercepting schools of herring as they make their way towards the spawning grounds. The domestic market for herring as food is limited, and as a result landings in the food and bait fishery have been between 600 to 1,200 tons annually (Chalmers 1993). This is a high value product generating significant revenues.

The commercial value of Pacific Herring in British Columbia has been greater than $40 million continuously since 1982, with a peak in 1979 of $150 million. The shift towards high value products such as herring roe has maintained a high commercial value while decreasing herring landings by about one fifth of catch rates during the low-value reduction fishery (Environment Canada 1998). The labour-intensive processing of high value products such as herring roe and other products has resulted in thousands of additional jobs and benefits for the province of British Columbia.

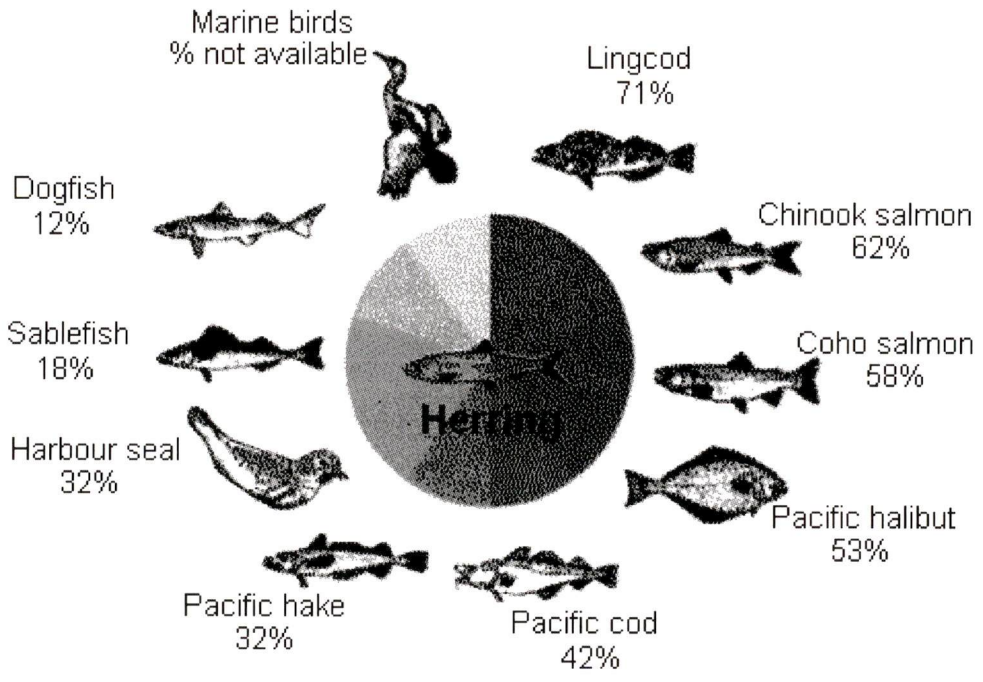
Life History

Pacific Herring (*Clupea pallasi*) is a small pelagic species that is found nearly continuously along the coastline of the northeastern Pacific Ocean ranging from Baja California in Mexico to the Beaufort Sea (Hourston & Haegele 1980). The center of abundance in the northeastern Pacific is along British Columbia's coastline between Dixon Entrance and Puget Sound. A small silvery fish, Pacific herring is the most abundant fish species in Canada's Pacific coastal waters (Environment Canada 1998). In addition to being an important commercial fish species, Pacific herring are central to the marine food web comprising 30-70% of the summer diets of adult Chinook and Coho

Salmon, Pacific Cod, lingcod, and harbour seals in British Columbia's southern waters. Herring eggs are also an important food source for migrating seabirds and grey whales.

Pacific herring have a maximum age of about 15 years and reach sexual maturity between the ages of three and five. After the fish have completed their first summer, but have yet to mature sexually, the fish congregate in to large school in the vicinity of the spawning grounds (Hourston & Haegele 1980). By September the schools of herring will move into the offshore feeding and wintering grounds. These are generally located off the mouths of Juan de Fuca and Hecate Straits (Hourston & Haegele 1980).

By the time individuals are three years old, they will start to become sexually mature and recruit into the spawning population. Age at recruitment tends to be correlated with latitude, with herring recruiting at younger ages in more southern waters and older ages in northern waters (Hourston & Haegele 1980). An individual spawning for the first time at three years of age will have achieved a mass of about 90 grams and a length of about 185 mm. The fish will grow rapidly in the next two years, from age three to five, gaining another 30 mm of length and increasing their mass by about 30 grams (Hourston & Haegele 1980). Adult herring can be found at depths of 100-150 m scattered in large schools along the 160 km coastline, off the mouth of the Juan de Fuca Strait. These aggregations may move north or south following food supply throughout the summer. Between October and December adult herring, and sexually mature recruits gather into larger schools and begin their annual migrations towards inshore spawning areas. Upon reaching the vicinity of spawning areas the fish stop feeding and remain in deep channels



Source: Fisheries and Oceans Canada, Nanaimo, B.C.

Figure 1.5: Contribution of Pacific herring to the diets of marine predators on the coast of British Columbia, Canada

and bays during the final stages of maturation before spawning (Hourston & Haegele 1980).

Spawning takes place in late winter to early spring with movement of mature fish into shallower waters, and up onto the spawning grounds. In the presence of suitable substrate (red algae, kelp, sea grass etc.) ritual spawning activities will occur in individuals of both sexes. Females will find suitable substrate, and deposit their eggs along its long axis, while males excrete milt into the water column creating large milky clouds indicative of herring spawning (Figure 1.6). A female will generally release ~1000 eggs in a single spawning act, and perform several hundred such acts in order to release her supply of 20,000 – 40,000 eggs. Both eggs and milt may be released in mid-

water spawning frenzies where fertilized eggs sink to the bottom covering rock substrates and marine vegetation. Large spawns may go on for several days, and spread out for miles involving upwards of 300 million fish (Hourston & Haegele 1980). After spawning, adult herring feed heavily while making the return migration to wintering and feeding grounds.

Stock Structure

Biologically a fish stock can be defined as a common and discrete breeding populations, which shows a common distribution, experiences similar sources and intensities of mortality, and shows common adaptive behaviors and morphologies. In most cases fish stocks are delineated by geography, commonly by spawning ground or feeding locations. By this definition many of the small local populations along the coast of British Columbia would be considered separate stocks; meaning there would be possibly thousands of small Pacific herring stocks along British Columbia's coastal waters. While an accurate description of stock structure is critical for the successful management of the resource, it would unrealistic for government agencies and scientists to manage many small populations, a problem not limited to British Columbia.

The debate of herring stock structure on Canada's west coast is generally composed of two sides; (i) herring populations represent "discrete stocks" which remain reproductively isolated; (ii) herring populations are interconnected by interactions between local populations creating a "metapopulation". Both of these scenarios have implications on herring stock management, and are being investigated by fisheries scientists. One implication of the discrete stocks concept is that overfishing, either by

itself or in addition to poor year classes, could permanently remove an entire stock of Pacific herring. Following the metapopulation model however, emigration and immigration of herring from different local populations means that areas where local populations have disappeared may have the ability to become re-colonized.

There has been evidence of a high degree of homing (~ 80%) in Pacific herring on Canada's west coast, however this is accompanied by a significant sharing of genetic material (~ 20%) between homing groups (Hay et al. 2001). This genetic sharing has made micro-satellite investigation into the discreteness of herring stocks difficult, as straying rates of Pacific herring are sufficiently high to homogenize any genetic variation one would expect if the stocks were truly discrete. As a result, much of the information concerning herring stock structure has been collected by large scale tagging studies conducted since 1936 (Hay et al. 2001). Further information on the stock structure, and population dynamics of Pacific herring would provide managers with a clearer view of the state of the resource, allowing for information-based management of the fishery.

Current Management Strategy

Pacific herring on Canada's west coast has been a pilot species for the objectives based fisheries management plan (OBFM) which has been implemented since 2001. The development of the OBFM plan was spurred on by the release of the 1997 Report on Atlantic Groundfish, and the 1999 Report on Shellfish released by Canada's Auditor General.

Figure 1.6: Aerial photo of herring spawn. Large milky clouds are releases of milt into the water column. *(Taken from DFO Website)*

Currently British Columbia's herring stocks are divided into five major management regions (Figure 1.7), each of which represents an associated group managed to be single stocks. These are the Prince Rupert District (PRD), Central Coast (CC), Queen Charlotte Islands (QCI), West Coast Vancouver Island (VCVI), and Strait of Georgia (GS). The herring fishery is managed following a conservative plan based on estimates of the mature stock biomass using an age-structure model. The total allowable catch for a management region is set at 20% of the forecasted mature stock biomass (Fisheries and Oceans Canada 2001). In order to protect stocks in years of poor environmental conditions the current management strategy also enforces a minimum stock biomass of 12,100 tonnes. If the forecasted mature stock biomass falls below this threshold the commercial fishery is closed for the season to allow for stock regeneration (Fisheries and Oceans Canada 2001). As a further conservative measure multiple fisheries are staggered within a management area to ensure that the entire years catch for a management area is not taken from a single group of herring.

Purpose of Study

The purpose of this study is to determine whether chemical information likely contained in the otoliths of Pacific herring on Canada's west coast can be accessed and interpreted in a fisheries management perspective using analytical methods available at the University of Victoria. The original experimental concept included the use of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for chemical analysis of the otoliths. This would allow for a broad selection of elements to be analyzed at very high precision, and low detection limits. The other benefit of LA-ICP-MS is that it allows for chemical quantification at very small (~30-50 micron) spatial

scales. As well the original experimental design included the characterization of chemical signals from two separate management areas based on the chemistry of young of the year otoliths sampled from the spawning grounds. This data would characterize stocks that originate from either Barkley Sound or the Strait of Georgia. These elemental characterizations determined from fish of known stock association may allow the separation of herring caught on the LaPerouse bank into Barkley Sound or Strait of Georgia populations.

A great deal of method development work was performed in order to confidently map chemical heterogeneity across the surface of solid samples. It was intended that the herring otoliths would be mapped in this way, and that the physical shape of the maps would give an indication of differing life histories between herring populations. In an effort to develop this elemental mapping, experiments were performed in order to determine the spatial resolution of the LA-ICP-MS at operating conditions used for otolith analysis.

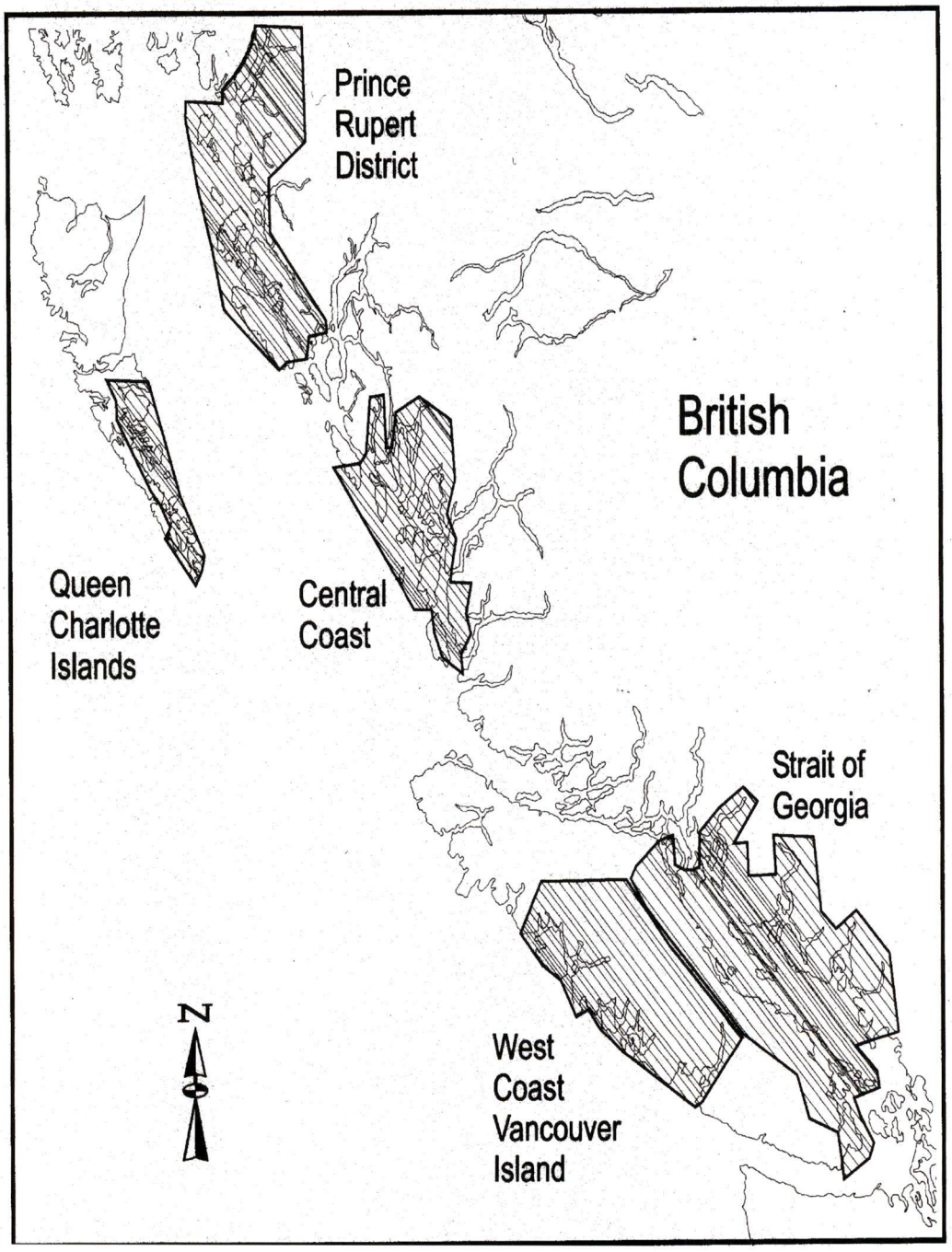


Figure 1.7: Pacific herring major stock assessment regions. British Columbia, Canada. (Taken from Schweigert 2002)

Due to instrument down time, and analytical difficulties LA-ICP-MS analysis was not an option for investigation into herring otolith chemistry. As a result other analytical methods were developed in order to generate a chemical dataset of Pacific herring otoliths. Methods of micro-digestion, and major cation analysis by ion chromatography were developed in order to investigate otolith chemistry. The purpose of this portion of the study was to quantify the concentrations of major cations in otoliths of Pacific herring and to interpret this information from a fisheries management, and otolith ecophysiology perspective. The goals of this portion of the study are to determine if major cation concentrations give some insights into the ecology, or school fidelity of Pacific herring, and to delineate groups of herring based on otolith concentrations. The results of this work are presented in Section 4.

CHAPTER 2

The spatial resolution of LA-ICP-MS line scans across heterogeneous materials such as fish otoliths and zoned minerals

Abstract

LA-ICP-MS line scans can provide equivalent or better information about the distribution of elements in heterogeneous solids than discrete spot analysis; and at much reduced time and cost. To do so however, the spatial resolution for given instrumentation and operating conditions must be known. Here, I present a quantitative and reproducible method that requires line scans across a sandwich of three glasses with varying certified concentrations of trace elements. To produce sufficient counting statistics, only Ca, Sr, Rb, and Pb were analysed. Raw data (counts per second) is reduced to "instantaneous concentration" and then filtered to produce concentration profiles that contain the same dimensions as the original data. The spatial resolution is empirically determined for Sr, Rb, and Pb based on these profiles by using a statistical "confidence" window.

Spatial resolution is controlled by the magnitude of concentration gradients, the direction of concentration shifts, the instrumental configuration and settings such as cell size and shape, and the speed of the scan. Spatial resolution is better by a factor of two for increasing concentration profiles than decreasing ones by a factor of 2. The relationship between the magnitude of the concentration gradient and the spatial resolution is linear. Therefore, once the range of concentration variation is known in any target, a minimum resolution can be determined from this linear relationship. The spatial resolutions of the

three elements examined did not differ significantly suggesting that element specific ablation behaviours are not a significant control on spatial resolution. A Sr concentration profile from a natural sample (fish otolith) is generated, and the resolutions from the glass sandwich are applied. For the concentrations observed and the settings and hardware used, a minimum spatial resolution of 50 μm was calculated. Concentration variations at smaller scales can be detected but not quantified.

Introduction

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is emerging as a preferred technique for the high precision analysis of trace elements in solid samples. This is in part due to the advantages laser ablation holds over dissolution techniques such as low probability of contamination, few limits on sample size, little to no sample preparation (depending on the application), small sample requirements (almost non-destructive), and for some elements, fewer matrix interferences. Digestion followed by ICP-MS solution analysis is superior to LA-ICP-MS for most bulk analysis, having limits of detection typically 2 to 3 orders of magnitude better and with much less concern over sample homogeneity.

The real advantage of LA-ICP-MS therefore, remains its spatial capabilities –and its ability to determine concentration gradients across small targets. This is typically performed one spot at a time and most method development efforts have focussed on this approach. Some lines of research however, such as fish otolith microchemistry, require continuous concentration profiles. Indeed, the daily increment added to a salmon otolith has a diameter of roughly 1 to 3 microns so a spot with a diameter of 50 microns yields an integrated signal equivalent to 50-16 of days of fish life. One hundred individual spots

would be required to produce a continuous life history for a four-year-old salmon, which would have a time resolution of months in the life history of the fish. This would be very time consuming and expensive, even for a single fish. For this reason we have investigated the utility of continuous lateral ablation scans across the surface of heterogeneous targets such as fish otoliths to increase spatial resolution and reduce the time and cost of analysis.

Prior to the use of beam-based technologies concentration profiles across solid samples were obtained by high precision milling, dissolution, and solution-based analysis. The use of beam based technologies such as ion microprobe or laser ablation remove some of the complications associated with chemical methods, however new variables are introduced. LA-ICP-MS has some significant calibration complications that arise from inter-element fractionation (Chen 1999) and so internal and external references are required. Even then there are questions about whether the relationships between elements (fractionation) are consistent across different matrices (Rodushkin et al. 2002a, Rodushkin et al 2002b, Russo et al. 2000). These problems impact the accuracy of analytical results more than precision. Concentration profiles using laser ablation can be achieved by either continuous ablation of a single crater creating a depth profile (Mason & Mank 2001, Stecher et al. 1996), or laterally by ablating a series of craters along the surface of the material (Hoffmann et al 2000). Continuous lateral ablation scans (henceforth called line scans) have also been employed for spatially resolved analysis (Wang et al. 1994) but have received comparatively little attention despite the fact that this technique seems well suited to investigating heterogeneous solids.

Line scans across homogenous matrices are relatively straightforward and even have some advantages over spot analysis. In line scans ablation pit geometry and depth become constant as the laser tracks across a homogenous target, eliminating some of the complications associated with spot analysis, such as changes in ablation yield and element fractionation that occur with increasing pit depth and Gaussian pit morphology (Eggins at al. 1998, Mank & Mason 1999). We observe this stability in instrument response for rasters across National Institute of Standards and Technology (NIST) glasses for example. However, line scans across heterogeneous targets such as gradually zoned minerals or fish otoliths are much more complex, making it more difficult to extract useful information from them.

One of the most complicated issues associated with line scans is quantitative determination of the spatial resolution. Processes such as mixing of heterogeneous materials at the ablation site, as well as mixing in the ablation chamber and gas lines makes reduction of line scan data a complex problem. In the present study we perform careful experiments on a multi-layered sandwich composed of three NIST glasses in order to constrain these complications and to develop a method for quantifying the spatial resolution that can be attained by any laser ablation system. To do this, we characterize the transition through chemically distinct regions and provide a method based on the statistical properties of the data to quantify the spatial resolution that can be attained with a commercially available laser ablation microprobe. Finally, we apply our experimental findings to a line scan across a natural target, a pacific Chum salmon (*Oncorhynchus keta*) otolith.

Methods

Glass Sandwich Construction

A glass sandwich was constructed using three layers of National Institute of Standards and Technology (NIST) standard reference materials (SRM). The SRMs used are trace element standards in a glass matrix, NIST 611, 613, and 615. These are available as circular wafers with a diameter of approximately 1 centimetre and a thickness of 1 millimetre. Wafers are cut into strips with a width of approximately 5 mm. SRM 611 is secured to a glass microscope slide using Krazy Glue®, then polished to a thickness of ~500 μm using a Buehler CARBIMET abrasive disc with a 600 grit size (14.5 μm). SRM 613 is subsequently secured to SRM 611 with Krazy Glue®. The procedure is repeated to mount the third SRM (615), which is also ground to 500 μm. The layer cake of glass wafers is turned on its side and embedded into Buehler EPOXIDE low temperature epoxy resin. The mount is polished using a series of Buehler CARBIMET and MICROCUT abrasive discs ranging from grit size 320 – 1200 (34.3 – 6.5 μm) in order to expose the three glass bands and remove surface contamination. The mount is then cleaned in an ultrasonic bath in de-ionized water for 5 minutes and allowed to air-dry. It's polished further using a Buehler TEXMET Polishing cloth impregnated with Buehler 0.25 μm METADI Supreme polycrystalline diamond suspension. Finally, the mount is re-cleaned in the ultrasonic bath and dried. The result is a circular disc of epoxy with a sandwich of three bands of NIST glass held within it (Fig. 2.1)

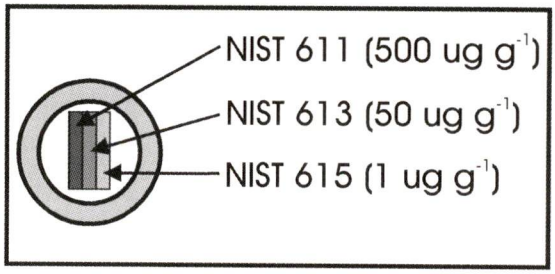


Figure 2.1: Schematic diagram of the glass sandwich in plan view showing the position of the three NIST glasses. The sandwich is held within an epoxy matrix in an acrylic ring (grey circle).

Instrument Optimization and Data Acquisition

Analysis is performed using a VG Elemental PQ II S+ ICP-MS. Laser ablation is conducted using a Merchantek UV frequency quadrupled Nd:YAG laser with an output of 266 nm and a maximum energy of 4 mJ. The laser is operated in Q-switch mode with a computer controlled pulse rate of 10 Hz for the optimization of the ICP-MS. Optimization of the plasma conditions and ICP-MS sensitivity are performed prior to any analytical experiments. Optimization is accomplished using NIST 613 SRM (nominal trace element concentration = ~50 $\mu g\ g^{-1}$). Nebulizer, auxiliary, and cooling gas flow rates, torch position, and lens settings are adjusted to achieve greatest signal intensity while NIST 613 is being ablated at a spot size of $\approx$ 50 μm with an output frequency of 10 Hz, and 70% power giving an output energy of ~2.2 mJ.

Laser operating conditions during line scans were slightly varied from tuning conditions to more closely simulate conditions used for analysis of fish otoliths. Operating conditions of the ICP-MS are shown in Table 2.1. For these analyses, the laser is operated in Q-switch mode with a pulse rate of 20Hz, spot size of 3 ($\approx$ 30 μm), at 50% power output. This results in an output energy of ~0.77 mJ. The lower energy output reduces the depth of penetration in otoliths producing data that more closely reflects the chemistry of the visible surface. This is done to minimize the uncertainties that arise from

the fact that otolith chemistry varies in all three dimensions. This is not a concern for the NIST glasses. The frequency of laser pulses is increased to provide material to the ICP-MS as continuously as possible. Line Scans are performed by tracking the laser across the three materials at a speed of 5.3 μm sec^{-1} using computer controlled stage automation. Data are collected in peak jumping mode with a dwell time of 10.24 milliseconds at one point per peak. Four isotopes ^{42}Ca, ^{85}Rb, ^{88}Sr, and ^{208}Pb were measured. ^{85}Rb, ^{88}Sr, and ^{208}Pb have certified values in all three NIST glasses. The matrix composition of all the NIST glasses is 12% CaO allowing calcium to be exploited as an internal reference element. Calcium is also the most appropriate internal reference element in the subsequent analysis of the otolith because of their aragonite mineralogy ($CaCO_3$). Data collection consists of 60 seconds blank to collect background intensities, followed by 240 seconds of ablation on the line scan. This creates a data table consisting of 6122 discrete intensity measurements (data slices) per isotope. The large number of data slices is collected to improve counting statistics for small segments of the concentration profile.

Data Reduction and Processing

Time resolved data collected in counts per second are normalized and reduced to concentration using a modification of the method outlined by Longerich et al. (1996) as follows. Raw ion counts are converted to concentrations using the following formula:

$$C_{an\ sam} = \frac{R_{an\ sam}}{S}$$

where R_{ansam} is the counts per second (CPS) of the analyte and S is the normalized sensitivity. The normalized sensitivity ($CPS/\mu g\ g^{-1}$) is determined based on calibration

Table 2.1: ICP-MS operating conditions.

Plasma	
Plasma gas	Argon
Forward power	1348 W
Reflected power	<3 W
Gas flow rates	
Nebulizer gas flow rate	1.246 L min^{-1}
Auxiliary gas flow rate	0.940 L min^{-1}
Cooling gas flow rate	14.00 L min^{-1}
Interface	
Sampling distance	16mm
Sample aperture	Nickel, 1.0 mm diameter
Skimmer aperture	Nickel, 0.7 mm diameter
Ion lens settings	
Extractor Lens	-177.5
Collector	-73.48
L1	1.01
L2	-45.27
L3	4.44
L4	4.52
Pole Bias	-10.6
Data acquisition	
Measurement mode	Peak Jumping
Dwell time	10.24 ms
Data acquisition time	300 s
Points per peak	1

standards, and corrects for variations in the mass of sample ablated. The normalized sensitivity (S) is determined based on the following equation:

$$S = \frac{CPS_{an\,cal}}{Con._{an\,cal}}\left(\frac{CPS_{is\,sam}}{CPS_{is\,cal}}\frac{Con._{is\,cal}}{Con._{is\,sam}}\right)$$

where CPS_{ancal} is the count rate of the analyte in the calibration material; $Con._{ancal}$ is the concentration of the analyte in the calibration material; CPS_{issam} and CPS_{iscal} are the counts per second of the internal standard in the sample and calibration material respectively; and $Con._{iscal}$ and $Con._{issam}$ are the concentrations of the internal standard in the calibration material and sample respectively. Applying the above to the raw counts

per second, minus the mean background intensity produces an "instantaneous concentration" for each data slice. Taking an average of a set of these instantaneous concentrations produces a concentration identical to that calculated using the Longerich et al. method. The difference in methods is simply in the order of operations. Longerich et al. take a mean value of the signal intensity counts, subtract from this the mean value of the background intensity counts, and then convert this into a single measure of concentration. In this study we subtract the mean background intensity from each data slice, and then calculate its discrete concentration. Because of noise, this alone is not a precise number. A statistically precise concentration is determined by taking the mean of a series of these measurements. The number of measurements used in calculating the mean and their variance determines the level of significance attained.

Normally, when analyzing unknowns, intensity counts for a standard reference material are obtained before and after analysis. These are used to calculate the normalized sensitivity, and to account for instrumental drift. In this case all three layers of the sample are standard reference materials and so sensitivity can be determined from the experimental data itself. Data from NIST 611 was used for this purpose.

To facilitate graphical methods, concentration profiles are filtered using a simple and elegant method described by Sinclair et al. (1998). The concentration profile is first filtered using a running eleven-point median to reduce noise, and then an eleven-point running average is applied to produce a smoothed profile containing an equal number of data points as the original chromatogram. Sinclair et al. found this method of filtering to be efficient at removing effects of outliers caused by the presence of dust or other particulates entering the plasma, and also found that more complex filtering methods

represented little or no improvement. In addition, they state that more complex methods simply create barriers to routine data processing. We concur with this perception.

The filtered concentration profiles can be graphically examined in order to characterize the transition from one glass to the next. This is useful but does not provide a repeatable and quantitative method to determine how quickly the instrument responds to shifts in composition – the spatial resolution. One can guess but not confidently determine the exact point of transition. To avoid arbitrary choices based on visual inspection, and to make the method repeatable, we employ a quantitative approach to determining spatial resolution. We define the maximum (best) spatial resolution as the minimum size of a target for which we can confidently determine a concentration while the laser is tracking along a line in 95% of the cases. In practical terms for this experiment, this is the distance the laser travels until we are 95% certain that we have crossed from one NIST glass to the next. To determine this distance, the mean observed concentrations of the pure NIST glasses (in the plateau regions when the laser is well inside a single glass) are enveloped by +/- 2 standard deviations of the concentration measurements for that glass. This defines a 95% confidence envelope. A statistically significant observation (outliers removed using the method of Sinclair et al. 1998) that lies outside this envelope is therefore due to the presence of another material in 95% of the cases. The distance the laser travels once the signal leaves the confidence envelope of one glass and enters the envelope of the next is therefore a non-arbitrary and repeatable measure of the spatial resolution. This distance will vary depending on instrument type and configuration but the method should be portable.

Results and Discussion

Spatial Resolution of the NIST glass sandwich

The calculated concentrations of strontium in the three glasses are within +/- 2.6% of certified values. Profiles of increasing strontium concentration (NIST 615<613<611) (Fig. 2.3) show a quick response to the changes in strontium concentration and then a new steady state is rapidly attained. The transition from NIST 615 to NIST 613, with a Δ [Sr] of 32.6 ppm, requires the laser to travel 32.1 μm for the signal to leave one 95% confidence envelope and enter the next. The transition from NIST 613 to NIST 611 (Δ [Sr] = 437.1 ppm) requires 67.41 μm. Distances are greater for transitions from high to low concentrations (Fig. 2.4). The initial response is rapid and similar to the profiles across increasing concentration gradients, however it takes longer before a new steady state is achieved within the subsequent confidence envelope. Transition from NIST 611 to NIST 613 (Δ [Sr] = -437.1 ppm) requires 157.26 μm and the transition from NIST 613 to NIST 615 (Δ [Sr] = -32.6 ppm) requires 38.94 μm. These results illustrate that instrument response to shifts in concentration is controlled by the magnitude, as well as the direction of the concentration gradient.

Instrument response for transitions across increasing concentrations of Sr is faster than that for decreasing concentrations by a factor of 2.03 and 2.51 for movements between NIST 615 and 613, and between NIST 613 and 611 respectively. The changing response time makes data reduction of heterogeneous samples somewhat complicated. A general approach that uses a single spatial resolution would have to employ the worst resolution to maintain confidence in all transitions. However, to create a portable tool

from this, a method for predicting the minimum and maximum spatial resolution for a target is discussed further in the section titled "Multi-element comparison".

Depth profiles versus lateral profiles (line scans)

Similar trends in magnitude and directional control have been observed when investigating the spatial resolution of depth profiling by LA-ICP-MS (Hoffmann et al. 2000) however the magnitude of the trends are very different. The depth resolution for shifts from high to low concentrations in depth profiles is two to three orders of magnitude slower (10 to 100 times) than for shifts from low to high concentration versus the roughly 2 times slower shown above for line scans. However spatial resolution in depth profiling for increases in concentration, at least at the beginning of the ablation (a shallow crater), can be as good as 3 μm - much better that the spatial resolution we attain here (Hoffmann et al. 2000). It is inappropriate to compare the two approaches however, as they are inherently useful for very different applications. For example, the complications (i.e. fractionation) associated with increasing pit depth in depth profiling would make it more or less impossible to depth profile through an otolith with a thickness of 1 cm, such as the one analysed in this study.

Multi-element comparison

Concentrations of Rb and Pb in NIST-615 were near our limits of detection, so results in that glass had poorer precision and poor accuracy compared to certified concentrations. Results across this transition (NIST 615-613) are therefore not as robust as those for Sr. Results for Rb and Pb in the high and mid concentration glasses (NIST 611 and NIST 613) were better and so with these we are able to examine differences in

spatial resolution across elements. For transitions from low to high concentration, NIST 613 to NIST 611 with a Δ [Rb] of 394.3 ppm and a Δ [Pb] of 387.4 ppm, the required lateral movement for the signal to move between 95% confidence envelopes is 91.17 μm and 79.88 μm for rubidium and lead respectively (Fig. 2.2). The spatial resolutions for decreasing signals (Fig. 2.3) are 214.2 μm and 196.21 μm for rubidium and lead respectively.

These data give the impression that the spatial resolution for Sr, Rb, and Pb are dissimilar and one may begin to imagine that this is related to their different ablation and transport behaviours, but in fact, this is not the case. If we normalize the spatial resolution to the magnitude of the change in concentration and group these by direction two linear relationships (one for increasing concentrations and one for decreasing concentrations) are observed onto which all 3 elements fall (Fig. 2.4). The linearity in the relationships and the fact that all three elements obey them is very encouraging. It suggests that spatial resolution is similar for many elements, at least those with similar characteristics to Rb, Sr, and Pb, and therefore may negate the need for complicated data reductions involving different resolutions for different elements. Of greater importance is the utility of these linear relationships in creating a portable tool for the "calibration" of line scans specific to the instrument settings and concentrations gradients observed in the sample. This "calibration" allows us to predict the attainable spatial resolution for a given heterogeneous target based on the performance of the laser ablation system when scanning the glass sandwich, as well as its observed concentration range.

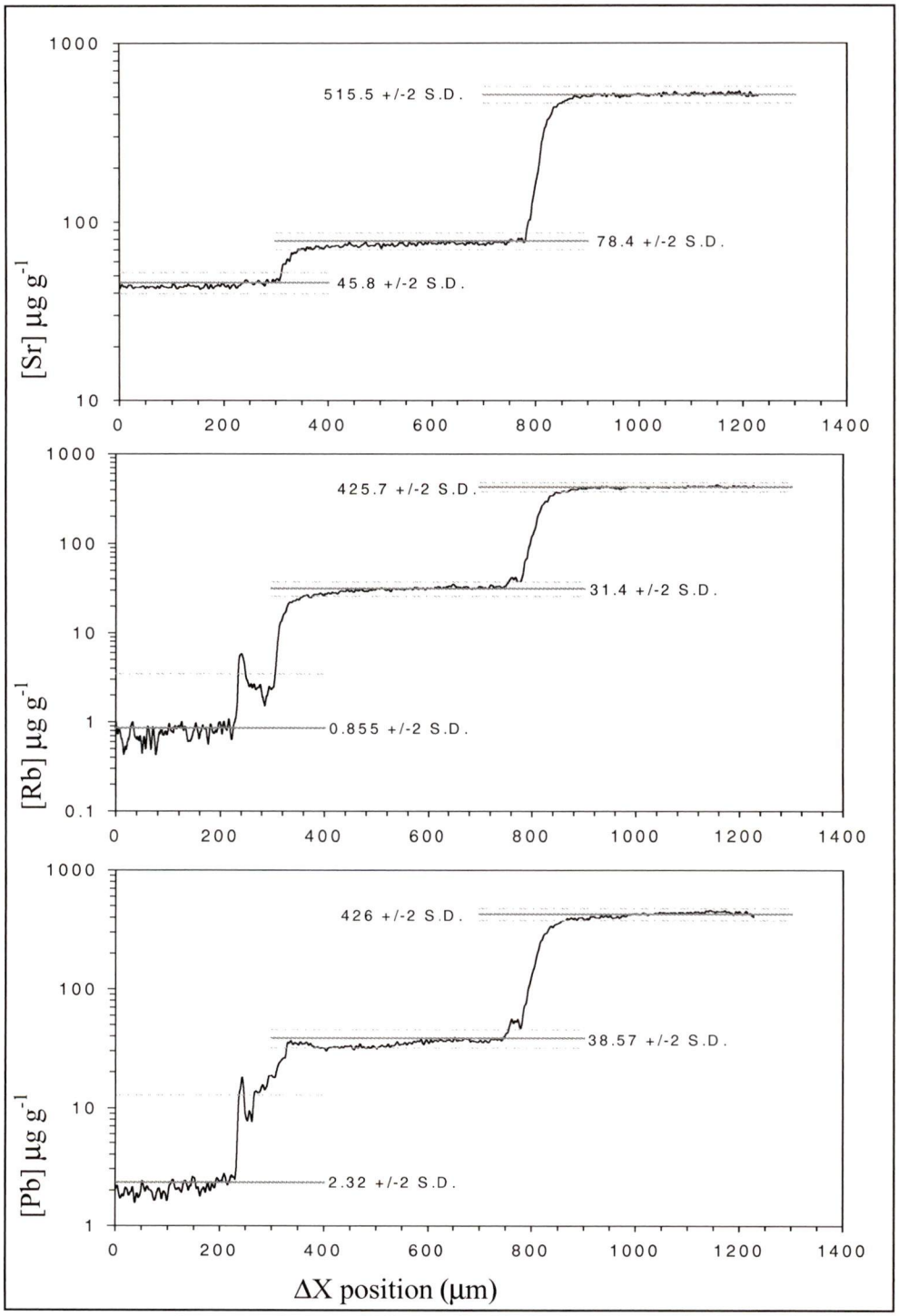


Figure 2.2: Filtered concentration profiles (µg g^{-1}) of Sr, Rb, and Pb moving from low to high concentration materials (NIST 615<613<611). The 95% concentration envelope surrounds measured concentrations of each NIST glass. Spatial resolution is determined by quantifying the lateral position of the laser from when the signal leaves one 95% envelope to when the signal enters the 95% envelope of the subsequent material.

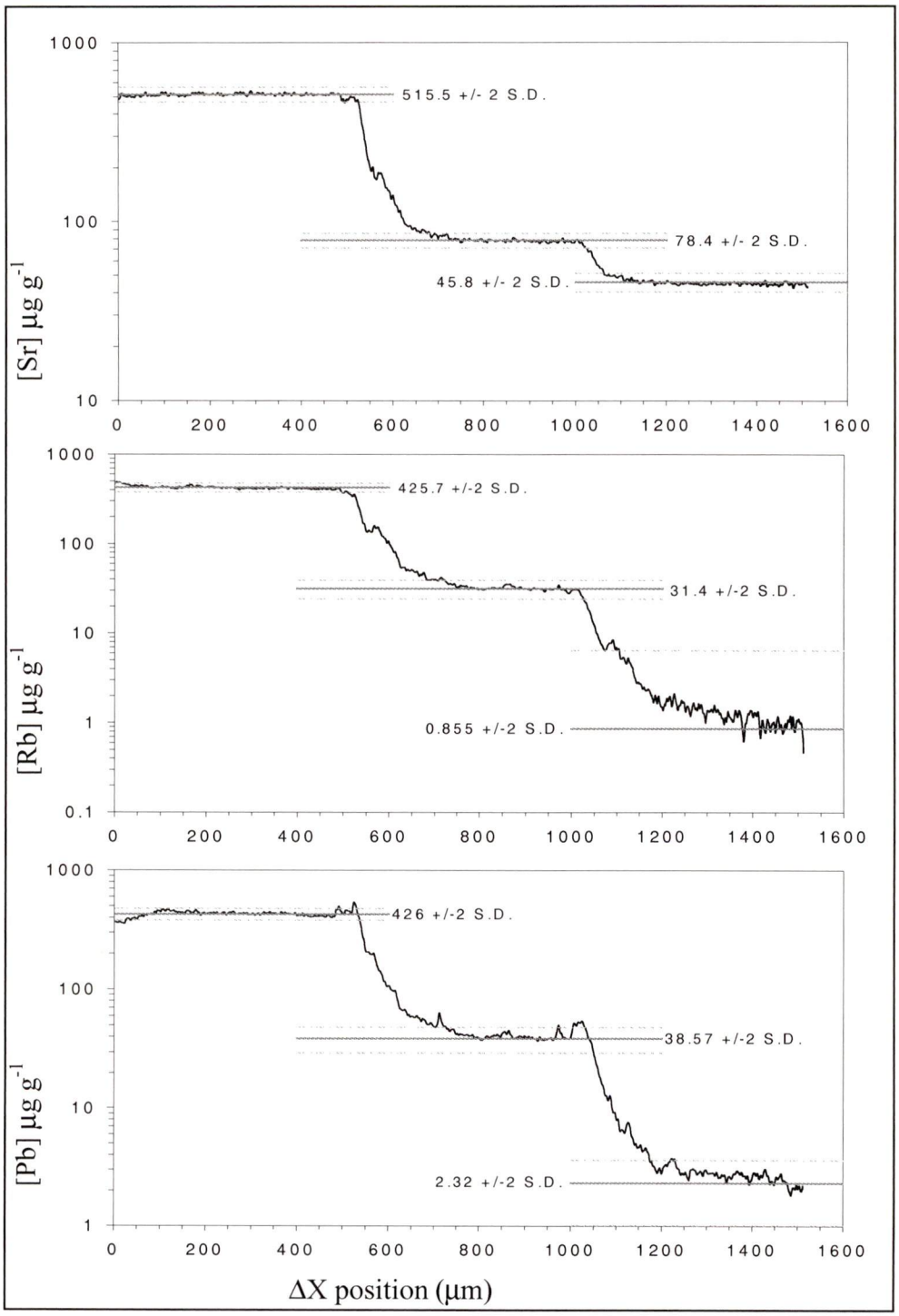


Figure 2.3: Filtered concentration profiles (µg g^{-1}) of Sr, Rb, and Pb moving from high to low concentration materials (NIST 611>613>615). The 95% concentration envelope surrounds measured concentrations of each NIST glass.

Although the linearity of the relationship between concentration gradient and spatial resolution is encouraging, caution will still need to be applied when transferring the results from the glass sandwich to different matrices. Recent work has shown that the transparency of materials can have a significant influence on the ablation yield per laser pulse and on the particle size distribution ejected from the ablation site, leading to increased fractionation in transparent samples (Russo et al. 2000, Guillong & Günther 2002). The penetration of laser energy into transparent samples allows a greater volume of material to be ablated per energy pulse. As a result there may be differences in the spatial resolutions of the glass sandwich and a similarly constructed gradient of a more opaque matrix. In order to determine the significance of this concern a similar experiment to this one needs to be performed using a sandwich constructed of a more opaque material.

Detection vs. quantification of concentration change

To provide a repeatable method that quantifies spatial resolution for line scans, we have suggested the statistical approach described above. Our hope is that this will provide the basis for a broadly accepted method that can be used in other laboratories. However, it must be noted that chemical variations in the target can be observed "qualitatively" across smaller distances than the spatial resolution determined by the statistical approach. Visual inspection of the line scan chromatograms (after normalization) indicates a nearly instantaneous response as new glass layers are encountered. However, if the size of a zone of different composition is less than the spatial resolution determined by our

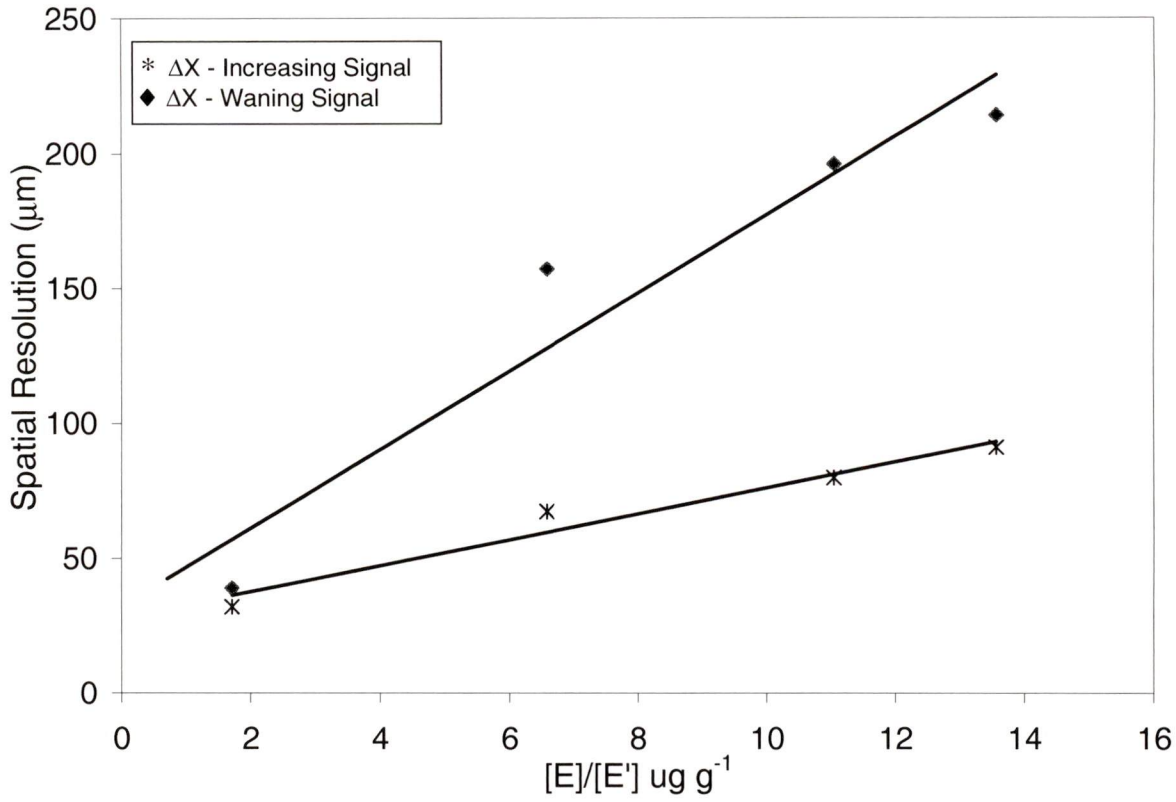


Figure 2.4: Influence of magnitude of the concentration gradient (x-axis) and direction (increasing vs. waning signals) on the spatial resolution (y-axis) of LA-ICP-MS. The x-axis is the ratio of elemental concentration between the NIST glasses at the two interfaces (e.g. $[Sr]_{611}/[Sr]_{613}$). Each line consists of two points for strontium, and one point for each of rubidium, and lead. The intercept of the two lines represents a non-arbitrary, measurable determination of laser spot size.

statistical method, for example a more concentrated strip in the NIST glass with a thickness of 1μm, the change in instrument response will never reach a steady state. Instead the instrument will report a continuously changing mixed signal that grows and fades as the laser tracks across the zone. Therefore, the concentration of the zone cannot be determined but its presence is easily detectable. LA-ICP-MS can therefore detect very small zones of chemical heterogeneity, however due to beam size, ablation cell dynamics, and mixing in gas lines, this can only be observed qualitatively. The minimum size of a chemically distinct zone that can be detected qualitatively is somewhere between the size

given by our statistical method (a maximum), and a minimum size that will depend on the zone's concentration. Very small zones may be detected if they are sufficiently concentrated.

Data Quality

In terms of data quality, one of the challenges in this experiment was to create a suitable target – a continuous target with distinct chemical horizons of known concentrations. This was mainly accomplished but at very small spatial scales, gaps between the NIST glasses are present (Figure 2.5). These gaps were unavoidable using the construction methods available to us, although great care was taken during the construction of the sandwich. The exact impact of the gaps is unknown, however it is certain that they impacted our results. Microscopic inspection of the glass sandwich (Fig. 2.5; 200 times magnification) reveals that the edges of the glass layers at the gaps were more damaged by the laser than other parts of the glass suggesting that ablation dynamics were different at these locations. The exact effects of this on the signal received by the ICP-MS are unknown. Undoubtedly, the ablation rate varied but providing the rate varied equally for Ca as for Sr, Rb, and Pb, these changes will be accounted for by normalizing to Ca. As such, we feel that the ablation dynamics are not of major concern for data quality.

A further complication is inconsistency of gap width. The gaps associated with the line scan moving from low to high concentrations are measured to be 29 μm and 3 μm for the transition between NIST 615 to 613, and NIST 613 to 611 respectively. For the line scan moving from high to low concentration, interface gaps between glass standards are

measured to be 22 μm and 3 μm for the transition between NIST 613 to 615 and NIST 611 to 613 respectively. Differences in gap width undoubtedly cause variations in the calculated spatial resolutions that are not related to instrument sensitivity or flushing rate of the ablation cell. Quantifying the contribution of the gaps to the line scan profiles would be very difficult, perhaps impossible, and so eliminating their contribution to the data would be arbitrary. We have therefore chosen to perform no correction but will state that the presence of the gaps cause the response times to be greater than they would be if the sandwich were seamless. As such, estimates of response times given here are inflated and those for a truly continuous media should be better.

Application to Natural Samples

In order to illustrate the utility of this experiment, the information derived from the glass sandwich is applied to a strontium concentration profile from a wild Pacific Chum salmon otolith. The otolith was removed from a post-spawn, deceased salmon taken from the Goldstream River near Victoria, British Columbia, Canada. The data obtained for the line scan of the otolith was reduced and filtered following the methods outlined above for the glass sandwich. The profile emanates from the centre of the otolith and radiates out to its edge and should therefore reflect exposures to varying concentrations of Sr through five years of the salmon's life, from birth to death. Indeed oscillations are apparent (Fig. 2.6). The oscillations have a Sr magnitude ratio of 1.3, slightly lower than the ratios for the transition from the NIST 615 and 613 glass standards. Extrapolating the linear relationship of spatial resolution for the waning signal, shown in Fig. 2.4 to 1.3 for the glass sandwich, we should be able to quantifiably detect

changes in concentration at a spatial resolution of 51 μm in the otolith. This corresponds to 34 days, or roughly one month of fish life for a 5-year-old fish (based on known life histories and major band counting).

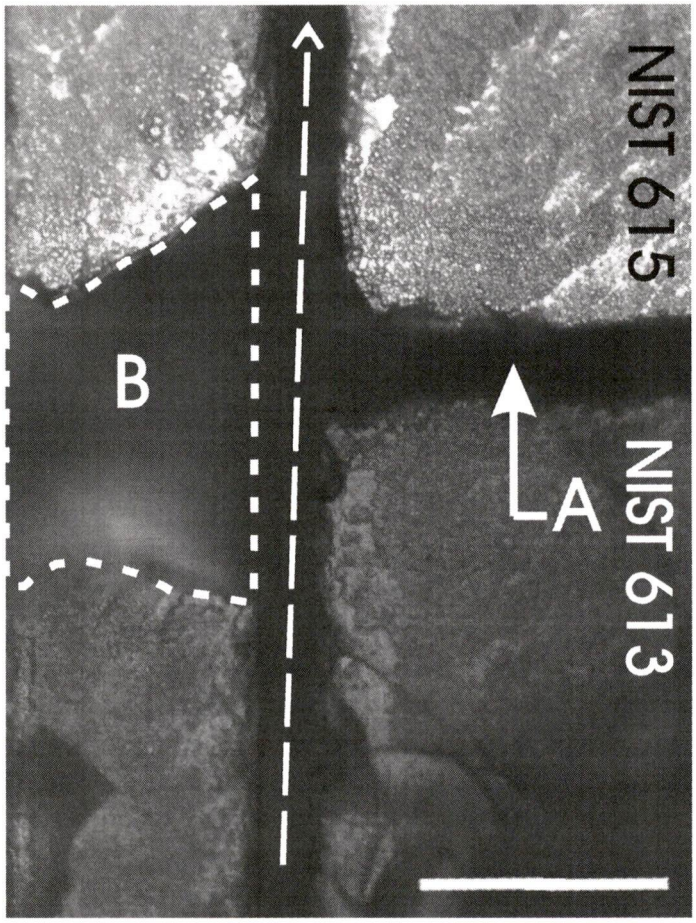


Figure 2.5: Photograph of line scan ablation track moving across interface of NIST 613 to NIST 615 under reflected and transmitted light (200 X magnification). Dashed arrow indicates location and direction of line scan. Position (A) shows the interface gap between the NIST 613 and 615 glasses, measured to be 21.65 μm. Location (B) shows area of large fractures surrounding interface gaps. Scale bar is 100 μm.

The oscillations visible in the Sr profile are therefore real variations in otolith strontium concentration that reflect environmental variations on a monthly scale. This allows us to re-construct a life history for this particular fish – very important information for fisheries

research. Strontium is considered a proxy for salinity and so initially we can see that the juvenile salmon moves from low salinity (river) to a higher salinity environment (estuary). Qualitatively this occurs after ~ 24 days, but at that time scale, shifts in concentration are detectable but concentration itself cannot be quantified because the signal is not able to reach a steady state at spatial scales below 50 μm. The salmon then remains in moderate salinity (perhaps near shore marine) for approximately 262 days (+\- 34 days) before moving to a higher salinity environment (perhaps off-shore, open ocean). Three "yearly" oscillations in strontium concentration then occur. These oscillations may be the product of movements between higher and lower salinities, perhaps near shore and offshore migrations, before returning to spawn. This is a plausible explanation, but there are in-fact many complications that need to be straightened out before this interpretation can be substantiated. For example, the Sr oscillations may be related to diet, salinity, temperature, or metabolism. Regardless, the example effectively illustrates that determining the spatial resolution of line scans is essential information for interpreting chemical variations in natural targets.

The development of a robust line scan method is important for other reasons as well. The profile shown in Figure 2.6 was produced from a single LA-ICP-MS analysis. It would require at least 37 individual spot analyses at a spot diameter of 50 μm to produce a continuous profile across the same otolith. Not only would this be much more time consuming and expensive than the line scan, but the data set produced would lack much

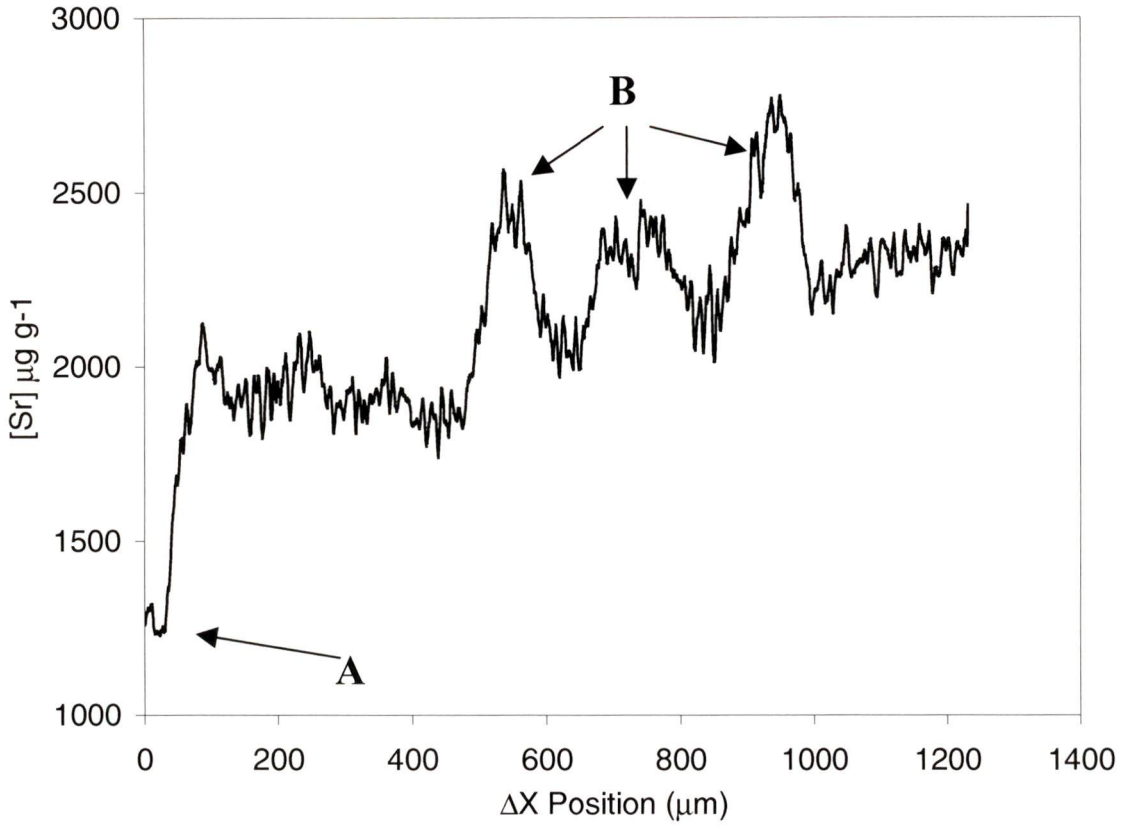


Figure 2.6: Strontium concentration profile from nucleus ($\Delta X = 0$ µm) to outer rim ($\Delta X \approx 1300$ µm) across a bisected saggital otolith from a Chum salmon *(Oncorhynchus keta)*. Movement of salmon from low concentration (fresh water) to higher concentration (estuarine) environment is evident in the strontium concentration profile (A). Oscillations in strontium concentration possibly associated with migration between near shore environments and the open ocean (B) are also evident.

of the detail. This is because the line scan retains the original dimensions of the raw data and therefore amounts to thousands of 50 µm spots, each centred on an individual data slice (raw data point), and can provide qualitative information about chemical variations that occur at scales smaller than 50 µm. Spot analysis, while still a useful tool in investigating chemical heterogeneity, will produce a concentration map with less resolution than line scans.

Conclusions

Element concentration profiles for Rb, Sr, and Pb were created by continuously ablating a line scan across the surface of three juxtaposed NIST glasses of varying trace element concentrations. Spatial resolution across concentration gradients is controlled by the magnitude and direction of the change in concentration. Profiles across increasing concentration gradients show better spatial resolution than decreasing ones by a factor of ~2. For either direction of change, the relationship between the magnitude of the concentration gradient and the spatial resolution is linear and so is predictable. The appropriate resolution for any target can, therefore, be based on the observed range of its concentrations and should be based on the decreasing concentration profile for the NIST glasses to ensure that resolution is not over-estimated.

Spatial resolutions for Sr, Rb, and Pb are similar suggesting that the ablation behaviour of different elements is not a significant control, and therefore a single resolution can realistically be applied to many elements. For our instrumentation and operating conditions, the spatial resolution at which the concentration of chemically distinct zones in a fish otolith could be reliably measured was 50μm. This can be considered a conservative estimate based on the observed concentration range in the otolith (a posteriori) and the results of the NIST glass sandwich analysis for the decreasing concentration profile. The results of the otolith line scan, however, show that line scans provide qualitative data at smaller spatial scales, which may be useful for characterizing heterogeneous targets. The "glass sandwich" method described here should determine the spatial resolution of most LA-ICP-MS instruments including future models, and is also applicable to other beam based analytical instruments.

CHAPTER 3

Otolith Mass, Growth Processes and Population Discrimination of Pacific Herring on Canada's West Coast As Determined from Micro-Digestion Ion Chromatography

Abstract

Concentrations of sodium, potassium, magnesium, calcium, and strontium of adult Pacific herring otoliths are determined by micro-digestion and ion chromatography for 126 individuals from three herring tows in management sub areas 6, 7, and 13 on the LaPerouse Bank off Vancouver Island, British Columbia, Canada. Herring are sampled on the feeding grounds well away from spawning areas. This is a novel approach to investigating population mixing and dynamics, and demonstrates the effectiveness of school discrimination by otolith chemistry. Due to their aragonitic composition, the concentration of calcium in otoliths is relatively invariant (+/- 2%). Analytical determination of the mass of Ca can therefore be used to accurately determine the mass of the otoliths. This method circumvents difficulties in weighing small (0.002 g) otoliths that are prone to holding a static charge and eliminates inaccuracies caused by variations in otolith humidity. Accurate determination of otolith mass is critical information for (i) the determination of the concentrations of other elements in the otolith, and (ii) observing relationships between elements and otolith mass. This method will be useful for species such as Pacific herring, or juvenile fish that typically have very small otoliths.

The concentrations of Na, Mg, and Sr show an inversely proportional relationship with otolith mass, which we ascribe to the changing ratio between the volume of endolymphatic fluid and otolith surface area that occurs during growth of the fish and

otolith. Early in life, this ratio is large and so the relative abundance, and hence availability, of elements that can mineralogically substitute into the otolith is large. During life, as the ratio of the volume of fluid that surrounds the otolith versus its surface area decreases, the relative abundance of elements like Na, Mg, and Sr, in the endolymphatic fluid and their availability, also decreases. In other words, the relative size of the reservoir of substitution elements becomes smaller with otolith size and this is reflected in otolith chemistry. This will occur predictably in like sized otoliths, regardless of age and implies that otolith chemistry should be normalized to a standard mass before comparisons are made.

Normalized concentrations of Na, K, and Sr are able to separate otoliths from sub area 7 from sub area 6 & 13 otoliths, while Mg concentrations are able to separate sub area 13 from sub areas 6 and 7. Combining element concentrations with element ratios improves discrimination. A two-step method using K\Na versus [Mg] and [Na] versus Mg\K discriminates all three otolith populations. The ability to chemically discriminate otoliths indicates that environmental conditions are imprinted in the otolith, and that herring schools are relatively discrete, with limited mixing between groups. As such, local populations will be vulnerable to decline due either to over fishing, or degradation/loss of spawning habitat.

Introduction

Otolith chemistry is an alternative option for school discrimination of Pacific herring. Otoliths are an acellular accretion of alternating layers of calcium carbonate (aragonite) and a protein layer, which occurs in the labyrinth of all teleost fishes

(Campana 1999). Elements involved in forming the otolith by chemical precipitation are likely derived primarily from the water in which the fish lives (Campana et al. 1994), while elemental composition of the fish's food plays only a minor role for marine species (Campana 1999). Contrary to other hard parts (bone, fin rays, scales etc.) otoliths grow throughout the life of the fish and are metabolically inert. Once deposited, elements that are incorporated into the otolith are unlikely to be resorbed or reworked (Campana et al. 1994). The precipitation of the otolith has been demonstrated to follow a diurnal pattern, showing yearly as well as daily banding, giving the otolith a powerful timekeeping quality (Campana 1999). The combination of the chronological nature of the otolith, and its ability to proxy for water chemistry create a continuous record of the fish's environment (a virtual flight data recorder for the history of the fish's life). Since the discovery of annular growth rings in otoliths in 1971 there has been an explosion of research into the use of otoliths as an information recorder. Several papers have been published dealing with topics ranging from stock identification (Campana et al. 1999; Thresher 1999), identification of migratory history (Volk et al. 2000; Radtke et al. 1998), reconstruction of environmental history (Gallahar & Kingsford 1996; Halden et al. 2000), and identification of pollution gradients (Hanson & Zdanowicz 1999).

The purpose of this study is to determine if information for school discrimination can be extracted from the otoliths of Pacific herring based on the concentrations of major elements within the otolith. In the present study we quantify the concentrations of five elements (Na, K, Mg, Ca, and Sr) within the otoliths of three schooling groups of Pacific herring using ion chromatographic techniques. The intention was that a chemical signature from two spawning areas in separate management regions

would be determined from young of the year herring. This information would allow for the assignment of stock based on adult otolith chemistry. This data was unavailable however, and so delineations can only be confidently made at the association, or schooling, level. An analytical technique for accurate and precise mass determination of otoliths is presented. The relationships between elements and otolith weight are examined to gain insight into the depositional controls of elements into the otolith, and the relationships between elements and element ratios are examined in order to determine whether populations can be discriminated based on their otolith chemistry.

Methods

Sample Collection

Adult herring were sampled as part of the Department of Fisheries and Oceans test fishery by mid-water trawl aboard the Canadian Coast Guard vessel W.E. Ricker from August 1st to 3rd 2001. Herring were sampled from herring tows 43, 48, and 51 from management sub areas 13, 6, and 7 on August 1st, 2nd, and 3rd respectively (Figure 3.1). Adult herring were sampled from a summer feeding area where populations are in close proximity. A subset of 50 individuals from three catches was taken for otolith removal from three mid-water trawls. Sampling away from spawning grounds is a novel approach in determining stock structure, as the majority of studies sample on or near spawning areas. Our sampling strategy removes the influence of geography, attempting to delineate stocks when they are most likely to mix. Herring trawls were geographically separated by a minimum distance of 37 kilometres between sub area 6 and 7, and a maximum distance of 78 kilometres between sub area 13 and 7. Adult herring were delivered to the dissection table immediately from the fish hopper, and otoliths were removed.

Otolith Removal

Otoliths were removed under a dissecting microscope following the "right between the eyes" method as described by Secor et al. (in Stevenson & Campana 1992) The head is severed from the rest of the body, and a mid-sagittal cut is made from the snout through the cranium. The cranial halves are separated, and the brain halves are removed to expose the labyrinth. Viewed through a dissecting microscope the sagittal otoliths can be clearly seen, and are removed using stainless steel curved forceps. Once removed, the otoliths are subject to a physical cleaning using a fine nylon orthodontic brush, and de-ionized water. This is done in an effort to remove any adhered tissue or fluids. The otoliths are wiped dry using a Kimwipe brand delicate task wiper, and placed in a clean dry 1.5 ml high-density polyethylene micro-centrifuge vial. Otoliths remain in the micro-centrifuge vials until digestion for ion chromatography. Dissection tools are cleaned with de-ionized water, and wiped dry between each sample.

Sample Preparation

Adult herring otoliths are digested whole for analysis of major cations by ion chromatography. Clean dry otoliths are transferred into pre-weighed 1.5 ml HDPE micro-centrifuge vials which have been rinsed five times with de-ionized water and dried in a laminar flow hood within a class 100 cleanroom. Otoliths are digested by adding 10 µL of 16N environmental grade HNO_3 using an Eppendorf® fixed volume pipette. The vials are sealed and centrifuged at 1000 rpm for fifteen minutes in order to keep acid and otolith material concentrated at the conical tip of the vial. The concentrated acid and otolith are allowed to sit for a minimum of 12 hours to ensure complete digestion,

although visual inspection of the solution showed no solids present after fifteen minutes. The concentrated solution is diluted to ~ 1 ml with deionized water by adding 1000 µL of deionized water using a fixed volume 1000 µL Eppendorf® pipette. The total weight of the vial plus solution is recorded, and the weight of the vial subtracted to determine the mass of the final solution.

Five procedural blanks are also prepared following the same procedure as the otolith digestions, in order to check for and make corrections for contamination that may arise from the reagents, containers, or handling procedures.

Ion Chromatography

Digested adult herring otoliths were analyzed for Na, K, Mg, Ca, and Sr using a Dionex DX 600 Ion Chromatography system. These elements are likely to substitute for calcium in the $CaCO_3$ as they are of similar size, and charge. The DX 600 was equipped with an AS50 Thermal Compartment, an EG40 Eluent Generator, a GP50 gradient pump, a CD25 conductivity detector, and an AS50 auto sampler. The instrument was operated using PeakNet 6 Chromatography Workstation software. The EG40 Eluent Generator is loaded with an EluGen EGC-MSA cartridge, which produced methanosulphuric acid eluent at a determined concentration as set by the operator. For cation analysis of the digested herring otoliths, method testing and development work prior to sample analysis determined that a concentration of 28 mM, with a flow rate of 1.2 ml/min., and an injection volume of 50 µL would yield optimized peak separation. These settings were used for the analysis of the digested herring otoliths. Operating conditions for analysis are summarized in Table 3.1.

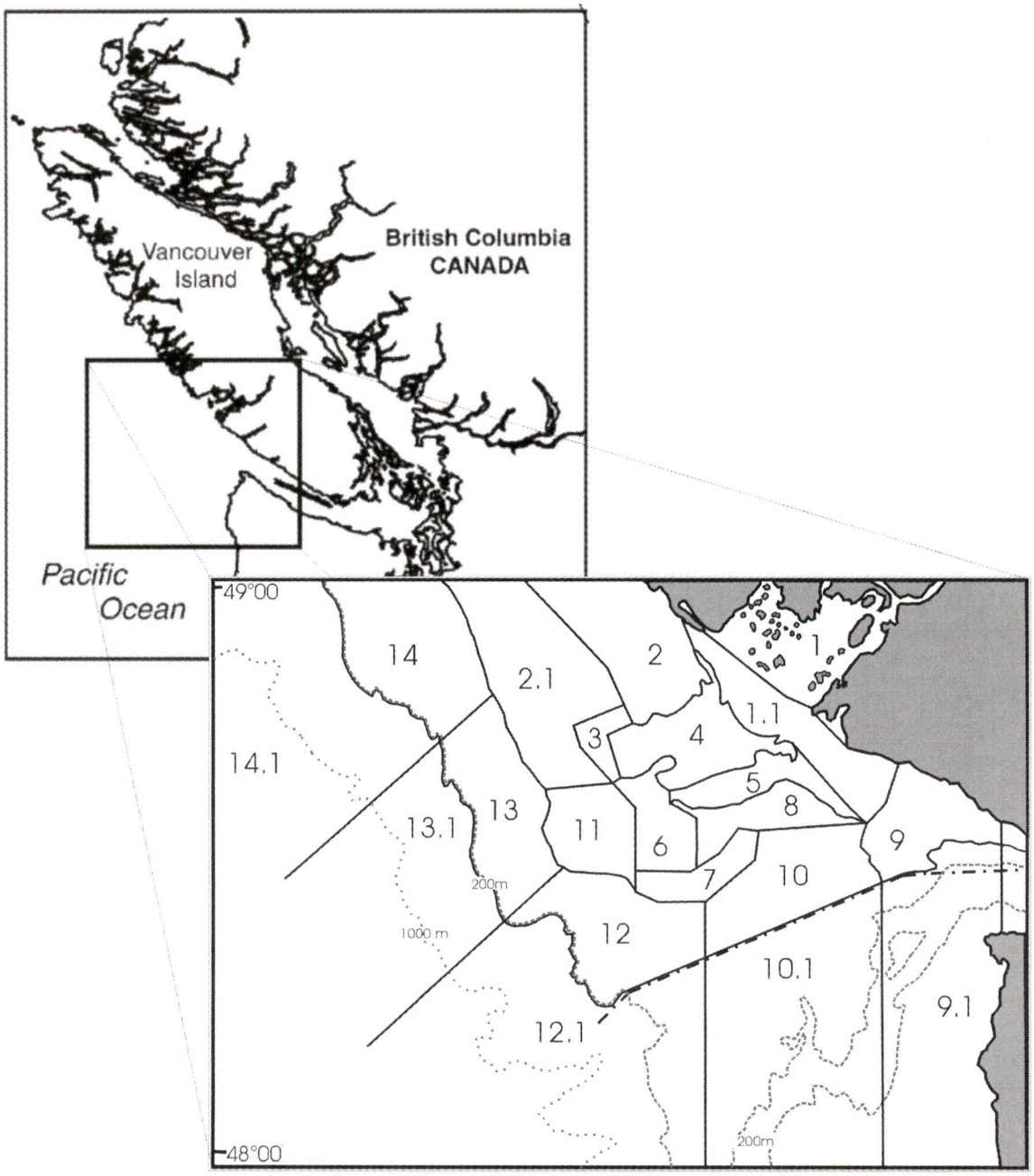


Figure 3.1: Map of sample locations. Expanded area shows LaPerouse Bank with management sub areas. 1000 m and 200 m depth contours are shown, as well as the Canada / United States border (dashed and dotted line).

Single element liquid standards were used to make a five element stock standard solution containing the major elements Li, Na, K, Mg, and Sr in the same relative concentrations as expected in the otolith. A separate standard was prepared for Ca as the high abundance of this element in otoliths (38.4 %) made its incorporation into the mixed standard stock solution impractical. The mixed element standard stock solution and the Ca stock solution are diluted to make up three standards each with concentrations surrounding those expected in the digested otolith solution (based on a suite of elements measured by ICPMS on digested herring otoliths shown in Table 3.2 and on literature values (Campana 1999)). Dilutions are performed gravimetrically to 0.00001 grams and are performed in a class 100 cleanroom. Concentrations of the elements in the stock solutions and the three standards are shown in Table 3.3.

Analysis is performed in randomized groups of 12 samples bracketed before and after by determination of the two groups of standards. Concentrations are determined by calibrating the peak areas of the unknowns to the standards that bracket each run. Bracketing each sample set with calibration standards easily accounts for instrumental drift. This is important, as the instrument was running continuously for ~ 245 hrs and some drift is expected over that time frame.

A measure of analytical precision is determined by the analysis of duplicate samples throughout the run and reported as relative standard deviation (RSD).

Otolith Mass Determination

Due to the small size of herring otoliths (approx. weight = 0.003 g), and static charge surrounding them, weights determined by conventional weighing are imprecise.

Table 3.1: Operating Conditions for DX 600 Ion Chromatograph.

Columns:	IONPAC CG16 Guard Column IONPACCS16 Analytical Column
Column Temp.	40 °C
Eluents:	28 mM methanosulphuric acid
Flow Rate:	1.2 mL / min.
Inj. Volume:	50 µL
Analysis Time:	45 min.
Detection:	Suppressed Conductivity, CSRS Ultra – 4mm
System Backpressure:	~ 1900 psi
Background Conductivity:	< 0.3 µS

Table 3.2: Concentration of major cations in digested herring otoliths determined by ICP-MS.

Mg ppm	Ca ppm	Rb ppm	Sr ppm
358.56 ± 2.4%	420676.64 ± 16%	2.35 ± 10%	1390.01 ± 10%

Table 3.3: Concentration of analytes (µg g^{-1}) in the standard solutions used for Ion Chromatography of digested herring otoliths.

	[Li]	[Na	[Mg]	[K]	[Ca]	[Sr]
Mix. Std. #1	0.0162	4.24	1.11	3.07	—	4.28
Mix. Std. #2	0.0382	10.00	2.61	7.22	—	10.09
Mix. Std. #3	0.0610	15.98	4.17	11.55	—	16.13
Ca #1	—	—	—	—	627.83	—
Ca #2	—	—	—	—	762.59	—
Ca #3	—	—	—	—	949.30	—

To circumvent this a chemical method was used for determining the mass of the otoliths. The concentration of calcium in the digested otolith solutions is determined by ion chromatography by calibrating the calcium peak areas with three calcium standards made of single element standard stock solutions (concentrations of standards used are displayed in Table 3.3). It is known that the otolith is composed of 96.2% by weight calcium carbonate (Campana et al. 1997), and as such is composed of 38.4% calcium. Knowing

the concentration of calcium in all solutions, and assuming an invariant calcium concentration of otoliths (38.4%) the mass of the otoliths can be mathematically determined.

This method of mass determination is valid when considering analytical error relative to the possible fluctuations of calcium in the otolith. The otolith is a relatively pure crystal structure, as compared to bone or other structures dominated by calcium carbonate, with ~ 2-3 % comprised of protein and the remaining ~1% inorganic impurities (Campana 1999). This means that the concentration of calcium can vary by +/- 1%, which is within the analytical precision of ion chromatography determinations of calcium (~ 2%); therefore these differences would go undetected by our analysis. As a result it is entirely valid to determine the mass of the otoliths based on an invariant concentration of calcium. Accurate determination of otolith mass is critical information for (i) the determination of the concentrations of other elements in the otolith, and (ii) observing relationships between elements and otolith mass. This method provides a level of accuracy and precision in mass determination that would be very difficult to achieve by simple weighing.

Statistics

Simple statistical tools are employed to aid in separation of populations based on major cation concentrations. Inspection of the data is performed by generating a scatter pot matrix (SPLOM) using Systat® V. 10.5. Analysis of variance (ANOVA) tests following a general linear model are performed on each of the four elements and all element ratios using herring sub-area as the treatment variable. Included are tables of

standardized residuals indicating unusual observations for each of the elements. The values of standardized residual are used to filter the data, removing those points determined to be unusual (an outlier). An unusual point is one whose standardized residual value is greater than two. This identifies outliers, which are removed from the data prior to investigation into the behaviour of elements, as well as discrimination of herring tows. Post-hoc analysis of the ANOVA results is performed using Tukey's procedure of studentized range distribution. All statistical tests are performed at the 95% confidence interval using Minitab™ V 13.32 statistical software. ANOVA and post-hoc Tukey results are presented in Appendices 1 & 2.

Weight Normalization

In order to compare otoliths of differing weights all chemical data are normalized to an idealized otolith weight of 0.003 g. This is discussed further in the section titled "Discrimination of Herring Tows".

Results and Discussion:

Quality Control

Analytical precision within calibration groups is good with mean RSDs of 1.79%, 1.84%, 1.16%, 2.14% and 1.15% for Na, K, Mg, Ca, and Sr respectively. Concentrations of Li are below detections limits of the current system, and as such are omitted from the data set.

Procedural blanks do not contain sodium, potassium, magnesium, calcium, or strontium in amounts greater than analytical uncertainty. As a result no correction for these elements is performed.

Behaviour of Elements

The behaviour of elements within otoliths was investigated by generating a SPLOM containing the weight of the otolith, all elements measured, and all possible element ratios for all data points. Otolith concentrations and element ratios are presented in Appendix 3. The relationships of elements to each other, and to the mass of the otolith give some insights into the processes that control the deposition and concentration of elements in the otolith. The inversely proportional relationship between weight and Na, Mg, and Sr concentration (Figure 3.2) poses the question; what process causes the concentration of substitution elements to decrease as the weight of the otolith increases?

Two mechanisms that can plausibly explain this relationship are: (1) that the otolith nucleus, which develops while the fish is still in its embryonic stage, is enriched in substitution elements such as NA, Mg, or Sr. Embryonic otolith enrichment could be caused by poor metabolic control of cations entering the egg and being incorporated into the otolith. Continued growth and maturation of the embryo leads to improved metabolic control over elements in the blood plasma, resulting in a suppressed concentration of substituting elements in subsequent layers of the otolith. The proportion of the otolith represented by the concentrated core steadily decreases as more and more relatively pure aragonite is precipitated onto the otolith throughout life. This leads to the inversely proportional relationship between concentration and otolith mass observed.

A second possibility is that ion availability to the otolith changes with otolith size. It has been documented that fish somatic and otolith growth are closely linked (Oozeki & Watanabe 2000), although this relationship has been shown to be discontinuous for some species under varying environmental conditions (Mosegaard et al. 1988). Previous

studies into the relationship between somatic and otolith growth indicate that a close relationship exists for herring species (Moksness 1992). We will assume that a similar relationship exists for Pacific herring on the West Coast of Canada.

As the otoliths increases in size, the ratio of the volume of endolymphatic fluid to the surface area of the otolith decreases and the mass of Ca that is deposited with each new layer increases. Early in life, this ratio is large and so the relative abundance - and hence availability - of elements that can mineralogically substitute into the otolith is large. Through life, as the ratio of the volume of fluid that surrounds the otolith versus its surface area decreases, the relative abundance of elements like Na, Mg, and Sr, in the endolymphatic fluid and their availability also decreases. In other words, the relative size of the reservoir of these elements becomes smaller with otolith size and is reflected in otolith chemistry.

While it's possible that both mechanisms may play a role, it is unlikely that the primordial region of an otolith is a significant factor. For the purpose of argument, this can be demonstrated using an idealized spherical otolith from a 2 year-old fish, comprised of 24 monthly growth bands, and with a total diameter of 5 millimetres. At a constant growth rate each of the 24 bands will increase the radius of the otolith by 0.11 millimetres. Based on the volume of the spheres, and using the reported density of aragonite (2.947 g/cm^3 Deer et al. (1992)) the mass of each monthly band can be calculated. If we use the first two months to represent the primordium, its total mass would be 0.00013 g, while the whole otolith would be 0.2271 grams. In other words, the primordium is only 0.06% of the otolith mass.

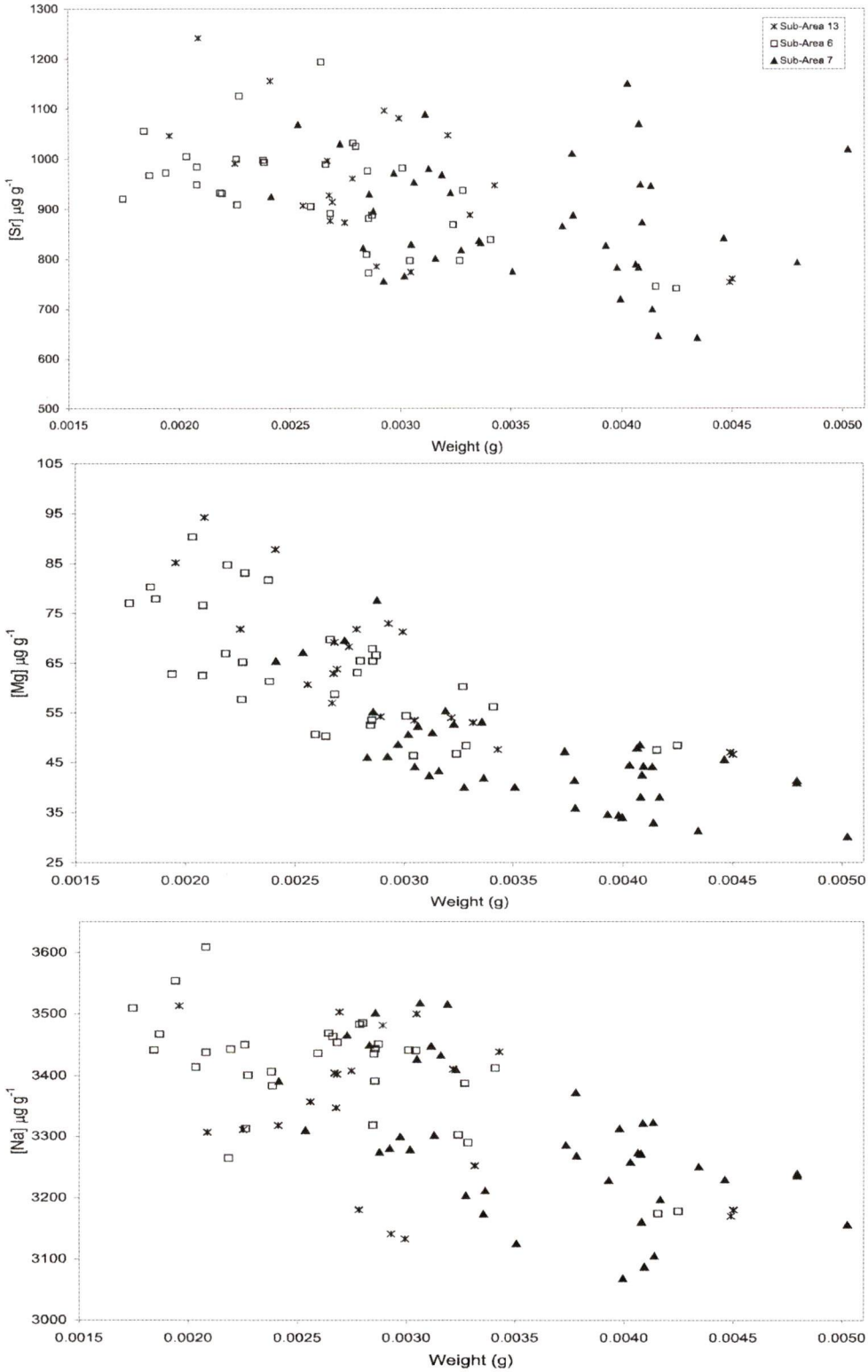


Figure 3.2: Inverse proportional relationships between the concentrations of sodium, magnesium, and strontium to otolith mass.

The total concentration of the otolith can be expressed as:

$$M_t[X_t] = M_p[X_p] + M_r[X_r]$$

where M_t is the mass of the total otolith, $[X_t]$ is the mean concentration of element X in the total otolith, M_p is the mass of the primordium, $[X_p]$ is the concentration of X in the primordium, M_r is the mass of the remainder of the otolith (Mt minus Mp), and $[X_r]$ is the concentration of X in the remainder of the otolith. Rearranging for the concentration of the primordium yields:

$$[X_p] = \frac{M_t[X_t] - M_r[X_r]}{M_p}$$

Using the mean concentration of sodium for the herring otoliths ([Na] = 3344.5 μg g^{-1}) for X_t and the masses from the idealized otolith, it is calculated that the primordium would need a sodium concentration 24 times higher or 79 mg g^{-1} (8%) in order to explain the observed inverse relationship between size and [Na]. This is unreasonable and so the primary control for the relationship must be the second mechanism – that ion availability to the otolith changes with the relative size of the ionic reservoir. The inverse proportional relationships for Na, Mg, and Sr, shown in Figure 3.2 are therefore not a product of ambient chemistry, but are instead a life stage phenomenon.

Edmonds et al. (in Secor et al. 1995) similarly found that concentrations of sodium, and potassium in Pilchard (*Sardinops sagax*) were inversely proportional to otolith weight. They ascribe this to growth rate with higher growth leading to more Na dilute otoliths. Rather than growth rate, we ascribe this phenomenon to the changing ratio

between endolymphatic fluid volume and otolith surface area. In other words, otoliths of the same weight will exhibit the same trend regardless of age or fish size.

The question that remains is whether or not ambient water chemistry imparts a signal on top of that caused simply by otolith size. In order to investigate this possibility the data must first be normalized to weight. In other studies, normalizing chemistry to weight for otoliths of Pink Snapper (*Pagrus auratus*) and Pilchard (*Sardinops sagax*) led to successful separation of their populations (Edmonds et al., in Secor et al. 1995).

Discrimination of Herring Tows

Concentrations are normalized to a standard otolith weight of 0.003 g following the formula:

$$[X]_n = \frac{M_t}{0.003}[X]_t$$

where $[X]_n$ is the normalized concentration of element X, M_t is the mass of the total otolith, and $[X]_t$ is the total concentration of element X in the otolith. This removes the influence of weight on concentration and allows all otoliths to be compared as though they were of equal weight.

Normalizing to weight requires some assumptions about the life history, physiology, and ecology of Pacific herring. Primarily we must assume that the incorporation of elements into the otoliths encounters the same metabolic controls regardless of the size of the fish. As well, we must assume that Pacific herring do not change trophic levels as they mature. Previous literature suggests that these assumptions are very reasonable for Pacific herring, as their diet consists of copepods, amphipods, and euphausiids from the juvenile stage onwards (Hourston & Haegele, 1980). If there were

evidence to suggest that these assumptions are not met, otoliths from distinct life phases (same trophic status for example) would have to be selected from the sample set for comparisons to be valid.

Data were sorted based on the three areas of capture (tows), and are treated as three populations for analysis. Summary statistics for the elements in the three tows are in Table 3.4. Differences exist in the means of all four elements between sampling sub area. Analysis of variance of the data indicates that the differences between means of the three groups are significant for all elements measured.

Post-hoc analysis of the ANOVA results using Tukeys test (summarized and graphically represented in Table 3.5) indicate that the normalized concentrations of sodium, potassium, and strontium in otoliths can separate herring sampled from sub area 7 from those caught in sub areas 6 & 13 but that sub area 6 cannot be separated from sub area 13. Magnesium concentration, on the other hand, is able to separate sub area 13 from sub areas 6 and 7, but cannot separate sub area 6 from sub area 7. While all elements showed positive results in the statistical analysis, only magnesium could discriminate between sub areas 6 and 13. However, its "power" to do so was relatively weak and so element ratios were investigated as a possibly more powerful tool to discriminate the populations.

A scatter plot matrix revealed that the ratios of K\Na and Mg\K separate populations well. ANOVA results for both ratios have p-value of <0.0001 indicating that both ratios have means that are statistically different at the 95% confidence level.

Table 3.4: Descriptive statistics of quantified concentrations grouped by sub area. These data are not normalized to otolith weight.

Sub Area 13					
	Wt. (g)	Na ppm	K ppm	Mg ppm	Sr ppm
Mean	0.0028	3348.58	900.58	72.51	995.56
Median	0.0027	3361.53	894.14	68.29	960.29
Standard Deviation	0.0008	156.26	44.37	25.82	186.43
Range	0.0033	640.39	183.25	87.50	612.97
Minimum	0.0012	2989.86	839.79	46.62	690.27
Maximum	0.0045	3630.25	1023.04	134.13	1303.24
Sub Area 6					
	Wt. (g)	Na ppm	K ppm	Mg ppm	Sr ppm
Mean	0.0027	3403.75	1023.07	64.33	934.27
Median	0.0027	3437.18	1017.19	62.80	936.74
Standard Deviation	0.0006	103.98	83.88	14.18	109.27
Range	0.0025	434.11	408.12	61.87	453.20
Minimum	0.0017	3174.15	859.42	46.37	740.47
Maximum	0.0042	3608.26	1267.55	108.23	1193.67
Sub Area 7					
	Wt. (g)	Na ppm	K ppm	Mg ppm	Sr ppm
Mean	0.0036	3281.34	961.06	46.49	893.21
Median	0.0036	3275.82	964.50	44.18	853.08
Standard Deviation	0.0007	135.56	67.63	10.53	151.71
Range	0.0026	562.01	262.14	47.42	801.88
Minimum	0.0024	2955.14	833.26	30.13	641.48
Maximum	0.0050	3517.15	1095.41	77.55	1443.37

Post-hoc analysis using Tukey's test for the ratio of K\Na indicate that sub area 13 is separate from sub areas 6 and 7, however sub areas 6 and 7 are not separable. The Mg\K ratio is able to separate all three populations. Results, and graphical representation, of the post hoc analysis of element ratios is displayed in Table 3.6.

Attempting to separate all three populations in a single step using a graphical representation is difficult, however a two-step process can be used to graphically illustrate the separate otolith populations. Plotting K\Na ratio versus [Mg] (two variables that separate sub area 13 from sub areas 6 and 7) isolates sub area 13 (Figure 3.3). A second

plot that discriminates between sub areas 6 and 7 can be generated using [Na] and the Mg\K ratio (Figure 3.4). Following the steps outlined in the flow diagram (Figure 3.5) one can therefore systematically identify the three herring populations based on their otolith chemistry. While this is possible with the three management sub areas sampled for this study, we expect that separation by chemical signature for a larger survey would be even greater for tows that capture fish from more distinct populations – ones with differing habitat/life history (eg. LaPerouse Bank versus Hecate Strait).

Management Implications

Homing can be described in two ways; (i) "natal homing"- an individual returns to it's natal spawning location by some geographic imprinting; (ii) "sexual homing" the return of a spawning fish to the same spawning area used in previous years (Hay et al. 2001). An example of natal homing is seen in many salmonid species where an imprinting of geographic location allows adult salmon to return to their natal spawning areas with a high degree of precision. Sexual homing requires that a spawning individual develop an association with a spawning group, or by developing an association with its first spawning location (not necessarily it's natal location). There is currently discussion surrounding the different homing mechanisms that may be in effect in different species, as well as the benefits of these behaviours. In order for either mode of homing to be utilized by a population, there must first be a movement away from spawning areas. Samples in this study were taken away from the spawning grounds, a novel approach in herring research as most tagging studies have occurred on or near the spawning areas. While the data presented here is not able to determine stock associations, it does indicate

school fidelity. The chemical differences which allow for school delineation also indicate that herring from the three sampling groups are also exposed to differing environmental conditions. This may be the result of residence in differing water masses (suggesting geographic divergence) however this cannot be determined conclusively.

The ability to separate herring schools by otolith chemistry indicates that an ambient environmental signature is recorded in the otoliths of Pacific herring. The distinct chemical signatures also shed some light on herring population dynamics. All herring in this study were sampled in fairly close proximity to one another (maximum distance = 78 km), and as a result distance barriers to straying are minimal. However, the fact that these three tows are chemically discrete indicates that, even in close proximity, straying rates are small and population homogenization does not occur. The degree of mixing on the summer feeding grounds appears small, and significant schooling associations are maintained during the feeding months, as well as during movement between spawning areas and feeding areas.

The overlapping compositions of some samples from each of the three tows generally agrees with the assertion of Hay et al. (2001) that there is some association between groups of herring. However, what is also apparent in the chemical data is that the life histories of the three populations are different and discrete. This is almost certainly the result of chemical and physical differences in the water masses that the three populations have been exposed to – temperature, salinity, and geochemical variations.

Table 3.5: P-values and graphical representation of the results of the Tukey post-hoc analysis of element concentrations grouped by sub area.

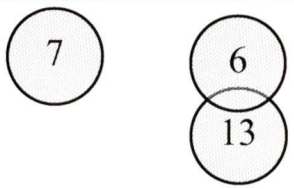
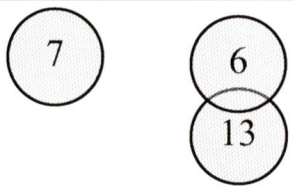
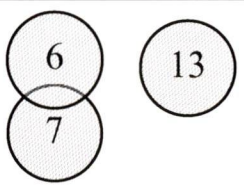
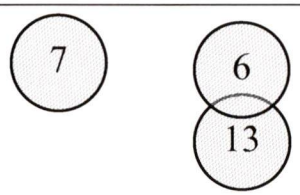
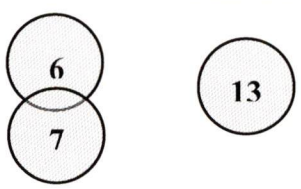
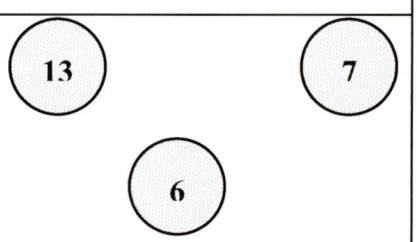
Response Variable	Pairwise Comparison	
[Na] ppm	|6 – 7| ⇒ P-value = 0.0000 |6 – 13|⇒ P-value = 0.9255 |7 – 13|⇒ P-value = 0.0000	
[K] ppm	|6 – 7| ⇒ P-value = 0.0000 |6 – 13|⇒ P-value = 0.3829 |7 – 13|⇒ P-value = 0.0000	
[Mg] ppm	|6 – 7| ⇒ P-value = 0.9753 |6 – 13|⇒ P-value = 0.0084 |7 – 13|⇒ P-value = 0.0034	
[Sr] ppm	|6 – 7| ⇒ P-value = 0.0000 |6 – 13|⇒ P-value = 0.3279 |7 – 13|⇒ P-value = 0.0004	

Table 3.6: P-values and graphical representation of the results of the Tukey post-hoc analysis of element ratios grouped by sub area.

Response Variable	P-Values	
[K] \ [Na]	|6 – 7| ⇒ P-value = 0.1301 |6 – 13|⇒ P-value = 0.0000 |7 – 13|⇒ P-value = 0.0000	
[Mg] \ [K]	|6 – 7| ⇒ P-value = 0.0004 |6 – 13|⇒ P-value = 0.0003 |7 – 13|⇒ P-value = 0.0000	

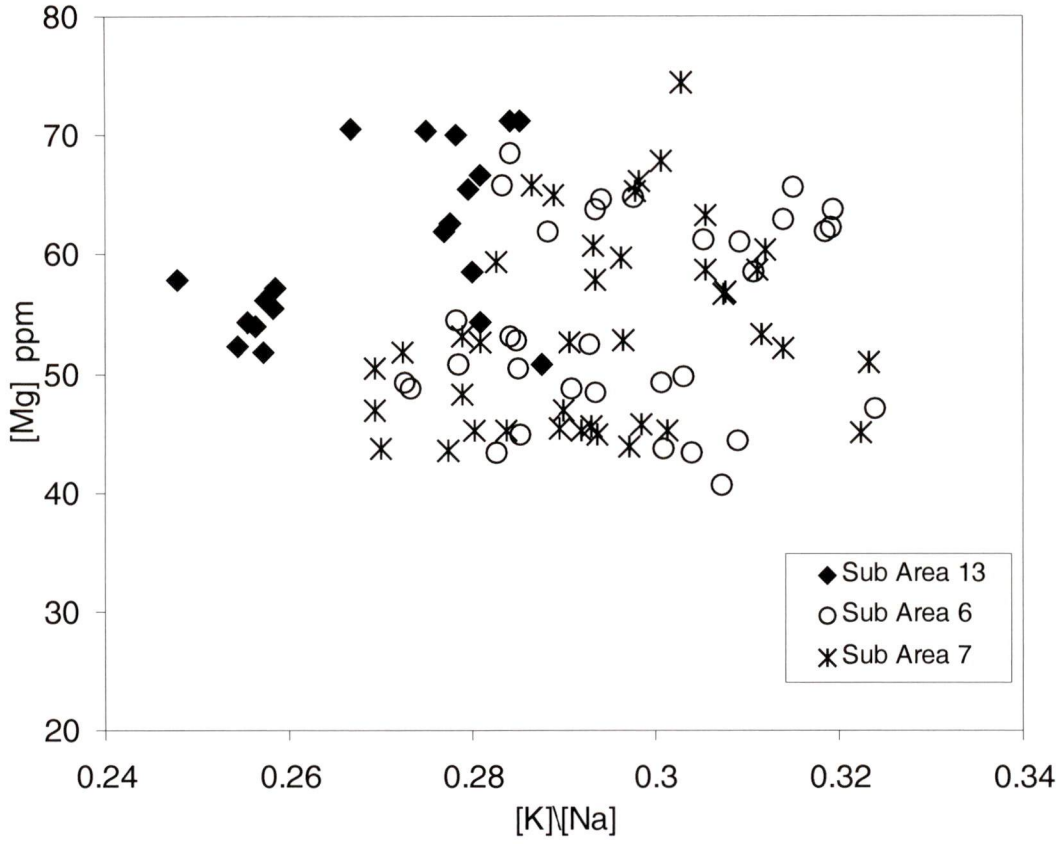


Figure 3.3: Scatter plot of magnesium concentration versus the potassium/sodium ratio. Plot clearly separate sub area 13 from sub areas 6 and 7. Sub areas 6 and 7 cannot be separated from each other based on this plot.

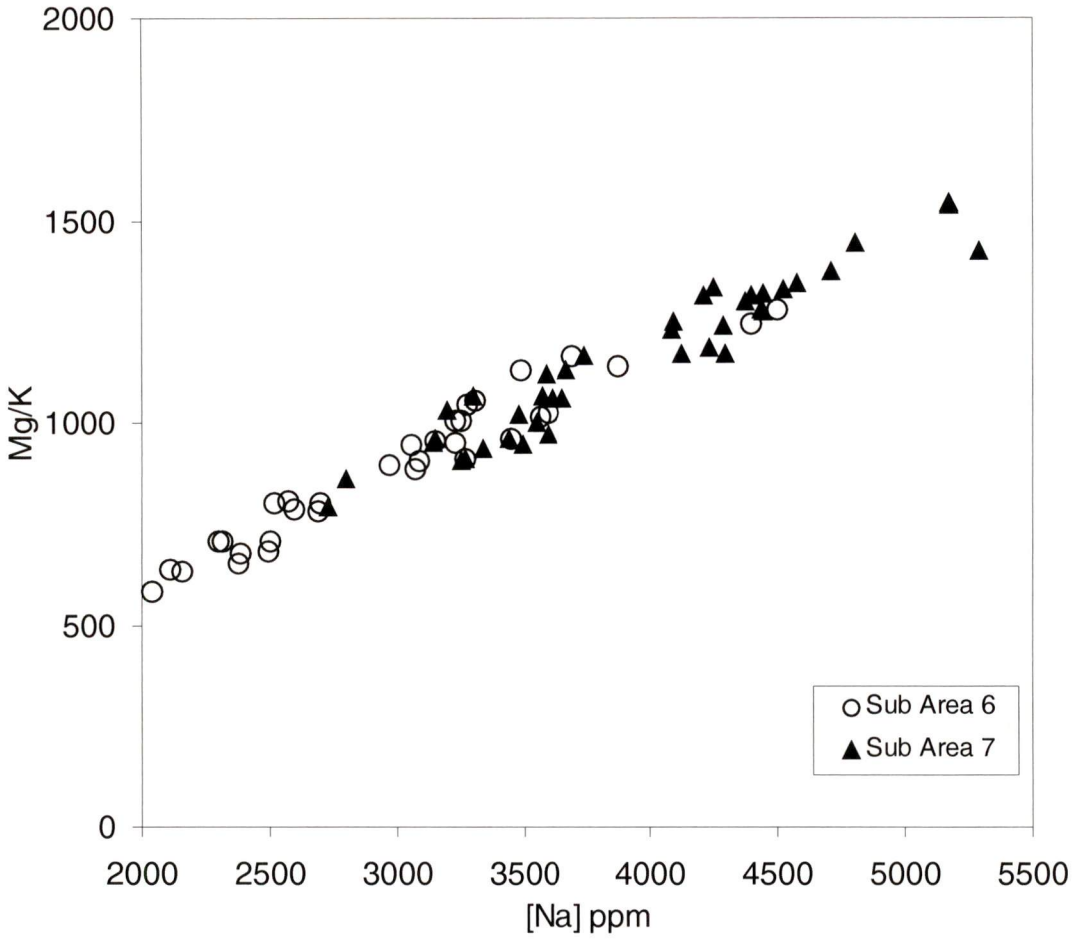


Figure 3.4: Scatter plot of Mg\K ratio versus the concentration of sodium. Separates sub areas 6 and 7.

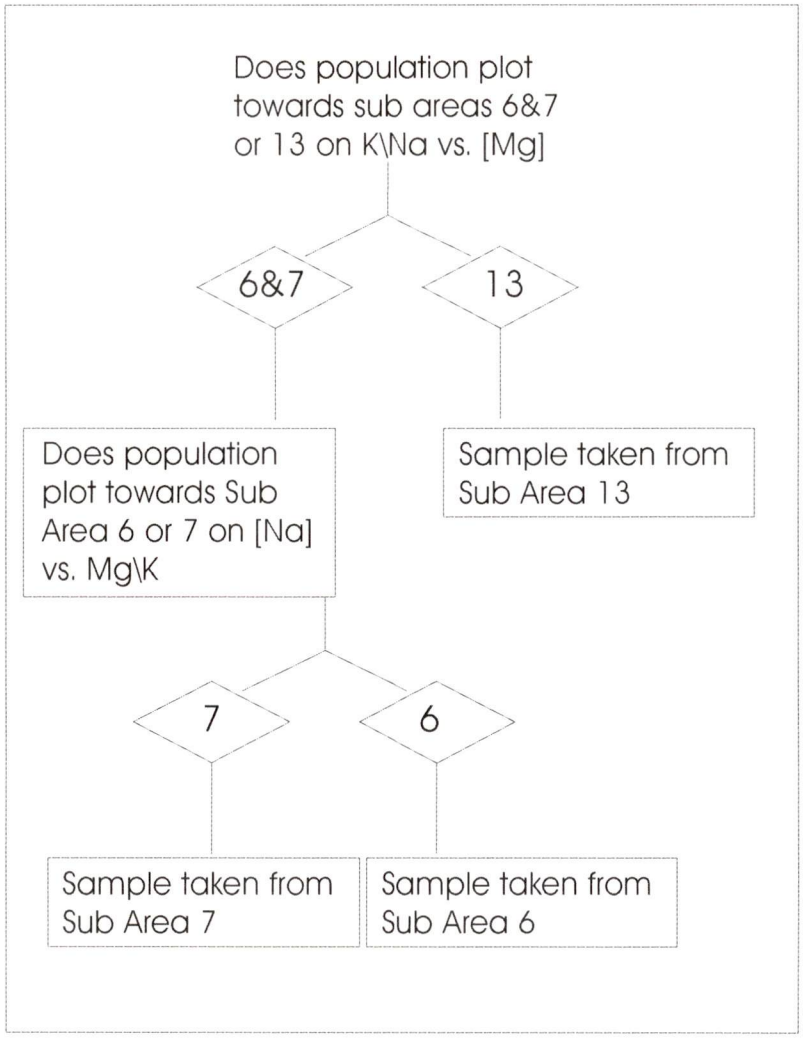


Figure 3.5: Flow chart of steps for chemically delineating otolith populations.

This may help explain population fluctuations at individual spawning grounds. It is known that herring spawns along Canada's west coast are not continuous over time (Hay & McCarter 1997). If open ocean schools of herring are as discrete as our data indicate, then it is possible that fish destined for an individual spawning ground can be largely removed by individual tows. This demonstrates that it would be inappropriate to manage the fishery as a single population distributed along the length of the British Columbia coastline. However, the association between schools also illustrates that it would be unrealistic to consider herring to be discrete populations tied to a single spawning ground. Rather a balance is required.

Currently the herring fishery is managed following a conservative plan based on estimates of the mature stock biomass, estimated by two models: an age-structure model and an escapement model. The total allowable catch for any of the five management areas on Canada's west coast is set at 20% of the forecasted mature stock biomass (Fisheries and Oceans Canada 2001). In order to protect stocks in years of poor environmental conditions the current management strategy also enforces a minimum stock biomass of 12,100 tonnes. If the forecasted mature stock biomass falls below this threshold the commercial fishery is closed for the season to allow for stock regeneration (Fisheries and Oceans Canada 2001). Furthermore, fisheries for gill net and seine fishing are separate, and staggered throughout the season. This is done in order to lessen the possibility of taking the season's quota from a single spawning location. Based on the level of homing demonstrated in this study, as well as others (Hay et al. 2001), this is a responsible approach to the management of this resource. The positive results of this investigation, which includes only major element chemistry, clearly shows that

Pacific herring are a suitable candidate species for the development of a management program incorporating otolith chemical data.

Conclusions

Concentrations of the major cations (sodium, potassium, magnesium, calcium, and strontium) in the otoliths of adult Pacific herring were quantified using ion chromatography. A sub-set of 50 individuals was taken from three herring tows sampling herring schools in fisheries management sub areas 13, 6, and 7 on the LaPerouse Bank of the western shore of Vancouver Island. The resulting database consisted of weight, and concentration data for 126 individuals from the three sub areas. The dataset was sorted based on herring tow and statistical tools were employed to investigate the nature of otolith cation concentrations, and to delineate herring populations.

Traditional weighing of small otoliths proved to be imprecise, and a robust chemical method for mass determination was developed. The known concentration of calcium in the otolith is divided by the concentration of calcium in the digested otolith solution to determine a dilution factor. This is subsequently applied to the concentration results for the other cations, as well as to the final solution weight in order to determine a precise otolith mass. This assumes invariant calcium concentration in all otoliths, a valid assumption considering analytical precision.

Ion chromatography provided some distinct advantages over inductively coupled plasma mass spectrometry (ICP-MS) techniques. Ion chromatography provides an inexpensive, highly precise method for the determination of major cations in digested otolith solutions. Further method development should see the suite of elements that can

be measured chromatographically increased to include some transition metals such as iron, copper, or zinc. The low cost, limited infrastructure, and ease of use make ion chromatography a superior technique as a tool for otolith analysis on large scales (coast wide management projects).

Concentrations of sodium, magnesium, and strontium show an inversely proportional relationship with otolith weight, likely the result of the constant change in the ratio of endolymphatic fluid volume to otolith surface area. This effect must be removed by normalizing otoliths to a standard weight before the chemistry of otoliths from different populations can be compared.

ANOVA and post-hoc analysis of the weight normalized concentrations shows that the three populations can be confidently separated by a combination of single element concentrations and element ratios of sodium, potassium, magnesium, and strontium. Sub area 7 can be discriminated from sub areas 6 and 13 based on the concentration of sodium, potassium, or strontium. Magnesium is the only element able to delineate sub area 13 from sub areas 6 and 7 based on concentration. The K/Na ratio is able to discriminate sub area 13 from sub areas 6 and 7, while the Mg/K ratio is able to discriminate all three populations.

A two-step process using a combination of concentration and ratio data most easily discriminates all three populations. The fact that these three populations can be separated by the chemical signal of their otoliths confirms that schools of herring are discrete, and that the degree of mixing associated with metapopulation dynamics is not sufficient to homogenize the signals (as is the case with genetic discrimination). As well this chemical discrimination provides evidence of differing life histories between groups

of herring. Herring on the LaPerouse Bank show a high degree of school fidelity, indicating that associations are maintained while on the feeding grounds, as well as during migrations between spawning and feeding areas. The limited degree of mixing seen in both tagging studies as well as this study suggests that commercial fishing in the open ocean can (by chance) have very significant impacts on herring returns to a particular breeding habitat. This may be an explanation for the sudden disappearance of herring from spawning grounds that had been occupied annually for several years.

This study illustrates the effectiveness of otolith chemistry to separate herring populations, and suggests that further development in otolith microchemistry can lead to an effective fisheries research and management tool for herring on Canada's west coast. The use of otolith chemistry for fisheries management depends on a robust understanding of the factors that cause variation in otolith chemistry between populations. As a result further study is required in order to determine the capacity of this tool to characterize Pacific herring stocks. The implementation of a management strategy that incorporates otolith chemistry will require considerable research directed at understanding the pathways of elements into the otoliths of Pacific herring and the factors that affect their concentration. In addition a frequent, and extensive sampling protocol will be required to generate a robust database of otolith chemistry throughout British Columbia's Pacific herring management areas.

Summary

Originally the goal of this project was to develop a method for the chemical discrimination of herring populations by analysis of herring otoliths by laser ablation inductively coupled mass spectrometry (LA-ICP-MS). A novel approach for delineating populations based on the physical shape of concentration profiles was conceptualized, and method development steps were followed. This method would identify divergence in herring life history by mapping chemical heterogeneity across the growth axis of the otolith. Divergence in chemical mapping would indicate a difference in water mass residency, and therefore indicate population differences.

In order to develop this method a glass sandwich of three National Institute of Standards and Technology standard reference materials of varying trace element concentrations was constructed. Element concentration profiles for rubidium strontium and lead were created by continuously ablating a line scan across the surface of the glass sandwich. The spatial resolution is empirically determined from these profiles by using a statistical "confidence" window. Concentration profiles indicate that spatial resolution across concentration gradients is controlled by the magnitude and direction of the change in concentration. Profiles across increasing concentration gradients show better spatial resolution than decreasing ones by a factor of ~2. For either direction of change, the relationship between the magnitude of the concentration gradient and the spatial resolution is linear, and therefore predictable. The appropriate resolution for any target can therefore be determined based on the observed range of its concentrations and should be based on the decreasing concentration profile for the NIST glasses to ensure that

resolution is not over-estimated. Spatial resolutions of Sr, Rb, and Pb are similar suggesting that the ablation behaviour of different elements is not a significant control, and therefore a single resolution can realistically be applied to many elements.

For our instrumentation and chosen operating conditions, the spatial resolution at which the concentration of chemically distinct zones in a fish otolith could be reliably measured was determined to be 50μm. This was based on the observed concentration range in the otolith (a posteriori) and the results of the NIST glass sandwich analysis for the decreasing concentration profile, and so can be considered a conservative estimate. The results of the otolith line scan obviously shows that that line scans do provide qualitative data at smaller spatial scales, which may be useful for characterizing heterogeneous targets.

Line scans can provide equivalent or better information about the distribution of elements in heterogeneous solids than discrete spot analysis; and at much reduced time and cost. The results of this study should be transferable to make a portable tool for the calibration of spatial resolution of laser ablation (ICP-MS) systems, as well as future systems. It is our hope that this approach will continue to develop, as it has inherent value in the investigation into heterogeneous solids.

Due to analytical complications, and instrument downtime, it was not possible generate concentration profiles, or quantify otolith chemistry by LA-ICP-MS for herring samples. As a result other analytical options were explored, and ion chromatography was determined to be a sensitive, precise, and accessible method for quantifying otolith chemistry. Concentrations of the major cations in the otoliths of 126 adult Pacific herring

were quantified using ion chromatography. The resulting database consisted of weight, and concentration data for 126 individuals from the three sub areas. The dataset was sorted based on herring tow and statistical tools were employed to investigate the nature of otolith cation concentrations, and to delineate herring populations.

Traditional weighing of small otoliths proved to be imprecise, and a robust chemical method for mass determination was developed. The known concentration of calcium in the otolith and the concentration in the digested solution (determined analytically) are used to mathematically determine the mass of the otolith. This assumes invariant calcium concentration in all otoliths, a valid assumption considering analytical precision. This is a novel approach to determining otolith mass, information critical to quantifying the concentration of elements in the otolith.

Concentrations of sodium, magnesium, and strontium show an inversely proportional relationship with otolith weight, likely the result of the constant change in the ratio of endolymph volume to otolith surface area. This effect must be removed by normalizing otoliths to a standard weight before the chemistry of otoliths from different populations can be compared.

ANOVA and post-hoc analysis of the weight normalized concentrations shows that the three populations can be confidently separated by a combination of single element concentrations and element ratios of sodium, potassium, magnesium, and strontium. A two-step process using a combination of concentration and ratio data most easily discriminates all three populations.

The chemical separation of these herring populations confirms that schools of herring are discrete, and that the degree of mixing associated with metapopulation

dynamics is not sufficient to homogenize the signals (as is the case with genetic discrimination). As well this chemical discrimination provides evidence of differing life histories between groups of herring. Herring on the LaPerouse Bank show a high degree of school fidelity, indicating that associations are maintained while on the feeding grounds, as well as during migrations between spawning and feeding areas.

Ion chromatography provides some distinct advantages over inductively coupled plasma mass spectrometry (ICP-MS) techniques. Ion chromatography provides an inexpensive, highly precise method for the determination of major cations in digested otolith solutions. Further method development should see the suite of elements that can be measured chromatographically increased to include some transition metals such as iron, copper, or zinc. The low cost, limited infrastructure, and ease of use make ion chromatography a superior technique as a tool for otolith analysis on large scales. This is a favourable technology for use by management organizations such as the Department of Fisheries and Oceans.

While our experimental design was of small scope, this study illustrates the effectiveness of otolith chemistry to separate herring populations, and suggests that further development in otolith microchemistry can lead to an effective research and management tool for herring on Canada's west coast. The use of otolith chemistry for fisheries management depends on a robust understanding of the factors that cause variation in otolith chemistry between populations. As a result further study is required in order to determine the capacity of this tool to characterize Pacific herring stocks. The implementation of a management strategy that incorporates otolith chemistry will require considerable research directed at understanding the pathways of elements into the otoliths

of Pacific herring and the factors that affect their concentration. In addition a frequent, and extensive sampling protocol will be required to generate a robust database of otolith chemistry throughout British Columbia's Pacific herring management areas.

References

Andrews A. H., G. M. Cailliet, and K. H. Coale. 1999. Age and growth of the Pacific grenadier (Coryphaenoides acrolepis) with age estimate validation using an improved radiometric ageing technique. *Can. J. Fish. Aquat. Sci.*, **56**: 1339-1350.

Arai T., T. Otake, and K. Tsukamoto. 2000. Timing of metamorphosis and larval segregation of the Atlantic eels *Anguilla rostrata* and *A. anguilla*, as revealed by otolith microstructure and microchemistry. *Marine Biology,* **137**:39-45.

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

Beacham T. D., J. F. Schweigert, C. MacConnachie, K. D. Le, K. Labaree, and K. M. Miller. 2001. Population structure of herring (*Clupea pallasi*) in British Columbia: an analysis using microsatellite loci. *Can. Stock Assessment Secretariat Res. Doc.* **2001/128**.

Bone Q., N. B. Marshall, and J. H. S. Blaxter. 1995. Biology of Fishes, 2nd Ed. Chapman & Hall, New York. p. 225-229.

Campana S.E. 1997. Use of radiocarbon from nuclear fallout as a dated marker in the otoliths of haddock *Melanogrammus aeglefinus*. *Mar. Ecol. Prog. Ser.*, **150**: 49-56.

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

Campana S.E. and J.D. Neilson. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.*, **42**: 1014-1032

Campana S.E., A. J. Fowler, and C. M. Jones. 1994. Otolith elemental fingerprinting for stock identification of Atlantic Cod (*Gadus morhua*) using laser ablation ICP-MS. *Can. J. Fish. Aquat. Sci.*, **51**: 1942-1950

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

Campana S.E., G. A. Chouinard, J. M. Hanson, and A. Fréchet. 1999. Mixing and migration of overwintering Atlantic Cod (*Gadus morhua*) stocks near the mouth of the Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.*, **56**: 1873-1881.

Campana S.E., G. A. Chouinard, J. M. Hanson, A. Fréchet, and J Brattey. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research,* **46**:343-357.

Chalmers D. D. 1993. Review of the 1991-1992 British Columbia herring fishery and spawn abundance. *Can. Ind. Rep. Fish. Aquat.,* No. **218**: viii + 133p.

Chen Z. X. 1999. Inter-element fractionation and correction in laser ablation inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.*, **14:** 1-7.

Chesney E.J., McKee B.M., Blanchard T., & Chan L. 1998. Chemistry of otoliths from juvenile menhaden *Brevoortia patronus*: evaluating strontium, strontium:calcium and strontium isotope ratios as environmental indicators. *Mar. Ecol. Prog. Ser.*, **171**:261-273.

Deer W. A., R. A. Howie, and J. Zussman. 1992. An introduction to the Rock-Forming Minerals. Longman Scientific & Technical, England.

Eggins S. M., L. P. J. Kinsley and J. M. G. Shelley. 1998. Deposition and element fractionation processes during atmospheric pressure laser sampling for analysis by ICP-MS. *Appl. Surf. Sci.*, **127-129**: 278-286.

Environment Canada.1998. Fisheries and Oceans Canada, *SOE Bulletin* No. **98-2**.

Fisheries and Oceans Canada. 2001. Fish stocks of the Pacific coast. Printed in Canada, ISBN 0-662-30042-4

Gallahar N.K., and M. J. Kingsford. 1996. Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: an experimental investigation. *J. Fish. Biol.*, **48**: 174-186.

Guillong M. and D. Günther. 2002. Effect of particle size distribution on ICP-induced elemental fractionation in laser ablation-inductively coupled plasma-mass spectrometry. *J. Anal. At. Spectrom.,* **17**: 831-837.

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

Hanson P.J., and V. S. Zdanowicz. 1999. Elemental composition of otoliths from Atlantic croaker along an estuarine pollution gradient. *Journal of Fish Biology*, **54**:656-668.

Hay D. and P. B. McCarter. 1997. Larval distribution, abundance, and stock structure of British Columbia herring. *J. Fish Biol.*, **51** (supp. **A**):155-175

Hay D. E., P. B. McCarter, and K. S. Daniel. 2001. Tagging of Pacific herring *Clupea pallasi* from 1936–1992: a review with comments on homing, geographic fidelity, and straying. *Can. J. Fish. Aquat. Sci.*, **58:**1356-1370.

Hoffmann E., H. Stephanowitz, E. Ullrich, J. Skole, C. Lüdke and B. Hoffmann. 2000. Investigation of mercury migration in human teeth using spatially resolved analysis by laser ablation-ICP-MS. *J. Anal. At. Spectrom.*, **15**: 663-667.

Hourston A. S., and C. W. Haegele. 1980. Herring on Canada's Pacific coast. *Can. Spec. Publ. Fish. Aquat. Sci.*, **48**: 23p

Kalish J.M. 1993. Pre- and post-bomb radiocarbon in fish otoliths. *Earth Planet Sci Lett* **114**: 549-554

Kingsford M. J., and B. M. Gillanders. 2000. Variation in concentrations of trace elements in otoliths and eye lenses of a temperate reef fish, *Parma microlepis*, as a function of depth, spatial scale, and age. *Marine Biology.*, **137**: 403-414.

Longerich H. P., S. E. Jackson and D. Günther.1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. *J. Anal. At. Spectrom.*, **11**: 899-904.

Mank A. J. G. and P. R. D. Mason. 1999. A critical assessment of laser ablation ICP-MS as an analytical tool for depth analysis in silica-based glass samples. *J. Anal. At. Spectrom.*, **14**: 1143-1153.

Mason P. R. D. and A. J. G. Mank. 2001. Depth-resolved analysis in multi-layered glass and metal materials using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *J. Anal. At. Spectrom.*, **16**: 1381-1388.

Mayer F.L. Jr., Marking L.L., Bills T.D. and G.E. Howe. 1994. Physico-chemical factors affecting toxicity in freshwater: hardness, pH, and temperature. In: Hamelink J.L., Landrum P.F., Bergman H.L. and W.H. Benson (eds) Bioavailability: physical, chemical, and biological interactions. Lewis Publishers, London, p 5-21

Milton D. A., S. R. Chenery, M. J. Farmer, and S. J. M. Blaber. Identifying the spawning estuaries of the tropical shad, terubok Tenualosa toil, using otolith microchemistry. *Mar. Ecol. Prog. Ser.*, **153**: 283-291.

Milton D. A., C. D. Tenakanai, and S. R. Chenery. 2000. Can the movements of barramundi in the Fly River Region, Papua New Guinea be traced in their otoliths? *Estuarine, Coastal and Shelf Science,* **50**: 855-868.

Moksness E. 1992. Validation of daily increments in the otolith microstructure of norwegian spring-spawning herring (clupea-harengus L). *ICES J. Mar. Sci.* **49**(2): 231-235

Mosegaard H., H. Svedang, and K. Taberman. 1988. Uncoupling of somatic and otolith growth rates in arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. *Can. J. Fish. Aquat. Sci.* **45**: 1514-1524

Moyle P. B., and J. J. Cech Jr. 1996. Fishes: An Introduction to Ichthyology, 3rd Ed. Prentice Hall. New Jersey, USA. p. 144-146.

Oozeki Y., and Y. Wanatabe. 2000. Comparison of somatic growth and otolith increment growth in laboratory reared larvae of Pacific saury, *Cololabis Saira*, under different temperature conditions. *Marine Biology*. **136**: 349-359

Olsson P.E., Kling P., and C. Hogstrand. 1998. Mechanisms of heavy metal accumulation and toxicity in fish. In: Langston W.J. and Bebianno M.J. (eds) Metal metabolism in aquatic environments. Chapman and Hall, London, p 321-350.

Panella G. 1971. Fish otoliths: daily growth layers and periodical patterns. *Science* **173**: 1124-1127.

Patterson H. M., S. R. Thorrold, and J. M. Shenker. 1999. Analysis of otolith chemistry in Nassau grouper (*Epinephelus striatus*) from the Bahamas and Belize using solution-based ICP-MS. *Coral Reefs*. **18**: 171-178.

Radtke R. L., J. B. Dempson, and J. Ruzicak. 1998. Microprobe analyses of anadromous Arctic char, *Salvelinus alpinus*, otoliths to infer life history migration events. *Polar Biol.* **19**: 1-8.

Rodushkin M. D., D. Axelsson, D. Malinovsky and D. C. Baxter. 2002. Analyte- and matrix-dependent elemental response variations in laser ablation inductively coupled plasma mass spectrometry. Part 1. The roles of plasma and ion sampling conditions. *J. Anal. At. Spectrom.* **17**: 1223-1230.

Rodushkin M. D., D. Axelsson, D. Malinovsky and D. C. Baxter. 2002. Analyte- and matrix-dependent elemental response variations in laser ablation indictively coupled mass spectrometry: Part 2. Implications for multi-element analyses. *J. Anal. At. Spectrom.* **17**: 1231-1239.

Russo R. E., X. L. Mao, O. V. Borisov and H. Liu. 2000. Influence of wavelength on fractionation in laser ablation ICP-MS. *J. Anal. At. Spectrom.* **15**: 1115-1120.

Secor D. H., J. M. Dean, and S. E. Campana [ed.]. 1995. Recent Developments in Fish Otolith Research. University of South Carolina Press. 655-670.

Sinclair D. J., L. P. J. Kinsley and M. T. McCulloch. 1998. High resolution analysis of trace elements in corals by laser ablation ICP-MS. *Geochim. Cosmochim. Acta*. **62**: 1889-1901.

Stecher III H.A., D. E. Krantz, C. J. Lord III, G. W. Luther III and K. W. Bock. 1996. Profiles of strontium and barium in *Mercenaria mercenaria* and *Spisula solidissima* shells. *Geochim. Cosmochim. Acta*. **60**: 3445-3456.

Stevenson D. K., and S. E. Campana [ed.]. 1992. Otolith microstructure examination and analysis. *Can. Spec. Publ. Fish. Aquat. Sci.* **117**: 19-57.

Thorrold S. R. S. e. Campana, C. M. Jones, and P. K. Swart. 1997. Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta*., **61**(14): 2909-2919.

Thorrold S. R., C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 1998. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). *Limnol. Oceanogr*., **43**(8): 1826-1835.

Thresher R.E. 1999. Elemental composition of otoliths as a stock delineator in fishes. *Fisheries Research*, **43**:165-204

Volk E.C., A. Blakley, S. L. Schroder, and S. M. Kuehner. 2000. Otolith chemistry reflects migratory characteristics of Pacific salmonids: Using otolith core chemistry to distinguish maternal associations with sea and freshwaters. *Fisheries Research*, **46**: 251-266.

Wang S., R. Brown and D. J. Gray. 1994. Application of laser ablation-ICPMS to the spatially resolved micro-analysis of biological tissue. *Appl. Spectrosc.* **48**: 1321-1325.

Ware D. M. and J. Schweigert. 2001. Metapopulation structure and dynamics of British Columbia herring. *Can. Stock Assessment Secretariat Res. Doc.* **2001/127**

Ware D. M., C. Tovey, D. E. Hay, and P. B. McCarter. 2000. Straying rates and stock structure of British Columbia herring. *Can. Stock Assessment Secretariat Res. Doc.* **2000/006**

Appendices

Appendix 1: ANOVA and Post-Hoc Tukey Tables for normalized otolith concentrations grouped by Sub Area. ANOVA follows a fixed general linear model, with three factors (sub areas 6, 7, and 13). All statistical tests performed at the 95% confidence limit.

Analysis of Variance for Na Norm, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SubArea	2	21611215	21611215	10805607	21.50	0.000
Error	103	51758254	51758254	502507		
Total	105	73369469				

Unusual Observations for Na Norm

Obs	Na Norm	Fit	SE Fit	Residual	St Resid
3	1342.04	3061.02	141.78	-1718.98	-2.47R
21	4744.20	3061.02	141.78	1683.17	2.42R
22	4771.64	3061.02	141.78	1710.61	2.46R
52	4498.75	2992.22	116.54	1506.52	2.15R
53	4394.39	2992.22	116.54	1402.17	2.01R

R denotes an observation with a large standardized residual.

Tukey Simultaneous Tests
Response Variable Na Norm
All Pairwise Comparisons among Levels of SubArea

SubArea = 6 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	942.61	158.1	5.9614	0.0000
13	68.80	183.5	0.3749	0.9255

SubArea = 7 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
13	-873.8	177.5	-4.922	0.0000

Appendix 1 (cont'd)

Analysis of Variance for K Norm, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SubArea	2	2110363	2110363	1055181	22.40	0.000
Error	103	4853012	4853012	47117		
Total	105	6963375				

Unusual Observations for K Norm

Obs	K Norm	Fit	SE Fit	Residual	St Resid
21	1305.01	825.98	43.41	479.03	2.25R
22	1327.29	825.98	43.41	501.31	2.36R
51	1643.06	900.62	35.69	742.44	3.47R

R denotes an observation with a large standardized residual.

Tukey Simultaneous Tests
Response Variable K Norm
All Pairwise Comparisons among Levels of SubArea

SubArea = 6 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	250.57	48.42	5.175	0.0000
13	-74.64	56.20	-1.328	0.3829

SubArea = 7 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
13	-325.2	54.36	-5.982	0.0000

Appendix 1 (cont'd)

Analysis of Variance for Mg Norm, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SubArea	2	854.60	854.60	427.30	6.33	0.003
Error	103	6949.33	6949.33	67.47		
Total	105	7803.93				

Unusual Observations for Mg Norm

Obs	Mg Norm	Fit	SE Fit	Residual	St Resid
93	74.3852	54.3236	1.2383	20.0616	2.47R

R denotes an observation with a large standardized residual.

Tukey Simultaneous Tests
Response Variable Mg Norm
All Pairwise Comparisons among Levels of SubArea

SubArea = 6 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	-0.3904	1.832	-0.2131	0.9753
13	6.4644	2.127	3.0398	0.0084

SubArea = 7 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
13	6.855	2.057	3.332	0.0034

Appendix 1 (cont'd)

Analysis of Variance for Sr Norm, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SubArea	2	1386265	1386265	693132	20.33	0.000
Error	103	3512461	3512461	34102		
Total	105	4898726				

Unusual Observations for Sr Norm

Obs	Sr Norm	Fit	SE Fit	Residual	St Resid
3	512.42	878.40	36.93	-365.98	-2.02R
79	1544.22	1063.67	27.84	480.55	2.63R
90	1705.60	1063.67	27.84	641.93	3.52R
101	1454.24	1063.67	27.84	390.57	2.14R

R denotes an observation with a large standardized residual.

Tukey Simultaneous Tests
Response Variable Sr Norm
All Pairwise Comparisons among Levels of SubArea

SubArea = 6 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	253.77	41.19	6.161	0.0000
13	68.50	47.81	1.433	0.3279

SubArea = 7 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
13	-185.3	46.25	-4.006	0.0004

Appendix 2: ANOVA and Post-Hoc Tukey Tables for element ration K/Na, and Mg/K grouped by Sub Area. ANOVA follows a fixed general linear model, with three factors (sub areas 6, 7, and 13). All statistical tests performed at the 95% confidence limit.

Analysis of Variance for K\Na, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SubArea	2	0.0150816	0.0150816	0.0075408	23.82	0.000
Error	103	0.0326063	0.0326063	0.0003166		
Total	105	0.0476879				

Tukey Simultaneous Tests
Response Variable K\Na
All Pairwise Comparisons among Levels of SubArea

SubArea = 6 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	-0.00774	0.003969	-1.950	0.1301
13	-0.03116	0.004606	-6.764	0.0000

SubArea = 7 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
13	-0.02342	0.004456	-5.255	0.0000

Analysis of Variance for Mg\K, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SubArea	2	0.0165435	0.0165435	0.0082717	30.65	0.000
Error	103	0.0277967	0.0277967	0.0002699		
Total	105	0.0443401				

Tukey Simultaneous Tests
Response Variable Mg\K
All Pairwise Comparisons among Levels of SubArea

SubArea = 6 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	-0.01467	0.003664	-4.004	0.0004
13	0.01732	0.004253	4.072	0.0003

SubArea = 7 subtracted from:

Level SubArea	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
13	0.03199	0.004114	7.775	0.0000

Appendix 3: Otolith weight, elemental concentrations, and element ratio data for herring otoliths, determined by ion chromatography. Samples with repeat rows indicated duplicate analysis for determination of analytical precision. Unusual observations as determined by statistical tests have been removed. This data has not been normalized to otolith weight.

Sample Name	Otolith wt. Grams	Sub Area	Na	K	Mg	Ca	Sr	K/Na	Mg/K
A-06	0.0042	13	2989.86	844.42	47.87	384400	690.27	0.282427	0.056687
A-12	0.0034	13	3437.76	965.38	47.57	384400	946.67	0.280817	0.049276
A-20	0.0012	13	3362.39	1023.04	130.42	384400	1283.83	0.304261	0.127482
A-32	0.0027	13	3404.04	978.87	56.95	384400	995.95	0.28756	0.058183
A-42	0.0028	13	3180.18	893.08	71.82	384400	960.29	0.280827	0.080414
A-44	0.0027	13	3346.80	861.09	62.91	384400	927.26	0.257286	0.073055
A-44	0.0026	13	3357.06	863.45	60.73	384400	906.37	0.257202	0.070335
A-54	0.0029	13	3140.63	895.52	72.92	384400	1096.14	0.285139	0.081429
A-54	0.0030	13	3132.53	890.35	71.29	384400	1081.07	0.284227	0.080075
A-56	0.0033	13	3252.08	910.38	53.03	384400	887.80	0.279938	0.058246
A-58	0.0027	13	3502.51	905.70	63.79	384400	913.78	0.258587	0.070432
A-62	0.0024	13	3317.41	884.66	87.80	384400	1155.74	0.266671	0.099249
A-64	0.0021	13	3306.32	924.35	94.18	384400	1241.56	0.27957	0.101889
A-70	0.0023	13	3310.59	848.69	71.85	384400	990.83	0.256357	0.084663
A-72	0.0020	13	3512.65	906.83	85.15	384400	1045.85	0.258162	0.093897
A-74	0.0029	13	3480.92	885.37	54.21	384400	784.92	0.25435	0.061225
A-74	0.0030	13	3499.51	894.14	53.47	384400	773.79	0.255505	0.059796
A-80	0.0032	13	3410.16	845.28	53.94	384400	1046.49	0.247872	0.063819
A-82	0.0027	13	3401.91	942.50	69.20	384400	876.15	0.277051	0.073418
A-82	0.0027	13	3407.76	946.26	68.29	384400	872.66	0.277679	0.072163
A-84	0.0045	13	3169.92	871.97	47.02	384400	753.40	0.275075	0.053928
A-84	0.0045	13	3180.15	884.60	46.62	384400	759.60	0.278163	0.052705
A-88	0.0016	13	3630.25	906.06	134.13	384400	1302.92	0.249587	0.148032
A-88	0.0016	13	3619.45	902.67	131.03	384400	1303.24	0.249394	0.145154
A-96	0.0022	13	3361.53	839.79	76.68	384400	1292.51	0.249823	0.091312
B-02	0.0027	6	3453.42	1011.12	58.76	384400	891.10	0.292788	0.058109
B-04	0.0019	6	3554.05	1183.38	70.68	384400	1049.11	0.332966	0.059729
B-06	0.0018	6	3441.14	1034.24	80.29	384400	1055.22	0.300552	0.077629
B-08	0.0026	6	3435.69	1033.85	50.60	384400	904.54	0.300916	0.048947
B-10	0.0028	6	3484.83	1077.42	65.46	384400	1024.53	0.309173	0.06076
B-10	0.0028	6	3482.38	1081.50	63.07	384400	1031.35	0.310563	0.058315
B-12	0.0021	6	3437.18	976.77	76.62	384400	983.61	0.284177	0.078438
B-12	0.0019	6	3553.72	1091.77	62.80	384400	971.99	0.307219	0.05752
B-14	0.0024	6	3383.52	983.99	61.31	384400	993.16	0.290818	0.062311
B-22	0.0024	6	3406.24	1013.37	81.68	384400	997.53	0.297506	0.080605
B-26	0.0023	6	3400.50	1067.15	83.08	384400	1125.55	0.31382	0.077853
B-28	0.0022	6	3442.39	1096.51	84.73	384400	930.77	0.318533	0.077274
B-30	0.0017	.06	3509.06	1000.50	77.07	384400	919.96	0.28512	0.077029
B-32	0.0028	6	3317.90	1005.38	52.48	384400	808.81	0.303018	0.052202

Appendix 3 (cont'd)

Sample Name	Otolith wt. grams	Sub Area	Na	K	Mg	Ca	Sr	K/Na	Mg/K
B-34	0.0029	6	3450.46	1101.60	66.55	384400	887.41	0.319262	0.060416
B-34	0.0029	6	3442.74	1098.38	65.41	384400	881.05	0.319043	0.059551
B-36	0.0026	6	3468.49	1071.41	50.29	384400	1193.67	0.308899	0.046938
B-38	0.0019	6	3466.94	1017.19	77.90	384400	966.88	0.293398	0.07658
B-40	0.0034	6	3412.25	1001.73	56.17	384400	838.16	0.293568	0.056073
B-46	0.0020	6	3413.46	1041.82	90.31	384400	1004.60	0.30521	0.086684
B-50	0.0029	6	3391.15	997.74	67.87	384400	772.10	0.294218	0.068028
B-52	0.0017	6	3448.86	1102.79	108.23	384400	1128.97	0.319755	0.098146
B-60	0.0033	6	3387.43	1067.37	60.22	384400	796.73	0.315096	0.056418
B-66	0.0023	6	3449.59	1048.06	57.67	384400	999.03	0.303823	0.055024
B-68	0.0033	6	3289.31	936.97	48.33	384400	936.74	0.284852	0.051579
B-74	0.0039	6	3214.41	1267.55	52.52	384400	788.81	0.394332	0.041435
B-74	0.0042	6	3177.79	903.19	48.38	384400	740.47	0.28422	0.053565
B-74	0.0042	6	3174.15	898.81	47.47	384400	744.66	0.283165	0.052815
B-76	0.0023	6	3312.25	903.15	65.24	384400	908.05	0.27267	0.07224
B-78	0.0027	6	3463.00	998.36	69.74	384400	988.70	0.288293	0.069851
B-80	0.0032	6	3302.27	941.03	46.69	384400	868.46	0.284964	0.049613
B-82	0.0030	6	3183.37	859.42	49.13	384400	803.26	0.269972	0.057163
B-86	0.0021	6	3608.26	1019.39	62.52	384400	948.05	0.282516	0.061335
B-88	0.0030	6	3440.04	1114.15	46.37	384400	796.27	0.323876	0.041615
B-88	0.0030	6	3440.96	957.45	54.36	384400	981.14	0.27825	0.056773
B-88	0.0029	6	3435.18	956.69	53.47	384400	975.55	0.278497	0.055892
B-96	0.0022	6	3264.32	892.36	66.92	384400	931.99	0.273367	0.074993
C-02	0.0049	7	2961.01	838.74	41.75	384400	808.43	0.28326	0.049774
C-02	0.0049	7	2955.14	833.26	41.05	384400	809.03	0.281971	0.04926
C-04	0.0032	7	3515.12	1093.73	55.32	384400	967.57	0.311149	0.050575
C-04	0.0031	7	3517.15	1095.41	52.29	384400	952.84	0.311448	0.047738
C-06	0.0040	7	3312.27	988.63	34.40	384400	782.31	0.298475	0.034791
C-100	0.0031	7	3301.44	920.50	50.97	384400	979.58	0.278819	0.055374
C-100	0.0030	7	3299.13	919.87	48.61	384400	970.72	0.278821	0.052844
C-12	0.0030	7	3277.75	1059.69	50.62	384400	765.49	0.323299	0.047771
C-12	0.0029	7	3280.03	1057.37	46.15	384400	755.69	0.322365	0.04365
C-18	0.0045	7	3229.52	971.13	45.54	384400	840.79	0.300705	0.046898
C-18	0.0048	7	3238.81	966.24	41.35	384400	792.20	0.298331	0.042793
C-20	0.0043	7	3250.61	949.31	31.26	384400	641.48	0.29204	0.032927
C-20	0.0048	7	3235.55	963.66	40.85	384400	792.31	0.297836	0.04239
C-22	0.0035	7	3124.91	906.21	40.03	384400	774.29	0.289994	0.044177
C-24	0.0034	7	3173.36	896.53	53.12	384400	836.17	0.282517	0.059255
C-26	0.0041	7	3104.86	899.13	32.88	384400	699.18	0.289589	0.036574

Appendix 3 (cont'd)

Sample Name	Otolith wt. grams	Sub Area	Na	K	Mg	Ca	Sr	K/Na	Mg/K
C-30	0.0040	7	3257.85	965.35	44.43	384400	1149.95	0.296314	0.046026
C-32	0.0041	7	3273.27	945.69	47.92	384400	789.18	0.288913	0.05067
C-32	0.0041	7	3271.32	937.46	48.48	384400	783.06	0.28657	0.051712
C-34	0.0037	7	3285.66	1003.68	47.18	384400	865.37	0.305472	0.047007
C-36	0.0040	7	3067.79	924.52	33.97	384400	719.43	0.301363	0.036746
C-38	0.0025	7	3309.60	1017.76	67.17	384400	1068.40	0.307516	0.065997
C-44	0.0030	7	3426.00	1006.35	44.12	384400	828.35	0.293738	0.043841
C-44	0.0032	7	3432.57	1006.09	43.30	384400	801.17	0.293101	0.043042
C-48	0.0027	7	3465.22	1058.77	69.57	384400	1028.81	0.305542	0.06571
C-50	0.0038	7	3267.79	926.94	35.81	384400	886.75	0.283659	0.038637
C-52	0.0041	7	3086.80	962.94	44.21	384400	872.59	0.311953	0.04591
C-54	0.0050	7	3155.69	850.01	30.13	384400	1018.31	0.269358	0.035441
C-56	0.0038	7	3372.64	1058.97	41.39	384400	1010.01	0.313989	0.039085
C-58	0.0032	7	3409.98	1048.29	52.69	384400	931.66	0.307418	0.050262
C-64	0.0029	7	3273.89	991.56	77.55	384400	895.19	0.302868	0.078207
C-68	0.0041	7	3322.34	974.62	44.15	384400	945.59	0.293354	0.045295
C-68	0.0041	7	3321.35	974.60	42.46	384400	948.42	0.293434	0.043571
C-70	0.0039	7	3228.28	904.92	34.54	384400	826.21	0.280309	0.038171
C-72	0.0034	7	3211.29	865.03	41.84	384400	832.04	0.269371	0.048369
C-72	0.0033	7	3203.64	864.83	40.03	384400	817.32	0.269953	0.046285
C-74	0.0031	7	3447.46	1024.62	42.33	384400	1088.57	0.29721	0.041316
C-78	0.0029	7	3359.60	891.20	59.45	384400	1443.37	0.265269	0.066703
C-80	0.0041	7	3160.27	861.09	38.05	384400	1069.47	0.272474	0.044191
C-84	0.0042	7	3196.67	948.10	38.07	384400	645.40	0.296591	0.040151
C-86	0.0024	7	3390.72	985.87	65.40	384400	924.20	0.290755	0.066335
C-88	0.0024	7	3454.24	987.96	64.08	384400	1193.94	0.286014	0.064864
C-90	0.0028	7	3448.97	956.82	46.01	384400	821.58	0.277422	0.048087
C-92	0.0029	7	3501.24	983.34	55.16	384400	928.91	0.280855	0.056095

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