USING OTOLITHS AS NATURAL TAGS TO STUDY STOCK STRUCTURE AND CONNECTIVITY OF ATLANTIC COD (*Gadus morhua*) IN NEWFOUNDLAND AND LABRADOR WATERS

GENEVIEVE D'AVIGNON

Using otoliths as natural tags to study stock structure and connectivity of Atlantic cod (*Gadus morhua*) in Newfoundland and Labrador waters

by

© Genevieve D'Avignon

A Thesis submitted to the

School of Graduate Studies

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Biology

Memorial University of Newfoundland

September 2013

St. John's Newfoundland

ABSTRACT

Atlantic cod (*Gadus morhua*) stock structure and dynamics are complex and remain a challenge to effective management. Otoliths have the ability to record the elemental signatures of a cod's environment. My research investigated the use of otolith chemistry to identify cod stocks within Newfoundland and Labrador waters, and to track cod movements and connectivity within and between stocks. Laser ablation inductively coupled mass spectrometer A-ICP-MS analyses provided precise and accurate measures for magnesium, mangnanesen, strontium , and barium concentrations. At large spatial scale, otolith elemental signatures and mean growth rate of fish were used to segregate cod into four spawning areas with 66-89% accuracy when the two closely related inshore sites were pooled. Elemental variations in the otoliths of cod in Newfoundland waters are associated with environmental and geographical conditions that enabled reassigning 89% of cod to their correct area at small spatial scale and the discrimination of inshore vs. offshore fish within the 3Ps stock complex. This study demonstrated that otolith chemistry shows great potential to identify the origin of cod and reconstruct inshore-offshore migrations, both important elements in managing fisheries.

ACKNOWLEDGEMENTS

I would like to thank everyone who contributed to the advancement of this project as every input is important and I could not have done it without your help. Thanks to my supervisor Dr George Rose who trusted me with a new project, welcomed my multiple questions, and supported me despite the difficulties. I consider myself very lucky to have worked alongside CFER staff (Ed, Wade, Gordon, Susan, Kiley, Tom, Juliana, Jennifer, Jonathan, and Sherrylynn) and students (Anna, Kate, Craig, Matt, Amin, Laura, Riley, Kyle, Victoria, and Darrell). You guys are a good crowd. You supported me throughout my research and offered your expertise and advice to help me when needed. Thanks to my committee members - Dr. Campana had the expert eye and his experience was instrumental to the success of this work while Dr Purchase brought a broader vision to the work at hand. I am also very grateful to Geoff Veinott and Ian Bradbury for helping with methodology, Clare Lewis for her help in the lab, and the CREAIT staff - Mike Tubrett, Jiggs, Glenn, and Rebecca for their supervision and advice with the LAICPMS. Thanks to: Pam King, Eugene Colbourne, Brian Gillett, Ryan Petty, Danny Boyce and Chris Canning for providing equipment, samples, lab material and expertise. Un gros merci à ma famille et amis qui étaient toujours là pour m'aider et m'encourager. Merci à Maxime qui ne m'a pas lâché malgré la distance et Sandrine qui m'a toujours encouragée.

Last but not least thanks to the Natural Science and Engineering Research Council of Canada, the Newfoundland and Labrador Department of Fisheries and Aquaculture, and Memorial University School of Graduate Studies for supporting this project.

Table of Contents

List of Tables

List of Figures

List of Symbols, Nomenclature or Abbreviations

CETAC: Canadian Education and Training Accreditation Commission

COSEWIC: Committee on the Status of Endangered Wildlife

CREAIT: Core Research Equipment and Instrument Training; A network provide major research equipment to Memorial University of Newfoundland researchers.

DFO: Department of Fisheries and Oceans of Canada

ICES: International Council for the Exploration of the Sea

FAO: Food and Agriculture Organization of the United Nation

FEBS 1: A sagittal otolith certified reference material procured from red snapper (*Lutjanus campechanus*) made by the National Research Council of Canada

LA-ICP-MS: Laser ablation inductively coupled plasma mass spectrometry

LAM-MAFIIC: Laser Microprobe laboratory of the Micro-Analysis Facility at Memorial University of Newfoundland.

NAFO: North Atlantic Fisheries Organisation

NIES 22: A sagittal otoliths certified reference material procured from the emperor red snapper (*Lutjanus sebae*) made by the National Institute for Environmental Studies

NIST: National Institute of Standards and Technology

NIST 612: Glass reference material produced by the National Institute of Standards and Technology (NIST)

NOAA: National Oceanic and Atmospheric Administration

NSERC: Natural Science and Engineering Research Council of Canada

MACS 1: Synthetic calcium carbonate reference material produced for micro-analysis by the United States Geological Survey

MPV: Most probable value. Notation used to report the mean value of MACS 1 measured for each element at the laser microprobe laboratory of Memorial University.

RSD: Relative Standard Deviation

USGS: United States Geological Survey

List of Appendices

Introduction

The understanding of population structure and connectivity of a marine species is essential for effective management (Clarke et al., 2009). In recent years, the idea that marine populations are open and widely connected has been increasingly questioned as some studies are showing clear evidence of homing behaviour (Robichaud, 2001; Robichaud and Rose, 2004) or isolation of groups of fish within a population due to separated spawning events (Beacham et al., 2002; Gibb and Gibb, 2007) .

Several methods can be employed to study connectivity. Genetic approaches are usually favorable to distinguish the different populations of a given species by looking at allele frequency or using mitochondrial DNA to clarify relationships among stock components (Ruzzante et al. 1999; 2001). However, non-genetic tags are better suited when fish movement is more frequent and over a small spatial scale (Thorrold et al., 2001), or for fish that are separated for a portion of their lives and return to spawn together (Elsdon et al. 2008). Artificial tags (satellite, PIT, implants, hydrostatic, etc.) are popular but expensive. Most require fish to survive until recapture and must have a good return rate to be cost-efficient and yield an adequate sample size (Fairclough et al., 2011). Natural tagging using scales, bones and otoliths is becoming more popular because they are easy to collect and can provide valuable information from a large number of fish and reveal spatial structure undetectable with other methods (Edmonds et al., 2001; Steer et al., 2009).

Recent reviews suggest otoliths are particularly good candidates to study connectivity because their chemical properties make them natural temporal and spatial data loggers (Elsdon et al., 2008; Campana and Casselman, 1993). Elements and isotopes are chronologically incorporated into the otolith according to the fish condition and environment (Campana, 1999; Elsdon et al., 2008). Fish sharing similar life histories and remaining with their respective spawning group should possess a similar otolith chemical composition (Campana and Gagne, 1995). The analysis of the chemical composition stored in the increments of an otolith can help identify spawning groups (Thorisson et al., 2011; Sévigny et al., 2009). In comparison with other tagging methods, otoliths can successfully distinguish fish groups that are not genetically different because they vary according to the environment and condition of fish (Campana et al., 1999). Thus, otolith composition has great potential to answer questions of migration, stock composition and connectivity.

Atlantic cod (*Gadus morhua*) has played an important role in the history, culture and economy of Eastern Canada. The northern cod fishery produced annual catches of over 200 000 tonnes from 1880-1960 (Hannesson, 1996). The arrival of large factory trawl ships along with changes in ocean ecosystem and climate conditions, led to rapid and catastrophic declines in cod numbers forcing the closing of the fisheries from Cape Cod to Newfoundland along the northeast coast of North America in the early 1990s (Rose, 2007). Though many fisheries have suffered the same fate, the collapse of the cod fisheries is recognized as one of the most devastating (Grafton et al., 2009) and a much cited example of failed fisheries management. It caused thousands of fishermen and

workers in the fish-processing industry to lose their jobs. To this day, cod populations in the Northwest Atlantic are classified as endangered by the Committee on the Status of Endangered Wildlife (COSEWIC; Schiermeier, 2003; COSEWIC, 2010). The collapse and slow recovery of the depleted cod stocks is attributed in part to reductions in population spatial distribution and connectivity (Atkinson et al., 1997; Rose, 1997). In the last few decades, changes in the population structure and dynamics of cod have been observed throughout the Newfoundland and Labrador region making it difficult to assess the state of the stocks.

Genetic studies revealed small scale population structure exist in Newfoundland and Labrador cod (Ruzzante et al., 1998; Ruzzante et al., 1999; Beacham et al., 2002) and lately, researchers propose they act as metapopulations (Smedbol and Wroblewski, 2002). Although they constitute different groups with different natural histories they can still interact and reproduce amongst themselves (Smedbol and Wroblewski, 2002) therefore connectivity between breeding groups may be crucial for maintaining stock health. Concepts of population ecology like "source and sink dynamic" (Pulliam 1988) and the "portfolio effect" (Tilman, 1996; Doak et al., 1998) described by Rose et al. (2011) for Atlantic cod may come into play where highly productive spawning groups can help repopulate other grounds. Effective management strategies to rebuild Newfoundland Atlantic cod (*Gadus morhua*) stocks therefore depend on a clear understanding of population structure, connectivity, and migration patterns of spawning aggregations contributing to the fisheries, and finding a reliable indicator to delineate cod groups.

Considering gene flow is not restricted among all spawning aggregations of cod in Newfoundland and Labrador waters, genetic markers are not as effective on their own to segregate spawning aggregations and study connectivity; two aspects of importance for rebuilding cod stocks. Despite extensive studies of Atlantic cod, the use of otoliths as a natural tagging mechanism has not been assessed along the Northeastern coast of Newfoundland. The purpose of this project was to assess the potential of using otoliths as natural tags to study connectivity among Newfoundland and Labrador spawning groups. My objectives were to measure and discriminate the otolith signatures of cod spawning groups at large, and small, spatial scales and to assess efficacy to determine fish movements and interactions.

Thesis Overview

This thesis examines the use of otoliths as natural tags to help distinguish cod spawning groups and assess the success of the method to study stock structure and connectivity between Newfoundland Atlantic cod groups. In the first chapter of the thesis, the theoretical approach for natural tagging using otoliths is reviewed and important considerations in its applications in fisheries science are outlined. In the second chapter, the precision and accuracy of the laser ablation inductively coupled plasma mass spectrometry system of the CREAIT laboratory at Memorial University is evaluated and its ability to detect the presence of nine commonly-used trace elements in otolith microchemical research is discussed. The results ensure the quality of the analyses and provide data to compare the elemental ratios quantified in Newfoundland and Labrador cod otoliths to similar otolith natural tagging studies in other marine ecosystems. In the third chapter, the most successful elemental ratios are used to distinguish four spawning aggregations of Newfoundland and Labrador cod at large spatial scales. Spatial and temporal variations in elemental ratios are examined to determine fingerprint stability and assess its success in discriminating groups and revealing past movements and connectivity. In the last chapter, the 3Ps NAFO division is used as a case study to investigate within-stock variability in otolith signature to determine whether elemental ratios can help track fish movements at smaller spatial scales.

Co-authorship Statement

I am the principal author of all research papers presented in this thesis. I was responsible for the planning, design, data analysis, and manuscript preparation. Dr. George A. Rose is second author on all papers, and provided conceptual suggestions and aid during all stages of the thesis, including editorial comments on previous drafts.

Publication and submission status:

Chapter 3 (D'Avignon and Rose) has been accepted for publication and will appear in the October 2013 issue of *Fisheries Research*

Chapter 4 (D'Avignon and Rose) will be submitted as a short communication in *Fisheries Research* or a similar journal

.

Chapter 1: Otoliths as natural tags in fisheries research

INTRODUCTION

The Atlantic cod (*Gadus morhua*) was an important player in the economy of eastern Canada and the collapse of its stocks is recognized as one of the most devastating failed fisheries management(Grafton et al., 2009). The collapse and slow recovery of the depleted cod stocks is attributed in part to the unintended exclusion of population distribution, dynamics, and connectivity from the management schemes of the time (Atkinson et al., 1997; Rose, 1997). This omission results from the challenge associated with measuring connectivity in species with complex stock structure (Smedbol and Wroblewski, 2002). Cod spread over large geographical scales, exploit different environments at each life stage (Bradbury et al., 2008), and mature fish migrate and mix with spawners of adjacent stocks - making assessments difficult.

In recent years, there has been increasing interest by international organizations (United Nation, FAO 2003; 2013; the International Council for the Exploration of the Sea, ICES 2012; 2013, National Oceanic and Atmospheric Administration, NOAA, 2013) and government agencies (United States, Busch et al., 2003; Canada, DFO 2013) to promote and favour an ecosystem-based approach to fisheries management. Achieving sustainable fisheries management requires a good understanding of the stock structure both spatially and how it evolves through time. Knowledge about connectivity among spawning groups

as well as interactions with and influences of the environment are also fundamental for efficient recovery of depleted stocks, like Atlantic cod.

The field of otolith chemistry has developed rapidly due to its potential for elucidating questions almost impossible to resolve in marine environments and has produced nearly 140 research publications in the last two years alone (Web of science). Among its uses, the advent of new technologies has permitted otoliths to become suitable natural markers to discriminate fish spawning groups that are not necessarily genetically distinguishable (Campana et al., 1999), identify the origin of fish in a mixed fishery (Campana et al., 1999; Jónsdóttir et al., 2007), and track movement of migrant species (Stransky et al., 2005; Morais et al., 2011).

The purpose of this work was to investigate the potential use and limitations of otolith chemistry as a natural tagging mechanism to distinguish Newfoundland and Labrador cod spawning groups. Information from these natural tags can provide insights on stock structure and distribution dynamics for rebuilding Atlantic cod stocks, an important economical asset in Atlantic regions.

Otoliths as Natural Markers

Otoliths (ear stones) are three small paired calcified structures in the inner ear canal of teleost fish that serve for balance (Campana, 2004) andhearing (Popper and Lu, 2000). Each pair has a different morphology. In adult fish, the sagitta is usually the largest

located near the astericus, and the last, the lapillus is the smallest. The size and shape of the otolith is species specific (L'Abée-Lund, 1988) and influenced by sex, age, year class, stock, and environment (Castonguay et al., 1991; Lombarte and Lleonart, 1993; Begg and Brown, 2000). Otolith shape and size differs amongst species and change according to the growth rate of the structure. In cod, the sagittal otolith is relatively large, making it easier to handle and use as a natural tag.

Natural tagging using genetics, scales, bones and otoliths have shown great successes in answering life history questions about important fish species. Otoliths are comparable to an airplane black box (Ben-Tzvi et al., 2007) because they can record temporal and spatial information permanently (Campana 1999; Elsdon et al., 2008). This is attributed to two general properties. First, they are acellular and metabolically inert so metabolic processes and fish health will not interfere with the material already deposited within the otolith. Second, otoliths are formed by continuous incorporation of isotopes to its calcium carbonate structure to creating a chronological record of fish condition and ambient environment conditions (Campana, 1999; Elsdon et al., 2008).

Considering that otoliths are present in the fish from the time of hatch to death and grow incrementally, it is possible to associate the composition of an increment to a specific event in the life of the fish. The capacity of otoliths to hold time-resolved lifetime environmental histories makes them a valuable tool to track individual fish in both space and time (Campana, 1999; Sturrock et al., 2012). Assuming spawning sites and habitats along their migration route consist of different physicochemical properties, it can be

possible to match otolith composition with these environments and reconstruct a time series of the areas visited by the fish during its lifetime (Campana, 1999). Similarly, elemental and isotopic fingerprinting can be applied to distinguish geographically separated groups and assess the degree of mixing between them (Campana and Gagne, 1995; Turan, 2006; Jónsdóttir et al., 2007).

Composition

Otoliths are almost pure calcium carbonate ($CaCO_3$) but also contain a variety of major, minor (>100ppm) and trace (<100ppm) elements (Campana, 1999). Calcium carbonate can be found under several crystal structures, the most common in otoliths being aragonite and vaterite (Brown and Severin, 1999; Melancon et al., 2005; Veinott et al., 2009). Both have the same initial chemical composition with 38-39% per weight of calcium (Campana 1999). Though pure aragonite otoliths are most common, otoliths can also exist as purely vaterite or a combination of both polymorphs (Melancon et al., 2005).

For studies using otoliths as natural tags, it is best to select otoliths of one pure polymorph because trace elements are not incorporated in the same proportions in aragonite and vaterite (Melancon et al., 2005; Veinott et al., 2009). In addition, vateritic portions of otoliths do not produce clear concentric rings in the sagittae of cod, causing difficulties in ageing fish. Correct ageing provides invaluable data on life history and can help reconstruct movements of fish at specific times in their lives when increments can accurately be related to a given season or year (Elsdon et al., 2008). Thus, the sagittae otolith of cod is most commonly used by researchers (Campana, 1999).

Elemental incorporation

The presence of elements other than calcium carbonate in the otolith matrix is a result of impurities and are thought to be incorporated to the otolith as a substitute for calcium (Sr, Mg, Li, Ba), by inclusion in the interstitidial space of the crystal, or as an association to the proteinaceous matrix (Campana, 1999). Though many hypotheses (elasticity, kinetic and physiological influences) have been proposed to explain incorporation mechanisms (Sturrock et al., 2012), the regulation of otolith elemental incorporation remains fundamental to understand how to use otolith microchemistry as natural tags.

Otolith calcification is a stepwise process: the elements are absorbed from the surrounding water and make their way through the blood plasma via the gills or intestine (Olsson et al., 1998). Given that otoliths are located in the inner ear, the elemental composition will depend on the availability of the elements in the water and diet, and the ability of these elements to move across four interfaces: environment-blood, blood-blood binding proteins, blood endolymph and endolymph-otolith (Sturrock et al., 2012). Some elements are physiologically discriminated against and rarely taken up by the fish (see Campana 1999). As a consequence, elements in the otolith will not necessarily be incorporated in proportion to their availability in the environment (Campana, 1999; Elsdon and Gillanders, 2003). Many environmental factors – pH, dissolved oxygen (Campana et al., 1999), temperature (Fowler et al., 1995; Bath et al., 2000), salinity (Elsdon, 2002; Martin and Thorrold, 2005), and biological factors- growth rate (Clarke and Friedland, 2004), diet (Buckel et al., 2004), ontogeny (Walther et al., 2010), and reproduction (Kalish, 1991) can influence elemental uptake.

Over 50 elements have been found in otoliths (Sturrock et al., 2012), but not all elements are valid indicators of the environment inhabited by the fish. For example, the concentrations of Ca, Na, K, Mg, and Cl differ significantly between freshwater and marine environments but this variation is not reflected in the otolith (Campana, 1999). Yet, it can still be possible to use these elements to differentiate fish among geographical locations if their concentrations are affected by other, spatially explicit factors (eg. temperature), and obtain information about the environmental conditions in which they lived. For example, Bath et al. (2000) demonstrated that elemental concentrations of Sr and Ba in spot croaker (*Leiostomus xanthurus*) reflect environmental conditions in pure aragonite otoliths. Other elements like Hg and Pb are associated with anthropogenic activities and therefore are more likely to exhibit higher concentrations near land or sources of runoff. The understanding of incorporation mechanism can explain the presence of specific elements in otoliths and help to identify movements of fish areas across areas or be used as a natural marker for cod aggregations.

THE EFFECT OF ABIOTIC AND BIOLOGICAL FACTORS

Ontogeny and diet

The formation of the otolith is influenced by the growth of the fish, so biological factors such as growth rate (Sadovy and Severin, 1994; de Pontual et al., 2003), diet (Walther and Thorrold, 2006), maturity and age (Kalish, 1989), can all affect the composition of the otolith. Fortunately, some of these factors can be controlled for and removed, at least

statistically, to avoid confounding interpretations of location signatures with differences in ontogeny. Dietary sources of elements are rarely examined but can cause changes in the concentration of certain elements in the otolith. For example, Zn concentration in otoliths of pink snapper consuming a Zn-enrich diet was higher than that of control fish (Ranaldi and Gagnon, 2008). Nonetheless, as long as fish with similar behaviour and ontogeny aggregate together, differences between individuals should not exceed differences among groups, thus their signature would enable discrimination among groups and movements over extreme environments.

Temperature

A review of the otolith literature revealed that the presence of some elements such as Sr, Ba (Elsdon and Gillanders, 2004) K, Na (Hoff and Fuiman, 1995), Mn (Miller, 2009), Mg, Zn and Fe (Campana, 1999) can be influenced by changes in temperature (Sturrock et al., 2012). In many controlled laboratory settings, the otolith concentration of Sr (Kalish, 1989; Martin et al., 2004) and Ba (Bath et al., 2000; Elsdon, 2002; DiMaria et al., 2010) increased with increasing temperature when water temperature tested varied from 12-28°C. However, water temperatures in these studies are well above those found in Newfoundland; therefore the trends observed may not be applicable in natural cold water environments. In fact, the effect of temperature on cold-water species experiencing sub-zero temperatures have been reported to be opposite to warm-water dwelling species (Bath et al., 2000; Elsdon, 2002; Martin et al., 2004), with several studies showing

negative influences of temperature on otolith concentrations (Townsend et al., 1992; Townsend et al., 1995).

In addition, the Sturrock et al. (2012) review of several experiments testing this effect suggested that relationships varied among species and interactions were found amongst factors such as temperature, growth rate and reproduction. Chemical analyses may not detect the true influence of each factor separately. This was demonstrated by a laboratory study on damselfish showing life history stage, temperature and food quantity interacted in their effect on the incorporation of Ba, while life history stage and food influenced the otolith Sr concentrations (Walther et al., 2010). Experiments testing how such factors (life history stage, temperature, food, fish growth, etc.) affect otolith elemental incorporation and how they interact with temperature are still needed to understand the presence of elements in cod otoliths.

Water chemistry and depth

Differences in chemistry have been observed between marine and freshwater environments and enabled fisheries biologists to reconstruct migration patterns of anadromous fish species (Thibault et al., 2010). For marine species, the uptake of spiked ambient water contributed to 83% of the Sr and 98% of the Ba concentrations found in the otoliths of juvenile fish, suggests the presence of these elements in the otolith are related to their abundance in the surrounding water (Walther and Thorrold, 2006; Martin and Thorrold, 2005). Variations in Sr:Ca can therefore be very powerful to detect changes

in salinity between extreme environments and plays a key role in predicting fish movements across salinity gradients (Elsdon, 2002; Martin and Thorrold, 2005).However, the potential for Sr:Ca changes to be driven by physiology in adult fish (Kalish, 1991; Brown and Severin, 2009) need to be investigated further.

Other elements like Ba, zinc (Zn) and cadmium (Cd) tend to exhibit a nutrient-type profile. Nutrients tend to become depleted near the surface and enriched with increasing depth, therefore these elements can help determine the depth at which fish lived for a given time. Depth may be an important factor to consider in otolith studies such as this, because cod are known to exploit different portions of the water column at different times of the year in the pre-spawning, spawning and post-spawning periods (Hussy et al., 2009) and according to their life stage (Rose, 2007). Eggs and larvae are pelagic, drifting under the influence of wind and currents (Pepin and Heibig, 1997; Bradbury et al., 2000). Juveniles less than 2 years of age are initiallypelagic then settle near the bottom, becoming increasingly pelagic in their habits as they grow. Finally at or near maturity, cod form spawning and migrating shoals that move between benthic and pelagic habitats to feed and reproduce (Rose, 2007). The exploitation of different locations and depths means that cod are exposed to different water chemistry, salinity and temperature throughout their lifetime. Significant changes in chemical concentration due to depth may confound interpretation of signatures in earlier life history of the. Consequently a change in depth may be correlated with several factors, both environmental and physiological, and could mask their effects. Untangling synergistic effects of multiple factors is complex

(Sturrock et al., 2012), especially when incorporation mechanism vary within two populations of the same species (Clarke et al., 2011).

Interaction among factors appears to be common (Secor et al., 1995; Elsdon, 2002) even in laboratory settings, thus it may be difficult to identify their effects in a variable and uncontrolled environment. However, a good understanding of the effects of abiotic and biotic factors, and their interactions is necessary before attempting to use otolith chemical signatures as natural tags to track movement of fish (Walther et al., 2010). In addition, a better understanding of element incorporation is necessary to identify potentially confounding changes in the signature that may accrue as a consequence of changes in physiology, life stage, depth, or temperature.

CONCLUSION

Otolith chemistry has been used as a natural tagging technique quite effectively in the last decade, and while its success is encouraging, some problems remain. Though otolith signatures of highly aggregated groups of fish can be distinguished; further controlled experiments are needed to understand why they differ. To achieve this, laboratory experiments need to test the interactions of physiochemical properties for fish of all ages and at each life stages. An ideal natural tag would include only trace elements that reflect environmental abundance, to provide a means to track fish based upon the environment at the location of the aggregation. For studies interested in group discrimination, an understanding of the processes governing elemental incorporation is not as important as for studies looking at smaller scale migration and movements. The greater complexity

involved with identifying a change in environment or individual condition, from a change in location involves a comprehensive understanding of element incorporation, the relevant physiochemical environments, and mechanisms and collection of more extensive datasets.

REFERENCES

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

Begg, G.A., Brown, R.W., 2000. Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. Trans. Am. Fish. Soc. 129(4), 935-945.

Ben-Tzvi, O., Abelson, A., Gaines, S. D., Sheehy, M. S., Paradis, G. L., Kiflawi, M., 2007. The inclusion of sub-detection limits LA-ICPMS data, in the analysis of otolith microchemistry, by use of a palindrome sequence analysis (PaSA). Limnol. Oceanogr. 5, 97-105.

Bradbury I., Snelgrove P., Fraser S., 2000. Transport and development of eggs and larvae of Atlantic cod, Gadus morhua, in relation to spawning time and location in coastal Newfoundland. Can. J. Fish. Aquat. Sci. 57(9), 1761-1772.

Brown, R., Severin, K., 1999. Elemental distribution within polymorphic inconnu (*Stenodus leucichthys*) otoliths is affected by crystal structure. Can. J. Fish. Aquat. Sci. 56(10), 1898-1903.

Buckel, J., Sharack, B., Zdanowicz, V., 2004. Effect of diet on otolith composition in *Pomatomus saltatrix*, an estuarine piscivore. J. Fish Biol. 64(6), 1469-1484.

Busch, W.-D.N., B.L. Brown, and G.F. Mayer (Eds). 2003. Strategic Guidance for Implementing an Ecosystem-based Approach to Fisheries Management. United States Department of Commerce, National Oceanic and Atmospheric Administration, NMFS, Silver Spring, MD 62p.

Campana, S.E., 2004. Photographic atlas of fish otoliths of the Northwest Atlantic Ocean. National Research Council Canada, Ottawa

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

Campana, S.E., Gagne, J.A., 1995. Cod stock discrimination using ICPMS elemental assays of otoliths, in: Secor, D.H., Dean, J.M., and Campana, S.E. Recent Developments in Fish Otolith Research.University of South California Press, Columbia, SC. pp: 671-691.

Campana, S.E., Casselman, J.M., 1993. Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. 50(5), 1062-1083.

Campana, S.E., Chouinard, G.A., Hanson, J.M., Fréchet, A., 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(10), 1873-1881.

Castonguay, M., Simard, P., Gagnon, P., 1991. Usefulness of Fourier-analysis of otolith shape of Atlantic mackerel *Scomber scombrus* stock discrimination. Can. J. Fish. Aquat. Sci.. 48(2), 296-302.

Clarke, L.M., Friedland, K.D., 2004. Influence of growth and temperature on strontium deposition in the otoliths of Atlantic salmon. J. Fish Biol. 65(3), 744-759.

Clarke, L.M., Thorrold, S.R., Conover, D.O., 2011. Population differences in otolith chemistry have a genetic basis in *Menidia menidia*. Can. J. Fish. Aquat. Sci. 68(1), 105-114.

de Pontual, H., Lagardière, F., Amara, R., Bohn, M., Ogor, A., 2003. Influence of ontogenetic and environmental changes in the otolith microchemistry of juvenile sole (Solea solea). J. Sea Res. 50, 199-211.

DFO., 2013. Principles of ecosystem-based fisheries management. Available at http://www.dfo-mpo.gc.ca/fm-gp/peches-fisheries/fish-ren-peche/sff-cpd/ecosys-back-fiche-eng.htm. Accessed on July 23, 2013.

DiMaria, R.A., Miller, J.A., Hurst, T.P., 2010. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. Environ. Biol. Fishes 89(3-4), 453-462.

Elsdon, T., 2002. Interactive effects of temperature and salinity on otolith chemistry: Challenges for determining environmental histories of fish. Can. J. Fish. Aquat. Sci. 59(11), 1796-1808.

Elsdon, T.S., Gillanders, B.M., 2004. Fish otolith chemistry influenced by exposure to multiple environmental variables. J. Exp. Mar. Biol. Ecol. 313(2), 269-284.

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

Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor, D.H., Thorrold, S.R., Walther, B.D., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Annu. Rev. 46, 297-330.

FAO Fisheries Department.The ecosystem approach to fisheries. FAO Technical Guidelines for Responsible Fisheries. 4, Suppl. 2. Rome, FAO. 2003. 112 p.

FAO.2013. The ecosystem approach to fisheries management. Fisheries and Aquaculture Department. Available at: http://www.fao.org/fishery/topic/13261/en. Accessed on July 23, 2013.

Fowler, A., Campana, S., Jones, C., Thorrold, S., 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS. Can. J. Fish. Aquat. Sci. 52(7), 1421-1430.

Hoff, G., Fuiman, L. 1995. Natural variation in elemental composition of sagittae from Red drum. J. Fish Biol. 47(6), 940-955.

Hussy, K., Nielsen, B., Mosegaard, H., Clausen, L.W., 2009. Using data storage tags to link otolith macrostructure in Baltic cod *Gadus morhua* with environmental conditions. Mar. Ecol. Prog. Ser. 378, 161-170.

ICES. 2012. ICES 2012 Annual Science Conference, Bergen, Norway.

ICES.2013. Report of the Working Group on Working Group on the Northwest Atlantic Regional Sea (WGNARS), 28 January – 1 February 2013, Dartmouth, Canada. ICES CM 2013/SSGRSP:03. 108 pp.

Jónsdóttir, I.G., Marteinsdottir, G., Campana, S.E., 2007. Contribution of different spawning components to the mixed stock fishery for cod in Icelandic waters. ICES J. Mar. Sci. 64(9), 1749-1759.

Kalish, J.M., 1989. Otolith microchemistry validation of the effects of physiology age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132(3), 151-178.

Kingsford, M., Gillanders, B., 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. Mar. Biol. 137(3), 403-414.

L'Abée-Lund, J., 1988. Otolith shape discriminates between Atlantic salmon, *Salmo salar* L, and brown trout, *Salmo trutta* L. J. Fish Biol. 33(6), 899-903.

Lombarte, A., Lleonart, J., 1993. Otolith size changes related with body growth, habitat depth and temperature. Environ. Biol. Fishes. 37(3), 297-306.

Martin, G.B., Thorrold, S.R., 2005. Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile Spot *Leiostomus xanthurus*. Mar. Ecol. Prog. Ser. 293, 223-232.

Martin, G.B., Thorrold, S.R., Jones, C.M., 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). Can. J. Fish. Aquat. Sci. 61(1), 34-42.

Melancon, S., Ludsin, S., Gagnon, J., Yang, Z., 2005. Effects of crystal structure on the uptake of metals by lake trout (*Salvelinus namaycush*) otoliths. Can. J. Fish. Aquat. Sci. 62(11), 2609-2619.

Miller, J.A. 2009. The effects of temperature and water concentration on the otolith incorporation of barium and manganese in Black rockfish *Sebastes melanops*. J. Fish Biol. 75(1), 39-60.

Morais, P., Dias, E., Babaluk, J., Antunes, C., 2011. The migration patterns of the European flounder *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae, Pisces) at the southern limit of its distribution range: Ecological implications and fishery management. J. Sea Res. 65(2), 235-246.

NOAA., 2013. Ecosystem Science. Available at: http://www.st.nmfs.noaa.gov/ecosystems/index . Accessed on July 23, 2013.

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

Pepin, P, and Helbig, J.A. 1997. Distribution and drift of Atlantic cod (Gadus morhua) eggs and larvae on the northeast Newfoundland shelf. Can. J. Fish. Aquat. Sci. 54: 670-685.

Ranaldi, M.M., Gagnon, M.M., 2008. Zinc incorporation in the otoliths of juvenile pink snapper (Pagrus auratus Forster): The influence of dietary and waterborne sources. J. Exp. Mar. Biol. Ecol. 360, 56-62.

Rose, G.A., 2009. Variations in the target strength of Atlantic cod during vertical migration. ICES J. Mar. Sci. 66(6), 1205-1211.

Rose, G.A., 2007. Cod : The ecological history of the north Atlantic fisheries. Breakwater Books, St. John's, Nfld.

Rose, G.A., Nelson, R.J., Mello, L.G.S., 2011. Isolation or metapopulation: whence and whither the Smith Sound cod? Can. J. Fish. Aquat. Sci. 68(1), 152-169.

Sadovy Y., Severin K., 1994. Elemental patterns in Red hind (*Epinephelus guttatus*) otoliths from Bermuda and Puerto Rico reflect growth-rate, not temperature. Can. J. Fish. Aquat. Sci. 51, 133-141.

Secor, D.H., Henderson-Arzapalo, A., Piccoli, P.M., 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J. Exp. Mar. Biol. Ecol. 192(1), 15-33.

Stransky, C., Garbe-Schoenberg, C. Guenther, D., 2005. Geographic variation and juvenile migration in Atlantic redfish inferred from otolith microchemistry. Mar. Fresh. Res. 56(5), 677-691.

Sturrock, A., Trueman, C., Darnaude, A., Hunter, E. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? J. Fish Biol. 81(2), 766-795.

Thibault, I., Hedger, R.D., Dodson, J.J., Shiao, J., Iizuka, Y., Tzeng, W., 2010. Anadromy and the dispersal of an invasive fish species (*Oncorhynchus mykiss*) in eastern Quebec, as revealed by otolith microchemistry. Ecol. Freshwat. Fish. 19(3), 348-360.

Townsend, D., Radtke, R., Malone, D., Wallinga, J., 1995. Use of otolith strontium-calcium ratios for hindcasting larval cod *Gadus morhua* distributions relative to water masses on Georges-bank. Mar. Ecol. Prog. Ser. 119(1-3), 37-44.

Townsend, D., Radtke, R., Corwin, S., Libby, D., 1992. Strontium-calcium ratios in juvenile Atlantic herring *Clupea harengus* L. otoliths as a function of water temperature. J. Exp. Mar. Biol. Ecol. 160(1), 131-140.

Turan, C., 2006. The use of otolith shape and chemistry to determine stock structure of Mediterranean Horse mackerel *Trachurus mediterraneus* (Steindachner). J. Fish Biol. 69(Suppl. C), 165-180.

Veinott, G.I., Porter, T.R., Nasdala, L., 2009. Using mg as a proxy for crystal structure and Sr as an indicator of marine growth in vaterite and aragonite otoliths of aquaculture Rainbow trout. Trans. Am. Fish. Soc. 138(5), 1157-1165.

Walther, B., Thorrold, S., 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. Mar. Ecol. Prog. Ser. 311, 125-130.

Walther, B.D., Kingsford, M.J., O'Callaghan, M.D., McCulloch, M.T., 2010. Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. Environ. Biol. Fishes. 89(3-4), 441-451.

Windle, M.J.S., Rose, G.A., 2007. Do cod form spawning leks? Evidence from a Newfoundland spawning ground. Mar. Biol. 150(4), 671-680.

Chapter 2: Assessing precision and accuracy of LA-ICP-MS in detecting trace elements in Atlantic cod otoliths

ABSTRACT

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has recently become the preferred method in fisheries research to access the life-history information stored in the growth increments of fish otoliths. Otolith chemical signatures can be used to understand population structure and connectivity of Atlantic cod (*Gadus morhua*) and help in the rebuilding of stocks. The use of LA-ICP-MS for detecting trace elements at

the edge of cod otoliths was evaluated and the analytical precision and accuracy of these elements were assessed and compared to other studies. Among the nine elements analysed, Mg, Mn, Cu, Sr, and Ba had 50% of scans above the detection limit and good precision (RSD $\leq$10%). Accuracy was within 7% of the most probable value for Cu, Sr, and Ba in MACS 1, but poor for lighter elements with concentrations of Mn falling 16% below MPV and overestimating Mg by 106%. Results show that Mg, Mn, Cu Sr and Ba are good candidates for elemental fingerprinting of spawning groups of cod in Newfoundland and Labrador, Canada.

Keywords: *LA-ICPMS, Precision, Accuracy, Limit of detection, Gadus morhua*

INTRODUCTION

In the last few decades, fisheries biologists have used sophisticated geochemical techniques to assess the properties of the otolith, a stone from the ear of teleost fish that records and stores elements and isotope ratios from the ambient environment in its growth increments (Campana, 1999). Different factors- ontogeny, geography, fish condition- may influence the presence of elements within the otolith (Campana, 1999; Gillanders, 2002a; Sturrock et al., 2012), but fish experiencing similar conditions tend to exhibit similar elemental signatures.

In various studies, otolith chemistry has revealed fish migration patterns and movements (Thibault et al., 2010; Campana et al., 1999; Thorrold et al., 1997), stock structure and connectivity (Fairclough et al., 2011; Bergenius et al., 2005; Gillanders, 2002b), and identified spawning and nursery sites (Vasconcelos et al., 2007; Thorrold et al., 1998). The composition of Atlantic cod (*Gadus morhua*) otoliths has the potential to serve as a valuable tool to understand connectivity patterns and help rebuild stocks in Newfoundland.

In the past, several microprobe techniques have been used to study otoliths: electron microprobe (Thibault et al., 2010; FitzGerald et al., 2004; Secor, 1992), proton-induced X-ray emission (PIXE; Syedang et al., 2010; Morris et al., 2003; Campana et al., 1997), inductively coupled plasma atomic emission spectrometry (ICP-AES; Turan, 2006; Begg et al., 1998; Kalish et al., 1996), inductively coupled plasma mass spectrometry (ICPMS)

by laser-ablation (LA; Bradbury et al., 2011; Campana et al., 1994), solution-based (SB; Vasconcelos et al., 2007; Gillanders and Kingsford, 1996) or isotope dilution methods (ID; Campana et al., 2000; Thorrold et al., 1998). However, recent work has demonstrated that measurements of otolith composition are sensitive to the methodology, instrumentation, laboratory and elements sampled (Geffen et al., 2013).

In order to use otoliths as natural tags of Newfoundland cod, it is important to choose an appropriate method to reliably quantify the elemental composition of an increment within the otolith and evaluate sensitivity, precision and accuracy of the results. LA-ICP-MS takes advantage of the chronological properties of the otolith and can quantify trace element concentrations at a very fine scale, allowing analysis of a specific period in the life of a fish - as short as a week depending on its size and growth rate (Jones and Chen, 2003). It is an ideal method for species like Atlantic cod that have a large lifetime geographical range and move to different environments at each life stage.

The goal of this research was to determine what trace elements can be quantified precisely and accurately in Atlantic cod otoliths captured in four areas around Newfoundland and Labrador using LA-ICP-MS.

METHODS

Sampling

Nearly 30 000 cod were captured between 1996 and 2003 as part of acoustic-trawl cod survey and research led by the NSERC Chair in Fisheries Conservation at the Marine Institute of Memorial University onboard several research vessels, mostly the *CCGS Teleost* and *CCGS Shamook*. Spawning aggregations of cod located with echo sounders were sampled with handlines or a bottom trawl, depending on depth and bathymetry. For each cod sampled, the date, weight (whole, gutted, stomach, liver, and gonad), length, sex, maturity stage, gill parasites were recorded. Otoliths were removed from the fish and stored dried for age determination by a professional reader. Among them, cod caught during the spawning period in 1998 and 1999 (April to July) on four well-established spawning areas (inshore and offshore) off Newfoundland and Labrador in Canada (Fig. 2.1). Cod growth (Fig. 2.2) and age-at-maturity (Fig.2.3) differs across sites. Hawke Channel fish are known reach smaller sizes than other southern spawning groups of cod (Fudge and Rose, 2008) and few fish were captured over the age of eight. Hawke Channel cod matures early, with 70% of cod being mature at four years (Fig. 2.3). Cod captured offshore in the 3Ps area were the last to reach maturity between the age of five and six (Fig. 2.3). Fish ranging between the age of four and seven years old were selected for otolith analysis (n=721) because the majority of fish captured were mature at this age, though some from HC and O3HC were immature. Otoliths from immature fish were kept in the analysis because they appear to follow the movements of the spawning aggregation and should have the same chemical signature.

Sample preparation

LA-ICP-MS detects trace elements at the microscale, hence is highly sensitive to contamination. The otoliths collected were cleaned of adhering tissues by brushing the surfaces with an acid-washed toothbrush and decontaminated by sonification in multiple baths of distilled, deionized and reverse-osmosis water (Campana et al., 2000). To expose annual growth rings, otoliths were weighed to the nearest 0.1 mg and embedded in epoxy (Buehler Epothin resin and hardener) before being sectioned transversely across the core using a Buehler Isomet slow-speed diamond-blade saw. Extra epoxy around the sections was removed and the sections were polished by hand with 30 μm then 3 μm lapping paper. To remove all possible residues left by the polish, all sections were rinsed and sonified in a series of baths as before. Sections were mounted on petrographic glass slides prior to LA-ICP-MS analysis (see Appendix I for a complete list of material and procedures).

Otolith analysis

A GEOLAS 193 nm excimer laser system was coupled to a Finnigan ELEMENT XR, a high resolution double-focusing magnetic sector inductively coupled plasma mass spectrometer, to analyze the charged particles released by the ablation of the otoliths (see Appendix II for details on the system used and calibrations). All LA-ICP-MS analyses were done at the Laser Microprobe Laboratory of the Micro-Analysis Facility (LAM-MAFIIC) at Memorial University of Newfoundland. A helium flow rate of 0.9 to 1.0 l/min was used to carry ablated material from the ablation cell to the ICP and an additional 0.75 l/min of argon gas was added after the ablation cell. Time resolved intensity data were acquired by peak-jumping in a combination of pulse-counting and

analog mode, depending on signal strength, with one point measured per peak for masses. The ICP-MS was tuned each day for maximum sensitivity using the National Institute of Standards and Technology (NIST) 612 glass standard. Oxides ThO/Th were monitored and were less than 0.5%. To ensure consistency, laser energy was approximately 3 J/cm^2, with a repetition rate of 10 Hz for 410 pulse/seconds. A pre-ablation sequence of 4-10 pulse/seconds was performedon each sample prior to quantification to eliminate potential surface contamination. Background was measured for 30 seconds before starting each ablation, which would last 41 seconds. For each run of 20 analyses, two NIST 612 glass and one MACS 1, a synthetic calcium carbonate USGS reference materialwith a similar matrix to coral, were sampled at the beginning and end of each run containing a maximum of 14 otoliths (see Appendix II for more details on the reference materials). Trace elements were chosen based upon the most common elements found in the literature. In each run, count per second (cps) data for ^{7}Li, ^{24}Mg,^{25}Mg, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{111}Cd, ^{137}Ba, ^{138}Ba, and ^{208}Pb were acquired. To avoid molecular interference in measuring the composition of some elemental ratios, two isotopes were selected for Mg (^{24}Mg and ^{25}Mg), Ca (^{43}Ca and ^{44}Ca), and Ba (^{137}Ba and ^{138}Ba). Lower mass isotopes of Mg and Ba are favoured by the analytical technique used; making their signal more robust against isobaric interference. To improve precision and accuracy, the ^{24}Mg,^{44}Ca, and ^{137}Ba isotopes were chosen.

The concentration of the analyte ($C_{AN_{SAM}}$) in the sample is a function of the count rate of the analyte ($R_{AN_{SAM}}$) over the normalized sensitivity (S) of the internal standard to the mass of the sample ablated (SAM) and was calculated according to Equation 1.

Equation 1: $$\boldsymbol{C_{AN_{SAM}}} = \frac{R_{AN_{SAM}}}{S}$$

Equation 2 shows the calculation made to determine the sensitivity (cps per unit of concentration) used in this study by the Lamtrace software (Longerich et al., 1996).

Equation 2: $$\boldsymbol{S} = \frac{R_{AN_{CAL}}}{C_{AN_{CAL}}}\left(\frac{R_{IS_{SAM}}}{R_{IS_{CAL}}}\frac{C_{IS_{CAL}}}{C_{IS_{SAM}}}\right)$$

Where:

$\boldsymbol{R_{AN_{CAL}}}$= count rate of the analyte in the calibration material

$C_{AN_{CAL}}$= concentration of the analyte in the calibration material

$\boldsymbol{R_{IS_{SAM}}}$= count rate of internal standard in the sample

$\boldsymbol{R_{IS_{CAL}}}$= count rate of internal standard in the calibration material

$C_{IS_{CAL}}$= concentration of the internal standard in the sample

$C_{IS_{SAM}}$= concentration of the internal standard in the calibration material

Calcium (^{44}Ca) is assumed to be homogeneous at 40 % in otoliths and is the internal standard used to correct for the differences in ablation yield and matrix effect between the otolith and the calibration material. Relative drift is assumed to occur linearly with time

therefore the NIST 612 glass standard was linearly interpolated between the first and last reading to correct for drift. The results obtained for the analyte concentrations are measured as element to Ca ratio in parts per million and were transformed into molar equivalents and reported as millimole of element to 1 mole of Ca (mmol/mol). MACS1 is made of coral and is reported to ablate in a similar manner to an otolith, so it was treated as an unknown to monitor accuracy and precision of the dataset.

Crater size

Fish growth varied among the four aggregations and among individual cod and can affect elemental incorporation on the otolih (Campana et al., 2000). Individual fish growth rate and incorporation patterns were not assessed for these fish so a fixed ablation size had to be used as an estimate to capture the otolith signature corresponding to the area where cod were captured. The width of the last growth increment of the otolith of each cod was measured by digital imagery (Olympus DP72 camera mounted on a SZX 16 stereoscope and CellSens software) along two axes from the end of the last opaque zone (the end of the summer band) to the time of capture (Fig. 2.4A). The last increment corresponds to seven to ten months of growth depending on time of capture. The dorsal edge of the otolith (location 1, Fig. 2.4A) was the widest and chosen for analysis. Considering cod aggregations overwinter and remain in each of the four areas until spawning (Sund, 1935; Rose, 1993; Dean et al., 2012), half of the average width measured (93 μm) for the last increment should correspond to a time where cod were in the area of capture. Three 40 μm-diameter spots were made on the edge of the otolith (Fig. 2.4B). For this system, laser spots between 30-50 μm were also found to yield results above the limit of detection

(LOD) without compromising spatial resolution and risking sampling material that was incorporated to the otolith before the fish was in the area of capture.

Otoliths are three dimensional structures so crater depth was controlled to ensure ablated material represented a signature of the area of capture. Ablation diameter (40 μm) was verified by scanning electron microscopy (SEM). Depths of a subsample of craters, were measured by equation 3, 44.49 μm ± 4.12 (95% confidence interval), using tilted SEM (FEI Quanta 400 SEM, Fig. 2.4C).

Equation 3: $$\boldsymbol{D = \frac{d}{tan\theta}}$$

D= depth of the ablation crater

d= diameter of the ablation crater at 0° tilt

θ= tilt angle of the stage of the SEM

DATA ANALYSIS

Data reduction and correction

Analyses employed the Convert and Lamtrace software (van Achterbergh et al., 2001; Jackson, 2001) following the procedures described by Longerich and Gunther (1996). Lamtrace allows selection of representative signal intervals, background subtraction, and internal standard correction for ablation yield differences. The software also corrects for drift in instrument sensitivity during the analytical session, and performs calculations converting count rates into concentrations by reference to the standards. Using this

software, trace element concentrations were calculated for each sample. The analyte concentrations had to be at least three standard deviations above background to be above the limit of detection (LOD). After corrections were made, the data were put into tabular and graphical form to best represent the composition of the material ablated. This process enables identification of heterogeneity (zoning, inclusions, contamination, etc.) within the ablation and selection of the portion of the background and signal to integrate. Signals that showed signs of external contamination or did not follow standard drift patterns were removed (see Appendix II. for troubleshooting procedures).

Low level trace element analysis is subject to contamination from the environment. Though otoliths were thoroughly decontaminated and pre-ablated to remove surface contamination, residues from polishing or trapped in cracks and imperfections of otolith surface can contaminate the signal. Graphical outputs of the acquisition signal in cps were reported over the ablation period (seconds) for each element to identify zoning patterns that can be attributed to contamination (see Appendix I and II). Ablations were also performed on the epoxy to record the presence of the elements of interest within it and ensure it is not a source of contamination.

Precision was determined by the relative standard deviation (RSD) measured per element and reported as an average for all runs. Analytical accuracy, the ability of a measuring instrument to give a true value (Potts, 1997), is normally calculated by measuring the mean concentrations of an element in a material and comparing it to the certified value. Considering certified values do not exist for MACS 1, its mean concentration measured

per element in all studies done by the LA-ICP-MS of the LAM-MAFIIC laboratory has been compiled and was used to assess accuracy. This long-term value will be referred to as the most probable value (MPV).

RESULTS

Common trace elements for natural tagging

A review of the published literature of the past two decades was done to identify studies that used ICP-MS to quantify otolith composition of marine and euryhaline fish (Table 2.1) to highlight the main elements used a total of 56 studies were found and 41 trace elements were analysed. More than 95% of the otolith is made of calcium carbonate (Campana et al., 1997; Payan et al., 2004) so calcium was measured in all studies. It is typically used as the internal standard so it was not examined in this review. Most studies began by sampling a wide array of elements but found only a few were useful for discriminating fish by otolith signatures (Arkhipkin et al., 2009; Bergenius et al., 2005; Campana and Gagne, 1995). Across studies, the most common elements analysed were Sr, Ba, Mn, Mg, Zn, Pb, Li, and Cu (Fig. 2.5).

Limit of detection

Among the elements reported, the nine elements selected to be quantified by LA-ICP-MS were also frequently used in other studies (Table 2.1). Li, Mg, Mn, Sr, and Ba were detected at the ppm level while Cu, Zn, Cd, and Pb were detected at the ppb level. Other than Cd with 80% of samples below LOD (n=721), all other elements were above LOD for at least 50% of the samples and 100% for Mg, Ca, Sr, and Ba (Table 2.2).

Precision and accuracy

Internal precision, MACS 1 RSD, was less than 5% for Mn, Zn, Sr, Ba and Pb and less than 10% for Cu and Cd. Poor precision was measured for Li with ~17% RSD. MACS 1 was within 10% of the MPV for Cu, Zn, Sr, Cd, and Ba (Table 2.3) indicating good analytical consistency of the LA-ICP-MS for these elements across studies. Concentrations measured for Li and Mn were below the MPV, suggesting that the abundance of these elements was underestimated during analyses. Concentrations of Mg and Pb were much higher than the MPV, reflecting poor accuracy. Concentrations obtained for MACS1 were also compared to expected values from Strnad et al. (2009) and results are reported in Table 2.4. Concentrations for Zn, Sr, Cd, and Ba were within expected ranges while values for Mn and Cu were higher. No values were found in the literature to compare MPV values obtained for Li and Mg.

DISCUSSION

The field of otolith chemistry has grown as technologies have permitted analyses of higher resolution and greater sensitivity. The otolith has become increasingly important to fisheries biologists, enabling them to derive information on the life history of fish, including migration patterns (Morais et al., 2011), nursery sites (Brown, 2006a), stock structure (Ferguson et al., 2011) and assessing the contribution of different stocks to the fisheries (Jónsdóttir et al., 2007). It is essential, however, that the measurements are sufficiently accurate and precise to provide a reliable indicator of the environment of the fish and serve as a natural tag for discriminating fish from different areas.

Among the nine elemental ratios analysed, only five showed good potential for otolith fingerprinting (Mg:Ca, Mn:Ca, Cu:Ca, Sr:Ca, and Ba:Ca) because they were well above detection limits and exhibited satisfactory precision and accuracy. These same five elements were also the most routinely sampled in recent studies (Table 2.1) due to their reliable precision in both laser and solution based analyses (Jones and Chen 2003; Ludsin et al., 2006). In addition, the results presented are encouraging because otolith Mg, Sr and Ba concentrations have shown promise of being influenced by temperature and salinity so may be good at discriminating Newfoundland and Labrador cod spawning areas.

Detection limit

Many individual spots were below LOD for Mn, Cu, Cd, and Pb, implying they are not abundant in the environment of cod or not taken up by the fish, so they cannot be reliably used as an otolith signature. However, no strict protocol is established to deal with data falling below LOD (Fairclough et al., 2011), given that it is an arbitrary threshold that often varies across laboratories (Beer et al., 2011; Ben-Tzvi et al., 2007). Detection limits (LOD) for a given element differ across laboratories because the type of laser, laser parameters, crater size, ablation rate and frequency, and background values are not always the same. For this study, the elemental ratio Cd:Ca was the only one discarded because more than 50% of samples were below LOD, a method described by Chittaro et al. (2006). Among all studies, only 9% analysed Cd and none found it to be a useful discriminant in otolith signatures because it was below LOD (Kingsford et al., 2009), had

poor RSD (Vasconcelos et al., 2007), or did not differ significantly between groups (Campana and Gagne, 1995). Thus, otolith Cu concentrations were also discarded from further analyses.

Precision and accuracy

Otolith standards or artificial reference materials are used to mimic the behaviour of the element matrix under ablation and allow laboratories to compare precision and accuracy of the elemental concentrations. In the present study MACS-1 was used as it is reported to be a good matrix match to otoliths, and its homogeneity was well tested at the LAM-MAFIIC laboratory. In addition, MACS 1 values obtained at the Memorial lab are within the expected range reported by other studies (DiMaria et al., 2010; Strnad et al., 2009).

Previous studies suggest that precision below 10% is adequate for elements sampled by ICP-MS (Geffen et al., 2013; Chittaro et al., 2006; Ludsin et al., 2006). All isotopes were satisfactory except for ^{25}Mg and Li and this may be due to poor instrument sensitivity.As a general rule, instrument sensitivity varies according to the isotope mass of the elements, improving as the mass increases and reaching good sensitivity around a mass of 85. Detection capabilities improve if an isotope is very abundant in the material sampled (Mike Tubrett personal comm.). For a given abundance in the otolith, heavy elements are easier to quantify than lighter elements by ICP-MS (Campana and Gagne, 1995). Li and Mg were the lightest elements sampled in the otolith and showed poorer precision than the heavier elements.

The RSD reported for Li was high (6.08%), therefore the concentrations of Li in MACS 1 and in the sampled otoliths are expected to be lower than the true value. This suggests the ICP-MS at the LAM-MAFIIC laboratory does not quantify Li well limitingits use in this work. Matrix interferences can be responsible for poor measurements of Li because elements with a lighter mass than the internal standard cannot pass through the ion lenses as efficiently as heavier elements (Mindak, 2008).

Recent studies recorded significant matrix effect during the analysis of Mg in synthetic and natural glasses (Norman et al., 2006), and the exact cause of fractionation needs to be studied (Janney et al., 2011). Similarily, the presence of Mg in otolith is more heterogeneous than for other elements so its reaction to ablation may also differ (Mike Tubrett pers. comm.). A high RSD was associated with both Mg isotopes. Mg concentrations in MACS 1 were substancially overestimated, being 106% higher than the MPV. The ICP-MS uses the mass of an isotope to measure its abundance in the otolith. However, the isotope of an element other than the analyte of interest (in this case Mg) can gain a single or doubly charged ion or form a molecular species with the same nominal mass as Mg. If it does, the instrument cannot tell the difference and assumes it is Mg (Mindak, 2008). Previous studies in the LAM-MAFIIC laboratory revealed more potential interference with ^{25}Mg than with ^{24}Mg. Potential isobaric interferences on isotopes of Mg include the molecular ions $^{12}C_2^+$,$^{12}C^{13}C^+$ and $^{12}C^{14}N^+$ as well as doubly-charged ^{48}Ca, ^{48}Ti, ^{50}Ti, and ^{52}Cr (Janney et al., 2011). These ions have not been monitored closely during aquisition, thus interference could have occurred and may explain >2x overestimation of Mg.

Contamination of the reference material can also lead to overestimation, though given less handling; it is less likely to occur than contamination of the samples. Very high concentrations of Pb, Zn and Cd were observed at the beginning of the ablation sequence for some of the samples suggesting surface contamination of the otoliths (Beer et al., 2011). Ablated otolith sections were examined and differences between triplicate analyses were assessed to detect anomalous concentrations or signals. If brief peaks in cps for an element was observed, it was excluded from the integration interval (Fig 2.6), and was not used to calculate the elemental concentration.

Ablation of epoxy can also contribute to high values of Zn or Pb (Jackson, 2001). The epoxy bases were randomly ablated to evaluate the potential for contamination.. A few cps were noted for ^{24}Mg and ^{43}Ca. These short peaks were not significant so the Lamtrace software reported no elements of interest above LOD in the epoxy.. As such, no contamination was assumed to come from the epoxy base. For future work, elemental spiking of the epoxy with indium (eg. Elsdon and Gillanders, 2005; Munroe et al. 2008) is recommended to ensure the concentrations measured are from the otolith and not from the resin.

With regard to choosing the best elements for further analyses, elements should be free from contamination, above detection limits for at least 50% of the samples and maintain both high precision and accuracy. However, when only one of the latter is possible (eg. ^{24}Mg or ^{25}Mg), precision is generally favoured when discrimination of different groups of fish is the objective so long as the measurement are made with the same instrument,

while accuracy must be the focus in comparisons of signatures across studies (or laboratories).

Conclusion

The uses of LA-ICP-MS are diverse and can be applied to fisheries research, particularly to otolith fingerprinting. This research used the analytical capacities of LA-ICP-MS to detect the presence of nine elements (Li, Mg, Mn, Cu, Zn, Sr, Cd, Ba, and Pb) on the last growth increment of the otolith of Atlantic cod. Li exhibited poor precision, Cd was generally below LOD, and Pb and Zn showed evidence of external contamination, so their concentrations were deemed unreliable. Generally, good accuracy and precision were observed for Mg, Mn, Cu, Sr, and Ba, making them the best candidates for future elemental fingerprinting studies of Atlantic cod, at least in the north-west Atlantic.

References

van Achterbergh, E., Ryan, C., Jackson, S., Griffin, W., 2001. Data reduction software for LA-ICPMS, in: Sylvester, P. Laser-Ablation-ICPMS in the Earth Sciences: Principles and Applications. Mineralogy Association of Canada, Ottawa, pp. 239-343.

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

Ashford, J., Jones, C., Hofmann, E., Everson, I., Moreno, C., Duhamel, G., Williams, R., 2005. Can otolith elemental signatures record the capture site of Patagonian toothfish

(*Dissostichus eleginoides*), a fully marine fish in the southern ocean? Can. J. Fish. Aquat. Sci. 62 (12), 2832-2840.

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

Beer, N. A., Wing, S. R., Swearer, S. E., 2011. Otolith elemental evidence for spatial structuring in a temperate reef fish population. Mar. Ecol. Prog. Ser. 442, 217-227.

Begg, G., Cappo, M., Cameron, D., Boyle, S., Sellin, M., 1998. Stock discrimination of School mackerel, *Scomberomorus queenslandicus*, and Spotted mackerel, *Scomberomorus munroi*, in coastal waters of eastern Australia by analysis of minor and trace elements in whole otoliths. Fish. Bull. 96(4), 653-666.

Ben-Tzvi, O., Abelson, A., Gaines, S. D., Sheehy, M. S., Paradis, G. L., Kiflawi, M., 2007. The inclusion of sub-detection limit LA-ICPMS data, in the analysis of otolith microchemistry, by use of a palindrome sequence analysis (PaSA). Limnol. Oceanogr. 5, 97-105.

Bergenius, M., Mapstone, B., Begg, G., Murchie, C., 2005. The use of otolith chemistry to determine stock structure of three epinepheline serranid coral reef fishes on the Great Barrier Reef, Australia. Fish. Res. 72(2-3), 253-270.

Bradbury, I. R., Campana, S. E., Bentzen, P., 2008. Otolith elemental composition and adult tagging reveal spawning site fidelity and estuarine dependency in rainbow smelt. Mar. Ecol. Prog. Ser. 368, 255-268.

Bradbury, I. R., DiBacco C., Thorrold, S. R., Snelgrove, P. V., Campana, S. E., 2011. Resolving natal tags using otolith geochemistry in an estuarine fish, rainbow smelt *Osmerus mordax*. Mar. Ecol. Prog. Ser. 433, 195-204.

Brown, J., 2006a.Classification of juvenile flatfishes to estuarine and coastal habitats based on the elemental composition of otoliths. Estuar. Coast. Shelf Sci. 66(3-4), 594-611.

Brown, J., 2006b. Using the chemical composition of otoliths to evaluate the nursery role of estuaries for English sole *Pleuronectes vetulus* populations. Mar. Ecol. Prog. Ser. 306, 269-281.

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

Campana, S.E., Gagne, J.A., 1995. Cod stock discrimination using ICPMS elemental assays of otoliths, in: Secor, D.H., Dean, J.M., and Campana, S.E. Recent Developments in Fish Otolith Research.University of South California Press, Columbia, SC. pp: 671-691.

Campana, S., Hanson, J., Frechet, A., Brattey, J., 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res. 46(1-3), 343-357.

Campana, S. E., Chouinard, G. A., Hanson, J. M., Fréchet, A., 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. 10, 1873-1881.

Campana, S., Thorrold, S., Jones, C., Guenther, D., Tubrett, M., Longerich, H., Jackson, S., Halden, N., Kalish, J., Piccoli, P., de Pontual, H., Troadec, H., Panfili, J., Secor, D., 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(9), 2068-2079.

Campana, S. E., Fowler, A. J., Jones, C. M., 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Can. J. Fish. Aquat Sci. 51(9), 1942-1950.

Campana, S. E., Valentin, A., Sevigny, D., Power, D., 2007. Tracking seasonal migrations of redfish (*Sebastes* spp.) in and around the Gulf of St. Lawrence using otolith elemental fingerprints. Can. J. Fish. Aquat. Sci. 64(1), 6-18.

Castro, B. G., 2007. Element composition of sardine (*Sardina pilchardus*) otoliths along the Atlantic coast of the Iberian peninsula. ICES J. Mar. Sci. 64(3), 512-518.

Chittaro, P. M., Klinger, T., Telmer, K., Sanborn, M., Morgan, L., 2010. Using otolith chemistry to investigate population structure of quillback rockfish in Puget sound. Northwest Sci. 84(3), 243-254,

Chittaro, P., Usseglio, P., Fryer, B., Sale, P., 2006. Spatial variation in otolith chemistry of *Lutjanus apodus* at Turneffe atoll, Belize. Estuar. Coast. Shelf Sci. 67(4), 673-680.

Chittaro, P., Usseglio, P., Fryer, B., Sale, P., 2005. Using otolith microchemistry of *Haemulon flavolineatum* (French grunt) to characterize mangroves and coral reefs throughout Turneffe atoll, Belize: Difficulties at small spatial scales. Estuaries 28(3), 373-381.

Clarke, L. M., Munch, S. B., Thorrold, S. R., Conover, D. O., 2010. High connectivity among locally adapted populations of a marine fish (*Menidia menidia*). Ecology 91(12), 3526-3537.

Clarke, L. M., Walther, B. D., Munch, S. B., Thorrold, S. R., Conover, D. O., 2009. Chemical signatures in the otoliths of a coastal marine fish, *Menidia menidia*, from the Northeastern United states: Spatial and temporal differences. Mar. Ecol. Prog. Ser. 384, 261-271.

Correia, A. T., Gomes, P., Goncalves, J. M. S., Erzini, K., Hamer, P. A., 2012. Population structure of the black seabream *Spondyliosoma cantharus* along the south-west Portuguese coast inferred from otolith chemistry. J. Fish. Biol. 80(2), 427-443.

DiMaria, R. A., Miller, J. A., Hurst, T. P., 2010. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. Environ. Biol. Fish. 89(3-4), 453-462.

Dorval, E., Jones, C. M., Hannigan, R., van Montfrans, J., 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Can. J. Fish. Aquat. Sci. 64(3), 411-424.

Elsdon, T.S., Gillanders, B.M., 2005. Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. Mar. Ecol. Prog. Ser. 285: 233-243.

Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., Limburg, K. E., Secor, D. H., Thorrold, S. R., Walther, B. D., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol: Annu. Rev. 46, 297-330.

Fairclough, D. V., Edmonds, J. S., Lenanton, R. C. J., Jackson, G., Keay, I. S., Crisafulli, B. M., Newman, S. J., 2011. Rapid and cost-effective assessment of connectivity among assemblages of *Choerodon rubescens* (labridae), using laser ablation ICP-MS of sagittal otoliths. J. Exp. Mar. Biol. Ecol. 403(1-2), 46-53.

Ferguson, G. J., Ward, T. M., Gillanders, B. M., 2011. Otolith shape and elemental composition: Complementary tools for stock discrimination of mulloway (*Argyrosomus japonicus*) in southern Australia. Fish. Res. 110(1), 75-83.

FitzGerald, J., Thorrold, S., Bailey, K., Brown, A., Severin, K., 2004. Elemental signatures in otoliths of larval Walleye Pollock (*Theragra chalcogramma*) from the northeast Pacific Ocean. Fish. Bull. 102(4), 604-616.

Forrester, G. E., Swearer, S. E., 2002. Trace elements in otoliths indicate the use of open-coast versus bay nursery habitats by juvenile California halibut. Mar. Ecol. Prog. Ser. 241, 201-213.

Fudge, S.B., Rose, G.A., 2008. Changes in fecundity in a stressed population: Northern cod (*Gadus morhua*) off Newfoundland, in: Kruse, G.H., Drinkwater, K., lanelli, J.N., Link, J.S., Stram, D.L., and Wespestad, V. Resiliency of Gadid Stocks to Fishing and Climate Change. Lowell and Wakefield Fisheries Symposia Series, Anchorage, AK. pp179-196.

Geffen, A.J., Morales-Nin, B., Perez-Mayol, S., Cantarero, A., Skadal, J., Tovar-Sanchez, A., 2013. Chemical analysis of otoliths: Cross validation between techniques and laboratorics. Fish. Rcs.143, 67-80.

Gibb, F. M., Gibb, I. M., Wright, P. J., 2007. Isolation of Atlantic cod (*Gadus morhua*) nursery areas. Mar. Biol. 151(3), 1185-1194.

Gillanders, B. M., 2002a. Temporal and spatial variability in elemental composition of otoliths: Implications for determining stock identity and connectivity of populations. Can. J. Fish. Aquat. Sci. 59(4), 669-679.

Gillanders, B. M., 2002b. Connectivity between juvenile and adult fish populations: Do adults remain near their recruitment estuaries? Mar. Ecol. Prog. Ser. 240, 215-223.

Gillanders, B. M., Kingsford, M. J., 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar. Ecol. Prog. Ser. 141(1-3), 13-20.

Hamer, P., 2003. Otolith chemistry of juvenile snapper *Pagrus auratus* in Victorian waters: Natural chemical tags and their temporal variation. Mar. Ecol. Prog. Ser. (Halstenbek) 263, 261-273.

Hamer, P., Jenkins, G., Gillanders, B., 2005. Chemical tags in otoliths indicate the importance of local and distant settlement areas to populations of a temperate sparid, *Pagrus auratus*. Can. J. Fish. Aquat. Sci. 62(3), 623-630.

Hanson, P., Koenig, C., Zdanowicz, V., 2004. Elemental composition of otoliths used to trace estuarine habitats of juvenile gag *Mycteroperca microlepis* along the west coast of Florida. Mar. Ecol. Prog. Ser. 267, 253-265.

Jackson, S. E., 2001. Lamtrace user's manual. School of Earth Sciences, Macquarie University, Sydney, Australia.

Janney, P.E., Ritcher, F.M., Mendybaev, R.A., Wadhwa, M., Georg, R.B., Watson, E.B., Hines, R.R., 2011. Matrix effects in the analysis of Mg and Si isotope ratios in natural and synthetic glasses by laser ablation-multicollector ICPMS: A comparison of single- and double-focusing mass spectrometers. Chem. Geol. 281, 26-40.

Jones, C., Chen Z., 2003. New techniques for sampling larval and juvenile fish otoliths for trace-element analysis with laser-ablation sector-field inductively-coupled-plasma

mass spectrometry (SF-ICP-MS). Institute of Marine Research, Postboks 1870 Nordnes N-5817 Bergen Norway

Jónsdóttir, I. G., Marteinsdottir, G., Campana, S. E., 2007. Contribution of different spawning components to the mixed stock fishery for cod in Icelandic waters. ICES J. Mar. Sci. 64(9), 1749-1759.

Kalish, J., Livingston, M., Schofield, K., 1996. Trace elements in the otoliths of New Zealand Blue grenadier (*Macruronus novaezelandiae*) as an aid to stock discrimination. Mar. Fresh. Res. 47(3), 537-542.

Kellison, G. T., Taylor, J. C., 2007. Demonstration and implications of habitat-specific chemical signatures in otoliths of juvenile summer flounder (*Paralichthys dentatus linnaeus*) in North Carolina. J. Fish Biol. 71, 350-359.

Kingsford, M. J., Hughes, J. M., Patterson, H. M., 2009. Otolith chemistry of the non-dispersing reef fish *Acanthochromis polyacanthus*: Cross-shelf patterns from the central great barrier reef. Mar. Ecol. Prog. Ser. 377, 279-288.

Longerich, H., Gunther, D., 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. J. Anal. At. Spectrom. 11(9), 899-904.

Longerich H., Gunther D., Jackson S., 1996. Elemental fractionation in laser ablation inductively coupled plasma mass spectrometry. Fresen. J. Anal. Chem. 355, 538-542.

Longmore, C., Fogarty, K., Neat, F., Brophy, D., Trueman, C., Milton, A., Mariani, S., 2010. A comparison of otolith microchemistry and otolith shape analysis for the study of spatial variation in a deep-sea teleost, *Coryphaenoides rupestris*. Environ. Biol. Fish. 89(3-4), 591-605.

Lo-Yat, A., Meekan, M., Munksgaard, N., Parry, D., Planes, S., Wolter, M., Carleton, J., 2005. Small-scale spatial variation in the elemental composition of otoliths of *Stegastes nigricans* (Pomacentridae) in French Polynesia. Coral Reefs 24(4), 646-653.

Ludsin, S., Fryer, B., Gagnon, J., 2006. Comparison of solution-based versus laser ablation inductively coupled plasma mass spectrometry for analysis of larval fish otolith microelemental composition. Trans. Am. Fish. Soc. 135(1), 218-231.

Milton, D. A., Chenery, S. R., Farmer, M. J., Blaber, S. J. M., 1997. Identifying the spawning estuaries of the tropical shad, *Terubok tenualosa toli*, using otolith microchemistry. Mar. Ecol. Prog. Ser. 153(1-3), 283-291.

Mindak, W.M., 2008. FDA Elemental Analysis Manual. Section 3.6.4 Inductively Coupled Plasma-Mass Spectrometer Available from: http://www.fda.gov/EAM3 (Accessed 2012 July 24).

Morais, P., Dias, E., Babaluk, J., Antunes, C., 2011. The migration patterns of the European flounder *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae, Pisces) at the southern limit of its distribution range: Ecological implications and fishery management. J. Sea Res. 65(2), 235-246.

Morris, J., Rulifson, R., Toburen, L., 2003. Life history strategies of striped bass, *Morone saxatilis*, populations inferred from otolith microchemistry. Fish. Res. 62(1), 53-63.

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

Norman, M.D., McCulloch, M.T., O'Neill, H.S., Yaxley, G.M., 2006. Magnesium isotopic analysis of olivine by laser-ablation multi-collector ICP-MS: composition dependent matrix effects and a comparison of the Earth and Moon. J. Anal. At. Spectrom. 21, 50–54.

Patterson, H., Thorrold, S., Shenker, J., 1999. Analysis of otolith chemistry in Nassau grouper (*Epinephelus striatus*) from the Bahamas and Belize using solution based ICP MS. Coral Reefs 18(2), 171-178.

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

Pearce, N. J. G., Perkins, W. T., Westgate, J. A., Gorton, M. P., Jackson, S. E., Neal, C. R., Chenery, S. P., 1997. A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. Geostandards Newslett. 21(1), 115-144.

Potts, P. J., 1987. Inductively coupled plasma-mass spectrometry, in: Handbook of Silicate Rock Analysis. Blackie, Glasgow, pp. 575-586.

Ruttenberg, B. I., Warner, R. R., 2006. Spatial variation in the chemical composition of natal otoliths from a reef fish in the Galapagos Islands. Mar. Ecol. Prog. Ser. 328, 225-236.

Ruttenberg B. I., Hamilton S. L., Warner R. R., 2008. Spatial and temporal variation in the natal otolith chemistry of a Hawaiian reef fish: Prospects for measuring population connectivity. Can. J Fish. Aquat. Sci. 65(6), 1181-1192.

Secor, D., 1992. Application of otolith microchemistry analysis to investigate anadromy in Chesapeake bay striped bass *Morone saxatilis*. Fish. Bull. 90(4), 798-806.

Sévigny, JM., Valentin, A., Talbot, A., and Ménard, N., 2009. Connectivity between Saguenay Fjord populations and those of the Gulf of St. Lawrence. J. Water Sci. 22(2), 315-339.

Silva, D. M., Santos, P., Correia, A. T., 2011. Discrimination of *Trisopterus luscus* stocks in Northern Portugal using otolith elemental fingerprints. Aquat. Living Res. 24(1), 85-91.

Standish, J. D., Sheehy, M., Warner, R. R., 2008. Use of otolith natal elemental signatures as natural tags to evaluate connectivity among open-coast fish populations. Mar. Ecol. Prog. Ser. 356, 259-268.

Stransky, C., Garbe-Schoenberg, C., Guenther, D., 2005. Geographic variation and juvenile migration in Atlantic redfish inferred from otolith microchemistry. Mar. Fresh. Res. 56(5), 677-691.

Strnad, L., Ettler, V., Mihaljevic, M., Hladil, J., Chrastny, V., 2009. Determination of trace elements in calcite using solution and laser ablation ICP-MS: Calibration to NIST SRM glass and USGS MACS carbonate, and application to real landfill calcite. Geostand. Geoanal. Res. 33(3), 347-355.

Syedang, H., Andre, C., Jonsson, P., Elfman, M., Limburg, K. E., 2010. Migratory behaviour and otolith chemistry suggest fine-scale sub-population structure within a genetically homogenous Atlantic cod population. Environ. Biol. Fish. 89(3-4), 383-397.

Tanner, S. E., Vasconcelos, R. P., Reis-Santos, P., Cabral, H. N., Thorrold, S. R., 2011. Spatial and ontogenetic variability in the chemical composition of juvenile common sole (*Solea solea*) otoliths. Estuar Coast. Shelf Sci. 91(1), 150-157.

Tanner, S. E., Reis-Santos, P., Vasconcelos, R. P., Franca, S., Thorrold, S. R., Cabral, H. N., 2012a. Otolith geochemistry discriminates among estuarine nursery areas of *Solea solea* and S. *senegalensis* over time. Mar. Ecol. Prog. Ser. 452, 193-203.

Tanner, S. E., Vasconcelos, R. P., Cabral, H. N., Thorrold, S. R., 2012b. Testing an otolith geochemistry approach to determine population structure and movements of European hake in the northeast Atlantic Ocean and Mediterranean sea. Fish. Res.125, 198-205.

Thibault, I., Hedger, R. D., Dodson, J. J., Shiao, J., Iizuka, Y., Tzeng, W., 2010.Anadromy and the dispersal of an invasive fish species (*Oncorhynchus mykiss*) in Eastern Quebec, as revealed by otolith microchemistry. Ecol. Fresh. Fish 19(3), 348-360.

Thorisson, K., Jónsdóttir, I. G., Marteinsdottir, G., Campana, S. E., 2011. The use of otolith chemistry to determine the juvenile source of spawning cod in Icelandic waters. ICES J. Mar. Sci. 68(1), 98-106.

Thorrold, S. R., Jones, C. M., Campana, S. E., 1997. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). Limnol. Oceanogr. 42(1), 102-111.

Thorrold, S., Jones, C., Swart, P., Targett, T., 1998. Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. Mar. Ecol. Prog. Ser. 173, 253-265.

Turan, C., 2006. The use of otolith shape and chemistry to determine stock structure of Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). J. Fish Biol. 69(Suppl. C), 165-180.

Vasconcelos, R. P., Reis-Santos, P., Tanner, S., Fonseca, V., Latkoczy, C., Guenther, D., Costa, M. J., Cabral, H., 2007. Discriminating estuarine nurseries for five fish species through otolith elemental fingerprints. Mar. Ecol. Prog. Ser. 350, 117-126.

Veinott, G., Porter, R., 2005. Using otolith microchemistry to distinguish Atlantic salmon (*Salmo salar*) parr from different natal streams. Fish Res. 71(3), 349-355.

Wang, Y., Ye, Z., Liu Q., Wang, W., Cao L., Shen, W., 2011. Otolith chemical signatures of spottedtail goby *Synechogobius ommaturus* in coastal waters of China. Chinese J. Oceanol. Limnol. 29(3), 640-646.

Warner, R., Swearer, S., Caselle, J., Sheehy, M., Paradis, G., 2005. Natal trace-elemental signatures in the otoliths of an open-coast fish. Limnol. Oceanogr. 50(5), 1529-1542.

TABLES

Table 2.1: Summary of reviewed publications using ICPMS for marine and euryhaline fish otolith chemistry analyses between 1992 and 2012

Species	Location	Ecosystem	Elements analysed	Method	Analysis	Authors
Abudefduf sordidus	Hawaiian island	Reef	Mg, Mn, Fe, Zn, Sr	LA-ICPMS	Otolith fingerprinting	Ruttenberg et al., 2008
Acanthochromis polyacanthus	Australia	Reef & shelf	Mg, Mn, Cu, Sr, Cd, Ba	SB-ICPMS	Otolith fingerprinting	Kingsford et al., 2009
Achoerodus viridis	Australia	Rocky reef	Mn, Cu, Zn, Sr, Ba	SB-ICPMS	Nursery site ID	Gillanders and Kingsford, 1996
Argyrosomus japonicus	S. Australia	Bay & coast	Na, Mg, Sr, Ba	LAICPMS	Stock structure	Ferguson et al., 2011
Cephalopholis cyanostigma	Australia	Reef	Al, Sc, Mg, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, Sr, As, Pd, Ba, Pb	SB-ICPMS	Stock structure	Bergenius et al., 2005
Choerodon rubescens	W. Australia	Coast	Mg, Mn, Cu, Zn, Sr, Zn, Rb, Ba, Pb	LA-ICPMS	Stock structure & connectivity	Fairclough et al., 2011
Citharichthys stigmaeus	California	Coast & estuary	Li, Mn, Sr, Ba	SB-ICPMS	Nursery site ID	Brown, 2006a
Coryphaenoides rupestris	North Atlantic	Deep sea	Li, Mg, Mn, Co, Ni, Cu, Zn, Sr, Ba, U	LA-ICPMS	Stock discrimination	Longmore et al., 2010
Cynoscion nebulosus	Chesapeake Bay	Seagrass	Mg, Mn, Sr, Ba, La	SB-ICPMS	Otolith vs. water signature	Dorval et al., 2007
Cynoscion regalis	USA	Estuary	Mg, Mn, Sr, Ba	ID-ICPMS	Nursery site ID	Thorrold et al., 1998
Dicentrarchus labrax	Portugal	Estuary	Li, Na, Mg, K, Mn, Ni, Cu, Zn, Sr, Cd, Ba, Pb	SB-ICPMS	Nursery site ID	Vasconcelos et al., 2007
Diplodus vulgaris	Portugal	Estuary	Li, Na, Mg, K, Mn, Ni, Cu, Zn, Sr, Cd, Ba, Pb	SB-ICPMS	Nursery site ID	Vasconcelos et al., 2007

Dissostichus eleginoides	South America & Antarctica	Estuary	Mg, Mn, Sr, Ba	LAICPMS	Fish origin	Ashford et al., 2005
Epinephelus fasciatus	Australia	Reef	Al, Sc, Mg, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, Sr, As, Pd, Ba, Pb	SB-ICPMS	Stock structure	Bergenius et al., 2005
Epinephelus striatus	Bahamas & Belize	Bay & coast	Zn, Sr, Ba, Pb	SB-ICPMS	Group discrimination	Patterson et al., 1999
Gadus macrocephalus	Kodiak Island, Alaska	Coastal	Li, Mg, Mn, Zn, Sr, Ba	LA-ICPMS	Effect of temperature & growth on signature	DiMaria et al., 2010
Gadus morhua	NW Atlantic	Coast & offshore	B, Mg, Si, Sc, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Br, Rb, Sr, Y, Mo, Ru, Rh, Cd, Cs, Ce, Ba, Eu, Pb	SB-ICPMS	Stock discrimination	Campana and Gagne, 1995
	NW Atlantic	Coast & offshore	Li, Mg, Mn, Sr, Ba	SB-ICPMS	Mixing & migration	Campana et al., 1999
	Gulf of St. Lawrence	Coast & offshore	Li, Mg, Mn, Sr, Ba, Zn	ID-ICPMS	Otolith fingerprinting	Campana et al., 2000
	Scotland	Coast	Mg, Mn, Ba	SB-ICPMS	Nursery site ID	Gibb et al., 2007
	Iceland	Coast & deep water	Li, Mg, Mn, Sr, Ba	SB-ICPMS	Stock contribution to fisheries	Jónsdóttir et al., 2007
	St. Lawrence & Saguenay	Fjord & estuary	B, Li, Mg, K, Na, Mn, Fe, Zn, Sr, Ba, Pb	ID-ICPMS SB-ICPMS	Connectivity	Sévigny et al., 2009
	Iceland	Coast	Mg, Mn, Sr, Ba	SB-ICPMS	Origin of spawners	Thorisson et al., 2011
Haemulon flavolineatum	Belize	Mangrove & Reef	Li, Mg, Cu, Zn, Rb, Sr, Sn, Ba, Pb	LA-ICPMS	Otolith fingerprinting	Chittaro et al., 2005
Lutjanus apodus	Belize	Mangrove & Reef	Li, Mg, Cu, Zn, Rb, Sr, Sn, Ba, Pb	LA-ICPMS	Otolith fingerprinting	Chittaro et al., 2006
Menidia menidia	New Jersey & Maine	Coast	Mg, Mn, Cu, Sr, Ba, Pb	LA-ICPMS	Otolith fingerprinting & connectivity	Clarke et al., 2009; 2010
Merluccius merluccius	Mediterranean	Coast	Mg, Mn, Sr, Ba	LA-ICPMS	Population	Tanner et al., 2012b

	Sea				structure & movement	
Micromesistius australis	SW Atlantic & SE Pacific	Shelf & slope	Sc, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Rb, Y, Ag, Cd, La, Pb, Bi, U	LA-ICPMS	Population structure	Arkhipkin et al., 2009
Micropogonias undulatus	USA Coast	Shelf	Mg, Mn, Zn, Sr	LA-ICPMS	Migration & movement	Thorrold et al., 1997
Mycteroperca microlepis	Florida ,USA	Coast	Li, Na, Mg, K, Mn, Cu, Sr, Ba, Pb	SB-ICPMS AAS-ICPMS[1]	Nursery sites ID	Hanson et al., 2004
Osmerus mordax	Newfoundland	Estuary	Sr, Ba	LA-ICPMS	Homing & movement	Bradbury et al., 2008
	Newfoundland	Coast & estuary	Mg, Mn, Sr, Ba	LA-ICPMS	Origin & dispersal	Bradbury et al., 2011
Pagrus auratus	Port Phillip Bay	Bay & estuary	Mn, Sr, Ba	LA-ICPMS	Otolith fingerprinting	Hamer 2003; Hamer et al., 2005
	Australia	Reef	Mg, Mn, Sr, Ba	LA-ICPMS	Nursery site ID	Gillanders, 2002a,b
Paralichthys californicus	S. California	Shelf	Cu, Pb	LA-ICPMS SB-ICPMS	Nursery site ID	Forrester and Swearer, 2002
Paralichthys dentatus	North Carolina	Estuary	Mg, Mn, Zn, Sr, Ba	SB-ICPMS	Habitat discrimination	Kellison and Taylor, 2007
Parapercis colias	New Zealand	Fjord	B, Li, Mg, P, S, Mn, Cu, Zn, Sr, Ba, Pb	LA-ICPMS	Population structure & connectivity	Beer et al., 2011
Platichthys flesus	Portugal	Estuary	Li, Na, Mg, K, Mn, Ni, Cu, Zn, Sr, Cd, Ba, Pb	SB-ICPMS	Nursery site ID	Vasconcelos et al., 2007
	NW-Iberian Peninsula	Coast & estuary	Sr	LA-ICPMS	Migration	Morais et al., 2011
Plectropomus leopardus	Australia	Reef	Al, Sc, Mg, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, Sr, As, Pd, Ba, Pb	SB-ICPMS	Stock structure	Bergenius et al., 2005
Pleuronectes vetulus	California	Coast & estuary	Li, Mn, Sr, Ba	SB-ICPMS	Nursery site ID	Brown, 2006b
Sardina pilchardus	Iberian Peninsula	Coast	Li, Na, Mg, K, Mn, Sr, Ba	SB-ICPMS	Stock discrimination	Castro, 2007
Sebastes atrovirens	California	Coast & island	Mg, Mn, Fe, Zn, Sr, Ba, Ce, Pb	LA-ICPMS	Origin & otolith fingerprinting	Warner et al., 2005

	California	Coast & offshore	Mg, Mn, Fe, Zn, Sr, Ba, Ce, Pb	LA-ICPMS	Otolith fingerprinting & connectivity	Standish et al., 2008
Sebestes fasciatus	Gulf of St. Lawrence	Shelf & offshore	Li, K, Mg, Mn, , Sr, Cd, Ba	ID-ICPMS	Migration	Campana et al., 2007
Sebastes maliger	Washington	Inshore	Mn, Zn, Sr, Ba	LA-ICPMS	Otolith fingerprinting	Chittaro et al., 2010
Sebastes marinus	North Atlantic	Coast & offshore	Li, Cu, Rb, Sr, Ba	LA-ICPMS SB-ICPMS	Migration	Stransky et al., 2005
	Gulf of St. Lawrence	Shelf & offshore	Li, K, Mg, Mn, , Sr, Cd, Ba	ID-ICPMS	Migration	Campana et al., 2007
Solea senegalensis	Portugal	Estuary	Li, Na, Mg, K, Mn, Ni, Cu, Zn, Sr, Cd, Ba, Pb	SB-ICPMS	Nursery site ID	Vasconcelos et al., 2007
	Portugal	Estuary	Li, Mg, Mn, Cu, Sr, Ba, Pb	LA-ICPMS	Nursery site ID & otolith fingerprinting	Tanner et al., 2011; 2012a
Solea solea	Portugal	Estuary	Li, Na, Mg, K, Mn, Ni, Cu, Zn, Sr, Cd, Ba, Pb	SB-ICPMS	Nursery site ID	Vasconcelos et al., 2007
	Portugal	Estuary	Li, Mg, Mn, Cu, Sr, Ba, Pb	LA-ICPMS	Otolith fingerprinting	Tanner et al., 2011; 2012a
Spondyliosoma cantharus	SW Portugal	Coast	Li, Mg, Mn, Ni, Cu, Zn, Sr, Ba, Pb	SB-ICPMS	Population structure	Correia et al., 2012
Stegastes beebei	Galapagos island	Island coast	Mg, Mn, Sr, Ba, Pb	LA-ICPMS	Larval dispersal	Ruttenberg and Warner, 2006)
Stegastes nigricans	French Polynesia	Coast	Li, Na, Mg, Mn, Sr, Y, Ba	SB-ICPMS	Population structure	Lo-Yat et al., 2005
Synechogobius ommaturus	China	Coast	Na, K, Mg, Mn, Co, Zn, Sr, Ba, Pb,	LA-ICPMS	Stock discrimination	Wang et al., 2011
Tenualosa toli	Borneo	Coast & estuary	Li, Na, Mg, Cu, Zn, Sr, Ba	LA-ICPMS	Spawning site ID	Milton et al., 1997
Theragra chalcogramma	Gulf of Alaska & Bering Sea	Coast & island coast	Mn, Sr, Ba	EPMA, LA-ICPMS	Otolith fingerprinting	FitzGerald et al., 2004
Trisopterus luscus	Portugal	Coast	Li, Mg, Mn, Ni, Cu, Zn, Sr, Ba, Pb	SB-ICPMS	Stock discrimination	Silva et al., 2011

Table 2.2: Percentage of samples below the limit of detection (LOD), mean LOD and mean elemental concentration across all otoliths analysed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Standard deviation is reported in brackets.

Isotope	Samples < LOD (%)	Mean LOD (ppm)		Mean concentration (ppm)	
^{7}Li	2.72	0.486	(0.21)	6.42	(3.6)
^{24}Mg	0.00	0.092	(0.05)	98.3	(13)
^{25}Mg	0.08	0.380	(0.21)	24.3	(15)
^{43}Ca	0.00	31.3	(15)	415040	(6303)
^{44}Ca	0.00	8.39	(4.2)	400800	-
^{55}Mn	48.27	0.093	(0.04)	0.39	(0.49)
^{63}Cu	21.95	0.061	(0.02)	0.80	(1.8)
^{66}Zn	2.39	0.052	(0.03)	2.12	(8.3)
^{88}Sr	0.00	0.093	(0.06)	3969	(859)
^{111}Cd	79.81	0.050	(0.03)	0.05	(0.10)
^{137}Ba	0.00	0.030	(0.02)	5.37	(2.9)
^{138}Ba	0.17	0.010	(0.01)	5.20	(2.9)
^{208}Pb	25.85	0.006	(0.01)	0.14	(0.37)

Table 2.3: Summary of analytical precision and accuracy. Estimates of precision are measured by relative standard deviation (%) of the reference material sampled. RSD $\leq$ 10% are generally accepted as reliable data. Accuracy estimates represent the percentage difference between the mean concentrations obtained for the reference material and the certified value. No certified values exist for MACS 1, so the laboratory long-term mean values measured for all other studies performed at the LAM-MAFIIC laboratory was used. Positive accuracy implies mean measured concentration was above the certified value while a negative accuracy indicates the mean was below the certified value.

	Precision (%RSD)	Accuracy
Li	16.92	-86.25
^{24}Mg	9.41	106.67
^{25}Mg	24.90	-4.23
^{43}Ca	2.30	0.37
^{44}Ca	0.00	0.00
^{55}Mn	2.92	-16.28
^{63}Cu	5.37	6.76
^{66}Zn	4.73	9.71
^{88}Sr	3.39	5.44
^{111}Cd	7.48	5.96
^{137}Ba	3.47	6.72
^{138}Ba	3.07	3.96
^{208}Pb	4.76	13.36

Table 2.4: Comparison of the long-term mean values reported as most probable value (MPV) obtained for MACS 1 (in ppm) at the LAM-MAFIIC laboratory at Memorial University using LA-ICP-MS and expected values reported by Strnad et al. (2009)

	MPV	Expected values
Li	18	-
Mg	60	-
Mn	155	100-130
Cu	137	50-100
Zn	114	100-150
Sr	222	200-240
Cd	103	100-150
Ba	127	100-150
Pb	143	100-150

FIGURES

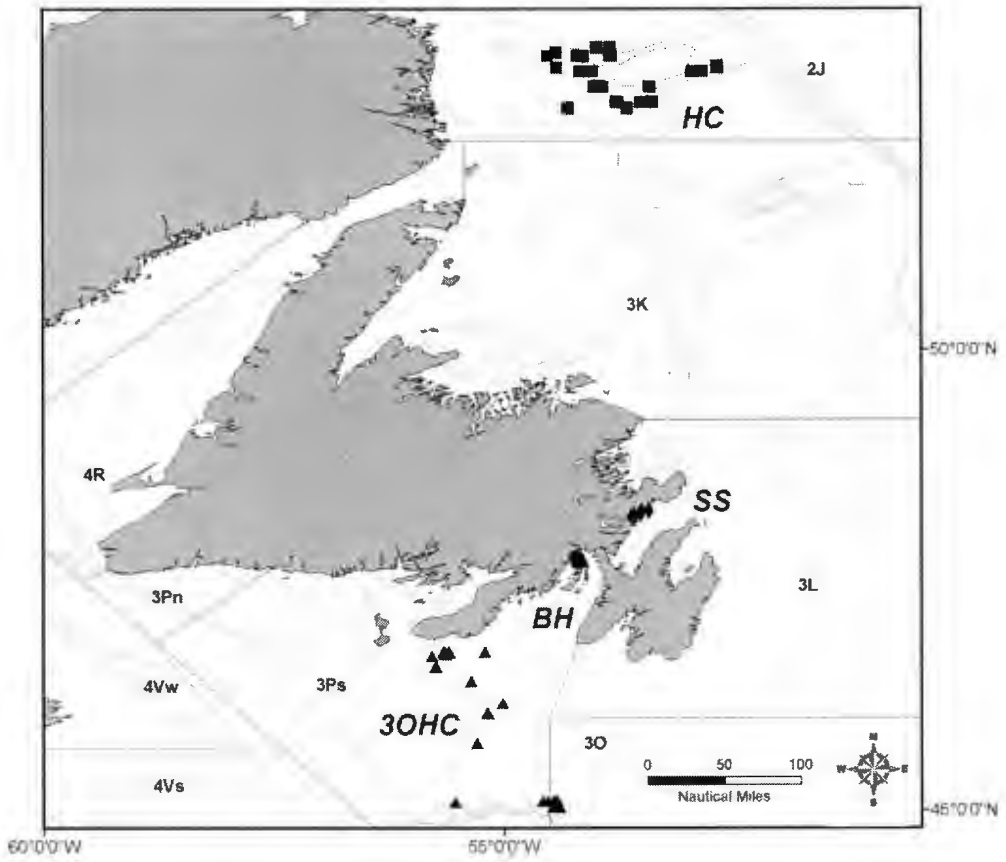


Figure 2.1: Map showing locations of fishing sets in each spawning area collected for this study. The dark symbols represent sampling locations for 1998 and empty symbols represent 1999 sampling locations. Spawning groups are Bar Haven (BH,●), Hawke Channel (HC, ■), Offshore 3Ps and Halibut Channel area (O3HC,▲), Smith Sound (SS, ♦). The O3HC region includes sampling sites near shore but outside of Placentia Bay leading to the Halibut Channel and along the shelf edge around Green Bank at the edge of 3O.

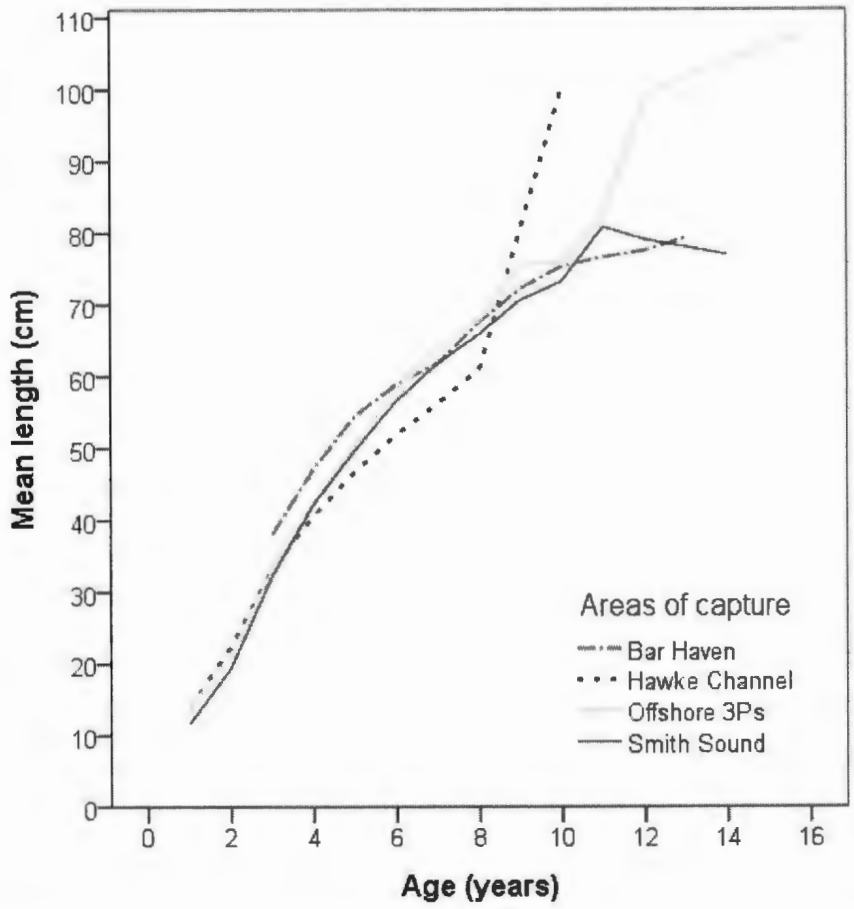


Figure 2.2: Cod growth expressed as the length (cm) of the fish upon capture as a function of its age estimated from the otolith increments. Each area of capture is represented by different line types. All growth rates are similar until fish reach the age of three. Hawke Channel growth rate slows between the age of three and eight.

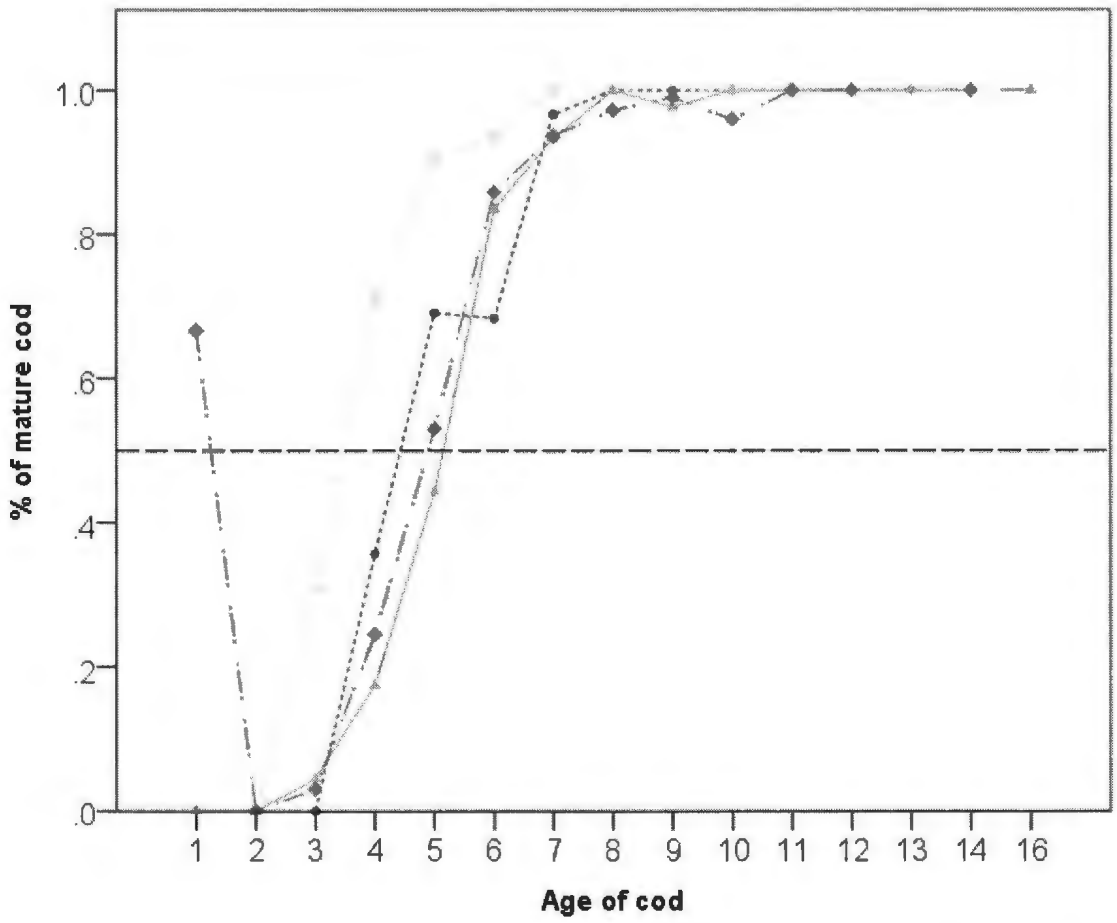


Figure 2.3: Cod maturity expressed as a the percentage of mature cod captured at each spawning area in relation to the age of the fish. The dashed line represent the age at which 50% of cod capture reaches maturity. Each spawning area is represented by a different line type and symbol; Bar Haven (●), Hawke Channel (■), Offshore 3Ps and Halibut Channel (▲), and Smith Sound (♦).

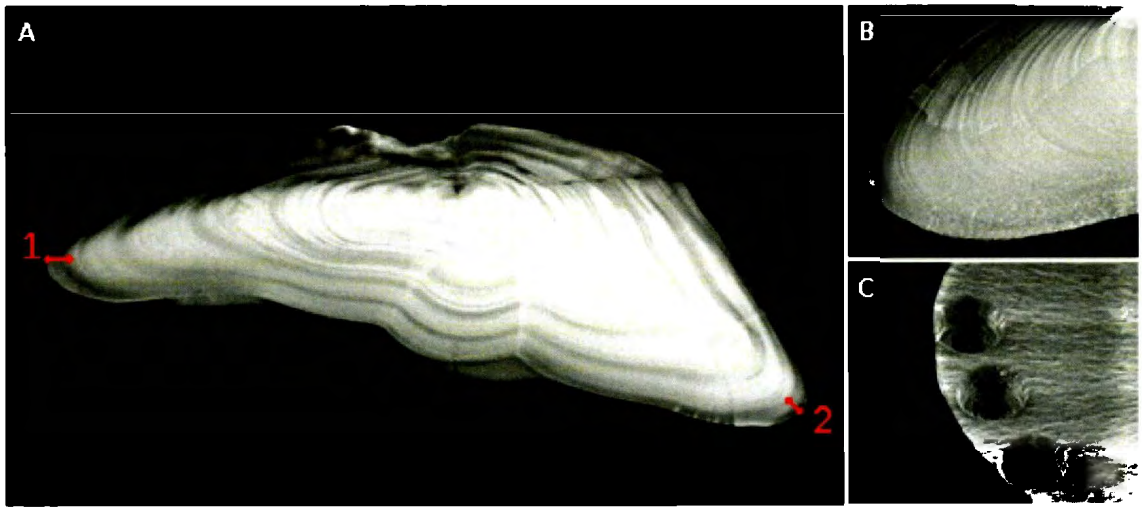


Figure 2.4: A. Otolith cross section showing width measurement locations under reflected light. Increment width was greatest on the dorsal side of the otolith at location 1 (93 µm). B. Laser beam sampled three 40µm-diameter spots at the edge of the otolith. Olympus SZX 16, 2.88X . C. Triplicate craters were measured for diameter and ablation depth using tilted scanning electron microscopy.

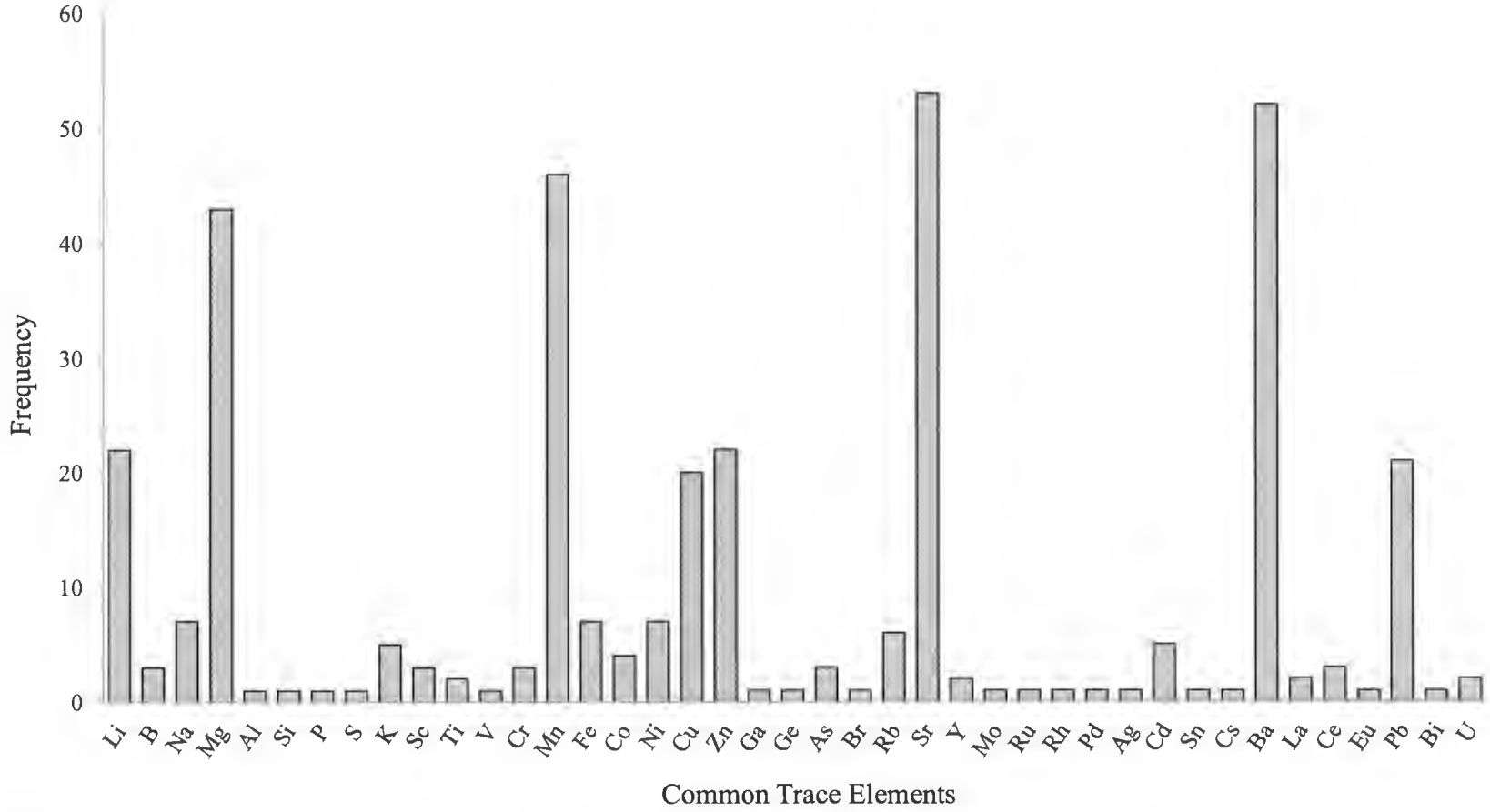


Figure 2.5: Frequency distribution of elements used by researchers to quantify otolith composition of marine fish with ICP-MS. The frequency of use for each element is reported. A total of 41 trace elements were analysed in 56 studies published in the last two decades. Ba, Mg, Mn and Sr were the most common with more than 40 studies reporting their use.

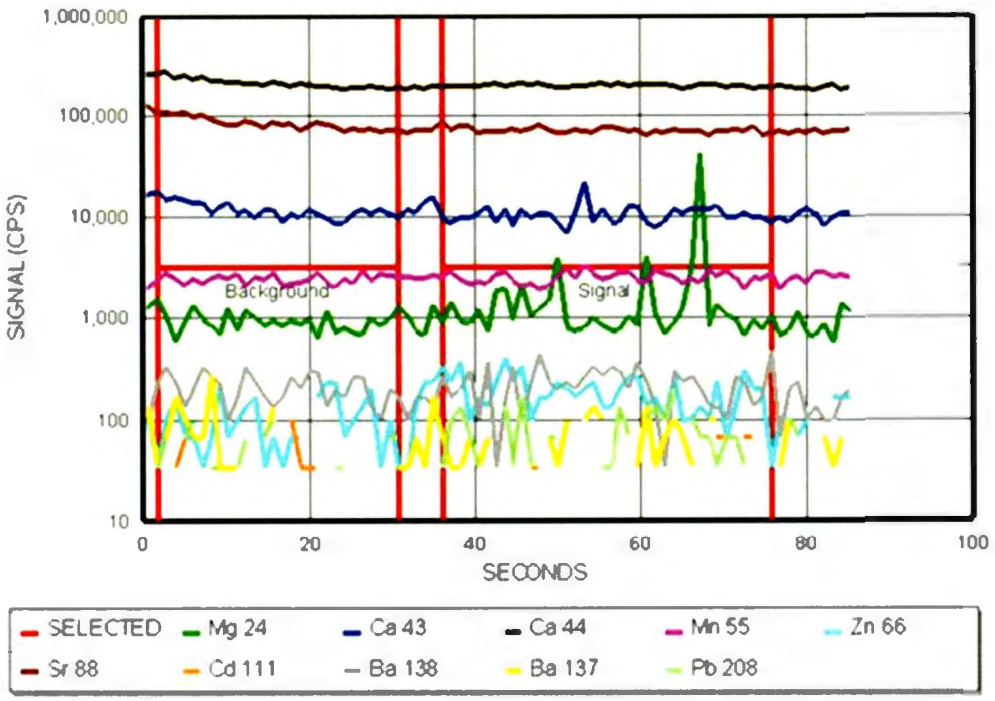


Figure 2.6: Signal (cps) per element obtained from the ablation of the epoxy surrounding the otolith. Only a few peaks of ^{24}Mg and ^{43}Ca were measured above the background values, though they were not significantly above the measured LOD to influence the concentration of these elements when calculated otolith elemental concentrations.

Chapter 3: Otolith elemental fingerprints distinguish Atlantic cod (*Gadus morhua*) spawning areas in Newfoundland and Labrador

ABSTRACT

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to analyse the otolith composition of four key spawning aggregations of cod in Newfoundland and Labrador waters in inshore, offshore, north and south locations. A group-specific fingerprint was established for each spawning location, using the elemental ratios of Mg, Mn, Sr, and Ba to Ca and fish growth rates. The fingerprint differed among groups but showed some variations through time. Cod of three cohorts collected in two consecutive years were correctly assigned to their spawning group with 66% accuracy and increased to 78% when inshore spawning areas were merged. Our findings suggest that otolith signatures coupled with growth rates have potential to enable tracking of cod movements over time, identification of distribution shifts that may occur with changing climate, and potentially determine the contribution of each spawning group to fisheries.

INTRODUCTION

Otoliths possess chemical properties that enable them to act as natural data loggers or tags (Campana, 1999; Elsdon et al., 2008), with trace element compositions providing a chronological signature reflecting the ambient environment and condition of the fish. Early studies used whole otolith signatures in attempts to differentiate fish from different regions, but more recently the use of laser ablation inductively coupled mass spectrometry (LA-ICP-MS) has allowed the quantification of otolith composition at a much finer spatial and temporal scale (Hamer, 2003). Otoliths from fish groups that are isolated spatially during at least a portion of their life cycle may have distinguishable otolith chemical signatures that allow classification by region (Campana and Gagne, 1995; Walther and Thorrold, 2006).

Since the decline of the Newfoundland and Labrador Atlantic cod (*Gadus morhua*) in the late 1980s and early 1990s, rebuilding of extant spawning groups has been highly heterogeneous (Rose, 2007). Some groups, such as the stock on the south coast of Newfoundland (NAFO subdivision 3Ps), and the newly appearing spawning group in Smith Sound on the NE coast (NAFO subdivision 3L), responded quickly after the moratorium on fishing imposed in the early 1990s, but others in adjacent regions did not (Brattey et al., 2009; DFO 2009). Adding further complexity, large scale distribution shifts were evident during this period (deYoung and Rose, 1993; Atkinson et al., 1997; Windle et al., 2012), and some spawning groups may have spilled-over into adjacent regions known to hold other groups (Brattey et al., 1999; Lawson and Rose, 2000; Rose et

al., 2011). Rebuilding prognoses and management of cod depends on knowledge of the spatial dynamics of spawning groups that at present is lacking. A central problem in addressing these issues is an inability to distinguish fish from the various spawning groups or regions.

Genetic studies of Newfoundland and Labrador cod have provided mixed results in separating spawning groups (Ruzzante et al., 2000; Rose et al., 2011), and on their own have not provided a useful natural tag of spawning location. In contrast, otolith elemental ratios have been used, although sparingly, to establish signatures for spawning groups of cod in the nearby Gulf of St. Lawrence (Campana et al., 2000), in the northwest Atlantic, including NAFO division 3O off Newfoundland (Campana et al., 1994), and on other species in Newfoundland waters (Bradbury et al., 2011). We hypothesized that chemical differentiation would allow fish from disparate cod spawning areas in the waters around Newfoundland and Labrador to be distinguished, and hence enable otolith microchemical signatures to be used to address ecological questions of group connectivity and distribution.

The goal of this study was to test the chemical differentiation hypothesis, to determine if otolith signatures of cod from key spawning areas in the waters of Newfoundland and Labrador would differ sufficiently to enable fish from these groups to be distinguished.

METHODS

Study Area

Historical data and tagging experiments indicated the persistence of cod spawning groups in several locations since the mid-1990s, of which four were chosen for this study: 1. offshore NAFO Subdivision 2J in Hawke Channel (HC) (Fudge and Rose, 2008a; Fudge and Rose, 2008b), 2. inshore NAFO Subdivision 3KL in Smith Sound (SS) (Rose, 2007), two locations within the NAFO 3Ps subdivision 3. inshore Placentia Bay around Bar Haven Island (BH), (Lawson and Rose, 2000; DFO, 2009), and 4. along the Halibut Channel and Green Bank continental shelf (O3HC) at the edge of 3O (Brattey, 1997; see Figure 3 1). These regions represented a wide range of north-south and inshore-offshore spawning locations with respect to the oceanographic and ecological conditions of the region. The HC area is characterized by cooler and enriched waters heavily influenced by the Labrador Current (Petrie and Anderson, 1983; Brown, 1999; Colbourne, 1999). SS is a fjord on the northeast coast of Newfoundland and influenced by both the Labrador Current and freshwater inputs from several rivers (Knickle and Rose, 2010). BH is also influenced by the diminished but still pervasive Labrador Current, but heavily influenced by freshwater inputs that have similar origin to those at SS (Lawson and Rose, 2000). The 03HC region is a mixing area of cold waters from the Labrador Current and warmer waters originating from the northward moving Gulf Stream (Colbourne, 1999). Sampling Atlantic cod in the four spawning areas were sampled by bottom-trawls between April and July of 1998 and 1999 (except at BH where fish were taken by hand-line, Figure 3.1). The majority of these fish were either in spawning condition or recently spent at all sites,

with some of the younger ages, especially at HC and O3HC being immature (Table 3.1). A total of three age classes, with at least one identical age at all locations were chosen for elemental analysis (Table 3.1). Otoliths were extracted from each fish and dried, with additional measurements taken, including weights, length, sex, and maturity stage.

Otolith preparation and analyses

One otolith from each sampled fish was selected for ageing by a professional reader while the other otolith was prepared for trace element analysis. This otolith was cleaned of any adherent tissues by brushing the surfaces with an acid-washed toothbrush and decontaminated by sonification in multiple solutions of distilled, deionized and reverse-osmosis baths (Campana et al., 2000, see chapter 2). To maintain consistency in otolith structure, those containing vaterite were discarded. A total of 659 aragonite otoliths were embedded in epoxy then sectioned transversely to expose the growth increments. For the microchemical analyses, the otoliths were randomly assigned into blocks with at least one otolith of each location and year per block to even out any effect of instrumental drift across samples.

Concentrations of ^{7}Li, ^{24}Mg, ^{25}Mg, ^{55}Mn, ^{43}Ca, ^{44}Ca, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{111}Cd, ^{137}Ba, ^{138}Ba, and ^{208}Pb were determined at the Laser Microprobe laboratory of Memorial University of Newfoundland, using a coupled 193 nm excimer laser and Finnigan ELEMENT XR. The laser sampled three 40μm spots at the margin of the last growth increment of each otolith– sampling the last three to six months of life of the fish (depending on fish growth

rate). To ensure consistency, laser energy was approximately 3 J/cm^2 with a repetition rate of 10 Hz for 410 pulse counts. Each sample was pre-ablated (4-10 pulses) prior to quantification to eliminate potential surface contamination. Mean ablation depth was 44.49 μm (40.37 μm to 48.62 μm).

National Institute of Standards and Technology (NIST) 612 glass served as an external standard to adjust for instrument mass bias. This standard was assayed twice both at the beginning and end of a block of 14 otoliths and linearly interpolated to correct for drift. A second reference material with a synthetic carbonate matrix similar to otoliths (United States Geological Survey MACS 1) was used to monitor the accuracy and precision of the analytical technique and was also assayed at beginning and end of each block. The data reduction Convert and Lamtrace spreadsheet programs were used to estimate elemental concentrations following the methods of Longerich and Gunther (1996). Elemental concentrations were reported as a ratio to the internal standard to account for differences in ablation yield. Calcium was assumed to make up 40.0% of the ablated material. Estimates of precision based on relative standard deviation (RSD) of MACS1 for each element to ^{44}Ca ratios (n=659 analyses) were 16.9% for Li, 9.41% for ^{24}Mg, 24.9% for ^{25}Mg, 2.92% for Mn, 5.37% for Cu, 4.73% for Zn, 3.39% for Sr, 7.48% for Cd, 3.47% for ^{137}Ba, 3.07% for ^{138}Ba, and 4.76% for Pb. Elements with lower molar mass showed poorer reproducibility and sensitivity and were excluded from the analyses if their RSD was more than 10% (Li and ^{25}Mg). Detection limits were calculated according to an algorithm described by Longerich et al. (1996) based on three standard deviations from the gas blank and adjusted to take into account ablation parameters (e.g., background

noise, length of signal, length of integrated interval) and are reported in ppm as 0.092 for ^{24}Mg, 0.093 for Mn, 0.061 for Cu, 0.052 for Zn, 0.093 for Sr, 0.050 for Cd, 0.030 for ^{137}Ba, 0.010 for ^{138}Ba, and 0.006 for Pb . Given there is no strict protocol for treating data falling below instrument LOD (Fairclough et al., 2011; Ben-Tzvi et al., 2007), elements were kept if more than 50% of samples were above LOD as described by Chittaro et al. (2006). Only Cd was below LOD for more than 50% of individual scans, so it was removed from further analyses. Pb and Zn showed evidence of external contamination and were also removed. Good detection, accuracy, and precise concentration estimates were established for ^{24}Mg, Mn, Cu, Sr, and ^{137}Ba.

STATISTICAL ANALYSIS

Basic diagnosis plots of each ratio were visually assessed for normality and homogeneity. Elemental ratios were ln-transformed prior to statistical analysis. Elemental concentrations below the ICP gas blank were removed (n=2) because they are negative and cannot be log-transformed.

Cod of different age classes and spawning groups are likely to have different growth rates (Sherwood et al., 2007: Wijekoon et al., 2009; Gjosaeter and Danielssen, 2011), and this may in turn influence the rate of incorporation of some elements during the formation of the otolith (Campana et al., 2000). To avoid confounding size-specific differences with stock-specific differences, analysis of covariance (ANCOVA) was used to quantify significant effects of length on the concentration of each elemental ratio (Campana et al., 2000). Length was significant in explaining elemental ratios (ANCOVA, $p< 0.001$, Table

3.2), but the nature of the effect differed among areas (Figure 3.2). The elemental concentrations of Mn and Sr showed significant relationships with length in all locations so the common within-group slope from the ANCOVA was subtracted to remove the effect of length. The effect of length on Ba:Ca was significant for HC alone. The relationship of Mg:Ca with length was positive for inshore locations and negative for offshore locations. For these elements the effect of length was not removed.

A multivariate analysis of variance (MANOVA) was performed on the elemental ratios to evaluate the influence of location, year of collection, maturity (mature vs. immature), cohort, and fish mean growth rate (cm/yr and kg/yr) on the otolith elemental signature. Cohorts were assigned based upon the age of the fish upon capture. Pillai's criterion was chosen as the test statistic because it is more robust for unequal sample size and the assumption of similar variance-covariance matrices (Johnson and Field, 1993).

A discriminant function analysis based upon the elemental concentrations and growth rates (cm/yr and kg/yr) was performed to test the ability of these discriminants to classify cod into their respective spawning area over a larger geographical scale for each year (1998, 1999) and for pooled data. Correct reassignment was evaluated for the three models using a jackknife (leave-one out) classification. To assess the variability in elemental signatures over time, two DFAs with jackknife cross-validation were performed using the signatures of one year (1998 or 1999) to predict group membership of individuals from the other.

RESULTS

Otolith signature

Four elements were selected to establish the chemical fingerprint of the otoliths, ^{24}Mg:Ca (Mg:Ca hereafter), Mn:Ca, Sr:Ca, and ^{137}Ba:Ca (Ba:Ca hereafter) because their concentrations differed significantly among groups (Figure 3.3). The multivariate elemental signatures of cod otoliths differed between locations (MANOVA, Pillai's Trace=0.462, F=29.3, $p < 0.001$), year of collection (Pillai's Trace=0.019, F=3.09, p= 0.015), cohort (Pillai's Trace=0.071, F=2.32, p= 0.001), and maturity status (Pillai's Trace=0.039, F=6.40, $p< 0.001$). Signatures were also related to the growth (cm/yr, kg/yr) of the fish (Pillai's Trace=0.018, F=2.95, p= 0.02 and Pillai's Trace=0.016, F=2.68, p= 0.031 respectively).

The variability in elemental concentration observed among spawning areas was greater than among years (Figure 3.4) and accounts for most of the variance (Table 3.3). Among elements, statistical relationships with the various factors varied. For example, the Mg concentration is almost equally influenced by location, the year of collection, fish cohort and maturity status but not by the mean growth rate, with a low explanation of variance (Table 3.3). Sr and Ba concentrations were most associated with location and maturity but not with fish mean growth rate. In contrast, Mn concentration was influenced by fish maturity status and growth rate (cm/yr and kg/yr) in addition to location.

Despite some differences is elemental concentrations between years (Figure 3.4), the multiple comparison Dunnett's T3 test indicated that each element could separate at least two of the four spawning groups when years were pooled (Figure 3.3). Sr and Ba, known to vary with environmental conditions, differed among spawning areas considering BH and SS as a single inshore site. Unexpected Sr and Ba concentrations were observed for the offshore HC spawning area with values more closely related to inshore (mainly BH) and estuarine values than fully marine offshore signatures. A clear inshore-offshore split was observed in Mn:Ca otolith concentrations with HC and O3HC cod expressing higher Mn concentrations than inshore cod (BH and SS) .

Spawning group classification success

Results of the MANOVA emphasized the impact of ontogeny and physiology on otolith elemental signatures, thus their quantitative estimates (growth rates) were included in the DFA model. The quadratic discriminant function analysis based on the Mg, Mn, Sr, and Ba ratios to Ca and growth rate (cm/yr and kg/yr) correctly assigned fish to their location of capture with 70%, 67% and 66% success for the 1998, 1999 and pooled data respectively (Table 3.4). Cross year classifications were less successful in separating all four groups, averaging 51%.

For the pooled data, eigenvalues of the first and second canonical roots were 0.955 and 0.342 and respectively accounted for 72.3% and 25.9% of the variance of the correlation variates (total of 98.2%). Fish growth rate (kg/yr) was the strongest discriminant for all functions followed by Sr and Ba. The first discriminant function segregated the inshore

from the offshore spawning areas while the second function separated the northern spawning area of HC from its southern counterparts (Figure 3.5).

The otolith signature of each cod was plotted based upon the two first canonical scores to provide a graphical representation of spawning area differences and visualize the multivariate fingerprints along with 50% confidence ellipsoids around the centroids of each distribution (Figure 3.5). Correct assignment was highest for offshore locations with 89% for HC in the north and 68% for O3HC in the south. Cod from BH was poorly classified (41%) resulting in a great overlap with other regions in the DFA plot. The BH signature was also largely responsible for the poor cross year classifications. Hence the two closely related inshore locations at BH and SS were pooled, which resulted in classification success of group signatures improving by 12%, and cross year classification by 14%.

Discussion

Effective rebuilding strategies for Newfoundland and Labrador cod stocks require knowledge of population structure, connectivity, and the migration patterns of the disparate spawning aggregations that contribute to the fisheries. The complex stock structure of Atlantic cod and evidence of metapopulations in this region (Smedbol and Wroblewski, 2002; Rose et al., 2011), coupled with the reality that few if any cod fisheries therein can be confined to a single spawning group, makes such knowledge essential. This is the first study to evaluate the use of otolith chemistry signatures as a

means to discriminate cod from some of these spawning areas. The results of this study demonstrate that this is possible, not only at the large scales of separation between HC and O3HC but between adjacent inshore and offshore fish, even within the same management unit if biological data (growth rates) are added to the model to account for variations attributable to cohort and age differences. Using LA-ICP-MS to provide a chemical signature corresponding to the time of capture, a specific fingerprint has been established for each spawning area from the pooled elemental ratios of Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca. Signatures along with mean growth rates enabled classification of fish to its area of capture with 66-89% accuracy when the two closely related inshore sites were pooled.

Spatial variations in signature

The identification of fish stocks, subgroups, or spawning aggregations relies on the assumption that otolith signatures vary according to the physico-chemical properties of the ambient environment (Campana, 1999; Elsdon et al., 2008). In the present study, location accounted for most of the individual variation in the elemental concentrations, but the effect of fish maturity and growth was also important and should not be ignored in future research using otolith chemical signatures.

The canonical discriminant functions provide clues as to the causes of the differences and similarities among otolith signatures of the various locations, with the first two functions explaining nearly 98% of the variation. Though the axes may result from a combination of factors, the first function separates the inshore spawning areas from their offshore

counterparts. The otolith signature of HC and O3HC, both offshore spawning areas but near the extremes of the north-south distribution of cod in these waters, exhibited negative scores, while signatures of the two inshore spawning areas showed positive scores.

The present results indicate that both Ba and Sr concentrations varied across salinity gradients, as was expected (Walther and Linburg, 2012; Campana 1999). Ba concentrations are generally higher in freshwater environments, while Sr concentrations assumed to be lower, with the exception of some marine systems (Walther and Limburg, 2012). The elemental ratio of Ba and Sr were dominant in the DFA. Dissolved Sr in freshwater systems originates from the bedrock geological composition and carried by rivers to the ocean (Walther and Limburg, 2012). Although BH and SS are located hundreds of km apart by sea, their waters are separated by a narrow landmass (<3 km at the closest point) and both have high inputs of freshwater from similar geological sources. Their common physical environments likely explains both the distinguishing of inshore vs. offshore locations and why pooling these sites increased successful classification considerably. It is evident from these results that otolith chemistry cannot resolve all spatial classifications, but requires differences in some environmental elements or conditions, which were for the most part lacking between SS and BH.

High Ba concentrations were observed for three spawning groups SS, HC and BH. Considering that freshwater inputs can significantly alter the elemental concentration in otoliths (DiMaria et al., 2010), the higher concentration of Ba:Ca observed for the inshore

spawning locations in Newfoundland and Labrador waters (SS, BH) is likely explained by the increase freshwater inputs at these sites. In contrast, the relatively high Ba values at the HC offshore spawning area is more difficult to explain. However, the dominant Labrador Current that influences the HC site has relatively low salinity (Petrie and Anderson, 1983; Smith et al., 1937), with a flow exceeding the freshwater input from the St. Lawrence River (Petrie and Buckley, 1996). The freshwater content and transport of the Labrador Current could explain why HC has a signature closer to the inshore spawning areas of SS and BH which also receive freshwater inputs. In addition, the sea-ice cover over the area during the winter varies from year to year and may affect the transport of freshwater in the years following an abundant sea-ice cover (Deser et al., 2002) and alter the abundance of dissolved elements. Accordingly, the influence of three currents (the Gulf Stream, the Labrador and the Gulf of St. Lawrence) on the physico-chemical properties of water around O3HC is unpredictable and may be responsible for the uncommonly low Sr concentration at this offshore location.

On the second canonical function, SS, BH and O3HC exhibit negative scores whereas HC exhibited a positive score. HC had the highest classification success with 89% correct assignment. Hawke Channel was the most northerly and deepest spawning area studied (>300m), which may have influenced this result. But its well-distinguished signature likely reflects its environment conditions that differ from the other areas studied. Moreover, HC cod also have natural histories and feeding habits that differ from fish in the other regions (Brown, 1999). In particular, the higher Mg:Ca content at HC could be related to maturation at a younger age (Anderson and Rose, 2001) and differing feeding

habits, with HC fish, at least during the time of this study, eating mostly Pandalid shrimp (Sherwood et al., 2007; Krumsick and Rose, 2012). These factors contribute to slower growth rates and lower condition than their southern counterparts (Fudge and Rose, 2008b). The concentration of Mg has previously been attributed to metabolic effects (DiMaria et al., 2010) or physiology (Sturrock et al., 2012). In addition to differences in natural history, HC residents are generally recognized as being genetically distinguishable from the other, more southerly distributed and more homogeneous groups studied (Rose et al. 2011). This implies less mixing of HC fish with the other groups than among the other three groups, which is consistent with its superior classification success.

Corrections for the effect of fish length on the elemental concentrations of Mn and Sr were essential prior to inter-site comparisons of Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca. It is noteworthy that no generalisations could be made on the impact of size on the concentrations of Mn:Ca and Sr:Ca because the relationships between the concentration of these elements and length varied across spawning groups. The factors that could have influenced the growth patterns are several, including water temperature, fish diet and maturity schedules (Kalish, 1989), and any or all may have played a role in elemental incorporation. Several studies have demonstrated the effect of somatic and otolith growth on Mg, Sr, and Ba incorporation which appears to be species dependent and show mixed relationships (Sadovy and Severin, 1994; Bath et al., 2000; Martin and Thorrold, 2005; Miller, 2009; DiMaria et al., 2010) with elemental ratios. My results are consistent with these reports and found that relationships between fish length and elemental concentration

can vary among populations of a species that inhabit different environments and have differing prey, condition and growth status.

The effect of ontogeny on the incorporation of elements onto the otolith is still unknown for many species. Walther et al., (2010) demonstrated Sr and Ba incorporation varied depending on life history stage in a tropical damselfish (*Acanthochromis polycanthus*) and this could also be true for cod because the effect of maturity was significant on all elemental ratios (Mg, Mn, Sr, and Ba) in this study. Accounting for growth may partially deal with this factor, as was done here, but further study of this aspect should be pursued as the present data were almost all mature fish.

Physiology may also impact the incorporation of specific elements (Mg, Mn) onto the otoliths (DiMaria et al., 2010; Sturrock et al., 2012). This could be the case for cod as both growth rates (length, weight) had significant influence on Mn concentrations. It is thus important to be cautious about future interpretations and uses of the elemental signatures obtained by combining fish of different ages, maturity stages and growth rates (Walther et al., 2010). It is important to note that growth rate (kg/yr) was the strongest discriminant factor across all functions and points to the benefits of adding biological information to discriminant models. Further studies evaluating the effect of ontogeny and comparing elemental signatures across life stages may be required before using otolith signatures on their own to infer cod movements and connectivity across spawning groups.

Temporal variations in signature

For an otolith signature to be used as a natural tag of fish of different origins, the signature observed at a location must be maintained over time. The data presented here indicate that variation across spawning areas was larger than variation between years. However, differences in otolith signatures across years were noted, and at least among the initial four groups cross year classification was poor, which calls into question the temporal stability of the otolith signatures of the spawning groups. The increased success of merging the similar inshore groups at SS and BH partially ameliorates that concern. Nevertheless, a longer study would be required to address this effect more thoroughly, in particular any drift in signatures that could occur with changing climate.

Reference

Anderson, J.T., Rose, G.A., 2001. Offshore spawning and year-class strength of northern cod (2J3KL) during the fishing moratorium, 1994-1996. Can. J. Fish. Aquat. Sci. 58(7), 1386-1394.

Atkinson, D.B., Rose, G.A., Murphy, E.F., Bishop, C.A., 1997. Distribution changes and abundance of Northern cod (Gadus morhua), 1981-1993. Can. J. Fish. Aquat. Sci. 54(Suppl. 1), 132-138.

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

Ben-Tzvi, O., Abelson, A., Gaines, S. D., Sheehy, M. S., Paradis, G. L., Kiflawi, M., 2007. The inclusion of sub-detection limit LA-ICPMS data, in the analysis of otolith microchemistry, by use of a palindrome sequence analysis (PaSA). Limnol. Oceanogr. 5, 97-105.

Bradbury, I.R., DiBacco, C., Thorrold, S.R., Snelgrove, P.V., Campana, S.E., 2011. Resolving natal tags using otolith geochemistry in an estuarine fish, Rainbow smelt Osmerus mordax. Mar. Ecol. Prog. Ser. 433, 195-204.

Brattey, J., 1997. Biological characteristics of Atlantic cod (*Gadus morhua*) from three inshore areas of northeastern Newfoundland. NAFO Sci. Coun. Stud. 29, 31-42.

Ruzzante, D.E., Taggart, C.T., Doyle, RW., Cook, D., 2001. Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size 1964-1994. Cons. Gent. 2 (3) 257-269.

Ruzzante, D.E., Taggart, C.T., Lang, S., Cook, D. 2000. Mixed-stock analysis of Atlantic cod near the Gulf of St. Lawrence based on microsatellite DNA. Ecol. Appl. 10(4), 1090-1109.

Ruzzante, D., Taggart, C., Cook, D.,1999. A review of the evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. Fish. Res. 43(1-3): 79-97.

Ruzzante, D., Taggart, C., Cook, D. (1998) A nuclear DNA basis for shelf- and bank-scale population structure in Northwest Atlantic cod (*Gadus morhua*): Labrador to Georges bank. Mol. Ecol. 7(12): 1663-1680.

Sadovy, Y., Severin, K., 1994. Elemental patterns in red hind (*Epinephelus guttatus*) otoliths from Bermuda and Puerto-Rico reflect growth-rate, not temperature. Can. J. Fish. Aquat. Sci. 51(1), 133-141.

Schiermeier, Q., 2003. Europe dithers as Canada cuts cod fishing. Nature. 423(6937), 212-212.

Secor, D. 1992. Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay Striped bass *Morone-saxatilis*. Fisheries Bulletin. 90(4), 798-806.

Secor, D.H., Henderson-Arzapalo, A., and Piccoli, P.M. 1995. Can otolith micro-chemistry chart patterns of migration and habitat utilization in anadromous fishes? Journal of Experimental Marine Biology and Ecology. 192(1), 15-33.

Sévigny, JM., Valentin, A., Talbot, A., and Ménard, N., 2009. Connectivity between Saguenay Fjord populations and those of the Gulf of St. Lawrence. J. Water Sci. 22(2), 315-339.

Sherwood, G., Fudge, S., Rose, G., 2007. Influence of diet on growth, condition and reproductive capacity in Newfoundland and Labrador cod (*Gadus morhua*): Insights from stable carbon isotopes (delta C-13). Deep-Sea Res. Part II-Trop. Stud. Oceanogr. 54(23-26), 2794-2809.

Silva, D.M., Santos, P., and Correia, A.T. 2011. Discrimination of *Trisopterus luscus* stocks in northern Portugal using otolith elemental fingerprints. Aquat. Liv. Res. 24(1), 85-91.

Smedbol, R., Wroblewski, J., 2002. Metapopulation theory and Northern cod population structure: Interdependency of subpopulations in recovery of a groundfish population. Fish. Res. 55(1-3), 161-174.

Standish, J.D., Sheehy, M., Warner, R.R., 2008. Use of otolith natal elemental signatures as natural tags to evaluate connectivity among open-coast fish populations. Mar. Ecol. Prog. Ser. 356, 259-268.

Steer, M.A., Fowler, A.J., Gillanders, B.M., 2009. Age-related movement patterns and population structuring in southern garfish, *Hyporhamphus melanochir*, inferred from otolith chemistry. Fish. Manag. Ecol. 16, 256-278.

Stransky, C., Garbe-Schoenberg, C., Guenther, D., 2005. Geographic variation and juvenile migration in Atlantic redfish inferred from otolith microchemistry. Mar. Fresh. Res. 56(5), 677-691.

Strnad, L., Ettler, V., Mihaljevic, M., Hladil, J., Chrastny, V., 2009. Determination of trace elements in calcite using solution and laser ablation ICP-MS: Calibration to NIST SRM glass and USGS MACS carbonate, and application to real landfill calcite. Geostand. Geoanal. Res. 33(3), 347-355.

Sturrock, A., Trueman, C., Darnaude, A., Hunter, E. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? J. Fish Biol. 81(2), 766-795.

Syedang, H., Andre, C., Jonsson, P., Elfman, M., Limburg, K. E., 2010. Migratory behaviour and otolith chemistry suggest fine-scale sub-population structure within a genetically homogenous Atlantic cod population. Environ. Biol. Fish. 89(3-4), 383-397.

Sylvester, P.J. 2001. Laser-ablation-ICPMS in the earth sciences: Principles and applications. Mineralogical Association of Canada, Ottawa, Ont., Canada.

Taggart, C. T., P. Penney, N. Barrowman, C. George., 1995. The 1954-1993 Newfoundland cod-tagging database: statistical summaries and spatial-temporal distributions. Can. Tech. Rep. Fish. Aquat Sci., 2042.

Tanner, S. E., Vasconcelos, R. P., Reis-Santos, P., Cabral, H. N., Thorrold, S. R., 2011. Spatial and ontogenetic variability in the chemical composition of juvenile common sole (*Solea solea*) otoliths. Estuar Coast. Shelf Sci. 91(1), 150-157.

Tanner, S. E., Reis-Santos, P., Vasconcelos, R. P., Franca, S., Thorrold, S. R., Cabral, H. N., 2012a. Otolith geochemistry discriminates among estuarine nursery areas of *Solea solea* and S. *senegalensis* over time. Mar. Ecol. Prog. Ser. 452, 193-203.

Tanner, S. E., Vasconcelos, R. P., Cabral, H. N., Thorrold, S. R., 2012b. Testing an otolith geochemistry approach to determine population structure and movements of European hake in the northeast Atlantic Ocean and Mediterranean sea. Fish. Res.125, 198-205.

Templeman, W., 1966. Marine resources of Newfoundland. Bull. Fish., Res. Bd Can., 154, 170pp.

Thibault, I., Hedger, R.D., Dodson, J.J., Shiao, J., Iizuka, Y., Tzeng, W., 2010. Anadromy and the dispersal of an invasive fish species (*Oncorhynchus mykiss*) in eastern Quebec, as revealed by otolith microchemistry. Ecol. Fresh. Fish. 19(3), 348-360.

Thorisson, K., Jónsdóttir, I.G., Marteinsdottir, G., Campana, S.E., 2011. The use of otolith chemistry to determine the juvenile source of spawning cod in Icelandic waters. ICES J. Mar. Sci. 68(1), 98-106.

Thorrold, S. R., Jones, C. M., Campana, S. E., 1997. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). Limnol. Oceanogr. 42(1), 102-111.

Thorrold, S.R., Latkoczy, C., Swart, P.K., Jones, C.M., 2001. Natal homing in a marine fish metapopulation. Science 291, 297-299.

Thorrold, S., Jones, C., Swart, P., Targett, T., 1998. Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. Mar. Ecol. Prog. Ser. 173, 253-265.

Tilman, D. 1996. Biodiversity: Population versus ecosystem stability. Ecology 77: 350–63.

Townsend, D., Radtke, R., Malone, D., Wallinga, J., 1995. Use of otolith strontium-calcium ratios for hindcasting larval cod *Gadus morhua* distributions relative to water masses on Georges-bank. Mar. Ecol. Prog. Ser. 119(1-3), 37-44.

Townsend, D., Radtke, R., Corwin, S., Libby, D., 1992. Strontium-calcium ratios in juvenile Atlantic herring *Clupea-harengus L* otoliths as a function of water temperature. J. Exp. Mar. Biol. Ecol. 160(1), 131-140.

Turan, C., 2006. The use of otolith shape and chemistry to determine stock structure of Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). J. Fish Biol. 69(Suppl. C), 165-180.

Vasconcelos, R.P., Reis-Santos, P., Tanner, S., Fonseca, V., Latkoczy, C., Guenther, D., Costa, M.J., Cabral, H., 2007. Discriminating estuarine nurseries for five fish species through otolith elemental fingerprints. Mar. Ecol. Prog. Ser. 350, 117-126.

Veinott, G., 2001. The use of laser ablation-ICP-MS in the environmental sciences in laser ablation-ICP-MS, in: Sylvester, P.J., Earth Sciences: Principles and applications. Mineralogical Association of Canada Short Course Series.

Veinott, G., Porter, R., 2005. Using otolith microchemistry to distinguish Atlantic salmon (*Salmo salar*) parr from different natal streams. Fish. Res. 71(3), 349-355.

Veinott, G.I., Porter, T.R., Nasdala, L., 2009. Using mg as a proxy for crystal structure and Sr as an indicator of marine growth in vaterite and aragonite otoliths of aquaculture Rainbow trout. Trans. Am. Fish. Soc. 138(5), 1157-1165.

Walther, B., Thorrold, S., 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. Mar. Ecol. Prog. Ser. 311, 125-130.

Walther, B.D., Kingsford, M.J., O'Callaghan, M.D., McCulloch, M.T., 2010. Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. Environ. Biol. Fishes. 89(3-4), 441-451.

Wang, Y., Ye, Z., Liu Q., Wang, W., Cao L., Shen, W., 2011. Otolith chemical signatures of spottedtail goby *Synechogobius ommaturus* in coastal waters of China. Chinese J. Oceanol. Limnol. 29(3), 640-646.

Warner, R., Swearer, S., Caselle, J., Sheehy, M., Paradis, G., 2005. Natal trace-elemental signatures in the otoliths of an open-coast fish. Limnol. Oceanogr. 50(5), 1529-1542.

Wijekoon, M.P., Puvanendran, V., Ings, D.W., Brown, J.A., 2009. Possible countergradient variation in growth of juvenile cod *Gadus morhua* from the northwest Atlantic. Mar. Ecol. Prog. Ser. 375, 229-238.

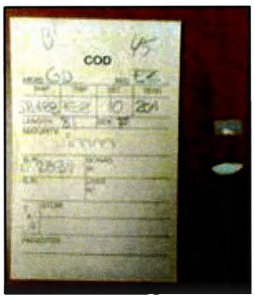
Windle, M.J.S., Rose, G.A., 2007. Do cod form spawning leks? Evidence from a Newfoundland spawning ground. Mar. Biol. 150(4), 671-680.

Windle, M.J.S., Rose, G.A., 2005. Migration route familiarity and homing of transplanted Atlantic cod (*Gadus morhua*). Fish. Res. 75(1-3), 193-199.

Windle, M.J.S., Rose, G.A., Devillers, R., Fortin, M.-J., 2012.Spatio-temporal variations in invertebrate-cod-environment relationships on the Newfoundland-Labrador shelf, 1995-2009. Mar. Ecol. Prog. Ser. 469, 263-278.

Appendix I: Materials and Procedures

OTOLITH PREPARATION



Material:

- ✓ Lead pencil
- ✓ Protective equipment (lab coat, goggles, gloves)
- ✓ Toothbrush
- ✓ Denatured ethanol (95%)

Procedures

1. Take one whole cod sagittal otolith from the collection of aged otoliths
2. Randomly assign each otolith to a group (14-21 otoliths/group)
3. Assign a letter code to each otolith group (A,B,C,…)
4. Add a number code for otoliths part of each group (A1, A2, A3, …)
5. This process randomizes samples to be analysed via LA-ICP-MS

Each group of otolith will be glued together on a petrographic slide and analysed via LA-ICP-MS. Randomization of samples prevents bias in measurement of chemical composition.

6. Put protective gloves and goggles if needed, wear lab coat
7. Take each otolith at a time and remove adhering tissue by soaking in distilled water and gently brushing with a toothbrush
8. Repeat the procedure with 95% ethanol

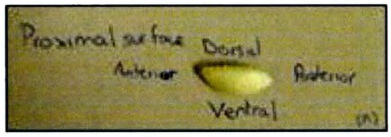
IMAGE ANALYSIS

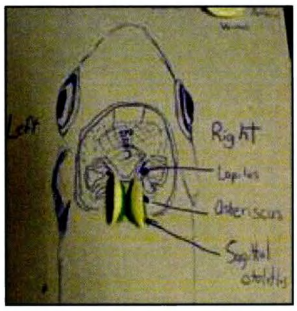
Material

- ✓ Digital stereomicroscope (Olympus SZ16 coupled to a DP72 camera)
- ✓ Black stage plate
- ✓ Light source
- ✓ Denatured ethanol (95%)
- ✓ Small petri dish
- ✓ Tape
- ✓ Image acquisition and analysis software (CellSens)

Procedures:

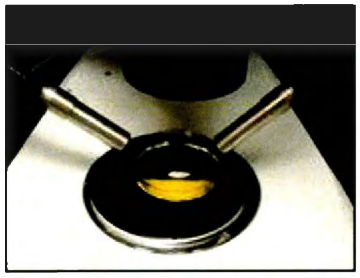
1. Determine if otolith is left or right





Note: Posterior side is usually the elongated part while the anterior, the ventral the longest arc.

2. Switch to dark field mode or add a black stage plate as a background
3. Place the otolith in a petri dish with 90%-95% ethanol
4. Begin with the distal side
5. Put the ventral side along the tape mark and the anterior side facing left
6. Use reflected light from above at a more or less 45 degree angle

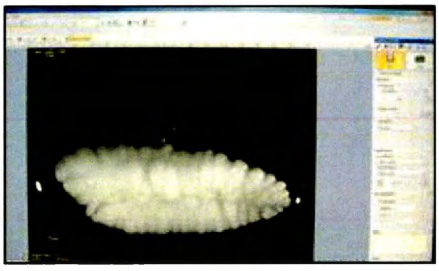


Try to avoid moving the reflected light once it is good, keep for all otoliths and keep the power low

7. Close all surrounding light for better results
8. Pull out the screw on the right hand side of the stereoscope to switch from stereoscope to camera mode
9. Open CellSens
10. Select the Acquisition tab→ Camera Control → Press the "LIVE" button
11. Use the 0.5 Lens and a magnification of 2 (maybe modified for large otoliths)

Image Adjustments in CellSens

12. Put VIEW to 100%
13. Adjust surrounding light if necessary
14. Select the ISO 200 parameter



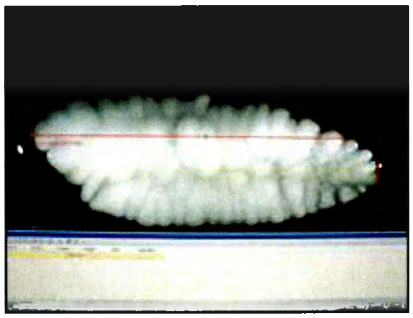
Keep the aperture of the iris in the middle or adjust slightly for better contrast

15. Use the fine knob adjustment to fine tune for same-size otoliths
16. Select greyscale 8 bit mode to capture the picture
17. Select either dark contrast in the DP72 section of camera control or customise greyscale by choosing G & B
18. Add focus tool by clicking on the second icon in the camera toolbar
19. Look at the screen to adjust the image

20. Note: since otolith is not flat, it is impossible to be in focus for all regions
- ✓ For the proximal side, try to see the sulcus asticus & the clearest contour definition as possible
- ✓ For the distal side, try to see as much detail and the contour of the spherical structures at the center of the otolith

21. Take a snap of the image (if the picture is good proceed, if not start over the image adjustments)
22. Save the picture by giving cod number, sampling group, left or right and side of otolith and spawning group as a name
23. Repeat step 3-20 for the proximal side

Measurements in Cell Sens



24. Select the measure tab→Arbitrary line
25. Click once to start taking the measure
26. Measure full length
27. Keep the horizontal line in a complete straight line
28. Click again to complete the measure
29. You should see it appear at the bottom in a table
30. Repeat steps 22-26 to measure the height of the otolith
31. Record in written form in the lab database & in the excel file
32. Record the magnification zoom, length and width
33. Select "Process" → "Enhancement"
    - Maximize contrast
    - Optimize contrast (allows you to play with grey, white & black balance)
34. Select the "Adjust display" tab
    - Contrast
    - Luminosity
    - Gamma balance
35. Select "Process" → "Sharpen mask"
36. Allows to remove noise around the contour of the otolith
37. Save the picture with modification in tiff format

MATERIAL DECONTAMINATION

Material:

- ✓ Protective equipment (lab coat, goggles, acid-resistant rubber gloves)
- ✓ Denatured ethanol (95%)
- ✓ NanoPure water (Distilled reverse osmosis water)
- ✓ Positive pressure chamber (Clean cell)
- ✓ Fume hood
- ✓ Large glass or polypropylene beakers (4 x 2L and 4L bath with lid)
- ✓ 12N Hydrochloric acid
- ✓ Plastic or Teflon tongs
- ✓ Plastic (polyethylene) vials (each vial can be used to decontaminate 3 otoliths)

Procedures:

1. Put protective equipment on
2. Soak all plastic equipment in 95% ethanol
3. Rinse with MilliQ/NanoPure* water
4. Put in plastic bag
5. Carry over to Clean Cell room

Clean Cell Room

6. Air dry in a positive pressure chamber

Acid Bath

7. Put on the acid-resistant rubber gloves, lab coat, & goggles

Under the fume hood

8. Dilute 12N HCL trace metal grade with MilliQ water, by adding the acid to the water (1:1 ratio)
9. Pour 800mL of MilliQ water into the 2L beakers
10. Pour 800 ml of 12N HCL in the beaker
11. Cover up the bath with a plastic bag (or lid)
12. Soak plastic equipment for at least 8 hours in 6N HCL
13. Use the plastic tongs to take items out of the bath
14. Rinse 3 times with Milli Q
15. Poor new MilliQ water in the small beaker and rinse the vials in it 2 times
16. Immerse the equipment for another 4 hours in Milli Q bath
17. Cover the beaker with a plastic bag (clean cell or bench top)
18. Rinse 3 times with Milli Q (as in step 12)
19. Air-dry in the Clean Cell

OTOLITH DECONTAMINATION

Material:

- ✓ Acid washed vials
- ✓ Acid washed toothbrush
- ✓ Distilled deionized reverse osmosis water (NanoPure or MilliQ)
- ✓ Positive pressure chamber (Clean cell)
- ✓ Permanent markers
- ✓ Rubber elastic bands
- ✓ Ultrasonic cleaner
- ✓ Plastic bags (for transport)

Procedures:

1. Handle all material with acid-washed instruments and wear plastic gloves
2. Put the whole otolith in a clean tube
3. Label the tube and its matching lid with the otolith group number
4. Fill the vial with Milli Q
5. Sonify the samples in groups of 5, so attach 5 tubes with an elastic band and place them in the ultrasonic bath for 5 minutes
6. Scrub the otolith with an acid-washed toothbrush under running Milli Q water for 1 minute
7. Triple rinse the otolith with Milli Q
8. Place the otolith back in its vial with fresh Milli Q water
9. Sonify for 3 minutes
10. Triple rinse with Milli Q
11. Place otoliths in their caps & air dry in Clean cell for 18-24 hours
12. The next day, close the lids and bring the otolith up to the lab to be weighted & embedded

WEIGHING OTOLITHS

Material:

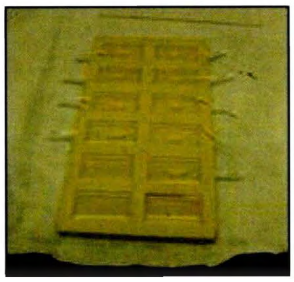
- ✓ Acid washed petri dish
- ✓ Acid washed forceps
- ✓ Top loading scale 0.001g

Procedures:

1. Turn on scale
2. Let it warm up for 1 hour before use
3. Place a petri dish on the scale
4. Tare scale with weight of petri dish (press Re-zero button)
5. Place the otolith to be weighed in the dish using plastic forceps
6. Wait until weight stabilises
7. Record weight of otolith

EMBEDDING

Material



- ✓ Fume hood
- ✓ Ultrasonic cleaner
- ✓ Protective equipment
- ✓ Paintbrush
- ✓ Frekote releasing agent
- ✓ Pencil and paper strips
- ✓ Epoxy resin and hardener (Epothin from Buehler)
- ✓ Plastic cups
- ✓ Popsicle sticks
- ✓ 12-well moulds
- ✓ Disposable plastic pipettes
- ✓ Toothpicks

Procedures:

Under the hood

1. Put lab gloves and lab coat, safety goggles if needed
2. Place paper towel on the bench of the fume hood to protect it
3. Prepare mold by paintbrushing it with a releasing agent (Frekote)
4. Put to dry

While waiting

5. Prepare labels for each sample & set aside otoliths for one mold
6. Labels are made using the group code (A1, A2, etc.)

When dried

7. Mix epoxy resin (Buehler Epothin resin) with epoxy hardener (Buehler Epothin hardener) in a 5:2 (35g : 14g gives enough epoxy for 12 wells)
8. Mix with popsicle stick for 2 to 3 minutes
9. Bring it over to the ultrasonic cleaner and leave it for 5 minutes

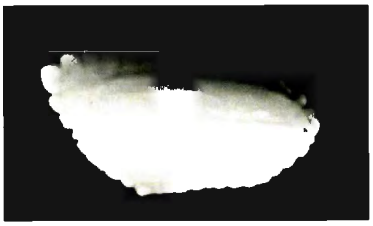
Be careful, the plastic cup may float so put something on top to make it stay in the bath without tipping over (plastic petri dish cover can be used with a weight on top)

While waiting

10. Put each label in the hole with its otolith, proximal side facing up

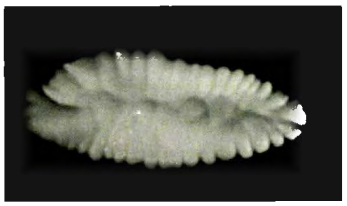
Remember always handle otolith using plastic clean (acid washed) material

Proximal cod otolith surface viewed under 0.5X magnification using Olympus SXZ16 stereoscope mounted with the DP72 camera.



11. Pour the epoxy in the main well leaving a little space
12. Remove bubbles stuck on the otolith using a toothpicks and pipettes,
13. Don't forget to turn over the otolith- it must be done quickly
14. Fill the rest of the well with epoxy
15. Place the otolith with the distal side up and the anterior tip on the left side
16. Make sure the otolith is straight in the well, it makes it easier to section

Distal cod otolith surface viewed under 0.5X magnification using Olympus SXZ16 stereoscope mounted with the DP72 camera.



17. Leave it to dry under the hood for 8-12 hours (see epoxy recommendations)
18. Remove from under the hood
19. Unmold (after 8-24 hours)
20. This epoxy hardens fast and otolith samples can be unmoulded after 8 hours. However it is recommended to let them dry for at least 24 hour before sectioning them.
21. Leave them to dry for 3 days (72 h) before sectioning

Again all our samples were cut after letting the epoxy dry 72 hours but trials were made and samples were can be cut in 24 hours if left to dry in an oven.

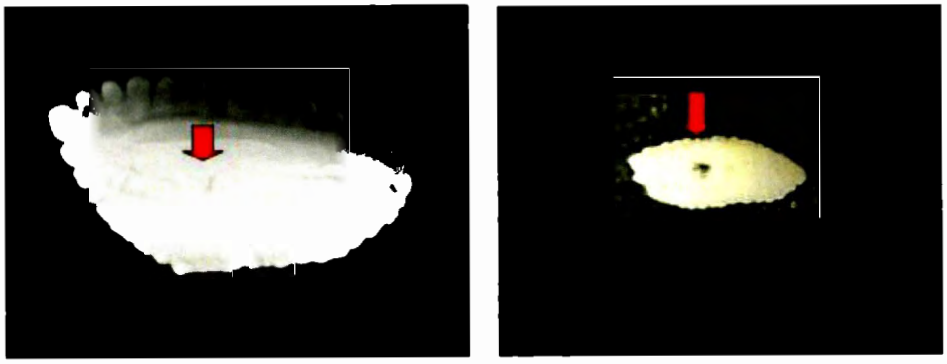
FINDING THE CORE FOR SECTIONING

Material

- ✓ Permanent marker
- ✓ Diamond pen (engraving tool)

Procedures:

The core can be found easily by looking at the proximal side. On the sulcus there should be a small depression located close to the middle but slightly towards the anterior end of the otolith along the groove (Figure 1). Draw a line with the engraving pen on the embedded otolith over the core and mark the two edges of the otolith where the cut should be with a permanent marker.



The proximal view of an otolith with the red arrow pointing to the core (on the left). The right image shows a marked core of an otolith and the location where the sectioning will occur.

SECTIONING

Material:

- ✓ Fume hood
- ✓ Protective equipment
- ✓ Paintbrush
- ✓ Absorbent paper
- ✓ Isomet slow speed diamond blade Buehler saw
- ✓ 3-4 diamond blades
- ✓ 0.6mm-1mm spacers
- ✓ Isomet fluid or water
- ✓ Callipers
- ✓ Permanent marker
- ✓ Diamond pen

Procedures:

1. Put absorbent paper underneath the Isomet saw to protect the bench top if required

2. Put together the sections of the Isomet low speed diamond saw

Assembling the slow speed saw for sectioning from left to right. Begin with long black stopper, then the large blade guard, the blade, a blade spacer (red arrow) of 1mm thickness or less, another blade, another blade spacer, a third blade, another large blade guard, the short black stopper and the screw (a 4th blade may be added to ensure the section was at the core). Blade spacers will determine the width of the section. Note: you should always tighten the screw with your fingers; if it is too tight it may over-use the blades.

3. Fill bath with Milli-Q water up to the first inner ring of the blade (or Isomet fluid)
4. The first time you cut, begin with a blank to test out the spacers' width and follow steps 5-10. Measure the width of the sections of the blank with a pair of callipers and make sure it fits with the spacer's width (1 mm or 0.6 mm spacers used at CFER).

For all samples:

5. Lift arm place the piece to section in the clamp
6. Put the flat portion of the piece towards the blade and keep the proximal surface facing up and the anterior part of the otolith to the left
7. Screw the clamp loosely
8. Verify the mark (indicating the core) and make sure it is aligned with the cut
9. Once aligned screw up the clamp, lower the arm and make sure it is a good fit
10. Lift the arm up and start the saw at speed between 6 -8
11. Make sure safety stop is at good distance
12. Lower the arm until it touches the blade and let gravity work its way through the sample
13. When it is nearly done, lower the speed to about 3
14. When the sample starts to wobble & water is coming through it

15. Stay close and make sure the saw stops when the cut is done if it stops before adjust the safety stop
16. Remove the outer part slowly to release the sections
17. You may need the scalpel to cut a few segments again
18. Repeat 7-13 with a new sample
19. While waiting, use the diamond pen to label the section & each part of the embedded otolith
20. The 1st section should be at the core while the 2nd cut is a little off. Be consistent, put the group code and add 1 for the first section & 2 for the second section (ex: A1 1, A12)
21. Cut the extra epoxy around the otolith off (be careful not to break the otolith)
22. Place section 1 into the labelled tube, the 2nd in an otolith envelope labelled with cod number and group number. Place the left over sample back in the bag with the tubes.

SECTION ANALYSIS AND MEASUREMENT

Material:

- ✓ Digital stereoscope
- ✓ Petri dish
- ✓ Denatured ethanol (95%)
- ✓ Diamond pen
- ✓ Permanent markers
- ✓ Jewelry polishing paper

Procedures:

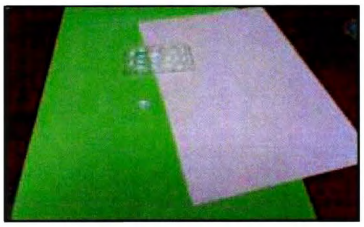
1. Look for the core under the stereoscope (on the section marked with a 1)
2. May need to polish sample if cannot find core
3. Proceed to imaging as for shape
4. Place the otolith sections in the petri dish
5. Fill with ethanol (90-95%)
6. Position the sulcus side down and the longest axis on the horizontal plane
7. Take a picture on both sides of the sample
8. If core is not seen, polish sample and snap pictures on both sides again

9. Label the picture using the group name , whether it is the core or the second section, side 1 (writing) or side 2 (reverse) and whether it was polished or not
    - ✓ JBB1_core_2p means JBB1 the section is from the core, on the reverse side and it was polished
    - ✓ A3_section_1 means sample is from group A, sample 3, the 2nd section was used and no polishing was done

10. Find the core (nucleus)
11. Count the number of increments seen (ageing)
12. Record whether the last increment is hyaline zone or not
13. Measure width and height of the entire section
14. Measure the width of the increments that will be assayed via LA-ICP-MS to have an idea of the laser spot size to use (last increment)
15. For best result choose three axes (long, short, near sulcus) and be consistent

POLISHING FOR IMAGE ANALYSIS

Material:



- ✓ Jewelry polishing paper 3µm and 30µm
- ✓ NanoPure or MilliQ water

Procedures:

1. Apply even and gentle pressure on sample
2. First use the green 30µm lapping paper (a few rubs)
3. Then finish with the pink 3µm lapping paper
4. Repeat if needed
5. Rinse with NanoPure or Milli Q water

PHOTO ENHANCEMENT

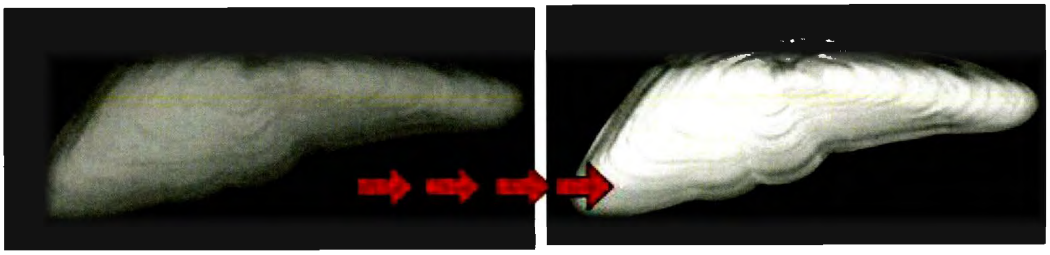
Material:

- ✓ Photoshop software
- ✓ Computer with good screen resolution

Procedures:

1. Open Photoshop

2. Select: Image→ Adjustments→ Levels→ Histogram
   - ✓ This expands the grey levels of the original image
3. Drag white end of histogram in to the point where the white of the otolith starts to register
4. Drag the black end of the histogram in until just before the edge of otolith to start to erode
5. Select: Filter ->Sharpen ->Unsharp mask*
   - ✓ Unsharp mask increases the contrast along the edges by detecting pixels that differ by the threshold
6. Set Unsharp Mask parameters to:
   - ✓ Threshold: 2
   - ✓ Radius (controls blur) : 10-20 (I used 15)
   - ✓ Amount (magnifies light and changes contrast levels): 120-160 (I used 150)



Troubleshoot & Tips

To minimize reflection of light on image, cover the otolith with ethanol -it changes the refraction index. Make sure no dust particles are floating over the otolith when snapping the picture. Don't adjust the image by looking too close to the screen, you should be at a minimum of 0.4m from it.

OTOLITH SECTION PREPARATION

Material

- ✓ Acid washed vials
- ✓ NanoPure or MilliQ water
- ✓ Ultrasonic cleaner
- ✓ Elastic bands
- ✓ Clean cell

Procedures:

1. Handle all material with acid-washed instruments and wear plastic gloves
2. Rinse the sections with Milli Q water
3. Put the sectioned otolith back in its 20 ml vial
4. Fill the vial with Milli Q
5. Rinse 3 times (close lids and shake vial softly)
6. Fill the vial with Milli-Q water
7. Attach 5 vials containing an otolith together with an elastic band and place them in the ultrasonic cleaner for 3 minutes
8. Rinse the otoliths with Milli-Q 3 times
9. Place the section the lid of the vials
10. Let section dry in the clean cell for at least 12 hours

LA-ICP-MS SLIDE PREPARATION

Material:

- ✓ Acid washed forceps
- ✓ Plastic gloves
- ✓ Clean plastic sheets
- ✓ Petrographic glass slides
- ✓ Paper and pencil
- ✓ Double sided tape
- ✓ Permanent felt pen
- ✓ Digital stereoscope
- ✓ NanoPure or MilliQ water
- ✓ Clean cell

Procedures:

1. Wear plastic gloves and use clean (acid-washed) plastic forceps
2. Take all the sectioned otoliths of a group (A, B, etc.)
3. Draw the contour of the ablation cell unto one petrographic glass slide
4. Cover it with plastic sheet to prevent contamination
    - ✓ This is your template to place otoliths on slides to be sampled
5. Place otolith sections of a group one at a time within the area marked by plastic covered slide – it represents the size of the ablation cell
6. The area to be sampled by LA-ICP-MS must fit within the marked area
7. Once all otolith sections are in place, draw a map of their position
8. Take a new slide

9. Mark the slide (top, bottom, right, left) and group letter with a permanent felt pen
10. Flip the slide over
11. Use double sided tape to cover the area ablation cell area
12. Hold the taped slide over the sections placed on the template
    - ✓ Ensure all otoliths are within the area of ablation (marked by circle on template)
13. Press the taped slide onto the otoliths to make them stick to the new slide
14. Place the completed slide over the template to ensure the edge of the otoliths to be sampled via LA-ICP-MS are still within the ablation area, sections may have shifted
15. Remove the otoliths that do not fit within the area
16. Re-draw a new map if needed
17. Place slides under the digital stereoscope and snap a picture – it will be used as am map during LA-ICP-MS sampling
18. Clean each slide by rinsing with Milli Q water 3 times
19. Bring the slides under
20.

LA-ICP-MS ANALYSIS

Material:

- ✓ Laser (Geolas system)
- ✓ Coupled to a plasma mass spectrometer (Finnigan Element XR)
- ✓ Standards (NIST 612 and MACS 1)
- ✓ Prepared slides
- ✓ Map of each slide
- ✓ Hooked tool
- ✓ Slide holder
- ✓ Allen key
- ✓ Helium, Nitrogen and Argon gas tanks
- ✓ Plastic gloves

Procedures:

Turning on LA-ICP-MS system (CREAIT laboratory)

1. In gas room, open the Helium and Nitrogen tanks
2. Return to MAF room and turn on Argon valve – behind the multicollector mass spectrometer
3. Put a parafilm on the plasma to protect it during activation of system and calibration
4. On the power panel, turn the red key first to activate system

5. Turn the black key on the power panel
6. Turn on the computer
7. Once computer is on, unplug the mouse and reconnect it
8. Return to the computer room and load the Thermo Finnigan folder
9. Minimize the network processor file once it has opened
10. Open the Instrument tab
11. Check the Argon symbol is green
12. Turn on the plasma by selecting the "On" button
13. Listen for a grinding sound as the plasma is switched on
14. Each section of the system will become green once they are ready- they pass from red to yellow to green

Tuning system

1. Open "Tuning" window
2. Enter today's date and save it
3. Ensure the plasma is disconnected from the system and covered with parafilm
4. Load the scan list – yellow graph
5. Select the Li_In_U_sd file
6. Change Lithium to Cobalt (Co)
7. Check GE
8. Press "Play" to initiate scan- all values must be black
9. Look at script at bottom to ensure settings are ok

Tuning Values

- ✓ U ≥300
- ✓ In ≥ 200
- ✓ Co is replaced by ^{44}Ca in the scan list and recorded in analog mode
- ✓ ^{44}Ca requires a minimum of 5

10. Ensure washout occurs rapidly
11. Return to MAF room, remove the parafilm and reconnect the plasma

Turn on Laser

1. Go to MAF room and pull out the stage of the ablation chamber
2. On the Geolas computer, enter the password to login
3. Open Geolas software
4. Run homing –check all windows
5. Once done, Open N2 valve, enter 4 in the left box
6. Select: "Adjust F"
7. Return to MAF room and push the stage in
8. Go to destination

Laser parameters

- ✓ Laser beam diameter: 40 μm
- ✓ Laser energy: 4J/cm2
- ✓ Repetition rate: 10Hz
- ✓ 410 pulse/s
- ✓ ±30 s gas blank collection
- ✓ ±41 s of ablation

To use NIST 612 for calibration

1. Turn on system and Geolas laser
2. Find the NIST 612 and create a new point
3. Select an end point and save it
4. In the position table, select both start and end point you just created
5. Copy selection – right click
6. Select the Scanning icon ↓↓
7. Select katelineh.scan
8. A window will appear to paste the points you copied
9. Press Yes to overwrite
10. Change the first 3 readings from the table to 0 (zero)
11. Set stage speed to 2 μm/s and energy to 3 J/cm2
12. Re-select the start position and press "go to"
13. Go in the macro menu (tab)
14. Select activate macro
15. Select kateline h
16. Start
17. Observe the bottom graphs and calibrate
18. The peaks should be as high as possible
19. Adjust X first, Y second and Z third, if issues are noticed
20. When tuning is ok stop scanning (square button) and save it

Otolith chemical composition analysis-sampling

1. Once system is on and calibrated
2. Place the slide to be analysed in the ablation cell
3. Push it down and make sure it is leveled
4. Make sure both standards are also in the cell
5. Close the cell by tightening the bolts with the Allen key
6. Return to the computer room and use the joystick or the arrows to move the automated stage and select areas to sample from your prepared slide

7. Create a new point for NIST 612, label it NIST and save it
8. Find MACS 1 and create a new point, label it MACS1 and save it
9. Find the location of each sample on the otoliths using the map you have drawn of the slide
10. Visually verify the position of each otolith by checking where the laser beam is pointing in the ablation cell – in the MAF room
11. Save each point according to the otolith letter code in the position table
12. Before saving ensure each position is in focus
13. You may need to adjust the stage plate height if samples are not in good focus
14. Once ready to sample, open a new sequence
15. Select a template in the form of <ssss>letter<nn> = au10b20
16. Au=letter of month, 10=date, letter= sequence, 20= order of sample in the sequence (1 = 1st, 18= 2nd to last; 1-20)
17. Select the method = will be established prior analysis by the technical staff, browse and select GDotoliths
18. Tune parameters: Select today's date
19. Blank – do not record anything
20. Calibration – select standards
21. Report – select Thermo_Chrom_Intensities
22. Save file

To begin the first sequence

1. Go to Customize, template, samples – you may need to select "change next" to modify a given sample (name it, etc.)
2. Drag the SMP icon to the template and select "Fill in" –you should have 20 samples in total
3. Save the sequence as in step 67, beginning with letter A for the first sequence of the day- each new sequence for a given date will follow the letters of the alphabet
4. Click on the blue flag set the sequence
5. The 1st, 2nd,19th and 20th (first 2 and last 2) samples are NIST 612 glass and serve to correct for drift
6. The 3rd and 18th samples are MACS1 which is treated as an unknown and is used to evaluate precision, accuracy and reproducibility of procedure
7. Sample 4 -17 are samples from the otoliths

To sample:

1. Go to the appropriate medium (NIST 612, MACS 1 or otolith)
2. Bring the area to sample into focus
3. Verify that the laser is ready

4. Click "Run" to begin the analysis
5. Open the graphical output showing the element counted per second over time
6. Start ablation by turning the laser on after 30 seconds have passed- this procedure collects the blank
7. Let the laser ablate the sample until the end- it should stop by itself
8. Observe the graphical output during acquisition and make sure the signal is clear and continuous
9. Stop the recording or repeat it if there is a sign of an abnormal signal
10. Each otolith edge are sampled 3 times
11. Keep a record of the order of sampling by assigning the ablation sequence label to a sample in the log book– write the otolith group code and its sampling location (1-3 from the sulcus), or the name of the standard sample if not an otolith
12. After a sample is successfully recorded, repeat 1-10 for the next sample in line
13. Click on STOP and CANCEL to stop the current sequence- all samples are automatically recorded unless you repeat the analysis of a sample
14. To rename a new file at the end of a sequence:
15. Click Cancel
16. Delete the first 3 rows- we will use the last 3 samples of the previous sequence for calibration and drift adjustments later on
17. Save the current file under the new sequence name- ex: au10c4
18. Adjust F of the laser at the end of every sequence
19. Verify resolution of signal and adjust the opening of the doors when needed
20. At the end of each day make a copy of the log book and shut down the system in the reverse order it was turned on

DATA ANALYSIS USING LAMTRACE

Material:

- ✓ Raw data files extracted using Convert software and saved in Lotus
- ✓ Lotus 123; requires Windows 95 to XP, 32 bit
- ✓ Lamtrace program and user guide
- ✓ A copy of the log book used to record the analyses

Procedures:

1. Install Lotus 123 on a compatible computer
2. Open Files converted to lotus under Lamtrace specification –see technician for details
3. Press CTRL + M to obtain the main menu

Configure LAMTRACE for use :

1. You must change the data file directory to your computer's directory
2. Follow step 1 in LAMTRACE manual or Open a file
3. Go to the tables tab
4. Search for the SPREADSHEET DEFAULTS
5. Enter the following:

Raw data directory	Directory were you have saved the raw data…on CREAIT lab computer
Combine file suffix (I, N, "")	i
File save directory	Directory where you want to save the files you modify
File xtract directory	Directory where you want to send the extracted file
Import run directory	Directory from which you are importing the run files
Import data range	impdata
Interval save file name	Directory of where the files are saved (end by /INTERVAL)
Normalising values	C1 chondrite
Threshold-1 setting, # of s.d. of bgd	10
Threshold-1 setting, min. signal	200
Threshold-2 setting, % max. signal	25%
Detection limit filter multiplier	1
Line style for signal graphs (L,B)	L (B=symbols+lines, L=lines only)
Line width for signal graphs (0-7)	2

It is possible to adjust for other parameters see LAMTRACE manual

Signal Processing/ Selecting intervals

1. CTRL+M→ DATA → SELECT→ 1
2. Enter the name of the sample – look at the record from the log book
3. Choose interval to integrate
4. The first parameter is to choose the beginning of the blank interval, the second is to set the end of the blank interval, the 3rd is to select where the ablation signal began and the last option set the end of the interval to be integrated
5. To set interval use up/down arrows and press return to select the interval and switch to the next- read the menu
   - ✓ Make sure background signal reflects real background signal
   - ✓ Avoid large peaks of important elements
   - ✓ If surface contamination seems to be present, exclude this segment from the interval selection

- ✓ Pressing CTRL during the interval selection process yields a menu that allows you to stop the task "BREAK", and save the work completed
- ✓ The system crashes often and you will lose all modifications unless you save your work. You can only do so by taking a BREAK. I suggest saving at least 2 times per sequence.
- ✓

Calculating output (in % weight or ppm)

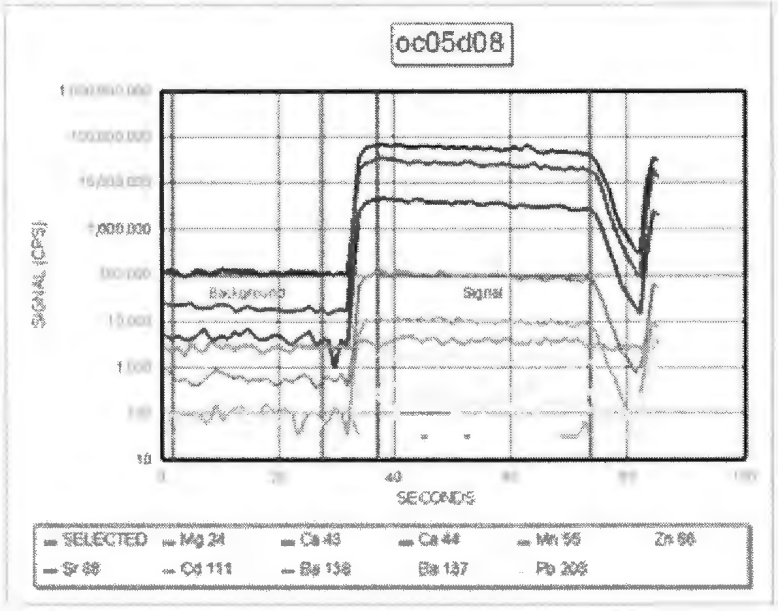
1. CTRL+M→ DATA → CALCULATE
2. Follow the instructions from the menu and press enter to move to the next step
3. The software assumes the standard used to calibrate are the first and last two samples of the sequence, so verify and if true say YES, if not SAY NO
4. If NO, it will guide you to select the position of the calibration material (NIST 612)
5. Select the standard used for calibration and for which you wish to have the true value (NIST 612 and MACS1)
6. Do not round results – NO
7. Once all steps are done, it will calculate the concentrations in ppm unless it was previously changed
8. To change %weight to ppm: Go to the TABLE tab and scroll down until you see a list of element and an option to record them in %weight or ppm

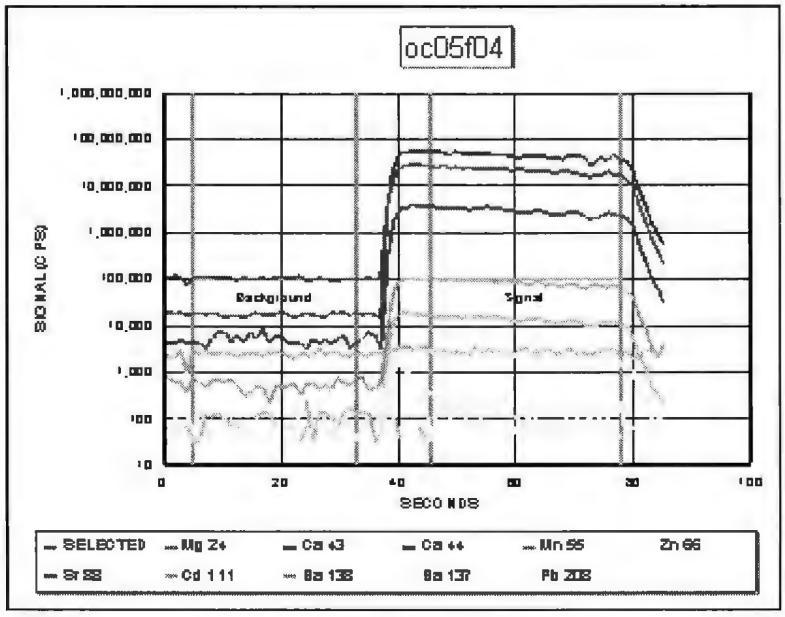
- ✓ Read through the lamtrace manual for more information

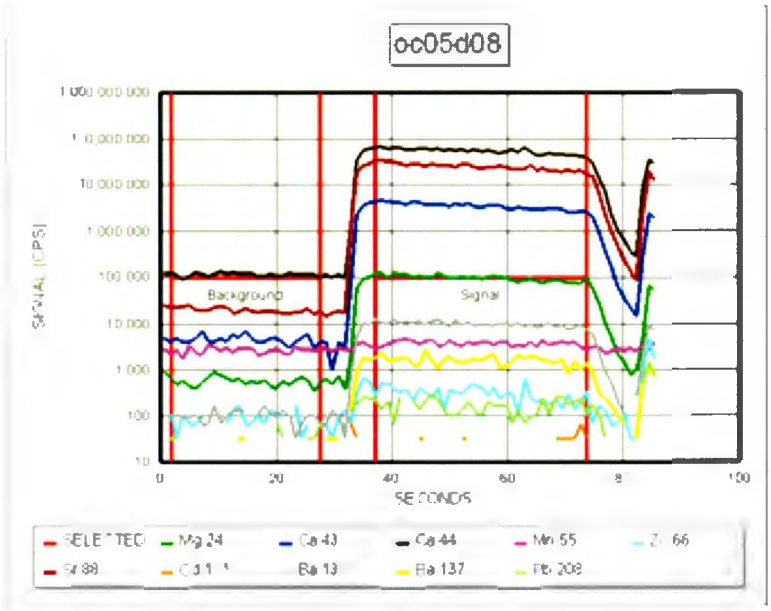
Examples of signal interpretation

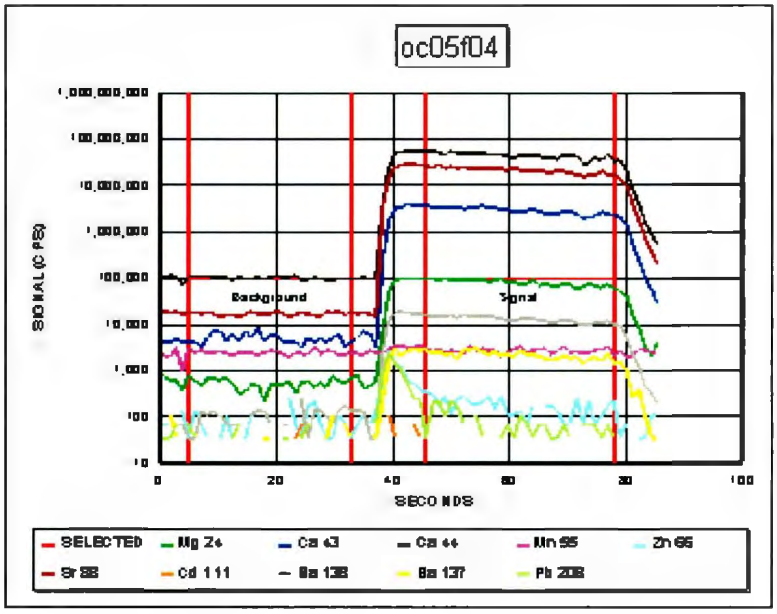
A good signal is observed when both the background and ablation counts over time are relatively stable (see figure oc05d08). The signal during ablation should be well above the background count per second recorded. Each element should have a distinct count per second value and should washout quickly after ablation has stopped because no more material is brought to the mass spectrometer.

Surface contamination by lead (Pb) and zinc (Zn) is observed at the beginning of the ablation of the otolith is observed in figure 0c05f04. The signal selected for integration (calibration) will not consider the portion of time where these anomalies were recorded (the red "signal" interval selected


oc05d08
SIGNAL (CPS)
Background
Signal
SECONDS
SELECTED
Mg 24
Ca 43
Ca 44
Mn 55
Zn 66
Sr 88
Cd 111
Ba 138
Ba 137
Pb 208


oc05f04
SIGNAL (C PS)
Background
Signal
SECONDS
SELECTED
Mg 24
Ca 43
Ca 44
Mn 55
Zn 66
Sr 88
Cd 111
Ba 138
Ba 137
Pb 208


oc05d08
SIGNAL (CPS)
SECONDS
Background
Signal
SELECTED
Mg 24
Ca 43
Ca 44
Mn 55
Sr 88
Ba 137
Pb 208


oc05f04
SIGNAL (CPS)
SECONDS
Background
Signal
SELECTED
Mg 24
Ca 43
Ca 44
Mn 55
Zn 66
Sr 88
Cd 111
Ba 138
Ba 137
Pb 208

Appendix II: Using laser probes to analyse otolith composition

The discovery of the record of growth increments has propelled the use of otoliths in fisheries biology forward and technological advances have further enabled the development of methods to extract the elements stored within these increments to study stock structure, dynamics, movements and migration of fish. Several microprobe techniques are employed and the specific characteristics of each type are dictated by the questions addressed and availability of otoliths.

Solution-based (SB; Vasconcelos et al., 2007; Gillanders and Kingsford, 1996) or isotope dilution (ID; Campana et al., 2000; Thorrold et al., 1998) inductively coupled mass spectrometry were well liked for stock discrimination or finding the origin of fish because they have greater detection power so can reveal a more complex otolith signature with better precision and accuracy (Jones and Chen, 2003; Campana and Gagne, 1995). Natural tagging relies on many assumptions; the most obvious being that the life period sampled must match a time when fish location is known (Elsdon et al., 2008). Addition of new material is perpetual throughout the life of the fish, therefore to capture the time period where the fingerprint corresponds to a spawning cycle, otoliths must be sampled at finer scale. Most groups of Atlantic cod around Newfoundland and Labrador are most aggregated during over-wintering and spawning, thus to use otolith as tags of a given group, it is best to sample the otolith increment representing the pre-spawning and spawning period. Sampling at the edge of otoliths of fish captured during the spawning season thus represents a signature of the aggregation. This requires known chronological

detail so the use of a laser is recommended because it can sample a given life-history event contained in daily growth increment.

The system

The LA-ICP-MS is a system made up of a laser and an ablation system. The excimer laser is based on the excited states of a noble gas and halide molecules (Durrant, 1999) that binds to create a ultra-violet light beam. The main process begins when the laser is projected onto a 45° angle mirror which deflects the light to the ablation cell that holds the sample. The platform under the sample is controlled by a computer and allows specific sections to be sampled by the reflected laser light. An inert gas is introduced to the ablation cell and electrically heated to form plasma, a gaseous fluid containing free ions and electrons, to carry the ablated material to the ICP (Durrant, 1999). At this point the ablated particles are vaporized, atomized and ionized further before reaching the mass spectrometer (Longerich, 2008).

Ablation occurs when the laser pulse converts the photon energy into heat and boils the surface of the material impacted. The vaporization of given elements will vary according to their own vaporization temperature and will occur at different rates (Durrant, 1999). Furthermore, the efficiency of transport depends on the size of the vaporized particles which take the shape of minuscule spherical droplets. An efficiency of more than 80% can be achieved for particles between 3nm-5nm in size (Durrant, 1999).

The mass spectrometer (MS) uses a magnetic sector analyser to separate ions spatially according to their mass to charge ratio (Longerich, 2008) and a vacuum in the chamber is used to lower the pressure and allow ions to travel farther. The charged particles travel at

a high velocity through the magnetic field and follow a curved trajectory which is determined by the nature of the element. Ions with a smaller mass to charge ratio bend more and hit the wall of the vacuum or flight tube, while ions with high mass to charge ratios do not bend as much and impact the outside of the flight tube.

The choice of laser and ICPMS is important however all recent systems perform well. In his review, Durrant (1999) points out some key points to consider when choosing a system and how to optimize its efficiency.

1. Lasers with a lower wavelength have better spatial resolution, lower fractionation and better analytical precision
2. Fixed-Q (free-running) pulse ablate more mass with the same energy as Q-switched pulses
3. Optimum defocusing for sensitivity is close to optimum defocusing for precision but depends on the material used
4. The greater the energy of the laser is, the more it will ablated material so more material is transported into ICP and thus a greater response for a given analyte concentration. However if too much material is ablated there is a risk of affecting the memory, blocking cones and create plasma disequilibrium.
5. Multiple shots of a few Hz is more stable and is more useful for quantification than single shot
6. Pre-ablation of sample prior to analysis increases analytical precision

Calibration

A commonly used method for LA-ICP-MS calibration is based on "element response factors" (Arrowsmith, 1987; Hager, 1989) where a ratio of the response of one element of interest is compared to an internal standard between a material of known composition and the unknown (Veinott, 2001). To improve accuracy and precision, Durrant (1999) further recommends performing response-concentration curves for each element of interest but this requires a set of matching standards. Best results are obtained when the internal standard concentration within the material is large so the signal is above background levels and can be detected easily. Also, its distribution must be homogeneous and its

concentration known. Considering otoliths are made of 96 % calcium carbonate, 3% organic material, and 1% impurities (Campana et al., 1997), calcium is often the medium of choice. An internal standard corrects for the matrix effect, drift in sensitivity, and the ablated volume (mass) from the sample in relation to the calibration material (Longerich and Gunther, 1996).

A matrix-effect can be observed when the chemical composition or physical nature of the sample that is introduced to the instrument differs from the standard and yields different results (Durrant, 1999; Veinott, 2001).The change in the relative proportion of chemical elements ablated during the LA-ICP-MS analysis can cause matrix dependent differences in relative sensitivities of the elements being recorded. In the case where this elemental fractionation behaviour is different between the sample and the calibration standard, matrix-matching of the standard to the sample should be applied if both accuracy and precision are wanted. However, if the elements of interest do not exhibit large differences in sensitivity in different matrices, the use of a matrix-matched internal standard is not necessary because accuracy will not be greatly affected (Sylvester, 2001).

Calibration for LA-ICP-MS analysis also involves the use of an external calibration material. Many criteria will help determine the choice of an adequate calibration material: it must be well characterized, contain the elements of interest with similar concentrations and be readily available and cheap (Durrant, 1999; Veinott, 2001; Veinott et al., 2009). It is also valuable for the research team to have experience with the standard and to have performed previous tests with this standard with the equipment to be used.

Otolith standards or artificial reference materials are used to mimic the behaviour of the element matrix under ablation and allow laboratories to compare precision and accuracy of the elemental concentrations. Although procedures and reference standards may differ among laboratories, most agree that to improve precision and accuracy, matrix-matching is recommended (Veinott, 2001; Durrant, 1999). Matrix matching means that the elemental matrix of the standard is similar to that of the sample being measured. Over the years, scientists have relied upon a typical glass standard for external calibration (NIST 610 or NIST 612) or produced their own standard to improve matrix-matching. Home-made standards often result in heterogeneity of the final product (Sylvester, 2001; Veinott, 2001) so certified otolith reference materials have been produced and made commercially available. Two certified otolith standards were made from red snapper otolith - a Japanese (NIES-022) and a Canadian (FEBS-1) reference powder. Using an otolith based standard may alleviate potential matrix problems and is becoming more widely used for otolith chemical analyses (Thorisson et al., 2011; Bradbury et al., 2011; Vasconcelos et al., 2007). Nevertheless, the performance of otolith-based and glass reference materials is still debated. One study reported that otolith reference materials resulted in a 3-5 fold improvement in detection limits as compared to the glass standards (CETAC) while others (Bellotto and Miekeley, 2000; Campana et al., 1997) did not show significant differences in the accuracy and precision of natural otolith and artificial powders. In addition, Tanner et al. (2011) attributed the high precision estimates of Mn:Ca to the mismatch in intensities between the reference material (NIES 22), the other otolith standard (FEBS1) and the common sole otoliths sampled. In this case, a matrix-effect likely occurred because the differences in the chemical composition or physical

nature of the sample introduced to the instrument and the standard affected the measures of individual elements (Veinott, 2001; Durrant, 1999). This suggests that differences between two materials are not always predictable and in some cases, the use of a matrix-matched internal standard does not improve accuracy or precision for some elements (Sylvester, 2001).

Reference material

The NIST 612 is a synthetic silicate glass standard well known for its stability and homogeneity. This standard is mainly composed of SiO_2, Al_2O_3, CaO and Na_2O, but also holds different levels of 61 trace elements (Jackson, 2001). The MACS-1 standard is a carbonate USGS reference material containing trace elements of Ca, Mg, Cr, Mn, Co, Ni, Cu, Zn, As, Sr, Cd, Ba, La, Ce, Nd, Pb, and U. Its carbonate dominant constitution is often used to study coral chemistry but it also makes good matrix match to otoliths. Its precision was well tested at the Memorial University laboratory and lies within one standard deviation for Mg, Mn, Co, Sr, Ba, and U (Mike Tubrett pers. comm.). Otolith-based reference materials are better matrix matched for all elements but are generally more expansive and available in smaller quantities. The FEBS-1 is certified for seven elements Ba, Ca, Li, Mn, Na, Sr with reference but uncertified values for Cd, Cu, Ni, Pb, Zn (Sturgeon et al., 2005) because they are not considered to be sufficiently homogenous within the population sampled. The NIES CRM 22 is certified for Ca, Na, Mg, K, Sr, and Ba with reference values provided for Cu, Zn, Cd and Pb (NIES 2000). A study done by the Canadian Education and Training Accreditation Commission (CETAC) demonstrated these otolith reference materials had a 3-5 times improvement in detection

limit as compared to the glass standards which may be very helpful to detect elements present in low concentrations in ppb or few ppm. However, other studies (Bellotto and Miekeley, 2000; Campana et al., 1997), did not show significant differences in the accuracy and precision of natural otolith and artificial powders. In addition, Geffen et al. (2013) compared sensitivity, accuracy and precision of these two otolith-based reference materials in two laboratories and found great variability in commonly used and more stable elements. Such findings seem to point towards the need for a revision of sampling methodology to improve accuracy of the results obtained before it is possible to share data uncritically across labs to compare chemical compositions.

PERFORMANCE AND PROBLEMS

Selecting otoliths

For studies concerned with using otoliths as natural tags, it is best to select otoliths of one pure polymorph because trace elements are not accreted in the same proportions in aragonite and vaterite (Melancon et al., 2005; Veinott et al., 2009). Concentrations of Sr and Ba are high and Mn and Mg are low in a purely aragonite otolith (Melancon et al., 2005; Veinott et al., 2009), while the opposite occurs in vaterite otolith. Bath et al. (2000) demonstrated only purely aragonite reflect the environmental conditions of the fish, so otoliths containing vaterite are not reliable spatial indicators and may cause error in discrimination. In addition, the vaterite portion of otolith tends to grow in a wavy pattern rather than concentric rings causing difficulties in ageing a fish with vaterite otoliths. Correct ageing provides invaluable data on fish life history and can help reconstruct movements of fish at specific times in their lives when increments can accurately be related to a given season or year (Elsdon et al., 2008). In cod, most researchers favor

using the sagittal otoliths because they are mainly composed of aragonite, the crystal form of calcium carbonate that is the most stable polymorph of calcium carbonate (Campana, 1999).

Several methods can be used to detect the presence of vaterite in an otolith. First, vaterite otoliths can generally be easily identified by their translucent coloration, larger size and uneven shape (Figure II.I, Melancon et al., 2005). Vaterite density is lower than aragonite, so a vaterite otolith weighs less though it may appear larger than an aragonite otolith from fish of a given age. This phenomenon was observed for Atlantic cod otoliths when only one of the two sagittal otoliths was made of vaterite and the other aragonite. Raman spectrometry and Ethylenediaminetetraacetic acid (EDTA) can assess the polymorph content of otoliths. Finally, the presence of Mg in high concentration in vaterite otoliths can serve as a good proxy for determining the presence of vaterite in an area of the otolith (Veinott et al., 2009). Regardless of the method employed to detect the polymorph, vaterite is generally discarded in studies aiming at identifying fish based on otolith chemical composition.

Choice of calibration material

The choice of standard varies depending on the type of analysis to be done. For this project several standards were reviewed including two otolith standards (matrix-matched) and one glass standard (non-matrix matched).The NIST 612 glass was selected as the external calibration standard for it has a diversity of elements above detection limits and is very homogeneous allowing better signal precision. Though the otolith standards may

have provided a closer matrix-match and better accuracy in sensitivity to some elements, they were not readily available and homogeneity of the pellets was questionable. Press pellets from otolith powders (or any powder) tend to be less homogeneous than powder dissolved in solution. The preferred method is thus to use a combination of solid-liquid calibration, performing solid ablation of the sampled otoliths (unknown) and using an aqueous solution of the otolith standard. The MAFIIC lab at Memorial University of Newfoundland (MUN) did not have previous experience with this technique, and had not performed precision tests, thus a solid-solid calibration was used.

Sample preparation and cell adjustments

The LAM allows any kind of preparation so long as it fits in the cell. The standard setup is to fasten the samples onto a petrographic slide or use the circular moulds provided. For a better performance of the system, it is recommended to make the ablation surface as flat as possible. Care must be taken when placing the samples in the cell to keep it leveled. The position of the laser is controlled by setting exact coordinates of a region to ablate or based upon visual localization. Cell movements are possible on a three dimensional plane but are limited. Focusing the laser unto the otolith sample to ablate was a problem when the cell was not straight, so adjustments were often performed to keep the cell perpendicular to the laser beam. This caused two problems; first the area to be ablated was not clearly visible therefore accuracy is lost and the area to be sampled may be off target. Scanning Electron Microscopy photos were taken of a portion of the samples to verify the position and state of the ablation crater. These photos were tilted to measure the circumference and depth of ablation craters and revealed some of the sampling was done

at an angle thus causing more variability in the depth and circumference of ablation craters. A second problem that may arise is poor focusing of the laser that will affect ablation yield as the calculations are based upon the assumption that the laser is in focus. The best solution to adjust the focus was to add paper strips underneath the cell plate to keep it leveled. Surface flatness may also be corrected by performing mechanical polishing of the samples to be placed in the cell rather than hand polishing individual otoliths then gluing them onto a slide. Such a procedure would increase uniformity of the surface of the samples to be analysed.

Our project required visual identification of the last growth increment and the edge of the otolith from the epoxy. Therefore a map of the slide holding the samples was taken with a DP72 Olympus camera from a SZ16 digital stereomicroscope prior to the installation of samples in the cell to help guide movement of the laser onto the ablation area (Figure II.II). Once the sample is well in place in the cell, localization of the sampling area was performed. Ablation areas for each otolith in the cell (approx. 20) were identified and coordinates were recorded.

Poor signal

Some otolith signals and sometimes the MACS1 signals were very spiky. These peaks or troughs can be a result of mechanical malfunctions of the setup. In our analysis, some peaks were observed when several particulates larger than normal were carried to the ICP causing a sudden sharp increase in counts for a given element. When the detector senses that a higher than normal count will come through, it may shut off for a few seconds to allow the particle to go through without risk to the plasma. Some troughs were observed

and were suspected to result from the shutting off of the detector to protect itself from potential damage. The effect of the passage of larger particulates was visible when all elements analysed peaked or dropped at the same time (Figure II.III). Signal with peaks and troughs are characterised by very high or very low counts of a given element for a few seconds and an immediate return to normal count values without drifting effects.

A second phenomenon was observed during the analysis, where spiky signals seem to reflect poor resolution of the system (Figure II.IV). The analysis of trace element requires low resolution and this option is often selected. However, there are three slits on a plate that control the resolution low, medium and high by the application of different argon pressure. A variation in argon pressure would cause a change in resolution which in turn would make the system unadjusted and lead to a spiky signal (Figure II.IV). To counter this potential effect, frequent changes in argon pressure were made to try and stabilize the flow. At the end of 2 or 3 runs, the resolution was switched from low to medium to high and back down again, each running for a few minutes. This maneuver was always successful at stabilizing the argon flow.

It is important to note that the occurrence of a few spikes (peaks or trough) does not typically influence statistical significance and does not change the results when sufficient amounts of signal are present because the data processing calculates the average count received over the entire sampling time. If there are significant peaks or trough over a long portion of the signal, revision and exclusion of the signal may be warranted.

Ablation at the edge of the otolith at times causes the epoxy also to be ablated. To ensure the epoxy did not constitute a source of contamination, holes were ablated through the epoxy several times and the composition of the epoxy was recorded. None of the

elements under study were above the background abundance therefore no contamination of epoxy was assumed to be present in the concentrations obtained from the otolith samples (Figure II.V). In the future, contamination of epoxy should be evaluated by spiking the epoxy with a rare element (like indium) and measure its concentration with each ablation as it is done in many other studies.

REFERENCE

Arrowsmith, P., 1987. Laser ablation of solids for elemental analysis by inductively coupled plasma mass-spectrometry. Anal. Chem. 59(10), 1437-1444.

Bellotto, V., Miekeley, N., 2000. Improvements in calibration procedures for the quantitative determination of trace elements in carbonate material (mussel shells) by laser ablation ICP-MS. Fres. J. Anal. Chem. 367(7), 635-640.

Campana, S.E., Gagne, J.A., 1995. Cod stock discrimination using ICPMS elemental assays of otoliths, in: Secor, D.H., Dean, J.M., and Campana, S.E. Recent Developments in Fish Otolith Research.University of South California Press, Columbia, SC. pp: 671-691.

Campana, S., Hanson, J., Frechet, A., and Brattey, J., 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res. 46(1-3), 343-357.

Campana, S., Thorrold, S., Jones, C., Guenther, D., Tubrett, M., Longerich, H., Jackson, S., Halden, N., Kalish, J., Piccoli, P., de Pontual, H., Troadec, H., Panfili, J., Secor, D., 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(9), 2068-2079.

CETAC. Studies of otolith reference materials using LA-ICP-MS. Available from: http://www.cetac.com/, accessed on April 3, 2010.

Durrant, S.F., 1999. Laser ablation inductively coupled plasma mass spectrometry: Achievements, problems, prospects. J. Anal. at. Spectrom. 14(9), 1385-1403.

Elsdon T.S., Wells B.K., Campana S.E., Gillanders B.M., Jones C.M., Limburg K. E., Secor D.H., Thorrold S.R., and Walther B.D., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Ann. Rev. 46, 297-330.

Geffen, A.J., Morales-Nin, B., Perez-Mayol, S., Cantarero, A., Skadal, J., Tovar-Sanchez, A., 2013. Chemical analysis of otoliths: Cross validation between techniques and laboratories. Fish. Res.143, 67-80.

Gillanders, B.M., Kingsford, M.J., 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar. Ecol. Prog. Ser. 141(1-3), 13-20.

Hager, J.W., 1989. Relative elemental responses for laser ablation inductively coupled plasma mass-spectrometry. Anal. Chem. 61(11), 1243-1248.

Jackson, S. E., 2001. Lamtrace user's manual. School of Earth Sciences, Macquarie University, Sydney, Australia.

Jones, C., Chen, Z., 2003. New techniques for sampling larval and juvenile fish otoliths for trace-element analysis with laser-ablation sector-field inductively-coupled-plasma mass spectrometry (SF-ICP-MS). Institute of Marine Research, Postboks 1870 Nordnes N-5817 Bergen Norway.

Longerich, H., 2008. Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) an introduction. In Laser ablation ICP–MS in the earth sciences: Current practices and outstanding issues. Edited by S. Paul. Mineral Association of Canada Short Course Volume 40, Vancouver, BC.

Longerich, H., Gunther, D., 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. J. Anal. At. Spectrom. 11(9), 899-904.

Melancon, S., Fryer, B.J., Ludsin, S.A., Gagnon, J.E., Yang, Z., 2005. Effect of crystal structure on the uptake of metals by lake trout (*Salvelinus namaycush*) otoliths. Can. J. Fish. Aquat. Sci. 62:2609-2619.

Sylvester, P.J., 2001. Laser-ablation-ICPMS in the earth sciences : Principles and applications. Mineralogical Association of Canada, Ottawa, Ont., Canada.

Tanner, S. E., Vasconcelos, R. P., Reis-Santos, P., Cabral, H. N., Thorrold, S. R., 2011. Spatial and ontogenetic variability in the chemical composition of juvenile common sole (*Solea solea*) otoliths. Estuar Coast. Shelf Sci. 91(1), 150-157.

Thorrold, S., Jones, C., Swart, P., Targett, T., 1998. Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. Mar. Ecol. Prog. Ser.. 173, 253-265.

Vasconcelos, R.P., Reis-Santos, P., Tanner, S., Fonseca, V., Latkoczy, C., Guenther, D., Costa, M.J., Cabral, H., 2007. Discriminating estuarine nurseries for five fish species through otolith elemental fingerprints. Mar. Ecol. Prog. Ser. 350, 117-126.

Veinott, G. 2001. The use of laser ablation-ICP-MS in the environmental sciences in laser ablation-ICP-MS, in: Sylvester, P.J., Earth Sciences: Principles and applications. Mineralogical Association of Canada Short Course Series.

Veinott, G.I., Porter, T.R., Nasdala, L., 2009. Using mg as a proxy for crystal structure and Sr as an indicator of marine growth in vaterite and aragonite otoliths of aquaculture Rainbow trout. Trans. Am. Fish. Soc. 138(5), 1157-1165.

FIGURES

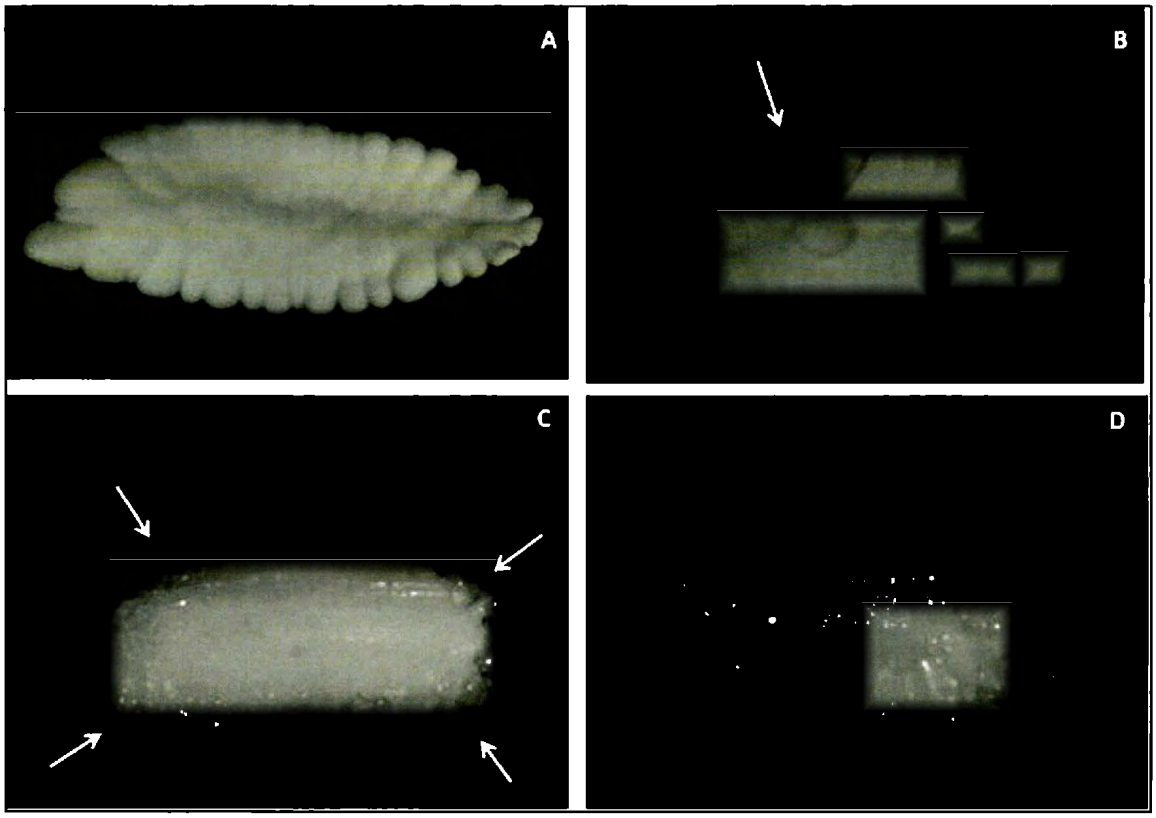


Figure II.I: Otolith of Atlantic cod with different calcium carbonate structure. A pure aragonite otolith (A), a mixture of vaterite and aragonite with a dominance of aragonite (B), an otolith with a core of aragonite and the last growth rings made of vaterite (C), and a pure vaterite otolith (D). Vaterite portions are more brittle and transparent and identified by arrows in B and C. All images were taken by a DP72 camera mounted on a SZ16 Olympus stereomicroscope.

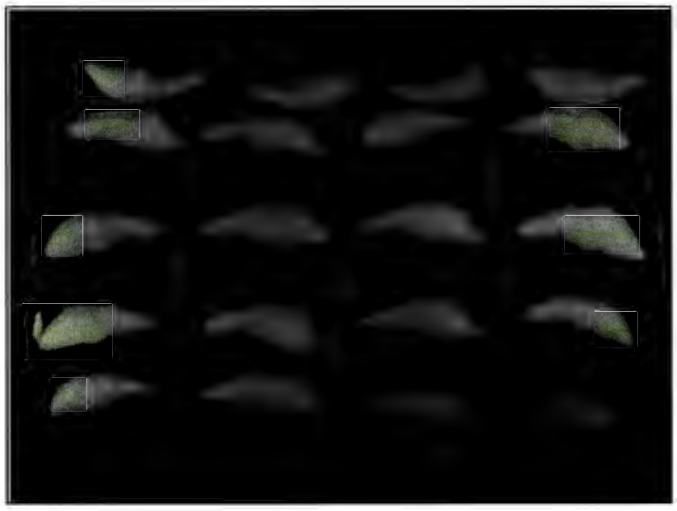


Figure II.II: Map for slide LM used for accurate sampling of the otolith and appropriate growth ring.

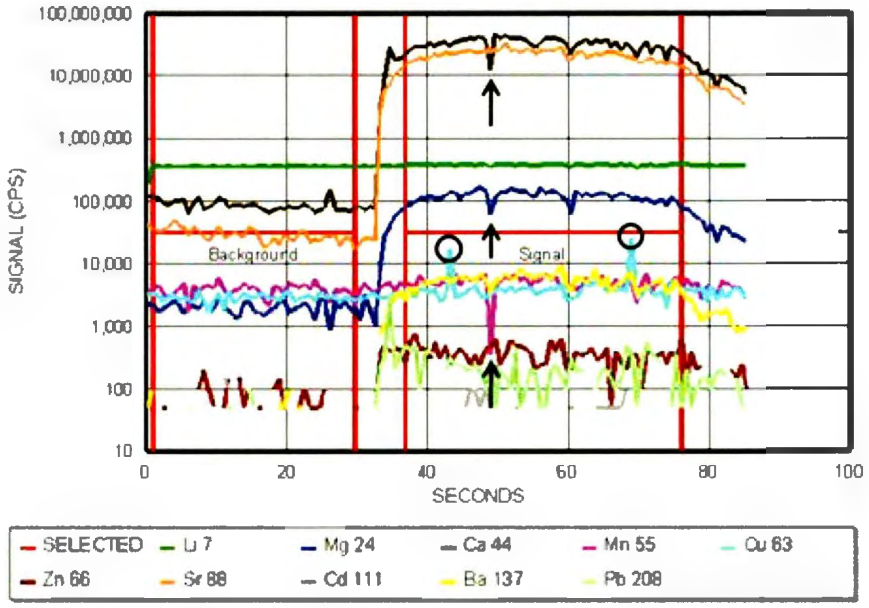


Figure II.III: Peaks (circle) caused by the passage of larger particulate and troughs (arrow) observed in a signal due to the shut off of the detector.

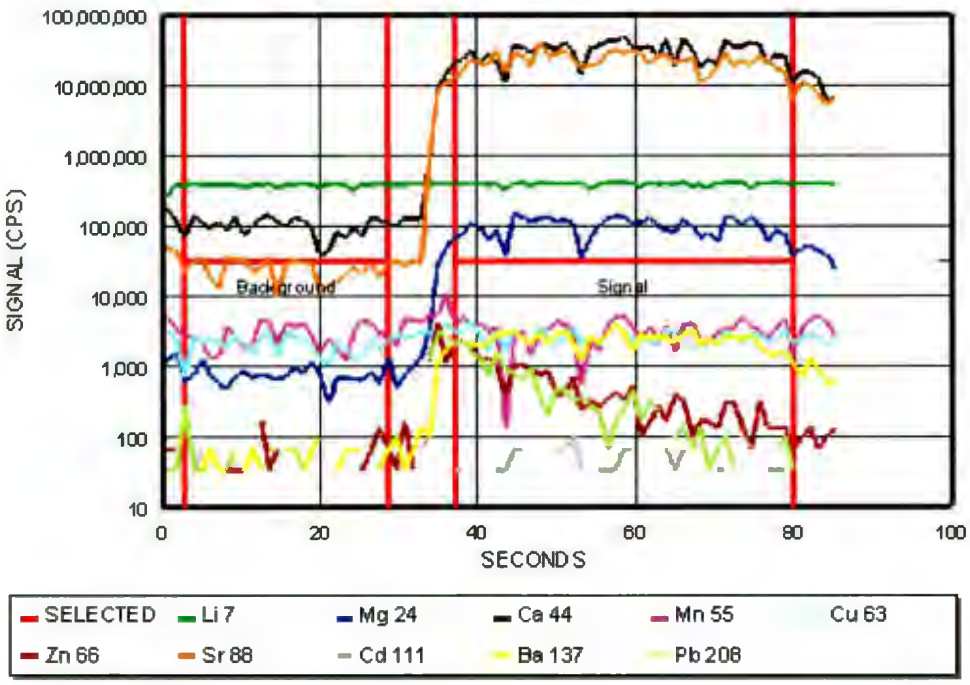


Figure II.IV: Signal read from the ICP is poor due to the variation in resolution caused by changes in argon pressure. Several peaks and troughs are observed for all elements. Note in this case there is also strong surface contamination by lead (Pb).

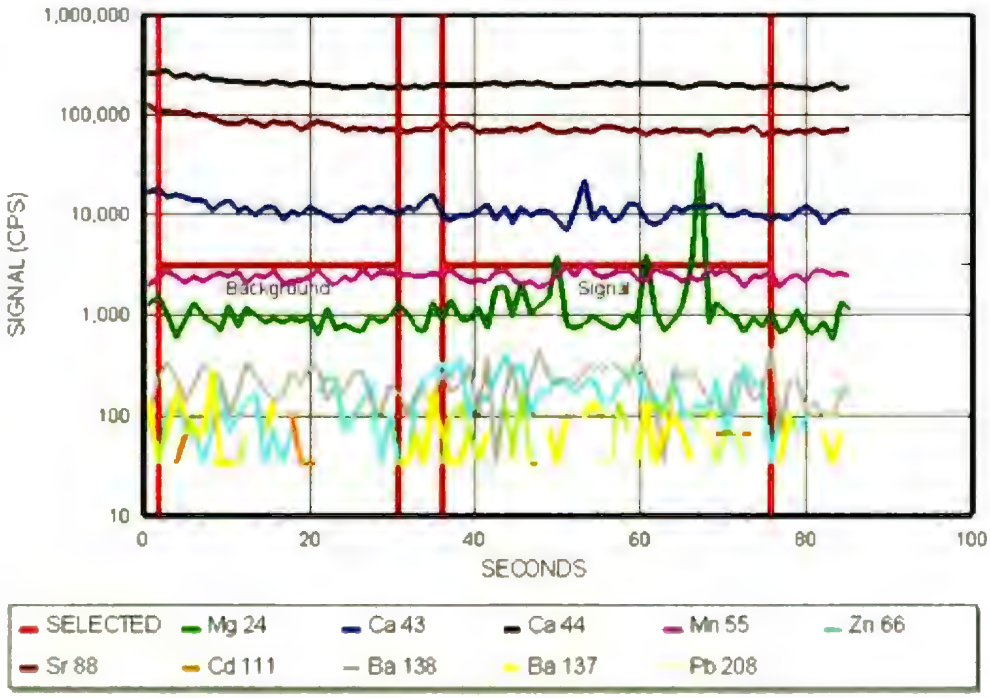


Figure II.V: Signal obtained from the ablation of the epoxy surrounding the otolith.

Brattey, J., Cadigan, N.G., Dwyer, K., Healey, B.P., Morgan, M.J., Murphy, E.F., Maddock Parsons, D., Power, D., 2009. Assessment of the cod (*Gadus morhua*) stock in NAFO division 2J + 3KL in 2009. Can. Sci. Advis. Res. Doc., 2009/061.

Brattey, J., Lawson, G., Rose, G., 1999. Seasonal migration patterns of Atlantic cod (Gadus morhua) in subdivision 3Ps based on tagging experiments during 1997-98. Can. Sci. Advis. Sec. Res. Doc., 99/37.

Brown, T.J., 1999. The Hamilton Bank-Hawke Channel region: potential as an offshore marine protected Area? A study to examine the physical, biological, economic and social characteristics of an offshore fishing area. M.M.S. Thesis, Marine Institute, Memorial University of Newfoundland.

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

Campana, S.E., Gagne, J.A., 1995. Cod stock discrimination using ICPMS elemental assays of otoliths, in: Secor, D.H., Dean, J.M., and Campana, S.E. (Eds). Recent Development in Fish Otolith Research. University of South California Press, Columbia, SC. pp.671-691.

Campana, S., Hanson, J., Frechet, A., Brattey, J. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res. 46(1-3), 343-357.

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

Chittaro, P., Usseglio, P., Fryer, B., Sale, P., 2006. Spatial variation in otolith chemistry of *Lutjanus apodus* at Turneffe Atoll, Belize. Estuar. Coast. Shelf Sci. 67(4), 673-680.

Colbourne, E. B., 1999. Oceanographic conditions in NAFO subdivisions 3Pn and 3Ps during 1997 and 1998 with comparisons to the long-term (1961-1990) average. Can. Sci. Advis. Sec. Res. Doc., 99/39.

Deser, C., Holland, M., Reverdin, G., Timlin, M., 2002. Decadal variations in Labrador sea ice cover and North Atlantic sea surface temperatures. J. Geophys. Res. 107, 3035.

deYoung, B., Rose, G.A., 1993. On recruitment and distribution of Atlantic cod (*Gadus-morhua*) off Newfoundland. Can. J. Fish. Aquat. Sci. 50(12), 2729-2741.

DFO, 2009. Stock Assessment of Northern (2J3KL) cod in 2009. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep., 2009/009.

DiMaria, R.A., Miller, J.A., Hurst, T.P., 2010. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, Gadus macrocephalus. Environ. Biol. Fishes. 89(3-4), 453-462.

Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor, D.H., Thorrold, S.R., Walther, B.D., 2008. Otolith chemistry to describe

movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Annu. Rev. 46, 297–330.

Fairclough, D. V., Edmonds, J. S., Lenanton, R. C. J., Jackson, G., Keay, I. S., Crisafulli, B. M., Newman, S. J., 2011. Rapid and cost-effective assessment of connectivity among assemblages of *Choerodon rubescens* (labridae), using laser ablation ICP-MS of sagittal otoliths. J. Exp. Mar. Biol. Ecol. 403(1-2), 46-53.

Fudge, S.B., Rose, G.A., 2008a. Life history co-variation in a fishery depleted Atlantic cod stock. Fish. Res. 92(1), 107-113.

Fudge, S.B., Rose, G.A., 2008b. Changes in fecundity in a stressed population: Northern cod (*Gadus morhua*) off Newfoundland, in: Kruse, G.H., Drinkwater, K., lanelli, J.N., Link, J.S., Stram, D.L., and Wespestad, V. Resiliency of Gadid Stocks to Fishing and Climate Change. Lowell and Wakefield Fisheries Symposia Series, Anchorage, AK. pp179-196.

Gjosaeter, J., Danielssen, D.S., 2011. Age, growth and otolith annulus formation of cod (*Gadus morhua*) in the risor area on the Norwegian Skagerrak coast during 1986-1996. Mar. Bio. Res. 7(3), 281-288.

Hamer, P., 2003. Otolith chemistry of juvenile snapper Pagrus auratus in Victorian waters: Natural chemical tags and their temporal variation. Mar. Ecol. Prog. Ser. 263, 261-273.

Johnson, C.R., Field, C.A., 1993. Using fixed-effects model multivariate analysis of variance in marine biology and ecology. Oceanogr. Mar. Biol Annu. Rev. 31, 117-221.

Kalish, J.M., 1989. Otolith microchemistry validation of the effects of physiology age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132(3), 151-178.

Knickle, D.C., Rose, G.A., 2010. Seasonal spawning and wind-regulated retention-dispersal of early life stage Atlantic cod (*Gadus morhua*) in a Newfoundland fjord. Fish. Oceanogr. 19(5), 397-411.

Krumsick, K., Rose, G.A., 2012. Atlantic cod (Gadus morhua) feed during spawning off Newfoundland and Labrador. ICES J. Mar. Sci. 69(10), 1701-1709.

Lawson, G., Rose, G., 2000. Seasonal distribution and movements of coastal cod (*Gadus morhua L.)* in Placentia Bay, Newfoundland. Fish. Res. 49(1), 61-75.

Longerich, H., Gunther, D., 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. J. Anal. at. Spectrom. 11(9), 899-904.

Longerich H., Gunther D., Jackson S., 1996. Elemental fractionation in laser ablation inductively coupled plasma mass spectrometry. Fresen. J. Anal. Chem. 355, 538-542

Martin, G.B., Thorrold, S.R., 2005. Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot Leiostomus xanthurus. Mar. Ecol. Prog. Ser. 293, 223-232.

Miller, J.A., 2009. The effects of temperature and water concentration on the otolith incorporation of barium and manganese in Black rockfish Sebastes melanops. J. Fish Biol. 75(1), 39-60.

Petrie, B., Buckley, J., 1996.Volume and freshwater transport of the Labrador Current in Flemish Pass. J. Geophys. Res. 101, 28335–28342

Petrie, B. Anderson, C., 1983. Circulation on the Newfoundland continental shelf. Atmosphere-Ocean, 21(2), 207-226.

Rose, G.A., 2007. Cod: The ecological history of the North Atlantic fisheries. Breakwater Books, St. John's, NL, Canada.

Rose, G.A., Nelson, R.J., Mello, L.G.S., 2011. Isolation or metapopulation: whence and whither the Smith Sound cod? Can. J. Fish. Aquat. Sci. 68(1), 152-169.

Ruzzante, D.E., Taggart, C.T., Lang, S., Cook, D., 2000. Mixed-stock analysis of Atlantic cod near the Gulf of St. Lawrence based on microsatellite DNA. Ecol. Appl. 10(4), 1090-1109.

Sadovy Y., Severin K., 1994. Elemental patterns in Red hind (*Epinephelus guttatus*) otoliths from Bermuda and Puerto Rico reflect growth-rate, not temperature. Can. J. Fish. Aquat. Sci. 51, 133-141.

Sherwood, G., Fudge, S., Rose, G., 2007. Influence of diet on growth, condition and reproductive capacity in Newfoundland and Labrador cod (*Gadus morhua*): Insights from

stable carbon isotopes (delta C-13). Deep-Sea Res. Part II-Trop. Stud. Oceanogr. 54(23-26), 2794-2809.

Smedbol, R., Wroblewski, J., 2002. Metapopulation theory and Northern cod population structure: interdependency of subpopulations in recovery of a groundfish population. Fish. Res. 55(1-3), 161-174.

Smith, E., Soule, F., Mosby, O., 1937. The Marlon and General Greene expeditions to Davis Strait and Labrador Sea, Bull. 19, Int. Ice Patrol Serv., Washington, D.C., 259 pp.

Sturrock, A., Trueman, C., Darnaude, A., and Hunter, E., 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? J. Fish Biol. 81(2), 766-795.

Walther, B.D., Limburg, K.E., 2012. The use of otolith chemistry to characterize diadromous migrations. J. Fish Biol. 81 (2): 796-825.

Walther, B., Thorrold, S., 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. Mar. Ecol. Prog. Ser. 311, 125-130.

Walther, B.D., Kingsford, M.J., O'Callaghan, M.D., McCulloch, M.T., 2010. Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. Environ. Biol. Fishes. 89(3-4), 441-451.

Wijekoon, M.P., Puvanendran, V., Ings, D.W., Brown, J.A., 2009. Possible countergradient variation in growth of juvenile cod *Gadus morhua* from the northwest Atlantic. Mar. Ecol. Prog. Ser. 375, 229-238.

Windle, M.J.S., Rose, G.A., Devillers, R., Fortin, M.-J., 2012.Spatio-temporal variations in invertebrate-cod-environment relationships on the Newfoundland-Labrador shelf, 1995-2009. Mar. Ecol. Prog. Ser. 469, 263-278.

TABLES

Table 3.1: Sampling location, year of collection, age of fish (year), sampling size (N) and total length of cod recorded in cm with their corresponding standard deviation (SD),the percentage of fish that were mature upon capture and the percentage of female cod used in the study.

Spawning area	Code	Year	Age	N	Length (cm)		%	%
					Mean	S.D.	Mature	Female
Inshore 3Ps- Bar Haven	BH	1998	5	32	53.09	4.71	72	41
			6	25	58.32	4.20	88	44
			7	24	60.08	4.87	100	13
		1999	5	26	50.38	5.51	58	58
			6	24	59.21	4.77	71	42
			7	31	60.19	4.84	92	55
Offshore 2J- Hawke Channel	HC	1998	3	30	34.30	3.51	50	40
			4	40	40.78	2.71	73	55
			5	32	46.06	3.50	91	44
		1999	3	15	31.80	4.72	13	60
			4	32	44.88	3.51	81	72
			5	34	49.18	3.31	97	62
Offshore 3Ps- Halibut Channel	3OHC	1998	4	30	42.47	4.25	10	57
			5	29	51.48	5.00	41	45
			6	30	55.87	6.18	77	63
		1999	4	23	43.22	4.47	17	61
			5	29	51.48	5.90	55	59
			6	27	57.07	6.45	89	63
Inshore 3KL- Smith Sound	SS	1998	5	25	51.52	4.00	52	52
			6	24	56.46	5.38	88	50
			7	24	60.00	3.21	92	58
		1999	5	19	50.42	2.54	47	63
			6	28	58.93	3.83	71	61
			7	26	64.15	4.48	92	65

Table 3.2: Parameters estimated from the ANCOVA model testing the effect of length including the intercept (b_0), the common within-group slope (b_1) and p-value for each elemental ratio. The effect of fish length was corrected by removing the common within-group slope from the elemental ratio value of Sr:Ca and Mn:Ca. No common slope was established for Mg:Ca and Ba:Ca because their relationships with length differed across locations.

	b_0	b_1	F	P
Ln Mg:Ca	-1.762	-0.00148	8.23	0.004
Ln Mn:Ca	-7.166	-0.02060	58.11	< 0.001
Ln Sr:Ca	0.3791	0.004910	26.05	< 0.001
Ln Ba:Ca	-6.999	0.008695	14.92	< 0.001

Table 3.3: Between subjects effect of the MANOVA examining otolith chemistry of Atlantic cod collected in four spawning areas (location) around Newfoundland, over two consecutive years. The effect of location, year, maturity, cohort, and the mean growth rate of fish (cm/yr, kg/yr) are evaluated.

	dF	MS	F	P	r^2
Mg:Ca					
Location	3	0.040	5.22	**0.001**	0.0219
Year	1	0.056	7.299	**0.007**	0.0101
Cohort	5	0.022	2.8033	**0.016**	0.0195
Maturity	1	0.114	14.72	< **0.001**	0.0206
Growth (length)	1	0.003	0.337	0.562	< 0.001
Growth (weight)	1	0.004	0.487	0.485	< 0.001
Error	645				
Mn:Ca					
Location	3	3.475	16.058	< **0.001**	0.0665
Year	1	0.595	2.750	0.098	0.0038
Cohort	5	0.162	0.747	0.588	0.0052
Maturity	1	0.844	0.3899	**0.049**	0.0054
Growth (length)	1	1.430	6.610	**0.010**	0.0091
Growth (weight)	1	1.863	0.8.606	**0.003**	0.0119
Error	645				
Sr:Ca					
Location	3	2.211	80.818	< **0.001**	0.254
Year	1	0.015	0.537	0.464	< 0.001
Cohort	5	0.024	0.875	0.497	0.0046
Maturity	1	0.329	12.029	**0.001**	0.0126
Growth (length)	1	0.002	0.066	0.798	< 0.001
Growth (weight)	1	0.031	1.116	0.291	0.0012
Error	645				
Ba:Ca					
Location	3	14.426	94.59	< **0.001**	0.2768
Year	1	9.24E-5	0.001	0.98	< 0.001
Cohort	5	0.395	2.592	**0.025**	0.0126
Maturity	1	0.639	4.192	**0.041**	0.0041
Growth (length)	1	0.293	1.920	0.166	0.0019
Growth (weight)	1	0.77	0.502	0.479	0.0049
Error	645				

Table 3.4: Jackknife classification matrix and percentages of Atlantic cod correctly assigned to the spawning area of capture using a discriminant function analysis model based upon otolith chemical signatures and fish mean growth rates (cm/yr, kg/yr). Correct classifications and total classification success (in %) are in bold. The upper panel of the table shows classification of four spawning groups BH, HC, O3HC, and SS, while the bottom panel shows classifications when BH and SS were pooled.

Jackknife classification matrix

	BH	HC	O3HC	SS	%
BH	**66**	20	28	47	41
HC	10	**162**	7	4	89
O3HC	23	23	**115**	7	68
SS	33	15	6	**92**	63
					66

	BH+SS	HC	O3HC		%
BH + SS	**245**	31	30		80
HC	19	**157**	7		86
O3HC	36	23	**109**		65
					78

FIGURES

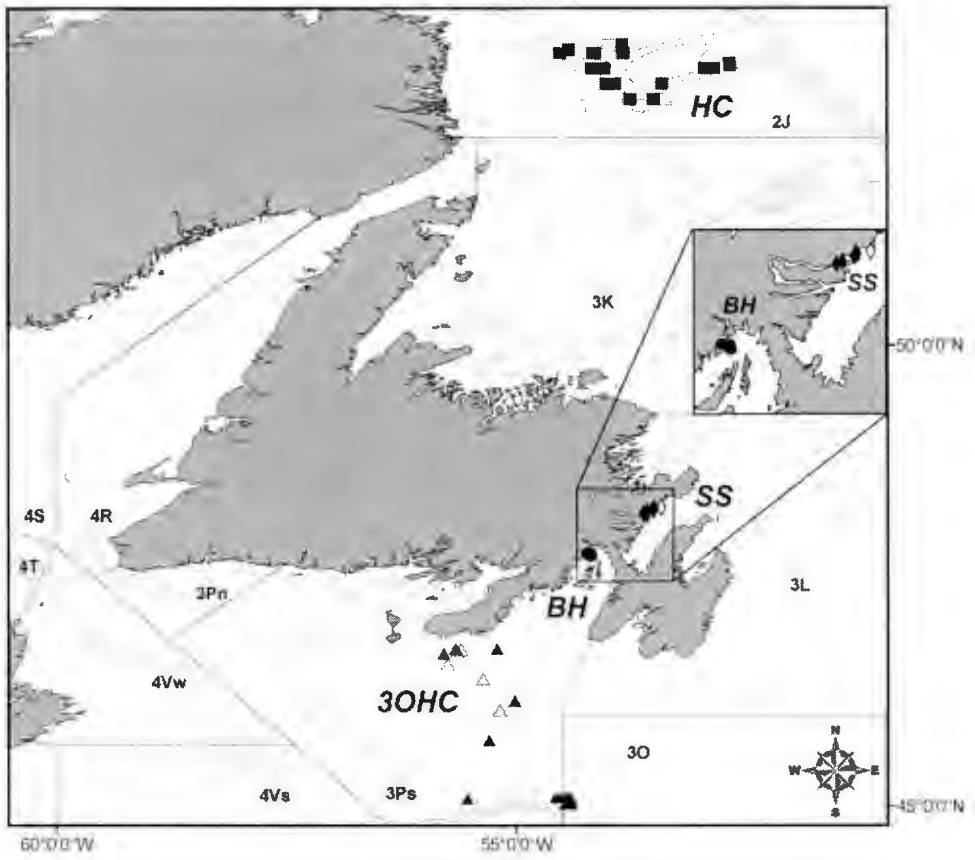


Figure 3.1 : Map showing locations of fishing sets in each spawning area collected for this study. The dark symbols represent sampling locations for 1998 and empty symbols represent 1999 sampling locations. Spawning groups are Bar Haven (BH, ●), Hawke Channel (HC, ■), Offshore 3Ps and Halibut Channel (O3HC, ▲), Smith Sound (SS, ♦). The O3HC region includes sampling sites near shore but outside of Placentia Bay leading to the Halibut Channel and along the shelf edge around Green Bank at the edge of 3O. The inset shows BH and SS sampling locations in more details.

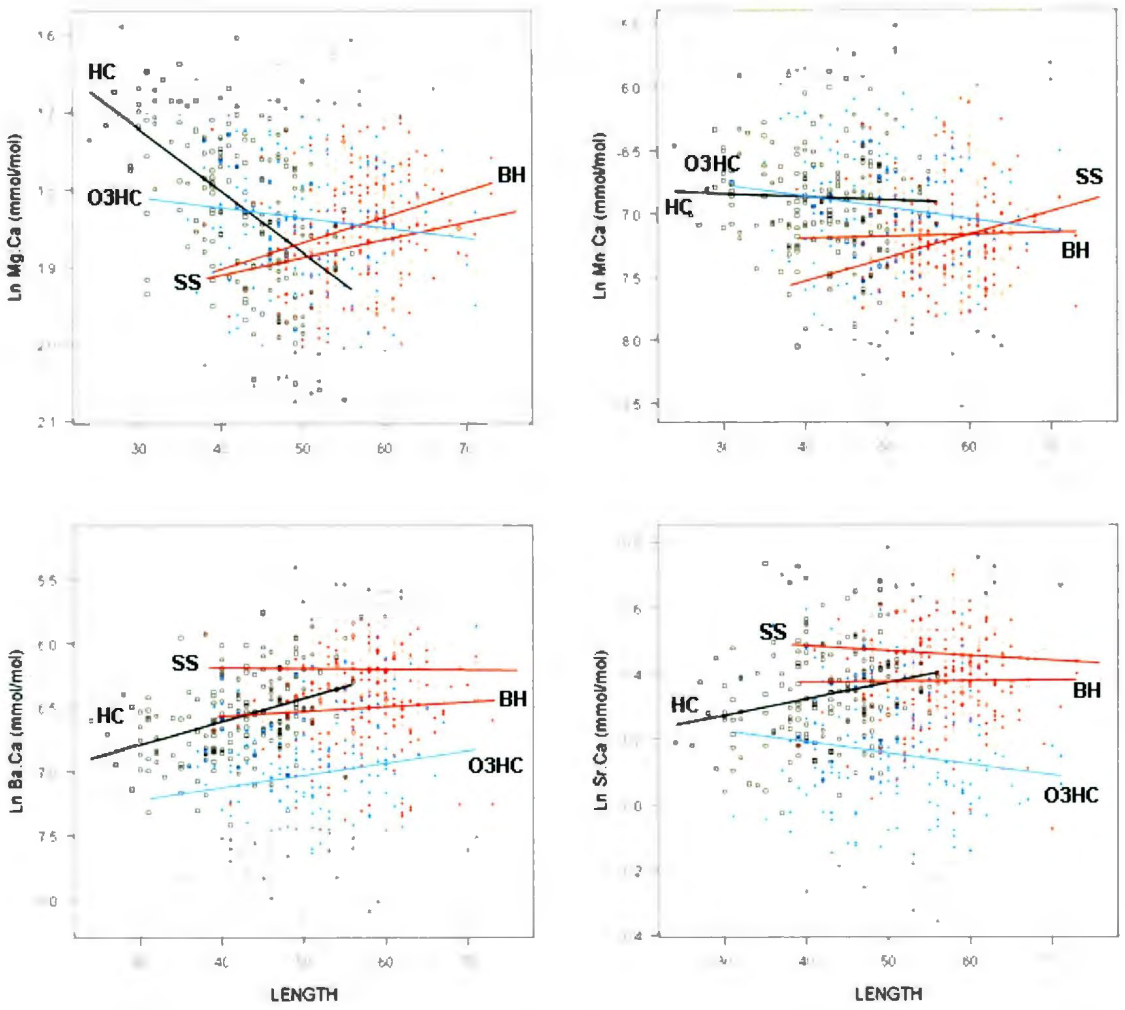


Figure 3.2: Scatterplots showing the relationship between otolith elemental concentrations (mmol/mol) and fish length at each spawning area BH (●), HC (□), O3HC (△), SS (◊). The elemental concentrations of Mn and Sr showed significant relationships with length in all locations so the pooled linear slope was subtracted to remove the effect of length. The effect of length on Mg and Ba differed across sites so elemental ratios were not length detrended.

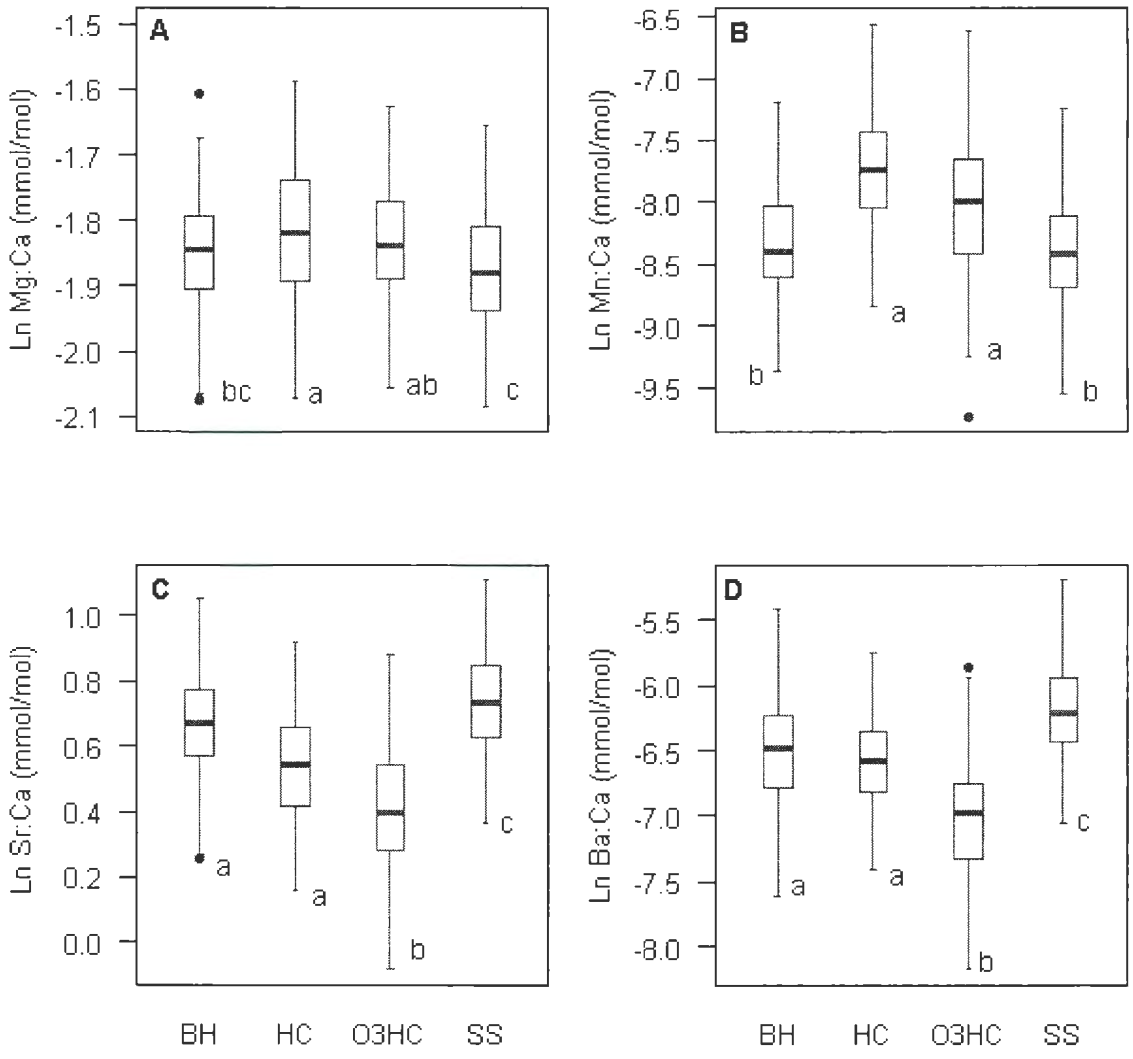


Figure 3.3: Boxplots showing the pooled elemental concentrations in mmol/mol of Atlantic cod otoliths by spawning area. (A) Ln Mg:Ca, (B) Ln Mn:Ca, (C) Ln Sr:Ca, (D) Ln Ba:Ca. Horizontal lines in the boxes represent the median, the top and bottom of the box show the 25th and 75th percentile, the whiskers the 5th and 95th percentiles and the filled circles represent the outliers; any value beyond the 95th percentile. Significant differences ($p \leq 0.05$) were measured by multiple comparison using Dunnett's T3 test and are expressed by letter codes (ex: for Ln Mg HC =a, so its concentration is the same as O3HC=ab, but differs from BH=bc and SS=c).

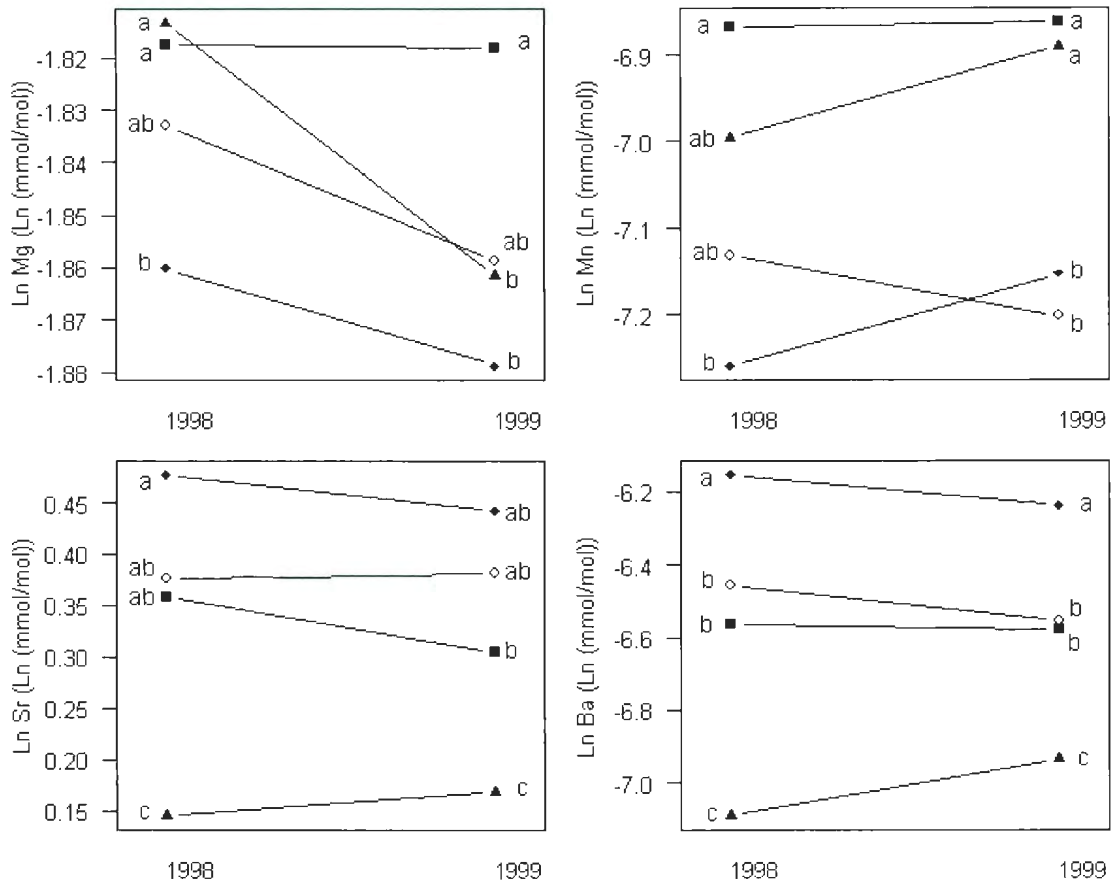


Figure 3.4: Interaction plot showing the difference in elemental concentration of cod otoliths among spawning locations per year. Spawning groups are BH (○), HC (■), O3HC (▲), SS (♦). Significant differences ($p \leq 0.05$) were measured by multiple comparison using Dunnett's T3 test and are expressed by letter codes (ex: for Ln Sr SS in 1998=a, so its concentration is the same as SS in 1999 = ab, but differs from O3HC in 1998 = b).

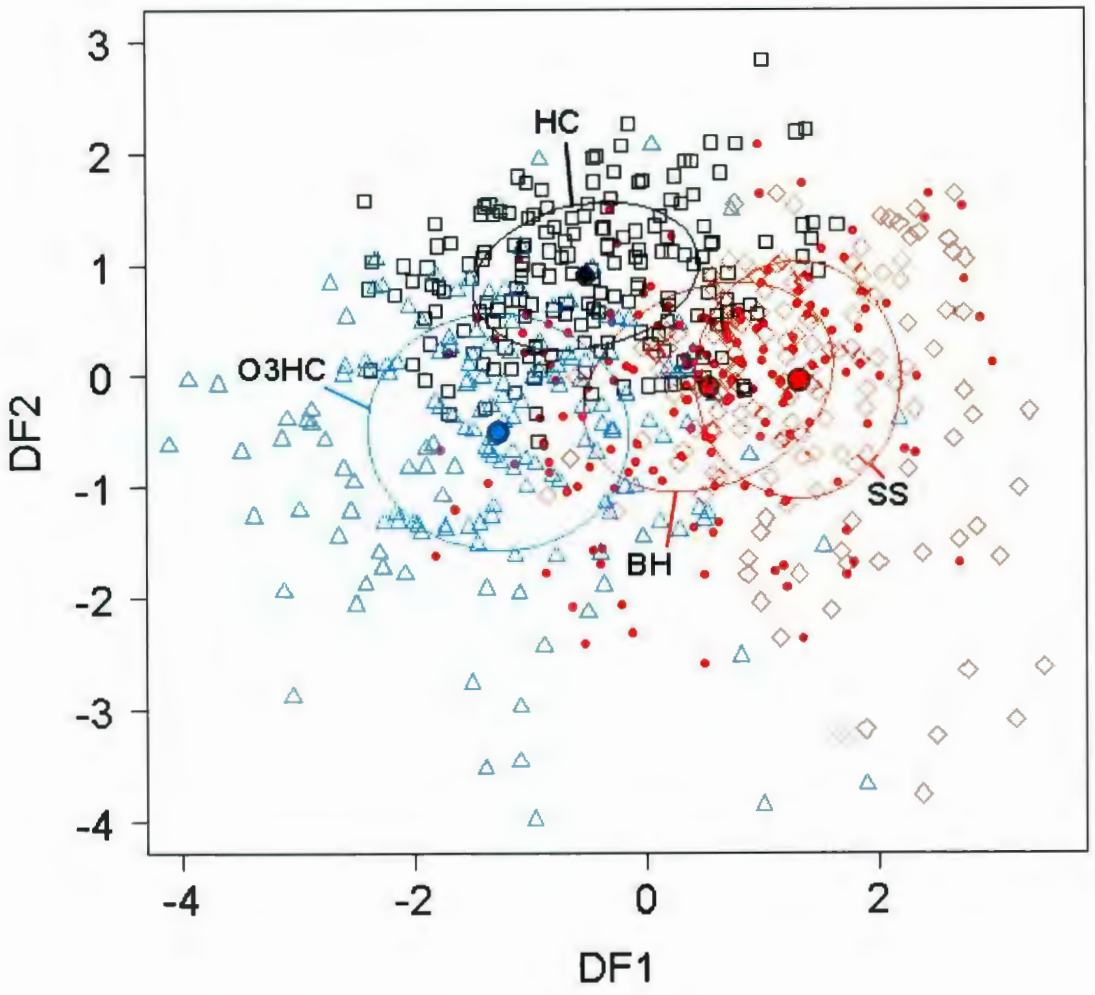


Figure 3.5: Elemental fingerprint of the four cod spawning group based upon the two first discriminant functions of pooled years. Spawning groups are BH (●), HC (□), O3HC (△), SS (◊). Each data point within an area represents signature of a single fish (n=659). A dark circle represents the centroid and the ellipse encompasses the 50% confidence interval of the distribution

Chapter 4: Otolith elemental signatures: Can Atlantic cod migrations be reconstructed in Newfoundland waters?

ABSTRACT

Otolith signatures of Atlantic cod (*Gadus morhua*) were examined along potential migration routes off the oceanographically structured southern coast of Newfoundland (NAFO Subdivision 3Ps). Signatures varied with the physiochemical properties of the environment (bathymetry, temperature, conductivity); cluster analyses suggested two (inshore, offshore) or three main areas (inshore, mid-shore, and offshore). Discriminant analyses of otolith elements (Mg, Mn, Ba, Sr) from fish sampled in these areas correctly classified 93% of cod from the inshore, 89.9% from the mid-shore and 58.6% of cod from the offshore. Considering only inshore-offshore locations and migration, mid- and offshore areas were pooled to yield an average cross-validated classification success of 93%. Distinguishable inshore-offshore otolith signature has the potential to help reconstruct the cross-shelf cod migration in 3Ps area.

INTRODUCTION

Atlantic cod (*Gadus morhua*) undertake seasonal migrations from offshore banks to and within the coastal zone in Newfoundland waters (Templeman, 1966; Rose, 1993). The consistency of these migrations has always varied, however, as reflected in the spatial variability of the fisheries over several centuries (Lear, 1984; Rose, 2007). More recently, with stock declines and heterogeneous rebuilding, this uncertainty has increased (e.g. Rose et al., 2011). Historically, tagging of fish has elucidated many of these phenomena (e.g., Taggart et al., 1995; Brattey et al., 1999; Robichaud and Rose, 2004), but recent restrictions on fisheries limit its usefulness. Telemetry provides an additional tool and has been used effectively to test some distribution hypotheses (e.g., Robichaud and Rose, 2001; Brattey et al., 2002; Brattey et al., 2008), but these methods are limited by high costs, logistics and the small number of fish that can be tagged. Natural tags that could provide an ontogenetic geographically-referenced signature would be invaluable to the fishery. One possible tag is based on otolith uptake of elements in seawater. Reconstruction of migration routes might be possible if the distributions of elements that are readily taken up in to the otolith differ along the route (Sturrock et al., 2012). For Atlantic cod (and many other species, see Sturrock et al., 2012), differences in otolith elemental content have been shown to distinguish fish resident in diverse areas (e.g. Campana et al., 1999; Jónsdóttir et al., 2007; D'Avignon and Rose, Chapter 3, 2013), but reconstruction of migration tracks using otolith chemistry remains largely untested.

The Atlantic cod stock complex on the south coast of Newfoundland (NAFO 3Ps, Figure 4.1) contains several spawning areas, both inshore and offshore, with putative migration patterns associated with feeding and spawning (Lawson and Rose, 2000; Robichaud and Rose, 2001; Brattey et al., 1999). A recent study has shown that fish located at an inshore and offshore spawning area could be distinguished using otolith elemental composition (D'Avignon and Rose, Chapter 3, 2013). In this follow-up study, the objective is to test whether otolith elemental composition might enable reconstruction of cod movements at finer scales from the offshore banks to the inshore region of Placentia Bay.

METHODS

Study region

The 3Ps cod stock was chosen as a case study because it is among the largest of Newfoundland and was at the centre of many tagging and telemetry studies analyzing migratory movements and spawning activity (Lawson and Rose 2000; Windle and Rose 2005). Cod in Placentia Bay in the 3Ps NAFO division migrate out of the Bay during feeding and experience strong homing behaviour –the majority of cod returns to the same spawning site year after year (Lawson and Rose 2000; Robichaud, 2001; Win dle and Rose 2005). Previous tagging experiment revealed high homing rates (87%) for cod in Placentia Bay (Lawson and Rose 2000) and identified a spawning aggregation in the Halibut Channel during the spring and summer months (Brattey et al,1999).

The study region has diverse oceanographic conditions (Figure 4.1). The eastern area adjacent to the Grand Bank and Placentia Bay is dominated by the cold (as low as -1.5^{o}C)

and relatively fresh Labrador Current, whereas the western and southern areas are influenced by warmer and more saline waters from the northward flowing Gulf Stream. (Colbourne, 1999; Bradbury et al, 2000). In April of 1998 and 1999 (Figure 4.1), the coastal areas of Placentia and Fortune Bays and the eastern St. Pierre Bank had salinities in the range of 32.5 to 33.0 (psu) and bottom temperatures near 0°C, while further south and west salinities increased to above 34 psu and temperatures to 8°C in some areas. Temperatures were above normal for areas with depths <200 m in 1998 and 1999 (Colbourne 1999; 2000).

Sampling

Cod were sampled at several sites along cod migration route in 3Ps from April to July in 1998 and 1999 with hand-lines or a bottom trawl, depending on depth and bathymetry (Figure 4.2). The regions ranged from inner Placentia Bay to the outer part of the Halibut Channel at the continental shelf edge, a range of over 200 km. For each cod captured, the date, weight, length, sex, maturity stage, and stomach contents were recorded (Table 4.1). Otoliths were removed, dried, and stored for age determination. At each fishing site, abiotic conditions (conductivity, temperature and depth) were recorded using a trawl-mounted Seabird 19 CTD.

One otolith from each fish was decontaminated and prepared for laser ablation inductively coupled mass spectrometry (LA-ICP-MS, see Campana et al. 1994) analysis to quantify the elemental cocentrations of Mg, Mn, Sr and Ba. Elemental concentrations were measured at the edge of the otolith (see chapter 2 for details), after Longerich and

Gunther (1996), and reported as a ratio to the internal standard Ca in ppm to account for differences in ablation yield. The calibration standard NIST 612 was used to adjust for instrument mass bias and MACS 1 was used to verify precision and accuracy. All elements analysed were above detection limit for more than 50% of individual analyses. Limits of detection (in ppm) were 0.092 for Mg, 0.093 for Mn, 0.093 for Sr, and 0.030 for Ba. Relative standard deviations were below 5% for Mn, Sr and Ba and below 10% for Mg.

Statistical Analysis

Basic diagnostic plots of each elemental ratio were visually assessed for normality and homogeneity. Each elemental concentration was ln-transformed. To avoid confounding size-specific differences with spawning group-specific differences, analysis of covariance (ANCOVA) was used to quantify and remove the effect of fish length on elemental concentrations (Campana et al., 2000). The effect of length was significant for Mg, Mn, and Ba concentraions (ANCOVA, $p< 0.001$), but not for Sr concentrations (ANCOVA, $p=0.580$). Otolith concentrations of Mn and Ba showed significant relationships with length in all locations, so the within-group linear slope (b_1*length) was substracted to remove the effect of length ($b_1= -0.023$ for Mn, $b_1=0.016$ for Ba). The relationship of otolith Mg:Ca with length was positive for inshore locations and negative for offshore locations, hence Mg:Ca was not length detrended.

Temperature, salinity and bathymetry of each fishing set were used as variables in a K-cluster analysis to regroup areas with similar physiochemical properties along cod migration route in 3Ps.Three geographical areas were identified (Figure 4.2). The first was located in inner Placentia Bay around Bar Haven island (K1, n=173), the second, off the Burin Peninsula and inner Halibut Channel (K2, n=120), and the last, at the southwestern tip of the Halibut Channel and continental shelf edge to the Grand Bank (K3, n= 29). Physiochemical properties differed among clusters (Table 4.1). K1 was characterised by the lowest salinity and cold shallow waters ranging between 0 and 4°C. K2 had a slightly higher conductivity than the other areas and greater depths (to 100m). K3 was the most saline with the deepest and warmest waters ranging between 6 and 8°C. These areas were used as a geographical template to test if fish sampled in these areas along the migration route could be distinguished by their otolith elemental signatures.

A multivariate analysis of variance (MANOVA) was performed on the elemental ratios to evaluate the influence of the environmental conditions based on location, age, sex, maturity (mature vs. immature), and fish mean growth rate (cm/yr) on the otolith elemental signature. Pillai's criterion was chosen as the test statistic because it is more robust for unequal sample size and the assumption of similar variance-covariance matrices (Johnson and Field, 1993).

A linear discriminant function analyses (DFA) with leave one out classification used on the otolith chemical concentrations of Mg, Mn, Sr and Ba at the otolith marginof cod

sampled from the environmentally-defined areas to test if otolith signatures differed along the inshore-offshore migration route. Analyses were performed using IBM SPSS Statistics 20. Priors were set as equal to group sizes of each cluster.

RESULTS

The multivariate elemental signatures of cod otoliths differed among clusters (MANOVA, Pillai's Trace=0.694, $F=37.6$, $p < 0.001$, Table 4.2). This suggests temperature, salinity and bathymetry of the geographical areas defined by the three clusters influence chemical composition of the otoliths of cod within 3Ps. Further analyses of the between subject effects revealed the variance of all elemental ratios is best explained by the physiochemical properties of their environment (Table 4.2). The age of the fish captured also influences the Mn:Ca concentration ($p=0.044$). Considering growth rate, sex and maturity were not significant, no biological factors were used in further analyses.

The DFA based upon the Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca otolith concentrations re-assigned cod to the three environmentally-defined areas along the migration route with an average of 88.8.% success (cross-validated estimate, Figure 4.3A).The highest classification accuracy was for K1 (Bar Haven, Placentia Bay) fish with 93.0% correctly re-assigned. The K2 area (Burin Peninsula and inner Halibut Channel) had 89.9% correct classification. The K3 area (offshore Halibut Channel and shelf edge) had the poorest classification success in this analysis (58.6%).

The first discriminant function (DF1) accounted for 93% of the variation in signatures and appears to distinguish geographical areas by their position along a hypothetical line from the head of Placentia Bay to the continental shelf edge (Figure 4.3A). In particular, K1 fish showed a strongly negative DF1 value while both K2 and K3 fish exhibited positive DF1 values (Table 4.2). Mg and Sr concentrations have the largest absolute correlation within DF1, while Mn and Ba dominated in DF2 (Table 4.3).

Considering that the DF1 separated fish along an axis from the head of Placentia Bay to the offshore shelf margins, which is similar to the extremes of putative inshore-offshore migration routes in this region, a second DFA was performed to evaluate the success of otolith chemical signatures to distinguish an inshore-offshore migration. In both areas otolith signatures re-assigned 93.1% of cod to their respective area (Figure 4.3B), improving total classification over the three areas by 4.3%.

Discussion

Otolith signatures in this study varied for the most part according to the physiochemical properties of the environment (temperature, conductivity and bathymetry), as proxies for the differing water masses in which the fish were located. This result followed the expectations of two basic assumptions of otolith elemental studies: that different environmental and water chemistry conditions exist for distinct geographical areas (Elsdon et al, 2008), and that elemental ratio incorporation reflects the ambient

environment in which the fish lives (Campana 1999; Martin and Thorrold, 2005; Walther and Thorrold, 2006).

The oceanic environment of the Newfoundland region provides a structured background that regulates some elemental contents of cod otoliths. Mg and Sr concentrations appear to vary at relatively small spatial scales in this ecosystem and hence are of particular use in reconstructions of location. Based on these differences, our study suggests that inshore to offshore and reverse migrations should be detectable within the otolith layers given locations are physiochemically different.

Even finer scale resolution of the locations of fish over time may also be possible in this ecosystem, as there was a gradient of DFA components from inshore to offshore. The offshore area, however, had greater variability than the inshore, which led to lower classification success. This area is suspected to be a mixing area of cod so this result was expected.

Otolith chemical signatures of the K1 inshore fish were more homogeneous (93% classification success) than the offshore fish. This may be attributed to their sedentary habits prior to capture. Cod spawn at K1 from March to July and stay within a relatively small area (Lawson and Rose 2000; Robichaud and Rose, 2004; Windle and Rose, 2005). These fish are likely exposed to the distinct inshore environment for longer periods than are the offshore fish that results in more consistent otolith composition. Fish spawning in the offshore areas, in contrast, likely move more during the pre-spawning and spawning

season (Brattey et al. 1999; Brattey and Healey 2003), as do offshore cod on the northeast coast of Newfoundland (e.g., Rose, 1993). Such movements limit the spatial resolution of otolith elemental reconstructions of location (Campana, 1999). Despite these sources of variation, fish from the offshore and inshore were differentiated with a high degree of success.

At present, the limit to the finest scale achievable is not known. My results confirm that this scale will be determined not only by elemental variation but by the rate of movement of fish with respect to the different water masses and the manner in which elements vary in the ocean and are incorporated into otoliths (Sturrock et al., 2012). Of note, the inclusion of carbon and oxygen isotope concentrations might lead to smaller scale resolution, as in the case of rainbow smelt (Bradbury et al, 2011); although for a fully marine species like cod this remains uncertain.

How physiochemical properties of the environment influenced otolith elemental ratios observed in this study remains unclear. The Mg:Ca ratio, which was the leading component of the first discriminant function, is normally associated with fish physiology and ontogeny (DiMaria et al., 2010; Sturrock et al, 2012) and not known to accrete in proportion to its presence in the environment. In contrast, the Sr:Ca ratio, also important in the first discriminant function, is thought to be representative of ambient elemental availability (Bath et al, 2000; Elsdon and Gillanders 2002; Walther and Thorrold, 2006). Though elemental ratios within the otolith (eg.Sr:Ca) are known to vary with temperature (Bath et al., 2000; Elsdon and Gillanders, 2002; DiMaria et al., 2010) and salinity (Martin

et al., 2004), further studies are needed to understand the effect of physiochemical properties on the incorporation of elements to the otolith to use otolith signature efficiently to track inshore-offshore migrations

In conclusion, the present study provides the first evidence that otolith elemental chemistry has potential to reconstruct inshore-offshore migrations of cod within their stock complexes in Newfoundland waters, and likely elsewhere where migration routes cross differing water-masses. Knowledge of such spatial dynamics is essential to fisheries management in the northwest Atlantic (Bradbury et al., 2008) and indeed universally (Botsford et al., 2009). The next step is to test reconstructions of seasonal elemental content of otoliths from fish that were known to have migrated.

REFERENCE

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

Botsford, L.W., White, J.W., Coffroth, M.-A., Paris, C.B., Planes, S., Shearer, T.L., Thorrold, S.R., Jones, G.P., 2009. Connectivity and resilience of coral reef metapopulations in marine protected areas: matching empirical efforts to predictive needs. Coral Reefs 28 (2), 327-337.

Bradbury, I.R., DiBacco, C., Thorrold, S.R., Snelgrove, P.V., Campana, S.E., 2011. Resolving natal tags using otolith geochemistry in an estuarine fish, Rainbow smelt *Osmerus mordax*. Mar. Ecol. Prog. Ser. 433, 195-204.

Bradbury I.R., Laurel B.J., Robichaud D., Rose G.A., Snelgrove P.V.R., Gregory R.S., Cote D., Windle M.J. S., 2008. Discrete spatial dynamics in a marine broadcast spawner: Re-evaluating scales of connectivity and habitat associations in Atlantic cod (*Gadus morhua L.*) in coastal Newfoundland. Fish. Res. 91(2-3), 299-309.

Bradbury I., Snelgrove P., Fraser S., 2000. Transport and development of eggs and larvae of Atlantic cod, *Gadus morhua*, in relation to spawning time and location in coastal Newfoundland. Can. J. Fish. Aquat. Sci. 57(9), 1761-1772.

Brattey, J., Healey, B., 2003. Updated estimates of exploitation from tagging of Atlantic cod (*Gadus morhua*) in NAFO Subdivision 3Ps during 1997–2003. Can. Sci. Advis. Sec.. Res. Doc., 2003/091.

Brattey, J., Healey, B., Porter, D., 2008. Northern cod (*Gadus morhua*) 16 years after the moratorium: new information from tagging and acoustic telemetry. Can. Sci. Advis. Sec. Res. Doc., 2008/047.

Brattey, J., Porter, D.R., George, C.W., 2002. Movements of Atlantic cod (*Gadus morhua*) in NAFO Subdiv. 3Ps and updated estimates of exploitation from tagging experiments in 1997-2002. Can. Sci. Advis. Sec. Res. Doc., 2002/097.

Brattey, J., Lawson, G., Rose, G., 1999. Seasonal migration patterns of Atlantic cod (*Gadus morhua*) in Subdivision 3 Ps based on tagging experiments during 1997-98. Can. Sci. Advis. Sec. Res. Doc., 99/37.

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

Campana S., Hanson J., Frechet A., Brattey J., 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res. 46(1-3), 343-357.

Campana S.E., Chouinard G. A., Hanson J. M., Fréchet A., 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(10), 1873-1881.

Colbourne, E.B., 2000. Oceanographic conditions in NAFO Subdivisions 3Pn and 3Ps during 1998 and 1999 with comparisons to the long-term (1961-1990) Average. Can. Sci. Advis. Sec. Res. Doc., 2000/49.

Colbourne, E.B., 1999. Oceanographic conditions in NAFO subdivisions 3Pn and 3Ps during 1997 and 1998 with comparisons to the long-term (1961-1990) average. Can. Sci. Advis. Sec. Res. Doc., 1999/39.

DiMaria R.A., Miller J.A., Hurst T.P., 2010. Temperature and growth effects on otolith elemental chemistry of larval pacific cod, *Gadus macrocephalus*. Environ. Biol. Fish. 89(3-4), 453-462.

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

Elsdon T.S., Wells B.K., Campana S.E., Gillanders B.M., Jones C.M., Limburg K. E., Secor D.H., Thorrold S.R., and Walther B.D., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Ann. Rev. 46, 297-330.

Jónsdóttir I. G., Marteinsdottir G., Campana S. E., 2007. Contribution of different spawning components to the mixed stock fishery for cod in Icelandic waters. ICES J. Mar. Sci. 64(9), 1749-1759.

Lawson G., Rose G., 2000. Seasonal distribution and movements of coastal cod (*Gadus morhua L.*) in Placentia Bay, Newfoundland. Fish. Res. 49(1): 61-75.

Lear, W.H., 1984. Discrimination of the stock complex of Atlantic cod *Gadus morhua* off southern Labrador and eastern Newfoundland Canada as inferred from tagging studies. J. Northw. Atlant. Fish. Sci. 5(2), 143-160.

Longerich H., Gunther D., 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. J. Anal. At. Spectrom. 11(9), 899-904.

Martin, G.B., Thorrold, S.R., 2005. Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile Spot *Leiostomus xanthurus*. Mar. Ecol. Prog. Ser. 293, 223-232.

Martin, G.B., Thorrold, S.R., Jones, C.M., 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). Can. J. Fish. Aquat. Sci. 61(1), 34-42.

Robichaud D., 2001. Multiyear homing of Atlantic cod to a spawning ground. Can. J. Fish. Aquat. Sci. 58(12), 2325-2329.

Robichaud D., Rose G., 2004. Migratory behaviour and range in Atlantic cod: Inference from a century of tagging. Fish Fish. 5(3), 185-214.

Rose G.A., 2007. Cod : The ecological history of the north Atlantic fisheries. Breakwater Books, St. John's, Nfld.

Rose, G.A., 1993. Cod spawning on a migration highway in the north-west Atlantic. Nature 366, 458-461.

Rose, G.A., Nelson, R.J., Mello, L.G.S., 2011. Isolation or metapopulation: whence and whither the Smith Sound cod? Can. J. Fish. Aquat. Sci. 68(1), 152-169.

Sturrock, A., Trueman, C., Darnaude, A., Hunter, E. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? J. Fish Biol. 81(2), 766-795.

Taggart, C. T., P. Penney, N. Barrowman, C. George., 1995. The 1954-1993 Newfoundland cod-tagging database: statistical summaries and spatial-temporal distributions. Can. Tech. Rep. Fish. Aquat Sci., 2042.

Templeman, W., 1966. Marine resources of Newfoundland. Bull. Fish., Res. Bd Can., 154, 170pp.

Walther, B., Thorrold, S., 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. Mar. Ecol. Prog. Ser. 311, 125-130.

Windle M.J.S., and Rose G.A., 2005. Migration route familiarity and homing of transplanted Atlantic cod (*Gadus morhua*). Fish Res. 75, 193-199.

TABLES

Table 4.1: Summary of environmental and biological data recorded for each fish caught in NAFO 3Ps subdivision in 1998 and 1999.

	Inshore Placentia Bay (K1)	Burin-Halibut Channel (K2)	Continental Shelf (K3)
Total sample size (n)	173	120	29
Age 4	0	38	16
Age 5	59	47	6
Age 6	55	35	7
Age 7	59	0	0
Fish condition			
Average Length (cm)	56.80 ± 6.12	49.93 ± 7.54	47.4 1 ± 8.76
Average Weight (kg)	1.59 ± 0.54	1.16 ± 0.54	0.99 ± 0.58
Proportion of mature fish	81.50%	41.70%	41.40%
Diet	Echinoderms, crustaceans and plants	Fish and crustaceans	Fish and crustaceans
Habitat Physico-chemistry			
Temperature range (°C)	0 to 4	<0 to 1	6 to 8
Conductivity range (psu)	32 to 32.4	32.5 to 34.4	32 to 34.7
Depth range (m)	23 to 74	82 to 121	145 to 256
Average otoltith elemental concentration			
Mg:Ca (mmol/mol)	0.13312	0.16774	0.19623
Mn:Ca (mmol/mol)	0.00094	0.00191	0.00125
Sr:Ca (mmol/mol)	1.44834	1.40091	1.33513
Ba:Ca (mmol/mol)	0.00130	0.00075	0.00082

Table 4.2: Multivariate analysis of variance testing the effect of environmental conditions of the clusters, age of cod, sex, maturity and growth rate (cm/year) on the otolith chemical composition of Atlantic cod in the 3Ps NAFO subdivision. The between subject effect test further describe the effect of each factor on each elemental ratio.

Multivariate Test	Pillai's trace	DF	F	P
Cluster	0.694	8	37.601	**<0.001**
Age	0.55	12	1.332	0.195
Sex	0.009	4	0.651	0.627
Maturity	0.027	4	1.926	0.106
Growth (cm/year)	1.306	4	0.18	0.268
Test of between subject effect	DF	MS	F	P
Ln Mg				
Cluster	2	1.771	161.7	**<0.001**
Age	1	0.011	1.026	0.382
Sex	1	0.001	0.116	0.733
Maturity	1	0.046	4.167	0.042
Growth (cm/year)	1	0.042	3.793	0.052
Ln Mn				
Cluster	2	14.68	35.16	**<0.001**
Age	1	1.123	2.737	**0.044**
Sex	1	0.387	0.942	0.333
Maturity	1	0.070	0.171	0.679
Growth (cm/year)	1	0.026	0.063	0.802
Ln Sr				
Cluster	2	0.197	6.208	**0.002**
Age	1	0.037	1.166	0.323
Sex	1	1.48E-5	0	0.983
Maturity	1	0.093	2.915	0.089
Growth (cm/year)	1	6.62E-5	0.002	0.964
Ln Ba				
Cluster	2	7.501	36.71	**<0.001**
Age	1	0.053	0.259	0.855
Sex	1	0.403	1.972	0.161
Maturity	1	0.024	0.117	0.732
Growth (cm/year)	1	0.208	1.02	0.313

Table 4.3: Upper table reports the unstandardized canonical discriminant functions evaluated at group mean for the discriminant function analysis based upon otolith chemistry of cod in three environmentally-defined areas (K1, K2, K3) along cod migration route in 3Ps. K1 show a negative DF1 while K2 and K3 are positive. Lower table shows the pooled within-groups correlations between the elemental ratios (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca) and the standardized canonical discriminant functions. Asterisk represent the largest absolute correlation between each variable and DF.

	DF1	DF2
Inshore Placentia Bay (K1)	-1.318	-0.067
Burin-Halibut Channel (K2)	2.318	-1.083
Continental Shelf (K3)	1.340	0.361
Ln Mg:Ca	*0.899	-0.388
Ln Mn:Ca	0.265	*0.627
Ln Sr:Ca	*-0.103	0.091
Ln Ba:Ca	-0.317	*-0.417

FIGURES

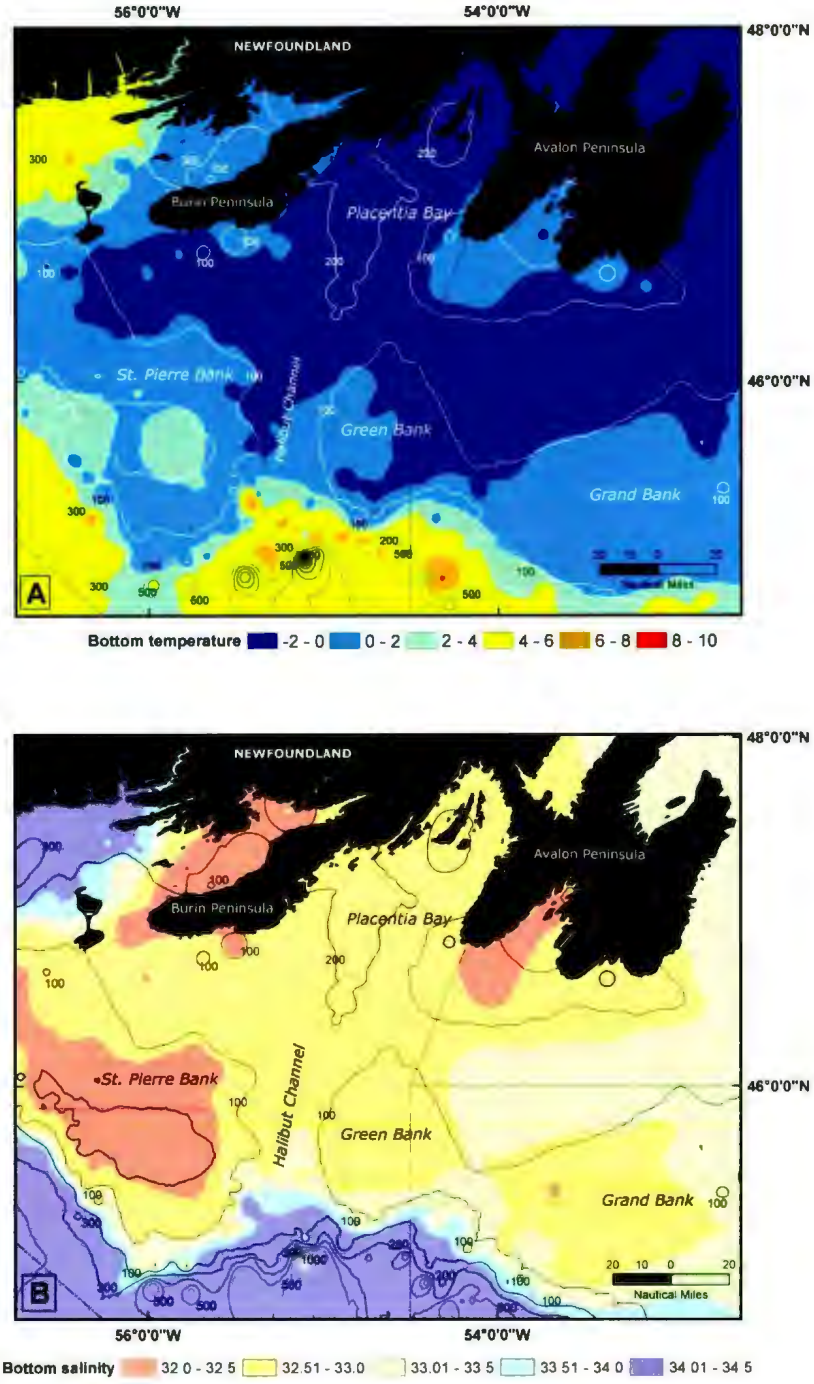


Figure 4.1: Average temperature (A) and average salinity (B) were recorded near the bottom of 3Ps NAFO subdivision from a trawl mounted Seabird 19 CTD between April and July of 1998 and 1999. Contour lines represent the bathymetry of the area and labels indicate depth.

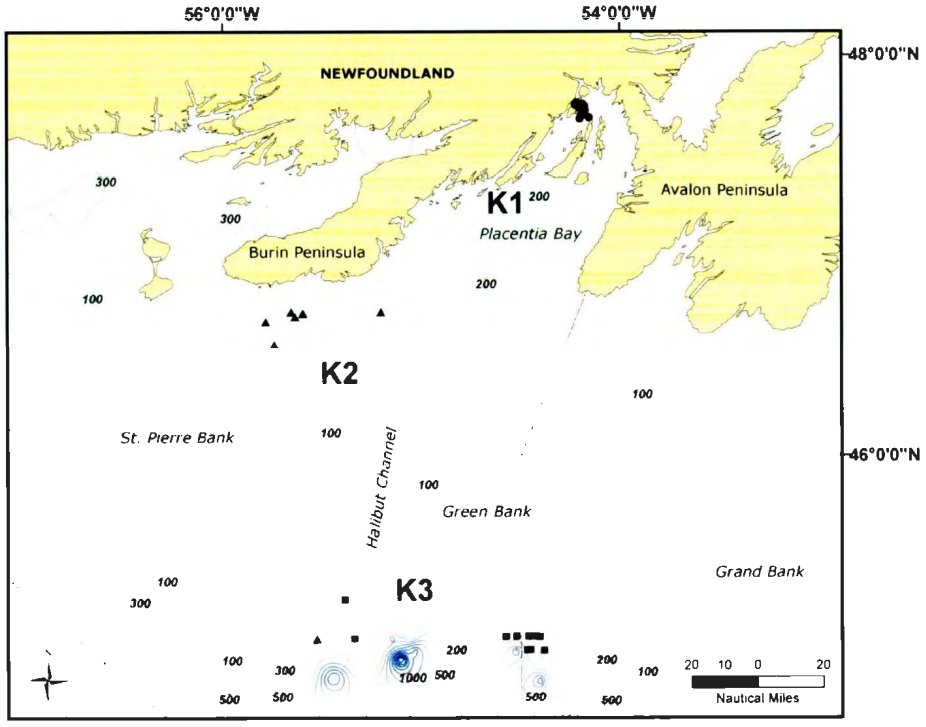


Figure 4.2 Map of 3Ps section with sampling locations in each of the three geographical areas determined by similarity in environmental conditions (cluster analysis of temperature, salinity and bathymetry). K1(●) has shallowest depths, temperatures below zero and low salinity, K2 (▲) sub-zero temperature and low salinity but is deeper, and K3 (■) is characterized by warmer and more saline waters with fish captured at greater depths.

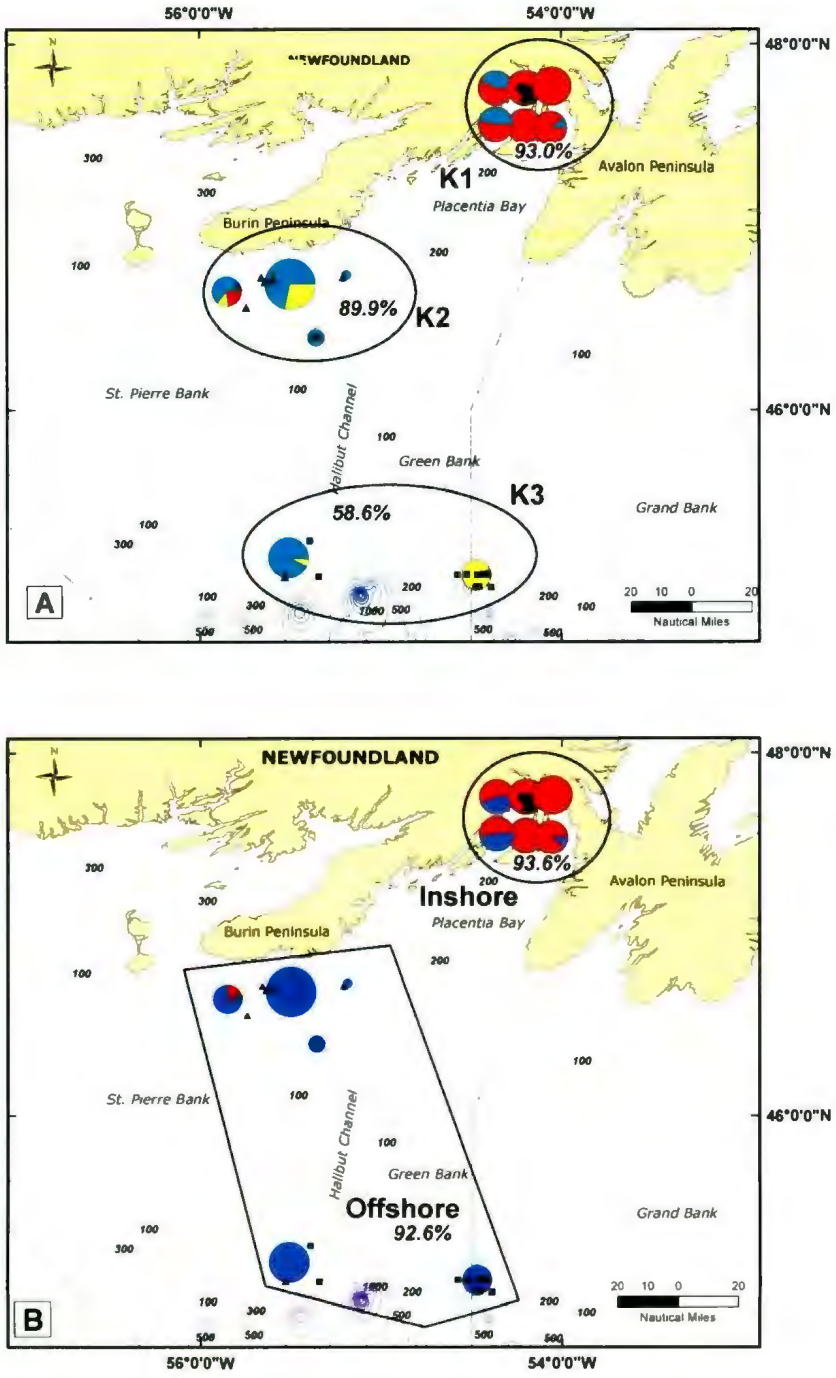


Figure 4.3: Map of 3Ps with the predicted classification (pie charts) of fish based upon otolith chemistry at individual sampling sites. K1 cod is red, K2 is blue and K3 is yellow. Panel A separates cod into three environmental-geographic areas K1 (●), K2 (▲), and K3 (■). Panel B segregates cod into inshore (K1) and offshore (K2 and K3) groups. Percentages indicate the proportion of correctly re-assigned fish by DFA. Pie charts are drawn in proportion to sample size (min. n=2).

Summary

Fish otoliths are natural tags that record important information on the life history of individual fish. Analysis of otolith signatures has potential to be a valuable tool for fisheries management in Canada. The use of sophisticated microprobe techniques (LA-ICP-MS) allowed sampling of the otolith to measure its composition and obtain a precise and accurate multi-element signature, that varied according to the recent environment and condition experienced by the fish. Otolith chemistry and mean growth rates distinguished three out of four spawning areas at large spatial scales. Inshore locations had similar signatures and could not be clearly segregated from each other using otolith elemental ratios of Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca. Merging the southern inshore sites resulted in good classification success (>65%) for all groups.

Important variations in elemental concentrations were attributed to inshore and offshore environments and ocean currents. My research has demonstrated that otolith chemistry can be used to identify Atlantic cod spawning groups in Newfoundland and Labrador waters at large scales if combined with growth rates (e.g., between NAFO divisions) and by itself for smaller scale migrations of cod where growth rates are more similar (e.g., between inshore and offshore areas in 3Ps). These results are encouraging because they suggest it is possible to use otoliths and their chemical signatures as natural tags to elucidate questions about stock structure, migration dynamics and connectivity in Atlantic cod groups off Newfoundland and Labrador. However, additional research should be carried out to identify the effects caused by ontogenetic and reproductive changes. A

novel finding of this research is that the combination of multiple cod age classes and maturity status necessitates the consideration of ontogeny (e.g., using mean growth rates as discriminants) on otolith signatures. Such work should prove beneficial to fisheries management and to stock recovery and rebuilding.

Bibliography

van Achterbergh, E., Ryan, C., Jackson, S., Griffin, W. 2001. Data reduction software for LA-ICPMS, in: Sylvester, P. Laser-Ablation-ICPMS in the Earth Sciences: Principles and Applications. Mineralogy Association of Canada, Ottawa, pp. 239-343

Anderson, J.T., Rose, G.A., 2001. Offshore spawning and year-class strength of Northern cod (2J3KL) during the fishing moratorium, 1994-1996. Can. J. Fish. Aquat. Sci. 58(7), 1386-1394.

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

Ashford, J., Jones, C., Hofmann, E., Everson, I., Moreno, C., Duhamel, G., Williams, R., 2005. Can otolith elemental signatures record the capture site of Patagonian toothfish (*Dissostichus eleginoides*), a fully marine fish in the southern ocean? Can. J. Fish. Aquat. Sci.. 62(12), 2832-2840.

Atkinson, D.B., Rose, G.A., Murphy, E.F., Bishop, C.A., 1997. Distribution changes and abundance of Northern cod (Gadus morhua), 1981-1993. Can. J. Fish. Aquat. Sci. 54(Suppl. 1), 132-138.

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

Beacham, T. D., Brattey, J., Miller, K. M., Le, K. D., Withler, R. E., 2002. Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. ICES J. Mar. Sci. 59(4): 650-665.

Beer, N.A., Wing, S.R., and Swearer, S.E., 2011. Otolith elemental evidence for spatial structuring in a temperate reef fish population. Mar. Ecol. Prog. Ser. 442, 217-227.

Begg, G.A., and Brown, R.W., 2000. Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. Trans. Am. Fish. Soc. 129(4), 935-945.

Begg, G., Cappo, M., Cameron, D., Boyle, S., Sellin, M., 1998. Stock discrimination of school mackerel, *Scomberomorus queenslandicus*, and spotted mackerel, *Scomberomorus munroi*, in coastal waters of eastern Australia by analysis of minor and trace elements in whole otoliths. Fish. Bull.. 96(4), 653-666.

Ben-Tzvi, O., Abelson, A., Gaines, S. D., Sheehy, M. S., Paradis, G. L., Kiflawi, M., 2007. The inclusion of sub-detection limit LA-ICPMS data, in the analysis of otolith microchemistry, by use of a palindrome sequence analysis (PaSA). Limnol. Oceanogr. 5, 97-105.

Bergenius, M., Mapstone, B., Begg, G., Murchie, C., 2005. The use of otolith chemistry to determine stock structure of three epinepheline serranid coral reef fishes on the Great Barrier Reef, Australia. Fish. Res. 72(2-3), 253-270.

Botsford, L.W., White, J.W., Coffroth, M.-A., Paris, C.B., Planes, S., Shearer, T.L., Thorrold, S.R., Jones, G.P., 2009. Connectivity and resilience of coral reef metapopulations in marine protected areas: matching empirical efforts to predictive needs. Coral Reefs 28 (2), 327-337.

Bradbury, I.R., Campana, S.E., Bentzen, P., 2008. Otolith elemental composition and adult tagging reveal spawning site fidelity and estuarine dependency in Rainbow smelt. Mar. Ecol. Prog. Ser. 368, 255-268.

Bradbury, I.R., Laurel, B.J., Robichaud, D., Rose, G.A., Snelgrove, P.V.R., Gregory, R.S., Cote, D., Windle, M.J.S., 2008. Discrete spatial dynamics in a marine broadcast spawner: Re-evaluating scales of connectivity and habitat associations in Atlantic cod (*Gadus morhua L.*) in coastal Newfoundland. Fish. Res. 91(2-3), 299-309.

Bradbury, I.R., DiBacco, C., Thorrold, S.R., Snelgrove, P.V., Campana, S.E., 2011. Resolving natal tags using otolith geochemistry in an estuarine fish, Rainbow smelt *Osmerus mordax*. Mar. Ecol. Prog. Ser. 433, 195-204.

Bradbury, I., Snelgrove, P., Fraser, S. 2000. Transport and development of eggs and larvae of Atlantic cod, *Gadus morhua*, in relation to spawning time and location in coastal Newfoundland. Can. J. Fish. Aquat. Sci.. 57(9), 1761-1772.

Brattey, J. 1997. Biological characteristics of Atlantic cod (*Gadus morhua*) from three inshore areas of northeastern Newfoundland. NAFO Scientific Council Studies.(29), 31-42.

Brattey, J., Healey, B., 2003. Updated estimates of exploitation from tagging of Atlantic cod (*Gadus morhua*) in NAFO Subdivision 3Ps during 1997–2003. Canadian Science Advisory. Secretariat. Research. Document., 2003/091.

Brattey, J., Healey, B., Porter, D., 2008. Northern cod (*Gadus morhua*) 16 years after the moratorium: new information from tagging and acoustic telemetry. Can. Sci. Advis. Sec. Res. Doc., 2008/047.

Brattey, J., Porter, D.R., George, C.W., 2002. Movements of Atlantic cod (*Gadus morhua*) in NAFO Subdiv. 3Ps and updated estimates of exploitation from tagging experiments in 1997-2002. Can. Sci. Advis. Sec. Res. Doc., 2002/097.

Brattey, J., Lawson, G., and Rose, G., 1999. Seasonal migration patterns of Atlantic cod (*Gadus morhua*) in Subdivision 3 Ps based on tagging experiments during 1997-98. Can. Sci. Advis. Sec. Res. Doc., 99/37.

Brown, J., 2006a.Classification of juvenile flatfishes to estuarine and coastal habitats based on the elemental composition of otoliths. Estuar. Coast. Shelf Sci. 66(3-4), 594-611.

Brown, J., 2006b. Using the chemical composition of otoliths to evaluate the nursery role of estuaries for English sole *Pleuronectes vetulus* populations. Mar. Ecol. Prog. Ser. 306, 269-281.

Brown, T.J. 1999. The Hamilton Bank-Hawke Channel region: potential as an offshore Marine Protected Area? A study to examine the physical, biological, economic and social characteristics of an offshore fishing area. M.M.S. Thesis, Marine Institute, Memorial University of Newfoundland.

Brown, R., and Severin, K. 1999. Elemental distribution within polymorphic inconnu (*Stenodus leucichthys*) otoliths is affected by crystal structure. Can. J. Fish. Aquat. Sci. 56(10), 1898-1903.

Buckel, J., Sharack, B., and Zdanowicz, V. 2004. Effect of diet on otolith composition in *Pomatomus saltatrix*, an estuarine piscivore. Journal of Fish Biology. 64(6), 1469-1484.

Busch, W.-D.N., B.L. Brown, and G.F. Mayer (Eds). 2003. Strategic Guidance for Implementing an Ecosystem-based Approach to Fisheries Management. United States Department of Commerce, National Oceanic and Atmospheric Administration, NMFS, Silver Spring, MD 62p.

Campana, S.E., 2004. Photographic atlas of fish otoliths of the Northwest Atlantic Ocean. National Research Council Canada, Ottawa.

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

Campana, S.E., Gagne, J.A., 1995. Cod stock discrimination using ICPMS elemental assays of otoliths, in: Secor, D.H., Dean, J.M., and Campana, S.E. Recent Developments in Fish Otolith Research.University of South California Press, Columbia, SC. pp: 671-691.

Campana, S.E., Casselman, J.M., 1993. Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. 50(5), 1062-1083.

Campana, S.E., Valentin, A., Sevigny, D., and Power, D. 2007. Tracking seasonal migrations of redfish (*Sebastes spp.*) in and around the Gulf of St. Lawrence using otolith elemental fingerprints. Can. J. Fish. Aquat. Sci. 64(1), 6-18.

Campana, S., Hanson, J., Frechet, A., Brattey, J. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res. 46(1-3), 343-357.

Campana, S.E., Chouinard, G.A., Hanson, J.M., and Fréchet, A. 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(10), 1873-1881.

Campana, S., Thorrold, S., Jones, C., Guenther, D., Tubrett, M., Longerich, H., Jackson, S., Halden, N., Kalish, J., Piccoli, P., de Pontual, H., Troadec, H., Panfili, J., Secor, D., 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(9), 2068-2079.

Campana, S.E., Fowler, A.J., Jones, C.M., 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Can. J. Fish. Aquat. Sci.. 51(9), 1942-1950.

Castonguay, M., Simard, P., Gagnon, P., 1991. Usefulness of Fourier-analysis of otolith shape of Atlantic mackerel *Scomber scombrus* stock discrimination. Can. J. Fish. Aquat. Sci. 48(2), 296-302.

Castro, B.G., 2007. Element composition of sardine (*Sardina pilchardus*) otoliths along the Atlantic coast of the Iberian Peninsula. ICES J. Mar. Sci. 64(3), 512-518.

Chittaro, P.M., Klinger, T., Telmer, K., Sanborn, M., Morgan, L., 2010. Using otolith chemistry to investigate population structure of Quillback rockfish in Puget Sound. Northwest Sci. 84(3), 243-254.

Chittaro, P., Usseglio, P., Fryer, B., Sale, P., 2006. Spatial variation in otolith chemistry of *Lutjanus apodus* at Turneffe Atoll, Belize. Estuar. Coast. Shelf Sci. 67(4), 673-680.

Chittaro, P., Usseglio, P., Fryer, B., Sale, P., 2005. Using otolith microchemistry of *Haemulon flavolineatum* (French grunt) to characterize mangroves and coral reefs throughout Turneffe Atoll, Belize: Difficulties at small spatial scales. Estuaries 28(3), 373-381.

Clarke, L.M., Friedland, K.D., 2004. Influence of growth and temperature on strontium deposition in the otoliths of Atlantic salmon. J. Fish Biol. 65(3), 744-759.

Clarke, L.M., Thorrold, S.R., Conover, D.O,. 2011. Population differences in otolith chemistry have a genetic basis in *Menidia menidia*. Can. J. Fish. Aquat. Sci. 68(1), 105-114.

Clarke, L.M., Munch, S.B., Thorrold, S.R., Conover, D.O., 2010. High connectivity among locally adapted populations of a marine fish (*Menidia menidia*). Ecology 91(12), 3526-3537.

Clarke, L.M., Walther, B.D., Munch, S.B., Thorrold, S.R., Conover, D.O., 2009. Chemical signatures in the otoliths of a coastal marine fish, *Menidia menidia*, from the Northeastern United States: Spatial and temporal differences. Mar. Ecol. Prog. Ser. 384, 261-271.

Colbourne, E.B., 2000. Oceanographic conditions in NAFO Subdivisions 3Pn and 3Ps during 1998 and 1999 with comparisons to the long-term (1961-1990) Average. Can. Sci. Advis. Sec. Res. Doc., 2000/49.

Colbourne, E.B. 1999. Oceanographic conditions in NAFO Subdivisions 3Pn and 3Ps during 1997 and 1998 with comparisons to the long-term (1961-1990) Average. Can. Sci. Advis. Sec. Res. Doc., 99/39. 18p.

Correia, A.T., Gomes, P., Goncalves, J.M.S., Erzini, K., Hamer, P.A., 2012. Population structure of the black seabream *Spondyliosoma cantharus* along the South-west Portuguese coast inferred from otolith chemistry. J. Fish Biol. 80(2), 427-443

COSEWIC. 2010. COSEWIC assessment and status report on the Atlantic cod Gadus morhua in Canada. Committee on the Status of Endangered Wildlife in Canada. xiii + 105pp. (www.sararegistry.gc.ca/status/status_e.cfm).

de Pontual, H., Lagardière, F., Amara, R., Bohn, M., Ogor, A., 2003. Influence of ontogenetic and environmental changes in the otolith microchemistry of juvenile sole (Solea solea). J. Sea Res. 50, 199-211.

deYoung, B., Rose, G.A., 1993. On recruitment and distribution of Atlantic cod (*Gadus morhua*) off Newfoundland. Can. J. Fish. Aqut. Sci. 50(12), 2729-2741.

DFO- Department of Fisheries and Oceans, 2013. Principles of ecosystem-based fisheries management. Available at http://www.dfo-mpo.gc.ca/fm-gp/peches-fisheries/fish-ren-peche/sff-cpd/ecosys-back-fiche-eng.htm. Accessed on July 23, 2013.

DFO-Department of Fisheries and Oceans. 2011. Stock Assessment of Subdivision 3Ps cod, October 2011. Can. Sci. Advis. Sec. Sci. Advis. Rep., 2011/079.

DFO-Department of Fisheries and Oceans. 2009. Stock Assessment of Northern (2J3KL) cod in 2009. Can. Sci. Advis. Sec. Sci. Advis. Rep., 2009/009.

DFO-Department of Fisheries and Oceans. 2005. Stock Assessment Report on Northern (2J3KL) cod. Newfoundland and Labrador Region. Can. Sci. Advis. Sec. Sci Advis. Rep., 2005/024.

DiMaria, R.A., Miller, J.A., Hurst, T.P., 2010. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. Environ. Biol. Fish. 89(3-4), 453-462.

Doak, D. F., D. Bigger, E. K. Harding, M. A. Marvier, R. E. O'Malley,Thompson, D., 1998. The statistical inevitability of stability–diversity relationships in community ecology. Am. Nat. 151: 264–76.

Dorval, E., Jones, C.M., Hannigan, R., van Montfrans, J.. 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Can. J. Fish. Aquat. Sci. 64(3), 411-424.

Edmonds, J., Caputi, N., Morita, M., 1991. Stock discrimination by trace-element analysis of otoliths of Orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. Mar. Fresh. Res. 42, 383-389.

Elsdon, T., 2002. Interactive effects of temperature and salinity on otolith chemistry: Challenges for determining environmental histories of fish. Can. J. Fish. Aquat. Sci. 59(11), 1796-1808.

Elsdon, T.S., Gillanders, B.M., 2005. Strontium incorporation into calcified structures: separating the effects of ambient water concentration and exposure time. Mar. Ecol. Prog. Ser. 285: 233-243.

Elsdon, T.S., Gillanders, B.M., 2004. Fish otolith chemistry influenced by exposure to multiple environmental variables. J. Exp. Mar. Biol. Ecol. 313(2), 269-284.

Elsdon, T.S., Gillanders, B.M., 2003. Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. Rev. Fish Biol. Fish. 13(3), 219-235.

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

Elsdon T.S., Wells B.K., Campana S.E., Gillanders B.M., Jones C.M., Limburg K. E., Secor D.H., Thorrold S.R., and Walther B.D., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Ann. Rev. 46, 297-330.

Fairclough, D. V., Edmonds, J. S., Lenanton, R. C. J., Jackson, G., Keay, I. S., Crisafulli, B. M., Newman, S. J., 2011. Rapid and cost-effective assessment of connectivity among assemblages of *Choerodon rubescens* (labridae), using laser ablation ICP-MS of sagittal otoliths. J. Exp. Mar. Biol. Ecol. 403(1-2), 46-53.

FAO Fisheries Department.The ecosystem approach to fisheries. FAO Technical Guidelines for Responsible Fisheries. 4, Suppl. 2. Rome, FAO. 2003. 112 p.

FAO. The ecosystem approach to fisheries management. Fisheries and Aquaculture Department. Available at: http://www.fao.org/fishery/topic/13261/en. Accessed on July 23, 2013.

Ferguson, G.J., Ward, T.M., Gillanders, B.M., 2011. Otolith shape and elemental composition: Complementary tools for stock discrimination of mulloway (*Argyrosomus japonicus*) in southern Australia. Fish. Res. 110(1), 75-83.

FitzGerald, J., Thorrold, S., Bailey, K., Brown, A., Severin, K., 2004. Elemental signatures in otoliths of larval walleye Pollock (*Theragra chalcogramma*) from the northeast Pacific Ocean. Fish. Bull. 102(4), 604-616.

Forrester, G.E., Swearer, S.E., 2002. Trace elements in otoliths indicate the use of open-coast versus bay nursery habitats by juvenile California halibut. Mar. Ecol. Prog. Ser. 241, 201-213.

Fudge, S.B., and Rose, G.A. 2008 a. Life history co-variation in a fishery depleted Atlantic cod stock. Fish. Res. 92(1), 107-113.

Fudge, S.B., Rose, G.A., 2008b. Changes in fecundity in a stressed population: Northern cod (Gadus morhua) off Newfoundland, in: Kruse, G.H., Drinkwater, K., lanelli, J.N., Link, J.S., Stram, D.L., and Wespestad, V. Resiliency of Gadid Stocks to Fishing and Climate Change. Lowell and Wakefield Fisheries Symposia Series, Anchorage, AK. pp179-196.

Geffen, A.J., Morales-Nin, B., Perez-Mayol, S., Cantarero, A., Skadal, J., Tovar-Sanchez, A., 2013. Chemical analysis of otoliths: Cross validation between techniques and laboratories. Fish. Res.143, 67-80.

Gibb, F.M., Gibb, I.M., Wright, P.J., 2007. Isolation of Atlantic cod (*Gadus morhua*) nursery areas. Mar. Biol. 151(3), 1185-1194.

Gillanders, B. M., 2002a. Temporal and spatial variability in elemental composition of otoliths: Implications for determining stock identity and connectivity of populations. Can. J. Fish. Aquat. Sci. 59(4), 669-679.

Gillanders, B. M., 2002b. Connectivity between juvenile and adult fish populations: Do adults remain near their recruitment estuaries? Mar. Ecol. Prog. Ser. 240, 215-223.

Gillanders, B.M., Kingsford, M.J., 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar. Ecol. Prog. Ser. 141(1-3), 13-20.

Gjosaeter, J., Danielssen, D.S., 2011. Age, growth and otolith annulus formation of cod (*Gadus morhua*) in the risor area on the Norwegian Skagerrak coast during 1986-1996. Mar. Bio. Res. 7(3), 281-288.

Grafton, R., Pham, V., 2009. Cod today and none tomorrow: The economic value of a marine reserve. Land Econ. 85(3), 454-469.

Hamer, P., 2003. Otolith chemistry of juvenile snapper *Pagrus auratus* in Victorian waters: Natural chemical tags and their temporal variation. Mar. Ecol. Prog. Ser. (Halstenbek) 263, 261-273.

Hamer, P., Jenkins, G., Gillanders, B., 2005. Chemical tags in otoliths indicate the importance of local and distant settlement areas to populations of a temperate sparid, *Pagrus auratus*. Can. J. Fish. Aquat. Sci. 62(3), 623-630.

Hannesson, R. 1996. Fisheries mismanagement: The case of the north Atlantic cod. Fishing News Books, Oxford; Cambridge, Mass.

Hanson, P., Koenig, C., Zdanowicz, V., 2004. Elemental composition of otoliths used to trace estuarine habitats of juvenile gag *Mycteroperca microlepis* along the west coast of Florida. Mar. Ecol. Prog. Ser. 267, 253-265.

Hoff, G., and Fuiman, L. 1995. Natural variation in elemental composition of sagittae from red drum. J. Fish Biol. 47(6), 940-955.

Hussy, K., Nielsen, B., Mosegaard, H., Clausen, L.W., 2009. Using data storage tags to link otolith macrostructure in Baltic cod *Gadus morhua* with environmental conditions. Mar. Ecol. Prog. Ser. 378, 161-170.

ICES. 2012. ICES 2012 Annual Science Conference, Bergen, Norway.

ICES. 2013. Report of the Working Group on Working Group on the Northwest Atlantic Regional Sea (WGNARS), 28 January – 1 February 2013, Dartmouth, Canada. ICES CM 2013/SSGRSP:03. 108 pp.

Jackson, S. E., 2001. Lamtrace user's manual. School of Earth Sciences, Macquarie University, Sydney, Australia.

Janney, P.E., Ritcher, F.M., Mendybaev, R.A., Wadhwa, M., Georg, R.B., Watson, E.B., Hines, R.R., 2011. Matrix effects in the analysis of Mg and Si isotope ratios in natural and synthetic glasses by laser ablation-multicollector ICPMS: A comparison of single- and double-focusing mass spectrometers. Chem. Geol. 281, 26-40.

Johnson, C.R., Field, C.A., 1993. Using fixed-effects model multivariate analysis of variance in marine biology and ecology. Oceanogr. Mar. Biol Annu. Rev. 31, 117-221.

Jones, C., Chen, Z., 2003. New techniques for sampling larval and juvenile fish otoliths for trace-element analysis with laser-ablation sector-field inductively-coupled-plasma mass spectrometry (SF-ICP-MS). Institute of Marine Research, Postboks 1870 Nordnes N-5817 Bergen Norway.

Jónsdóttir, I.G., Marteinsdottir, G., Campana, S.E., 2007. Contribution of different spawning components to the mixed stock fishery for cod in Icelandic waters. ICES J. Mar. Sci. 64(9), 1749-1759.

Kalish, J.M., 1989. Otolith microchemistry validation of the effects of physiology age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132(3), 151-178.

Kalish, J., Livingston, M., Schofield, K., 1996. Trace elements in the otoliths of New Zealand blue grenadier (*Macruronus novaezelandiae*) as an aid to stock discrimination. Mar. Fresh. Res. 47(3), 537-542.

Kellison, G.T., Taylor, J.C., 2007. Demonstration and implications of habitat-specific chemical signatures in otoliths of juvenile summer flounder (*Paralichthys dentatus linnaeus*) in North Carolina. J. Fish Biol. 71, 350-359.

Kingsford, M.J., Hughes, J.M., Patterson, H.M., 2009. Otolith chemistry of the non-dispersing reef fish *Acanthochromis polyacanthus*: Cross-shelf patterns from the central Great Barrier Reef. Mar. Ecol. Prog. Ser. 377, 279-288.

Kingsford, M., Gillanders, B. 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. Mar. Biol. 137(3), 403-414.

Knickle, D.C., Rose, G.A., 2010. Seasonal spawning and wind-regulated retention-dispersal of early life stage Atlantic cod (*Gadus morhua*) in a Newfoundland fjord. Fish. Oceanogr. 19(5), 397-411. .

Krumsick, K., Rose, G.A., 2012. Atlantic cod (Gadus morhua) feed during spawning off Newfoundland and Labrador. ICES J. Mar. Sci. 69(10), 1701-1709.

L'Abée-Lund, J., 1988. Otolith shape discriminates between Atlantic salmon, *Salmo salar L*, and brown trout, *Salmo trutta L.* J. Fish Biol. 33(6), 899-903.

Lawson, G., Rose, G., 2000. Seasonal distribution and movements of coastal cod (*Gadus morhua L.*) in Placentia Bay, Newfoundland. Fish. Res. 49(1), 61-75.

Lear, W.H., 1984. Discrimination of the stock complex of Atlantic cod *Gadus morhua* off southern Labrador and eastern Newfoundland Canada as inferred from tagging studies. J. Northw. Atlant. Fish. Sci. 5(2), 143-160.

Lombarte, A., Lleonart, J., 1993. Otolith size changes related with body growth, habitat depth and temperature. Environ. Biol. Fishes. 37(3), 297-306.

Longerich, H., Gunther, D., 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. J. Anal. At. Spectrom. 11(9), 899-904.

Longerich H., Gunther D., Jackson S., 1996. Elemental fractionation in laser ablation inductively coupled plasma mass spectrometry. Fresen. J. Anal. Chem. 355, 538-542.

Longmore, C., Fogarty, K., Neat, F., Brophy, D., Trueman, C., Milton, A., Mariani, S., 2010. A comparison of otolith microchemistry and otolith shape analysis for the study of spatial variation in a deep-sea teleost, *Coryphaenoides rupestris*. Environ. Biol. Fish. 89(3-4), 591-605.

Lo-Yat, A., Meekan, M., Munksgaard, N., Parry, D., Planes, S., Wolter, M., Carleton, J., 2005. Small-scale spatial variation in the elemental composition of otoliths of *Stegastes nigricans* (Pomacentridae) in French Polynesia. Coral Reefs 24(4), 646-653.

Ludsin, S., Fryer, B., Gagnon, J., 2006. Comparison of solution-based versus laser ablation inductively coupled plasma mass spectrometry for analysis of larval fish otolith microelemental composition. Trans. Am. Fish. Soc. 135(1), 218-231.

Martin, G.B., Thorrold, S.R., 2005. Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*. Mar. Ecol. Prog. Ser. 293, 223-232.

Martin, G.B., Thorrold, S.R., Jones, C.M., 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot *Leiostomus xanthurus*. Can. J. Fish. Aquat. Sci. 61(1), 34-42.

Melancon, S., Ludsin, S., Gagnon, J., Yang, Z., 2005. Effects of crystal structure on the uptake of metals by lake trout (*Salvelinus namaycush*) otoliths. Can. J. Fish. Aquat. Sci. 62(11), 2609-2619.

Miller, J.A., 2009. The effects of temperature and water concentration on the otolith incorporation of barium and manganese in black rockfish *Sebastes melanops*. J. Fish Biol. 75(1), 39-60.

Milton, D.A., Chenery, S.R., Farmer, M.J., Blaber, S.J.M., 1997. Identifying the spawning estuaries of the tropical shad, *Terubok tenualosa toli*, using otolith micro-chemistry. Mar. Ecol. Prog. Ser. 153(1-3), 283-291.

Mindak. W.M. 2008. FDA Elemental Analysis Manual. Section 3.6.4 Inductively Coupled Plasma-Mass Spectrometer Available from: http://www.fda.gov/EAM3 (Accessed 2012 July 24).

Morais, P., Dias, E., Babaluk, J., Antunes, C. 2011. The migration patterns of the European flounder *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae, Pisces) at the

southern limit of its distribution range: Ecological implications and fishery management. J. Sea Res. 65(2), 235-246.

Morris, J., Rulifson, R., Toburen, L., 2003. Life history strategies of striped bass, *Morone saxatilis*, populations inferred from otolith microchemistry. Fish Res. 62(1), 53-63.

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

NOAA., 2013. Ecosystem Science. Available at: http://www.st.nmfs.noaa.gov/ecosystems/index . Accessed on July 23, 2013.

Norman, M.D., McCulloch, M.T., O'Neill, H.S., Yaxley, G.M., 2006. Magnesium isotopic analysis of olivine by laser-ablation multi-collector ICP-MS: composition dependent matrix effects and a comparison of the Earth and Moon. J. Anal. At. Spectrom. 21, 50–54.

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

Patterson, H., Thorrold, S., Shenker, J., 1999. Analysis of otolith chemistry in Nassau grouper (*Epinephelus striatus*) from the Bahamas and Belize using solution based ICP MS. Coral Reefs. 18(2), 171-178.

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

Pearce, N. J. G., Perkins, W. T., Westgate, J. A., Gorton, M. P., Jackson, S. E., Neal, C. R., Chenery, S. P., 1997. A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. Geostandards Newslett. 21(1), 115-144.

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

Potts, P. J. 1987. Inductively coupled plasma-mass spectrometry, in: Handbook of Silicate Rock Analysis. Blackie, Glasgow, pp. 575-586

Pulliam, H.R., 1988. Sources, sinks, and population regulation. Am. Nat. 132(5), 652-661.

Ranaldi, M.M., Gagnon, M.M., 2008. Zinc incorporation in the otoliths of juvenile pink snapper (Pagrus auratus Forster): The influence of dietary and waterborne sources. J. Exp. Mar. Biol. Ecol. 360, 56-62.

Rao, A., Outhouse L-A., Gregory D., 2009. Special marine area in Newfoundland and Labrador: Areas of interest in our marine backyards. (CPAWS-NL) Canadian Parks and Wildlife Society, Newfoundland and Labrador. Available at: http://cpaws.org/uploads /pubs/report_nlmarineguide.pdf, Accessed on September 4, 2011.

Robichaud, D., 2001. Multiyear homing of Atlantic cod to a spawning ground. Can. J. Fish. Aquat. Sci. 58(12), 2325-2329.

Robichaud, D., Rose, G., 2004. Migratory behaviour and range in Atlantic cod: Inference from a century of tagging. Fish Fish. 5(3), 185-214.

Rose, G.A., 2009. Variations in the target strength of Atlantic cod during vertical migration. ICES J. Mar. Sci. 66(6), 1205-1211.

Rose, G.A., 2007. Cod: The ecological history of the North Atlantic fisheries. Breakwater Books, St. John's, Nfld. pp591.

Rose, G.A. 1997. The trouble with fisheries science! Rev. Fish Biol. Fish. 7(3), 365-370.

Rose, G.A., 1993. Cod spawning on a migration highway in the north-west Atlantic. Nature 366, 458-461.

Rose, G.A., Nelson, R.J., Mello, L.G.S., 2011. Isolation or metapopulation: whence and whither the Smith Sound cod? Can. J. Fish. Aquat. Sci. 68(1), 152-169.

Ruttenberg, B.I., Warner, R.R., 2006. Spatial variation in the chemical composition of natal otoliths from a reef fish in the Galapagos Islands. Mar. Ecol. Prog. Ser. 328, 225-236.

Ruttenberg, B.I., Hamilton, S.L., Warner, R.R., 2008. Spatial and temporal variation in the natal otolith chemistry of a Hawaiian reef fish: Prospects for measuring population connectivity. Can. J. Fish. Aquat. Sci. 65(6), 1181-1192.