UTILITY ASSESSMENT OF OTOLITH MICROCHEMISTRY FOR SALMON FISHERY MANAGEMENT AND MP-AES ANALYTICAL SYSTEM FOR FOOD SAFETY IN LAKE MELVILLE, LABRADOR

by

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Abstract

Atlantic salmon (*Salmo salar*) Food, Social, and Ceremonial (FSC) fishery in Lake Melville, Labrador, is an important food source for Indigenous communities living around Lake Melville. The feasibility of using analytical tools to provide information for fishery management and to enable healthcare authorities to ensure food security and safety was assessed in this thesis. First, the feasibility of using otolith elemental concentrations as natural markers of natal river were investigated by using Na, Mg, K, Mn, Zn, Rb, Sr, and Ba concentrations in juvenile and adult salmon. Juvenile salmon were sampled in 11 watersheds flowing into Lake Melville, while adult salmon samples were collected from the FSC fishery. Discrete juvenile otolith elemental composition among watersheds was demonstrated by linear discriminant function analysis, reassigning juveniles accurately to their natal river 89 % (range 73 % - 100 %) of the time on average. Natal river of adults was inferred based on the juvenile dataset. Most of the adult salmon were assigned to the Kenamu River, but the assignment performances seemed to be limited by the interannual and spatial variability of the natal rivers' chemistry. The results obtained demonstrated that the use of otolith microchemistry as a tool for providing information on natal river for fishery management and ensure food security in Lake Melville is promising but further research is required.

Second, the feasibility of using microwave plasma-atomic emission spectrometry (MP-AES) to quantify mercury at low concentrations in Lake Melville salmon muscle tissue was assessed. The method developed was validated by using certified reference material (DORM-3) and by mercury quantification of the salmon tissue performed by an accredited laboratory using cold vapor-atomic fluorescence spectrometry (CV-AFS) and by MP-AES. Mercury concentrations quantified by MP-AES were not significantly different than those obtained by an accredited laboratory, indicating that MP-AES can accurately quantify mercury. The MP-AES limit of detection (0.005 $\mu g\ g^{-1}$ wet weight) is well below the mercury consumption limit (0.2 $\mu g\ g^{-1}$ wet weight) recommended by Health Canada for FSC fisheries. Our results also demonstrated that the Atlantic salmon in Lake Melville are safe to consume (range 0.04 – 0.07 $\mu g\ g^{-1}$ wet weight) and will serve as references for monitoring this fishery after the impoundment of Muskrat Falls. Finally, we concluded that MP-AES is a suitable analytical system for mercury quantification to assess and monitor food safety.

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List of Abbreviations and Symbols

ANOVA – Analysis of variance

Ba – Barium

$CaCO_3$ – Calcium carbonate

CRM – Certified reference material

CV – Canonical variable

CV-AFS – Cold vapor-atomic fluorescence spectrometry

CV-MP-AES – Cold vapor microwave plasma-atomic emission spectrometry

FSC – Food, Social, and Ceremonial

Hg – Mercury

K – Potassium

LA-ICP-MS – Laser ablation inductively coupled plasma mass spectrometer

LDA – Linear discriminant function analysis

LOD – Limit of detection

MAD – Median absolute deviation

MANOVA – Multivariate analysis of variance

MeHg – Methylmercury

Mg – Magnesium

Mn – Manganese

MP-AES – Microwave plasma-atomic emission spectrometry

Na – Sodium

$NaBH_4$ – Sodium borohydride

Rb – Rubidium

RSD – Relative standard deviation

SD – Standard deviation

Sr – Strontium

Zn – Zinc

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Chapter 1: Introduction and Overview

1.1 Context and Objectives

Atlantic salmon is an important food source for Indigenous communities, tightly linked to their social, cultural, spiritual, economic and nutritional well-being (Brice-Bennett 1977, Government Nunatsiavut 2016). Lake Melville, a semi-enclosed estuarine fjord located in central Labrador (Bobbitt and Akenhead 1982), supports an important Atlantic salmon Food, Social, and Ceremonial (FSC) fishery from the three Indigenous groups (Innu, Inuit and Southern Inuit) which represent 60 % of the region's population (Statistics Canada 2017). The FSC fishery in Lake Melville harvests Atlantic salmon from different river populations (mixed-stock) (Bradbury et al. 2018) and a lack of information on the salmon mixed-stock causes food security concern for Indigenous communities. Identifying natal rivers is fundamental to understanding salmon population dynamics and structure to developing appropriate fishery management practices and to protecting the resource (Hart and Reynolds 2002, Cadrin and Secor 2009). Salmon populations often show different biological characteristics and productivity (Prévost et al. 2003, Crozier et al. 2004). Therefore, information collected from one salmon population may not be representative of another population. These differences among salmon populations make the management of a fishery exploiting multiple river populations (mixed-stock) challenging (Crozier et al. 2004) and can lead small populations with low productivity to extinction (Hilborn and Walters 1992). Lake Melville's fishery is managed based on four index rivers (English River, Muddy Bay Brook, Southwest Brook, and Sand Hill River) located outside the Lake Melville watershed on the coast of Labrador (DFO 2016), which may not be representative of salmon populations dynamics in the Lake Melville watershed. Furthermore, salmon populations in Lake Melville maybe genetically distinct compared to Labrador coastal populations. Verspoor (2005) hypothesized that juvenile salmon collected in Cape Caribou River, a tributary of Lake Melville, were genetically different from coastal Labrador salmon populations, including two of the index rivers used in Labrador fishery management. Using novel developments in sequencing, genotyping, and bioinformatics, several genetic studies were recently initiated to investigate Vespoor's hypothesis (Bradbury et al. 2018, Sylvester et al. 2018). Three recent studies have confirmed that salmon captured in 12 tributaries of Lake Melville are genetically different from coastal Labrador populations (Jeffery et al. 2017, Bradbury et al. 2018). Bradbury et al. (2018) also concluded that Lake Melville's mixed-stock fishery is principally harvesting salmon originating from two clusters of rivers within Lake Melville's watershed: Crooked-Red Wine Rivers and Caroline-Traverspine-Kenamu Rivers. These results accentuate the importance of gathering information on the relative importance of the salmon natal river and their contribution to the mixed-stock FSC fishery.

In my thesis, instead of using genetic techniques, we used otolith microchemistry as a natural marker to infer natal river of adult salmon from the Lake Melville FSC fishery. Otolith microchemistry is an increasingly common technique to delineate fish stocks and infer natal origins of adult fish to its their natal river or tributary (Walther et al. 2008, Marklevitz et al. 2011, Olley et al. 2011, Martin et al. 2013). Rapidity, cost-effectiveness, and simplicity are the principal advantages of using otolith microchemistry instead of tagging, telemetry or genetic techniques (Elsdon et al. 2008, Pangle et al. 2010, Fairclough et al. 2011, Sturrock et al. 2012). Tagging studies require a great tagging/sampling effort due to low recovery rate (Elsdon et al. 2008, Pangle et al. 2010, Fairclough et al. 2011), while telemetry can be time consuming in the field . Genetic sample preparation requires more time and manipulations before analysis, is expensive and genetic markers usually identify populations at broader scale (multiple natal rivers grouped together) than does otolith microchemistry (Martin et al. 2015, Taillebois et al. 2017, Bradbury et al. 2018). Another advantage of using otoliths that may sometimes be overlooked but is highly useful, is that samples can be stored for years without fear of degradation. The efficiency of otolith microchemistry has been successfully demonstrated to infer the natal origin of Atlantic salmon and other anadromous fish (see section 1.2.2 for further information) (Wells et al. 2003, Olley et al. 2011, Veinott et al. 2012, Martin et al. 2013).

Mercury concentration in water increases following hydroelectric dam impoundment and its associated risk for food safety is also a critical concern in Lake Melville. In the 1970s, the Churchill Falls hydroelectric dam was built on the upper Churchill River, approximately 300 km upstream from Lake Melville (Anderson 2011). Since 2014, a new hydroelectric dam is currently being constructed and impoundment of the reservoir began in November 2016 at Muskrat Falls on the lower Churchill River, approximately 40 km upstream from Lake Melville (Calder et al. 2016). Increases of methylmercury in aquatic food chains associated with the decomposition of organic matter following reservoir impoundment have been recognized since 1974 and are well documented worldwide (Smith et al. 1974, Hylander et al. 2006, Larssen 2010, Anderson 2011) (see section 1.2.3

for further information). Coupled with atmospheric mercury emissions from anthropogenic sources at lower latitudes (Macdonald et al. 2000, 2003, Clarkson 2002) and increasing local anthropogenic activities other than hydroelectric damming (i.e., mining, oil exploitation, and transportation), rising mercury concentration in northern regions is expected (Pirkle et al. 2016). Mercury is recognized as a priority contaminant in northern regions due to its bioaccumulation potential, toxicity, and probability of consumption by northern Indigenous peoples who rely on country foods (AMAP 2011, 2015). Therefore, developing local, rapid, and cost-effective analytical strategies to monitor food safety in northern regions is vital, particularly for the Indigenous communities.

The Microwave Plasma-Atomic Emission Spectrometer (MP-AES) has great potential for increasing research capacity in northern regions due to its reduced operational cost, multi-elemental capacity, and simple manipulations compared to standard analytical techniques including atomic absorption spectrometry (AAS) and inductively coupled plasma-mass spectrometry (ICP-MS) (Niedzielski et al. 2015). However, MP-AES technology has, to the best of our knowledge, no well-established protocols for quantifying mercury at trace levels (environmental level) and its performance (accuracy and precision) is yet to be determined.

The overall objective of my thesis was to assess the potential of using analytical chemistry tools to provide fundamental information on Atlantic salmon in Lake Melville for fishery management, food security, and food safety. In Chapter 2, I evaluated the feasibility of using otolith microchemistry (Na, Mg, K, Mn, Zn, Rb, Sr, Ba) to infer adult

Atlantic salmon natal river from the mixed-stock fishery in the Lake Melville watershed. Otoliths sampled from juvenile and adult salmon otolith captured in rivers and the Lake Melville estuary from FSC fishery, respectively, were analyzed by Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and linear discriminant function analysis was used to distinguish and infer the natal rivers.

Chapter 3 described my assessment of the performance of MP-AES for mercury quantification at low concentration in salmon muscle tissue in order to provide a rapid, local, and cost-effective tool for assessing food safety. Although mercury is not the only trace element of health concern in northern regions, it was the focus of this chapter since it is an issue of special concern in Lake Melville area. The performances of the MP-AES were investigated by comparing mercury quantified in adult salmon muscle tissue sampled from the FSC fishery in Lake Melville to mercury concentrations obtained by a certified laboratory.

1.2 Background

1.2.1 Study area: Lake Melville

Hamilton Inlet is a coastal body of water located in central Labrador (Fitzhugh 1972) and is comprised of Groswater Bay and Lake Melville (Fig. 1.1). The term Hamilton Inlet represents different water bodies in local usage and official terminology (Fitzhugh 1972). This thesis uses the official terminology and the term Hamilton Inlet. Lake Melville is made up of three basins: Goose Bay, Lake Melville proper, and The Backway and is connected to Groswater Bay and the Labrador Sea by a shallow constriction called The Narrows near the town of Rigolet.

Lake Melville's watershed is important for Atlantic salmon as it provides freshwater spawning, nursery and rearing habitats and saltwater foraging habitats (Anderson 1985, Reddin et al. 2010). Lake Melville is 180 km long with a maximum width of 35 km, and has an average depth of 85 m (maximum depth: 256 m; Bobbitt and Akenhead 1982). This semi-enclosed estuarine fjord is characterized by a permanent stratification on its entire surface (3,069 km^2) (Bobbitt and Akenhead 1982). At the Narrows, cold salt water from the Labrador Sea enters Lake Melville. At the western end, Lake Melville receives most of its freshwater inputs from four major rivers: Churchill River, Naskaupi River, Kenamu River, and Goose River (Backus 1957, Bobbitt and Akenhead 1982, Anderson 1985). The Churchill River is the largest river draining the Labrador plateau (120,000 km^2) and contributes more than 60 % of the freshwater inflow to Lake Melville through Goose Bay (Anderson 2011, Schartup et al. 2015). At the western end of Lake Melville, Grand Lake receives freshwater inputs from five rivers before outflowing into Lake Melville. The rich freshwater inputs bring nutrients to the cold salt water, creating a dynamic environment that supports high productivity and species diversity (Wells et al. 2017). Lake Melville's unique habitat resulted in it being identified as an Ecological and Biological Significant Area (EBSA) by the Canadian Science Advisory Secretariat (CSAS) (Wells et al. 2017).

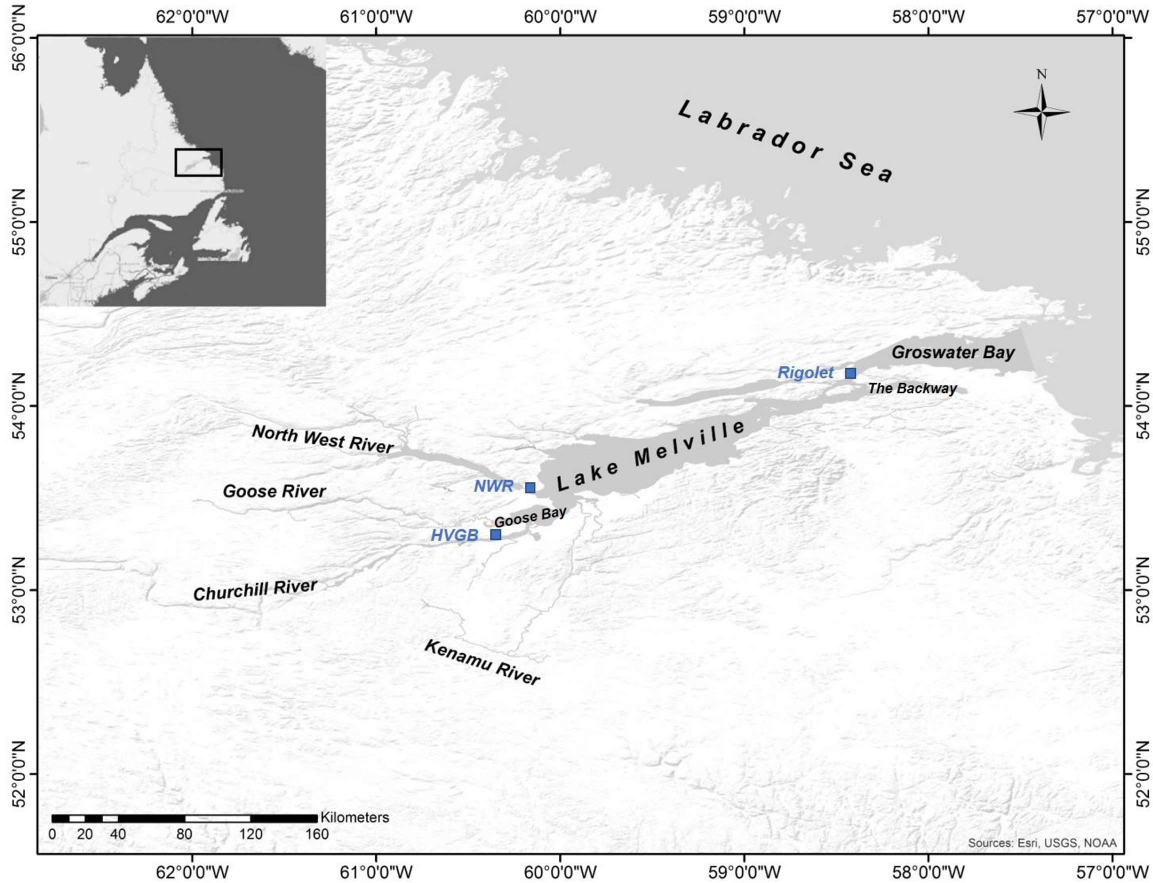

Fig. 1.1. The study area showing Hamilton Inlet and the four major tributaries of Lake Melville. In blue, HVGB: Happy Valley-Goose Bay town, NWR: North West River town, Rigolet town.

Dense conifer forest and wetlands can be found in the lowlands surrounding Lake Melville, while barrens lands and lichen-covered areas are found at higher altitudes (Notzl et al. 2013). Lake Melville is located in the Grenville geological province of the Canadian Shield and its watershed bedrock is primarily composed of granitic-gneiss, anorthosite, arkose, and gabbro (Wardle et al. 1997).

1.2.2 Salmon fishery management tool: Otolith microchemistry analysis

Otoliths are inner ear bones of teleost fish used for sound detection, maintaining balance, and orientation (Payan et al. 2004, Popper and Fay 2011). The otolith is a crystalline structure made of ~97 % calcium carbonate ($CaCO_3$); growing daily and incorporating minor and trace elements in the accreted layers (Pannella 1971, Campana 1999, Campana and Thorrold 2001). Elements comprising the otolith are mainly absorbed from the water via the gills or intestines then transferred to the blood plasma, and to the endolymph (Campana 1999, Payan et al. 2004). The material forming the otolith is precipitated from the endolymph on to the exterior surface during the life of the fish (Campana and Neilson 1985, Campana 1999, Payan et al. 2004). Therefore, the otolith provides information on the life history of the fish (Campana 1999). Teleost fish such as Atlantic salmon have three pairs of otoliths: sagittae, lapilli, and asterisci. The sagittal otoliths are usually the largest of the three and the most used in natural marker studies (Campana and Thorrold 2001, Kerr and Campana 2013).

The potential of using the otoliths as a natural marker for natal river in anadromous fish has been greatly explored and proven successful in several studies (Walther et al. 2008, Olley et al. 2011, Veinott et al. 2012, Martin et al. 2013). The utilization of elemental composition depends on several assumptions (reviewed in Elsdon et al. 2008). In particular, three key factors allow the utilization of the otolith as a natural marker. First, the elemental composition must be preserved in the structure during the life of the fish. Otoliths are acellular and metabolically inert through the life of the fish; they are not reabsorbed during times of metabolic stress (Campana and Neilson 1985). Second, to use the otolith as natural markers of fish population, there must be significant spatial variation in water chemistry and other physical parameters (i.e., temperature) that influence the composition of the otolith structure. Consequently, fish inhabiting different environments exhibit different otolith elemental compositions (Elsdon et al. 2008). Atlantic salmon otolith microchemistry is influenced by river water chemistry, which in turn is influenced by the geology of the drainage basin (Kennedy et al. 2000, Wells et al. 2003, Friedrich and Halden 2008). The latter affects river water chemistry through the weathering of the till, bedrock and soil cover of the watershed (Brezonik and Arnold 2011). Third, the residence time must be sufficient for the otolith to register the water's elemental signature and allow quantification (Elsdon et al. 2008).

Depending of the region, adult salmon usually spawn in fresh water in October and November, egg hatching usually begins soon after spring freshet and juveniles rear in fresh water for three to six years until they reach the smolt stage and migrate to sea (Anderson 1985, Klemetsen et al. 2003, DFO and MNRF 2008). Typically, salmon mature at sea and forage between one up to three years before returning to their natal river to spawn (Klemetsen et al. 2003). Migration from fresh water to salt water can be visually identified by looking at a cross-section of an adult otolith (Fig. 1.2). On a cross-section of adult otolith, the center (core) of the otolith represents the maternal signature and the edge of the otolith represents the most recent accretion. In Fig. 1.2, otolith layers are visible and the change in coloration indicates the downstream migration to salt water. By analyzing the otolith microchemistry, the end of natal river elemental signature is identified by a sharp increase in Sr and a simultaneous decrease in barium (Ba) concentrations, indicating the downstream migration from fresh water to salt water habitats (Kraus and Secor 2004, Elsdon and Gillanders 2005, Zimmerman 2005). Therefore, in river systems showing distinct water chemistry, it is possible to infer natal river of adult fish by analyzing the elemental composition of the freshwater signature (juvenile phase) in an adult otolith and compare it to juvenile otolith signatures collected from nearby rivers.

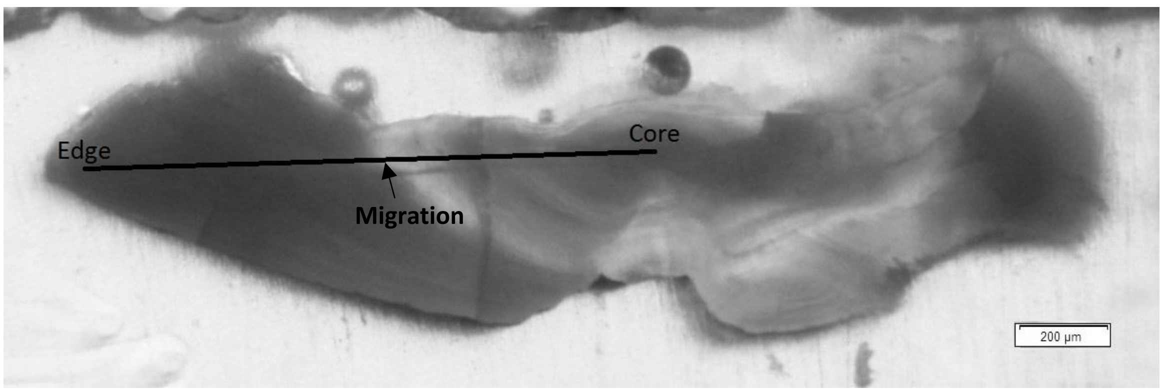

Fig. 1.2. Photography of a cross-section of an adult otolith with visible layers of accretion under 2.88x magnification. The core of the otolith is the maternal signature and the edge is the most recent material accretion. The change of coloration indicates the downstream migration to salt water.

In general, non-essential elements (i.e., strontium and barium) are used in otolith microchemistry techniques since they are less physiologically regulated. However, essential elements (i.e., sodium and potassium) can be useful as markers if their concentrations vary significantly among groups (Campana 2005). Non-essential elements such as Sr and Ba are frequently used due to the strong correlation between elemental concentrations in water and otoliths (Bath et al. 2000, Wells et al. 2003, Martin et al.

2013). However, other elements have been used in discrimination studies, such as lithium (Li), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), rubidium (Rb), and zinc (Zn), to improve discrimination power (e.g., Edmonds et al. 1989, Thorrold et al. 1998, Veinott and Porter 2005, Arkhipkin et al. 2009, Walther and Thorrold 2009, Cuveliers et al. 2010, Loewen et al. 2015).

Otolith elemental (minor and/or trace elements) concentrations are quantified with an inductively coupled plasma mass spectrometer (LA-ICP-MS). Following simple preparation of the otolith (see Chapter 2), the desired surface of the otolith is ablated by the laser, which vaporizes the elements. The ablated particles are entrained by a flux of argon (Ar) to an inductively coupled plasma torch (ICP) where the elements are ionized (Skoog et al. 2013). The ions are directed to the mass spectrometer, which separates the ions based on their mass-to-charge ratio (*m/z*) and quantifies the elements according to the intensity of the signal (count per second, cps). Transformation of the data based on the otolith ^{43}Ca normalised concentration is then required to obtain elemental concentrations (µg g^{-1}) (Longerich et al. 1996). Statistical analyses such as discriminant function or machine learning method (i.e., random forest) is then performed on the otolith elemental concentrations of the groups (Olley et al. 2011, Gahagan et al. 2012, Mercier et al. 2012, Veinott et al. 2012, Martin et al. 2013, Loewen et al. 2015).

1.2.3 Food safety: Mercury analysis with microwave plasma – atomic emission spectrometry

Mercury is a toxic element which can be lethal at high exposure and has neurological and reproductive effects (World Health Organization 1990, Tchounwou et al. 2003, Hong et al. 2012). Its ability to pass the blood-brain and blood-placenta barriers and affect the central nervous system makes mercury a priority contaminant to human health (UNEP 2013). Northern regions (Arctic and Sub-Arctic) are exposed to increasing mercury concentrations, mostly produced and released by anthropogenic sources at lower latitudes and then transported to northern regions (Macdonald et al. 2000, 2003, AMAP 2011). Water impoundment associated with hydroelectric development can also result in elevated mercury levels (Bodaly et al. 1984, Anderson et al. 1995).

Mercury is naturally present in the environment (air, water, soil, bedrock, and vegetation) under three forms: inorganic (Hg^{2+}), elemental (Hg^0), and methylmercury CH_3Hg^+, its most toxic form. When an area is flooded, the organic matter decomposes and releases non-methylated mercury into the water, where it becomes bioavailable for methylating bacteria to produce methylmercury (Ullrich et al. 2001). Methylmercury is readily bioaccumulated and biomagnified through the aquatic food chain (Braune et al. 2015). The bioaccumulation process by which methylmercury is absorbed by organisms causes an increasing mercury concentration during the life of the organisms (AMAP 2011). The biomagnification process by which species from a higher trophic level will have greater mercury concentrations than species from a lower trophic level (i.e., seal eating fish versus fish eating crustacean) results usually in top-of-the-food-chain animals such as predator fish and marine mammals having the highest mercury concentrations (AMAP 2011). Although Lake Melville is located downstream (~300 km) of the Churchill Falls hydroelectric development, estuarine fish species in Lake Melville, Rainbow smelt (*Osmerus mordax*) and Sea-run brook trout (*Salvelinus fontinalis*), were affected by the impoundment of the Smallwood Reservoir (Anderson 2011). The time required to return to mercury background level after impoundment was over 20 years for rainbow smelt and less than 28 years for sea trout (Anderson 2011). Adult Atlantic salmon feed on crustaceans and small fish at sea (Sheehan et al. 2012), as do sea trout in Lake Melville (Scott and Crossman 1998, Anderson 2011). Atlantic salmon's trophic level is similar to that of sea trout and its shorter exposure to mercury due to its feeding far out to sea would suggest that mercury concentration in Lake Melville's Atlantic salmon is probably currently at background level. However, impoundment of the reservoir for a new hydroelectric development at Muskrat Falls on the Lower Churchill River has begun (October 2016). Mercury concentration in water and organisms living in Lake Melville is expected to increase following the impoundment (Schartup et al. 2015, Calder et al. 2016). While adult Atlantic salmon are less likely to be affected than resident species that feed solely within the estuary, there remains significant concern among the Indigenous fishers for whom this species represents a significant FSC fishery.

Schartup et al. (2015) demonstrated that most of the mercury found in Lake Melville originates from rivers and that the upper meters of the estuarine water column are enriched with methylmercury from the freshwater inputs and bacterial activity. Lake Melville is highly efficient at methylating mercury due to a thin layer at the interface of the brackish water (well oxygenated with high content of terrestrial organic matter) and salt water layers (Schartup et al. 2015) (Fig. 1.3).

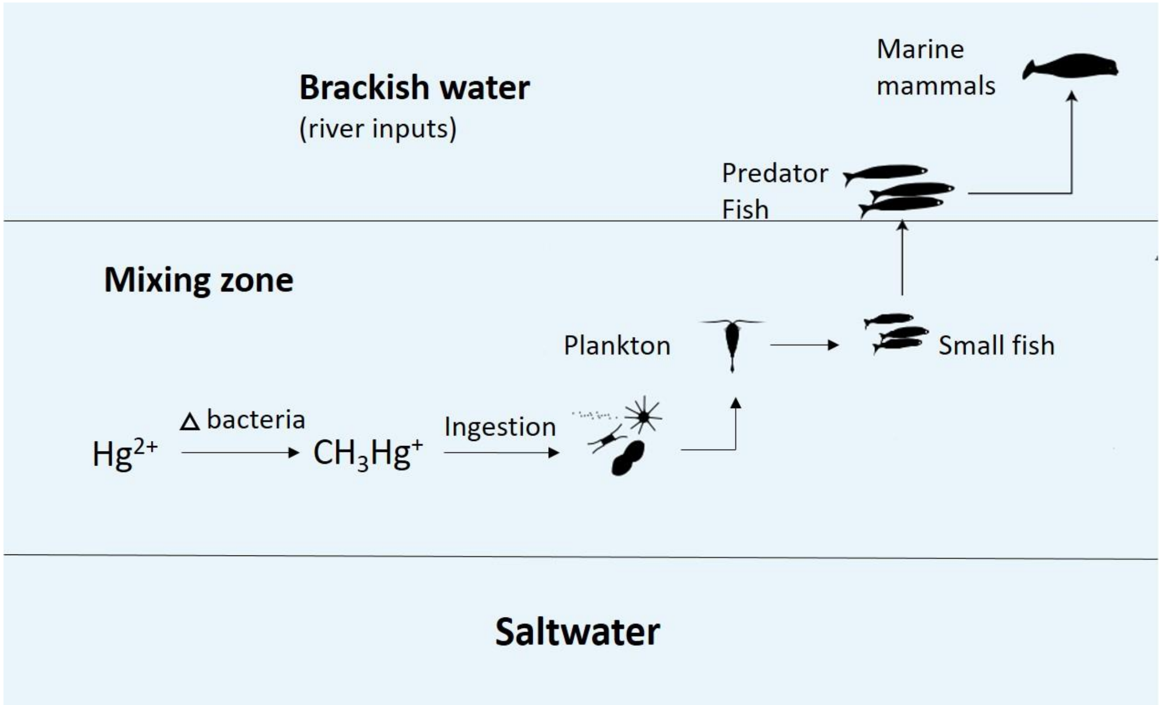

Fig. 1.3. Conceptual diagram representing mercury methylation, bioaccumulation, and biomagnification in the aquatic food chain of the Lake Melville estuary. Mercury (Hg^{2+}) from fresh water inputs is methylated by bacteria in the mixing zone to produce methylmercury (CH_3Hg^+) that is bioaccumulated and biomagnified through the food chain. Different water layers are not to scale and taxa are not restricted to certain water layers. Adapted from AMAP 2011, Schartup et al. 2015.

The microwave plasma-atomic emission spectrometer (MP-AES) is an analytical system for quantifying elements in a solution that could allow the development of mercury analytical capacity in northern regions. To analyze a solid matrix such as fish tissue, the samples must be digested to a solution by using an acid reagent and heat. The solution can then be pumped and is either nebulized, to form fine particles, or transformed to their volatile form (i.e., mercury volatile state Hg^0). The elements are entrained by the flux of nitrogen and are excited (higher energy level) by the microwave-induced plasma torch (Skoog et al. 2013). When the elements return to their normal state, the energy is emitted as photons (visible or ultraviolet light) (Skoog et al. 2013). The wavelengths (nm) are element-specific and are used to quantify the elements by the charge coupled device (CCD) detector. Although microwave-induced plasma technology has existed since the 1950s, it was not until the 1990s that the microwave-induced plasma torch was developed (Broekaert and Siemens 2004), and in 2011 a system using the microwave-induced nitrogen plasma torch coupled to an atomic emission spectrometer was commercialized (MP-AES by Agilent Technologies). This analytical system operates using a nitrogen generator, therefore, reducing costs and challenges related to transport and provision of other gases (e.g., argon and acetylene) (Hammer 2008, Balaram et al. 2013). Atomic absorption spectroscopy (AAS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) analytical systems are commonly used for elemental analysis at low cost. MP-AES is a cost-effective analytical system compared to inductively coupled plasma systems (ICP-MS and ICP-AES) using argon gas and the multi-elemental capability offer more possibilities than the traditional AAS. Its use also requires only simple and minimal manipulation of the samples. The analytical performance of the MP-AES was reported superior to AAS (Li et al. 2013, Niedzielski et al. 2015, Lima et al. 2015) and comparable to ICP-AES (Hammer 2008, Li et al. 2013, Zhao et al. 2015). Since the commercialization of the MP-AES, the performance of the analytical system has been assessed for at least 30 elements in different inorganic and organic matrices such as geological materials, fertilizer, water, crude oil, wine, plants, and leather (Balaram et al. 2013, Li et al. 2013, Kamala et al. 2014, Nelson et al. 2015, Niedzielski et al. 2015, Lima et al. 2015, Karlsson et al. 2015, Zhao et al. 2015, Tanabe et al. 2016). To the best of our knowledge, mercury quantification has been successfully performed in two studies. One study quantified mercury contained in inorganic fertilizer and the other in leather (Lima et al. 2015, Zhao et al. 2015). This methodology has not yet been validated for quantifying mercury in fish tissue.

To reduce health risks from mercury, Health Canada established the maximum level for total mercury in commercial fish sales at 0.5 $\mu g\ g^{-1}$ wet weight (ww) and 0.2 $\mu g\ g^{-1}$ for subsistence fisheries (Health and Welfare Canada 1979). In order to be useful for northern regions, MP-AES must have lower detection and quantification limits than the maximum level of Health Canada's subsistence fishery mercury guidelines. Since Atlantic salmon in Lake Melville have low mercury concentrations (0.07 ± 0.02 $\mu g\ g^{-1}$ ww, Li et al. 2016) in their muscle tissue, they are suitable for use to evaluate MP-AES performance at low concentrations.

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Co-authorship statement

The initial concept was designed by my co-supervisors (Dr. Marie Clément and Dr. Richard St-Louis) and the research team working on the genetics of Atlantic salmon in Lake Melville prior to the begin of my masters and during my first semester. During my first and second semesters, I completed a literature review and wrote the research proposal. The details of the project design of the MP-AES performances assessment in Chapter 3 were decided during discussions with my co-supervisors.

Chapter 2 was originally part of a doctoral project and most of the practical aspects of the research were organized by student Jonathan Pearce under the supervision of Dr. Marie Clément and Dr. Ian Bradbury (Fisheries and Oceans Canada). Otolith preparation and analysis methodology were suggested by Dr. Geoff Veinott (Fisheries and Oceans Canada) and followed Jenke (2002) procedures; and water samples were analyzed at the Earth Resources Research Analysis laboratory of Memorial University of Newfoundland. Statistical analyses described in this thesis were performed by myself with guidance from my co-supervisors and Dr. Geoff Veinott. Practical aspects of Chapter 3 were organized and performed by myself, except sampling, which was performed with the help of Jonathan Pearce, Lianna Rice, and Joshua Adams.

This thesis manuscript consolidates the research work described above and has been prepared by myself. The manuscript has been reviewed and improved following recommendations suggested by my committee members, Dr. Geoff Veinott and Dr. Ian

Bradbury. Finally, the third chapter has been accepted with revision by the International Journal of Environmental Analytical Chemistry and I am the principal author.

Chapter 2: Otolith microchemistry as a tool for inferring a mixed-stock fishery's origin on a large spatial scale, Lake Melville watershed, Labrador

Abstract

We evaluated the feasibility of using otolith elemental concentrations (Na, Mg, K, Mn, Zn, Rb, Sr, and Ba) for inferring natal rivers of Atlantic salmon (*Salmo salar*). Juvenile salmon were sampled from 11 river watersheds flowing into Lake Melville and adult Atlantic salmon were sampled from the Food, Social, and Ceremonial fishery in Lake Melville (Labrador, Canada). The linear discriminant function analysis (LDA) cross-validation reassigned the juveniles accurately to their natal river 89 % of the time on average (range 73 % - 100 %). Adult natal rivers were inferred based on the juvenile dataset and the majority of the salmon were assigned to Kenamu River. Four rivers (Main Brook, Mulligan, Peters, and Red Wine) had no adult assigned to them. Nearly all adults, except one, failed the squared Mahalanobis distance test for true membership. Although otolith microchemistry is a promising technique to provide information on the natal river, the capacity to infer the natal rivers of adult salmon appeared to be limited in the Lake Melville watershed and further research is required. The likely cause of this result was the water chemistry interannual variability of the natal river and discrete environments within river watersheds. Data on otolith microchemistry interannual variability and spatial variability within river watersheds are required to advance the technique in the Lake Melville watershed. Further data on river watersheds such as geology, vegetation cover,

water chemistry, and data on salmon life-history and movements would allow linking the factors influencing otolith microchemistry to the otolith microchemistry observed.

Résumé

Nous avons évalué la faisabilité d'utiliser les concentrations des éléments (Na, Mg, K, Mn, Zn, Rb, Sr et Ba) dans les otolites comme marqueurs naturels afin de déterminer les rivières natales de Saumons de l'Atlantique (*Salmo salar*) adultes provenant de la pêche de subsistance dans l'estuaire Lac Melville (Labrador, Canada). La fonction discriminante linéaire (LDA) a classifié correctement les juvéniles provenant de 11 rivières différentes à leurs rivières natales avec un taux de succès moyen de 89 % (73 % - 100 %). La majorité des saumons adultes ont été assignés à la rivière Kenamu. Aucun saumon adulte n'a été assigné à quatre rivières (Main Brook, Mulligan, Peters et Red Wine). La capacité à déterminer les rivières natales des saumons adultes semble être limitée dans le bassin versant du Lac Melville. Des données sur la variation interannuelle et spatiale à l'intérieur des bassins versants des rivières devront être recueillies afin d'améliorer la technique. Ensuite, plus de données sur la géologie, le couvert végétal et la chimie de l'eau de rivière des bassins versants, ainsi que des données sur l'historique du poisson et de ses mouvements, permettront de lier les facteurs influençant la chimie des otolithes aux concentrations en éléments observées.

Keywords: Otolith, microchemistry, Atlantic salmon, Natal river, subsistence fishery

2.1 Introduction

Lake Melville is a large (3,000 km^2) subarctic estuarine fjord in Labrador (Canada) connected to the Labrador Sea. Lake Melville supports an important aboriginal Food, Social, and Ceremonial (FSC) mixed-stock fishery used by three Indigenous groups (Innu, Inuit, and Southern Inuit). Salmon stock status, structure, and river-specific contributions to the mixed stock remain largely unknown in the Lake Melville watershed despite the importance of the FSC fishery to local food security and culture. Lake Melville's fishery is currently managed based on four coastal index rivers located outside the Lake Melville watershed (DFO 2016) as part of Salmon Fishing Area 1 (SFA1). However, these index rivers may not be representative of Lake Melville's salmon populations since genetic studies indicate that salmon of Lake Melville are locally adapted and genetically distinct from coastal salmon (Verspoor 2005, Bradbury et al. 2014, 2018). Essentially, it was, until recently, unknown whether the FSC fishery harvests salmon that originate from a few specific rivers or if the fishing effort is evenly distributed among many rivers that sustain the Lake Melville salmon mixed-stock.

A fishery that exploits multiple river populations poses challenges for management because the populations can differ in life history characteristics (i.e., length-at-age, timing of sexual maturation), reproductive strategies, productivity, and conservation status (Crozier et al. 2004). Management that does not consider individual population dynamics can expose small, localized populations to high exploitation and possible extirpation (Hilborn and Walters 1992). Thus, identifying mixed-stock FSC fishery composition within the Lake Melville watershed is critical for developing sound management practices for sustainable fishery and food security for Indigenous communities. This information is particularly important in Labrador today, as the fish habitat is experiencing changes due to industrial development (i.e., climate change, hydroelectric development, and mining). Identifying specific river(s) contributing to and maintaining Lake Melville's salmon mixed-stock is also critical for establishing key rivers to be monitored for stock assessment purposes and determining essential rivers to protect during Labrador's ongoing economic development.

The chemical composition of fish otoliths (otolith microchemistry) can be used as natural markers because it reflects the environmental conditions that those fish experienced in the past. This is an increasingly common approach to infer the natal origin of migratory fish (Campana et al. 2000, Elsdon et al. 2008). Otolith microchemistry is mainly influenced by physical and chemical parameters of the surrounding water (Fowler et al. 1995), while water chemistry is, in turn, influenced by the geology of the watershed (Kennedy et al. 2000, Wells et al. 2003, Friedrich and Halden 2008). Therefore, in river systems with distinctive water chemistry, the otoliths of juvenile fish such as Atlantic salmon can have a distinct elemental composition (Veinott and Porter 2005, Martin et al. 2013a). For anadromous fish, the downstream migration from freshwater to saltwater habitats can be detected by a sharp increase in strontium (Sr) and a simultaneous decrease in barium (Ba) concentrations in the otolith (Kraus and Secor 2004, Elsdon and Gillanders 2005, Zimmerman 2005). Furthermore, as the otolith made of calcium carbonate ($CaCO_3$) is an inert material, its elemental composition is stable during the life of a fish and is not resorbed in times of metabolic stress (Campana and Neilson 1985). Therefore, by using the freshwater signature (juvenile stage) registered in adult otoliths, it is possible to infer a fish’s natal river (Walther et al. 2008, Olley et al. 2011, Martin et al. 2013b).

Strontium and barium are frequently used as natural markers in otoliths due to the strong correlation between elemental concentrations in water and in otoliths (Bath et al. 2000, Wells et al. 2003, Martin et al. 2013a). Magnesium and manganese are also commonly used to improve discrimination power (e.g., Thorrold et al. 1998, Veinott and Porter 2005, Walther and Thorrold 2009). Many other elements have also been used in discrimination studies, such as lithium (Li), sodium (Na), potassium (K), zinc, and rubidium (Edmonds et al. 1989, Campana et al. 2000, Arkhipkin et al. 2009, Cuveliers et al. 2010, Martin et al. 2013b, Loewen et al. 2015). Although these essential elements are subject to strong physiological regulation, they can be useful to differentiate population if their concentration varies significantly among groups (Campana 2005).

The use of the freshwater signature in adult anadromous fish to infer natal river has been successfully demonstrated for American shad (*Alosa sapidissima*) in the York River on the Atlantic coast of the USA (Walther et al. 2008), brown trout (*Salmo trutta*) in four river watersheds on the East coast of Newfoundland in Canada and in the Motueka River watershed in New Zealand (Olley et al. 2011, Veinott et al. 2012), and Atlantic salmon in the Ardour River watershed in south France (Martin et al. 2013b) using magnesium (Mg), manganese (Mn), zinc (Zn), rubidium (Rb), strontium, and/or barium concentrations in otoliths.

In this study, we conducted otolith microchemistry analysis in juvenile and adult Atlantic salmon to infer the natal river of adult salmon captured in Lake Melville's FSC fishery. We first evaluated the feasibility of using the otolith microchemistry of juvenile Atlantic salmon to distinguish rivers across the watershed of the Lake Melville estuary. Using the database of juvenile otolith elemental signatures, we then inferred the natal river of adult salmon sampled in Lake Melville's FSC fishery to determine mixed-stock composition.

2.2 Method

2.2.1 Study area

Lake Melville is a subarctic fjord and part of the Hamilton Inlet, a coastal inlet in Labrador (Bobbitt and Akenhead 1982). Lake Melville provides drainage for nearly half of the Labrador region's watersheds (~ 149,000 km^2 including the Churchill River watershed) (Reddin et al. 2010, Anderson 2011). However, the accessible drainage area for salmon spawning and rearing is ~30,000 km^2 (including Lake Melville's surface area of ~3,000 km^2) (see Appendix A). The Lake Melville watershed is characterized by diverse geological surface bedrock and vegetation cover (Wardle et al. 1997, Notzl et al. 2013) and is located in the Grenville province of the Canadian Shield. Its watershed is mainly made of four different bedrock types, with the largest being a complex of granite-gneiss. To the south, the Mealy Mountains are mainly made of anorthosite with barrens and alpine vegetation cover (Wardle et al. 1997, Notzl et al. 2013). There are also sporadic presences of arkose and gabbro in the Lake Melville watershed (Wardle et al. 1997). Vegetation cover in the Lake Melville watershed varies from dense conifer forest to barrens and includes wetlands and lichen-shrub covered areas (Notzl et al. 2013).The diversity in geological surface bedrock in the Lake Melville watershed should allow differentiating natal river based on otolith microchemistry analysis.

2.2.2 Juvenile otolith sampling

Juvenile salmon (mean fork length = 11.7 cm; range 4.2 – 17.0 cm) were sampled from 11 river watersheds flowing into Lake Melville, Labrador, during summer 2013 (August) and 2014 (June to August) (Fig. 2.1, Table 2.1). Due to the low water conductivity and the associated low electrofishing capture efficiency in Labrador rivers, most (~81 %) of the juvenile salmon were captured by angling. The target sample sizes were 50 juveniles across two sampling sites (25 juveniles per site) per river drainage area (minimum distance between sites was 0.5 km), except in Cape Caribou River, Caroline Brook, and Sebaskatchu River, where only one site (25 juveniles) was sampled.

The majority of the rivers supporting Atlantic salmon mixed-stock in the Lake Melville watershed were sampled. Overall, 10 of the 13 salmon rivers identified by the Department of Fisheries and Oceans Canada (DFO) were sampled in the main stem or in one of their tributaries (Reddin et al. 2010). Rivers sampled included the Kenamu, Traverspine, Caroline Brook (tributary of Churchill River), Peters (tributary of Goose River), Cape Caribou, Susan, Red Wine (tributary of Naskaupi River), Crooked, Sebaskatchu, and Mulligan (Reddin et al. 2010). In addition, Main Brook (not listed as a salmon river by Reddin et al. 2010) was also sampled. Only one juvenile was captured in the English River (identified as a salmon river), but this population was assumed to be small due to impassable waterfalls near the river mouth (Anderson 1985, Reddin et al. 2010). Two other salmon rivers (Kenemich River and Beaver River) were not sampled in the current study due to time limitations and costs associated with reaching suitable juvenile habitats. Both are considered small salmon rivers. Finally, seven tributaries of Lake Melville were also fished: an unnamed brook (east of Main Brook), Caravalla Brook, Etagaulet River, Louse River, Shoal River, Peter Jackie Brook, and McKenzie River. However, no juvenile salmon were captured in these tributaries.

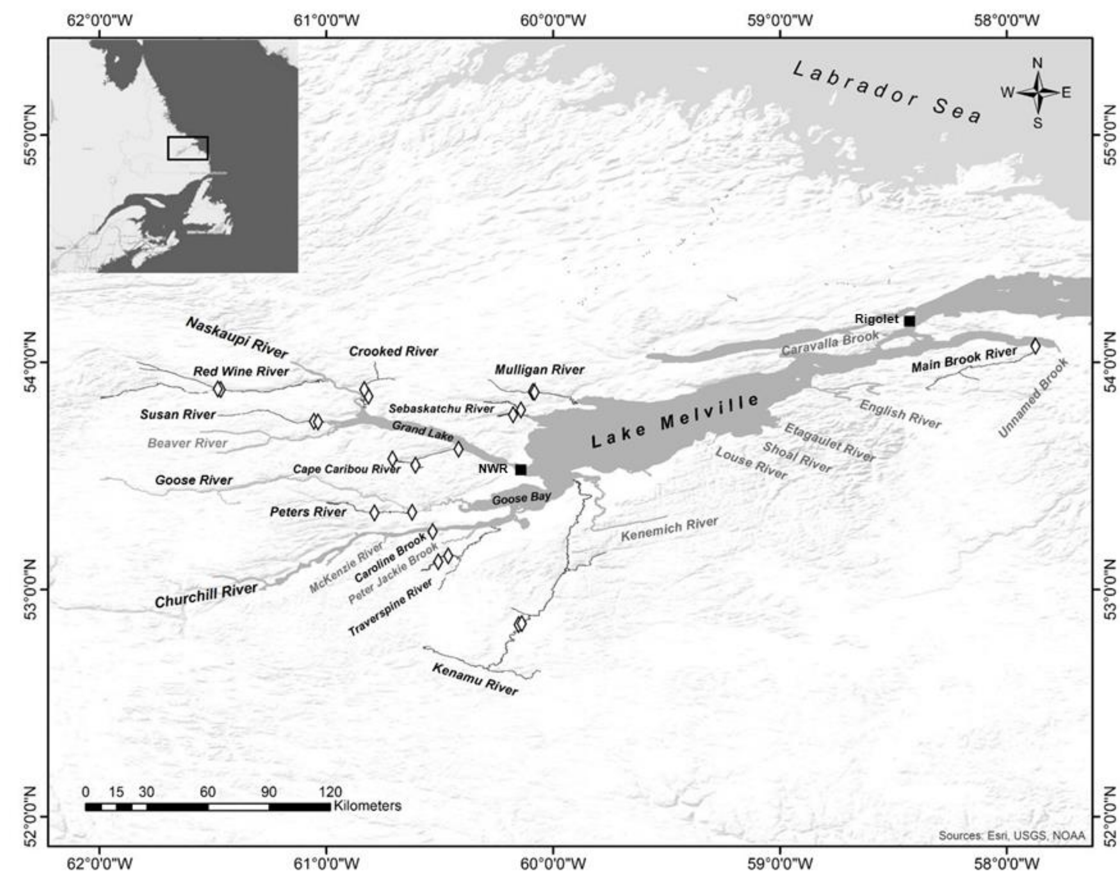

Fig. 2.1. Map of surveyed locations for juvenile Atlantic salmon in the Lake Melville watershed area, Labrador. Diamonds represent the sampling locations established in the 11 tributaries of Lake Melville. River names written in grey represent rivers not sampled, black squares represent the town of North West River (NWR) on the West end and Rigolet at the East end of Lake Melville.

Captured juveniles were measured (fork length, cm), weighed (g), euthanized and kept on ice until further processing could be completed at the Labrador Institute research station located in North West River. There, the sagittal otoliths were extracted, cleaned with ultrapure water, dried, and stored in individually labelled plastic vials at room temperature.

Table 2.1. Juvenile salmon sample size, average weight, length and range (in parenthesis) collected at each site within the Lake Melville watershed in 2013 and 2014.

Sampling locations	Sample size site 1	Sample size site 2	Average (range) weight (g)	Average (range) length (cm)
Caroline Brook	18	-	18.70 (2.88 - 28.85)	11.8 (6.1-14.3)
Cape Caribou River	19	-	17.37 (8.11 - 25.68)	12.3 (9.8-13.8)
Crooked River	23	19	17.21 (2.70 - 34.26)	12.0 (6.6-15.3)
Goose River	21	23	21.94 (1.85 - 51.63)	12.0 (5.6-17.0)
Kenamu River	25	22	10.22 (0.67 - 32.79)	9.0 (4.2-13.8)
Main Brook	20	19	11.24 (4.54 - 28.56)	10.5 (7.7-14.3)
Mulligan River	22	21	19.60 (4.43 - 34.59)	12.4 (7.5-14.9)
Naskaupi River	20	19	11.73 (4.03 - 23.58)	10.7 (8.1-13.8)
Sebaskatchu River	23	-	26.55 (19.45 - 35.81)	14.8 (13.6-16.0)
Susan River	25	16	15.18 (6.92 - 24.86)	11.6 (9.1-13.6)
Traverspine River	22	22	15.49 (7.61 - 26.17)	11.5 (8.9-14.6)

2.2.3 Water collection and analysis

Spatial differences in the water chemistry among rivers were assessed by sampling river water during summer 2014. Water samples were collected on the left side, in the middle, and on the right side of the river channel at each juvenile sampling site (Fig. 2.1) using a cleaned 1L Nalgene bottle. Water was filtered on site using a peristaltic pump and a polycarbonate in-line filter holder containing a geofilter™ cellulose acetate and cellulose nitrate flatstock filter membrane. Samples were acidified to 1 % using concentrated ultrapure HNO_3, kept on ice in the field and frozen upon return to the research station. The water samples were analyzed by the Earth Resources Research Analysis (TERRA) laboratory at Memorial University of Newfoundland using an ICP-MS (PerkinElmer ELAN DRCII). Five elements (Ba, Mg, Mn, Rb, and Sr) were transformed to element:Ca ratios.

2.2.4 Adult otolith sampling

A total of 321 adult salmon (288 small < 63 cm, 30 large ≥ 63 cm, and 3 without length recorded) were sampled from the FSC fishery in Lake Melville from July 14th to August 15th, 2014 (Fig. 2.2). Upon fishers' arrival to the wharves in Rigolet and North West River, fishing locations were recorded, salmon were measured (fork length, cm), and sagittal otoliths were extracted and placed in individually identified plastic vials. As with the juvenile samples, adult otoliths were thereafter cleaned with ultrapure water, dried, and stored in the plastic vials until analysis.

2.2.5 Otolith preparation and laser ablation analysis

Laser ablation coupled to an inductively coupled plasma-mass spectrometer (LA-ICP-MS) was used to determine the freshwater elemental signature in adult and juvenile salmon. Adult otoliths were first embedded in epoxy resin, air dried overnight, cut to 0.5 mm thickness with a Buehler Isomet low speed saw as per Jenke (2002), then polished (silicon carbide abrasive sheet 2000 grit) until growth annuli were clearly visible under magnification (2.88x). Adult otolith sections and juvenile whole otoliths were randomly mounted on glass slides using two-sided tape. Whole juvenile otoliths were placed sulcus side-down on the glass slide. Analyses of nine elements (Na^{23}, Mg^{24}, K^{39}, Ca^{44}, Mn^{55}, Zn^{66}, Rb^{85}, Sr^{88} and Ba^{137}) were performed at the Laser Ablation Microprobe laboratory of Memorial University of Newfoundland using a Geolas 193 nm excimer laser system coupled to a Finnigan Element-XR double focusing sector field inductively coupled plasma-mass spectrometer. The laser was fired at a rate of 10 Hz with an energy of 3 J $cm^{2\ -1}$, its beam size was 40 µm in diameter and the surface of the otolith was ablated at a speed of 10 µm s^{-1}. Before each ablation, a pre-ablation of the area under investigation was performed to remove potential contamination of the otolith surface. Adult otoliths were analyzed by ablating a line, perpendicular to the growth annuli, from the core to the outer edge along the axis of maximum growth. To avoid analyzing the maternal signal at the center of the otolith, juvenile otoliths were analyzed by ablating the distal surface of the otolith, which represents the most recent material accreted in raster mode (200 µm X 160 µm). The National Institute of Standards and Technology (NIST) 612 glass served as an external standard to monitor and correct instrumental drift. This standard was ablated twice at the beginning and the end of a block of 14 otoliths. Between each NIST 612 glass analysis, a second reference material made from a synthetic carbonate matrix, similar to an otolith (United State Geological Survey MACS 1), was ablated to monitor the accuracy and precision of the instrument. Raw count data from the LA-ICP-MS analysis were transformed to elemental concentrations (µg g^{-1}) based on the Ca^{43} signal used as an internal standard to account for the difference in ablation yield by using LAMTRACE software developed by Memorial University of Newfoundland (Longerich et al. 1996).

2.2.6 Statistical analysis

To assess whether there was a variation among water element:Ca ratios among rivers, ANOVAs were performed. To determine if there was a relationship between element:Ca ratios in water samples and elemental concentrations in juvenile otoliths, linear regressions were performed with the element:Ca ratios in the water as independent variables and juvenile otolith chemistry as the dependent variable.

Juvenile and adult otoliths that were composed of vaterite, which was determined based on their unusually low concentration of Sr combine with high concentration of Mg and by a glassier appearance (Tomas and Geffen 2003, Melancon et al. 2005), were discarded. Juvenile otolith microchemistry was also screened for extreme outliers using the Median Absolute Deviation (MAD) test with a threshold value of 4 (Leys et al. 2013). The MAD is less sensitive to outliers and more robust than standard deviation-based methods (Leys et al. 2013).

To assign adult salmon to their natal river, a discriminant function analysis was used based on the juvenile otolith microchemistry. First, assumptions of the juvenile dataset normality and homogeneity of variance were assessed by visual inspection of the residuals. Data were $\log_{10}$ transformed to correct for non-normality. Next, to test if the variance among rivers was greater than the variance among sites within rivers, a nested MANOVA with Pillai test was performed. Then, a LDA was performed on the juvenile otolith elemental $\log_{10}$ transformed concentration with the “MASS” package of the R software following Borcard et al. (2011). To evaluate the performances of the model created with LDA, a cross-validation procedure was performed which reassigns individuals to their natal river using both training and holdout datasets.

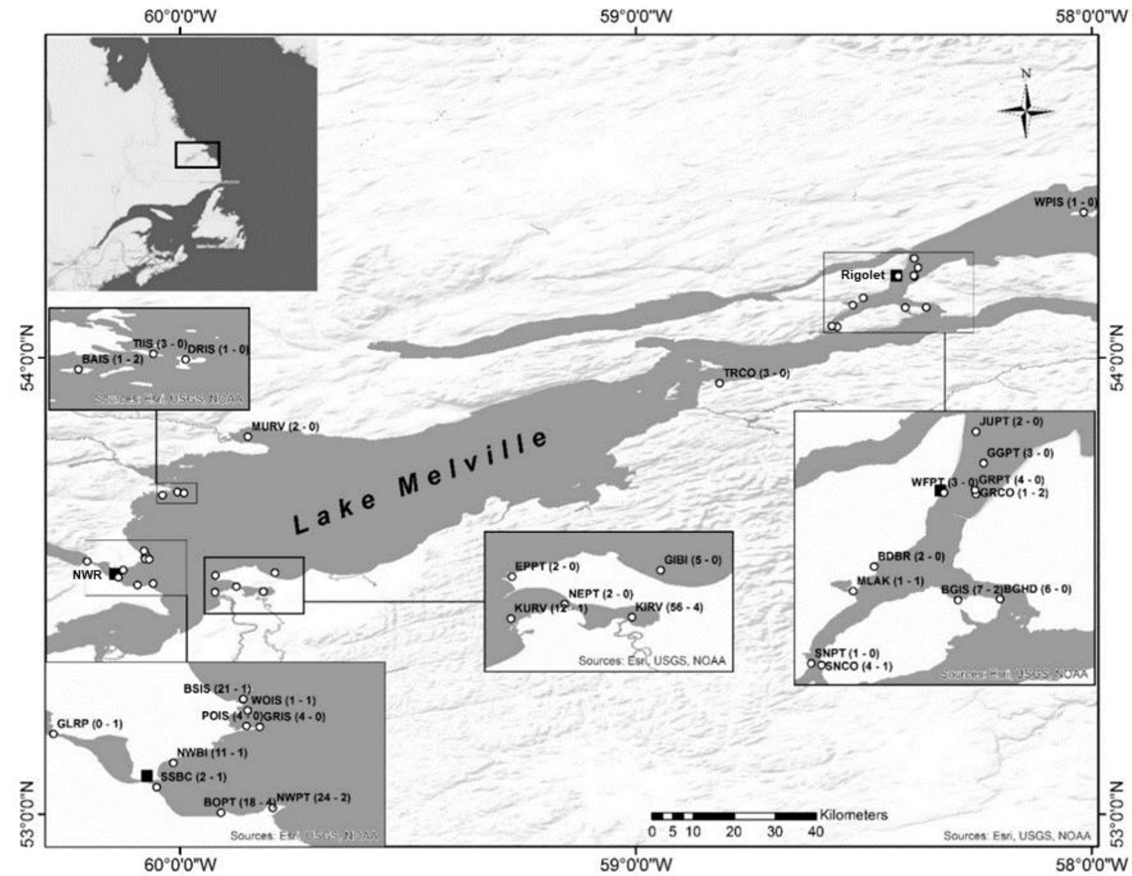

Fig. 2.2. Fishing locations of the adult salmon sampled from the Lake Melville FSC Fishery. Numbers in parenthesis indicate the number of small-large salmon captured; fishing location was unknown for 88 salmon, black squares represent the towns of North West River (NWR) on the West shore and Rigolet at the East end of Lake Melville. See Table 2.4 for complete fishing location names with their code reference.

The natal river fingerprint in adult otoliths was considered to be the 100 µm of otolith growth just prior to a sharp increase of Sr concentration and a simultaneous sharp decrease in Ba concentration, indicating the transition from freshwater to saltwater (Kraus and Secor 2004, Elsdon and Gillanders 2005, Zimmerman 2005) (Fig. 2.3). Means of the eight elemental concentrations ($\log_{10}$ transformed) in the 100 µm before saltwater migration were used as independent variables in the LDA model.

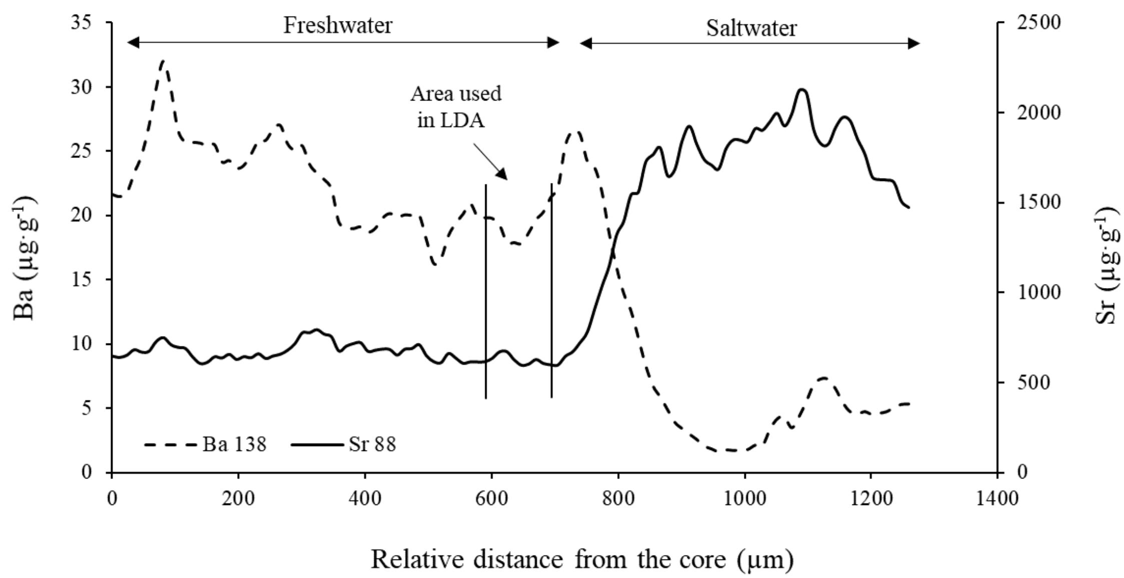

Fig. 2.3. Example of Sr and Ba concentrations (µg g^{-1}) profiles in an otolith cross-section from an adult salmon otolith showing a sharp increase in Sr and decrease in Ba and indicating the transition from freshwater to saltwater.

To confirm that the adults "truly" belong to the natal river assigned by the LDA, two methods were used: (1) posterior probabilities which suggests the most likely natal river and (2) squared Mahalanobis distance, which gives a measurement of the likelihood of belonging to the natal river inferred. Specifically, it is a multi-dimensional measure of the distance between the adult and the river centroid (Olley et al. 2011). First, posterior probabilities of the adult predictions have to be greater than the mean posterior probabilities of the juvenile group predicted minus two standard deviations. Second, the squared Mahalanobis distance of the adults from the predicted group's centroid, calculated only with the canonical variable(s) accounting for 90 % of the discrimination, had to be less than the average squared Mahalanobis distance of the predicted group from their centroid plus two standard deviations.

2.3 Results

2.3.1 River water chemistry

Analysis of water samples revealed a wide range of element:Ca ratios that were representative of freshwater habitat and allowed clear discrimination of freshwater growth from saltwater water growth (Fig. 2.4). There were variations in the water chemistry among rivers for each element:Ca ratio tested ($P < 0.001$). Otolith and water composition showed significant positive linear regressions for only Sr and Ba ($P < 0.01$), indicating that the proportion of Sr and Br in otoliths could be influenced by the ambient water (Fig. 2.5).

2.3.2 Juvenile salmon otolith microchemistry

Among the 11 rivers sampled, a large range of elemental concentrations was observed in juvenile salmon otoliths (Fig. 2.6). The eight elements analyzed in the otoliths were found to vary among rivers ($P < 0.001$). The multivariate composition of the otolith also varied within rivers ($P < 0.001$).

Furthermore, discrimination among the 11 rivers using the eight elements from the juvenile otolith was possible with LDA. The two first canonical variables explained 67 %

of the variation in the data, while the four first canonical variables explained ~90 % of the variation. The absolute value of the coefficients of the canonical variables indicated the relative importance of the eight variables to discriminate among the rivers (Table 2.2). The first variable (CV1) was primarily driven by Sr, the second (CV2) by K, and the third and fourth (CV3-4) by Na, indicating that Sr was the element with the greatest influence

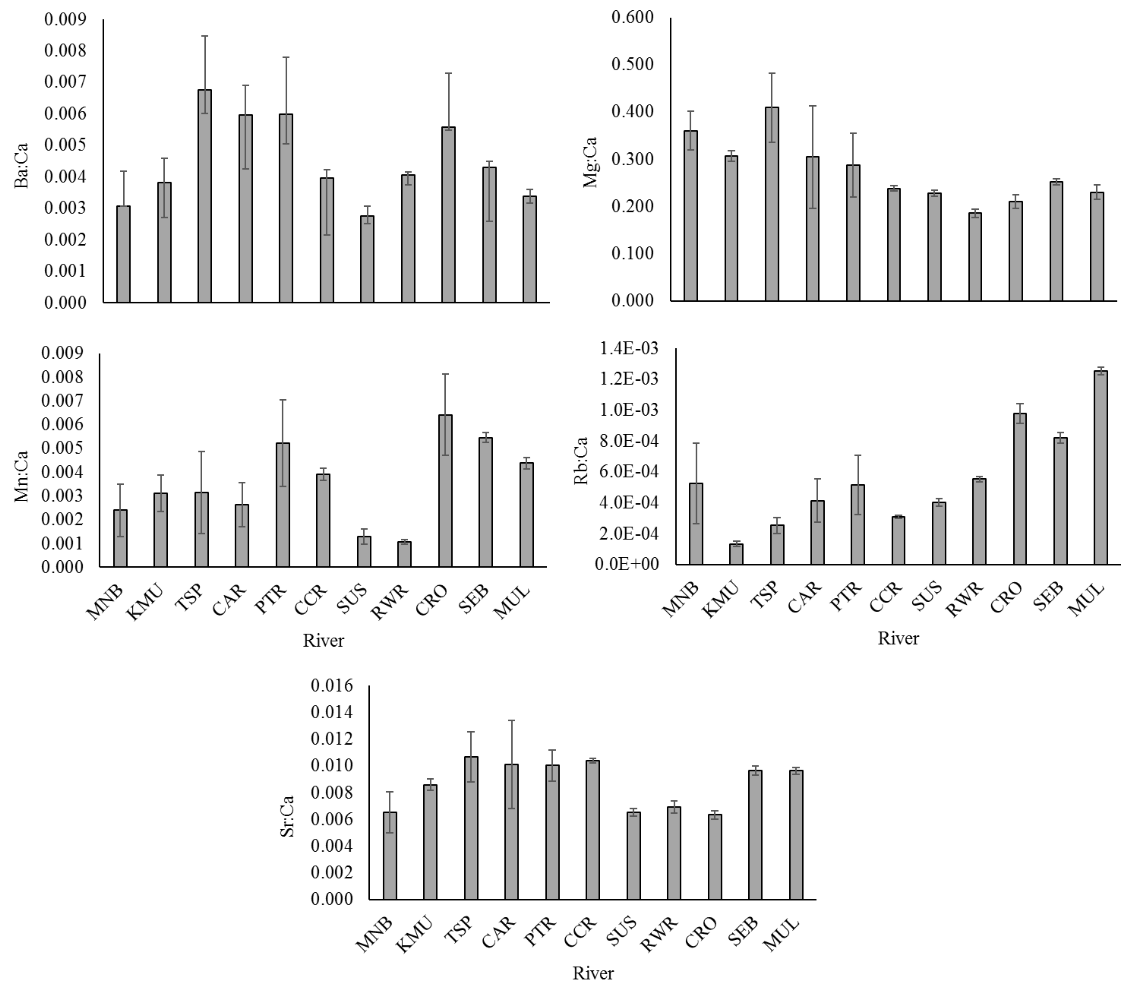

Fig. 2.4. Mean element:Ca concentration ratios (µg L^{-1}) measured in water samples collected in 11 rivers, Lake Melville, Labrador. Natal rivers are ordered clockwise from the Lake Melville entrance. MNB = Main Brook, KMU = Kenamu River, TSP =

Traverspine River, CAR = Caroline Brook, PTR = Peters River, CCR = Cape Caribou River, SUS = Susan River, RWR = Red Wine River, CRO = Crooked River, SEB = Sebaskatchu River, and MUL = Mulligan River. Error bars represent the standard deviations.

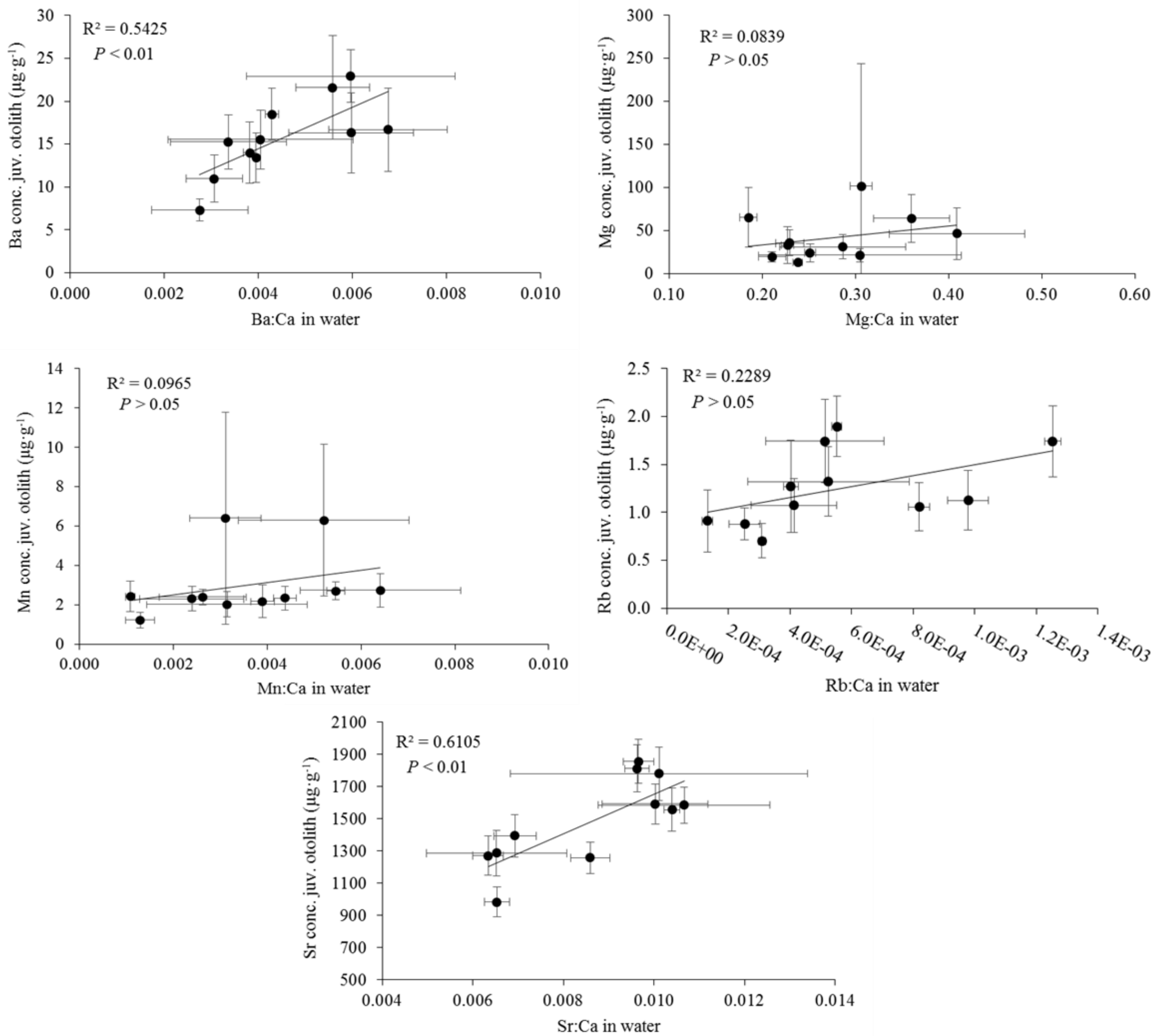

Fig. 2.5. Relationship between mean element:Ca concentration ratios (µg L^{-1}) in water samples and elemental concentrations (µg g^{-1}) in juvenile Atlantic salmon otoliths from 11 rivers, Lake Melville, Labrador. Error bars represent the standard deviations.

in discrimination among rivers, followed by K and Na. Other variables such as Mn, Rb, and Ba also seemed to contribute moderately. Mg and Zn were the smallest contributors. However, removing Mg and Zn reduced the average performance of the cross-validation by 6 %. On average, the LDA cross-validation accurately classified 89 % (range 73 % - 100 %) of the juveniles to their correct natal river (Table 2.3). Peters River and Main Brook River watersheds showed the lowest classification success, with 73 % and 77 % respectively. Juveniles collected in the Peters River watershed were mainly misidentified as from Red Wine River, Sebaskatchu River, or Mulligan River. Juveniles captured in the Main Brook River watershed were mainly misidentified as from Kenamu River, Traverspine River, or Red Wine River. When the two first canonical variables of the LDA (representing ~90 % of the discrimination) based on the juvenile otolith elemental signature were plotted, most of the rivers were clearly distinct (Fig. 2.7).

2.3.3 Determination of adult natal river

Natal river of adult salmon (n = 321) was inferred using LDA based on the juvenile otolith elemental signatures from 11 rivers. More than half (60 %) of the adult salmon were assigned to the Kenamu River, 26 % to Traverspine River, 5 % to Susan River, and 10 % of the adults were spread between Caroline Brook, Cape Caribou River, Crooked River, and Sebaskatchu River (Table 2.4). No adult salmon was assigned to four of the 11 rivers: Main Brook, Peters River, Red Wine River, and Mulligan River. Most of the adult salmon were captured around the towns of North West River (57 %) and Rigolet (15 %). Adult salmon assigned to the Kenamu River watershed were captured at nearly (90 %) all fishing locations. Proportions of fish assigned to Kenamu River (61 %, 64 % and 51 %), Traverspine River (35 %, 20 % and 34 %) and Susan River (4 %, 3 %, 3 %) were similar at both areas (Rigolet and North West River) and from unknown fishing locations, respectively.

Table 2.2. Canonical variable (CV) coefficients for linear discriminant function analysis (LDA) performed on juvenile Atlantic salmon otolith microchemistry collected in 11 rivers.

Elements	CV1	CV2	CV3	CV4	CV5	CV6	CV7	CV8
Na	-4.7645	6.8849	-25.1774	-12.6236	-19.7649	-19.7256	1.7623	30.4529
Mg	-0.4606	1.2382	-1.4816	0.2557	0.1480	2.6771	3.3901	2.6164
K	-1.0403	-10.7167	-5.5887	4.3471	-4.8470	2.3744	2.2897	-12.3340
Mn	2.9294	-2.5472	2.4626	5.4863	1.8189	-1.5629	1.42076	1.2403
Zn	-0.4727	1.4259	-0.2476	-0.0941	0.0083	0.7129	-5.0897	-0.8392
Rb	4.1166	7.6115	7.0655	0.7799	-2.1121	-1.2839	-1.4361	-1.6697
Sr	28.7382	9.7911	-11.7274	0.7733	6.7646	0.8908	-1.18772	0.7242
Ba	-2.1827	-7.5292	6.9476	-5.3279	-5.2782	2.8102	0.0088	-0.0956

Table 2.3. Number (percent) of juvenile Atlantic salmon from each natal river correctly classified by the cross-validation procedure performed on the otolith microchemistry (log_{10} transformed) using a linear discriminant function analysis (LDA). Numbers in parenthesis next to the river names indicate sample size.

	Main Brook	Kenamu River	Traverspine River	Caroline Brook	Peters River	Cape Caribou River	Susan River	Red Wine River	Crooked River	Sebaskatchu River	Mulligan River	**Correctly reassigned**
Main Brook (39)	30 (**77**)	2 (0)	2 (0)	-	-	1 (0)	1 (0)	3 (0)	-	-	-	**77%**
Kenamu River (46)	1 (0)	41 (**87**)	2 (0)	-	-	3 (0)	-	-	-	-	-	**87%**
Traverspine River (44)	-	1 (0)	36 (**82**)	4 (0)	-	3 (0)	-	-	-	-	-	**82%**
Caroline Brook (18)	-	-	1 (0)	17 (**94**)	-	-	-	-	-	-	-	**94%**
Peters River (44)	-	-	-	-	32 (**73**)	1 (0)	-	3 (0)	-	4 (0)	4 (0)	**73%**
Cape Caribou River (19)	-	-	1 (0)	-	-	18 (**95**)	-	-	-	-	-	**95%**
Susan River (41)	-	-	-	-	-	-	41 (**100**)	-	-	-	-	**100%**
Red Wine River (39)	3 (0)	-	1 (0)	-	1 (0)	-	-	34 (**87**)	-	-	-	**87%**
Crooked River (42)	-	-	-	-	-	1 (0)	-	-	41 (**98**)	-	-	**98%**
Sebaskatchu River (23)	-	-	1 (0)	-	2 (0)	-	-	-	-	20 (**87**)	-	**87%**
Mulligan River (43)	-	-	-	-	-	-	-	1 (0)	-	1 (0)	41 (**95**)	**95%**
Average												**89%**

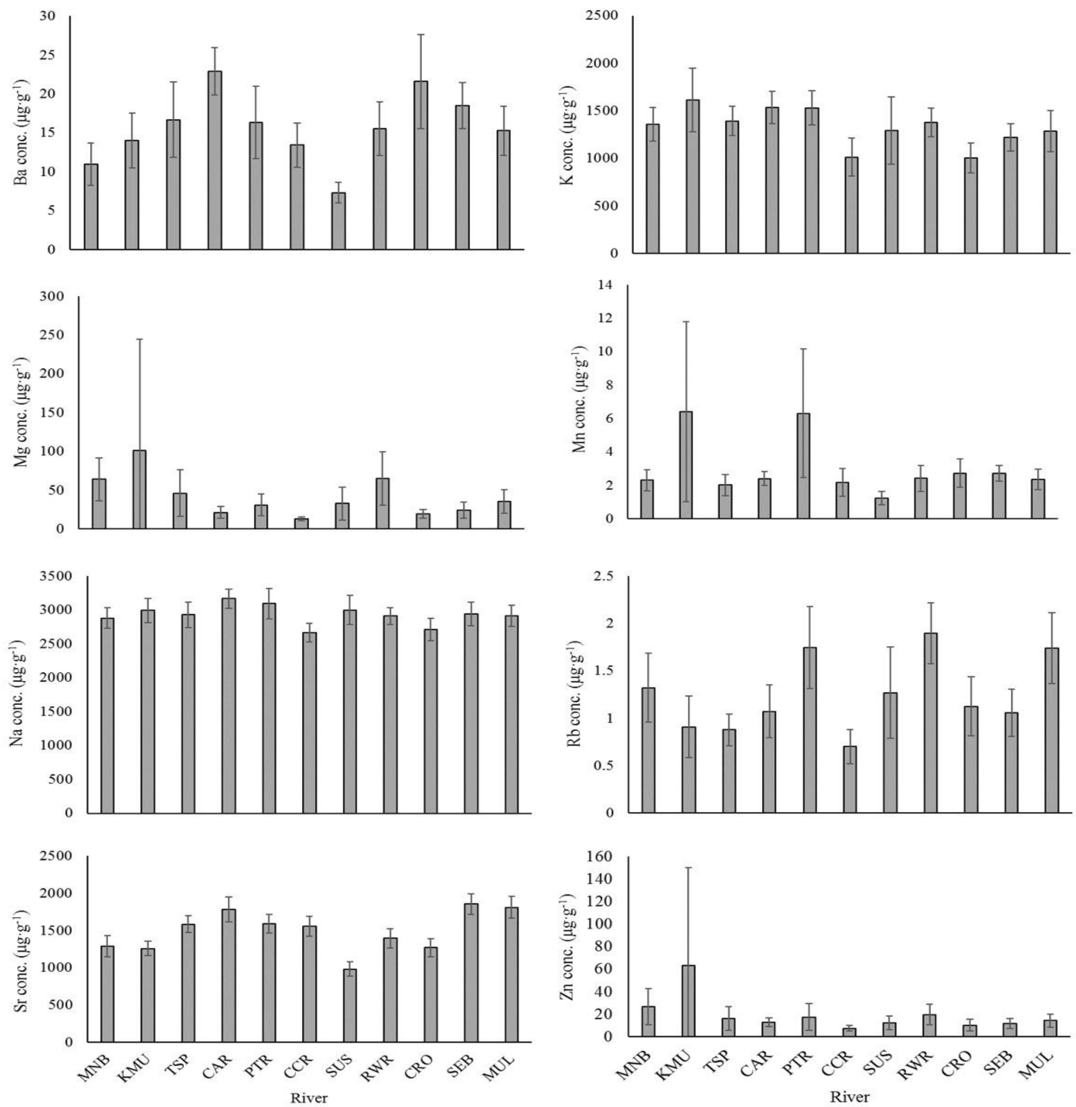

Fig. 2.6. Mean elemental concentrations (µg g^{-1}) measured in juvenile Atlantic salmon otoliths sampled in 11 rivers, Lake Melville, Labrador. Natal rivers are ordered clockwise from the Lake Melville entrance. MNB = Main Brook, KMU = Kenamu River, TSP = Traverspine River, CAR = Caroline Brook, PTR = Peters River, CCR = Cape Caribou River, SUS = Susan River, RWR = Red Wine River, CRO = Crooked River, SEB = Sebaskatchu River, and MUL = Mulligan River. Error bars represent the standard deviations.

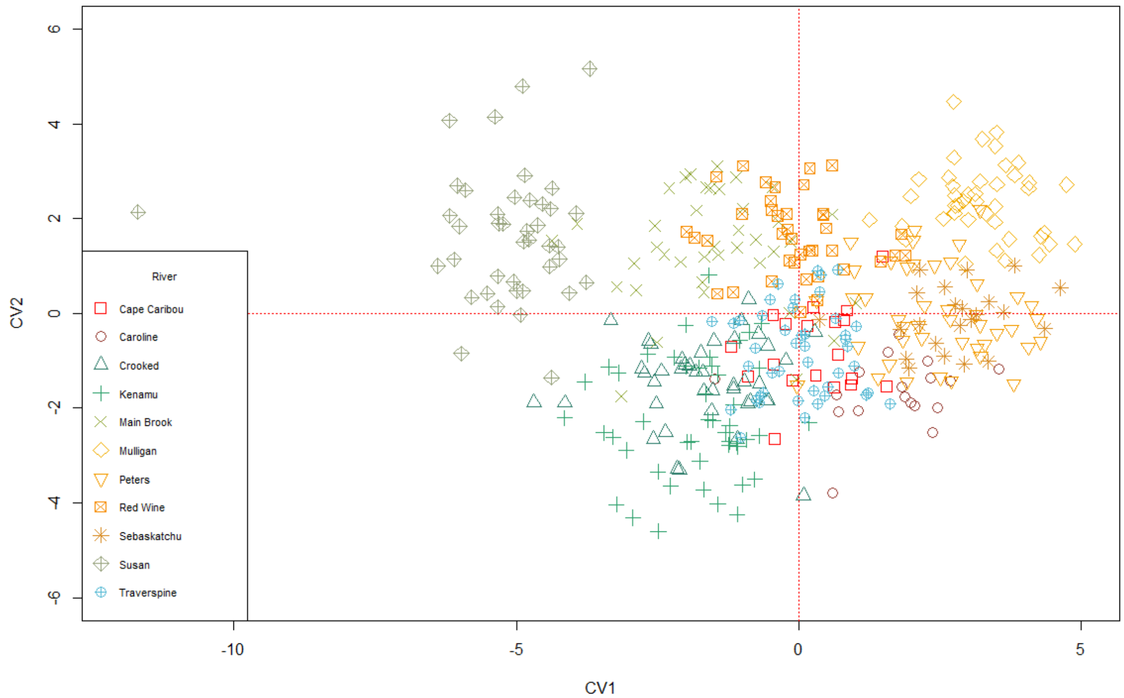

Fig. 2.7. Plots representing the linear discriminant function analysis (LDA) performed on juvenile Atlantic salmon otolith microchemistry grouped by river using the two first canonical variables (CV1-2).

Adult salmon posterior probabilities were higher than 70 % for 78 % of the fish, which indicated that most of the adults had a high probability of belonging to the group predicted. Two adult salmon assigned to Caroline River and three assigned to Susan River failed the posterior probabilities test (Table 2.4). However, the threshold value for posterior probability calculated with the juvenile for Susan River was high (97 %) and the three fish assigned to Susan River that failed it had posterior probabilities of 77 %, 86 %, and 94 %, which are high posterior probabilities. However, all predictions failed the squared Mahalanobis distance test for true membership except one individual assigned to Caroline Brook. The posterior probabilities indicate which juvenile group is the most similar to the adult, while the squared Mahalanobis distance measured the distance between the adult and the predicted group centroid. Poor overlapping between juvenile clusters and adults was observed (Fig. 2.8). Thus, if an individual was assigned to a natal river with high posterior probability (>90 %), it was far from the group centroid and failed the squared Mahalanobis distance test, indicating that the otolith microchemistry method for inferring natal river of adult Atlantic salmon appears to be limited in the Lake Melville watershed.

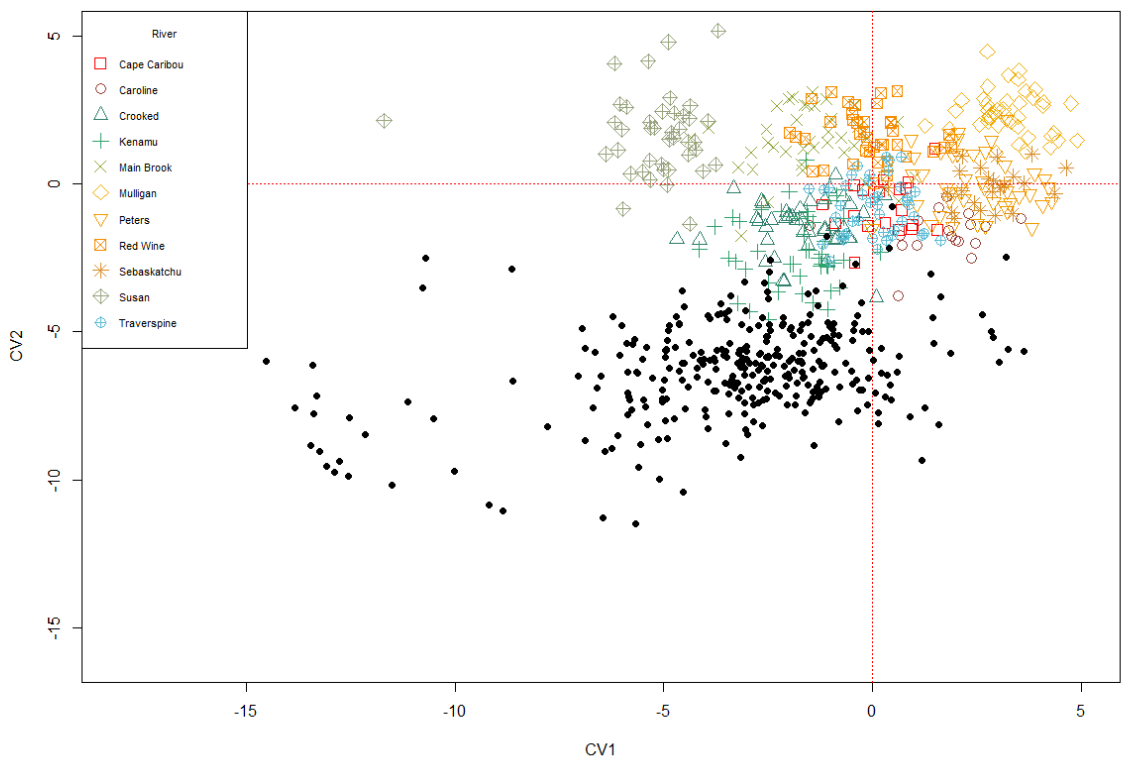

Fig. 2.8. Plots representing adult Atlantic salmon (black dots) and juveniles grouped by river determined by linear discriminant function analysis (LDA) using the first two canonical variables (CV1-2).

Table 2.4. Summary of adult Atlantic salmon captured in the FSC fishery and assigned to a river using linear discriminant function analysis (LDA) based on juvenile otolith microchemistry. Numbers in brackets indicate the number of adults captured at this location. Natal rivers are ordered clockwise from the Lake Melville entrance. Fishing location names in italic are located at the Lake Melville entrance.

		Natal stream										
Code	Fishing locations	Main Brook	Kenamu River	Traverspine River	Caroline Brook	Peters River	Cape Caribou River	Susan River	Red Wine River	Crooked River	Sebaskatchu River	Mulligan River
BAIS	Bakeapple Island (3)		2					1				
BDBR	*Black Duck Brook (2)*		2									
BGHD	*Big Head (6)*		5	1								
BGIS	*Big Island (9)*		4	5								
BOPT	Bottle Point (22)		11	2	2			4*		2	1	
BSIS	Butter and Snow Island (21)		15	5				1				
EPPT	Epinette Point (2)		2									
GGPT	*Gunning Point (3)*		1	1				1				
GIBI	Gillard's Bight (5)		3	1			1					
GLRP	Grand Lake Rapids (1)		1									
GRCO	*Grassy Cove (3)*		2					1				
GRIS	Green Island (4)		3	1								
GRPT	*Gunners Point (4)*		4									
JUPT	*Juniper Point (2)*			2								
KURV	Kenamu River mouth (59)		38	16	1*		4					
KIRV	Kenemich River mouth (13)		8	4						1		
MLAK	*Mulligak (2)*		1	1								
MURV	Mulligan River mouth (2)			2								
NEPT	Net Point (2)		1				1					
NWBI	North West Bight (12)		10		2							
NWPT	North West Point (26)		18	5				3*				
POIS	Pork Island (4)		1	1	1					1		
SNCO	*Snooks Cove (10)*		6	4								
SNPT	*Snooks Point(1)*		1									
SSBC	Sheshatshiu Beach (3)		2					1				
TDIS	Tickle and dry Island (4)		1	1	1			1				
TRCO	Trout Cove (2)		2									
WFPT	*Wolfrey's Point (3)*		2	1								
WOIS	Woody Island (2)		1				1					
WPIS	*West Pompeys Island (1)*			1								
	Unknown (88)		45	30	5*		3	3		1	1	
	Total per river	0	192	84	12	0	10	16	0	5	2	0
	Proportion	**0%**	**60%**	**26%**	**4%**	**0%**	**3%**	**5%**	**0%**	**2%**	**1%**	**0%**

* 2 and 3 adult salmon failed the posterior probability test for Caroline Brook from Kenamu River mouth (1) and Unknown finshing location (1) and Susan River from Bottle point (1) and North West point (2)

2.4 Discussion

The otolith elemental composition of juvenile Atlantic salmon collected in 11 salmon river watersheds was specific to the river of origin. LDA cross-validation correctly reassigned juvenile salmon to their natal river with an average of 89 % (range:73 % to 100 %). Only two rivers had a reassignment rate below 80 % (Main Brook, 77 % and

Peters River, 73 %). In LDA cross-validation, the juvenile training dataset was the same as the test dataset, which created a positive bias in the reassignment results (Miller and Miller 2010). Reassignment accuracy similar to ours was observed in juvenile Atlantic salmon sampled in 12 tributaries within one river watershed in Southern France (average 80 %, range: 20 % to 100 %, Martin et al. 2013b). Veinott and Porter (2005) obtained an average reassignment rate of 92 % (range 84 %-100 %) in juvenile Atlantic salmon sampled in the main stem of three river watersheds in Newfoundland, Canada. These results demonstrated that the first step to infer adult Atlantic salmon was successful; juvenile Atlantic salmon otolith elemental composition can be used as a marker of natal river in Lake Melville watershed.

Most of the adult assignments passed the posterior probability test with high probability (>70 % for 78 % of the assignment). Kenamu River was identified as the river producing most (~60 %) of the adults sampled in the FSC fishery, however, poor overlap was observed between juvenile and adult elemental compositions when plotting LDA and nearly all adults (except one) failed the squared Mahalanobis distance test for true membership. Thus, it was not possible to determine the relative importance of salmon rivers or to confirm that Kenamu River is the river contributing most to Lake Melville Atlantic salmon mixed-stock in this study. The results contrast with other studies that have successfully inferred natal river of salmonids based on otolith microchemistry techniques (Marklevitz et al. 2011, Veinott et al. 2012, Martin et al. 2013b, Zimmerman et al. 2013). There was no report of adult assignment failing the statistic validation of their membership in past studies. Also, Mulligan River is acknowledged as the second most important river for Atlantic salmon in the Lake Melville watershed and no adult was reassigned to this river. Three other rivers (Main Brook, Peters, and Red Wine) has also no adult assigned to them, yet these rivers are known to be salmon rivers (Reddin et al. 2010). These results seem to indicate that the application of the current method is limited to determine the origin of the adult salmon harvested in the FSC fishery and further research is required. Sampling salmon captured by recreational fishers in rivers could have been used to validate the results. However, obtaining a high number of samples (enough to validate the technique) would have been difficult due to the relatively small recreational fishery in these rivers.

Inferring natal origin from a mixed-stock composition using otolith microchemistry analysis is based on several assumptions (summarized in Table 2.5). The first assumption was that the analytical method did not induced any bias and created the offset between juvenile and adult otolith microchemistry (Elsdon et al. 2008). In this study, juvenile and adult otoliths were randomly analyzed at the same time and over a short period of time (a few weeks) to limit instrument drift. Therefore, it was unlikely that there was systematic error in the analyses and any random error that might have occurred would be spread among juveniles and adults equally. The second assumption was that the otolith elemental composition accreted during the juvenile was preserved in the adult otolith. Otoliths are acellular and metabolically inert through the life of the fish, i.e., they are not reabsorbed in times of metabolic stress (Campana and Neilson 1985). Therefore, adult otolith areas corresponding to the juvenile growth phase analyzed in this study should have the same elemental composition as at the time of the accretion.

The third assumption was that each river exhibits a unique chemical signature (Elsdon et al. 2008). Clear discrimination among rivers by otolith microchemistry analysis depends on the amount of time the fish spent in an environment with sufficiently different physicochemical properties (Elsdon et al. 2008). Studies from Zimmerman et al. (2013) and Marklevitz et al. (2011) highlighted the importance of geological distinction and fish residence-time among streams or watersheds for high accuracy in predictions. Zimmerman et al. (2013), using elemental composition and strontium isotopic ratio, demonstrated that adult classifications of Chum salmon were less accurate than with Coho salmon due to shorter freshwater residence time after hatching. In Lake Melville, Atlantic salmon usually spend three to four years in rivers before migrating to the marine environment, and the adult freshwater signature was clearly visible in every otolith cross-section analyzed. Marklevitz et al. (2011) demonstrated that at large spatial scales (Lake Huron watershed), higher classification rates could be reached when the assignment was performed according to geological regions (grouping multiple rivers together) rather than to specific rivers. Trace element composition and concentrations in river water are correlated to the geology of the watershed. The weathering of rocks, minerals, and soil in the watershed are the main source of trace elements in river water (Brezonik and Arnold 2011). The Lake Melville watershed is geologically variable, containing different type of rocks and formed at different geological periods (Wardle et al. 1997, Notzl et al. 2013). In our study, the juvenile otolith cross-validation mean accuracy (89 %) was high and suggested distinct underlying geology among river watersheds.

A fourth assumption was that the juvenile otolith microchemistry dataset was representative of the river watersheds from which the adults were sampled (Campana et al. 2000, Gillanders 2005, Elsdon et al. 2008). All natal rivers of the adult salmon sampled must be represented in the juvenile dataset. If a natal river is missing in the juvenile dataset and an adult originating from this missing river has been collected, it could lead to the adult misclassification to a known group (Gillanders 2005). In our study, 11 of the 14 salmon river watersheds were sampled. The three salmon river watersheds not sampled represent small populations (Anderson 1985). Although juvenile salmon were sampled in the majority of rivers supporting salmon mixed-stock in Lake Melville, rivers that are located outside Lake Melville and near its outflow were not sampled (Tom Luscombe Brook, Partridge Point Brook, and Double Mer). Fifty-two (16 %) adult salmon were caught at the outflow of Lake Melville (Rigolet area). It is possible that these salmon were not entering Lake Melville but instead were migrating to spawn in rivers located outside Lake Melville. However, there was no apparent difference in assignment between salmon caught in the Rigolet and North West River areas. As for salmon caught inside Lake Melville, the majority of these adult salmon were reassigned to Kenamu River (61 %) and Traverspine River (35 %). Furthermore, a genetic study on the same Atlantic salmon samples and salmon sampled outside Lake Melville did not assign salmon to rivers outside the Lake Melville watershed (Bradbury et al. 2018). Therefore, the number of natal rivers represented in the juvenile dataset is unlikely to be the reason for the adults failing the test for true membership.

Although the majority of watersheds supporting salmon mixed-stock were sampled, only one tributary per watershed was sampled. Therefore, the fourth assumption of representativeness may have been violated in this study (Elsdon et al. 2008). It is important to acknowledge the large spatial scale of this study and the limitations of otolith elemental technique. Lake Melville is a large estuary with an extensive, accessible watershed for salmon spawning and rearing (~30,000 km^2) (Reddin et al. 2010) and includes at least 14 salmon river watersheds, each with multiple tributaries. If the river watershed is highly heterogenic, one sampling location may not be representative of another sampling location. Significant otolith elemental composition differences among tributaries within a river watershed have been widely demonstrated in past studies (Wells et al. 2003, Olley et al. 2011, Martin et al. 2013b). The accuracy of prediction is closely related to geological distinctions among watersheds and heterogeneity among tributaries within a watershed. Inferring natal river at the watershed scale is possible when the variation among river watersheds is more important than within the watersheds. Zimmerman et al. (2013) demonstrated that otolith elemental variation among watersheds was greater than within watershed variation by sampling juveniles in four tributaries located in two river watersheds. This study obtained higher classification accuracy when inferring natal origin at the watershed level. However, it also demonstrated that a watershed is not necessarily homogenous. Fish should be sampled from multiple tributaries within a watershed to ensure that the sampling sites do not represent discrete environmental habitats within a river watershed (Elsdon et al. 2008). In our study, juvenile salmon were sampled in the main stem or in one tributary of major salmon rivers (e.g., Red Wine River, which is a tributary of the Naskaupi River and Peters River, a tributary of the Goose River). Also, it is possible that sampling locations were more representative of a discrete environment within the watershed which could have led the adult assignment to fail the true membership test. For example, Main Brook River watershed has a heterogenous surficial geology (Wardle et al. 1997) and sampling location was in only one type of surficial geology close to the river mouth. Therefore, it is possible that adult salmon sampled in Lake Melville in this study were not from the specific locations where juveniles were sampled. Increasing the number of sites in order to include more than one tributary and main stem within the river watersheds in Lake Melville could potentially improve the assignment success. However, in Lake Melville's watershed, multiple rivers are located in remote areas with limited access to the main stem or tributaries, and it is not feasible to sample every tributary. Previous studies successfully inferred natal river without sampling every tributary within a watershed (Walther et al. 2008, Zimmerman et al. 2013). In the Lake Melville watershed, descriptions of the river watersheds' geology should be first performed to identify which tributaries should be sampled to obtain a representative otolith microchemistry dataset for the river watershed.

A fifth assumption was that interannual variability in otolith microchemistry is minimal or accounted for. Interannual variability in otolith microchemistry could have an impact on assignment accuracy and should be assessed and/or included in the dataset (Gillanders 2002, Elsdon et al. 2008, Walther and Thorrold 2009). Differences in precipitation, water flow and water temperature influence water chemistry and otolith microchemistry (Fowler et al. 1995, Bath et al. 2000, Martin et al. 2013a). Temporal variability in anadromous and non-anadromous fish otolith have been reported for Mg, Mn, Sr, Rb, and Ba (Feyrer et al. 2007, Olley et al. 2011, Martin et al. 2013a). Although studies have reported significant interannual variability in otolith elemental composition in freshwater habitats (rivers), it was not significant when compared to spatial variability (Feyrer et al. 2007, Olley et al. 2011, Martin et al. 2013a). In contrast, Walther et al. (2008) found important interannual variability of Mg, Mn, and Ba in juvenile American shad otoliths, which limited their capacity to infer adult natal using these elements. Furthermore, the fifth assumption implies that the age of individuals is correctly identified in order to compare it to the appropriate dataset. Adult salmon sampled in this study had left their natal river for one to three years before migrating again to Lake Melville and left in different years. If there was variation in water chemistry in the river since the adult left the river, the current juvenile baseline would not be representative of the natal river signature in the adult otolith. Gillanders (2002) reported that the same year of accretion of the juvenile and adult otolith needed to be analyzed for accurate classification. Therefore, multiple years of juvenile collections before adult sampling should have been performed to ensure that it is representative of the adult material analyzed. However, it is not always possible to compare to the same accretion year. In Walther and Thorrold (2009), it was concluded that an alternative would be to restrict the dataset to temporally stable elements and pool juvenile otoliths from more than one year. In our study, there was no assessment of the interannual variability in otoliths or limitation of its impact. Juveniles and adults were sampled once during the summer of 2013 or 2014. The offset of the adults from the juvenile clusters in the LDA plot and the resulting failure of the true membership test could be caused by interannual variability that was not assessed in this study. This hypothesis could be verified by analyzing juvenile otoliths with a drilling method (Veinott et al. 2012), scanning the juvenile growth phase on adult otolith cross-sections with the LA-ICP-MS, or by water sampling during multiple years. Furthermore, with an adult otolith cross-section it could be possible to match the year ring with elemental composition for a more detailed interannual variability analysis.

A sixth assumption was that the adult sampling effort was representative of all juvenile groups and the Lake Melville mixed-stock. Four of the 11 rivers had no salmon assigned to them, including the Mulligan River, which is known to be an important salmon river. However, similar results were obtained using genetics on the same salmon samples (Bradbury et al. 2018). The genetic analyses did not assign adult salmon to Main Brook and Peters rivers and only a few salmon to Mulligan River (Bradbury et al. 2018). However, Red Wine River was identified as part of the same group as Crooked River using the genetic analyses and a high number of adult salmon was assigned to their group. Fishing effort was concentrated near the town of North West River (57 % of adults) and would be more representative of salmon from the Grand Lake (Cape Caribou, Susan, Red Wine, Crooked rivers) and Goose Bay areas (Kenamu, Traverspine, Caroline, Peters rivers). However, adult salmon (15 % of those collected from fishers) were also captured near the outflow of Lake Melville and these salmon should include salmon from all the Lake Melville watershed. Salmon were also collected at different locations farther (middle of Lake Melville) from the two main fishing areas around Rigolet and North West River towns. Therefore, there is no obvious bias that might explain that no adult salmon were assigned to four rivers. In 2014, the FSC fishery harvested 6,841 salmon in the Lake Melville watershed. The sampling size (n = 321) of this study represent 5 % of the salmon harvested and it is possible that salmon from these four rivers were not sampled. Also, samples were collected during the upstream migration peak, but there is a possibility that salmon migrate to Main Brook, Mulligan, Peter, and Red Wine rivers at a different time and were not captured during the sampling period. For these four rivers, a telemetry study would be an efficient technique to gather information on salmon behavior and might explain why none of these salmon were captured in this study.

In summary, this preliminary study on the feasibility of using the otolith microchemistry to infer natal river of adult Atlantic salmon in Lake Melville indicates that otolith microchemistry of juveniles was distinct across salmon rivers in the Lake Melville watershed. However, the use of otolith microchemistry as a tool for inferring the natal rivers of adult salmon sampled from the FSC fishery appeared to be limited for the Lake Melville watershed. Adults were assigned with high posterior probabilities but failed the squared Mahalanobis distance test for true membership. We hypothesize that the most likely explanations would be that (1) the juvenile dataset is potentially representative of discrete habitats within the river watershed, therefore not representative of the entire watershed and the adults sampled were not from these specifics locations. (2) Otolith interannual variability is potentially creating significant difference between adult otolith freshwater signature and juvenile otolith signature, confounding the ability to infer adult natal river. However, the results obtained in this study are similar to results obtained by genetic analyses on the same salmon samples (Bradbury et al. 2018). Therefore, even if the adult assignments failed the test for true membership, the method has high potential for determining natal river and that with improvement could be a helpful tool for fishery management.

Table 2.5. Summary of the assumptions followed for otolith microchemistry. “Likely” means that the assumption was likely met based on our work or the literature. “Unlikely” means that it is unlikely that the assumption was being met and that it requires further research or complementary research.

	Assumptions	Likelihood of being met
1	Analytical method did not bias the otolith microchemistry	Likely
2	Otolith material is not reabsorbed during its life or in time of metabolic stress	Likely
3	River exhibit unique chemical signatures and the residence-time allow accretion of the signature	Likely
4	Juvenile otolith microchemistry was representative of the adults' natal river	Likely
	Juvenile otolith microchemistry was representative of the whole river watershed	Unlikely
5	Temporal variation was accounted for	Unlikely
6	Adult sampling effort was representative of the juvenile groups and Lake Melville salmon population	Unlikely

A study comparing river watershed geology and water chemistry in salmon rivers in the Lake Melville watershed could have provided useful information to determine representative juvenile sampling locations and to identify whether some regions and/or tributaries have distinct geology that would have improved the interpretation of the results. The information could have allowed us to link factors influencing otolith microchemistry such as the water chemistry and the observed otolith microchemistry.

Further work is required to improve the representativeness of the juvenile dataset by addressing interannual and spatial variability within and among watersheds. Interannual variability should be assessed to validate or invalidate the assumption that it is creating the offset between juvenile and juvenile growth phase in adult otoliths and also to determine which elements are stable through time. If interannual variability is causing the offset between juvenile and adult signatures, then increasing the temporal scale of the juvenile elemental signature would reduce the impact of the interannual variability on the natal river predictions (Walther and Thorrold 2009). Also, by assessing the interannual variability, it could be possible to identify trends between and within years and to integrate it into the statistical treatment.

The interest of using otolith microchemistry as a natural marker of natal river looks less appealing when its rapidity and cost-efficiency is reduced by the great sampling effort that is required for a representative dataset. However, the genetic study performed on the same Atlantic salmon demonstrated that Crooked and Red Wine rivers and Caroline, Kenamu, and Traverspine rivers had similar genetics and could be considered as one group (Bradbury et al. 2018). In this study, the juvenile otolith microchemistry clearly distinguished between the Crooked and Red Wine rivers and among the Caroline, Kenamu, and Traverspine rivers. Therefore, using the otolith microchemistry could provide a tool for determining the contribution of each river-specific population to the mixed-stock FSC fishery. In a context where natal origins need to be inferred at a fine scale, this accuracy could counterbalance the effort required for sampling.

Following this research, a telemetry study is currently being performed by DFO on the Lake Melville Atlantic salmon populations (i.e., migration timing, and movement inside Lake Melville, natal homing, spawning locations) in order to provide context to these results and those of the genetic study and to potentially refine the otolith microchemistry sampling methodology.

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Chapter 3: Facilitating local analysis in northern regions: Microwave Plasma-Atomic Emission Spectrometry for mercury determination in wild Atlantic salmon

Abstract

An analytical procedure was developed to quantify mercury concentration in wild Atlantic salmon (*Salmo salar*) muscle tissue by cold-vapor microwave plasma-atomic emission spectrometry (CV-MP-AES) with microwave-assisted acid digestion. Muscle samples were collected from the Atlantic salmon Food, Social, and Ceremonial fisheries in Lake Melville, Labrador (Canada). Muscle samples were digested with nitric acid and hydrogen peroxide, mercury was stabilized with thiourea, reduced with $NaBH_4$, and quantified by CV-MP-AES. Analysis of fish protein certified reference material (CRM, DORM-3) by CV-MP-AES was used to assess the accuracy and precision of the procedure. CRM recovery averaged 88 %, with a relative standard deviation of less than 8 %. The limits of detection were as low as 0.22 µg L^{-1} in solution, which translates to 0.02 µg g^{-1}. Mercury concentrations in salmon muscle tissue quantified by CV-MP-AES were not significantly different from results obtained by cold vapor-atomic fluorescence spectrometry (CV-AFS) from an accredited laboratory. Our results indicated that the CV-MP-AES procedure is appropriate for the quantification of mercury at background levels (range 0.15 – 0.29 µg g^{-1} dry weight) in wild fish of Labrador.

Keywords

Mercury, Microwave Plasma-Atomic Emission Spectrometry, Atomic Fluorescence Spectrometry, Cold-vapor, Fish muscle

3.1 Introduction

Northern regions (Arctic and subarctic) are exposed to increasing mercury concentrations, mostly produced and released by anthropogenic sources at lower latitudes and then transported to northern regions [1–3] or locally by hydroelectric development [4,5]. Inorganic mercury, which also occurs naturally, is methylated by bacteria in aquatic environments to its most toxic form (methylmercury) [6]. Methylmercury is readily bioaccumulated and biomagnified through the aquatic food chain [7], resulting in increased mercury concentrations in fish, including the traditional food sources of Indigenous communities [2]. Mercury has a wide range of adverse effects on human health and monitoring mercury concentrations in food sources is critical [2,8].
Lake Melville is a large (3,000 km^2) subarctic estuarine fjord in Labrador that supports important Food, Social, and Ceremonial (FSC) fisheries. The dynamic and rich environment of Lake Melville is creating favorable conditions for mercury methylation, thus facilitating bioaccumulation in the food chain [9].

A small number of studies already quantified total mercury concentrations in muscle tissue in Lake Melville's anadromous Arctic char (*Salvelinus alpinus*), Atlantic salmon, and brook trout (*Salvelinus fontinalis*) averaging 0.062 ± 0.042 $\mu g\ g^{-1}$ wet weight (ww), 0.073 ± 0.020 $\mu g\ g^{-1}$ ww, and 0.10 ± 0.034 $\mu g\ g^{-1}$ ww, respectively [10]. Total mercury concentration averaged 0.04 $\mu g\ g^{-1}$ ww in anadromous Arctic char collected along the coast of Labrador [11]. In the Canadian Arctic, mercury concentrations were below 0.05 $\mu g\ g^{-1}$ ww in anadromous arctic char [12] and ranged from 0.015 to 0.25 $\mu g\ g^{-1}$ ww in Northern Dolly Varden (*Salvelinus malma*) [13]. Svalbard arctic char mercury concentrations ranged from 0.039 to 0.44 μg g^{-1} ww [14]. Health Canada assumes that 100 % of mercury in fish muscle is in the most toxic form (methylmercury) [15], therefore total mercury is used as a conservative estimate of methylmercury concentrations.

Northern regions have limited analytical capacity and mercury quantification is usually not performed locally. Local analytical capacity could benefit northern communities by making community-based research more feasible, and in turn help communities to define, prioritize and address their scientific questions. Increasing mercury research capacity at research stations located in northern regions is now being facilitated by the commercialization of the Microwave Plasma-Atomic Emission Spectrometry (MP-AES) system [16]. Although the microwave-induced plasma technology has existed since the 1950s, it was not until the 1990s that the microwave-induced plasma torch was developed [17], and in 2011 a system using the microwave-induced plasma torch coupled to an atomic emission spectrometer was commercialized (MP-AES by Agilent Technologies). This analytical system operates using a nitrogen generator, therefore, reducing costs and challenges related to transportation and supply of other gases (e.g., argon and acetylene) [16,18]. MP-AES is a cost-effective analytical system compared to inductively coupled plasma systems (ICP-MS and ICP-AES) and the multi-elemental capability offers more possibilities than traditional atomic absorption spectrometry (AAS). Since the commercialization of the MP-AES, a wide range of elements have been quantified using this system in geological materials [16,19], inorganic fertilizer [20], water, soil, sediment [21], crude oil [22], sunflowers [23], agricultural materials [24], wine [25], and leather and fur [26]. Overall, the analytical performance of the MP-AES was reported superior to AAS [19,20,24] and comparable to ICP-AES [18,24,26].

Cold vapor is a well established technique that enables mercury determination with analytical systems that require volatile compounds (AAS or AES) [27,28]. A reducing agent (sodium borohydride, $NaBH_4$ or tin chloride, $SnCl_2$) in an acid matrix is used to generate cold-vapor mercury, transforming mercury in solution (Hg^{2+}) to its volatile form (Hg^0). Both reducing agents have been successfully applied for mercury determination by CV-MP-AES [20,26]. A common problem linked to mercury determination is the memory effect caused by the adsorption of mercury on the surfaces of the sample introduction system, which leads to an increase in signal strength during the analysis and results in long wash-out time, poor accuracy, and reliability between analyses even at relatively low concentration (1-5 µg L^{-1}) [29–31]. A number of different procedures have been proposed to solve this problem, such as the addition of gold [30], a sulphur containing compound (e.g. 2-mercaptoethanol, L-cysteine, thiourea) [30–32], or a mix of Triton X-100, ammonia, and ethylene diaminetetra-acetic acid (EDTA) [33,34]. For cost, toxicity, and simplicity related reasons, analytical procedures using gold, 2-mercaptoethanol, and Triton X-100 should be excluded when working in northern regions.

To the best of our knowledge, there have been no studies that have assessed the accuracy and precision of mercury determination in fish tissue with a MP-AES analytical system. This study aims to evaluate the potential of using thiourea to stabilize mercury in sample solution after digestion and $NaBH_4$ as reducing agent to accurately and precisely quantify total mercury in wild Atlantic salmon from Lake Melville by CV-MP-AES. The capacity of CV-MP-AES for measuring total mercury at concentrations below the consumption limit (maximum concentration) for mercury in fish recommended by Health Canada for subsistence fishery (0.2 μg g^{-1} ww) [35] is investigated. The proposed procedure has been developed for quantifying total mercury concentration.

3.2 Method

3.2.1 CRM and reagents

The certified reference materials (CRM), DORM-3 (fish protein), were purchased from the National Research Council Canada, NRCC.

Reducing agent solution of 0.1M sodium hydroxide (NaOH, Anachemia, VWR, Radnor, USA) and 1 % w/v sodium borohydride ($NaBH_4$, Fisher Scientific, Hampton, USA) was freshly (< 1h) prepared before CV-MP-AES analysis. A solution of 10 % w/v thiourea (CH_4N_2S, Fisher Scientific, Hampton, USA) was used to stabilize mercury in sample solutions. A stock standard solution of Hg (1000 mg·L^{-1}) for ICP-MS analytic grade was purchased from Fluka Analytical (Sigma-Aldrich, St. Louis, USA). Trace metal grade nitric acid trace metal grade (15.9 M HNO_3, Ultrex II, J.T. Baker, Center Valley, USA) and hydrogen peroxide trace metal grade (≥30 % H_2O_2, TraceSELECT®, Sigma-Aldrich, St. Louis, USA) were used as reagents in microwave digestion.

3.2.2 Apparatus

Samples were digested with a MARSXpress microwave digestion system (CEM Corporation, Matthews, USA). Digested samples were analyzed with an Agilent Technologies 4200 MP-AES (Santa Clara, USA) equipped with a multi-mode sample introduction system (MSIS) and an autosampler (Thermo Fisher Scientific, CETAC, ASX-520, Waltham, USA). The sample and reducing agent solutions were pumped separately with the MSIS, mixed after the peristaltic pump with a mixing tee, and then connected to the spray chamber (Fig. 3.1). The unused sample lines to the nebulizer and MSIS were capped to prevent nebulizer gas from escaping. The MP-AES operating conditions following manufacturer recommendations are listed in Table 3.1. The rinse, sample uptake, stabilization, and read times were increased to ensure accurate and precise quantification. Viewing position and nebulizer flow were optimized automatically by the software instrument before each calibration. The nitrogen plasma gas was supplied by a 4107 Nitrogen generator (Agilent Technologies). Data were acquired using MP Expert version 1.5.0.6545 software provided by Agilent Technologies.

Table 3.1. MP-AES operating conditions for mercury analysis

Instrument parameter	
Nebulizer	Concentric
Spray chamber	MSIS
Nebulizer flow rate (L/min)	0.50-0.55
Nitrogen consumption (L/min)	20
Read-time (s)	10
Number of replicates	3
Rinse time (s)	60
Sample uptake delay (s)	45
Stabilization time (s)	30
Pump speed (rpm)	15
Sample flow rate (mL/min)	~2.0
Reducing agent flow rate (mL/min)	~2.0
Viewing position	0-10
Hg wavelength (nm)	253.652

3.2.3 Samples and sample preparation

Muscle tissue samples from 15 adult Atlantic salmon were obtained from the FSC fisheries in Lake Melville near the town of North West River (Labrador) during summer 2014. Salmon were measured (fork length, cm), sampled for muscle tissue on the dorsal area behind the head, stored in a clean Nalgene bottle (50 mL), kept on ice during sampling period (2-3 hours), and stored at -20 °C. At the end of the field season, frozen samples were sent to the chemistry laboratory of the biology, chemistry, and geography department at the Université du Québec à Rimouski.

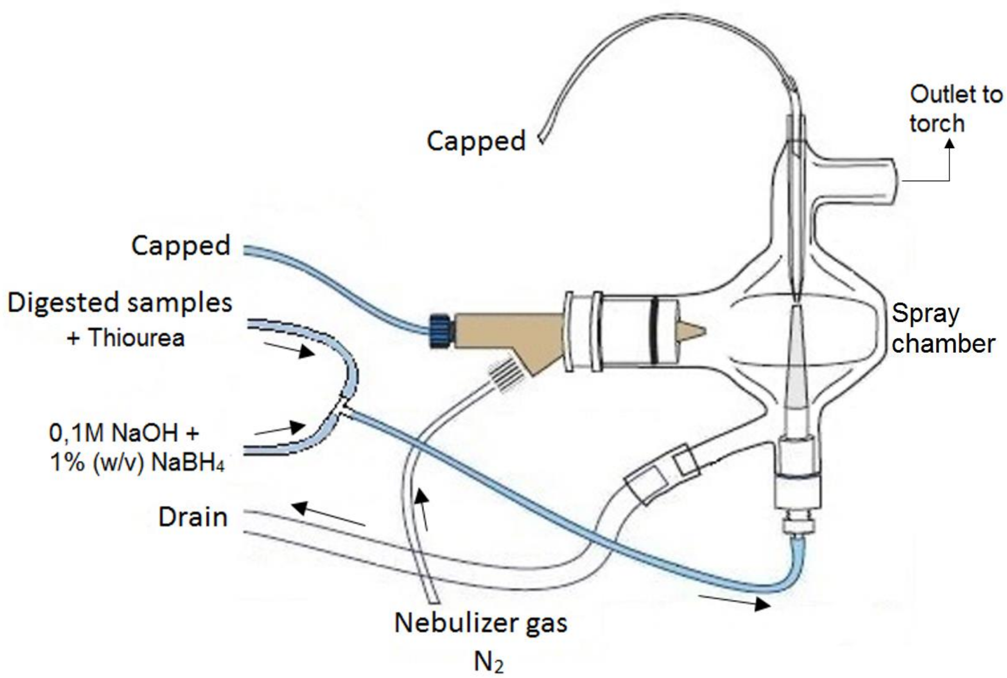

Fig. 3.1. Schematic diagram of the MSIS for cold-vapor mercury analysis with MP-AES. The digested samples with 1 % thiourea solution and reducing agent solutions are pumped separately, mixed at the mixing tee, and then enter the spray chamber. Volatile Hg^0 is transported by the nebulizer gas to the torch.

In laboratory, samples were rinsed with ultrapure water, frozen at -80 °C, freeze-dried (Labconco, Kansas, USA), homogenized (VirTis model "45"), and stored at -20 °C. For CV-MP-AES analyses, salmon sub-samples (approximately 0.5 g) were accurately (± 0.1 mg) weighed into 55 mL TFM® vessels (CEM Corporation, MARSXpress). To each sub-sample, 10 mL of nitric acid and 1 mL of hydrogen peroxide were added before being tightly capped. The sub-samples were then digested at high temperature and under pressure with a microwave digestion system for a total of 30 min in two different steps with the power set at 800 W. The first step was a ramp temperature for 20 min to 160 °C.

Temperature was maintained at 160 °C for 10 min during step 2. Once cooled to room temperature, digested samples were transferred into cleaned (10 % HNO_3 and ultrapure water) 50 mL polypropylene conical centrifuge tubes. Sample volumes were then adjusted to 40 mL with ultrapure water and stored in a refrigerator until analysis. For quality control, a procedural blank (HNO_3 and H_2O_2) was included in each digestion run. Also, for assessing accuracy and precision, five sub-samples of the CRM were digested as previously described for salmon muscle samples.

Before CV-MP-AES analyses, 1 mL of the 10 % w/v thiourea solution was added to each digested salmon, CRM, and procedural blank (9 mL) and allowed to react for 30 min. The thiourea reacted with the digested sub-samples and formed a white precipitate. However, preliminary tests showed that the precipitation process did not affect the mercury concentrations in solution. After precipitation, the solutions were filtered with a 25 mm diameter 0.45 µm PTFE membrane syringe filter (VWR, Radnor, USA).

Sub-samples of our 15 salmon freeze-dried and homogenized muscle samples were sent to the Lakehead University Environmental Laboratory (LUEL; Thunder Bay, Ontario), accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA) to ISO 17025 for result comparison and validation. The LUEL determined total mercury concentrations in salmon muscles using EPA method 1631 with cold vapor-atomic fluorescence spectrometry (CV-AFS). The results obtained were compared to the CV-MP-AES results with a paired t-test.

3.2.4 MP-AES analysis

Before analyses, the MP-AES system was purged with nitrogen for a period of 3 hours and mercury quantifications were performed under nitrogen atmosphere. Seven levels of calibration ranging from 0 to 10 µg L^{-1} were selected to match the expected concentrations. Calibration solutions contained ~2.8 M HNO_3 and 1 % w/v thiourea to mimic the sample matrix. To assess accuracy and precision of the procedure, the CRM digested sub-samples were analyzed first, followed by the salmon digested sub-samples and the procedural blanks. The system was rinsed for 1 min with 10 % HNO_3 ultra trace grade between each analysis.

The monitoring of mercury signal intensity after injecting calibration solution at 10 µg L^{-1} of mercury, followed by the rinsing solution, demonstrated that memory effect is not occurring with this method. The signal rapidly decreased after the beginning of system rinsing and after 60 seconds the intensity had stabilized to the background noise level, indicating that thiourea successfully stabilizes mercury, and that rinsing solution and time are sufficient to eliminate mercury from the system.

The calibration solution of 10 μg L^{-1} was the highest mercury concentration entering the system. To assess if memory effect was occurring at this mercury concentration, the mercury emission signal was monitored during and after the injection of the calibration solution (10 μg L^{-1}), followed by the rinsing solution. The signal rapidly decreased after the beginning of the system rinsing and after 60 seconds the intensity had stabilized to the background noise level, indicating that thiourea successfully stabilizes mercury and that rinsing solution and time are sufficient to eliminate mercury from the system. The limit of detection (LOD) was calculated from the regression line of the calibration as described in Miller and Miller [36]. Accuracy and precision of the procedure were verified using t-tests on CRM certified value and the experimental mean value of mercury obtained by CV-MP-AES.

3.3 Results and discussion

3.3.1 LOD

The LODs obtained for the analysis of total mercury with the MP-AES were similar or lower than the limits of detection reported for AAS [20] or ICP-AES [26]. Laboratory work lasted for more than one year, during which time period high reproducibility was achieved by performing calibration curves and obtaining excellent linearity with correlation coefficients of at least 0.999 (Table 3.2). LOD was as low as 0.22 μg L^{-1} and is similar to what Lima et al. [20] found (LOD: 0.3 μg L^{-1}) when measuring mercury in inorganic fertilizer with CV-MP-AES using $SnCl_2$ as reducing agent. The lowest LOD obtained in this study translated to 0.018 μg g^{-1} dw with a freeze-dried muscle tissue mass of 0.50 g. The LOD of this study is lower to what was previously found (LOD of

2.0 µg g^{-1} dw) by Zhao et al. [26] when measuring mercury in leather and fur with CV-MP-AES using $NaBH_4$ as reducing agent.

Since samples were freeze-dried for conservation purposes, the concentration in dry weight obtained had to be converted to wet weight to compare with the Health Canada standard for mercury in fish. Based on an estimated 75 % water content for fish muscle [37], the highest mercury concentration observed in salmon muscle was 0.071 µg g^{-1} ww, which is well under the Canadian consumption limit for mercury in fish (0.2 µg g^{-1} ww) set by Health Canada for subsistence fishery [35]. Furthermore, when the LOD (0.02 µg g^{-1} dw) is converted to wet weight (0.005 µg g^{-1} ww), the developed CV-MP-AES procedure is able to detect mercury at a level well below the Canadian consumption limit for mercury in fish.

Table 3.2. Limits of detection (LOD) calculated from the calibration curves. The experiments were performed in June 2015 (JN2015) and August 2016 (AU2016).

Calibration curve (conc. range µg·L^{-1})	Correlation coefficient	Intercept (b)	Slope (m)	SD ($S_{y/x}$)	LOD (y_1) (µg·L^{-1})	LOD* (µg·g^{-1})
JN2015 (0-10)	0.99986	-22.29	239.29	17.86	0.22	0.018
AU2016 (0-10)	0.99978	-11.74	215.73	19.60	0.27	0.022
$y_1 = 3S_{y/x} + b$						

* Based on a 0.50g sample mass

3.3.2 Accuracy and precision

Results obtained for both CRM and salmon muscle analyses show that MP-AES can accurately and precisely quantify mercury concentrations with the analytical procedure used. Total mercury concentrations in CRM and salmon muscle tissue measured with CV-MP-AES were well above the LOD determined (Table 3.3Table 3.4). The CV-MP-

AES procedure showed high accuracy and precision with a recovery percentage averaging 88 % ± 8.6 % and a relative standard deviation (RSD) of 7.8 % for the five CRM sub-samples (Table 3.3). These results indicate that variability in the measurements are related more to sample treatment than to system variability. Procedures using CV-MP-AES have been reported to have 95 to 101 % recovery with RSD of 1.6 to 3.8 % with certified reference materials [20,26]. Although the recovery percentage was lower in this study, no significant difference (t_{10} = 2.23, $p > 0.05$) was observed between the CRM certified value and CV-MP-AES experimental values. Furthermore, recovery percentage and RSD values meet the recommendation for analysis in trace amounts (µg g^{-1}) as described in AOAC [38]

Table 3.3. Accuracy and precision of the developed CV-MP-AES procedure for mercury quantification in five CRM (DORM-3, fish protein, certified value 0.382 ± 0.060 µg g^{-1} dry weight) sub-samples by three repeated measurements. [a] The 95 % confidence interval (CI) was calculated on the five replicate mean values and for the mean recovery value. The relative standard deviation (RSD) is based on the standard deviation of the mean values of the five replicates.

Replicates	Mean mercury ± CI (µg·g^{-1} dw)	Recovery (%)	RSD (%)
CRM-1	0.315 ± 0.010	82	1.0
CRM-2	0.356 ± 0.007	93	0.7
CRM-3	0.347 ± 0.010	91	0.0
CRM-4	0.360 ± 0.010	94	1.4
CRM-5	0.301 ± 0.025	79	3.6
Mean[a]	0.336 ± 0.032	88 ± 8.6	7.8

The 15 salmon sampled ranged in length from 51 to 85 cm (Table 3.4). The mercury concentrations in salmon muscle samples quantified by CV-MP-AES and CV-AFS (accredited laboratory) ranged from 0.15 to 0.29 µg g^{-1} dw and from 0.16 to 0.25 µg g^{-1} dw, respectively. In comparison to mercury concentrations obtained by CV-AFS, the relative error of the mercury concentration in salmon muscle determined by CV-MP-AES varied from 1.19 to 19.0 % with an average of 7.97 % and no significant difference was observed between mercury concentrations quantified by both procedures ($t_{14} = 2.06$, $p > 0.05$). Although relative error was as high as 19 % for one sample, our procedure was compared to two standardized procedures and no significant difference was observed. This indicates that our procedure is suitable for accurately and precisely quantifying mercury at low concentration.

Total mercury concentrations in Atlantic salmon, Arctic char, and Brook trout in Lake Melville have been reported to vary between 0.062 and 0.105 µg g^{-1} ww [10]. The accuracy, precision and detection limit obtained indicate that our procedure is appropriate to quantify mercury background concentrations in salmonids in Lake Melville. Mercury concentrations found in Arctic salmonids vary from 0.001 to 0.6 µg g^{-1} ww [12–14]. With the parameters used, the LOD is slightly higher than what can be found in some of the fish muscle in the Arctic. However, it is possible to simply increase the muscle quantity digested or to dilute less after digestion to ensure that the concentration in the digested sample is higher than the LOD. Thus, with the high sensitivity, accuracy, and precision demonstrated in this study, the CV-MP-AES is an appropriate procedure to quantify mercury for northern regions.

Table 3.4. Mercury concentrations (μg g^{-1} dry weight) in wild Atlantic salmon muscle samples analyzed by CV-AFS (accredited laboratory) and CV-MP-AES. The relative error (%) was calculated from the MP-AES compared to the accredited laboratory results.

Salmon	Length (cm)	CV-AFS Hg (μg·g^{-1})	CV-MP-AES Hg (μg·g^{-1})	Relative error (%)
A	51	0.168	0.200	19.0
B	55	0.189	0.204	7.94
C	56	0.157	0.172	9.55
D	56	0.174	0.186	6.90
E	56	0.229	0.206	10.0
F	57	0.196	0.215	9.69
G	57	0.171	0.187	9.36
H	57	0.172	0.185	7.56
I	59	0.225	0.199	11.6
J	61	0.159	0.174	9.43
K	64	0.210	0.214	1.90
L	75	0.163	0.150	7.98
M	82	0.191	0.221	15.7
N	83	0.253	0.256	1.19
O	85	0.246	0.285	15.9
Mean				7.97

3.4 Conclusions

An analytical procedure for mercury quantification in Atlantic salmon (*Salmo salar*) muscle using CV-MP-AES was developed and its performance was evaluated. Analysis of CRM and salmon muscle demonstrated that the procedure developed can accurately and precisely quantify mercury in fish samples. No significant difference was found between mercury concentrations in wild Atlantic salmon determined by CV-AFS (accredited laboratory) and by CV-MP-AES; indicating that the procedure developed can be used to accurately quantify mercury at background level in northern regions. As shown in this study, due to the low operating cost, use of nitrogen for plasma, and high sensitivity, accuracy, and precision, MP-AES analytical systems could be implemented in northern regions more easily than other analytical systems (e.g., ICP-AES, AAS). It also represents a rapid, economical, and safe technique to assess mercury concentrations in biological matrices similar to Atlantic salmon muscle tissue. This analytical strategy could facilitate data gathering for northern regions by reducing analytical cost and easing and enhancing communication between scientists and northern communities.

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Chapter 4: Conclusion

4.1 Summary of findings

My work demonstrated that otolith microchemistry is a promising technique to provide information on the natal river of Lake Melville Atlantic salmon for fishery management, but further research is required. There was a clear distinction among river watersheds using the juvenile otolith microchemistry. Adult natal rivers were inferred based on the juvenile dataset and the majority of the salmon were assigned to Kenamu River which concurred with the genetic results (Bradbury et al. 2018). However, the adult origins inferred did not statistically belong to the river assigned. This work demonstrated that the actual juvenile dataset should be completed with information on otolith microchemistry interannual and spatial variability within the 11 river watersheds used in my project to accurately infer adult natal river.

Mercury concentration can be quantified in Lake Melville's Atlantic salmon muscle tissue with MP-AES analytical system. MP-AES procedure assessed in my work can quantify low mercury concentration ~0.02 $\mu g\ g^{-1}$ ww which is ten times lower than the consumption limit for mercury in fish (0.2 $\mu g\ g^{-1}$ ww) recommended by Health Canada for a subsistence fishery. Therefore, MP-AES is a suitable analytical system to monitor food safety of salmon muscle. Furthermore, our results also demonstrated that mercury concentrations in Lake Melville Atlantic salmon (range 0.04 – 0.07 $\mu g\ g^{-1}$ ww) are well below Health Canada consumption limit.

4.2 Relevance and future work

Atlantic salmon are an important part of country food for Indigenous communities, but are also related to the social, cultural, spiritual, economic and nutritional well-being of the Indigenous communities living around Lake Melville. Developing tools to gather information (data) on salmon for fishery management and for fish contamination assessment to ensure food security and safety for these Indigenous communities is highly significant.

First, the information obtained on the utilization of otolith microchemistry as a tool for inferring mixed-stock fishery origin in Lake Melville represented an important preliminary step to providing data for better understanding Lake Melville salmon populations. It also identified the next steps to take to improve the technique. My work demonstrated the possible limitations of the otolith microchemistry technique caused by the interannual and spatial variability within river watersheds. Therefore, the interannual variability of the juvenile otolith microchemistry and its spatial variability among tributaries of the same river watershed should be assessed and included in the dataset. Information on interannual variability will validate or invalidate the assumption that the interannual variability of the juvenile otolith microchemistry is causing the offset between freshwater signatures of juveniles and adults. Otolith microchemistry spatial variability among tributaries within a river watershed should be assessed to determine the extent to which sampling locations (in the tributary or at the mouth of the river) are representative of the whole river watershed otolith microchemistry.

Future projects should also include a comparison of the salmon river watershed geology and water chemistry variability in the Lake Melville watershed. The geology of the watershed influences the water chemistry, which influences the otolith microchemistry. This information could allow determination of representative juvenile sampling locations, classification of regions or rivers that could have similar geology, and identification of distinct areas within a river. Furthermore, if the information on watershed geology and water chemistry is compared to the observed otolith microchemistry it would provide information on which element:Ca ratios to use for a representative dataset of each river or group of rivers, with good separation of the signature among rivers in Lake Melville watershed.

Otolith microchemistry and genetics are complementary techniques to delineate fish populations (Campana and Thorrold 2001, Feyrer et al. 2007). A genetic study by Bradbury et al. (2018) on the same Atlantic salmon sampled in this study demonstrated that few rivers (Crooked/Red Wine Rivers and Caroline/Kenamu/Traverspine) had similar genetics and could be considered as one group. Otolith microchemistry was able to distinguish among the Caroline, Kenamu and Traverspine Rivers and between Crooked and Red Wine Rivers and could therefore be used to assess the proportion of salmon assigned to their group originating from each river. The juvenile and adult otoliths sampled and used to infer salmon natal river for the Lake Melville FSC fishery in my work are the same salmon used in Jeffery et al. (2017), Bradbury et al. (2018), Sylvester et al. (2018). In future, a more comprehensive comparison between results from the genetic studies and otolith microchemistry will be performed and will provide a unique opportunity which has rarely been done in the past (Miller et al. 2005, Bradbury et al. 2008, Martin et al. 2015) to compare both techniques for describing spatial distributions of Lake Melville's Atlantic salmon populations.

Further work on movement of Lake Melville salmon within the Lake Melville watershed, such as upstream migration timing, adult movement inside Lake Melville, juvenile movement inside the watershed, and downstream migration timing, using telemetry would also provide useful information to link the ecology of Lake Melville's salmon to the otolith microchemistry observed, not to mention complementary information for FSC mixed-stock fishery management.

Based on previous studies of the impact of the impoundment of a hydroelectric reservoir and past impoundment on the Churchill River, it is known that mercury concentrations will rise in Lake Melville after the impoundment of the Muskrat Falls reservoir on the Lower Churchill River (main tributary of Lake Melville) (Anderson et al. 1995, Anderson 2011, Schartup et al. 2015). Calder et al. (2016) surveyed mercury concentrations in different species supporting Indigenous country food sources in Lake Melville and determined the frequency with which they were consumed. Based on a statistical model, the authors used these values to predict mercury peak concentration in the most consumed species and the health risk for Indigenous people. Compared to Atlantic salmon, which migrate at sea and reduce their exposure to mercury in Lake Melville, brook trout and lake trout remain in Lake Melville all year long and will have higher exposure to mercury, resulting in higher mercury concentrations in their tissues. Also, species higher up the food chain such as seals will have higher mercury concentration increases than fish due to biomagnification. Therefore, the MP-AES could answer the need for rapid and local monitoring of the safety of country food for Lake Melville communities.

Mercury is not the only metal or metalloid of concern in country food. Arsenic, cadmium, lead, and selenium can also have adverse effects on human health and can be quantified along with a wide range of elements using MP-AES (Niedzielski et al. 2015, Zhao et al. 2015, Tanabe et al. 2016). The methods developed here for analyzing element concentrations in fish muscle tissue and other country food species may allow the extension of food safety assessments to these elements of concern.

Although the use of the MP-AES was evaluated in the context of northern regions in my study, the analytical system could be of use for other communities that are in remote areas and have a high reliance on country foods and/or communities that have a desire to be integrated or independent in science.

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Appendix A. Salmon river watersheds flowing into Lake Melville

Table A.1. Total river watershed area (km^2) of Lake Melville region and their accessible drainage area to salmon as spawning and rearing habitats. (Reddin et al. 2010, Anderson 2011) Note that Peters River and Red Wine River is a tributary of the Goose River and Naskaupi River, respectively.

River	Total (km^2)	Accessible to salmon (km^2)
Beaver River*	1 878	1 624
Cape Caribou River	546	546
Caroline Brook	N/A	~1 **
Crooked River	2 391	2 391
English River*	640	33
Goose River	3 432	1 938
Kenamu River	4 403	4 403
Kenemich River*	699	699
Main Brook	N/A	N/A
Mulligan River	1 062	1 062
Naskaupi River	12 691	11 422
Sebaskatchu River	580	580
Susan River	363	363
Traverspine River	728	613
Total	29 413	25 674

* Rivers not sampled in this study

** Estimated from our observation