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ELEMENTAL FINGERPRINTS OF OTOLITHS FROM SMOLT OF ATLANTIC SALMON, *Salmo salar* Linnaeus, 1758, FROM THREE MARITIME WATERSHEDS: NATURAL TAG FOR STOCK DISCRIMINATION

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ABSTRACT

The utility of otolith elemental fingerprints for discriminating sub-regional stocks of Atlantic salmon was examined. Otoliths were removed from Atlantic salmon smolts collected from three individual river watersheds in the Canadian Maritimes during spring and analyzed for 27 elements using inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma emission spectrometry (ICP-ES). Calcium and minor and trace elements were precisely measured in whole otoliths at concentrations well above detection limits. Six elements (Ba, Pb, Li, Mn, Rb, and Tl) were significantly different among watersheds. Linear discriminate function analysis based on otolith elemental concentrations of Li, Mn, Rb, and Tl correctly classified smolts to their river of origin with an average accuracy of 73%. At a slightly greater spatial scale of large watersheds, correct mean classification rate was 92% based on a fingerprint of four elements (Ba, Li, Mn, and Rb). Results indicate that elemental fingerprints of otoliths can be used to discriminate among river management stocks which may be important in the future since dried or frozen stored otoliths retain their signature indefinitely and otoliths are often available from previous studies. Otolith elemental fingerprints would be effective as a natural tag of a river system or biogeoclimatic zone of origin when applied to the study and management of Atlantic salmon in the North Atlantic Ocean.

INTRODUCTION

Effective fisheries management demands the prediction of temporal trends in population abundance and productivity so that sustainable biological limits on harvesting can be set (Hilborn and Walters 1992).

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Anadromous salmon species form reproductively isolated populations, which are managed as distinct units or stocks, yet they are distributed over a vast area (i.e., oceans) outside of managementareas (i.e., rivers) for a large portion of their life. Recently, a more holistic approach to salmon management has been suggested, one that considers the ocean conditions which primarily determine spawning recruitment (Bisbal and McConnaha 1998). An essential component of this management strategy is a thorough understanding of the spatial and temporal marine distribution of individual river management stocks. Data collection, however, required to describe life histories of specific river stocks is complicated by the need to discriminate each stock from the mixed assemblage of fish being sampled in the ocean or in some fisheries.

Each year, juvenile anadromous Atlantic salmon, *Salmo salar* Linneaus, 1758, of appropriate size, undergo smoltification and migrate downriver to the sea (McCormick *et al*.1998). Post-smolts emigrating from different watersheds move through common migration corridors (Shelton *et al*. 1997; Lacroix *et al*. 2005; Byron *et al*. 2014) and then distribute over broad oceanic regions (Holm et al 2000; Soto *et al*. in review). During feeding migrations in the North Atlantic Sub-polar Gyre, populations become aggregated and mixed together (Dadswell *et al*. 2010). After 1–5 years of marine residency, mixing of both regional stock groupings (Reddin 1985; Jacobsen *et al*. 2001) and the two continental stock complexes (Møller Jensen 1980a, 1980b) occurs as North American and European origin Atlantic salmon undertake trans-Atlantic migrations between ocean feeding grounds and freshwater spawning rivers.

The anadromous life cycle and long distant migratory behavior of Atlantic salmon presents many opportunities for their interception by fisheries at sea and coastal and estuarine salmon fisheries indiscriminately harvest individuals from different river stocks that can vary greatly in productivity and which can lead to a significant decline in recruitment or even collapse of some management units (Crozier *et al*. 2004). While Canadian and American commercial Atlantic salmon fisheries have been terminated (Chase 2003), other commercial and subsistence fisheries continue to operate in the exclusive economic zones: France (Saint Pierre and Miquelon), Denmark (Greenland), Norway, Ireland and the United Kingdom (UK; ICES 2009). Also, there are recreational angling fisheries in many rivers of Atlantic Canada, Iceland, Ireland, the UK, the Nordic countries, and Russia. Determining the geographic origin of migrating individuals to

estimate the degree of mixed stock composition of trans-boundary and straddling stock fisheries remains an important management issue throughout the North Atlantic.

During the past decade, advances in analytical techniques used to identify and quantify the chemical composition of biological samples have encouraged the application of elemental and isotopic marks in the tissues of fishes as an alternative method to test stock discrimination (Campana 2005). Both calcified and non-calcified fish tissues can incorporate elements and isotopes characteristic of physiological, dietary, or ecological events that individual fish have experienced. These events impart a signature of past exposure in the tissue structure which allows for relative chemical information to be collected

(Elsdon and Gillanders 2003a). Indeed, elemental and isotopic signatures in a variety of Atlantic salmon tissues have been used to retrospectively detect migrations and infer distributions (Tucker *et al*. 1999; Spares *et al*. 2007), and distinguish populations (Flem *et al*. 2005; Veinott and Porter 2005; Martin *et al*. 2013). Selecting the appropriate tissue to analyze is essential since chemical composition can differ among tissue types (Gillanders 2001). Tissue structures that are not susceptible to metabolic reworking are required to link chemical signatures to discreet time periods in the environmental record.

Sagittal otoliths, usually the largest of the three otolith pairs, are composed predominately of calcium carbonate crystallized in the form of polycrystalline aragonite (Campana 1999). As fish grow, otolith formation occurs by the sequential layering of calcium carbonate and protein precipitated from the endolymphatic fluid surrounding the otolith to form a crystalline-proteinaceous matrix (Campana and Nielsen 1985). Calcium carbonate is the dominate component (>90%), followed by protein (<8%), with the remainder being non-organic impurities such as minor (> ppm) and trace (< ppm) elements and their isotopes (1–2%). Campana (1999) reported that a total of 31 elements had been detected in otoliths with the majority occurring at low concentrations (<10 ppm) and presumably more are awaiting detection as new analytical techniques that improve properties of accuracy, precision, and sensitivity of analysis are developed. Still, detection of either a given element or isotope in the otolith is not simply a function of instrumentation because the presence and concentration are influenced by numerous environmental factors (Elsdon and Gillanders 2004) and the physiology of the individual (Kalish 1989).

To reconstruct environmental histories or discriminate among mixed groups of fish which have inhabited different past environments, elements under strong physiological control (e.g., C, O, H, N, S, Cl, K, and P) may be of minimal use since they have been found to vary little among populations (Campana 1999). Elements that have been shown to reflect environmental availability are predominately the alkaline earth metals (e.g., Ca, Sr, Ba; Farrell and Campana 1996; Bath *et al*. 2000; Elsdon and Gillanders 2002, 2003b) and their isotopes (e.g., $^{87}Sr/^{86}Sr$; Kennedy *et al*. 2002), some of the alkaline metals (e.g., Li; Milton and Chenery 2001), and, to a lesser extent, the transition metals (e.g., Mn; Dorval *et al*. 2005). For freshwater fish, the primary source of elemental uptake occurs via water passing over the gills, which is influenced by relative Ca concentration in the water (Campana 1999). For some elements assimilated into the otolith matrix such as Sr and Ba, elemental concentration relative to Ca may be the most indicative measure of environmental availability for fish residing in freshwater as opposed to the absolute concentration of the element (Campana 1999). In studies focusing on fish population dynamics in both lotic and lentic freshwater systems, referencing elemental concentrations to Ca has proven extremely successful for identifying geographic origins (Wells *et al*. 2003a; Brazner *et al*. 2004b; Martin *et al*. 2013).

The underlying premise of this approach is based on two key properties of the otolith: the growth of the otolith is continuous from before the time of hatch to the time of death and material deposited onto the growing surface of the otolith is unlikely to be reworked or resorbed due to its metabolically inert and acellular nature (Campana and Neilson 1985), and (2) the calcium carbonate, trace elements and isotopes that comprise greater than 90% of the otolith in the fish are mainly derived from the ambient water (Campana 1999). This implies that the chemical and physical composition of the environment is reflected in the otoliths of individual fish providing a permanent record of exposure structured by age. Therefore, concentrations of elements and isotopes incorporated into increments of the otolith corresponding to the juvenile life stage provide a natural tag or fingerprint (Campana *et al*. 1994) specific to a particular natal water mass which can be used to discriminate among stocks of fish from different origins.

In our study, the utility of otolith chemistry as a method for identifying Atlantic salmon to river of origin was assessed. Specifically, the objectives were to (1) quantify the variation in trace element chemistry in the otoliths of Atlantic salmon smolts from three

different watersheds in the Canadian Maritimes and (2) test the ability of the differences in otolith elemental concentrations among river stocks to accurately classify individual Atlantic salmon smolts to river of origin. Smolts were sampled for two reasons: (1) to ensure that the characterization of otolith elemental fingerprints for discrete reference groups were accurate by removing the possibility of stock mixing prior to sample collection since smolt remain in, or at least near, natal rivers until migration into fully marine waters (Cunjak *et al*. 1989); and (2) to allow for analysis of the entire juvenile portion of the otolith corresponding to the period of time before hatch until the period of time just prior to marine residency thus deriving elemental fingerprints characteristic of the entire freshwater life history stage.

The rationale to base elemental fingerprints on the entire freshwater phase was to provide an approach to sampling the otoliths of ocean migrating Atlantic salmon for determination of stock identity with high replication capabilities and to use a part of the salmon that preserves the stock identity and can be easily stored without damage. In most commercial and angling fisheries the salmon are processed quickly leaving only the head containing the otoliths available for scientific study (Tucker *et al*. 1999; Spares *et al*. 2007). Additionally, fisheries are often remote (Labrador, Greenland, etc.) and freezing, which destroys DNA for stock analysis, is used to maintain the samples. Bulk examination of otolith chemical composition should provide a level of resolution adequate for accurately classifying individual salmon to river of origin while at the same time offering an economic and time-efficient means of analysis (Campana *et al*. 1995b).

STOCK WATERSHED DESCRIPTIONS

The Department of Fisheries and Oceans Canada (DFO) utilizes the index river monitoring program in the Canadian Maritime provinces to evaluate the status of Atlantic salmon stocks in nearby or similar rivers located in the same biogeoclimatic zone (Chadwick 1995; DFO 2003).

The Miramichi River, New Brunswick, Canada is part of DFO's Gulf Region and is the largest river system in the New Brunswick Southeast Gulf biogeoclimatic zone (DFO 2003, 2006; Fig 1). The river is approximately 250 km in length and consists of two main branches, the Northwest and the Southwest, which drain watersheds of 3900 km^2 and 7700 km^2, respectively (DFO 2001a). Both the Northwest

Miramichi River and the Southwest Miramichi River originate in the Ordovician, Silurian, and Devonian granitic and volcanic rocks of the north central Miramichi highlands and flow predominately eastward where they join below the head of tide in the Maritime Plain which is underlain with sandstones, conglomerates, and siltstones from the Pennsylvanian period or earlier (Chiasson 1995).

The Margaree River, Nova Scotia, Canada is also part of DFO's Gulf Region and is the principal river in the Nova Scotia Northumberland Strait and western Cape Breton Island biogeoclimatic zone (DFO 2003, 2006; Fig 1). The Margaree River is comprised of two main branches that drain a combined area of 1165 km^2 and have a combined length of 120 km. The Southwest branch originates at Lake Ainslie and flows through Carboniferous sedimentary rock (Barr and Reaside 1989) in a northerly direction to join the Northeast branch flowing south from its headwaters, which drain the highlands of Cape Breton. The Northeast branch intersects crystalline and metamorphic rock before it joins with the Southeast branch (MacNabb *et al*. 1976).

METHODS

All 97 Atlantic salmon smolts sampled were collected during the spring smolt migration period (Table 1). Fish were captured with trap-nets in the Northwest Miramichi River at Cassilis, New Brunswick and in the Southwest Miramichi River at Millerton, New Brunswick and with a rotary screw trap near the head of the tide in the constricted main stem of the Margaree River (Clément *et al*. 2007; Fig 1). They were then placed together in plastic bags according to river and date of collection and frozen until otoliths were removed.

Table 1 Summary information and descriptive statistics for Atlantic salmon smolts sampled from three watersheds in the Canadian Maritimes. Mean ± SE age, fork length, and otolith mass are given.

Watershed	Biogeoclimatic zone	Number of fish	Age (yr)	Fork length (cm)[a]	Otolith mass (mg)[b]
Margaree	SFA 1	38	2.4 ± 0.1	12.4 ± 0.2	1.5 ± 0.1
Northwest Miramichi	SFA 16	29	2.3 ± 0.1	12.7 ± 0.2	1.6 ± 0.1
Southwest Miramichi	SFA 16	30	2.3 ± 0.1	12.4 ± 0.2	1.5 ± 0.1

[a] Fork length based on measurements taken from frozen-thawed fish.
[b] Otolith mass is the calcium-referenced mass used for elemental analysis.

Otolith preparation – Sagittal otoliths were removed, cleaned, and prepared for elemental analysis using a modification of the decontamination protocol described in Campana *et al*. (2000). All otoliths were handled with only high-density plastic materials (polyethylene and polypropylene), and all were pre-cleaned with trace-metal grade reagents. All steps to clean, then protect otoliths from potential contaminants were performed under a laminar flow, positive pressure fume hood lined with polyethylene sheeting that had been previously

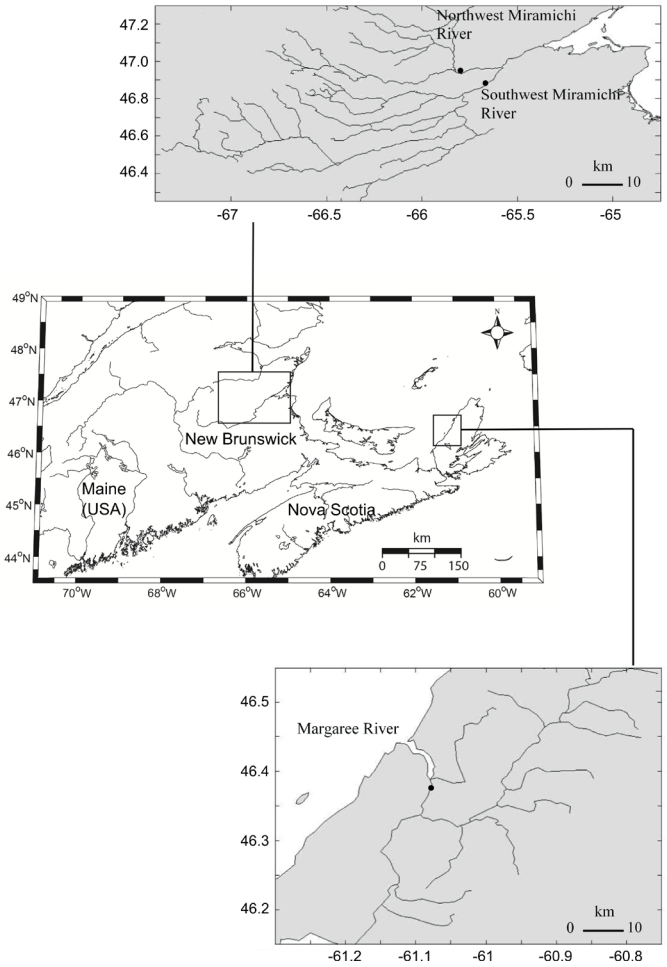

Fig 1 Map of the watersheds in the Canadian Maritimes examined in this study, showing Atlantic salmon smolt collection sites (•).

cleaned with 95% non-denatured ethanol, although 18 MΩ doubly deionized water ($DDIH_2O$) was used rather than Super Q water. Disposable polyethylene gloves were worn at all times when handling equipment and specimens and exchanged regularly.

Smolts were thawed in the laboratory for 3 h prior to dissection and examined for adipose fin clips that indicate hatchery origin. No smolts had fin clips. Fork length, to the nearest 0.1 cm, was recorded for each individual (Table 1). Sagittal otolith pairs were removed from all fish with acid-washed forceps, placed in acid-washed polypropylene caps, submersed in $DDIH_2O$, and any remaining biological tissue removed with fine tipped non-metallic, acid-washed forceps under a dissecting microscope. Right otoliths were used for age analysis and were air dried for 24 h before storage in acid-washed 2 ml polyethylene micro-centrifuge tubes. Left otoliths were used for elemental analysis and were placed in acid-washed 50 ml polypropylene vials for further cleaning which included immersion in $DDIH_2O$ and sonification for 5 min, triple rinsing in $DDIH_2O$, a 5 min soaking in ultra-pure 3% hydrogen peroxide, another 5 min sonification in $DDIH_2O$, followed by a triple rinse in $DDIH_2O$. Left otoliths were then placed in acid-washed polypropylene caps and air dried under the laminar flow, positive pressure fume hood for 24 h. Once dried, each otolith was weighed to the nearest 0.1 mg (Table 1). To remove any potential surface contamination from the weighing process, otoliths were triple-rinsed in $DDIH_2O$ and air-dried under the laminar flow, positive pressure fume hood for a final 24 h. Decontaminated left otoliths were stored in labeled, capped, acid-washed 5 ml polypropylene vials to await elemental analysis.

Empty vials were also prepared for blank corrections and to calculate limits of detection (LOD) with the same decontamination procedure carried out on the left otoliths. All vials were sealed within acid-washed polyethylene bags and forwarded to the Research and Productivity Council Laboratory (RPC) in Fredericton where all elemental analyses were conducted. At the RPC, decontaminated left otoliths were digested into solution in the original acid-washed containers with high-purity nitric acid in preparation for elemental analysis. After the initial reaction subsided, the samples were warmed in an oven (80ºC) for 15 min to complete sample dissolution. After cooling, samples were diluted to a final volume of 2 ml with deionized water.

Age analysis – Age was determined by counting annuli on polished thin sections of right otoliths. Otoliths were chosen for age

determination because fish were not stored individually after collection and scale fidelity was lost. Otoliths were mounted sulcus side up on glass micro-slides with cyanoacrylate glue and allowed to dry for 2 h then polished to the mid-plane with 12 μm and 3 μm adhesive-backed metallurgical aluminum oxide (Al_2O_3) lapping film attached to a smooth glass plate. To enhance annuli visibility, otoliths were further polished with 0.3 μm lapping film. Because otoliths were small in size, only one side of the otolith required polishing to fully reveal annuli. The resulting otolith thin sections were then viewed under a compound microscope with transmitted light at 100x magnification and annuli were counted according to guidelines outlined by Chilton and Beamish (1982). Otoliths were aged on two separate occasions without previous knowledge of the specimens' estimated age or individual fork length. When the two age readings agreed, that age was assigned to the individual. When the two age readings disagreed, the ageing protocol was repeated until agreement. Self-comparison tests of otolith age estimates were conducted to measure within reader precision and age reproducibility (Campana *et al*. 1995a).

Elemental analysis – The use of solution-based assays was adopted to analyze the whole otoliths of smolts. The classification power of the elemental fingerprint is an important consideration for investigations involving lotic freshwater since the concentrations of some elements and isotopes in surface waters can often exhibit low spatial variability. Therefore, a suite of trace elements were analyzed as opposed to a single isotopic ratio. This multi-element fingerprint should maximize the observed differences between groups and increase classification accuracy.

All otolith sample solutions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma emission spectrometry (ICP-ES). With small sample sizes (mean sample size was ≈ 1.4 mg) greater analytical uncertainty for elements that are close to the detection limits was a concern, therefore, isotope dilution (ID) was not used since data improvement (increased accuracy, precision, and sensitivity) provided by ID would have been minimal (Campana *et al*. 1995b). In addition, simultaneous analysis by ICP-ES would have been made impossible since ID requires spikes of stable isotopes to be added to sample solutions. ICP-MS analysis was carried out using a Thermo X-7 ICP-MS instrument equipped with a high-pressure injection interface designed for high sensitivity.

The instrument was operated in a manual sampling mode to reduce analysis time and sample consumption. Indium (In) was used as an internal standard for ICP-MS analysis for all elements where µl of 40 µg/l ^{49}In was added to the final diluted volume of 2 ml for all samples after sample digestion in preparation for elemental analysis. Quantification was based upon external standardization. The only target element that was not assayed on ICP-MS was Fe, which is subject to molecular ion interferences in this instrument.

ICP-ES analysis was conducted using a Varian Vista Pro ICP-ES instrument. Quantification was by external standardization using matrix-matched standards. The solutions were manually sampled using a naturally aspirated micro-concentric nebulizer. Laboratory blanks, pooled otolith laboratory control material (LCM), and matrix-matched standards were analyzed concurrently with the samples at regular intervals throughout both the ICP-MS and ICP-ES analytical runs. Variability of the blanks indicated the ultimate detection limit for each element and the pooled otolith LCM provided a measure of within-run precision. Results from the matrix-matched standards and laboratory blanks were used for dynamic drift correction. Otolith samples were randomized across collection locations with respect to assay sequence to remove sample order bias. Finally, for most elements, multiple isotopes (ICP-MS) and multiple emission lines (ICP-ES) were used for analytical verification and to help detect potential molecular ion interferences.

A total of 27 elements were targeted for analysis by ICP-MS and ICP-ES. Based on preliminary analysis of element concentrations and element interferences significant data were obtained for 14 elements (Table 2). For elements analyzed by both methods and when multiple emission lines or isotopes yielded comparable data, preferred data sources were chosen on the basis of element sensitivity, known interferences, calibration stability, and background values (Table 2). Raw sample data were corrected for blank concentrations based upon the measured solution concentration factored with the average mass of all the samples. A proportional correction based upon the relative mass of the individual sample to the average otolith mass was used. For very small otoliths (<2.0 mg) Ca concentration is probably a more accurate determination of the actual amount of sample. Consequently, all reported element concentrations and subsequent data comparisons are based upon a sample mass normalized to a constant Ca concentration calculated from blank-corrected solution concentrations of Ca

Table 2 Limits of detection (LOD) and precision (CV) for significant elements in otoliths of Atlantic salmon smolts analyzed by ICP-MS and ICP-ES.

Instrument	Element	LOD[a] (µg/g)	CV[b] (%)	MAR[c] BCMC (µg/g)	NWM[d] BCMC (µg/g)	SWM[e] BCMC (µg/g)	BCMC> LOD (number of rivers)
ICP-MS	Ba	0.02	12.7	36.96	5.72	11.44	3
	Pb	0.0204	18.5	0.0201	0.0253	0.0255	2
	Li	0.004	5.0	0.020	0.053	0.056	3
	Mg	0.3	5.1	104.1	45.8	34.0	3
	Mn	0.02	3.5	5.07	8.91	13.29	3
	Rb	0.002	6.0	0.467	0.71	0.727	3
	Sr	0.04	9.0	898.07	622.34	996.01	3
	Tl	0.00006	7.9	0.04457	0.06052	0.04184	3
	U	0.00007	54.6	0.00102	0.00030	0.00027	3
	Zn	0.4	10.9	66.5	39.3	36.3	3
ICP-ES	B	0.6	13.2	0.3	0.5	0.5	0
	Ca	824	1.4	400000	400000	400000	3
	K	1	2.7	832	922	905	3
	Na	7	1.1	2974	2896	3052	3

[a] LOD based on nine laboratory blanks.
[b] CV based on seven pooled otolith laboratory control material.
[c] MAR BCMC represents blank corrected mean concentration within the Margaree River.
[d] NWM BCMC represents blank corrected mean concentration with the Northwest Miramichi River.
[e] SWM BCMC represents blank corrected mean concentration with the Southwest Miramichi River.

(Table 1).This approach corrects for weighing errors and produces accurate estimates of sample mass for all samples since the otolith is composed of nearly pure $CaCO_3$ (40% Ca by weight; Campana 1999). LOD for both ICP-MS and ICP-ES (LOD = 3 SD in µg/g of otolith mass) were below mean concentrations for all elements at each location, except for B and Pb (Table 2). Estimates of precision (coefficient of variation [CV = x SD/mean]) based on seven samples of pooled otolith LCM ranged from as low as 1.1% for Na to as high as 54.6% for U (Table 2). Since no strict criteria exist for retaining elements in these types of studies, Brazner *et al*. (2004b) recommended other studies involving otolith elemental fingerprints apply the following conditions: the blank corrected mean concentration for at least one sample site had to exceed the LOD, and the CV of replicate assays of standards had to be less than 30%. Upon applying these criteria to this study B and U were eliminated while Ba, Pb, Li, Mg, Mn, K, Rb, Na, Sr, Tl, and Zn were retained for statistical analyses. Calcium was

not included in subsequent analyses as it was only used as a sample weight reference to calculate concentrations of other elements.

Statistical analysis – Concentrations of individual elements in otoliths of smolts were grouped by collection and screened for normality of distribution (Shapiro-Wilk test) and homogeneity of variance (Bartlett's test). To normalize within group distributions of data, concentrations of Ba and Sr were cosine transformed, concentrations of Pb and Mn were log transformed, concentrations of Li and Tl were square root transformed, and concentrations of Mg, K, Na, and Zn were sine transformed. Concentrations of Rb were normally distributed but were log transformed to meet the assumption of equal variances across sample groups. Data points abnormally separated from the main data distributions with the potential to distort stock discrimination results were then removed. These included three Li values, two from the Margaree River data set and one from the Northwest Miramichi River data set.

Analyses of otolith elemental fingerprints were done using both univariate and multivariate analysis of variance (ANOVA). Differences between otolith elemental concentrations among individual watersheds were compared with a Bonferroni-corrected multiple comparison procedure. Elements significant in the univariate ANOVAs were entered into a multivariate analysis of variance (MANOVA) using Pillai's Trace as the test statistic as it is the most robust to violations of homogeneity and multivariate normality (Wilkinson *et al*. 1996).

Linear Discriminant Function Analysis (LDFA), using only those elements entered into the MANOVA, was employed to assess the ability of otolith elemental fingerprints to predict river of origin of individual Atlantic salmon smolts. A backward stepwise LDFA classification method tested the significance of the contribution of each variable (i.e., element) for discriminating across groups (i.e., rivers) using *F*-statistics, estimated by the discriminant analysis procedure. Backward stepwise LDFA enters all variables selected but retains only those elements with significant *F*-to-remove values (Wilks' Lambda, $P < 0.05$; Wilkinson *et al*. 1996). For comparative purposes, a forward variable entry approach was undertaken as well and no difference in classification accuracy or *F*-statistics was observed. Tolerance statistics were used to measure the correlation of elements used in the LDFA model. Tolerance values range from 0 to 1 with a low value indicating that the contribution of an element to the LDFA model is somewhat dependent on at least one other element (Wilkinson *et al*.

1996). A cross-validation algorithm (SYSTAT 2004), which uses a bias decreasing jackknife technique, was used to approximate stock discrimination accuracy of smolts from the three watersheds.

Discriminant analysis also aided in the graphical visualization of otolith elemental fingerprints in multivariate space. Scores for the first two canonical variates were calculated and plotted along with 95% confidence ellipses around the centroids of each group to illustrate significant multivariate differences in otolith elemental concentrations among rivers. All statistical analyses were carried out using SYSTAT software Version 11 (SYSTAT 2004).Data values are stated as mean ± SE unless otherwise indicated.

RESULTS

Age class ranges for Atlantic salmon smolts sampled from the Margaree River, the Northwest Miramichi River, and the Southwest Miramichi River were age 2 to age 4 (2.4 ± 0.1 yrs), age 2 to age 3 0.1 ± 2.2) yrs), and age 2 to age 0.1 ± 2.3) 3 yrs),respectively (Table 1). The age 2 smolt class dominated the Margaree River (n = 23), the Northwest Miramichi River (n = 21), and the Southwest Miramichi River (n = 22) samples. Only one age-4 fish was sampled and was from the Margaree River. There was 96% agreement from initial double readings of all otoliths with an average CV of 1.2%. Only four smolts required further age scrutiny with age differences between readings for those fish being one year.

Previous studies have documented fish age and size related differences in concentrations for some elements within the otolith, which can be confused for stock specific differences (Campana *et al*. 1995b, 2000). It was important to identify if such differences in samples among these management river stocks existed. ANOVA revealed no significant difference in length ($df = 2, 94, P = 0.339$) or age ($df = 2, 94, P = 0.348$) in fish collected from the watersheds (Table 1), but lengths may have been affected by storage protocols (Armstrong and Stewart 1997) and ages could not be validated (Campana 2001). Hence, otolith mass was used as a proxy for fish size and fish age (Campana *et al*. 1999). To internally verify this proxy, otolith mass (mg) was regressed separately on fish length (cm) and fish age (yr) – with one Margaree River outlier (stem and leaf plot; SYSTAT 2004). Least absolute deviations (LAD) regression using a modified version of the simplex

algorithm was selected to compute estimates of regression coefficients as it is robust to extreme observations present in the data set of the dependent variable and may provide better estimates of regression coefficients when outliers exist (SYSTAT 2004). Despite producing r^2 values which were low (0.169; Equation 1) and moderate (0.401; Equation 2), respectively, both relationships were significant (df = 2, 94, $F > 19.3$, $P < 0.001$) indicating that otolith mass reliably reflected the range of smolt lengths and ages observed (Fig 2). Fish length is ostensibly a function of otolith mass only as the constant spans zero.

Fish Length = 0.126 · otolith mass (± 0.029) - 0. (±0.357)
(Equation 1)

Fish Age = 0.420 · otolith mass (± 0.053) + 0. (±0.126)
(Equation 2)

Differences in otolith mass among watershed were not significant (ANOVA, df = 2, 94, $F = 0.558$, $P = 0.574$), suggesting that the relationships between elemental concentration and otolith mass (and by inference fish size and fish age) did not have an important influence.

Despite an insignificant relationship, an effect of otolith mass on concentrations of individual elements across groups and within groups was identified. A significant relationship was observed for only Sr and Tl with Sr concentrations negatively correlated with otolith mass across river stocks (ANCOVA, df = 1, 95, $F = 5.483$, $P = 0.021$) and Tl concentrations positively correlated with otolith mass within the Southwest Miramichi River (ANCOVA, df = 1, 28, $F = 5.269$, $P = 0.029$). Initially, concentrations of these elements were otolith mass detrended (Campana *et al*. 2000), but repeated statistical analyses performed without detrending element concentrations resulted in identical conclusions and an insignificant difference in otolith mass among samples was not expected to confound any stock-specific differences in otolith elemental concentrations. Thus, all results reported here are based on undetrended data.

Non-transformed mean (± SE) concentrations of the eleven elements retained for univariate and multivariate statistical analyses ranged from as high as 2 (± 34) μg/g for Na to as low as 0.0 (± 0.0036) μg/g for Pb, when examined across all rivers (Table 2). Most notably were the significant Zn concentrations in these samples (49.02 ± [3.25]; Table 2). When comparisons were made among river stocks, concentrations

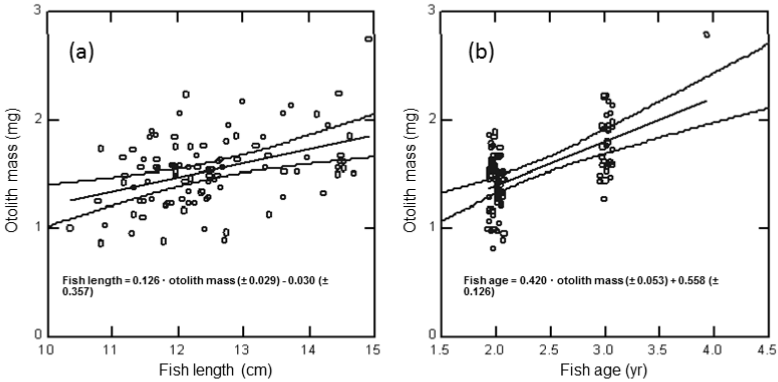

Fig 2 **Regression relationships between (a) otolith mass (mg) and fish length (cm) and (b) otolith mass (mg) and fish age (yr) for Atlantic salmon smolts sampled. Otolith mass (mg) is the calcium-referenced weight used for elemental analysis. Slight random jitter has been applied to the data due to overlapping points (SYSTAT 2004). Plotted above and below the regression lines are 95% confidence intervals.**

of Pb, Li, K, Rb, Na, and Tl in otolith samples from the Northwest Miramichi River were observed to be highest (6 of 11 elements) and four were alkali metals (Li, K, Rb, and Na; Table 2). Conversely, concentrations of Pb, Li, Mn, K, and Rb in Margaree River samples were lowest (5 of 11 elements) with the lowest concentrations overall measured in three of these five (Li, K, and Rb; Table 2).

Transformed mean concentrations for Ba, Pb, Li, Mn, Rb, and Tl (6 of 11 elements) differed significantly among watersheds (ANOVA, df = 2, 91, 94, $F > 4.133$, $P < 0.02$; Fig 3). Notable differences among river stocks were low Rb values for the Margaree River and high Tl values for the Northwest Miramichi River (Figs 3e and 3f). Mn was the only element that differed significantly among all three rivers (ANOVA, Bonferroni correction, df = 2, 94, $P < 0.029$; Fig 3d).

Concentrations of Pb were significantly different only between the Margaree River and Northwest Miramichi River (ANOVA, Bonferroni correction, df = 2, 94, $P = 0.04$; Fig 3b). Li and Rb differed significantly between both the Margaree River and Northwest Miramichi River (ANOVA, Bonferroni correction, df = 2, 91, 94, $P \leq 0.000$) and the Margaree River and Southwest Miramichi River (ANOVA Bonferroni correction, df = 2, 91, 94, $P \leq 0.000$; Figs 3c and 3e). The only elements to exhibit significantly different concentrations between the Northwest Miramichi River and the Southwest

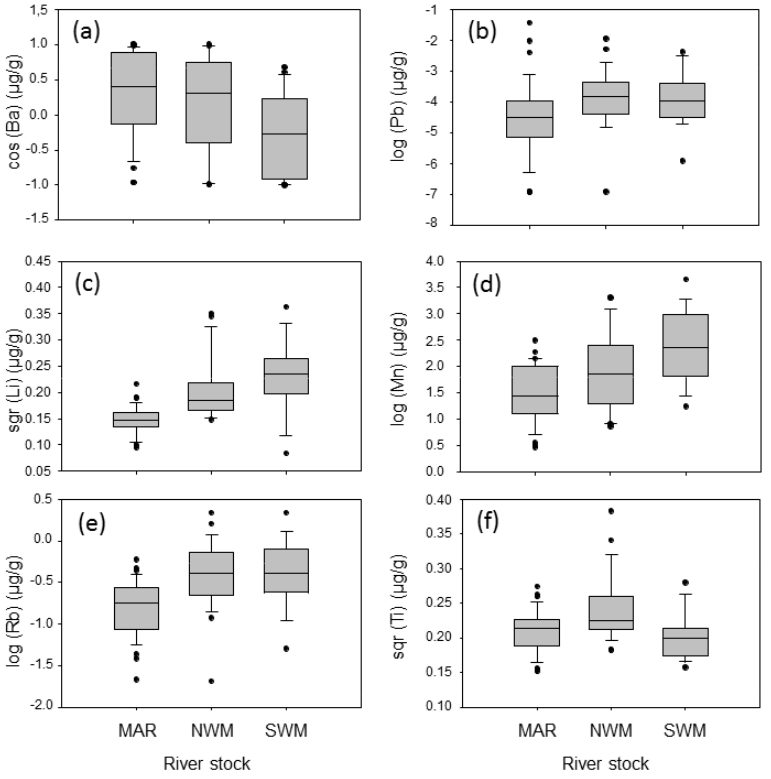

Fig 3 **Box plots of transformed (a) Ba concentrations (*µ*g/g), (b) Pb concentrations (*µ*g/g), (c) Li concentrations (*µ*g/g), (d) Mn concentrations (*µ*g/g), (e) Rb concentrations (*µ*g/g), and (f) Tl concentrations (*µ*g/g) in otoliths of Atlantic salmon smolts sampled from the Margaree River (MAR), Northwest Miramichi River (NWM), and Southwest Miramichi River (SWM).**

Miramichi River were Ba (ANOVA, Bonferroni correction, df = 2, 94, $P = 0.041$) and Tl (ANOVA, Bonferroni correction, df = 2, 94, $P \leq 0.000$; Figs 3a and 3f).

Omnibus river stock of origin differences were also observed when otolith elemental concentrations were analyzed as multivariate fingerprints (MANOVA, Pillai's trace, df = 12, 174, $F = 9.369$, $P \leq 0.000$). Concentrations of Pb differed significantly less among rivers when analyzed by MANOVA (Pillai's trace, df = 2, 91, $F = 3.813$, $P = 0.026$) than when analyzed by ANOVA (df = 2, 94, $F = 4.134$, $P = 0.019$).

Overall, MANOVA identified all six elements that differed significantly among rivers in univariate analyses as significant contributors

to by-river stock separation of elemental fingerprints in multivariate space, and this discrimination was even more distinct using a four-element (Li, Mn, Rb, and Tl) stepwise LDFA model (Wilk's lambda, $P \leq 0.000$). Ba and Pb were removed from the LDFA model because they were considered insignificant contributors to separation of the groups.

The first canonical variable (Factor 1) of the LDFA model captured the greatest amount of the difference among river management stocks (86%). Factor 1 best separated the Margaree River samples from the two Miramichi River groups. Canonical scores for Factor 1 were -1.332 (Margaree River), 0.195 (Northwest Miramichi River), and 1.416 (Southwest Miramichi River). Correlations between Factor 1 and individual elements were high for Mn (0.85) and Rb (0.84) and between the second canonical variable (Factor 2) and Tl (0.96). Based on high F-statistics, elements in the LDFA model in order of importance (first listed being of highest importance) were Mn (19.59), Rb (15.71), Tl (10.36), and Li (6.17; Table 3). Tolerance statistics for all four elements were greater than 0.64 (range 0.684 to 0.963; Table 3) indicating elements in the LDFA model were not highly correlated with other elements. Li had the highest degree of independence from other elements in its contribution to the LDFA model (Table 3).

Equality of group means examined using between groups *F*-ratio values revealed that measured group centroid distances were greatest between the Margaree River and the Southwest Miramichi and least between the Northwest Miramichi River and the Southwest Miramichi River. Distances between group means were also compared visually using a plot of the two canonical variables with 95% confidence ellipses centered on the centroid of each group (Fig 4). Canonical variable

Table 3 Summary of discriminant analysis (stepwise jackknife procedure) performed on elemental concentrations in otoliths of Atlantic salmon smolts sampled from three watersheds the Canadian Maritimes.

Element	*F*-statistic[a]	Tolerance[b]
Li	6.17	0.963
Mn	19.59	0.738
Rb	15.71	0.648
Tl	10.36	0.869

[a] *F*-statistic signifies importance of each element.
[b] Tolerance measures correlation of elements (0.00 = high correlation, 1.00 = low correlation).

scores for the Margaree River and the Southwest Miramichi River had less overlap relative to canonical scores for the Margaree River and the Northwest Miramichi River (Fig 4). Considerably more overlap existed for otolith elemental fingerprints between the two Miramichi River stocks. Spread of canonical scores for the Margaree River stock was low while distributions of canonical scores in discriminant space for both of the Miramichi River stocks were relatively wide in comparison (Fig 4).

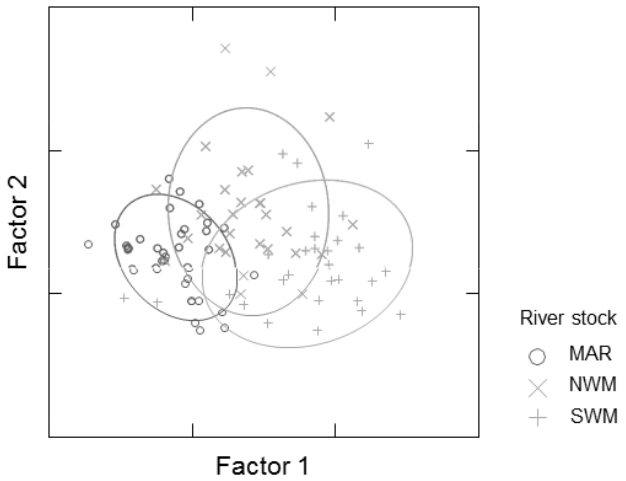

Fig 4 **Plot of canonical variable scores summarizing otolith elemental fingerprints of Atlantic salmon smolts sampled from the Margaree River (MAR), Northwest Miramichi River (NWM), and Southwest Miramichi River (SWM). Based on discriminant analysis of the four most significant predictor elements (Mn, Rb, Tl, and Li [ordered from most to least significant based on F-statistics]). Observations for each river illustrated with 95% confidence ellipses centered on group centroids.**

Overall classification success for the four-element stepwise LDFA model using a cross-validated jackknife classification technique was relatively high at 73%, with the highest accuracy for the Margaree River (92%) and lowest accuracy for the Northwest Miramichi River (46%; Table 4). The majority of fish misclassified from both the Northwest Miramichi River and the Southwest Miramichi River were classified as Margaree River fish whereas relatively few Margaree River fish were misclassified (3 of 36; Table 4).

Considerable overlapping distributions of scores in the canonical variable plot (Fig 4) and a relatively low *F*-ratio value between the

Table 4 **Classification matrix resulting from discriminant analysis (stepwise jackknife procedure) performed on elemental concentrations in otoliths of Atlantic salmon smolt sampled from three watersheds in the Canadian Maritimes. Results based on elements identified by discriminant analysis as significant predictor variables (Li, Mn, Rb, and Tl).**

	Predicted Watershed			
Actual Watershed	**MAR**	**NWM**	**SWM**	**% Correct**
Margaree	33	2	1	92
Northwest Miramichi	9	13	6	46
Southwest Miramichi	4	3	23	77
Total	46	18	30	73

Northwest Miramichi River and the Southwest Miramichi River suggested that otolith elemental fingerprints would be better discriminators at larger spatial scales where both Miramichi River stocks were components of a single group. To explore this hypothesis, samples were grouped according to major river system of origin and data were analyzed following the same elemental and statistical methods described for data grouped by management river stocks. An extremely high 92% mean cross-validated classification accuracy was achieved based on a significant (LDFA, Wilk's lambda, $P \leq 0.000$) four-element model that included Ba, Li, Mn, and Rb (Table 5).Classification accuracy was higher for the Miramichi River (93%) compared to the Margaree River (89%) with four fish from each river being misclassified (Table 5). Examination of *F*-statistics in LDFA revealed Ba was the most helpful variable in discriminating smolts from the two river systems followed by Rb, Li, and Mn (Table 6). Tolerance statistics indicated contributions of the four individual elements to group separation were highly independent (range 0. to 0.976; Table 6). These four elements along with Pb were also found to be significant contributors to separation among large river systems in multivariate space (MANOVA, Pillai's trace, df = 5, 90, $F = 35.943$, $P < 0.009$).

DISCUSSION

The variation in the elemental composition of whole otoliths of Atlantic salmon smolts was sufficient to discriminate among three watersheds in the Canadian Maritimes with 73% accuracy. Otolith elemental fingerprints when two watersheds were considered discriminated smolts with an accuracy of 92%. Mean classification accuracy

Table 5 **Classification matrix resulting from discriminant analysis (stepwise jackknife procedure) performed on elemental concentrations in otoliths of Atlantic salmon smolts sampled from two watersheds in the Canadian Maritimes. Results based on elements identified by discriminant analysis as significant predictor variables (Ba, Li, Mn, and Rb).**

Actual Watershed	Predicted Watershed		% Correct
	Margaree	Miramichi	
Margaree	33	4	89
Miramichi	4	55	93
Total	38	58	92

Table 6 **Summary of discriminant analysis (stepwise jackknife procedure) performed on elemental concentrations in otoliths of Atlantic salmon smolts sampled from two watersheds in the Canadian Maritimes.**

Element	*F*-statistic[a]	Tolerance[b]
Ba	41.05	0.976
Li	16.35	0.974
Mn	12.61	0.859
Rb	21.07	0.845

was lower when samples were grouped by river management stocks compared to when samples were grouped by large river systems partly due to the low classification rate (46%) of Northwest Miramichi River smolts. Some fish captured in the Northwest Miramichi could have been spawned in the Southwest Miramichi River and later dispersed into the Northwest branch during the latter portion of their freshwater residency. A more likely possibility is that smolts misclassified between the two Miramichi River branches by the LDFA is a reflection of the similar bedrock geology underlying the basins of both tributaries (Chiasson 1995). There were more cases, however, where fish collected from the Northwest Miramichi were incorrectly assigned to the Margaree River stock rather than the Southwest Miramichi stock. Quite probably there is substantial variability in the physicochemical character of the Northwest Miramichi watershed.

The most important variables for discriminating fish originating from the different watersheds were Ba, Rb, Li, and Mn, whereas the most significant predictors of river stock membership were Mn, Rb, Tl, and Li. In discriminating groups of fish in lotic and lentic freshwater systems, the best and most common explanatory variables have been Sr, Ba, Mn, and Mg (Thorrold *et al*. 1998; Wells *et al*. 2003a; Brazner *et al*. 2004a). Otolith concentrations of Sr and Mg were not

significantly different among watersheds, but both Mn and Ba contributed the greatest to group separation in the river management stock and large river system of origin LDFA models, respectively.

Observed differences in Ba concentrations probably reflect differences in Ba:Ca concentrations in the ambient waters of the three rivers at the time of deposition. Walther and Thorrold (2006) assessed the relative contributions of water and food sources to concentrations of Sr and Ba incorporated into the otoliths of a saltwater fish and found that almost all Ba (98%) deposited in the otolith aragonite was controlled by seawater chemistry. A correlation between water composition and uptake of Ba in the otoliths of freshwater fish has also been documented under both field and laboratory conditions (Wells *et al*. 2003a; Elsdon and Gillanders 2005), and variation in ambient Ba concentrations among and within freshwater environments was influenced primarily by the geology of the surrounding rock (Guay and Falkner 1998, Martin *et al*. 2013). Higher Ba:Ca concentrations in Margaree River otoliths, and presumably Margaree River water, than the Miramichi River, probably derive from the underlying geology of the Maritime Basin in eastern Canada (Kontak *et al*. 2006), particularly Ba-F mineral veins near the Lake Ainslie system within western Cape Breton Island (Kontak and Macdonald 1995).

Factors contributing to the observed variations in otolith Mn concentrations are not as clear. In his review, Campana (1999) presented evidence for an environmental effect on relative concentrations of Mn in freshwater and saltwater fish otoliths. Using new generation ICP-MS instruments, however, with the ability to quantify trace elements such as Mn with improved resolution, recent research has consistently revealed no correlation between water temperature, salinity, or ambient Mn abundance and otolith Mn:Ca ratios (Martin and Thorrold 2005; Hamer and Jenkins 2007), save for Dorval *et al*. (2005) who demonstrated a positive correlation between the Mn:Ca ratio in water and otolith.

Physiological processes are likely important to the incorporation of Mn:Ca ratios into otoliths based on obvious differences in otolith accretion rate (Hamer and Jenkins 2007) which is thought, in salmonids, to be coupled with factors related to metabolic rate (Wright 1991) including growth rate (Yamamoto *et al*. 1998), water temperature (Wright *et al*. 2001), oxygen concentration (Waller *et al*. 1997), and even life history strategy (Metcalfe *et al*. 1995). Mn is a highly regulated trace element (Bendell-Young and Harvey 1986)

essential for growth and metabolism in fishes (Lall 2002), which are two physiological parameters known to differ among stocks of Atlantic salmon with different geographic origins (Seppänen *et al*. 2009). Also, hydrological conditions varied among the watersheds across years when sampled smolts were rearing in natal freshwater habitats (DFO 1999, 2000, 2001b). Therefore, the observed significant differences in otolith Mn concentrations could be linked to physiology through the environmental availability of dissolved oxygen and ambient temperature.

Information on Rb, Li, and Tl is scarce. Concentrations of the alkali metal Li in fish otoliths and Rb in sea lamprey, *Petromyzon marinus* Linneaus, 1758, statoliths may reflect physical and chemical characteristics of the ambient environment (Campana *et al*. 2000; Brothers and Thresher 2004). Variations in otolith Li and Rb in relation to the proximity of specific rock types and mineral deposits (Friedrich and Halden 2008; Halden and Friedrich 2008) suggest the environment is the primary factor influencing the uptake of some of the alkali metals. Still, these relationships appear to be species-specific, indicating some physiological role in deposition (Chittaro *et al*. 2006). Li and Rb showed similarities across samples from different management stocks and freshwater systems suggesting local geology and ambient water chemistry were the principal factors influencing otolith concentrations of these elements.

Mean concentrations of Tl from the Northwest Miramichi River were elevated compared to mean concentrations for the Southwest Miramichi River and the Margaree River. It is possible that ambient concentrations of Tl in some areas of the Northwest Miramichi were high due to industrial sources during the time period of juvenile residency. Base metal mining operations have existed on a few tributaries of the Northwest Miramichi River since the 1960s and Tl normally occurs in discharged effluents from this industry (Zitko 1995). Arslan (2005) speculated that should variations in otolith Tl concentrations be detected it would be mainly due to differences in environmental availability of this element since the biological utility of Tl to the fish would be minimal and the affinity of the calcium carbonate otolith to Tl appears to be low. Stable isotopes of Pb and Se have also been detected in the otoliths of fish taken from aquatic environments significantly influenced by anthropogenic inputs (Spencer *et al*. 2000; Halden and Friedrich 2008).

Data on all environmental conditions for the time period of juvenile residence in natal rivers were not available to definitively support the premise that the ambient physicochemical environment primarily influenced otolith composition. A number of laboratory and field studies have documented a high correlation between otolith and water concentrations of some elements as influenced by temperature and salinity (Bath *et al*. 2000; Elsdon and Gillanders 2003b, 2004; Dorval *et al*. 2005) but it is unlikely that smolt size or age contributed significantly to differences in elemental fingerprints since there was no significant difference in smolt length, smolt age, or otolith mass of individuals collected among locations.

The successful application of otolith elemental fingerprints requires that three assumptions be met: (1) there are characteristic and reproducible markers for each group, (2) all possible groups contributing to the group mixture have been characterized, and (3) the marker remains stable over the interval between characterization and mixing (Campana 2005).Our elemental fingerprints appear to satisfy or display the potential to satisfy all three assumptions. One of the requirements of the first assumption has certainly already been met in that the fingerprints are characteristic markers of each of the rivers or area stocks examined. Also, they have already proven to be partly reproducible through the constancy of the smolt age and size ranges across stocks and river systems ensuring that elemental concentrations did not need to be detrended to remove any age or size effects (Campana *et al*. 2000).

Another aspect of reproducibility is the ability of the researcher to identify the correct growth increments to be sampled. Basing reference fingerprints on whole otoliths of smolts is advantageous since the smolt growth zone in otoliths of adult salmon is distinct (Kennedy *et al*. 2002) and can be used as a mark for where to initiate milling of the juvenile portion of the otolith for subsequent elemental analysis.

With regard to satisfying the second assumption, establishing sufficiently different otolith elemental fingerprints for all possible anadromous salmon river stocks contributing to themixed stocks in the North Atlantic Ocean is probably not practical considering there are more than 2100 individual rivers in North America and Europe with variable stock status (Crozier *et al*. 2004).Distinct elemental fingerprints for all possible groups at the meso-scale level of population organization (i.e., major river systems or biogeoclimatic zones) is a more realistic expectation.

The final assumption of marker stability between characterization and mixing is presumed met for elemental fingerprints based on analysis of the otolith core. Because of its metabolically inert nature, the composition of the otolith is not susceptible to resorption nor can it be reworked (Campana and Neilson 1985). Therefore, the otolith should maintain a permanent record of the physicochemical environment experienced by an individual during its lifetime (Campana 1999).

For Atlantic salmon, limiting elemental assays to the smolt portion of the otolith restricts the basis for the fingerprint to only elemental exposure and modification during an individual's freshwater life history. Concentrations of elements incorporated into the growing surface of the otolith from the time before hatch to the time of smoltification would then remain stable even after extended periods at sea and participation in multiple spawning events. The stability of the chemical composition of scales over the same interval is not as likely, especially after the juvenile period, maturation, and spawning (Wells *et al*. 2003b).

While stability in otolith core elemental composition is assumed, temporal persistence in otolith elemental fingerprints among multiple smolt year classes is not. Variation in elemental fingerprints between years was not assessed here, but some inter annual variability should be expected (Gillanders 2002).The magnitude of that variability would depend upon the chemical and physical dynamics of the aquatic environment (Elsdon and Gillanders 2006). Considering the range of sea-ages for Atlantic salmon, elemental concentrations would need to remain relatively stable over a 3-5 year period in order for reference fingerprints to be based on a single smolt year-class. While long-term (4-13 years) persistence is not commonly observed (Campana *et al*. 2000), combinations of some elements have maintained constancy in concentrations over shorter intervals of 2-5 years (Campana *et al*. 2000; Morris *et al*. 2003).

Another advantage of using otoliths is that they can be stored dry or frozen for long periods of time without loss of information. Because Atlantic salmon taken in many fisheries are processed immediately isolated recreational and commercial fisheries are difficult to sample for stock-specific characteristics unless scientific personnel are on hand (Tucker *et al*. 1999; Spares *et al*. 2007).Salmon heads are usually removed during processing and these can be frozen, hence preserving the otoliths for later study.

The results of our study demonstrate that otolith elemental fingerprints can be used to discriminate Atlantic salmon of different origins at an appropriate scale for studying population dynamics and managing stocks. Based on significant differences in the elemental composition of whole otoliths of smolts, mean classification accuracies were moderately high and extremely high for river management stocks and large river systems, respectively. Previous studies attempting to discriminate among stocks with the aid of natural marks have also achieved high classification rates, but not at the resolution needed to identify oceanic factors influencing differences in recruitment patterns which have been shown to act at regional scales (Friedland 1998; Friedland and Reddin 2000; Niemelä *et al*. 2004). More recent studies employing chemical marks have been able to discriminate among individual hatchery or stream populations (Flem *et al*. 2005; Veinott and Porter 2005; Martin *et al*. 2013), but may not meet the assumptions required to act as natural tags of origin for the purpose of ocean research and management. A metabolically stable natural tag of individual watersheds or large river system of origin would contribute considerably to research on marine factors that influence stick dynamics of Atlantic salmon. Such natural tags would also be useful to delineate and protect marine habitats critical for recruitment, estimating proportions of stocks subject to separate management regimes in mixed stock fisheries, and identifying salmon belonging to stocks which are not legally allowed to be exploited.

Conversely, the chemical and statistical analyses of smolt otoliths described in our study do show potential as a natural tag of large river system or biogeoclimatic zone of origin. Moreover, quantifying naturally occurring stable isotope ratios in otoliths (Kennedy *et al*. 2005) and analyzing the resultant isotopic signatures in concert with trace elemental fingerprints may increase classification accuracies further and satisfy the assumptions of mixed-stock analyses with even greater ease (Thorrold *et al*. 2001; Walther *et al*. 2008). First, however, future studies attempting to delineate the stock structure of Atlantic salmon in the marine environment should focus on further validating the use of smolt otolith elemental fingerprints as natural tags of large river system or biogeoclimatic area of origin. This may require cataloguing a library of otolith elemental fingerprints over time for each defined population unit (Gillanders 2002). Once accomplished, researchers should be able to characterize spatial and temporal meso-scale stock structure of ocean migrating salmon by analyzing otolith cores.

Depending on where research or fishing efforts are directed, the relative proportions of various stock groups utilizing known marine nurseries, staging areas, and feeding grounds (Reddin 1985; Reddin and Short 1991; Jacobsen *et al*. 2001) could be more accurately determined at a finer level of population organization. Considering recent research, which indicates trans-Atlantic migrations for North American salmon stocks (Spares *et al*. 2007; Dadswell *et al*. 2010; Soto *et al*. in review) and the endangered status of many river populations in adjacent regions, the development of empirical methods to accurately discriminate stocks in the North Atlantic Ocean at a scale appropriate for effective research and management is extremely important to the conservation and restoration of this species.

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