

Using otolith microchemistry to reconstruct habitat use of American eels *Anguilla rostrata* in the St. Lawrence River–Lake Ontario system

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Accepted for publication July 8, 2015

Abstract – Catadromy among freshwater eels is increasingly recognised as being facultative, with some individuals carrying out growth exclusively in brackish or coastal marine waters, or switching between brackish or marine waters and freshwater habitats. In an attempt to reconstruct habitat use of yellow-stage American eels in a large river-lake ecosystem, trace element line scans were obtained, using LA-ICP-MS, from the otoliths of 110 eels sampled at various locations throughout the St. Lawrence River–Lake Ontario (SLRLO) system. Elemental profiles for strontium (Sr), barium (Ba), manganese (Mn) and magnesium (Mg) enabled us to distinguish three chemical signatures that appear to represent three distinct habitats within the SLRLO. Of these, one was shown to likely correspond to the brackish estuary (high strontium values). The other two signatures, characterised by low strontium but variable concentrations of barium and manganese, may correspond to habitats within the main-stem St. Lawrence River and one or more of its tributaries. Most (78%) of the switches among habitats occurred within the first four years after recruitment suggesting an increasing likelihood for eels to maintain residence in one habitat as they grow older. This suggests that tributaries may provide important habitats for American eels during the first several years after recruiting to the SLRLO. In addition, our results suggest that a small proportion American eels in the SLRLO can undertake movements on the order of at least 200 km during the early growth stage. This information has important implications for the management and conservation of this species in the system.

Key words: anguilla; otolith; microchemistry; St. Lawrence; movement

Introduction

The American eel, *Anguilla rostrata*, is a wide-ranging facultatively catadromous fish that inhabits freshwater, brackish water and coastal marine habitats on the western margin of the North Atlantic Ocean (Scott & Crossman 1974). Panmictic spawning (Côté et al. 2013) occurs offshore within the subtropical gyre of the North Atlantic, in the south-western Sargasso Sea, approximately 500–1000 km south-west of Bermuda (Schmidt 1923; McCleave et al. 1987; Kleckner & McCleave 1988). Like other members of the Anguillidae family (Tsukamoto & Aoyama 1998), oceanic currents transport leptocephalus larvae

to continental shelves throughout the species' range, where they metamorphose into glass eels and begin actively migrating towards coastal waters. Upon reaching the coast, glass eels continue to migrate upstream in watersheds while slowly becoming pigmented (Tesch 2003). The young sexually undifferentiated pigmented eels, also termed elvers, represent the earliest stage of the eel's life in continental waters. The traditionally held view was that this extended growth phase – the yellow eel stage – was carried out exclusively in freshwater before eventually embarking on the return-leg migration to their oceanic spawning site. However, a number of recent studies on several freshwater anguillid eel species

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have collectively reshaped our understanding by demonstrating that this typical catadromous lifecycle is not obligatory (Tsukamoto & Arai 2001; Tzeng et al. 2002; Limburg et al. 2003; Daverat & Tomas 2006). In fact, certain yellow eels never enter freshwater, instead spending their entire growth stage in brackish or marine waters although it is not known how common these behaviours are for the American eel. Moreover, some individuals switch between freshwater and brackish or marine habitats on one or multiple occasions during the yellow phase (Daverat et al. 2006). This same pattern of behaviour was reported for American eels in eastern Quebec (Thibault et al. 2007), Nova Scotia (Jessop et al. 2002) and in north-eastern United States (Morrison & Secor 2004). Such observations illustrate the extent of plasticity that characterises habitat use among anguillid eels. Daverat et al. (2006) noted that eels residing in the upper reaches of rivers were less likely to use estuarine or marine habitats. Furthermore, eels using brackish or marine habitats appear to show higher rates of growth than those residing exclusively in freshwater (Daverat et al. 2006). This is consistent with the current theory suggesting that such behavioural plasticity has evolved to allow eels to exploit brackish and marine habitats that tend to be more productive than freshwater habitats at higher latitudes (Edeline 2007).

The Great Lakes–St. Lawrence River basin is one of the major hydrological systems in North America and drains vast areas of eastern North America, including the Great Lakes. Unlike most large rivers of the world, the geochemical properties of the St. Lawrence River (SLR) more closely resemble those of typical lacustrine systems with relatively low levels of dissolved minerals (Yang et al. 1996). In addition, several natural enlargements, often termed fluvial lakes, are located along its course (Lac Saint-François, Lac Saint-Louis and Lac Saint-Pierre) (Yang et al. 1996; Vincent & Dodson 1999). The St. Lawrence River, together with Lake Ontario (SLRLO), supports important production areas and growth habitats for American eels and accounts for a large proportion (19%) of the runoff across the American eel's entire range (Castonguay et al. 1994). The upper reaches of the SLRLO lie a considerable distance from the spawning grounds in the Sargasso Sea (>3500 km), and eels recruiting there must travel much further than conspecifics recruiting to the Gulf of St. Lawrence tributaries or more southerly locations throughout the species' range. Virtually all (99%) eels that recruit naturally to the SLRLO are females (Dutil et al. 1985; Couillard et al. 1997) and are considerably larger and longer lived (about 20 years) than individuals elsewhere (Jessop 2010). Despite the unique characteristics of the species in

the SLRLO, the existence of alternative migratory strategies has never previously been investigated, and limited information is available describing such behavioural traits during the yellow phase within this system.

The challenge for studies attempting to characterise the migratory and movement patterns of long-lived fishes lies in the difficulty in obtaining retrospective information covering the entire life of the individuals. Compounding potential variation due to age is the large geographical area of the SLRLO system that includes a multitude of tributaries and lakes, a fluvial estuary ultimately draining into a brackish estuary. Otoliths, small aragonite accretions of the inner ear of teleosts, have been used by investigators to retrospectively determine information relating to fish ecology (Kalish 1989; Campana 1999; Kraus & Secor 2004). Recent advances in the capabilities of modern probes have allowed an increased number of researchers to use chemical information from otoliths to address questions of increasing complexity about habitat use and ecology, such as population structure or migration patterns (Elsdon et al. 2008; Kerr & Campana 2014). Most studies have involved analysing Sr/Ca profiles from otoliths in an attempt to retrace movements of diadromous fishes across habitats exhibiting coarse differences in salinity (i.e. rivers, estuaries and coastal areas) (Secor et al. 1995; Daverat et al. 2006). Several different probes can be used to extract this type of chemical data from otoliths (Campana 1999). Among these, the use of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been a cost-effective and time-efficient means to obtain the concentration profiles of multiple minor and trace elements running from the core to the edge of the otoliths covering the entire life of a fish (Sanborn & Telmer 2003). The general objective of this study was therefore to obtain multi-element otolith line scans from eels collected at multiple sites in the SLRLO system, ultimately attempting to link chemical variation therein to residence in distinct habitats. More specifically, we aimed to determine whether certain elemental profiles in otoliths could be used to quantitatively establish discrete signatures and then relate these signatures to specific habitats used by yellow-stage eels in the SLRLO system.

Materials and methods

Eel sampling

A total of 110 eels were sampled at six different locations in the SLRLO system between September 2010 and October 2011 (Fig. 1). Eels sampled from Lac Saint-Louis and Lake Champlain were captured at

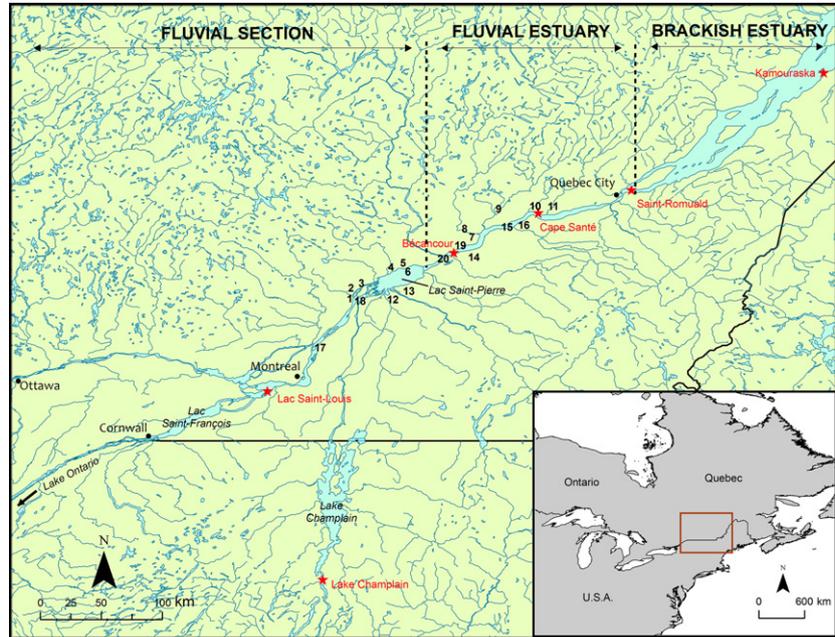


Fig. 1. The Saint Lawrence River Lake Ontario system including Lake Champlain. Dashed lines mark the limits between the fluvial river, fluvial estuary and brackish estuary. Red star (★) represents eel capture location. Numbers 1–20 represent Quebec MRN water sampling locations (see Table 3 for mean concentration values of Ba and Mn for each station).

night using an electro-fishing vessel in July 2011. Eels sampled from Lac Saint-Pierre, Cap Santé and Saint-Romuald were obtained from commercial fishermen using fyke nets between October 2010 and August 2011. Although most of the individuals sampled were yellow-stage eels, the majority of those captured at Saint-Romuald were likely silver eels, given that they were captured late in the season (October 2010). Furthermore six silver eels from the brackish estuary were obtained from the Quebec Ministry of Wildlife and Natural Resources (MRNF) during their downstream migration in October 2011. These individuals had been previously marked with PIT (passive integrated transponder) tags upstream from the Moses-Saunders dam in the vicinity of Cornwall, Ontario (approximately 400 km upstream from the brackish estuary), and we assumed to have carried out most of their growth in Lake Ontario. All eels were stored frozen and allowed to thaw before extracting otoliths and recording total length and body mass.

Preparation of otoliths

Sagittal otoliths were extracted using acid-washed plastic or Teflon-coated forceps (Fine Science Tools®, North Vancouver, BC, Canada). Once extracted, otoliths were rinsed in three successive baths of de-ionised water and any remaining tissue was mechanically removed using acid-washed forceps. Cleaned otoliths were then allowed to dry for 24 h and subsequently stored in acid-washed plastic Eppendorf tubes. Each otolith was individually embedded in an epoxy resin and hardener mixture

(©Freeman Manufacturing and Supply Company) and sectioned in the transverse plane just above the core using a low-speed Isomet saw and a diamond wafering blade from ©Buehler, Whitby, ON, Canada. The core of the sectioned otolith was subsequently exposed by progressively sanding with 1200 down to 400-grit sanding paper. This involved frequent verification under a binocular microscope to ensure otoliths were not sanded past the core. Finally, the surface of the otolith surface was smoothed and polished using 3 μm lapping film to better visualise the core and growth rings.

Multi-elemental line scans of otoliths and ageing

Prepared embedded otoliths were mounted onto a petrographic slide (15–20 per slide), sonicated in ultrapure water for 5 min and dried under a laminar flow fume hood for 24 h. Each slide was then placed inside the ablation cell of a Resolution M-50 Excimer ArF laser (© Resonetics, Nashua, NH, USA). The laser pulsing was set to 15 Hz at 4 $\text{mJ}\cdot\text{pulse}^{-1}$, a beam size of 75 μm and a displacement speed of 15 $\mu\text{m}\cdot\text{s}^{-1}$. The ablated material was transported to a 7700 \times model ICP-MS (© Agilent, Santa Clara, CA, USA) using helium as a carrier gas and then ionised using high temperature argon plasma. The laser trajectory was set, individually for each otolith, to scan from the ventral to dorsal margins (or vice versa) passing through the core. Raw data were outputted as an intensity signal measured in $\text{counts}\cdot\text{s}^{-1}$ for ${}^7\text{Li}$, ${}^{11}\text{B}$, ${}^{24}\text{Mg}$, ${}^{27}\text{Al}$, ${}^{29}\text{Si}$, ${}^{44}\text{Ca}$, ${}^{47}\text{Ti}$, ${}^{55}\text{Mn}$, ${}^{56}\text{Fe}$, ${}^{57}\text{Fe}$, ${}^{59}\text{Co}$, ${}^{61}\text{Ni}$, ${}^{63}\text{Cu}$, ${}^{64}\text{Zn}$, ${}^{69}\text{Ga}$, ${}^{75}\text{As}$, ${}^{88}\text{Sr}$, ${}^{95}\text{Mo}$, ${}^{107}\text{Ag}$, ${}^{110}\text{Cd}$, ${}^{111}\text{Cd}$, ${}^{133}\text{Cs}$, ${}^{138}\text{Ba}$, ${}^{140}\text{Ce}$, ${}^{202}\text{Hg}$,

^{208}Pb and ^{238}U . Before each otolith, the blank gas was run for 30 s to determine background counts. Before and after scanning a series of 10 transects, we ran a 30 s laser scan across the standard material (NIST 610) to correct for temporal drift in the sensitivity of the mass spectrometer. Following laser analyses, otoliths were prepared for ageing analyses. For this, each otolith was polished with an aluminium oxide powder, etched with a 5% EDTA solution and stained using a 0.01% toluidine blue solution following the procedure used by Verreault (2002) to enhance annuli. Digital images of stained otoliths were captured using a binocular microscope. These images (example shown in Fig. 1) were then used to age each otolith by counting the annuli after the elver check to the edge.

Otolith data analysis

Raw data, output as intensity in $\text{counts}\cdot\text{s}^{-1}$, were converted into instantaneous mass fractions expressed in $\mu\text{g}\cdot\text{g}^{-1}$ (parts per million – ppm) for each element using NIST 610 (matrix-matched standard reference material) as a calibration standard and ^{44}Ca as an internal normalisation standard for quantification of elemental mass fractions assuming a calcium concentration of 40% by weight in the otolith. Traditionally, otolith microchemistry studies have expressed elemental profiles as a ratio of calcium as ions are generally incorporated as function of ambient calcium (Campana et al. 1995). In this study, mass fractions were used instead as element/Ca ratio data were not available for water samples and the interpretations would be similar. Given that the aim of this study was to characterise the movements of individuals during the continental portion of their lifecycle, elemental transects were truncated so as to exclude the high strontium section recorded inside the elver check that correspond to the leptocephalus and glass eel stages prior to recruitment to continental waters. Strontium values are several times greater before the elver check and drop abruptly at the onset of the elver stage, even for eels that have yet to enter freshwater (Lin et al. 2009). Tzeng (1996) and Otake et al. (1994) demonstrated that this drastic drop is, at least in part, related to ontogeny rather than exposure to lower ambient Sr concentrations. A drop in Sr concentrations after a shift from brackish water to freshwater is generally accompanied by an increase in Ba. All elemental profiles were chosen to begin at the position along each transect after which this pronounced reduction in strontium values and increase in Ba occurred. Outlier data points occurring along the transect profiles for the elements analysed were defined as any value greater than three times the interquartile of the mean for the preceding seven, and

subsequent 7 data points. Such values were likely to be an artefact of dust or other particulate impurities interacting with the laser and were replaced with the median value for those 14 points. We did not retain any elements whose transect profiles contained values below the limits of quantification (LOQ). The latter corresponds to five times the mean blank concentration for the element. Moreover, elements for which transect profiles showed little to no trends were not retained.

The elemental profiles for Sr, Ba, Mn and Mg, as opposed to the other elements not examined, each exhibited profiles with variation in the trends of elemental concentrations. Therefore, a quantitative method was used to determine where transitions occurred in the profiles of these four elements for each otolith transect. Hedger et al. (2008) highlighted the importance of using a quantitative method to establish where changes occur along the sequence of elemental profiles in otoliths. They compared the application of three algorithms on Sr:Ca profiles of American eels in an attempt to classify movements between fresh, brackish and sea water. The local zoning algorithm, developed by Webster (1973), was chosen for the analyses in this study given its ability to account for multiple variables simultaneously. The algorithm consisted of running a split-moving window across each of the four elemental profiles (Sr, Ba, Mn and Mg) for each otolith to generate a series of positions along its transect where a break in the chemical profile occurred. An R code written by Rossiter (2009) was used to run the algorithm along the elemental profiles of each otolith. The window width was individually set for each transect to correspond to 2/3 of the position at which the autocorrelation function fell to zero. Running the split window across each transect for the four elements simultaneously in this manner enabled us to divide each of these into a series of sections for which chemical signatures were statistically uncorrelated. The mean values for the selected elements within each section were subsequently calculated. To further reduce the complexity and variation in the data, we performed a hierarchical cluster analysis using Ward's minimum variance method (Ward 1963) on the total number of mean concentration values within each section for each retained element for all otoliths. Three criteria were used to determine the number of clusters resolved from the hierarchical cluster dendrogram: the Pseudo-F criterion (Calinski & Harabasz 1974), Cubic Clustering criterion (Sarle 1983) and the Pseudo- t^2 criterion (Duda & Hart 1973). A principal component analysis (PCA) was performed on the mean concentrations for each section, and these were plotted in multivariate space. We performed a one-way ANOVA followed by pair-wise t -tests on the data for each ele-

ment setting the assigned group as a fixed factor. This allowed us to determine whether the groups differed with respect to each of the mean concentration of each of the four elements. Prior to each ANOVA, data were tested performed Kolmogorov–Smirnov tests for normality (in each case $P > 0.05$) and Bartlett's test of equal variance. When the variances were not equal, the data were inverse, square root, cubic root or log-transformed depending on which transformation achieved equal variance. We then calculated the numbers of times within one otolith transect that a switch between these three groups occurred and at what age this switch occurred.

Quantification of water elemental concentrations

To obtain a snapshot of a whole river profile, water samples were collected by staff of the Great Lakes Laboratory of Fisheries and Aquatic Sciences (GLLFAS) at various sites between the head of the St. Lawrence River and the Gulf of St. Lawrence during the summer of 2009 well after the spring freshet. The concentrations of various elements were analysed following the methodology used by Dove et al. (2012). An upstream–downstream profile for Sr, Ba, Mn and Mg concentrations was generated from the GLLFAS sampling to obtain an overall snapshot of longitudinal variation for those elements along the St. Lawrence. No quantitative analyses were performed on this data given that only one data point was available for each sample site. To provide more in-depth data on seasonal and spatial variation for Ba and Mn, data from water samples collected by the Quebec Ministry of Natural Resources (MRN) at various sites in the St. Lawrence River and a number of its tributaries (see Fig. 1) were used (note: dissolved Sr and Mg data was not available). This information was used to complement GLLFAS data and serve as potential means of linking the chemical patterns observed in the otoliths to specific environments or geographic locations. Water samples were collected almost monthly between May 2008 and October 2012 following the methodology of (CEAEQ 2014). Each sampling site was assigned to one of three categories: (i) main-stem St. Lawrence River, (ii) north-shore tributaries and (iii) south-shore tributaries. Data were verified to ensure normality and an ANOVA was then performed to compare mean dissolved concentrations for both Ba and Mn between the three categories using month as the repeated measure. Subsequently, pair-wise t -tests allowed us to determine which of the three categories differed with respect to the concentration of each of the two elements. To understand finer-scale variation in the mean concentrations of Ba and manganese, ANOVAs were used to compare sites within each of the three

categories for the same two elements. Once again, pair-wise t -tests allowed us to determine which sites differed with respect to mean dissolved concentrations of Ba and Mn.

Results

Characterisation of chemical signatures in otoliths

Elemental profiles for Li, B, Ti, Ga, Mo, Ag, Cs, Ce, Hg and U comprised concentration values that fell below the limits of detection and/or limits of quantification. Moreover, otolith line scan profiles for Al, Si, Fe, Fe, Co, Ni, Cu, Zn, As, Cd, Cd and Pb showed no obvious trend. Therefore, the profiles for the above-mentioned elements were not further considered. In contrast, the profiles for Sr, Ba, Mn and Mg were all above the limits of detection and limits of quantification and exhibited varying patterns across the otolith transects. Consequently, the profiles for these four trace elements were retained for subsequent analyses.

Using the split-moving window algorithm, a total of 633 sections were obtained for all otoliths. Otolith transects encompassed a minimum of two sections and a maximum of ten (a mean of five sections per otolith). Each of the three aforementioned criteria (Pseudo- f , Cubic Clustering and Pseudo- t^2) resolved three distinct groups from the cluster analysis performed on the means of each element for all sections (Fig. 2a). Using a principal component analysis, each section's assigned group (1, 2 or 3) was plotted in multivariate space (Fig. 2b). The first principal component showed a strong negative correlation with Ba, Mn and Mg (PC1 loadings: -0.496 , -0.615 , -0.613 respectively) while the second component was strongly correlated with Sr (PC2 loading; 0.985). Together, the first two principal components (PC1 and PC2) explained approximately 70% of the total variation. For each of the four elements, significant differences among the three signatures were detected. More specifically, we detected significant differences for Sr among the three groups (Table 1 $F = 270.95$, $df = 2$, $P < 0.001$). The first group referred to as signature 1 had a mean Sr concentration approximately three times greater than that of signatures 2 and 3 (pair-wise t -test, $P < 0.001$) (Table 1). Signature 2 had a slightly greater mean Sr concentration than signature 3 (pair-wise t -test, $P = 0.0012$).

Similar to Sr, concentrations of Ba differed among all three signatures ($F = 188.37$, $df = 2$, $P < 0.001$) with signature 1 having the lowest barium concentrations, signature 3 an intermediate concentration and signature 2 the highest Ba concentrations (pair-wise t -tests, $P < 0.001$). In addition, significant differences in Mn concentrations among the three signatures were also detected ($F = 203.51$, $df = 2$, $P < 0.001$);

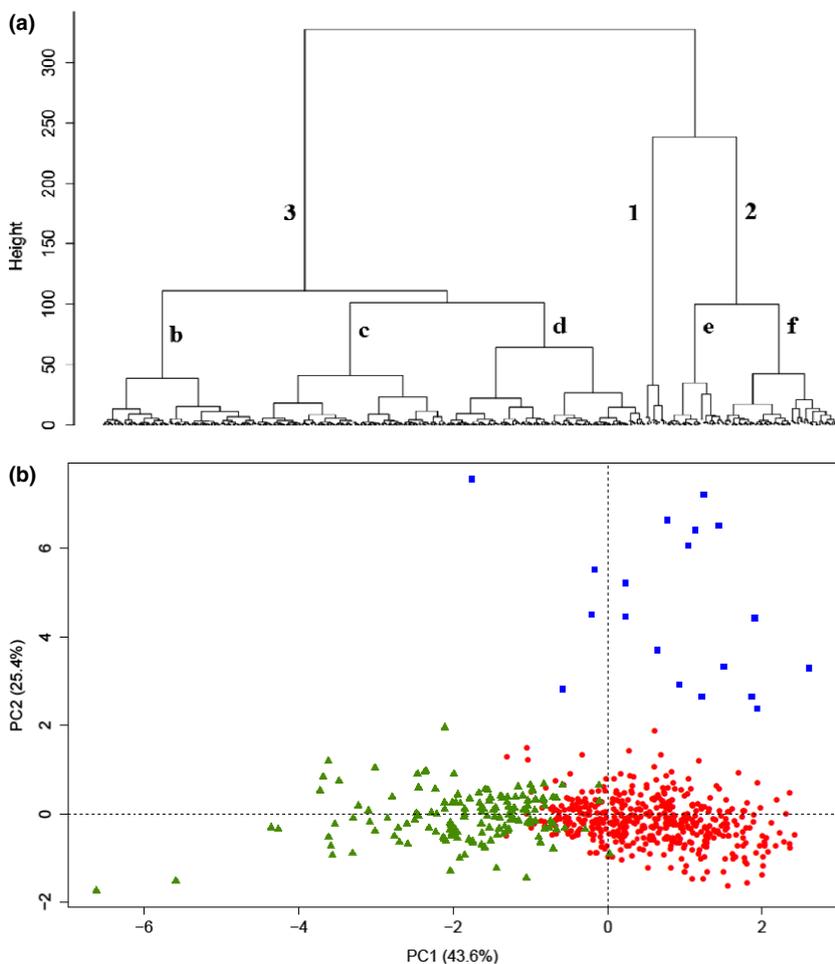


Fig. 2. Cluster Analysis performed on concentrations of four elements within each section: (a) Dendrogram illustrating the three main clusters and five sub-clusters. (b) PC scores for each of the sections plotted in multivariate space (■ cluster 1; ▲ cluster 2; ● cluster 3). Ba, Mn and Mg are all strongly negatively correlated with PC1 while Sr shows a strong positive correlation with PC2. PC1 and PC2 combine to explain approximately 69% of the variation.

Table 1. Mean mass fractions in $\mu\text{g}\cdot\text{g}^{-1}$ and standard deviations for Sr, Ba, Mn and Mg for each of the three signatures formed in the first cluster analysis.

Signature	Mean [Sr] (\pm SD)	Mean [Ba] (\pm SD)	Mean [Mn] (\pm SD)	Mean [Mg] (\pm SD)
1	1876 (\pm 423.6)	1.65 (\pm 0.93)	6.25 (\pm 1.60)	28.1 (\pm 7.36)
2	661.8 (\pm 126.1)	5.59 (\pm 0.19)	10.30 (\pm 1.25)	35.5 (\pm 5.06)
3	625.2 (\pm 119.9)	2.84 (\pm 0.21)	6.05 (\pm 3.90)	28.3 (\pm 5.57)

signature 2 had a mean Mn concentration almost two times greater than signatures 1 and 3 (pair-wise *t*-tests, $P < 0.001$) that did not differ from each other. Significant differences between all three signatures for Mg concentrations were detected ($F = 99.34$, $df = 2$, $P < 0.001$). The Mg concentration was significantly different between signature 1 and 3 ($P < 0.001$). Signature 2 had a higher mean Mg value than signatures 1 and 3 (pair-wise *t*-tests, $P < 0.001$) although no differences were found between the latter two groups. From here onwards, all three signatures will be referred to as habitats 1, 2 and 3.

To investigate finer patterns of variation in the otolith data, we also examined subclusters formed immediately under the three main signatures in the cluster hierarchy. In total, five subclusters were considered. Habitat 3 was comprised of three subclusters (*b*, *c*

and *d*) while habitat 2 included subclusters *e* and *f* (see Fig. 2a). Mean concentrations for Ba, Mn and Mg were compared between subclusters *e* and *f* of habitat 2. This was repeated for subclusters *b*, *c* and *d* of habitat 3. Pair-wise *t*-tests were used *post hoc* when required. Subcluster *e* showed significantly higher mean Mn and significantly lower mean Ba than sub-cluster *f* ($P < 0.001$). No difference was detected for Mg between both subclusters. Significant differences for mean Mn, Ba and Mg were detected between subclusters *b*, *c* and *d* (Table 2).

Characterisation of chemical signatures in the environment

General longitudinal trends in salinity and elemental concentrations for Sr, Ba, Mn and Mg are presented in

Table 2. Mean mass fractions in $\mu\text{g}\cdot\text{g}^{-1}$ for Ba, Mn and Mg for each subcluster within the two freshwater signatures.

Original signature	Subcluster	Mean [Mn] (\pm SD)	Mean [Ba] (\pm SD)	Mean [Mg] (\pm SD)
Signature 2	e	13.9 (\pm 4.49)	4.02 (\pm 1.11)	35.4 (\pm 4.57)
	f	8.51 (\pm 1.81)	6.37 (\pm 2.20)	35.6 (\pm 6.03)
Signature 3	b	5.64 (\pm 0.90)	3.53 (\pm 1.37)	27.7 (\pm 4.13)
	c	7.42 (\pm 0.97)	2.77 (\pm 0.87)	32.9 (\pm 3.75)
	d	5.37 (\pm 0.83)	2.18 (\pm 0.74)	25.1 (\pm 4.02)

Fig. 3. Sr concentrations were low throughout the fluvial section of the river as were salinity values. Strontium values increased dramatically after Saint-

Jean-Port-Joli (see linear distance from Lake Ontario in Fig. 3), the first site within the brackish estuary with a recorded salinity of 3.3 psu. The opposite pattern was observed for Ba with higher values in the fluvial section although there is some spatial variation in the Ba concentration within this section. Mn concentrations were higher in the fluvial section but exhibit some variation, particularly downstream from Montreal, where values peaked within the fluvial estuary before dropping in the brackish estuary. Finally, Mg concentrations increased by two orders of magnitude in the brackish estuary over that of the fluvial section.

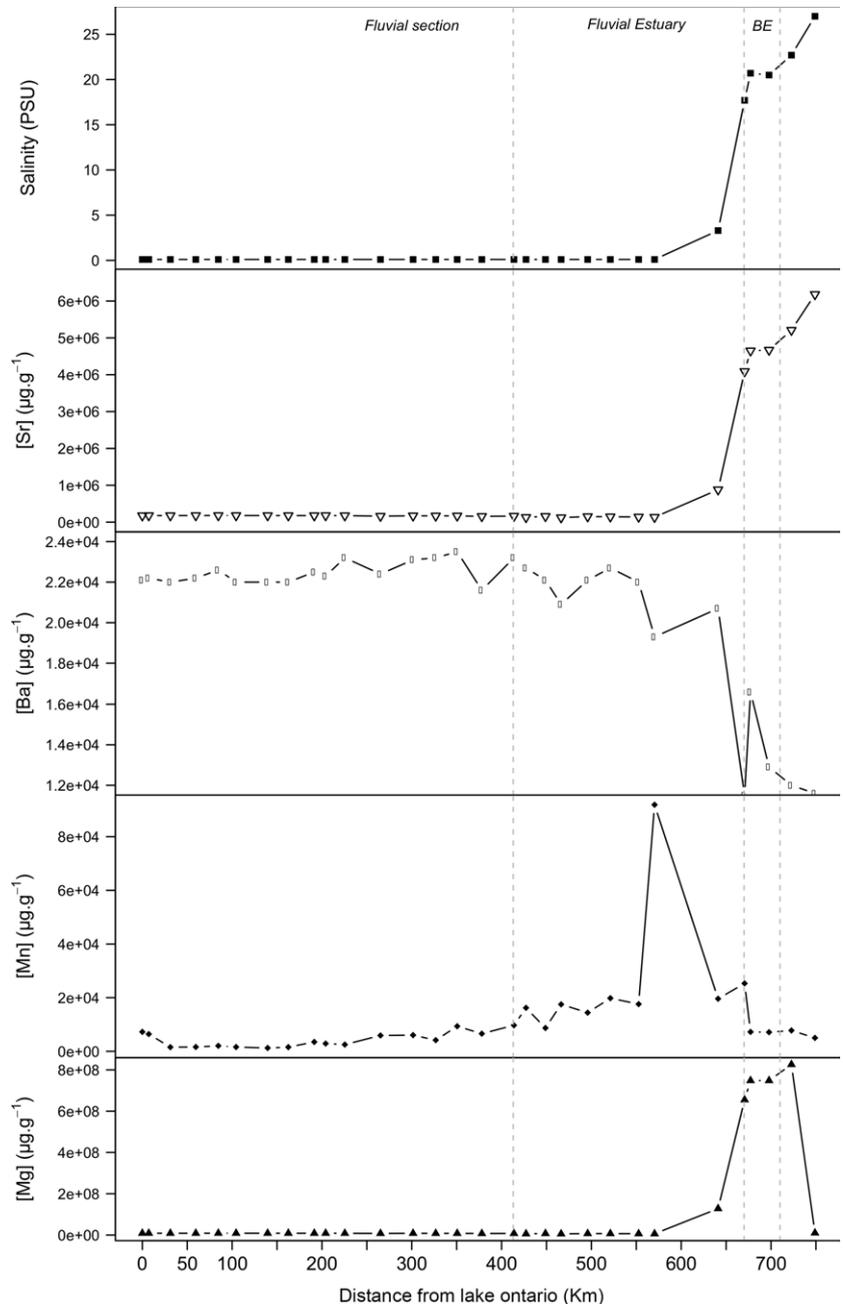


Fig. 3. Longitudinal snapshot profile for salinity, Sr, Ba, Mn and Mg in the St. Lawrence River from the Great Lakes Laboratory of Fisheries and Aquatic Sciences data. Dashed lines show approximate delineation of fluvial, fluvial estuarine and brackish estuarine sections.

Finer-scale patterns in elemental variation are presented in Table 3. Overall, concentrations of Ba and Mn were clearly variable between tributaries (north- and south-shore) and main-stem SLR sites. With respect to Mn, both north- and south-shore tributaries showed significantly greater ($P < 0.001$) mean concentrations than those observed in the main stem of the SLR (26.7 and 25.9 vs. $1.82 \mu\text{g}\cdot\text{l}^{-1}$) (Table 3). On the other hand, mean Ba concentration was found to be significantly greater for north-shore tributaries than the main-stem SLR ($P < 0.05$) while no difference was detected between the latter and south-shore tributaries. However, when looking at the mean Ba concentrations for specific north-shore tributaries, many of the values were in fact lower than the values in main-stem SLR sites (Table 3). The extremely elevated ($281 \mu\text{g l}^{-1}$) mean concentration for one of the tributaries (La Petite Rivière Yamachiche, station 5 in Table 3) explains why, as a whole, north-shore tributaries showed significantly higher mean Ba concentrations. No differences were detected for mean dissolved Ba or Mn between north-shore and south-shore tributaries (Table 3). We detected no significant differences in mean Ba and Mn concentrations between sampling sites of the main-stem SLR besides Tracy North that had significantly higher mean Mn concentration ($P < 0.001$). For north-shore tributaries, La Petite Rivière Yamachiche had a significantly higher mean Ba concentration than all of the other tributaries while no differences were detected for any of the tributaries with respect to Mn (Table 4). Finally, with the exception of Yamaska and Gentilly rivers (stations 16 and 14 respectively in Table 3), significant differences in mean Ba were found between all of the south-shore tributaries. Yamaska and Saint-François (station 15 in Table 4) rivers had significantly lower mean Mn than the other tributaries.

Patterns of habitat use observed in otoliths

Of the 110 eels sampled, only 11 (10%) used habitat 1, the brackish estuary, and of these, all were sampled at the three most downstream sites (five eels from Bécancour, three from Cap Santé and three from Saint-Romuald) (Table 5). However, when taking into account only individuals sampled at these three downstream sites, this represents 18.6% of individuals. The vast majority (90%) of eels enter freshwater in the first year after the transition from the glass eel phase to the elver phase and remain in freshwater (Table 5). Three alternative patterns were observed that differed from this typically catadromous behaviour (Fig. 4). Seven individuals spent an extended period of time (1–5 years) in the brackish estuary before entering freshwater (habitats 2 or 3). Three individuals entered freshwater and subsequently made a brief incursion

Table 3. Mean dissolved Ba and Mn concentration data from water samples collected at sites along the main-stem Saint Lawrence River, north shore tributaries and south shore tributaries. Distribution of sampling sites for each category is presented in Fig. 5.

Region	Station #	Mean Mn ($\mu\text{g}\cdot\text{L}^{-1}$) ($\pm\text{SD}$)	Mean Ba ($\mu\text{g}\cdot\text{L}^{-1}$) ($\pm\text{SD}$)
North Shore	1	53.2 (± 24.9)	31.0 (± 4.94)
	2	27.2 (± 13.0)	11.6 (± 2.73)
	3	33.7 (± 22.4)	9.45 (± 2.29)
	4	10.3 (± 2.28)	7.88 (± 0.93)
	5	37.5 (± 24.0)	281 (± 307)
	6	19.0 (± 3.10)	10.8 (± 3.10)
	7	39.5 (± 12.8)	11.7 (± 2.88)
	8	12.8 (± 3.03)	10.0 (± 1.23)
	9	20.5 (± 3.86)	7.88 (± 2.02)
	10	15.8 (± 2.48)	15.0 (± 4.10)
	11	11.5 (± 3.07)	25.3 (± 4.13)
South Shore	12	17.7 (± 13.0)	24.2 (± 5.10)
	13	13.8 (± 7.81)	10.5 (± 3.39)
	14	62.7 (± 11.4)	26.2 (± 15.3)
	15	61.0 (± 46.5)	41.3 (± 12.0)
	16	32.0 (± 49.2)	77.7 (± 9.56)
	SLR Main-stem	18 South	1.39 (± 0.96)
18 Middle		2.22 (± 1.09)	19.47 (± 1.55)
18 North		3.73 (± 1.90)	17.00 (± 2.42)
20 South		1.32 (± 0.77)	20.24 (± 1.09)
20 Middle		1.54 (± 1.27)	20.06 (± 1.14)
20 North		2.37 (± 1.66)	18.76 (± 1.64)
19 South		1.50 (± 1.15)	19.41 (± 1.50)
19 Middle		1.39 (± 1.01)	19.76 (± 0.97)
19 North		1.80 (± 1.22)	19.24 (± 1.56)
17 Middle		1.19 (± 0.77)	21.35 (± 0.63)
17 North		1.69 (± 0.63)	20.59 (± 0.83)
17 South	1.63 (± 0.83)	21.82 (± 0.77)	

Table 4. Fish size and age data from eels captured at each of the six locations: sample sizes, mean values $\pm\text{SD}$ for length (TL), body mass (BW) and age with respect to sample location.

Location	N	TL (mm)	BW (g)	Age (years)
Lake Ontario	6	952 (± 87)	1872 (± 531)	18 (± 5)
Lake Champlain	22	477 (± 99)	231 (± 158)	6 (± 1)
Lac Saint-Louis	23	765 (± 82)	1142 (± 500)	14 (± 2)
Bécancour	20	857 (± 144)	1403 (± 681)	15 (± 5)
Cap Santé	18	647 (± 69)	523 (± 282)	7 (± 4)
Saint-Romuald	21	858 (± 58)	1279 (± 268)	19 (± 7)

into the brackish estuary before finally returning to freshwater. One individual, captured at Cap Santé, spent an extended period in the brackish estuary before entering freshwater. This was followed by a return to the brackish estuary after 4 years and, finally, a return to freshwater 1 year later. None of the individuals from Lake Ontario, Lac Saint-Louis or Lake Champlain made use of habitat 1. Nearly half (44.5%) the eels used habitat 2 at some time during their growth stage whereas 100% of the eels used habitat 3 (Table 5). Use of habitat 2 varied geographically, from a minimum at Bécancour to a maximum in

Table 5. The proportions of eels, with respect to sampling location, having used each of the three habitats during their growth phase.

Capture site	Distance upstream from brackish estuary (km)	Used Habitat 1 (%)	Used Habitat 2 (%)	Used Habitat 3 (%)
Lake Ontario (Lake)	605	0	83.3	100
Lake Champlain (Lake)	475	0	90.9	100
Lac Saint-Louis (Fluvial Lake)	350	0	65.2	100
Bécancour (Fluvial Estuary)	185	25.0	30.0	100
Cap Santé (Fluvial Estuary)	105	17.6	70.6	100
Saint-Romuald (Fluvial Estuary)	50	14.3	52.4	100
Total percentage of individuals	–	10.0	44.5	100

Table 6. The proportions of eels, with respect to sampling location, having made zero, one, two and three switches between each of the two FW habitats during their growth phase.

Capture site	Number of individuals	0 switches (%)	1 switch (%)	2 switches (%)	3 switches (%)
Lake Ontario	6	16.7	66.7	16.7	0
Lake Champlain	23	4.5	45.6	27.3	22.7
Lac Saint-Louis	23	34.8	52.2	4.3	8.7
Bécancour	20	65.0	25.0	10.0	0
Cap Santé	18	11.1	66.7	5.6	16.7
Saint-Romuald	21	52.4	14.3	23.8	4.8
Total (All Sites)	110	32.7	41.8	14.5	10.0

Lake Champlain. Overall, 28.2% of eels sampled did not switch habitats after the elver check (Table 6). While one individual from Lake Champlain made five habitat switches, the eels sampled appeared to make a maximum of three habitat switches; 41.8% of all individuals made one switch as opposed to 14.5% that made two switches and only 10.0% that made three switches. Of the eels that made one habitat switch, 87.5% moved from habitat 2 to 3. Of those that made two switches, 55% moved from habitat 2 to 3 and back to 2 while 25% moved from habitat 3 to 2 and back to 3. Most (90.1%) of the individuals that made three habitat switches moved from habitat 2 to 3 to 2 and back to 3. The vast majority of individuals (72/77 or 93.5%) that made at least one habitat switch made their first habitat switch within the first 4 years after the elver check (Table 7). Furthermore, the majority of eels (22/29 or 75.9%) having made two or more switches made their second switch within the first 5 years after the elver check (Table 7) while the same was true for 81.8% (9/11) of individuals that made their third switch (Table 7).

Table 7. Ages of individuals at which first, second and third habitat switches were estimated. Each of these three categories is not mutually exclusive (i.e. individuals that made more than one habitat switch are included in more than one category). Total number (*N*) of individuals for each of the categories is also listed.

Age (year)	1	2	3	4	5	6	7	8≥
Age at 1st Switch								
Number of individuals (<i>N</i> = 77)	39	16	7	10	3	–	–	2
Age at 2nd Switch								
Number of Individuals (<i>N</i> = 29)	5	7	6	4	1	2	2	2
Age at 3rd Switch								
Number of Individuals (<i>N</i> = 11)	1	3	3	2	1	–	–	1

Discussion

Brackish estuarine habitat

Based on otolith microchemical data, we succeeded in quantitatively distinguishing one chemical signature from the otoliths of sampled eels with a significantly higher Sr and lower Ba concentration. Given the coarse longitudinal profile exhibited by Sr and Ba within the SLR and its brackish estuary, this signature referred to as habitat 1 is estuarine in nature. Only a small proportion of sampled eels (10%) made use of this estuarine habitat after recruiting to the system as elvers. However, these individuals were sampled at Bécancour, Cap Santé and Saint-Romuald; the three most downstream sites that respectively lie 185, 105 and 50 km upstream from the brackish estuary. Accounting only for eels sampled at these three downstream sites, 18% of the individuals sampled there made use of the brackish estuary after recruiting to the system as elvers. Consistent with this finding, a large-scale acoustic telemetry study conducted in 2010 and 2011 in the St. Lawrence River reported unidirectional downstream movements towards the upper brackish estuary by approximately 16% of tagged yellow eels (Béguer-Pon et al. 2015). It is interesting to note that each of the three aforementioned sites is located downstream of Lac Saint-Pierre, placing them inside the fluvial estuary and under tidal influence. In contrast, the otoliths of individuals captured from Lake Ontario and Lac Saint-Louis, both further upstream, showed no evidence of those eels having made use of the brackish estuarine habitat after having initially recruited to the system as elvers. Instead, those individuals exhibited typically catadromous behaviour characterised by exclusive residence in freshwater. In the case of stocked eels from Lake Champlain, there is no evidence of them having made extensive downstream movements to the brackish estuary. Of the 11 individuals that made use of the brackish water habitat, the dominant behaviour observed consisted of an initial prolonged residence in the brackish estuary lasting between 1

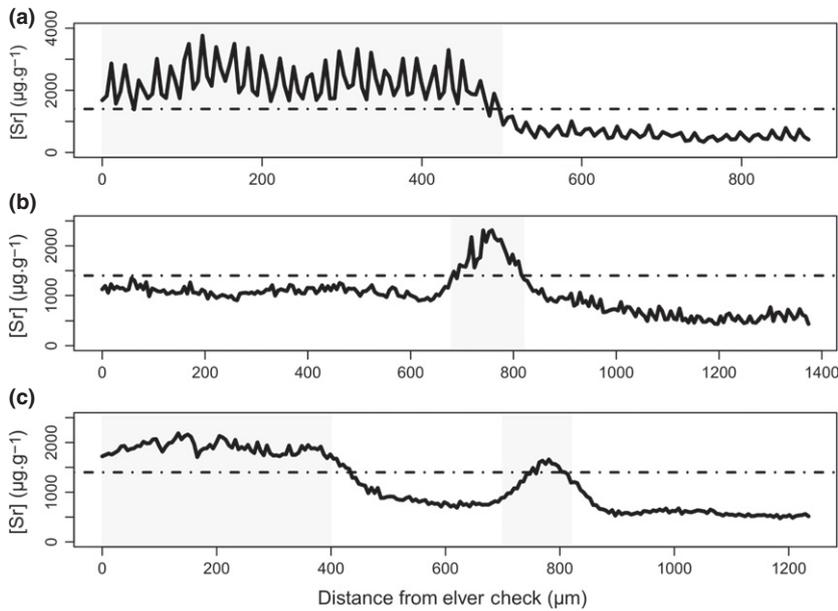


Fig. 4. Three alternative migration tactics derived from variation in Sr concentrations along cross-sectional otolith line scans using LA-ICP-MS. Light gray area correspond to *Habitat 1*: (a) prolonged residence in the brackish estuary before entering freshwater; (b) recruitment to freshwater followed by a brief incursion into the brackish estuary; (c) migrant fish moving more than once between the brackish estuary and freshwater. The dashed line is the presumed threshold between freshwater and brackish water.

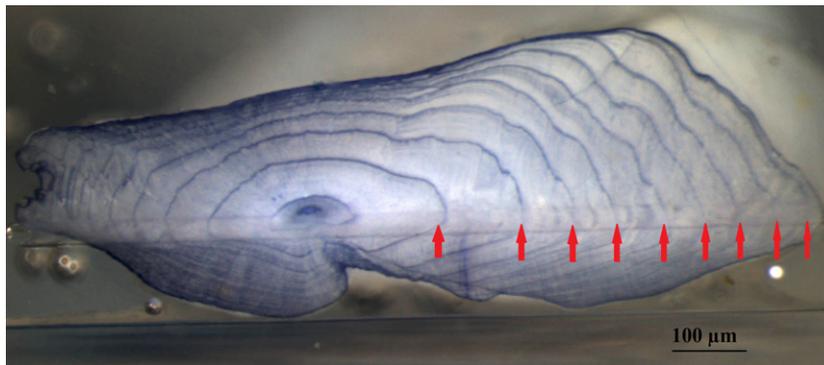


Fig. 5. Image of a stained eel otolith sectioned and polished in the transverse plane. Red arrows indicate growth rings and represent 1 year's worth of growth. The age estimate for this individual is 9 years. Laser transect is clearly visible across the surface and passing through the core.

and 5 years before eventually entering and remaining in freshwater. Three individuals showed evidence of an excursion to the brackish estuary after having initially entered freshwater followed by a return to freshwater. Interestingly, one individual sampled at Cap Santé exhibited 'nomadic' behaviour, moving back and forth between the brackish estuary and freshwater sections of the system on more than one occasion. More specifically, this individual spent 3 years in the brackish estuary before entering freshwater. After spending 4 years in freshwater, it made a brief return to the brackish estuary before finally returning to freshwater. If eels exhibiting this 'nomadic' behaviour experience higher turbine and/or fishery mortality than their more sedentary congeners, this might explain their under-representation in the sampling of this study. Generally, however, the behaviour patterns observed suggest that a small proportion of eels recruiting to the St. Lawrence River do, in fact, make use of downstream brackish water habitats as has been documented for conspecifics

elsewhere. Based on analyses using Sr:Ca profiles, Daverat et al. (2006) noted that the vast majority of European and Japanese eels sampled in the upper reaches of watersheds were freshwater residents that showed no evidence of residence in brackish or coastal waters. This same trend was observed for the American eel in the Hudson River where all individuals sampled 190 km upstream from the mouth of the brackish estuary were freshwater residents (Morrison & Secor 2004). Similarly, the results of this study suggest that eels colonising the upper reaches of the SLRLO remain exclusively in freshwater. Nevertheless, the fact that individuals from Bécancour made use of the brackish estuary suggests that naturally recruiting eels in the St. Lawrence River can undertake movements of at least 200 km during their growth stage. Given that our study did not target yellow-stage eels within the brackish estuary, we cannot discount the possibility that a proportion of eels entering the St. Lawrence system never enter the fluvial section at all, instead carrying out their entire

growth phase in brackish habitats. American eels are reported to inhabit many estuaries throughout the United States (Nelson et al. 1991; Jury et al. 1994; Stone et al. 1994). Since Daverat et al. (2006)'s review on facultative catadromy among temperate anguillid eels, several works have also reported this for tropical species suggesting that this observed life-cycle plasticity is a more general trait than previously reported among anguillid eels (Arai & Chino 2012).

Freshwater habitats

Contrary to the brackish estuary habitat, two of the three habitats characterised – habitats 2 and 3 – showed a much lower Sr signature, and most probably represent habitats within freshwater. Nevertheless, these two habitats differed with respect to their Ba, Mn and Mg signatures. Habitat 2 had significantly greater mean concentrations for each of these elements compared to habitat 3. Although the majority of individuals sampled in this study were shown to have made use of these two habitats, linking otolith signatures to discrete geographical areas is far less straightforward. Two initial observations are evident with respect to the two freshwater habitats. Firstly, all of the sample sites included eels that made use of habitat 3 and, except for individuals sampled at Bécancour, the majority of eels (>50%) at each site made use of habitat 2 which was characterised by significantly higher mean concentrations of Ba, Mn and Mg. The waters of the SLR exhibit some cross-sectional variation in their geochemical properties owing to the stratification of different water masses entering the river from several of its major tributaries (i.e. Ottawa River and Richelieu Rivers) along the north and south shores respectively (Frenette et al. 2003). The different water masses result from differences in the underlying geology of the catchments drained by the tributaries entering the SLR. Yang et al. (1996) and Rondeau et al. (2005) reported that the tributaries draining Precambrian shield rocks of the north shore of the SLR are relatively low in dissolved minerals, whereas southern shore tributaries drain limestone dolomite that is relatively high in dissolved minerals. The water data analysed and presented in this study showed dissolved Mn and Ba to be significantly higher in both north-shore and south-shore tributaries when compared with values for the main-stem SLR. With respect to Mn, this result supports the first interpretation that switches between habitat 3 (significantly lower Mn) and habitat 2 (significantly higher Mn), inferred from the otolith data, represent movements between the main-stem SLR and its tributaries. Although we observed significantly higher mean dissolved Ba concentrations between main-stem SLR and north-shore tributaries

as a whole, in reality most of the north-shore tributary sites exhibited Ba concentrations similar to those of main-stem SLR sites. The extremely elevated mean concentration observed at La Petite Rivière Yamachiche (see station 5 in Table 3) explains the significant difference detected between those groups. When comparing sites within each of the three categories, our results suggest that there is little longitudinal and cross-sectional variation in dissolved Mn and Ba along the main-stem SLR as dissolved concentrations for these elements were not found to differ statistically. Mean Mn concentrations did not vary between north-shore tributaries. However, for the south-shore tributaries, the Yamaska (station 15) and Saint-François Rivers (station 16) had significantly lower mean dissolved Mn when compared with other tributaries and mean dissolved Ba was significantly different between all tributaries except for Yamaska and Gentilly (station 14) rivers. Consequently, these results might suggest that switches between subclusters *e* and *f*, detected in the otolith data, represent the use of, and movement between, different south-shore tributaries. Although this interpretation is speculative, Clark et al. (2005) reported similar observations for Arctic Grayling in freshwater catchments of northern British Columbia. In their study, Mn was used successfully as a habitat tracer and allowed them to infer movements based on otolith line scans between tributaries with high Mn concentrations and open-water areas with lower Mn concentrations. The use of tributaries by American eels during the growth phase has previously been observed. Machut et al. (2007) reported elevated densities of yellow-stage American eels in the tributaries of the Hudson River. These densities were much greater than those for the main stem of the Hudson River suggesting that tributaries might represent important habitats for juvenile eels during the first several years in a large river system such as the St. Lawrence or Hudson rivers. Tributaries of the St. Lawrence River were reported to provide historically important habitats to the species although the construction of numerous dams and hydroelectric facilities has been associated with contraction of recruitment to the lower reaches of tributaries in recent times (MacGregor et al. 2010). That similar habitat-use patterns were also observed for eels sampled in Lake Champlain raises the possibility that switches between habitats 2 and 3 correspond to movements between the lotic waters (habitat 3) of Lake Champlain proper and the waters of its tributaries (habitat 2). However, this interpretation is purely speculative as no water data from the Lake Champlain system were analysed.

Another plausible interpretation with respect to the nature of both freshwater habitats is related to marshland biogeochemistry and hypoxia more generally

(Limburg et al. 2015). Recall habitat 2 exhibited a significantly greater mean Mn concentration as compared to habitat 3. Under normal conditions, manganese mostly occurs in an insoluble oxidised form in freshwater systems. However, intense biological activity, brought about by bacterial decomposition in wetland soils, plays an important role in increasing the concentration of dissolved Mn^{2+} ions wetland pore waters (Kuenzler et al. 1980; Santschi 1988). Although reduced in historical extent and threatened by a number of human-related activities, wetlands and marshlands occur throughout the SLR, notably along the shores of Lac Saint-Pierre. Wetlands are also present along the shores of Lake Champlain. In this context, habitat 2, characterised by significantly higher concentrations of Mn, might possibly represent a marshland signature. The GLLFAS water data presented in this study show an increase in the dissolved concentration of Mn within the fluvial section of the SLR between Montreal and the brackish estuary where relatively large areas of marshlands occur. Although this data set only provides a snapshot of dissolved Mn in the SLR, it does provide further evidence to the hypothesis that habitats 2 and 3 represent marshland signature and 'open-water' signature respectively.

Although Elsdon & Gillanders (2002) and Walther et al. (2008) have discussed the challenges with respect to the use of Mn and Mg as habitat tracers in fish otoliths, a number of studies carried out during the last 10 years have successfully managed to use Mn as a habitat tracer in otoliths to infer habitat use or site of origin. Notably, Arslan & Secor (2005) successfully managed to use Mn concentrations in American eel otoliths, among other elements, to discriminate among different sites in the Hudson River. For other fish species such as Artic Grayling, Yellow Perch, and Brown Trout, Mn concentrations in otoliths were successfully used as a habitat tracer to discriminate among streams and tributaries in freshwater catchments from northern British Columbia, the Great Lakes and New Zealand respectively (Clark et al. 2005; Pangle et al. 2010; Olley et al. 2011). Together, these studies support the above-mentioned interpretations for freshwater habitat use based on concentration profiles for Mn in the otoliths and variation of Mn in the SLR system. It is important to note, however, that in the face of the limited available data for Mg and the previously reported challenges regarding its use as a habitat tracer, interpretations in this study based on Mg are much weaker.

Patterns of habitat use

Based on chemical data from otoliths and the fact that chemical signatures of otoliths represent uptake

from water (Campana et al. 1995), our results suggest that St. Lawrence eels move between aquatic habitats that vary in their geochemical signatures. However, it is important to note that we cannot exclude the possibility that the observed chemical variation in the otoliths is the result of an ontogenetic or physiological signal (Elsdon et al. 2008). Nevertheless, given the observed variation pattern in Mn and Ba in the environment, the majority of these movements occur in the first 4 years after recruiting to the system as elvers. This is consistent with observations for other anguillid eels, including the American eel, where most habitat switches between freshwater and brackish water occurred before the age of five (Daverat et al. 2006). Furthermore, this is also consistent with the current model of eel recruitment to the upper reaches of the St. Lawrence and Lake Ontario. Castonguay et al. (1994) discussed evidence that the 750 km journey from the Gulf of St. Lawrence as glass eels to the Moses-Saunders dam in the upper St. Lawrence River lasts around 4 years. Moreover, the mean age of yellow eels migrating upstream at the Moses-Saunders dam was estimated at 5 years in the 1980s and 6 years in 2006–2007 (Casselmann 2003; Casselman 2008). Dutil et al. (1989) reported similar findings for yellow eels recruiting to the Petite-Trinité, a small river on the north shore of the Gulf of St. Lawrence. Should changes in the chemical signatures in the otolith reflect changes in habitats; our results suggest that these generally occur within a restricted window of time early during the eel's growth stage. The lack of variation in otolith chemistry observed thereafter suggests a more sedentary behaviour although the relatively large beam size used in this study reduces the ability to resolve temporal patterns later in life as otolith annuli are progressively less spaced apart. Nevertheless and consistent with this proposed behavioural trend, acoustic telemetry work by Béguet-Pon et al. (2015) found that, aside from a small proportion of individuals making unidirectional downstream movements to the brackish estuary, most yellow eels tagged at two sites in the St. Lawrence River showed little evidence of large-scale movement within the system.

Conclusion

This study presents important information relevant to the ecology of the American eel in one of the largest drainages within the species' distribution. Using statistical techniques designed for profile analysis, this study goes beyond simply retracing movements between brackish and freshwater habitats. Although limited in its ability to resolve the finer-scale nature of the two freshwater otolith signatures, we propose two, nonmutually exclusive, interpretations. The first

of these suggests that eels are perhaps exploiting and/or moving between, tributaries and main-stem SLR habitats while the second suggests that they are perhaps making use of, and moving between, marshlands and open-water habitats. In both instances, Mn, Mg and Ba signatures were significantly different between the two within-freshwater otolith signatures that we interpreted as different habitats. Although our interpretations based on Mg are limited given the lack of water data and the challenges reported with its use as a habitat tracer in otoliths, the information we present for the other three elements provides evidence in support of our interpretations. Acquiring a more detailed and refined understanding of habitat use and movement patterns of American eels in the St. Lawrence River is fundamental to further exploring these interpretations. Hopefully, this will lead to a clearer understanding of the species' ecology during the growth phase in the Saint Lawrence River–Lake Ontario system and ultimately better-inform the management and conservation of the species.

Acknowledgments

Funding for this project was provided to Julian J. Dodson and Martin Castonguay as part of the Ocean Tracking Network, a Strategic Network Grant funded by the Natural Sciences and Engineering Research Council of Canada and the Canadian Foundation for Innovation. The authors acknowledge Québec-Océan for financial and material support. We also wish to thank Simon Bernatchez for assisting in both the field and the laboratory work. We are extremely grateful for the support from Guy Verreault and his team (MRNF) by providing us with eels from Cap Santé and Lake Ontario as well as by sharing eel otolith ageing expertise. We thank Daniel Hatin and his team (MRNF) for helping us obtain eels from Lac Saint-Louis. We are also grateful to Shawn Good (USFWS Rutland, Vermont) for generously providing us with eels he captured in Lake Champlain and allowing us to use his laboratory facilities. We thank Denise Deschamps and Yanick Soulard (MRNF) for their technical assistance in the preparation and sectioning of otolith samples. Finally, we would like to thank Dany Savard, Angélique Lazartigues and Olivier Morissette (UQAC) for their help with LA-ICP-MS analyses.

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