Discriminating natal origin of spawning adult sea lamprey (Petromyzon marinus) in Lake Champlain using statolith elemental signatures

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A B S T R A C T
Sea lamprey (Petromyzon marinus) is a nuisance species in the Great Lakes and Lake Champlain. Information about tributary contributions to the spawning adult phase is critical for appropriate allocation of efforts to control this species. We examined the accuracy of statolith elemental composition to identify the natal origin (i.e., individual rivers or clusters of rivers) of 33 known-origin adults from the Lake Champlain basin. To do so, we first established natal origin chemical signatures using the statoliths of 238 larvae from the same basin. Using laser-ablation inductively coupled plasma mass spectrometry, the 238 larvae originating from 12 streams and one delta were discriminated with a classification accuracy of 57% (range: 25–80%) and 70% (range: 29–80%) when individual streams and groups of streams were considered respectively, highlighting the potential of statolith microchemistry to identify natal origins. However, the assignment of natal origin for adults was overwhelmingly incorrect. Using a maximum likelihood procedure, 88% of the adults were assigned to a cluster of three streams and one delta, while only 3% of these individuals were known to originate from this particular cluster. More research is required to understand the low classification accuracy of sea lamprey adults and validate the use of statolith microchemistry to identify sea lamprey natal origin.

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Introduction

The sea lamprey (Petromyzon marinus) is a nuisance species in the Laurentian Great Lakes and Lake Champlain and has caused major damage to economically and ecologically important fishes, especially lake trout (Salvelinus namaycush) and other salmonines (Smith and Tibbles, 1980). Sea lamprey larvae are filter feeders that inhabit burrows in the soft sediments of their natal streams for four to six years (Moore and Mallat, 1980; Potter, 1980). At the end of the larval phase, individuals transform into parasitic-phase sea lampreys that migrate into the open lake to feed on large-bodied fishes (Farmer, 1980; Swink, 2003). After 12 to 18 months as parasites, adults migrate to streams for spawning. Sea lamprey population control efforts in the Great Lakes and Lake Champlain have relied heavily on the periodic treatment of streams with chemical lampricides to kill larvae (Christie et al., 2003; Marsden et al., 2003; Smith and Tibbles, 1980).

More streams contain sea lamprey larvae than can be treated, due to limited resources. Streams are selected for lampricide treatment based on (i) the assessment of larval population and size structure, (ii) the prediction of the proportion of sea lamprey larvae within a given stream that is likely to undergo metamorphosis into fish parasites in the following year and (iii) treatment cost (Christie et al., 2003; Fenichel and Hansen, 2010; Treble et al., 2008). However, larval abundance estimates do not translate into numbers of spawning-phase sea lampreys produced because of complex in-stream processes and differences in the survival of newly transformed parasitic-phase lampreys. Factors such as larval density may affect survival and growth in streams (Dawson and Jones, 2009; Morman, 1987; Rodríguez-Muñoz et al., 2003) so that not all larvae metamorphose into parasitic-phase sea lampreys. Similarly, not all parasitic-phase sea lampreys will contribute to the spawning adult population, although the differences in open lake survival of parasitic-phase sea lampreys from different streams are not well known (Jones, 2007). Thus, treating a stream with high larval densities that will not be translated into parasitic-phase lampreys is a poor use of scarce resources. A better understanding of the tributary production of adult sea lampreys could improve the control program.
Natural geochemical tags in calcified structures, especially otoliths, have been widely used to track fish migration and assess natal origin (Campana, 1999; Elsdon et al., 2008). Otoliths are calcium-carbonate concretions in the teleost sensory system that grow continuously incorporating elements from the surrounding waters in the process and that are metabolically inert (Campana and Thorrold, 2001). Consequently, fish growing in chemically distinct waters will record unique signatures in their otoliths that reflect those habitats.

Statoliths in sea lampreys are considered as primitive otoliths (Gauldie, 1996; Lychakov, 1995). Elemental composition of statoliths in sea lamprey larvae varies geographically (Brothers and Thresher, 2004; Hand et al., 2008). Accuracy of classifying larvae to their tributaries reached 88.9% for larvae from five different source locations in the Lake Huron watershed (Brothers and Thresher, 2004), and averaged 82% among larvae from 13 streams located in lakes Michigan, Huron and Superior with individual stream accuracies ranging from 31% to 100% (Hand et al., 2008). However, the potential for using statolith elemental signatures to identify the natal tributaries of sea lamprey adults is not well known, mostly because having adults of known-origin is rare. Only one study to date has tested the use of statolith microchemistry to identify the natal origin of known-origin adult sea lampreys from the Lake Huron watershed (Brothers and Thresher, 2004). In that study, 18 adult sea lampreys were assigned to their natal river with limited success (44%) despite high assignment accuracy for the larvae from the same river. Herein, we further examine the use of statolith microchemistry as a tool to identify natal origins of adult sea lampreys by extending the study of Brothers and Thresher (2004) to a different system (i.e., Lake Champlain), with larger larval and adult sample sizes collected from a greater number of natal streams. The elemental composition of statoliths in larvae from tributaries of Lake Champlain was analyzed to test whether statolith chemistry varies geographically. Then, samples from known-origin adults were used to determine the accuracy of using statolith elemental fingerprints to assign sea lamprey adults to their natal origin.

**Material and methods**

**Sea lamprey collection**

Sea lamprey sampling focused on Lake Champlain, which lies between New York and Vermont, USA, and Quebec, Canada (Fig. 1). Ten to 56 sea lamprey larvae were collected from each of 12 tributaries to Lake Champlain and one tributary delta during lampreicide treatments or by electrofishing. Because larvae were collected as part of different research projects, larvae used in this study were collected in summer 2002 through summer 2005 (Table 1, Fig. 1) and specimens were preserved in two ways. Among the 238 larvae collected, 137 were immediately frozen and 101 were preserved in 95% ethanol (Table 1).

We acquired known-origin adult sea lampreys from a tagging study conducted by Howe et al. (2006). In that study, recently metamorphosed adult lampreys were captured in the fall of 2001 and 2002 from five tributaries to Lake Champlain: Lewis Creek, Malletts Creek, Pike River, Morpion Stream and Saranac River (Fig. 1). All lampreys were marked with coded wire tags and released back into their stream of collection. Thirty-three tagged lampreys were recaptured in 2003 and 2004 as spawning-phase sea lamprey during their upstream migration or from nests during spawning. One lamprey each originated from Morpion Stream and Saranac River, two from Pike River, nine from Malletts Creek, and 20 from Lewis Creek. All adults were frozen after collection.

**Statolith preparation**

Using the methods described by Hand et al. (2008), sagittal statoliths were dissected from the left and right otic sacs of each individual in a Class-100 clean room and sonicated for five minutes in a Petri dish floating on Milli-Q ultrapure water in an ULTRASONic cleaner (model 57X; Ney Dental, Inc., Bloomfield, Connecticut). Statoliths were then transferred with a glass probe to a clean Petri dish where they were rinsed three times in Milli-Q water. All laboratory apparatus in contact with the statoliths were acid-washed prior to use (Ludsin et al., 2006).

The method for statolith preparation differed depending on the sea lamprey life stage. Because larvae were collected from their natal streams, the stream chemical signature is represented by the entire statolith. Larval statoliths were mounted on their base using Scotch double-sided tape (3 M, St. Paul, Minnesota) on a petrographic microscope slide. Larval statoliths were ablated by traversing their entire width, from the apex to the base on the opposite side.

For the statoliths of adults, only the portion of the statoliths deposited during the larval stage was of interest. Statoliths grow in a conical shape, with the oldest material found at the apex of the statoliths and the most recently deposited material found at the base (Carlström, 1963; Lychakov, 1995). Thus, the post-larval stage material is expected to be found at the base of the statoliths of adults. To properly extract data only from the larval portion, a mid-sagittal section of the statoliths of adults was prepared. Specifically, statoliths were mounted in crystal bond (Structure Probe, Inc., West Chester, PA) on a strip of transparency film, with the median plane of the statolith parallel to the film. Mounted statoliths were then ground perpendicular to their base until their banding patterns were exposed. Prepared statoliths were then placed on a clean glass slide using Scotch double-sided tape. Statolith sections were ablated along a transect from the apex to the base. The statolith material along this transect was deposited during stream residency as larvae and during the residency in Lake Champlain as fish parasites. Because statolith size increases at a decreasing rate, with limited statolith growth after the larval stage (Brothers, 1987; Meeuwis and Bayer, 2005), the information recorded from the apex to three-quarters of the way down to the base was assumed to be an accurate representation of the material deposited during stream residency at the larval stage. Consequently, only this portion of the statoliths of adults were considered.

**Statolith analysis**

Statoliths were analyzed for a suite of elements using an inductively coupled plasma-mass spectrometry ICP-MS (Thermo Elemental X7; Thermo Fisher Scientific Inc., Waltham, Mass.) coupled with a Continuum® Sirelite® solid-state Nd:YAG laser (wavelength = 266 nm, maximum power = 40 mJ, pulse rate = 20 Hz, primary beam width = 6 mm; Continuum Inc., Santa Clara, Calif.) following the techniques outlined by Hand et al. (2008).

A typical acquisition consisted of a 60 s measurement of the gas blank before the laser was switched on, followed by 100 s of measurement with the laser on and statolith material being ablated. Outputs from laser-ICP-MS were counts per second. After ablation, we chose the time intervals over which to integrate the background (measured as gas blank) and the statolith ablation count rates. For larvae, statolith data integration was started when the laser hit the statolith and was terminated when the laser started to sample the Scotch tape. For adult statoliths, data were integrated over the first three-quarters of the time interval between the hit of the statolith and the hit of the crystal bond, to ensure that only material deposited at the larval stage was integrated in the signal. Calcium was used as an internal standard to account for ablation-yield differences. A Microsoft excel™ macro was then used to calculate background-corrected signals, average the data down to one value per isotope per statolith, and convert the counts per second into concentrations.

To calibrate analytical sensitivity, estimate measurement precision, and to account for instrumental drift, a reference standard (National Institute of Standards and Technology [NIST] 610) was run in pairs prior to and after every ten statoliths. A coefficient of
variation (CV = standard deviation/mean × 100) was calculated for each element in each run. Among the 10 elements analyzed, only those with 90% or more of the samples above the limits of detection and with an average coefficient of variation less than 10% were used in this study (Ludsin et al., 2006). Six elements met these criteria: magnesium (Mg), manganese (Mn), zinc (Zn), rubidium (Rb), strontium (Sr) and barium (Ba) (Table 2). All elemental concentrations were natural log transformed to normalize the data.

Outlier identification and data corrections due to the effects of preservation on Rb

Any single larval data point (an elemental concentration for an individual sea lamprey) that was greater than three standard deviations from the mean for its respective tributary was considered to be an outlier. Less than 2% of the data were outliers. We evaluated the influence of outliers by running the analysis described in this study twice: using a dataset that included the original outlier values, and using a dataset where outliers were replaced with a random value generated using a normal distribution from the mean and standard deviation of the element for that tributary, a method known for artificially increasing the precision of the measurements (Quinn and Keough, 2002). Because the two approaches led to similar conclusions, the influence of outlier values was considered negligible and the original outlier values were kept in the analysis.

As some larvae were preserved in 95% ethanol while others were frozen (Table 1), the effects of the mode of preservation on statolith chemistry were tested using the larvae collected from Lewis Creek VT in summer 2002, as Lewis Creek had the most abundant supply of larvae at the time (Howe, 2006). Among the 56 larvae from Lewis Creek, 29 were randomly selected and immediately preserved in 95% ethanol while the remaining 27 were immediately frozen. Only Rb concentrations were affected by the mode of preservation (Howe, 2006), in agreement with Hand et al. (2008). In both studies, Rb concentrations were higher for larvae preserved in ethanol than those that were frozen.

To remove the effect of preservation method, an analysis of variance (ANOVA) was performed using the natural log of Rb concentration of all individuals (whatever their stage or their natal stream) as the dependent variable and the mode of preservation (ethanol versus frozen) as the independent variable.
the independent variable. The aim of this ANOVA was to partition the total variation into portions associated with the explanatory factor (here, the mode of preservation) and residuals (the variation not explained by the mode of preservation). Variation due to stream differences was expressed in the residuals. Thus, in this study, statolith elemental signatures from different streams were characterized using the residuals of the ANOVA rather than the natural log of Rb concentrations. Low statolith Rb concentrations were represented by highly negative residuals. High Rb concentrations were represented by highly positive residuals. Such a statistical approach has been used in other studies (Burge, 2004).

Natal origin assignment for larvae

A quadratic discriminant function analysis (DFA) was used to determine the accuracy with which sea lamprey larvae could be assigned to their natal origin. A quadratic DFA was appropriate because this procedure does not assume homogeneity of variance–covariance matrices (McGarigal et al., 2000). The DFA uses a jackknife cross-validation procedure to determine classification accuracy. Two discriminant analyses were conducted to test the effects of grouping rivers on classification accuracy. A first discriminant analysis was performed using larvae from all 12 tributaries and the single delta kept separate (grouping A). A second discriminant analysis was performed using clustered tributaries of geographic proximity and similar geologic drainages (grouping B). For grouping B, the separation of chemical signatures was primarily driven by variations in Rb along the northeastern end of Lake Champlain, while most of the eastern side (Vermont) drains the Green Mountains. The northern tributaries of the Adirondacks – Salmon, Ausable, Saranac rivers and the Saranac delta – were clustered together based on geographic proximity (cluster B2). The southern tributaries of the Adirondacks – Mill and Mount Hope brooks – were grouped together (cluster B3). Finally, Morpion Stream, Pike River and Missisquoi River, all flowing into Missisquoi Bay at the northeastern end of Lake Champlain, were assigned to the same group (cluster B7) (Fig. 1, Table 1). Geographic differences in elemental signatures among rivers and groups of rivers were visualized using canonical discriminant analysis. Canonical variate coefficients were used to assess the relative importance of each variable to the observed separation among rivers and groups of rivers.

Statistical analyses were performed using R software (R Development Core Team, 2010). The packages MASS and ade4 were used to perform the discriminant analysis and the canonical discriminant analysis, respectively (Dray and Dufour, 2007; Venables and Ripley, 2002).

Validation of natal origin assignments of adults

A maximum likelihood estimation (MLE) procedure (HISEA; Millar, 1987) was used to determine the natal origin of the 33 known-origin adult sea lampreys. In this procedure, the adults were treated as the stock mixture of unknown origin. The baseline was the statolith elemental signatures of the 238 larvae from the seven reference populations presented in grouping B. Stock mixtures and associated standard deviations were calculated in bootstrap mode by resampling the baseline 500 times with replacement. The MLE algorithm does not identify origins of individual fish but estimates the proportions of each reference population in the unknown mixture. The predicted proportions of adults from each reference population were compared to the known proportions.

To test the stability of statolith composition between larvae and the larval portion of the statoliths of adults from the same stream, two-sample t-tests were used to compare elemental concentrations between the two groups. We did not apply a Bonferroni correction as sample sizes for larvae and adults were not equal (Dray and Dufour, 2007; Venables and Ripley, 2002). The comparisons were restricted to Lewis Creek and Malletts Creek due to the very low number of adults coming from the three other streams. The level of statistical significance was set at α = 0.05.

Results

When tributaries were kept separate, larvae were assigned to their natal origin with an average accuracy of 57.1% (range: 25.0%–80.4%) (Table 3a). Clustering tributaries by geographic proximity and similar geologic drainages (grouping B) improved the average classification accuracy to 70.2% (range: 29.4%–80.4%) (Table 3b). Lewis Creek was the best discriminated stream regardless of the grouping (Table 3a, b). For grouping B, the separation of chemical signatures was primarily driven by variations in Rb along the first canonical variate and by variations in Sr along the second canonical variate (Table 4). Canonical variate 1 discriminated mostly between Lewis Creek (low Rb) and cluster B3 (Mill and Mount Hope brooks, high Rb). Canonical variate 2 mostly separated cluster B7 (Morpion Stream, Pike and Missisquoi rivers, high Sr) from the Great Chazy River and cluster B2 (northern New York tributaries, low Sr) (Fig. 2, Table 5).

Classification accuracy of the known-origin adults was extremely poor. The MLE procedure assigned 88% of the sea lamprey adults to their natal origin with an average accuracy of 57.1% (range: 25.0%–80.4%) (Table 3a). Clustering tributaries by geographic proximity and similar geologic drainages (grouping B) improved the average classification accuracy to 70.2% (range: 29.4%–80.4%) (Table 3b). Lewis Creek was the best discriminated stream regardless of the grouping (Table 3a, b). For grouping B, the separation of chemical signatures was primarily driven by variations in Rb along the first canonical variate and by variations in Sr along the second canonical variate (Table 4). Canonical variate 1 discriminated mostly between Lewis Creek (low Rb) and cluster B3 (Mill and Mount Hope brooks, high Rb). Canonical variate 2 mostly separated cluster B7 (Morpion Stream, Pike and Missisquoi rivers, high Sr) from the Great Chazy River and cluster B2 (northern New York tributaries, low Sr) (Fig. 2, Table 5).
Table 3
Cross-validation summary from the quadratic discrimination function analysis for classifying Lake Champlain sea lamprey larvae to their natal origin for groupings A (panel a) and B (panel b). Rows represent the known streams or clusters of origin, columns are predicted streams or clusters of origin. Reported values are percent classification with the number of individuals in each classification (in parentheses). Accurate classifications are shown in bold, on the diagonal.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Great Chazy R.</td>
<td>71.4</td>
<td>0.0</td>
<td>0.0</td>
<td>14.3</td>
<td>4.8</td>
<td>0.0</td>
<td>0.0</td>
<td>9.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>(21)</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Poultney R. (17)</td>
<td>5.9</td>
<td>5.9</td>
<td>0.0</td>
<td>0.0</td>
<td>11.8</td>
<td>0.0</td>
<td>29.4</td>
<td>11.8</td>
<td>5.9</td>
<td>29.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Lewis Cr. (56)</td>
<td>1.8</td>
<td>5.4</td>
<td>0.0</td>
<td>1.8</td>
<td>3.6</td>
<td>0.0</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
<td>80.4</td>
<td>0.0</td>
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</tr>
<tr>
<td>Malletts Cr. (16)</td>
<td>0.0</td>
<td>12.5</td>
<td>0.0</td>
<td>0.0</td>
<td>6.3</td>
<td>6.3</td>
<td>18.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>56.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Missiquoi R. (12)</td>
<td>8.3</td>
<td>8.3</td>
<td>0.0</td>
<td>0.0</td>
<td>8.3</td>
<td>0.0</td>
<td>25.0</td>
<td>16.7</td>
<td>8.3</td>
<td>25.0</td>
<td>8.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Morpion Str. (18)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>22.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>66.7</td>
<td>11.1</td>
</tr>
<tr>
<td>Pike R. (14)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>7.1</td>
<td>0.0</td>
<td>14.3</td>
<td>0.0</td>
<td>7.1</td>
<td>21.4</td>
<td>0.0</td>
<td>50.0</td>
<td>57.1</td>
</tr>
<tr>
<td>Overall accuracy</td>
<td>66.7</td>
<td>23.8</td>
<td>0.0</td>
<td>7.1</td>
<td>0.0</td>
<td>14.3</td>
<td>0.0</td>
<td>7.1</td>
<td>21.4</td>
<td>0.0</td>
<td>50.0</td>
<td>57.1</td>
</tr>
</tbody>
</table>

Table 4
Coefficients for canonical discriminant analysis performed on the natural log concentrations in Lake Champlain sea lamprey larval statoliths collected from the sources presented in grouping B.

<table>
<thead>
<tr>
<th></th>
<th>Canonical Variate 1</th>
<th>Canonical Variate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln (Mg)</td>
<td>−0.37</td>
<td>0.38</td>
</tr>
<tr>
<td>Ln (Mn)</td>
<td>−0.17</td>
<td>−0.04</td>
</tr>
<tr>
<td>Ln (Zn)</td>
<td>0.48</td>
<td>−0.03</td>
</tr>
<tr>
<td>Residuals Ln (Rb)</td>
<td>0.62</td>
<td>0.42</td>
</tr>
<tr>
<td>Ln (Sr)</td>
<td>0.40</td>
<td>0.95</td>
</tr>
<tr>
<td>Ln (Ba)</td>
<td>−0.17</td>
<td>0.48</td>
</tr>
<tr>
<td>Cumulative proportion</td>
<td>0.38</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Figure 2. Canonical discriminant analysis of sea lamprey larvae statolith signatures from the seven Lake Champlain sources (rivers and cluster of rivers) presented in grouping B. Source symbols are as follows: cluster B2 (●), cluster B3 (△), cluster B7 (+), Great Chazy River (○), Lewis Creek (□), Malletts Creek (■), Poultney River (×). Tributaries in each cluster (B2, B3 and B7) are listed in Table 1.
The performance of classification using MLE is affected by factors such as sample size in the baseline (e.g., larvae from each stream), sample size in the group mixture (e.g., adults), and the number of classification variables (e.g., elements) (Millar, 1987, 1990; Wood et al., 1989). But more importantly, accurate prediction of natal origin using natural tags strongly relies on three criteria: (i) each group in the baseline should be characterized by specific and reproducible markers; (ii) all possible groups contributing to the group mixture should be characterized; and (iii) group-specific markers should be stable over the interval between characterization (baseline) and mixing (group mixture) (Campana et al., 2000).

According to the first criterion defined by Campana et al. (2000), statolith chemistry among larvae from different tributaries should be characterized by specific and reproducible markers. The wide range of larval stream assignment accuracy (from around 25 to 80%) indicates that in the Lake Champlain watershed some streams present more specific and reproducible markers than others, which shows that the statolith microchemistry approach is not necessarily appropriate to depict the chemical characteristics for all streams. Interestingly, even adults from streams with highly specific signature showed poor success in natal origin assignment. This discrepancy is well illustrated by Lewis Creek. With a classification accuracy of 80%, larvae from Lewis Creek present specific markers. They mostly differ from the larvae of other streams by their relatively low statolith Rb and Zn concentrations. However, none of the adults originating from Lewis Creek were successfully assigned to this river. Consequently, factors other than the ability to discriminate among the geographic locations in the reference groups (larvae) explain the unsuccessful natal origin identification for adult sea lamprey.

According to the second criterion defined by Campana et al. (2000), all the groups contributing to the group mixture should be characterized in the baseline. Uncharacterized groups of fish present in the stock mixture could be mistakenly interpreted and assigned to a group that was characterized in the baseline (Gillanders, 2005). Consequently, an exhaustive identification of potential sources is critical (Waldman and Fabrizio, 1994). Our study meets this criterion: although we did not sample all streams that contain sea lamprey larvae, the five streams from which the adults originated were known and characterized in the baseline.

### Table 5

Sea lamprey statolith elemental signatures for larvae (L) and adults (A) from the seven Lake Champlain sources of grouping B. Median (min–max) concentrations in ppm are presented for all elements except Rb for which the residuals of the ANOVA between ln(Rb) and the mode of preservation, as explained in the Material and methods section, are shown.

<table>
<thead>
<tr>
<th>Natai origin</th>
<th>Mg × 10²</th>
<th>Mn</th>
<th>Zn</th>
<th>Rb residuals</th>
<th>Sr</th>
<th>Ba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Chazy River (L)</td>
<td>45.3 (32.7–243.5)</td>
<td>48.8 (343.4–274.4)</td>
<td>19.3 (0.4–77.3)</td>
<td>0.1 (–0.5–0.7)</td>
<td>341.3 (263.0–1169.3)</td>
<td>23.9 (126–792)</td>
</tr>
<tr>
<td>B2 (L)</td>
<td>56.3 (36.0–76.5)</td>
<td>63.0 (25.9–250.7)</td>
<td>6.7 (0.7–428.5)</td>
<td>0.5 (–1.0–1.8)</td>
<td>582.0 (329.2–3190.4)</td>
<td>24.9 (10.0–142.4)</td>
</tr>
<tr>
<td>B2 (A)</td>
<td>47.6</td>
<td>114.5</td>
<td>18.2</td>
<td>1.2</td>
<td>482.4</td>
<td>42.6</td>
</tr>
<tr>
<td>B3 (L)</td>
<td>34.7 (21.9–73.6)</td>
<td>35.4 (169–742)</td>
<td>25.2 (20–75.4)</td>
<td>0.6 (0.0-0.3)</td>
<td>6750.0 (368.9–1645.5)</td>
<td>21.9 (50–429.5)</td>
</tr>
<tr>
<td>B3 (A)</td>
<td>10.0</td>
<td>0.3</td>
<td>4.3</td>
<td>1.2</td>
<td>564.5</td>
<td>42.6</td>
</tr>
<tr>
<td>Poulney R. (L)</td>
<td>59.30 (36.4–105.9)</td>
<td>75.4 (408.8–112.0)</td>
<td>11.0 (1.4–64.5)</td>
<td>−0.4 (–0.9–0.3)</td>
<td>6760.0 (537.3–1760.8)</td>
<td>19.8 (50–416.4)</td>
</tr>
<tr>
<td>Lewis C. (L)</td>
<td>55.3 (42.8–70.2)</td>
<td>54.8 (34.4–105.0)</td>
<td>1.9 (0.2–33.6)</td>
<td>−1.1 (–2.4–0.5)</td>
<td>6196.0 (411.8–1256.0)</td>
<td>24.8 (9.3–191.1)</td>
</tr>
<tr>
<td>Lewis C. (A)</td>
<td>74.1 (54.6–89.5)</td>
<td>61.6 (46.7–110.3)</td>
<td>46.8 (1.9–259.7)</td>
<td>1.1 (0.6–1.5)</td>
<td>6529.0 (4746–1387.3)</td>
<td>35.6 (18.3–120.2)</td>
</tr>
<tr>
<td>Malletts C. (L)</td>
<td>54.8 (313–335.7)</td>
<td>48.1 (323–643)</td>
<td>21.9 (1.0–66.7)</td>
<td>0.0 (–0.6–0.7)</td>
<td>6293.0 (3466–1825.6)</td>
<td>8.9 (4.5–46.3)</td>
</tr>
<tr>
<td>Malletts C. (A)</td>
<td>63.2 (51.1–90.7)</td>
<td>76.4 (503.1–182.2)</td>
<td>64.1 (29–222.8)</td>
<td>1.4 (0.9–1.6)</td>
<td>6935.0 (613.3–1033)</td>
<td>19.8 (14.5–48.2)</td>
</tr>
<tr>
<td>B7 (L)</td>
<td>47.4 (33.1–169.1)</td>
<td>53.7 (169–218.6)</td>
<td>18.6 (0.7–123.3)</td>
<td>−0.6 (–2.0–0.7)</td>
<td>10635.0 (462.7–1853.8)</td>
<td>20.8 (7.8–63.7)</td>
</tr>
<tr>
<td>B7 (A)</td>
<td>64.4 (62.4–65.0)</td>
<td>74.3 (498.9–995)</td>
<td>414.3 (1219.9–954.4)</td>
<td>0.7 (0.6–1.2)</td>
<td>15806.0 (9167–1677.3)</td>
<td>62.0 (26.5–96.6)</td>
</tr>
</tbody>
</table>

Fig. 3. Percent composition estimates derived from mixed-stock analysis (grey bars) and percent composition of known-origin sea lamprey adults from Lake Champlain (white bars). B2 = cluster B2, B3 = cluster B3, B7 = cluster B7, GCR = Great Chazy River, LEW = Lewis Creek, MAL = Malletts Creek, POUL = Poulney River.

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### Table 6

Probabilities associated with the t-test comparisons of statolith chemistry between sea lamprey larvae and adults originating from Lewis Creek and Malletts Creek. A t-test was performed for each element and each river using the log-natural transformations for all elements except for Rb where the residuals of the ANOVA between ln(Rb) and the mode of preservation were considered. Significant effects (P < 0.05) are indicated by asterisks.

<table>
<thead>
<tr>
<th>Element</th>
<th>Stream of origin</th>
<th>Lewis Creek</th>
<th>Malletts Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>&lt;0.001*</td>
<td>0.826</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.015*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>&lt;0.001*</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td>Rb residuals</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>0.786</td>
<td>0.483</td>
<td></td>
</tr>
<tr>
<td>Ba</td>
<td>0.001*</td>
<td>0.001*</td>
<td></td>
</tr>
</tbody>
</table>
The third criterion of Campana et al. (2000) requires that group-specific markers remain stable over the interval between the larval stage (used for group characterization) and the adult stage (for which natal origin has to be assigned). The comparison of statolith chemistry between larvae and adults revealed that the concentrations of most elements vary between the two life stages, with the most notable differences reported for Rb and to a lesser extent for Zn and Ba. Therefore, the criterion of stability was not met for all of the group-specific markers. In their study, Brothers and Thresher (2004) reported higher variability in Rb and Zn concentrations for adults than for larvae from the Black Mallard river (Lake Huron tributary), although the differences between the two life-stages were not significant. It is important to emphasize that the higher Rb concentrations in adults are not an artifact of preservation method. Frozen samples exhibit lower Rb concentrations than alcohol-preserved samples (Hand et al., 2008; Howe, 2006). Adults were frozen so their higher Rb concentrations cannot be an effect of the preservation method. Conversely, Sr did not differ between the two life stages from the same streams, suggesting that the stability criteria was met for this element. The effects of the non-stability of group-specific markers on our ability to identify natal origin of adult sea lamprey depend on the amplitude of the variation and the power of elements to discriminate among lampreys from different streams. Rubidium, which shows the most notable change between larvae and adults for Lewis Creek and Mallets Creek, is among the most important elements for larval discrimination in our study as well as others (Brothers and Thresher, 2004; Hand et al., 2008).

The stability of group-specific markers might be affected in at least two ways. First, investigations into the natural tag properties of otoliths revealed that elemental composition of individuals from the same stream may vary among years, in relation to annual changes in water chemistry (Gillanders, 2002b; Schaffer and Winkelman, 2008). Such temporal variations might complicate natal origin identification. As an example, natal origin discrimination success of one-year-class juvenile striped bass Morone saxatilis from two tributaries of Lake Texoma (Oklahoma-Texas) was lower when the discriminant functions were based on individuals from another year-class compared to individuals from the same year-class (Schaffer and Winkelman, 2008). To account for temporal variability, it is often recommended to match cohorts between fish used in the baseline to characterize the potential natal origins and fish of unknown origin (Gillanders, 2002a; Walther and Thorrold, 2009). Because sea lamprey age estimation is still problematic (Dawson et al., 2009), the sea lamprey larvae and adults from our study could not be assigned to any cohort. However, we can offer an estimation of their stream residency time. The adults from our study were first captured and tagged in fall 2001 and fall 2002, when they were in their stream of origin (Howe et al., 2006). Assuming that the larval phase in Lake Champlain sea lamprey lasts for four years before metamorphosis (Marsden et al., 2003), the adult sea lampreys were in their natal streams as larvae from 1997 to 2001 or from 1998 to 2002. The larvae from this study were collected from tributaries in 2002, 2003, 2004, and 2005 and they had probably spent a few years (less than four) in these streams before collection. Consequently, the adults might have been in streams as larvae at the same time as the larvae collected in this study. However, the duration of this overlap remains unknown and could be limited. The uncertainties associated with the temporal variability of stream-specific signatures are also amplified by our approach itself. Stream chemical signatures were characterized using measurements in the entire statoliths of sea lamprey larvae, integrating material deposited over the several years of larval stream residency. The extent to which the statolith chemical variability integrated over these years accurately depicts the variability occurring over a longer time period is unknown. In addition, as statolith size increases at a decreasing rate (Meeuwig and Bayer, 2005), it is very likely that earlier years contributed more to stream-specific chemical signatures than older years. Consequently, the extent to which temporal variability affects our ability to identify natal origin of adult sea lamprey remains unclear.

Second, group-specific markers can also be affected by ontogenic effects. Unlike otoliths (Campana and Neilson, 1985), the absence of reworking of previously deposited statolith material has never been demonstrated. Two lines of evidence suggest the potential for chemical reworking of the statoliths. First, statoliths are made of apatite, a basic form of calcium phosphate (Carlström, 1963). The same mineral is found in teeth, bones and scales (Ikoma et al., 2003; Pasteris et al., 2008), for which resorption has been shown (Kacem et al., 1998; Witten et al., 2000). Second, Barker et al. (1997) reported cases where statoliths were found in larvae but they were not systematically present in metamorphosing lampreys from the same stream, suggesting that statolith resorption might be related to stream calcium content. Resorption in bones or scales is often associated with events of intense physiological stress (Kacem et al., 1998). For sea lamprey, metamorphosis and sexual maturation are events of intense physiological changes (Larsen, 1980; Youson, 2003) that might induce restructuring of the statolith.

The results of this study invite further research regarding statolith chemical stability through ontogeny and, at this time, we do not recommend using statolith microchemistry to classify adults to a natal site. Because the sea lamprey control program in Lake Champlain and the Great Lakes would greatly benefit from the natal origin identification of sea lamprey adult spawners, by improving the prioritization of streams to be treated with chemical lampricides for example, we strongly recommend pursuing the efforts to validate this approach.

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References


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