GREAT LAKES FISHERY COMMISSION

2006 Project Completion Report[1]

# Micro-elemental Analysis of Statoliths as a Tool for Tracking Tributary Origins of Sea Lamprey

by:

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**ABSTRACT:**

The analysis of otolith micro-elemental composition has been a valuable tool for differentiating among local spawning populations, and identifying origins of recruits to the fishery. Herein, we explored whether the analysis of sea lamprey (*Petromyzon marinus*) statolith micro-elemental composition by laser-ablation inductively-coupled plasma-mass spectrometry (LA-ICP-MS) could be used as a tool to 1) discriminate among sea lamprey larvae collected from Lake Huron tributaries, and 2) classify a mixed-sample of unknown-origin parasites or spawners back to their natal source. By providing the GLFC with an alternate means, to labor-intensive tagging studies, to determine relative contributions of parasites and spawners from various spawning tributaries, we sought to enhance the GLFC's ability to prioritize sea lamprey control efforts. As part of this effort, we analyzed statoliths of larvae collected in 45 Lake Huron tributaries during 2004 and 2005, as well as 72 female spawners collected in the Thessalon and Opequeoc rivers during 2005. Our analyses were conducted at three different classification scales: by geologic zone (n = 4 zones), by watershed (n = 9 watersheds), and by individual stream (n = 45 streams). Similar to a previous GLFC pilot study conducted using Proton Induced X-ray Emission (PIXE), we found that LA-ICP-MS analysis of statolith micro-elemental composition could be used to reliably differentiate among individuals produced in different regions (i.e., geologic zones, watersheds, streams), with the level of successful discrimination varying with classification scale. Regardless of the scale of classification, rubidium (Rb), strontium (Sr), manganese (Mn), and barium (Ba) were always most important for discrimination, with zinc (Zn), magnesium (Mg), and lead (Pb) being less useful. Analysis of water and sediment samples from 31 Lake Huron and Lake Champlain tributaries helps to understand these differences in utility for discrimination in that concentrations of Rb, Sr, Mn, and Ba in larval statoliths and ambient water were correlated, whereas concentrations of Zn, Mg, and Pb were not. Analysis of left-right statolith pairs also demonstrate that Zn, Mg, and Pb were not analytically stable in our analyses for reasons that are not entirely clear. Ultimately, using Rb, Sr, Mn, and Ba in maximum-likelihood estimation analyses conducted on a mixed-stock of adult spawners collected in 2005, we found that the sources contributing the greatest proportion of adults to our mixed sample were: 1) the Southern Lake Superior geologic zone (located north of Lake Huron); 2) the Wanipiti-French watershed; and 3) Beavertail Creek, Mississagi River, Lauzon Creek, Garden River, and Musquash River. However, these results need to be viewed with some caution, given are lack of success in accurately typing a sample of known-origin (tagged) spawners back to their natal streams in parallel study conducted in Lake Champlain. In discussing these results, we identify analytical and research needs that could further support our findings, and advance the use of statolith microchemistry as a means to identify natal origins of parasitic and adult sea lamprey in Lake Huron.

**PRESS RELEASE:**

Scientists at the University of Windsor (Carrol Hand and Brian Fryer), NOAA's Great Lakes Environmental Research Laboratory (Stuart Ludsin), and the University of Vermont (Ellen Marsden) are using features in the "ears" of sea lamprey, an invasive species in the upper Great Lakes, to find out which streams are producing lampreys that become parasites on fish.

Sea lampreys have been devastating Great Lakes fish populations for over 60 years. For nearly 50 years, the Great Lakes Fishery Commission has been working to reduce this damage by controlling sea lamprey populations, mostly by poisoning larval sea lamprey in their originating streams. Streams are targeted because sea lampreys spend up to six years in their natal stream as larvae, whereas they only spend the last year of their lives in the lake as parasites on fish before returning to streams to reproduce and die. Information about which streams produce the most parasites and spawning adults would improve the success of the control program, and reduce costs and use of lampricides.

The research team is using a state-of-the-art analysis, known as laser-ablation inductively-coupled plasma-mass spectrometry, to look at the trace-metal chemistry of sea lamprey ear 'stones', also called statoliths. Essentially, this instrument sends a laser beam through a microscope and ionizes the statolith into a gas. This gas is then brought into an instrument that measures the trace-metal composition.

Statoliths accumulate trace materials from the water in which the lampreys live. Therefore, each lamprey should have a chemical 'signature' that is unique to the stream in which it lived as a larvae. By analyzing the chemical composition of statoliths of parasites captured in the open lake or reproducing adults captured in streams, and matching it to stream-specific chemical signatures developed from larvae, it is possible to determine from which stream each individual originated. In instances where streams are chemically similar to one another, it may be possible to at least identify the watershed or general geographic area where each individual was produced. Microchemical data were collected from larval sea lamprey captured in 45 streams in the Lake Huron watershed and 23 streams in the Lake Champlain watershed. Then, adult sea lampreys from each lake were examined to determine their stream of origin.

The scientists were able to discriminate many streams from each other using the chemical data. It was, however, more challenging to determine the origins of the adult spawners. The general areas from which sea lamprey originated in Lake Huron were: 1) the Southern Lake Superior geologic zone (located north of Lake Huron); 2) the Wanipiti-French River watershed; and 3) Beavertail Creek, Mississagi River, Lauzon Creek, Garden River, and Musquash River.

Further work needs to be done to test the accuracy of their findings and to find other ways, such as stable isotopes, to help distinguish among streams. Even so, the team is optimistic that this method will ultimately prove to be a useful tool in the battle against invasive sea lamprey in the Great Lakes.

**SUMMARY STATEMENT:**

There were three primary objectives to our research:

1. *Determine whether statolith elemental signatures differ among larvae produced in different Lake Huron streams;*
2. *Quantify relative contributions of parasitic and spawning lamprey from important production tributaries in Lake Huron; and*
3. *Develop relationships to predict statolith elemental chemistry from water chemistry.*

## Report Layout

Below, we summarize the research conducted for each objective. Much of the work conducted as part of Objective 1 has been incorporated into a submitted (Appendix 1) and soon-to-be-submitted (Appendix 2) manuscript. Thus, our summary of Objective 1 is brief, and the reader is referred to Appendices 1 and 2 for more details. Objectives 2 and 3 have not yet been incorporated into manuscript format. As such, for both Objectives 2 and 3, we provide a more detailed discussion of the background rationale, methods, results, and implications in this Summary Statement section. Finally, following the summary of Objective 3, but before the Appendices, we provide a bulleted overview section that provides a synopsis of our conclusions and recommendations to the GLFC.

## Objective 1 Summary

The results for Objective 1 are contained within two drafted manuscripts, one that is in review at *Canadian Journal of Fisheries and Aquatic Sciences* (Appendix 1) and another that should be submitted

during winter 2006-2007 (Appendix 2). We feel that we more than accomplished the work we set out to do as part of this objective, including conducting a variety of additional analyses that were not originally proposed. Appendix 1 provides a preliminary assessment of the usefulness of this technique for discrimination, and includes **1) a rigorous protocol for preparing and processing statoliths for micro-elemental composition using laser-ablation inductively-coupled plasma-mass spectrometry (LA-ICP-MS), 2) findings that demonstrate both freezing and ethanol preservation can be used as methods to store statoliths, 3) a statistical determination of minimum sample size of larvae that should be used to characterize stream-specific signatures (i.e., n = 15 larvae/stream), 4) support for our conclusion that LA-ICPM-MS offers a better tool for quantifying micro-elemental composition of statoliths in the Great Lakes (but perhaps not other systems) than PIXE (as used by Brothers and Thresher 2004), and 5) evidence to indicate that micro-elemental composition of statoliths differs among larval production streams in many of the Great Lakes, including lakes Huron, Superior, and Michigan.**

Appendix 2 focuses more intensively on our ability to discriminate among 45 Lake Huron tributaries for which we had larvae (collections were made by both the Canadian Department of Fisheries and Oceans and US Fish and Wildlife Service during 2004 and 2005). In an effort to maximize the potential value of our results to the GLFC, we explored our ability to discriminate larval production areas at three classification scales: by geologic zone (n = 4 geologic zones), by watershed (n = 9 watersheds), and by individual stream (n = 45 streams). **Overall, our results demonstrate that statolith microchemistry holds promise for differentiating among sea lamprey larval production tributaries in Lake Huron, but that this technique will not offer perfect discrimination for all potential sources at each classification scale.** Specifically, conducting linear discriminant function analyses with a suite of seven elements (Rb, Sr, Mn, Ba, Zn, Mg, and Pb), we were able to differentiate a) 2 of 4 geologic zones (Grenville and younger Paleozoic) with ~80% accuracy, b) 3 of 9 watersheds with > 80% accuracy (East Lake Huron = 100%; Wanipiti-French = 100%; Titabawasee = 82%), and c) 23 of 45 individual streams with >80% classification accuracy (Appendix 2). Importantly, 8 of these 23 streams (i.e., Albany Creek, Blue Jay Creek, Manitou River, Serpent River, Thessalon River, Browns Creek, Gawas River, and Garden River) were Category I streams (i.e., streams with high larval abundance; Morse et al. 2003, Young and Klar 2003) that demonstrated > 90% classification accuracy. In this initial analysis, we were only able to discriminate the St. Marys River from all other rivers with 67% accuracy (Appendix 2).

Knowing that the GLFC was most interested in being able to distinguish important larval production tributaries, we conducted an additional set of analyses aimed at improving our ability to discriminate the most important producers of larvae. Using GLFC Annual Reports on Sea Lamprey Control from 2002-2005 (and unpublished data from 2006) as a guide (not just Morse et al. 2003 and Young and Klar 2003, per above), we compiled a new list of 18 important production tributaries (Table 1). To potentially improve our ability to discriminate these streams from others, we ran a weighted hierarchical cluster analysis (Euclidean distance measure, Ward's method), using the mean elemental concentrations (weighted by sample size). We ran two sets of analysis, one with all seven elements (Rb, Sr, Ba, Mn, Mg, Zn, and Pb) and one with only the four most reliably measured elements (Rb, Sr, Ba, and Mn; see Objectives 2 and 3 below). We then used a linkage distance cutoff of 10 (7-element analysis) and 6.5 (4-element analysis) to create 1- or 2-stream clusters (Table 1), with which we could then attempt to discriminate using linear discriminant function analysis (LDFA). By forming these 1- and 2-stream clusters, the number of groups to be discriminated was reduced from 45 streams to 40 streams/stream pairs and 34 streams/stream pairs for the 7-element and 4-element analyses, respectively. Only in one instance were two "important" tributaries paired together (i.e., the Saginaw and Rifle rivers in the 4-element analysis; see Table 1).

The LDFAs conducted on these 40 and 34 stream groups resulted in an average correct classification rate of 68.4% and 59.5%, respectively, which was not all that different from our overall classification rate when all streams were treated individually (68%; see Appendix 2). In comparing the classification rates

for our 18 important tributaries (again, some of them were clustered with another stream; see Table 1), we found that clustering streams generally did not have a major effect when comparing results from analyses with all seven elements (Table 1); the only obvious change was an increase in the classification accuracy of the St. Marys River from 67% to 80% when this river was clustered with Elliot Creek (Table 1). We also found that the classification accuracy of the St. Marys River (when paired with Elliot Creek) could be improved to 91.1%, if up to 3-stream clusters (linkage distance cutoff of 20) were allowed to be formed (C. Hand, unpub. data). For the 4-element analyses, classification accuracies increased for 11 of the 18 streams, did not change for three of them, and actually decreased for four of them, when up to two-stream clusters were allowed to be formed (Table 1). Further, while we saw a decrease in our ability to correctly distinguish the Cheboygan and Thessalon rivers, we found an overall increase in the number of streams that were classified correctly between 70% to 90% of the time and a reduction in streams that were classified correctly less than 50% of the time (Table 1).

Ultimately, our suite of findings from Objective 1 highlights two important considerations for future statolith microchemistry research. First, **the analysis of micro-elemental concentrations is imperfect when it comes to discriminating among all potential sources of larvae.** One reason is that many of the streams within a watershed or within a geologic zone are exposed to the same groundwater or upstream pollutant sources. A second reason for this limitation is inter-annual variation in stream-specific signatures that likely arises because of variation in runoff and pollution that influence water chemistry (see Objective 3 results below). Owing to issues associated with inter-annual variability in stream-specific signatures, we **encourage the GLFC to consider collecting larvae from all larval production streams within the same year when possible.**

Indeed, our analyses (Appendix 2) and the analysis of otolith microchemistry data from other systems (Fabrizio 2005) have clearly demonstrated improved discrimination success when data from multiple years are not combined. The use of stable isotopes, which do not vary through time among tributaries (Kennedy et al. 2000), also might help reduce the impact of inter-annual variation. We also encourage continued efforts to better quantify the magnitude of inter-annual variation within streams. Doing so would help put our two years of data into context, and also help determine an "average" stream signature for Lake Huron tributaries. In turn, this average steam signature might improve our ability to correctly type back individuals to natal streams, given that the larval statolith signature represents up to six years of stream residence, not just one or two years.

Second, our ability to discriminate important production streams improves when more elements are included in the analysis (Table 1). Thus, **future statolith microchemistry research should consider combining techniques for quantifying elemental concentrations that complement one another.** One recommendation would be to first analyze statoliths with PIXE because it is non-destructive and can quantify some elements (e.g., Fe, Hg, Pb) better than LA-ICP-MS. Afterwards, the same statolith could be analyzed with LA-ICP-MS, which has its analytical advantages (e.g., higher precision, an ability to quantify Ba). In this way, a wider range of elements could be quantified, which likely would improve discrimination abilities. Another recommendation would be to combine the analysis of elemental concentrations with analysis of statolith stable isotope composition.

*Table 1. Classification accuracies (% Correct) from linear discriminant function analyses conducted on larval sea lamprey collected in 45 Lake Huron streams during 2004-2005. Only our 18 most important larval production streams are listed. Analyses were conducted using either seven elements (Rb, Sr, Ba, Mn, Mg, Zn, and Pb) or four elements (Rb, Sr, Ba, and Mn). In addition to conducting analyses with individual streams (n = 45; columns with "No clustering"), we used a hierarchical cluster analysis to group up two streams into a common cluster, based on their elemental composition (columns with "Clustering"). Stream names in the "2-Stream Cluster" columns indicate the other stream in that cluster. With exception of the Saginaw and Rifle River, both of which are considered an important production stream, the other listed streams are not considered important producers of larvae, and hence, is not listed. A blank in the "2-Stream Cluster" column indicates that stream is its own cluster in the analysis.*

| **Larval Production Stream** | **2- Stream Cluster (7 elements)** | **% Correct No Clustering (7 elements)** | **% Correct Clustering (7 elements)** | **2- Stream Cluster (4 elements)** | **% Correct No Clustering (4 elements)** | **% Correct Clustering (4 elements)** |
|---|---|---|---|---|---|---|
| Black Mallard R. | | 75.0 | 75.0 | With Gordon Cr. | 75.0 | 36.0 |
| Cheboygan R. | | **85.7** | **85.7** | With Mindemoya R. | **92.9** | 72.7 |
| Devils R. | | 53.3 | 53.3 | With Browns Cr. | 20.0 | 50.0 |
| French R. | | **100.0** | **100.0** | | **80.0** | **86.7** |
| Garden R. | | **93.3** | **93.3** | | **100.0** | **100.0** |
| Little Munuscong R. | | 40.0 | 40.0 | | 60.0 | 60.0 |
| McKay Cr. | | 73.3 | 73.3 | | 33.3 | 46.7 |
| Mississagi R. | With Hessel Cr. | 40.0 | 56.7 | With Hessel Cr. | 6.7 | 33.3 |
| Naiscoot R. | | 36.7 | 36.7 | With Magnetewan R. | 33.3 | 73.3 |
| Nottawassaga R. | | **80.0** | **80.0** | | 60.0 | 63.3 |
| Pine R. | | 10.5 | 5.3 | With Lauzon Cr. | 0.0 | 29.4 |
| Rifle R. | | 30.8 | 38.5 | With Saginaw R. | 23.1 | 53.7 |
| Saginaw R. | | 60.7 | 57.1 | With Rifle R. | 32.1 | 53.7 |
| Sauble R. | | **86.7** | **86.7** | | **93.3** | **93.3** |
| Spanish R. | With Grace Cr. | 66.7 | 53.3 | | 46.7 | 40.0 |
| St. Marys R. | With Elliot Cr. | 66.7 | **80.0** | With Elliot Cr. | 60.0 | 73.3 |
| Tawas R. | | 73.3 | 73.3 | | 56.7 | 60.0 |
| Thessalon R. | With Gawas R. | **100** | **96.7** | | **93.3** | **86.67** |

## Objective 2 Summary

Background. Objective 2 focused specifically on determining what role individual Lake Huron tributaries play in contributing parasitic- and spawning-phase sea lamprey, with initial emphasis placed on important larval production streams such as the St. Marys River. Our initial goal was to determine the origins of adults and spawners captured during 2004 and 2005. No parasites were provided to us because any that were collected were being used for the Hammond Bay Biological Station tagging program. Also, no spawners were processed from 2004; the entire 2004 sample was unfortunately lost somewhere in transition between NOAA-GLERL and the University of Windsor. From 2005, we processed statoliths from adult females captured as part of the sterile male release trapping program in Thessalon and Opequeoc rivers. We also received adult sea lamprey captured in the St. Marys River; however, their statoliths were severely degraded upon arrival and could not be used. *We recently received a large sample of 2006 female adult spawners that we still plan to analyze during December 2006.*

Methods. In total, we processed 72 spawning adult females collected by the US Fish and Wildlife Service during summer 2005 from the Thessalon and Opequeoc rivers. All samples were provided frozen.

All statolith preparation took place in a Class 100 clean room. Before statolith removal, the fish were partially thawed, the total length of each individual was measured (TL; to nearest cm), and the head was removed and refrozen. To extract the statoliths, we partially thawed the fish, and used a kitchen knife to make multiple slices perpendicular to the anterior dorsal fin and adjacent to the gill slits, until the two auditory labyrinths were exposed. Both left and right statoliths were then removed on a clean glass slide using clean glass probes. Removed statoliths were transferred using a clean glass probe to a drop of MQW in a clean, covered Petri dish. Rinsed statoliths were then mounted in crystal bond media on a strip of transparency film, with the base perpendicular to the film. Mounted statoliths were then ground using 0.3-µm aluminum-oxide lapping paper, ultrasonically cleaned, and then rinsed in a similar fashion as larvae (Appendices 1 and 2). Cleaned statoliths were then placed on a clean glass slide using double sided Scotch© tape. All glassware was cleaned prior to use, and we used the same LA-ICP-MS analytical and data-handling procedure as for larvae (see Appendices 1 and 2).

In the absence of parasites or adult spawners of known-origin from Lake Huron, we used known-origin adults, collected as part of a parallel Lake Champlain Sea Grant-funded program (Marsden, lead PI), to test our ability to correctly classify individuals back to their natal tributaries. In Lake Champlain, individuals that were tagged as transformers in 2001-2002 were collected as spawners (using portable assessment traps placed in 18 streams) during 2002-2005. In total, 19 known-origin (tagged) sea lampreys were processed and analyzed using identical procedures and instrumentation as the Lake Huron fish. Additionally, all Lake Champlain streams were sampled for larvae as part of the Sea Grant project and by the US Fish and Wildlife Service; stream-specific signatures were developed for 19 of them (no larvae were captured in 4 streams). A detailed description of the Lake Champlain statolith microchemistry component is provided in Appendix 3.

Previous work has demonstrated that instrument bias can be problematic when doing trace-metal work (Campana et al. 1997). Because the University of Windsor's trace-metal analytical laboratory underwent an overhaul during late 2005, being moved into a new floor of the Great Lakes Institute for Environmental Research, we conducted a comparison of left-right statolith pairs form larvae to ensure that samples processed before the move could be reliably compared to all samples processed after the move. For that analysis, we compared elemental concentrations of 82 statolith pairs from larvae collected across numerous Lake Huron streams sampled during 2004 and 2005, as well as several Lake Champlain streams. For each of the seven elements (Rb, Sr, Ba, Mn, Zn, Mg, and Pb) that were important for discriminating among streams, we used linear regression analysis to assess the relationship between statoliths processed before the move to their second statoliths processed after the move. Specifically, we tested whether the slope of this relationship differed from an expected 1:1 line, and if not, whether the intercept differed from zero. All data were $\log_{10}$ transformed to achieve normality, except

for Pb, which was arcsin-square root-transformed (Kolmogorov-Smirnov normality test: all $p > 0.20$). Additionally, outliers (relative standard deviation > 3.5) were removed prior to analysis. In total, we removed 4 outliers for Rb, 3 outliers for Sr, and 1 outlier for Pb.

We used a maximum-likelihood estimation (MLE) method (Campana et al. 1999) to determine the most probable sources of the 72 adult spawners from Lake Huron and the 19 tagged fish from Lake Champlain. Essentially, an MLE approach uses the statistical distribution of elemental signatures of each potential production stream to estimate the proportion of each reference stream population in our mixed sample of unknown adults (Campana et al. 1999). This method is preferred to other classification methods, such as LDFA, especially when the number of potential spawning stocks is high (Millar 1990, Fabrizio 2005). The major assumption with this method is that all potential spawning tributaries are represented in our reference group, otherwise the estimate might be biased because all individuals are designated a stream of origin.

For the Lake Huron adults, we ran two MLE analyses for each level of classification: geologic zone, watershed, individual stream, and 1- or 2-stream clusters. For each classification scale, one set of analyses was run with normal, unaltered elemental data, and a second with a correction for Mn (see below). Hence, when the proportions of the mixed-stock are reported for any level of classification, we report two values for each level (Table 2).

Results. Analysis of left-right otolith pairs indicates major problems for some elements, but not others. We found a strong, positive relationship between concentrations of Rb, Sr, and Ba in statolith pairs wherein the slope did not differ from a 1:1 line and the intercept did not differ from zero (Figure 1a,b,c). Thus, there was no need to make any corrections for these elements. We also found that the slope of the Mn relationship did not differ from a 1:1 line; however, the intercept was significantly less than zero (intercept = -0.29 ppm), indicating that the new ICPMS estimates were slightly higher across the entire range of concentrations than statoliths processed before the move (Figure 1d). Unfortunately, estimates of Mg, Zn, and Pb appear unreliable, given that the slopes for each element were significantly less than one and the intercepts were significantly greater than zero (Figure 1e,f,g). The relationships for Mg, Zn, and Pb also explained $\leq$ 25% of the variance in our data (Figure 1e,f,g). As such, we did not use these three elements in our MLE analyses of our unknown-origin and known-origin lampreys; determination of adult origins was only made using Rb, Sr, Ba, and Mn, which fortunately, happened to be the four elements most useful for discriminating among our 45 Lake Huron streams (Appendix 2). To help assess any potential bias associated with our estimation of Mn, we ran our mixed-sample analyses of Lake Huron unknowns and Lake Champlain tagged fish with and without a correction factor (i.e., the intercept being subtracted from measured Mn values of adults analyzed during 2006).

Our MLE analysis of 72 Lake Huron spawning females indicates that the Southern Superior geologic zone (located north of Lake Huron, and east of the St. Mary's River) appears to have contributed the most individuals (64% to 70%) in our mixed sample (Table 2). This region contains some important (Category I) production streams, including the Mississagi River, Thessalon River, and Garden River (Table 2). In addition, the Spanish River, a potentially large producer of sea lamprey, also resides within this geologic zone. The Grenville geologic zone, which is located on the northeast coast of Georgian Bay and contains two Category I streams (Naiscoot and French rivers), also contributed between 16% and 22% of the individuals to the mixed sample of unknown adults (Table 2). The Older Paleozoic zone, which contains the St. Mary's River (and 29 other streams), was estimated to contribute only 9% - 10% of the fish to our mixed sample (Table 2).

At the watershed-level, our analyses suggest that the Wanapiti-French watershed, which was represented by only the French River in our analyses, was the single biggest contributor to our mixed sample with 49% to 55% of the samples coming from this watershed (Table 2). Importantly, this watershed and the French River were discriminated with 100% accuracy in both the watershed and individual-stream analyses, with average posterior classification probabilities > 0.82 (Appendix 2). Thus, our confidence in

this assignment is high. The East Georgian Bay watershed was the next most important, contributing 30% to 32% of the individuals to our mixed sample. This watershed also contained Category I production streams, including the Naiscoot and Nottawassaga rivers. Although not Category I producers, the Boyne, Magnetewan, and Musquash rivers also are located in the East Georgian Bay watershed, and also are found in the seemingly important Grenville geologic zone (Table 2). The St. Marys watershed, which contains the St. Mary's River and the Little Munuscong River, was estimated to contribute only 3% - 6% of the fish to our mixed sample (Table 2).

At the individual-stream level, eight streams contributed approximately 85% of the mixed sample of unknowns. These streams were—from most to lest important—Beavertail Creek (18% to 23%), Mississagi River (12% to 16; Category I stream), Garden River (10% to 18%; Category I stream), Musquash River (9% to 11%), Lauzon Creek (9% to 12%), Naiscoot River (7% to 9%; Category I stream), Rifle River (4%; Category I stream), and Timber Bay Creek (3% to 8%) (Table 2). Thus, based on this analysis, the majority of historically important larval production streams, including the St. Marys River, did not contribute many individuals to this mixed sample (Table 2).

Our analysis of the mixed sample using our 1- and 2-stream clusters (and four elements) produced similar results as the individual stream analysis (Table 2). Specifically, Beavertail Creek was again the most important contributor (14% to 22%), followed by the Garden River (15% to 22%), Musquash River (13% to 15%), Hessel Creek and Mississagi River cluster (9% to 12%), Lauzon and Pine River cluster (8%), Timber Bay Creek (5% to 10%), Magnetewan and Naiscoot River cluster (5% to 6%), and the Rifle and Saginaw River cluster (4%). The importance of the St. Marys River and Elliot Creek cluster was minimal (~1%), regardless of whether Mn was corrected for or not (Table 2).

Analysis of known-origin (tagged) individuals from Lake Champlain was rather disappointing. The MLE analysis did not correctly identify any adults to individual streams. When streams were clustered by adjacency, the classification suggested that nearly all of the samples originated from the Saranac River (n = 1), Mount Hope Brook, Putnam Creek, Mill Brook, and Salmon River. However, based on tagging information, we know these samples originated from the Saranac River (1), Morpion Stream, Pike River, Mallets Creek, and Lewis Creek. Even after clustering streams by geologic drainage (cluster D in Table 3 in Appendix 3), which allowed for the best classification accuracies of Lake Champlain streams (ranging between 65% and 100% for all drainages; see Table 6 in Appendix 3), our classification accuracy was < 5%. The MLE analysis suggested that 92% to 100% of the samples should have come from cluster C1, which consisted of the Saranac River and Saranac River Delta, Ausable River, and the Salmon River. Overall, only one individual, the one tagged in the Saranac River, was likely correctly classified (Appendix 3).

Discussion. Analysis of the 72 Lake Huron spawning adults indicates that important larval production streams may not be contributing to the adult spawning population as much as larval abundance estimates would indicate. While some Category I streams (e.g., Mississagi, Garden, Naiscoot, and Rifle rivers) were indeed to be found to be contributing to our mixed sample, nearly all others, including the St. Marys River, were found largely unimportant. Instead, several non-Category I streams were suggested as being important, including Beavertail Creek, Musquash River, Lauzon Creek, and Timber Bay Creek. **If our findings prove to be accurate, they clearly suggest caution when relying on historical larval abundance patterns as an indicator of the relative importance a particular stream.**

Assuming that we can actually trust the accuracy of assignment of stream origins (see below), there are two likely reasons that some non-Category I streams might be contributing disproportionately to our adult mixed sample. First, survival rates may not be constant among streams, owing to density-dependent effects. For example, high in-stream larval abundance theoretically could lead to reduced in-stream survival through food or habitat limitation, or perhaps reduced growth (Rodriquez-Munoz et al. 2003, Zerrenner and Marsden 2005), which could negatively affect post-transformation survival. Conversely,

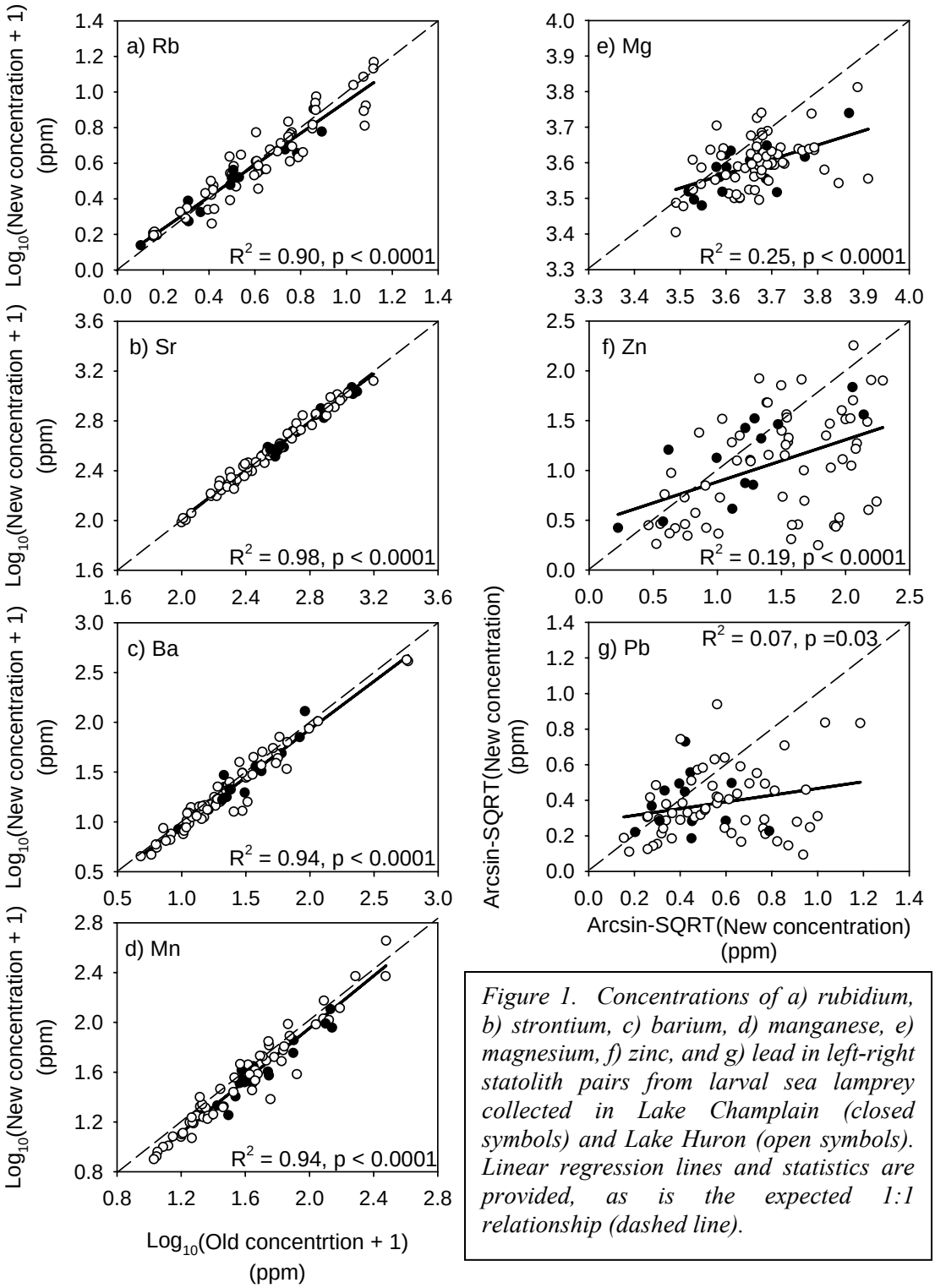


*Figure 1. Concentrations of a) rubidium, b) strontium, c) barium, d) manganese, e) magnesium, f) zinc, and g) lead in left-right statolith pairs from larval sea lamprey collected in Lake Champlain (closed symbols) and Lake Huron (open symbols). Linear regression lines and statistics are provided, as is the expected 1:1 relationship (dashed line).*

streams with few larvae may have high growth rates (Rodriquez-Munoz et al. 2003, Zerrenner and Marsden 2005) that confer a survival advantage both pre- and post-metamorphosis. Although evidence for density-dependent growth and survival has been equivocal in the Great Lakes(Jones et al. 2003), we feel that more research is needed to get a better handle on density-dependent effects on growth and survival in Lake Huron.

A second potential biological reason for the unexpected high contributions from non-category I streams may relate to lampricide treatment schedules. For example, Beavertail Creek, which contributed 17%-23% of the individuals in our sample, has not been treated since 2000. Likewise, as far as we can tell, other important contributors, including the Garden River (10%-22%) and Timber Bay Creek (5%-10%), have not been treated since 2001. Interestingly, we also found that many of the historically important (Category I) larval production streams that were treated during 2004 (e.g., St. Marys , Sauble, Cheboygan, Devils, and Little Munuscong rivers), contributed no individuals to our mixed sample. The only exceptions were the Naiscoot River, which contributed ~10% of the individuals, and the Mississagi River, which contributed 12% to 16% of the individuals. Further, the remaining nine streams that were treated during 2004, for which we also had a signature, contributed $\leq$ 7% (combined), with six of them contributing no individuals. **Thus, it appears that larval treatments are generally successful in reducing contributions to the next year's batch of adult spawners. By contrast, a long gap between treatments for some streams (e.g., Beavertail Creek, Garden River, Timber Bay Creek) appears to allow detectable contributions of larvae to adult stages.**

Unfortunately, owing to the poor results from our MLE analysis of known-origin (tagged) adults and spawners, **our results need to be viewed with some caution.** Again, our success rate of correctly assigning tagged fish to their natal sites in Lake Champlain was 0% at the individual stream level, and only 5% when nearest streams were clustered, despite a good ability to discriminate larvae among nearly all streams (average = ~75%; Appendix 3). As for the cause of our inability to correctly assign adults, we are not quite certain. However, we offer several potential explanations.

Inter-annual variability in statolith signatures (per Appendix 2) might limit our ability to properly type back individuals to their natal stream. With exception of the Great Chazy River, for which we had samples from two years, Lake Champlain stream-specific signatures were based on one year of data from each stream (Appendix 3). By contrast, the larval material in our tagged adults represented up to six years of larval growth (and six years of chemical variation). Thus, if it so happened that the chemical signature in that stream for that year was atypical, then our ability to accurately type back adults would decline.

**Inter-annual variation in elemental signatures clearly poses a real limitation to this approach, and as such, we strongly encourage future researchers try to minimize its effects by 1) sampling all tributaries within the same year, 2) conducting studies over multiple years so as to better understand the magnitude of inter-annual variation within and among streams and to allow for development of 'average' elemental signatures for streams that might correlate better with larval material in statoliths from adults, and 3) combine the analysis of elemental data with an approach that is not time-variant, such as stable isotope analysis (e.g., Kennedy et al. 2000).**

Inter-annual variation, however, does not help to explain why nearly all of the tagged fish were typed back to the Saranac River or Saranac River delta (Appendix 3). Instead, it may be that we are not properly removing the chemical signature associated with non-stream residence, and that this post-transformation signature associated with residence in Lake Champlain proper is swamping the larval signature. While we know of no definitive way to determine whether the larval core is being properly exposed (and the post-metamorphosis layers removed), the fact that we are getting detectable variation in Lake Huron adults seemingly rules out the possibility that our processing procedure (e.g., polishing with aluminum- oxide lapping film) is contaminating the statolith by leaving contamination that swamps real environmental variation. We also sought advice from Ed Brothers, who has experience processing sea

*Table2. Results from maximum likelihood estimation (MLE) analyses of a mixed sample of 72 unknown-origin spawning females collected in Lake Huron tributaries during 2005. Only the elements Rb, Sr, Ba, and Mn were used. Map refers to the location in Figure 1 of Appendix 2. GEO, WAT, and CLU refer to the geologic zone, major watershed, and 1- or 2-stream cluster for that stream, respectively (streams with a similar letter in the CLU column are a cluster; a blank in this column indicates a cluster with only that stream). MLE results for the four different scales of classification (GEO, WAT, CLU, and STR) are presented. The two values in each column represent the proportion that natal source contributed to the mixed sample of unknowns when Mn was not corrected and corrected for in our analysis, respectively. For the MLE results for GEO, WAT, and CLU analyses, proportions are only presented for the first stream within that grouping. Instances in which proportions do not sum to one are due to rounding error. GEO acronyms: OP = Older Paleozoic, GV = Grenville, SS = Southern/Superior, YP = Younger Paleozoic. WAT acronyms: C-P = Carp-Pine, ELH = East Lake Huron, NLH = North Lake Huron, LLO = Long Lake Ocqueoc, W-P = Wanipitai-French, STM = St. Marys, EGB = East Georgian Bay, A-R = Aues Gres-Rifle, TI = Titabawasee.*

| Map | Stream (STR) | GEO | WAT | CLU | MLE GEO | MLE WAT | MLE CLU | MLE STR |
|---|---|---|---|---|---|---|---|---|
| 1 | Albany Cr. | OP | C-P | | 0.10, 0.09 | 0.05, 0.02 | 0.03, 0.03 | 0.03, 0.03 |
| 2 | Beavertail Cr. | OP | C-P | | | | 0.17, 0.22 | 0.18, 0.23 |
| 3 | Bighead R. | OP | ELH | | | 0.00, 0.00 | 0.00, 0.00 | 0.00, 0.00 |
| 4 | Black Mallard R. | OP | LLC | J | | 0.00, 0.00 | 0.00, 0.00 | 0.00, 0.00 |
| 5 | Blue Jay Cr. | OP | NLH | G | | 0.00, 0.00 | 0.00, 0.00 | 0.00, 0.00 |
| 6 | Boyne R. | GV | EGB | | 0.22, 0.16 | 0.32, 0.30 | 0.01, 0.00 | 0.00, 0.00 |
| 7 | Browns Cr. | OP | NLH | D | | | 0.00, 0.00 | 0.00, 0.00 |
| 8 | Caribou Cr. | OP | C-P | C | | | 0.01, 0.02 | 0.03, 0.02 |
| 9 | Cheboygan R. | OP | LLC | I | | | 0.00, 0.00 | 0.00, 0.00 |
| 10 | Devils R. | OP | LLC | D | | | | 0.00, 0.00 |
| 11 | Echo R. | SS | NLH | | 0.64, 0.70 | | 0.00, 0.00 | 0.00, 0.00 |
| 12 | Elliot Cr. | OP | LLC | A | | | 0.01, 0.01 | 0.01, 0.02 |
| 13 | French R. | GV | W-F | | | 0.55, 0.49 | 0.03, 0.02 | 0.00, 0.00 |
| 14 | Garden R. | SS | NLH | | | | 0.15, 0.22 | 0.10, 0.18 |
| 15 | Gawas R. | OP | NLH | G | | | | 0.00, 0.00 |
| 16 | Gordon Cr. | OP | NLH | J | | | | 0.00, 0.00 |
| 17 | Grace Cr. | OP | LLC | | | | 0.00, 0.00 | 0.00, 0.00 |
| 18 | Hessel Cr. | OP | C-P | K | | | 0.12, 0.08 | 0.00, 0.00 |
| 19 | Koshkawong R. | OP | NLH | C | | | | 0.00, 0.00 |
| 20 | Lauzon Cr. | SS | NLH | E | | | 0.08, 0.08 | 0.12, 0.09 |
| 21 | Little Munuscong R. | OP | STM | | | 0.03, 0.06 | 0.00, 0.00 | 0.00, 0.00 |
| 22 | Magnetewan R. | GV | EGB | H | | | 0.06, 0.05 | 0.00, 0.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 23 | Manitou R. | OP | NLH | | | | 0.00, 0.00 | 0.00, 0.00 |
| 24 | McKay Cr. | OP | C-P | | | | 0.01, 0.01 | 0.01, 0.01 |
| 25 | Mindemoya R. | OP | NLH | I | | | | 0.00, 0.00 |
| 26 | Mississagi R. | SS | NLH | K | | | | 0.16, 0.12 |
| 27 | Musquash R. | GV | EGB | | | | 0.13, 0.15 | 0.09, 0.11 |
| 28 | Naiscoot R. | GV | EGB | H | | | | 0.09, 0.07 |
| 29 | Nottawasaga R. | OP | EGB | | | | 0.00, 0.00 | 0.00, 0.00 |
| 30 | Pine R. | OP | C-P | E | | | | 0.01, 0.03 |
| 31 | Rifle R. | YP | A-R | B | 0.04, 0.04 | 0.00, 0.00 | 0.04, 0.04 | 0.04, 0.04 |
| 32 | Saginaw R. | YP | TI | B | | 0.04, 0.04 | | 0.00, 0.00 |
| 33 | Sauble R. | OP | ELH | | | | 0.00, 0.00 | 0.00, 0.00 |
| 34 | Schmidt Cr. | OP | LLC | | | | 0.00, 0.00 | 0.00, 0.00 |
| 35 | Serpent R. | SS | NLH | F | | | 0.00, 0.00 | 0.00, 0.00 |
| 36 | Silver Cr. | OP | EGB | | | | 0.02, 0.02 | 0.03, 0.02 |
| 37 | Spanish R. | SS | NLH | | | | 0.00, 0.00 | 0.00, 0.00 |
| 38 | St. Marys River | OP | STM | A | | | | 0.00, 0.00 |
| 39 | Steeles Cr. | OP | C-P | | | | 0.00, 0.00 | 0.00, 0.00 |
| 40 | Tawas R. | YP | A-R | | | | 0.00, 0.00 | 0.00, 0.00 |
| 41 | Thessalon R. | SS | NLH | | | | 0.00, 0.00 | 0.00, 0.00 |
| 42 | Timber Bay Cr. | OP | NLH | | | | 0.10, 0.05 | 0.08, 0.03 |
| 43 | Trout Cr. | OP | C-P | F | | | | 0.00, 0.00 |
| 44 | Trout R. | OP | LLC | | | | 0.00, 0.00 | 0.00, 0.00 |
| 45 | Watson Cr. | OP | NLH | | | | 0.02, 0.01 | 0.02, 0.00 |

lamprey statoliths (Brothers and Thresher 2004), and we attempted a number of different preparation and ablation procedures, settling on the one we believed to be both efficient and most likely to succeed. **While we feel our statolith processing protocol is sound, we do feel that more effort is needed to ensure that statolith processing is not responsible for our inability to correctly type back tagged individuals.**

An alternative explanation for a seeming consistency for the chemical signature in our tagged Lake Champlain fish is that statoliths are not as inert as otoliths. For example, metamorphosis, maturation, spawning, or even the act of tagging these fish may cause statolith resorption, or induce an overwhelming chemical change in the statolith. Indeed, previous otolith research has demonstrated that physiology can affect micro-elemental composition (Kalish 1989), including some of our work with Lake Erie lake trout *Salvelinus namaycush* (Ludsin et al. 2004). Unfortunately, no research has been conducted to test whether statoliths are truly inert and that no re-working of the apatite matrix occurs, as with their otolith analogues. By analogy with human tooth enamel (Curzon and Featherstone 1983), statoliths may be made up of an array of hyroxyapatite microcrystals that may be variably (by element) susceptible to chemical loss or gain with ageing. Such a finding might help explain why some elements (e.g., Rb, Sr,

Mn, and Ba) appear stable in statoliths, but others (e.g., Mg, Zn, and Pb) do not (see Figure 1). Indeed, studies on human teeth indicate that key elements for sea lamprey discrimination (Sr and Mn) are stable.

Along the same line, we have no way of knowing how our results would have differed, had a mixed sample of known-origin (tagged) parasites been evaluated instead of spawners. It is quite possible that the physiological demands/costs associated with maturation, migrating upstream to spawn, and/or spawning itself may cause statoliths to break down or degrade, thus causing the discrepancy between larval signatures from larvae and tagged adults. Although we can only speculate, perhaps this kind of degradation is the reason the unknown adults from the St. Marys River could not be used in this study. Further, had a sample of known-origin (tagged) parasites been used to test our abilities to correctly classify unknowns, we might come up with a more encouraging result (i.e., higher classification success). **Clearly, more mechanistic work is required to determine whether statoliths are indeed inert, and if and when any reworking of the apatite matrix occurs. Further, while the possibility that some reworking of statoliths occurs due to maturation and/or spawning activities does provide some optimism in that classification of parasites might not be problematic, it also underscores the need to treat the results of our mixed sample of unknowns with caution.**

One other important finding that emanates from this work is the need to ensure stability of the LA-ICP-MS. Previous research has documented that instrumentation error can be quite large, and caution is needed when comparing results between labs or between years (Campana et al. 1997). **As such, it should be mandatory of all future statolith (and otolith) chemistry researchers to verify that the instrument being used is producing consistent results through time.** As was clear from our comparison of statolith pairs, our instrument was indeed providing consistent readings for several elements, including Rb, Sr, Ba, and Mn (the latter, however, with a slight bit of bias). As for Mg, Zn, and Pb, we are not fully certain as to why statolith pairs were so drastically different. To assess potential bias associated with the instrument, we compared the coefficients of variation (CV=standard deviation/mean * 100) of all NIST samples run before and after the move of GLIER's trace-metal laboratory. Given the constancy of Rb, Sr, Ba, and Mn, we expected the CVs for these elements to be low both before and after the lab moved, whereas we expected the CVs of Mg, Zn, and Pb to be higher before and/or after the move. However, for all seven elements, the CVs were < 5.25% before and after the move (and even when all data were combined). Thus, it doesn't appear that instrument bias was the source of the differences in our paired statolith analyses.

As for the cause of the discrepancy in Mg, Zn, and Pb, we can only speculate. One possibility is that statoliths are simply not stable for these elements, and that these elements are re-worked during periods of stress such as metamorphosis, maturation, or spawning. Or, perhaps statoliths are comprised of an array of hyroxyapatite microcrystals, similar to what is found in human tooth enamel, wherein these elements may be susceptible to chemical loss or gain with ageing (sensu Curzon and Featherstone 1983).

A second explanation is potential contamination of the second statoliths due to their storage in an acid-washed glass vial for 1 to 2 yr after initial extraction. Perhaps there was some exchange of Zn into the apatite matrix, given that Zn is a major component of glass. Although a paired t-test did demonstrate a significantly higher level of Zn in the second statolith than the first ($p < 0.05$), there were many instances in which Zn levels in the first statolith were greater than those in the second statolith (see Figure 1f). Additionally, if glass contamination was truly problematic, we also would expect higher Ba levels in the second statolith, given that Ba also is a major element in glass. This clearly was not the case, as the relationship between Ba levels in statolith pairs was near perfect (Figure 1c).

More likely, we feel that discrepancies between statolith pairs is either due to non-homogeneity in how Zn, Mg, and Pb are distributed throughout statoliths and/or due to the laser ablating different zones within statolith pairs. Given that larval statoliths can represent up to six years of the life of a larva that has been residing in a stream exposed to varying levels of runoff and pollution, it seems highly probable that elemental composition would not be completely homogeneous throughout the larval portion of the

statolith. Thus, even slight deviations in which part of the statoliths were ablated could lead to major differences in statolith elemental composition. This, however, does not explain why Rb, Sr, Ba, and Mn would be so constant, but not Mg, Zn, and Pb. Although we cannot even begin to speculate as to why this discrepancy exists, we feel that finding the answer is important because Mg, Zn, and Pb can help improve ability to discriminate among larval production streams, as was evident from our analysis of Lake Champlain production streams (Appendix 3).

## **Objective 3 Summary**.

Background. The use of otolith (statolith) chemistry as a tool to distinguish individuals produced in different locations is based on the premise that elements are incorporated into the calcium-carbonate (phosphate) matrix in proportion to their abundance in the surrounding environment (Campana 1999, Thresher 1999). Indeed, positive relationships between water and otolith chemistry have been identified for many elements (e.g., Mg, Sr, Ba, Cu; Farrell and Campana 1996, Thorrold et al. 1998, Bath et al. 2000, Milton and Chenery 2001). In an effort to help better understand why some elements were more important for discriminating among streams than others, we sought to compare elemental concentrations in water samples with elemental concentrations in statoliths of larvae collected from the same streams. Additionally, because sea lamprey larvae spend their time either in or near bottom sediments in rivers, we also collected sediment samples, which might help understand variation in statolith signatures among streams. Our ultimate hope was that we would find strong predictive relationships between water (or sediment) chemistry and statolith chemistry, which would preclude the need to collect larvae annually to characterize spawning-site signatures (i.e., we simply could collect water and predict statolith chemistry), thus saving both time and money.

Methods. To explore statolith-sediment-water relationships, we collected water and sediment samples once during October, November, or December 2005 in 24 Lake Huron tributaries (Figure 2). Likewise, we collected water and sediment samples from 27 Lake Champlain streams (spanning the entire circumference of the lake) monthly during June through August, with collections being made in four streams during September (see Figure 1 in Appendix 3 for sampling locations; water and sediments were collected in all streams). Water samples also were collected every two weeks between June and September from three streams in the Lake Champlain watershed (Mallett's Creek, Sunderland Brook, and the Winooski River) to provide a finer-scale view of stability in water chemistry.

Water samples were filtered in the field following EPA protocols, and stored frozen in acid-washed vials until analysis. Filtered water samples were then acidified using trace grade $HNO_3$. The volume of acid added was equal to 1% of the total volume of water (0.6ml acid to 60 ml of water). The resulting solution was analyzed by ICP-MS and/or ICP Optical Emmission Spectrometry (OES).

Sediment samples were stored frozen in clean plastic bags until analysis. Sediment samples were prepared for analysis according to a cold acetic acid extraction protocol. Sediment was sieved through *5μm* nylon mesh, after which samples were weighed, and 0.50 g was placed in a 50 ml centrifuge tube. Twenty (20) ml of 5% acetic acid was then added to the centrifuge tube, and the tube was shaken for 24 hr at room temperature. After 24 hr, the tubes were centrifuged into a pellet at 5000 RPM for 10 min, and the contents of the tube were filtered (without disturbing the pellet) into a 125 ml bottle. The filter paper was rinsed two times with 2% $HNO_3$, three times with Milli Q water (MQW) and three times with 5% acetic acid. Milli-Q water was added to the solution until the final solution weight was equal to 50.00g Prepared samples were then run on the ICP-MS (OES) to analyze for metals. In addition to samples, each sample batch included three method blanks, one internal reference sample, and two certified reference materials.

To develop predictive relationships between statolith and water/sediment chemistry, we conducted forward stepwise linear regressions for each of the seven elements used for discriminating among streams

(Rb, Sr, Mn, Ba, Zn, Mg, Pb). For the analyses, we only included water and sediment data from streams for which we also had statolith microchemical information (n = 38 streams; n = 7 streams had water, but no sediment, data). In these models, mean statolith elemental concentration (with Ca used as an internal standard) was the dependent variable, and mean water and sediment elemental concentration (standardized by the associated ambient calcium concentration) were used as independent (predictor) variable(s). For an element to be included and remain in a regression model, it had to have an F-value $\geq 1$ to enter the model, and a p-value $< 0.05$ to remain in the model. All elemental data were square-root transformed to achieve normality (Kolmogorov-Smirnov test for normality: all $p \geq 0.05$), residuals were checked for outliers (only one was removed for the analysis with Ba; all final standardized residuals < 3.5), and residuals from the regressions were checked for normality.

To better assess stability of stream-specific signatures, we ran a forward stepwise linear discriminant function analysis (LDFA; F = 1 and tolerance = 0.01 to enter the model) on the Lake Champlain water chemistry data (n = 27 streams), using individual monthly measurements (n = 3-4 measurements per stream) of all seven elements. High classification accuracies for streams would indicate that water chemistry varied little among streams during June through September.

We also used annual historical water chemistry data from the U.S. Geological Survey National Stream Water-Quality Monitoring Networks to explore whether sufficient differentiation in water chemistry among Lake Huron sea lamprey production tributaries existed over a long-term basis. Although many

*Figure 2. Map of Lake Huron water and sediment sampling stations during 2005.*

Lake Huron tributaries were monitored at some point in time, complete water chemistry records exist for only seven Lake Huron tributaries after 1982 (see Figure 3; n = 2-6 replicates/tributary/year). In addition, no water chemistry data exist for most locations after 1993, although Saginaw River water chemistry data exist through 2000.

We contrasted these seven rivers on the basis of six trace elements (Mn, Zn, Sr, Ba, Fe, Pb) using LDFA and a multivariate generalized linear model (MGLM). These elements were chosen for the completeness of their historical record. Because trace elements compete with calcium (Ca) for incorporation into the calcium-carbonate (phosphate) matrix of otoliths (statoliths), all data were standardized against ambient Ca concentrations before use in analyses (Campana 1999).

Results. Analysis of water, sediment, and statolith micro-elemental data from lakes Huron and Champlain demonstrate that statolith elemental concentrations were related to ambient water and/or sediment chemistry for most elements, with water chemistry being more strongly related to statolith chemistry for all elements except Zn and Pb. Specifically, we found a single-variable model with only water data was the best predictor of rubidium ($R^2 = 0.33$; $F = 17.8$, $p = 0.0002$, $n = 38$), strontium ($R^2 = 0.48$; $F = 32.7$, $p < 0.0001$, $n = 38$), and barium ($R^2 = 0.31$; $F = 16.0$, $p = 0.0003$, $n = 38$) concentrations (Figure 3). Manganese concentrations in water also were a good predictor of those in statoliths ($R^2 = 0.43$; $F = 27.0$, $p < 0.0001$, $n = 38$; Figure 3); however, addition of Mn concentrations in sediments improved the predictive relationship ($R^2 = 0.52$; $F = 15.1$, $p < 0.0001$, $n = 31$). Magnesium in both water and sediments was not a good predictor of Mg in statoliths ($R^2 = 0.12$; $F = 2.2$, $p = 0.16$, $n = 31$), whereas

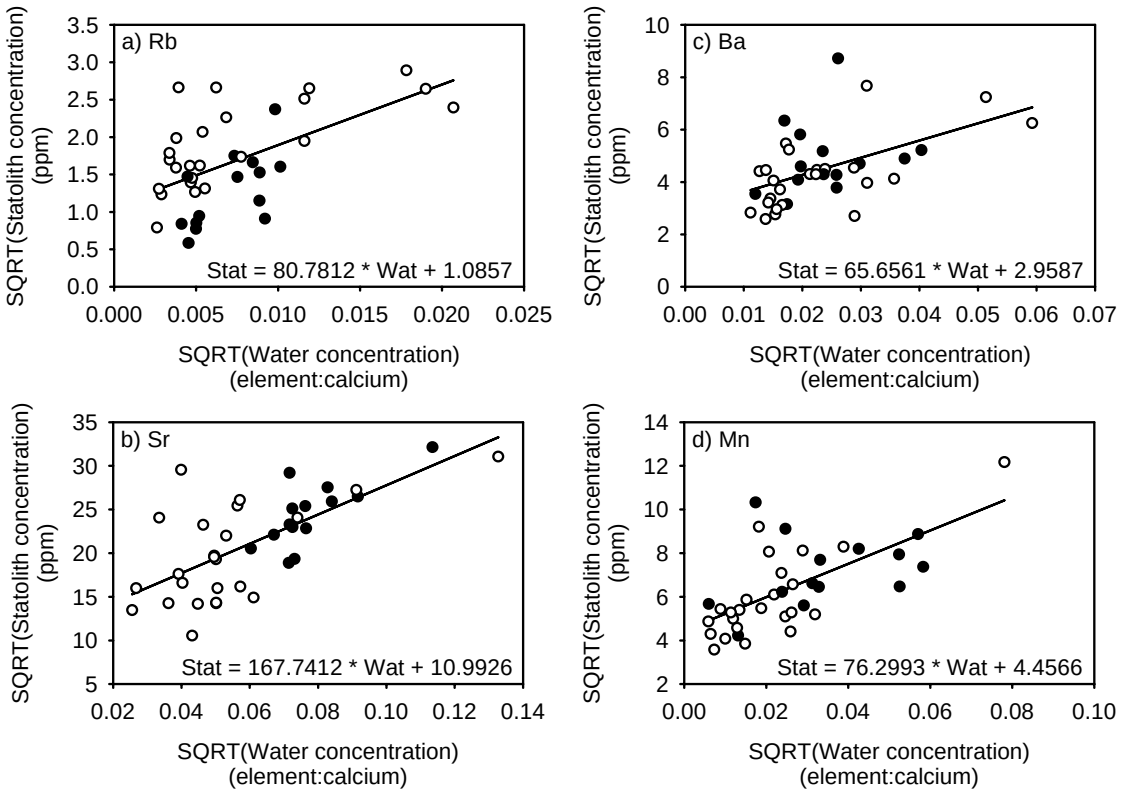


*Figure 3. Relationship between concentrations of a) rubidium, b) strontium, c) barium, and d) manganese in water versus statoliths from Lake Huron (open symbols) and Lake Champlain (closed symbols) tributaries. Data for both axes were square root-transformed. Linear regression equations that predict statolith (Stat) chemistry from water chemistry (Wat) are provided.*

sediment chemistry was the best, but still rather poor, predictor of concentrations of lead ($R^2 = 0.17$; F = 6.0, p = 0.02, n = 31) and zinc ($R^2 = 0.16$; F = 5.6, p = 0.02, n = 31) in statoliths

Results from the LDFA conducted on Lake Champlain streams indicate that good discrimination was possible and that stream-specific signatures were largely stable during June through September. Specifically, we found that 95% of the variation in the June-September water chemistry data could be explained with knowledge of four roots. The first root explained 38% of the variation in our data, and most strongly correlated with Ba (r = 0.60). Magnesium was most strongly correlated with the second root (r = 0.61), which explained 36% of the variation in our data. The third root explained another 16% of the variation, and was strongly correlated with Rb (r = -0.69), whereas the fourth root explained 5% of the variation and was correlated with Sr (r = -0.85). Overall, we could discriminate the 27 streams with average classification accuracy of 86%, with 14 of 27 streams having 100% classification accuracy.

Plots of water chemistry data collected every two weeks from our three focal Lake Champlain streams (Mallett's Creek, Sunderland Brook, and the Winooski River) further illustrate within-stream stability, and differences among streams in terms of elemental concentrations (Figure 4). For example, Rb concentrations were consistently highest in the Winooski River followed by Sunderland Brook and then Mallets Creek (Figure 4a). By contrast Sunderland Brook had the highest Mg levels of the three streams, with Winooski River having the lowest (Figure 4e). Obvious differences among these three streams also were found for Mn (Figure 4d).

Our analyses of long-term Lake Huron water chemistry data demonstrate differences among rivers (MGLM, $p < 0.001$). Of the elements examined, univariate tests (within the context of the larger MGLM) revealed differences in Ba, Fe, and Zn among sites (all $p < 0.0002$). Most notably, the St. Marys River had higher levels of Zn than all other rivers (unequal sample size Tukey's hsd test, all pairwise $p < 0.0004$), and higher concentrations of Ba than the Au Sable, Pigeon, and Thunder Bay rivers (unequal sample size Tukey's hsd test, all pairwise $p < 0.01$).

Owing to differences in water chemistry, our ability to discriminate rivers was high (Figure 5). Although differences among all elements were highly significant in our LDFA (all $p < 0.001$), some were more important than others. Ranked from most-to-least important were Sr (F = 81.2), Zn (F = 41.8), Mn (F = 21.9), Ba (F = 14.6), Fe (F = 6.0), and Pb (F = 1.4). Overall, the first three LDFA roots (axes) explained 95% of the variation in the data.

The first LDFA root (axis) explained 53% of the variation among tributaries (root $\lambda = 7.8$; root $\chi^2$=450.4, df = 36, $p < 0.00001$), and was positively correlated with Sr (r = 0.76) and negatively correlated with Zn (r = -0.59). Thus, tributary-years (points) on the right side of LDFA root 1 were higher in Sr and lower in Zn than those on the left side of this axis (Figure 5). The second LDFA root also was highly significant ($R^2$=0.30, root $\lambda = 4.5$; $\chi^2 = 273.8$, d = 25, $p < 0.00001$), and negatively correlated with both Sr (r=-0.65) and Ba (r=-0.67). Thus, tributary-years located at the top of Figure 5 were lower in Sr and Ba than those located near the bottom. Finally, LDFA root 3 ($R^2 = 0.15$, root $\lambda$=1.9; $\chi^2$=136.5, df=16, $p < 0.00001$) was negatively correlated with Mn (r = -0.92). Using these discriminant functions, we could classify tributaries quite accurately. Our overall classification success was 88.6%, with individual tributaries as follows: Thunder Bay River = 100%, St. Clair River = 100%, Au Sable River = 91.7%, St. Marys River = 90.9%, Pigeon River = 83.3%, Rifle River =81.8%, and Saginaw River = 78.9%.

Discussion. The results from our analysis of water and sediment chemistry versus statolith chemistry are both revealing and encouraging. First, it appears that statolith elemental composition is more strongly influenced by water chemistry than sediment chemistry. Thus, even though sea lampreys burrow in stream sediments and feed on detritus as larvae, their statolith chemistry is mostly influenced by water chemistry. Consequently, future sampling of sediments may not be as critical as sampling water when attempting to predict statolith chemistry from water chemistry. Second, our analyses also help to understand why some elements were more useful for discrimination than others. Specifically, we found that concentrations of Rb, Sr, Mn, and Ba—the elements that were most useful for discriminating sea

lamprey larvae in Lake Huron—in statoliths and the ambient water were positively related, confirming the underlying tenet of otolith microchemisty as a discrimination tool. By contrast, concentrations of Zn, Mg, and Pb were only weakly related (at best) between water/sediments and statoliths.

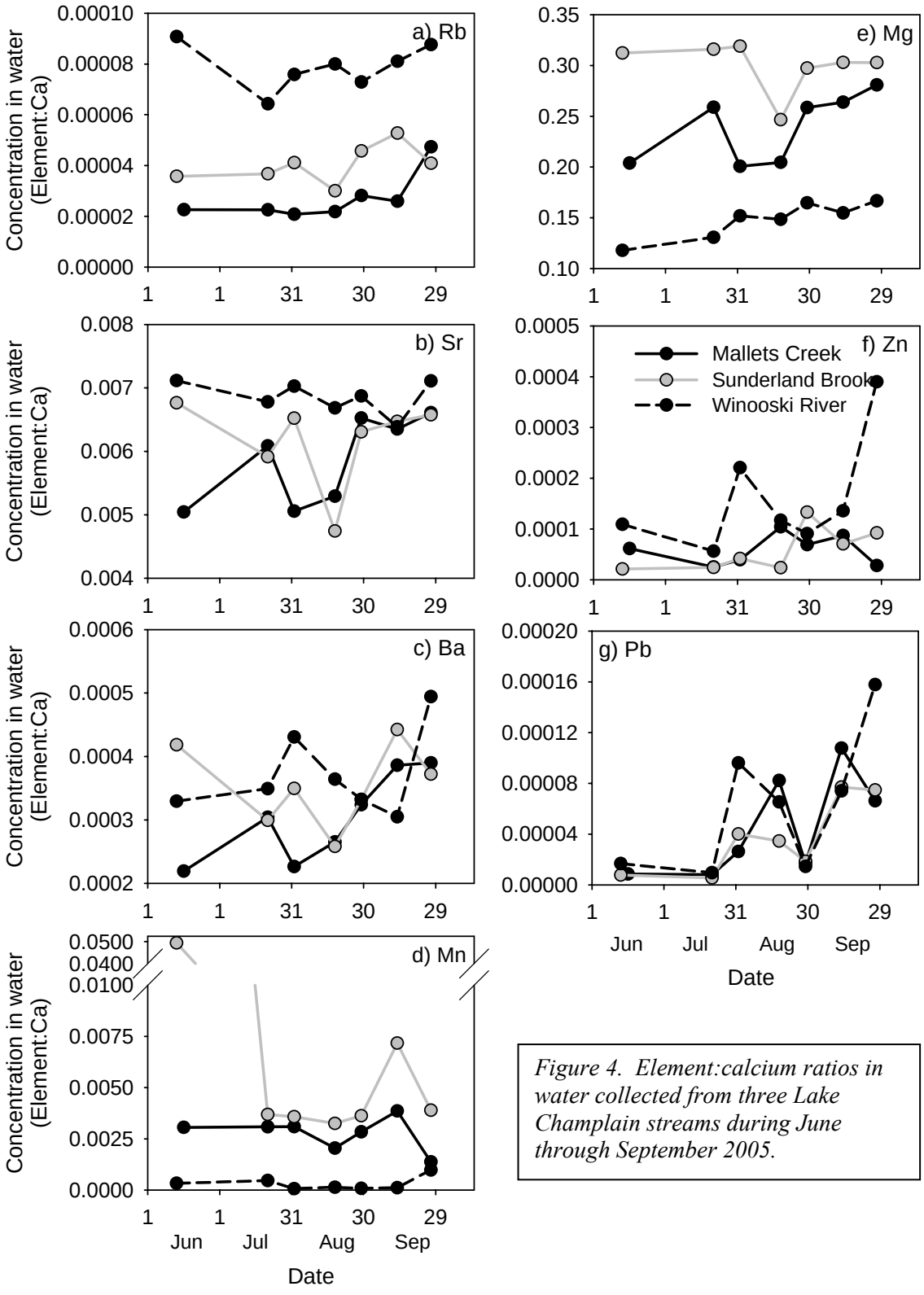


*Figure 4. Element:calcium ratios in water collected from three Lake Champlain streams during June through September 2005.*

The difference in the value of these elements for discrimination is not all that surprising. Rubidium, Sr, Mn, and Ba concentrations in otoliths and statoliths are more apt to reflect ambient concentrations than elements such as Zn and Mg because the former elements are not as physiologically regulated as Zn and Mg (Campana 1999, Thresher 1999). Thus, even though our analysis of water chemistry data demonstrated that 1) Mg had the potential to be a good discriminator in Lake Champlain (see Figure 4e), and 2) Zn had the potential to improve discrimination in Lake Huron (Figure 5), the high likelihood that these elements are physiologically regulated (i.e., do not reflect water chemistry) may limit value for sea lamprey discrimination.

In the time-series samples from Lake Champlain, all elements were relatively stable over the 14 weeks of sampling in water samples; however, there was considerable variation in Ba, Pb, Mn, Zn, and U in sediments from the Winooski River, and in Ba and U in sediments from the Mallets Creek and Sunderland Brook. This variation may point to inter-sample variation rather than temporal variation. Flowing water, by its nature, integrates whole-stream chemistry, and we chose days when flows were relatively stable, so that water chemistry would not be affected by high-flow events. Sediments, on the other hand, may have substantial small-scale spatial variation, such that samples at the same time in the same location may vary substantially.

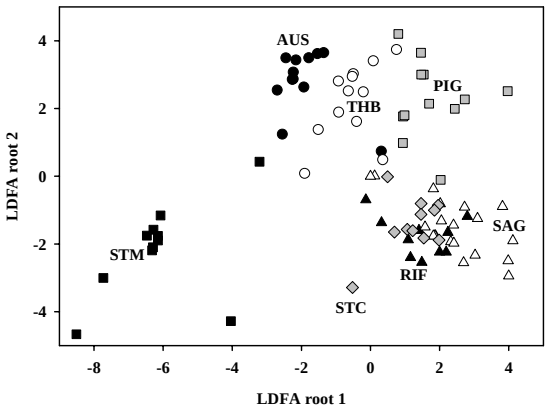


*Figure 5. Linear discriminant function analysis (LDFA) results of historical water chemistry data collected in seven Lake Huron tributaries, ranked from northernmost to southernmost: St. Marys River above Sault Ste. Marie, MI (STM, ■; 1982-1992); Thunder Bay River near Alpena, MI (THB, ○; 1982-1993); Au Sable River near Au Sable, MI (AUS, ●; 1982-1993); Rifle River near Sterling, MI (RIF, ▲; 1982-1992); Saginaw River at Saginaw, MI (SAG, Δ; 1982-2000); Pigeon River near Caseville, MI (PIG, ■; 1982-1993); and St. Clair River at Port Huron, MI (STC, ◆; 1982-1992). Root 1 was negatively correlated with Zn and positively correlated with Sr, whereas Root 2 was negatively correlated with Sr and Ba.*

**These results ultimately suggest that stream-specific water chemistry may actually be fairly stable, which would greatly facilitate the use of statolith micro-elemental concentrations as a means to discriminate potential sea lamprey production tributaries.** Two sets of evidence support this notion. First, the LDFA conducted on Lake Champlain water chemistry data during June through September indicate stability during at least the summer period, given that 14 of 27 streams could be discriminated with 100% success. Most important to discrimination were Mg, Ba, Rb, and Sr in that order. Secondly, our analysis of historical Lake Huron water chemistry data demonstrates long-term stability in stream-specific water chemistry of the subset of seven streams investigated.

Importantly, however, the results of our analyses of the long-term Lake Huron water chemistry data somewhat counter our findings in our analysis of statoliths collected from many of the streams sampled during 2004 and 2005 (see Table 9 in Appendix 2). This discrepancy is most likely due to the small number of streams included in the water chemistry analysis, given that our ability to discriminate streams using statolith micro-elemental composition generally improved when only streams sampled in the same year were included in the same analysis (C. Hand, unpublished data). Clearly, the more streams included in an analysis, the greater the likelihood that another stream will have a similar chemistry, which would reduce the ability to discriminate among them.

However, there may also be long-term stability in water chemistry, and 2004 or 2005 may be an anomalous year in terms of water chemistry. As such, we strongly encourage continued collections of

both water and larvae so as to better understand the magnitude of inter-annual variation that is possible. It very well may be that differences among streams are largely constant and that we see large inter-annual variation in stream-specific signatures in only a small fraction of years. Additionally, we also strongly suggest that all larval production streams be sampled in the same year, if possible, which will negate much of the potential effect of inter-annual variability on discrimination abilities (Fabrizio 2005).

## Summary and Recommendations

1. The micro-elemental composition of statoliths can discriminate among larval production sources with good (but not perfect) classification success at the stream, watershed, and geologic scales.
    - We strongly encourage that micro-elemental analyses using LA-ICP-MS be supplemented with stable isotope analyses and/or other micro-elemental approaches (e.g., PIXE) so as to increase the number of factors (e.g., additional elements, stable isotopes) that could be used to discriminate streams.
    - We also suggest that sampling all tributaries within the same year be conducted, if possible, so as to reduce issues associated with inter-annual variability in stream-specific signatures.
2. Statolith elemental composition appears to be highly influenced by water (but not sediment) chemistry, and long-term water chemistry data from Lake Huron suggest that long-term stability in elemental signatures exists.
    - We recommend continued monitoring of stream-specific statolith micro-elemental signatures so as to better understand the magnitude of inter-annual variation within and among streams, and place our two years of data into context.
    - Given the strength of the relationship between our point estimate(s) of water chemistry and an integration of up to six years of statolith chemistry, we recommend that water samples be collected more frequently in future studies in an effort to develop a stronger predictive relationship of statolith chemistry from water chemistry. Quite possibly, stream-specific elemental signatures could be determined from water chemistry.
3. Results from our analysis of a mixed sample of unknown adult spawners indicate that **both** historically important larval production tributaries and less-important larval production tributaries contributed individuals to our mixed sample.
    - While our results should be heeded with caution (see next point below), if correct, they suggest that estimates of larval abundance may not be the best indicator of relative contributions of spawners from streams.
4. We were unable to validate use of statolith micro-elemental signatures to correctly classify adult spawners back to their natal stream, using a mixed-sample of known-origin (tagged) individuals.
    - While it is possible that our mixed-sample results are correct, they need to be accepted with caution.
    - We recommend several future research activities that could improve our ability to correctly classify individuals to their natal stream:
        - Reduce issues associated with inter-annual variability in stream-specific signatures by collecting statolith data over multiple years so as to allow for development of ‘average’ elemental signatures for streams that might correlate better with larval material in statoliths from adults or parasites.

- Further explore whether our current methods for adult statolith processing is responsible for our inability to correctly type back tagged individuals, using known-origin fish, including both parasites and adults.
- Explore whether statoliths are as inert as otoliths, or if reworking of the statolith occurs with metamorphosis, maturation, spawning, or simply aging by conducting mixed-sample analyses on known-origin (tagged) sea lamprey collected during various life stages (e.g., transformers, parasites, spawners).

5. Comparison of left-right statolith pairs demonstrated good correspondence for some, but not all, elements.
   - We strongly encourage that any future statolith (or otolith) microchemistry investigation should conduct similar analyses to test stability of elemental concentrations, given that instrumentation error or reworking of statolith/otolith material could occur.
6. Overall, our ability to discriminate among Lake Huron streams using larval statolith signatures indicates a lot of promise for this technique.
   - We encourage continued research into the potential application of this tool.

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**DELIVERABLES:**

**Refereed Manuscripts**

Hand, C.P., S.A. Ludsin, B.J. Fryer, and J.E. Marsden. *in review*. Development of statolith microchemistry as a technique for discriminating among sea lamprey (*Petromyzon marinus*) spawning tributaries in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences.

**Theses and Dissertations**

Hand, C. 2006. Micro-elemental analysis of statoliths as a tool for tracking the stream origins of sea lamprey (*Petromyzon marinus*) in Lake Huron. Master's thesis. University of Windsor, Windsor, ON, Canada.

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Howe, E.A., C.P. Hand, J.E. Marsden, S.A. Ludsin, and B.J. Fryer. 2006. Tributary contributions to the parasitic and spawning adult population of sea lamprey (*Petromyzon marinus*) in Lake Champlain using elemental signatures. Final report to the Lake Champlain Basin Program.

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## APPENDIX 1

Hand, C.P., S.A. Ludsin, B.J. Fryer, and J.E. Marsden. *in review*. Development of statolith microchemistry as a technique for discriminating among sea lamprey (*Petromyzon marinus*) spawning tributaries in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences.

## Abstract

Fishery management agencies are seeking ways to identify natal origins of parasitic- and spawning-phase sea lamprey (*Petromyzon marinus*) so control efforts can be prioritized. We develop laser-ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS) as a technique to quantify elemental concentrations in larval sea lamprey statoliths, and explore the use of statolith microchemistry as a tool to discriminate among larval sea lamprey production streams. Our analyses demonstrate that: 1) traversing across the statolith with the laser is preferable to drilling down through its apex; 2) preserving specimens in 95% ethanol versus freezing them has minimal effects on elemental concentrations; 3) a minimum of 15 individuals per stream should accurately depict stream-specific statolith elemental signatures; and 4) LA-ICP-MS is preferable to particle-induced X-ray emission (PIXE) for statolith analysis, based on higher precision, lower cost, reduced sampling time requirements and wider availability. Using LA-ICP-MS, we could discriminate among larvae from 12 streams located in lakes Michigan, Huron and Superior with 80% classification accuracy, indicating this tool holds promise for determining natal origins of sea lamprey in the Great Lakes.


## Introduction

Sea lamprey (*Petromyzon marinus*) invaded the upper Laurentian Great Lakes during the early 20$^{th}$ century, following construction of the Welland Canal (Lawrie 1970). Owing to their devastating impact on economically and ecologically important species such as lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*) throughout the Great Lakes (Weise and Pajos 1998), fishery management agencies (led by the Great Lakes Fishery Commission) have implemented strategies to control sea lamprey populations throughout the lakes. These strategies include use of lampricides, physical barriers to block spawning adults, sterile-male-release programs, and trapping of spawners. Currently, application of lampricides (TFM, granular Bayluscide) to larval production streams is the most commonly used control method, wherein streams with the highest density of larvae are typically targeted for treatment (Smith and Tibbles 1980). One potential limitation of using larval abundance to prioritize streams for lampricide treatment is that larval mortality may not be constant among streams, and therefore, larval abundance may not be the best predictor of stream-specific contributions of parasites to open lake populations (Slade et al. 2003). Owing to sea lamprey remaining a continual problem despite continued lampricide application to larval production streams, as well as uncertainty concerning the assumption that streams contribute parasites to the open lake in proportion to their larval abundances, fishery management agencies are still seeking a definitive way to quantify the relative contributions of parasites and spawners from spawning tributaries.

Tagging of larvae offers one possible method of identifying from which natal streams parasites and spawning adults originate. Artificial tags, however, are impractical for several reasons, the least of all being acquisition of transformer stage lamprey for tagging. Once sea lampreys have been tagged, there is a distinct likelihood that, even if tags are not lost physically, tagged individuals would not be recovered in sufficient numbers to provide reliable estimates of mortality. This likelihood is compounded by the fact that sea lamprey do not home to their natal streams (Bergstedt et al. 1993; Bergstedt and Seelye 1995), thus limiting the use of adult spawners as an indicator of stream origin.

An alternative to artificial tags is the use of natural tags, including both genetics and otolith microchemistry. However, use of genetics as a tool to identify natal origins of sea lamprey is impractical since populations in the upper Great Lakes have been present for less than a century and adult lamprey do not home to their natal streams (Bergstedt et al. 1993; Bergstedt and Seelye 1995). Consequently, it is unlikely that sufficient genetic differentiation is present among sea lamprey production tributaries (Bergstedt et al. 1993).

The more feasible natural-tag alternative is otolith microchemistry. Otoliths are the calcareous structures located in the inner ear of teleost fish, used for hearing and balance (Pannella 1971). Otolith microchemistry data have been used to address many fisheries-related problems, including stock delineation, tracking migration pathways, and reconstructing habitat-use patterns (Campana 1999; Thresher 1999). Three unique properties of otoliths allow them to be used for this type of research: 1) they are metabolically inert (exhibit no reworking once layers are set down); 2) they continue to grow even when somatic growth is non-existent; and 3) their elemental composition reflects the physical and chemical environment in which the fish has resided in the past (Campana 1983; Campana and Thorrold 2001).

Herein, we explore the potential extension of otolith microchemistry to statoliths, a calcified inner-ear concretion found in sea lamprey that also exhibits annual banding patterns (Volk 1986; Barker et al 1997). Statoliths are analogous to the otoliths found in teleost fish (Carlstrom 1963). However, whereas otoliths are composed of calcium carbonate (usually aragonite), and typically continue growing in proportion to body size throughout the life of the fish, statoliths are: 1) made of calcium phosphate (apatite); 2) small relative to otoliths from teleost fish (statoliths average ~50 μm across, even in adults, which is equivalent in size to larval otoliths from teleost fish); and 3) the only calcified structures found in an otherwise cartilaginous body. Statoliths also differ from otoliths of most teleost fishes in that the majority of the statolith material reflects the larval environment. This disparity occurs because of the long time (3 to 7 yr) sea lampreys spend burrowing as larvae in stream sediments, and short time (< 2 yr) spent as parasites and adults during which little post-metamorphosis statolith growth occurs (Volk 1986).

Recently, Brothers and Thesher (2004) used particle induced x-ray emission (PIXE) analysis to demonstrate that statolith microchemistry could be used to discriminate larvae collected from four Lake Huron tributaries. We build upon this previous work by using laser-ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS), a method that allows examination of trace elements at very low levels (parts per billion), even in larval fish otoliths (Ludsin et al. 2006). In determining whether LA-ICP-MS can be reliably used for analysis of larval sea lamprey statoliths and for discriminating among spawning tributaries from 3 of the 5 Laurentian Great Lakes (Huron, Michigan, and Superior), we explore factors that might influence the data, including laser-ablation technique, larval preservation method, number of individuals needed to accurately characterize a stream, and instrumental method (e.g., PIXE vs. LA-ICP-MS. In addition, we use our findings to discuss the potential application of statolith microchemistry as a tool for identifying natal origins of sea lamprey in the Great Lakes.

## Methods

**Field collections.** Sea lamprey larvae were collected from 17 streams within the Lake Huron, Lake Michigan, and Lake Superior watersheds (Figure 1) by the Canadian Department of Fisheries and Oceans (Sault St. Marie, ON) and the U.S. Fish and Wildlife Service (Marquette and Ludington, MI field stations), and one stream (Lewis Creek) from Lake Champlain (NY-VT). All larval sea lamprey collections were made using electrofishing in the summers of 2004 and 2005 during assessment surveys in streams recently treated with lampricide. All larvae collected were stored in Nalgene® bottles containing 95% ethanol, except for the subset of larvae used in the comparison of preservation methods (see below), which were stored frozen.

**Statolith preparation.** Statolith processing occurred in a Class 100 clean room. Prior to statolith removal, the total length (TL; to nearest 1 mm) of each individual was measured, after which the head was removed. Bilaterally dissected heads were soaked in ultra-pure milli-Q water (MQW), and then both left and right statoliths were removed on a clean glass slide using clean glass probes. Removed statoliths were transferred, using another clean glass probe, to a drop of MQW in a covered Petri dish. After six sets of statoliths had been removed, the covered Petri dishes were sonicated for 5 min, using an ULTRAsonik™ sonicator (model 57X; Ney Dental Inc. Bloomfield, CT, USA) filled with MQW.

Sonicated statoliths were then cleaned, and rinsed three times with MQW. Afterwards, statoliths were mounted dorsal side up to a slide with Scotch™ double sided tape for LA-ICP-MS analysis (Ludsin et al. 2006). No statolith surface removal was necessary as larvae were captured in their natal streams, and therefore the desired signature is present throughout the entire statolith (Volk 1986).

All glassware and plastic-ware used were acid washed prior to use. Acid washing consisted of preliminary cleaning using Nitrox® soap, followed by a 24-hr immersion in 13% nitric acid solution, a 24-hr immersion in ultra-pure MQW water, and three final rinses with MQW. Finally, everything was dried for 24-hr under a Class 100 laminar-flow fume hood.

**Experimental set-up and data analysis.** Toward further developing micro-elemental analysis of statoliths as a tool for discriminating among sea lamprey production streams, we compared: 1) two laser-ablation approaches (drilling down through the central apex versus ablating across the entire statolith); 2) the effects of ethanol vs. freezer storage as a preservation technique; 3) the likely minimum sample size needed to accurately represent a stream population; and 4) differences between our LA-ICP-MS results and the PIXE results of Brothers and Thresher (2004). In addition, we also determined whether stream-specific statolith elemental signatures differed among three of the Great Lakes (Huron, Michigan and Superior), as well as within them.

**LA- ICP-MS analysis.** Statoliths were analyzed using a Thermo Elemental X7 ICPMS, coupled with a Continuum solid state ND:YAG laser (wavelength: 266nm; max. power: 40mJ; pulse rate: 20Hz; primary beam width: 6mm). With exception of the subset of 10 statoliths that were ablated through the central apex, elemental concentrations were determined by traversing the entire width of the statolith. Given the small size of statoliths, the laser power was reduced to < 1 mJ and the beam diameter to 2 mm , which, when focused onto the sample through a 10x lens, resulted in a ablation spot size of ~10 μm. By beginning and ending each laser transect on the double-sided tape, we could easily determine where the statolith began and ended in our analysis because mass 120, representative of a carbon molecular ion (instrumentally measured as $^{120}Sn$) would spike noticeably when the laser hit the tape (Ludsin et al. 2006).

A glass reference standard (NIST 610) was analyzed before and after every 16 samples (n = 2 replicates before and after), which allowed for quantification and correction of instrumental drift. This same standard also was used to determine precision in estimating elemental concentrations. The Argon carrier gas (i.e., background) was analyzed for 60 s before every sample, allowing limits of detection (LODs) to be calculated for individual samples (Ludsin et al. 2006).

We quantified 11 elements (not including calcium, Ca) (Table 1), but only seven met our criteria for inclusion in analysis: magnesium (Mg), manganese (Mn), zinc (Zn), rubidium (Rb), strontium (Sr), barium (Ba) and lead (Pb). These criteria included 1) a coefficient of variation that was < 10% for individual isotopes (measured in NIST 610 standards), and 2) no more than 20% of the samples with concentrations below detection limits (LOD) for a stream. If a sample concentration was below its own LOD, the missing value was replaced with a randomly generated value between zero and the LOD for that specific sample. To correct for ablation yield differences that resulted from varying laser spot size, we used calcium (measured as $^{43}Ca$) as the internal standard, given that this element comprises a relatively large, constant (39.9% by wt) proportion of the statolith apatite ($Ca_5(PO_4)_3(OH)$). For subsequent analyses in which only elemental concentrations are presented, we used $Sr^{88}$ and $Ba^{138}$ to estimate concentrations of strontium and barium, respectively.

**Comparison of laser-ablation approaches**. Statoliths grow in a conical shape, with the tip being the oldest material and the base being the most recently deposited (Brothers and Thresher 2004). To determine whether the same elemental signature is found throughout the entire statolith, we compared elemental concentrations in statolith pairs, wherein one statolith was ablated by traversing across its entire width and the second statolith was ablated by drilling down (vertically) through its apex. Left and right statolith pairs from larvae collected in the Sauble River and Lauzon Creek (n = 5 individuals per stream; triangles symbols on Figure 1) were compared, using univariate paired t-tests ($\alpha = 0.05$). To determine

the best approach for ablating statoliths with LA-ICP-MS, we compared differences in 1) average elemental concentrations, 2) average limits of detection, and 3) average statolith dwell times (i.e., analysis durations). No data transformations were necessary (Kolmogorov-Smirnov test for normality, all $p \geq 0.20$).

**Comparison of statolith preservation methods.** Due to logistical difficulties in freezing larvae while in the field and difficulties (and cost) associated with preserving parasites and adults in ethanol, it is not practical for the agencies that collect sea lamprey (DFO and USFWS) to use a single preservation technique for all life stages. Thus, we tested whether preservation in 95% ethanol versus freezing has a significant effect on the trace elemental concentrations in statoliths. Although preservation in ethanol does not appear to affect trace elemental concentrations in juvenile and adult teleost fish (Milton and Chenery 1998; Proctor and Thresher 1998; Hedges et al. 2004), we wanted to ensure that ethanol preservation versus freezing also has no effect on statoliths. To do so, we used fish from both the Big Manistee River (Lake Michigan, square symbol on Figure 1) and Lewis Creek (Lake Champlain). In both streams, 60 larvae were collected: half were frozen and the other half was preserved in 95% ethanol. Two sample t-tests ($\alpha = 0.05$) were used to determine if elemental concentrations differed due to preservation technique. All data were log-transformed to achieve normal distributions (Kolmogorov-Smirnov test for normality, all $p \geq 0.20$). Mean total length was not significantly different between preservation methods (frozen: 74.7 mm, ethanol: 79.3 mm; $p = 0.23$).

**Comparison of stream sample sizes.** To determine the minimum sample size required to adequately characterize stream-specific signatures, we used larvae from Silver Creek, Lake Huron (n=49: diamond symbol on Figure 1). The minimum representative sample size was determined via bootstrapping, wherein random sub-samples consisting of 10, 15, 20, 25, 30 and 40 fish were drawn (n= 500 replicates per sample size). We compared individual element concentrations using a multivariate one-way analysis of variance (MANOVA, $\alpha = 0.05$). Subsequently, we conducted a one-way ANOVA on individual elements ($\alpha = 0.05$). We log-transformed Mn, Zn, Rb and Sr, and took the reciprocal of Mg, Ba and Pb to normalize the data (Kolmogorov-Smirnov test for normality, all $p \geq 0.20$).

**Comparison of PIXE versus LA-ICP-MS.** Brothers and Thresher (2004) used PIXE to discriminate among sea lamprey larvae collected in four Lake Huron tributaries (n= 4-18 individuals per site): St. Mary's River (two sites), Pigeon River, Rifle River, and Black Mallard River. We collected larvae from the same rivers (but different sites), except for substituting Loeb Creek for the nearby Pigeon River (open circle symbols on Figure 1). To evaluate similarities in our results, we compared untransformed values for both methods. We then performed two linear discriminant function analyses (LDFA) to compare results between the two instrumental methods.

**Among- and within- lake comparisons.** To explore whether sufficient elemental variation exists in statoliths to differentiate among individuals spawned in different streams in the Great Lakes, we analyzed sea lamprey larvae from 12 tributaries spanning three Great Lakes (Figure 1). Collection sites included two tributaries from Lake Superior (Bad River and Brule River), four tributaries from Lake Michigan (Boardman River, Ford River, Loeb Creek, White River) and six tributaries from Lake Huron (Black Mallard Creek, Musquash River, Nottawasaga River, Rifle River, Saginaw River, St. Mary's River) with 10-25 individuals per stream. The ability to discriminate among streams was tested using a forward stepwise LDFA. For an element to be included in the final model, its F-value had to exceed 1 and its tolerance had to be > 0.01. Classification accuracies for individual streams were determined by a jackknifing procedure, conducted as part of the LDFA. All data were log-transformed to ensure normality (Kolmogorov-Smirnov test for normality, all $p \geq 0.20$). Mean total length did significantly differ among streams (range: 68- 130 mm; $p = 0.000$); however, there was no meaningful correlation between an individual's total length and individual statolith elemental concentrations ($r < 0.28$ for all seven elements).

## Results

**Comparison of laser-ablation approaches**. For the seven elements analyzed (Mg, Mn, Zn, Rb, Sr, Ba and Pb), no significant differences were found between ablation methods for Mn, Zn, Rb, Ba, and Pb (Table 2). However, significant, differences were found for Mg (<500 ppm difference) and Sr (<20 ppm difference), with concentrations higher for statoliths that were ablated across versus down through the apex. The LODs did not differ between methods for any element (Table 2). By contrast, however, we found that the average (± 1 SE) ablation analysis time was significantly longer for statoliths that were traversed across (22.9 ± 8.3 seconds; ranging from 12.7 to 39.8 seconds) versus those that were ablated down through the apex (9.4 ± 3.1 seconds; ranging from 4.2 to 14.9 seconds: $p < 0.0002$).

**Comparison of statolith preservation methods**. For both Lewis Creek and the Big Manistee River, no differences were found for Mg, Mn, Zn, Sr, Ba, and Pb in statoliths stored in 95% ethanol versus frozen (univariate t-tests, all $p > 0.05$). However, for both streams, there was a small but significant difference in Rb, which was higher for larvae preserved in 95% ethanol versus those that were frozen (univariate t-tests, both $p \leq 0.005$); the average difference was 0.552 ppm for the Big Manistee River and 0.120 ppm for Lewis Creek.

**Comparison of stream sample sizes**. Analysis of the means generated from bootstrapped samples of varying size (n = 10, 15, 20, 24, 30 and 40 individuals) demonstrated no differences in site-specific signatures (MANOVA: $p = 0.28$). However, univariate tests revealed differences for Zn and Pb such that a sample size of 10 produced different results for Zn and Pb than for all larger sample sizes (ANOVA Tukey's post-hoc test: both $p \leq 0.01$), whereas no differences were found among larger sample sizes (15 to 40 individuals) for any element (including Zn and Pb).

**Comparison of PIXE versus LA-ICP-MS**. Brothers and Thresher (2004) used PIXE to quantify 10 elements (not including Ca), finding all elements (i.e., Rb, Zn, Pb, Mn, Sr, Fe, Hg, Cu and Ni) to be reliable with exception of Ba. We quantified 11 elements (Table 1), but found that only seven of them (i.e., Mg, Mn, Rb, Sr, Zn, Ba, and Pb) were useable. A comparison of those elements that were analyzed by both PIXE and LA-ICP-MS from the same streams (n = 4), revealed that LA-ICP-MS estimates of Rb, Sr, Pb and Ba were in the same general range as those of Brothers and Thresher (2004); however, for each of these elements, the measured concentrations were lower with LA-ICP-MS than with PIXE, and the measured concentration data were tighter with LA-ICP-MS (Figure 2). Manganese differed from other elements in that LA-ICP-MS produced lower concentrations and smaller ranges than PIXE for two streams (St. Mary's River and Loeb Creek), whereas the opposite was true for the other two streams (Black Mallard River and Rifle River; Figure 2). Zinc concentrations also were comparable between methods, though LA-ICP-MS demonstrated a potential for contamination, as indicated by a couple of extreme, high concentrations in the Black Mallard River and Loeb Creek (Figure 2).

Brothers and Thresher (2004) also ran a LDFA to learn whether the St. Mary's River, an important larval sea lamprey production stream, could be discriminated from the other three streams (all located in lower Michigan). They were able to accurately classify 89% of the fish to their natal stream. In their LDFA analysis, the first two roots were dominated by Rb, with additional roots showing Fe, Mn, Zn, and Sr as being important for discrimination. In our similar analysis, our average classification accuracy for the four streams was 88 %. We also found our first two roots, which explained 95% of the variation our data, were dominated primarily by Rb, with both Sr and Mn being important. Thus, both studies concur that Rb is the most important discriminator, regardless of method, and that Mn and Sr are also important.

Brothers and Thresher (2004) also successfully discriminated the St. Mary's River from the lower peninsula of Michigan (the other three streams combined). In fact, they were able to discriminate with 94% accuracy, with only 1 of 18 St. Mary's River larvae misclassified as from the Lower Peninsula. Likewise, only 1 of 17 samples from the Lower Michigan peninsula was misclassified as a St. Mary's River fish. We found similar (89%) discrimination success, with only 3 of 35 Lower Peninsula Michigan fish classified as St. Mary's River individuals and only 4 of 30 St. Mary's River fish misclassified.

**Among- and within- lake comparisons**. A LDFA conducted using 12 streams from lakes Superior, Huron, and Michigan allowed us to classify larvae back to their correct stream with an average accuracy of 80%, with individual stream accuracies ranging from 31% to 100% (Table 4). Seven of the 12 streams had ≥ 80% correct reassignment, including two streams from Lake Superior, one from Lake Michigan, and four from Lake Huron (Table 4). In Figure 4, we illustrate the difference between two streams with good separation (Brule River and Black Mallard River) and two streams that consistently were misclassified as one another (Rifle River and Saginaw River). Inclusion of all seven elements (Mg, Mn, Zn, Rb, Sr, Ba, Pb) in our model suggests that all were important for explaining variation among streams; however, we found that Sr, Rb, and Mn were the most important elements, dominating LDFA roots 1 through 3, which explained 86% of the variation in the data (Table 5). Magnesium, Pb and Zn were largely unimportant for discriminating sites, being most related to axes that explained relatively little variation (< 2%), and Ba was only slightly more important (Table 5).

## Discussion

Our investigation indicates promise for statolith microchemistry as a technique to discriminate among sea lamprey produced in different streams, similar to the results of Brothers and Thresher (2004). Further, as we explain below, this technique seems robust in that there appears to be considerable flexibility in sample size requirements and methods for statolith preservation, ablation, and analysis (LA-ICP-MS versus PIXE).

**Comparison of laser-ablation approaches**. We found no difference between concentrations of Mn, Zn, Rb, Ba, and Pb in statoliths that were ablated across (horizontal traverse) versus those that were ablated through the apex (vertically). This result was expected, given that larval sea lampreys burrow into stream sediments for the entire larval period, so that their statoliths should uniformly represent the larval chemical environment (Volk 1986). While we did observe statistical differences for both Mg and Sr between ablation approaches, these differences are likely unimportant as the average differences were small (<500 ppm for Mg; < 20 ppm for Sr) relative to variation in average elemental signatures among streams from throughout the Great Lakes (Mg range: 3,083 to 5,586 ppm; Sr range: 111 to 1,114 ppm) (Hand 2006). While mean Sr values are analytically indistinguishable (strong overlap of SE, Table 2), concentrations for traversing across the statolith were consistently higher, resulting in a significant difference. Additionally, these slight differences may be due to heterogeneity in elemental concentrations across the statolith, which has been observed even in otoliths from small teleost fish larvae (Ludsin et al. 2006).

Visual inspection of LODs between ablation approaches suggests that the vertical ablation produces higher LODs than those obtained by traversing the width of the statolith, which is intuitive given that 1) average dwell times on statoliths were ~3-fold longer when traversing across (i.e., more material is ablated), and 2) our sample LODs are in part based on the amount of material ablated (i.e., LODs decrease as ablated material increases; Ludsin et al. 2006). Importantly, however, the differences in LODs were not statistically significant. These findings ultimately suggest that both techniques could be used to ablate statoliths, yet we recommend using a horizontal traverse (and used it for all of our remaining analyses) because 1) it may lead to lower LODs for less abundant elements, and 2) it allows more of the larval statolith to be analyzed, including both base and apex material.

**Comparison of statolith preservation methods.** The issue of preservation is of particular concern for the use of statolith microchemistry as a tool, since it is logistically difficult and cost-prohibitive for regulatory agencies to preserve adults and larvae using the same method. For larvae it is difficult, due to space and energy limitations, to carry a cooler to remote field locations, while for adult collections it is not cost-effective to store bodies in 95% ethanol due to their large size. Fortunately, analysis of statoliths from the two storage techniques demonstrated only minor differences between freezing and 95% ethanol storage. Specifically we found no differences in concentrations of Mg, Mn, Zn, Sr, Ba, and Pb, which is

consistent with findings from previous otolith/statolith investigations (Milton and Chenery 1998; Proctor and Thresher 1998; Hedges et al. 2004; Brothers and Thresher 2004). The one exception is that Rb was slightly higher in ethanol-preserved fish than in ones that were frozen. Although this difference is statistically significant, it is small enough in magnitude that it should not prove to be biologically significant. Our two test streams support this idea, given that the mean difference in Rb between freezing versus ethanol storage within our two study streams was < 0.53 ppm, whereas the mean difference in Rb between our two test streams was > 1.4 ppm. Additionally, Hand (2006) found that the average Rb concentrations from > 40 streams across Lake Huron ranged from 0.63 ppm to 8.36 ppm, indicating that differences of < 0.5 ppm due to storage technique are unlikely to play a major role in discriminating among streams. While we still would recommend that all specimens, both adults and larvae, be preserved in the same manner, if possible, our results suggest that the current practice of preserving larvae in ethanol and adults in a freezer is acceptable.

**Comparison of stream sample sizes.** The GLFC is currently seeking a cost-effective method for identifying which natal streams are producing the most parasitic- and spawning-phase sea lamprey. Owing to the large size of the Laurentian Great Lakes, applying this technique will require comparison of a large number of streams (e.g., Lake Huron has > 60 tributaries draining into it). For this reason, identifying the smallest possible sample size that will still accurately represent individual streams would be of great benefit, by reducing processing time and costs. While our multivariate analysis indicates that a sample size of 10 individuals should be sufficient, a different result might have been obtained had elements such as Zn and Pb, both of which varied in our univariate analyses (i.e., sample size of 10 differed from all larger sample sizes), been more important for discriminating among sites. Given the fact that both Zn and Pb concentrations in small samples (n = 10) were significantly different from all larger sample sizes when analyzed with univariate tests, but that no differences were found for sample sizes of 15 to 40 individuals for any element, we highly recommend using a minimum sample size of 15 fish per stream. Additionally, we recommend that future investigations examine the minimum sample size needed to detect differences among sites, given that we only looked at differences within a single stream.

**Comparison of PIXE versus LA-ICP-MS.** Overall, comparison of our results with those of Brothers and Thresher (2004) indicate that both PIXE and LA-ICP-MS are suitable for trace-elemental analysis of statoliths, but also that neither is perfect. For both methods, concentrations of elements were comparable; though LA-ICP-MS consistently exhibited a tighter range of concentrations (i.e., was more precise) for several elements, including Rb, Sr, Pb and Ba. In addition, LA-ICP-MS consistently produced lower concentrations of Rb, Sr, Pb and Ba than PIXE, which may indicate sample contamination stemming from the apparently less rigorous cleaning protocol used by Brothers and Thresher (2004) than we used, rather than differences arising from analytical technique. By the same token, occasional high Zn values (>600 ppm) arising from LA-ICP-MS also may be due to sample contamination (Brophy et al. 2003; Ludsin et al. 2006), not analytical error, even after rigorous cleaning.

A number of previous studies have demonstrated that both PIXE and LA-ICP-MS are capable of analyzing a large number of elements at low concentrations. Likewise, both techniques can be limited by individual element LODs. For example, although PIXE was capable of estimating Fe concentrations in statoliths (Brothers and Thresher 2004), LA-ICP-MS was not, owing to its low abundance in statoliths and high LOD. Similar difficulties in estimating Fe concentrations in teleost otoliths have been demonstrated (Campana et al. 1997). Conversely, LA-ICP-MS was able to precisely and accurately estimate Ba, an element PIXE is notoriously unable to analyze at low levels (Campana et al. 1997; Brothers and Thresher 2004). Given the strengths and weaknesses of both approaches, we recommend that researchers take care in deciding which technique to use, and glean information from previous studies. For example, previous otolith microchemical research in the Great Lakes has demonstrated that Ba is an important element for discriminating among yellow perch *Perca flavescens* spawning locations in Lake Superior and Lake Erie (Brazner et al. 2004; Ludsin et al. 2006). As such, future research investigations involving yellow perch (on these systems) may want to use LA-ICP-MS to ensure that Ba

is reliably quantified. By contrast, investigations where elements such as Hg, Ni, and Fe may be important, might want to lean toward using PIXE.

Three other major differences between analyzing statoliths using PIXE versus LA-ICP-MS are notable: 1) time required for analysis; 2) invasiveness of the technique; and 3) cost and availability. Excluding sample preparation time, PIXE requires between 10-15 min per sample, whereas LA-ICP-MS requires < 3 min. This difference in time may prove crucial, particularly when taking into account the number of replicates required per location to apply the technique to fisheries management. PIXE is a relatively non-destructive technique, while LA-ICP-MS destroys the sample as it ablates it. Thus, PIXE can allow for statoliths/otoliths to be examined for other purposes (e.g., aging, growth determination) after analysis, whereas LA-ICP-MS requires these determinations to be made prior to analysis (or use of the second statolith/otolith). In addition to these technical differences between methods, a major advantage of LA-ICP-MS is the wider availability of machines and lower cost for analysis than PIXE, logistics that will greatly influence the implementation of statolith microchemistry as a technique by fisheries management agencies. Ultimately, while both techniques are suitable for this type of analysis, we recommend the use of LA-ICP-MS when analyzing trace elements in sea lamprey statoliths because of the savings of time and money, and because of the likely importance of Ba for discrimination in freshwater systems.

**Among- and within- lake comparisons.** Our analysis of sea lamprey-producing streams from lakes Huron, Superior, and Michigan demonstrates promise for statolith microchemistry as a tool for discriminating among spawning tributaries within the Great Lakes. Overall, individual streams from these three lake basins could be discriminated with about 80% classification accuracy. Importantly, however, our ability to discriminate among lakes, when all streams within a lake were grouped into a single category (i.e., Huron vs. Superior vs. Michigan) was only about 60% (Hand 2006), indicating that stream elemental signatures *within* a lake basin also vary substantially such that no single stream could represent an entire Great Lake. The fact that we could discriminate among individual streams within lakes Huron, Michigan, and Superior with almost 20% higher accuracy demonstrates the fact that stream-specific signatures indeed differ within each of these systems. The differences in stream-specific signatures within a lake indicate that factors such as local geology, watershed runoff, and pollutant sources may overwhelm regional, basin-wide geology. This finding suggests that sufficient variation may exist to discriminate among natal streams of parasitic- and spawning- phase sea lampreys in any single Great Lake, as supported by previous otolith microchemistry studies that have successfully discriminated among local spawning locations within the Great Lakes. For example, Brazner et al. (2004) found that age-0 yellow perch from different Lake Superior wetlands could be discriminated with an average classification accuracy of 76%, while Ludsin et al. (2006) found this species could discriminate larval yellow perch from different spawning areas in Lake Erie with 69-100% accuracy.

Our ability to discriminate among streams was not perfect; several streams share a common signature. This commonality may be dictated by regional environmental factors that influence a stream, such as local geology or location in a watershed, as the larval period is spent burrowing in sediment. For example, in our analysis of 12 Great Lakes tributaries, four of them (Loeb Creek, Rifle River, Saginaw River, and White River) located in close proximity to one another in the lower peninsula of Michigan were consistently misclassified as one another, most likely because they shared a common geological source. By contrast, two streams (Nottawasaga River and Musquash River) located in relatively close proximity around Georgian Bay, but in different watersheds and geology, were not misclassified as one another and were almost perfectly differentiated. In addition, if we look back on the LDFA from the PIXE/LA-ICP-MS comparison, we see that the St. Mary's River discriminates slightly more accurately when compared to 11 other streams (90% accuracy) than when compared to a conglomerate of Lower Peninsula of Michigan streams (86% accuracy). From these results, we can see that relative geographical location holds little bearing over our ability to discriminate, but the importance of geology and watershed influences can be significant. Further, the addition of more distinct site-specific signatures (rather than a geographical generalization) increases our ability to discriminate important production streams.

## Conclusions

Overall, our results indicate that statolith microchemistry shows strong potential as a technique for discriminating among individual sea lamprey produced in different spawning tributaries, especially those of the Great Lakes. Although PIXE is a valid method of analyzing for trace-elemental composition of statoliths, and should be used in investigations where elements such as Hg, Ni, and Fe are likely to be important, we advocate using LA-ICP-MS over PIXE for freshwater investigations (including statolith microchemistry) because 1) it more precisely measures concentrations of elements oftentimes important for discrimination (e.g., Rb, Sr, Pb and Ba), 2) it is more widely available, and 3) it is faster and has lower analytical costs, In using LA-ICP-MS, we recommend a minimum sample size of 15 individuals per stream, and suggest that both freezing or 95% ethanol can be used to preserve fish (and still be comparable). Finally, despite its promise as a tool for discriminating among local sea lamprey production tributaries, our findings also suggest that statolith microchemistry may not provide perfect discrimination for all streams, owing to the likely influence of local geology and watershed effects. We therefore recommend further research to fully explore 1) the impact of these factors on discrimination abilities, 2) other potential limitations of the application of the technique (e.g., inter-annual variability, physiological effects), and 3) whether other approaches (e.g., statolith Sr isotope analysis) could be used in combination with statolith elemental concentrations to improve our ability to discriminate among individual streams.

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**Table 1.** Isotopes used for quantification (not including calcium) using LA-ICP-MS. Mean limits of detection (LOD) were calculated based on all sample runs. The coefficient of variation (CV), as determined from NIST 610 standards, is the average for all runs, and was calculated by dividing standard deviations of runs by their means. The percentage of samples greater than detection limits for a stream **(% > LOD )** also is provided. Isotopes shaded in gray met our criteria for inclusion in analysis.

| Isotope | $^{7}$Li | $^{25}$Mg | $^{55}$Mn | $^{57}$Fe | $^{66}$Zn | $^{85}$Rb | $^{86}$Sr | $^{88}$Sr | $^{137}$Ba | $^{138}$Ba | $^{140}$Ce | $^{208}$Pb | $^{238}$U |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Element LOD** | | | | | | | | | | | | | |
| **(ppm)** | 0.750 | 13.6 | 0.466 | 77.9 | 0.671 | 0.291 | 2.09 | 0.234 | 0.516 | 0.143 | 0.575 | 0.528 | 0.023 |
| **CV (%)** | 6.03 | 3.52 | 3.18 | 12.9 | 6.32 | 5.25 | 2.50 | 2.22 | 3.33 | 3.52 | 3.67 | 6.16 | 5.39 |
| **% > LOD** | 3 | 100 | 100 | 73 | 96 | 99 | 100 | 100 | 100 | 100 | 32 | 82 | 4 |

**Table 2.** Comparison between average elemental concentrations (ppm ± SE) and average limits of detection (LOD ± SE) of paired statoliths ablated across versus down through the apex (N=10 pairs), using LA-ICP-MS. Significant values are bolded.

| Element | Method | Concentration | p | LOD | p |
|---|---|---|---|---|---|
| Mg | Across | 3792 ± 147.7 | **0.022** | 9.72 ± 0.79 | 0.412 |
| | Down | 3324 ± 156.5 | | 10.6 ± 1.16 | |
| Mn | Across | 23.8 ± 1.53 | 0.995 | 0.405 ± 0.015 | 0.059 |
| | Down | 23.8 ± 2.60 | | 0.446 ± 0.029 | |
| Zn | Across | 20.7 ± 6.23 | 0.564 | 0.576 ± 0.058 | 0.097 |
| | Down | 29.2 ± 12.7 | | 0.833 ± 0.130 | |
| Rb | Across | 3.06 ± 0.69 | 0.451 | 0.145 ± 0.007 | 0.164 |
| | Down | 3.21 ± 0.74 | | 0.157 ± 0.009 | |
| Sr | Across | 367 ± 51.4 | **0.027** | 0.300 ± 0.044 | 0.488 |
| | Down | 348 ± 48.8 | | 0.371 ± 0.104 | |
| Ba | Across | 19.7 ± 7.01 | 0.515 | 0.077 ± 0.030 | 0.504 |
| | Down | 22.5 ± 10.7 | | 0.054 ± 0.008 | |
| Pb | Across | 0.202 ± 0.05 | 0.944 | 0.024 ± 0.004 | 0.366 |
| | Down | 0.206 ± 0.09 | | 0.02 ± 0.002 | |

**Table 3.** Standardized canonical scores for LDFA roots 1 and 2, from analysis of St. Mary's River versus three other streams in the lower peninsula of Michigan, using LA-ICP-MS. The cumulative proportion of variation also is provided. Bolded values indicate values most strongly associated with each root.

| Element | Root 1 | Root 2 |
|---|---|---|
| Rb | **-0.891** | 0.094 |
| Sr | **1.223** | -0.273 |
| Mn | -0.349 | **-0.797** |
| Ba | -0.695 | 0.119 |
| Zn | -0.336 | -0.578 |
| Pb | 0.333 | 0.634 |
| Mg | 0.164 | -0.167 |
| Cumulative Proportion | 0.641 | 0.955 |

**Table 4.** Classification matrix for 12 streams from the watersheds of lakes Huron (H), Michigan (M), and Superior (S). Numeric designations to the left of the stream names correspond to those along the top. Letters following stream names (H, M, S) denote which lake the stream drains into (see Figure 1; note, numbers do not correspond between this figure and Figure 1). Correct classifications are shown in bold font, with sample sizes (N) for each stream located along the bottom. Streams 1-4 were also used in the comparison of PIXE and LA-ICP-MS.

| | | | Classification accuracies | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Stream** | **% Correct** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | Black Mallard R. (H) | 100 | **12** | | | | | | | | | | | |
| 2 | Loeb Cr. (M) | 60 | | **6** | 1 | | 1 | 1 | | 1 | | | | |
| 3 | Rifle R. (H) | 31 | | 2 | **4** | | | | | | | 1 | 4 | 2 |
| 4 | St. Mary's R. (S/H) | 90 | | 1 | | **27** | | 1 | | 1 | | | | |
| 5 | Bad R. (S) | 80 | | | | 1 | **12** | 1 | | | | 1 | | |
| 6 | Boardman R. (M) | 60 | | 1 | | | | **6** | 1 | | | 1 | | 1 |
| 7 | Brule R. (S) | 100 | | | | | | | **15** | | | | | |
| 8 | Ford River (M) | 82 | 1 | | | | | | | **9** | | | 1 | |
| 9 | Musquash R. (H) | 100 | | | | | | | | | **25** | | | |
| 10 | Nottawasaga R.(H) | 93 | | | | | 1 | | | | | **28** | | 1 |
| 11 | Saginaw R. (H) | 61 | | | 4 | | | | | 3 | | | **17** | 4 |
| 12 | White R. (M) | 70 | | 1 | 1 | | | | | | | | 1 | **7** |
| | **Average % correct** | 80 | | | | | | | | | | | | |
| | | **N** | 13 | 11 | 10 | 28 | 14 | 9 | 16 | 14 | 25 | 31 | 23 | 15 |

**Table 5.** Factor correlations from canonical analysis of 12 streams within the watersheds of Lake Huron (n=6), Lake Michigan (n=4), and Lake Superior (n=2). The cumulative proportion of variation explained by each axis is presented. Bolding indicated the elements most strongly correlated with that root.

| Element | LDFA Roots | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Rb** | 0.422 | **-0.732** | -0.341 | 0.269 | -0.307 | 0.056 | 0.018 |
| **Sr** | **0.656** | 0.346 | -0.396 | -0.324 | 0.041 | -0.128 | 0.412 |
| **Mn** | **0.588** | -0.371 | **0.611** | -0.146 | 0.120 | -0.318 | 0.077 |
| **Ba** | 0.179 | -0.113 | -0.291 | **-0.786** | 0.161 | -0.404 | 0.254 |
| **Mg** | 0.142 | -0.041 | 0.229 | -0.567 | **-0.608** | 0.368 | -0.315 |
| **Pb** | -0.007 | -0.289 | 0.077 | -0.257 | 0.372 | **0.715** | 0.441 |
| **Zn** | -0.132 | -0.147 | 0.261 | -0.182 | -0.353 | 0.084 | **0.853** |
| **Cumulative Proportion** | 0.442 | 0.741 | 0.861 | 0.942 | 0.981 | 0.995 | 1.000 |

## FIGURE LEGENDS

Figure 1. Sample site locations in Lake Huron, Lake Michigan and Lake Superior (designated as H, M and S, respectively). Streams are: 1) Sauble River (H), 2) Lauzon Creek (H), 3) Big Manistee River (M), 4) Silver Creek (H), 5) Loeb Creek (M), 6) St. Mary's River (S/H), 7) Black Mallard River (H ), 8) Rifle River (H), 9) Bad River (S), 10) Brule River (S), 11) Boardman River (M), 12) Saginaw River (H), 13) Nottawasaga River (H), 14) Musquash River (H), 15) Ford River (M), and 16) White River (M). Longitude is denoted on the x-axis; latitude on the y-axis.

Figure 2. Concentrations of magnesium (Mg), zinc (Zn), rubidium (Rb), strontium (Sr), lead (Pb), and barium (Ba), as measured by PIXE (from Brothers and Thresher 2004) and LA-ICP-MS, from four Lake Huron and Lake Michigan streams. The streams are: St. Mary's River (top row), Black Mallard River ($2^{nd}$ row), Rifle River ($3^{rd}$ row) and Pigeon River (PIXE)/Loeb Creek (LA-ICP-MS) (bottom row).

Figure 3. Linear discriminant function analysis root 1 versus root 2, which explained 74% of the variation among the four Lake Huron streams.

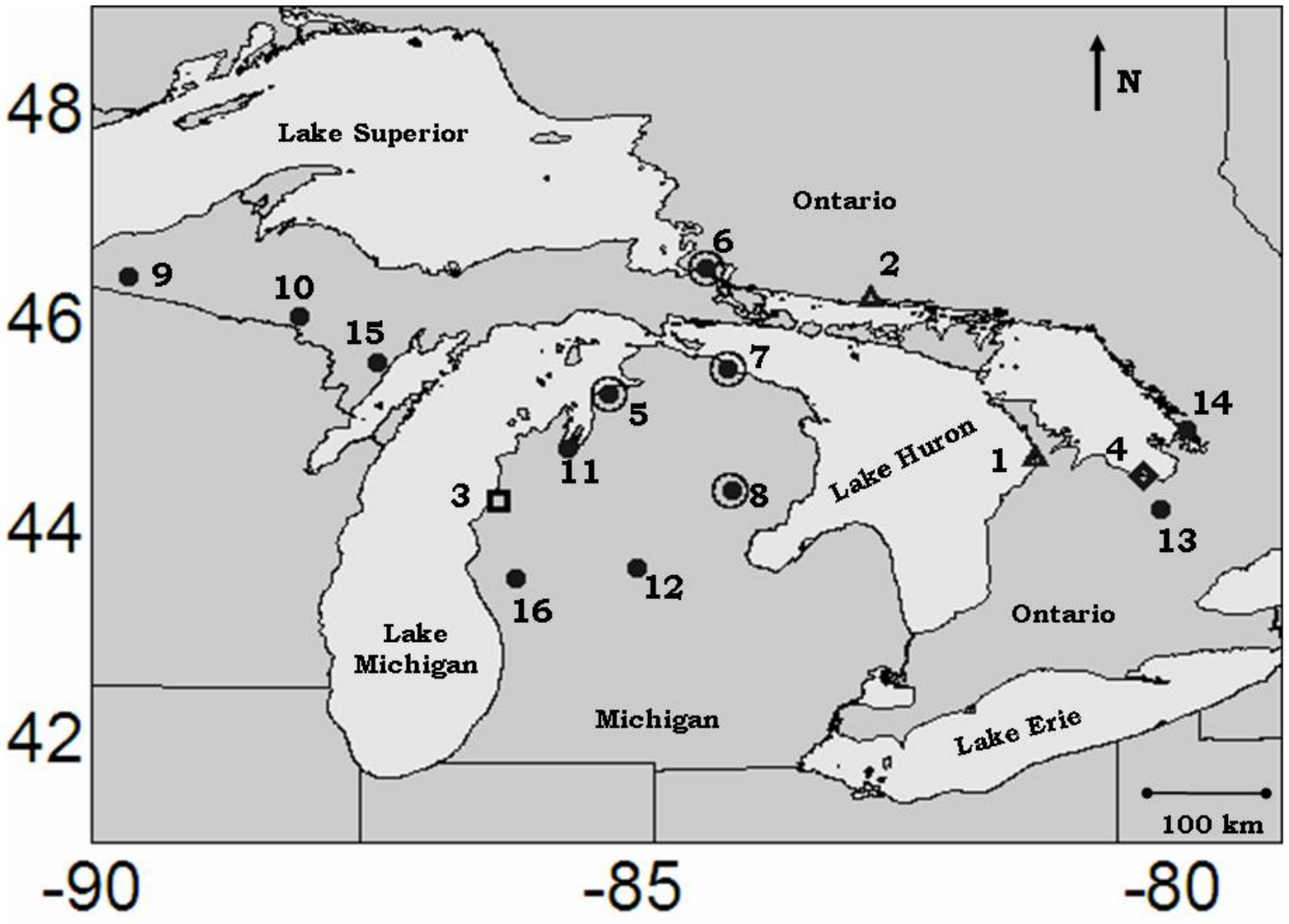
48
46
44
42
-90
-85
-80
N
Lake Superior
Ontario
Lake Huron
Lake Michigan
Michigan
Ontario
Lake Erie
100 km
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

Figure 1

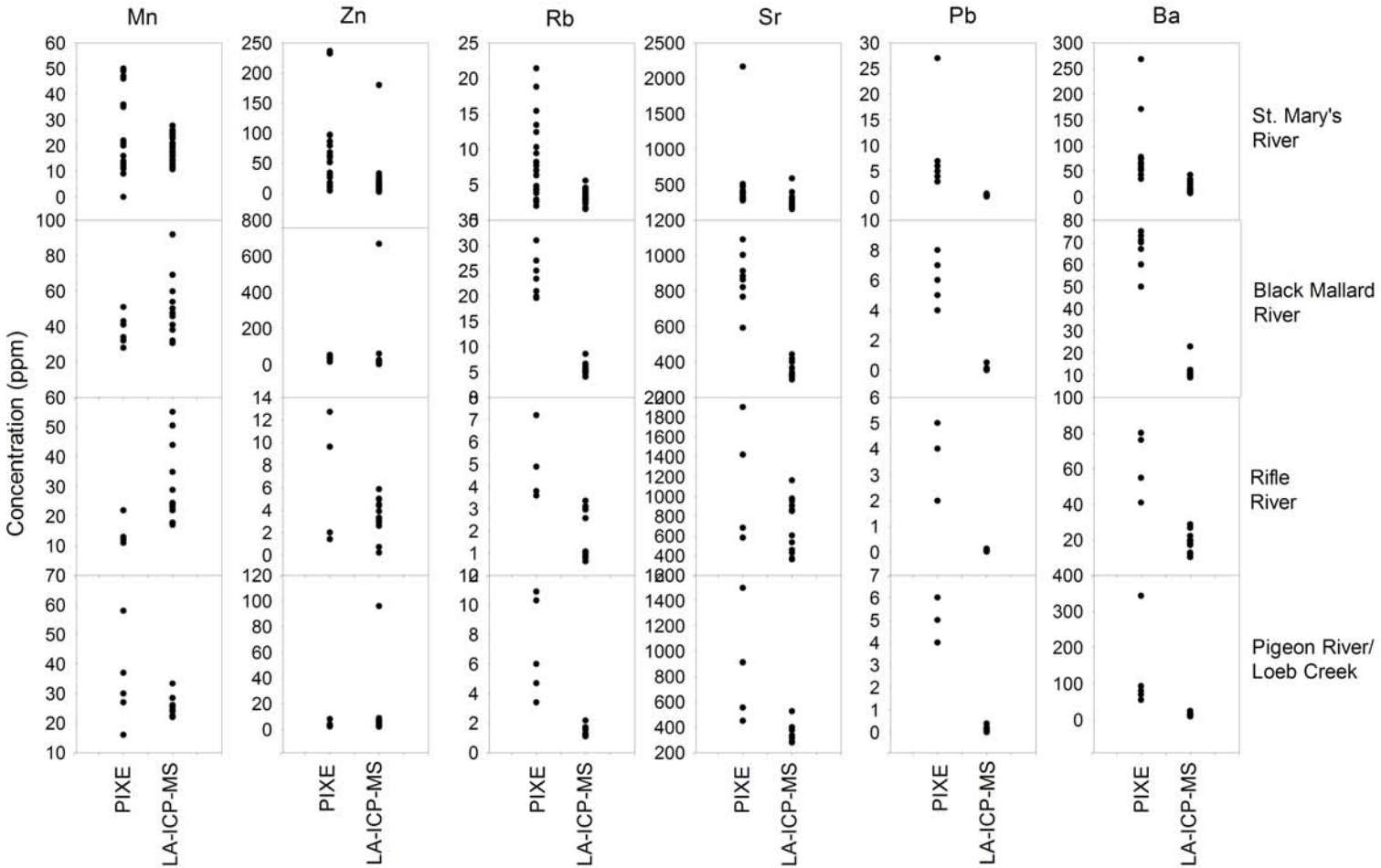
Mn
Zn
Rb
Sr
Pb
Ba
Concentration (ppm)
PIXE
LA-ICP-MS
St. Mary's River
Black Mallard River
Rifle River
Pigeon River/ Loeb Creek

Figure 2

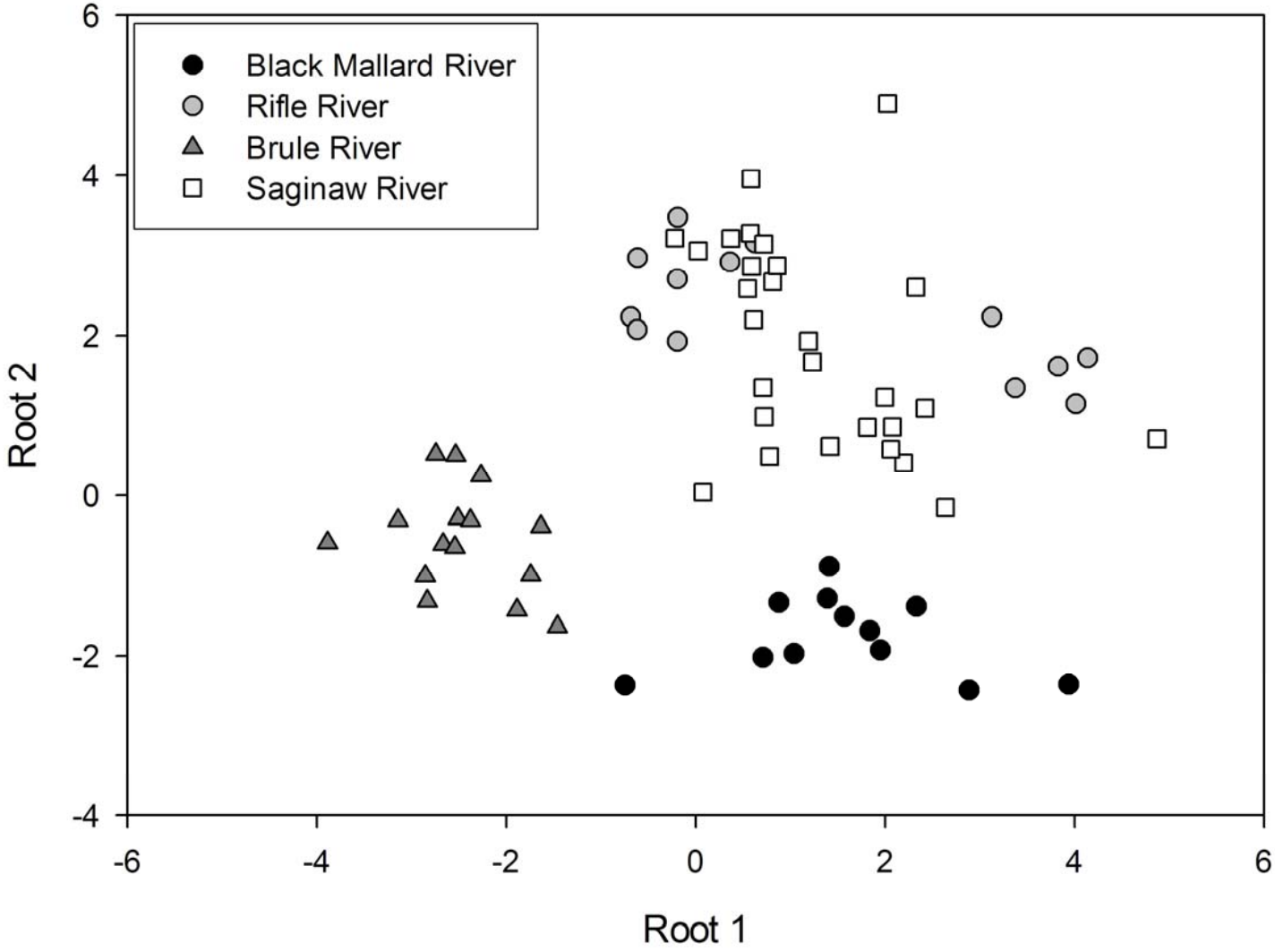
Black Mallard River
Rifle River
Brule River
Saginaw River
Root 2
Root 1
6
4
2
0
-2
-4
-6
-4
-2
0
2
4
6

Figure 3

## APPENDIX 2

Hand, C.P., S.A. Ludsin, B.J. Fryer, and J.E. Marsden. 2006. Ability of statolith microchemistry to discriminate amongst sea lamprey (*Petromyzon marinus*) larvae from Lake Huron. Pages 41-80 in C. Hand's M.Sc. thesis. Micro-elemental analysis of statoliths as a tool for tracking the stream origins of sea lamprey (*Petromyzon marinus*) in Lake Huron. University of Windsor, Windsor, ON, Canada.

## Introduction

Sea lamprey (*Petromyzon marinus*) first invaded the upper four Laurentian Great Lakes following construction of the Welland Canal in the early 20th century (Lawrie 1970). Since that time, sea lampreys have adapted and extended their range to include all of the Great Lakes, where their impact on native fish communities has been devastating (Weise and Pajos 1998). The magnitude and extent of their impact resulted in the creation of the Great Lakes Fishery Commission (GLFC), a bi-national agency that oversees sea lamprey control efforts throughout the Great Lakes. These efforts include the creation of physical and electrical barriers to prevent upstream migration of spawners, trapping of adult spawners, sterile male release programs, and application of lampricides (i.e. granular bayluscide; 3-trifluoromethyl-4-nitrophenol TFM) to larval production streams (Smith and Tibbles 1980). In turn, the abundance of parasitic phase sea lampreys declined by as much as 90% in some lakes leading up to 1970, only to rebound and increase dramatically since that time in other systems (Young et al. 1996). As such, millions of dollars are still spent annually on these various control efforts. Given the expense of these efforts, the GLFC is seeking a reliable method to identify important tributary sources of sea lamprey so that control efforts can be better prioritized (Schleen et al. 2003).

Since sea lamprey control efforts began in the 1950s, the primary focus has been on either preventing spawning, accomplished by stopping upstream migration of spawning adults with barriers and releasing sterile males into the environment, or killing larvae prior to metamorphosis, accomplished by applying lampricides to known production streams (Smith and Tibbles 1980). Focusing control efforts on the larval stage is most sensible, however, since while most Great Lakes fish have short larval stages (from weeks to months), sea lampreys spend 3-7 years of their lives as larvae in a single tributary, only emerging as parasitic juveniles in the open lake during their final 1-2 years of life (Potter 1980; Manion and McLain 1971; Quintella et al. 2003*)*.

Unfortunately, lampricides have drawbacks, including costs for purchase and application, and mortality of non-target species (McLaughlin et al. 2003). Owing to these costs, the GLFC is seeking methods to allow them to focus and prioritize control efforts such that only tributaries that contribute large numbers of parasitic and spawning phase sea lampreys are targeted. Currently, the primary method used to prioritize streams that will undergo lampricide treatment is an intensive survey of the larval distribution in streams (a Quantitative Assessment Survey, QAS). The QAS is based mostly on the presence of larvae and the length frequency distribution of larvae, both of which have proven difficult to quantify, particularly for large streams. This approach also assumes that all larvae will progress to the parasitic phase (i.e., survival is equal among streams), and therefore, the number of parasites emerging from streams correlates precisely with the number of larvae produced in those streams (Slade et al. 2003). In turn, streams with the highest larval density generally get highest priority for lampricide treatment (Smith and Tibbles 1980). Given the strong likelihood that larval survival varies among streams, the GLFC is seeking other methods of identifying important sources of parasitic and spawning lampreys.

Artificial tags offer one alternative for determining the differential contribution of larval production streams to parasitic and spawning adult populations. Toward this end, Bergstedt et al. (1993) developed a coded wire tagging program for Great Lakes sea lamprey. However, as with all artificial tagging programs in large systems, the likelihood of short-term success is low, owing to the difficulty and expense of tagging and recovering sufficient numbers of individuals. Recovering tagged individuals also seems especially difficult for sea lamprey, given that individuals do not home to their natal streams (Bergstedt and Seelye 1995).

'Natural' tags offer an easier and more cost effective approach, since every individual is tagged at birth, (i.e., no application is required). Two methods are common in fisheries research: genetics and otolith microchemistry. The use of genetic markers for sea lamprey in the Great Lakes is not particularly feasible since populations are recently established (only present since ~1920s), so they have not have developed a clear genetic structure, and sea lamprey do not home to their spawning streams, thereby preventing

genetic differentiation among populations (Smith and Tibbles 1980). Indeed, Jacobson et al. (1984) found sea lamprey larvae to be undesirable for stock identification studies, given that allelic frequency variation within drainages was greater than among them.

Owing to the limitations of genetic approaches, the other natural tag, otolith microchemistry (statolith microchemistry in the case of sea lampreys), offers more promise. Statoliths are the calcified ear stones found in sea lampreys, analogous to the otoliths found in teleost fish (Carlstrom 1963). Whereas otoliths are comprised of calcium carbonate (usually aragonite), and typically continue growing in proportion to body size throughout the life of the fish, statoliths are made of calcium phosphate (apatite), are the only calcified structures found in an otherwise cartilaginous body, and do not grow substantially post-metamorphosis (Volk 1986). Preliminary work by Brothers and Thresher (2004) concluded that not only is it possible to use scanning proton microprobe (micro-PIXE) to discriminate among Lake Huron streams using statolith micro-elemental composition, but the St. Mary's River, a major producer of sea lampreys in Lake Huron, could be easily distinguished from other tributaries (Brothers and Thresher 2004). More recently, Hand et al. (submitted) explored the potential of laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS) to quantify statolith microchemistry in Great Lakes sea lamprey, and arrived at a similar conclusion to Brothers and Thresher (2004). However, in both of these sea lamprey statolith investigations, less than 12 Lake Huron production streams were investigated, whereas >60 potential production streams exist in the Lake Huron watershed. As such, whether individual streams can be sufficiently discriminated from enough other streams to benefit sea lamprey control efforts in Lake Huron remains unknown.

Herein, we build on these two previous statolith microchemistry investigations by exploring variation in statolith micro-elemental signatures among 45 Lake Huron larval production streams. In addition to determining how well individual streams can be discriminated from one another, we explore the effects of local geology and watershed characteristics on our results, as well as quantify inter-annual variability in stream-specific signatures. Ultimately, we provide recommendations concerning the future use of this technique to eventually identify natal origins of parasitic- and spawning-phase sea lamprey in Lake Huron and other Great Lakes.

## Materials and Methods

**Field collections.** Sea lamprey larvae were collected from 45 Lake Huron tributaries by the Canadian Department of Fisheries and Oceans (Sault St. Marie, ON) and the U.S. Fish and Wildlife Service (Marquette, MI and Luddington, MI field stations) (Figure 1). All larvae were collected via electrofishing during post-lampricide surveys (biological collection and quantitative assessment surveys (QAS)) in 2004 and 2005. Larval samples were provided to us stored in 95% ethanol, which does not appear to affect trace elemental concentrations in sea lamprey statoliths (Brothers and Thresher 2004; Hand et al. submitted). For this study, we processed 6 to 30 individuals per stream, deviating from the suggested 15 samples (Hand et al. submitted) when a limited number of samples were available, there was more than one year of data, or $^{120}Sn$ concentrations were high enough to suggest possible contamination (Table 1).

**Larval sample preparation.** All statoliths were prepared in a Class 100 clean room. Prior to removal, the total length (TL; to nearest mm) of each individual was measured, after which both statoliths were removed and rigorously cleaned according to the protocol in Hand et al. (submitted). Afterwards statoliths were mounted dorsal side up to a glass slide with Scotch™ double sided tape (Ludsin et. al. 2006). Statoliths were analyzed with LA-ICP-MS, using a Thermo Elemental X7 ICPMS coupled to a Continuum solid state ND:YAG laser (wavelength: 266nm; max power: 40mJ; pulse rate: 20 Hz; beam width: 6mm). We quantified 11 elements with LA-ICP-MS (Table 1) by traversing the entire width of the statolith, only finding the following seven (not including calcium) suitable for analysis, using the criteria of Ludsin et al. (2006) and Hand et al. (submitted): magnesium (Mg), manganese (Mn), zinc (Zn), rubidium (Rb), strontium (Sr), barium (Ba) and lead (Pb).

**Data analysis.** To identify the resolution at which streams can be discriminated, and also to determine the effect of watershed and geology bedrock record on our discrimination ability, we used forward stepwise linear disriminant function analysis (LDFA; Statistica software, Statsoft Inc., Tulsa, OK). We ran three separate LDFAs to explore our ability to discriminate individuals collected in different groupings: 1) geologic zones (n=4 zones: older Paleozoic, Southern/Superior, Grenville and younger Paleozoic (Figure 2)); 2) major watershed (n=9 watersheds: Lone Lake Ocqueoc, North Lake Huron, Carp-Pine, Aues Gres-Rifle, East Georgian Bay, St. Mary's, Titabawasee, East Lake Huron and Wanipiti-French (Figure 1)); and 3) streams (n=45 streams) (Table 2; Figure 1). In each LDFA, our suite of seven elements (Table 1) was used to predict group membership. In addition, because larval sea lamprey total length varied among streams (ANOVA: $F= 14.21$, $p= 0.00$; Table 1), we used average stream total length as a covariate in all analyses (Ludsin et al. 2006) to guard against potential physiological effects on site-specific signatures. For entry into our model, a predictor had to have a minimum F-value and tolerance equal to one. Classification accuracies were determined by a jackknifing procedure, conducted as part of a default stepwise LDFA. In instances where an element was below the sample limit of detection (LOD; see Ludsin et al. 2006 for its determination), we replaced each missing value with a random value generated from between that sample's LOD and 0 (using a uniform probability distribution).

All elements were natural-log transformed prior to analysis to normalize the data (Kolmogorov-Smirnov test for normality: all $p>0.20$). Alpha levels were set at 0.05.

## Results

**Geology.** When streams were grouped according to geologic zone, Sr, Mn, Rb and Zn were most important for discrimination, all being negatively correlated with LDFA root (axis) 1 (Table 3). Strontium also was positively related to LDFA root 2, while Rb also was negatively related to it (Table 3). In total, the first two roots explained 93% of the variation among geologic zones. Root 3, which explained the remaining variation, was dominated by Mn (negatively related) and total length (TL) (positively related). Lead also was statistically significant in our model, but its relative importance for explaining variation among streams within different geologic zones was minimal.

Using geologic zones as a grouping variable, we were able to correctly classify 69.7% of the individuals, with classification success for individual zones ranging from 66% to 81% (Table 4). From analysis of our classification matrix, we could distinguish the Grenville effectively from both younger Paleozoic and older Paleozoic due to high levels of Mn, Rb and Sr and low Zn, and we could distinguish the younger Paleozoic from Grenville and Southern/Superior due to low Mn and Rb and high Sr and Zn values (Table 4; Figure 3). By contrast, fish from older Paleozoic substrates were consistently mistaken for all other zones, owing to the large range of elemental concentrations in individuals collected throughout this region (Mn: 7-360 ppm, Zn: 0.05-1704 ppm, Rb: 0.4-11 ppm, Sr:95-1468 ppm). Ultimately, the two geologic zones we could reliably discriminate from all others were the Grenville (81%) and the younger Paleozoic (78%) (Table 4).

To assess our confidence in these classifications, we averaged the posterior probabilities for each correctly classified individual by geologic zone. This analysis suggests that we can have confidence in our classifications, especially for the Grenville zone (average posterior probability = 0.89; Table 4).

**Major watershed.** When the fish were grouped by major watershed, all seven elements and TL were included (and significant) in the final model (Table 5). However, TL was largely unimportant, being correlated with LDFA root 5, which explained less than 4% of the variation among watersheds (Table 5). Similarly, Ba, Pb, and Mg were relatively unimportant, being correlated with LDFA roots 5, 6 and 7, which combined explained less than 6% of the variation among watersheds (Table 5; Figure 4). Thus, similar to our analysis of geologic zones, Sr, Rb and Mn were the most important elements. The first two

roots were dominated by Sr (strongly, negatively correlated to LDFA root 1) and Rb (strongly, positively correlated to LDFA root 2) and described ~73% of the variation, whereas LDFA root 3 was most highly (positively) correlated with Mn (Table 5).

For the nine major watersheds, the overall classification accuracy was 48%, with success for individual watersheds ranging from ~31% (Lone Lake Ocqueoc) to 100% (Wanipiti-French and East Lake Huron) (Table 6). From analysis of misclassifications, we could clearly distinguish 1) the Wanipitai-French (located near Georgian Bay; Figure 1) from most other watersheds based on high Mn and Rb and low Sr values, 2) the East Lake Huron (located near Georgian Bay; Figure 1) from most others due to low Mn, Rb and Sr values, and 3) the Titabawasee (located in the lower peninsula of Michigan; Figure 1) from all but the Aues-Gres Rifle (also located in the lower peninsula of Michigan; Figure 1) due to intermediate Mn, low Rb and high Sr. Lone Lake Ocqueoc, North Lake Huron and Carp-Pine, all of which are located in Michigan or along the top of the basin (Figure 1), had the worst classification success (Table 6). All three watersheds were frequently mistaken for one another, as well as the St. Mary's, due to similar intermediate levels of Sr and Rb (Table 6; Figure 4). Many sea lamprey were misclassified to the St. Mary's watershed, but the St. Mary's watershed discriminated fairly well (70% correct), most likely due to low Rb and Sr values. Ultimately, we could fully distinguish both the Wanipitai-French and East Lake Huron watersheds from all others based on high Rb and Mn and low Sr, and low Mn, Sr and Rb, respectively (Table 6; Figure 4).

As with the LDFA based on geologic zones, we assessed our confidence in our classification success by averaging posterior probabilities for correctly classified individuals in each watershed. Average posterior probabilities ranged from a less certain 0.43 (North Lake Huron) to fairly certain 0.85 (Wanipitai-French) (Table 6). Thus, even though individuals were re-classified with 100% accuracy for the Wanipitai-French and East Lake Huron watersheds, our confidence in these classifications is a bit lower (Table 6).

**Individual Streams.** Similar to previous analyses, all factors were significant in our final model, with Mn, Rb, Sr and Zn once again being the most important for discriminating among streams (Table 7). Manganese, Rb and Sr dominated the first three LDFA roots, with Mn and Sr positively related to LDFA root 1, Rb positively and Sr negatively related to LDFA root 2 and Mn positively and Sr negatively related to LDFA root 3 (Table 7). Sixty-four percent of the variation among streams was explained by the first two roots, with an additional 15% explained with LDFA root 3. Total length (TL) and Ba were both important as well, dominating roots 4 and 5, respectively, and explaining a combined 12% of the variation among streams (Table 7). Magnesium and Pb, dominating LDFA roots 7 and 8, were relatively unimportant, as combined they described only 5% of the variation among streams (Table 7).

Overall, we were able to classify fish back to their natal streams with 68% accuracy. Individual stream classification rates ranged from 6% to 100%, with four streams exhibiting perfect classification: Browns Creek (#7 on Figure 1), French River (13), Manitou River (23) and Thessalon River (41) (Table 8). Of the 45 streams, 11 had $\leq$ 60% correct classification accuracy, seven had between 60 and 70%, four had between 70 and 80%, nine had between 80 and 90%, and 14 had 90% or higher (Table 8). Due to the impracticality of graphing all individuals for each of the 45 streams (1000+ samples), we instead provide a ternary plot of the mean Sr, Rb and Mn concentrations for each of the streams that demonstrates how well some streams discriminate (Figure 5). The streams with 100% correct classification had similar concentrations for the most significant elements, with all values being intermediate except for low Mn and Zn in the Manitou River (23), low Sr in the Thessalon River (41), and high Rb and low Sr in the French River (13) (Table 2). As shown in figure 1, the four streams come from 3 different geologic zones, one older Paleozoic (Manitou River (23)), two Southern/Superior (Browns Creek (7) and Thessalon River (41)) and one Grenville (French River (13)).

Using two separate data sources, a case study of Lake Huron 1979 to 1999 (Morse et al. 2003) and the 2003 GLFC Sea Lamprey Annual Report (Young and Klar 2003), we were able to determine streams which have historically been major producers in Lake Huron. Morse et al. (2003) indicate that 32 of our

45 streams are considered Category 1 streams (i.e., highly productive for sea lampreys). Of these 32 streams, we are able to discriminate eight with 90% or higher accuracy, including Albany Creek (1), Blue Jay Creek (5), Manitou River (23), Serpent River (35), Thessalon River (41), Browns Creek (7), Gawas River (15) and Garden River (14). All of these streams, with the exception of Albany Creek, are located either in the Canadian Shield (Grenville or Southern/Superior) or on Manitoulin Island (older Paleozoic). Figure 6 demonstrates how well those 8 streams discriminate from one another. Albany Creek, in particular, has been slated as a large producer, and it was completely discriminated with high Mn, low Rb and intermediate Sr and Zn, with the exception of one sample misclassified as Gordon Creek (16) (also an important producer) (Table 2).

Two additional streams that have been designated as important producers are the Cheboygan River (9) and the St. Mary's River (38) (Morse et al. 2003). The Cheboygan River, located in the lower peninsula of Michigan, was classified with 86% accuracy, and had low Mn and Rb concentrations and intermediate Sr and Zn concentrations (Table 2). Two Cheboygan River samples were misclassified, one as the Mindemoya River (25) and one as the Manitou River (23), both of which also exhibit low Mn. The St. Mary's River, acknowledged as the single leading producer of parasites and spawners, was misclassified as McKay Creek (24) several times, and vice versa. Both streams have low levels of Sr, and comparable low levels of Rb and Mn (Table 2).

The 2003 GLFC Sea Lamprey Annual Report (Young and Klar 2003) estimated larval year class size for 2003, and 14 of our streams are on that list as Category 1 streams. Of those 14 streams, six with large estimated populations have ≤ 60% classification accuracy, while three streams have 80-90%. Of the poorly classified streams, the Echo (11) and Mississagi Rivers (26) are of particular concern, the Echo having < 7% correct classification (and nearly 10,000 larvae estimated in 2003), while the Mississagi had 40% classification, but nearly 430,000 larvae estimated (Young and Klar 2003). The Echo River was characterized by intermediate concentrations for all elements, and was misclassified as any of 14 different streams, without any particular pattern as to location. The stream with which the Echo River was most misclassified was Grace Creek (17), a category 3 stream (not very productive) in the Lower Peninsula. The Mississagi River, in contrast, was characterized by high levels of Mn, Rb and Sr, but intermediate levels of Zn. The Mississagi River was misclassified as 6 other streams, but four of those streams are category 2 (less productive for sea lamprey), such that only two samples were classified as other streams of concern (Albany Creek (1) and Black Mallard River (4)). Figure 7 shows both poorly classified streams, and the streams they are misclassified as, illustrating the overlap.

Analysis of average posterior probabilities of correct classifications for each stream demonstrates that 25 streams had posterior probabilities ≥ 0.7, while the remaining 20 had between 0.24 and 0.69 likelihood of being classified to that particular stream. A distinct pattern emerged for posterior probabilities, where we were generally very confident in our classifications for streams that also exhibited good discrimination (> 70%), but were much less confident in our streams with low classification rates. This is illustrated by a $R^2$ value of 0.58 for correct classification versus posterior probability.

**Inter-annual variation.** Closer inspection of our individual stream analysis revealed that nearly half of the streams with larvae sampled during both 2004 and 2005 (5 of 11 streams) had classification accuracies less than 60%. To assess the potential influence of inter-annual variation in stream-specific signatures on our ability to discriminate, we removed one year of data from a stream sampled during both 2004 and 2005, and re-ran the LDFA without it. We did this for both years for all 11 streams sampled in both 2004 and 2005 (22 analyses in total) and then compared classification accuracies for those streams when only 2004 data, 2005 data or both years of data were included (Table 9).

Overall, our results suggest that inter-annual variation in stream-specific signatures may reduce our discrimination abilities, given that classification accuracies were potentially much higher when one year of data had been removed from analysis. For example, the Echo River (11) had ~7% correct classification with both years, but increased to ~67% when only 2005 data were present and increased to 60% when

2004 data were present. This pattern was also evident in the Naiscoot River (28) (improved by 16% when either year was removed), the Trout River (44) (improved by 10% in both cases) and the St. Mary's River (38) (improved by 26% when only 2005 data and by 6% when only 2004 data) (Table 9). Nine of the 11 streams had classification accuracies that stayed the same or improved when one year of data was removed. In contrast, the Tawas River (40) improved by 20% with only 2004 data, but decreased by 13% with only 2005 data. The Pine River (30) was the prime example where both an increase and a decrease occurred, with classification accuracy improving to 100% with only 2004 data, but decreasing to 0% with only 2005 (Table 9). With few exceptions, there was a clear increase in classification accuracy when one year of data (whether 2004 or 2005) was removed.

## Discussion

Sea lampreys have been a major problem in the Great Lakes for over a century. Current control efforts have substantially improved the situation, but there is still a need for a method to identify important production tributaries. With this project we tested statolith microchemistry as a tool, determining our ability to discriminate individuals produced in different streams in different geologic zones, and watersheds. Below, we discuss the effectiveness of this technique for discriminating known production tributaries, and then possible effects of inter-annual variability on our ability to discriminate. Afterwards, we use our findings to provide recommendations to those interested in using statolith microchemistry as a tool to identify natal origins of sea lamprey in the Great Lakes.

Regardless of how the data were grouped (geology, watershed or stream), Mn, Rb, and Sr consistently dominated the first several LDFA roots, and thus were the most important elements for discriminating among individuals produced in different areas. Our results are supported by Brothers and Thresher (2004), who found the same three elements to be the primary site discriminators in their pilot work with micro-PIXE, and found Pb and Ba to be relatively unimportant. Brothers and Thresher (2004) also found Zn to be somewhat important, especially for distinguishing the St. Mary's River (38) from other streams. By contrast, Zn was relatively unimportant in our analyses, perhaps due to the many streams we sampled, but Brothers and Thresher (2004) did not, and those streams having similar levels of Zn to those of the St. Mary's River. Further, because Zn can change due to physiology and fish size (Renfro et al. 1975), it also may be possible that high levels of Zn relative to other systems in Brothers and Threshers (2004) work may have been due to physiology and not water chemistry. The fact that Zn and TL are positively correlated to one another, and also highly correlated with LDFA root 4 in our individual stream analysis (Table 7), supports this contention.

The consistent pattern of important elements is very clearly influenced by geology. In our results we saw high values for all three major elements in the Grenville (Table 4), results which are consistent with the high Rb and areas of high Sr expected for Precambrian rocks. In contrast, for limestones we expect high Sr and Zn, both of which we found in the older and younger Paleozoic zones. While these generalities direct what we can expect for these geologic zones, the areas are large and do not take into account local influences of water and sediments.

While the important elements for discrimination were fairly consistent regardless of how the data were grouped, our classification accuracy varied drastically. We were able to discriminate with fair accuracy both geological zones and individual streams; however, at the watershed level, our accuracy was generally poor. This fact is due to the tendency of one watershed to incorporate multiple geologic zones, such that fish being classified collectively are exposed to a wide array of geologic influences. Importantly, while geology does appear to be a driving influence on our ability to discriminate, high classification accuracies for more than a dozen streams also indicates the significant influence of local water chemistry.

Aside from Brothers and Thresher (2004) and Hand et al. (submitted), no previous work has been done on statolith microchemistry as a method for discriminating among natal streams of sea lamprey. However, previous work with teleost fish has explored the ability of otolith elemental composition to discriminate among individuals produced in different spawning areas of the Great Lakes. For example, similar to our work, Brazner et al. (2004) found Sr and Mn to be important for discriminating among age-0 yellow perch (*Perca flavescens)* produced in different Lake Superior wetlands, though they also found Ba, Mg and K to be important. Likewise, Ludsin et al. (2006) found Sr, Ba, Zn and Mg to be useful for discriminating larval yellow perch produced in different spawning areas in Lake Erie. In both studies site discriminators were consistent with what we found, with the exception of K, which we did not analyze.

The usefulness of statolith microchemistry as a technique should not be defined by the average classification accuracy, but rather how well individual production streams can be discriminated. Our ability to discriminate eight major production streams with 90% or greater accuracy (i.e., Albany Creek (1), Blue Jay Creek (5), Manitou River (23), Serpent River (35), Thessalon River (41), Browns Creek (7), Gawas River (15) and Garden River (14)) is very promising, particularly when three of these eight streams are classified perfectly (100%) and have high posterior probabilities of classification. Thus, we are optimistic that our results can provide immediate value to the GLFC efforts to identify contributions from these streams.

Unfortunately, other important production streams appear more problematic. For example, the Cheboygan River (9) had a successful classification rate of 86%, yet the misclassified fish were consistently classified as originating from other important production tributaries. Likewise the Echo River (11) also was consistently misclassified as other major producers. The St. Mary's River (38), which is the largest producer of parasitic sea lamprey in Lake Huron (Young and Klar 2003), could only be discriminated with 67% success, oftentimes being confused with McKay Creek (24), also an important producer of sea lamprey (Morse et al. 2003). Thus, whereas Brothers and Thresher (2004) found that the St. Mary's River could easily be discriminated from three streams in the lower peninsula of Michigan (Rifle River (31), Black Mallard River (4), and Pigeon Creek), it is likely the case that the St. Mary's River cannot be discriminated well using just elemental concentrations when the majority of other Lake Huron streams are included in the analysis.

In a study by Kennedy et al. (2000), Sr isotopes were found to be stable over time, and allow for effective discrimination (83%) among juvenile salmon. Strontium isotopes were stated to be the most effective for several different reasons, but most applicable to this study is that $^{87}Sr/^{86}Sr$ ratios arise from differences in bedrock geology. Preliminary data suggest this usefulness for discrimination extends to statolith microchemistry, as stream water from our different geologic zones exhibit distinct Sr isotopic signatures (Fryer, unpublished data).

Because it is not feasible to sample all sea lamprey-producing streams in the Lake Huron watershed every year, a potential limitation to using statolith elemental concentrations to discriminate among production areas is inter-annual variability in stream-specific signatures. From our analyses of streams sampled in multiple years, it is evident that inter-annual variation is affecting classification accuracy. Several of our most poorly classified streams were ones that were sampled over multiple years. Further, classification rates almost universally improved when one year was removed, indicating that variation in site-specific signatures between years can be greater than signatures among streams sampled in the same year.

Previous otolith microchemistry work with teleost fishes also has documented temporal variation in site-specific signatures, with variation occurring at daily, weekly, monthly, seasonal and annual time scales. For example, Elsdon and Gillanders (2006), in their exploration of water samples in Gulf St. Vincent, Australia, found that Ca, Ba, Sr and Mn concentrations varied on the scale of days and weeks, while Sr also varied seasonally. Hatje et al. (2001) also found that Cu, Pb and Zn varied on the scale of hours and days in their study of 3 rivers draining into the Port Jackson Estuary, Australia. An important conclusion in this second study was that small scale variability becomes more important as the size of the natural

system decreases (Hatje et al 2001). In a freshwater study performed on the Mississippi River, it was found that Mn, Zn and Pb were highly variable seasonally, while Rb and Ba were fairly stable seasonally (Shiller 1997). No inter-annual variability was found, though Shiller (1997) suggested that hydrologic factors such as mixing of tributaries or changes in discharge rates did have an impact on inter-annual variability.

For our study, we only had a small subset of streams (n=11) with two years of data; however, we observed at least some inter-annual variation for each of them, despite the fact that they varied tremendously in both location and size. Further, Young and Klar (2003) projected that 8 of the 11 streams for which we had two years of data would produce parasitic sea lamprey the following year, a further illustration of the need to continue exploring the influence of inter-annual variation. Overall, before this technique is implemented, this phenomenon needs to be examined further, as our evidence of inter-annual variability could be due to real variation in site-specific signatures, due to varying water chemistry, or due to samples coming from different locations within the stream. Clearly, assessment of how site-specific signatures vary over a longer time span (5 to 10 years) would allow us to place our observed variation in a better context.

## Conclusions

Herein, we explored statolith microchemistry as a technique to identify natal origins of sea lamprey, and ultimately help prioritize sea lamprey control efforts in Lake Huron. Overall, our results demonstrate promise, given that we could accurately discriminate a substantial number of streams within the Lake Huron basin, many of which have been identified as likely contributors of parasitic- and spawning-phase sea lamprey, based on QAS estimates of larval abundance (Young and Klar 2003). However, statolith microchemistry seems far from perfect as a technique, as demonstrated by our inability to successfully discriminate some other important production tributaries (i.e., the St. Mary's River (38) and Echo River (11)). Also, inter-annual variation in site-specific stream signatures poses a major problem, since it will prohibit the development of statolith microchemical 'libraries' with only a few years of data (i.e., larvae would need to be sampled every year on a continuous basis to develop a signature). Most certainly, however, the potential of the technique would increase with the ability to sample larvae from all of the production streams in a single year. We also suggest that future studies explore the possibility of integrating discrimination techniques, such as analysis of elemental composition and stable isotopes and/or larval growth rates. In this way, localized effects from water chemistry/pollution or the larval growth environment can be captured with analysis of elemental concentration and growth rate, respectively, and large-scale effects due to geology could be captured using isotope data. Work by Kennedy et al. (2000) has shown that $^{87}Sr/^{86}Sr$ ratios are effective for discriminating among juvenile salmon from 18 different tributaries, and Fryer (unpublished data) has found isotopic signatures to be distinct for a selection of streams scattered around Lake Huron. Since Sr isotopes are directly attributable to the underlying bedrock, differences among statolith signatures that we have found to be strongly influenced by local geology should be reflected in the $^{87}Sr/^{86}Sr$ ratios. Ultimately, the use of Sr isotopes (Kennedy et al. 2000) would effectively deal with the primary problem we have identified with the technique, i.e., inter-annual variability. We are optimistic that combining isotopic data (or perhaps larval growth data, as recorded in statoliths) with already fairly distinctive elemental signatures would provide the GLFC with a means to prioritize sea lamprey control efforts throughout the Great Lakes without unwanted assumptions about larval survival.

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**Table 1.** Isotopes quantified (not including calcium) using LA-ICP-MS. Mean limit of detection (LOD) was calculated based on all samples run. The coefficient of variation (CV) is the average for all runs, and was calculated as mean divided by standard deviation of NIST-610 standards. Isotopes shaded in gray met our criteria for inclusion in analysis.

| **Isotope** | **$^{7}$Li** | **$^{25}$Mg** | **$^{55}$Mn** | **$^{57}$Fe** | **$^{66}$Zn** | **$^{85}$Rb** | **$^{86}$Sr** | **$^{88}$Sr** | **$^{137}$Ba** | **$^{138}$Ba** | **$^{140}$Ce** | **$^{208}$Pb** | **$^{238}$U** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean LOD (ppm)** | 0.750 | 13.6 | 0.466 | 77.9 | 0.671 | 0.291 | 2.09 | 0.234 | 0.516 | 0.143 | 0.575 | 0.528 | 0.023 |
| **CV (%)** | 6.03 | 3.52 | 3.18 | 12.9 | 6.32 | 5.25 | 2.50 | 2.22 | 3.33 | 3.52 | 3.67 | 6.16 | 5.39 |
| **% above LOD** | 3.1 | 100 | 100 | 72.7 | 96.0 | 99.1 | 100 | 100 | 100 | 100 | 32.0 | 82.4 | 4.2 |

**Table 2.** Information on our study streams, including location on Figure 1, year sampled, larvae processed (N), mean total length of larvae ± 1 SD (TL ± SD), geologic zone, watershed designation, and mean stream concentrations (± 1 SD) of Mg, Mn, Zn, Rb, Sr, Ba, and Pb.

| Map | Stream | Year | # samples | TL± SD | Geologic Age | Major Watershed |
|---|---|---|---|---|---|---|
| 1 | Albany Cr. | 2005 | 12 | 92.3 ± 27.5 | Older Paleozoic | Carp-Pine |
| 2 | Beavertail Cr. | 2004 | 15 | 78.9 ± 20.3 | Older Paleozoic | Carp-Pine |
| 3 | Bighead R. | 2005 | 15 | 91.0 ± 23.1 | Older Paleozoic | East Lake Huron |
| 4 | Black Mallard R. | 2005 | 12 | 84.8 ± 7.9 | Older Paleozoic | Lone Lake Ocqueoc |
| 5 | Blue Jay Cr. | 2005 | 15 | 67.6 ± 11.8 | Older Paleozoic | North Lake Huron |
| 6 | Boyne R. | 2005 | 15 | 60.9 ± 18.4 | Grenville | East Georgian Bay |
| 7 | Browns Cr. | 2005 | 9 | 144 ± 8 | Older Paleozoic | North Lake Huron |
| 8 | Caribou Cr. | 2004 | 15 | 99.1 ± 23.7 | Older Paleozoic | Carp-Pine |
| 9 | Cheboygan R. | 2005 | 14 | 107 ± 13 | Older Paleozoic | Lone Lake Ocqueoc |
| 10 | Devils R. | 2004 | 15 | 114 ± 21 | Older Paleozoic | Lone Lake Ocqueoc |
| 11 | Echo R. | 2004/2005 | 30 | 91.8 ± 42.9 | Southern/Superior | North Lake Huron |
| 12 | Elliot Cr. | 2004 | 15 | 109 ± 16 | Older Paleozoic | Lone Lake Ocqueoc |
| 13 | French R. | 2005 | 15 | 146 ± 13 | Grenville | Wanipitai-French |
| 14 | Garden R. | 2005 | 15 | 77.7 ± 18.4 | Southern/Superior | North Lake Huron |
| 15 | Gawas R. | 2005 | 15 | 135 ± 7 | Older Paleozoic | North Lake Huron |
| 16 | Gordon Cr. | 2005 | 13 | 51.8 ± 30.6 | Older Paleozoic | North Lake Huron |
| 17 | Grace Cr. | 2004 | 15 | 72.4 ± 22.9 | Older Paleozoic | Lone Lake Ocqueoc |
| 18 | Hessel Cr. | 2004 | 15 | 99.9 ± 27.6 | Older Paleozoic | Carp-Pine |
| 19 | Koshkawong R. | 2005 | 6 | 62.0 ± 32.3 | Older Paleozoic | North Lake Huron |
| 20 | Lauzon Cr. | 2004 | 15 | 127 ± 10 | Southern/Superior | North Lake Huron |
| 21 | Little Munuscong R. | 2004 | 10 | 122 ± 18 | Older Paleozoic | St. Marys |

| | | | | | | |
|---|---|---|---|---|---|---|
| 22 | Magnetewan R. | 2004 | 15 | 78.5 ± 21.4 | Grenville | East Georgian Bay |
| 23 | Manitou R. | 2005 | 15 | 95.4 ± 18.9 | Older Paleozoic | North Lake Huron |
| 24 | McKay Cr. | 2004 | 15 | 117 ± 9 | Older Paleozoic | Carp-Pine |
| 25 | Mindemoya R. | 2004/2005 | 30 | 82.5 ± 16.1 | Older Paleozoic | North Lake Huron |
| 26 | Mississagi R. | 2004 | 15 | 120 ± 20 | Southern/Superior | North Lake Huron |
| 27 | Musquash R. | 2004/2005 | 25 | 103 ± 30 | Grenville | East Georgian Bay |
| 28 | Naiscoot R. | 2004/2005 | 30 | 75.3 ± 32.7 | Grenville | East Georgian Bay |
| 29 | Nottawasaga R. | 2004/2005 | 30 | 114 ± 22 | Older Paleozoic | East Georgian Bay |
| 30 | Pine R. | 2004/2005 | 19 | 91.9 ± 40.0 | Older Paleozoic | Carp-Pine |
| 31 | Rifle R. | 2005 | 13 | 82.2 ± 23.7 | Younger Paleozoic | Aues Gres-Rifle |
| 32 | Saginaw R. | 2004/2005 | 28 | 95.9 ± 20.6 | Younger Paleozoic | Titabawasee |
| 33 | Sauble R. | 2004 | 15 | 108 ± 15 | Older Paleozoic | East Lake Huron |
| 34 | Schmidt Cr. | 2004 | 15 | 110 ± 14 | Older Paleozoic | Lone Lake Ocqueoc |
| 35 | Serpent R. | 2005 | 15 | 131 ± 6 | Southern/Superior | North Lake Huron |
| 36 | Silver Cr. | 2004 | 15 | 108 ± 36 | Older Paleozoic | East Georgian Bay |
| 37 | Spanish R. | 2004 | 15 | 91.3 ± 9.8 | Southern/Superior | North Lake Huron |
| 38 | St. Marys R. | 2004/2005 | 30 | 103 ± 22 | Older Paleozoic | St. Marys |
| 39 | Steeles Cr. | 2004 | 15 | 105 ± 17 | Older Paleozoic | Carp-Pine |
| 40 | Tawas R. | 2004/2005 | 30 | 97.8 ± 21.6 | Younger Paleozoic | Aues Gres-Rifle |
| 41 | Thessalon R. | 2005 | 15 | 144 ± 11 | Southern/Superior | North Lake Huron |
| 42 | Timber Bay Cr. | 2004/2005 | 30 | 89.2 ± 16.9 | Older Paleozoic | North Lake Huron |
| 43 | Trout Cr. | 2004 | 15 | 79.9 ± 36.6 | Older Paleozoic | Carp-Pine |
| 44 | Trout R. | 2004/2005 | 30 | 80.5 ± 24.4 | Older Paleozoic | Lone Lake Ocqueoc |
| 45 | Watson Cr. | 2005 | 15 | 73.7 ± 8.9 | Older Paleozoic | North Lake Huron |

**Table 2 (continued)**

| Map | Mg | Mn | Zn | Rb | Sr | Ba | Pb |
|---|---|---|---|---|---|---|---|
| 1 | 4090 ± 1090 | 186 ± 93 | 39.7 ± 31.7 | 2.81 ± 0.96 | 382 ± 168 | 26.8 ± 6.1 | 0.134 ± 0.103 |
| 2 | 3080 ± 511 | 25.9 ± 3.2 | 4.69 ± 8.93 | 7.09 ± 1.27 | 181 ± 56 | 19.4 ± 23.7 | 0.052 ± 0.030 |
| 3 | 4240 ± 538 | 16.6 ± 3.6 | 1.62 ± 1.15 | 0.63 ± 0.16 | 310 ± 119 | 7.98 ± 4.96 | 0.055 ±0.035 |
| 4 | 4150 ± 288 | 50.4 ± 17.0 | 157 ± 488 | 5.64 ± 1.16 | 358 ± 49 | 11.6 ± 3.7 | 0.120 ± 0.133 |
| 5 | 3300 ± 410 | 23.7 ± 8.2 | 2.11 ± 1.56 | 1.94 ± 0.34 | 203 ± 44 | 6.68 ± 2.62 | 0.044 ± 0.020 |
| 6 | 3810 ± 358 | 76.5 ± 22.6 | 0.76 ± 0.47 | 4.95 ± 0.42 | 1110 ± 115 | 32.0 ± 6.4 | 0.044 ± 0.022 |
| 7 | 4300 ± 575 | 29.9 ± 6.8 | 7.44 ± 5.77 | 2.84 ± 0.22 | 307 ± 19 | 19.3 ± 4.4 | 0.320 ± 0.165 |
| 8 | 3760 ± 531 | 52.9 ± 13.2 | 12.3 ± 5.9 | 4.30 ± 1.04 | 285 ± 86 | 21.2 ± 9.2 | 0.071 ± 0.032 |
| 9 | 4190 ± 706 | 10.6 ± 2.8 | 11.6 ± 14.5 | 1.74 ± 0.25 | 311 ± 55 | 11.2 ± 3.0 | 0.804 ± 0.770 |
| 10 | 4380 ± 638 | 24.9 ± 5.8 | 20.4 ± 8.3 | 2.88 ± 0.38 | 388 ± 90 | 13.8 ± 4.8 | 0.134 ± 0.129 |
| 11 | 3860 ± 606 | 33.6 ± 10.2 | 7.39 ± 10.2 | 3.31 ± 1.57 | 425 ± 209 | 14.7 ± 6.9 | 0.102 ± 0.087 |
| 12 | 3520 ± 486 | 18.4 ± 2.4 | 38.7 ± 21.1 | 3.19 ± 0.65 | 255 ± 67 | 16.4 ± 6.3 | 0.290 ± 0.137 |
| 13 | 4240± 526 | 50.2 ± 9.7 | 11.7 ± 14.2 | 6.31 ± 1.04 | 255 ± 32 | 17.0 ± 3.1 | 0.195 ± 0.076 |
| 14 | 3490 ± 417 | 23.3 ± 6.9 | 11.3 ± 14.3 | 10.1 ± 1.0 | 486 ± 160 | 53.4 ± 104 | 0.072 ± 0.034 |
| 15 | 4460 ± 569 | 29.1 ± 5.8 | 13.8 ± 6.2 | 2.62 ± 0.29 | 201 ± 12 | 7.61 ± 0.84 | 0.140 ± 0.044 |
| 16 | 3370 ± 635 | 51.1 ± 27.9 | 7.65 ± 12.2 | 4.04 ± 2.62 | 402 ± 107 | 17.1 ± 10.1 | 0.071 ± 0.056 |
| 17 | 3260 ± 509 | 29.9 ± 4.9 | 4.01 ± 7.78 | 5.12 ± 1.08 | 372 ± 82 | 19.8 ± 25.7 | 0.138 ± 0.142 |
| 18 | 5590 ± 469 | 83.3 ± 10.5 | 32.5 ± 18.2 | 6.29 ± 1.06 | 550 ± 132 | 39.7 ± 39.2 | 0.201 ± 0.097 |
| 19 | 3510 ± 562 | 37.2 ± 6.8 | 19.9 ± 28.4 | 4.28 ± 0.72 | 203 ± 21 | 19.9 ± 8.9 | 0.090 ± 0.690 |
| 20 | 4400 ± 623 | 29.7 ± 9.7 | 39.2 ± 19.4 | 6.14 ± 1.13 | 572 ± 138 | 34.6 ± 7.1 | 0.339 ± 0.193 |
| 21 | 4110 ± 642 | 67.7 ± 41.9 | 65.1 ± 52.6 | 2.45 ± 0.85 | 237 ± 50 | 16.0 ± 5.0 | 0.224 ± 0.135 |
| 22 | 4340 ± 604 | 183 ± 73 | 8.09 ± 11.3 | 8.35 ± 2.87 | 829 ± 165 | 59.6 ± 14.6 | 0.239 ± 0.084 |
| 23 | 3780 ± 388 | 17.1 ± 3.6 | 2.88 ± 2.21 | 3.32 ± 0.28 | 449 ± 104 | 11.7 ± 4.3 | 0.062 ± 0.029 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 24 | 3780 ± 624 | 27.8 ± 11.2 | 24.9 ± 10.0 | 1.72 ± 0.21 | 205 ± 44 | 9.76 ± 3.53 | 0.141 ± 0.064 |
| 25 | 3990 ± 504 | 12.8 ± 3.4 | 6.82 ± 9.41 | 2.11 ± 0.64 | 382 ± 158 | 11.3 ± 6.8 | 0.135 ± 0.143 |
| 26 | 5180 ± 809 | 84.7 ± 47.9 | 30.5 ± 12.5 | 7.02 ± 1.49 | 646 ± 691 | 58.8 ± 101 | 0.273 ± 0.228 |
| 27 | 4580 ± 653 | 65.0 ± 29.4 | 7.58 ± 9.87 | 6.99 ± 1.31 | 741 ± 61 | 39.0 ± 9.3 | 0.269 ± 0.281 |
| 28 | 3970 ± 730 | 148 ± 130 | 12.9 ± 37.3 | 8.36 ± 2.07 | 964 ± 221 | 52.4 ± 23.9 | 0.282 ± 0.802 |
| 29 | 4020 ± 523 | 14.8 ± 3.1 | 9.91 ± 16.5 | 1.52 ± 0.39 | 483 ± 149 | 20.2 ± 9.8 | 0.070 ± 0.056 |
| 30 | 3930 ± 942 | 33.5 ± 15.3 | 26.0 ± 45.0 | 4.98 ± 3.46 | 485 ± 289 | 31.5 ± 23.0 | 0.152 ± 0.146 |
| 31 | 3980 ± 854 | 29.5 ± 12.7 | 3.44 ± 1.65 | 1.71 ± 1.08 | 680 ± 281 | 18.5 ± 5.4 | 0.066 ± 0.033 |
| 32 | 4570 ± 578 | 34.4 ± 19.2 | 9.94 ± 9.00 | 1.60 ± 0.76 | 579 ± 192 | 18.4 ± 11.8 | 0.152 ± 0.210 |

**Table 3.** Correlations between elements and LDFA roots (axes) for geologic zones. The percentage of variation that each axis (root) explained is provided in the bottom row.

| | **Root 1** | **Root 2** | **Root 3** |
|---|---|---|---|
| Sr | **-0.640** | **0.710** | 0.160 |
| Rb | **-0.512** | **-0.576** | 0.219 |
| Mn | **-0.571** | -0.175 | **-0.476** |
| Zn | 0.145 | 0.182 | 0.338 |
| TL (mm) | 0.007 | -0.084 | **0.532** |
| Pb | -0.127 | 0.078 | 0.131 |
| Mg | -0.076 | 0.168 | -0.112 |
| Ba | -0.524 | 0.234 | -0.016 |
| Cum.Prop | 0.73 | 0.93 | 1.00 |

**Table 4.** Background information on geologic zones, including sample size per region (N), percent of correct classifications, average posterior probability, and mean values plus one standard deviation for key important elements.

| Geologic Zone | N | % correct | posterior probability | Mn | Rb | Sr | Zn |
|---|---|---|---|---|---|---|---|
| Grenville | 100 | 81.00 | 0.89 | 107 ± 92 | 7.20 ± 2.13 | 804 ± 300 | 8.84 ± 22.2 |
| Younger Paleozoic | 71 | 78.87 | 0.78 | 27.8 ± 14.6 | 2.05 ± 0.93 | 722 ± 615 | 26.9 ± 40.5 |
| Southern/Superior | 120 | 68.33 | 0.67 | 40.6 ± 29.6 | 5.45 ± 2.48 | 478 ± 294 | 17.8 ± 18.0 |
| Older Paleozoic | 500 | 66.40 | 0.71 | 35.2 ± 33.6 | 3.53 ± 2.11 | 322 ± 162 | 22.8 ± 90.9 |

**Table 5.** Correlations between elements and LDFA roots (axes) for watersheds. The percentage of variation that each axis (root) explained is provided in the bottom row.

| | Root 1 | Root 2 | Root 3 | Root 4 | Root 5 | Root 6 | Root 7 | Root 8 |
|---|---|---|---|---|---|---|---|---|
| Sr | **-0.721** | 0.660 | -0.130 | 0.085 | -0.140 | 0.016 | -0.022 | 0.027 |
| Rb | 0.438 | **0.832** | 0.000 | 0.123 | 0.140 | -0.253 | -0.067 | -0.116 |
| Mn | -0.003 | 0.433 | **0.598** | **0.521** | -0.052 | -0.326 | -0.022 | 0.273 |
| Zn | 0.090 | -0.111 | -0.343 | **0.528** | -0.364 | -0.320 | **0.590** | -0.004 |
| TL (mm) | 0.134 | -0.046 | 0.129 | -0.241 | **-0.669** | 0.076 | **0.671** | 0.029 |
| Ba | -0.155 | 0.590 | -0.009 | 0.476 | -0.424 | 0.324 | -0.308 | -0.148 |
| Pb | 0.029 | 0.039 | -0.263 | 0.034 | **-0.721** | **-0.620** | -0.144 | -0.044 |
| Mg | -0.195 | -0.006 | 0.255 | 0.083 | -0.227 | -0.407 | 0.317 | **-0.757** |
| Cum.Prop | 0.41 | 0.73 | 0.86 | 0.94 | 0.97 | 0.99 | 1.00 | 1.00 |

**Table 6.** Background information on watersheds, including sample size per region (N), percent of correct classifications, average posterior probability, and mean values plus one standard deviation for key important elements.

| Watershed | N | % correct | posterior prob | Mn | Rb | Sr | Zn |
|---|---|---|---|---|---|---|---|
| East Lake Huron | 30 | 100.00 | 0.82 | 23.6 ± 8.9 | 0.74 ± 0.25 | 282 ± 92 | 7.23 ± 18.0 |
| Wanipiti-French | 15 | 100.00 | 0.85 | 50.2 ± 9.7 | 6.31 ± 1.05 | 255 ± 32 | 11.7 ± 14.2 |
| Titabawasee | 28 | 82.14 | 0.63 | 34.4 ± 19.2 | 1.60 ± 0.76 | 579 ± 192 | 9.94 ± 9.00 |
| St. Marys | 40 | 70.00 | 0.55 | 31.4 ± 29.6 | 2.87 ± 0.98 | 226 ± 81 | 47.6 ± 146 |
| East Georgian Bay | 130 | 64.62 | 0.79 | 83.5 ± 90.9 | 5.84 ± 3.06 | 754 ± 274 | 16.7 ± 42.0 |
| Aues Gres-Rifle | 43 | 58.14 | 0.75 | 23.5 ± 8.4 | 2.33 ± 0.92 | 814 ± 765 | 38.0 ± 48.5 |
| Carp-Pine | 121 | 39.67 | 0.54 | 60.7 ± 54.8 | 4.83 ± 2.44 | 351 ± 239 | 19.2 ± 25.0 |
| North Lake Huron | 268 | 33.58 | 0.43 | 33.4 ± 23.8 | 4.43 ± 2.36 | 379 ± 235 | 12.0 ± 16.9 |
| Lone Lake Ocqueoc | 116 | 31.90 | 0.45 | 28.6 ± 13.9 | 3.25 ± 1.42 | 305 ± 84.5 | 38.1 ± 159 |

**Table 7.** Correlations between elements and LDFA roots (axes) for all 45 streams. Elements most highly correlated with each axis are bold, and indicate their importance in discrimination (bottom). The percentage of variation that each axis (root) explained is provided in the bottom row.

| | Root 1 | Root 2 | Root 3 | Root 4 | Root 5 | Root 6 | Root 7 | Root 8 |
|---|---|---|---|---|---|---|---|---|
| LnMn | **0.697** | -0.116 | **0.675** | 0.172 | -0.041 | 0.013 | 0.074 | -0.090 |
| LnRb | **0.685** | **0.566** | -0.263 | -0.247 | -0.207 | -0.064 | 0.172 | 0.066 |
| LnSr | 0.419 | **-0.668** | **-0.477** | -0.005 | 0.269 | 0.161 | -0.225 | -0.027 |
| LnZn | 0.024 | 0.093 | -0.048 | **0.789** | 0.093 | **-0.594** | -0.060 | -0.009 |
| TL (mm) | -0.059 | 0.140 | -0.035 | **0.738** | -0.183 | **0.505** | -0.298 | 0.231 |
| LnBa | 0.394 | -0.094 | -0.150 | 0.035 | **0.829** | 0.190 | 0.068 | 0.290 |
| LnMg | 0.058 | -0.205 | -0.040 | 0.384 | -0.332 | -0.060 | **0.545** | **0.628** |
| LnPb | 0.068 | 0.037 | -0.193 | 0.574 | 0.126 | 0.103 | **0.639** | -0.439 |
| Cum.Prop | 0.44 | 0.64 | 0.80 | 0.87 | 0.92 | 0.95 | 0.98 | 1.00 |

**Table 8.** Posterior probabilities and classification accuracies for 45 Lake Huron streams.

| % Correct | Stream | Mean posterior probability |
|---|---|---|
| 6.67 | Echo River | 0.30 |
| 10.53 | Pine River | 0.24 |
| 30.77 | Gordon Creek | 0.72 |
| 30.77 | Rifle River | 0.63 |
| 33.33 | Trout Creek | 0.39 |
| 36.67 | Naiscoot River | 0.56 |
| 40.00 | Little Munuscong River | 0.79 |
| 40.00 | Mississagi River | 0.55 |
| 53.33 | Devils River | 0.61 |
| 53.33 | Timber Bay Creek | 0.56 |
| 56.67 | Trout River | 0.68 |
| 60.00 | Grace Creek | 0.72 |
| 60.00 | Hessel Creek | 0.61 |
| 60.00 | Schmidt Creek | 0.60 |
| 60.71 | Saginaw River | 0.59 |
| 64.00 | Musquash River | 0.69 |
| 66.67 | Spanish River | 0.57 |
| 66.67 | St. Marys River | 0.63 |
| 73.33 | Caribou Creek | 0.57 |
| 73.33 | McKay Creek | 0.58 |
| 73.33 | Tawas River | 0.83 |
| 75.00 | Black Mallard River | 0.70 |
| 80.00 | Beavertail Creek | 0.76 |
| 80.00 | Elliot Creek | 0.72 |
| 80.00 | Magnetewan River | 0.79 |
| 80.00 | Mindemoya River | 0.69 |
| 80.00 | Nottawasaga River | 0.70 |
| 83.33 | Koshkawong River | 0.68 |
| 85.71 | Cheboygan River | 0.93 |
| 86.67 | Sauble River | 0.89 |
| 86.67 | Silver Creek | 0.91 |

| | | |
|---|---|---|
| 91.67 | Albany Creek | 0.98 |
| 93.33 | Bighead River | 0.88 |
| 93.33 | Blue Jay Creek | 0.77 |
| 93.33 | Boyne River | 0.96 |
| 93.33 | Garden River | 0.85 |
| 93.33 | Gawas River | 0.69 |
| 93.33 | Lauzon Creek | 0.84 |
| 93.33 | Serpent River | 0.89 |
| 93.33 | Steeles Creek | 0.87 |
| 93.33 | Watson Creek | 0.83 |
| 100.00 | Browns Creek | 0.76 |
| 100.00 | French River | 0.88 |
| 100.00 | Manitou River | 0.59 |
| 100.00 | Thessalon River | 0.79 |

**Table 9.** Eleven streams with data collected in both 2004 and 2005. Shown are classification accuracies for individual streams when both years of data are present, when 2004 data are removed and when 2005 data are removed.

| | % Correct | | |
|---|---|---|---|
| **Stream** | **2004 and 2005** | **2005 only** | **2004 only** |
| Echo River | 6.67 | 66.7 | 60.0 |
| Pine River | 10.5 | 0.00 | 100 |
| Naiscoot River | 36.7 | 53.3 | 53.3 |
| Timber Bay Creek | 53.3 | 73.3 | 53.3 |
| Trout River | 56.7 | 66.7 | 66.7 |
| Saginaw River | 60.7 | 84.6 | 60.0 |
| Musquash River | 64.0 | 86.7 | 80.0 |
| St. Marys River | 66.7 | 93.3 | 73.3 |
| Tawas River | 73.3 | 60.0 | 93.3 |
| Mindemoya River | 80.0 | 86.7 | 86.7 |
| Nottawasaga River | 80.0 | 93.3 | 80.0 |

## Figure Legends

Figure 1. Map of sample locations for 45 Lake Huron streams, and their respective locations in nine major watersheds (those 9 watersheds are labeled). The streams include: 1) Albany Creek, 2) Beavertail Creek, 3) Bighead River, 4) Black Mallard River, 5) Blue Jay Creek, 6) Boyne R, 7) Browns Creek, 8) Caribou Creek, 9) Cheboygan River, 10) Devils River, 11) Echo River, 12) Elliot Creek, 13) French River, 14) Garden River, 15) Gawas River, 16) Gordon Creek, 17) Grace Creek, 18) Hessel Creek, 19) Koshkawong River, 20) Lauzon Creek, 21) Little Munuscong River, 22) Magnetewan River, 23) Manitou River, 24) McKay Creek, 25) Mindemoya River, 26) Mississagi River, 27) Musquash River, 28) Naiscoot River, 29) Nottawasaga River, 30) Pine River, 31) Rifle River, 32) Saginaw River, 33) Sauble River, 34) Schmidt Creek, 35) Serpent River, 36) Silver Creek, 37) Spanish River, 38) St. Marys River, 39) Steeles Creek, 40) Tawas River, 41) Thessalon River, 42) Timber Bay Creek, 43) Trout Creek, 44) Trout River, and 45) Watson Creek.

Figure 2. Map of geologic zones in Lake Huron watershed. (source: www.epa.gov)

Figure 3. LDFA root 1 versus root 2 for geologic zones. Sr, Rb and Mn decrease along the x-axis. Strontium increases on root 2, whereas Rb decreases along it.

Figure 4. LDFA root 1 versus root 2 for watersheds. which combined explain 73% of the variation among watersheds. Samples decrease in Sr along the x-axis and increase Rb along the y-axis.

Figure 5. Ternary graph of mean Rb, Mn (divided by 10) and Sr (divided by 100) concentrations (ppm) for all 45 streams analyzed.

Figure 6. LDFA root 1 versus root 2 for eight major production streams with between 90% and 100% classification accuracy. Mn and Rb increase along the x-axis. Rubidium increases along root 2, whereas Sr decreases along it.

Figure 7. LDFA root 1 versus root 2 for the Echo River and Mississagi River, along with the streams they are consistently misclassified with (Grace Creek, Albany Creek and Black Mallard River). Mn and Rb increase along the x-axis. Rubidium increases along root 2, whereas Sr decreases along it.

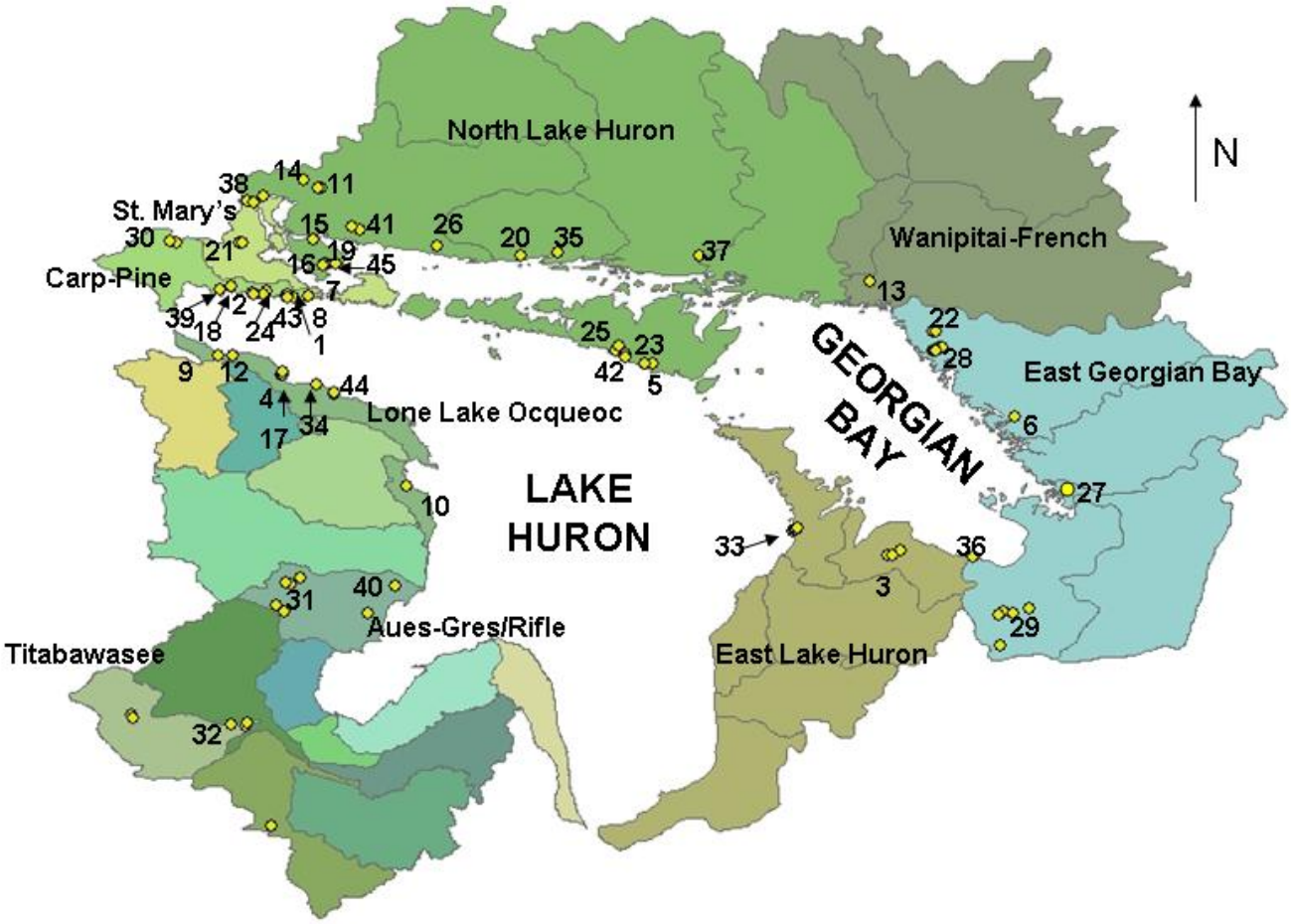
North Lake Huron
N
St. Mary's
Carp-Pine
Wanipitai-French
GEORGIAN BAY
East Georgian Bay
Lone Lake Ocqueoc
LAKE HURON
Aues-Gres/Rifle
Titabawasee
East Lake Huron
38
14
11
15
41
26
20
35
37
30
21
19
16
45
7
13
22
39
18
2
24
43
8
1
25
23
28
42
5
9
12
44
4
34
17
6
10
27
33
36
3
40
31
29
32

Figure 1

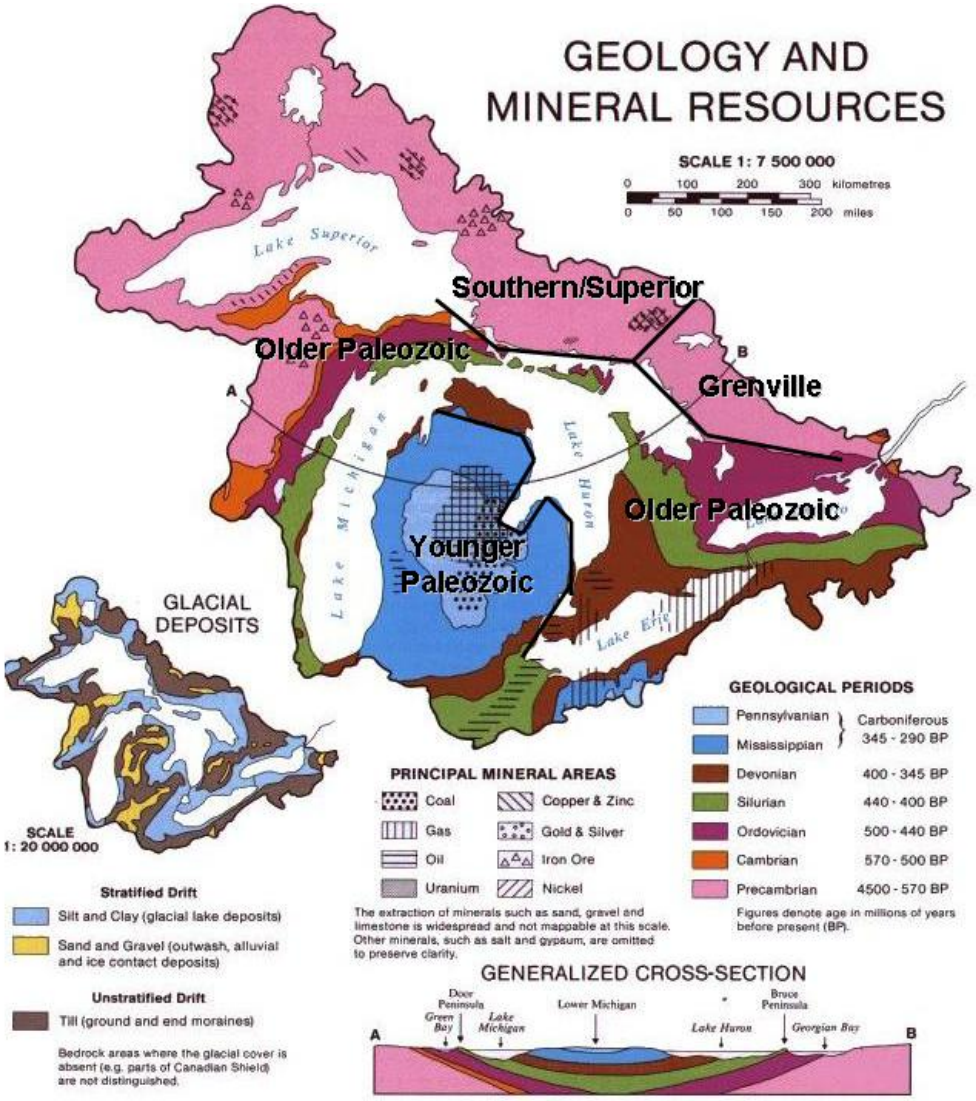
GEOLOGY AND
MINERAL RESOURCES
SCALE 1: 7 500 000
0 100 200 300 kilometres
0 50 100 150 200 miles
Lake Superior
Southern/Superior
Older Paleozoic
Grenville
A
B
Lake Michigan
Lake Huron
Younger Paleozoic
Older Paleozoic
Lake Erie
GLACIAL DEPOSITS
SCALE 1: 20 000 000
Stratified Drift
Silt and Clay (glacial lake deposits)
Sand and Gravel (outwash, alluvial and ice contact deposits)
Unstratified Drift
Till (ground and end moraines)
Bedrock areas where the glacial cover is absent (e.g. parts of Canadian Shield) are not distinguished.
PRINCIPAL MINERAL AREAS
Coal
Gas
Oil
Uranium
Copper & Zinc
Gold & Silver
Iron Ore
Nickel
The extraction of minerals such as sand, gravel and limestone is widespread and not mappable at this scale. Other minerals, such as salt and gypsum, are omitted to preserve clarity.
GEOLOGICAL PERIODS
Pennsylvanian
Mississippian
Carboniferous 345 - 290 BP
Devonian 400 - 345 BP
Silurian 440 - 400 BP
Ordovician 500 - 440 BP
Cambrian 570 - 500 BP
Precambrian 4500 - 570 BP
Figures denote age in millions of years before present (BP).
GENERALIZED CROSS-SECTION
Door Peninsula
Green Bay
Lake Michigan
Lower Michigan
Lake Huron
Bruce Peninsula
Georgian Bay
A
B

Figure 2

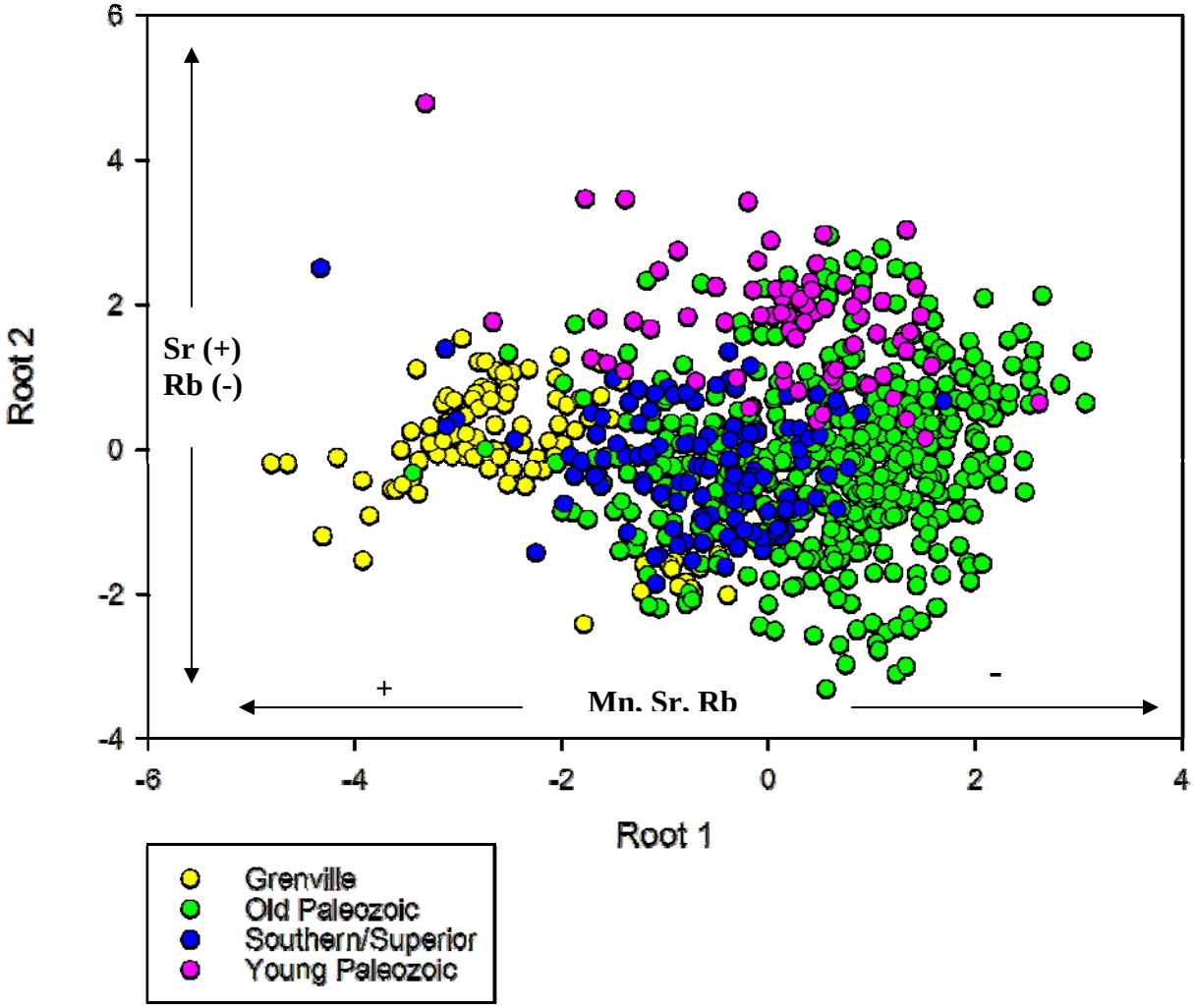
6
4
2
0
-2
-4
-6
-4
-2
0
2
4
Root 2
Root 1
Sr (+)
Rb (-)
+
-
Mn. Sr. Rb
Grenville
Old Paleozoic
Southern/Superior
Young Paleozoic

Figure 3

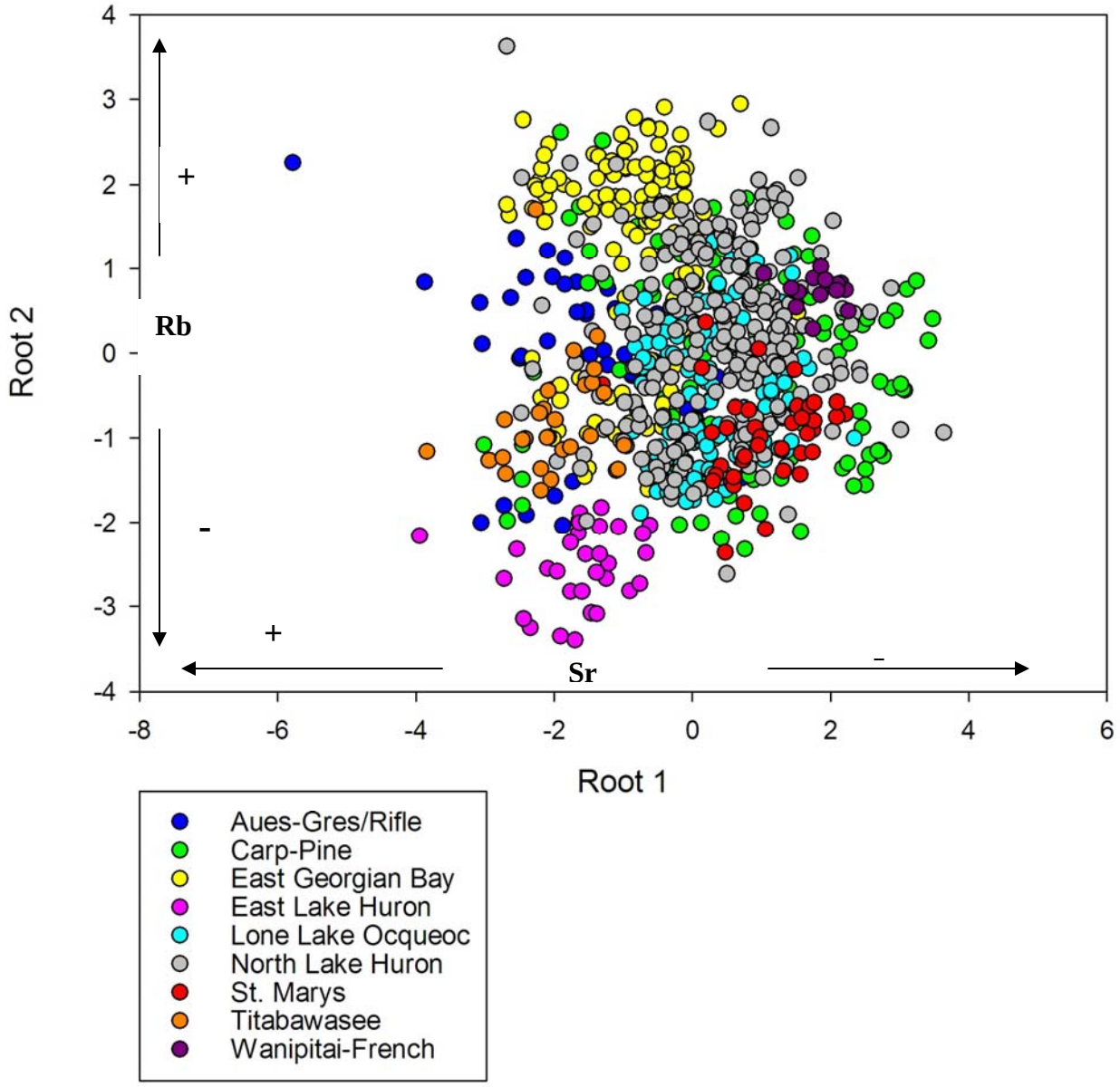
Root 2
Root 1
Rb
Sr
+
-
+
-
Aues-Gres/Rifle
Carp-Pine
East Georgian Bay
East Lake Huron
Lone Lake Ocqueoc
North Lake Huron
St. Marys
Titabawasee
Wanipitai-French

Figure 4

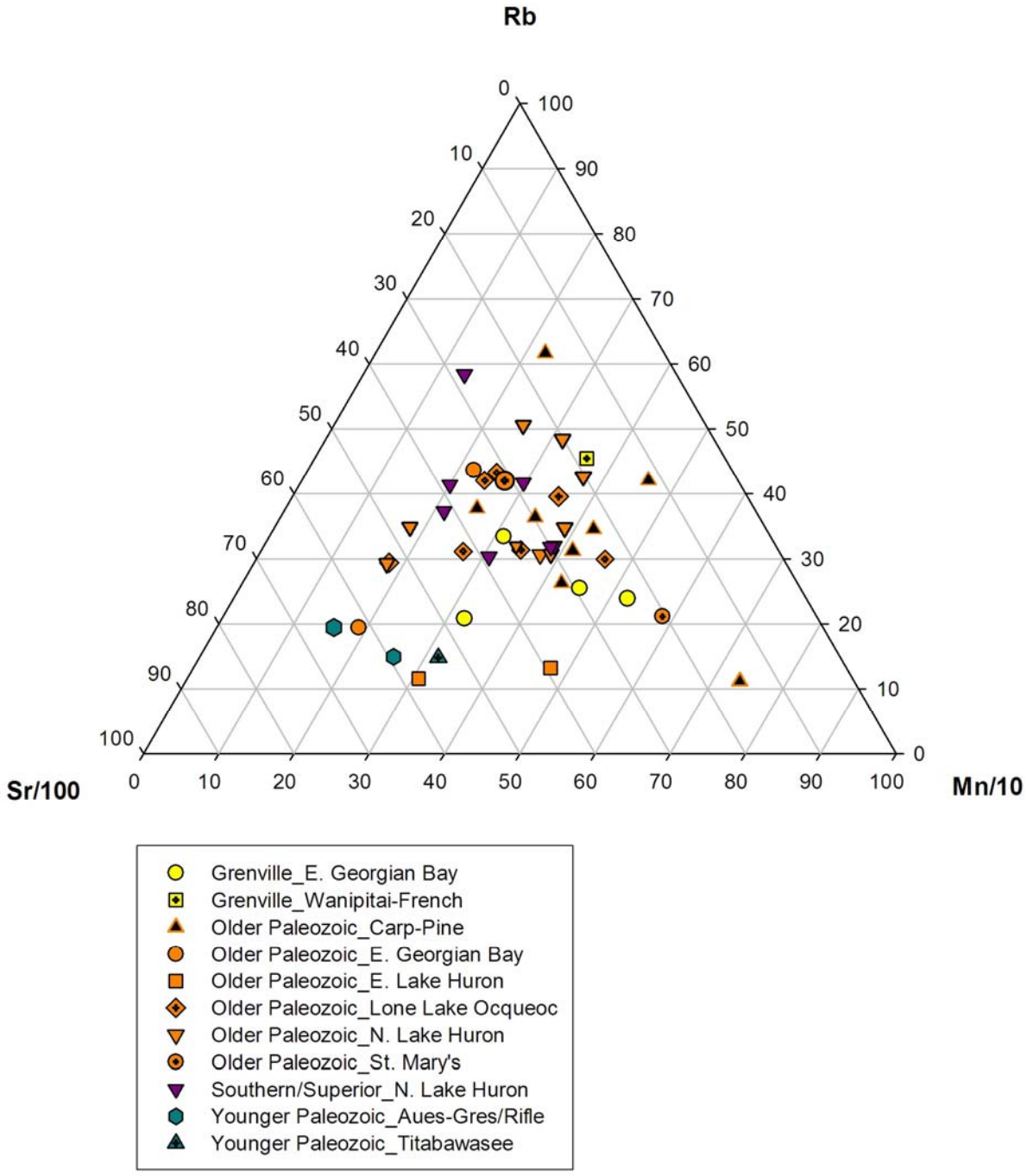
Rb
Sr/100
Mn/10
0
10
20
30
40
50
60
70
80
90
100
Grenville_E. Georgian Bay
Grenville_Wanipitai-French
Older Paleozoic_Carp-Pine
Older Paleozoic_E. Georgian Bay
Older Paleozoic_E. Lake Huron
Older Paleozoic_Lone Lake Ocqueoc
Older Paleozoic_N. Lake Huron
Older Paleozoic_St. Mary's
Southern/Superior_N. Lake Huron
Younger Paleozoic_Aues-Gres/Rifle
Younger Paleozoic_Titabawasee

Figure 5

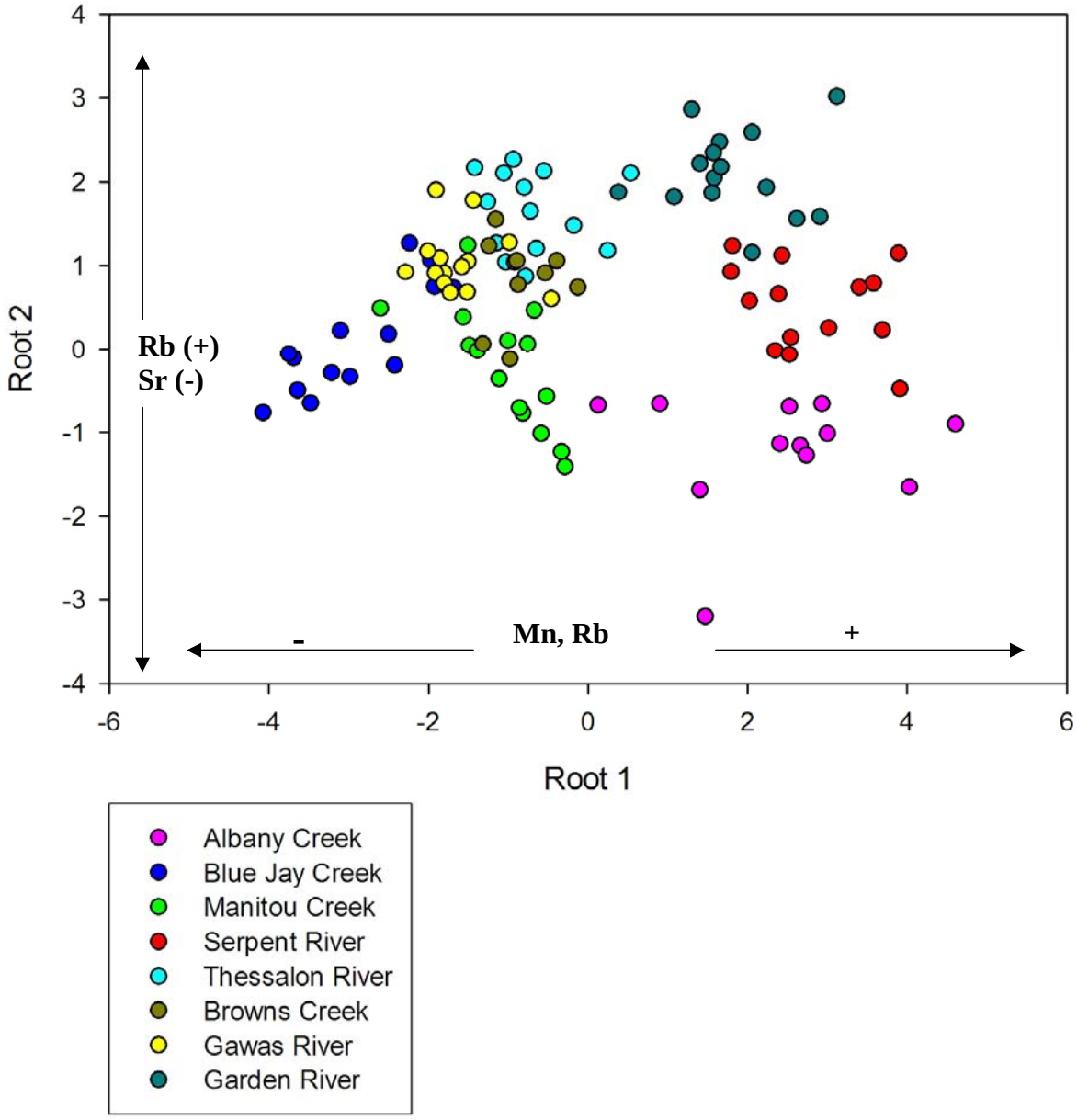
Root 2
Root 1
Rb (+)
Sr (-)
-
Mn, Rb
+
Albany Creek
Blue Jay Creek
Manitou Creek
Serpent River
Thessalon River
Browns Creek
Gawas River
Garden River

Figure 6

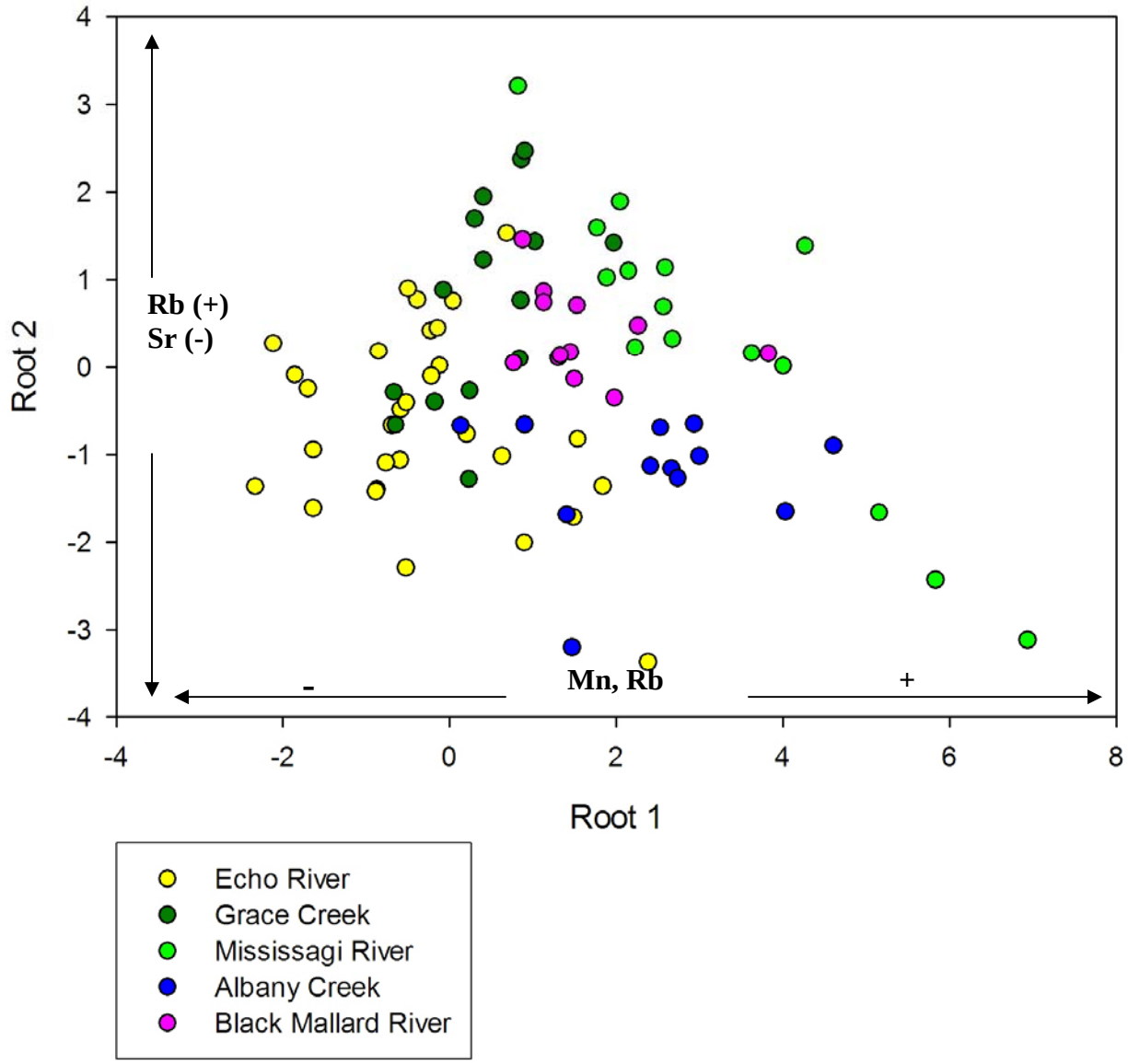
Root 2
Root 1
Rb (+)
Sr (-)
-
+
Mn, Rb
Echo River
Grace Creek
Mississagi River
Albany Creek
Black Mallard River

Figure 7

## APPENDIX 3

Howe, E.A., C.P. Hand, J.E. Marsden, S.A. Ludsin, and B.J. Fryer. In prep. Tributary contributions to the parasitic and spawning adult population of sea lamprey (*Petromyzon marinus*) in Lake Champlain using elemental signatures. Target Journal: Canadian Journal of Fisheries and Aquatic Sciences.

## Abstract

In Lake Champlain, sea lamprey (*Petromyzon marinus*) has been at nuisance levels since the 1970s, hindering restoration of the salmonid sport fishery. Better knowledge of the contribution of sea lamprey from each tributary to parasitic and spawning phases would ensure the appropriate allocation of management resources to control their populations. Toward this end, we have been adapting otolith microchemistry, a tool that has been used to assess fishery stock structure, to evaluate stock structure of sea lamprey populations in the Lake Champlain basin. We quantified the elemental composition in statoliths of sea lamprey collected from 19 tributaries and one delta in Lake Champlain using laser-ablation inductively coupled plasma mass spectrometry, and then used discriminant analysis to classify the lamprey by tributary and determine correct classification probabilities. Five groupings consisting of different clusters of tributaries were evaluated to assess the utility of clustering tributaries together based on adjacency and geologic similarity, and to reduce variation and potential biases in the data. The best grouping consisted of three clusters of tributaries (n=4 per cluster) and seven isolated tributaries. Correct classifications of streams and clusters within this grouping ranged from 64.0 to 100.0% ($\bar{x}$ = 80.7 ± 11.45 SD, n = 290). Correct classification rates among groupings ranged from 69.3 – 77.2%, indicating that these groupings were not necessarily an advantage, from a management perspective, given the loss in resolution of tributary contributions when tributaries were clustered. Spawning-phase lamprey tagged as transformers in their natal streams (n = 34) were used to validate assignment of lamprey to streams using microelemental data. One of 34 samples was correctly classified to its natal tributary (2.4%). These results indicate that there may be material deposited on the statolith during the parasitic-phase that masks the larval signature. Further work is needed to identify a technique that will access the larval signature of statoliths from parasitic- and spawning-phase sea lamprey without risk of contamination from material deposited post-metamorphosis.

## Introduction

The sea lamprey (*Petromyzon marinus*) is a primitive fish that has been implicated in the demise of the salmonid fishery of the Laurentian Great Lakes and Lake Champlain (Smith and Tibbles 1980; Christie and Goddard 2003b). Since the early 1950s, a significant effort has been put forth to control or limit the growth of these nuisance populations (Smith and Tibbles 1980; Christie and Goddard 2003b). With finite resources available, however, sea lamprey control programs must ensure that the efficiency of each control action is optimized. The control program for all Great Lakes and for Lake Champlain relies heavily on the application of chemical pesticides (lampricides) to tributaries, where sea lamprey spend four to six years as sedentary bottom feeders before metamorphosing into a parasitic-phase (Applegate 1950; Swink 1991; Bence *et al.* 2003). In order for this control method to be most effective, knowledge of which tributaries are contributing the highest proportions of sea lamprey to the parasitic-phase population must be obtained so that treatment can be prioritized for these areas (Bergstedt and Seelye 1995; Howe *et al.* In review).

Current prioritization for lampricide treatments relies heavily on estimates of larval density, length frequency, and likelihood of metamorphosis to predict the number of lamprey that each tributary will produce in a given year (Christie et al. 2003; Henson et al. 2003; Slade et al. 2003). In this method, managers must assume that survivorship from the tributary to the lake is equal among all tributaries. This assumption, however, has not been validated. It is possible that survival to the parasitic phase may be significantly higher in some tributaries than others – for example, sea lamprey from tributaries that drain into locations in the main lake with an abundance of preferred prey may have higher survivorship probability than those in tributaries emptying into locations with low prey abundance and sub-optimal habitat. If these differences do exist, then the necessity and efficiency of some lampricide treatments is called into question. An increased knowledge of the tributary contributions of sea lamprey to the overall population could result in the optimization of the control program, allowing management resources to be redirected toward tributaries that produce the greatest numbers of parasitic- and spawning-phase sea lamprey, or to other facets of the control program.

Tracking of sea lamprey from the larval to the parasitic phase is essential if we are to ascertain differing rates of survivorship. Natural marking, which relies on characteristics of the species itself, has been used to track animals in many different settings – examples include use of genetic markers in species conservation (Milligan et al. 1994), stable isotopes to track current and historical migratory routes of birds (Bearhop et al. 2005; Rocque and Winker 2005), and trace element (or microelement) analysis in fisheries (e.g., Campana et al. 2000; Campana and Thorrold 2001). Fisheries managers have been able to garner information about the origin of fish stocks by analyzing the microelemental characteristics of otoliths in marine (Campana et al. 1994; Humphreys et al. 2005), estuarine (Gillanders and Kingsford 1996; Gillanders 2005), and freshwater fishes (Brazner et al. 2004a; Brazner et al. 2004b). More recently, work has been done to explore the suitability of microelemental analysis for stock discrimination in species containing statoliths (Zacherl *et al.* 2003; Brothers and Thresher 2004). The metabolically inert otolith of most fishes, and the statolith of sea lamprey, incorporates elements from the surrounding environment. Otoliths are composed primarily of aragonite, a calcium carbonate ($CaCO_3$) matrix, whereas statoliths are apatite ($CaPO_4$). In each matrix, additional elements present in the local environment, such as barium (Ba), strontium (Sr), and rubidium (Rb), are deposited onto the aragonite or apatite matrix. Layers of material are added to the otolith or statolith on a daily basis, essentially creating a diary of where the individual animal has been (Campana 2005; Meeuwig and Bayer 2005b). This natural tag has many benefits, but perhaps most important is that *all* animals are marked, so the expense and challenge of mark-recapture is eliminated. In addition, natural tags do not affect the behavior or survival of the animals so there are fewer external impacts on the animal's activities.

Several techniques have been developed to examine elemental compositions for otoliths; while no one technique has been proven significantly superior to another, some are more effective for addressing

specific hypotheses and examining specific elements (see Campana *et al.* 1997; see Campana 2005). Examining trace elements, such as those present in sea lamprey statoliths, requires a technique with very high precision and accuracy (Campana et al. 1997). Laser-ablation inductively-coupled plasma mass spectrometry (LA-ICPMS) is a technique that has been increasingly used in stock discrimination studies because of very low detection limits (as low as parts per quadrillion), creating the ability to detect a wide range of elements precisely and accurately (Campana et al. 1997; Ludsin et al. 2006). Proton-induced X-ray emission (PIXE) is an alternative technique to ICPMS that was used in a recent study to analyze sea lamprey ammocoete statoliths collected from the Great Lakes (Brothers and Thresher 2004). With the PIXE analysis, Brothers and Thresher (2004) were able to successfully discriminate and classify larval sea lamprey from four out of five different locations, two of which were within a single tributary (Brothers and Thresher 2004). Using the discriminant functions from the PIXE analysis, they correctly classified 8 out of 18 (44%) adult sea lamprey of known tributary origin. Campana et al. (1997) found that PIXE and LA-ICPMS have similar limits of detection and precision levels, although for different elements. For example, in the Brothers and Thresher (2004) study, their PIXE analysis did not consistently detect barium (Ba), and the LA-ICPMS analysis in the study described in this manuscript did not consistently detect iron (Fe).

The life cycle of the sea lamprey is such that an individual spends several years (usually 4-6) in the sediments of a tributary prior to migrating to the lake. We hypothesize that this long sedentary residence will create a stable record of the elemental composition of the given tributary within the statolith of the lamprey, often resulting in a unique signature that reflects the geology of a local drainage basin. In some cases, tributaries themselves may not be uniquely identifiable, but clusters of tributaries in shared watersheds may be. This study evaluates the potential of elemental signatures to identify the natal tributaries of spawning-phase populations. Samples from adults of known tributary origin are then used to determine the ability of the classification functions generated from the larval tributary signatures to correctly classify adult sea lamprey to their natal tributary.

## Methods

*Field collections*

Three to 23 larval sea lamprey were collected from each of 19 tributaries to Lake Champlain and one tributary delta during lampricide treatments or via electrofishing from summer 2002 through fall 2005 (Table 1, Figure 1). All specimens were identified to species level (*P. marinus*), enumerated, and either immediately frozen or preserved in 95% ethanol (USP grade).

*Statolith preparation*

We followed the techniques described by Ludsin et al. (2006) for dissection and removal of teleost otoliths. Sagittal statoliths were dissected from the left and right otic sacs of each sea lamprey in a Class-100 clean room, sonicated for five minutes in a Petri dish floating on Milli-Q ultrapure water in a ULTRAsonik 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. One statolith from each statolith pair was randomly selected, mounted with Scotch double-sided tape (3M, St. Paul, Minnesota) on a petrographic microscope slide and dried under a laminar-flow hood for 24-48 h prior to being analyzed. The second statolith was dried and stored in a clean vial as an alternate sample. All laboratory apparatus in contact with the statoliths was acid-washed prior to use (Ludsin et al. 2006).

The statoliths were analyzed using a laser ablation infrared plasma-mass spectrometer (LA-ICPMS), following techniques outlined in Ludsin et al. (2006). The laser beam is concentrated on the statolith, at which point the desired statolith material is ablated (ionized). The concentrations of the gaseous elements are measured by mass spectrometer. Laser power was set to 1.10 Kvolts; all other settings were identical to those described in Ludsin et al. (2006). The ablation from the double-sided tape yields a large spike

similar to tin (Sn), indicating the beginning and end of each burn, with the material from the statolith in between. Reference standards (National Institute of Standards and Technology [NIST] 610 or 620) were run in pairs prior to and after every ten statoliths to estimate the precision of the machine and account for drift over time. The criteria for inclusion of an element in the statistical analysis were limits of detection (LOD) > 80% and coefficient of variation for level of precision (CV) < 10.5% (Table 2). Calculation of these criteria followed the methodology described in Ludsin et al. (2006).

The sea lamprey statolith is of a conical shape, with new material deposited at the base of the cone (Volk 1986). Thus, in a statolith extracted from a parasitic- or spawning-phase sea lamprey, the larval material is near the apex of the cone. As with many fishes, annual bands (annuli) that form on the statolith can be used to determine the age of the individual (Volk 1986). During analysis of spawning-phase samples, laser transects were set to sample the first three to four years of larval material, spanning the upper third of the statolith (closest to the apex).

To test for effects of sample preservation, 52 larval sea lamprey were collected from Lewis Creek, VT on 11 August 2005. Twenty-five larval sea lamprey were randomly selected and immediately preserved in 95% ethanol, and the remaining 27 were immediately frozen. Statoliths were extracted from 15 frozen sea lamprey after 40 days; the remaining statoliths (frozen and ethanol-preserved) were extracted after 89 days. Elemental concentrations were log-normal transformed, and tested for significant differences in duration of storage. Statoliths were extracted using the technique described below (see *Statolith preparation*). Once extracted and analyzed, the mean elemental concentrations in the statoliths of the lamprey from both preservation methods were tested for differences (t-test). Optimally, a paired analysis would have been conducted for which the left and right statoliths from each fish would be preserved separately. However, this type of analysis is extremely challenging in a practical sense, due to the minimal physical separation of the otic sacs in the head cavity. To check for inter-annual temporal stability in statolith microchemistry within streams, statoliths were collected from the Great Chazy River, NY in 2003 and 2004 and the means of each element for the respective collection year were compared using a t-test.

*Statistical analyses*

Linear discriminant function analysis (LDFA) was used to group the lamprey into tributaries and determine the misclassification probabilities (Hill 1959). All statistical analyses were performed using Statistica v. 7.1 (Statsoft, Inc., Tulsa, OK). Any data point that was more than 3 standard deviations from the mean for its respective tributary was considered to be an outlier and the data point was replaced with a random value generated using a normal distribution from the mean and standard deviation of the element for that tributary (Ludsin et al. 2006). Missing data points (i.e., data points below detection) within any tributary were replaced with a data point generated in the same fashion as those for outliers. All elemental concentrations were log-normal transformed to normalize the data prior to LDFA analysis. Forward stepwise regression was used to determine which elements were useful in discrimination among the tributaries. Classification rates were generated using the cross-validation procedure in Statistica, which follows a standard jackknife (leave-out) method.

Five discriminant analyses were conducted to determine which groupings of the statoliths yielded the highest correct classification rates (Table 3, Figure 2). Groupings were created based on geographic proximity and geologic similarity. In Grouping A, all larval collections were kept separate (i.e., tributaries were separate as well as the collections from the Great Chazy River in 2003 and 2004). Grouping B combined the samples from the 2003 and 2004 collections in the Great Chazy River. Grouping C combined three main-stem tributaries with their secondary tributaries (or delta) – Saranac River with its delta, Missisquoi R. with Youngman Brook, and Pike River with Morpion Stream (Table 3). Grouping D clustered tributaries of similar geologic drainages. Much of the western side (New York side) of Lake Champlain is drained by the Adirondack mountain range, where the geology is quite different than that of the Green Mountains. The Missisquoi River watershed of northwestern Vermont

and southern Quebec is also quite different from the Green Mountains. Given this, the tributaries on the New York side of Lake Champlain were clustered together based on geographic proximity – Saranac, Salmon, and Ausable Rivers were assigned to Cluster 1, and the southern tributaries of the Adirondacks – Boquet, Mullen, Mill, Putnam, and Mount Hope – were assigned to Cluster 2. All tributaries that drain into Missisquoi Bay were assigned to Cluster 3. For Grouping E, tributaries in similar geographic regions of Vermont were also clustered together – LaPlatte and Winooski Rivers, Malletts Creek with Trout/Stonebridge Brooks. For this grouping, the Missisquoi River drainage was separated from the Pike River drainage to improve resolution of predictions for sea lamprey coming from this region (Table 3).

A post-hoc power analysis was used to determine whether, given the variability of the data within each element for each cluster, the sample sizes used in this study were large enough to detect a significant difference among the clusters using the given elements. A several means 1-way ANOVA power calculation was used to determine the power for each microelement used in this study using Statistica v. 7.1 (Statsoft, Inc., Tulsa, OK).

*Adult classification and method validation*

Samples extracted from parasitic- and spawning-phase sea lamprey of known origin were used to validate the classification functions derived from the LDFA. Sea lamprey transformers were marked with coded wire tags and released into their natal tributaries, and then recaptured as parasites or spawning adults (Chapter3). Statoliths from two parasitic-phase sea lamprey (one in the 2002 and one in the 2003 parasitic-phase cohort) and 32 spawning-phase sea lamprey of known tributary origin (15 from the 2002 parasitic-phase cohort, 17 from the 2003 parasitic-phase cohort) were processed and analyzed using the same methods as those for larval statoliths. These 34 individuals were then used as unknown samples in the LDFA analysis to predict their natal tributary. These predictions were then compared to their true natal tributary, and correct classification rates were generated based on these predictions. An additional 36 samples from spawning-phase sea lamprey (2004 parasitic-phase cohort) of unkown origin were also analyzed.

## Results

*Tributary discrimination*

Of the ten elements detected by the LA-ICPMS, six were consistently detected in all tributaries, and fell within the criteria for inclusion into the data analysis (Table 2). Lead (Pb) was detectable in several tributaries, and consequently was used as a covariate (presence or absence in each sample).

Tests for differences among storage intervals for frozen samples indicated that storage time does not have a significant effect on concentrations of the elements used in this analysis (t-tests, df =15, 10, $p > 0.05$ for all elements). Tests for differences between elemental concentrations in samples preserved in ethanol and frozen samples indicated that there was a significant difference in concentrations of rubidium, but not any other elements (Rb; df = 25 ethanol, 27 frozen, $t = 4.12$, $p < 0.001$). To address this difference in rubidium concentrations between preservation methods, predicted values from an ANOVA of the logN(Rb) against the preservation method (freezing or ethanol) were subtracted from the original data set, yielding the residual value, and thereby removing the effect of preservation method. The residual values for logN(Rb) were then used in the discriminant analysis.

The test for differences in elements between the 2003 and 2004 transformer cohorts in the Great Chazy river indicated significant differences for Mg ($df = 21$, $p < 0.005$; Table 4). Significant differences were also found for Mg, Mn, Zn, and Ba between Pike River and secondary tributary Morpion Stream while Rb and Sr were marginal (Table 5). Three elements were found to be significantly different in the tests between Saranac River and its delta, and Missisquoi River and its secondary tributary, Youngman Brook (Table 4).

Using the log-normalized concentrations from the six elements and the Pb covariate, Grouping D had the highest correct classification rate at 77.2% (224 out of 290 samples; Table 3). Results from the forward stepwise regression indicated that all elements were important for tributary discrimination, although Rb was by far the most important (Table 5a). The first four discriminant functions (roots) explained 95.8% of the variation in the discriminant model (Table 5b). The standardized coefficients of the canonical variables (roots) indicate that Rb carried the most weight for root 1, which also explained 63.5% of the variance. The second root was weighted mostly by Sr, and explained another 18.6% of the variance. The weights of the elements in the third and fourth roots were mostly carried by Mg and Mn for root 3 and Zn and Pb for root 4 (Table 5b). Root 1 was strongest for discriminating Lewis Creek and Cluster 2 (Saranac, Ausable, Salmon rivers) from the other tributaries (Table 5c; Figure 2), and the second root discriminated for the Great Chazy, Cluster 1, Winooski R., and Cluster 3 (Figure 2). Root 3 served to further discriminate Cluster 2 and the Winooski R., and root 4 best discriminated the LaPlatte River and Malletts Creek (Table 5c).

The classification matrix can be used to predict to which tributaries sea lamprey are most likely to be correctly assigned, as well as probabilities for incorrect predictions. In grouping D, the lowest classification rate (64.0%) was for the C3 cluster, from which samples were incorrectly classified into C1 and all Vermont drainages. However, only three samples from other tributaries were incorrectly assigned to the C3 cluster (Poultney R. and Malletts Cr.; Table 6). Similarly, the classification function for the Poultney River incorrectly assigned samples to other Vermont tributaries (Green Mountain drainage), but not to any New York (Adirondack drainage) tributaries. Classification rates within New York and Vermont (including Quebec) drainages were very good. Within Vermont tributaries, 157 out of 166 samples (94.6%) were correctly classified into Vermont tributaries, and 111 of 124 samples (89.5%) collected from New York tributaries were correctly classified into New York tributaries (Table 6).

The power analysis was conducted using Grouping D on the raw values of the six elements. Of these six elements, Ba had the lowest power (0.60), follwed by Zn (0.85). Power for all other elements was > 0.99.

*Adult classification and method validation*

Using Grouping D, only one (2.94%) of the known-origin samples was correctly classified to its natal tributary, Cluster 1 (Saranac River, with 99.8% probability). Overall, 30 of the 34 samples were classified to Cluster 1. Two samples (Malletts Creek and Pike River) were assigned to the Winooski River, and two samples (Morpion Stream and Lewis Creek) were assigned to Cluster 2. One sample from the Pike River had a 1.2% probability of being correctly classified into Cluster 3, all other samples had 0.00% probability of being correctly classified into their natal tributary. Classification results were similar for known-origin samples for all other groupings.

In addition to the 34 adult sea lamprey of known origin, 36 adults of unknown origin were also analyzed. As with the known samples, the majority of the unknown origin samples were assigned to Cluster 1 (22, or 61.1%). The remaining 14 samples were equally divided between Cluster 2 and the Winooski River.

## Discussion

This study demonstrates that tributaries within the Lake Champlain drainage basin can be discriminated using trace element analysis of statoliths from larval sea lamprey. However, we were unsuccessful in validating the method for correct identification of natal streams of parasitic- or spawning-phase sea lamprey.

The task of identifying natal tributaries of parasitic-phase sea lamprey in Lake Champlain is a standard mixed-stock analysis problem. Mixed-stock analysis is used to predict the proportion of source stocks that are part of an unknown mixture population. A classic example of this application is with Pacific salmon, where considerable resources have been allocated to the maintenance of tributary-specific stock populations. These individual populations migrate to the Pacific Ocean, where they mix with populations from other stocks, and these mixed populations are then harvested. Information about the proportion of

each stock within the harvest population allows fishery managers to allocate conservation efforts to the proper tributaries to achieve their fishery goals (Wood *et al.* 1987; Smouse *et al.* 1990).

The performance of mixed stock analyses primarily depends on five factors: the number of characters used to discriminate stocks, inclusion of all component stocks in the baseline dataset, the degree of separation among stocks, accuracy of description of stocks, and the number of fish per stock in the mixture (Millar 1987; Wood *et al.* 1987; Campana *et al.* 2000). The first three of these, in our study, appear to be robust; further work is needed on analytical methods to resolve the components of the mixture.

*Discriminating characters*

The number of discriminating characteristics and separation among stocks determine the amount of information available to accurately estimate the mixture composition. A review of the published literature indicates a broad range in the number of elements required to achieve discrimination; the number of elements used for discrimination in this study (6) falls in the middle of the spectrum (cf. Zacherl *et al.* 2003; three elements; Brothers and Thresher 2004; nine elements; Humphreys *et al.* 2005, five elements).

*Separation among baseline stocks*

The number of fish examined from each stock determines the level of sample error, and accordingly, the level of sample noise within the discriminatory model (Wood *et al.* 1987). As with most studies, the necessary sample size is dependent upon the measured variation within each parameter of the statistical model, and the objectives of the study. Sample sizes in this study ranged from three to 23 base samples per stock. While the range of sample sizes was enough to achieve a level of accuracy that is suitable for management purposes (77%), those tributaries for which the sample size was less than 10 exhibited higher variation within each element than those with larger sample sizes, with the exception of the Ausable River. Even with a sample size of 15, the variation in Rb for the Ausable River was quite large, causing a higher misclassification rate for this tributary than for others with comparable sample sizes (Appendix I). Increased sample size for the Poultney River and the cluster of tributaries that drain into Missisquoi Bay might reduce the variance in these systems and ultimately increase the overall classification accuracy of the baseline samples. The power analysis indicated that the sample sizes used in this study were more than sufficient to account for the variability within the data for each element (with the exception of barium); however, given the constraints of the power analysis, the sample size was assumed to be uniform for each cluster (n=15) although this was not the case for all clusters. Increasing the sample size within each cluster should improve the resolution of the analysis and therefore reduce the misclassification rate for several of the clusters used in this study.

*Inclusion of all stocks*

A concern for any project in which individuals are to be classified into a predefined group is to ensure that all groups potentially contributing to the adult population (i.e., tributaries contributing sea lamprey to the parasitic- and spawning phase populations) are adequately represented within the baseline samples from which the classification functions are generated (Wood *et al.* 1987; Waldman and Fabrizio 1994; Gillanders 2005). Sea lamprey populations have been detected in 24 tributaries in the Lake Champlain basin; we were unable to collect larvae from the Boquet River and Beaver Brook due to recent lampricide applications, which removed 95% or more of the larval populations, and samples from the Lamoille and Little Ausable rivers could not be analyzed due to laboratory difficulties. These tributaries missing from the baseline data may lead to incorrect assignments of parasitic and adult sea lamprey to their natal streams (Waldman *et al.* 1997).

*Accuracy of baseline stock description*

One technique to reduce the noise within the discriminatory model (i.e., to increase the discrimination by reducing the variance within the baseline stocks) is to combine similar stocks, such as those in close geographic proximity, which will help to reduce variance around the estimate and bias in baseline data when similar stocks contribute differently to the mixture population (Millar 1987). In this study, combining sea lamprey from similar tributaries ("stocks") in terms of geographic proximity and geologic similarities improved the classification accuracy (5.8% improvement), but at the cost of losing resolution to the individual tributary level. The 77.2% correct classification rate for Grouping D is on par with published classification rates from other studies of similar design, and indicates that this technique may be useful for identifying tributary origin of parasitic- and spawning-phase sea lamprey (Brazner *et al.* 2004a; Brothers and Thresher 2004). Tributary classification accuracy using the six elements and Pb covariate described in this study ranged from 64.0 – 100.0%, ( $\bar{x}$ = 80.7 ± 11.45 SD; Table 6).

Grouping these main stem tributaries with their secondary tributaries improves the classification ability by 4.1% (Table 3). Grouping B, with all tributaries separate, had a correct classification rate of 71.4%, while the best classification rate (Grouping D) was 77.2%. From a management perspective, the loss in resolution (or discrimination) among tributaries for Grouping D may not be worth the 5.8% gain in correct classification probability. The ability to discern sea lamprey from the Saranac delta and the river proper or from the Ausable and Salmon rivers, and to distinguish Morpion Stream from the Pike River, could be very important in terms of allocation of management resources for these tributary systems, as each of these separate populations can be managed in very different ways. For example, if the majority of parasitic- or spawning-phase sea lamprey were found to have come from Cluster 1 of Grouping D, then management efforts could be focused on that region of tributaries. However, if Grouping B were used in this scenario, then management efforts could be focused directly on the source, say, Saranac delta, rather than on the suite of tributaries in Cluster 1. Additionally, main-stem and secondary tributary populations may overlap to some extent, given the typical downstream progression of ammocoetes over time (Hardisty and Potter 1971; Quintella *et al.* 2005). There is a strong possibility that many of the larvae collected in the Pike River were actually spawned in Morpion Stream, as nearly the entire larval habitat in the Pike River is downstream of the mouth of Morpion Stream. The t-test results comparing the elemental concentrations found in Morpion Stream samples to those from the Pike River indicate that the two populations may in fact be different, and thus should be managed separately.

*Mixture classification*

Despite good discrimination among the tributaries with the baseline data, the correct classification rate of 2.4% for spawning-phase sea lamprey of known origin is not useful. The elemental concentrations in statoliths of spawning-phase sea lamprey appear to be affected by parasitic-phase material that accumulates in this period of rapid growth, masking the true tributary signature. For example, concentrations of Rb in Lewis Creek were similar to several tributaries in Vermont, but significantly lower than all of the tributaries that drain the Adirondacks in New York (Appendix I). However, the concentrations of Rb in statoliths from all spawning-phase adults (Lewis Creek-origin and others) were significantly higher than even those concentrations measured in samples from the New York tributaries. This likely explains why most of the spawning-phase samples were assigned to Cluster 1 or Cluster 2, and suggests that there is some event that occurs during the parasitic growth period that incurs high Rb deposition on the statolith.

Correct assignment of adults requires better methods to separate the larval signature from parasitic- and spawning-phase statoliths. Grinding and polishing statoliths from parasitic- and spawning-phase sea lamprey may remove additional material deposited during the parasitic growth period, and improve the assignment of these samples to their natal tributary. This technique was used by Brothers and Thresher (2004), and they successfully classified 44% of spawning-phase sea lamprey of known origin. Studies comparing the effect of grinding, polishing, and other sample cleaning techniques on fish otoliths have

found that these methods affect some elements, but not others (Campana *et al.* 2000). However, these evaluations were on Atlantic cod (*Gadus morhua*), which do not undergo the near-exponential growth periods in their life cycles seen in sea lamprey. This period of parasitic growth in sea lamprey may incorporate enough material from either the open lake or their prey to completely mask the larval signature within the statolith. Recent work has demonstrated that material deposited in the otolith core is significantly different from the marginal material for many species, and consequently fish stocks can be discriminated if the core material can be analyzed separately from the marginal material (Thorrold *et al.* 1997; Campana 1999; Fowler *et al.* 2005). However, species in these studies have longer adult life spans than the parasitic phase of the sea lamprey life cycle (usually several years, vs. the 12 – 18 months for sea lamprey). In our study, the statoliths of both larval and spawning-phase sea lamprey were ablated along a transect spanning the base of the statolith (new material) to the apex (oldest material). It was assumed that the duration of the parasitic-phase is short enough to limit the amount of material deposited post-metamorphosis. Given the results of the validation samples, this assumption was likely incorrect.

Few studies have validated microchemistry predictions using marked individuals of known origin, aside from that reported by Brothers and Thresher (2004). Generally speaking, most studies using microelemental analyses of otoliths as biological tracers are validated by using stocks sampled during times of known aggregations, in species known to have high fidelity to spawning grounds (e.g. Waldman *et al.* 1997; Campana *et al.* 2000); other studies have used the technique simply to identify how stocks may be structured (e.g. Fowler *et al.* 2005). Thus the amount of precision and accuracy required in the validation step is determined by the objective of the study. For our study, the ability to accurately predict the natal tributary of a sea lamprey of unknown origin is crucial to the utility of this technique as a management tool.

This study sets the stage for sea lamprey identification by confirming that microelemental data yield good discrimination among tributaries. Further research into the deposition of material during the parasitic portion of the sea lamprey life cycle, and appropriate methods of statolith analysis, are required before this technique can be used to accurately predict the natal tributary for sea lamprey of unknown origin.

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Table 1. Tributaries sampled for larval sea lamprey, with most recent QAS survey year and respective larval and transformer estimates (if available), discharge, sample date, and collection method. Tributaries are listed in counter-clockwise orientation around the lake, beginning with the northwest corner.

| Tributary | State | Survey year[a] | Estimated no. ammocoetes[a] | Estimated no. transformers[a] | Most recent treatment | Collection date | Collection method |
|---|---|---|---|---|---|---|---|
| Great Chazy R. | NY | 2003 | 253,101 | na | 2004 | Fall 2003 | Electrofishing |
| | | | | | | Summer 2004 | TFM |
| Saranac R. | NY | ns | ns | ns | 1992 | Summer 2005 | Electrofishing |
| Saranac R. delta | NY | 2003 | 450,000 | na | 2003 | Fall 2004 | TFM |
| Salmon R. | NY | 2005 | 62,161 | 347 | 2002 | Fall 2002 | TFM |
| Little Ausable R. | NY | 2005 | 164,781 | na | 2002 | Summer 2002 | Electrofishing |
| Ausable R. | NY | 2005 | 648,532 | na | 2002 | TFM 2002 | TFM |
| Boquet R. | NY | 2002 | 58,300 | 176 | 2003 | Summer 2005 | Electrofishing |
| Beaver Br. | NY | 2002 | 1,235 | na | 2003 | ns | ns |
| Mill Br. | NY | ns | na | na | none | Summer 2004 | Electrofishing |
| Mullen Br. | NY | 2001 | 1,275 | na | none | Summer 2004 | Electrofishing |
| Putnam Cr. | NY | 2005 | 101,906 | 4,131 | 2002 | Fall 2002 | Electrofishing |
| Mt. Hope Br. | NY | 2002 | 53,208 | 1,468 | 2003 | Fall 2004 | TFM |
| Poultney R. | VT/NY | 2000 | 107,594 | 955 | 1996 | Summer 2004 | Electrofishing |
| Hubbardton R. | VT | 2000 | 648 | na | 1996 | ns | ns |
| Lewis Cr. | VT | 2005 | 59,292 | 948 | 2002 | Summer 2002 | Electrofishing |
| LaPlatte R. | VT | 2002 | 2,502 | na | none | Summer 2005 | Electrofishing |

Table 1. continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Lamoille R. | VT | 2005 | 38,791 | na | none | Summer 2005 | Electrofishing |
| Winooski | VT | 2002 | 33,062 | na | 2004 | Fall 2004 | TFM |
| Sunderland | VT | 2000 | 119 | na | none | Summer 2004 | Electrofishing |
| Mallets Cr. | VT | 2005 | 4,442 | 342 | none | Summer 2003 | Electrofishing |
| Trout Br. | VT | 2005 | 2,253 | na | 1995 | Summer 2004 | Electrofishing |
| Missisquoi R. | VT | 2004 | 16,732 | 16,732 | none | Summer 2003 | Electrofishing |
| Youngman Br. | VT | 2001 | 7,768 | 588 | none | Fall 2002 | Electrofishing |
| Pike R. | QUE | 2000 | 69,719 | 8,300 | none | Summer 2004 | Electrofishing |
| Morpion St. | QUE | 2000 | 39,366 | 436 | none | Summer 2004 | Electrofishing |

[a] USFWS, unpublished data (2005)

[b] Fisheries Technical Committee (2001), unless otherwise noted.

[c] USGS average September stream flow

ns - not sampled

na - information not available

Table 2. Limit of detection (LOD), coefficient of variation (CV), and the percentage of samples analyzed that were higher than the LOD for each element. Values highlighted in boldface indicate the elements that met the selection criteria for potential inclusion in the analysis.

| Element | LOD | Mean CV | % > LOD |
|---|---|---|---|
| $^{7}$Li | 1.167 | **5.0** | 5% |
| $^{25}$Mg | 16.658 | **1.9** | **100%** |
| $^{55}$Mn | 0.467 | **1.4** | **100%** |
| $^{66}$Zn | 2.720 | **7.5** | **80%** |
| $^{85}$Rb | 0.185 | **4.0** | **100%** |
| $^{86}$Sr | 2.650 | **1.0** | **100%** |
| $^{138}$Ba | 0.111 | **1.0** | **100%** |
| $^{140}$Ce | 0.031 | **1.1** | 70% |
| $^{208}$Pb | 0.053 | **8.3** | **90%** |
| $^{238}$U | 0.017 | **2.7** | 45% |

Table 3. Tributary groupings used for discriminant analysis in this study with correct classification rates for each grouping. Groupings are indicated by same number under column header.

| | | Grouping | | | | |
|---|---|---|---|---|---|---|
| | Total # samples | A | B | C | D | E |
| Great Chazy R. 2003 | 15 | 1 | 1 | 1 | 1 | 1 |
| Great Chazy R. 2004 | 8 | 2 | 1 | 1 | 1 | 1 |
| Saranac R. | 16 | 3 | 2 | 2 | C1 | C1 |
| Saranac R. delta | 19 | 4 | 3 | 2 | C1 | C1 |
| Ausable R. | 15 | 5 | 4 | 3 | C1 | C1 |
| Salmon R. | 15 | 6 | 5 | 4 | C1 | C1 |
| Mill Br. | 18 | 7 | 6 | 5 | C2 | C2 |
| Mullen Br. | 5 | 8 | 7 | 6 | C2 | C2 |
| Putnam Cr. | 3 | 9 | 8 | 7 | C2 | C2 |
| Mount Hope Br. | 10 | 10 | 9 | 8 | C2 | C2 |
| Poultney R. | 17 | 11 | 10 | 9 | 4 | 3 |
| Lewis Cr. | 56 | 12 | 11 | 10 | 5 | 4 |
| LaPlatte R. | 3 | 13 | 12 | 11 | 6 | 5 |
| Winooski | 15 | 14 | 13 | 12 | 7 | 5 |
| Mallets Cr. | 17 | 15 | 14 | 13 | 8 | 6 |
| Trout Br. | 8 | 16 | 15 | 14 | 9 | 6 |
| Youngman Br. | 4 | 17 | 16 | 15 | C3 | 7 |
| Missisquoi R. | 14 | 18 | 17 | 15 | C3 | 7 |
| Morpion St. | 18 | 19 | 18 | 16 | C3 | 8 |
| Pike R. | 14 | 20 | 19 | 16 | C3 | 8 |
| Total samples | 290 | | | | | |
| # correctly classified | | 201 | 207 | 212 | 224 | 221 |
| Correct classification rate (%) | | 69.3 | 71.4 | 73.1 | 77.2 | 76.2 |

Groupings:
A - all separate
B - Great Chazy 2003 & 2004 combined
C - B + Saranac R & delta, Missisquoi R./Youngman Br., Pike R./Morpion St. combined
D - C + Saranac R./Ausable R./Salmon R., Mill Br./Mullen Br./Putnam Cr./Mt Hope Br.
E - D + Winooski/LaPlatte R., Malletts Cr./Trout Br.

Table 4. Tests for differences in elemental concentrations of sea lamprey statoliths collected from the Great Chazy River by sample year (2003 & 2004), the Pike River/ Morpion Stream, Saranac R. and Saranac delta, and Missisquoi R./ Youngman Br. Significant differences are highlighted in boldface ($\alpha = 0.05$).

| Element | Mean value | | # samples | | t-value | df | p |
|---|---|---|---|---|---|---|---|
| | Chazy 2003 | Chazy 2004 | Chazy 2003 | Chazy 2004 | | | |
| **Log(Mg)** | **8.57** | **8.27** | **15** | **8** | **3.12** | **21** | **<0.0051** |
| Log(Mn) | 3.92 | 3.90 | 15 | 8 | 0.18 | 21 | <0.8578 |
| Log(Zn) | 2.80 | 2.74 | 15 | 8 | 0.20 | 21 | <0.8425 |
| Log(Sr) | 5.98 | 5.78 | 15 | 8 | 2.04 | 21 | <0.0539 |
| Log(Ba) | 3.23 | 3.00 | 15 | 8 | 1.43 | 21 | <0.1686 |
| **Log(Rb) residuals** | **0.98** | **1.34** | **15** | **8** | **-2.75** | **21** | **<0.0120** |
| | Pike R. | Morpion St. | Pike R. | Morpion St. | | | |
| **Log(Mg)** | **8.55** | **8.39** | **14** | **18** | **-2.31** | **30** | **<0.0278** |
| **Log(Mn)** | **4.06** | **3.63** | **14** | **18** | **-3.43** | **30** | **<0.0017** |
| **Log(Zn)** | **3.63** | **2.18** | **14** | **18** | **-4.52** | **30** | **<0.0001** |
| Log(Sr) | 6.92 | 7.09 | 14 | 18 | 1.81 | 30 | <0.0799 |
| **Log(Ba)** | **2.83** | **3.41** | **14** | **18** | **4.08** | **30** | **<0.0003** |
| Log(Rb) residuals | 0.90 | 0.72 | 14 | 18 | -1.60 | 30 | <0.1197 |
| | Saranac R. | Saranac delta | Saranac R. | Saranac delta | | | |
| **Log(Mg)** | **8.60** | **8.43** | **16** | **19** | **-2.67** | **33** | **<0.0117** |
| Log(Mn) | 4.45 | 4.23 | 16 | 19 | -1.73 | 33 | <0.0934 |
| Log(Zn) | 2.55 | 2.76 | 16 | 19 | 0.39 | 33 | <0.6969 |
| **Log(Sr)** | **6.31** | **5.99** | **16** | **19** | **-5.49** | **33** | **<0.0001** |
| **Log(Ba)** | **3.32** | **2.81** | **16** | **19** | **-4.43** | **33** | **<0.0001** |
| Log(Rb) residuals | 2.02 | 2.04 | 16 | 19 | 0.24 | 33 | <0.8149 |
| | Missisquoi R. | Youngman Br. | Missisquoi R. | Youngman Br. | | | |
| **Log(Mg)** | **8.58** | **8.37** | **14** | **4** | **2.20** | **16** | **<0.0425** |
| Log(Mn) | 4.47 | 4.51 | 14 | 4 | -0.24 | 16 | <0.8111 |
| **Log(Zn)** | **2.10** | **0.25** | **14** | **4** | **2.35** | **16** | **<0.0316** |
| Log(Sr) | 6.66 | 6.26 | 14 | 4 | 1.90 | 16 | <0.0760 |
| Log(Ba) | 2.69 | 2.65 | 14 | 4 | 0.19 | 16 | <0.8535 |
| **Log(Rb) residuals** | **0.70** | **1.69** | **14** | **4** | **-3.89** | **16** | **<0.0012** |

Table 5. Results from linear discriminant function and canonical analyses. a) forward stepwise regression for variable selection ($\alpha < 0.05$ to enter), b) standardized coefficients for canonical variables, including the eigenvalues for each root and cumulative proportion of the variance explained with the addition of each root, and c) means of the canonical variables. Important variables for each root in (b) and (c) are in boldface.

a)

| Element | Steps | df | F to enter | P to enter |
|---|---|---|---|---|
| Log(Rb), residuals | 1 | 9 | 102.56 | <0.001 |
| Log(Sr) | 2 | 9 | 19.39 | <0.001 |
| Log(Mg) | 3 | 9 | 21.92 | <0.001 |
| Log(Zn) | 4 | 9 | 12.13 | <0.001 |
| Log(Ba) | 5 | 9 | 10.67 | <0.001 |
| Log(Mn) | 6 | 9 | 9.61 | <0.001 |
| binary for Pb | 7 | 9 | 6.61 | <0.001 |

b)

| Element | Root 1 | Root 2 | Root 3 | Root 4 |
|---|---|---|---|---|
| Log(Mg) | 0.340 | -0.571 | **-0.478** | 0.228 |
| Log(Mn) | 0.096 | 0.079 | **-0.734** | 0.025 |
| Log(Zn) | -0.377 | 0.080 | 0.070 | **0.720** |
| Log(Sr) | -0.180 | **1.179** | -0.245 | -0.330 |
| Log(Ba) | 0.161 | -0.631 | 0.285 | -0.238 |
| Log(Rb), residuals | **-0.955** | -0.210 | -0.224 | -0.179 |
| Pb (covariate) | 0.223 | -0.119 | 0.038 | **-0.457** |
| Eigenvalue | 4.435 | 1.301 | 0.562 | 0.394 |
| Cum. Prop. | 0.635 | 0.821 | 0.901 | 0.958 |

c)

| Tributary cluster | Root 1 | Root 2 | Root 3 | Root 4 |
|---|---|---|---|---|
| Great Chazy R. | 0.126 | **-1.910** | 0.819 | **1.122** |
| C1 | -1.473 | **-1.195** | -0.801 | -0.289 |
| C2 | **-3.431** | 0.549 | **1.285** | -0.506 |
| Poultney R. | 1.874 | 0.357 | -0.428 | 0.139 |
| Lewis Cr. | **3.066** | -0.256 | 0.295 | -0.591 |
| LaPlatte R. | 1.923 | 0.076 | -0.037 | -0.936 |
| Winooski R. | -1.968 | **1.464** | **-1.348** | 0.491 |
| Malletts Cr. | 0.412 | 0.640 | 0.010 | **1.148** |
| Trout Br. | 1.091 | -0.445 | 0.722 | **1.482** |
| C3 | 0.418 | **1.613** | -0.158 | 0.120 |

Table 6. Classification matrix for Grouping D. Rows are actual streams of origin, columns are predicted streams of origin. Correct classifications are highlighted in boldface.

| Tributary/Cluster | # Samples | % Correct | Great Chazy R. | C1 | C2 | Poultney R. | Lewis Cr. | LaPlatte R. | Winooski R. | Malletts Cr. | Trout Br. | C3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Great Chazy R. | 23 | 91.30 | **21** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| C1 | 65 | 76.92 | 2 | **50** | 3 | 2 | 0 | 1 | 4 | 3 | 0 | 0 |
| C2 | 36 | 88.89 | 0 | 2 | **32** | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Poultney R. | 17 | 64.71 | 0 | 0 | 0 | **11** | 2 | 1 | 0 | 2 | 0 | 1 |
| Lewis Cr. | 56 | 76.79 | 3 | 0 | 0 | 4 | **43** | 4 | 0 | 2 | 0 | 0 |
| LaPlatte R. | 3 | 100.00 | 0 | 0 | 0 | 0 | 0 | **3** | 0 | 0 | 0 | 0 |
| Winooski R. | 15 | 80.00 | 0 | 2 | 1 | 0 | 0 | 0 | **12** | 0 | 0 | 0 |
| Malletts Cr. | 17 | 76.47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **13** | 2 | 2 |
| Trout Br. | 8 | 87.50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | **7** | 0 |
| C3 | 50 | 64.00 | 0 | 3 | 0 | 5 | 1 | 3 | 2 | 3 | 1 | **32** |
| Total | 290 | 77.24 | 26 | 58 | 36 | 22 | 46 | 12 | 20 | 24 | 11 | 35 |

## Figure Legends

Figure 1. Map of Lake Champlain and tributaries sampled in this study.

Figure 2. Canonical plot of tributary groupings B, C and F, with 95% confidence ellipses.

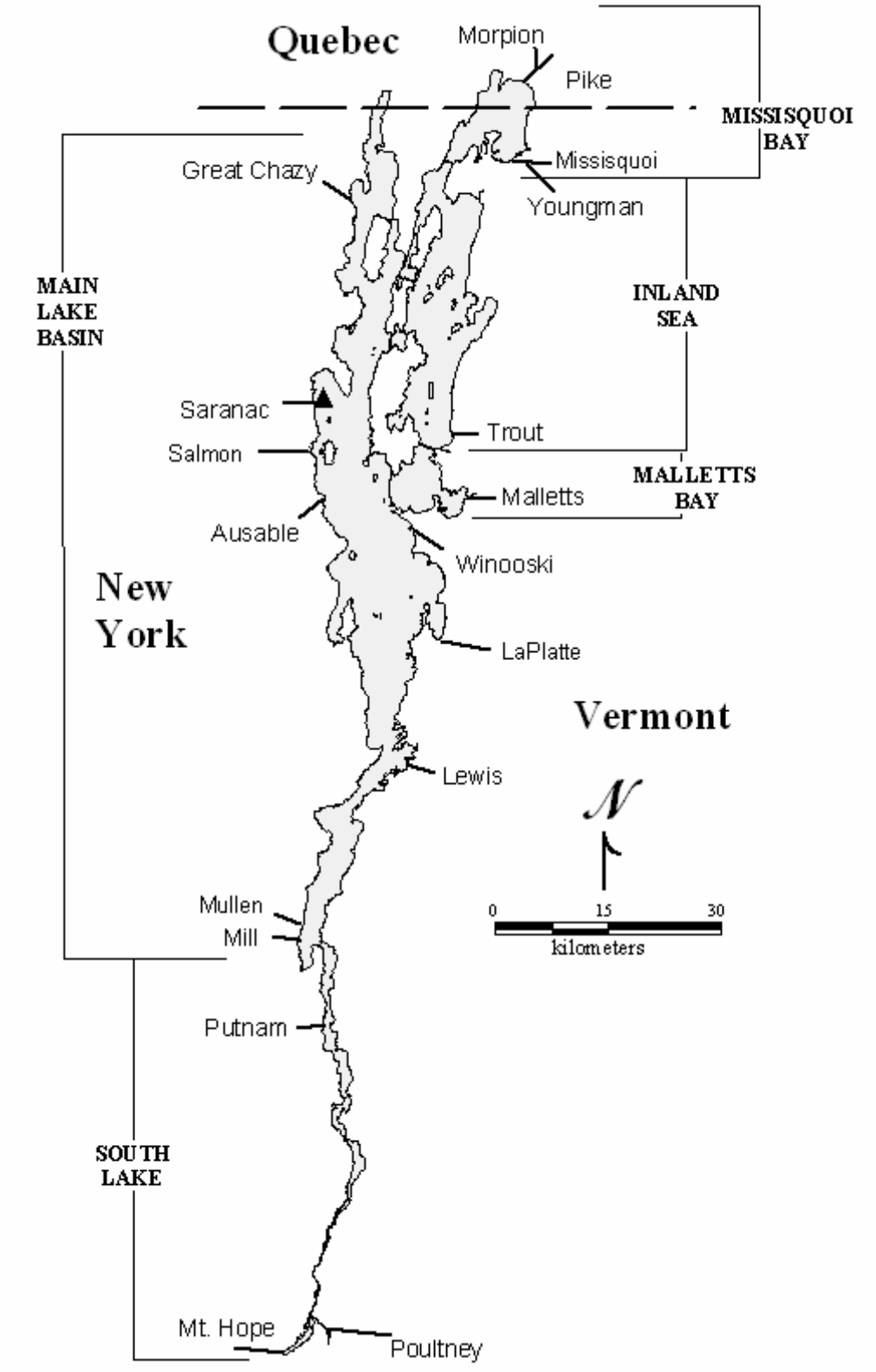

Quebec
Morpion
Pike
MISSISQUOI BAY
Missisquoi
Great Chazy
Youngman
MAIN LAKE BASIN
INLAND SEA
Saranac
Trout
Salmon
MALLETTS BAY
Malletts
Ausable
Winooski
New York
LaPlatte
Vermont
Lewis
N
0
15
30
kilometers
Mullen
Mill
Putnam
SOUTH LAKE
Mt. Hope
Poultney

**Figure 1.**

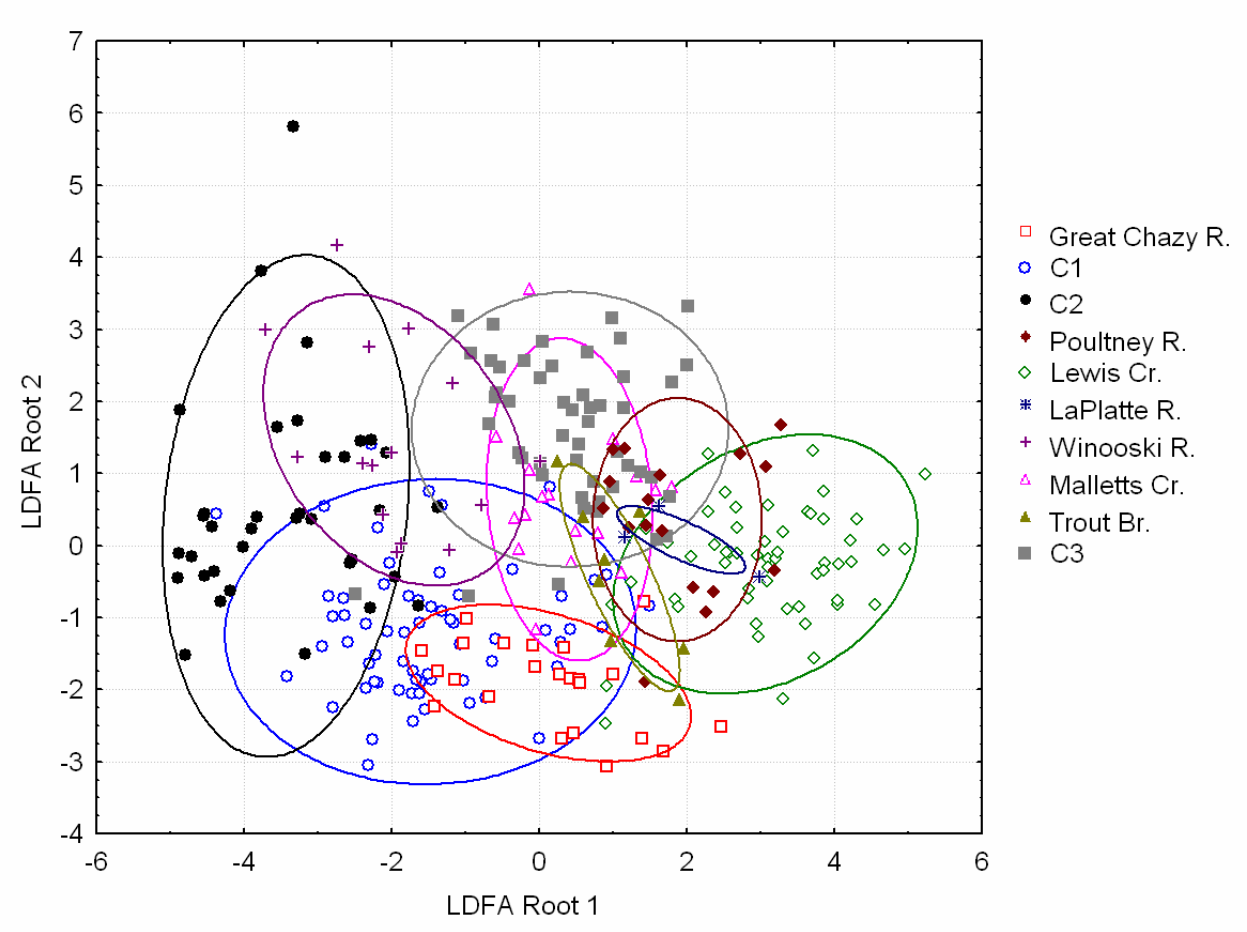
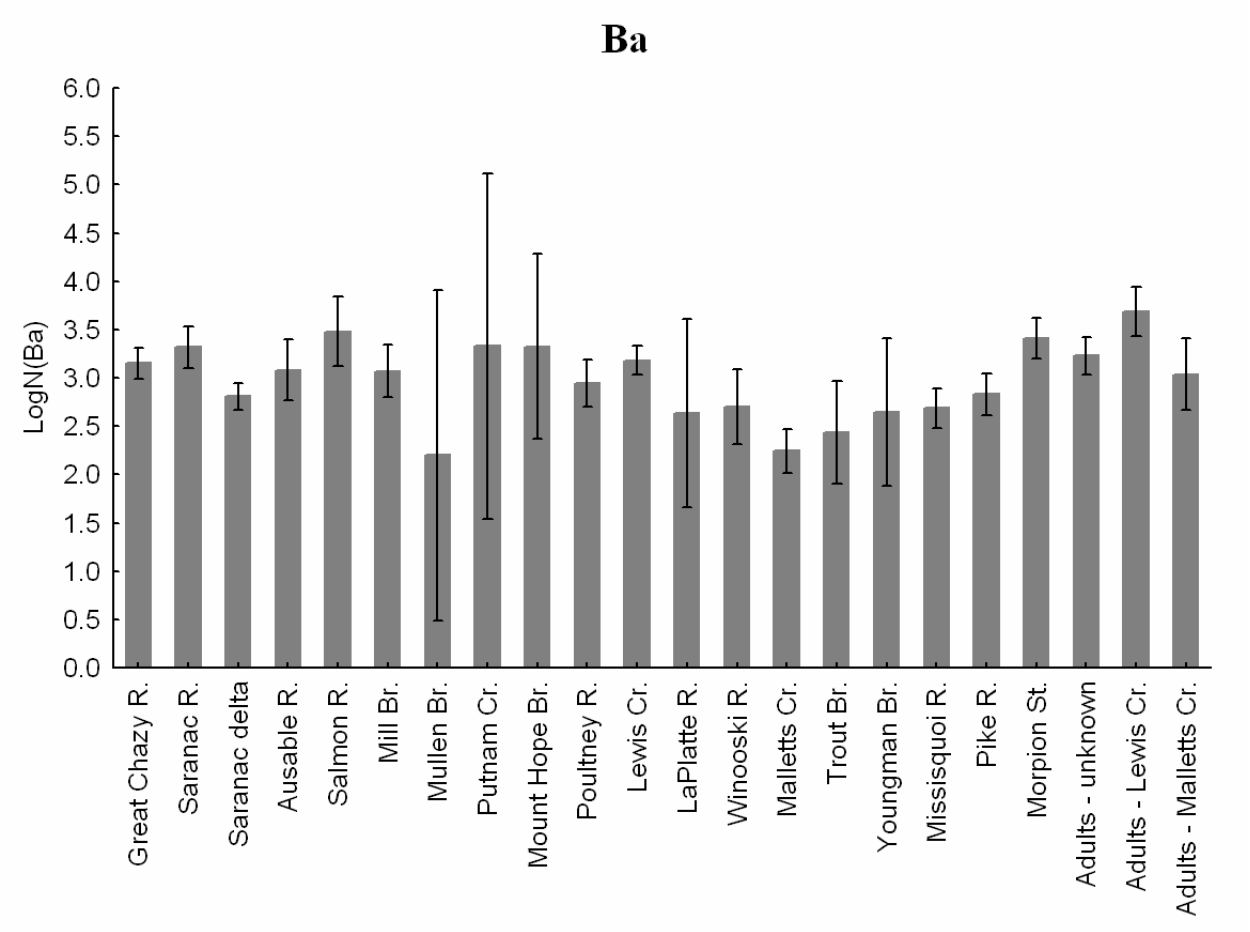
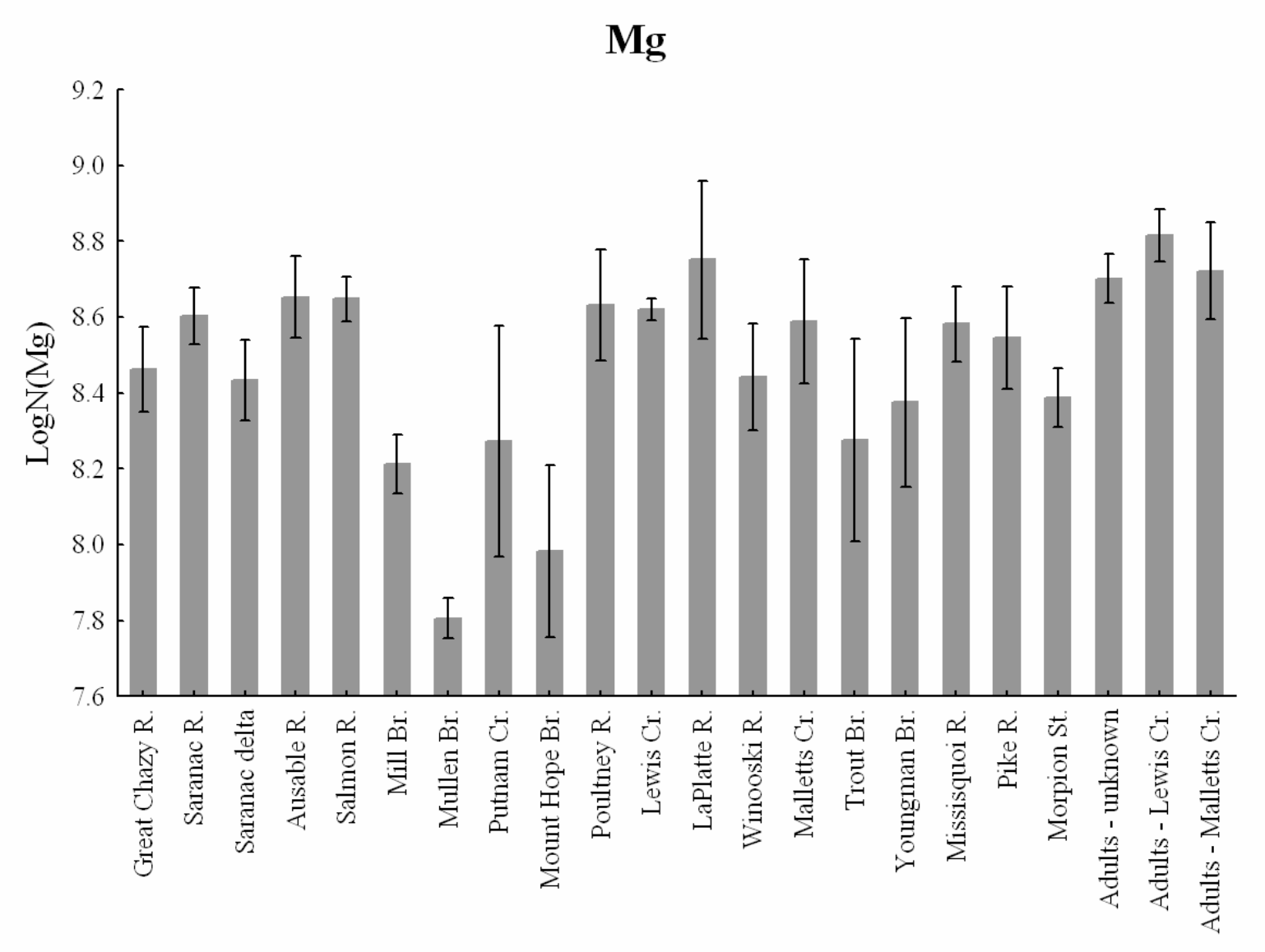
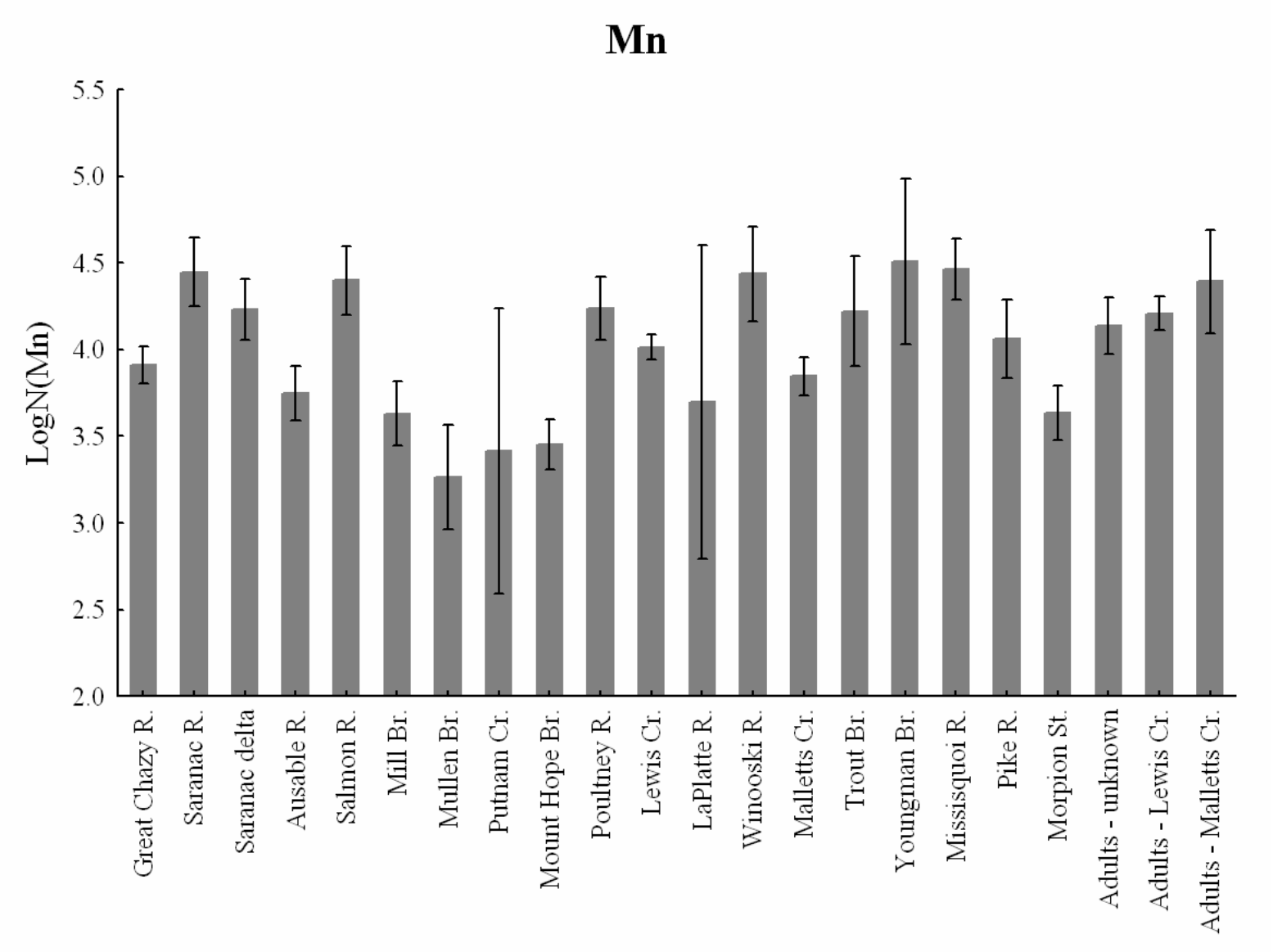

LDFA Root 2
LDFA Root 1
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-6
-4
-2
0
2
4
6
Great Chazy R.
C1
C2
Poultney R.
Lewis Cr.
LaPlatte R.
Winooski R.
Malletts Cr.
Trout Br.
C3

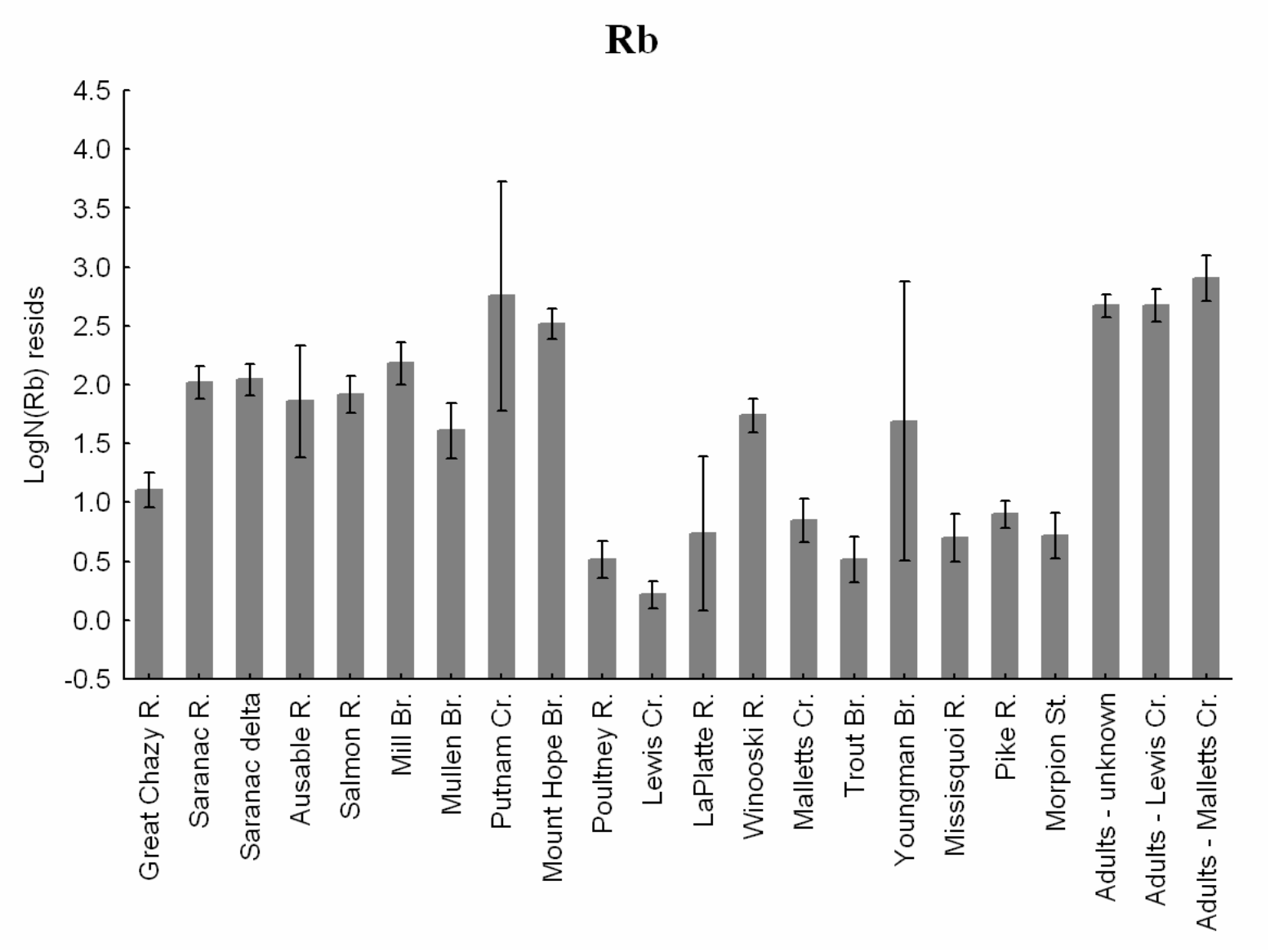
**Figure 2.**

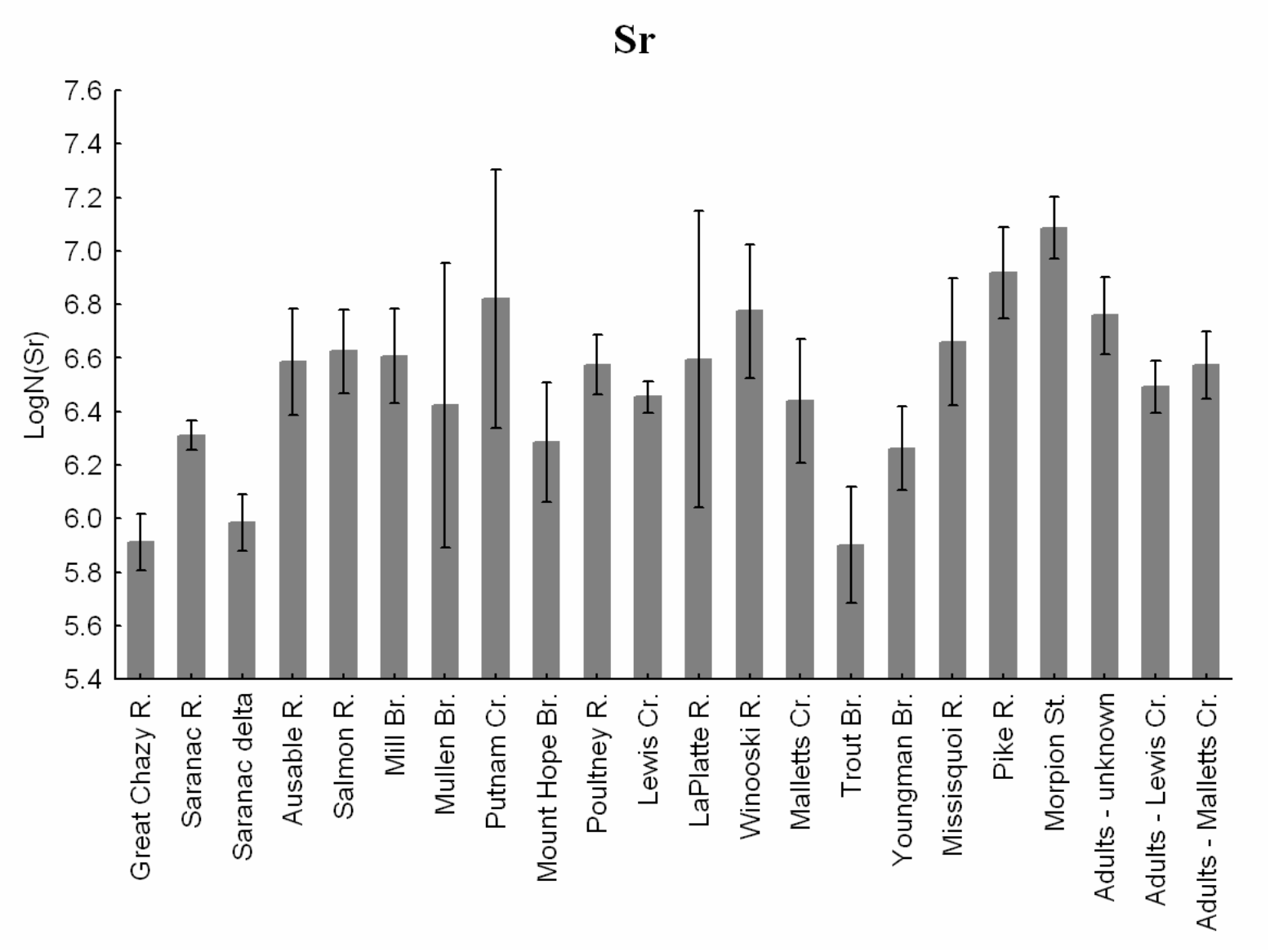
Appendix I. Weighted mean elemental concentrations (□g/g) for each of the tributaries, with spawning-phase samples from Lewis and Malletts creeks and from unknown origin. Error bars are 95% CI.

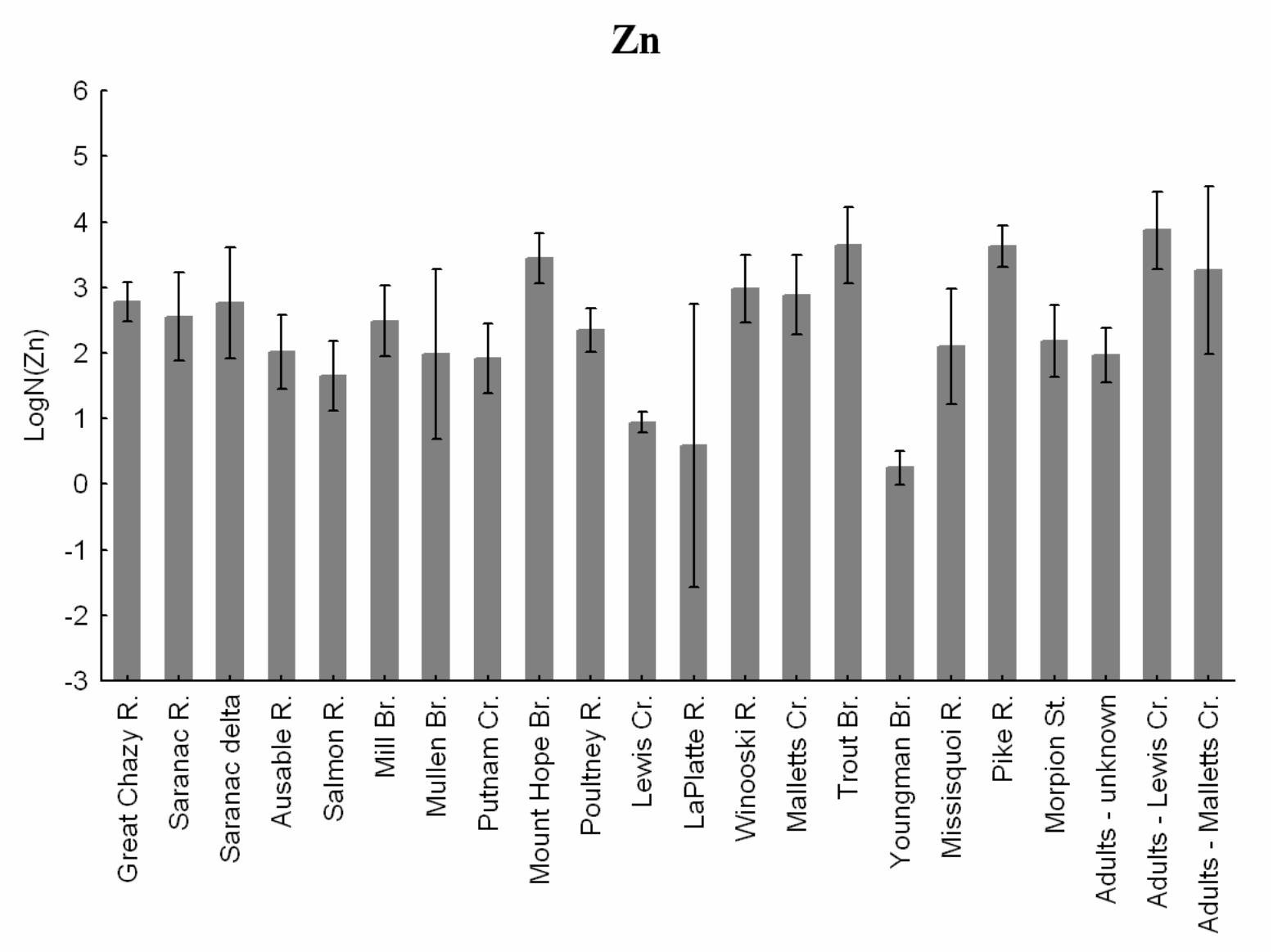



Mg
LogN(Mg)
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
Great Chazy R.
Saranac R.
Saranac delta
Ausable R.
Salmon R.
Mill Br.
Mullen Br.
Putnam Cr.
Mount Hope Br.
Poultney R.
Lewis Cr.
LaPlatte R.
Winooski R.
Malletts Cr.
Trout Br.
Youngman Br.
Missisquoi R.
Pike R.
Morpion St.
Adults - unknown
Adults - Lewis Cr.
Adults - Malletts Cr.


Mn
LogN(Mn)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Great Chazy R.
Saranac R.
Saranac delta
Ausable R.
Salmon R.
Mill Br.
Mullen Br.
Putnam Cr.
Mount Hope Br.
Poultney R.
Lewis Cr.
LaPlatte R.
Winooski R.
Malletts Cr.
Trout Br.
Youngman Br.
Mississquoi R.
Pike R.
Morpion St.
Adults - unknown
Adults - Lewis Cr.
Adults - Malletts Cr.


Rb
LogN(Rb) resids
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Great Chazy R.
Saranac R.
Saranac delta
Ausable R.
Salmon R.
Mill Br.
Mullen Br.
Putnam Cr.
Mount Hope Br.
Poultney R.
Lewis Cr.
LaPlatte R.
Winooski R.
Malletts Cr.
Trout Br.
Youngman Br.
Missisquoi R.
Pike R.
Morpion St.
Adults - unknown
Adults - Lewis Cr.
Adults - Malletts Cr.


Sr
LogN(Sr)
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
Great Chazy R.
Saranac R.
Saranac delta
Ausable R.
Salmon R.
Mill Br.
Mullen Br.
Putnam Cr.
Mount Hope Br.
Poultney R.
Lewis Cr.
LaPlatte R.
Winooski R.
Malletts Cr.
Trout Br.
Youngman Br.
Missisquoi R.
Pike R.
Morpion St.
Adults - unknown
Adults - Lewis Cr.
Adults - Malletts Cr.


Zn
LogN(Zn)
6
5
4
3
2
1
0
-1
-2
-3
Great Chazy R.
Saranac R.
Saranac delta
Ausable R.
Salmon R.
Mill Br.
Mullen Br.
Putnam Cr.
Mount Hope Br.
Poultney R.
Lewis Cr.
LaPlatte R.
Winooski R.
Malletts Cr.
Trout Br.
Youngman Br.
Missisquoi R.
Pike R.
Morpion St.
Adults - unknown
Adults - Lewis Cr.
Adults - Malletts Cr.