Technical Report No.48

Lake Champlain
Basin Program

Tributary contributions to the parasitic and spawning populations of sea lamprey (*Petromyzon marinus*) in Lake Champlain using elemental signatures

Prepared by
Howe, E.A., C.P Hand, J.E. Marsden, S.A. Ludsin, and B.J Fryer,
Rubenstein School of Environment and Natural Resources,
University of Vermont; Great Lakes Institute for Environmental
Research, University of Windsor; National Oceanic and
Atmospheric Administration, Great Lakes Environmental
Research Laboratory.

for
Lake Champlain Basin Program

March 2006

PUBLICATION SERIES

THIS PROGRAM IS SPONSORED BY U.S.E.P.A. AND THE STATES OF NEW YORK AND VERMONT.

Tributary contributions to the parasitic and spawning adult population of sea lamprey (*Petromyzon marinus*) in Lake Champlain using elemental signatures

Eric A. Howe[1], Carrol P. Hand[2], J. Ellen Marsden[1], Stuart A. Ludsin[3], and Brian J. Fryer[2]

[1]Rubenstein School of Environment and Natural Resources
81 Carrigan Dr., University of Vermont
Burlington, VT 05405

[2]Great Lakes Institute for Environmental Research
University of Windsor, 2990 Riverside Dr. West
Windsor, ON, Canada, N9B 3P4

[3]National Oceanic and Atmospheric Administration
Great Lakes Environmental Research Laboratory
2205 Commonwealth Blvd.
Ann Arbor, MI 48105 USA

March, 2006

Table of Contents

List of Tables

List of Figures

Executive Summary

The sea lamprey (*Petromyzon marinus*) is a primitive fish that has been implicated in the loss of the salmonid fishery of the Laurentian Great Lakes and Lake Champlain. Significant effort has been put forth into controlling or limiting the growth of this nuisance population since the early 1950s. The sea lamprey control program for all of the Great Lakes and Lake Champlain relies heavily on the application of chemical pesticides (lampricides) to tributaries to manage the sea lamprey population. Current prioritization for lampricide treatments relies on estimates of larval density, length frequency, and likelihood of metamorphosis to predict the number of parasitic-phase lamprey that each tributary will produce in a given year. Managers must assume that survivorship from the tributary to the lake is equal among all tributaries. Survival to the parasitic-phase may be significantly higher in tributaries that empty directly into locations in the main lake with an abundance of preferred prey, whereas lamprey in tributaries that empty into a lake region that has low prey abundance may have a lower chance of survival. If this is the case, then some lampricide treatments may not be necessary, or need to be as effective, to maintain the parasite population at the desired level. Optimization of the control program would allow resources to be redirected toward other tributaries that have the highest survivorship to the parasitic stage, or to other facets of the sea lamprey control program. The life cycle of the sea lamprey is such that an individual spends several years in the sediments of a tributary prior to migrating to the lake. This extended sedentary residence creates a record of the elemental composition of the tributary within the statolith of the lamprey, which can then be used to predict which tributary an individual of unknown origin came from.

The objective of this study is to determine whether larval origins of sea lamprey can be identified using trace elements in their statoliths. This objective requires a technique with very high precision and accuracy. 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.

There are currently 21 primary tributaries and several secondary tributaries in Lake Champlain from which sea lamprey have been collected. In this report we document results from statoliths collected from 12 primary tributaries, 2 secondary tributaries, and one tributary delta. Number of analyzed statoliths ranged from 3 to 15 per tributary. The goal of this project is to analyze 15 statoliths from each of 22 tributaries. The tributaries were analyzed separately, and as groupings based on geographic proximity.

Statolith concentrations of six elements (rubidium, strontium, magnesium, manganese, barium, zinc) were used in a linear discriminant function and classification analysis. Rubidium and strontium were the most important elements for discriminating among the locations. The grouping that had the Pike River clustered with its secondary tributary Morpion Stream, Missisquoi River clustered with its secondary tributary Youngman Brook, and the three tributaries in the Port Henry area of the lake (Mullen and Mill Brooks, Putnam Creek) clustered together had the lowest misclassification rate (34 out of 151; 22.5%), although the other groupings were not very different (range to 28.5%).

With a full analysis including statoliths from all tributaries (including larger sample sizes), discrimination among tributaries should improve significantly, enabling analysis of trace element composition within statoliths of parasitic and adult lampreys to provide a record of the contribution of each tributary system (or perhaps region) within the lake to the parasitic and spawning adult sea lamprey populations in Lake Champlain.

Subsequent to completion of this project, statoliths from one parasitic-phase sea lamprey and 33 spawning-phase sea lamprey of known tributary origin 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. For this analysis, tributary streams were grouped by geographic proximity and larvae were assigned to groups. Only one (2.94%) of the known-origin samples was correctly classified to the group containing its natal tributary, the Saranac River. Overall, 30 of the 34 samples were classified to this group. The results indicate that the analysis reflects microelemental composition of the parasitic (lake-resident) portion of the statolith, which should be relatively homogeneous among all lamprey, rather than the larval portion. Further work is being conducted to resolve the larval microelemental signature from parasitic-phase and spawning-phase statoliths.

Introduction

The sea lamprey (*Petromyzon marinus*) is a primitive fish that has been implicated in the loss of lake trout in the Laurentian Great Lakes and has hindered restoration of salmonid populations in Lake Champlain (Christie and Goddard 2003; Smith and Tibbles 1980). Significant effort has been put forth into controlling or limiting the growth of this nuisance population since the early 1950's (Christie and Goddard 2003; Smith and Tibbles 1980). The sea lamprey control program for all Great Lakes and for Lake Champlain relies heavily on the application of chemical pesticides (lampricides) to tributaries to manage the sea lamprey population. Sea lamprey spend the first three to four years of their life cycle in tributaries as sedentary filter feeders before metamorphosing into a parasitic phase in which they feed on blood and body fluids of host fish (Applegate 1950; Bence et al. 2003; Swink 1991). With finite resources available, sea lamprey control programs must ensure that the efficiency of each control action is maximized. Knowledge of which tributaries are contributing high proportions of sea lamprey to the parasitic population is crucial to the success of the sea lamprey control program (Bergstedt and Seelye 1995; Howe et al. in review).

Current prioritization for lampricide treatments relies on estimates of larval density, length frequency, and likelihood of metamorphosis to predict the number of parasitic-phase lamprey that each tributary will produce in a given year (Christie et al. 2003; Henson et al. 2003; Slade et al. 2003). Managers must assume that survivorship from the tributary to the lake is equal among all tributaries. This assumption, however, has not been validated (Howe et al. in review). Survival to the parasitic-phase may be significantly higher in tributaries that empty directly into locations in the main lake with an abundance of preferred prey, whereas lamprey in tributaries that empty into a lake region that has low prey abundance may have a lower chance of survival. If this is the case, then some lampricide treatments may not be necessary, or need to be as effective, to maintain the parasite population at the desired level. Optimization of the control program would allow resources to be redirected toward other tributaries that have the highest survivorship to the parasitic stage, or to other facets of the sea lamprey control program.

Natural marks have been used to track animals in many different settings, such as genetic marks 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 analysis in fisheries (e.g., Campana et al. 2000; Campana and Thorrold 2001). Trace elements (or microelements) have allowed fisheries management programs to garner information on origins of fish stocks using the otoliths of marine (Campana et al. 1994; Humphreys et al. 2005), estuarine (Gillanders 2005; Gillanders and Kingsford 1996), and freshwater fishes (Brazner et al. 2004a; Brazner et al. 2004b). More recently, work has been done exploring the suitability of microelemental analysis for stock discrimination in species containing statoliths (Brothers and Thresher 2004; Zacherl et al. 2003). The metabolically inert otolith of most fishes, and the statolith of lampreys, incorporates elements from the surrounding environment. Layers of material are added to the otolith (or statolith) on a daily basis, essentially creating a diary of where the fish has been (Campana 2005; Meeuwig and Bayer 2005). The primary elements used for stock discrimination are Sr, Ba, Mn, Fe, and Pb, and occasionally Li, Mg, Cu, and Ni. The benefits of natural tags are numerous; the most important benefit is that all animals are marked, so the expense and challenge of marking large numbers of animals and finding marked individuals is eliminated. In addition, the tags do not affect the behavior or survival of the animals.

Several techniques have been utilized to examine elemental compositions for otoliths; while no one technique is superior to another, some are more effective for addressing specific hypotheses and examining specific elements (see Campana 2005; Campana et al. 1997). The objectives of this study examine trace elements, thus requiring 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). Brothers and Thresher (2004) used proton-induced X-ray emission (PIXE) to analyze sea lamprey ammocoete statoliths collected from the Great Lakes. They were able to successfully discriminate and classify larval sea lamprey from four out of five different locations; two of the locations were within a single tributary (Brothers and Thresher 2004). Campana et al. (1997) found that PIXE and LA-ICPMS have similar minimum detection limits and precision, albeit 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 this study 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 the long sedentary residence of the sea lamprey in tributaries to Lake Champlain will create a stable record of the elemental composition of the tributary within the statolith of the lamprey. Often these unique signatures are a reflection on the geology of a basin. In some cases, tributaries themselves may not be uniquely identifiable, but groups of tributaries with a common watershed may be. This study evaluates the application of elemental signatures to sea lamprey populations in Lake Champlain to distinguish the lake's tributaries and then identify those tributaries or geological regions in the Lake Champlain basin that contribute the highest numbers of sea lamprey to the parasitic and spawning adult populations. The objectives of this project are:

1. Characterize statolith elemental signatures among larvae produced in different tributary streams of Lake Champlain (specifically, funding was provided by LCBP to analyze lamprey from 6 tributaries).
2. Test whether we can accurately classify adults to their natal streams using individuals tagged as transformers.

Populations of larval sea lamprey have been found in 22 tributaries in Lake Champlain at varying abundances (Table 1). Based on population estimates from the quantitative assessment sampling (QAS) surveys for larval sea lamprey (Christie et al. 2003; Slade et al. 2003), the tributaries that are most likely to be major contributors to Lake Champlain are the Ausable, Great Chazy, Little Ausable, and Poultney rivers (based on ammocoete estimates) and Pike River, Putnam Creek, and Mount Hope Brook (based on transformer estimates; Table 1). Statoliths from 14 tributaries have been analyzed to date; not all larvae sampled from each tributary have yet been analyzed. Ultimately, statoliths will be analyzed from as many as 15 sea lamprey collected from each of the 19 tributaries listed in Table 1. An additional three tributaries had populations of lamprey that were either eliminated by trapping, or were too small to obtain samples.

Significant delays in analysis of samples were encountered due to malfunction, repair, and upgrading of the ICPMS at the University of Windsor, and consequent waiting list for use of the instrument. As a consequence, analysis of parasitic and adult statoliths was not possible during the period of this grant. However, the results of the complete larval analyses including conclusions about the potential for this method for identification of larval origins is included in this report.

Methods

Field collections

Three to fifteen larval lamprey were collected from each of 14 tributaries to Lake Champlain and one tributary delta during lampricide treatments or via electrofishing (Table1, Figure 1). Two different electrofishing units were used (Table 1); backpack electrofishing gear described by Slade et al. (2003) and a georator with an aluminum canoe described by Weisser (1994). Collection dates spanned from summer 2002 through fall 2005 (Table 1). Once collected, all specimens were identified to species level (*P. marinus*), enumerated, and either immediately frozen or preserved in ethanol. Lamprey were stored for > 120 days prior to statolith extraction using the methods described below.

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.

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 on top of a 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 that came 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). 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 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) > 90% and coefficient of variation for level of precision (CV) < 10% (Table 3). Calculation of these criteria followed the methodology described in Ludsin et al. (2006).

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.). 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 mean value for that group (Ludsin et al. 2006). Overall, 2.5% of all data points were replaced using this protocol; replacements for individual elements ranged from 0.7 to 5.3%, and 5.9 to 25% for single elements in individual streams, with the exception of rubidium in Morpion Stream (43%). Elemental concentrations were log10 transformed to normalize the data prior to LDFA analysis. Six discriminant analyses were conducted to determine which groupings of the statoliths yielded the lowest misclassification rates (Table 2, Figure 2). 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. The remaining four groupings were combinations of several tributaries that are geographically adjacent (Table 2).

Results

Of the ten elements detected by the LA-ICPMS, six fell within the criteria for inclusion into the data analysis (Table 3). Using these six elements, Grouping C had the lowest misclassification rate at 22.52% after $\log_{10}$ transformation to normalize the data (Table 2). A measure of Wilk's   is used to indicate the ability to discriminate or separate the tributaries. Values for Wilk's   range from 0 to 1.0, with 0.0 representing complete separation, 1.0 as no separation. For the individual variables, partial Wilk's   values are calculated, and these values are used to interpret the importance of each variable in the discrimination analysis (Table 4a). The element contributing the most to the discrimination was Rb (0.30) and the element contributing the least was Mg (0.716; Table 4a). Tolerance values were highest for Rb and lowest for Sr, indicating that Rb is the least redundant element in terms of its contribution to the discriminant model (Table 4a). The first four discriminant functions (roots) explained 98.0% of the variation in the discriminant model (Table 4b). The standardized coefficients of the canonical variables (linear discriminant function roots) indicate that log10(Rb) carried the most weight for Root 1, which also explained 58.5% of the variance. The second root was weighted mostly by log10(Sr), and explained another 28.4% of the variance. The weights of the elements in the third and fourth roots were mostly carried by the log10(Mn) and log10(Zn), respectively. The cumulative proportion of the variance for the fourth root was 98.0% (Table 4b). The first root was strongest for discriminating Mount Hope Brook from the other tributaries (Table 4c; Figure 2c), and the second root discriminated for the Saranac delta, Great Chazy River, and the Quebec tributaries (Table 4c; Figure 2c). The last two roots had weaker discriminating power than the first two, but they served to discriminate the Winooski River and Lewis Creek, respectively (Table 4c).

Of the tests for differences of the means between the lamprey collected from the Great Chazy River in 2003 and 2004, only Rb was significantly different among the six elements ($t = -5.11$, $df = 15$, $p < 0.0001$; Table 5a). The test for differences of the means among statolith compositions in the Pike River and its secondary tributary, Morpion Stream, indicated that there were significant differences for log10(Zn), log10(Sr), and log10(Ba) (Table 5b).

Discussion

This study indicates that there are sufficient microchemical differences among tributary streams in the Lake Champlain basin to allow reasonable discrimination of larvae from among streams. Discriminant function analysis of the trace elements contained within sea lamprey statoliths appears to serve as a useful technique to classify sea lamprey origin by tributary. Grouping C had the lowest misclassification rate of the six groupings that were explored (Table 2). Tributary classification accuracy using the six elements described in this study ranged from 50.0 - 95.7%, with a mean of 77.5% (Table 6). This classification rate is lower than several published studies using similar techniques (Brothers and Thresher 2004; Campana et al. 2000), but not out of range for studies of this nature (Brazner et al. 2004a). The probability of incorrectly predicting which tributary a sample originated from is clearly correlated to the sample size of the baseline population. Larger sample sizes will improve our ability to discriminate among the tributaries. Based on the results from this exercise, the best model (Grouping C) more accurately classified samples into tributaries from which there was a larger sample size. For example, the Saranac River (n = 15) had one misclassified sample (into the Great Chazy River) and 14 correctly classified samples. No samples from other tributaries were incorrectly classified into the Saranac River (Table 6). However, tributaries with smaller sample sizes, such as Lewis Creek (n = 3) have large variances, increasing the possibility for misclassification. This example is well demonstrated in Figure 2, where the 95% confidence ellipse for Lewis Creek is so large that it exceeds the scale in each of the figures and is not visible.

Another concern for any project in which individuals are to be classified into a predefined group is ensuring that all groups contributing to the adult population (i.e., tributaries contributing sea lamprey to the parasite/adult population) are adequately represented within the baseline samples from which the classification functions are generated (Gillanders 2005; Waldman and Fabrizio 1994; Wood et al. 1987). From this, the data set used in this study will not accurately predict the tributary origin of sea lamprey collected as adults because there are several tributaries missing from this data set (i.e., Lamoille R., Boquet R., Beaver Br.) which might contribute lamprey to the population mixture. The final set of data should include all of these tributaries.

For this data set, the grouping of the tributaries does not appear to clearly affect the ability to discriminate sea lamprey origins. Groupings C, D, and E all have tributaries that were grouped together as geographic groupings. The misclassification rate for Grouping C is the lowest of these three groupings; however, the rates of the other two groupings are not far behind (22.52, 28.48, and 27.15%, respectively; Table 2). Grouping C contains three sets of combined tributaries; the first is the Missisquoi River and its secondary stream Youngman Brook, the second is Mullen Brook, Mill Brook, and Putnam Creek, all of which are in geographic proximity on the New York shore in the south-central basin of the lake and share similar land-use patterns, and the third is the Pike River and its secondary tributary, Morpion Stream in Quebec. There is a strong possibility that the samples collected in the Pike River are actually natal to Morpion Stream, as nearly the entire larval habitat in the Pike River is downstream of the mouth of Morpion Stream, and consequently lamprey moving downstream out of Morpion Stream may have been collected as Pike River samples. The t-test results comparing the elemental concentrations found in Morpion Stream samples to those from the Pike River are somewhat inconclusive. The log10 mean values are significantly different for Zn, Sr, and Ba, but not for Mg, Mn, and Rb (Table 4b). The reasons for these differences are unexplainable at this time.

For microelemental analysis to be useful for management of sea lamprey in Lake Champlain, it is necessary to be able to identify streams that are major contributors to the mixed population of parasites and adults in the lake, and those that do not contribute at all, with a reasonable level of confidence. This level of confidence is not fixed, but depends on the results of the analysis – for example, if the misclassification rate is high but consistent (i.e., misclassified individuals from stream A are always assigned to stream B), then managers can be reasonably confident that control of both streams will encompass the highest contributing streams. Given the range of sample sizes analyzed to date, microelemental analysis appears to successfully discriminate sea lamprey among most tributaries. Currently, the tributaries most likely to be misclassified are the Poultney River and the Missisquoi R./Youngman Br. system. Each of these tributaries had correct classification rates of 50.0%, mostly due to overlap with one another. Malletts Creek also had a low classification rate of 58.3%. These three tributary systems are not near each other geographically (in fact, they represent the southern, northern, and central parts of the lake, respectively), but large portions of their watersheds are used for agricultural purposes. Agricultural land use in each of these three watersheds may contribute to similar hydrological and chemical patterns, due to use of similar crops and fertilizers, and consequently may have similar elemental compositions within their substrates.

With a complete analysis of statoliths representing all of the tributaries in which larval sea lamprey are present in Lake Champlain, this technique may be a powerful tool for identifying sea lamprey origins. This tool promises to become a useful guide for the sea lamprey management program in Lake Champlain by predicting which regions of the lake are contributing significant numbers of sea lamprey to the parasitic and spawning populations in Lake Champlain. Future work includes completion of analysis of the remaining samples and streams, validation of the method using known-origin parasites and adults, and integration of stream assignment data into a life history model of sea lamprey. These data will improve accuracy of the life history model by providing more accurate parameters for survival of lamprey from individual streams; currently, it is only possible to parameterize the model under the assumption that all larval lamprey from every stream have an equal probability of survival to the parasitic and adult stages (Howe et al. unpublished data).

Acknowledgements

This study was funded by the Lake Champlain Basin Program, USFWS, and the Great Lakes Fishery Commission. We thank C. Martin of the USFWS for his assistance in obtaining funding. For their aid in collection of sea lamprey for this project, we thank the USFWS Essex Junction office, NYSDEC, S. Smith, J. Fricke, and A. Zerrenner. We thank C. Martin and W. Bouffard of the USFWS for their help with the logistics of this study in its early stages and for the QAS data in Table 1, and J. Gagnon for his assistance with the ICPMS at GLERL in Windsor, Ontario

Literature Cited

Applegate, V. C. 1950. Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. U.S. Fish and Wildlife Service, Special Scientific Report - Fisheries 55. Washington, D.C.

Bearhop, S., Fiedler, W., Furness, R. W., Votier, S. C., Waldron, S., Newton, J., Bowen, G. J., Berthold, P. and Farnsworth, K. 2005. Assortative mating as a mechanism for rapid evolution of a migratory divide. Science 310(5747):502-504.

Bence, J. R., Bergstedt, R. A., Christie, G. C., Cochran, P. A., Ebener, M. P., Koonce, J. F., Rutter, M. A. and Swink, W. D. 2003. Sea lamprey (*Petromyzon marinus*) parasite-host interactions in the Great Lakes. J. Great Lakes Res. 29 (Suppl. 1):253-282.

Bergstedt, R. A. and Seelye, J. G. 1995. Evidence for lack of homing by sea lampreys. Trans. Am. Fish. Soc. 124:235-239.

Brazner, J. C., Campana, S. E. and Tanner, D. K. 2004a. Habitat fingerprints for Lake Superior coastal wetlands derived from elemental analysis of yellow perch otoliths. Trans. Am. Fish. Soc. 133:692-704.

Brazner, J. C., Campana, S. E., Tanner, D. K. and Schram, S. T. 2004b. Reconstructing habitat use and wetland nursery origin of yellow perch from Lake Superior using otolith elemental analysis. J. Great Lakes Res. 30(4):492-507.

Brothers, E. and Thresher, R. 2004. Statolith chemical analysis as a means of identifying stream origins of lampreys in Lake Huron. Trans. Am. Fish. Soc. 133(5):1107-1116.

Campana, S. E. 2005. Otolith elemental composition as a natural marker of fish stocks. In Stock Identification Methods: Applications in Fishery Science, eds. S. X. Cadrin, K. D. Friedland, and J. R. Waldman. pp. 227-245 Boston, MA, Elsevier Academic Press.

Campana, S. E., Chouinard, G. A., Hanson, J. M., Frechet, A. and Brattey, J. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res. 46(1-3):343-357.

Campana, S. E., Fowler, A. J. and Jones, C. M. 1994. Otolith elemental fingerprinting for stock identification of Atlantic Cod (*Gadus morhua*) using laser-ablation ICP-MS. Can. J. Fish. Aquat. Sci. 51(9):1942-1950.

Campana, S. E. and Thorrold, S. R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. 58(1):30-38.

Campana, S. E., Thorrold, S. R., Jones, C. M., Gunther, D., Tubrett, M., Longerich, H., Jackson, S., Halden, N. M., Kalish, J. M., Piccoli, P., de Pontual, H., Troadec, H., Panfili, J., Secor, D. H., Severin, K. P., Sie, S. H., Thresher, R., Teesdale, W. J. and Campbell, J. L. 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Can. J. Fish. Aquat. Sci. 54(9):2068-2079.

Christie, G. C., Adams, J. V., Steeves, T. B., Slade, J. W., Cuddy, D. W., Fodale, M. F., Young, R. J., Kuc, M. and Jones, M. L. 2003. Selecting Great Lakes streams for lampricide treatment based on larval sea lamprey surveys. J. Great Lakes Res. 29 (Suppl. 1):152-160.

Christie, G. C. and Goddard, C. I. 2003. Sea Lamprey International Symposium (SLIS II): Advances in the integrated management of sea lamprey in the Great Lakes. J. Great Lakes Res. 29 (Suppl. 1):1-14.

Gillanders, B. M. 2005. Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. Estuar. Coast. Shelf Sci. 64(1):47-57.

Gillanders, B. M. and Kingsford, M. J. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Mar. Ecol. Prog. Ser. 141(1-3):13-20.

Henson, M. P., Bergstedt, R. A. and Adams, J. V. 2003. Comparison of spring measures of length, weight, and condition factor for predicting metamorphosis in two populations of sea lamprey (*Petromyzon marinus*) larvae. J. Great Lakes Res. 29 (Suppl. 1):204-213.

Hill, D. R. 1959. Some uses of statistical analysis in classifying races of American shad (*Alosa sapidissima*). Fish. Bull. 59:269-286.

Howe, E. A., Marsden, J. E. and Bouffard, W. in review. Movement of sea lamprey in the Lake Champlain basin. J. Great Lakes Res.

Humphreys, R. L., Campana, S. E. and DeMartini, E. E. 2005. Otolith elemental fingerprints of juvenile Pacific swordfish *Xiphias gladius*. J. Fish Biol. 66(6):1660-1670.

Ludsin, S. A., Fryer, B. J. and Gagnon, J. E. 2006. Comparison of solution-based versus laser ablation inductively coupled plasma mass spectrometry for analysis of larval fish otolith microelemental composition. Trans. Am. Fish. Soc. 135:218-231.

Meeuwig, M. H. and Bayer, J. M. 2005. Morphology and aging precision of statoliths from larvae of Columbia River basin lampreys. N. Am. J. Fish. Manag. 25(1):38-48.

Milligan, B. G., Leebensmack, J. and Strand, A. E. 1994. Conservation genetics - beyond the maintenance of marker diversity. Mol. Ecol. 3(4):423-435.

Rocque, D. A. and Winker, K. 2005. Use of bird collections in contaminant and stable-isotope studies. Auk 122(3):990-994.

Slade, J. W., Adams, J. V., Christie, G. C., Cuddy, D. W., Fodale, M. F., Heinrich, J. W., Quinlan, H. R., Weise, J. W., Weisser, J. W. and Young, R. J. 2003. Techniques and methods for estimating abundance of larval and metamorphosed sea lampreys in Great Lakes tributaries. J. Great Lakes Res. 29 (Suppl. 1):137-151.

Smith, B. R. and Tibbles, J. J. 1980. Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan and Superior: history of invasion and control, 1936-1978. Can. J. Fish. Aquat. Sci. 37:1780-1801.

Swink, W. D. 1991. Host-size selection by parasitic sea lampreys. Trans. Am. Fish. Soc. 120:637-643.

Waldman, J. R. and Fabrizio, M. C. 1994. Problems of stock definition in estimating relative contributions of Atlantic striped bass to the coastal fishery. Trans. Am. Fish. Soc. 123(5):766-778.

Weisser, J. W. 1994. Response of larval sea lamprey (*Petromyzon marinus*) to electric stimulus. M.A. thesis, Northern Michigan University, Marquette, MI.

Wood, C. C., McKinnell, S., Mulligan, T. J. and Fournier, D. A. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. 44:866-881.

Zacherl, D. C., Manriquez, P. H., Paradis, G., Day, R. W., Castilla, J. C., Warner, R. R., Lea, D. W. and Gaines, S. D. 2003. Trace elemental fingerprinting of gastropod statoliths to study larval dispersal trajectories. Mar. Ecol. Prog. Ser. 248:297-303.

Table 1. Tributaries sampled in this study, with most recent QAS survey year and respective larval and transformer estimates (if available), discharge (estimated values are in parentheses), sample date, quantity analyzed to date (total samples collected in parentheses) 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	September mean discharge (m^3/s)[b]	Access (km)[b]	# Samples	Collection date	Collection method
Great Chazy R.	NY	2003	253,101	na	2004	2.46	33.2	10 (30)	Fall 2003	Electrofishing
								7 (40)	Summer 2004	TFM
Saranac R. delta	NY	2003	450,000	na	2003	na	na	15 (30)	Fall 2004	TFM
Salmon R.	NY	2005	62,161	347	2002	0.62	6.4	- (15)	Fall 2002	TFM
Little Ausable R.	NY	2005	164,781	na	2002	0.60	9.8	- (30)	Summer 2002	Electrofishing
Ausable R.	NY	2005	648,532	na	2002	9.60	11.3	- (30)	Fall 2002	TFM
Mullen Br.	NY	2001	1,275	na	none	(<0.300)	na	11	Summer 2004	Electrofishing
Mill Br.	NY	Ns	na	na	none	na	na	13	Summer 2004	Electrofishing
Putnam Cr.	NY	2005	101,906	4,131	2002	0.40	8.4	3 (50)	Fall 2002	Electrofishing
Mt. Hope Br.	NY	2002	53,208	1,468	2003	(0.14 - 0.28)	1.4	10 (30)	Fall 2004	TFM
Poultney R.	VT/NY	2000	107,594	955	1996	2.61	16.9	12	Summer 2004	Electrofishing
Lewis Cr.	VT	2005	59,292	948	2002	1.19	15.3	3 (25)	Summer 2002	Electrofishing
LaPlatte R.	VT	2002	2,502	na	none	0.40	5.3	- (3)	Summer 2005	Electrofishing
Winooski	VT	2002	33,062	na	2004	20.39	17.7	10 (50)	Fall 2004	TFM
Mallets Cr.	VT	2005	4,442	342	none	(0.14 - 0.28)	2.7	12 (30)	Summer 2003	Electrofishing

Table 1. continued

Lamoille R.	VT	2005	38,791	na	none	18.10[c]	na	- (15)	Summer 2005	Electrofishing
Trout Br.	VT	2005	2,253	na	1995	(< 0.28)	2.1	8	Summer 2004	Electrofishing
Missisquoi R.	VT	2004	16,732	16,732	none	18.12	12.9	10 (15)	Summer 2003	Electrofishing
Youngman Br.	VT	2001	7,768	588	none	(0.14 - 0.28)	1.8	4	Fall 2002	Electrofishing
Pike R.	QUE	2000	69,719	8,300	none	2.07	13.2	9 (30)	Summer 2004	Electrofishing
Morpion St.	QUE	2000	39,366	436	none	(0.14 - 0.42)	27.5	14 (30)	Summer 2004	Electrofishing

[a] USFWS, unpublished data (2005)

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

[c] USGS average September stream flow

na - information not available

Table 2. Tributary groupings used for discriminant analysis in this study with misclassification rates for each grouping. Groupings are indicated by same number under column header.

	Grouping					
	A	B	C	D	E	F
Great Chazy R. 2003	1	1	1	1	1	1
Great Chazy R. 2004	2	1	1	1	1	1
Saranac R. delta	3	2	2	2	1	2
Mill Br.	4	3	3	3	2	3
Mullen Br.	5	4	3	3	2	4
Putnam Cr.	6	5	3	3	2	5
Mount Hope Br.	7	6	4	4	3	6
Poultney R.	8	7	5	4	3	7
Lewis Cr.	9	8	6	5	4	8
Mallets Cr.	10	9	7	6	5	9
Winooski R.	11	10	8	7	6	10
Trout Br.	12	11	9	8	7	11
Youngman Br.	13	12	10	9	8	12
Missisquoi R.	14	13	10	9	8	13
Morpion St.	15	14	11	10	9	14
Pike R.	16	15	11	10	9	14
# misclassified (out of 151):	37	36	34	43	41	36
Misclassification rate (%):	24.5	23.8	22.5	28.5	27.2	23.8

Table 3. Coefficient of variation (CV), limit of detection (LOD), and the percentage of samples analyzed that were higher than the LOD. The first six elements met the selection criteria for potential inclusion in the analysis.

		CV	LOD	%>LOD
Magnesium	Mg25	2.080	1.479	100.0
Manganese	Mn55	2.615	12.685	95.8
Zinc	Zn66	6.672	0.481	100.0
Rubidium	Rb85	4.898	0.034	100.0
Strontium	Sr86	4.168	0.918	100.0
Barium	Ba138	2.277	0.047	100.0
Lithium	Li7	5.0	1.167	5.0
Cesium	Ce140	1.1	0.031	70.0
Lead	Pb208	8.3	0.053	90.0
Uranium	U238	2.7	0.017	45.0

Table 4. Results from linear discriminant function and canonical analyses. a) discriminant function analysis summary of importance of the elements for discrimination, b) standardized coefficients for canonical variables and c) means of the canonical variables. Table headings in (a) are Wilk's , a measure of the model discriminatory power with the respective variable in the model (values close to zero indicate high discriminatory power), partial Wilk's , a measure of the unique contribution of the respective variable to the discriminatory power of the model, F-remove, a standard F-value converted from the Wilk's with corresponding p-value, and tolerance, redundancy of the respective variable in terms of its discriminatory power with other variables in the model (values close to zero indicate redundancy).

a)

log10(element)	Wilk's	Partial Wilk's	F-remove	p-value	Tolerance
log10(Rb)	0.066	0.300	31.557	0.000000	0.904
log10(Sr)	0.063	0.314	29.462	0.000000	0.631
log10(Mn)	0.041	0.489	14.093	0.000000	0.866
log10(Zn)	0.031	0.635	7.761	0.000000	0.780
log10(Ba)	0.031	0.651	7.245	0.000000	0.686
log10(Mg)	0.028	0.716	5.345	0.000001	0.820

b)

Element	Root 1	Root 2	Root 3	Root 4
log10(Mg)	0.430	-0.430	0.080	-0.358
log10(Mn)	0.604	-0.211	0.785	0.062
log10(Zn)	-0.556	-0.194	-0.275	0.775
log10(Rb)	-0.899	-0.117	0.471	-0.134
log10(Sr)	0.039	1.228	0.217	0.072
log10(Ba)	-0.134	-0.664	-0.412	-0.448
Eigenvalue	4.943	2.400	0.646	0.289
Cumulative proportion	0.585	0.869	0.946	0.980

c)

Tributary group	Root 1	Root 2	Root 3	Root 4
Great Chazy R.	0.666	-2.495	-0.766	-0.154
Malletts Cr.	1.373	0.555	-0.359	0.386
Mount Hope Br.	-4.448	-0.441	-0.086	0.224
Port Henry area	-2.657	0.847	-0.303	-0.299
Quebec area	0.766	2.007	-0.605	0.183
Missisquoi/Youngman	2.611	0.404	0.990	-0.379
Poultney R.	2.888	0.066	0.141	-0.366
Saranac R. delta	-1.234	-2.206	1.066	0.113
Trout Br.	2.056	-1.517	-0.813	1.276
Winooski R.	-0.293	1.272	1.738	0.430
Lewis Cr.	1.520	-0.937	-0.703	-2.300

Table 5. Test for differences in elemental concentrations of sea lamprey statoliths collected from the Great Chazy River by sample year (2003 & 2004, a) and from the Pike River/ Morpion Stream (b). Significant differences are highlighted in bold.

a)

	Mean concentration (ppm)				
Element	2003	2004	t-value	df	p-value
log10(Mg)	3.70	3.59	2.03	15	0.0610
log10(Mn)	1.69	1.79	-0.89	15	0.3858
log10(Zn)	1.32	1.25	0.42	15	0.6774
log10(Rb)	**0.00**	**0.25**	**-5.11**	**15**	**0.0001**
log10(Sr)	2.54	2.51	0.82	15	0.4260
log10(Ba)	1.40	1.32	0.90	15	0.3805

b)

	Mean concentration (ppm)				
Element	Morpion	Pike	t-value	df	p-value
log10(Mg)	3.625357	3.688944	-1.67983	21	0.107807
log10(Mn)	1.574846	1.696935	-1.82445	21	0.082347
log10(Zn)	**1.156533**	**1.492096**	**-2.37433**	**21**	**0.027192**
log10(Rb)	0.040527	0.036542	0.07719	21	0.939206
log10(Sr)	**3.053475**	**2.949453**	**2.32887**	**21**	**0.029932**
log10(Ba)	**1.441670**	**1.142255**	**4.55241**	**21**	**0.000174**

Table 6. Classification matrix of tributaries in Grouping C using linear discriminant function analysis. Actual streams of origin are in rows, predicted streams of origin are in columns. Correct classifications are highlighted in bold.

Tributary	Percent correct	Chazy	Malletts	Mount Hope	Port Henry area	Quebec	Missisquoi/ Youngman	Poultney	Saranac Delta	Trout	Winooski	Lewis
Great Chazy R.	88.2	**15**	0	0	0	0	0	1	1	0	0	0
Malletts Cr.	58.3	0	**7**	0	0	3	2	0	0	0	0	0
Mount Hope Br.	90.0	0	0	**9**	1	0	0	0	0	0	0	0
Port Henry area	77.8	0	0	2	**21**	0	0	0	3	0	1	0
Quebec area	95.7	0	1	0	0	**22**	0	0	0	0	0	0
Missisquoi/Youngman	50.0	0	1	0	0	1	**7**	4	0	0	1	0
Poultney R.	50.0	0	0	0	0	1	5	**6**	0	0	0	0
Saranac R. delta	93.3	1	0	0	0	0	0	0	**14**	0	0	0
Trout Br.	75.0	1	0	0	0	0	0	1	0	**6**	0	0
Winooski R.	80.0	0	0	0	1	0	1	0	0	0	**8**	0
Lewis Cr.	66.7	1	0	0	0	0	0	0	0	0	0	**2**

List of Figures.

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

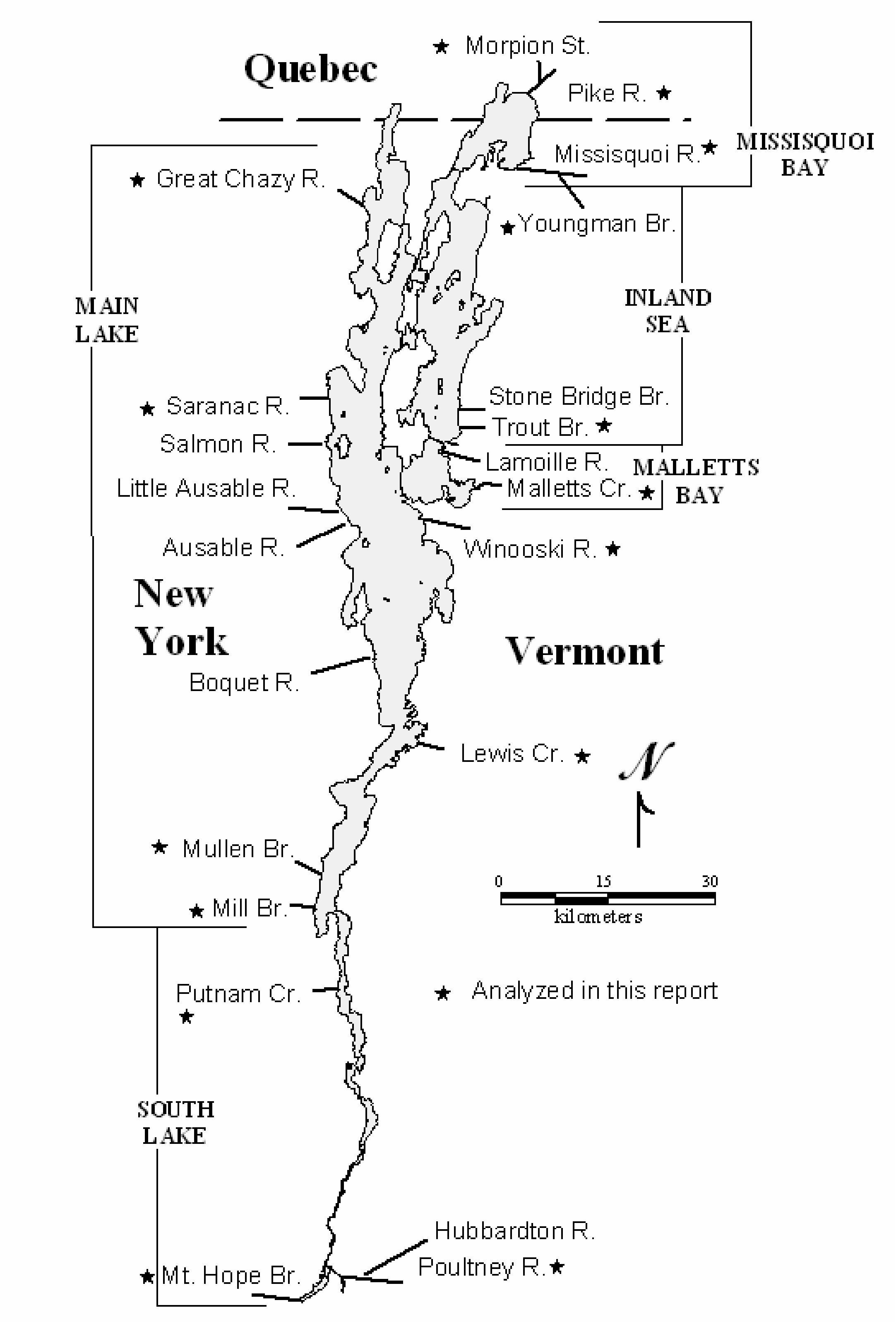
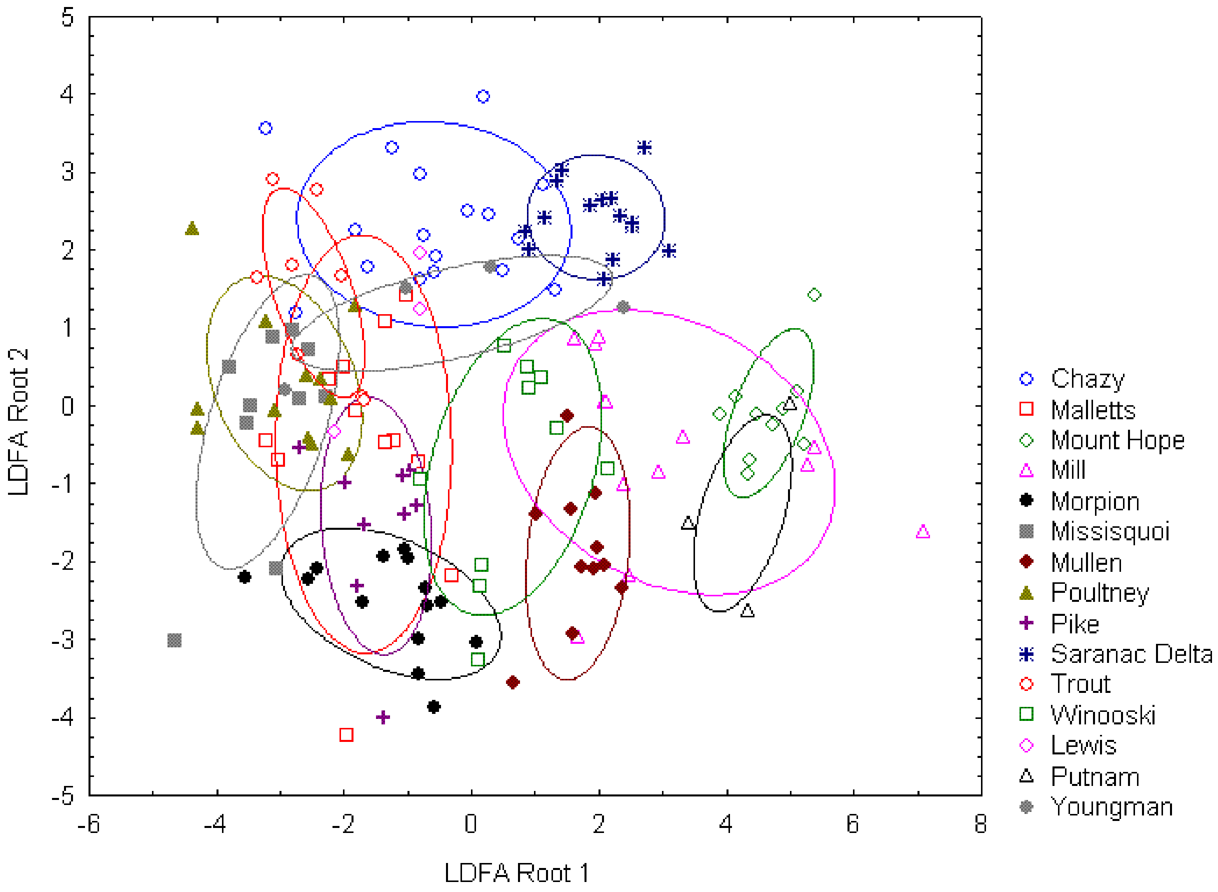
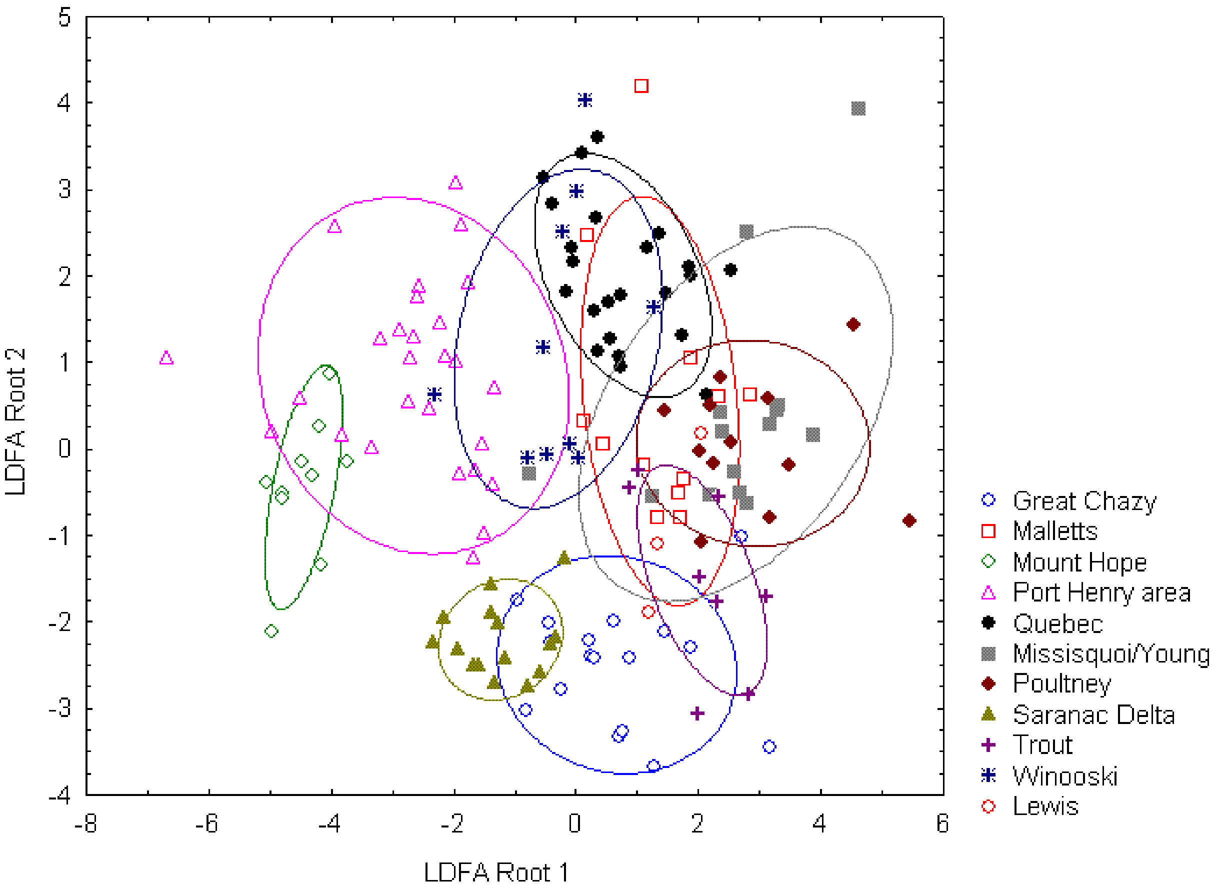


Figure 2. Canonical plots of the five tributary groupings, with 95% confidence ellipses.

2a. Canonical plot of Grouping B.



2b. Canonical plot of Grouping C. Port Henry area = Mill Brook, Mullen Brook and Putnam Creek; Quebec = Pike River and Morpion Stream.



2c. Canonical plot of Grouping F.