

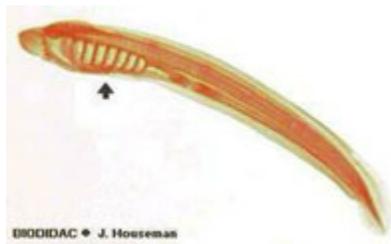
# Micro-Elemental Analysis of Statoliths as a Tool for Tracking Tributary Origins of Sea Lamprey

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## Overview

During 2004, collections of larval, parasitic, and adult Lamprey were made from numerous tributaries of Lake Huron, as well as the open lake, by our partners (US Fish and Wildlife Service and Canadian Department of Fisheries and Oceans). Larvae (n = 15 per stream) from ~30 Lake Huron streams have been processed using laser-ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Our preliminary results, which were presented in a poster at the annual American Fisheries Society meeting in Anchorage, AK, suggest that site discrimination will be possible for many streams, based upon statolith micro-elemental composition, but not for others. Further analysis, however, is needed to determine which sites can be discriminated. Similar larval, parasitic, and adult Sea Lamprey collections were made by the USFWS and DFO during 2005, which will be analyzed during FY06.



## Background

Of the numerous anthropogenic perturbations experienced by the Laurentian Great Lakes during the past century, arguably the most costly, from both ecological and economic standpoints, has been invasion of the Sea Lamprey (*Petromyzon marinus*). By parasitizing large-bodied native fishes, Sea Lampreys have greatly altered the ecology of the Laurentian Great Lakes, as well as other coolwater systems within the surrounding basin (e.g., Lake Champlain). Of primary importance has been the reduction of native salmonines, including Lake Trout (*Salvelinus namaycush*) and Lake Whitefish (*Coregonus clupeaformis*), which underwent dramatic declines during the 1940s and 1950s (Smith and Tibbles 1980). In fact, Sea Lamprey predation played a major role in causing the extirpation of Lake Trout in lakes Ontario, Erie, Huron, and Michigan (Cornelius et al. 1995, Elrod et al. 1995, Eshenroder et al. 1995, Holey et al. 1995).

Large-bodied fishes such as Lake Trout and Lake Whitefish are of vital ecological, economic, and cultural importance in the Great Lakes. As such, the Great Lakes Fishery Commission (GLFC), in partnership with both U.S. and Canadian resource management agencies, has developed an integrated Sea Lamprey management program designed to suppress Sea Lamprey populations in the Great Lakes, and ultimately allow for the rehabilitation and continued protection of these and other important salmonines (GLFC 2001). Critical to the success of this effort is development of a control program that minimizes the costs expended for Sea Lamprey control relative to the economic, ecological, and social benefits associated with protecting fish from Sea Lamprey predation (GLFC 2001). To make progress toward development of a cost-effective control program, a major effort has been undertaken to understand Sea Lamprey life history (physiology, ecology, and behavior), as well as describe the geographical distribution of Sea Lamprey production in the Great Lakes.

Historically, Sea Lamprey control efforts have focused on blocking upstream migration of adults to spawning sites using barriers, and application of lampricides (e.g., TFM, Bayluscide) to spawning tributaries. These chemicals kill Sea Lamprey as larvae, before they metamorphose into parasites (Smith and Tibbles 1980). Overall, the use of barriers and lampricides helped reduce Sea Lamprey populations by as much as 90% in the Great lakes between the 1940s and 1970 (Young et al. 1996). In turn, these efforts, combined with stocking programs and restrictions on harvest, allowed for re-establishment of Lake Trout populations in all of the Great Lakes (Cornelius et al. 1995, Elrod et al. 1995, Eshenroder et al. 1995, Hansen et al. 1995, Holey et al. 1995).

Unfortunately, however, in recent decades Sea Lamprey populations in the Great Lakes have begun to increase, especially in Lake Huron, to levels on par with those before control efforts were established (Eshenroder et al. 1995, Young et al. 1996). Much of this increase in parasitic-phase Sea Lamprey is apparently due to production from the St. Marys River, the connecting corridor between Lake Superior and Lake Huron, where ~66% of all suitable spawning habitat in the Great Lakes exists (Eshenroder et al. 1987, Young et al. 1996). Although habitat rehabilitation in the St. Marys River may have played a role in this increase in Sea Lamprey in the northern basin (Ferreri et al. 1995), the documented increase since the late 1970s is more likely due to enhanced abundance of potential prey (e.g., Lake Trout, bloater chub *Coregonus hoyi*) for recently transformed Sea Lamprey (Young et al. 1996). Owing to the continued presence of Sea Lamprey in the Great Lakes, mortalities due to Sea Lamprey predation remain high in most lakes (Hansen et al. 1995, Holey et al. 1995, Schneider et al. 1996), especially in northern Lake Huron where Sea Lamprey are responsible for a larger proportion of Lake Trout mortality than all other sources of mortality combined (Sitar et al. 1999).

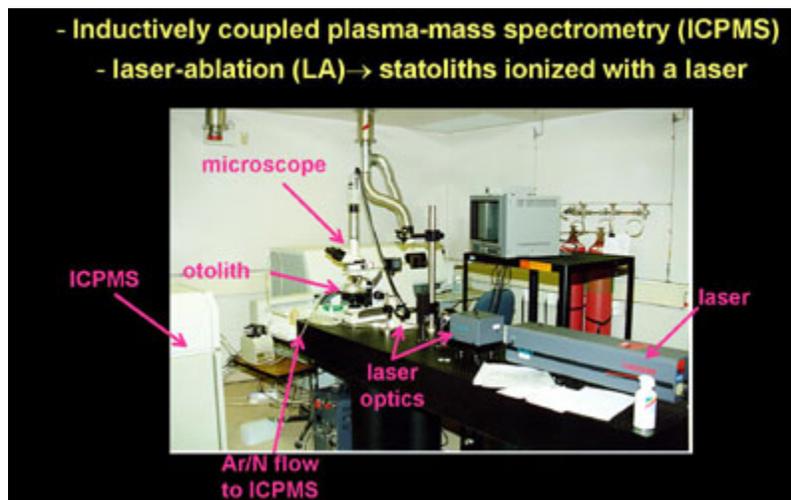
Given the persistence of Sea Lamprey populations in the Great Lakes, continuation of control efforts is essential, which is problematic because of high associated costs (e.g., lampricide applications cost ~\$15 million annually). These economic concerns, as well as concerns about the potential for development of resistance to lampricides, potential effects of lampricides on non-target species, and the negative stigma associated with applying large quantities of a chemical pesticide to tributaries, have prompted the GLFC to seek ways to reduce the use of lampricides (GLFC 2001). Toward this end, efforts have been undertaken to optimize control

strategies so that lampricide treatments (and other more experimental control efforts, such as sterile male release) are used where and when they would be most effective. A significant step forward has been development of the Integrated Management of Sea Lamprey process, which ultimately generates a ranked list of tributaries for lampricide treatment based on cost-effectiveness (i.e., dollars expended to kill each larva). Although numerous tributary-specific variables are considered in these rankings (e.g., amount of lampricide and labor needed for treatment, amount of spawning habitat, estimated economic-injury levels), a major factor determining which spawning locations receive treatment is the estimate of larval Sea Lamprey abundance in each location. Thus, a tributary with a large estimated larval Sea Lamprey population would be ranked as a higher priority for control than a tributary with a small population.

There are two major drawbacks to relying heavily on larval abundance for these prioritizations. First, Quantitative Assessment Survey (QAS) sampling, which is conducted by both the U.S. Fish and Wildlife Service (USFWS) and the Canadian Department of Fisheries and Oceans (DFO) to estimate Sea Lamprey population sizes, is operationally difficult in large rivers (e.g., St. Marys, Spanish, and Mississagi rivers). Consequently, confidence in estimates of larval Sea Lamprey population sizes in large rivers is not great, and hence, an understanding of the potential contributions of parasites from these rivers remains largely unknown (M. Steeves, DFO, pers. comm.). Second, prioritizing tributaries according to larval abundance estimates makes the implicit assumption that the number of transformers (and ultimately future parasitic- and spawning-stage Lamprey) that emerge from a tributary is correlated with larval abundance such that tributaries with a large larval population will contribute more individuals to parasitic populations than those with a small number of larvae. In other words, this approach assumes that survival rates of larvae to the parasitic and adult life stages are equal across all tributaries. Because tributary-specific survival rates are largely unknown, and quite possibly vary among tributaries, owing to habitat differences, this approach seems limited. Realistically, two tributaries with very different larval Sea Lamprey populations could contribute equal numbers of parasites due to factors such as competition (high larval production x high mortality = low larval production x low mortality). Thus, there is a strong need to develop alternative methods to prioritize tributaries for control efforts that are not constrained by assumptions concerning larval survival. Such methods ideally would have the potential to provide a definitive understanding of which spawning locations contribute both parasites and spawners, as well as be cost-effective. Clearly, until a true understanding of the relative contribution of each tributary to the parasitic population is obtained, the GLFC cannot be assured that its control measures are being implemented in the most cost-effective manner possible.

Herein, we propose to continue development of a rather novel technological approach, statolith microchemistry, as a tool to determine which local spawning tributaries contribute to parasitic- and spawning-stage Sea Lamprey populations in Lake Huron. First, we seek to provide a definitive answer as to whether statolith elemental concentrations can be used as a tool to identify sources of Sea Lamprey in Lake Huron, which no study has currently done. Given the geographical scope of our study and technologies available in Fryer's laboratory, we are confident in our ability to do this. Second, assuming that statolith microchemistry holds up to its potential, we will seek to quantify the relative contribution of parasites and spawners from

spawning tributaries important to Great Lakes management agencies, including the St. Marys River. This river is of specific interest because historically it has been the primary source of Sea Lamprey production in the Great Lakes (Young et al. 1996). In addition, a major control effort, consisting of the application of granular Bayluscide, trapping of adult Sea Lamprey, and release of sterile males (GLFC 2001), has recently been undertaken in the St. Marys River. Although preliminary data suggest that the larval population has been reduced by as much as 45% in this river after initial (1999) application of Bayluscide, a true assessment of whether the St. Marys River control effort has changed the relative contribution of adult spawners and parasites in Lake Huron remains unknown. We fully expect that estimation of relative contributions from other Lake Huron tributaries, including difficult-to-sample large rivers (e.g., Spanish and Mississagi; M. Steeves, DFO, pers. comm.), will be possible as well. Finally, taking advantage of the stability of water chemistry in Lake Huron tributaries (see below), we will attempt to quantify relationships between water and statolith chemistry in hopes of eliminating any future need for annual larval sampling from spawning tributaries. Ultimately, this would reduce the expense associated with using this technology for estimation of tributary contributions of Sea Lamprey to the open lake.

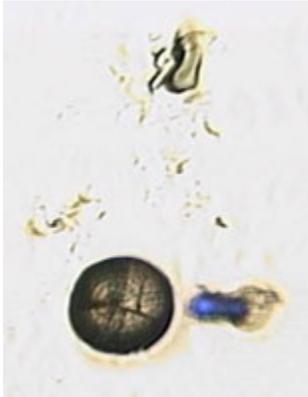


### ***Inductively Coupled Plasma Mass Spectrometer (ICPMS)***

#### **Scientific Rationale**

The analysis of otolith micro-elemental composition has been a valuable tool for differentiating between local spawning populations, and identifying origins of recruits to the fishery. Building on two pilot investigations conducted in lakes Champlain and Huron, we will determine whether trace elements incorporated into Sea Lamprey statoliths during larval stream residence can be used to discriminate among local populations in Lake Huron. Assuming discrimination is possible, we will begin to identify origins of parasites and spawners in Lake Huron, focusing on tributaries (e.g., St. Marys River) important to US and Canadian agencies. We also will develop predictive relationships between water and statolith chemistry, which eventually could help eliminate the need to sample larvae annually to develop stream-specific signatures. By using statolith elemental “signatures” as a natural environmental tag, we aim to provide an alternative

means to identify spawning origins of spawners and parasites, which currently can only be acquired using labor-intensive tagging studies that yield low tag returns. Beyond helping assess whether recent Sea Lamprey control efforts (e.g., St. Marys River) have been successful, an ability to identify natal origins would pave the way for studies aimed at understanding attributes (e.g., stream-specific growth rates, sex ratios, and movement) that promote survival to parasitic and spawning stages, as well as help better target streams for control measures.



## **Accomplishments**

### **Objective 1:**

Determine whether statolith elemental signatures differ among larvae produced in different Lake Huron streams.

### **Larval Sea Lamprey Collections**

To develop characteristic statolith elemental signatures for Lake Huron streams, larvae were collected from numerous Lake Huron tributaries (Figure 1) by the US Fish and Wildlife Service (Marquette and Ludington stations) and the Department of Fisheries and Oceans (Sault Ste. Marie). Similar collections in tributaries surrounding both Lake Michigan and Huron have already begun for 2005. Importantly, these collections will allow us to explore inter-annual

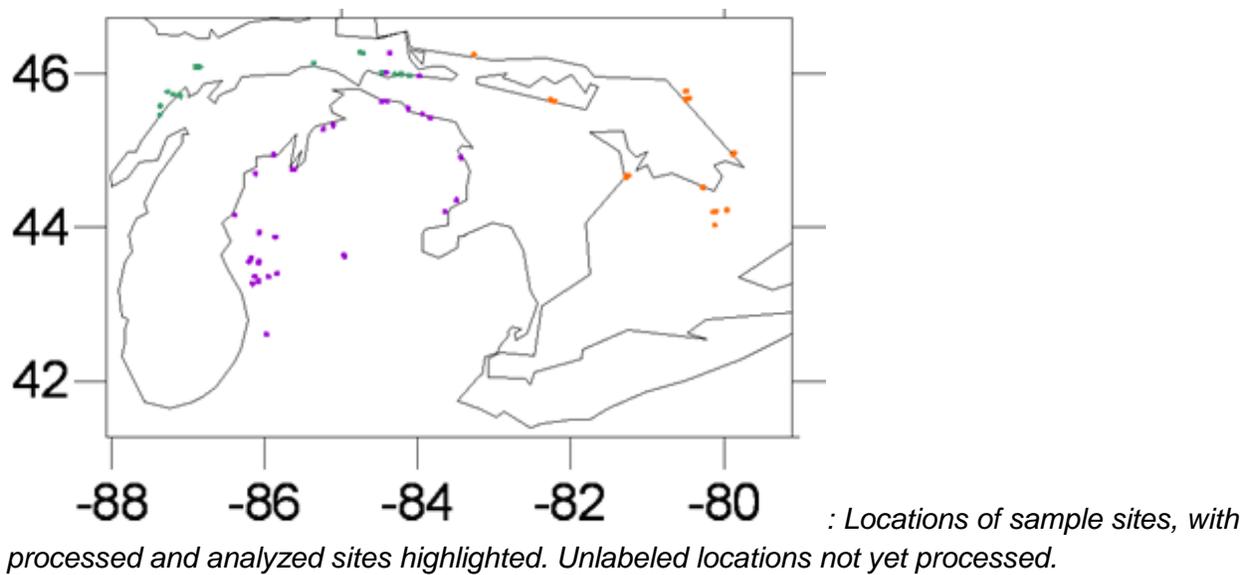
variability in stream signatures, as well as fill in gaps in sampling because not all Lake Huron streams were sampled during 2004.

We currently have analyzed larval Sea Lamprey from 30 streams surrounding Lake Huron and Lake Michigan (Ford River, St. Mary's River, Silver Creek, Mississagi River, Hessel Creek, Tawas River and Trout Creek) ( $n = 15$  per stream). We fully expect that all samples, from both 2004 and 2005, will be processed and analyzed by January 2006.

### **Preliminary Results**

Preliminary results from the seven streams (Figure 1) processed thus far are encouraging. In total, 8 of 13 elements analyzed were above detection limits (i.e., concentrations in statoliths

were sufficiently high to be reliably detected by our ICPMS). More importantly, we found several elements (rubidium, manganese, strontium, and zinc) were valuable for discriminating among streams (Table 1; Figure 2). Using these 8 elements, we could reliably discriminate most streams; 6 of 7 streams could be discriminated (using Linear Discriminant Function Analysis, LDFA) with  $\geq 85\%$  accuracy, whereas the seventh stream (Mississagi River) could only be discriminated from other with 50% accuracy (Table 2). As we increase the number of streams analyzed at any given time, it is likely that other elements will become valuable for discrimination purposes. Further, as we increase sample sizes for each stream-ideal sample sizes will be determined objectively, using a rigorous bootstrapping analysis to look at how sample sizes within streams influence characteristic elemental signatures-we are hopeful that our discrimination abilities will increase (e.g., we will be able to discriminate the Mississagi River from others as well).



### **Objective 2:**

Quantify relative contributions of parasitic and spawning Lamprey from important production tributaries.

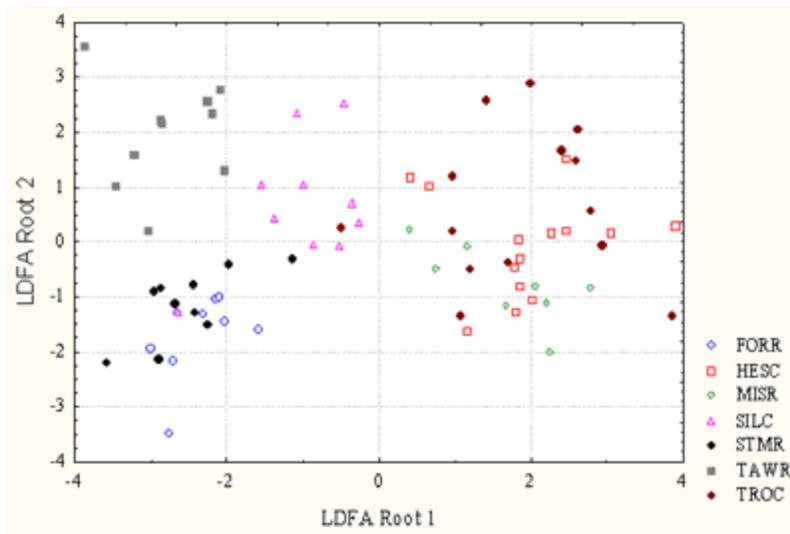
We will address this objective once the preliminary work on the larvae has been completed, since without results from our first objective, we will have no basis for determining the origins of parasites and spawners. However, to this point, we have secured both parasites and spawners from Lake Huron from both commercial fishing operations and agent trapping efforts (the USFWS, DFO, and USGS were instrumental in securing these collections). We also will receive parasites and spawners from Lake Huron, as well as Lake Michigan, during 2005. The Lake Michigan adults and parasites will prove invaluable to (hopeful) future efforts to conduct a similar study in Lake Michigan (per some discussions with Chuck Krueger).

One assumption we are making is that storage method has no effect on elemental composition of statoliths, given that larvae are stored in ethanol and adults/parasites are stored frozen. To explore the effects of storage method on statolith elemental composition, we analyzed larvae ( $n = 14$  stored frozen;  $n = 15$  stored in 95% ethanol) collected from Browns Bayou in the Big Manistee River. Briefly, we found no significant differences between frozen and ethanol preserved fish for magnesium, manganese, zinc, barium, lead or strontium (two-sample t-tests; all  $p > 0.05$ ). We did, however, a significant, difference between storage methods for rubidium. Importantly, however, the difference, although statistically significant, was small (ethanol mean + 1 SD =  $2.10 \pm 0.49$  ppm; ethanol mean + 1 SD =  $2.76 \pm 0.62$  ppm) relative to the variation in rubidium that has been documented across Lake Huron and Lake Michigan streams (range in Rb across the seven streams processed thus far is 2.47 to 7.09 ppm). Thus, we are optimistic that any biases associated with storage method will be negligible. We will further assess the robustness of these results by performing a similar suite of analyses on larvae collected in Lake Champlain this summer.

### **Objective 3:**

Develop relationships to predict statolith elemental chemistry from water chemistry.

This objective will be met first with some preliminary work on Lake Champlain, followed by comparative work on Lake Huron. Water and sediment samples from Lake Champlain will be collected by Carrol Hand during the week of June 13-18, brought back and analyzed at the lab in Windsor. Specifically, Carrol will sample all 22 Sea Lamprey producing streams in Lake Champlain for both water chemistry and sediments. Additional collections in these same streams will be made again during fall 2005 and spring 2006 (after snowmelt) to assess inter-annual variability in signatures. Collections of water chemistry and sediment samples around Lake Huron also will be made during summer 2005, which will supplement collections being made by other agencies (USGS). Ultimately, we will compare our water and sediment chemistry results to Sea Lamprey statolith micro-elemental composition to develop partition coefficients.



**Figure 2:** Canonical analysis of Sea Lamprey larvae (only Roots 1 and 2 presented, which explained a combined 78% of the variation in the data). See Table 1 for an explanation of elemental concentrations, and Figure 1 for an explanation of stream acronyms.

**Table 1:** Correlation of elemental concentrations with Linear Discriminant Function Analysis axes (roots). Elements most highly correlated with each axis are highlighted in gray, and indicate their importance in discrimination. For example, Manganese (Mn) and Rubidium (Rb) were most important for explaining variation along Root 1 (horizontal axis; positive coefficients indicate that Mn and Rb increase from left to right along Root 1; see Figure 2). Root 2 was positively correlated with Strontium (Sr), indicating that individuals at the top of Figure 2 were higher in Sr than those at the bottom (see Figure 2). The percentage of variation that each axis (root) explained is provided in the bottom row.

Element	Root 1	Root 2	Root 3	Root 4
Magnesium (Mg)	0.20	-0.24	-0.33	-0.55
Manganese (Mn)	0.56	-0.22	-0.34	0.16
Zinc (Zn)	-0.07	0.35	-0.54	-0.56
Rubidium (Rb)	0.62	0.19	0.44	-0.58
Strontium (Sr)	0.11	0.73	-0.22	0.27
Tin (Sn)	-0.12	-0.06	0.03	-0.08
Barium (Ba)	0.15	0.12	-0.27	0.12
Lead (Pb)	-0.03	0.00	-0.04	-0.61
<b>Cumulative % variance explained</b>	<b>0.63</b>	<b>0.78</b>	<b>0.91</b>	<b>0.96</b>

**Table 2:** Classification matrix for larvae processed at seven Lake Huron/Michigan sampling locations. Our ability to discriminate among individuals (larvae) from different streams was quite good. With the exception of Mississagi River (MISR) individuals, we could discriminate among streams with > 85% accuracy. In the case of the Mississagi River, individuals were misclassified as Hessel Creek (HESC) individuals in half the cases (5 of 10). Individuals classified correctly are highlighted in gray. See Figure 1 for stream acronym definitions.

	Percent Correct	FORR	HESC	MISR	SILC	STMR	TAWR	TROC	Total
FORR	88.9	8	0	0	0	1	0	0	9
HESC	86.7	0	13	1	0	0	0	1	15
MISR	50.0	0	5	5	0	0	0	0	10
SILC	90.0	1	0	0	9	0	0	0	10
STMR	90.0	1	0	0	0	9	0	0	10
TAWR	90.0	1	0	0	0	0	9	0	10
TROC	93.3	0	0	0	1	0	0	14	15
Total	84.8	11	18	6	10	10	9	15	79

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