GREAT LAKES FISHERY COMMISSION

Research Completion Report[1]

A Feasibility Study to Determine theImmediate Source of Carbon Filtered by *Petromyzon Marinus* Ammocoetes from the Root River, Sault Ste. Marie, Through use of the Stable Carbon Isotope Analysis

by

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Abstract

The feasibility of using stable isotope analysis to determine the immediate source of carbon filtered by *Petromyzon marinus* ammocoetes from the Root River, Sault Ste. Marie was studied using stable carbon and nitrogen isotopes. A variety of samples including sea lamprey ammocoetes, detritus, algae, leaf litter, fine particulate organic matter, and aquatic plants were collected to determine their isotopic compositions. As well, the purpose of the study was to identify any relationships existing between sea lamprey ammocoetes and their potential food sources based on carbon isotope ratios. Samples were collected in August of 1994 from three sites, two of which were situated on the Root River and one of which was situated on Crystal Creek, a tributary of the main Root River. All sites were well-forested, and the range in stream widths was 3.5 to 6 meters.

Site to site differences were observed for the majority of the categories of samples collected. Generally, the most depleted $\delta^{13}C$ values were found to exist at the Crystal Creek tributary site, while the most enriched $\delta^{13}C$ values were observed for the upstream site. Analyses of $\delta^{13}C$ for aquatic algae and terrestrially derived leaf litter showed them to be distinct from one another in all three sites. High variability in $\delta^{13}C$ values for each component of samples collected precluded the use of a quantitative mixing model, and hence the ability to compare between sites and to determine the relative importance of allochthonous and autochthonous materials in the diets of sea lamprey ammocoetes.

Since no significant difference was found to exist between the $\delta^{13}C$ values of detrital and leaf litter samples, detritus samples were therefore thought to be primarily composed of vascular

plant detrital material, as opposed to amorphous biofilm. Similarly, FPOM was composed mainly of allochthonous matter and not dislodged algae being transported downstream.

Sea lamprey ammocoete $\delta^{13}C$ values were unusually enriched relative to their potential food sources, preventing a clear interpretation of relationships existing between sea lamprey ammocoetes and their potential food sources. Three possible reasons for the large enrichment pattern of sea lamprey ammocoetes observed in all 3 sites were suggested. First of all, some physiological aspect specific to sea lamprey may be blurring or altering their isotope signal. Secondly, an unrecognized carbon source may be causing the excessive ^{13}C enrichment observed in the sea lamprey ammocoetes. Finally, the suspected food sources for sea lamprey ammocoetes may not have been appropriately sampled and analyzed in this study.

Stable nitrogen isotope analyses of sea lamprey ammocoetes from the downstream site and the tributary site indicate that they occupy one trophic level above the base of the food chain.

These results indicate that further studies are needed in order to explain the enrichment observed in sea lamprey ammocoete $\delta^{13}C$ values. The enrichment factor must be explained before further stable isotope analyses can be properly used as a means of identifying relationships between the lamprey and their potential food sources.

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Introduction

Since the establishment of the sea lamprey (*Petromyzon marinus*) in the upper Great Lakes, there has been a need to examine and understand its life history. Much attention has been focused on the nonparasitic, stream-dwelling ammocoete stage in order to formulate means of controlling populations throughout the Great Lakes. More specifically, the biology of larval lamprey is being researched in order to understand some of the variance seen in larval survival, growth, and rate of metamorphosis. An understanding of the food chain may lead to an explanation of stream habitat preference, ammocoete densities, larval lamprey growth rates and length of larval life. Young et al. (1990) have examined the relationships between physical/chemical factors and ammocoete presence/absence in streams, ammocoete relative abundance, and growth. Their results suggest that between stream differences in lamprey presence are largely a function of stream-bed particle size, or other variables correlated with particle size and related to habitat. Also, the most significant factors affecting lamprey abundance are stream size, gradient and local geology. Young et al. (1990) identified several factors affecting habitat suitability which is not constant but varies with temperature, pH and food supply. The relation between food supplies and ammocoete presence/absence in streams, larval lamprey relative abundance, and growth have not been examined in past research. All aspects of ammocoete habitat suitability must be examined and considered together so that one can begin to account for the discrepancies and variation observed between streams.

The main purpose of this study is to determine the immediate source of carbon filtered and assimilated by sea lamprey (*Petromyzon marinus*) ammocoetes through the use of stable carbon and nitrogen isotope analysis. Further studies could associate food sources to habitat

preference, stream preference, ammocoete densities, larval lamprey growth rates and duration of growth. Such a study could help biologists to identify stream characteristics which encourage successful colonization by sea lamprey. As well, transect sampling of stable isotopes in sediments, plants and animals, including sea lamprey ammocoetes, may allow scientists to identify gradients and boundaries of biogeochemical processes and cycles that may not otherwise be apparent (Peterson and Fry, 1987). The three objectives of this study were as follows:

1. Collect sea lamprey ammocoetes and other relevant species from three sites on the Root River, Sault Ste. Marie.
2. Determine if the stable carbon isotope ratio is the same in ammocoetes from upstream, downstream and tributary collection sites on the Root River.
3. Examine stable nitrogen isotope measurements for ammocoetes in order to identify the different levels of the food chain involved in ammocoete feeding. Nitrogen analysis of food categories will be done for the downstream site only.

In the past, gut content analysis, observations of feeding, and radioisotopes as tracers have been utilized to identify food resources used by aquatic consumers (Rounick and Winterbourn, 1986). Because of the complex nature of natural food webs and the possibility of multiple contributors of primary carbon sources, these methods do not always provide explicit answers (Rounick and Winterbourn, 1986).

Stable carbon isotope analysis provides an alternative approach to identifying important food resources in aquatic ecosystems. This technique uses differences in the natural abundances

of the stable carbon isotopes ^{13}C and ^{12}C as tracers which move through food chains with small or predictable alteration (Rounick and Winterbourn, 1986). Stable carbon isotope ratios of ^{13}C and ^{12}C are referred to as $\delta^{13}C$ values, and are defined as a parts per thousand or per mil (‰) difference between a sample and a standard reference material. The δ values are measures of the amounts of heavy and light isotopes in a sample (Peterson and Fry, 1987). Samples enriched in the heavy isotope have higher or less negative $\delta^{13}C$ values, whereas samples depleted in the ^{13}C isotope are lighter and have more negative $\delta^{13}C$ values (Fry and Sherr, 1984). Comprehensive overviews of stable isotope theory, methods, and implementation can be found in Fry and Sherr (1984), Peterson and Fry (1987), Rounick and Winterbourn (1986), and Rundel et al. (1989).

Stable carbon isotope analysis provides several advantages over traditional methods. First of all, isotopic measurements provide an independent means of evaluating diet that can supplement stomach content analysis (Fry and Sherr, 1984). Stomach content analyses yield only the materials ingested by an organism over a rather short time interval. By contrast, stable carbon isotope data reflect the materials actually assimilated into an organism. Analyses are performed on body tissues that are built up from the diet over time, hence the food materials are incorporated into the tissue (Fry and Sherr, 1984). Furthermore, stomach content analysis requires considerable sampling in order to account for seasonal changes in diet composition. Biomass carbon effectively summarizes an animal's recent feeding history, and therefore the ratio of ^{13}C to ^{12}C in a consumer's tissue reflects long-term diet (Rounick and Winterbourn, 1986). Finally, traditional methods such as gut content analysis require that too many animals be sacrificed, whereas stable carbon isotope analysis requires fewer dead specimens.

The use of stable isotopes in ecosystem analysis is especially useful in that stable isotope data can be used to clarify food webs through both source information ($\delta^{13}C$) and process information ($\delta^{15}N$) (Peterson and Fry, 1987). Stable carbon isotope food web studies are based on the presumption that animals have carbon isotopic values similar to their diets, within approximately $\pm 2‰$ (Fry and Sherr, 1984). $\delta^{13}C$ values seem to show modest increases between 0.0 and 1.0‰ per trophic level, the small enrichment probably a result of carbon isotopic fractionation during assimilation or respiration (Peterson and Fry, 1987). In order to use carbon isotope ratios obtained for sea lamprey as a means of identifying dietary intakes, several assumptions must be made (Jackson and Harkness, 1987). First of all, one must assume that the food sources of interest have different $\delta^{13}C$ values than other potential food sources. Second, one must assume that $\delta^{13}C$ values do not change drastically with time, or due to external environmental factors which may also change with time. Third, that the $\delta^{13}C$ values obtained for sea lamprey within any given area can be considered as representative, with little spatial variation. Fourth, that the $^{13}C/^{12}C$ ratio of sea lamprey tissues accurately reflects the mean ratio of the carbon assimilated from all food sources. Finally, that the sea lamprey diet does not change more rapidly than can be detected by analysis of their carbon isotope ratios.

Stable nitrogen isotopes in ecosystem analysis can be used to clarify food webs through process information, in that $\delta^{15}N$ values provide an estimate of trophic level (Fry, 1991; Peterson and Fry, 1987). Nitrogen isotopic values increase by 10 to 15‰ in most food webs, indicative of the presence of 3 to 5 consecutive trophic transfers. Each transfer boosts the ^{15}N content by 3 to 5‰ (Peterson and Fry, 1987) due to the loss of ^{14}N by animals during nitrogen metabolism (Gu et al., 1994). Stable nitrogen isotope ratios of ^{15}N and ^{14}N are referred to as $\delta^{15}N$ values,

and are also defined as a parts per thousand or per mil (‰) difference between a sample and a standard reference material. As with stable carbon isotope ratios, the δ nitrogen values are measures of the amounts of heavy and light isotopes in a sample (Peterson and Fry, 1987). Stable nitrogen isotopes are therefore used under the assumption that each level in the food chain has a distinct ^{15}N value (Gu et al., 1994; Fry, 1991; Peterson and Fry, 1987).

In studies such as this, the combined result of using two or more stable isotope tracers will allow for the identification of important primary food sources, and will facilitate identification of pathways through which food energy is transferred throughout the food web.

Ammocoete Feeding

Larval lampreys are suspension/filter feeders which inhabit burrows in soft sediments of streams (Moore and Mallatt, 1980; Bowen, 1992). Since ammocoetes are filter feeders, much of their food is suspended material. It has been shown that their food is drawn from the water immediately above the substrate where there are high densities of microorganisms (Moore and Mallatt, 1980). Since lamprey cannot always consume suspended food, for example when closed off in their burrows, food may be drawn from the water, or from the water and sediments depending on the surrounding environmental conditions and the activity patterns of the ammocoete.

Larval lampreys feed by trapping small, water-borne particles in mucus within the pharynx (Mallatt, 1981). A unidirectional respiratory current transports particulate material through a ring of coarse oral cirri into the pharynx (Bowen, 1992). The cirri act as a sieve to prevent larger particles (> 340 mm) and algal filaments from entering the pharyngeal chamber.

These particles are periodically expelled as they accumulate on the cirri. Particles which pass through the cirri are incorporated into a mucous complex in the pharynx and aggregate as they are passed by cilia to the intestine (Mallatt, 1981). Therefore, the mucous complex serves several functions in trapping, aggregating, and transporting food particles. (Bowen, 1992).

The diet of larval lamprey has been reported to consist largely of algae (primarily diatoms), organic detritus, and bacteria (Bowen, 1992). Although these components of the diet vary according to season, amorphous organic detritus has been shown to be the principle component of the diet and probably most important to the nutrition of the ammocoetes (Bowen, 1992). This was evident in the study done by Bowen (1992) where organic detritus averaged 97.75% of diet AFDW (ash-free-dry-weight), with algae making up 2.15% and bacteria limited to 0.10%. Other suggested food sources include benthic organisms to a varying degree (Moore and Mallatt, 1980). Although the importance of these various food items is not explicitly known, it was found that there was some selective feeding based on particle size of different species of algae (Moore and Mallatt, 1980). In general, the size distribution of ingested particles is independent of the size of the larval lamprey (Moore and Mallatt, 1980).

It is not known if there is an optimal temperature for food intake in ammocoetes, although Moore and Mallatt (1980) reported that the feeding rates of ammocoetes in northern temperate zone streams vary seasonally and, to a lesser extent, with food density. Ammocoetes consume more as food abundance increases except at high concentrations of food. In this they are similar to suspensions feeders. In contrast to other suspension feeders, the filtering rates of ammocoetes do not begin to decline until food concentrations are much higher than 10 mg/L. The metabolic rate of ammocoetes is low which results in a low filtration rate. This implies that

the water filtered must provide a relatively high concentration of food to allow for growth (Moore and Mallat, 1980).

Finally and perhaps most important to this study, ammocoetes, like most aquatic herbivores, digest only part of the food in their guts. This inefficient utilization of food may be attributable to the thick cellulose walls that many species of algae possess, and due to the ash, cellulose, and lignin of detritus, all of which are not easily broken down by digestive enzymes (Moore and Mallatt, 1980; Bowen, 1992). Therefore, it is important to test the feasibility of using stable carbon and nitrogen analyses to determine what food sources have been assimilated into the bodies of sea lamprey ammocoetes rather than simply ingested.

Methods

Sampling sites

Three sites on the Root River located in Sault Ste. Marie Ontario were chosen: upstream on the main river (UP - Site 1); downstream on the main river (DN - Site 2); and on Crystal Creek, a tributary of the Root River (TRI - Site 3) (see Figure 1). All sites were chosen primarily based on three criteria: the accessibility of the site; that suitable ammocoete habitat was found within 200 meters above or below the site; and that the site fulfilled one of the requirements of being either located upstream or downstream on the main, or on one of the tributaries. The upstream and downstream sites are approximately 10 km apart. The upstream site was approximately 3.5m in width at the sampling location, whereas the downstream site was 6m in width, and the tributary site was 4.5m in width.

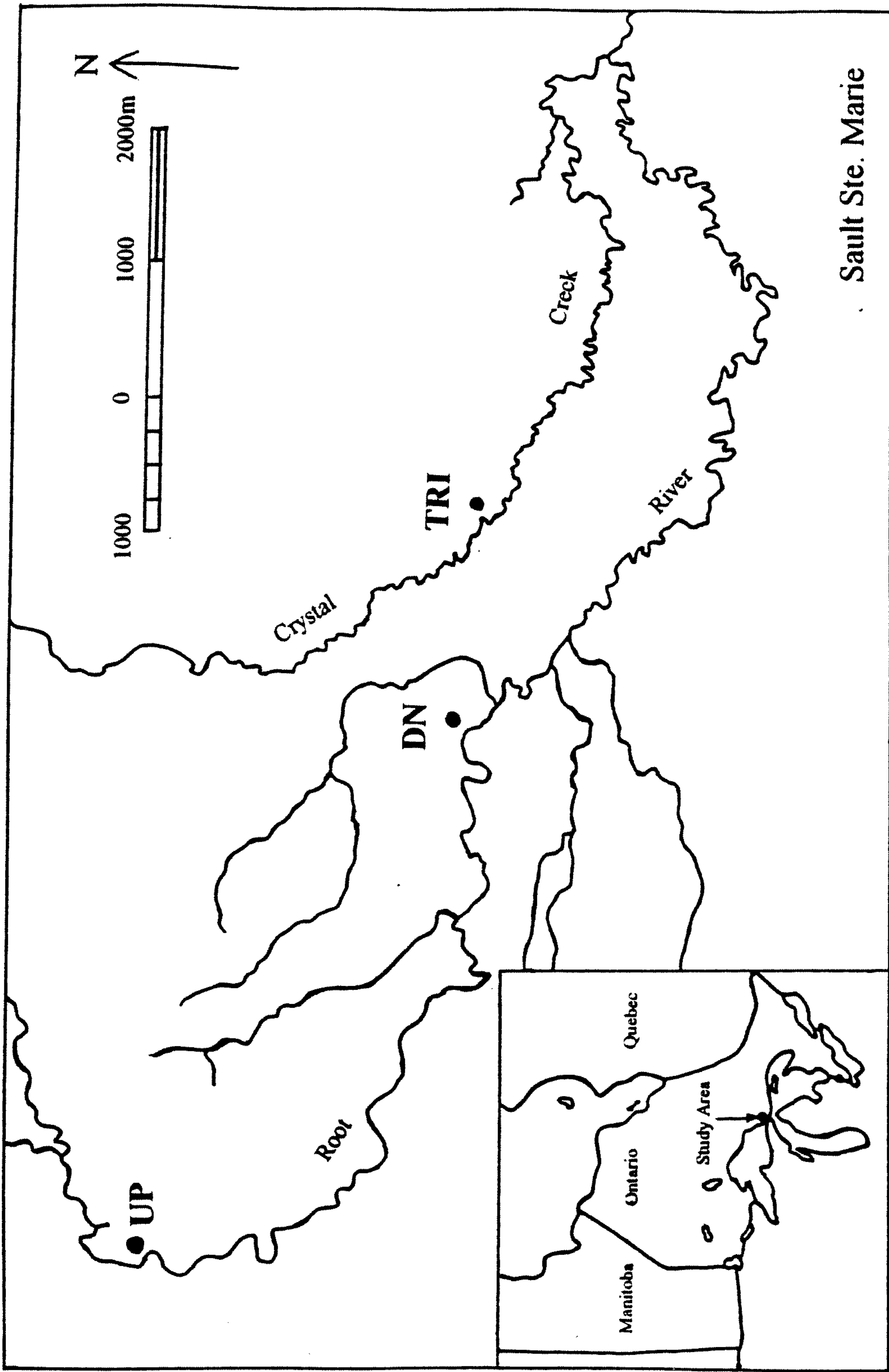


Figure 1. Map of the Root River Study Area showing the position of the 3 study sites.

All of the three sites were forested with some degree of canopy cover. Tree cover surrounding the three sites is representative of the Great Lakes-St. Lawrence Forest Region (Hosie, 1979), characterized by Red Pine (*Pinus resinosa* Ait.), Red Maple (*Acer rubrum* L.), Striped Maple (*Acer pensylvanicum* L.), and White Elm (*Ulmus americana* L.). Other prevalent species include Eastern White Cedar (*Thuja occidentalis* L.), White Spruce (*Picea glauca* (Moench) Voss), Black Spruce (*Picea mariana* (Mill.) B.S.P.), Balsam Poplar (*Populus balsamifera* L.), White Birch (*Betula papyrifera* Marsh.), and Speckled Alder (*Alnus rugosa* (Du Roi) Spreng).

All of the samples were collected in mid-August, and all samples for each site were collected at the same time on the same day in August.

Sampling Methods

At each of the three sites, five or six categories of specimens were collected. Larval sea lamprey were collected using battery operated backpack DC electrofishing gear. The samples of sea lamprey ammocoetes (SLA) were placed in plastic bags and immediately frozen. SLAs were identified to species in the laboratory based on methods described in Auer (1982) and Vladykov and Kott (1980). Fine Particulate Organic Matter (FPOM) was collected from the water column onto pre-combusted glass micro-fibre filters (Whatman GF/C, pore size = 0.45 mm) by filtering water collected in a 20 liter Nalgene jug. Positive pressure created by a hand-held bicycle pump was used to force water through the filtering apparatus. The sample filters from each site were wrapped in aluminum foil, placed in plastic bags and frozen. Detritus from the stream bed was collected in a fine mesh net when rocks were moved and/or their surfaces

scraped. The samples were then placed in plastic bags and frozen. Samples of submerged aquatic macrophytes and epilithic algae were either hand picked from the stream bottom or scraped from the surface of rocks. The samples were then placed in plastic bags and frozen. Decomposing leaf litter from the stream bottom was gathered by hand, placed in plastic bags and frozen. As well, fresh leaves were also collected for comparison.

Methodology for the preparation of samples for stable carbon and nitrogen isotope analysis

For stable carbon isotope analysis, all samples must be free of carbonates. As a result, all samples were acidified with 10% HCl to remove carbonates and washed with distilled water (Heemskerk and Diebolt, 1994), prior to being freeze-dried under vacuum at < -50°C for at least 12 hours depending on the moisture content of the sample. After drying, samples were contained in sealed plastic bags until isotope analysis could be done. Prior to analysis, samples were finely ground to a homogeneous state.

Invertebrates inadvertently collected with the samples were removed before analysis. Any obvious large woody debris, algae, or leaf litter was extracted from the detritus samples. Algal samples should be free of all terrestrial detritus to produce accurate algal isotopic signatures, although the ability to separate between algae and detritus was considered to be difficult by Junger and Planas. (1994). The inability to remove terrestrially-derived detritus from an algal sample might cause the sample $\delta^{13}C$ value to be more or less depleted than the true value (Doucett, 1994).

In the isotope analysis, several whole sea lamprey ammocoetes from each site were ground up and prepared. Since carbon acts to integrate dietary input, DeNiro and Epstein

(1978) have identified the use of whole animals for the analysis of carbon as the method which allows for the most accurate estimate of the $\delta^{13}C$ value of the diet. By contrast, suborganismic components provide a less accurate estimate of the $\delta^{13}C$ value of the diet since these values reflect various sources and isotopic fractionations during assimilation (Tieszen et al., 1983). Furthermore, the analysis of several lamprey collected at one locality can further improve the accuracy of the estimated $\delta^{13}C$ values by reducing any variation among individuals (DeNiro and Epstein, 1978). In this study, several SLAs per site were ground up together to be analyzed. Although pooling the carbon of SLA gives no information about the variance of isotope ratio between individuals, it does show the average isotopic composition of the lamprey collected at the each site at a given time, and therefore their average carbon dependence (Rosenfeld and Roff, 1992). One limitation of the study is that guts of the lamprey were not removed before analysis, and as a result food in their guts may have represented contamination (DeNiro and Epstein, 1978). In order to make a comprehensive analysis of the primary source of carbon filtered by sea lamprey ammocoetes, it has been suggested by Jackson and Harkness (1987) that single collection samples may not provide an adequate basis for obtaining and interpreting carbon isotope ratios for any previously unstudied taxa or sites due to seasonal variation in $\delta^{13}C$ values. Therefore, within-site spatial and seasonal variation in $\delta^{13}C$ values should also be investigated.

Methodology for carbon isotope analysis

For stable carbon isotope analysis of organic samples, the Breakseal Combustion Method in Pyrex glass tubes was used following the method described by Heemskerk and Diebolt (1994). Generally, this involves placing approximately 10mg of each of the organic samples in pre-

combusted tubes with pre-treated cupric oxide and pre-treated silver wire. The tubes and their contents are then sealed under vacuum and combusted to convert organic carbon to CO_2. Breakseals are then cracked open on a standard vacuum line (following Technical Procedure 22.0, Environmental Isotope Laboratory (EIL), Department of Earth Sciences, University of Waterloo). Purified CO_2 is cryogenically separated from water vapor and other non-condensable gases. The purified CO_2 samples are then analyzed on a Micromass 903E triple collector ratio mass spectrometer or a VG Prism Series II. Stable carbon isotope ratios of ^{13}C to ^{12}C are expressed as $\delta^{13}C$ and are defined as a parts per thousand difference from a standard reference material where:

$$\delta^{13}C = \frac{(^{13}C/^{12}C_{sample}) - (^{13}C/^{12}C_{standard}) \ x \ 1000}{(^{13}C/^{12}C_{standard})}$$

The primary standard used for reporting $\delta^{13}C$ values is PDB, a marine limestone, however the supply of this reference material has been exhausted (Fry and Sherr, 1984). Presently, measurements are made relative to secondary standards which have been referenced to the PBD isotopic composition. Most biological materials are depleted in ^{13}C relative to PBD, and therefore have negative $\delta^{13}C$ values. Specifically, EIL uses tank gases for working standards in the mass spectrometers, which are calibrated using the international reference materials available. Analytical precision of replicate samples in the EIL are ± 0.2‰ (working limits) and ± 0.3‰ (control limits).

Methodology for nitrogen isotope analysis

Nitrogen gas was isolated from the samples for measurement of $\delta^{15}N$ by mass spectrometry, using the procedure described by Flatt and Heemskerk (1994). The process of stable nitrogen isotope analysis involves a breakseal combustion method, similar to that used in stable carbon isotope analysis. Samples are sealed in a tube under vacuum and combusted in a breakseal at 850 °C, using copper metal granules, copper oxide for the reduction of the nitrogen, and calcium oxide for the removal of CO_2 and water by absorption. The purified N_2 which remains in the combusted tubes is then injected directly on a 602D mass spectrometer using a tube cracker. Reference gas used is ultra high purity (UHP) nitrogen gas. $^{15}N/^{14}N$ ratios relative to the international standards atmospheric nitrogen are calculated: $\delta^{15}N$ = 0.0‰, where:

$$\delta^{15}N = \frac{(^{15}N/^{14}N_{sample}) - (^{15}N/^{14}N_{standard}) \ x \ 1000}{(^{15}N/^{14}N_{standard})}$$

Once again, analytical precision of replicate samples in the EIL are ± 0.2‰ (working limits) and ± 0.3‰ (control limits).

Statistical Analysis

To test the hypothesis that there were no differences in $\delta^{13}C$ values among categories of samples collected within sites, isotope ratios were analyzed per site using either a one-way analysis of variance or the nonparametric Kruskal-Wallis one-way analysis of variance (ANOVA). Unless otherwise specified, all tests were done and interpreted at an alpha level of

0.05. Due to the fact that the $\delta^{13}C$ values recorded for the upstream site violated the main assumptions of the ANOVA, the log, square-root, and Box-Cox transformations were applied to the data. None of the transformations were successful and therefore the nonparametric Kruskal-Wallis one-way ANOVA was used to test the hypothesis that there were no differences in $\delta^{13}C$ values among categories of samples collected within the upstream site (site 1). The result of this test should be interpreted with care. Multiple comparisons were performed as described by Zar (1974, pp.156).

To test the hypothesis that there were no differences in $\delta^{13}C$ values among categories of samples collected within the downstream site (site 2), isotope ratios were analyzed by a one-way ANOVA. The residuals of the ANOVA were tested for homogeneity of variance using the Levene's test ($F_{4,31} = 2.589$, $p = 0.056$), and as well were tested for normality by visual inspection of a normal-quantile plot. There was insufficient evidence to reject the null hypotheses that the variances of the residuals were homogeneous and that they were normally distributed. Following significant probabilities in the overall ANOVA, planned non-orthogonal contrasts were performed. Since these contrasts were non-orthogonal, the Dunn-Šidák approach was used to reduce the maximal probability of a type-1 error for each contrast so that, after all contrasts have been made, the overall experiment-wise error rate was still at the pre-specified alpha level of 0.05. Therefore a' = 0.0085, and a contrast must have p £ 0.0085 before the null hypothesis can be rejected.

To test the hypothesis that there were no differences in $\delta^{13}C$ values among categories of samples collected within the tributary site (site 3), isotope ratios were analyzed by a one-way ANOVA. Prior to the ANOVA, the data were transformed by a Box-Cox transformation. The

residuals of the ANOVA were tested for homogeneity of variance using the Levene's test ($F_{4,9}$ = 1.953, p = 0.186), and as well were tested for normality by visual inspection of a normal-quantile plot. There was insufficient evidence to reject the null hypotheses that the variances of the residuals were homogeneous and that they were normally distributed. Following significant probabilities in the overall ANOVA, planned non-orthogonal contrasts were performed. Since these contrasts were non-orthogonal, the Dunn-Šidák approach was used to compute $\alpha' = 0.0085$. Therefore once again, a contrast must have $p \leq 0.0085$ before the null hypothesis can be rejected.

All statistical analyses were performed using SYSTAT ver. 5.04 (Wilkinson, 1992). The Box-Cox transformation was performed by the BIOM program BASTAT.

Results

$\delta^{13}C$ Values

Site 1 (upstream site)

The hypothesis that there were no differences in $\delta^{13}C$ values among categories of samples collected within the upstream site was tested by a nonparametric one-way Kruskal-Wallis ANOVA. Significant differences amongst food categories exist within this site (H = 14.68, p = 0.012) (Table 1). Multiple comparisons showed that (the rank sums from the Kruskal-Wallis test are arranged in increasing order of magnitude):

Leaf Litter FPOM Algae Detritus SLA Aquatic Plant

where categories underscored by the same line were not significantly different at a = 0.05.

Due to the very small sample sizes and the fact that assumptions of the ANOVA model were violated by this data, interpretations of the data for this site should be made with caution.

Table 1. Stable carbon isotope ratios ($\delta^{13}C$) of the six categories of samples collected at Site 1, the upstream site. Included are the means and the standard errors (SEM). The statistical results are from a nonparametric one-way Kruskal-Wallis ANOVA and are significant at $p < 0.05$.

Site 1	$\delta^{13}C$ (‰)					
	SLA	Detritus	Algae	Leaf Litter	FPOM	Aquatic Plant
Mean	-20.411	-24.430	-23.829	-29.867	-26.456	-23.216
SEM	0.096	0.451	0.179	0.015	1.408	0.039
n	3	3	2	2	2	5

H-statistic = 14.68

$p = 0.012$

Site 2 (downstream site)

The hypothesis that there were no differences in $\delta^{13}C$ values among categories of samples collected within the downstream site was tested by a one-way ANOVA. Significant differences amongst categories exist within this site ($F = 205.015$, $p < 0.0001$) (Table 2). To identify

differences between categories of samples, non-orthogonal contrasts were carried out. As a result, the Dunn-Šidák approach was used to compute $\alpha' = 0.0085$. Therefore, a contrast must have $p \leq 0.0085$ before the null hypothesis can be rejected. No significant difference was not found to exist between SLA and algae ($F = 4.962$, $p = 0.033$), suggesting that SLA are feeding on algae. As well, no significant difference was found to exist between detritus and leaf litter ($F = 1.390$, $p = 0.247$), indicating the importance of allochthonous inputs to detritus. Significant differences were found to exist between SLA and detritus ($F = 529.196$, $p < 0.0001$) and between SLA and FPOM ($F = 129.223$, $p < 0.0001$), implying that detritus and FPOM are not primary food sources for the SLAs. As well, significant differences were found between algae and leaf litter ($F = 282.173$, $p < 0.0001$) depicting a difference between allochthonous and autochthonous components of the food web. Finally, significant differences were found between FPOM and Algae ($F = 80.609$, $p < 0.0001$).

Table 2. Stable carbon isotope ratios ($\delta^{13}C$) of the five categories of samples collected at Site 2, the downstream site. Included are the means and the standard errors (SEM). The statistical results are from a one-way ANOVA and are significant at $p < 0.05$.

Site 2	$\delta^{13}C$ (‰)				
	SLA	Detritus	Algae	Leaf Litter	FPOM
Mean	-21.943	-26.844	-22.466	-27.152	-25.147
SEM	0.159	0.157	0.230	0.072	0.134
n	10	10	7	5	4

F-statistic = 205.015

p = < 0.0001

Site 3 (Crystal Creek Tributary Site)

The hypothesis that there were no differences in $\delta^{13}C$ values among categories of samples collected within the tributary site was tested by a one-way ANOVA. Significant differences amongst categories exist within this site ($F = 33.825$, $p < 0.0001$) (Table 3). Once again, to identify differences between categories of samples, non-orthogonal contrasts were carried out. As a result, the Dunn-Šidák approach was used to compute $\alpha' = 0.0085$. Therefore, a contrast must have $p \leq 0.0085$ before the null hypothesis can be rejected. There was no significant difference between detritus and leaf litter ($F = 0.584$, $p = 0.464$), once again indicating the importance of allochthonous inputs to detritus. Significant differences were found to exist between SLA and detritus ($F = 54.755$, $p < 0.0001$) and between SLA and algae ($F = 115.973$, $p < 0.0001$), implying that both detritus and algae, the two most important known food sources of SLA, were not detected as food sources. As well, significant differences were

found between algae and leaf litter ($F = 11.778$, $p = 0.007$), depicting a difference between allochthonous and autochthonous components of the food web. Finally, significant differences were found between SLA and FPOM ($F = 13.093$, $p = 0.006$) and between FPOM and Algae ($F = 38.348$, $p < 0.0001$).

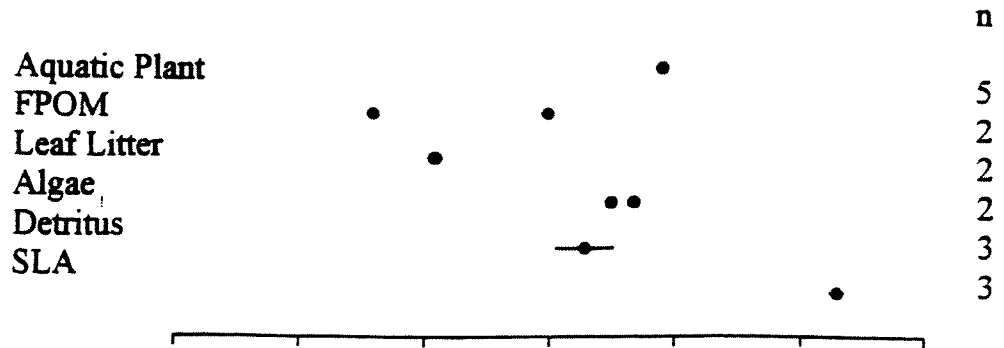
Table 3. Stable carbon isotope ratios ($\delta^{13}C$) of the five categories of samples collected at Site 3, the tributary site on Crystal Creek. Included are the means and the standard errors (SEM). The statistical results are from a one-way ANOVA and are significant at $p < 0.05$.

SITE 3			$\delta^{13}C$ (‰)		
	SLA	DETRITUS	ALGAE	LEAF LITTER	FPOM
Mean	-23.004	-27.397	-30.022	-27.859	-25.821
SEM	0.675	0.237	0.258	0.299	0.651
n	4	4	2	2	2

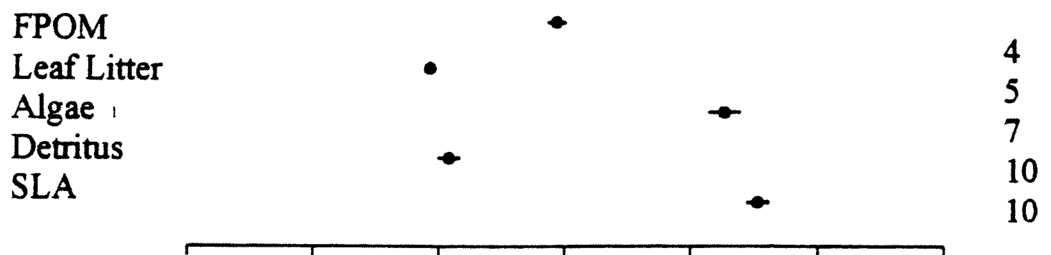
F-statistic = 33.825

p = < 0.0001

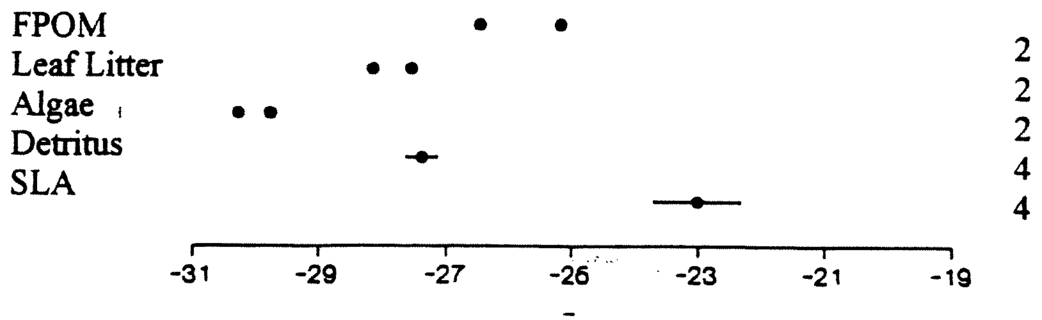
Root River
Upstream Site



Downstream Site



Crystal Creek
Tributary Site



$\delta^{13}C$ Values (‰)

Figure 2. The mean $\delta^{13}C$ values (‰) for each of the categories of samples collected at each of the 3 sites. The sample sizes (n) of each category are included on the right of the graphs. Standard error bars are included for values where n > 2. SLA = sea lamprey ammocoetes.

The mean $\delta^{13}C$ values for each of the categories of samples collected for each site are depicted in Figure 2. High variability in $\delta^{13}C$ values amongst the categories of samples collected precluded the use of a quantitative mixing model, and hence the ability to compare between sites and to determine the relative importance of allochthonous and autochthonous materials in the diets of sea lamprey ammocoetes. However, several trends depicted in Figure 2 can be observed and should be noted. First of all, the mean $\delta^{13}C$ values for the SLAs are similar in all sites, ranging from -23.004 in the tributary site to -20.411 in the upstream site. The carbon ratios for detritus vary slightly, but again are quite similar from site to site with means ranging from -27.397 in the tributary site to -24.430 in the upstream site. By contrast, mean algae $\delta^{13}C$ values vary widely from -30.022 in the tributary site to -22.466 in the downstream site. The mean leaf litter $\delta^{13}C$ values are similar between sites ranging from -29.867 in the upstream site to -27.859 in the tributary site. Finally, the mean $\delta^{13}C$ values for FPOM range from -26.456 in the upstream site to -25.147 in the downstream site. With the exception of FPOM, all categories collected for isotope analysis from the tributary site were more depleted in ^{13}C than the same categories in the other two sites. As well, with the exceptions of FPOM and algae, all categories collected for isotope analysis from the upstream site are more enriched in ^{13}C, then are the same categories in the other two sites.

$\delta^{15}N$ Values

$\delta^{15}N$ values were difficult to obtain for most of the categories sampled in this study due to the amount of sample required to obtain sufficient nitrogen. In Table 4, the raw $\delta^{15}N$ values are presented for the categories for which it was possible to compute $\delta^{15}N$ values. The

decomposing leaf litter $\delta^{15}N$ value is near to that of atmospheric N_2 (≈ 0‰) at 0.63. The SLA $\delta^{15}N$ values for both the tributary and downstream sites are fairly similar, with values ranging from 2.72 at the tributary site to 3.45 at the downstream site. With increasing trophic level, $\delta^{15}N$ enrichment has been reported in the area of +2 to 4 per mil. This enrichment in ^{15}N results from the preferential loss of ^{14}N by animals during nitrogen metabolism (Gu et al. 1994). The low $\delta^{15}N$ values for SLA might imply a diet which includes autotrophic matter or material at the base of the food chain.

Table 4. Stable nitrogen isotope ratios ($\delta^{15}N$) of samples of sea lamprey ammocoetes (SLA) and leaf litter collected from the tributary site (3) and the downstream site (2).

SITE AND COMPONENT	$\delta^{15}N$ VALUE (‰)
Site 3 SLA	3.04
Site 3 SLA	2.72
Site 2 SLA	3.45
Site 2 Leaf Litter	0.63

Discussion

Carbon Sources

The observed $\delta^{13}C$ values for leaf litter in all three sites were fairly close to the average of -28‰ expected for terrestrial C_3 plants (Rosenfeld and Roff,1992), in that the mean $\delta^{13}C$ values for site 1, 2, and 3 were -29.87, -27.15, and -27.86‰ respectively. Many species of terrestrial C_3, or Calvin pathway plants, have similar carbon isotope ratios since they make use of the same photosynthetic pathway and the same carbon source, being atmospheric CO_2 which is approximately constant at -7‰ (Rounick and Winterbourn, 1986). Although the $\delta^{13}C$ values for leaf litter are reasonably similar between sites, variability does exist since a mix of leaves collected from the stream floor were analyzed together. The leaf litter $\delta^{13}C$ values were considered to be representative of terrestrial C_3 plants, and the mean leaf litter $\delta^{13}C$ values for each site were used for comparison against algal values later in this discussion.

Algal samples should have been free of all terrestrial detritus in order to produce accurate algal $\delta^{13}C$ values, however the collection of pure algal samples is difficult. Although variability of algal $\delta^{13}C$ values is not high within sites, variability between sites is great. The mean algal carbon isotope ratios for sites 1, 2, and 3 are -23.83, -22.47, and -30.02‰ respectively. This suggests that the algal isotope ratio is site specific and dependent on local conditions. This observation is in accordance with those made in numerous other food web studies where aquatic plants have had highly variable $\delta^{13}C$ values, as compared to terrestrial plants. Some studies have found that algal $\delta^{13}C$ values are more enriched relative to terrestrial leaf litter (Winterbourn et al., 1986; Junger and Planas, 1994), while other studies have shown more depleted $\delta^{13}C$ algal values (Rounick and Hicks, 1985; Rosenfeld and Roff, 1992). In this study, mean $\delta^{13}C$ values for algal samples were both more enriched (-22.47‰) and more depleted (-30.02‰), compared to terrestrial leaf litter. The most depleted algal $\delta^{13}C$ values were found in the Crystal Creek tributary site (site 3), whereas the most enriched algal $\delta^{13}C$ values were found in the downstream site (site 2). Junger and Planas (1994) described similar enrichment patterns in algal $\delta^{13}C$ values, and they suggested that these differences were due to changes in physical, geological, and chemical parameters associated with the stream gradient. More specifically, based on observations made by Fry and Sherr (1984), algal $\delta^{13}C$ will depend on several factors: the physiology of the plant and the isotopic discrimination of the particular pathway used during carbon fixation; the isotopic composition of dissolved inorganic carbon in the stream water; and, diffusion barriers related to current velocity.

Dissolved inorganic carbon in groundwater may become ^{13}C-depleted due to respiration of terrestrial carbon during percolation through the soil, thereby resulting in algae with more

negative $\delta^{13}C$ values in spring-fed streams (Rosenfeld and Roff, 1992). Although groundwater dissolved inorganic carbon $\delta^{13}C$ values were not measured as part of this study, it is possible that Crystal Creek receives higher inputs of ^{13}C-depleted groundwater than does the main Root River. This could help to explain the more negative $\delta^{13}C$ values for algae sampled at site 3.

As a result of the variability in algal $\delta^{13}C$ values, conclusions pertaining to the carbon dependence of sea lamprey ammocoetes at a given site will be based on the algal isotope ratio specific to the particular site in question. The differences in algal isotope ratios are consistent enough to make inferences with regards to carbon dependence of sea lamprey ammocoetes at the different sites.

In all three sites in this study, aquatic algae and terrestrial leaf litter $\delta^{13}C$ values were distinctly different from one another. This is indicative that a measurable isotopic distinction does exists between allochthonous and autochthonous inputs in this study.

The observed $\delta^{13}C$ values for detritus samples varies between sites. The mean detritus carbon isotope ratio for sites 1, 2, and 3 are -24.43, -26.84, and -27.40‰ respectively. As was noted for leaf litter and algal $\delta^{13}C$ values, the most depleted detrital $\delta^{13}C$ values are found in samples taken from the Crystal Creek tributary site (site 3), whereas the most enriched $\delta^{13}C$ values were found in the upstream site (site 1). This suggests that the detritus isotope ratio is somewhat site specific and dependent on local conditions.

Bowen (1992) identified two types of detritus present in streams. The first type is essentially biofilm which is amorphous, translucent, and individual particles are often < 100 mm in diameter. More specifically, this type of detritus is microbial biofilm which is composed of the accumulation of organic extracellular polymer matrix with embedded microbial cells on

submerged surfaces such as rocks, logs, and living and dead vascular plants. This material is considered detritus because most of it is non-living matter. By contrast, vascular plant detritus is morphous, shows remnants of cellular structure, is often opaque, and is usually > 100 mm. Therefore, according to these definitions the detritus collected in this study is most likely a combination of the two, although composed mainly of the later type of vascular plant detritus. As established earlier, there was no significant difference between detrital $\delta^{13}C$ values and leaf litter $\delta^{13}C$ values in sites 2 and 3. This reflects the domination of C_3 plants at sites 2 and 3, and hence the vascular plant nature of the detritus. Detritus isotope ratios and leaf litter isotope ratios do differ in site 1, implying that this detritus contains perhaps slightly less allochthonous materials and more autochthonous matter than is present in sites 2 and 3.

The observed $\delta^{13}C$ values for FPOM were similar between sites. The mean FPOM carbon isotope ratio for sites 1, 2, and 3 were -26.46, -25.15, and -25.82‰ respectively. These values suggest that all FPOM samples were composed primarily of allochthonous carbon. As discussed earlier, the catchment vegetation consists of C_3 plants whose $\delta^{13}C$ values are approximately 27‰. Bowen (1992) has also suggested that dislodged fragments of biofilm can be transported downstream as part of the suspended particulate matter, thereby contributing to the dead particulate organic matter pool. Therefore, in this study FPOM and algal $\delta^{13}C$ values were statistically contrasted, and it was found that these two categories were significantly different in all three sites. Once again this implies that all FPOM samples were composed primarily of allochthonous carbon. It should however be recognized that the composition of FPOM is likely to vary spatially and seasonally (Rounick et al., 1982). For example, more depleted FPOM δ^{13} values will result as autumn-shed leaves are processed into FPOM.

Alternately, when the contribution of autochthonous material to FPOM increases, its carbon signature will move towards sources such as aquatic macrophytes or algae.

One species of aquatic plant was gathered at the upstream site (site 1). Although it is not believed that sea lamprey ammocoetes feed on aquatic plants (unless perhaps in the form of detritus), these were sampled as an autochthonous source. The mean aquatic plant $\delta^{13}C$ value was -23.22‰, which is very similar to the mean algal $\delta^{13}C$ value at the same site (-23.83‰). Factors that may affect the isotopic composition of algae might also effect those of other aquatic plants (Fry and Sherr, 1984).

The observed $\delta^{13}C$ values for SLAs were fairly similar between sites. The mean SLA carbon isotope ratio for sites 1, 2, and 3 were -20.41, -21.94, and -23.00‰ respectively. The most depleted SLA $\delta^{13}C$ mean value was found in the tributary site (site 3), whereas the most enriched SLA $\delta^{13}C$ mean value was found in the upstream site (site 1). This enrichment pattern corresponds to that observed for detrital, leaf litter, and generally for the algae and FPOM $\delta^{13}C$ mean values.

Past studies have shown that SLAs tend to feed primarily on algae (diatoms), organic detritus, and bacteria (Morman et al., 1980; Bowen, 1992). Although most SLA feeding studies have focused primarily on the algal component of the diet, Bowen (1992) has shown that algae actually make up little of the diet. Bowen (1992) has also found that algal abundance within the diets of SLA varied seasonally. For example, algal abundance was greatest in May/June and September in his study area when the spring and autumn diatom blooms were expected. As canopies closed in the summer, the abundance of algae in the diet decreased until canopies began to reopen in September (Bowen, 1992). The abundance of diatoms in the Root River at the time

of this study is unknown.

When Bowen examined the gut contents of ammocoetes, it appeared that organic detritus was the primary source of nutrition in ammocoete diets. Algae and bacteria are important to the diet in that the biofilm they produce appears to be the source of organic detritus upon which ammocoetes feed. Therefore, variation associated with the contribution of organic detritus to ammocoete diets appears to be related to the annual cycle of algal production (Bowen, 1992). As Moore and Mallatt (1980) have pointed out, the oral cirri of the ammocoete filtration apparatus will only allow selection based on particle size (5 - 340 mm). Therefore, Bowen (1992) has suggested that algae and bacteria are probably ingested incidentally with detritus, and this would account for their presence in stomach content analyses. As well, Moore and Beamish (1973) have shown that sea lamprey ammocoetes pass 45 to 90% of diatoms they ingest through their digestive tract unharmed at 17.8°C and 0°C, respectively. It has also been shown that ammocoetes do not seem to have an effective mechanism for breaking down bacteria which enter the gut (Bowen, 1992). Based on these findings, it would be reasonable to conclude that bacteria and algae for the most part are not assimilated by ammocoetes and it would appear that algal and bacterial biomass alone do not provide the energy required to support ammocoete nutrition. Bowen (1992) suggests from this evidence that organic detritus is the primary source of nutrition for SLAs, and biofilm appears to be the source of this organic detritus.

The results of this study do not support Bowen's (1992) suggestion in regard to the primary source of carbon filtered by SLAs in the Root River. First of all, even allowing for some enrichment due to processing, it appears as though SLAs are not feeding on detritus since SLA and detrital $\delta^{13}C$ values were too different in all three sites. The same conclusion can be

made for SLA and FPOM $\delta^{13}C$ values although the differences are less. SLA and algal $\delta^{13}C$ values were also quite different in sites 1 and 3, although in site 2 SLA $\delta^{13}C$ values were only slightly enriched. Therefore, the only conclusion that can be drawn with regard to ammocoete feeding history is that SLAs at site 2 appear to be feeding on algae.

Assuming that the proper food sources have been collected and analyzed in this study, the next question to be asked is why then are SLA $\delta^{13}C$ values so enriched and why are their potential food source(s) $\delta^{13}C$ values so depleted? This same question became evident in a study done by Doucett (1994). Doucett used stable isotope analysis to determine food pathways in the Miramichi River System, New Brunswick. Although not incorporated into his study, he analyzed a total of 18 lamprey ammocoetes from three different sites, collected in July/August of 1992. The mean lamprey ammocoete $\delta^{13}C$ values found in each of the three sites of Doucett's study were -22.65, -21.83, and -22.19‰. By direct examination of the $\delta^{13}C$ values of all other samples analyzed in Doucett's study (i.e. algae, FPOM, and so on), once again the lamprey ammocoetes appear to be unusually enriched relative to their potential food sources. In both studies it appears as though the food source of lamprey ammocoetes is of an autochthonous source as opposed to an allochthonous source, however a large gap exists between the ammocoetes and their potential food source(s) $\delta^{13}C$ values. Several species of fish were also analyzed in Doucett's study, all of which had more depleted $\delta^{13}C$ values and were closer to their potential food source $\delta^{13}C$ values. Recent work on food webs has shown that carbon becomes more ^{13}C enriched as it proceeds through a food chain (Rounick and Hicks, 1985). This enrichment occurs since consumers generally are more enriched than their food source, and therefore top predators should be the most ^{13}C enriched animals in the food web. In Doucett's

study, this would not have been the case since the lamprey ammocoetes had values more enriched than Atlantic Salmon for example. Although fish were not sampled and analyzed in my study, similar findings may be expected.

Why then the inability to determine the primary source of carbon filtered by SLA in the Root River? There are three possible reasons for the large gap in carbon isotope ratios of SLAs and their potential food sources. First of all, there is perhaps some physiological aspect of lamprey which is blurring the isotope signal. For example, SLA ammocoetes are known to contain relatively high concentrations of lipid in their bodies. The amount of fat per individual SLA rises annually in the early summer, however it usually remains more or less constant at approximately 4% of the wet body weight of the animal (Potter, 1980). At the end of the larval life prior to metamorphosis a large buildup of lipid occurs, and therefore there is little in the way of protein production. Stable carbon isotope ratios of different tissues have been studied in the past, and it has been found that fat tissue of an organism is approximately 3.0‰ more depleted in ^{13}C than its diet. As well, fat tissue showed the largest departure from dietary ^{13}C. (Tieszen et al., 1983; Rounick and Winterbourn, 1986). Such depleted ^{13}C values are to be expected since lipid synthesis discriminates against ^{13}C (DeNiro and Epstein, 1977). Although the SLA $\delta^{13}C$ values are enriched and not depleted, the combination of lipids and some other physiological aspect, or some other entirely different physiological characteristic specific to SLAs could be working to blur or alter isotope signals. The large gap between the SLA $\delta^{13}C$ values and their potential food source $\delta^{13}C$ values implies that some sort of 'Sea Lamprey Ammocoete Stable Carbon Isotope Correction Factor' is required in order to correctly interpret the primary source of carbon filtered by sea lamprey ammocoetes through the use of stable

carbon isotope analysis.

The second possible reason for the excessive ^{13}C enrichment found in SLA is that it may be caused by an unrecognized carbon source. This material which could represent this carbon perhaps was not available to be sampled at the time of study, but which may have been held in 'isotopic memory' in the SLA tissue. Further studies would be required to estimate the $\delta^{13}C$ turnover time, enabling one to predict how long tissue retains the $\delta^{13}C$ of previously assimilated foods. (Rounick and Hicks, 1985). This explanation was offered by Rounick and Hicks (1985) who encountered a similar problem in that the $\delta^{13}C$ values they obtained for fish and the $\delta^{13}C$ values of potential invertebrate prey showed a greater than expected difference between the two trophic levels.

The third and final possible reason for the large variation observed between $\delta^{13}C$ values of SLAs and their potential food source is that the appropriate food source(s) were not appropriately sampled and used in the stable carbon isotope analyses. This concern is based on findings made by Bowen (1992) which were referred to earlier in this discussion. In short, he suggested that organic detritus is the primary source of nutrition for lamprey ammocoetes, and that biofilm appears to be the source of this organic detritus. By biofilm he is referring to microbial amorphous biofilm (both algal and bacterial), or the accumulation of organic extracellular polymer matrix with microbial cells on submerged rocks, logs, living and dead vascular plants. This material is considered detritus because it is mostly composed of dead organic matter. In contrast he is not referring to vascular plant detritus, generally consisting of larger particles, showing remnants of cellular structure. It has already been established that in this study, the detritus sampled and analyzed was probably composed more of vascular plant

detritus than it was of detritus derived from amorphous biofilm. This is based on the fact that there was no significant difference found to exist between leaf litter and the detritus samples obtained for all three sites.

Nitrogen Analysis

The ability of $\delta^{15}N$ values to identify an animal's trophic position has been well studied (Fry, 1991; Gu et al., 1994). Higher trophic positions can be recognized since $\delta^{15}N$ values enrich on average +2 to 4 per mil at each successive level in the food chain. SLA analyzed from both the tributary and the downstream sites possessed more enriched $\delta^{15}N$ values than leaf litter. This suggests that the SLAs occupy a trophic level above the primary producers, or one level up from the base of the food chain as one might expect.

Conclusions

The results of this study revealed several trends amongst sites and within the categories of samples collected at each site. Generally, the most depleted $\delta^{13}C$ values were found at the Crystal Creek tributary site (site 3). By contrast, the most enriched $\delta^{13}C$ values were found in the upstream site on the main Root River.

Algae and aquatic plant carbon isotope ratios were found to be site specific and dependent on local conditions. Aquatic algae and terrestrial leaf litter had distinctly different $\delta^{13}C$ values, therefore indicating a measurable differentiation between allochthonous and autochthonous inputs to the Root River.

Detrital samples collected in this study were found to be composed of a combination of

amorphous biofilm and vascular plant detritus, however the primary constituent was vascular plant material. The fact that there was no significant difference between detrital $\delta^{13}C$ values and leaf litter $\delta^{13}C$ values in sites 2 and 3 suggests that the detrital samples collected were primarily composed of allochthonous inputs. Detrital $\delta^{13}C$ values for site 1 were slightly more enriched, thereby suggesting that perhaps the detrital samples collected at this site may have included a slightly larger amount of autochthonous material.

FPOM was composed primarily of allochthonous material. Although it had been suggested that dislodged fragments of biofilm could also be transported downstream as particulate matter, FPOM and algal $\delta^{13}C$ values were found to be significantly different. The composition of FPOM will however vary spatially and seasonally.

Generally, sea lamprey ammocoete $\delta^{13}C$ values were found to be very much more enriched as compared to their potential food sources. Although it is recognized that consumers are usually enriched relative to their diets, an unusually large gap existed between the sea lamprey ammocoetes and their potential diets. Three possible reasons are provided for this unexpected enrichment. First of all, some physiological aspect specific to sea lamprey ammocoetes could have blurred or altered the isotope signals as they are analyzed. It is suggested that with further study, some sort of 'Sea Lamprey Ammocoete Stable Carbon Isotope Correction Factor' may have to be applied to the $\delta^{13}C$ values before the data can be meaningfully interpreted. The second possible reason for the inability to determine strong relationships between sea lamprey ammocoetes and their diets could be the result of an unrecognizable carbon source. Finally, it has been suggested that sea lamprey ammocoetes feed primarily on amorphous organic detritus, produced by biofilm. As a result, perhaps the appropriate food

source(s) were not collected and analyzed in this study.

Through the use of stable nitrogen isotope analysis it has been found that sea lamprey ammocoetes occupy one trophic level up from the base of the food chain as one might expect.

Overall, the main conclusion of this study is that stable isotope studies provide both an effective and efficient means of identifying and understanding food webs and the interactions between producers and consumers. Stable carbon isotope analysis used alone or in conjunction with more traditional methods such as stomach content analysis can provide a clear and comprehensive description of the food sources an animal has assimilated into its body. Isotope analyses are particularly important for organisms such as sea lamprey ammocoetes, since much is not yet known about their biology and feeding habits. Further studies should be done and should focus on spatial and season differences within streams relating to sea lamprey ammocoetes. Suggestions for such studies might include: larger sample sizes; research and refinement with respect to the manner in which samples of potential food sources are collected; the addition of samples of invertebrates and other fish species in the analyses in order to allow for comparisons of isotope ratios, and hence better analyses of the entire food web; and, a more comprehensive set of corresponding nitrogen isotope ratios, which in turn will aid in estimating trophic levels and process information.

References

Auer, N. (ed.). 1982. Identification of Larval Fishes of the Great Lakes Basin with Emphasis on Lake Michigan Drainage. Great Lakes Fishery Commission Special Publication, 3. pp.23 - 37 (Chapter by L. A. Fuiman).

Bowen, S. 1992. Identification of the nutritional resource supporting growth in larvae of two lamprey species in the Great Lakes Basin. Great Lakes Fishery Commission Research Completion Report (unpublished). 23pp.

DeNiro, M.J., and S. Epstein. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197: 261 - 263.

DeNiro, M.J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42: 495 - 506.

Doucett, R. 1994. Stable isotope analysis of food pathways in the Miramichi River system, New Brunswick. M.S. Thesis, University of Waterloo, Waterloo, Ontario. 104 pp.

Flatt, H., and A.R. Heemskerk. 1994. ^{15}N In Dissolved Nitrate. Technical Procedure 12.0, Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario. 8pp.

Fry, B. 1991. Stable isotope diagrams of freshwater food webs. Ecology 72(6): 2293 - 2297.

Fry, B., and E.B. Sherr. 1984. $\delta^{13}C$ Measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science 27: 13 - 47.

Gu, B., D.M. Schell, and V. Alexander. 1994. Stable carbon and nitrogen isotopic analysis of the plankton food web in a subarctic lake. Can. J. Fish. Aquat. Sci. 51: 1338 - 1344.

Heemskerk, A.R., and P. Diebolt. 1994. Breakseal Organic Combustion. Technical Procedure 22.0, Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario. 11pp.

Hosie, R.C. 1979. Native Trees of Canada (Eighth Edition). Minister of Supply and Services Canada, Canada.

Jackson, D., and D.D. Harkness. 1987. The use and interpretation of $\delta^{13}C$ values as a means of establishing dietary composition. Oikos 48: 258 - 264.

Junger, M., and D. Planas. 1994. Quantitative use of stable carbon isotope analysis to determine the trophic base of invertebrate communities in a boreal forest lotic system. Can. J. Fish. Aquat. Sci. 51: 52 - 61.

Mallatt, J. 1981. The suspension feeding mechanism of the larval lamprey *Petromyzon marinus*. J. Zool. 194: 103 - 142.

Moore, J.W., and F.W.H. Beamish. 1973. Food of larval sea lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*). J. Fish. Res. Bd. Can. 30: 7 - 15.

Moore, J.W., and J.M. Mallatt. 1980. Feeding of larval lamprey. Can. J. Fish. Aquat. Sci. 37: 1658 - 1664.

Morman, R.H., D.W. Cuddy, and P.C. Rugen. 1980. Factors influencing the distribution of sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can. J. Fish. Aquat. Sci. 37: 1811 - 1826.

Peterson, B.J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18: 293 - 320.

Potter, I.C. 1980. Ecology of Larval and Metamorphosing Lampreys. Can. J. Fish. Aquat.

Sci. 37: 1641 - 1657.

Rosenfeld, J.S., and J.C. Roff. 1992. Examination of the carbon base in southern Ontario streams using stable isotopes. J. N. Am. Benthol. Soc. 11(1): 1 - 10.

Rounick, J.S., M.J. Winterbourn, and G.L. Lyon. 1982. Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. Oikos 39: 191 - 198.

Rounick, J.S., and B.J. Hicks. 1985. The stable carbon isotope ratios of fish and their invertebrate prey in four New Zealand rivers. Freshwater Biology 15: 207 - 214.

Rounick, J.S., and M.J. Winterbourn. 1986. Stable carbon isotopes and carbon flow in ecosystems. Bioscience 36(3): 171 - 177.

Rundel, P.W., J.R. Ehleringer and K.A. Nagy (eds.) 1989. Stable isotopes in ecological research. Springer-Verlag, New York, N.Y. 525pp.

Tieszen, L.L., T.W. Boutton, K.G. Tesdahl, and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57: 32 - 37.

Vladykov, V.D., and E. Kott. 1980. Description and key to metamorphosed specimens and ammocoetes of Petromyzonidae found in the Great Lakes Region. Can. J. Fish. Aquat. Sci. 37: 1616 - 1625.

Wilkinson. L. 1992. SYSTAT: The system for statistics. Evanston, Illinois: SYSTAT, Inc.

Winterbourn, M.J., J.S. Rounick, and A.G. Hildrew. 1986. Patterns of carbon resource utilization by benthic invertebrates in two British river systems: a stable carbon isotope study. Arch. Hydrobiol. 107: 349 - 361.

Young, R.J., K.A. Houston, J.G. Weise, and J.R.M. Kelso. 1990. The effect of environmental variables on the population dynamics of sea lamprey, *Petromyzon marinus*. Can. Tech. Rep. Fish. Aquat. Sci., 1736. i-v + 33pp.

Zar, J.H. 1974. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, N.J. USA. 620p.

Appendix

Carbon and Nitrogen isotope ratios of all samples used in this study.

Table A1. Stable carbon and nitrogen isotope ratios for all samples from all three sites in 1994.

Site	Category	13C / 12C
1	SLA	-20.320
1	SLA	-20.602
1	SLA	-20.310
1	Detritus	-24.470
1	Detritus	-25.191
1	Detritus	-23.630
1	Algae	-23.650
1	Algae	-24.007
1	Leaf Litter	-26.852
1	Leaf Litter	-26.882
1	FPOM	-25.048
1	FPOM	-27.863
1	Aquatic Plant	-23.210
1	Aquatic Plant	-23.191
1	Aquatic Plant	-23.359

1	Aquatic Plant	-23.118
1	Aquatic Plant	-23.299
2	SLA	-21.977
2	SLA	-22.398
2	SLA	-22.514
2	SLA	-21.771
2	SLA	-22.436
2	SLA	-22.590
2	SLA	-21.471
2	SLA	-21.461
2	SLA	-21.481
2	SLA	-21.331
2	Detritus	-26.420
2	Detritus	-26.722
2	Detritus	-27.608
2	Detritus	-27.801
2	Detritus	-26.604
2	Detritus	-26.250
2	Detritus	-26.557
2	Detritus	-26.785

2	Detritus	-26.987
2	Detritus	-26.709
2	Algae	-23.171
2	Algae	-22.245
2	Algae	-23.413
2	Algae	-21.826
2	Algae	-22.083
2	Algae	-22.526
2	Algae	-21.998
2	Leaf Litter	-27.140
2	Leaf Litter	-26.960
2	Leaf Litter	-27.090
2	Leaf Litter	-27.170
2	Leaf Litter	-27.400
2	FPOM	-25.338
2	FPOM	-25.270
2	FPOM	-25.228
2	FPOM	-24.752
3	SLA	-24.586
3	SLA	-21.540

3	SLA	-22.310
3	SLA	-23.580
3	Detritus	-26.839
3	Detritus	-27.550
3	Detritus	-27.960
3	Detritus	-27.240
3	Algae	-29.764
3	Algae	-30.280
3	Leaf Litter	-28.157
3	Leaf Litter	-27.560
3	FPOM	-26.472
3	FPOM	-25.170

Site	Category	15N / 14N
2	SLA	3.45
2	Leaf Litter	0.63
3	SLA	3.04
3	SLA	2.72