Assessing Food and Nutritional Resources of Native and Invasive Lamprey Larvae Using Natural Abundance Isotopes

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Graduate School of The Ohio State University

By

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2012

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Abstract

Lampreys (Family: Petromyzontidae) are primitive jawless fishes that have been identified in the fossil record from at least 360 million years ago. However, human impacts have frequently restricted native lamprey populations, reduced their numbers and contracted their ranges. In contrast, in the Laurentian Great Lakes invasion by the parasitic lamprey species *Petromyzon marinus* (sea lamprey) caused large scale ecosystem changes until human control efforts significantly reduced *P. marinus* numbers. Although lampreys have played a vital role in numerous ecosystems, there is currently limited information on the sources of food and nutrition during the longest stage of their life cycle (i.e., the ammocoete stage). If conservation and restoration strategies for native lamprey, and control strategies for invasive sea lamprey, are to be optimized and cost-effective, a fundamental understanding of the factors contributing to the growth, nutrition and survival of ammocoetes is critically needed. Prior conclusions and interpretations on lamprey ammocoete diet have focused on gut-content analysis studies, and our understanding of ammocoete diet and nutrition are therefore limited by older and largely non-quantitative techniques.

Natural abundance isotopes represent a potentially more robust and quantitative approach than gut content analysis for assessing the types of organic materials supporting an organism's somatic growth and energetic maintenance. In

the present study we used natural abundance stable isotopes ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$) to estimate the major sources of autochthonous and allochthonous organic matter (OM) to the diets and nutrition of three species of ammocoetes (i.e., two native species, *Lampetra aepyptera*, *Lethenteron appendix*, and one invasive species, *Petromyzon marinus*) collected in 2010 from rivers in Michigan (USA) and Ohio (USA).

For all three species of lamprey, ammocoete $\delta^{13}C$ increased from ~-32‰ to ~-22‰ with increasing animal length and was strongly positively correlated with animal C:N. Ammocoete $\delta^{15}N$ suggested that ammocoetes are primary consumers of primary producers and detrial materials. Ammocoete $\delta^{2}H$ values showed a strong negative relationship with both animal length and C:N. The observed size-dependent shifts in isotopic values suggest that ammocoete diet changes during growth over the extended ammocoete phase of the lamprey life cycle. However, the possibility cannot be ruled out that some part of the ontogenetic shift in ammocoete isotopic values may be under the influence of non-trophic (i.e., internal) mechanisms that obscure food source signatures.

Contributions of different potential dietary and nutritional sources to ammocoetes were estimated using Bayesian modeling of animal and potential food source isotopic signatures. The model findings indicate significant subsidization of the animals' nutrition by allochthonous terrestrial sources of fresh

plant and soil OM (20-90%), and that aquatic microalgae was the dominant autochthonous (aquatic) subsidy to ammocoete nutrition (i.e., up to 80%), especially in larger and presumably older ammocoetes. Aquatic macrophytes and diagenetically altered sediment OM also contributed to ammocoete diet (2-70%), but contributions were highly variable and influenced by site. The more accurate identification and quantification of allochthonous and autochthonous food and nutritional subsidies to the ammocoete stage provide important information on potential key factors affecting the overall life history of both native and non-native lamprey, and support the growing body of evidence of the significant role of allochthonous terrestrial OM to a range of lotic and aquatic consumers.

Dedication

This thesis is dedicated to my wife Caitlin and to my family, both of whom have been supportive and open to my interest (or rather, obsession) with lampreys. Especially notable is my wife, who was generous enough to go sampling with me and encouraged me throughout.

Acknowledgements

Thanks to my adviser, Dr. James Bauer, for providing me the opportunity to work on whatever topic I was interested in and generously providing financial and other resources to make it possible. Also thanks to Drs. Stuart Ludsin and Meg Daly for allowing me to ask them many questions and make use of their labs and equipment whenever I needed to. Amy Barrett, who assisted with my project and showed me the ropes in the lab. This thesis was made possible by the help of two dedicated undergraduates, Steven Loeffler and Katie Everson. Thanks are due to the other members of the Bauer lab including Amber Bellamy and Dr. Katie Hossler. Great thanks and much appreciation is also due to the Fish Division at The Ohio State University (OSU), and especially Marc Kibbey, who was instrumental in providing Ohio sampling site locations and helped with field collections. Also thanks is due to the Great Lakes Fishery Commission for providing sampling site locations in Michigan and allowing me to work with them during preliminary collections. Finally, I want to thank numerous other graduate students and faculty members of the OSU Department of Evolution, Ecology and Organismal Biology, who have helped me in numerous ways over the course of my M.S. Thesis. Finally, we thank Dr. Andrea Grottoli and Yohei Matsui and other members of the OSU Stable Isotope Facility for their assistance in preparation and analyses of stable isotope samples.

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Introduction

Invasion of the Laurentian Great Lakes by the parasitic sea lamprey (*Petromyzon marinus*) in 1921 (Lawrie 1970) fundamentally altered the structure and function of this globally important freshwater ecosystem. Sea lamprey most likely invaded Lakes Erie, Huron, Michigan and Superior from Lake Ontario via the Welland canal (Ontario, Canada; Lawrie 1970; Waldman et al. 2004). Juvenile sea lampreys parasitize fish by extracting body fluids and have had devastating effects on a number of important native fish species, in particular lake trout (*Salvelinus namaycush*), the preferred host, which declined to record lows in all of the Great Lakes by 1965 (Hardisty and Potter 1971). By 1969, human control efforts had significantly reduced sea lamprey numbers throughout all of the Great Lakes (Christie 1974).

However, the major portion of the life cycle of sea lamprey is spent in natal stream sediments as a filter-feeding larval form (i.e., the ammocoete stage) for as long as 17 years (Manion and Smith 1978; Purvis 1979; Potter 1980; Potter et al. 1986). In contrast, the combined parasitic juvenile and adult stages of sea lamprey are only 1-1.5 years long (Clemens et al. 2010). Despite the duration and

likely key nature of the ammocoete stage to sea lamprey recruitment, survival, and overall life history, little is known about the feeding ecology and nutrition of this stage. Identification of the nutritional resources supporting the extended larval stage of this primitive fish family could potentially provide insight into its evolutionary success, while also allowing for better predictability of ammocoete habitat and more efficient control measures in non-native settings. The reclusive nature of ammocoetes has in part complicated identification and quantification of their food and nutritional resources. In addition, the observational methods historically employed to visually identify and quantify ingested items within the ammocoete gut upon dissection have largely been ambiguous and non-quantitative (Moore and Mallatt 1980; Sutton and Bowen 1994) and provide at best a general indication of the food resources ingested, rather than assimilated by, the animals (Grey et al. 2002; Michener and Kaufman 2007).

Early studies of sea lamprey ammocoete food and nutrition using gut content analysis (GCA) described the presence of "microscopic organisms" in the diet but downplayed the role of far more dominant detrital organic matter (OM), and suggested that ammocoetes filtered and expelled the detrital material before ingesting the microorganisms (Applegate 1961). Other GCA studies identified microalgae, in particular diatoms, as being critical to the diet of sea lamprey ammocoetes (Manion 1967; Moore and Beamish 1973; Moore and Mallatt 1980). However, Manion (1967) also noted that sea lamprey ingestion of diatoms correlated with diatom abundance in the water column and suggested diatoms would therefore be of only seasonal dietary value. The importance of diatoms to

sea lamprey ammocoete nutrition was further questioned by Sutton and Bowen (1994) who found that >92% of the ammocoete gut consisted of undifferentiated amorphous detrital material. These workers also calculated that even if ammocoetes assimilated diatoms with 100% efficiency, diatoms could account for at most ~4% of ammocoete metabolic demand (Sutton and Bowen 1994).

In contrast to visual identification methods, natural abundance isotopic analysis represents a potentially more robust and quantitative approach for assessing the sources of OM supporting the somatic growth and energetic maintenance of an organism or population (Solomon et al. 2011; Roach et al. 2011). Naturally occurring isotopes of C, N, H, O and S in OM may be used to both qualitatively and quantitatively assess different OM sources supporting organism nutrition and through food webs and even ecosystems, provided the isotopes of these elements can be measured in both the potential food sources and the target organisms (Peterson and Fry 1987; Post 2002; Michener and Lajtha 2007). In biological systems, the lighter isotope of an element is utilized at the biochemical level to a slightly greater extent than the heavier isotope due to mass-dependent effects, resulting in isotopic fractionation (Hoefs 2004; Michener and Lajtha 2007). In addition, ingested versus assimilated food source(s) may be differentiated by isotopic examination of both gut contents and animal tissues (Rounick and Winterbourn 1986; Melville and Connolly 2003). Simultaneous use of multiple natural isotopes can further help resolve with far greater accuracy the dietary and nutritional sources of a given species and with greater resolution and accuracy than single isotopes (Peterson and Fry 1987; Caraco et al. 2010; Cole et

al. 2011). Natural abundance stable isotopes can be used to help establish at which trophic level organisms are feeding. For example, $\delta^{15}N$ is known to fractionate at ~3‰ for each trophic level while $\delta^{13}C$ fractionates only ~0-1‰ per trophic level (Peterson and Fry 1987).

Our goal was to identify and quantify the dominant food resources contributing to sea lamprey ammocoete diet and nutrition in watersheds of Lakes Michigan and Huron. We hypothesized that: 1) sea lamprey ammocoete natural abundance isotopic values would reflect a diet composed to a significant extent of allochthonous detrital terrestrial OM, owing to the dominance of this material in most streams and rivers of North America (Cummins 1974; Vannote et al. 1980; Cole and Caraco 2001), and 2) ammocoete diet and nutrition would vary spatially (e.g., as a function of different watershed types) and temporally (i.e., seasonally) as the relative abundances and quality of different potential food sources shift.

Materials and Methods

Study Sites

P. marinus ammocoetes were collected from two rivers on the lower peninsula of northern Michigan, USA (Jordan River and Pigeon River; Figure 1). The Jordan is a 4th order river that is 53 km in length and drains a watershed of 405 km^2 (Michigan Department of Natural Resources 1972). The dominant land uses in the Jordan River watershed are mixed deciduous secoND–growth forest (~70%) and agricultural (27%), with little urban development (<1%; Hay and Meriwether 2004). The Pigeon is a 3rd order river that is 68 km in length and

drains a watershed of 360 km^2 (Michigan Department of Natural Resources 1982). The Pigeon River watershed is considered one of Michigan's most pristine, with essentially 100% of the land being mixed deciduous secoND–growth forest with little development or agriculture of any kind (Michigan Department of Natural Resources 2007). The Pigeon River was sampled in May and October 2010 within 2nd order reaches and the Jordan was sampled in June and October 2010 within both 2nd and 3rd order reaches (Table 1, Figure 1).

Streams known to harbor *P. marinus* ammocoetes have been targeted for chemical control in the Great Lakes region since 1958 (Smith and Tibbles 1980; Christie and Goddard 2003), and both the Jordan and Pigeon Rivers are periodically drip-treated with 3-triflouromethyl-4-nitrophenol (TFM) to kill ammocoetes. The Pigeon and Jordan Rivers were last treated in July 2007 and September 2007, respectively. Therefore, both rivers are assumed to have been repopulated by spawning adults no earlier than spring 2008. As a result, during our sampling in May and June 2010, only two year classes likely existed, however, by October 2010, young-of-year (YOY) ammocoetes may have been present from spawning in spring 2010.

Ammocoete Collection

Ammocoetes were collected following standard electrofishing procedures using a backpack electrofisher (model ABP-2MP-600V, Electrofishing LLC) as described by Moser et al. (2007) and following the manufacturer's recommendations. Upon emergence from their burrows, ammocoetes were netted and rinsed of any sediment before being placed in a sampling container. Within

60 min of collection ammocoetes were wrapped in pre-baked (500ºC for 4 hours) sheets of aluminum foil, sealed in an airtight plastic bag, placed on dry ice in the field, and kept frozen in the lab until processing. Dissection of guts in the laboratory, and lack of significant fecal material in the sampling containers, confirmed that significant defecation had not occurred between animal collection and freezing. Because similarities in ammocoete morphology make identification by morphology alone equivocal (Vladykov and Kott 1980; Page and Burr 1991), fin clips of frozen ammocoetes were taken in the lab for microsatellite genetic confirmation that individuals were sea lamprey.

Collection of Ammocoete Potential Food Sources

Suspended Particulate Organic Matter (SPOM). Riverine SPOM is defined as material that is retained by a ~0.8 μm nominal pore size filter (Cole et al. 2006; Caraco et al. 2010). It is derived from diverse sources but can include bacteria, plankton, fresh and detrital vegetation, terrestrial soil material and resuspended stream sediments (Cole and Caraco 2001; Hoffman et al. 2008). SPOM samples were collected at each site by pumping stream water from a pre-cleaned (10% HCl) polycarbonate collection carboy using a peristaltic pump equipped with 10% HCl cleaned silicone tubing. Stream water from the carboy was filtered through a pre-baked (525ºC for 4 hours) 47 mm quartz fiber QMA filter (Whatman; 0.8 μm nominal pore size) that was immediately frozen until processing in the lab.

Sediment Samples. Samples of sedimentary organic matter (SOM) were collected from streambeds using cut-off 60 cc plastic syringes to a depth of ~8 cm

along the banks of pools in each stream in which ammocoete sampling occurred. Following collection, the bottom and top of each core were covered with a sheet of pre-baked (500ºC for 4 hours) aluminum foil and the core was frozen upright on dry ice in an airtight plastic bag. Upon return to the lab the cores were stored at −20ºC until processing and analysis.

Size-fractionated SOM. Ammocoetes are known to select material they ingest on the basis of particle size (Moore and Mallatt, 1980; Yap and Bowen, 2003) and therefore may size-fractionate SOM. To more fully identify the characteristics of the SOM, the top 4 cm of a sediment core from each sampling date was resuspended in distilled-deionized (DI) water. The resuspended material was then gravity-filtered in series through five pre-cleaned (10% HCl) Nitex mesh sieve filters (353, 163, 63, 35 and 10 μm) and then by gentle vacuum through a QMA filter (0.8 μm). Particles from the Nitex mesh were then transferred onto separate pre-baked (525ºC for 4 h) quartz fiber QMA filters using gentle vacuum to remove DI water. The filters were dried and stored in desiccators until processing and analysis.

Soil Samples. To identify potential terrestrial soil contributions to stream SPOM and SOM, terrestrial soils were collected within ~10 m of the stream bank (October 2010 only) by excavating a side of the bank to a depth of ~30 cm using a clean spade. Soil was collected from different horizons using a clean trowel, wrapped in pre-baked aluminum foil, frozen on dry ice in the field and stored at -20ºC until processing.

Aquatic Plants and Terrestrial Vegetation. In low-productivity aquatic

environments terrestrial vegetation often dominates allochthonous sources of OM (Jones et al. 1998; Bauer and Bianchi 2011; Bianchi and Bauer 2011). The dominant species of living terrestrial and aquatic vegetation in the vicinity of each sampling site in the two rivers were collected by identifying the most abundant local species and handpicking leaf material using clean nitrile gloves. Samples were stored in airtight plastic bags and frozen on dry ice in the field and stored at -20ºC until processing.

Dissolved Inorganic Carbon (DIC). DIC in most freshwaters is primarily comprised of dissolved CO_2 gas and bicarbonate ions (Rounick and Winterbourn 1986; Peterson and Fry 1987; Kalff 2002) and is fixed by aquatic primary producers (e.g., phytoplankton and submerged aquatic vegetation) during photosynthesis (Finlay 2001; Cole et al. 2006; Caraco et al. 2010). By applying well-established isotopic fractionations of dissolved CO_2 by primary producers, the $\delta^{13}C$ values of DIC can serve as a proxy for the $\delta^{13}C$ of submerged aquatic plants and phytoplankton (Canuel et al. 1995; Chanton and Lewis 1999; Finlay 2001; Caraco et al. 2010). Stream water for DIC samples was pumped using bubble-free techniques into pre-baked (450ºC for 4 h) gas-tight sealed serum bottles (125 ml; Wheaton Co.) poisoned with 200 µl of saturated $HgCl_2$ solution and previously purged using ultra-high purity N_2 gas. Samples were stored at room temperature in the dark until extraction at the laboratory.

Water Column Sampling

Basic water quality parameters were measured at each site during each sampling time. These parameters included pH, dissolved oxygen, conductivity

(only during the second sampling), and temperature and were measured using Fisher Scientific AP 61 portable pH meter, YSI 550A DO meter, and YSI Model 30 conductivity meter, respectively.

Sample Preparation and Natural Abundance Isotope Analyses

Ammocoete Body Tissue and Ingested Gut Material. Muscle tissue and a fin clip (see *Genetic Confirmation of Ammocoetes* below) from each ammocoete were surgically removed following isotopic clean protocols. Muscle tissue was dissected from the third segment (the segment containing the 50-75% length of gut, see below) of each ammocoete, unless there was too little muscle tissue for analysis. In this case muscle from the second segment was also dissected. For ammocoetes <3.5 cm in length, muscle from segments 1-4 was dissected. The samples of muscle tissue were dried at 60ºC for 48 h, homogenized by grinding, and stored in pre-baked glass scintillation vials in a polycarbonate dessicator maintained at <10% relative humidity until analysis.

The gut was removed from the body of each animal and the length was measured. The extracted gut was cut into four equal lengths and each section was extruded onto a clean sheet of pre-baked aluminum foil. The gut contents were then dried at 60ºC for 48 h and stored in baked glass scintillation vials in a dessicator as above for ammocoete muscle samples until analysis.

Genetic Confirmation of Ammocoete Genus

While juvenile and adult lampreys are easily identified to species by visual means, similarities in ammocoete morphology make identification by morphology alone equivocal (Vladykov & Kott, 1980; Page & Burr, 1991), and unambiguous

identification is only possible by genetic analyses. Microsatellites are short (1-6 base pairs) tandemly repeating segments of DNA found throughout eukaryotic genomes (Tautz, 1989; Weissenbach *et al.*, 1992; Roder *et al.*, 1998), and have been developed to establish whether an individual is a genus of native lamprey (*Ichthyomyzon* or *Lampetra*) or the invasive *P. marinus* (Filcek *et al.*, 2005).

Fin clips were taken from each individual following clean procedures and DNA was extracted using the QIAGEN DNeasy Kit. Following extraction, DNA concentrations were established with a UV/Visible spectrophotometer. DNA extracted in this manner is amplified by a microsatellite loci (Pmaμ9), following the procedure of Filcek et al. (2005). Once amplified, each extracted DNA sample was run on 1.5% agarose gel at 85 V. Gels were soaked in 2 $mg \cdot ml^{-1}$ of ethidium bromide for 15 min, and then de-stained for 10 min in deionized water. After de-staining, gels were fluoresced under a UV light to visualize bands and determine whether the ammocoete was a native species or the invasive *P. marinus*.

Potential Food Sources. SPOM filters were dried (60 ºC, 24 h) and then fumed (concentrated HCl, 24-48 h) in a clean glass dessicator to remove inorganic C. Following acidification, filters were placed under vacuum for 24-48 h to remove any residual acid fumes and then dried for 24 h at 60 ºC. The filters were then cut into quarters using an acetone-cleaned razor blade and stored in a dessicator as above for ammocoete muscle samples until analysis.

Stream sediment cores for SOM analyses were sectioned into 1 cm increments with a clean razor blade. The section closest to the water-sediment interface was divided into two 0.5 cm increments. Sediment sections were acid

fumed following the procedure described above for SPOM filters. Each sample was then homogenized and stored in pre-baked (500 ºC, 4 h) glass scintillation vials in a dessicator as above for ammocoete muscle samples until analysis. Size-fractionated stream SOM samples were dried at 60ºC on pre-baked QMA filters and then treated in the same manner as SPOM filters for preparation for analysis. Terrestrial soil samples were processed in the same manner as stream sediments.

Aquatic and terrestrial plant tissues were dried to constant mass at 60ºC. After drying, each sample was homogenized by grinding and stored in a dessicator as above for ammocoete muscle samples until analysis. DIC samples were extracted by acidification with 0.2 ml of 85% Phosphoric acid and cryogenic collection of CO_2 on a vacuum extraction line using ultra-high purity He gas to sparge and strip the samples. Following extraction and purification, sample CO_2 was stored in clean 6 mm Pyrex gas-tight sealed tubes at room temperature until isotopic analyses. All DIC samples after extraction had a pH <2.

Isotopic Analyses. Subsamples of each sample type were packed in tin capsules and analyzed for $\delta^{13}C$ and $\delta^{15}N$ at the University of California, Davis (PDZ Europa ANCA-GSL (EA) attached to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS)) or at The Ohio State University's Stable Isotope Biogeochemistry Laboratory (Costech EA with continuous flow by CONFLOIII attached to a Finnigan Delta Plus IV IRMS). Standard deviations for replicate analyses of standards using both instruments were $\leq$ 0.2‰ for $\delta^{13}C$ and $\leq$ 0.3‰ for $\delta^{15}N$. Separate subsamples were packed in silver capsules and analyzed for deuterium ($\delta^{2}H$) by a DELTA plus XL IRMS attached to a ConFlo II and using a

Carlo Erba NC2100 EA at the Northern Arizona University, Colorado Plateau Stable Isotope Laboratory (CPSIL). The standard deviation for replicate standards and samples for this instrument was $\leq 2‰$ for δ^2H.

Statistical Analyses

All statistical analyses were performed using Minitab® for Windows (version 15; MINITAB Inc, State College, PA, U.S.A.). Length data were square root transformation before analysis. We used an ANCOVA to test for differences between slopes of regression fit to independent variables. Separate ANOVA's of $\delta^{13}C$, $\delta^{15}N$ and δ^2H were used to test for significant effects of site, date and their interactions in ammocoete and potential nutritional sources. In addition, ANOVA's of plants included type (i.e., aquatic versus terrestrial) and ANOVA's of sediment and soil included depth. Tukey's test for pair wise comparisons was performed for any factor found to have significance.

Isotope Mass Balance Model

We used the Bayesian stable isotope mixing model MixSIR (Moore and Semmens 2008) to estimate contributions of potential food sources to ammocoete nutrition. MixSIR allows for the incorporation of uncertainties of source isotopic values and fractionation estimates to better predict food source dependence and confidence of the importance of a given dietary item to the organism (Moore and Semmens 2008; Kim et al. 2011). Ammocoetes and their potential nutritional resources were modeled separately by site.

Isotopic signatures for ammocoetes and potential nutritional sources were determined from measurements of samples collected in the present study with a

few exceptions and modifications. While macroalgal samples were collected in the present study, these samples were epilithic and contained large amounts of non-living detrital OM and mineral materials. Therefore, we estimated a range of potential $\delta^{13}C$ signatures for "pure" microalgae based on our stream water DIC $\delta^{13}C$ values (Table 2), microalgae $\delta^{13}C$ values and DIC-microalgae $\delta^{13}C$ relationships from the literature (Finlay et al. 1999; Finlay 2001), and C_3 submerged aquatic macrophyte $\delta^{13}C$ values from our study. Microalgae were assumed to have $\delta^{15}N$ values similar to submerged aquatic macrophytes (Boon and Bunn 1994; Cole et al. 2005), and means and standard deviations of microalgal $\delta^{15}N$ were drawn from those data. Because microalgal $\delta^{2}H$ values are influenced by latitude (Hobson and Wassenaar 1997), we estimated microalgae values by using published differences between the $\delta^{2}H$ of aquatic microalgae and $\delta^{2}H$ for terrestrial plants (-67‰; Doucett et al. 2007; Finlay et al. 2010) measured in our study. The $\delta^{2}H$ values we estimated for microalgae in our Michigan streams (~-220‰) were consistent with other published microalgae $\delta^{2}H$ values found in temperate U.S. watersheds (Doucett et al. 2007; Caraco et al. 2010; Cole et al. 2011). The largest calculated standard deviation across all other potential food sources measured at our study sites was used in the model as the standard deviation for microalgal $\delta^{2}H$.

On the basis of previous studies (Mallatt 1982; Sutton and Bowen 1994), we assumed ammocoetes were one trophic level above primary producers and followed published fractionation values of 0.4± 1.3‰ for $\delta^{13}C$, 3.4 ±1.0‰ for

$\delta^{15}N$ (Post 2002), and 0 ± 10‰ for $\delta^{2}H$ (Hobson et al. 1999; Jardine et al. 2005). The model was run with $\geq 1x10^{6}$ iterations, which resulted in an importance ratio of <0.001 and >1,000 posterior draws in all cases, following recommended guidelines for determining if the model output has estimated true posterior distributions (Moore and Semmens 2008). MixSIR was not used to estimate contributions of potential food and nutritional sources at Jordan River 2 because of the absence of any $\delta^{2}H$ data at this site, which significantly lowers the resolution of the model.

Results

Stream Water Parameters

Ammocoetes collected in this study were found in cool (<20°C), highly oxygenated (>9.5 $mg \cdot L^{-1}$) stream waters (Table 1). Sampling streams were weakly acidic to basic (pH 6.5-9), and had conductivities between 201.1-214.0 $\mu S \cdot cm^{-1}$ (at 25°C). Water visibility was >1.5m at all sampling locations (*pers. observation*).

Microsatellite Confirmation

During this study, 58 of the 62 animals captured were confirmed by microsatellite markers to be invasive sea lamprey ammocoetes (Table 3). Figure 2 shows an example agarose gel with a control animal (*Lethenteron appendix*, Mad River, Ohio) in lane 2 and unknown animals from the Jordan River, Michigan, in lanes 3-9. Lane 4 was not scored as a native species from this gel and was re-run to confirm that the animal was not a sea lamprey.

Ammocoete Size Distributions

Sea lamprey ammocoetes across all sampling sites and times had a mean length of 4.3 cm (SD ±2.2 cm, range: 1.4-10.7 cm; Table 4, Figure 3a). Mean lengths across all sites and sampling times did not differ (ANOVA, p=0.33). However, during October sampling, the mean length of YOY animals was greater in the Pigeon River (mean=3.0 cm; SD ±0.2 cm, range: 2.7-3.1 cm) than for sites in the Jordan River (mean=1.8 cm, SD ±0.2 cm, range: 1.4-2.1 cm; ANOVA, p<0.001; Figure 4). Ammocoete length and wet mass across all sites were correlated (p <0.0001; Figure 4), and sites did not differ significantly (p>0.05).

C:N of Ammocoete Tissue

Ammocoete muscle tissue C:N data were pooled because an ANOVA found no effect of site (p=0.62), date (p=0.43) or their interaction (p=0.78). Ammocoete C:N showed a bimodal distribution (Figure 3b), and had a mean of 7.1 (SD ±1.6, range: 4.6-11.0) across all sites and times. The first mode had a mean C:N of 6.4 (SD ±0.9), while the second mode had a mean of 9.7 (SD ±0.6). C:N was positively related to ammocoete length across all sampling sites and time (R^2=0.41, p<0.001, Figure 5a).

Isotopic Signatures of Ammocoetes

Mean $\delta^{13}C$ values of ammocoete muscle tissues across all sizes and sampling times did not differ between sites (ANCOVA, p=0.32; Table 4). In spite of three ammocoetes having larger-than-normal residuals, $\delta^{13}C$ values were positively related to animal length for all sampling sites and times (R^2=0.45, p<0.001, Figure 5b).

The $\delta^{15}N$ values of ammocoete muscle tissue were not associated with animal length at any site (Jordan River 1, p=0.46; Jordan River 2, p=0.21; Pigeon River, p=0.27; Figure 5c) but were different between sites (ANOVA, p<0.001). All sites means differed from one another. The mean $\delta^{15}N$ for Jordan River 1 animals was 4.0‰ (SD ±0.7‰, range: 3.0 to 6.1‰), while Jordan River 2 averaged 2.8‰ (SD ±0.6‰, range: 1.9 to 4.1‰), and Pigeon River averaged 4.7‰ (SD ±0.6‰, range: 3.7 to 5.5‰).

Mean $\delta^{2}H$ values of ammocoetes did not differ between Jordan River 1 and the Pigeon River (ANOVA, p=0.58). $\delta^{2}H$ values were significantly inversely related to animal length (R^2=0.37, p=0.001; Figure 5d) for the Pigeon River site and Jordan River site 1 in October 2010.

Isotopic Signatures of Ammocoete Potential Food Sources

Aquatic and Terrestrial Plants. The mean $\delta^{13}C$ of Jordan River 1 aquatic macrophytes across sampling times was -29.1‰ (SD ±3.1‰), while the means for Jordan River 2 and the Pigeon River across sampling times were -28.1‰ (SD ±2.5‰) and -30.75‰ (SD ±1.7‰), respectively (Table 5, Table 6). For terrestrial plants, mean $\delta^{13}C$ values were -30.8‰ (SD ±2.2‰), -29.8‰ (SD ±1.8‰), and -29.1‰ (SD ±2.0‰) for Jordan River 1, Jordan River 2 and the Pigeon River, respectively (Table 5,Table 6).The $\delta^{13}C$ values did not differ between sites (ANOVA, p =0.40) or plant type (i.e., aquatic vs. terrestrial; ANOVA, p =0.13). Aquatic and terrestrial plants across all sites had a mean $\delta^{13}C$ value of -29.5‰ (SD ±2.5‰, range:-34.68 to -23.15‰).

Mean $\delta^{15}N$ for aquatic macrophytes across sampling times for the Jordan River was 3.2‰ (SD ±2.2‰, range: -1.1 to 6.5‰), and for terrestrial plants was -0.3‰ (SD ±2.4‰, range: -4.0 to 4.8‰; Table 5, Table 6). The mean $\delta^{15}N$ for aquatic macrophytes from the Pigeon River across sampling times was 5.7‰ (SD ±1.1‰, range: 4.9 to 7.4‰) and for terrestrial plants was 1.8‰ (SD ±2.4‰, range: -2.6 to 5.5‰). The $\delta^{15}N$ values across all times did not vary between sites (ANOVA, p=0.01) or plant type (i.e., aquatic vs. terrestrial; ANOVA, p<0.001).

Mean $\delta^{2}H$ values of aquatic macrophytes and terrestrial plants across sampling times did not differ between sites (ANOVA, p=0.07) or plant type (ANOVA, p=0.15). The mean $\delta^{2}H$ value for both sites at which $\delta^{2}H$ was measured for aquatic macrophytes (i.e., Jordan River 1 and Pigeon River) was -157‰ (SD ±18‰, range: -183 to -120‰; Table 5, Table 6).

Stream DIC. The mean $\delta^{13}C$ of stream DIC (the carbon source used by submerged aquatic plants and microalgae) did not vary between sites and had an overall mean across all sites and sampling times of -11.4‰ (SD ±0.4‰, range: -13.8 to -10.7‰; Table 2). Jordan River 1 mean $\delta^{13}C$ of DIC was -11.2‰ (SD ±0.2‰), while at Jordan River 2 and the Pigeon River it was -11.1‰ (SD ±0.4‰) and -11.8‰ (SD ±1.3‰), respectively, across both sampling times. These site means did not differ from each other (ANOVA, p=0.61).

Stream SPOM. The relatively small sample number for isotopic analyses of SPOM precluded meaningful statistical analysis. SPOM was similar across all sites and times with a mean $\delta^{13}C$ of -28.5‰ (SD ±0.4‰, range:-29.1 to -28.2‰; Table 7) and a mean $\delta^{15}N$ of 3.4‰ (SD ±0.7‰, range: 2.3 to 4.0‰). $\delta^{2}H$ values

were only measured at two sites. For Jordan River 1, SPOM had a measured δ^2H of -128‰ while for the Pigeon River it was -106‰.

Stream Sediments. SOM was isotopically similar across all sites, depths and times (Table 8). The mean $\delta^{13}C$ for SOM at Jordan River 1 was -26.4‰ (SD ±2.8‰), -26.7‰ (SD ±2.1‰) at Jordan River 2, and -26.0‰ (SD ±1.2‰) for the Pigeon River across sampling times. The mean $\delta^{13}C$ of SOM did not differ between sites (ANOVA, p=0.15) or depths (ANOVA, p=0.47), and the overall mean was -27.4‰ (SD ±2.1‰).

The mean $\delta^{15}N$ of SOM across sampling times was 3.2‰ (SD ±0.6‰) for Jordan River 1, 2.8‰ (SD ±0.4‰) for Jordan River 2, and 2.8‰ (SD ±0.3‰) for Pigeon River, with an overall mean across all sites and times of 3.0‰ (SD ±0.5‰; Table 8). An ANOVA of SOM $\delta^{15}N$ also showed no effect of site (p=0.12) or depth (p=0.13). Because of the smaller number of δ^2H measurements and their similarity across all sediment depths, the δ^2H values for all SOM samples were averaged yielding a mean δ^2H of -144‰ (SD ±13‰, range:-126 to -160‰).

Terrestrial Soils. Terrestrial surface soil OM had $\delta^{13}C$ values that varied between -29.0‰ and -28.2‰ (Table 9), with an overall mean of -27.2‰ (SD ±1.4‰) for all sites. An ANOVA of soil OM found no effect of site (p=0.59) or depth (p=0.05) on $\delta^{13}C$. Soil OM $\delta^{15}N$ values also showed no effect of site (p=0.15) or depth (p=0.05), and the overall mean for all sites and depths was 1.4‰ (SD ±2.2‰). δ^2H values for soil OM were similar between sites and had a mean value for all sites and depths of -132‰ (SD ±7.6‰, range:-123 to -141‰).

Size-fractionated Aquatic Sediments. The $\delta^{13}C$ of SOM in size fractionated aquatic sediments showed a small (1.1‰) but significant difference in mean values (p=0.004; Table 10, Figure 6), but no evidence for site or a site-size fraction interaction (p=0.32 and p=0.81, respectively). A single value was removed from analysis because it was >10‰ from all other samples from the site and was identified as an outlier (Grubb's test, p<0.05). $\delta^{15}N$ values of size-fractionated sediment OM samples showed no differences between sites (p=0.49), size fractions (p=0.20), or their interaction (p=0.68). The overall mean $\delta^{15}N$ was 2.4‰ (SD ±0.9‰, range:-0.65 to 3.9‰).

Isotopic Signatures of Ammocoete Gut Contents

Ammocoete gut content (GC) OM was analyzed for $\delta^{2}H$ only, since GC material was highly limited due the small sizes of the majority of animals. In addition, some GC samples were pooled (Table 11) to achieve adequate sample sizes. With the exclusion of a single anomalous $\delta^{2}H$ value (-208‰) the mean $\delta^{2}H$ of GC for all gut segments was -150‰ (SD±16‰), and no significant difference existed in the $\delta^{2}H$ of GC among gut segments (Table 11). With or without the anomalous value included, a 1-way within subjects ANOVA found that ammocoete GC $\delta^{2}H$ did not show a significant effect of individual ammocoete or between the interaction of gut segment and individual ammocoete (p>0.05; Table 11).

Discussion

Natural abundance stable isotopes (in particular $\delta^{13}C$ and $\delta^{15}N$) have been

used in a limited number of previous studies to assess nutritional resources for sea lamprey juveniles and adults (Drevnick et al. 2006; Harvey et al. 2008) and ammocoetes (Doucett 1994; Hollett 1995). Hollett (1995) reported that sea lamprey ammocoetes from several Lake Huron tributaries in Ontario, Canada were more enriched in ^{13}C ($\delta^{13}C$ as high as –20.3‰) than any of the potential food sources identified (i.e., aquatic plants, fine SPOM, leaf litter, algae, and detritus), indicating that the dominant food sources for ammocoetes were not fully constrained using $\delta^{13}C$ alone. Doucett (1994) found similarly enriched and unconstrained $\delta^{13}C$ values of sea lamprey ammocoetes from the Miramichi River system, Ontario relative to potential food sources measured. $\delta^{15}N$ analyses of ammocoetes and their potential food resources, although limited in scope, suggested that ammocoetes were one trophic level above primary producers (e.g., phytoplankton and aquatic plants; Hollet 1995). From these previous studies, it is apparent that the stable isotopic signatures of ammocoetes require additional examination in order to explain the factors controlling them.

Size -Dependant Changes in Ammocoete C:N and Isotopic Signatures

Both C:N and $\delta^{13}C$ values of ammocoetes increased as a function of animal size (Figure 5a and b), and as a result C:N was positively correlated with ammocoete $\delta^{13}C$, following a logarithmic relationship (Figure 7a). This finding contrasts with other studies, which demonstrated an inverse relationship between animal muscle C:N and $\delta^{13}C$ due to increasing lipid content and the generally lower $\delta^{13}C$ of lipids. In contrast to $\delta^{13}C$, the $\delta^{2}H$ of ammocoetes was inversely related to C:N (Figure 7b), in general agreement with the expectation that animal

C:N is a predictor of lipid content (Hobson et al. 1999; Soto et al. 2011) because lipids are essentially devoid of N and more depleted in ^{2}H than proteins. The $\delta^{13}C$ and $\delta^{2}H$ of ammocoetes were correlated (Figure 7c) suggesting that physiological mechanisms that are driving $\delta^{13}C$ values may be driving $\delta^{2}H$.

The increase in ammocoete $\delta^{13}C$ as a function of animal size (Figure 5b) may be the result of two main factors: 1) animal $\delta^{13}C$ (and potentially $\delta^{2}H$) is influenced by one or more physiological mechanisms, or 2) animals shift with increasing size to more ^{13}C-enriched and ^{2}H-depleted food sources relative to smaller animals. In contrast to both $\delta^{13}C$ and $\delta^{2}H$ values, the $\delta^{15}N$ of ammocoete muscle tissue did not change as a function of length (Figure 5c) or and only marginally with C:N at a single site (Figure 8). If ammocoete $\delta^{13}C$ tracks carbon assimilated from food sources, we would expect other isotopic values to converge towards the same food sources. In contrast, if the mechanism is a physiological one, the isotopic values of ammocoete muscle tissues may not directly reflect food sources without additional information on how the isotopic values are being altered during digestion and assimilation (Gannes et al. 1997).

Ammocoetes that are not preparing for metamorphosis accumulate lipids (as high as 8% by wet mass) in the spring and then metabolize them throughout the remainder of the year (reaching as low as 1% by wet mass; O'Boyle and Beamish 1977; Potter 1980). All the ammocoetes in this study should have had similar lipid content, because they were too young and too small (<3 years, <12 cm) to begin metamorphosis (Purvis 1979). The$\delta^{15}N$ values of tissue were

not expected to be correlated with C:N, as N is not depleted by lipid synthesis (DeNiro and Epstein 1977).

Harvey et al, (2008) also showed that sea lamprey transformers (animals that have recently undergone metamorphosis but not yet fed parasitically) have normally distributed C:N values with a mean of 7.4 (SD ±1.7, n=50), which is not significantly different from the mean C:N (7.1, SD ± 1.6) in the present study (Student's t-test, P=0.29). However, transformers collected in the fall have been found to have about twice the lipid stores of ammocoetes (O'Boyle and Beamish 1977). As a result, these data suggest that C:N may not be a reasonable proxy for sea lamprey ammocoete lipid content.

If ammocoete $\delta^{13}C$ is corrected for lipid content on the basis of the animals' C:N following Post *et al*. (2007), the resultant $\delta^{13}C$ values become more elevated (as high as -15.5‰) and even less plausible given the potential food sources in these streams. Therefore, ammocoete $\delta^{13}C$ values were not further corrected for lipid or other potential compositional changes in our models of diet composition. Although lipids do not appear to provide a satisfying explanation of ammocoete $\delta^{13}C$ and $\delta^{2}H$ values, other physiological mechanisms may underly or contribute in addition to lipid effects.

Assuming that ammocoete $\delta^{13}C$ and $\delta^{2}H$ ultimately reflect their food and nutritional sources, then the isotopic shifts with body size (Figure 5b and d) suggest that the animals consume different (i.e., isotopically unique) food sources during growth. Smaller ammocoetes have more depleted $\delta^{13}C$ (~-32‰, Figure 5b) values and more enriched $\delta^{2}H$ values (~-180‰; Figure 5d), which both suggest a

greater reliance on fresh terrestrial and/or aquatic OM (e.g., fresh or decaying plant material). The enrichment in $\delta^{13}C$ (~-23‰) and depletion in $\delta^{2}H$ (~–220‰) of larger ammocoetes suggests a reliance on proportionately greater microalgal (water column and/or benthic) production as no other known food source exist that approaches these values (Finlay 2001; Finlay et al. 2010; Caraco et al. 2010). Limited epilithic microalgal samples were able to be collected in this study (Table 4); however, these samples were observed to contain large proportions of detrital material and not representative of "pure" microalgae (also confirmed by isotopic measurements of these samples; Table 6). Nonetheless, estimates of microalgal $\delta^{13}C$ from our $\delta^{13}C$-DIC values (Table 2), and of microalgal δ^{13} (-12‰ to -34‰) and $\delta^{2}H$ (-136‰ to -264‰; Finlay et al. 1999, 2010) measurements from the literature, fully constrain the observed $\delta^{13}C$ enrichment and $\delta^{2}H$ depletion of large ammocoetes in the present study (also see *Estimates of Nutritional Subsidies to Sea Lamprey Ammocoetes* below for additional explanation).

Isotopic Comparison of Ammocoete Gut Material and Muscle Tissue

The $\delta^{2}H$ values of ammocoete gut OM (mean -173‰) were similar to values of aquatic and terrestrial plants (~-160‰; Table 5, Table 6) suggesting that ammocoete GC are dominated by recently deposited detrital components of plants (Figure 9), rather than by diagenetically altered sediment and soil OM components. Ammocoete muscle was more depleted in $\delta^{2}H$ than either GC ($\Delta\delta^{2}H$=-34‰, where $\Delta\delta^{2}H=\delta^{2}H_{ammocoete} - \delta^{2}H_{GC}$), aquatic macrophytes ($\Delta\delta^{2}H$= -38‰), or terrestrial plants ($\Delta\delta^{2}H$=-59‰), suggesting that the animals may digest

and assimilate specific dietary components of ingested OM, extracting ^{2}H-depleted forms of OM for their nutrition. Although there appears to be ^{2}H enrichment toward the posterior of the ammocoete gut (Table 11, Figure 9), this was marginally non-significant (p=0.06, when a single deviant value was removed) along the length of the gut. Nonetheless, these data suggest that ^{2}H-depleted materials may be extracted during gut passage and ^{2}H-enriched materials may be passed along the gut and excreted. While we cannot rule out the possibility that ingested material was influenced by animal material (mucous, gut lining, etc.) when GC were extracted, accounting for this would result in even greater divergence of ammocoete muscle tissues and GC $\delta^{2}H$ values, thus making our observed gut-animal differences conservative. Although the ammocoetes analyzed for GC were variable in length (5.6-10.7 cm), the gut content $\delta^{2}H$ values were similar (Table 11), supporting a possible physiological explanation.

Estimates of Nutritional Subsidies to Sea Lamprey Ammocoetes

As noted previously, $\delta^{13}C$ and $\delta^{2}H$ values of ammocoetes (particularly larger animals) could not be fully constrained without the inclusion of another potential food source (i.e., "pure" microalgae) in our models (Figure 10a-f, Figure 11), because large ammocoetes were more enriched in $\delta^{13}C$ and depleted in $\delta^{2}H$ than all measured sources. Ammocoete isotopic values were fully constrained (excluding a single individual at the Pigeon River) by the isotopic signatures of directly measured (all sources except microalgae) and indirectly estimated (i.e., "pure" microalgae) potential food sources. Ammocoete $\delta^{13}C$ and $\delta^{15}N$ were not correlated either overall or at individual sites (Figure 10a and b, Figure 11). On

the basis of their $\delta^{15}N$ values, ammocoetes are therefore deduced to consume low trophic level food sources with a broad range of $\delta^{13}C$ values (Figure 10a and b, Figure 11). Interestingly, ammocoete $\delta^{15}N$ values were influenced by site (Figure 5c), suggesting that one or more food sources were variable in $\delta^{15}N$ between sites, and likely explained by differences in land use (e.g., forested vs. agricultural) and $\delta^{15}N$ values of associated N inputs (Lake et al. 2001; Anderson and Cabana 2005). In support of this, we found that terrestrial plant, aquatic plant, and terrestrial soil $\delta^{15}N$ values were variable between sites and to a similar extent as ammocoete $\delta^{15}N$ values from the same sites (Table 5, Table 4, Table 6, Table 9, Figure 10a, b, e, f, Figure 11).

To estimate the contributions of the potential food and nutritional resources to sea lamprey ammocoetes, we used the $\delta^{13}C$, $\delta^{2}H$, and $\delta^{15}N$ values of aquatic macrophytes, microalgae and terrestrial vegetation as the fundamental forms of fresh, contemporary primary production in our Bayesian model. We also included terrestrial soil and aquatic sediment OM as potential sources of nutrition. While both soils and sediments are expected to contain the same initial plant sources, diagenetic alteration may impart different isotopic signatures to them than fresh plant materials (Fogel and Tuross 1999). While SPOM was expected to be derived from the other sources (i.e., terrestrial and aquatic plants, soils and sediments; Cole and Caraco 2001; Hoffman et al. 2008), it was actually poor at explaining ammocoete isotopic values due to its anomalously enriched $\delta^{2}H$ values (mean -117‰; Figure 10c-f). This has also been found in a companion study of

Ohio, USA streams (Evans and Bauer, *submitted manuscript b*), suggesting that SPOM is unique from the other potential food sources in this study. Preliminary modeling with SPOM found that it specifically contributed <5% of ammocoete nutrition in all of our models, and thus it was not explicitly included in our final model.

Modeling of potential food source contributions using MixSIR estimated that ammocoetes from all sites relied on significant and similar amounts of both microalgae (35 - 64%) and fresh terrestrial plant derived OM (33 - 65%) to subsidize their nutritional needs (Table 12, Figure 12). Overall, ammocoetes from Jordan River 1 and Pigeon River had similar median terrestrial OM subsidies (44.3% and 40.4%, respectively), while microalgae had slightly lower median contributions for Jordan River 1 (42.4%; Figure 12a) compared to Pigeon River (57.4%; Figure 12b) ammocoetes (Table 12). Macrophytes contributed a maximum of 14% of ammocoete nutrition, while digenetically altered and more aged soil and sediment OM contributed $\leq$ ~10% to ammocoetes from both streams (Table 12, Figure 12).

Assuming that the size-dependent increases in ammocoete $\delta^{13}C$ and decreases in $\delta^{2}H$ (Figure 5b and d) are due to ontogenetic shifts in diet and nutrition, then ammocoete reliance on microalgae increases with length, while terrestrial plant dependence decreases proportionally at both sites (Table 12, Figure 12). For Jordan River 1, the model results indicate a progressive increase in microalgal subsidies across ammocoete size classes from 23.5% to 60.9% (i.e., a 37% increase), with a corresponding decrease (32%) in terrestrial plant

subsidies from 49.9% to 18.1% (Table 12, Figure 12a). Pigeon River ammocoetes showed a similar increase (31%) in the microalgal subsidy with increasing size (i.e., from 52.9% to 84.0%) and decrease (32%) in the terrestrial plant subsidy (i.e., from 40.3% to 6.0%) between the smallest and largest size classes, however, the intermediate size class was more similar to the smallest size class in both microalgal and terrestrial plant subsidies (Table 12, Figure 12b). These strong apparent ontogenetic increases in the proportions of microalgal vs. terrestrial plant OM subsidizing the largest ammocoetes may reflect their greater requirements for presumably higher quality nutritional resources as they approach metamorphosis.

As noted previously, it is possible that $\delta^{13}C$ and $\delta^{2}H$ are not entirely trophically controlled (see *Size -Dependant Changes in Ammocoete C:N and Isotopic Signatures*). Laboratory experiments in which ammocoetes are raised on food sources of known isotopic composition may provide valuable information in this regard (see, e.g., Shirakawa et al. 2009; Limm and Power 2011). If our model findings are confirmed, these results indicate a) that sea lamprey ammocoetes overall, and in particular smaller and younger animals, are reliant to a significant degree upon allochthonous OM production in order to subsidize and sustain their growth but also utilize comparable autochthonous contributions, from at least certain aquatic primary producers (i.e., microalgae); and b) that the strong ontogenetic increases in the proportions of microalgal vs. terrestrial plant OM subsidizing the largest ammocoetes may reflect a greater requirement for higher quality food compared to smaller ammocoetes.

Comparison of Isotope-Based Estimates of Sea Lamprey Ammocoete Nutrition to Prior Studies

Prior work on ammocoetes of different lamprey species generally focused on visual identification and bomb calorimetry of ammocoete GC (Table 13). Early workers identified algae (Creaser and Hann 1928; Manion 1967; Moore and Beamish 1973) as important to ammocoete diets (and therefore likely nutrition), but more recent work (Moore and Mallatt 1980; Sutton and Bowen 1994; Mundahl et al. 2005) has cast doubt upon its importance often citing the detrital component that dominates the gut by volume (Table 4). However, not all workers have agreed, suggesting that algae is still the dominant food source contributing to ammocoete nutrition (Moshin and Gallaway 1977; Yap and Bowen 2003), even though quantitative evidence of this has been lacking. As suggested by the offset in ammocoete gut OM vs. muscle tissues δ^2H values (Fig. 5), ammocoetes must internally digest and assimilate in one or more ways materials that are more depleted in δ^2H than the ingested material. In addition, the resemblance of ammocoete muscle δ^2H to δ^2H values of microalgae (Fig. 6c-f) further suggests that, even though algae may be quantitatively relatively scarce in the diet, they can still be an important contributor to ammocoete nutrition.

Also in contrast to most previous studies of ammocoete food and nutritional resources (Table 13), our findings (Table 12, Figure 12) suggest that the detrital component supporting ammocoete growth is largely derived from relatively fresh terrestrial plant sources. Since fresh terrestrial OM is influenced by land use, further research should evaluate how land use impacts ammocoete

isotopic signatures and nutritional subsidies by both allochthonous and autochthonous food sources. Our estimates of as much as one-half to two-thirds of ammocoete nutrition being subsidized by allochthonous terrestrial sources of OM in the Pigeon and Jordan Rivers, respectively, are consistent with estimates of allochthony to other aquatic organisms using natural abundance isotopic approaches, including zooplankton (2-77%; Cole et al. 2011; Karlsson et al. 2012), detritivorous fish (0-68%; Babler et al. 2011), and invertebrate filter feeders (e.g., Simuliidae, 32-52%; Rasmussen 2010). The unique lifestyle of the sea lamprey ammocoete as a functional benthic filter feeder for the majority of the animal's life is predicted to be an important aspect of both the availability and utilization of a significant proportion of allochthonous materials.

Quantitative assessment of the nutritional subsidies to the ammocoete portion, and within this extended larval stage, of the sea lamprey life cycle is important because ammocoete growth can be variable between rivers (Holmes 1990; Morkert et al. 1998; Griffiths et al. 2001), which may partly result from the rate at which ammocoetes ingest, digest and assimilate more labile OM. Ammocoete diet and nutrition may also be influenced by factors such as land use type which can have a profound impact on the inputs of both the absolute and relative amounts of allochthonous and autochthonous food resources. In addition, sea lampreys are an important member of many freshwater communities, and fundamental aspects of the prolonged ammocoete stage are still poorly understood. Better understanding the basic life history of sea lamprey, especially the feeding ecology of the ammocoete stage, may provide both further insights to

the factors limiting ammocoete growth and sea lamprey recruitment, and, potentially, better management tools for controlling this invasive species in the Great Lakes and other systems. Finally, the evolutionary and adaptive success of sea lampreys may, at least in part, be due to a) the ability of the larval phase to take advantage of a variety of food and nutritional resources, including terrestrial OM that dominates most lotic systems, b) ontogenetic shifts in diet and nutrition throughout the larval stage, and c) plasticity in the duration of the larval phase.

Chapter 2: Constraining Nutritional Resources Supporting Native Larval Lamprey in the Ohio River (USA) Watershed Using Multiple Stable Isotopes

Introduction

Lamprey are a group (Order: Petromyzontiformes) of primitive jawless fishes that are predominantly distributed in temperate to arctic regions of the northern hemisphere (Joseph 2006). Lampreys have historically been important members of many fish communities and were widely distributed throughout their range (Hardisty and Potter 1971a; Hardisty 2011). However, many populations of native lampreys are currently threatened because of anthropogenic activities which have degraded habitat for all life stages and impeded the migration of juveniles and adults (Renaud 1997; Close et al. 2002; Mesa and Copeland 2009). Larval lampreys (also known as ammocoetes) may be particularly vulnerable to human activities, in part because they require as long as 1-2 decades to complete this life stage (Manion and Smith 1978; Farmer 1980; Mundahl et al. 2005).

Ammocoetes grow in low-flow sandy or silty stream sediments filter feeding (Morman et al. 1980; Beamish and Lowartz 1996) for an average of 5 ± 2 years (Purvis 1979; Potter et al. 1986; Mundahl et al. 2005), although in some tributaries metamorphosis to the juvenile stage has been reported to occur in as

little as 2 (Morkert et al. 1998) or as long as 17 years (Manion and Smith, 1978). Water temperature, stream productivity and ammocoete density may all factor into the observed variability in the length of the ammocoete life stage, however, the relative impacts of these factors are not well-established (Holmes 1990; Rodriguez-Munoz et al. 2001).

While considerable recent interest has developed in lamprey conservation (Renaud 1997; Ojutkangas et al. 1998; Close et al. 2002), there is relatively little understanding of ammocoete diets and nutritional sources exist. Identification of the nutritional resources supporting ammocoetes are critically needed in order to understand lamprey impacts on their ecosystems, including other benthic organisms and communities, the and to establish efficient conservation strategies of this primitive fish family. Previous dietary studies of native lamprey larvae have focused on semi-quantitative gut-content analysis techniques (Sutton and Bowen 1994; Yap and Bowen 2003; Mundahl et al. 2005). Analysis of gut contents provides little information on the amounts and forms of ingested materials that are assimilated by an organism (Grey et al. 2002; Michener and Kaufman 2007). As a result of the limitations of these previous approaches, our knowledge of feeding and nutrition in the ammocoete stage of all lamprey species is at best ambiguous and largely qualitative in nature.

Natural abundance isotopes represent a potentially far more robust and quantitative approach than gut content analysis for assessing the types of organic matter supporting an organism's somatic growth and energetic maintenance. Naturally occurring isotopes of C, N, H, O and S in organic materials may be used

to follow these elements through organisms, food webs, and even ecosystems, provided the distributions of the isotopes can be measured in both the relevant potential food sources and the target organisms (Rounick and Winterbourn 1986; Peterson and Fry 1987; Post 2002). In biological systems, the lighter isotope of an element is processed at the enzymatic level to a slightly greater extent than the heavier isotope due to mass-dependent effects, resulting in isotopic fractionation (Hoefs 2004; Michener and Lajtha 2007). For example, $\delta^{15}N$ is known to fractionate at ~3‰ for each trophic level while $\delta^{13}C$ fractionates 0-1‰ per trophic level (Peterson and Fry 1987). Therefore, the ability to estimate both food source and trophic level further expands the usefulness of an isotopic approach.

The goals of the present study were to identify and quantify food and nutritional resources supporting the critical ammocoete stage of two native species (*Lampetra aepyptera* and *Lethenteron appendix*) of non-parasitic lamprey common throughout their range in Ohio (Page and Burr 1991). We predicted that: 1) *Lampetra aepyptera* and *Lethenteron appendix* ammocoete natural abundance isotopic values would reflect a diet composed primarily of allochthonous terrestrial detrital organic matter (OM) which dominates the majority of temperate inland waters (Jones et al. 1998; Bauer and Bianchi 2011; Bianchi and Bauer 2011), and that 2) ammocoete diets and nutrition would vary both temporally (e.g., seasonally) and spatially (e.g., as a function of different watersheds).

Materials and Methods

Study Sites

Lampetra aepyptera (Least brook lamprey, LBL) ammocoetes were collected from the Clear Fork River in eastern Ohio (Figure 13a) and *Lethenteron appendix* (American brook lamprey, ABL) ammocoetes were collected from the Mad River in western Ohio (Figure 13b). Both the Clear Fork and Mad Rivers are within the greater Ohio/Mississippi River basin. The Clear Fork River is a 3rd order river that drains a watershed of 512 km^2 (Table 14). Sampling was conducted on a 1st (Clear Fork River 2, CFR2) and 3nd (Clear Fork River 1, CFR1) order stretch of the river. The Clear Fork River watershed has ~47% agricultural land use, ~41% forest cover and ~12% urban development (Table 15; Office of Policy, Research and Strategic Planning 2009). The Mad River is a 3rd order river that drains a watershed of 164 km^2 (Table 14). Sampling was conducted within both 2nd (Mad River 2, MR2) and 3rd (Mad River 1, MR1) order reaches of the Mad River. The Mad River watershed is dominated by agricultural land use (81%), and has ~18% forest cover (Table 15; Ohio EPA 2005). Both rivers were sampled in July and November 2010.

Ammocoete Collection

Ammocoetes were collected using a backpack electrofisher (model ABP-2MP-600V, Electrofishing LLC) following electrofishing procedures described by Moser et al. (2007) and the manufacturer's recommendations. Upon emergence from their burrows, ammocoetes were immobilized, netted, rinsed and wrapped individually in pre-baked (500ºC for 4 hours) sheets of aluminum foil before

being sealed in a self sealing plastic bag and placed on dry ice. Upon return to the laboratory ammocoetes were frozen at -20ºC until they were processed.

Collection of Potential Ammocoete Food Sources

Suspended Particulate Organic Matter (SPOM). SPOM is defined as material that is retained by a ~0.8 μm nominal pore size filter (Cole et al. 2006; Caraco et al. 2010). SPOM is composed of numerous sources and may include bacteria, plankton, detrital vegetation, and soil material (Cole and Caraco 2001; Hoffman et al. 2008). SPOM samples were collected at each site by filtering stream water through a pre-baked (525ºC for 4 hours) 47 mm quartz fiber QMA filters (Whatman; 0.8 μm nominal pore size) using a peristaltic pump equipped with 10% HCl cleaned silicone tubing. Filters with collected SPOM were immediately placed in pre-baked aluminum foil and frozen until processing.

Stream Sediment Organic Matter (SOM). Sediment cores, consisting of cut-off 10% HCl cleaned 60 cc plastic syringes, were collected to a depth of ~8 cm from pools in which ammocoetes were collected in undisturbed locations. Following collection, the bottom and top of the core were covered with pre-baked (500ºC for 4 hours) aluminum foil and the core was placed upright on dry ice in a self sealing plastic bag. Upon return to the lab the cores were stored at −20ºC until processing and analysis.

Soil Samples. Soils were excavated within ~10m of the stream to a depth of ~30 cm using a spade, wrapped in pre-baked aluminum foil and frozen on dry ice in the field. Soils were stored at -20ºC in the lab until processing.

Aquatic Macrophytes and Terrestrial Vegetation. The dominant species of

terrestrial and aquatic vegetation were collected by identifying abundant local species and handpicking leaf material using clean nitrile gloves. Samples were stored in individual self sealing plastic bags and frozen on dry ice until return to the lab and storage at -20ºC, after which they were processed.

Dissolved Inorganic Carbon (DIC). Stream water samples for DIC analysis were pumped using bubble-free techniques into pre-baked (450ºC for 4 hours) gas-tight sealed serum bottles (125 mL; Wheaton Co.) poisoned with 200 µl of saturated $HgCl_2$ solution and previously purged using ultra-high purity N_2 gas. The sample bottles were stored at room temperature in the dark until extraction in the laboratory.

Natural Abundance Isotope Analyses

Ammocoete Muscle Tissue. Muscle tissue from each ammocoete were surgically removed following isotopic clean protocols. Muscle tissue was dissected from the muscle on the third segment (the segment containing the 50-75% length of gut) of the ammocoete, unless there was too little muscle tissue for analysis, in which case muscle from the second segment was also dissected. For ammocoetes that were <3.5cm long, muscle from segments 1-4 was dissected. The muscle tissue was dried at 60 ºC for 48 hours, homogenized by grinding, and stored in pre-baked glass scintillation vials placed in a dessicator (<~10% relative humidity) until isotopic analysis.

Potential Food Sources. Aquatic and terrestrial plant tissues were dried to constant mass at 60ºC. After drying each sample was homogenized by grinding and stored in pre-cleaned scintillation vials in a dessicator as above for muscle

tissues.

SPOM filters were dried at 60ºC for 24 hours, and then fumed with stock HCl for 24-48 hours, in a clean glass dessicator, to remove inorganic C. Following acid fuming, filters were placed under vacuum for 24-48 hours to remove residual acid fumes and then dried for 24 hours at 60 ºC. After drying, the filters were cut into quarters using a solvent cleaned razor blade and stored in a clean polycarbonate dessicator until isotopic analysis.

Thawed stream sediment cores were sectioned by extruding the frozen core with a plunger and dividing the core into 1 cm increments with a clean metal razor, except for the segment closest to the water-sediment interface, which was divided into two 0.5 cm increments. Each segment was dried at 60ºC in pre-baked glass petri dishes for 24 hours, and then fumed with concentrated HCl for 24-48 hours to remove inorganic C. Following acidification samples were placed under vacuum for 24-48 hours to remove any residual acid fumes. Samples were then homogenized and stored in pre-cleaned glass scintillation vials in a dessicator until isotope analysis. Terrestrial soil samples were processed in the same manner as stream sediments.

DIC samples were extracted by acidification with 0.2 mL of 85% Phosphoric acid to a pH<2 and cryogenic collection of CO_2 on a vacuum extraction line using ultra-high purity He gas to strip the samples. Following extraction, sample CO_2 was stored in clean gas-tight glass ampoules (pre-baked 6 mm Pyrex) at room temperature until isotope analysis.

Isotopic Analysis. Subsamples of each sample type were packed in tin

capsules for analysis of $\delta^{13}C$ and $\delta^{15}N$ at the University of California, Davis (PDZ Europa ANCA-GSL (EA) attached to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS)) or at the Ohio State University's Stable Isotope Biogeochemistry Laboratory (Costech EA with continuous flow by CONFLOIII attached to a Finnigan Delta Plus IV IRMS). The standard deviations for replicates of standards for these instruments was $\leq$ 0.2‰ for $\delta^{13}C$ and $\leq$ 0.3‰ for $\delta^{15}N$. A quarter of each was combusted for $\delta^{13}C$ and $\delta^{15}N$ analysis by EA-IRMS.

Separate subsamples of select samples were packed in silver capsules and analyzed for deuterium ($\delta^{2}H$) by a DELTA plus XL RIMS attached to a ConFlo II and using a Carlo Erba NC2100 EA at the Northern Arizona University, Colorado Plateau Stable Isotope Laboratory (CPSIL). The standard deviation for replicates samples for this instrument was $\leq$ 2‰ for $\delta^{2}H$.

Water Column Physical Measurements

Basic water quality parameters were measured at each site during each sampling time and were pH (Fisher Scientific Portable Laboratory), dissolved oxygen (YSI Model 55), conductivity (YSI Model 30, only during the November sampling) and temperature.

Statistical Analysis

An ANOVA of $\delta^{13}C$ that included species, dates, the interactions of species and site (species was tested as a nested factor since only one species was found at each site), and the interaction of species date and site, as well as the covariates length and C:N, was performed. In addition, a separate ANOVA of $\delta^{15}N$, that did not include the covariate of length, but that included the covariate

C:N, and the effects of site, date, species' interaction with site, and the interaction of species, date and site, was performed. Since SOM was analyzed from different depths that could be isotopically unique (in addition to the influence of site and date), we included depth as a factor.

Isotope Mass Balance Model

We used the Bayesian stable isotope mixing model MixSIR (Moore and Semmens 2008) to estimate contributions of potential food sources to native lamprey ammocoetes. MixSIR offers the ability to incorporate uncertainty of a source's isotopic value into a mixing model to better express the predicted contributions and uncertainty of food source contributions to an organism (Moore and Semmens 2008; Kim et al. 2011). The ammocoetes and potential nutritional resources for each site were modeled separately. The isotopic means and standard deviations of potential sources were drawn only from samples collected at the site being modeled except in the case of microalgae. Macroalgae samples were used in place of microalgae signatures in our study. However, we had limited numbers of clean reliable macroalgae sources, so we supplemented our data with literature values. We also estimated a range of potential microalgae $\delta^{13}C$ signatures based on our stream water DIC $\delta^{13}C$ values (Table 16), DIC-microalgae $\delta^{13}C$ relationships from the literature (Finlay et al. 1999; Finlay 2001), macroalgae $\delta^{13}C$ values, and C_3 submerged aquatic macrophyte $\delta^{13}C$ values from our study. For $\delta^{15}N$ we used the macroalgae values, and at sites without macroalgae samples, microalgae were assumed to have $\delta^{15}N$ equal to other aquatic macrophytes and means and standard deviations were drawn from our data. $\delta^{2}H$ values were drawn

from our own data, if a site did not have macroalgae sample, the mean macroalgae value at the paired site was used. Since macroalgae δ^2H samples were limited, the largest calculated standard deviation of δ^2H for any source at the site was used in the model as the standard deviation of microalgae (~16‰).

Ammocoetes are considered primary consumers (Mallatt 1982; Sutton and Bowen 1994) and therefore for our model we assumed they were one trophic level above primary producers and followed published fraction values from the literature of 0.4± 1.3‰ for $\delta^{13}C$, 3.4 ±1.0‰ for $\delta^{15}N$ (Post 2002), and 0 ± 20‰ for δ^2H (Hobson et al. 1999; Jardine et al. 2005). All sites were resampled $\geq 1x10^5$ times, which resulted in an importance ratio of <0.001 and >1,000 posterior draws in all cases, following the recommended guidelines for determining if the model output has estimated true posterior distributions (Moore and Semmens 2008).

Results

Stream Water Parameters

The ammocoetes collected in this study were from cool (<23°C), highly oxygenated (>9.5 $mg \cdot L^{-1}$, >97% saturation) waters (Table 14). Stream waters were neutral to slightly basic (7.12-8.22) and had conductivities between 165.8-345.3 $\mu S \cdot cm^{-1}$ (at 25°C). In addition, water clarity was high at all sampling sites and the stream bottom was always visible at the maximum depths (~2m) of each stream (T. Evans, *personal observation*).

Least Brook and American Brook Lamprey Ammocoete Characteristics

LBL collected in this study had a bimodal distribution, with a mean length of 7.9±3.8 cm (Table 17, Figure 14a) and ranged in size from 1.8 to14.6 cm. ABL had a mean length of 14.5±2.7 cm, and ranged from 8.0-18.9 cm (Table 18, Figure 14b). ABL appeared to have a bi-modal distribution, with a large decrease in the collection of animals larger than 17 cm (Table 18, Figure 14b). Length-mass correlations (Figure 15) were unique for the two species, and while they did not have different intercepts (ANCOVA, $F_{1,69=}0.16$, p=0.69), these relationships did have different slopes (ANCOVA, $F_{1,69=}98.08$, p<0.0001).

The C:N values for LBL were right-skewed with a mode at 4.5 (Figure 16a). ABL C:N had three distinct peaks centered around 4.7, 6.5 and 8.4 (Figure 16b). C:N was significantly correlated positively with length only at CFR1 (R^2=0.29, p=0.005; Figure 17a) and at CFR2 ($R^{2=}0.35$, p=0.008). For the Mad River, ABL length was significantly positively correlated to C:N only at MR2 (R^2=0.24, p=0.028; Figure 17b), but not at MR1 ($R^{2=}0.05$, p=0.38).

The $\delta^{13}C$ values of ammocoetes were positively correlated with animal length at both CFR1 (R^2=0.35, p=0.03; Figure 17c) and CFR2 (R^2=0.48, p=0.001). The correlation of $\delta^{13}C$ to length was not significant at MR1 (R^2=0.04, p=0.43), however, at MR2 the relationship was correlated with length (R^2=0.27, p=0.012; Figure 17d). There were no correlations at any site between $\delta^{15}N$ and animal length (p>0.55 at all sites; Figure 18). Ammocoete δ^2H values were significantly correlated with length at CFR1 (R^2=0.89, p=0.005; Figure 19a) and

MR1 (R^2=0.65, p=0.029; Figure 19b), but not at MR2 (R^2=0.28, p=0.18; Figure 19b).

Species Comparisons

LBL and ABL ammocoetes had different means (ANOVA, $F_{1,63}$=19.71, p<0.0001), and dates also had different means ($F_{1,63}$=47.74, p<0.0001). The interaction of species and site was significant ($F_{2,63}$=23.25, p<0.0001) but the interaction of species, date and site was not ($F_{3,65}$=2.59, p=0.06). Both length ($F_{1,63}$=35.59, p<0.0001) and C:N ($F_{1,63}$=34.85, p<0.0001) were significant.

Species ($F_{1,64}$=35.03, p<0.0001) and date ($F_{1,64}$=14.39, p=0.0003) were both significant, but only the interaction of species and site ($F_{2,64}$=20.15, p<0.0001) was significant. The interaction of species, date and site was not significant ($F_{3,64}$=1.67, p=0.18), while the covariate (C:N) did have a significant effect on $\delta^{15}N$ ($F_{1,64}$=12.81, p=0.0007).

Isotopic Characteristics of Potential Food and Nutritional Sources

Aquatic primary producers. Aquatic macrophytes had a mean $\delta^{13}C$ of -30.7‰ (±0.7‰, SD) at CFR1, -30.9‰ at CFR2 (only a single aquatic plant was collected at this site; Table 19, Table 20), -33.4‰ (±1.8‰) at MR1, and -29.2‰ (±2.5‰) at MR2 (Table 21). CFR1 macroalgae had a mean $\delta^{13}C$ of -26.3‰ (±2.7‰), while at MR2 a single macroalgal sample had a $\delta^{13}C$ value of -33.0‰. CFR1 aquatic macrophytes had a mean $\delta^{15}N$ of 19.9‰ (±4.3‰) and the single sample at CFR2 had a $\delta^{15}N$ of 7.4‰ (Table 19, Table 20). In addition, macroalgae samples from CFR1 had a mean $\delta^{15}N$ of 7.6‰ (±2.1‰). At MR1 aquatic macrophytes had a

mean $\delta^{15}N$ value of 10.8‰ (±1.1‰), and at MR2 the mean macrophytic $\delta^{15}N$ was 6.0‰ (±0.4‰; Table 21). The aquatic macroalgae sample at MR2 had a $\delta^{15}N$ of 7.5‰. The mean $\delta^{2}H$ value of aquatic macrophytes at CFR1 was -124‰ (±14‰; Table 19, Table 20) while at MR1 and MR2 the mean $\delta^{2}H$ values were -120‰ (±6‰) and -111‰ (±20‰), respectively (Table 21). Samples of macroalgae at CFR1 and MR2 had $\delta^{2}H$ values of -194‰ and -171‰, respectively.

Terrestrial Plants. Terrestrial C_3 plant mean $\delta^{13}C$ values were -30.4‰ (±1.3‰), -30.6‰ (±1.7‰), -31.2‰ (±1.0‰), -30.6‰ (±1.4‰) at the CFR1, CFR2, MR1 and MR2, respectively (Table 19, Table 20, Table 21). The $\delta^{15}N$ of terrestrial C_3 plants was 4.2‰ (±3.0‰) at CFR1, 2.1‰ (±1.7‰) at CFR2, 2.8‰ (±2.3‰) at MR1, and 3.3‰ (±3.1‰) at MR2. The mean $\delta^{2}H$ of terrestrial plants at CFR1 was -120‰ (±15‰), at MR1 it was -120‰ (±6‰), and at MR2 it was -135‰ (±15‰; Table 19, Table 20, Table 21).

Stream Water Dissolved Inorganic Carbon (DIC). The $\delta^{13}C$ of DIC (the carbon source used by submerged aquatic plants and algae) was very similar at all four sites. The mean $\delta^{13}C$ values -11.0‰ at Clear Fork 1, -11.9‰ at Clear Fork 2, -11.4‰ at MR1 and -11.8‰ at MR2 (Table 16).

Stream Water Suspended Particulate Organic Matter (SPOM). SPOM had a mean $\delta^{13}C$ of -31.6‰ (±0.2‰) at CFR1, -29.5‰ (±1.9‰) at CFR2, -32.0‰ (±1.7‰) at MR1, and -31.1‰ (±0.1‰) at MR2 (Table 22). The means of $\delta^{15}N$ values across the four sites was 4.9‰ (±0.8‰), 5.4‰ (±1.6‰), 3.9‰ (±2.5‰), and 4.5‰ (±0.1‰), at the CFR1, CFR2, MR1, and MR2 respectively. Only three

δ^2H SPOM values were measured in this study, and were -113‰, -107‰ and -95.9‰ CFR1, MR1 and MR2, respectively.

Aquatic Sedimentary Organic Matter (SOM). An ANOVA showed that sediment depth ($F_{8,11}$=3.87, p=0.021) and site ($F_{3,11}$=80.13, p<0.001) were significant. The interaction of site and depth was marginally significant ($F_{16,11}$=2.72, p=0.05). However, pairwise comparisons of sediment depths found that only one significant difference in means existed, which was between the 4-5 cm depth and the 0-0.5 cm depth (p=0.021). Site was significant (p<0.0001), with all pairwise comparisons having different means except that between MR2 and CFR1 (p=0.72). The means of δ^{13}C at CFR1 and CFR2 were -24.3‰ (±2.0‰) and -27.5‰ (±0.6‰) respectively (Table 19, Table 23). The mean δ^{13}C at MR1 was -23.3‰ (±3.8‰), the mean δ^{13}C at MR2 was -24.1‰ (±4.6‰; Table 21).

A separate ANOVA with δ^{15}N of SOM as the response and site, depth, and the interaction of site and depth found no significant influence of site ($F_{3,11}$=80.13, p=0.11), depth ($F_{8,11}$=0.41, p=0.89), or their interaction ($F_{16,11}$=1.54, p=0.24). However, sites were considered separately during food source contribution modeling and their means are reported here. The mean SOM δ^{15}N values for the four sites were 4.3‰ (±0.8‰) at CFR1, 3.5‰ (±1.1‰) at CFR2, 4.2‰ (±1.1‰) at MR1, and 3.6‰ (±0.8‰) at MR2.

SOM δ^2H data were extremely limited and an ANOVA was not appropriate for these data. SOM had a mean δ^2H of -120‰ (±1‰) at CFR1, -129‰ (±5‰) at MR1, and -126‰ (±5‰) at MR2 (Table 19, Table 23).

Terrestrial Soils. The mean $\delta^{13}C$ soil organic matter at was -29.0‰ (±1.7‰) at CFR1, -28.1‰ (±1.5‰) at CFR2 (Table 19, Table 24), -23.3‰ (±3.8‰) at MR1, and -23.3‰ (±5.4‰) at MR2 (Table 21). The mean $\delta^{15}N$ of soil organic matter was 2.0‰ (±2.0‰), 3.9‰ (±1.6‰), 4.0‰ (±1.3‰), and 3.6‰ (±2.1‰) at CFR1, CFR2 (Table 19), MR1, and MR2 (Table 21), respectively. The mean $\delta^{2}H$ of soil organic matter was -124‰ (±13‰) at CFR1, -124‰ (±4‰) at MR1, -101‰ (±18‰; Table 21, Table 24).

Discussion

Ammocoete Size and C:N Distributions

Length distributions of ammocoetes from the Clear Fork River suggest that these animals grew more slowly than those from the Mad River (Figure 14). LBL ammocoetes from the Clear Fork River had a broader range of sizes than ABL ammocoetes from the Mad River, possibly a result of higher water temperatures in the Clear Fork River (22.6-22.8°C) than the Mad River (16.3-20.2°C) during the July sampling (Table 14). The higher Clear Fork temperatures were outside of the range thought to be optimal for ammocoete growth (17.8-21.8°C; Holmes and Lin 1994), likely slowing overall growth. However, at the Mad River temperatures were within the optimal growing temperatures allowing ammocoetes to have a longer growing season.

In addition to animal size, lipids are important in controlling ammocoete metamorphosis (Lowe et al. 1973; Moore and Potter 1976; O'Boyle and Beamish 1977). Although we did not measure lipids directly, the C:N of aquatic animals

(including many fish species) has been be used as a proxy for estimating lipid content (Post et al. 2007). One strategy to accumulate lipids for metamorphosis is known from the sea lamprey (*Petromyzon marinus*) where ammocoetes maintain low levels of lipids until they approach their maximum size, at which point they cease significant growth and rapidly accumulate lipids ("all or none" strategy; O'Boyle and Beamish 1977). The other strategy, identified in *Lampetra fluviatilus* and *L. planeri*, is for animals to accumulate lipids annually without depleting them to initial levels, until adequate accumulation of lipids allows metamorphosis to occur ("rising tide" strategy; (Moore and Potter 1976).

While LBL are more closely related to *L. fluviatilus* (Docker et al. 1999; White and Martin 2009) than to sea lamprey, LBL in the present study had a C:N distribution suggesting they may follow the "all or none" strategy employed by sea lamprey (Figure 16a). The majority of LBL ammocoetes had relatively low C:N ($\leq$ 5.0), but the largest animals had higher C:N values ($\geq$ 7.0; Figure 17a). It is not surprising that elevated C:N was not limited to large ammocoetes as ammocoetes may metamorphose over a wide range of sizes (Hardisty 1961; Purvis 1970; Bird and Potter 1979). The positive correlation of C:N to length of LBL (Figure 17a) suggest that the minimum size for metamorphosis is ~10 cm, similar to reported values (Docker and Beamish 1991, 1994).

In contrast to LBL ammocoetes, the C:N vs. length distribution of ABL ammocoetes in this study suggests they may utilize the "rising tide" approach of lipid storage (Figure 16b). The three C:N maxima may result from sequential years of increasing lipid content during animal growth. In addition, the C:N peak

at ~8.5 is broader than the other two peaks, suggesting that multiple year classes may begin to overlap during what has been called the "arrested growth" stage (Leach 1946; Hardisty and Potter 1971b). That is, as ABL ammocoetes of different age classes attain the lipid levels required for metamorphosis, older individuals that fail to reach the necessary lipid levels may still be able to maintain relatively high levels of lipids until the following year. In addition, rapidly growing younger ABL ammocoetes may overtake older ammocoete in size and exaggerate the C:N peak at ~8.4 (Figure 16b).

The observed C:N distribution for ABL ammocoetes (Figure 16b) suggests that young-of-year (YOY) animals were captured at both Mad River sites. The minimum C:N for ABL (4.1) was not different from the minimum C:N for LBL (4.1), suggesting that we successfully captured YOY of both species and potentially offers a way to estimate age in ammocoetes for species utilizing the "rising tide" strategy of lipid accumulation. The weak relationship between C:N and length, which was only significant at the MR2 site for ABL (Figure 17b), is in agreement with our current understanding that length is a poor proxy for ammocoete age, particularly between different streams or even different reaches of the same stream (Potter 1970; Kelso and Todd 1993; Quintella et al. 2003).

Isotopic Shifts Associated with Ammocoete Growth

The $\delta^{13}C$ values of LBL and ABL ammocoetes increased with animal length (Table 17, Table 18, Figure 17c and d), suggesting that the animal's food source changed during growth. Small (<6 cm) LBL ammocoetes had relatively depleted $\delta^{13}C$ values (~-29‰ to ~-24‰; Table 17, Figure 17c) suggesting that

fresh terrestrial plant-derived OM was important to their nutrition. While we cannot reliably establish age in LBL ammocoetes, (see *Ammocoete Size and C:N Distribution*) these animals were likely YOY recruiting into the population (Figure 14a). In contrast, the $\delta^{13}C$ values of larger (>6cm) LBL ammocoetes (~-25‰ to ~-21‰;Table 17, Figure 17c) suggest a relatively greater reliance on ^{13}C-enriched food sources (e.g. microalgae or terrestrial soil OM; Finlay et al. 2010; Cole et al. 2011). $\delta^{13}C$ correlations with C:N were logarithmic and site was not important (Figure 20a), in contrast to the linear relationships fit by site (Figure 17c). The elevated ammocoete C:N values (~6 to ~10; Table 17, Figure 17a) primarily form CFR1 drove the correlation (Figure 20a), and suggests C:N in LBL does not well explain $\delta^{13}C$ values of ammocoetes.

ABL ammocoetes from the Mad River grew rapidly enough that animal length was a very weak proxy for age (see *Ammocoete Size and C:N Distribution*), so it is not unexpected that $\delta^{13}C$ would not correlate well with length (Table 18, Figure 17d). However, when ammocoete $\delta^{13}C$ is correlated with C:N, which may be useful as an age proxy, $\delta^{13}C$ is well explained ($R \geq 0.8$) and positively correlated at both sites. ABL ammocoetes with lower C:N (<5, and likely younger) were even more depleted in $\delta^{13}C$ (~-30‰ to ~-27‰; Figure 20b) than ammocoetes with higher C:N ($\geq$5, and likely older) were more enriched (~-27‰ to ~-22‰; Table 18, Figure 20b), suggesting a greater reliance on more $\delta^{13}C$ enriched food sources (i.e., microalgae or aged OM) as ABL ammocoetes increased in C:N (and possibly age).

The range of $\delta^{15}N$ values of LBL ammocoetes (>50‰ range; Table 17, Figure 18a, Figure 20c) suggest that N sources to the Clear Fork River are highly variable. The Clear Fork River watershed is evenly divided between agriculture (47%) and forested (41%) land uses, with smaller amounts of urban development (12%; Table 15). Each of these land use types and their dominant forms of nutrient N may influence the $\delta^{15}N$ values of terrestrial and aquatic OM (Lake et al. 2001; Anderson and Cabana 2005). In contrast to $\delta^{13}C$, site differences were not observed for $\delta^{15}N$ of ammocoetes in the Mad River (Figure 18b), suggesting that N sources to the Mad River are more similar and continuous along the study reach than the Clear Fork. This is supported by the dominance of land use in the Mad River watershed by agriculture (81%) with smaller amounts of forested land use (18%), in contrast to the great amount of mixed land use in the Clear Fork (Table 15). Fertilizers in cropland are known to have $\delta^{15}N$ values of ~+3 to +~12‰ which is consistent with our measured ammocoete $\delta^{15}N$ values at both sites (i.e., after accounting for enrichment from fractionation; Table 18, Figure 20d; Chang et al. 2002). In addition, $\delta^{15}N$ of ABL ammocoetes was negatively correlated with their C:N (Figure 20d), suggesting ammocoetes incorporate more ^{15}N-depleted material into their diet as they grow.

Ammocoete $\delta^{2}H$ was negatively correlated with length only at CFR1 (R^2=0.89; Figure 19a) and MR1 (R^2=0.65; Figure 19b), and those sites had different best fit lines. However, $\delta^{2}H$ was better explained by C:N (R=0.95), and accounted for species and sites (Figure 21a). Because lipid content has been found

to correspond to C:N for a range of organisms, including fish (Smith and Epstein 1970; Hobson et al. 1999; Soto et al. 2011), the inverse relationship between ammocoete δ^2H and C:N suggests that C:N in our study is a proxy for lipids. This relationship (Figure 21a) further suggests that ammocoetes may not shift nutritional sources as they grow or age, in contrast to the observed shifts in $\delta^{13}C$ (Figure 17c, d, Figure 20a and b) and $\delta^{15}N$ in Mad River ABL (Figure 20d). However, $\delta^{13}C$ and δ^2H values are significantly correlated (Figure 21b), suggesting that food sources influencing δ^2H are also driving $\delta^{13}C$ signatures. Microalgae subsidies to ammocoete nutrition potentially offer a way to explain this relationship (Figure 21b) since it is enriched in ^{13}C and depleted in ^2H (Doucett et al. 2007; Finlay et al. 2010; Caraco et al. 2010).

$\delta^{13}C$ is also known to be influenced by the lipid content of the tissues measured (DeNiro and Epstein 1977; Post et al. 2007), and ammocoetes have been found to have relatively high lipid contents in their muscle that are size and age dependent (up to 18% by wet mass; Moore and Potter 1976; O'Boyle and Beamish 1977; Beamish and Legrow 1983). In addition, our δ^2H correlation with C:N (Figure 21a) is supportive of the assertion that C:N was a good proxy for lipids (Figure 21a). However, the observed direct relationship of $\delta^{13}C$ enrichment with C:N (Table 17, Table 18, Figure 20a and b) and therefore lipid content, does not follow the predicted relationship of decreasing $\delta^{13}C$ as a function of increasing lipid content and C:N (Kiljunen et al. 2006; Post et al. 2007; Abrantes et al. 2012). A recent study of sea lamprey ammocoetes (Evans and Bauer,

submitted manuscript *a*) also found unusually high $\delta^{13}C$ enrichment (as high as –22‰) with increasing C:N , suggesting that this finding may be common to ammocoetes in general. While ammocoete $\delta^{15}N$ values were negatively correlated with C:N in ABL from the Mad River (Figure 20d), lipids are known to have low N content and are not expected to influence $\delta^{15}N$ values (DeNiro and Epstein 1977).

Several aspects of ammocoete feeding ecology may help explain the unexpected relationship between ammocoete $\delta^{13}C$ and C:N. First, ammocoetes are unique among filter feeding benthic organisms, in that they rely on high suspended particulate loads and low pumping rates (30 $mL \bullet g^{-1} \bullet h^{-1}$; Mallatt 1982) for efficient feeding (Mallatt 1984). Although ammocoetes are traditionally defined as filter feeders (Malmqvist and Brönmark 1982; Richardson et al. 2010; Bettaso and Goodman 2010), their feeding ecology likely reflects an ancient vertebrate feeding mode that has few good present-day analogues (Mallatt 1981, 1984). Related to this is the continuous re-ingestion of the mucous strand, used to capture food particles and comprises ~8% of the animal's gut content (Yap and Bowen 2003), may cause a progressive enrichment in the ^{13}C of this material, which is presumably ultimately assimilated. Second, one of the dominant food and nutritional sources of ammocoetes (see *Isotopic Mixing Model Estimates of Ammocoete Nutritional Subsidies*, and Evans and Bauer, submitted manuscript *a*), microalgae, tend to have relatively high lipid contents, and concomitant ^{13}C-enriched and ^{2}H-depleted isotopic signatures (Hobson et al. 2004; McKechnie 2004; Doucett et al. 2007). Therefore, if larger ammocoetes preferentially feed on

and assimilate microalgal materials, this could help explain the observed patterns in the size-dependent changes in ammocoete C:N, $\delta^{13}C$ (Table 17, Table 18, Figure 17) and $\delta^{2}H$ (Figure 19) and the correlations between these parameters (Table 17, Table 18, Figure 20, Figure 21). Finally, it is also possible that ammocoete isotopic values may not directly reflect food source contributions (also see Evans and Bauer, submitted manuscript *a*) because of metabolic pathways that obscure the food source signature (Pinnegar et al. 2001).

Isotopic Mixing Model Estimates of Native Ammocoete Nutritional Subsidies

Isotope-isotope plots of ammocoetes and their potential food sources suggest that Clear Fork River and Mad River animals derived their nutrition from multiple sources of OM at all sampling sites (Figure 22a-f, Figure 23a-d). In all cases ammocoetes were constrained by the measured potential nutritional source isotopic means and errors. We used a Bayesian model (Moore and Semmens 2008) to estimate the contributions of these potential dietary and nutritional resources to ammocoetes which included the following: 1) fresh terrestrial vegetation, 2) terrestrial soil OM, 3) aquatic macrophytes, 4) microalgae, and 5) aquatic sediment OM. While soils and sediments contain terrestrial and aquatic plant-derived materials, respectively, these materials may undergo significant post-depositional diagenetic alteration and aging (Fogel and Tuross 1999; Hoefs 2004) that alters the availability and reactivity of the OM to organisms. Therefore soil and sediment OM were used as independent sources in the model. SPOM, however, was not included in our models because it is a heterogeneous composite of all of the other five potential sources (Cole and Caraco 2001; Hoffman et al.

2008), and graphically SPOM does not well explain the isotopic signatures of ammocoetes at any site (Table 19, Table 21, Figure 22, Figure 23). In addition, preliminary modeling that included SPOM weakened the model's ability to distinguish source contributions and resulted in broad contributions from many sources. For these reasons, SPOM was not used explicitly it in our final model.

Model findings indicate that allochthonous subsidies were important to ammocoete nutrition. Fresh terrestrial vegetation was quantitatively important (9-54%) to ammocoete nutrition at all sites except for LBL from CFR1 (2% terrestrial vegetation; Table 25, Figure 22, Figure 23, Figure 24). Terrestrial soil OM displayed a broad range of median contributions (3-68%) to ammocoete nutrition across all sites (Table 25, Figure 24), but also indicated that this presumably more refractory source was still an important subsidy to ammocoete nutrition. Terrestrial soil OM was eroding from different depths and likely at different rates, potentially influencing the importance of this potential food source, through availability and nutritional quality, to ammocoetes. In terms of autochthonous forms of nutrition, microalgal subsidies to ammocoete nutrition were generally next in importance after fresh terrestrial vegetation (Table 25, Figure 24). Model estimates indicated that native lamprey ammocoetes relied on microalgae for 6-39% for support of their biomass across all four sites (Table 25, Figure 24). ABL ammocoetes from MR1 had the lowest contribution of microalgae (6.4%), likely because MR1 ammocoete $\delta^{13}C$ values were not as enriched and were not as depleted in $\delta^{2}H$ as other sites (Figure 22a-d, Figure 23a and b). The model estimated that LBL ammocoetes (CFR1 and CFR2 sites) did

not rely on aquatic macrophytes for more than ~6% of their nutritional needs (Table 25, Figure 24). However, contributions of aquatic macrophytes to ABL ammocoetes were higher (20%) at MR1, which may be a result of the large beds of macrophytes growing adjacent to ammocoete populations at this site (T. Evans, *personal observation*). Conversely, at other sites aquatic macrophytes were either scarce or were not present (Table 25; T. Evans, *personal observation)*. Contributions of microalgae to ABL at MR2 were the highest of any site (39%). In contrast to terrestrial soils, aquatic sediment OM was quantitatively important for ammocoetes only at CFR2 (52%) and had low contributions at other sites (<10%; Table 25, Figure 24). The CFR2 site was located adjacent to an agricultural field in a headwater that down cut deeply (>2m) into the soil (T. Evans, *personal observation*). Therefore, local aquatic sediment OM at this site may have been primarily composed of diagenetically altered terrestrial soils exposed by erosive forces.

Assuming that the size-dependent increases in ammocoete $\delta^{13}C$ and decreases in $\delta^{2}H$ (Figure 17c and d, Figure 19) are due to ontogenetic shifts in diet and nutrition, then native lamprey ammocoete reliance on microalgae generally increased with length, while other nutritional sources decreased proportionally with size (Table 25). For LBL from the Clear Fork, microalgae went from a marginal nutritional subsidy (<10%) in the smallest size class to a significant subsidy in the largest size classes at both sites (71% at CFR1 and 29% at CFR2; Table 25). At CFR2, regardless of size, there were large contributions to ammocoete nutrition from aquatic sediment OM (20 -30%), terrestrial soil OM

(18-23%) and terrestrial vegetation OM (12-19%; Table 25). Microalgal sources however, increased from 5% in ammocoetes 0-4 cm in length (and likely YOY) to 29% in the largest ammocoetes >8 cm in length (Table 25). These data suggest that ammocoetes at CFR2 are reliant on diverse nutritional resources to support their growth and development, but still increase dependence on microalgal sources as they increase in length.

At the Mad River sites, increases in length increased ammocoete reliance on microalgae only at MR2 (Table 25). At MR1, microalgal contributions actually decreased marginally in importance from 11 to 8% with increasing size, but at MR2 microalgal contributions increased from 14 to 40% as ammocoete length increased (Table 25). Terrestrial vegetation OM increased in importance with ABL ammocoete size at MR1 from 35% to 55% and at MR2 from 21% to 43% (Table 25). However, it should be noted that only a single individual was small enough to be estimated in the 0-10cm group at MR1, making this estimate somewhat tentative.

A previous study of ABL ammocoetes in Minnesota (USA) confirmed that undifferentiated detritus made up >85% of the ingested material by volume and that there were no statistically significant seasonal differences in this (Mundahl et al. 2005). Our models also suggest that microalgae, although rare in ammocoete guts (Sutton and Bowen 1994) is nutritionally important to larger and likely older ammocoetes as they prepare for metamorphosis, suggesting that ammocoetes may selectively digest the relatively rarer microalgal material at higher efficiencies than terrestrial plant materials and soil and sediment OM . Finally, the importance

of both terrestrial plants and microalgae to ammocoete diet and nutrition support the contention that seasonal differences in both allochthonous and autochthonous primary production (Holmes 1990; Morkert et al. 1998; Griffiths et al. 2001), while not explicitly examined in this study, may be an important factor to ammocoete nutrition on seasonal and annual bases (Yap and Bowen 2003; Mundahl et al. 2005).

Comparison of Ammocoete Elemental and Isotopic Characteristics

Prior elemental and isotopic studies in which ammocoetes are the organism of study are limited (Hollett 1995; Shirakawa et al. 2009; Limm and Power 2011, Evans and Bauer, submitted manuscript *a*), and even published isotopic data reported because of the incidental collection of ammocoetes, is rare (Table 26; Bilby et al. 1996; Riel et al. 2006; Bergfur et al. 2009; Marty et al. 2009). C:N distributions of invasive sea lamprey (*Petromyzon marinus*) ammocoete populations in two Michigan rivers had a mean of 7.1 and a range of 4.6-11.0 (Table 26; Evans and Bauer, submitted manuscript *a*). Sea lamprey ammocoetes are known to use an "all or none" strategy to store lipids (O'Boyle and Beamish 1977) but their C:N distribution did not well match the C:N distributions of native LBL (mean=5.7, range: 4.1 to 10.3; Figure 16a) or even ABL (mean=6.3, range: 4.1 to 9.4; Figure 16b) ammocoetes.

In contrast to sea lamprey, Pacific lamprey (*Entosphenus tridentatus*) ammocoetes were found to have low C:N values (4.8), similar to the lowest values in the present study (4.1), with a limited range suggesting lipid accumulation strategy similar to LBL (Table 26). Work on the biochemical

composition of ammocoetes would help to establish the C pools contributing to these variations in C:N values. C:N was significantly and positively correlated with length in SL (Evans and Bauer, submitted manuscript *a*), LBL (Figure 17c) and at one site for ABL (Figure 17d) in the present study. These correlations suggest that lipid stores in ammocoetes generally increase as size increases to facilitate metamorphosis (O'Boyle and Beamish 1977; Potter 1980), but differences in C:N distributions (Figure 16) and correlations (Figure 17a and b; Evans and Bauer, submitted manuscript *a*) between species suggest potential avenues of research.

The $\delta^{13}C$ and $\delta^{15}N$ values of ammocoetes from different studies suggest that isotopic differences in source materials in different systems, including those due to differences in hydrology and land use, can influence ammocoete isotopic values (Table 26). Interestingly, the range of $\delta^{13}C$ values in ammocoetes from published studies is as large as or larger than the paired $\delta^{15}N$ value's range (Table 26). The larger range in $\delta^{13}C$ compared to $\delta^{15}N$ in ammocoetes suggest that ammocoete ontogenetic shifts in diet are widespread, but could also be interpreted to indicate that internal metabolic alteration of internal ammocoete $\delta^{13}C$ signatures occurs across lamprey species. Only two studies to date have correlated ammocoete isotopic values to C:N and/or length (the present study, and Evans and Bauer, submitted manuscript *a*). In both studies, $\delta^{13}C$ and $\delta^{2}H$, and to a more limited extent $\delta^{15}N$, were shown to co-vary significantly with C:N and/or length. Therefore, long-term controlled laboratory feeding experiments using food

sources of know isotopic signatures are needed to establish if ontogenetic shifts are truly occurring.

Comparison of Nutritional Subsidies to Ammocoetes of Native and Invasive Lamprey

To estimate the contributions of allochthonous and autochthonous sources of nutrition to lamprey ammocoetes of different species, we assumed that terrestrial plants and soil OM were the primary allochthonous sources. In addition, while soil OM is ultimately derived from terrestrial plant materials, soil OM may be significantly altered in terms of its age, diagenetic status (and inherent OM molecular composition), and hence, reactivity (Fogel and Tuross 1999; Hoefs 2004). Therefore, terrestrial plants and soil OM were considered as separate allochthonous nutritional sources. Aquatic macrophytes, microalgae and aquatic sediment OM were considered to be autochthonous sources of nutrition, and similar to terrestrial soil OM, aquatic sedimentary OM was further considered a separate autochthonous source due to diagenetic and reactivity considerations. This approach allows for the most conservative estimate of allochthonous subsidization of ammocoete nutrition because aquatic sediments (assumed to be entirely autochthonously derived here) may actually contain significant amounts of both terrestrial plant and soil OM due to hydrological erosion and movement of these materials from the water shed to the study streams.

Findings from the present study of native lamprey ammocoetes, and a previous study on invasive sea lamprey ammocoetes (Michigan, USA; Evans and Bauer, submitted manuscript *a*), indicate that ammocoete nutrition and growth is

subsidized by multiple allochthonous and autochthonous sources that include significant allochthonous (i.e., combined fresh terrestrial plant and soil OM) sources (as high as ~41-70% at CFR1; Table 27). The single exception to this was for CFR2 where ammocoetes were less reliant on allochthonous sources (~20%) primarily due to a great contribution of aquatic sediment OM to autochthonous sources of nutrition (Table 27). As noted above, however, aquatic sediment OM is the most challenging of all of the five potential nutritional sources to characterize in terms of its autochthonous or allochthonous origins. Therefore, the lower allochthonous estimate for CFR2 is conservative and probably higher, as are the allochthonous subsidies to ammocoetes for the other sites for the same reasons. In addition, larger ammocoetes of both native and invasive species tend to have greater $\delta^{13}C$ and lower $\delta^{2}H$ values than smaller ammocoetes (Table 4,Table 17, Table 18, Table 27, Figure 5, Figure 16, Figure 19) and hence we have deduced that larger ammocoetes have greater autochthonous subsidies.

If, however, the isotopic shifts in larger ammocoetes are not entirely driven by ontogenetic shifts in food and nutritional resources as we have assumed for purposes of the model (see section *Isotopic Shifts Associated with Ammocoete Growth*) but include one or more internal mechanisms causing the isotopic shifts, then the allochthonous subsidy estimates to larger ammocoetes (and hence to the overall populations of each species of ammocoete as a whole) will be even larger than the estimates in Table 27. Given these considerations, it is reasonable to conclude that both native and invasive lamprey ammocoetes rely for about half or more of their nutritional subsidies from allochthonous (terrestrial) sources.

Clearly, however, autochthonous sources are a significant and indispensible part of ammocoete nutrition as well, and we thus further conclude that both allochthonous and autochthonous sources are important (Table 27).

It is noteworthy that allochthonous vs. autochthonous subsidies to ammocoete nutrition were not obvious between species, whether native or invasive, or between different streams (Table 6), leading us to conclude the ammocoete nutrition is similar across a variety of species and systems. Land use may still play an important role in ammocoete nutrition, but may be better estimated at local scales.

Findings that lamprey ammocoetes have significant nutritional subsidies by allochthonous sources (as much as 70%; Table 27) contrast with earlier studies of ammocoete gut content that concluded that ammocoete nutrition was dominated by sources such as microalgae and bacteria (Manion 1967; Moore and Beamish 1973; Potter et al. 1975). These findings are consistent with prior work that has estimated allochthonous subsidies to other aquatic organisms using natural abundance isotopic approaches, including zooplankton (2-77%; Cole et al. 2011; Karlsson et al., 2011), detritivorous fish (0-68%; Babler et al. 2011), invertebrate benthic filter feeders (e.g., Simuliidae, 32-52%; Rasmussen 2010), chironomids (63%, Karlsson et al., 2011), and in estimates to multiple species in a temperate river (40-60%, Cole and Solomon, in press). The unusual benthic feeding behavior and extended ammocoete stage of the lamprey life cycle may be an important factor controlling the availability of allochthonous forms of nutrition to these primitive fishes and the nutritional support of growth and development.

In addition, the similar nutritional resources of both native (this study) and invasive (Evans and Bauer, submitted manuscript *a*) lamprey, as well as between different species of native lamprey, further suggest that resource overlap may occur at sites were multiple species are present. Additional studies are therefore needed to better understand and establish if competitive effects on diets and nutritional resources are present in co-occurring species of ammocoetes.

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Appendix A: Tables

Site	Date	TFM Treatment	Water Temp. (^{0}C)	DO (mg•L^{-1})	pH	Conductivity (µS•cm^{-1} at 25°C)	Species Collected
Jordan River 1	June 10 2010	Sept 2007	13-15	>10	7.0-8.0	ND	*P. marinus* & native genera
	Oct 3 2010		8.2	10.21	8.28	205.1	*P. marinus* & native genera
Jordan River 2	June 10 2010	Sept 2007	13-15	>10	6.5-7.0	ND	*P. marinus* & native genera
	Oct 4 2010		6.9	12.27	8.17	201.1	*P. marinus*
Pigeon River	May 19 2010	July 2007	14.6	11.07	8.69	ND	*P. marinus*
	Oct 2 2010		10.6	9.61	8.23	214.0	*P. marinus*

Table 1. Site and ammocoete characteristics of the Jordan and Pigeon Rivers (Michigan, USA) sampled in the present study. ND– not determined.

Sample ID	Site	Date	$\delta^{13}C$ (‰)
JR1-15	Jordan River 1	June 10 2010	-11.23
JR1-13	Jordan River 1	June 10 2010	-11.07
JR1F-6B	Jordan River 1	Oct 3 2010	-11.41
JR2-1	Jordan River 2	June 10 2010	-11.29
JR2-2	Jordan River 2	June 10 2010	-11.43
JR2F-4A	Jordan River 2	Oct 4 2010	-10.70
P1-16	Pigeon River 1	May 19 2010	-10.86
P1-14	Pigeon River 1	May 19 2010	-11.08
PR1F-1A	Pigeon River 1	Oct 2 2010	-13.75
PR1F-2B	Pigeon River 1	Oct 2 2010	-11.42

Table 2. Dissolved inorganic carbon data from the Jordan and Pigeon Rivers measured in the present study.

Site	Collection Date	ID	Primer Name (*Pma*µ9)	Sea Lamprey
Jordan River 1	June 10 2010	J1 S6	250	Y
Jordan River 1	June 10 2010	J1 S7	250	Y
Jordan River 1	June 10 2010	J1 S8	250	Y
Jordan River 1	June 10 2010	J1 S9	250	Y
Jordan River 1	June 10 2010	J1 S10	None	N[a]
Jordan River 1	June 10 2010	J1 S11	250	Y
Jordan River 1	June 10 2010	J1 S12	250	Y
Jordan River 1	June 10 2010	J1 S13	250	Y
Jordan River 1	June 10 2010	J1 S14	250	Y
Jordan River 1	Oct 3 2010	J1F S1	250	Y
Jordan River 1	Oct 3 2010	J1F S2	None	N[a]
Jordan River 1	Oct 3 2010	J1F S3	250	Y
Jordan River 1	Oct 3 2010	J1F S4	250	Y
Jordan River 1	Oct 3 2010	J1F S5	250	Y
Jordan River 1	Oct 3 2010	J1F S6	250	Y
Jordan River 1	Oct 3 2010	J1F S7	250	Y
Jordan River 1	Oct 3 2010	J1F S8	250	Y
Jordan River 1	Oct 3 2010	J1F S9	250	Y
Jordan River 1	Oct 3 2010	J1F S10	250	Y
Jordan River 1	Oct 3 2010	J1F S11	250	Y
Jordan River 1	Oct 3 2010	J1F S12	250	Y
Jordan River 1	Oct 3 2010	J1F S13	250	Y
Jordan River 1	Oct 3 2010	J1F S14	None	N[a]
Jordan River 1	Oct 3 2010	J1F S15	250	Y
Jordan River 1	Oct 3 2010	J1F S16	250	Y
Jordan River 1	Oct 3 2010	J1F S17	250	Y
Jordan River 1	Oct 3 2010	J1F S18	None	N
Jordan River 1	Oct 3 2010	J1F S19	250	Y
Jordan River 1	Oct 3 2010	J1F S20	250	Y
Jordan River 2	June 10 2010	J2 S17	None	N[a]
Jordan River 2	June 10 2010	J2 S18	250	Y
Jordan River 2	June 10 2010	J2 S19	250	Y
Jordan River 2	June 10 2010	J2 S20	250	Y
Jordan River 2	Oct 4 2010	J2F S1	250	Y

Continued

Table 3. Genetic confirmation of ammocoetes using a microsatellite marker. Superscript a denotes ammocoetes identified as being from a native lamprey genus and removed from further analysis in the study. Superscript b denotes ammocoetes that were from known populations of American brook lamprey (*Lethenteron appendix*) and used as control animals for genetic testing.

Table 3 continued

Site	Collection Date	ID	Primer Name (*Pma*μ9)	Sea Lamprey
Jordan River 2	Oct 4 2010	J2F S2	250	Y
Jordan River 2	Oct 4 2010	J2F S3	250	Y
Jordan River 2	Oct 4 2010	J2F S4	250	Y
Jordan River 2	Oct 4 2010	J2F S5	250	Y
Jordan River 2	Oct 4 2010	J2F S6	250	Y
Jordan River 2	Oct 4 2010	J2F S7	250	Y
Jordan River 2	Oct 4 2010	J2F S8	250	Y
Jordan River 2	Oct 4 2010	J2F S9	250	Y
Jordan River 2	Oct 4 2010	J2F S10	250	Y
Pigeon River	May 19 2010	P1 S1	250	Y
Pigeon River	May 19 2010	P1 S2	250	Y
Pigeon River	May 19 2010	P1 S3	250	Y
Pigeon River	May 19 2010	P1 S4	250	Y
Pigeon River	May 19 2010	P1 S5	250	Y
Pigeon River	Oct 2 2010	P1F S1	250	Y
Pigeon River	Oct 2 2010	P1F S2	250	Y
Pigeon River	Oct 2 2010	P1F S3	250	Y
Pigeon River	Oct 2 2010	P1F S4	250	Y
Pigeon River	Oct 2 2010	P1F S5	250	Y
Pigeon River	Oct 2 2010	P1F S6	250	Y
Pigeon River	Oct 2 2010	P1F S7	250	Y
Pigeon River	Oct 2 2010	P1F S8	250	Y
Pigeon River	Oct 2 2010	P1F S9	250	Y
Pigeon River	Oct 2 2010	P1F S10	250	Y
Pigeon River	Oct 2 2010	P1F S11	250	Y
Pigeon River	Oct 2 2010	P1F S12	250	Y
Pigeon River	Oct 2 2010	P1F S13	250	Y
Pigeon River	Oct 2 2010	P1F S14	250	Y
Mad River 1 (OH)	July 1 2010	MR1 A1	None	N[b]
Mad River 1 (OH)	July 1 2010	MR1 A2	None	N[b]

Sample ID	Site	Collection Date	Length (cm)	Wet Mass (g)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR 1 S6M	JR 1[a]	June 10 2010	3.3	0.2000	7.17	-25.78	5.16	ND
JR 1 S13M	JR 1	June 10 2010	3.4	0.1379	7.29	-27.57	4.04	ND
JR 1 S8M	JR 1	June 10 2010	3.9	0.1790	5.21	-28.42	4.07	ND
JR 1 S9M	JR 1	June 10 2010	4.0	0.2674	6.66	-26.89	4.37	ND
JR 1 S12M[b]	JR 1	June 10 2010	4.3	0.3100	6.14	-27.81	4.69	ND
JR 1 S12M[b]	JR 1	June 10 2010	4.3	0.3100	6.13	-27.83	4.79	ND
JR 1 S7M	JR 1	June 10 2010	4.9	0.3630	6.51	-26.70	3.99	ND
JR 1 S14M	JR 1	June 10 2010	5.5	0.5160	9.29	-24.91	3.74	ND
JR 1 S11M	JR 1	June 10 2010	5.6	0.5900	9.64	-25.56	3.06	ND
JR 1F S20M	JR 1	Oct 3 2010	1.4	0.0067	5.02	-29.57	6.08	ND
JR 1F S8M	JR 1	Oct 3 2010	1.7	0.0169	5.58	-30.48	3.54	-197
JR 1F S15M	JR 1	Oct 3 2010	1.7	0.0062	5.77	-29.67	4.06	ND
JR 1F S9M	JR 1	Oct 3 2010	1.9	0.0181	5.08	-32.08	3.55	ND
JR 1F S19M	JR 1	Oct 3 2010	1.9	0.0212	6.03	-29.88	3.05	-176
JR 1F S12M	JR 1	Oct 3 2010	3.3	0.1379	6.29	-28.45	3.38	-183
JR 1F S12M	JR 1	Oct 3 2010	3.3	0.1379	6.29	-28.45	3.38	-183
JR 1F S13M	JR 1	Oct 3 2010	3.8	0.1404	5.53	-28.73	3.85	ND

Continued

Table 4. Ammocoete data for the Jordan and Pigeon Rivers measured in the present study. Ammocoete JR 2F S5M and JR 2F S9M were mislabeled between each other and means of these animal length/mass are reported instead of individual length/mass. [a]Jordan River 1 site, [b]Replicate analyses of the same sample, [c]Jordan River 2 site, [d]Ammocoete JR 2F S5M and JR 2F S9M were mislabeled between each other. Means of animal lengths and mass listed, [e]Pigeon River site. ND–not determined.

Table 4 continued

Sample ID	Site	Collection Date	Length (cm)	Wet Mass (g)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR 1F S3M	JR 1	Oct 3 2010	3.9	0.1790	8.33	-25.57	4.28	-204
JR 1F S4M	JR 1	Oct 3 2010	4.0	0.1764	9.28	-25.08	5.07	-192
JR 1F S17M	JR 1	Oct 3 2010	4.2	0.2165	5.89	-28.81	3.52	-191
JR 1F S7M	JR 1	Oct 3 2010	4.3	0.2595	6.88	-27.01	3.74	-204
JR 1F S10M	JR 1	Oct 3 2010	4.3	0.2132	6.64	-26.15	3.67	-195
JR 1F S11M	JR 1	Oct 3 2010	4.6	0.2998	5.91	-27.67	4.33	-194
JR 1F S16M	JR 1	Oct 3 2010	4.7	0.3228	7.67	-25.46	3.81	-208
JR 1F S6M	JR 1	Oct 3 2010	6.0	0.5064	6.68	-27.36	3.71	-199
JR 1F S5M	JR 1	Oct 3 2010	7.1	0.7192	6.18	-30.22	4.44	-210
JR 1F S1M	JR 1	Oct 3 2010	7.4	0.9882	9.81	-25.25	3.70	-203
JR 2 S18M	JR 2[c]	June 10 2010	2.5	0.0683	7.70	-25.14	2.46	ND
JR 2 S20M	JR 2	June 10 2010	5.6	0.4924	7.06	-26.52	3.23	ND
JR 2 S19M	JR 2	June 10 2010	6.3	0.7304	9.04	-24.88	2.81	ND
JR 2F S7M	JR 2	Oct 4 2010	1.4	0.0127	5.38	-29.60	4.11	ND
JR 2F S2M	JR 2	Oct 4 2010	1.8	0.0166	5.01	-30.26	3.26	ND
JR 2F S4M	JR 2	Oct 4 2010	1.8	0.0218	7.00	-30.27	2.95	ND
JR 2F S3M	JR 2	Oct 4 2010	1.9	0.0266	6.55	-28.00	2.89	ND
JR 2F S6M	JR 2	Oct 4 2010	2.0	0.0200	9.82	-27.46	2.53	ND
JR 2F S5/9M[d]	JR 2	Oct 4 2010	2	0.02	5.64	-31.57	2.80	ND
JR 2F S5/9M[d]	JR 2	Oct 4 2010	2	0.02	4.84	-30.75	3.19	ND
JR 2F S10M	JR 2	Oct 4 2010	2.1	0.0331	7.19	-27.14	2.23	ND

Continued

Table 4 continued

Sample ID	Site	Collection Date	Length (cm)	Wet Mass (g)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR 2F S8M	JR 2	Oct 4 2010	5.3	0.3592	7.07	-28.18	1.88	ND
JR 2F S1M	JR 2	Oct 4 2010	8.0	1.1670	11.03	-23.50	2.20	ND
PR 1 S2M	PR 1[e]	May 19 2010	4.2	0.3400	6.43	-24.92	3.85	ND
PR 1 S5M	PR 1	May 19 2010	4.2	0.3300	8.04	-23.22	4.82	ND
PR 1 S4M	PR 1	May 19 2010	5.2	0.5200	7.56	-26.27	5.49	ND
PR 1 S3M	PR 1	May 19 2010	6.3	0.6800	6.32	-27.62	5.44	ND
PR 1 S1M	PR 1	May 19 2010	7.0	1.0200	6.80	-27.35	4.76	ND
PR 1F S6M	PR 1	Oct 2 2010	2.7	0.0571	6.42	-27.25	4.52	-186
PR 1F S7M	PR 1	Oct 2 2010	2.9	0.0538	4.57	-30.93	4.88	-165
PR 1F S1M	PR 1	Oct 2 2010	3.0	0.0526	6.59	-25.88	5.27	-195
PR 1F S5M	PR 1	Oct 2 2010	3.1	0.0834	8.37	-26.30	5.13	-185
PR 1F S8M	PR 1	Oct 2 2010	3.1	0.0759	5.16	-29.71	5.38	-221
PR 1F S11M	PR 1	Oct 2 2010	3.1	0.0635	5.27	-28.02	5.12	-197
PR 1F S2M	PR 1	Oct 2 2010	5.1	0.2700	6.46	-26.12	3.87	-194
PR 1F S10M	PR 1	Oct 2 2010	5.5	0.4277	7.23	-26.06	4.53	-197
PR 1F S13M	PR 1	Oct 2 2010	5.6	0.6982	7.42	-25.50	3.88	-204
PR 1F S14M	PR 1	Oct 2 2010	6.1	0.6415	6.54	-27.95	3.68	-198
PR 1F S12M	PR 1	Oct 2 2010	8.6	1.4436	9.73	-23.06	5.38	-213
PR 1F S3M	PR 1	Oct 2 2010	8.7	1.4930	9.90	-24.36	4.90	-198
PR 1F S4M	PR 1	Oct 2 2010	9.4	1.7949	9.04	-23.73	4.12	-230
PR 1F S9M	PR 1	Oct 2 2010	10.7	2.3607	10.42	-22.49	3.94	-211

Source	Jordan River 1			Jordan River 2			Pigeon River		
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
Aquatic Macrophytes	-29.1±3.1 (8)	3.8±2.6 (8)	-165±14 (5)	-28.1±2.5 (5)	2.4±1.0 (5)	ND	-29.3±3.7 (5)	5.6±1.3 (5)	-161±16 (5)
Terrestrial Plants	-30.8±2.2 (10)	-0.8±2.6 (10)	-163±18 (5)	-29.8±1.8 (9)	0.2±2.1 (9)	ND	-29.1±2.0 (11)	1.8±2.4 (11)	-140±14 (5)
Microalgae[a]	-26.3±8.3	3.8±2.6	-230±18	-24.4±9.0	2.4±1.0	ND	-25.6±7.6	5.6±1.3	-207±16
Aquatic Sediment	-26.4±2.8 (20)	3.2±0.6 (20)	-142±1 (3)	-26.7±2.1 (18)	2.8±0.4 (18)	ND	-26.0±1.2 (15)	3.0±0.7 (19)	-152±11 (2)
Terrestrial Soil	-26.8±1.9 (3)	2.5±1.0 (3)	-134±11 (3)	-27.5±1.3 (3)	0.2±3.0 (3)	ND	-26.6±0.9 (3)	1.5±2.5 (3)	-130±5 (4)
SPOM	-28.3 (1)	3.7 (1)	-128 (1)	-28.5 (1)	2.3 (1)	ND	-28.6±0.6 (2)	3.8±0.2 (2)	-106 (1)

Table 5. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values for potential food and nutritional resources to sea lamprey ammocoetes from the Jordan River and Pigeon Rivers (Michigan, USA) measured in the present study. All values (in per mil, ‰) are reported as mean±SD (n); ND–not determined.

Sample ID	Site	Collection Date	Plant Type	Plant Species	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR1 AqV1	JR 1[a]	June 10 2010	Aquatic	Unidentified	8.50	-30.65	5.21	ND
JR1 AqV2	JR 1	June 10 2010	Aquatic	Unidentified	23.27	-28.23	6.48	ND
JR1 AqV3	JR 1	June 10 2010	Aquatic	*Eleocharis* sp.	12.14	-25.96	-1.01	ND
JR1 TerV1	JR 1	June 10 2010	Terrestrial	Hardwood	53.80	-26.89	-0.23	ND
JR1 TerV2	JR 1	June 10 2010	Terrestrial	*Thuja occidentalis*	13.71	-30.38	2.61	ND
JR1 TerV3	JR 1	June 10 2010	Terrestrial	*Abies balsamifera*	66.54	-30.95	-3.11	ND
JR1 TerV4	JR 1	June 10 2010	Terrestrial	*Picea* sp.	48.32	-30.04	-2.13	ND
JR1 TerV5	JR 1	June 10 2010	Terrestrial	*Larix laricina*	39.34	-30.85	-2.67	ND
JR1F AqV1	JR 1	Oct 3 2010	Aquatic	*Elodea* sp.	12.26	-30.81	4.16	-146
JR1F AqV2[b]	JR 1	Oct 3 2010	Aquatic	Aquatic Moss	27.53	-34.50	5.28	-182
JR1F AqV2[b]	JR 1	Oct 3 2010	Aquatic	Aquatic Moss	ND	ND	ND	-178
JR1F AqV3	JR 1	Oct 3 2010	Aquatic	Macroalgae	17.15	-26.69	4.57	-162
JR1F AqV4	JR 1	Oct 3 2010	Aquatic	*Ludwigia* sp.	8.05	-30.54	4.92	-176
JR1F AqV5	JR 1	Oct 3 2010	Aquatic	*Eleocharis* sp.	23.82	-25.57	0.37	-161
JR1F TerV1	JR 1	Oct 3 2010	Terrestrial	*Larix laricina*	80.78	-31.29	-2.94	-166
JR1F TerV2	JR 1	Oct 3 2010	Terrestrial	Family: Lamiaceae	30.57	-34.68	4.80	-176
JR1F TerV3	JR 1	Oct 3 2010	Terrestrial	*Picea*	51.10	-32.89	-1.44	-135
JR1F TerV4[b]	JR 1	Oct 3 2010	Terrestrial	*Thuja occidentalis*	50.83	-27.98	-1.92	-159
JR1F TerV4[b]	JR 1	Oct 3 2010	Terrestrial	*Thuja occidentalis*	ND	ND	ND	-156
JR1F TerV5	JR 1	Oct 3 2010	Terrestrial	*Solidago* spp.	32.97	-31.84	-0.50	-181

Continued

Table 6. Aquatic and terrestrial plant elemental and isotopic data for the Jordan and Pigeon Rivers in the present study. [a]Jordan River 1 site, [b]Replicate analyses of the same sample, [c]Jordan River 2 site, [d]Pigeon River site, ND–not determined.

Table 6 continued

Sample ID	Site	Collection Date	Plant Type	Plant Species	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR 2 AqV1	JR 2[c]	Oct 3 2010	Aquatic	*Elodea* sp. and *Vallis* sp.	14.54	-26.79	2.59	ND
JR2 TerV1[b]	JR 2	Oct 3 2010	Terrestrial	*Physocarpus opulifolius*	25.68	-29.97	0.75	ND
JR2 TerV1[b]	JR 2	Oct 3 2010	Terrestrial	*Physocarpus opulifolius*	25.56	-30.07	0.24	ND
JR2 TerV1[b]	JR 2	Oct 3 2010	Terrestrial	*Physocarpus opulifolius*	64.87	-29.33	1.20	ND
JR2 TerV2	JR 2	June 10 2010	Terrestrial	*Poa* sp.	25.25	-28.76	2.43	ND
JR2 TerV3	JR 2	June 10 2010	Terrestrial	*Thuja occidentalis*	61.19	-26.95	-0.42	ND
JR2 TerV4	JR 2	June 10 2010	Terrestrial	*Abies balsamifera*	25.91	-30.05	0.25	ND
JR2F AqV1	JR 2	Oct 4 2010	Aquatic	*Elodea* sp.	10.44	-29.19	3.22	ND
JR2F AqV2[b]	JR 2	Oct 4 2010	Aquatic	*Eleocharis* sp.	18.69	-24.43	0.91	ND
JR2F AqV2[b]	JR 2	Oct 4 2010	Aquatic	*Eleocharis* sp.	18.62	-24.43	0.55	ND
JR2F AqV3	JR 2	Oct 4 2010	Aquatic	*Ranunculus longirostris*	11.99	-29.29	3.10	ND
JR2F AqV4	JR 2	Oct 4 2010	Aquatic	*Ludwigia* sp.	10.56	-30.72	2.55	ND
JR2F TerV1	JR 2	Oct 4 2010	Terrestrial	*Thuja occidentalis*	46.82	-27.15	-1.86	ND
JR2F TerV2	JR 2	Oct 4 2010	Terrestrial	Grass	28.69	-30.37	3.12	ND
JR2F TerV3	JR 2	Oct 4 2010	Terrestrial	*Abies balsamifera*	53.75	-30.87	-3.95	ND
JR2F TerV4	JR 2	Oct 4 2010	Terrestrial	Hardwood	18.70	-31.97	0.90	ND
JR2F TerV5	JR 2	Oct 4 2010	Terrestrial	*Solidago* sp.	20.03	-31.98	0.71	ND
PR1 AqV1	PR 1[d]	May 19 2010	Aquatic	*Vallis* and *Ludwigia*	9.74	-30.37	4.88	ND
PR1 TerV1	PR 1	May 19 2010	Terrestrial	*Caltha palustris*	15.98	-27.92	4.82	ND
PR1 TerV2	PR 1	May 19 2010	Terrestrial	*Viburnum* sp.	19.44	-26.53	-0.41	ND
PR1 TerV3	PR 1	May 19 2010	Terrestrial	Grass	15.68	-30.47	5.52	ND

Continued

Table 6 continued

Sample ID	Site	Collection Date	Plant Type	Plant Species	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
PR1 TerV4	PR 1	May 19 2010	Terrestrial	*Abies* sp.	70.56	-29.84	-2.61	ND
PR1 TerV5[b]	PR 1	May 19 2010	Terrestrial	Hardwood	17.75	-29.35	0.92	ND
PR1 TerV5[b]	PR 1	May 19 2010	Terrestrial	Hardwood	18.73	-29.27	1.25	ND
PR1 TerV6[b]	PR 1	May 19 2010	Terrestrial	Fern	11.33	-27.24	0.12	ND
PR1 TerV6[b]	PR 1	May 19 2010	Terrestrial	Fern	ND	ND	ND	ND
PR1F AqV1	PR 1	Oct 2 2010	Aquatic	*Eleocharis* sp.	ND	ND	ND	-183
PR1F AqV2[b]	PR 1	Oct 2 2010	Aquatic	*Ludwigia* sp.	20.25	-33.07	5.31	-144
PR1F AqV2[b]	PR 1	Oct 2 2010	Aquatic	*Ludwigia* sp.	20.12	-33.24	4.70	ND
PR1F AqV3	PR 1	Oct 2 2010	Aquatic	*Vallisneria americana*	15.20	-29.10	7.44	-159
PR1F AqV4	PR 1	Oct 2 2010	Aquatic	*Ludwigia* sp.	9.61	-30.37	4.93	-159
PR1F TerV1	PR 1	Oct 2 2010	Terrestrial	Grass	34.40	-28.11	3.56	-152
PR1F TerV2	PR 1	Oct 2 2010	Terrestrial	*Cystopteris bulbifera*	22.96	-30.86	1.96	-120
PR1F TerV3[b]	PR 1	Oct 2 2010	Terrestrial	*Thuja occidentalis*	60.28	-27.28	0.40	-147
PR1F TerV3[b]	PR 1	Oct 2 2010	Terrestrial	*Thuja occidentalis*	ND	ND	ND	-146
PR1F TerV4	PR 1	Oct 2 2010	Terrestrial	Family: Asteraceae	15.27	-33.16	2.46	-150
PR1F TerV5[b]	PR 1	Oct 2 2010	Terrestrial	*Ulmus* sp.	23.20	-29.68	2.50	-138
PR1F TerV5[b]	PR 1	Oct 2 2010	Terrestrial	*Ulmus* sp.	ND	ND	ND	-126

Sample ID	Site	Collection Date	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR1F A[a]	Jordan River 1	Oct 3 2010	15.21	-28.30	3.66	-131
JR1F A[a]	Jordan River 1	Oct 3 2010	ND	ND	ND	-126
JR2F A	Jordan River 2	Oct 4 2010	11.32	-28.49	2.32	ND
PR1 B	Pigeon River	May 19 2010	14.05	-29.06	3.69	ND
PR1F A[a]	Pigeon River	Oct 2 2010	13.47	-28.22	3.96	-105
PR1F A[a]	Pigeon River	Oct 2 2010	ND	ND	ND	-107

Table 7. Suspended particulate organic matter (SPOM) elemental and isotopic data for the Jordan and Pigeon Rivers in the present study. [a]Replicate analyses of the same sample, ND–not determined.

Sample ID	Site	Collection Date	Sediment Depth (cm)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR1 AqS1	Jordan River 1	June 10 2010	0.0-0.5	19.24	-27.24	3.15	ND
JR1 AqS2	Jordan River 1	June 10 2010	0.5-1.0	ND	-28.72	3.43	ND
JR1 AqS3	Jordan River 1	June 10 2010	1.0-2.0	22.25	-27.63	3.46	ND
JR1 AqS4	Jordan River 1	June 10 2010	2.0-3.0	21.74	-27.65	3.64	ND
JR1 AqS5	Jordan River 1	June 10 2010	3.0-4.0	15.60	-28.12	3.68	ND
JR1 AqS6	Jordan River 1	June 10 2010	4.0-5.0	15.79	-24.71	3.98	ND
JR1 AqS7	Jordan River 1	June 10 2010	5.0-6.0	11.56	-28.99	3.94	ND
JR1 AqS8	Jordan River 1	June 10 2010	6.0-7.0	7.97	-27.68	2.10	ND
JR1 AqS9	Jordan River 1	June 10 2010	7.0-8.0	31.27	-25.02	4.17	ND
JR1 AqS10	Jordan River 1	June 10 2010	8.0-9.0	ND	ND	3.64	ND
JR1F AqS1	Jordan River 1	Oct 3 2010	0.0-0.5	22.53	-26.97	1.91	-126
JR1F AqS2	Jordan River 1	Oct 3 2010	0.5-1.0	21.28	-25.15	2.65	-154
JR1F AqS3	Jordan River 1	Oct 3 2010	1.0-2.0	20.26	-26.82	2.96	ND
JR1F AqS4	Jordan River 1	Oct 3 2010	2.0-3.0	19.99	-26.69	3.08	ND
JR1F AqS5	Jordan River 1	Oct 3 2010	3.0-4.0	18.22	-25.30	2.88	ND
JR1F AqS6	Jordan River 1	Oct 3 2010	4.0-5.0	19.58	-27.60	3.27	ND
JR1F AqS7	Jordan River 1	Oct 3 2010	5.0-6.0	20.07	-27.33	3.34	ND
JR1F AqS8	Jordan River 1	Oct 3 2010	6.0-7.0	20.42	-27.29	3.60	ND
JR1F AqS9	Jordan River 1	Oct 3 2010	7.0-8.0	19.88	-27.19	3.10	ND
JR1F AqS10	Jordan River 1	Oct 3 2010	8.0-9.0	22.12	-25.16	2.60	-145

Continued

Table 8. Aquatic sediment organic matter elemental and isotopic data for the Jordan and Pigeon Rivers in the present study. ND–not determined.

Table 8 continued

Sample ID	Site	Collection Date	Sediment Depth (cm)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR2 AqS1	Jordan River 2	June 10 2010	0.0-0.5	24.80	-28.20	3.13	ND
JR2 AqS2	Jordan River 2	June 10 2010	0.5-1.0	19.13	-24.56	3.45	ND
JR2 AqS3	Jordan River 2	June 10 2010	1.0-2.0	ND	-28.54	3.04	ND
JR2 AqS4	Jordan River 2	June 10 2010	2.0-3.0	ND	ND	2.89	ND
JR2 AqS5	Jordan River 2	June 10 2010	3.0-4.0	ND	ND	2.61	ND
JR2 AqS6	Jordan River 2	June 10 2010	4.0-5.0	ND	ND	2.48	ND
JR2 AqS7	Jordan River 2	June 10 2010	5.0-6.0	ND	ND	2.68	ND
JR2 AqS8	Jordan River 2	June 10 2010	6.0-7.0	12.19	-28.87	3.96	ND
JR2 AqS9	Jordan River 2	June 10 2010	7.0-8.0	ND	-28.64	2.98	ND
JR2 AqS10	Jordan River 2	June 10 2010	8.0-9.0	ND	ND	2.77	ND
JR2F AqS1	Jordan River 2	Oct 4 2010	0.0-0.5	20.83	-27.34	2.50	ND
JR2F AqS2	Jordan River 2	Oct 4 2010	0.5-1.0	20.16	-27.41	2.80	ND
JR2F AqS3	Jordan River 2	Oct 4 2010	1.0-2.0	18.47	-27.32	2.64	ND
JR2F AqS4	Jordan River 2	Oct 4 2010	2.0-3.0	17.21	-27.33	2.84	ND
JR2F AqS5	Jordan River 2	Oct 4 2010	3.0-4.0	18.66	-27.12	2.82	ND
JR2F AqS7	Jordan River 2	Oct 4 2010	5.0-6.0	22.49	-26.66	2.46	ND
JR2F AqS8	Jordan River 2	Oct 4 2010	6.0-7.0	22.33	-22.38	2.49	ND
JR2F AqS9	Jordan River 2	Oct 4 2010	7.0-8.0	29.87	-23.85	3.16	ND
PR1 AqS1	Pigeon River 1	May 19 2010	0.0-0.5	18.68	-27.03	3.16	ND
PR1 AqS1	Pigeon River 1	May 19 2010	0.0-0.5	18.68	-27.03	3.16	ND
PR1 AqS2	Pigeon River 1	May 19 2010	0.5-1.0	19.67	-26.52	3.08	ND

Continued

Table 8 continued

Sample ID	Site	Collection Date	Sediment Depth (cm)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
PR1 AqS3	Pigeon River 1	May 19 2010	1.0-2.0	18.33	-26.91	2.60	ND
PR1 AqS4	Pigeon River 1	May 19 2010	2.0-3.0	20.80	-26.59	2.54	ND
PR1 AqS5	Pigeon River 1	May 19 2010	3.0-4.0	17.62	-26.15	2.47	ND
PR1 AqS6	Pigeon River 1	May 19 2010	4.0-5.0	19.14	-24.92	2.85	ND
PR1 AqS7	Pigeon River 1	May 19 2010	5.0-6.0	19.88	-24.93	2.84	ND
PR1 AqS8	Pigeon River 1	May 19 2010	7.0-8.0	21.93	-25.61	2.97	ND
PR1 AqS9	Pigeon River 1	May 19 2010	8.0-9.0	21.09	-25.26	2.74	ND
PR1F AqS1	Pigeon River 1	Oct 2 2010	0.0-0.5	17.27	-28.56	2.71	-145
PR1F AqS2	Pigeon River 1	Oct 2 2010	0.5-1.0	ND	ND	3.02	-160
PR1F AqS3	Pigeon River 1	Oct 2 2010	1.0-2.0	18.45	-26.85	2.25	ND
PR1F AqS4	Pigeon River 1	Oct 2 2010	2.0-3.0	35.66	-24.34	3.05	ND
PR1F AqS5	Pigeon River 1	Oct 2 2010	3.0-4.0	31.78	-24.57	2.56	ND
PR1F AqS6	Pigeon River 1	Oct 2 2010	4.0-5.0	ND	ND	-0.14	ND
PR1F AqS7	Pigeon River 1	Oct 2 2010	5.0-6.0	ND	ND	4.89	ND
PR1F AqS8	Pigeon River 1	Oct 2 2010	6.0-7.0	ND	ND	4.04	ND
PR1F AqS9	Pigeon River 1	Oct 2 2010	7.0-8.0	ND	ND	4.28	ND
PR1F AqS10	Pigeon River 1	Oct 2 2010	8.0-9.0	ND	ND	2.36	ND
PR1F AqS11	Pigeon River 1	Oct 2 2010	9.0-10.0	17.82	-26.76	0.63	-131
PR1F AqS12	Pigeon River 1	Oct 2 2010	10.0-11.0	ND	ND	2.28	ND

Sample ID	Site	Collection Date	Sample Depth (cm)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR1F TerS0	Jordan River 1	Oct 3 2010	0	ND	ND	1.60	-122
JR1F TerS5	Jordan River 1	Oct 3 2010	5	23.82	-25.84	2.95	-141
JR1F TerS31	Jordan River 1	Oct 3 2010	31	ND	ND	5.42	-141
JR2F TerS0	Jordan River 2	Oct 4 2010	0	ND	-29.66	-2.95	ND
JR2F TerS20	Jordan River 2	Oct 4 2010	20	15.84	-26.25	2.71	ND
JR2F TerS53	Jordan River 2	Oct 4 2010	53	10.50	-27.36	0.90	ND
PR1F TerS0	Pigeon River	Oct 2 2010	0	ND	-28.22	0.05	-129
PR1F TerS18	Pigeon River	Oct 2 2010	18	ND	-26.70	4.18	-136
PR1F TerS30	Pigeon River	Oct 2 2010	30	ND	ND	2.15	-131
PR1F TerS52[a]	Pigeon River	Oct 2 2010	52	ND	ND	1.52	-122
PR1F TerS52[a]	Pigeon River	Oct 2 2010	52	ND	ND	ND	-126

Table 9. Terrestrial soil organic matter elemental and isotopic data for the Jordan and Pigeon Rivers in the present study.
[a]Replicate analyses of the same sample, ND– not determined.

Sample ID	Site	Collection Date	Size fraction	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR1 C363[a]	Jordan River 1	June 10 2010	>363 µm	35.0	-27.11	2.28	ND
JR1 C363[a]	Jordan River 1	June 10 2010	>363 µm	33.9	-27.23	2.38	ND
JR1 C163[a]	Jordan River 1	June 10 2010	163-363 µm	21.1	-27.09	3.51	ND
JR1 C163[a]	Jordan River 1	June 10 2010	163-363 µm	20.9	-27.17	2.87	ND
JR1 C63[b]	Jordan River 1	June 10 2010	63-163 µm	21.4	-27.14	3.04	ND
JR1 C35	Jordan River 1	June 10 2010	35-63 µm	20.0	-27.17	2.76	ND
JR1 C10	Jordan River 1	June 10 2010	10-35 µm	20.0	-27.24	2.94	ND
JR1 CS	Jordan River 1	June 10 2010	0.2-10 µm	11.8	-27.82	3.92	ND
JR1F C363	Jordan River 1	Oct 3 2010	>363 µm	27.8	-26.94	2.03	-144
JR1F C163	Jordan River 1	Oct 3 2010	163-363 µm	21.1	-26.78	2.82	-134
JR1F C63[b]	Jordan River 1	Oct 3 2010	63-163 µm	19.8	-26.77	2.90	-130
JR1F C35	Jordan River 1	Oct 3 2010	35-63 µm	19.6	-26.81	2.67	-120
JR1F C10	Jordan River 1	Oct 3 2010	10-35 µm	18.7	-27.37	2.72	ND
JR1F CS	Jordan River 1	Oct 3 2010	0.2-10 µm	11.6	-27.59	0.59	ND
JR1F CS	Jordan River 1	Oct 3 2010	0.2-10 µm	12.2	-27.90	2.27	ND
JR1F CS	Jordan River 1	Oct 3 2010	0.2-10 µm	12.6	-27.80	1.39	ND
JR2 C363	Jordan River 2	June 10 2010	>363 µm	59.7	-15.10	-1.35	ND
JR2 C163	Jordan River 2	June 10 2010	163-363 µm	21.2	-24.99	1.75	ND
JR2 C63[a]	Jordan River 2	June 10 2010	63-163 µm	18.8	-26.35	2.03	ND
JR2 C63[a]	Jordan River 2	June 10 2010	63-163 µm	27.0	-26.83	2.21	ND
JR2 C35	Jordan River 2	June 10 2010	35-63 µm	19.1	-27.14	3.53	ND

Continued

Table 10. Size-fractionated aquatic sediment organic matter data for the Jordan and Pigeon Rivers in the present study.
[a]Replicate analyses of the same sample, [b]Sample may have been mislabeled and maybe JR1 C163, ND– not determined.

Table 10 continued

Sample ID	Site	Collection Date	Size fraction	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
JR2 C10[a]	Jordan River 2	June 10 2010	10-35 µm	18.2	-27.22	2.12	ND
JR2 C10[a]	Jordan River 2	June 10 2010	10-35 µm	18.4	-27.16	2.68	ND
JR2 CS	Jordan River 2	June 10 2010	0.2-10 µm	13.3	-27.75	0.99	ND
JR2F C363[a]	Jordan River 2	Oct 4 2010	>363 µm	19.8	-27.22	3.17	ND
JR2F C363[a]	Jordan River 2	Oct 4 2010	>363 µm	19.5	-27.17	3.14	ND
JR2F C163	Jordan River 2	Oct 4 2010	163-363 µm	21.0	-26.99	2.79	ND
JR2F C63	Jordan River 2	Oct 4 2010	63-163 µm	20.4	-26.41	2.63	ND
JR2F C35	Jordan River 2	Oct 4 2010	35-63 µm	20.2	-26.45	2.38	ND
JR2F C10	Jordan River 2	Oct 4 2010	10-35 µm	18.8	-27.08	1.47	ND
JR2F CS	Jordan River 2	Oct 4 2010	0.2-10 µm	14.5	-27.38	0.56	ND
PR1 C363	Pigeon River 1	May 19 2010	>363 µm	24.3	-26.55	3.44	ND
PR1 C163	Pigeon River 1	May 19 2010	163-363 µm	19.3	-26.96	1.39	ND
PR1 C63	Pigeon River 1	May 19 2010	63-163 µm	16.5	-27.01	1.85	ND
PR1 C35	Pigeon River 1	May 19 2010	35-63 µm	17.1	-26.77	2.96	ND
PR1 C10	Pigeon River 1	May 19 2010	10-35 µm	16.9	-27.00	2.85	ND
PR1 CS	Pigeon River 1	May 19 2010	0.2-10 µm	11.9	-27.74	2.94	ND
PR1F C363[a]	Pigeon River 1	Oct 2 2010	>363 µm	21.1	-27.06	2.54	-129
PR1F C363[a]	Pigeon River 1	Oct 2 2010	>363 µm	21.8	-27.24	2.39	ND
PR1F C163	Pigeon River 1	Oct 2 2010	163-363 µm	19.7	-27.07	2.53	-131
PR1F C63	Pigeon River 1	Oct 2 2010	63-163 µm	18.1	-26.45	2.69	-119
PR1F C35	Pigeon River 1	Oct 2 2010	35-63 µm	17.1	-26.88	3.16	-118
PR1F C10	Pigeon River 1	Oct 2 2010	10-35 µm	17.3	-27.29	2.92	ND
PR1F CS	Pigeon River 1	Oct 2 2010	0.2-10 µm	11.3	-28.10	-0.65	ND

Sample ID	Site	Collection Date	Gut Segment[a]	δ^2H (‰)
PR1F S3 G1	Pigeon River 1	Oct 2 2010	1	-177
PR1F S3 G2	Pigeon River 1	Oct 2 2010	2	-178
PR1F S3 G3	Pigeon River 1	Oct 2 2010	3	-168
PR1F S14 G1	Pigeon River 1	Oct 2 2010	1	-164
PR1F S13+S14 G2[b]	Pigeon River 1	Oct 2 2010	2	-169
PR1F S13+S14 G3[b]	Pigeon River 1	Oct 2 2010	3	-156
PR1F S4+S9+S12 G1[c]	Pigeon River 1	Oct 2 2010	1	-185
PR1F S4+S9+S12 G2[c]	Pigeon River 1	Oct 2 2010	2	-208
PR1F S4+S9+S12 G3[c]	Pigeon River 1	Oct 2 2010	3	-166
PR1F S4+S9+S12 G4[c]	Pigeon River 1	Oct 2 2010	4	-155

Table 11. δ^2H values of Pigeon River sea lamprey ammocoete gut segment organic matter measured in the present study. [a]Gut segments are defined as described in *Materials and Methods: Ammocoete Body Tissue and Ingested Gut Material*, [b]S13+S14: the same gut segments from ammocoetes 13 and 14 were pooled in order to produce adequate sample size for analysis, [c]S4+S9+S12: the same gut segments from ammocoetes 4, 9 and 12 were pooled in order to produce adequate sample size for analysis.

Site	Ammocoete Size	N	Aquatic Macrophytes	Microalgae	Aquatic Sediments	Terrestrial Plants	Terrestrial Soils
Jordan River 1	All sizes	15	4.5 (0.4-14.3)	44.3 (35.0-50.0)	2.9 (0.2-10.1)	44.3 (33.2-54.7)	3.2 (0.3-11.0)
	0-3 cm	2	9.1 (0.8-34.7)	23.5 (4.5-45.6)	5.6 (0.5-19.8)	49.9 (17.9-73.8)	6.0 (0.5-20.7)
	3-6 cm	10	6.0 (0.6-18.0)	43.3 (35.4-51.6)	3.8 (0.3-13.1)	39.4 (26.4-51.2)	4.3 (0.4-14.2)
	>6 cm	3	5.9 (0.5-21.6)	60.9 (46.6-75.4)	4.5 (0.3-16.6)	18.1 (2.1-40.1)	4.8 (0.4-17.2)
Pigeon River	All sizes	14	0.4 (0.0-1.9)	57.4 (51.6-63.7)	0.6 (0.0-2.5)	40.4 (33.8-46.4)	0.5 (0.0-2.4)
	0-4 cm	6	1.4 (0.1-5.6)	52.9 (43.2-63.0)	1.8 (0.1-7.4)	40.3 (7.3-51.2)	1.9 (0.1-25.0)
	4-8 cm	4	1.3 (0.1-5.5)	49.1 (36.5-62.0)	1.6 (0.1-6.2)	44.8 (2.4-58.1)	1.7 (0.1-33.2)
	>8 cm	4	1.2 (0.1-5.1)	80.4 (65.9-91.9)	2.0 (0.2-8.5)	6.0 (0.4-28.0)	3.9 (0.2-20.7)

Table 12. Bayesian model estimates of potential nutritional sources to sea lamprey ammocoetes in the Jordan and Pigeon Rivers (Michigan, USA) using MixSIR. Values are median percent contributions of each listed source to ammocoetes. Values in parentheses are 5%-95% confidence intervals of median contributions of each potential source. Ammocoete size groupings were chosen to reflect likely age classes such that the smallest group (i.e., 0-3 cm and 0-4 cm for Jordan River 1 and Pigeon River, respectively) is all YOY individuals, and the largest group is likely comprised of age-2 individuals (i.e., > 6 cm and > 8 cm).

Reference	Species	Length (mm)	Primary Food Source Identified	Other Food Source(s)	System	Approach
Creaser & Hann, 1928	*Lethenteron appendix*	39-121	Algae	Protozoa, nothing from stream bed	Michigan, USA	Description of GC
Wigley, 1959	*Petromyzon marinus*	NR	Algae	"Periphyton", Protozoa	New York, USA	Description of GC
Manion, 1967	*P. marinus*	33-35, 70-72	Algae (Diatoms)		Michigan, USA	Enumeration of diatoms in GC
Moore & Beamish, 1973	*P. marinus*[a], *L. appendix*	0->105	Algae (98.6-100%)		Ontario, Canada	Enumeration of algae in anterior 25% of GC
Potter *et al*., 1975	*Mordacia mordax*	>100	Algae	Protozoans, rotifers, nematodes	New South Wales, Australia	Enumeration of groups in GC
Moshin & Gallaway, 1977	*Ichthyomyzon gagei*	40-170	Algae (97.7-100%)	Rotifers (0-2.3%)	Texas, USA	Enumeration of groups in GC
Moore & Mallat, 1980	Many species	Multiple sizes	Detritus	Algae (<1.5%[c])	Multiple sites in Europe	Review of literature
Sutton & Bowen, 1994	*P. marinus, I. fossor*	60-155	Detritus (94.5-99.8%)	Algae (0.2-5.5%), Bacteria (<1%)	Laurentian Great Lakes	Quantification of GC materials
Yap & Bowen, 2003	*I. fossor*	>70	Aquatic seston		Michigan, USA	BC of anterior and posterior 10% of GC
Mundahl *et al*., 2005	*L. appendix*	90-179	Detritus (86.0-92.6%)	Algae (7.3-14.0%)	Minnesota, USA	Quantification of posterior 10% of GC

Table 13. Estimates of potential nutritional sources contributing to ammocoete diets by GC analysis. [a]Both anadromous and invasive populations were included. Unless otherwise noted, estimates were by visual examination, GC-gut content, BC- bomb calorimetry.

Site	Date	Water Temp. (^{0}C)	DO (mg•L^{-1})	pH	Conductivity (µS•cm^{-1} at 25°C)	Species Collected
CFR1	July 8 2010	22.6	12.07	7.14	ND	*Lampetra aepyptera*
	Nov 7 2010	4.8	12.58	8.13	281.6	*L. aepyptera*
CFR2	July 8 2010	22.8	9.69	7.12	ND	*L. aepyptera*
	Nov 7 2010	5.3	12.83	8.13	165.8	*L. aepyptera*
MR1	July 1 2010	16.3	11.43	7.14	ND	*Lethenteron appendix*
	Nov 6 2010	7.0	12.68	8.22	345.3	*L. appendix*
MR2	July 1 2010	20.2	10.2	7.27	ND	*L. appendix*
	Nov 6 2010	4.0	14.58	8.22	232.4	*L. appendix*

Table 14. Characteristics of the Clear Fork and Mad River sites (Ohio, USA) sampled in this study. ND–not determined, CFR1–Clear Fork River 1 site; CFR2–Clear Fork River 2 site; MR1–Mad River 1 site; MR2–Mad River 2 site.

Site	Length (km)	Stream Order	Avg. Discharge (m^3/s) [a]	Watershed Area (km^2) [a]	Watershed Use (%)[a]		
					Forested	Urban	Agriculture
Clear Fork River	59	3	5.65	51,200	41	12	47
Mad River	100	3	20.57	164,000	18	1	81

Table 15. Characteristics of the Clear Fork and Mad Rivers and their associated watersheds. [a]Data obtained from the Ohio (USA) EPA and Ohio Department of Development.

Sample ID	Site	Date	Concentration (mmols C•L^{-1})	$\delta^{13}C$ (‰)
CF1-6	Clear Fork 1	July 8 2010	4.63	-10.97
CF1-9	Clear Fork 1	July 8 2010	4.76	-11.57
CF1F-1	Clear Fork 1	Nov 7 2010	2.76	-10.3
CF2-4	Clear Fork 2	July 8 2010	1.46	-12.16
CF2-19	Clear Fork 2	July 8 2010	1.38	-11.65
MR1-8	Mad River 1	July 1 2010	7.13	-11.47
MR1-10	Mad River 1	July 1 2010	7.23	-11.32
MR2-5	Mad River 2	July 1 2010	6.67	-11.81
MR2-7	Mad River 2	July 1 2010	6.67	-11.80

Table 16. DIC concentration and $\delta^{13}C$ data for the Clear Fork and Mad Rivers in the present study.

Sample ID	Site	Date	Total Length (cm)	Wet Mass (g)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF1 A1M	Clear Fork 1	July 8 2010	8.4	1.6310		5.87	-25.34	4.69	ND
CF1 A2M	Clear Fork 1	July 8 2010	10.0	2.3414		8.79	-23.57	4.54	ND
CF1 A3M	Clear Fork 1	July 8 2010	10.3	2.5018		6.45	-23.95	3.24	ND
CF1 A4M	Clear Fork 1	July 8 2010	11.6	4.0751	1	9.64	-24.41	6.55	ND
CF1 A4M	Clear Fork 1	July 8 2010	11.6	4.0751	2	10.30	-24.19	6.37	ND
CF1 A5M	Clear Fork 1	July 8 2010	12.1	4.3106		9.67	-24.76	6.68	ND
CF1 A6M	Clear Fork 1	July 8 2010	12.1	4.3399		5.79	-25.64	6.04	ND
CF1 A7M	Clear Fork 1	July 8 2010	10.0	2.4702		5.55	-24.34	3.66	ND
CF1 A8M	Clear Fork 1	July 8 2010	9.0	1.8472		10.29	-23.65	4.77	ND
CF1F A1M	Clear Fork 1	Nov 7 2010	11.1	2.5401		6.69	-24.67	3.75	-175
CF1F A2M	Clear Fork 1	Nov 7 2010	13.2	5.2892		8.37	-23.09	7.31	-207
CF1F A3M	Clear Fork 1	Nov 7 2010	9.3	1.8088		4.41	-28.42	4.95	-137
CF1F A4M	Clear Fork 1	Nov 7 2010	7.1	0.9126	1	4.08	-29.35	7.67	-142
CF1F A4M	Clear Fork 1	Nov 7 2010	7.1	0.9126	2	4.51	-26.54	7.37	ND
CF1F A5M	Clear Fork 1	Nov 7 2010	7.9	1.1903		4.26	-26.77	6.15	-133
CF1F A6M	Clear Fork 1	Nov 7 2010	12.1	4.3399		7.60	-24.23	4.77	-194
CF2 A1M	Clear Fork 2	July 8 2010	11.9	3.6924		4.90	-22.88	7.40	ND
CF2 A2M	Clear Fork 2	July 8 2010	8.2	1.5573		5.28	-24.16	7.35	ND
CF1F A3M	Clear Fork 1	Nov 7 2010	9.3	1.8088		4.41	-28.42	4.95	-137
CF1F A4M	Clear Fork 1	Nov 7 2010	7.1	0.9126	1	4.08	-29.35	7.67	-142

Continued

Table 17. Ammocoete size, elemental and stable isotopic data from the Clear Fork River in the present study. ND–not determined.

Table 17 continued

Sample ID	Site	Date	Total Length (cm)	Wet Mass (g)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF1F A4M	Clear Fork 1	Nov 7 2010	7.1	0.9126	2	4.51	-26.54	7.37	ND
CF1F A5M	Clear Fork 1	Nov 7 2010	7.9	1.1903		4.26	-26.77	6.15	-133
CF1F A6M	Clear Fork 1	Nov 7 2010	12.1	4.3399		7.60	-24.23	4.77	-194
CF2 A1M	Clear Fork 2	July 8 2010	11.9	3.6924		4.90	-22.88	7.40	ND
CF2 A2M	Clear Fork 2	July 8 2010	8.2	1.5573		5.28	-24.16	7.35	ND
CF2 A3M	Clear Fork 2	July 8 2010	8.2	1.3147		4.39	-24.04	7.27	ND
CF2 A4M	Clear Fork 2	July 8 2010	14.6	7.1407	1	7.92	-22.99	6.87	ND
CF2 A4M	Clear Fork 2	July 8 2010	14.6	7.1407	2	7.70	-23.21	6.72	ND
CF2 A5M	Clear Fork 2	July 8 2010	3.0	0.0849		5.73	-24.12	5.11	ND
CF2 A6M	Clear Fork 2	July 8 2010	1.8	0.0074		4.19	-24.86	6.86	ND
CF2 A7M	Clear Fork 2	July 8 2010	2.2	0.0242		4.12	-25.19	7.25	ND
CF2 A8M	Clear Fork 2	July 8 2010	2.2	0.0214		4.24	-26.68	5.93	ND
CF2F A1M	Clear Fork 2	Nov 7 2010	1.5	0.0865		4.23	-27.79	7.79	ND
CF2F A2M	Clear Fork 2	Nov 7 2010	1.8	0.1334		4.14	-27.93	8.48	ND
CF2F A3M	Clear Fork 2	Nov 7 2010	1.6	0.1100		4.09	-28.71	6.91	ND
CF2F A4M	Clear Fork 2	Nov 7 2010	4.6	2.1823		4.15	-25.52	8.42	ND
CF2F A5M	Clear Fork 2	Nov 7 2010	5.2	3.4060		4.19	-23.96	7.68	ND
CF2F A6M	Clear Fork 2	Nov 7 2010	4.8	2.4163		4.41	-24.47	7.85	ND
CF2F A7M	Clear Fork 2	Nov 7 2010	1.6	0.0989		4.31	-27.82	9.04	ND
CF2F A8M	Clear Fork 2	Nov 7 2010	5.4	4.0824		5.74	-23.69	7.00	ND
CF2F A9M	Clear Fork 2	Nov 7 2010	1.7	0.0838		4.11	-28.83	6.86	ND

Continued

Table 17continued

Sample ID	Site	Date	Total Length (cm)	Wet Mass (g)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF2F A10M	Clear Fork 2	Nov 7 2010	2.7	0.4341		4.11	-24.24	9.01	ND
CF2F A11M	Clear Fork 2	Nov 7 2010	1.7	0.1347		4.75	-28.86	8.23	ND

Sample ID	Site	Date	Total Length (cm)	Wet Mass (g)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
MR1 A1M	Mad River 1	July 1 2010	14.2	4.8029		7.83	-27.03	7.02	ND
MR1 A2M	Mad River 1	July 1 2010	13.1	4.4024	1	6.04	-27.19	6.88	ND
MR1 A2M	Mad River 1	July 1 2010	13.1	4.4024	2	6.63	-27.14	6.82	ND
MR1 A3M	Mad River 1	July 1 2010	16.3	7.6225		6.04	-27.06	7.64	ND
MR1 A4M	Mad River 1	July 1 2010	9.6	1.6282		5.16	-28.48	7.54	ND
MR1 A5M	Mad River 1	July 1 2010	13.8	4.5953		4.91	-27.70	8.24	ND
MR1 A6M	Mad River 1	July 1 2010	15.6	6.0833	1	6.74	-27.14	8.13	ND
MR1 A6M	Mad River 1	July 1 2010	15.6	6.0833	2	6.83	-27.19	8.22	ND
MR1 A7M	Mad River 1	July 1 2010	16.5	7.0460		7.94	-25.36	7.23	ND
MR1 A8M	Mad River 1	July 1 2010	16.0	7.3196		6.12	-26.63	7.89	ND
MR1 A9M	Mad River 1	July 1 2010	14.7	5.8084		4.66	-27.35	7.91	ND
MR1 A10M	Mad River 1	July 1 2010	16.6	7.5106	1	5.14	-27.95	7.89	ND
MR1 A10M	Mad River 1	July 1 2010	16.6	7.5106	2	5.21	-27.98	7.72	ND
MR1 A11M	Mad River 1	July 1 2010	15.2	6.2020		7.65	-25.51	8.05	ND
MR1 A12M	Mad River 1	July 1 2010	13.2	3.9593		5.48	-27.56	7.41	ND
MR1F A1M	Mad River 1	Nov 6 2010	9.5	1.2275		4.35	-28.80	8.44	-138
MR1F A2M	Mad River 1	Nov 6 2010	12.3	2.3103		4.22	-28.67	7.75	-141
MR1F A3M	Mad River 1	Nov 6 2010	18.8	9.8929	1	5.35	-28.70	7.73	-169
MR1F A3M	Mad River 1	Nov 6 2010	18.8	9.8929	2	ND	ND	ND	-167
MR1F A4M	Mad River 1	Nov 6 2010	15.7	5.8119		4.07	-29.31	7.77	-142
MR1F A5M	Mad River 1	Nov 6 2010	14.5	4.5771		4.18	-29.60	8.16	-150

Continued

Table 18. Ammocoete size, elemental and stable isotopic data from the Mad River in the present study. ND–not determined.

Table 18 continued

Sample ID	Site	Date	Total Length (cm)	Wet Mass (g)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
MR1F A6M	Mad River 1	Nov 6 2010	16.4	6.2426		4.65	-28.68	7.96	-149
MR1F A7M	Mad River 1	Nov 6 2010	15.8	6.6330		4.10	-29.51	8.00	-147
MR1F A8M	Mad River 1	Nov 6 2010	15.5	6.554	1	6.39	-26.58	7.87	-183
MR1F A8M	Mad River 1	Nov 6 2010	15.5	6.554	2	6.25	-26.64	7.92	ND
MR2 A1M	Mad River 2	July 1 2010	13.6	4.2639		6.86	-25.27	8.21	ND
MR2 A2M	Mad River 2	July 1 2010	16.7	7.5363		8.88	-22.81	6.84	ND
MR2 A3M	Mad River 2	July 1 2010	16.3	9.9044		8.19	-23.21	7.34	ND
MR2 A4M	Mad River 2	July 1 2010	16.2	7.9160		9.44	-23.69	7.35	ND
MR2 A5M	Mad River 2	July 1 2010	18.9	11.8361		8.97	-22.96	6.99	ND
MR2 A6M	Mad River 2	July 1 2010	8.6	1.2842		8.37	-26.09	7.21	ND
MR2 A7M	Mad River 2	July 1 2010	17.9	8.9422		8.66	-25.68	7.46	ND
MR2 A8M	Mad River 2	July 1 2010	16.6	7.5400		7.65	-25.25	7.91	ND
MR2 A9M	Mad River 2	July 1 2010	16.1	7.3308		8.27	-24.34	7.47	ND
MR2 A10M	Mad River 2	July 1 2010	12.6	3.2283		6.72	-24.83	7.24	ND
MR2 A11M	Mad River 2	July 1 2010	16.3	8.2314		8.52	-24.67	7.51	ND
MR2 A13M	Mad River 2	July 1 2010	10.9	2.0893		6.15	-26.55	7.97	ND
MR2F A1M	Mad River 2	Nov 6 2010	16.5	6.8843		4.27	-27.50	8.34	-139
MR2F A2M	Mad River 2	Nov 6 2010	13.3	4.1335	1	6.43	-24.72	6.52	-184
MR2F A2M	Mad River 2	Nov 6 2010	13.3	4.1335	2	6.42	-24.66	6.42	ND
MR2F A3M	Mad River 2	Nov 6 2010	8.0	0.9972		4.15	-27.63	8.26	-131
MR2F A4M	Mad River 2	Nov 6 2010	16.7	7.852		6.20	-26.05	7.56	-181

Continued

Table 18 continued

Sample ID	Site	Date	Total Length (cm)	Wet Mass (g)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
MR2F A5M	Mad River 2	Nov 6 2010	10.0	1.7967		4.36	-27.16	7.85	-135
MR2F A6M	Mad River 2	Nov 6 2010	12.7	3.7388		5.58	-25.23	7.34	-165
MR2F A7M	Mad River 2	Nov 6 2010	14.2	4.4627		7.20	-23.17	7.26	-177
MR2F A8M	Mad River 2	Nov 6 2010	16.0	6.753		4.73	-26.80	7.59	-159

	Clear Fork River 1 (CFR1)			Clear Fork River 2 (CFR2)		
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$
Terrestrial Plants	-30.4±1.3 (9)	4.2±3.0 (9)	-120±15 (6)	-30.6±1.7 (10)	2.1±1.7 (10)	ND
Aquatic Macrophytes	-30.7±0.7 (2)	19.9±4.3 (2)	-124±14 (2)	-30.9±1.7 (1)	7.4±1.7 (1)	ND
Microalgae	-24.6±9.3 (2)	7.6±2.1 (2)	-194±15 (1)	-24.5±9.1*	7.4±1.7*	ND
Aquatic Sediment	-24.3±2.0 (8)	4.3±0.8 (8)	-120±1 (3)	-27.5±0.6 (15)	3.5±1.1 (15)	ND
Terrestrial Soil	-29.0±1.7 (3)	2.0±2.0 (3)	-124±13 (3)	-28.1±1.5 (3)	3.9±1.6 (3)	ND
SPOM	-31.6±0.2 (2)	4.9±0.8 (2)	-113 (1)	-29.5±1.9 (2)	5.4±1.6 (2)	ND

Table 19. Mean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of potential dietary and nutritional sources for least brook lamprey ammocoetes from the Clear Fork River sites (CFR1 and CFR2) used in food source modeling in the present study. All values (in per mil, ‰) are reported as mean±SD (n). *Estimated; see text for details, ND–not determined.

Sample ID	Site	Date	Plant Type	Species	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF1 AqV1	Clear Fork River 1	July 7 2010	Aquatic	Eplithic filamentous algae	1	12.21	-24.75	5.75	ND
CF1 AqV1	Clear Fork River 1	July 7 2010	Aquatic	Eplithic filamentous algae	2	12.54	-24.07	6.61	ND
CF1 TerV1	Clear Fork River 1	July 7 2010	Terrestrial	*Aesculus glabra*	1	24.89	-29.59	1.76	ND
CF1 TerV1	Clear Fork River 1	July 7 2010	Terrestrial	*Aesculus glabra*	2	24.78	-29.57	2.19	ND
CF1 TerV2	Clear Fork River 1	July 7 2010	Terrestrial	Grass	1	13.02	-32.18	8.29	ND
CF1 TerV2	Clear Fork River 1	July 7 2010	Terrestrial	Grass	2	12.88	-32.16	8.14	ND
CF1 TerV3	Clear Fork River 1	July 7 2010	Terrestrial	*Acer saccharum*	1	39.18	-29.89	-0.09	ND
CF1 TerV3	Clear Fork River 1	July 7 2010	Terrestrial	*Acer saccharum*	2	38.13	-29.91	-0.55	ND
CF1F AqV1	Clear Fork River 1	Nov 7 2010	Aquatic	Eplithic filamentous algae	1	11.24	-28.27	9.09	-192
CF1F AqV1	Clear Fork River 1	Nov 7 2010	Aquatic	Eplithic filamentous algae	2	ND	ND	ND	-196
CF1F AqV2	Clear Fork River 1	Nov 7 2010	Aquatic	*Ludwigia* sp.	1	27.10	-31.09	23.35	-133
CF1F AqV2	Clear Fork River 1	Nov 7 2010	Aquatic	*Ludwigia* sp.	2	27.97	-31.17	22.56	ND
CF1F AqV3	Clear Fork River 1	Nov 7 2010	Aquatic	Fallen terrestrial leaves	1	64.68	-28.47	1.06	-146
CF1F AqV3	Clear Fork River 1	Nov 7 2010	Aquatic	Fallen terrestrial leaves	2	62.91	-29.49	0.80	ND
CF1F AqV4	Clear Fork River 1	Nov 7 2010	Aquatic	*Cyperaceae* sp.	1	19.38	-30.19	17.16	-114
CF1F AqV4	Clear Fork River 1	Nov 7 2010	Aquatic	*Cyperaceae* sp.	2	19.17	-30.15	16.69	ND
CF1F TerV1	Clear Fork River 1	Nov 7 2010	Terrestrial	*Stellaria* sp.		11.52	-29.85	7.17	-111
CF1F TerV2	Clear Fork River 1	Nov 7 2010	Terrestrial	*Acer neguando*	1	22.89	-29.94	4.90	-113
CF1F TerV2	Clear Fork River 1	Nov 7 2010	Terrestrial	*Acer neguando*	2	22.35	-29.99	4.41	ND
CF1F TerV3	Clear Fork River 1	Nov 7 2010	Terrestrial	Grass	1	13.06	-32.25	5.75	-147
CF1F TerV3	Clear Fork River 1	Nov 7 2010	Terrestrial	Grass	2	12.79	-32.43	5.09	ND
CF1F TerV4	Clear Fork River 1	Nov 7 2010	Terrestrial	*Urticaceae* sp.		11.15	-31.54	6.70	-129
CF1F TerV5	Clear Fork River 1	Nov 7 2010	Terrestrial	*Rosa multiflora*	1	29.63	-29.11	3.08	-132
CF1F TerV5	Clear Fork River 1	Nov 7 2010	Terrestrial	*Rosa multiflora*	2	30.94	-29.06	2.91	-132
CF2 TerV1	Clear Fork River 2	July 7 2010	Terrestrial	Impatiens	1	17.98	-33.60	1.97	ND
CF2 TerV1	Clear Fork River 2	July 7 2010	Terrestrial	Impatiens	2	18.12	-33.47	2.31	ND
CF2 TerV2	Clear Fork River 2	July 7 2010	Terrestrial	*Salix* sp.		20.73	-30.11	3.48	ND
CF2 TerV3	Clear Fork River 2	July 7 2010	Terrestrial	Grass		16.08	-31.02	0.00	ND

Continued

Table 20. Aquatic and terrestrial vegetation elemental and stable isotopic data for the Clear Fork and Mad Rivers in the present study. ND–not determined.

Table 20 continued

Sample ID	Site	Date	Plant Type	Species	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF2 TerV4	Clear Fork River 2	July 7 2010	Terrestrial	*Rosa multiflora*	1	22.27	-31.25	4.64	ND
CF2 TerV4	Clear Fork River 2	July 7 2010	Terrestrial	*Rosa multiflora*	2	22.36	-31.39	4.05	ND
CF2 TerV4	Clear Fork River 2	July 7 2010	Terrestrial	*Rosa multiflora*	3	22.06	-31.18	4.40	ND
CF2F AqV1	Clear Fork River 2	Nov 7 2010	Aquatic	Fallen terrestrial leaves	1	33.97	-28.87	3.30	ND
CF2F AqV1	Clear Fork River 2	Nov 7 2010	Aquatic	Fallen terrestrial leaves	2	35.80	-28.49	3.88	ND
CF2F AqV2	Clear Fork River 2	Nov 7 2010	Aquatic	*Ludwigia* sp.	1	11.06	-30.95	7.42	ND
CF2F AqV2	Clear Fork River 2	Nov 7 2010	Aquatic	*Ludwigia* sp.	2	11.11	-30.85	7.35	ND
CF2F TerV1	Clear Fork River 2	Nov 7 2010	Terrestrial	Hardwood, tree	1	17.75	-30.02	0.16	ND
CF2F TerV1	Clear Fork River 2	Nov 7 2010	Terrestrial	Hardwood, tree	2	18.37	-28.67	0.77	ND
CF2F TerV2	Clear Fork River 2	Nov 7 2010	Terrestrial	*Rosa multiflora*	1	25.16	-30.40	3.54	ND
CF2F TerV2	Clear Fork River 2	Nov 7 2010	Terrestrial	*Rosa multiflora*	2	24.85	-30.37	4.04	ND
CF2F TerV3	Clear Fork River 2	Nov 7 2010	Terrestrial	Grass	1	14.79	-29.29	2.31	ND
CF2F TerV3	Clear Fork River 2	Nov 7 2010	Terrestrial	Grass	2	14.97	-29.04	2.94	ND
CF2F TerV4	Clear Fork River 2	Nov 7 2010	Terrestrial	*Solidago* sp.	1	20.05	-29.71	-0.34	ND
CF2F TerV4	Clear Fork River 2	Nov 7 2010	Terrestrial	*Solidago* sp.	2	21.01	-28.63	0.18	ND
CF2F TerV5	Clear Fork River 2	Nov 7 2010	Terrestrial	*Lonicera* sp.	1	32.42	-33.37	0.43	ND
CF2F TerV5	Clear Fork River 2	Nov 7 2010	Terrestrial	*Lonicera* sp.	2	33.54	-32.92	1.64	ND
MR1 AqV1	Mad River 1	July 1 2010	Aquatic	*Ludwigia* sp.		12.00	-33.49	11.20	ND
MR1 AqV2	Mad River 1	July 1 2010	Aquatic	*Eleocharis* sp.		18.56	-33.69	11.67	ND
MR1 AqV3	Mad River 1	July 1 2010	Aquatic	*Vallisneria americana*	1	14.68	-33.27	8.67	ND
MR1 AqV3	Mad River 1	July 1 2010	Aquatic	*Vallisneria americana*	2	14.23	-33.31	8.47	ND
MR1 TerV1	Mad River 1	July 1 2010	Terrestrial	Grass		16.71	-32.96	6.47	ND
MR1 TerV2	Mad River 1	July 1 2010	Terrestrial	*Crataegus* sp.	1	26.02	-30.64	1.58	ND
MR1 TerV2	Mad River 1	July 1 2010	Terrestrial	*Crataegus* sp.	2	26.33	-30.40	1.76	ND
MR1 TerV3	Mad River 1	July 1 2010	Terrestrial	*Acer saccharum*	1	19.88	-30.73	1.66	ND
MR1 TerV3	Mad River 1	July 1 2010	Terrestrial	*Acer saccharum*	2	20.41	-30.77	1.30	ND
MR1 TerV4	Mad River 1	July 1 2010	Terrestrial	*Ulmus* sp.		17.60	-30.67	6.51	ND
MR1F AqV1	Mad River 1	Nov 6 2010	Aquatic	*Ludwigia* sp.		9.47	-30.02	11.40	-153
MR1F AqV2	Mad River 1	Nov 6 2010	Aquatic	*Vallisneria americana*	1	28.84	-35.45	10.89	-153

Continued

Table 20 continued

Sample ID	Site	Date	Plant Type	Species	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
MR1F AqV2	Mad River 1	Nov 6 2010	Aquatic	*Vallisneria americana*	2	29.17	-35.76	10.51	ND
MR1F AqV3	Mad River 1	Nov 6 2010	Aquatic	*Eleocharis* sp.		15.54	-34.15	11.34	-127
MR1F TerV1	Mad River 1	Nov 6 2010	Terrestrial	Unidentified		ND	ND	ND	-122
MR1F TerV2	Mad River 1	Nov 6 2010	Terrestrial	*Acer* sp.		20.00	-30.73	3.19	-123
MR1F TerV3	Mad River 1	Nov 6 2010	Terrestrial	Fallen terrestrial leaves	1	66.00	-30.98	1.81	-110
MR1F TerV3	Mad River 1	Nov 6 2010	Terrestrial	Fallen terrestrial leaves	2	ND	ND	ND	-112
MR1F TerV4	Mad River 1	Nov 6 2010	Terrestrial	*Rosa multiflora*		43.41	-29.63	-0.41	-127
MR1F TerV5	Mad River 1	Nov 6 2010	Terrestrial	Shrub		27.45	-31.93	3.10	-118
MR2 AqV1	Mad River 2	July 1 2010	Aquatic	*Eleocharis* sp.		11.74	-26.31	5.70	ND
MR2 TerV1	Mad River 2	July 1 2010	Terrestrial	*Platanus occidentalis*		28.76	-32.23	1.22	ND
MR2 TerV2	Mad River 2	July 1 2010	Terrestrial	Grass		16.40	-30.86	7.62	ND
MR2 TerV3	Mad River 2	July 1 2010	Terrestrial	*Morus alba*		23.11	-28.46	1.16	ND
MR2 TerV4	Mad River 2	July 1 2010	Terrestrial	*Maclura pomifera*		21.14	-29.13	-0.28	ND
MR2F AqV1	Mad River 2	Nov 6 2010	Aquatic	Eplithic hair algae	1	17.39	-32.96	7.27	-171
MR2F AqV1	Mad River 2	Nov 6 2010	Aquatic	Eplithic hair algae	2	16.91	-32.95	7.19	ND
MR2F AqV1	Mad River 2	Nov 6 2010	Aquatic	Eplithic hair algae	3	17.16	-32.93	7.96	ND
MR2F AqV2	Mad River 2	Nov 6 2010	Aquatic	Fallen terrestrial leaves		42.51	-29.23	2.31	-121
MR2F AqV3	Mad River 2	Nov 6 2010	Aquatic	*Ludwigia* sp.	1	15.73	-30.36	6.51	-125
MR2F AqV3	Mad River 2	Nov 6 2010	Aquatic	*Ludwigia* sp.	2	ND	ND	ND	-124
MR2F AqV4	Mad River 2	Nov 6 2010	Aquatic	*Myriophyllum* sp.		23.24	-30.96	5.92	-97
MR2F TerV1	Mad River 2	Nov 6 2010	Terrestrial	*Lonicera* sp.	1	32.71	-30.75	-1.17	-132
MR2F TerV1	Mad River 2	Nov 6 2010	Terrestrial	*Lonicera* sp.	2	29.07	-31.28	-0.83	ND
MR2F TerV2	Mad River 2	Nov 6 2010	Terrestrial	*Aster prenanthoides*		26.75	-33.28	6.31	-124
MR2F TerV3	Mad River 2	Nov 6 2010	Terrestrial	Grass		30.51	-30.53	4.78	-148
MR2F TerV4	Mad River 2	Nov 6 2010	Terrestrial	Grass	1	14.97	-30.75	3.39	-159
MR2F TerV4	Mad River 2	Nov 6 2010	Terrestrial	Grass	2	13.99	-30.89	3.20	ND
MR2F TerV5	Mad River 2	Nov 6 2010	Terrestrial	*Rosa multiflora*		23.19	-30.32	7.27	-126

	Mad River 1 (MR1)			Mad River 2 (MR2)		
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$
Terrestrial Plants	-31.2±1.0 (9)	2.8±2.3 (9)	-120±6 (5)	-30.6±1.4 (10)	3.3±3.1 (10)	-135±15 (6)
Aquatic Macrophytes	-33.4±1.8 (6)	10.8±1.1 (6)	-144±15 (3)	-29.2±2.5 (3)	6.0±0.4 (3)	-111±20 (2)
Microalgae	-26.8±12.5*	10.8±1.1*	-171±15*	-25.5±10.6 (1)	7.5±0.4 (1)	-171±20 (1)
Aquatic Sediment	-18.0±3.9 (8)	4.2±1.1 (8)	-129±5 (3)	-24.1±4.6 (8)	3.6±0.8 (8)	-126±5 (3)
Terrestrial Soil	-23.3±3.8 (3)	4.0±1.3 (3)	-124±4 (3)	-23.3±5.4 (3)	3.6±2.1 (3)	-101±18 (3)
SPOM	-32.0±1.7 (2)	3.9±2.5 (2)	-107 (1)	-31.1±0.1 (2)	4.5±0.1 (2)	-95.9 (1)

Table 21. Mean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of potential dietary and nutritional sources to American brook lamprey ammocoetes from the Mad River sites (MR1 and MR2) used in food source modeling in the present study. All values (in per mil, ‰) are reported as mean±SD (n). *Estimated; see text for details.

Sample ID	Site	Date	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF1 A	Clear Fork River 1	July 8 2010	6.384	-31.68	5.49	ND
CF1F B	Clear Fork River 1	Nov 7 2010	5.617	-31.43	4.35	-113
CF2 B	Clear Fork River 2	July 8 2010	6.793	-28.12	6.56	ND
CF2F A	Clear Fork River 2	Nov 7 2010	6.434	-30.83	4.32	ND
MR1 B	Mad River 1	July 1 2010	7.917	-30.86	5.63	ND
MR1F A	Mad River 1	Nov 6 2010	3.515	-33.23	2.14	-107
MR2 C	Mad River 2	July 1 2010	7.692	-31.01	4.54	ND
MR2F A	Mad River 2	Nov 6 2010	7.031	-31.18	4.40	-96

Table 22. Suspended particulate OM elemental and stable isotopic data for the Clear Fork and Mad Rivers in the present study. ND–not determined.

Sample ID	Site	Date	Sample Depth (cm)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF1 AqS1	Clear Fork River 1	July 8 2010	0.0-0.5	1	14.86	-24.41	3.12	ND
CF1 AqS1	Clear Fork River 1	July 8 2010	0.0-0.5	2	13.38	-23.01	3.77	ND
CF1 AqS1	Clear Fork River 1	July 8 2010	0.0-0.5	3	18.73	-19.74	3.42	ND
CF1 AqS2	Clear Fork River 1	July 8 2010	0.5-1.0		15.47	-23.62	5.11	ND
CF1 AqS3	Clear Fork River 1	July 8 2010	1.0-2.0		15.48	-23.53	3.89	ND
CF1 AqS4	Clear Fork River 1	July 8 2010	2.0-3.0		19.04	-21.01	5.89	ND
CF1 AqS5	Clear Fork River 1	July 8 2010	3.0-4.0		19.45	-17.82	3.80	ND
CF1 AqS6	Clear Fork River 1	July 8 2010	4.0-5.0	1	22.55	-17.83	4.62	ND
CF1 AqS6	Clear Fork River 1	July 8 2010	4.0-5.0	2	24.96	-17.87	4.83	ND
CF1 AqS7	Clear Fork River 1	July 8 2010	5.0-6.0		33.61	-12.08	4.44	ND
CF1F AqS1	Clear Fork River 1	Nov 7 2010	0.0-0.5		14.03	-26.36	4.12	-122
CF1F AqS2	Clear Fork River 1	Nov 7 2010	0.5-1.0		14.17	-26.94	3.78	-119
CF1F AqS3	Clear Fork River 1	Nov 7 2010	1.0-2.0		15.25	-25.66	4.64	ND
CF1F AqS4	Clear Fork River 1	Nov 7 2010	2.0-3.0		16.06	-24.77	3.72	ND
CF1F AqS5	Clear Fork River 1	Nov 7 2010	3.0-4.0	1	17.55	-17.69	2.39	ND
CF1F AqS5	Clear Fork River 1	Nov 7 2010	3.0-4.0	2	17.42	-18.90	4.99	ND
CF1F AqS5	Clear Fork River 1	Nov 7 2010	3.0-4.0	3	37.78	-10.69	2.17	ND
CF1F AqS6	Clear Fork River 1	Nov 7 2010	4.0-5.0		21.20	-17.47	3.02	ND
CF1F AqS7	Clear Fork River 1	Nov 7 2010	5.0-6.0		41.82	-12.84	3.54	ND

Continued

Table 23. Aquatic sediment OM elemental and stable isotopic data for the Clear Fork and Mad Rivers in the present study. ND–not determined.

Table 23 continued

Sample ID	Site	Date	Sample Depth (cm)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF1F AqS8	Clear Fork River 1	Nov 7 2010	6.0-7.0		32.48	-11.48	6.33	-120
CF2 AqS1	Clear Fork River 2	July 8 2010	0.0-0.5		12.12	-27.57	3.22	ND
CF2 AqS2	Clear Fork River 2	July 8 2010	0.5-1.0		13.61	-27.79	3.24	ND
CF2 AqS3	Clear Fork River 2	July 8 2010	1.0-2.0		13.14	-27.25	3.18	ND
CF2 AqS4	Clear Fork River 2	July 8 2010	2.0-3.0		11.46	-26.81	2.46	ND
CF2 AqS5	Clear Fork River 2	July 8 2010	3.0-4.0		12.26	-26.71	4.00	ND
CF2 AqS6	Clear Fork River 2	July 8 2010	4.0-5.0	1	14.80	-26.80	4.54	ND
CF2 AqS6	Clear Fork River 2	July 8 2010	4.0-5.0	2	13.51	-26.56	1.18	ND
CF2 AqS7	Clear Fork River 2	July 8 2010	5.0-6.0	1	11.84	-26.99	2.91	ND
CF2 AqS7	Clear Fork River 2	July 8 2010	5.0-6.0	2	12.97	-27.18	3.72	ND
CF2 AqS8	Clear Fork River 2	July 8 2010	6.0-7.0		13.35	-27.47	6.33	ND
CF2F AqS1	Clear Fork River 2	Nov 7 2010	0.0-0.5		15.39	-28.31	3.54	ND
CF2F AqS2	Clear Fork River 2	Nov 7 2010	0.5-1.0		15.34	-28.15	3.20	ND
CF2F AqS3	Clear Fork River 2	Nov 7 2010	1.0-2.0		15.57	-28.28	2.99	ND
CF2F AqS4	Clear Fork River 2	Nov 7 2010	2.0-3.0		18.84	-27.89	2.74	ND
CF2F AqS5	Clear Fork River 2	Nov 7 2010	3.0-4.0		19.90	-28.22	2.32	ND
CF2F AqS6	Clear Fork River 2	Nov 7 2010	4.0-5.0		12.25	-26.79	5.77	ND
CF2F AqS7	Clear Fork River 2	Nov 7 2010	5.0-6.0		15.68	-27.01	2.74	ND
MR1 AqS1	Mad River 1	July 1 2010	0.0-0.5	1	71.45	-8.46	4.82	ND
MR1 AqS1	Mad River 1	July 1 2010	0.0-0.5	2	78.83	-5.91	3.35	ND
MR1 AqS1	Mad River 1	July 1 2010	0.0-0.5	3	84.62	-4.90	3.69	ND

Continued

Table 23 continued

Sample ID	Site	Date	Sample Depth (cm)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
MR1 AqS2	Mad River 1	July 1 2010	0.5-1.0		309.14	-1.38	3.69	ND
MR1 AqS3	Mad River 1	July 1 2010	1.0-2.0		211.87	-2.72	0.38	ND
MR1 AqS4	Mad River 1	July 1 2010	2.0-3.0		195.78	-3.70	0.08	ND
MR1 AqS5	Mad River 1	July 1 2010	3.0-4.0	1	169.38	-3.64	-2.18	ND
MR1 AqS5	Mad River 1	July 1 2010	3.0-4.0	2	152.85	-4.03	0.78	ND
MR1 AqS6	Mad River 1	July 1 2010	4.0-5.0		237.06	-2.04	-0.74	ND
MR1 AqS7	Mad River 1	July 1 2010	5.0-6.0		152.06	-3.81	0.72	ND
MR1 AqS8	Mad River 1	July 1 2010	6.0-7.0	1	87.86	-4.97	4.00	ND
MR1 AqS8	Mad River 1	July 1 2010	6.0-7.0	2	112.02	-4.10	1.46	ND
MR1F AqS1	Mad River 1	Nov 6 2010	0.0-0.5	1	27.28	-15.28	4.53	-129
MR1F AqS1	Mad River 1	Nov 6 2010	0.0-0.5	2	29.77	-16.19	3.65	ND
MR1F AqS2	Mad River 1	Nov 6 2010	0.5-1.0		23.67	-20.90	3.01	-124
MR1F AqS3	Mad River 1	Nov 6 2010	1.0-2.0		25.36	-17.38	4.26	ND
MR1F AqS4	Mad River 1	Nov 6 2010	2.0-3.0		26.39	-16.22	6.67	ND
MR1F AqS5	Mad River 1	Nov 6 2010	3.0-4.0		25.91	-17.81	4.15	ND
MR1F AqS6	Mad River 1	Nov 6 2010	4.0-5.0	1	29.68	-13.76	3.73	ND
MR1F AqS6	Mad River 1	Nov 6 2010	4.0-5.0	2	17.64	-23.76	3.03	ND
MR1F AqS6	Mad River 1	Nov 6 2010	4.0-5.0	3	32.83	-14.76	4.33	ND
MR1F AqS7	Mad River 1	Nov 6 2010	5.0-6.0	1	28.83	-15.61	3.93	ND
MR1F AqS7	Mad River 1	Nov 6 2010	5.0-6.0	2	26.71	-15.96	3.65	ND
MR1F AqS9	Mad River 1	Nov 6 2010	7.0-8.0		20.35	-26.29	3.35	-134

Continued

Table 23 continued

Sample ID	Site	Date	Sample Depth (cm)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
MR2 AqS1	Mad River 2	July 1 2010	0.0-0.5		41.43	-11.41	3.22	ND
MR2 AqS2	Mad River 2	July 1 2010	0.5-1.0		35.49	-13.50	2.71	ND
MR2 AqS3	Mad River 2	July 1 2010	1.0-2.0		40.43	-12.38	2.35	ND
MR2 AqS4	Mad River 2	July 1 2010	2.0-3.0		43.40	-12.51	2.39	ND
MR2 AqS5	Mad River 2	July 1 2010	3.0-4.0		42.98	-13.60	1.65	ND
MR2 AqS6	Mad River 2	July 1 2010	4.0-5.0		36.97	-18.01	1.87	ND
MR2 AqS7	Mad River 2	July 1 2010	5.0-6.0		59.16	-11.11	0.54	ND
MR2 AqS8	Mad River 2	July 1 2010	7.0-8.0		83.97	-7.74	-0.45	ND
MR2 AqS9	Mad River 2	July 1 2010	8.0-9.0		48.93	-12.82	0.68	ND
MR2F AqS1	Mad River 2	Nov 6 2010	0.0-0.5		13.95	-27.28	3.55	-121
MR2F AqS2	Mad River 2	Nov 6 2010	0.5-1.0		13.75	-27.84	3.69	-126
MR2F AqS3	Mad River 2	Nov 6 2010	1.0-2.0		13.65	-27.75	4.35	ND
MR2F AqS4	Mad River 2	Nov 6 2010	2.0-3.0		14.22	-28.08	3.03	ND
MR2F AqS5	Mad River 2	Nov 6 2010	3.0-4.0		15.47	-25.14	4.92	ND
MR2F AqS6	Mad River 2	Nov 6 2010	4.0-5.0		20.82	-21.10	3.77	ND
MR2F AqS7	Mad River 2	Nov 6 2010	5.0-6.0		27.76	-16.50	3.33	ND
MR2F AqS8	Mad River 2	Nov 6 2010	7.0-8.0		27.98	-18.86	2.31	-131

Sample ID	Site	Date	Sample Depth (cm)	Analytical Replicate	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
CF1F TerS0	Clear Fork River 1	Nov 7 2010	0		34.61	-30.65	0.03	-130
CF1F TerS2	Clear Fork River 1	Nov 7 2010	2		15.82	-29.08	2.07	-108
CF1F TerS18	Clear Fork River 1	Nov 7 2010	18		12.66	-27.19	3.99	-133
CF2F TerS0	Clear Fork River 2	Nov 7 2010	0		29.32	-29.59	2.01	ND
CF2F TerS3	Clear Fork River 2	Nov 7 2010	3		13.44	-27.98	4.63	ND
CF2F TerS20	Clear Fork River 2	Nov 7 2010	20	1	12.17	-27.05	4.90	ND
CF2F TerS20	Clear Fork River 2	Nov 7 2010	20	2	20.18	-27.99	-0.86	ND
CF2F TerS20	Clear Fork River 2	Nov 7 2010	20	3	11.81	-26.29	4.93	ND
MR1F TerS0	Mad River 1	Nov 6 2010	0		28.37	-27.54	2.68	-121
MR1F TerS10	Mad River 1	Nov 6 2010	10		16.62	-20.24	4.29	-124
MR1F TerS30	Mad River 1	Nov 6 2010	30		14.22	-22.15	5.16	-128
MR2F TerS0	Mad River 2	Nov 6 2010	0		27.11	-28.31	1.20	-119
MR2F TerS2	Mad River 2	Nov 6 2010	8		14.21	-23.83	4.39	-84
MR2F TerS3	Mad River 2	Nov 6 2010	30		19.02	-17.62	5.14	-101

Table 24. Terrestrial soil OM data for the Clear Fork and Mad Rivers in the present study. ND–not determined.

Sample Site	Group [c]	N	Terrestrial Vegetation	Terrestrial Soil	Aquatic Macrophytes	Microalgae	Aquatic Sediment
Clear Fork River 1[a]	All sizes	6	1.8 (0.1-7.6)	68.0 (54.4-79.9)	0.8 (0.1-3.1)	25.5 (14.1-38.1)	2.1 (0.2-8.3)
	0-10 cm	3	21.8 (2.1-55.1)	48.6 (11.9-73.1)	2.2 (0.2-8.0)	8.7 (1.1-20.9)	15.6 (2.0-36.0)
	>10 cm	3	4.2 (0.3-26.1)	14.2 (1.0-51.1)	0.8 (0.1-3.3)	70.9 (41.7-87.5)	3.1 (0.2-13.0)
Clear Fork River 2[a]	All sizes	11	9.4 (0.8-34.1)	10.4 (0.8-45.3)	1.5 (0.1-5.5)	22.5 (17.1-29.6)	51.8 (12.0-71.9)
	0-4 cm	5	18.9 (3.0-40.5)	22.8 (2.6-52.0)	19.5 (5.0-34.7)	5.0 (0.5-15.7)	29.6 (4.9-56.6)
	4-8 cm	1	15.0 (1.4-44.6)	18.9 (1.7-54.0)	19.1 (2.0-48.2)	12.7 (1.3-39.8)	20.1 (1.8-55.0)
	>8 cm	5	11.8 (1.0-34.6)	17.9 (1.7-48.2)	5.1 (0.4-16.3)	29.1 (19.7-41.9)	28.8 (3.8-57.6)
Mad River 1[b]	All sizes	7	53.5 (41.5-67.1)	8.7 (0.8-24.1)	19.8 (4.8-30.8)	6.4 (0.7-20.4)	8.9 (1.1-18.2)
	0-10 cm	1	34.7 (6.6-62.0)	18.0 (1.6-52.9)	14.6 (1.5-36.0)	11.3 (1.0-30.4)	12.3 (1.2-38.2)
	>10 cm	6	55.0 (41.6-69.3)	8.0 (0.7-24.7)	17.5 (2.8-30.3)	7.5 (0.8-23.4)	8.4 (0.9-18.3)
Mad River 2[b]	All sizes	7	46.8 (34.5-57.6)	2.8 (0.2-10.5)	3.9 (0.4-10.9)	38.7 (29.5-46.8)	5.1 (0.4-18.4)
	0-10 cm	2	21.0 (2.9-48.9)	13.0 (1.3-35.2)	22.0 (2.7-50.4)	13.9 (1.8-32.2)	22.0 (2.7-50.3)
	>10 cm	5	42.9 (27.4-58.3)	3.1 (0.2-12.0)	2.4 (0.2-9.7)	40.4 (27.3-51.8)	7.1 (0.6-25.9)

Table 25. Median percent contributions of potential dietary and nutritional sources to native lamprey ammocoetes estimated by Bayesian modeling. Values in parenthesis are the 5-95% median percent contributions. [a]Sites where least brook lamprey (*Lampetra aepyptera*) were present. [b]Sites where American brook lamprey (*Lampetra appendix*) were present. [c]Ammocoete size groupings were chosen to create groups roughly based on age from size distributions.

Site	Reference	Location	Length (cm)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{2}H$ (‰)
			Sea Lamprey (*Petromyzon marinus*)[a, b]				
Jordan River 1	Evans and Bauer in prep. a	Michigan, USA	4.0±1.6 (25)	6.8±1.4 (25)	-27.6±2.0 (25)	4.0±0.7 (25)	-197±10 (13)
Jordan River 2	Evans and Bauer in prep. a	Michigan, USA	3.3±2.2 (13)	7.2±1.9 (13)	-27.9±2.5 (13)	2.8±0.6 (13)	ND
Pigeon River	Evans and Bauer in prep. a	Michigan, USA	5.5±2.4 (19)	7.3±1.6 (19)	-26.1±2.2 (19)	4.7±0.6 (19)	-200±16 (14)
Root River	Hollett 1995	Ontario, Canada	NR	NR	-21.92±1.10 (18)	3.07±0.37 (3)	ND
			Least Brook Lamprey (*Lampetra aepyptera*)				
Clear Fork River 1	Evans and Bauer in prep. b	Ohio, USA	10.3±1.8 (14)	7.0±2.1 (14)	-25.0±1.6 (14)	5.3±1.4 (14)	-165±32 (6)
Clear Fork River 2	Evans and Bauer in prep. b	Ohio, USA	6.2±4.0 (19)	4.7±0.9 (19)	-25.6±2.1 (19)	7.4±1.0 (19)	ND
			River Lamprey (*Lampetra fluviatilis*)[a]				
Rhine River (1996-98)	van Riel et al 2006	Nijmegan, Netherlands	9.0-13.0 (3)	NR	-22.53±0.60 (3)	9.81±0.38 (3)	ND
Rhine River (2001-03)	van Riel et al 2006	Nijmegan, Netherlands	1.5-8.5 (3)	NR	-28.11±2.12 (3)	9.61±0.96 (3)	ND
			American Brook Lamprey (*Lethenteron appendix*)				
Mad River 1	Evans and Bauer in prep. b	Ohio, USA	14.6±2.3 (19)	5.5±1.3 (19)	-27.8±1.2 (19)	7.8±0.4 (19)	-148±10 (7)
Mad River 2	Evans and Bauer in prep. b	Ohio, USA	14.4±3.1 (20)	7.0±1.7 (20)	-25.2±1.5 (20)	7.5±0.5 (20)	-159±22 (8)
Batchawana River	Marty et al. 2008	Ontario, Canada	NR	NR	-24.8±1.1 (2)	3.0±0.1 (2)	ND
			Japanese Lamprey (*Lethenteron japanicum*)[a]				
Ishikari River	Shirakawa et al. 2009	Hokkaido, Japan	1-5 (6)	NR	-18.91±1.73 (6)	1.23±0.33 (6)	ND
			Pacific Lamprey (*Entosphenus tridentatus*)[a]				
Snoqualmie River	Bilby et al. 1996	Washington, USA	NR	NR	-27.8±1.2 (4)	6.4±1.4 (4)	ND
Eel River	Limm and Power 2011	California, USA	8.2±0.5 (104)	4.8 (104)	-23.4±0.2 (15)	2.7±0.1 (15)	ND

Table 26. Mean length, C:N, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values of ammocoetes from the present study and other published data on wild populations of ammocoetes. All values are reported as mean±SD (n), all values are reported from non-lipid extracted tissue. [a]Denotes a parasitic species; all others are non-parasitic. [b]Denotes an invasive species in the areas of the cited study. ND–not determined, NR–not reported.

Site	Species	Land use (%)			Autochthonous	Allochthonous	Reference
		Forested	Agriculture	Urban	(%)	(%)	
Clear Fork River 1	*Lampetra aepyptera* [a]	41	47	12	28.4 (14.4-49.5) [d]	69.8 (54.5-87.5)	This study
Clear Fork River 2	*Lampetra aepyptera* [a]	41	47	12	75.8 (19.2-107.0)	19.8 (1.6-79.4)	This study
Mad River 1	*Lethenteron appendix* [b]	18	81	1	35.1 (6.6-69.4)	62.2 (42.3-91.2)	This study
Mad River 2	*Lethenteron appendix* [b]	18	81	1	47.7 (30.3-76.1)	49.6 (34.7-68.1)	This study
Jordan River 1	*Petromyzon marinus* [b]	72	27	1	51.7 (35.6-74.4)	47.5 (33.5-65.7)	Evans and Bauer (*submitted*)
Pigeon River	*Petromyzon marinus* [b]	93	7	0	58.4 (51.6-68.1)	40.9 (33.8-48.8)	Evans and Bauer (*submitted*)

Table 27. Median percentage contributions of autochthonous and allochthonous nutritional sources to native (Ohio, USA) and invasive (Michigan, USA) lamprey ammocoetes estimated by Bayesian modeling. [a]Native least brook lamprey ammocoetes. [b]Native American brook lamprey ammocoetes. [c]Invasive sea lamprey ammocoetes. [d]Values in parenthesis are the 5-95% median percent contributions. Note that % values are created by summation of minimum and maximum estimates of median percentage contributions derived from Bayesian modeling and may therefore add up to be >100% or < 100%.

Appendix B: Figures

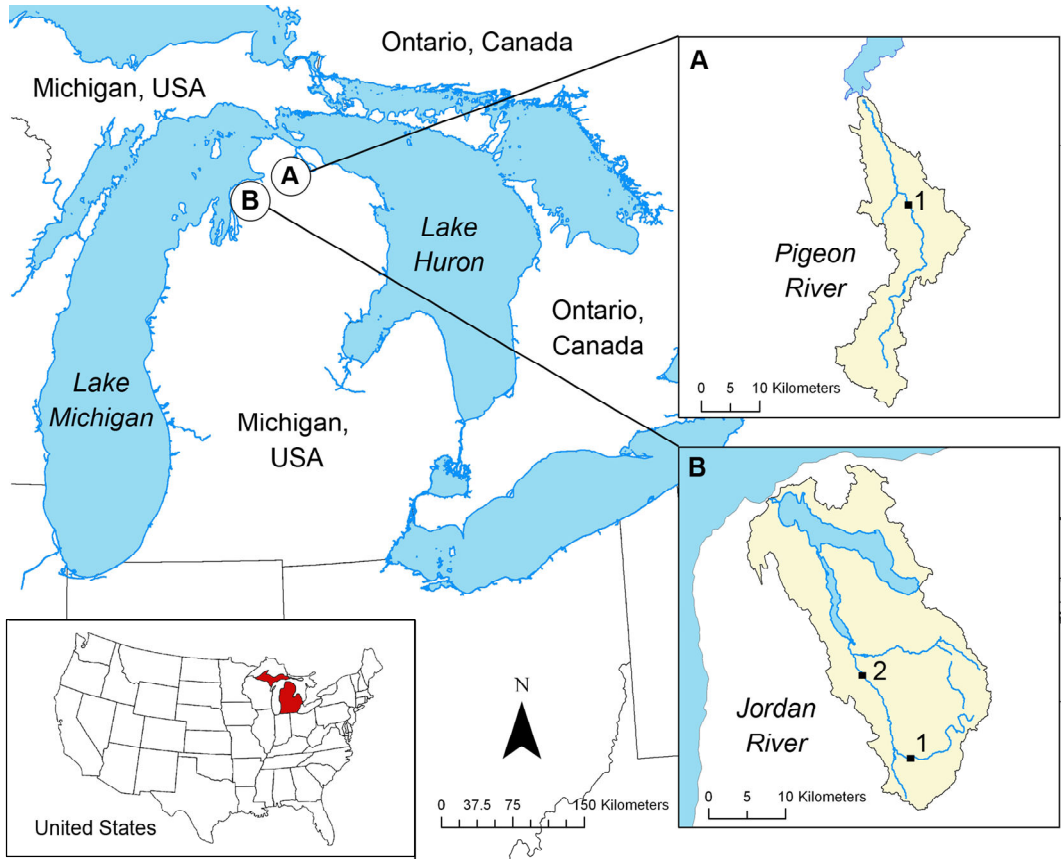

Figure 1. Site locations on the (a) Pigeon River and (b) Jordan River sampled in the present study.

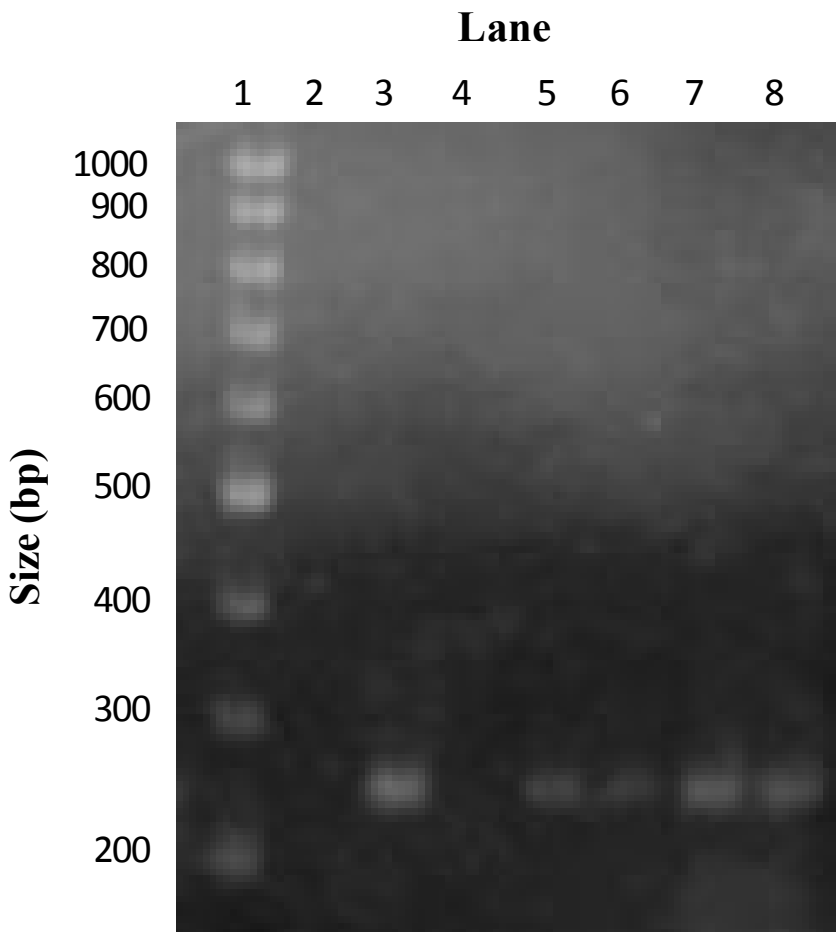

Figure 2. Electrophoretic agarose gel displaying a known control ammocoete (Lethenteron appendix, lane 2) and ammocoetes of unknown genera (lanes 3-9) collected from the Jordan River 1 site on Oct 3, 2010. Lanes 3 and 5-9 were scored as sea lamprey(*Petromyzon marinus*). Lane 4 was re-run on a separate gel and amplified in a separate reaction to confirm that no band was present. Lane 1 contains different molecular weight markers, with associated size of bands indicated. bp–number of base pairs.

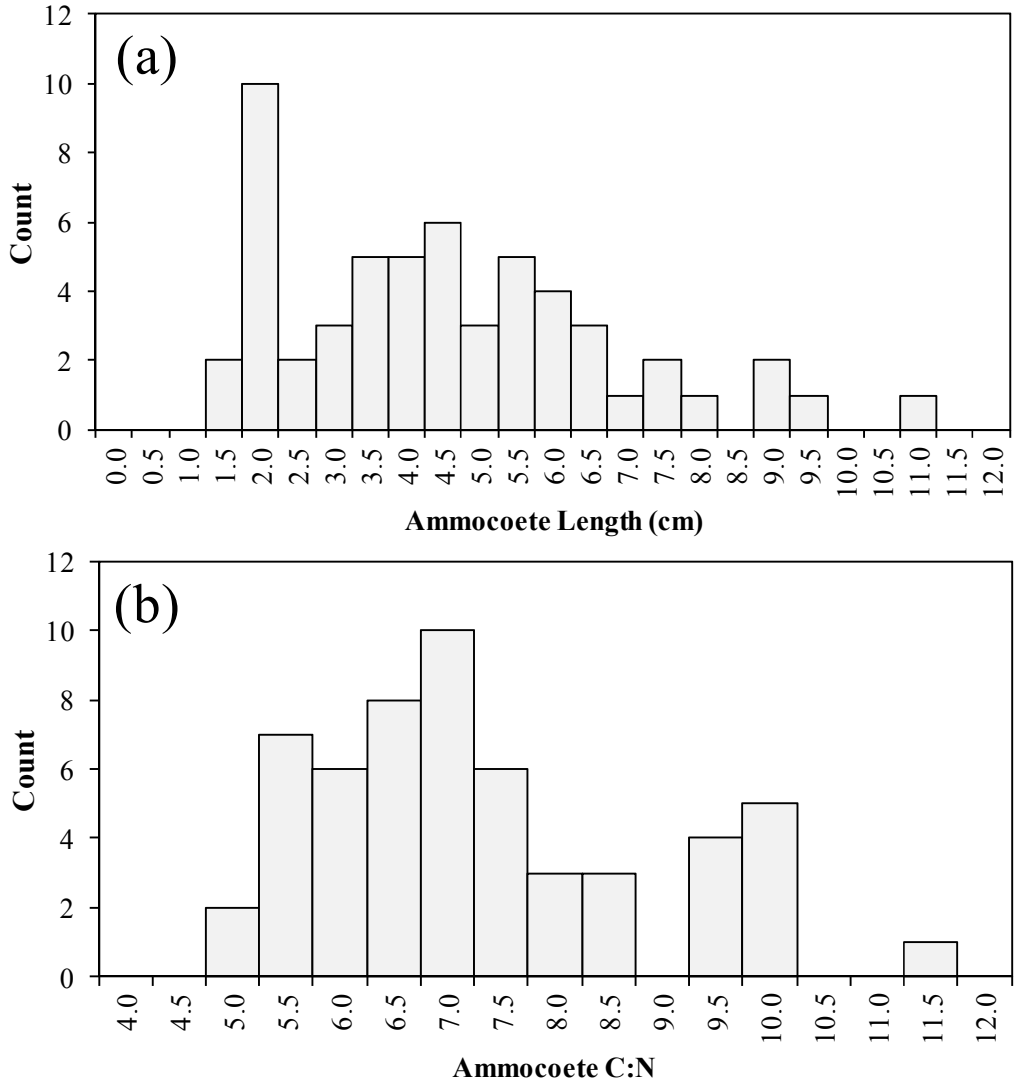

Figure 3. (a) Distribution of sea lamprey (*Petromyzon marinus*) ammocoete sizes for animals captured and analyzed in the present study. Large (>10 cm) animals were rare at all of the sites sampled because of chemical treatment of streams every 3-5 years to control sea lamprey by extirpating the larvae from the streams. Small (<4 cm) ammocoetes were likely young-of-year (YOY). (b) Distribution of C:N in ammocoete muscle tissue.

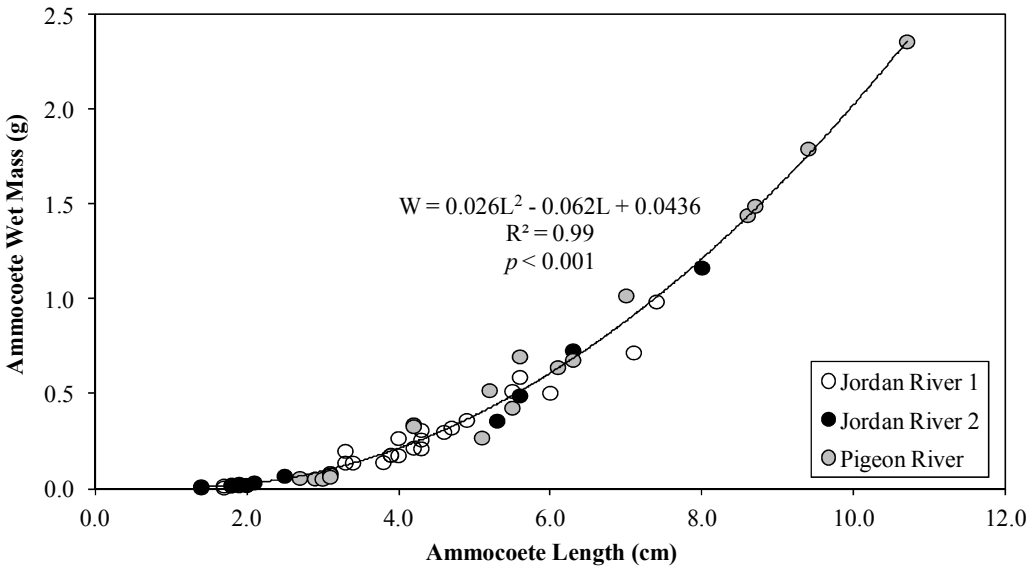

Figure 4. Sea lamprey ammocoete wet mass versus animal length across all sampling sites and times, with best fit line shown.

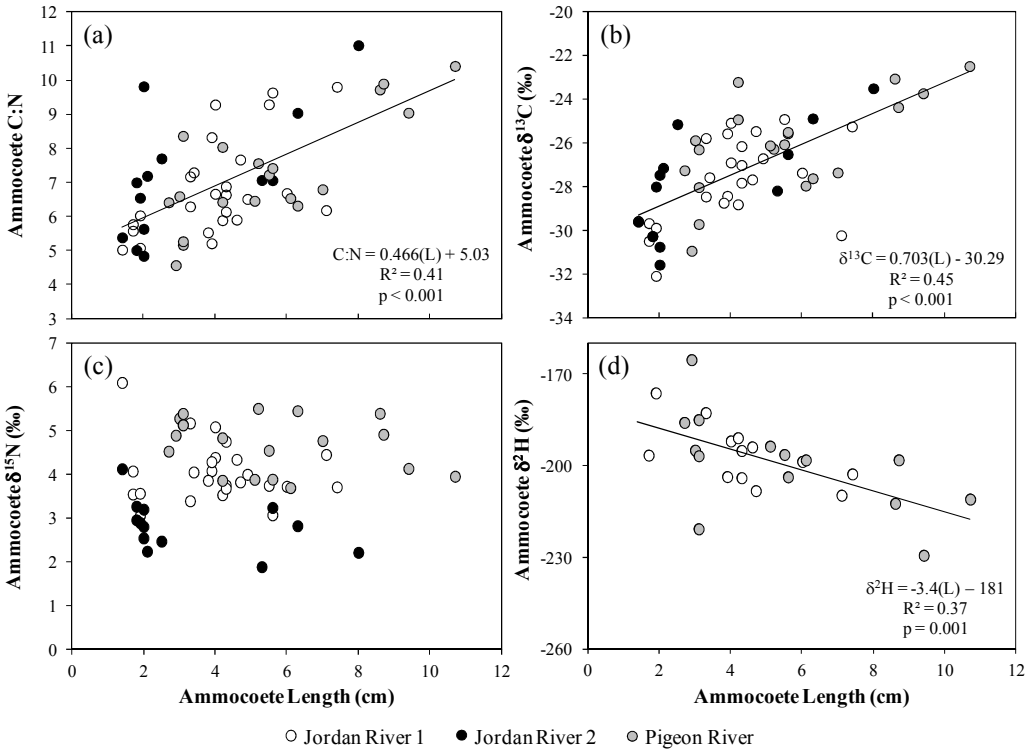

Figure 5. Sea lamprey ammocoete muscle tissue (a) C:N, (b) $\delta^{13}C$, (c) $\delta^{15}N$ and (d) $\delta^{2}H$ versus animal length for all sampling sites and times in the present study.

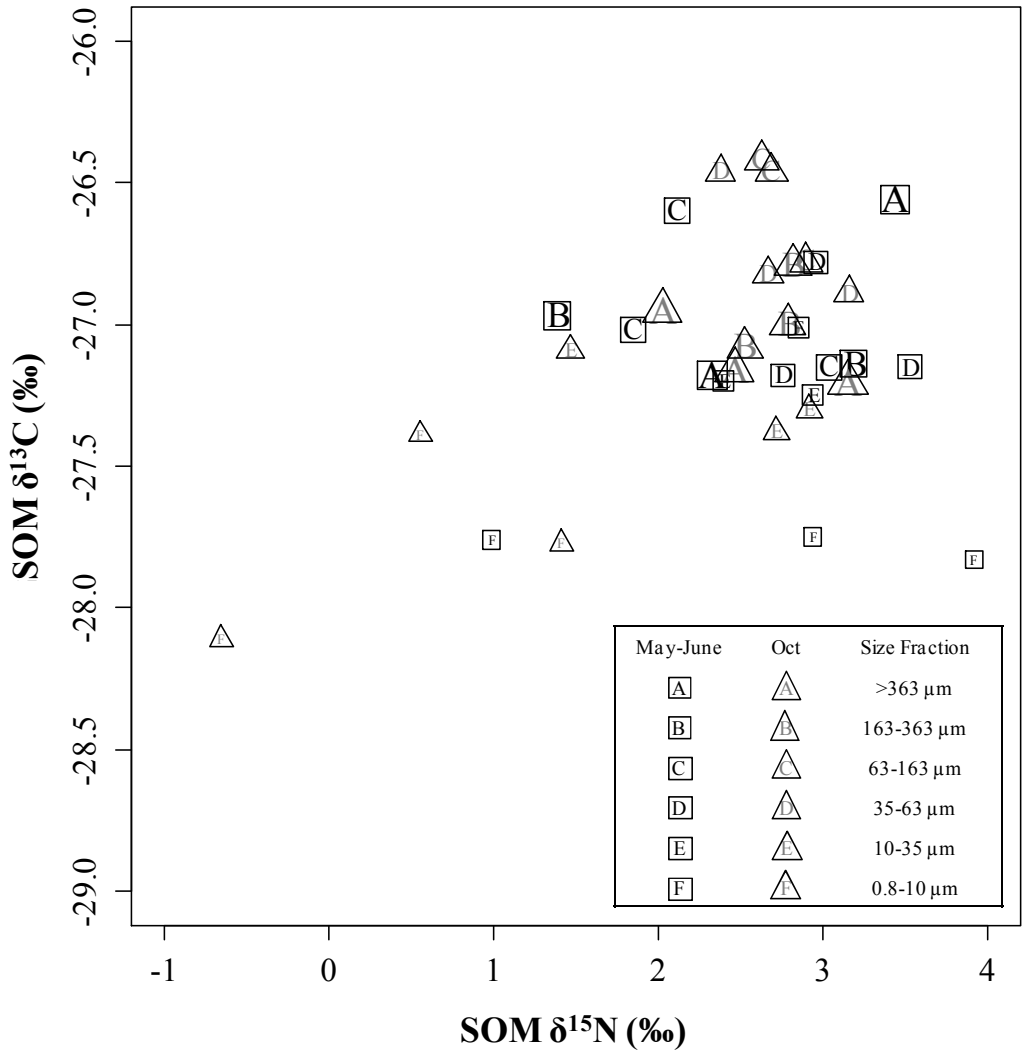

Figure 6. $\delta^{13}C$ versus $\delta^{15}N$ of size-fractionated aquatic sediment organic matter (SOM) in 2010. A single value at Jordan River 2 ($\delta^{13}C$=-15.2 and $\delta^{15}N$=-1.35) is not displayed. Letters refer to different size fractions and are scaled to help visually differentiate size fractions in the plot (see *Materials and Methods* for further explanation).

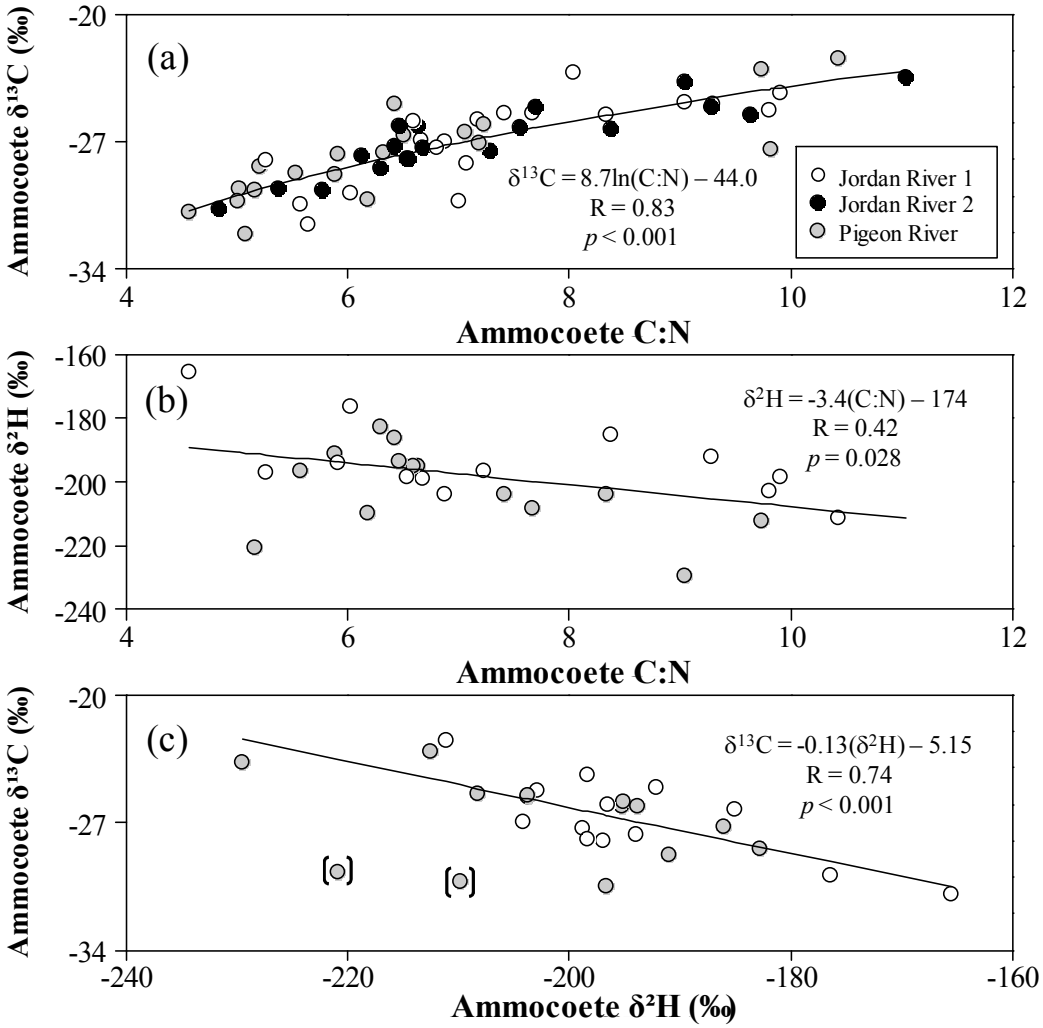

Figure 7. (a) $\delta^{13}C$ versus C:N and (b) $\delta^{2}H$ versus C:N (c) $\delta^{13}C$ versus $\delta^{2}H$ of sea lamprey ammocoete muscle tissue for all sampling sites and times in the present study. Samples in brackets were removed from best fit line, even though their inclusion still allowed the relationship to be significant ($P < 0.05$).

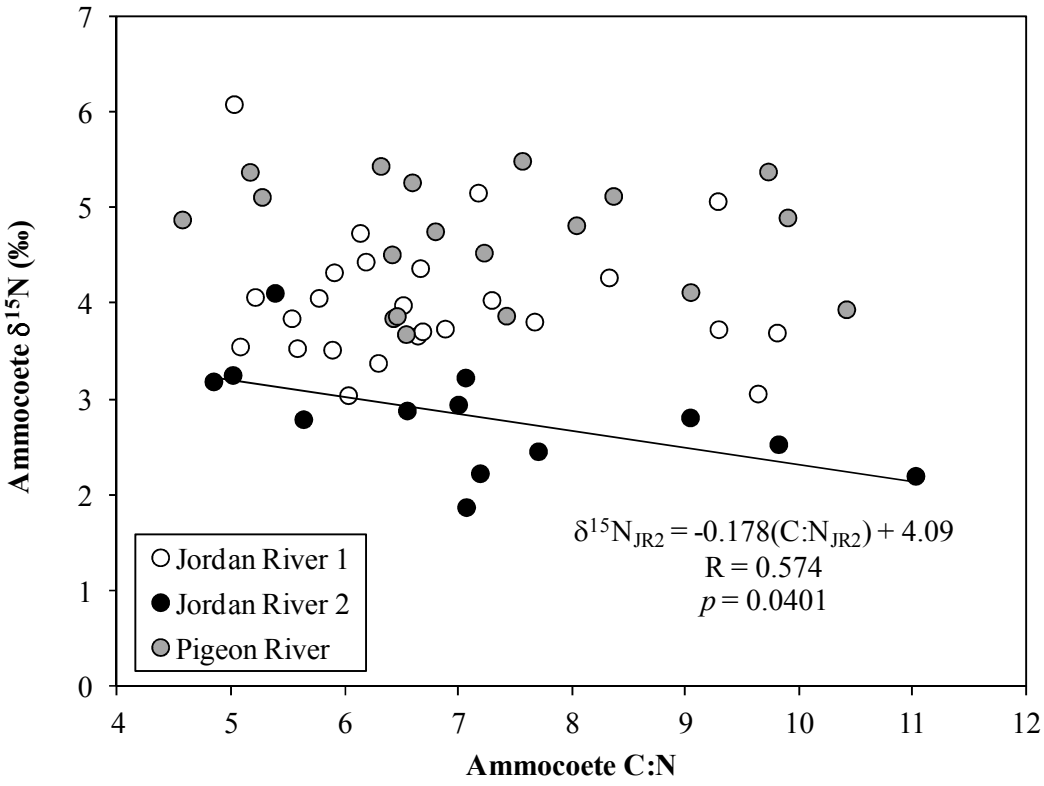

Figure 8. $\delta^{15}N$ versus C:N of sea lamprey ammocoete muscle by site, with best fit lines shown. Correlations for Jordan River 1 and Pigeon River sites are not shown as they were not significant ($p>0.05$).

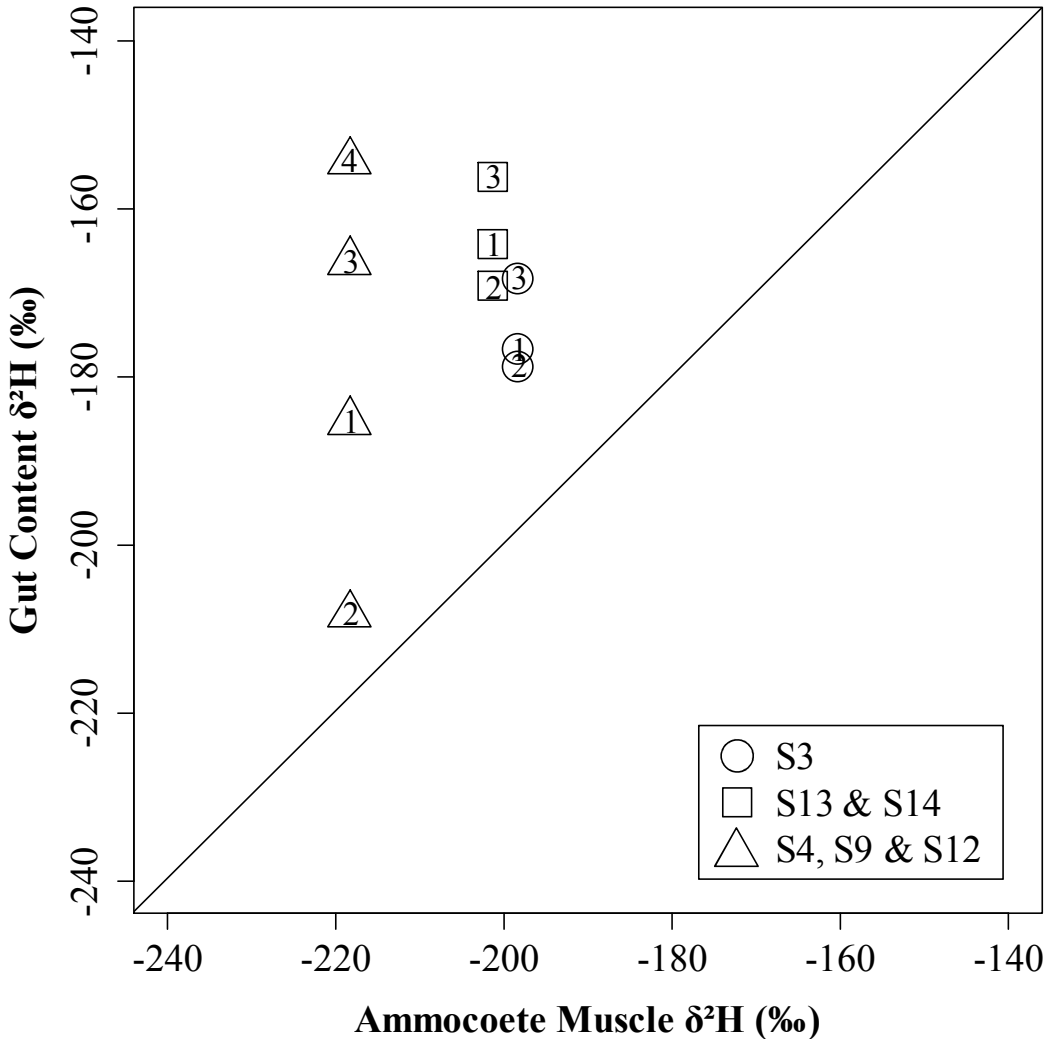

Figure 9. δ^2H of sea lamprey ammocoete gut content OM vs. muscle tissue from the Pigeon River in October 2010. Solid line represents 1:1 correspondence. Numbers in circles, squares and triangles in plot are the gut segments from which values were measured. The identifiers in the key (S3, S13, S14, etc.) refer to individual ammocoetes. S13+S14: the same gut segments from ammocoetes 13 and 14 were pooled in order to produce adequate sample size for analysis; S4+S9+S12: the same gut segments from ammocoetes 4, 9 and 12 were pooled to produce adequate sample size for analysis.

Figure 10. Stable isotope ratios of sea lamprey ammocoetes and their potential food and nutritional sources (mean ± SD). $\delta^{13}C$ vs. $\delta^{15}N$ for (a) Jordan River 1 and (b) Pigeon River; $\delta^{13}C$ vs $\delta^{2}H$ for (c) Jordan River 1 and (d) Pigeon River; $\delta^{15}N$ vs. $\delta^{2}H$ for (e) Jordan River 1 and (f) Pigeon River. Microalgae values were not measured directly but were developed using our measurements of $\delta^{13}C$-DIC and literature predictions, macrophytes $\delta^{15}N$ and published literature values for $\delta^{2}H$ (see Methods for full description). All values are raw data, without fractionation corrections applied. In panels e and f SPOM does not have standard error bars because only single samples were measured for $\delta^{2}H$ at each site.

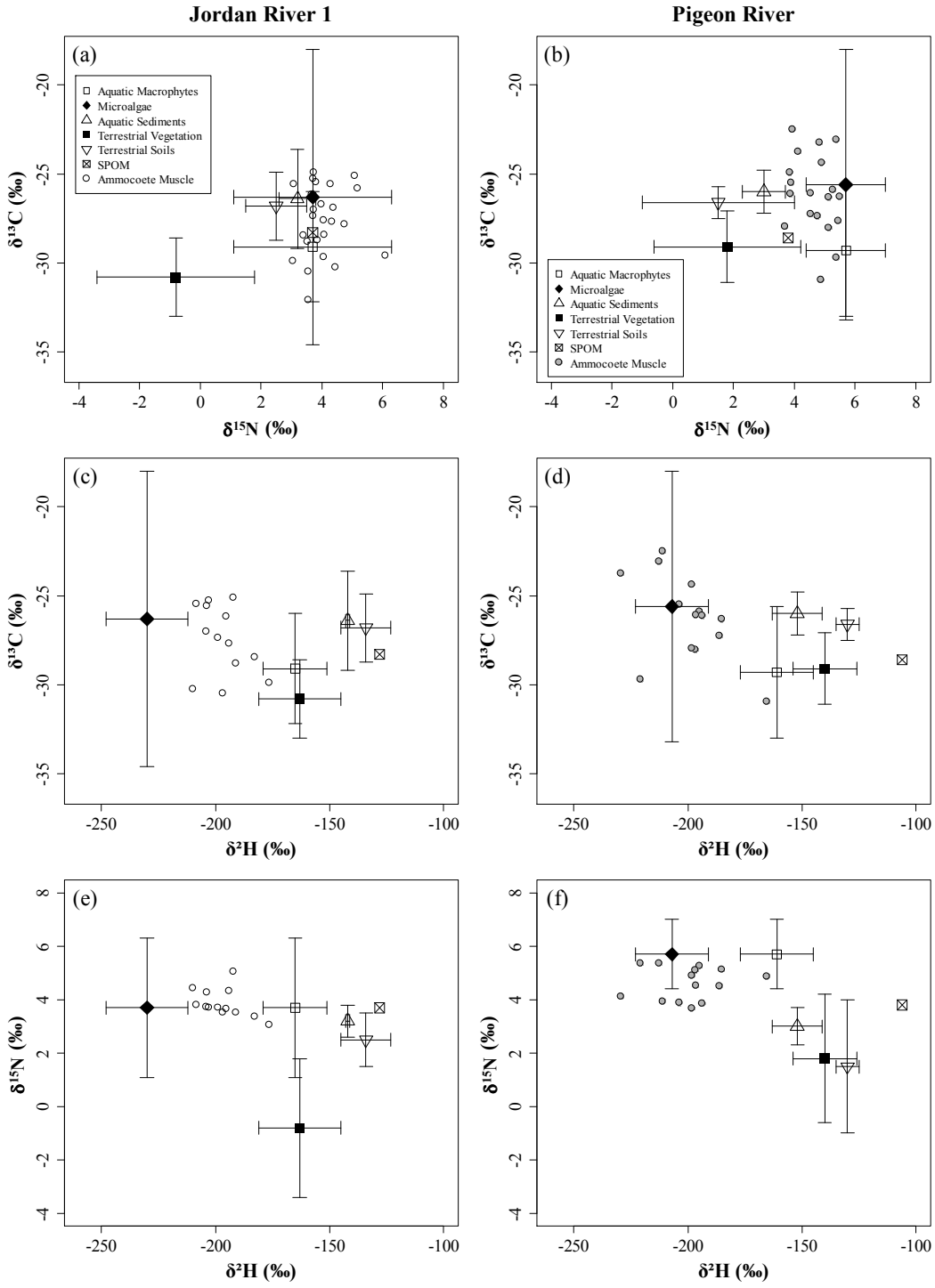

Figure 10. Isotope vs. isotope plots of sea lamprey from the Jordan River 1 and the Pigeon River.

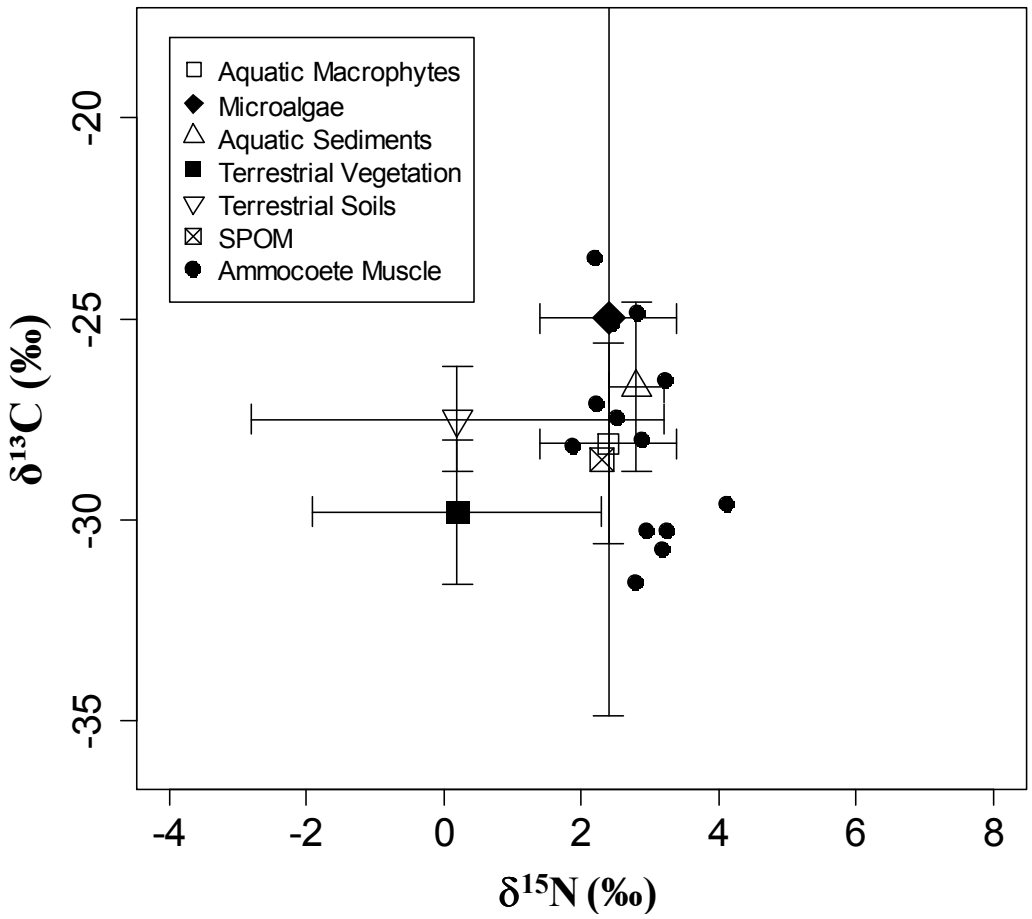

Figure 11. $\delta^{13}C$ vs. $\delta^{15}N$ of sea lamprey ammocoetes and potential food and nutritional sources for Jordan River 2 site. Symbols represent mean values and bars represent ± 1 SD of means. Only significant relationships are plotted.

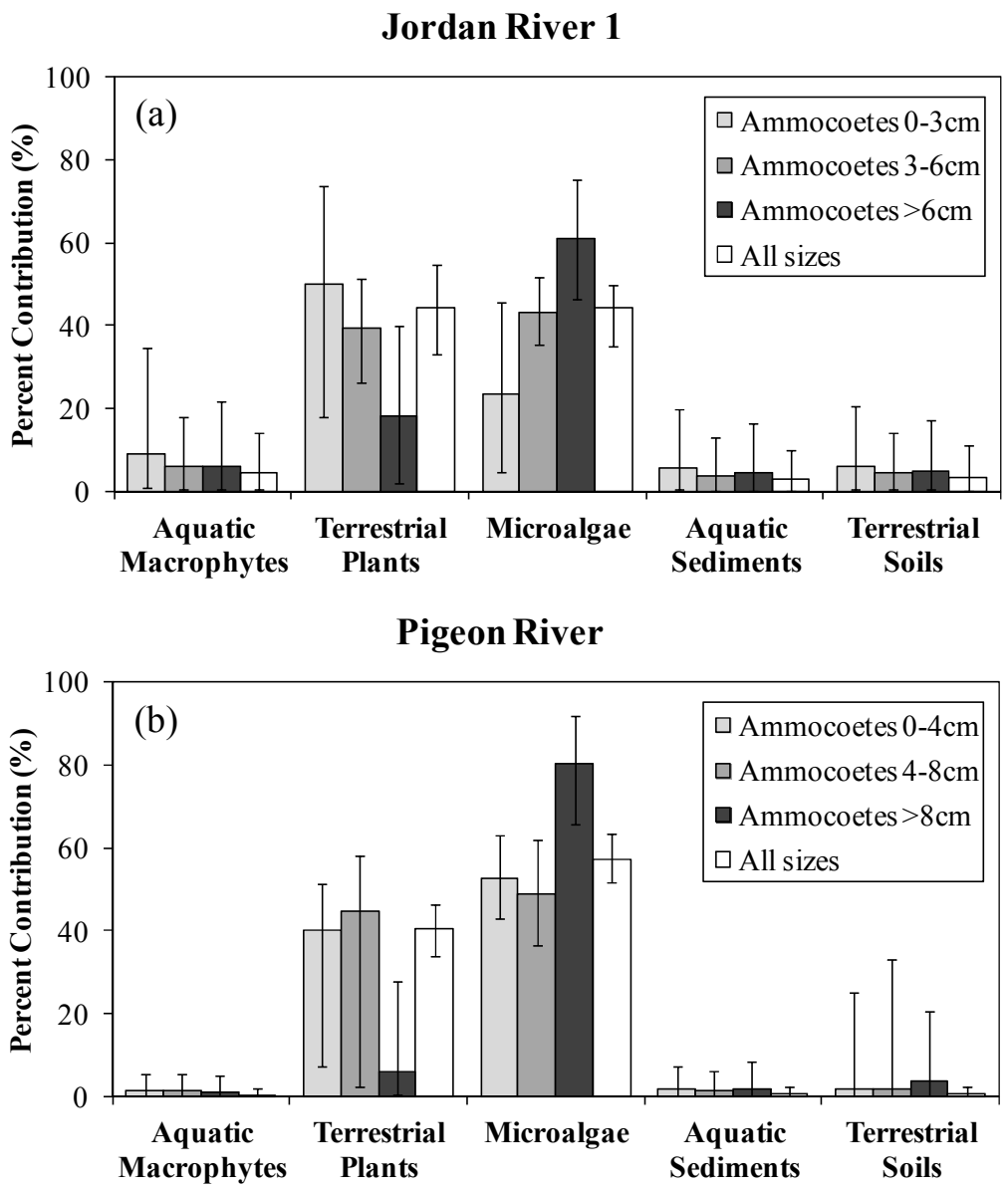

Figure 12. Median percent contributions of different nutritional resources to sea lamprey ammocoetes of different size classes (and likely age classes) from a) the Jordan River 1 and b) the Pigeon River calculated by the Bayesian model, MixSIR. Lower and upper error bars correspond to 5% and 95% posterior proportional contributions respectively.

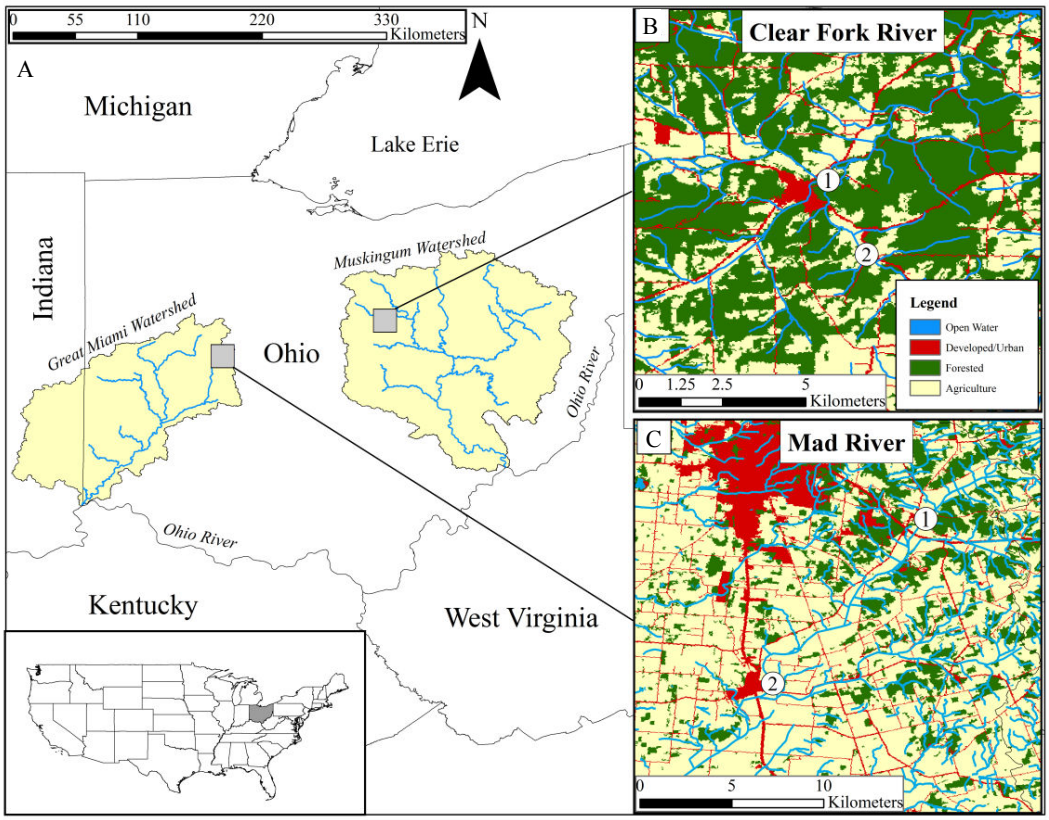

Figure 13. Study sites and sampling locations. (a) The Great Miami and Muskingum River watersheds in which the (b) the Clear Fork River and (c) the Mad River watersheds and locations of sampling sites in the present study are indicated. Least brook lamprey (*Lampetra aepyptera*) were collected in the Clear Fork River and American brook lamprey (*Lethenteron appendix*) were collected in the Mad River.

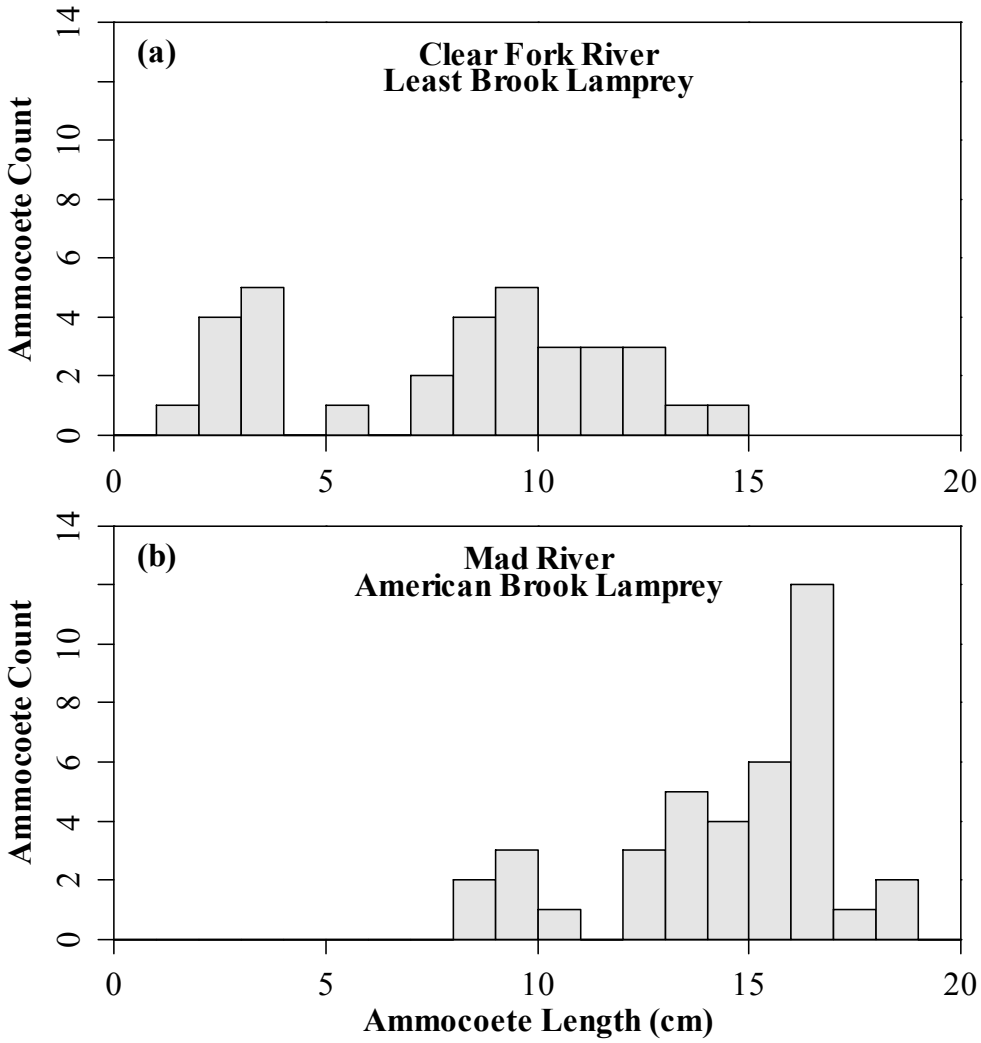

Figure 14. Size distributions of native ammocoetes captured in the present study at (a) Clear Fork River (Least brook lamprey, *Lampetra aepyptera*) and (b) Mad River (American brook lamprey, *Lethenteron appendix*) sites.

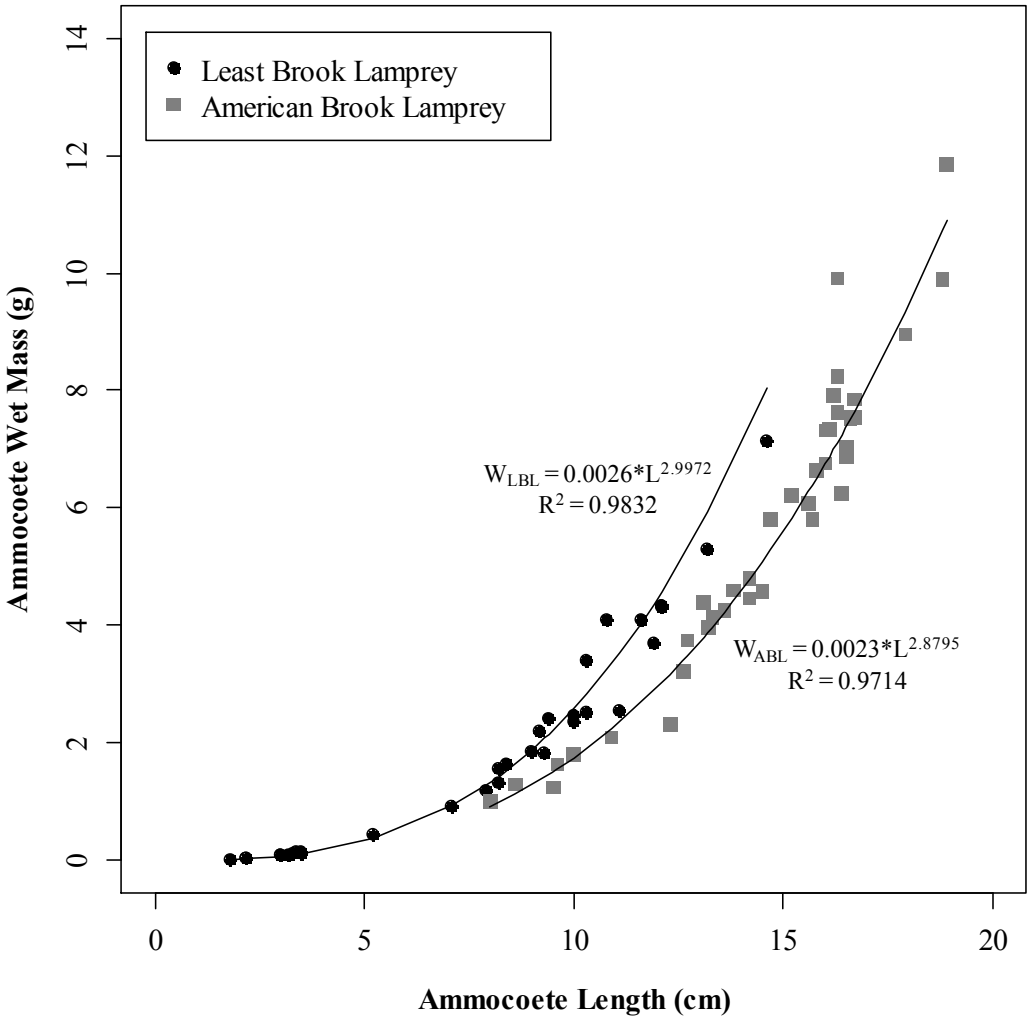

Figure 15. Mass-length relationships for least brook lamprey (Clear Fork River) and American brook lamprey (Mad River) ammocoetes collected in the present study.

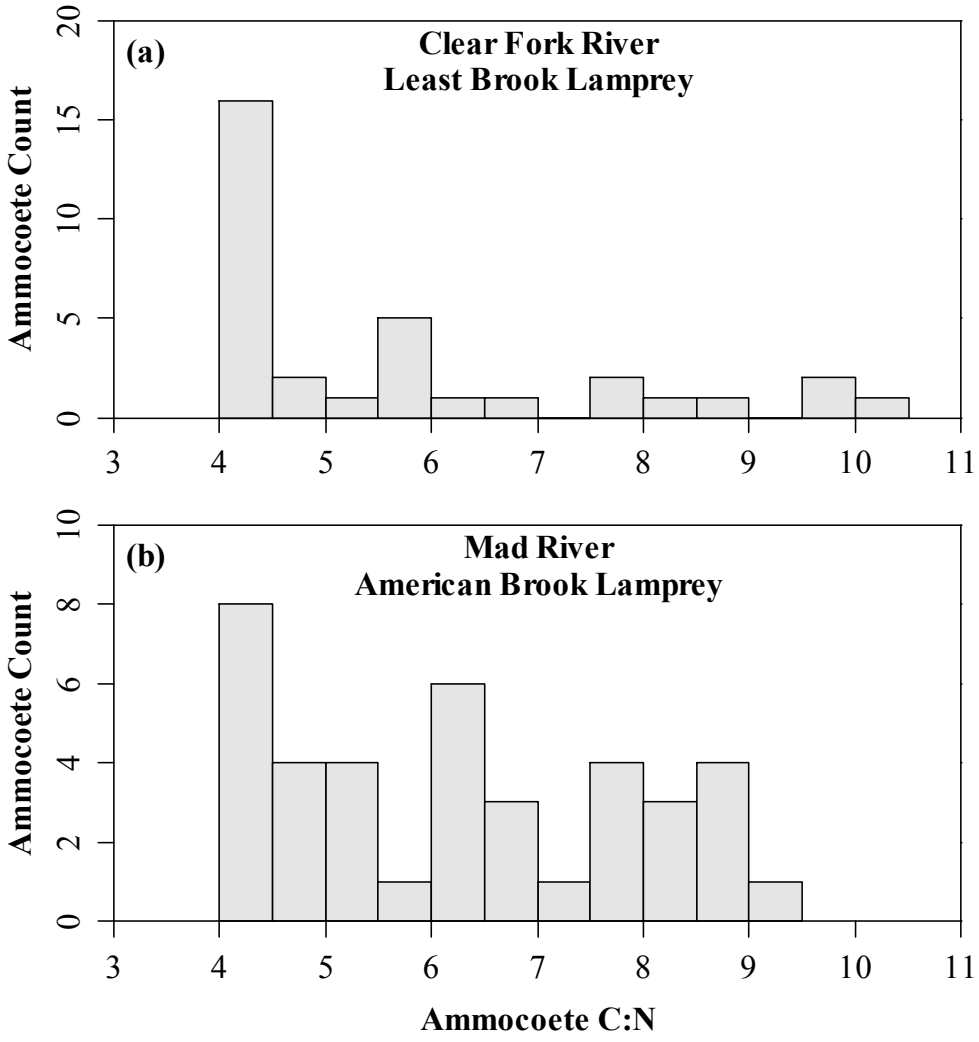

Figure 16. C:N distributions of (a) least brook lamprey from the Clear Fork River and (b) American brook lamprey from the Mad River.

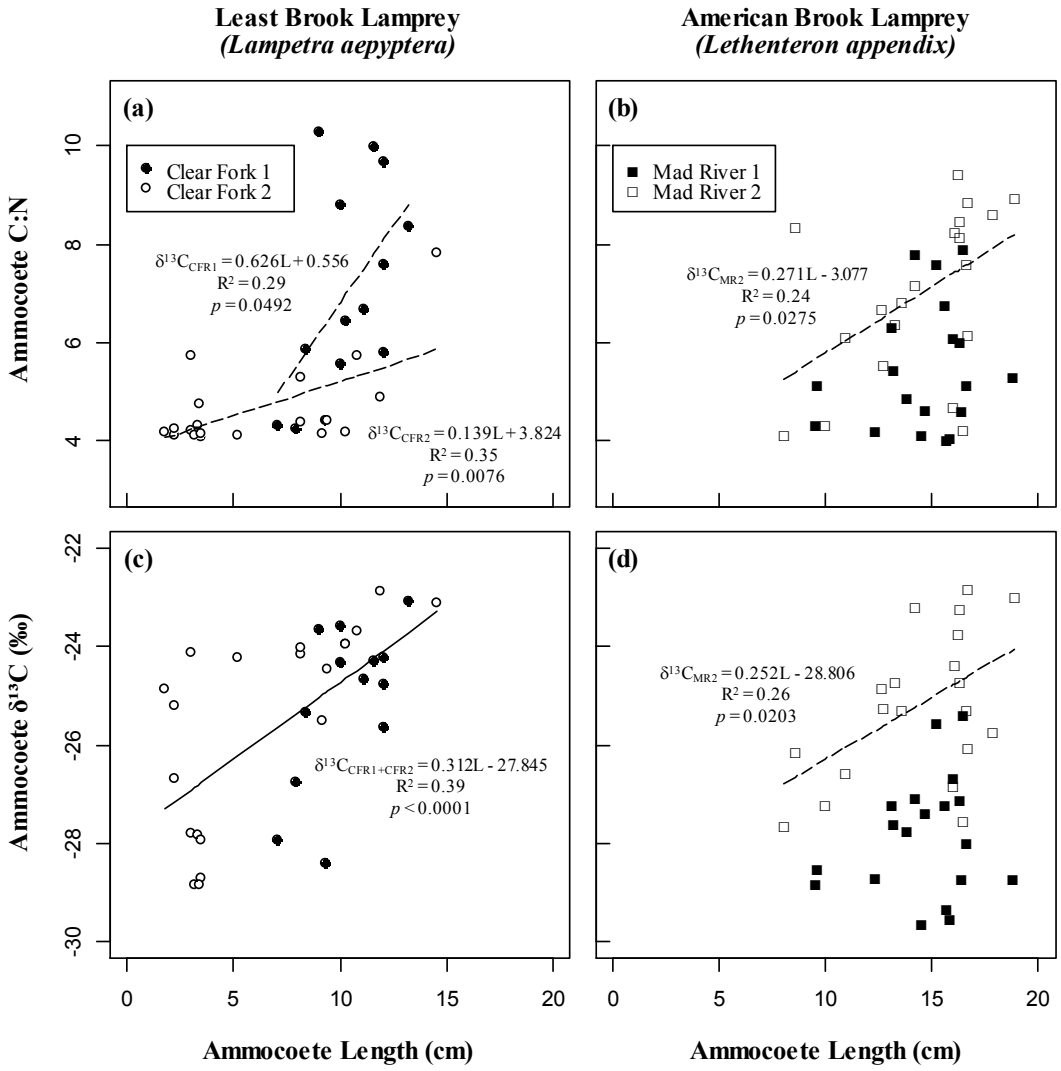

Figure 17. Ammocoete C:N vs. length for (a) the Clear Fork River and (b) the Mad River study sites, and ammocoete $\delta^{13}C$ values vs. length at (c) the Clear Fork River and (d) the Mad River study sites. Significant correlations are plotted, with dashed lines of best fit representing relationships plotted by site. If site was not found to be significant then overall correlations were plotted as solid lines of best fit.

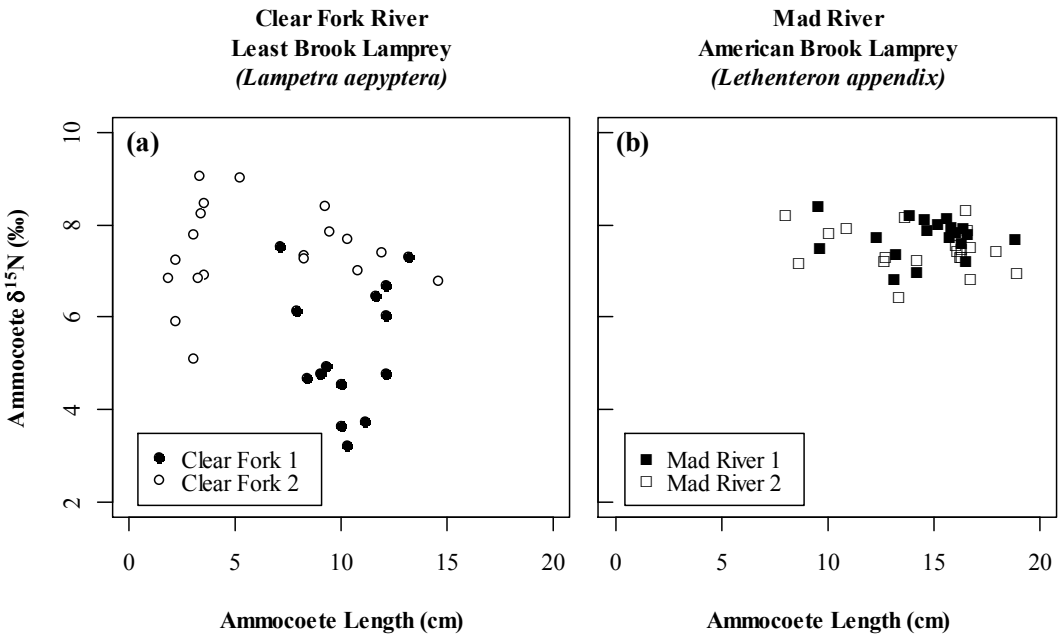

Figure 18. Ammocoete $\delta^{15}N$ vs. length for (a) the Clear Fork River and (b) the Mad River study sites. There were no significant correlations (p>0.05).

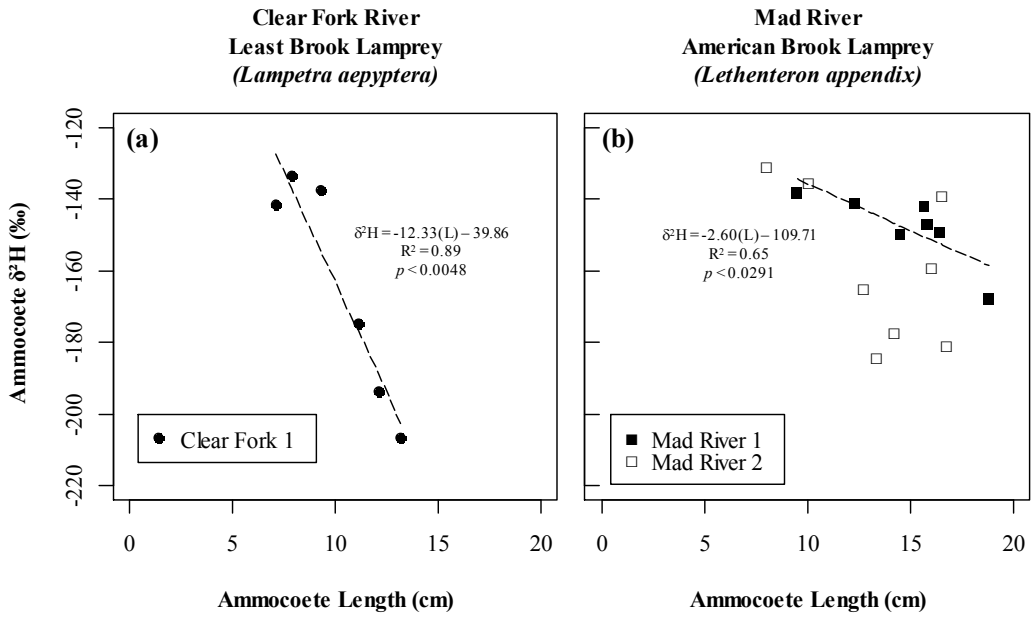

Figure 19. Ammocoete δ^2H vs. length for (a) the Clear Fork River and (b) the Mad River study sites. Significant correlations (p>0.05) are plotted with dashed lines of best fit representing relationships plotted by site.

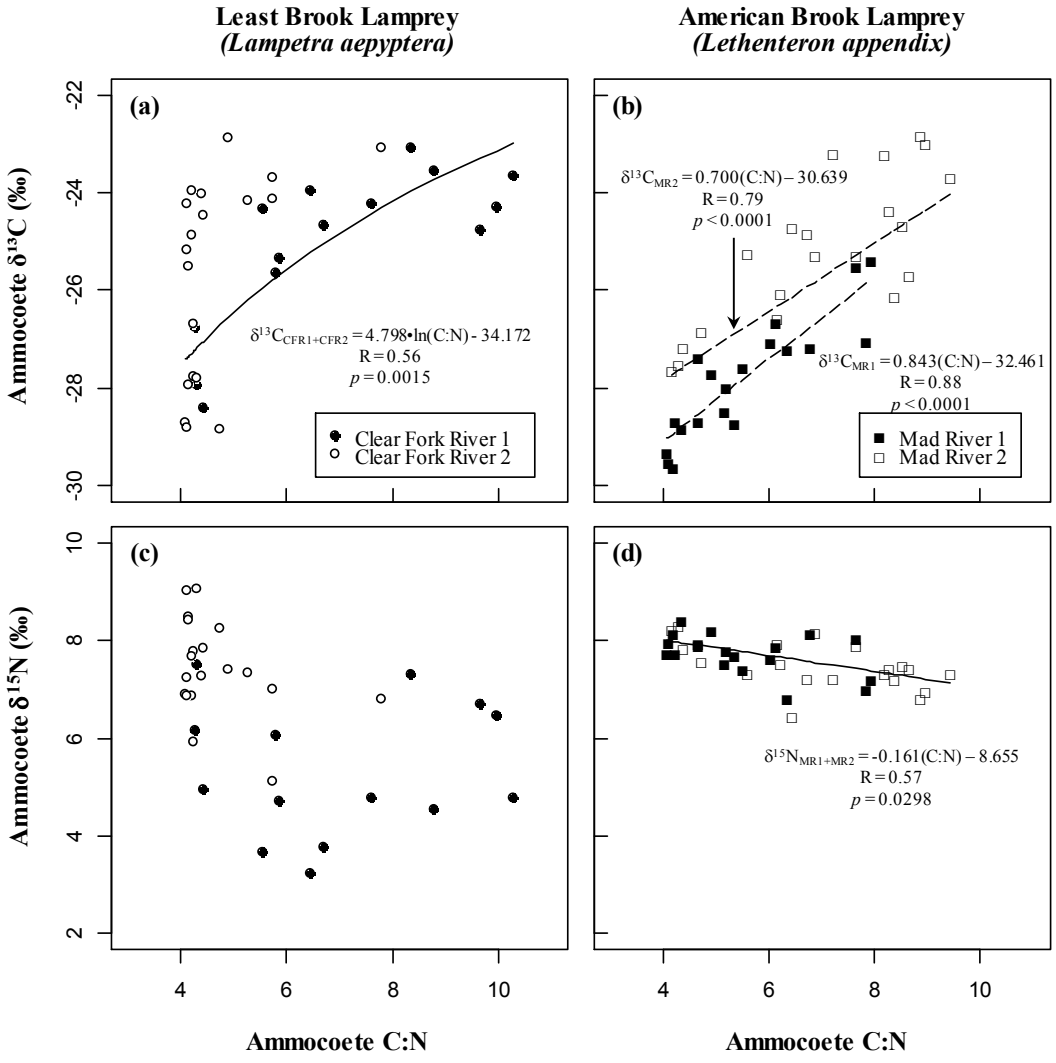

Figure 20. $\delta^{13}C$ vs. C:N of (a) least brook lamprey ammocoetes from Clear Fork River study sites and (b) American brook lamprey ammocoetes from Mad River study sites, $\delta^{15}N$ vs. C:N (c) least brook lamprey ammocoetes from Clear Fork River study sites and (d) American brook lamprey ammocoetes from Mad River study sites. Only significant relationships are plotted, with dashed lines representing correlations fit by site and solid lines representing correlations fitted with sites combined.

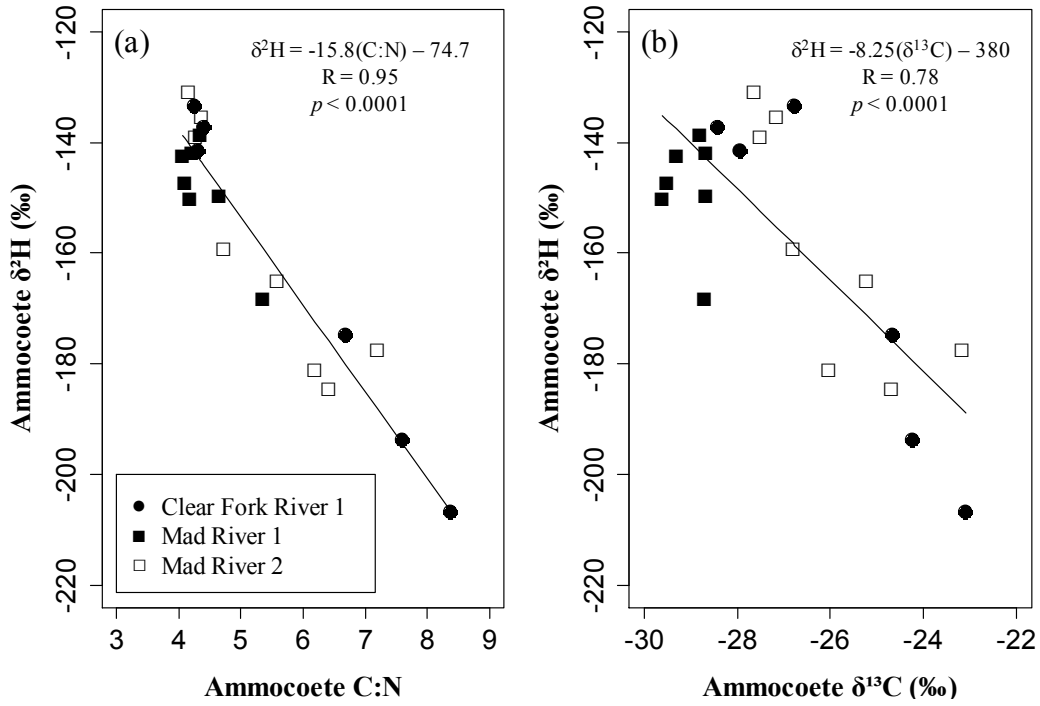

Figure 21. Ammocoete (a) δ^2H vs. C:N and (b) δ^2H vs. $\delta^{13}C$ for all sites at which both measurements were made.

Figure 22. Isotope-isotope plots of least brook lamprey and American brook lamprey ammocoetes from the Clear Fork River 1 and Mad River study sites, along with their potential nutritional resources(means ± SD). . $\delta^{13}C$ vs. $\delta^{15}N$ for (a) Clear Fork River 1 and (b) Mad River 1; $\delta^{13}C$ vs $\delta^{2}H$ for (c) Clear Fork River 1 and (d) Mad River 1; $\delta^{15}N$ vs. $\delta^{2}H$ for (e) Clear Fork River 1; and (f) Mad River 1. Samples of pure microalgae were limited, therefore an isotopic range for these samples was developed using both our data and published literature values (see text for full description).

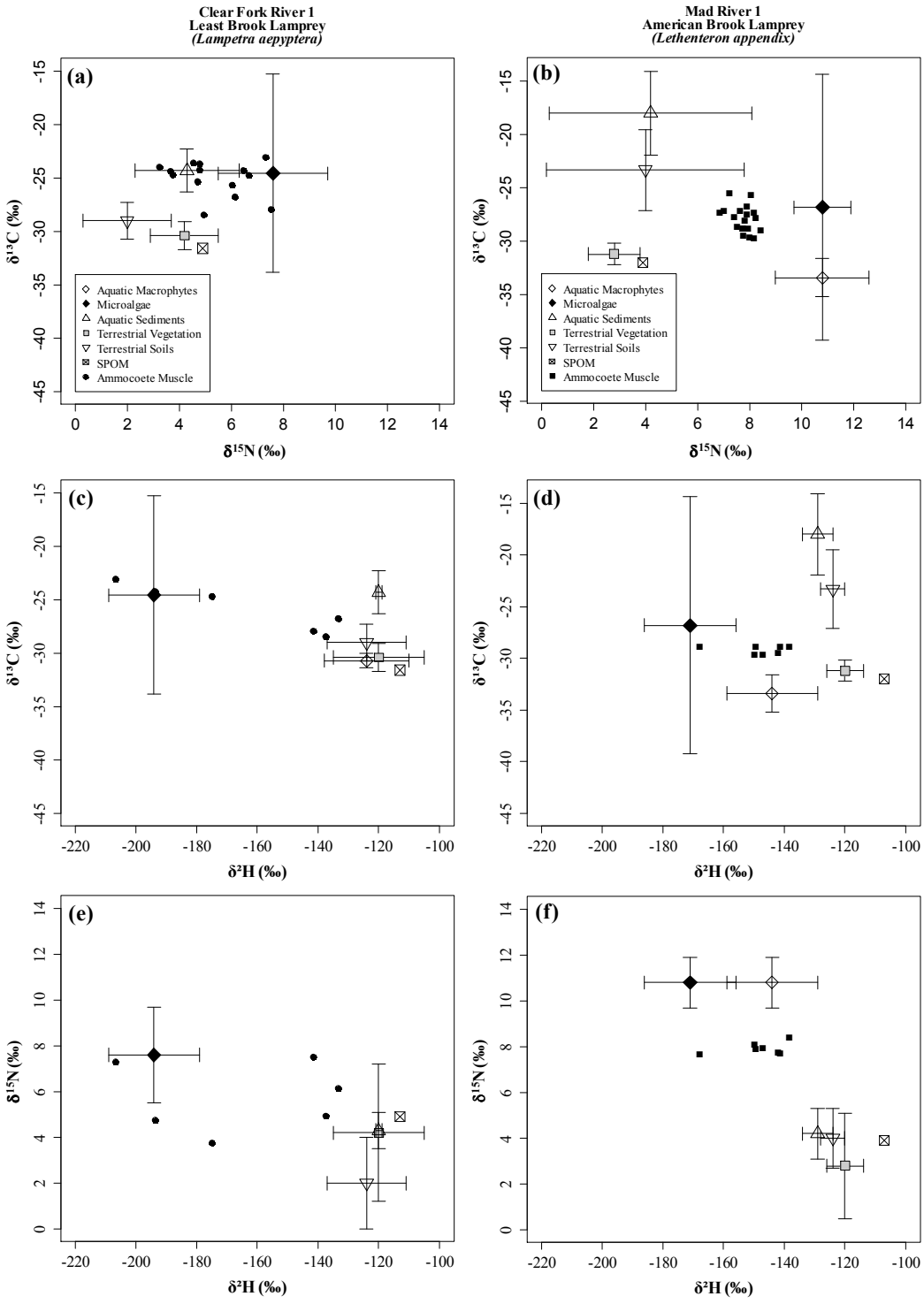

Figure 22. Isotope-isotope plots of least brook lamprey and American brook lamprey ammocoetes from the Clear Fork River 1 and Mad River 1 study sites, along with their potential nutritional resources(means ± SD). See page 147 for full caption.

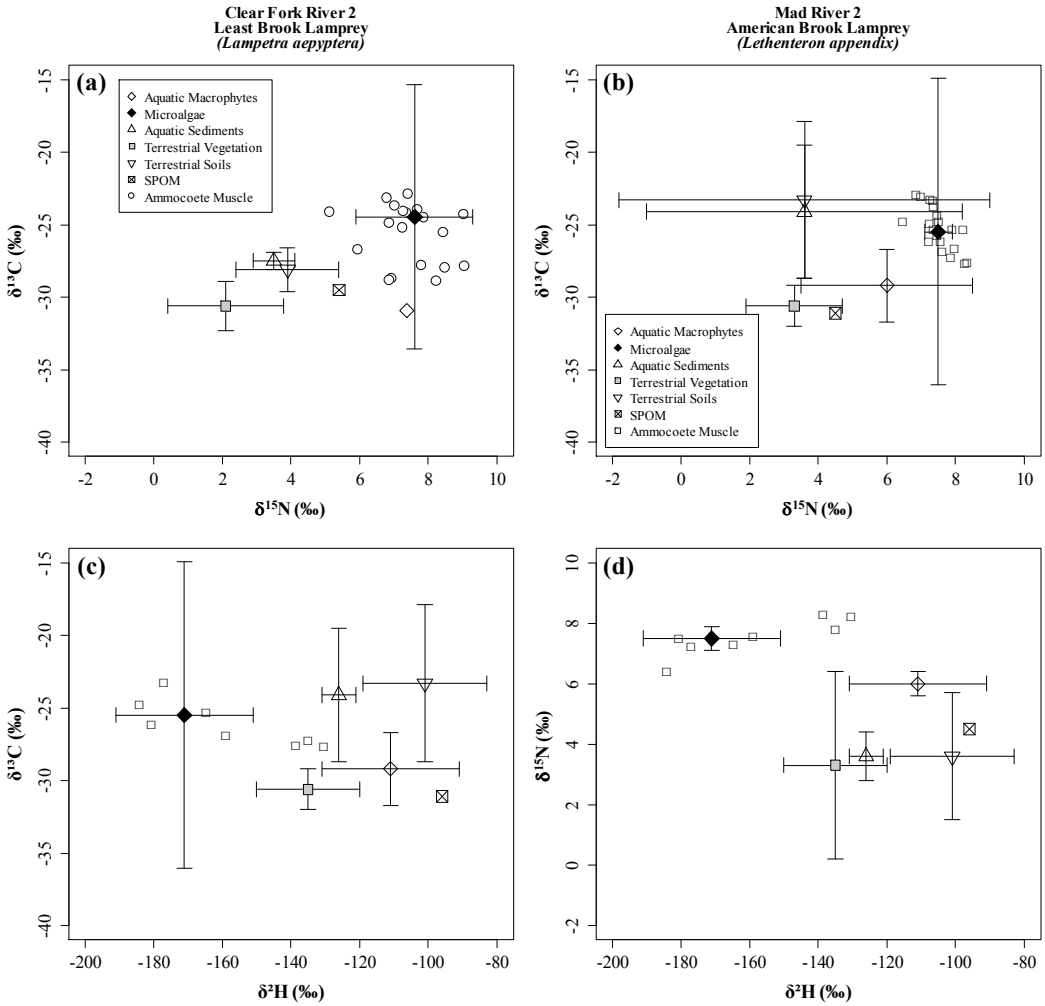

Figure 23. Isotope-isotope plots of least brook and American brook lamprey ammocoetes and their potential nutritional sources (mean ± SD) for the Clear Fork River 2 and Mad River 2 study sites. $\delta^{13}C$ vs. $\delta^{15}N$ for (a) Clear Fork River 2 and (b) Mad River 2; $\delta^{13}C$ vs $\delta^{2}H$ for (c) Mad River 2 and $\delta^{15}N$ vs. $\delta^{2}H$ for (d) Mad River 2. Samples of pure microalgae were limited, therefore an isotopic range for these samples was developed using both our data and published literature values (see text for full description).

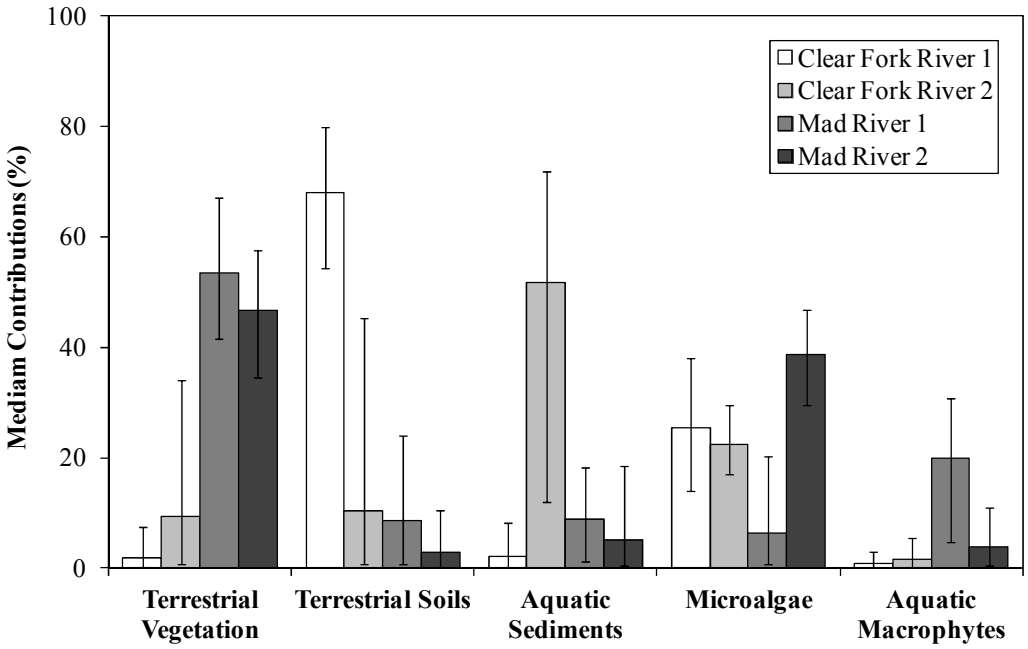

Figure 24. Median nutritional source contributions to ammocoetes from the CFR and MR calculated by the Bayesian model, MixSIR. Lower and upper error bars are the 5% to 95% posterior proportional contributions respectively.