Identification of the nutritional resources of larval sea lamprey in two Great Lakes tributaries
using stable isotopes

Thomas M. Evans^{a,1} and James E. Bauer^b

^aAquatic Biogeochemistry Laboratory, Department of Evolution, Ecology, and Organismal
Biology, The Ohio State University, 318 W 12th Ave, Columbus, OH 43210,

^bAquatic Biogeochemistry Laboratory, Department of Evolution, Ecology, and Organismal
Biology, The Ohio State University, 318 W 12th Ave, Columbus, OH 43210,

*Corresponding author: tevens03@syr.edu, Office number: 315-470-6804, Cell number: 215-
692-2027, Fax number: 315-470-6934

*Current Address: State University of New York, College of Environmental Science and
Forestry, 144 Illick Hall, 1 Forestry Drive, Syracuse, NY, 13210

Abstract

The sea lamprey (*Petromyzon marinus*) is an important invasive parasitic species in the Laurentian Great Lakes, but the nutritional subsidies supporting the protracted filter feeding ammocoete stage are not well established. We used stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to determine the major sources of autochthonous (aquatically produced) and allochthonous (terrestrially produced) organic matter (OM) to the nutrition of ammocoetes collected in 2010 from the Pigeon and Jordan Rivers in Michigan (USA). Ammocoete $\delta^{13}\text{C}$ was positively correlated with animal length and C:N ratio, but $\delta^{15}\text{N}$ was not correlated with either, suggesting that ammocoetes are primary consumers. We used a Bayesian model (MixSIR) to estimate the contributions of potential nutritional sources to ammocoetes. Estimates suggest that aquatic sediments were most important to ammocoete nutrition (median contributions ranged from 49-51%). Aquatic plants, including macrophytes and algae, were also important to ammocoete nutrition with a median contribution of 29%. Terrestrial plants were generally of lesser but still significant importance to ammocoetes, with a median contribution of 19-39%. Our findings generally agree with those of previous studies that have found that inputs of detrital and recently living OM from aquatic primary producers both provide an important source of nutrition for ammocoetes. The present study provides more quantitative estimates of the different forms of OM supporting ammocoete nutrition and biomass.

Keywords: Sea lamprey, stable isotopes, Laurentian Great Lakes, MixSIR, autochthonous; allochthonous

Introduction

Invasion of the Laurentian Great Lakes by the parasitic sea lamprey (*Petromyzon marinus*) has fundamentally altered the structure and function of this ecosystem (Christie et al., 2003). The majority of the sea lamprey life cycle is spent as a sediment-dwelling, filter-feeding larva (i.e., the ammocoete stage) (Manion and Smith, 1978; Potter, 1980). The methods historically employed to visually identify ingested items within the ammocoete gut have suggested that algae are an important source of ammocoete nutrition (Moore and Mallatt, 1980; Mundahl et al. 2005; Sutton and Bowen, 1994). However, visually based methods cannot identify either the composition of the large detrital component in guts or the nutritional resources assimilated, rather than simply ingested, 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 the far more dominant detrital organic matter (OM) (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) noted that the ingestion of diatoms was correlated with diatom abundance in the water column, and suggested diatoms are only seasonally important. Later work by Sutton and Bowen (1994) and later Mundahl et al. (2005) examined the entirety of the gut, and these authors argued that while algae could be important to ammocoete nutrition, ammocoete gut content was generally dominated by undifferentiated amorphous detrital material. For example, Sutton and Bowen (1994) found that >92% of the ammocoete gut consisted of detrital organic matter.

Stable isotopic analysis (SIA) represents a potentially more robust and quantitative

approach than GCA for assessing the nutritional source(s) of OM to organisms (Solomon et al., 2011). Naturally occurring isotopes of C and N in OM may be used to both qualitatively and quantitatively assess OM sources supporting an organism's nutrition, provided the isotopes of these elements can be measured in both the potential food sources and the consumer (Michener and Lajtha, 2007; Peterson and Fry, 1987; Post, 2002). Simultaneous use of multiple isotopes can better resolve the nutritional sources supporting consumers (Caraco et al., 2010; Cole et al., 2011; Peterson and Fry, 1987). Some naturally occurring stable isotopes can also be used to help establish at which trophic level organisms are feeding. For example, $\delta^{15}\text{N}$ fractionates at $\sim 3\text{‰}$ for each trophic level providing wide differentiation, while $\delta^{13}\text{C}$ fractionates only $\sim 0\text{--}1\text{‰}$ per trophic level (Peterson and Fry, 1987).

The objectives of the present study were to identify and quantify the dominant nutritional resources contributing to sea lamprey ammocoete biomass in two Laurentian Great Lakes tributaries. We predicted that sea lamprey ammocoete isotopic values would reflect nutritional contributions not only from aquatic OM, but also to a significant extent from allochthonous terrestrially derived detrital OM, owing to the dominance of terrestrially derived OM in streams and rivers (Cole et al., 2007; Cole et al., 2011).

Methods

Site Description

Sea lamprey ammocoetes were collected from two rivers on the lower peninsula of Michigan, USA (Jordan River and Pigeon River; Fig. 1). The Jordan River is a tributary of Lake Michigan, and the Pigeon River flows into Lake Huron. Samples were collected from a single site in the Pigeon River in May and October of 2010, while at the Jordan River samples were collected from two sites in June and October of 2010. Both the Jordan and Pigeon Rivers are

Fig. 1

periodically drip-treated with 3-trifluoromethyl-4-nitrophenol (TFM) by the Great Lakes Fishery Commission to kill ammocoetes, and 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 also have been present from spawning in spring 2010. The watersheds of both rivers are dominated by forest (>70%) with little to no urban development (<5%) (Jin et al., 2013).

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 (500°C for 4 h) sheets of aluminum foil, sealed in an airtight plastic bag, placed on dry ice in the field, and kept frozen until processing. Because similarities in ammocoete morphology make identification by morphology alone equivocal (Vladykov and Kott, 1980), 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

Aquatic Sediment Samples. Samples of aquatic sedimentary organic matter (SOM) were collected from streambeds using cut-off 60 cc plastic syringes to a depth of ~8 cm in each stream in which ammocoete sampling took place. Following collection, the bottom and top of each core were covered with a sheet of pre-baked (500°C for 4 h) aluminum foil and the core was frozen upright on dry ice in an airtight plastic bag. Back in the laboratory, cores were stored at -20°C

until processing and analysis.

To more fully identify the particle size-dependent 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 sieves were then transferred onto separate pre-baked (525°C 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.

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

Terrestrial and Aquatic Vegetation. The dominant species of living terrestrial plants (e.g., *Thuja occidentalis*, *Abies balsamifera*, *Larix laricina*) in the vicinity of each sampling site in the two rivers were collected after identifying the most abundant local species and handpicking leaf material using clean nitrile gloves. Samples of dominant aquatic macrophytes (e.g., *Ludwigia* spp., *Eleocharis* spp., *Vallisneria americana*) were collected similarly, and included only submerged aquatic vegetation. In two cases we collected filamentous algae. We chose not to collect biofilms because they are typically a heterogeneous mix of various sources, and because of the limited algal samples collected we assumed algae had similar isotopic values to aquatic macrophytes. Samples of vegetation were stored in pre-baked foil and placed in airtight plastic bags and frozen until processing.

Sample Processing and Stable Isotope Analyses

Ammocoetes were thawed before their total length (to the nearest millimeter) and weight (to the nearest 0.01g) were measured. Muscle tissue and a fin clip from each ammocoete were surgically removed using acetone cleaned forceps and baked aluminum foil to store samples. The samples of muscle tissue were dried at 60°C for 48 h, homogenized by grinding with an acetone cleaned mortar and pestle, and then stored in pre-baked glass scintillation vials in a polycarbonate desiccator maintained at <10% relative humidity until analysis. Aquatic and terrestrial plant samples were treated in the same manner as ammocoete muscle tissue.

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 (concentrated HCl, 24-48 h) in a clean glass desiccator 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°C. Each sample was then homogenized and stored in pre-baked (500°C for 4 h) glass scintillation vials in a desiccator as above for ammocoete muscle samples until analysis. Size-fractionated stream SOM samples were dried at 60°C on pre-baked QMA filters and then treated as for SOM. Terrestrial soil samples were processed in the same manner as SOM.

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

Statistical Analyses

All statistical analyses were performed using the R statistical system (R Development Core Team, 2011). We used ANCOVAs to test for differences between slopes of regression fit to independent variables. Separate ANOVAs of ammocoete length, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ were used to test for significant effects of site, date and their interactions in ammocoetes. Tukey's test for pairwise comparisons was performed for any factor found to have significance. To determine which potential dietary sources could be distinguished isotopically for isotopic modeling we used separate MANOVAs of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for each site. Following the MANOVAs, separate ANOVAs and Tukey's tests were run to determine the direction of the effect, if any.

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 potential dietary source isotopic values, consumer isotopic values, and isotopic fractionation estimates to better predict food source dependence as well as the confidence of the contribution of a given potential dietary source to the organism (Kim et al., 2011; Moore and Semmens, 2008). These developments have improved upon algebraic solutions and models, the most notable and widely used being IsoSource, which does not produce standardized error measures (Phillips and Gregg, 2003). Ammocoetes and their potential nutritional resources were modeled separately for each sampling site.

To estimate the contributions of the measured potential food and nutritional resources to larval sea lamprey, we used the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of aquatic plants and terrestrial vegetation (Table 1) as the primary forms of fresh, contemporary primary production in our Bayesian model (see Methods for details). At the Pigeon River and Jordan River 1 sites, we also included aquatic

Table 1

sediment OM as a potential source of nutrition because multiple comparisons from a MANOVA suggested aquatic sediments could be distinguished from other sources. At Jordan River 2, aquatic sediments overlapped too strongly with aquatic plants, and terrestrial soils overlapped too strongly with terrestrial plants to be discriminated. As a result, aquatic sediments and terrestrial soils were subsequently removed from the model. While aquatic sediments are expected to contain the same or similar initial plant sources, diagenetic alteration of sediment OM may impart different isotopic signatures to these plant-derived materials compared to fresh plant materials (Fogel and Tuross, 1999). SIA of different sediment particle size fractions and depths showed little isotopic difference in aquatic SOM or terrestrial soil OM across the measured depths (data not shown). Therefore, each of these sources was reduced to a single source in the final model.

On the basis of previous studies (Mallatt, 1982; Sutton and Bowen, 1994), and the fact that ammocoetes are filter feeders, we assumed ammocoetes were one trophic level above primary producers and established fractionation values based upon an ammocoete feeding study of Pacific lamprey (*Entosphenus tridentatus*) (Uh et al., 2014) and published values for a broad range of organisms (Post, 2002). We used the following fractionation values in our model: $0.4 \pm 6\text{‰}$ for $\delta^{13}\text{C}$ and $1.2 \pm 1.5\text{‰}$ for $\delta^{15}\text{N}$. Fractionation values are the difference between a consumer's isotopic value and that of its dietary source value (Post, 2002). Uninformative priors were used (i.e., we did not specify likely food source contributions prior to our model runs) and the model was run with 1×10^6 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). To test the robustness of the models, we varied the fractionation values by $\pm 50\%$ and reran the models.

Results

Ammocoete Size Distributions

During this study, 58 of the 62 animals captured were confirmed by microsatellite markers to be invasive sea lamprey ammocoetes. Sea lamprey ammocoetes across all sampling sites and times had a mean length of 43 mm (± 22 SD; Fig. 2). Mean lengths did not differ between sampling dates ($df=1$, $F\text{-value}=0.88$, $p=0.35$), but did differ between sites ($F\text{-value}=5.0$, $df=2$, $p=0.01$) at the Pigeon River and Jordan River 2 (Tukey test, $p=0.011$). During the October sampling the mean length of presumed YOY animals was greater in the Pigeon River (30 ± 2 mm, SD) than for sites in the Jordan River (18 ± 2 mm, SD; $df=2$, $F\text{-value}=72$, $p<0.001$; Fig. 2).

Fig. 2

C:N Ratio of Ammocoete Tissue

Ammocoete muscle tissue C:N values were pooled because an ANOVA found no effect of site ($df=2$, $F\text{-value}=0.48$, $p=0.62$), collection date ($df=1$, $F\text{-value}=0.64$, $p=0.43$) or their interaction ($df=2$, $F\text{-value}=0.57$, $p=0.57$). Ammocoete C:N ratio had an overall mean of 7.1 (± 1.6 SD) across all sites and times (Fig. 2b) and was positively related to ammocoete length ($df=55$, $r^2 = 0.41$, $p<0.001$; Fig. 3a).

Isotopic Signatures of Ammocoetes

Mean $\delta^{13}\text{C}$ values of ammocoete muscle tissue across all sizes and sampling times (Fig. 3b) did not differ between sites (ANCOVA, $df=2$, $F\text{-value}=0.45$, $p=0.64$) but were significantly positively related to animal length for all sampling sites and times ($df=55$, $r^2=0.45$, $p<0.001$; Fig. 3b). The $\delta^{15}\text{N}$ values of ammocoete muscle tissue were not related to animal length at any site ($df=1$, $F\text{-value}=1.6$, $p=0.27$; Fig. 3c) but were different between sites ($df=2$, $F\text{-value}=35$, $p<0.001$). The mean $\delta^{15}\text{N}$ for Jordan River 1 animals was 4.0‰ (± 0.7 SD), while Jordan River 2

Fig. 3

animals averaged 2.8‰ (± 0.6 SD), and at the Pigeon River animals averaged 4.7‰ (± 0.6 SD). Overall, ammocoete $\delta^{13}\text{C}$ values were positively correlated with their C:N ratio, following a logarithmic relationship ($\text{df}=55$, $r^2=0.69$, $p<0.001$, Fig. 4). $\delta^{15}\text{N}$ showed no relationship to C:N ratio ($\text{df}=55$, $r^2=0.02$, $p=0.30$; data not shown).

Fig. 4

MANOVA's of Food Sources

MANOVA to determine if food sources could be discriminated by $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ values at each site were tested (see Fig. 5a-c for mean isotopic values and standard deviations of potential dietary sources at each site). All MANOVAs were significant (Pigeon River: $\text{df}=3$, Wilk's number=0.33, $p<0.0001$; Jordan River 1: $\text{df}=3$, Wilk's number=0.30, $p<0.0001$; Jordan River 2: $\text{df}=3$, Wilk's number=0.39, $p=0.0005$), indicating that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of two or more sources could be distinguished at each site. Terrestrial soils overlapped isotopically with many other potential dietary sources, and could not be distinguished as a unique source at any site (Table 2). Aquatic and terrestrial plants were distinguishable based on their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at all sites (Table 2). However, only at Pigeon River and Jordan River 1 were aquatic sediments considered different enough from other potential sources to be included in the model.

Table 2

Estimates of Allochthonous and Autochthonous Nutritional Subsidies to Larval Sea Lamprey

Modeling of potential nutritional subsidies using MixSIR estimated that sea lamprey ammocoetes relied on similar amounts of aquatic plants and aquatic sediments at the Pigeon River and Jordan River 1 (Fig. 6a, b). At Jordan River 2 aquatic plants (which would also be included in aquatic sedimentary sources) had a median contribution of 61% (range: 43-77%) to ammocoete nutrition (Fig. 6c). Contributions from living terrestrial plant materials were lower at all sites (average median contribution: 27%, range: 19-39%) (Fig.6). Ammocoetes from Jordan River 1 and Pigeon River were similar to one another, while those at Jordan River 2 appeared

similar if the aquatic plant source is reflective of all aquatic sources (i.e., aquatic plants and aquatic sediment OM) at other sites (Fig. 6). Assuming aquatic plants (and aquatic sediments where they could be distinguished isotopically) were entirely autochthonous and terrestrial plants were allochthonous sources, ammocoetes are primarily dependent on autochthonous subsidies for growth and development ($78 \pm 26\%$ at Pigeon River, $81 \pm 25\%$ at Jordan River 1, $61 \pm 16\%$ at Jordan River 2).

Fig. 5

Varying the fractionation values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by 50% in either direction had only moderate effects on the model's predicted contributions from different food sources (Fig. 6). When fractionation values were reduced by 50%, the importance of aquatic plants and aquatic sediments increased in importance in all models, with a concomitant decrease in terrestrial plant contributions (Fig. 6). In models in which the fractionation values were increased by 50% from the estimated fractionation values, all source contributions were more similar (Fig. 6). It should be noted that the largest differences within a single source were $<10\%$, and the average difference between median contributions was 6% ($\pm 2\%$ SD, $n=16$).

Discussion

Size-Dependent Changes in Larval Lamprey C:N and Isotopic Signatures

Both C:N and $\delta^{13}\text{C}$ values of ammocoetes increased as a function of animal size (Fig. 3a, b), and C:N was positively correlated with ammocoete $\delta^{13}\text{C}$, following a logarithmic relationship (Fig. 4). This finding contrasts with other studies that have shown an inverse relationship between C:N and $\delta^{13}\text{C}$ in muscle tissue for a range of aquatic organisms (which, however, did not include larval lamprey), presumably as a result of increasing lipid content with animal size and the correspondingly lower $\delta^{13}\text{C}$ of lipids generally (Kiljunen et al., 2006; Post et al., 2007).

The elevated C:N values (i.e., those greater than ~7) reported in the present study are not anomalous for sea lamprey ammocoetes, as Harvey et al (2008) also found that sea lamprey transformers (animals that have recently undergone metamorphosis but have not yet fed parasitically) have normally distributed C:N values and a mean C:N of 7.4 ± 1.7 (SD, n=50). The C:N values reported by these workers were not significantly different from the mean C:N (7.1 ± 1.6 , SD, n=59) in the present study (Student's t-test, $p=0.29$).

Fig. 6

Similar to the present study, elevated $\delta^{13}\text{C}$ values (as high as -20‰) were previously observed for ammocoetes from several Lake Huron (Ontario, Canada) tributaries (Hollett, 1995); these ammocoetes were more enriched in ^{13}C ($\delta^{13}\text{C}$ as high as -20.3‰) than any of the potential food sources identified (i.e., aquatic plants, fine SPOM, leaf litter, algae, and detritus). If our ammocoete $\delta^{13}\text{C}$ values are corrected for presumed lipid content on the basis of the animals' observed C:N values following Post et al. (2007), the resultant ammocoete $\delta^{13}\text{C}$ values become even more elevated (as high as -15.5‰) and ammocoete $\delta^{13}\text{C}$ becomes even less realistic given the range of $\delta^{13}\text{C}$ values of potential food sources in our study rivers. Furthermore, lipid correction of $\delta^{13}\text{C}$ values has been found to not be applicable to all species and tissue types (Kiljunen et al., 2006).

Finally, ammocoete $\delta^{13}\text{C}$ values in the present study are unlikely to be the result of a maternal $\delta^{13}\text{C}$ signature. Female sea lamprey have elevated $\delta^{13}\text{C}$ (as high as -18‰) and $\delta^{15}\text{N}$ (as high as 15‰) values (Harvey et al 2008) while the smaller ammocoetes examined had lower $\delta^{13}\text{C}$ values than larger, and likely older, animals (Fig. 3b). All likely had lower $\delta^{15}\text{N}$ values than female sea lamprey (Fig. 5). In addition, maternal contributions even to small animals would be limited because sea lamprey eggs are small (~1 mm), and ammocoete feeding on drastically different dietary sources than adult females would result in dilution and/or turnover of

ammocoete C:N shortly after hatching. For these reasons, and our inability to fully explain the observed relationship between $\delta^{13}\text{C}$ and C:N ratio (Fig. 6a) without additional data (e.g., compound-specific $\delta^{13}\text{C}$ measurements of lipids) in our study, we chose not to correct ammocoete $\delta^{13}\text{C}$ values for lipids or other potential compositional changes in our models of diet composition. On the basis of our findings it is clear that additional studies are needed (for example, under controlled laboratory conditions using potential dietary sources of known isotopic composition), and analysis of specific tissue types (e.g., total lipid extracts and compound-specific $\delta^{13}\text{C}$ analyses of lipids) to fully understand this relationship.

The $\delta^{13}\text{C}$ values of consumers ultimately reflect their food and nutritional sources. The isotopic shifts with ammocoete body size (Fig. 3b) therefore suggest that the animals consume and/or assimilate different (i.e., isotopically unique) food sources during growth. Smaller ammocoetes had lower $\delta^{13}\text{C}$ values ($\sim -32\text{‰}$, Fig. 3b) suggesting a greater reliance on terrestrial plants as fresh or recently decaying plant material. For larger ammocoetes, the enrichment in ^{13}C by as much as 7-8‰ (Fig. 3b) suggests a greater reliance on aquatic plant biomass, as there is no other known potential food source that could account for these values (Caraco et al., 2010; Finlay, 2001; Finlay et al., 2010).

In contrast to $\delta^{13}\text{C}$ values, the $\delta^{15}\text{N}$ values of ammocoete muscle tissue did not reveal relationships with animal length (Fig. 3c) or C:N (data not shown). Ammocoete $\delta^{15}\text{N}$ was also not correlated with $\delta^{13}\text{C}$ (Fig. 5), as might be expected if ammocoetes shift dietary and nutritional sources with increasing age and size. A correlation between C:N values and the $\delta^{15}\text{N}$ values of ammocoetes is not expected as ^{15}N is not fractionated during lipid synthesis (DeNiro and Epstein, 1977). The lower degree of variability in ammocoete $\delta^{15}\text{N}$, in contrast to $\delta^{13}\text{C}$, may result from factors such as 1) minimal fractionation of ^{15}N during biosynthesis, as has been

reported for other detritivores (Vanderklift and Ponsard, 2003), and/or 2) ammocoetes fractionating or discriminating against ^{13}C at a higher rate than ^{15}N . In controlled feeding studies, for example, ammocoetes were shown to fractionate the ^{13}C of their lipid-extracted body tissues significantly (~ -3 to $\sim 7\text{‰}$) relative to known individual dietary sources (algae, yeast, and salmon), and variation in ammocoete $\delta^{13}\text{C}$ values was $\sim 2\text{--}3\text{‰}$ greater than for $\delta^{15}\text{N}$ values (Uh et al., 2014). The greater relative variability of $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ values has been reported from other studies of larval lamprey (Marty et al., 2009; Van Riel et al., 2006; Shirakawa et al., 2009) and juveniles before they feed parasitically (Harvey et al., 2008), and requires further investigation to fully understand its cause.

Estimates of Nutritional Subsidies to Sea Lamprey Ammocoetes

As noted previously, large ammocoetes were more enriched in ^{13}C than small ammocoetes (Fig. 3b), suggesting possible ontogenetic shifts in larval diet and nutrition. Spawning of sea lamprey occurs in late spring (Beamish, 1980; Morman et al., 1980), and newly hatched ammocoetes initially seek out fine-grained OM-rich sediments, transitioning to sand beds once they grow large enough to burrow quickly in them (Pletcher, 1968). Therefore, small ammocoetes would have access to large amounts of OM-rich sediments and are predicted to depend on these sources until metamorphosis to parasitic juveniles.

On the basis of their $\delta^{15}\text{N}$ values, ammocoetes are deduced to consume and assimilate low trophic level food sources with a broad range of $\delta^{13}\text{C}$ values (Fig. 5). Interestingly, ammocoete $\delta^{15}\text{N}$ values were influenced by site (Fig. 3c), suggesting that one or more food sources were variable in $\delta^{15}\text{N}$ between sites, and most likely explained by differences in land use (e.g., forested vs. agricultural) and $\delta^{15}\text{N}$ values of associated N inputs (Anderson and Cabana, 2005; Lake et al., 2001). In support of this, we found that terrestrial plant, aquatic macrophyte,

and terrestrial soil $\delta^{15}\text{N}$ values were variable between sites and to a similar extent as ammocoete $\delta^{15}\text{N}$ values from the same sites (Fig. 5).

Our model findings (Fig. 5) also suggest that the detrital component supporting ammocoete growth in our tributaries is derived primarily from aquatic sediments and to a lesser (but still substantial) extent terrestrial plant biomass and detritus. Prior studies have often been at odds over whether ammocoete nutrition was primarily the result of rarer algae cells or the more abundant detrital fraction (Table 3). Assuming that our estimates of aquatic macrophytes were sufficient for estimating algal isotopic signatures (France, 1995), the present study generally agrees with prior work by Sutton and Bowen (1994) and Mundahl et al. (2005) that allochthonous biomass provides critical nutrition for ammocoete growth and development, but stresses the high importance of presumably algal biomass ammocoetes utilize (Fig. 6). Ammocoetes also depend on detrital subsidies arising from other primary producers (e.g., terrestrial) to meet their needs. This dependence on other, and presumably less nutritious, materials may come at the cost of slower growth rates and longer times to metamorphosis. Our work suggests that streams with high aquatic primary productivity, and/or or more labile detrital dietary sources (as in Morkert et al. 1998), will result in a higher ammocoete growth rates, shorter times to metamorphosis, and shorter times to migration of juveniles streams.

Our estimates that half or more of ammocoete nutrition is driven by autochthonous sources of OM and are consistent with estimates of autochthony in other aquatic organisms using natural abundance isotopic approaches, including zooplankton (23-98%) (Cole et al., 2011; Karlsson et al., 2012), detritivorous fish (32-100%) (Babler et al., 2011), and invertebrate filter feeders (e.g., Simuliidae, 48-68%) (Rasmussen, 2010). Therefore, while algae or recently produced macrophyte detritus may be relatively scarce in ammocoete diet, these findings

collectively suggest that these nutritional resources may be disproportionately important contributors to ammocoete nutrition.

The limited sample size ($n=58$), primarily composed of animals between 20-100 mm, the similarity of the sample sites, the assumption in our mixing models that algal isotopic values are similar to those of aquatic macrophytes, and the time frame of sampling, and may all constrain the conclusions we may draw from this work. A broader examination of ammocoetes from different watersheds (with different dominant land uses), and samples from different times of the year may help confirm the relative importance of autochthonous (i.e., algae and aquatic plants) vs. allochthonous (i.e., terrestrial plants and soils) to ammocoete nutrition and growth. However, if our model findings are generally representative of ammocoete nutritional resources, these results indicate that a) smaller and younger larval sea lamprey may be reliant, to a significant degree, upon autochthonous OM production in order to subsidize and sustain their growth, and b) ammocoetes, as they grow, may depend on increasing allochthonous contributions from terrestrial plants and soils, which is even more likely in watersheds where aquatic primary production is limited. Additional experiments in which ammocoetes of different ages are raised on food sources of known isotopic composition may provide valuable supporting information in this regard (e.g., Limm and Power, 2011; Shirakawa et al., 2009; Uh et al., 2014).

Quantitative assessment of the nutritional subsidies to the ammocoete stage of the sea lamprey life cycle is important because ammocoete growth has been found to be highly variable among natal streams (Griffiths et al., 2001; Holmes, 1990; Morkert et al., 1998). This may result, in addition to the quality of the available nutritional resources, from the rates at which ammocoetes of different size and age ingest, digest and assimilate different dietary materials. Ammocoete diet and nutrition may also be influenced by factors, such as land use type, which

can have a profound impact on the availability of allochthonous and autochthonous food resources. Better understanding of the basic life history of sea lamprey, especially the feeding ecology of the ammocoete stage, may provide further insights to the factors limiting ammocoete growth and sea lamprey recruitment.

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Table 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of potential food and nutritional resources to sea lamprey ammocoetes from the Jordan and Pigeon Rivers (Michigan, USA) in the present study. Values (in per mil, ‰) are reported as mean $\pm$ SD (n). Values in brackets represent numbers of replicates, n, for each sample type.

Source	Jordan River 1		Jordan River 2		Pigeon River	
	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Aquatic Plants	-29.1 $\pm$ 3.1 (8)	3.8 $\pm$ 2.6 (8)	-28.1 $\pm$ 2.5 (5)	2.4 $\pm$ 1.0 (5)	-29.3 $\pm$ 3.7 (5)	5.6 $\pm$ 1.3 (5)
Terrestrial Plants	-30.8 $\pm$ 2.2 (10)	-0.8 $\pm$ 2.6 (10)	-29.8 $\pm$ 1.8 (9)	0.2 $\pm$ 2.1 (9)	-29.1 $\pm$ 2.0 (11)	1.8 $\pm$ 2.4 (11)
Aquatic Sediments	-26.4 $\pm$ 2.8 (20)	3.2 $\pm$ 0.6 (20)	-26.7 $\pm$ 2.1 (18)	2.8 $\pm$ 0.4 (18)	-26.0 $\pm$ 1.2 (15)	3.0 $\pm$ 0.7 (19)
Terrestrial Soil	-26.8 $\pm$ 1.9 (3)	2.5 $\pm$ 1.0 (3)	-27.5 $\pm$ 1.3 (3)	0.2 $\pm$ 3.0 (3)	-26.6 $\pm$ 0.9 (3)	1.5 $\pm$ 2.5 (3)

Table 2. P-values for all possible contrasts from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ MANOVA analyses of potential nutritional sources to ammocoetes, separated by site.

Site	Source	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
		Aquatic Sediment	Terrestrial Plant	Terrestrial Soil	Aquatic Sediment	Terrestrial Plant	Terrestrial Soil
Jordan River 1	Aquatic Plant	0.084	0.57	0.59	0.91	0.00003*	0.72
	Aquatic Sediment		0.0008*	0.99		0.000007*	0.90
	Terrestrial Plant			0.13			0.042*
Jordan River 2	Aquatic Plant	0.59	0.45	0.98	0.94	0.033*	0.14
	Aquatic Sediment		0.009*	0.94		0.0003*	0.021*
	Terrestrial Plant			0.35			1.00
Pigeon River	Aquatic Plant	0.026*	1.00	0.42	0.0055*	0.0002*	0.011*
	Aquatic Sediment		0.0045*	0.98		0.17	0.57
	Terrestrial Plant			0.39			1.00

*value significant at $\alpha=0.05$

Table 3. Summary of estimates of potential nutritional sources contributing to ammocoete diets from studies using gut content analysis. Unless otherwise noted, estimates were by visual examination.

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

Mundahl et al., 2005	<i>L. appendix</i>	90-179	Detritus (86.0-92.6%)	Algae (7.3-14.0%)	Minnesota, USA	Quantification of posterior 10% of GC
<hr/>						
GC-gut contents						
BC- bomb calorimetry						

Figure Captions

Fig 1. Locations of sample collection sites on the (a) Pigeon River and (b) Jordan River, Michigan, USA in the present study.

Fig 2. Distribution of sea lamprey (*Petromyzon marinus*) ammocoete lengths for animals captured and analyzed in the present study. Dark gray represents ammocoetes collected in May or June, and light gray represents ammocoetes collected in October.

Fig 3. Sea lamprey ammocoete muscle tissue (a) C:N ratio, (b) $\delta^{13}\text{C}$, and (c) $\delta^{15}\text{N}$ as a function of animal length in the present study, with significant correlations shown. Open circles are ammocoetes from the Jordan River 1, gray circles denote Jordan River 2, and black circles are the Pigeon River.

Fig 4. $\delta^{13}\text{C}$ vs. C:N ratio of sea lamprey ammocoete muscle tissue in the present study. The open circles are Jordan River 1, the black circles are Jordan River 2, and the gray circles are the Pigeon River.

Fig 5. $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ of sea lamprey ammocoetes and their potential food and nutritional sources (mean $\pm$ SD) for (a) Pigeon River, (b) Jordan River 1; and (c) Jordan River 2. All isotope values are raw data without isotopic fractionation factors applied.

Fig 6. Proportional contributions of the four most important potential food and nutritional sources identified for ammocoetes at the three sampling sites in the present study as calculated

by the Bayesian model, MixSIR. Lower and upper error bars correspond to 5% and 95% posterior probabilities, respectively. The light gray bar represents contributions calculated using the low fractionation value model (50% less than the estimated fractionation value)), the white bar represents contributions calculated using the estimated fractionation value, and the dark gray bar represents contributions calculated using the high fractionation value model (50% greater than the estimated fractionation value).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6