



# Variability in sea lamprey fatty acid profiles indicates a range of host species utilization in Lake Michigan

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## ABSTRACT

Despite being a “top predator”/parasite in the Great Lakes, knowledge of sea lamprey feeding ecology remains hindered by methodological constraints. Particularly, our knowledge of sea lamprey dietary habits is likely biased as it relies primarily on wounding rates of commercially and recreationally caught fish. Biochemical methods provide a means to extract diet information from sea lamprey themselves, and therefore provide a more objective assessment of sea lamprey feeding ecology. Of particular interest is the use of fatty acid profiles to qualitatively describe foraging patterns of sea lamprey. Adult sea lamprey were captured throughout the Lake Michigan basin during spring spawning migrations into rivers, and muscle tissues were analyzed for fatty acid profiles. Exploratory multivariate analyses were used to investigate variation in fatty acid profiles among captured individuals and to compare these to profiles of potential host species. In general, we noted a large variability in fatty acid profiles suggesting a broad spectrum of host species targeted by sea lamprey. Comparing sea lamprey fatty acid profiles with published data on host species, we concluded that sea lamprey feed on a wide variety of host species.

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## Introduction

Despite targeted programs by the Great Lakes Fishery Commission to reduce sea lamprey (*Petromyzon marinus*) abundance, parasitism remains an impediment to restoration efforts of native fish populations (Dexter et al., 2011). Relative frequency of visible wounds are used as a primary indicator of sea lamprey–host interactions, however, variation in mortality rates among different host species may lead to a lack of appreciation for the diversity of species supporting sea lamprey populations (Bence et al., 2003). Adult sea lamprey returning to spawn in the Great Lakes vary dramatically in size, suggesting a wide variation in feeding rate and diet quality (Bergstedt and Swink, 1995). As wounding rates generally remain above target levels and native host populations remain below target levels, undocumented sea lamprey–host interactions may hinder the effectiveness of basin-wide management efforts (Stapanian and Madenjian, 2007). Direct observation of sea lamprey foraging is challenging due to high host mortality (>40% Swink, 2003), propensity of deceased fish to sink (Bergstedt and Schneider, 1988; Schneider et al., 1996), and rapid digestion of liquid diets (Jorgensen and Kitchell, 2005). As such, assessing biochemical trophic markers in sea lamprey may elucidate previously underappreciated sea lamprey–host interactions within Lake Michigan and beyond.

Fatty acid biomarkers provide dietary information where direct observations are impractical. Fatty acids incorporate into consumers in patterns reflective of prey assemblages consumed over periods of months (Budge et al., 2006; Iverson, 2009). Like many fish species, sea lamprey accumulate and store lipid reserves (triacylglycerols) in their body wall muscles (Plisetskaya, 1980; Bird and Potter, 1983b; Sheridan, 1988). Despite a reduction in lipid content of sea lamprey carcasses (eviscerated carcasses) over the course of a 65 km migration, Araújo et al. (2013) reported that only changes in anteiso-15:0, 18:0, and 20:1(n-7, 9, 11) corresponded with distance migrated. Other fatty acids in their data set either did not appear to change in proportions or changes did not correlate with distance migrated suggesting migration activities do not have a large or consistent effect on fatty acid profiles of sea lamprey muscles. A similar study suggested that fatty acids of muscle tissue between sea lamprey caught at river mouths on the Iberian peninsula were not consistently different from those caught at sites >60 km inland (Lança et al., 2011). Visceral fat and fat stored within the liver are primarily used to support up-stream spawning migrations (Araújo et al., 2013), and have been shown to be greatly altered during starvation periods (Bird and Potter, 1983b). Thus, muscle tissue can provide a sample to explore fatty acids that is not as likely to be altered by migration activities compared to lipid profiles in other tissues.

Of the large lipid reserves in sea lamprey muscle tissues, neutral lipids comprise a larger fraction (>60%) than polar lipids (Lança et al., 2011; Lança et al., 2013). The neutral lipid fraction has been shown to

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represent fatty acids of dietary origin to a higher degree than polar lipids (Iverson, 2009; Lazzarotto et al., 2015). As such, fatty acid profiles derived from sea lamprey muscle tissues should represent the fatty acid composition of their diets (Plisetskaya, 1980; Lança et al., 2011). Although up-stream migrants have begun to mature, and likely physiologically alter in preparation to enter freshwater streams, analysis of salmonids undergoing parr-smolt transformation indicates that smolt fatty acid profiles reflect parr diets (Bell et al., 1997; Tocher et al., 2000). Through fatty acid analysis of muscle tissues from anadromous Atlantic sea lamprey returning to spawn, distinct feeding strategies have been described among and within populations (Lança et al., 2011; Lança et al., 2013). Specifically that a segment of the European sea lamprey population uses more demersal species as hosts compared to a general reliance on pelagic productivity (Lança et al., 2013). In a similar vein, we sought to evaluate if fatty acid profiles of muscle tissues could provide insights into foraging habits of the Lake Michigan sea lamprey population.

As sea lamprey are not known to exhibit homing behavior (Bergstedt and Seelye, 1995; Howe et al., 2013), we hypothesized that fatty acid profiles of up-stream migrants would not be specific to location of capture. Consistent with other studies of early migrant sea lamprey, we also hypothesized that there would be no differences in fatty acid profiles between sexes (Pinela et al., 2009; Lança et al., 2011; Lança et al., 2013). We also hypothesized that within the Lake Michigan sea lamprey population, significantly different groups of adult sea lamprey fatty acid profiles could be found. The presence of statistically dissimilar groups of sea lamprey fatty acid profiles that are not associated with migration distance, location of capture, or sex would indicate differences in individual's foraging habits. The converse would be all individuals specializing on one host species or a similar host assemblage, for which we would expect to see relatively homogenous fatty acid profiles with few discernable differences between any individuals.

## Methods

### Field collections

The U.S. Fish and Wildlife Service operates live traps at barrier dams throughout the Great Lakes basin as part of routine population monitoring for the Sea Lamprey Control Program. Sea lamprey migrating upstream were captured at dams located around Lake Michigan (Fig. 1). Separate live traps were located in-stream at each of five sites, where approximately 20 sea lamprey were kept for <2 weeks before sampling (Table 1). Streams selected had barrier dams that were relatively close to Lake Michigan (<20 km) in order to reduce the effects migratory activity may have on fatty acid profiles. If there were effects of migration we would see differences between the furthest upstream sites ~19 km and the sites closer to the lake ~2.5 km during analyses.

Sea lamprey were euthanized according to recommendations by the American Veterinary Medical Association (>10 min without opercula movement; Leary et al., 2013) using a bath of stream water containing an overdose (>250 mg/l) of tricaine methanesulfonate (MS-222). Sea lamprey were subsequently measured (mm) and weighed (g), an approximately 5-g muscle flank sample was excised and sex determined for each individual. Tissue samples were stored on dry ice in individual labelled bags until stored at  $-80^{\circ}\text{C}$  prior to biochemical analyses.

### Laboratory analysis

Total lipids were extracted from a 1.0 g sample of homogenated tissue with chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene as an antioxidant (Iverson et al., 1957), a method that performs well on wet fish tissues (Iverson et al., 2001). The organic solvent was evaporated under nitrogen gas and the lipid content was determined gravimetrically. Fatty acid methyl esters (FAMES) were prepared following the methods of Metcalfe and Schmitz (1961). Fatty



Fig. 1. Streams from which adult sea lamprey were captured during spawning migrations from Lake Michigan.

acids profiles were determined using an Agilent Technologies 7890A GC system with Agilent Technologies 7693 Autosampler and Agilent Technologies 5975C inert XL EI/CI MSD with Triple-Axis detector (Agilent Technologies, Inc., Santa Clara, CA). The capillary column is an Omegawax 250 Fused Silica Capillary Column with 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$  film thickness (Supelco, Bellefonte, PA). Helium was used as a carrier gas. The oven temperature was programmed from 175  $^{\circ}\text{C}$  for 26 min to 205  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C}$  per min, and then held at 205  $^{\circ}\text{C}$  for 24 min. The rate of helium carrier gas flow was 1.8 ml/min. The source and analyzer temperature of the MS was set at 230  $^{\circ}\text{C}$ . FAMES were separated on an Omegawax<sup>TM</sup> 250 fused silica capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$  film thickness, Supelco, Bellefonte, PA).

Prior to transmethylation, a known amount of nonadecanoate acid (19:0, Nu-Check Prep Inc., Elysian, MN), proportional to the amount of total lipids detected (8 mg per 50 mg of lipids) was added as an internal standard. The individual FAMES were identified by comparing the retention times of authentic standard mixtures (FAME mix 37 components, Supelco) and with known spectrographic patterns of FAMES. The FAMES quantification was made by comparing their peak areas

Table 1

Location of traps, distance traps were located upstream from Lake Michigan, date of collection, and sample numbers of sea lamprey.

Creek Name	Distance (km)	Date sampled	Male	Female
Betsie	19.3	June 3, 2014	17	14
Boardman	2.4	June 3, 2014	7	11
Manistique	2.4	June 2, 2014	8	11
Peshtigo	18.0	June 2, 2014	12	11
Trail creek	6.9	May 25, 2014	6	19

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