Sea Lamprey (*Petromyzon marinus*) in the Cheboygan River, Michigan, Watershed: Parasitic Feeding Ecology and Origin of Adults

by

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Abstract

Historic and recent evidence suggests that a landlocked population of Sea Lamprey (*Petromyzon marinus*) complete their life cycle in the upper Cheboygan watershed. In this watershed, the fish communities of Burt and Mullett lakes support the parasitic-stage for this population, while the surrounding tributaries provide spawning and larval rearing habitat. The Cheboygan River lock and dam system serves as a partial barrier between this 'upper-river' population and Sea Lamprey that feed in Lake Huron. Despite this barrier, Lake Huron Sea Lamprey still may escape into the 'upper-river' and utilize the same spawning grounds as those from Burt and Mullett lakes. The objectives of this study were to determine (1) what fishes Sea Lamprey feed on in Burt and Mullett lakes and (2) whether spawning-phase Sea Lamprey from Lake Huron can be distinguished from those from Burt and Mullett lakes through morphological characteristics and stable isotopes. Results indicate that 'upper-river' Sea Lamprey feed on 'less desirable' fishes when compared to their Lake Huron counterparts. Parasitic-phase Sea lamprey from the 'upper river' did not differ significantly in size relative to Sea Lamprey from Lake Huron as a group. However, when stratified by month of capture, 'upper-river' Sea Lamprey were significantly larger than parasites from Lake Huron. Spawning-phase Sea Lamprey differed significantly in size with Lake Huron Sea Lamprey being larger than those of unknown origins. Stable isotope data showed that 'upper-river' Sea Lamprey heads had significantly lower deuterium (δ^2H) and $\delta^{18}O$ values than those from Lake Huron. Therefore, measurement of total length and weight of spawning-phase Sea Lamprey and deuterium from Sea Lamprey heads should be a useful method for managers to distinguish between spawning-phase Sea Lamprey as either originating from Lake Huron or from the 'upper-river', allowing for more accurate population estimates of landlocked Sea Lamprey from Burt and Mullett lakes, aiding in their control.

Introduction

The Sea Lamprey (*Petromyzon marinus*) is a parasitic, anadromous fish native to the Atlantic coasts of Europe and North America (Applegate 1950; Hansen *et al.* 2016). As juveniles, they feed as external parasites on fish for approximately 12 to 18 months (Bergstedt and Swink 1995a). They migrate up streams to spawn, and then die afterward (Applegate 1950). Hatched larval Sea Lamprey, or ammocoetes, burrow into soft substrate and spend two to seven years as sedentary filter feeders before metamorphosing into parasitic-phase juveniles and migrating back to the Atlantic Ocean (Applegate 1950; Kao *et al.* 1997; Morkert *et al.* 1998; Drevnick *et al.* 2006; Swink and Johnson 2014). Like many anadromous fish, Sea Lamprey can tolerate freshwater throughout their life cycles, which occasionally results in the establishment of landlocked populations when return access to the ocean becomes blocked or carries excessive risk of mortality, but sufficient resources are available (e.g. spawning habitat and hosts) (Zydlewski and Wilkie 2013; Johnson *et al.* 2016). This is the case in the Laurentian Great Lakes, as well as in some sub-basins within the Great Lakes, including Lake Champlain and the Finger lakes (Applegate 1950; Twohey *et al.* 2003; Holbrook 2015; Johnson *et al.* 2016). As a result, Great Lakes fishes have been impacted by the establishment of Sea Lamprey throughout the basin and several native fish species have even been extirpated (Sitar *et al.* 1997; Christie and Goddard 2003; Cline *et al.* 2013; Siefkes *et al.* 2013).

Sea lamprey have resided in Lake Ontario longer than any of the other Laurentian Great Lakes and are sometimes considered native or naturalized to Lake Ontario (Applegate 1950; Waldman *et al.* 2004, 2009). Other evidence suggests that they may have entered through the Erie Canal (Mandrak and Crossman 1992; Smith 1995; Eshenroder 2009, 2014). The first verified record of breeding occurred in 1835 in Duffins Creek, a Lake Ontario tributary (Lark 1973; Smith 1995; Christie and Goddard 2003). Movement into Lake Erie was later facilitated by improvements in the Welland Canal (Eshenroder and Burnham-Curtis 1999); the first adult Sea Lamprey was captured in Lake Erie 1921 (Applegate 1950). From there, Sea Lamprey spread rapidly, occupying all five of the Great Lakes by 1938 (Hansen *et al.* 2016).

Once established in the Great Lakes, Sea Lamprey dramatically impacted economically important fisheries through parasitism of commercially and culturally important fishes (Lawrie 1970; Smith 1972; Christi 1974; Smith and Tibbles 1980; Stapanian *et al.* 2006). Declines in Lake Trout (*Salvelinus namaycush*) (Lawrie 1970), Lake Whitefish (*Coregonus clupeaformis*) (Smith and Tibbles 1980) and Burbot (*Lota lota*) (Stapanian *et al.* 2006) throughout the basin spurred the Canadian and U.S. governments to create the Great Lakes Fisheries Commission (GLFC), which was tasked with reducing Sea Lamprey populations through mechanical, chemical and biological control measures (Applegate *et al.* 1961; Smith and Tibbles 1980; Bergstedt and Twohey 2007; McDonald and Kolar 2007; McLaughlin *et al.* 2007). This binational collaboration is considered one of the most intense efforts to reduce the numbers of a vertebrate pest (Lawrie 1970); annual costs of Sea Lamprey control totaled approximately USD $10 million (Lavis *et al.* 2003), and now can surpass USD $20 million (N. Johnson, USGS Hammond Bay Biological Station, personal communication).

Various control methods are utilized throughout the Great Lakes Basin. Mechanical control measures include dams and weirs that limit adult Sea Lamprey movement, reducing the number of stream miles available for spawning adults (Lavis *et al.* 2003). In areas where Sea Lamprey can access spawning habitat, Sea Lamprey control is administered after considering number of larvae present, their size, and the cost of treatment. Sea Lamprey production is estimated through electrofishing surveys of stream reaches, which provide larval population estimates, size of larvae and probability larvae will metamorphose in the upcoming year. If a stream is selected for treatment, selective pesticides (lampricides) are then applied, targeting ammocoetes (Christie et al. 2003). Adults are also trapped to estimate population size, evaluate barriers, and lampricide effectiveness (Christie and Goodard 2003; Holbrook *et al.* 2014). However, control efforts can be met with diminishing returns (Christie *et al.* 2003), due to a variety of factors including larvae residual to treatment (Holmes 1990), increased survival of parasitic-phase Sea Lamprey due to restoration of preferred hosts (e.g. Lake Trout) (Twohey *et al.* 2003), and potential contributions from landlocked populations.

Burt and Mullett lakes in Michigan (**Figure1**) are inland lakes within the Cheboygan River watershed that are large enough to support a population of landlocked Sea Lamprey (Applegate 1950; Holbrook 2015; Johnson *et al.* 2016). Individuals in this population (hereinafter referred to as 'upper-river' Sea Lamprey) can spend the entirety of their life cycle within Burt and Mullett lakes, but may also migrate out to Lake Huron to feed (Johnson *et al.* 2016). This 'upper-river' population shares spawning grounds within the Cheboygan River watershed with Sea Lamprey that feed as juveniles in Lake Huron (hereinafter referred to as 'Lake Huron' Sea Lamprey) (Johnson *et al.* 2016). This overlap of spawning habitat complicates control of Sea Lamprey within northern Michigan. While these two populations are separated by a dam and lock system approximately two kilometers from the mouth of the Cheboygan River (**Figure 1**), the upper reaches of the Cheboygan River watershed have remained infested with Sea Lamprey (Holbrook *et al.* 2014; Johnson *et al.* 2016).

Holbrook *et al.* (2014) showed that while the existing dam and lock system, as well as Sea Lamprey traps, have the potential to act as an effective barrier to Sea Lamprey migration, the possibility of escapement into the upper Cheboygan River exists. Holbrook *et al.* (2014) estimates that 0-2% of adult Sea Lamprey in the lower Cheboygan River watershed could have passed upstream of the dam and lock. With an estimated population size of 21,828 – 29,300 adult Sea Lamprey in the Cheboygan River, zero to 514 individuals could have escaped (Holbrook *et al.* 2014). These uncertainties present Sea Lamprey control agents with difficult decisions with regard to Sea Lamprey in the Cheboygan River watershed: should costly improvements to the dam and lock system be made to fortify this Sea Lamprey barrier? Can 100% blockage of Sea Lamprey migration to the 'upper-river' be achieved in this system?

These questions are amplified because the Cheboygan River watershed is an important area with regard to Sea Lamprey control, as it is one of the largest Sea Lamprey producing streams in the Great Lakes (Holbrook *et al.* 2014; Sullivan and Adair 2014). Roughly 425,000 m^2 of preferred larval habitat exists upstream of the Cheboygan River dam and lock (Holbrook *et al.* 2014). To perform efficient control measures and evaluate their effectiveness, accurate population estimates are necessary. Sea Lamprey abundance is primarily estimated by capturing spawning-phase Sea Lamprey (Applegate 1950; Schleen and Klar 2000; Mullett *et al.* 2003; Swink 2003). Current population estimates for 'upper-river' spawning Sea Lamprey are less than 200 individuals, and therefore, they may be susceptible to control tactics that reduce reproduction like trapping and sterile male release (Hanson and Manion 1980). Eradication may also be feasible (Johnson *et al.* 2016) by harnessing a variety of control methods hinged on the concept of integrated pest management, including lampricide application, trapping and the sterile-male-release-technique (Hanson and Manion 1980; Christie and Goddard 2003; Klassen *et al.* 2004; Johnson *et al.* 2016). Eradicating the 'upper-river' population would reduce control costs; lampricide treatments takes place once every four years at a cost of USD $100,000 annually (Johnson *et al.* 2016). Eradication efforts may only make sense though, if we know that Sea Lamprey are capable of escaping upstream of the

Cheboygan River lock and dam system. Studying this small 'upper-river' population to better understand inland lake Sea Lamprey ecology could help inform a method of eradication for application across the Great Lakes.

Johnson *et al.* (2016) concluded that a small population of Sea Lamprey spawned in the upper Cheboygan watershed and some may have completed their life cycle in the 'upper-river'. This conclusion is supported by the presence of Sea Lamprey wounds on fish from Burt and Mullett lakes, presence of adult Sea Lamprey above the Cheboygan dam and lock prior to the seasonal opening of the lock, small differences in statolith microchemistry when comparing adults from the 'upper-river' to those of Lake Huron, and differences in total length between populations (Johnson *et al.* 2016). But several questions remain, such as: what damage Sea Lamprey are causing to fishes in Burt and Mullett lakes and what is the primary source of the spawning stock of Sea Lamprey in the 'upper-river'? Are spawning Sea Lamprey primarily composed of individuals that completed their life cycle in the 'upper-river' or from individuals that originated from Lake Huron and entered the 'upper-river' by bypassing the lock and dam? Answering these two questions will help managers set targets for Sea Lamprey control in this system and inform control strategies. Therefore, utilizing the framework described in Johnson *et al.* (2016), this study explores parasitic Sea Lamprey feeding ecology in the upper Cheboygan watershed, and evaluates methods for distinguishing the 'upper-river' spawning adults from those that feed in Lake Huron, escape around the lock and dam, and spawn in the upper Cheboygan watershed.

Sea lamprey originating in the 'upper-river' versus Lake Huron may differ physically and physiologically due to differences in fish communities. Burt and Mullett lakes primarily support cool water fishes such as Northern Pike (*Esox lucius*), Smallmouth Bass (*Micropterus dolomieu*), Walleye (*Sander vitreus*), and Yellow Perch (*Perca flavescens*), which belong to a variety of feeding guilds: piscivores, pelagic planktivores, insectivores, and benthivores (Hanchin *et al.* 2005). Cold water fishes such as landlocked populations of Rainbow Trout (*Oncorhynchus mykiss*), Brown Trout (*Salmo trutta*), and recovering populations of Lake Sturgeon (*Acipenser fulvescens*) can also be found in Burt and Mullett lakes (Hanchin *et al.* 2005; Johnson *et al.* 2016). In contrast, Lake Huron is dominated by cold water salmonids, such as Lake Trout, Chinook Salmon (*Oncorhynchus tshawytscha*), Rainbow Trout, Coho Salmon (*Oncorhynchus kisutch*) and Pink Salmon (*Oncorhynchus gorbuscha*) (Roseman *et al.* 2014). While Sea Lamprey do exhibit feeding preferences towards large salmonids (Coutant 1977; Swink 1993; Swink 2003), they are generalist feeders (Silva *et al.* 2014; Happel *et al.* 2017). Therefore, I hypothesized that differences in available host species may lead to differences in size, condition factor and biochemical markers between the two populations.

Isotopic values of Sea Lamprey tissues are influenced by diet as well as the waters that they occupy. The analysis of stable isotopes and trophic interactions has relied heavily on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), which are derived solely from diet (DeNiro and Epstein 1981; Hobson *et al*. 1999; Ehleringer *et al.* 2008; Vander Zanden *et al.* 2016). Analyses of other stable isotopes, such as deuterium ($\delta^{2}H$) and oxygen ($\delta^{18}O$), have historically been used in climate sciences to study hydrological cycles by tracing water origin or to reconstruct past climates (Clark and Fritz 1997). However, these isotopes have recently been applied in animal studies through the linkage of spatial patterns in amount weighted precipitation and $\delta^{2}H$ and $\delta^{18}O$ values in animal tissues (Vander Zanden *et al.* 2016). This has made it possible to track movements and origin of a broad range of animals (Bowen *et al.* 2005). Deuterium and $\delta^{18}O$ composition is influenced by diet and environmental water, resulting in the application of these isotopes as a tracer of organismal food and resource use (Vander Zanden *et al.* 2016).

Jasechko *et al.* (2014) demonstrated through the analysis of deuterium that surface water flowing into Lake Huron (i.e., the Cheboygan River watershed) was more negative than water analyzed from Lake Huron. Therefore, I hypothesized that 'upper-river' Sea Lamprey may have different isotopic composition when compared to Sea Lamprey known to originate from Lake Huron. If proven correct, the analysis of isotopic composition of Sea Lamprey tissue may be a more reliable and cost effective tool to determine if Sea Lamprey are escaping past the barrier at the Cheboygan River dam and lock. Increased knowledge of the effectiveness of this barrier can assist managers with developing more accurate population estimates of 'upper-river' Sea Lamprey and ultimately assist with control efforts of Sea Lamprey in the upper Cheboygan watershed.

My objectives were to: (1) determine what fishes Sea Lamprey are feeding on in Burt and Mullett lakes, and the damage they cause within these lakes and how it compares with Lake Huron; and to (2) determine if physiological differences exist between the two populations, and if they can be used to distinguish Sea Lamprey that completed their life cycle in the 'upper-river' from those from Lake Huron. To accomplish Objective 1, fishes from Burt and Mullett lakes were inspected for lamprey inflicted wounds. Objective 2 was accomplished through a comparison of Sea Lamprey biometrics and isotopic composition to test if differences in host/prey communities affect Sea Lamprey physiology.

Methods

Study System

Burt and Mullett lakes are located within the northern portion of Michigan's lower peninsula, and are part of the Cheboygan River watershed (**Figure 1**). Burt Lake has a surface area of 6,930 ha and a maximum depth of 22.2 m. Mullett Lake is slightly larger, with a surface area of approximately 7,025 ha, and a maximum depth of 36.5 m (Laarman 1976). Mullett Lake is drained by the Cheboygan River along its northern lobe, where it heads northeast to Lake Huron. The Indian River flows from Burt to Mullett Lake. This system makes up a portion of the inland waterway, which also contains Black Lake (4,092 ha), another substantial inland lake. Alverno Dam on the Black River (**Figure 1**) inhibits Sea Lamprey spawning migration, resulting in an absence of Sea Lamprey in Black Lake (Johnson *et al.* 2016).

Burt and Mullett lakes are considered mesotrophic, and they experience moderate recreational fishing pressure (Schrouder 1976; Tipp of the Mitt Watershed Council 2002). The Crooked and Maple rivers feed into Burt Lake on its western side, and the Sturgeon River enters on the south side (Hanchin *et al.* 2005), along with several smaller streams. Mullett Lake is fed by the Indian River, the Little Pigeon and Pigeon rivers, and Mullett Creek.

Many of the tributaries that feed Burt and Mullett lakes contain or have contained larval native lampreys (*Lethenteron appendix* and *Ichthyomyzon* spp.) as well as larval Sea Lamprey (Applegate 1950; Johnson *et al.* 2016). However, native lamprey populations are generally restricted to areas of the watershed that are not treated with lampricide, since lampricide will kill all lamprey species (McLaughlin *et al.* 2003; Sullivan *et al.* 2003). Since 2010 only the Pigeon (including Little Pigeon), Sturgeon (including Little Sturgeon) and Maple rivers have contained larval native and Sea lampreys (Johnson *et al.* 2016). Since the discovery of Sea Lamprey within the Cheboygan River watershed, numerous lampricide treatments have been applied to the infested streams (Sullivan and Adair 2014; Johnson *et al.* 2016). The Pigeon, Sturgeon and Maple rivers were most recently treated during late summer of 2016, using a combination of liquid and solid 3-trifluromethyl-4-nitrophenol (TFM) as well as Bayluscide in the Sturgeon River lentic, after a larval population likely exceeding 100,000 was found during survey efforts (Mullet and Sullivan 2017).

Angler Surveys

To determine which fishes Sea Lamprey feed on in Burt and Mullett lakes (Objective 1), recreationally caught fish from Burt and Mullett lakes were examined for wounds and these data were used to construct wounding rates. Surveys were conducted by the author on Burt Lake from May through August, 2016. Access points along Burt Lake were targeted, specifically Burt Lake State Park, Maple Bay Boat Ramp and Sturgeon Bay Boat Ramp (**Figure 1**). In an effort to encounter as many anglers as possible, multiple sites were typically visited in a given day. Angler catches were inspected as they returned from outings for lamprey wounds, with the angler's permission. Total lengths (mm) of all fishes observed were recorded. Any fish which exhibited signs of lamprey wounding, as classified by King (1980), were of special interest and pictures of wounding were taken. Wounds with a diameter greater than 20 mm were classified as Sea Lamprey wounds. Wounds smaller than 20 mm were also recorded, but were believed to be inflicted by native sea lamprey (e.g. Silver Lamprey) (Johnson *et al.* 2016). Anglers were also encouraged to self-report wounding utilizing a wound-identification handout which was distributed to anglers encountered at boat ramps and/or left on vehicles (**Figure 2**). Coupled with this effort, fliers designed to target anglers not utilizing water craft were posted at popular access points along the Maple, Pigeon and Sturgeon rivers, as well as Veteran's Pier fishing access point in Indian River, Michigan (**Figure 3**). These fliers were modeled after those distributed by Applegate (1950). The author did not personally conduct surveys on Mullett Lake during the summer of 2016 as to not confound a Michigan Department of Natural Resources creel survey. Nonetheless, a Mullett Lake angler (Steven Philip) provided wounding data from fish caught. Angler survey data were combined with wounding information collected by U.S. Geological Survey, Hammond Bay Biological Station (HBBS) for Burt and Mullett lakes spanning from 2013 to 2019, as part of their upper Cheboygan River Sea Lamprey observation program. In this thesis, only wounding data from 2016 are reported.

HBBS staff conducted presentations at angler meetings and posted fliers at bait shops in the area. Fliers requested that resource users send pictures of wounded fish alongside a meter stick and any lampreys attached to fish to HBBS. Photos of wounded fish were evaluated based on the aforementioned criterion (greater than 20 mm vs. less than 20 mm) and classified accordingly. If a parasitic juvenile Sea Lamprey was captured from Mullett or Burt lakes, it was frozen and stored at HBBS for future inspection. From these angler survey results, Sea Lamprey wounding rates of fishes were compiled and then statistically analyzed between the two lakes through a test of equal or given proportions (H_o: proportions or probabilities within two groups are the same; RStudio Team 2016).

Specimen collection

Morphological characteristics from Sea Lamprey were collected to determine if significant differences in size and condition exist between the two populations, and if differences could aid in distinguishing the two populations (Objective 2). Sea Lamprey undergo dramatic physical and biological changes during spawning runs, which may influence total length and/or weight (Applegate 1950; Araújo *et al.* 2013). For this reason, total lengths, weights and condition factor of Sea Lamprey were compared based on life cycle (i.e., spawning-phase vs. parasitic-phase) and origin. All parasitic-phase lamprey were classified as either 'upper-river' or Lake Huron Sea Lamprey. Origin of these Sea Lamprey is known as they were captured while feeding in Burt/Mullett lakes or in Lake Huron. Adult spawning-phase Sea Lamprey captured in the

Pigeon, Sturgeon or Maple rivers were either classified as originating from Lake Huron or had unknown origins. These two groups could be distinguished in the field because spawners from Lake Huron were marked via fin clippings by HBBS staff, while Sea Lamprey with unknown origins did not have fin clippings. Sea Lamprey with unknown origins could have completed their life cycle in the upper river or escaped around the lock and dam and therefore originated from Lake Huron.

For all Sea Lamprey (i.e., spawning- and parasitic-phase Sea Lamprey) total length (mm), weight (g), and sex was recorded. Condition factor (W_r) was derived from length and weight using the equation:

$$Wr = \frac{Weight\ (g)}{10^{-4.70251+(2.63133*LOG10(Length[mm]))}}$$

from Schneider *et al.* (2000). Mean total length, weight and condition factor or Lake Huron and 'upper-river' Sea Lamprey were analyzed for significant differences (i.e., $p < 0.05$) via two sample t-tests after testing for equal variance (RStudio Team 2016). Sea Lamprey with unknown origin were not statistically compared to 'upper-river' or Lake Huron Sea Lamprey since individuals from both groups could be represented in the unknowns.

Spawning-phase Sea Lamprey

Adult spawning-phase Sea Lamprey were captured during the summers of 2013 through 2019 during their upstream migration by employees of HBBS. Spawning-phase males captured below the Cheboygan River dam (known Lake Huron source) were marked via fin clippings, and released at the mouths of the Maple, Pigeon and Sturgeon rivers as part of a separate study conducted by HBBS. Pairs of trap nets were placed within the rivers, oriented with the trap opening facing downstream, to capture upstream migrating Sea Lamprey. Captured Sea Lamprey were examined for the presence of fin clippings; when fin clippings were absent, the Sea Lamprey origin was classified as unknown (i.e., could be from the landlocked population or could be from Lake Huron if it escaped through the dam/lock system). From 2013 to 2019, a total of 24 Sea Lamprey with unknown origins were captured. Total length (mm) (measured from the beginning of the oral disk to the end of the caudal fin) and total mass (g) of all Sea Lamprey was recorded. Sex was recorded for unmarked individuals. Additional marked male Sea Lamprey (n = 9) were captured in the Maple River during nighttime nest surveys, conducted by the author, from June to August 2016. These surveys were conducted on the Maple River because the Lake Kathleen Dam (**Figure 1**) aggregated Sea Lamprey below it, allowing for relatively easy capture of Sea Lamprey when compared to the Pigeon and Sturgeon rivers. One marked spawning-phase Sea Lamprey was also supplied by residents along the Sturgeon River, who captured this in July of 2016. A total of 455 spawning-phase Sea Lamprey were collected, 431 of which originated from Lake Huron and 24 with unknown origins (**Table 1**)

Spawning-phase Sea lamprey of unknown origins are assumed to be either from the 'upper-river' or from Lake Huron, making it difficult to group them as one entity. Nevertheless, statistical analyses (two sample t-tests, testing for variance) comparing spawning-phase Sea Lamprey were conducted in order to understand trends. Lengths and weights of Sea Lamprey of unknown origins were also plotted with spawners from Lake Huron. Normal confidence ellipses (95%) are overlaid on top of scatterplots in an attempt to compare Lake Huron Sea Lamprey to those with unknown origins. This qualitative comparison was done to determine if any of the Sea Lamprey with unknown origin fall outside of the confidence ellipse associated with Lake Huron spawners, and could be spawning-phase Sea Lamprey from the 'upper-river'. Combination box plots and scatterplots were also used to compare total length, weight and condition factor of spawning-phase Sea Lamprey of unknown origin to spawners from Lake Huron.

Parasitic-phase Sea Lamprey

Parasitic-phase Sea Lamprey found attached to fishes, and in one case, to the hull of a boat, were also collected from Burt and Mullett lakes by anglers and turned over to HBBS (Burt Lake, n = 2; Mullett Lake, n = 5). Fisheries and Oceans Canada and HBBS donated Sea Lamprey that were known to feed in Lake Huron, in 2015 (n = 139) and 2016 (n = 14), respectively. In total, parasitic-phase Sea Lamprey accounted for 160 specimens, with 143 from Lake Huron, and seven from Burt and Mullett lakes (**Table 1**). Mean total length, weight, and condition factor were analyzed for significant differences between the two groups, to compare all parasitic-phase Sea Lamprey. However, Bergstedt and Swink (1995b) demonstrated that growth of parasitic-phase Sea Lamprey differs significantly throughout the year. Periods of rapid growth were observed in early June, and then again in early October. For this reason, a seasonal comparison of morphological characteristics for parasitic-phase Sea Lamprey was also conducted, focusing on Sea Lamprey caught in August and September. These two months were selected due to the fact that the majority of 'upper-river' Sea Lamprey were caught within this timeframe (August = 4, September = 2).

Sample Preparation

Stable isotopes (δ^2H and $\delta^{18}O$) from Sea Lamprey were analyzed to determine if significant differences in isotopic composition exists between 'upper-river' and Lake Huron Sea Lamprey, due to differences in available hosts and water chemistries (Objective 2). Additionally, isotopic composition of potential Sea Lamprey hosts from Burt and Mullett lakes were prepared and analyzed to further understand isotopic composition of 'upper-river' organisms.

A total of 52 Sea Lamprey individuals were utilized in stable isotope analysis (**see Appendix A**). We prioritized Burt and Mullett lakes specimens (n = 7), and unknown spawning-phase Sea Lamprey (n = 10) due to funding constraints. The tissue types used in stable isotope analysis included the heart, liver, adipose tissue, and head. Therefore, multiple samples were run (e.g., heart, liver, adipose, and head tissues) from each individual when possible. However, analysis of heads is presented here as heads were the only available tissue type for the majority of Sea Lamprey with unknown origins (**see Appendix A**). Heads were utilized because HBBS has collected and stored them (-80C) since 2013. The assumption is heads and/or adipose tissue can be utilized as a surrogate for other tissue types (e.g. heart, liver), which are commonly used in stable isotope analyses (Harvey and Kitchell 2000; Happel *et al.* 2016), but require dissection of specimens.

Tissues were excised from carcasses, weighed, and stored in a -80^{o} C freezer (Shaikh 1986; Käkelä *et al.* 2005; Budge *et al.* 2006). Tissues were later lyophilized for at least 48 hours, and then reweighed to determine water content (Lança *et al.* 2014; Foley *et al.* 2016). Prior to analysis, tissue samples were ground into a fine dust under liquid nitrogen and returned to the lyophilizer for an additional 24 hours to remove any moisture absorbed during processing. Unfortunately, through the process of preparing tissue samples for analysis several samples were deemed insufficient to run as they were too small to accurately and effectively be analyzed for stable isotopes. The majority of these samples were heart and liver tissue (**see Appendix A**).

Potential host species were also prepared for stable isotope analysis to further compare isotopic composition of organisms from Burt and Mullett lakes and their influence on Sea Lamprey isotopic values (**see Appendix B**). Whole fish were homogenized following the process outlined by Benville and Tindle

(1970). Samples were lyophilized for at least 48 hours, or until no changes in sample weight were observed.

Stable Isotope Analysis

Sea Lamprey

Aliquots of approximately 0.5 mg of tissue were 'tin-balled' using silver caps (Drevnick *et al.* 2006) and run in triplicate through a Thermo Scientific EA Isolink. The EA system (Thermo Scientific Flash IRMS) was comprised of a high-temperature conversion reactor (glassy carbon with alumina sheath) maintained at 1300 ˚C, and a molecular sieve GC column (Restek Molesieve 5A, 60/80 mesh, 2m x 2mm ID) maintained isothermally at 70 ˚C. A small amount of pure silver wool at the exit of the reactor sorbed any resulting H_2S and an ascarite/magnesium perchlorate trap was placed before the GC column to sorb any cyanide evolved during the pyrolytic reaction. Carrier gas was ultrahigh-purity He at 80 mL min-1. The resolved CO and H_2 gases were transferred to a Thermo Scientific Delta V gas isotope ratio mass spectrometer by means of a continuous flow inlet (Conflo IV) with a 90% pre-split to avoid saturating the detectors. The mass spectrometer was tuned daily with pure reference gases (CO and H_2), H_3^+ correction and instrument precision ('on-off' testing) and linearity assessments were performed daily. Prior to each analysis, peak centering was performed to correct for small deviations in the ion beam. Certified reference materials (USGS-42, CBS, and KHS) were used to calibrate raw delta values to the VSMOW (Vienna Standard Mean Ocean Water) scale.

Isotopic composition of parasitic- and spawning-phase Sea Lamprey heads from Lake Huron were analyzed to determine if life cycle influences H and O isotopes. Deuterium and $\delta^{18}O$ values from Lake Huron Sea Lamprey heads were then compared to those from the 'upper-river'. Statistical analyses were conducted via two sample t-tests, accounting for variance (RStudio Team 2016). To further investigate differences physiological differences between 'upper-river' and Lake Huron Sea Lamprey, linear regression models comprised of deuterium values and weight (g) were constructed. These variables were used because they meet the assumption of homogeneity of variances. An analysis of covariance (ANCOVA) was then performed examining slope and y-intercepts (JMP Pro Statistical Discovery™, Version 14.0, SAS Institute, Inc., 2014). Size-adjusted least-squares means of deuterium from heads of Lake Huron and 'upper-river' Sea Lamprey were then compared via a two sample t-tests to examine if significant differences (i.e. $P < 0.05$) exists (JMP Pro Statistical Discovery™, Version 14.0, SAS Institute, Inc., 2014)

Significant differences in H and O isotopic composition have been observed in inter- and intra-cellular water (Kreuzer-Martin *et al.* 2005), meaning that a consumer's body is comprised of heterogeneous local water body pools (Vander Zanden *et al.* 2016). As a result, isotopic composition of each tissue type (i.e., heads, hearts, livers, and adipose tissue) was compared within groups (i.e., Lake Huron, 'upper-river', and unknown) to determine if significant differences (i.e., $P < 0.05$) do exist within specimens; results are presented in **Appendix A**. Isotopic composition of potential host species from Burt and Mullett lakes were also compared to those of 'upper-river' and Lake Huron Sea Lamprey via two sample t-tests, testing for equal variance (RStudio Team 2016) (**see Appendix B**).

Host Species

Potential host species of parasitic-phase Sea Lamprey were collected from Burt (n = 15) and Mullett (n = 4) lakes (**Table 2**). Fishes either were provided from anglers encountered during angler surveys, were caught by the author or were provided by HBBS. All fishes provided by anglers and HBBS were filleted prior to collection so weights do not represent the actual weights of the fishes (**Table 2**). Isotope data from potential hosts from Burt and Mullett lakes was combined with ‘upper-river’ Sea Lamprey isotope data, and published isotope data from Lake Huron groundwater (Jasechko *et al.* 2014) in a stable isotope mixing model to determine the influence prey has on isotopic composition of ‘upper-river’ Sea Lamprey. Apportions for the stable isotope mixing model were calculated based on the following equations:

$$H_2O_{apportion} = \frac{\mu\delta^2H\ SEL_{UR} - \mu\delta^2H\ Prey_{UR}}{\mu\delta^2H\ H_2O_{UR} - \mu\delta^2H\ Prey_{UR}}$$

$$Prey_{apportion} = 1 - H_2O_{apportion}$$

Contributions to uncertainty in the stable isotope mixing model were then calculated using the following equations:

$$H_2O_{uncertainty} = \left(\frac{\mu\delta^2H\ Prey_{UR} - \mu\delta^2H\ SEL_{UR}}{(\mu\delta^2H\ H_2O_{UR} - \mu\delta^2H\ Prey_{UR})^2}\right)^2 \times \left(\sigma_{H_2O_{UR}}\right)^2$$

$$Prey_{uncertainty} = \left(\frac{\mu\delta^2H\ SEL_{UR} - \mu\delta^2H\ H_2O_{UR}}{(\mu\delta^2H\ H_2O_{UR} - \mu\delta^2H\ Prey_{UR})^2}\right)^2 \times \left(\sigma_{H_2O_{UR}}\right)^2$$

$$SEL_{uncertainty} = \frac{1}{(\mu\delta^2H\ H_2O_{UR} - \mu\delta^2H\ Prey_{UR})^2} \times \left(\sigma_{H_2O_{SEL}}\right)^2$$

where SEL_{UR} refers to values derived from ‘upper-river’ parasitic-phase Sea Lamprey, $Prey_{UR}$ values from potential ‘upper-river’ hosts and H_2O_{UR} refer to isotopic values for Lake Huron groundwater presented by Jasechko *et al.* (2014). A mixing model was not constructed for Lake Huron Sea Lamprey due to lack of published δ^2H values on fishes from Lake Huron.

Results

Angler Survey

To understand parasitic feeding habits of ‘upper-river’ Sea Lamprey (Objective 1), 381 fishes from Burt Lake were examined during summer 2016 (**Table 3**). Of the fish inspected, 17 exhibited lamprey inflicted wounds (5%) with 15 of the 17 wounds likely being caused by Sea Lamprey. Silver lamprey (*Ichthyomyzon unicuspis*) were found attached to two fish (Lake Sturgeon and White Sucker (*Catostomus commersonii)*), and a Northern Pike had three wounds, two of which were likely from Silver Lamprey, and one from Sea Lamprey. Walleye were the most abundant fish observed during angler surveys (n = 157), representing 41% of all fish observed from Burt Lake. Walleye also exhibited the highest Sea Lamprey wounding rate (8%) of all fish observed from Burt Lake (**Figure 4**). The second most abundant species from Burt Lake was Yellow Perch (n = 132), representing 35% of all fishes inspected. No Sea Lamprey wounds were observed on Yellow Perch from Burt Lake. Northern Pike (n = 48) comprised 13% of fish inspected during Burt Lake angler surveys, and exhibited a wounding rate of 2%. All other species inspected during Burt Lake angler surveys represented less than 10% of fish inspected and exhibited no signs of Sea Lamprey wounding. The sole exception was a Brook Trout caught in the Maple River which the angler thought had a Sea Lamprey wound, but the observation could not be verified (**Table 3**).

Self-reported wounding data from Mullett Lake in 2016 yielded a total of 530 fishes inspected, including 29 wounded fishes. Of the wounded fishes, 20 were believed to exhibit wounds from Sea Lamprey and 9 from native lamprey (**Table 3**). Northern Pike (n = 340) represented 64% of all inspected fish from Mullett Lake and exhibited a Sea Lamprey wounding rate of 3%. Walleye (n = 129) were the second most common species inspected from Mullett Lake, representing 24% of all fish; none were observed with wounds. Rainbow Trout (n = 58) made up 11% of all fish observed in Mullett Lake and exhibited a wounding rate of 12%. Other fish observed in Mullett Lake with Sea Lamprey wounds included two Cisco and one Yellow Perch. For each of these species a wounding rate of 100% was observed, but the only reason individuals from these species were reported was because they had a wound so the wounding rate is meaningless in this case.

Wounding rate of Northern Pike (X = 0.11, $P = 0.74$) and Rainbow Trout (X = 0.41, $P = 0.52$) did not differ significantly between Burt and Mullett lakes, whereas wounding rates on Walleye in Burt Lake was higher than Mullett Lake (X = 0.02, $P < 0.001$).

Length-weight

Spawning-phase Sea Lamprey

Spawning-phase Sea Lamprey were categorized into two groups: those from Lake Huron (n= 431) and those with unknown origins (n = 24). Sea Lamprey with unknown origins are likely either from Lake Huron or from Burt and/or Mullett lakes. Total length (mm) of Lake Huron spawning-phase Sea Lamprey (n = 431, mean = 491 mm, SD = 37 mm) was significantly larger than that of spawners of unknown origin (n = 24, mean = 440 mm, SD = 58 mm) (T-statistic = 4.19, $P < 0.001$). Lake Huron spawners (n = 431, mean = 249 g, SD = 49 g) also had significantly higher weights (g) than unknown spawners (n = 24, mean = 198 g, SD = 59 g) (T-statistic = 4.92, $P < 0.001$). Despite these significant differences in size, no significant differences in condition factor (Wr) were found between Lake Huron spawners (n = 431, mean = 1.04, SD = 0.20) and spawners of unknown origins (n = 24, mean = 1.04, SD = 0.29) (T-statistic = 1.08, $P = 0.29$).

As a group, many of the unknown Sea Lamprey appear to fall within the 95% confidence ellipse associated with Lake Huron Sea Lamprey (e.g. MR2.2015, PIR2.2018, PIR4.2018, PIR1.2015, MR1.2015, MR1.2014, PIR1.2013, PIR1.2018, MR1.2013, MR2.2015, STR1.2018, STR1.2019, STR2.2019, STR3.2019, STR6.2019) (**Figure 5**). However, several of the unknown Sea Lamprey also fall outside of the 95% confidence ellipse corresponding to Lake Huron Sea Lamprey (e.g. PIR2.2017, PIR1.2017, STR1.2017, PIR3.2018, STR1.2013, PIR2.2013, PIR1.2019, STR4.2019, STR5.2019) (**Figure 5**). These individuals were smaller, with regard to total length and weight, when compared to Lake Huron spawners. Categorization of individuals from the group of unknowns as either Lake Huron or not from Lake Huron (i.e. potential 'upper-river' Sea Lamprey) based on morphological characteristics can be done to a certain degree. Sea Lamprey with unknown origins as a group, appear to align closely to spawners from Lake Huron. However, a few individuals fall below the 95% confidence intervals when analyzing total length and weight (e.g. PIR1.2017, PIR2.2017, STR5.2017) and two individuals (e.g. STR1.2013, STR1.2017) when only comparing weight (**Figure 6a**). Unknowns appear to be tightly grouped with spawning-phase Lake Huron Sea Lamprey when comparing condition factor (Wr), with all individuals falling within the confidence interval for Lake Huron spawners (**Figure 6b**).

Parasitic-phase Sea Lamprey

Morphology characteristics from 'upper-river' and Lake Huron Sea Lamprey were analyzed to determine if significant differences in size, possibly attributed to differences in potential hosts and abiotic conditions, exists between the two groups. Parasitic Sea Lamprey from Lake Huron (n = 153, mean = 325 mm, SD = 111 mm) and the 'upper-river' (n = 7, mean = 324 mm, SD = 111 mm) did not differ significantly in total length (T-statistic = 0.01, P = 0.99) when comparing individuals captured year-round. Parasitic-phase Sea Lamprey from Lake Huron (n = 153, mean = 91 g, SD = 81 g) and the 'upper-river' (n = 7, mean = 93, SD = 66 g) exhibited no significant differences in weight (g) (T-statistic = 0.07, P = 0.94) when comparing Sea Lamprey captured year-round. Similarly, parasitic Sea Lamprey from Lake Huron (n = 153, mean = 0.82, SD = 0.20) and the 'upper-river' (n = 7, mean = 0.83, SD = 0.25), captured year-round, did not differ significantly when examining condition factor (Wr) (T-statistic = 0.19, P = 0.86) (**Figure 7**).

Parasitic-phase Sea Lamprey from Lake Huron (n = 74) and the 'upper-river' (n = 5) did not differ significantly in total length (T-statistic = 1.29, P = 0.20), weight (T-statistic, = 1.45, P = 0.15) or condition factor (T-statistic = 1.45, P = 0.08) when comparing those captured in August or September (**Figure 8**).

Stable Isotope Analysis

Parasitic- vs. spawning-phase Sea Lamprey from Lake Huron

Isotope data were compared between parasitic- and spawning-phase Sea Lamprey captured from Lake Huron to determine if life stage influences δ^2H and δ^{18}O values of the four different tissue types. Results from heads are presented here (**Figure 9**), because data from heads are used for comparisons among Lake Huron Sea Lamprey, 'upper-river' Sea Lamprey, and lamprey of unknown origins. Results for hearts, livers and adipose tissue can be found in **Appendix A**. For δ^2H, heads from parasitic- and spawning-phase Lake Huron Sea Lamprey did not differ significantly (T-statistic = 0.65, P = 0.52). For δ^{18}O, heads from spawning-phase Sea Lamprey had higher mean δ^{18}O values than parasitic-phase Sea Lamprey (T-statistic = 3.78, $P <$ 0.001). Insignificant results for δ^2H indicate that heads collected from different stages in a Sea Lamprey's life can be used interchangeably, increasing sample size and therefore statistical power.

Lake Huron vs. 'Upper-river' Sea Lamprey

The above results demonstrate that deuterium values are not influenced by Sea Lamprey life stage, but δ^{18}O values are significantly different based on life cycle. As a result, δ^2H values from heads of parasitic- and spawning-phase Lake Huron Sea lamprey were combined to increase sample size and statistical power. Deuterium values from Lake Huron (parasitic- and spawning-phase) and 'upper-river' Sea Lamprey differed significantly (T-statistic = 4.33 $P <$ 0.001) with 'upper-river' Seal Lamprey exhibiting more negative δ^2H values (**Figure 10**). Due to significant differences in δ^{18}O values when comparing life cycle, δ^{18}O values from Lake Huron spawning- and parasitic-phase were not combined. Instead, a one-way ANOVA was conducted to determine the effect that origin and life cycle have on δ^{18}O values from Lake Huron spawners, Lake Huron parasites and 'upper-river' parasites. Oxygen isotopes differed significantly based on origin and life cycle (F2,45 = 9.40, $P <$ 0.001). Post hoc comparisons using the Tukey HSD test indicated that δ^{18}O values of heads from Lake Huron spawning-phase Sea Lamprey differed significantly from δ^{18}O values of 'upper-river' Sea Lamprey as well as δ^{18}O values from Lake Huron parasites, with 'upper-river' Sea Lamprey having lower δ^{18}O values (**Figure 10**).

Despite significant differences observed between means of δ^2H and δ^{18}O for Lake Huron and 'upper-river' Sea Lamprey, ranges of δ^{18}O values do overlap between the two groups. As a result δ^{18}O values may be an unreliable parameter when comparing Lake Huron and 'upper-river' Sea Lamprey. As an alternative, deuterium values of heads and whole body weight (g) from Lake Huron (parasitic- and spawning-phase) and 'upper-river' Sea Lamprey were plotted to determine if the relationship is significant (**Figure 11**). For Sea Lamprey originating from Lake Huron a significant relationship (y = -0.12x – 74.94; $P <$ 0.05, R^2 = 0.15) between deuterium values of heads and whole body weight (g) was observed; deuterium values of heads and whole body weight (g) from 'upper-river' Sea Lamprey also exhibited a significant relationship (y = -0.12x – 131.06; $P < 0.05$, $R^2 = 0.67$) (**Figure 11**). Because both relationships are significant, an ANCOVA was performed comparing the slopes and y-intercepts of each linear model. Slopes of the two linear models (**Figure 11**) were not significantly different (ANCOVA part1; F3,44 < 0.001, $P < 0.001$). However, significant differences in y-intercepts (ANCOVA part2; F2,45 = 30.472, $P < 0.001$) were observed, indicating that there may be significant differences in deuterium values of heads from Lake Huron and 'upper-river' Sea Lamprey. Heads from Lake Huron and 'upper-river' Sea Lamprey differed significantly (T-statistic = 2.01, $P < 0.05$) when comparing least-squares means of deuterium values, when adjusted for weight. 'Upper-river' Sea Lamprey head δ^2H values were more negative (-156.44) when compared to Lake Huron Sea Lamprey (-100.35).

Grouping Sea Lamprey of unknown origin

Isotopic composition of Sea Lamprey tissue can be highly variable (**see Appendix A**). Samples analyzed from Sea Lamprey with unknown origin were limited to heads (with the exception of adipose tissue from one individual (**see Appendix A**) due to low capture rates of these Sea Lamprey. As a result of these two constraints, heads were the only tissue type used when comparing Sea Lamprey of unknown origin to those from Lake Huron, and 'upper-river' Sea Lamprey. No statistical analyses were conducted due to the bi-modal distribution of these unknown lamprey (i.e., either from Lake Huron or the 'upper-river'). Instead isotopic composition of heads from Sea Lamprey with unknown origin were visually compared heads from 'upper-river' Sea Lamprey and Lake Huron Sea Lamprey, spawning- and parasitic-phase.

Comparison of 'upper-river' and Lake Huron Sea Lamprey isotopic composition show that δ^{18}O values overlap for the two groups and may not be a reliable tool to categorize 'unknowns' (**Figure 12**). These inferential statistics do illustrate that there is a relationship between size and δ^2H and that the difference δ^2H values from 'upper-river' and Lake Huron Sea Lamprey, when adjusted for size, are real (**Figure 11**). Using this knowledge, the relationship between δ^2H and weight was used to group Sea Lamprey with unknown origins (**Figure 13**). Only one individual, MR2.2015, falls within the 95% confidence ellipse associated with 'upper-river' Sea Lamprey. The remaining five individuals (PIR1.2017, MR2.2014, MR1.2014, MR1.2015 and PIR1.2015) fall within the 95% confidence ellipse associated with Lake Huron Sea Lamprey, but PIR1.2015 and MR1.2015 fall on the edge of both 95% confidence ellipses.

An additional tool which can be utilized to help categorize Sea Lamprey of unknown origin involves looking at when these individuals were captured during their spawning runs and when the lock on the Cheboygan River opened for the season (**Table 4**). Several unmarked Sea Lamprey were captured either before or on the day the lock was opened for the season (e.g. MR1.2013, PIR1.2015, PIR1.2017) or before the first marked Sea Lamprey from Lake Huron was captured (e.g. PIR1.2013, MR1.2014, MR1.2015, STR1.2017, STR1.2019, STR2.2019, STR3.2019). For lamprey which were captured before the lock was operational, two of the three individuals were classified as 'upper-river' Sea Lamprey either based on morphological characteristics (PIR1.2017) or stable isotope results (PIR1.2015), but not by both methods. Stable isotope results are not available for the third individual (MR1.2013), but morphological characteristics indicate that it was closely linked to Lake Huron spawning-phase Sea Lamprey (**Figure 5**). Unknowns captured in HBBS nets before arrival of the first marked Sea Lamprey from Lake Huron were categorized as 'upper-river' individuals based on morphological characteristics (STR1.2017) or stable isotope results (MR1.2015). MR1.2014, which arrived two days before the first marked Lake Huron Sea Lamprey, was classified as from Lake Huron based on morphological characteristics and stable isotope results, but when referencing the length-weight comparison (**Figure 5**) it is near the edge of the 95% confidence ellipses. Results from stable isotope analyses show that it is closely related to the mean isotopic composition of 'upper-river' Sea

Lamprey when examining δ^2H and $\delta^{18}O$ (**Figure 12**), but falls in both cases it was near or on the edge of the 95% confidence ellipses for length-weight and δ^2H-weight well within the 85% confidence ellipse associated with Lake Huron spawning-phase Sea Lamprey when looking at weight adjusted δ^2H values (**Figure 13**). Stable isotope results are not available for PIR1.2013, which arrived six days before the first marked Lake Huron Sea Lamprey was captured in HBBS nets.

Stable Isotope composition of potential host species

Deuterium (δ^2H) and oxygen ($\delta^{18}O$) values from potential host species of Sea Lamprey were compared to those of heads from the Lake Huron and 'upper-river' Sea Lamprey (**Figure 14**). These results provide further information on isotopic composition of organisms living in the two study systems. Initial assessment of **Figure 14** shows that $\delta^{18}O$ and δ^2H values of potential host species from the 'upper-river' are tightly clustered, with regard to means of 'upper-river' and Lake Huron Sea Lamprey.

Significant differences in δ^2H values were observed when comparing isotopic composition of Lake Huron Sea Lamprey to potential hosts from the 'upper-river' (T-statistic = 7.08, $P < 0.001$) (**Figure 14**). No significant differences were observed in δ^2H values when comparing 'upper-river' Sea Lamprey to potential hosts from the 'upper-river' (T-statistic = 1.06, $P = 0.30$).

Values of $\delta^{18}O$ were significantly different when comparing 'upper-river' Sea Lamprey to potential hosts from the 'upper-river' (T-statistic = 3.65, $P < 0.001$) and Lake Huron Sea Lamprey to potential hosts from the 'upper-river' (T-statistics = 2.97, $P = 0.004$) (**Figure 14**).

A stable isotope mixing model shows that 11.1% of δ^2H composition in 'upper-river' Sea Lamprey comes from water (i.e. Burt and Mullett lakes) while 88.9% comes from potential hosts from the 'upper-river' (**Table 5**). The model also shows that contributions to uncertainty in the δ^2H data for 'upper-river' Sea Lamprey is primarily (86.8%) due to variation in δ^2H values from potential 'upper-river' hosts (**Figure 14**)

Intra-group differences in isotopic composition of tissue types

Isotope data of the four tissue types were compared within three groups (i.e. Lake Huron spawners, Lake Huron parasites and 'upper-river' parasites) to determine if δ^2H and $\delta^{18}O$ values differ based on which organ/tissue is used for analysis (**see Appendix A**) . These results could be useful for future work in determining the best tissue type or types for stable isotope analysis.

Discussion

An examination of wounded fishes from Burt and Mullett lakes provided insight on inland lake Sea Lamprey feeding habits. Though Sea Lamprey are generalist feeders (Silva *et al.* 2014; Happel *et al.* 2017), they exhibit some preference towards large Salmonids (Coutant 1977; Swink 1993; Swink 2003). This 'preference' is linked to preferred thermal regimes of hosts and Sea Lamprey, as opposed to palatability. Paucity of preferred host species (e.g. Lake Trout, Coregonus spp.) in Burt and Mullett lakes may lead Sea Lamprey to feed on less desirable hosts such as Walleye, Northern Pike and one Yellow Perch in Mullett Lake (**Table 3**). In contrast, Lake Huron supports substantial populations of salmonids (Roseman *et al.* 2014) leading to Lake Huron Sea Lamprey parasitizing Lake Trout and other salmonids (Sitar *et al.* 1997). Laboratory experiments have shown that Sea Lamprey will feed on Walleye but that they are least preferred in the presence of more desirable host species (e.g. Splake (*Salvelinus fontinalis* x *Salvelinus namaycush)*, White Suckers, Lake Whitefish, and Burbot) (Farmer and Beamish 1973; Farmer *et al.* 1975, 1977). Sea Lamprey seldom attach and feed on Walleye in laboratory settings, instead they attack the Walleye and they detach quickly (Farmer and Beamish 1973).

While this study showed that 'upper-river' Sea Lamprey do feed on non-preferred hosts, Rainbow Trout from Mullett Lake exhibited the highest wounding rate (**Table 3**) of any species observed (excluding species with fewer than 10 observations). Rainbow Trout were one of the few salmonids observed during these angler surveys and may serve as a primary host in Burt and Mullett lakes, as they occupy deeper, colder waters during summer months. Cisco were also observed in Mullett Lake and all three sampled fish exhibited Sea Lamprey wounds (**Table 3**).

It appears that differences in fish community structure have led to different feeding strategies, when comparing 'upper-river' and Lake Huron Sea Lamprey. Kitchell and Breck's (1980) foraging hypothesis postulates that in situations of low prey density Sea Lamprey may 'choose' to act more as a predator than a parasite, feeding for extended periods of time on one individual host, leading to host mortality. If this is the case in Burt and Mullett lakes, it would be difficult to determine which fishes Sea Lamprey are feeding on as they may die and sink to the bottom. This process could result in lower wounding rates, as observed by fisheries managers. Black Bay, Lake Superior serves as another Great Lakes example where Sea Lamprey have adjusted to low densities of preferred hosts by feeding on less desirable hosts such as suckers (Catostomus spp.) (Harvey et al. 2008). Even though 'upper-river' Sea Lamprey are feeding on non-preferred hosts, these fishes constitute top predators in Burt and Mullett lakes and are economically important fishes. This is consistent with findings from across the Great Lakes basin in which Sea Lamprey derive their energy from top predators (Bence *et al.* 2003; Harvey *et al.* 2008).

Adaptation to low density of preferred hosts within the 'upper-river' can have physiological consequences for 'upper-river' Sea Lamprey. If 'upper-river' Sea Lamprey are feeding for prolonged periods on singular hosts, this could slow growth rates as nutritional quality of host blood declines rapidly after Sea Lamprey attachment (Farmer *et al.* 1975). Johnson *et al.* (2016) found that 'upper-river' Sea Lamprey were significantly smaller than those that feed in Lake Huron in terms of total length, as well as several other morphometrics. However, Johnson *et al.* (2016) classified 'upper-river' Sea Lamprey as unmarked spawning-phase Sea Lamprey. This study defines 'upper-river' Sea Lamprey as parasitic-phase Sea Lamprey known to feed in Burt and/or Mullett lakes. Using this definition, analyses showed there were no significant differences between the two populations with regard to total length, weight, or condition factor (**Figure 7**). This initial comparison utilizes Sea Lamprey caught at all times of year, however as outlined above, Sea Lamprey growth rates differ depending on season. Utilizing season of capture as a filter, no significant differences were found when comparing only Sea Lamprey captured in the fall (**Figure 8**).

Sea Lamprey feeding increases during autumn months, typically resulting in higher growth rates as potential hosts occupy warmer waters during the autumnal partial circulation (Ruttner 1963; Bergstedt and Swink 1995b). However, if Burt and Mullett lakes are cooling faster than Lake Huron, as Johnson *et al.* (2016) postulates, then 'upper-river' Sea Lamprey growth rates for autumn caught lamprey should be lower, resulting in smaller individuals. This does not appear to be the case in Burt and Mullett lakes. While no significant differences in size were found, 'upper-river' Sea Lamprey were, on average, larger than their Lake Huron counterparts, when comparing Sea Lamprey caught at the same time of year (e.g., August through September). Johnson *et al.* (2016) also hypothesized that availability and/or density of prey could influence Sea Lamprey size, resulting in smaller individuals from the 'upper-river'. However, Johnson *et al.* (2016) acknowledged that Burt and Mullett lakes are actually more productive than Lake Huron, potentially leading to higher prey density and/or availability. While ‘upper-river’ Sea Lamprey are feeding on less desirable hosts for prolonged periods of time, density of hosts may be higher in Burt and Mullett lakes, allowing ‘upper-river’ Sea Lamprey to keep pace with those from Lake Huron. However, Burt and Mullett lakes experience faster cooling in the fall, when compared to Lake Huron, potentially resulting in a quicker decline in growth rates for ‘upper-river’ Sea Lamprey leading to smaller spawning-phase ‘upper-river’ Sea Lamprey. Here, this theory is corroborated by significant differences in size when comparing Lake Huron spawning-phase Sea Lamprey to spawning-phase Sea Lamprey with unknown origins, with those from Lake Huron being larger. As previously stated, Sea Lamprey of unknown origins could either be from Lake Huron or Burt/Mullett lakes, so these results may not be representative of true population trends for ‘upper-river’ spawning-phase Sea Lamprey. Further investigation of size differences is needed to determine if there are significant differences between parasitic-phase Sea Lamprey from Burt and Mullett lakes and those which feed in Lake Huron, as only seven ‘upper-river’ individuals were analyzed, and therefore may not accurately represent the ‘upper-river’ population.

While no significant differences were found when comparing ‘upper-river’ and Lake Huron parasitic-phase Sea Lamprey, morphological characteristics of spawners in the upper Cheboygan watershed could be useful for managers attempting to categorize unknown spawners. Overall, comparison of morphological characteristics from Lake Huron spawners and spawners with unknown origins were inconclusive when lumping the unknowns as one group. However certain individuals with unknown origin appear to be more similar in size to Lake Huron spawners. **Figure 5** shows that 14 of the 24 unknown individuals have lengths and weights more similar to Lake Huron spawners than ‘upper-river’ parasites, leaving nine individuals (i.e., PIR1.2017, PIR2.2017, STR5.2017, STR1.2017, STR1.2013, PIR2.2013, PIR3.2018, PIR1.2019 and STR4.2019) that more closely align with ‘upper-river’ parasites. When looking at each metric individually, categorization of unknowns is less clear (**Figure 6**). Three unknowns (i.e., PIR1.2017, PIR2.2017, and STR5.2017) fall below the first quartile of total length for Lake Huron spawners while five (i.e., STR1.2013, PIR1.2017, PIR2.2017, STR1.2017 and STR5.2017) are below the third quartile for weight of Lake Huron spawners (**Figure 6a**). Based on these results, three unknowns (i.e., PIR1.2017, PIR2.2017, and STR5.2017) could be categorized as not from Lake Huron, and potentially from the ‘upper-river’ population. However, comparison of condition factor between the two populations shows that only one unknown, PIR2.2017, falls outside of the range for Lake Huron spawners. Definitive conclusions from these observations are difficult to make, but it does appear that several of the unknowns could potentially be from the ‘upper-river’ population. Analysis of stable isotope composition may be more useful when attempting to group Sea Lamprey with unknown origin as either from Lake Huron or from Burt/Mullett lakes.

Results of our stable isotope analyses of heads from these Sea Lamprey provide slightly different insights into origin than those provided by length and weights. As a group, ‘upper-river’ Sea Lamprey exhibited more negative δ^2H values and lower δ^{18}O values than Lake Huron parasites, with significant differences seen when comparing heads (**Figure 10**). Similarly, potential hosts form the ‘upper-river’ had significantly more negative δ^2H values than Sea Lamprey from Lake Huron (**Figure 14**). These two comparisons demonstrate that the organisms from Burt and Mullett lakes have significantly more negative δ^2H values than those which spend the majority of their lives in Lake Huron. Before attempting to group Sea Lamprey with unknown origins as either from the ‘upper-river’ or Lake Huron populations, it must be noted that when analyzing the four tissue types, isotopic composition of δ^2H and δ^{18}O differed within each source group based on tissue type, highlighting differences in isotope composition throughout an individual's body (**see Appendix A**). These differences could be attributed to rates of metabolic water production which exceed the rates of isotopic equilibration across cell membranes (Kreuzer-Martin *et al.* 2005; Vander Zanden *et al.* 2016).

Results show that mean isotopic values of δ^2H for parasitic-phase Sea Lamprey from Lake Huron were more negative when compared to spawning-phase Lake Huron lamprey, with significant differences in deuterium values for adipose tissue (**see Appendix A**). Average deuterium values increased by 44%, 18% and 5% for adipose, heart and head tissues, respectively, when comparing Lake Huron parasites to Lake Huron spawners. A similar trend is observed with regard to $\delta^{18}O$, with parasitic-phase lamprey from Lake Huron having lower average values than spawners from Lake Huron, and significant differences in $\delta^{18}O$ values for heads. Trends in $\delta^{18}O$ may be explained by differences in water temperature experienced by parasitic- and spawning-phase Lake Huron Sea Lamprey. Isotopic values for $\delta^{18}O$ are influenced by abiotic factures such as temperature, with lower values found in warmer waters and higher values in cooler waters (Newsome *et al.* 2007). For all tissue types, parasitic-phase Sea Lamprey from Lake Huron had lower $\delta^{18}O$ values than Lake Huron spawners. Water temperatures within the Maple, Pigeon, and Sturgeon rivers are influenced by groundwater, and could be colder than Lake Huron in the early fall, when many of the parasitic-phase Sea Lamprey from Lake Huron were captured. While spawners spend a relatively small amount of time in these tributaries, they cease feeding, resulting in the only inputs of δ^2H and $\delta^{18}O$ coming from the water they occupy. However, sample sizes for some of these tissue types were small (n = 6) so results here may not be representative of true population trends. Deuterium values of livers were the one exception, with more negative values for spawners. This can likely be attributed to physiological changes which occur as lamprey move upstream to spawn (Kott 1971; Beamish *et al.* 1979; Araújo *et al.* 2013; Happel *et al.* 2016).

These disparities between Lake Huron spawning- and parasitic-phase individuals shows that utilizing 'upper-river' parasites and Lake Huron spawners to categorize unknowns is imperfect. Utilization of $\delta^{18}O$ values for this comparison is not worthwhile due to significant differences observed between Lake Huron spawning- and parasitic-phase Sea Lamprey. Nevertheless, certain conclusions with regard to origin can be drawn when utilizing δ^2H and weight (g). Four of the six unknown Sea Lamprey (MR1.2014, MR2.2014, MR1.2015 and PIR1.2017) align more closely to the weight adjusted isotopic composition of Lake Huron spawners; one unknown (MR2.2015) fall within the 95% confidence interval for 'upper-river' Sea Lamprey, and one unknown (PIR1.2015) could be classified as either from Lake Huron or Burt/Mullett lakes (**Figure 13**). Predictions of origin based on weight adjusted δ^2H values do not align precisely with interpretations of length-weight comparisons. For example, unknown individual MR2.2015 falls clearly within the 95% confidence interval for Lake Huron spawners when looking at length-weight (**Figure 5**), but can be categorized as an 'upper-river' Sea Lamprey when using weight adjusted δ^2H values of heads (**Figure 13**). Conversely, unknown individual PIR1.2017 can be categorized as an 'upper-river' Sea Lamprey based on analysis of length, weight and condition factor (**Figure 5, Figure 6**) but aligns more closely with Sea Lamprey from Lake Huron when discussing stable isotopes (**Figure 13**).

It is difficult to know what the isotopic composition of 'upper-river' spawners would look like, but it can be assumed that similar trends observed when comparing Lake Huron spawners to Lake Huron parasites could exist within the 'upper-river' population. If this is the case, isotopic values of δ^2H and $\delta^{18}O$ for 'upper-river' spawners would increase in value. Similarly, weights of parasitic-phase Sea Lamprey from

Lake Huron were lower than those from spawning-phase Sea Lamprey from Lake Huron; this trend may also exist in the 'upper-river' population.

Analyses of morphological characteristics and stable isotopes allows for the categorization of unknowns as either 'upper-river' or Lake Huron Sea Lamprey to an extent. Another tool that can be used to validate these results is date of capture (**Table 4**). Three unknowns (MR1.2013, PIR1.2015, PIR1.2017) were captured either the day the lock was opened or before the lock was operational. Assuming the Cheboygan River lock and dam system is impassable for Sea Lamprey before seasonal operation, these three Sea Lamprey likely belong to the 'upper-river' population. Results from analysis of morphological characteristics indicate that MR1.2013 and PIR1.2015 are Lake Huron lampreys while PIR1.2017 is from the 'upper-river'. Stable isotope results were only available for PIR1.2015 and PIR1.2017. Deuterium and $\delta^{18}O$ values indicated that PIR1.2015 was an 'upper-river' lamprey while analysis of deuterium and weight (g) showed that PIR1.2015 fell right on the edge of the 95% confidence ellipses associated with Lake Huron spawners and 'upper-river' Sea Lamprey, while both methods of stable isotope classification indicated that PIR1.2017 originated from Lake Huron Lamprey (**Figure 13**). Seven other Sea Lamprey (PIR1.2013, MR1.2014, MR1.2015, STR1.2017, STR1.2019, STR2.2019, STR3.2019) were captured before the first marked spawning-phase Sea Lamprey from Lake Huron captured in HBBS nets, indicating they may have originated in Burt or Mullett lakes. Analysis of morphological characteristics categorized three of these individuals as 'upper-river' Sea Lamprey: STR1.2017, STR4.2019, STR5.2019. Stable isotope results were only available for two of these early arrivers (MR1.2014, MR1.2015). Analysis of $\delta^{2}H$ vs. $\delta^{18}O$ grouped both MR1.2014 and MR1.2015 as potential 'upper-river' lampreys as they both had isotopic values close to mean isotopic values of 'upper-river' Sea Lamprey (**Figure 12**). However, deuterium vs. weight analysis grouped MR1.2014 and MR1.2015 as originating from Lake Huron, with the caveat that MR1.2015 is near the edge of the 'upper-river' 95% confidence ellipses (**Figure 13**).

However, the utilization of date of capture as a means to compare stable isotope and morphological characteristics analyses relies on two assumptions being met: (1) Sea Lamprey can only bypass the lock and dam system via escapement through the lock and (2) spawning-phase Sea Lamprey captured in 'upper-river' tributaries moved into the 'upper-river' as mature adults. While passage above the Cheboygan River lock and dam system most likely occurs while the lock is operational, Sea Lamprey could potentially move into the 'upper-river' during certain operating conditions, when flow through the spillway is suitable for upstream migration. While this scenario is possible, it has not been documented and Holbrook (2015) determined that the lock provided the most plausible route for upstream migration. Assumption two assumes that unmarked individuals captured in HBBS nets reached sexual maturity before escaping past the lock and dam system and continued directly to spawning grounds. It is possible that juveniles from Lake Huron could have moved into the 'upper-river' and then matured there, meaning their date of capture is inaccurate. This scenario though seems unlikely as adult Sea Lamprey migration is cued by the detection of pheromones released by larvae, and other sexually mature adults, and juvenile Sea Lamprey would not respond to this (Teeter 1980).

Utilizing date of capture as a means to validate the two methods tested for distinguish populations is valuable, but illustrates the high level of uncertainty associated with these data. Of the unknowns which arrived before the first lock opening and were analyzed via morphological characteristics and stable isotopes, two of them (PIR1.2015, MR1.2015) were categorized as possible non-Lake Huron spawners by stable isotopes while only one (PIR1.2017) was categorized as a spawning-phase Sea Lamprey from Lake Huron, based on both methods of stable isotope analyses. These results are far from conclusive, but show that a combination of stable isotopes and size may be more reliable method for distinguishing Sea Lamprey which share spawning grounds in the upper Cheboygan River watershed, but larger sample sizes are needed to validate these preliminary findings.

Moving forward, results from this study could assist fisheries managers in their attempts to eradicate Sea Lamprey from the upper Cheboygan River watershed. Knowledge of a Sea Lamprey population in the upper Cheboygan River has existed since Applegate's (1950) seminal work on Sea Lamprey life history in northern Michigan. Johnson *et al.* (2016) provided further evidence of distinct populations above and below the Cheboygan River lock and dam through analysis of wounded fishes, morphological characteristics and statolith microchemistry. However, Johnson *et al.* (2016) did not provide a method for distinguishing individuals from these two populations when they are most readily captured: during their spawning migration. With ongoing efforts to eradicate Sea Lamprey from the upper Cheboygan River watershed, ability to construct accurate population estimates will become more important. By utilizing weight-adjusted δ^2H values for captured Sea Lamprey, fisheries managers could determine the efficacy of their population control efforts. If eradication efforts are successful, and can be monitored and evaluated through population estimates derived from stable isotope and weight data, removal of many of the dams within the Cheboygan River watershed will benefit native fishes, without increasing the length of stream that requires treatment with lampricide (Johnson *et al.* 2016).

Conclusions

To further understand the feeding ecology of Sea Lamprey in Burt and Mullett lakes, fall angler surveys should be conducted during peak Sea Lamprey feeding (Kitchell and Breck 1980). Angler surveys may not be the most reliable approach as sport fish are targeted, leaving out potential preferred host (e.g. Cisco, Lake Sturgeon, Rainbow Trout, Catostomidae) of Sea Lamprey in Burt and Mullett lakes. To better understand the feeding ecology of Sea Lamprey in these inland lakes a more exhaustive study focused on a full assessment of Burt and Mullett lakes' fish communities', with an emphasis on Sea Lamprey wounds should be conducted. Fatty acid analysis of 'upper-river' Sea Lamprey tissue and potential hosts would also provide more quantitative insight into Sea Lamprey feeding ecology in Burt and Mullett lakes (e.g. Budge *et al.* 2006; Daly *et al.* 2010; Happel *et al.* 2015). A study of this nature could pinpoint which fishes are acting as the preferred hosts of Sea Lamprey in Burt and Mullett lakes. A thermal history of preferred hosts could then be constructed and bioenergetics models (Kitchell and Breck 1980) could be applied to 'upper-river' Sea Lamprey to better understand factors influencing their growth.

The ability to categorize unmarked spawning-phase Sea Lamprey moving into the upper Cheboygan watershed proved difficult. Definitive conclusions were limited by small sample sizes, high intra-group isotopic value variability (**see Appendix A**), and imperfect comparison groups. Nevertheless, distinct differences between the δ^2H values of heads of Sea Lamprey known to feed in Lake Huron and those which feed in Burt and Mullett lakes were observed (**Figure 10**). For this study heads were utilized out of necessity, but may not be the most appropriate tissue to use for analysis. Results showed that isotopic values of hearts and livers from parasitic- and spawning-phase Lake Huron Sea Lamprey did not differ significantly (**see Appendix A**). This indicates that either hearts or livers may be more suitable for building defined isotopic signatures for 'upper-river' and Lake Huron Sea Lamprey as comparison of parasitic- and spawning-phase Sea Lamprey can be conducted. Nevertheless, these results can assist fisheries managers in developing a defined isotopic signature range for 'upper-river' Sea Lamprey, and assist with population estimates utilized for Sea Lamprey control. While removal of Sea Lamprey heads in the field may serve as an efficient method for collecting tissue for biochemical analysis, the continued collection of a variety of organs for analysis is recommended, or analysis of the entire organism; stored at -20˚C until they are ready for analysis or at -80˚C if samples need to be stored for an extended period of time (Harvey and Kitchell 2000). Future studies could also incorporate other stable isotopes (e.g. C, N, S) as well as fatty acids to further define biochemical markers of 'upper-river' Sea Lamprey as well as organisms which live in Burt/Mullett lakes and Lake Huron. More refined biochemical signatures for these two systems will allow for more confidence in assigning unknowns as either from the 'upper-river' or from Lake Huron.

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Figures

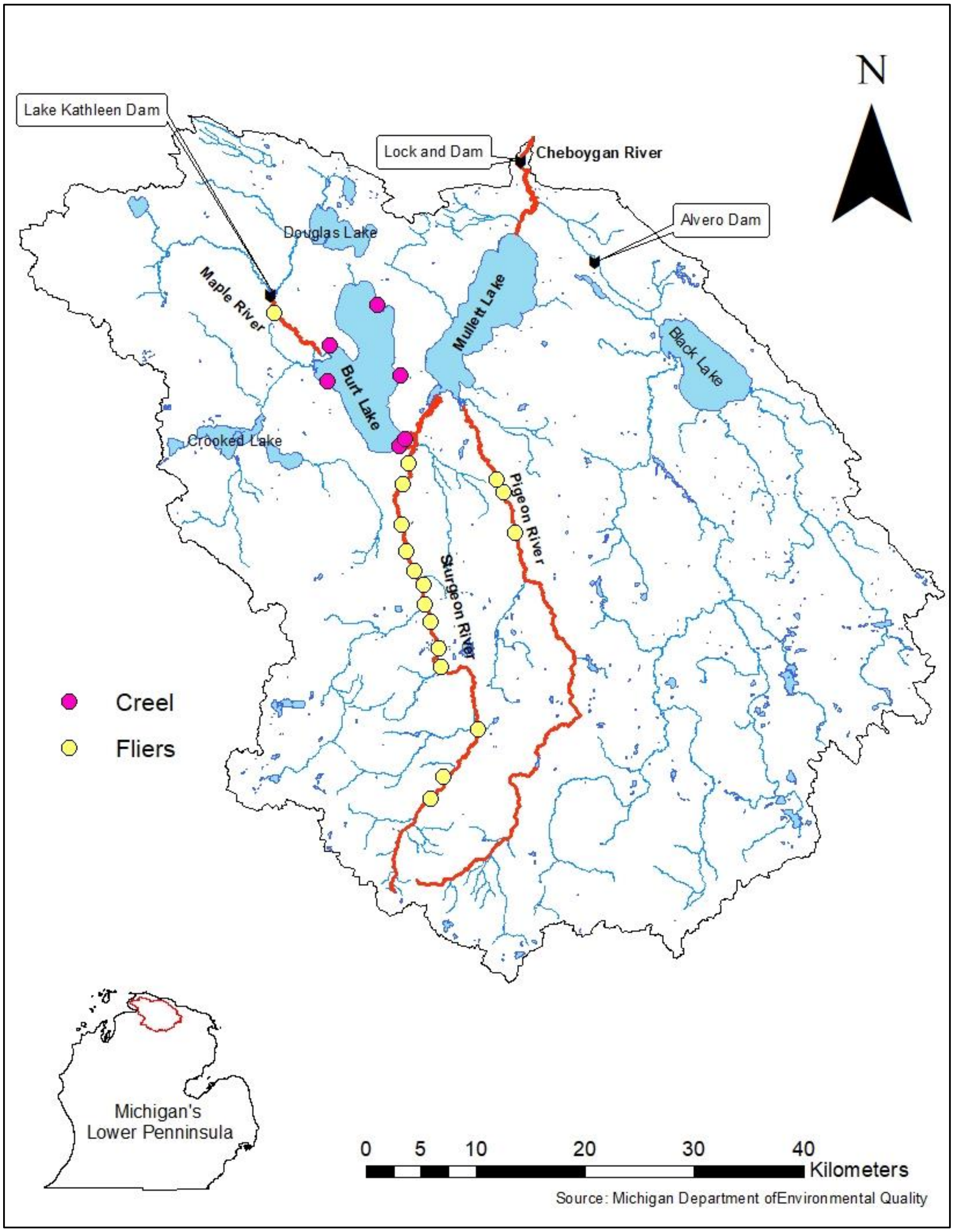
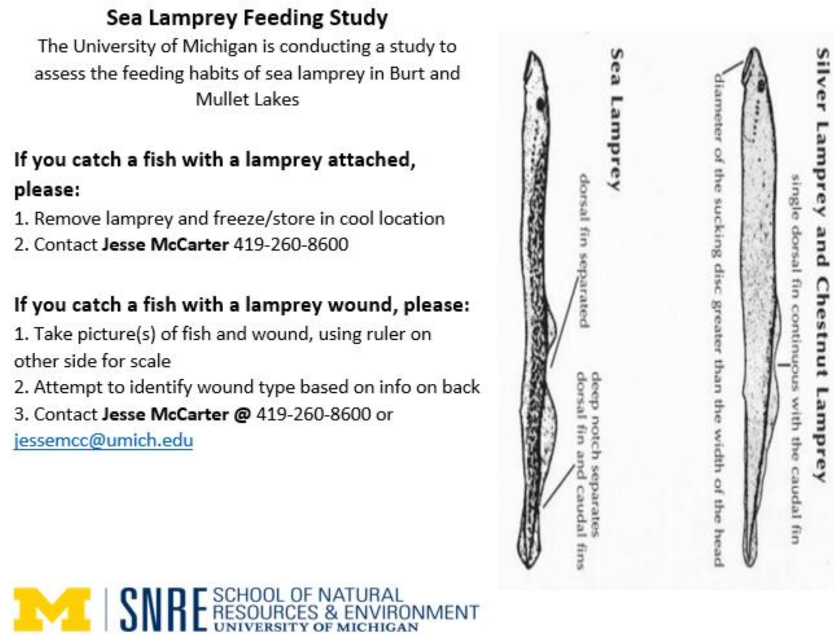


Figure 1. Cheboygan River watershed located in Northern Michigan's lower peninsula. All major lakes, rivers and dams within the Cheboygan River watershed are labeled. The Lake Kathleen dam has been removed as of fall 2018. Stream segments in red indicate areas traditionally infested with larval Sea Lamprey. Locations of creel/angler surveys to examine recreationally caught fish for lamprey wounds, as well as locations of fliers posted to prompt 'self-reporting' of wounded fish are denoted by colored circles.

a)



b)

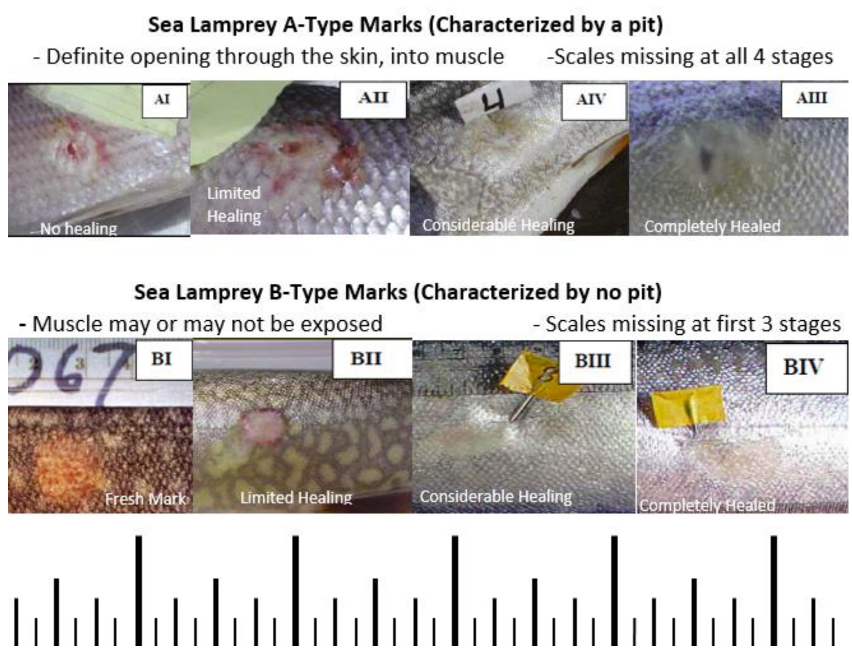


Figure 2: Handouts distributed to anglers or left on vehicles prompting anglers to self-report any potential lamprey wounds. The front (a) of the handout provides directions on how to report either a fish with a lamprey attached or a fish with suspected lamprey wounds. The back (b) provides examples of the various classifications of Sea Lamprey wounds according to King (1980).

ANGLERS!

YOUR COOPERATION IS REQUESTED!

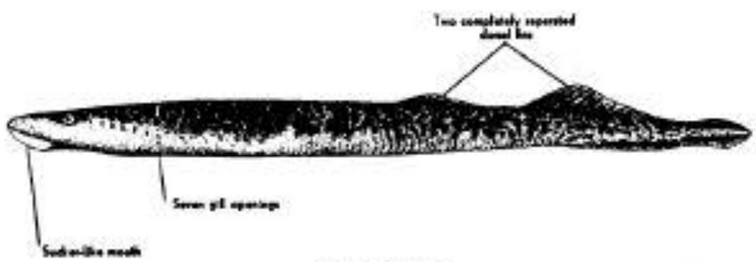
The University of Michigan is conducting a study to assess the feeding habits of sea lamprey in Burt and Mullet Lakes, and the Maple, Pigeon and Sturgeon Rivers. We are requesting anglers to report any lamprey or lamprey wounds found on captured fish.

If you catch a fish with a sea lamprey attached, please:

1) Remove sea lamprey and freeze/store lamprey in cool location
2) Contact Jesse McCarter (419-260-8600 or jessemcc@umich.edu)

If you catch a fish with sea lamprey wounds, please:

1) Take picture(s) of fish and wound, with something for scale
2) Contact Jesse McCarter (419-260-8600 or jessemcc@umich.edu)



SEA LAMPREY

Adult sea lampreys usually will be more than a foot long. They usually appear mottled with brown and black on the backs and they may have a somewhat golden tint.

Photo credits: Applegate, 1950 (left); M.Gaden/GLFC (top); Ted Treska/USFWS (bottom)

SNRE SCHOOL OF NATURAL RESOURCES & ENVIRONMENT UNIVERSITY OF MICHIGAN

Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu	Jesse McCarter 419.260.8600 jessemcc@umich.edu

Figure 3: Informational fliers were posted at various fishing locations/access points to prompt anglers to self-report any potential lamprey wounds. Directions on what to do if a lamprey is found attached to a fish or if potential wounds were observed were present. The investigators contact information was also provided in convenient tear-off tabs.

Figure 4: Example of Sea Lamprey (*Petromyzon marinus*) wound found on Walleye (*Sander vitreus*).

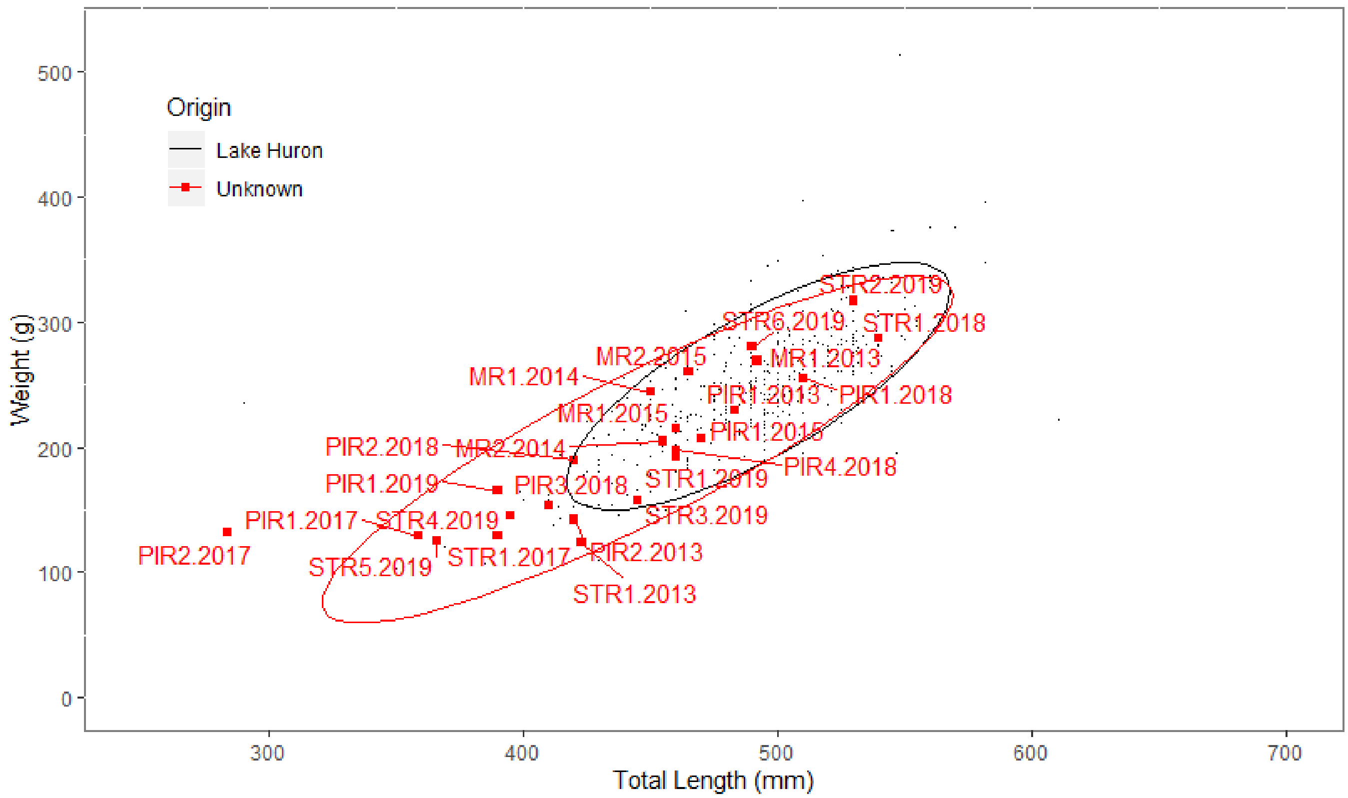


Figure 5: Total lengths (mm) and weights (g) of spawning-phase Sea Lamprey from Lake Huron and Sea Lamprey of unknown origins. Normal confidence ellipses (95%) are overlaid on top of scatter plot. Individuals of unknown origin are labeled using a code for the stream they were captured in (e.g. Maple River = MR; Pigeon River = PIR; Sturgeon River = STR); number indicating sequence of capture in a given year and the year they were captured.

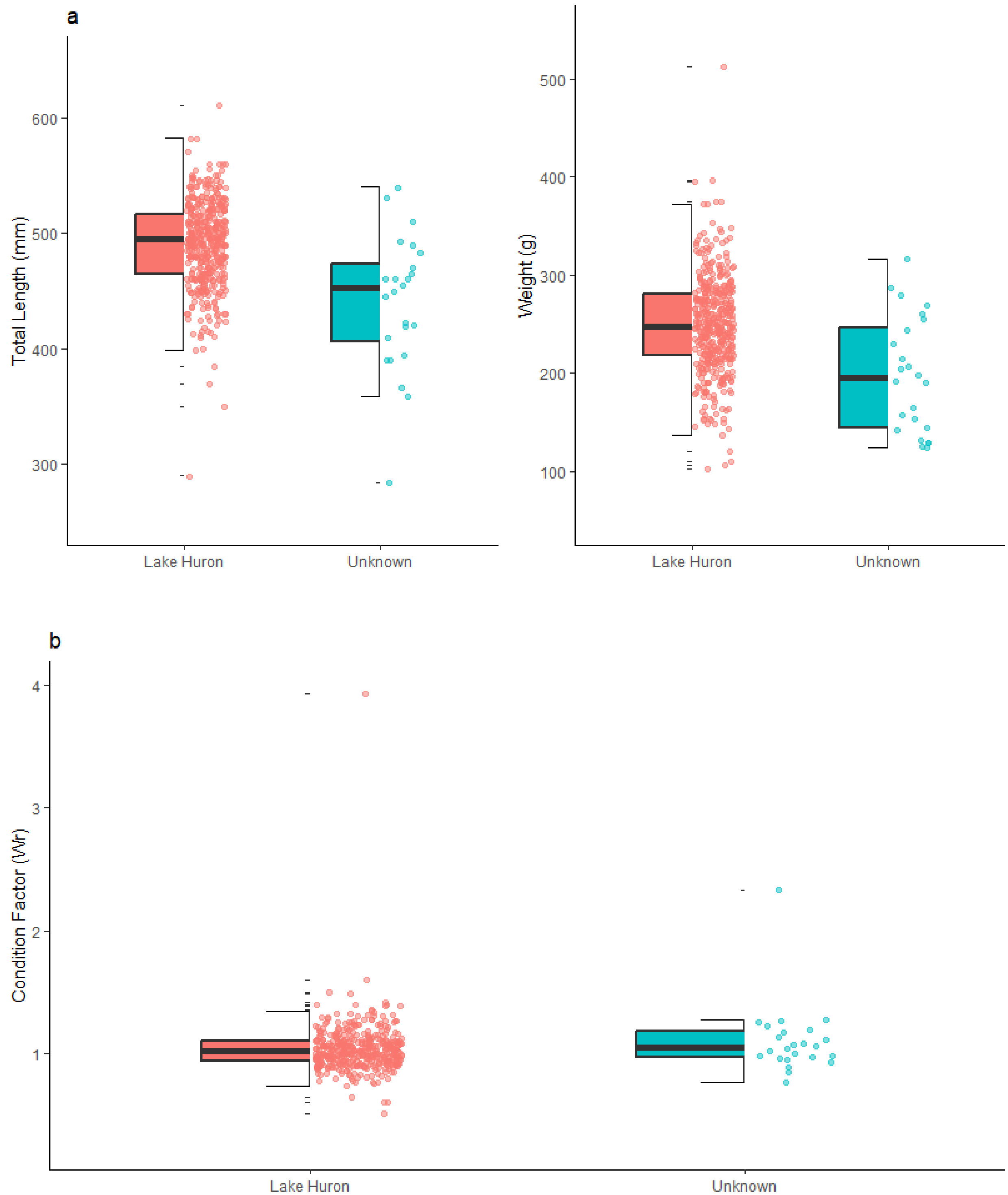


Figure 6: (a) Box and whisker plots comparing total length (mm) and weights (g) of spawning-phase Sea Lamprey from Lake Huron (n = 431; mean TL = 491 mm, SD = 37 mm; mean Wt = 249 g, SD = 49 g) and Sea Lamprey with unknown origins (n = 24; mean TL = 438 mm, SD = 59 mm; mean Wt = 194 g, SD = 58 g). (b) Condition factor (Wr) of spawning-phase Lake Huron Sea Lamprey (n = 431, mean = 1.04, SD = 0.19) and Sea Lamprey with unknown origins (n = 24, mean = 1.11, SD = 0.30).

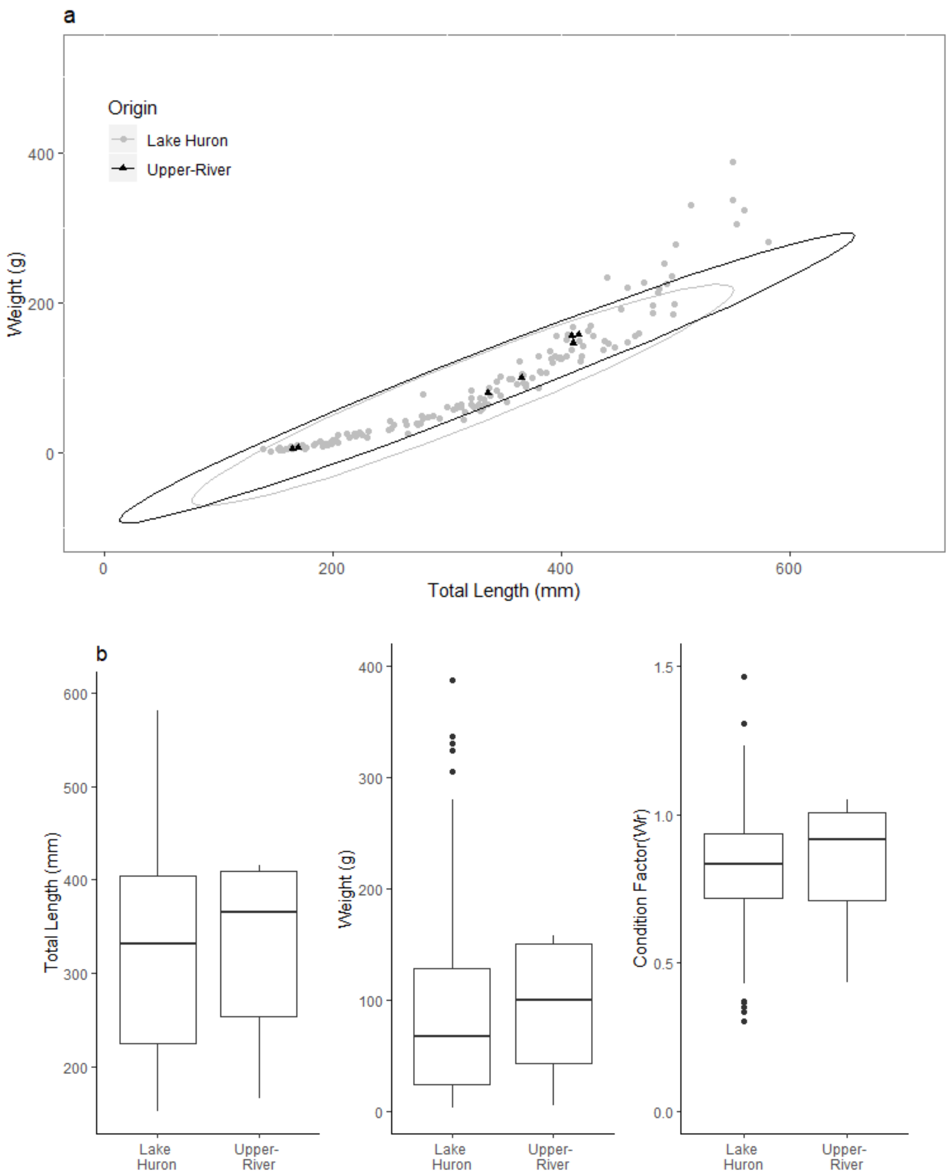


Figure 7: a) Total lengths (mm) and weights (g) of parasitic-phase Sea-Lamprey from Lake Huron and the upper Cheboygan watershed, collected any time of year. Normal confidence ellipses (95%) are overlaid on top of scatter plot. b) No significant differences (T-statistic = 0.01, *P* = 0.99) were observed when comparing total length (mm) of parasitic-phase Sea Lamprey known to have originated in Lake Huron (n = 153; mean = 325 mm; SD = 111 mm) and those known to feed in Burt and Mullett lakes (n = 7; mean = 324 mm; SD = 111 mm). No significant differences were observed when comparing weights (g) (T-statistic = 0.07, *P* = 0.94) of Sea Lamprey from Lake Huron (mean = 91 g, SD = 81) to Sea Lamprey from Burt and Mullett lakes (mean = 93 g, SD = 66) or condition factor (Wr) (T-statistic = 0.19, *P* = 0.86) with Sea Lamprey from Lake Huron having lower condition factors (mean = 0.82, SD = 0.20) than 'upper-river' Sea Lamprey (mean = 0.83, SD = 0.25).

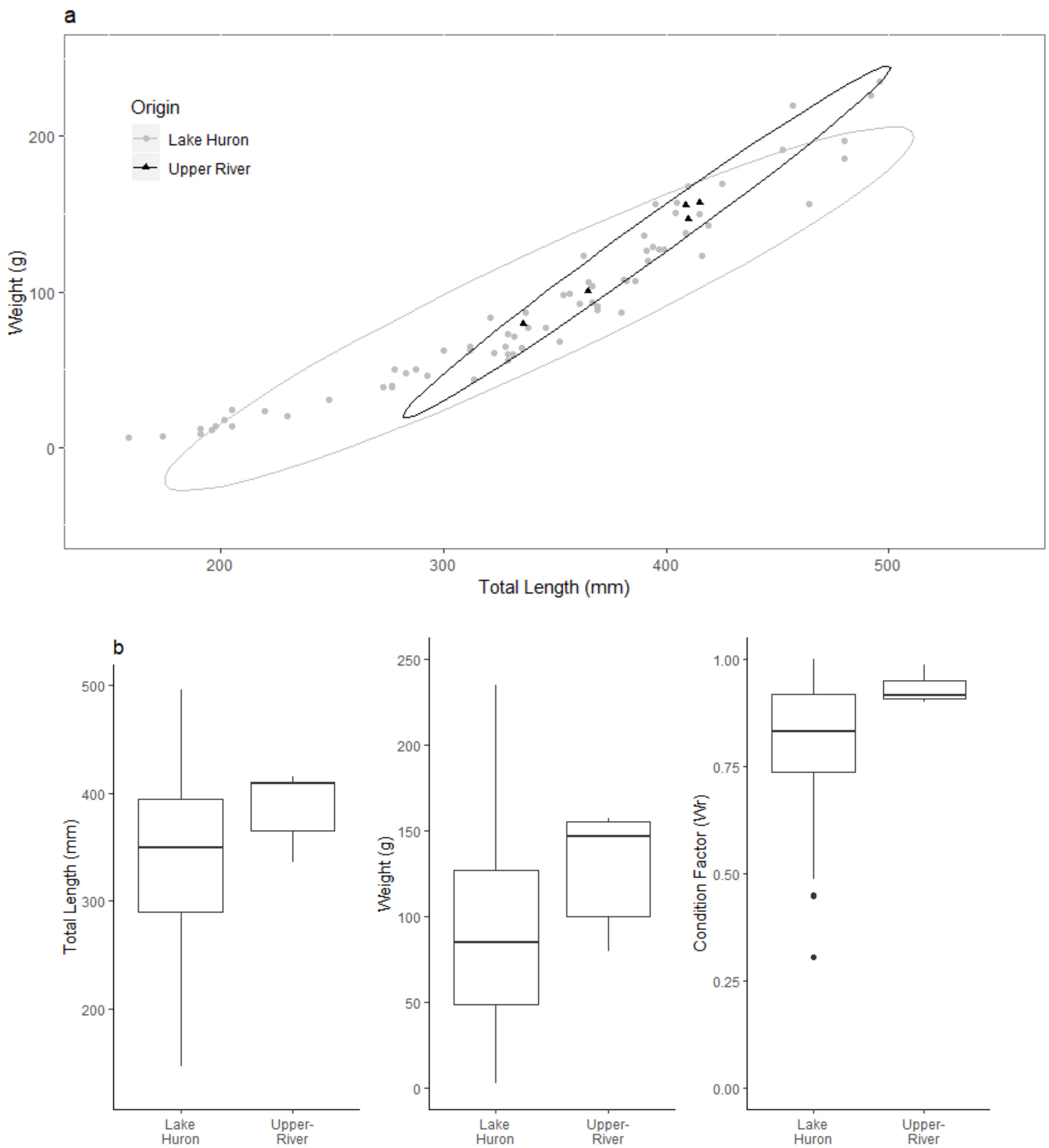


Figure 8: (a) Total lengths (mm) and weights (g) of parasitic-phase Sea-Lamprey from Lake Huron and the Burt and Mullett lakes ('upper-river') collected during the months of August and September. Normal confidence ellipses (95%) are overlaid on top of scatter plot. b) No significant differences (T-statistic = 1.29, *P* = 0.20) were observed when comparing total length (mm) of parasitic-phase Sea Lamprey, captured in August and September and known to have originated in Lake Huron (n = 74; mean = 338 mm; SD = 84 mm), and those known to have fed in Burt and Mullett lakes (n = 5; mean = 387 mm; SD = 35 mm); or when comparing weights (g) (T-statistic = 1.45, *P* = 0.15) of Sea Lamprey from Lake Huron (mean = 90 g, SD = 57) to 'upper-river' Sea Lamprey (mean = 123 g, SD = 36) or condition factor (Wr) (T-statistic = 1.80, *P* = 0.08) with Sea Lamprey from Lake Huron having lower condition factors (mean = 0.08, SD = 0.17) than 'upper-river' Sea Lamprey (mean = 0.98, SD = 0.07*).*

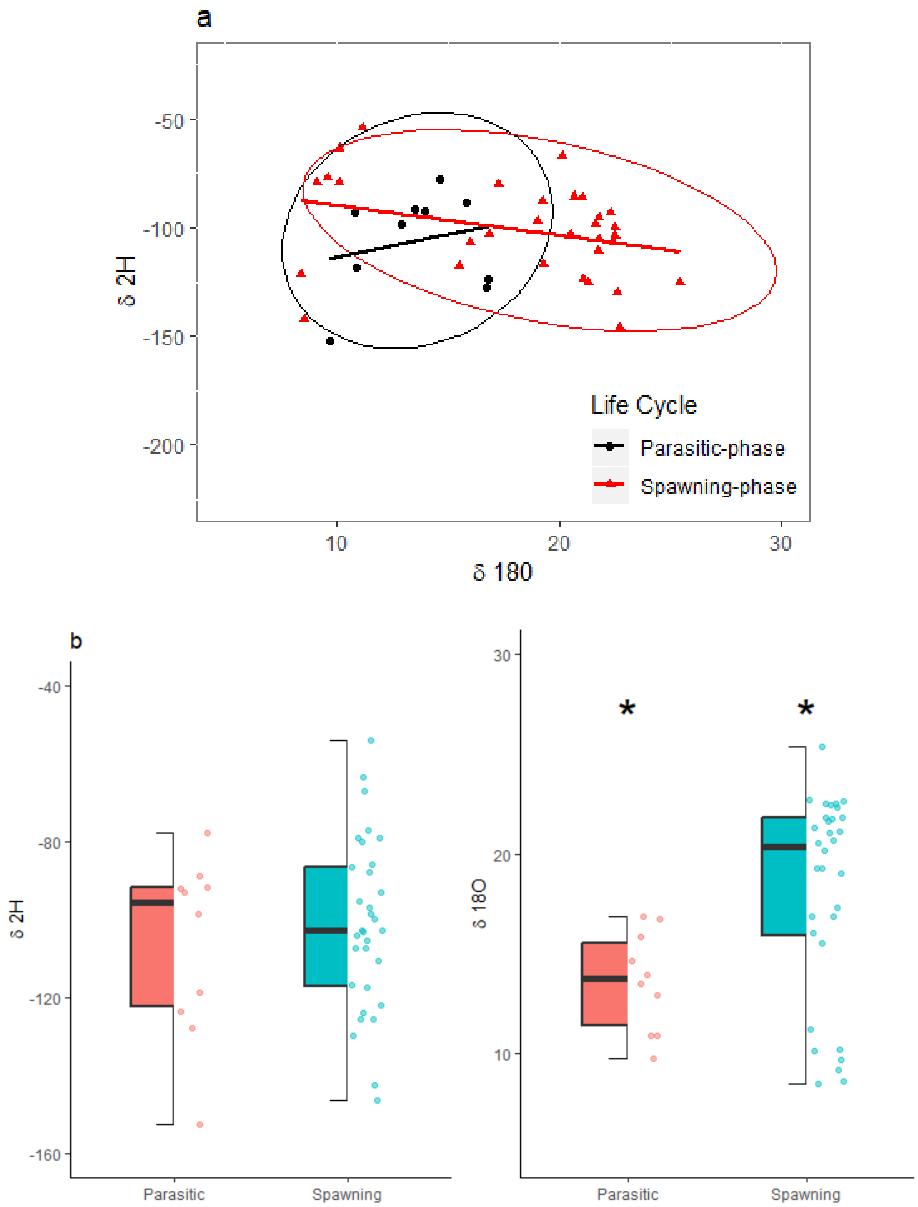


Figure 9: (a) Isotopic values of deuterium (δ^2H) and oxygen ($\delta^{18}O$) for heads of parasitic- and spawning-phase (n = 10, n = 32, respectively) Sea Lamprey known to feed in Lake Huron. Normal confidence ellipses (95%) are overlaid on scatter plot. (b) Box and whisker plots of deuterium (δ^2H) and oxygen ($\delta^{18}O$) stable isotopes for heads of parasitic- and spawning-phase Sea Lamprey from Lake Huron. No significant differences (T-statistic = 0.65, $P = 0.52$) in δ^2H values were observed between spawning-phase (mean = -101.13, SD = 21.93) and parasitic-phase (mean = -106.38, SD = 23.07) Sea Lamprey from Lake Huron. Significant differences (T-statistic = 3.78, $P < 0.001$) in $\delta^{18}O$ values were observed, with parasitic-phase Sea Lamprey exhibiting lower values (mean = 13.60, SD = 2.51) than spawning-phase Sea Lamprey (mean = 18.12, SD = 5.07).

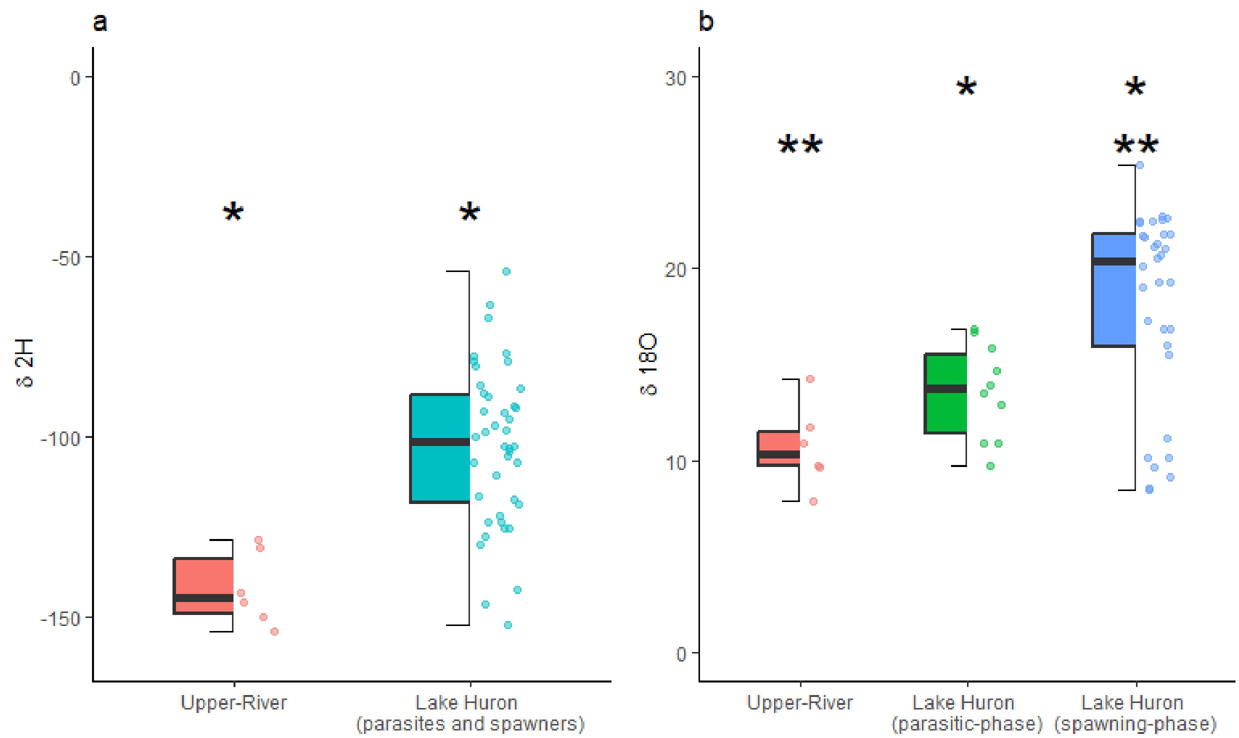


Figure 10: Isotopic values of deuterium (δ^2H) (a) and oxygen ($\delta^{18}O$) (b) for heads of parasitic-phase Sea Lamprey known to feed in Burt and/or Mullett lakes ('upper-river') and parasitic- and spawning-phase Sea Lamprey from Lake Huron (n = 6, n = 42, respectively). 'Upper-river' Sea Lamprey exhibited significantly lower δ^2H values when compared to Lake Huron parasitic- and spawning-phase Sea Lamprey (T-statistic = 4.33, $P < 0.001$). Values for $\delta^{18}O$ from Sea Lamprey heads differed significantly when comparing across groups ($F_{2,45} = 9.40$, $P < 0.001$).

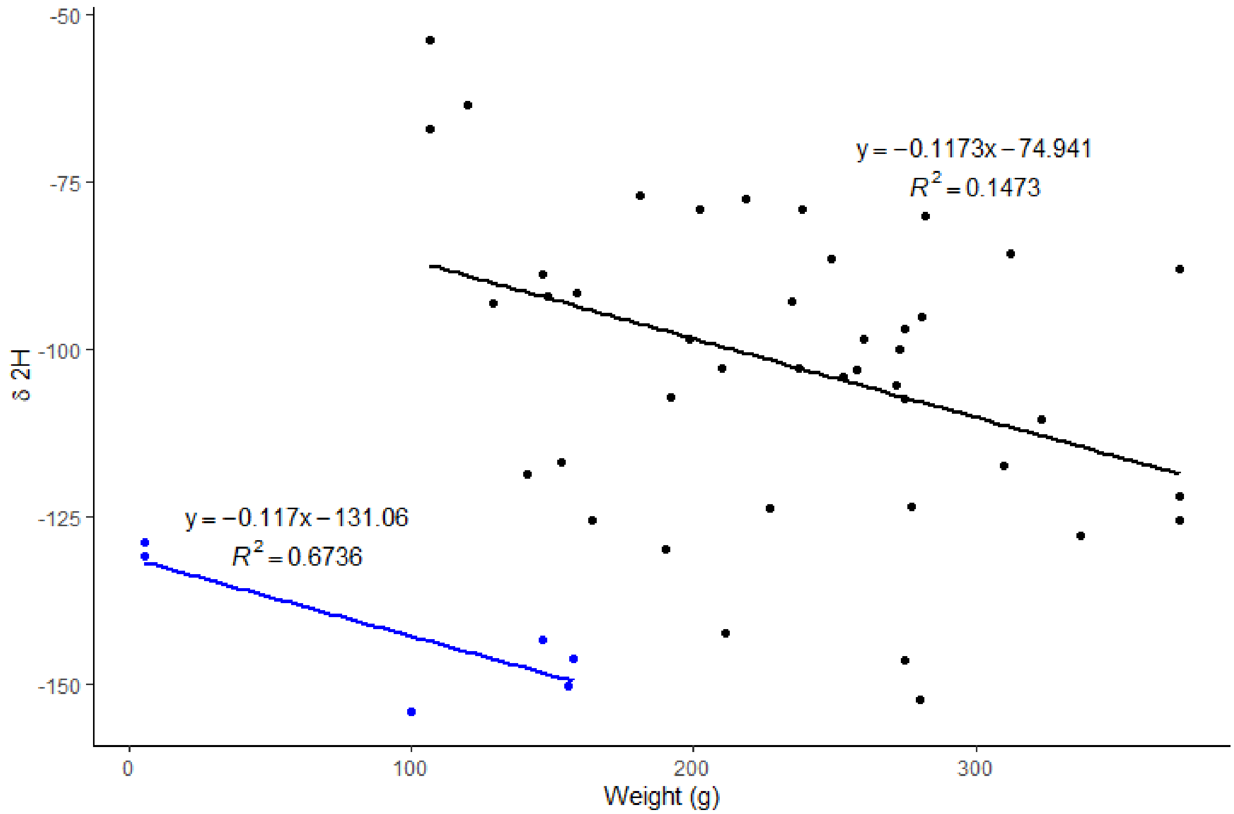


Figure 11: Deuterium (δ2H) versus mass of whole body (weight (g)) for parasitic-phase Sea Lamprey from Burt and/or Mullett lakes (blue circles), and parasitic- and spawning-phase Sea Lamprey known to originate from Lake Huron (black circles).

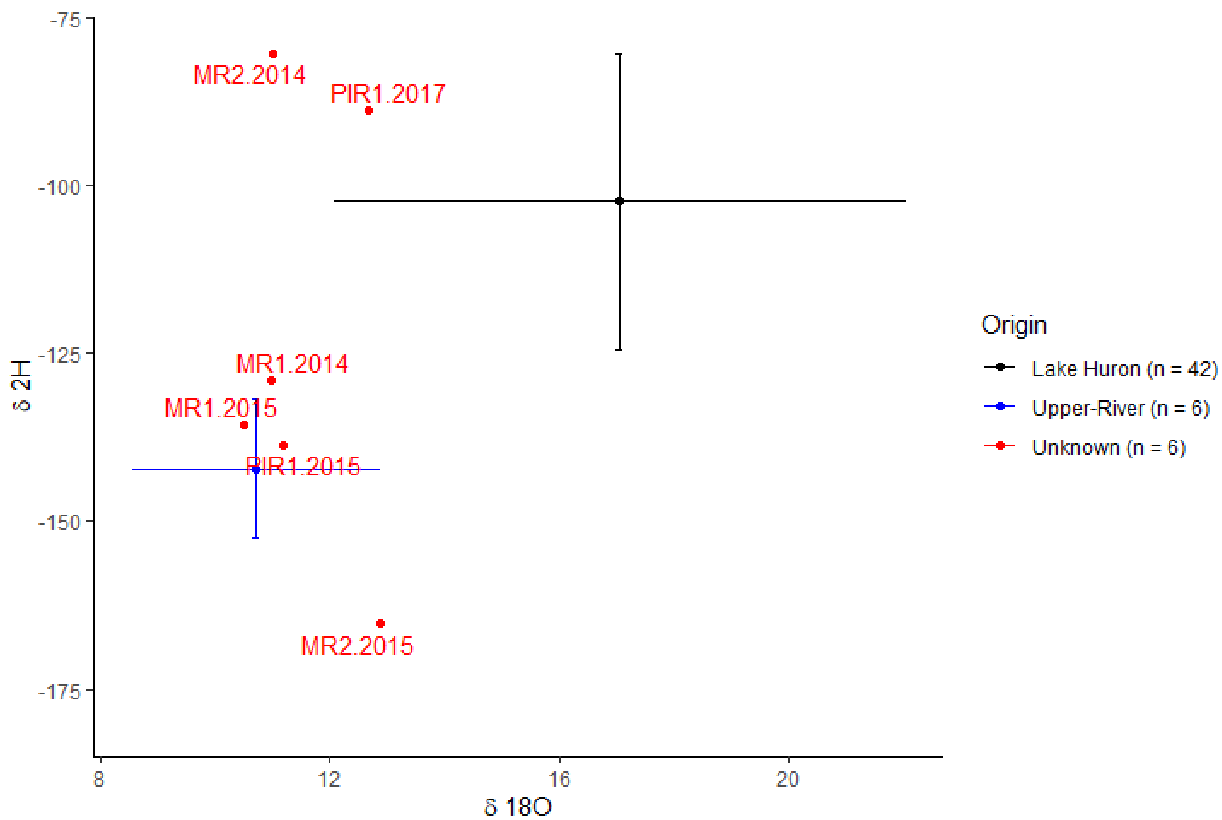


Figure 12: Mean deuterium (δ^2H) vs $\delta^{18}O$ values for heads of Sea Lamprey from Lake Huron (parasitic- and spawning-phase), Burt and Mullett lakes('upper-river') and those of unknown origins with error bars of 1 S.D. Individuals of unknown origin are labeled using a code for the stream they were captured in (e.g. Maple River = MR; Pigeon River = PIR; Sturgeon River = STR); number indicating sequence of capture in a given year and the year they were captured.

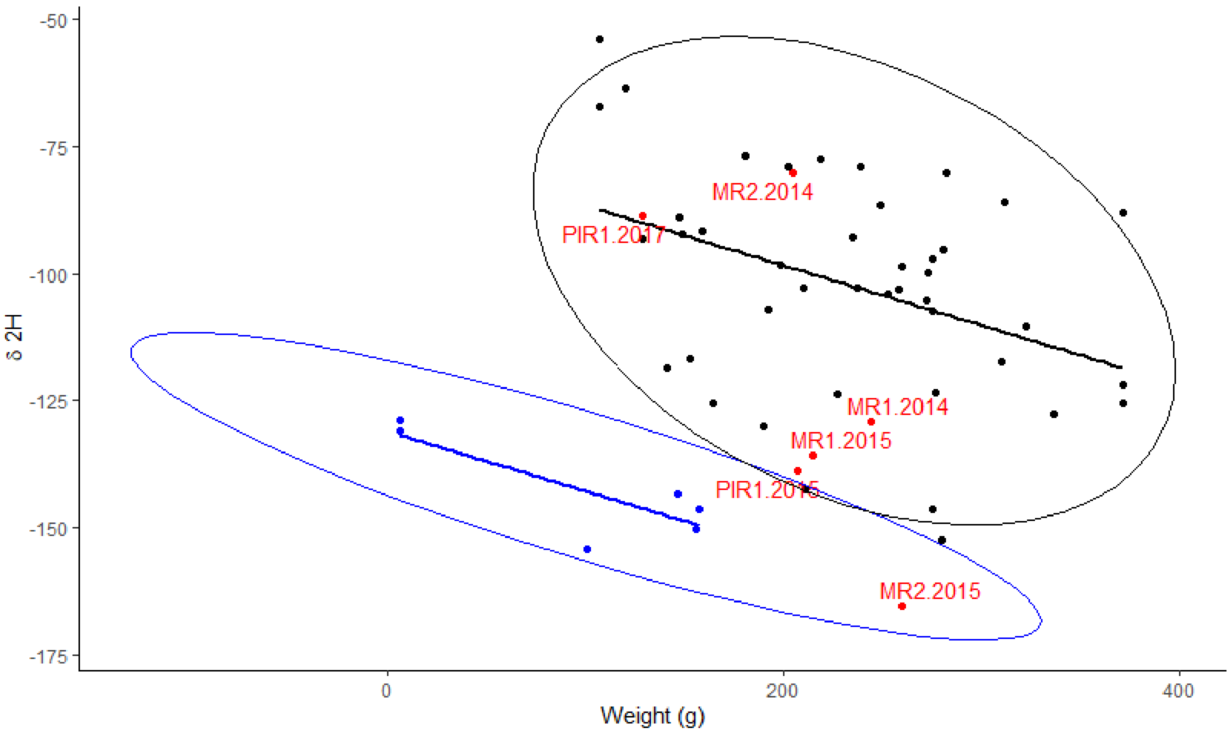


Figure 13: Deuterium (δ^2H) versus mass of whole body (weight (g)) for parasitic-phase Sea Lamprey from Burt and/or Mullett lakes (blue circles), parasitic- and spawning-phase Sea Lamprey known to originate from Lake Huron (black circles) and Sea Lamprey of unknown origins (red circles). Individuals of unknown origin are labeled using a code for the stream they were captured in (e.g. Maple River = MR; Pigeon River = PIR; Sturgeon River = STR), number indicating sequence of capture in a given year, and the year they were captured and normal confidence intervals (95%) are overlaid on scatter plot.

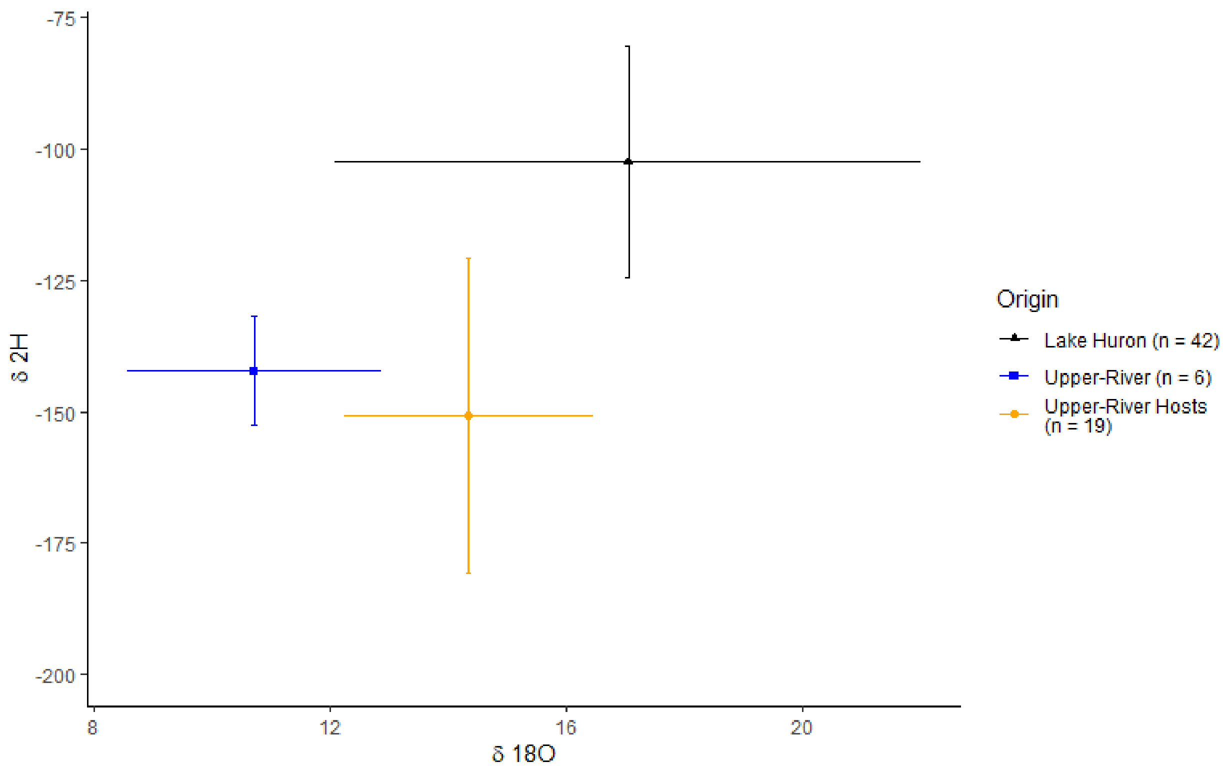


Figure 14: Mean δ^2H and $\delta^{18}O$ values and 1 S.D. error bars for Lake Huron Sea Lamprey (parasitic- and spawning-phase) (black), parasitic-phase Sea Lamprey from Burt and/or Mullett lakes (blue) and potential host species from Burt and Mullett Lakes (orange).

.

Tables

Table 1: A total of 494 Sea Lamprey specimens, collected from 2013 to 2019, were analyzed for differences in total length (mm), weight (g) and condition factor. Parasitic juvenile Sea Lamprey accounted for 160 of the specimens analyzed, with 153 having originated from Lake Huron and seven from the upper Cheboygan River watershed (i.e., Burt and Mullett lakes). The remaining 454 Sea Lamprey were spawning adults captured in the upper Cheboygan River watershed. These spawning adults either originated from Lake Huron (n = 431) or have unknown origins (n = 24).

	Origin			
Life Stage	**Lake Huron**	**Upper Cheboygan River**	**Unknown**	**Total**
Parasitic juvenile	153	7	-	160
Spawning adult	431	-	24	455
Total	**584**	**7**	**24**	**615**

Table 2: A total of 19 potential Sea Lamprey host species were collected from Burt and Mullett lakes for stable isotope analysis during the summer of 2016. One fish (Lake Herring) was collected during the winter of 2017 and included in this analysis. USGS Hammond Bay Biological Station (HBBS) also provided potential host species collected from anglers. Total length (mm) and frozen weight (g) were recorded when possible.

Common Name	Scientific Name	Total Length (mm)	Frozen Weight (g)	Origin	Source
Brown Trout	*Salmo trutta*	200	80	Burt Lake	Author
Rainbow Trout	*Oncorhynchus mykiss*	-	882	Mullett Lake	HBBS
Rainbow Trout	*Oncorhynchus mykiss*	-	91	Mullett Lake	HBBS
Yellow Perch	*Perca flavescens*	235	671	Burt Lake	Angler
Lake Herring	*Coregonus artedi*	-	125	Mullett Lake	HBBS
Walleye	*Sander vitreus*	404	424	Burt Lake	Author
Walleye	*Sander vitreus*	492	770	Burt Lake	Angler
Yellow Perch	*Perca flavescens*	178	75	Burt Lake	Angler
Yellow Perch	*Perca flavescens*	189	50	Burt Lake	Angler
Walleye	*Sander vitreus*	457	40	Burt Lake	Angler
Largemouth Bass	*Micropterus salmoides*	400	833	Burt Lake	Author
Walleye	*Sander vitreus*	457	566	Burt Lake	Author
Northern Pike	*Esox lucius*	445	411	Burt Lake	Angler
Yellow Perch	*Perca flavescens*	222	92	Burt Lake	Angler
Rock Bass	*Ambloplites rupestris*	197	107	Burt Lake	Angler
Yellow Perch	*Perca flavescens*	180	42	Burt Lake	Angler
Northern Pike	*Esox lucius*	610	841	Burt Lake	Angler
Rainbow Trout	*Oncorhynchus mykiss*	-	1298	Mullett Lake	HBBS
Walleye	*Sander vitreus*	464	549	Burt Lake	Angler

Table 3: Wounding rates of fishes from Burt and Mullett lakes. Data for Burt Lake were derived from angler surveys conducted during the summer of 2016, and data for Mullett Lake were provided by USGS, Hammond Bay Biological Station.

	Species	Fish Inspected	SEL Wounds	Native Wounds	SEL Wounding Rate	Native Wounding Rate
Mullett Lake	Northern Pike (*Esox lucius*)	340	10	9	2.9%	2.7%
	Rainbow Trout (*Oncorhynchus mykiss*)	58	7	0	12.1%	0%
	Walleye (*Sander vitreus*)	129	0	0	0%	0%
	Cisco (*Coregonus spp.*)	2	2	0	100%	0%
	Yellow Perch (*Perca flavescens*)	1	1	0	100%	0%
	Total	**530**	**20**	**9**	**3.8%**	**1.7%**
Burt Lake	Brook Trout (*Salvelinus fontinalis*)	1	1	0	100%	0%
	Brown Trout (*Salmo trutta*)	1	0	0	0%	0%
	Lake Sturgeon (*Acipenser fulvescens*)	1	0	1	0%	100%
	Largemouth Bass (*Micropterus salmoides*)	2	0	0	0%	0%
	Northern Pike (*Esox lucius*)	48	1	2	2.1%	4.2%
	Rock Bass (*Ambloplites rupestris*)	3	0	0	0%	0%
	Smallmouth Bass (*Micropterus dolomieu*)	32	0	0	0%	0%
	Rainbow Trout (*Oncorhynchus mykiss*)	3	0	0	0%	0%
	Walleye (Sander vitreus)	157	13	0	8.3%	0%
	White Sucker (*Catostomus commersonii*)	1	0	1	0%	100%
	Yellow Perch (*Perca flavescens*)	132	0	0	0%	0%
	Total	**381**	**15**	**4**	**3.9%**	**1.1%**

Table 4: Stream and date of capture of unmarked adult Sea lampreys in the upper Cheboygan River, date of the first lock opening, and the fewest days marked lampreys took to reach a tributary net from a release location immediately above the dam (Transit time). For a given capture date and transit time, earliest escapement date past the lock and dam was estimated for each unmarked sea lamprey (Escapement date). Bolded escapement dates are those that likely occurred before the first lock opening. Total length, weight, maturity and isotopic values for H and O from heads are also presented.

Capture Location	Specimen ID	Total Length (mm)	Weight (g)	Sexually Mature	δ^2H	$\delta^{18}O$	Capture Date	First Lock Opening	Transit Days	Escapement Date
Pigeon	PIR1.2013	483	230	N	-	-	8-May-13	3-May-13	9	**30-Apr-13**
Pigeon	PIR2.2013	420	142	Y	-	-	31-May-13	3-May-13	9	23-May-13
Sturgeon	STR1.2013	423	124	N	-	-	30-May-13	3-May-13	19	9-May-13
Maple	MR1.2013	492	269	N	-	-	3-May-13	3-May-13	30	**4-Apr-13**
Maple	MR1.2014	450	244	Y	-128.94	10.98	13-Jun-14	17-May-14	30	**14-May-14**
Maple	MR2.2014	455	205	Y	-80.38	11.00	20-Jun-14	17-May-14	30	21-May-14
Pigeon	PIR1.2015	470	207	N	-138.65	11.17	4-May-15	15-May-15	9	**26-Apr-15**
Maple	MR1.2015	460	215	Y	-135.60	10.50	8-Jun-15	15-May-15	30	**8-May-15**
Maple	MR2.2015	465	260	Y	-165.24	12.88	16-Jun-15	15-May-15	30	17-May-15
Pigeon	PIR1.2017	359	129	N	-88.74	12.67	28-Apr-17	6-May-17	9	**19-Apr-17**
Pigeon	PIR2.2017	284	132	Y	-	-	31-May-17	6-May-17	9	22-May-17
Sturgeon	STR1.2017	390	129	N	-	-	18-May-17	6-May-17	19	**30-Apr-17**
Pigeon	PIR1.2018	510	255	N	-	-	7-Jun-18	1-May-18	9	29-May-18
Pigeon	PIR2.2018	420	190	Y	-	-	15-Jun-18	1-May-18	9	6-Jun-18
Pigeon	PIR3.2018	410	153	Y	-	-	18-Jun-18	1-May-18	9	9-Jun-18
Pigeon	PIR4.2018	460	198	Y	-	-	18-Jun-18	1-May-18	9	9-Jun-18
Sturgeon	STR1.2018	540	287	Y	-	-	3-Jun-18	1-May-18	19	15-May-18
Pigeon	PIR1.2019	390	165	Y	-	-	30-May-19	16-May-19	9	21-May-19
Sturgeon	STR1.2019	460	192	N	-	-	24-May-19	16-May-19	19	**5-May-19**
Sturgeon	STR2.2019	530	317	N	-	-	26-May-19	16-May-19	19	**7-May-19**
Sturgeon	STR3.2019	445	157	N	-	-	28-May-19	16-May-19	19	**9-May-19**
Sturgeon	STR4.2019	395	145	Y	-	-	13-Jun-19	16-May-19	19	25-May-19
Sturgeon	STR5.2019	366	125	Y	-	-	13-Jun-19	16-May-19	19	25-May-19
Sturgeon	STR6.2019	490	280	Y	-	-	1-Jul-19	16-May-19	19	12-Jun-19

Table 5: Values used for stable isotope mixing model to quantify contribution of potential hosts from Burt and Mullett lakes runoff to Lake Huron to isotopic composition of 'upper-river' Sea Lamprey.

	'Upper-River' Sea Lamprey	**'Upper-River' Hosts**	**Runoff to Lake Huron** (Jasechko *et al.* 2014)
Mean δ^2H	-142.21	-150.70	-74.00
Source proportion	-	0.89	0.11
Uncertainty	0.018	0.12	0.00017

Appendices

Appendix A: Tissue analyzed for stable isotope analysis

Table 1: Four different tissue types (head, heart, liver, and adipose tissue) excised from Sea Lamprey were analyzed for isotopic composition of δ^2H and $\delta^{18}O$. A total of 52 individual Sea Lamprey were utilized, resulting in 104 samples being run. Samples lost during preparation are represented in parenthesis.

Year	Tissue type	Lake Huron		Upper Cheboygan River	Unknown
		Parasitic juvenile	Spawning adult	Parasitic juvenile	Spawning adult
2013	Head	-	-	1	-
n = 3 (1)	Heart	-	-	- (1)	-
	Liver	-	-	1	-
	Adipose	-	-	1	-
2014	Head	-	10	1 (1)	2
n = 18 (2)	Heart	-	-	2	-
	Liver	-	-	1 (1)	-
	Adipose	-	-	2	-
2015	Head	-	11	1	3
n = 18	Heart	-	-	1	-
	Liver	-	-	1	-
	Adipose	-	-	1	-
2016	Head	9	11	3	-
n = 59 (9)	Heart	5 (1)	6	- (1)	-
	Liver	5 (2)	3 (3)	1 (1)	-
	Adipose	8	6	2 (1)	-
2017	Head	1	-	-	1
n = 6 (2)	Heart	1	-	-	- (1)
	Liver	1	-	-	- (1)
	Adipose	1	-	-	1

Table 2: Mean deuterium (δ^2H) and oxygen ($\delta^{18}O$) values for Sea Lamprey tissue (adipose tissue, heads, hearts and livers) from Sea Lamprey known to feed in Lake Huron and the upper Cheboygan River, as well as those with unknown origins.

Origin	Tissue type	Mean δ^2H	Stand. Dev.	Mean $\delta^{18}O$	Stand. Dev.
Lake Huron spawning-phase	Adipose (n = 6)	-98.32	34.18	11.67	1.25
	Head (n = 32)	-101.13	21.93	18.12	5.07
	Heart (n = 6)	-83.89	25.23	12.08	0.98
	Liver (n = 3)	-146.81	6.66	12.86	0.38
Lake Huron parasitic-phase	Adipose (n = 8)	-177.47	17.73	10.18	4.44
	Head (n = 10)	-106.38	23.07	13.60	2.51
	Heart (n = 6)	-103.38	23.52	10.83	1.67
	Liver (n = 6)	-134.75	32.06	12.07	1.47
Upper-River	Adipose (n = 6)	-185.67	14.38	14.06	4.10
	Head (n = 6)	-142.21	10.30	10.71	2.15
	Heart (n = 3)	-115.67	12.35	11.06	1.62
	Liver (n = 4)	-144.28	18.29	11.91	1.78
Unknown	Adipose (n = 1)	-144.78	-	9.40	-
	Head (n = 6)	-122.93	32.29	11.53	0.99
	Heart	-	-	-	-
	Liver	-	-	-	-

Appendix B: Stable isotope composition of potential host species

Table 1: Mean deuterium (δ^2H) and oxygen ($\delta^{18}O$) values for heads from Sea Lamprey known to feed in Lake Huron, those known to feed in the upper Cheboygan River, those with unknown origins and whole bodies from potential hosts from Burt and Mullett lakes.

Origin	Tissue type	Mean δ^2H	Stand. Dev.	Mean $\delta^{18}O$	Stand. Dev.
Lake Huron spawning-phase	Head (n = 32)	-101.13	21.93	18.12	5.07
Lake Huron parasitic-phase	Head (n = 10)	-106.38	23.07	13.60	2.51
Upper-River	Head (n = 6)	-142.21	10.30	10.71	2.15
Unknown	Head (n = 6)	-122.93	32.29	11.53	0.99
Potential Hosts (Burt and Mullett lakes)	Whole body (n = 19)	-150.70	29.84	14.35	2.12