

**EVALUATING MATE PREFERENCE IN ADULT FEMALE SEA
LAMPREY**

AND

**APPLICATION OF THE GROWING DEGREE DAY METRIC TO
REVEAL PATTERNS OF GROWTH AMONG LARVAL SEA LAMPREYS**

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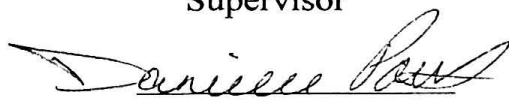
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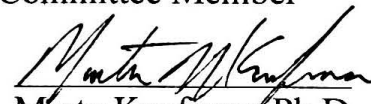
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MATE PREFERENCE AND LARVAL GROWTH OF GREAT LAKES SEA
LAMPREY (*PETROMYZON MARINUS*) IN A WARMING CLIMATE

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ABSTRACT

MATE PREFERENCE AND LARVAL GROWTH OF GREAT LAKES SEA LAMPREY (*PETROMYZON MARINUS*) IN A WARMING CLIMATE

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Sea lamprey (*Petromyzon marinus*) are parasitic pests in the Great Lakes. Once sea lamprey started to have a negative impact on important game fish populations, management efforts began. More information on how sea lamprey choose mates and how larval sea lamprey grow could give more insight on how to better manage their populations.

Increased temperatures due to global climate change may result in increased growth of individuals, higher count of eggs, higher quality of eggs, and higher sperm production. I presented an average-sized ovulating female with the choice of a small or large spermiating male in a two-way mate preference experiment. Trials were conducted and investigated whether stream side bias, male or female activity, or the presence of male odor upstream affected the female's preference. Results showed the female sea lamprey had a mesocosm side bias and females preferred to be in front of the small male when male odor was released.

Improving the accuracy of larval sea lamprey growth models would benefit management strategies by providing better predictions as to when metamorphosis could occur. The primary technique used to establish growth of sea lamprey within the control program is the use of an incomplete growing degree day (GDD) metric, where average

daily growth across a latitudinal gradient during the warmer months is used to predict time of metamorphosis. I tested a complete GDD metric in which the number of year-round growing degree days for each age-class of sea lamprey population tested was calculated. Water temperatures were obtained as much as possible during the larval growth time frame for each stream examined. For streams in which I did not have water temperature, I placed data loggers in streams to record the temperature every hour for one year. Air temperatures were then obtained from weather station locations closest to the mouth of the river for the same year. A relationship between the air and water temperature for each stream was established from this year's data. Air temperature were then obtained from weather stations closest to each stream during the periods of larval growth, and air temperature was used to predict water temperature larval sea lamprey experienced. A generalized linear model was used to determine the relationship between the response variable, lamprey length-at-age, and one or more predictors, which included log-transformed GDD, log-transformed calendar days, stream, and lake. The best fit model, which used basin wide data, was log-transformed calendar days and lake. The results show that GDD was the best predictor for Lake Ontario and calendar days were the best predictor for Lakes Huron and Michigan to determine growth of sea lamprey. Calendar days and GDD both predicted length-at-age for Lake Superior populations equally well.

DEDICATION

I would like to dedicate this completed thesis to my fiancé, Steven Weier, my best friend Shirlene Armstrong, and my late grandfather, Donald Leaym, for their continual support in my education, loving me, and believing in my dreams.

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INTRODUCTION

Sea lamprey (*Petromyzon marinus*) are a parasitic invasive fish species in the Laurentian Great Lakes (Hansen et al. 2016). Invasive sea lamprey entered into the Great Lakes from the Atlantic Ocean (Ciereszko et al 2000, Hansen et al 2016) and were first found in Lake Ontario in 1835 (Hansen et al. 2016). Sea lamprey entered Lake Erie through the Welland Canal (Hansen et al. 2016) and were found in Lake Erie in 1921, Lake Michigan in 1936, Lake Huron in 1937, and Lake Superior in 1938 (Applegate 1950, Smith and Tibbles 1980, Hansen et al. 2016). Establishment of sea lamprey occurred quickly which resulted in large scarring rates and mortality of lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), catostomids (*Catostomus* spp. and *Moxostoma* spp.), walleye (*Sander viteus*), and rainbow trout (*Oncorhynchus mykiss*; Smith and Tibbles 1980). By 1953, Lake Michigan lake trout catches decreased and in 1959 the fishery in Lake Huron collapsed (Smith and Tibbles 1980). Sea lamprey control began in the 1950s with mechanical and electrical barriers (Smith and Tibbles 1980, Hansen et al. 2016). Beginning in the 1960's, the primary method of control was lampricide using 3-trifluoromethyl-4-nitrophenol, called TFM, which was applied to streams to kill larval lamprey (Smith and Tibbles 1980, Hansen et al. 2016). This technique is still the primary method of removing sea lamprey populations today. In 1954, the Great Lakes Fishery Commission was established to control sea lamprey, advance science, and help agencies work together (GLFC 2015).

The life cycle of sea lamprey begins by mature male and female lamprey entering tributaries from the Great Lakes to spawn in the late spring and early summer (Applegate 1950, Cochran et al. 2011). The male will arrive first and begin building a nest in an area

with large water worn rocks and sand before the arrival of a female (Breder 1966, Smith and Tibbles 1980). Lamprey move rocks to create a nest by placing their buccal disk on the surface and swimming to move the rock (Hagelin and Steffner 1958, Malmqvist 1983). Female sea lampreys have been observed helping to build nests in some cases (Applegate 1950, Beamish and Neville 1992), or swimming around the nest restlessly in other cases (Hagelin and Steffner 1958). Once nests are ready, the female attaches herself to a stone at the top of the nest extending her body into the nest (Hagelin and Steffner 1958). The male lamprey will then attach himself to the top of her head and press his body tightly to hers (Applegate 1950, Hagelin and Steffner 1958). The male's tail will loop near the female's genital opening and they will both release sperm and eggs while shaking their bodies rapidly (Hagelin and Steffner 1958, Beamish and Neville 1992). A single female produces 60,000 eggs per spawning event, and males can milt for several spawning events (Ciereszko et al 2000). Adult lamprey die shortly after reproduction is complete (Applegate 1950, Breder 1966).

The fertilized eggs deposited by the adults adhere to the sand in the nest (Hagelin and Steffner 1958, Breder 1966). Sea lamprey eggs will hatch after 10 to 12 days. Larval sea lamprey will develop gills and a buccal hood before emergence from the nest (Hansen et al. 2016). When the larvae emerge, they will drift downstream where they will burrow into muddy and silty areas of the stream bed (Applegate 1950, Swink and Johnson 2011). Larval lamprey live in the stream bed for 3 to 7 years feeding on detritus (Morkert et al. 2011, Swink and Johnson 2014). Once larval sea lamprey have reached a minimum size of 100 mm, they will undergo metamorphosis into parasitic juveniles and migrate back into the Great Lakes (Applegate 1950, Morkert et al. 2011). Larval sea lamprey will

develop gills and a buccal hood before emergence from the nest (Hansen et al. 2016). Juveniles will live within the lakes feeding on host species for 1 to 1.5 years and then migrate back into tributaries to spawn (Applegate 1950, Swink and Johnson 2014). During the parasitic life stage, sea lamprey will attach themselves to a fish with its buccal disk and bore a hole within the host to feed off its blood and body fluids (Applegate 1950, Smith and Tibbles 1980). In 1954, the Convention on Great Lakes Fisheries held a meeting to discuss the need to work together to combat sea lamprey, which established the Great Lakes Fishery Commission (Smith and Tibbles 1980, GLFC 2015).

There are increasing concerns with climate change affecting host-parasite interactions and invasive species. Global climate change is predicted to increase parasitic feeding, growth, and fecundity of sea lampreys in the Great Lakes (Cline et al. 2014). Currently, an individual juvenile sea lamprey can kill up to 18 kilograms of fish over the 12 to 18 months they feed (GLFC 2015). Changes in average adult sea lamprey size correlate with longer growing seasons, which represent increased feeding and growth with warming waters. Changing thermal regimes and precipitation may also affect natural barriers against sea lamprey. An increase in temperatures in the Great Lakes could result in an increased level of larval development (Cline et al. 2014). Increased temperature is also strongly related to shorter periods of egg incubation and increased larval sea lamprey growth (Holmes 1990, Holmes and Youson 1998). Increased growth of larval sea lamprey would allow larval sea lamprey to be able to more quickly reach the size needed to enter metamorphosis. Changes in parasite phenology and life cycle completion rates would result in an increase in direct mortality of host species (Cline et al. 2014). Finally, fecundity is expected to increase in sea lamprey, partly because large fish not only have a

greater body volume for holding eggs, but also because they may devote a greater fraction of surplus energy to egg production than smaller mature fish (Lester et al. 2004, Edeline et al. 2007).

Increased size of adult female sea lamprey could result in more eggs, higher quality eggs, and also a longer search time for a mate (Andersson 1994), while increased size of adult males should improve nest quality and defense, pheromone production, and sperm production (Andersson 1994, Oliverira et al. 2000). A female's ability to have increased fecundity could be due to her preferences for her mate's secondary sexual characteristics (Darwin 1872, Andersson 1994). Female choice allows the female to choose a mate that will provide her with the best opportunity to pass on desired traits that her mate possesses to her offspring (Andersson 1994, Eberhard 1996). Fecundity selection and sexual selection are the major evolutionary forces that select for larger body size in many organisms (Bisazza and Pilastro 1997, Blanckenhorn 2000, Liao and Lu 2011, Blaul and Ruther 2012). Since body size correlates with many physiological and fitness characteristics (Blanckenhorn 2000), larger body size may increase a male's reproductive success (Andersson 1994, Cothran 2007, Labonne et al. 2009, Kehl et al. 2015). The reproductive success of female sea lamprey is lower than in males (Manion and Hanson 1980), thus females may be more selective when choosing mates to try to ensure higher fitness of their offspring (Labonne et al 2009, Alcock 2013).

Because the growth rate of sea lamprey is expected to increase with global climate change, growth models of sea lamprey, particularly in the longest larval stage, are needed that reflect growth of larval sea lamprey in a warming climate. Current models that are used to determine larval sea lamprey growth are dependent on calendar time,

which does not consider variables such as temperature (Neuheimer and Taggert 2007). These growth models are used in more complex models that allow for the evaluation of a range of sea lamprey control strategies, which are used to determine the best course of action for the control of Great Lakes sea lamprey. Models are valuable tools for fisheries management but can misinform managers when they fail to account properly for uncertainty (Schnute and Richards 2001). Growth rates of larval sea lamprey vary among individuals and groups of lamprey in different areas of a stream due to variables such as water temperature and population density (Morkert et al. 2011). Growing degree days (GDD) are an index of ambient thermal energy that relates directly to an ectotherm's cumulative metabolism (Venturelli et al. 2010) and has been shown to be a reliable predictor of growth and development in some ectotherms. The GDD metric has been shown to have greater explanatory power than conventional time-based methods in an assessment of growth in nine species of fish and may be a useful metric in explaining the growth of larval sea lamprey in a warming climate (Neuheimer and Taggert 2007).

To understand how a warming climate may ultimately contribute to changes in fecundity and growth of sea lamprey, this study i) evaluates the preferences of adult female sea lamprey by presenting each female individually with the simultaneous choice of a small or large adult male in a two-way mate preference experiment with and without the addition of the odor of sexually mature males upstream; and ii) compares the complete GDD metric with conventional time-based methods in their ability to explain the growth of larval sea lampreys in streams experiencing widely contrasting temperatures across the basin.

Chapter 1

EVALUATING MATE PREFERENCE IN ADULT FEMALE SEA LAMPREYS

Abstract

Sea lamprey (*Petromyzon marinus*) are a parasitic invasive species in the Great Lakes. Increased temperatures due to global climate change may result in increased growth of individuals, higher count of eggs, higher quality of eggs, and higher sperm production. If females discern external indicators of male quality (e.g., body size, pheromone production, etc.), then females may exhibit a preference for mating with larger males as a means to increase their reproductive success. I tested to see whether ovulating females exhibit a preference for larger spermiating males in a mesocosm in a tributary of Lake Huron. I presented each average-sized ovulating female with the choice of a small or large spermiating male in a two-way mate preference experiment. Males were situated upstream of the female in separate netted boxes partially buried in the stream bed, while the female was in a partially buried net that allowed her to spend more time with the male she preferred. Ten-minute trials were conducted, and trials also investigated whether side bias, male or female activity, or the presence of upstream male odor affected the female's preference. A multinomial logistic regression was used to determine which variables affected female mate preference. Females exhibited a mesocosm side bias and spent more time with small males when upstream male odor was present. According to the results of this study, sea lamprey size was not a factor when it came to females choosing a mate. These results illustrate that sea lamprey preferences may be based on other factors that can be explored in future studies.

Introduction

Choosy females prefer traits in mates that indicate health, viability, and sexual attractiveness based on healthy mates, good genes, and runaway selection theories. The healthy mate theory states that a male's appearance and courtship influence a female's choice in a mate to avoid passing diseases to her offspring. The good genes hypothesis indicates that a female will evaluate a potential partner's traits to determine if they would benefit her offspring. The sexy son's hypothesis indicates a female will choose a mate that has traits that are attractive, and these traits can be passed to offspring to make them more likely to reproduce (Alcock 2013). Ultimately, females will choose a mate that possesses characteristics that provide greater fitness to her offspring (Cote and Hunte 1989, Aspbury and Basolo 2002). Characteristics that can increase a male's chances of reproductive success are sperm count, motility, aggressiveness, persistence, weaponry, fat content, body mass, and body size (Kehl et al. 2015). Intra and intersexual selection help form male characteristics that females favor, such as body size (Andersson 1994, Eberhard 1996, Aspbury and Basolo 2002).

Body size is an important characteristic that is correlated with physiology and fitness characters (Andersson 1994, Blanckenhorn 2000), and body size can serve as a basis for female mate choice (Liao and Lu 2011). Larger body size is beneficial to males to defend territories or nesting sites, for example, and compete with other males (Cote and Hunte 1989, Blanckenhorn 2000). Larger-sized males of some species have been found to be more reproductively successful (Oliveira et al. 2000, Aspbury and Basolo 2002, Labonne et al. 2008). This study investigates mate preference in the invasive Great Lakes sea lamprey. Specifically, whether female sea lampreys exhibit a preference for

mating with larger males as a means to increase their reproductive success, as even marginal increases in zygote production can result in large changes in recruitment (Myers 2002). This is an important question, as increased temperatures due to global climate change is predicted to result in increased growth of female and male sea lamprey, which can mean more eggs, higher quality eggs, and increased sperm production (Andersson 1994, Ciereszko et al. 2000, Mainka et al. 2010, Cline et al. 2014). Increased reproductive success of sea lamprey should increase the number of sea lamprey in the population, which will require increased allocation of resources to sea lamprey control efforts to maintain sea lamprey abundance at current levels.

Female sea lamprey have a limited number of spawning events, which makes choosing a good mate important for successful reproduction. An average-sized female sea lamprey can produce around 60,000 eggs (Applegate 1950), but sperm availability and quality are likely regulators of fertilization success, with maximum fertilization rates of sea lamprey eggs obtained at a sperm:egg ratio of 50,000:1. Male sea lamprey can have multiple milts, and larger males may have increased sperm production (Ciereszko et al. 2000). Estimated reproductive success of female sea lamprey is very low and is governed in part by deposition or retention of eggs in the nest and fertilization success (Manion and Hanson 1980). Manion and Hansen (1980) suggests that only 14% of eggs remain in the nest after spawning, with nest site greatly contributing to the number of eggs in the nest. Sea lamprey will construct nests for the survival of their offspring, with males usually constructing the nest first before the female arrives (Applegate 1950, Hagelin and Steffer 1958, Docker 2015). High quality nesting sites are usually occupied by larger males (Hagelin and Steffer 1958). Once arriving to the desired location, lamprey will move

rocks using their oral disk to create a nest for spawning (Applegate 1950, Hagelin and Steffer 1958, Docker 2015).

Determining if ovulating adult females display a preference for larger spermiating males would be valuable for sea lamprey control. Known mate preferences of adult females could be used to evaluate if sea lamprey use cues other than pheromones to choose a mate. Mature spermiating male sea lamprey release pheromones that induce preference for ovulating female sea lamprey to come to the nest (Johnson et al. 2005, Docker 2015, Hansen 2016). The male pheromone may be used to increase attractiveness to increase fecundity (Docker 2015, Hansen 2016). Possible mate preferences can be used for understanding sea lamprey reproduction and management opportunities. I propose to examine whether adult females exhibit preferences for male characteristics (body size, pheromone production) that are thought to correlate with sperm production, and how those preferences manifest in pre-spawning behavior, such as help with nest construction and tail fanning (Docker 2015).

Methods

Study Area

The study site location was the Trout River near Rogers City, Michigan (45.431991, -83.841621; Figure 1). The Trout River is a tributary of Lake Huron in Presque Isle County, Michigan. The Trout River was last treated with lampricide in 2011 (GLFC Sea Lamprey Control Map, 2015). The stream bed in the Trout River has habitat preferences that mature sea lamprey choose such as sand, gravel, and pebbles (Smith and Tibble 1980), and this stream has also been chosen previously in other studies on sea

lamprey (Swink and Johnson 2014, Brant 2015). Discharge was not measured in the study area, but 7.4 km upstream the discharge ranged from 0.133 m³/sec to 1.232 m³/sec between June 1st and July 9th, 2017 (Skye Fissette, personal communication). The mating behavior observations were conducted in a section of the Trout River that was 2.1 km upstream of the mouth of the river.

Collecting sea lamprey

Sea lamprey were taken from holding tanks from at Hammond Bay Biological Station (HBBS) where they were held after being collected by United State Fish and Wildlife (USFWS) and United States Geological Survey (USGS) staff from assessment traps in streams across the Great Lakes Basin. Sea lamprey were placed into three different flow-through tanks (1.06 m H x 2.14 m L x 0.91 m W) at HBBS for small males, large males, and females. Each storage container was aerated by an air stone to allow sufficient oxygen requirements for sea lamprey. The water in the sea lamprey holding tanks at the biological station was drawn directly from Lake Huron, with water temperature, read from a temperature sensor at HBBS, ranging from 9° to 15° C from June 1st to July 15th, 2017. Cages (1.4 m H x 1.5 m L x 0.8 m W) that allowed water to flow through were placed 15 m downstream from the experimental site to mature sea lamprey in the Trout River. Sexually immature sea lampreys mature faster when placed in Great Lakes tributary streams with higher temperatures than the water at HBBS. Individuals that did become mature at HBBS were also brought to the cages in the Trout River at least a day prior to being used in experiments so they could acclimate to the stream. Sea lamprey sexual maturity was determined by stroking the ventral side of each

sea lamprey near the genital opening to see if females expressed eggs or males expressed sperm (a cloudy liquid).

Male and female sea lamprey were held at HBBS for up to ten days before being moved to the cages 15 m downstream of the experimental site. Sea lamprey were transported in coolers filled with Lake Huron water that was aerated at all times, with males and females placed in separate coolers. Ten lampreys were placed into these cages twice a week from June 2nd to July 10th, 2017. The cages were zip tied onto a tie rod and placed within a 1.5 m deep area of the river. The three cages were labeled to distinguish the different sea lamprey groups. The males were placed downstream of the females. Temperatures were taken daily and recorded. Cages were checked daily for mortalities. If lamprey appeared to have fungal growth, they were removed from the cages and taken back to HBBS.

Determination of size classes of sea lamprey

To examine the preference of ovulating females for large or small spermiating males, a large number of sea lamprey had to be measured to determine appropriate size ranges of small, average-sized, and large individuals. Sea lamprey that were used to determine different size classes for large and small male were collected from holding tanks at HBBS during our 2016 field season on June 15th, 2016. A sample of 238 male and 300 female sea lampreys collected by USFWS and USGS staff from assessment traps in streams across the Great Lakes Basin were measured in order to establish the different size classes. Sea lamprey were measured to the nearest millimeter and weighed to the nearest gram. Once measurements were completed, they were input into Microsoft Excel

and a histogram was generated, which approximated a normal distribution. I calculated a z score to determine the number of standard deviations above or below the mean a particular length was. Males that were of a length ≥ 1 standard deviation below the mean were defined as small (≤ 420 mm), and those that were ≥ 1 standard deviation above the mean were defined as large (≥ 505 mm). Females that were within 0.5 standard deviations from the mean were determined to be of average size (452 mm to 493 mm).

Experimental setup

The one female sea lamprey that would be used in experiments for the day was placed within one plastic acclimation cage that was located 7.6 m downstream of the experimental site 15 minutes before the experiment began. The plastic cage (5.39 m H x 3.44 m L x 4.87 m W) allowed for water to flow through and was attached to tie rods pounded into the stream bed (0.5 m). The top of the basket had a plastic grid that was fastened with zip ties to keep it in place.

A three-box fish net (Number NHB14-4, Memphis Net and Twine Co., Memphis, Tennessee) was used in our trials, which was meant to keep each male lamprey separated and visible to our female lamprey during the test (Figure 2). The dimensions for the two fish nets were 1.22 m H x 1.22 m L x 1.22 m W with a 0.95 cm mesh size. The other fish net box had dimensions of 1.22 m H x 2.43 m L x 1.22 m W with a 0.95 cm mesh size. Fence posts were pounded into the substrate in all four outer corners and three posts were placed in the middle of the net to keep the boxes in the streambed. Zip ties were used to secure the net to the fence posts. Rocks were taken from the shore and river and placed inside of the net to cover the netted bottom. The rock layer ranged from 15 cm to 36 cm

deep and rocks ranged in size from 3.14 cm to 238.76 cm. The female was in the larger netted box downstream and could choose which male she wished to spend more time orientated towards. The two separate males were in their own separate netted box located upstream of the female.

To determine whether female sea lamprey would change their preference in the presence of additional male odor, I set up a system to introduce male odor upstream of either male. A Grainger peristaltic chemical metering pump (85MJH2A1STG1, Minimum flow: 0.8 gpd, Maximum flow: 17.0 gpd, Maximum pressure: 100 psi, Maximum viscosity: 1500 cP, Lake Forest, Illinois) was placed on the side of the streambed. Tubing (0.32 cm) was connected from the pump to the inside of each male netted box. Tubing length for the netted box closer to the shore was 3.04 m and the tubing for the farther side of the netted box was 5.4 m. Metal washers were placed around the tubing to keep them submerged. Male odor, or spermiating male washings (SMW), was created by placing five spermiating males into 20 L of water for four hours (Johnson et al. 2009, Brant et al. 2013). SMW were stored in 3.78 L containers and placed in a freezer. To thaw the SMW, the 3.78 L container was taken out of the freezer and placed in tanks of water at HBBS for ten hours before use. The rate that SMW were pumped out of the peristaltic pump into the experimental area during some trials was 0.95 mL per hour, or 0.016 mL per minute.

Experimental Trials

The mating preference trials were conducted from June 6th to July 12th, 2017. Experiments began in the morning by moving one female, one large male, and one small

male from their acclimation cages into the netted boxes. Males and females were placed into netted boxes at 3-minute intervals with the males being transferred first. I allowed the lampreys to acclimate to their netted boxes for 30 minutes. After acclimation, trials were conducted, with each experiment consisting of 6 trials using one adult female sea lamprey, one small male, and one large male. Trial A began by recording the behaviors of both males and the female for a ten-minute period with no added spermiating male washings. Two observers used Sony ICD-PX440 audio recorders to log notes and behaviors of the sea lamprey via continuous behavior collection using an ethogram of sea lamprey reproductive behaviors (Table 1). One observer recorded the behaviors of the female sea lamprey, while another observer recorded the behavior of the two male sea lamprey. The behaviors were each distinguished as an active and non-active behavior (Table 1). Active behaviors were categorized as any actions that involved movement and non-active behaviors were categorized as no movement from the sea lamprey. Once the ten-minute Trial A was over, it was determined which male the female spent more time oriented toward (the male whose netted box she spent the most time oriented in front of). Behavioral data for Trial B was then collected for ten minutes in the same manner as in Trial A. SMW was released on the side the female was less oriented with during Trial A. Behavioral data for Trial C was then collected for ten minutes in the same manner as Trials A and B. SMW was released on the side the female was most oriented with during Trial A. Once Trial C was completed, the two males were switched and placed into the opposite netted area. Another acclimation period of 30 minutes occurred. Trials D, E, and F were then conducted in the same manner as Trials A, B, and C, respectively. The same

female was used in all 6 trials to test for repeatability of the female's preference and whether the females exhibited a side bias.

Audio recordings

The audio recordings were consolidated in a program called Solomon Coder 170322 (András Péter, <http://solomoncoder.com>). In Solomon Coder, buttons were created for the behaviors that were listed in the ethogram (Table 1). Buttons were also created for whether the female was on the small male side or large male side and if she was on the left or right side of the stream. Time intervals on the program were set to record data every second. The male sea lamprey audio files were listened to and their behaviors were recorded using the same behavior buttons. Each audio file was listened by two different observers to check for mistakes. The recorded information was organized by how long the female spent with each male, what behaviors the lampreys exhibited, if the behaviors of lamprey were active or non-active, if the SMW were on or off, if the large male was on the right or left side, and if it was the first set of trials (A, B, C) or second set of trials (D, E, F), which trials repeatability of behavior by the female. .

Data Analysis

A multinomial logistic regression was used to predict a nominal dependent variable (female in front of the small or large male) for multiple independent variables (female repeat, female activity, right side large male, SMW on or off, SMW right or left, small male activity, large male activity). These variables tested whether or not female's exhibited a side bias, if SMW influenced mate choice, if male activity affected female preference, and if a female would prefer the same male during a repeatability test. We

present parameter estimates and confidence levels of those estimates, as well as estimated marginal means. This analysis was carried out using the multinomial logistic regression command in SPSS® version 24 (IBM Corp., 2011). All factors mentioned were tested in a full model which was

$$\ln(F_V/F_i) = \alpha + R_s + S_0 + S_s + F_R + F_a + S_a + L_a$$

α = intercept

F_v = Female oriented toward large male

F_i = Female oriented toward small male

R_s = Right side large male

S_0 = SMW (if it is on or off)

F_r = Female repeatability

F_a = Female activity

S_a = Small male activity

L_a = Large male activity

Results

A total of 50 experiments were conducted, however, 10 experiments could not be used due to sea lamprey escaping or death that occurred before an experiment would begin. Thus, we collected data on a total of 40 experiments were conducted from June 6th to July 12th, 2017, with one ovulating female sea lamprey, one small spermiating male, and one large spermiating male used for each experiment. The average lengths of experimental females were 473 mm (standard deviation (SD) = 9.38 mm). The average length for the small experimental males was 398 mm (SD = 12.84 mm), and the average length for the large experimental males was 533 mm (SD = 16.89 mm). The average temperature during the study was 18.1 °C (SD = 2.072 °C). The temperature on the Trout River during the study ranged from 13.9°C to 21.9°C. Twenty-six gallons of SMW was used over the 40 experiments, an average of 0.65 gallons per test.

The global model included right side large male, SMW, SMW side, female activity, female repeat, small male activity, and large male activity. Females spent more time with smaller males when SMW was added to the side in which the small males occupied. Females did exhibit a mesocosm side bias, preferring to stay on the left side (when facing upstream) of the mesocosm (Table 2; Figures 3 and 4). There is uncertainty as to the exact relationship between the other variables (SMW side, female activity, female repeat, small male activity, large male activity) and which male the female was in front of because the 95% confidence intervals for the regression coefficients included both positive and negative values (Table 2).

Discussion

In this study, female sea lamprey exhibited a side bias, preferring the left side of the mesocosm to the right side. Additionally, female sea lamprey spent more time with smaller males versus larger males when upstream male odor (SMW) was added.

The mesocosm side bias exhibited in the study may have been due to slight differences in stream features across the mesocosm. The left side of the stream was favored more by the female sea lamprey than the right side. The left side of the experimental area had some available shade especially later in the study due to leaf out from trees, and the left side of the stream was slightly deeper (5 cm) compared to the right. The right side of the experimental area did not provide shade. Sea lamprey normally mate when water temperature ranges are between 10 and 20 °C. Once temperatures reach above 26 °C sea lamprey cannot survive (Hansen et al. 2016). During our study, temperatures were within the suitable range for sea lamprey mating to occur. By staying out of the direct sunlight, sea lamprey might be able to prolong their life

longer to possibly find a mate. Avoiding fatigue and high temperatures would make the left side of the experimental area a more favorable side for female sea lamprey to survive.

When the female sea lamprey had the option of being in front of the small male or large male, she would choose to be on the side of the small male when the SMW was being pumped on the side of the small male. Our results determined that when the small male was on the left and the SMW was released on the left, she spent more time with the small male.

Sea lamprey have an olfactory system that they use for identifying spawning habitats, avoiding risks, and attracting mates. Male sea lamprey will release pheromones to attract female sea lamprey to their nesting sites (Wagner et al. 2011). According to a study done by Buchinger et al. (2017), there is a negative relationship between mass-adjusted pheromone signaling and total body mass. This study found that females moved towards the odor of smaller males over larger males, and females exhibited similar courtship behaviors in nests that had been treated with large and small male odors. This indicates that the pheromone signal of small males containing components that facilitate nest entry matches that of large males, and even attracts more females (Buchinger et al. 2017). Buchinger et al. (2017) indicates that a better understanding of mate choice in sea lamprey will reveal whether females are deceived into perceiving small males as large, or if differences in pheromone signaling are the result of compensation by small males, or a cost for large males.

The lack of information about both sea lamprey mate preference as well as general mating behavior made it difficult to find literature that supported either if small or large males were more suitable mates. I used a two-way mate preference experimental set

up in the field during the sea lamprey mating season. This study is a beginning in understanding how sea lamprey may exhibit potential preferences in mates and possible ways to evaluate these behaviors.

Table 1. Ethogram created with possible sea lamprey behaviors. All behaviors were characterized as active or non-active.

Behavior	Description	Active or Non Active
Tail fanning	Rapid beating of the caudal fin at the substrate, with sand being swept to the side or into the water column	Active
Rock moving	using oral disk to move rock from one area to another	Active
Attaching	using oral disk to attach to side of net or rock	Non Active
Resting	No movement from lamprey for a duration from two minutes and forward	Non Active
Out of Sight	Not being able to see sea lamprey from observer's view	N/A
Other	Undocumented behavior that sea lamprey preforms	N/A
Forward Swimming	Largely done by the caudal fin with the pectoral fish being used for changing directions	Active
Searching	Slow or moderate swimming by a fish that is neither interacting directly with other fish nor showing a recognizable motor pattern	Active
Body Shake	Lateral shaking of the entire body, two or three times in rapid succession.	Active
Close-swim	Near a nest site, when a female enters. Male moves rapidly towards female. Both fish expand their fins	Active
Skim	Male moving its venter above the substrate. Body vibrates slightly, and head is held a bit higher than the rest of the body. Females exhibit same pattern, though her venter rests directly against substrate	Active
Spawning	Skimming, with release of eggs and milt. Can be a long or short occurrence. Short breaks occur during spawning. Spawning terminated by female who left nest not to return	Active

Table 2. Regression coefficient estimates (B) and upper and lower 95% confidence limits for estimates.

Variables	B	95% CL
<i>Right side large male + small male activity + large male activity + SMW + SMW side + Female activity + Female repeat</i>		
Right side large	1.141	0.930 – 1.352
Female repeat	-0.115	-0.325 – 0.095
SMW	0.558	0.117 – 0.996
SMW side	-0.214	-0.463 – 0.036
Female activity	0.067	-0.145 – 0.279
Small male activity	-0.066	-0.324 – 0.192
Large male activity	-0.241	-0.491 – 0.007

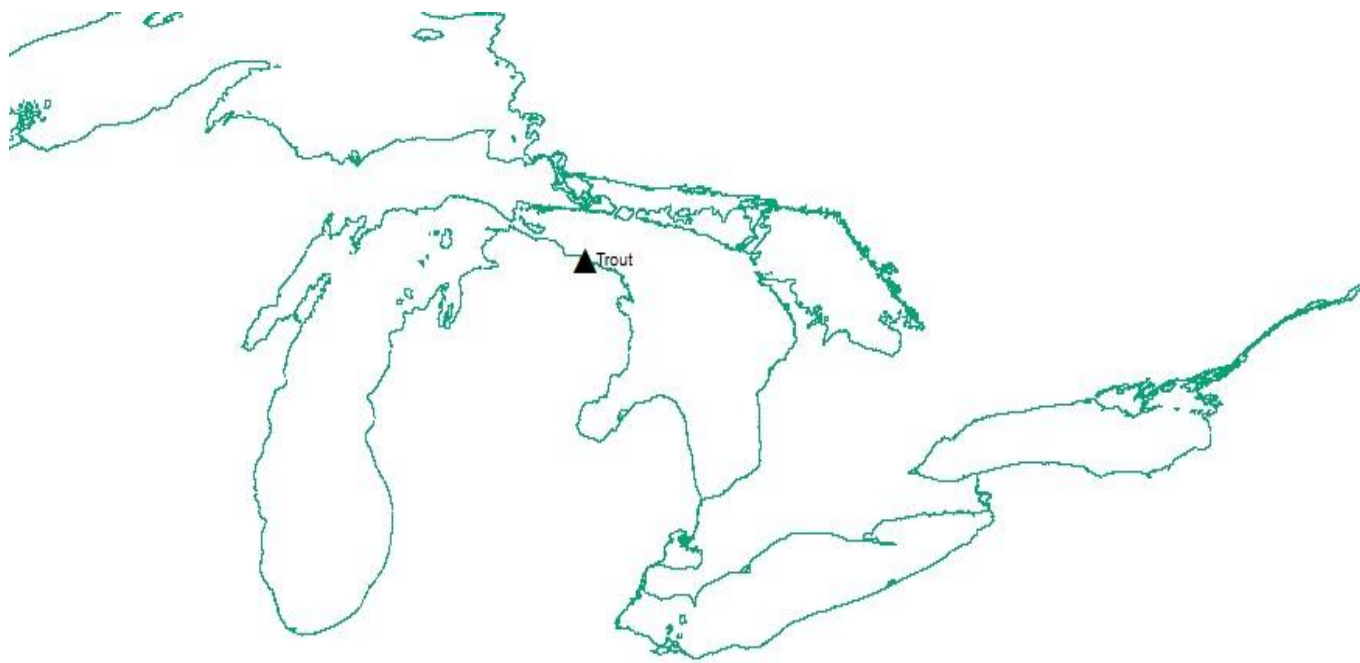


Figure 1. The Trout River is the location of the stream used to perform trials to determine mate preference of female sea lamprey.

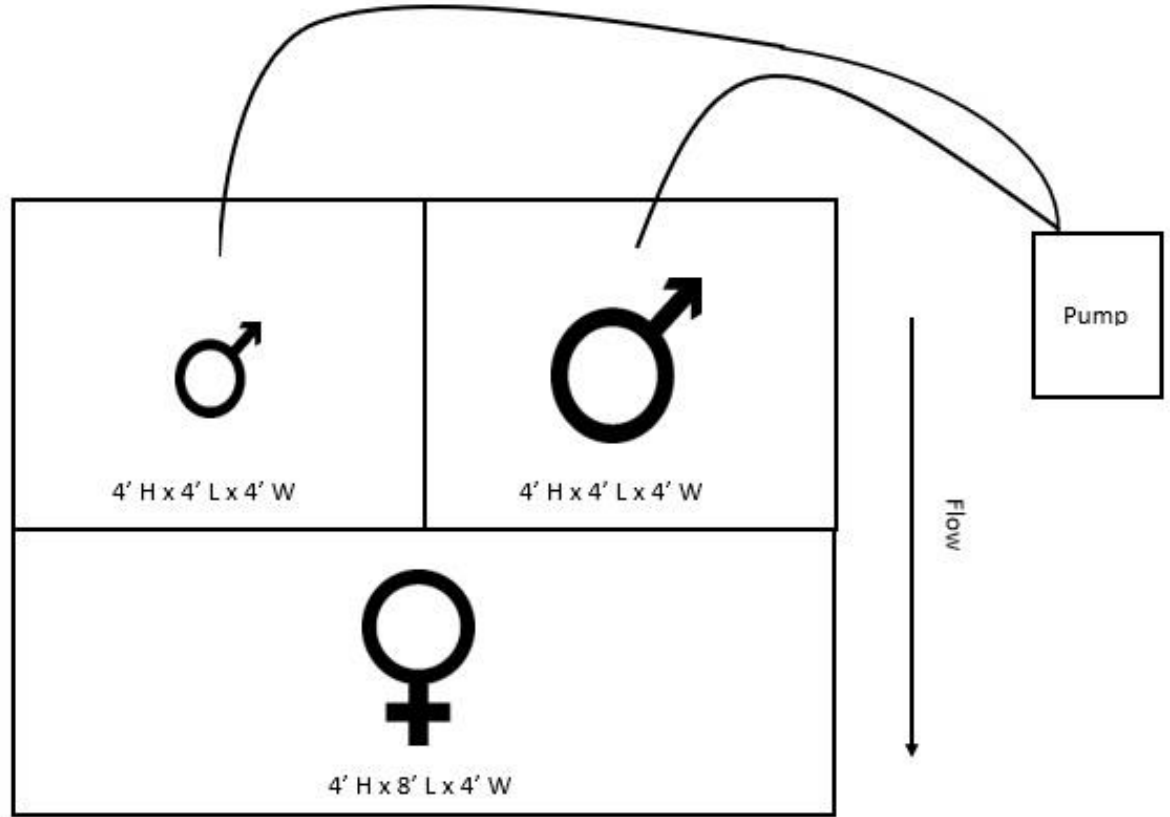


Figure 2. The experimental setup for assessment of female preference for male size (top view). The image depicts the areas where the female and the two males are in their netted boxes, the direction of stream flow, and where the peristaltic chemical metering pump was positioned.

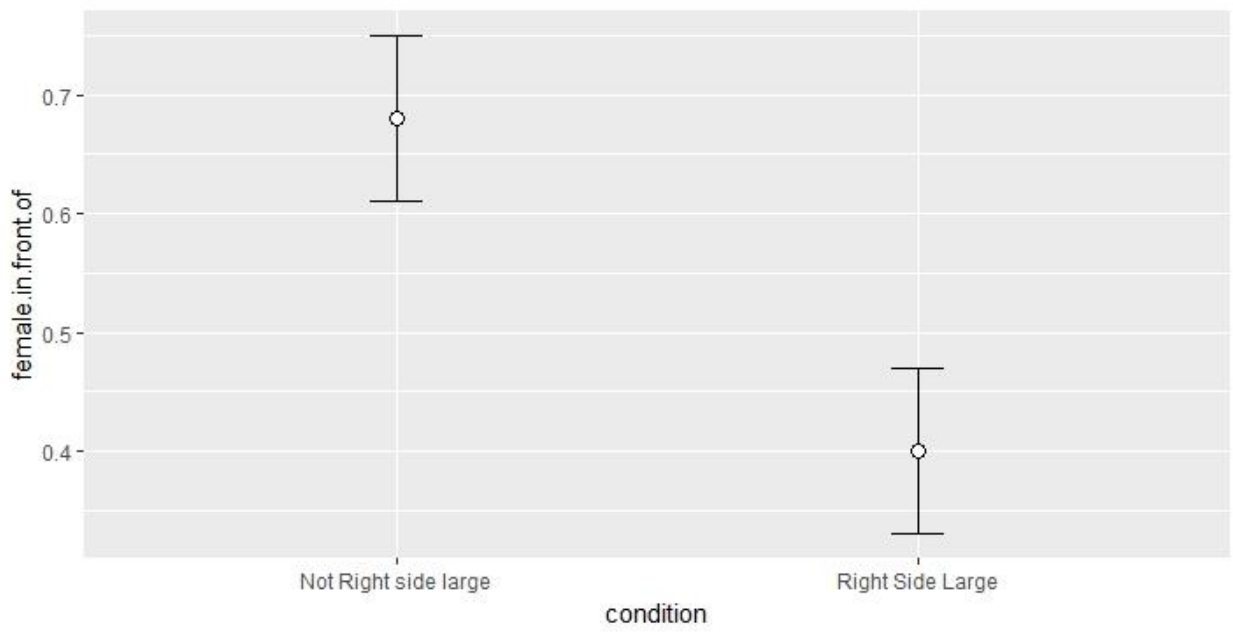


Figure 3. Estimated marginal means plot of the significant variable right side large, indicating that the female preferred to be on the left side of the mesocosm. Estimated marginal means plots indicate the mean response for a variable, adjusted for any other variables in the model.

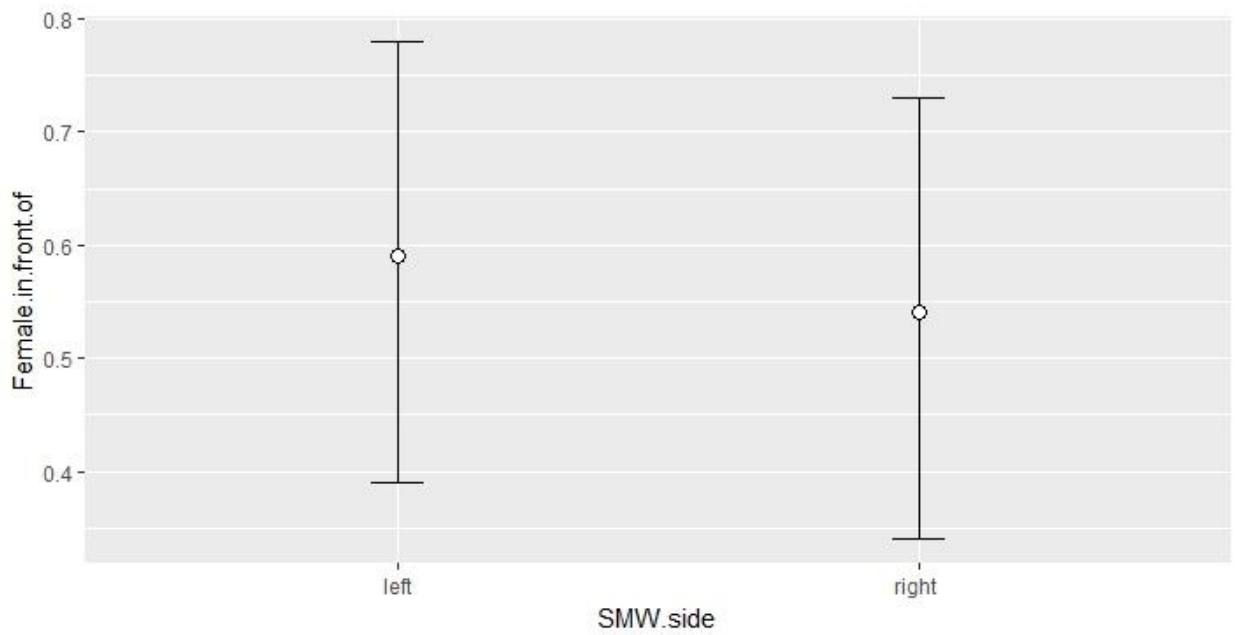


Figure 4. Estimated marginal means plot of the significant variable of spermiating male washings (SMW) being released on the right or left side of the stream. Female preferred the pheromone being released on the left.

Chapter 2

APPLICATION OF THE GROWING DEGREE DAY METRIC TO REVEAL PATTERNS OF GROWTH AMONG LARVAL SEA LAMPREY

Abstract

Invasive sea lamprey (*Petromyzon marinus*) have negative effects on important game fish in the Great Lakes region. Improving the accuracy of larval sea lamprey growth models would benefit management strategies by better predicting the timing of metamorphosis to the damaging parasitic stage. The primary technique used to describe fish growth is the von Bertalanffy growth equation. However, the growing degree day metric (GDD) may allow for better predictions of growth during the sea lampreys' larval life stage. I used larval growth data from Jones et al. (2003) and Dawson and Jones (2009) of larvae collected between the years 1998 and 2005 from across the Great Lakes basin. Water temperatures were obtained as much as possible during the larval growth time frame for each stream. In streams where water temperature was not available, I deployed temperature loggers from July 17th, 2016 to July 20th, 2017 and obtained air temperature from nearby weather stations for the same time period to establish a relationship between air and water temperature. This allowed me to predict water temperatures experienced by larvae in each of our study streams during the larval growth timeframe. The data was log-transformed to be able to use linear models to determine if calendar days or growing degree days (GDD) better predicted growth. A generalized linear model was used to determine the relationship between the response variable (log-transformed length at age) and either log-transformed calendar days or GDD. I also tested streams and lake as predictors in each model to determine the model that best predicted

larval growth. Larval growth was best predicted using basinwide data by a model containing log-transformed calendar days and lake. However, larval growth in Lake Ontario (the warmest lake) was best predicted by growing degree days.

Introduction

Improving the accuracy of larval sea lamprey growth models would benefit management strategies by improving predictions of when metamorphosis may occur. The primary method of controlling sea lamprey involves applying lampricides to streams before sea lamprey metamorphose and emigrate out of the streams into the lakes to parasitize fish, and streams are ranked for lampricide treatment based on the cost per expected larvae killed (Christie et al. 2003). Thus, to improve the selection of streams to be treated with lampricide a better understanding of larval sea lamprey growth rates is needed (Hansen et al 2003). Current fish growth models use calendar time to predict growth (Grebeldinger 2008, Dion and Hughes 2011, Uphoff et al 2013). However, there could be other possible factors that contribute to growth such as population density, stream productivity, and temperature (Jones et al 2003, Neuheimer and Taggart 2007, Venturelli et al 2010).

The greatest impediment to most fish growth models, including the pervasive von Bertalanffy growth function (VBGF; von Bertalanffy 1938) is their dependence on calendar time to explain length variation (i.e., length-at-age; Neuheimer and Taggart 2007). Such dependence explicitly ignores time-dependent and physiologically meaningful variables (Neuheimer and Taggart 2007). Growing degree days (GDD) are a common temperature index that allows development to be correctly scaled to the

physiology that drives ectotherm growth and development (Neuheimer and Taggart 2007, Grebeldinger 2008, Venturelli et al 2010, Dion and Hughes 2011, Uphoff et al 2013). Sea lamprey growth has been estimated by sea lamprey control agents using an incomplete GDD metric. Sea lamprey are assumed to only grow within the spring and summer season, and populations in the same latitudinal range are assumed to all experience the same linear growth pattern. However, the growth of known-age populations were not accurately estimated using this incomplete method (H. Dawson, personal communication).

A more rigorous, complete GDD metric should be used that assesses growth throughout the year by summing average air temperature above 5 °C (minimum temperature thought to be required for any growth to occur). Incorporating predictors that likely affect larval sea lamprey growth such as lake or stream (which may be more or less productive) to better predict growth in this life stage. Using a GDD metric that takes into consideration a minimum temperature required for growth to occur should allow for better predictions of growth during the sea lampreys' larval life stage. Results from several studies (Neuheimer and Taggart 2007, Grebeldinger 2008, Venturelli et al. 2010) show that fish length was predictable using a complete GDD metric. Due to high predictability observed in other studies, a complete GDD metric may allow for a more accurate prediction of larval sea lamprey growth. Further, results from GDD could reveal if climate change may influence the recruitment of parasitic sea lamprey by increasing the growth rate of larvae (King et al 1999, Neuheimer and Grokjer 2012).

If water temperature larval sea lamprey experienced was absent, air temperature can be used as a substitute (Stefan and Preud'homme 1993, Pilgrim et al. 1998). Water

temperature has been predicted from air temperature in studies when water temperature was unavailable (Stefan and Preud'homme 1993, Pilgrim et al 1998, Webb et al. 2003, Stoneman and Jones 2011). Water and air temperatures have a statistical relationship, and the sensitivity and explanatory power of simple water–air temperature regression models based on daily data can be improved by incorporation of a lag. Air temperature was used as an independent variable of stream temperature because it can be a surrogate for changes in heat that affect the water surface (Webb et al. 2003).

In this study I used data from Jones et al. (2003) and Dawson and Jones (2009) of larvae collected from multiple years across the Great Lakes basin, in which larvae were aged by years since last stream treatment or age was known. A statistical model described in Dawson and Jones (2009) used length-frequency data to determine the mean length-at-age using a von Bertalanffy growth function. I predicted water temperature from air temperature for streams where water temperature was unavailable and determined whether variation in larval length-at-age was better predicted using the complete GDD metric or calendar days. I also evaluated whether adding predictors such as lake or stream improved predictions of larval length-at-age.

Methods

For this study, I used larval sea lamprey data from Jones et al. (2003) and Dawson and Jones (2009) where spawning-phase sea lamprey were introduced above barriers in streams or established themselves in streams after lampricide treatments, and larvae were sampled in subsequent years. Thus, I know the approximate time of birth and burrowing date (the start of exogenous feeding) for larval sea lamprey in 35 streams across the Great

Lakes Basin, and length and weight of larval sea lamprey at time of sampling (Figure 1). Multiple age-classes were established in these streams, and age-classes were separated using larval length in an objective, likelihood-based model described in Dawson and Jones (2009) and Potts et al. (2015). This model assumed that larval sea lamprey grew according to a von Bertalanffy growth function, and that individual variation in length increased linearly with age. The length-at-age distribution and cohort group were recorded for each year of data in each stream. The mode of larval length for each length-at-age distribution was used as the mean larval length for each age-class. I combined the data across years for each stream to mimic a mixed-age population, and had the model predict the length distribution for each age class for each year for which I had data (Table 1).

Where water temperature was unavailable during the larval growth period, a relationship between air temperature and water temperature needed to be established. First, air temperature was obtained from the closest National Oceanic and Atmospheric Administration (NOAA) or Environment Canada weather station to each stream mouth for the period from July 17th-20th, 2016 to July 17th-20th, 2017. This data included the following information: weather station ID, weather station name, date, air temperature maximum, fixed air temperature maximum, air temperature minimum, and fixed air temperature minimum. Using ArcGIS, the latitude and longitude of each stream mouth was collected and recorded, as well as the location of the nearest weather station to each stream. If air temperature data from the afore mentioned period was not available from the closest weather station, the next closest weather station was used. Water temperature for each stream during the period July 17th-20th, 2016 to July 17th-20th, 2017 was

collected by placing a temperature logger (Onset HOBO U22 Water Temperature Pro v2, Bourne, Massachusetts) in each stream with latitude and longitude and landmarks recorded. HOBOWare software (Onset Computer Corporation., Bourne, Massachusetts) was used to set up the temperature loggers, with the loggers set to record the temperature every hour. I deployed the temperature loggers in a way to keep the temperature logger under water and reduce the risk of losing the temperature logger. Specifically, a 30-cm piece of PVC pipe was used as a vessel to hold the temperature logger. A metal bolt (25.4 cm) was placed through the PVC pipe, and a chain (.6 m) was placed on the bolt inside of the PVC pipe with a metal nut placed on each end. The temperature logger was placed on the metal bolt and sat between the two nuts. The chain was connected to either a tree or into the ground next to the stream by pounding a metal stake into the ground. Each temperature logger was labeled with the name of the stream on the outside of the PVC pipe. The latitude and longitude of where each temperature logger was placed was recorded using a Garmin Gpsmap handheld GPS (Oregon 300, Schaffhausen, Switzerland). Water samples were collected from each stream from July 17th – 20th 2017. Each water sample was stored within a 354-mL container within a cooler until it could be placed in a refrigerator. Water samples were taken to Hammond Bay Biological Station (HBBS) and titration was used to determine alkalinity, as alkalinity is often used as a surrogate for stream productivity which can affect growth. Low alkalinity was determined to be 0-89 mg/L, moderate alkalinity was 90-157 mg/L, and high alkalinity was 158-225 mg/L.

I examined whether water temperatures can be approximated as a linear function of air temperature as expressed in equation 1 below

(1)
$$T_w = T_a - L$$

where T_w = water temperature, T_a = air temperature, and L = lag. I evaluated the amount of lag that was best (0 days, 1 day, or 2 days) between air temperature and water temperature during the period in which water temperature was collected by temperature loggers. To do this, I evaluated average R^2 across all streams when incorporating the three different lag times. The time lag with the highest average R^2 value across all streams was used in the equations for each stream to predict water temperature from air temperature when water temperature was not available. I used these relationships calculated for each stream to predict water temperature from air temperature during each year for which larval growth data was collected. In order to figure out the GDD each age-class of sea lamprey experienced in each stream, I summed up the average daily water temperature above 5 °C for all days each age-class of lamprey had been exogenous feeders. Sea lamprey larvae become exogenous feeders once they burrow, and sea lamprey burrowing dates were determined by Department of Fisheries and Oceans Canada, which was based on stream latitude. Because larvae were collected over a period of multiple days, I calculated a minimum GDD and a maximum GDD, from exogenous feeding to earliest and latest collection dates, respectively. Data was log-transformed to better predict using generalized linear models.

Generalized linear models were carried out using the generalized linear model command in R Foundation for Statistical Computing (R Core Team, 2013, Vienna, Austria). Generalized linear models were used to determine the best fit between (log-transformed length-at-age) and calendar days or GDD. I also tested the predictor

variables lake and stream in each model. Equation 2 below shows the model with calendar days and the other predictors, lake and stream.

$$(2) \quad \ln(L_L) = \alpha + L_C + L_I + S_I$$

α = intercept

L_L = Log-transformed
Length-at-age

L_I = Lake Identification

L_C = Log-transformed calendar
days

S_I = Stream Identification

I also tested the reduced models and followed the same procedure for GDD calculations (Table 3). Akaike information criteria with small sample size correction (AICc) was used to evaluate the performance of each of the candidate models (Burnham and Anderson, 2002). These variables (Lake Identification and Stream Identification) were important to test because they could be significant factors that change how growth of larval sea lamprey is calculated. Number of parameters, AICc weights, and strength of evidence were calculated for each of the models.

Results

Length-at-age vs. age for all sea lamprey populations shows the different larval growth rates by lake (Figure 2). Of the 22 temperature loggers that were deployed, a total of 19 were retrieved, five from Lake Michigan streams, six from Lake Huron streams, one from a Lake Ontario stream, and six from Lake Superior streams. Results from alkalinity testing does not show a clear relationship with growth rate of sea lamprey (Figure 3). Alkalinity was only tested on water samples collected on one day in each stream during the duration of the study. The time lag with the highest average R^2 value across all streams was the one day time lag (Table 2). The best fit model using basin wide

data was log-transformed calendar days and lake (Table 3). Log-transformed calendar days and stream was the second best fit model (Table 3). The third best fit model was log-transformed GDD and lake (Table 3). Model regression coefficient estimates, exponentiated regression coefficients, and upper and lower 95% confidence intervals were reported for the best fit models (Table 4). Rivers that had a net positive or negative effect on log-transformed length-at-age for the second best fit model were Au Sable, Au Gres, Big Garlic, Duffins, Grafton, Hog Island, Misery, and Trout rivers (Table 4).

I also tested the effects of log-transformed calendar days and log-transformed GDD on log-transformed length-at-age with only populations from each individual lake. When Lake Superior was analyzed as a reduced model, it showed no difference between using GDD or calendar days as the best predictor of larval growth (Table 5). An AICc difference between two models of 2 or less provides little evidence for one model over the other (Burnham and Anderson 2002). The best predictor for larval growth for Lake Ontario was the GDD metric (Table 5). Lake Michigan and Lake Huron were both better predicted by using calendar days (Table 5).

Discussion

When comparing lake to stream, lake was the better predictor of sea lamprey growth. One possible reason why lakes could be better predictors of growth are variables such as latitude and longitude, pH, and conductivity. Fortin et al. (1996) looked at regional determinants of growth and body condition of lake sturgeon (*Acipenser fulvescens*). The results from the study showed that growth and body condition were different when it came to lakes and rivers that were at different latitudes and longitudes. Growth rates decreased when the mean annual air temperature decreased. Increasing

latitudes and temperature were the best predictors for a complete data set. Important factors that also affected the body condition of lake sturgeon were pH and conductivity. Fish production was generally higher in more mineralized, buffered, alkaline waters and at higher mean annual temperatures (Fortin et al. 1996). These same factors could be affecting why lake was a better predictor for sea lamprey growth than stream.

The best fit model for comparing larval lamprey growth was calendar days and lake (Table 5). This is contrary to other studies. Venturelli et al. (2010) discovered that using GDD metric on populations of walleye (*Sander vitreus*) represented a better model of fish growth than previously used models. Neuhemier and Taggart (2007) also used GDD to look at growth rates of Arctic grayling (*Thymallus arcticus*) to determine if von Bertalanffy could be replaced by GDD. The results showed that GDD was a better predictor (Neuhemier and Taggart 2007). When looking at individual lakes, Lake Ontario was the warmest lake and was best predicted by GDD. GDD may prove to be a better model in a warming climate. Neuhemier and Grokjer (2012) studied how a warming climate might affect the growth of North Sea Atlantic cod (*Gadus morhua*) using GDD metric to compare year classes. The results from the study first noted that temperatures had a significant increase between years 1979 to 2010. North Sea Atlantic cod growth trends showed that growth was affected by temperature and explained why North Sea Atlantic cod became mature earlier later years (Neuhemier and Grokjer 2012). Warmer temperatures could explain why GDD works better in warmer waters (Lake Ontario). Global warming may increase the chance of GDD metric working better to predict sea lamprey growth (Lake Ontario). Increased rate of sea lamprey maturity would cause

negative effects in the Great Lakes ecosystem. If sea lamprey are able to metamorphosis faster, it would result in an increase in recruitment.

Table 1. The years that rivers were visited to collect information of larval lamprey growth from Jones (2003) and Dawson and Jones (2009).

River name	Years of larval growth
Albany	1998-2000
Au Gres	1999-2000
Au Sable	1998
Betsie	1998-1999
Betsy	1998
Big Carp	1999-2005
Big Garlic	1999-2005
Big Manistee	1998
Bowmanville	2000-2001
Carp	1998-2005
Deer	1999-2000
Devil	1999
Duffins	2000-2001
Firesteel	1999
Grafton	2000-2005
Hog Island	2000
Koshkawong	1999
Little Manistee	1998-1999
Miners	2000
Misery	1997-2000
Ocqueoc	2000
Ogemaw	2000-2001
Ogontz	2000
Pancake	1998
Port Britain	2000-2005
Rock	1997-2000
Sterling	1999
Sterling Valley	1999
Stokely	1998-1999
Thessalon (Bridgeland)	1998-2000
Trout	1999
Wolf	1998-1999

Table 2. Calculated lag day (R^2) values for sampled rivers. Day lags of 0, 1, and 2 were calculated comparing water to air temperatures.

River Name	Lag Day 0	Lag Day 1	Lag Day 2
Albany	0.8489	0.8661	0.8873
Au Gres	0.9014	0.8672	0.8322
Au Sable	0.8517	0.8514	0.8515
Betsie	0.7585	0.7668	0.7763
Betsy	0.8412	0.8674	0.8857
Big Carp	0.7727	0.7755	0.76295
Big Garlic	0.7426	0.7741	0.8077
Big Manistee	0.8194	0.8393	0.8818
Bowmanville	0.81535	0.80175	0.77445
Bridgeland	0.7361	0.73815	0.74085
Carp	0.7847	0.7911	0.786
Deer	0.8375	0.892	0.9168
Devils	0.8423	0.8567	0.8245
Duffins	0.8505	0.8542	0.84
Firesteel	0.8441	0.8242	0.7973
Furnace	0.7833	0.793	0.8076
Grafton	0.853	0.8669	0.84295
Hogs Island	0.8968	0.8979	0.8588
Koshkawong	0.7271	0.72965	0.7301
Misery	0.8478	0.8641	0.8384
Ocqueoc	0.7508	0.7568	0.7604
Ogemaw	0.9179	0.8687	0.8167
Ogontz	0.8575	0.8517	0.8429
Pancake	0.71065	0.7113	0.70885
Port Britain	0.89545	0.89645	0.86195
Rock	0.8056	0.8184	0.8291
Sterling	0.8548	0.8979	0.8883
Sterling Valley	0.8548	0.8979	0.8883
Stockley	0.8425	0.8469	0.828
Trout	0.7906	0.7884	0.7834
Wolf	0.73985	0.74235	0.73565
Averages	0.81856	0.82562	0.81893

Table 3. Listing of candidate models fit for comparing log-transformed calendar days and log-transformed growing degree day metric. The table includes AICc values, AICc differences (ΔAICc), number of parameters (K), AICc weights (ω), and strength of evidence for each of the models in ascending order of ΔAICc . The dependent variable is log transformed length-at-age (mm).

Explanatory variables	AICc	ΔAICc	K	ω	Strength of evidence
Log transformed calendar days + lake	-229.23	1	4	0.00078	1
Log transformed calendar days + stream	-227.67	1.565	4	0.00122	0.638977636
Log transformed gdd + lake	-218.56	10.673	4	0.00831	0.146631687
Log transformed calendar days	-215.52	13.711	3	0.01067	0.778426081
Log transformed gdd + stream	-210.08	19.157	4	0.01491	0.715717492
Log transformed gdd	-189.2	40.038	4	0.03116	0.478470453
<i>Log-transformed calendar days + stream</i>					
Stream (Misery)	-50.562	178.672	4	0.13906	0.224086594
Stream (Big Garlic)	-49.865	179.369	4	0.1396	0.996114156
Stream (Hog Island)	-16.092	213.142	4	0.16588	0.84154695
Stream (Au Sable)	-16.024	213.21	4	0.16594	0.999681066
Stream (Grafton)	-15.603	213.631	4	0.16626	0.998029312
Stream (Trout)	-15.272	213.962	4	0.16652	0.998452996
Stream (Au Gres)	0.034	229.268	4	0.17843	0.933239702
Stream (Duffins)	29.959	259.193	4	0.20172	0.884545493

Table 4. AICc model-averaged regression coefficient estimates (B) and upper and lower 95% confidence limits for best fit models fit of log-transformed calendar days. Streams are those with a Exp. 95% CL (antilog of the log-transformed 95% CL of the parameter estimate) that indicated a net positive or negative effect on log-transformed length-at-age.

Variables	B	95% CL	Exp. B	Exp. 95% CL
<i>Log-transformed calendar days + lake</i>				
Log transformed calendar days	0.364	0.364 – 0.413	1.439074214	1.439 – 1.511
Lake 1 (Superior)	0.344	0.295 – 0.392	1.410578636	1.343 – 1.479
Lake 2 (Michigan)	-0.164	-0.222 – -0.106	0.848742022	.800 – 0.899
Lake 3 (Huron)	-0.137	-0.216 – -0.059	0.871970226	.805 – 0.942
Lake 5 (Ontario)	-0.223	-0.285 – -0.140	0.800114849	0.752 – 0.869
<i>Log-transformed calendar days + stream</i>				
Stream (Misery)	0.158	-3.124 – -1.457	1.171166195	0.043 – 0.232
Stream (Big Garlic)	0.229	-1.897 – -1.181	1.257342039	0.150 – 0.306
Stream (Hog Island)	0.134	-2.146 – -1.112	1.14339282	0.116 – 0.328
Stream (Au Sable)	0.165	-2.526 – -1.386	1.179393119	0.079 – 0.250
Stream (Grafton)	0.139	-2.617 – -1.419	1.1491241	0.073 – 0.241
Stream (Trout)	0.023	-3.016 – -1.519	1.02326654	0.048 – 0.218
Stream (Au Gres)	0.195	-.270 – -1.273	1.215310986	0.763 – 0.279
Stream (Duffins)	0.14	-2.882 – -1.492	1.150273799	0.056 – 0.224

Table 5. Reduced models tested each lake individually to determine which growth model worked best for each individual lake.

Lake	Number of stream years	GDD (AICc)	Calendar Days (AICc)
Superior	125	-126.2098	-124.1109
Michigan	22	-31.13518	-56.61443
Huron	34	-31.13518	-56.61443
Ontario	27	-45.74424	-24.47676

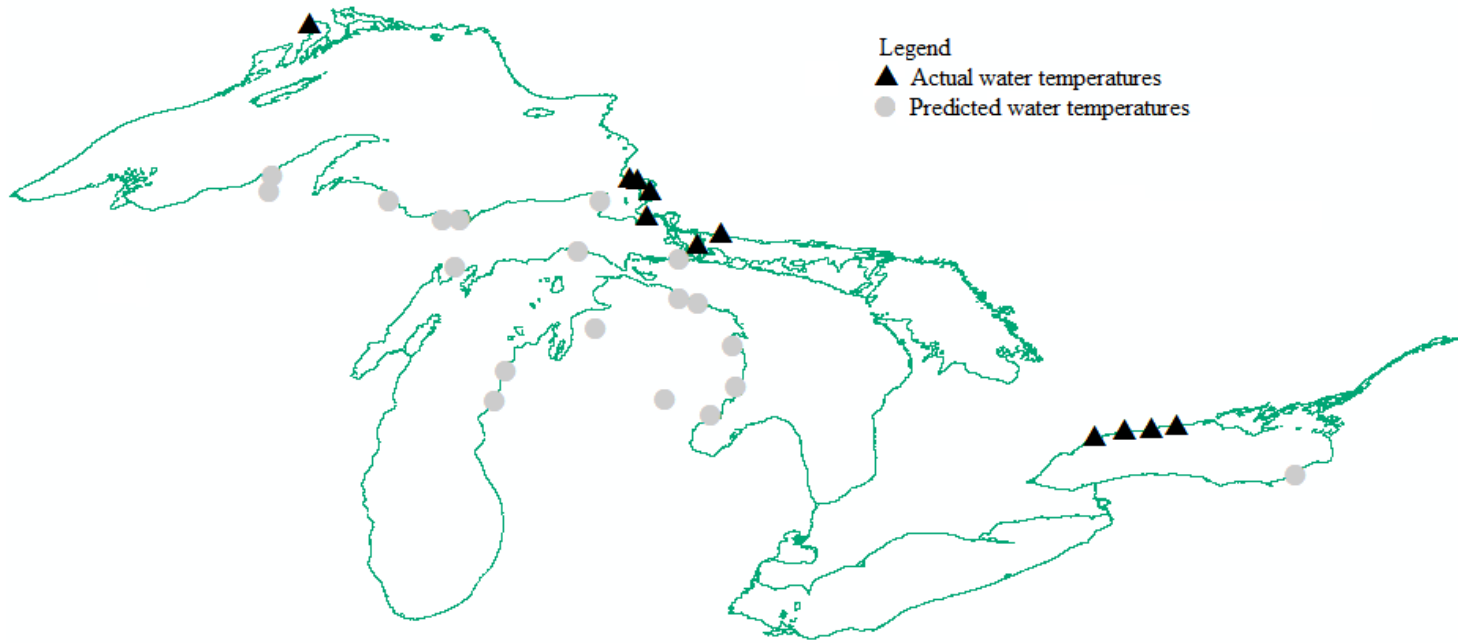


Figure 1. River locations from American and Canadian study sites. Triangles show rivers that actual water temperatures were collected from. Circles represent rivers that had air temperatures to predict the water temperatures.

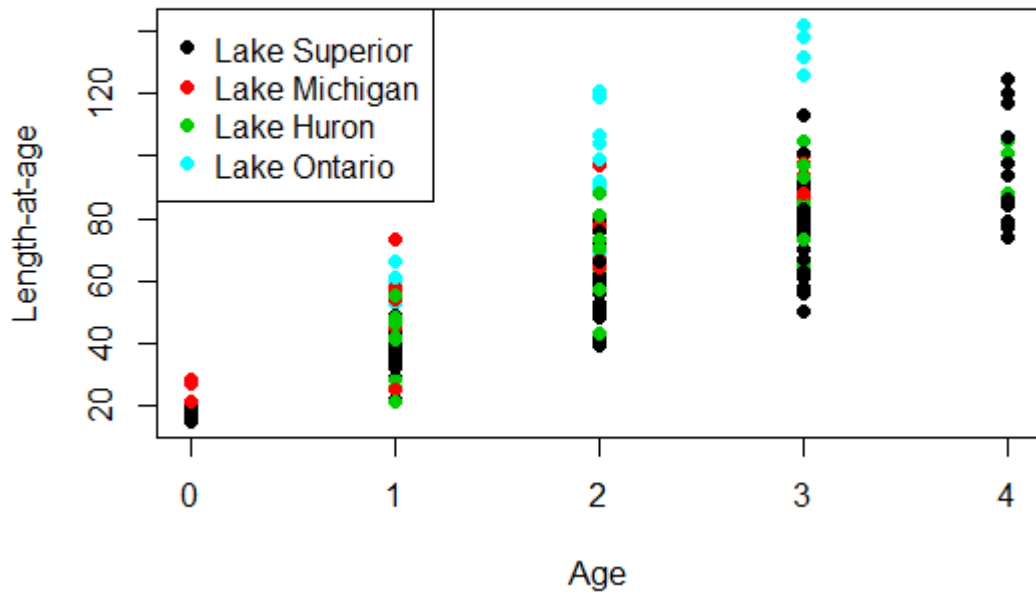


Figure 2. Length-at-age vs. age for all populations of sea lamprey. Colors represent the four different Great Lakes that we tested.

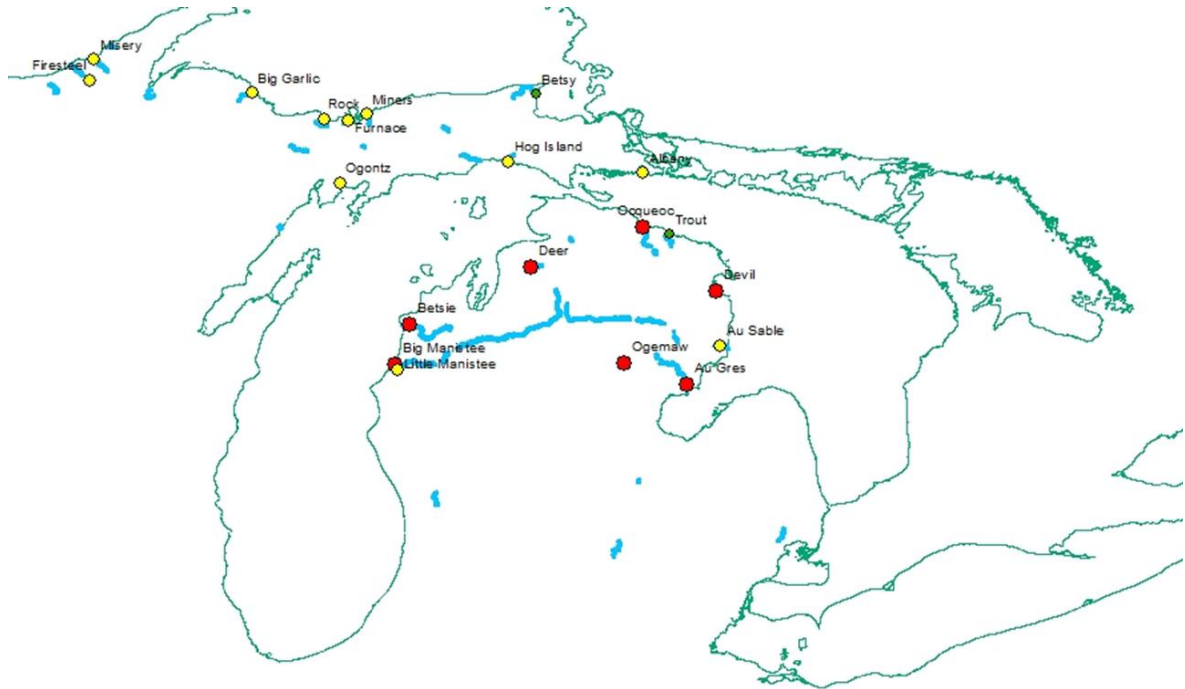


Figure 3. River locations from American study sites. Colors represent different levels of alkalinity. Green is low alkalinity, yellow is moderate alkalinity, and red is high alkalinity.

MANAGEMENT RECOMMENDATIONS

Based on our initial results and lack of prior research involving sea lamprey mate preference and mate behavior conducted, I would recommend another study be conducted investigating ovulating adult female sea lamprey mate preference. Additional studies need to be conducted that look at if females have a size preference in males, and what other factors contribute to mate preference in females. Assessing sea lamprey mating overall would be beneficial because little is known about their mating process.

With recent evidence from Buchinger et al. (2017) supporting mass-adjusted pheromone signaling of smaller males, researchers using SMW as a sea lamprey attractant should consider the size of the males used to create the washings. In Johnson et al. (2009), their methods used five spermiating male sea lamprey of no particular size placed in 20 L of water to create SMW. I created SMW for my experimental design using the same method, as mass-adjusted pheromone signaling of smaller males had not yet been recorded in the literature. Using small or large spermiating male washings to evaluate whether SMW could change the preference of ovulating female sea lamprey may have provided more insight into factors that contribute to mate preference in females.

Based on results from my GDD study, I learned that using a complete GDD metric produced a better predictor of larval sea lamprey growth in warmer waters (Lake Ontario). In the coldest lake, Lake Superior, there was no real difference between a model using calendar days or the complete GDD metric model in predicting larval sea lamprey growth. This is likely due to the fact that the complete GDD metric provides a more accurate representation of larval sea lamprey growth in warmer or colder waters,

where growth differs from the average growth based on just the number of calendar days of life. As the climate warms, and in heterogeneous environments across the basin, the use of a complete GDD metric by sea lamprey control agencies to figure out when larval sea lamprey could metamorphose would be advantageous.

Sea lamprey growth can be influenced by stream productivity, and although alkalinity has been used as a surrogate for productivity, no discernable pattern appeared when comparing alkalinity levels and growth in streams across the basin. However, measuring alkalinity at different points of the year may provide a more accurate representation of alkalinity for each river, which could be related to the amount of larval growth observed in each river. Then, adding alkalinity into the complete GDD metric may improve the prediction of larval sea lamprey growth.

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