INVESTIGATING NEW METHODS TO IMPROVE AGEING AND STUDY MOVEMENT PATTERNS OF LARVAL GREAT LAKES SEA LAMPREY (*PETROMYZON MARINUS*) POPULATIONS

BY

DANIELLE D. POTTS

Masters Degree Thesis

Submitted to
University of Michigan-Flint
in partial fulfillment of the requirements
for the degree of

MASTERS OF SCIENCE

Department of Biology

2012

ABSTRACT

INVESTIGATING NEW METHODS TO IMPROVE AGEING AND STUDY MOVEMENT PATTERNS OF LARVAL GREAT LAKES SEA LAMPREY (*PETROMYZON MARINUS*) POPULATIONS
BY

Danielle D. Potts

Sea lampreys (*Petromyzon marinus*) are parasitic pests in the Great Lakes which have negatively impacted game fish populations. Management efforts to control sea lamprey populations throughout the Great Lakes began in the 1950s and continue today. The primary control technique used is the application of lampricides to streams to kill larvae before they become parasites. A better understanding of larval sea lamprey growth rates, age determination, and habitat preference is greatly needed to improve both selection of streams for lampricide application and to inform models of sea lamprey population dynamics.

Otoliths have been used to estimate age in teleost fish through annuli counts and otolith size metrics. Lampreys do not have otoliths, having instead an analogous structure called a statolith. Determining age based on statolith annuli counts has been found to be imprecise and inaccurate. Therefore, I evaluated whether statolith size is correlated with sea lamprey larva, also known as ammocoete, age using known-age populations of ammocoetes from two contrasting Great Lakes streams. Statolith width was found to be the measurement that better distinguished the age-classes within the populations. When combining length-frequency and statolith width data into a likelihood-based statistical model I was able to more accurately assess ammocoete population age composition for one known-age population than when using only length-frequency data.

Studies of the biology and ecology of Great Lakes sea lampreys (*Petromyzon marinus*) may require an effective means of tagging larvae to track individual movements to better control

this pest species. I evaluated the feasibility of using passive integrated transponder (PIT) and visible implant (VI) Alpha tags in larval sea lampreys at least 100 mm and 85 mm in length, respectively. The use of PIT tags in lampreys as small as 100 mm in length is not suggested unless a tag burden of less than 5% can be achieved. Until a better method of wound closure is found to limit VI Alpha tag loss in lampreys, the use of these tags is not suggested in lampreys as small as those used in this study.

If habitat preference, depth distribution, or in-stream distribution changes as larvae approach metamorphosis, as anecdotal evidence suggests, there is a potential for bias in the current ranking surveys which make the assumption that larval lampreys occupy habitat in the same proportion irrespective of size. To monitor the movement and preferred habitats of large larvae, I marked and released larvae approaching metamorphic size and subsequently tracked their movements. I used three different tagging methods, including passive integrated transponders (PIT) tags, visible implant (VI) Alpha tags, and visible implant elastomer (VIE) tags to mark larvae. Using PIT telemetry and electrofishing, I was able to locate 11% of our released ammocoetes. It was found that the larger ammocoetes moved further distances downstream from release locations than the smaller ammocoetes.

DEDICATION

I would like to dedicate this completed thesis to my husband and family for their continual support of furthering my education and understanding my draw to studying unique animals.

ACKNOWLEDGEMENTS

I first thank my advisor Dr Heather Dawson for all of her support and guidance throughout my graduate program. Her advice, enthusiasm, and assistance every step of the way kept me focused and made me challenge myself to learn and explore new things. I also thank my committee members Ernest Szuch and Teresa Yoder-Nowak for their support, advice, and continual belief in me every step of the way. Thank you also to John Cohoon, Josh Beaulaurier, and Katie Clugston for their help in data collection, company, and light-heartedness that they provided while in the field. I was lucky to have such great assistants.

Thank you to Larry Atherton from University of Michigan-Flint for all of his help in ordering equipment, obtaining funding, and the endless paperwork that he assisted with. Thank you also to Mike Jones, Iyob Tsehaye and Sharon Reasoner at Michigan State University. I thank Sharon Reasoner for organizing availability and pick up of field equipment. Thank you to Dr Mike Jones for the use of his field equipment, advice, and all his help with the statistical model and analyzing data for chapter 1. Thank you also to Iyob Tsehaye for all of his help with altering AD Model Builder to work for my study.

I also thank everyone at the USGS Hammond Bay Station for all of their advice, assistance, and support, especially Nick Johnson, Kathy Jones, and Roger Bergstedt. I am grateful to Lisa O'Connor for helping us with the sea lamprey larvae tagging. Thanks to Dave Keffer, Jeff Slade from U.S. Fish & Wildlife Service, and Steve Yancho from the National Park Service. A special thanks to the staff at Platte River Campground for your hospitality. I also thank Homestead Resort in Glen Arbor, MI for allowing us the use of their property to access the river. Thank you to Jeremy Summerville and Doug Kotnik from Precision Laser & Instrument Inc., and Joe Delli from W. Nuhsbaum Inc. for all of their equipment and technical assistance.

A special thank you to my husband Jeff for all of his support and patience, I could not have done it without you.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

CHAPTER 2

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

INTRODUCTION

Sea lampreys (*Petromyzon marinus*) are a parasitic invasive fish species that were a contributing factor in the collapse of lake trout (*Salvelinus namaycush*), whitefish (*Coregonus clupeaformis*), deepwater cisco (*Leucichthys johannae*) and blackfin cisco (*Coregonus nigripinnis*) populations in the Great Lakes during the 1940s and 1950s (Morman et al. 1980). Sea lamprey entered the Great Lakes from the Atlantic Ocean through a series of shipping canals built to connect the Upper Great Lakes with the Atlantic Ocean (Morman et al. 1980). By 1938 the sea lampreys had invaded all of the Great Lakes, and by the early 1960s lake trout fishery harvests had declined to 2% of pre-invasion levels (Schneider et al. 1996). Beginning in the 1950s and continuing today, management efforts work to control sea lamprey populations throughout the Great Lakes.

The life cycle of sea lamprey in the Great Lakes begins in lake tributaries where fertilized eggs hatch into small, wormlike larvae called ammocoetes which burrow into soft stream bottoms to feed on detritus for 3 to 6 years (Potter 1980). After ammocoetes reach a certain size (~120 mm), they metamorphose into parasitic juveniles and migrate into the Great Lakes where they feed for 12 to 20 months. Mature adults then migrate into streams to spawn, after which they die. The complete life cycle of the sea lamprey, from egg to adult, can take an average of 5 to 8 years to complete.

Juvenile sea lamprey damage their host by attaching to the fish with a suction-cup mouth and rasping a hole through the fish's scales and skin with their tongue so they can feed on its blood and body fluids. During its parasitic lifetime (1 - 2 years) a single sea lamprey has been estimated to destroy between 6.6 kg and 18.9 kg of hosts from May through September (Swink 2003). Bence et al. (2003) calculated values of 1.36, 1.32, and 0.75 lake trout deaths per sea

lamprey in lakes Superior, Huron, and Michigan, respectively. In the Great Lakes, the invasive sea lampreys have no commercial value and no natural predators, and therefore no natural population controls. Due to destruction of valuable fish stocks and the adverse effect of sea lampreys on the ecological balance of fish species in the Great Lakes, the Great Lakes Fishery Commission (GLFC) was established in 1955 by a treaty between Canada and the United States. Their mission is to coordinate efforts to formulate and implement a program to eradicate or minimize sea lamprey populations in the Great Lakes (Pearce et al. 1980).

The sea lamprey control program uses several techniques to reduce abundance of sea lampreys during different stages of their life cycle. The primary technique involves applying a lampricide (3-trifluoromethyl-4-nitrophenol), called TFM, to streams to kill larval lamprey while they are burrowed in the stream bottom. Lampricide removes larvae from Great Lakes tributaries before they undergo metamorphosis, become parasites, and migrate out to the lakes to parasitize Great Lakes fishes (Smith and Tibbles 1980; Brege et al. 2003). TFM is effective at killing ammocoetes, and has a minimal impact on other fish species, aquatic plants, invertebrates, and wildlife (Dahl et al. 1980). Lampricide treatments are believed to remove between 95 and 99% of the ammocoetes from treated streams when effective. Currently, due to the cost of TFM, only about 250 Great Lakes tributaries are treated with lampricide at regular intervals (Steeves 2002). Because the cost of lampricide prohibits treating all streams that will produce parasitic sea lampreys each year, information on larval sea lamprey abundance, growth, survival, and distribution in streams is used to rank streams for lampricide treatment based on the number of larvae approaching metamorphosis that could be killed per dollar. Through biological assessments and careful TFM use, the GLFC and its agents have successfully reduced sea lamprey populations in the Great Lakes by 90% from peak population levels. In 1995, the GLFC

adopted an Integrated Pest Management Strategy, which included defining targets for control that optimize benefits, use of quantitative methods and systems approaches, and application of alternative methods of control (Christie and Goddard 2003).

The GLFC have developed a management strategy that utilizes assessment information on larval sea lamprey abundance and distribution in streams to select a set of streams for lampricide treatment each year (Christie et al. 2003; Slade et al. 2003). Current assessment techniques and population estimates make the assumption that larval lampreys occupy habitat in the same proportion irrespective of size or life history stage. If habitat preference or in-stream distribution changes as larvae approach metamorphosis, as anecdotal evidence suggests, there is a potential for bias in ranking surveys used for the stream selection process. Streams are ranked for lampricide treatment, in part, on electrofishing catches in stratified, habitat-based surveys. These ranking surveys are conducted at reference stations subjectively determined by the managing agents to be representative of the reach as a whole (Anderson 2006), where what is considered to be the highest quality larval habitat available at each access site is sampled. By monitoring transformer and large ammocoete movement and habitat preference, the ability of ranking surveys to provide precise indexes of population abundance and size distribution could be evaluated.

Another way to improve the stream selection process is to find a more accurate way of aging ammocoete sea lamprey. Variability of growth rates has been seen among populations, sections of stream, and even over time (Hansen 2003). The growth rates of sea lamprey ammocoetes are affected by multiple forces including the water temperature, productivity of the stream, and stream ammocoete density. Being able to accurately estimate ammocoete age would benefit management strategies by allowing managers to better predict the timing of

metamorphosis (Christie and Goddard 2003). Treble et al. (2008) found that knowledge of ammocoete age improved length-based predictions of metamorphosis probability for individual sea lampreys. Models that better predict metamorphosis will enable managers to find the most cost-effective method and timing to treat streams with lampricide (Christie and Goddard 2003). Further, greater accuracy in age determination would improve models of sea lamprey stock-recruitment relationships, which are important to determining the expected effectiveness of non-chemical control tactics such as adult trapping (Dawson 2007). Creating a standard protocol to more objectively age ammocoete sea lamprey would be very valuable for sea lamprey control.

Even though data that require age composition data, such as ammocoete growth and survival, would be helpful in ranking streams for treatment, the use of these data sets have been discouraged by the unreliability of age-assessment methods for ammocoetes. Determining age based on visual assessments of length-frequency distributions of sea lamprey body length is subjective and uncertain, and determining age based on counts of annuli on statoliths has been found to be imprecise and inaccurate (Dawson et al. 2009). However, statoliths are the analogous structure in lampreys to otoliths in teleost fishes, and otolith size is more correlated with fish age than is fish length. A known-age population of ammocoetes could be used to evaluate whether statolith size is correlated with ammocoete age. By combining length-frequency and statolith size data in a statistical model, the accuracy of an objective, statistical method for ammocoete age assessment that combines length-frequency and partial age composition data could be evaluated.

In an effort to contribute to knowledge of the ecology of sea lamprey, and improve the selection of streams for treatment by reducing the uncertainty in larval abundance estimates, a better understanding of habitat preference, frequency of movements, and age of larvae approaching metamorphosis must be gained. To address this issue, this study proposes to: 1)

improve aging of ammocoetes by combining length-frequency and statolith size data; 2) use PIT telemetry to monitor transformer and large ammocoete movement and habitat preference; and 3) investigate the feasibility of using various implanted tags to monitor the behavior of ammocoetes.

Chapter 1

ESTABLISHING A RELATIONSHIP BETWEEN STATOLITH SIZE AND AGE OF LARVAL GREAT LAKES SEA LAMPREYS

Abstract

A better understanding of larval sea lamprey growth rates and age determination is greatly needed to improve both selection of streams for lampricide application and to inform models of sea lamprey population dynamics. Growth rates of sea lamprey larvae (ammocoetes) are affected by multiple factors and therefore vary between streams, within sections of streams, and over time. Thus, determining age based on visual assessments of length-frequency distributions is subjective and uncertain. Otoliths have been used to estimate age in teleosts through annuli counts and otolith size metrics. Lampreys do not have otoliths, having instead an analogous structure called a statolith. Determining age based on statolith annuli counts has been found to be imprecise and inaccurate (Dawson et al. 2009). Therefore, I evaluated whether statolith size is correlated with ammocoete age using known-age populations of ammocoetes from two contrasting Great Lakes streams. I used a morphometric system to measure length, width, and height of statoliths from these known-age populations. Statolith width was found to be the measurement that better distinguished the age-classes within the populations. A likelihood-based statistical model was used to assess ammocoete population age composition. When combining length-frequency and statolith width data in the model I was able to more accurately assess ammocoete population age composition for one known-age population than when using only length-frequency data.

Introduction

Being able to accurately estimate larval age would benefit management strategies by allowing managers to better predict the timing of metamorphosis (Christie and Goddard 2003). Natural variation in recruitment, growth rates, and survival of larval sea lampreys makes it impossible to predict with certainty when each stream will require treatment to prevent the downstream migration of parasitic juveniles (Anderson 2006). Therefore, each year a group of candidate streams is assessed to determine which streams have the largest populations of large larvae relative to their treatment cost and thus should be prioritized for treatment (Slade et al. 2003). Variability of growth rates of sea lamprey larvae (ammocoetes) has been seen among populations, sections of stream, and even over time (Hansen 2003). The growth rates of sea lamprey ammocoetes are affected by multiple forces including water temperature, stream productivity, and ammocoete density of the stream. Models that better predict metamorphosis will enable managers to find the most cost-effective method and timing to treat streams with the chemical control program (Christie and Goddard 2003). For example, Treble et al. (2008) found that knowledge of ammocoete age improved length-based predictions of metamorphosis probability for individual sea lampreys. Further, greater accuracy in age determination would improve models of sea lamprey stock-recruitment relationships, which are important in determining the expected effectiveness of non-chemical control tactics such as adult trapping (Dawson 2007).

Creating a standard protocol to more objectively age ammocoete sea lamprey would be valuable for sea lamprey control. Even though age composition data such as ammocoete growth and survival would be helpful in ranking streams for treatment, the use of these data have been discouraged by the unreliability of age-assessment methods for ammocoetes. Determining larval

sea lamprey age based on visual assessments of length-frequency distributions is subjective and uncertain (Dawson et al. 2009). Determining age based on counts of annuli on larval sea lamprey statoliths, the analogous structure to teleost otoliths, has been found to be imprecise and inaccurate (Dawson et al. 2009). Interpreting annuli formation on statoliths can also be more difficult than observing annuli on otoliths in teleost fishes due to limitations imposed by the chemical composition and size of statoliths (Dawson et al. 2009). However, Beamish and Medland (1988) state that statolith growth patterns appear to be tightly correlated to sea lamprey larvae body size and growth rates. Thus, just as otolith size can be used to infer fish age (Francis and Campana 2004), and has been used as a predictor in previous studies (Boehlert 1985; Pawson 1990; Worthington et al. 1995), statolith size could potentially be used to infer larval lamprey age.

A known-age population of ammocoetes could be used to evaluate whether statolith size is correlated with ammocoete age. By combining length-frequency and statolith size data in a statistical model, the accuracy of an objective, statistical method for ammocoete age assessment that combines length-frequency and partial age composition data could be evaluated. I propose to evaluate whether statolith size is correlated with larval age using a known-age population of ammocoetes from two contrasting Great Lakes streams established in a previous study (Dawson et al. 2009). I also propose to evaluate a statistical method for ammocoete age assessment that combines length-frequency data with partial age composition data (using statolith size to infer age) to more accurately assess ammocoete population age composition.

Methods

Known age population of ammocoetes

Statoliths were obtained from known-age ammocoete populations established in a previous study by Dawson et al. (2009). Spawners were released above barriers in the Big Garlic River, a cold, low-alkalinity (mean alkalinity = 52mg/L $CaCO_3$) tributary of Lake Superior, and in Ogemaw Creek, a warmer, high-alkalinity (mean alkalinity = 175mg/L $CaCO_3$) tributary of Lake Huron (Fig. 1), in 2002 and 2003 respectively (adult sea lampreys spawn only once before they die). The rivers were then sampled for ammocoetes every summer through 2007, producing ages of 1, 2, 3, 4, and 5 for Big Garlic River and 1, 2, 3, and 4 for Ogemaw Creek. Ammocoetes were measured and their statoliths removed and transferred into individual wells filled with immersion oil for 9-18 days to improve visibility. They were then assigned unique random numbers, sealed in Crystal Bond™ adhesive, and placed on microscope slides (Dawson et al. 2009).

Statolith measurements

In order to take measurements of the statoliths, I used a morphometric system, which included a digital microscope camera and software (W. Nuhsbaum 2012), a digital microscope, and computer to measure the length, width, and height of each prepared statolith. The Crystal Bond™ adhesive was heated until softened and the statolith manipulated into the position needed for measurement with assistance of a compound microscope. The slide was then transferred to a microscope fitted with the Q-Imaging Micropublisher camera to capture the image. Measurements of the statolith image were then taken using the Q-Imaging Professional Version 7.0 software (2012) on the computer (W. Nuhsbaum 2012). Statolith length and width

measurements were taken from the top angle while the statolith was flat on its bottom. The statolith is flat when sides appear to be relatively symmetrical (Fig. 2). To measure statolith length, I measured down the middle of the statolith, and the width was measured at the widest point (Fig. 2a). The height measurement was collected by then placing the statolith on its side and measuring from the tallest point straight down (Fig. 2b).

When taking length and width measurements it is important to make sure that the statolith is as flat on its bottom as possible. If it is not, this will lead to significantly different measurements, especially in statolith width. The best way to ensure that the statolith is flat is to make sure that the edges of both sides are equally in focus. Collecting the statolith length measurement by measuring in the middle of the statolith does not always yield the greatest length of that statolith, but does allow for consistency among statoliths.

A one-way ANOVA was used to test for differences between statolith measurements (length, width, and height) between each of the age classes for both the Big Garlic River and Ogemaw Creek. This analysis was carried out using the ANOVA command in SPSS® version 20 (IBM Corp., 2011).

Estimating proportion at age

In this study I used an objective, likelihood-based statistical model successfully used previously by Dawson et al. (2009) to evaluate a statistical method for ammocoete age assessment. The model combines length-frequency data with partial age composition data (in this study I use statolith size to infer ammocoete age). This method was developed and reported by Francis and Campana (2004), with a reparameterization after Schnute and Fournier (1980). It was then amended by Fournier (1983) to include age composition information from a sample of fish from which a calcified structure was removed.

This method requires assumptions about how the fish in the population grow and how individual growth rates vary. I assumed that lengths for each age-class are normally distributed and that their standard deviation increases as a linear function of age (ammocoetes have been shown to follow this growth pattern). I also assumed that the mean length at age of the ammocoetes followed a von Bertalanffy growth function (growth slows with age). These assumptions allow the prediction of the length-at-age composition of a mixed-age population, which can then be compared to actual data. Using maximum likelihood methods, parameters describing growth and variation in growth that best fit observed data were obtained, and used to infer population age composition (Dawson et al. 2009). I estimated the most likely proportions at each age using a multinomial log-likelihood function implemented in AD Model Builder (Otter Research 2000).

I used statolith size to infer age by measuring the length, width, and height of at least one hundred statoliths from a subsample of the ammocoetes whose total lengths were also measured, and including the statolith metric in the model which most distinctly separated the age classes. I used a one-way ANOVA and Tukey post-hoc comparisons to determine which metric more distinctly separated the age-classes of the populations. This statolith metric was included in the model by incorporating the data using the growth function that this metric followed (e.g., von Bertalanffy growth function, linear growth function) and by adding a second multinomial likelihood term to the objective function. I assumed that statolith widths for each age-class are normally distributed and that their standard deviation increases as a linear function of age.

The precision and accuracy of the model estimates were evaluated. Precision of the model estimates of proportion-at-age was evaluated by deriving approximate confidence limits from likelihood profiles. Accuracy of the model estimates was tested by estimating the

proportion at age of the two known-age populations when ammocoete samples from several years were combined into a single length-frequency data set (Dawson et al. 2009). I used the statistical model to estimate the proportion at age using the ammocoete length-frequency information alone and then combined with the chosen statolith metric data. The estimates were then compared to the known proportion at age of the two populations.

Results

Statolith measurements

A total of 333 statoliths were examined. There were 58 statoliths in which no measurements were collected due to an unreadable or broken sample. Of the remaining statoliths, there were 119 from the Big Garlic River and 104 from Ogemaw Creek in which at least one measurement was collected. A total of 52 additional statoliths of unknown ages from another river were also included to eliminate any reader bias (i.e., knowledge of age-classes of the known-age populations).

An increase in average ammocoete length as they age is seen in both Big Garlic River and Ogemaw Creek known age ammocoetes. Growth slows with age in each population, thus ammocoete growth follows a von Bertalanffy growth function (Fig. 3). An increase in statolith width with age was observed in both known-age populations (Fig. 4).

Length-frequency distribution graphs of statolith widths for all ages combined and with each age class individually for Big Garlic River (Fig. 5) and Ogemaw Creek (Fig. 6) show distribution of width measurements among the known age samples. Statoliths of 4 year old ammocoetes from Big Garlic River as well as 3 year old ammocoetes from Ogemaw Creek were

lost due to a temporary change in preservation methods; statoliths were left in immersion oil too long while being examined for annuli rings at Michigan State University by Heather Dawson.

A one-way ANOVA was run between each of the four age classes for Big Garlic River and found that statolith length, width, and height measurements all differ significantly between age classes ($p < .05$). Statolith length, $F\,(2, 108) = 8.045$, $p < .0001$, width, $F\,(3, 113) = 17.796$, $p < .0001$, and height, $F\,(3, 88) = 3.889$, $p = .012$, measurements were all found to differ significantly between age classes. Tukey post-hoc comparisons for statolith length were only significantly different between age classes 1 and 5, $p = .001$, and 2 and 5, $p < .0001$. The Tukey post-hoc comparisons for statolith width showed that each of the age classes, 1, 2, and 3, differed significantly from age class 5, $p < .0001$. The only significant differences between age class for statolith height were between age classes 3 and 5, $p = .009$. Statolith height was only measureable for a portion of the statoliths due to difficulty of positioning.

A one-way ANOVA was run between each of the three age classes for Ogemaw Creek and found that statolith length, width, and height measurements all differed significantly between age classes ($p < 0.05$). Statolith length, $F\,(2, 99) = 42.437$, $p < .0001$, width, $F\,(2, 100) = 69.760$, $p < .0001$, and height, $F\,(2, 80) = 19.361$, $p < .0001$, measurements were each found to differ significantly between the age classes. Tukey post-hoc comparisons for statolith length found significant differences between all age classes, ages 2 and 4 from age 1, $p < .0001$, and age class 2 from 4, $p = .001$. The Tukey post-hoc comparisons of the statolith width between age classes show that age class 1 was significantly smaller than both age 2, $p < .0001$, and age class 4, $p < .0001$. Statolith widths of age class 2 were also significantly different than those of age class 4, $p < .0001$. Tukey post-hoc comparisons between age classes of statolith height found age 1 to differ significantly from age 2, $p = .0001$, and age 4, $p < .0001$, as well as age 2 to differ

significantly from age 4, $p = .0028$. Statolith height was only measureable for a portion of the statoliths due to difficulty of positioning.

Estimating proportion at age

Estimated proportions of age were similar to the true proportions of age for both the Big Garlic River and Ogemaw Creek when using only ammocoete length data in the model. When using both ammocoete length and statolith width (to infer age) data, the model was more accurately able to estimate proportion at age for all age classes of ammocoetes from Ogemaw Creek (Fig. 6), but not those from Big Garlic River. For Big Garlic River, ages 1, 4, and 5 were better estimated using only ammocoete length data, while estimates for ages 2 and 3 were better estimated when using both length and statolith width data (Table 1). By adding the statolith measurement data, the precision of the model's estimate of the proportion of age 2 ammocoetes from Ogemaw Creek was improved, as shown by the likelihood profiles for the estimate of proportion at age 2, with and without statolith information (Fig. 7). This was not the case for the Big Garlic River.

Statolith growth patterns appear to be tightly correlated to ammocoete body size and growth rates. Therefore, I used the same growth function and standard deviations in the model for the statolith width data as I did for the ammocoete length data. One of the assumptions made for the purpose of the model was that the standard deviations increase as a linear function of age. Although standard deviations of ammocoete length were observed to increase with age, standard deviations of statolith width were actually larger at younger ages (Fig. 9). Efforts to increase standard deviation values for both ammocoete length and statolith width resulted in the model not converging to a solution.

Discussion

In this study, I found that the increase of statolith growth slowed as age increased (Fig. 2). This was also true for ammocoete body growth (Fig.1). These growth patterns are common of fish species and have been found in other studies examining calcified structures such as Boehlert (1985) and Anderson et al. (1992).

Statolith width was found to be the best statolith metric from which to infer ammocoete age. Statoliths vary greatly in shape. Some appear to grow more in height as they age, while others grow more in length. However, statolith width appears to widen symmetrically from the apex as they increase in age no matter the shape of the statolith.

When statolith width and ammocoete length frequency data were combined in a statistical model, proportion-at-age estimates were improved for Ogemaw Creek, but not for Big Garlic River (Table 1). Statolith widths were significantly different between each age class only in Ogemaw Creek (Fig. 6); this likely provided the model with more information on the separation of age classes and lead to a more accurate determination of the proportion-at-age. These results could be due to the difference in ammocoete and statolith growth between Ogemaw Creek, a faster growing, warm, high alkalinity stream, and the Big Garlic River, a slower growing, cold, low alkalinity stream (Fig. 1). A study by Lombarte and Lleonart (1993) found that species living in cold waters had calcified structures that were smaller and thinner than those living in warmer waters. They also found that water temperature was an important factor in regulating otolith growth in teleost fish.

Using statolith measurements instead of annuli counts to age ammocoetes eliminates the reader bias which ran as high as 30% in studies conducted by Meeuwig & Bayer (2005) and

Dawson et al. (2009). In this study, the proportion-at-age estimates for Ogemaw Creek were not only more accurate, but also more precise using all available information; as evidenced by a comparison of likelihood profiles for the estimate of proportion-at-age 2, with and without inclusion of statolith width data (Fig. 8). Francis and Campana (2004) also found that when aging fish, a mixture analysis model combining both otolith information and fish length was a better predictor of age than using either of them individually.

In order to get the model to converge to a reasonable solution, I used standard deviations around ammocoete length and statolith width that were smaller than observed standard deviations of the known age populations. However, the standard deviations still increased with age, to reflect typical fish growth. While I do see similar growth patterns between ammocoete body length and statolith size (measured using width), there is a greater variation around statolith size at age 1 than any other age for both Big Garlic River and Ogemaw Creek. This variation in statolith size observed in the first year of growth of sea lamprey is in contrast to variation seen in otolith size in the first year of growth of teleost fish. Metin and Ilkyaz (2008) observed a von Bertalanffy growth pattern for otolith growth similar to that of fish growth. However, this growth pattern was only seen in fast growing species or the juveniles of slow growing species.

As mentioned above, proportion-at-age estimates for Ogemaw Creek were improved when combining statolith width data with ammocoete length data; however this was not the case for Big Garlic River (Table 1). Francis and Campana (2004) and Metin and Ilkyaz (2008) both state that otolith weight was a better predictor of fish age than otolith length. In fact, Boehlert (1985) and Anderson et al. (1992) also found that while the growth of otolith length slows as fish age, the otolith weight continues to increase due to deposited material on the otolith surface. Otolith weight, instead of otolith length, is suggested to be a stronger relationship with fish age,

especially in slow growing fish populations (Metin and Ilkyaz 2008). Sea lamprey statoliths are smaller than otoliths from teleosts fish. In sea lamprey with an average length of 148 mm, statoliths were an average of 0.63 mm long (Hollett 1998). However, in contrast, arctic cod averaging 136 mm in body length had otoliths of 6.5 mm in length; walleye pollock 234 mm in length had otoliths 5.7 mm long (Short et al. 2006). This difference in size between the otolith and statolith makes obtaining the weight of a statolith very difficult.

Interpreting statoliths is a time consuming process and requires skilled technicians familiar with the procedure. In order to collect statolith measurements, the statolith must first be carefully manipulated into the correct position. Once the statolith image is captured, it must then be rotated into the correct orientation to ensure that all measurements are accurate and consistently taken (Fig. 2). If the statolith is not in the correct orientation or measurements are inconsistent between statoliths, this method can lead to high error rates and inconsistent results. Due to the variability of statolith shapes and sizes, the time it takes for a skilled technician to collect one statolith measurement varies between 5 and 20 minutes, increasing to as long as 30 or more minutes to collect all three statolith measurements (length, width, and height). If this method were to be used by management agencies it would not only be time consuming, but also reasonably expensive.

Previous studies have found that using length frequency analysis and statolith information in the form of annuli counts are unreliable and inaccurate (Dawson et al. 2009). This study found that combining statolith measurement data with length frequency information slightly improved estimates of proportion-at-age for one known-age population, but not both. Collecting statolith measurements was found to be time consuming, expensive, and required skilled technicians. In conclusion, due to the inconsistency of improving proportion-at-age

estimates and the overall cost of using this method, I would not recommend the use of statolith measurements in determining age populations in ammocoetes.

Table 1 True proportions at age and those estimated by the model using ammocoete length data alone and length and statolith width data combined for known-age sea lamprey populations in Big Garlic River and Ogemaw Creek

Age-Class	True Proportion	Estimated using length data only	Estimated using length and statolith width data
		Big Garlic River	
1	0.123	0.119	0.134
2	0.330	0.154	0.175
3	0.240	0.364	0.335
4	0.153	0.150	0.143
5	0.155	0.212	0.214
		Ogemaw Creek	
1	0.212	0.190	0.196
2	0.228	0.137	0.172
3	0.168	0.303	0.246
4	0.392	0.369	0.386

Fig. 1 Locations of the streams used to create known age populations of sea lamprey to establish a relationship between age and statolith size

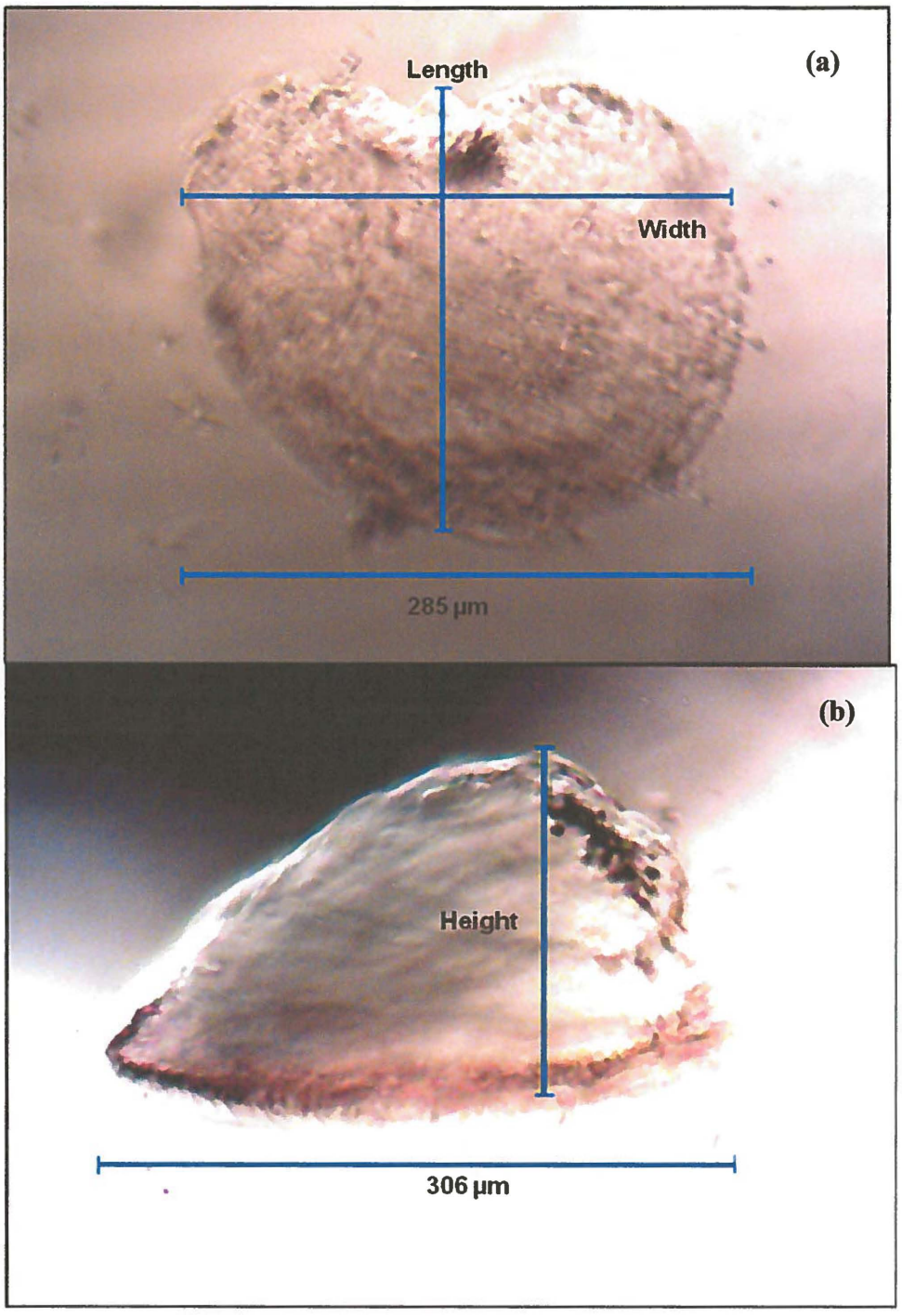

Fig. 2 Digital images of statoliths from ammocoetes; (a) statolith length and width measured from the dorsal view and (b) statolith height measured from the lateral view

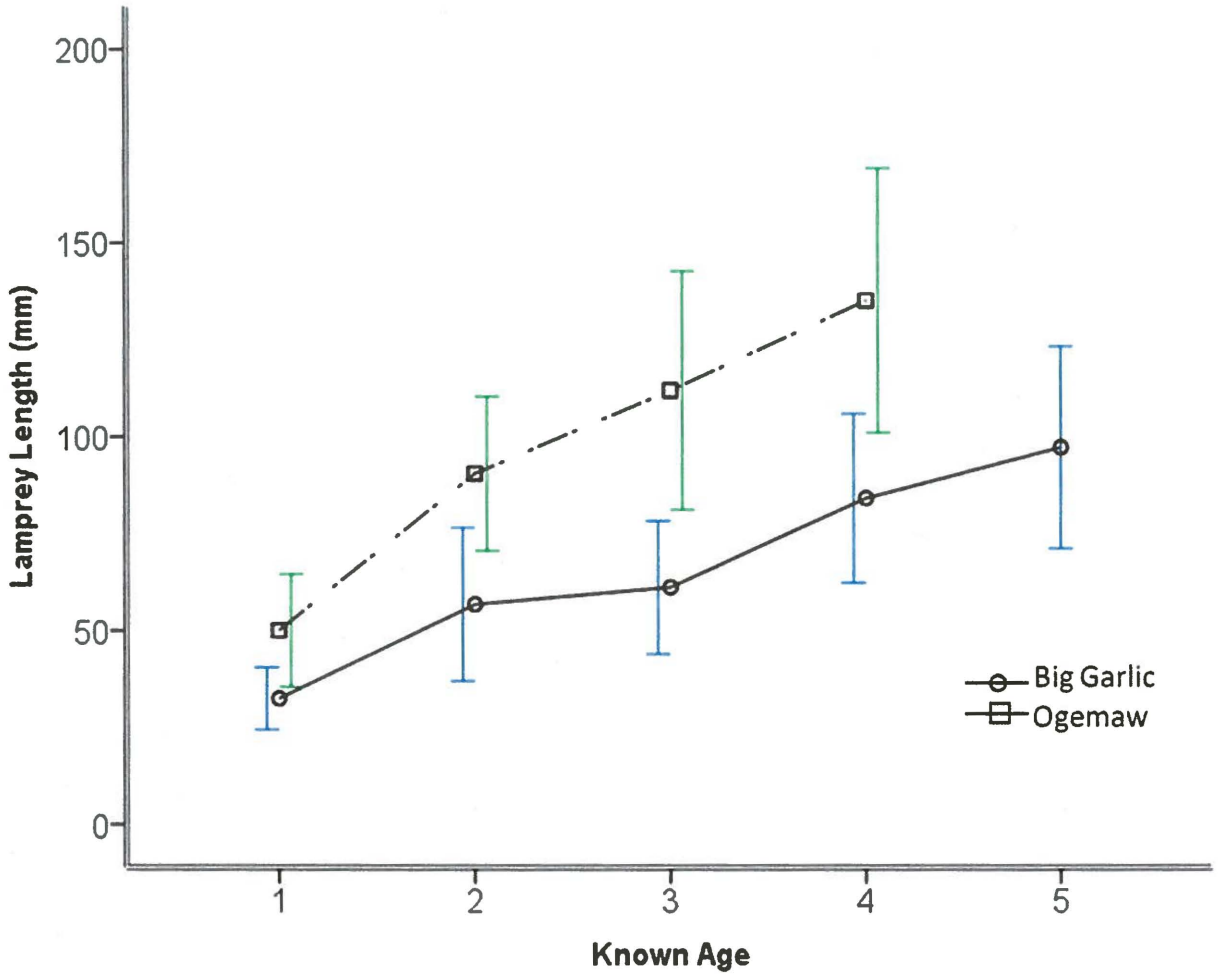

Fig. 3 Ammocoete length at age for both Big Garlic River and Ogemaw Creek (error bars indicate standard deviation)

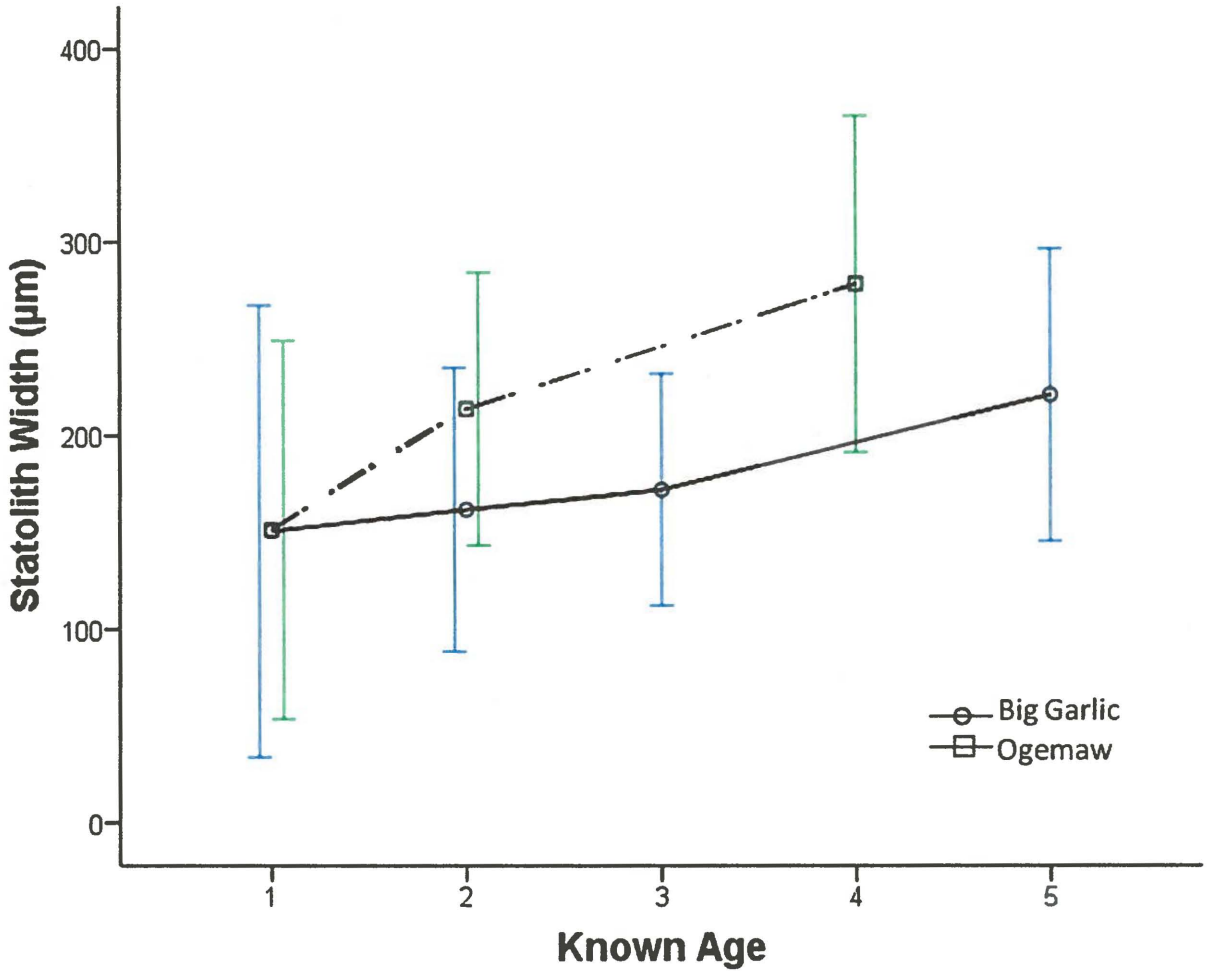

Fig. 4 Ammocoete statolith width at age for both Big Garlic River and Ogemaw Creek (errors bars indicate standard deviation)

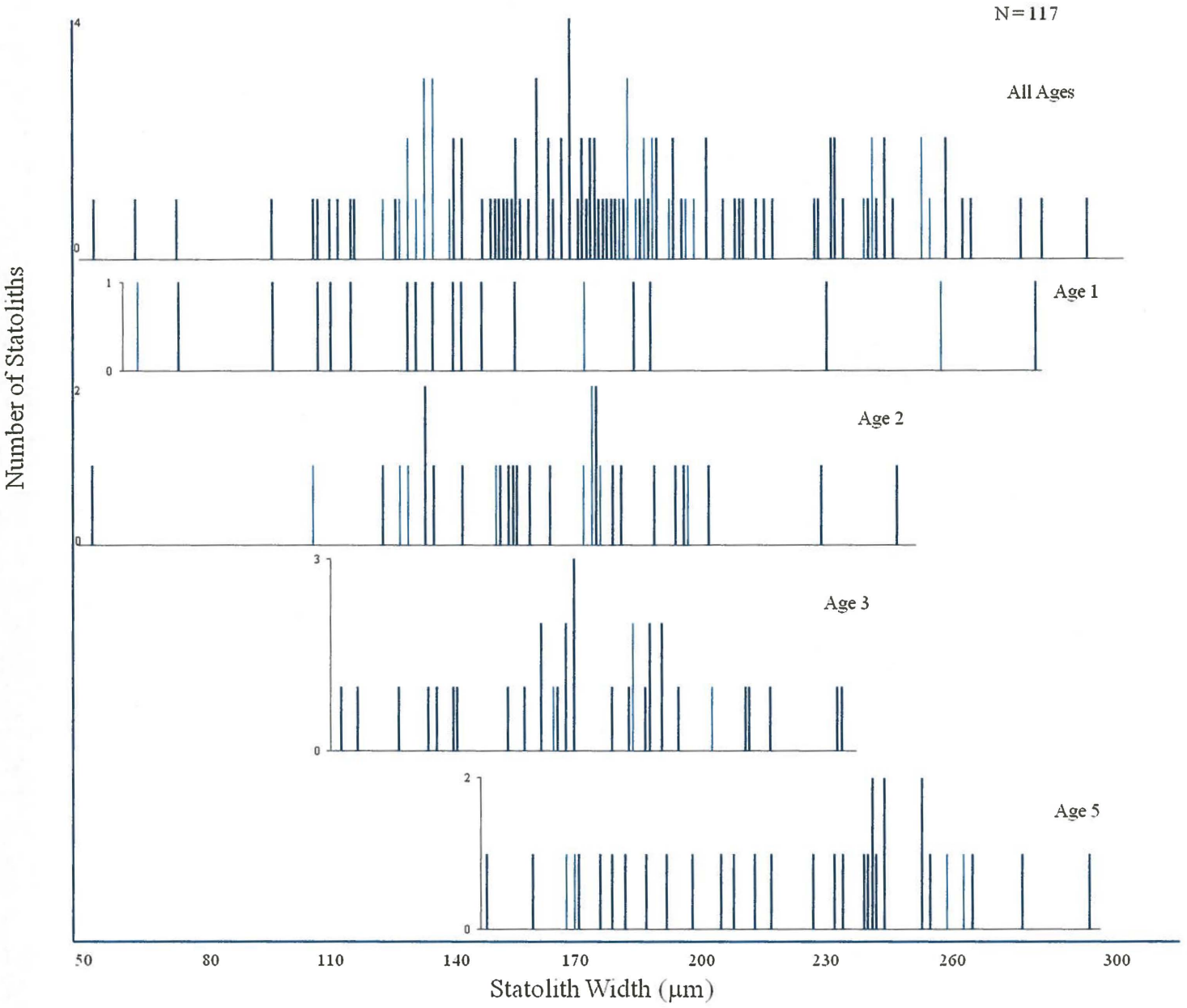

Fig. 5 Length-frequency distributions of statolith widths for all ages combined and for age 1, 2, 3, and 5 individually for a known age population of sea lamprey ammocoetes from Big Garlic River. N equals the total number of statoliths measured

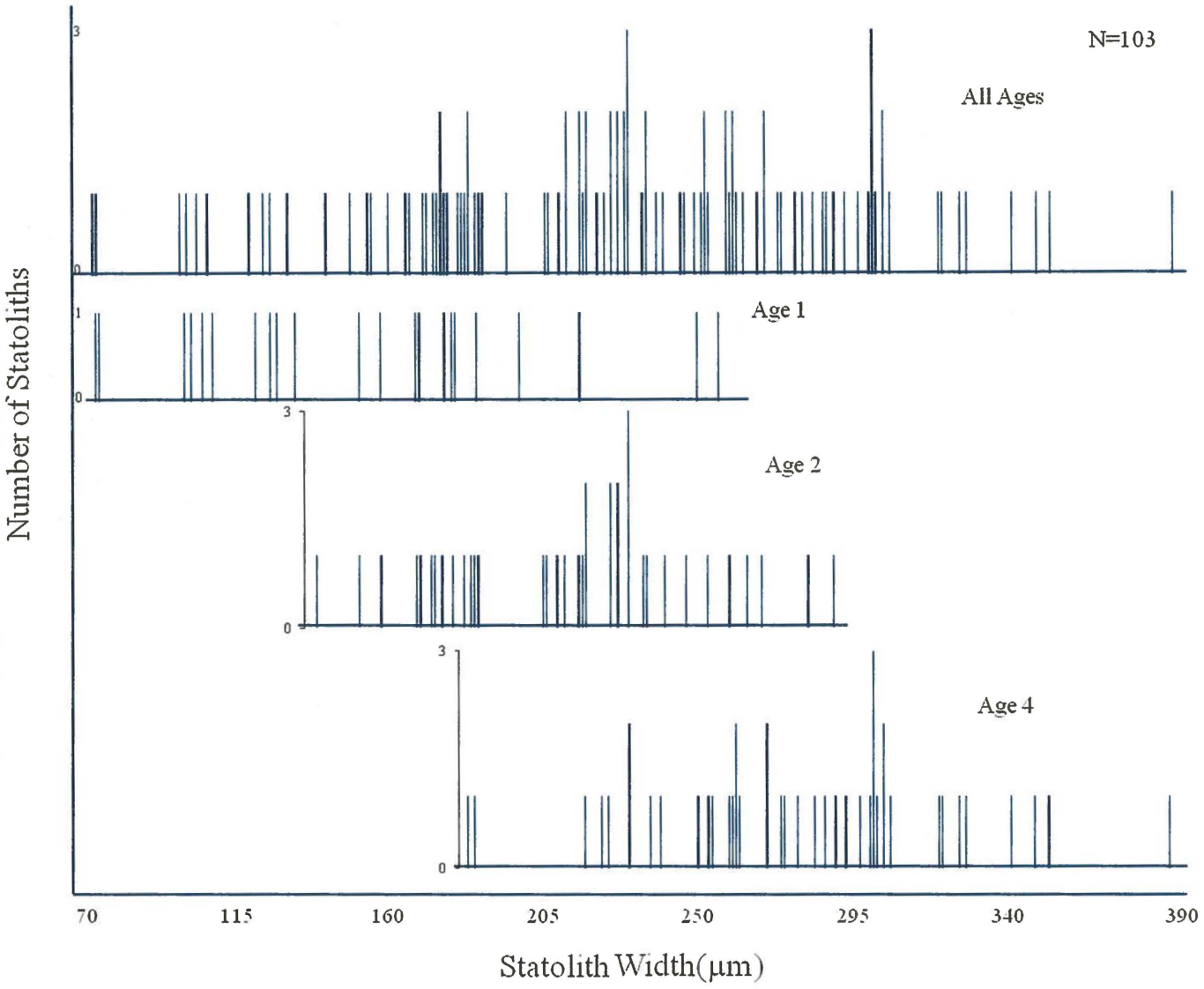

Fig. 6 Length-frequency distributions of statolith widths for all ages combined and for age 1, 2, and 4 individually for a known age population of sea lamprey ammocoetes from Ogemaw Creek. N equals the total number of statoliths measured

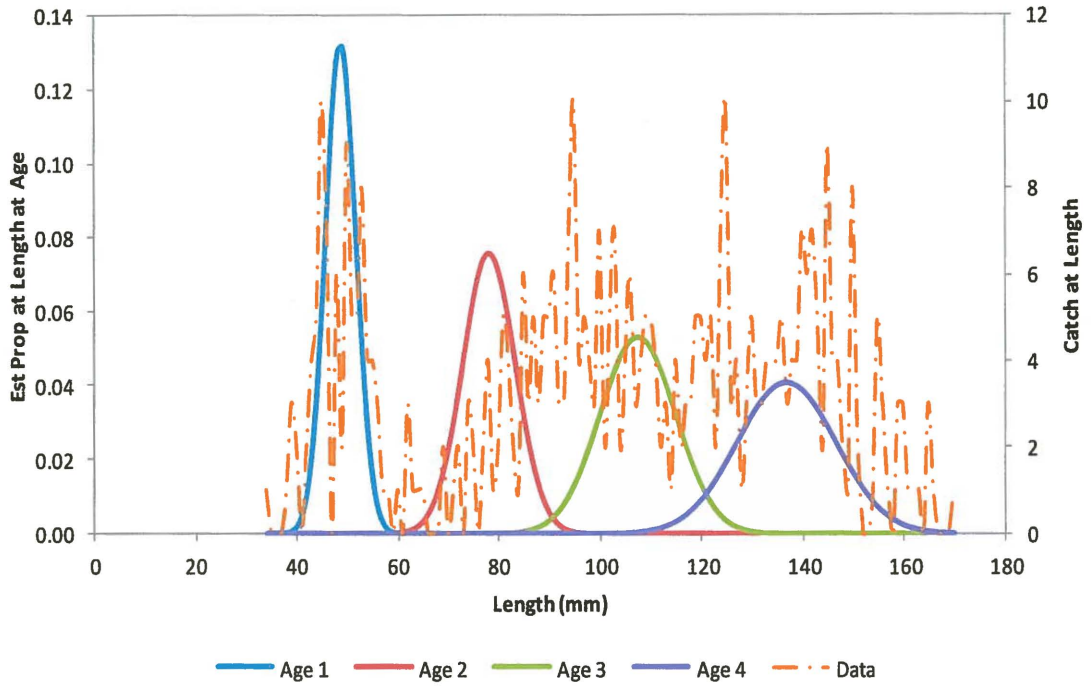

Fig. 7 Length-frequency data for the known age ammocoete population from Ogemaw Creek (orange lines) and the approximate age-class distribution produced by the model using both ammocoete length and statolith width data to indicate proportion of ages 1, 2, 3, and 4

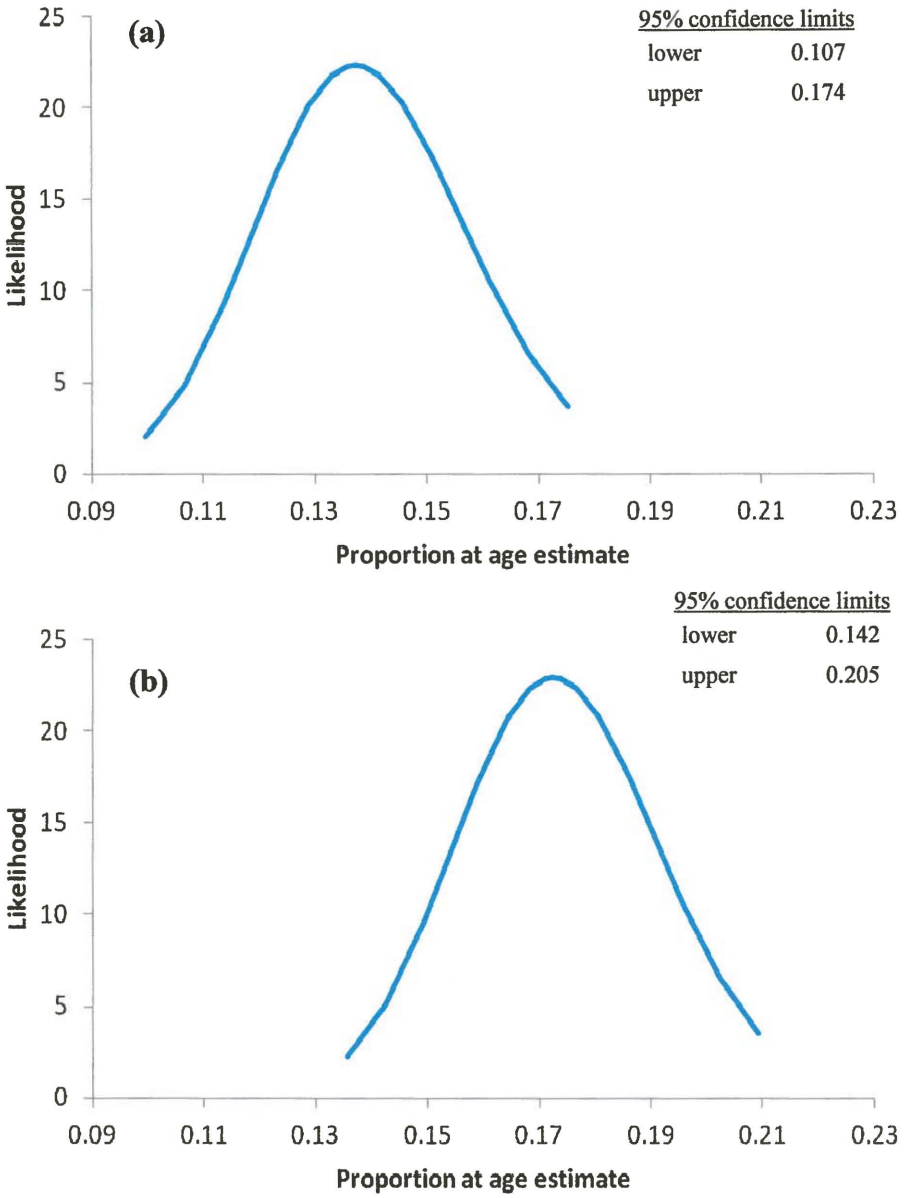

Fig. 8 Likelihood profiles for the model estimates of the proportion-at-age 2 for the Ogemaw Creek population using (a) ammocoete length data and (b) both ammocoete length and statolith width data. True proportion-at-age 2 is 0.228

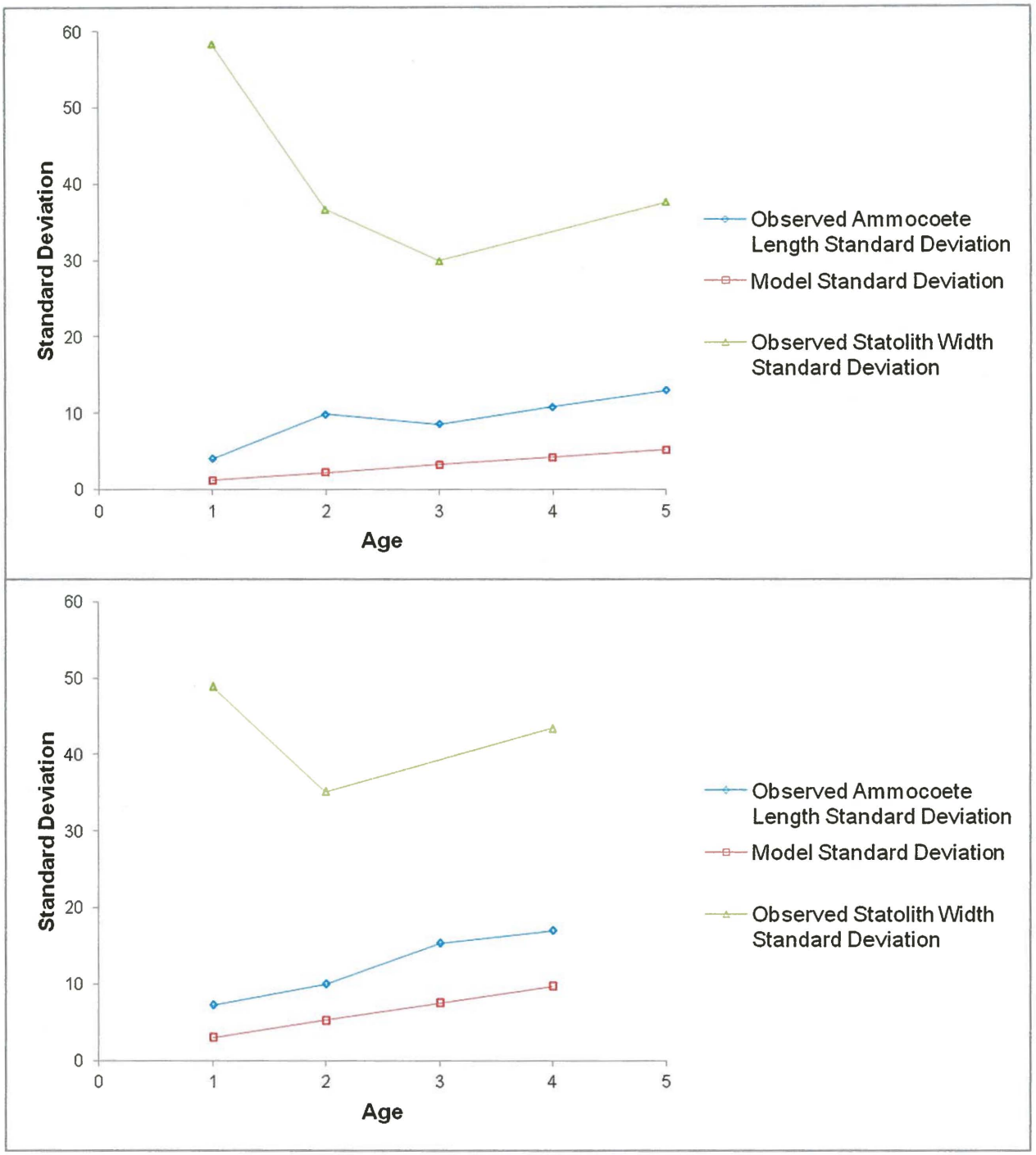

Fig. 9 Known standard deviation for ammocoete length and statolith width as well the standard deviation used in the statistical modeling program for both Big Garlic River (a) and Ogemaw Creek (b). Statolith of age-4 ammocoetes from Big Garlic River and age-3 ammocoetes from Ogemaw Creek were not available to be measured

Chapter 2

USE OF MULTIPLE TAGGING METHODS INCLUDING PIT TELEMETRY TO MONITOR SMALL SCALE MOVEMENTS AND HABITAT SELECTION OF LARVAL SEA LAMPREY

Abstract

A more complete understanding of the demographics and movement of sea lamprey (*Petromyzon marinus*) at the larval stage is needed in order to improve control of this Great Lakes invasive. The primary technique used to control sea lamprey is the application of lampricides to streams to kill larvae before they become parasites. Because costs prohibit all streams from being treated, control agents perform ranking surveys of populations of large larvae to inform the stream selection process. If habitat preference, depth distribution or in-stream distribution changes as larvae approach metamorphosis, as anecdotal evidence suggests, there is a potential for bias in the current ranking surveys which make the assumption that larval lampreys occupy habitat in the same proportion irrespective of size. Evidence suggests that larvae do move further downstream as they approach metamorphosis; however the rate of this movement and the types of habitat that they occupy is still unknown. To monitor the movement and preferred habitats of large larvae, I marked and released larvae approaching metamorphic size and subsequently tracked their movements. I used three different tagging methods, including passive integrated transponder (PIT) tags, visible implant (VI) Alpha tags and visible implant elastomer (VIE) tags to mark larvae. I released marked larvae into different habitats and locations in the stream, and subsequently determined their movements using PIT telemetry and electrofishing. I was able to locate 11% (17) of released, tagged ammocoetes, 10 of which had

relocated to a new burrowed location. I witnessed no movement into Type II substrate and found that as the size of ammocoetes increased, so did their distance moved downstream.

Introduction

Streams are ranked for lampricide treatment in part on electrofishing catches in stratified, habitat-based surveys, with treatment schedules targeting those tributaries expected to contain the most large (>100 mm) sea lamprey larvae, or ammocoetes. Currently, due to the cost of lampricides, only a portion of the streams that will produce parasitic sea lampreys each year are treated (Steeves 2002). Information on larval sea lamprey abundance, growth, survival, and distribution in streams is used to rank streams for lampricide treatment based on the number of larvae approaching metamorphosis that could be killed per dollar.

As larvae grow, habitat preference appears to shift towards substrate made up of larger particles (Sullivan 2003); however, little is known about individual movements of larvae or transformers within the stream. Anecdotal evidence suggests additional changes in habitat preferences and depth as metamorphosis occurs (Jones 2007). As larvae increase in size, movement towards the mouth of the stream and lentic area is also noted, but the rate of this movement and the habitats occupied during this migration is unknown (Jones 2007). If habitat preference changes as larvae approach metamorphosis, there is a potential for bias in the stream ranking surveys used to select streams for the application of lampricides. A better understanding of timing, distance, habitat preference, and frequency of movements of large larvae may reduce uncertainty in estimates of larval abundance.

In order to monitor the movement and habitat preferences of ammocoetes, they ideally need to be individually marked and relocated with minimal disturbance. However, the current

methods of tagging sea lamprey of this size use coded wire tags which do not allow for relocation without disturbance (Bergstedt et al. 2003). Quintella et al. (2005) successfully accomplished this through the use of Passive Integrated Transponder (PIT) telemetry to monitor tagged ammocoetes (>125 mm) with accuracy and minimal disturbance to the larval movement patterns. The authors released tagged larvae, and with the use of a PIT Telemetry antenna, relocated the animals *in-situ* without disturbing the burrowed larvae.

Since lampricide treatment schedules target those tributaries expected to contain the most large larvae (>100 mm), I was interested in determining fine-scale spatial movements and assessing microhabitat preferences of larvae of approximately 100 mm in size.
Our objectives in this study were to 1) establish a tagged population of large larvae and transformers (>85 mm) in a stream and 2) monitor the movements and habitat preferences of the tagged population.

Materials and Methods

Tagging Equipment

Three different tags were used in this study: Passive Integrated Transponder (PIT) tags, Visible Implant (VI) Alpha tags, and Visible Implant Elastomer (VIE) tags. PIT tags are cylindrical glass capsules that weigh 0.1g and are 9 mm x 2 mm (Biomark) surgically inserted inside the body cavity. Visible Implant (VI) Alpha tags are small (1.2 mm x 2.7 mm), orange fluorescent tags with a black alphanumeric code, visible through the skin to allow for individual identification of animals (NMT 2010). VI Alpha tags are inserted under the skin using a VI Alpha Tag Injector (NMT 2010). Visible Implant Elastomer (VIE) tags are colorful fluorescent two-part silicon based materials that are injected beneath the skin as a liquid that quickly cures

into a pliable, biocompatible solid (NMT 2011). These tags are inserted using a 1 mL, 29-gauge needle and can be easily seen with the naked eye or with the help of a UV light (NMT 2011).

To monitor the location of the PIT tagged ammocoetes while still burrowed I used a portable PIT tag reader unit (FS2001F-ISO) (Biomark 2008) with BP Portable Antenna, capable of reading full duplex, 134.2 kHz tags. The reader displays the identification code of a tag when detected.

Tagging Procedure

Ammocoetes were collected from the Platte River in Honor, Michigan in June 2011 (Fig. 1). Larvae were then placed in coolers containing continuously aerated stream water and transported to the U.S. Geological Survey Hammond Bay Biological Station in Millersburg, MI the same day of capture. Once at the station, larvae were transferred to 37.9-L aquarium tanks (50 cm x 25.2 cm x 29 cm) at densities of no more than 25 larvae per tank. Aquariums contained approximately 75 mm of beach sand as a burrowing substrate and were aerated, and supplied with flowing Lake Huron water at ambient temperature (approximately 20°C) at a rate of 500 ±75 ml/min. Lampreys were fed baker's yeast (75 g of dry yeast per 25 lampreys twice a week). For all tagging procedures ammocoetes were anaesthetized using 1 ml 2-phenoxyethanol l/L water in groups of 3-6 individuals. Once activity level ceased, larvae were weighed to the nearest 0.1 g (±0.1 g) and measured to the nearest millimeter (±1 mm) prior to the tagging procedure. Also, prior to the tagging procedure, metamorphic stage (ranging from 1 to 7 and based on the development and enlargement of the eyes and appearance of teeth inside the mouth cavity) was determined using detailed illustrations from Youson and Potter (1979).

Ammocoetes (N=68) at least 110 mm in length were implanted with PIT tags on 7-8 July 2011 and 22 July, 2011. Larvae were placed on their dorsal side and a 3mm scalpel incision was made on the ventral side at the widest point of the body (approximately 40% of the total length from the anterior end). The PIT tag (0.1 g, 9 mm X 2 mm, Biomark, 134.2 kHz ISO FDXB) was then inserted by hand into the body cavity through the opening. The incision was then sewn up using a single monofilament absorbable suture (Byosin® Glycomer 631, USP 4-0). Each tagging procedure lasted approximately 3 minutes.

Ammocoetes (N=40) at least 85 mm in length were implanted with VI Alpha tags on 7-8 July, 2011. Ammocoetes were placed on their dorsal side and the VI Alpha Tag Injector (NMT 2010) was inserted underneath the skin on their ventral side (approximately 40% of the total length from the anterior end), bevel-side up, and the VI Alpha tag (1.2 mm x 2.7 mm, NMT) was inserted. The incision was then closed with a single stitch of monofilament absorbable suture (Byosin® Glycomer 631, USP 4-0). Each procedure lasted approximately 3 minutes.

Ammocoetes (N=95) at least 77 mm in length were implanted with VIE tags on 7-8 July, 2011. Larvae were placed on their dorsal side and a 1 mL, 29-gauge needle was inserted underneath the skin on their ventral side to insert the VIE tags (NMT 2011, fluorescent pink and blue). Lamprey were split into four groups and tagged with one of four different patterns in order to identify the group: red anterior - blue posterior far (RABP Far), red anterior – blue posterior close (RABP Close), blue anterior – red posterior far (BARP Far), or blue anterior – red posterior close (BARP Close). Each procedure lasted approximately 3 minutes.

After the tagging procedures, all tagged ammocoetes were placed in well-aerated tanks containing water chilled to 6-8°C for approximately 24 hours to inhibit fungal growth and reduce movement to aid in tag retention. Larvae were then returned to aforementioned aquarium tanks

supplied with Lake Huron water at ambient temperature (approximately 20°C). Larvae were held for at least eight days (22 days for those tagged on 7-8 July, 2011) to allow for healing of the incision.

Field Study

The tagged ammocoetes were released into five predetermined locations located along the last 1.6 km of the Crystal River in Glen Arbor, Michigan on 1 August 2011 (Fig.1). Locations varied by preferred (Type I) or acceptable (Type II) habitat and distance from the mouth of the river. Habitats were typed using the same methods used by the sea lamprey control agents, which is based on substrate type and particle size (Mullett and Bergstedt 2003). Type I substrate consists of fine sand/silt particulates while Type II substrate is made up of a more coarse sand and small pebbles. Each release location had both preferred and acceptable habitat available nearby. The tagged ammocoetes were released using a 5 gallon bucket, with the bottom removed, that was inserted into the substrate prior to release and held until all animals had completely burrowed. A total of 150 tagged larvae were released: 25 with PIT tags, 31 with VI Alpha tags, and 94 with VIE tags (Table 1). The 25 PIT tagged larvae were split up between four of the release locations in order to monitor movement and habitat selection throughout the entire study site (Table 1).

The locations of the released PIT-tagged larval lamprey were assessed at two week intervals using a portable pit tag reader above the substrate so as to not disturb the burrowed location of the lamprey. Since larvae are thought to primarily move downstream, all burrowable habitats 5 m upstream and at least 30 m downstream from each release site were scanned for PIT-tagged larvae. The position of any animal located was then logged with a GPS unit

(Trimble® GeoXH 6000). On 26 September 2011, 56 days after release, I attempted to relocate and collect tagged larvae using both a portable PIT tag reader and a backpack electroshocker. In order to relocate as many animals as possible, I expanded our final search by surveying all burrowable habitat 10 m upstream and 40 m downstream from the release locations. All locations in which tagged larval lamprey were found were marked with the GPS unit, and distance from release site and habitat substrate selection were noted. A thorough survey of the entire study area (approximately 1.6 km), as described in Jones et al. (2003), was then conducted by shocking burrowable habitat at randomly spaced transects both to calculate larval abundance and habitat availability of the study area, as well as locate tagged lamprey that could have moved further downstream.

A simple linear regression of distance moved vs. larval length was performed to determine if movement toward the mouth of the stream was observed as larvae increase in size, and whether length explained a significant proportion of variance in distance moved.

Results

Tagging Procedure

A total of 203 ammocoetes were tagged using the three methods: PIT, VI Alpha, and VIE tags. The average length of the 68 larvae tagged with the PIT tags was 124 mm (110 mm-162 mm). PIT tags comprised up to 8.1% of the length and up to 5.6% of the body mass of the ammocoetes. Of the 68 larvae implanted with PIT tags, 25 individuals both survived the surgery and retained the tag and were subsequently released. The average length of the 40 larvae tagged with the VI Alpha tags was 103 mm (85 mm-118 mm). Of these 40 VI Alpha tagged larvae, 31 individuals both survived the implant and retained the tag and were then released. The average

length of the 95 larvae tagged with the VIE tags was 100 mm (77 mm-117 mm). All of the 95 larvae tagged with the VIE tags survived the surgery, retained their tags, and were later released.

Field Study

Using both of the relocation procedures I was able to relocate 17 out of the 150 released lampreys (11.3%): 2 VI Alpha-tagged, 6 VIE-tagged, and 9 PIT-tagged larvae.

During the first two weeks of the experimental period, I was able to locate 9 of 25 PIT-tagged larvae, most of which had moved from their original release location. There were no additional movements made by the nine after the first two weeks except in one case. With that individual larva, I observed two separate downstream movements over the course of the experiment. The tagged animals that were never relocated were assumed to have left the release location and the surrounding area.

At the end of the experimental period, the Crystal River was surveyed and it was found that 92% of the burrowable habitat was made up of Type I substrate and only 8% Type II substrate. Three of the release locations were in Type I substrate and two in Type II substrate. Of the 17 larvae that were relocated, 11 were found in Type I and 6 in Type II. A total of 10 out of the 17 relocated larvae moved from their original release locations; 5 tagged larvae moved from Type II into Type I, 5 moved from Type I back into Type I, and 0 moved from Type I into Type II or from Type II into Type II (Table 2).

Movement, defined as a change in location of at least 1 m from the original location, was observed in 10 of the 17 relocated lamprey larvae. A positive relationship between distance moved and larval length was observed (Fig. 2). Larval length significantly predicted distance moved, $\beta=0.587$, $t(8)=7.892$, $p<0.001$. Larval length also explained a significant proportion of

variance in distance moved, Adjusted R^2=0.872, $F(1,9)$=62.286, $p<0.001$. Prior to their release, all tagged larvae were assigned a metamorphic stage and then staged again after they were relocated. No larvae that were relocated at the end of the study had advanced in metamorphic stage. Three larvae were categorized as Stage 1 (lengths 148 mm – 162 mm) and all of the others Stage 0 (ammocoete) (lengths 77 mm – 143 mm). Larvae, in the ammocoete stage (N=8) in which movement was recorded, moved 1.97 m - 6.58 m from their original release location. Larvae, that were Stage 1 (N=2), in which movement was recorded, moved 33.77 m and 44.78 m from their original release location. Distances moved were then graphed against the length of the ammocoetes (Fig. 2).

Discussion

The field trials of this study support the observation that as ammocoetes increase in size they increase their movements downstream toward the mouth and lentic areas of the stream (Jones 2007). The downstream movements of the larger sea lamprey were significantly greater than those of the smaller larvae. Figure 2 shows that as ammocoete length increased the distance traveled increased as well.

There has also been anecdotal evidence that has suggested that as ammocoetes grow and begin to metamorphose, habitat preference shifts toward substrate made up of larger particles (Sullivan 2003). The availability of suitable substrate for burrowing is an important factor for larvae movement distribution (Morman et al. 1980; Young et al. 1990; Ojutkangas et al. 1995; Beamish & Lowartz 1996; Almeida & Quintella 2002). This study did not find evidence of movement into substrate with large particles as the larvae increased in size. However, the burrowable habitat in the stream in which the study was conducted was primarily composed of

the preferred habitat, Type I substrate (92%). A survey of the entire study area of the stream was conducted and found that the density of ammocoetes was low; only 0.1 ammocoete/m^2 of Type I substrate. In the Great Lakes region, a moderate to high density of ammocoetes is considered to be > 5 larvae/m^2 (Steeves et al. 2003). Due to the fact that our study stream was composed of predominately Type I substrate, we were unable to test the habitat preference between Type I and Type II. Similar research was conducted by Smith et al. (2011) when looking at the habitat selection habits of least brook lamprey (*Lampetra aepyptera*). They found that both large and small least brook lamprey ammocoetes selected fine sand habitat both non-randomly and disproportionate to availability (Smith et al. 2011). It was also noted that even though larger ammocoetes were found in course sand substrate more often than smaller ammocoetes, both small and large selected fine sand over all other types of substrate when given the choice (Smith et al. 2011).

As found in previous studies, the use of the portable passive transponder detection system showed to be a valuable technique in studying the movements of ammocoetes. This system allowed for the study of larvae movement without increased handling or disruption of their burrowed locations. Quintella et al. (2005) suggested use of this system for this type of study noting that limitations would include use in water <1m deep, increased time to effectively search the study area, and difficulty of tagging larvae smaller than 120 mm in length. This study has shown that while this detection system works well in shallow waters, the time to efficiently search the study area is great. A better system to use may include setting up several stationary cross river PIT antennas throughout the study area to allow for detection of movement as PIT-tagged animals traveled downstream. This set up has been successfully used in monitoring PIT-tagged adult sea lamprey as they traveled upstream by Luehring et al. (2011). Multiple stationary

detection sites could also lead to an increase in data collection as it could decrease the number of tagged animals that go undetected.

In conclusion, our results suggest that larger ammocoetes relocate more often and travel further distances downstream while moving. However, future studies could more accurately document this movement by using a larger sample size and a stationary PIT tag detection system that would be able to determine movements that our study was unable to detect.

Table 1 Details about each of the five release locations on the Crystal River; including how many of each tag type were released, as well as the substrate type of each release location

Release Location Information		
Site ID	Substrate Type	Tagged Animals Released
A	I	31 VI Alpha
B	II	6 PIT; 24 VIE
C	I	6 PIT; 23 VIE
D	II	6 PIT; 23 VIE
E	I	7 PIT; 24 VIE

Table 2 Details about the movements of the tagged ammocoetes at each of the five release locations

Release Site	Release Substrate	Number Located	Number Moved	Relocated Substrate	Distance Moved
A	I	2	2	I	2.8 m
B	II	1	0	II	0 m
C	I	1	1	I	33.8 m
D	II	10	5	I & II	2.7 m - 44.78 m
E	I	3	2	I	5.4 m - 6.6 m

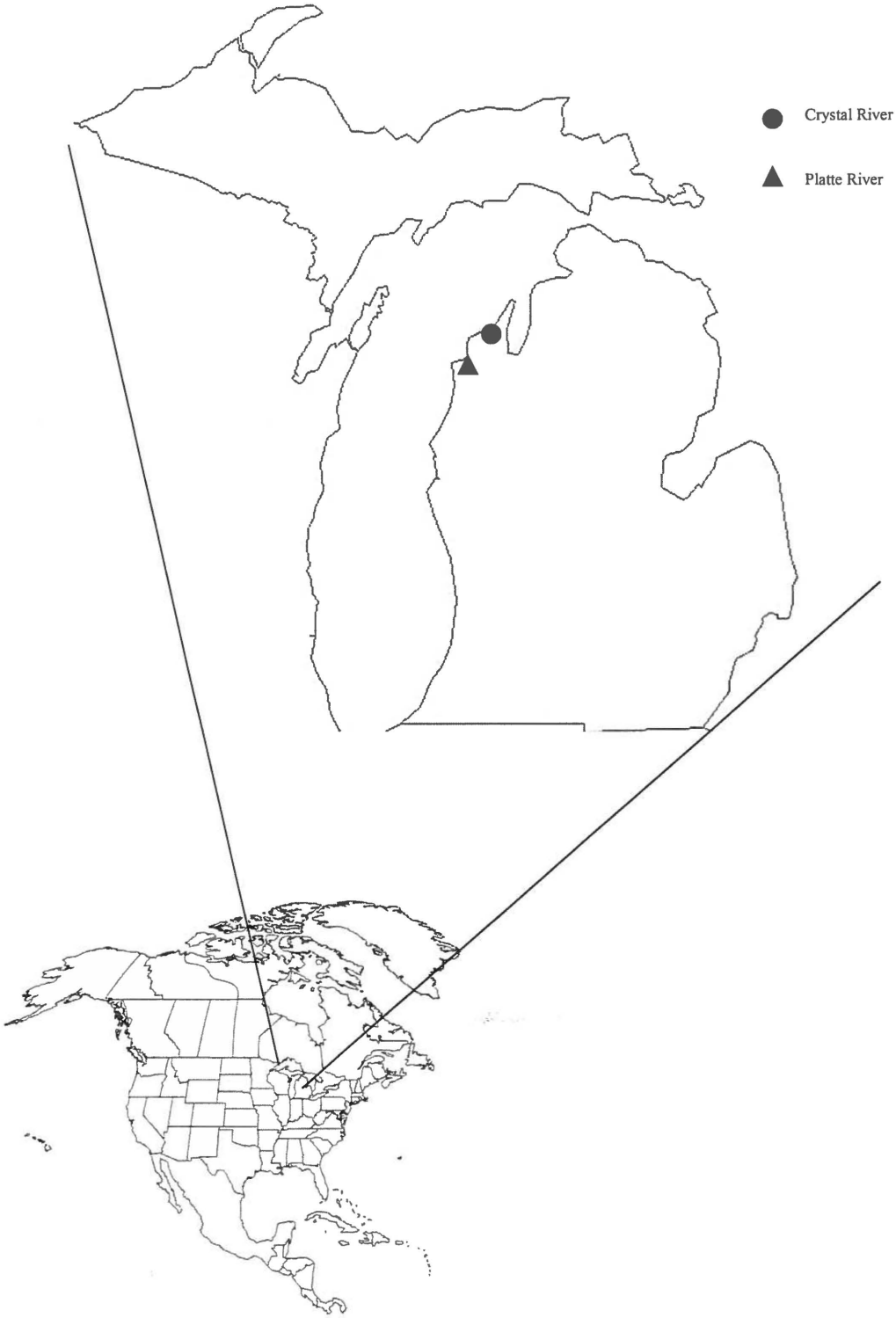

Fig. 1 Map of the Platte River, ammocoetes collection source, and the Crystal River, the location of the study

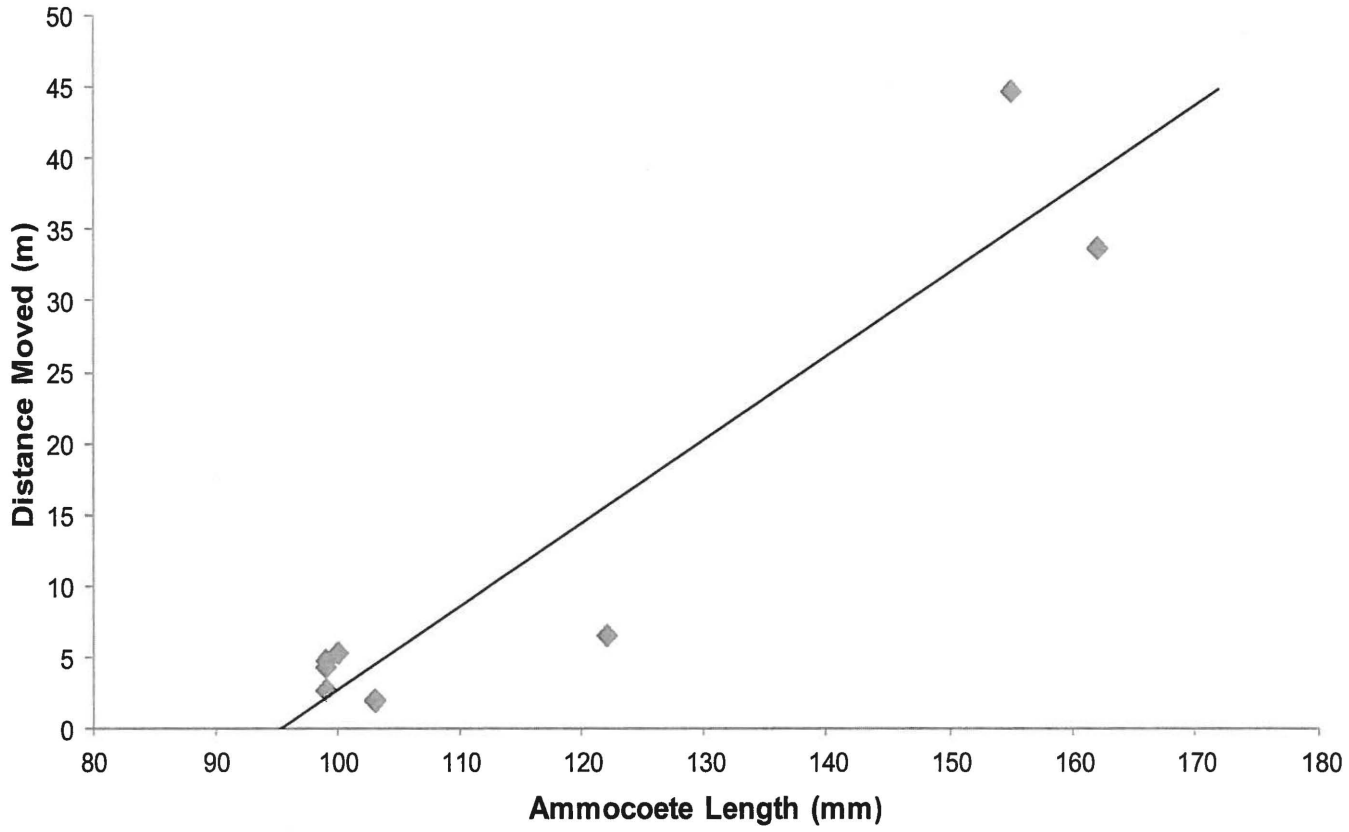

Fig. 2 Distance moved (m) from the release locations compared to ammocoete length (mm). Adjusted R^2=0.872, F(1,9)=62.286, p<0.001

Chapter 3

USING IMPLANTED TAGS IN LARVAL SEA LAMPREYS: MORTALITY, DETECTION, AND LONGEVITY

Heather A. Dawson[1], Danielle D. Potts[1], and Lisa M. O'Connor[2]

[1]University of Michigan-Flint, Biology Department, 303 E. Kearsley St. Flint, MI 48502

[2] Fisheries and Oceans Canada, Great Lakes Laboratory for Fisheries and Aquatic Sciences, 1219 Queen St East, Sault Ste. Marie, Ontario P6A 2E5

Abstract

Studies of the biology and ecology of Great Lakes sea lampreys (*Petromyzon marinus*) may require an effective means of tagging larvae to track individual movements to better control this pest species. We evaluated the feasibility of using passive integrated transponder (PIT) and visible implant (VI) Alpha tags in larval sea lampreys at least 100 mm and 85 mm in length, respectively. In 2010, larval sea lampreys (N=49) were tagged with 13 mm PIT tags using either an injection needle or insertion after a small incision. Within 24 hours lampreys suffered 100% mortality. In 2011, larval sea lampreys (N=67) were tagged with 9 mm PIT tags by making a small incision and then inserting the tag. Within three weeks lampreys experienced 49% mortality and 7.5% tag loss. In 2010, larval sea lampreys (N=41) tagged with VI Alpha tags suffered 7.5% mortality and 69% tag loss over a period of 228 days. In 2011, larval sea lampreys (N=40) tagged with VI Alpha tags suffered 0% mortality and 27.5% tag loss over a period of 22 days. The use of PIT tags in lampreys as small as 100 mm in length is not suggested unless a tag burden of less than 5% can be achieved. Since this study was conducted smaller PIT tags have

become available, which are shorter in length, thinner in diameter, and weigh less; use of these tags would result in approximately ¼ of the tag burden experienced by lampreys in our study. Until a better method of wound closure is found to limit VI Alpha tag loss in lampreys, the use of these tags is not suggested in lampreys as small as those used in this study.

Introduction

Sea lampreys (*Petromyzon marinus*) are an invasive species in the Laurentian Great Lakes that feed on teleost fishes. Sea lampreys are controlled primarily through the use of selective toxicants (lampricides) which are applied to tributaries of the Great Lakes where the filter-feeding larvae rear, prior to larvae undergoing metamorphosis (to the parasitic feeding stage) and migrating to the Lakes. Streams are ranked for lampricide treatment, in part, on electrofishing catches in stratified, habitat-based surveys, with treatment schedules targeting those tributaries expected to contain the most large lampreys (>100 mm). Current assessment techniques and population estimates make the assumption that larval sea lampreys occupy habitat in the same proportion irrespective of size or life history stage. However, Sullivan (2003) found that as ammocoetes grow their preference appears to shift towards larger particle sizes. Little is known about actual movements of individual ammocoetes, except for very young (age-0) animals (Derosier et al. 2007).

There are few tagging technologies available for live, individual marking prior to larval metamorphosis, which can occur at sizes as small as 120 mm and 3.0 g (Youson 1993). Quintella et al. (2005) found that passive integrated transponder (PIT) telemetry using 12 mm tags is suitable for examining movements of larval sea lampreys (125 -181 mm) in river beds *in-situ*, without disturbance. However, treatment schedules target those tributaries expected to contain

the most large lampreys (>100 mm), thus there is interest in the movements of smaller larvae than were used in the Quintella et al. (2005) study. Larval lampreys of this size have a relatively small body cavity in addition to their small overall size, which severely restricts internal tag implantation (Schreck et al. 2000). However, the increasing availability of smaller PIT tags (9 mm) potentially makes these tags an option in tracking larval sea lampreys as small as 100 mm as a way to determine their fine-scale spatial movements and assess their microhabitat preferences. Additionally, other tags such as visible implant (VI) Alpha tags (Northwest Marine Technology, Inc. (NMT) Shaw Island, Washington) could allow for the external identification and tracking of individual larval sea lampreys; however, the use of VI Alpha tags has not been evaluated in larval lampreys.

Sea lamprey larval dispersal has received little attention other than in early observational studies (Applegate 1950, 1961; Manion and McLain 1971), and observational methods alone do not provide an adequate description of how individuals are distributed in nature (Gaines and Bertness 1993). Ammocoetes probably redistribute frequently, either because of physical events such as bedload movement during high flows or dewatering at low flows, or simply to seek better habitats for feeding (Jones 2007). If habitat preference, depth distribution or in-stream distribution changes as larvae approach metamorphosis, there is a potential for bias in ranking surveys which inform the stream selection process. To individually track larvae prior to metamorphosis, we first needed to determine the types of tags that would be suitable for larval lampreys of a small size with a relatively small body cavity. Thus, our objectives in this study were to examine the feasibility of tagging larval sea lampreys as small as 100 mm with PIT tags and as small as 85 mm with VI Alpha tags by evaluating their short-term mortality and rates of tag detection and retention in the laboratory.

Methods

Larval sea lampreys were obtained from the Mineral River, Ontonagon County, Michigan with an AbP-2 backpack electroshocker during May 2010. Larvae 85 mm and greater in length were transported to the Hammond Bay Biological Station, Millersburg, MI. Lampreys were held in 37.9 L aquarium tanks (50 cm x 25.2 cm x 29 cm) at densities of no more than 25 per tank, and all aquariums contained approximately 75 mm of beach sand as a burrowing substrate. Tanks were aerated, and flowing Lake Huron water at ambient temperature was supplied at a rate of 500 ±75 ml/min. Lampreys were fed baker's yeast throughout the holding and experimental period (75 g of dry yeast per 25 lampreys twice a week).

2010 PIT Tagging procedure.

Larval sea lampreys of a minimum length of 100 mm were removed from their holding tanks on 30-31 August 2010 in groups of 5 and anesthetized in a 250 mg/L solution of MS-222 (tricaine methanesulfonate) and water (sodium bicarbonate was added to the solution to bring the pH to 7.0). Once activity level ceased, lampreys were measured to the nearest 1 mm and weighed to the nearest 0.1 g.

Lampreys were split into four groups and implanted with PIT tags (0.1 g, 13 mm x 2.15 mm, Texas Instruments®, supplied by Oregon RFID, ISO 11784/11785) by either incision or injection, or received an incision or injection needle puncture without receiving a tag (control groups). Lampreys in the incision group (N=15) were placed on their dorsal side and a 3 mm scalpel incision was made on the ventral side at the widest point of the body (approximately 40% of the total length from the anterior end). A tag was inserted by hand into the body cavity

through the opening. A single monofilament absorbable suture was used to close the incision (Byosin® Glycomer 631, USP 4-0). The incision control group (N=5) received an incision and a single suture, but no tag was placed into the body cavity. Lampreys in the injection group (N=14) were placed on their dorsal side and a 1 mm scalpel incision was made at the widest point of the body on the ventral side to allow for insertion of a needle. A 12-gauge PIT tag injector needle was inserted into the opening with the bevel side up and the tag was inserted into the body cavity. A single monofilament absorbable suture was used to close the incision (Byosin® Glycomer 631, USP 4-0). The injection control group (N=7) received an incision, a needle puncture, and a single suture, but no tag was placed into the body cavity. Experimental procedures lasted approximately 8 min, while control procedures lasted 5 min.

After tagging, lampreys recovered for 4-5 days in well-aerated tanks containing water chilled to 6-8°C and were then returned to holding tanks at ambient Lake Huron water temperature (12.5°C). Throughout the experiment mortalities were checked for daily on the surface of the substrate. Dead animals were removed from the tank, frozen, and measured to the nearest mm at a later time. During the experimental period, 31 August 2010 to 16 April 2011, lampreys were checked individually for tag detectability and retention on four occasions. Lampreys with unreadable or lost tags were removed from the tanks.

2011 PIT Tagging procedure.

The 2011 tagging procedure was performed as a part of another study; therefore no controls were performed. The methods used were refined based on the results from the previous year. Larval sea lampreys (N= 49) at least 110 mm in length were removed and tagged on 7-8 July 2011. Another 18 larval sea lampreys, with a minimum length of 115 mm, were tagged on 22 July 2011. They were anesthetized in groups of three in a solution of 1 ml/L 2-phenoxyethanol

and water, as used in Quintella et al. (2005). Once activity level ceased, lampreys were measured to the nearest mm and weighed to the nearest 0.1 g.

Larval lampreys were implanted with PIT tags (0.1 g, 9 mm X 2 mm, Biomark, 134.2 kHz ISO FDXB), which were smaller tags than those used in 2010. The lampreys were placed on their dorsal side and a small incision was made just off center of the widest point of their body on the ventral side. The PIT tag was inserted by hand into the body cavity. The incision was then closed using a single monofilament absorbable suture (Byosin® Glycomer 631, USP 4-0). Each procedure lasted approximately 3 minutes.

After tagging, lampreys recovered for two days in well-aerated tanks containing water chilled to 6-8°C and were then returned to holding tanks at ambient Lake Huron water temperature (approximately 20°C). Lampreys tagged on 7-8 July were held for 22 days and those tagged on 22 July for 10 days prior to release. Lampreys were checked for tag readability and tag loss prior to release.

2010 VI Alpha Tagging procedure.

Larval sea lampreys at least 90 mm in length were removed from their holding tanks on 30-31 August 2010 in groups of five and anesthetized in a 250 mg/L solution of MS-222 (tricaine methanesulfonate) and water (sodium bicarbonate was added to the solution to bring the pH to 7.0). Once activity level ceased, lampreys were measured to the nearest mm and weighed to the nearest 0.1 g. Lampreys were split into two groups and implanted with VI Alpha tags (1.2 mm x 2.7 mm, NMT, black letters with fluorescent orange background) or were punctured with a VI Alpha Tag Injector (NMT 2010) without receiving a tag (control group). Lampreys in the experimental group (N=41) were placed on their dorsal side and the VI Alpha Tag Injector was inserted underneath the skin on the ventral side, bevel-side up, and the tag was inserted (NMT

2010). Control groups (N=14) received the same treatment, but no tag was placed in the body. 3M™ Vetbond™ Tissue Adhesive (n-butyl cyanoacrylate) was used to close the wound created by the injector. Both experimental and control procedures lasted approximately 1 min.

After tagging, lampreys recovered for 4-5 days in well-aerated tanks containing water chilled to 6-8°C and were then returned to holding tanks at ambient Lake Huron water temperature (12.5°C). Throughout the experiment mortalities were checked for daily on the surface of the substrate. Dead animals were removed from the tank, frozen, and measured to the nearest mm at a later time. During the experimental period, 31 August 2010 to 16 April 2011, lampreys were checked individually for tag detectability and retention on four occasions. Lampreys with unreadable or lost tags were removed from the tanks.

2011 VI Alpha Tagging procedure.

The 2011 tagging procedure was performed as a part of another study; therefore there were no control animals. The methods used were refined based on the results from the previous year. On 8 July 2011 larval sea lampreys (N= 40) at least 85 mm in length were anesthetized in groups of six in a solution of 1 ml/L 2-phenoxyethanol and water, measured, and weighed.

Lampreys were tagged with a unique VI Alpha identification tag (1.2 mm x 2.7 mm, NMT, black letters with fluorescent orange background) with a VI Alpha Tag Injector (NMT 2010). The lampreys were positioned on their dorsal side and a tag was inserted on the ventral side of their body under the skin with the VI Alpha Tag Injector (NMT 2010). Based on results from the previous year, modifications were made to the 2010 tagging method by sewing the wound created by the injector closed with a single stitch of monofilament absorbable suture (Byosin® Glycomer 631, USP 4-0). Each procedure lasted approximately 3 minutes.

After tagging, lampreys recovered for 2 days in well-aerated tanks containing water chilled to 6-8°C and were then returned to holding tanks at ambient Lake Huron water temperature (approximately 20°C). Lampreys were held for 22 days prior to release. Lampreys were checked for tag readability and tag loss prior to release.

Results

2010 PIT Tag Effects.

The lengths of PIT-tagged sea lampreys ranged from 100 to 135 mm (mean=110 mm, N = 29) and were significantly different than lengths of untagged lampreys (100-108 mm; mean=103 mm, N = 12; independent sample t test, p=0.006). However, both tagged and untagged lampreys experienced 100% mortality within 5 days post-surgery. Mortality of tagged lampreys was 100% within 24 hours. The tags comprised up to 13% of the length and up to 7.7% of the body mass of lampreys. Control groups experienced a 50% mortality rate within 24 hours and a 100% mortality rate within 5 days post-surgery. Internal hemorrhage was observed in the majority of lampreys and seemed to be the most likely cause of death.

2011 PIT Tag Effects.

Lengths of PIT-tagged sea lampreys ranged from 110 mm to 162 mm (mean=124 mm, N=67). The tags comprised up to 8.1% of the length and up to 5.6% of the body mass of lampreys. Lampreys had a 49% mortality rate within three weeks. Internal hemorrhage was observed in the majority of lampreys, and seemed to be the most likely cause of death. There was no significant difference in lengths of lampreys who survived to be released vs. those that died. A 7.5% tag loss was noted during the 22 day holding period.

2010 VI Alpha Tag Effects.

The lengths of VI Alpha-tagged sea lampreys ranged from 90 to 131 mm (mean=104 mm, N=41) and were similar to lengths of untagged lampreys (95-112 mm; mean=103 mm, N=14; independent sample t test, p=0.58). Confirmed mortality of tagged lampreys was 9.5%. One tagged lamprey escaped and thirteen tagged lampreys were unaccounted for at the end of the study; they either were unobserved mortalities which decomposed in the sand substrate or they escaped via the outlet of the flow-through water system. Untagged lampreys were all alive and accounted for at the end of the study. When checked 33 days after tagging, five lampreys were observed to have had shed their tags. When checked 228 days after tagging an additional six lampreys had shed their tags. At the conclusion of the study 13 of 41 lampreys (32%) had retained their tags, which were all readable.

2011 VI Alpha Tag Effects.

The lengths of the VI Alpha tagged larval lampreys ranged from 85 mm to 118 mm (mean=103 mm, N=40). During the 22 day holding period 0% mortality was observed. A 27.5% tag loss was noted during the 22 day holding period with an additional 10% of tagged individuals having tags with identification numbers that were indistinguishable.

Discussion

Total mortality was observed in larval sea lampreys implanted with 13 mm PIT tags and nearly half the lampreys died that were tagged with 9 mm PIT tags. Typically the effect of a tag is assumed to be relative to the ratio of the tag weight to overall weight of the organism (tag

burden). Brown et al. (2010) found that the survival of implanted juvenile Chinook salmon (*Oncorhynchus tshawytscha*) smaller than 11.1 g (average tag burden, 6.7%) were negatively affected by the implantation or presence of an acoustic microtransmitter and PIT tag. We observed 100% mortality of larvae within 24 hours using 13 mm tags (average tag burden, 5.0%) and 49% mortality of larvae within a three week period using 9 mm tags (average tag burden, 3.6%). Quintella et al. (2005) observed 14% mortality of larval sea lampreys ranging from 2.9 to 6.3 g in an average recovery period of 81 days using 12 mm tags (average tag burden, 2.3%). Much of the mortality is likely due to the size of the tag relative to the size of the animal as larval sea lampreys have a relatively small body cavity (Schreck et al. 2000). In some lampreys in which we implanted PIT tags we observed a loss of blood flow to the body posterior to the tag location. However, some of the mortality experienced by animals in 2010 was attributable to the increased handling time and inexperience with incision making and suturing, as total mortality of the control group suggests. Mueller et al. (2006) concluded that (post-metamorphic) juvenile Pacific lampreys (*Entosphenus tridentatus*) greater than 120 mm total length are suitable for being implanted with PIT tags that were 12 mm in size or smaller (2.2% mortality over a 40 day recovery period). Mueller et al. (2006) did not report weight of their experimental animals, but it is important to note that large lipid reserves are required for lamprey to undergo the process of metamorphosis (Bird and Potter 1981). Juvenile (metamorphosed) sea lampreys harbor more lipid stores than larvae (Youson et al. 1979) which could influence their ability to survive tag implantation. Lipid stores are utilized but not depleted to fuel morphogenesis as metamorphosis proceeds (Lowe et al. 1973; Youson et al. 1979) with the primary sites of storage for lipids in lampreys being the fat column, nephric fold, beneath the skin, and in regions surrounding the body cavity (Youson et al. 1979). Since this study was conducted PIT tags can be found as small

as 8 mm in length with a diameter of 1.25 mm and weight of 0.024g; use of these tags would result in approximately ¼ of the tag burden experienced by lampreys in our study. The use of smaller PIT tags such as these in larval lampreys as small as 100 mm would most likely result in mortalities lower than those observed in this study.

We chose not to use the injection needle to insert PIT tags in 2011, as it was thought to add to the potential for injury from the tagging process. The needle requires space within the body cavity in addition to that required by the tag, and this space is very limited in larval lampreys. However, Mueller et al. (2006) found that in juvenile Pacific lampreys tags could be efficiently injected into the body cavity by using an injection needle, perhaps due to the increased fat stores and weight of these animals relative to their larval form.

This is the first study to evaluate the use of VI Alpha tags in lampreys and we were able to successfully tag larval sea lampreys as small as 85 mm; however, during the holding period significant tag loss was observed and in 2011 some retained tags were unreadable. VI Alpha tags were used in wild brook trout (*Salvelinus fontinalis*) and tags remained legible over a year; however, tag retention rates were approximately 50% for brook trout 130-160 mm in total length (TL) and 100% for brook trout 200 mm TL or greater (Bryan and Ney 1994). Another study using VI Alpha tags in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) indicated that fish length was the most significant variable that positively influenced tag retention (Shepard et al. 1996). McMahon et al. (1996) recommend placing another batch mark or tag on VI Alpha-tagged fish to retain information on fish as tag retention may approach only 50% for some salmonids over a period of a year. Tag loss of VI Alpha tags in larval sea lampreys could potentially be decreased by finding a better implantation site on the body. In this study we found it difficult to close the wound that was opened by subcutaneous injection of VI Alpha tags. In

2010 we tried using a tissue adhesive, and in 2011 we attempted to suture the wound. Neither method provided satisfactory wound closure, nor prevented the tag from being lost through the initial injection site once the animal became active. In some fish the ideal injection site has been found to be surrounding the eye or within the adipose eyelid tissue (NMT 2010), but larval lampreys do not have eyes or a pocket of adipose tissue in the vicinity where the eyes will develop. It is possible that VI Alpha tags may not be suitable for use in larval lampreys.

Tags that would identify individual larval sea lampreys as small as 100 mm would provide the opportunity to assess their fine-scale spatial movements and microhabitat preferences. Tags of this size would be useful for many other small bodied fishes and fish of similar body morphometric, such as American eel and hagfish. Coded wire tags have been successfully used to track groups of out migrating juvenile sea lampreys (Bergstedt and Seelye 1995; Howe et al. 2006) and group movement within rivers over time (Weise and Pajos 1998). PIT tags and VI Alpha tags provide unique identifiers to individual lampreys, with PIT-tagged animals able to be detected *in-situ* in river beds without disturbance (Quintella et al. 2005). Implantable tags could be used in larval lampreys; however, the tag burden must be lower than 5% (PIT), and implantation and wound closure (PIT and VI Alpha) requires refinement.

MANAGEMENT RECOMMENDATIONS

Based on our initial results, I would recommend another study be conducted investigating the movement and habitat preference of larval sea lamprey ammocoetes. Smaller PIT tags have been developed since this study took place. These new, smaller PIT tags would most likely result in lower mortality rates and therefore, generate a larger tagged population of ammocoetes. I would recommend this study be conducted in a semi-remote stream, with little human presence, with relatively similar proportions of Type I and Type II habitat, and a natural stream flow. This study was conducted on a stream regularly used for human recreation and where water levels were controlled by a dam.

Also the use of a stationary PIT tag detection system made up of cross river PIT antennas, similar to the system used by Luehring et al. (2011) would be able to better detect movements of PIT-tagged ammocoetes downstream, especially any large scale movements made. This would potentially lead to an increase in data collection as it could decrease the number of tagged animals that go undetected.

While using statolith measurement data along with ammocoete length frequency data did improve proportion-at-age estimates for one of the streams, it did not for the second stream. I would recommend looking at the differences between the two streams in order to determine the reason(s) for the difference in results. If it is found that the differences in estimates are due to temperature of the streams, and therefore, speed of ammocoete growth, I would recommend developing statistical models that analyze colder and warmer streams individually.

A study that examined statolith annuli rings conducted by Dawson et al. (2009) found that using annuli rings along with length frequency data better predicted proportion-at-age

estimates for a colder, slow growing ammocoete population over a warmer, fast growing ammocoete population. I would suggest looking at ammocoete length frequency data along with statolith annuli counts and measurement data. If the combination of these three types of data does not improve the proportion-at-age estimates over the combinations of two of the data groups, perhaps analyzing colder and warmer streams separately is the answer.

The time, skill level, and overall cost of the methods needed to extract and analyze statoliths is great. Statolith extraction from ammocoetes takes between 10 – 25 minutes depending on skill level of the technician. Statoliths must then be prepared for at least nine days in immersion oil. Collecting statolith measurements then takes another 5 – 30 minutes depending on skill level. It will be important for management agencies to take this into consideration.

REFERENCES

Almeida PR, Quintella BR (2002) Larval habitat of the sea lamprey (*Petromyzon marinus L.*) in the River Mondego (Portugal). In Freshwater Fish Conservation: Options for the Future (Collares-Pereira MJ, Coelho MM, Cowx IG, eds). Oxford: Fishing News Books, Blackwell Science, pp. 121–130

Anderson GJ (2006) Improving larval sea lamprey assessment in the Great Lakes using adaptive management and historical records. MS Thesis, Michigan State University

Anderson JR, Morison AK, Ray DJ (1992) Age and growth of Murray cod, *Maccullochella peelii* (Perciformes: Percichthyidae), in the lower Murray–Darling Basin, Australia, from thin-sectioned otoliths. Aust. J. Mar. Freshw. Res. 43: 983–1013

Beamish FWH, Lowartz S (1996) Larval habitat of American brook lamprey. Can J Fish Aquat Sci 53: 693–700

Beamish FWH, Medland TE (1988) Age determination for lampreys. Trans Am Fish Soc 117:63-71

Bergstedt RA, Seelye JA (1995) Evidence for lack of homing by sea lampreys. Trans Am Fish Soc 124:235–239

Bergstedt et al. (2003) Mark-recapture population estimates of parasitic sea lampreys (*Petromyzon marinus*) in Lake Huron. J Gt Lakes Res 29: 226-239

Biomark, Inc. (2008). Retrieved from http://biomark.com. Accessed 1 June 2011

Bird DJ, Potter IC (1979) Metamorphosis in paired species of lampreys, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch): 1. A description of the timing and stages. Zool J Linnean Soc 65:127–143

Boehlert GW (1985) Using objective criteria and multiple regression models for age determination in fishes. Fish Bull 83:103-117

Brege DC, Davis DM, Genovese JH, McAuley TC, Stephens BE, Westman RW (2003) Factors responsible for the reduction in the quantity of the lampricide, TFM, applied annually in streams tributary to the Great Lakes from 1979 to 1999. J Gt Lakes Res 29(Suppl. 1): 500-509

Brown RS, Harnish RA, Carter KM, Boyd JW, Deters KA, Eppard MB (2010) An evaluation of the maximum tag burden for implantation of acoustic transmitters in juvenile Chinook salmon. N Am J Fish Manag 30:499–505

Bryan RD, Ney JJ (1994) Visible implant tag retention by and effects on condition of a stream population of brook trout. N Am J Fish Manag 14(1):216-219

Christie GC, Goddard CI (2003) Sea Lamprey International Symposium (SLIS II): Advances in the integrated management of sea lamprey in the Great Lakes. J Gt Lakes Res 29 (Suppl 1):1-14

Dahl FH, McDonald RB (1980) Effects of control of the sea lamprey (*Petromyzon marinus*) on migratory and resident fish populations. Can. J. Fish. Aquat. Sci 37:1886-94

Dawson HA (2007) Recruitment dynamics of Great Lakes sea lamprey populations (Petromyzon marinus) and implications for integrated pest management. PhD Dissertation, Michigan State University, East Lansing, MI, USA

Dawson HA, Jones ML (2009) Factors affecting recruitment dynamics of Great Lakes sea lamprey (*Petromyzon marinus*) populations. J Gt Lakes Res 35:353-360

Derosier AL, Jones ML, Scribner KT (2007) Dispersal of sea lamprey larvae during early life: relevance for recruitment dynamics. Environ Biol Fish 78:271–284

Fournier DA (1983) Use of length and age data for estimating the age structure of a collection of fish. Can Spec Publ Fish Aquat Sci 66:206-208

Francis RICC, Campana SE (2004) Inferring age from otolith measurements: a review and a new approach. Can J Fish Aquat Sci 61:1269-1284

Hansen MJ, Adams JV, Cuddy DW, Richards JM, Fodale MF, Larson GL, Ollila DJ, Slade JW, Steeves TB, Young RJ, Zerrenner A (2003) Optimizing Larval Assessment to Support Sea Lamprey Control in the Great Lakes. J Gt Lakes Res 29 (Suppl. 1):766-782

Howe EA, Marsden JE, Bouffard W (2006) Movement of sea lamprey in the Lake Champlain basin. J Gt Lakes Res 32:776-787

Hollett AK (1998) Condition factor and statolith aging in assessment of metamorphosis in sea lamprey (*Petromyzon marinus*) in the Great Lakes. Master's thesis. University of Toronto, Toronta, Ontario

IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp

Jones ML (2007) Toward improved assessment of sea lamprey population dynamics in support of cost-effective sea lamprey management. J Gt Lakes Res 33(Special Issue 2):35–47

Jones ML, Bergstedt RA, Twohey MB, Fodale MF, Cuddy DW, Slade JW (2003) Compensatory mechanisms in Great Lakes sea lamprey populations: implications for alternative controls. J Gt Lakes Research 29 (Supplement 1):113-129

Lombarte A, Lleonart J (1993) Otolith size changes related with body growth, habitat depth and

temperature. Environ. Biol. Fish. 37: 297-306

Lowe DA, Beamish FWH, Potter IC (1973) Changes in the proximate body composition of *Petromyzon marinus* (L.) during larval life and metamorphosis. J Fish Biol 5:673-682

Luehring MA, Wagner MC, Li W (2011) The efficacy of two synthesized sea lamprey sex pheromone components as a trap lure when placing in direct competition with natural male odors. Biol Invasions 13: 1589-1597

McMahon TE, Dalbey SR, Ireland SC, Magee JP, Byorth PA (1996) Field evaluation of visible implant tag retention by brook trout, cutthroat, rainbow trout, and Arctic grayling. N Am J Fish Manag 16(4):921-925

Meeuwig MH, Bayer JM (2005) Morphology and aging precision of statoliths from larvae of Columbia River basin lampreys. N Am J Fish Manag 25:38-48

Metin G, Ilkyaz AT (2008) Use of otolith length and weight in age determination of poor cod (Trisopterus minutus Linn., 1758). Turkish J. Zool. 32: 293-297

Morman RH, Cuddy DW, Rugen PC (1980) Factors influencing the distribution of sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37: 1811–1826

Mueller RP, Moursund RA, Bleich MD (2006) Tagging juvenile Pacific lamprey with passive integrated transponders: methodology, short-term mortality, and influence on swimming performance. N Am J Fish Manag 26: 361-366

Mullett KM, Bergstedt RA (2003) Agreement Among Observers Classifying Larval Sea Lamprey (*Petromyzon marinus*) Habitat. J. Gt. Lakes Res. 29: 183-189

NMT (Northwest Marine Technology, Inc.) (2010) Instructions for NMT's new visible implant alpha tags. Retrieved from http://nmt.us. Retrieved 1 July 2010

NMT (Northwest Marine Technology, Inc.) (2011) Instructions for 10:1 Visible Implant Elastomer. Retrieved from http://nmt.us. Retrieved 1 June 2011

Ojutkangas E, Aronen K, Laukkanen E (1995) Distribution and abundance of river lamprey (Lampetra fluviatilis) ammocoetes in the regulated river Perhonjoki. Regul Rivers: Res & Manag 10: 239–245

Otter Research (2000) An introduction to AD Model Builder, version 6.0.2, for use in nonlinear modeling and statistics. Otter Research Ltd, Nanaimo, British Columbia, Canada

Pawson MG (1990) Using otoliths weight to age fish. J Fish Biol 36:521-531

Pearce WA, Braem RA, Dustin SM, Tibbles JJ (1980) Sea lamprey (Petromyzon marinus) in the

lower Great Lakes. Can. J. Fish. Aquat. Sci. 37:1802-10

Potter IC (1980) Ecology of larval and metamorphosing lampreys. Can J Fish Aquat Sci 37:1641-1657

Quintella BR, Andrade NO, Espanhol R, Almeida PR (2005) The use of PIT telemetry to study movements of ammocoetes and metamorphosing sea lampreys in river beds. J Fish Biol 66:97–106

Schneider CP, Owens RW, Bergstedt RA, O'Gorman R (1996) Predation by sea lamprey (*Petromyzon marinus*) on lake trout (*Salvelinus namaycush*) in southern Lake Ontario, 1982-1992. Can J Fish Aquat Sci 53:1921-32

Schnute J, Fournier D (1980) A new approach to length-frequency analysis: growth structure. Can J Fish Aquat Sci 37:1337-1351

Schreck CB, Fitzpatrick MS, Lerner DL (2000) Determination of passage of juvenile lamprey: development of a tagging protocol. Oregon Cooperative Fish and Wildlife Research Unit, Oregon State University, Corvallis, OR

Shepard BB, Robison-Cox J, Ireland SC, White RG (1996) Factors influencing retention of visible implant tags by westslope cutthroat trout inhabiting headwater streams of Montana. N Am J Fish Manag 16(4):913-920

Short JA, Gburski CM, Kimura DK (2006) Using otolith morphometrics to separate small walleye pollock *Theragra chalcogramma* from Arctic cod *Boreogadius saida* in mixed samples. Alaska Fish Res Bull. 12(1): 147-152

Slade JW, Adams JV, Christie GC, Cuddy DW, Fodale MF, Heinrich JW, Quinlan HR, Weise JG, Weisser JW, Young RJ (2003) Techniques and methods for estimating abundance of larval and metamorphosed sea lampreys in Great Lakes tributaries, 1995 to 2001. J Gt Lakes Res 29 (Suppl 1):137–151

Smith BR, Tibbles JJ (1980) Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: history of invasion and control, 1936-78. Can J Fish Aquat Sci 37: 1780-1801

Steeves TB (2002) Uncertainty in estimating sea lamprey (*Petromyzon marinus*) abundance in Great Lakes tributaries. Master's Thesis, Michigan State University, East Lansing, MI

Steeves TB, Slade JW, Fodale MF, Cuddy DW, Jones ML (2003) Effectiveness of using backpack electroshocking gear for collecting sea lamprey (*Petromyzon marinus*) larvae in Great Lakes tributaries. J Gt Lakes Res 29(Suppl 1):161–173

Sullivan WP (2003) Substrate as a correlate of density and distribution of larval sea lampreys in

streams. Master's thesis, Guelph, ON Canada: University of Guelph

Swink WD (2003) Host selection and lethality of attacks by sea lamprey (*Petromyzon marinus*) in laboratory studies. J. Gt Lakes Res 29(Suppl. 1): 307-319

Treble AJ, Jones ML, Steeves TB (2008) Development and evaluation of a new predictive model for metamorphosis of Great Lakes larval sea lamprey (*Petromyzon marinus*) populations. J Gt Lakes Res 34:404-417

W. Nuhsbaum, Inc. (2010). Retrieved from http://www.nuhsbaum.com/optical-equipment-michigan.php. Retrieved 1 June 2010

Weise JG, Pajos TA (1998) Intraspecific competition between larval sea lamprey year-classes as Salem Creek was recolonized, 1990–1994, after a lampricide application. N Am J Fish Manag 18:561–568

Worthington DH, Doherty PA, Fowler AJ (1995) Variation in the relationship between otolith weight and age: implications for the estimation of age of two tropical damselfish *(Pomacentrus moluccensis and P. wardi)* Can J Fish Aquat Sci 52:233-242

Young RJ, Kelso JRM, Weise JG (1990) Occurrence relative abundance, and size of landlocked sea lamprey (*Petromyzon marinus*) ammocoetes in relation to stream characteristics in the great lakes. Can J Fish Aquat Sci 47: 1773–1778

Youson JH, Lee J, Potter IC (1979) The distribution of fat in larval, metamorphosing, and adult anadromous sea lampreys, *Petromyzon marinus* (L.), Can J Zool 57:237-246

Youson JA, Holmes JA, Guchardi JA, Seelye JG, Beaver RE, Gersmehl JE, Sower SA, Beamish FWH (1993) Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 50: 2448-2456

Youson JH, Potter IC (1979) A description of the stages in metamorphosis of the anadromous sea lamprey (*Petromyzon marinus*). Can J Zool 57(9): 1808-1817