

CHOOSING STREAMS FOR SEA LAMPREY CONTROL:
USING ALTERNATIVE MODELS OF METAMORPHOSIS TO
OPTIMIZE THE STREAM SELECTION PROCESS

By

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A THESIS

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

MASTER OF SCIENCE

Department of Fisheries and Wildlife

2006

ABSTRACT

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Control of sea lamprey (*Petromyzon marinus*) in the Great Lakes is primarily achieved through the application of lampricide to streams harbouring abundant populations of larvae. For lampricide treatments to be efficient, streams are prioritized for treatment based on forecasts concerning the escapement of recently-metamorphosed parasitic juveniles relative to the cost of treating each stream. Computer simulations confirmed that while this is the most effective method to employ when the actual number of lamprey is known, uncertainty associated with assessment surveys and the use of predictive models of metamorphosis decreases the efficiency of this method. Building on laboratory experiments that suggest the accumulation of lipids is a critical stage prior to the onset of metamorphosis, I adapted a non-invasive method to estimate lipid content in larval sea lamprey. Predicted lipid content was combined with other biotic and abiotic stream measurements in a mark-recapture study to investigate the relative importance of various factors influencing metamorphosis. The predictive metamorphic model that was developed was evaluated for its ability to accurately estimate transformer abundance and compared with the performance of other models used in the stream selection process. The results suggest that the incorporation of stream-specific measures can greatly improve our ability to accurately predict metamorphosis, allowing for better stream treatment decisions, and further reductions in sea lamprey abundance.

DEDICATION

This work is dedicated to the memory of my mother Jane, and her sister Catherine, both of whom lived difficult lives that were cut far too short, but whose impact on my life was immeasurable and continues to this day.

ACKNOWLEDGEMENTS

I would like to thank first and foremost my advisor, Dr. Michael Jones, for his guidance and encouragement throughout the course of this project, and for making graduate school the enjoyable experience that it was. I would also like to acknowledge the contributions of my advisory committee, Dr. Dan Hayes and Dr. Patrick Muzzall, for their advice and encouragement. I am also indebted to Dr. Mike Wilberg and Dr. Ty Wagner for countless statistical consultations, and for having the patience to guide me through them all. I would like to thank all of my lab mates in the Jones-Bence lab, for all of their valued input, encouragement, and much-needed distractions. I would also like to thank Sarah Haan, my undergraduate assistant, for her tireless efforts. From reading coded wire tags and aging statoliths, to measuring piles of dead lampreys, I never once heard her complain. Gretchen Anderson, Nathan Miller, and Dr. Renate Snider provided invaluable editorial comments and suggestions during the preparation of this thesis.

I wish to thank all of my friends and co-workers at the sea lamprey control centre in Sault Ste Marie, Ontario, for their support, particularly those who provided assistance with the mark-recapture portion of this study. Special thanks go to the Canadian and U.S. treatment crews for all of their assistance and tolerance of my meddling through two field seasons. I would also like to acknowledge former Jones lab member and the PI on this project, Mike/Todd Steeves, for his endless encouragement, as well as logistical and technical support.

I need to thank friends and family, without whose support and encouragement I surely would have given up and returned to life as a fishing guide. To my dad and Helen: for all of your support, for providing the occasional weekend retreat away from all things

lamprey, and for the random unsolicited deposits into my ever-dwindling bank account at the most opportune moments. To my adopted family Sean, Gaylyn, Griffin, and Quinn: for keeping me grounded and reminding me what is important in life. And to Fraser and Nancy: for their friendship, support, and endless generosity; as well as for making sure that I always had a home in the Soo to return to. I am extremely grateful to you all.

And finally, to my girlfriend Stacy, who infected me along with the rest of the department with her enthusiasm for cheese and all things Wisconsin. Who would have guessed that all of those meetings at F.A.R.T. would have turned out like this? While we supported one another through the whole process, I still think you got the short end of the stick... as well as a short Canadian *to boot... eh?*

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THESIS INTRODUCTION

Sea lamprey biology

Sea lampreys (*Petromyzon marinus*) are the largest and most voracious of the world's lamprey species, although landlocked populations are somewhat smaller than their anadromous counterparts (Scott & Crossman 1973). Native to the Atlantic Ocean and surrounding waters, sea lampreys were present in low numbers in the waters of the St. Lawrence, Lake Champlain, and Lake Ontario prior to 1920 (Scott & Crossman 1973). However, with the construction of the Erie and Welland Canals, sea lampreys were able to by-pass natural barriers and gained access to the upper Great Lakes. Once in the upper lakes, their distribution and numbers expanded dramatically (Smith & Tibbles 1980; Christie & Goddard 2003). Their continued persistence and impacts on fish communities in the Great Lakes serve as a constant reminder to the risks associated with exotic species invasions.

In the spring, the semelparous adults use pheromones from larval populations and other unknown cues to locate and migrate up tributaries suitable for spawning, where they spawn in gravel riffles when water temperatures approach 15 °C (Manion & Hanson 1980; Bjerselius *et al.* 2000; Sorensen & Vrieze 2003). Males construct crescent-shaped nests of gravel and rubble, and release sex pheromones to attract females to the nest (Li *et al.* 2003). During spawning, eggs are released, fertilized, and drift to the back of the nest, where they adhere and become covered by substrate (Potter 1980). The eggs hatch after approximately 18-21 days and the free-swimming larvae drift passively downstream with the current, burrowing into the soft sediments of depositional areas, which are generally comprised of a mixture of silt, sand, and detritus (Applegate 1950; Potter 1980).

Although the length of the larval phase can be highly variable, sea lamprey of the Great Lakes typically remain as larvae in their natal streams for 2 – 7 years, where they filter feed on a variety of minute plant and animal material (Scott & Crossman 1973; Potter 1980; Youson 2003). The length of the larval phase is thought to be related to the rate of growth, which itself is highly variable and influenced by environmental and demographic characteristics of the natal stream (Purvis 1980; Youson *et al* 1993). Once larvae attain a minimum length (typically around 120 mm for landlocked sea lampreys of the Great Lakes), changes in metabolic processes alter their growth patterns; shifting from linear growth through protein anabolism to growth in mass through lipid accumulation (Potter 1980; Holmes & Youson 1994; Youson 2003). This pre-metamorphic stage has often been referred to as an arrested growth phase, because larvae show declining rates of linear growth, while their lipid content increases from an average of 4 to 14% (wet weight) (Lowe *et al* 1973; Potter 1980; Holmes & Youson 1994; Henson *et al* 2003). It is assumed that these fat reserves support the ammocoetes through the non-trophic stage of metamorphosis (transformation) until they are able to obtain their first blood meal; typically a period lasting between 4 to 10 months (Youson & Potter 1979; Potter 1980).

Sea lampreys represent one of the few vertebrate species that undergo a true metamorphosis (Wald 1958), which is a required developmental stage that prepares the organism for life in a new environment. Prior to the discovery of individuals with characteristics intermediate to larvae and adult lamprey (Müller 1856), larval lampreys were considered to be separate species (genus *Ammocoetes*) from their adult equivalents. The myriad of physical and biochemical changes that sea lamprey undergo as part of their

transformation into parasitic adults has been well researched and summarized by Youson (1980 & 2003). Although driven by internal changes (primarily decreases in thyroid hormone levels) as lamprey prepare to shift from sedentary filter feeding to an open-water, parasitic feeding lifestyle, external changes are used to divide the process of metamorphosis into seven developmental stages (Youson & Potter 1979; Youson 1980). These external changes include: development and enlargement of the eyes (stages 1-7), modification of branchiopores (stage 3), development/enlargement of dorsal and caudal fin surfaces (stages 4-7), changes in body pigmentation and proportion (stages 5-7), and development of oral apparatus (stages 4-6), which includes the formation of the oral disc, fimbriae, teeth, tongue-like piston, and infraoral lamina (Youson 1980). Virtually all internal systems undergo some form of change, with the transformation of the liver and development of the kidneys occurring early in the process, and systems related to osmoregulation, feeding, and digestion occurring in the latter stages (Youson 1980). Fundamental to these internal changes is the change in structure and function of various proteins from larval to adult forms (e.g. albumins), as well as a redistribution of iron within the body, which is thought to be key to post-metamorphic development (Youson 2003). During the entire process of metamorphosis, which may last as long as ten months, lipids are used as the primary source of energy.

In landlocked populations of the Great Lakes, metamorphosis is typically initiated in late June/early July (Manion & Stauffer 1970; Youson & Potter 1979). While the process of metamorphosis is surprisingly synchronous, even among landlocked and anadromous populations, the length of time from initiation of metamorphosis until feeding is highly variable and seems to be more dependent on high water levels, either

the fall or following spring, to facilitate migration out to the lakes (Manion & Smith 1978; Potter 1980).

Once in the lakes, less is known about their specific ecology. Recently metamorphosed lampreys are thought to head to deep water and then slowly work their way to shore as they grow and mature (Scott & Crossman 1973). Lamprey will typically remain in the open lakes for approximately 20 months, feeding on the blood, bodily fluids, and products of tissue cytolysis from a variety of hosts (Farmer 1980). It is thought that lamprey locate their hosts through a combination of vision and olfactory senses (Farmer 1980). Although lampreys have been shown to prefer lake trout (*Salvelinus namaycush*) and other salmonid species (Wells & McLain 1973), they have been found to attack a wide range of other species (Farmer & Beamish, 1973; Farmer 1980; Christie & Kolenosky 1980). Lampreys also tend to select larger hosts over smaller hosts, and will avoid lake trout under 600mm in length (Farmer & Beamish 1973; Swink 2003). In a study of attachment sites, Farmer and Beamish (1975) found that lampreys preferred to attach below the lateral line, between the head and the caudal peduncle. They also found that of lampreys that attached in this manner, over half were found near the pectoral fin insertion.

Lampreys “suck on” to a host by generating a vacuum within their oral disc. This is achieved by creating a seal where the oral disc meets the host and suction is generated through a change in volume of the buccal funnel (Lennon 1954; Farmer 1980). Despite abundant teeth lining the oral disc, it is a series of denticles located on 2 plates attached to the tongue-like piston, used to wrasp a hole in the flesh of the fish, which inflicts the majority of the initial tissue damage (Farmer 1980). Once attached and the external

integument has been penetrated, lamprey secrete lamphredin from the buccal glands, which possess anticoagulant and tissue cytolytic properties to facilitate feeding (Farmer 1980).

Fish possess only 3% of their total weight as blood (relative to 7-9% in mammals), making them especially vulnerable to lamprey predation (Holmes & Donaldson 1969; Farmer 1980). As lamprey remove blood, their hosts attempt to compensate by manufacturing additional red blood cells (RBCs) and leukocytes, as well as maintaining osmotic pressure by increasing the water content of the blood and transferring ions from other body compartments (Lennon 1954). While it appears that fish can usually maintain leucocyte levels and osmotic pressure, Lennon (1954) found that white suckers (*Catostomus commersoni*) experienced an 84% reduction in RBC count relative to uninjured fish. A corresponding increase in the water content of the fish's blood, from 84.5 to 96.4% was also observed (Lennon 1954). This decrease in RBC concentrations and increases in water content, serving to lower the overall energy content of the blood, may cause some lamprey to detach prematurely, prior to killing the host (Lennon 1954). Thus it appears that fish parasitized by lamprey die from an inability to maintain RBC numbers and regular oxygen transport to the rest of the body. The rate at which fish succumb to lamprey attacks is both seasonal and highly variable (Bergstedt & Swink 1995; Swink 2003).

Studies of feeding lamprey in the laboratory have indicated that feeding rates and the duration of attachment of sea lampreys vary with the size of the lamprey, size of the host, and water temperature (Farmer 1980; Bence *et al* 2003). At 10°C, Farmer *et al.* (1975) calculated the mean feeding rate to be equal to 25% of the lamprey's wet body

weight of blood/day. Mortality of the host therefore depends on the amount of available blood. Fish that had close to 100% of their blood volume (per day) withdrawn from them only survived two days, whereas fish that had 10% of their blood volume withdrawn per day seemed to survive indefinitely (Farmer *et al.* 1975). Based on their calculations, it appears that the host-to-lamprey weight ratio must be in the order of 40:1 for hosts to survive lamprey attacks (Farmer *et al.* 1975). Not all attachments, however, result in feeding. Farmer (1980) found that 37% of all attachments did not involve feeding, but may represent a method for satiated lampreys to conserve swimming energy. A review of research conducted on the effects of lamprey parasitism (Swink 2003) indicated that between 40-80% of attachments end in host mortality. Early laboratory studies placed the average weight of trout or salmon killed over the course of a lamprey's lifetime as high as 16.8 kg (~40 lbs.) (Parker & Lennon 1956). However, more recent laboratory studies and individual bioenergetics modeling (IBM) have suggested that this value is highly variable, ranging between 6.8 and 19.3 kg of host killed per sea lamprey over a season (Swink 2003; Madenjian *et al.* 2003, Bence 2003). Recent studies have also suggested that host mortality is highly dependent on several factors, including mean daily temperature, host size, and host species (Swink 2003). Furthermore, based on the recovery of lake trout carcasses from the bottom of Lake Huron, it appears that lamprey-induced mortality is not constant, but instead spikes in early October to November (Bergstedt & Swink 1995). This spike in mortality is thought to be related to increases in blood consumption by lamprey, potentially in response to water temperature, gonad maturation, or the attainment of an optimal feeding size (Bergstedt & Swink 1995; Madenjian *et al.* 2003). Increased mortality may also be related to increased opportunities

for lamprey attachment as salmonid species aggregate in the fall to spawn (Madenjian *et al.* 2003).

Sea lamprey control

The history of the invasion into the Great Lakes by the parasitic sea lamprey and its subsequent impacts on both fisheries resources and the ecosystem as a whole has been well documented (Smith & Tibbles 1980; Pearce *et al.* 1980; Heinrich *et al.* 2003; Larson *et al.* 2003; Lavis *et al.* 2003; Morse *et al.* 2003; Sullivan *et al.* 2003). The Great Lakes Fishery Commission (GLFC) was formed in 1954 as a result of the invasion, to coordinate research and control efforts in the bordering U.S. states and Canadian Provinces (Christie & Goddard 2003). The GLFC operates in the United States and Canada through its agents, the U.S. Fish and Wildlife Service (USFWS) and the Department of Fisheries and Oceans (DFO) respectively. Initial attempts at control focused on restricting access to spawning streams by the blockage and removal of adults during their spawning migrations. After almost 20 years of this practice, however, the number of migrating adult sea lampreys captured at these barriers had not declined (Smith & Tibbles 1980; Christie & Goddard. 2003).

Effective lamprey control was not achieved until the discovery and first application of the larval lampricide 3-triflouromethyl-4-nitrophenol (TFM) in 1958, which marked the beginning of the chemical treatment program that continues today. Sea lamprey lack the ability possessed by other fish species to detoxify TFM and excrete it from their tissues, thus TFM is selective for lamprey while having minimal long-term effects on the aquatic community (Howell *et al.* 1980; Hubert 2003). Since the discovery

of TFM's selective toxicity for lamprey, an estimated 1223 chemical treatments were performed on 334 streams, leading to a 92% reduction in overall sea lamprey abundance (Smith & Tibbles 1980). Initially, decisions regarding stream treatments were made subjectively, based on the observed presence or absence of substantial numbers of large larvae in electrofishing surveys. In 1995, the GLFC adopted a quantitative assessment and stream ranking methodology. Streams were now objectively selected for lampricide treatment based on a cost-benefit analysis of the estimated potential number of metamorphosing larvae relative to the cost of treating a specific stream.

Quantitative assessment surveys (QAS) produce estimates of larval density and the extent of available larval habitat in each stream. These data are used in a computer-based model (ESTR: Empirical Stream Treatment Ranking System) to compile larval assessment data and generate cost-benefit ratios to prioritize streams for lampricide treatment across the basin. This method was developed in an attempt to optimize efficiency and maximize suppression of the population; however, recent indices of abundance suggest that lamprey numbers may be on the rise in a number of the lakes (Lavis *et al.* 2003; Sullivan *et al.* 2003). It is unclear if these increases are attributable to reductions in lampricide usage, natural variations in lamprey populations, or whether inaccuracies in the models used to select streams for treatment have also played a role.

Today, the rehabilitation of native fish populations and a multi-billion dollar recreational fishery are reliant on continuing lamprey control efforts (GLFC 2003). The GLFC must balance an effective lamprey control program with the objectives of fisheries managers from across the lakes, in the face of shrinking financial resources and public concern regarding the use of pesticides. The control program is ever improving and

evolving, searching for new and more efficient ways to control the abundance of sea lampreys and minimize their impact on Great Lakes fish communities.

Predicting Metamorphosis

An independent review of the methods used in the larval assessment and stream ranking process (Hansen *et al.* 2002) identified several sources of uncertainty that may frustrate efforts to select streams optimally. One source of uncertainty lies in the probability of metamorphosis model that ESTR uses to convert lamprey lengths from larval surveys into forecasts of transformer production. This model is based on solely on the length of the larval lamprey and is reliant on several assumptions that have not been evaluated using field studies to see how inaccuracies in its estimates may affect our overall ability to control sea lampreys. Other predictive models have since been developed that attempt to identify larvae in the lipid accumulation stage prior to metamorphosis by using combinations of length, weight and condition factor measurements (Holmes & Youson 1994); however these models failed to show any improvement in predictive ability.

The goal of this thesis is to answer several questions surrounding lamprey metamorphosis and its suitability for use to rank streams for treatment. First of all, focusing control efforts on metamorphosis seems like an optimal method to minimize the effects of lamprey parasitism. Is this really the case, given the uncertainty that exists in predicting metamorphosis? In chapter 1, I address this question by using computer simulations to simulate lamprey population, assessment, and treatment cycles and to

incorporate variability in each cycle to see how it impacts on our ability to suppress parasitic abundance over the long term.

Despite the fact that the accumulation of lipids appears to be an essential developmental stage prior to the onset of metamorphosis, direct estimates of lipid content have not been used in any previous metamorphic models. In Chapter 2, I explore the use of various non-invasive methods commonly used to estimate body composition parameters and develop a model for the direct estimation of lipid content in larval sea lampreys.

In chapter 3, I include estimates of lipid content with a suite of individual- and stream-level measurements to not only identify parameters that affect the individual metamorphic likelihood, but to also examine environmental factors that might cause variation in metamorphic rates among streams. A new model of metamorphosis is developed and evaluated relative to current models used in the stream ranking process. The results of the modeling work in Chapter 1 will be used to qualitatively evaluate the utility of this new model and provide sea lamprey managers with guidance regarding which criteria for ranking streams will lead to the greatest overall effect on parasitic abundance well into the future.

CHAPTER I

A STOCHASTIC LIFE HISTORY MODEL TO EVALUATE TREATMENT RANKING STRATEGIES FOR CONTROLLING SEA LAMPREY IN THE GREAT LAKES

ABSTRACT

A stochastic computer simulation model was developed, based in part on the empirical stream treatment ranking (ESTR) software that the Great Lakes Fishery Commission uses to rank streams for lampricide treatment. One of the purposes of this model was to investigate how different stream ranking strategies perform at limiting parasitic lamprey abundance as the magnitude of uncertainty in the larval assessment data upon which they are based was varied. The simulation results suggest that the current method of ranking streams, based on the forecasted production of recently metamorphosed juveniles is not optimal. A ranking strategy that focuses on large larvae (>100 mm) without the use of metamorphic models has less associated uncertainty and leads to better decisions regarding which streams to treat. In addition, improved performance resulted from the removal of treatment cost from the ranking process, which increased the frequency with which large lamprey-producing streams were treated. The modeling results suggest that the implementation of these two methods concurrently would lead to a superior overall reduction in parasitic lamprey abundance, relative to current ranking methodologies, given typical levels of uncertainty surrounding assessment data. This analysis is meant to assist the GLFC in attaining an optimal level of lampricide suppression and hopefully lead to further reductions in the reliance on lampricide treatments as the primary control method.

INTRODUCTION

In 1995, the Great Lakes Fishery Commission (GLFC) adopted an Integrated Pest Management Strategy, fashioned after successful insect pest control programs, to combat sea lamprey infestation in the Great Lakes (Sawyer 1980; Christie and Goddard 2003). This decision fundamentally changed the way biologists assessed and controlled lampreys across the basin, shifting the emphasis from CPUE-style assessment surveys and subjective stream treatment decisions to a more rigorous quantitative method (Slade *et al.* 2003). Adopting quantitative assessment methods facilitated the evaluation and improvement of alternative control strategies, and provided an objective basis for making stream treatment decisions. As part of this process, the Empirical Stream Treatment Ranking (ESTR) program was developed. ESTR is a computer-based model that is used to generate short-term forecasts of population demographics and estimates of future stream-level parasitic juvenile production, based on data from quantitative assessment surveys (QAS) collected throughout the year. Refinement of control techniques and continuing research into assessment methods have served to improve the ESTR model and stream ranking process since their inception (Christie *et al.* 2003; Steeves *et al.* 2003).

One of the drawbacks of the current ESTR system is that it fails to address the significant variability and uncertainty inherent in the stream ranking process (Hansen *et al.* 2003). This uncertainty is introduced through measurement error (i.e., inaccurate estimates of larval habitat and abundance), as well as process error (i.e. inaccuracies in the various parameter estimates such as larval survival and probability of

metamorphosis). Consequently, the estimates of abundance that it produces often do not accurately reflect actual lamprey abundance for a majority of streams (Steeves 2002).

Because of the uncertainty associated with the current stream ranking methodology, it has been suggested that criteria more robust to sources of uncertainty in assessment data should be used to prioritize streams for lampricide treatment (Hansen *et al.* 2003). Ideally, an evaluation of different ranking criteria would be accomplished within an adaptive management framework (Walters 1986), applying different stream selection criteria to each lake and then monitoring the effects of the lampricide treatments on the parasitic population in the lake. However, the temporal and spatial scale required for such an evaluation, coupled with political and financial limitations, pose large challenges for such an approach. As an alternative, a realistic computer simulation model was developed to mimic the entire sea lamprey life-cycle and control program within Lake Michigan. This model has previously been used to explore variability in the sea lamprey stock recruitment relationship and efficacy of alternative control strategies (Dawson & Jones 2005); in this chapter I used the model to evaluate the impact of uncertainty in assessment data, using different stream ranking strategies, on our ability to control sea lamprey abundance.

Simulation models can provide unique insights into biological processes in the face of complex problems (Peck 2004). Several studies (Hansen *et al.* 2003; Steeves 2002) have suggested that the deterministic nature of current stream ranking methods is hindering lamprey control efforts. Simulation of the lamprey life-cycle, assessment, and treatment processes allow us to see how uncertainty at the level of the survey plot or stream reach can ultimately affect our ability to control the number of parasitic lamprey

in the Great Lakes. Although the use of models in the natural sciences is not without limitations (Oreskes *et al.* 1994), the model used here is built upon empirical data and knowledge accrued from five decades of lamprey control.

The objective of this study was to compare different criteria for prioritizing streams for lampricide treatment using stochastic simulations, and evaluate these methods based on the parasitic population that results when selected streams are treated by the model. Based on the recommendations of Hansen *et al.* (2003), I first evaluated the use of the current ranking method (parasitic juveniles (transformers) killed per unit of treatment cost) against other ranking criteria, such as the number of larvae or large larvae (>100 mm) killed per treatment dollar spent. I also assessed the utility of ranking streams based on estimates of overall lamprey abundance, as opposed to dividing those estimates by stream treatment cost, as occurs presently.

METHODS

Using a stochastic age-structured population model, I evaluated different criteria to rank streams for lampricide treatment based on estimates of either overall larval abundance, large larvae abundance, or transformer abundance. I also compared the use of total abundance estimates relative to abundance estimates adjusted for treatment costs as criteria for prioritizing streams for lampricide treatment. Given a specified level of uncertainty and a specific treatment ranking criterion, the abundance of parasitic lamprey within Lake Michigan was forecast for 100 years. For each model iteration, the average parasitic abundance over the final 10 years (years 91-100) was recorded. To capture the variability inherent in the stochastic model, each simulation was repeated 1000 times.

Model Description:

The simulation model I used is based on the structure of the ESTR system currently used by the GLFC to prioritize streams for lampricide treatment, and borrows much of the underlying data and parameter values from that model. The model was derived from the ESTR model and developed at Michigan State University (MSU), so it acquired the acronym MUSTR (Michigan State University Stream Treatment Ranking model). While the ESTR model uses stream data from across the Great Lakes basin to produce basin-wide rank lists, the MUSTR model is presently limited to streams flowing into Lake Michigan. However, the MUSTR model differs from the ESTR model, in that it simulates the entire lamprey life-cycle, from the parasitic population in the lake, to developing larvae in nursery streams, over multiple generations. It couples this with virtual larval assessment, stream ranking and treatment programs (Figure 1; Dawson & Jones 2005). The model is stochastic because it includes density independent variation in the stock-recruitment relationship and uncertainty in larval assessment surveys. The latter were simulated by adding the appropriate amount of variation to the simulated “true” abundance, based on research documenting the uncertainty associated with larval assessment methodologies (Figures 2 & 3; Steeves 2002).

Model Components

(1) Spawner Allocation

Each simulation began with 100,000 spawning adults. Spawners were allocated to the suite of Lake Michigan streams based proportionally on the total area of

available larval habitat in a stream and the number of larval lampreys contained in that stream (i.e., these two factors were given equal weight in the calculations).

(2) Recruitment of Age-1 Larvae

A stochastic, Ricker stock-recruitment relationship was used to determine the number of progeny that would result from the number of spawners allocated to each stream each year. This relationship was based on data combined from several sources which provided estimates of spawner abundance and the subsequent recruitment to age one of larval lampreys, and incorporated a measure of density-independent variability around that relationship (Jones *et al.* 2003; Dawson & Jones 2005).

(3) Larval Survival and Growth

Once the numbers of larvae resulting from a spawning event were determined, they were assigned to age-specific length-frequency bins. A constant rate of larval survival (see model tuning) and a stream-specific growth rate (based on values from the ESTR model) were applied to these age/length frequency bins to grow larvae over successive years and adjust overall stream demographics for natural mortality.

(4) Metamorphosis

The length-based probability of metamorphosis model used in ESTR for Lake Michigan was applied to the larval length bins for each stream to generate the number of animals that would metamorphose that year (Hansen *et al.* 2003; Slade *et al.* 2003). A constant rate of transformer survival was applied to this stage to determine the final number of recently metamorphosed juveniles that would leave the system and join the pool of parasitic individuals in the lake, provided the stream is not treated. Another

constant survival rate was applied to this total pool of parasites in order to determine the number of individuals that would survive to spawn, closing the cycle.

(5) Larval Assessment

Population estimates generated from larval assessment surveys were modelled by adding uncertainty to the true number of larvae or metamorphosing lamprey present in each stream. This was accomplished by adding an error term that was drawn independently from a gamma distribution possessing a specified coefficient of variation (CV). The gamma distributions and range of CVs used were obtained from an analysis by Steeves (2002), who used a Monte Carlo simulation approach, using the ESTR model and larval assessment data from nine different streams, to quantify the distribution of abundance estimates for three lamprey life stages (larvae, big larvae, and transformers) (Figures 2 & 3). A gamma distribution was used because the distributions generated did not fit normal or log-normal distributions well. The coefficients of variation (represented as minimum, average and maximum uncertainties) for each life stage used in the analysis relate to the range in uncertainties found in the abundance estimates for the nine different streams used in Steeves study (2002).

(6) Stream Ranking and Treatment

Using simulated assessment data, different criteria were used to prioritize streams for lampricide treatment. Streams were treated in order of decreasing priority until the treatment budget was exhausted. The treatment budget was held constant at \$2.06 million, the amount that is currently spent on lampricide control in Lake Michigan. Lamprey mortality resulting from simulated treatments was calculated for each

stream using treatment efficacy estimates (generally 95 to 99%) from the ESTR model, which are based on treatment history and judgement by experienced treatment personnel (Smith & Tibbles 1980; Heinrich *et al.* 2003).

Lamprey surviving treatment (residuals), lampreys from streams not treated, and lampreys belonging to an “untreatable pool” were added to the whole-lake population and then allocated to streams for spawning the following year. The untreatable pool represented lampreys in untreatable stream reaches or lentic areas at the mouths of streams that are presently untreated by the control program. The allocation of lamprey to the untreatable pool and the population dynamics applied to lamprey in the pool were the same as for treated streams. The size of the untreatable pool was set at 10% of the available lake-wide larval habitat.

Model Calibration

Model parameters were adjusted to produce an average parasitic population similar to that presently estimated for Lake Michigan based on a series of assumptions. The deterministic ESTR model estimates that there are roughly 10,000 parasitic lampreys that should either survive stream treatments or leave untreated streams annually. However, according to recent mark-recapture estimates, the spawner abundance is thought to be closer to 150,000. Other simulations have suggested that this discrepancy between mark-recapture estimates of parasitic abundance and the number forecast by ESTR could be a result of the assessment uncertainty I am modeling here (M. Jones, unpublished data). If this is true, then the MUSTR model should produce a population

close to what ESTR predicts when I assume no error associated with larval assessments, and produce a parasitic population similar to what mark-recapture estimates suggest are in the lake, under an average amount of uncertainty, as determined by Steeves (2002) (Figure 3). Accordingly, I adjusted the larval survival parameter for the MUSTR model to achieve this outcome.

Treatment Ranking Algorithms

- (1) Number of transformers killed per treatment dollar: This algorithm is currently used by the GLFC to rank streams, and treats streams until the treatment budget is exhausted based on the predicted number of transformers a specific stream will produce, divided by the projected cost of treating that stream. Streams are then ranked in order of decreasing benefit-cost ratio.
- (2) Number of large larvae killed per treatment dollar: To avoid the uncertainty associated with estimates of transformation, this algorithm focuses on larvae of a specified size (≥ 100 mm). The numbers of larvae in each stream that are projected to reach 100 mm by the end of the year are divided by the estimated treatment costs for treating each stream. The streams are ranked based on this benefit-cost ratio and treated in order until the treatment budget is exhausted.
- (3) Number of larvae killed per treatment dollar: This is the simplest of all the ranking algorithms because it does not require models of growth or probability of metamorphosis, this method simply divides the total number of estimated larvae, regardless of size, by the projected treatment costs for each stream. Streams across

the basin are then ranked and treated in order of decreasing benefit-cost ratio until the treatment budget is exhausted.

(4) *Most Kill-Transformers*: This algorithm ranks streams based on the predicted abundance of transformers alone, and does not incorporate an estimate of treatment cost. Streams are treated in order of decreasing transformer abundance until the treatment budget is exhausted.

(5) *Most Kill-Big Larvae*: Similar to the algorithm above, this ranking procedure avoids the use of metamorphic models, using estimates of abundance for larvae ≥ 100 mm, without consideration of treatment costs, and prioritizes streams for treatment based on these abundance estimates

The different ranking criteria were evaluated by comparing the median population (from the last ten years of the simulation) of parasites that resulted after 100 years of treating streams selected using a specific criterion. Median abundance was used over mean abundance due to the skewed distribution (non-normal) of the simulated outcomes. I also compared algorithms by examining the frequency that each individual stream was treated. For this latter analysis, the same sequence of random numbers was applied to each scenario, thereby removing the influence of random recruitment events from the variability observed among the different methods.

RESULTS

Results of the simulation modeling confirm that if we knew exactly how many recently metamorphosed lampreys would leave each stream every year, then prioritizing

streams based on these numbers would be the most effective method to control the parasitic population of sea lampreys in the Great Lakes (Figures 4 & 5). Even with minimal levels of uncertainty, ranking streams using a cost/ kill ratio based on the estimated transformer abundance achieves the greatest reduction in parasite numbers. As uncertainty in these estimates increases however, using ranking criteria based on either the total number of estimated larvae or the number of estimated large larvae becomes increasingly advantageous (Figures 4 & 5).

Comparisons of ranking criteria that either do or do not account for treatment costs also suggest that given perfect knowledge, ranking streams using a cost/kill method achieves optimal levels of lamprey control (Figure 6). As uncertainty in estimates of abundance increase, the optimal method switches to one that does not incorporate measures of treatment cost (Figure 6). Putting these two concepts together, at an average level of uncertainty, the simulations suggest that ranking streams by using overall abundance of large larvae performs, on average, at least equally, if not better than criteria using predicted overall abundance of transformers (Figures 5 & 6).

Breaking these results down from the whole lake to the individual stream level, the two cost/kill methods tend to treat large streams less frequently than the two most kill methods (Tables 1-2; Figures 7-9). Although either method (cost/kill or most kill) treats every stream numerous times over the course of 100 years, the most kill method tends to treat the very large streams more often, in exchange for a few less treatments on smaller streams, relative to using a cost/kill criterion. For example, over the course of a 100 year cycle, the Muskegon River, which is by far and away the largest lamprey producer in Lake Michigan, is treated an additional 3 times using a transformer-most kill algorithm,

and an additional 6 times using a large larvae-most kill algorithm, relative to algorithms incorporating treatment cost (Tables 1 & 2). Furthermore, comparing the two most kill techniques, using big larvae (which has less average uncertainty surrounding estimates of abundance) tends to treat the large lamprey producing streams more often than using predicted transformer abundance as the criteria (Figure 9).

DISCUSSION

The MUSTR model, like all models, is a simplistic version of a complex, real-world problem. While it does not account for all the intricacies of the larval assessment, stream ranking, and lampricide treatment cycles, it does allow for the exploration of stochastic processes that the deterministic nature of the ESTR system ignores. The results of the simulation modeling provided insights into the way uncertainty propagates through the stream ranking process and how it affects our ability to control sea lamprey populations.

While the modeling exercise supported the current approach of using the cost of a stream treatment divided by the estimated transformer abundance of that stream, for prioritizing streams for lampricide treatment, this result is reliant on having accurate estimates of how many transforming larvae each stream would produce. The simulation results suggest that as uncertainty in larval assessment estimates increases, there are two ways to reduce the impact that uncertainty has on the stream ranking process. First, by shifting the focus of the ranking criteria from estimates of transformer abundance to estimates of large larvae, uncertainty is reduced by avoiding the use of metamorphic

models, which are known to add significant amounts of uncertainty to larval estimates (Hansen *et al.* 2003; Steeves 2002).

Secondly, by switching from a cost/kill to an overall abundance criterion, large rivers, which have the most variability associated with larval estimates (Hansen *et al.* 2003), tend to be treated more often (Table 1(A & 2(A)). The improved lamprey suppression that results from this method originates from the fact that the cost (in parasitic lamprey escapement) is high when you fail to treat a large stream (due to inaccurate abundance estimates which incorrectly suggest that it would not be cost effective). The cost is less when you mistakenly do not treat a small stream that could have been treated. By removing the cost of treating large streams from the equation, the ranking process becomes more robust to the variation in larval densities and estimates of abundance on large streams.

While the structure of the MUSTR model did not allow for the direct determination of an optimal size criterion for either of the big larvae algorithms, simulated parasitic abundance did decline sharply once length criteria ≥ 100 mm were used to select streams for treatment. Setting this criterion too high might lead to the escapement of parasitic juveniles from fast growing streams. Lowering the size criteria produced a gradient of results intermediate to those observed using either the 100 mm criterion or a raw larval abundance (any size) criterion. As metamorphosis in larval lamprey from the Great Lakes typically does not occur unless the animal has attained a length of 120 mm by the spring of the year (Manion & Stauffer 1970), depending on when larval surveys were conducted, a minimum size criteria somewhere between 100 and 120 mm seems appropriate.

Estimates of uncertainty that were used to simulate variation in assessment surveys were generated by Steeves (2002), who used Monte Carlo simulations to add variability to assessment data at all stages of the deterministic ESTR model, including the estimated parameters for the length-based probability of metamorphosis curves. The large amount of uncertainty introduced by the prediction of metamorphosis relative to other ranking criteria (i.e. larvae or large larvae) (Figure 3) highlights the potential that improvements in metamorphic models may hold. This is even more apparent when looking at the results of using either of the transformer-based (kill/\$ or most kill) algorithms. When the uncertainty surrounding estimates of transformer abundance is decreased from average to minimal levels, the resulting median parasitic abundance is greatly reduced (Figures 4 & 5). While estimates of lamprey abundance, regardless of size, will always contain some level of uncertainty, reductions in the amount of uncertainty surrounding the prediction of metamorphosis could lead to great improvements in the accuracy of stream treatment decisions.

That being said, unless a more precise model of metamorphosis can be developed, in the long run, better levels of suppression can be achieved by using estimates of larvae exceeding 100 mm in length as a criterion for ranking streams, rather than predicted transformer abundance. Additionally, costs associated with treating each stream should not be considered during the compilation of a stream treatment list, as this tends to bias the selection towards smaller, more cost effective streams. This point highlights a problem with the current assessment protocols. Under QAS, a stream reach receives the same level of assessment effort regardless of its size or the size of the whole river. If a “most kill” criterion ranks large streams more regularly, even when uncertainty may be

high, there may be times when large streams will be treated when they don't require it. Given the greater treatment cost associated with those streams, why is the same proportion of assessment effort allocated to these larger streams? My results suggest that reducing assessment uncertainty in these larger streams, perhaps by allocating effort based on available larval habitat or potential larval abundance might be a preferred method when compared with an equal allocation of effort regardless of stream/reach size.

The MUSTR model represents a simplification of the entire Lake Michigan lamprey life/treatment cycle and is reliant on numerous assumptions about lamprey life history processes. As such, the specific parasitic abundances that are projected to result from a given management decision are highly suspect. However, the modeling results do suggest that, all other things being equal, better levels of lamprey control can be achieved by using ranking criteria that have less overall uncertainty associated with them. Furthermore, the inclusion of a treatment cost consideration can bias the selection process towards smaller, seemingly more cost effective streams, especially when the uncertainty associated with population estimates for large streams is high.

Table 1(A). Comparison of infested area and treatment cost for the ten rivers treated more often when the Transformer Most Kill algorithm was used, relative to the Transformer Kill/\$ algorithm.

River Name	Infested Area (m ²)	Treatment Cost	Average Number Treatments /100 year cycle	Average Number Treatments with Kill/\$
White River	2,294,592	\$460,091	23.1	22.3
Pere Marquette River	4,367,408	\$901,073	20.0	17.5
Muskegon River	10,694,838	\$1,316,224	19.1	15.8
Big Manistee River	3,506,405	\$1,243,345	17.9	14.5
Paw Paw River	2,145,189	\$638,247	17.3	15.6
Manistique River	5,161,522	\$873,164	17.1	14.1
Kalamazoo River	1,111,665	\$766,901	16.0	13.5
Galien River	228,959	\$397,639	14.6	14.1
Platte River	426,352	\$403,224	13.1	12.7
Whitefish River	1,685,366	\$522,773	12.8	11.6

Table 1(B). Comparison of of infested area and treatment cost for the ten rivers treated more often when the Transformer Kill/\$ algorithm was used, relative to the Transformer Most Kill algorithm.

River Name	Infested Area (m ²)	Treatment Cost	Average Number Treatments /100 year cycle	Average Number Treatments with Kill/\$
Bailey Creek	13,665	\$8,011	23.7	18.9
Pentwater River	229,164	\$131,968	20.9	17.8
Lincoln River	580,634	\$189,041	20.3	18.2
Burns Ditch	232,193	\$133,290	20.1	16.9
Trail Creek	184,495	\$99,203	20.0	15.9
Pipestone Creek	340,000	\$69,992	19.9	15.5
Crystal River	143,988	\$23,932	19.7	13.1
State Creek	38,070	\$25,396	19.5	11.8
Peshtigo River	1,088,653	\$213,666	19.2	17.8
Hickory Creek	240,000	\$66,530	19.1	14.6

Table 2(A). Comparison of infested area and treatment cost for the ten rivers treated more often when the Big Larvae Most Kill algorithm was used, relative to the Big Larvae Kill/\$ algorithm.

River Name	Infested Area (m²)	Treatment Cost	Average Number Treatments /100 year cycle	Average Number Treatments with Kill/\$
Muskegon River	10,694,838	\$1,316,224	21.8	15.9
Big Manistee River	3,506,405	\$1,243,345	20.5	14.2
Pere Marquette River	4,367,408	\$901,073	24.3	20.4
Manistique River	5,161,522	\$873,164	20.3	16.7
Kalamazoo River	1,111,665	\$766,901	14.0	10.8
Ford River	4,348,996	\$692,844	11.6	8.8
Paw Paw River	2,145,189	\$638,247	18.3	15.7
Whitefish River	1,685,366	\$522,773	13.7	12.4
White River	2,294,592	\$460,091	26.3	25.9
Millecoquins River	335,850	\$412,898	6.8	6.6

Table 2(B). Comparison of infested area and treatment cost for the rivers treated more often when the Big Larvae Kill/\$ algorithm was used, relative to the Big Larvae Most Kill algorithm.

River Name	Infested Area (m²)	Treatment Cost	Average Number Treatments /100 year cycle	Average Number Treatments with Kill/\$
Bailey Creek	13,665	\$8,011	25.0	18.1
Crystal River	143,988	\$23,932	24.1	12.7
Betsie River	1,295,256	\$139,504	23.6	18.4
Lincoln River	580,634	\$189,041	21.7	17.1
Pipestone Creek	340,000	\$69,992	21.6	13.5
N. Branch Pentwater River	229,164	\$131,968	21.5	14.2
Burns Ditch	232,193	\$133,290	21.0	13.9
Peshtigo River	1,088,653	\$213,666	20.6	17.0
Trail Creek	184,495	\$99,203	20.4	12.1
Hickory Creek	240,000	\$66,530	20.3	12.0

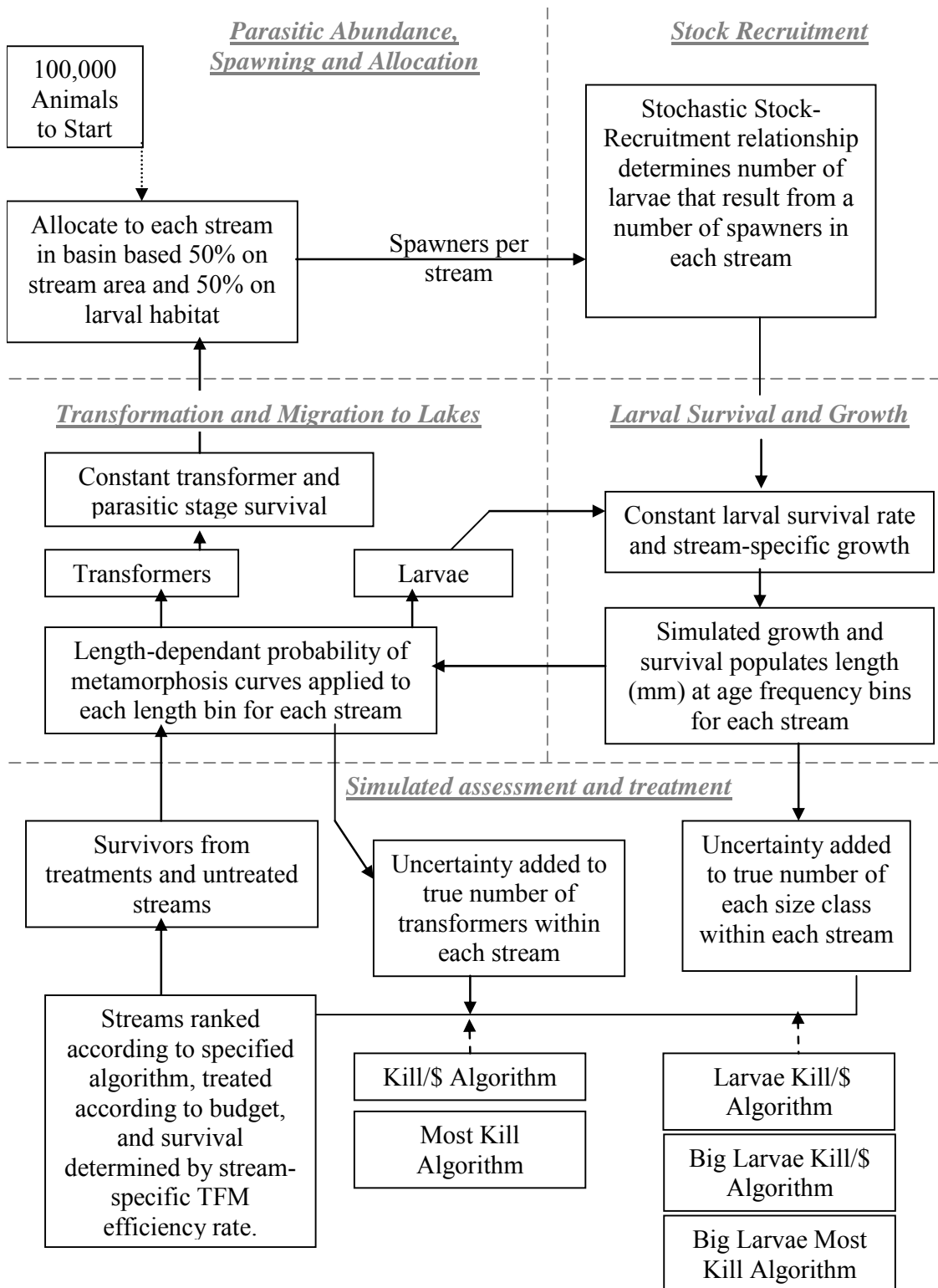
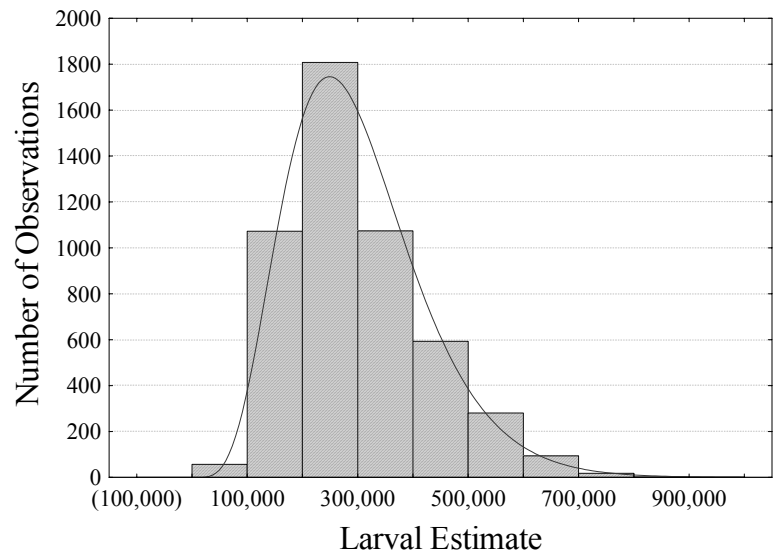
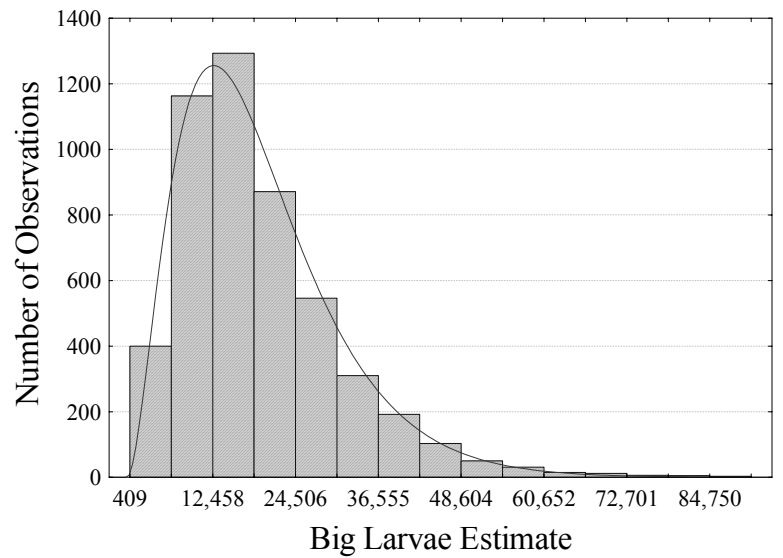
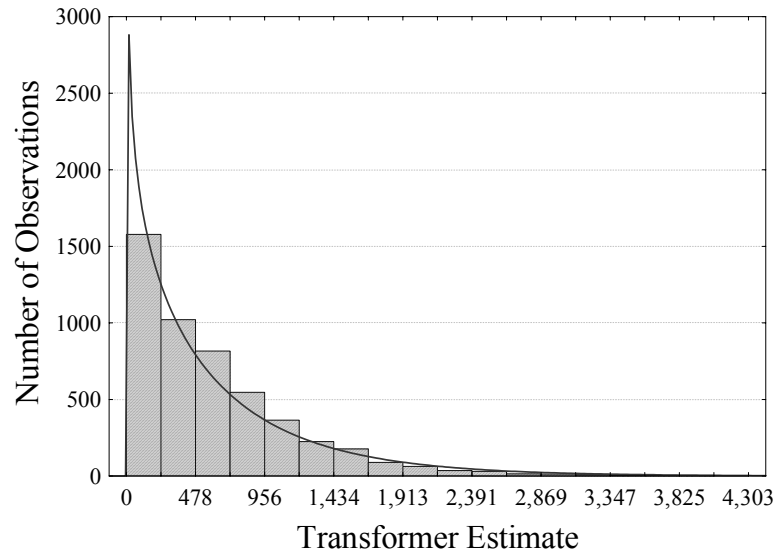


Figure 1. Flow chart illustrating life-cycle and assessment/treatment simulations of the MUSTR model.

Figure 2. Results from one of nine rivers of Monte Carlo simulations which propagated the error inherent in assessment surveys and the ESTR model.

Parameters describing each of the three curves, (from nine different rivers; Steeves 2002) were used to describe and simulate the error structure surrounding estimates of three life history stages of Great Lakes sea lamprey.

Data from Steeves (2002)



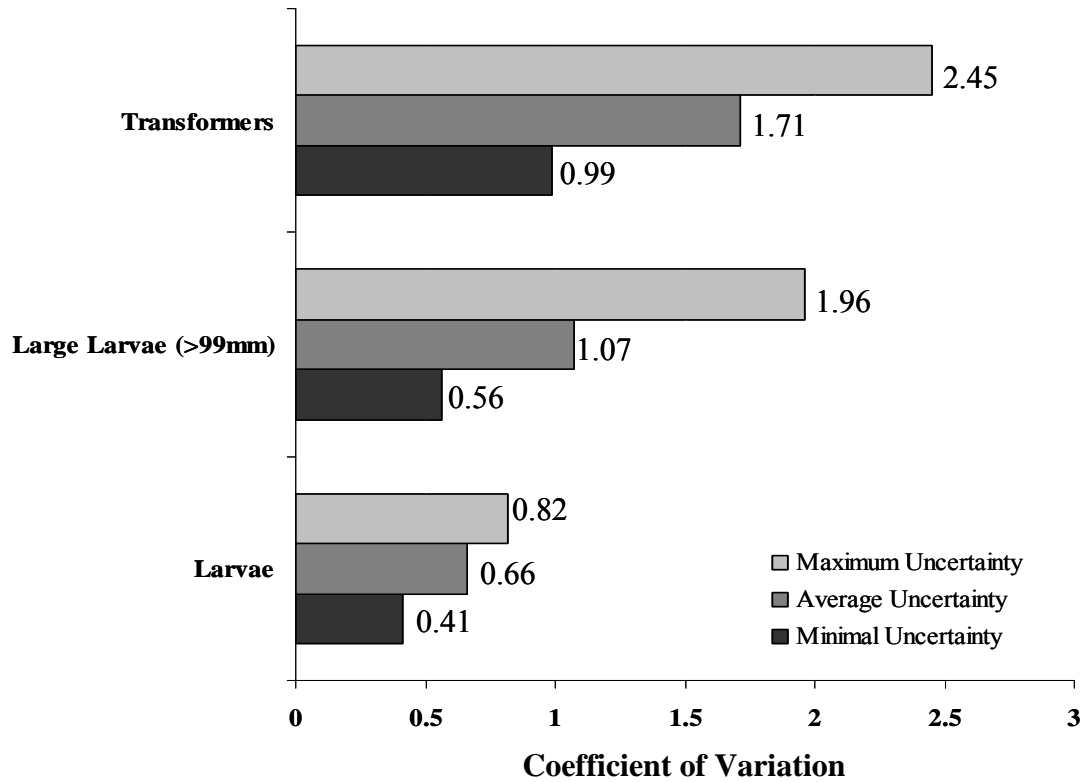


Figure 3. Comparison of the levels of uncertainty associated with larval assessment estimates for different lamprey life stages, from Monte Carlo simulations performed on assessment data from nine Great Lakes streams (Steeves 2002). Numbers represent the coefficient of variation for abundance estimates.

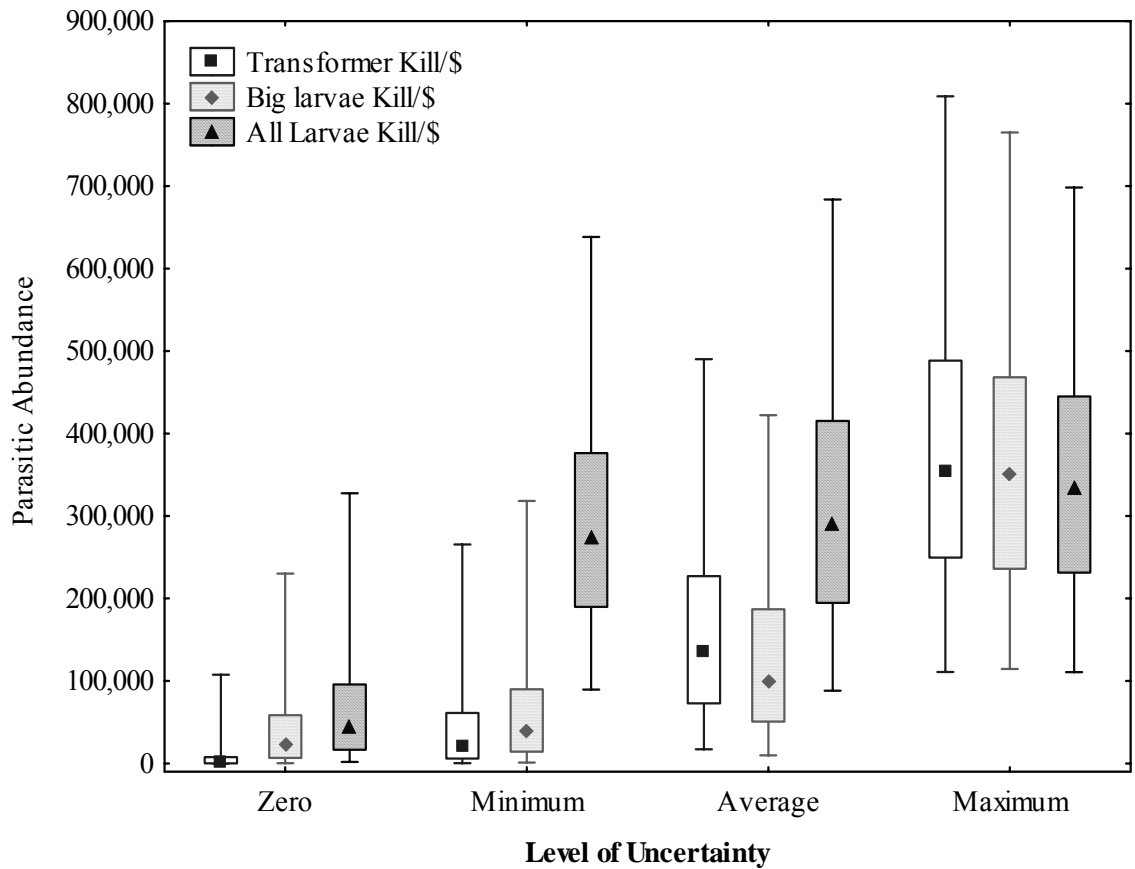


Figure 4. Simulated median parasitic population from the last ten years of 1000, 100 year stochastic simulations, using the predictions of larval, big larval, and transformer abundance divided by the cost of treating each stream to rank larval nursery streams for lampricide treatment. Points represent the median parasitic abundance, whereas boxes and whiskers represent the 25 to 75 and 2.5 to 97.5 percentiles respectively. Minimum, average, and maximum uncertainty are based on results from Monte Carlo simulations performed by Steeves (2002) using assessment data from nine Great Lakes streams.

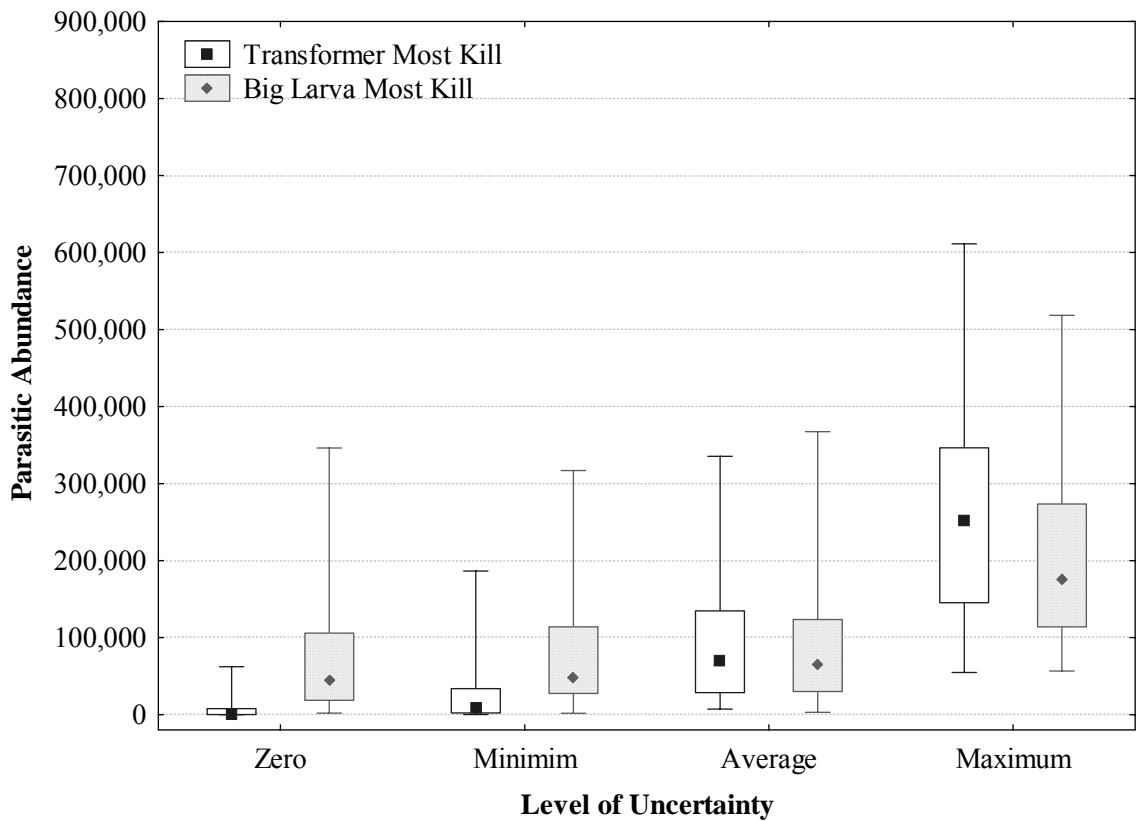


Figure 5. Simulated median parasitic population from the last ten years of 1000, 100 year simulations, with the same set of random stock-recruitment events used in both simulations. Stream ranking criteria consisted of using predicted abundances of big larvae or transformers, without standardizing for treatment cost. Points represent median parasitic abundance, whereas boxes and whiskers represent the 25 to 75 and 2.5 to 97.5 percentiles respectively. Minimum, average, and maximum uncertainty are based on results from Monte Carlo simulations performed by Steeves (2002) using assessment data from nine Great Lakes streams.

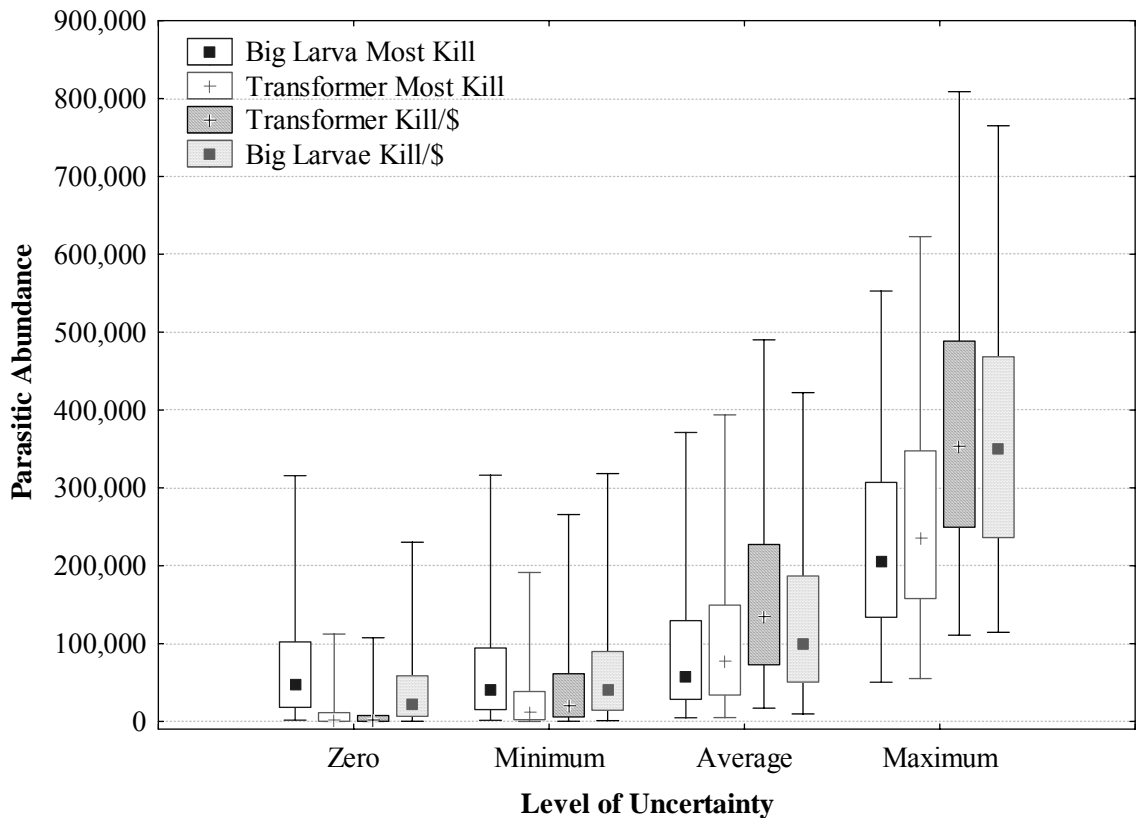


Figure 6 Simulated median parasitic population from the last ten years of 1000, 100 year stochastic simulations, combining estimates of both big larvae and transformers, as well as raw abundance estimates and estimates standardized for treatment cost, to rank streams for priority of lampricide treatment. Points represent median parasitic abundance, whereas boxes and whiskers represent the 25 to 75 and 2.5 to 97.5 percentiles respectively. Minimum, average, and maximum uncertainty are based on results from Monte Carlo simulations performed by Steeves (2002) using assessment data from nine Great Lakes streams.

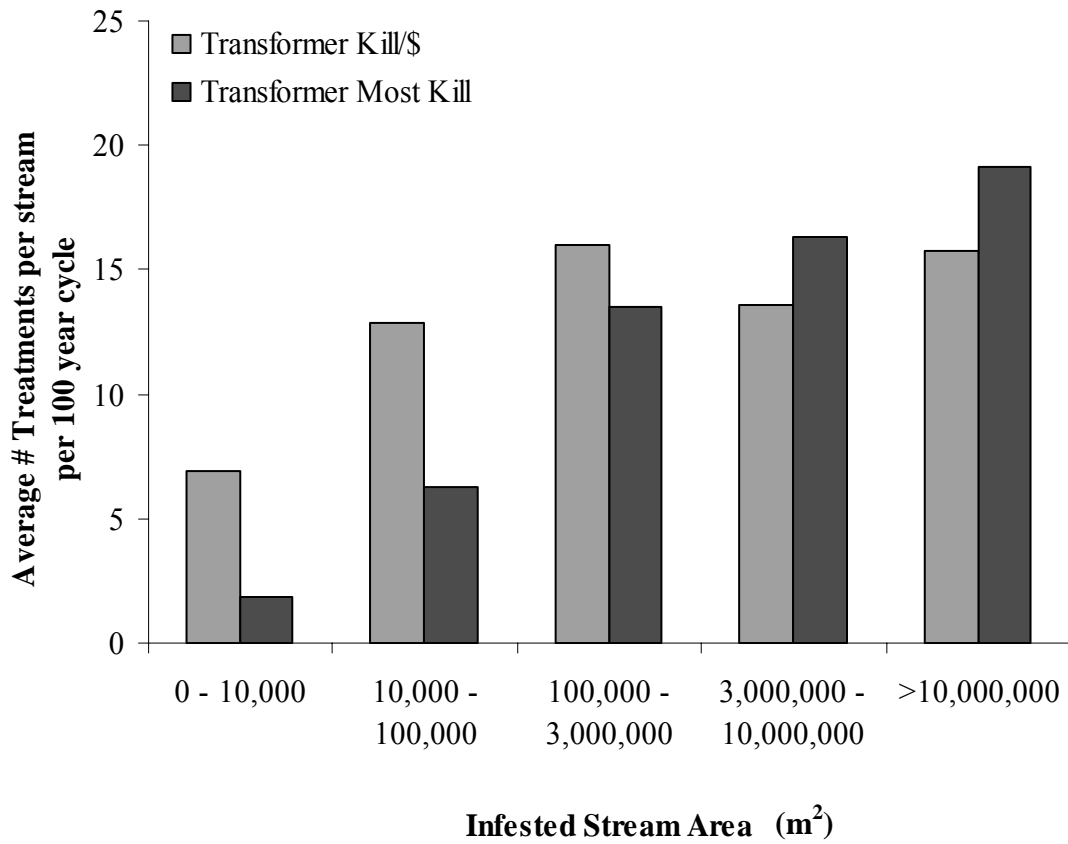


Figure 7. The average number of treatments per stream per 100 year simulation cycle on streams with increasing amounts of available larval habitat, using either a Transformer Kill/\$ or a Transformer Most Kill ranking criteria.

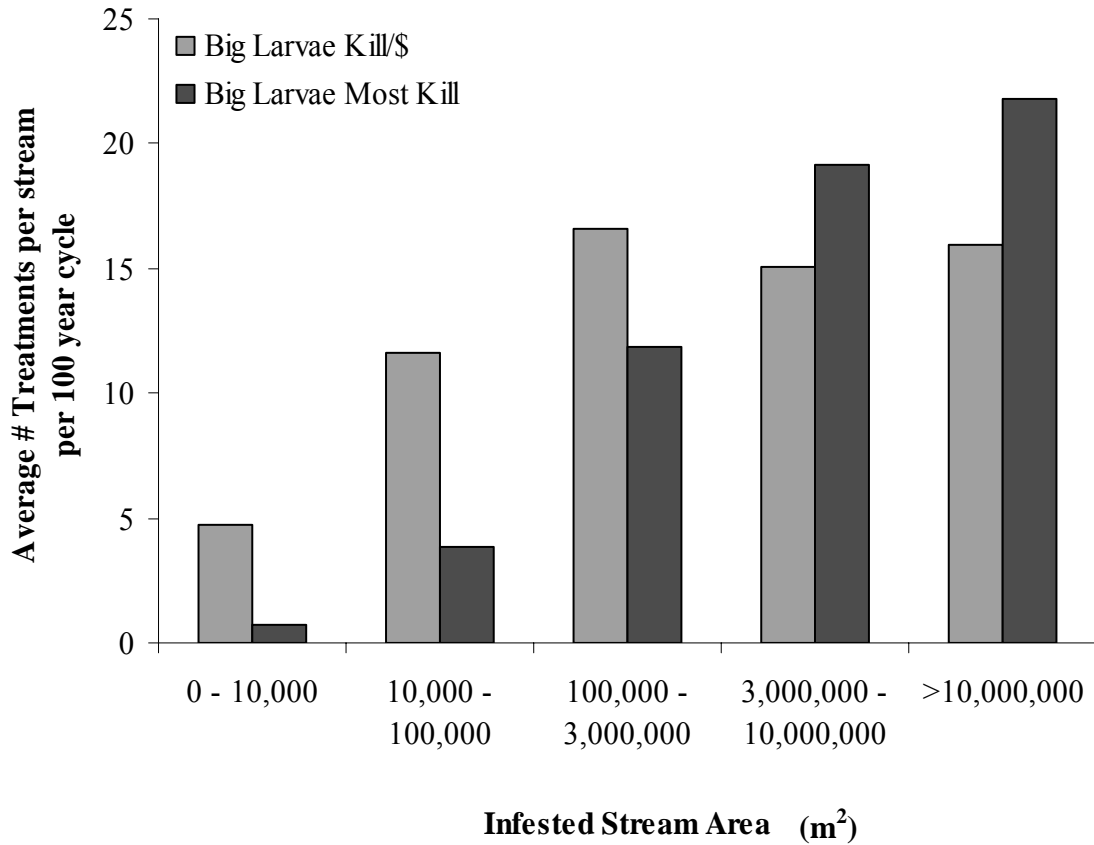


Figure 8. The average number of treatments per stream per 100 year simulation cycle on streams with increasing amounts of available larval habitat, using either a Big Larvae Kill/\$ or a Big larvae Most Kill ranking criteria.

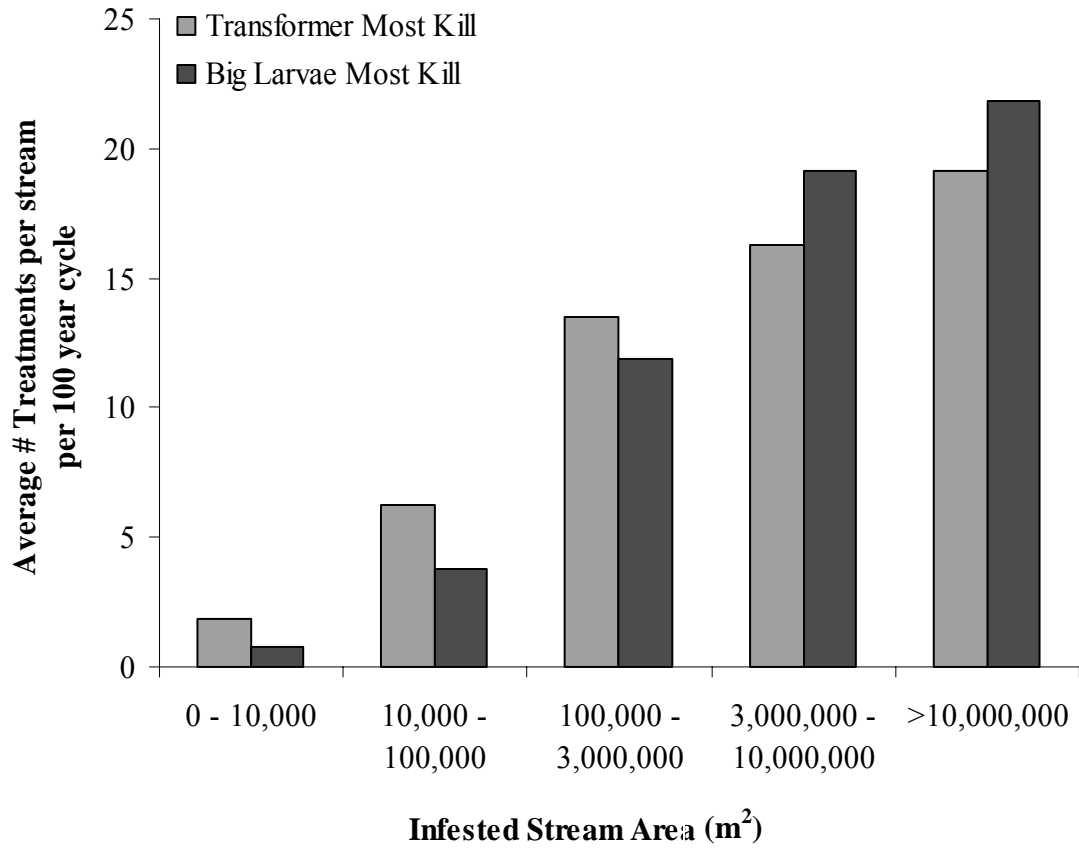


Figure 9. The average number of treatments per stream per 100 year simulation cycle on streams with increasing amounts of available larval habitat, using Transformer- and Big Larvae-Most Kill ranking Criteria.

CHAPTER II

NON-INVASIVE ESTIMATION OF LIPID CONTENT IN GREAT LAKES LARVAL SEA LAMPREYS

Abstract

The accumulation of lipids in larval sea lampreys (*Petromyzon marinus*) has been suggested to be critical in determining when larvae are ready to enter into metamorphosis. To facilitate an investigation into the relationship between metamorphosis and lipid content, a method to non-invasively estimate lipid levels in larval sea lampreys was developed. Using lipid weight as the dependent variable, standard length, weight, and condition factor measurements were combined with displacement, buoyancy, hydrostatic-weight and Total Body Electrical Conductivity (TOBEC) measurements as exploratory variables in a series of multiple regressions. Corrected AIC values were used to determine the best non-invasive model from a list of potential models. In the end, three models were developed: one TOBEC-based non-invasive model, one model based on other non-invasive measurements, and one invasive model that incorporated water content with other non-invasive measurements. The TOBEC method predicted lipid weight slightly better ($R^2=0.67$) than the other non-invasive model ($R^2=0.60$), but was far less accurate when compared with an invasive model that included a measure of water content ($R^2=0.94$). While the non-invasive TOBEC-based model was able to produce estimates of lipid content in larval sea lampreys from four Great Lakes streams, whether the accuracy of these estimates is sufficient to differentiate between lamprey that are likely to metamorphose and those that are not, remains to be seen.

INTRODUCTION

The estimation of lipid content and investigations into the role lipids play in the life history of an organism have been growing in prominence within the fields of ecology and fisheries management. An animal's lipid content is often a reflection of its overall health (Adams 1999; Hwang 1989) and can have implications on growth (Arndt 2000; Johnsson et al. 2000), maturation (Silverstein et al. 1998; Shearer & Swanson 2000), survival (Biro *et al.* 2004; Howell & Baynes 1993), and reproductive success (Gillooly & Baylis 1999; Craig *et al.* 2000), as well as reflect changes in environmental conditions or prey availability (Hutchings 1994; Finstad *et al.* 2002; Brown & Murphy 2004). Numerous studies have documented methods for estimating lipid content in various species (Speakman 2001; Fischer et al. 1996; Crossin & Hinch 2005) or reported on the cause and effects of atypical lipid levels on populations (Howell & Baynes 1993).

In larval sea lampreys (*Petromyzon marinus*), increased lipid levels have been linked to the initiation of metamorphosis, the process by which they transform from innocuous filter-feeders to parasites of fish (Lowe *et al.* 1973; Potter 1980). To prevent the economic damage to Great Lakes Fisheries that goes along with this change in feeding behaviour, biologists concentrate a majority of their management efforts on preventing populations from reaching this parasitic stage. The ability to predict what proportion of a stream's larval population is likely to transform in a given year is important to lamprey control on the Great Lakes because nursery streams across the basin are targeted for lampricide treatment based on forecasts of the potential number of recently-metamorphosed juveniles (often called transformers) they would produce. Despite the use of a variety of alternative control strategies (e.g., barriers, trapping, sterile

male release), control of parasitic lamprey populations is primarily accomplished through the application of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to tributaries containing larval lamprey populations, thereby reducing the number of recently metamorphosed juveniles that migrate down to the lakes every year (Christie *et al.* 2003).

During the larval phase, sea lampreys live burrowed in the soft sediments of tributaries to the Great Lakes. Larvae vary in the number of years they require to grow and mature to a point where they are ready to metamorphose. As a consequence of this long duration in larval phase, only those streams where the metamorphosis of a larval cohort is imminent need to be treated in any given year. Given limited resources, the problem for fisheries managers becomes how to decide which streams to treat each year to achieve maximum lamprey suppression. The Great Lakes Fishery Commission (GLFC) and its agents use population demographics from individual streams, determined annually from intensive assessment surveys, to predict the number of larvae expected to enter into metamorphosis the following year. Together with information on stream-specific treatment costs, these predictions are then used to rank streams across the basin for lampricide treatment. This method relies on a predictive model of metamorphosis that uses larval length to determine the likelihood of larvae to transform (Hansen *et al.* 2003). Based on back-calculated lengths from both larvae and transformers collected during numerous past lampricide treatments, this model has not been adequately evaluated, relies on questionable assumptions regarding larval growth, and ignores any stream- or year-specific variability (Hansen *et al.* 2003).

Several studies have found that larvae preparing to enter into metamorphosis exhibit an increase in lipid content from a baseline of about 4% to a peak of around 14%,

before lipid levels fall again as the animals enter a non-trophic state during metamorphosis (Lowe *et al.* 1973; Potter 1980; Holmes & Youson 1994; Henson *et al.* 2003). This accumulation of lipids is thought to prepare the animals for the energy demands of metamorphosis, which may take as long as 4 to 10 months (Potter *et al.* 1978).

Several attempts have been made to identify these pre-metamorphic larvae by using indices of condition (i.e., Fulton's condition factor, $CF = \text{Weight}/\text{Length}^3 \times 10^6$) to distinguish individuals that have begun to accumulate lipids from those that have not (Holmes & Youson 1994; Hollett 1998; Henson *et al.* 2003). These studies have been mostly unsuccessful, probably because condition factor does not partition total mass into lean mass and fat mass (Hayes and Shonkwiler 2001). This problem is further confounded by the inverse relationship that exists in the body between percent lipid and percent water, so that increases in lipids are not necessarily translated into an increase in mass (Hartman & Brandt 1995). The inability to develop a predictive model that accurately identifies which animals have begun to accumulate lipids, and thus be expected to transform the following year, has added a considerable amount of uncertainty to the stream ranking process (Christie *et al.* 2003; Hansen *et al.* 2003).

Non-invasive methods for estimating lipid content can provide information regarding the immediate fitness of an animal without requiring it to be killed. Non-invasive methods also allow for repeated measures to be made and thus document changes in energy reserves over time (Speakman 2001). In the case of larval lampreys, a non-invasive method for estimating lipid content would allow for the determination of the role lipids play, if any, as indicators as to an individual's likelihood to enter into

metamorphosis in preparing an individual larva for metamorphosis. While laboratory analyses have documented increases in lipid content (Holmes & Youson 1994), this was done through physical extraction of lipids, and so the metamorphic outcome of an individual animal was not definitively linked to prior lipid levels. If a non-invasive method to determine lipids in sea lamprey larvae can be developed, then the importance of lipids in metamorphosis can be confirmed, and their use as predictors of the occurrence of metamorphosis can be tested. If lipids are established as a reliable predictor of metamorphosis, perhaps then other invasive methods for determining lipid content, (i.e. ones that provide more accurate measures of lipid content) could be used by the lamprey control program to predict the proportions of larval populations that are likely to metamorphose.

Studies of other fish species have attempted to use Total Body Electrical Conductance (TOBEC) to estimate the proportions of lipid and lean muscle (e.g., Jaramillo *et al.* 1994; Lantry *et al.* 1998; Novinger & Martinez Del Rio 1999). Since the electrical conductivity of lipids is only 4-5% that of lean tissue, the TOBEC method uses variations in electrical impedance, caused by placing animals within a low-frequency electrical field, to generate an index that is directly related to the amount of lean tissue and inversely proportional to the lipid mass the animal contains (EM-Scan Inc. 1993; Piasecki *et al.* 1995; Scott *et al.* 2001). Despite the fact that the ability of TOBEC to predict lipid content in these previous studies met with mixed results, the important role lipids appear to play in sea lamprey metamorphosis warranted an investigation into the potential application of this technique for use in the sea lamprey control program.

This study encompasses two years of field and laboratory work. Initially a single non-invasive model, centered on the TOBEC measure, was developed by comparing measurements taken in the field with lipids chemically extracted from lampreys in the laboratory. In the second year of analysis, buoyant weight (weight suspended in water), hydrostatic weight (difference between weight in air and weight in water), and displacement (amount of water displaced when lamprey was submerged) measurements were added as potential surrogates for the costly and time-consuming TOBEC method. The addition of these measures necessitated the development of three models of lipid prediction, each increasing in the level of complexity. Two of the models are based entirely on non-invasive techniques, while the third includes a direct measure of the animal's water content. The first two models were developed for use in field experiments exploring the use of lipids as predictors of metamorphic status in sea lamprey. The third model was developed with the intent that, should lipids prove valuable in predicting lamprey metamorphosis, this model could be used to more accurately estimate lipids, and thus metamorphic rates, for use within the sea lamprey control program.

METHODS

Study Sites

In 2004, larval sea lampreys were collected in the spring from Soper Creek (Latitude 44° 58' 31", Longitude 79° 41' 3"), a tributary to Lake Ontario; and in the fall from the Pancake River (Latitude 47° 58' 33", Longitude 85° 40' 45"); a tributary to Lake Superior (Figure 1). Collections were made using backpack electrofishing gear (University of Wisconsin Engineering Technical Services, Model ABP-II) and the

lampreys were kept alive in aerated coolers containing sufficient sediment for burrowing, during their transport back to a laboratory at Michigan State University.

In 2005, during a TFM treatment, additional larvae were collected from Silver Creek (Latitude 44° 20' 53", Longitude 83° 29' 30") and Juniata Creek (Latitude 43° 24' 39", Longitude 83° 29' 06"), both tributaries to Lake Huron (Figure 1). Actively swimming larvae were netted within the downstream end of the lampricide block, measured for weight (± 0.01 g), length (± 1 mm), and frozen for later analysis.

Laboratory Analysis

During the first round of analysis, live sea lamprey larvae from Soper Creek were anesthetized, measured for total length (± 1 mm) and weight (± 0.01 g), placed in a right lateral recumbent position on a carrier plate, and inserted into a TOBEC scanning chamber (EM-Scan Model SA-3000 Small Animal Body Composition Analyzer, EM-Scan Inc., Springfield, IL, 62704-5026; Figure 2). The animal was left in the scanner for approximately 15-20 seconds, or until measurements appeared to stabilize (i.e. $> \pm 1/5$ seconds). The animal was then removed and the corresponding E-value recorded. The E-value represents an index of electrical impedance due to the composition of the tissue within the EM-Scan chamber. This procedure was repeated three times for each specimen. A pilot experiment undertaken prior to starting field work indicated that an average of three scans produced the same value as the average of five-scans, so a three scan method was adopted (Appendix A). This method has been implemented in previous studies as well (Bai *et al.* 1994). Based on the results of several studies indicating that the TOBEC method is highly sensitive to differences in body shape and alignment (Novinger

& Martinez Del Rio, 1999; Lantry *et al*, 1999), care was taken to orient and align lampreys within the EM-Scan chamber in an identical manner during each successive scan.

Additional measurements of displacement, buoyant weight and hydrostatic weight were taken from larvae collected from the Pancake River. Lampreys collected from Juniata and Silver creeks could not be transported back to the laboratory alive, as they were collected during a lampricide treatment and quickly perished. Instead, these animals were frozen on site and non-invasive measurements, excluding TOBEC, were performed at a later date.

A measure of displacement for each animal was obtained by individually submerging larval lampreys in a 25mL graduated cylinder, half-filled with water, and recording the amount of water displaced (± 0.1 mL). Buoyant weight was determined by measuring the residual weight of larvae suspended in a column of water. Hydrostatic weight was calculated as the difference between the animal's weight in air and its buoyant weight.

To test the utility of each of the non-invasive measures in predicting lipid content, lipid extractions were performed on individual lamprey. Larval lampreys were first desiccated in a drying oven for 48 hours and the amount of water they contained was recorded before each lamprey was individually homogenized using a mortar and pestle. Samples were then returned to the drying oven for an additional 24 hours to remove any residual water. Once thoroughly dehydrated and homogenized, the Soxtec method (AOAC International, 2000; Reynolds & Kunz 2001) was then used to extract lipids from lamprey tissues. This method involved the submersion of tissue samples in boiling

petroleum ether, causing the lipids within the samples to dissolve, and then recovering the lipids by evaporating the ether. The remaining lipids were then weighed to the nearest 0.001 gram. The standard protocol was altered slightly by adding two glass-wool cleaning stages, in an attempt to recover as much of the homogenized material from the mortar and pestle as possible. Due to the limited amount of material remaining after desiccation and homogenization (<2 g /lamprey), replicate samples from individual larvae could not be obtained.

In addition to the direct measurements described above, a length-standardized TOBEC ($\sqrt{Length \times MeanTOBEC}$) value and Fulton's condition factor ($CF = \frac{W}{L^3} \times 10^6$) were evaluated for their ability to predict lipid weight.

Model Development

Using a General Linear Model approach within Statistica (Version 7, StatSoft Inc., Tulsa, OK, USA), three models, varying in their complexity and level of invasiveness, were evaluated using a mixed-model analysis. The first model, referred to here as the TOBEC-based model, combined the non-invasive measurements of length, weight, condition factor, average and length-standardized TOBEC values as fixed effects to predict the weight of lipid in sea lamprey larvae. An alternate non-invasive model included displacement, buoyancy, and hydrostatic-weight variables in addition to standard length, weight, and condition measurements (as fixed effects). A third model incorporated measures of water content, along with the other variables from the alternate model. River was modeled as a random effect in each model to account for the correlation of characteristics relating to lampreys originating from the same river. For

each model, both lipid weight and percent lipid were modeled separately as response variables to see if there was more statistical support for the use of one over the other.

The entire suite of variables for each model was used in a best subsets analysis as fixed effects, while modeling river as a random effect. Corrected Akaike Information Criterion (AIC_C) values were used to rank potential models in order of statistical support, with the best model possessing the lowest AIC_C (Burnham & Anderson 2004). All models whose AIC_C values were more than two greater than the top model were removed from further consideration, since this suggests there is considerably less statistical support for those models (Burnham & Anderson 2004). Models possessing highly correlated variables were identified by analyzing their variance inflation factors (VIF) and models with VIFs greater than ten were removed from further consideration, as this indicated that at least some of the variables were highly correlated and provided redundant information (Quinn & Keough, 2002).

Model Evaluation

Twenty-two larval sea lampreys were selected at random from the total of 132 animals in this study and removed from model development. Larvae were selected from each of the four rivers in proportion to their frequency of occurrence in the dataset. This subset of the data was then used to evaluate the derived models by applying the parameter estimates from each of the models to data that was not included in the development of the models.

RESULTS

A total of 132 sea lampreys larvae were collected from four different rivers for lipid extractions over the course of this study (Tables 1 & 2). Lipid extractions were performed on larvae ranging from 100 to 167 mm in length, and 1.81 to 6.79 g in weight. Lipids extracted from individual lamprey ranged from 0.05 to 0.99 g, which translated to a range of 2.7 to 23.2% of total body weight.

Using simple linear regressions, a significant linear relationship between lipid weight and each of the predictive variables was observed, with the exception of condition factor (Table 1). The measure of weight accounted for almost 59% of the variation in lipid content, followed closely by displacement and a length-standardized TOBEC value. A negative correlation between percent lipid (dry weight) content and percent water content was observed throughout the analysis (Figure 3).

TOBEC-based Model

Only lampreys collected from Soper Creek (n=36) and the Pancake River (n=60) were used to develop the TOBEC model, since TOBEC measures were not performed on lampreys from the other two rivers. The results of the model selection procedure, using lipid weight as the dependent variable, indicated that there were three models with relatively equal support (difference between AIC_C values <2) (Table 3(a)). Each of the three models contained both measures of TOBEC, but varied in the inclusion of stream and condition factor. Based on a high coefficient of determination and statistically significant parameter estimates, a model that contained condition factor, average TOBEC, and length-standardized TOBEC was selected as the best model (Table 4a).

When percent lipid was used as the dependent variable, a similar model was obtained but was less supported by the data ($R^2=0.28$), so the model using lipid weight as the dependent variable was chosen. While the model with lipid weight as the dependent variable accounted for 66.6% of the variation in lipid content, the slope of the line of best fit diverged significantly ($p>0.05$) from the one to one line, indicating that the model overestimated lipid content at low lipid levels and underestimated lipid content in lamprey with high lipid content. Confirmation of this model using the separate dataset showed a strong association with lipid weights observed from chemical extraction ($R^2=0.74$, Figure 4), however, the same pattern of over/under-estimation at low/high lipid levels was observed. The random effect of river was not significant in this model (Table 4(a)).

Alternate Non-Invasive Model

Analysis of the alternate non-invasive model was performed using lampreys from Pancake River, Silver and Juniata Creeks. The model selection procedure, followed by the removal of models with highly correlated variables, selected a single model. This model consisted of weight and buoyant weight as fixed effects and river as a random effect, although it was not significant (Table 4(b)). This model had a coefficient of determination ($R^2=0.60$) slightly less than that of the TOBEC-based model. As with the TOBEC model, predicted lipid weights from the alternate non-invasive model diverged from observed weights for lamprey at either extreme of lipid content. The results of the model validation procedure provided a correlation of 0.74 (Figure 5).

Similar to the TOBEC-based model, a parallel analysis using percent lipid as the response variable was explored, but this model showed greater variance about the regression line ($R^2=0.22$) and so the model using lipid weight as the dependent variable was selected.

Invasive Model

Both non-invasive models were modified by the inclusion of water weight and percent water to analyze the effectiveness of an invasive model. As two separate datasets existed, two separate analyses were performed. An analysis involving data excluding the TOBEC variables selected a model containing weight, condition factor, buoyant weight, and percent water as fixed effects ($R^2=0.95$). A significant random effect of river was not found (Tables 2b and 3c). An analysis based on data that included TOBEC measurements produced three possible models, two containing TOBEC measures. A model consisting of weight, condition factor, and percent water was selected because the inclusion of TOBEC measurements did not increase the R^2 value appreciably (Table 3(c)). This model had a coefficient of determination of 0.97. Since the R^2 values for both invasive models were within 2% of one another, the model developed from the larger dataset was selected as the overall invasive model (Table 3(c); Figure 6).

Results of the model confirmation indicated a strong positive correlation between observed and predicted values from the model ($R^2=0.96$) as is illustrated in Figure 6. In addition, predicted lipid weights from the invasive model did not differ from measured lipid content for lamprey with either low or high lipid content.

DISCUSSION

Although a TOBEC-based model was selected from several different methods investigated in this study, none of the non-invasive methods provided predictions of lipid content that were highly correlated with the extracted lipid content and both noninvasive models provided an underestimate of lipid content for lamprey with high lipid levels. The TOBEC method explained 6.7% more of the variation in lipid content relative to the non-invasive model, however the TOBEC method was both costly and time consuming to use. The results of the alternate model development suggest that simple and inexpensive non-invasive techniques, such as measuring buoyant weight, can be used to roughly estimate lipid content in larval lamprey. For both models, accurate estimates of lipid content were not obtained until the animal was dried and a measure of water content was added.

The proportions of lipids extracted in this study (Table 1), as well as the relationship between percent lipid (dry weight) and percent water (Figure 3) were consistent with the findings of others (Potter 1980; Holmes & Youson 1994; Jonsson & Jonsson 1998). The relationship between percent lipid and percent water supports the concept that as lipids are accumulated, water is displaced from the body. It is likely this replacement of one component for another that has confounded the use of condition factor to separate pre-metamorphic from non-metamorphic larvae in earlier studies (Youson *et al.* 1993; Hollett 1998; Henson *et al.* 2003). The strength of this relationship also illustrates why the addition of percent water to the invasive model greatly improves its explanatory power relative to the two non-invasive models. Unfortunately, the validation of lipid content as a predictor of metamorphic status requires a non-invasive

model to substantiate its utility (through mark-recapture studies; see Chapter 3) before more accurate invasive methods can be employed.

Failure of the TOBEC method to produce a model that precisely predicts lipid content in larval sea lamprey is consistent with the results of other studies involving fish. Various researchers have attempted to use TOBEC to non-invasively estimate lipid content in a wide variety of freshwater species, with TOBEC accounting for 56.6 to 90.3% of the variation in lipid content (Jaramillo *et al.* 1994; Fischer *et al.* 1996; Novinger & Martinez Del Rio 1999). Together with the results of this study, these findings suggest that the TOBEC method, while capable of providing rough estimates and documenting general trends in lipid content, should not be used when accurate and precise predictions of lipid content are required (Scott *et al.* 2001).

The inability of the TOBEC-based model to produce accurate predictions of lipid content may be related to variability in the temperature and/or body proportions of larval lampreys at the time of measurement. Several authors have documented that TOBEC values increase as the temperature of the organism increases (Piasecki *et al.* 1995; Gillooly & Baylis 1998). Since lampreys are ectothermic, their body temperature varies with that of the ambient environment. As lampreys were collected and measured in variable conditions over the course of this study, it was not possible to maintain a constant temperature at the time of scanning for all of the lampreys analyzed.

Although the TOBEC small animal chamber used in this study was the smallest available and best suited to the dimensions of larval lampreys, portions of the heads and/or tails of some of the larger larvae protruded from the scanning chamber. In contrast, even the largest animals did not possess a cross-sectional area equal to at least

half that of the scanning chamber, as is recommended (EM-Scan 1993; Scott *et al.* 2001). A review of nine other studies using the TOBEC method on aquatic species found that the average minimum size of animals used was approximately 100 grams; with the smallest individual weighing 10 g (Bai *et al.* 1994). By comparison, the largest larva used in this study was roughly half that size, only 6.79 g. Perhaps a more accurate model could be developed if a scanning chamber, better suited for the dimensions of sea lamprey larvae, could be constructed. However, Lantry *et al.* (1999) suggest that TOBEC should not be used if the study animal is undergoing mass or compositional changes. This would preclude the use of the TOBEC method to predict lipids in larval lampreys about to undergo metamorphosis altogether, since metamorphosis and the period leading up to it represent a major shift in metabolism and composition of larval lampreys. Instead, my results support the published findings of other researchers in suggesting that the TOBEC method is not suitable for use in accurately predicting lipid content of aquatic species (Novinger & Martinez Del Rio 1999; Scott *et al.* 2001).

While it is questionable whether the accuracy of either the TOBEC or alternative model are sufficient to distinguish between larvae of different metamorphic status, they represent the only (and thus most accurate) methods for non-invasively estimating lipid content in larval sea lamprey that have been developed to date. Until other methods are developed and evaluated, the utility of lipid content as a predictor of a larva's likelihood to enter into metamorphosis will be based on estimates provided by these equations. Research into new methodologies that may be better suited for use with larval lamprey is currently being conducted and may lead to improvements in our ability to accurately and non-invasively estimate lipid content in the near future.

Bioelectrical Impedance Analysis (BIA) and the use of microwaves have recently been reported in the primary literature (Van Marken Lichtenbelt 2001; Cox & Hartman 2005) as new and potentially more accurate methods for non-invasively determining various components of body composition. BIA, while similar in concept to TOBEC, is slightly invasive and involves the insertion of two electrodes, which measure the resistance imparted on a mild electrical current applied to the specimen (Van Marken Lichtenbelt 2001). Cox & Hartman (2005) validated the method using brook trout (*Salvelinus fontinalis*) and suggested the method was most appropriate for fusiform body types, such as larval lampreys.

Using a handheld microwave energy meter, Crossin and Hinch (2005) were able to rapidly estimate the energy content of spawning sockeye salmon (*Oncorhynchus nerka*) and document declining energy reserves as they sampled fish at various points along their upstream spawning migration. Their results suggest that this same method could be used to rapidly and accurately obtain lipid estimates in lampreys, perhaps obviating the need for complicated, multivariable models.

While these new methods show promise, it will be the level of precision in lipid estimation required to accurately separate pre-metamorphic from non-metamorphic larvae that will determine their utility. An accurate method of estimating lipid content non-invasively, that can be used within the framework of a study that tracks the fate of individual fish, is required before the use of lipids to predict metamorphosis can be definitively supported. Any new method for estimating lipid content will need to be evaluated against these standards before its use within the sea lamprey control program can be incorporated.

Table 1. Summary statistics of measured and calculated variables used in the development of predictive models of lipid content in larval sea lamprey. Coefficients of determination (r^2) are for simple linear regressions of each variable regressed against lipid weight.

	n	Min	Max	Mean (\pm SD)	r^2
<i>Measured Values</i>					
Length (mm)	132	100.00	167.00	132.86 (\pm 14.37)	0.511
Weight (g)	132	1.81	6.79	3.51 (\pm 1.11)	0.588
Mean TOBEC	96	345.33	620.67	511.11 (\pm 49.70)	0.261
Buoyancy (g)	96	0.00	0.19	0.09 (\pm 0.04)	0.102
Displacement (ml)	96	1.70	6.00	3.27 (\pm 1.00)	0.575
Water Content (g)	132	1.29	5.12	2.49 (\pm 0.82)	0.391
Lipid Weight (g)	132	0.05	0.99	0.43 (\pm 0.22)	---
Percent Lipid	132	2.7	23.2	12.4 (\pm 3.99)	---
Percent Water	132	61.0	83.6	72.7 (\pm 4.21)	0.928
<i>Calculated Values</i>					
Condition Factor	132	1.14	1.82	1.46 (\pm 0.12)	0.002 [†]
Length Standardized TOBEC	96	193.13	309.75	255.83 (\pm 21.85)	0.543
Hydrostatic Weight (g)	96	1.84	6.56	3.47 (\pm 1.12)	0.539
Length Standardized Hydrostatic Weight (g)	96	14.61	33.10	21.47 (\pm 4.54)	0.533

[†] indicates a statistically insignificant relationship ($p < 0.0005$)

Table 2. Sources of lamprey and a summary of measurements taken on lamprey used for lipid extractions, lipid model development, and model validation.

(A All lamprey collected for lipid extraction and model development)

Source Stream	n	Length (± 1 SD)	Weight (± 1 SD)	Lipid Weight (± 1 SD)
Juniata Creek	18	150 \pm 7.78 mm	4.96 \pm 0.80 g	0.52 \pm 0.18 g
Pancake River	60	128 \pm 10.47 mm	3.07 \pm 0.77 g	0.38 \pm 0.21 g
Soper Creek	36	129 \pm 14.23 mm	3.39 \pm 1.01 g	0.46 \pm 0.20 g
Silver Creek	18	140 \pm 15.71 mm	3.77 \pm 1.27 g	0.44 \pm 0.28 g

(B Lamprey excluded from model development and used for validation only)

Source Stream	n	Length (± 1 SD)	Weight (± 1 SD)	Lipid Weight (± 1 SD)
Juniata Creek	3	151 \pm 3.51 mm	5.02 \pm 0.27 g	0.49 \pm 0.08 g
Pancake River	10	130 \pm 12.18 mm	3.23 \pm 0.90 g	0.46 \pm 0.26 g
Soper Creek	6	133 \pm 19.94 mm	3.70 \pm 1.40 g	0.58 \pm 0.30 g
Silver Creek	3	145 \pm 24.95 mm	4.32 \pm 2.22 g	0.55 \pm 0.31 g

Table 3. Top models from best-subsets model selection procedures for the development of invasive and non-invasive models of lipid estimation. The model selected in each case is marked in bold.

(A Top three TOBEC-based Models

Model Parameters	df	AICc	Multiple R ²
(Average TOBEC) + (Length Standardized TOBEC) + (Stream)	3	-121.807	0.646
(Condition Factor) + (Average TOBEC) + (Length Standardized TOBEC) + (Stream)	4	-121.283	0.666
(Condition Factor) + (Average TOBEC) + (Length Standardized TOBEC)	3	-121.159	0.666

(B Top three invasive models, excluding TOBEC parameters

Model Parameters	df	AICc	Multiple R ²
(Weight) + (Bouyant Weight) + (Percent Water)	3	-244.78	0.946
(Weight) + (Condition Factor) + (Bouyant Weight) + (Percent Water)	4	-244.33	0.948
(Weight) + (Condition Factor) + (Bouyant Weight) + (Percent Water) + (Stream)	6	-244.06	0.948

(C Top three invasive models including TOBEC parameters

Model Parameters	df	AICc	Multiple R ²
(Weight) + (Condition Factor) + (Percent Water)	3	-318.51	0.971
(Weight) + (Condition Factor) + (Length-standardized TOBEC) + (Percent Water)	4	-317.44	0.972
(Weight) + (Condition Factor) + (Mean TOBEC) + (Percent Water)	4	-316.67	0.971

Table 4. Parameter estimates, standard errors, and p-values of the predictor variables from a mixed model analysis of the three models of lipid prediction.

A) TOBEC-based model parameter estimates ($R^2=0.67$).

Fixed Effects	Parameter Estimate	Standard Error	p-value
Intercept	-1.5136	0.2470	<0.0001
Condition Factor	0.1590	0.1255	0.2091
Average TOBEC	-0.0030	0.0006	<0.0001
LS-TOBEC	0.0124	0.0014	<0.0001
Random Effect	Variance Estimate	Standard Error	p-value
River	0.0005	0.0013	0.6970

B) Alternative non-invasive model parameter estimates $R^2=0.60$.

Fixed Effects	Parameter Estimate	Standard Error	p-value
Intercept	-0.2755	0.0731	<0.0001
Weight	0.2436	0.0221	<0.0001
Buoyant Weight	-3.1461	0.7158	<0.0001
Random Effect	Variance Estimate	Standard Error	p-value
River	0.0056	0.0065	0.3908

C) Invasive model parameter estimates ($R^2=0.95$).

Fixed Effects	Parameter Estimate	Standard Error	p-value
Intercept	2.0525	0.1490	<0.0001
Weight	0.1419	0.0100	<0.0001
CF	0.1298	0.0658	0.0525
Buoyant Weight	-0.6971	0.3118	0.0284
Percent Water	-3.0501	0.1480	<0.0001
Random Effect	Variance Estimate	Standard Error	p-value
River	0.0003	0.0005	0.5975

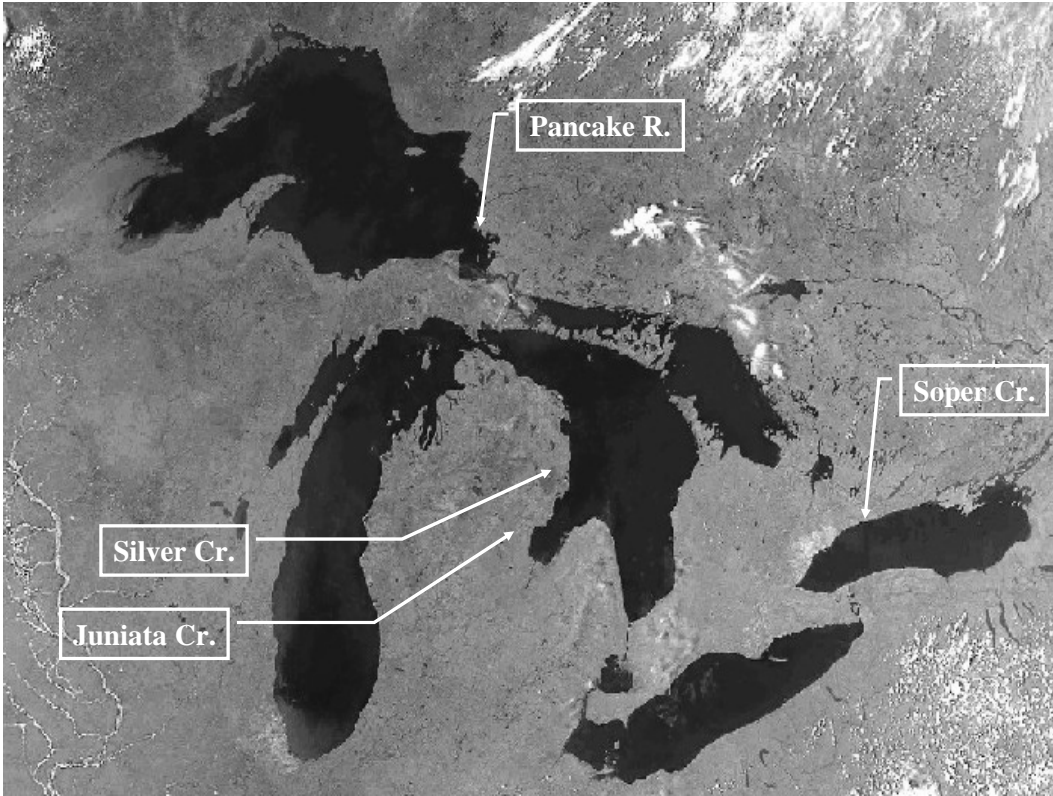


Figure 1. Locations of the four rivers used to collect larval sea lampreys for lipid analysis and development of a predictive lipid model.

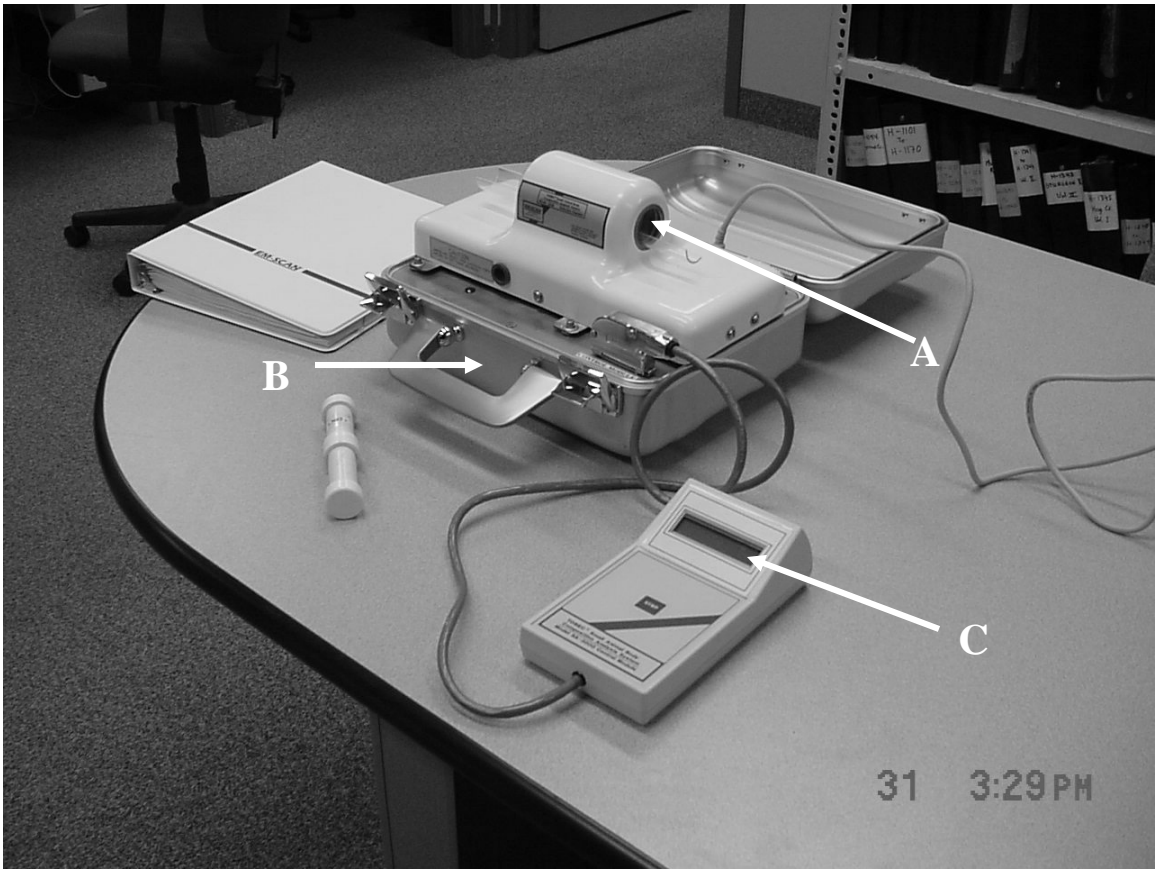


Figure 2. Picture of the EM-Scan Model SA-3000 Small Animal Body Composition Analyzer (EM-Scan Inc., Springfield, IL, 62704-5026) used to measure TOBEC. The unit is composed of (A) the scanning chamber, (B) the base unit and (C) the control unit.

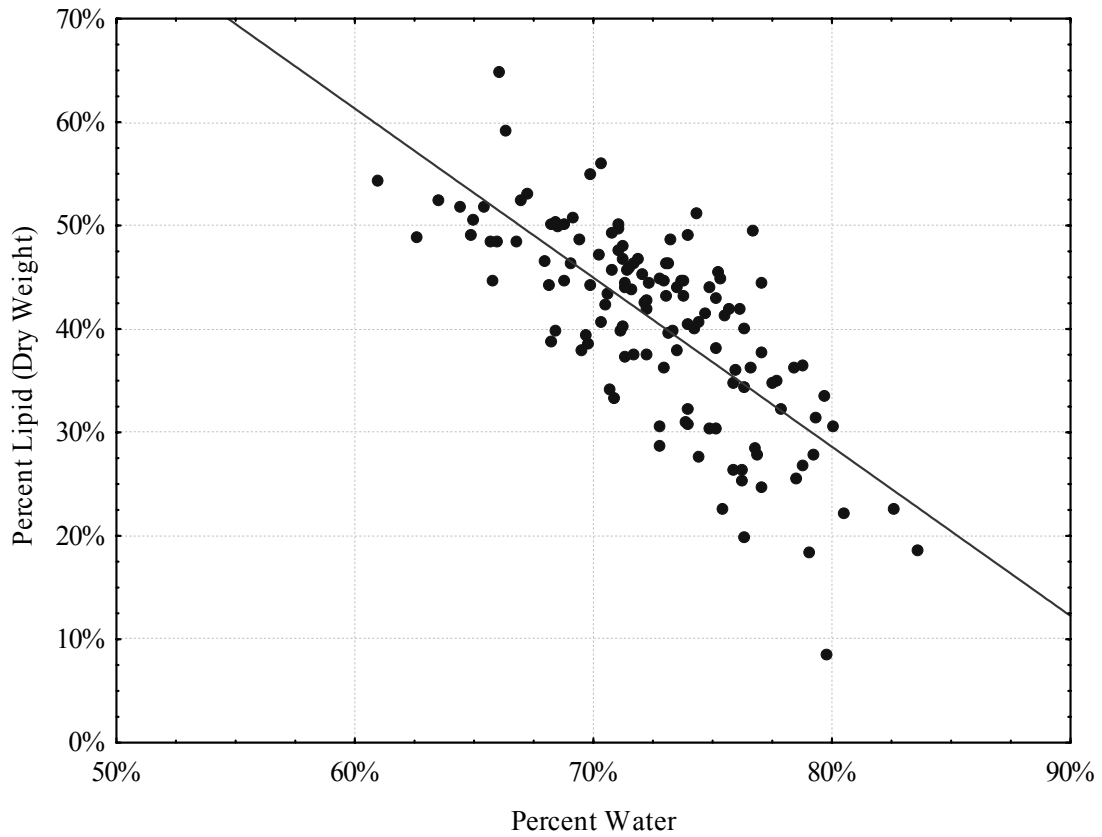


Figure 3. Correlation between percent body water and percent lipid (dry weight) based on the dry weight of larval sea lampreys ($r^2=0.5434$, $p<0.00001$).

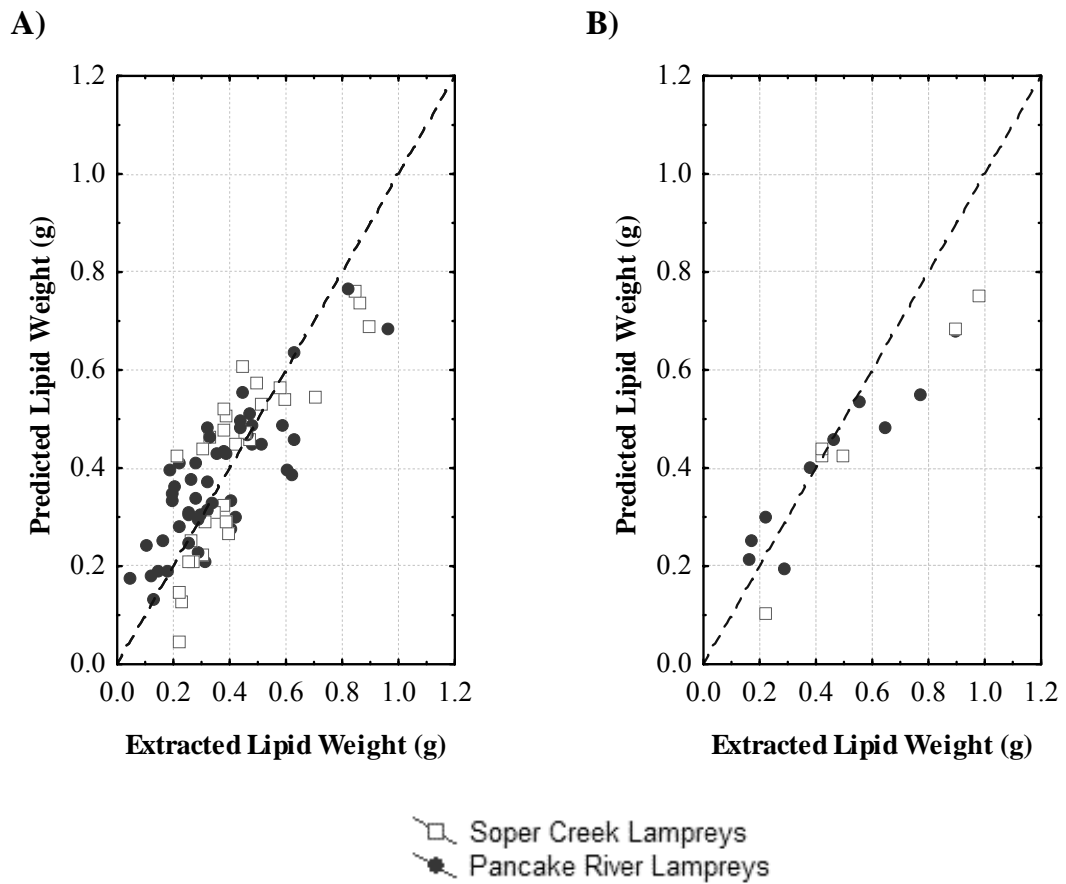


Figure 4. Observed versus predicted values for lipid weight of larval lampreys from the TOBEC-based model, using (A) all the data used to develop model ($R^2=0.67$) and (B) a subset of the data, set aside for model validation ($r^2=0.74$). The dashed line indicates perfect correlation.

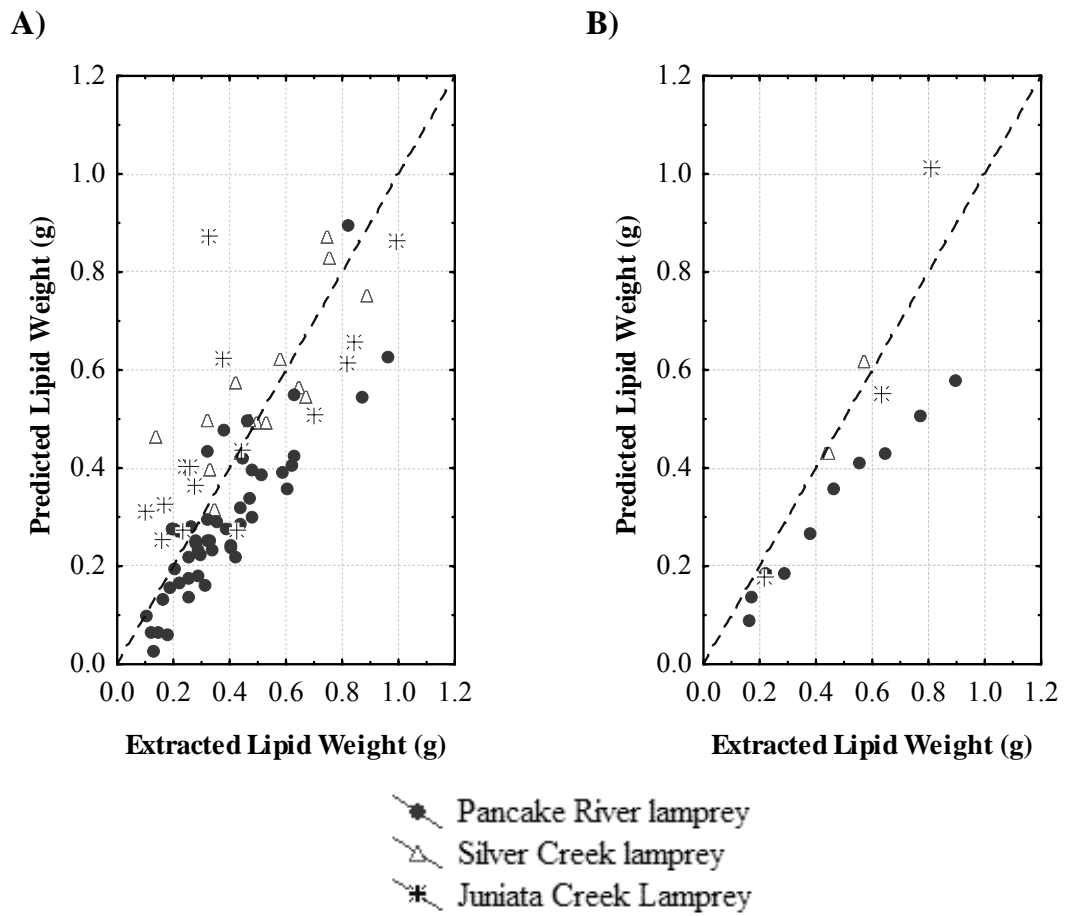


Figure 5. Observed versus predicted values for lipid weight of larval sea lampreys from the alternative non-invasive model, using (A) all the data used to develop the model ($R^2=0.60$) and (B) a subset of the data, set aside for model validation ($r^2=0.57$). The dashed line indicates perfect correlation.

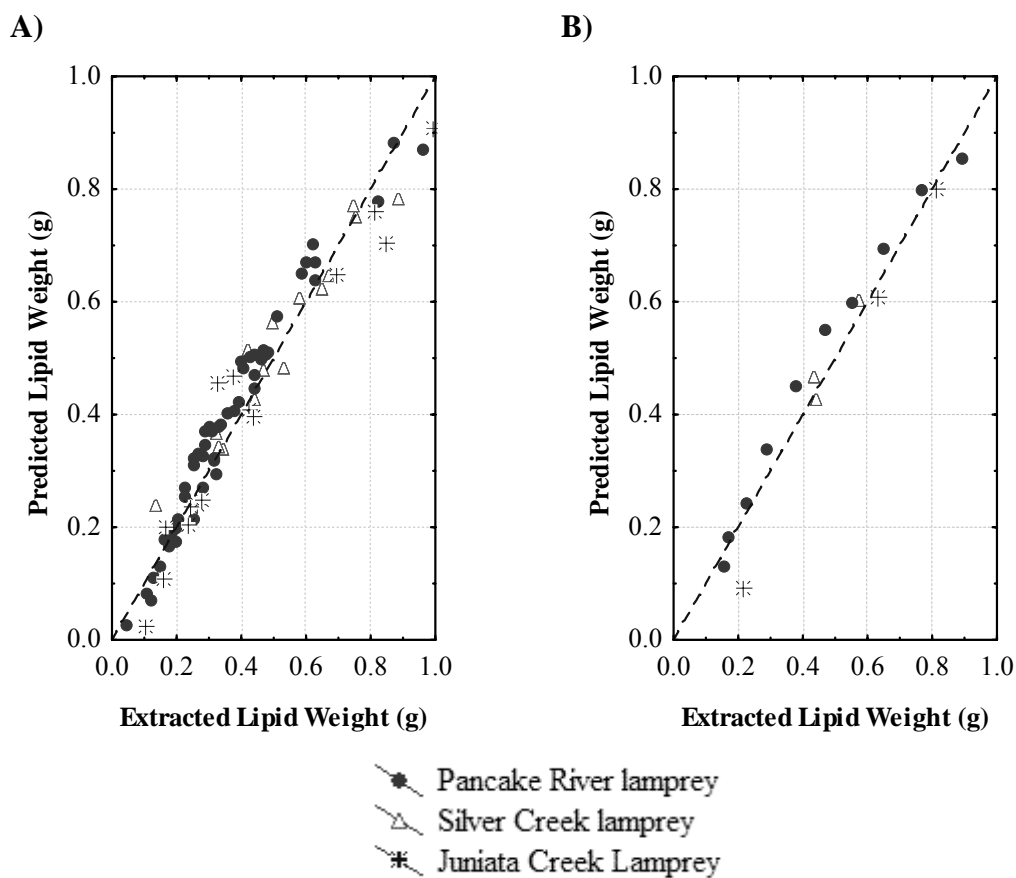


Figure 6. Observed versus predicted values for lipid weight of larval sea lampreys from the invasive model (that includes percent water), using (A) all the data used to develop the model ($R^2=0.95$) and (B) a subset of the data, set aside for model validation ($r^2=0.96$). The dashed line indicates perfect correlation.

CHAPTER III

DEVELOPMENT AND ASSESSMENT OF A PREDICTIVE MODEL OF METAMORPHOSIS.

ABSTRACT

Length-based models currently used by the GLFC to predict metamorphosis in larval sea lamprey lack the accuracy to be effectively used as ranking criteria to prioritize streams for lampricide treatment. A study to develop and assess a new method for predicting the likelihood of metamorphosis was undertaken. A mark-recapture technique, involving the marking of individual lamprey with uniquely coded wire tags, was used to combine information regarding individual and stream level parameters collected in year T , with knowledge of metamorphic outcome of lamprey recaptured in year $T+1$. One of the models developed demonstrated excellent predictive capabilities and highlighted the importance of weight, age, sex, stream temperature, and larval density on influencing when individual lamprey are likely to transform. While this model was informative, it was not applicable for use within the stream ranking methodology, because measures of sex, age, and stream temperature would be too difficult to obtain for regularly assessed streams across the Great Lakes. A second model was developed, limited to parameters easily obtained by fisheries biologists, consisting of length, condition factor, alkalinity, lamprey production stream category, latitude, longitude, and number of years since the stream was last treated. While this model produced predictions that were much more accurate than the model currently used to predict the future abundance of metamorphosed juveniles, it should be evaluated using additional mark-recapture studies before being adopted for use within the stream ranking protocols used by the Great Lakes Fishery Commission to allocate lamprey control resources.

INTRODUCTION

In his classic paper from 1950, Applegate described the complete life history of sea lampreys in the Great Lakes; from harmless burrowing filter-feeders, through the juvenile parasitic feeding phase, to the adult spawning phase. His work served to focus preliminary control efforts on two vulnerable stages in the life history: spawning adults and larvae. Early in the control program, limited reductions in spawning success were achieved using electrical weirs and physical barriers, the latter of which are still in use today (Smith & Tibbles 1980; Christie & Goddard 2003). However, true reductions in the parasitic population of the Great Lakes was not achieved until 1958, with the first application of the newly discovered lampricide 3-trifluoromethyl-4-nitrophenol (TFM), which greatly reduced the escapement of parasitic juveniles from treated nursery streams (Smith & Tibbles 1980; Christie & Goddard 2003).

In response to the sea lamprey invasion, the U.S. and Canada signed the Convention on Great Lakes Fisheries in 1954, which established the Great Lakes Fishery Commission (GLFC) to oversee the sea lamprey control program in the Great Lakes on both sides of the border (Christie & Goddard 2003). From the inception of the lampricide treatment program until 1995, decisions regarding which streams should be treated with TFM were made subjectively, based on the presence and size distribution of larval lampreys, as well as the number of years since the stream was last treated. To facilitate a more objective methodology for making pesticide application decisions and to optimize the effectiveness of the treatment program, the GLFC switched their assessment and stream ranking protocol in 1995. This new methodology, called Quantitative Assessment Surveys (QAS), relied more on quantitative measures of larval density and population

demographics. The implementation of QAS allowed for model-based estimation of density and overall larval abundance in a stream, as well the proportion of a population that would metamorphose (or transform) into parasitic juveniles. To develop these latter estimates, fisheries managers collected thousands of larvae and recently metamorphosed juveniles (transformers) during lampricide treatments. These specimens were measured to generate length-frequency histograms, which were back-calculated to represent the population size structure at the end of the previous year (i.e., when assessments are traditionally performed), using assumptions about larval growth. These data were then fitted to a logistic model that related larval length to the probability of metamorphosis. In the original analysis, 14 logistic curves were generated to represent different regions of the Great Lakes. A more recent analysis of these regional curves found that the variability in metamorphic rates was greater within regions than among regions, so the GLFC reduced the number of regional curves from 14 to 2 (Hansen *et al.* 2003); one for the upper lakes (Lakes Huron, Michigan, and Superior) and one for the lower lakes (Lakes Ontario and Erie).

Using specifically designed software (Empirical Stream Treatment Ranking (ESTR)), survey data are combined with information on stream-specific or region-specific larval growth rates and metamorphic probability to generate estimates of transformer abundance in streams across the Great Lakes basin. Streams are then ranked for lampricide treatment based on a cost/kill basis, calculated as the estimated number of transformers divided by the projected cost of treating each stream.

During the development of the ESTR model, no attempt was made to confirm the growth assumptions on which the two regional metamorphic models rely, or to test the

overall suitability of these models for use in the stream ranking process. QAS and the stream ranking protocol were also implemented in such a way that the ensuing effect on the parasitic population could not be readily determined. At about the same time as the introduction of QAS, the GLFC adopted a goal of reducing lampricide use, and embraced an integrated pest management philosophy, which involved the development of various alternative control strategies and changes to lampricide application tactics to suppress parasitic sea lamprey abundance while at the same time attempting to reduce reliance on TFM and its impacts on non-target species (e.g. lake sturgeon). However, the parasitic abundance of lampreys in some of the Great Lakes actually increased following the introduction of these new strategies. The concurrent implementation of several new control strategies and reductions in lampricide applications clouded the causal relationship between the implementation of the new QAS methodology and stream ranking protocol and subsequent changes in parasitic lamprey abundance.

Hansen *et al.* (2003) evaluated the larval assessment and stream ranking program and concluded that the estimation of metamorphosis from assessment data introduces significant uncertainty into the stream ranking process. They recommended that efforts to reduce this uncertainty be made through further research into factors contributing to variation in metamorphic rates, or that a more robust stream ranking methodology be developed by eliminating the need for metamorphic models altogether.

Collections made at the time of lampricide treatment, as well as numerous field and laboratory studies, have documented that length at metamorphosis can be highly variable among streams and within streams from year to year (Manion & Stauffer 1970; Purvis 1980; Morkert *et al.* 1998). Near the end of the larval phase, linear growth appears

to slow, and metabolism shifts to the accumulation of lipids (Lowe *et al.* 1973; Youson *et al.* 1979). This phase of reduced or “arrested” growth (Holmes & Youson 1997) complicates the sole use of length as a metamorphic predictor, because two groups of lampreys in different metamorphic states may exist in a single length-class: those that recently attained that size, and those that attained their size earlier, and have been accumulating energy reserves (lipids) but not increasing in length. Potter (1980) documented an increase in lipid content of larval sea lampreys from approximately 4%, up to 14% prior to the onset of metamorphosis. While a length-based model clearly would not capture this shift in body composition, other metamorphic models have attempted to identify this stage of lipid accumulation by using various measures of condition (e.g., Fulton’s condition factor = $\text{weight}/\text{length}^3 \times 10^6$) (Ricker 1975; Nash *et al.* 2006). Unfortunately, there is often an inverse relationship between lipids and water content in fishes, and thus increases in lipid content are not necessarily reflected in proportional increases in mass (Youson *et al.* 1993; Holmes & Youson 1994; Jonas *et al.* 1996). As a result, these models have not performed well at predicting metamorphosis across streams within the Great Lakes (A. Treble, unpublished data).

Other research has investigated the effects of larval density on the rate of metamorphosis. Although it has been suggested that abiotic factors may also play a significant role in the variability of growth and metamorphosis, high larval densities have been shown to negatively affect growth, survival, and age at metamorphosis (Morman 1987; Murdoch *et al.* 1992; Rodriguez-Munoz *et al.* 2003). Larval densities also affect the size and sex ratios of transforming lampreys, with populations from high larval densities showing a greater proportion of males in the population and a smaller average

size at metamorphosis (Zerrenner & Marsden 2005). These density-dependent effects contribute to the overall variation in metamorphic rates and confound metamorphic models used within the lamprey control program, where larval populations routinely go from high densities to low densities during the course of a stream treatment and re-establishment cycle.

Several studies (Purvis 1979; Docker & Beamish 1994) have suggested that the sex ratios of sea lamprey populations are also determined by density-dependent effects, with low density lamprey populations producing a higher proportion of females. This was well documented during the early years of the control program, when lampricide applications greatly reduced the overall abundance of lampreys in the Great Lakes, and the sex ratios of adults changed from predominantly male to predominantly female across the basin (Heinrich *et al.* 1980; Torblaa & Westman 1980). Studies of isolated populations have also shown increases in the percentages of female transformers from one year to the next (Manion & Smith 1978; Zerrenner & Marsden 2005), presumably due to decreasing larval densities as lampreys metamorphose and leave the stream. Since there is no evidence that TFM is selectively toxic to a particular sex (Purvis 1979), this suggests that males may be transforming earlier than females and/or that density-dependent effects (at low densities) lead to the production of more females. While studies (Hardisty 1965; Wicks *et al.* 1998; Lowartz & Beamish 2000) have shown atypical or unfixed sexual development in larval lampreys (i.e., males could be changing into females), the tendency of males to transform earlier relative to females of the same population is consistent with other fish species, where the added resource investment required for ovary development delays maturation (Fleming 1998).

Age is yet another variable that potentially could influence the likelihood of larvae to metamorphose. While recently-transformed lampreys are generally thought to be one year older than larvae of a similar size from the same population (Purvis 1980), it is believed that metamorphosis is more closely related to size, lipid reserves, and stream temperature than it is to age (Purvis 1980, Morkert *et al.* 1998). Furthermore, the determination of age through the analysis of length-frequency distributions has proven troublesome (Beamish & Medland 1988; Barker *et al.* 1997), likely due to overlap in the size distribution of larval lamprey from different year classes. This may partially explain why length is not an accurate predictor of metamorphosis. Because of the difficulties associated with rapidly assigning age and sex during field surveys, it is unlikely that the use of either sex or age within a predictive model of metamorphosis would be feasible in a large-scale management program. However, if metamorphic rates are found to be strongly influenced by either sex or age, then attempts to get accurate predictions of metamorphosis based on other measures may be futile.

Although photoperiod, which serves as a cue for many biological processes in nature, was not found to play a significant role in metamorphosis (Youson *et al.* 1993), other abiotic factors have been hypothesized to influence metamorphic rates. A stream's temperature regime has been cited by numerous researchers as one of the most important determinants of metamorphic rate, not only by influencing growth and metabolism, but also by acting as a cue to initiate transformation (Youson *et al.* 1993; Holmes & Youson 1998). Laboratory studies have demonstrated the need for cold winter temperatures followed by a spring warming period to initiate metamorphosis (Youson *et al.* 1993; Holmes *et al.* 1994), and that temperature effects are especially critical during the six

week period leading up to the onset of metamorphosis (Purvis 1980; Holmes & Youson 1998). This requirement for a seasonal temperature cycle is not unique to lampreys. Many temperate species require a cold winter thermal period followed by warming temperatures in the spring to initiate various developmental processes (Hokanson 1977).

Edaphic measurements have been linked to the variability in size and age at metamorphosis of larval lampreys as well. Young *et al.* (1990) found that lampreys in hard water streams grew faster and thus transformed earlier than those of soft water streams. This finding was supported by Griffiths *et al.* (2001) who found that conductivity accounted for some of the variation in growth among streams and that mean length at metamorphosis was negatively correlated with conductivity. Stream chemistry is largely determined by the geological characteristics of the catchment; these characteristics vary regionally throughout the Great Lakes Basin, for example leading to a preponderance of low alkalinity streams north of Lake Superior and high alkalinity streams surrounding Lake Ontario (Smith & Tibbles 1980). In addition, the location of a stream within the Great Lakes is related to the length of the growing season and the average annual temperature.

Using computer simulations, an analysis of the uncertainty intrinsic within the assessment and stream selection process was undertaken (refer to Chapter 1). This analysis suggested that, given perfect assessment information (i.e., no uncertainty), ranking streams using predicted numbers of transformers is the best way to select streams for treatment. As uncertainty in estimates of transformer abundance increase however, there comes a point where selecting streams using other methods becomes preferable. Given the demonstrated effectiveness of targeting metamorphosing larvae when we have

accurate estimates of their abundance, reasonable attempts should be made to lower the uncertainty surrounding predictive models of metamorphosis. While the results of the simulation modeling suggest that the current level of lamprey suppression could be improved if we switched the stream ranking methodology to one focusing on large larvae, the results also suggest that if the amount of uncertainty surrounding metamorphic estimates can be lowered, than targeting metamorphosing lampreys is the more optimal approach to take.

The current regional length-based logistic model used by the GLFC to predict transformation only distinguishes between streams from the upper or lower Great Lakes. The other predominant metamorphosis model, which I refer to as the Minimum Criteria (MC) model (Holmes & Youson 1994; Hollett 1998; Henson *et al.* 2003), also makes no attempt to address the variability inherent in stream productivity, larval growth, or metamorphic rates. This model sets criteria for length (>120 mm), weight (>3.0 g), and condition factor (>1.45) before metamorphosis can occur and applies these criteria across all streams in the Great Lakes equally. Both of these models ignore the variation in metamorphic rates that exists between streams. For an effective model of metamorphosis to be developed, measures that can differentiate between streams possessing different larval and metamorphic conditions need to be incorporated.

Unlike the development of the existing length-based metamorphic model, the objective of this study was to develop a predictive model of metamorphosis in Great Lakes sea lampreys based on direct measurements of individual lampreys before and after transformation, as well as associated stream-specific characteristics. The ability to individually identify lamprey during a mark/recapture allows for specific measurements

of each larva (observed at year T) to be linked directly to a specific metamorphic outcome (observed at year $T+1$), without the need for assumptions regarding growth or other factors that may influence the onset of metamorphosis. Also, because previous research has emphasized the importance of lipid content in determining the metamorphic preparedness of larvae, this study incorporated direct estimates of lipid content in addition to indirect measures. By combining individual mark-recapture data with stream- and year-specific measures of temperature and water chemistry parameters, some of the variation associated with metamorphosis in the Great Lakes may be explained. This study should enable us to improve our ability to predict metamorphosis and rank streams for lampricide treatment by reducing the uncertainty associated with converting estimates of larval densities to predictions of transformer abundance.

METHODS

Field Methods

A mark-recapture method was employed to document the occurrence of metamorphosis in populations of larval sea lampreys from different streams. Larval lampreys were collected in late summer using ABP-II DC backpack electrofishing gear (University of Wisconsin Engineering Technical Services, Madison, WI), measured for length (± 1 mm) and weight (± 0.01 g), and finally injected with a coded wire tag (CWT) before being released. Tagged lampreys were recaptured the following fall during a lampricide treatment, when the entire lamprey population of the stream could be easily collected with fyke and scap nets.

Nine streams were selected from the Great Lakes basin (Table 1, Figure 1) in consultation with USFWS and DFO sea lamprey biologists to provide contrast in

geography, as well as other biotic and abiotic parameters that may influence metamorphosis. Based on recent larval assessment surveys, streams with relatively abundant large larvae (>100 mm) were considered as candidate streams. Streams were chosen based on an expectation of a fall treatment the following year.

Between the August and October of 2003, collections of larval lampreys measuring >100 mm in length were made from the Pancake River, Dawson Creek, Little Sandy Creek, and Bowmanville Creek. Animals were anaesthetized, measured for length, weight, and scanned for total body electrical conductivity (TOBEC) using an EM-Scan Model SA-3000 Small Animal Body Composition Analyzer (EM-Scan Inc., Springfield, IL, 62704-5026). Larvae were injected with a CWT, following the methodology described in Bergstedt et al. (1993), and released back into their natal streams. A similar procedure was used between August and October in 2004 on larval lampreys from the Root River, Crystal Creek, Juniata Creek, Silver Creek, and Ceville Creek. A total of 609 larvae were marked and released in 2003, with an additional 916 marked and released in 2004, for a grand total of 1525 tagged larval lamprey (Table 2). Following the release of tagged larvae, temperature loggers (HOBO Water Temp Pro, Onset Computer Corporation, Pocasset, MA, USA) were installed within the release area and set to record water temperature every 4 hours.

The year following the marking of larvae, streams were visited in the fall during a scheduled lampricide treatment. The entire study section of the stream was walked during and after the treatment, using crews of six to eight people, from the uppermost point where marked animals were released, to the downstream limit of wadable water. Larvae and transformers were collected using long-handled scap nets while animals were either

actively swimming in the lampricide block or lying dead on the bottom. In addition, fyke nets were placed at suitable points throughout the study area to collect dead and dying animals as they drifted downstream. After the treatment was completed, individual lampreys were scanned for the presence of CWTs, using a Northwest Marine Coded Wire Tag V-Detector (Northwest Marine Technology, Shaw Island, WA, USA). Those containing a CWT were measured for length (± 1 mm) and weight (± 0.01 g) and then frozen for tag extraction, sex determination, and statolith extraction back in the laboratory.

Temperature loggers were retrieved and their data downloaded. In two instances, loggers were lost, so temperature data from nearby streams were used. On the Root River, temperature data from a logger installed in a tributary, Crystal Creek, were used as surrogate measurements. On Little Sandy Creek, temperature data were obtained for two nearby streams (Little Salmon and Grindstone Creeks) and the daily average for the two streams was used to represent the temperature profile for Little Sandy Creek (Andrew Hallett, DFO Sea Lamprey Control, unpublished data). Water chemistry parameters (alkalinity and pH) were collected from lampricide treatment crews at the time of treatment, whereas conductivity measures for each stream were determined by calculating the average measurement from larval surveys performed over the previous 5 years (Jeff Slade, USFWS; Fraser Neave, DFO, unpublished data).

Laboratory Methods

Using the V-Detector, lampreys were continuously sectioned until each CWT was retrieved. Tags were gently cleaned, mounted between two magnetic brass pencils, and

read using a stereoscopic dissecting microscope. Tag numbers of recaptured lampreys were then matched with measurements taken the year prior.

Statoliths were used to determine the age of recaptured lampreys, following procedures described in Hollett (1998). Statoliths are structures analogous to otoliths in teleost fish, which exhibit a banding pattern as a result of the reduction in growth during the winter months, and as such can be used to determine the age of larval lampreys (Beamish & Medland 1988). Using a standard dissection scalpel, a longitudinal cut was made along the entire length of the head, bisecting it. Jeweler's forceps were then used to remove any excess tissue and expose the otic capsule in each half. The fine tips of the forceps were used to pierce the otic capsule and remove the statolith, which is encased within a membranous material. This was then transferred to a microscope slide, water applied, and using a dissecting pin, statoliths teased out of the membranous sac and cleaned off. Statoliths were then transferred to a multiwell plate containing immersion oil for a period of 10-15 days, to improve the transparency and clarity of the annuli. After the 10 to 15 day waiting period, statoliths were removed from the multiwell plates and transferred to a depression slide filled with immersion oil. The number of annuli they contained was determined by manipulating the statolith under a dissecting microscope until a lateral aspect was achieved, allowing for clear enumeration of annuli. Once aged in oil, statoliths were mounted on numerically coded slides using a small amount of Crystal Bond adhesive. Crystal BondTM, a clear acetone-soluble adhesive, can be reheated repetitively, and allowed for the repositioning of the statolith during aging. Statoliths were then aged by three separate people, using a compound microscope, without knowledge of the life stage, source stream, or previous age assignments. The interpreted

ages were then compared, and statoliths with less than 50% agreement were removed from further analysis.

Recaptured lampreys were also examined to determine if there was any difference in metamorphic rates between male and female larvae, following procedures described in Docker and Beamish (1994). Portions of recaptured lampreys were cross-sectioned while frozen and examined under the microscope for the presence of ovaries. The remaining portion of the lamprey was fixed in a 10% formalin solution for verification purposes. In either fixed or frozen samples, if the lamprey was in good condition and lacked observable ovaries, and the presence of oocytes could not be confirmed with further dissection, the lamprey was classified as male. Several specimens, especially from samples collected during the first year, were classified as undetermined because the internal organs were indistinguishable, due either to an advanced state of decomposition at time of recapture or to problems related to their subsequent preservation.

TOBEC measurements were combined with condition factor for each larva, using a model developed for the non-invasive estimation of lipid content (see Chapter 2) to generate estimates of lipid weight. Estimates of both lipid weight and percent lipid were combined with length, weight, condition factor, sex, and age measurements as potential individual-level predictor variables for metamorphosis.

Data collected from temperature loggers were used to generate several exploratory temperature variables. For each stream, the number of days within a suitable temperature range for metamorphosis (between 9 and 25°C (Youson & Holmes 1998), the number of days within $\pm 2^\circ\text{C}$ of the optimal temperature (21°C, (Youson and Holmes 1998), the average temperatures for each of the three months leading up to

metamorphosis (April, May, June), and the overall mean annual temperature were calculated. A measure of the spring warming rate was also included, calculated as the average daily increase in water temperature, starting when streams reached the lower thermal limit of 9 °C, and ending when the stream approached within 2°C of the suggested optimal temperature for metamorphosis (as some streams did not reach an optimal temperature of 21 °C) (Youson & Holmes 1998).

Based on larval assessment and treatment data from the lamprey control program, estimates of the average larval density in type-1 (optimal) and type-2 (satisfactory) habitat (Dustin *et al.* 1989; Slade *et al.* 2003), along with the number of years since each stream was last treated, were added to the list of predictor variables. Streams were also categorized based on the regularity with which they produced transformers, and this categorization was also added to the list. Category one streams tend to have regular treatment intervals and rapid recruitment after treatment, whereas category three streams exhibit irregular treatment and lamprey production cycles (G. Anderson, unpublished data). A list of all the variables included in this analysis is provided in Table 3.

Statistical Methods

Differences in age, growth rates, and average size between larvae and transformers, as well as between males and females, were examined using analysis of covariance (ANCOVA), with river as a random effect, to allow for the non-independent nature of measurements taken from lampreys originating from the same river.

An exploratory analysis of the potential individual- and stream-level explanatory variables was performed using a best-subsets multiple logistic regression technique with

Statistica (Version 7, StatSoft Inc., Tulsa, OK, USA). Since many of the predictor variables were highly correlated and could not be used together in the same model, a multivariate analysis was initially employed. Both full and partial Principal Components Analysis (PCA) were used to see if components made of linear combinations of the predictor variables could add to the explanatory power of predictive models (Quinn & Keough 2002). Full PCA involved the reduction of the entire dataset to seven principal components, which were regressed against metamorphic state. The partial PCA involved combinations of principal components and raw variables in a best subsets regression. Corrected Akaike's Information Criterion (AIC_C) values were used to compare models, as sample size relative to the number of possible parameters was low (Burnham & Anderson 2004). Because AIC_C values can only be used to distinguish between models differing by more than a value of two, variance inflation factors (VIF) were used to remove models that contained highly correlated variables from the list of possible models. Model averaging was also used to develop a model that was a hybrid of the top models (Burnham & Anderson 2004). The Kappa statistic (κ) (Cohen 1960) was used to select the final model and is determined by an analysis of observed/predicted-correct/incorrect matrix.

The Kappa statistic was chosen over Chi-square, binomial, or Fisher's exact tests as the criterion to select the best overall model because these statistics compare the relative frequencies of larvae/transformer predictions, without regard for the correct number of individual predictions. Kappa measures the degree of agreement between observed and predicted values on an individual basis (Norman & Streiner 2003; Mullett & Bergstedt 2003). It also accounts for chance agreements (i.e., a model could predict

100% larvae and due to the preponderance of larvae in most collections, would exhibit a moderate degree of accuracy by chance), by subtracting the proportion of correct predictions expected by chance from the overall number of correct prediction (Kundel & Polansky 2003). Details regarding the calculation of the Kappa statistic are presented in Figure 2.

Once the best model was chosen, its ability to predict metamorphosis in larval sea lampreys was compared with that of two other prevalent metamorphic models, using the Kappa statistic. These two models were: (1) the regional length-based probability of metamorphosis model currently used in ESTR by the GLFC (Christie *et al.* 2003); and (2) the MC model, which sets minimum thresholds for length, weight, and condition factor before metamorphosis will occur (Holmes & Youson 1994; Hollett 1998; Henson *et al.* 2003). The number of correct predictions overall, the number of correct transformer predictions specifically, and the Kappa statistic were used to compare the overall performance of each model relative to the other two.

Since the main purpose of this study was to develop a model that could be used within the framework of the stream ranking process, a second model analysis was performed to develop a management-oriented model. In this analysis, the suite of variables used was limited to those that could be readily collected by fisheries managers or those that would not exhibit substantial yearly variation. I obtained stream-specific data from DFO and the USFWS which pertained to streams (Figure 3) from a similar mark-recapture study that was performed in 1995/1996 (Hollett 1998). This allowed me to combine the two data sets and increase the number of observations with which to develop the management model.

Once the top biological and management-based models were selected, the dataset was entered into the PROC GLIMMIX macro within SAS (SAS Institute Inc., Cary, NC 27513), incorporating a binomial error structure, to generate the parameter estimates, standard errors and p-values of the fixed effects, as well as the variance estimate and standard error for the random effect of river in each model.

RESULTS

A total of 212 larvae were recaptured from the 1525 that were marked in 2003 and 2004 (Table 2). An additional 56 lamprey were marked and 2 subsequently recaptured from Dawson Creek (a tributary to Lake Superior). However, due to the onset of winter prior to sufficient marking could be completed and poor collecting conditions at the time of treatment a year later, this stream was not included in the analysis

The model for estimating lipid weight in larval lampreys, based on the TOBEC method (see Chapter 2), was able to predict the specific metamorphic outcome in approximately 50% of the recaptured animals, based on a 0.3 g criterion (Figure 4). A similar result was obtained using percent lipid (Figure 4).

Most of the recaptured lampreys demonstrated some amount of growth between tagging and recapture (although some showed negative growth), regardless of whether or not they entered metamorphosis, and growth increments declined as the size of the animal increased (Figure 5). This appeared to hold true within each stream as well, although not all relationships were significant due to limited sample sizes obtained from some streams. Age is naturally positively correlated with length, as lamprey continue to grow throughout their larval phase, however, age (and thus length) exhibited a significant

negative relationship with the average rate of growth ($p < 0.05$). When data from individual streams were pooled, daily average growth rate (calculated as the change in length of an individual from the time of marking to recapture divided by the number of days between release and recapture) was significantly less for larvae that metamorphosed relative to those that did not ($p < 0.001$). Weight and length of larvae and transformers were significantly different ($p < 0.05$).

Analysis of the recaptured lampreys indicated that of the 142 larvae, 85 were classified as female and 47 as male (10 were indistinguishable). Of the 70 recently metamorphosed lampreys, 53 were female and 12 were male, and 5 were impossible to differentiate. The sex ratios from seven of the eight rivers varied from between 50 and 92% female; however the sex ratio from Ceville Creek was inverted, with 70% of the larvae being classified as male (Figure 6). There were significantly more female transformers relative to larvae; however statistical significance of this finding disappears when lampreys of undetermined sex are considered. I did not find evidence of differential transformation rates between males and females, with the average age at metamorphosis not being significantly different between the sexes ($p > 0.05$).

Growth rates and the size of lampreys were related to several environmental factors. There was a significant trend for the average daily growth rate of the lamprey population to increase as stream location became more southerly and/or easterly ($p < 0.0001$). Growth was positively correlated with stream conductivity, alkalinity, pH, and negatively correlated with stream category. Stream latitude was highly correlated with all of the temperature and edaphic variables. Average daily growth was not

significantly related to any of the average water temperature parameters used in this analysis ($p > 0.05$).

The location of the stream was correlated with numerous environmental parameters (Table 4). Latitude was positively correlated with the number of extreme temperature days (< 9 or $> 25^{\circ}\text{C}$) and the slope of the spring warming curve, and negatively correlated with all other temperature and stream-level variables. Longitude was negatively correlated with most temperature and stream-level variables (Table 4).

Biological Model Analysis

Since I was not able to obtain statoliths from 33 of the 212 recaptured lampreys, I was unable to estimate age for these animals, and thus the number of lampreys available for the development of the biological model analysis was reduced to 179. The best-subsets model selection procedure, using the full suite of variables collected during the course of this study, produced a list of 26 potential models, all with comparable statistical support, as demonstrated by having AIC_C values within a range of two (Burnham & Anderson 2004). This list was further reduced to 22 models by analyzing the variance inflation factors (VIF) of all the models and removing those whose variables demonstrated excessive co-linearity (Table 5). A Principal Components Analysis (PCA) did not provide any additional predictive capability (i.e., models with larger Kappa values), so the results presented hereafter will only focus on the analysis involving the raw variables.

The relative ability to predict metamorphosis and a kappa statistic were calculated for each model (Table 5), since AIC_C values are generally not able to further distinguish

between models exhibiting a range of AIC_C values of less than two. However, because two models remained that possessed equal Kappa values and only differed by the specific temperature parameter they contained, the one with the lowest AIC_C was chosen. This resulted in the selection of a model that contained a measure of an individual lamprey's weight, age, and sex, as well as the stream-level effects of the number of days between 9 and 25°C, stream longitude, and average larval density in type 2 habitat. This model produced predictions that were 93.3 % correct overall, with proper classification of metamorphosing lamprey occurring in 87.5% of the recaptured animals (Table 5).

Akaike weights were generated using differences in the AIC_C values of the top models and these weights were combined with parameter estimates to generate a composite model (Burnham & Anderson 2004). However, this composite model exhibited a low Kappa statistic ($\kappa = 0.64$) and poor predictive capability (only 58.3% transformers correctly predicted), so the model was discarded. Akaike weights were useful, however, in illustrating variable importance by summing variable weights across all of the models (Figure 7). This analysis highlighted the importance of weight, age, and stream longitude measurements in particular, in predicting the probability of metamorphosis.

A mixed model analysis failed to find a significant random effect of river, indicating that the inclusion of temperature, location and larval density estimates was able to account for the variability between rivers in metamorphic rate (Table 6(A))

Management Oriented Model

The model selected above contained variables (age, gender, year-specific temperature) that are not practical for use as a management tool in predicting metamorphosis. As a result, a concurrent analysis was performed, this time limiting the explanatory variables to those that could readily be obtained by management agencies and that would not be significantly affected by yearly variation. Since age was not included in this analysis, the full dataset of 212 recaptured lampreys was used. Addition of data from Hollett (1998) further increased the overall size of the dataset to 315 lampreys. The addition of these data increased the number of rivers in this analysis to 11.

As was the case in the biological model analysis, inclusion of PCA did not add any explanatory power, so the results reported here are limited to an analysis of the first-order variables only. The results of the model selection procedure on the combined dataset produced a list of three models with AIC_C values that differed by less than two (Table 7). A model consisting of length, condition factor, stream alkalinity, stream category, stream latitude and longitude, as well as the number of years since the stream was last treated, possessed the highest Kappa value. This model was able to correctly predict the metamorphic outcome of individual larvae 86.27% of the time.

As was the case with the biological model, a mixed model analysis showed that the inclusion of the stream-specific parameters in the selected management model was able to account for inter-stream variability in metamorphic rate (Table 6(B)).

Comparison with alternate predictive models of metamorphosis

Comparison of the predictive ability of the biological model with that of the ESTR-based model and the MC model suggested that the biological model was much more accurate at predicting metamorphosis overall. The number of predicted larvae and transformers were closer to observed values (Figure 8(A), and the number of correct individual predictions, a more accurate evaluation of model performance, was higher with the biological model as well (Figure 8(B). Comparison of the kappa statistic for each model indicated that the biological model was 54% more accurate than the ESTR model and 68% more accurate than the MC model, relative to a random assignment of metamorphic state. Not only did the biological model perform better overall, but it produced more accurate predictions relative to the other two models for every stream in the analysis (Table 8).

A direct comparison of the management model with the biological model was not possible, as the sample size was increased at the same time sex, age, and stream temperature parameters were removed from the dataset. While all models explored in this analysis were successful at identifying larvae that would not undergo metamorphosis (93 to 99%), the ability to determine which larvae would metamorphose varied greatly (13 to 81%). Model performance was generally lower for the management model relative to the biological model, but the management model remained a better overall predictor of metamorphic outcome when compared with the ESTR or MC models (13.3% and 51.1% improvement over ESTR and MC models respectively). A stream by stream analysis of model output indicated that the management model provided equal or more accurate

predictions of metamorphosis on 8 of 11 streams, whereas the ESTR model produced equal or better results on only 5 of 11 streams (Table 9).

DISCUSSION

Biological Model

Incorporating stream-specific variables within a predictive model of metamorphosis improved the predictive capabilities of both the biological and management based models, relative to models currently in use today. Although the biological model developed could not be used within a management context to determine probability of metamorphosis, it does shed light on some of the factors that have made accurately predicting the occurrence of metamorphosis in larval sea lampreys difficult. The prevalence of weight and age in all of the top models (Table 5) supports the idea that larval lampreys need to attain a critical mass before metamorphosis can occur, and that when presented with two animals of similar size (length or weight), the older individual is more likely to transform. In addition, every model in the list of 22 top models (Table 5) contains some measure of stream temperature, location, and estimated larval density. The numerous combinations of temperature, larval density, and stream-level variables that make up the list of models suggest that while these parameters are important, the dataset needs to be expanded to provide greater contrast to identify which of these measurements exert the most influence over metamorphic rates. The importance of additional data became evident during the development of the management oriented model, where the addition of a supplementary dataset from Hollett (1998) greatly reduced the number of potential models. Additional data provided greater contrast between stream-specific

variables and thus clarified which specific variables provide the greatest explanatory power.

The common occurrence of temperature variables in the list of potential models confirmed the generally held belief that metamorphic rates are influenced by stream temperature (Purvis 1980; Holmes & Youson 1994; Holmes & Youson 1998). Some measure of stream temperature was present in all 22 of the top models (Table 5). Since Holmes and Youson (1998) determined that the optimal temperature for metamorphosis is around 21°C, it was not surprising to find that the temperature variable representing the number of days on each stream that the temperature was within a 2°C range of this value was so prevalent in the top models. The number of days in each stream that lampreys spent within a range between the approximate upper and lower limits of metamorphosis (9 to 23°C; Holmes & Youson 1998) also weighted heavily (Figure 7) in the list of models and was the temperature parameter selected in the top model. Both of these measures would affect metabolism and larval growth rates and likely serve to reflect the among stream differences in these processes. Given previous research that has suggested that temperature exposure in the month immediately prior to the onset of metamorphosis (i.e., June), it was surprising that May or June average temperatures did not appear in any of the top models.

Another aspect of stream temperature is its ability to act as a cue for metamorphosis. Holmes *et al.* (1994) suggested that outside of its influence on metabolism and growth, water temperature, especially a long cold period followed by a rise in temperature in the spring, acts as a critical cue for metamorphosis. These results were based on two groups of lampreys: one group kept at constant temperature; the other

at ambient. While this is critical in understanding some of the underlying mechanisms of metamorphosis, it is doubtful that this parameter can be used to predict metamorphosis, as all streams within the Great Lakes experience seasonal temperature fluctuations to some degree. Although a parameter measuring the slope of the spring warming curve was found in one of the top biological models (Table 5), it would be interesting to see, using data from an additional set of streams, if this parameter would be able to differentiate between among streams with different metamorphic rates.

An estimate of larval density in type-2 habitat was included in seven of the top ten biological models, which supports the concept that density influences the rates of growth and metamorphosis (Manion & Smith 1978; Zerrenner & Marsden 2005), as well as the notion that larger, pre-metamorphic larvae may seek out Type-2 habitats, which consist of a higher proportion of coarse substrate (Sullivan 2003). Although not reflected in the relative importance of individual parameters (Figure 7), an estimate of larval density (in either type-1 or type-2 habitat) was present in every one of the 22 top biological models. This is of special relevance to the lamprey control program, which regularly causes great changes in larval density within streams through lampricide treatments, and thus may add a great deal of the variability in metamorphic rate within a single stream. It also suggests that density estimates derived from QAS surveys may have value outside of their use in ESTR and may be used in combination with other variables to more accurately predict metamorphosis.

Due to the positive correlation between temperature and latitude, it was not surprising that latitude was so prevalent in many of the top biological models, as it is highly correlated with stream temperature parameters. The prominence of longitude in all

of the top models, however, was an unexpected result. While all of the stream temperature parameters were highly correlated with latitude, longitude was either positively or negatively correlated with most of the edaphic measurements (Table 4). In addition, moving east to west within the Great Lakes is equivalent to moving upstream (and up in latitude) simply due to the orientation of the lakes, and so longitude is correlated with latitude in this way. With this in mind, perhaps the prevalence of longitude in the list of potential models should not be so surprising, as it acts as a surrogate measure for many environmental variables. This correlation of geographic coordinates with various stream thermal and hydro-chemical characteristics suggests that the use of stream geographic coordinates may provide an easily adopted method for explaining some of the variability inherent in metamorphic rates in different areas of the Great Lakes.

Management Model

Although it appears that the management-oriented model was not able to predict metamorphosis as consistently or with such a high degree of accuracy as the biological model, this conclusion is somewhat confounded by the inclusion of supplemental data in the development of the management model. While the management model remained a better predictor of metamorphosis overall (relative to the ESTR and MC models), the relative performance of this model was slightly more variable among individual streams, relative to the biological model. While the management model was equal to or superior to the ESTR model on 8 of 11 streams, it is possible that this result would have been improved had recapture rates been higher. Some of the rivers produced numbers of

recaptured lamprey that were insufficient to compare all three models, since a correct predicted value for both larvae and transformers must be achieved before a non-zero Kappa value can be calculated (Figure 2). This was the case for all three rivers where the management model was not the top model, indicating that further mark-recapture studies would be advisable to confirm the superior ability of the management model.

At first glance, the biological and management models do not appear to be related, as the top models in each category only share longitude as a common variable. However, a closer look reveals some similarities in model structure. The management model contains a length measurement instead of the weight variable contained in the biological model. This is not a major change however, since length and weight are highly correlated ($r^2=0.96$, $p<0.05$). As length and weight are too highly correlated to be included together, the addition of condition factor is needed to explain how weight varies with length within the population. While age was removed as a potential predictor variable in the management model, it seems reasonable that, on regularly treated streams, the number of years since the last treatment occurred could serve as an effective surrogate for age, since they are significantly correlated ($p<0.05$); the management model reflects this. The loss of a temperature parameter appears to have been compensated for by the addition of alkalinity and latitude, both of which are highly correlated ($p<0.05$) with mean annual stream temperature and both of which add information regarding the productivity of the stream.

The inclusion of the lamprey production stream category in every one of the top management models was also a surprising result. Originally designed to distinguish between streams that demonstrated varying rates of recruitment, growth, and regularity of

treatment, it appears that this categorization has some relevance for predicting metamorphic rates as well. It is likely that in the management model, stream category functions in a similar manner to latitude or longitude in that it summarizes a whole suite of environmental, and in this case population level effects that are common to a group of streams. While the current stream designations were made subjectively by lamprey control biologists, research is underway to identify demographic differences in stream specific data, to define which streams belong in which category (G. Anderson, unpublished data). Perhaps this refinement will add to this variables explanatory power.

Improving on the Management Model

The biological model emphasized the importance of age, sex and temperature parameters in a predictive model. While it would not be feasible to determine the age and sex of larvae collected during larval assessments, inclusion of a stream temperature variable into the management model may be plausible. Using temperature logging devices, various temperature indices for all lamprey producing streams could be obtained. An analysis of which temperature variable would be most appropriate would need to be conducted. This analysis would include whether an index of temperature could differentiate between the metamorphic rates of different streams. The amount of yearly variation surrounding the temperature index would also need to be determined. If a temperature index varied substantially from one year to the next, then its inclusion as a predictor variable in a metamorphic model is unlikely.

The research done to date on the role lipids play in preparing lampreys for metamorphosis (Lowe *et al.* 1973; Potter 1980; Holmes & Youson 1994) suggests that

this above all other individual-level measurements should be the best indicator of a lamprey's likelihood to metamorphose. The complete absence of this parameter within any of the models developed during this analysis suggests, as discussed in the preceding chapter, that the model used to non-invasively estimate lipid content in this study lacked sufficient accuracy to be included as an explanatory variable. Any information it did provide was likely captured by the inclusion of length and condition factor in the management model. However, the ability of even this rough estimate of lipid content on its own to distinguish between metamorphic and non-metamorphic larvae (Figure 4), suggests that more accurate models may improve upon this result. While the lipid analysis in the preceding chapter explored several non-invasive methods used in other areas of ecology, new technologies have since been reported (Crossin & Hinch 2005; Cox & Hartman 2005) that may be better suited to estimate lipid content non-invasively in sea lamprey, and thus establish the utility of using lipid mass as a predictor for metamorphosis.

Problems with Current Models

The data from this study refute the existence of an arrested-growth phase, as most lampreys demonstrated some form of linear growth, regardless of metamorphic outcome. While recaptured animals did not show a significant change in weight related to their weight at time of marking, larval lamprey did show a significant trend of decreasing linear growth as a function of length at the time of marking (Figure 5). The variability of the observed growth as a function of both size and age also calls into question the use of simple linear growth models to statistically grow assessment collections forward to a

common end date, as is currently done in the ESTR model. The growth rates used in those calculations, aside from being highly variable from year to year, are based on the observed growth of a re-established cohort following a treatment (i.e. when growth rates are highest due to low densities), and could overestimate the amount of growth that large larvae actually experience (Hansen *et al.* 2003). Instead, these results suggest that a nonlinear, Von Bertalanffy growth model (Ricker 1975), which accounts for decreasing linear growth with increasing size, would be more appropriate for use in calculating the amount of growth to statistically adjust survey collections.

With the exception of Ceville Creek, the sex ratios of recaptured lampreys were skewed towards female (Figure 6), which is consistent with the findings of other studies, and is indicative of a lamprey population that is attempting to compensate for control-induced reductions in abundance by producing an excess of females (Wicks *et al.* 1998; Jones *et al.* 2003). The 70% male: 30% female ratio observed in Ceville Creek may be related to lampreys within the stream becoming highly concentrated during the summer months when low water levels restrict available habitat in that stream. Ceville Creek was by far the smallest of the streams in this study and is prone to extreme low water levels during the summer months (unpublished survey data, USFWS Marquette Biological Station). This may be evidence of density-dependent sex determination, or related to some unmeasured environmental variable. The tendency for high-density populations to be disproportionately male has implications for the effect of density on metamorphosis, as males tend to transform at a smaller size (Docker & Beamish 1994; Zerrenner & Marsden 2005). This correlation between density and sex may help to explain the

prevalence of density estimates in all of the models, but why sex is only in some of the top models.

Previous model evaluations have relied on a basic comparison of observed versus predicted frequencies of larvae and transformers in recaptured collections (Youson *et al.* 1993; Holmes & Youson 1994). For ongoing development and refinement of predictive models to occur, a standardized method of directly comparing the performance of one model to another needs to be adopted. The use of raw prediction frequencies is not suitable for this type of comparison and can lead to deceiving results if the frequency of incorrect predictions is not taken into account. Although some controversy exists surrounding the use of the Kappa statistic (Ker 1991), when used for the evaluation of a simple 2×2 matrix, it provides a superior method for evaluating and directly comparing model performance. Unlike other comparative statistics, standard errors and confidence intervals can also be calculated using Kappa (Kundel & Polansky 2003). Predictive models of metamorphosis also seem to be biased towards accurate predictions of larvae that do not enter into metamorphosis, with less accuracy in identifying individuals that do transform. As collections are usually skewed toward having more larvae than transformers, this can lead to a deceiving measure of overall agreement. An assessment of how accurately predictive models correctly forecast the number of transformers should also be incorporated in model evaluation, since errors in transformer estimates have a higher cost associated with them as a result of the importance put upon them in the stream selection process.

Table 1. List and locations of streams used for the mark-recapture of larval sea lampreys in the Great Lakes. Note that Dawson Creek, a tributary to Lake Superior was initially included in the mark-recapture study, but was dropped due to a poor marking effort and subsequent poor return of animals.

Stream	Lake	Latitude	Longitude
Pancake River	Superior	46°57'36"	84°39'46"
Bowmanville Creek	Ontario	43°53'16"	78°39'51"
Little Sandy Creek	Ontario	43°38'23"	76°10'08"
Root River	Huron	46°32'28"	84°12'24"
Crystal Creek (tributary to the Root River)	Huron	46°33'39"	84°14'21"
Silver Creek	Huron	44°20'53"	83°29'30"
Juniata Creek (tributary to the Cass River)	Huron	43°24'38"	83°29'06"
Ceville Creek	Michigan	45°59'44"	84°21'46"

Table 2. Number of marked and recaptured sea lamprey larvae by stream, including the year of marking and total numbers.

Stream	Number Marked	Number Recaptured	Number Larvae	Number Transformers	Recapture Rate
Pancake River ¹	144	10	4	6	6.9%
Bowmanville Cr. ¹	344	50	12	38	14.5%
Little Sandy Cr. ¹	121	22	11	11	18.2%
Root River ²	254	11	6	5	4.3%
Crystal Creek ²	170	15	13	2	9.4%
Silver Creek ²	182	40	37	3	22.0%
Juniata Creek ²	168	37	34	3	22.0%
Ceville Creek ²	142	27	25	2	19.0%
Totals	1525	212	143	70	14.0%

¹ denotes streams with larvae marked in 2003 and recaptured in 2004.

² denotes streams with larvae marked in 2004 and recaptured in 2005.

Table 3. List of variables evaluated for use in a predictive model of metamorphosis in Great Lakes sea lamprey populations.

Individual-level predictors

Length (mm)
Weight (g)
Condition Factor
Predicted Lipid (g)*
Predicted Percent Lipid (g)*
Sex*
Age*

Stream-level predictors

Mean Annual Stream Temperature*
Number of days <9 or >25 °C*
Number of days between 9 and 25 °C*
Number of days between 19 and 23 °C*
Mean April Temperature*
Mean May Temperature*
Mean June Temperature*
Spring Warming Slope*
Average Daily Growth*
Estimated Mean Larval Density in Type 1 habitat
Estimated Mean Larval Density in Type 2 habitat
Lamprey Production Stream Category
Conductivity
Alkalinity
pH
Stream Drainage Area
Latitude of stream mouth
Longitude of stream mouth
Number of years since last lampricide treatment

* indicates variables that were used only in the biological model analysis. All other variables were used in both the biological and management based models.

Table 4. Correlation of stream geographic location with environmental parameters used to estimate probability of metamorphosis in this study.

Parameter	Latitude		Longitude	
	Postively Correlated	Negatively Correlated	Postively Correlated	Negatively Correlated
Mean Annual Temperature		-0.75		-0.21
Number of extreme temperature days (<9 or >25°C)	0.76		0.33	
Number of days between 9 and 25°C		-0.77		-0.38
Number of days between 19 and 23°C		-0.32		-0.13*
Mean April Temperature		-0.60		-0.14
Mean May Temperature		-0.58		-0.67
Mean June Temperature		-0.25	0.30	
Spring Warming Slope	0.49		0.77	
Lamprey Production Stream Category	0.12*		0.42	
Conductivity		-0.85		-0.24
Alkalinity		-0.65		-0.24
pH		-0.87		-0.39
Stream Drainage Area		-0.11*		-0.66
Latitude			0.53	
Longitude		-0.53		

Table 5. List of top biological models based on corrected AIC values. Models are ranked in decreasing order of model predictive capability according to each model's kappa statistic.

Model Variables	df	AICc	Percent Overall Larvae Correct		Percent Transformers Correct		Kappa Statistic
			Correct	Percent	Correct	Percent	
(Weight) + (Age) + (# Days between 9 and 25 C) + (Longitude) + (Larval Density in Type-2 habitat) + (Sex)	7	70.38	93.30%	95.42%	87.50%	87.50%	0.829
(Weight) + (Age) + (Mean Annual Temperature) + (Longitude) + (Larval Density in Type-2 habitat) + (Sex)	7	71.79	93.30%	95.42%	87.50%	87.50%	0.829
(Weight) + (Age) + (Mean Annual Temperature) + (Longitude) + (Larval Density in Type-1 habitat) + (Sex)	7	71.55	92.74%	94.66%	87.50%	87.50%	0.816
(Weight) + (Age) + (# Days between 9 and 25 C) + (# Days between 19 and 23 C) + (Longitude) + (Larval Density in Type-2 habitat) + (Sex)	8	72.33	92.74%	96.18%	83.33%	83.33%	0.811
(Weight) + (Age) + (# Days between 19 and 23 C) + (Latitude) + (Longitude) + (Larval Density in Type-2 habitat)	6	72.03	92.74%	96.95%	81.25%	81.25%	0.809
(Weight) + (Age) + (# Days between 9 and 25 C) + (Longitude) + (Larval Density in Type-2 habitat) + (Average Daily Growth) + (Sex)	8	72.27	92.18%	95.42%	83.33%	83.33%	0.798
(Weight) + (Age) + (# Days between 19 and 23 C) + (Alkalinity) + (Longitude) + (Larval Density in Type-2 habitat)	6	70.93	92.18%	96.18%	81.25%	81.25%	0.795
(Weight) + (Age) + (# Days between 19 and 23 C) + (pH) + (Longitude) + (Larval Density in Type-2 habitat)	6	71.13	92.18%	96.18%	81.25%	81.25%	0.795
(Weight) + (Age) + (# Days between 9 and 25 C) + (# Days between 19 and 23 C) + (Longitude) + (Larval Density in Type-1 habitat)	6	71.46	92.18%	96.18%	81.25%	81.25%	0.795
(Weight) + (Age) + (# Days between 19 and 23 C) + (pH) + (Longitude) + (Larval Density in Type-1 habitat)	6	71.61	92.18%	96.18%	81.25%	81.25%	0.795
(Weight) + (Age) + (# Days between 19 and 23 C) + (Alkalinity) + (Longitude) + (Larval Density in Type-1 habitat)	6	71.73	92.18%	96.18%	81.25%	81.25%	0.795
(Weight) + (Age) + (# Days between 19 and 23 C) + (Latitude) + (Longitude) + (Larval Density in Type-1 habitat)	6	72.12	92.18%	96.18%	81.25%	81.25%	0.795
(Weight) + (Age) + (# Days between 9 and 25 C) + (Longitude) + (Larval Density in Type-2 habitat)	5	71.01	91.62%	95.42%	81.25%	81.25%	0.782
(Weight) + (Age) + (# Days between 19 and 23 C) + (Slope of Spring Warming Trend) + (Longitude) + (Larval Density in Type-2 habitat)	6	71.15	91.62%	95.42%	81.25%	81.25%	0.782
(Weight) + (Age) + (# Days between 19 and 23 C) + (Latitude) + (Longitude) + (Larval Density in Type-2 habitat) + (Sex)	8	71.39	91.62%	95.42%	81.25%	81.25%	0.782
(Weight) + (Age) + (# Days between 9 and 25 C) + (Longitude) + (Larval Density in Type-1 habitat)	5	71.67	91.62%	95.42%	81.25%	81.25%	0.782
(Weight) + (Age) + (# Days between 19 and 23 C) + (Longitude) + (Larval Density in Type-1 habitat)	5	71.29	91.62%	96.18%	79.17%	79.17%	0.779
(Weight) + (Age) + (# Days between 19 and 23 C) + (Longitude) + (Larval Density in Type-1 habitat) + (Sex)	7	72.09	91.62%	96.18%	79.17%	79.17%	0.779
(Weight) + (Age) + (# Days between 19 and 23 C) + (pH) + (Longitude) + (Larval Density in Type-2 habitat) + (Sex)	8	71.19	91.06%	94.66%	81.25%	81.25%	0.769
(Weight) + (Age) + (# Days between 19 and 23 C) + (Alkalinity) + (Longitude) + (Larval Density in Type-2 habitat) + (Sex)	8	71.39	91.06%	94.66%	81.25%	81.25%	0.769
(Weight) + (Age) + (# Days between 9 and 25 C) + (Longitude) + (Larval Density in Type-1 habitat) + (Sex)	7	71.52	91.06%	94.66%	81.25%	81.25%	0.769
(Weight) + (Age) + (Mean Annual Temperature) + (Longitude) + (Larval Density in Type-1 habitat)	5	71.96	91.06%	94.66%	81.25%	81.25%	0.769

Table 6. Parameter estimates, standard errors, and p-values from mixed model analysis involving variables from the top predictive models of metamorphosis.

A) Biological Model

Effect	Parameter Estimate	Standard Error	p-value
<i>Fixed Effects:</i>			
Intercept	-21.652	18.579	0.2964
Weight	4.024	0.083	<0.0001
Age	3.093	0.879	0.0006
# Days between 9 and 25°C	-0.021	0.178	0.9059
Estimated Mean Larval Density in Type-2 Habitat	-0.100	0.333	0.7643
Sex (Male)	1.775	1.947	0.3632
Sex (Female)	2.659	1.915	0.1668
Sex (Undetermined)	---	---	---
	Variance Estimate	Standard Error	p-value
<i>Random Effects:</i>			
Stream	12.266	9.301	>0.05

B) Management Model

Effect	Parameter Estimate	Standard Error	p-value
<i>Categorical Effects:</i>			
Lamprey Production Stream Category	NA	NA	NA
<i>Fixed Effects:</i>			
Intercept	-40.628	29.079	0.2119
Length	0.204	0.031	<0.0001
Condition Factor	2.927	2.415	0.227
Alkalinity	0.008	0.01	0.392
Latitude	1.956	0.977	0.046
Longitude	0.978	0.427	0.023
# years since last treated	0.582	0.518	0.262
	Variance Estimate	Standard Error	p-value
<i>Random Effects:</i>			
Stream	2.527	2.162	>0.05

Effect	Parameter Estimate	Standard Error	p-value
<i>Categorical Effects:</i>			
Lamprey Production Stream Category	NA	NA	NA
<i>Fixed Effects:</i>			
Intercept	-40.628	29.079	0.2119
Length	0.204	0.031	<0.0001
Condition Factor	2.927	2.415	0.227
Alkalinity	0.008	0.01	0.392
Latitude	1.956	0.977	0.046
Longitude	0.978	0.427	0.023
# years since last treated	0.582	0.518	0.262
	Variance Estimate	Standard Error	p-value
<i>Random Effects:</i>			
Stream	2.527	2.162	>0.05

Table 7. Top three models from best subsets analysis on data from this study combined with data from Hollett (1998).

Model Variables	df	AICc	Percent		Kappa Statistic
			Overall Correct	Larvae Correct	
(Length) + (Condition Factor) + (Alkalinity) + (Lamprey Stream Category) + (Latitude) + (Longitude) + (# years since last treated)	7	182.81	86.27%	91.15%	0.681
(Length) + (Alkalinity) + (Lamprey Stream Category) + (Latitude) + (Longitude) + (# years since last treated)	6	181.24	85.21%	90.63%	0.656
(Length) + (Alkalinity) + (Lamprey Stream Category) + (Latitude) + (Longitude) + (# years since last treated) + (Average Larval Density in Type-2 habitat)	7	183.13	84.86%	90.10%	0.649

Table 8. Stream by stream comparison of predictive capability of the biological model with that of the ESTR and MC models (n=179).

Stream	Model	Correct Larval Predictions	Correct Transformer Predictions	Misclassified Specimens	Kappa Statistic
Bowmanville Creek	Biological	11	26	2	0.880
<i>(12 Larvae)</i>	ESTR	10	13	16	0.246
<i>(27 Transformers)</i>	MCM	12	4	23	0.097
Ceville Creek	Biological	24	2	0	1.000
<i>(24 Larvae)</i>	ESTR	21	0	5	-0.102
<i>(2 Transformers)</i>	MCM	24	0	2	0
Crystal Creek	Biological	12	1	1	0.632
<i>(12 Larvae)</i>	ESTR	11	0	3	-0.105
<i>(2 Transformers)</i>	MCM	11	1	2	0.417
Juniata Creek	Biological	32	1	3	0.357
<i>(33 Larvae)</i>	ESTR	32	0	4	-0.043
<i>(3 Transformers)</i>	MCM	33	0	3	0
Little Sandy Creek	Biological	5	1	1	0.588
<i>(6 Larvae)</i>	ESTR	5	1	1	0.588
<i>(1 Transformers)</i>	MCM	6	0	1	0
Pancake River	Biological	2	5	2	0.526
<i>(4 Larvae)</i>	ESTR	3	1	5	-0.047
<i>(5 Transformers)</i>	MCM	4	1	4	0.182
Root River	Biological	6	4	1	0.814
<i>(6 Larvae)</i>	ESTR	6	0	5	0
<i>(5 Transformers)</i>	MCM	5	0	6	-0.179
Silver Creek	Biological	33	2	2	0.637
<i>(34 Larvae)</i>	ESTR	32	1	4	0.275
<i>(3 Transformers)</i>	MCM	34	0	3	0

Table 9. Stream-by-stream comparison of predictive capability of the management model with that of the ESTR and MC models, using the pooled dataset (n=315).

Stream	Model	Correct Larval Predictions	Correct Transformer Predictions	Misclassified Specimens	Kappa Statistic
Bowmanville Creek <i>(12 Larvae)</i> <i>(38 Transformers)</i>	Management	11	29	10	0.554
	ESTR	12	23	15	0.424
	MC	12	6	32	0.083
Ceville Creek <i>(25 Larvae)</i> <i>(2 Transformers)</i>	Management	25	1	1	0.649
	ESTR	25	1	1	0.649
	MC	25	0	2	0.000
Crystal Creek <i>(13 Larvae)</i> <i>(2 Transformers)</i>	Biological	13	1	1	0.634
	ESTR	13	0	2	0.000
	MC	12	1	2	0.423
Juniata Creek <i>(34 Larvae)</i> <i>(3 Transformers)</i>	Management	34	0	3	0
	ESTR	31	2	4	0.444
	MC	34	0	3	0
Little Sandy Creek <i>(11 Larvae)</i> <i>(11 Transformers)</i>	Management	11	7	4	0.636
	ESTR	11	4	7	0.364
	MC	11	1	10	0.091
Pancake River <i>(4 Larvae)</i> <i>(6 Transformers)</i>	Management	3	5	2	0.583
	ESTR	4	0	6	0.000
	MC	4	1	5	0.138
Root River <i>(6 Larvae)</i> <i>(5 Transformers)</i>	Management	4	4	3	0.459
	ESTR	6	1	4	0.214
	MC	5	0	6	-0.179
Silver Creek <i>(37 Larvae)</i> <i>(3 Transformers)</i>	Management	36	1	3	0.362
	ESTR	36	1	3	0.362
	MC	37	0	3	0
Oshawa Creek <i>(2 Larvae)</i> <i>(21 Transformers)</i>	Management	0	21	2	0
	ESTR	1	17	5	0.184
	MC	2	9	12	0.115
Gordon Creek <i>(63 Larvae)</i> <i>(7 Transformers)</i>	Management	59	4	7	0.478
	ESTR	56	5	9	0.458
	MC	52	3	15	0.176
Wilmot Creek <i>(7 Larvae)</i> <i>(3 Transformers)</i>	Management	0	3	7	0
	ESTR	6	3	1	0.783
	MC	2	2	6	-0.034

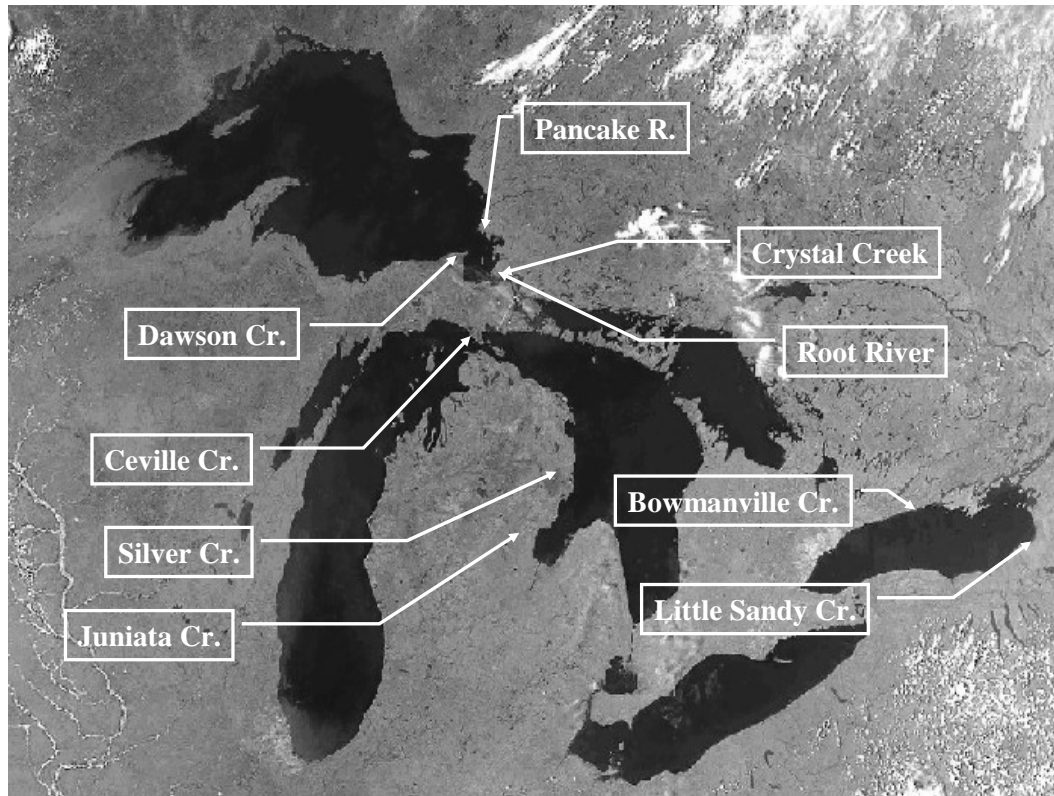


Figure 1. Location of the nine study streams selected for this study. Note that Dawson Creek, a tributary to the Two-Hearted River and Lake Superior was dropped from the analysis due to poor marking and subsequent recapture results.

General Formula:

$$\kappa = \frac{P_0 - P_e}{1 - P_e} \qquad SE(\kappa) = \sqrt{\frac{P_0(1 - P_0)}{n(1 - P_e)^2}}$$

Where P_0 is the overall proportion of correct predictions, P_e is the proportion of correct predictions expected by chance, and n is the overall number of predictions.

Using a 2×2 Contingency Table:

		<u>PREDICTED</u>			
		Larva	Transformer		
<u>OBSERVED</u>	Larva	A	B	f_1	
	Transformer	C	D	f_2	
		n_1	n_2		

$$\kappa = \frac{2(AD - BC)}{n_1 f_2 + n_2 f_1}$$

Interpretation of the Kappa Statistic:

κ Value	Strength of Agreement beyond Chance
<0	Poor
0-0.20	Slight
0.21-0.40	Fair
0.41-0.60	Moderate
0.61-0.80	Substantial
0.81-1.00	Almost perfect

Taken from Landis & Koch 1977

Figure 2. Calculation and interpretation of the Kappa (κ) statistic based on the relationship between observations and model predictions.

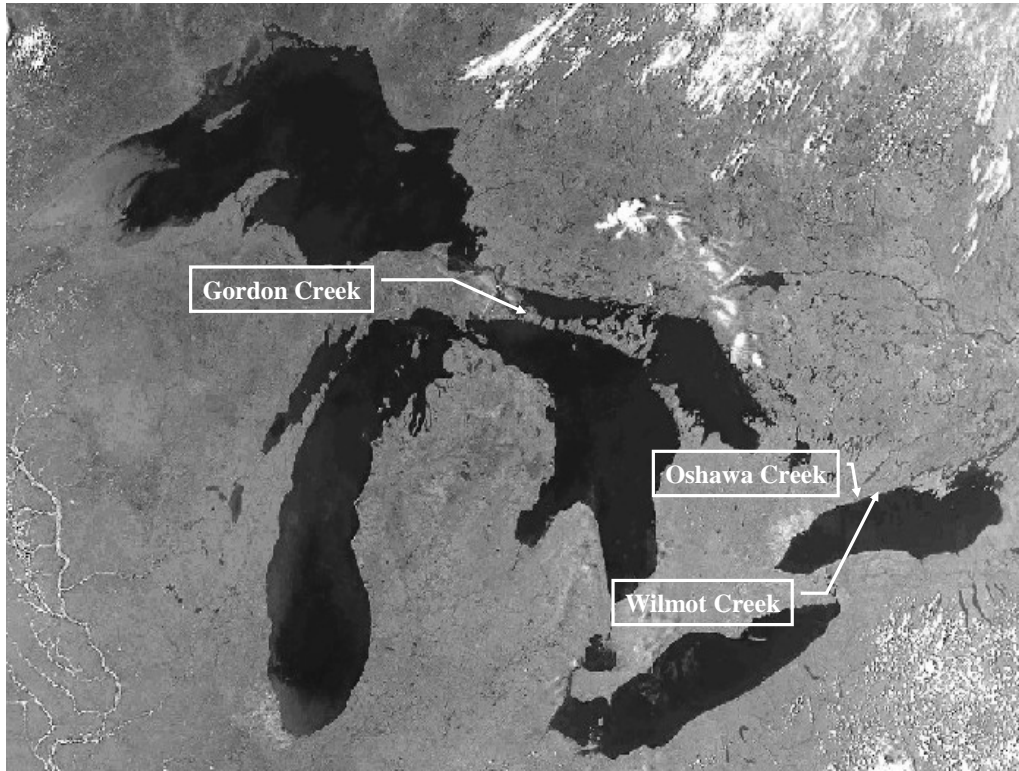


Figure 3. Location of the three study streams used in a 1995-96 mark recapture analysis (Hollett 1998) that were added to the dataset for the development of the management model.

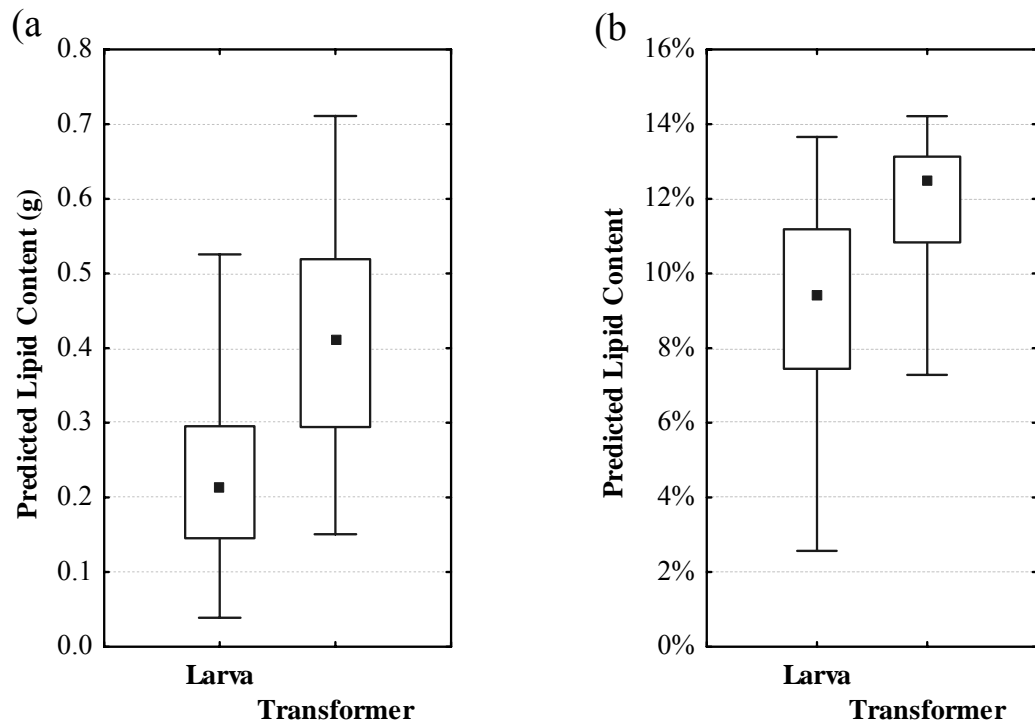


Figure 4. Ability of predicted lipid content ((a) lipid weight or (b) percent lipid (wet weight)) to predict the occurrence of metamorphosis. Points represent median values, whereas boxes and whiskers represent the 25 to 75 and 2.5 to 97.5 percentiles, respectively.

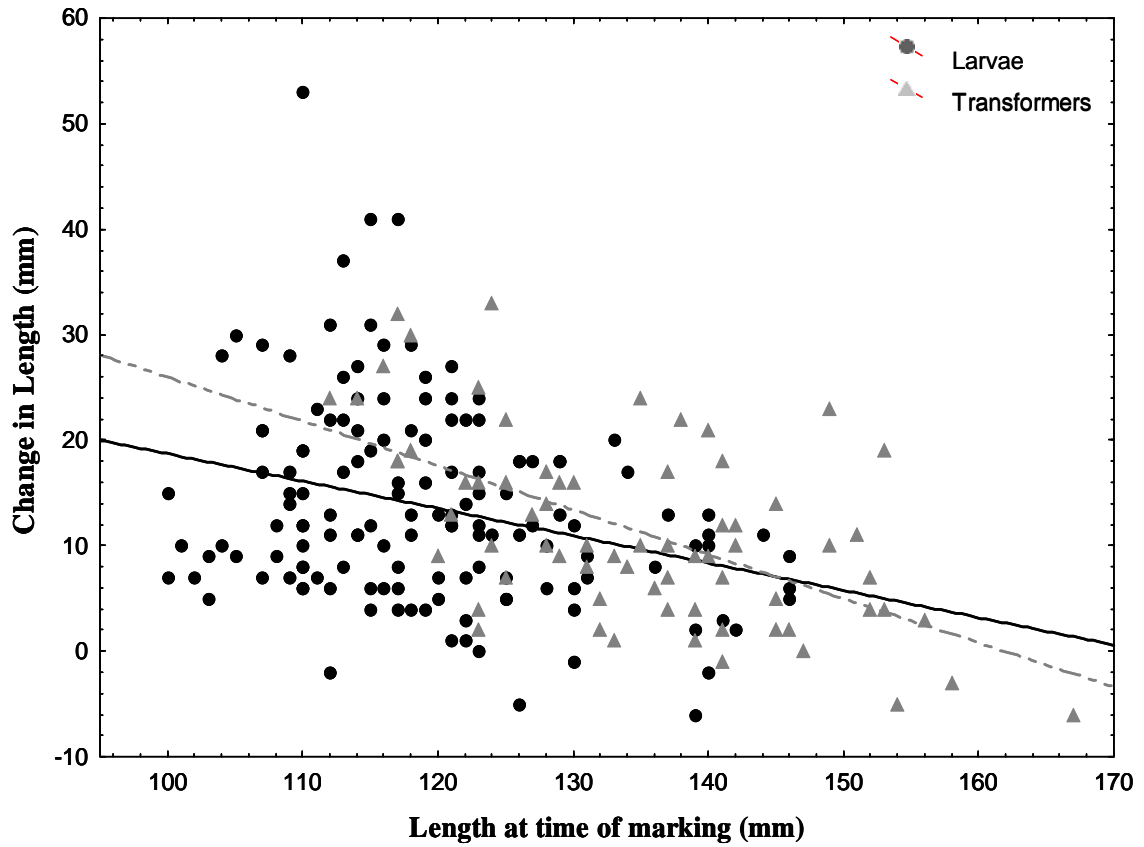


Figure 5. Declining linear growth in recaptured sea lamprey as a function of size for both metamorphosing (grey broken line) and non-metamorphosing (black solid line) larvae ($r^2=0.33$, $p<0.0001$ and $r^2=0.29$, $P<0.001$, respectively).

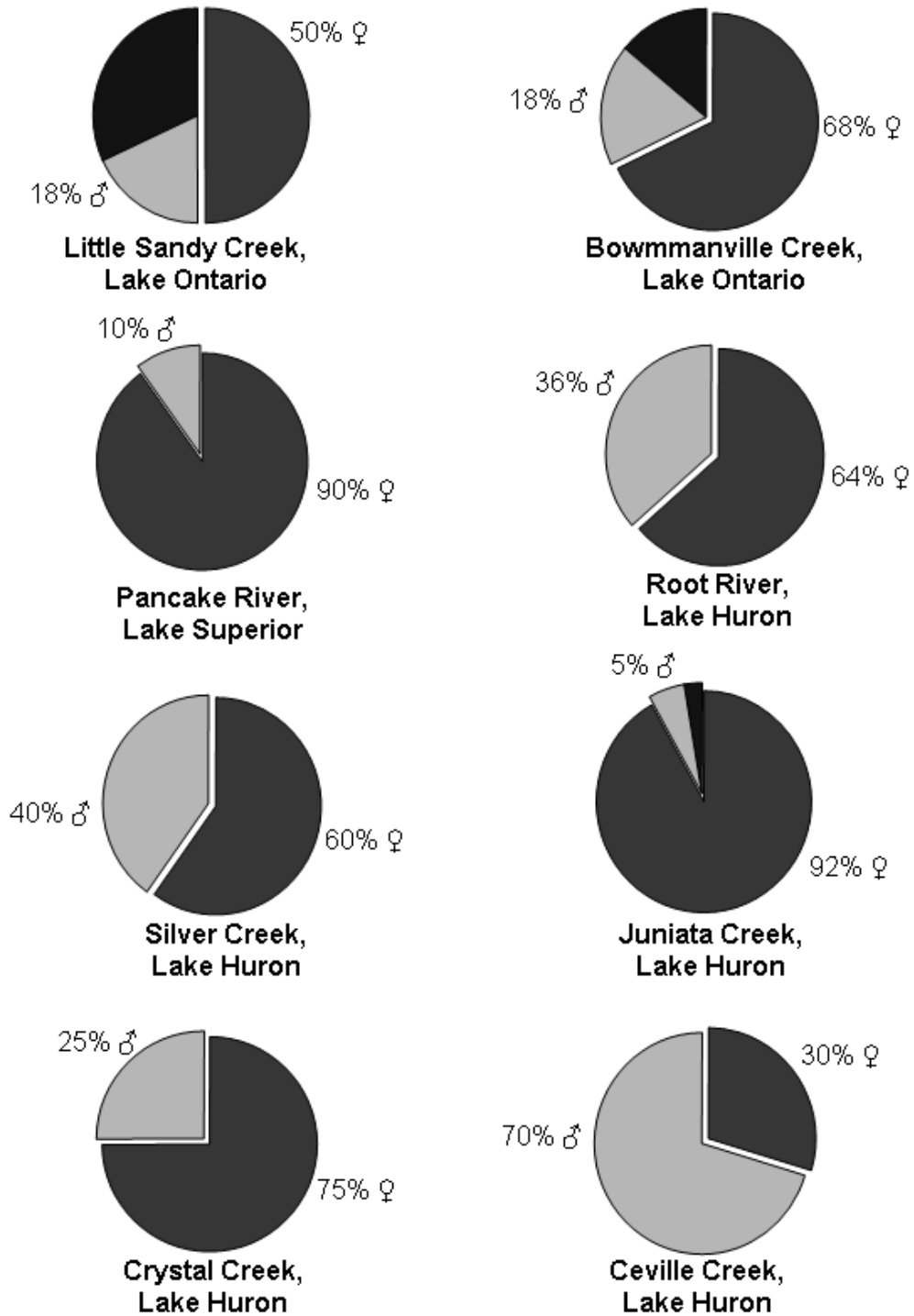


Figure 6. Sex ratios observed in recaptured larvae from each of the eight streams used in this analysis. Dark wedges indicate lampreys where sex was not able to be determined from microscopic examination of the gonads.

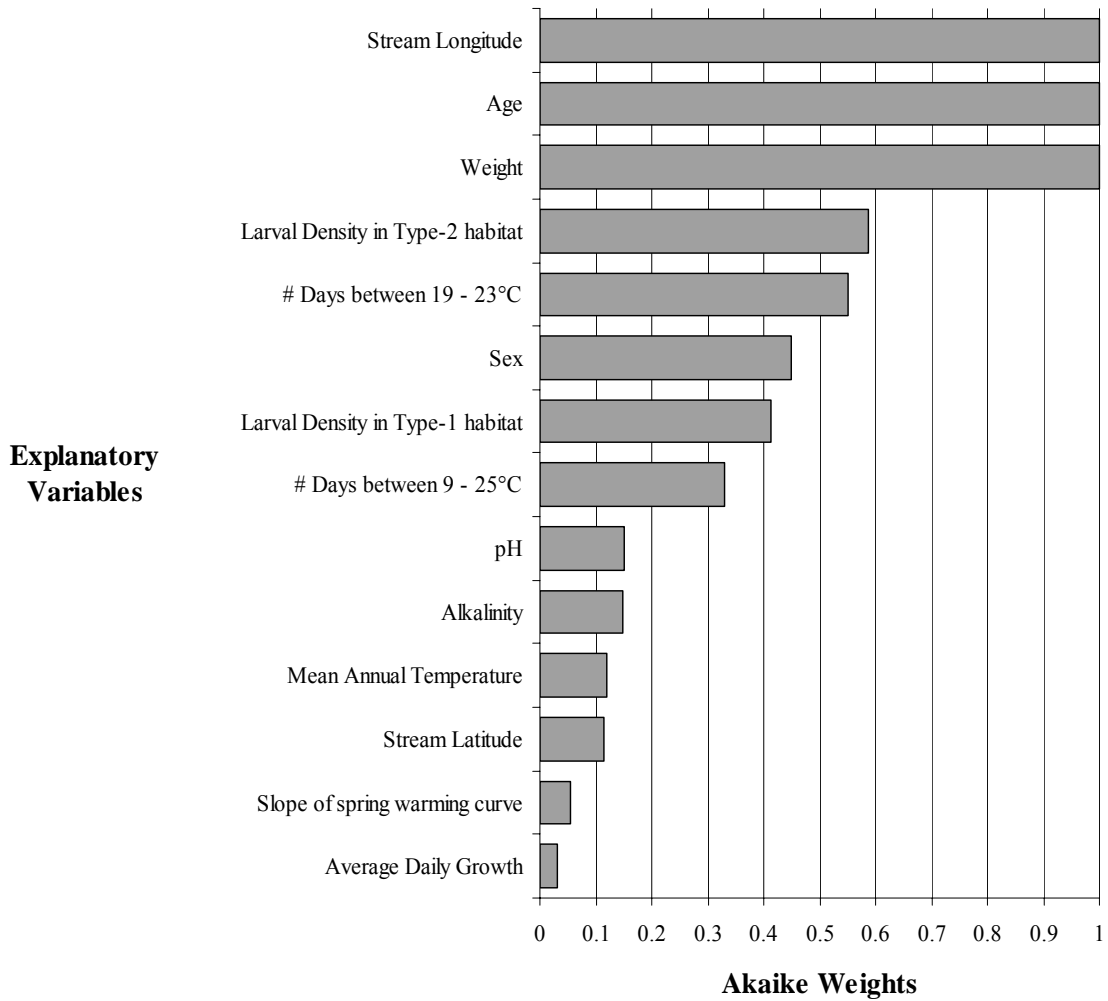


Figure 7. Relative importance, based on Akaike weights, of predictor variables from the top biological models for the prediction of metamorphosis. An akaike weight of one indicates the parameter was present in all of the top models.

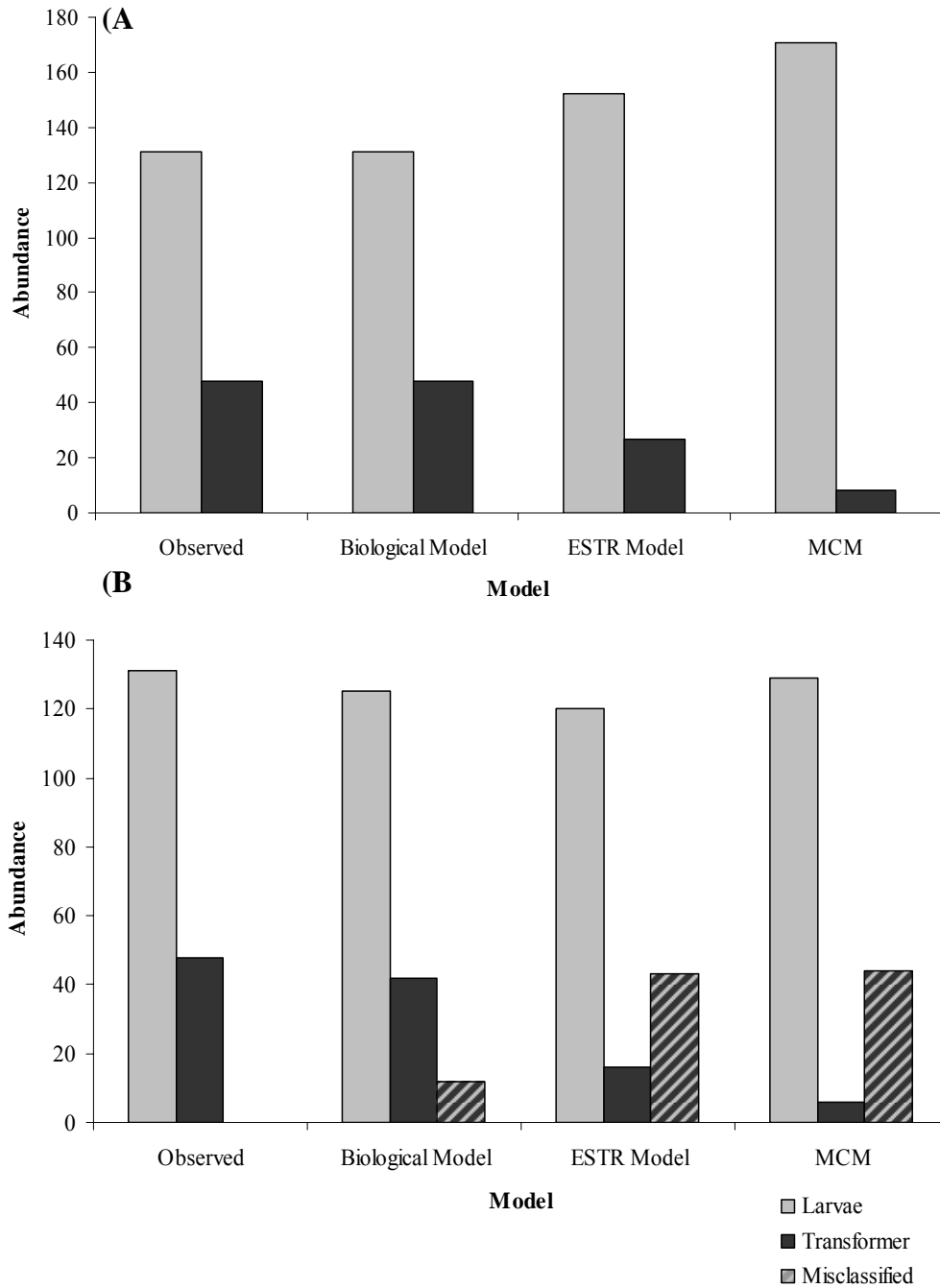


Figure 8. Observed versus predicted values from the biological model compared with those of the ESTR and MCM models. Graph (A shows raw predictions, whereas graph (B shows the number of correct predictions for each, along with the numbers of misclassified individuals

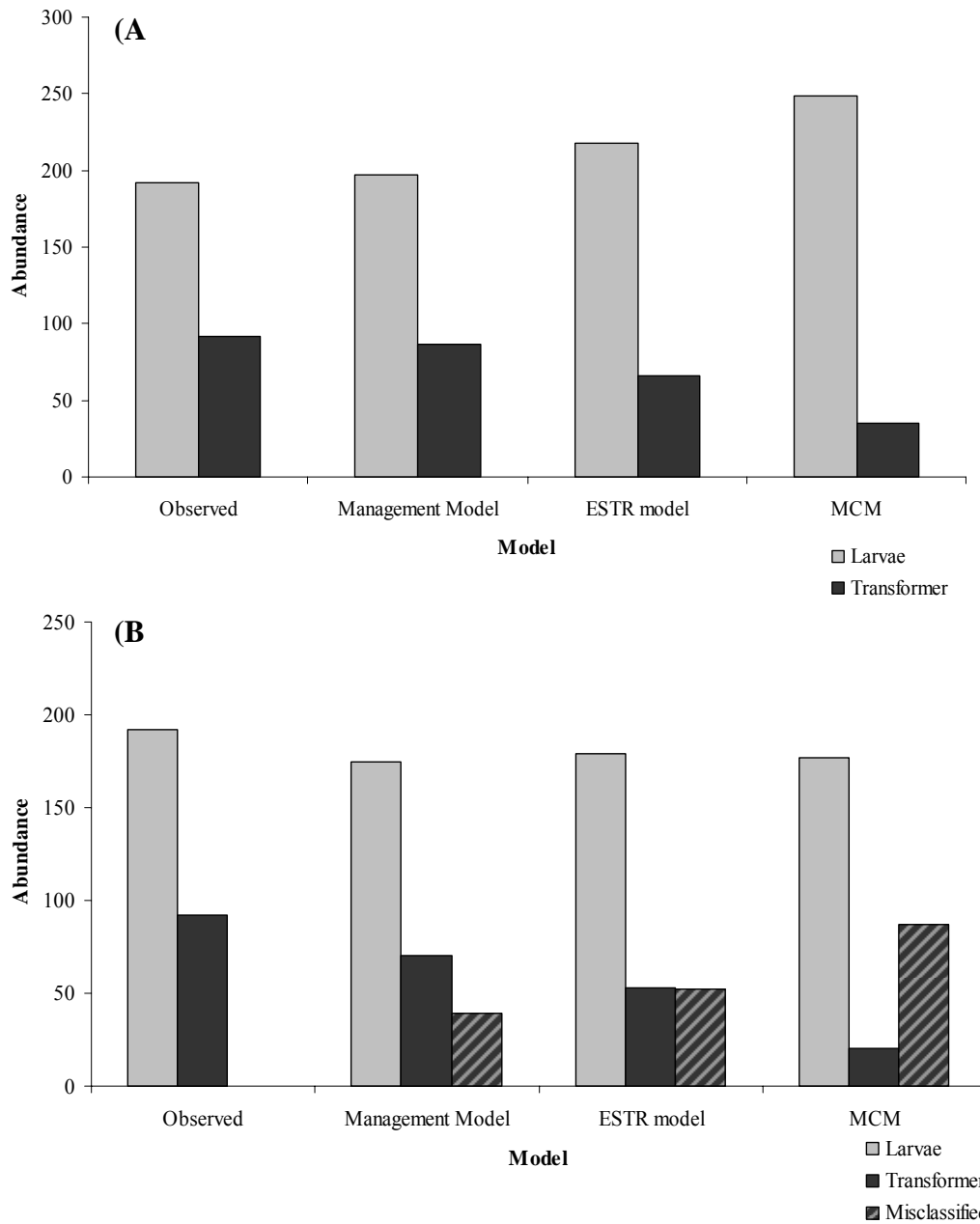


Figure 9. Observed versus predicted values from the management model compared with those of the ESTR and MCM models. Graph A represents raw predictions, whereas graph B shows the number of correct predictions for each, along with the numbers of misclassified in.

MANAGEMENT RECOMMENDATIONS

Based on the results of the simulation modeling (chapter 1), I recommend switching the stream selection criteria from the current method of using the ratio of estimated transformer abundance relative to the cost of stream treatment, to a more simple method based on the total abundance of large larvae (>100 mm). Ranking streams for treatment in this manner tends to treat the really large lamprey producing streams more frequently, resulting in lower overall escapement of parasitic juveniles to the lakes. A cost/kill method will often fail to treat large streams when smaller less expensive streams appear more cost-effective to treat, but this will result in an overall increase in larval lamprey abundance, especially when uncertainty in the assessments of large streams is high.

Results of computer simulations also suggest that given the accuracy of current metamorphic models, using estimates of larvae >100 mm in length, without the use of metamorphic models, will reduce uncertainty in the stream ranking process and may achieve better levels of control. The results of the metamorphic model development (Chapter 3) suggest that improvements in our ability to predict metamorphosis can be achieved by incorporating stream specific parameters that are linked to temperature regime, productivity, and population demographic factors. However, any new predictive model should be rigorously evaluated prior to its incorporation into the stream ranking process.

The management model developed (Chapter 3) should be evaluated using additional mark-recapture studies. An analysis similar to the one performed by Steeves (2002) should be conducted to determine the amount of error associated with using this

new metamorphic model. The error associated with this model could then be integrated into a stream selection simulation analysis to compare the results directly with those reported here (Chapter 1). Should the new metamorphic model lower the uncertainty surrounding transformer forecasts, it could be that a most kill algorithm using predictions of metamorphic lamprey would lead to better overall suppression of parasitic lampreys relative to the big larvae-most kill method. Given the emphasis recently placed on evaluating larval estimates through the use of mark-recaptures studies during lampricide treatments (Hansen *et al.* 2003), it seems plausible that additional information and refinement of the metamorphic model could be achieved by modifying a portion of these mark-recapture studies to include measurements of individual lamprey and coded wire tag implantation at the time of marking. Selecting streams where the discrepancy in transformer estimates between different metamorphic models are the greatest may lead to a rapid improvement in our ability to accurately predict metamorphosis.

The results of the metamorphic model development also suggest a means by which metamorphic prediction could be improved. As research continues into the factors that cause differences in recruitment and lamprey production from stream to stream (H. Dawson & G. Anderson, unpublished data), environmental and demographic processes that explain variation in these characteristics may also be applicable to metamorphic models as well. It is conceivable that streams exhibiting differences in growth and recruitment rates may also show similar patterns in metamorphosis. If this is the case, perhaps predictive models of metamorphosis could also be developed for each stream category.

The prevalence of temperature parameters in the biological model (chapter 3) suggests that research into how much inter-annual variation exists in stream temperature indices in individual streams would be warranted. If temperature indices that are fairly consistent over time can be developed and evaluated, their addition to a management-oriented model would likely yield improvements in its predictive capability, based on the superior predictive capability observed in the biological model.

The frequency of density estimates in the top biological and management models (Chapter 2, Tables 4 & 5) suggest that the data obtained by QAS surveys could be an important component in explaining variation in metamorphic rates. Research is currently underway (G. Anderson, unpublished data) that explores the potential replacement of QAS with a more rapid index of abundance. While this method may free up resources for additional lampricide treatment, it is important to acknowledge that the loss of density information may affect more than just the stream ranking process.

Finally, despite the failure of predictions of lipid content to play a role in either of the predictive models developed in Chapter 3, the ability of the non-invasive lipid model developed in Chapter 2 to separate metamorphic from non-metamorphic larvae (Figure 4) suggests that further research into this area would be worthwhile. Bioelectric impedance analysis and handheld microwave energy meters represent two possible techniques to get accurate measurements of lipid content. If either of these methods could provide a rapid method to estimate lipid content in larval lampreys collected during assessment surveys, this could lead to great improvements in our ability to predict metamorphosis.

APPENDIX A
COMPARISON OF A 5-SCAN VERSUS 3-SCAN TOBEC METHOD.

The owner's manual for the EM-Scan unit (EM-SCAN Inc., 1993) recommended that 5 scans be performed per individual. I found that the time required to perform 5 scans limited the number of larval lampreys that could be scanned and marked within a reasonable amount of time, and thus limited the number of animals released in the mark-recapture portion of that study. I compared the ability of a 3 scan procedure to capture the average E value relative to that of a 5 scan average. My findings for larval sea lampreys (see figure on next page) are consistent with those of Bai *et al* (1994) in determining that 3 scans provided an accurate average TOBEC value and that performing 5 scans did not significantly increase the predictive capability (coefficient of regression for 3 scan average regressed against 5 scan average was 0.9858, n=665). As a result, all methods and analysis in this study are based on a 3 scan average of the EM-Scan device. By reducing the required number of scans from 5 to 3, more lampreys were able to be scanned and injected with coded wire tags (refer to Chapter 3), thereby increasing the number of marked animals in the river and potentially improving the recapture rate the following year.

APPENDIX A (Continued)

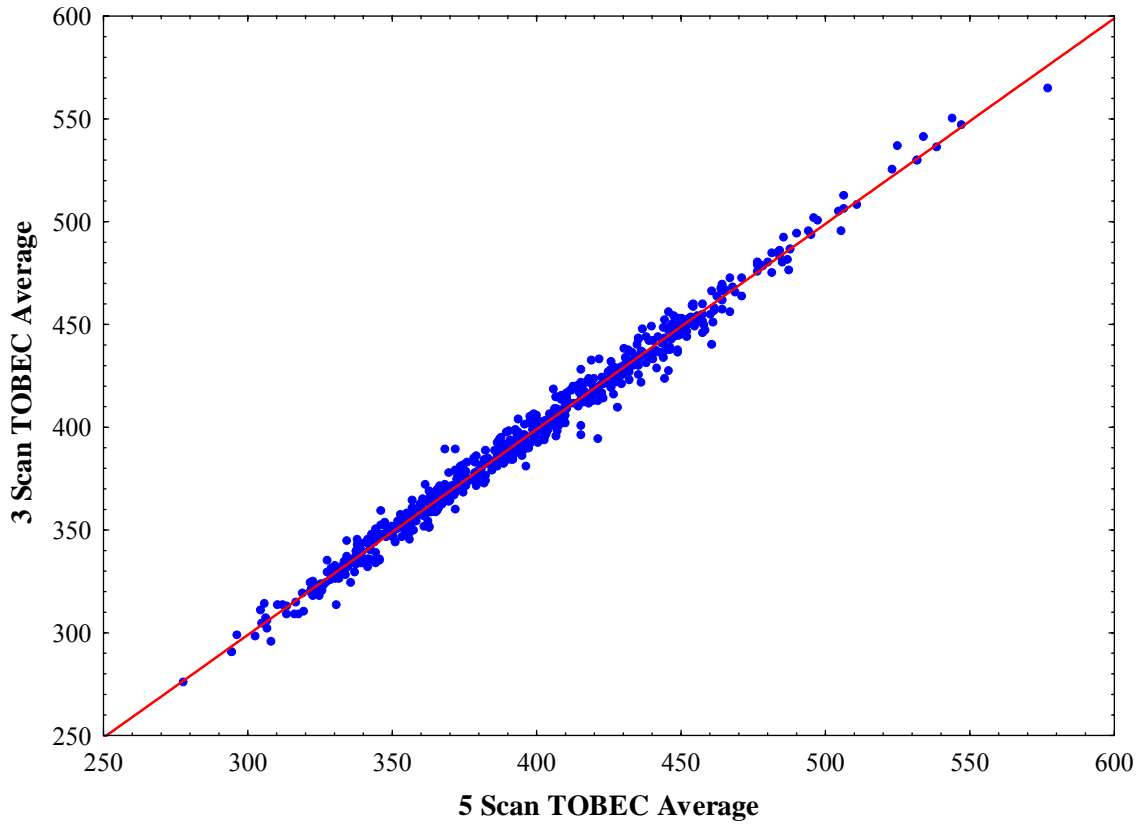


Figure 1. Plot showing the correlation between the E-values obtained using a 5-scan TOBEC method with those obtained using a 3-scan method. E-values from the 3-scan method were highly correlated ($r^2=0.986$) with those from the 5-scan method. The solid line represents the 1:1 line.

APPENDIX B

COMPARISON OF BACK-CALCULATED METAMORPHOSIS CURVES WITH THOSE GENERATED USING MARK-RECAPTURE DATA

As discussed in Chapter 3, the current ESTR model used to select streams for lampricide treatment currently relies on two length-based predictive models of metamorphosis; one for upper lakes tributaries and one for lower lakes tributaries. These two curves rely on length as the sole predictor of metamorphosis and required assumptions regarding larval growth to allow for the back calculation of population length frequency distributions. The following two graphs compare the two ESTR curves with two different length-based models which were derived using measured growth and transformation rates from the mark-recapture study conducted in Chapter 3. While the results of Chapter 3 indicate that better predictive models can be developed by including other individual and stream level variables, the following graphs are presented to illustrate the effect that the assumptions regarding larval growth used to develop the current ESTR models have on our ability to predict metamorphosis and effectively rank streams for lampricide treatment.

Appendix B (Continued)

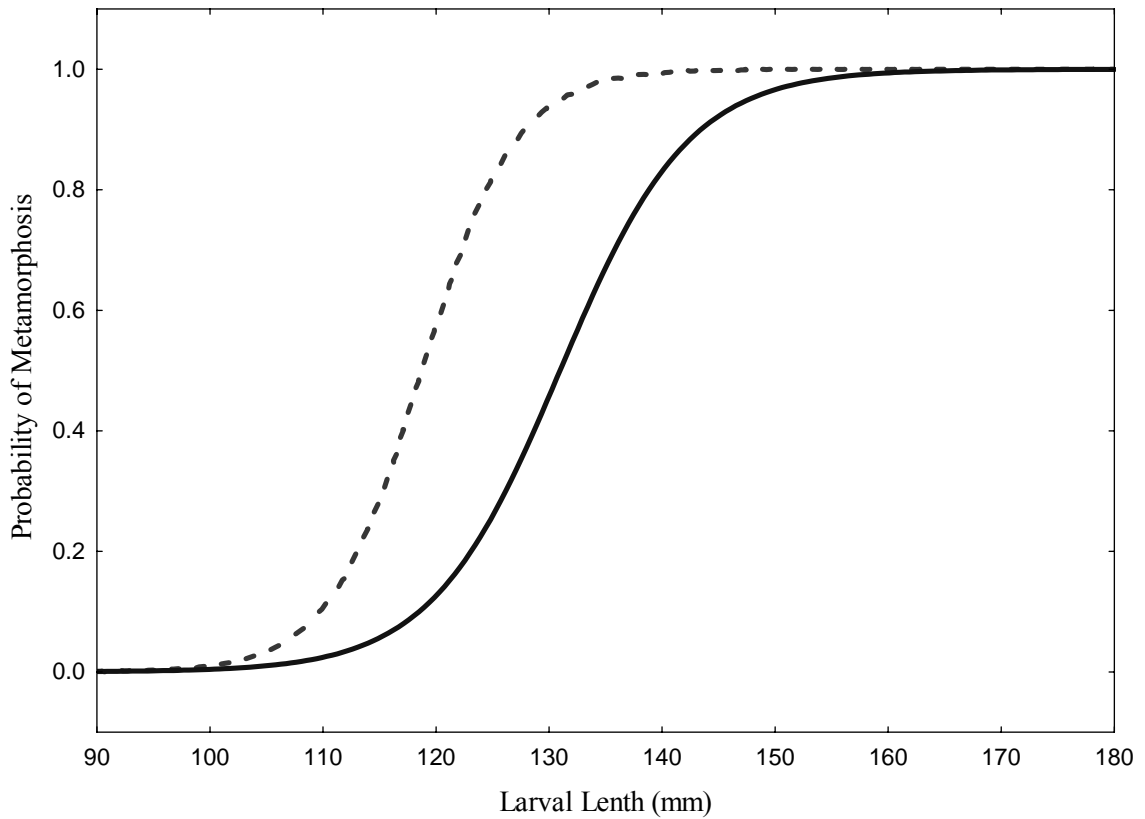


Figure 1. A comparison of the ESTR lower lakes probability of metamorphosis curve (solid line), with the curve generated using mark-recapture data (dotted line) and thus avoiding the use of assumptions required for the back-calculation of collection lengths. The current lower lakes ESTR curve greatly underestimates the metamorphic rates for lamprey from streams in this study.

Appendix B (Continued)

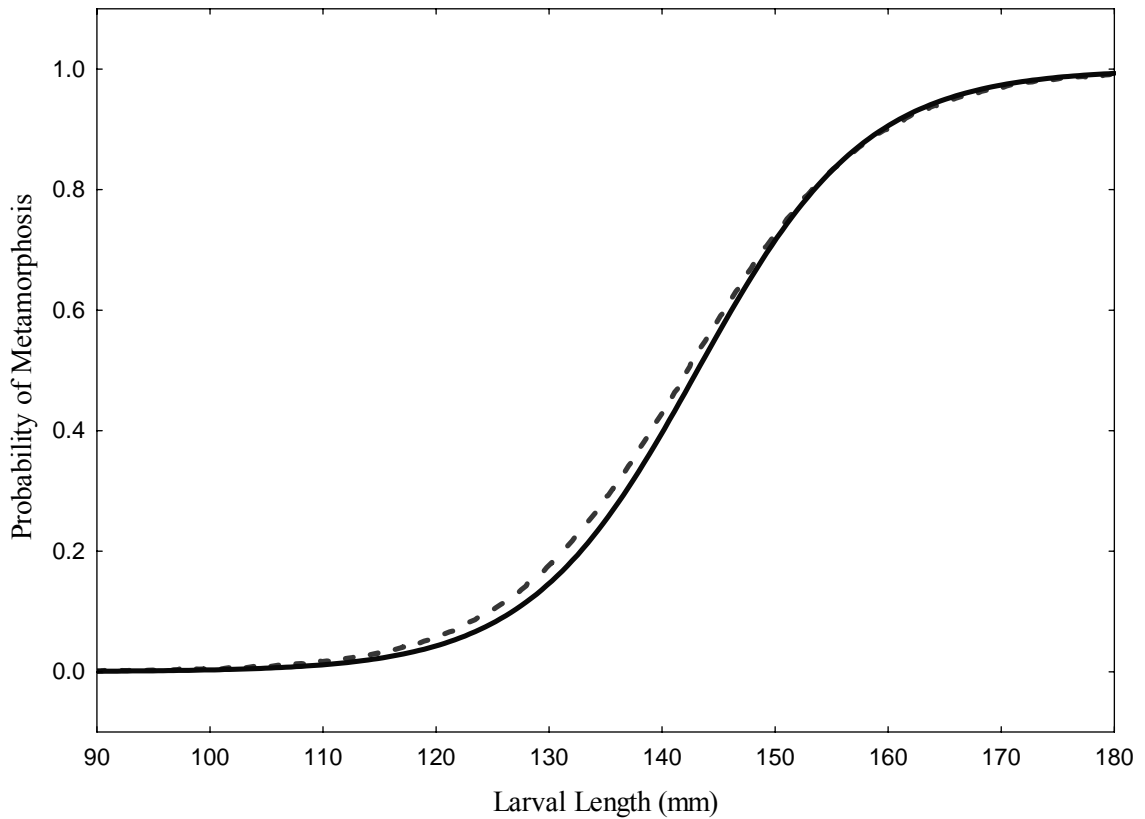


Figure 2. A comparison of the ESTR upper lakes probability of metamorphosis curve (solid line), with the curve generated using mark-recapture data (dotted line) and thus avoiding assumptions required for the back-calculation of collection lengths. It appears that for upper lakes streams, the ESTR curve closely approximates the length to metamorphosis relationship observed in mark-recapture collections. Length is the sole independent variable in these models.

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