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## Metabolic Scaling Physiology and the Uptake, Elimination and Toxicity of TFM (3-trifluoromethyl-4-nitrophenol) to Invasive Sea Lampreys (*Petromyzon marinus*)

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Metabolic Scaling Physiology and the  
Uptake, Elimination and Toxicity of TFM  
(3-trifluoromethyl-4-nitrophenol) to  
Invasive Sea Lampreys (*Petromyzon  
marinus*)

by

Laura Ruth Tessier

(Bachelor of Science, Honours Program, Biology, Trent University, 2013)

A thesis

Submitted to the Department of Biology

Faculty of Science

in partial fulfillment of the

thesis requirement for the degree of

Master of Science in Integrative Biology

Wilfrid Laurier University

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## **AUTHOR'S DECLARATION**

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

## Abstract

Sea lampreys (*Petromyzon marinus*) have a complex life cycle that involves a parasitic phase in which they feed on the blood and bodily fluids of large piscivorous fishes. Following the invasion of the Great Lakes by sea lampreys in the early 20<sup>th</sup> century, sea lamprey parasitism contributed to major declines in the populations of commercial and sports fisheries in that basin. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM), developed in the late 1950s, is now routinely applied to tributaries of the Great Lakes to control sea lamprey populations. This lampricide is selectively toxic to larval sea lampreys, which typically reside as burrow-dwelling filter-feeders in streams. Although the TFM concentrations commonly applied to streams and rivers is 1.2 - 1.5 times the 9 h LC<sub>99,9</sub> (minimum lethal concentration; MLC) of the larvae, surviving residual sea lamprey may be observed after treatments. The underlying causes for “residuals” are poorly understood, however. The main goal of my M.Sc. thesis was to determine how body size and life stage influenced TFM uptake, excretion and survival of larval sea lampreys following TFM exposure. Because rates of oxygen consumption ( $\dot{M}O_2$ ) are inversely proportional to body size in animals, I predicted that correspondingly lower rates of ventilation would result in lower rates of TFM uptake and greater TFM tolerance in larger compared to smaller larval sea lampreys. As predicted, smaller lampreys had exponentially higher rates of  $\dot{M}O_2$  and TFM uptake compared to larger animals. I also predicted that due to higher metabolic demands, more active post-metamorphic sea lampreys would have higher TFM uptake rates compared to larval sea lampreys. Surprisingly, both  $\dot{M}O_2$  and TFM uptake rates were similar in size matched larval and post-metamorphic sea lampreys. Body mass was also correlated with TFM elimination,

following intraperitoneal injection of TFM, but there were no differences in the TFM clearance rates between larval and post-metamorphic sea lampreys. Finally, toxicity tests indicated that larval sea lamprey with greater mass and condition factor (CF) were able to survive exposure to TFM longer than smaller sea lampreys. Collectively, these results suggest that large larval sea lampreys may be more tolerant to TFM, suggesting that streams containing high densities of large larvae may be at risk for increased residual sea lamprey following TFM treatment. Thus, it would be advisable to take precautions to reduce the chances of residuals when TFM is applied to streams with high densities of large larvae.

## **Acknowledgements**

I would like to acknowledge and thank my supervisor, Dr. Michael Wilkie, for his continual patience, advice and supervision during this thesis. I appreciate my committee members Dr. Allison McDonald and Dr. Jonathan Wilson for their support and guidance. I am thankful for the assistance in collecting animals from the US Fish & Wildlife Services, as well as to the staff at Hammond Bay Biological Station, Karen Slaght, Dr. Nick Johnson, and Dr. Michael Hansen for the use of their facilities to hold animals and perform experiments. I would like to thank the Great Lakes Fisheries Commission for financial support. I am grateful for all my lab members and their support and assistance in conducting experiments. Finally, I would like to thank friends and family for their support and patience during the completion of this thesis.

## **Dedication**

I dedicate this thesis to my Grandfather, Roger Tessier, who taught me to fish when my brothers weren't interested.

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#### *(Petromyzon marinus)*

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# Chapter 1

## Sea Lamprey Biology and the Invasion of the Great Lakes

### 1.1 The Life Cycle and Physiology of the Sea Lamprey

Anadromous sea lampreys (*Petromyzon marinus*) begin their life-cycle burrowed in the soft sediments of freshwater streams and rivers as filter-feeding larvae called ammocoetes for 3-7 years (Lowe et al. 1973; Youson and Potter 1979; Henson et al. 2003; Figure 1-1). They feed by filtering detritus and bacteria using their oral hoods to direct water into the oral cavity and across the gills, trapping food on mucus secreted by an endostyle (Potter 1980). After the animals have grown and acquired sufficient lipid stores, they then enter the non-trophic period of metamorphosis. In Great Lake's sea lampreys, metamorphosis usually occurs after the larvae have reached approximately 120 mm in length and attained a mass of 3 g, yielding a condition factor of 1.5 or greater (Youson et al. 1993; Holmes et al. 1994). The onset of metamorphosis coincides with a rise, then a fall, in thyroid hormone (TH) concentration (Gross and Manzon 2011). There are 7 distinct developmental stages that occur during metamorphosis, which culminates in a juvenile, free-swimming parasitic sea lamprey that feeds on the blood of teleost fishes (Youson and Potter 1979; Figure 1-2).

Stage 1 metamorphosis is characterized by a slight darkening and enlarging of the eye spots that are present throughout the larval stage (Youson and Potter 1979). By stage 2, the feeding and breathing apparatus also changes as the sea lamprey switches from a flow-through (one way) mode of ventilation to a tidally ventilated gill. Stages 3 – 7 of metamorphosis result in the transformation of the oral hood into a cartilage-ringed oral disc, with numerous teeth and a rasping tongue, which allow the parasitic lamprey to latch onto

fishes, and penetrate the body cavity wall to extract the blood. Metamorphosis is also accompanied by the presence of a more prominent dorsal fin develops in preparation for a more active free-swimming parasitic stage, and the internal organs (liver, gut and kidney) are re-structured as the lamprey become hematophagous (Tetlock et al. 2012; Youson and Potter 1979; Figure 1-2). After metamorphosis, the juvenile parasitic sea lamprey migrates from its natal stream to a larger body of water where it feeds on the blood of fishes for one to two years, and then returns to a suitable stream to spawn and then die (Potter 1980). Sea lampreys are panmictic (Swink and Johnson 2014), relying on chemical signals (pheromones) secreted by larval sea lampreys and migrating males to locate spawning habitat (Li et al. 2007; Meckley et al. 2014). The search for proper spawning habitat results in random mating of the sea lampreys, as observed by the heterozygous population (Bryan et al. 2005). Migration of the parasitic sea lampreys in the Great Lakes occurs either in the spring or late fall, with no survival or growth advantage between groups of migrants (Swink and Johnson 2014).

Larval sea lampreys generally have a low metabolic rate, defined by relatively low rates of oxygen consumption ( $\dot{M}O_2$ ) and nitrogenous waste excretion rates (Lewis 1980; Wilkie et al. 1999, 2001). Following metamorphosis, however,  $\dot{M}O_2$  and nitrogenous waste excretion increase to levels more reflective of an active teleost fish (Lewis 1980; Wilkie et al. 2006). However, larval sea lampreys are capable of significantly increasing their rates of  $\dot{M}O_2$  following sporadic bursts of activity (Wilkie et al. 2001). Moreover,  $\dot{M}O_2$  tends to scale allometrically in larval sea lamprey, with weight specific rates of  $\dot{M}O_2$  declining with body size (Wilkie et al. 2001).

## 1.2 The Great Lakes Invasion by Sea Lamprey

Sea lampreys are native to the Atlantic Ocean, but were present in Lake Ontario in the 1800's, possibly entering from the Hudson River via the Erie Canal (Lawrie 1980). However, they were prevented from entering Lake Erie due to the presence of Niagara Falls (Lawrie 1980; Eshenroder 2014). Following modifications to the Welland Canal in the early 1900s, they subsequently by-passed the falls and gained access to Lake Erie and the upper Great Lakes (Hartman 1973; Lawrie 1980; Figure 1-3). The first sea lampreys were observed in Lake Erie in the 1920's, before migrating to the upper Great Lakes where they were discovered in Lake Huron in 1937 (Applegate 1950). The sea lampreys then quickly spread to the other Great Lakes where they became established and founded the current population that exists today (Smith and Tibbles 1980; Sullivan et al. 2003).

The sea lamprey invasion of the Great Lakes caused massive declines in valuable sport and commercial fishes (Smith and Tibbles 1980). By the early 1950's, sea lamprey parasitism, combined with overfishing, contributed to the collapse or near-collapse of a number of Great Lakes fisheries, including the lake trout (*Salinus namaycush*) fishery (Stein and Goddard 2008). Injuries to fish from the parasitic sea lamprey can cause infections and the loss of blood with a juvenile parasitic sea lamprey able to consume up to 30% of its body weight in blood per day (Farmer et al. 1975), leading to mortality rates of 60% for the host fish (Swink 2003; Patrick et al. 2009). By the 1940's, populations of native lake trout and walleye (*Sander vitreus*) were extirpated from the Lake Huron basin (Eshenroder 1992). Indeed, Swink (2003) has estimated that a single parasitic juvenile sea lamprey can kill up to 18 kg of lake trout.



Because sea lamprey targeted top-predators in the Great Lakes, it also led to a massive explosion of alewife (*Alosa pomolobus*) and rainbow smelt (*Osmerus mordax*) populations, and regular die-offs of these fishes. These die-off events fouled shorelines and beaches and contributed to declines in water quality (GLFC 2011). In response to the crisis, the Canadian and American governments launched an integrated sea lamprey control program, overseen by the Great Lakes Fisheries Commission (GLFC) (GLFC 2011). One method of SLC that arose from these efforts was the use of lampricides to suppress sea lamprey populations, with the goal of restoring fish populations in the Great Lakes.

As a result of lampricide treatments, sea lamprey populations declined and efforts began in the 1970s to restock the keystone predators (lake trout) into the Great Lakes (Eshenroder 1995). This, combined with water quality improvements including decreases of phosphorus, helped improve conditions for lake trout populations to recover in the Great Lakes (Eshenroder 1995). Today, lake trout stocking programs (1981 – 2012), combined with reductions in sea lamprey populations, have resulted in the restoration of lake trout populations (Johnson et al. 2015).

### **1.3 History of Sea Lamprey Control in the Great Lakes**

The GLFC was formed by international treaty between Canada and the United States in 1955 (GLFC 2011). The goals of the GLFC are to develop measures to reduce sea lamprey populations through the use of barriers, traps and chemical control. The GLFC is also responsible for maintaining the Great Lakes fisheries for both commercial and recreational use. The first attempts to control sea lamprey populations were physical barriers set-up in the 1950's (Lawrie 1970). These barriers, while successful in preventing the sea lampreys from exploiting new territory and spreading, also caused harm to other migratory fishes, such as sturgeon (*Acipenseridae*) (Pess et al. 2014).

In addition to barriers, scientists tested over 6,000 chemicals to find one that would be selectively toxic to sea lamprey. In the 1960's, Applegate and colleagues identified 3-trifluoromethyl-4-nitrophenol (TFM) as the most effective and selective potential lampricide (Applegate et al. 1961). The specificity of TFM to sea lampreys is due to the relative inability of sea lampreys to detoxify TFM compared to non-target fishes (Boogaard et al. 2003; Hubert 2003; McDonald and Kolar 2007). Fish detoxify TFM in the liver through the conjugation of TFM to TFM-glucuronide (Figure 1-4; Lech and Statham 1975; Kane et al. 1993). The bio-transformed TFM glucuronide is then excreted via the digestive tract or in the urine (Lech and Statham 1975; Hunn and Allan 1974; Shultz et al. 1979).

TFM applications began in the 1950s and are still currently used in larval sea lamprey-infested streams at regular intervals as part of the integrated SLC program. Streams are selected for lampricide treatments based on prior surveys done by sea lamprey assessment crews, who identify the most densely populated waters (Irwin et al. 2012). Each

year, approximately 50 000 kg of TFM is used (Ellis and Mabury 2000), costing approximately \$8 million dollars annually (Fisheries and Oceans Canada 2013). Before treating a stream, the 9 h minimum lethal concentration (MLC) of TFM (MLC, where 99.9% mortality is observed to larval sea lamprey) is determined based on water pH and alkalinity (Bills et al. 2003; McDonald and Kolar 2007). Because TFM is a weak acid with a pKa of 6.07, higher proportions of its more lipophilic, un-ionized form are present at lower pH (Hubert 2003; Figure 1-5). Streams are typically treated with 1.2 – 1.5 times the MCL for a 9 h exposure period (McDonald and Kolar 2007).

The toxic mode of action of TFM appears to be the same in sea lamprey and non-target fishes such as the rainbow trout (*Oncorhynchus mykiss*), taking place through the inhibition of oxidative phosphorylation in the mitochondria (Birceanu et al. 2011; Figure 1-6), which forces the animal to rely on the anaerobic production of ATP (Birceanu et al. 2009, 2014). ATP is the main energy currency in the body, and mainly produced in the mitochondria via oxidative phosphorylation. Protons ( $H^+$ ) are pumped through Complexes I, III and IV, through the conversion of NADH to  $NAD^+$  and the conversion of  $O_2$  to  $H_2O$ , creating a proton gradient across the inner mitochondrial membrane called the proton motive force (Figure 1-6). At Complex V, the ATP synthase, the  $H^+$  flows down its electrochemical gradient, releasing the energy required to phosphorylate ADP to ATP (Figure 1-6). TFM likely interferes with this process by acting as a protonophore, targeting the inner membrane of the mitochondria, where it causes a dissipation of the proton motive force between the intermembrane space of the mitochondria and the mitochondrial matrix, resulting in less ATP

production. In the meantime, the electron transport chain continues to futilely pump protons across the membrane, consuming high amounts of O<sub>2</sub> (Birceanu et al. 2011; Figure 1-6).

Due to the mismatch between ATP supply and demand that results from TFM exposure, glycogen reserves become depleted and lactate concentrations increase in tissues including the brain and liver due to increased reliance on anaerobic glycolysis (Birceanu et al. 2009; Clifford et al. 2012). However, these organs cannot sustain anaerobic glycolysis for a prolonged period of time because it results in metabolic acidosis (decreased tissue pH) and a rapid depletion of glycogen reserves. These glycogen reserves are needed to anaerobically generate ATP to meet the demands of the central nervous system (brain) and other systems; depletion leads to hypoglycemia and subsequently mortality (Birceanu et al. 2009).

Despite the high toxicity of TFM, not all sea lampreys die following TFM exposure, undermining sea lamprey control efforts (McDonald and Kolar 2007). However, the abiotic and biotic factors that allow sea lampreys to survive TFM treatments remain unresolved. This study will focus on physiological characteristics of sea lampreys that could enable some sea lampreys to survive lampricide treatments.

Despite the significant decreases in energy stores that occur during TFM exposures, some sea lampreys are able to almost fully recover from short-term (4 – 6 h) exposures to the LC<sub>99.9</sub> of TFM in as little as 2 - 4 h, with brain ATP, PCr, lactate and glycogen levels all returning to control levels by 12 h (Clifford et al. 2012). Because TFM treatments typically last up to 12 h, to ensure that larval sea lamprey are exposed to at least a 9 h block of TFM at 1.2 - 1.5 times the MLC (McDonald and Kolar 2007), a sea lamprey that can partially escape

the full treatment cycle of lampricide, could potentially recover from its effects (Clifford et al. 2012). Aside from the length of time a sea lamprey is exposed to TFM, other factors that may influence the probability of survival include body size and energy stores, life cycle stage, and individual metabolic rates.

#### **1.4 Metabolic Scaling Related to Toxicity**

Metabolic rates are known to scale to body size to the power of  $2/3 - 3/4$  (Glazier 2005), and may also be influenced by life stage (Lewis 1980). Metabolic rates can be measured to determine the amount of stress an organism is experiencing, and can give insight as to its overall health and energetics. Organisms that are stressed will raise their metabolic rates, while organisms that are at rest will have a lower baseline rate to maintain metabolic demands. For example, when an organism is exposed to a toxin it may have to increase its metabolic rate in order to be able to detoxify and eliminate the substance (Klinger et al. 2015). Metabolic rates can be measured directly using calorimetry, which is the amount of heat produced by an organism over time, but this method is not feasible for ectothermic organisms such as fish. A more practical method is to measure oxygen consumption rates ( $\dot{M}O_2$ ), which is directly proportional to metabolic rate (Goolish 1991).

Reductions in mass specific metabolic rate (as measured by  $\dot{M}O_2$ ) are generally associated with increases in body size, which would result in lower rates of oxygen consumption and reduced ventilation of the gills in sea lamprey (Lewis 1980). The overarching goal of my thesis was to relate changes in  $\dot{M}O_2$  arising from differences in body

size and life stage to differences in TFM uptake, clearance and tolerance to TFM. Based on this principle I predicted that larger lampreys would have lower mass specific rates of TFM uptake, and a greater tolerance to TFM than smaller animals. In addition, the metabolic rates of sea lampreys have also been reported to increase after sea lampreys transform from a sedentary filter-feeding larvae to free-swimming juvenile animals (Lewis 1980). With a greater  $\dot{M}O_2$ , I therefore predicted that juvenile sea lampreys would also have higher rates of TFM uptake and clearance.

### **1.5 Thesis Objectives**

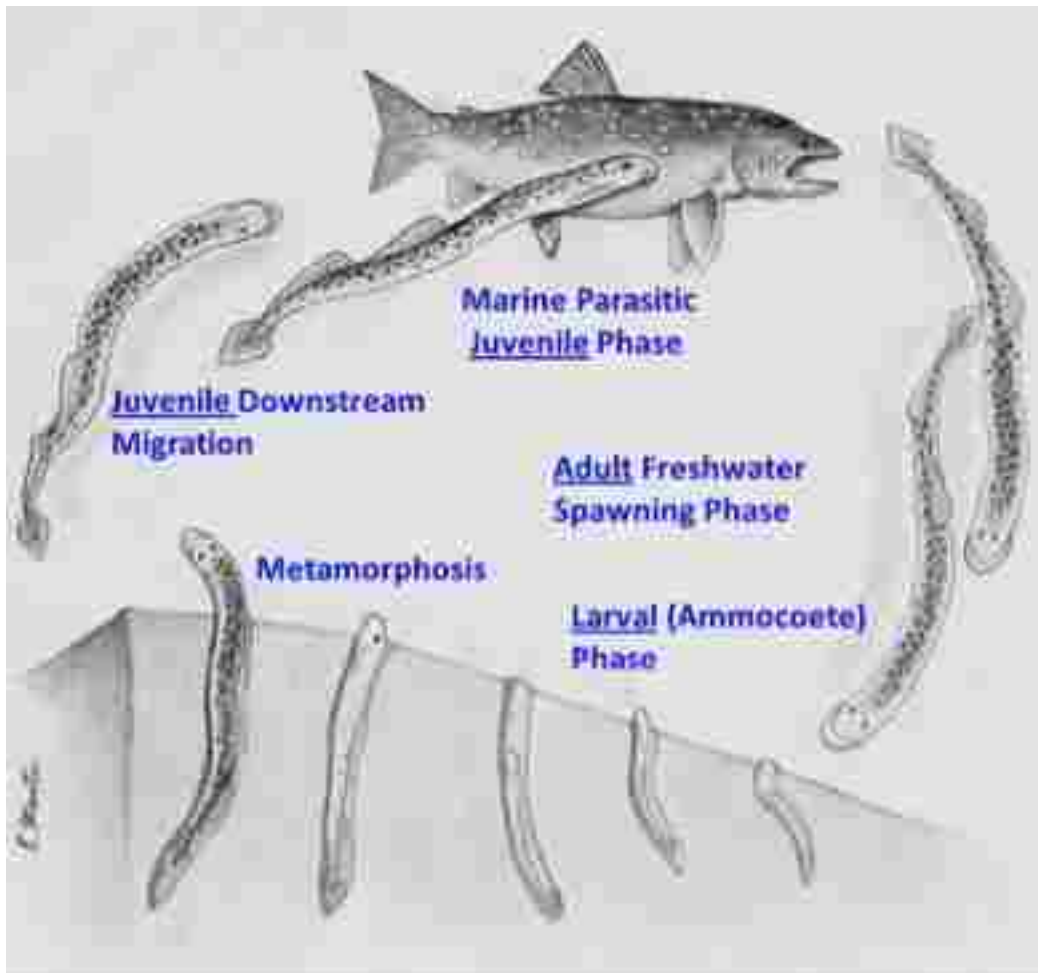
To characterize how metabolic rate is related to TFM sensitivity in the sea lampreys the objectives of my thesis were to:

- 1) Determine how differences in body size affected  $\dot{M}O_2$ , and TFM uptake and excretion by larval sea lampreys;
- 2) Ascertain if differences in the rates of TFM uptake with body size were reflected by differences in the tolerance of larval sea lampreys to TFM;
- 3) Elucidate how metamorphosis influences  $\dot{M}O_2$ , TFM uptake and excretion in larval sea lampreys compared to post-metamorphic sea lampreys.

To address these objectives, static respirometry was used to quantify rates of  $\dot{M}O_2$  in larval sea lamprey of various body sizes (50 – 130mm in length), as well as in size-matched juvenile parasitic and larval sea lampreys. Radio-labeled TFM ( $^{14}C$ -TFM) was then used to track rates of TFM uptake in the same animals, so that direct comparisons could be made to

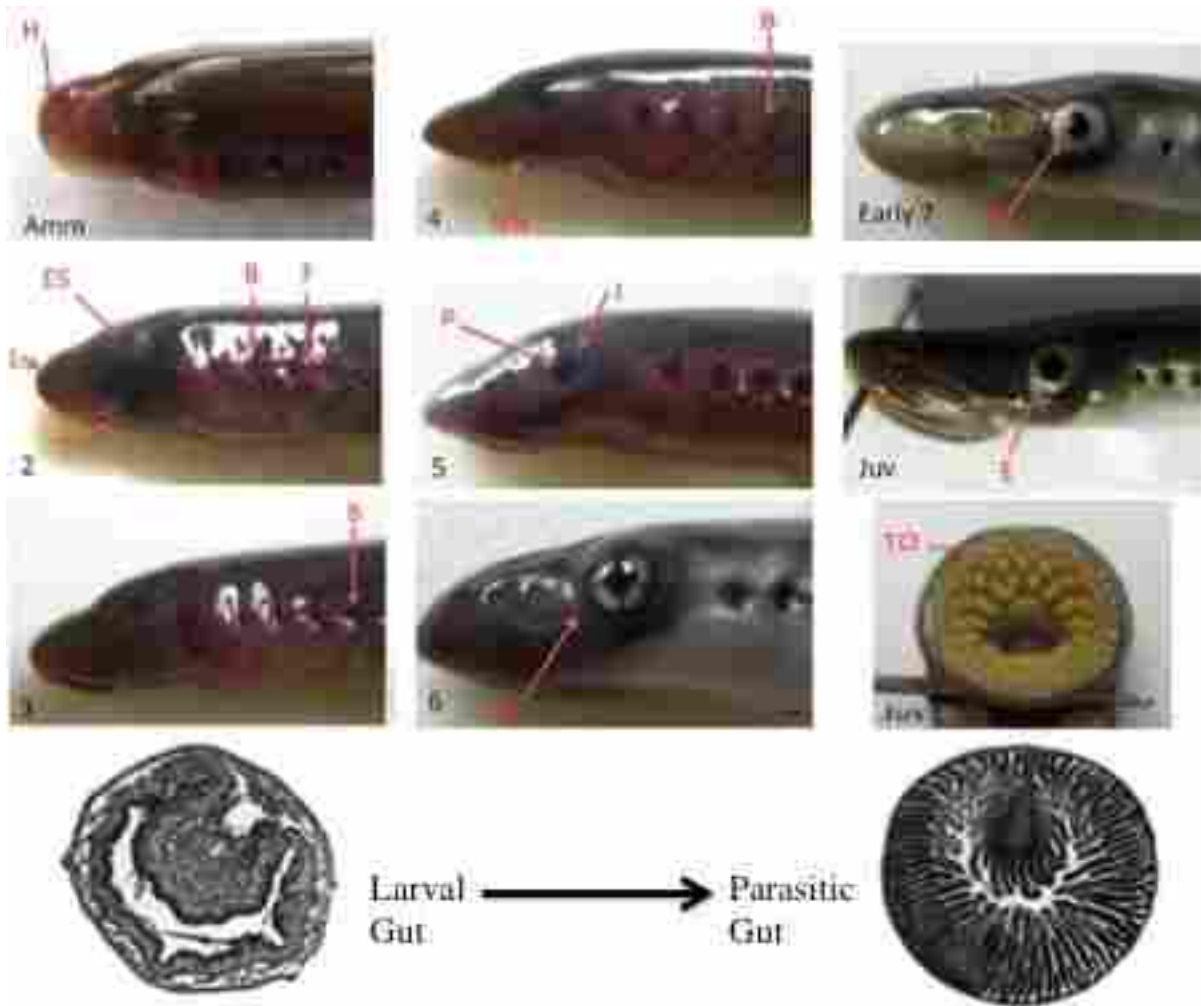
$\dot{M}O_2$ . Radio-labeled  $^{14}C$ -TFM was also used to measure TFM elimination rates in sea lampreys injected with the radio-labeled lampricide. To determine if body size affected TFM tolerance, toxicity tests were performed to determine how body size was related to the  $LC_{50}$ ,  $LC_{99.9}$ ,  $LT_{50}$  and survival time of larval sea lamprey exposed to TFM.

## 1.6 Figures

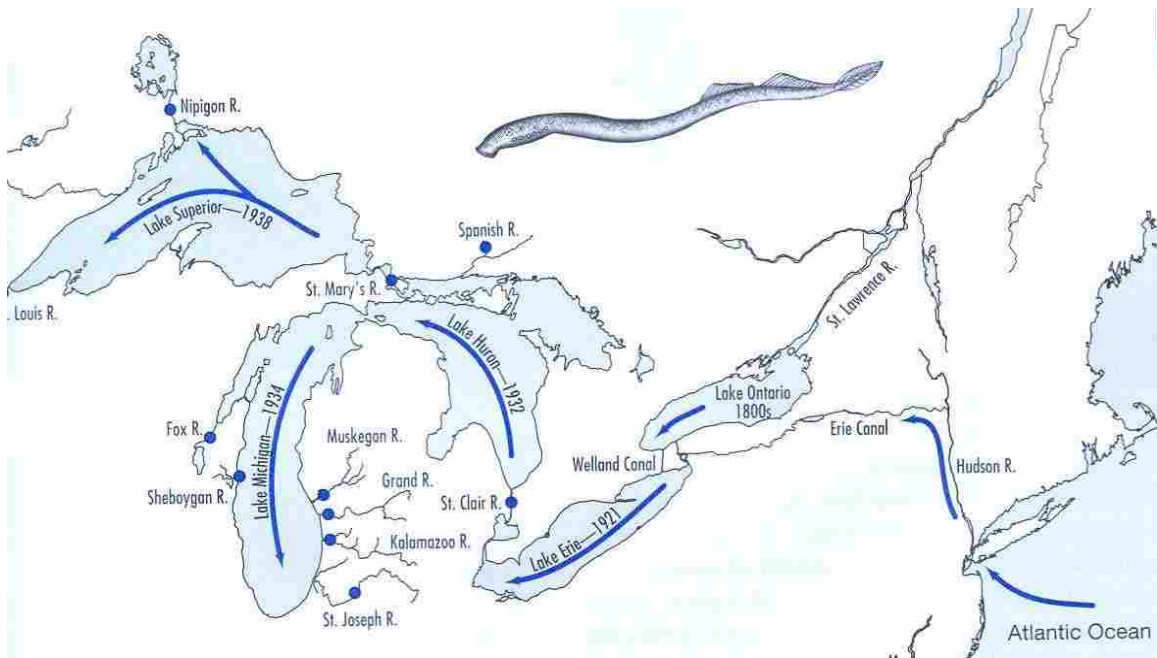


**Figure 1-1: Sea lamprey life cycle.** Larval sea lampreys are relatively sedentary and live burrowed in the substrate of freshwater streams and rivers filter-feeding on detritus and algae for 3 – 7 years. The larvae then metamorphosis into free-swimming juvenile sea lampreys, which migrate downstream to larger bodies of water, where they attach to larger fishes and feed on their blood and bodily fluids. After 12 – 20 months of feeding, sea lampreys become migrate back to suitable streams to spawn and then die. From Wilkie (2011).

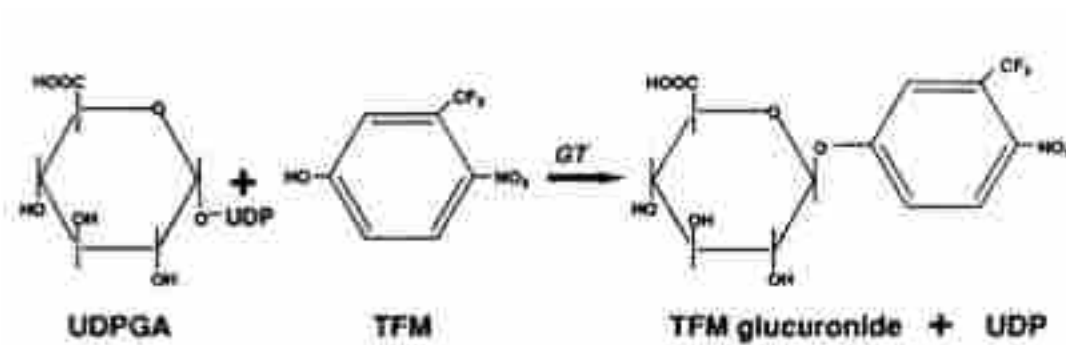




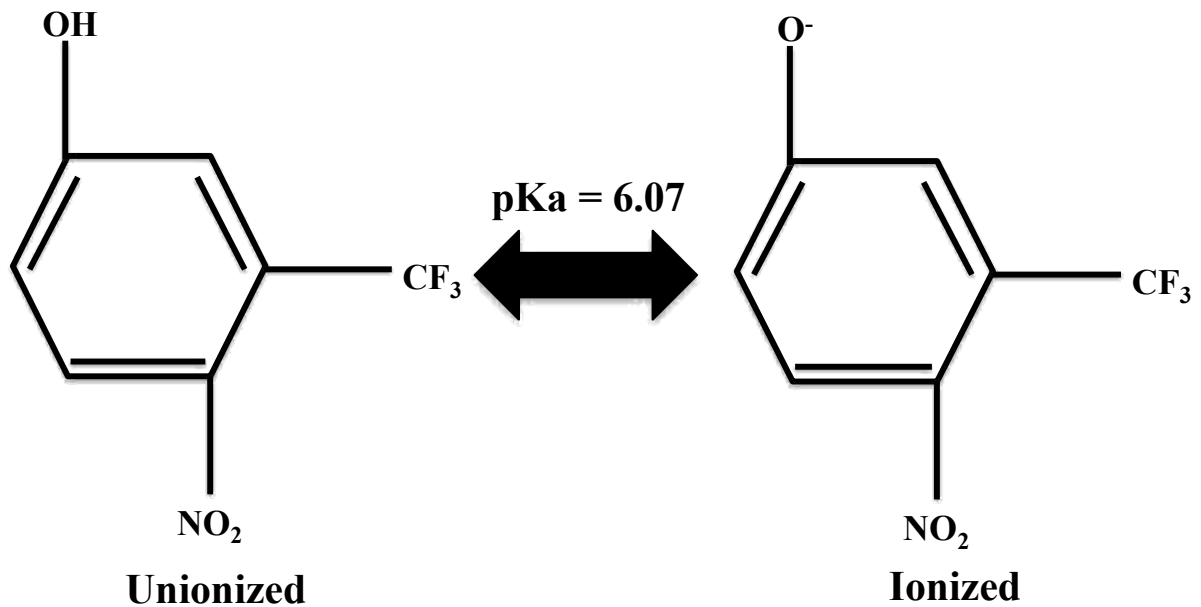
**Figure 1-2: Stages of metamorphosis.** The seven stages of metamorphosis of sea lampreys, from larvae (ammocete Amm) to juvenile parasitic sea lampreys (Juv). Notable is the development of a complex eye (E) with its pupil (P), the restructuring of the gills (B), as well as the development of the oral disc (TO) with its rasping teeth and tongue from the oral hood (H). Internal modifications occur as the animal switches from a filter feeding diet of detritus to the blood and bodily fluids of teleost fishes. From Tetlock et. al (2012) and Neal (2013).



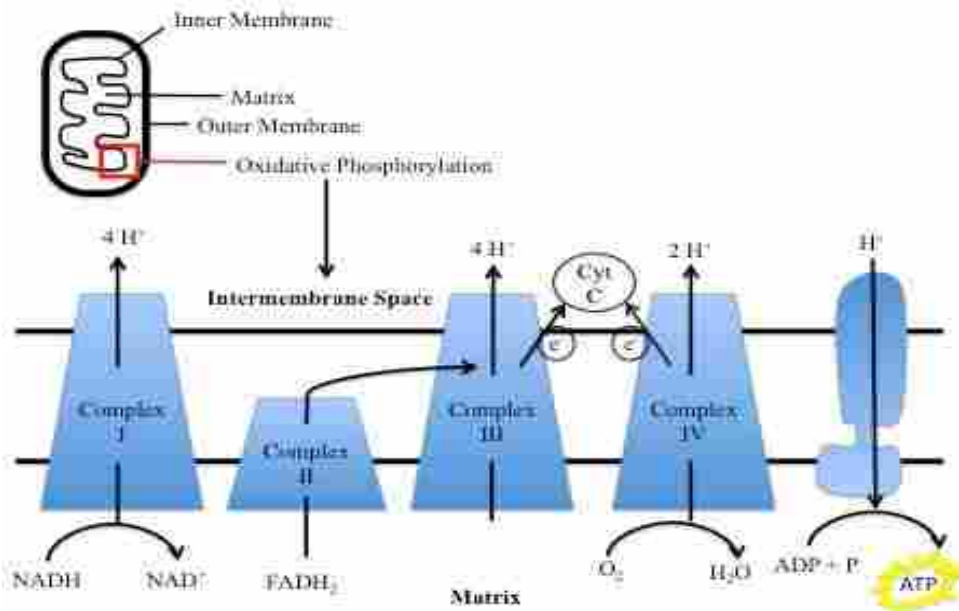
**Figure 1-3: The sea lamprey invasion of the Great Lakes.** Sea lampreys were either native to Lake Ontario, or entered via Erie Canal from the Hudson River in the early 1800's. They were first observed in Lake Erie in the 1920's after modifications were made to the Welland Canal, which allowed the lamprey to circumvent Niagara Falls. They subsequently invaded Lake Huron and the upper Great Lakes in the 1930's and 40's (Applegate 1950; Sullivan et al 2003). Figure from Fishing Wisconsin, Wisconsin Department of Natural Resources (2003).



**Figure 1-4. Detoxification of TFM.** Conjugation of TFM and UDPGA (UDP-glucuronic acid) to form soluble TFM-glucuronide and UDP, which is then eliminated via biliary or urinary excretion by non-target fishes, but not lampreys. From Kane et al. (1993).



**Figure 1-5: Speciation of TFM.** TFM in its un-ionized (left) and ionized (right) forms. The pKa of TFM is 6.07, the pH at which 50% of the TFM is ionized and 50% un-ionized (Hubert 2003). The un-ionized lipid soluble form of TFM is predicted to easily cross the gills, leading to increased TFM toxicity at lower pH.



**Figure 1-6: ATP Production by Oxidative Phosphorylation.** Diagram of mitochondria showing the inner membrane, the site of oxidative phosphorylation and the electron transport chain. Protons are pumped across the inner mitochondrial membrane using energy from the passage of electrons through the electron transport chain (ETC) at protein complexes I, III, and IV. The electrons (reducing equivalents) are supplied by  $\text{NADH}^+$  and  $\text{FADH}_2$ , which is mainly produced in the citric acid cycle. The final electron acceptor is Complex IV, in which  $\text{O}_2$  combines with  $\text{H}^+$  to form  $\text{H}_2\text{O}$  (aerobic respiration). As the protons in the intermembrane space flow into the mitochondrial matrix down their electrochemical gradient (the proton motive force) via the ATP synthase (Complex V), energy is released and harnessed to add a phosphate group to ADP, forming ATP. In the presence of TFM, the proton motive force breaks down, leading to decreased ATP production, along with increased passage of electrons through the electron transport chain and increased rates of oxygen consumption by the mitochondria (Alberts et al. 2004).

## Chapter 2

# Effects of Body Size and Life History Stage on the Uptake and Excretion of the Lampricide 3-Trifluoromethyl-4-Nitrophenol (TFM) by Invasive Sea Lamprey (*Petromyzon marinus*)

### 2.1 Introduction

The invasion of the Great Lakes by sea lampreys (*Petromyzon marinus*) in the early 20<sup>th</sup> century decimated commercial and recreational fisheries in the basin (Lawrie 1980). A series of integrated pest management strategies including barriers to the migration of adult spawning sea lampreys, trapping, sterile male release and the application of lampricides to streams hosting populations of the larval sea lampreys were subsequently implemented to bring the sea lamprey populations under control (Applegate 1961; Bergstedt and Twohey 2007; McLaughlin 2007; McDonald and Kolar 2007). Lampricides remain a key element of the sea lamprey control program in the Great Lakes, in which streams infested with larval sea lamprey are treated every 3 – 4 years with 3-trifluoromethyl-4-nitrophenol (TFM), which targets multiple generations of sea lampreys with a single application (McDonald and Kolar 2007). However, the use of this strategy also means that there will be wide variations in the sizes of sea lamprey present during a TFM treatment due to the different ages of the larvae, although it is not known how this influences TFM treatment effectiveness. Indeed, streams are surveyed and selected for treatment with TFM based on a ranking system, which prioritizes streams with large larvae and therefore high proportions of metamorphosing animals (Hansen et al. 2003).

It is unknown how body size influences sea lamprey sensitivity to TFM, but as in other animals, metabolic rates are inversely related to body size in larval sea lampreys (Lewis 1980; Wilkie et al. 2001). Thus, corresponding rates of oxygen consumption ( $\dot{M}O_2$ ) and gill ventilation would be expected to be higher leading to greater rates of TFM accumulation per unit body mass in smaller larval sea lamprey. The goal of the current study was to determine how body size dependent differences in the metabolic rate ( $\dot{M}O_2$ ) of larval sea lamprey influenced their rates of TFM uptake and excretion, and their ability to survive a typical TFM treatment. Differences in tolerance to TFM based on body size may be an important factor to consider when selecting streams for lampricide treatments, or for determining the probability of residuals after applications.

As an organism's size increases, its mass specific metabolic rate increases allometrically, to the power of 0.75 (Goolish 1991; Rao et al. 1995). This is demonstrated by the equation  $Y = aM^b$ , where ( $Y$ ) is respiration rate (or other physiological variable of interest), ( $a$ ) is a constant, ( $M$ ) is the mass (g), and ( $b$ ) is the experimentally determined scaling exponent (Gillooly et al. 2001; Glazier 2005). This scaling exponent ( $b$ ) represents the allometric relationship between an organism's size and its metabolic rate. Metabolic rate can be quantified directly using calorimetry, but  $\dot{M}O_2$  is typically measured as a proxy for metabolic rate in fishes (Nelson 2016). The main objective of the present study was to test the prediction that  $\dot{M}O_2$  and TFM uptake rates of sea lamprey scaled allometrically to body mass, making larger larval sea lamprey more tolerant and likely to survive TFM treatments.

Another factor that could affect sea lamprey sensitivity to TFM is life stage. Following metamorphosis, the feeding apparatus, gills and internal organs are completely

restructured as the animals enter the parasitic, blood-feeding phase of their life cycle (Youson 1980; Wilkie 2011). Metabolic rate is also thought to increase following metamorphosis (Lewis 1980), which would likely be accompanied by increases in gill ventilation due to their greater O<sub>2</sub> demands. Thus, another goal of the present study was to test the hypothesis that post-metamorphic sea lampreys had higher  $\dot{M}O_2$  and rates of TFM uptake than comparably sized larval sea lamprey.

The clearance of TFM by larval sea lampreys could also be influenced by body size. While other fish have shown the ability to detoxify TFM through the excretion of TFM-glucuronide, which is generated in the liver through the process of conjugation (Lech and Statham 1975; Kane et al. 1993), the inability of sea lamprey to use this process means that the elimination of TFM would mainly depend upon the passive diffusion of TFM across the gills following re-introduction into the clean (TFM-free) water, when favourable blood-water TFM diffusion gradients were restored (Clifford et al. 2012). Under such conditions, higher rates of gill ventilation and greater surface area: volume ratios in smaller larval sea lampreys should theoretically allow them to clear TFM more quickly from their bodies following prolonged (9 – 12 h) exposure to TFM. To test this possibility, the rates of TFM clearance were measured in different size groups of larval sea lampreys following TFM exposure.



## 2.2 Methods and Materials

### 2.2.1 Sea lamprey collection and holding

Larval sea lampreys were collected using backpack, pulsed DC electrofishing (ABP-2 Electrofisher, Electrofishing Systems, LLC, Madison, WI, USA) in the spring of 2014 from streams draining into Lake Michigan by United States Fish and Wildlife personnel, and held at the Hammond Bay Biological Station in flowing Lake Huron water (ambient lake temperature 14 – 16°C; 1 L min<sup>-1</sup>) for approximately three months. The animals were then shipped by overnight courier to Wilfrid Laurier University (WLU) in approximately 30 L of oxygen-saturated water contained in sealed high gauge plastic bags contained within 70 L coolers. After arrival, the sea lampreys were transferred to 110 L tanks, continuously receiving aerated WLU well-water (pH = 7.8 – 8.1; T = 14 – 16°C) at a flow rate of 1.0 - 2.0 L minute<sup>-1</sup>. The bottom of each tank was lined with sand to a depth of 4 - 5 cm to provide the animals with burrowing substrate, and the larvae were fed a slurry of baker's yeast once per week (1.0 g yeast per sea lamprey; Holmes and Youson 1994).

Parasitic sea lamprey were either reared in the laboratory, or supplied courtesy of Dr. Barb Zielinski (Department of Biology, University of Windsor, Windsor, Ontario). Parasitic sea lamprey provided from University of Windsor were permitted to feed on rainbow trout (*Oncorhynchus mykiss*) for 2 – 3 weeks prior to their arrival at WLU, where feeding ceased and they were used in experiments after 2 weeks acclimation to WLU water. The parasitic lampreys reared in the lab came from the same pool of larval sea lampreys described above, and their likelihood of undergoing metamorphosis was determining by measuring body size.

The larval sea lamprey deemed likely to enter metamorphosis (length > 120 mm; mass > 2.5 g) (Holmes et al. 1994), were moved to static 50 L aquaria prior to start of metamorphosis. The aquaria were filled with aerated WLU well water and kept at a temperature of 16 - 18°C; with weekly water changes to ensure there was no build-up of ammonia. No substrate or feeding was required for either the metamorphosing or parasitic (Windsor and Laurier animals) sea lampreys at WLU.

All experiments were approved by the Wilfrid Laurier Animal Care Committee, and followed the guidelines and principles of the Canadian Council of Animal Care (CCAC).

### **2.2.2 Experimental set-up and design of respirometers**

All experiments were completed using a flow-through system comprised of a 40 L head tank to which well water was pumped from a lower sump (40 L; reservoir), which was continuously receiving and collecting well-water. In the reservoir and head tank, well-water was vigorously aerated, which maintained O<sub>2</sub> at > 90% saturation. The well-aerated water then flowed from the head tank via flow-splitters into individual 100 mL respirometer chambers at a rate of 10 mL per minute per chamber. The chambers were immersed in a water bath, which maintained water temperature between 12 – 14°C. Each chamber contained a cylindrical mesh insert that was elevated 4 cm off the bottom of the chamber using 1 cm diameter polypropylene syringe barrel cut to the appropriate length, which enclosed a small (5 mm) magnetic stir bar to mix the water during oxygen consumption measurements. For  $\dot{M}O_2$  determination, the chambers were filled with 1 g of aquarium cotton, to provide burrowing substrate to calm the animals (Wilkie et al. 2001). Previous studies have

demonstrated that absence of burrowing substrate can result in up to two-fold higher  $\dot{M}O_2$  in unburrowed compared to burrowed larval lampreys (Potter and Rogers 1972; Wilkie et al. 2001).

Under control conditions or when measuring TFM uptake, the chambers were covered by a lid, through which a 5 mm in diameter inflow tube could be inserted plus openings for two polyethylene 0.58 mm [Polyethylene Intramedic Clay Adams Brand, 0.58 mm diameter (PE50)] airlines that continuously aerated water in the chamber. During  $\dot{M}O_2$  measurements, the respirometry chambers were sealed with a black rubber stopper, through which two 21-gauge needles connected to external 3-way polyethylene stopcock valves. The chambers were submersed under water to prevent atmospheric  $O_2$  from entering the chamber. Surgical tubing (PE50) fitted over the tip of the needles, extended below the mesh stage, which supported the animals in the chamber to facilitate water sampling with minimal disturbance to the animals. Water samples were collected from the sampling port of one stopcock valve, using a 1 mL disposable syringe attached to the luer tip connector of the valve, while the second port had a 10 mL syringe attached to it and was left open to replace water that was removed from the chamber (2 x 1 mL) for measurement of water  $PO_2$ .

The same chambers were used as flux chambers to measure  $^{14}C$ -TFM uptake and excretion rates, but it was not necessary to seal the lids with rubber stoppers and cotton substrate was not included in the chambers to ensure that there was adequate mixing of the chemical in the container. Instead, the chambers were continuously aerated to ensure mixing and oxygenation of the water as described above.

### 2.2.3 Experimental Protocols

#### *Series 1 – Effects of Body Size on <sup>14</sup>C-TFM Uptake and Oxygen Consumption ( $\dot{M}O_2$ )*

To determine how body size influenced metabolic rate in larval sea lampreys,  $\dot{M}O_2$  was measured using the closed system respirometers described above. The day before experiments, four size groups (50 – 70 mm, 71 – 90 mm, 91 – 110 mm, 111 – 130 mm; N = 8 per group; body mass range 0.1 – 3.0 g) of larval sea lamprey, were transferred to the respirometers, to which water was continuously flowing at a rate of 10 mL per minute. The next day, water flow to each respirometer was cut-off, and the containers were submerged in water to prevent atmospheric O<sub>2</sub> from entering the chamber during sampling (Cech and Brauner 2011). Water samples (2 X 1 mL) were then withdrawn using gas tight Hamilton syringes (2 X 1 mL) at the beginning of the experiment, and at 0.5 and 1.0 h. The first sample (1 mL) at each sample period was discarded to eliminate dead-space water in the siphon tube, followed by measurement of water PO<sub>2</sub> using the second 1 mL sample. Two consecutive measures of water O<sub>2</sub> were made (2 X 500  $\mu$ L) using an acrylic flow-through cell coupled to a fiber optic oxygen probe (Integr Planar Oxygen Sensor FTC-PSSt3), which was connected to a Fibox 3 oxygen meter (PreSens Regensburg, Germany; Software PST3v602 version 5.32). The PO<sub>2</sub> in each respirometer was never allowed to decrease below 100 Torr to prevent hypoxia (Cech and Brauner 2011).

Following  $\dot{M}O_2$  determination (1 h), the rubber stopper was removed from each container, and replaced with the lid through which water and airlines could be inserted. After 30 minutes of water replacement, water flow was cut-off, and the water volume in the container adjusted to 100 mL, followed by the addition of 0.5  $\mu$ Ci of <sup>14</sup>C-TFM mixed with

sufficient cold TFM to yield an exposure concentration of 4.6 mg L<sup>-1</sup> TFM (the 12 h TFM LC<sub>50</sub>; Birceanu et al. 2009). Prior to addition of <sup>14</sup>C-TFM and TFM, a background (T = -0.25 h) water sample was collected, followed by a 0.25 h mixing period and subsequent samples at 0 h, 1 h and 3 h for determination of the <sup>14</sup>C-TFM radioactivity and the total “cold” (non-radioactive) concentration of TFM in the water. Following the final water sample at 3 h, the larval sea lampreys were terminally sampled with an overdose of the anesthetic tricaine methanesulfonate (1.5 g L<sup>-1</sup> MS-222) buffered with 3 g L<sup>-1</sup> sodium bicarbonate, and processed for quantification of beta radioactivity as described below.

### *Series 2 – Relationship Between Body Size and TFM Clearance*

The TFM elimination rates of different size groups of larval sea lampreys (see Series 1; N = 8 per group) were based on the appearance of <sup>14</sup>C-TFM radiation in the water after the animals were administered <sup>14</sup>C-TFM labeled TFM by interperitoneal injection (IP) at a dose of 100 nmol TFM g<sup>-1</sup> wet weight per animal. This dose of TFM represents the approximate total concentration of TFM that is reached in larval sea lampreys experiencing mortality during TFM exposure (Le Claire 2014). The mean specific activity of the solution was 99 CPM nmol<sup>-1</sup>. Immediately following TFM administration, the larval sea lamprey were transferred to flux chambers containing 100 mL of TFM-free water, and the animals were left for 1 h to allow the TFM to become evenly distributed in the tissues. This was followed by the collection of water samples (10 mL) at 0, 1, 2, 4, 6, 8, 12, 22 and 24 h to measure the appearance of <sup>14</sup>C-TFM radioactivity in the water. Survival was assessed regularly using a tail pinch, and animals that did not survive were removed from the experiment. Mortality rates post-injection were approximately 10% after 24 h. Fresh water was added between time

periods at 4 h and 12 h, and the volume in each container was adjusted to 100 mL.

Temperature (12 – 14°C) and water pH (7.8 – 8.1) was recorded and monitored to ensure all conditions were similar between the replicates.

*Series 3 – Effects of Life History Stage on TFM Uptake, Clearance and Oxygen Consumption*

To quantify differences in metabolic rates of sea lampreys at various life stages, each sea lamprey was acclimated to an individual darkened 100 mL flow-through exposure container for 12 h prior to the experiment. Experiments were conducted from October – November 2015. Sea lampreys were size-matched, and larval (N = 8 October; N = 8 November) stages 6 – 7 (N = 8) and non-feeding juvenile parasitic sea lampreys (N = 8; reared at WLU lab) were used. Larval sea lampreys were used in both October (with stages 6 – 7 transformers) and November (with the post-metamorphic juvenile parasitics) as controls due to the timing of sea lamprey metamorphosis. No differences in  $\dot{M}O_2$  or TFM uptake rates were detected in larval sea lampreys measured in October and November, and therefore any temporal influences on life stage  $\dot{M}O_2$  and TFM uptake rates were excluded.

In March 2015, larval sea lampreys (N = 10) were size matched with juvenile sea lampreys (N = 8; courtesy of Dr. B. Zielinski, University of Windsor); which were not fed in the 2 weeks preceding experiments. To quantify life stage differences in TFM clearance rates, larval and juvenile parasitic sea lampreys were administered IP injections of  $^{14}C$ -TFM, inspected for leakage of TFM solution around the injection site, and then placed in TFM-free water. It was assumed that there was either little or no biotransformation or detoxification of TFM because we previously only detected traces of this metabolite in sea lampreys exposed

to TFM (LeClaire 2014). After experiments, sea lampreys were euthanized with an overdose of anesthetic as described earlier, and the carcasses processed to determine the amount of residual, whole body TFM that was not excreted.

#### **2.2.4 Tissue processing for determination of tissue and water radioactivity**

Following experiments, the carcasses of sea lampreys subjected to  $^{14}\text{C}$ -TFM exposure were immediately washed in cold, concentrated TFM ( $50 \text{ mg L}^{-1}$ ) to remove any bound  $^{14}\text{C}$ -TFM on the surface of the animal, and then again in well water. The animals were then weighed and transferred to 50 mL polypropylene centrifuge tubes (Falcon Tube, Epindorf) to which 10 mL of 1M nitric acid were added. Each carcass was digested for 48 h at  $60^\circ\text{C}$  and vortexed for 30 seconds every 12 h to ensure thorough mixing of the sample. After the carcass was fully digested, it was centrifuged for 5 minutes at 5,000 rpm, and 2 mL of the supernatant was drawn off and transferred to a scintillation vial (7 mL) to which 4 mL of Ultima Gold organic scintillation cocktail (Perkin Elmer, Walktham, MA, USA) was added, and thoroughly mixed. Samples were left in the dark for 12 h, to minimize chemiluminescence, followed by the measurement of beta radioactivity in counts per minute (CPM) using a Beckman-Coulter, LC 6500 Multipurpose Scintillation Counter (Beckman-Coulter, Fullerton, CA, USA). The beta radioactivity of water samples (2 mL) was determined on samples mixed with 4 mL Optiphase “Hisafe” 2 aqueous scintillation cocktail (Perkin Elmer, Waltham, MA, USA), after being left overnight in the dark to minimize chemiluminescence. Quench curves, constructed using tissue and water samples spiked with known amounts of  $^{14}\text{C}$ -TFM were used to determine the efficiency of the scintillation counter.

## 2.2.5 Calculations

### *M<sub>O<sub>2</sub></sub> and TFM Uptake*

The following equation, modified from Cech (1990), was used to calculate  $\dot{M}O_2$  ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ ):

$$\dot{M}O_2 = \frac{(C_{O_2(i)} - C_{O_2(f)}) * V}{(M)(\Delta T)} \quad (1)$$

Where  $C_{O_2(i)}$  is the concentration ( $\mu\text{mol L}^{-1}$ ) of  $O_2$  in the water at the beginning and  $C_{O_2(f)}$  is the concentration at the end of the measuring period ( $\mu\text{mol L}^{-1}$ );  $V$  is the volume of the respirometer (L),  $M$  is the mass of the sea lamprey (g), and  $\Delta T$  is the duration of the measurement period (h).

The rate of TFM uptake ( $\text{nmol g}^{-1} \text{h}^{-1}$ ) was calculated using the following equation:

$$\text{TFM Uptake} = \frac{\text{CPM g}^{-1}}{(\text{MSA} * \Delta T)} \quad (2)$$

Where  $\text{CPM g}^{-1}$  is the radioactivity of the tissue measured in counts per minute  $\text{g}^{-1}$  wet mass,  $\text{MSA}$  is the specific activity of the water samples ( $\text{CPM nmol}^{-1}$ ), and  $\Delta T$  is the time of exposure (h).

### *TFM Clearance Rates*

After quantifying TFM uptake rates, the TFM clearance rates were then determined based on the appearance of  $^{14}\text{C}$ -TFM radiation in water samples taken from the containers



holding the IP injected lampreys over time as they were in TFM-free water. The following equation was used to calculate  $^{14}\text{C}$ -TFM excretion rates:

$$J_{^{14}\text{C-TFM}}^{\text{out}} = \frac{(\text{CPM}_i - \text{CPM}_f) * V}{M * \Delta T} \quad (3)$$

Where  $\text{CPM}_i$  and  $\text{CPM}_f$  are the radioactivity of the water samples (measured in CPM) at the beginning and end of each flux, respectively, and  $M$ ,  $V$  and  $\Delta T$  are as previously stated.

The TFM efflux (clearance) rate was calculated using the  $J_{^{14}\text{C-TFM}}$ , from the following equation:

$$\text{TFM efflux (clearance) rate} = \frac{J_{^{14}\text{C-TFM}}^{\text{out}}}{\text{MSA}} \quad (4)$$

Where  $\text{MSA}$  is the mean specific activity of the  $^{14}\text{C}$ -TFM injected into the sea lamprey in  $\text{CPM nmol}^{-1}$  TFM (Evans 1967; Wilkie et al. 2006).

The percentage of injected TFM cleared by each sea lamprey was compared using the following equation:

$$\% \text{ TFM clearance} = \frac{[(^{14}\text{C-TFM}_i - ^{14}\text{C-TFM}_f)] * 100}{^{14}\text{C-TFM}_i} \quad (5)$$

Where  $\text{TFM}_i$  is the initial  $\text{nmol TFM g}^{-1}$  injected, based on the  $\mu\text{L}$  of solution injected into the sea lamprey body corrected by the measured percentage retained in the body 5 minutes post-injection ( $57 \pm 15\%$ ), and  $\text{TFM}_f$  is the measured  $\text{nmol g}^{-1}$  left in the sea lamprey after 24 h.

### *Allometric Scaling Equations*

To determine metabolic scaling exponents, the whole body  $\dot{M}O_2$  ( $\mu\text{mol h}^{-1}$ ) and TFM uptake rates ( $\text{nmol h}^{-1}$ ) were calculated from modifications to equations (1) and (2) respectively, where:

$$\dot{M}O_2 = \frac{(C_{O_2(i)} - C_{O_2(f)}) * V}{(\Delta T)} \quad (6)$$

$$\text{TFM Uptake} = \frac{\text{CPM}}{(\text{MSA} * \Delta T)} \quad (7)$$

The whole body  $\dot{M}O_2$  and TFM data was plotted on a log-log scale and the bivariate line of fit was plotted using least squares regression (JMP version 12.0.1). The following equations were required to determine the mass-scaling relationships between whole body  $\dot{M}O_2$  and TFM uptake in sea lampreys:

$$\log Y = \log a + b \log M \quad (9)$$

Where  $Y$  = dependent variable (either whole body  $\dot{M}O_2$  or TFM), ( $a$ ) is the proportionality constant [given as the y-intercept in equation (8)], ( $b$ ) is the scaling exponent (given as the slope in the previous equation) and  $M$  is the mass (g).

### *Statistical Analysis*

Log-transformed data and non-transformed linear data were analyzed using linear regression (least squares regression; JMP version 12.0.1; SAS Institute Inc. 2015; Cary, NC). Data in histograms was presented as the mean  $\pm$  the standard error of the mean (SEM).

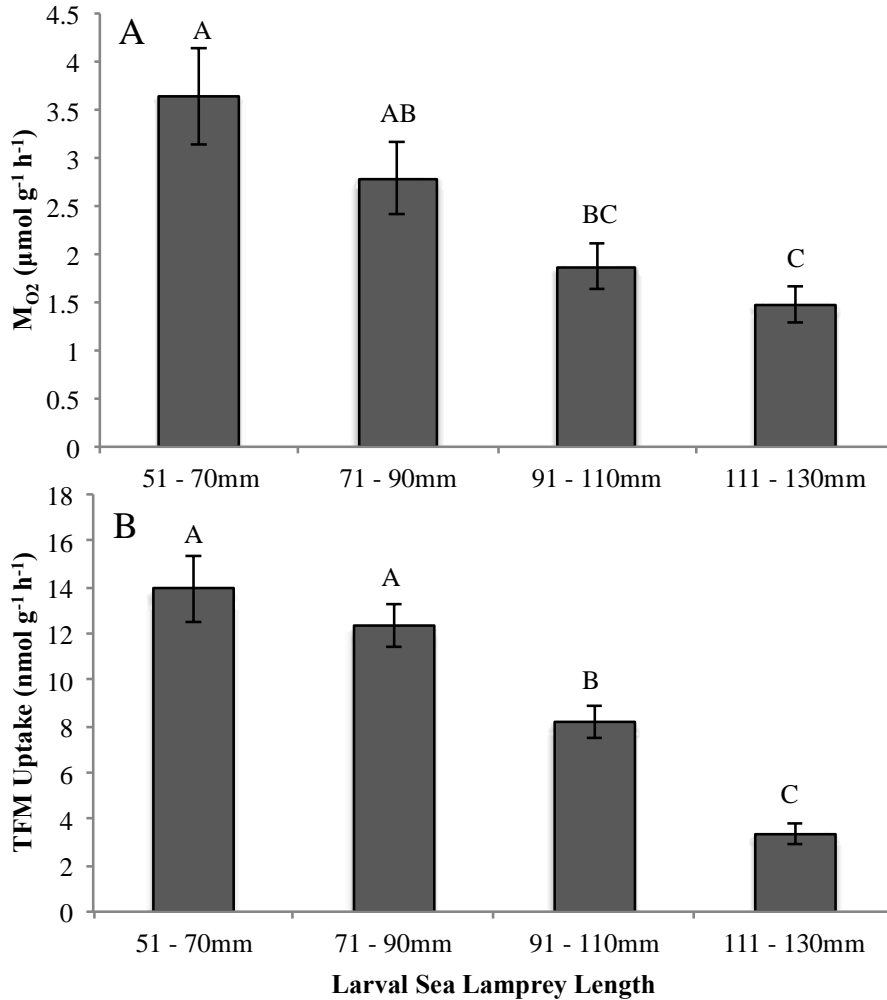
Normally distributed data with equal variances (Shapiro-Wilk's test for normality and Welch's test for homogenous variance) were analyzed with parametric repeated measures analysis of variance (ANOVA  $\alpha = 0.05$ ). The non-parametric Kruskal-Wallis test was used for non-normally distributed.

## 2.3 Results

### *Series 1 – Effects of Body Size on <sup>14</sup>C-TFM Uptake and Oxygen Consumption ( $\dot{M}O_2$ )*

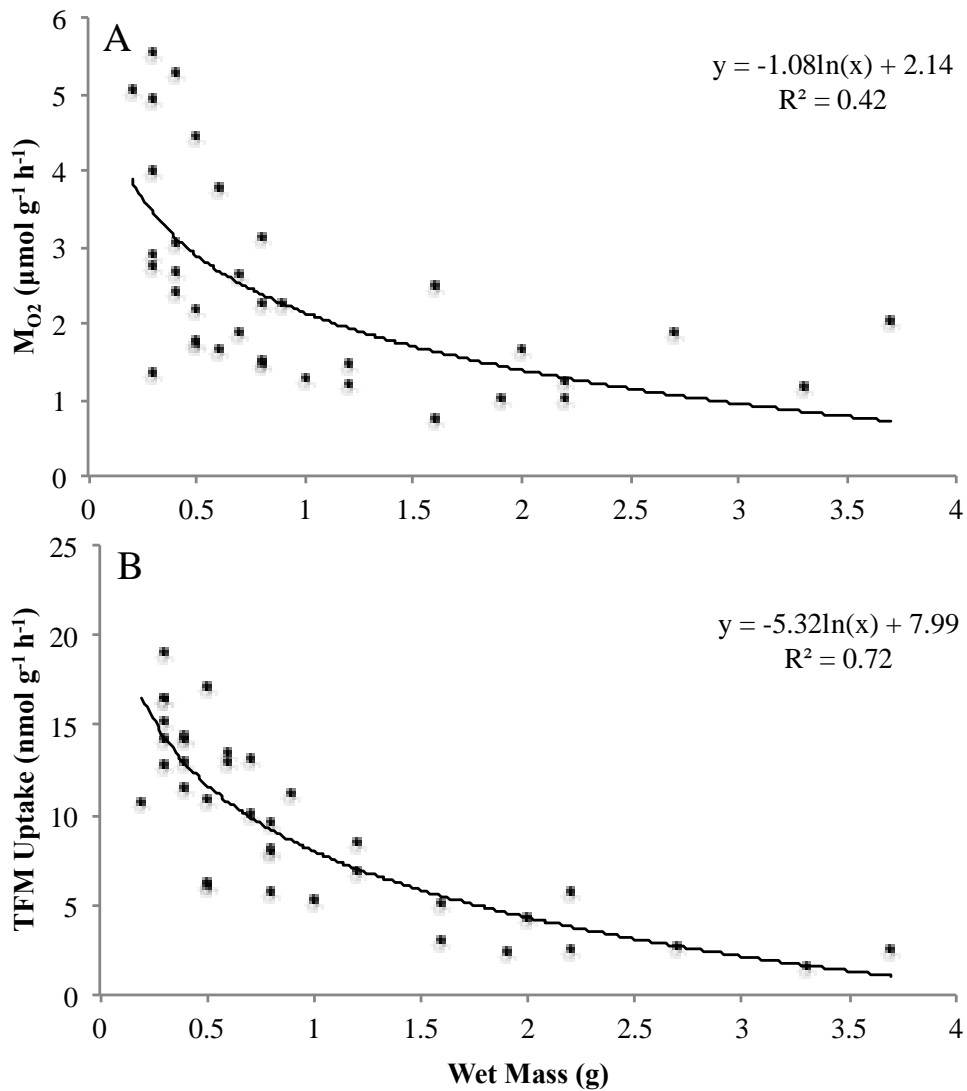
Preliminary <sup>14</sup>C-TFM uptake experiments were conducted in order to determine a suitable length of time to expose larval sea lampreys to TFM. The rates of <sup>14</sup>C-TFM uptake were initially highest after 1 h of <sup>14</sup>C-TFM exposure to 5.4 mg L<sup>-1</sup> TFM, but also the most variable, compared to the narrower range of <sup>14</sup>C-TFM uptake rates observed after 2 and 4 h of TFM exposure ( $R^2 = 0.23$ ). This decreasing trend in <sup>14</sup>C-TFM uptake rates was not found to be significant ( $P = 0.08$ ), however. Accordingly, rates of TFM uptake were determined over 3 h time intervals in the present study. Preliminary exercise respirometry was also completed on 6 larval sea lamprey (0.1 – 1.4 g) following 5 minutes of exhaustive exercise, to test the sensitivity in the oxygen consumption method. At rest, larval sea lampreys had an average  $\dot{M}O_2$  of  $2.0 \pm 0.7 \mu\text{mol g}^{-1} \text{h}^{-1}$ , but immediately following exercise  $\dot{M}O_2$  was 2-fold greater, approaching  $4.0 \pm 0.9 \mu\text{mol g}^{-1} \text{h}^{-1}$ . After 1 h,  $\dot{M}O_2$  peaked at  $5.0 \pm 1.2 \mu\text{mol g}^{-1} \text{h}^{-1}$ , before decreasing to approximately  $3.0 \pm 0.6 \mu\text{mol g}^{-1} \text{h}^{-1}$  after 3 h post-exercise (data not shown).

The mass specific  $\dot{M}O_2$  ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ ) and TFM uptake rates ( $\text{nmol g}^{-1} \text{h}^{-1}$ ) of larval sea lampreys were inversely related to the length of sea lamprey (Figure 2-1).  $\dot{M}O_2$  was highest in the shortest group (50 – 70mm), averaging  $3.6 \pm 0.5 \mu\text{mol g}^{-1} \text{h}^{-1}$  ( $N = 8$ ), and steadily decreased as body length increased, averaging  $1.48 \pm 0.2 \mu\text{mol g}^{-1} \text{h}^{-1}$  in the largest group ( $N = 9$ ; Figure 2-1). Parallel trends were observed for TFM uptake, which averaged  $13.9 \pm 1.4 \text{nmol g}^{-1} \text{h}^{-1}$  in the animals of shortest length, steadily decreasing as body length increased (Figure 2-1).



**Figure 2-1: Relationship between (A) rates of oxygen consumption ( $\dot{M}_{O_2}$ ) and (B) TFM uptake rate vs. sea lamprey length (mm).**  $\dot{M}_{O_2}$  was quantified for 1 h using static respirometry in WLU well water (temperature 14 – 16°C; pH 7.8 – 8.1), prior to the addition of  $^{14}\text{C}$ -TFM (4.6 mg L<sup>-1</sup>), to which sea lampreys were exposed to for 3 h. Data analyzed by one-way ANOVA and presented as the mean  $\pm$  1 standard error of the mean (N = 9 per size group). There were no statistical differences between bars sharing the same letter (P > 0.05).

The mass specific  $\dot{M}O_2$  of larval sea lampreys was also inversely correlated with body mass, decreasing exponentially as body mass increased (Figure 2-2A;  $R^2 = 0.42$ ). Log transformed plots of the mass specific  $\dot{M}O_2$  with body mass (g) showed that there was a strongly significant negative linear correlation (Figure 2-3A;  $R^2 = 0.44$ ;  $P < 0.001$ ). An identical, but stronger, relationship was observed for the log of mass specific TFM uptake rates, which also decreased exponentially with increasing body mass (Figure 2-2B;  $R^2 = 0.72$ ), with a very strong linear relationship between the log body masses vs. log TFM uptake (Figure 2-3C;  $R^2 = 0.77$ ;  $P < 0.0001$ ).



**Figure 2-2: Relationship between mass specific rates of (A) oxygen consumption ( $\dot{M}_{O_2}$ ) and (B) TFM uptake and sea lamprey wet mass.**  $\dot{M}_{O_2}$  was quantified for 1 h using static respirometry in WLU well water (temperature 14 – 16°C, pH 7.8 – 8.1), prior to the addition of  $^{14}\text{C}$ -TFM ( $4.6 \text{ mg L}^{-1}$ ), to which sea lampreys were exposed to for 3 h (N = 36; 0.2 – 3.7 g).

Both whole body  $\dot{M}O_2$  ( $\mu\text{mol h}^{-1}$ ) and TFM uptake rates ( $\text{nmol h}^{-1}$ ) scaled allometrically as described by the classic  $Y = aM^b$  relationship, where  $Y$  is the dependent variable ( $\dot{M}O_2$  or TFM uptake),  $a$  is the proportionality constant,  $M$  is the mass (g) and  $b$  is the slope of the line (scaling exponent). To derive these empirical values, the whole body rates of  $\dot{M}O_2$  and TFM uptake rates were plotted on a double logarithmic scale. The log whole body  $\dot{M}O_2$  vs. log body mass relationship (Figure 2-3B) was described by the formula:

$$\log(\dot{M}O_2) = 0.277 + 0.561 * \log(\text{Mass}(g)) \quad (10)$$

Where  $b$  was 0.56 and  $a$  (the y-intercept) equaled 0.277. Taking the antilog of  $a$  ( $10^{0.277}$ ), and rearranging equation 10 then yielded the classic power equation:

$$\dot{M}O_2 = 1.89 * M^{0.57} \quad (11)$$

The same approach was taken to create a model for whole body TFM uptake rates (Figure 2-3D), which was described by:

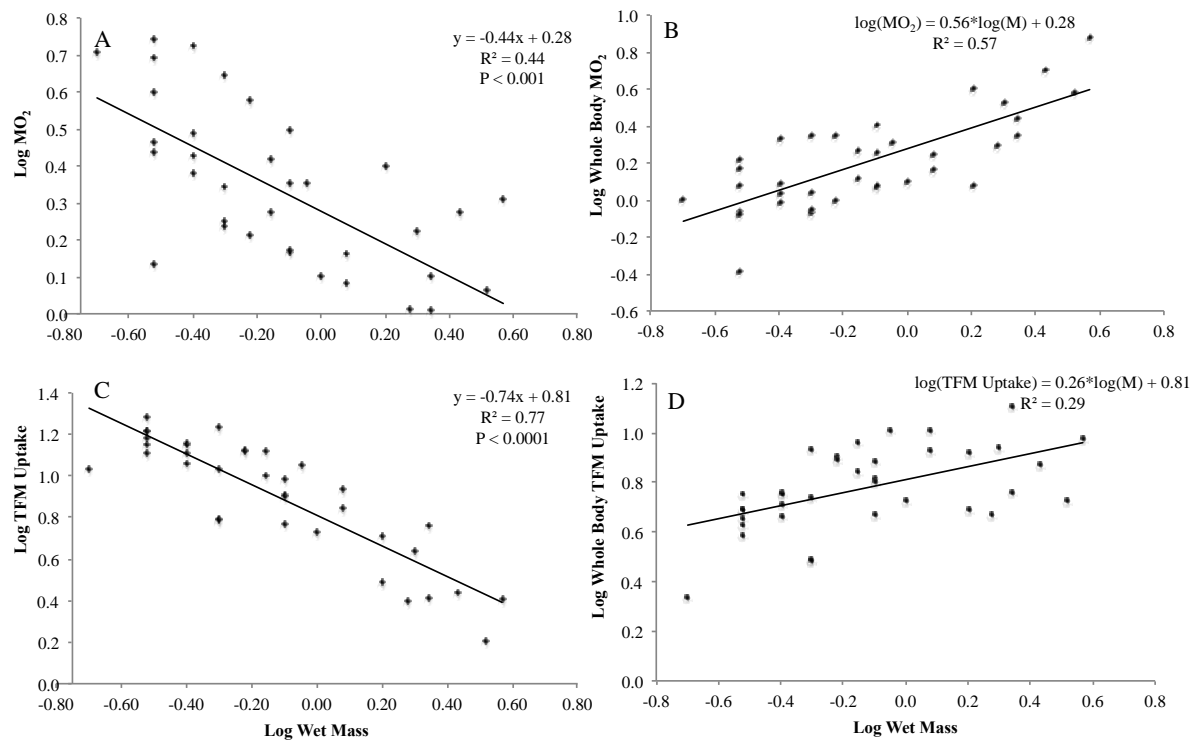
$$\log(\text{TFM Uptake}) = 0.81 + 0.26 * \log(\text{Mass}(g)) \quad (12)$$

which was equivalent to:

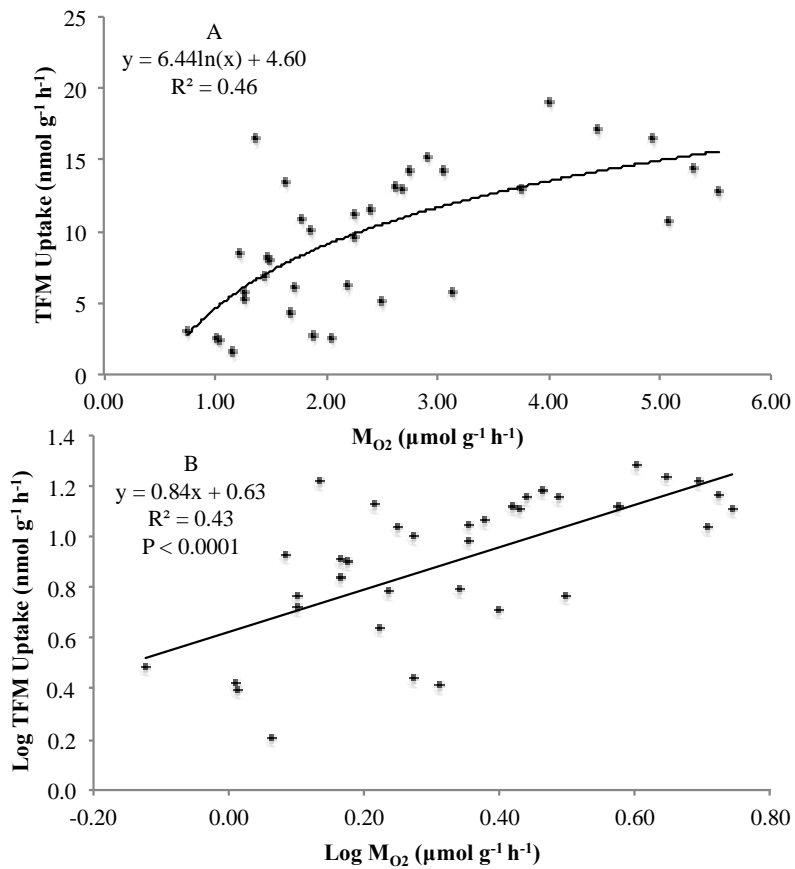
$$\text{TFM Uptake} = 6.47 * M^{0.26} \quad (13)$$

There was also a strong proportional relationship between mass specific  $\dot{M}O_2$  ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ ) and mass specific TFM uptake ( $\text{nmol g}^{-1} \text{h}^{-1}$ ) (Figure 2-4;  $R^2 = 0.41$ ).





**Figure 2-3: Log transformed mass specific and whole body  $\dot{M}O_2$  and TFM uptake rates vs. larval sea lamprey body mass.** Regression analysis of (A) log mass specific  $\dot{M}O_2$  with linear regression and subsequent (B) log whole body  $\dot{M}O_2$ ; (C) log mass specific TFM uptake rates; (D) log whole body TFM uptake rates in larval sea lampreys (N = 36; 0.2 – 3.7 g; WLU well water; 4.6 mg L<sup>-1</sup> TFM).

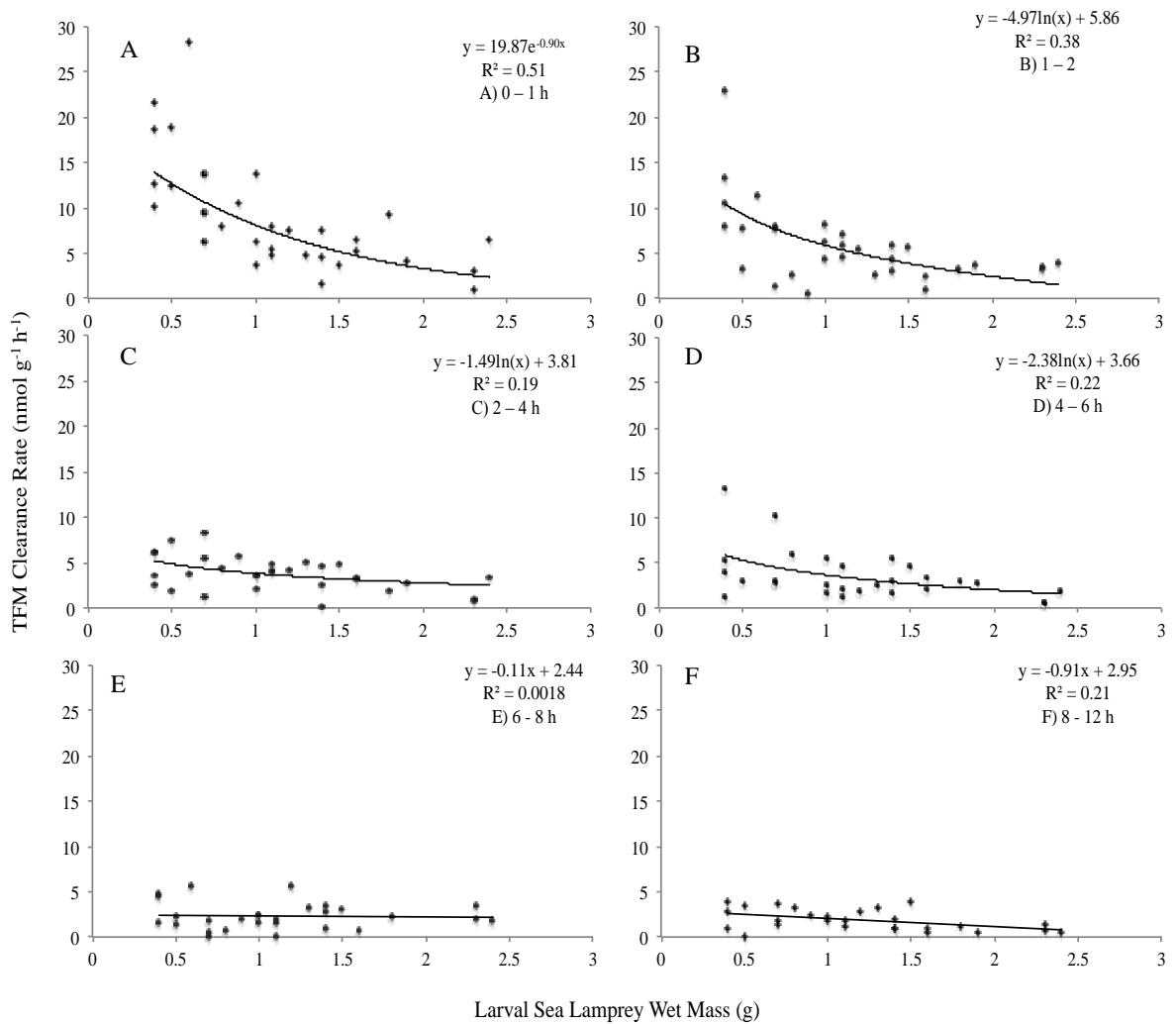


**Figure 2-4: TFM uptake rates vs.  $\dot{M}_{O_2}$ .** Mass specific rates of TFM uptake vs. resting  $\dot{M}_{O_2}$  of larval sea lampreys expressed linearly (A) and following log-log transformation (B). Data is graphed as individual data points, with the line of best fit and equation of the line shown in each panel (N = 36; 0.2 – 3.7 g; WLU well water; 4.6 mg L<sup>-1</sup> TFM).

### *Series 2 – Relationship Between Body Size and Post-TFM Clearance*

The mean amount of TFM injected averaged  $57 \pm 2 \text{ nmol g}^{-1}$ , estimated based on the actual  $\mu\text{L}$  of  $^{14}\text{C}$ -TFM solution and the MSA that was injected, and a retention rate of  $57 \pm 15\%$ .  $^{14}\text{C}$ -TFM retention was determined by preliminary experiments where six larval sea lampreys were injected with the target  $100 \text{ nmol g}^{-1}$  and then euthanized 5 minutes post-injection and the carcasses processed to determine the amount of  $^{14}\text{C}$ -TFM retained.

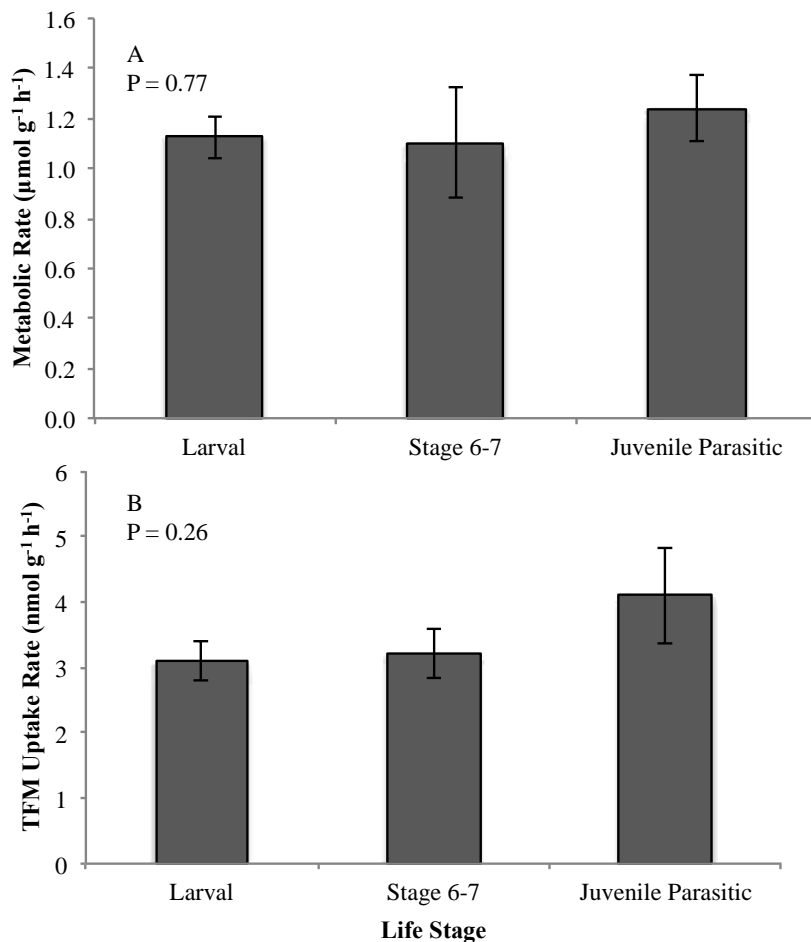
The highest TFM excretion rates were observed during the first hour following  $^{14}\text{C}$ -TFM administration, and then exponentially decreased with time (Figure 2-5). The overall average TFM clearance rates across all body sizes was  $9.0 \pm 1.1 \text{ nmol g}^{-1} \text{ h}^{-1}$ , and by the 8 - 12 h flux had decreased to  $1.9 \pm 0.2 \text{ nmol g}^{-1} \text{ h}^{-1}$ . The relationships between the TFM clearance rate and body size were observed over 8 flux periods, from 0 – 12 h post-injection. The highest TFM clearance rate was in a 0.6 g larval lamprey, with a TFM clearance rate of  $28 \text{ nmol g}^{-1} \text{ h}^{-1}$ , observed during the 0 – 1 h period. In contrast, the largest sea lamprey (2.4 g) had a TFM clearance rate of only  $6.5 \text{ nmol g}^{-1} \text{ h}^{-1}$  during this flux.



**Figure 2-5: Relationship between TFM clearance rates and body mass of larval sea lampreys.** TFM clearance rates of larval sea lampreys ranging in size from 0.4 – 2.4 g (N = 38) were measured over different time intervals, following TFM administration via intraperitoneal (IP) injection. Measurements were conducted over: (A) 0 – 1 h, (B) 1 – 2 h, (C) 2 – 4 h, (D) 4 – 6 h, (E) 6 – 8 h, (F) 8 – 12 h and 12 – 24 h (data not shown). Water levels in each container were replenished to 100 mL using WLU well water (temperature 14 – 16°C; pH 7.8 – 8.1) at 4 and 12 h.

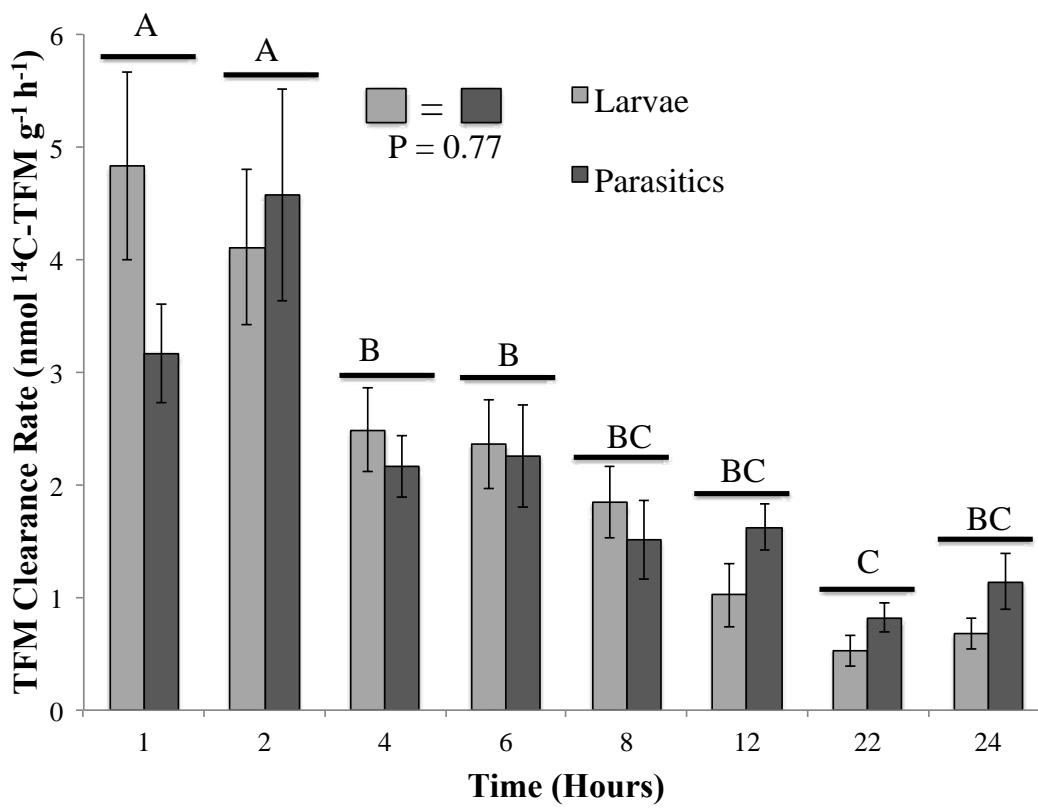
*Series 3 – Effects of Life History Stage on Oxygen Consumption, TFM Uptake, and Clearance*

There were no statistically significant differences in the rates of  $\dot{M}O_2$  or TFM uptake in larval sea lampreys sampled in October compared to November. Accordingly, all of the larval data were pooled for statistical analysis. No significant differences in  $\dot{M}O_2$  were observed between any of the life stages examined. In larval sea lampreys,  $\dot{M}O_2$  averaged  $1.1 \pm 0.1$ , compared to  $1.1 \pm 0.2$  in late metamorphosis (stage 6 – 7) and  $1.2 \pm 0.4 \mu\text{mol g}^{-1} \text{h}^{-1}$  in parasitic juvenile sea lamprey (Figure 2-6A;  $P = 0.77$ ). Similarly, the rate of TFM uptake in larval sea lampreys was  $3.1 \pm 0.3 \text{ nmol g}^{-1}\text{h}^{-1}$ , which was not statistically different from rate of  $3.2 \pm 0.4 \text{ nmol g}^{-1}\text{h}^{-1}$  measured in the late metamorphosing or in the juvenile parasitic lampreys, which had a TFM uptake rate of  $4.1 \pm 0.7 \text{ nmol g}^{-1}\text{h}^{-1}$  (Figure 2-6B;  $P = 0.26$ ).



**Figure 2-6: Influences of life stage on TFM uptake and  $\dot{M}\text{O}_2$ .** Sea lampreys at three life stages were size matched and rates of (A)  $\dot{M}\text{O}_2$  and (B) TFM uptake rates were compared. Data is shown as the mean  $\pm$  one standard error of the mean (larval, N = 16; stage 6 – 7, N = 8; juvenile parasitic, N = 8). No significant differences were observed for  $\dot{M}\text{O}_2$  or TFM uptake rates according to life stage ( $P > 0.05$ ).

In size-matched larval sea lampreys administered (IP) injections of a target concentration of  $100 \text{ nmol TFM g}^{-1}$ , however, this volume was corrected since preliminary experiments indicated only  $57 \pm 15\%$  of the injected TFM remained inside the lamprey 5 minutes post-injection (data not shown). The actual amount of TFM injected and retained in the parasitic lampreys was  $40 \pm 1 \text{ nmol g}^{-1}$  of TFM, and in the larvae  $45 \pm 2 \text{ nmol g}^{-1}$  of TFM. The highest TFM clearance rates in both larval and juvenile sea lampreys were observed during the first 2 h of depuration in TFM-free water, and gradually declined, averaging  $1 - 2 \text{ nmol g}^{-1} \text{ h}^{-1}$  through to 8 h before stabilizing between 12 - 24 h, when the majority of TFM had been cleared (Figure 2-7). However, larval sea lampreys had excreted significantly more TFM compared to juvenile parasitic sea lampreys during this time period, averaging  $93 \pm 2\%$  versus  $86 \pm 2\%$ , respectively, which was significant according to T-test ( $P = 0.001$ ; data not shown).



**Figure 2-7: Influences of life stage on TFM clearance rates.** Larval (N = 10) and juvenile parasitic sea lampreys (N = 8) were injected with  $^{14}\text{C}$ -TFM, and placed in TFM-free water, followed by the collection of water samples (10 mL) at -1, 0, 1, 2, 4, 6, 8, 12, 22 and 24 h. Data is shown as the mean TFM clearance rate  $\pm$  1 SEM. Sample periods sharing the same letter are not significantly different from each other ( $P > 0.05$ ); there were no statistically significant differences between life stage and overall TFM clearance rates ( $P = 0.77$ ).



## 2.4 Discussion

Smaller sea lampreys take-up TFM faster than larger ones, which is at least partially explained by their much higher mass specific rates of  $\dot{M}O_2$ . This could have important implications in streams with high numbers of relatively large larval sea lampreys, which could be more tolerant of TFM due to slower rates of TFM accumulation. TFM clearance rates were less dependent on the body size, but smaller animals did have higher TFM clearance rates after the first 6 h than the larger animals following TFM injection, which could promote more rapid recovery of smaller than larger animals if TFM applications were interrupted due to weather, mechanical or changes in water flow. Surprisingly, life stage did not influence  $\dot{M}O_2$  or rates of TFM uptake, although larval sea lampreys were able to clear more TFM compared to parasitic sea lampreys over 24 h following depuration in TFM-free water.

### *Effects of Body Size on Oxygen Consumption ( $\dot{M}O_2$ ) and TFM Uptake*

The rates of TFM uptake in larval sea lamprey increased in a less than proportional manner with body size, resulting in much greater mass specific rates of TFM uptake in smaller animals, which decreased exponentially as they got larger (Figure 2-1, 2-2, 2-3). This allometric relationship, in which body size and TFM uptake change non-proportionally, is similar to the relationship between metabolic rate and body mass. In most animals, metabolic rate ( $\dot{M}O_2$ ) is a power function of body mass (Randall et al. 1997), as described by the well-known relationship:

$$Y = aM^b \quad (14)$$

Where Y is the dependent variable ( $\dot{M}O_2$ ), M is the mass of the animal,  $b$  is the universal mass (aka. scaling) exponent which corrects for changes in  $\dot{M}O_2$  with body mass,  $a$  is a proportionality constant that is empirically determined from the equation of the line generated in log-log plots of  $\dot{M}O_2$  vs. M (Kleiber 1947; Peters 1983; Glazier 2013). In its logarithmic form, equation (14) becomes:

$$\log Y = \log a + b \cdot \log M \quad (15)$$

which describes the linear relationship that results when curvilinear data relating  $\dot{M}O_2$  to body mass are log-transformed (present study) or plotted on a log-log scale. Relationships where  $b = 1$  are described as isometric, meaning that  $\dot{M}O_2$  increases in direct proportion to body mass. The classic work of Kleiber (1932) demonstrated that  $b$  is actually nearer to 0.75 when the  $\dot{M}O_2$  of many groups of mammals are compared to one another, meaning that whole body  $\dot{M}O_2$  increases in a less than proportionate manner with body size. In other

words, smaller animals have a relatively higher  $\dot{M}O_2$  compared to larger animals, as best illustrated by the classic mouse-elephant curve depicting the relationship between the body mass and mass specific  $\dot{M}O_2$  of very small mammals (e.g. mouse) through to very large mammals (e.g. elephant), which is known as Kleiber's Law (Kleiber 1947). As numerous studies have demonstrated, Kleiber's Law also applies to poikilothermic animals, including many species of fish and lampreys (Lewis 1980; Hughes 1984; Goolish 1991). This is especially interesting that this scaling relationship is true even between homeothermic animals, which have higher metabolic demands due to their internal regulation of body temperature and homeostasis compared to poikilothermic animals, which use considerably less energy to meet their metabolic demands (Glazier 2005).

Oxygen consumption ( $\dot{M}O_2$ ) is often used as an indirect measurement of metabolic rates, and has been quantified for multiple lamprey species (Potter and Roger 1970; Hill and Potter 1974; Wilkie et al. 2001). In larval lampreys, however, it is important to provide burrowing substrate during  $\dot{M}O_2$  measurements, as rates of  $\dot{M}O_2$  can be up to two-fold higher in unburrowed compared to burrowed larvae (Potter and Rogers 1972; Wilkie et al. 2001). In the present study, diffuse aquarium cotton (1 g) was added to each respirometer, which tends to calm these burrow-dwelling creatures (Potter and Rogers 1972; Wilkie et al. 2001).

The resting metabolic rate of larval sea lampreys ( $\dot{M}O_2$ ) scaled allometrically as described by the power relationship:

$$\dot{M}O_2 = 1.89 * M^{0.56} \quad (11)$$

These findings are in general agreement with previous studies, but the mass exponent,  $b = 0.56$ , was lower than expected. In *Ichthyomyzon hubbsi* (Hill and Potter 1970),  $b = 0.72$ ,  $0.78$  in *Lampetra planeri* (Potter and Rogers 1972), and  $b = 0.83$  in larval sea lamprey ranging in mass from 1.3 – 3.9 g (Wilkie et al. 2001). On the other hand,  $a = 1.89$  in the present larval sea lampreys, which was near values (1.74 and 1.62) reported in larval *I. hubbsi* and *L. planeri* (Lewis 1980).

The uptake of TFM also increased allometrically, as described by the power equation:

$$\text{TFM Uptake} = 6.47 * M^{0.26} \quad (13)$$

In contrast to  $\dot{M}O_2$ , however,  $b$  was much less for TFM uptake, averaging 0.26. This indicates that as the mass of the lamprey exposed to TFM increased, TFM uptake rates were higher, but the increase was proportionately far less than observed for  $\dot{M}O_2$  (compare equations 11 and 13). In other words, this suggests that TFM uptake is disproportionately greater in smaller larval sea lamprey than would be predicted based solely on metabolic rate. The proportionality constant,  $a$ , for TFM uptake was also much higher than for  $\dot{M}O_2$ , at 6.47. The proportionality constant can be used to compare scaling relationships between groups or data sets. For example, a physiological factor may be proportionally lower in one species compared to another, yet still share the same scaling relationship. In this case, the

proportionality constants,  $a$ , would be different between the species while the scaling exponents,  $b$ , would be similar.

Because TFM uptake can be predicted using the power relationship developed from this study (equation 11), it may be possible to apply this relationship to predict TFM accumulation and the likelihood of residual sea lampreys in streams containing different size ranges (and ages) of animals. For instance, in streams where the size structure of the population is known from sea lamprey assessments, it may eventually be possible to predict the time-course of TFM loading and corresponding TFM burdens in different size groups of animals, which could be used to predict the likelihood of residual sea lampreys. In streams with many large sea lampreys, it may also be advisable to extend treatment times to ensure that enough lampricide is accumulated and retained for sufficient times, to ensure that larger larval sea lamprey succumb to the lampricide. On the other hand, treating more streams with larger numbers of smaller lamprey may make sense, because they tend to take-up and accumulate TFM at exponentially higher rates than larger animals, which may also improve the efficiency of lampricide. Therefore, rather than treating streams with high numbers of large, pre-metamorphic sea lampreys, as is presently the case using the ESTR system, it may make sense to treat streams more frequently or earlier in the sea lampreys life cycle to ensure a more complete eradication of smaller animals. Further studies are required to analyze the cost:benefit analysis of such a strategy, however.

*Physiological Determinants of TFM Uptake in Different Sizes of Larval Sea Lamprey*

Lipid soluble compounds (lipophilic) are easily taken-up via the gills, then diffuse into the blood stream and tissues of the fish. TFM in its un-ionized form is lipophilic, which can be quantified based on the log octanol:water partition coefficient ( $K_{ow}$ ). Chemicals with a log  $K_{ow}$  greater than 3 are highly lipophilic. With a log  $K_{ow}$  of 2.77, TFM is moderately lipophilic (Schmitt et al. 2000). The whole body uptake of other lipophilic chemicals also scales with fish body mass to the power of 0.75 (Peters 1983; Sijm et al. 1995). For instance, the rate uptake constant ( $Y$ ) derived for fish exposed to a range of lipophilic (hydrophobic) chemicals such as the phenols, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene, 2,3,5-trichloroanisole increased in accord with the predicted body size relationship (Sijm et al. 1995). Thus, the ability to use scaling exponents to predict uptake of lipophilic chemicals based on the body size scaling relationships is not just restricted to TFM.

Higher rates of xenobiotic uptake are likely due to the greater respiration rates and larger gill surface area to body mass ratios of smaller vs. larger fishes (Erickson et al. 2006; Mackay et al. 2014). In sea lampreys, however, there are no published data on how gill surface area changes with growth in the larval sea lamprey. Nevertheless, fishes with higher  $\dot{M}O_2$  also tend to have greater gill surface area and higher ventilation rates, which have all been correlated with greater uptake of lipophilic organic substances, similar to TFM (McKim and Erickson 1991; Brauner et al. 1994; Blewitt et al. 2013). For example, rainbow trout took up significantly more 1,2,4,5-tetrachlorobenzene when  $\dot{M}O_2$  rates increased with exercise (Brauner et al. 1994). Higher resting  $\dot{M}O_2$  was also correlated with an increase in the uptake

and accumulation of 17 $\alpha$ -ethynylestradiol in the mummichug *Fundulus heteroclitus* (Blewitt et al. 2013) and the methylmercury uptake in the tilapia *Oreochromis niloticus* (Want et al. 2011).

In addition to routine (resting) measures, changes in metabolic rate can also influence the toxicity of different substances (Baas and Kooijman 2015; Blewitt et al. 2013; Rodgers and Beamish 1981). For instance, organisms with high metabolic rates such as *Daphnia magna* and *Gammarus pulex* have been shown to be most sensitive to toxicants such as the pesticides chlorpyrifos, malathion, carbaryl, and carbofuran (Baas and Kooijman 2015). A multitude of physiological factors including temperature, feeding, activity levels and body mass can also affect  $\dot{M}O_2$  (Carrasco-Navarro et al. 2015). Larval lampreys are relatively sedentary creatures with  $\dot{M}O_2$  levels that are significantly lower than many comparably sized teleost fishes (Lewis and Potter 1980; Wilkie et al. 2001). However, they are capable of substantially increasing their  $\dot{M}O_2$  following brief bursts of activity, which might occur during intense swimming or even burrowing (Wilkie et al. 2001). Life stage could also potentially affect TFM uptake, because  $\dot{M}O_2$  has been shown in other lamprey species (*L. planeri* and *L. fluviatilis*) to increase following metamorphosis to levels comparable with active teleost fish (Lewis and Potter 1980; Lewis 1980).

Higher  $\dot{M}O_2$  results in greater ventilatory flow of water across the gills (Hughes 1984; Gonzalez and McDonald 1992), which can therefore increase the rate of TFM delivery and accumulation. Oxygen, and likely TFM, are taken-up across the gill lamellae, which is related to the rate of lamellar water flow, which can be calculated using the following equation from Erickson et al. (2006):

$$\text{lamellar water flow} = \frac{\dot{M}O_2}{[O_2] * (O_2 \text{ uptake efficiency})} \quad (16)$$

Where the  $\dot{M}O_2$  ( $\mu\text{mol g}^{-1} \text{ h}^{-1}$ ) of a fish is divided by the concentration of oxygen in the water  $[O_2]$  ( $\mu\text{mol mL}^{-1}$ ) multiplied by oxygen uptake efficiency (percentage of  $O_2$  removed from the water by the fish, which varies between 50 - 80%) (Erickson et al. 2006). A larger fish with greater metabolic demands will have a lower mass-specific  $\dot{M}O_2$  compared to a smaller organism, which would result in lower ventilatory flow of water across the gills and lower rate of TFM uptake during lampricide treatment (Cao et al. 2014; Hughes 1984; Sijm et al. 1995).

In agreement with these theoretical predictions,  $\dot{M}O_2$  and TFM uptake were closely correlated in the larval sea lamprey, indicated by the positive correlation between  $\dot{M}O_2$  and TFM uptake rates ( $R^2 = 0.41$ ; Figure 2-4A). It would therefore be very informative to measure ventilatory flow in larval sea lampreys exposed to TFM, to better understand how TFM is entering the animals and to determine how uptake is influenced by body size. While there is a very strong correlation between metabolic rates and sensitivity to toxicants, body size may only be indirectly related to toxicity. This would indicate that the metabolic rates of species are more likely a driving factor for toxicity compared to body size (Baas and Koojiman 2015). With smaller organisms having higher respiration rates, exposing a small fish to a lower concentration of toxicants may still result in a toxic threshold being reached internally at the same rate as a larger fish exposed to higher concentrations (Mackay et al. 2014).



To determine which was the better predictor of TFM uptake in differently sized sea lampreys, the relative effects of and strength of each relationship can be quantified and compared using the standard least squares method. The log body size of larval sea lampreys was tightly correlated to the uptake rates of TFM ( $P < 0.0001$ ;  $R^2 = 0.77$ ; Figure 2-3C), while the log  $\dot{M}O_2$  was more variable but still significantly correlated ( $P < 0.0001$ ;  $R^2 = 0.43$ ; Figure 2-4B). Therefore, the mass of the larval sea lamprey was a better predictor for TFM uptake rates rather than the  $\dot{M}O_2$  of the lamprey.

#### *Relationship Between Body Size and TFM Clearance*

In the early stages of depuration in TFM-free water (0 – 1 h, 2 – 4 h), smaller larval sea lampreys were able to excrete TFM at a faster rate compared to larger larval sea lamprey. This was also likely because of the greater gill surface: body surface area of smaller animals, which would facilitate passive diffusion of TFM from the body. However, it should be noted that the branchial surface area and body surface area were not quantified in the present study, but this should be addressed in future studies. The skin could also have made a small contribution to TFM unloading, particularly in smaller larval sea lampreys, which have a very thin epidermis, and the presence of capillaries in the dermal layers of the epithelium (Potter et al. 1996).

Excretion via the renal routes is another possibility, but excretion via this pathway would likely require TFM to be bio-transformed to its more water soluble glucuronide conjugate, as reported for coho salmon (*Oncorhynchus kisutch*) and largemouth bass

(*Micropterus salmoides*) (Hunn and Allen 1975; Schultz et al. 1979). For many xenobiotics in fish, including phenolic compounds such as TFM, glucuronidation is the most important type of conjugation for detoxification and elimination (Clarke et al. 1991). This Phase II metabolic process mainly takes place in the liver, where glucuronic acid is added to drugs, toxins, toxicants, steroid molecules and other compounds by UDP-glucuronosyltransferase. The glucuronidated compound is then excreted via the bile or urine (Clarke et al. 1991). However, there is little evidence to suggest that sea lampreys have the capacity to detoxify and excrete TFM in this manner (Hunn and Allen 1974; Lech and Statham 1975; Kane et al. 1993). This suggests that renal TFM excretion only plays a minor, if any, role in larval sea lamprey. Because TFM excretion is likely limited to passive diffusion of the un-ionized parent compound across the gills, and to a lesser extent the skin, it likely explains why the total elimination of TFM is much slower in sea lamprey than in rainbow trout, which are capable of conjugating TFM to TFM-glucuronide (Kane et al. 1993; Hubert et al. 2002; Birceanu et al. 2014; LeClair 2014). Indeed, LeClair (2014), using high performance liquid chromatography (HPLC) to quantify the TFM burden in sea lamprey exposed to TFM, reported that the half-life for TFM elimination was 1.8 h in rainbow trout, compared to 7.7 h in sea lamprey. As well, trace amounts of TFM were still detectable in sea lamprey tissues even after 24 h (LeClair 2014).

Despite the unlikelihood of TFM detoxification to glucuronidated-TFM, larval sea lampreys were able to excrete more than 90% of the injected  $^{14}\text{C}$ -TFM, regardless of body size, within 24 h ( $P = 0.11$ ; data not shown). The rapid reduction in internal TFM concentrations following depuration in TFM-free water may help explain why larval sea

lampreys are able to rapidly restore internal energy stores following short-term TFM exposure. Clifford et al. (2012) demonstrated that larval sea lampreys fully restore internal glycogen stores and phosphocreatine within 2 – 4 h following short-term (4 – 6 h) exposure to the 12 h LC<sub>100</sub> of TFM. Due to TFM's uncoupling effects on mitochondrial oxidative phosphorylation (Birceanu et al. 2011), TFM exposure results in greater reliance and reductions in these anaerobic energy reserves to make up for short-falls in ATP supply (Viant et al. 2001; Birceanu et al. 2009; Clifford et al. 2012; Henry et al. 2015). Thus, despite their inability to detoxify TFM, sea lampreys are resilient to short-term TFM exposure. In the early stages of post-TFM exposure, it also appears that smaller sea lamprey can initially clear TFM at a faster rate than larger larval sea lampreys. However, further tests are required in order to determine if this actually increases survival following short-term TFM exposure.

#### *Effects of Life History Stage on Oxygen Consumption ( $\dot{M}O_2$ ) TFM Uptake and Elimination*

The life stage of sea lampreys did not have an effect on TFM uptake or  $\dot{M}O_2$  of the sea lamprey in this study. Previous work has indicated that in the free-swimming parasitic stage,  $\dot{M}O_2$  increased compared to relatively sedentary larval lamprey (Lewis 1980). Reasons for the apparently higher  $\dot{M}O_2$  of juvenile compared to larval sea lampreys were thought to be related to the transition from a relatively sedentary, burrow-dwelling life style to a more active, free-swimming life stage (Lewis 1980; Wilkie 2011). Indeed, activity levels tend to increase in lampreys following metamorphosis, particularly in the evening due to the development of a distinct circadian following metamorphosis (Claridge and Potter 1975).

These observations also contrast the previous observations of Lewis and Potter (1977), who reported that  $\dot{M}O_2$  approximately doubled in the European river lamprey (*L. fluviatilis*) and brook lampreys (*L. planeri*) by stage 6 metamorphosis.

As the larval animals were provided with burrowing substrates in this, and in previous studies focusing on *L. fluviatilis*, *L. planeri* and sea lamprey (Lewis and Potter 1977; Wilkie et al. 2001), it is unlikely that  $\dot{M}O_2$  was overestimated. Indeed, the  $\dot{M}O_2$  of resting larval sea lampreys was about twice as high in the absence of cotton burrowing substrate in the previous study done by Wilkie et al. (2001). However, other factors such as keeping the animals in the dark for the duration of  $\dot{M}O_2$  measurements and/or making measurements during the day might also have helped calm both larval and post-metamorphic sea lampreys. However, the absence of a distinct photoperiod may have muted the diurnal (nightly) fluctuations in  $\dot{M}O_2$  that are known to increase in juvenile and adult lampreys (Lewis 1980). Inter-species differences between sea lampreys and the European river and brook lampreys cannot be ruled out either.

The structure of the gills, presumed to be the main route of TFM uptake, also changes markedly during metamorphosis (Lewis 1980; Rovainen 1996). In larval lampreys, the gill is unidirectionally ventilated with water entering the pharynx via the oral hood before crossing the gills and exiting via the branchiopores. Following metamorphosis, however, the gills are tidally ventilated with water being actively pumped out of the re-structured branchiopores (gill pouches) which makes it possible for the animals to irrigate the gills while attached to a fish during feeding or when anchored to the substrate (Lewis and Potter 1980; Rovainen 1996; Baghdadaln and Wilson 2014). Despite these changes in gill structure following

metamorphosis, it should be noted that the secondary lamella, which are the sites of gas exchange on the gill filaments, are similar in larval and post-metamorphic lampreys. Indeed, the actual surface area of the gills relative to body size does not change, despite increased activity levels following metamorphosis (Lewis and Potter 1976; Rovainen 1996; Baghdadwala and Wilson 2014). In the absence of any change in functional gill surface area or  $\dot{M}O_2$ , it is perhaps not surprising the metamorphosis failed to result in any change in the rates of TFM uptake by sea lampreys. However, further study including more detailed morphological and ultrastructural analysis of lamprey gill structure and function during metamorphosis, including measurements of branchial surface area, are still required to validate this prediction. It would also be informative to determine how the metabolic scope, the ability of the animals to increase  $\dot{M}O_2$  to meet increased metabolic demands (e.g. more rapid swimming), changes following metamorphosis.

While there were no differences between the life stages of larval and parasitic sea lamprey in the  $\dot{M}O_2$  or TFM clearance rates over time, after 24 hours it was determined that the larval sea lamprey had excreted slightly more TFM compared to the parasitic sea lampreys. This could indicate that larval sea lampreys had a greater capacity to excrete TFM from their bodies. In fact, the total  $\text{nmol g}^{-1}$  of TFM that was retained in the carcasses of the lampreys after the 24 h was significantly higher in the juvenile parasitic lampreys ( $6.7 \pm 0.8 \text{ nmol g}^{-1}$ ) compared to the larval lamprey ( $3.1 \pm 0.2 \text{ nmol g}^{-1}$ ;  $P = 0.003$ ; data not shown).

Particularly in the larval life stage of different species of lampreys, it appears that cutaneous respiration occurs at a higher rate in the larval stages compared to the free-swimming life stage, with the skin being significantly thinner ( $70 \mu\text{m}$ ) in larvae compared to

adult river lamprey (250 – 300  $\mu\text{m}$ ) (Czopek and Sawa 1971; Downing and Novales 1971).

It is possible that  $^{14}\text{C}$ -TFM was able to pass through the skin of the larval sea lamprey easier than the parasitic sea lamprey, leading to the overall increased ability of larval sea lamprey to clear more TFM after 24 h than in the juvenile sea lamprey. Future studies isolating the gills from cutaneous routes of excretion could be implemented to explore this hypothesis.

The internal modifications that take place during metamorphosis result in the loss of the sea lamprey's gallbladder, bile ducts and bile canaliculi (Youson 2003; Tetlock et al. 2012). While it has been found that sea lampreys have a very low capacity to detoxify TFM (Lech and Statham 1975), the loss of these organs may have implications on their already limited ability to excrete TFM using the renal pathways (Kane et al. 1993). In teleost fishes, the gall bladder is where detoxified TFM-G collects in the bile prior to excretion. Schultz et al. (1979) noted an accumulation of TFM-glucuronide in the bile, up to 1400 times higher than the external concentration. Although the capacity to glucuronidated TFM is very low in sea lamprey (Kane et al. 1993), perhaps the loss of bile-ducts in post-metamorphic sea lampreys impairs the already limited ability sea lampreys have to detoxify and excrete any TFM that accumulates in the bile during exposure. However, more experiments, including quantification of the TFM concentration in the bile, are required to test this hypothesis.

## 2.5 Conclusions

In conclusion, the lower TFM uptake rates of larger larval sea lampreys suggests that they are a more likely source of residuals following TFM treatment than smaller animals. With a much higher mass specific  $\dot{M}O_2$ , smaller sea lampreys likely have greater ventilation rates and relatively greater branchial surface area per unit body mass, which leads to greater TFM uptake. Because TFM uptake, like  $\dot{M}O_2$ , scales allometrically in larval sea lampreys, it is possible to model how body mass influences TFM uptake using the power function (eqn. 10;  $TFM\ Uptake = 6.47M^{0.26}$ ) to predict how much and how quickly TFM is accumulated during lampricide treatments. It should be noted, however, that this relationship is also likely to be dose-dependent and the present study only measured TFM uptake at a single concentration. Thus, further studies addressing the allometric relations between TFM uptake and body mass are now required at different TFM doses. It may also be prudent to evaluate the current selection of streams for lampricide treatments, and incorporate body size relationships into treatment protocols. For example, streams could be treated with lampricide more frequently to prevent larval lampreys from reaching large body sizes, or the length of lampricide treatment could be extended when there are high numbers of large larvae present. Finally, increased diligence may also be helpful to guard against high numbers of residual sea lampreys in streams with high populations of large larval sea lampreys.

## Chapter 3

### Relationship Between Larval Sea Lamprey Body Size and TFM Tolerance

#### 3.1 Introduction

Many rivers and streams flowing into the Great Lakes are treated with lampricides on a regular basis in order to eradicate populations of larval sea lampreys (*Petromyzon marinus*) prior to the initiation of metamorphosis, which ultimately reduces the number of parasitic sea lampreys entering the lakes (McDonald and Kolar 2007). The most common lampricide is TFM (3-trifluoromethyl-4-nitrophenol), but it may be mixed with 1 – 2% niclosamide (2'5-dichloro-4' nitrosalicylanilide) to reduce TFM requirements or to treat sea lampreys in fast flowing, deep or lentic environments (McDonald and Kolar 2007). Streams are ranked for lampricide applications based on the Empirical Stream Treatment Ranking (ESTR) system, which considers sea lamprey density, habitat, body size, year class and estimated production (number of metamorphosing sea lamprey). Candidate streams that have high densities of large larval sea lampreys that are predicted to undergo metamorphosis the following summer are selected and prioritized for treatment each year (Hansen et al. 2003). A successful treatment has a larval sea lamprey mortality rate of 90 - 100% (Adair and Sullivan 2013; Johnson et al. 2014), but there can be “residual” sea lampreys that survive treatment, undergo metamorphosis, and then migrate into the Great Lakes where they feed on valuable sport, commercial and culturally significant fish populations (McDonald and Kolar 2007). Barring sudden changes in environmental conditions such as fluctuations in water flow or water pH, which can affect TFM speciation, uptake and toxicity (Bills et al. 2003; Hlina 2015), the



underlying causes of residuals are generally not well understood. Better knowledge of the factors that contribute to residual sea lampreys is therefore key to improving lampricide treatment effectiveness to limit unanticipated damage to Great Lakes fisheries.

Because sea lamprey treatments generally occur at regular intervals every 3 – 4 years, TFM targets multiple generations of sea lampreys. As a result, there can be wide variation in body size, from less than 0.1 g to greater than 4.0 g for body mass and 1 – 140 mm for body length (Applegate 1950; Potter et al. 1978). Yet, there is little information on how variation in body size influences the sensitivity of sea lampreys to TFM. As demonstrated in Chapter 2, rates of TFM uptake scale allometrically in larval sea lamprey, with mass specific rates of TFM uptake decreasing with body mass (Chapter 2). This suggests that larger larval sea lampreys would be less susceptible to TFM-induced mortality, which could therefore influence the success rate of a TFM treatment. Indeed, streams with larger sea lampreys are often selected for chemical treatments when using the ESTR system, due to the fact that yields of parasitic sea lamprey from those streams will likely be greater (Hansen et al. 2003).

It is widely recognized that body size significantly influences sensitivity of different animals and pests to toxicants and pesticides (Kevan and Dixon 1996; Mackay et al. 2014; Baas and Kooijman 2015). Because copper toxicity is greater in smaller than larger rainbow trout, Howarth and Sprague (1978) applied a correction factor to reflect differences in the 96 h  $LC_{50}$  for copper with body size, where  $LC_{50} = \text{constant} * \text{Weight}^{0.3477}$ . Other variables related to body size, such as lipid content, surface area: volume ratios, and mass-specific metabolic rates, which may facilitate increased uptake of toxicants, have also been

incorporated into predictive models of xenobiotic toxicity (Lassiter and Hallam 1989; Kiffney and Clemets 1996).

The other factor, besides metabolic rate that may explain body size relationships in tolerance to toxicants is the lipid composition of an organism. As a larval sea lamprey approaches metamorphosis, they accumulate large amounts of lipid (Lowe et al. 1973) to sustain them through this non-trophic period, which lasts up to 4 months (Holmes and Youson 1994; Kao et al. 1997). Animals with larger reserves of these energy stores are generally able to survive longer during toxicant exposures (Lassiter and Hallam 1989). In sea lampreys, the size of the lipid pool is reflected by a greater condition factor (CF =  $((\text{mass}/\text{length}^3) \times 10^6)$ , which is calculated using both body mass and length measurements (Holmes and Youson 1994). Larval sea lampreys with a CF > 1.5 in the autumn, are more likely to enter metamorphosis the following summer (Holmes and Youson 1994). The effect of lipid content and CF on TFM sensitivity has not yet been elucidated in larval sea lampreys, however.

Due to the fact that there is a strong body size relationship with the lipid and glycogen content in larval sea lampreys (V. Hoytfox and M.P Wilkie 2012 unpublished findings) and the mass specific rates of TFM uptake are greater in smaller animals (Chapter 2), the goal of the present chapter was to test the hypothesis that larger sea lampreys would be able to survive TFM treatments longer than smaller sea lampreys and/or those with a lower CF. To test this hypothesis, acute toxicity tests were performed to quantify the lethal concentrations of TFM needed to cause mortality in 50% (LC<sub>50</sub>) and 99.9% (LC<sub>99.9</sub>) [minimum lethal concentrations (MLC)] in different size groups of sea lamprey over 12 h exposure periods to

TFM. The time to death (h), as measured by the time needed for 50% of sea lamprey to die at given concentrations of TFM ( $LT_{50}$ ), and the mean survival time in different sized sea lampreys were also quantified.

## **3.2 Methods and Materials**

### **3.2.1 Sea lamprey collection and holding conditions**

Larval sea lampreys were collected in June 2015 using pulsed-DC backpack electrofishers (ABP-2 Electrofisher, Electrofishing Systems, LLC, Madison, WI, USA) from the Black River, Michigan, which drains into Lake Huron. The larvae were immediately anesthetized with 0.5 g tricaine methanesulfonate (MS-222) plus 1.0g of sodium bicarbonate, sorted by body length (to the nearest 1.0 mm) into separate (70 L) coolers filled with approximately 40 L of aerated Black River water (Temp = 17.5 – 20°C; conductivity = 387 – 415  $\mu\text{S cm}^{-1}$ ) and then transported by pick-up truck to the Hammond Bay Biological Station, Millersburg, MI. After arrival, the larvae were then distributed according to body length (50 – 70mm, 71 – 90mm, 91 – 110mm, and 111 – 130mm) into four separate 200 L tanks continually receiving filtered, aerated Lake Huron water (pH = 8.0 – 8.15; hardness = 150  $\text{mg L}^{-1}$  as  $\text{CaCO}_3$ ; dissolved oxygen = 80 – 99 % saturation) at a rate of 2  $\text{L min}^{-1}$ . The bottom of each tank was lined with 4 – 5 cm of sand to provide burrowing substrate for the larvae. The animals were acclimated to the holding tanks for 2 weeks prior to the toxicity tests in July 2015. Larval sea lampreys were not fed prior to experiments.

All toxicity tests were performed at the Hammond Bay Biological Station in Millersburg, MI. All procedures were approved by the Wilfrid Laurier University Animal Care Committee and followed Canadian Council of Animal Care guidelines.

### 3.2.2 Toxicity Test Protocols

The acute toxicity tests on the different size ranges of larval sea lampreys (50 – 70mm, 71 – 90 mm, 91 – 110 mm, 111 – 130 mm) were preceded (1 – 2 days) by a range finder experiment, during which separate groups of larval sea lampreys (N = 10 per tank) were exposed to a different TFM concentrations for 24 h, and the data was compiled to select the appropriate range of TFM concentrations to which the animals would be exposed to in the acute toxicity tests (Table 1).

Each acute toxicity test followed American Society for Testing and Materials (ASTM International) guidelines (ASTM 2007). Prior to each test, larval sea lamprey were gently removed from their respective holding tanks, and acclimated to the experimental conditions overnight in 30 L glass aquaria, but without cotton or sand (burrowing substrate). At the same time, 16 L glass aquaria were filled with Lake Huron water dosed with sufficient TFM (from 3 g L<sup>-1</sup> field grade stock) to achieve the appropriate TFM test concentrations (1 control tank, plus each pre-determined TFM concentrations for each size group in triplicate; recorded in Appendix). The concentrations of TFM in the water were measured immediately after preparation, and immediately before the addition of test animals at 0800 the next day, and after 12 h and 24 h to ensure that TFM concentrations were stable (Appendix: Tables 2 – 5). Water pH was measured using a handheld pH meter (pH 11 meter, Oakton Instruments, IL, USA), to ensure that pH was maintained at pH 8.10 (Appendix: Tables 1 – 5). Because pH was relatively stable, tank water did not require manual pH adjustment. Temperature was maintained between 10 – 12°C by immersing the aquaria a water bath, in which water was re-

circulated using a 0.1 Amp, 115 V Model 1-AA pump and temperature controlled using a Minneapolis-Honeywell Temperature Controller (Minneapolis, MN).

After the toxicity tests, surviving lamprey were euthanized with an overdose of anesthetic after 24 h (1.5 g L<sup>-1</sup> tricaine methanesulfonate buffered in 3 g L<sup>-1</sup> sodium bicarbonate). Both the euthanized, surviving larvae and the dead larval sea lamprey were snap-frozen in liquid nitrogen and then stored at -80°C.

### **3.2.3 Analytical Methods**

Water samples were collected 12 h prior to the toxicity tests, and at 0 h, 12 h, and 24 h for a total of 4 water samples from each tank. Precision standards were made (0, 0.5, 1.0, 2.0, 3.0, 5.0 and 7.0 mg L<sup>-1</sup> TFM) using powdered 99.9% TFM (Sigma-Aldrich Chemical Co., St. Louis, MO) and filtered Lake Huron water, and a standard curve was constructed by measuring the absorbance of each sample spectrophotometrically (Genesys 6 spectrophotometer; Thermo Electron Corporation, MA, USA) at a wavelength of 395 nm (Department of Fisheries and Oceans, Sea Lamprey Control Center, Standard Operating Procedures IOP: 012.4).

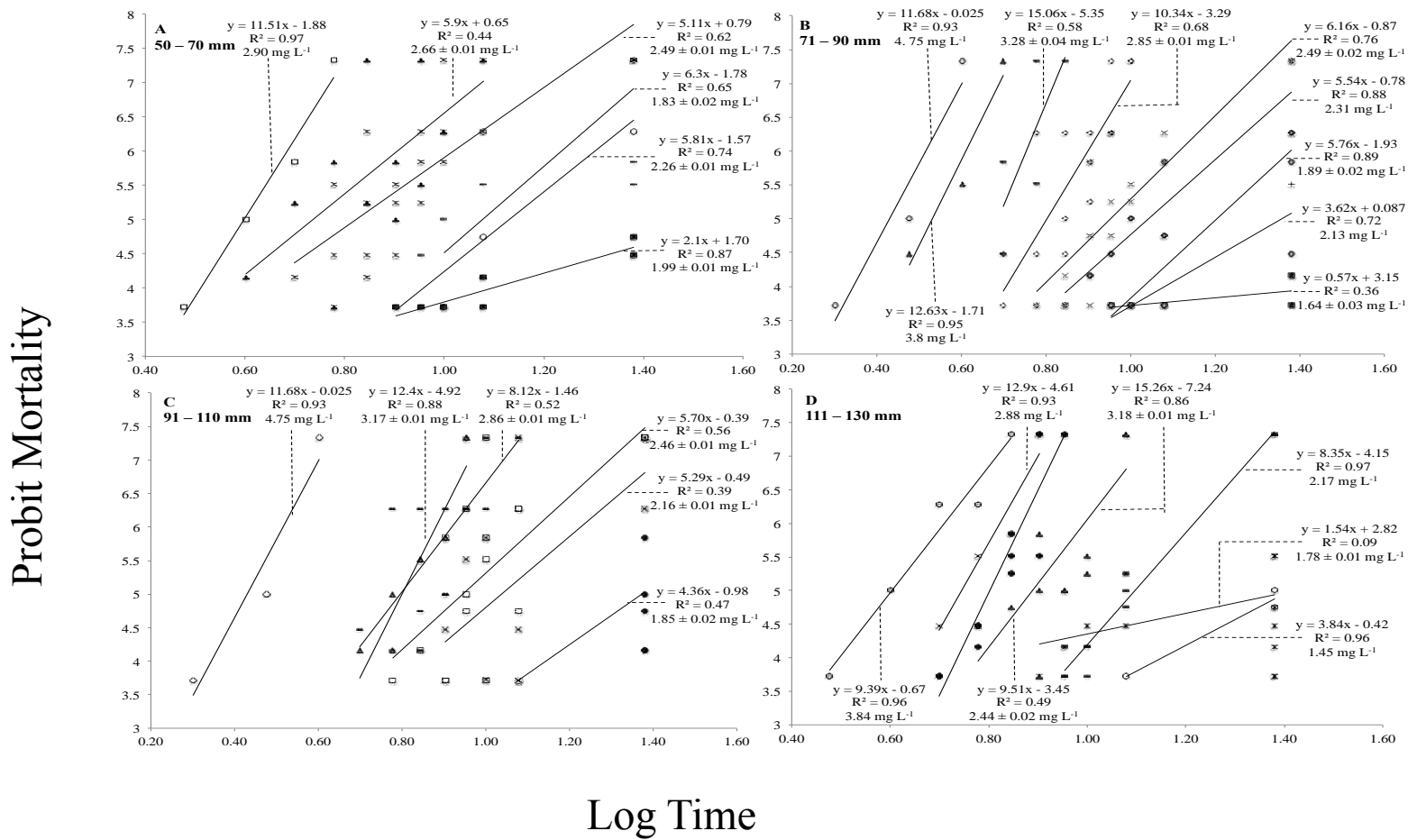
The LC<sub>50</sub> and LC<sub>50</sub> values with the respective 95% CI (Confidence Intervals) were calculated by probit analysis using the statistical program CETIS (Comprehensive Environmental Toxicity Information Scientific Software, McKinley, CA, USA), and confirmed with simple probit analysis in the statistical program JMP (version 12.0.1; SAS Institute Inc. 2015; Cary, NC). Simple probit analysis through a nonlinear fit in normal regression was used to determine LT<sub>50</sub> values with their corresponding 95% CI in the

statistical program JMP (version 12.0.1; SAS Institute Inc. 2015; Cary NC) and confirmed with probit analysis using Finney's table (Finney 1952). The differences in the 95% CI were used to assess statistical differences between  $LC_{50}$ ,  $LC_{99.9}$  and  $LT_{50}$  values for each size group. Linear regression with the standard least squares method was used to correlate larval sea lamprey body size with survival times during the toxicity tests using the commercial software package JMP (JMP version 12.0.1; SAS Institute Inc. 2015; Cary, NC).

### **3.3 Results**

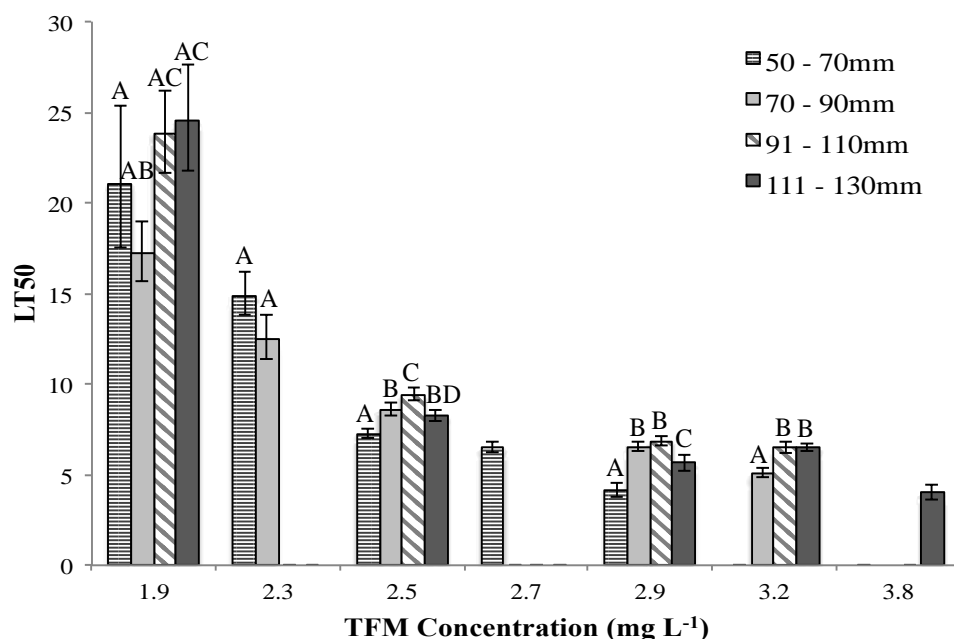
The toxicity of TFM at various to four size groups of sea lampreys was assessed using log-probit analysis (Figure 3-1). No changes in the slope of probit mortality vs. log time relationship were observed at a given concentration. It was notable, however, that the slopes of the probit mortality vs. log time relationship were steepest at higher doses of TFM, which is consistent with higher rates of mortality that were observed compared to the lowest concentrations (Figure 3-1).





**Figure 3-1: Probit mortality versus log time for different size ranges of larval sea lampreys exposed to TFM.** Sizes ranged from (A) 50 – 70mm; n = 160, (B) 71 – 90 mm; n = 180, (C) 91 – 110 mm; n = 170, (D) 111 – 130 mm; n = 120. Concentrations of TFM ( $\text{mg L}^{-1}$ ) and equations of each line are noted individually on each panel. All tests were done in Lake Huron water ( $\text{pH} = 8.1 \pm 0.01$ ; alkalinity =  $460 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$ ; temperature =  $12.1 \pm 0.05$ ).

From the log-probit analysis, the  $LT_{50}$  values for each of the four larval size groups were calculated from concentrations of TFM ranging from 1.9 – 3.8 mg L<sup>-1</sup> (Figure 3-2). Not surprisingly, the  $LT_{50}$  values were inversely proportional to TFM concentration, decreasing from the lowest to the highest TFM concentration (Figure 3-2). In only some cases, the  $LT_{50}$  at given TFM concentrations was directly proportional to body size. For instance, at 1.9 mg L<sup>-1</sup>, the 91 – 110 mm and 111 – 130 mm size groups had significantly longer  $LT_{50}$ 's ( $24 \pm 2$  h and  $25 \pm 3$  h, respectively) than the 71 – 90 mm group of animals ( $17 \pm 2$  h; Figure 3-1). However, due to the much greater variability of the data, the smallest size group, 50 – 70mm, did not have an  $LT_{50}$  value at 1.9 mg L<sup>-1</sup> that was different from any other group ( $21 \pm 4$  h). At TFM concentrations of 2.5, 2.9 mg L<sup>-1</sup>, and 3.2 mg L<sup>-1</sup>, the smallest size group (50 – 70 mm) had significantly shorter  $LT_{50}$ 's compared to the other groups (Figure 3-2).



**Figure 3-2: Relationship between body length and the  $LT_{50}$  of larval sea lampreys**

**exposed to TFM.**  $LT_{50}$  values were calculated using simple probit analysis, confirmed with Finney's table (1952), for four size groups of larval sea lamprey. Data is shown as the  $LT_{50}$  with the upper and lower 95% CI at each concentration of TFM ( $mg L^{-1}$ ). Bars at each concentration that share the same letter and whose CI overlap were not considered

significantly different from one another. Sample sizes were unequal, the number of tanks ( $N = 10$  larval sea lamprey per tank) for each size group at each concentration were as follows:

**1.9  $mg L^{-1}$ :** 50 – 70 mm ( $N = 2$ ), 71 – 90 mm ( $N = 3$ ), 91 – 110 mm ( $N = 6$ ), 111 – 130 mm ( $N = 4$ );

**2.3  $mg L^{-1}$ :** 50 – 70 mm ( $N = 4$ ), 71 – 90 mm ( $N = 2$ );

**2.5  $mg L^{-1}$ :** 50 – 70 mm ( $N = 3$ ), 71 – 90 mm ( $N = 3$ ), 91 – 110 mm ( $N = 4$ ), 111 – 130 mm ( $N = 3$ );

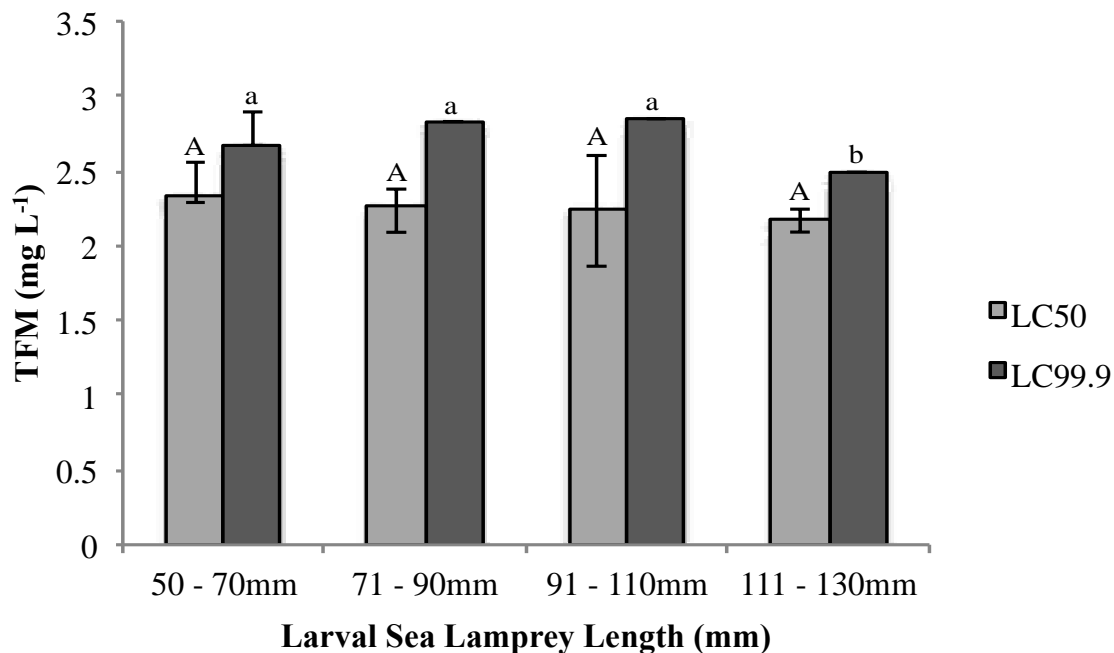
**2.7  $mg L^{-1}$ :** 50 – 70 mm ( $N = 3$ );

**2.9  $mg L^{-1}$ :** 50 – 70 mm ( $N = 2$ ), 71 – 90 mm ( $N = 3$ ), 91 – 110 mm ( $N = 3$ ), 111 – 130 mm ( $N = 1$ );

**3.2  $mg L^{-1}$ :** 71 – 90 mm ( $N = 2$ ), 91 – 110 mm ( $N = 2$ ), 111 – 130 mm ( $N = 3$ );

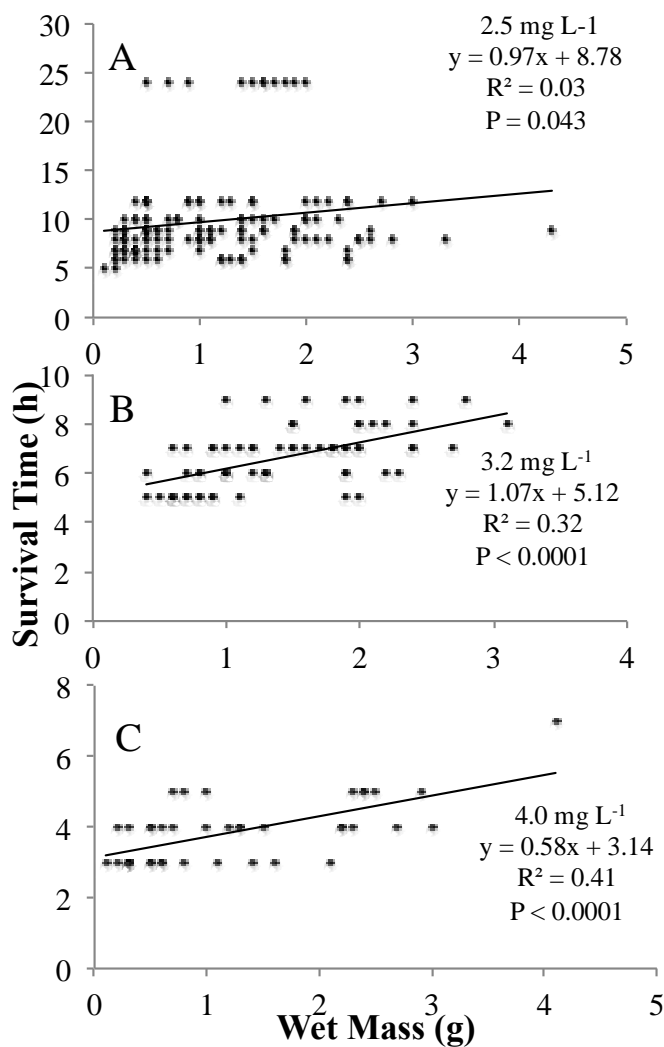
**3.8  $mg L^{-1}$ :** 111 – 130 mm ( $N = 1$ ).

The 12 h LC<sub>50</sub> of TFM for larval sea lampreys, based on body length (50 – 130 mm), ranged from 2.17 - 2.33 mg L<sup>-1</sup>, with overlapping 95% CIs (Figure 3-3). The 12 h LC<sub>99,9</sub> of TFM for larval sea lampreys was highest in the 91 – 110 mm group (2.84 mg L<sup>-1</sup>, 95% CI = 2.84 - 2.94 mg L<sup>-1</sup>), which was not significantly different from the 50 – 70 mm group (2.66 mg L<sup>-1</sup>, 95% CI = 2.42 - 2.66 mg L<sup>-1</sup>) or the 71 – 90 mm size group (2.83 mg L<sup>-1</sup>, 95% CI: 2.83 - 2.83 mg L<sup>-1</sup>). However, the LC<sub>99,9</sub> of TFM in the 111 – 130 mm group had a surprisingly lower LC<sub>99,9</sub> compared to the smaller sea lampreys (50 – 110 mm) (2.48 mg L<sup>-1</sup>, 95% CI = 2.48 - 2.48 mg L<sup>-1</sup>; Figure 3-3). Because complete mortalities were observed in the LC<sub>99,9</sub> measurements, there was very little variation in the CI (Figure 3-3).



**Figure 3-3: The 12 h LC<sub>50</sub> and the 12 h LC<sub>99.9</sub> compared to different body lengths of larval sea lamprey exposed to 3-trifluoromethyl-4-nitrophenol (TFM) (50 – 70 mm N = 20; 71 – 90 mm N = 19; 91 – 110 mm N = 22; 111 – 130 mm N = 22). For each sample size, N = 1 represents one tank with ten larval sea lampreys. All measurements were made in moderately hard Lake Huron water (pH = 8.1 ± 0.01; alkalinity = 460 mg L<sup>-1</sup> as CaCO<sub>3</sub>; temperature = 12.1 ± 0.05). Values expressed as ± the 95% confidence interval (CI). Bars with overlapping CI are not significantly different and share the same letter (upper case for LC<sub>50</sub> and lower case for LC<sub>99.9</sub>). LC<sub>99.9</sub> values with small CI are due to complete mortality of tanks at that concentration.**

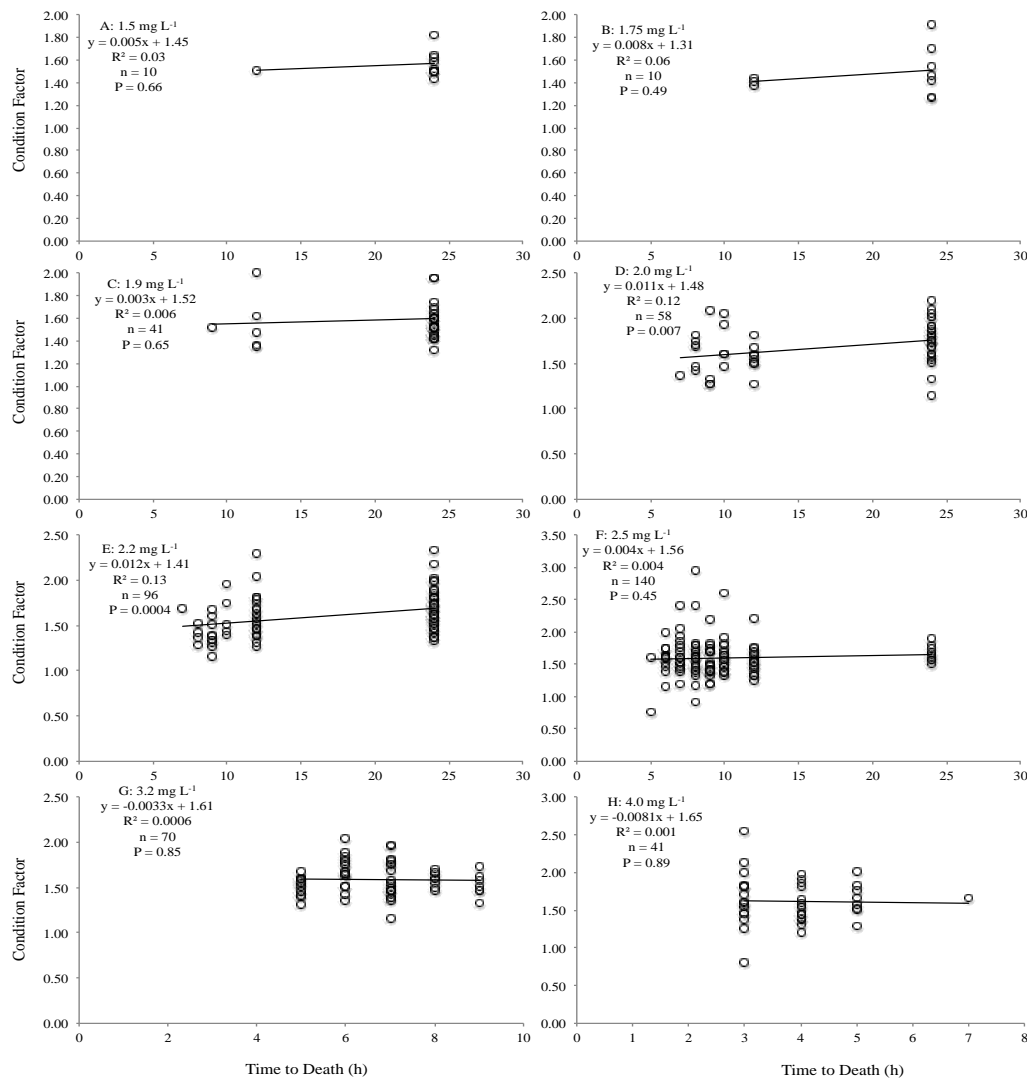
The clearest relationships between TFM sensitivity and body size were obtained by comparing body mass to survival time. Regression analysis demonstrated that TFM survival time was directly proportional to body mass at TFM exposure concentrations of 2.5 mg L<sup>-1</sup>, 3.2 mg L<sup>-1</sup> and 4.0 mg L<sup>-1</sup> (Figure 3-4). Notably, the strength of the linear relationship increased as the TFM exposure concentration increased. At 2.5 mg L<sup>-1</sup>, the slope of the line was (0.97), with a weak correlation coefficient (R<sup>2</sup>) of only 0.03 (Figure 3-4A). However, at 3.2 mg L<sup>-1</sup>, the slope of the line was slightly steeper (1.07), but the strength of the linear relationship (R<sup>2</sup> = 0.32) was much stronger (Figure 3-4B). At the highest concentration of 4.0 mg L<sup>-1</sup>, the slope of the relationship between survival time and body mass was 0.58, with an R<sup>2</sup> of 0.41 (Figure 3-4C).



**Figure 3-4: Relationship between larval sea lamprey body mass and survival times during TFM exposure.** The survival times of larval sea lampreys are depicted at the following concentrations: A) 2.5 mg L<sup>-1</sup> (N = 140); B) 3.2 mg L<sup>-1</sup> (N = 70) and C) 4.0 mg L<sup>-1</sup> (N = 41). Toxicity tests were performed in Lake Huron water (pH = 8.1 ± 0.01; alkalinity = 460 mg L<sup>-1</sup> as CaCO<sub>3</sub>; temperature = 12.1 ± 0.05).

Interestingly, there were no differences in the CF of larval sea lampreys and the length of time they were able to survive TFM exposure (data not shown). Similar trends were generally observed when the data was analyzed using least squares analysis, except at exposure concentrations of 2.0 and 2.2 mg L<sup>-1</sup>, where larval sea lamprey with a higher CF survived longer compared to those with a lower CF (Figure 3-5D; 3-5E). At all other concentrations, even the highest (4.0 mg L<sup>-1</sup>), there was no relationship between condition factor and time to death during TFM exposure (Figure 3-5).





**Figure 3-5: Relationship between condition factor (CF) and survival time of individual larval sea lampreys exposed to TFM for 24 h.** In these experiments, larval sea lampreys were exposed to the following concentrations of TFM, with the time to death (hours survived) compared with the sea lamprey's CF, in each figure panel: A) 1.5 mg L<sup>-1</sup> (N = 10); B) 1.75 mg L<sup>-1</sup> (N = 10); C) 1.9 mg L<sup>-1</sup> (N = 41); D) 2.0 mg L<sup>-1</sup> (N = 58); E) 2.2 mg L<sup>-1</sup> (N = 96); F) 2.5 mg L<sup>-1</sup> (N = 140); G) 3.2 mg L<sup>-1</sup> (N = 70); H) 4.0 mg L<sup>-1</sup> (N = 41). Linear regression using standard least squares method was used at each concentration to determine correlations between CF and time to death (h). All tests were done in Lake Huron water (pH= 8.1 ± 0.01; alkalinity = 460 mg L<sup>-1</sup> as CaCO<sub>3</sub>; temperature = 12.1 ± 0.05).

### 3.4 Discussion

The present study demonstrates that while the overall LC<sub>50</sub> and LC<sub>99.9</sub> were similar for sea lampreys regardless of body size, the larger sea lampreys tended to survive for longer periods of time when exposed to identical concentrations of TFM. While the LC<sub>99.9</sub> was statistically lower in the longest sea lamprey body size group (111 – 130 mm), this is unlikely to be physiologically relevant due to the lack of variation in the CI. This suggests that larger sea lampreys with increased mass, or those with a faster growth rate, would have a survival advantage compared to smaller animals when subjected to TFM applications in the field, increasing the risk of residual sea lampreys. The greater survival times of larger larval sea lampreys could be due to lower mass specific metabolic rates, which would be accompanied by lower rates of branchial ventilation and therefore TFM uptake (Chapter 2). The higher metabolic rate in smaller fish can be correlated to the simple estimate that a decrease in mass by a factor of 10 will generally result in an increased respiration rates by a factor of 5 (Mackay et al. 2014).

The main site of TFM uptake is likely at the gills of the sea lamprey, which was supported in Chapter 2 when the highest TFM uptake rates were observed in smaller fish with higher  $\dot{M}O_2$ . In such instances, the smaller sea lampreys would accumulate and reach a critical threshold of internal TFM concentration at a faster rate compared to larger sea lampreys. This would result in the sea lampreys with shorter body lengths (Figure 3-2) and smaller body masses (Figure 3-4) having a decreased survival time when exposed to TFM, which was observed in the present study.

As with many dose-dependency relationships, at higher concentrations of TFM, the  $LT_{50}$  values were shorter (Figure 3-2). When the time to death (h) was plotted by body mass (g) of the larval sea lampreys, a positive linear correlation with toxicity emerged (Figure 3-4). At three concentrations of TFM (2.5, 3.2 and 4.0 mg L<sup>-1</sup>) larval sea lampreys with the greatest body mass were also found to survive the longest. Given the Empirical Stream Treatment Ranking (ESTR) system, streams that have large larval sea lampreys are predicted to have higher productivity, in which many sea lampreys are likely to undergo metamorphosis. These rivers and streams are then given the highest priority for lampricide treatments (Johnson 1987; Christie and Goddard 2003; McDonald and Kolar 2007). However, the selection of streams with large animals for lampricide treatments may be counter-productive and increase the risk of residuals if larger sea lampreys have a survival advantage when exposed to TFM.

Larger animals with greater lipid contents and energy reserves have been known to have increased tolerance to toxicants – coined as “survival of the fittest” by Lassiter and Hallam (1990). Mackay et al. (2014) also proposed that lipid content and body mass were linked to differences in the sensitivity of animals to organic chemicals. While metabolites were not compared in this study, it is possible that longer survival times for the larger sea lampreys was due to a greater lipid content, which has been associated with increased body size (Lowe et al. 1973; Potter et al. 1978). However, the relationship between lipid stores and toxicity is complex. Having more lipid stores in general can contribute to increased tolerance to toxicants because it takes longer for the body to absorb enough chemical to reach a critical body residue (CBR), leading to death (Mackay et al. 2014). Accordingly, TFM would

accumulate at a slower rate per unit mass of lipid compared to the smaller sea lampreys, and therefore survive longer (Figures 3-2 and 3-4). While the amount of TFM in the sea lamprey's bodies was not directly measured in this study, it would be relatively straightforward to measure lipid content and TFM burden in sea lampreys using HPLC.

The uptake and subsequent toxicity of a toxicant is influenced by its log  $K_{ow}$  (Erickson et al. 2006). Lipophilic toxicants, such as methyl-Hg with a  $K_{ow}$  1.7 – 2.5 (Halbach 1985, Major et al. 1991), readily dissolve and accumulate in fatty tissues (Hendriks and Heikens 2001). However, with a log  $K_{ow}$  of 2.77 (Schmitt et al. 2000), TFM has moderate lipid solubility and would be less likely to accumulate in this manner (Huckins et al. 1997). Indeed, the retention of TFM by non-target organisms such as rainbow trout and catfish (*Ictalurus punctatus*) is relatively short (Hubert et al. 2001), with 90 – 99% of TFM being cleared from fish muscle tissue within 6 – 12 h of recovery (Vue et al. 2002). Indeed, the biological half-life of TFM ranges from approximately 1.8 h in rainbow trout and approximately 2.5 h in lake sturgeon (LeClair 2014).

Lipids, or triacylglycerols, contain fatty acids, which can be oxidized via the citric acid cycle (Kreb's cycle). Because TFM uncouples oxidative phosphorylation (Niblett and Ballantyne 1976; Birceanu et al. 2011), it seems unlikely that greater lipid reserves would allow sea lampreys to more effectively counter the impairing effects that TFM has on ATP production. Sea lampreys and non-target organisms rely more on anaerobic fuels such as phosphocreatine (PCr) and glycolysis to generate ATP during TFM exposure. Indeed, phosphocreatine reserves in the brain and muscle of both lamprey and trout are greatly reduced during TFM exposure due to increased rates of creatine phosphokinase-mediated

hydrolysis of PCr to sustain ATP supply (Viant et al. 2001; Wilkie et al. 2007; Birceanu et al. 2009; Henry et al. 2015). However, anaerobic energy reserves such as glycogen and PCr are finite and cannot sustain ATP supply indefinitely (Hochachka 1990), which eventually results in mortality during TFM exposure (Birceanu 2009).

The depletion of PCr and glycogen stores would be expected to be faster in smaller compared to larger fishes, due to their greater mass specific metabolic (ATP) demands and lower baseline levels of these energy stores. In the rainbow trout, Ferguson et al. (1993) found a strong body size influence on the metabolic energy stores in the white muscle of rainbow and that the depletion of energy reserves such as glycogen and phosphocreatine, as well as acid-base disturbances, were proportional to body mass following exercise. Similar observations were made for fish under hypoxia stress (Nilsson and Ostlund-Nilsson 2008). Thus, a larger lamprey would also be able to survive longer when exposed to TFM because they may also have greater glycogen and/or PCr stores, allowing them to rely on anaerobic respiration for longer than a smaller sea lamprey. Indeed, V. Hoytfox and M.P. Wilkie (2012 unpublished findings) reported that the whole body glycogen and lipid stores were positively correlated with body size in larval sea lampreys, which was also related to survival during TFM exposure.

A drawback of increased reliance on anaerobic energy reserves is that increased glycolysis leads to metabolic acidosis, along with increased lactate production (Hochachka 1990), which could also lead to mortalities (Nilsson and Ostlund-Nilsson 2008). It would also be important for the larval sea lamprey to counter such acid-base disturbances in order to survive TFM exposure. Ferguson et al. (1993) also demonstrated that the white muscle buffer

capacity of larger trout was greater than in smaller fish, enabling them to withstand greater reductions in white muscle pH following exercise, which is more intensive in larger fish. The buffer capacity of the plasma and muscle of larval lampreys is known, but it would be informative to establish if a similar relationship exists in these animals.

As larval sea lamprey grow and approach metamorphosis, there is an increase in their condition factor (CF), where  $CF = (W/L^3) \times 10^6$ , with  $W$  and  $L$  representing larval weight (g) and length (mm), respectively (Potter et al. 1978; Holmes and Youson 1994). This increase in lipid stores allows the sea lamprey to survive the period of starvation that accompanies the metamorphosis from a microphagous lifestyle as a larvae to hematophagous parasite as a juvenile (Potter et al. 1978; Youson 2003). It would be informative to determine if immediately pre-metamorphic animals are more tolerant to TFM than non-transforming animals.

In the present study, pH was maintained around 8.0 – 8.15. At this pH during a typical field treatment, a stream would likely be treated with 1.2 – 1.5 times the MLC of TFM for that pH range, which would be approximately 3.4 – 7.0 mg L<sup>-1</sup> (Bills et al. 2003; McDonald and Kolar 2007). Interestingly, when sea lampreys were exposed to higher concentrations of TFM that were reflective of an actual stream treatment levels (~ 4.0 mg L<sup>-1</sup>), the larger sea lampreys survived significantly longer than the small larval sea lampreys (Figure 3-4). While a larger larval sea lamprey exposed to TFM may survive longer than its smaller conspecifics, the MLC where death is ultimately observed may be unaffected if the period of exposure is sufficiently long. Indeed, the time course over which death occurred was still within the time frame typically allotted to TFM treatments in the field. This was

particularly true in fish exposed to 4.0 mg L<sup>-1</sup> TFM, in which the relationship between TFM survival time and body size was strongest, but death also took place within 7 h, which is well below the 9 h window of TFM exposure routinely used to treat lamprey-infested streams (Hansen et al. 2003; McDonald and Kolar 2007). However, this could have implications for larger larval sea lampreys surviving treatments, especially if exposure to TFM was transiently interrupted.

In addition to being able to survive longer during a lampricide exposure, it has been demonstrated that sea lamprey rapidly recover from 4 – 6 h of TFM exposure (Clifford et al. 2012). As well, not all sea lamprey will be exposed to lampricide for the desired 9 h; some lamprey may remain burrowed, or in riffles or pools of the stream where the TFM does not adequately penetrate. In these cases, the large larval sea lamprey may be able to survive treatments. Given the relationships between TFM uptake and tolerance with body size, it would be informative to determine if larger larval sea lampreys recover more quickly from short-term TFM exposure.

### **3.5 Conclusion**

Tolerance to TFM in larval sea lampreys involves a combination of abiotic and biotic factors. This study quantified the influences of body size with the survival of larval sea lampreys exposed to a range of TFM concentrations, including concentrations that are comparable to those used during actual lampricide treatments (1.2 – 1.5 times MLC). Using the ESTR system, streams with the largest larval sea lampreys are currently selected for lampricide treatments; But, because larger larval sea lamprey are

more likely to survive a TFM treatment, this approach may paradoxically result in a greater risk of residual sea lampreys due to their relatively lower rates of TFM uptake (Chapter 2), and their ability to withstand toxic concentrations of TFM for longer periods. Thus, it would be advisable to be cognizant of the greater TFM tolerance of larger larval sea lampreys when selecting streams for TFM treatment, and possibly even consider altering the timing of treatments to take advantage of the greater sensitivity of larval sea lampreys earlier in their life cycle. Another advantage of such an approach is that TFM treatments could be more efficient by reducing the amount of TFM required to achieve desired mortality rates.



### 3.6 Appendix

**Table 1: Measured concentrations of TFM and pH in acute toxicity tests.** N = 10 per tank for each size group; total number of larval sea lampreys = 360. Temperature ranged from 11.8 – 12.5°C, and dissolved oxygen (DO) varied from 90 – 102%.

<b>Nominal TFM (mg L<sup>-1</sup>)</b>	<b>Actual TFM (mg L<sup>-1</sup>)</b>	<b>pH</b>
0	0	7.94 – 8.12
0.5	0.46 ± 0.01	7.99 – 8.14
1.0	0.93 ± 0.01	7.98 – 8.09
1.5	1.43 ± 0.02	7.95 – 8.11
2.0	1.86 ± 0.02	7.93 – 8.11
2.5	2.42 ± 0.03	8.01 – 8.14
3.0	2.90 ± 0.01	7.96 – 8.14
4.0	3.81 ± 0.01	8.00 – 8.11
5.0	4.79 ± 0.01	7.90 – 8.12

**Table 2: Measured concentrations of TFM to which difference size groups of larval sea lampreys were exposed to during acute TFM toxicity tests.** Size group: 50 – 70mm, acute toxicity test. Larval sea lampreys were N = 10 per tank. Temperature ranged from 10.9 – 12.8°C and dissolved oxygen (DO) varied from 84 – 99%.

<b>Nominal TFM (mg L<sup>-1</sup>)</b>	<b>Actual TFM (mg L<sup>-1</sup>)</b>	<b>pH</b>	<b>N</b>
0	0	8.11 – 8.13	50
1.0	1.01 ± 0.02	8.04 – 8.16	50
1.4	1.40 ± 0.06	8.08 – 8.16	50
1.6	1.61 ± 0.03	8.13 – 8.20	30
1.75	1.77 ± 0.02	8.10 – 8.13	40
1.9	1.98 ± 0.01	8.04 – 8.14	30
2.1	2.08 ± 0.04	8.05 – 8.14	40
2.25	2.29 ± 0.01	8.02 – 8.13	30
2.5	2.50 ± 0.0	8.03 – 8.10	30
2.7	2.66 ± 0.03	8.03 – 8.12	30

**Table 3: Measured concentrations of TFM to which difference size groups of larval sea lampreys were exposed to during acute TFM toxicity tests.** Size group: 71 – 90 mm, acute toxicity test. Larval sea lampreys were N = 10 per tank. Temperature ranged from 11.0 – 13.4 – 12.8°C and dissolved oxygen (DO) varied from 83 – 100%.

<b>Nominal TFM (mg L<sup>-1</sup>)</b>	<b>Actual TFM (mg L<sup>-1</sup>)</b>	<b>pH</b>	<b>N</b>
0	0	8.05 – 8.13	20
1.1	1.12 ± 0.03	8.01 – 8.14	30
1.5	1.47 ± 0.03	8.05 – 8.13	30
1.75	1.71 ± 0.05	8.04 – 8.20	30
1.9	1.93 ± 0.03	8.00 – 8.10	30
2.1	2.13 ± 0.01	8.07 – 8.13	20
2.3	2.31 ± 0.01	8.07 – 8.09	20
2.5	2.51 ± 0.06	8.01 – 8.07	20
2.8	2.83 ± 0.01	8.07 – 8.08	20
3.2	3.26 ± 0.08	8.02 – 8.08	20

**Table 4: Measured concentrations of TFM to which difference size groups of larval sea lampreys were exposed to during acute TFM toxicity tests. Size group: 91 – 110 mm, acute toxicity test. Larval sea lampreys were N = 10 per tank. Temperature ranged from 10.8 – 12.1°C and dissolved oxygen (DO) varied from 90 – 100%.**

<b>Nominal TFM (mg L<sup>-1</sup>)</b>	<b>Actual TFM (mg L<sup>-1</sup>)</b>	<b>pH</b>	<b>N</b>
0	0	8.01 – 8.14	20
1.3	1.28 ± 0.04	8.01 – 8.09	40
1.6	1.58 ± 0.05	8.02 – 8.14	40
1.8	1.83 ± 0.03	8.01 – 8.12	50
2.1	2.15 ± 0.02	8.01 – 8.12	40
2.5	2.46 ± 0.03	8.01 – 8.09	30
2.8	2.84 ± 0.00	8.03 – 8.08	20
3.2	3.17 ± 0.04	8.00 – 8.14	20

**Table 5: Measured concentrations of TFM to which difference size groups of larval sea lampreys were exposed to during acute TFM toxicity tests.** Size group: 111 – 130mm acute toxicity test. Larval sea lampreys were N = 10 per tank. Temperature ranged from 10.5 – 12.3°C and dissolved oxygen (DO) varied from 98 – 100%.

<b>Nominal TFM (mg L<sup>-1</sup>)</b>	<b>Actual TFM (mg L<sup>-1</sup>)</b>	<b>pH</b>	<b>N</b>
0	0	8.06 – 8.11	20
0.75	0.77 ± 0.00	8.14	10
1.2	1.17 ± 0.02	8.04 – 8.13	30
1.4	1.44 ± 0.00	8.07	10
1.7	1.73 ± 0.02	8.04 – 8.14	40
1.8	1.85 ± 0.01	8.04 – 8.15	30
2.2	2.17 ± 0.01	8.06 – 8.17	30
2.5	2.48 ± 0.03	8.06 – 8.09	20
3.2	3.18 ± 0.02	8.04 – 8.09	30

## Chapter 4

### Integration of Biotic Factors into Methods of Sea Lamprey Control

#### 4.1 Introduction

Lampricide treatments are 90 – 100% effective (Smith and Swink 2003), but residual sea lampreys that survive treatments remain a challenge. The present study has revealed that sea lamprey body sizes may be a useful predictor of TFM tolerance, which could be used to predict the likelihood of residual sea lampreys in streams designated for lampricide applications. Currently, streams are evaluated and selected for lampricide treatment based on larval density, habitat, cost of treatment, and estimated parasitic sea lamprey production (Christie et al. 2003; Hansen et al. 2003). As well, abiotic factors such as temperature, pH, alkalinity, season, and water flow, are considered during lampricide applications (Hubert 2003; McDonald and Kolar 2007; Scholefield et al. 2008). However, this thesis has indicated that biotic factors also have a place in sea lamprey control, both in selecting streams for lampricide treatment and during the application of lampricide. By demonstrating that body size is inversely proportional to rates of TFM uptake and excretion, resulting in larger animals having greater TFM tolerance, body size and metabolic rates should be incorporated into sea lamprey control. Below, I propose how this information could be used to increase lampricide effectiveness, and to help prioritize sea lamprey infested streams for TFM treatment.

## **4.2 Evaluation of methods for selecting streams for lampricide treatments**

The annual budget for sea lamprey control is greater than 15 million US dollars (Hansen and Jones 2008). Optimizing the timing, application process, and targeting sea lampreys when they are most vulnerable and least likely to survive as residuals is key in balancing the limited resources available for the SLC program. The methods used to select streams for lampricide treatments are regularly evaluated and updated according to recent treatment success and the results of ongoing research. Streams do not need lampricide treatments yearly because sea lampreys typically remain in the larval phase for 3 - 7 years prior to metamorphosis (Applegate 1950; Potter 1980; Holmes and Youson 1994). This allows for flexibility in treatment programs, and allows for evaluations of stream productivity to be assessed and streams to be prioritized for lampricide applications, based on larval density and estimated productivity (which streams are likely to have the most transforming sea lampreys) (Christie et al. 2003; Slade et al. 2003; Hansen and Jones 2008). The two current stream survey methods are QAS and RA (quantitative assessment sampling and rapid assessment, respectively) (Hansen and Jones 2008). Using the QAS method, data is provided based on the density, size, distribution, and habitat (Slade et al. 2003). Currently, the results of the QAS are then combined with the Empiric Stream Treatment Ranking system (ESTR), which predicts the number of transformers per stream and determines which streams should be treated to maximize the cost: benefit ratio of lampricide treatment resources (Hansen and Jones 2008). Data collected with QAS is costly because it involves intensive assessment of larval sea lamprey populations in the streams (Slade et al. 2003). The second type of sampling, RA, evaluates the first type of habitat encountered at a site, as opposed to the best

available habitat in QAS, and compares cost per kill for larvae > 100 mm, while QAS incorporates a transformer estimate and calculates the basis for ranking based on cost per kill of transformers (Hansen and Jones 2008).

In the present study, smaller larval sea lamprey with higher mass specific metabolic rates took up TFM faster than larger ones, and also died sooner during TFM exposures. Thus, it may be useful to treat streams with lampricide before there are high densities of large pre-metamorphic lamprey, as they may be more likely to end up as residuals. Rather than increasing TFM concentrations, extending the TFM exposure period when large larval sea lampreys are present may be a more effective way to achieve target mortality rates, as it was demonstrated a larger larval sea lamprey body size correlates to an increased survival time.

#### **4.3 Integrating Sea Lamprey Physiology into the Sea Lamprey Control Program**

The sea lamprey control program uses an integrated pest management approach, which incorporates three strategies to suppress invasive sea lamprey populations in the Great Lakes: physical, alternative, and chemical (Figure 4-1). Physical measures include traps and barriers that prevent sea lampreys from reaching desirable spawning habitats (Larson et al. 2003). Even barriers and dams that were originally built in the 1800's for alternative purposes such as sawmills and water management have proved to be effective at preventing sea lampreys from exploiting new territories (Larson et al. 2003). Alternative experimental methods for controlling sea lamprey include pheromones, to either attract sea lampreys into traps from which they can be collected and removed, or to send signals to make spawning habitat appear unusable (Li et al. 2007; Figure 4-1). Chemical control using lampricides remains the most effective method of sea lamprey control in the Great Lakes, although



research is currently on-going to continually improve the efficiency of lampricide treatments, and reduce residual sea lampreys.

The findings of the present study investigated the likelihood of residual sea lampreys can be predicted based on these three physiologic characteristics:  $\dot{M}O_2$ , body size and life stage (Figure 4-1). First,  $\dot{M}O_2$  and body size were strongly correlated to both themselves and TFM uptake rate. The allometric scaling equations formulated for both  $\dot{M}O_2$  and TFM uptake can be used to predict the amount of TFM a larval sea lamprey will take up during a lampricide treatment (Figure 4-1). This could potentially help sea lamprey control officials to infer approximately how much TFM may be required to achieve target mortality rates during a sea lamprey treatment; Allowing a minimum amount of TFM to be applied, which would be both cost-effective, and may help protect non-target species from unnecessarily high concentrations of TFM.

As well, this study found increased survival times with larval sea lamprey body size. Therefore, one method to incorporate body size into sea lamprey control may be to consider increasing TFM exposure times during a lampricide treatment when large larvae are present and likely to end up as residuals; increasing TFM concentrations would not be as effective because no differences were found between the  $LC_{50}$  and  $LC_{99.9}$  of larval sea lampreys (Figure 4-1). For life stage, while it was found that larval sea lampreys could clear more TFM after 24 hours compared to parasitic sea lampreys, there were no differences in  $\dot{M}O_2$  or TFM uptake rates. In the future this would be a very informative experiment to better determine how life stage affects TFM sensitivity. However, it would be difficult to target and

treat metamorphosing sea lampreys as they are migrating downstream and into the Great Lakes.

#### 4.4 Conclusion and Further Directions

Using Kleiber's Law (Kleiber 1947), the scaling relationships relating for TFM uptake rates and  $\dot{M}O_2$  of larval sea lampreys were quantified. The power equation described relates mass to metabolic rate is  $\dot{M}O_2 = aM^b$ , where  $\dot{M}O_2$  is the metabolic rate (dependent variable, also the *TFM uptake rate* in Ch. 2),  $a$  is the proportionality constant (y-intercept),  $M$  is the mass (independent variable) and  $b$  is the scaling exponent (slope) (Kleiber 1947; Goolish 1991; Glazier 2005). Future studies should focus on the mechanisms behind the allometric relationships of TFM uptake and clearance with sea lamprey's mass (g) and  $\dot{M}O_2$  ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ ), and could do so by incorporating TFM response experiments as well as variations in temperature.

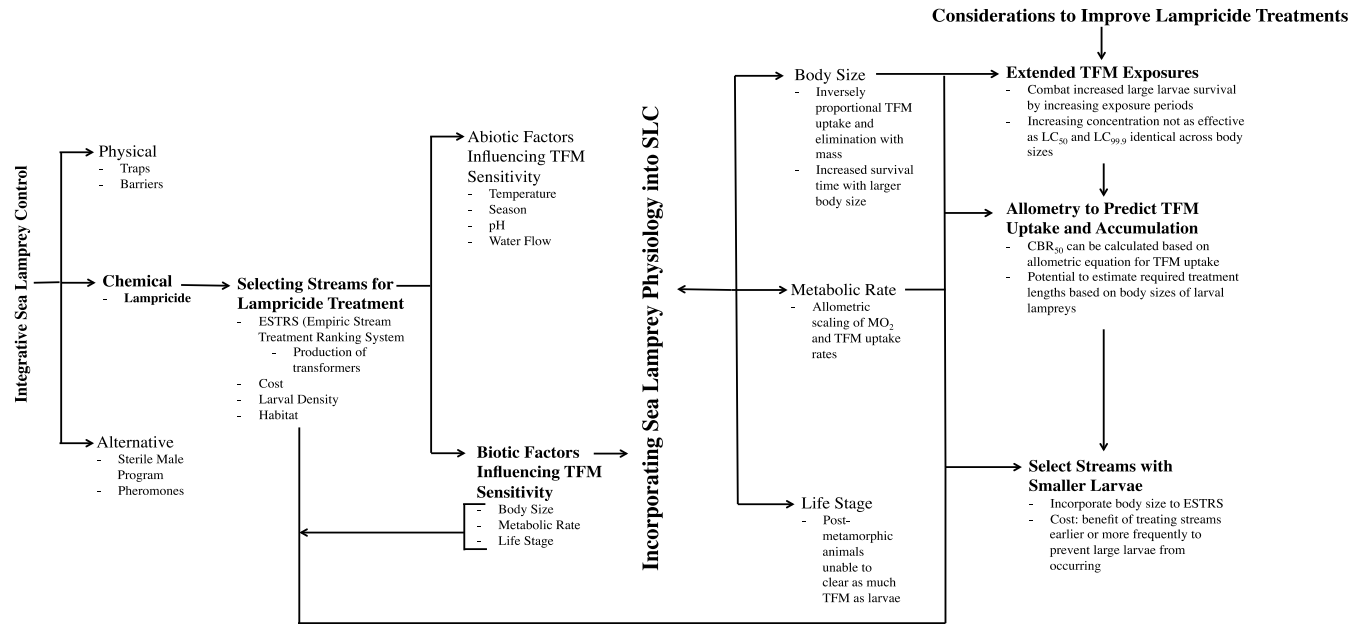
To calculate the amount of TFM being taken up by a sea lamprey over time, the scaling relationship of TFM uptake rates should include both TFM doses and temperature. Experiments to quantify TFM uptake rates should be performed at several temperatures to reflect ambient stream temperatures according to season, as well as a range of concentrations that would be applicable in the field. The present study only quantified TFM uptake rates at the  $LC_{50}$  of WLU well water; but TFM uptake is dose dependent and there is a positive correlation between the concentration of TFM with TFM uptake rates (TFM ranged from 0 – 26  $\text{mg L}^{-1}$ ;  $y = 1.4x + 2.8$ ;  $R^2 = 0.94$ ;  $P < 0.0001$ ; Smits (unpublished); data not shown). The

power scaling equation could be quantified at multiple concentrations of TFM, to test how concentration affects the allometric relationship between TFM uptake and mass.

As well, lipid analysis of larval sea lampreys may provide further data on how TFM is distributed within the body of a sea lamprey; and answer the question of whether increased lipids could act as a reservoir for lipophilic TFM, preventing it from circulation and protecting the brain and other vital organs from its perturbing effects. Using this approach, it would be possible to calculate the CBR<sub>50</sub> for TFM in larval sea lampreys. The model *TFM Uptake* =  $6.47M^{0.26}$  would be used to predict the amount of TFM a sea lamprey is taking up over time according to mass, and therefore an accurate estimate of the time it will take for the average sea lamprey to reach CBR<sub>50</sub> during a TFM treatment at the specified target concentration could be calculated. This data could be incorporated into the duration of a TFM application to reduce residual sea lampreys or possibly decrease the required treatment time (Figure 4-1).

From this, the incorporation of biotic factors of sea lampreys can be considered for sea lamprey control (Figure 4-1). The strong correlation between body size and  $\dot{M}O_2$  with TFM clearance may have implications on the ideal lampricide exposure times, as well as selection of streams for lampricide treatments. The goal of this research was to provide physiologic considerations for sea lamprey control, in order to ensure the efficiency of TFM treatments.

## 4.5 Figures



**Figure 4-1: Incorporating sea lamprey physiology into an integrative sea lamprey control program.** In addition to abiotic factors, such as water chemistry and flow, biotic factors can also be integrated into the sea lamprey control program. These include the body size and metabolic rates of sea lampreys, which markedly influence the rates of TFM uptake and sensitivity. By incorporating these biotic factors into the selection of streams for chemical control (TFM application) sea lamprey control agents may be able to predict which waters have a greater likelihood of residuals, so that appropriate measures (e.g. adjustment of treatment time and/or frequency) can be made to minimize such risks.



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