Investigation into the Ecological Costs of Sea Lamprey Control on Lake Sturgeon and Ammocoete Predators using Olfactory Techniques

A thesis presented to

The Faculty of Graduate Studies

of

Lakehead University

by

KATHRINE SAKAMOTO

In partial fulfillment of requirements
for the degree of
Master of Science in Biology

July 12, 2015

ABSTRACT

Fish that feed or travel in low light conditions particularly rely on their chemical senses, such as olfaction, for survival. Exposure to toxicants at concentrations lower than those causing mortality can have detrimental effects on olfactory senses. My research studied sea lamprey control from two ecological perspectives. The first was to determine if the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) affects the olfactory capabilities and behaviour of young-of-the-year (YOY) lake sturgeon (*Acipenser fulvescens*), reduces food consumption and induces a change in blood glucose and lactate. My methods utilized electro-olfactography (EOG), behavioural trials and blood analysis. The second part of my study investigated the attraction of lake sturgeon to the scent of lamprey ammocoetes as a food source, using chemosensory baits in four northwestern Ontario locations. Laboratory exposure of YOY lake sturgeon to TFM caused a reduced olfactory response to L-alanine, taurocholic acid and a food cue. It also reduced attraction to the scent of food and food consumption in the same species. Exposed fish were active for a higher percentage of time, but with slower acceleration. Fish were able to detect the scent of TFM, but did not significantly avoid it, which may expose fish to the full toxic effects. A number of small aquatic predators were attracted to ammocoete-conditioned baits. Healthy populations of these species may benefit sea lamprey control and help to restore ecological processes that would improve the functional performance of the Laurentian Great Lakes ecosystem.

LAY SUMMARY

Faculty and students in the Department of Biology are bound together by a common interest in explaining the diversity of life, the fit between form and function, and the distribution and abundance of organisms. This thesis helps to explain the ability of young-of-the-year (YOY) lake sturgeon to detect cues using olfaction, and how that ability is diminished after exposure to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM). It also demonstrates that YOY lake sturgeon exposed to TFM eat less. Although YOY lake sturgeon are able to detect the odour of TFM, they do not avoid it and so may be subjected to the full detrimental effects. Also, this thesis helps to identify potential lamprey ammocoete predators that use olfaction to help locate ammocoetes.

ACKNOWLEDGEMENTS

This research would not have been possible without the financial support from the Ontario Ministry of Natural Resources (OMNR) Species at Risk Branch SARRFO program, the Lake Superior Advisory Committee, Black Bay Fish and Game Club, Thunder Bay District Stewardship Council, Northwestern Ontario Sportsmen's Alliance, North Shore Steelhead Association, Thunder Bay Salmon Association, and the North Lake Superior Graduate Thesis Award. Thanks go to Dr. Greg Pyle and Dr. Stephen Hecnar for their faith in me, support for this research and their endless review of funding proposals and other related documents. I could not have made this happen without Dr. Bill Dew, whose positive outlook, patience, knowledge and support helped to ensure good scientific methodology and made it fun. Thanks to field assistants Karen Schmidt and Greg Kilroy. Thanks to Dr. Edward Baker and Nathan Barton for their cooperation in providing sturgeon and accommodating this research at the Black River Streamside Hatchery. Thanks also to the Dupuis and Paradis families, Black Bay Fish and Game Club and Lakehead Region Conservation Authority for access to field sites. Thanks to Fritz Fischer and the Upper Great Lakes Management Unit for assistance with ammocoetes collection and the OMNR Fire Program for loan of equipment. Thanks to all the folks that provided support and inspiration: Dr. Brian McLaren, Mike Friday, Kimberly McNaughton, Rob Swainson, Pat Furlong, Dr. Walter Momot, Dr. Tim Haxton, Dr. Tom Pratt, Dr. Peter Colby, Al Willcocks and Dr. Rob Mackereth. Finally, thanks to my husband Norm and my family for their patience and support. I am truly humbled by all of these amazing people.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Fish rely on olfaction to assist them in many ecologically important life processes such as finding food, assessing habitat quality, homing/migration, evaluating predation risk, finding mates and recognizing kin and conspecifics (Hara 1975, Smith 1992, Wisenden 2000, Daghfous et al. 2012). Fish that live in low light conditions and/or feed or travel nocturnally particularly rely on their chemical senses (Carr et al. 1996, Daghfous et al. 2012). Impairment of olfactory senses can lead to disruption of activities crucial for survival of fish (Scott and Sloman 2004). Impairment can occur from exposure to toxicants at levels well below those causing mortality (Norris et al. 1999, Mirza et al. 2009, Green et al. 2010). Many studies on a number of fish species indicate that ecologically relevant exposures to common pollutants such as metals and pesticides can interfere with fish olfaction and disrupt processes that are critical for survival and reproductive success (Tierney et al. 2010). The effect of lampricides, used for sea lamprey control, on olfaction in fish has not been studied.

Sea lamprey control began in the mid-1950s to selectively kill the stream-living larvae (ammocoetes) of sea lamprey (*Petromyzon marinus*), and has been successful in reducing sea lamprey predation on lake trout and other valued fishes in the Laurentian Great Lakes (Great Lakes Fishery Commission (GLFC), 2011A). The decline in lake trout populations was caused by predatory sea lamprey, in combination with overfishing in some locations (Coble et al. 1990). Sea lampreys may be indigenous to Lake Ontario, though this is hotly debated (Waldman et al. 2004, Bryan et al. 2005, Eshenroder 2009). The widening and deepening of the Welland Canal from 1913 onward led to their invasion of the other upstream Great Lakes. Sea lampreys reached Lake Erie by 1921, Lakes Michigan and Huron by 1932, and Lake Superior by 1946 (Holeck et al. 2004).

Lampricide is a primary component of sea lamprey control, made up of 3-trifluoromethyl-4-nitrophenol (TFM) or a combination of TFM and 2', 5-dichloro-4'-nitrosalicylanilide (Bayluscide) (Brege et al. 2003). Lampricide is applied to streams as a single block of lampricide for 12 or more hours, at concentrations up to 1.5 times the minimal lethal concentration (MLC) required to kill 99.9% of lamprey ammocoetes, to allow for attenuation and dilution of the block of lampricide (McDonald and Kolar 2007, Pratt et al. 2012).

TFM is bright yellow and has a distinct odour to humans. The application of TFM usually occurs over a 12 hour period during spring and summer, but may also occur in fall for other reasons, such as to protect lake sturgeon by allowing them to reach a larger size before treatment (GLFC 2012A, GLFC 2014). Application procedures suggest no particular time of day or night (GLFC 2012B). The effects of the colour and odour of TFM on fish behaviour have not been studied.

The toxicity of TFM in lamprey ammocoetes results from the failure of adenosine triphosphate (ATP) supply to match ATP demand. The mechanism of toxic action is such that the lampricide interferes with oxidative ATP production by mitochondria, causing rapid depletion of energy stores in vital, metabolically active tissues such as the liver and brain, a decline in blood glucose levels and a rise in blood lactate levels, ultimately leading to neural arrest and death (Birceanu et al. 2009). The same mechanism of toxic action of TFM has also been found to occur in rainbow trout (*Oncorhynchus mykiss*) (Birceanu et al. 2011). I am unaware if this mechanism of toxic action has been tested on other fish species.

Although TFM is selectively toxic to lampreys, some other fish may be killed. (Dahl and McDonald 1980). Lake sturgeon (*Acipenser fulvescens*), black bullhead (*Ameiurus melas*), channel catfish (*Ictalurus punctatus*), white suckers (*Catostomus commersonii*), longnose suckers (*Catostomus catostomus*) and northern pike (*Esox*

lucius) are among the most sensitive (Dahl and McDonald 1980, Boogaard et al. 2003, McDonald and Kolar 2007). Notable lampricide-induced mortalities in Great Lakes tributaries include five instances of 10,000 to 30,000 sucker mortalities, two instances of 1,000 and 5,000 walleye mortalities, one instance each of 12,500 northern pike, 5,000 carp, 10,000 brown bullheads (*Ameiurus nebulosus*) and 2,000 brown trout (*Salmo trutta*) mortalities (summarized in Dahl and McDonald 1980). High mortality of small forage fish species in streams has occasionally occurred as well (Dahl and McDonald 1980). The forage fish most often affected are common shiner (*Notropis cornutus*), johnny darter (*Etheostoma nigrum*), longnose dace (*Rhinichthys cataractae*), blacknose dace (*Rhinichthys atratulus*), spottail shiner (*Notropis hudsonius*), brook stickleback (*Culaea inconstans*) and sculpin (*Cottus spp.*). Stonecat (*Noturus flavus*) have undergone a dramatic depletion in the tributaries of the southwest corner of Lake Superior due to lampricide treatments (Dahl and McDonald 1980). Trout-perch (*Percopsis omiscomaycus*), logperch (*Percina caprodes*), bullhead (*Ictalusus spp.*) and mudminnow (*Umbra limi*) are also sometimes adversely affected by lampricides (Dahl and McDonald 1980). Juvenile lake sturgeon less than 1 cm long are more sensitive to TFM than lamprey ammocoetes in laboratory exposures (McDonald and Kolar 2007) and exposure to TFM by young-of-the-year (YOY) lake sturgeon has caused 20 to 50% mortality (Boogaard et al. 2003, Pratt et al. 2012). Although the lethal effects on other fish are known, the sub-lethal effects on other fish species have not been studied.

Impacts on olfaction combined with changes in behaviour can be particularly critical. Evolution has shaped the morphology and physiology of fish whose hydrodynamic, physiological, trophic, reproductive and behavioural traits are integrated and adapted to a particular location (Matthews 1998). Changes in animal movement such as frequency or speed of locomotion following exposure to a toxicant have been considered as biomarkers of contaminant exposure (Werner and Anholt 1993, Martel

and Dill 1995, Dingle and Holyoak 2001, Marentette et al. 2012). I am unaware of any studies investigating the impacts of TFM on the behaviour of fish.

Although sea lamprey are viewed in a negative light in the Great Lakes, native lamprey in other parts of the world are prized as a food or considered an important part of the ecosystem (Close et al. 2002, Kircheis 2004, Brown et. al. 2009). There are a number of fish species that prey on eggs, ammocoetes or adults of lamprey species which are either Great Lakes species, or from the same genus, including sturgeon (*A. baeir* and *A. transmontanus*), catfish (*I. punctatus* and *I. noturus phaeus*), walleye (*Sander vitreus*), brown trout, northern pike, burbot (*Lota lota*), dace and chubs (*Nocomis biguttatus*) (Scott and Crossman 1973, Cochran 2009). Further supporting that fish are natural ammocoetes predators is the use of ammocoetes of many lamprey species as bait (Scott and Crossman 1973). All of the above-noted fish taxa share with lamprey a preference for cool or cold water, and may come in contact with lamprey in the Great Lakes if they share additional habitat preferences. Sturgeon and catfish are benthic feeding stream fish that feed at night when spawning lamprey and ammocoetes are most active. Perhaps some Great Lakes fish species prey on lamprey ammocoetes. Although there is no expectation that ammocoetes predators would eliminate sea lamprey from the Great Lakes, knowledge of this behaviour may be a helpful tool in the future to assist in sea lamprey control management.

Lamprey ammocoetes inhabit fine sand and silt in the banks, deep pools and back eddies of rivers. They tend to passively drift downstream during storm events or by burrowing over three to six years before metamorphosis, and therefore also occur off the mouths of lamprey-spawning streams along the leading edge of an alluvial fan (Hardisty and Potter 1971, Gilderhus and Johnson 1980, Lee and Weise 1989, Fodale et al. 2003, Mullett and Bergstedt 2003). Ammocoetes are most active at night when they feed,

occasionally coming entirely from the bottom sediments (Quintella et al. 2005, Hardisty 2006).

Sturgeon use shallow areas with low current velocity and sandy substrates for nursery habitat (Benton et al. 2005). Juvenile sturgeon (YOY to 6-7 years of age) congregate in large schools in shallow river mouths (Peterson et al. 2007). Adult sturgeon are bottom dwellers found on productive shoals in lakes or river deltas (Rusak and Mosindy 1997, Auer 1999, Haxton 2002, Dick et al. 2006). Thus, there is likely some overlap in ammocoete and sturgeon habitat.

Lake sturgeon in the Great Lakes is classified as threatened by the Committee on the Status of Species at Risk in Ontario (OMNR 2011). Lake St. Clair and the St. Clair River are two areas in the Great Lakes where sturgeon populations are considered healthy and stable (OMNR 2011). The Sea Lamprey Control program has estimated a population of 150,000 ammocoetes in the St. Clair River based on ammocoete habitat(GLFC 2011B), however the numbers of ammocoetes found during assessments has been generally low (GLFC 2005 to 2014). Perhaps the presence of a healthy predator population is having an influence on ammocoetes numbers.

Confirmation of lake sturgeon preying on ammocoetes would be very difficult to detect using traditional gut content methods. Very few studies investigate the stomach contents of lake sturgeon. In addition, ammocoetes have no hard tissue and are consumed at night, so they may be digested by the time sturgeon are caught and processed. However, the study of the attraction of lake sturgeon to the scent of lamprey ammocoetes using chemosensory baits may provide an opportunity to investigate whether lake sturgeon are attracted to the scent of lamprey ammocoetes.

My research investigated the effect of the lampricide TFM (Alfa Aesar, United Kingdom) on olfaction and behaviour in (YOY) lake sturgeon (*Acipenser fulvescens*) and the attraction of lake sturgeon to the scent of lamprey ammocoetes. First I report on

sub-lethal effects of TFM on olfaction, behaviour, activity, blood glucose and lactate in hatchery-reared YOY lake sturgeon. The second part of the study investigates the attraction of lake sturgeon to chemosensory stimuli (food cues) derived from lamprey ammocoetes. I hypothesized that TFM would reduce the olfactory capabilities of YOY lake sturgeon, and that lake sturgeon would be attracted to the scent of lamprey ammocoetes. My objectives were to determine:

1. if exposure to environmentally relevant concentrations of TFM affects:
    a. the olfactory response of YOY lake sturgeon to standard chemosensory stimuli (L-alanine, TCA, food cues);
    b. the activity levels and behavioural response to food cues of YOY lake sturgeon;
    c. the appetite of YOY lake sturgeon;
2. if YOY lake sturgeon can detect the odour of TFM;
3. if YOY lake sturgeon avoid, are attracted to, or have no reaction to water containing environmentally relevant concentrations of TFM during darkness or daylight;
4. if exposure to environmentally relevant concentrations of TFM affects YOY lake sturgeon blood glucose and lactate levels;
5. if YOY lake sturgeon are attracted to substrate conditioned by lamprey ammocoetes under natural conditions;

This research required multiple related tests linking neurological responses to behavioural responses. I used electro-olfactography (EOG) to measure the electrical activity of the receptors on the epithelium of the olfactory rosette, which is the interface

between the external environment and neurological system, making the receptors a probable target for waterborne contaminants. Electro-olfactography is an electrophysiological technique that measures the amplitude of an odour-evoked extracellular field potential. The electro-olfactogram records the negative electrical potential at the surface of the olfactory epithelium of vertebrates (Scott and Scott-Johnson 2002). An inhibition of the EOG response to standard chemosensory cues in TFM-exposed sturgeon indicates impairment at the olfactory epithelium.

Teleosts such as sturgeon have two olfactory organs located in the dorsal part of the snout. Each olfactory organ consists of an olfactory chamber, containing an olfactory rosette, connected to the exterior via the anterior and posterior nares. Water flows into the anterior naris, exposing the olfactory rosette to the circulation of water and odour cues in the olfactory chamber, then the water flows out the posterior naris. The olfactory rosette is composed of a series of olfactory lamellae, which are covered with three types of olfactory sensory neurons (OSNs); ciliated, microvillous and crypt OSNs. Each of the three types of OSNs transmits information related to specific behavioural functions along a particular olfactory tract bundle to the olfactory bulb of the brain (Figure 1 (i)). Ciliated OSNs respond to information related to predation risk and migration, microvillous OSNs to information related to food detection and crypt OSNs to information related to reproduction (Laberge and Hara 2001, Hamdani and Doving, 2007) (Figure 1 (ii)).

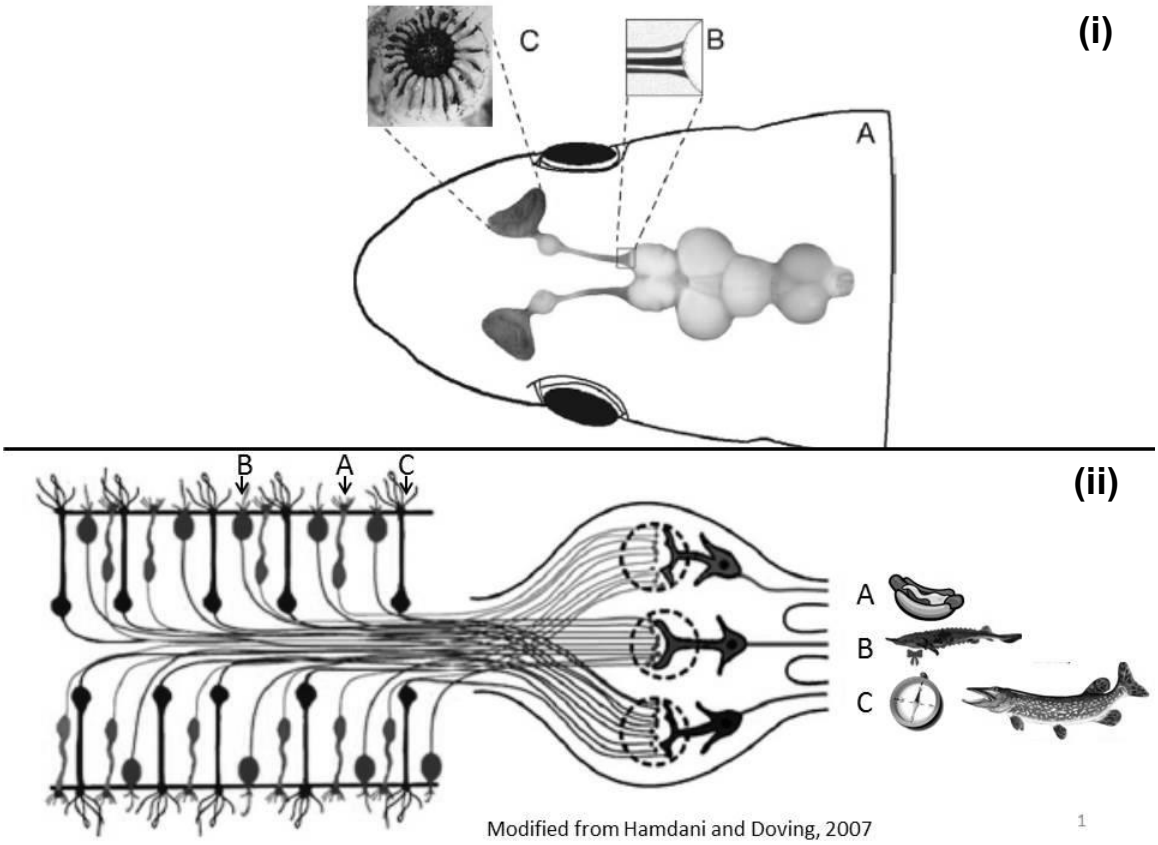

Figure 1. (i) Overview of the fish olfactory system (A) Dorsal view of the head of a crucian carp (*Carassius carassius*) showing the brain and olfactory system. (B) Schematic drawing of the olfactory tract as it enters the telencephalon, demonstrating three distinct bundles. (C) Micrograph of the olfactory rosette of a young-of-the-year lake sturgeon. (ii) Schematic diagram illustrating three parallel pathways from the lamellae of the olfactory rosette. Microvillous sensory neurons (A) collect information about food. Crypt cells (B) collect information about reproduction. Ciliated sensory neurons (C) collect information about migration and predator alarm.

Toxicants may impair specific classes of olfactory sensory neurons in the olfactory epithelium (Dew et al. 2014). To study the effect of TFM on microvillous sensory neurons, L-alanine was used as an odour cue in EOG assays. Multiple studies report that L-alanine is very effective at inducing food searching behaviour in Persian and Russian sturgeon (*Acipenser gueldenstadtii)*, Siberian sturgeon (*A. baerii)*, green sturgeon (*A. medirostris)*, and stellate sturgeon (*A. stellatus)* (Kasumyan, 1999,

Shamushaki et al. 2011). The bile salt taurocholic acid (TCA) was used to test the ciliated sensory neurons, which are tuned to migration and alarm cues (Lo et al. 1994, Hamdani and Doving 2007).

Behavioural analysis provided a more insightful evaluation of the ecological effects of this toxicant, which helped to determine if there was a behavioural change that coincided with olfactory impairment. Blood glucose and lactate analysis was performed to give a hint as to whether TFM has the same effect on the ATP energy pathway in lake sturgeon as it does in sea lamprey ammocoetes and rainbow trout. If sturgeon fail to avoid TFM exposure, they may suffer toxic consequences, especially given the documented reports of non-target species being affected by TFM exposure. Measuring glucose and lactate may serve as an early warning biomarker owing to TFM's known mechanism of toxic action. This work will address the obvious question that if sturgeon fail to avoid TFM, do they suffer toxicity? Impaired glucose and lactate profiles could suggest that they do. This work was not meant to replace the type of thorough analysis conducted by Birceanu et al. (2009) and Birceanu et al. (2011) but merely to identify that further analysis may be warranted on YOY lake sturgeon as well.

I used underwater camera censuses to test sturgeon's response to the scent of lamprey ammocoetes in natural conditions, which is an accepted and widely used method for both ecological and fishery-based field surveys (Assis et al. 2013). Underwater monitoring of chemosensory baits is used to investigate fish species occurrences and densities, as well as attraction to scented underwater baits (Bassett and Montgomery 2011, Assis et al. 2013, Hardinge et al. 2013, Santana-Garcon et al. 2014). Since nocturnal fish such as sturgeon are an important part of aquatic ecosystems, infrared light was used to detect fish at night. Infrared light is known to be undetectable by fish and has been successfully used by others to observe and record

marine fish (Kobayashi et al. 2002; Bassett and Montgomery 2011). I also used minnow traps baited with conditioned substrate, which attracts aquatic predators (Wasylenko et al. 2014). However, using this method eliminated the ability to differentiate day and night feeding predators and limited the types and size of fish predators.

MATERIALS AND METHODS

FISH

With the exception of the study of lake sturgeon in natural conditions, this research was conducted on YOY lake sturgeon at the Black River Streamside Sturgeon Rearing Facility near Onaway, Michigan during August of 2013 and 2014. Sturgeon lengths ranged from 85 to 182 mm (mean 130 ± 19.8 mm SD) and mass ranged from 2.7 to 26.7 g (mean 9.3 ± 4.0 g SD); n = 193. Water temperatures during 2013 research ranged from 21.1 to 22.8°C, and from 17.2 to 17.8°C during 2014 research. The alkalinity of the water was 180 mg/L $CaCO_3$. The pH of the water was 8.1 without aeration, and varied slightly from 8.5 to 8.7 with aeration. Fish were fed bloodworms three times per day, reduced to once a day, two days prior to the commencement of experiments. This research was conducted under the approval of the Lakehead University Animal Care Committee (protocol numbers 08-2013, 10-2013 and 09-2014) and was CCAC compliant.

TFM EXPOSURES

Fish were exposed to TFM in tanks containing 5 L of water, with an air stone to provide aeration. The amount of TFM used replicated 1.0 x MLC used by Sea Lamprey Control to kill 99.9 % of larval sea lamprey, which is dependent on the alkalinity and pH of the water, as described in Bills et al. (2003). The toxicity of TFM, which is an acid, increases with lower pH of the water (Hunn and Allen 1974). The concentration of TFM used for fish exposures in my study ranged from 6.5 mg/L (pH 8.5) to 8.6 mg/L (pH 8.7). Fish remained in the exposure bath for 12 hours to simulate TFM exposure during a typical stream treatment. All assays were conducted after the exposures, with no

additional TFM added during the trials. All experiments and interpretations were based on nominal TFM concentrations.

ELECTRO-OLFACTOGRAPHY (EOG)

The EOG methods were modified from those described by Green et al. (2010). A schematic of the EOG set up is provided in Figure 2. Each fish was anaesthetized and tested for an electro-physiological response to L-alanine, TCA, food, TFM and water blank. These olfactory cues were administered in random order with a minimum of 90 seconds of rest between cues. Water was used to irrigate the olfactory chamber during the rest period to keep the tissue moist. Each odour cue was tested three times on each fish, and the raw EOG amplitude results were averaged. The water blank average was subtracted from the average response to the other cues, to correct for any non-specific response, then the result was divided by the mean overall response of the control fish to the corresponding odour cue, to yield the mean corrected EOG response.

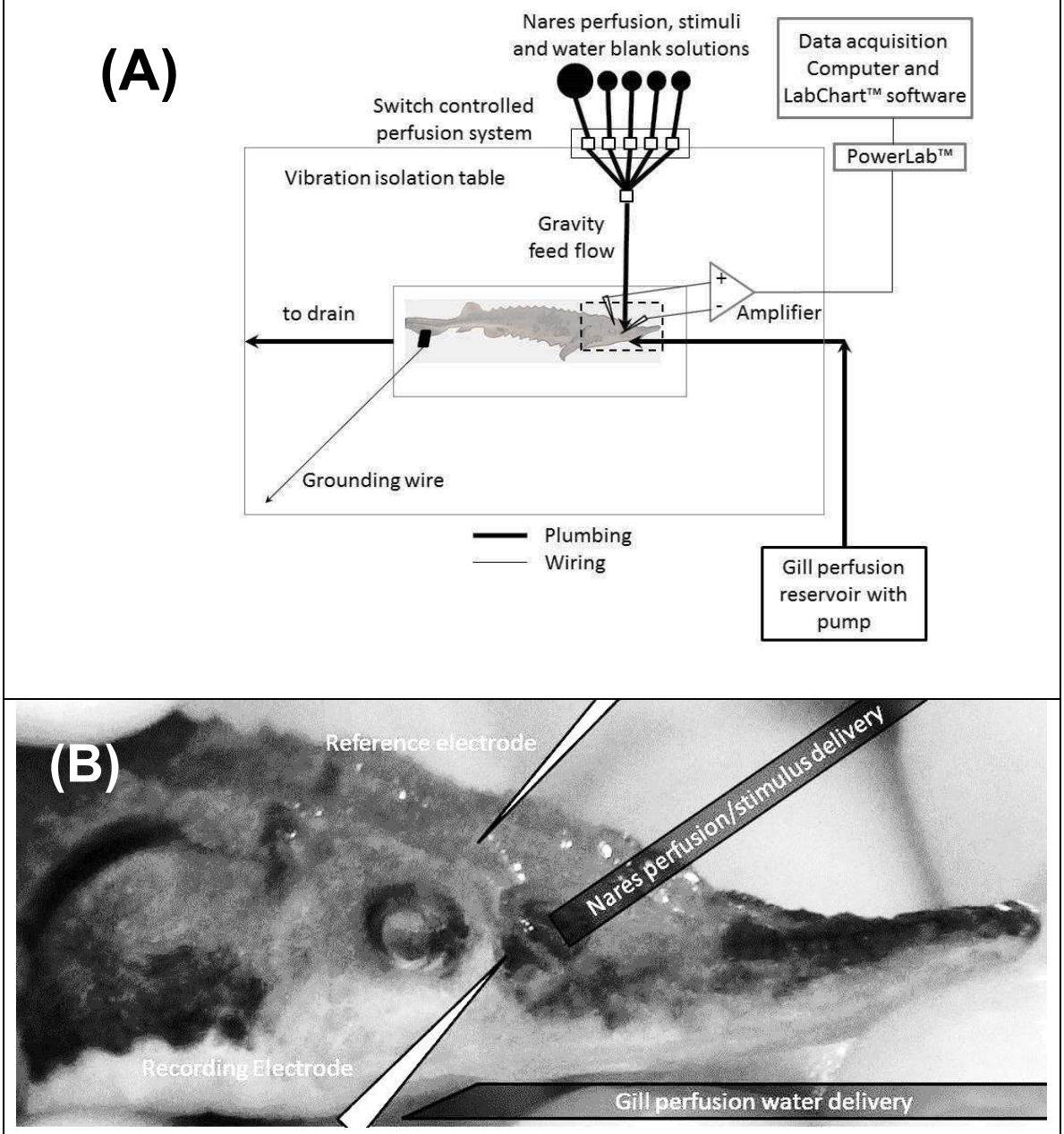

Figure 2. The electrophysiological recording system used to measure the response to odour cues from the sensory epithelium of YOY lake sturgeon in the form of an electro-olfactogram (EOG). (A) Schematic diagram showing the major components of the apparatus. The dashed box denotes the area shown in more detail in (B). (B) Photograph showing the head of a YOY lake sturgeon and the positioning of the gills perfusion water delivery tube, the nares perfusion/stimulus delivery tube and the reference and recording glass electrodes.

The olfactory response of YOY lake sturgeon was characterized prior to the commencement of the trials, as no previous EOG work on sturgeon had been recorded in scientific literature. Three concentrations each of L-alanine (10^{-2}, 10^{-3} and 10^{-4} M) and TCA (10^{-3}, 10^{-4} and 10^{-5} M) were tested, providing a range above and below the concentrations commonly used in EOG studies on other fish species. All of these concentrations proved to be within detection and saturation limits, so the commonly used concentrations of 10^{-3} L-alanine and 10^{-4} TCA were employed in this study.

The anaesthetic bath was prepared using 100 mg/L tricaine methanesulphonate (MS-222) buffered to the pH of the water. A maintenance dose of MS-222 at 50 mg/L, also buffered to the pH of the water, was used to perfuse the gills in order to keep the fish anaesthetized while on the EOG rig. Water from the hatchery was used in the anaesthetic bath, the recovery bath, the gill perfusion water and to irrigate the olfactory epithelium.

The food cue was prepared from 0.5 grams of bloodworms stirred in 1 L of hatchery water for 30 minutes using a stir plate set to 350 rpm. The solution was filtered through aquarium polyester filter wool. A water blank cue consisting of hatchery water was stirred and filtered in the same manner.

The TFM cue was prepared at a concentration of 3.7 mg/L, (1.0 MLC based on pH 8.1 and alkalinity 180 mg/L as $CaCO_3$) as described in Bills et al. (2003). The pH of the water and subsequent concentration of TFM used here was less than that used in the TFM exposure baths due to a rise in pH in the exposure baths when the water was aerated. The EOG response to TFM was not blank corrected so that the response could be compared to the blank response, to determine if the response to TFM is non-specific.

Food Consumption Behavioural Trials

To study the effects of TFM on the behaviour of YOY lake sturgeon, it would be useful to know if there is a change in the amount of food eaten after exposure to TFM, which may impact on their survival in a natural environment. Exposed fish were compared to control fish, using bloodworms as the food source. Bloodworms were the food fed to YOY sturgeon at the hatchery, therefore the fish were conditioned to respond to them as food. Each YOY lake sturgeon (n = 10) was placed in 5 L of aerated hatchery water, along with 2 g of bloodworms. Fish were left to feed for 1.5 hours, then removed from the bin along with any faeces. The remaining food was filtered out of the water through a sieve, then re-weighed.

Behavioural Maze Trials

It is important for the survival of fish in a natural environment, to gain an understanding of whether exposure to TFM affects the ability to detect the scent of food and if fish can detect and react to a block of TFM in the water. Results that correspond with EOG results support the linkage between behaviour and the neurophysiological response of the olfactory tissue. The ability to detect the scent of food is critical for survival of fish species that rely on olfaction when feeding at night or in murky water. The ability to detect and avoid TFM would be advantageous for fish, to avoid any potential negative effects from TFM exposure. Behavioural maze trials provide an arena to study the behavioural response to a chemosensory stimulus by giving fish the opportunity to choose an end containing stimulus or an end containing a water blank (Fig. 3). Five litres of hatchery water was used in each maze. A camera was placed above the maze to observe and record fish behaviour. Once a fish was placed in the maze, a fabric sheet was placed over the entire unit, to limit extraneous distractions that may affect the fish. Fish were acclimated to maze conditions by restricting their access

to the distal reaches of the maze where the chemosensory cues were ultimately delivered. A five-minute acclimation period was used to calm the fish and allow them to return to normal swimming behaviour. After the acclimation period the cues were released, the acclimation chamber removed, and the behaviour observed for the following 10 minutes. The location of the fish in the maze was recorded every 10 seconds (stimulus arm, blank arm or middle of maze). The arm that received the chemosensory stimulus was randomly assigned to ensure the fish had no preferential bias to a particular arm of the maze. Any fish that was recorded in the middle of the maze for half of the trial or longer was removed from analysis. The mazes were washed with Fisher Brand™ Sparkleen™ detergent and rinsed thoroughly with hatchery water before every trial.

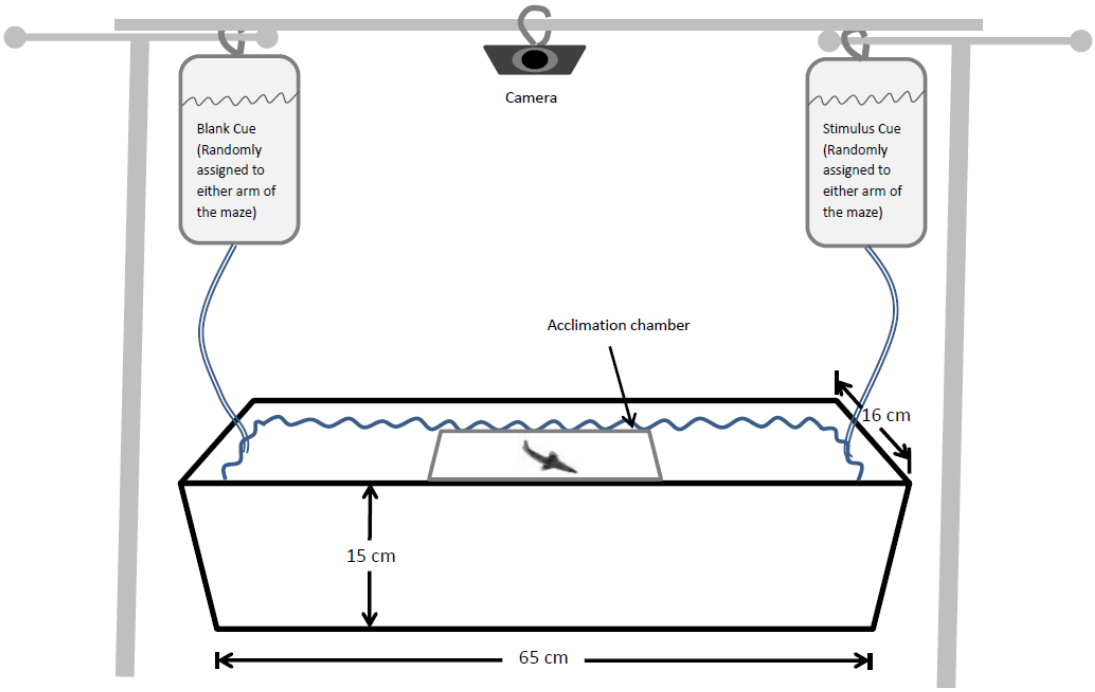

Figure 3. Diagram of behavioural maze used for response to food cues and TFM.

To determine the minimal concentration of food cue that would elicit a behavioural response in control YOY lake sturgeon, four bloodworm cue concentrations (0.50, 0.40, 0.35 and 0.30 g/L) were prepared by stirring the weighed amount of bloodworms in 1 L of hatchery water for 30 minutes. The conditioned water was filtered through aquarium polyester filter wool to remove any particles. A water blank cue was prepared in exactly the same manner as the food cue except no bloodworms were added to the solution. The stimulus cue and water blank cue were placed in intravenous (IV) bags located at the opposite distal ends of the maze and delivered simultaneously to the maze through 6 mm (i.d.) silicone airline tubing. The trial concentrations were tested on 10 fish in a preliminary trial. Each cue was also tested at two drip rates (70 – 100 drips per minute (3.5 – 5.0 mL/min) and 120 – 150 drips per minute (6.0 – 7.5 mL/min)). A distinct behavioural response was detected at a minimum bloodworm cue concentration of 0.35 g/L, with a drip rate of 70-100 drips per minute. This amount of flow attracted initial test fish to the scented cue if they swam into the stimulus arm of the maze, with minimal attraction to the flow of water from either the stimulus or blank cue tubes. The same concentration was maintained in the behavioural maze trials and delivered at the same flow rate to test the response to a food cue by YOY sturgeon exposed to TFM. The maze and camera (webcam) were covered with a white sheet and the lights in the building were left on, to reduce distractions but allow natural light into the maze.

To determine if YOY lake sturgeon were attracted to, avoided or had no reponse to TFM, a behavioural maze study was conducted to mimic an encounter with a block of TFM similar to a typical TFM stream treatment. The cue was prepared and delivered to produce a block of TFM on one half of the maze at 1 x MLC. An initial stock solution was prepared using 92.5 mg of TFM in one litre of hatchery water. For each trial 100 mL of the stock solution was poured into one end of the behavioural maze and allowed to

disperse towards the middle of the maze. A 100 mL water blank cue, consisting of hatchery water, was simultaneously added to the opposite end of the maze. The acclimation chamber was removed when the TFM had dissipated about two-thirds of the way towards the middle of the maze so that the concentration of TFM encountered would be no greater than 1.5 x MLC, the maximum amount that may be encountered in the field. Trials simulating daylight conditions, when colour and odour may influence behaviour, had the lights in the building on and a white cotton sheet over the maze and camera. Trials simulating darkness, when only odour may influence behaviour, had the lights in the building off, windows covered and a black cotton sheet over the maze and camera, to reduce distractions and block light from entering the maze. A monochrome camera with infrared lighting was hung over the maze to record the response to TFM during both daylight and darkness.

ACTIVITY ANALYSIS

Considering that TFM exposure could potentially affect the locomotory performance of fish, I analyzed the percentage of time the fish were active, as well as their acceleration and velocity using the control and exposed fish video recordings from the food cue trials. LoliTrack™ software,version 4.1.0, (Loligo Systems, Tjele, Denmark) was used to perform the analysis. The percent time active calculates the amount of active and inactive time. Activity was identified when the fish moved a distance larger than a pixel between video frames and was measured as a percentage of the total time. Acceleration was calculated video frame by frame, in cm/m^2. The average acceleration is calculated from positive acceleration values only. The average velocity was calculated from the speed of movements video frame by frame, in cm/s, from positive velocity values only.

Blood Glucose and Lactate Trials

Blood glucose and lactate analysis was performed on YOY lake sturgeon to give a hint as to whether TFM has the same effect on the ATP energy pathway in lake sturgeon as it does in sea lamprey ammocoetes and rainbow trout. Human blood glucose and lactate meters were utilized for this analysis, as they have been used successfully by others on fish (Wells and Pankhurst 1999, Eames et al. 2010, Brown et al. 2008). A 10 µL sample of blood was drawn from the caudal vein of fish that were either exposed to TFM or unexposed, directly behind the anal fin, using a 29 G needle. Blood glucose and lactate concentrations were measured using test strips, an Accu-Chek™ (Roche, Indianapolis, IN, USA) blood glucose meter and Lactate Plus™ (Nova Biomedical, Waltham, MA, USA) lactate meter. The recording range of the blood glucose meter was 0.6 to 33.3 mmol/L. The recording range of the lactate meter was 0.3 to 25.0 mmol/L. No anaesthetics were used in blood sampling to avoid any influence of anaesthetic on the results. Manual restraint and a gentle flow of water over the fish's head and upper body provided a calming effect while blood was drawn.

Chemosensory Bait Assay

For this portion of the research I used chemosensory baits to observe if lake sturgeon in a natural environment were attracted to the scent of lamprey ammocoetes. Lamprey ammocoetes were captured in the Cypress (2013) (48°55'49"N, 87°52'8"W) (25 ammocoetes) and Wolf (2014) (48°49'8"N, 88°31'10"W) (41 ammocoetes) Rivers (Fig. 4), by electrofishing or the use of a shovel and modified dipnets, in shallow water with fine sand and silt substrate. Ammocoetes were kept in an aerated 72 L tank with approximately 20 L of clean sand and 40 litres of water, to create sand conditioned with their scent. The density of ammocoetes (25 to 40 ammocoetes/0.5 m^2 surface area)

held in the tank used to condition the sand was within the reported densities used by others in ammocoetes research (Swink 1995). Control sand was treated in the same manner, but did not contain ammocoetes. The sand was conditioned for at least 2 days before use. The 2-day minimum conditioning time was longer than the 24 hours used by others to successfully condition olfactory stimuli with live organisms (Ward et al. 2005, Martin et al. 2010, Ward and Currie 2013).

Chemosensory bait study locations were either known lake sturgeon habitat or had fine sand and silt substrates. The four sites were the Cypress River (2013), Black Sturgeon River (2014), Kaministiquia River (2014) and the Mission Island embayments (2014) (Fig. 4).

Chemosensory baits were constructed of fibreglass screening sewn with fishing line, holding approximately one litre of conditioned or control substrate. The baits were placed on the river bottom in a quiet location in the river away from the main current in water depths ranging from 30 to 110 cm. Aquatic organism activity near the bait was recorded for 30 minutes four times a day, in early morning (between 6 and 8 AM), mid-day (between noon and 2 PM), dusk (between 9 and 11 PM) and night (between midnight and 2 AM), using a Delta Vision B&W underwater video camera with infrared lighting (Sea-View Underwater Technologies, Everett, WA, USA). The time of day, genus or family and number of individuals investigating the bait was recorded. Fresh bait was used for each recording. Control and stimulus baits were tested at each location for each of the time periods, then the baits were positioned at a new location at least 20 m away. The underwater camera methodology was utilized on the Cypress River in 2013, for one recording during mid-day of both the control and stimulus baits, before heavy rains and high water prevented any further study in 2013. This methodology was also utilized in 2014 on the Kaministiquia River for six control

recordings (2 dusk, 2 midnight, 1 dawn and 1 mid-day) and four stimulus recordings (1 dusk, 1 midnight, 1 dawn and 1 mid-day).

Camera malfunction necessitated an alternate methodology to monitor fish attracted to the baits in 2014. I used methods similar to Wasylenko et al. (2014), placing two minnow traps on the river/embayment bottom at each sampling site approximately 2 to 3 m apart with the open ends facing each other so that fish had the choice of entering a trap containing control bait or stimulus bait. Minnow traps were located away from the main current, in water depths ranging from 30 to 110 cm. Chemosensory baits were constructed in the same manner as described earlier, but were half the size. Traps were inspected for aquatic organisms after 24 hours. Trapped organisms were counted and either immediately identified or photographed, then released. The locations of the traps were switched and left for another 24 hours to eliminate any positional bias. The traps were then moved to a new location at least 20 m away and the process repeated. The minnow trap methodology was used in the Black Sturgeon River and the Mission Island embayments. Six pairs of baited minnow traps (1 with control bait and 1 with stimulus bait) were used for 2 days at each location (total n = 24 per treatment).

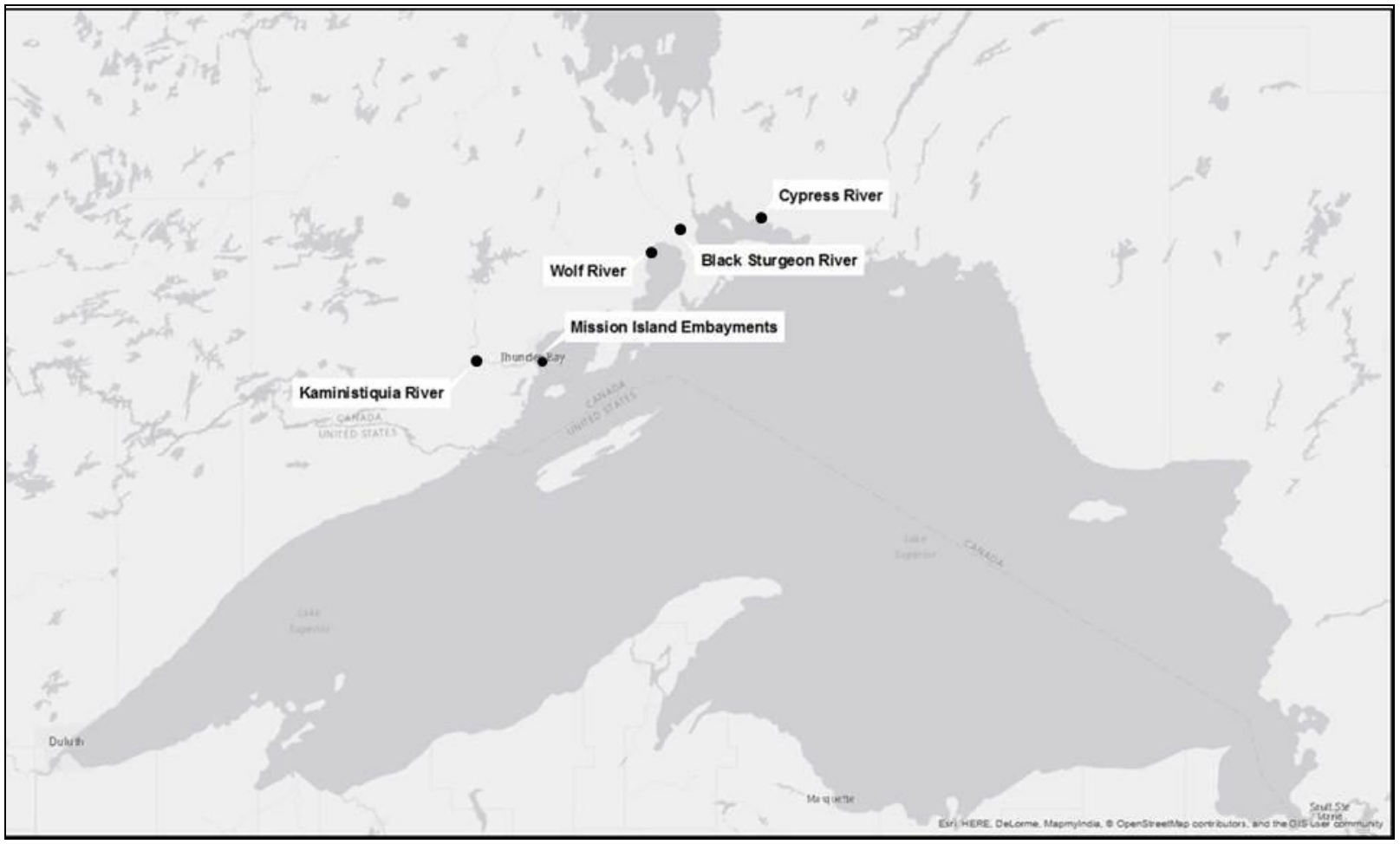

Figure 4. Location of chemosensory bait research on the Cypress River, Black Sturgeon River, Kaministiquia River and Mission Island Embayment (Embayment) and lamprey ammocoetes collection sites from the Cypress and Wolf Rivers, near the north shore of Lake Superior, Ontario, Canada.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics software (IBM Corp 2013). The EOG responses to L-alanine, TCA and food were measured in the same fish (i.e., three dependent variables, four including a control) and were therefore analyzed using MANOVA to compare responses from exposed and unexposed sturgeon. The food consumption trials compared consumption rates between exposed and unexposed animals using t-tests for independent samples. Behavioural maze trials and EOG response to TFM were analyzed using t-tests for paired samples, except when data were not normally distributed, when Wilcoxon's signed rank test was used. Any differences in the velocity, acceleration and percent of time active between the control and exposed fish were tested using independent t-tests. Blood glucose comparisons were analyzed using Mann-Whitney U tests. A Pearson's chi-squared test was used to compare the number of organisms attracted to control and ammocoete-conditioned baits. For all analyses, significance was set at $\alpha = 0.05$. Reports are in mean ± standard errors, unless otherwise stated.

RESULTS

EOG RESPONSE TO L-ALANINE, TCA AND FOOD CUES

The EOG response of YOY sturgeon exposed to TFM showed diminished response to L-alanine (10^{-3} M) (52%), TCA (10^{-4} M) (64%) and food cues (80%) which were not quite significant due to small sample size and relatively high variation in control animal EOG responses ($\boldsymbol{F}_{2,4} = 5.97$, $\boldsymbol{p} = 0.06$ (Figs. 5 and 6)).

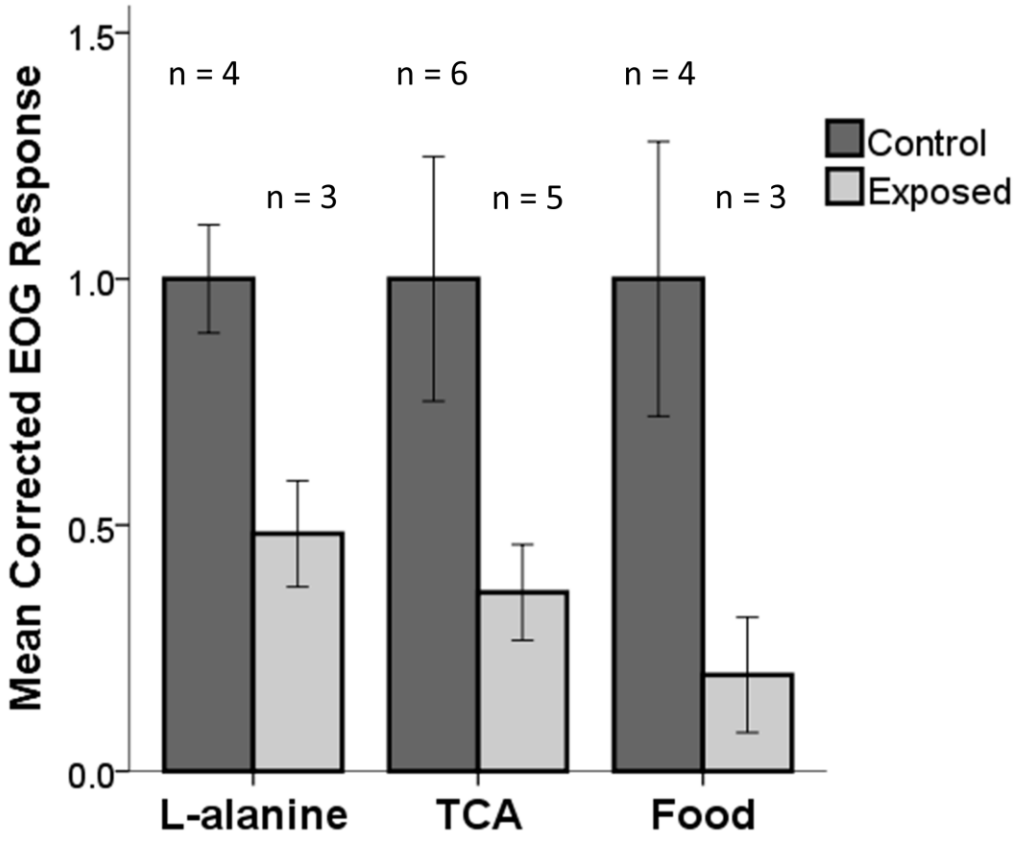

Figure 5. Comparison of the mean corrected EOG response to 10^{-3} M L-alanine, 10^{-4} M TCA, and food cue of hatchery reared YOY lake sturgeon held in clean water (control) or water containing TFM at 1 x MLC. Exposed fish were held for 12 hours in nominal TFM concentrations of 6.5 mg/L (water pH 8.5), 7.5 mg/L (water pH 8.6) or 8.6 mg/L (water pH 8.7). Control fish were held in clean water for 12 hours. All fish were tested in hatchery water. Error bars denote +/- one standard error.

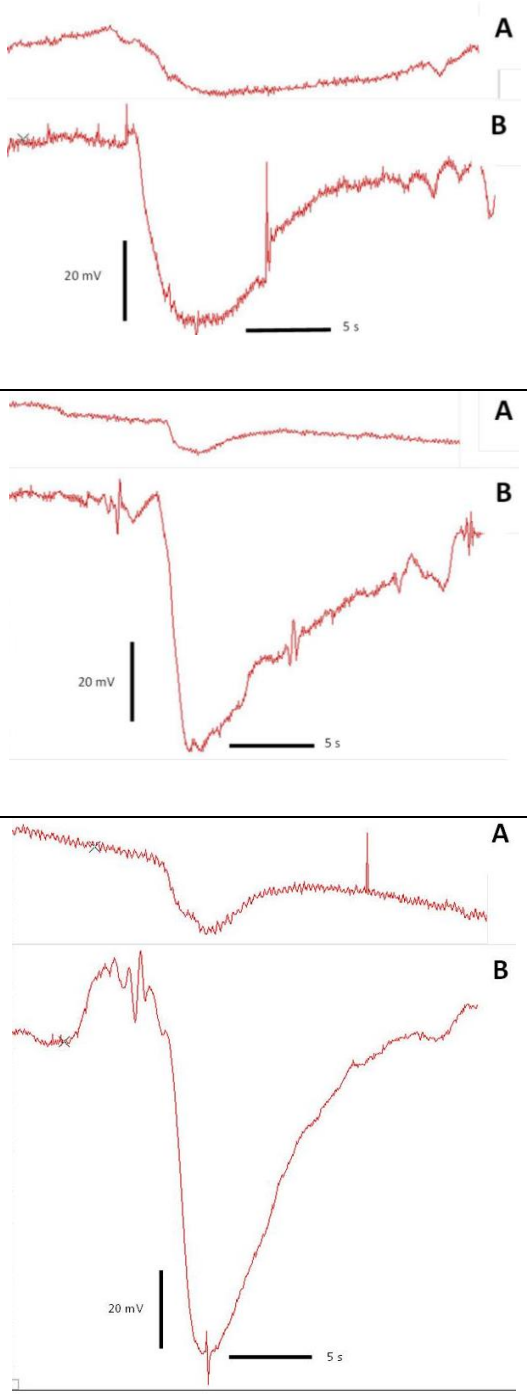

Figure 6. Representative EOG traces for exposed (A) and control fish (B) for (i) 10^{-3} M L-alanine, (ii) 10^{-4} M TCA and (iii) a food cues.

BEHAVIOURAL RESPONSE TO FOOD CUES

When given the choice between an arm containing a food cue or hatchery water, the control fish spent 66% more time in the food cue arm relative to the water blank arm (t_{13} = 2.64, p = 0.02, Fig. 7). The response of fish exposed to TFM was different, showing no significant difference between the time spent in the food cue arm relative to the arm containing a water blank cue (t_{19} = 0.06, p = 0.95, Fig. 7).

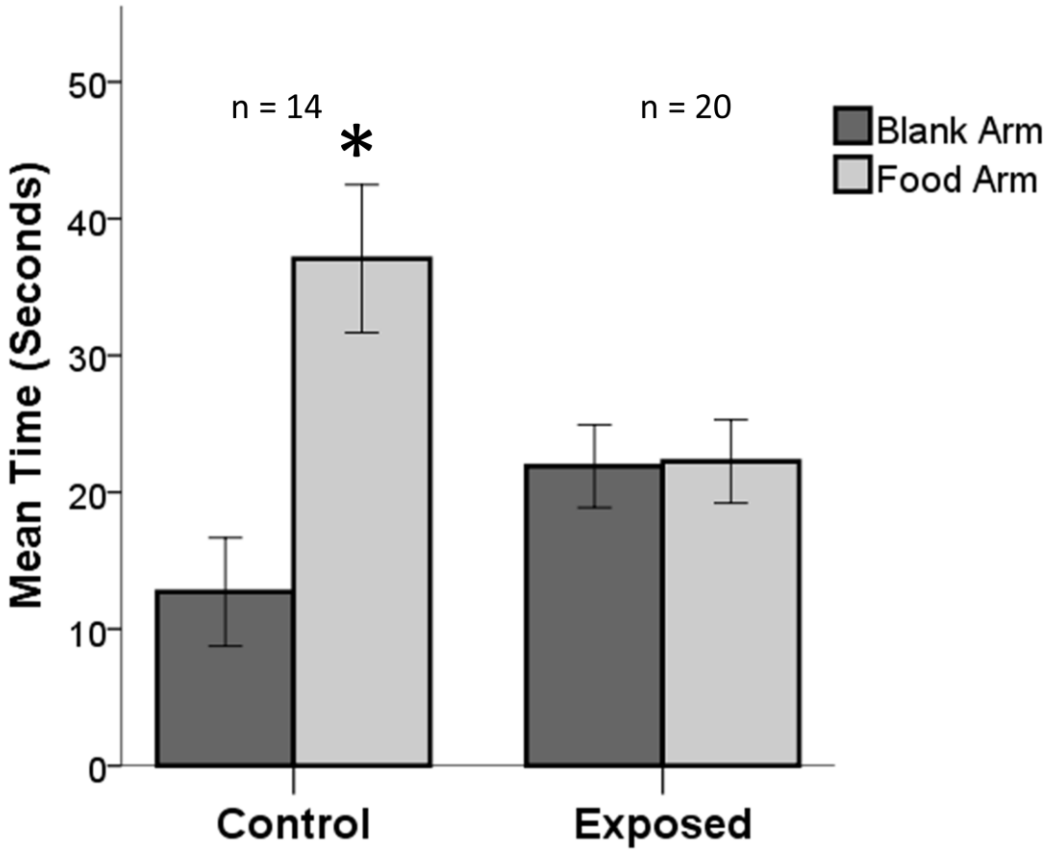

Figure 7. Comparison of the behavioural response to a food cue and a water blank cue by hatchery reared YOY lake sturgeon held in clean water or water containing TFM at 1 x MLC. Exposed fish were held for 12 hours in nominal TFM concentrations of 6.5 mg/L (water pH 8.5), 7.5 mg/L (water pH 8.6) or 8.6 mg/L (water pH 8.7). Control fish were held for 12 hours in clean water. All fish were tested in hatchery water. An asterisk denotes a significant difference between the time spent in the food cue end of the maze versus the water blank end. Error bars denote +/- one standard error.

FOOD CONSUMPTION

Fish exposed to TFM ate 70% less than controls ($t_{18} = 6.55$, $p < 0.01$; Fig. 8).

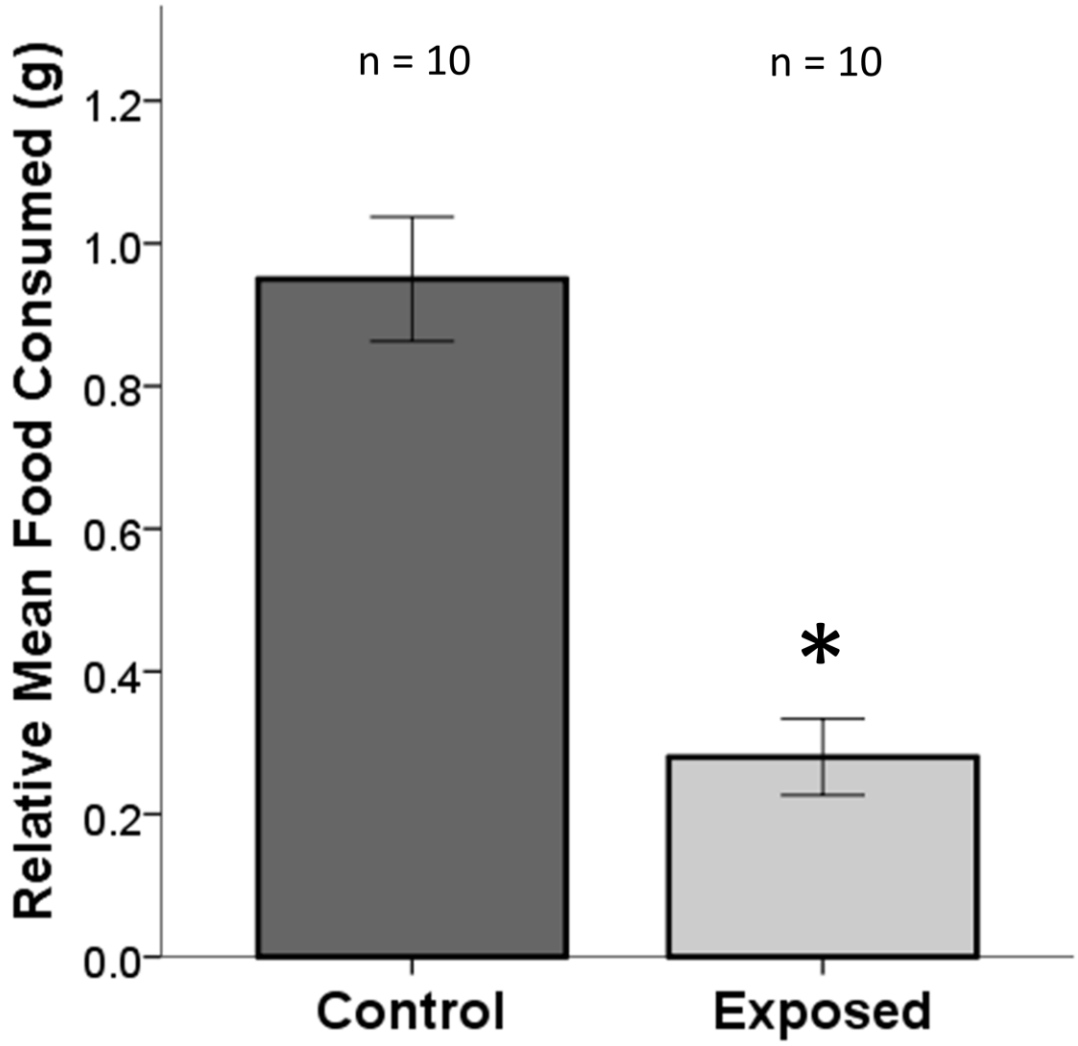

Figure 8. Comparison of the mean food consumed after 1.5 hours of feeding for YOY lake sturgeon. Exposed fish were held for 12 hours in nominal TFM concentrations of 6.5 mg/L (water pH 8.5), 7.5 mg/L (water pH 8.6) or 8.6 mg/L (water pH 8.7). Control fish were held for 12 hours in clean water. All fish were tested in their native hatchery water. An asterisk denoted a significant difference. Error bars denote +/- one standard error.

EOG AND BEHAVIOURAL RESPONSE TO TFM

Fish had 120% greater EOG responses to TFM, compared to a water blank (t_2 = -6.00, p = 0.03; Fig. 9).

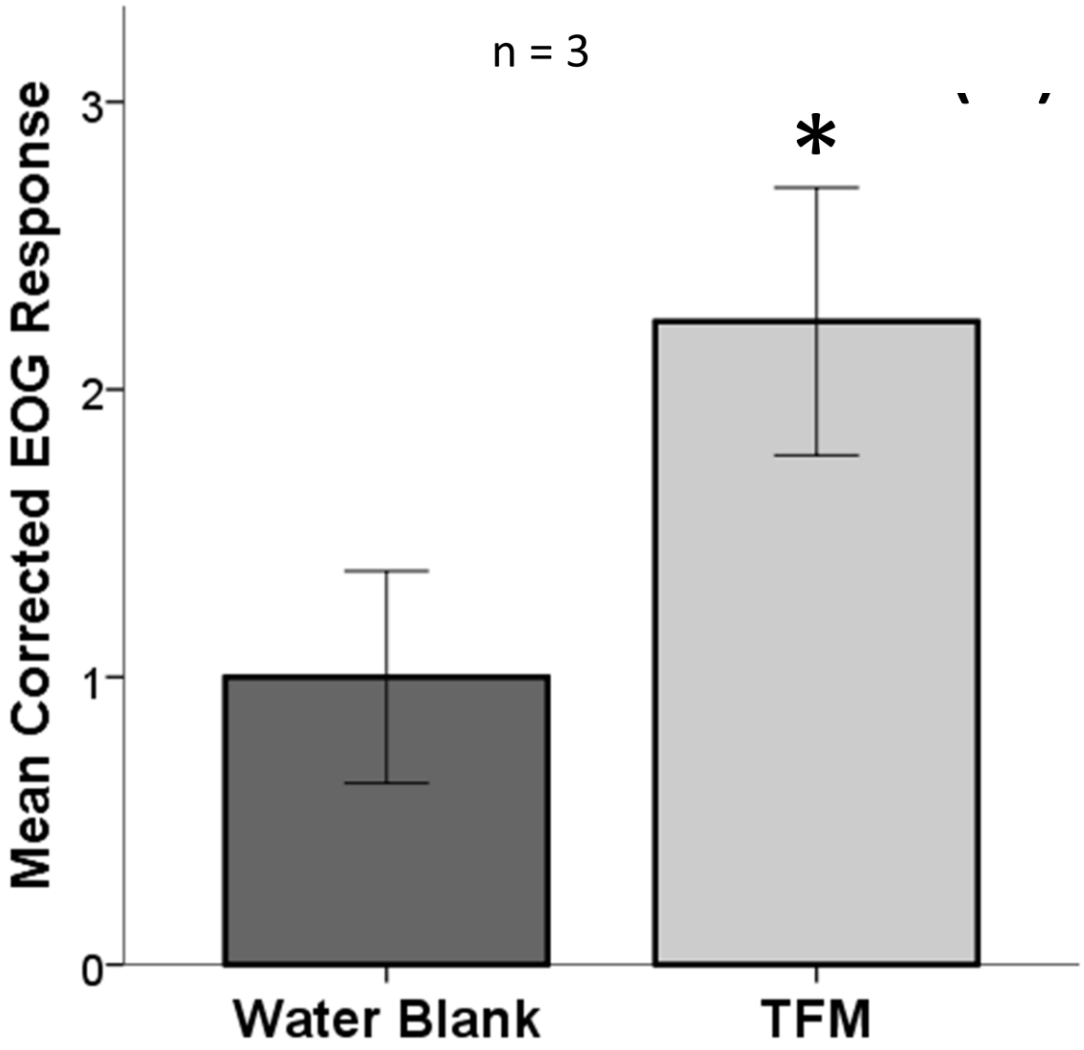

Figure 9. Comparison of the mean corrected EOG response to 3.7 mg/L of TFM (in water pH 8.1) and distilled water (Blank Cue) on hatchery reared YOY lake sturgeon. An asterisk denotes a significant difference. Error bars denote +/- one standard error.

BEHAVIOURAL RESPONSE TO TFM

When presented with an arm containing TFM and an arm containing water blank, there was no significant difference between the time fish spent in either arm for both the trials in daylight (t_{15} = 0.470, p = 0.64, Fig. 10) and in darkness ($\boldsymbol{t}_{16}$ = -1.419, $\boldsymbol{p}$ = 0.175, Fig. 10).

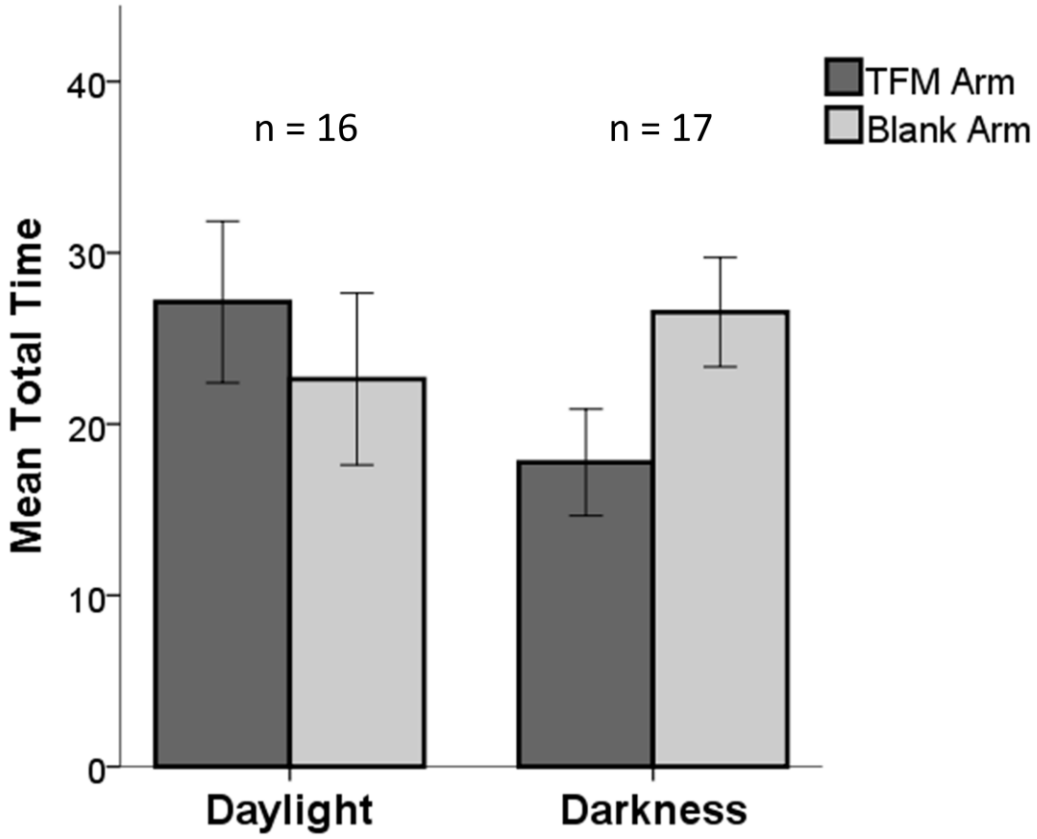

Figure 10. A comparison of the behavioural response to a TFM cue and a water blank cue by hatchery reared YOY lake sturgeon. All fish were tested in hatchery water. An asterisk denotes a significant difference between the time spent in the TFM arm versus the water blank arm. Error bars denote +/- one standard error.

ACTIVITY ANALYSIS

The fish exposed to TFM were 79% more active ($\boldsymbol{t}_{32}$ = -2.96 $\boldsymbol{p}$ < 0.01; Fig. 11), but accelerated 19% more slowly ($\boldsymbol{t}_{32}$ = 2.01 $\boldsymbol{p}$ = 0.05: Fig. 12) and had a 16% slower

velocity (t_{32} = 1.83 ***p*** = 0.08; Fig. 13). The slower velocity was not statistically significant due to the variability in the data for both the control and exposed fish.

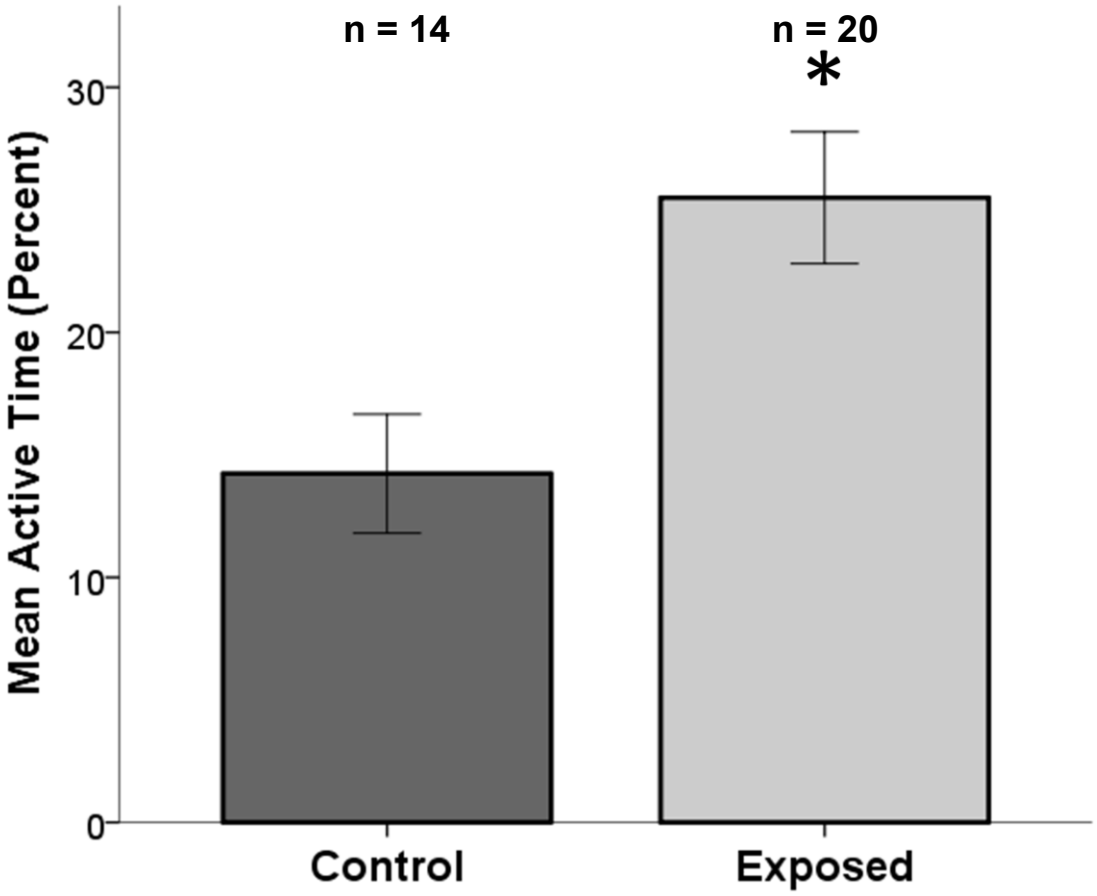

Figure 11. Effects of TFM on the percent of active time of YOY lake sturgeon. Exposed fish were held for 12 hours in nominal TFM concentrations of 6.5 mg/L (water pH 8.5), 7.5 mg/L (water pH 8.6) or 8.6 mg/L (water pH 8.7). Control fish were held for 12 hours in clean water. An asterisk denotes a significant difference between control fish and other category. Data are expressed as the mean +/- one standard error.

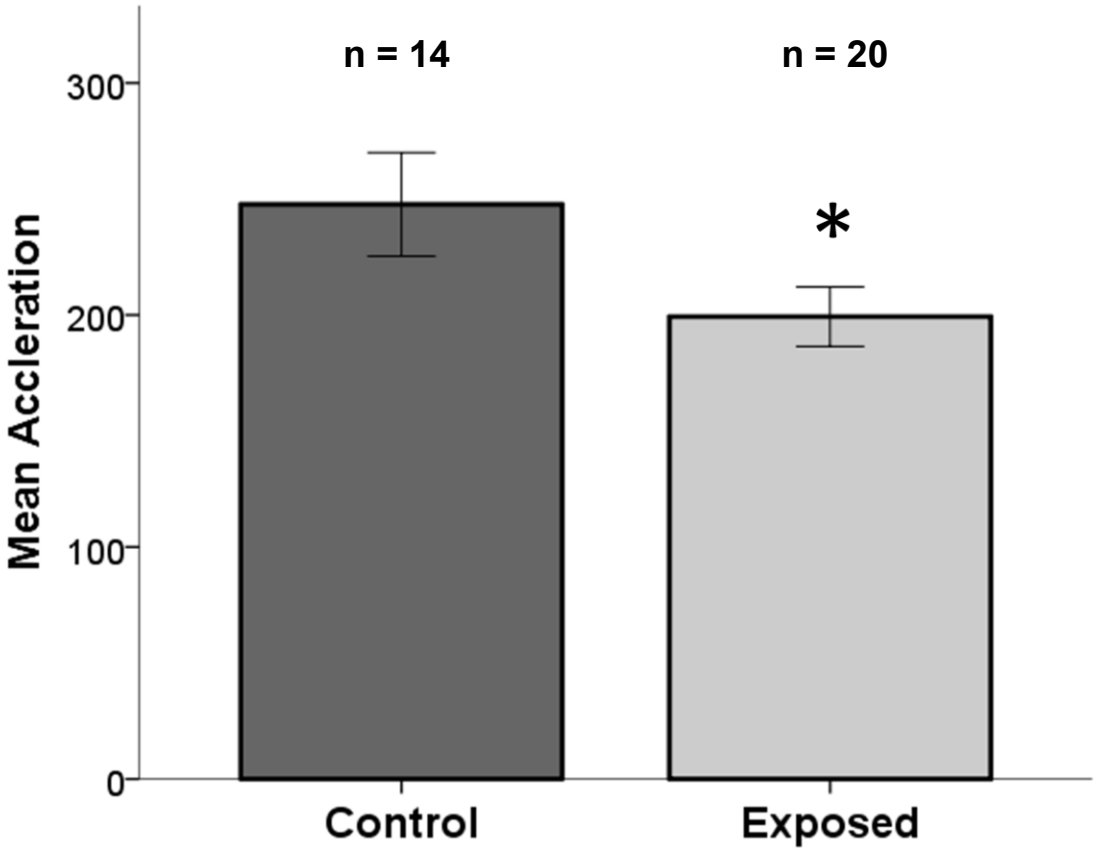

Figure 12. Effects of TFM on acceleration of YOY lake sturgeon. Exposed fish were held for 12 hours in nominal TFM concentrations of 6.5 mg/L (water pH 8.5), 7.5 mg/L (water pH 8.6) or 8.6 mg/L (water pH 8.7). Control fish were held for 12 hours in clean water. An asterisk denotes a significant difference between control fish and other category. Data are expressed as the mean +/- one standard error.

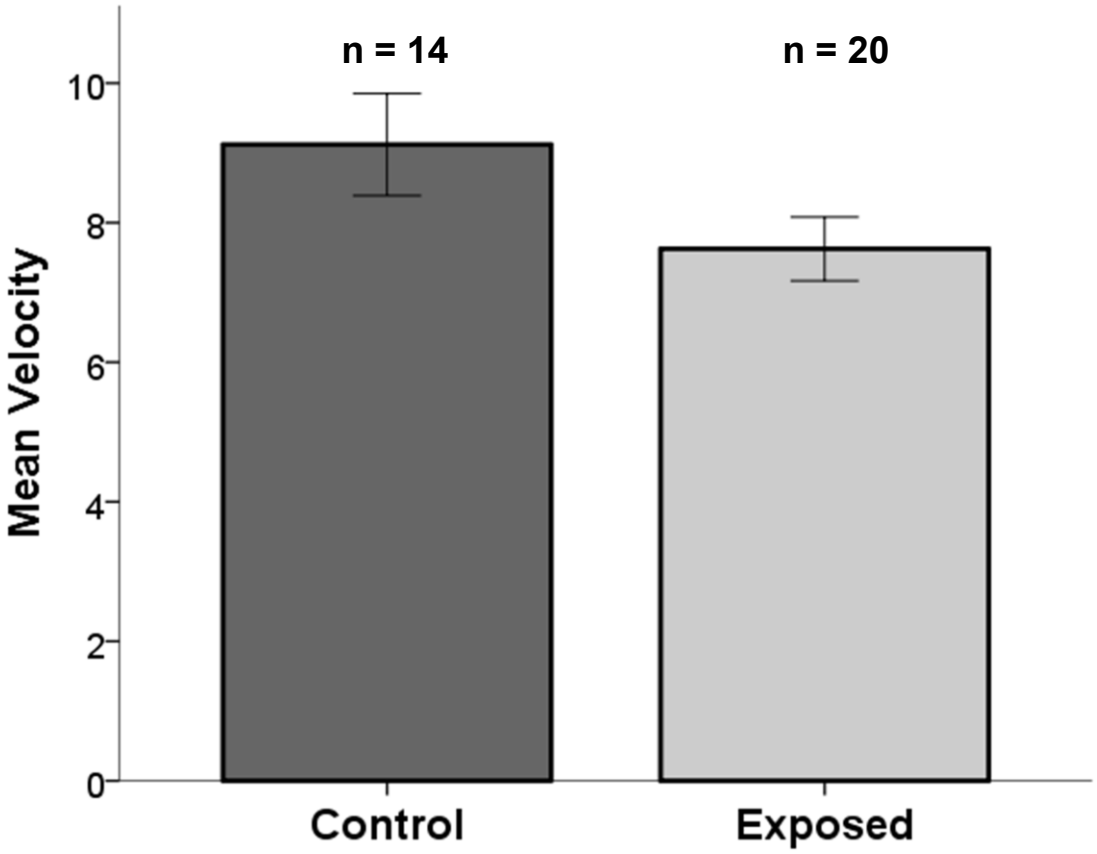

Figure 13. Effects of TFM on the velocity of YOY lake sturgeon. Exposed fish were held for 12 hours in nominal TFM concentrations of 6.5 mg/L (water pH 8.5), 7.5 mg/L (water pH 8.6) or 8.6 mg/L (water pH 8.7). Control fish were held for 12 hours in clean water. Data are expressed as the mean +/- one standard error.

BLOOD GLUCOSE AND LACTATE

Applied at environmentally relevant concentrations (1.0 MLC), TFM induced mortality in eight of the ten exposed fish. There were no further deaths after blood was drawn.

There was no statistical difference in blood glucose concentrations between fish exposed to TFM and control fish (**U** = 2.5, ***p*** = 0.12; Fig. 14). However, this lack of difference may be attributable to the small sample size (n = 2) of the exposure group owing to mortalities.

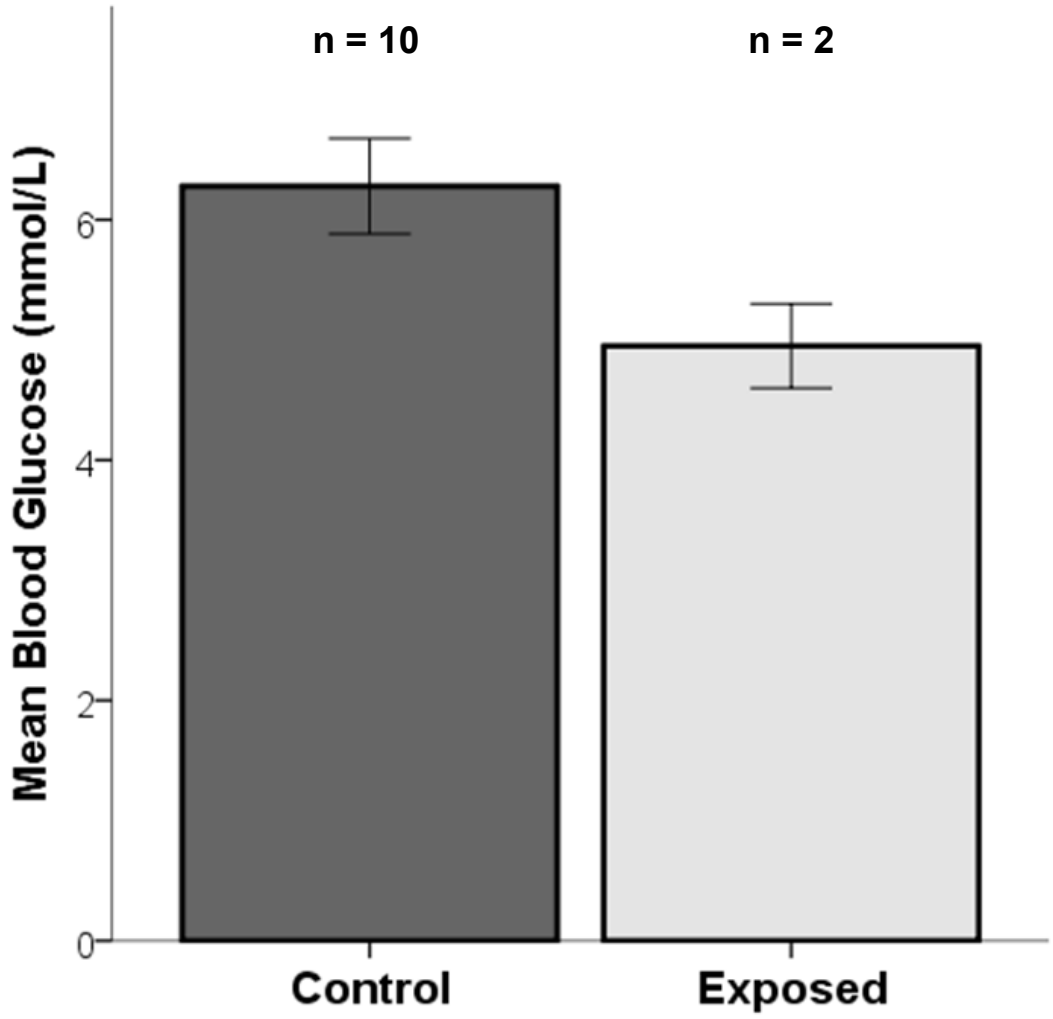

Figure 14. Comparison of the effects of TFM on blood glucose levels of hatchery reared YOY lake sturgeon. Exposed fish were held for 12 hours in nominal TFM concentrations of 6.5 mg/L (water pH 8.5), 7.5 mg/L (water pH 8.6) or 8.6 mg/L (water pH 8.7). Control fish were held for 12 hours in clean water. Error bars denote +/- one standard error.

There was no detectable difference in blood lactate concentrations between TFM-exposed and control fish because all but one reading was at or below the detection limit (DL) of the instrument (DL = 0.3 mmol/L).

CHEMOSENSORY BAIT ASSAYS

More visits by potential predators occurred for lamprey ammocoete-conditioned baits, than for control baits ($\chi^2_1 = 19.70$, $p < 0.01$; Fig. 15). Individuals observed at the conditioned baits included nineteen sticklebacks (*Gasterosteidae spp.*), seven crayfish (*Orconectes spp.*), four trout (*Oncorhynchus spp.*), one shiner (*Notropis spp.*) and one sculpin (*Cottus spp.*), whereas only crayfish were observed at the control baits. Sticklebacks, trout and shiner were observed in the Cypress River using the underwater camera. Crayfish and sculpin were observed in minnow traps in the Mission Island embayment and crayfish were observed in minnow traps in the Black Sturgeon River. No sturgeon were observed at any baits.

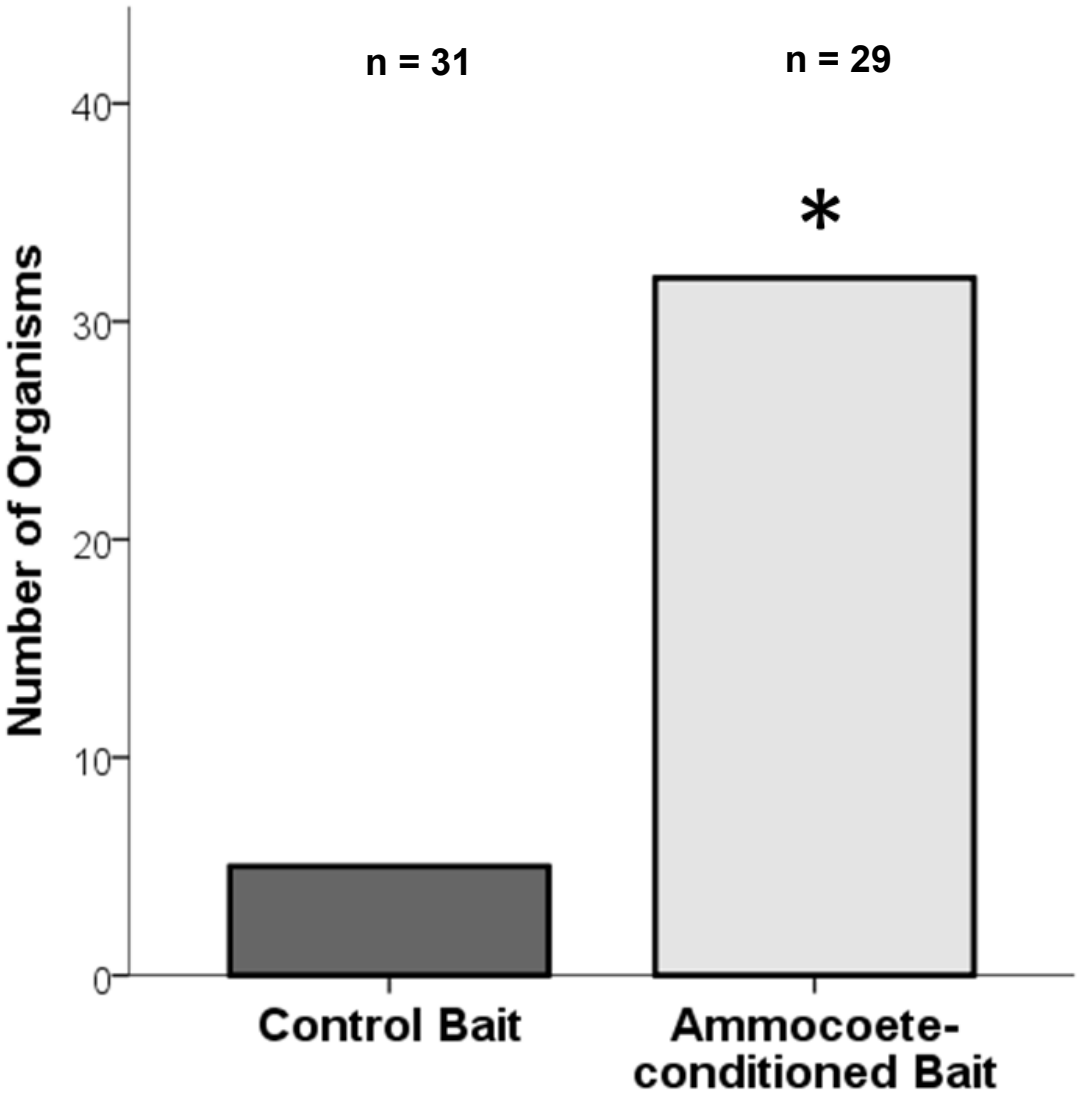

Figure 15. Number of organisms attracted to chemosensory baits conditioned with lamprey ammocoetes compared to control (unconditioned) baits. An asterisk denotes a significant difference between control fish and other category. Data are expressed as the mean +/- one standard error.

DISCUSSION

EFFECTS OF EXPOSURE TO TFM

Both neurophysiological and behavioural assays demonstrated sub-lethal effects of the lampricide TFM on YOY lake sturgeon. The neurophysiological results (via EOG measurements) suggest that YOY lake sturgeon exposed to TFM had an impaired response to L-alanine, taurocholic acid and food cues. Behaviour trials showed that fish exposed to TFM had difficulty detecting food cues and ate less food. Fish exposed to TFM were active for longer periods of time, with a reduction in acceleration. Although able to detect the scent of TFM in EOG assays, YOY sturgeon were unable to avoid it in behavioural trials.

Exposure to aquatic contaminants has been found to impair olfaction and alter behaviour in other fish species (Tierney et al. 2010). For example, environmentally relevant copper concentrations impaired olfaction in fathead minnows (*Pimephales promelas*) and reduced behavioural responses to food stimuli (Green et al. 2010). An environmentally realistic mixture of pesticides reduced the EOG response in rainbow trout (*Oncorhynchus mykiss*) (Tierney et al. 2008). The water-soluble herbicide glyphosate produced EOG reductions in coho salmon (*Oncorhynchus kisutch*) (Tierney et al. 2006). Although the detrimental effects of metals and organic pollutants have been studied for many years, there has been no research on the effects of TFM on any component of chemosensation in fish. It is possible that other Great Lakes tributary fishes may be affected by TFM as well. An increasing number of studies have found that low, non-toxic concentrations of chemicals have potential to disrupt the transfer of chemical information, which could have implications for ecosystem functioning and conservation management (Lürling and Scheffer 2007). Bayluscide, the other

component to some lampricide assessments and treatments, has not been studied and may also have an impact on chemosensation and behaviour. Further research is needed to fully understand and interpret the impacts of lampricide treatment on fish behaviour and ecosystem functioning.

The specificity of L-alanine to microvillous olfactory sensory neurons (OSNs) and TCA to ciliated OSNs allowed me to determine that exposure to TFM at environmentally relevant concentrations caused impairment to both types of sensory neurons. The EOG responses to an amino acid (L-alanine) and bile salt (TCA) are consistent with many other studies examining the effects of exposure to environmentally relevant levels of pesticides and metals on olfaction in fish (summarized in Tierney et al. 2010). However, not all pesticides and metals are toxic to both olfactory sensory neurons studied here. Nickel was found to impair microvillous OSNs, while copper impaired ciliated OSNs in fathead minnows and yellow perch (Dew et al. 2014). Impairment of both of these OSNs may increase the impact of TFM on the behaviour of YOY lake sturgeon.

Since microvillous OSNs are known to respond to amino acids (Sato and Suzuki 2001) related to food seeking behaviour (Hamani and Doving 2007), the altered behavioural responses to the scent and consumption of food may be connected to the impairment of microvillous OSNs. The impaired EOG response to the food cue further supports this connection. Ciliated OSNs are known to respond to bile salts such as TCA (Sato and Suzuki 2001), which are used in migration and alarm response (Hamdani and Doving 2007). The impairment of ciliated OSNs to TCA raises concern that there may also be a behaviour change related to migration and alarm response. Sturgeon are known to have a third type of OSN called ‘crypt cells’ (Hansen and Finger 2000) which respond to sex pheromones (Hamdani and Doving 2007). My results suggest there is a general olfactory impairment which may affect these OSNs as well. Further work is

required to determine if TFM affects chemosensory detection of sex cues and associated behaviours.

The results of activity analysis revealed an increased amount of active time, combined with a decrease in acceleration in exposed fish. Swimming traits can be critical to the survival of young fish and in combination with impaired chemosensory traits, may affect their ability to survive. Modification of olfactory and swimming traits caused by TFM may have an impact on the survival of YOY lake sturgeon in their environs. There are few studies on the effects of other aquatic toxicants on behavioural traits of fish. Juvenile rainbow trout had an impaired alarm response after exposure to copper nanoparticles, eliminating their typical freeze response and reducing swimming activity (Sovová et al. 2014). Mummichog (*Fundulus heteroclitus*) embryos exposed to methylmercury swam greater distances, which increased predation, potentially by attracting the predator to the increased activity (Weis and Weis 1995). Reduced swimming behaviour has also been related to failure to avoid predation in this same species (Zhou and Weis 1998). The change in swimming behaviour found in YOY lake sturgeon in my study may increase their risk of predation by attracting attention to increased movement and reducing their ability to escape due to slower acceleration. This risk may be increased if their ability to detect predators has been diminished due to olfactory impairment.

I found that YOY lake sturgeon were able to detect but did not avoid the scent of TFM. This is not a typical response, as most metals and pesticides cause an avoidance response in fish (Tierney et al. 2010). However, a few studies found no reaction to environmentally relevant concentrations of toxicants, as found here. Sheepshead minnow (*Cyprinodon variegatus*) had no reaction to Dursban (chlorpyrifos), Malathion or Sevin (carbaryl) insecticides (Hansen 1969) or Aroclor (PCB mix) (Hansen et al. 1974).

Mosquitofish (*Gambusia affinis*) had no reaction to the insecticides Endrin (Hansen et al. 1972) and DDT (Kynard 1974). Golden shiners (*Notemigonus crysoleucas*) had no reaction to either cadmium or selenium (Hartwell et al. 1989). The inability to avoid a toxicant may result in real biological effects. In this case, YOY lake sturgeon may not avoid exposure to TFM and subsequent injury, even when given a choice, resulting in abnormal behaviour that could impact their growth and survival.

Applied at environmentally relevant concentrations, TFM induced mortality in eight of the ten exposed YOY lake sturgeon assigned to blood glucose and lactate assays. Death of YOY lake sturgeon from exposure to TFM has been found in other studies as well. The concentrations of TFM that produced 50% mortality in a study by Boogaard et al. (2003) were at or near the minimal lethal concentrations required for effective control of lamprey ammocoetes. A more recent study found 20% mortality at similar concentrations of TFM (Pratt et al. 2012). My study based TFM concentrations on the same criteria used by others; the minimal lethal concentration required for effective control of lamprey ammocoetes, following tables provided in Bills et al. (2003). I exposed forty-five YOY sturgeon to this concentration of TFM, but had no other deaths due to exposure to TFM. The overall mortality in my study was 18%, which is very close to the mortality found by Pratt et al. (2012).

The blood glucose levels measured in this study were within the range found by others in sturgeon species using older fish (Cataldi et al. 1998, Webb et al. 2007, DiMarco et al. 2011, Aramli et al. 2014). Lactate levels were low to non-detectable in both control and exposed fish. Lactate readings found in this study were comparable to those found by Webb et al. (2007) on pallid sturgeon (*Scaphirhynchus albus*), but lower than those found in other sturgeon species (Barton et al. 2000, Kieffer et al. 2001, Baker et al. 2005).

This research was conducted with a limited number of fish over a short period of time at a seasonal facility, so study of the duration of these effects of TFM on YOY lake sturgeon could not be researched and is unknown at this time. All of my assays were conducted in clean water and produced measurable changes in EOG response and behaviour, demonstrating that olfactory recovery was not immediate. Other studies have found a wide variation in the recovery of fish to toxicants. Yellow perch (*Perca flavescens*) showed a rapid olfactory recovery from metal contaminated lakes when exposed to clean water for only a few hours (Azizishirazi et al. 2013). In contrast, cadmium caused cell death in the olfactory tissue of developing zebrafish embryos (*Danio rerio*) (Blechinger et al. 2007). Alkyl benzene sulphonate surfactants caused impaired olfaction in yellow bullhead (*Ictalurus natalis*) that was not repaired within 6 weeks of exposure (Bardach et al. 1965). Future research is required to determine the duration of impaired olfactory and behavioural responses of YOY lake sturgeon from exposure to TFM so that the full potential impacts on the local ecosystem can be assessed. The degree and duration of impairment may vary with the age of the fish, and therefore also requires further study.

CHEMOSENSORY BAITS

There was a significant difference in the number of organisms observed at the ammocoete-conditioned baits compared to the control baits. However, no sturgeon were observed at either type of bait. Although sturgeon were the focus of this research, I have shown that other aquatic predators; sticklebacks, crayfish, trout, shiners and sculpin are attracted to the scent of lamprey ammocoetes.

All of these species are carnivorous, known to eat a variety of aquatic insect larvae, small fish and fish eggs (Scott and Crossman 1973, Momot 1995). Stickleback, shiner, trout and sculpin species have also been reported to prey on ammocoetes and/or

eggs of lamprey species including sea lamprey (Cochran 2009). Crayfish are known to be a keystone predator, resorting to herbivory only when animal protein sources are exhausted (Momot 1995). All of these species are known to use olfaction to locate food (Willman et al. 1994, Hara 2006, Johannesen et al. 2012, Quinn et al. 2012, Wasylenko et al. 2014). Ammocoetes of all lamprey species inhabit similar habitats (Hardisty 2006). Invasion by sea lamprey into the upper Great Lakes replaced the ammocoetes of native species with sea lamprey ammocoetes in some locations (Torblaa and Westman 1980). Predation on ammocoetes by fish predators may have occurred prior to the invasion of sea lamprey into the upper Great Lakes as observed in other places in the world (Cochran 2009).

Mortality of sticklebacks, trout, shiners and sculpins during TFM treatments has been well-documented, along with other potential ammocoete predators such as catfish species (*Ictalurid spp*.), sucker species (*Catostomus spp*.) and mudpuppies (*Necturus maculosus*) (Dahl and McDonald 1980, Gilderhus and Johnson, 1980, Boogaard et al. 2003). Assessments of sea lamprey ammocoetes numbers using Bayluscide have also been known to cause mortalities of bottom-dwelling minnow species among others (Dahl and McDonald 1980). Crustaceans are known to be very resistant to TFM, and crayfish are the most resistant of crustaceans (Gilderhus and Johnson 1980). It is plausible that the olfactory abilities of the survivors may be impaired in a similar manner to my findings in YOY sturgeon in this research, which would make it more difficult to find food. More research is needed to fully understand the ecological effects of TFM and Bayluscide on predator species in Great Lakes tributaries.

If there is a reduction in the number of ammocoete predators and a diminished ability to find food, there could be an increased number of ammocoetes surviving to adulthood, inflating the counts during ammocoetes assessments. The abundance of sea

lamprey ammocoetes increased after initial successive sea lamprey control treatments in some streams supporting this hypothesis (Torblaa and Westman 1980).

Other research has shown that the ability of a species to invade new habitat can decrease with increased species richness and density of resident species (Stachowic et al. 1999, Shurin 2000). More predator species reduce the avenues of escape open to prey (Levin et al. 2009). Even the non-consumptive effects of predators can influence growth and maturity of prey, causing prey to move and forage less, leading to less energy gain and slowed growth (Abrams 1982, Houston et al. 1993, Werner and Anholt 1993, McPeek 2004, Bolnick and Preisser 2005, Preisser et al. 2005, Mittelbach 2012). An increased mortality risk in the juvenile stage, such as ammocoetes, can favour earlier maturity, which means maturing at a smaller size (Abrams and Rowe 1996). This effect could lead to an increase in the number of adults being preyed upon as their smaller size may be more suitable for consumption by a greater number of predators. Sea lamprey in the Great Lakes are larger since the commencement of chemical treatments (Torblaa and Westman 1980). This may be a due to the reduction of numbers and efficiency of predators due to chemical treatments used for sea lamprey control.

The lack of sturgeon or other large benthic feeders attracted to the ammocoete conditioned bait may have been due to factors in the study design. This study used baits that consisted of only olfactory cues and did not contain live organisms. Fish may use a combination of senses to find food in addition to olfaction, such as auditory and electrosensory cues. Olfaction may help to orient a predator to a recent location of prey, but the addition of auditory and electrosensory cues would add information about the actual presence of prey. Also, since ammocoetes are known to move by hydrologic processes during storm events, they may be carried in deeper water by the current, and consumed by predators in pools; not in shallow water. Further investigation into the

attraction of native benthic stream predators to lamprey ammocoetes and eggs would be a worthwhile endeavour to gain a better understanding regarding their potential role in Great Lakes ecosystems.

CONCLUSIONS

The goal of this research was to increase the knowledge related to sea lamprey control from an ecological perspective, but no one research project can answer all of the questions. The sub-lethal impacts of TFM on chemosensation and behaviour in other aquatic species still needs to be discovered, looking in particular at the duration of these effects and ontogenic factors. Also, a better understanding is needed of the ability of native predator species to include ammocoetes as prey, so that they can be used to support sea lamprey control management.

There are very few examples of alien species that have been successfully eradicated once an invasion is well established. Ecosystem management, including the enhancement of predator species populations, may target the overall condition of the ecosystem rather than the individual alien species, by focusing on ecosystem processes. I hope that my research will add to the knowledge of Great Lakes ecosystems and be useful in the development of more ecologically based sea lamprey control management.

REFERENCES

Abrams, P.A. 1982. Functional responses of optimal foragers. *American Naturalist* **120**: 382-390.

Abrams, P.A., Rowe, L. 1996. The effects of predation on the age and size of maturity of prey. *Evolution* **50**: 1052-1061.

Aramli, M.S., Kalbassi, M.R., Nazari, R.M. 2014. Selected biochemical parameters in plasma of blood and semen of Persian sturgeon, *Acipenser persicus*. *Comparative Clinical Pathology* **23**: 1241-1245.

Assis, J., Claro, B., Ramos, A., Boavida, J., Serrão, E.A. 2013. Performing fish counts with a wide-angle camera, a promising approach reducing diver's limitations. *Journal of Experimental Marine Biology and Ecology.* **445**: 93-98.

Auer, N.A. 1999. Population characteristics and movements of lake Sturgeon in the Sturgeon River and Lake Superior. *Journal of Great Lakes Research* **25**: 282-293.

Azizishirazi, A., Dew, W.A., Forsyth, H.L, Pyle, G.G. 2013. Olfactory recovery of wild yellow perch from metal contaminated lakes. *Ecotoxicology and Environmental Safety*. **88**: 42-47.

Baker, D.W., Wood, A.M., Litvak, M.K., Kieffer, J.D. 2005. Haematology of juvenile *Acipenser oxyrinchus* and *Acipenser brevirostrum* at rest and following forced activity. *Journal of Fish Biology* **66**: 208-221.

Bardach, J.E., Fujiya, M., Holl, A., 1965. Detergents: effects on the chemical senses of the fish *Ictalurus natalis*. *Science* **148**: 1605–1607.

Barton, B.A., Bollig, H., Hauskins, B.L., Jansen, C.R. 2000. Juvenile pallid (*Scaphirhynchus albus*) and hybrid pallid X shovelnose (*S. albus X platorynchus*) sturgeons exhibit low physiological responses to acute handling and severe confinement. *Comparative Biochemistry and Physiology* **126A**: 125-134.

Basset, D.K., Montgomery, J.C. 2011. Investigating nocturnal fish populations *in situ* using baited underwater video: with special reference to their olfactory capabilities. *Journal of Experimental Marine Biology and Ecology* **409**: 194-199.

Benton, A.C., Sutton, T.M., Elliott, R.F., Meronek, T.G. 2005. Seasonal movement patterns and habitat preferences of age-0 lake sturgeon in the lower Pestigo River, Wisconsin. *Transactions of the American Fisheries Society* **134**: 1400-1409.

Bills, T.D., Boogaard, M.A., Johnson, D.A., Brege, D.C., Scholefield, R.J., Westman, R.W., Stephens, B.E. 2003. Development of a pH/alkalinity treatment model for applications of the lampricide TFM to streams tributary to the Great Lakes. *Journal of Great Lakes Res*earch **29**: 510-520.

Birceanu, O., McClelland, G.B., Wang, Y.S., Wilkie, M.P. 2009. Failure of ATP supply to match ATP demand: The mechanism of toxicity of the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), used to control sea lamprey (*Petromyzon marinus*) populations in the Great Lakes. *Aquatic Toxicology.* **94**: 265-274.

Birceanu, O., McClelland, G.B., Wang, Y.S., Brown, J.C.L., Wilkie, M.P. 2011. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) uncouples mitochondrial oxidative phosphorylation in both sea lamprey (*Petromyzon marinus*) and TFM-tolerant rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology, Part C* **153**: 342-349.

Blechinger, S.R., Kusch, R.C., Haugo, K., Matz, C., Chivers, D.P., Krone, P.H. 2007. Brief embryonic cadmium exposure induces a stress response and cell death in the developing olfactory system followed by long-term olfactory deficits in juvenile zebrafish. *Toxicology and Applied Pharmacology* **224**: 72-80.

Bolnick, D.I., Preisser, E.I. 2005. Resource competition modifies the strength of trait-mediated predator-prey interactions: a meta-analysis. *Ecology* **86**: 2771-2779.

Boogaard, M.A., Bills, T.D., Johnson, D.A. 2003. Acute toxicity of TFM and TFM/Niclosamide mixture to selected fish, including lake sturgeon (*Acipenser fulvescens*) and mudpuppies (*Necturus maculosus*), in laboratory and field exposures. *Journal of Great Lakes Research* **29**: 529-541.

Brege, D.C., Davis, D.M., Genovese, J.H., McAuley, T.C., Stephens, B.E., Westman, R.W. 2003. Factors responsible for the reduction in quantity of the lampricide, TFM, applied annually in streams tributary to the Great Lakes from 1979 to 1999. *Journal of Great Lakes Research* **29**: 500-509.

Brown, J.A., Watson, J., Bourhill, A., Wall, T. 2008. Evaluation and use of the Lactate Pro, a portable lactate meter, in monitoring the physiological well-being of farmed Atlantic cod (*Gadus morhua*). *Aquaculture* **285**: 135-140.

Brown, L.R., Chase, S.D., Mesa, M.G., Beamish, R.J., Moyle, P.B. 2009. Biology, Management and Conservation of Lampreys in North America (Ed.). *Proceedings of the American Fisheries Society Symposium 72.* American Fisheries Society, Bethesda, Maryland, USA.

Bryan, M.B., Zalinski, D., Filcek, K.B., Libants, S., Li, W., Scribner, K.T. 2005. Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus*) in North America as revealed by microsatellite genotypes. *Molecular Ecology* **14**: 3757-3773.

Carr, W.E.S., Netherton, J.C., Gleeson, R.A., and Derby, C.D. 1996. Stimulants of feeding behavior in fish: analyses of tissues of diverse marine organisms. *Biological Bulletin* **190**: 149-160.

Cataldi, E., Di Marco, P., Mandich, A., Cataudella, S. 1998. Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): effects of temperature and stress. *Comparative Biochemistry and Physiology Part A*, **121**: 351-354.

Close, D.A., Fitzpatrick, M.S., Li, H.W. 2002. The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. *Fisheries* **27**: 19-25.

Coble, D.W., Bruesewitz, R.E., Fratt, T.W., Scheirer, J.W. 1990. Lake Trout, Sea Lampreys and Overfishing in the Upper Great Lakes: A Review and Reanalysis. *Transactions of the American Fisheries Society* **119**: 985-995.

Cochran, P.A. 2009. Predation on Lampreys. *American Fisheries Society Symposium* **72**: 139-151.

Daghfous, G., Green, W.W., Zielinski, B.S., and Dubuc, R. 2012. Chemosensory-induced motor behaviors in fish. *Current Opinion in Neurobiology.* **22**: 223-230.

Dahl, F.H., McDonald, R.B. 1980. Effects of control of the sea lamprey (*Petromyzon marinus*) on migratory and resident fish populations. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 1886-1894.

Dew, W.A., Azizishirazi, A., Pyle, G.G. 2014. Contaminant-specific targeting of olfactory sensory neuron classes: connecting neuron class impairment with behavioural deficits. *Chemosphere* **112**: 519-525.

Dick, T.A., Jarvis, S.R., Sawatzky, C.D., Stewart, D.B. 2006. *The lake sturgeon, Acipenser fulvescens, (Chondrostei: Acipenseridae): an annotated bibliography.* Canadian Technical Report of Fisheries and Aquatic Sciences 2671. Central and Arctic Region. Fisheries and Oceans Canada. Winnipeg, Manitoba.

Dingle, H., Holyoak, M. 2001. The evolutionary ecology of movement. In: Fox, C.W., Roff, D.A., Fairbairn, D.J. (eds) Evolutionary ecology: concepts and case studies. Oxford University Press, Toronto, pp 247-261.

Di Marco, P., Priori, A., Finoia, M.G., Petochi, T., Longobarki A., Donadelli, V., Marino, G. 2011. Assessment of blood chemistry reference values for cultured sturgeon hybrids (*Acipenser naccarii* female *X Acipenser baerii* male). *Journal of Applied Ichthyology* **27**: 584-590.

Eames, S.C., Philipson, L.H., Prince, V.E., Kinkel, M.D. 2010. Blood sugar measurement in zebrafish reveals dynamics of glucose homeostasis. *Zebrafish* **7**: 205-213.

Eshenroder R.L. 2009. Comment: Mitochondrial DNA analysis indicates sea lampreys are indigenous to Lake Ontario. *Transactions of the American Fisheries Society* **138**: 1178-1189.

Fodale, M.F., Bronte, C.R., Bergstedt, R.A., Cuddy, D.W., Adams, J.V. 2003. Classification of lentic habitat for sea lamprey (Petromyzon marinus) larvae using a remote seabed classification device. *Journal of Great Lakes Research* **29**: 190-203.

Gilderhus, P.A., Johnson, B.G.H. 1980. Effects of Sea Lamprey (*Petromyzon marinus*) control in the Great Lakes on aquatic plants, invertebrates and amphibians. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 1895-1905.

GLFC 2005. *Sea Lamprey Control in the Great Lakes 2004: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, http://www.glfc.org/sealamp/ANNUAL_REPORT_2004.pdf

GLFC 2006. *Sea Lamprey Control in the Great Lakes 2005: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, http://www.glfc.org/sealamp/ANNUAL_REPORT_2005.pdf

GLFC 2007. *Sea Lamprey Control in the Great Lakes 2006: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, http://www.glfc.org/sealamp/ANNUAL_REPORT_2006.pdf

GLFC 2008. *Sea Lamprey Control in the Great Lakes 2007: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, http://www.glfc.org/sealamp/ANNUAL_REPORT_2007.pdf

GLFC 2009. *Sea Lamprey Control in the Great Lakes 2008: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, http://www.glfc.org/sealamp/ANNUAL_REPORT_2008.pdf

GLFC 2010. *Sea Lamprey Control in the Great Lakes 2009: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, http://www.glfc.org/sealamp/ANNUAL_REPORT_2009.pdf

Great Lakes Fishery Commission (GLFC) 2011A. *Strategic Vision of the Great Lakes Fishery Commission 2011-2020*. Great Lakes Fishery Commission, Ann Arbor, Michigan, 48150-1563. 17p.

GLFC 2011B. *Sea Lamprey Control in the Great Lakes 2010: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, http://www.glfc.org/sealamp/ANNUAL_REPORT_2010.pdf

GLFC 2012A. *Protocol for Application of Lampricides to Streams with Populations of Young-of-the-Year Lake Sturgeon (Acipenser fulvescens).* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, TOP:011.9A http://www.glfc.org/sealamp/sop/top/top%20011A%20sturgeon%20protocol.pdf.

GLFC 2012B. *Procedures for the Application of TFM* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA, TOP:012.4. http://www.glfc.org/sealamp/sop/top/top%20012%20TFM%20application.pdf

GLFC 2012C. *Sea Lamprey Control in the Great Lakes 2011: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA,

http://www.glfc.org/sealamp/ANNUAL_REPORT_2011.pdf

GLFC 2013. *Sea Lamprey Control in the Great Lakes 2012: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA,
http://www.glfc.org/sealamp/ANNUAL_REPORT_2012.pdf

GLFC 2014. *Sea Lamprey Control in the Great Lakes 2013: Annual Report to the Great Lakes Fishery Commission.* Great Lakes Fishery Commission, Ann Arbor, Michigan, USA,
http://www.glfc.org/pubs/misc/2013SLCAnnualReportFinal.pdf

Green, W.W., Mirza, R.S., Wood, C.M., Pyle, G.G. 2010. Copper binding dynamics and olfactory impairment in fathead minnows (*Pimephales promelas*). *Environmental Science and Technology* **44**: 1431-1437.

Hamdani, E.H., Doving, K.B. 2007. The functional organization of the fish olfactory system. *Progress in Neurobiology.* **82**: 80-86.

Hansen, A., Finger, T.E. 2000. Phyletic distribution of crypt-type olfactory receptor neurons in fishes. *Brain Behaviour and Evolution* **55**: 100 -110.

Hansen, D.J. 1969. Avoidance of pesticides by untrained sheepshead minnows. *Transactions of the American Fisheries Society* **98**: 426-429.

Hansen, D.J., Matthews, E., Nall, S.L., Dumas, D.P. 1972. Avoidance of pesticides by untrained mosquitofish, *Gambusia affinis*. *Bulletin of Environmental Contamination and Toxicology* **8**: 46-51.

Hansen, D.J., Schimmel, S.C., Matthews, E. 1974. Avoidance of aroclor® 1254 by shrimp and fishes. *Bulletin of Environmental Contamination and Toxicology* **12**: 253-256.

Hara, T.J. 1975. Olfaction in fish. *Progress in Neurobiology* **5**: 271-335.

Hara, T.J. 2006. Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. *Journal of Fish Biology* **68**: 810-825.

Hardinge, J., Harvey, E.S., Saunders, B.J., Newman, S.J. 2013. A little bait goes a long way: the influence of bait quality on a temperate fish assemblage sampled using stereo-BRUVs. *Journal of Experimental Marine Biology and Ecology* **449**: 250-260.

Hardisty, M.W., Potter, I.C. 1971. The behaviour, ecology and growth of larval lampreys. In *The Biology of Lampreys*, Vol. 1 (Eds.) pp. 127-206. London: Academic Press.

Hardisty, M.W. 2006. *Lampreys: Life without jaws.* (Eds.) Forrest Text. ISBN 0-9550740-6-1 Sŵn y Nant, Tresaith, Cardigan, Ceredigion SA43 2JG, UK. 272 p.

Hartwell, S.I., Jin, J.H., Cherry, D.S., Cairns, J. 1989. Toxicity versus avoidance response of golden shiner, *Notemigonus crysoleucas*, to five metals. *Journal of Fish Biology* **35**: 447-456.

Haxton, T.J. 2002. An assessment of lake sturgeon in various reaches of the Ottawa River. *Journal of Applied Ichthyology* **18**: 449-454.

Holeck, K.T., Mills, E.L., MacIsaac, H.J., Dochoda, M.R., Colautti, R.I., Ricciardi, A. 2004. Bridging troubled waters: biological invastions, transoceanic shipping and the Laurentian Great Lakes. *BioScience* **54**: 919-929.

Houston, A.I., MacNamara, J.M., Hutchinson, J.M.C. 1993. General results concerning the trade-off between gaining energy and avoiding predators. *Philosophical Transactions of the Royal Society of London B* **341**: 375-397.

Hunn, J.B., Allen, J.L. 1974. Movement of drugs across the gills of fishes. *Annual Review of Pharmacology and Toxicology* **14**: 47-54.

IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Johannesen, A., Dunn, A.M., Morrell, L.J. 2012. Olfactory cue use by three-spined sticklebacks foraging in turbid water: prey detection or prey location? *Animal Behaviour* **84**: 151-158.

Kasumyan, A.O. 1999. Olfaction and taste senses in sturgeon behaviour. *Journal of Applied Ichthyology* **15**: 228-232.

Kieffer, J.D., Wakefield, A.M., Litvak, M.K. 2001. Juvenile sturgeon exhibit reduced physiological responses to exercise. *Journal of Experimental Biology* **204**: 4281-4289.

Kircheis, F.W. 2004. Sea Lamprey White Paper. U.S. Fish & Wildlife Service, Gulf of Maine Coastal Program. http://www.fws.gov/GOMCP/pdfs/lampreyreport.pdf

Kobayashi, R., Endo, M., Yoshizaki, G., Takeuchi, T. 2002. Sensitivity of tilapia to infrared light measured using a rotating striped drum differs between two strains. *Nippon Suisan Gakkai* **68**: 645-651.

Kynard, B. 1974. Avoidance behaviour of insecticide susceptible and resistant populations of mosquitofish to 4 insecticides. *Transactions of the American Fisheries Society* **103**: 557-561.

Laberge, F., Hara, T.J. 2001. Neurobiology of fish olfaction: a review. *Brain Research Reviews* **36**: 46-59.

Lee, D.S., Weise, J.G. 1989. Habitat selection of lentic larval lampreys: preliminary analysis based on research with a manned submersible. *Journal of Great Lakes Research* **15**: 156-163.

Levin, S.A., Carpenter, S.R., Godfray, H.C.J., Kinzig, A.P., Loreau, M., Losos, J.B., Walker, B., Wilcove, D.S., Morris, C.G. 2009. (Eds.) *The Princeton Guide to Ecology*. Princeton University Press, Princeton, New Jersey.

Lo, Y.H., Bellis, S.L., Cheng, L.J., Pang, J., Bradley, T.M., Rhoads, D.E. 1994. Signal transduction for taurocholic acid in the olfactory system of Altantic salmon. *Chemical Senses* **19**: 371-380.

Lürling, M., Scheffer, M. 2007. Info-disruption: pollution and the transfer of chemical information between organisms. *Trends in Ecology and Evolution* **22**: 374 – 379.

Marentette, J.R., Tong, S., Wang, G., Sopinka, N.M., Taves, M.D., Koops, M.A., Balshine, S. 2012. Behavior as a biomarker? Laboratory versus field movement in round goby (*Neogobius melanostomus*) from highly contaminated habitats. *Ecotoxicology* **21**: 1003-1012.

Martel, G., Dill, L.M. 1995. Influence of movement by coho salmon (*Oncorhynchus kisutch*) parr on their detection by common mergansers (*Mergus merganser*). *Ethology* **99**: 139-149.

Martin, C.W., Fodrie, F.J., Heck Jr., K.L., Mattila, J. 2010. Differential habitat use and antipredator response of juvenile roach (*Rutilus rutilus*) to olfactory and visual cues from multiple predators. *Oecologia* **162**: 893-902.

Matthews, W.J. 1998. *Patterns in freshwater fish ecology.* (Eds.) Kluwer Academic Press, Norwell, Massachusetts.

McDonald, D.G., Kolar, C.S. 2007. Research to guide the use of lampricides for controlling sea lamprey. *Journal of Great Lakes Research* **33**: 20-34.

McPeek, M.A. 2004. The growth/predation risk trade-off: so what is the mechanism? *American Naturalist* **163**: E88-E111.

McDonald, D.G., Kolar, C.S. 2007. Research to guide the use of lampricides for controlling sea lamprey. *Journal of Great Lakes Research* **33**: 20-34.

Mittelbach, G.G. 2012. *Community Ecology.* (Eds.) Sinauer Associates Inc., Sunderland, MA.

Mirza, R.S., Green, W.W., Connor, S., Weeks, A.C.W., Wood, C.M., Pyle, G.G. 2009. Do you smell what I smell? Olfactory impairment in wild yellow perch from metal-contaminated waters. *Ecotoxicology and Environmental Safety* **72**: 677-683.

Momot, W.T. 1995. Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science* **3**: 33-64.

Mullett, K.M., Bergstedt, R.A. 2003. Agreement among observers classifying larval sea lamprey (Petromyzon marinus) habitat. *Journal of Great Lakes Research* **29**: 183-189.

Norris, D.O., Donahue, S., Dores, R.M., Lee, J.K., Maldonado, T.A., Ruth, T., Woodling, J.D. 1999. Impaired adrenocortical response to stress by brown trout, Salmo trutta, living in metal-contaminated waters of the Eagle River, Colorado. *General and Comparative Endocrinology* **113**: 1–8.

OMNR 2011. Recovery Strategy for Lake Sturgeon (Acipenser fulvescens) – Northwestern Ontario, Great Lakes-Upper St. Lawrence River and Southern Hudson Bay-James Bay populations in Ontario. Ontario Recovery Strategy Series. Prepared for the Ontario Ministry of Natural Resources, Peterborough, Ontario. vii + 77 pp.

Peterson, D.L., Vecsei, P., Jennings, C.A. 2007.

Ecology and biology of the lake sturgeon; a synthesis of current knowledge of a threatened North American Acipenseridae. *Review of Fish Biology and Fisheries* **17**: 59-76.

Pratt, T.C., O'Connor, L.M., Steeves, T.B., Stephens, B., Boogaard, M., Kaye, C. 2012. *In situ assessment of lampricide toxicity to age-0 lake sturgeon.* 2012 Project Completion Report. Great Lakes Fishery Commission 38 p.

Preisser, E.I., Bolnick, D.I., Benard, M.F. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology* **86**: 501-509.

Quinn, T.P., Dittman, A.H., Barrett, H., Cunningham, C., Bond, M.H. 2012. Chemosensory responses of juvenile Coho salmon, *Oncorhynchus kisutch*, Dolly

Varden, *Salvelinus malma*, and sculpins (*Cottus* spp.) to eggs and other tissues from adult Pacific salmon. *Environmental Biology of Fishes* **95**: 301-307.

Quintella, B.R., Andrade, N.O., Espanhol, R., Almeida, P.R. 2005. The use of PIT telemetry to study movements of ammocoetes and metamorphosing sea lampreys in river beds. *Journal of Fish Biology* **66**: 97-106.

Rusak, J.A., Mosindy, T. 1997. Seasonal movements of lake sturgeon in Lake of the Woods and the Rainy River, Ontario. *Canadian Journal of Zoology* **74**: 383-395.

Santana-Garcon, J., Newman, S.J., Harvey, E.S. 2014. Development and validation of a mid-water baited stereo-video technique for investigating pelagic fish assemblages. *Journal of Experimental Marine Biology and Ecology* **452**: 82-90.

Sato, K., Suzuki, N., 2001. Whole-cell response characteristics of ciliated and microvillous olfactory receptor neurons to amino acids, pheromone candidates and urine in rainbow trout. *Chemical Senses* **26:** 1145–1156.

Scott, G.R., Sloman, K.A. 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicology* **68**: 369-392.

Scott, J.W., Scott-Johnson, P.E. 2002. The electroolfactogram: a review of its history and uses. *Microscopy Research and Technique* **58**: 152-160.

Scott, W.B., Crossman, E.S. 1973. *Freshwater fishes of Canada.* (Eds.) *Fisheries Research Board of Canada Bulletin 184.*

Shamushaki, V.A.J., Abtahi, B., Kasumyan, A.O. 2011. Olfactory and taste attractiveness of free amino acids for Person sturgeon juveniles, Acipenser persicus: a comparison with other acipenserids. *Journal of Applied Ichthyology.* **27**: 241-245.

Shurin, J.B. 2000. Dispersal limitation, invasion resistance and the structure of pond zooplankton communities. *Ecology* **81**: 3074-3086.

Smith, R.J.F. 1992. Alarm signals in fishes. *Reviews in Fish Biology and Fisheries* **2**: 3–63.

Sovová, T., Boyle, D., Sloman, K.A., Pérez, C.V., Handy, R.D. 2014. Impaired behavioural response to alarm substance in rainbow trout exposed to copper nanoparticles. *Aquatic Toxicology* **152**: 195-204.

Stachowicz, J.J., Whitlatch, R.B., Osman, R.W. 1999. Species diversity and invasion resistance in a marine ecosystem. *Science* **286**: 1577-1578.

Swink, W. D. 1995. *Effect of larval sea lamprey density on growth.* Project Completion Report. Great Lakes Fishery Commission. 17 p.

Tierney, K.B., Ross, P.S., Jarrard, H.E., Delaney, K.R., Kennedy, C.J. 2006. Changes in juvenile coho salmon electro-olfactogram during and after short-term exposure to current-use pesticides. *Environmental Toxicology and Chemistry* **25**: 2809-2817.

Tierney, K.B., Sampson, J.L., Ross, P.S., Sekela, M.A., Kennedy, C.J. 2008. Salmon olfaction is impaired by an environmentally realistic pesticide mixture. *Environmental Science and Technology* **42**: 4996-5001.

Tierney, K.B., Baldwin, D.H., Hara, T.J., Ross, P.S., Scholz, N.L. 2010. Olfactory toxicity in fishes. *Aquatic Toxicology* **96**: 2-26.

Torblaa, R.L., Westmand, R.W. 1980. Ecological impacts of lampricide treatments on sea lamprey (*Petromyzon marinus*) ammocoetes and metamorphosed individuals. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 1835-1850.

Waldman, J.R., Grunwald, C., Roy, N.K., Wirgin, I.I. 2004. Mitochondrial DNA analysis indicates sea lampreys are indigenous to Lake Ontario. *Transactions of the American Fisheries Society* **133**: 950-960.

Ward, A.J.W., Currie, S. Shoaling fish can size-assort by chemical cues alone. *Behavioural Ecology and Sociobiology* **67**: 667-673.

Ward, A.J.W., Holbrook, R.I., Krause, J., Hart, P.J.B. 2005. Social recognition in sticklebacks: the role of direct experience and habitat cues. *Behavioral Ecology and Sociobiology* **57**: 575-583.

Wasylenko, B.A., Callaghan, D.T., Blanchfield, P.J., Pyle, G.G. 2014. *Salvelinus namaycush* spawning substratum attracts egg predators and opportunists through chemosensory cues. *Journal of Fish Biology* **84**: 1562-1573.

Webb, M.A.H., Allert, J.A., Kappenman, K.M., Marcos, J., Feist, G.W., Schreck, C.B., Shackleton, C.H. 2007. Identification of plasma glucocorticoids in pallid sturgeon in response to stress. *General and Comparative Endocrinology* **154**: 98-104.

Werner, E.E., Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *American Naturalist* **19**: 460-470.

Weis, J.S., Weis, P. 1995. Swimming performance and predator avoidance by mummichog (*Fundulus heteroclitus*) larvae after embryonic or larval exposure to methylmercury. *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 2168–2173.

Wells, R.M.G., Pankhurst, N.W. 1999. Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. *Journal of the World Aquaculture Society* **30**: 276-284.

Werner, E.E., Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *American Naturalist* **142**: 242-272.

Willman, E.J., Hill, A.M., Lodge D.M. 1994. Response of three crayfish congeners (*Orconectes spp.*) to odors of fish carrion and live predatory fish. *American Midland Naturalist* **132**: 44-51.

Wisenden, B.D. 2000. *Scents of danger: the evolution of olfactory ornamentation in chemically-mediated predator–prey interactions.* In: Espmark, Y., Amundsen, T., Rosenqvist, G. (Eds.), Animal Signals: Signalling and Signal Design in Animal Communication. Tapir Academic Press, Trondheim, Norway, pp. 365–386.

Zhou, T., Weis, J.S. 1998. Swimming behavior and predator avoidance in three populations of *Fundulus heteroclitus* larvae after embryonic and/or larval exposure to methylmercury. *Aquatic Toxicology* **43**: 131–148.